INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.

University Microfilms International

300 N. ZEEB ROAD, ANN ARBOR, MI 48106
18 BEDFORD ROW, LONDON WC1R 4EJ, ENGLAND

HRABIE, JOSEPH ANTHONY

THE CONVERSION OF ASPERULOSIDE TO AN ANALOG OF THE COREY LACTONE-ALDEHYDE INTERMEDIATE FOR PROSTAGLANDINS

City University of New York PH.D. 1981

THE CONVERSION OF ASPERULOSIDE TO AN ANALOG OF THE COREY LACTONE-ALDEHYDE INTERMEDIATE FOR PROSTAGLANDINS

by

JOSEPH ANTHONY HRABIE

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1981

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirements for the degree of Doctor of Philosophy.

5/4/81
date

William Forst Berkowitz
Chairman of the Examining Committee

7 may 1981
date

David C. Locke
Executive Officer

Jack C. Day

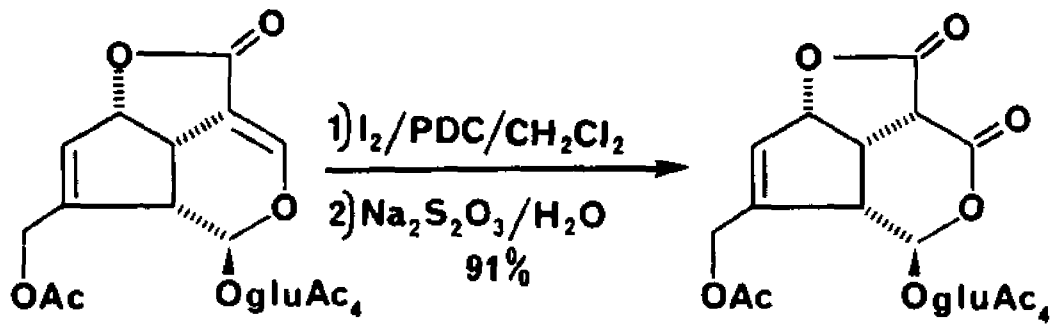
Arthur D Baker

Supervisory Committee

The City University of New York

ABSTRACT

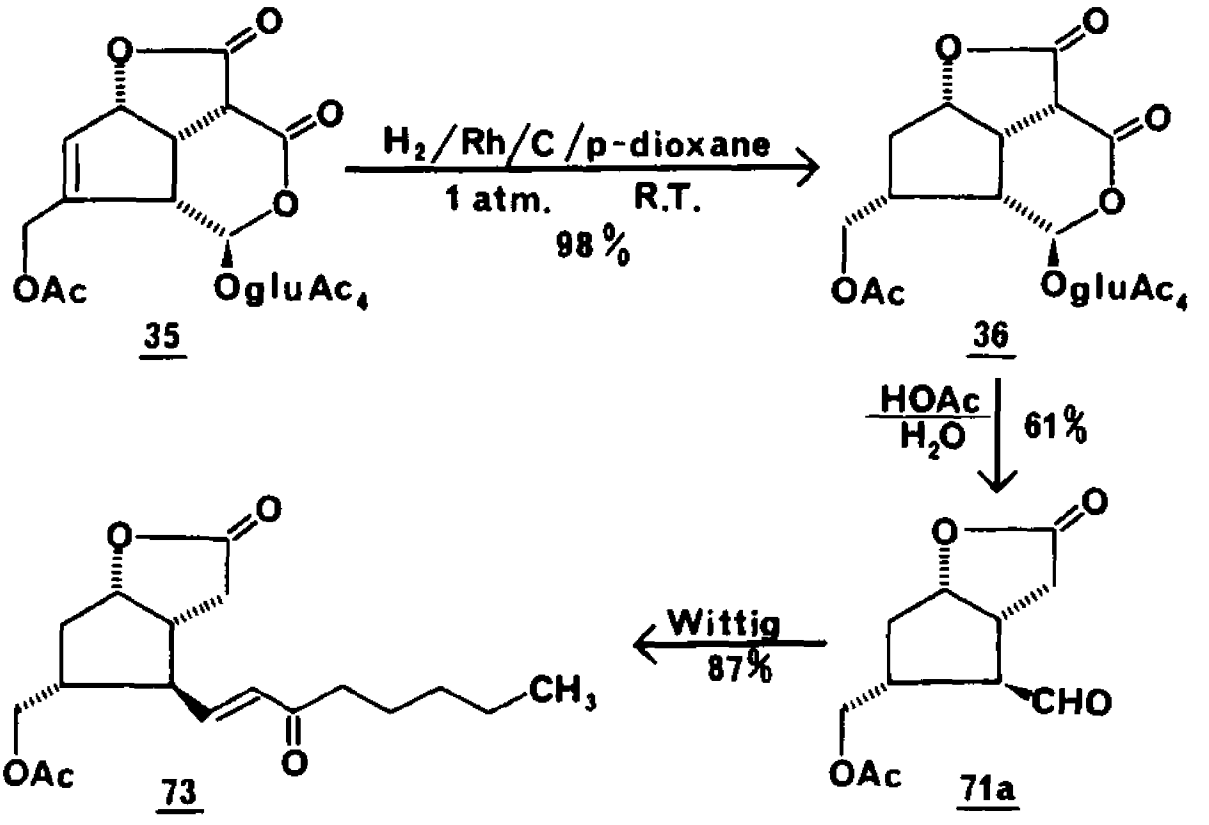
A three-step conversion of asperuloside tetraacetate (34) to the 11-deoxy-11-hydroxymethyl analog 71a of the Corey lactone aldehyde has been developed.



34 35

The key intermediate, dilactone 35, was formed from asperuloside tetraacetate in one step by treatment with iodine/pyridinium dichromate followed by washing with sodium thiosulfate solution. This was then converted to aldehyde 71a by hydrogenation over rhodium on carbon and hydrolysis in refluxing aqueous acetic acid. Wadsworth-Emmons reaction of 71a with dimethyl 2-oxoheptylphosphonate gave 73 in excellent overall yield (from 34).

Since previous workers have converted 71b to PGE_2, $PGF_{2\alpha}$ and their 11-hydroxymethyl homologs, application of similar procedures to 71a should produce the same prostaglandins.



Extensive investigation of the chemistry of asperuloside resulted in the preparation of the previously unreported dibromide (65) and tertiary alcohol (64) as well as other derivatives of this iridoid.

TO MY PARENTS

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the members of the Queens College Chemistry Department for their help throughout the course of this work.

In particular, I thank Professor William Berkowitz for his guidance and encouragement through the years.

I also thank Professor George Axelrad for his friendship, encouragement and advice throughout my undergraduate and graduate education.

Thanks also to my fellow graduate students at City University for making life in the lab. and classroom both painless and enjoyable. In particular, I thank (now Dr.) Satish Choudhry for his help and friendship.

CONTENTS

LIST OF SCHEMES

LIST OF FIGURES

Chapter I

INTRODUCTION TO THE PROSTAGLANDINS

The story of the prostaglandins is now widely known. It stands as the classic example of the benefits to be derived from scientists' pursuit of explanations for what might at first seem to be only trivial curiosities. It also shows the merit of conducting scientific research over the entire spectrum of natural phenomena rather than concentrating the effort in only a few areas which might seem most promising at any one moment in history. The following account is not intended to be all-inclusive and the interested reader is encouraged to seek out the other prostaglandin histories which are available [1].

1.1 EARLY HISTORY OF THE PROSTAGLANDINS

In 1930, two gynecologists observed that fresh human semen induced strips of uterine tissue to relax or contract and noted that the type of response depended upon whether or not the tissue donor had borne children [2]. Shortly thereafter, Goldblatt [3] and von Euler [4] reported that extracts from sheep vesicular glands and human seminal plasma exhibited powerful physiological effects. Specifically, the extracts caused a significant fall in blood pressure when injected

into various animals and stimulated a variety of smooth muscle segments to contract. This effect was at first attributed to some combination of substances already known (adrenaline, histamine and acetylcholine) which had previously been shown to exhibit similar biological activity [5]. Intrigued by the unparalleled potency of the new extracts, von Euler applied the then new technique of using specific inhibitors to differentiate between biologically active substances and showed that the activity of the extracts was a result of some as yet unknown substance rather than an exotic mixture of the already known materials [6]. Von Euler also noted that the amount of this new substance found in any of the other organs of the sheep was always less than one percent of that found in the prostate gland and he named the new substance "prostaglandin" in the mistaken belief that it was produced only in this gland. He was able to convert the chloroform extract of the sheep vesicular glands into a water soluble barium salt which could be isolated as a stable amorphous powder. This material exhibited potent biological activity but was still a mixture of substances and resisted further analysis by the admittedly inadequate techniques available at that time [7].

After these pioneering studies, very little was done to extend prostaglandin research for over twenty years. This was undoubtedly due to several factors acting in concert. The scarcity of material and lack of suitable methods for

purification and characterization certainly played a large role in delaying further work. Another contributing factor was undoubtedly the great promise shown by the early research in the areas of the antibiotics and steroid hormones which were discovered at about the same time [8].

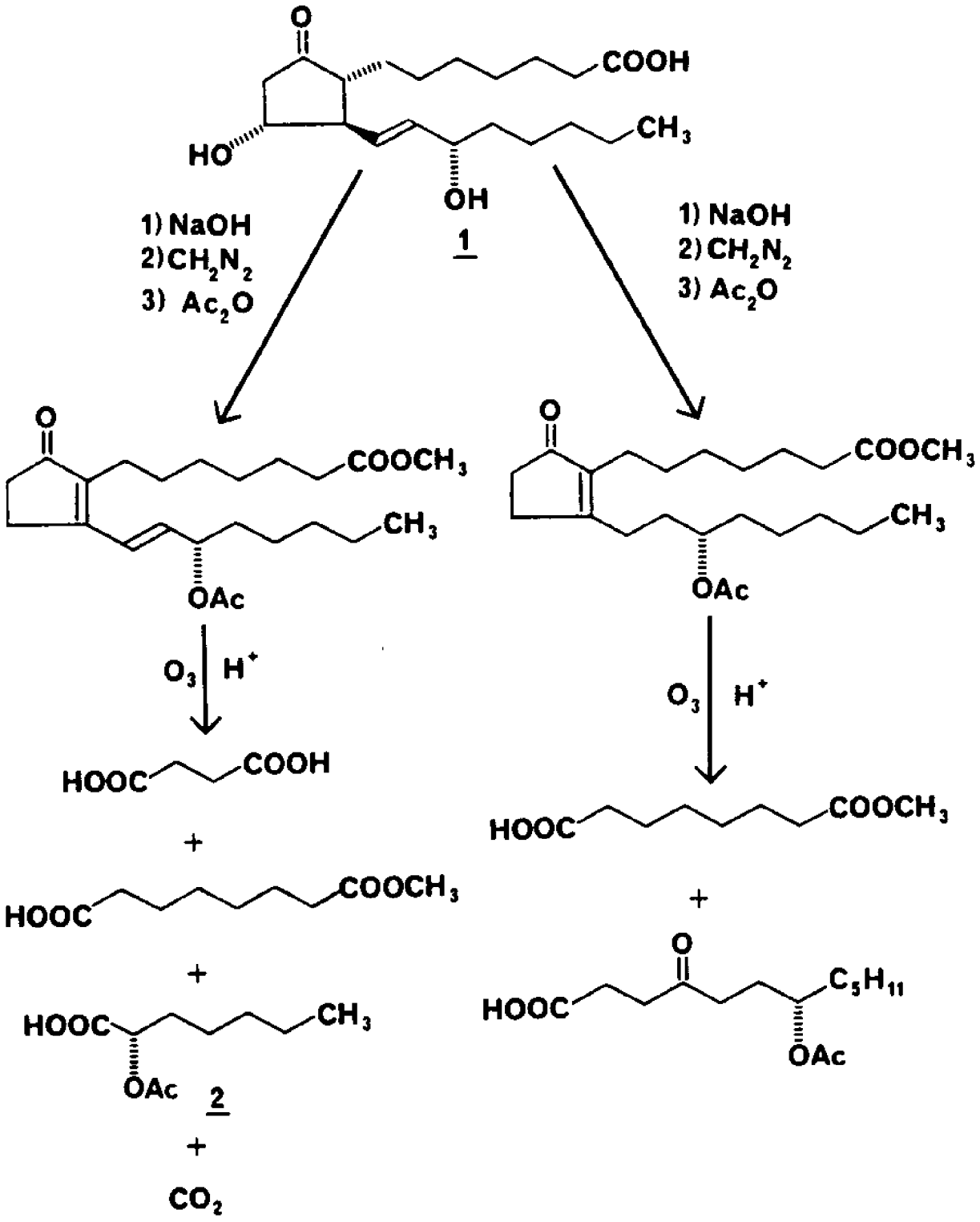
Interest in "prostaglandins" revived in the early nineteen sixties after Bergstrom and co-workers had shown "prostaglandin" to be an entire family of closely related compounds. They were able to isolate the two predominant compounds from the extracts of the sheep vesicular glands as colorless needle-shaped crystals and to show that they were twenty-carbon acids differing by one unit of unsaturation [9,10,11]. They also showed that the biological activity of von Euler's amorphous solid was due mostly to the presence of these two compounds. These workers also laid the foundation for the prostaglandin nomenclature still used today when they decided to call the ether soluble prostaglandin PGE and the other PGF since it was soluble in a "fosfate" (the Swedish word) buffer. By the mid-sixties, Bergstrom and co-workers had isolated over a dozen different prostaglandins from human seminal plasma and had determined their structures by an ingenious combination of instrumental and chemical methods [12].

Structure work on the prostaglandins began immediately after the isolation of several milligrams of pure crystal-

line PGE and PGF (later to be known as PGE_1 and $PGF_{1\alpha}$). Infrared spectroscopy and microhydrogenation showed that both contained one trans double bond and that PGE contained a cyclopentanone whereas PGF did not. Borohydride reduction of PGE produced two isomeric compounds which were given the names $PGF_{1\alpha}$ and $PGF_{1\beta}$. $PGF_{1\alpha}$ was shown to be identical to the PGF isolated from sheep vesicular glands. The structures were then deduced by studying the products of the oxidative ozonolysis of PGE_1 (1), dihydro-PGE_1 and their methyl esters as shown in Scheme 1. Reaction products were identified by a combination of gas chromatography and mass spectrometry. After the structure was known, the stereochemistry was deduced from an x-ray analysis of the halobenzoates of $PGF_{1\beta}$ [13,14] and the correct absolute configuration determined by relating the configuration of L-2-hydroxyheptanoic acid to that of the corresponding acetate (2 in Scheme 1) obtained from the ozonolysis of acetylated PGE_1. The structures of the other prostaglandins have likewise been deduced through chemical interconversion and liberal use of modern instrumental techniques. The tremendous diversity of the natural variations of the basic twenty-carbon skeletal unit of the prostaglandins will be obvious from a discussion of the nomenclature which has come into general use in this field.

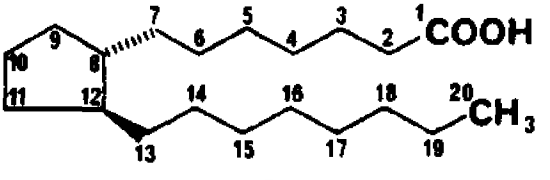
SCHEME 1

Degradation of PGE_1

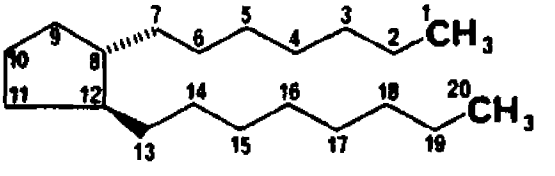


1.2 NOMENCLATURE OF THE PROSTAGLANDINS

As soon as the diversity of the prostaglandins became apparent, it was recognized that a systematic but communicable nomenclature system was necessary. Such a nomenclature system would have to convey the vast amount of structural information needed to uniquely specify any one prostaglandin in a way that made it useful to scientists from many different disciplines with many different goals in mind. A standard Chemical Abstracts type nomenclature would not only be too cumbersome but would also obscure the details most necessary in developing the structure-activity relationships necessary for the medicinal applications of the prostanoids. Although most chemists would acknowledge that such a rigidly systematic system of nomenclature is necessary for efficient information storage and retrieval, few would question the need for a second more humanistic method. Such a practical system has developed based on the saturated, unsubstituted twenty-carbon carboxylic acid called prostanoic acid (3) and



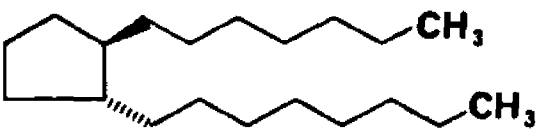
3



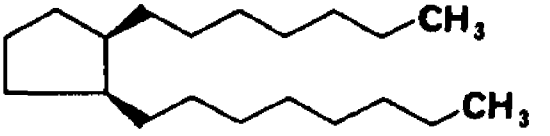
4

the analogous hydrocarbon, prostane (4). Although the natural prostaglandins known to date all have the same side chain configuration as these two reference compounds, synthetic prostanoids have been produced which have differing

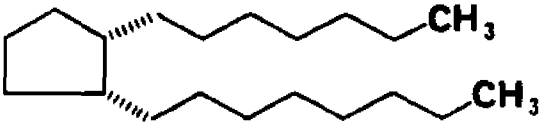
stereochemistries at these positions. These are named after the other three stereoisomeric hydrocarbons ent-prostane



5



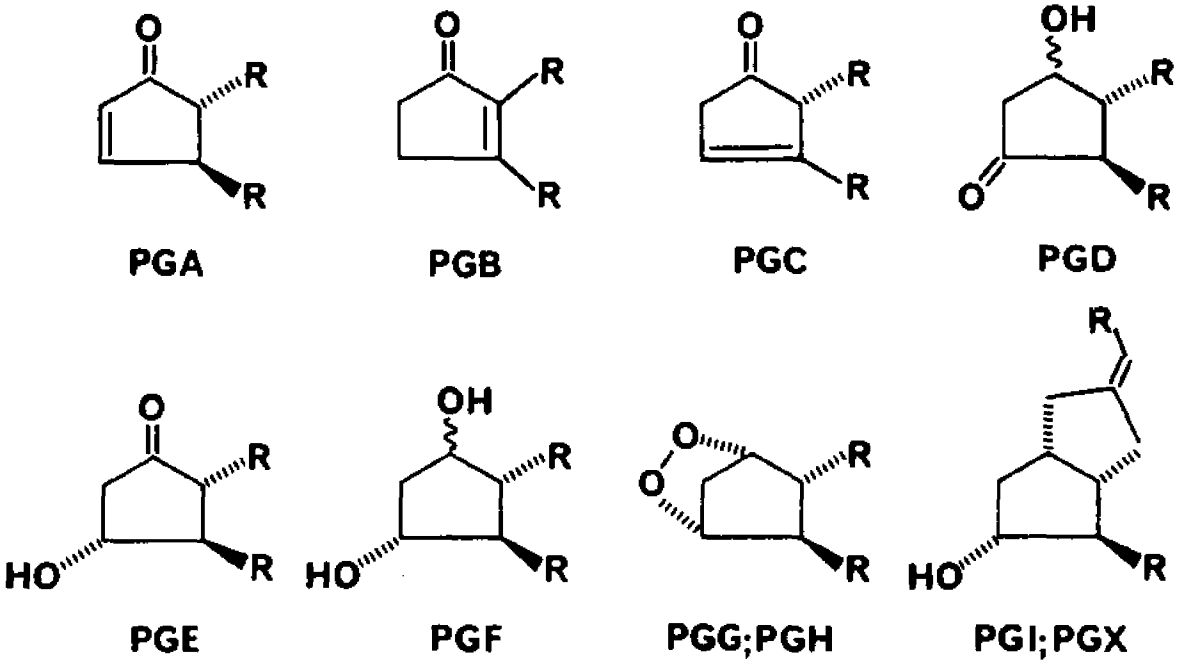
6



7

(5), isoprostane (6) and ent-isoprostane (7). Note that the prefix "ent" is used to denote the enantiomer (at C-8 and C-12) of the named compound.

The prostaglandins are then grouped into families based upon the nature of the substituents of the cyclopentane ring. The names of these families still reflect the original assignments by von Euler based on solubilities and reactivities. Thus, PGE is ether soluble, PGF is phosphate soluble, PGA is formed on acid treatment of PGE and PGB is formed on base treatment of PGE. The other designations simply fill in the alphabet. The number of double bonds in the rest of the molecule exclusive of any in the ring is then designated by a subscript after the letter abbreviation. Those prostaglandins designated by a subscripted "1" have only a trans double bond between carbons thirteen and fourteen of the prostanoic acid system. Prostaglandins designated by a

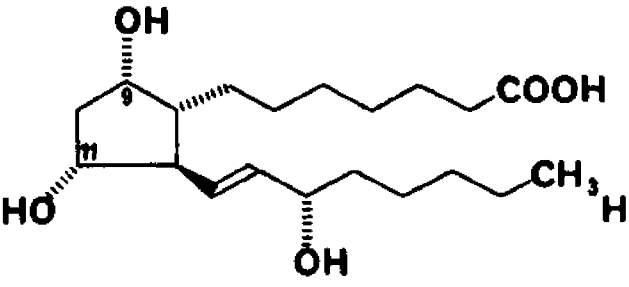


subscripted "2" also contain a cis-double bond at the 5,6 position and those designated by a subscripted "3" have an additional cis-double bond at the 17,18 position.

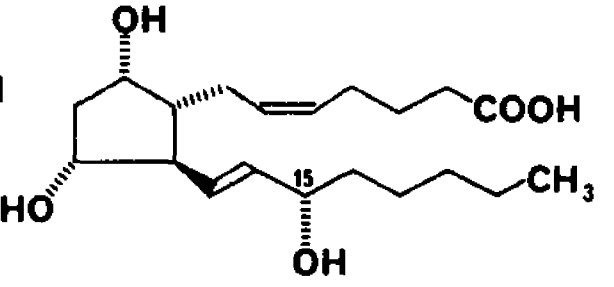
The stereochemistry of the cyclopentane substituents, if variable, is designated as an additional subscript. The Greek letter "alpha" is used if the substituent is below the ring plane and "beta" is used if it is above. Obviously, this system only works if the structures are all drawn with the side chains extending to the right. Thus, PGF_{α} and PGF_{β}

are as shown and the subscripts refer to the hydroxyl at C-9

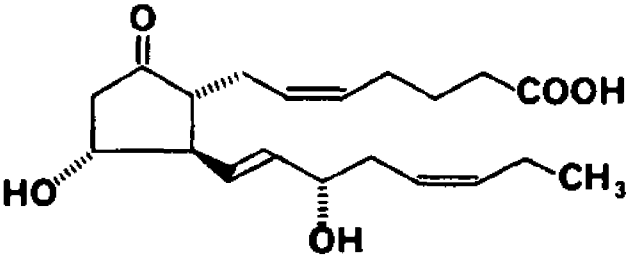
since this is the one which varies in nature. The structures and designations of $PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_3 shown here should



$PGF_{1\alpha}$

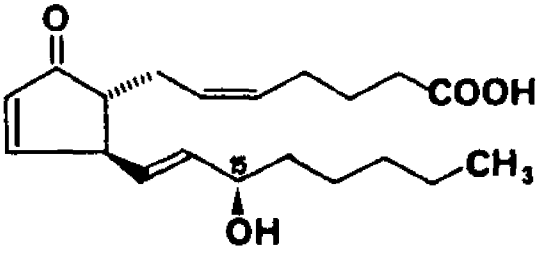


$PGF_{2\alpha}$



PGE_3

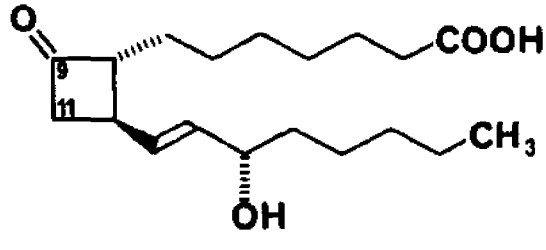
serve as useful examples. Note that this system relies heavily on the user's knowledge of the natural configurations of the prostaglandins since it does not designate these. If it is necessary to designate a configuration other than natural, this is usually done in one of two ways using prefixes.



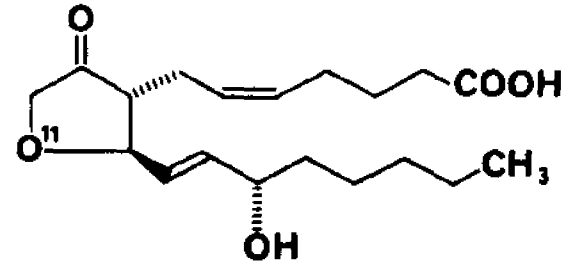
8

For example, compound 8 would be called either 15-epi-PGA_2 or (15R)-PGA_2 since it does not have the usual (15S or α) hydroxy configuration.

This system has served well for the natural prostaglandins but as research in synthesis produces many variations of these basic structures, the number of prefixes used to designate these prostanoids may very well become cumbersome. Each change in the basic prostaglandin is denoted by a prefix consisting of a number giving the position of the change followed by the appropriate designation for the change. Thus, the prefixes dihydro if one double bond is absent, deoxy if a hydroxy is removed, oxo if a carbonyl is inserted, oxa if a carbon is replaced by an oxygen, aza if by a nitrogen, nor if it is removed with no replacement and homo if a carbon is inserted, have been used (among oth-



9



10

ers). As examples, note 10-nor-11-deoxy PGE (9) and 11-deoxy-11-oxa PGE_2 (10). Nevertheless, this nomenclature system has many advantages when compared with the usual Chemical Abstracts designations. For example, the Chemical Abstracts systematic name for $PGF_{2\alpha}$ (structure shown earlier) is (5Z,9α,11α,13E,15S)-9,11,15-trihydroxyprosta-5,13-dien-1-oic acid and it is not easy to see the key differences between this and (5Z,13E,15R)-15-hydroxy-9-oxoprosta-5,10,13-trien-1-oic acid (the CA designation for compound 8;

(15R)-PGA_2) at a quick glance! Extensive discussions of the prostaglandin nomenclature system as it relates to synthetic analogs have been given by Nelson [15] and Anderson [16] and will not be repeated in detail here.

Two anomalies which have recently arisen in this nomenclature system deserve mention. First, the astute observer will note that the prostaglandin endoperoxides, despite having the same ring substituents, are designated by two series (PGG and PGH). Actually, PGG is the precursor to PGH and as such has a 15-hydroperoxy group which is reduced to give the ultimate 15-hydroxy of PGH and the other prostaglandins. Since the nomenclature that has developed has no provision for easily accommodating these two variations in the same series, they were given the different designations. Second, PGI (also known as prostacyclin) does not have the usual side chain arrangement. It is the newest member of the prostaglandin family having been discovered only in nineteen seventy six [17]. It was originally known as PGX since it was obviously different from all other prostaglandins but it took some time for the actual structure to be determined. PGX is no longer used as a designation for this system.

1.3 OCCURRENCE, BIOSYNTHESIS AND ACTIVITY

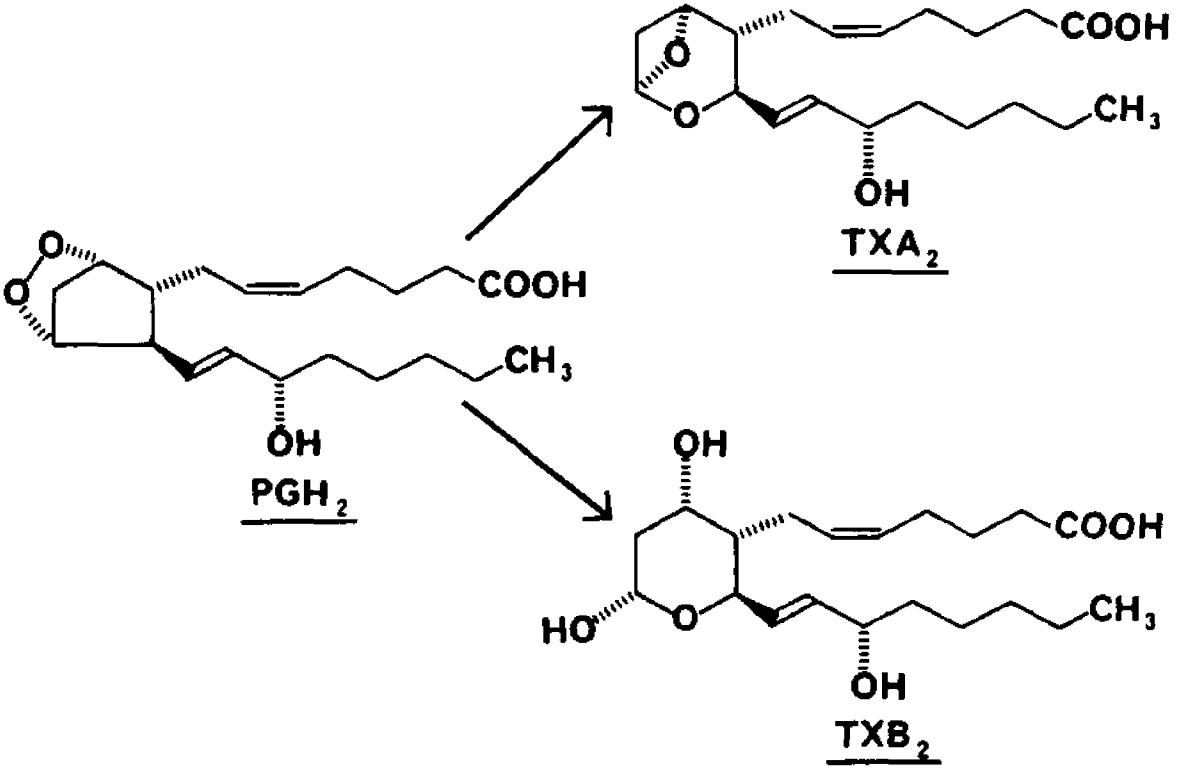
Since the initial work with human seminal plasma, prostaglandins have been detected in many tissues and cell types (including brain, kidney, lung, ovary, uterus, intestine,

stomach, pancreas and eye) from virtually all mammals. The richest known mammalian source is still human seminal fluid which contains about 300 micrograms of prostaglandins (mixture of 13 types) per milliliter. The concentrations in other mammalian tissues are generally less than one microgram per gram. Detection is hampered by the fact that the prostaglandins are not stored in the cells but are synthesized as needed in response to various stimuli and are then rapidly metabolized. In addition, they are so potent it is estimated that an adult human produces no more than one or two milligrams per day [18] and most other animals produce less than this. Nevertheless, it is believed that prostaglandins are produced by all mammalian cells [19].

Prostaglandins have also been found in various lower animals such as mussels, corals, lobsters, frogs and some fish [8]. Recently, the first isolation of prostaglandins (PGE_2 and $PGF_{2\alpha}$) from a plant (the red alga Gracilaria lichenoides) has been reported [20]. Unfortunately, this plant only contains approximately 0.02% (wet weight) of these prostaglandins. The richest source of prostaglandins is the soft coral Plexaura homomalla (Esper) which is found in the Caribbean and contains as much as one percent by weight of a mixture of prostaglandins (mostly PGA_2, PGE_2, 15-epi PGA_2 and 15-epi PGE_2) which varies in composition with the source of the coral [21]. To date, this is the only natural source of prostaglandin precursors which has been intensively stud-

ied as a practical means of supplying the large quantities needed for pharmaceutical purposes.

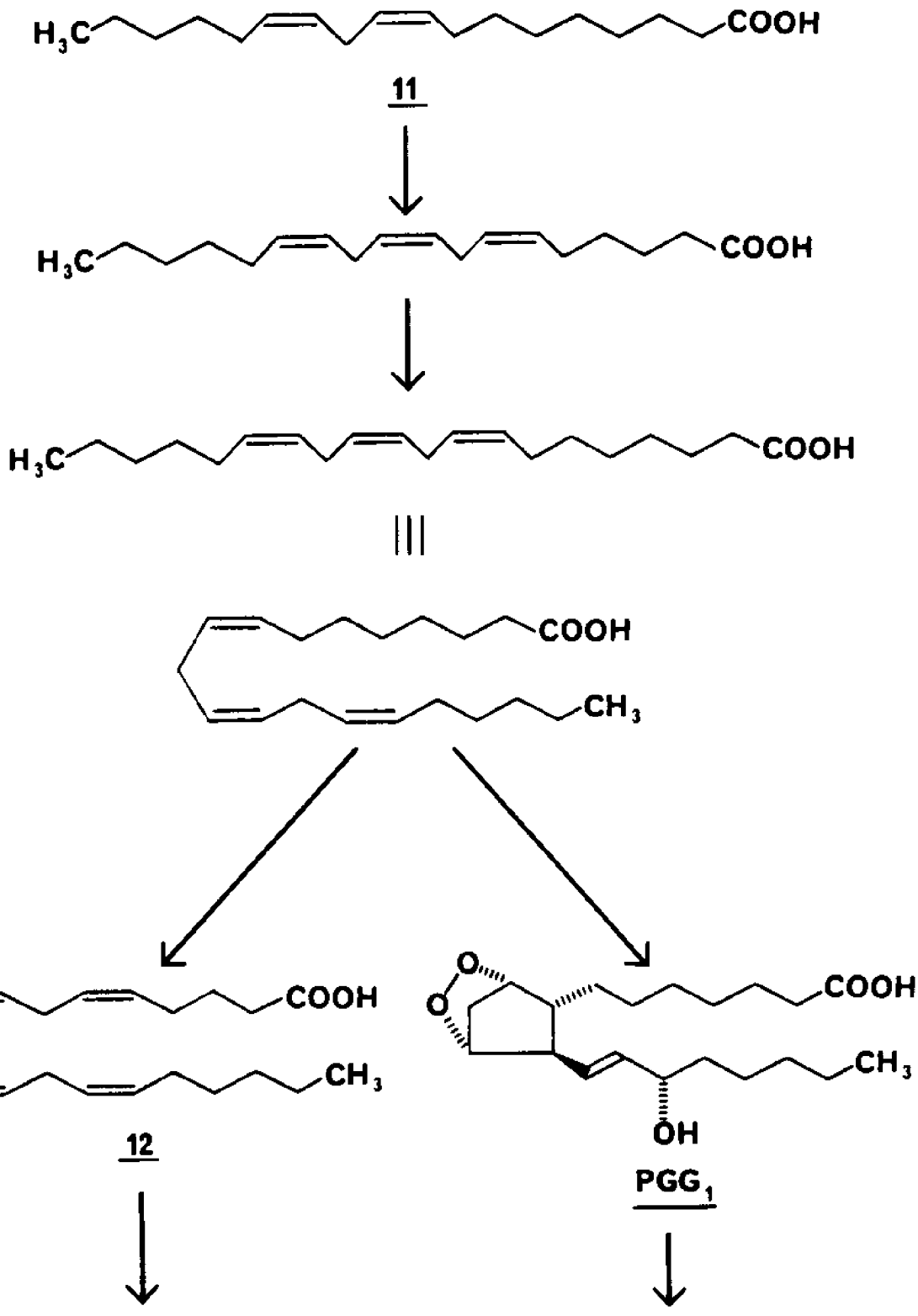
Prostaglandins are known to be synthesized in cell membranes from simple fatty acids. Although not all the biosynthetic pathways have been traced in detail, the E and F series are known to form from the essential fatty acid linoleic acid (11; 9E,12E-octadecadienoic acid) as shown in Scheme 2. Note that the prostaglandin endoperoxides (PGG and PGH) are intermediates in these pathways. They have also been implicated as key intermediates in the synthesis of



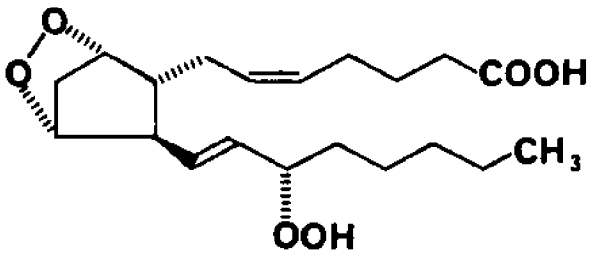
prostacyclin (PGI) and the thromboxanes (TXA_2 and TXB_2) from arachidonic acid (12; 5,8,11,14-eicosatetraenoic acid). These pathways have been deduced by the usual methods using radioactive label incorporation (see [8,12]).

SCHEME 2

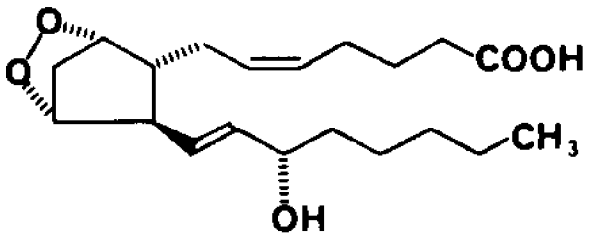
Biosynthesis of the Prostaglandins



12



PGG_2



PGH_2

PGE_2

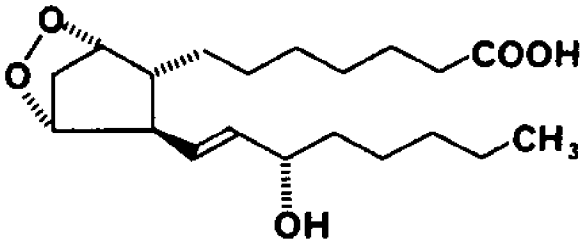
+

PGI_2 ⟶ TXA_2

+

$PGF_{2\alpha}$ ⟶ TXB_2

PGG_1



PGH_1

PGE_1

+

$PGF_{1\alpha}$

Since the prostaglandins are rapidly metabolized, it is not surprising that their principal function is considered to occur in the cell membrane where they are synthesized. They are thought to play a key role as regulators of intracellular metabolism through control of the cellular activity caused by the arrival at the cell membrane of hormone-messengers from other parts of the body [22]. In this respect, they have been implicated in tissue auto-defense mechanisms which result in such manifestations as fever, vomiting, pain and inflammation. Indeed the anti-inflammatory agents indomethacin, phenylbutazone and aspirin are believed to act by inhibiting the synthesis of certain prostaglandins [23].

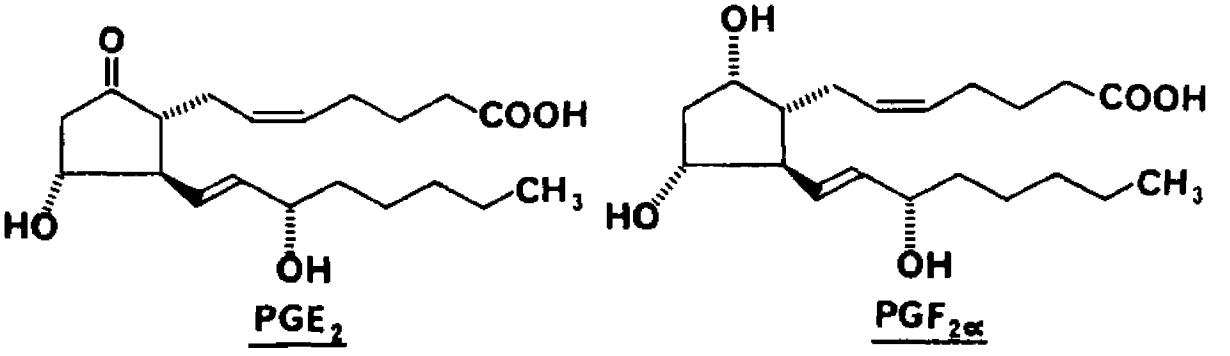
Prostaglandins have been found to stimulate smooth muscle contraction, open bronchial tubes, control pituitary hormone release, block the breakdown of fats, inhibit stomach acid production and constrict the pupil of the eye [24]. In addition, various series have been shown to affect the normal functioning of the respiratory, gastric, digestive, renal, reproductive, nervous, endocrine and cardiovascular systems [8]. They also play key roles as inhibitors and promotors of platelet aggregation and as intermediates in thyroid response to hormonal stimulation.

The wide range of effects exhibited by the natural prostaglandins at such extremely low concentrations and in so many diverse types of tissues makes them prime targets as a

source of new medicinal compounds. In fact, prostaglandins have been investigated for use in abortions and artificial inseminations as well as to control asthma, ulcers, high blood pressure, arthritis and various central nervous disorders. Unfortunately, the very characteristics which have prompted the intense study of these compounds also make such study incredibly difficult. The natural prostaglandins occur in such small quantities that even they must be synthesized "artificially" if medicinally useful amounts are to be available. Even then, researchers have found such broad activity that synthetic analogs which exhibit greater specificity must be found if prostaglandins are to become routine clinical medicines. Thus, synthesis of prostaglandins and their analogs (called "prostanoids") has developed at a rapid pace.

1.4 SYNTHESIS OF PROSTAGLANDINS

The prostaglandins present a formidable synthetic challenge.



PGE_2 $PGF_{2\alpha}$

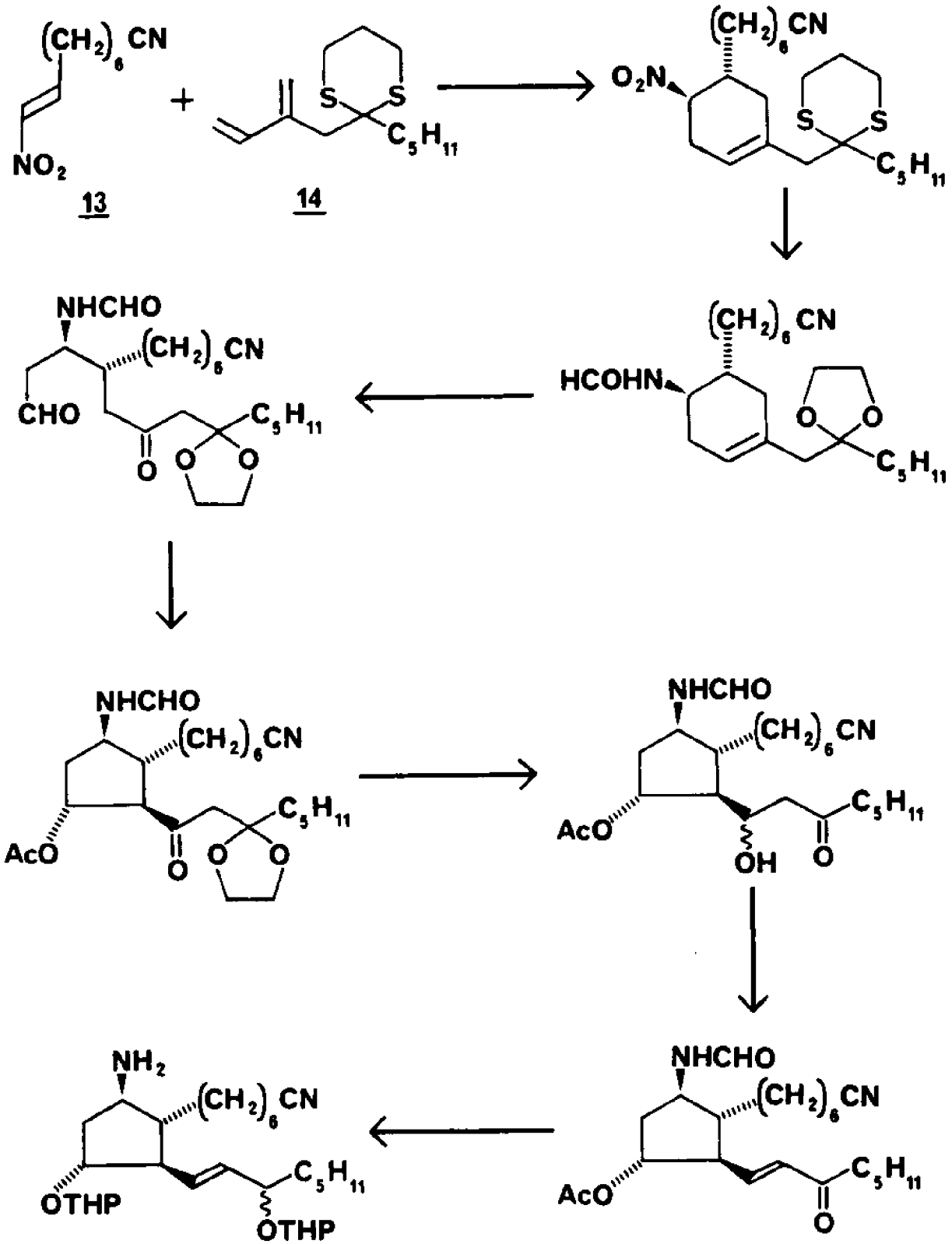
The two main series, PGE_2 and $PGF_{2\alpha}$, contain four and five chiral centers respectively as well as two side chain double bonds. Many of these compounds contain sensitive function-

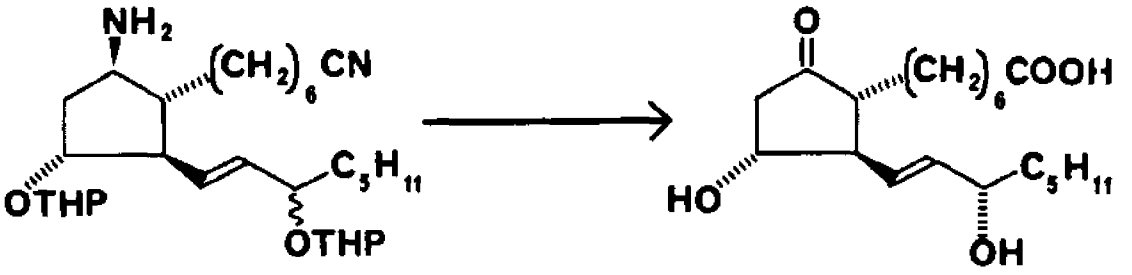
alities such as the β-ketol function of PGE_2 (acid and base labile) and the less stable enone of the PGA family (subject to double bond migration). In addition, stereochemical control at carbon fifteen (usually bearing a hydroxyl) is difficult since it is so far removed from the other rigid parts of the molecule.

Given these complications, it is surprising that less than ten years elapsed between the isolation of the first pure crystals of PGE_1 and the first unambiguous total synthesis of a prostaglandin (racemic PGE_1) by E. J. Corey in 1968 [25]. This synthesis centers around the Diels-Alder reaction of compounds 13 and 14 which must themselves be prepared from cyclooctene (6 steps gives 13 in 60% yield) and 2-bromomethyl-1,3-butadiene (reaction with 2-lithio-2-n-amyl-1,3-dithiane; 70%). The synthesis is long, not stereospecific, and proceeds in low overall yield. In addition, it is limited to the synthesis of the one prostaglandin and those which can be made from it (reduction of the 9-keto group yields racemic $PGF_{1\alpha}$ and $PGF_{1\beta}$). The formation of racemic mixtures and the subsequent need for resolution is the main reason that most of the syntheses designed since these first attempts have started either with cyclic starting materials or various chiral acyclic precursors found in nature (i.e. sugars). It is worth noting, however, that the first synthesis of an optically pure prostaglandin (again PGE_1 and again by Corey [26]) involved resolution by recrystalliza-

SCHEME 3

The First Synthesis of Racemic Prostaglandins (Corey 1968)





dl-PGE$_1$

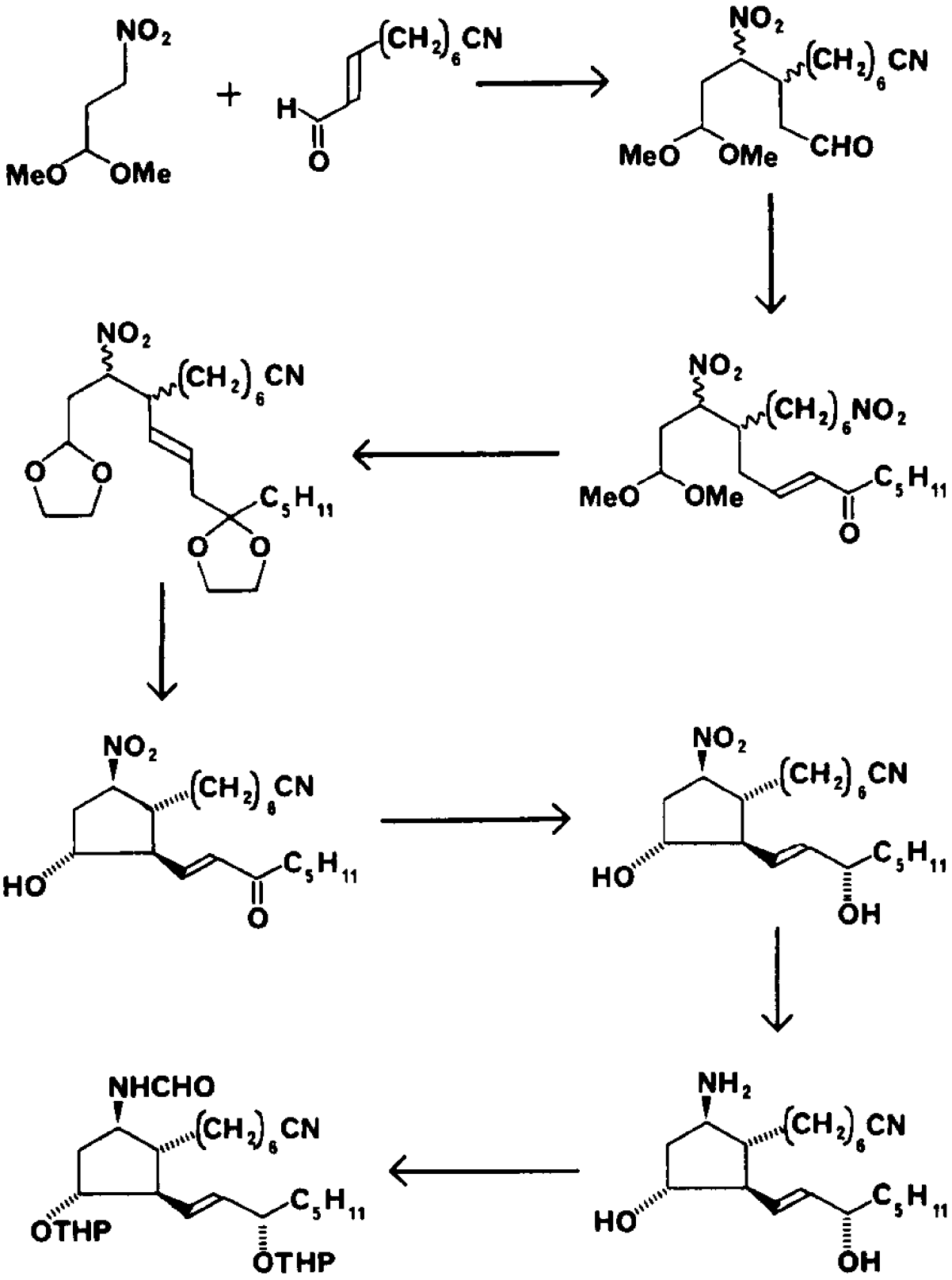
tion of the diastereomeric salt formed from 15 (Scheme 4) and (-)-α-bromocamphor-π-sulfonic acid [27].

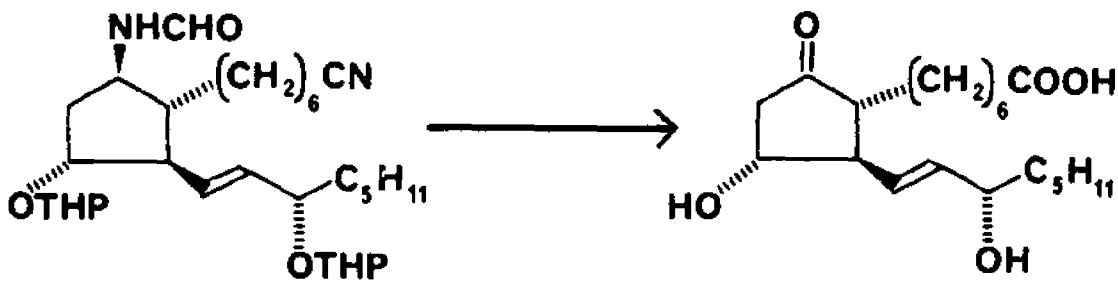
Although this synthesis was significant at the time (1969) because it was the first, it also served to push research in other directions since it could obviously not provide the vast amounts of material needed for study. At about this time, Weinheimer and Spraggins discovered that the soft coral Plexaura homomalla (Esper) contained large amounts of prostaglandins [21,28] of the A type, as mentioned earlier. This stimulated extensive research on the conversion of this type to the more active E and F types. Unfortunately, the yields for these interconversions are not incredible (ca. 50%) and while work with this coral still continues, it now appears that its range is so small that it would be an expensive source even if fully developed. In any event, the number of prostanoids available in this way will be limited.

Fortunately, syntheses from cyclic precursors have yielded more encouraging results than either method above. The

SCHEME 4

The First Synthesis of Optically Active Prostaglandins



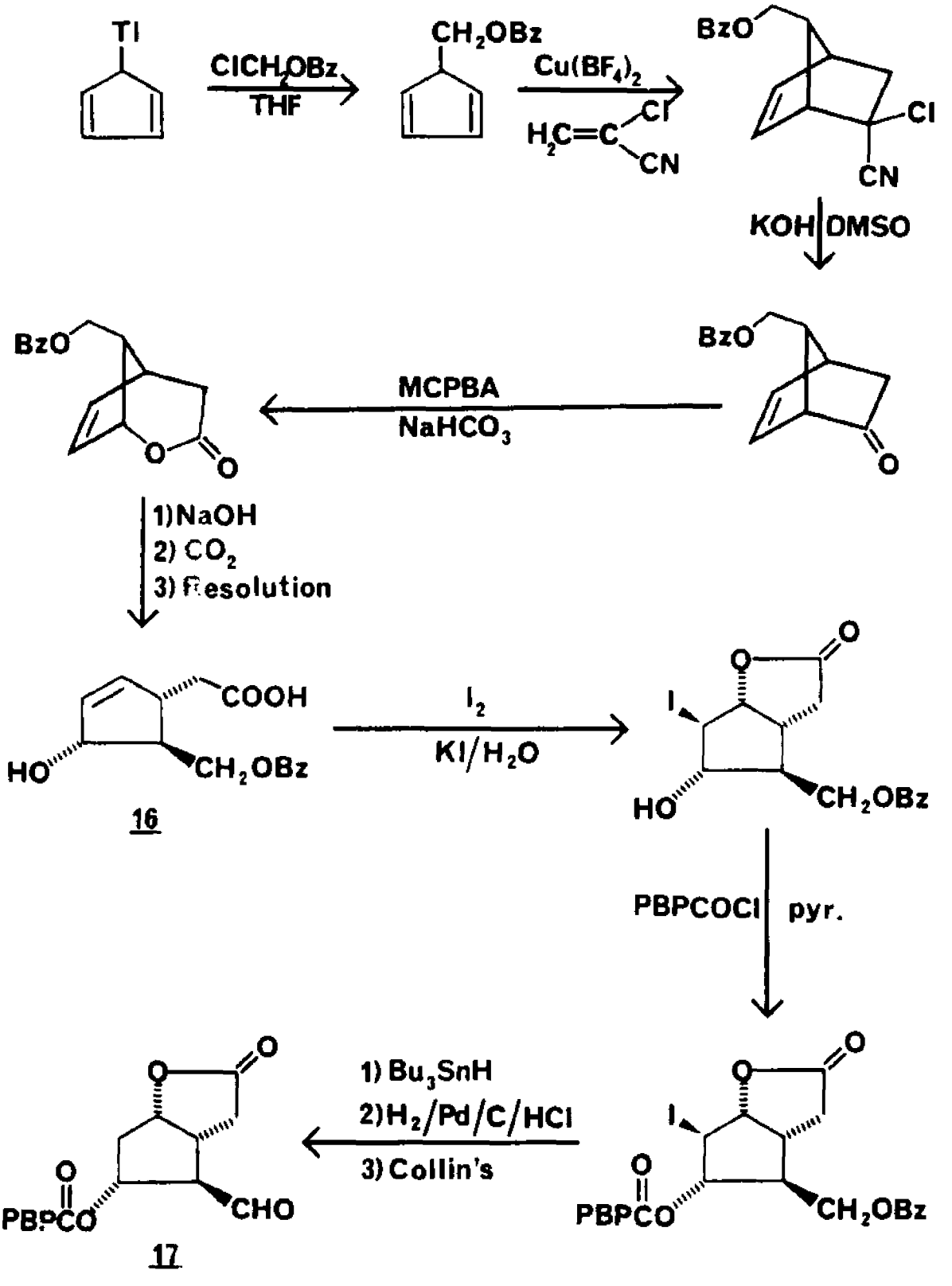


(-)-PGE$_1$

most dramatic of these is Corey's bicycloheptane approach which has been widely acclaimed for its versatility and potential industrial applicability [29,30,31]. This synthesis is perhaps the ultimate solution to the problem of generating the four contiguous asymmetric centers of the cyclopentane ring in the F prostaglandins. The cyclopentane nucleus is embedded, along with its key chiral centers and the necessary latent functionality, in a bicyclo[2.2.1]heptane system. Cleavage of one bond then generates, in the correct stereochemistry, the functionality necessary for further elaboration. Although other syntheses have been based around this general approach, the Corey route shown in Schemes 5 and 6 is nothing short of miraculous. By formation of carboxylic acid 16, he has provided an early means of resolution via the (+)-amphetamine or (+)-ephedrine salt of this compound and at the same time provided a way to introduce the fourth ring chiral center through iodolactonization. Further elaboration results in the formation of the now-famous Corey lactone-aldehyde (17), an intermediate which al-

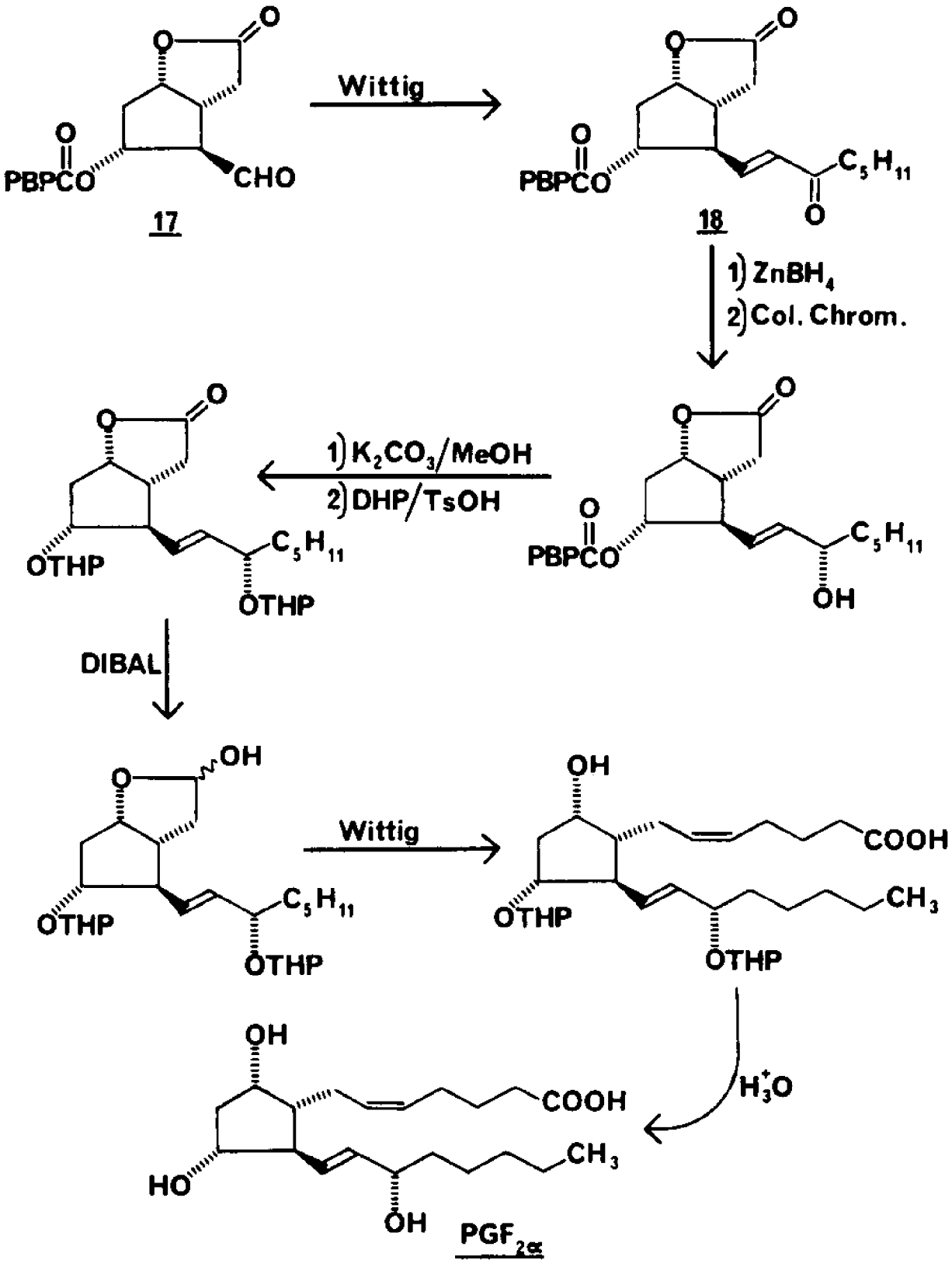
SCHEME 5

Corey's Lactone-Aldehyde Synthesis



SCHEME 6

Elaboration of the Lactone-Aldehyde to Prostaglandins

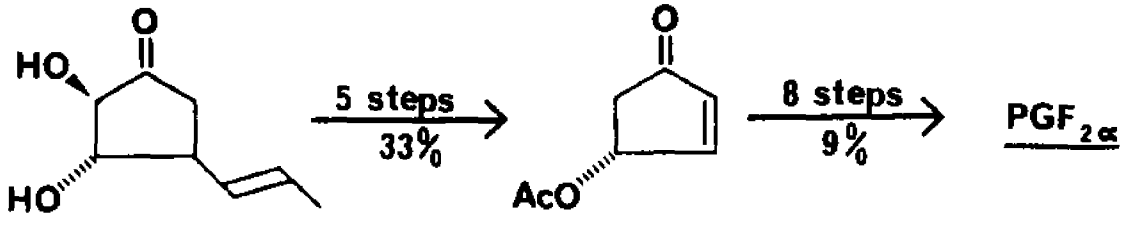


lows the selective introduction of both side chains by modified Wittig reactions. Using this method, Corey synthesized PGE_2 in 23% overall yield and $PGF_{2\alpha}$ in 27% overall yield (from cyclopentadiene).

This synthesis is so practical that it has been extended by workers from other universities as well as from industry. Several of these extensions have been modifications of the original bicycloheptane itself but most have involved alternate approaches to the lactone-aldehyde since it may be converted into a wide variety of side chain altered prostanoids. Corey has even gone so far as to determine that if the p-biphenyl urethane group is used to protect the hydroxyl at carbon eleven, compound 18 can be reduced selectively by thexyl limonyl borohydride to yield 92% S alcohol at liquid nitrogen temperature. He has also formed the bicycloheptane precursor using a Lewis acid-catalyzed asymmetric Diels-Alder reaction with (S)-(-)-pulegone acrylate as the dienophile so as to avoid the need for a resolution. However, this is considerably more costly than the original route since the pulegone is first synthesized from (-)-citronellol.

The thought of starting with a natural resolved compound as a means of avoiding the resolution step is attractive and prostaglandins have been produced starting from L-rhamnose [32], D-glyceraldehyde [33], D-glucose [34], (+)-D-tartaric

acid [35] and S-malic acid [36]. Prostaglandin intermediates have even been synthesized from a metabolite (terrein) of



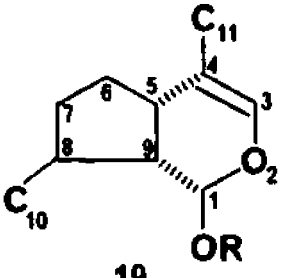
Terrein

the fungus Aspergillus fischerii [37,38] although in an overall yield of less than three percent. In fact, low yields have plaqued all of this type of work with the possible exception of Johnson's synthesis of the Corey lactone-aldehyde from S-malic acid which proceeds in 30% overall yield. We believe that we have discovered a class of optically active natural products which closely resembles the Corey lactone-aldehyde and may give high yields of many different prostaglandin intermediates without need for optical resolution or expensive reagents and without the need to develop a new strategy for each change in ring substitution.

Chapter II

INTRODUCTION TO THE IRIDOIDS

The iridoids are a group of naturally occurring cyclopentanoid terpene derivatives having the cyclopentano[c]pyran

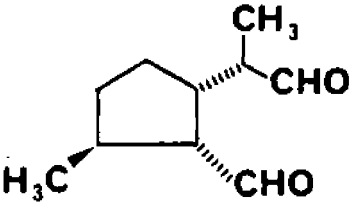


19

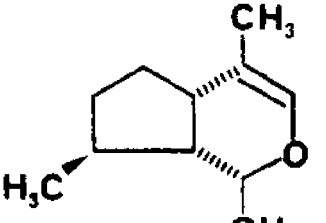
ring system (19) as the common structural feature.

2.1 HISTORY, OCCURRENCE, STRUCTURE AND ISOLATION

There are now over forty iridoids of known structure. Most are β-D-glucosides (19; R = β-D-glucose) but some have no glucose (19; R = H or R) and recently several diglycosides (19; R = β-D-glucose, another sugar attached as a cyclopentane ring substituent) have been reported [39,40]. The name



20



21

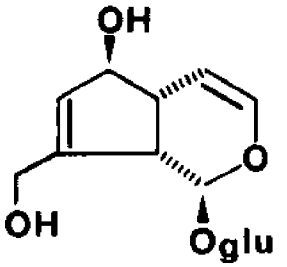
"iridoid" is used to show the relationship of these com-

pounds to iridodial (20) which exists as the hemiacetal (21) and is considered the parent compound of the group [41]. Some of the structural variations presented by the iridoids are shown in Figure 1 on the following page. Those iridoids which possess the β-glucosyloxy group on C-1 are referred to as iridoid glucosides and these are the main subject of this work.

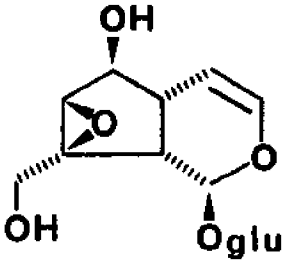
The iridoid glucosides have been known since the early nineteenth century although not usually by their current names. They first attracted the attention of chemists and botanists because of the intense blue color formed when plants containing them are exposed to acidic conditions or allowed to dry after collection. This instability is characteristic of the aglucones which are formed under acidic conditions and by enzymatic degradation. Neither the mechanism of the formation of the blue polymeric material nor its structure is known even though this is the characteristic used to distinguish the iridoids from other plant products and has been widely employed in the search for new members of the group.

Well over a century passed between the isolation of the first crystalline iridoid (verbanalin isolated in 1835 [42]) and the first structure elucidation (plumieride in 1958 [43]). This was largely due to the complexity of these structures, their instability and the lack of instrumental

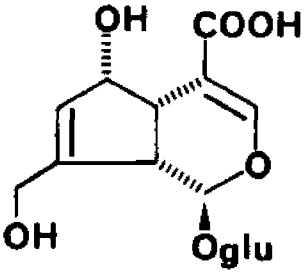
Figure 1: Structures of Some Representative Iridoids



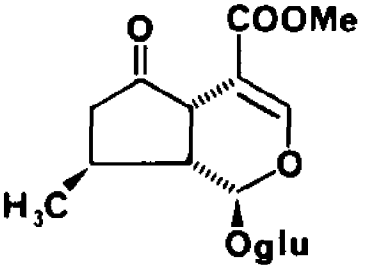
Aucubin



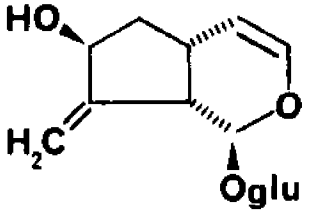
Catalpol



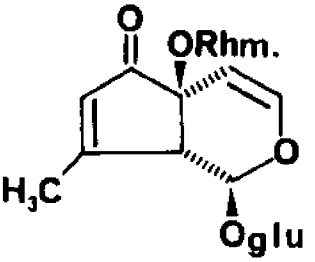
Daphylloside



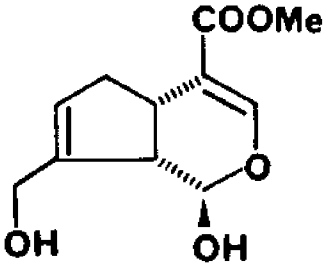
Verbenalin



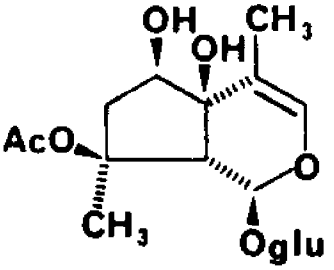
Antirrid



Teucardoside



Genepin

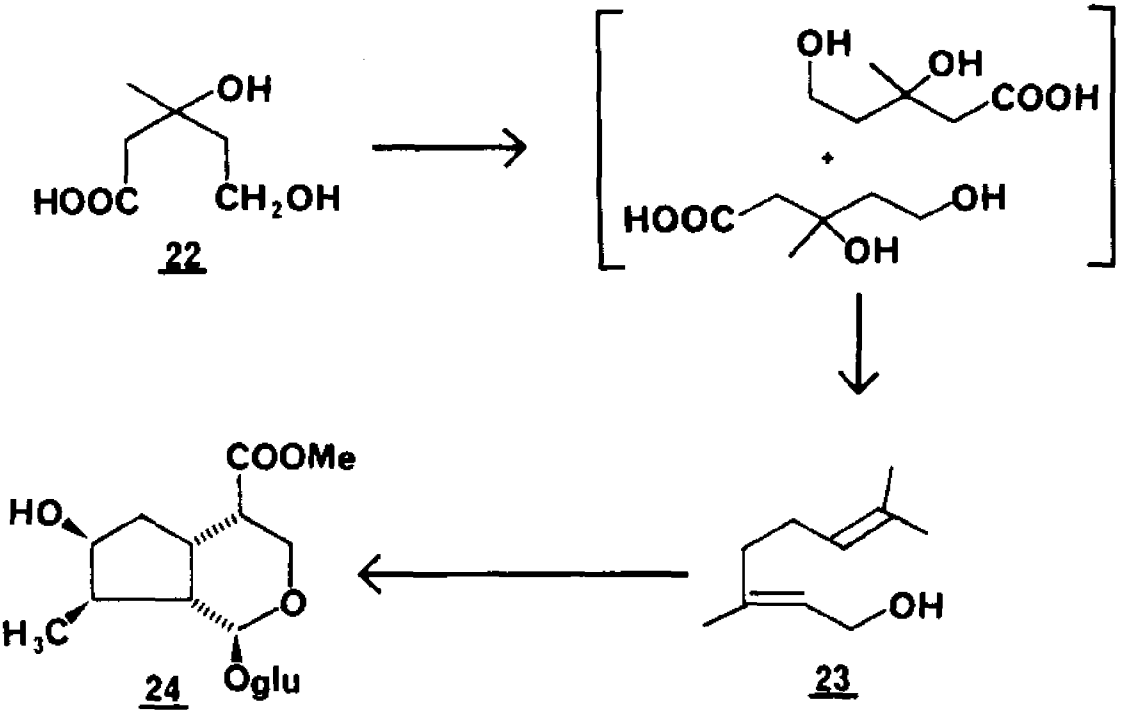


Lamioside

techniques in this time period. The structure proofs rely heavily on chemical degradation but also involve the interconversion of several iridoids of similar structure. More recently, the usual modern instrumental methods have verified proposed structures and allowed rapid determination of new ones. A detailed discussion of the structure proofs for many iridoids has been given elsewhere [44]. After the correct ring system was recognized, the correct structures of many of the then-known iridoids were determined in a relatively short period of time and studies of the botanical occurrence, biosynthesis and biosynthetic roles of these compounds were begun.

The iridoids have been found to be amazingly widespread in the plant kingdom. They have been isolated from all types of plant tissue (leaves, seeds, fruit, bark, twigs and even roots) from hundreds of species of dicotyledonous trees and shrubs. Isolation is done by extraction with hot solvent followed by various purification methods as discussed later for asperuloside, the specific iridoid of interest in this work. Yields are generally in the range of 0.1 to 1.0% based upon the weight of fresh (undried) plant parts and have been found to vary with the time of harvest. Although the plants have generally been freshly harvested, several isolations from preserved leaves have been reported. Column chromatography is sometimes (but not always) required either as a preliminary or final purification step.

Isolation and degradation of iridoids from various plants fed a number of radioactive precursors has led to a general outline of the biosynthesis of these compounds although the exact routes to most are not well understood. As shown ap-



plied to loganin (24), the iridoids are formed from two molecules of mevalonic acid (22) by way of geraniol (23). Additional carbons are supplied by L-methionine and sodium acetate [49,50].

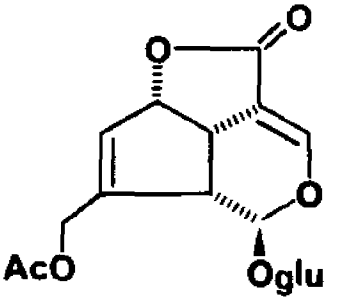
Similarly, the use of radioactive (carbon-14) labelled loganin has shown that this iridoid is the origin of parts of the carbon skeleton of several of the alkaloids [47,48]. When plants were fed labelled loganin, radioactive catharanthine, serpentine, ajmalicine, cordifoline and quinine were isolated. Other alkaloids are also believed derived from lo-

ganin by still-to-be determined pathways. Thus, the iridoids obviously play an important role in plant defense since in addition to serving as alkaloid precursors, many of these compounds have a very bitter taste as well (although admittedly some iridoid-containing plants are used as food for zoo animals).

Although iridoids are present in a number of folk medicines [44] and several were investigated as antibiotics in the nineteen fifties [49,50], they do not at present have any practical medicinal value.

2.2 ASPERULOSIDE

Asperuloside (25) has been known since 1848 under such names

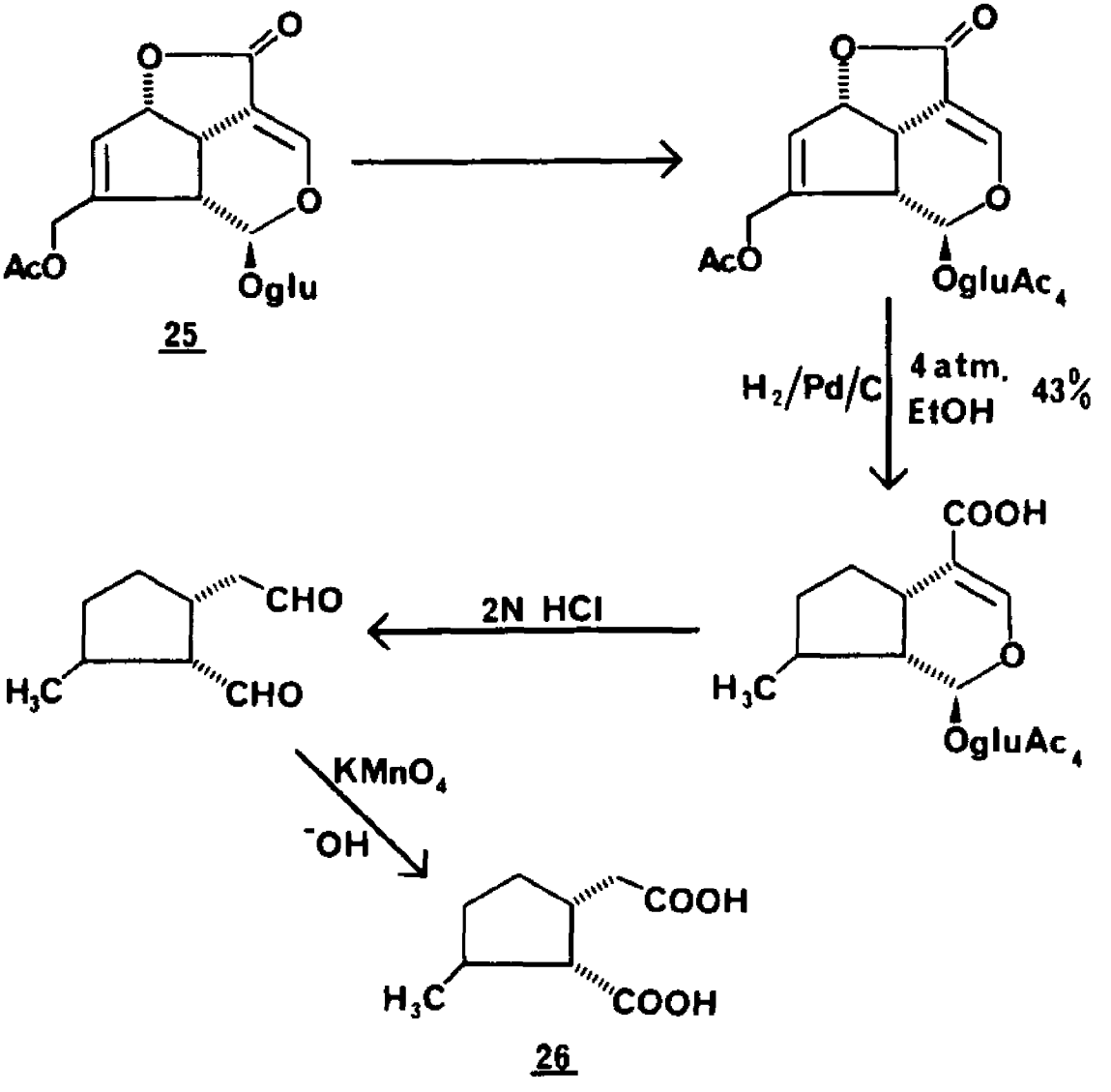


25

as chlorogenin, rubichloric acid and alstonin [44]. It was given its current name by Herissey in 1925 after he had isolated it from Asperula odorata and shown that his material was the same as all the previous materials [51]. Although Herissey recognized the glycosidic nature of the compound, a dozen years passed before it was shown to be a glucoside [52] and it was not until fifteen years after this that the

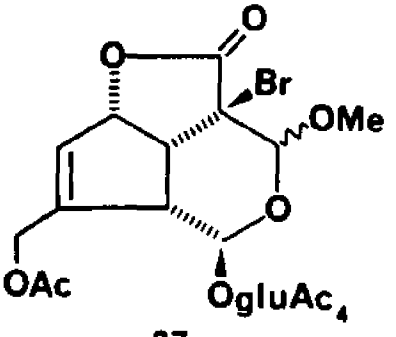
SCHEME 7

Degradation of Asperuloside

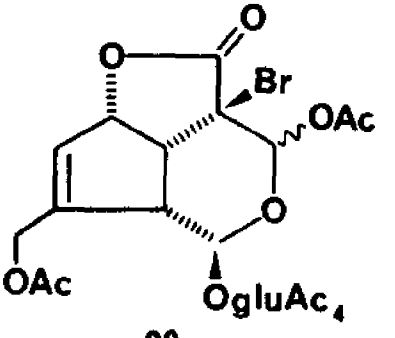


correct formula was deduced by Briggs and Nicholls [53]. These workers also carried out the chemical degradations (Scheme 7) that eventually led to the correct structure of asperuloside although they were at first misled by the ultraviolet absorption of the compound and its tetraacetate. The correct structure (25) was first proposed by Grimshaw

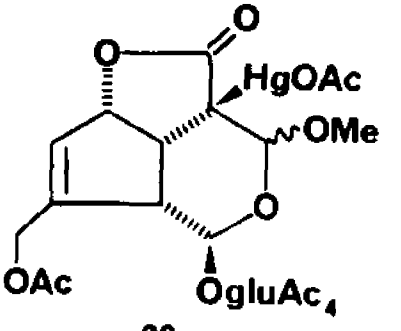
[54] in 1961 after the structure of aucubin had been elucidated (these two compounds had been considered closely related for many years). Briggs then reexamined the compound [41] and published the conclusive structural evidence [55]. This included the degradation to the known diacid 26 proving the cis ring fusion but not definitively establishing the absolute configuration at C-5 and C-9. It also included the preparation of the standard derivatives of enol ethers, namely, the bromomethoxide 27, bromoacetoxylate 28 and me-



27



28

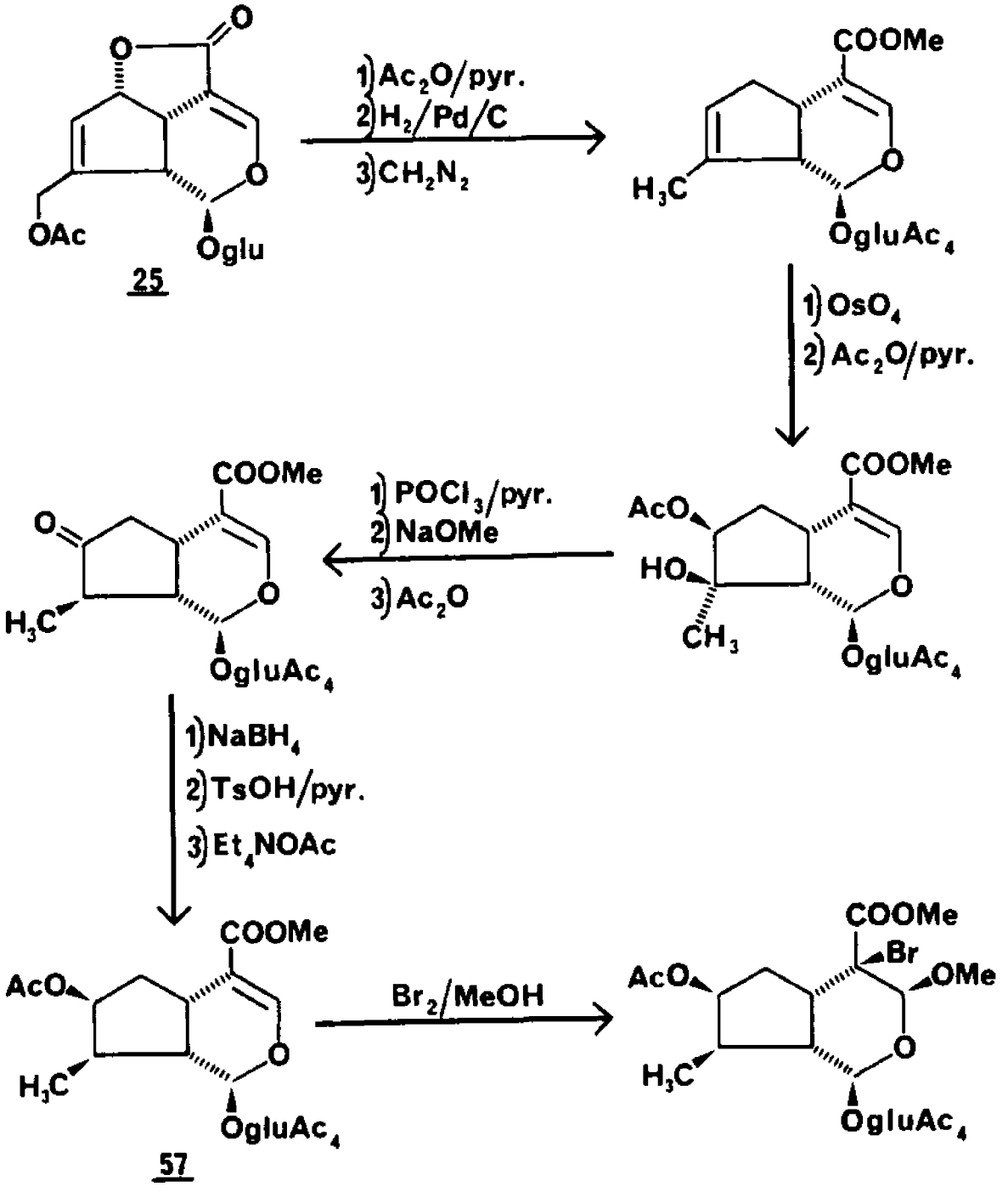


29

thoxymercuriacetate 29. Noticeably absent from this paper is any mention of the corresponding bromohydrin since it had been used so effectively in the structure work on aucubin. Final proof of the absolute configuration of asperuloside is based upon the absolute configuration of loganin pentaacetate which was established [56] by X-ray crystallographic analysis of its bromomethoxide. Since asperuloside has been converted to loganin pentaacetate [57] having an optical rotation identical to that obtained from naturally occurring loganin, its absolute configuration at the ring junction must be the same and is as drawn (see Scheme 8).

SCHEME 8

Conversion of Asperuloside to Loganin Pentaacetate Bromomethoxide



25

57

Asperuloside is widespread in nature. It has been found in over a hundred species of land plants (mostly shrubs and bushes) in concentrations ranging from 0.1 to 12% by weight of fresh plant material [44]. The actual isolated yield has been found to vary with the time of harvest as well as the age of the plant parts (young new leaves and stems have a higher content) [58]. Although fresh (undried) plant samples seem to give the highest yields, samples of Coprosma solandri collected by Captain Cook in 1769 still gave positive results almost two centuries later [44]. The application of modern freeze-drying methods would seem to hold great promise for improved yields but no attempts at such isolations have been reported to date. The methods of isolation that have been used include extraction with boiling water, acetone, alcohol and ethyl acetate either directly or with a Soxhlet apparatus [59,60]. The solutions are frequently treated with calcium carbonate to neutralize acids present in the plants and with a hydrocarbon such as toluene to prevent formation of mold and fungus during the work-up since the major part of the extracts consists of various sugars. Purification of the crude extracts varies widely depending on the plant source but generally involves concentration in vacuo (this results in a water solution since the water from the undried plants remains) followed by one of three purification methods. The iridoid may be extracted into an organic solvent, washed with water or dilute base to remove the sug-

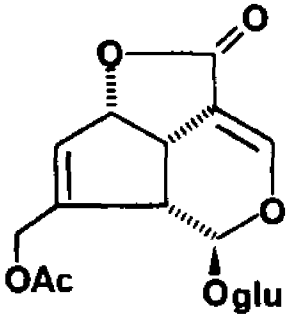
ars and tannins and then be isolated by crystallization from wet acetone or ethanol. It may also be isolated by adsorption onto charcoal, washing with water and eluting with 71% ethanol followed by concentration and recrystallization. Still another method (and the method we have chosen) is to use either partition chromatography and/or regular column chromatography to separate the asperuloside from other iridoids and plant sugars followed by recrystallization to remove the last traces of chlorophyll and other plant pigments.

Due to the sensitivity of asperuloside to both acids and bases, it is not surprising that very little chemistry (other than that needed for the structure proof) of this compound has been investigated. The only additional work with asperuloside has involved its conversion to loganin as a means of interrelating the structures of the two iridoids as previously discussed. The goal of our work in this area has been to elaborate the chemistry of asperuloside (and thereby that of the other, less sensitive, iridoids) so that a synthetically useful transformation to prostanoids would be achieved.

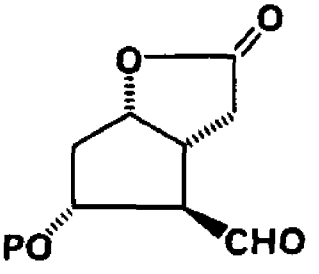
Chapter III

PROPOSAL AND SYNTHETIC STRATEGY

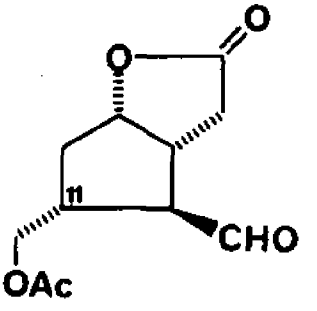
The iridoid glucosides are optically active and have the same absolute configuration as the prostaglandins. In particular, asperuloside (25) has the skeleton of the Corey



25



17



30

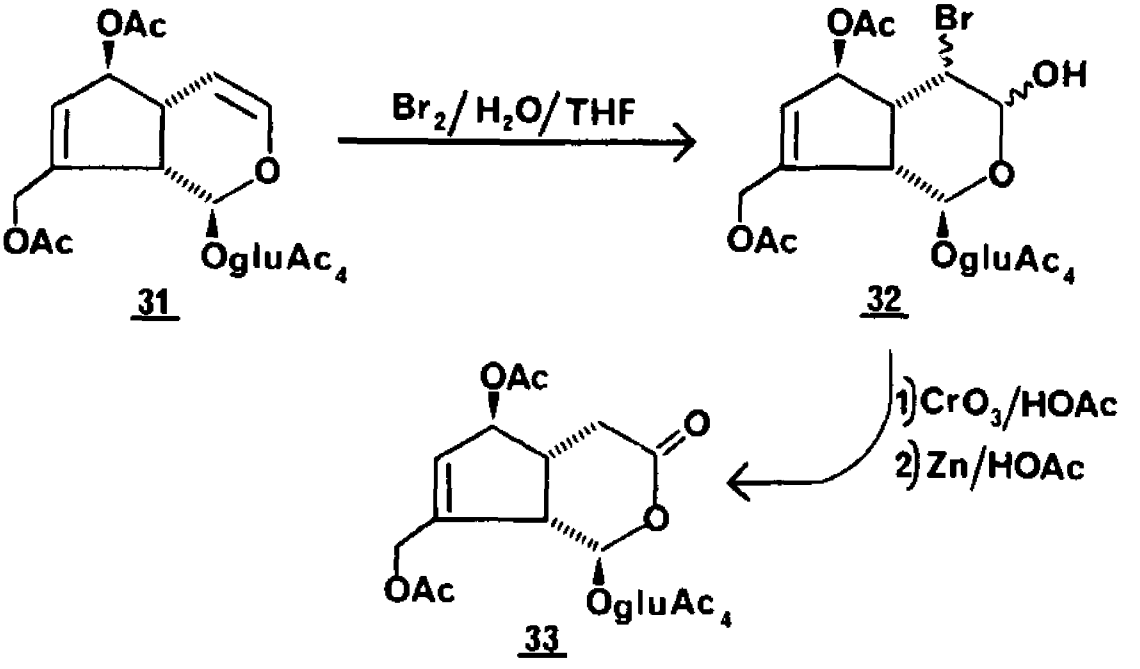
lactone-aldehyde (17) embedded in its aglucone although other iridoids (see Figure 1) do not show as obvious a resemblance. We believe that the conversion of asperuloside to the 11-hydroxymethyl analog (30) of the Corey intermediate could well be the best source of supply of the 11-hydroxymethyl analogs of PGE_2 and $PGF_{2\alpha}$. These analogs are considerably more stable than the natural materials and have thus been the subject of intense study. In addition to providing an important source of these compounds without the need for resolution, a synthetic scheme for this conversion should be designed so that it is readily applicable to the majority of the other iridoid systems, thus providing a source of ring

altered prostanoids. We believe that we have developed such a synthesis and in this chapter a broad overview of our strategy is discussed.

Any manipulation of the iridoid glucosides must take into consideration the general instability of the group toward even weak acids. In addition, asperuloside is also sensitive to base. Previous workers have overcome these problems by using two distinct methods, one oxidative and one reductive, to remove the double bond of the aglucone which apparently causes the instability.

The reductive route involves catalytic hydrogenation of all double bonds present. Unfortunately, since most of the work was done at least twenty years ago, the catalysts used also often caused hydrogenolysis of any allylic acetate or lactone groups in the compounds studied. Thus, none of this previous chemistry would be of much value in the types of syntheses contemplated. Nevertheless, we could foresee a study to search for suitable hydrogenation conditions since it would be necessary to reduce the cyclopentene regardless of the route chosen.

In contrast, the oxidative route was used quite successfully by Schmid [61] as part of the structure proof of aucubin hexaacetate (31) although he gave no information about the yields. This proved that the bromohydrin (32), bromolactone and lactone (33) of at least one iridoid were stable

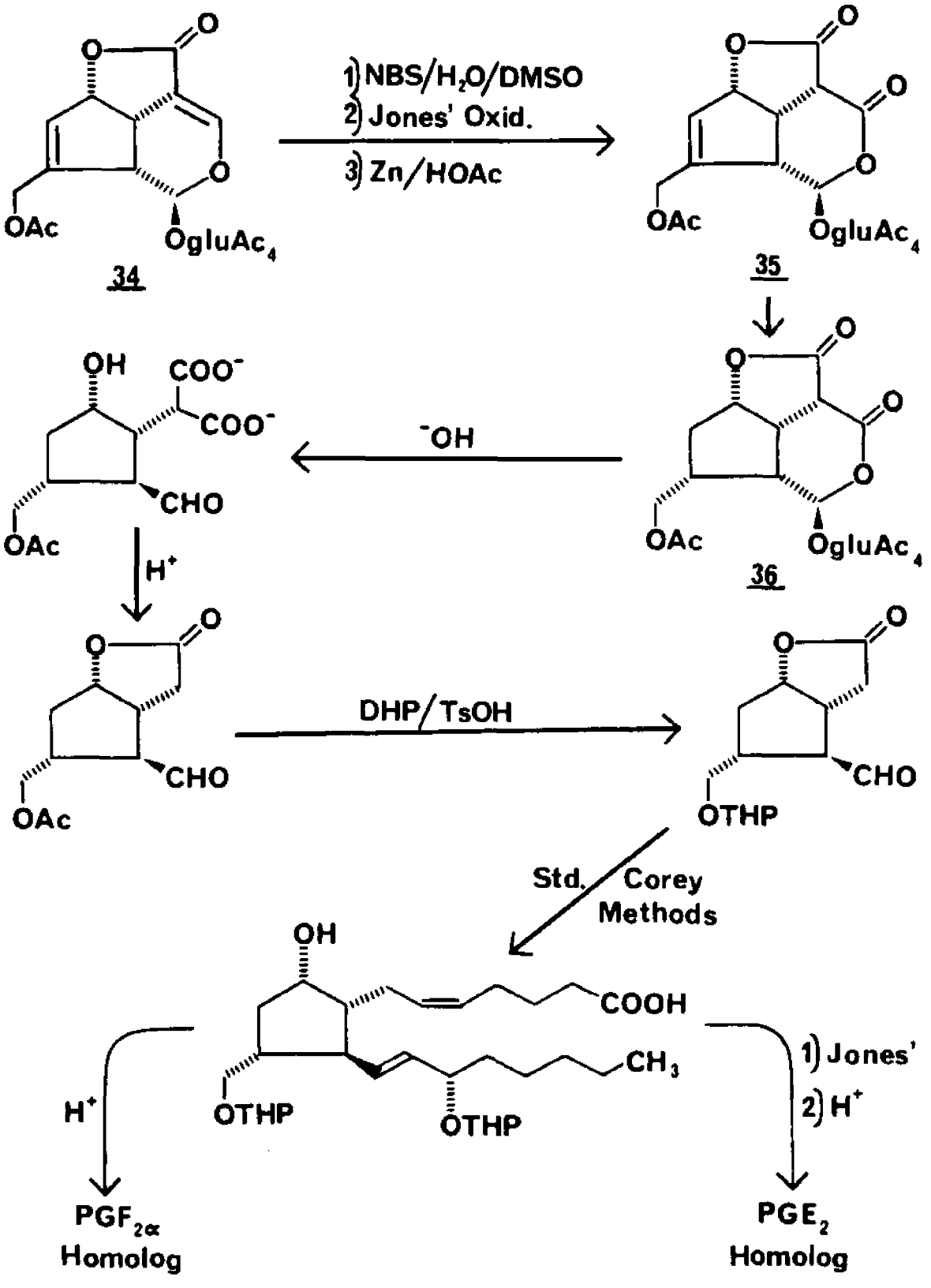


compounds and showed an important method for stabilizing the enol ether system common to all iridoids.

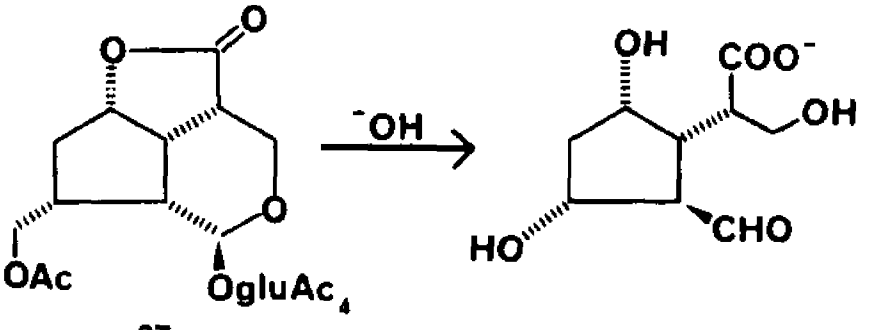
Stabilization is not the only problem involved in the conversion of asperuloside to the desired intermediates. One will observe that asperuloside has an extra carbon (C-3) which must be removed and that the orientation at C-9 must be changed to reflect the trans orientation of the prostanoid side chains. Also, the hydrogenation of the 7,8-double bond must occur from the beta face (and without hydrogenolysis as mentioned above) to give the correct stereochemistry at C-8 (throughout this thesis, the iridoid numbering system is used in referring to any compound which still has the glucose attached and the prostanoid system is used once the glucose is removed). Our initial plan to solve these problems centered upon the oxidation route for several reasons.

SCHEME 9

Planned Conversion of Asperuloside to Prostaglandins

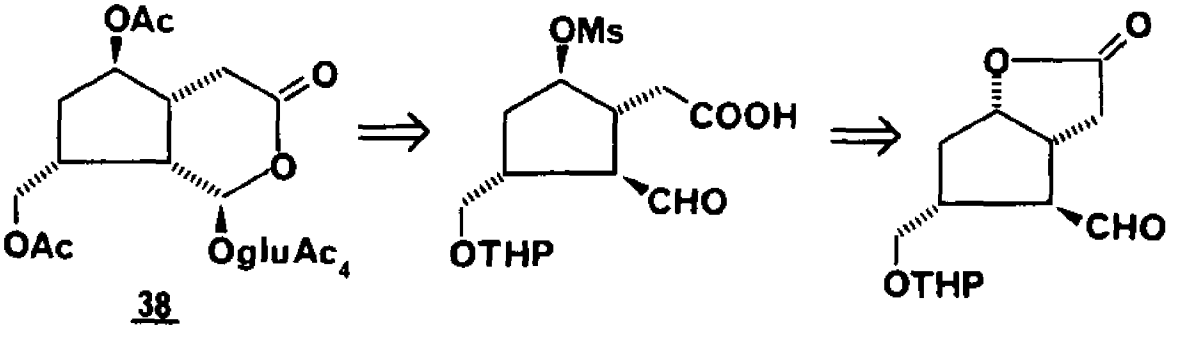


First, it was more intensively studied in the literature although not for asperuloside. Second, as shown in Scheme 9, the oxidation of C-3 to a carboxylic acid, which is a derivative of malonic acid, enables its removal by decarboxylation. Third, we had doubts about the ease with which we



37

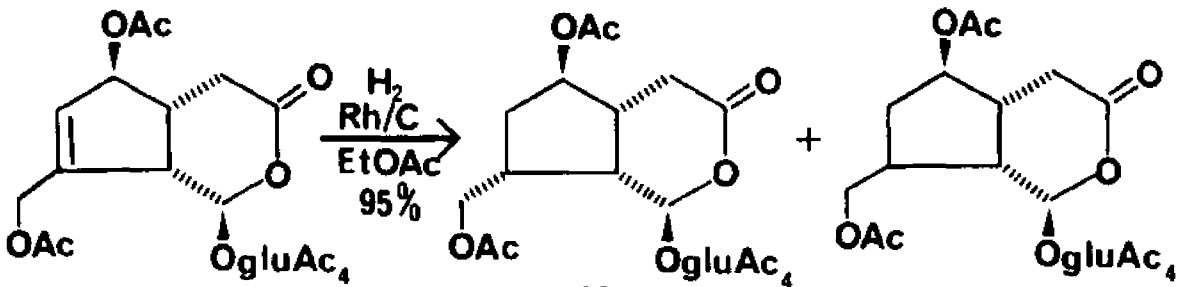
could convert the tetrahydro derivative (37) to the desired aldehyde and at the same time remove C-3 as the carboxylic acid. Finally, we desired a method applicable even to iridoids which do not have the carbonyl at C-11 and, at least



38

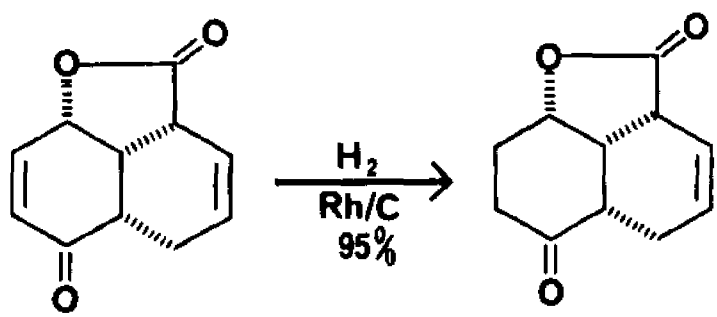
in theory, oxidation of C-3, hydrolysis and then subsequent lactonization is an excellent way to form Corey-like intermediates from these iridoids as shown for dihydro aucubin lactone (38). This has actually been accomplished in our laboratory (see Chapter V and Scheme 16).

We based our optimism for the successful hydrogenation of asperuloside tetraacetate dilactone 35 to dihydro asperuloside tetraacetate dilactone 36 (Scheme 9) on the previous success in our laboratory in the hydrogenation of aucubin



33 38 39

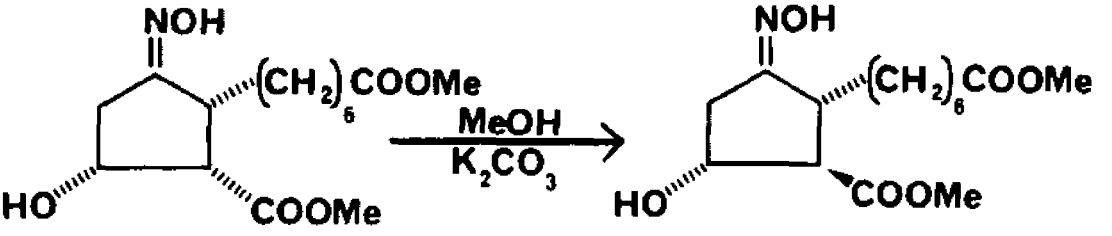
lactone (33) to a mixture of two epimeric dihydrolactones (38 and 39) in high yield without hydrogenolysis of the allylic acetates [62]. We were not concerned about the predominance of hydrogenation from the alpha face since asperuloside is so much more hindered on this side than aucubin that we expected to get at least a majority of the product with the correct stereochemistry. Evidence that the lactone would survive also comes from the reported high yield hydrogena-



40

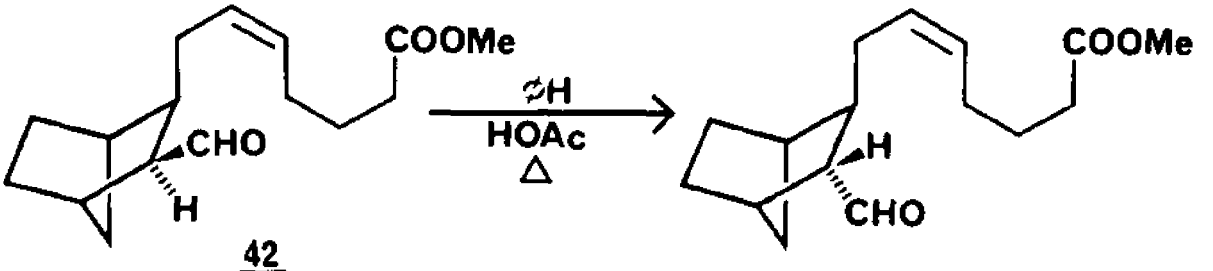
tion of 40 [63] using rhodium on carbon. Similarly, we were not concerned about the epimerization of C-9 since there is ample evidence that this would assume the more stable

trans-trans arrangement once it became epimerizable. Exam-



41

ples of this are the epimerization of compound 41 in basic



42

solution [64] and the epimerization of 42 in acetic acid [65]. Nor were we concerned with the final steps from the lactone-aldehyde to the prostanoids since we expected our analog to mimic the behavior of Corey's (see Scheme 6). Thus we felt that it was the formation of asperuloside tetraacetate lactone (35) which was the key to the success of the synthesis.

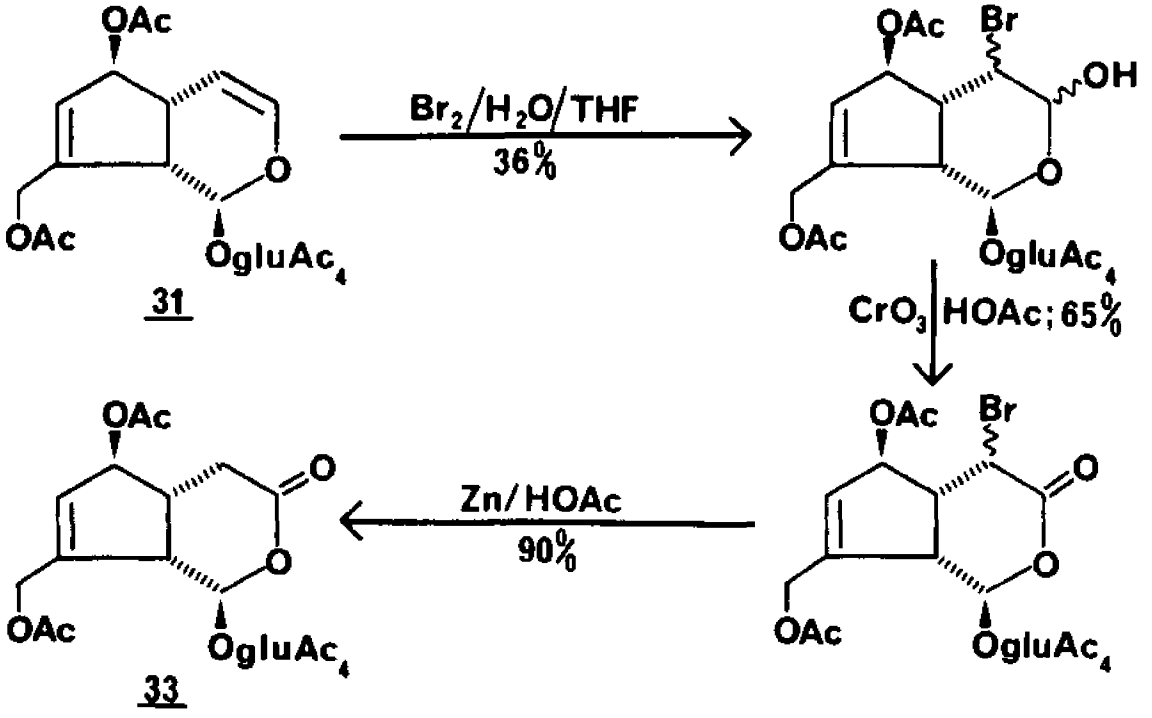
Since this conversion is the most important of the synthesis, it is enlightening to present a brief survey of the methods available for achieving this result. Throughout this section it must be kept in mind that not all the meth-

ods discussed are directly applicable to asperuloside since the conditions may be prohibitive (too acidic or basic), the reagents too expensive and the regioselectivity may be questionable. Specific methods that have been chosen for testing can be seen in the section covering the results of our work.

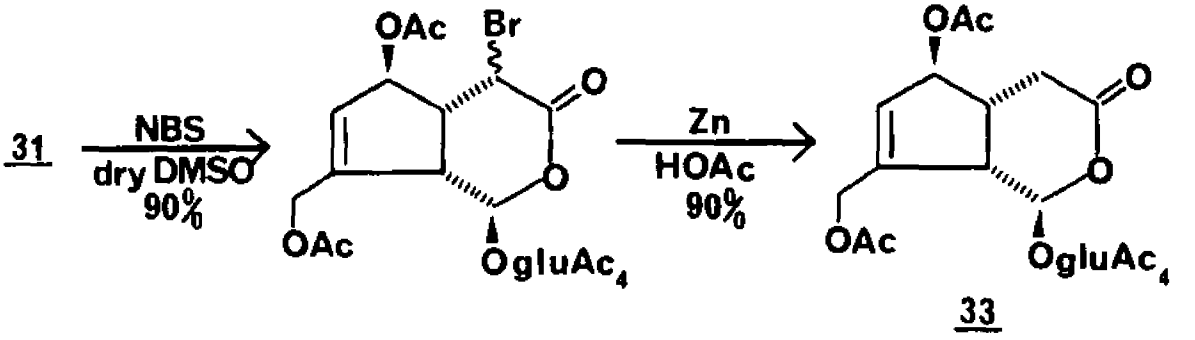
Chapter IV

CONVERSION OF THE ENOL ETHER SYSTEM TO A LACTONE

As previously mentioned, the first procedure applied to the conversion of the enol ether system of the iridoids to a lactone was the Schmid preparation of aucubin hexaacetate lactone via the bromohydrin. Unfortunately, work in our lab-



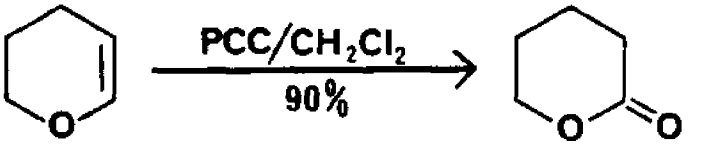
oratory has shown this to be a low yield (about 20% overall) sequence. Work in our laboratory improved the yield substantially through use of the Dalton [66] bromohydrin procedure (NBS/wet DMSO, 61%) and the Jones oxidation ($CrO_3/H_2SO_4/H_2O$, 90%) and subsequently, through the use of dry DMSO, a two step conversion of aucubin hexaacetate to its lactone was



33

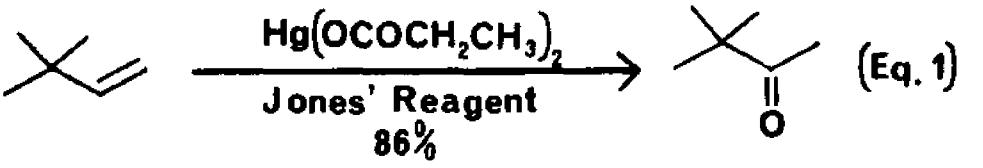
developed which proceeded in better than 80% yield [62]. This was the most promising method for effecting this conversion when we began our work on asperuloside but there are others worthy of mention which had not yet been applied to the iridoids.

The number of one step conversions of alkenes (and thus enol ethers) to ketones (thus lactones) is severely limited since most methods require the isolation of an intermediate compound. In fact, there has been only one example reported in the literature of a direct conversion of an enol ether to



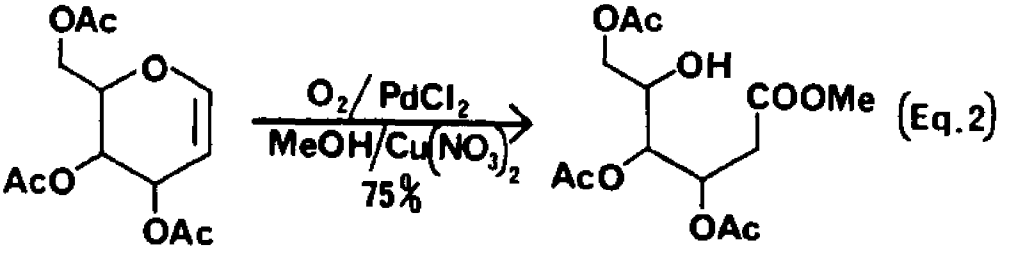
43

a lactone. This was the oxidation of 43 to δ-valerolactone with pyridinium chlorochromate [67]. While this procedure does not work for olefins of normal nucleophilicity (i.e. simple double bonds), there are several examples of oxidations of olefins which should be easily applicable to enol ethers. These include the mercury (II) catalyzed oxidation



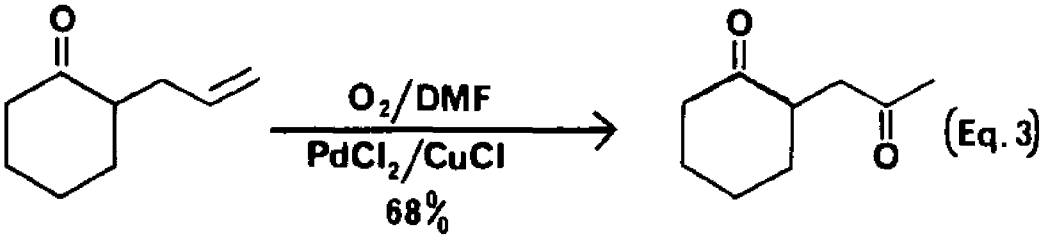
(Eq. 1)

with Jones' reagent (Eq. 1) as developed by Whitesides [68],



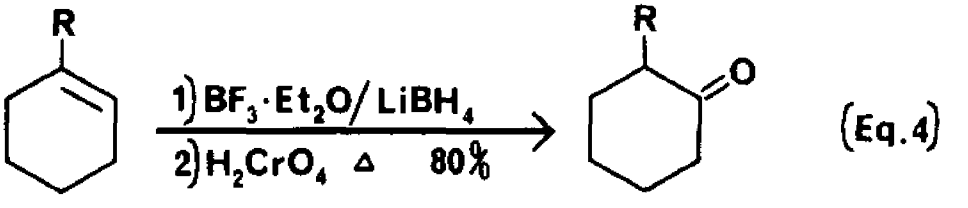
(Eq. 2)

a variation of the Wacker oxidation developed by Gaudemer and Deslongchamps (Eq. 2; [69,70]) and the analogous reac-



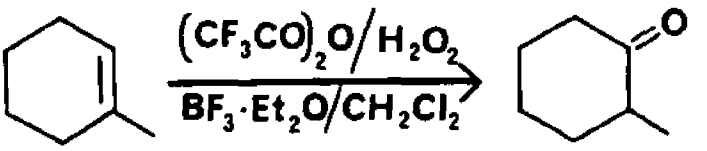
(Eq. 3)

tion with cuprous chloride in DMF (Eq. 3; [71]). Also included in this category is the procedure developed by Brown



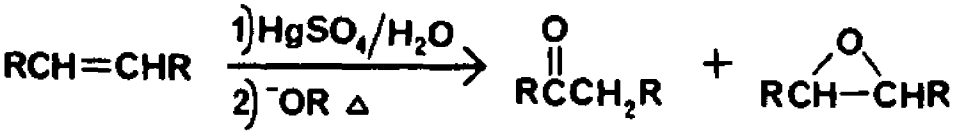
(Eq. 4)

and Garg [72] for in-situ oxidation of the borane (Eq. 4) as well as the epoxidation procedure of Hart and Lerner (Eq. 5; [73]). In addition, two methods of effecting an oxymercura-

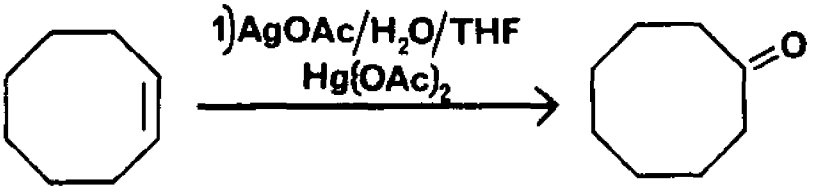


(Eq. 5)

tion-demercuration so as to achieve a carbonyl rather than the usual hydroxyl are known although the yields are not



(Eq. 6)

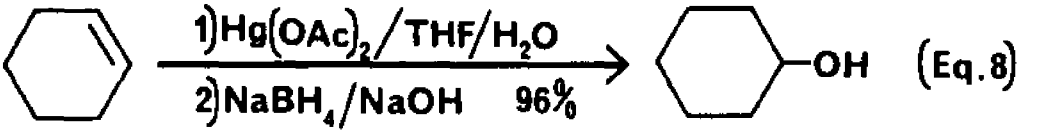


(Eq. 7)

good. These are shown in Equations 6 [74] and 7 [75]. Olefins have also been oxygenated directly to the ketones using oxygen and rhodium tris(triphenylphosphine) chloride but in low yield (20%) [76]. One additional method, but one which would most likely suffer from low yield, would be to hydrogenate the enol ether and then oxidize the ether with ruthenium tetroxide either directly [77] or catalytically using sodium metaperiodate [78].

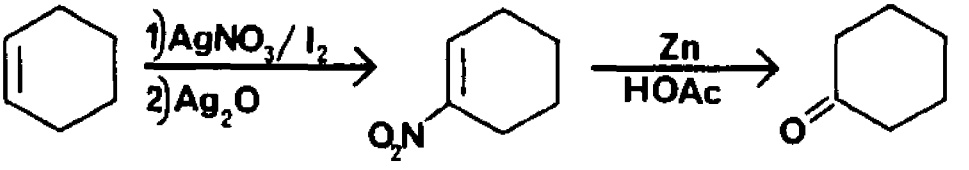
In addition to the method (above) developed in our laboratory, there are several two step sequences which might be used to achieve this conversion. The most obvious is hydroboration-oxidation to yield the alcohol followed by oxida-

tion to the ketone but this would obviously not give the desired product in our scheme. However, the solvomercura-



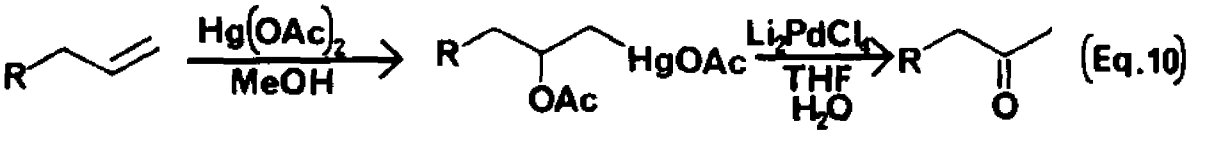
(Eq.8)

tion-demercuration reaction (Eq. 8) developed by Brown and Geoghegan [79] might prove useful as the source of alcohol which would then be oxidized in a second step with Jones reagent. Mercuric nitrate has also been used in this reaction [80]. Other methods include the reaction of the alkene with cyanogen azide to give the cyanoiminoketone followed by acid hydrolysis to the ketone [81], reaction with nitrosyl chloride/acetic anhydride to give the α-chloroketoxime followed by reduction with chromous acetate to give the ketone [82]



(Eq.9)

and nitration with iodine-silver nitrate-silver oxide (Eq. 9) to give the nitroalkene which could then be reduced to the ketone [83]. Still another two step method is the conversion of the alkene to the methoxymercuriacetate followed

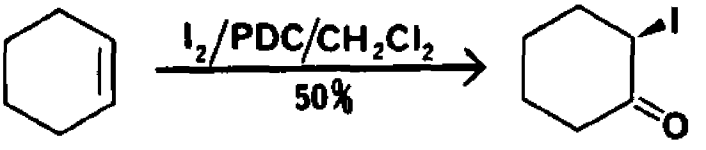


(Eq.10)

by oxidative demercuration [84] as shown in Equation 10. It

is worth noting that the methoxymercuriacetate of asperuloside tetraacetate is a known compound although it has only been prepared in moderate yield.

One final example of a two step conversion, which proved to be extremely important to this project, appeared recently. It is the formation of α-iodoketones from alkenes in one



step as shown [85]. Zinc and acetic acid reduction may then be used to complete the sequence. This is actually just a less expensive version of the same reaction which had previously been achieved in comparable yield with iodine and silver chromate [86] and in lower yield [87] with chromyl chloride (to give the α-chloroketone).

The reaction sequences of longer than two steps which could be devised to convert an alkene to a ketone are too numerous to cover completely and in any event this would not be very enlightening. Several of the three step methods do deserve mention. The "usual" sequence as given for the iridoids above may be varied substantially. The bromohydrin of the first step may be a hydroxy-mercuriacetate, the oxidation may be done with any of dozens of reagents (see results and discussion section) and the reduction can use chromium acetate [88], sodium cyanoborohydride [89] or tributyltin

hydride [90] instead of zinc and acetic acid. Another widely-used three step procedure involves the epoxidation of the olefin using a peracid followed by epoxide opening and oxidation of the resultant alcohol. In the case of asperuloside, this would certainly fail, if not due to the other (cyclopentene) double bond then certainly due to the likelihood of forming the wrong (tertiary) alcohol. One other three step procedure is conceivable. This is the conversion of the alkene to a dihalide followed by oxidation of the less hindered secondary halide (at C-2) via a nucleophilic displacement with trimethylamine-N-oxide [91], chromate ion [92] or even possibly DMSO [93] to give the haloester which could then be reduced as above.

Thus, there are a considerable number of methods which might accomplish the conversion of the enol ether system of the iridoids to a lactone (ester) and the prospects for finding a method applicable to the majority of iridoids seemed good.

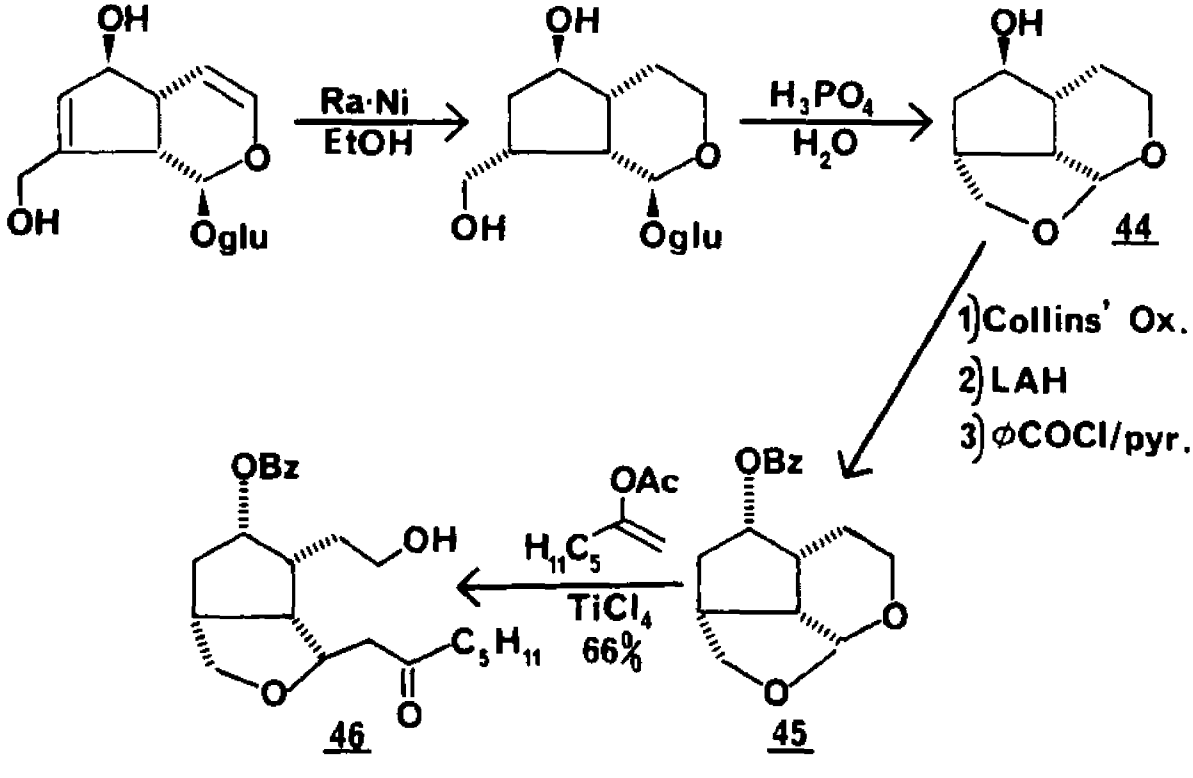
Chapter V

PREVIOUS WORK ON OBTAINING PROSTANOIDS FROM IRIDOIDS

Other work on this type of conversion has been conducted simultaneously with our own by Ohno and co-workers of Toray Industries in Japan. They have converted aucubin to various prostanoids by two methods, both quite different from the route proposed above and based upon the stabilization of the iridoid by reduction of the double bonds.

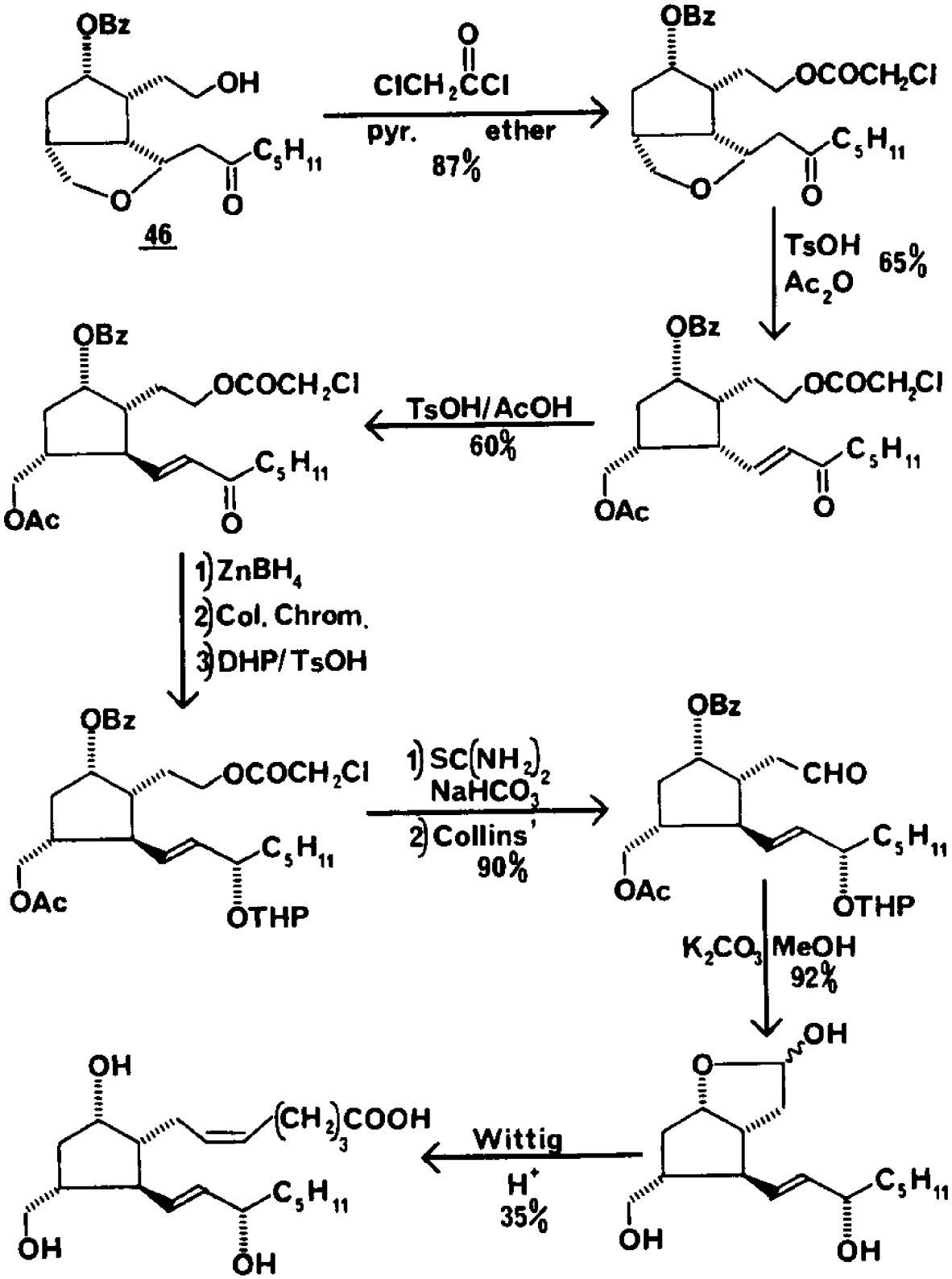
SCHEME 10

Ohno's First Synthesis of Intermediates for PG's from Aucubin



SCHEME 11

Ohno's Synthesis of (+)-11-deoxy-11α-hydroxymethyl $PGF_{2\alpha}$



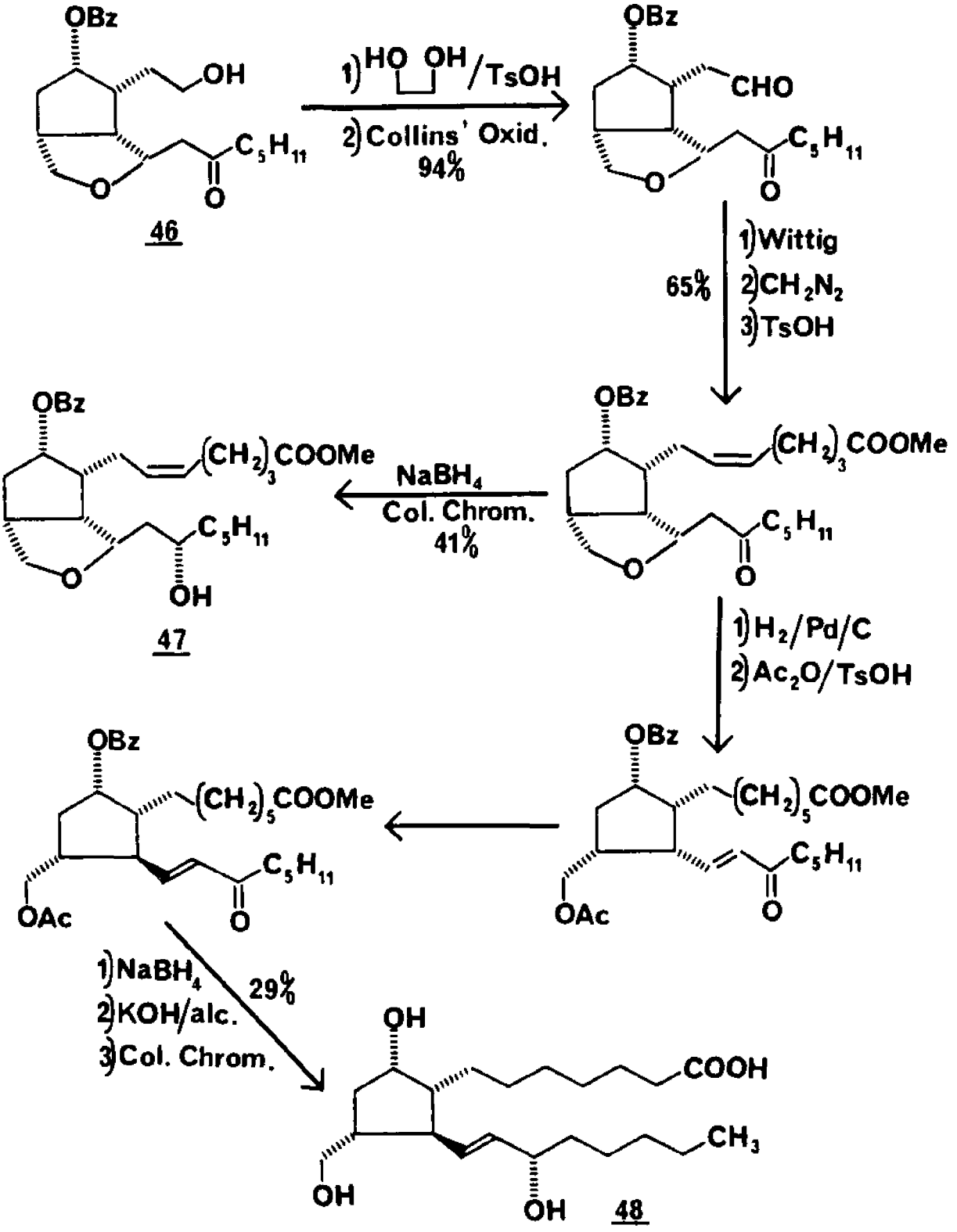
The first route [94] involves the synthesis of a key intermediate 46 by titanium tetrachloride promoted aldol condensation (Mukaiyama reaction) of acetal 45 with the appropriate enol acetate (2-acetoxy-1-heptene) as shown in Scheme 10. As in all of Ohno's syntheses, aucubin was stabilized by reduction with Raney nickel and subsequently hydrolyzed in aqueous phosphoric acid to produce tetrahydroanhydroaucubigenin (44) in unstated yield. The configuration at C-6 was then inverted, the alcohol protected and the aldol condensation carried out to give 46. These workers have converted this intermediate to 11-deoxy-11α-hydroxymethyl analog of $PGF_{2\alpha}$ as shown in Scheme 11 and have also synthesized prostanoids 47 and 48 from this material (Scheme 12; [95,96]).

The second route (Scheme 13) devised by these workers [97] involves conversion of the same cyclic acetal 44 to a monothioacetal 49 which was then converted to the protected (benzoate) 11-hydroxymethyl analog (50) of the Corey aldehyde. This was then converted to the natural prostaglandins (PGE_2 and $PGF_{2\alpha}$) as well as analogs as shown in Scheme 14 (p. 59). This route has also been adapted so that the "top side chain" (C-8) can be added first [98]. This produced the prostanoid 52 as shown in Scheme 15 (p. 60).

In addition to this Japanese group, one other team of researchers has been working on the synthesis of prostanoids from iridoids. Weinges and co-workers in Germany [99] have

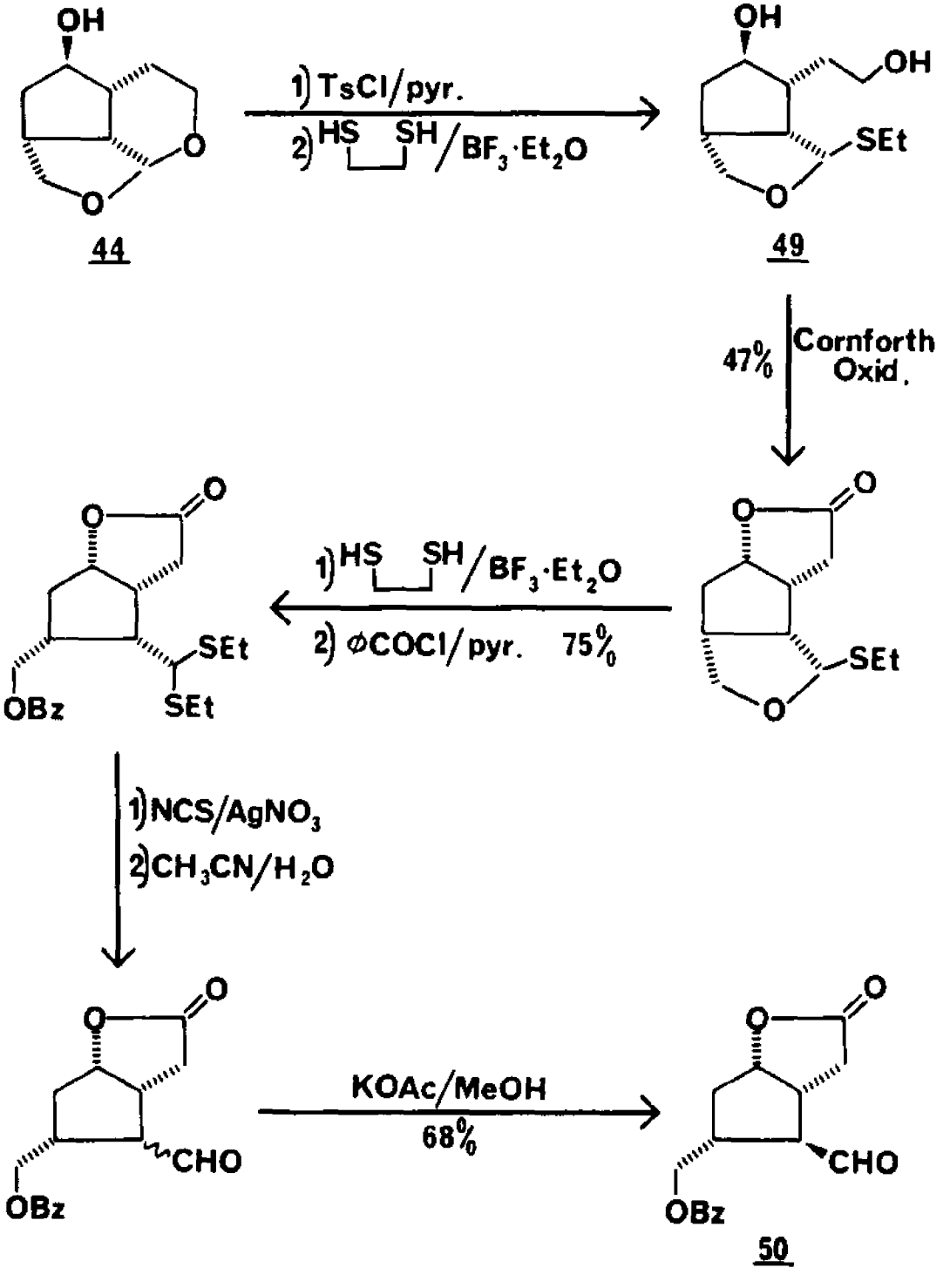
SCHEME 12

Prostanoids From Ohno's First Route

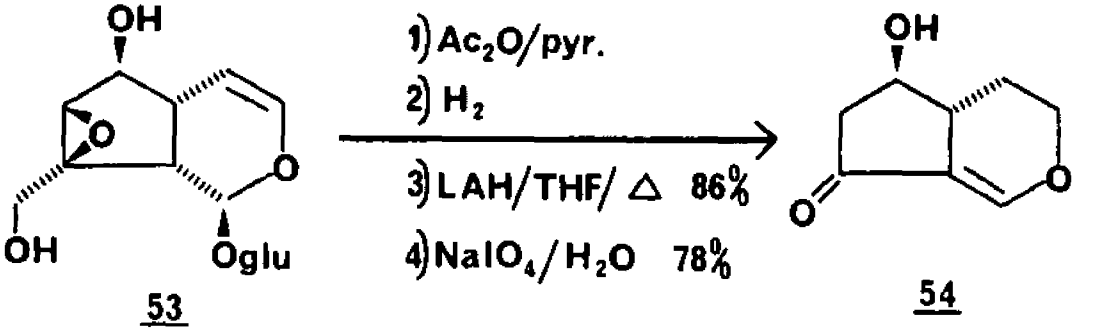


SCHEME 13

Ohno's Synthesis of a Corey Aldehyde Analog



converted catalpol (53) to the keto enol ether 54, an inter-



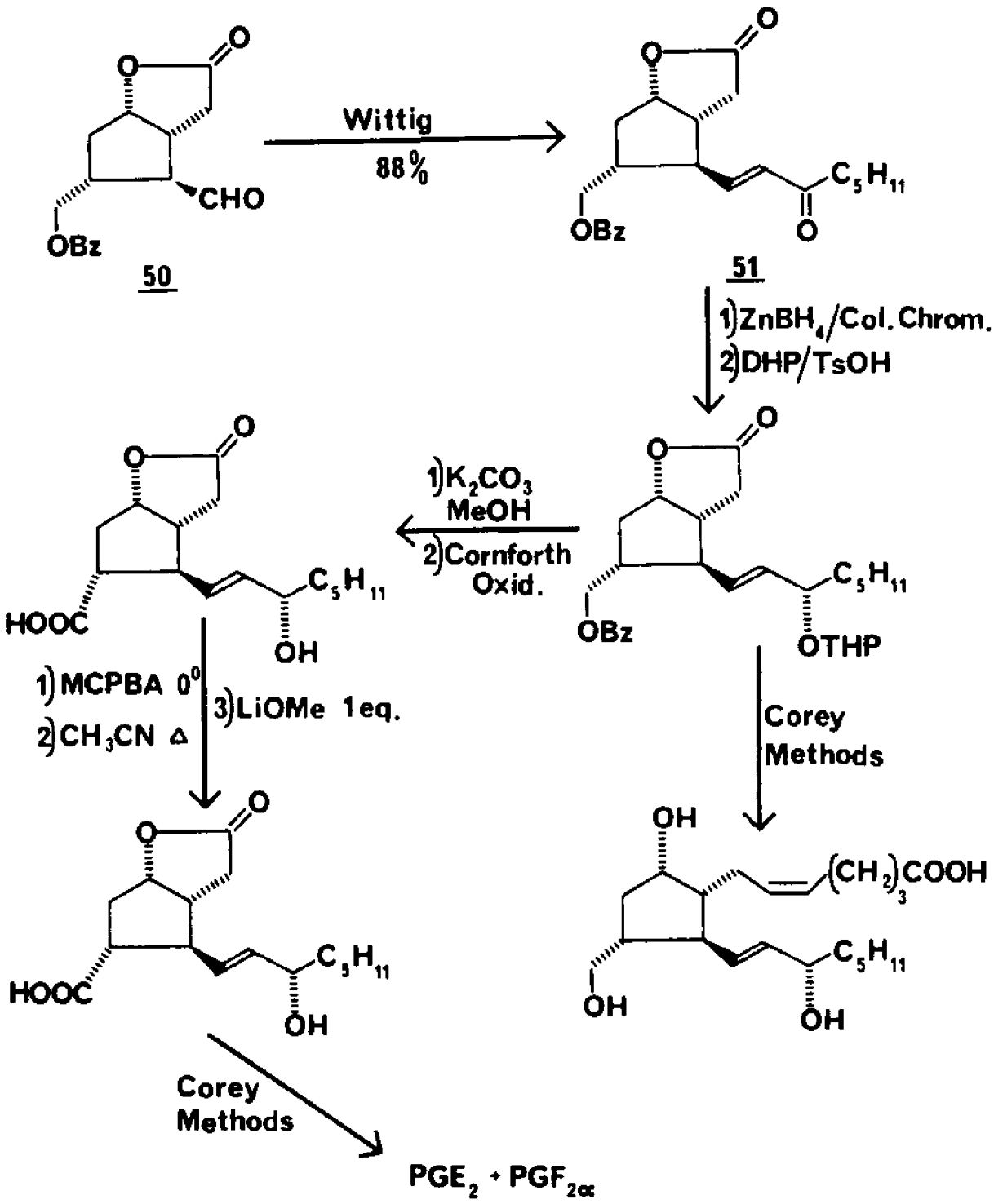
53 54

mediate from which they hope to synthesize prostanoids and other natural products. However, they have not reported any further results.

Previous work in our laboratory [62,100] has been directed at the conversion of aucubin to prostanoids (Scheme 16, p. 61) but has followed the oxidative route rather than a route similar to either of the above. Unfortunately, the highest yield leads to the 11-epi intermediates since the hydrogenation occurs (predominantly) from the wrong side. Nevertheless, the final product (55 in Scheme 16) is a valid prostanoid precursor similar to those derived by Ohno's second route.

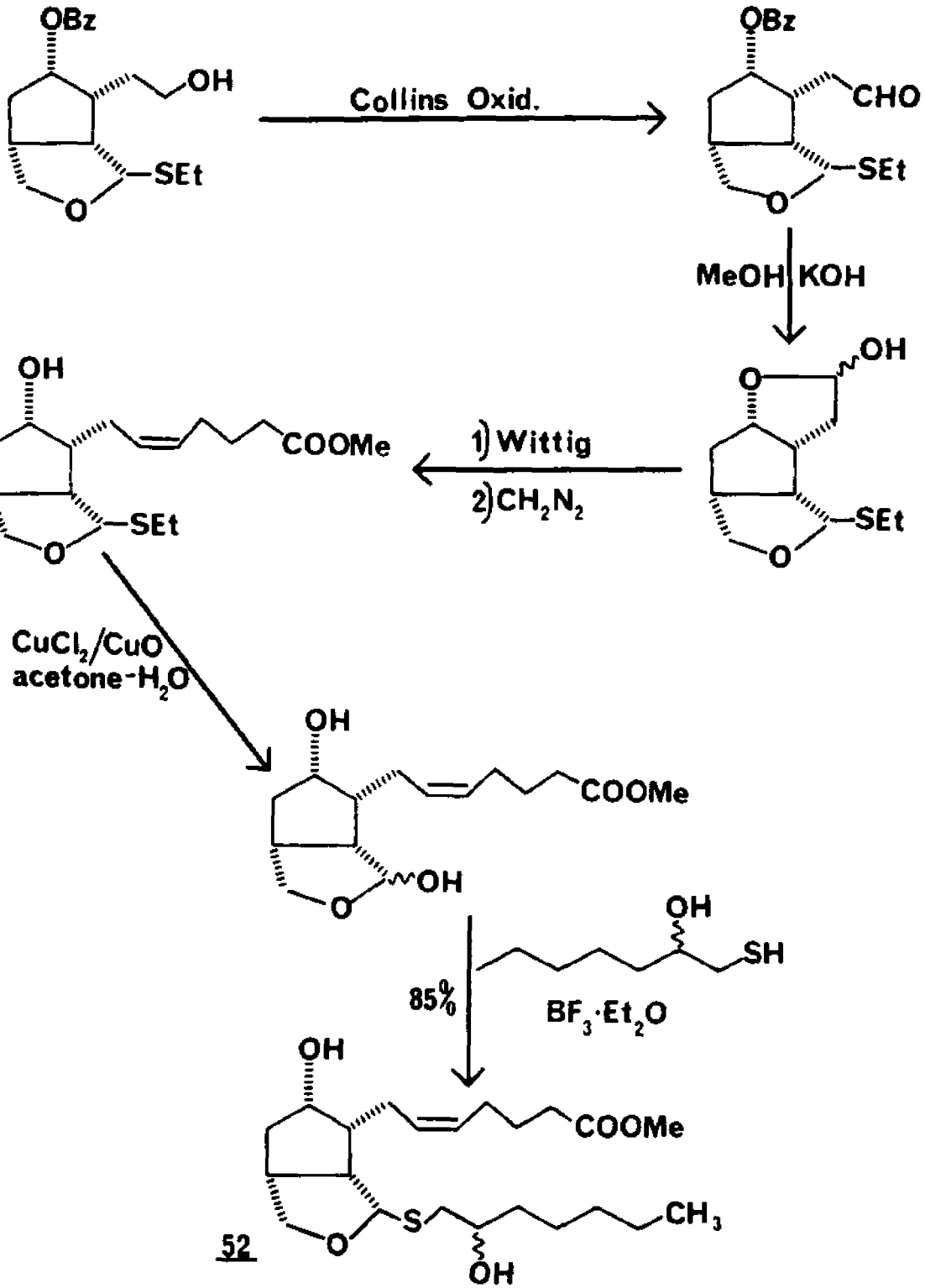
SCHEME 14

Prostanoids From Ohno's Corey Aldehyde Analog



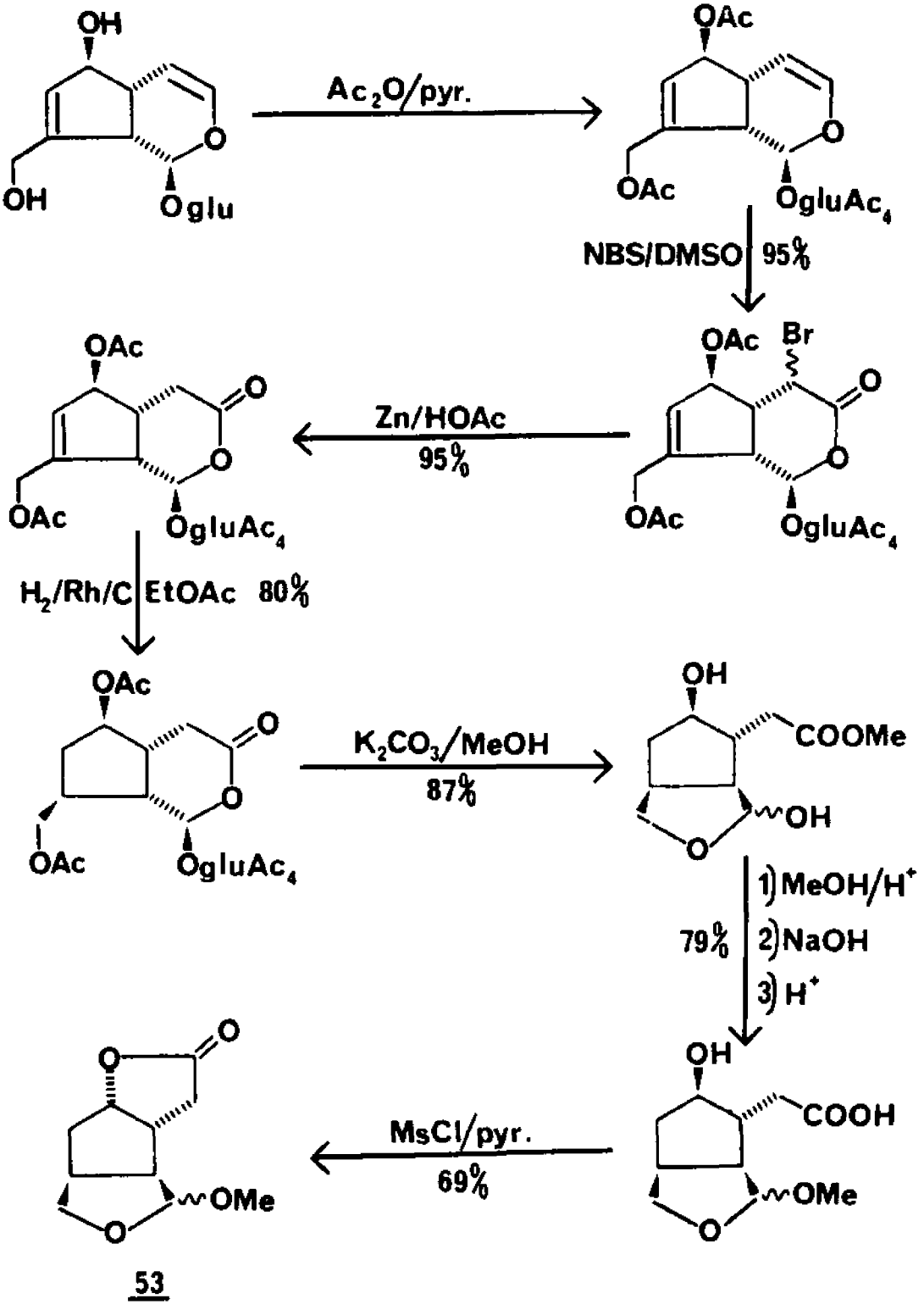
SCHEME 15

Prostanoids From Ohno's Monothioacetal Intermediate



SCHEME 16

The Prostanoid Intermediate of Berkowitz et. al.



53

Chapter VI

RESULTS AND DISCUSSION

The conversion of asperuloside to prostaglandin intermediates that has actually been developed is even shorter and more convenient than originally visualized. The detailed discussion of our results appears in this chapter and a summary of the actual synthesis developed is given in the following chapter.

6.1 ISOLATION OF ASPERULOSIDE

In any large scale synthetic work, the aim is to find a "readily available" starting material and a suitable supplier. In this case, asperuloside (the starting material) is widely available (see Chapter II) but the location of a suitable bulk supplier (i.e. plant species) was a considerable challenge. The perfect source would be common, inexpensive, high in iridoid content, low in sugars, available year-round and fast growing. The isolation procedure selected must be amenable to large scale work thus eliminating from consideration those methods (see Introduction) which employ volatile and/or toxic solvents or clarifying agents.

With these considerations in mind, the procedure developed by Duff and co-workers [101] was used for the initial

survey and isolation of asperuloside. We first selected plant species readily available in our climate. Thus, we first surveyed Liquidambar styraciflua which grows in abundance in the parks of New York City and is known as the sweet gum tree. The fresh plants were separated into batches of leaves, stems and fruit (called "sweet gum balls"). The stems and fruits were broken open as well as possible and each batch was then covered with water. These were then boiled for varying lengths of time (between 15 min. and several hours) and filtered crudely to remove the bulk of the plant mass. Comparison of the TLC behavior of these extracts (using various developing solvents) with a known sample (kindly provided by Dr. J. M. Bobbitt, University of Connecticut at Storrs) did not show any material exhibiting the characteristic bright blue color of asperuloside on heating with sulfuric acid. Further purification of the extracts by various chromatographic methods also failed to produce any asperuloside-like material. Similar results were obtained with samples of the leaves and stems of Escallonia rubra.

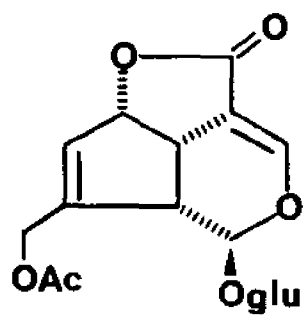
The crude extracts from Escallonia fradesi (known as the "pink princess") leaves and twigs did show some asperuloside and these were concentrated to a thick brown sludge which was then stirred with about twice its weight of Celite. This brown paste was further dried in vacuo for three days to yield a brown powder which was then extracted with acetone. Since most of the plant tannins and sugars are water soluble

but not acetone soluble, they remained on the Celite and concentration of the acetone extract gave a light brown oil which showed a strong blue spot for asperuloside on TLC. This oil could not be induced to crystallize and so the crude material was acetylated (acetic anhydride/pyridine) and chromatographed on silica gel (1:1 ethyl acetate:hexane eluent). This procedure resulted in the isolation of a small amount (less than 0.005% by weight of fresh plant) of asperuloside tetraacetate which was identical to an authentic sample from the laboratories of L. H. Briggs (generously provided by The University of Auckland, New Zealand).

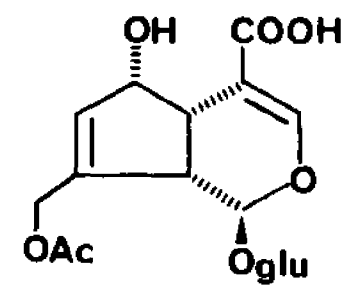
More promising results were shown by the aqueous extracts of the leaves, stems and fruits of Coprosma repens (also known as Coprosma baueri). This is a rather large evergreen shrub which is used as a hedge in many of the southern states and is grown in California as food for the primates at the world-famous San Francisco Zoo. Although the intense blue color formed on treatment of asperuloside with sulfuric acid makes quantitative evaluation of thin layer chromatography data impossible, our qualitative observations suggest that the fruits (red berries about one centimeter in diameter) have the highest content. Of course, the fruit is not available on a year-round basis and so cannot provide the desired source of material. Several isolation methods involving the twigs and leaves both separately and together have been developed and applied to large scale (as much as

40 kg. of plant at one time) extraction. Coprosma repens has been the only source used for the present work and from here on, any reference to plants or plant parts should be understood to refer exclusively to this species unless explicitly stated.

As discussed earlier, isolation techniques used for any iridoid vary with the plant source. The first method we developed for this situation was very similar to the method used by Lemieux to purify other plant extracts [102]. The filtered aqueous extracts (typically about a hundred liters of solution per 10 kg. of plant) were concentrated onto Celite and purified by liquid-liquid partition chromatography using water saturated with 1-butanol as the stationary phase and 1-butanol saturated with water as the moving phase. The brown oil thus obtained was hygroscopic and proved to be a mixture of asperuloside (25) and the known product of hydro-

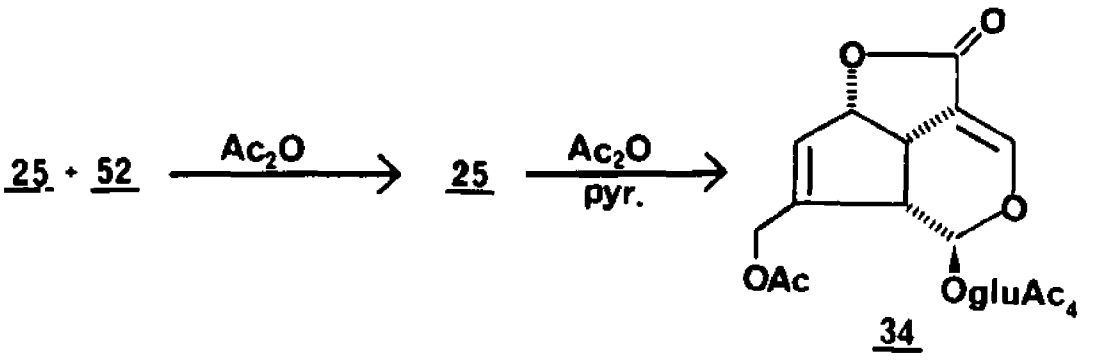


25



56

lysis of the δ-lactone (56, asperulosidic acid). This mixture was acetylated without further purification to yield a tan powder which could then be cleaned up using silica gel chromatography. Using an eluting solvent of 1:1 ethyl ace-



34

tate:hexane, the plant tannins remained nearly motionless at the origin and concentration of the fractions followed by recrystallization gave pure asperuloside tetraacetate (34) in 0.2% yield based on the weight of fresh plant parts.

Although this procedure is a general one which we have also used in the isolation of aucubin from Aucuba japonica [100], it is long and requires two column chromatographies one of which is very large-scale. The main reason that so much chromatographic purification is required is that water extracts so many materials from the plant. Briggs has reported successful isolations using ethyl acetate and acetone [53] without the need for chromatography. The key to these methods is that asperuloside is only partly soluble in these solvents while other plant compounds are either totally insoluble or extremely soluble. Thus, on concentration and cooling of the extract, the iridoid precipitates and most other impurities remain in the plant parts or in solution.

We have taken advantage of this to eliminate the first (partition) chromatography while still using water for the

initial (large scale) extraction. The filtered brown aqueous extract was concentrated onto Celite and dried in vacuo to a free-flowing brown powder which was then extracted with acetone. Concentration of the acetone afforded a thick brown oil which was acetylated and then purified as before on a silica gel column to give pure asperuloside tetraacetate in yields comparable to those of the "two column" method.

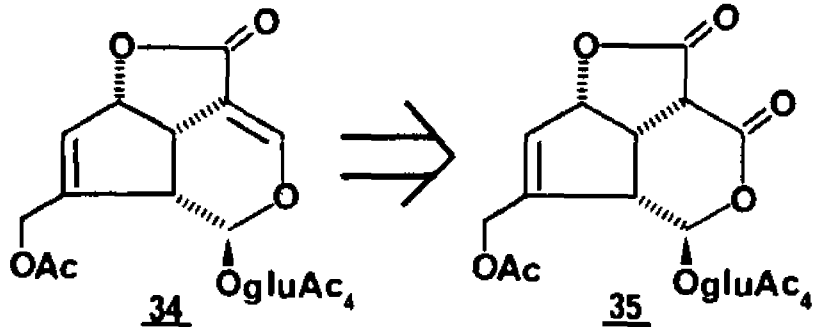
Direct extraction of the plant parts with refluxing acetone held the promise of eliminating all need for chromatography and this was easily accomplished on a pilot-plant scale thanks to Dr. William Schrieber (IFF in Union Beach, N.J.) and Dr. Vaskin Paraganian (McNeil Labs. in Washington, Pa.). The acetone extracts thus obtained were dark green and unfortunately still contained significant amounts of water since the plants could not be dried before use. Concentration of this extract (which turns brown in the process) and cooling resulted in the precipitation of a brown sludge rich in asperuloside. This was filtered, rinsed with hexane to remove remaining chlorophylls and then acetylated. Purification by silica gel chromatography was still necessary and again resulted in pure asperuloside tetraacetate in comparable yield.

The use of fresh undried plant parts would thus seem to constrain us to procedures using at least one chromatographic separation. Since air drying of Coprosma repens leaves

results in formation of healthy colonies of mold and oven drying destroys the iridoid (the leaves turn black), vacuum drying is indicated. It proved impossible to dry huge batches of plant material in this way although this would not present any problem on an industrial scale (as is shown by the many types of freeze-dried coffee on the market). Even so, a one kilogram batch of leaves was dried at room temperature under high vacuum for two days (58% reduction in weight) and then extracted with acetone. Concentration of the acetone, cooling, filtration and washing with hexane afforded a good yield (0.3% of undried plant weight) of asperuloside as a light green powder.

It should be noted that the acetone extracts of undried leaves are very "dirty" due to the huge quantity of water present and on several occasions, a second column chromatography was required to purify this material. Extraction of just the twigs is much cleaner and gives yields comparable with those from leaves alone or mixed parts while requiring only one chromatography. Twig extractions also give material which is easier to handle and less bulky than extractions of leaves. They also require less time, smaller apparatus and use less solvent. Thus, at least on a non-industrial basis, it is actually more expedient to discard the leaves and work only with the twigs and stems. Indeed, it is probably for this reason that other workers [53] have used only the bark of the plant as their source.

6.2 SYNTHESIS OF ASPERULOSIDE TETRAACETATE LACTONE



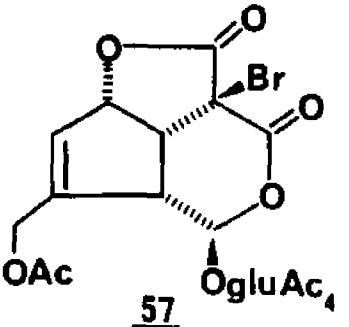
34 35

Our first synthetic goal was the conversion of asperuloside tetraacetate (34) to asperuloside tetraacetate lactone (35) in high yield. The methods available for this conversion have been reviewed in Chapter IV.

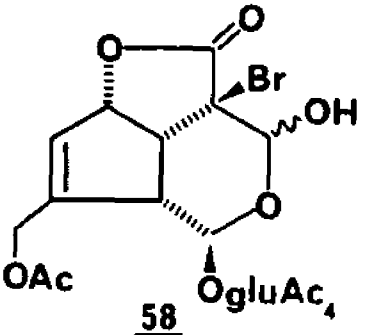
Previous experience with the NBS/dry or wet DMSO reaction led us to choose this method for our preliminary studies. Application of the NBS/dry DMSO reaction to asperuloside tetraacetate afforded a mixture of two compounds (R_f values 0.53 and 0.32 developed with 4:1 ether:ethyl acetate) which co-precipitated as a white amorphous solid when the reaction mixture was poured into water. Attempts to recrystallize this material resulted in decomposition to a thick brown tar which then showed only one mobile compound on TLC (precoated silica gel plates with 4:1 ether:ethyl acetate developing solvent, visualized by spraying with 10% sulfuric acid in methanol) and a large amount of decomposition product at the origin. Similarly, any attempt to separate the two compounds by column chromatography resulted in the decomposition of the slower-moving material and although the faster-moving

compound survived intact, it was so hopelessly contaminated by the decomposition products that purification seemed impossible.

The fact that we were obtaining a mixture of two products did not really disturb us since it is reasonable to expect



57



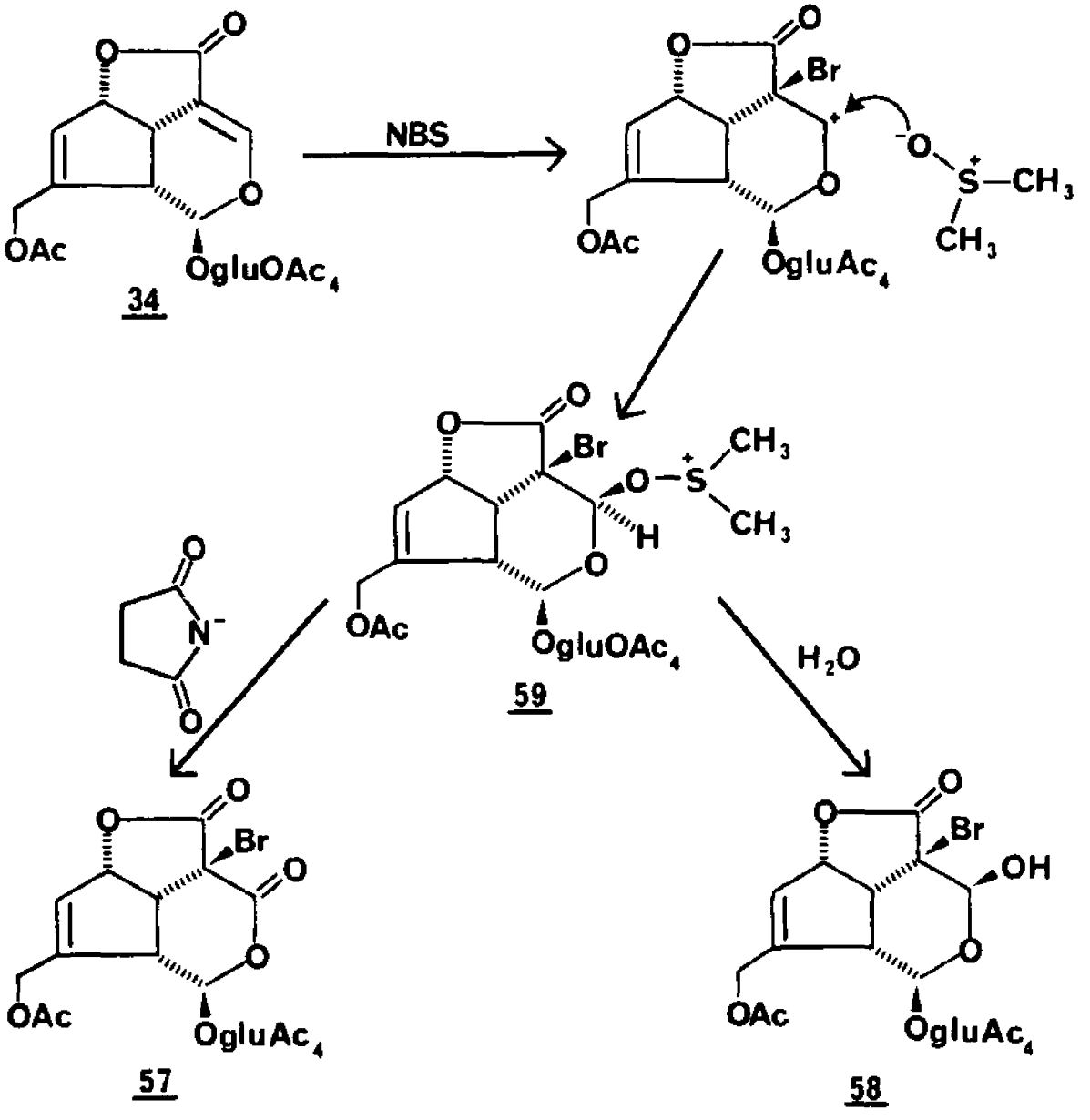
58

that in addition to the expected asperuloside tetraacetate bromolactone (57), some of the corresponding bromohydrin (58) could form if the system was not rigorously dry. However, even when extra precautions were taken to exclude moisture, the unstable (slower) compound was the major product (although quantitative results were not possible). Even more puzzling was the fact that when the Dalton bromohydrin procedure ([61], wet DMSO) was used, there seemed to be only a small change in the ratio of the products. The wet reaction afforded a slight increase in the amount of the unstable product but a significant amount of the fast compound was still formed.

If indeed the two products from this reaction are 57 and 58, it would be possible to rationalize this result on the

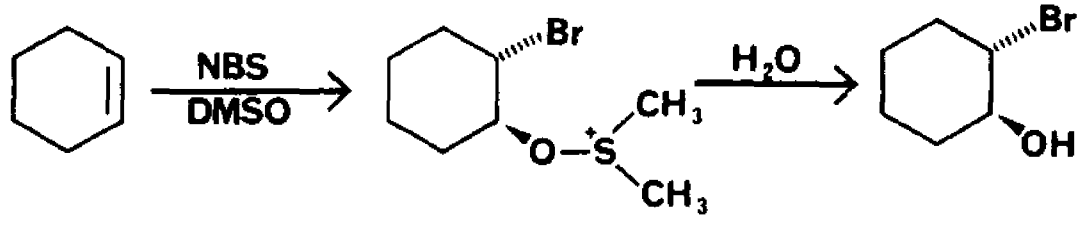
SCHEME 17

Possible Mechanism to Explain the Formation of 57 and 58



basis of the accepted mechanisms of the Dalton reaction [103] and of the DMSO-mediated oxidations [104] as shown in Scheme 17. This mechanism features a competition between attack of the sulfoxonium intermediate (59) by water (at the sulfur) and attack by succinimide ion (at either the methyl

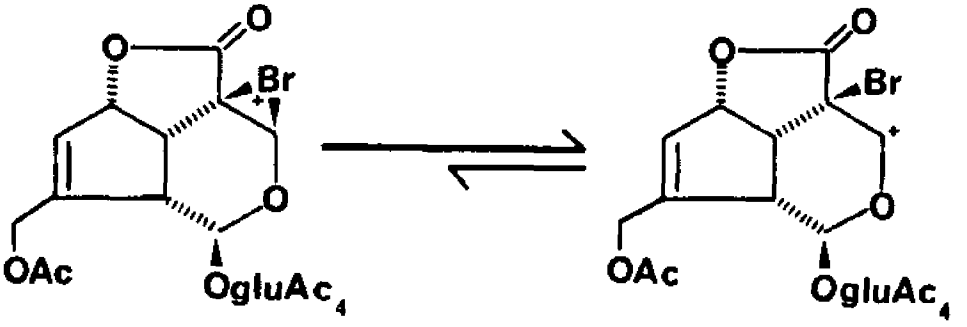
or the "acetal" hydrogen). The formation of large amounts of bromohydrin 58 even in the initial absence of water could be explained if the sulfoxonium intermediate was stable enough to survive until the reaction was poured into water for the work-up. This is quite plausible since succinimide is a large base and steric hindrance might prevent it from rapidly attacking the intermediate. Our confidence in this explanation was bolstered by the fact that the α-bromosulfoxonium



intermediate expected from treatment of cyclohexene with NBS/DMSO has been isolated as a stable tetraphenylboride salt [105]. This salt decomposed to bromohydrin upon addition of water and to bromoketone upon addition of trimethylamine [106]. We attempted to add this base in our reaction even though the asperuloside system is not stable to basic conditions and the expected decomposition to brown tar occurred rapidly.

It seems appropriate to mention the stereochemistry of these reactions at this point. The Dalton reaction has been found to give exclusively trans products [103] resulting from the backside attack of an initially formed bromonium ion. Asperuloside is so "folded" that it is reasonable to expect that the bromine will attack exclusively from the

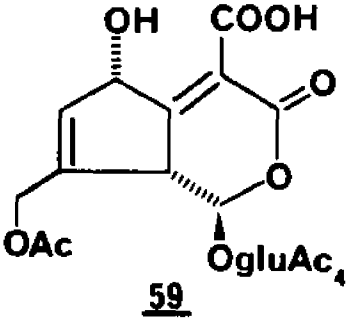
less-hindered side which is the side above the plane of the paper in all drawings used here (also known as the "β-face" of the molecule). Furthermore, since this is an enol ether, the bromonium ion should suffer attack at only the carbon alpha to the ether linkage. However, the ether oxygen may so stabilize the free carbonium ion that a bridged ion is not



present. If this is true, the stereochemistry of addition to the double bond would be syn since the nucleophile would undoubtedly also approach from the far less hindered beta face (note that the bromomethoxide of loganin was found to be cis by X-ray). The presence of the lactone carbonyl would seem to ensure this mode of attack. Thus, we are reasonable in our expectation that only one bromohydrin and one bromolactone should form (with stereochemistries as shown for 57 and 58).

Thus, since we had good reason to suspect that the two compounds were indeed the desired bromolactone and bromohydrin (and we had every reason to suspect that the slower (unstable) compound was the latter), we attempted the oxidation of the amorphous solid with Jones' reagent. This resulted in

the disappearance of the unstable (slower) compound, no change in the faster compound and the appearance of a new, very polar material which remained at the origin in the usual TLC solvent but could be moved if an alcohol-based solvent was used for developing. These compounds differed sufficiently in polarity so that they could be separated by fractional crystallization from methylene chloride/ether. Upon standing, fairly pure fast compound crystallized while only slightly impure "polar compound" remained in the mother liquor. Recrystallization of the fast compound from ethanol afforded crystalline asperuloside tetraacetate bromolactone 57 in 10% yield. The polar compound was recrystallized from



59

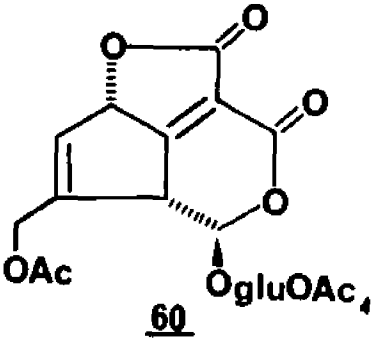
1:3 ethyl acetate:hexane and appears to have structure 59 (yield 25%). Structure 59 is supported by several facts:

1. Elemental analysis shows the absence of bromine.

2. That same analysis is correct for this structure.

3. The proton NMR shows a broad peak at 8.3δ with an area of slightly less than two hydrogens. This vanishes upon addition of deuterium oxide.

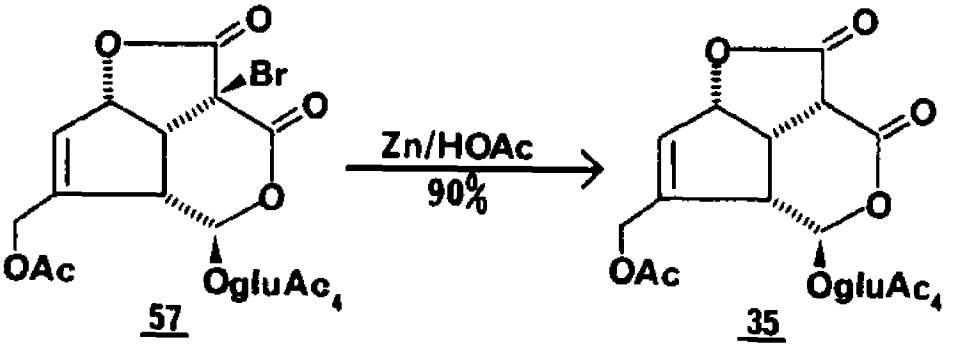
The formation of this compound may be rationalized if one visualizes initial elimination of HBr from the bromohydrin followed by oxidation of the now perhaps more readily acces-



60

sible lactol. This lactone (60) might then be sufficiently more strained than the desired bromolactone 57 to undergo hydrolysis in the aqueous sulfuric acid formed when the quenched Jones' oxidation mixture was poured into water. Of course, it is impossible to explain why a bromine alpha to two carbonyls would be less labile than one which is alpha to a single carbonyl. Nevertheless, we continued this line of research since the fast material (unchanged on oxidation) was definitely the desired bromolactone.

Treatment of bromolactone (57) with zinc and acetic acid



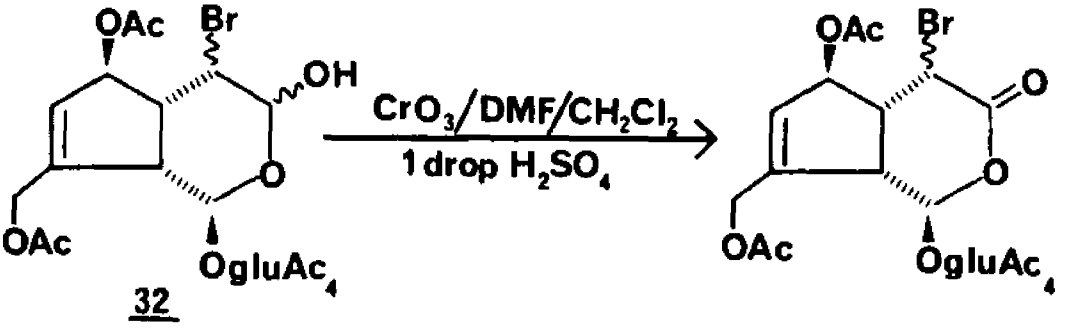
57 35

afforded an excellent yield of asperuloside tetraacetate

lactone (35). Unfortunately, treatment of the mixture obtained from the NBS/DMSO reaction resulted in a dark blue solution which contained only traces of lactone and no other identifiable compounds. If this reaction was run in the cold, asperuloside tetraacetate could be identified as a transient intermediate by TLC.

Since we still believed that the unstable compound was the bromohydrin, we attempted to oxidize the mixture using a wide variety of reagents and conditions. It must be remembered that the starting material for this work is not available commercially and it was thus necessary to use it sparingly. For this reason, the oxidations were followed by thin-layer chromatography and high pressure liquid chromatography but were not performed on a large enough scale to enable the isolation and thorough characterization of each product. Even so, the results seemed to fall neatly into two categories. There were those reagents which simply did nothing to the mixture until it decomposed (with "decomposition times" varying from less than five minutes to over a week) due to its instability, and those which seemed to leave the bromolactone intact (or increase its amount very slightly) but converted the other material to various highly polar products (arising from hydrolysis of the ɣ-lactone and various degrees of oxidation). The former included the Pfitzner-Moffatt reagent (DMSO/DCC/PTFA, [107]), DMSO/acetic anhydride [108], CrO_3/DMF/H_2SO_4 [109] and activated manganese

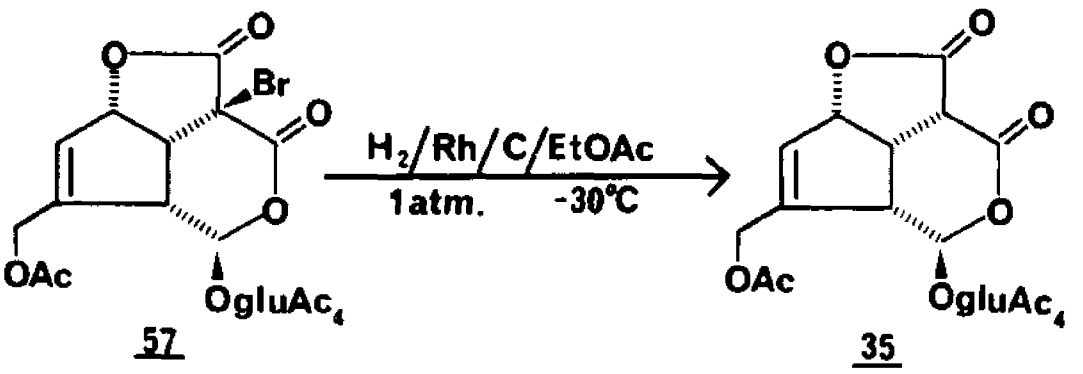
dioxide [110]. The latter included chromium trioxide in glacial acetic acid [111], pyridinium chlorochromate [112], pyridinium dichromate [113] and nitrogen tetroxide [114]. The failure of the chromium trioxide/DMF complex was espe-



32

cially surprising since we had applied this method to aucubin as a model system and had obtained excellent yields (>90%) by varying the original procedure so as to use methylene chloride as the solvent rather than dimethylformamide. In addition to these methods, oxidation by the Ratcliffe modification [115] of the Collins procedure [116] for use of the Sarett reagent ($CrO_3 \cdot 2$ pyr. [117]) resulted in the formation of significant amounts of asperuloside tetraacetate which then decomposed in the usual manner.

At this time, it appeared that the major source of instability in our mixture was the bromine. Since removal with zinc and acetic acid produces the desired lactone only at the expense of the major product, this method obviously could not be tolerated. We thus began a search for a more suitable reducing agent. Catalytic hydrogenation of the pure bromolactone (57) at -20°C produced the lactone (35) in quantitative yield. However, the mixture as obtained from



57 35

the NBS/DMSO reaction turned deep green under these conditions and none of the desired product could be detected. Presumably, the hydrobromic acid which is generated not only poisons the catalyst but also decomposes the less stable of our compounds. Since we knew that unlike previous workers [118], it was not practical for us to start with some base present to capture the acid as formed, it did not seem useful to pursue this type of reduction further.

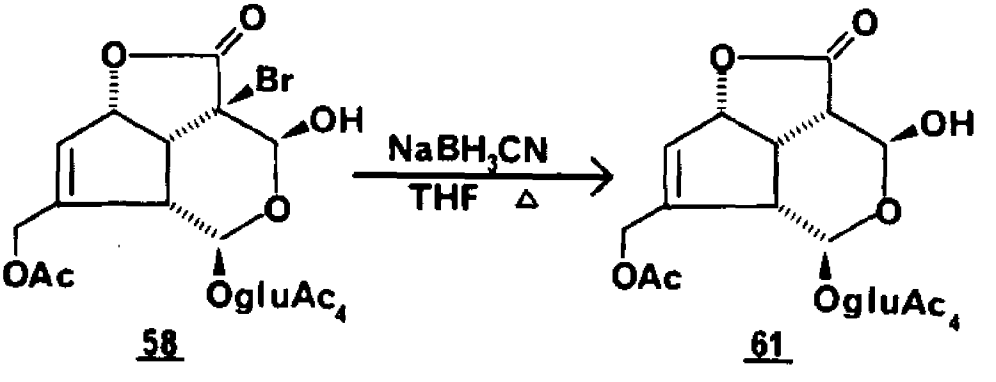
The use of chromous acetate [119,120] resulted in the formation of a small amount of the lactone from this mixture but it too did not successfully stabilize the other material (which seemed to remain unchanged until decomposition in the work-up).

Treatment of the amorphous solid obtained from the NBS/DMSO reaction plus the light brown oil obtained on extraction of the mother liquor (methylene chloride) with sodium cyanoborohydride [84] resulted in a mixture of stable, crystalline compounds. Separation by HPLC resulted in the isolation of asperuloside tetraacetate lactone (20% from as-

peruloside tetraacetate) and a stable derivative of the other material (47%) which we named "compound X". Preliminary analysis of "X" showed:

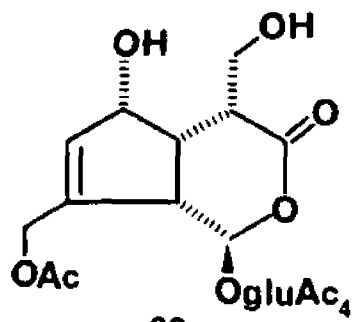
1. Elemental analysis was correct for asperuloside tetraacetate plus the elements of H_2O.
2. A single hydroxyl (by IR and NMR).
3. All the acetates remained (by C-13 NMR).
4. The cyclopentane double bond (between C-7 and C-8) was still present (by C-13 NMR).
5. The γ-lactone remained intact (by C-13 NMR).

These facts further enhanced our belief that we did indeed get the bromohydrin (58) from NBS/DMSO and that removal of

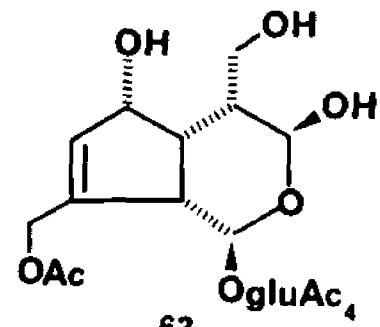


58 61

the halogen afforded asperuloside tetraacetate lactol (61). Unfortunately, treatment with sodium cyanoborohydride resulted in only modest yields of these materials accompanied by two very polar side products which we believe to result from reduction of the γ-lactone to the diol forming 62 and



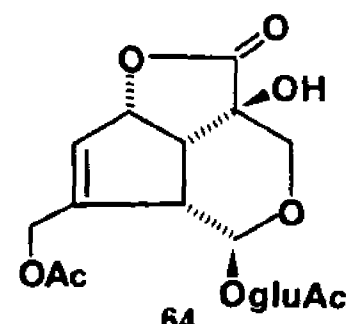
62



63

63. While cyanoborohydride has not been used for this purpose, the use of borohydride to reduce lactones is widespread [121,122]. These side products were not purified for full characterization.

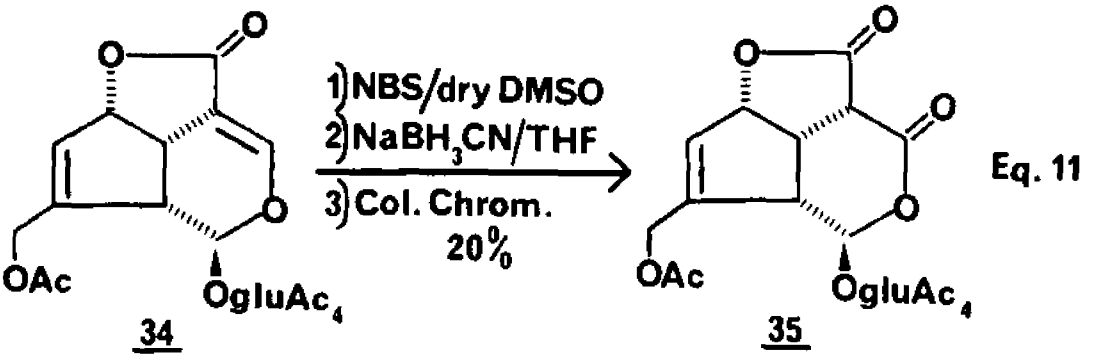
Now that the apparent source of instability had been removed, "X" was oxidized under a wide variety of conditions. Results were indeed at variance with those obtained earlier for the unstable precursor of "X". After lengthy exposure, Jones' reagent, pyridinium dichromate and pyridinium chlorochromate caused hydrolysis of the δ-lactone with no formation of the desired product. No reaction or decomposition was observed with DMSO/DCC/PTFA [107], manganese dioxide [110], barium manganate [123], NCS/DMS [124] and gaseous oxygen over platinum [125]. It no longer seemed probable that "compound X" was the desired lactol 61. Indeed, the best



64

guess at this point was that it was the tertiary alcohol 64. Detailed studies of the structure of this compound have verified that it is this tertiary alcohol. This analysis was conducted after the hydrogenation of the remaining double bond and will therefore be discussed in a later section.

Although we could not do anything to improve the yield of

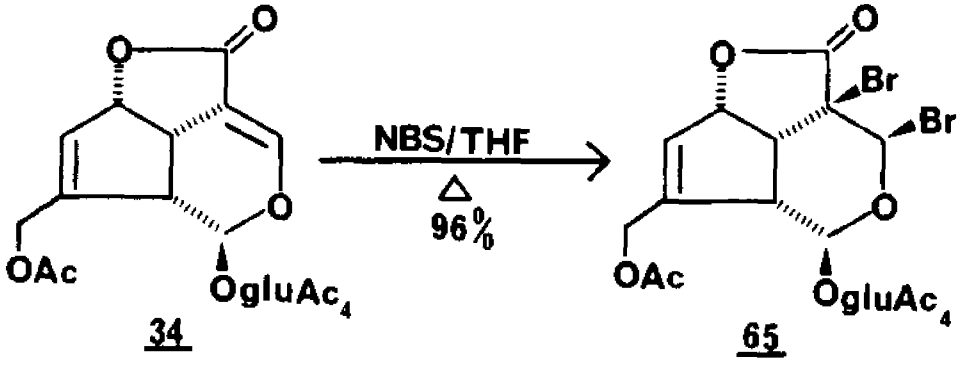


34 35 Eq. 11

desired lactone 35 at this time, it was reasonable to expect that this could be done eventually and our operating plan was to proceed with the rest of the synthesis while attempting various other routes to the lactone. In the interest of clarity, our various attempts to obtain this key intermediate in higher yield are all summarized here first. However, it must be remembered that most of the actual synthetic work discussed later was done on a very small scale with lactone obtained as in Equation 11.

Various other procedures were investigated in an attempt to prepare bromohydrin 58. Included among these were "known" methods such as bromine in wet tetrahydrofuran [61] and NBS in wet dimethoxyethane [126] as well as more speculative

methods such as NBS in a wide range of solvent not considered conventional for this purpose (ethyl acetate, dioxane, 1:1 DMSO:THF and acetone; wet and dry solvents used). The only promising reaction was the use of NBS in dry dioxane in the presence of traces of butylated hydroxytoluene (a widely used antioxidant). The reaction required the use of a large excess of NBS and did not proceed in the same way if shielded from the light of the room. So much bromine was present in the mixture that it was immediately submitted to column



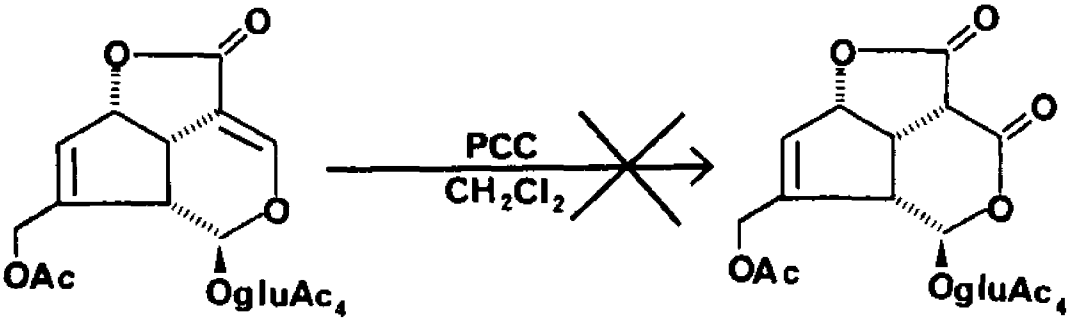
34 65

chromatography as the only work-up. This afforded an excellent yield of asperuloside tetraacetate dibromide (65) as a white crystalline material. The stereochemistry of the halogen-bearing carbons is not certain but is drawn as expected for this system. This material is stable for several weeks in a refrigerator but turns dark blue if left several days at room temperature.

Selective bromination of asperuloside tetraacetate had previously eluded Briggs and Cain [60] so we had not considered it as a viable alternative to bromohydrin formation. After it was in hand, the nucleophilic displacement of the

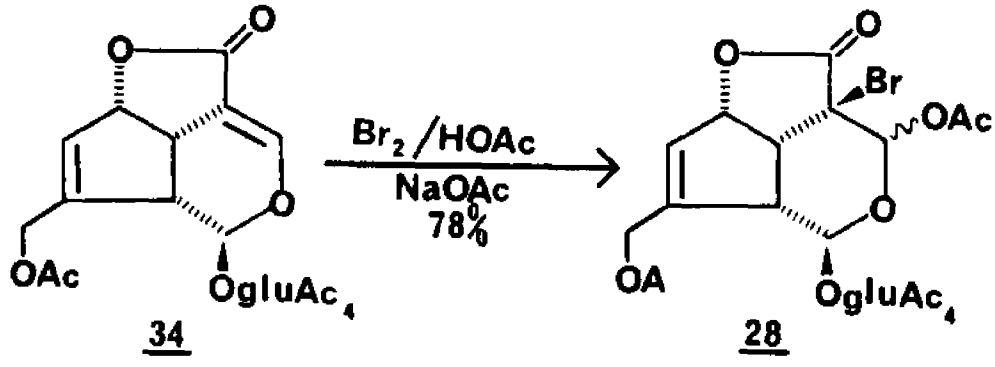
halide at C-3 was attempted (see Chapter IV). Unfortunately, the system does not withstand the basic conditions resulting from the use of trimethylamine-N-oxide and only a brown tar results. Heating in dry DMSO did give some reaction after 24 hours. Interestingly, the product resembled (TLC) the material isolated from the NBS/DMSO procedure described above. Nothing further was done to identify the products of this reaction since it was slow and thus considerable decomposition occurred before all starting material vanished. When the dibromide was refluxed over polymer-supported chromate ion [127], no reaction was observed until decomposition occurred. These attempts were abandoned at this point since, although the yield is excellent, the dibromide-forming reaction is messy.

Equally unsuccessful was an attempt to synthesize the lactone in one step using pyridinium chlorochromate [67].



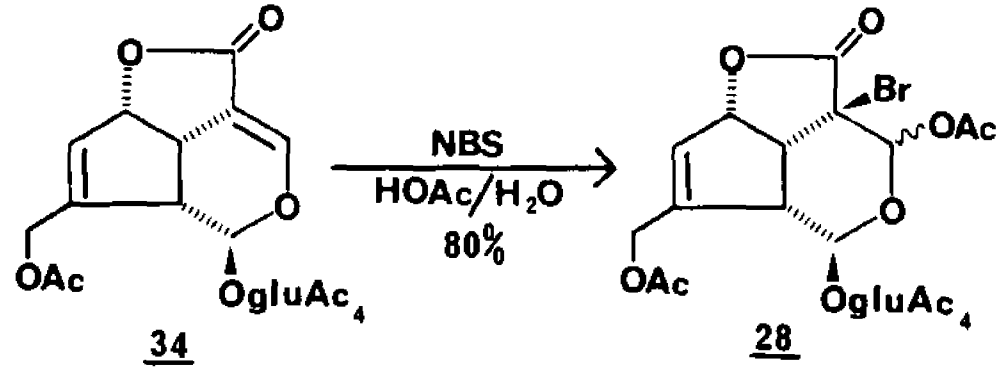
This oxidation is reported to be quite specific for enol ethers (see Chapter IV). However, although we were able to duplicate the original work, our system did not react at all with this reagent.

The preparation of asperuloside tetraacetate bromoacetate



34 28

(28) has been reported [41]. We were able to duplicate this procedure without difficulty and were also able to effect

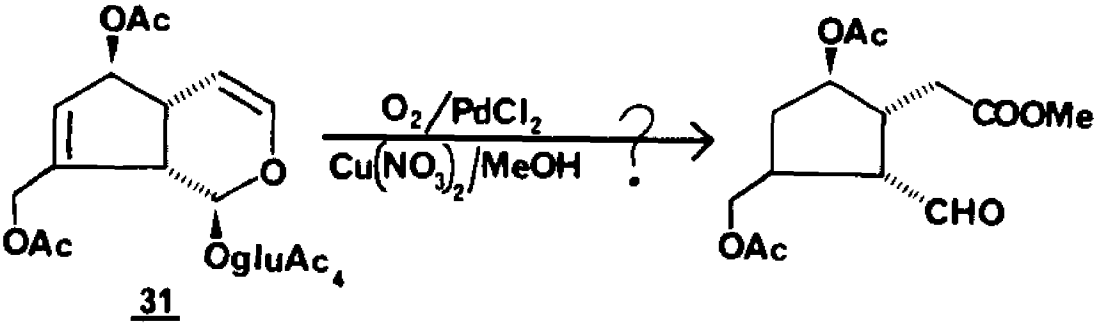


34 28

the same conversion in comparable yield using NBS in place of elemental bromine without the need to add sodium acetate. The fact that no bromohydrin (by HPLC) is formed even in the presence of water concentrations as high as 50% is certainly amazing and is one indication of the strange behavior of the asperuloside system. Attempts to remove the halogen with zinc in acetic acid resulted in the formation of asperuloside tetraacetate which was slowly converted to the blue polymer. Reduction with sodium cyanoborohydride reformed a small amount of the starting material, small amounts of two other products and two major products apparently resulting

from reduction of the lactone. Similarly, reduction using chromium (II) acetate produced a mixture containing at least five substances. This conversion was thus not straightforward and, given the moderate yield of bromoacetoxylate, was soon abandoned.

Since aucubin hexacetate (31) was more readily available to us than asperuloside, several of the more speculative conversions were tried with this material but were never applied to asperuloside since they showed little promise. They are mentioned here in the last paragraph before the successful route simply as a matter of record. Pyridinium hydrobromide perbromide has been reported to be more desirable than bromine [128] as a brominating agent. Our attempts to replace NBS and elemental bromine by this reagent in the various bromohydrin reactions went without success. Also

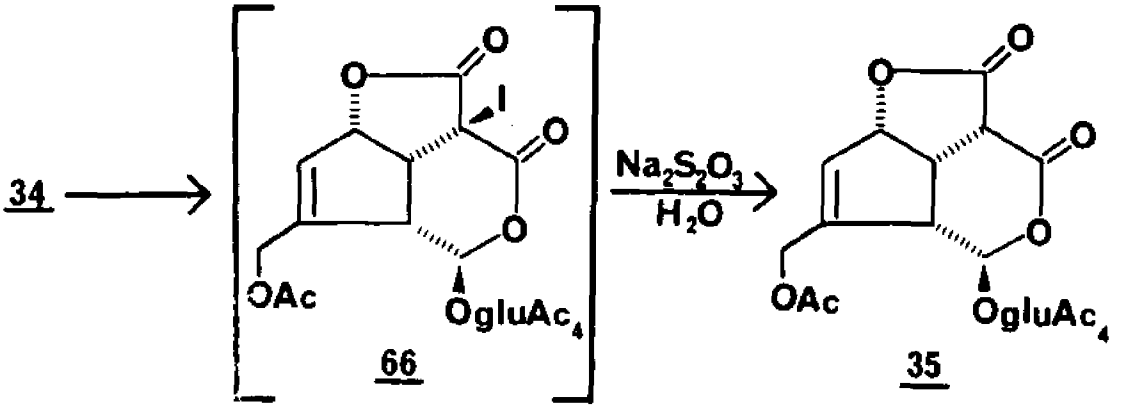


31

unsuccessful was an attempt to apply the Wacker oxidation to aucubin as shown here (compare to Eq. 3 in Chapter IV). This process gave only slow reaction and showed no less than eight products after one week. Similarly disappointing was the mercuric propionate catalyzed Jones' oxidation of aucubin (again, see Chapter IV) hexaacetate. After several days,

HPLC analysis showed mostly unreacted starting material plus a small amount of the desired lactone with small amounts of a number of other products. Since asperuloside would never survive in the presence of Jones' reagent for this length of time, the use of this method was also abandoned.

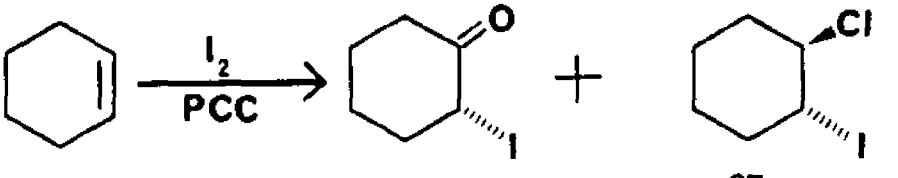
The much sought after lactone (35) was finally synthesized in excellent yield in one step by combining the then-



new method for the preparation of iodoketones and the standard reaction of iodide ion with thiosulfate. Although the Italian workers [80] obtained only a 50% yield of iodoketone from cyclohexene (see Chapter IV), application of this reaction to our compounds gave an overall yield of 91% for the two-reaction process. Unlike these workers, we used a relatively large excess of iodine and pyridinium dichromate. It was also found to be beneficial to add crushed molecular sieves to the reaction rather than the pellets recommended previously. Even though the iodine is alpha to two carbonyls and is thus easily removed by washing the filtered reaction mixture with thiosulfate solution, the iodolactone (66) is sufficiently stable to allow isolation and recrystalliza-

tion. It gave a satisfactory analysis and its spectra (IR and NMR) were identical to those of the bromolactone isolated previously. Mixed melting point of the lactone obtained in this way with that prepared by the NBS/DMSO method verified that they were indeed identical. In addition to the obvious advantages of this one-step procedure, the main advantage is that the product crystallizes directly from the concentrated reaction mixture (combined with the back extracts of the thiosulfate solution) without the need for further purification.

Some comment concerning the mechanism of this reaction is necessary. Piancatelli and co-workers [80] suggest that the intermediate "olefin-iodine complex" is a bridged iodonium

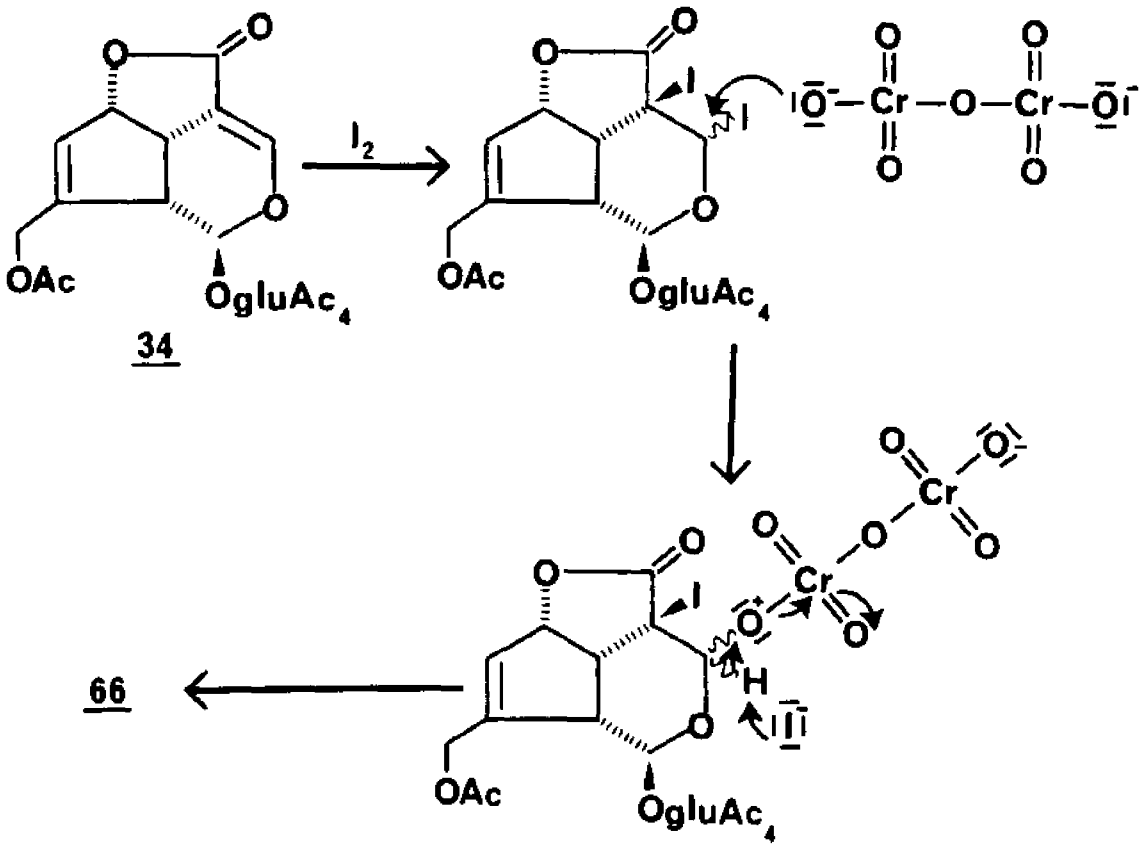


67

ion because they obtained trans 1-chloro-2-iodo-cyclohexene (67) as the main product when PCC was used in place of PDC. This is consistant with back side nucleophilic attack of the chromate ion on a cyclic iodonium ion. However, this is not the same reaction and is in conflict with our observations. These workers may not have prepared the diiodides of the compounds they studied. The dibromide of asperuloside tetraacetate had been prepared in our laboratory and would be expected to have properties similar to the diiodide. When the

SCHEME 18

Suggested Mechanism for the Formation of ATA Lactone with Iodine/PDC



reaction of asperuloside tetraacetate with iodine/PDC was monitored by TLC, a transient intermediate was observed (R_f value 0.35 developed with 4:1 ether:ethyl acetate) which behaved as expected for the diiodide. This was also the product formed in the absence of PDC although no attempts at its isolation have been made. We thus believe that, at least in the case of asperuloside tetraacetate, the reaction proceeds via initial formation of a diiodide and subsequent nucleophilic attack on the iodine-bearing carbon by dichromate to

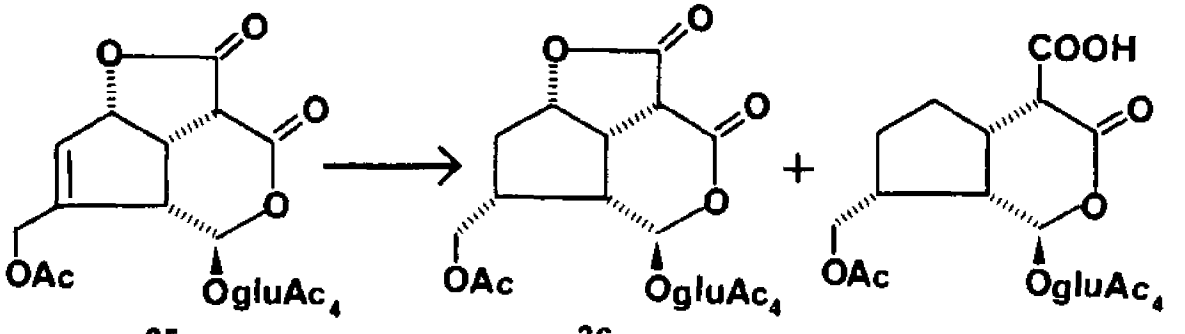
yield the product as shown in Scheme 18. The stereochemistry of the diiodide intermediate cannot be given as definite and obviously, if this is the intermediate in the simpler cases reported in the original paper, it would have to be a cis addition of iodine to give the reported products. In fact, the generation of a cis diiodide would explain the relatively low yields reported for simple cycloalkenes since this intermediate can presumably undergo simultaneous "double replacement" by dichromate followed by oxidative ring cleavage. However, note that diiodide formation is not necessary to explain these low yields since once the iodolactone has formed, the alpha halogen is extremely susceptible to nucleophilic displacement! This same line of reasoning explains why the yield in our case is so dramatically improved over the simple cycloalkenes since our tertiary iodine is on a highly hindered carbon. It also explains why the reaction would fail entirely for simple linear olefins (as has been reported) since the key requirement for success would be that there be some difference in the reactivity of the two iodine-bearing carbons toward nucleophilic displacement. Studies with linear olefins which are highly hindered at one of the olefinic carbons would be useful in determining the role of steric hindrance versus electronic effects in this reaction (both are favorable in our case).

In further defense of the nucleophilic substitution mechanism, we note that such reactions have been used previously

to form carbonyls from halides with chromate ion in the presence of crown ethers [129]. The only problem encountered was the relative insolubility of the inorganic chromium compounds and this was of course the major impetus for Corey's development of pyridinium dichromate.

6.3 HYDROGENATION OF ASPERULOSIDE TETRAACETATE LACTONE AND COMPOUND X

Attempted hydrogenation of both asperuloside tetraacetate lactone and the tertiary alcohol (which we still refer to as compound X) under conditions identical to those used with aucubin (Rh/C, ethyl acetate; see Chapter III) gave extensive hydrogenolysis. Since the resulting polar products



35 36

still contained all five acetates, they undoubtedly resulted from cleavage of the allylic lactone as shown for asperuloside tetraacetate lactone (35). Only one hydrogenation product formed and at this point it was assumed to have the stereochemistry shown based upon addition of hydrogen to the least hindered side of the alkene. The ratios of these products did not vary with temperature so a search for a more suitable solvent was conducted.

Hydrogenolysis is generally avoided by a combination of four methods [130]:

1. Use of dilute solutions which are well stirred.

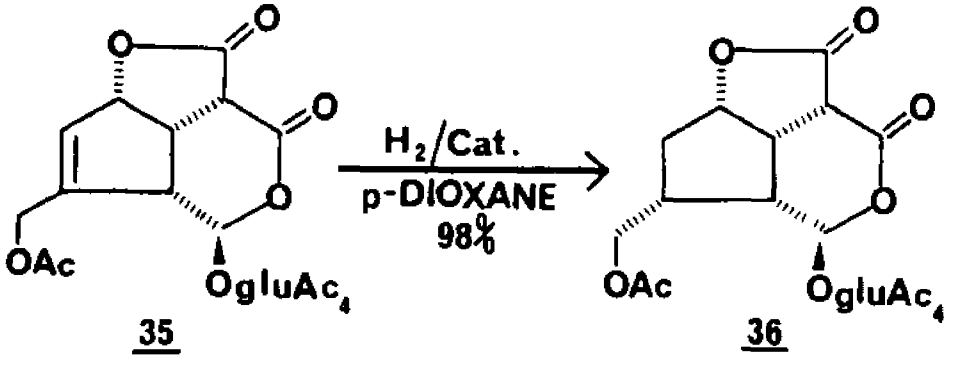
2. Use of rhodium or ruthenium catalysts.

3. Changing to a solvent of lower dielectric constant.

4. Reducing the temperature of the hydrogenation.

Since varying the temperature did not help and ruthenium catalysts require high pressures, we were restricted to trying various solvents at high dilution. We tried mixtures of ethyl acetate/hexane, chloroform/hexane and tetrahydrofuran/hexane. Each was better (less hydrogenolysis) than the one before but still unacceptable. This was also the case with pure tetrahydrofuran, chloroform and t-butyl alcohol.

Commercial p-dioxane gave no reaction but when it was purified by passage through a column of basic alumina and distilled from lithium aluminum hydride it gave outstanding re-



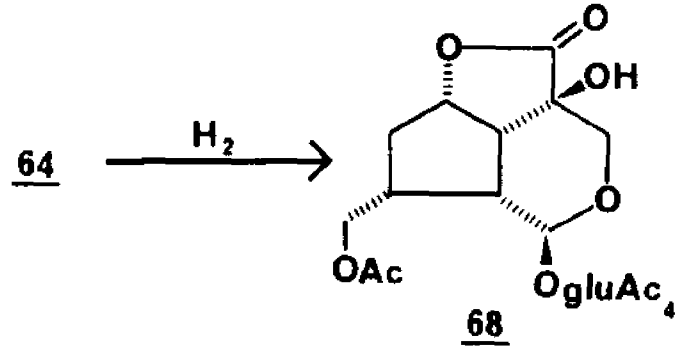
35 36

sults. Asperuloside tetraacetate lactone (35) was converted

to dihydro asperuloside tetraacetate lactone (36) in virtually quantitative yield at room temperature and pressure. Careful chromatographic purification of this material revealed the presence of less than 1% of one other hydrogenation product, probably the result of hydrogenation from the hindered side of the molecule.

The stereochemistry of the product was verified by high field proton NMR (400 MHz.). With the aid of double irradiation, every proton of dihydro asperuloside tetraacetate lactone could be assigned with certainty (see the section on identification of products below). The four contiguous cis protons of the cyclopentane ring all showed equal coupling to each other with a coupling constant of 8 Hz. as expected for an all cis structure.

Hydrogenation of the tertiary alcohol in the same way af-



68

forded an excellent yield of dihydro product 68 which was positively identified by high field proton (400 MHz.) and carbon (67 MHz.) NMR with the aid of double irradiation and off-resonance decoupling techniques.

These hydrogenations proceeded in only two hours and despite the use of very dilute solutions, they could be done on a multi-gram scale using a closed-system mechanical stirrer. Since recently manufactured rhodium on carbon tended to give inconsistent results (yields varying from 75% upwards), the use of rhodium on alumina was investigated. This modification gave yields of 83% of pure dihydrolactone after removal of the hydrogenolysis products by a short silica gel column chromatography.

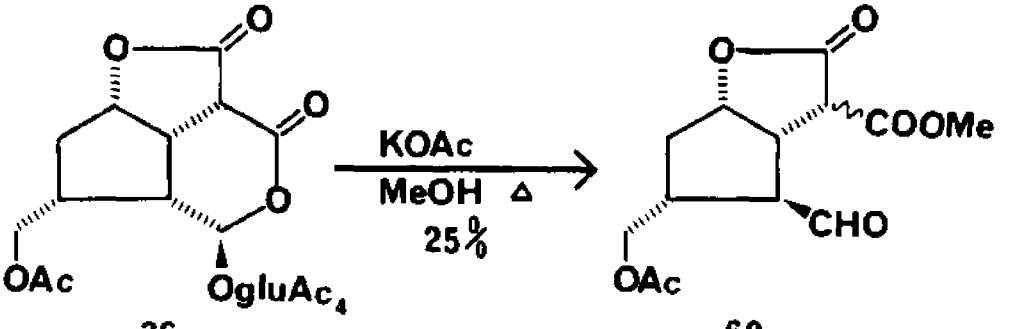
Dioxane which had only been passed through a column of alumina (which had been dried in an oven under vacuum unless it was recently received) worked just as well as material distilled from lithium aluminum hydride. Solvent which was distilled from sodium metal gave inferior results for unknown reasons. This was true even if it had been passed through alumina before distillation. Presumably the purification not only removes water and peroxides but also the sodium diethyldithiocarbamate used as a preservative by the manufacturers. This is important since sulfur compounds are severe poisons for rhodium catalysts.

6.4 HYDROLYSES OF THE DIHYDROLACTONE AND THE DIHYDRO TERTIARY ALCOHOL

The most desirable conditions for the hydrolysis of these glucosides are those which are mild enough to leave the acetate on the aglucone and yet allow its easy separation from

the various possible glucose products (there are seventeen glucose mono-, di- and triacetates possible).

While hydrolysis with potassium carbonate in methanol is the most common method, it suffers from the fact that it is non-selective. When this was tried with dihydro asperuloside tetraacetate lactone it proved impossible to isolate any substantial amount of product. Hydrolysis with potassi-

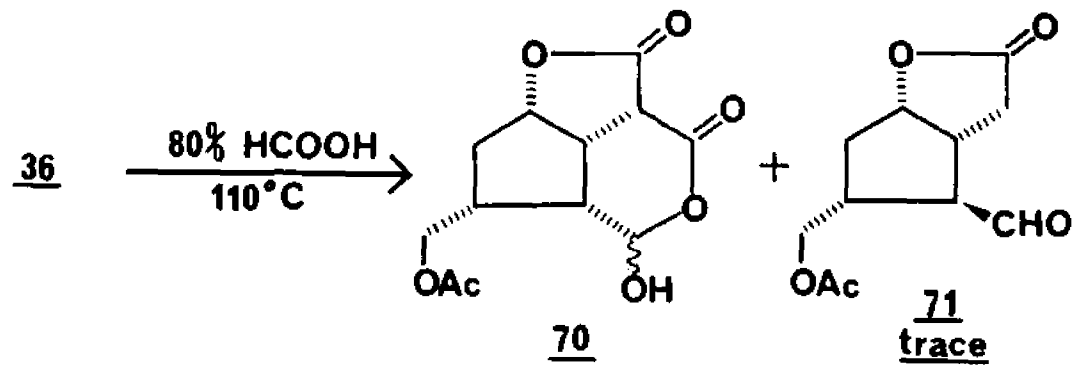


36 69

um acetate in methanol afforded a low yield of the aldehyde ester 69 after purification by column chromatography to remove remaining glucose tetraacetate and various other glucose acetates. The aldehyde is drawn in its more stable position ("up") based upon the work of Ohno [97] who used this procedure to epimerize the aldehyde which his synthesis produced. This intermediate was not further pursued due to the low yield and non-trivial chromatography required.

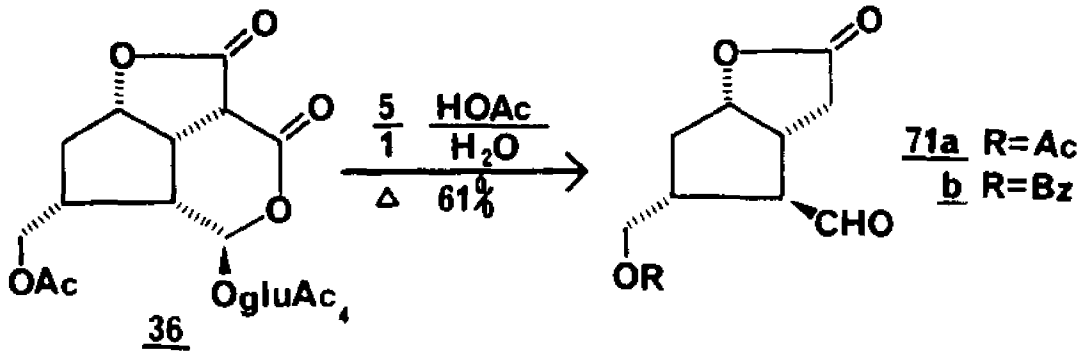
Hydrolyses using potassium hydrogen sulfate or concentrated hydrogen chloride in refluxing acetonitrile afforded very low yields of aldehydic products but caused much decomposition and were also not pursued.

Treatment of the dihydrolactone with refluxing 5:1 acetic acid:water or 80% formic acid gave much more promising results. Hydrolysis for 30 minutes in 80% formic acid affords aglucone 70 in excellent yield after purification by column



chromatography on silica gel to remove the glucose tetraacetate. Very minor amounts of three other glucose derivatives were also present (by HPLC) but were polar enough to cause no problem in the separation. This reaction also formed traces of the desired 11-deoxy-11-hydroxymethyl analog (71) of the Corey aldehyde. Longer reaction times caused an increase in the amount of aldehyde and glucose with a corresponding decrease in the amounts of glucose tetraacetate and aglucone 70. However, this reagent was too vigorous to allow isolation of the aldehyde after a lengthy heating period since it began to decompose. Of course, aglucone 70 is a viable intermediate in its own right but experiments with acetic acid gave 71 in improved yield so work with 70 was deferred.

Hydrolysis of 36 in refluxing (c. 115°C) 5:1 acetic acid:water for 15-18 hours in dilute solution afforded alde-



36

hyde 71a in 61% yield. The majority of the glucose tetraacetate is completely deacetylated in the course of this reaction and the work-up does not require chromatography since glucose is so water soluble it is separated in the extraction. Actually, for analytical purposes, column chromatography was used since this aldehyde is an oil and thus even trace impurities cannot be removed by other means. The yield of this reaction is only moderate due to the length of time this sensitive system must be exposed to hot acidic conditions. Even so, it is remarkable if one considers the fact that four reactions are actually occurring:

1. Hydrolysis of the glucoside to yield an aglucone hemiacylal, probably 70.

2. Decomposition of the hemiacylal to the parent aldehyde acid.

3. Decarboxylation of the resulting malonic acid derivative.

4. Epimerization of the aldehyde to the more stable form.

and at least three undesirable reactions are minimized:

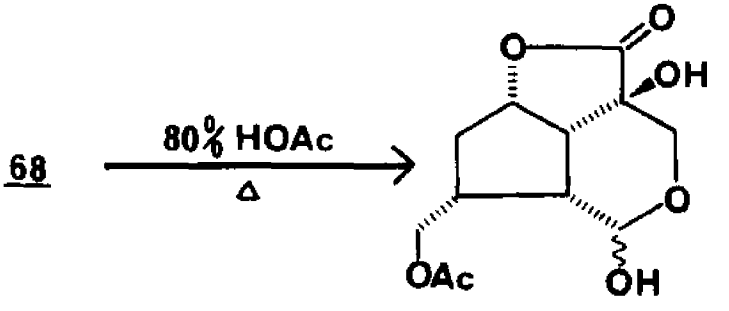
1. Hydrolysis of the one remaining acetate.

2. Hydrolytic opening of the lactone.

3. Dimerization or oxidation of the aldehyde.

Since Ohno and co-workers have converted benzoate 71b to both natural PGE_2 and $PGF_{2\alpha}$ and their synthetic 11-deoxy-11-hydroxymethyl analogs, use of their procedures (see Scheme 13) should give similar results with our aldehyde (71a). The next step toward this goal is the addition of the bottom side chain by Wadsworth-Emmons modification of the Wittig reaction and we decided to undertake this reaction as a means of verifying the stereochemistry of the aldehyde.

As a means of simplifying the low-field proton NMR, the tertiary alcohol was also hydrolyzed as above. This gave low

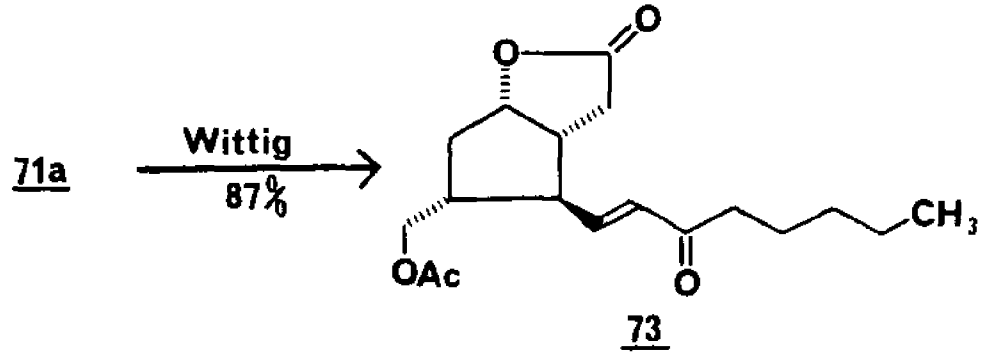


72

yields of the aglucone (72) with no aldehyde present. As expected, the NMR of 72 did not show two virtually identical hemiacetal protons but did show a sharp singlet of two protons at the expected position for an ether.

6.5 WADSWORTH-EMMONS REACTION OF ALDEHYDE 71A

The Wittig reaction of 71a with the sodium derivative of 2-oxoheptylphosphonate was done as previously described by Corey [131] except that granules of sodium hydride were used instead of a dispersion in mineral oil. This afforded a good



73

yield (87% as opposed to Ohno's reported 88% yield) of enone 73. This product was optically active (rotation similar to that of the benzoate reported by Ohno) and showed the appropriate trans coupling constant (15 Hz.) for a trans double bond. It was identical (by IR, NMR and HPLC) to a sample of the same material produced by an entirely different route [132] in our laboratory.

6.6 IDENTIFICATION OF PRODUCTS

Since in this type of work the identification of the various glucosidic intermediates is far from a trivial matter, a special section to discuss briefly the data supporting several of the key structures seems relevant. All new compounds gave satisfactory elemental analysis but it must be recognized that with compounds of this molecular weight, this does not pinpoint accurately the number of hydrogens in the molecule. Thus, spectroscopic techniques become even more important as a means of tracking unsaturation. In addition to the data summarized here, actual copies of many of the spectra are included in the Appendices at the end of the text and detailed data is in the Experimental Chapter.

6.6.1 Asperuloside Tetraacetate Lactone, Bromolactone and Iodolactone

The verification of these structures rests first on the chemical evidence that they survive oxidation intact, the absence of the carbon-carbon double bond stretch at 1659 cm^{-1} (this is its location in asperuloside tetraacetate) in the infrared and the absence of hydroxyl both in the IR and NMR. In addition, carbon-13 NMR shows seven carbonyl carbons and two alkene carbons in the lactone. One of the carbonyl carbons stands out from the rest at 162.6 ppm (downfield from internal TMS) as would be expected for a six-membered ring lactone. Proton NMR shows the absence of the C-3 vinylic proton which resonates at 7.23 ppm in asperuloside tetra-

Figure 2: Proton NMR Shifts of Asperuloside Tetraacetate Iodolactone

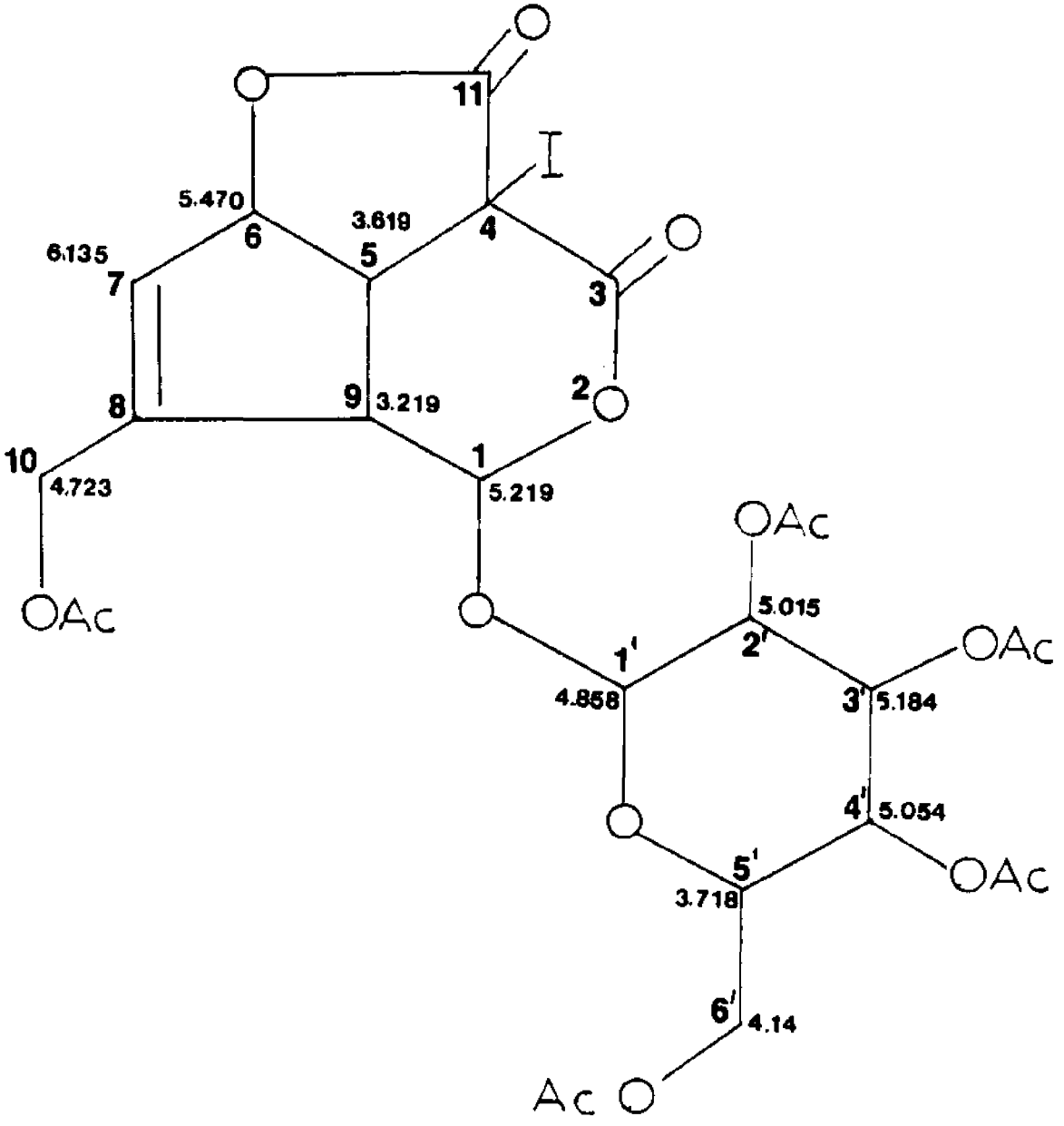
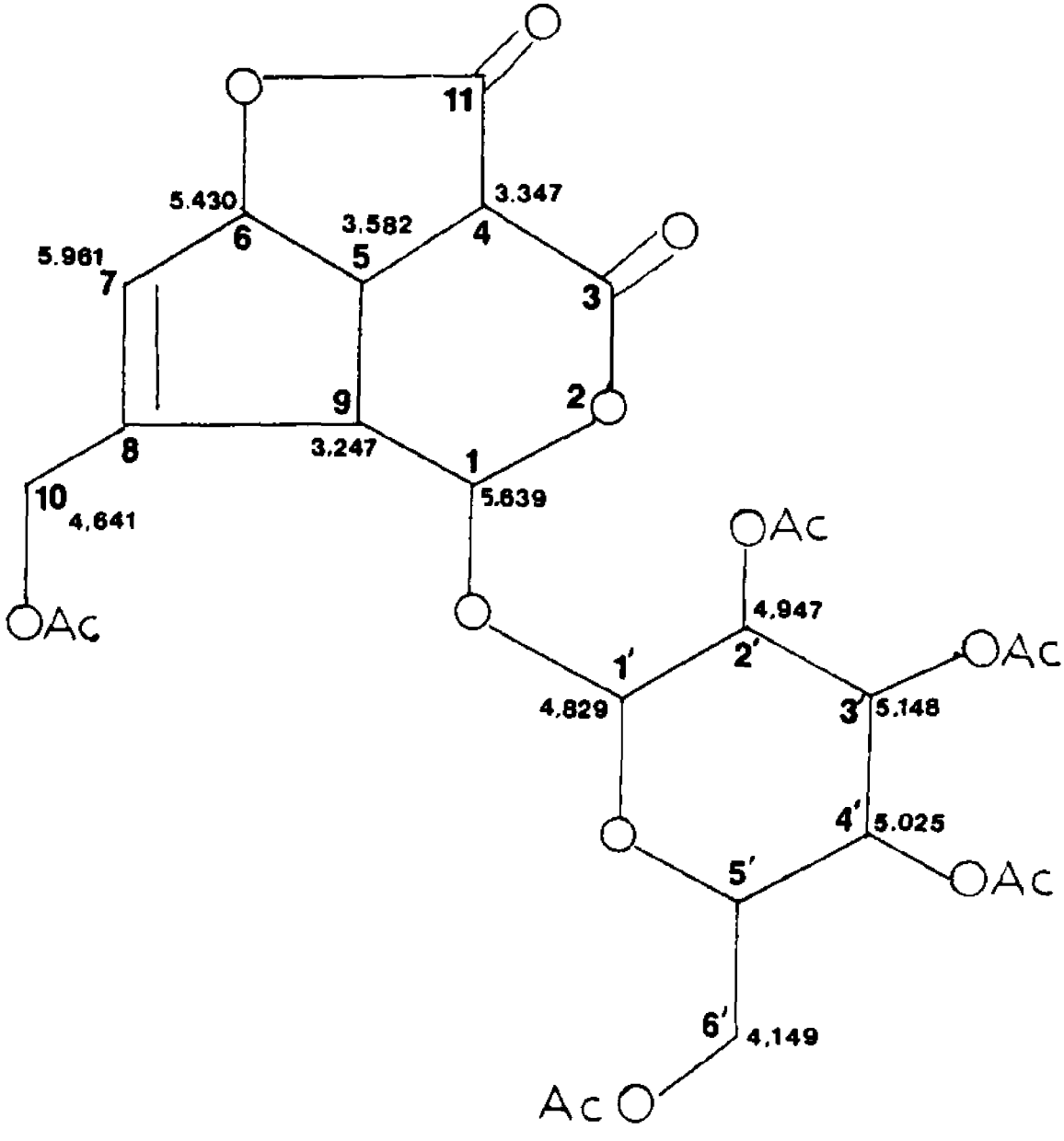
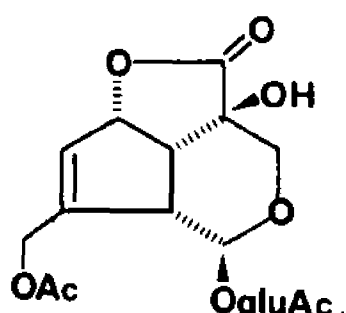


Figure 3: Proton NMR Shifts of Asperuloside Tetraacetate Lactone

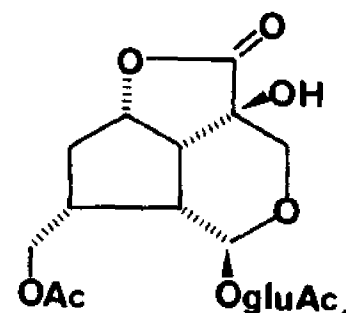


acetate. The 400 MHz. proton NMR of both the iodolactone and lactone were obtained and could be completely assigned as shown in Figures 2 and 3. As in all cases studied, the five acetates gave well-resolved singlets but could not be assigned and are not even shown in these figures (they are all between 1.9 and 2.1 ppm, specific values given in experimental sections). The subtle conformational effects which cause the H_{10a} and H_{10b} protons to appear as an AB quartet in the lactone but as a singlet in the iodolactone are worthy of note but not comprehensible. Notice that equal coupling around the cyclopentene ring frequently causes doublets of doublets to degenerate to "triplets" and this is a valuable means for verifying ring stereochemistry.

6.6.2 Compound X (The Tertiary Alcohol) and its Dihydro Product



64



68

These products presented the greatest challenge as far as identification is concerned. Early proton NMR results at 60 MHz. showed the presence of one exchangeable hydroxyl proton and the absence of the vinylic proton on C-3 but could not locate the hydroxyl or establish the presence of the

Figure 4: Proton NMR Shifts of AIA Tertiary Alcohol (64)

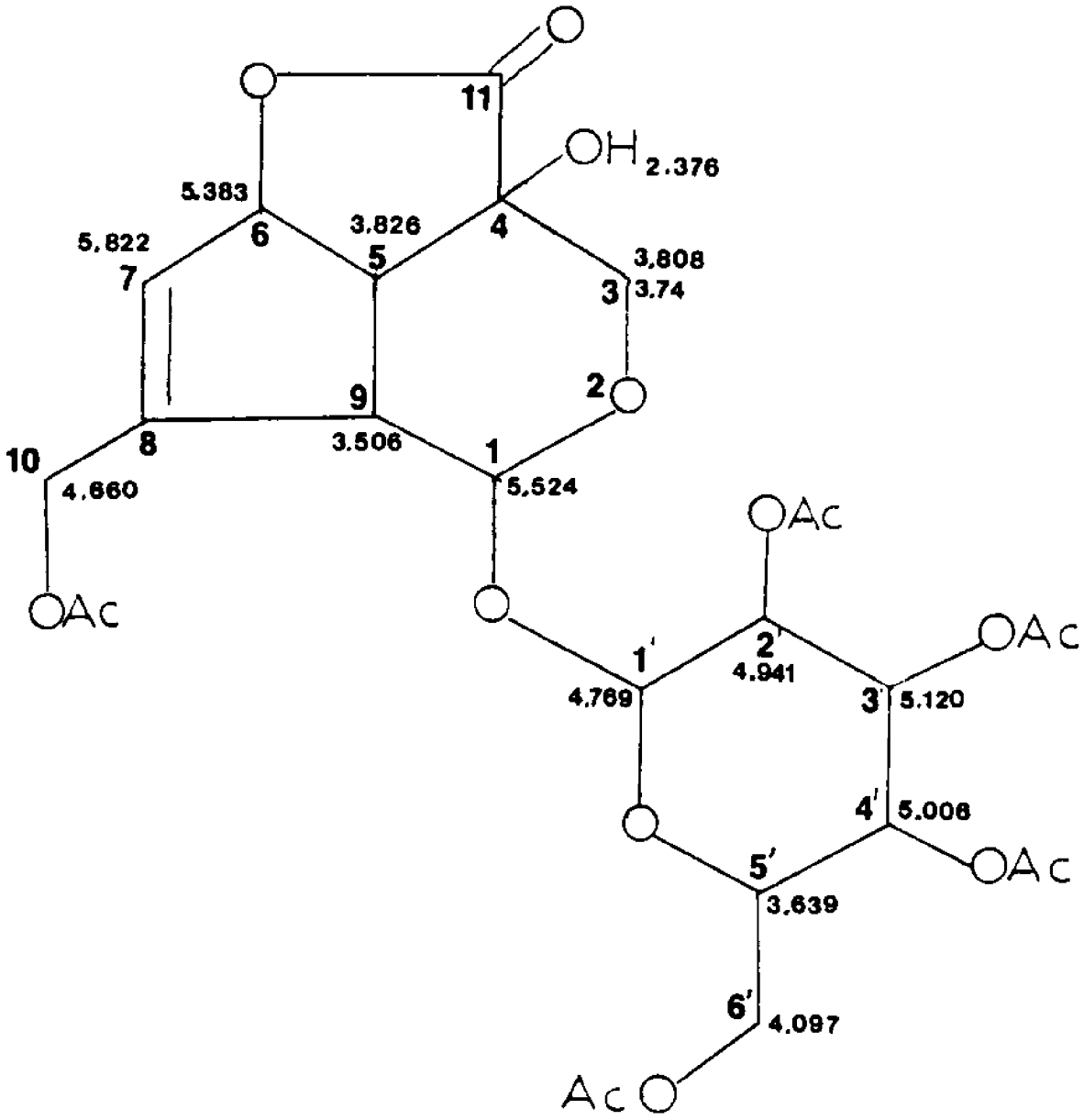
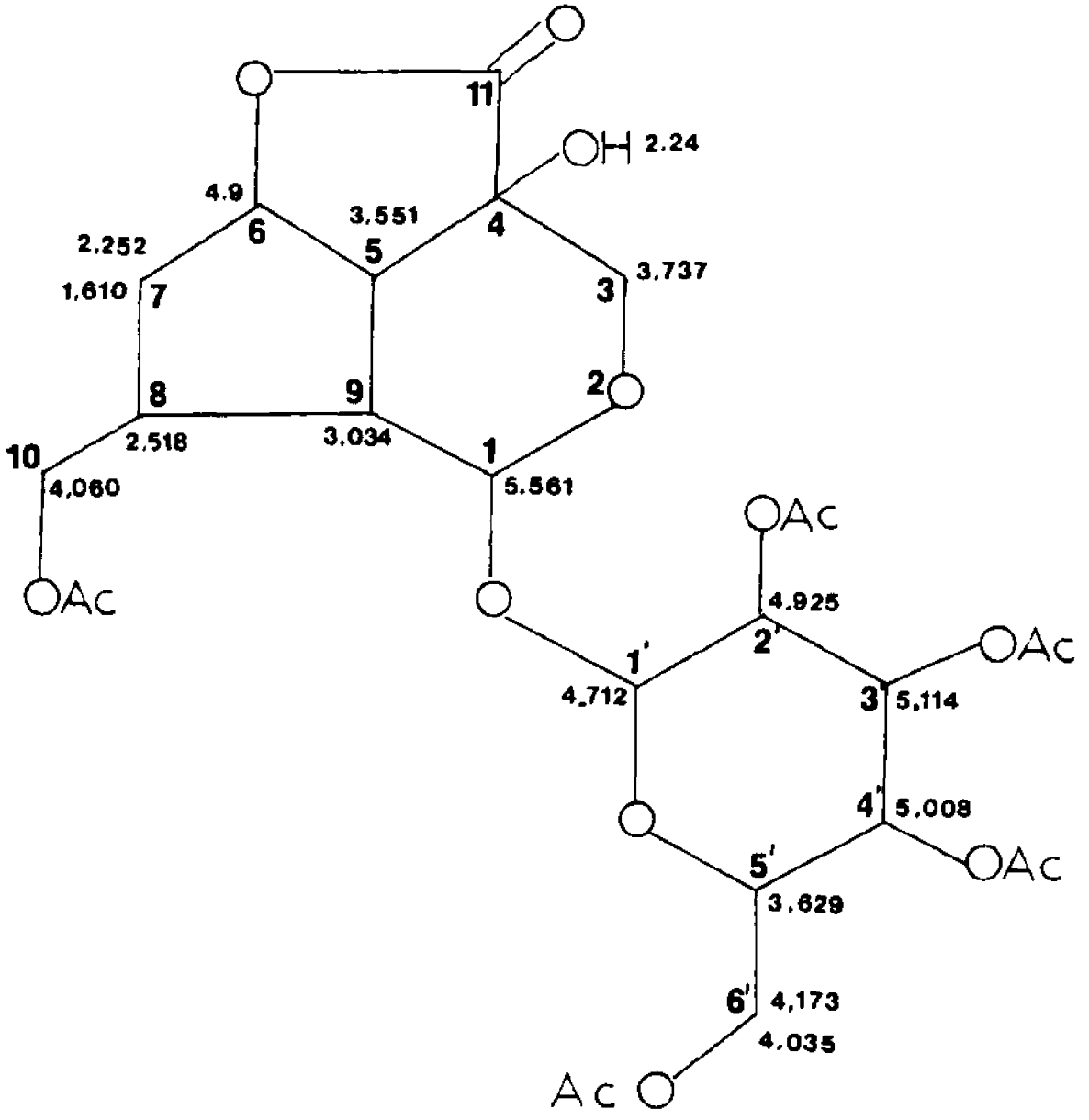


Figure 5: Proton NMR Shifts of Dihydro ATA Tertiary Alcohol (68)

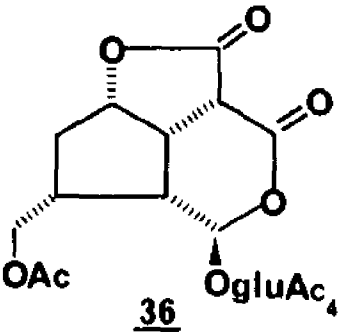


C7-C8 double bond with certainty. Carbon spectra at 25 MHz. proved that this double bond remained intact and showed the presence of only six carbonyls. One of these was at significantly higher field (174.2 ppm) than the rest thus giving the first indication that the compound was indeed an alpha-hydroxylactone.

The high field proton NMR of these products was not straightforward. The two protons on C-3 appear as an ABX pattern in the unsaturated tertiary alcohol due to their non-equivalence and long range coupling to the hydroxyl proton (which is a broad doublet of doublets). In the dihydro product, these two protons appear as a tight doublet while the hydroxyl has a complex pattern. Addition of deuterium oxide or selective irradiation at the frequency of the hydroxyl proton causes the signal from these two hydrogens to change to a sharp singlet. The remainder of these proton spectra could be assigned without difficulty with the aid of double irradiation and this is summarized in Figures 4 and 5.

Final confirmation of these structures resulted from an analysis of the carbon spectra at 68 MHz. Broadband decoupling followed by a gated decoupled spectrum clearly showed the quaternary carbon at 90.21 ppm and also revealed the presence of four methylenes in the dihydro tertiary alcohol as expected.

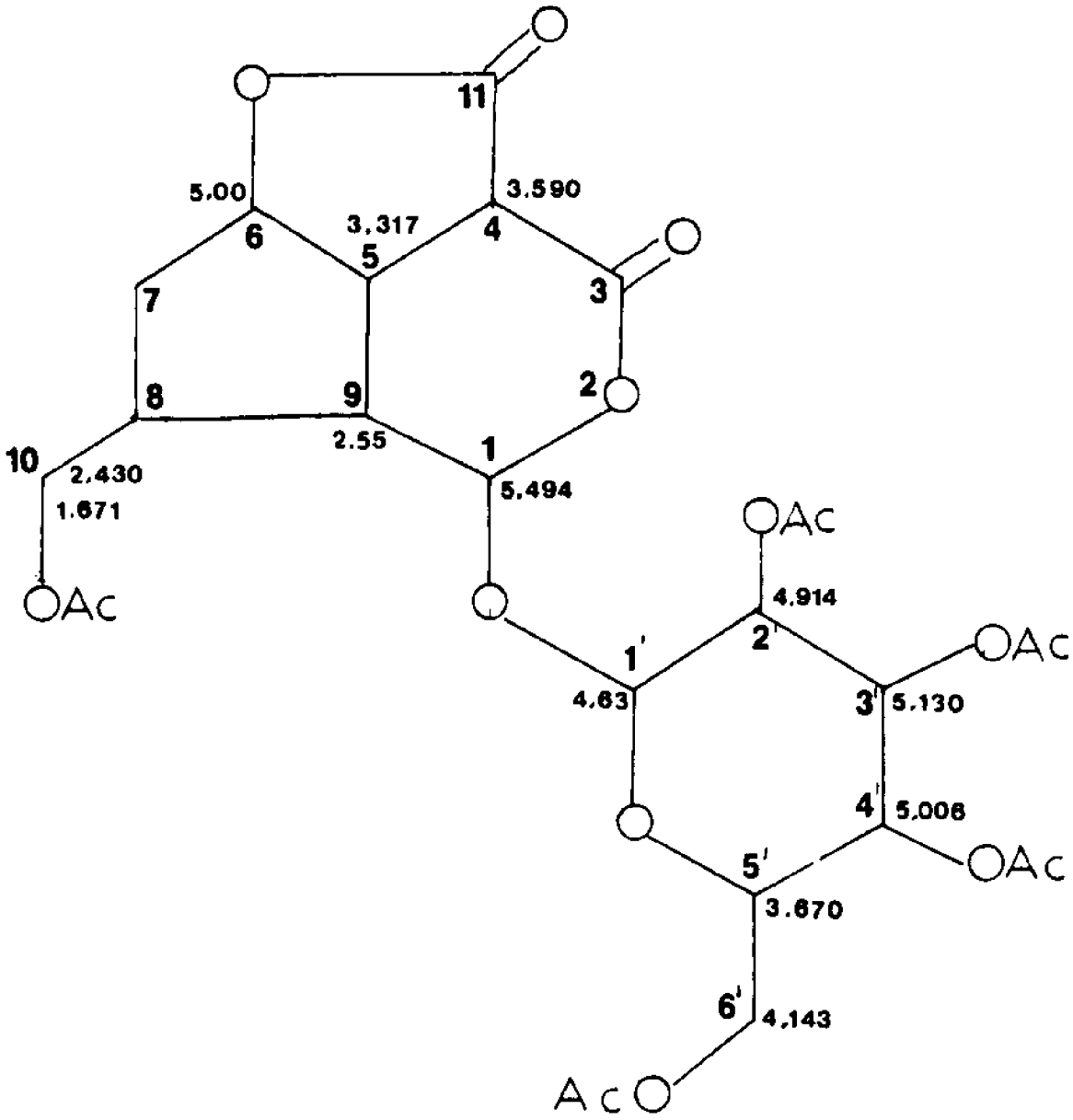
6.6.3 Dihydro Asperuloside Tetraacetate Lactone



36

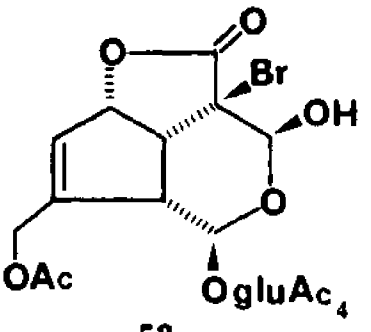
This compound presented no difficulty but is worthy of mention because it is the only iridoid glucoside derivative we have yet encountered in which the 10a and b protons appear as an ABX pattern and the 6' protons of the glucose do not (see Figure 6). Of course, this is due to subtle conformational effects which are not easily explained. This is an excellent example of why assignments in the iridoid field should not be accepted until verified by double irradiation since many of the early studies were done before this technique came into use.

Figure 6: Proton NMR Shifts of Dihydro AIA Lactone (36)

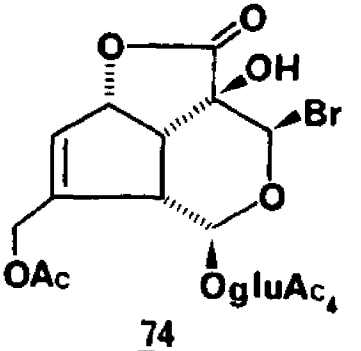


6.7 THE NBS/DMSO REACTION OF ASPERULOSIDE TETRAACETATE UNRAVELLED

It now seems certain that this reaction gives neither the



58

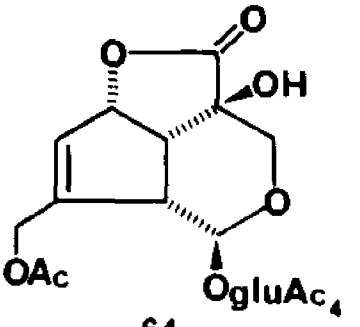


74

desired bromohydrin (58) nor the bromohydrin of opposite regiochemistry (74). Since it proved impossible to isolate this material or even to purify the mixture which contained it, this conclusion is of necessity based upon indirect evidence and chemical intuition. The "correct" bromohydrin 58 can be ruled out for several reasons:

1. There is no reason to suspect that it would not be oxidized successfully to the bromolactone which has been shown to be stable to several of the oxidizing agents tried.

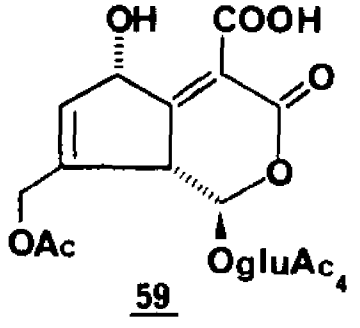
2. Even if oxidation did not proceed for some mysterious reason, there is no reason to expect that reduction of this material with sodium cyanoborohydride would give the tertiary alcohol (64) whose structure has been proven.



64

3. Since the bromohydrin of a closely related system (aucubin, see earlier discussion) has been prepared and is stable, there is no reason to expect the bromohydrin of asperuloside to be otherwise, especially since the dibromide of asperuloside is also stable.

Admittedly, this bromohydrin could, at least in theory,

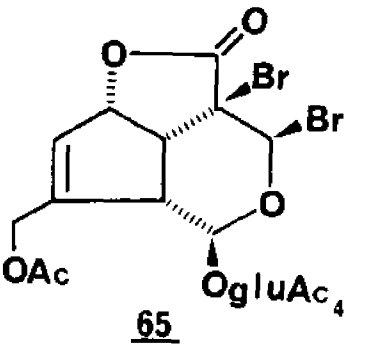


59

produce 59 as its oxidation product with Jones' reagent but this is also unlikely since the bromine of the bromolactone should certainly be even more labile than that of 58.

The bromohydrin of opposite regiochemistry (74) is very unappealing in terms of electronic factors and may also be ruled out by the following considerations:

1. It is difficult to see how 59 could be derived from

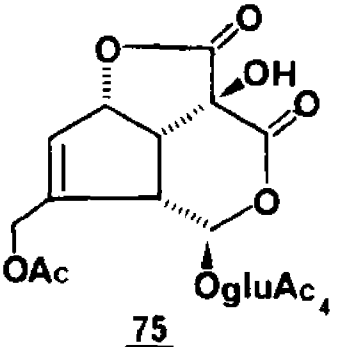


65

74 by Jones' oxidation in light of the fact that dibromide 65 is inert to these conditions.

2. Similarly, since both the dibromide 65 and the tertiary alcohol 64 are stable, there is no reason that 74 should be unstable.

3. If our mechanism for the formation of the iodolactone is correct, compound 74 would be oxidized by PDC to



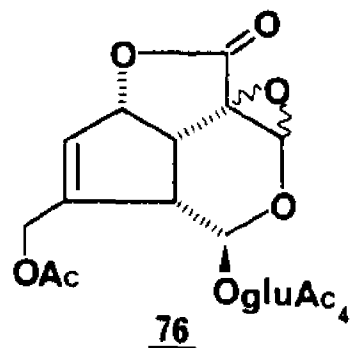
75

give the α-hydroxylactone 75 and it is not. Admittedly, this postulate should be tested by treating the dibromide with PDC and we have not yet done so.

4. Since the bromomethoxide and bromoacetoxylate of asperuloside tetraacetate are known and do not form

with this regiochemistry, there is no reason to believe that the bromohydrin would do so either.

All of this reasoning points to the epoxide 76 as the un-



76

stable NBS/DMSO reaction product. This could form the isolated Jones' oxidation product and is supported by the following additional arguments.

1. Epoxides have been obtained previously as products in the oxymercuration-demercuration process (see Eq. 6 in Chapter IV) and the investigators proposed an intramolecular "back-side" attack by oxygen on the mercury-bearing carbon to explain the results [74].

2. Although the borohydrides do not generally reduce epoxides, some reductions of strained epoxides have been reported [132] and reduction of the less hindered C-O bond was observed. This would explain our results.

3. In addition, the neighboring oxygen should make this epoxide much easier to reduce and also favors the observed product.

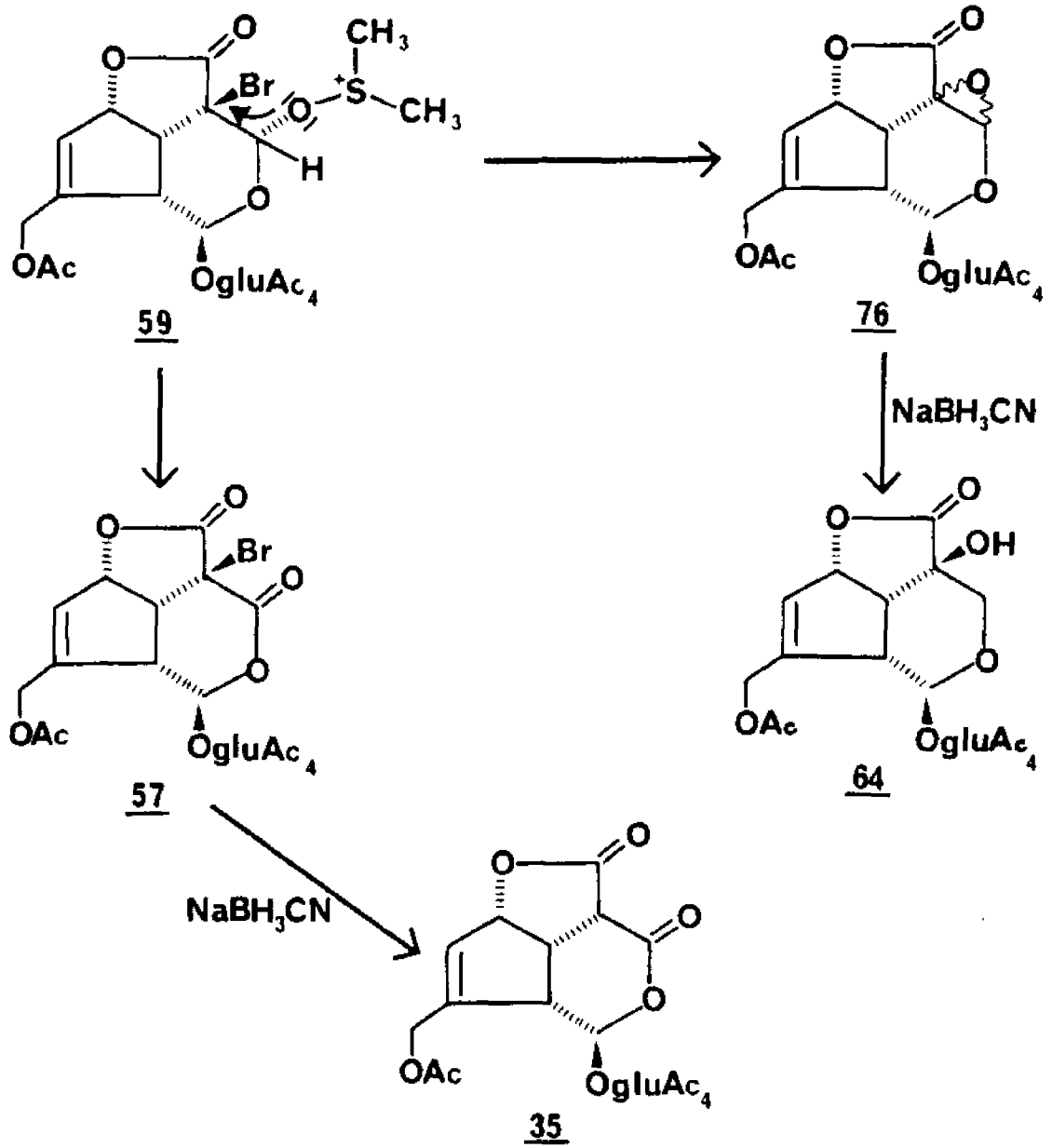
4. The unstable compound remained unchanged on treatment with chromous acetate thus suggesting that there was no halogen present in the molecule.

Thus, we suspect that the NBS/DMSO reaction proceeds via the bromosulfoxonium salt 59 as previously postulated (Scheme 17) but that the two pathways competing for this intermediate are not as shown earlier. Rather, the competition is between the intramolecular displacement of bromine by a pair of electrons from oxygen and the intermolecular process leading to the bromolactone (as shown in Scheme 19).

It is necessary to add one disclaimer to this entire discussion. The stereochemistries of all the products are not known for sure and the representations given here are merely for convenience and should not be taken as definite. In addition, it was not possible to tell if dimethylsulfide was the only sulfur-containing product of this reaction. It is possible that the sulfoxonium intermediate giving rise to the epoxide did not decompose to product until the reaction mixture was poured into water (it became very warm at this point if not cooled in an ice bath). Needless to say, the reason that this process happens in the case of asperuloside and not for aucubin is not known.

SCHEME 19

The Reaction of Asperuloside Tetraacetate with NBS/DMSO

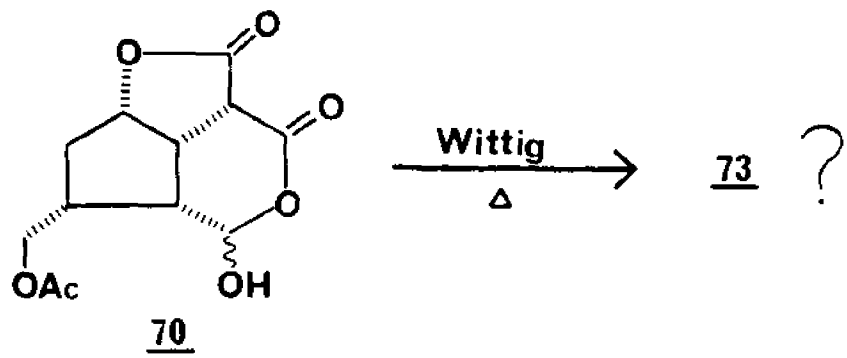


6.8 SUGGESTIONS FOR FUTURE WORK

Obviously, further work can be done to attempt to prove the mechanism just described. Treatment of asperuloside tetraacetate with m-chloroperbenzoic acid may produce the epox-

ide in a pure state. Also, as mentioned, the dibromide and diiodide should be investigated in order to gain further insight into the reactions of these systems.

Furthermore, the aglucone 70 may be a better prostanoid intermediate than the aldehyde 71a since it is formed in



70

higher yield and might undergo the Wadsworth-Emmons reaction followed by decarboxylation in one step (or 2 steps without isolation of the intermediate carboxylic acid) to give 73 in higher yield than by the aldehyde route.

In addition, it would obviously be satisfying to carry the synthesis all the way through to the prostanoids even though Ohno has already done this for his intermediates.

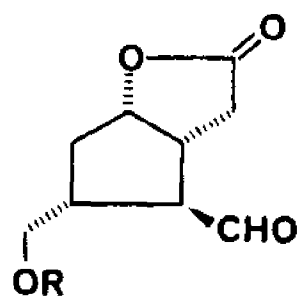
Chapter VII

SUMMARY AND CONCLUSION

The goal of this research has been to develop a practical synthetic route from iridoids to prostanoids. Indeed, the conversion of asperuloside tetraacetate (34) to the 11-deoxy-11-hydroxymethyl analog (71a) of the Corey aldehyde as shown in Scheme 20 is just such a route.

The synthesis is the shortest (3-step) synthesis of a Corey aldehyde analog yet devised and requires no resolution. The only column chromatography required is that used for the isolation of the iridoid and this may be avoided by changing the plant source.

Since the conversion of benzoate 71b to PGE_2 and $PGF_{2\alpha}$

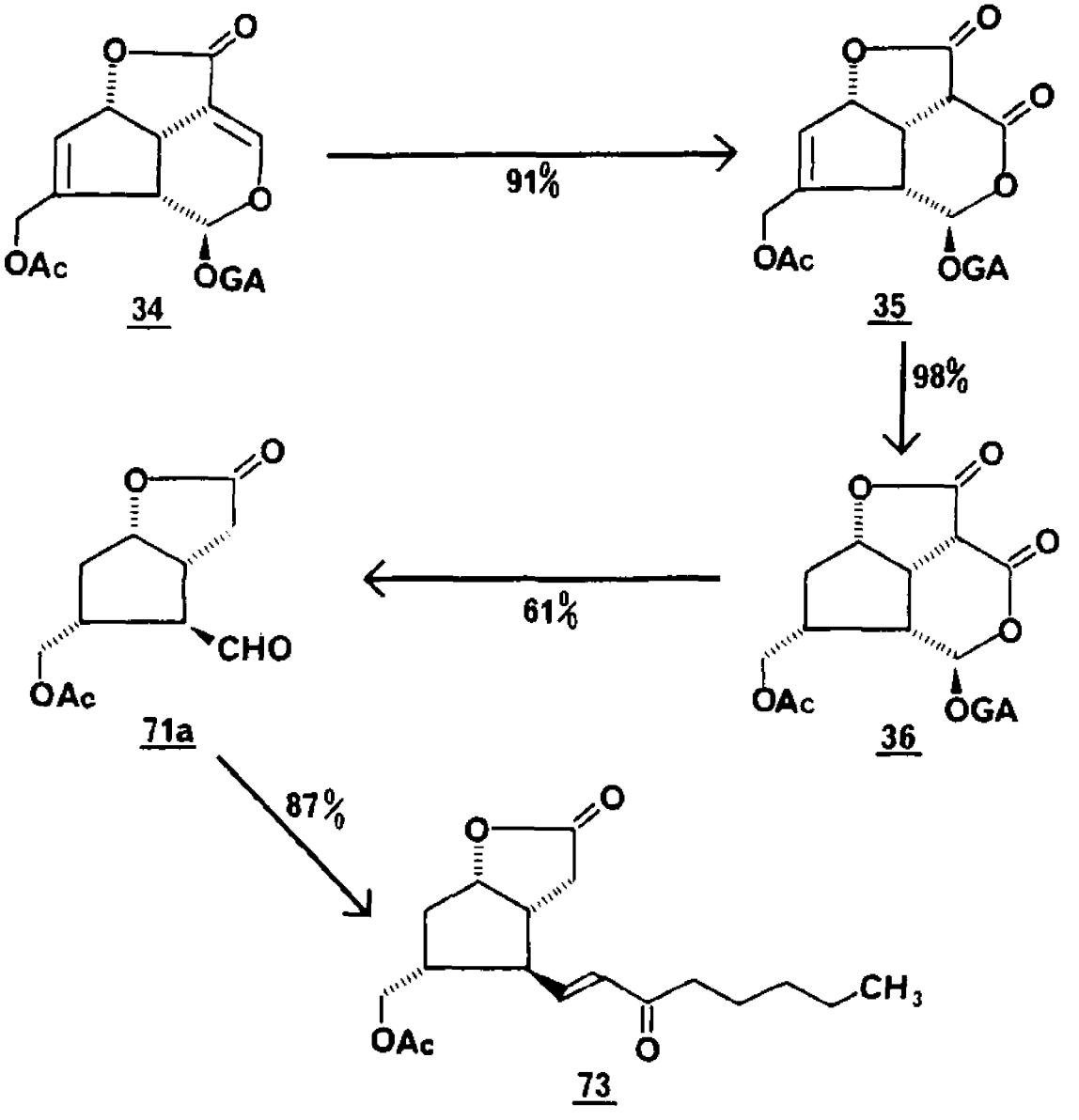


71a R=Ac
b R=Bz

has been previously reported (see Scheme 14), conversion of 71a to these same prostaglandins should be straightforward. The first step in this process (Wittig reaction to produce

SCHEME 20

Synthesis of Prostaglandin Intermediates Developed by This Research



73) gave yields comparable to those reported for the corresponding benzoate.

This synthesis is efficient (overall yield in excess of 50%) and uses readily available and relatively non-toxic re-

agents. The actual synthesis (independent of isolation of the iridoid) can be accomplished on a multi-gram scale in only three days and requires no special precautions.

Furthermore, this sequence would appear to be applicable to all the known iridoids thus presenting an excellent opportunity for the synthesis of a host of new prostanoids. The only possible change in procedure which may be required is the addition of a step to remove the iodine from those iridoids not having a carbonyl at carbon eleven (zinc and acetic acid has been shown effective for aucubin).

In conclusion, a short, simple and efficient conversion of asperuloside to a key prostanoid intermediate has been developed which should be applicable to all iridoid glucosides with little modification.

Chapter VIII

EXPERIMENTAL

Melting points were determined in open capillaries using a Thomas-Hoover Uni-melt apparatus and are uncorrected. Routine proton spectra were recorded at 60 MHz on a Varian EM 360 instrument with TMS as an internal standard. High field NMR spectra were obtained at Rockefeller University (220 MHz), the Southern New England High Field NMR facility (270 MHz) at Yale University or the University of South Carolina Magnetic Resonance Laboratory (400 MHz). NMR data is reported in parts per million downfield from TMS with the multiplicity (d = doublet; t = triplet usually actually a collapsed double doublet; s = singlet; ABq = AB quartet; br = broad; m = multiplet) and assignment (see numbering systems of Figures 2-6) in parentheses. Infrared spectra were recorded on a Perkin Elmer IR 598 which was calibrated against polystyrene. Absorptions are reported in reciprocal centimeters with intensity in parentheses (s = strong; m = moderate; w = weak; br = broad). Optical rotations were measured with a Perkin Elmer model 141 polarimeter. Carbon spectra were recorded at 25 MHz on a JEOL PS/PFT-100 spectrometer in deuteriochloroform with TMS as an internal standard or at 68 MHz at the Southern New England High Field NMR facility at Yale University.

Silica gel thin layer chromatography was performed using E Merck precoated plates (no. 5763-9H; silica gel 60). Unless otherwise noted, these were developed with 4:1 ether:ethyl acetate and visualized by spraying with 10% sulfuric acid in methanol and heating to 350°C. Analytical high pressure liquid chromatographic analyses were done using a Waters Associates (Milford, Mass.) system consisting of two 4 mm by 30 cm microporasil silica columns in series, a 6000 SDS pump, U6K injector and model 401 differential refractometer. Analyses were performed at a flow rate of 2 mL/min. using solvent I (1:1 ethyl acetate:hexane) or II (2:1 ethyl acetate:hexane) as noted. Preparative HPLC separations were done on silica columns using a Waters Prep 500 instrument. Large scale (10 cm) column chromatography equipment was obtained from Glenco (Houston, Texas).

Hydrogenation catalysts were obtained from Engelhard Industries, Newark, New Jersey. Catalyst purchased prior to 1975 seemed superior.

Dimethyl sulfoxide was dried by distillation from calcium hydride at reduced pressure and storage over 4A molecular sieves. Dioxane was purified by passage through a column of basic alumina (Brockmann activity grade I), stored in the dark and used within 48 hours. Methylene chloride was distilled from phosphorous pentoxide and stored over 4A molecular sieves. N-bromosuccinimide was recrystallized from chlo-

roform, dried in vacuo and stored in the dark and in the refrigerator. All other chemicals and solvents were used as purchased.

Coprosma repens plant parts were purchased from Hines Wholesale Nurseries, Inc., Los Angeles, California. Extracts were concentrated at water aspirator pressure using a "cyclone" circulatory evaporator available from Scientific Glass Apparatus Co., Bloomfield, New Jersey.

Microanalyses were performed by Spang Microanalytical (Eagle Harbor, Mich.) or Galbraith (Knoxville, Tenn.) Laboratories.

8.1 ISOLATION OF ASPERULOSIDE TETRAACETATE

As previously discussed, three isolation procedures were developed and used on a large scale. Each will be presented here.

8.1.1 From Leaves and Twigs by the Procedure of Duff

A total of 8.45 Kg of leaves, stems and berries of Coprosma repens was boiled with 30 L of water for one hour. This was filtered crudely through a Buchner funnel without filter paper and the plant residue was boiled with an additional 30 L of water for two hours. The combined extracts were filtered through a Celite pad and concentrated under reduced pressure (with a cyclone evaporator) to 3.5 L. A small amount of tol-

uene was added to retard bacterial growth during storage at room temperature before concentration. The concentrated solutions were stored in the refrigerator. The concentrate was slurried with 630 g of Celite 535, concentrated to dryness under reduced pressure in a rotary evaporator and finally dried under high vacuum overnight. The resulting tan solid was placed atop a partition chromatography column prepared as follows [102]: Approximately 1.8 Kg of Celite 535 was mixed with an equal weight of water saturated with 1-butanol and allowed to stand overnight at room temperature. This stationary phase was then slurried with 1-butanol saturated with water, added to a 10 cm diameter column and solvent was forced through at a pressure of 10 psi (compressed air) until settling ceased. The loaded column was eluted with 1-butanol saturated with water and 500 mL fractions collected at the rate of one every 10 min. (10 psi air pressure). Asperuloside (R_f = 0.72 in 2:1 ethanol:acetone) appeared in fractions 12-40 accompanied by asperulosidic acid (R_f = 0.83, same solvent), glucose and several plant tannins. The leading and trailing fractions were dark brown while the central fractions were yellow. Concentration of the central fractions and cooling afforded hygroscopic tan crystals which were not isolated for this reason. The combined, concentrated fractions were dried in vacuo overnight yielding a thick brown oil which was stirred vigorously with 300 mL acetic anhydride at room temperature overnight. At this

point, only asperuloside (blue) and various dark gray glucose spots appeared on TLC. To this stirred solution was added 150 mL pyridine with cooling in an ice bath to keep the temperature below 50°C. After the exotherm had subsided this was allowed to stir at room temperature overnight. This was then poured into a mixture of 200 mL water, 25 mL concentrated HCl and 300 g ice with stirring (the whole was immersed in an ice bath as well). The resulting brown solution and precipitate was extracted with chloroform (3 x 250 mL). This was placed in a large beaker and neutralized with saturated sodium bicarbonate solution until foaming ceased (efficient stirring was essential as was occasional cooling in an ice bath). The solution was then transferred to a separatory funnel and separated. The chloroform extract was washed once with brine then concentrated to 75 mL and diluted with 250 mL of anhydrous ethyl ether. Upon standing overnight in the refrigerator, tan powdery asperuloside tetraacetate precipitated. Filtration, washing with ether and drying in vacuo afforded 21.8 g of tan powder (0.25% based on fresh plant weight. This was stored in the refrigerator and purified as needed using a silica gel column chromatography done as follows: A column was prepared by loading 210 g of Baker 60-200 mesh silica gel into a 4 cm OD column in 1:1 ethyl acetate:hexane and packing with a slight air pressure to give a height of 47 cm. Atop this was placed 40 g of the same silica gel onto which 7.00 g of the above crude asper-

uloside tetraacetate had been deposited (slurried in acetone and rotary evaporated to dryness). The column was eluted with the same solvent under a slight air pressure and fractions of 250 mL were collected. Asperuloside tetraacetate appeared in fractions 8-30 while the brown tannins remained fixed at the origin. Concentration of these fractions and recrystallization from absolute ethanol afforded pure asperuloside tetraacetate (5.91 g) as white crystals. Based upon this result, the overall yield of iridoid from fresh plant parts is 0.21% (m.p. 150-151°C; lit. m.p. 154°C [55]).

^{1}H NMR (220 MHz, $CDCl_3$, ppm): 2.113, 2.097, 2.045, 2.018, 2.001 (all s, $OCOCH_3$); 7.23 (d, H_3, $J_{1,9}$ = 1.5 Hz); 5.76 (br. s, H_7); 5.69 (s, H_1); 5.51 (br. d, H_6); 5.253 (t, $H_{3'}$, equal coupling to 2' and 4'); 5.106 (t, $H_{4'}$, equal coupling to 3' and 5'); 5.014 (t, $H_{2'}$; equal coupling to 1' and 3'); 4.91 (d, $H_{1'}$, $J_{1',2'}$ = 8.1 Hz); 4.66 (ABq, H_{10} a and b); 4.25 (eight line ABX, $H_{6'}$ a and b); 3.80 (ddd, $H_{5'}$); 3.50 (br. t, H_5); 3.26 (m, H_9).

^{13}C NMR (25 MHz, $CDCl_3$, ppm): 170.4; 170.0; 169.8; 169.2; 169.1; 169.1 (s, C=O); 147.6 (d, C_3); 141.4 (s, C_8); 128.8 (d, C_7); 105.4 (s, C_4); 95.9 (d); 91.6 (d); 83.9 (d); 72.2; 72.2; 70.5; 68.2; 61.6; 60.3; 43.4 (d); 36.0 (d); 20.6 (q, five overlapping CH_3 groups).

IR ($CHCl_3$, cm^{-1}): 3020 (m); 2960 (w); 1750 (br, vs); 1659 (s).

HPLC: Solvent I retention time 11.4 min. Solvent II: T_r = 6.3 min.

TLC: R_f = 0.40, intense blue spot.

8.1.2 From Leaves and Twigs Without Partition Chromatography

A total of 3.2 Kg of Coprosma repens leaves and stems was extracted with boiling water as above. The extract was concentrated onto 217 g Celite 535 and dried in vacuo overnight to give a free-flowing brown powder. This was then slurried with 1.5 L of acetone overnight with good stirring. The acetone was filtered and the slurrying process repeated twice more with fresh acetone. The combined acetone filtrates were concentrated to dryness in vacuo and treated as above (acetylation, column chromatography on silica gel and recrystallization). This afforded 6.4 g pure asperuloside tetraacetate (0.19% by weight of fresh plant parts) upon recrystallization from chloroform/ether.

8.1.3 From Twigs with Acetone Extraction

A total of 6.84 Kg of Coprosma repens twigs was boiled with one 10 L portion of acetone and this green solution was concentrated to 75 mL. Upon cooling in the refrigerator, a brown sludge appeared and was filtered and washed with a small amount of cold acetone. The sludge was washed repeatedly with hexane to remove chlorophylls and was then dried

and treated as before (acetylation and column chromatography on silica gel). The mother liquor was concentrated onto silica gel and washed with hexane. This was then extracted with absolute ethanol and the extract concentrated and treated in the same way as the brown sludge. After recrystallization from chloroform/ether, the overall yield of pure tetraacetate by this method is 0.18% (12.1 g).

8.2 PREPARATION OF ASPERULOSIDE TETRAACETATE BROMOLACTONE

Asperuloside Tetraacetate Bromolactone (57): A solution of 1.00 g asperuloside tetraacetate (1.72 mmole) in 10.0 mL dry DMSO was prepared in a flame-dried apparatus and to this stirred solution was added 0.489 g dry recrystallized N-bromosuccinimide (2.75 mmole; 1.6 equiv.). After being stirred at room temperature for 90 minutes, the yellow solution was slowly poured into 100 mL cold, distilled water with vigorous stirring. The resulting suspension was allowed to sit in an ice bath for one hour. Filtration and drying in vacuo yielded a white amorphous powder (0.9033 g) which could be stored indefinitely in the cold. The mother liquor was extracted with methylene chloride (5 x 25 mL) and the combined organic layers were washed once with brine. Drying over anhydrous magnesium sulfate, filtration and concentration in vacuo afforded 0.36 g of light yellow oil still having the odor of DMSO. This oil was combined with the powder and dissolved in 20 mL acetone which was cooled in an ice bath. To

this was added 4.0 mL Jones' reagent (prepared by dissolving 6.7 g chromium trioxide in 12.5 mL water, adding 5.8 mL concentrated sulfuric acid with cooling and then adding enough water to dissolve the precipitate), the ice bath was removed and stirring at room temperature was continued for 40 minutes. The reaction was quenched with methanol, poured into 75 mL cold water and extracted with methylene chloride (4 x 50 mL). The combined organic layers were washed with water and brine, dried over magnesium sulfate and concentrated in vacuo to a white puffy semi-solid (0.99 g). Recrystallization from methylene chloride/ether afforded white crystals of impure bromolactone (0.34 g) which was purified by passage through a short silica column (with 1:1 ethyl acetate:hexane) followed by recrystallization from 95% ethanol to yield 0.197 g of pure bromolactone (17% of theoretical). m.p. 220-221 d.

Analysis: Calculated for $C_{26}H_{29}O_{16}Br$: C: 46.11; H: 4.28; Br: 11.80. Found= C: 45.96; H: 4.19; Br: 11.88.

^1H NMR (60 MHz, $CDCl_3$, ppm): 2.10, 2.08, 2.08, 2.04, 2.00 (all s, $OCOCH_3$); 6.1 (br. s, H_7); 5.48 (dd, H_6, $J_{5,6}$ = 6 Hz, $J_{6,7}$ = 2 Hz); 4.8-5.4 (m, 4H, $H_{1'}, H_{2'}, H_{3'}, H_{4'}$); 4.72 (s, H_{10} a and b); 4.15 (m, $H_{6'}$ a and b); 3.5-3.8 (m, $H_{5'}$, $H_{6'}$ and H_5); 3.25 (br. t, H_9).

IR ($CHCl_3$, cm^{-1}): 3020 (m); 2950 (w); 1790 (s); 1755 (br, vs).

HPLC: Solvent I: T_r = 9.3 min.

TLC: R_f = 0.43; yellow-green spot.

8.3 ISOLATION OF OXIDATION PRODUCT 59

Asperulosidic Acid Lactone (59): The mother liquor from the above bromolactone recrystallization was purified by concentration in vacuo and recrystallization from ether. A final crystallization from ethyl acetate/hexane afforded pure asperulosidic acid lactone (0.216 g; 19%). m.p. 180-180.5°C

Analysis: Calculated for $C_{26}H_{29}O_{17}$: C: 50.90; H: 4.76. Found: C: 50.95; H: 4.62.

^{1}H NMR (60 MHz, $CDCl_3$, ppm): 2.10, 2.10, 2.08, 2.01, 1.99 (all s, $OCOCH_3$); 8.3 (br. s, COOH, OH, D_2O labile); 5.73 (br. s, H_1); 5.63 (s, H_7); 5.45 (br. d, H_6); 4.7-5.3 (m, 4H); 4.6 (br. s, H_{10} a and b); 3.4-4.3 (complex, 6H, unassigned).

IR ($CHCl_3$, cm^{-1}): 3500 (m); 3020 (w); 1750 (br. vs).

TLC: R_f = 0.00, gray-black spot.

8.4 PREPARATION OF ASPERULOSIDE TETRAACETATE LACTONE AND TERTIARY ALCOHOL

Asperuloside Tetraacetate Lactone (35) and Tertiary Alcohol (64): A solution of 2.00 g asperuloside tetraacetate (3.44 mmole) in 10 mL dry DMSO was prepared and to this was added

1.04 g of recrystallized N-bromosuccinimide (5.84 mmole; 1.7 equiv.). After being stirred at room temperature for 90 minutes, the yellow solution was poured into 100 mL cold distilled water with vigorous stirring which was continued with cooling in an ice bath for one hour. Filtration and drying in vacuo yielded a white amorphous powder (1.63 g; 70%). Extraction of the mother liquor with methylene chloride (4 x 25 mL) followed by concentration in vacuo yielded additional material as a clear colorless oil (0.81 g; 35%) which turned brown slowly as it was allowed to dry in vacuo overnight at room temperature. The above materials were combined and dissolved in 40 mL tetrahydrofuran. To this was added 0.328 g sodium cyanoborohydride (recrystallized grade; 5.22 mmole) and the solution was refluxed for two hours. The slightly yellow slurry was then poured into 350 mL saturated sodium chloride and extracted with methylene chloride (5 x 150 mL). After drying over magnesium sulfate, filtration, concentration and final drying in vacuo gave 2.07 g of puffy white semi-solid (101%) mixture of 35 and 64. These were separated using preparative HPLC with an eluting solvent of 5:1 chloroform:acetonitrile. Concentration of the fractions in vacuo and recrystallization from methylene chloride/ether afforded pure lactone 35 (0.404 g; 19.8%) and pure tertiary alcohol 64 (0.949 g; 47%).

Lactone 35: m.p. 141-142°C

Analysis: Calculated for $C_{26}H_{30}O_{16}$: C: 52.17; H: 5.05. Found: C: 51.95; H: 5.07.

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 2.035, 2.022, 1.991, 1.962, 1.931 (all s, $OCOCH_3$); 5.961 (t, H_7, slight allylic coupling); 5.639 (d, H_1, $J_{1,9}$ = 3.91 Hz); 5.430 (br. d, H_6); 5.148 (t, $H_{3'}$); 5.025 (t, $H_{4'}$); 4.947 (dd, $H_{2'}$, $J_{1',2'}$ = 8.06 Hz, $J_{2',3'}$ = 9.52 Hz); 4.829 (d, $H_{1'}$); 4.641 (ABq, H_{10} a and b, $\Delta\delta$ (AB)= 31.25 Hz, $J_{10a,10b}$ = 14.64 Hz); 4.149 (eight line ABX, $H_{6'}$ a and b, $\Delta\delta$ (AB)= 53 Hz, $J_{6'a,6'b}$ = 12.45 Hz, $J_{5',6'a}$ = 4.88 Hz, $J_{5',6'b}$ = 1.95 Hz); 3.703 (d[dd], $H_{5'}$, $J_{4',5'}$ = 9.76 Hz); 3.582 (dt, H_5, $J_{4,5}$ = 10.74 Hz, $J_{5,6}$ = $J_{5,9}$ = 7.5 Hz); 3.347 (d, H_4); 3.247 (m, H_9).

^{13}C NMR (25 MHz, $CDCl_3$, ppm): 170.5, 170.2, 169.9, 169.5, 169.4, 168.9, 162.6 (all C=O); 143.4 (C_8); 129.5 (C_7); 97.5; 97.4; 97.3; 85.6; 72.4; 70.8; 67.9; 61.5; 60.4; 45.7; 43.8; 37.7; 20.6 (five identical CH_3 acetate groups).

IR ($CHCl_3$, cm^{-1}): 3020 (m); 2960 (w); 1792 (s); 1755 (br. vs).

HPLC: Solvent II: T_r = 7.9 min.

TLC: R_f = 0.33, gray-black spot.

Tertiary Alcohol 65: m.p. 161-161.5°C

Analysis: Calculated for $C_{26}H_{32}O_{16}$: C: 52.00; H: 5.37. Found: C: 51.84; H: 5.38.

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 2.018, 2.016, 1.999, 1.952, 1.923 (all s, $OCOCH_3$); 5.822 (t, H_7, slight long range coupling); 5.524 (s, H_1); 5.383 (br. d, H_6); 5.120 (t, $H_{3'}$); 5.006 (t, $H_{4'}$); 4.941 (dd, $H_{2'}$, $J_{1',2'}$ = 8.30 Hz, $J_{2',3'}$ = 8.06 Hz); 4.769 (d, $H_{1'}$); 4.660 (s, H_{10} a and b); 4.097 (eight line ABX, $H_{6'}$ a and b, $\Delta\delta$ (AB)= 53 Hz, $J_{6'a,6'b}$ = 12.45 Hz, $J_{5',6'a}$ = 5.13 Hz, $J_{5',6'b}$ = 1.95 Hz); 3.826 (t, H_5); 3.778 (eight line ABX, H_3 a and b, $J_{3a,3b}$ = 11.47 Hz, long range coupling to the OH); 3.639 (d[dd], $H_{5'}$, $J_{4',5'}$ = 10.01 Hz); 3.506 (br. d, H_9); 2.376 (br. dd, OH, D_2O labile, concentration dependent).

^{13}C NMR (25 MHz, $CDCl_3$, ppm): 174.3, 170.7, 170.3, 170.3, 169.5, 169.5 (all C=O); 145.1 (C_8); 128.6 (C_7); 104.0; 95.6; 90.2; 85.6; 73.9; 72.2; 71.3; 68.2; 63.3; 61.9; 61.8; 61.0; 47.4; 20.7 (five identical acetate CH_3 groups).

IR ($CHCl_3$, cm^{-1}): 3540 (br.,w); 3020 (m); 2950 (w); 1750 (br.,vs).

HPLC: Solvent II: T_r = 8.6 min.

TLC: R_f = 0.29, gray-black spot.

8.5 PREPARATION OF ASPERULOSIDE TETRAACETATE DIBROMIDE (65)

Asperuloside Tetraacetate Dibromide (65): A solution of 0.200 g asperuloside tetraacetate (0.344 mmole) in 4 mL of tetrahydrofuran (distilled from LAH) and 0.004 g butylated

hydroxytoluene was prepared and to this was added 0.306 g recrystallized N-bromosuccinimide (5 equiv.). This was heated at reflux for 30 minutes and rapidly cooled to room temperature. A column was prepared by loading 68.5 g of 60-200 mesh silica gel (J. T. Baker) into a 2.5 cm OD column in 3/1 ether/pentane to give a height of 40 cm. The cool yellow reaction mixture was placed atop this column and eluted with the same solvent under a slight air pressure. Concentration of the middle fractions afforded 0.244 g of clear colorless oil (96%) which was crystallized from ether/pentane affording 0.200 g of white amorphous dibromide (79%). m.p. 85 d.

Analysis: Calculated for $C_{26}H_{30}O_{15}Br_2$: C: 42.07; H: 4.07; Br: 21.53. Found: C: 42.06; H: 4.14; Br: 21.67.

^{1}H NMR (60 MHz, $CDCl_3$, ppm): 2.13, 2.13, 2.08, 2.01, 2.01 (all s, $OCOCH_3$); 6.40 (s, H_7); 6.09 (br. s, H_1); 5.42 (br. s, H_5); 4.7-5.1 (m, 5H); 4.70 (br. s, H_{10} a and b); 4.15 (m, H_6, a and b); 3.3-3.8 (m, 3H).

IR ($CHCl_3$, cm^{-1}): 3020 (w); 1775 (s); 1750 (br.,vs).

HPLC: Solvent I: T_r = 7.8 min.

TLC: 2:1 chloroform:acetonitrile R_f = 0.66 blue-green spot.

8.6 PREPARATION OF ASPERULOSIDE TETRAACETATE BROMOACETOXYLATE (28)

Asperuloside Tetraacetate Bromoacetoxylate (28): A solution of 0.05 g asperuloside tetraacetate (0.086 mmole) in 1.0 mL glacial acetic acid and 1.2 ml distilled water was prepared and to this was added 0.0245 g recrystallized N-bromosuccinimide (1.6 equiv.). After 30 minutes at room temperature, the light yellow reaction mixture was poured into 8 mL of ice-cold water and extracted with methylene chloride (3 x 3 mL). This was dried over magnesium sulfate, filtered and concentrated in vacuo to yield a colorless oil. Crystallization from chloroform/ether afforded white needles. Yield 0.49 g (80%). m.p. 178-179°C (Lit. m.p. 178.5-179.5°C [55]). Since mixed melting point showed this to be identical with known material and we did not pursue this path, no spectral data is available.

8.7 PREPARATION OF ASPERULOSIDE TETRAACETATE LACTONE WITH IODINE/PDC

Asperuloside Tetraacetate Lactone (35): A solution of 1.00 g asperuloside tetraacetate (1.72 mmole) in 20 mL methylene chloride was prepared in a 3-neck flask equipped with an efficient mechanical stirrer and a reflux condenser. To this was added a large quantity (typically 6 g although weighing was not necessary) of powdered 4 molecular sieves and then 1.31 g iodine (3.0 equiv.). After 5 minutes at room temperature, 4.45 g pyridinium dichromate (7.0 equiv.) was added

and the reaction was refluxed in an oil bath at 57°C. After 6 hours at reflux, the cooled reaction mixture was poured into 40 mL ethyl acetate and filtered through a little anhydrous magnesium sulfate. The filter cake was rinsed with an additional 50 mL ethyl acetate. The combined organic filtrates were poured into 100 mL 15% sodium thiosulfate solution in a separatory funnel and shaken vigorously for 15 seconds. The aqueous layer was back extracted with methylene chloride (4 x 50 mL) and the combined organic layers dried over calcium sulfate. Filtration, concentration in vacuo and recrystallization from chloroform/ether afforded 0.93 g lactone (90.8%) identical to that previously prepared (mixed m.p., IR and NMR).

8.8 ISOLATION OF ASPERULOSIDE TETRAACETATE IODOLACTONE

Asperuloside Tetraacetate Iodolactone (66): The reaction was performed exactly as in preparing the lactone (above) except that after filtration, the organic solution was concentrated in vacuo to a gray powder which was recrystallized twice from chloroform/ether. A small amount of decolorizing carbon was used in the second crystallization affording pure iodolactone in 24% yield as white needles. m.p. 209-211 d.

Analysis: Calculated for $C_{26}H_{29}O_{16}I$: C: 43.11; H: 4.03; I: 17.52. Found: C: 43.37; H: 4.08; I: 17.73.

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 2.095, 2.055, 2.055, 2.009, 1.981 (all s, $CCOCH_3$); 6.135 (s, H_7); 5.470 (dd, H_6, $J_{5,6}$ = 5.86 Hz); 5.219 (d, H_1, $J_{1,9}$ = 8.545 Hz); 5.184 (t, $H_{3'}$); 5.054 (t, $H_{4'}$); 5.014 (dd, $H_{2'}$, $J_{1',2'}$ = 8.06 Hz); 4.858 (d, $H_{1'}$); 4.723 (s, H_{10} a and b); 4.140 (eight line ABX, $H_{6'}$ a and b, $\Delta\delta$ (AB)= 38.6 Hz, $J_{6'a,6'b}$ = 12.21 Hz, $J_{6'a,5'}$ = 5.37 Hz, $J_{5,6'b}$ = 2.20 Hz); 3.718 (d[dd], $H_{5'}$); 3.619 (dd, H_5); 3.219 (t, H_9).

IR ($CHCl_3$, cm^{-1}): 3020 (m); 2950 (w); 1780 (s); 1755 (br.,vs).

HPLC: Solvent I: T_r = 10.4 min.

TLC: R_f = 0.43 yellow-green spot.

8.9 PREPARATION OF DIHYDRO ASPERULOSIDE TETRAACETATE LACTONE

Dihydro Asperuloside Tetraacetate Lactone (36). Method A: A solution of 2.00 g asperuloside tetraacetate lactone (3.36 mmole) in 500 mL purified p-dioxane was prepared and to this was added 2.00 g 5% rhodium on carbon. The mixture was attached to a standard atmospheric pressure hydrogenation apparatus and equipped with a closed system (magnetically coupled) mechanical stirrer and was hydrogenated for four hours (hydrogen uptake 1.2 times theoretical) at which time there was no additional hydrogen uptake. TLC of this solution showed only the one product. The suspension was first fil-

tered through a sintered glass funnel to recover the bulk of the catalyst. A little decolorizing carbon was then added and this filtered through a Celite pad. Rotary evaporation and drying in vacuo yielded a clear colorless oil (1.97 g; 98%). Crystallization from chloroform/ether afforded white needles of pure dihydrolactone (1.85 g; 92%). m.p. 186.5-187°C

Analysis: Calculated for $C_{26}H_{32}O_{16}$: C: 52.00; H: 5.37. Found: C: 51.81 H: 5.37.

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 2.019, 1.987, 1.982, 1.962, 1.924 (all s, $OCOCH_3$); 5.494 (d, H_1, $J_{1,2}$ = 6.35 Hz); 5.130 (t, $H_{3'}$); 5.006 (t, $H_{4'}$); 5.000 (dt, H_6); 4.914 (dd, $H_{2'}$, $J_{1',2'}$ = 8.06 Hz); 4.828 (d, $H_{1'}$); 4.143 (d, $H_{6'}$ a and b) 4.246, 4.040 (eight line ABX centered at 4.143, H_{10} a and b, $\Delta\delta$(AB)= 82 Hz, $J_{10a,10b}$ = 11.72 Hz, $J_{8,10a}$ = 6.59 Hz, $J_{8,10b}$ = 6.35 Hz); 3.670 (dt, $H_{5'}$); 3.590 (d, H_4, $J_{4,5}$ = 10.74 Hz); 3.317 (dt, H_5, equal coupling to H_6 and H_9); c. 2.55 (m, H_9); 2.552 (m, H_8); 2.430 (dt, H_{7a}); 1.671 (d[dd], H_{6b}).

IR ($CHCl_3$, cm^{-1}): 3020 (m); 2960 (w); 1790 (br.,s); 1752 (br.,vs).

HPLC: Solvent II: T_r = 11.4 min.

TLC: R_f = 0.18 gray-black spot; Using 9/1 chloroform/methanol R_f = 0.66.

Dihydro Asperuloside Tetraacetate Lactone (36). Method B: A solution of 2.00 g asperuloside tetraacetate lactone (3.36 mmole) in 500 mL purified p-dioxane was prepared and to this was added 2.00 g 5% rhodium on alumina. This was hydrogenated as in Method A but for only three hours and then filtered through a Celite pad. TLC showed some hydrogenolysis products so this was concentrated onto a little silica gel and chromatographed on a short (15 cm) column of the same silica using 1:1 ethyl acetate:hexane as the eluent. Concentration and drying in vacuo afforded 1.66 g of clear colorless oil (83%) which was crystallized from chloroform/ether to yield 1.53 g (77%) of pure dihydrolactone identical in all respects to that obtained above.

8.10 PREPARATION OF DIHYDROASPERULOSIDE TETRAACETATE TERTIARY ALCOHOL (68)

Dihydroasperuloside Tetraacetate Tertiary Alcohol (68): A solution of 2.00 g asperuloside tetraacetate tertiary alcohol (3.36 mmole) in 500 mL purified p-dioxane was prepared and to this was added 2.00 g 5% rhodium on carbon. This was hydrogenated at atmospheric pressure using the usual apparatus and a closed system mechanical stirrer. After two hours, no additional hydrogen uptake was evident and the reaction mixture was filtered through a Celite pad. TLC showed a small amount of hydrogenolysis product at the origin so this was passed through a short column of silica using 1:1 ethyl acetate:hexane as eluent. Concentration and drying in

vacuo afforded 1.76 g (88%) of clear colorless oil which could be crystallized from chloroform/ether to yield dense cubic white crystals (several crops yield 1.44 g, 70%). m.p. 162-163°C

Analysis: Calculated for $C_{26}H_{34}O_{16}$: C: 51.83; H: 5.69. Found: C: 51.75; H: 5.74.

^{1}H NMR (400 MHz, $CDCl_3$, ppm): 2.013, 2.006, 1.970, 1.952, 1.921 (all s, $OCOCH_3$); 5.560 (s, H_1); 5.114 (t, $H_{3'}$); 5.008 (t, $H_{4'}$); 4.925 (dd, $H_{2'}$); c. 4.91 (m, H_5, hidden partly by $H_{2'}$); 4.712 (d, $H_{1'}$, $J_{1',2'}$ = 8.30 Hz); 4.104 (eight line ABX, $H_{6'}$ a and b, $\Delta\delta$ = 55.2 Hz, $J_{6'a,6'b}$ = 12.69 Hz, $J_{5,6'a}$ = 5.13 Hz, $J_{5,6'b}$ = 1.95 Hz); 4.060 (d, H_{10} a and b, $J_{8,10}$ = 8.06; 3.737 (dd, H_3 a and b, becomes singlet upon addition of D_2O); 3.629 (d[dd], $H_{5'}$); 3.551 (t, H_5); 3.034 (t, H_9); 2.518 (m, H_8); 2.251 (dt, H_{7a}); 2.24 (m, OH, D_2O labile); 1.610 (m, H_{7b}).

IR ($CHCl_3$, cm^{-1}): 3540 (br.,w); 3020 (m); 2960 (w); 1775 (s); 1750 (br.,vs).

HPLC: Solvent I: T_r = 10.6 min.

TLC: R_f = 0.27 gray-black spot.

8.11 PREPARATION OF DIHYDRO ASPERULOSIDE TETRAACETATE LACTONE AGLUCONE

Dihydro Asperuloside Tetraacetate Lactone Aglucone (70): A solution of 0.200 g dihydro asperuloside tetraacetate lactone (0.33 mmole) in 30 mL of 90% formic acid was prepared and refluxed for 30 minutes in an oil bath at 110°C. This was then concentrated to about 5 mL, poured into 20 mL distilled water and extracted with methylene chloride (4 x 5 mL). After drying over magnesium sulfate, the combined organic extracts were concentrated onto 5 g silica gel, placed atop a 1.4 cm OD column loaded with the same silica gel to a height of 33 cm and eluted with 4:1 ether:ethyl acetate. Upon concentration of the appropriate fractions a clear colorless oil (0.090 g, 90%) was obtained. No attempt was made to crystallize this product or obtain an analysis since we did not work with it further.

^{1}H NMR (60 MHz, $CDCl_3$, ppm): 2.05 (s, $OCOCH_3$); 5.81 (d, H_1); 4.90 (m, H_6); 4.2 (s, H_{10} a and b); 3.6 (d, H_4); 3.3 (m, H_5); 2.3-2.7 (m, 3H); 1.6 (m, H_{7a}); 4.9 (br., OH).

IR ($CHCl_3$, cm^{-1}): 3500 (br.,w); 3020 (m); 2950 (w); 1775 (s); 1735 (br.,s).

HPLC: Solvent I: T_r = 11.6 min.

TLC: R_f = 0.17 gray-black spot.

8.12 PREPARATION OF DIHYDRO ATA TERTIARY ALCOHOL AGLUCONE (72)

Dihydro Asperuloside Tetraacetate Tertiary Alcohol Aglucone (72): A solution of 0.100 g dihydro asperuloside tetraacetate tertiary alcohol (0.16 mmole) in 10 mL of 5:1 glacial acetic acid:water was prepared and refluxed in an oil bath at 110°C for 20 hours. This was cooled to room temperature, poured into 15 mL water and extracted with methylene chloride (6 x 10 mL). The combined extracts were dried over magnesium sulfate, filtered and concentrated onto a little 60-200 mesh silica gel. A 1.4 cm OD column was loaded with the same silica gel to a height of 33 cm in 5:1 ether:ethyl acetate. The silica bearing the extract was placed atop this column and eluted with the same solvent under a slight air pressure. Concentration of the appropriate fractions gave the aglucone as a white solid (0.018 g, 41%) which was not further purified for analysis.

^{1}H NMR (60 MHz, $CDCl_3$, ppm): 2.05 (s, $OCOCH_3$); 5.8 (d, H_1); 4.9 (m, H_6); 4.9 (br., OH, D_2O labile); 4.3 (s, H_{10} a and b); 3.7-4.0 (m, H_3 a and b); 3.3-3.7 (m, 2H); 2.9 (br., OH, D_2O labile); 2.2-2.4 (m, 2H); 1.4 (m, 1H).

IR ($CHCl_3$, cm^{-1}): 3540 (br.,m); 3020 (m); 2950 (w); 1775 (s); 1735 (br.,s).

TLC: R_f = 0.25 gray black spot.

8.13 PREPARATION OF THE COREY ALDEHYDE ANALOG (71A)

11-Deoxy-11-Hydroxymethyl Analog of Corey's Lactone Aldehyde (71a): A solution of 0.500 g dihydro asperuloside tetraacetate lactone (0.83 mmole) in 100 mL of 5:1 glacial acetic acid:water was prepared and refluxed at 116°C for 18 hours. The reaction mixture was then placed on a rotary evaporator and its volume reduced to 10 mL. This was poured into 90 mL distilled water and extracted with methylene chloride (5 x 20 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated onto 3 g of 60-200 mesh silica gel. This was placed atop a column prepared by loading 45 g of the same silica gel into a 2.5 cm OD column in 5:1 ether:ethyl acetate and eluted with the same solvent under a slight air pressure. Concentration of the appropriate fractions and drying in vacuo gave the aldehyde (0.114 g, 61%) as a pale yellow oil.

Analysis: Calculated for $C_{11}H_{14}O_5 \cdot H_2O$: C: 54.09; H: 6.60. Found: C: 54.09; H: 6.64.

^{1}H NMR (60 MHz, $CDCl_3$, ppm): 2.08 (s, $OCOCH_3$); 9.75 (s, CHO); 5.00 (m, H_6); 4.15 (br. dd, H_9 a and b); 3.15 (t, H_2); 2.2-2.9 (m, 5H); 1.9 (m, H_3).

IR ($CHCl_3$, cm^{-1}): 3020 (m); 2950 (w); 1775 (s); 1735 (br.,s).

HPLC: Solvent II: T_r = 8.6 min.

TLC: R_f = 0.23 gray-black spot.

8.14 WITTIG REACTION OF ALDEHYDE 71A

Wittig Reaction of Aldehyde 71a to Produce Enone 73: A suspension of 0.0212 g sodium hydride granules (0.884 mmole; Alfa-Ventron) in 4.0 mL dry dimethoxyethane (distilled from LAH) was prepared in a flask which was under a positive pressure of dry nitrogen. A solution of 0.210 g dimethyl 2-oxoheptylphosphonate (1.07 equiv., c. 200 uL, Aldrich) in 1.0 mL dry DME was injected into this stirred suspension and stirring continued at room temperature for one hour while a voluminous white precipitate formed. This was cooled in an ice bath and a solution of 0.1000 g aldehyde 71a (0.442 mmole) in 1.0 mL dry DME was injected all at once. Stirring was continued in an ice bath for 30 minutes followed by two and a half hours at room temperature. The reaction was then neutralized with glacial acetic acid (120 uL) and concentrated directly onto 2.5 g of 60-200 mesh silica gel. This was placed atop a column prepared by loading 45 g of the same silica into a 2.9 cm OD column in 1:1 ethyl acetate:hexane (height 33 cm) and eluted with the same solvent under a slight air pressure. Fractions of 25 mL were collected and fractions 7-16 combined and concentrated to yield the enone (0.125 g; 87.3%) as a pale yellow oil identical in every respect to a sample prepared by another route which gave a good analysis.

^{1}H NMR (220 MHz, $CDCl_3$, ppm): 6.613 (dd, H_{13}, $J_{12,13}$ = 8.45 Hz, $J_{13,14}$ = 15.44 Hz); 6.183 (d, H_{14}); 4.974 (m, H_9);

4.045 (eight line ABX, H_{21} a and b, $\Delta\delta$ = 45 Hz, $J_{21a,21b}$ = 11.4 Hz, $J_{11,21a}$ = 4.41 Hz, $J_{11,21b}$ = 5.2 Hz); 2.56-2.77 (m, 2H); 2.518 (t, 2 H_{16}); 2.3-2.4 (m, 1H); 2.227 (m, H_{11}); 2.024 (s, $OCOCH_3$); 1.82 (m, H_8); 1.602 (m, $2H_{17}$); 1.28 (m, $4H_{18,19}$); 0.886 (t, $3H_{20}$).

IR ($CHCl_3$, cm^{-1}): 3020 (m); 2960 (s); 2930 (s); 2875 (m); 2860 (m); 1760 (vs); 1740 (vs); 1695 (s); 1630 (s).

TLC: R_f = 0.55 gray-black spot.

Optical Rotation: $[\alpha]_D^{25}$ = -28° (chloroform).

Appendix A

PARTIAL LIST OF ABBREVIATIONS

ATA.....asperuloside tetraacetate

DCC.....N,N'-Dicyclohexylcarbodiimide

DME.....dimethoxyethane

DMF.....N,N-dimethylformamide

DMS.....(di)methyl sulfide

DMSO.....dimethyl sulfoxide

glu.....glucose

NBS.....N-bromosuccinimide

OAc.....acetate

OBz.....benzoate

PCC.....pyridinium chlorochromate

PDC.....pyridinium dichromate

PG.....prostaglandin(s)

PTFA.....pyridinium trifluoroacetate

pyr.....pyridine

Rhm.....rhamnose

THF.....tetrahydrofuran

THP.....tetrahydropyran

Appendix B

SELECTED CURRENT CHEMICAL ABSTRACTS INDEX NAMES

Asperuloside Tetraacetate Bromolactone (57):
4-[(Acetyloxy)methyl]-7a-bromo-4a,5,7a,7b-tetrahydro-5-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione

Asperuloside Tetraacetate Lactone (35):
[2aS-(2aα,4aα,5α,7aα,7bα)]-4-[(Acetyloxy)methyl]-tetrahydro-5-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione

Dihydro Asperuloside Tetraacetate Lactone (36):
[2aS-(2aα,4α,4aα,5α,7aα,7bα)]-4-[(Acetyloxy)methyl]-hexahydro-5-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione

Dihydro Asperuloside Tetraacetate Lactone Aglucone (70):
4-[(Acetyloxy)methyl]hexahydro-5-hydroxy-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione

Enone 73:
5-[(Acetyloxy)methyl]hexahydro-4-(3-oxo-1-octenyl)-2H-cyclopenta[b]furan-2-one

Corey Aldehyde Analog 71a:
5-[(Acetyloxy)methyl]hexahydro-4-formyl-2H-cyclopenta[b]furan-2-one

Asperuloside Tetraacetate Tertiary Alcohol (64):
[2aS-(2aα,4aα,5α,7bα)]-4-[(Acetyloxy)methyl]-7a-hydroxy-5-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]-1H-2,6-dioxacyclopent[c,d]indene-1-one

Asperulosidic Acid Lactone (59):
[1S-(1α,5β,7aα)]-7-[(Acetyloxy)methyl]-1,3,5,7a-dihydro-5-hydroxy-1-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]cyclopenta[c]pyran-3-one-4-carboxylic acid

Appendix C

SELECTED IUPAC NAMES

Asperuloside Tetraacetate Bromolactone (57):
(2aS,4aS,5R,7bR)-7a-Bromo-5-(β-D-glucopyranosyloxy)-4a,5,7a,7b-tetrahydro-4-(hydroxymethyl)-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione pentaacetate

Asperuloside Tetraacetate Lactone (35):
(2aS,4aR,5R,7aR,7bS)-5-(β-D-glucopyranosyloxy)tetrahydro-4-(hydroxymethyl)-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione pentaacetate

Dihydro Asperuloside Tetraacetate Lactone (36):
(2aS,4R,4aR,5R,7aR,7bS)-5-(β-D-glucopyranosyloxy)-hexahydro-4-(hydroxymethyl)-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione pentaacetate

Dihydro Asperuloside Tetraacetate Lactone Aglucone (70):
(2aS,4R,4aR,7aR,7bS)-Hexahydro-5-hydroxy-4-(hydroxymethyl)-1H-2,6-dioxacyclopent[cd]indene-1,7(2aH)-dione 4-acetate

Enone 73:
(3aS,4S,5R,6aS)-Hexahydro-5-(hydroxymethyl)-4-[(E)-(3-oxo-1-octenyl)]-2H-cyclopenta[b]furan-2-one 5-acetate

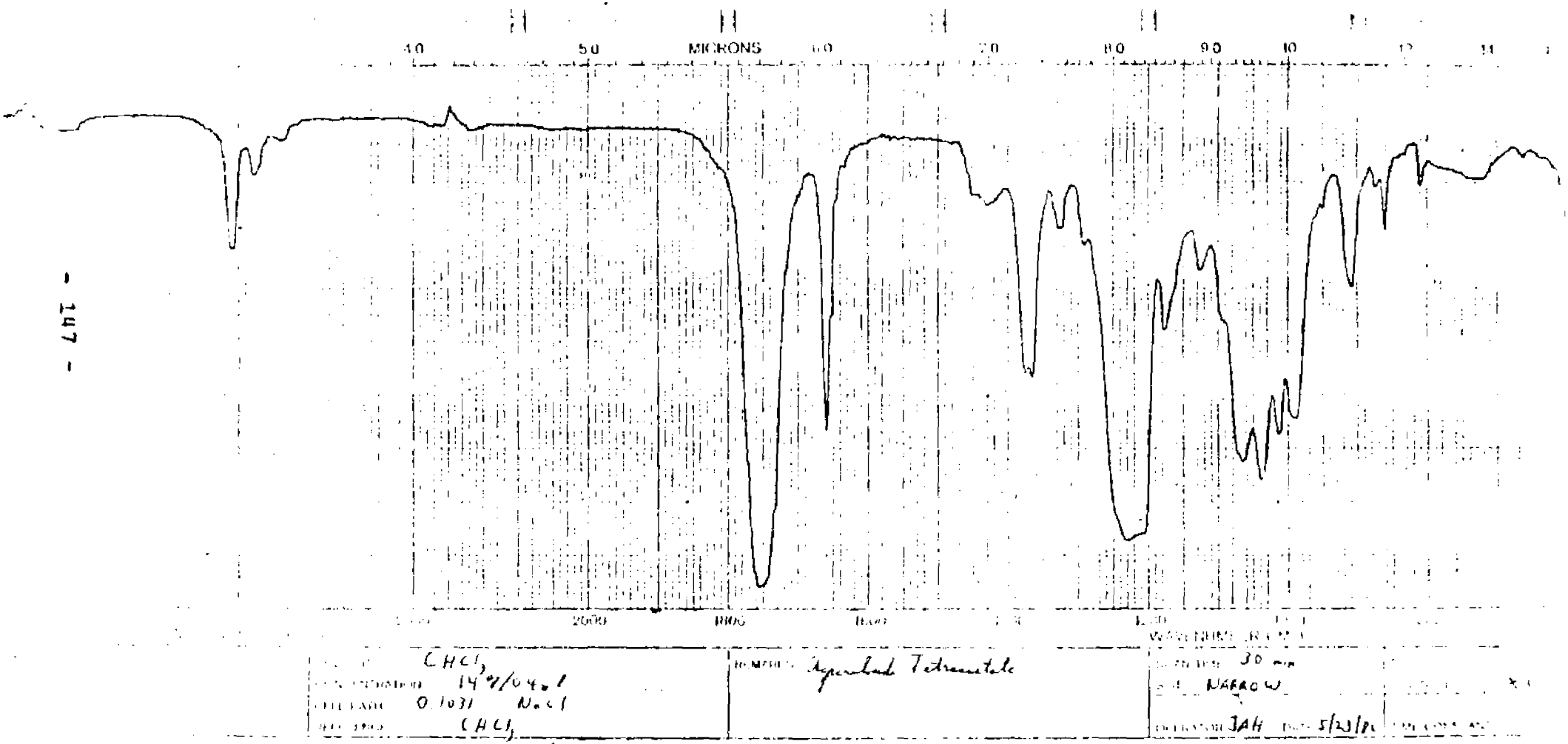
Corey Aldehyde Analog 71a:
(3aS,4S,5R,6aS)-Hexahydro-5-(hydroxymethyl)-4-formyl-2H-cyclopenta[b]furan-2-one 5-acetate

Asperuloside Tetraacetate Tertiary Alcohol (64):
(2aS,4aR,5S,7bS)-5-(β-D-Glucopyranosyloxy)tetrahydro-4-(hydroxymethyl)-7a-hydroxy-1H-2,6-dioxacyclopent[cd]inden-1-one, 2',3',4',6',7-pentaacetate

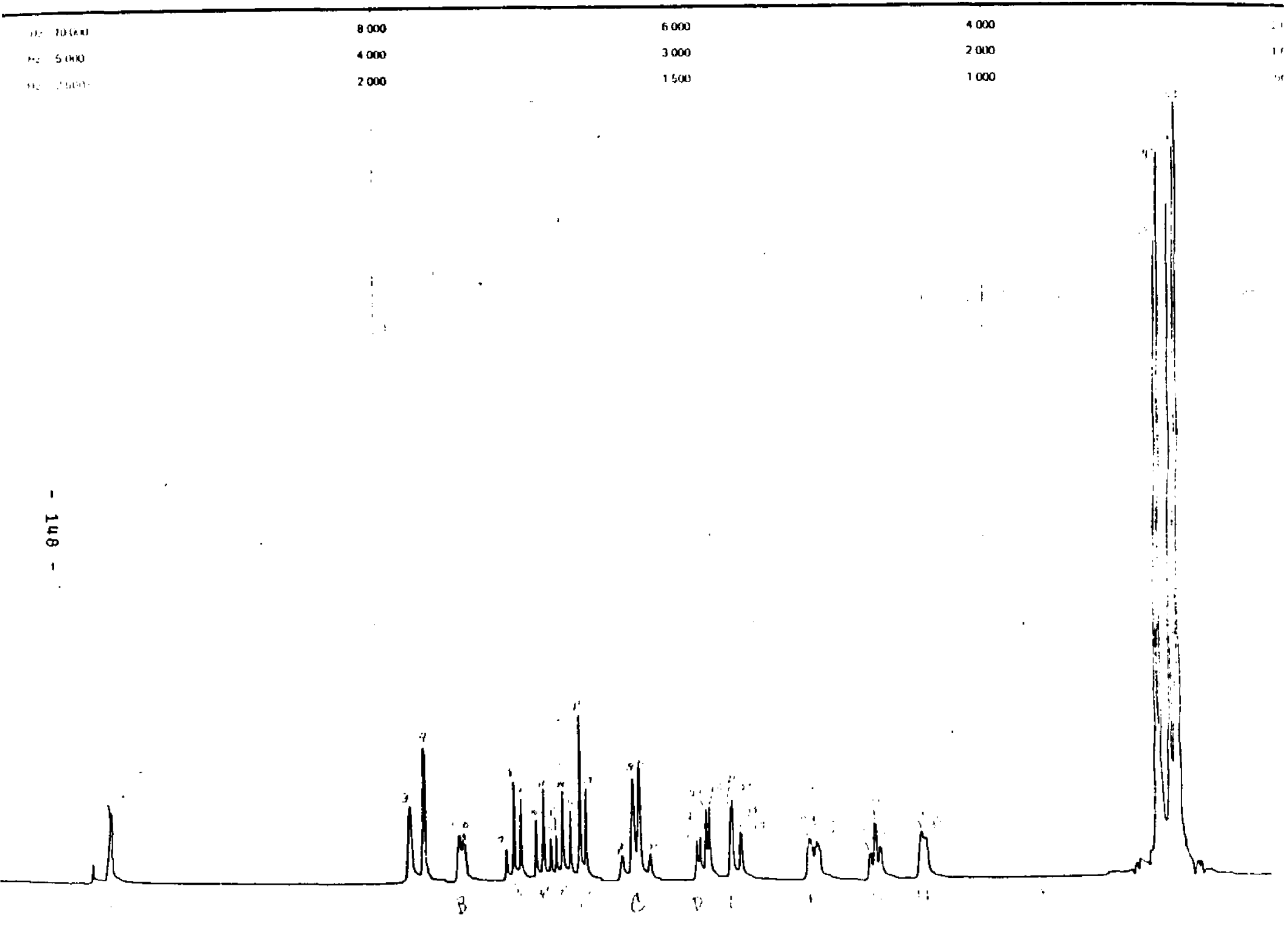
Asperulosidic Acid Lactone (59):
(1S,5S,7aR)-1-(β-D-Glucopyranosyloxy)-1,3,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)cyclopenta[c]pyran-3-one-4-carboxylic acid, 2',3',4',6',7-pentaacetate

Appendix D

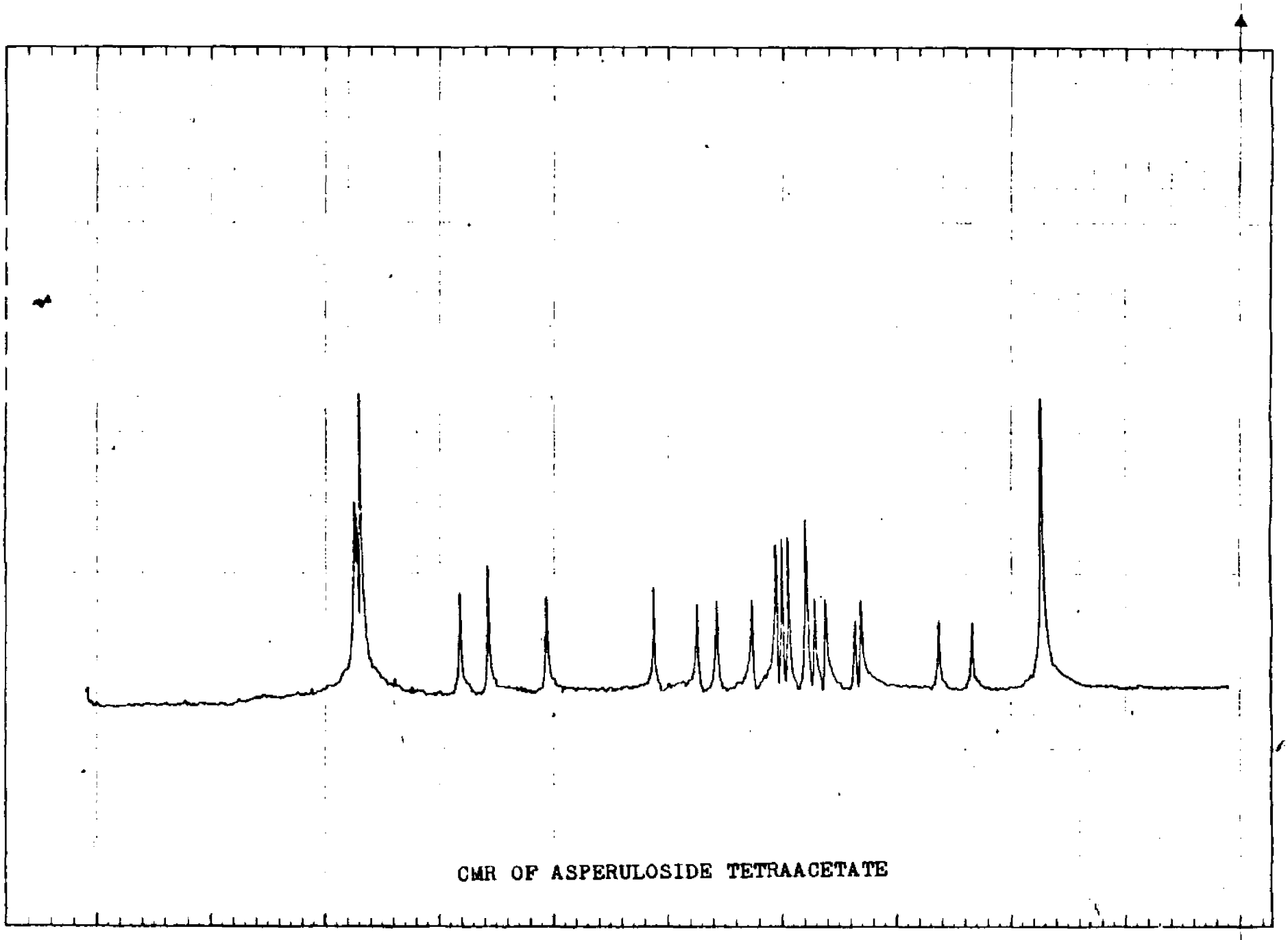
IR, NMR AND CMR SPECTRA OF ASPERULOSIDE TETRAACETATE



IR OF ASPERULOSIDE TETRAACETATE



NMR OF ASPERULOSIDE TETRAACETATE

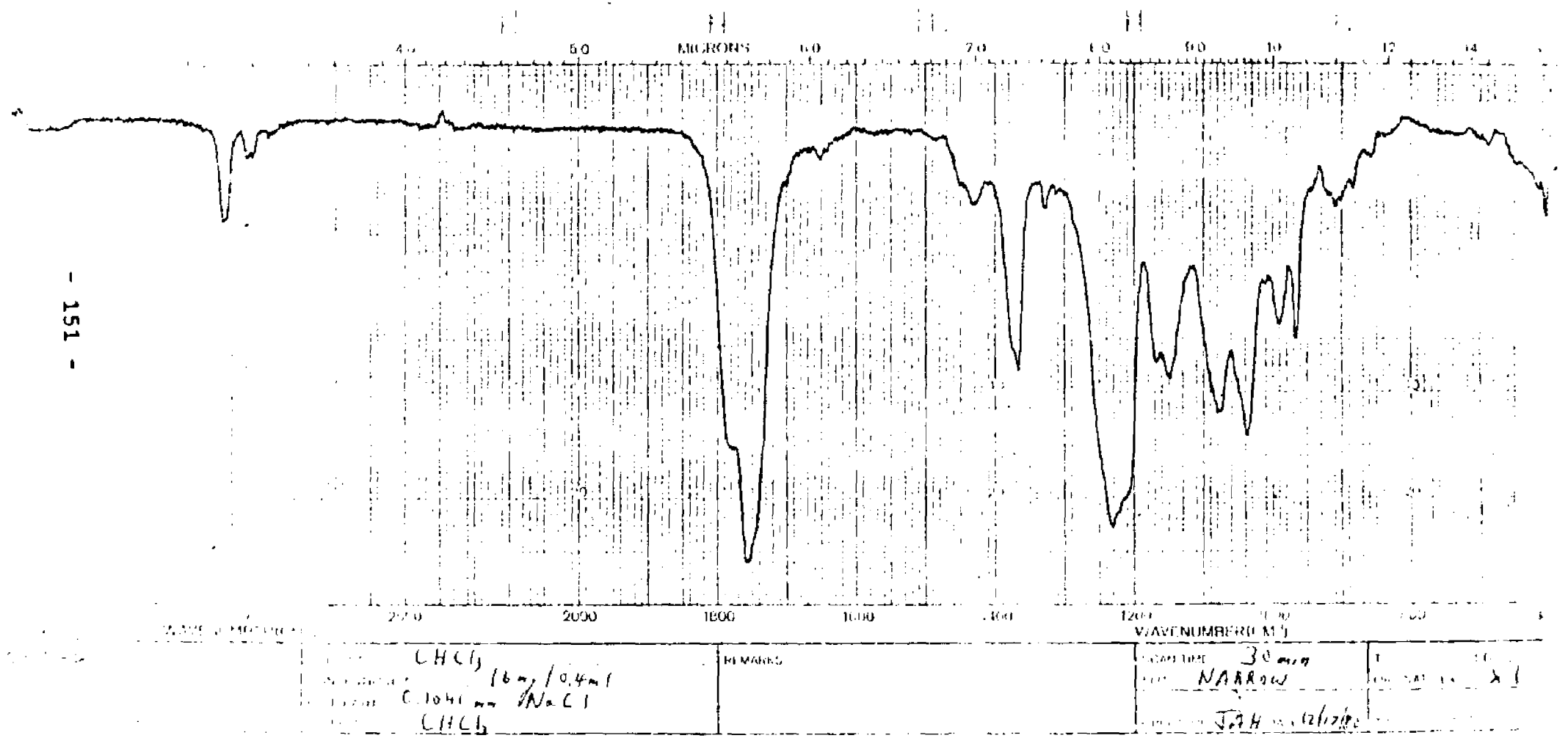


CMR OF ASPERULOSIDE TETRAACETATE

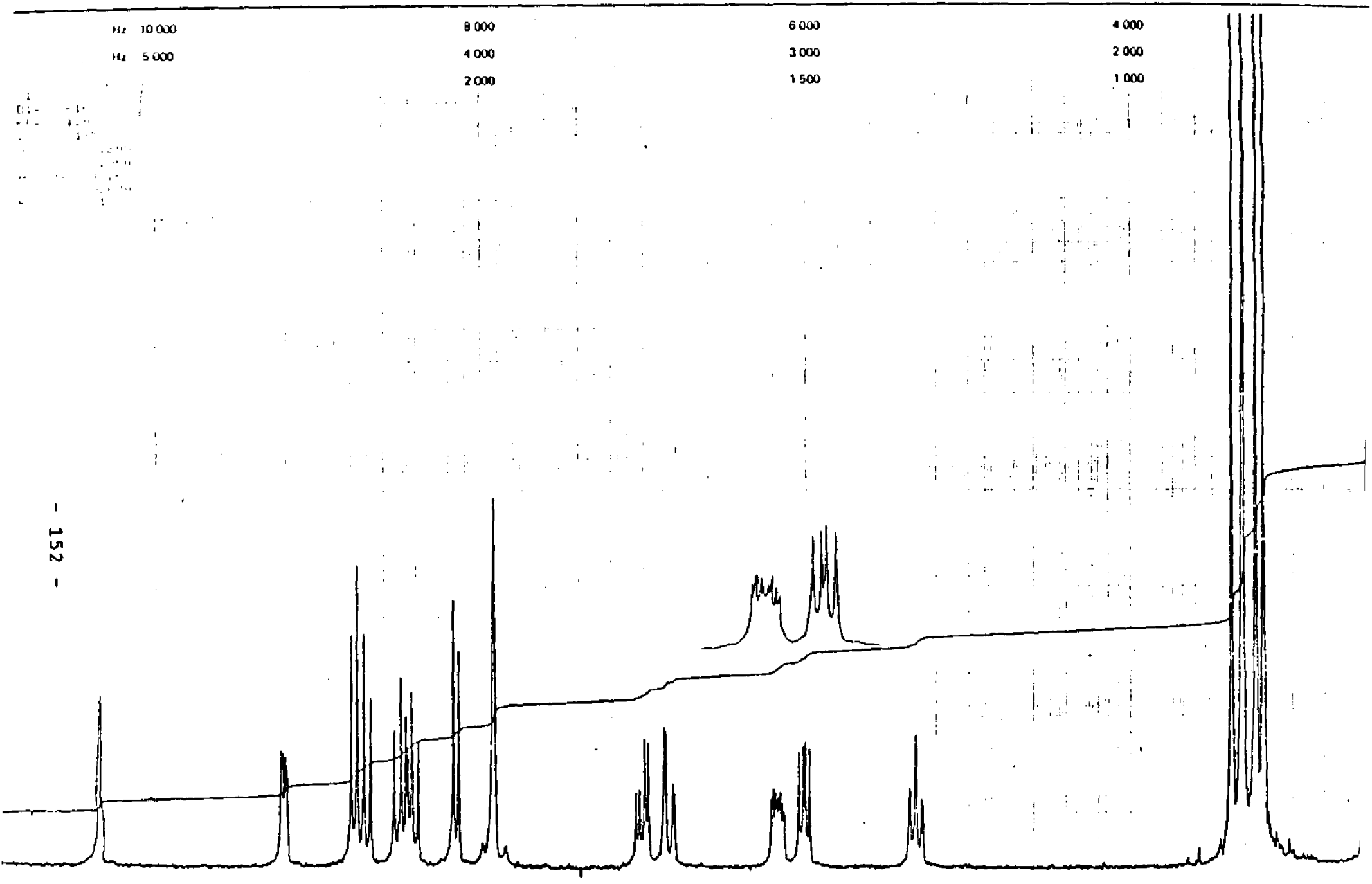
PFT 100

Appendix E

IR AND NMR OF IODOLACTONE (66)



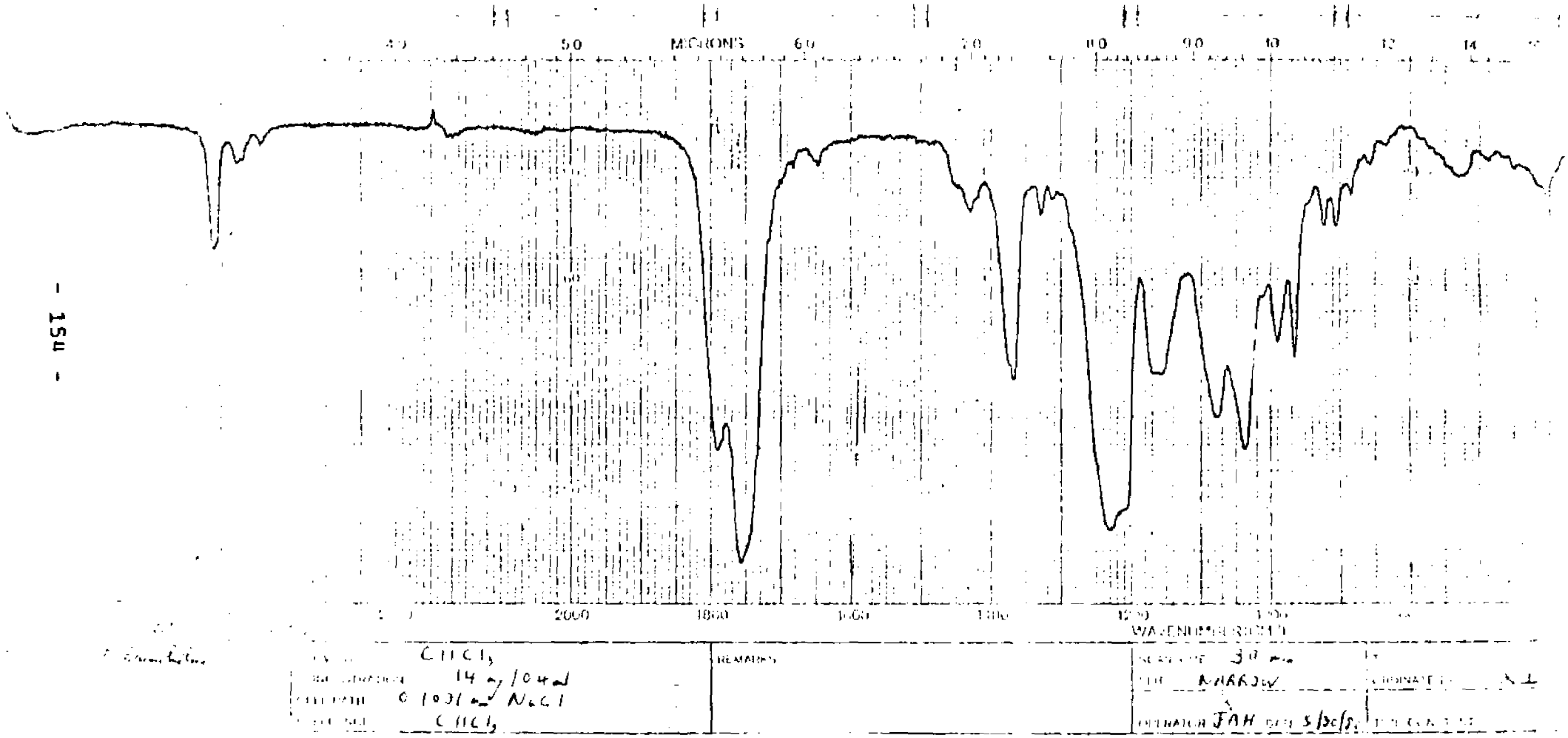
IR OF IODOLACTONE (66)



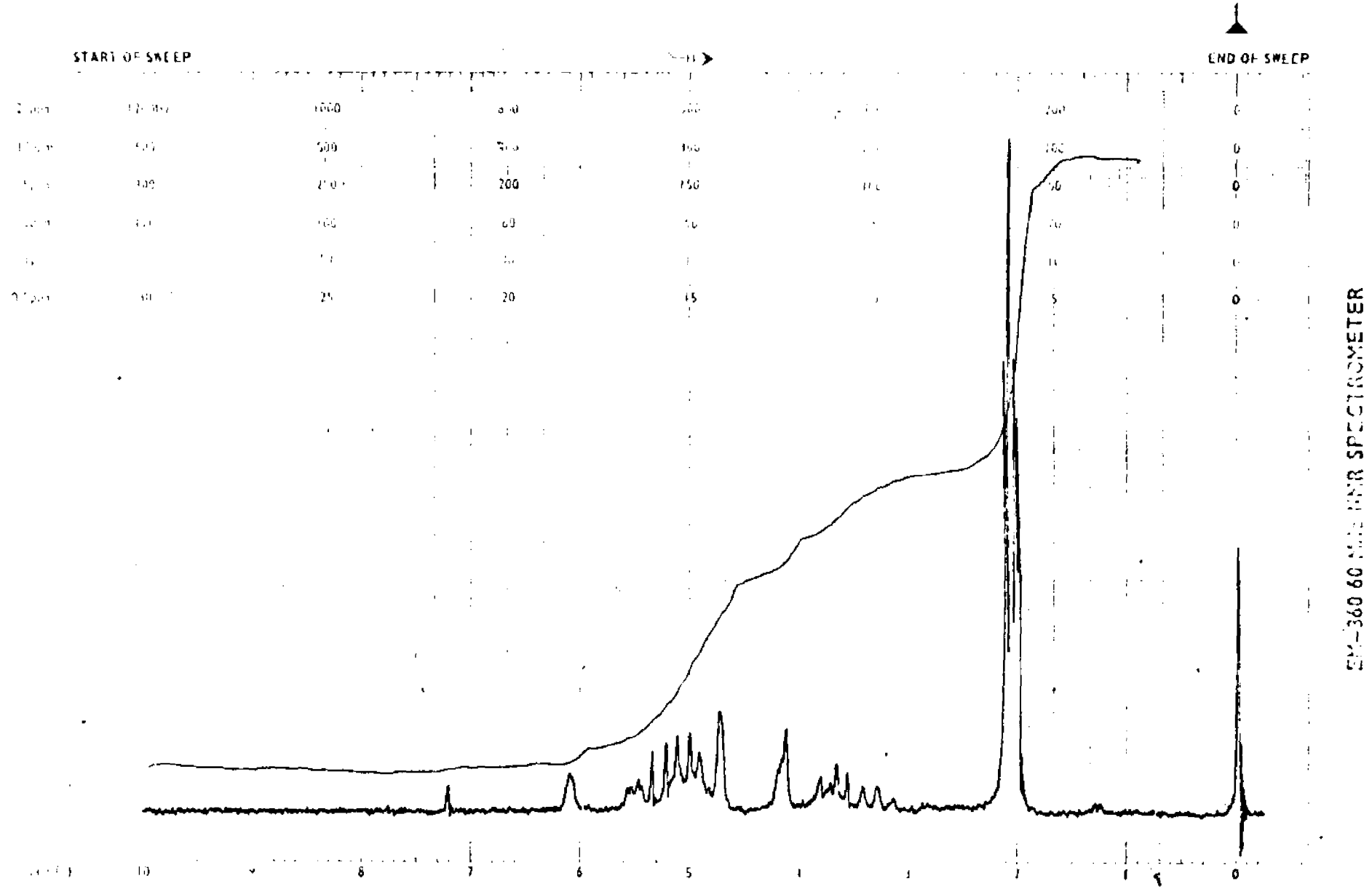
NMR OF IODOLACTONE (66)

Appendix F

IR AND NMR OF BROMOLACTONE (57)



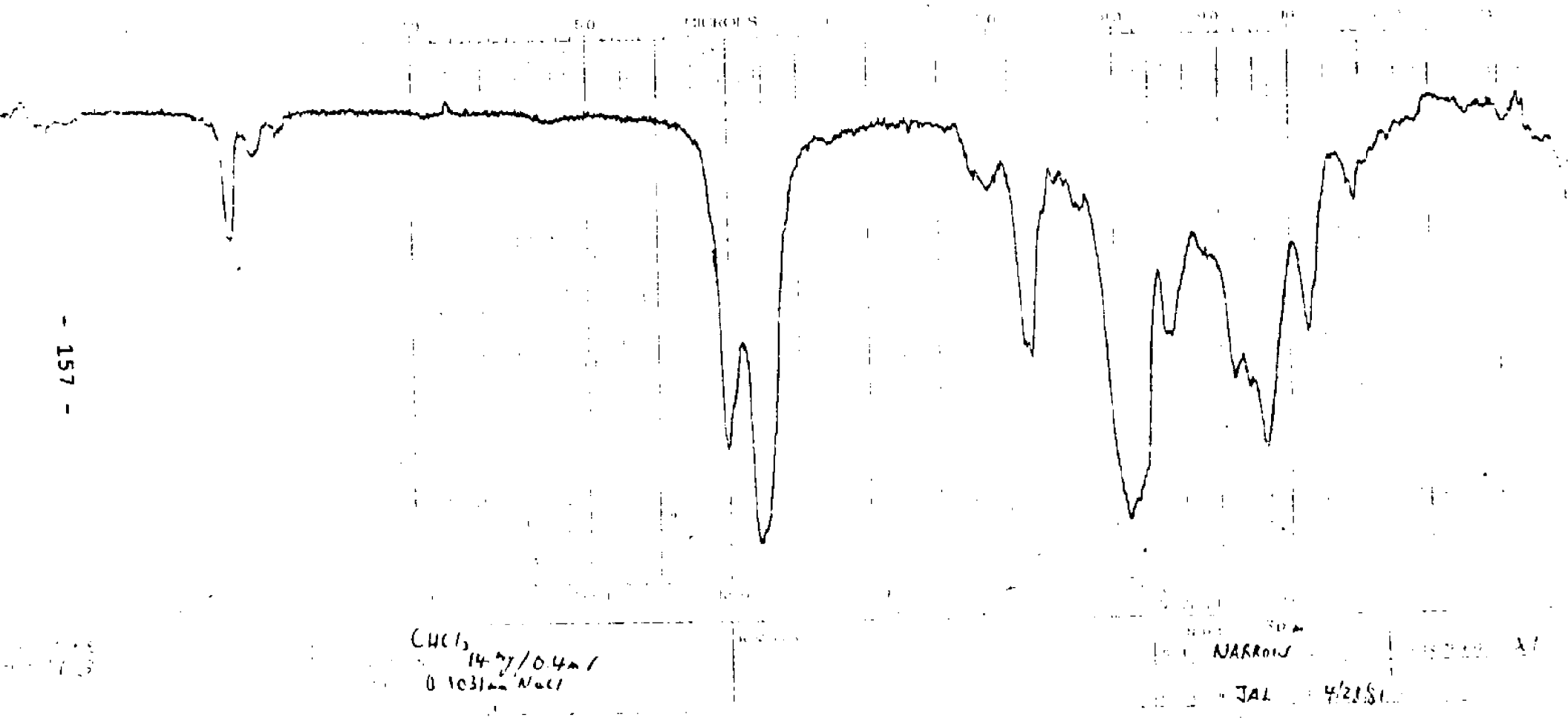
IR OF BROMOLACTONE (57)



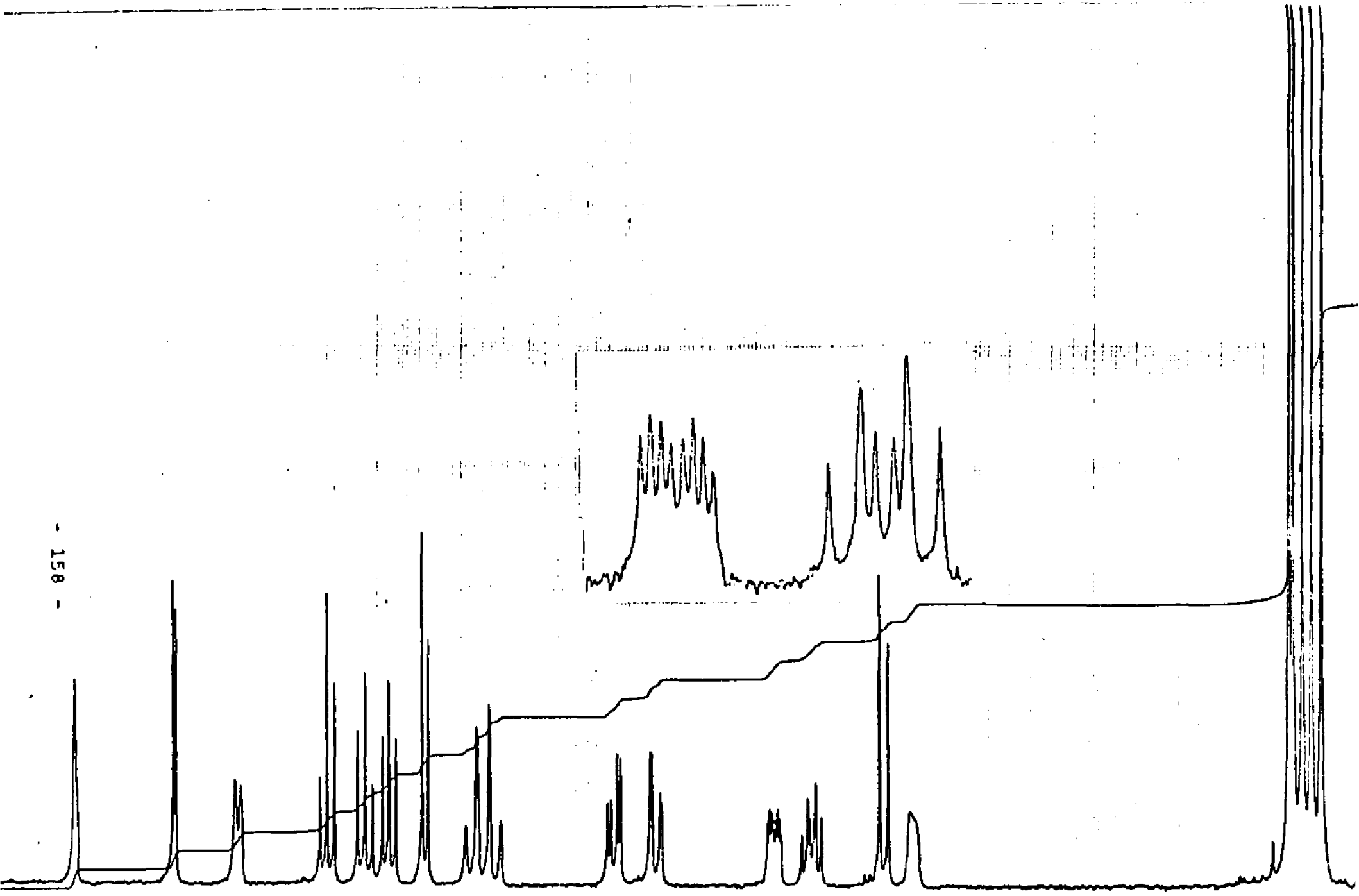
NMR OF BROMOLACTONE (57)

Appendix G

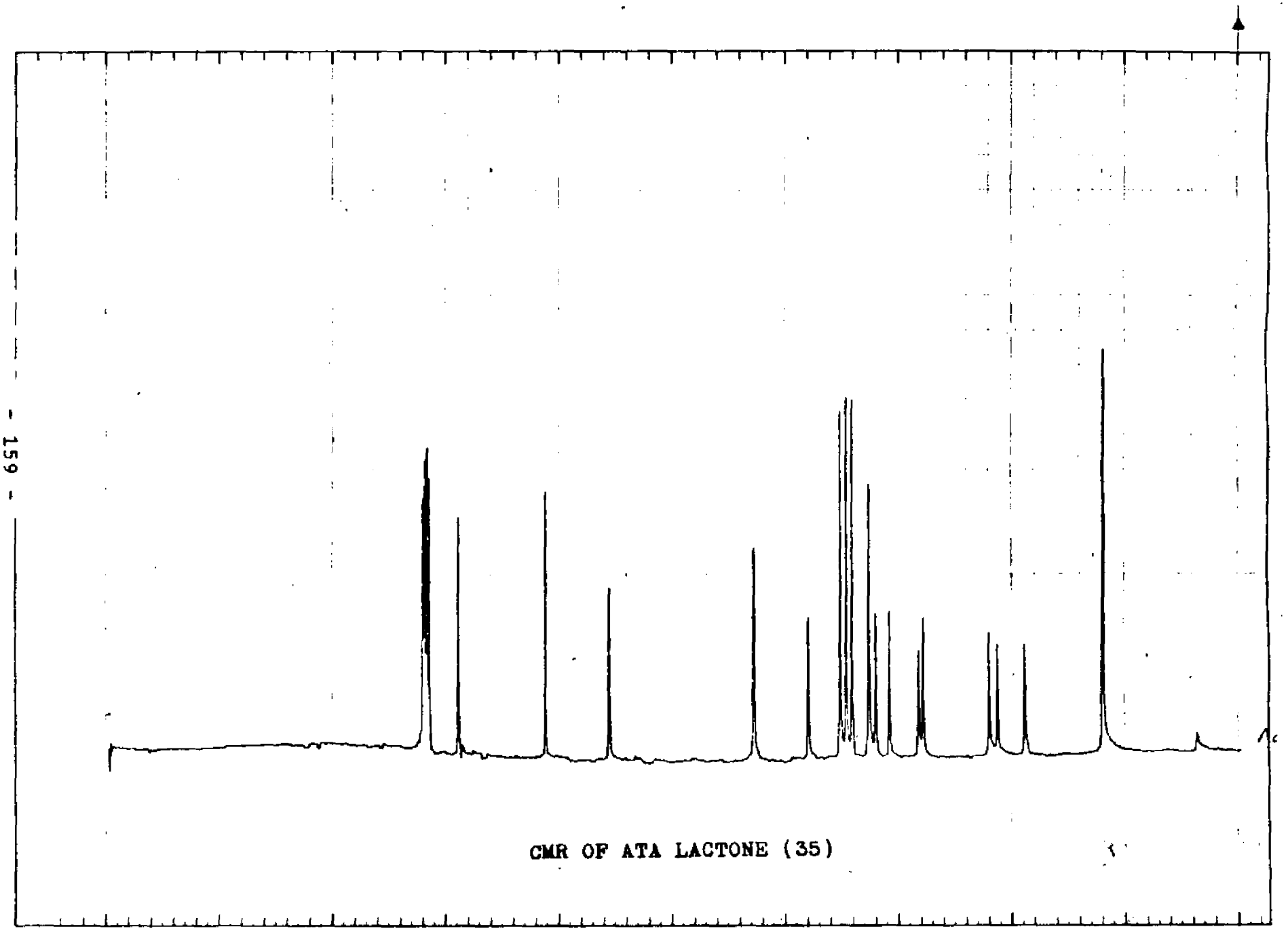
IR, NMR AND CMR OF ATA LACTONE (35)



IR OF ATA LACTONE (35)



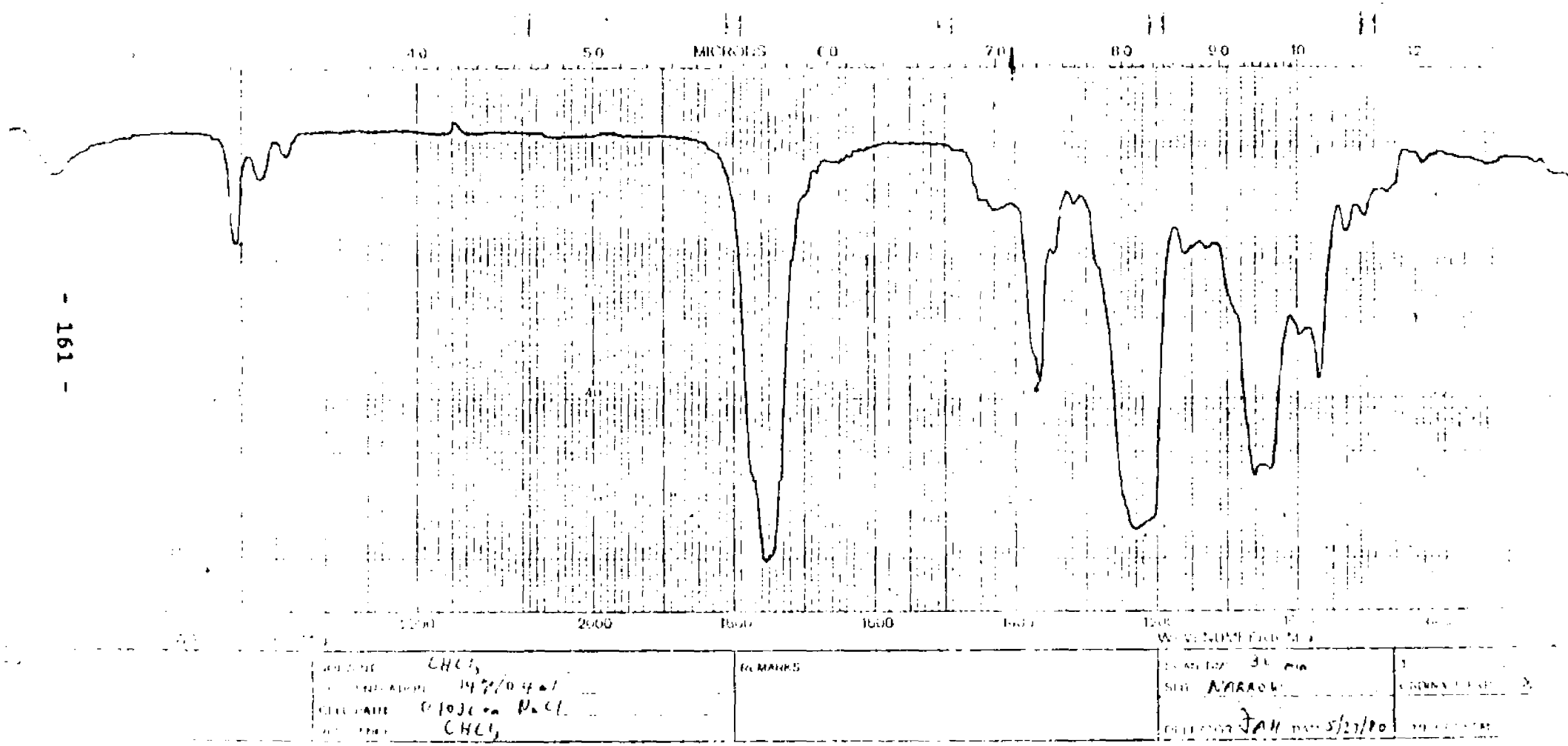
NMR OF ATA LACTONE (35)



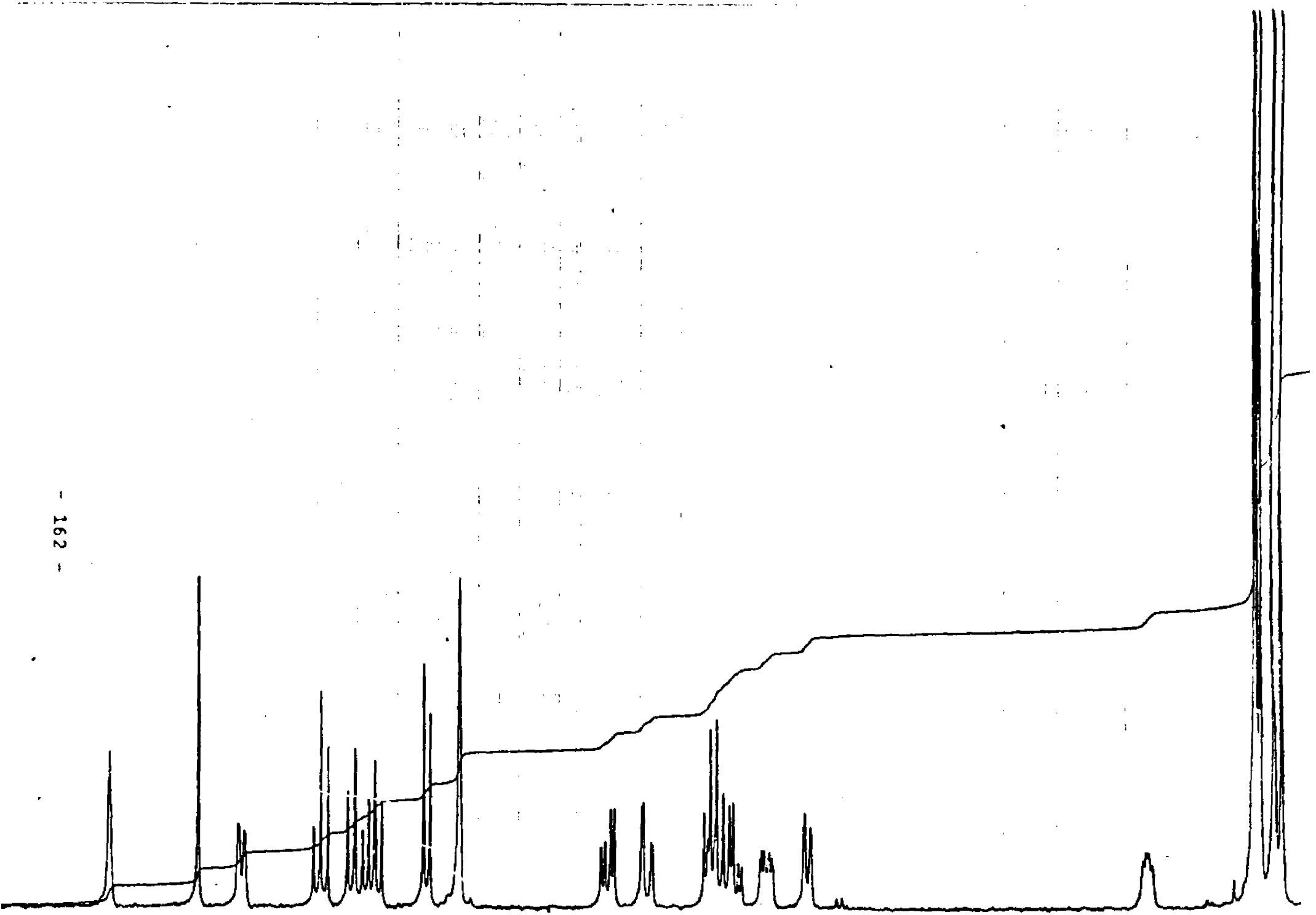
CMR OF ATA LACTONE (35)

Appendix H

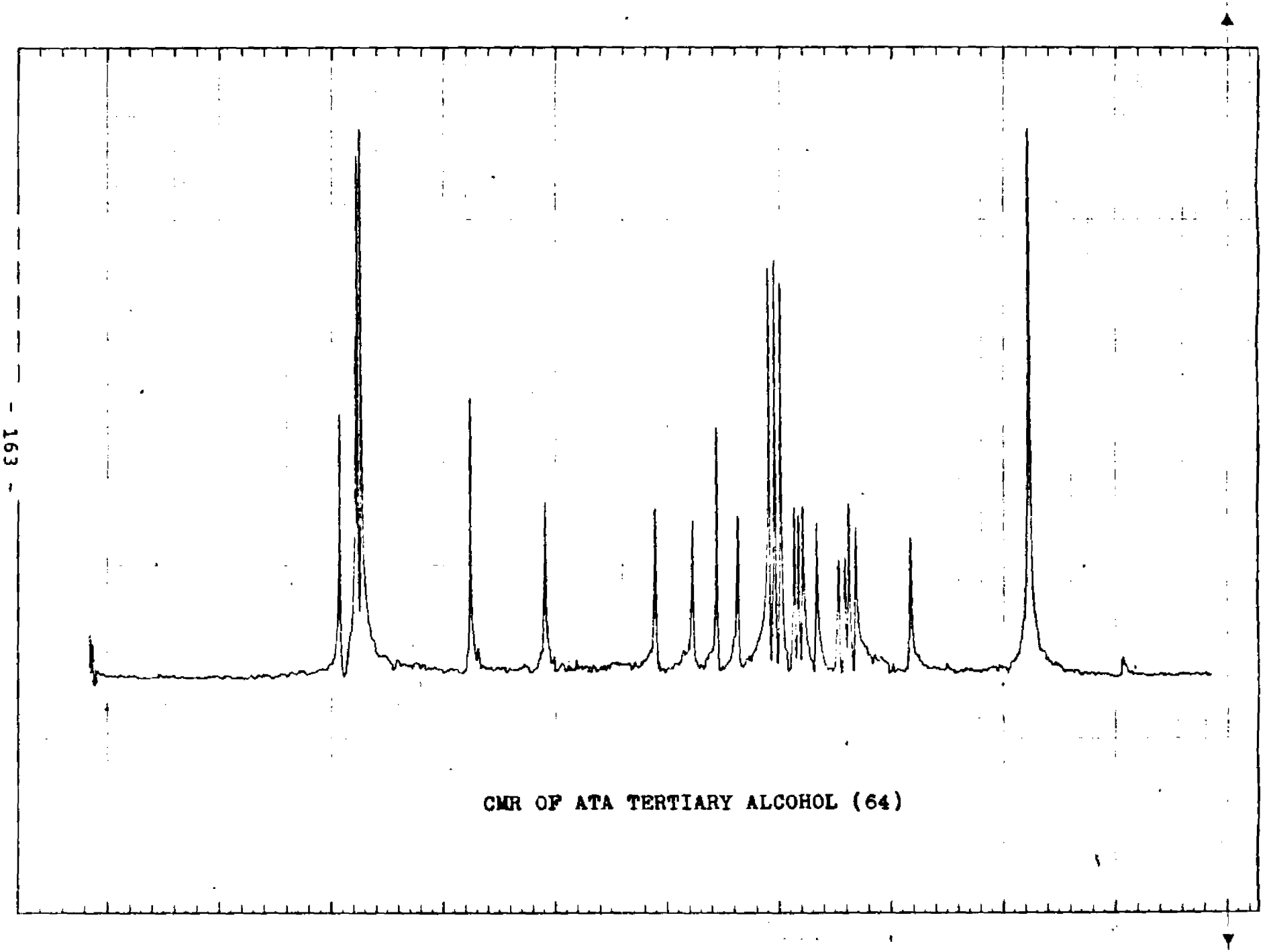
IR, NMR AND CMR OF ATA TERTIARY ALCOHOL (64)



IR OF ATA TERTIARY ALCOHOL (64)



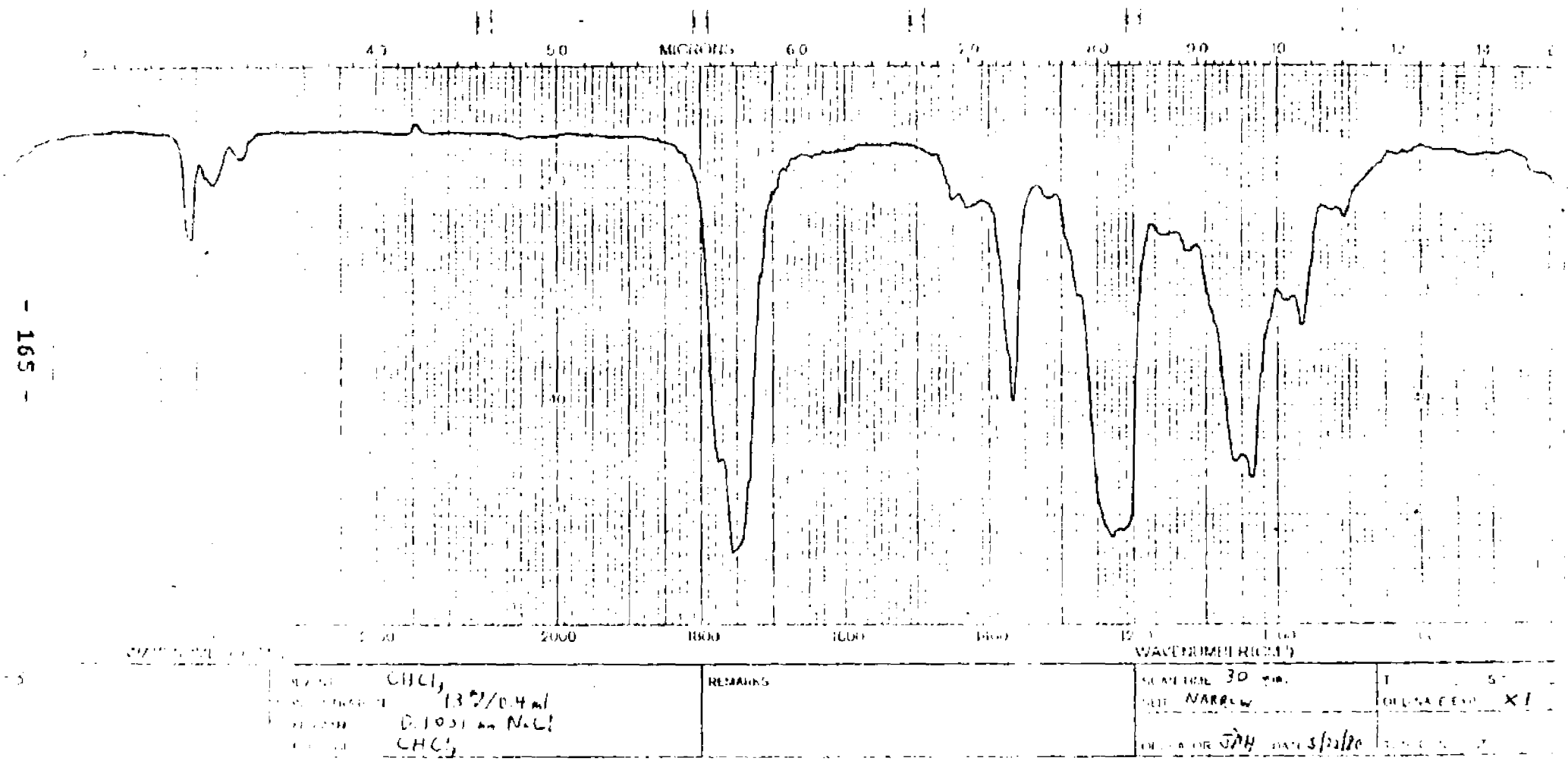
NMR OF ATA TERTIARY ALCOHOL (64)



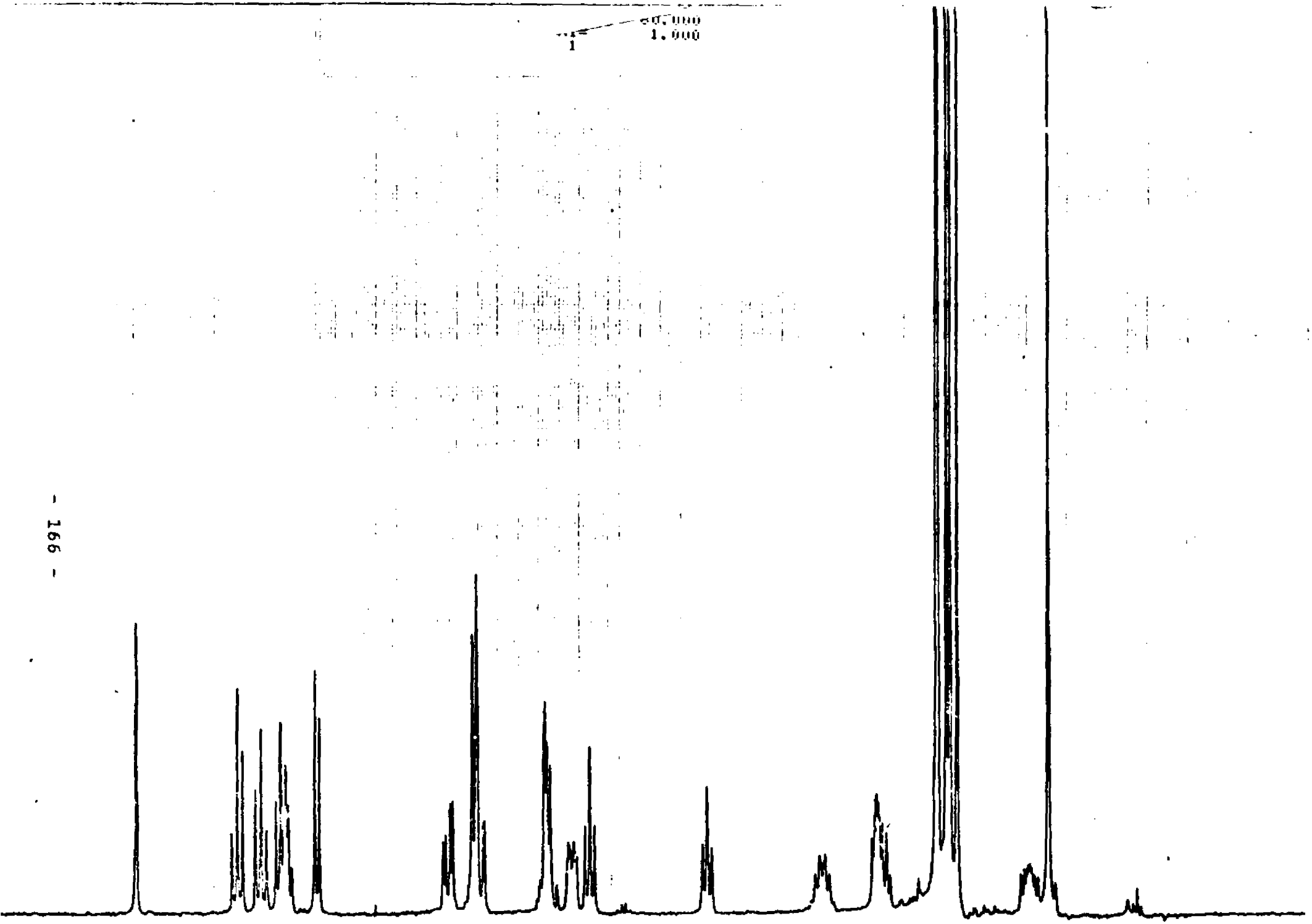
CMR OF ATA TERTIARY ALCOHOL (64)

Appendix I

IR, NMR AND CMR OF DIHYDRO ATA TERTIARY ALCOHOL (68)

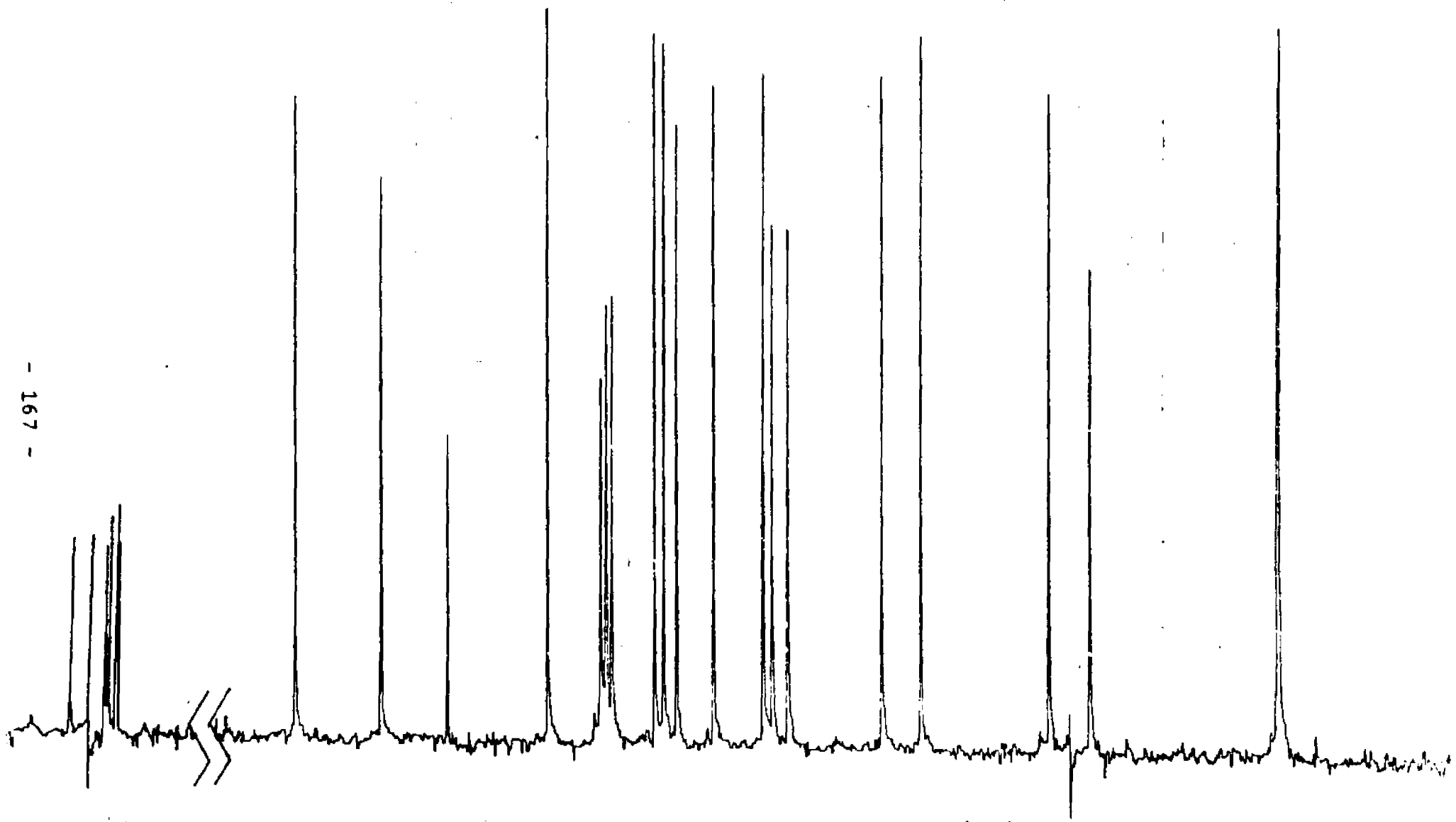


IR OF DIHYDRO ATA TERTIARY ALCOHOL (68)



NMR OF DIHYDRO ATA TERTIARY ALCOHOL (68)

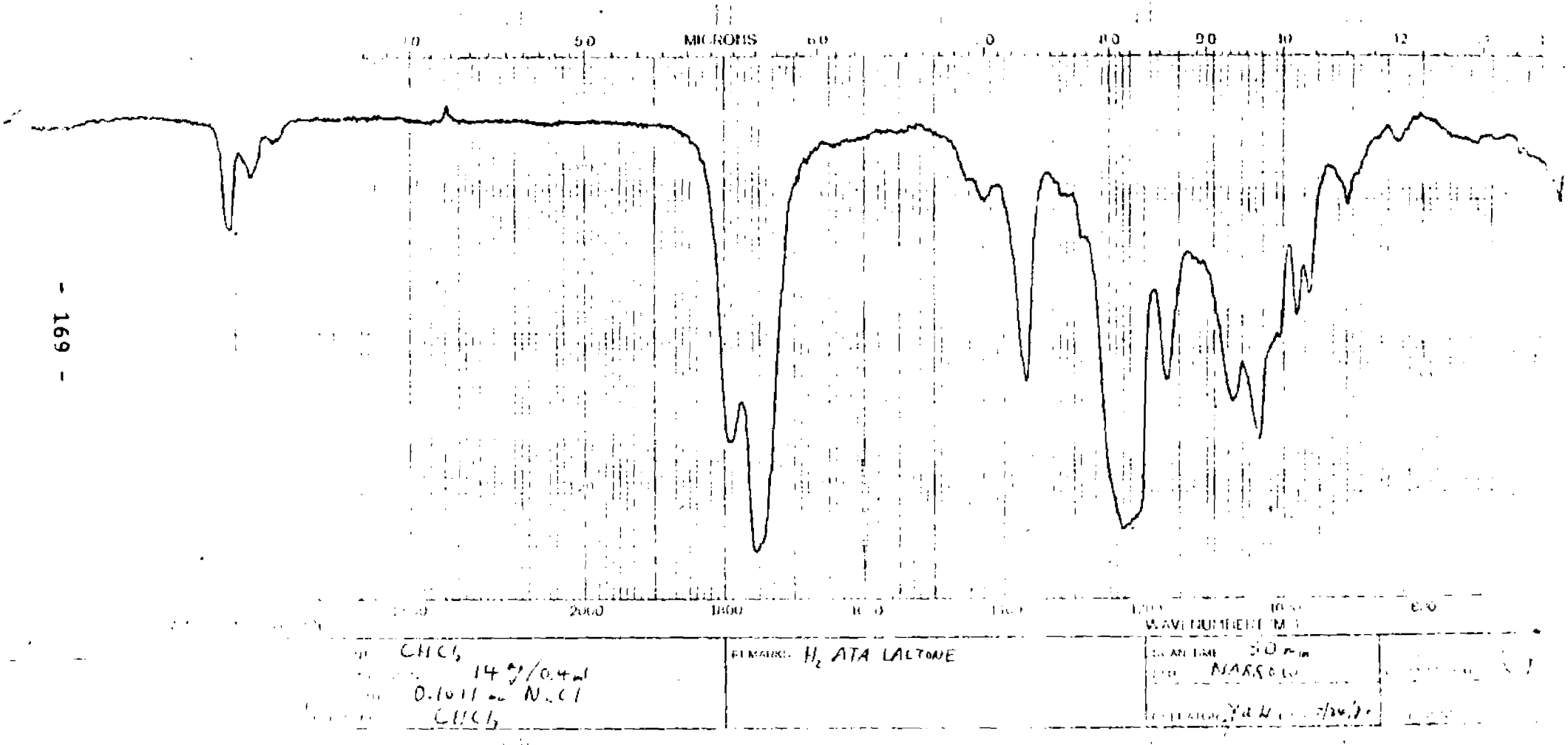
Hz	10 0	6 000	4 000	2 000
Hz	5 0(	3 000	2 000	1 000
Hz	2 5(	1 500	1 000	500



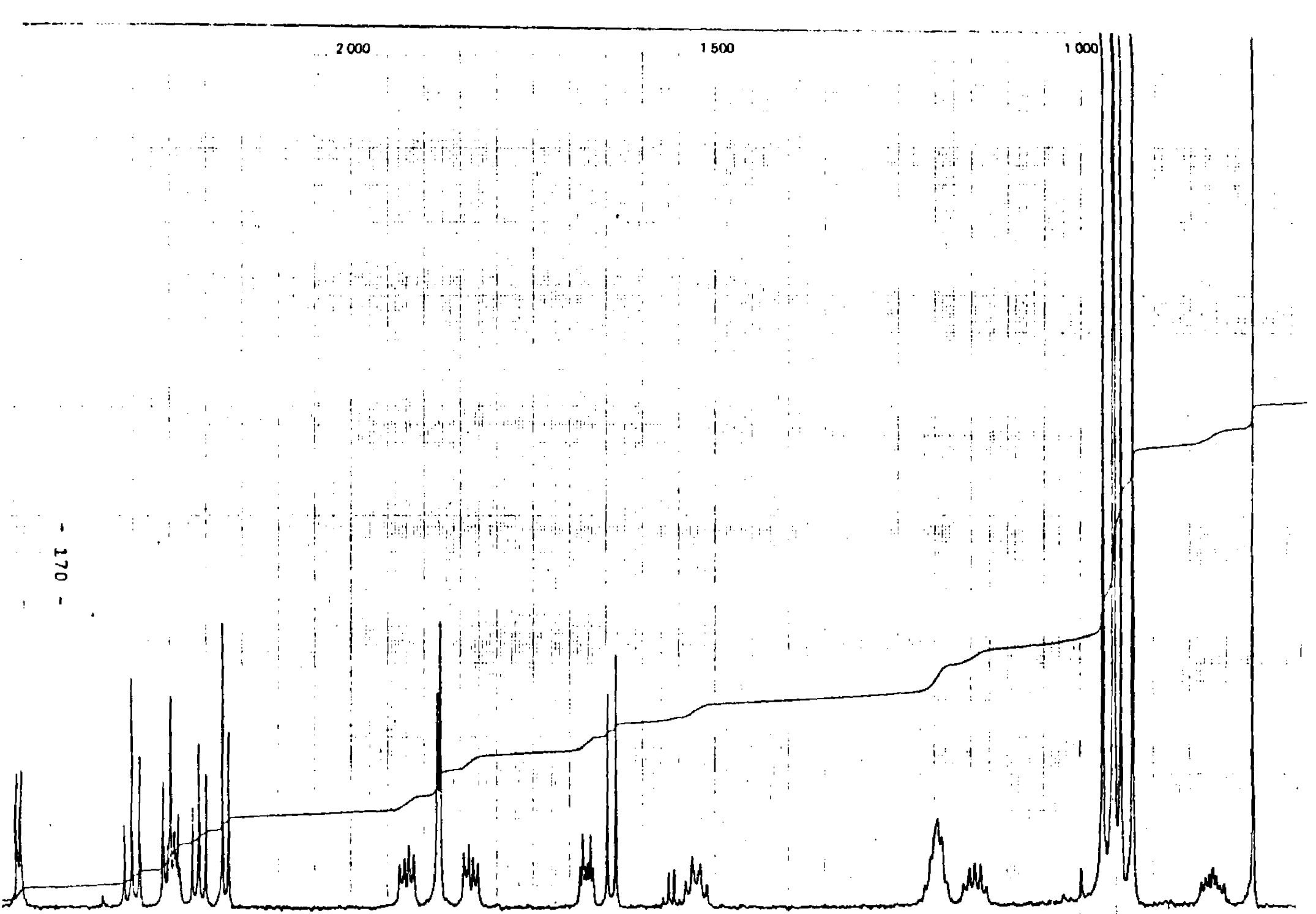
CMR OF DIHYDRO ATA TERTIARY ALCOHOL (68)

Appendix J

IR AND NMR OF DIHYDRO AIA LACTONE (36)



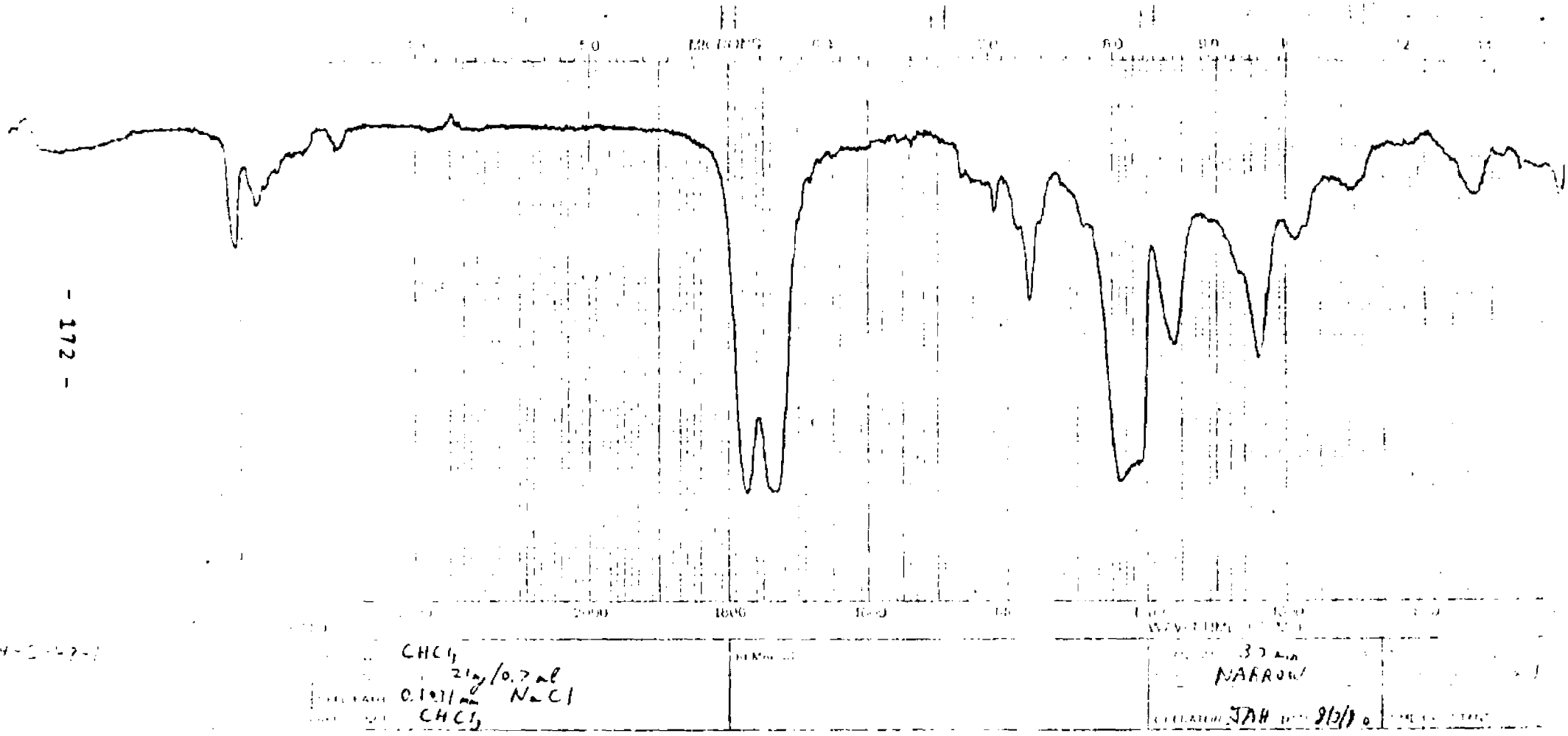
IR OF DIHYDRO ATA LACTONE (36)



NMR OF DIHYDRO ATA LACTONE (36)

Appendix K

IR AND NMR OF ALDEHYDE 71A



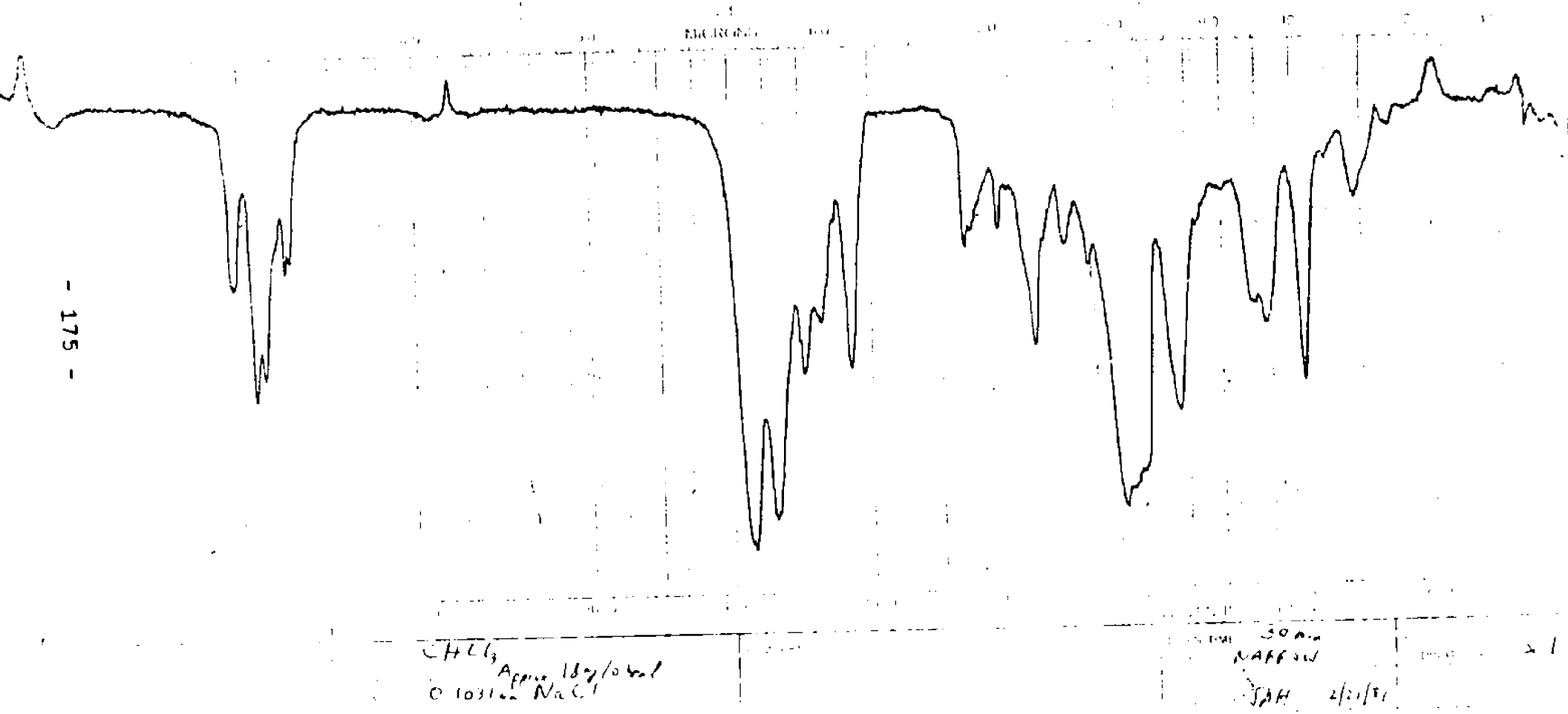
IR OF ALDEHYDE 71A

NMR OF ALDEHYDE 71A

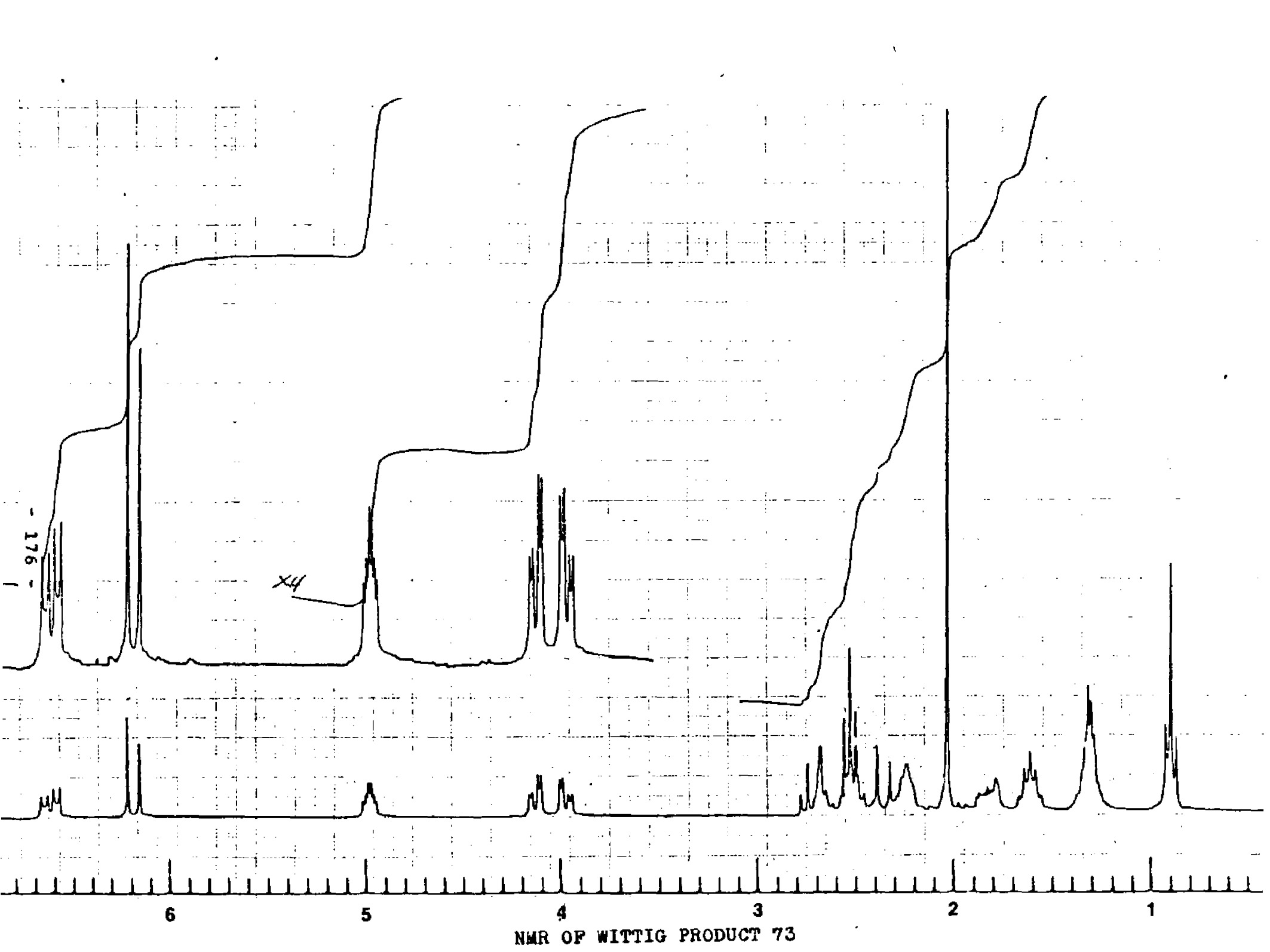
EM-360 60 MHz NMR SPECTROMETER

Appendix L

IR AND NMR OF WITTIG PRODUCT 73



IR OF WITTIG PRODUCT 73



NMR OF WITTIG PRODUCT 73

REFERENCES

1. (a) Bindra, J. S. and Bindra, R.; "Prostaglandin Synthesis", Academic Press: New York, 1977, pp. 7-22. (b) Pike, J. E.; Sci. Am., 225, 84 (1971).

2. Kurzrok, R. and Lieb, C.; Proc. Soc. Exp. Biol. Med., 28, 268 (1930).

3. Goldblatt, M. W.; J. Soc. Chem. Ind. Lond., 52, 1056 (1933).

4. von Euler, U. S.; Naunyn Schniedebergs Arch. Exp. Path. Pharmak., 175, 78 (1934).

5. Cockrill, J. R., Miller, E. G. and Kurzrok, R.; Am. J. Physiol., 112, 557 (1935).

6. von Euler, U. S.; J. Physiol. Lond., 88, 213 (1936).

7. von Euler, U. S.; Scand. Arch. Physiol., 81, 65 (1939).

8. Crabbe, P., Ed.; "Prostaglandin Research", Academic Press: New York, 1977, pp. 1-13.

9. Bergstrom, S. and Sjovall, J.; Acta Chem. Scand., 11, 1086 (1957).

10. Bergstrom, S. and Sjovall, J.; Acta Chem. Scand., 14, 1693 (1960).

11. Bergstrom, S. and Sjovall, J.; Acta Chem. Scand., 14, 1701 (1960).

12. Bergstrom, S.; Science, 157, 382 (1967).

13. Abrahamson, S., Bergstrom, S. and Samuelsson, B.; Proc. Chem. Soc., 1962, 332.

14. Abrahamson, S.; Acta Cryst., 16, 409 (1963).

15. Nelson, N. A.; J. Med. Chem., 17, 911 (1974).

16. Anderson, N.; Anals N. Y. Acad. Sci., 1971, 14.

17. Moncado, S., Gryglewski, R., Bunting, S. and Vane, J. R.; Nature (London), 263, 663 (1976).

18. Willis, A. L., Vane, F. M., Kuhn, D. C., Scott, C. G. and Petrin, M.; Prostaglandins, 8, 453 (1974).

19. Rawls, R. L.; Chem. and Eng. News, 52, 18 (1974).

20. Gregson, P. P., Marwood, J. F. and Quinn, R. J.; Tet. Lett., 1979, 4505.

21. Schneider, W. P., Hamilton, R. D. and Rhuland, L. E.; J. Am. Chem. Soc., 94, 2122 (1972).

22. Pike, J. E.; Sci. Am., 225, 84 (1971).

23. Horton, E. W.; Sci. Prog. Oxf., 63, 335 (1976).

24. Crosley, N. S.; Chem. and Ind., 1976, 337.

25. Corey, E. J., Anderson, N. H., Carlson, R. M., Paust, J., Vedejs, E., Vlattas, I. and Winter, R. E. K.; J. Am. Chem. Soc., 90, 3245 (1968).

26. Corey, E. J., Vlattas, I., Anderson, N. H. and Harding, K.; J. Am. Chem. Soc., 90, 3247 (1968).

27. Corey, E. J., Vlattas, I. and Harding, K.; J. Am. Chem. Soc., 91, 535 (1969).

28. Weinheimer, A. J. and Spraggins, R.; Tet. Lett., 1969, 5185.

29. Corey, E. J., Weinshenker, N. W., Schaaf, T. K. and Huber, W.; J. Am. Chem. Soc., 91, 5675 (1969).

30. Corey, E. J., Koelliker, U. and Neuffer, J.; J. Am. Chem. Soc., 93, 1489 (1971).

31. Corey, E. J., Albonico, S. M., Koelliker, U., Schaaf, T. K. and Kumar, R.; J. Am. Chem. Soc., 93, 1491 (1971).

32. Stork, G. and Raucher, S.; J. Am. Chem. Soc., 98, 1583 (1976).

33. Stork, G. and Takahashi, T.; J. Am. Chem. Soc., 99, 1275 (1977).

34. Stork, G., Takahashi, T., Kawamoto, I. and Suzuki, T.; J. Am. Chem. Soc., 100, 8272 (1978).

35. Ogura, K., Yamashita, M. and Tsuchihushi, G.; Tet. Lett., 1976, 759.

36. Paul, K. G., Johnson, F. and Favara, D.; J. Am. Chem. Soc., 98, 1285 (1976).

37. Mitscher, L. A., Clark, G. W. and Hudson, P. B.; Tet. Lett., 1978, 2553.

38. Stork, G. and Isobe, M.; J. Am. Chem. Soc., 97, 6260 (1975).

39. Bianco, A., et. al.; Gazz. Chim. Ital., 109, 561 (1979).

40. Ruhdorfer, J. and Rimpler, H.; Tet. Lett., 22, 839 (1981).

41. Briggs, L. H., Cain, B. F., LeQuesne, P. W. and Shoolery, J. N.; Tet. Lett., 1963, 69.

42. Geiger, P. L.; Ann., 14, 206 (1835).

43. Halpern, O. and Schmid, H.; Helv. Chim. Acta, 41, 1109 (1958).

44. Bobbitt, J. M. and Segebarth, K. P.; "The Iridoid Glycosides and Similar Substances" in "Cyclopentanoid Terpene Derivatives", Taylor, W. I. and Battersby, A. R., Eds., Marcel Dekker: New York (1969).

45. Yeowell, D. A. and Schmid, H.; Experientia, 20, 250 (1964).

46. Battersby, A. R., Kapil, R. S. and Southgate, R.; J. Chem. Soc. Chem. Commun., 1968, 131.

47. Battersby, A. R., Brown, R. T., Kapil, R. S., Martin, J. A. and Plunkett, A. O.; J. Chem. Soc. Chem. Commun., 1966, 888, 890.

48. Loew, P. and Arigoni, D.; J. Chem. Soc. Chem. Commun., 1968, 137.

49. Rombouts, J. E. and Links, J.; Experientia, 12, 78 (1956).

50. Little, J. E. and Johnstone, D. B.; Arch. Biochem. Biophys., 30, 445 (1951).

51. Herissey, H.; Compt. Rend., 180, 1695 (1925).

52. Juillet, A., Susplugas, Y. and Massa, V.; J. Pharm. Chim., 27, 56 (1938).

53. Briggs, L. H. and Nicholls, G. A.; J. Chem. Soc., 1954, 3940.

54. Grimshaw, J.; Chem. Ind. (London), 1961, 403.

55. Briggs, L. H., Cain, B. F., LeQuesne, P. W. and Shoolery, J. N.; J. Chem. Soc., 1965, 2595.

56. Lentz, P. J. and Rossman, M. G.; J. Chem. Soc. Chem. Commun., 1969, 1269.

57. Inouye, H., Yoshida, T. and Tobita, S.; Tet. Lett., 1968, 2945.

58. Trim, A. R.; Biochem. J., 50, 319 (1952).

59. Trim, A. R. and Hill, R.; Biochem J., 50, 310 (1952).

60. Briggs, L. H. and Cain, B. F.; J. Chem. Soc., 1954, 4182.

61. Wendt, M. W., Haegele, W., Simonitsch, E. and Schmid, H.; Helv. Chim. Acta, 43, 1440 (1960).

62. Berkowitz, W. F., Sasson, I., Sampathkumar, P. S., Hrabie, J., Choudhry, S. and Pierce, D.; Tet. Lett., 1979, 1641.

63. Roy, S. K. and Wheeler, D. M. S.; J. Chem. Soc., 1963, 2155.

64. Finch, N. and Fitt, J. J.; Tet. Lett., 1969, 4639.

65. Shimomura, H., Sugie, A., Katsube, J. and Yamamoto, H.; Tet. Lett., 1976, 4099.

66. Dalton, D. R., Dutta, V. P. and Jones, D. C.; J. Am. Chem. Soc., 90, 5498 (1968).

67. Piancatelli, G., Scettri, A. and D'Auria, M.; Tet. Lett., 1977, 3483.

68. Rogers, H. R., McDermott, J. X. and Whitesides, G. M.; J. Org. Chem., 40, 3577 (1975).

69. Goudard, M., Gaudemer, F. and Gaudemer, A.; Bull. Soc. Chim. Fr., 1973, 577.

70. Deslongchamps, P.; Tetrahedron, 31, 2463 (1975).

71. Tsuji, J., Shimizu, I. and Yamamoto, K.; Tet. Lett., 1976, 2975.

72. Brown, H. C. and Garg, C. P.; J. Org. Chem., 27, 2938 (1962).

73. Hart, H. and Lerner, L. R.; J. Org. Chem., 32, 2669 (1967).

74. Argoumanian, H., Aune, J. P., Guitard, J. and Metzger, J.; J. Org. Chem., 39, 3445 (1974).

75. Moon, S. and Waxman, B. H.; J. Org. Chem., 34, 1157 (1969).

76. Dudley, C. and Read, G.; Tet. Lett., 1972, 5273.

77. Berkowitz, L. M. and Rylander, P. N.; J. Am. Chem. Soc., 80, 6682 (1958).

78. Parikh, V. M. and Jones, J. K. N.; Can. J. Chem., 43, 3452 (1965).

79. Brown, H. C. and Geoghegan, P. J.; J. Org. Chem., 35, 1844 (1970).

80. Krow, G. R. and Fan, D. M.; J. Org. Chem., 39, 2674 (1974).

81. Marsh, F. D. and Hermes, M. E.; J. Am. Chem. Soc., 86, 4506 (1964).

82. Corey, E. J. and Richman, J. E.; J. Am. Chem. Soc., 92, 5276 (1970).

83. Hassner, A., Kropp, J. E. and Kent, G. J.; J. Org. Chem., 34, 2628 (1969).

84. Rodeheaver, G. T. and Hunt, D. F.; J. Chem. Soc. Chem. Commun., 1971, 818.

85. D'Ascoli, P., D'Aurio, M. D., Nucciarelli, L., Piancatelli, G. and Scettri, A.; Tet. Lett., 21, 4521 (1980).

86. Cardillo, G. and Shimizu, M.; J. Org. Chem., 42, 4268 (1977).

87. Sharpless, K. B., Teranishi, A. Y. and Backvall, J. E.; J. Am. Chem. Soc., 99, 3120 (1977).

88. Hanson, J. R.; Synthesis, 1974, 1.

89. Lane, C. F.; Synthesis, 1975, 135.

90. Kuivila, H. G.; Synthesis, 1970, 499.

91. Franzen, V. and Otto, S.; Ber., 94, 1360 (1961).

92. Cardillo, G., Orena, M. and Scandri, S.; J. Chem. Soc. Chem. Commun., 1976, 190.

93. Kornblum, N., Jones, W. J. and Anderson, G. J.; J. Am. Chem. Soc., 81, 4113 (1959).

94. Naruto, M., Ohno, K., Naruse, N. and Takeuchi, H.; Tet. Lett., 1979, 251.

95. Naruto, M., Ohno, K., Naruse, N. and Takeuchi, H.; Chem. Lett., 1978, 1423.

96. Naruto, M., Ohno, K., Naruse, N. and Takeuchi, H.; Chem. Lett., 1979, 1015.

97. Naruto, M., Ohno, K. and Naruse, N.; Chem. Lett., 1978, 1419.

98. Ohno, K. and Naruto, M.; Chem. Lett., 1980, 175.

99. Weinges, K., von der Eltz, H. and Tran-Viet, D.; Angew. Chem. Int. Ed. Eng., 19, 628 (1980).

100. Berkowitz, W. F., Sasson, I., Sampathkumar, P. S., Hrabie, J., Choudhry, S. and Pierce, D.; J. Org. Chem., submitted for publication.

101. Duff, R. B., Bacon, J. S. D., Mundie, C. M., Farmer, V. C., Russell, J. D. and Forrester, A. R.; Biochem. J., 96, 1 (1965).

102. Lemieux, R. V.; Methods in Carbohydrate Chemistry, 1, 45 (1962).

103. Dalton, D. R. and Dutta, V. P.; J. Chem. Soc., B, 1975, 85.

104. Epstein, W. W. and Sweat, F. W.; Chem. Rev., 67, 247 (1967).

105. Torssell, K.; Tet. Lett., 1966, 4445.

106. Torssell, K.; Acta Chem. Scand., 21, 1, (1967).

107. Epstein, W. W. and Sweat, F. W.; Chem. Rev., 67, 247 (1967).

108. Albright, J. D. and Goldman, L.; J. Am. Chem. Soc., 87, 4214 (1965).

109. Snatzke, G.; Ber., 94, 729 (1961).

110. Fatiadi, A. J.; Synthesis, 65, 133 (1976).

111. Wendt, W., Haegele, W. H., Simonitsch, E. and Schmid, H.; Helv. Chim. Acta, 43, 1440 (1960).

112. Corey, E. J. and Suggs, J. W.; Tet. Lett., 1975, 2647.

113. Corey, E. J. and Schmidt, G.; Tet. Lett., 1979, 399.

114. Field, B. D. and Grundy, J.; J. Chem. Soc., 1955, 1110.

115. Ratcliffe, R. and Rodehorst, R.; J. Org. Chem., 35, 4000 (1970).

116. Collins, J. C., Hess, W. W. and Frank, F. J.; Tet. Lett., 1968, 3363.

117. Poos, G. I., Arth, G. E., Beyler, R. E. and Sarett, L. H.; J. Am. Chem. Soc., 75, 422 (1953).

118. Freifelder, M.; "Practical Catalytic Hydrogenation", Wiley Interscience: New York, 1971.

119. Bachi, M. D., Epstein, J. W., Mingly, Y. H. and Loewenthal, H. J. E.; J. Org. Chem., 34, 126 (1969).

120. Hanson, J. R.; Synthesis, 1974, 1.

121. Brown, H. C. and Shubba Rao, B. C.; J. Am. Chem. Soc., 78, 2582 (1956).

122. Woodward, R. B., Gostelli, J., Ernest, I., Friary, R. J., Nestler, G., Raman, H., Sitrin, R., Suter, Ch. and Whitesell, J. K.; J. Am. Chem. Soc., 95, 6853 (1973).

123. Firouzabad, H. and Ghaderi, E.; Tet. Lett., 1978, 839.

124. Corey, E. J. and Kim, C. U.; J. Am. Chem. Soc., 94, 7586 (1972).

125. Lee, D. G. in "Oxidation Techniques and Applications in Organic Chemistry", Vol. 1, Augustine, R. L., Ed., Marcel Dekker: New York, 1971, pp. 77-79.

126. Corey, E. J., Petrzilka, M. and Ueda, Y.; Helv. Chim. Acta, 60, 2294 (1977).

127. Cardillo, G., Orena, M. and Sandri, S.; Tet. Lett., 1976, 3965.

128. Fieser, L. F. and Fieser, M.; "Reagents for Organic Synthesis", Vol. 1, John Wiley and Sons, Inc.: New York, 1967, p. 967.

129. Cardillo, G., Orena, M. and Sandri, S.; J. Chem. Soc. Chem. Commun., 1976, 190.

130. Augustine, R. L.; "Catalytic Hydrogenation", Marcel Dekker: New York, 1965.

131. Bindra, J. S. and Bindra, R.; "Prostaglandin Synthesis", Academic Press: New York, 1977, p. 201.

132. Berkowitz, W. F. and Choudhry, S. C.; Tet. Lett., 22, 1075 (1981).

133. Stevens, C. L., Cahoon, J. M., Potts, T. R. and Pillai, P. M.; J. Org. Chem., 37, 3130 (1972).