AN INVESTIGATION OF THE TOXIC PLANT-RAYLESS GOLDENROD (Aplopappus heterophyllus)

By

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AN INVESTIGATION OF THE TOXIC PLANT-RAYLESS GOLDENROD
(APLOPAPPUS HETEROPHYLLUS)

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CHAPTER I

HISTORICAL

During the last century, a disease known as "milksickness" in humans and "trembles" in animals was reported (1) in various parts of the central United States. This disease was invariably fatal and occasionally reached epidemic proportions, sometimes wiping out most of the inhabitants of a settlement. It was early suspected (2,3) that animals which contracted "trembles" by foraging on a poisonous plant passed the disease on to humans through the milk of an affected cow. White snakeroot was shown to be the plant responsible for the disease in the central states.

Couch, in a series of investigations (1,4) in the late 1920's, undertook the extraction of white snakeroot plant to find the responsible toxin. His animal feeding experiments with the plant demonstrated that fresh, green plants were necessary and that the various species of white snakeroot did not all possess the same degree of toxicity.

After collecting and grinding the plant, Couch extracted it with 95% ethanol. The extract was evaporated to leave a dark green residue which was shown to be toxic to sheep and cattle. The water-soluble portion of the extract was not toxic. The toxin was, however, extractable into 50% hot ethanol and following that into 30% hot ethanol.

No further purification could be accomplished by solvent extraction; therefore the toxin was saponified. The ether extract of the saponified material contained the toxic material with a considerable

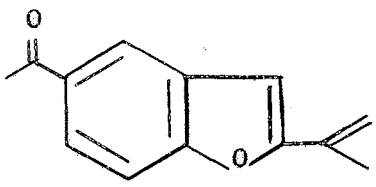
increase in purity, most of the weight having been removed as base-soluble fatty acids. The ether-soluble material yielded a non-toxic solid, m.p. 148-9°, as well as a yellow, viscous, toxic oil. All attempts to further purify the oil were unsuccessful and the material was considered to be pure. Elemental analysis and molecular weight determinations of the toxic oil indicated the molecular formula to be $C_{16}H_{22}O_3$, $[\alpha]_D$ -21°. Bromine titration showed the presence of two double bonds; no crystalline derivatives were obtained upon reaction with hydroxylamine or phenylhydrazine, and a negative Schiff's test indicated the probable absence of an aldehyde group. The toxin was stable to boiling water, but decomposed upon distillation, even at pressures less than one millimeter. From these data Couch asserted the toxin to be an aromatic alcohol with a side chain containing two double bonds. He named the material "tremetol".

In the early part of this century a disease clinically identical with "milksickness" appeared in the southwestern part of the United States However, this section of the country is devoid of white snakeroot. It was established that rayless goldenrod (Aplopappus heterophyllus) was responsible for "milksickness" in the southwestern part of the United States (5). Couch (4,6) showed by the extraction process used on white snakeroot that "tremetol" was also present in rayless goldenrod. Goldenrod, unlike white snakeroot, retains its toxicity when it has been dried. For unknown reasons Couch apparently stopped his work on "tremetol" and did not publish any further work after 1933.

The next reported attempt to isolate and identify the toxic material from rayless goldenrod was by Dermer (7,8,9,10) and his students. They found that "tremetol" was not a single compound, but a complex mixture. They attempted to further fractionate the oil using a Hickman molecular still; the oil was separated into various toxic fractions, but no pure

toxic compound was obtained by using this technique. During the next twenty years work on these poisonous plants apparently ceased, and fortunately, "milksickness" became less of a problem with the passage of time for two reasons. First, farmers were educated to recognize and eradicate the poisonous plant and secondly, milk was consumed on a local level less frequently as milk went into large dairy pools where any toxin-containing milk became diluted.

Recently, Bonner and co-workers (11,12,13) reported the results of their reinvestigation of white snakeroot. By the use of chromatography, these workers found that white snakeroot "tremetol" could be separated into a number of components. Three closely related ketones, tremetone (II), dehydrotremetone (I) and hydroxytremetone (III) were identified by chemical degradation (13,14) and the structures of tremetone and dihydrotremetone were confirmed by synthesis (15,16,17). These proved to be toxic to goldfish and tremetone, the most abundant constituent, was suspected as being the active toxin in white snakeroot. However, the toxicity of tremetone to higher animals has not been verified.



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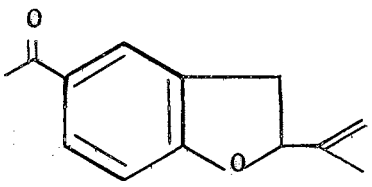

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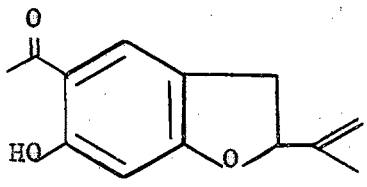


III

The problem of the chemical nature of the toxin of the rayless goldenrod plant, responsible for "trembles" in cattle and "milksickness" in humans, lay dormant from the late 1930's until 1961, when the present investigation was initiated.

CHAPTER II

DISCUSSION OF RESULTS

The yellow oil, "tremetol", isolated by Couch (1,4) was not a pure compound as Couch believed, but a complex mixture. The fact that the active material had shown only slight volatility, either with steam or under reduced pressure, indicated that it was either a high-boiling oil or a solid which for some reason had not crystallized.

DeGraw (11,12) modified the original procedure of Couch because of certain mechanical difficulties encountered by Couch, and achieved some success; however, his procedure still involved a long solvent-extraction step. It was decided in this investigation that a further modification might provide the toxic material without the lengthy solvent-extraction procedures used by Couch and DeGraw. However, enough material was separated by Couch's procedure for use in comparison with the material obtained by other procedures.

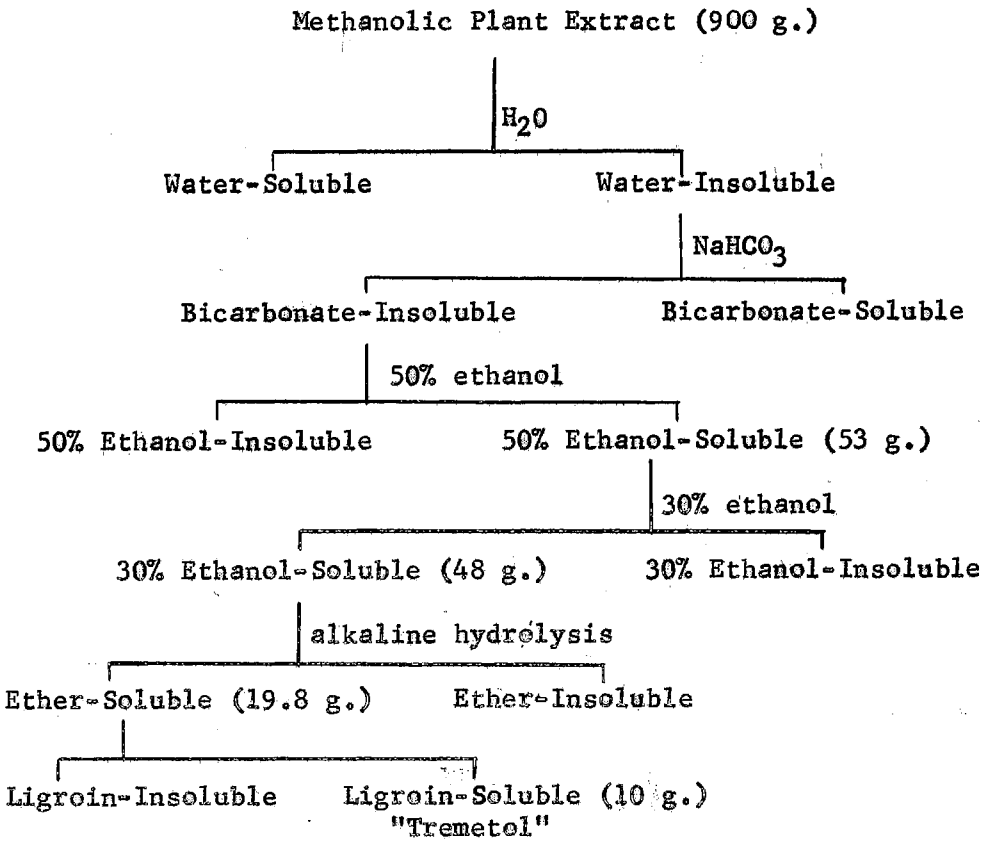
ISOLATION

Separation of plant extract by Couch's procedure (chart A)

Rayless goldenrod was collected during the month of August while in full bloom just north of the Pecos River in New Mexico. The entire plant was cut at ground level, dried and then ground in a hammer mill. The ground meal was extracted with methanol in a large Soxhlet extractor; the methanol solution was cooled overnight and then filtered to remove the precipitated waxes. The methanol filtrate was evaporated at reduced pressure, and the residue extracted with boiling water, the insoluble material being allowed to settle and then the aqueous solution decanted.

CHART A

SEPARATION BY COUCH PROCEDURE



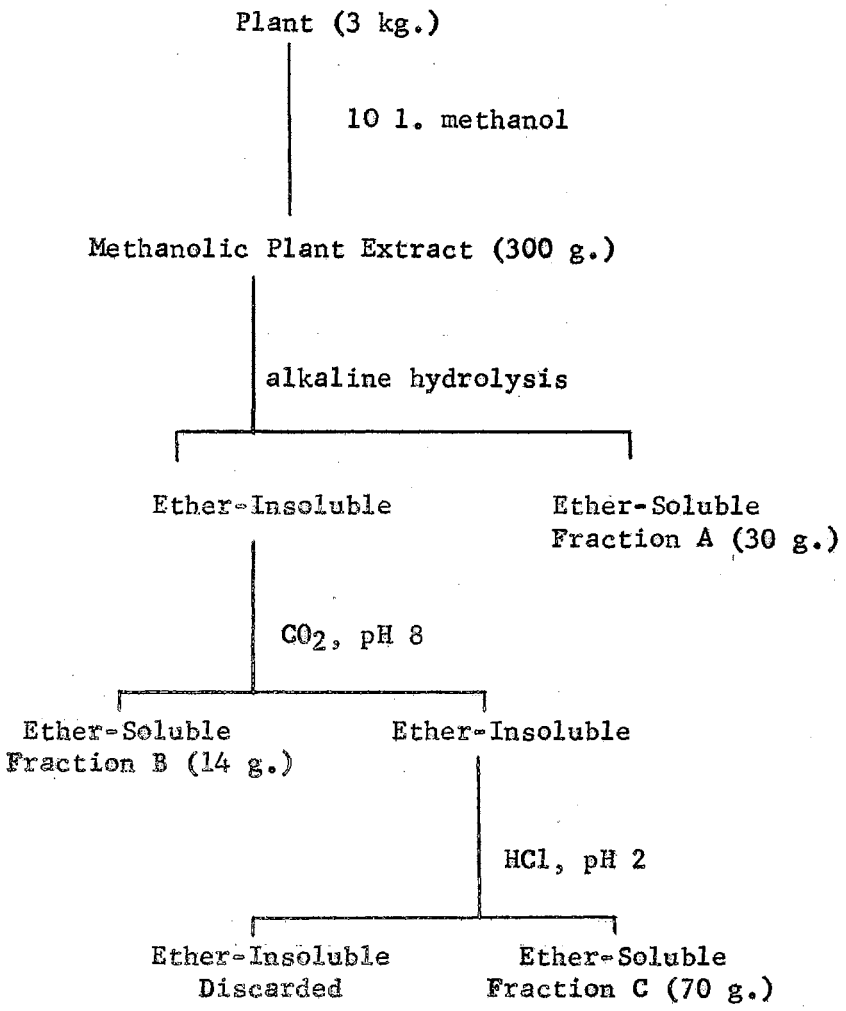
Although it took some time for the insoluble material to settle, it was not necessary to centrifuge the aqueous solution as DeGraw (11) had indicated. The insoluble residue was then extracted with 5% sodium bicarbonate to remove acids; the residue was then extracted with hot 50% ethanol. After evaporation of the ethanol, the residue was separated from the aqueous supernatant and subjected to a 30% ethanol extraction in the same manner. The material thus obtained was hydrolyzed with 5% methanolic potassium hydroxide and the resulting solution extracted with ether. The ether solution was diluted with ligroin, which precipitated a small amount of colored material. The ether-ligroin filtrate was evaporated to give a viscous yellow oil that was similar to the "tremetol" described by Couch (1,4). This procedure differs from that of Couch only in that Couch used 95% ethanol to extract the plant instead of methanol.

Modified extraction procedure (chart B)

The ground plant meal was extracted with methanol in the same manner as described above. The methanolic plant extract was hydrolyzed with 5% methanolic potassium hydroxide and the resulting solution extracted with ether. The ether was evaporated to leave a dark red oil, fraction A, which was assumed to contain Couch's "tremetol" as well as a number of other compounds. Gas chromatography indicated that fraction A contained all of the compounds present in Couch's "tremetol", obtained as described above, and in addition a number of additional compounds were present. The remaining aqueous solution was saturated with carbon dioxide and the solution extracted with ether. The ether was evaporated to leave a dark oil, fraction B. The remaining aqueous solution was further acidified with hydrochloric acid and then extracted with ether. The latter ether extract was evaporated to leave

CHART B

SEPARATION BY
MODIFIED PROCEDURE



a black viscous oil, fraction C.

Although "tremetol" had been shown to be toxic to sheep and cattle (4,6), the amount of material necessary for testing these large animals precluded their use for testing the many fractions expected from chromatography. For this reason the use of bacteriological tests to monitor the separation seemed most appropriate. These tests were performed by Dr. Grula and his associates of the Department of Microbiology. Seventeen bacterial cultures were selected at random from a stock collection for initial screening with the crude toxin; these included both Gram-positive and Gram-negative bacteria of several genera having different nutritional requirements and metabolic activities. All cells were grown on nutrient agar slants for 20 hours at 30°. Organisms were agitated and washed from the slants with sterile physiological saline and one drop of this solution was added to sterile tubes of nutrient broth. After mixing, two drops of crude toxin were added and the cultures shaken again and incubated at 30° on a reciprocating shaker. Control cultures containing no toxin were inoculated simultaneously. Presence or absence of growth was observed visually after 19 hours of incubation. The results are given in Table I.

When incubation was continued to 66 hours the three organisms Staphylococcus albus, Streptococcus lactis, and Sarcina lutea were still unable to grow. Since the crude toxin showed only slight solubility in the nutrient broth, organisms shown to be susceptible in the liquid assay were further screened using the following procedure. Organisms were grown and washed from slants as described above and a few drops of the given culture then were added aseptically to tubes of nutrient agar cooled to 50°. After mixing, the melted and seeded agar was aseptically poured into a Petri dish and the agar allowed to solidify at room temperature. The crude

toxin dissolved in ethyl ether was then deposited in a small area (about 7 mm. diameter) on strips of Whatman No. 1 chromatography paper and the solvent evaporated under a stream of warm air. The strips of paper then were overlaid on the seeded agar and allowed to remain during subsequent incubation at 30°. Toxicity was determined by removing the paper and observing the growth inhibition in the area where the crude toxin had been in contact with the organism. Responses were recorded after 19 and 43 hours. In this procedure, three of the organisms exhibited excellent growth inhibition (B. cereus, Staph. albus and Coryneb. hoagi). B. subtilis, Strep. lactis and Chromo. violaceum were less susceptible and S. lutea was resistant. Although growth of three organisms were inhibited by the crude toxin both in liquid and solid agar assay, B. cereus was chosen as assay organism. This choice was based on hardiness and ease of cultivation. Further studies have revealed that the crude toxin is bacteriostatic for B. cereus.

SEPARATION OF FRACTION A

Procedure I (chart C)

Fraction A was partitioned on Florisil with methanol as the stationary phase and ligroin as the mobile phase. Two of the early fractions obtained by elution with the mobile phase gave identical partially crystalline solids, which showed strong conjugated carbonyl and aromatic bands in their infrared spectra similar to those reported (11) for dehydrotremetone (I). This solid (m.p. 81-82°) was designated ketone I, and was found to inhibit bacterial growth. Continued elution with the ligroin phase afforded only 75% of the material placed on the column. No other crystalline or

TABLE I

ANTIBACTERIAL ACTIVITY OF CRUDE TOXIN

Test organism	Inhibition (-) or growth (+) after 19-hr. incubation
Streptococcus lactis	-
Escherichia coli B	+
Bacillus cereus	-
Aerobacter cloaceae	+
Bacillus subtilis	-
Chromobacterium violaceum	-
Erwinia caratovora	+
Pseudomonas aeruginosa	+
Salmonella gallinarum	+
Alcaligenes faecalis	+
Corynebacterium hoagi	-
Aerobacter aerogenes	+
Micrococcus lysodeikticus	+
Proteus vulgaris	+
Sarcina lutea	-
Serratia marcescens	+
Staphylococcus albus	-

bacterial growth-inhibiting material was obtained. The remaining 25% of the material on the column was eluted with the stationary phase; this material, fraction G, inhibited bacterial growth.

The material eluted by the stationary phase, fraction G, was chromotographed (absorption) on Florisil. A small amount of material was eluted with benzene, but did not inhibit bacterial growth. About one-third of fraction G was eluted with ether. The ether-eluted fraction caused strong bacterial growth inhibition and its infrared spectrum showed strong hydroxyl, carbonyl and aromatic bands. The infrared spectrum was similar in the fingerprint region to that of ketone I, and the fraction was designated ketone III (toxol)*. The material that remained on the column was eluted with chloroform and then methanol, however, neither of these eluates inhibited bacterial growth.

When fraction G was chromatographed on alumina, a small amount of bacterially inactive material was eluted with benzene. About 70% of the material was eluted with benzene-chloroform (1:1) and this material inhibited bacterial growth. The remaining material was eluted with chloroform and methanol; neither of these fractions inhibited bacterial growth. The benzene-chloroform eluent was partitioned on Florisil; toxol was the only material obtained that inhibited bacterial growth.

Although this separation was similar to that used by DeGraw (11) and gave two toxic compounds, it was by no means shorter or more convenient than the procedure used by Couch. The following method was found to give a clean separation of the methanolic extract into a number of well-defined fractions.

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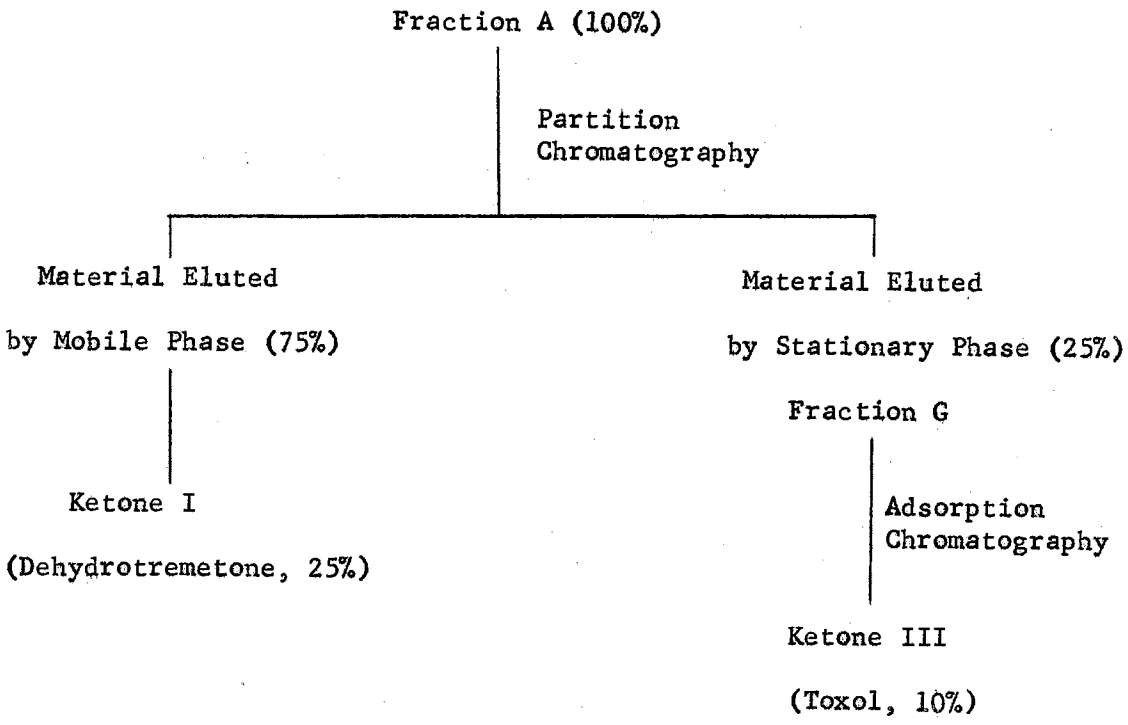
* Named in this laboratory

CHART C

SEPARATION OF FRACTION A

PROCEDURE I



Procedure II (chart D)

In Couch's procedure, the steam-volatile material was removed during the water extraction of the plant extract; however, fraction A in our above separation still contained the steam-volatile materials. Therefore, removal of any steam-volatile material was expected to give, besides the steam-volatile fraction, a fraction that contained an increased concentration of toxic material.

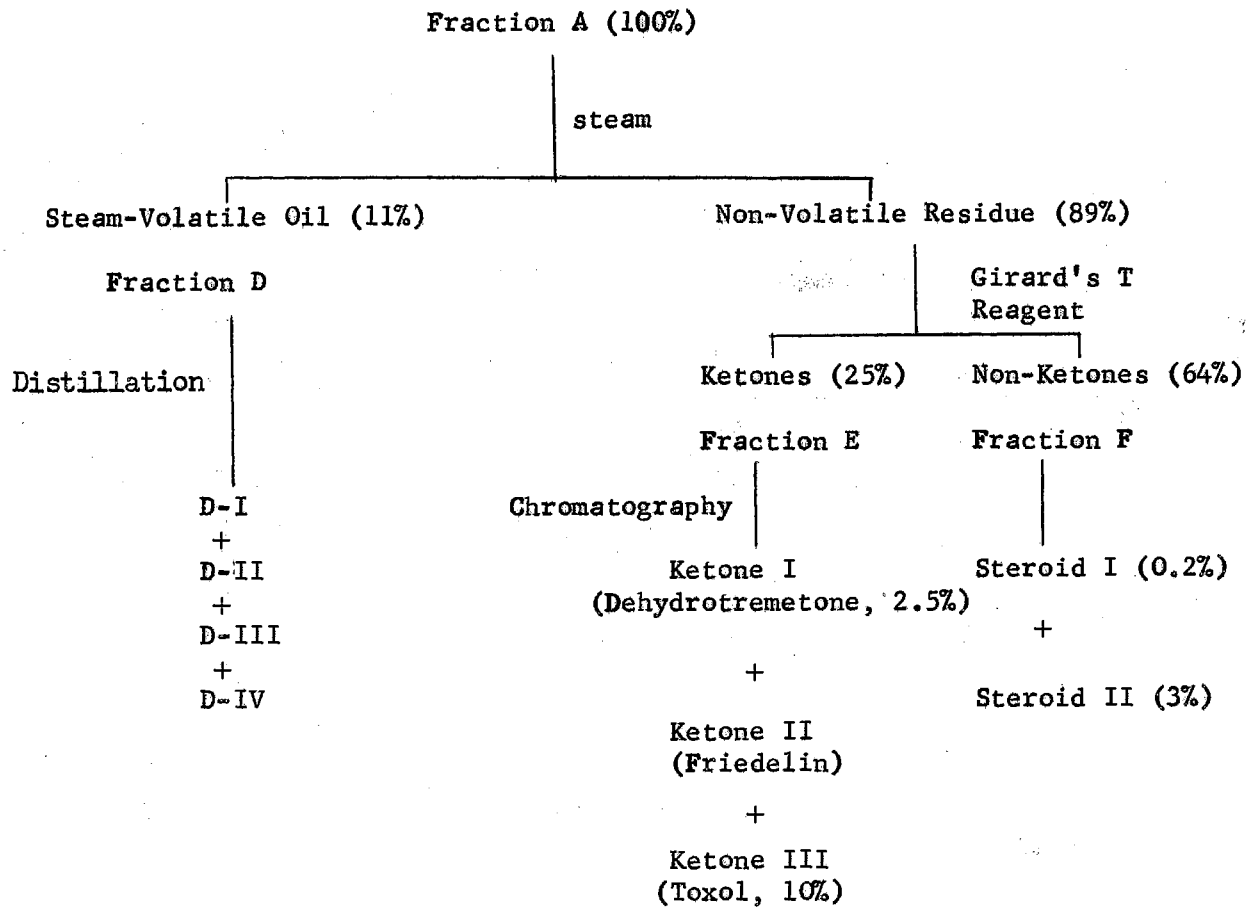
Fraction A was suspended in water and the mixture exhaustively steam distilled. The distillate was then continuously extracted with ether and the ether was carefully removed to avoid loss of the volatile oil. The residue was designated fraction **D**. The non-volatile residue was recovered from the aqueous solution by ether extraction.

The non-volatile material was separated into a ketonic and a non-ketonic fraction by treating the material with Girard's T reagent in methanol. The non-ketonic material, which did not react with this reagent, was removed by diluting the methanol solution with an aqueous solution of sodium carbonate and extracting the resulting solution with ether. Evaporation of the ether gave the non-ketonic fraction **F**. The ketonic material was then obtained by acidification of the aqueous carbonate solution with hydrochloric acid (pH 2) and extracting the solution with ether. Evaporation of the ether gave the ketonic fraction E. If any aldehydes were present, they would not have been recovered owing to the difficulty in hydrolyzing the product obtained from the reaction of an aldehyde with Girard's T reagent.

CHART D

SEPARATION OF FRACTION A

PROCEDURE II



SEPARATION OF INDIVIDUAL FRACTIONS

Steam-volatile fraction D

The steam-volatile material, fraction D, was fractionally distilled using a rough, rapid separation to avoid prolonged heating of the high-boiling components. Four fractions were collected: fraction D-I, b.p. 40-55° (0.05 mm.); fraction D-II, b.p. 55-65° (0.05 mm.); fraction D-III, b.p. 65-75° (0.05 mm.); and fraction D-IV, b.p. 75-95° (0.05 mm.). The small amount of residue remaining was discarded.

Fraction D-I was then refractionated through a 25 x 0.5 cm. Todd column. Four fractions were collected from the column: fraction D-Ia, b.p. 65-70° (1 mm.); fraction D-Ib, b.p. 70-73° (1 mm.); fraction D-Ic, b.p. 73-75° (1 mm.); and fraction D-Id, b.p. 75-80° (1 mm.). A fifth fraction was obtained by removal of the Todd column and distilling the residue at low pressure to give fraction D-Ie, b.p. 65-70° (0.08 mm.). The various fractions were analyzed by gas chromatography using a 10% silicone rubber column; the first two fractions were similar in composition, differing only in the proportions of the various components. The third fraction exhibited three peaks, all different from the peaks exhibited by the first two fractions, and the fourth showed two peaks which were different from any of the peaks exhibited by the previous fractions. The last fraction was a complex mixture.

Ketone fraction E

Ketonic fraction E was chromatographed on alumina (activity II). The ligroin eluent yielded a crystalline solid having an infrared spectrum indicating that it was dehydrotremetone (ketone I). The material eluted with ligroin-benzene (1:1) inhibited bacterial growth while the remaining fractions (benzene, chloroform, and methanol eluent) contained no bacterial-inhibiting material.

The ligroin-benzene eluted fraction was rechromatographed on alumina (activity I). When the column was treated with a large quantity of benzene a small quantity of crystalline material, ketone II, was obtained. Further elution with benzene-chloroform (1:1) afforded the greater portion of material placed on the column. This latter material inhibited bacterial growth and its infrared spectrum indicated that it was toxol. The remainder of the material on the column was eluted with chloroform and did not inhibit bacterial growth.

Non-ketone fraction F

Non-ketonic fraction F was chromatographed on alumina (activity II). Upon elution with ligroin a crystalline solid (steroid I) was obtained from the eluent, the infrared spectrum of which showed a strong hydroxyl band but no other distinctive features. The material was only slightly soluble in most common organic solvents. This substance could also be obtained during the separation of the ketonic fraction E and the non-ketonic fraction F with Girard's T reagent, if the aqueous carbonate solution was extracted with an equal volume of ether. Under these conditions steroid I separated at the interface. If the aqueous carbonate solution was repeatedly extracted with ether all of the solid dissolved in the ether and was then

obtained by chromatography as indicated above. Another steroid (steroid II; $C_{29}H_{48}O$; m.p. 152-156°; $[\alpha]_D$-9°, $CHCl_3$) has been isolated from rayless goldenrod and appears to be isomeric with "α" spinasterol.*

PURIFICATION AND PROPERTIES OF INDIVIDUAL COMPONENTS

Fraction B

When a methanolic solution of fraction B was treated with a saturated solution of lead acetate in methanol, an immediate precipitate was obtained. The precipitate was collected by filtration and then suspended in methanol. The solution was saturated with hydrogen sulfide and the resulting lead sulfide was removed. The filtrate was evaporated to leave a solid residue which, after recrystallization from methanol, gave m.p. 68-70°. The infrared spectrum was identical with that of stearic acid; the mixed melting point with an authentic sample of stearic acid was not depressed.

The filtrate from the lead acetate treatment was diluted with water and extracted with ether and the ether evaporated. The residue so obtained was esterified with an ethereal solution of diazomethane and the resulting solution was analyzed by gas chromatography using a 5% SE-30 column. Only two compounds were detected, the retention times of which corresponded to those of methyl myristate (81%) and methyl laurate (19%).

Fraction C

A solution of fraction C in methanol was treated with a solution of boron trifluoride in methanol and after heating for a short time the solution was diluted with water and made basic with potassium hydroxide. The

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* L. H. Zalkow and G. Cabat, unpublished results

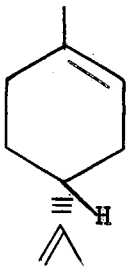
basic solution was extracted with ether and on evaporation the first ester fraction was obtained as a black viscous residue. The basic solution was acidified with hydrochloric acid and extracted with ether; evaporation of the ether gave an acid fraction as a dark viscous residue. A portion of this residue was dissolved in ether and treated with an ethereal solution of diazomethane to give the second ester fraction.

When the esters thus obtained were analyzed by gas chromatography, using a 5% SE-30 and a Craig polyester column, the first ester fraction was shown to contain: methyl hexanoate (32.4%), methyl octanoate (28.0%), methyl laurate (15.1%), methyl myristate (7.5%), methyl palmitate (7.7%), and methyl linoleate (8.3%). The second ester fraction was shown to contain: methyl hexanoate (11.9%), methyl octanoate (7.7%), methyl laurate (13.3%), methyl myristate (11.1%) and methyl palmitate (56.0%).

d-Limonene

When the methanol recovered from the extraction of the ground rayless goldenrod was diluted with an equal volume of water, the solution became milky. This milky solution was extracted with ligroin until the aqueous solution became clear. After removal of the ligroin, gas chromatography of the residue using a 5% SE-30 column showed one major component (60%). The residue was then distilled at 45-60° (4.5 mm.) and gas chromatography indicated that the distillate consisted of about 80% one component. The distillate was redistilled at 52-55° (4.5 mm.) and gas chromatography indicated that the distillate was about 95% pure. The retention time was the same as the retention time of limonene and the infrared and nuclear magnetic resonance spectra were identical to those of an authentic sample

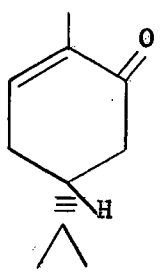
of limonene. The optical rotation, $[\alpha]_D$+145.1°, was close to that reported (18)($[\alpha]_D$+126.8°) for d-limonene (IV).



IV

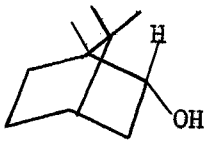
Steam-volatile fractions D-Ia and D-Ib

Because fractions D-Ia and D-Ib showed similar gas chromatograms they were combined and chromatographed on alumina. Five fractions were eluted in the following solvents: ligroin, ligroin-benzene (1:1), benzene, benzene-chloroform (1:1) and chloroform. The ligroin fraction showed four peaks by gas chromatography using a 10% silicone rubber column; one of these peaks had the same retention time as limonene. The benzene and the ligroin-benzene fractions were complex mixtures. About 90% of the benzene-chloroform fraction was a single compound, which had retention time the same as l-carvone (V). The infrared spectrum was nearly identical with the infrared spectrum of an authentic sample and the optical rotation ($[\alpha]_D$-58°), was similar to that reported (19)($[\alpha]_D$-62°) for l-carvone.



V

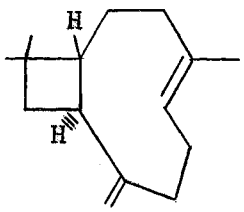
The chloroform fraction gave a semi-crystalline solid which could not be purified by vacuum sublimation. It was easily recrystallized from ligroin at -70° to give m.p. 207-208°; reported for borneol (20) m.p. 204-205°. A mixed melting point with an authentic sample of borneol was not depressed. The infrared spectrum was identical to the infrared spectrum of an authentic sample and the optical rotation ($[\alpha]_D$-25.4°) was similar to that reported for borneol (VI)(21)($[\alpha]_D$-33.1°).



VI

Fraction D-Id

Fraction D-Id showed two peaks by gas chromatography, using a 10% silicone rubber column, in the approximate ratio of 4:1. The larger peak had the same retention time as caryophyllene. Caryophyllene, VII, has been previously isolated in this laboratory from rayless goldenrod*; therefore, no attempt was made to obtain the pure hydrocarbon. The other fractions obtained from the steam-volatile oil were not investigated.

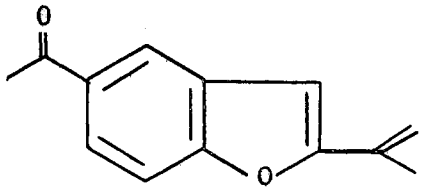


VII

* L. H. Zalkow and G. Cabat, unpublished results

Ketone I

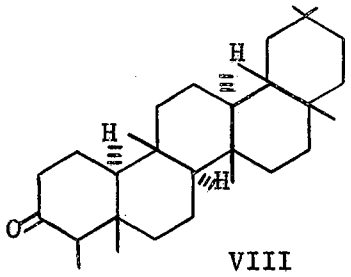
Ketone I was purified by repeated recrystallization from ligroin; m.p. 85-86°; reported for dehydrotremetone, I, (11,12) m.p. 87.5-88.5°. The infrared spectrum was the same as that reported (11).



I

Ketone II

Ketone II was a crystalline solid as obtained from the chromatography column. A sharp melting point, 245-246°, indicated that the material was probably pure and no attempt was made to purify it further. The infrared spectrum showed a carbonyl band at 5.82 μ as the only distinctive feature in the spectrum. The material gave a negative tetranitromethane test, and had an optical rotation of $[\alpha]_D$-21°. The nuclear magnetic resonance spectrum indicated seven distinct methyl groups and an eighth probably present, and showed no vinylic protons. The integration of the nuclear magnetic resonance spectrum indicated that the material contained a total of fifty protons. This data suggested that the material was a saturated pentacyclic triterpene (22) such as friedelin (VIII) and the melting point and optical rotation were the same as those reported (23) for friedelin. The oxime was prepared and after recrystallization from chloroform-ethanol gave m.p.



VIII

277-278° (d), $[\alpha]_D$+57.7°; reported (23) m.p. 280-281° (d), $[\alpha]_D$+56°. The enol benzoate was prepared and recrystallized from chloroform-ethyl acetate; m.p. 257-258°, $[\alpha]_D$+57.1°, reported (23) m.p. 255-256°, $[\alpha]_D$+57.1°.

Steroid I

Steroid I isolated either from the separation of the ketonic, non-ketonic fractions or from the chromatography of the non-ketonic fraction was only slightly soluble in common organic solvents. However, it was easily soluble in pyridine, from which it was recrystallized; m.p. 286-288° with previous melting at 265°, $[\alpha]_D$-16.5°. The infrared spectrum (plate I) exhibited a strong hydroxyl band at 2.9 μ as the only distinctive feature. Steroid I gave a negative tetranitromethane test. The structural elucidation is presented below.

Toxol

Toxol was distilled at 110° (0.05 mm.) and the distillate solidified after standing at room temperature for several days. Repeated recrystallization from ligroin at 0° afforded the analytical sample; m.p. 52-53°, $[\alpha]_D$-25.1°. The analysis and boiling point (110°, 0.05 mm.) indicated the formula to be $C_{13}H_{14}O_3$. The ultraviolet spectrum showed peaks at 223 mμ (log ε 4.02) and 273 mμ (log ε 4.13) and the infrared spectrum (plate II) contained a strong hydroxyl band at 3.0μ, a strong carbonyl band at 5.98 μ and a number of aromatic bands. The nuclear magnetic resonance spectrum (plate III) indicated the presence of a methyl ketone by the signal at δ2.36, and a methyl group on a double bond by a signal at δ1.66; in addition, the nuclear magnetic resonance spectrum showed aromatic signals at δ6.72 and δ7.7 and a doublet at δ4.89, the latter signal

integrated for five protons. The structure elucidation is presented below.

STRUCTURAL STUDIES

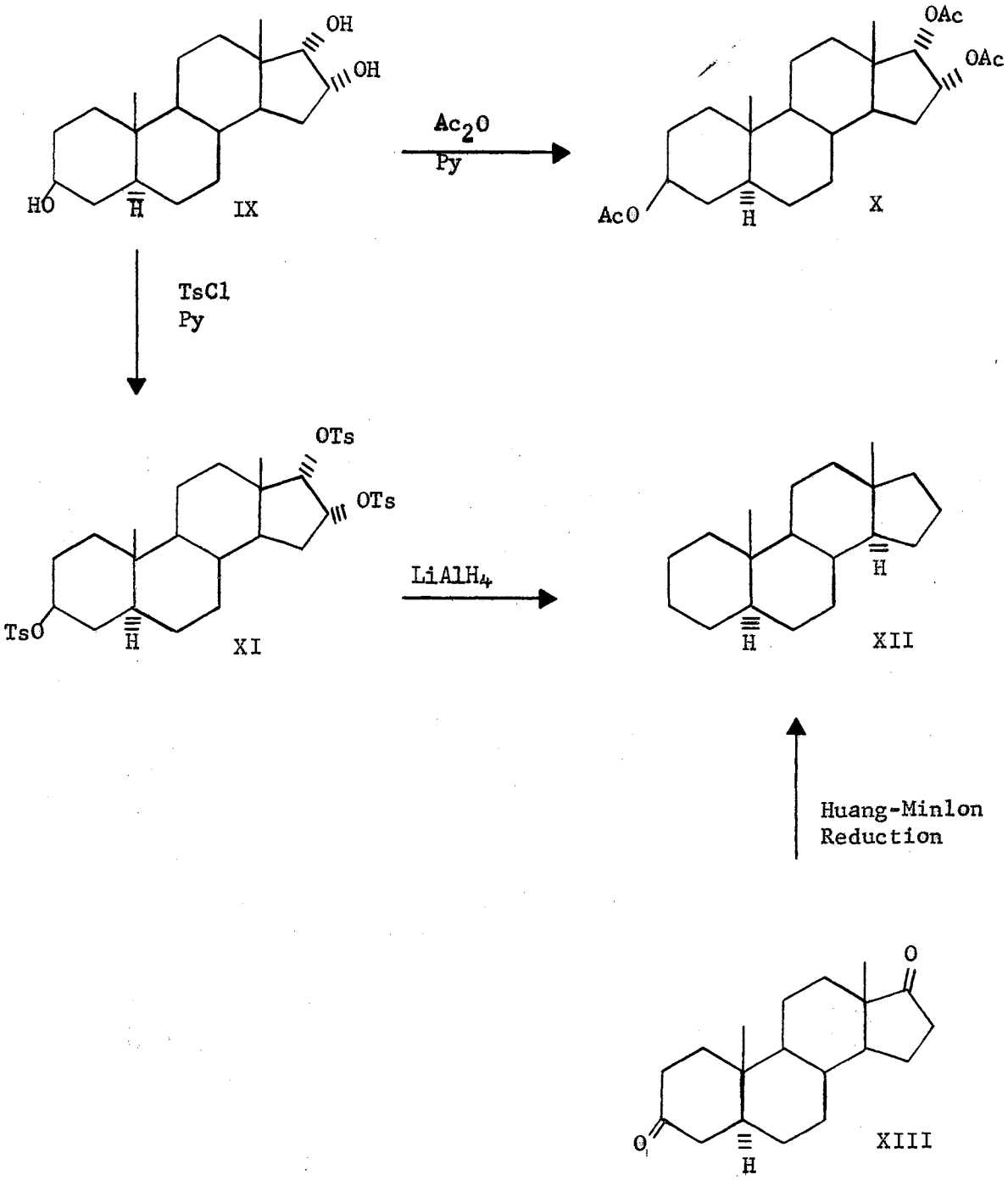
Steroid I

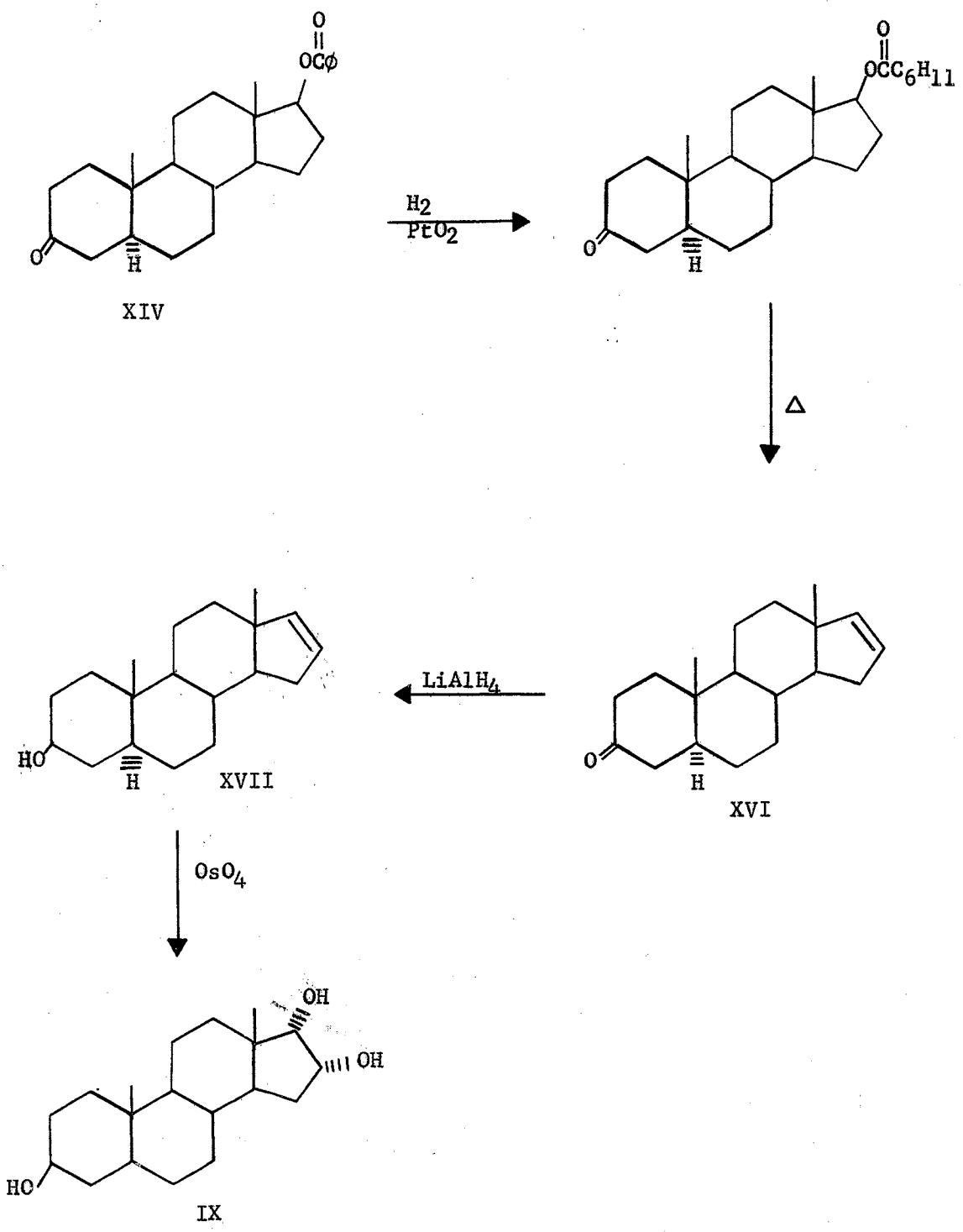
Steroid I readily gave an acetate (m.p. 168-169°, $[\alpha]_D$+8.1°), for which the nuclear magnetic resonance spectrum (plate IV) indicated a ratio of three acetate groups to two bridgehead methyl groups. Steroid I was shown to be an androstane derivative by its conversion to 5α-androstane (XII) by preparation of the tritosylate (XI) followed by hydrogenolysis with lithium aluminum hydride. An authentic sample of 5α-androstane was prepared by the Huang-Minlon (24) reduction of 5α-androstane-3,17-dione (XIII). The two samples of 5α-androstane gave identical melting points alone and on admixture, and identical gas chromatograms (using a 5% SE-30 column) were obtained for each individually and on admixture. Ruzicka, Prelog and Wieland (25) had previously reported the preparation of 5α-androstane-3β, 16α, 17α-triol (IX) (m.p. 265-266°, $[\alpha]_D$-19.4°) and their triol gave a triacetate (X) (m.p. 165°, $[\alpha]_D$+10.4°). Owing to the discrepancy observed in the melting points of the reported 5α-androstane-3β,16α,17α-triol and that isolated from rayless goldenrod, Ruzicka's synthesis (25,26,27) was repeated (28). 17β-Hydroxy-5α-androstan-3-one 17-benzoate (XIV) was readily hydrogenated to 17β-hydroxy-5α-androstan-3-one 17-hexahydrobenzoate (XV), which on pyrolysis gave Δ^{16}-5α-androstan-3-one (XVI). Reduction of the latter compound with lithium aluminum hydride gave Δ^{16}-5α-androstan-3β-ol (XVII), which on treatment with osmium tetroxide gave 5α-androstane-3β,16α,17α-triol (IX) identical in infrared spectrum and melting point with that isolated from rayless goldenrod. It was found that 5α-androstane-3β,16α,17α-triol exhibited two melting points, one at 265-268° and the other

at 288°, if the material is allowed to resolidify after first melting.

Huffman and Lott (29,30) suggested that the product obtained by hydroxylation of Δ^{16}-5α-androstan-3β-ol (XVII) with osmium tetroxide was 5α-androstane-3β, 16β, 17β-triol, since it was not identical with the product they obtained by reduction of Butenandt's triol (31), which they believed to be Δ^{5}-androstane-3β,16β,17β-triol. However, their work was based on the assumption that the biologically active estradiol and the corresponding androstane-3,17-diol had the 17α-configuration. It was later shown by Heusser (32) and Goldberg (33) that the estradiol and the corresponding androstane possess the 17β-configuration. The configurations presented by Huffman and Lott are therefore in error and, as stated by Gallagher (34), need to be inverted at C_{16} and C_{17} to be correct. The correct configuration in both the estrane and the androstane series have been given by Fieser and Fieser (35), and Shoppee (36) and Gallagher (34).

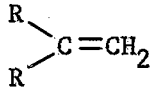
Although 5α-androstane-3β,16α, 17α-triol may have been isolated previously by Butler (10) from rayless goldenrod since he reported the presence of an unidentified sterol of m.p. 258° in the residue left after distillation of the crude toxin, this is believed to be the first report of its isolation from any natural source. Its presence in the plant kingdom is particularly interesting because of its close relationship to the urinary steroids such as 5α-androstane-3α,16α,17β-triol, 5β-androstane-3α,16α,17β-triol and Δ^{5}-androstane-3β,16α,17β-triol, which have been isolated from urine and from testicular extracts.


OH
OH
HO
H
IX
Ac2O
Py
OAc
OAc
AcO
H
X
TsCl
Py
OTs
OTs
TsO
H
XI
LiAlH4
H
H
XII
Huang-Minlon
Reduction
O
O
H
XIII


O
OCØ
O
H
XIV
H2
PtO2
O
OCC6H11
O
H
Δ
LiAlH4
HO
H
XVII
O
H
XVI
OsO4
OH
OH
HO
IX

Toxol

The similarity of the infrared spectrum of toxol with the reported (11) spectra of tremetone (II), dehydrotremetone (I) and hydroxytremetone (III) indicated that toxol was probably a benzofuran derivative. The molecular formula suggested by analysis, $C_{13}H_{14}O_3$, indicated that toxol was isomeric with hydroxytremetone; their non-identity was, however, apparent from the fact that hydroxytremetone exhibited no hydroxyl band in its infrared spectrum while toxol exhibited a strong hydroxyl band. The nuclear magnetic resonance spectrum of toxol (plate III) indicated that it was a methyl ketone and possessed a methyl group on a double bond; both of these features are also characteristic of the three ketones mentioned above. A doublet centered at δ4.9 in the n.m.r. spectrum showed five protons by integration; however, the nature of the five protons were not immediately apparent. If toxol was a benzofuran with a methyl ketone, then only three carbon atoms, one oxygen function and the remaining hydrogen atoms need be located. Elemental analysis indicated seven modes of unsaturation; six of these would be accounted for by a dihydrobenzofuran ring system and the methyl ketone leaving one, presumably a double bond, to be accounted for. With only two methyl groups indicated by the n.m.r. spectrum, the remaining three carbons must carry the other double bond and one methyl group; if the double bond were in the furan ring then the molecule would contain either three methyl groups or at least one methylene group, which could be seen in the n.m.r. spectrum. The n.m.r. spectrum showed a doublet at δ4.9 which was thought to correspond to two olefinic protons of the



type but the infrared spectrum did not show the characteristic terminal methylene band at 11.2 μ (37). Structure XVIII has been shown to represent toxol (38) on the basis of the following evidence.

The acetate (XIX) was easily obtained by treatment with acetic anhydride and pyridine as a colorless oil, $[\alpha]_D$-76°. The infrared spectrum showed no hydroxyl band, but contained an ester carbonyl band at 5.75 μ as well as an unsaturated ketone band at 5.99 μ. Elemental analysis indicated the formula to be $C_{15}H_{16}O_4$, as expected for a monoacetate of $C_{13}H_{14}O_3$. The n.m.r. spectrum (plate V) of the acetate clearly showed that toxol contained a secondary hydroxyl group by the characteristic paramagnetic shift (~100 cps.) of one proton in going from the alcohol to its acetate (39). The proton showing this shift is attached to the carbon atom containing the hydroxyl group and appeared as a doublet (J= 3 cps.) centered at δ5.9 in the acetate indicating that it was flanked by only one adjacent proton. The doublet centered at δ4.9 in toxol and in toxol acetate integrated for only three protons in the acetate, while in toxol it integrated for five. Both toxol and its acetate gave positive iodoform tests, confirming the presence of a methyl ketone indicated by the n.m.r. spectra. Toxol also formed a 2,4-dinitrophenylhydrazone ($C_{19}H_{18}N_4O_6$, m.p. 176-177°).

The positions of substitution on the aromatic ring were shown by oxidation of toxol with either 5% potassium permanganate in acetone or chromium trioxide in acetic acid to 5-acetylsalicylic acid (XXIV), m.p. 208-210°. The infrared spectrum and the melting point were identical with those of an authentic sample. The 5-acetylsalicylic acid obtained from toxol was treated with sodium hypoidite and the resulting 4-hydroxy-

isophthalic acid (XXV) was identified by its melting point, 304-307°; reported (40) m.p. 305-306°. These acids accounted for nine of the carbon atoms and two of the oxygen atoms and indicated that toxol was at least similar to the ketones isolated recently by DeGraw (10).

Hydrogenation of toxol with 5% palladium-on-charcoal resulted in the uptake of four moles of hydrogen per mole of ketone to give a mixture of products which were not identified. When 2% palladium-on-calcium carbonate was used as the hydrogenation catalysis 1.6 moles of hydrogen per mole of ketone were absorbed. Elemental analysis of the product, isolated by distillation, indicated that hydrogenation had produced a mixture of the expected dihydro product and a product that resulted from hydrogenolytic removal of the hydroxyl group. The ease of hydrogenolysis suggested that the hydroxyl group was either benzylic or allylic.

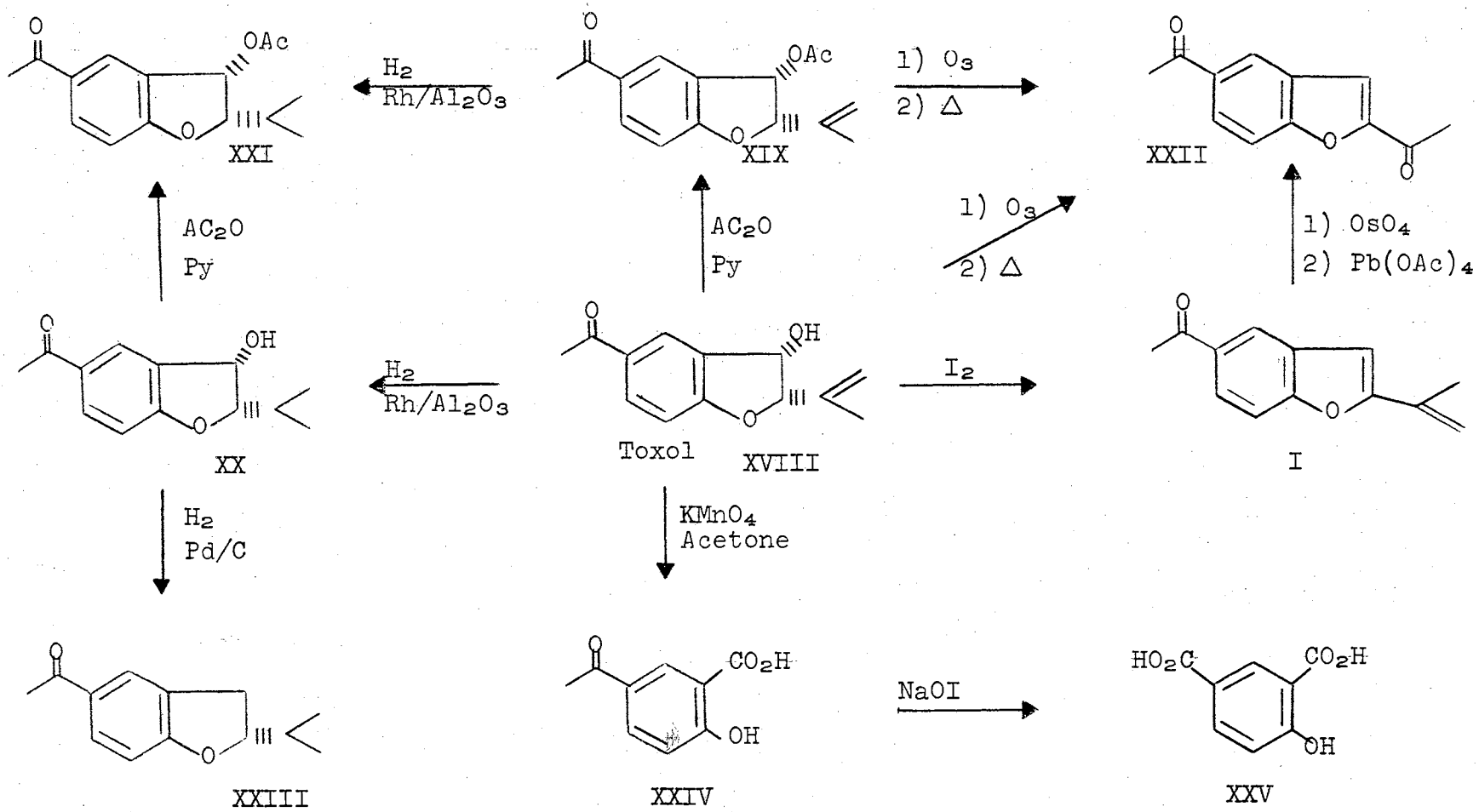
Hydrogenation of toxol with 5% rhodium-on-alumina resulted in the uptake of one mole of hydrogen per mole of ketone, to give the desired dihydro product (XX), $[\alpha]_D$-107°. The n.m.r. spectrum (plate VI) showed a doublet (J=6 cps.) centered at δ4.2 which integrated for one proton. This signal apparently shifted from δ4.9 in the unsaturated ketone indicating that the proton was allylic in toxol; the position also indicated that it was attached to a carbon atom that also contained an oxygen atom. The doublet still centered at δ4.9 integrated for two protons compared to five in toxol.

Dehydration of toxol or pyrolysis of its acetate was expected to give dehydrotremetone. Surprisingly, a variety of reagents, hydrochloric acid in dioxane, sulfuric acid in acetic acid and pyrolysis of the acetate did not give dehydrotremetone but rather products were obtained which had identical infrared spectra, but different melting points. However, iodine

at 120° gave a small amount (9%) of dehydrotremetone as well as a product that had the same infrared spectrum as the products obtained by the other procedures. When dehydrotremetone itself was treated with iodine a product was obtained that again had an infrared spectrum which was the same as the infrared spectra of the products obtained from toxol. Photolysis of dehydrotremetone in ligroin gave a crystalline solid, m.p. 175-177°, the infrared spectrum of which was the same as that of the products obtained as mentioned above from toxol. The molecular weight (1394 and 1388) of the latter product, determined by the Rast method, indicated that the material was a polymer with an average of seven units (M.W. 1400) in the chain. It thus appeared that the material obtained from toxol by the various methods was a polymer of differing molecular weights and hence different melting points but identical infrared spectra.

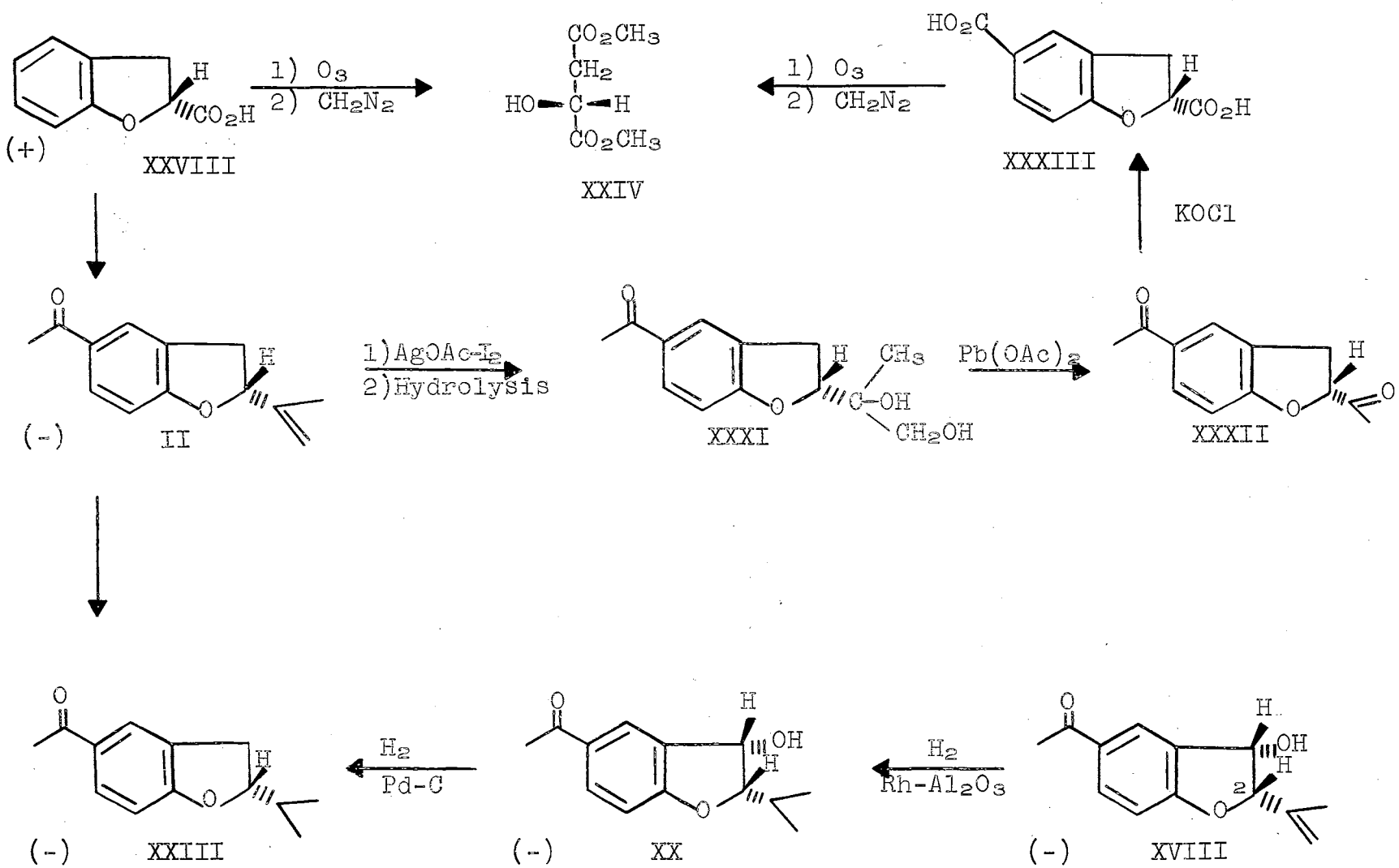
When toxol or its acetate was ozonized at -70°, a 35% yield of formaldehyde, isolated as its dimedone derivative, was obtained. The large fragment from the ozonolysis was distilled; the resulting distillate, m.p. 140-141° after recrystallization from methanol, showed two nearly identical methyl ketone signals and four aromatic protons in its n.m.r. spectrum. This spectrum indicated that the ozonolysis product had lost water (acetic acid in the case of the acetate) during distillation. The product was identified as 2,5-diacetylbenzofuran (XXII). Dehydrotremetone was treated with osmium tetroxide and then lead tetraacetate to give 2,5-diacetylbenzofuran, which was identical with that obtained from toxol. These data indicated that formula XVIII represented the structure of toxol.

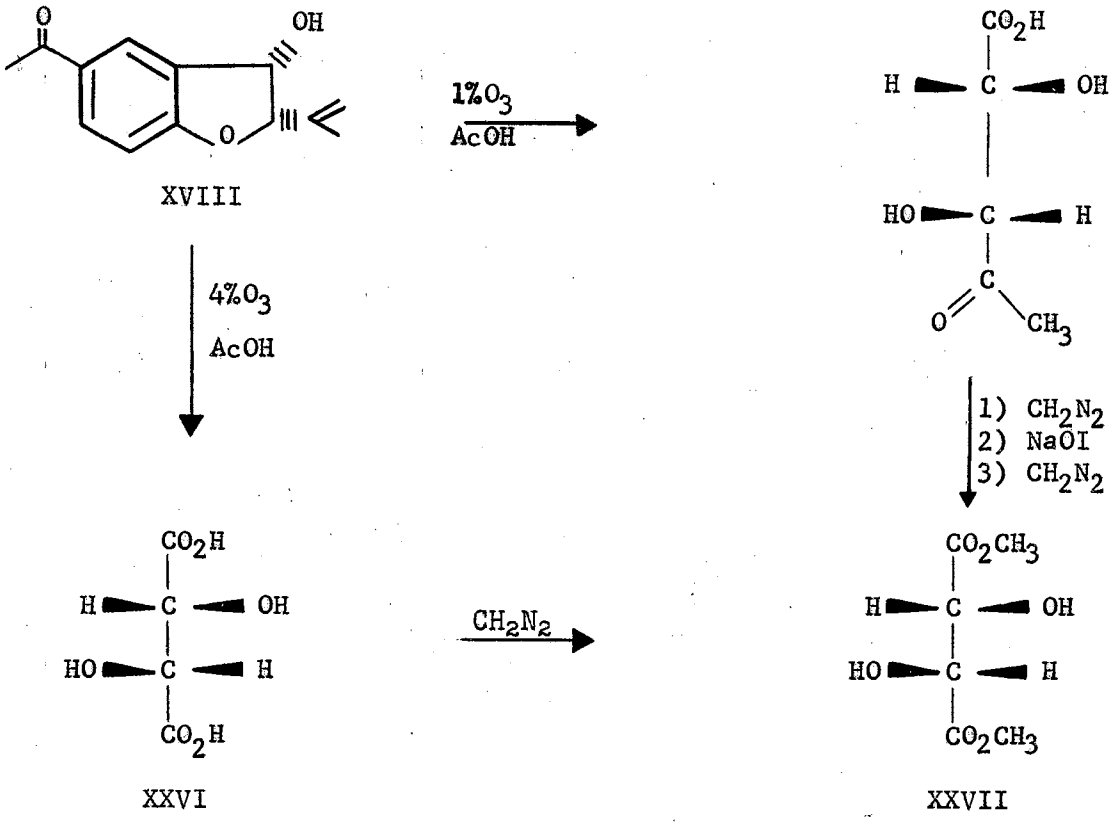
After the structure of toxol had been determined, the next consideration was its absolute configuration. Büchi (41) and Nakazaki (42)


OAc
XXI
H_2
Rh/Al_2O_3
XIX
1) O_3
2) Δ
XXII
AC_2O
Py
1) O_3
2) Δ
1) OsO_4
2) $Pb(OAc)_4$
OH
XX
Toxol
XVIII
I_2
I
H_2
Pd/C
$KMnO_4$
Acetone
CO_2H
OH
XXIII
XXIV
NaOI
HO_2C
XXV

independently determined the absolute configuration of dihydrotubaic acid (XXXVII), a degradation product of rotenone (see page 35). Ozonolysis of this compound in acetic acid destroyed the benzene ring, but did not destroy the asymmetric center. The similarity of toxol and tubaic acid indicated that the absolute configuration of toxol could be determined in the same manner. When toxol was ozonized with a stream of about 1% ozone in oxygen, a ketoacid was obtained. This acid was converted to L(+) dimethyl tartarate (XXVII) by treatment with sodium hypoiodite followed by diazomethane (43). When toxol was ozonized with a stream of about 4% ozone in oxygen, L(+) tartaric acid (XXVI) was obtained. Treatment of this acid (XXVI) with diazomethane gave L(+)dimethyl tartrate which showed a plain negative optical rotatory dispersion curve (44) as previously described (45).

The ease with which toxol underwent hydrogenolysis indicated that it could be converted into dihydrotremetone, which had been obtained by hydrogenation of tremetone. Toxol was hydrogenated using rhodium-on-alumina catalyst to give dihydrotoxol which was then hydrogenolyzed with palladium-on-charcoal to give (-)dihydrotremetone (XXIII), the infrared spectrum of which was identical with the spectrum of material obtained by hydrogenation of dehydrotremetone.


(+)
XXVIII
1) O3
2) CH2N2
XXIV
1) O3
2) CH2N2
XXXIII
KOCl
(-)
II
1)AgOAc-I2
2)Hydrolysis
XXXI
Pb(OAc)2
XXXII
(-)
XXIII
H2
Pd-C
(-)
XX
H2
Rh-Al2O3
(-)
XVIII



XXVI XXVII

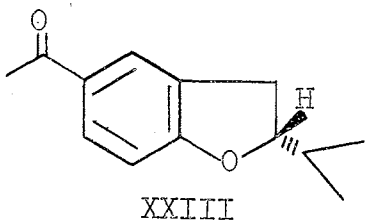
The absolute configuration of (+)dihydrocoumarilic acid was suggested by Fredga (46), on the basis of plant physiological tests, and by Sjöberg (47), on the basis of optical rotatory dispersion, as being the mirror image of that shown in XXVIII. The absolute configuration of toxol was determined chemically and toxol was subsequently converted into (-) dihydrotremetone which itself had been obtained by hydrogenation of (-) tremetone. Tremetone was also synthesized from (+)dihydrocoumarlic acid (17). This sequence of reactions thus indicated that the absolute configuration of (+)-dihydrocoumarilic acid is as shown in XXVIII. During the synthesis of tremetone it was reported (17) that (+)-dihydrocoumarilic acid gave (-) ethyl dihydrocoumarilate. This change in sign of rotation in going from the acid to its ester seemed unusual and for this reason this observation was rechecked. Dihydrocoumarilic acid was resolved by the method of Fredga (46) and the (+) acid esterified as previously

described (17) to give as previously reported (-) ethyl dihydrocoumarilate.

As a check on the absolute configuration of tremetone, Bonner (48) treated tremetone with silver acetate and iodine followed by hydrolysis to obtain the (-) glycol (XXXI). This glycol was treated with lead tetraacetate to give (-) 2,5-diacetyldihydrobenzofuran (XXXII) which with potassium hypochlorite gave (-) dihydrobenzofuran-2,5-dicarboxylic acid (XXXIII). When this diacid was ozonized in acetic acid and then treated with diazomethane, D (+) dimethyl malate (XXXIV) was obtained, thus confirming the configuration as had been shown by the conversion of toxol to (-) dihydrotremetone.

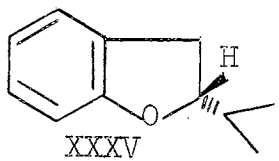
Sjöberg (48) converted (+)-dihydrocoumarilic acid to its methyl ester with diazomethane and ozonolysis of this ester followed by treatment with diazomethane gave D (+) dimethyl malate (XXXIV). This indicated that the conclusion arrived at earlier (47) by interpretation of rotatory dispersion curves was incorrect.

Hill (48) started with rotenone (XXXIX) of known absolute configuration at C(5'), and converted it into tubaic acid (XXXVIII) which was hydrogenated to give dihydrotubaic acid (XXXVII), then decarboxylated and finally treated with p-toluenesulfonyl chloride to give (-) dihydrobutanol p-toluenesulfonate (XXXVI). Treatment of the latter with Raney nickel in ethanol gave (-) 2-isopropyldihydrobenzofuran (XXXV); this was treated with trifluoroacetic anhydride and acetic acid to give (-) dihydrotremetone (XXIX). The latter interconversion provided additional confirmation for the absolute configuration of dihydrotremetone and therefore for toxol and tremetone as well.



XXIII

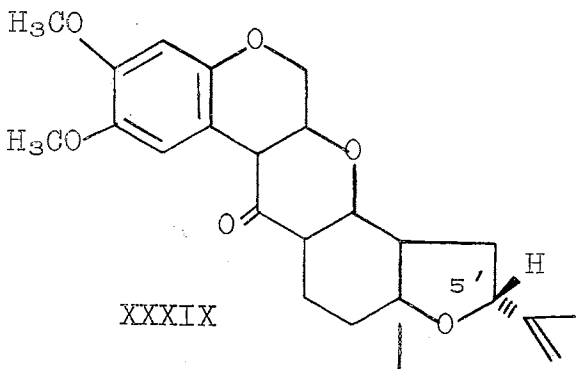
$(CF_3CO)_2O$



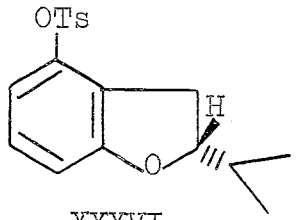
XXXV

H_2

Ni



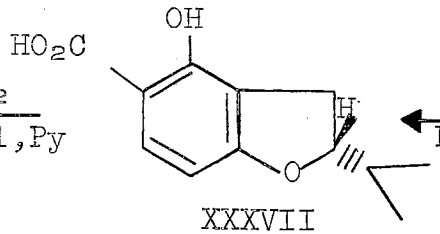
XXXIX



XXXVI

1)-CO_2

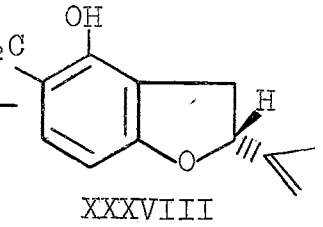
2)TsCl,Py



XXXVII

H_2

Pd-C



XXXVIII

BIOLOGICAL TESTING

The chromatographic separation was followed by microbiological assay. Pure toxol could be identified by a combination of paper chromatography and microbiological assay. Chromatography on Whatman No. 1 paper using the solvent system methanol-isopropyl alcohol-95% ethanol (85:10:5) gave a single spot of R_f 0.81. This spot exhibited quenching with u.v. light and inhibited growth of B. cereus. Under the same conditions, dehydrotremetone and toxol acetate showed R_f 0.60 and R_f 0.84 respectively. It was found that 200 μg. of any one of the three compounds mentioned above deposited in an area of about 7 mm. on Whatman No. 1 paper was sufficient to inhibit growth of B. cereus; growth inhibition was restricted to the area of compound deposition, owing to low water solubility. Judging from the completeness of growth inhibition, toxol is more toxic than either toxol acetate or dehydrotremetone at equal concentrations. No other materials in the chromatographic separation exhibited growth inhibition of B. cereus.

Since toxicity toward bacteria does not necessarily indicate toxicity toward higher animals, it was decided to test several of the fractions obtained during the separation. When chopped plant was mixed with regular feed, to make the plant palatable, and fed to a sheep, the animal developed "trembles" 28 days after the feeding started; it died two days later. The methanolic plant extract was fed to a sheep for four days; on the fourth day the sheep appeared sick and on the fifth day a typical case of "trembles" was apparent, and death occurred on the sixth day. The death of these two sheep demonstrated that the plant was toxic and that the methanol was extracting the toxic material. Two of the fractions obtained by Couch's

separation procedure (Chart A), the 30% ethanol fraction and the ether-soluble fraction, were tested. The 30% ethanol-soluble fraction was fed to a sheep for twelve days; the sheep developed "trembles" and was sacrificed for pathological studies. The sheep that was fed the ether-soluble fraction became weak on the eighth day, but recovered even though feeding of the ether-soluble fraction was continued. When fraction A (Chart B) was fed to a sheep there was no noticeable effect on the sheep. Toxol was fed to two sheep without any effect. The fact that toxol did not produce "trembles" with sheep indicated that it is probably not the toxic constituent of rayless goldenrod.

SYNTHETIC STUDIES

The synthesis of toxol or dihydrotoxol was undertaken to complete work on the structural aspects of these molecules. However, various difficulties were encountered which made the synthesis an elusive goal that has not been reached.

3-Coumarone (XL) was prepared as previously described (49) by Fries rearrangement of phenyl chloroacetate, followed by sodium acetate treatment. When 3-coumarone was treated with acetone and zinc chloride in methanol, by the procedure of Shriner (50), 2-isopropylidene-3-coumarone (XLI) ($C_{11}H_{10}O_2$, m.p. 86-87°) was obtained in 70% yield; the n.m.r. spectrum showed two methyl groups on a double bond (δ 2.05 and δ 2.30). When ethyl 2-acetoxycoumarilate (XLVII), prepared as previously described (51, 52, 53), was treated with methylmagnesium iodide, 2-isopropylidene-3-coumarone (XLI) was obtained in 60% yield. Hydrogenation of 2-isopropylidene-3-coumarone with rhodium-on-alumina catalyst gave 2-isopropyl-3-coumarone (XLII); the n.m.r. spectrum showed two isopropyl methyl groups (δ 0.83, $J \cong 6$ and

δ 1.60, J≃6) and a single proton as a doublet at δ 4.61 (C-2). Sodium borohydride reduction of either 2-isopropylidene-3-coumarone or 2-isopropyl--3-coumarone gave 2-isopropyl-3-hydroxycoumaran (XLIII) ($C_{11}H_{14}O_2$, m.p. 105-106°), for which the infrared spectrum showed a strong hydroxyl band at 2.95 μ and no carbonyl band; the n.m.r. spectrum showed two isopropyl methyl groups centered at δ 1.05 (triplet, J≃6 cps.), a quartet at δ 3.68 (C-2 proton) and a doublet (J=5 cps) centered at δ 4.58 (C-3 proton).

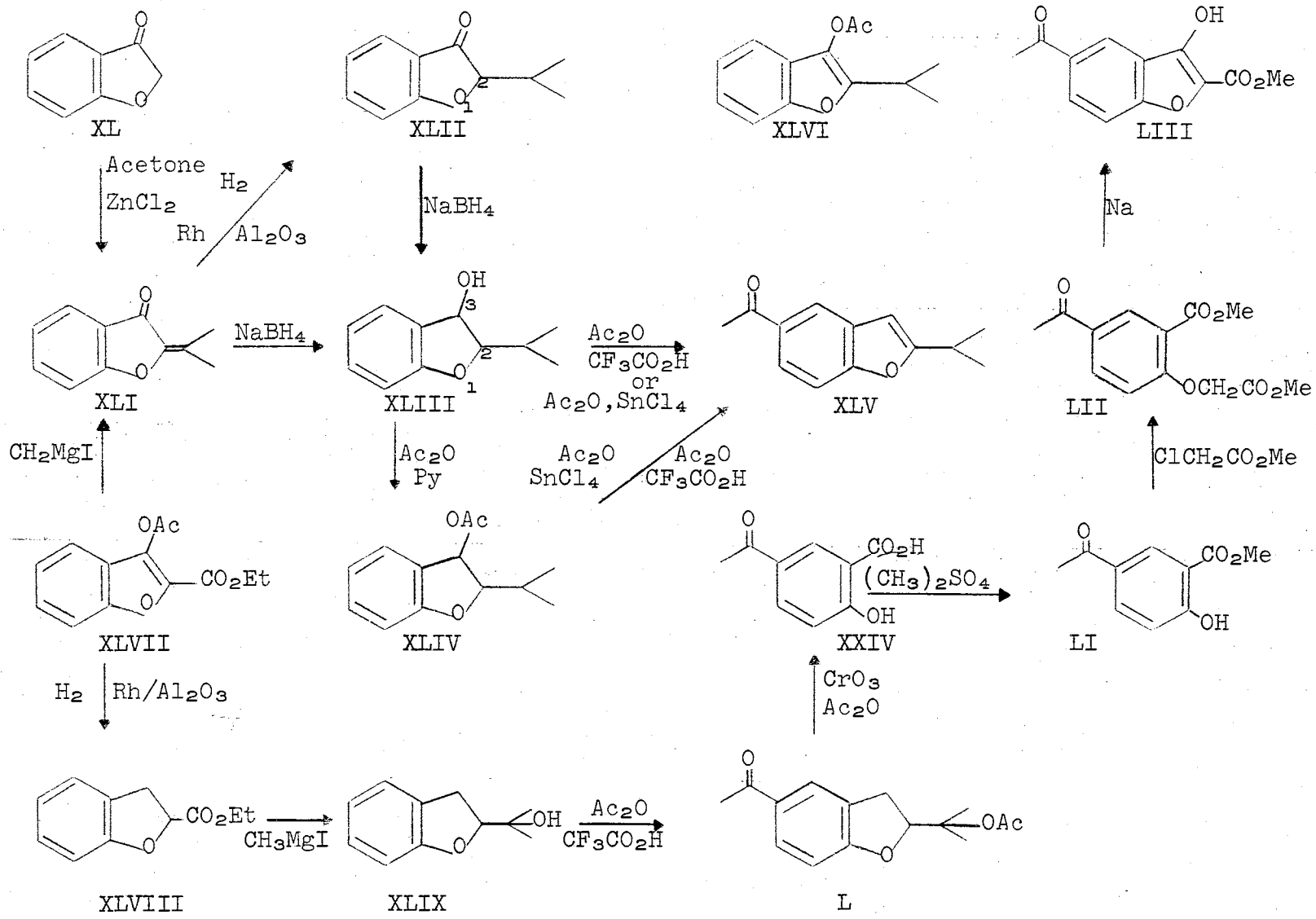
The acetylation of 2-isopropyl-3-hydroxycoumaran (XLIII) was expected to give dihydrotoxol acetate, which could then be hydrolyzed to give dihydrotoxol. However, treatment with acetic anhydride and trifluoroacetic acid as solvent and catalyst (54) gave 2-isopropyl-5-acetylbenzofuran (XLV) in 80% yield, the infrared spectrum of which was the same as that of the material obtained as previously reported (11). It was therefore thought that a non-protonic Friedel-Craft's catalyst might be less likely to dehydrate the benzylic alcohol during the acetylation reaction. However, when 2-isopropyl-3-hydroxycoumaran was acetylated with acetic anhydride and stannic chloride in carbon disulfide, 2-isopropyl-5-acetylbenzofuran (XLV) was again obtained in 75% yield. The ease of dehydration of the benzylic hydroxyl group is well known (55); therefore 2-isopropyl-3-hydroxycoumaran was acetylated with acetic anhydride in pyridine to give 2-isopropyl-3-acetoxycoumaran (XLIV) ($C_{13}H_{16}O_3$), the infrared spectrum of which showed the presence of no hydroxyl group. When 2-isopropyl-3-acetoxycoumaran was treated with either acetic anhydride and trifluoroacetic acid or acetic anhydride and stannic chloride in carbon disulfide, 2-isopropyl-5-acetylbenzofuran (XLV) was again obtained.

Since both the 3-hydroxy and the 3-acetoxy groups were readily eliminated during acetylation reactions, it was thought that the use of 2-iso-

propyl-3-acetoxybenzofuran (XLVI) would solve the problem. 2-Isopropyl-3-acetoxybenzofuran was prepared by treatment of 2-isopropyl-3-coumarone with acetic anhydride and trifluoroacetic acid and by treatment with acetic anhydride and perchloric acid in carbon tetrachloride (56). When 2-isopropyl-3-acetoxybenzofuran was treated with acetic anhydride and stannic chloride in carbon disulfide the only product obtained after the usual workup was 2-isopropyl-3-coumarone (XLII).

When ethyl 2-acetoxycoumarilate (XLVII) was hydrogenated using rhodium-on-alumina as catalyst, ethyl dihydrocoumarilate (XLVIII) was obtained in 95% yield; the infrared spectrum was identical with the spectrum of ethyl dihydrocoumarilate prepared as previously described (57,17). Treatment of ethyl dihydrocoumarilate with methylmagnesium iodide gave 2-(2'-dihydrobenzofuryl)-2-propanol (XLIX), which was acetylated with acetic anhydride and trifluoroacetic acid as previously described (11) to give 2-(5'-acetyl-2'-dihydrobenzofuryl)-2-propyl acetate (L). Oxidation of L with chromium trioxide in acetic anhydride at 0° was expected to give the benzylic ketone (58), but instead gave 5-acetylsalicylic acid (XXIV), the melting point ($212-214^{\circ}$) and infrared spectrum of which were identical with that of a sample prepared as previously described (59).

Methyl 5-acetylsalicylate (LI) was prepared by treating 5-acetylsalicylic acid with either alkaline dimethyl sulfate or diazomethane in ether; the n.m.r. spectrum showed the presence of a methyl ketone (δ 2.43) and a methyl ester (δ 3.98). Treatment of this ester with methyl chloroacetate and sodium ethoxide in absolute ethanol gave dimethyl O-carboxymethyl-5-acetylsalicylate (LII) ($C_{13}H_{14}O_6$, m.p. $103-104^{\circ}$); the n.m.r. spectrum showed the presence of a methyl ketone (δ 2.54), two methyl esters (δ 3.80 and 3.91) and two methylene protons (δ 4.77). Treatment of this diester with sodium in refluxing toluene gave methyl 3-hydroxy-


XL
XLII
XLVI
LIII
Acetone
ZnCl2
H2
Rh
Al2O3
NaBH4
Na
XLI
XLIII
XLV
LII
NaBH4
Ac2O
CF3CO2H
or
Ac2O,SnCl4
CH2MgI
Ac2O
Py
Ac2O
SnCl4
Ac2O
CF3CO2H
ClCH2CO2Me
XLVII
XLIV
XXIV
LI
(CH3)2SO4
H2
Rh/Al2O3
CrO3
Ac2O
XLVIII
XLIX
L
CH3MgI
Ac2O
CF3CO2H

5-acetylcoumarilate (LIII) ($C_{12}H_{10}O_5$, m.p. 95-96°); the n.m.r. spectrum showed the presence of a methyl ketone (δ 2.47) and a methyl ester (δ 3.88). An attempt to prepare the acetate of methyl 3-hydroxy-5-acetylcoumarilate with acetic anhydride in pyridine gave 90% recovery of the starting ester; Barton's procedure (56) for the preparation of enol acetates also gave only starting material in 85% yield, and trifluoroacetic acid and acetic anhydride again gave 85% recovery of starting material. An attempt to prepare the benzoate with sodium hydride and benzoyl peroxide in benzene as previously described for enol benzoates (60) gave only starting material. The reason that methyl 3-hydroxy-5-acetylcoumarilate could not be esterified is not apparent since ethyl 3-hydroxycoumarilate is easily acetylated with acetic anhydride in pyridine.

If methyl 3-hydroxy-5-acetylcoumarilate could have been acetylated, then the haloform reaction followed by treatment with methyl magnesium iodide and reduction with sodium borohydride would be expected to give 2-isopropyl-3-hydroxy-5-carboxydihydrobenzofuran. Acetylation of this compound would give 2-isopropyl-3-acetoxy-5-carboxydihydrobenzofuran (LV) which could then be converted into dihydrotoxol (XX) with methyl lithium.

Dehydrotremetone (I) was hydrogenated using palladium-on-charcoal catalyst to give dihydrotremetone (XXIII) (11). The ketal of dihydrotremetone was prepared with ethylene glycol and p-toluenesulfonic acid in benzene and was then treated with N-bromosuccinimide in carbon tetrachloride to give 2-isopropyl-5-acetylbenzofuran (XLV) after hydrolysis. Apparently the intermediate bromide underwent dehydrobromination to give the benzofuran derivative.

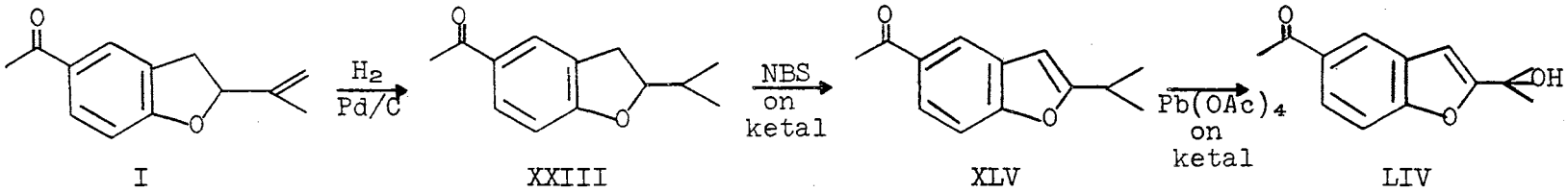
It was thought that lead tetraacetate might cause an allylic shift of the double bond in the five membered ring and form the benzylic acetate.

This acetate could then be hydrolyzed and the double bond hydrogenated to give dihydrotoxol.

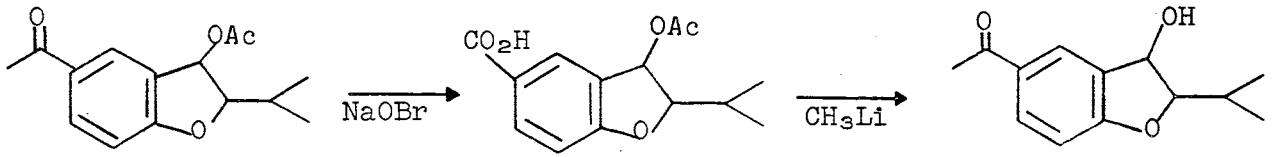
The ketal of 2-isopropyl-5-acetylbenzofuran (XLV), prepared by the usual method, was treated with lead tetraacetate in refluxing benzene following the procedure of Cavill and Soloman (61). The product was hydrolyzed with 5% hydrochloric acid to remove the ketal, which also resulted in hydrolysis of the acetate. The n.m.r. spectrum of the product indicated that it was a mixture of starting material and 2(5'-acetyl-2'-benzofuryl)-2-propanol (LIV); the paramagnetic shift of the isopropyl methyl groups from δ 0.85 and δ 0.95 to δ 1.33 and δ 1.43 indicated that a hydroxyl group was introduced as shown.

Dihydrotoxol acetate (XXI) readily reacted with sodium hypobromite to give acid LV, which was treated with an excess of methyllithium in ether. Ammonium chloride was used to decompose the complex whereupon dihydrotoxol (XX) was obtained in 60% yield. The fact that LV did not lose water during the workup indicated that dihydrotoxol could be synthesized if acid LV could be prepared.

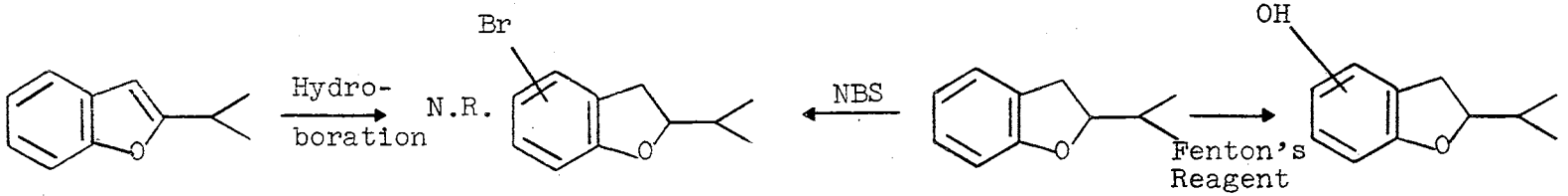
An attempt to hydroborate 2-isopropylbenzofuran (LVI), prepared as previously described (15), using the procedure of Brown (62), resulted in recovery of starting material in 85% yield. Treatment of 2-isopropyldihydrobenzofuran (LVII), prepared as previously described (15), with N-bromosuccinimide in carbon tetrachloride gave LVIII. The n.m.r. spectrum of LVIII indicated two benzylic protons at δ 3.26 and the integration indicated the presence of only three aromatic protons, thus suggesting that the bomine atom was attached to the aromatic ring and not the benzylic position as desired. The product obtained when 2-isopropyldihydrobenzofuran (LVII) was treated with Fenton's reagent (63) showed a strong hydroxyl band in its infrared spectrum at 2.9 μ and the n.m.r. spectrum showed two benzylic



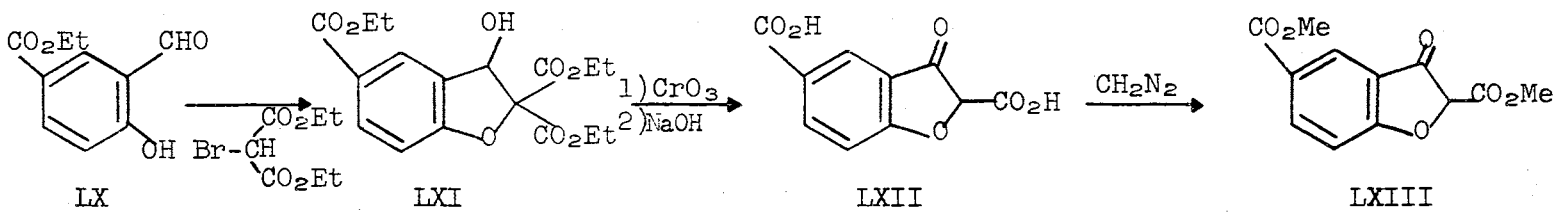
I XXIII XLV LIV



XXI LV XX



LVI LVIII LVII LIX



LX LXI LXII LXIII

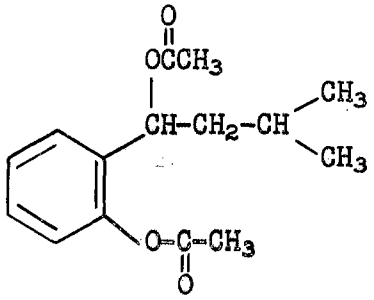
protons at δ 3.18; the integration indicated only three aromatic protons. This product was soluble in dilute sodium hydroxide, but insoluble in bicarbonate solution, indicating that the hydroxyl group was phenolic. Structure LIX is suggested for this product.

Treatment of ethyl 4-hydroxyisophthalaldehydate (LX), prepared as previously described (64), with diethyl bromomalonate and potassium carbonate in 2-butanone, as described by Duro (65), gave triethyl 3-hydroxycoumaran-2,2,5-tricarboxylic acid (LXI) in 90% yield. Oxidation of this triester with Jones' reagent (66) followed by hydrolysis with 20% sodium hydroxide gave 3-coumarone-2,5-dicarboxylic acid (LXII) which was stable to 300°. Treatment of LXII with an ether solution of diazomethane gave the diester LXIII; the n.m.r. spectrum indicated protons of two methyl ester groups (δ 3.93, δ 3.96). Attempts to prepare the enol acetate of diester LXIII using the procedure of Hagemeyer (67) with either p-toluenesulfonic acid or concentrated sulfuric acid as catalyst and isopropenyl acetate as reagent and solvent gave only the starting diester in nearly quantitative yield. When the diester LXIII was treated with ethylene glycol and p-toluenesulfonic acid in benzene in an attempt to prepare the ketal, only starting diester was recovered in about 90% yield.

The diester LXIV was prepared by treating salicylaldehyde with two molar equivalents of isobutylmagnesium bromide followed by acetylation of the product with acetic anhydride in pyridine. An attempted Fries rearrangement with aluminum chloride in nitrobenzene resulted in recovery of the starting material. Attempted acylation with either trifluoroacetic acid and acetic anhydride or acetic anhydride and stannic chloride also gave starting material.

The various attempts to synthesize either toxol or dihydrotoxol were

unsuccessful because of the ease of elimination of a 3-hydroxy or 3-acetoxy group in dihydrobenzofurans and the lack of reactivity of a 3-coumarone which has a carbonyl group attached at C-5.



LXIV

CHAPTER III

EXPERIMENTAL

All infrared spectra were recorded with a Beckman IR-5 infrared spectrometer using sodium chloride optics; solids were taken in potassium bromide pellets, and liquids as films on sodium chloride crystals. All nuclear magnetic resonance spectra were recorded on a Varian A-60 n.m.r. spectrometer; the samples were approximately 10% (w/v) solutions in either carbon tetrachloride or deuteriochloroform with tetramethylsilane (TMS) as an internal standard ($\delta \equiv 0$) and results are reported as dimensionless "chemical shift" units (δ). Gas chromatography data was obtained using either an Aerograph Hy-Fi gas chromatograph with a hydrogen flame detector or an Aerograph model A-300 gas chromatograph with a thermal detector. Melting points were obtained on a Fisher-Johns melting point apparatus, or in a sealed capillary using a Thomas-Hoover apparatus and are uncorrected. Analyses were performed by Midwest Micro Lab, Inc., Indianapolis, Indiana.

EXTRACTION AND SEPARATION

Collection, grinding and alcohol extraction of rayless goldenrod

Rayless golden rod was collected in August while in full bloom just north of the Pecos River on U. S. highway 70 in New Mexico. The entire plant was cut at ground level, dried by air or forced draft and then ground in a hammer mill with a twenty-mesh screen. The ground

meal was extracted continuously with methanol in a large Soxhlet extractor. In a typical run three kilograms of ground plant were extracted for forty-eight hours with approximately ten liters of methanol. The methanol was removed from the extractor and placed in a cold room at 4° overnight. The precipitated waxes were removed by filtration through glass wool. The filtrate was concentrated on a steam bath with the aid of a water aspirator to a viscous dark green oil (500 ml.).

SEPARATION OF METHANOLIC PLANT EXTRACT

Separation by Couch procedure (chart A, p. 5)

The viscous dark green residue from the methanolic plant extraction (900 g.) was extracted with boiling water in a large flask. The solution was then cooled, to allow the insoluble material to settle, and decanted and this process repeated until no further water-soluble material remained.

The water-insoluble material was extracted with 5% sodium bicarbonate solution using mechanical stirring, and then allowed to stand for thirty minutes to allow the insoluble material to settle. The aqueous solution was decanted and this procedure repeated until all of the sodium bicarbonate-soluble material had been removed.

The bicarbonate-insoluble material was exhaustively extracted with 50% boiling ethanol. The soluble material was recovered from the alcoholic solution by distillation of the ethanol and then decanting the water that remained.

The material soluble in 50% ethanol was extracted with 30% boiling ethanol in successive portions until all of the soluble material had been removed. The soluble material was recovered from the alcoholic solution by distillation of the alcohol as described above. In this manner, 48 g.

of dark residue were obtained.

The above mentioned dark-colored residue (27 g.) was hydrolyzed with 5% potassium hydroxide in 250 ml. of 50% aqueous methanol After removal of the alcohol, the remaining solution was diluted with water and extracted with ether. The ether solution was dried ($MgSO_4$), and evaporated to give 11 g. of a light orange, viscous oil.

This residue (11 g.) was dissolved in 30 ml. of ether and then diluted with 120 ml. of ligroin*. The insoluble material was filtered off and the filtrate evaporated to dryness. This process was repeated until no more material precipitated upon the addition of ligroin.

The residue obtained by evaporation of the ligroin solution was dissolved in ether and washed with a 2% potassium hydroxide solution and then water. After drying ($MgSO_4$), the ether was evaporated to give 10 g. of light yellow oil ("tremetol").

Procedure I (chart B, p. 7)

The viscous dark green residue from the methanolic plant extraction (300 g.) was dissolved in 1.5 liters of 50% aqueous methanol containing 105 g. of potassium hydroxide. The solution was refluxed for eight hours, then concentrated to about 800 ml. with the aid of a water aspirator. The residue was diluted with three liters of water and continuously extracted with ether. The ether extract was dried ($MgSO_4$) and then evaporated to give 30 g. of red viscous oil (Fraction A).

The aqueous solution remaining after the removal of fraction A was saturated with carbon dioxide (pH 8) and extracted continuously with

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* Ligroin refers to petroleum ether, b.p. 40-60°, unless otherwise indicated.

ether, until the ether layer was colorless. The ether solution was dried ($MgSO_4$) and evaporated to give 14 g. of black residue (Fraction B).

The aqueous solution that remained after the extraction of fraction B was acidified with hydrochloric acid (pH 2). The acidic solution was continuously extracted with ether until the ether phase was colorless. The ethereal solution was dried ($MgSO_4$), then evaporated to leave 70 g. of a black residue (Fraction C). The remaining aqueous solution was discarded.

SEPARATION OF FRACTION A

Procedure I (chart C, p. 12)

Florisil (300 g., Floridin Company) was suspended in a solution prepared by saturating 95% methanol with ligroin (b.p. 60-80°, stationary phase) then poured into a column and allowed to stand overnight in the solution. The column was then washed with ligroin saturated with 95% methanol (mobile phase) in order to remove any excess of the stationary phase. Fraction A (20 g.) was added to the column which was then eluted with 100 ml. fractions of the mobile phase. Fractions 3 and 4 deposited a crystalline solid when the solvent was removed (ketone I, m.p. 72-78°; 81-82° after recrystallization from methanol water 3:1). The infrared spectrum of this solid showed a strong conjugated carbonyl band and aromatic bands similar to those reported (10) for dehydrotremetone, II. This solid exhibited bacterial growth inhibition. A total of 1500 ml. of the mobile phase yielded 15 g. of material. The remaining 5 g. of material was obtained by washing the column with the stationary phase and was designated fraction G. It likewise exhibited bacterial growth inhibition.

Procedure II (chart D, p. 14)

Steam-volatile oils (Fraction D)

Fraction A (30 g., chart B, p. 7) was suspended in 1.5 g. of water and the mixture exhaustively steam distilled. The distillate was then continuously extracted with ether. After drying ($MgSO_4$), the ether extract was concentrated by distillation through a 25 cm. x 0.5 cm. tantalum spiral Todd column and gave 3.3 g. of oily residue. The non-volatile material was recovered from the aqueous solution remaining after steam distillation by extraction with ether. The latter ether extract was dried ($MgSO_4$) and the solvent evaporated to give 26.6 g. of non-volatile material.

Separation of non-volatile fraction A with Girard's T reagent

A solution of 26.6 g. of the above residue, 15 g. of Girard's T reagent and 3 ml. of acetic acid in 200 ml. of methanol was refluxed for one hour. The solution was cooled, poured into 500 ml. of 10% sodium carbonate solution and then extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 19 g. of viscous oil, non-ketone fraction F. The aqueous carbonate solution was acidified with hydrochloric acid (pH 2), stirred for two hours and then extracted with ether. The ether extract was dried ($MgSO_4$) and then evaporated to give 7.5 g. of viscous oil, ketone fraction E.

SEPARATION OF INDIVIDUAL FRACTIONS

Steam-volatile fraction D

Steam-volatile fraction D (100 g.) was fractionally distilled to give four fractions with an eight-inch by one-half-inch Vigreux column.

fraction D-I	b.p. 40-55°	(0.05 mm.)	31%
fraction D-II	b.p. 55-65°	(0.05 mm.)	27%
fraction D-III	b.p. 65-75°	(0.05 mm.)	25%
fraction D-IV	b.p. 75-95°	(0.05 mm.)	16%
			99%

About one gram of undistilled residue remained and was discarded.

Fraction D-I was then refractionated through a 25-cm. x 0.5-cm. tantalum-spiral Todd column. Four fractions were obtained and a fifth was obtained by removal of the Todd column and distilling the residue at low pressure.

fraction D-Ia	b.p. 65-70°	(1.0 mm.)	29%
fraction D-Ib	b.p. 70-73°	(1.0 mm.)	13%
fraction D-Ic	b.p. 73-75°	(1.0 mm.)	15%
fraction D-Id	b.p. 75-80°	(1.0 mm.)	30%
fraction D-Ie	b.p. 65-70°	(1.0 mm.)	12%
			99%

About one-half gram of undistilled residue remained and was discarded.

Gas chromatography (Aerograph Hy-Fi) using a 10% silicone rubber column (10' x 1/8") at 180° (hydrogen flow 30 ml./min., nitrogen flow 30 ml./min.) indicated that fractions D-Ia and D-Ib were mixtures of similar composition, differing only in the relative amounts of the components. Fraction D-Ic exhibited three peaks of comparable size,

but different from those in D-Ia and D-Ib. Fraction D-Id showed only two peaks, both of which were different than any of those of the previous fractions, in the ratio of about 4:1, and fraction D-Ie was a complex mixture.

Ketone fraction E

Ketone fraction E (29 g.) was chromatographed on 500 g. of activity II Merck acid-washed alumina. The column was eluted with solvents of increasing polairy until less than 50 mg./100 ml. was eluted.

eluent	weight of eluate, g.
ligroin	2.9
ligroin-benzene(1:1)	15
benzene	1
chloroform	7
methanol	3
total recovered	28.9

When the solvent from the ligroin fraction was evaporated, the residue solidified, and the infrared spectrum of this solid, m.p. 78-80°, was the same as the infrared spectrum of ketone I (dehydrotremetone). The fractions were tested for bacteriostatic action, but only ketone I and the ligroin-benzene fraction exhibited toxicity. The ligroin-benzene fraction was then rechromatographed on 500 g. of activity I Merck acid-washed alumina.

eluent	volume	weight of eluate, g.
benzene	1500 ml.	0.2
benzene-chloroform(1:1)	750 ml.	13
chloroform	1000 ml.	1
	total recovered	14.2

The above benzene fraction deposited crystals, m.p. 246-8°, when the solvent was removed, and these were designated ketone II. The benzenechloroform fraction gave a viscous yellow oil, while the chloroform fraction gave a dark red oil. The fractions were tested for bacteriostatic action and only the benzene-chloroform fraction showed toxicity. The infrared spectrum of this fraction indicated a strong hydroxyl band, a strong conjugated carbonyl band and aromatic bands similar to those in ketone I. This material was designated ketone III (toxol).

Non-ketone fraction F

Non-ketone fraction F (50 g.) was chromatographed on 500 g. of Merck acid-washed activity II alumina. The ligroin fraction (750 ml.) deposited 600 mg. of crystalline solid, m.p. 265-70°, when the solvent was removed. The infrared spectrum of this solid contained a strong hydroxyl band and bands similar to those found in steroids. The remaining material eluted from the column was not investigated.

This same material could be obtained during the separation of the ketonic and the non-ketonic fractions. When the aqueous carbonate solution was extracted with an equal volume of ether, a solid precipitated at the interface. This solid was separated by filtration. Its infrared spectrum indicated that it was the same as the material described above. If the carbonate solution was repeatedly extracted with ether all of the solid dissolved in the ether and the steroid was then obtained by chromatography as indicated above. This material was referred to as Steroid I.

Fraction G (chart C, p. 11)

Fraction G (34 g.) was chromatographed (adsorption) on 300 g. of Florisil. The column was eluted with solvents of increasing polarity until less than 50 mg./100 ml. was eluted.

eluent	weight of eluate, g.
benzene	1
ether	11
chloroform	15
methanol	6
total	33

The ether fraction was the only fraction that inhibited bacterial growth. The infrared spectrum of material obtained on evaporation of the ether fraction was nearly the same as the spectrum of toxol obtained from fraction E (see p. 16).

When fraction G (22 g.) was chromatographed (adsorption) on 300 g. of alumina (act. I) only the benzene-chloroform (1:1) fraction (16 g.) inhibited bacterial growth. This fraction was rechromatographed (partition) on 300 g. of Florisil, prepared by adding 185 ml. of 95% methanol saturated with ligroin (b.p. 60-80°, stationary phase) to the Florisil and allowing the mixture to equilibrate overnight and then packing the column. Toxol was eluted with ligroin saturated with 95% methanol (mobile phase) in fractions 11-25 (250-ml. fractions); 8.2 g. was obtained. These fractions inhibited bacterial growth and the other fractions did not.

PURIFICATION AND PROPERTIES OF INDIVIDUAL COMPONENTS

Fraction B (chart B, p. 7)

To a solution of 14 g. of fraction B in 30 ml. of methanol was added 20 ml. of a saturated solution of lead acetate in methanol. The resulting precipitate was removed by filtration, then suspended in 50 ml. of methanol and a stream of hydrogen sulfide was passed through the suspension for one hour. The resulting lead sulfide was removed by filtration, and the methanol filtrate evaporated to dryness to give 8 g. of a solid. Recrystallization of this solid from methanol gave 6.5 g., m.p. 68-70°; when mixed with an authentic sample of stearic acid, m.p. 69-70°. The infrared spectrum was identical with the infrared spectrum of stearic acid previously reported (68).

The filtrate remaining after removal of the lead stearate was diluted with 500 ml. of water, acidified with hydrochloric acid and extracted with ether. The ether was dried ($MgSO_4$) and evaporated to leave 6 g. of a dark green oil. This oil was dissolved in ether and treated with an ether solution of diazomethane; the excess diazomethane was allowed to evaporate. The solution was analyzed by gas chromatography using a 10' x 1/4" 5% SE-30 column at 225° (helium flow rate 75 ml./min.). Only two peaks were detected; the retention times corresponded to the retention times of methyl myristate (81%) and methyl laurate (19%) as determined by injection of authentic samples.

Fraction C (chart B, p. 7)

A solution of 70 g. of fraction C in 200 ml. of methanol was added to 400 ml. of a solution containing 65 g. of BF_3/500 ml. methanol. The solution was refluxed for 15 minutes, cooled, diluted with 600 ml. of water, made basic with potassium hydroxide (pH 10), then extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 28.8 g. of viscous oil. The basic solution was acidified with hydrochloric acid (pH 2) and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 40 g. of a waxy residue. A solution of 2 g. of this residue in 20 ml. of ether was treated with an ether solution of diazomethane. The excess diazomethane was allowed to evaporate. The esters prepared both with BF_3 in methanol and with diazomethane were analyzed by gas chromatography.

	Columns		
Esters	10' x 1/4" 5% SE-30 He flow 73 ml./min.	10' x 1/4" Craig polyester succinate He flow 75 ml./min.	Avg. per cent
by BF_3/CH_3OH	retention time (sec.)		
Methyl hexanoate	80	98	32.4
Methyl octanoate	91	157	28.0
Methyl laurate	120	210	15.1
Methyl myristate	165	345	7.5
Methyl palmitate	270	645	7.7
Methyl linoleate	420	-	8.3

by diazomethane

Methyl hexanoate	80	98	11.9
Methyl octanoate	91	157	7.7
Methyl laurate	120	210	13.3
Methyl myristate	165	345	11.1
Methyl palmitate	270	645	56.0

d-Limonene

The methanol that was recovered from the extraction of rayless goldenrod was diluted with an equal volume of water. The resulting milky solution was extracted continuously with ligroin. The ligroin extract was dried ($MgSO_4$) and the solvent distilled through a 50 cm. x 1.5 cm. Vigreux column. Gas chromatography of the residue (5% SE-30, at 90°, H_2 flow 30 ml./min., N_2 flow 30 ml./min.) showed one major peak, about 60%, and several smaller peaks of about equal concentration. The material was distilled, b.p. 45-60° (4.5 mm.), and the distillate shown to consist of about 80% one component by gas chromatography using the conditions outlined above. The distillate was redistilled, b.p. 52-55° (4.5 mm.), and this distillate was at least 95% one component as indicated by gas chromatography. The retention time was the same as the retention time of an authentic sample of limonene. The infrared spectrum and the n.m.r. spectrum were the same as the corresponding spectra of limonene. The optical rotation, $[\alpha]_D$+145.1° (c, 4.2; ethanol), was similar to that reported (18) $[\alpha]_D$+126.8 for d-limonene (IV).

Steam-volatile fractions D-Ia and D-Ib (see p. 19)

Because fractions D-Ia and D-Ib showed similar gas chromatograms they were combined (19 g.) and chromatographed on 500 g. of Merck acid-washed alumina (act. I). The column was treated with solvents of increasing polarity until less than 50 mg./100 ml. was eluted.

eluent	weight of eluate g.
ligroin	3.4
ligroin-benzene (1:1)	0.3
benzene	4.8
benzene-chloroform (1:1)	0.75
chloroform	9.0
Total	18.25

The ligroin fraction showed four peaks in its gas chromatogram (10' x 1/8", 10% silicone rubber column, at 180°, Aerograph Hy-Fi, H_2 flow 30 ml./min., N_2 flow 30 ml./min.); one of these peaks had the same retention time as authentic linonene (alone and on admixture). The ligroin-benzene and benzene fractions were complex mixtures. The benzene-chloroform fraction gave a compound that was about 90% pure; the retention time of this was the same as the retention time of carvone (alone and on admixture). The infrared spectrum was nearly identical with that of an authentic sample. The optical rotation ($[\alpha]_D$-58° (c, 1.8; ethanol), reported for carvone (19), $[\alpha]_D$-62°) indicated that the material was l-carvone (V).

The chloroform fraction gave a semi-crystalline solid which could not be purified by vacuum sublimation. It was easily recrystallized from

ligroin at -70° to give m.p. 207-208° (sealed capillary), reported for borneol (20) m.p. 204-205°. A mixed melting point with an authentic sample of borneol was not depressed. The infrared spectrum was identical with the infrared spectrum of an authentic sample. The optical rotation ($[\alpha]_D$-25.4°, c, 2.3; methanol) was similar to that reported (21), $[\alpha]_D$-33.1, for l-borneol (VI).

Fraction D-Id (see p. 20)

Fraction D-Id showed two peaks in its gas chromatogram (10' x 1/8" silicone rubber, Aerograph Hy-Fi, at 180°, H_2 flow 30 ml./min., N_2 flow 30 ml./min.) in the approximate ratio of 4:1. The larger peak had the same retention time as caryophyllene. Caryophyllene (VII) has been isolated from rayless goldenrod*; therefore, no attempt was made to further purify the caryophyllene. The other fractions obtained from the steam-volatile oil were not investigated.

Ketone I, Dehydrotremetone

Ketone I (4 g.) obtained by chromatography of fractions E and G was dissolved in 10 ml. of ligroin and the solution cooled overnight at 0°. The resulting crystals were filtered out to give 2.9 g.; recrystallization from ligroin gave 2.5 g., m.p. 85-86°; reported for dehydrotremetone (11,12) m.p. 87.5-88.5°. The infrared spectrum was the same as that reported (11).

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* L. H. Zalkow and G. Cabat, unpublished results.

Ketone II, Friedelin

Ketone II, obtained by chromatography of fraction E, was a crystalline solid as obtained from the chromatography column. When the crystals were washed with ligroin all color was removed; the melting point (245-246°) indicated that the material was pure, and so no further purification was attempted. Ketone II gave a negative tetranitromethane test and had an optical rotation of $[\alpha]_D$-21° (c, 1.1; $CHCl_3$). Reported for friedelin (23): m.p. 245-246°, $[\alpha]_D$-21°.
λ_{max}^{KBr} 5.82, 6.86, 7.18 μ; n.m.r.: seven peaks between δ0.9 and δ1.4.

Oxime of ketone II

A solution of 33 mg. of ketone II in 20 ml. of absolute ethanol, 20 ml. of benzene, 1 ml. of pyridine and 80 mg. of hydroxylamine hydrochloride was refluxed for two hours. The solution was cooled, diluted with water, extracted with ether and the ether extract dried ($MgSO_4$) and evaporated. The residue was recrystallized from chloroform-ethanol to give 16 mg., m.p. 277-278° (d), $[\alpha]_D$+57.7° (c, 0.91; $CHCl_3$). Reported for the oxime of friedelin (23): m.p. 280-281° (d), $[\alpha]_D$+56°.

Enol benzoate of ketone II

A solution of 50 mg. of ketone II in 1 ml. of benzoyl chloride was heated at 185° for two hours, cooled and diluted with 5 ml. of absolute ethanol. The resulting crystals were collected by filtration; recrystallized from chloroform-ethyl acetate gave 25 mg., m.p. 257-258°, $[\alpha]_D$+57.1° (c, 0.85; $CHCl_3$). Reported for the enol benzoate of friedelin (23): m.p. 255-256°, $[\alpha]_D$+57°.

Steroid I

Steroid I (IX), isolated either by separation of the ketonic and non-ketonic fractions or by chromatography of the non-ketonic fraction F, was difficultly soluble in common organic solvents. However, it was easily soluble in pyridine from which it was recrystallized to give m.p. 286-288° with previous melting at 265°, $[\alpha]_D$-16.5° (c, 0.72; $CHCl_3$). Steroid I gave a negative tetranitromethane test. The limited solubility of steroid I prohibited obtaining its n.m.r. spectrum; λ_{max}^{KBr} 2.9 μ.

Toxol

Toxol obtained by chromatography of either fraction E or fraction G was distilled at 110° (0.05 mm.). The distillate solidified on standing at room temperature for a week. The solid material was recrystallized from ether-ligroin to give m.p. 50-51°; an analytical sample was obtained by recrystallization repeatedly from ether-ligroin to give m.p. 52-53°, $[\alpha]_D$-25.1° (c, 1.34; methanol).

Anal. Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.64; H, 6.51.

λ_{max}^{KBr} 3.0, 5.98, 6.03, 6.22 μ.; λ_{max} 223 mμ, log ε 4.02; λ_{max} 273 mμ. log ε 4.13; n.m.r. δ1.66 (methyl on double bond), δ2.36 (methyl ketone), δ4.95 and δ4.84 (5 protons), δ6.72 and δ7.70 (aromatic).

STRUCTURAL STUDIES

Structure of Steroid I

Steroid I triacetate (X)

A solution of 12.5 mg. of steroid I (IX) in 1 ml. of pyridine and 0.5 ml. of acetic anhydride was stirred overnight. The solution was diluted with 10 ml. of water and the resulting crystals collected by filtration. Recrystallization from methanol gave 14 mg. of steroid I acetate, m.p. 168-169°, $[\alpha]_D$ +8.1° (c, 0.91; $CHCl_3$), λ_{max}^{KBr} 5.7 μ.

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.22; H, 8.78. Found: C, 69.34; H, 8.94.

Preparation of 5α-Androstane (XII) from steroid I

To a solution of 50 g. of steroid I in 20 ml. of pyridine was added 0.5 g. of p-toluenesulfonyl chloride and the solution was stirred over night. After dilution with water, the resulting precipitate was collected by filtration. The precipitate was dissolved in 20 ml. of ether and 200 mg. of lithium aluminum hydride added; the solution was then stirred for twenty-four hours, the complex destroyed with water and 10% sulfuric acid, and the solution extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give a waxy solid. This solid was dissolved in 20 ml. of ligroin and the solution washed with concentrated sulfuric acid to remove any oxygenated or unsaturated compounds, then washed with water. The ligroin solution was dried ($MgSO_4$) and the solvent removed to give 6 mg. of 5α-androstane, m.p. 45-46°, reported (23) m.p. 48-49°.

Preparation of 5α-Androstane (XII) from 5α-androstane-3,17-dione*

To a solution of 15 mg. of 5α-androstane-3,17-dione in 25 ml. of diethylene glycol was added 3 ml. of 95% hydrazine and the solution was refluxed for twenty-four hours. After dilution with water and extraction with ether, the ether extract was dried ($MgSO_4$) and evaporated to give 10 mg. of 5α-androstane (XII), which showed, after recrystallization from ligroin, m.p. 46-47° alone and on admixture with that obtained from steroid I. Gas chromatography (Aerograph Hy-Fi, 10' x 1/8", 5% SE-30 column; 220°, H_2 flow 30 ml./min.; N_2 flow 30 ml./min.) gave only one peak (retention time 8 min.) with either the material obtained from steroid I, or the material obtained from the dione or admixture of the two.

5α-Androstan-17β-ol-3-one-17-hexahydrobenzoate (XV)

A solution of 1.5 g. of 5α-androstan-17β-ol-3-one 17-benzoate (XIV) in 100 ml. of acetic acid was hydrogenated in the presence of 200 mg. of PtO_2 at atmospheric pressure; slightly more than three molar equivalents of hydrogen were absorbed in 1.5 hours. The catalyst was removed by filtration and 1 g. of chromium trioxide in 10 ml. of 80% aqueous acetic acid was added and the solution stirred for two hours. The solution was diluted with one liter of a saturated solution of sodium carbonate, then extracted with chloroform. The chloroform extract was dried ($MgSO_4$) and evaporated to give 1.2 g. of residue. The residue was recrystallized from

* Donated by Dr. J. Martin, Microbiology Department, Oklahoma State Univ.

chloroform-methanol to give 5α-androstan-17β-ol-3-one 17-hexahydrobenzoate (XV), m.p. 138-139°, $[\alpha]_D$+25°(c, 0.79; $CHCl_3$), reported (25) m.p. 138-139°, $[\alpha]_D$+26°.

Δ^{16} 5α-androsten-3-one (XVI)

The hexahydrobenzoate was heated at 265° and 150 mm. pressure for four hours, after which 10 ml. of 10% potassium hydroxide in 95% ethanol was added and the solution refluxed for four hours. The solution was diluted with water and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 1 g. of a glassy solid. This was chromatographed on 20 g. of Merck acid-washed alumina (act. III); elution of the column with ligroin-benzene (3:1) afforded 600 mg. of solid. Sublimation of this material at 115° (0.05 mm.) gave 150 mg. of Δ^{16} 5α-androsten-3-one (XVI), m.p. 140-141°, $[\alpha]_D$+35° (c, 7.3; $CHCl_3$); reported (26) m.p. 140-141°, $[\alpha]_D$+38°.

Δ^{16} 5α-Androsten-3β-ol (XVII)

To a solution of 100 mg. of Δ^{16} 5α-androsten-3-one (XVI) in 10 ml. of ether was added 100 mg. of lithium aluminum hydride and the solution stirred for four hours at room temperature. The complex was decomposed with 5 ml. of water and 10 ml. of 5% hydrochloric acid. The solution was extracted with chloroform, the chloroform extract was dried ($MgSO_4$) and evaporated. The resulting residue was recrystallized from methanol and then sublimed at 100° (0.05 mm.) to give 40 mg. of Δ^{16}-5α-androsten-3β-ol (XVII), m.p. 126-127°, $[\alpha]_D$+16.1° (c, 1.6; $CHCl_3$); reported (10) m.p. 127°, $[\alpha]_D$+15.9°.

5α-Androstane-3β,16α,17α-triol (IX)

To a solution of 25 mg. of Δ^{16}-5α-androsten-3β-ol (XVII) in 2.0 ml. of absolute ether was added 100 mg. of osmium tetroxide in 2.0 ml. of ether containing 100 mg. of pyridine, and the solution allowed to stand overnight. The solid which had precipitated was filtered out and then dissolved in 2.0 ml. of 1N sodium hydroxide containing 100 mg. of mannitol and the solution was stirred for five hours at room temperature. The resulting solid was collected by filtration and dissolved in 200 ml. of ether; the ether solution was washed with 5% potassium hydroxide then water and finally dried ($MgSO_4$) and evaporated to give 17 mg. of crystalline 5α-androstane-3β,16α,17α-triol (IX), m.p. 284-286° with previous melting at 265°, $[\alpha]_D$-17.1° (c, 0.75; $CHCl_3$), reported (24) m.p. 265°, $[\alpha]_D$-19.4°. The infrared spectrum was identical with that of steroid I obtained from rayless goldenrod and the melting point was not depressed when the two samples were mixed.

Structure of Toxol

Toxol acetate (XIX)

Toxol acetate (XIX) was prepared by dissolving toxol (XVIII) in 10 ml. of pyridine and 2 ml. of acetic anhydride and stirring the solution overnight. The solution was diluted with 100 ml. of water and extracted with ether. The ether extract was washed with 5% hydrochloric acid, then with water and finally dried ($MgSO_4$) and evaporated to give a viscous yellow oil. Distillation of this oil gave the acetate as a colorless oil,

b.p. 70-75° (0.05 mm.), $[\alpha]_D$-76.4° (c, 1.4; methanol) λ_{max}^{film} 5.75, 5.95, 6.21, 7.92 and 8.15 μ; n.m.r. δ1.75 (methyl on double bond), δ2.01 (methyl ketone), δ2.40 (acetate methyl), δ4.90 (center of multiplet, 3 protons: 2 vinylic, 1 on ether carbon), δ5.93 (doublet, J=3 cps., proton under acetate), δ6.8 and 7.8 (multiplets, 3 aromatic protons).

Anal. Calcd. for $C_{15}H_{16}O_4$: C, 69.17; H, 6.19. Found: C, 69.41; H, 6.47.

2,4-Dinitrophenylhydrazone of toxol

The 2,4-dinitrophenylhydrazone of toxol was prepared by adding 67 mg. of toxol in 10 ml. of methanol to 90 ml. of a solution prepared by dissolving 3 g. of 2,4-dinitrophenylhydrazine in 270 ml. of methanol and 30 ml. of concentrated hydrochloric acid. The precipitated derivative was filtered out and then chromatographed on Merck acid-washed alumina (act. I). The derivative was eluted with benzene and then recrystallized from ethanol-water (4:1) to give 105 mg. of the derivative, m.p. 176-177°. The infrared spectrum showed no carbonyl band. λ_{max} 389 mμ, log ε 4.39.

Anal. Calcd. for $C_{19}H_{18}N_4O_6$: C, 57.29; H, 4.55; N, 14.07. Found: C, 57.14; H, 4.75; N, 13.75.

Iodoform test on toxol and toxol acetate

Treatment of toxol in dioxane-water (1:1) with 10% sodium hydroxide and a solution containing 1 g. of iodine and 2 g. of potassium iodide in 10 ml. of water gave a yellow precipitate. The isolated precipitate had m.p. 117-118°; mixed m.p. with an authentic sample of iodoform was 117-118°.

When toxol acetate was treated similarly, it also gave a precipitate of iodoform, identified by melting point and mixed melting point with an authentic sample.

Dehydration of toxol with hydrochloric acid in dioxane

A solution of 10 ml. of 5% hydrochloric acid was added to a solution of 216 mg. of toxol in 10 ml. of dioxane and the resulting solution was refluxed for twenty-four hours. The solution was then cooled and diluted with 100 ml. of water and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 199 mg. of a yellow oil. This oil was distilled at 100° (0.05 mm) to give a distillate which solidified. Recrystallization from ether-ligroin (2:1) gave m.p. 105-108°. The infrared spectrum was very similar to the infrared spectrum of dehydrotremetone, m.p. 85-86°.

Dehydration of toxol with sulfuric acid in acetic acid

Toxol (100 mg.) was dissolved in 10 ml. of acetic acid; to this solution was added 3 drops of concentrated sulfuric acid, and the solution was heated on a steam bath for 10 minutes. After dilution with 150 ml. of water and extraction with ether, the extract was dried ($MgSO_4$) and evaporated to leave 75 mg. of an oil. The oil could not be distilled at 100° (0.05 mm.) and attempts to crystallize the material failed. The infrared spectrum was identical with that of the material obtained by treating toxol with hydrochloric acid.

Pyrolysis of toxol acetate

A solution of 278 mg. of toxol acetate in 10 ml. of benzene was passed through a 1.0 x 25 cm. column packed with glass helices at 225° in a nitrogen atmosphere. The solution was then washed with water and dried ($MgSO_4$) and the solvent evaporated to give 200 mg. of a yellow oil. This oil was distilled at 95° (0.05 mm.); the infrared spectrum of the distilled oil was the same as the infrared spectrum of the material obtained by treating toxol with acid.

Treatment of toxol with iodine. Preparation of Dehydrotremetone.

Toxol (900 mg.) was heated at 120° in the presence of a crystal of iodine for 30 minutes. The mixture was cooled and dissolved in benzene, and then chromatographed on 50 g. of Merck acid-washed alumina (act. I). Dehydrotremetone (81 mg.) was eluted with benzene and identified by melting point (84-85°) and by infrared spectral comparison with an authentic sample. The material eluted with chloroform (540 mg.) had an infrared spectrum which was the same as the infrared spectrum of the material obtained by treating toxol with acid.

Treatment of dehydrotremetone with iodine

Dehydrotremetone (1.0 g.) was heated at 120° with a crystal of iodine for 30 minutes. The solution was cooled and dissolved in benzene, and then chromatographed on 50 g. of Merck acid-washed alumina (act. I). The material eluted with chloroform (600 mg.) had an infrared spectrum that was the same as the infrared spectrum of the material obtained by treating toxol with acid.

Photolysis of dehydrotremetone

A solution of 2 g. of dehydrotremetone in 400 ml. of ligroin was photolyzed using a 200-watt mercury arc lamp with a quartz filter, for four hours; during the photolysis a crystalline solid precipitated continuously. This solid was removed by filtration; when the filtrate was concentrated to 50 ml., a small amount of additional precipitate was obtained. This was collected by filtration and added to the original precipitate, m.p. 175-177°. The infrared spectrum was identical with that of the material obtained by treatment of toxol with acid. The molecular weight, determined by the Rast method, was 1394 and 1388, indicating that the material was a polymer with seven units of dehydrotremetone in the chain (M.W. 1400).

Hydrogenation of toxol with palladium-on-charcoal

A solution of 723 mg. of toxol in 20 ml. of 95% ethanol was hydrogenated at atmospheric pressure using 75 mg. of 10% palladium-on charcoal as catalyst. The solution absorbed 3.84 moles of hydrogen per mole of ketone. The catalyst was removed by filtration and the solvent evaporated at reduced pressure. The infrared spectrum of the product showed no carbonyl band. This material was not investigated further.

Hydrogenation of toxol with palladium-on-calcium carbonate

A solution of 511 mg. of toxol in 20 ml. of 95% ethanol was hydrogenated at atmospheric pressure using 50 mg. of 2% palladium-on calcium carbonate as catalyst. The solution absorbed 1.61 moles of hydrogen per

mole of ketone. The catalyst was removed by filtration and the solvent evaporated at reduced pressure to give 420 mg. of a viscous oil. The oil was distilled at 110° (0.05 mm.). Elemental analysis of the distillate indicated that it was a mixture of dihydrotoxol and a material that had undergone hydrogenolysis.

Anal. Found: C, 73.54; H, 7.81, which corresponds to 60% $C_{13}H_{16}O_3$ and 40% $C_{13}H_{16}O_2$.

Hydrogenation of toxol with rhodium-on-alumina

A solution of 534 mg. of toxol in 20 ml. of 95% ethanol was hydrogenated at atmospheric pressure using 62 mg. of 5% rhodium-on-alumina as catalyst. The solution absorbed one mole of hydrogen per mole of ketone. The catalyst was removed by filtration and the solvent evaporated at reduced pressure to give 412 mg. of a viscous oil. The oil was distilled at 90-95° (0.05 mm.) to give dihydrotoxol (XX), $[\alpha]_D$-107° (c, 1.1; methanol). λ_{max}^{film} 2.96, 6.01 and 6.22 μ. n.m.r. δ0.97 (doublet, J=7 cps., isopropyl group), δ2.35 (methyl ketone).

Dihydrotoxol acetate (XXI)

To a solution of 200 mg. of dihydrotoxol in 20 ml. of pyridine was added 2 ml. of acetic anhydride and the solution stirred overnight, then diluted with 100 ml. of water and extracted with ether. The ether extract was washed with 5% hydrochloric acid, water, then dried ($MgSO_4$) and evaporated to give 195 mg. of viscous oil. This oil was distilled at 100-105° (0.05 mm.) to give dihydrotoxol acetate (XXI), $[\alpha]_D$-164° (c, 1.7; methanol).

λ_{max}^{film} 5.75, 5.98 and 6.21 μ.

The same material was obtained when toxol acetate was hydrogenated using rhodium-on-alumina as catalyst.

2,4-Dinitrophenylhydrazone of dihydrotoxol

A solution of 25 mg. of dihydrotoxol in 10 ml. of methanol was added to 20 ml. of a solution containing 1 g. of 2,4-dinitrophenylhydrazine in 90 ml. of methanol and 10 ml. of hydrochloric acid. The precipitated derivative was collected by filtration, dissolved in benzene and chromatographed on Merck acid-washed alumina (act. I) and then recrystallized from ethanol, m.p. 215-216°. The infrared spectrum contained no carbonyl band.

Anal. Calcd. for $C_{19}H_{18}O_5N_4$: C, 59.67; H, 5.03. Found: C, 59.76; H, 5.35.

Oxidation of toxol to 5-acetylsalicylic acid (XXIV)

To a solution of 180 mg. of toxol in 10 ml. of acetone was added 50 ml. of 5% potassium permanganate in acetone solution and the solution was heated for thirty minutes on a steam bath. After dilution with 100 ml. of 5% hydrochloric acid, a saturated solution of sodium sulfite was added slowly until the solution became colorless. The acetone was distilled out, and the aqueous solution extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 74 mg. of 5-acetylsalicylic acid (XXIV), m.p. 208-210°, mixed with an authentic sample, m.p. 206-207°. The infrared spectra of the two samples were identical.

To a solution of 160 mg. of toxol in 10 ml. of acetic acid was added 790 mg. of chromium trioxide. The solution was stirred for twenty-four hours, then diluted with water and extracted with ether. The ether extract was washed with water and then dried ($MgSO_4$) and evaporated to give 20 mg. of 5-acetylsalicylic acid (XXIV), m.p. 207-209°, mixed m.p. 206-207° with an authentic sample. The infrared spectrum was identical with the spectrum of an authentic sample.

4-Hydroxyisophthalic acid (XXV) from 5-acetylsalicyclic acid

To a solution of 50 mg. of 5-acetylsalicylic acid in 20 ml. of 20% sodium hydroxide was added an excess of a solution containing 1 g. of iodine and 2 g. of potassium iodide per 10 ml. of water. The resulting precipitate was removed by filtration and the filtrate acidified with hydrochloric acid; sodium bisulfite was added to remove the excess iodine. The precipitated acid was collected by filtration and recrystallized from ethanol to give 4-hydroxyisophthalic acid (XXV), m.p. 304-307°, reported (40) m.p. 305-306°.

Ozonolysis of toxol

A stream of approximately 1% ozone in oxygen was passed through a solution of 294 mg. of toxol in 5 ml. of dihloromethane at -70° until the solution became blue. The solution was then warmed to room temperature and 10 ml. of water and 0.5 g. of zinc dust were added; the solution was stirred for four hours and then extracted with water. The aqueous extract was added to 10 ml. of a saturated solution of dimethone. The resulting precipitate was collected by filtration and gave a 38% yield

of the dimethone derivative of formaldehyde, m.p. 191-192° alone and on admixture with an authentic sample.

The dichloromethane solution was dried ($MgSO_4$) and evaporated to give 215 mg. of a yellow oil. Distillation of this oil at 140° (0.04 mm.) gave a solid distillate. Recrystallization from methanol-water (3:1) gave 2,5-diacetylbenzofuran (XXII), m.p. 139-140°, alone or on admixture with an authentic sample prepared from dehydrotremetone. λ_{max}^{KBr} 5.98 μ.; n.m.r. δ2.63 and δ2.66 (methyl ketones).

Anal. Calcd. for $C_{12}H_{10}O_3$: C, 71.27; H, 4.98. Found: 71.10; H, 5.03.

Ozonolysis of toxol acetate

A solution of 273 mg. of toxol acetate in 40 ml. of tetrahydrofuran was ozonized with a stream of about 1% ozone in oxygen at -70° until the solution turned blue. The solution was warmed to room temperature and 50 ml. of water and 0.5 g. of zinc dust were added and the solution stirred for four hours and then extracted with ether; the aqueous phase was added to 10 ml. of a saturated solution of dimethone. The resulting precipitate was filtered to give a 33% yield of the dimethone derivative of formaldehyde, m.p. 191-192° alone and on admixture with an authentic sample.

The tetrahydrofuran phase was dried ($MgSO_4$) and the solvent evaporated to give 205 mg. of an oil. The oil was distilled at 140° (0.04 mm.) to give a solid distillate. Recrystallization from methanol-water gave 2,5-diacetylbenzofuran (XXII), m.p. 139-140° alone and on admixture with an authentic sample. The infrared and n.m.r. spectra were identical with

those of an authentic sample.

2,5-Diacetylbenzofuran from Dehydrotremetone

A solution of 767 mg. of dehydrotremetone in 15 ml. of dioxane containing 6 drops of pyridine was added to a solution of 1.0 g. of osmium tetroxide in 15 ml. of dioxane. After standing in the dark for 12 days hydrogen sulfide was passed through the solution for 1 hr. and the solution filtered. The precipitate was washed with hot ethyl acetate and the combined filtrates were concentrated. The residue was then taken up in 25 ml. of acetic acid to which 1.5 g. of lead tetraacetate was added. After standing overnight, the solution was diluted with 200 ml. of water, and then neutralized with sodium bicarbonate and finally extracted with ether. The ether layer was extracted with 5% potassium hydroxide to remove phenolic material and after drying over sodium sulfate was evaporated to give 251 mg. (35%) of 2,5-diacetylbenzofuran which after recrystallization from methanol-water had m.p. 139-140°.

Anal. Calcd. for $C_{12}H_{10}O_3$: C, 71.27; H, 4.98. Found: C, 71.10; H, 5.03.

The n.m.r. spectrum showed the four aromatic protons in the region 450 to 500 c.p.s. downfield from tetramethylsilane and the protons of the two acetyl groups appeared as two peaks (total of 6 protons) at δ2.62 and δ2.65.

Dihydrotremetone (XXIII) from dihydrotoxol

A solution of 500 mg. of dihydrotoxol in 20 ml. of 95% ethanol was

hydrogenated at atmospheric pressure using 65 mg. of 10% palladium-on-charcoal catalyst. After the uptake of one molar equivalent of hydrogen, the hydrogenation was stopped. The catalyst was removed by filtration and the solvent evaporated at reduced pressure. The residue was dissolved in 50 ml. of ether, and the solution poured through a 0.5 x 2 cm. column of Merck acid-washed alumina (act. I). The solvent was evaporated and the resulting residue distilled at 65° (0.05 mm.) to give 97 mg. of dihydrotremetone (XXIII), $[\alpha]_D$-43° (c, 2.71; ethanol), reported (12) $[\alpha]_D$-47°. The infrared spectrum was identical with the spectrum of racemic dihydrotremetone obtained by the hydrogenation of dehydrotremetone using 10% palladium-on-charcoal catalyst.

Ozonolysis of toxol in acetic acid with ~1% ozone

A stream of about 1% ozone in oxygen was passed through a solution of 1.56 g. of toxol in 60 ml. of acetic acid at room temperature for 25 hours. A 30% solution of hydrogen peroxide (8 ml.) was then added and the solution stirred for 18 hours, then palladium-on-charcoal was added to destroy the excess hydrogen peroxide and the solution stirred for two hours. After filtration, the solvent was evaporated at reduced pressure to give a viscous residue. The residue was esterified with diazomethane in ether and the excess diazomethane was allowed to evaporate. The ether was evaporated and the resulting residue was added to 25 ml. of a 20% sodium hydroxide solution and enough iodine added to impart the color of iodine to the solution. The solution was filtered to remove iodoform and the filtrate extracted with ether to remove dimethyl oxalate. The aqueous

solution was acidified (pH 5) with hydrochloric acid and sodium bisulfite was added until the solution became colorless; the solution was then acidified (pH 1) and extracted continuously with ether. The ether extract was dried ($MgSO_4$) and evaporated; the residue was esterified with excess diazomethane in ether. The excess diazomethane was removed and the solvent evaporated to give 250 mg. of a light yellow oil. This oil was chromatographed on 50 g. of silica gel; 150 mg. of (+)-dimethyl tartrate was eluted with chloroform-acetone (1:1). The infrared and the n.m.r. spectra were identical with the spectra of an authentic sample of dl-dimethyl tartrate; the (+)-dimethyl tartrate gave a plain negative rotatory dispersion curve (c, 0.056; dioxane), $[\alpha]_{589\ m\mu}$ +15.59°, $[\alpha]_{450\ m\mu}$ +8.66°, $[\alpha]_{400\ m\mu}$ -8.66°, $[\alpha]_{260\ m\mu}$ -8.06° as previously described (44,45).

Ozonolysis of toxol in acetic acid with ~ 4% ozone

A stream of oxygen containing about 4% ozone was passed through a solution of 2.1 g. of toxol in 25 ml. of acetic acid at room temperature for 25 hours; a solution of 8 ml. of 30% hydrogen peroxide was then added and the solution stirred for 25 hours, whereupon palladium-on-charcoal was added and the solution stirred an additional two hours. After filtration, the acetic acid was removed at reduced pressure to leave a viscous residue. Oxalic acid was removed from the residue by sublimation at 90° (0.5 mm.) for 12 hours. The remaining residue was decolorized by dissolving in 2 ml. of water and heating with charcoal, after which filtration and concentration gave 110 mg. of (+)-tartaric acid, which after recrystallization from water had m.p. 170-171°, $[\alpha]_D$ +8.40°, (c, 0.032; water),

reported (69) m.p. 170°, $[\alpha]_D$+12° (20% aqueous solution).

This (+)-tartaric acid was esterified by treatment with an ethereal solution of diazomethane. After distillation at 65° (1.2 mm.) (+)-dimethyl tartrate was obtained, $[\alpha]_D$+10.81° (c, 0.021; methanol), reported (44) $[\alpha]_D$+13.82 (methanol). The infrared spectrum was identical with that of an authentic sample of dl-dimethyl tartrate.

BIOLOGICAL TESTING OF SHEEP

The individual sheep were selected at random from a flock of 40 to 50 sheep.* The various fractions were administered either by drenching or by gelatin capsule. The whole plant was mixed with alfalfa hay to induce the sheep to eat the plant. The results of the tests are shown in table II.

TABLE II

BIOLOGICAL TESTING OF SHEEP

Wt. (lbs.) of sheep	Material Fed	Daily Dose (lb. equiv. of plant per 100 lbs. of sheep)	Total Dose Fed (lb. equiv. of plant per 100 lbs. of sheep)	Effect
49.5	Toxol	0.5	7	none
44.0	fraction A	1.75	24.5	none
63.5	dried plant	0.69	15	Trembles (death)

* The selection of the sheep and the administration of the various fractions was performed by Richard Goodrich of the Animal Husbandry Department, Oklahoma State University. The sheep were provided by the Animal Husbandry Department through the courtesy of Dr. Tillman.

TABLE II (Continued)

Wt. (lbs.) of sheep	Material Fed	Daily Dose (lb. equiv. of plant per 100 lbs. of sheep)	Total Dose Fed (lb. equiv. of plant per 100 lbs. of sheep)	Effect
53.5	methanolic plant ext.	4.2	16.8	Trembles (death)
85.8	Toxol	2.8	16.8	none
43.5	30% ethanol soluble	5.65	62.1	none
48.0	ether sol. fraction from hydrol. of 30% ethanol solubles	4.5	49.5	Trembles (sacrificed)

SYNTHETIC STUDIES

2-Isopropylidene-3-coumarone (XLI)

A solution of 5 g. of 3-coumarone (XL), prepared as previously described (49), 5 g. of zinc chloride, 25 ml. of acetone and 5 ml. of methanol was refluxed for three hours. The reaction mixture was diluted with 200 ml. of water and extracted with ether; the ether extract was dried ($MgSO_4$) and the solvent evaporated to give 3.5 g. of 2-isopropylidene-3-coumarone (XLI), which gave after repeated sublimation m.p. 86-87°, λ_{max}^{KBr} 5.84, 6.02 and 6.21 μ; n.m.r. δ2.05 and δ2.30 (methyl groups on double bond), δ7.0 to 7.5 (aromatic, 3 protons).

Anal. Calcd. for $C_{11}H_{10}O_2$: C, 75.98; H, 5.78. Found: C, 76.29; H, 5.85.

To a solution prepared from 23 g. of magnesium and 146 g. of methyl iodide in one liter of ether was added 35 g. of ethyl 3-acetoxycoumarilate (XLVII), prepared as previously described (53), and the solution stirred overnight. The complex was decomposed with 500 ml. of water and 250 ml. of 10% hydrochloric acid and then extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated. The resulting residue was distilled at 110° (0.1 mm.) and the solid distillate sublimed to give 2-isopropylidene-3-coumarone (XLI), m.p. 86-87°; the infrared spectrum was identical with that of the material described above.

2-Isopropyl-3-coumarone (XLII)

A solution of 5 g. of 2-isopropylidene-3-coumarone in 20 ml. of 95% ethanol was hydrogenated at atmospheric pressure using 500 mg. of 5% rhodium-on-alumina catalyst. The uptake of hydrogen ceased after the absorption of one molar equivalent. The catalyst was removed by filtration and the solvent evaporated at reduced pressure to give 5 g. of 2-isopropyl-3-coumarone (XLII) as a viscous oil. λ_{max}^{film} 5.82, 6.21 and 6.72 μ; n.m.r.: δ0.83 (doublet, J=6 cps., methyl group), δ1.60 (doublet, J=6 cps., methyl group), δ4.61 (doublet, J=3 cps., 1 proton) and δ7.0 to 7.5 (aromatic, 4 protons).

2-Isopropyl-3-hydroxycoumaran (XLIII) from 2-isopropyl-3-coumarone (XLII)

A solution of 5 g. of 2-isopropyl-3-coumarone and 1 g. of sodium borohydride in 50 ml. of methanol was refluxed for two hours, then 20 ml. of acetone was added and the solution stirred overnight. The solution was concentrated to 15 ml. and then diluted with 100 ml. of water

and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated to give 3.6 g. of viscous oil. Distillation of this oil at 120° (0.1 mm.) gave a solid distillate; repeated sublimation of this solid gave 2-isopropyl-3-hydroxycoumaran, m.p. 105-106°. λ_{max}^{KBr} 2.95, 6.22 and 6.73 μ; n.m.r.: δ1.05 (triplet, J=6.5 cps., isopropyl group), δ3.68 (quartet, one proton), δ4.60 (doublet, J=5 cps., 2 protons) and δ7.0 to 7.5 (aromatic, 4 protons).

Anal. Calcd. for $C_{11}H_{14}O_2$: C, 74.16; H, 7.92. Found: C, 74.03; H, 7.88.

2-Isopropyl-3-hydroxycoumaran (XLIII) from 2-isopropylidene-3-coumarone XLI

A solution of 5 g. of 2-isopropylidene-3-coumarone and 1 g. of sodium borohydride in 50 ml. of methanol was refluxed for two hours and then diluted with 20 ml. of acetone and the solution stirred overnight. The solution was concentrated to 15 ml. and then diluted with 80 ml. of water and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 3.8 g. of viscous oil. This oil was distilled at 120° (0.1 mm.); the solid distillate was sublimed to give 2-isopropyl-3-hydroxy-coumaran (XLIII), m.p. 105-106°. The infrared spectrum was identical with the infrared spectrum of the material obtained by the reduction of 2-isopropyl-3-coumarone; mixed melting point gave no depression.

Preparation of 2-isopropyl-5-acetylbenzofuran (XLV) using trifluoroacetic acid

To a solution of 5 ml. of acetic anhydride in 10 ml. of trifluoroacetic

acid was added 3.2 g. of 2-isopropyl-3-hydroxycoumaran and the solution was stirred at room temperature for four hours. After pouring into a solution containing an excess of sodium carbonate the reaction mixture was extracted with ether. The ether extract was dried ($MgSO_4$) and then evaporated to give 2.8 g. of a viscous oil. This residue was distilled at 100° (0.1 mm.) to give 2-isopropyl-5-acetylbenzofuran (XLV), λ_{max}^{film} 5.99, 6.48 and 6.62 μ (11).

Preparation of 2-isopropyl-5-acetylbenzofuran (XLV) using stannic chloride

To a solution of 2.0 g. of 2-isopropyl-3-hydroxycoumaran and 2.8 g. of acetic anhydride in 20 ml. of carbon disulfide at 0° was added 10 g. of stannic chloride and the solution stirred for 40 minutes. The solution was diluted with 20 ml. of water and 20 ml. of 20% hydrochloric acid, and then extracted with ether. The ether extract was dried ($MgSO_4$) and then evaporated to give 1.7 g. of viscous oil. This oil was distilled at 100° (0.1 mm.) to give 2-isopropyl-5-acetoxybenzofuran (XLV). The infrared spectrum was identical with that of the material obtained as described above.

2-Isopropyl-3-acetoxycoumaran (XLIV)

To a solution of 14 g. of 2-isopropyl-3-hydroxycoumaran in 125 ml. of pyridine was added 25 ml. of acetic anhydride and the solution stirred overnight then diluted with 1.5 l. of water and finally extracted with ether; the ether extract was washed with 5% hydrochloric acid, dried ($MgSO_4$) and then evaporated. The resulting residue was distilled at 86-88° (0.5 mm.) to give 11 g. of 2-isopropyl-3-acetoxy-coumaran (XLIV)

as a colorless oil, λ_{max}^{film} 5.72, 6.19, 6.22 and 6.74 μ; n.m.r.: δ1.00 (J=6 cps., methyl group), δ1.20 (J=6 cps., methyl group), δ2.02 (acetate methyl group), δ6.25 (J=6 cps., 1 proton) and δ7.0 to 7.5 (aromatic, 4 protons).

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.88; H, 7.32. Found: C, 70.31; H, 6.78.

Preparation of 2-isopropyl-5-acetylbenzofuran from 2-isopropyl-3-acetoxycoumaran using trifluoroacetic acid

A solution of 2.2 g. of 2-isopropyl-3-acetoxycoumaran in 5 g. of trifluoroacetic acid and 2.5 g. of acetic anhydride was stirred for four hours at room temperature. The reaction mixture was poured into a solution containing an excess of sodium bicarbonate and stirred for 30 minutes, then extracted with ether and the ether extract was dried ($MgSO_4$) and evaporated. The resulting residue was distilled at 100° (0.1 mm.) to give 1.3 g. of 2-isopropyl-5-acetylbenzofuran (XLV). The infrared spectrum was the same as that of the material obtained from 2-isopropyl-3-hydroxycoumaran.

Preparation of 2-isopropyl-5-acetylbenzofuran (XLV) from 2-isopropyl-3-acetoxycoumaran using stannic chloride

To a solution of 1.6 g. of 2-isopropyl-3-acetoxycoumaran in 20 ml. of carbon disulfide and 2.5 ml. of acetic anhydride at 0° was added 9 g. of stannic chloride and the solution stirred for 40 minutes then diluted with 50 ml. of 10% hydrochloric acid and extracted with ether; the ether extract was dried ($MgSO_4$) and then evaporated. The resulting residue was

distilled at 100° (0.1 mm.) to give 1.1 g. of 2-isopropyl-5-acetylbenzofuran (XLV) of identical infrared spectrum with that previously obtained.

2-Isopropyl-3-acetoxybenzofuran (XLVI)

A solution of 3 g. of 2-isopropyl-3-coumarone in 50 ml. of carbon tetrachloride and 6 drops of 70% perchloric acid was stirred for two hours, then successively washed with water, 5% potassium hydroxide and water and dried ($MgSO_4$) and the solvent evaporated. The resulting residue was distilled at 95° (0.2 mm.) to give 1.8 g. of 2-isopropyl-3-acetoxybenzofuran (XLVI), λ_{max}^{film} 5.58, 6.21 and 6.90 μ.

A solution of 2 g. of 2-isopropyl-3-coumarone in 5 ml. of trifluoroacetic acid and 2.5 ml. of acetic anhydride was stirred for four hours, then poured into 100 ml. of water containing an excess of sodium bicarbonate and stirred for 30 minutes. The solution was extracted with ether, the ether dried ($MgSO_4$) and evaporated. The residue was distilled at 95° (0.2 mm.) to give 1.5 g. of 2-isopropyl-3-acetoxybenzofuran (XLVI); the infrared spectrum was identical with the spectrum of the material described above.

Preparation of 2-isopropyl-3-coumarone (XLII) from 2-isopropyl-3-acetoxybenzofuran

To a solution of 1.8 g. of 2-isopropyl-3-acetoxybenzofuran in 50 ml. of carbon disulfide and 2 ml. of acetic anhydride was added 3 ml. of stannic chloride and the solution stirred for 40 minutes, then diluted with 50 ml. of 10% hydrochloric acid and extracted with ether; the ether extract was dried ($MgSO_4$) and evaporated. The resulting residue was distilled at 115° (0.1 mm.) to give 1.1 g. of 2-isopropyl-3-coumarone

(XLII), of identical infrared spectrum with that of material described above.

Resolution of dihydrocoumarilic acid

A solution of 10 g. of dihydrocoumarilic acid, prepared as previously described (57,17), and 28.4 g. of brucine in 150 ml. of water was heated to boiling with enough ethanol to effect solution. The solution was cooled slowly and the resulting crystals isolated by filtration and recrystallized six times from water. The salt was decomposed by dissolving it in water and acidifying (pH 2) with 10% sulfuric acid; the solution was extracted with ether, the ether dried ($MgSO_4$) and evaporated to give 500 mg. of (+)-dihydrocoumarilic acid; after recrystallization from methanol it gave m.p. 113-114°, $[\alpha]_D$+11° (c, 1.95; ethanol), reported (46) m.p. 113-114°, $[\alpha]_D$+22°.

(-) Ethyl dihydrocoumarilate

A solution of 500 mg. of (+)-dihydrocoumarilic acid in 10 ml. of absolute ethanol and two drops of concentrated sulfuric acid was refluxed for six hours then diluted with 100 ml. of water and extracted with ether; the ether extract was washed with water, dried ($MgSO_4$) and evaporated. The resulting residue was distilled 110° (0.5 mm.) to give (-)-ethyl dihydrocoumarilate, $[\alpha]_D$-5.3° (c, 1.03; benzene), reported (17) $[\alpha]_D$-11°.

(+)-Ethyl dihydrocoumarilate (XLVIII)

An ethanolic (50 ml.) solution of 1.8 g. of ethyl 3-acetoxycoumarilate (XLVII), prepared as previously described (51,52,53), was hydrogenated at

atmospheric pressure using rhodium-on-alumina as catalyst. The uptake of hydrogen ceased after two molar equivalents had been absorbed. The catalyst was removed by filtration and the solvent evaporated at reduced pressure. The resulting residue was distilled at 110° (0.5 mm.) to give 1.6 g. of ethyl dihydrocoumarilate (XLVIII); the infrared spectrum was the same as the spectrum of a sample prepared as previously described (17).

2-(2'-Dihydrobenzofuryl)-2-propanol (XLIX)

To a solution prepared by adding 10 g. of methyl iodide to 1 g. of magnesium in 100 ml. of ether was added 5 g. of ethyl dihydrocoumarilate in 20 ml. of ether. The reaction mixture was stirred overnight, diluted with 100 ml. of a saturated solution of ammonium chloride, and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated; the resulting residue was distilled at 70-75° (0.5 mm.) to give 3 g. of 2-(2'-dihydrobenzofuryl)-2-propanol (XLIX). The infrared spectrum was the same as the spectrum of the material prepared as previously described (11).

2-(5'-Acetyl-2'-dihydrobenzofuryl)-2-propyl acetate (L)

To a solution of 3 g. of 2-(2'-dihydrobenzofuryl)-2-propanol in 5 ml. of acetic acid at 0° was added 4 g. of trifluoroacetic acid and the solution warmed to room temperature and stirred for four hours. The reaction mixture was poured into 100 ml. of water containing an excess of sodium bicarbonate and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 2.9 g. of a solid residue. Recrys-

tallization from methanol gave 2-(5'-acetyl-2'-dihydrobenzofuryl)-2-propyl acetate (L), m.p. 94-95°, reported (11) m.p. 95-96°.

Oxidation of 2-(5'-acetyl-2'-dihydrobenzofuryl)-2-propyl acetate (L)

To a solution of 5 g. of 2-(5'-acetyl-2'-dihydrobenzofuryl)-2-propyl acetate in 25 ml. of acetic acid and 25 ml. of acetic anhydride at 0° was added 6 g. of chromium trioxide in 25 ml. of acetic anhydride and the solution stirred for one hour. The reaction mixture was diluted with 500 ml. of water and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 3.5 g. of 5-acetylsalicylic acid (XXIV), m.p. 214-215°, reported (59) m.p. 213-214°; the infrared spectrum was the same as the spectrum of the material obtained as previously described (59).

Methyl 5-acetylsalicylate (LI)

To a solution of 180 g. of 5-acetylsalicylic acid and 44 g. of sodium hydroxide was added 127 g. of dimethyl sulfate and the solution stirred for two hours at 0°. The solution was then heated on a steam bath for 15 minutes, cooled and extracted with ether. The ether extract was washed with 5% sodium bicarbonate and then extracted with 10% sodium hydroxide. The basic extract was acidified (pH 2) with hydrochloric acid and the resulting precipitate collected by filtration to give 18 g. of methyl 5-acetylsalicylate (LI); after sublimation, m.p. 60-61°; λ_{max}^{KBr} 5.88, 5.96, 6.31 and 6.94 μ; n.m.r. δ2.43 (methyl ketone) and δ3.98 (methyl ester).

The same ester was obtained when 5-acetylsalicylic acid was treated with an ethereal solution of diazomethane.

Anal. Calcd. for $C_{10}H_{10}O_4$: C, 62.27; H, 5.19. Found: C, 62.47; H, 5.47.

Dimethyl O-carboxymethyl-5-acetylsalicylate (LII)

To a solution of 3.2 g. of sodium methoxide in 100 ml. of absolute methanol was added 9.5 g. of methyl 5-acetylsalicylate and then 6 g. of methyl chloroacetate and the solution refluxed for 24 hours. The methanol was removed at reduced pressure and the resulting residue diluted with 200 ml. of water and extracted with ether. The extract was washed successively with 20% aqueous potassium hydroxide and water, then dried ($MgSO_4$) and the solvent evaporated. The resulting residue was recrystallized from ether-ligroin (1:1) and then sublimed at 95° (0.05 mm.) to give 2.1 g. of dimethyl O-carboxymethyl-5-acetylsalicylate (LII), m.p. 103-104°; λ_{max}^{KBr} 5.65, 5.85, 5.99 and 6.25μ; n.m.r. δ2.54 (methyl ketone), δ3.80 and 3.91 (methyl esters) and δ4.77 (two protons).

Anal. Calcd. for $C_{13}H_{14}O_6$: C, 58.78; H, 5.27. Found: C, 59.16; H, 5.41.

Methyl 3-hydroxy-5-acetylcoumarilate (LIII)

To a solution of 8.5 g. of dimethyl O-carboxymethyl-5-acetylsalicylate in 50 ml. of toluene was added 1 g. of sodium and the solution refluxed until all of the molten sodium had dissolved. The mixture was cooled, 50 ml. of water was added and the solution extracted with 10% sodium hydroxide. The basic extract was acidified (pH 2) with hydrochloric acid and the resulting precipitate collected by filtration. Sublimation at 90° (0.05 mm.) gave 7 g. of methyl 3-hydroxy-5-acetyl-

coumarilate (LIII), m.p. 95-96°; λ_{max}^{KBr} 3.01, 5.91 and 6.01 μ; n.m.r. δ2.47 (methyl ketone) and δ3.88 (methyl ester).

Anal. Calcd. for $C_{12}H_{10}O_5$: C, 63.12; H, 4.83. Found: C, 62.93; H, 4.94.

Attempts to esterify methyl 3-hydroxy-5-acetylcoumarilate (LIII)

To a solution of 1 g. of methyl 3-hydroxy-5-acetylcoumarilate in 20 ml. of pyridine was added 2 ml. of acetic anhydride and the solution stirred overnight. The solution was diluted with 200 ml. of water and extracted with ether; the ether extract was extracted with 10% sodium hydroxide. The basic extract was acidified (pH 2) and the resulting precipitate collected by filtration to give 900 mg. of starting material, identified by its infrared spectrum.

A solution of 1 g. of methyl 3-hydroxy-5-acetylcoumarilate in 50 ml. of carbon tetrachloride, 2 ml. of acetic anhydride and 6 drops of 70% perchloric acid was stirred for two hours. The reaction mixture was extracted with 10% sodium hydroxide and the basic extract was acidified (pH 2) with hydrochloric acid. The resulting precipitate (850 mg.) was identified as starting material by its infrared spectrum.

A solution of 1 g. of methyl 3-hydroxy-5-acetylcoumarilate in 5 ml. of acetic anhydride and 2 ml. of trifluoroacetic acid was stirred for three hours. The solution was poured into 100 ml. of water containing an excess of sodium bicarbonate and extracted with ether; the ether extract was dried ($MgSO_4$) and evaporated to give 850 mg. of starting material, identified by its infrared spectrum.

To a solution of 1 g. of methyl 3-hydroxy-5-acetylcoumarilate in 50 ml. of benzene was added 0.5 g. of sodium hydride and the 1.5 g. of

benzoyl peroxide and the solution stirred for two days at room temperature. Excess sodium hydride was decomposed with ethanol, and the solution was extracted with 10% sodium hydroxide; the basic extract was acidified (pH 2) with hydrochloric acid. The resulting precipitate (700 mg.) was identified as starting material by its infrared spectrum.

Dihydrotremetone (XXIII) from dehydrotremetone (I)

A solution of 1.5 g. of dehydrotremetone in 20 ml. of 95% ethanol was hydrogenated at atmospheric pressure using 10% palladium-on-charcoal as catalyst. The uptake of hydrogen ceased after the absorption of two molar equivalents. The catalyst was removed by filtration and the solvent evaporated at reduced pressure; the resulting residue was distilled at 100° (0.1 mm.) to give 1.3 g. of dihydrotremetone (XXIII), the infrared spectrum of which was the same as that reported (11).

Reaction of N-bromosuccinimide with dihydrotremetone ketal

A solution of 1 g. of dihydrotremetone in 50 ml. of benzene and 20 ml. of ethylene glycol and 100 mg. of p-toluenesulfonic acid was refluxed for two days. The water was removed as formed with a Dean-Stark trap. The solution was poured into 200 ml. of water containing an excess of sodium bicarbonate and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated. The resulting residue was dissolved in 50 ml. of carbon tetrachloride to which was added 880 mg. of N-bromosuccinimide and 100 mg. of benzoyl peroxide and the solution refluxed for four hours. The succinimide was removed by filtration and the filtrate washed with water and then the solvent was evaporated. The residue was

dissolved in 50 ml. of 50% methanol and 10 ml. of 10% hydrochloric acid and the solution refluxed for one hour. The reaction mixture was diluted with 200 ml. of water and extracted with ether; the ether extract was dried ($MgSO_4$) and the solvent evaporated to give 500 mg. of 2-isopropyl-5-acetylbenzofuran (XLV), identified in infrared spectrum with the same material described above.

Reaction of lead tetraacetate with 2-isopropyl-5-acetylbenzofuran ketal

A solution of 1 g. of 2-isopropyl-5-acetylbenzofuran in 50 ml. of benzene and 25 ml. of ethylene glycol and 100 mg. of p-toluenesulfonic acid was refluxed for two days; the water was removed as formed with a Dean-Stark trap. The reaction mixture was poured into 200 ml. of water containing an excess of sodium bicarbonate and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated. The residue was dissolved in 50 ml. of acetic acid to which was added 2 g. of lead tetraacetate and the solution heated for three hours at 75° and then stirred overnight at room temperature. The reaction mixture was diluted with 300 ml. of water and extracted with ether; the ether extract was washed with 10% sodium hydroxide, dried ($MgSO_4$) and the solvent evaporated. The resulting residue was dissolved in 50 ml. of 50% methanol and 10 ml. of 10% hydrochloric acid and the solution refluxed for one hour. The solution was diluted with 200 ml. of water and extracted with ether; the ether extract was dried ($MgSO_4$) and the solvent evaporated to give 600 mg. of a viscous oil. The infrared spectrum showed a strong hydroxyl band at 2.9 μ. The n.m.r. spectrum indicated a mixture of starting material and 2-(5'-acetyl-2'-benzofuryl)-2-propanol (LIV),

the presence of the latter compound was indicated by the two signals at δ1.33 and δ1.43 in its n.m.r. spectrum.

Reaction of sodium hypobromite on dihydrotoxol acetate

To a solution prepared by adding 3 g. of bromine to 50 ml. of 20% sodium hydroxide was added 1.5 g. of dihydrotoxol acetate in 10 ml. of dioxane and the solution stirred for two hours. After extraction with ether, the aqueous solution was acidified (pH 2) with hydrochloric acid, then extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give acid LV. This acid was added to a solution of 6 g. of methyllithium in 100 ml. of ether and the solution refluxed for 24 hours. The reaction mixture was diluted with 100 ml. of a saturated ammonium chloride solution and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated to give 600 mg. of dihydrotoxol (XX), identified by its infrared spectrum.

Attempted hydroboration of 2-isopropylbenzofuran (LVI)

Diborane, prepared by addition of 2.2 g. of sodium borohydride in 20 ml. of diglyme to 6.7 g. of boron trifluoride etherate in 20 ml. of diglyme, was added in a nitrogen stream to a solution of 2.9 g. of 2-isopropyl-benzofuran, prepared as previously described (62), in 100 ml. of ether and the solution stirred overnight at room temperature. The excess diborane was decomposed with 20 ml. of water and 10 ml. of 3 N sodium hydroxide and 3 ml. of 30% hydrogen peroxide were added and the solution stirred for three hours. The ether layer was washed with water, dried ($MgSO_4$) and the solvent evaporated to give 2.5 g. of starting

material, identified by its infrared spectrum.

The reaction of N-bromosuccinimide with 2-isopropyldihydrobenzofuran (LVII)

To a solution of 4.8 g. of 2-isopropyldihydrobenzofuran, prepared as previously described (15) in 50 ml. of carbon tetrachloride was added 5.2 g. of N-bromosuccinimide and 100 mg. of benzoyl peroxide and the solution refluxed for two hours. The succinimide was removed by filtration and the filtrate washed with water, dried ($MgSO_4$) and evaporated to dryness. The residue (LVIII) was dissolved in benzene and the solvent concentrated at reduced pressure. The residue gave a positive Beilstein test. The n.m.r. spectrum showed two benzylic protons at δ3.26 and integration indicated only three aromatic protons.

Reaction of Fenton's reagent (63) with 2-isopropyldihydrobenzofuran

To a solution of 3.4 g. of 2-isopropyldihydrobenzofuran in 30 ml. of dioxane-water (1:1) was added 30 ml. of a 10% ferrous sulfate solution and 25 ml. of 3% hydrogen peroxide. The ferrous sulfate and hydrogen peroxide solutions were added dropwise, the ferrous sulfate being always kept in slight excess. After addition was complete, the solution was stirred for 30 minutes, diluted with 200 ml. of water and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated to give 3.2 g. LIX. The infrared spectrum showed a strong hydroxyl band at 2.9 μ; the n.m.r. spectrum showed two benzylic protons at δ3.18, but integration indicated only three aromatic protons. The product was soluble in 5% sodium hydroxide, but insoluble in 5% sodium bicarbonate.

Triethyl 3-hydroxycoumaran-2,2,5-tricarboxylate (LXI)

To a solution of 10 G. of ethyl 4-hydroxyphthalaldehydate (LX), prepared as previously described (64,65), in 150 ml. of 2-butanone was added 7 g. of potassium carbonate and then 14 g. of diethyl bromomalonate and the solution refluxed for six hours, as previously described (65). The solution was concentrated to 25 ml., suspended in 200 ml. of water and acidified (pH 2) with 10% sulfuric acid and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated to give 16 g. of triethyl 3-hydroxycoumaran-2,2,5-tricarboxylate (LXI), m.p. 96-98°, λ_{max}^{film} 2.95, 5.76-5.90, 6.22 μ. Reported for 3-hydroxycoumaran-2,2-diethyl-5-methyl-tricarboxylate, m.p. 94-95° (65).

3-Coumarone-2,5-dicarboxylic acid (LXII)

To a solution of triethyl 3-hydroxycoumaran-2,2,5-tricarboxylate (18 g.) in 250 ml. of acetone at 0° was added 14 ml. of Jones' reagent (4 moles per ml.) (66) over a period of one hour. The solution was diluted with 500 ml. of water and extracted with ether; the ether extract was washed with water, dried ($MgSO_4$) and the solvent evaporated. The resulting residue was dissolved in 150 ml. of 50% aqueous methanol containing 30 g. of sodium hydroxide and the solution refluxed for 16 hours. The solution was diluted with 200 ml. of water and acidified (pH 2) with hydrochloric acid. The resulting precipitate was collected by filtration and recrystallized from methanol to give 8 g. of 3-coumarone-2,5-dicarboxylic acid (LXII), m.p. $> 300°$, λ_{max}^{KBr} 5.85, 5.88, 6.27 μ.

Dimethyl 3-coumarone-2,5-dicarboxylate (LXIII)

An ethereal solution of 8 g. of 3-coumarone-2,5-dicarboxylic acid was treated with an excess of diazomethane in ether. The excess diazomethane was evaporated and the solvent removed. The resulting residue was recrystallized from methanol to give 8 g. of dimethyl coumarone-2,5-dicarboxylate (LXIII), m.p. 130-140°, λ_{max}^{KBr} 5.78, 5.85 and 5.90μ; n.m.r. δ3.90 and δ3.94 (methyl esters).

Anal. Calcd. for $C_{12}H_{10}O_6$: C, 57.50; H, 4.02. Found: C, 57.73; H, 4.11.

Attempts to prepare dimethyl 3-acetoxybenzofuran-2,5-dicarboxylate

A solution of 1 g. of dimethyl 3-coumarone-2,5-dicarboxylate (LXIII) in 25 ml. of isopropenyl acetate and 100 mg. of p-toluenesulfonic acid was refluxed for three hours. The solution was concentrated to 10 ml. by slow distillation of the solvent, then poured into 200 ml. of water containing an excess of sodium bicarbonate and finally extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated to give 920 mg. of starting material, identified by its infrared spectrum.

A solution of 1 g. of dimethyl 3-coumarone-2,5-dicarboxylate in 25 ml. of isopropenyl acetate and two drops of concentrated sulfuric acid was refluxed for three hours. The solution was concentrated to 10 ml. by slow distillation of the solvent, then poured into 200 ml. of water containing an excess of sodium bicarbonate and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated to give 947 mg. of starting material, identified by its infrared spectrum.

Attempts to prepare dimethyl 3-coumarone-2,5-dicarboxylate ketal

A solution of 1 g. of dimethyl 3-coumarone-2,5-dicarboxylate in 25 ml. of benzene and 25 ml. of ethylene glycol and 100 mg. of p-toluenesulfonic acid was refluxed for two days under a Dean-Stark trap; however, very little water was obtained. The solution was poured into 200 ml. of water containing an excess of sodium bicarbonate and extracted with ether. The ether extract was dried ($MgSO_4$) and the solvent evaporated to give 910 mg. of starting material, identified by its infrared spectrum.

Preparation of 1-(O-Hydroxyphenyl)-3-methyl-1-butanol diacetate (LXIV)

To a solution of 100 g. of isobutyl bromide and 17 g. of magnesium in 500 ml. of ether was added 37 g. of salicylaldehyde and the solution stirred for one hour. The complex was decomposed with 250 ml. of water and 500 ml. of a saturated solution of ammonium chloride and extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated. The residue was dissolved in 400 ml. of pyridine and 100 ml. of acetic anhydride and the solution stirred overnight. The solution was diluted with 2 l. of water and extracted with ether. The ether ectract was washed with water and 5% hydrochloric acid, dried ($MgSO_4$) and evaporated. The residue was distilled at 100° (0.2 mm.) to give 54 g. of a colorless liquid whose infrared and n.m.r. spectra indicated it to be LXIV.

Attempted Fries rearrangement of LXIV

To a solution of 8 g. of aluminum chloride in 50 ml. of nitrobenzene

was added 10 g. of LXIV and the solution heated at 60° for one hour. The complex was decomposed with 50 ml. of 10% hydrochloric acid and the nitrobenzene steam distilled. The aqueous residue was extracted with ether, the ether dried ($MgSO_4$) and evaporated. The infrared spectrum of the residue was the same as the starting material.

Attempted acylation of LXIV

A solution of 10 g. of LXIV in 5 ml. of trifluoroacetic acid and 5 ml. of acetic anhydride was stirred overnight. The solution was poured into 50 ml. of a saturated solution of sodium carbonate and then extracted with ether. The ether extract was dried ($MgSO_4$) and evaporated to give 8 g. of starting material.

To a solution of 10 g. of LXIV in 50 ml. of carbon disulfide was added 10 g. of stannic chloride and the solution refluxed for 30 minutes. The complex was decomposed with 50 ml. of 10% hydrochloric acid and extracted with ether; the ether extract was dried ($MgSO_4$) and evaporated to give 7 g. of starting material.

Plate I

Infrared Spectrum of Steroid I: 5α-Androstane-3β,16α,17α-Triol

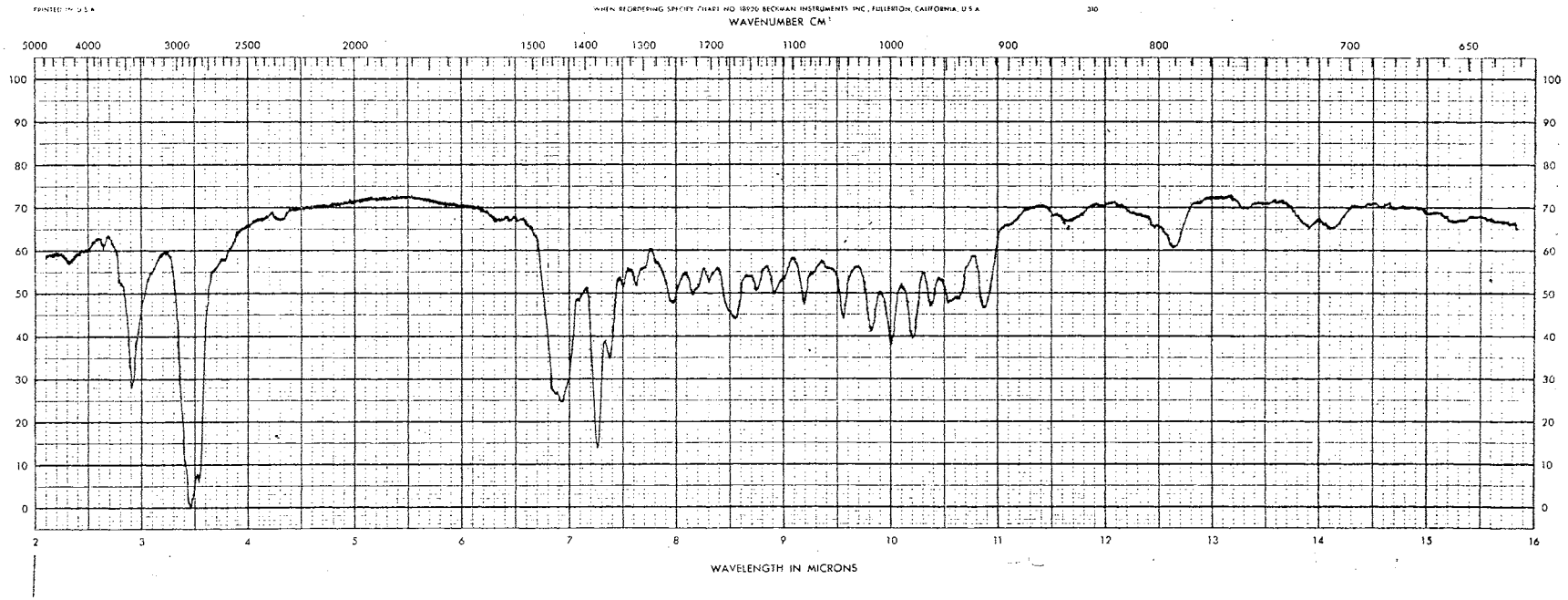


Plate II

Infrared Spectrum of Toxol

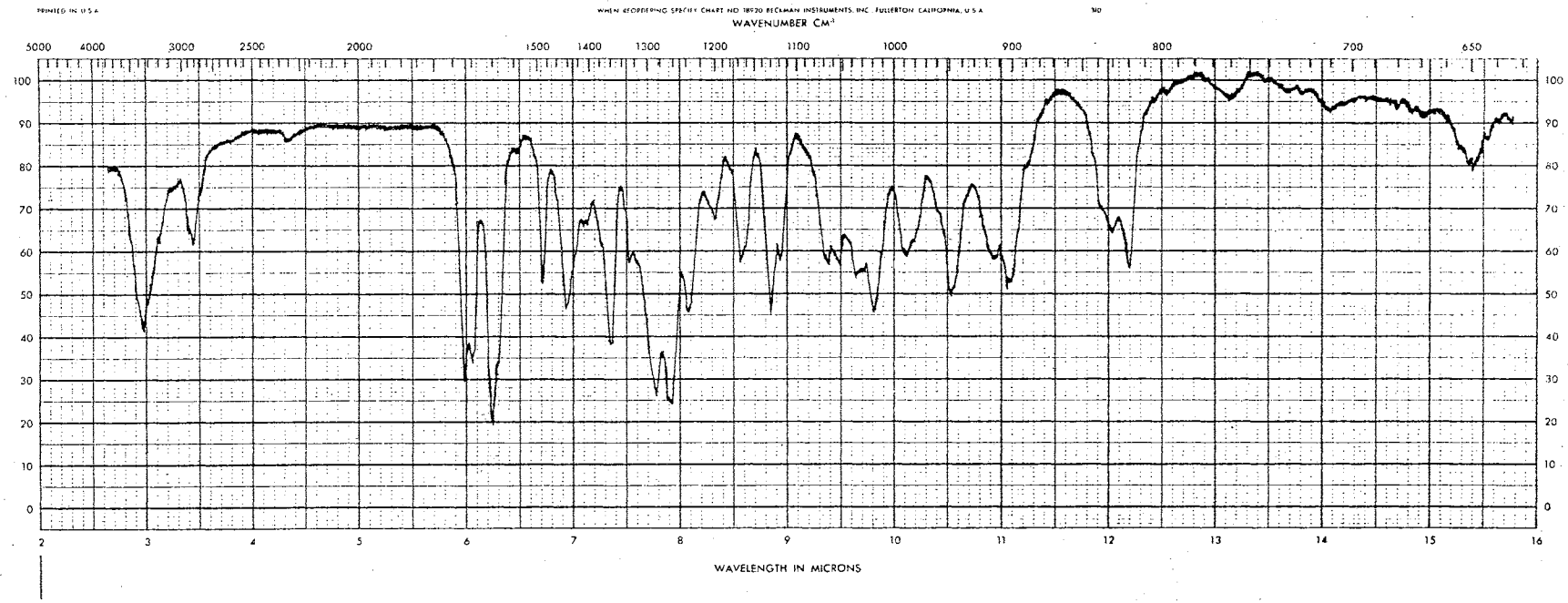
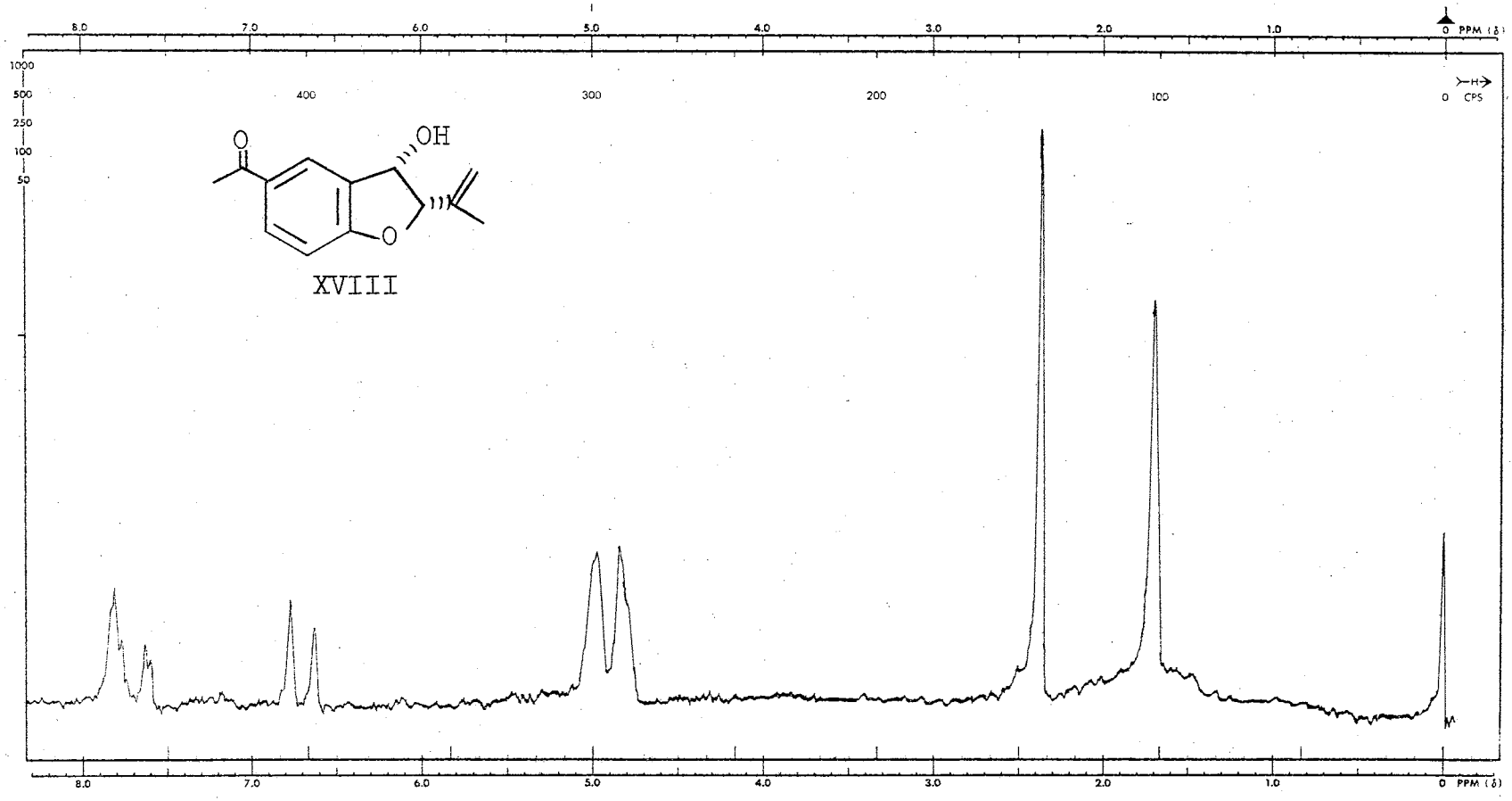


Plate III

Nuclear Magnetic Resonance Spectrum of Toxol



XVIII

Plate IV

Nuclear Magnet Resonance Spectrum of Steroid I
Acetate: 5α-Andostane-3β,16α,17α-Triol Triacetate

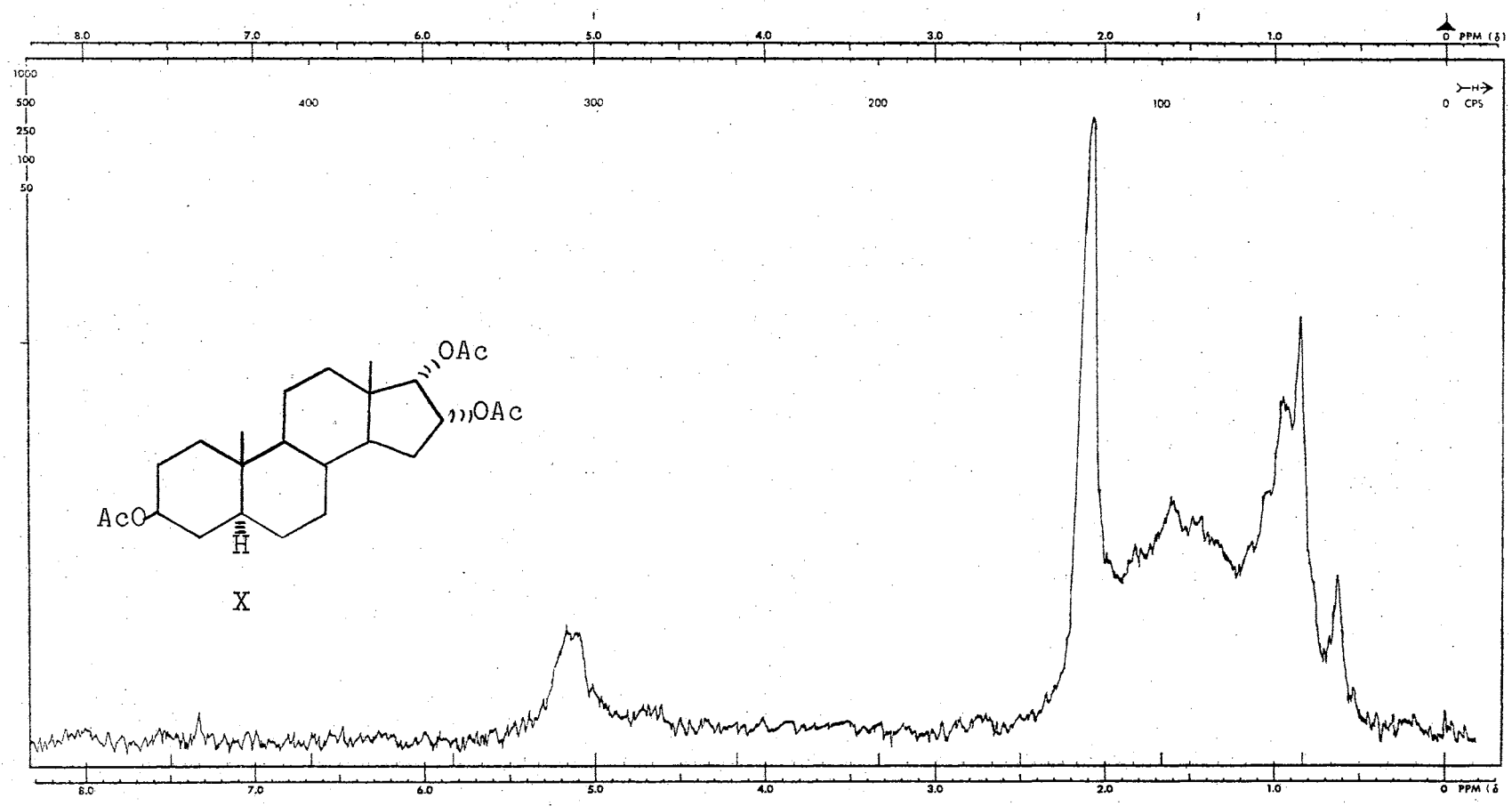


Plate V

Nuclear Magnetic Resonance Spectrum of Toxol Acetate

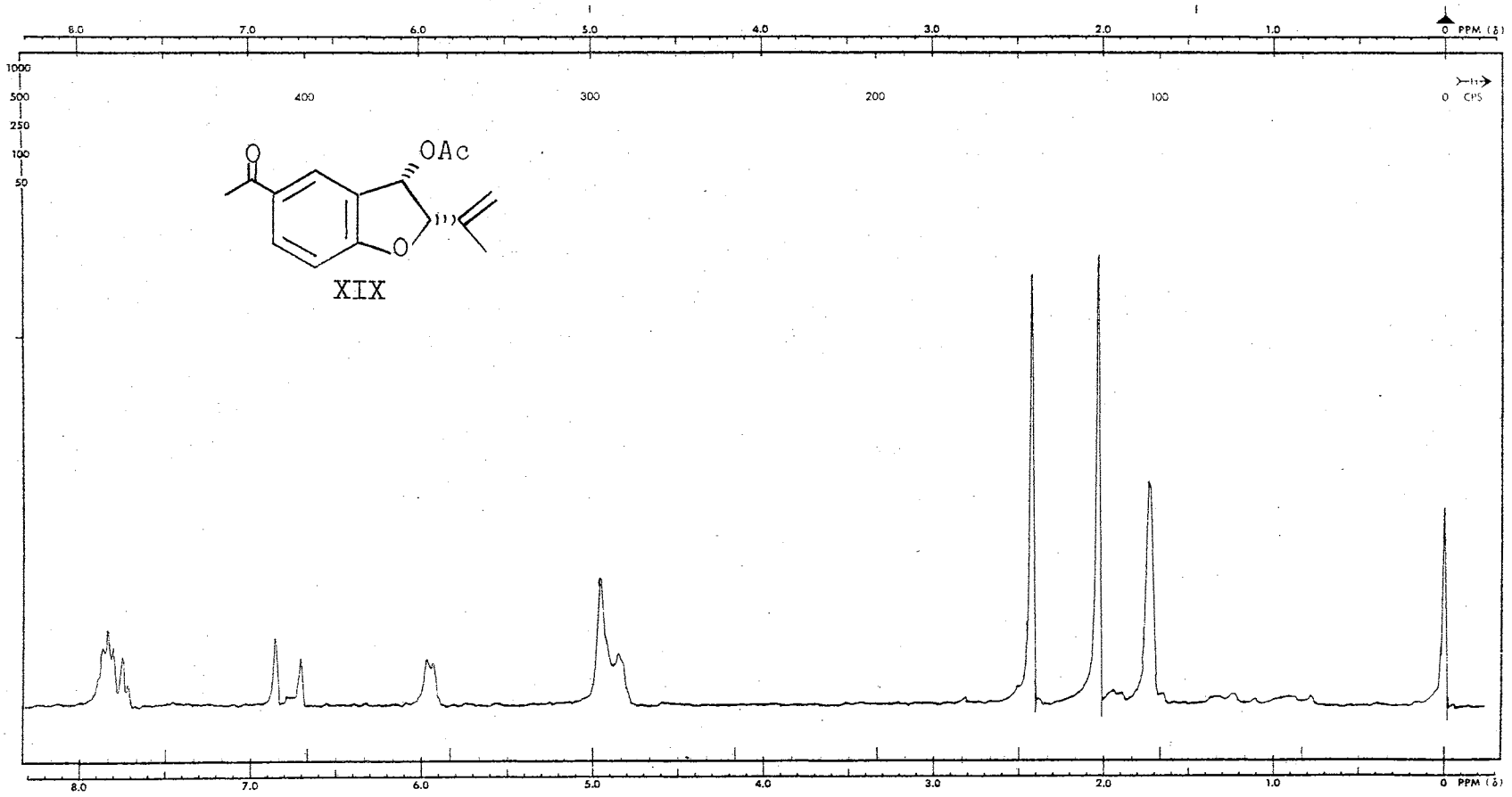
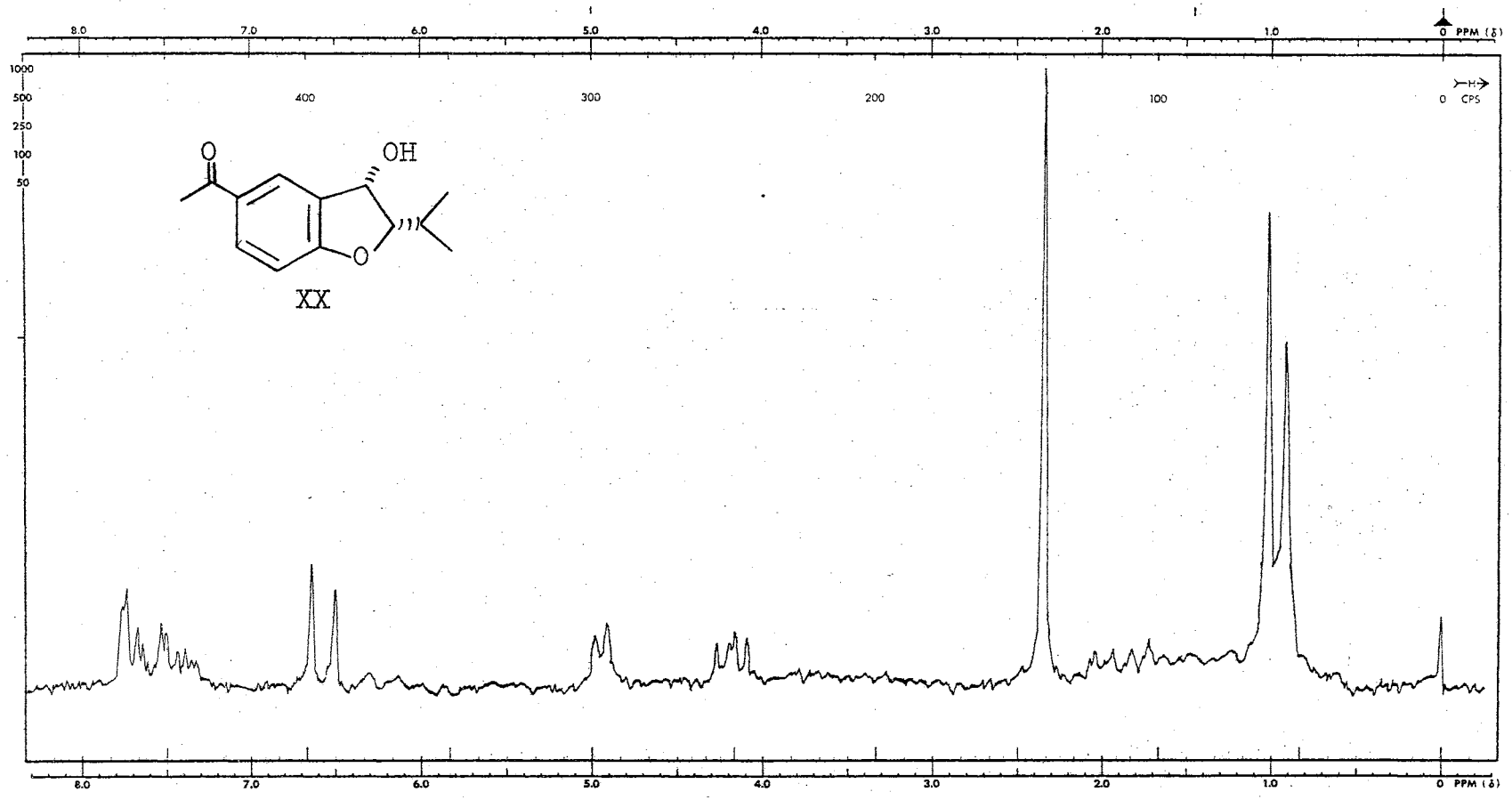


Plate VI

Nuclear Magnetic Resonance Spectrum of Dihydrotoxol



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