A STUDY OF THE TOXIC PLANT RAYLESS GOLDENROD

(APLOPAPPUS HETEROPHYLLUS)

By

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CHAPTER I

HISTORICAL AND INTRODUCTION

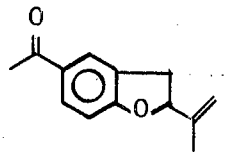
Applopappus heterophyllus, more commonly known as rayless goldenrod, is a plant native to several regions of the southwestern part of the United States, including New Mexico, Colorado and Arizona. Marsh, Roe and Clawson[1] established that rayless goldenrod was responsible for "trembles" in animals and "milksickness" in humans and calves. These diseases have also been shown to arise from Eupatorium urticaefolium (white snakeroot) in the central part of the United States.

Rayless goldenrod is a perennial plant growing six inches to three feet tall and headed with yellow flowers. It looks similar to goldenrod, its hay fever-producing cousin of the genus Solidago, which is native to most of North America.

Couch[2,3] showed by an extraction process that "tremetol" was present in both rayless goldenrod and white snakeroot. This was assumed to be the toxic component. Dermer and his students[4,5,6,7] found, however, that rayless goldenrod "tremetol" was not a pure compound; they found it to be a complex mixture.

Bonner and co-workers[8,9,10] obtained tremetone (1), dehydrotremetone (2) and hydroxytremetone (3) from white snakeroot "tremetol". Zalkow and co-workers[11,12] obtained 2 and a similar but previously unreported compound, toxol (4), from the alcoholic extract of rayless goldenrod processed in a manner similar to that described by Couch to

obtain "tremetol".



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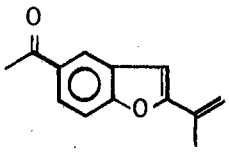

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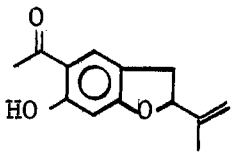

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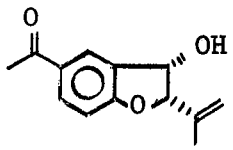
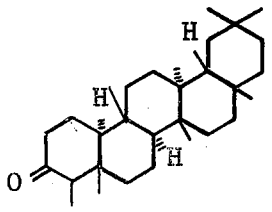


4

During the course of this investigation, 4, friedelin (5), 5α-androstane-3β,16α,17α-triol (6), 2,5-diacetylbenzofuran (7) and two unknown alcohols, A and B, have been isolated. Alcohol A[13,15], 5[14], and 6[13] have been previously reported in the plant extract and 7 has been synthesized[16].



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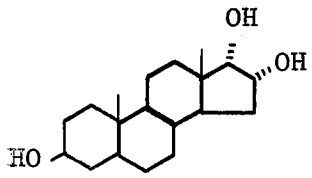

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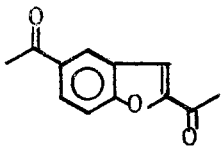

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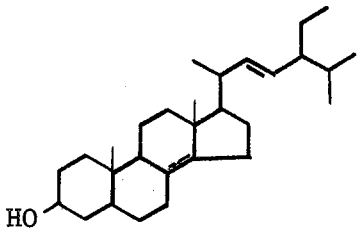
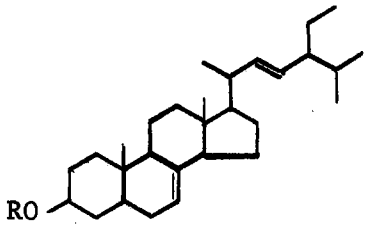


8

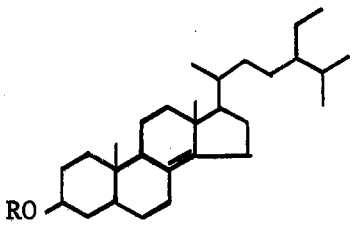
The isolation of 6 is of interest because it is believed to be the first time an androgen has been obtained from a plant source.

The unknown alcohol A is interesting because of its close relationship to α-spinasterol, a sterol which was once thought[17] to be stigmasta-8(14):22-dien-3β-ol (8), but which was later shown by Barton[18] and Fieser[19] to be stigmasta-7:22-dien-3β-ol (9). The acetates of 9[18], β-spinasterol[20], δ-spinasterol[20], and γ-spinasterol[21] may be converted

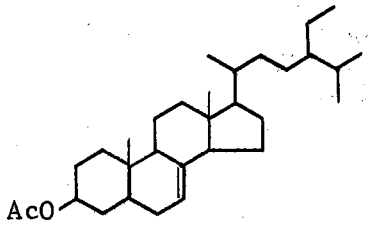
to α-spinastenyl acetate (10) upon hydrogenation in acetic acid in the presence of Adams' catalyst (PtO_2). The acetate of 9 may be converted to Δ^7-spinastenyl acetate (11) by hydrogenation in a neutral solvent.



9 R = H
13 R = $COCH_3$



10 R = $COCH_3$
14 R = H



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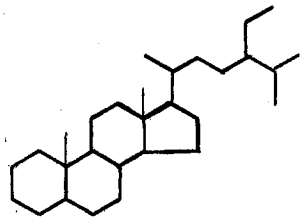


12

The structure of 8 has been suggested for isospinasterol[22] and also for γ-spinasterol[23]. Isospinasterol is formed by the hydrolysis of the chloroacetate of α-spinasterol. γ-Spinasterol is formed by the acid hydrolysis of α-spinasterol esters. Georg[23] suggests that γ-spinasterol is actually a mixture of compounds with the double bond only partially isomerized to the 8(14) position. He believes that in the formation of the chloroacetic ester of 9 the double bond may be partially isomerized to the fourteen position. Takeda, Kubota and Matsui[24] have found β-spinasterol to be a mixture of 9 and 13.

The ketone, 7, is important because of its close relationship to 2, 4 and the other compounds found in "tremetol".

CHAPTER II

RESULTS AND DISCUSSION

Collection and Extraction of the Plant

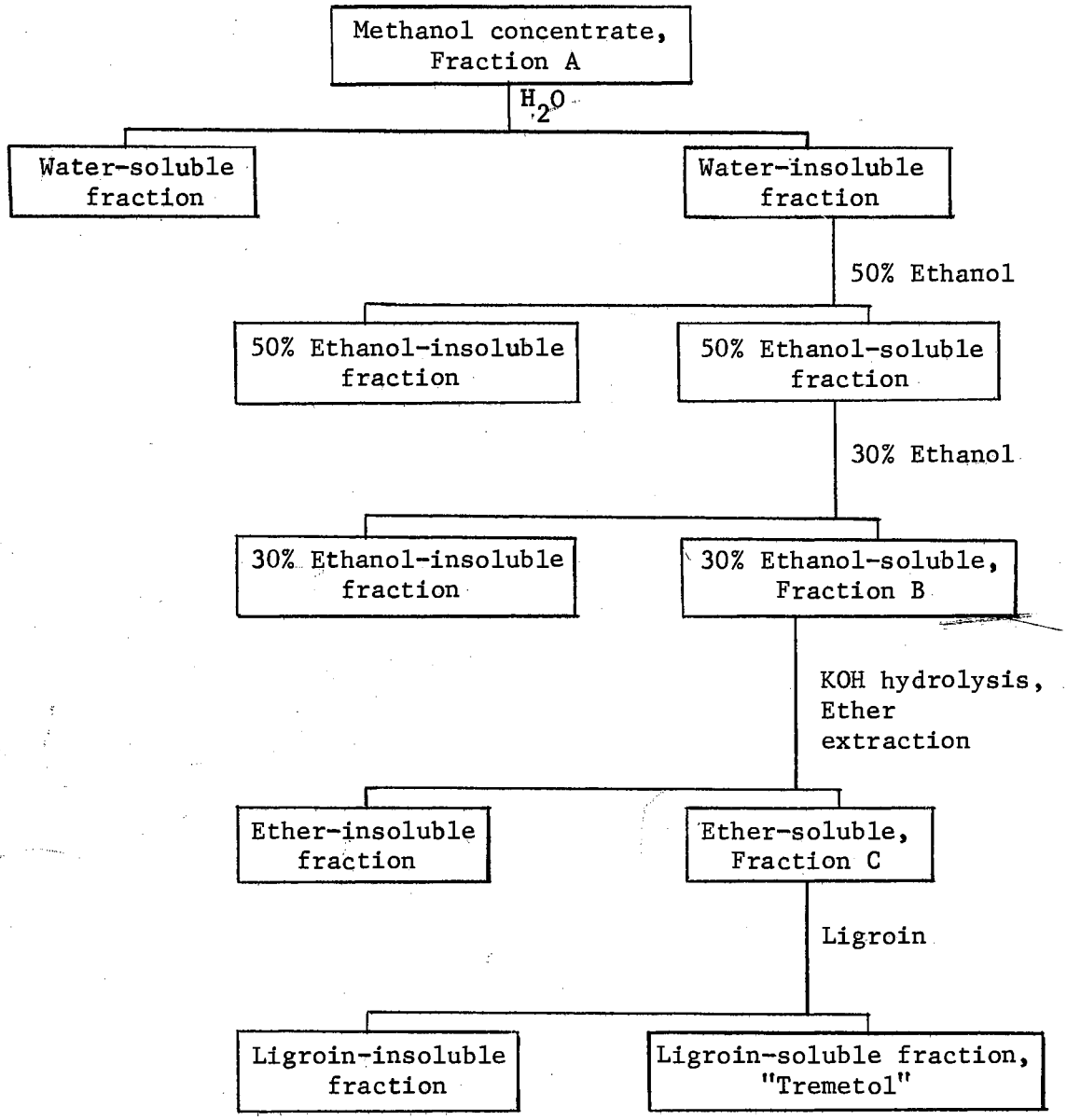
Rayless goldenrod was collected during August, 1963, and in August, 1964, in the general area of Roswell, New Mexico. The plant was cut at ground level, dried, and ground. The ground plant material was extracted with methanol in Soxhlet extractors. The solvent was removed, yielding a dark-colored oil, fraction A.

Separation of Fraction A by the Couch Procedure (Chart A)

The concentrated methanol extract, fraction A, was extracted in boiling water, the insoluble oil was settled by the addition of a small amount of chloroform, and the supernatant water layer was decanted. The chloroform was boiled off, and the residue was extracted with boiling fifty percent ethanol. This solution was filtered through glass wool. The fifty percent ethanol solution was diluted until it contained thirty percent ethanol. The solution was boiled and filtered through a glass wool filter. The ethanol was boiled from the solution. A small amount of chloroform was added to the resulting aqueous solution, which was stirred and allowed to stand overnight until the emulsion separated. The water was decanted and the chloroform was evaporated, leaving a residue, fraction B. Fraction B was hydrolyzed with five percent methanolic potassium hydroxide; the resulting solution was

CHART A

SEPARATION BY THE COUCH PROCEDURE



extracted with ether. The ether solution was washed with water, dried and concentrated to yield fraction C. The ligroin (bp 30-60°)-soluble portion of fraction C should be essentially equivalent to Couch's "tremetol".

Separation of Fraction A by an Alternate Procedure (Chart B)

Fraction A was hydrolyzed with five percent methanolic potassium hydroxide, and the resulting solution was extracted with ether. The ether solution was washed with water, dried, and concentrated to yield fraction D.

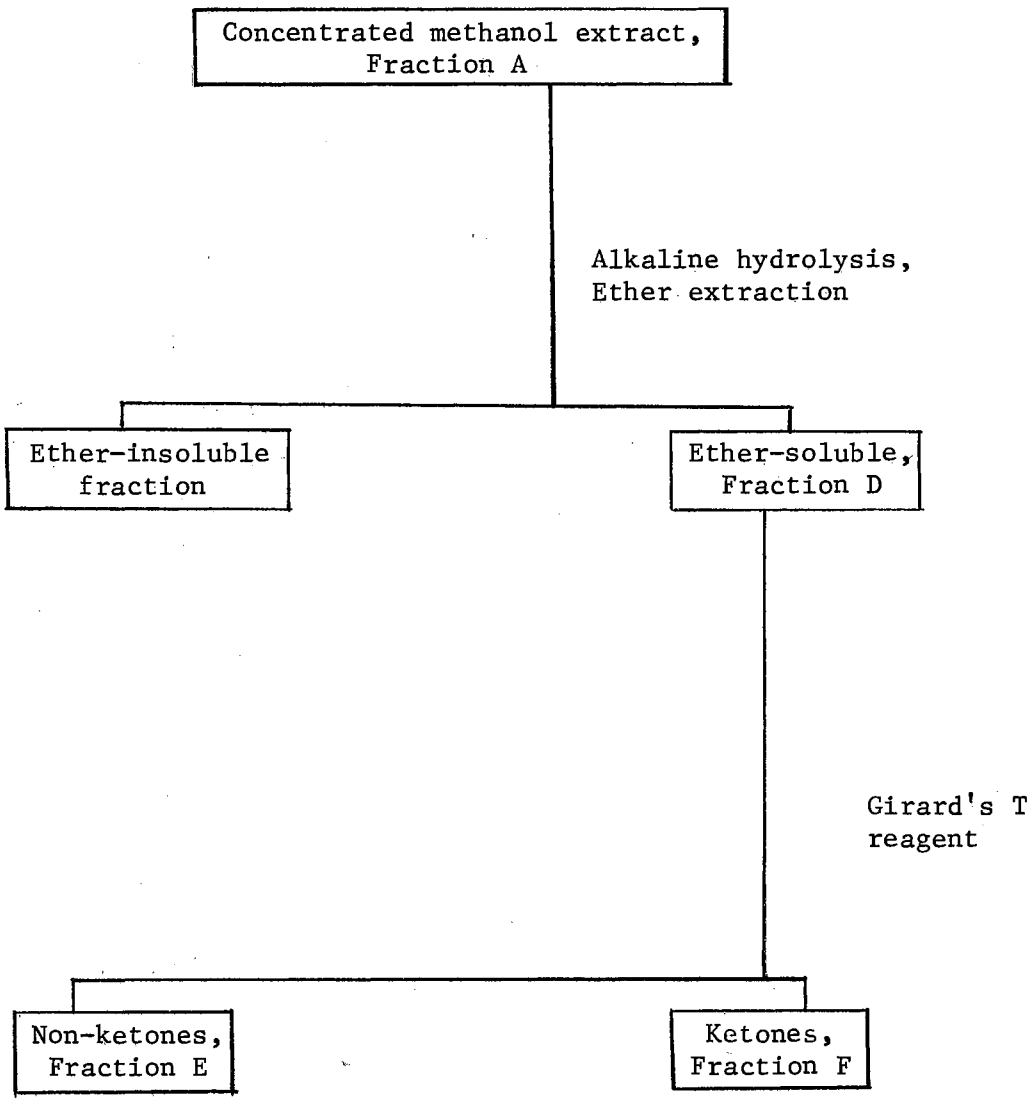
Fraction D was partially separated into ketonic and non-ketonic fractions by the use of Girard's T reagent in methanol. The methanol solution was diluted with an aqueous solution of sodium carbonate and the resulting solution was extracted with ether. Evaporation of the ether gave the non-ketonic fraction E. The aqueous solution was acidified carefully to pH 2 and extracted with ether. Evaporation of the ether gave fraction F.

Chromatography of Fraction B

Fraction B (Chart A) was extracted with petroleum ether. The petroleum ether-soluble fraction was chromatographed over activity I alumina. From this chromatography 5, 6, 7 and an unknown compound were isolated. Friedelin (5) was eluted from the column with benzene-ether (1:1 and 4:1). When the solvent was changed to benzene-ether (3:2), 6 was eluted as a crystalline solid but contaminated with 5. No pure compound was isolated with benzene-ether (1:1), but when the solvent was changed to benzene-ether (4:6) a sweet-smelling, green band was eluted.

CHART B

SEPARATION OF FRACTION A BY AN ALTERNATE PROCEDURE



The green viscous residue remaining after the solvent had been removed slowly crystallized to pale yellow crystals melting at 139°. The infrared spectrum of these crystals indicated that it was an aromatic ether, possibly a benzofuran. A comparison of the melting point and the infrared spectrum of this compound showed it to be identical to 7 obtained in the degradation of 4 by Zalkow, Burke, Cabat and Grula.[11] Since this identification, 7 has been synthesized by Zalkow and Ramming.[16]

The portion of the material eluted with ether contained a compound melting at 128°. The infrared spectrum of this compound indicated that it was an alcohol with no double bonds. The nmr spectrum of the compound was consistent with this assumption. The alcohol gave a negative tetranitromethane test and formed an acetate. The nmr spectrum showed it to be a diacetate. The unknown alcohol will be called alcohol B.

The chromatography of fraction B seems to be an efficient way to obtain 5, 7 and alcohol B.

Chromatography of Fraction E (Chart C)

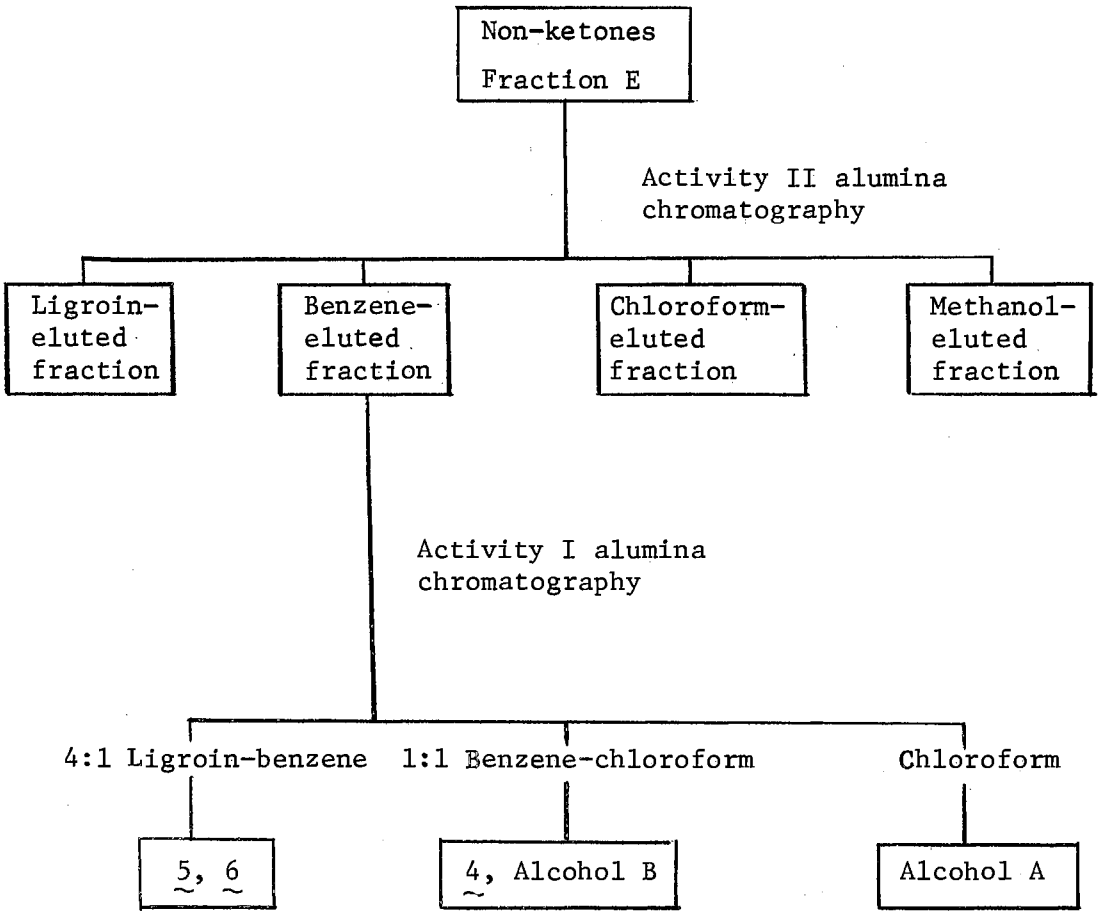
Fraction E was the unsaponifiable portion of the methanol extract which has had some of the ketones removed by the use of Girard's T reagent. From the chromatography of fraction E several compounds were isolated: 4, 5, 6, unknown alcohol A, and unknown alcohol B.

The petroleum ether soluble portion of fraction E was chromatographed over activity II alumina. Four major fractions were collected: a petroleum ether fraction, a benzene fraction, a chloroform fraction and a methanol fraction.

The benzene fraction was re-chromatographed over activity I

CHART C

CHROMATOGRAPHY OF FRACTION E



alumina. The material eluted with ligroin (bp 30-60°)-benzene (4:1) contained a white solid which was removed, recrystallized from acetone, and washed with ligroin to yield a white crystalline solid melting at 286-288° after a slight softening at 265°. The infrared spectrum of this compound was identical to that of 6 isolated from this plant by Zalkow and Burke[13]. The final wash with ligroin seems to be necessary to remove the last traces of 5. The fraction eluted with benzene-chloroform (1:1) contained a small amount of alcohol B along with a small amount of a liquid giving an infrared spectrum identical[14] to that of 4.

The fraction eluted with chloroform slowly crystallized. The solid was recrystallized from acetone and dried to yield an unknown sterol (alcohol A) previously isolated in this laboratory[15] and thought to be 8.

The chromatography of fraction E is a fairly good way to isolate 5, 6, unknown alcohol A, and unknown alcohol B, but the yield of 4 is low owing to the partial removal of ketones with Girard's T reagent.

Chromatography of Fraction F

Fraction F is the ketonic fraction from the Girard's T separation (see Chart B). This fraction was investigated by Burke.[14] Fraction F was chromatographed over activity I alumina. The fraction eluted with chloroform yielded 4.

Purification and Properties of Individual Components

Unknown Alcohol A

The non-ketonic fraction E (Chart B) was chromatographed over

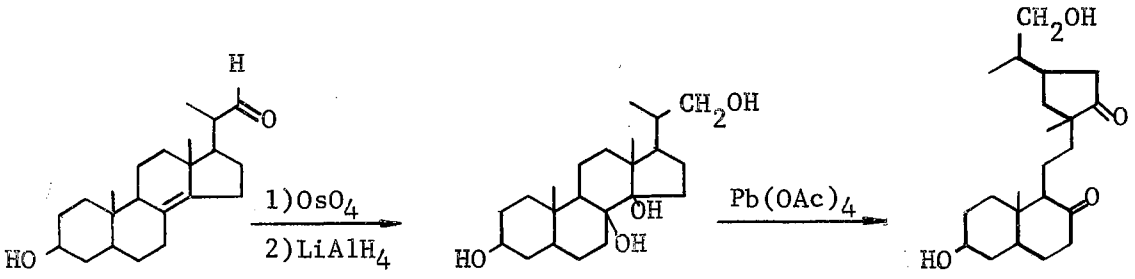
activity II alumina (see Chart C). Four fractions were collected. The benzene fraction was chromatographed over activity I alumina. The fraction eluted with chloroform slowly crystallized. The solid was recrystallized from methanol to give a solid melting at 154-156°. It was recrystallized from acetone and dried under vacuum overnight to yield a solid melting at 161-162°. The infrared spectrum of the compound indicated that it was an unsaturated alcohol. It will be called alcohol A. The nmr spectrum of the compound was similar to the one of dihydroergosterol.[25] In order to prove the skeletal structure, Cabat[15] hydrogenated the double bonds in the presence of platinum and perchloric acid. The saturated stenol was oxidized with chromic anhydride and reduced by the Wolff-Kishner[26] method to give a saturated hydrocarbon. Gas chromatography showed the hydrocarbon to be stigmastane (12).

When the acetate of the sterol was hydrogenated in ether in the presence of platinum oxide, only one equivalent of hydrogen was taken up. The resulting compound still gave a positive tetranitromethane test. This indicated that the sterol had two double bonds, only one of which was easily hydrogenated. After purification, the mono-unsaturated acetate was found to have a melting point of 113-114° and $[\alpha]_D$+22.6°. The acetate was hydrolyzed to give an alcohol melting at 112°, $[\alpha]_D$+11.1°. This evidence indicates that the mono-unsaturated stenol is 9.

The hydrogenation of 13 in the presence of platinum and acetic acid has been found[18] to produce 10. Under these conditions, the Δ^{22} double bond is hydrogenated while the Δ^{7} double bond shifts to $\Delta^{8(14)}$. When 13 is hydrogenated in the presence of platinum and ether, the double bond stays in the 7-position to yield 11. The analogy of this

reaction to that of alcohol A would indicate that the unknown compound has one double bond in the 8(14) position. More data of Cabat[15] tends to confirm these conclusions and to locate the other double bond. Ozonolysis of the alcohol and the destruction of the ozonide with zinc and water followed by steam distillation of the products yielded a volatile carbonyl compound which was oxidized with Tollens' reagent to form an acid. The amide of this acid was found to be 2-ethyl-3-methyl-butyramide. This fixed the double bond at the 22-position.

The unknown alcohol A appears, therefore, to be 8. More data of Cabat is in agreement with this conclusion. The nuclear fragment of the sterol was treated with osmium tetroxide and the osmate ester was reduced with lithium aluminum hydride. The glycol so produced was oxidized to a diketone with lead tetraacetate. The infrared spectrum gave a doublet at 1734 and 1725 cm^{-1} which could be due to 5- and 6-membered cyclic ketone absorption bands. This could occur only if the double bond had been in the 8(14) position.



All these data indicate that alcohol A has the structure 8. However, mass spectral data obtained from both the time-of-flight (TOF) and magnetic deflection instruments show the following:

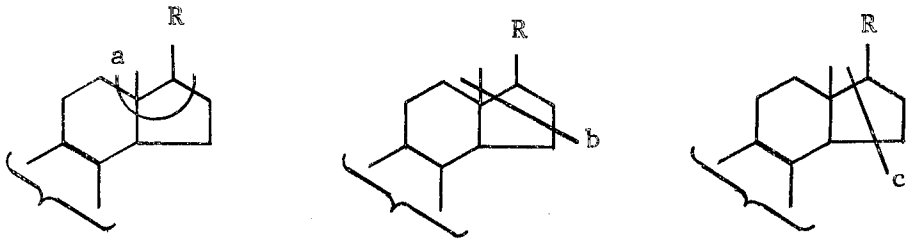
I. Two components are present--one of molecular weight 414 and the other of 412. Molecular ions for both components were seen in the

TOF spectrum.

II. The spectrum from the magnetic deflection instrument showed M-18 peaks at 396 and 394. These peaks indicate the presence of an OH group.

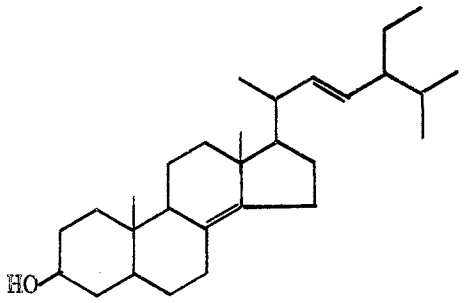
III. Only one M-18-R peak was observed in the TOF spectrum. This peak, caused by the removal of HOH and the side chain, had a mass of 255, indicating the tetracyclic structure has one OH group and one double bond.

IV. The position of neither the OH group nor the double bond could be determined. However, the absence of a large peak at M-42-R in the TOF spectrum and the absence of M-42-18-R in the magnetic-deflection spectrum suggests that one of these functional groups is in rings C or D. Steroids which do not have unsaturation in rings C or D commonly undergo[27] one of the following splitting patterns to give M-42-R:



V. The side chain of one compound (MW = 412) has an empirical formula of $C_{10}H_{19}$ and the other (MW = 414) a formula of $C_{10}H_{21}$.

From the mass spectral data, therefore, the following new conclusions may be drawn: Two components, 8 and 14, are present in a ratio of about 1:1.



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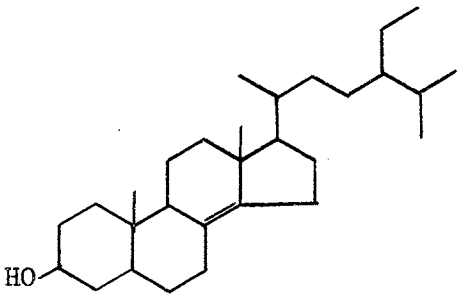


14

The isolation of 14 upon hydrogenation and the indication of unsaturation in ring C or D indicate that the mixture consists of 8 and 14. It is possible, however, that the mixture is 9 and 14.

2,5-Diacetylbenzofuran

The benzene-soluble portion of fraction B was chromatographed over activity I alumina. When the solvent was gradually changed from benzene to benzene-ether (4:6), a sweet-smelling, green band of material was eluted from the column. Green crystals formed from the viscous residue after the solvent was removed. Recrystallization afforded pale yellow crystals melting at 139° and giving an infrared spectrum showing a strong α,β-unsaturated ketone band. Several bands indicated the presence of an aromatic ring and possibly an aromatic ether structure. Because several benzofurans had been found in rayless goldenrod extracts, such a structure was suspected for this ketone. The high melting point suggested that if this compound was a benzofuran it would most likely be symmetrical. 2,5-Diacetylbenzofuran (7) and 3,6-diacetylbenzofuran were considered because of their symmetry. Zalkow and Burke[11] reported 7, m.p. 139-140°, as a degradation product of toxol. Therefore, 7 was regarded as the probable structure of the

compound. The spectra of the materials obtained by both methods were compared by Zalkow and Ramming[16] and found to be identical. A subsequent synthesis by Zalkow and Ramming confirmed the assignment as 7.

Friedelin (5)

Friedelin (5) was isolated from rayless goldenrod by Zalkow and Burke[14] through chromatography of the ketone fraction F. The high content of 5 in rayless goldenrod extract, fraction A, makes it an important compound in several other fractions. When fraction B was chromatographed over activity I alumina, 5 was isolated from fractions eluted with benzene-ether (9:1 through 3:2). When non-ketonic fraction E was chromatographed over activity I alumina, 5 was obtained over the same range of solvent polarities. The large range of solvents which elute 5 causes it to be an impurity in several fractions. The fact that 5 can be isolated from a non-ketonic fraction illustrates its complicating effect in the purification of other compounds eluted from columns along with it.

5α-Androstane-3β,16α,17α-triol (6)

The triol 6 was first isolated in rayless goldenrod from the chromatography of the non-ketonic fraction E using activity II alumina. The benzene fraction was concentrated, dissolved in petroleum ether and chromatographed over activity I alumina. The fractions eluted with petroleum ether-benzene (4:1) contained a white solid which was found to be impure 6. This same impure material may be isolated from the interface in the Girard's T separation of fraction D. It can also be isolated from the benzene-ether (3:2) fraction from the chromatography

of fraction B.

When 6 was isolated by any of these three ways, however, it showed some degree of carbonyl absorption in its infrared spectrum. This carbonyl band was probably due to 5 which could be removed by recrystallization from acetone followed by washing the crystals with petroleum ether or by extraction of the crystals with petroleum ether. This final wash was necessary to remove the last traces of 5. Since the carbonyl absorption of 5 is so intense, the absence of any carbonyl band in the sample probably assures that it has been completely removed. After this final wash 6 was found to exhibit a transition at 265° followed by melting at 286-288°. When 5 was present, the mixture melted at 265°.

Toxol (4)

Toxol (4) was first isolated from the ketonic fraction F from rayless goldenrod by Zalkow and Burke[18]. It can also be isolated from fraction E, partially freed of ketones by the use of Girard's T reagent. The benzene fractions from the activity II alumina chromatography of fraction E were rechromatographed over activity I alumina. The material eluted with benzene-chloroform (1:1) contained 4 along with alcohol B. Toxol (4) was distilled from this fraction and was found to give a melting point and infrared spectrum identical to the values reported by Burke for 4 isolated from fraction F.

Alcohol B

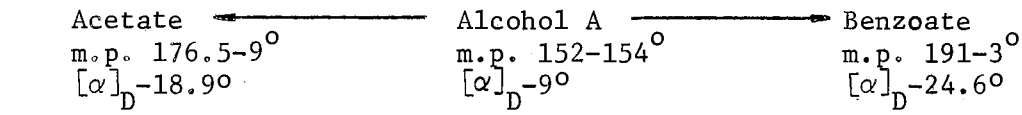
Alcohol B may be isolated through the chromatography of fraction B. The material eluted from activity I alumina with ether contained

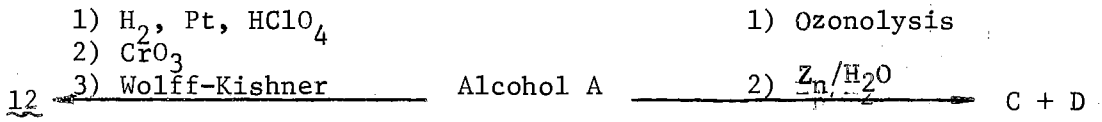
alcohol B. It was also isolated along with 4 in the chromatography of non-ketonic fraction E. It was separated from 4 by recrystallization from acetone. Toxol is more soluble in acetone.

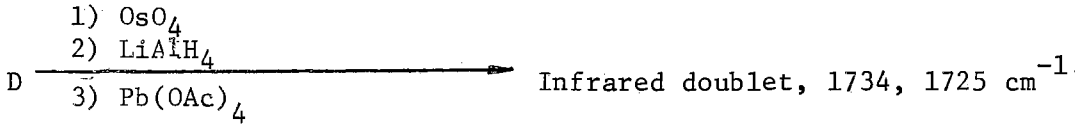
Reactions of the Compounds

Alcohol A

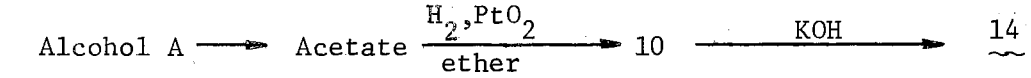
The following chart shows the reactions carried out by Cabat[15] on alcohol A.







In the present study, the following additional reactions were carried out.



Melting points and optical rotation values were used to identify 10 and 14. The hydrogenation step was incomplete. Successive recrystallizations were used to purify the products.

Reactions of Toxol (4)

Toxol (4) was acetylated to form an acetate with an infrared spectrum and a boiling point essentially the same as that of the acetate obtained by Burke.[14]

Dihydrotoxol was formed by the hydrogenation of 4 in the presence of rhodium on alumina.

Dihydrotoxol acetate was formed by the acetylation of dihydrotoxol.

Reactions of Alcohol B

Alcohol B, mp 128.6-129.5°, was treated with acetic anhydride and pyridine to form an acetate, mp 62-66°. The acetate showed two peaks in the acetate-methyl region of its nmr spectrum. The loss of acetic acid was incomplete after an attempted pyrolysis at 400°C. An attempt was made to dehydrate alcohol B with $POCl_3$ but no pure compound could be isolated. When alcohol B was oxidized with Jones' reagent in acetone, the product obtained gave an infrared spectrum indicating the presence of a carboxylic acid and possibly a ketone or a lactone.[28]

Alcohol B was mixed with camphor and the molecular weight was obtained from the melting point depression of the mixture. The molecular weight was found to be 275-311.

The mass spectrum of alcohol B was obtained. The highest intensity peak in the high resolution mass spectrum was found to be at m/e = 245.2267. This peak could only arise from $C_{18}H_{29}$. Another large peak is at m/e = 263.2365. This can be due only to $C_{18}H_{31}O$ when nitrogen is absent and cannot be the molecular ion peak. The molecule must have lost a fragment with an odd mass number in order to give rise to the 263-peak. An elemental analysis indicated that three oxygen atoms were

present in the compound. A fragment peak at m/e = 61 can only come from $C_2H_5O_2$ (C_5H, C_4H_{13}, C_3H_9O, and CHO_3 can probably be ruled out). The fragments of $C_{18}H_{31}O$ and $C_2H_5O_2$ could both arise from a compound $C_{20}H_{36}O_3$. The elemental analysis is consistent with this formula. The $C_2H_5O_2$ fragment indicates that two of the oxygen atoms are very close to one another. A 1,2-glycol would yield a fragment $HO\overset{+}{=}CH-CH_2OH$, mass 61. The mass spectral data indicate, therefore, that the compound has a molecular formula of $C_{20}H_{36}O_3$ and that two of the oxygen atoms probably form a 1,2-glycol group. The third oxygen atom is lost as a water molecule. It probably forms a third hydroxyl group.

CHAPTER III

EXPERIMENTAL

All infrared spectra were recorded with a Beckman IR-5 or a IR-5A infrared spectrophotometer. Solids were taken in potassium bromide pellets, and liquids were taken as films on sodium chloride plates. All nuclear magnetic resonance spectra were obtained on a Varian A-60 nmr spectrometer. The solids were sampled as nearly saturated solutions in carbon tetrachloride or deuteriochloroform with tetramethylsilane (TMS) as an internal standard ($\delta = 0$) and results are reported as dimensionless "chemical shift" units (δ). Mass spectra were obtained from a Consolidated 21-103C magnetic deflection spectrometer, or on a Bendix time-of-flight spectrometer. A high resolution mass spectrum was obtained on a Consolidated Electrodynamics 21-110 spectrometer. Melting points were obtained on a Fisher-Johns melting point apparatus or in a capillary tube using a Thomas-Hoover apparatus and are uncorrected. Analyses were performed by Midwest Micro Lab, Inc., Indianapolis, Indiana.

Collection, Extraction and Separation

Rayless goldenrod was collected during August, 1963, and in August, 1964, in the general area of Roswell, New Mexico. The plants were collected while in full bloom, bagged, and transported to Stillwater, Oklahoma. The plants were dried in air and ground in

hammermills with 8-20 mesh screens. The ground plant material was extracted with methanol in large Soxhlet extractors and 12-l flasks. In each extraction about three kg of plant material was extracted with about 10 l of methanol for a period of 70-100 hours, depending upon the reflux rate. Whenever cold weather and cold water permitted high reflux rates to be used, the plant material could be completely extracted in 3 days. The methanol extracts were concentrated by the use of a steam-heated distillation apparatus to a volume of about 500-700 ml per 10 l of extract. The residue obtained is called fraction A.

Separation of Fraction A by the Couch Procedure (Chart A)

Fraction A was extracted in boiling water, the insoluble oil was settled by the addition of a small amount of chloroform, and the supernatant water layer was washed from the chloroform layer by trickling water into the water layer, just above the interface, forcing the layer to overflow the container. This washing was continued until the overflow was clear. The water was decanted, the chloroform was boiled off, and the residue was extracted with boiling 50% ethanol. This solution was filtered through glass wool, and the filtrate was diluted until it contained 30% ethanol. This solution was boiled, and once more the mixture was poured through a loose glass wool filter. The ethanol was boiled from the solution and the resulting aqueous solution was mixed with chloroform, extracted and allowed to stand overnight until the emulsion separated. The water was decanted and the chloroform was evaporated, leaving a residue, fraction B. When separated in this manner, about 50 g of fraction B could be obtained from each 800-900 g of fraction A.

Fraction B was saponified by boiling a solution containing about 10% fraction B, 40% methanol, 45% water, and 5% KOH for 6-8 hours. After the hydrolysis, the methanol was removed by distillation and the remaining solution was diluted with water and allowed to cool. The aqueous solution was extracted with ether and the ether fraction was dried over magnesium sulfate. The dried ether solution was concentrated to yield fraction C. In a typical run, about 20 g of fraction C was obtained from 50 g of fraction B.

Separation of Fraction A by an Alternate Procedure (Chart B)

Fraction A was hydrolyzed by boiling a solution containing about 10% fraction A, 5% KOH, 40% methanol, and 45% water for 8 hours. The solution was concentrated to about one-half of the original volume, diluted with water, cooled and extracted with ether. The ether extracts were washed with water, dried over magnesium sulfate, and concentrated to yield fraction D.

Fraction D was partially separated into ketonic and non-ketonic fractions by the use of Girard's T reagent. To a solution of 30 g of fraction D in 250 ml of methanol, 20 g of Girard's T reagent and 4 ml of acetic acid were added. The solution was boiled for one hour under reflux conditions. The solution was allowed to cool and was poured carefully into 500 ml of 10% sodium carbonate solution. The aqueous solution was then extracted with ether and the ether solution was dried over magnesium sulfate and concentrated to yield about 23 g of non-ketonic fraction E. The carbonate solution was acidified with hydrochloric acid to pH 2, stirred for three hours, and then extracted with ether. The ether solution was washed with water, dried over magnesium

sulfate and concentrated to yield about 7 g of ketonic fraction F.

Chromatography of Fraction B

Fraction B was extracted with benzene. The benzene-soluble material (61% of fraction B) was placed onto a chromatography column. A saturated solution containing about 250 g of material was chromatographed over 10 kg of acid-washed, activity I alumina (Merck). Fractions were collected as the solvent was gradually changed stepwise from benzene to ether to methanol. With the help of Charles Raming, the chromatography was carried out, non-stop, for about 40 hours, until all of the important fractions had been collected. The fractions were taken to dryness and allowed to stand so that crystallization could occur in those fractions which were pure enough to warrant it. Friedelin (5) was isolated as 2% of fraction B from the fractions eluted with 9:1 benzene-ether, 4:1 benzene-ether and 3:2 benzene-ether and it was identified by its mp (245-246°) and its infrared spectrum. These were identical to the mp and infrared spectrum of 5 isolated by Burke[14] by the chromatography of fraction F. An alcohol was also isolated from the fraction eluted with 3:2 benzene-ether. This alcohol (1% of fraction B) was extracted with petroleum ether and recrystallized from acetone to yield 6, previously isolated by Burke[14]. No pure compound was isolated from the fractions eluted from the column with 1:1 benzene-ether but when the solvent was changed to 4:6 benzene-ether a dark green band was eluted from the column. When the solvent was removed, a sweet-smelling, viscous green residue was left, which slowly crystallized to form pale green crystals. The crystals (1.1% of fraction B) were recrystallized to yield yellow crystals melting at 139°. This

compound gave an infrared spectrum indicating the presence of an α,β-unsaturated ketone and possibly an aromatic ether structure. The structure 7 was suggested for the compound.

The fractions eluted from the column with ether (1.5% of fraction B) yielded a compound melting at 128°. The infrared spectrum of this compound indicated that it was an alcohol with no double bonds. The nmr spectrum was consistent with this assumption. The alcohol gave a negative tetranitromethane test. It will be called alcohol B.

Chromatography of Fraction E (Chart C)

Fraction E is the unsaponifiable portion of the methanol extract which has had some of the ketones removed by the use of Girard's T reagent. From the chromatography of fraction E several compounds were isolated, including 4, 5, 6, 8, alcohol A, and alcohol B.

The petroleum ether-soluble portion of fraction E was chromatographed over activity II alumina. Four major fractions were collected--a petroleum ether fraction, a benzene fraction, a chloroform fraction and a methanol fraction. The benzene fraction was rechromatographed over activity I alumina. The material eluted with benzene-ligroin (1:4) contained a white solid which was removed. Recrystallized from acetone, and washed with ligroin to yield a white crystalline solid melting at 286-288° after a slight softening at 265°. This melting point and the comparison of the infrared spectrum to that of 6 previously isolated in the chromatography of fraction B indicated that 6 could also be isolated in the chromatography of fraction E. The fractions eluted with benzene-chloroform (1:1) contained an oil with an infrared

spectrum similar to that of 4, previously isolated by Zalkow and co-workers.[11] When this oil was dissolved in a small amount of acetone and allowed to stand, however, white crystals formed in the solution. These were recrystallized to yield alcohol B. The concentrated mother liquor was distilled under vacuum to yield 4. The fractions eluted with chloroform slowly crystallized. The crystals were recrystallized to yield alcohol A.

Chromatography of Fraction F

Fraction F, the ketonic fraction from the Girard's T separation, was extensively investigated by Burke.[14] The fraction eluted from an activity I alumina chromatography with chloroform yielded 4.

Purification and Properties of Individual Components

Unknown Alcohol A

The non-ketonic fraction E (chart B) was chromatographed over activity II alumina. Four fractions were collected: a petroleum ether fraction, a benzene fraction, a chloroform fraction and a methanol fraction. The benzene fraction was chromatographed over activity I alumina. The fraction eluted with chloroform slowly crystallized. The solid was recrystallized from methanol to yield a white crystalline solid melting at 154-156°. It was recrystallized from acetone and dried under a vacuum overnight to give a solid melting at 161-162°. The infrared spectrum shows strong absorption at 970 cm^{-1}, indicating the presence of a trans-disubstituted double bond.

Time-of-flight and magnetic deflection mass spectra have been obtained. Data from these spectra are given in the appendices.

2,5-Diacetylbenzofuran (7)

The benzene-soluble fraction of fraction B was chromatographed over activity I alumina. When the solvent was gradually changed from benzene to benzene-ether (4:6), a sweet-smelling, green band of material was eluted from the column. The green crystals which remained after the removal of solvent were recrystallized to yield pale yellow crystals melting at 139° and giving an infrared spectrum with strong absorption bands at ν_{Max}^{KBr} 1660, 1600, and 1290 cm^{-1}. From these data, the compound was identified as 7.

Friedelin (5)

When fraction B was chromatographed over activity I alumina, 5 was isolated from fractions eluted with benzene-ether (9:1 through 3:2). When non-ketonic fraction E was chromatographed over activity I alumina, 5 was isolated from the fractions eluted over the same range of solvent polarities. Friedelin was identified by its melting point (245-246°) and the identity of the infrared spectrum to that of the compound isolated by Burke[22].

5α-Androstane-3β,16α,17α-triol (6)

Fraction E was chromatographed over activity II alumina. The benzene fraction was concentrated, dissolved in petroleum ether, and chromatographed over activity I alumina. The fractions eluted with petroleum ether-benzene (4:1) contained a white solid melting at 265° and found to be 6.

Fraction B was chromatographed over activity I alumina. The fraction eluted with benzene-ether (3:2) contained 6.

When the ketonic and non-ketonic portions of fraction D were separated by means of Girard's T reagent, 6 was isolated from the interface, which had the appearance of an emulsion. The triol 6 was also found crystallized on the sides of the extractor flasks and in the tubes of the extractor during the original extraction of rayless goldenrod.

When 6 is isolated in any of these ways, however, it shows some carbonyl absorption in the infrared spectrum. This carbonyl band was due to 5 and could sometimes be removed by washing the crystals with petroleum ether, followed by recrystallization from acetone and rewashing until the carbonyl band disappeared.

After all of 5 had been removed, the melting point of 6 was found to be 286-288° after a slight softening at 265°.

Toxol (4)

Fraction F was chromatographed over activity I alumina. The fractions eluted with chloroform were concentrated and distilled under vacuum. The cut obtained at 110° (0.05 mm) was allowed to stand until it crystallized. It was recrystallized from 1:1 ether-ligroin and was found to have a melting point of 51°.

Fraction E was chromatographed over activity II alumina. The fractions eluted with benzene were concentrated and chromatographed over activity I alumina. The material eluted with benzene-chloroform (1:1) contained 4, which was isolated by distillation and recrystallization as above. The infrared spectrum was found to be identical to that of 4 isolated from fraction F.

Alcohol B

Fraction B was chromatographed over activity I alumina. The fractions eluted with ether contained a white crystalline solid, alcohol B, melting at 128.5-129.5°.

Fraction E was chromatographed over activity II alumina. The fractions eluted with benzene were concentrated, dissolved in petroleum ether, and chromatographed over activity I alumina. The fraction eluted with benzene-chloroform (1:1) contained alcohol B, along with 4. The viscous concentrate was dissolved in hot acetone and allowed to cool until crystals formed. The crystals were filtered from the solution and recrystallized from acetone to yield alcohol B, melting at 128.6-129.5°. In some cases, it was necessary to wash with ligroin to remove 5. The infrared spectrum of alcohol B shows strong absorption bands at 2.95, 3.45, 8.95, 9.10, 9.28, 9.40, and 9.70 μ. The nuclear magnetic resonance spectrum indicates the absence of aromatic or olefinic protons. The mass spectrum of the compound obtained on a Consolidated 21-110 high resolution spectrometer is given in the appendices. Alcohol B is probably a tricyclic triol, $C_{20}H_{36}O_3$. Anal. Calculated for $C_{20}H_{36}O_3$: C, 74.03; H, 11.18; O, 14.79. Found: C, 73.93; H, 10.92; O, 15.15.

Reactions of the Compounds

Reactions of Alcohol A

Alcohol A, 431 mg (m.p. 156-157°), was heated in a refluxing solution of 2.4 ml of acetic anhydride and 0.7 ml of pyridine. The reaction mixture was cooled to 0°C, poured into 10 ml of water and treated

with sodium carbonate until evolution of carbon dioxide ceased. The mixture was extracted with ether and the extract was washed with 1 N HCl, with 10% Na_2CO_3 and finally with water. The ether solution was dried over magnesium sulfate and concentrated to yield a crystalline product which was chromatographed over activity I alumina to yield 452 mg of acetate: theoretical yield, 475 mg. The acetate was found to have a melting point of 175-177°.

A solution of 500 mg of the acetate of alcohol A in ether was hydrogenated at one atmosphere in the presence of 100 mg of PtO_2. About one equivalent (55 ml) of hydrogen was taken up. The reaction mixture was filtered and concentrated to yield 435 mg of product containing two major products. Successive recrystallizations from acetone yielded 100 mg which melted at 113-114° and showed no infrared absorption at 970 cm^{-1}, and 50 mg which melted at 172-176° and did show infrared absorption at 970 cm^{-1}. The latter product was assumed to be starting material.

The hydrogenated acetate which had been recrystallized from acetone was found to have an optical rotation of +11.1° and a melting point of 114-115°.

An alcoholic solution containing 32.5 mg of the hydrogenated acetate was added to 1:1 KOH/H_2O and boiled for 30 minutes. The volatile material was distilled from the aqueous solution which was then extracted with ether to yield 25 mg of alcohol, mp 111-112°, $[\alpha]_D$+22.6°. From these melting points and optical rotations, the hydrogenated sterol was found to be 14. α-Spinastenol (14) is reported[18] to have mp 112-113°, $[\alpha]_D$+23° and α-spinastenyl acetate (10) to have mp 116-117°, $[\alpha]_D$+12°.

Reactions of Toxol (4)

One-half gram of 4 was added to a solution containing 5 ml of pyridine and 1 ml of acetic anhydride and the mixture was heated overnight at the refluxing temperature. The reaction mixture was cooled to ice temperature and was added to 10 ml of Na_2CO_3 solution. The aqueous solution was extracted with ether, and the ether portion was washed with 1N HCl, 10% Na_2CO_3 and water. The extract was dried over magnesium sulfate and concentrated to yield a viscous residue which was distilled at 65-70° (0.05 mm). The acetate was found to have an identical spectrum to that of toxol acetate isolated by Burke.[14]

Six hundred mg of 4 in 25 ml of 95% ethanol was hydrogenated at one atmosphere in the presence of 60 mg of Rh on Al_2O_3. The solution was filtered and concentrated to yield 521 mg of oil, which was chromatographed over activity I alumina. The fraction eluted with ether was distilled at 0.04 mm and the fraction distilling at 86-90° was collected. Thin-layer chromatography showed one spot. Toxol (4) was found to have a different R_f value using 0.025-cm-thick coats of silica gel on plates with benzene as a solvent. The infrared spectrum of dihydrotoxol was found to be identical to that of dihydrotoxol isolated by Burke.[14]

A solution of 210 mg of dihydrotoxol in 21 ml of pyridine and 2.1 ml of acetic anhydride was stirred overnight. The mixture was diluted with water, and was extracted with ether. The ether solution was washed with Na_2CO_3 until CO_2 evolution ceased and washed with HCl until it was acid to litmus. Finally, the solution was washed with water, dried over $MgSO_4$, and concentrated. The residue was distilled at 96-110° (0.04 mm) to yield a viscous oil giving one thin-layer

chromatography spot. The infrared spectrum was identical to that of the acetate of dihydrotoxol isolated by Burke.[14]

Reactions of Alcohol B

A solution containing 47 mg of alcohol B, 0.242 ml of acetic anhydride and 0.074 ml of pyridine was heated at the reflux temperature for five hours. After cooling to ice temperature, the mixture was diluted with 1 ml of water and slowly treated with 0.24 g of sodium carbonate. After neutralization was complete, the solution was extracted three times with ether. The combined extracts were washed with two portions of 5% HCl, one portion of Na_2CO_3 and a portion of water. The extract was dried over $CaCl_2$ and concentrated to yield 50 mg of acetate, mp 62-66°. This acetate showed two acetate methyl peaks in its nmr spectrum. An attempt was made to pyrolyze the acetate over Pyrex helices at 400°C. The product obtained had an infrared spectrum identical to that of the starting acetate.

An attempt was made to dehydrate alcohol B with $POCl_3$, but no pure compound could be isolated.

Adding Jones' reagent[28] to alcohol B in acetone oxidized it to a mixture which was chromatographed over activity I alumina. The ether fraction was concentrated to give a mixture which appeared to contain a carboxylic acid. The infrared spectrum showed strong carbonyl absorption and a broad OH band. An alcohol solution of the sample was added to a solution of KI and KIO_3 in water. A yellow color was formed in the solution. The addition of starch caused the appearance of a blue color. This test indicates the presence of an acid. A blank test made on the alcohol was negative. A shoulder on the infrared carbonyl

absorption band indicated the presence of a ketone or a lactone.

A sample of 0.0226 g of alcohol B was placed in a test tube with 0.1025 g of camphor. The test tube was immersed into an oil bath at 180° to melt the mixture. The solution was thoroughly mixed, removed, cooled and the solid was ground in a mortar. The melting point of the mixture was taken in capillaries. Five samples gave melting points of 148-152°. The melting point of pure camphor was found to be 178.4°. From these data, the molecular weight of the alcohol was calculated to be 275-311.

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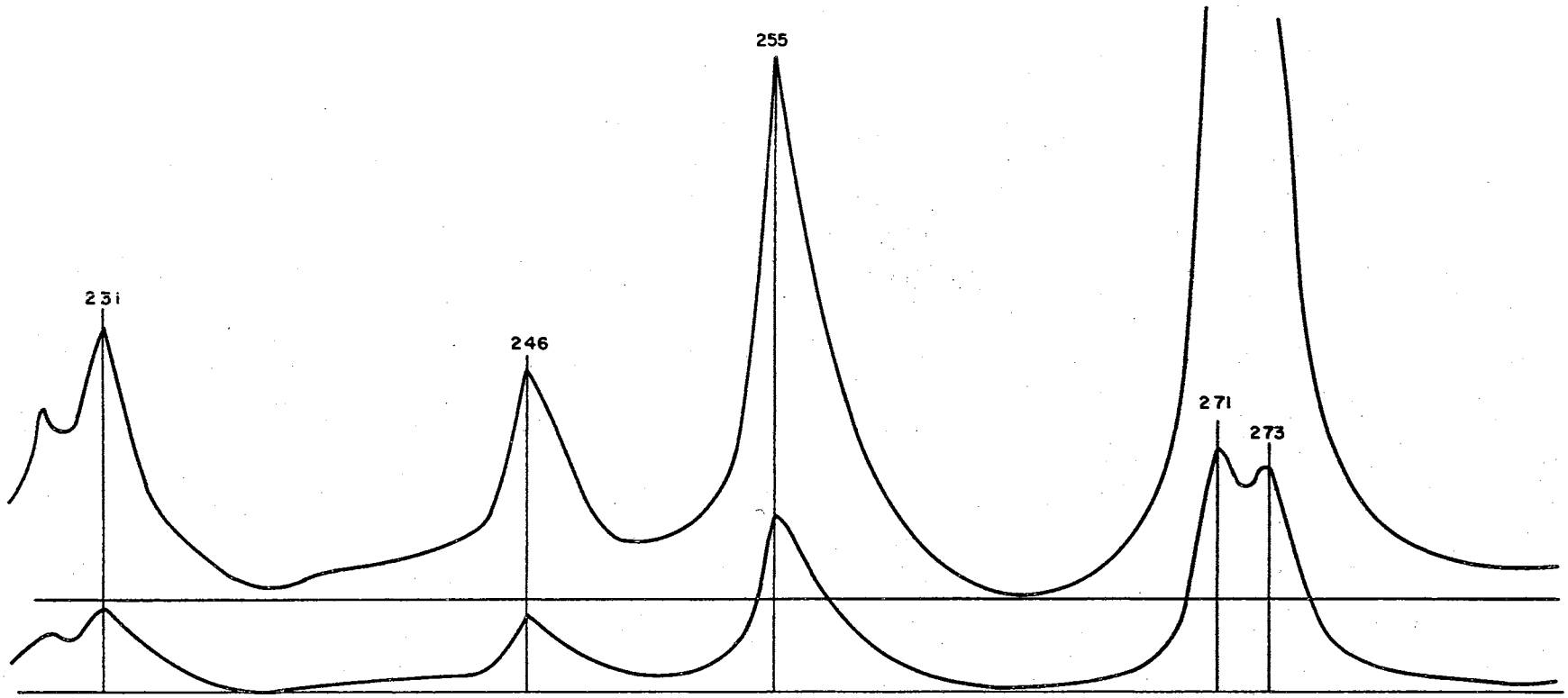

Plate I. Time-Of-Flight Mass Spectrum of Alcohol A, m/e 231-273.

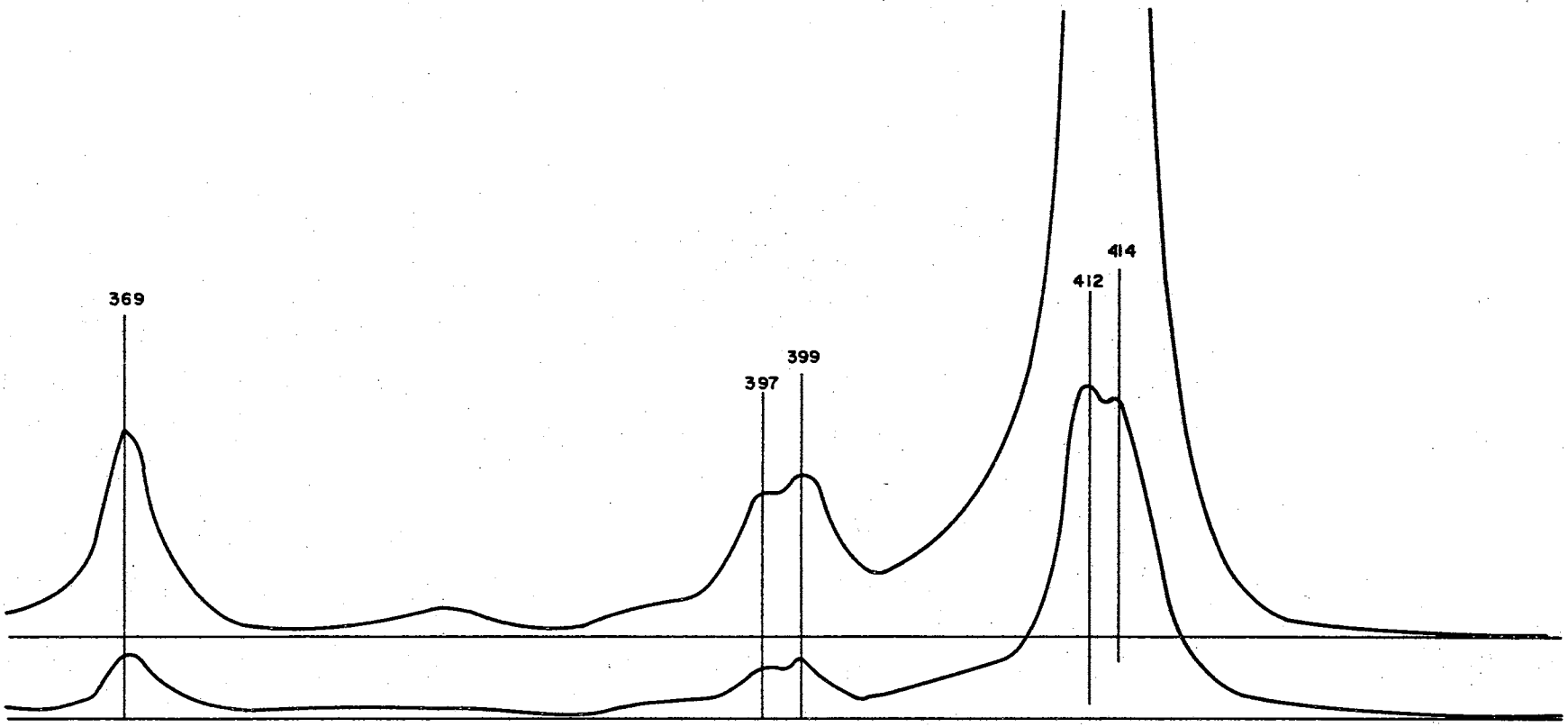

Plate II. Time-Of-Flight Mass Spectrum of Alcohol A, m/e 369-414.

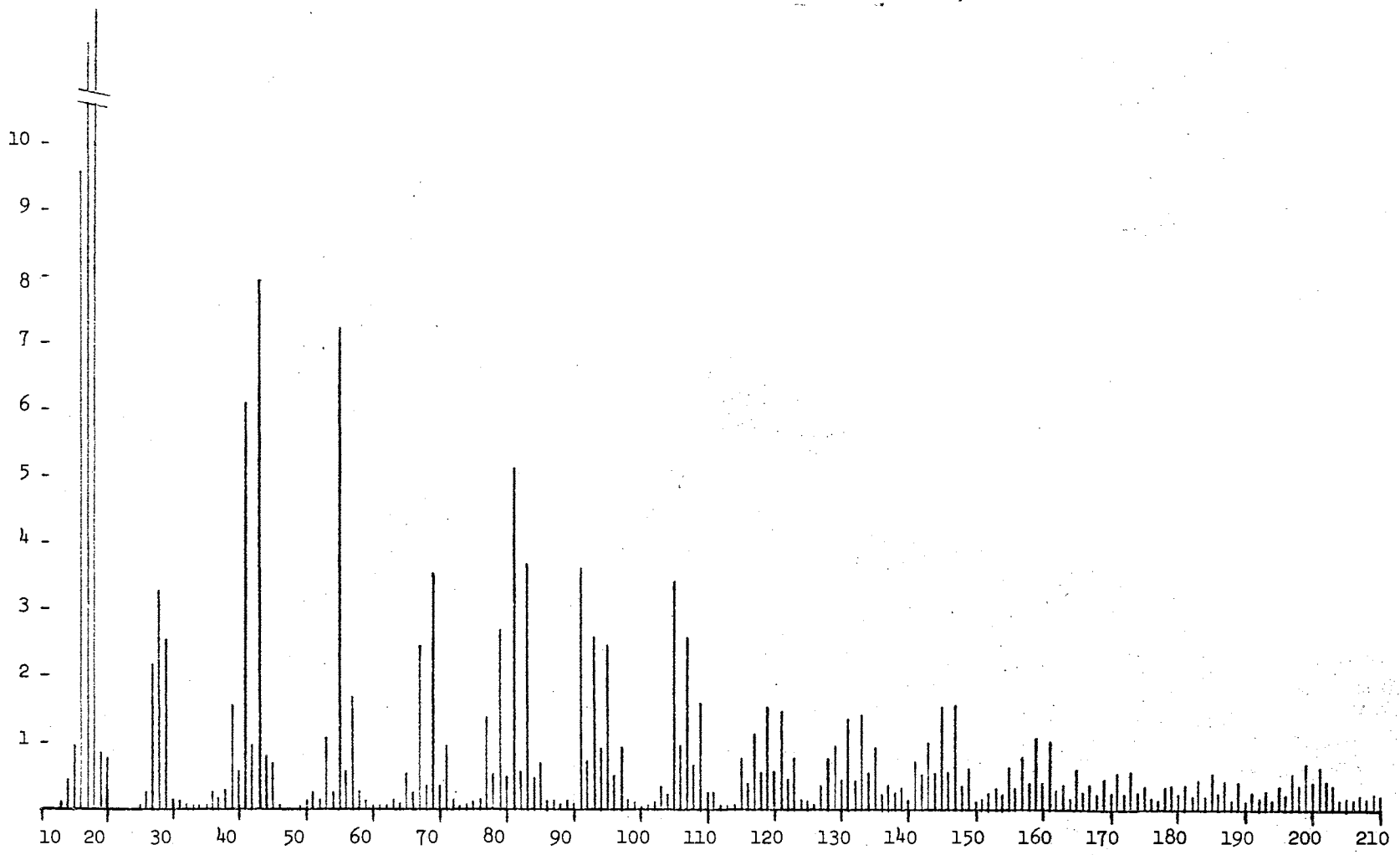

Plate III. Mass Spectrum of Alcohol A, m/e 10-210.

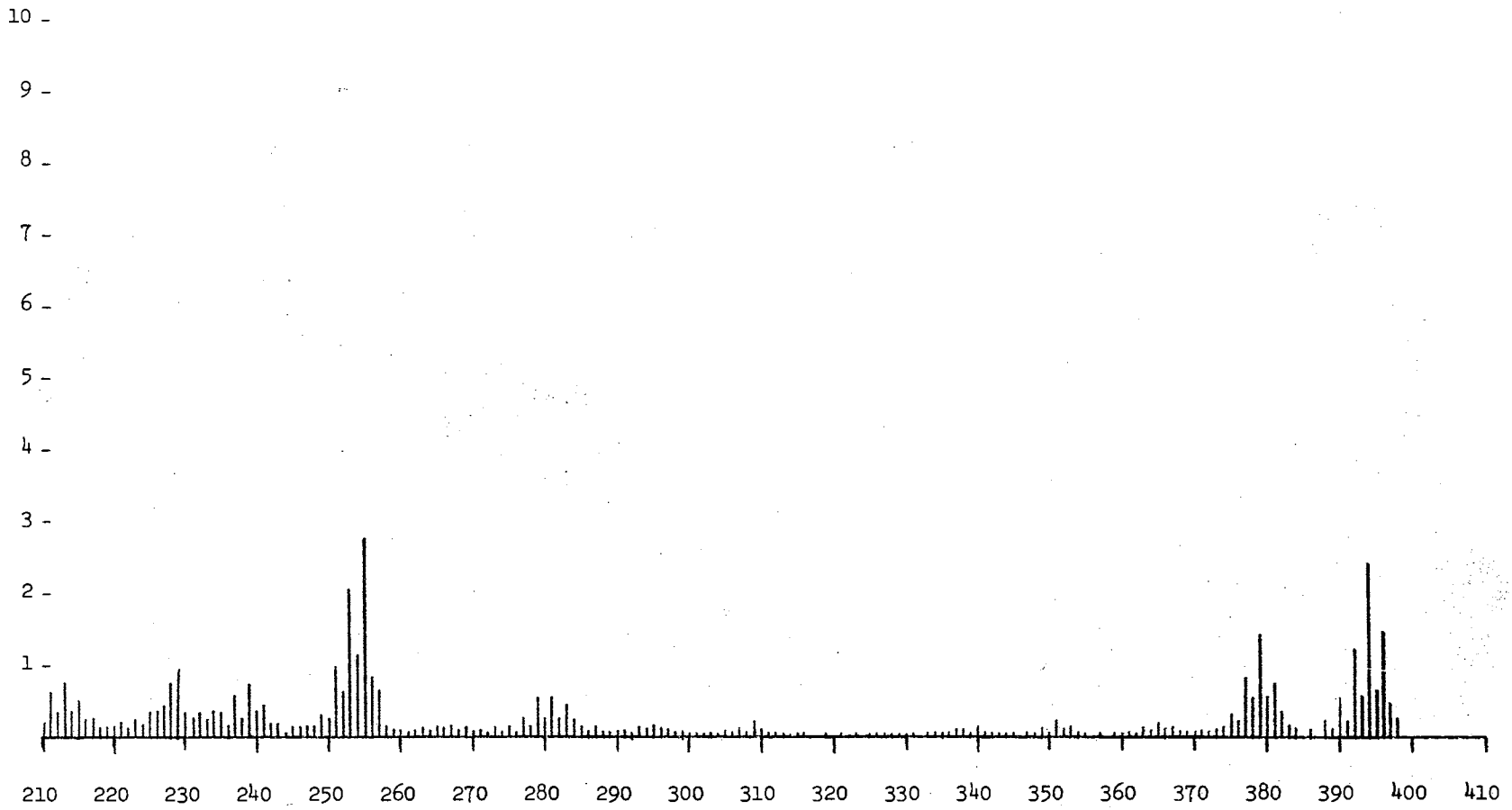

Plate IV. Mass Spectrum of Alcohol A, m/e 210-410.

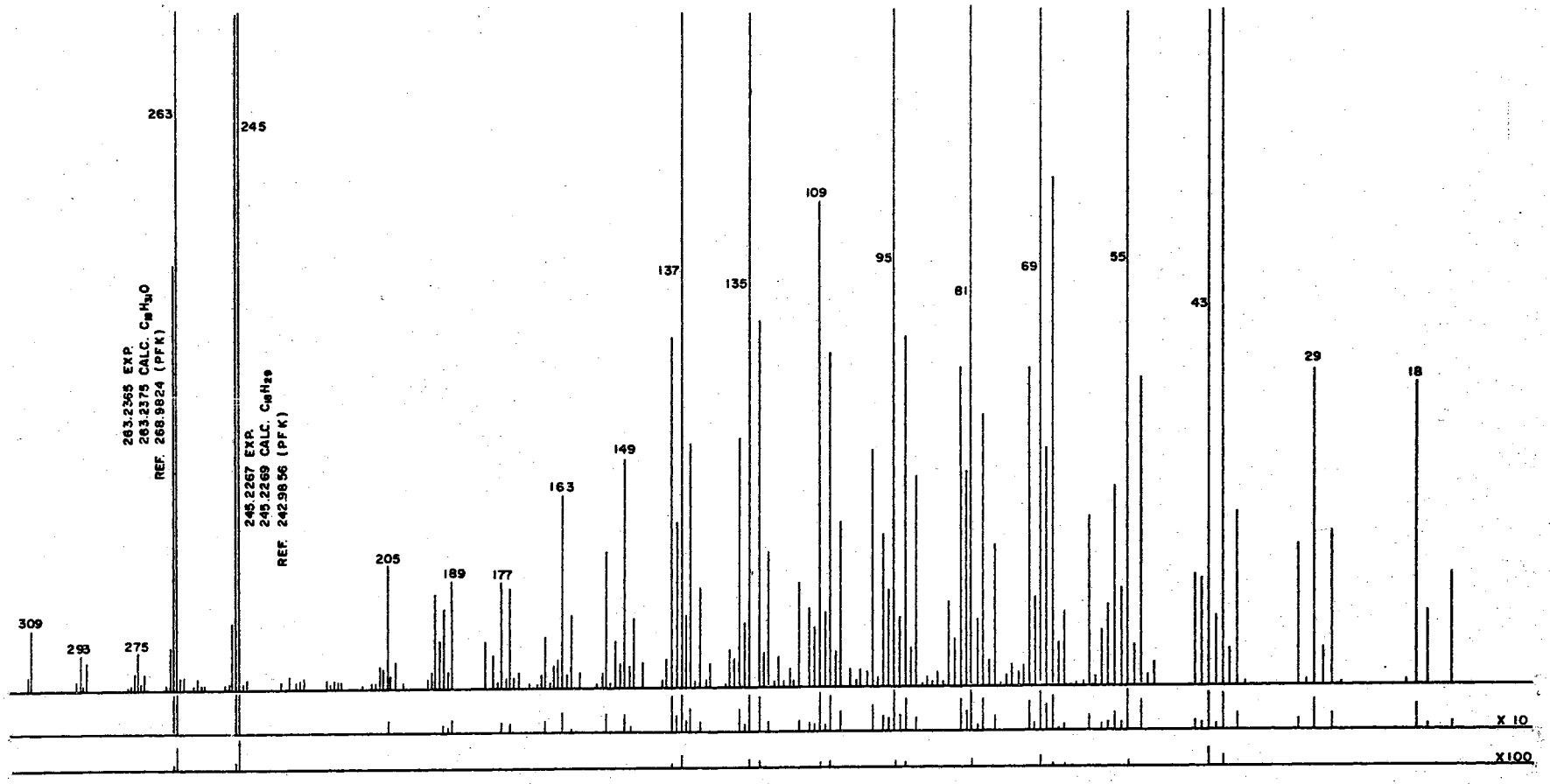

Plate V. High Resolution Mass Spectrum of Alcohol B.

VITA

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