GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF RESEARCH ADMINISTRATION

Date: 7 September 1965

RESEARCH PROJECT INITIATION

Project Title: The Toxic Principle of Aplopappus Heterophyllus Blake

Project No.: B-1556

Project Director: Dr. Leon H. Zalkow

Sponsor: National Institute of General Medical Sciences, NIH, Bethesda, Md.

Agreement Period: From 1 September 1965 until 31 August 1966

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Bethesda, Maryland 20014

Reports Required

Interim progress - when application is made for continuation or renewal support (Form PHS-2590)
Final - upon completion of project.

Assigned to: School of Chemistry

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A. Summary Page

Title: The Toxic Principle of Aplopappus heterophyllus Blake

Grant No.: GM-13694 and GM-08346

Principal Investigator: Dr. L. H. Zalkow

Sponsoring Institution: Georgia Institute of Technology

Period Covered: September 30, 1963 - March 1, 1966

Date of Preparation: March 21, 1966

Summary:

Rayless goldenrod (Aplopappus heterophyllus), a toxic plant indigenous to the southwestern United States, has been found to contain four benzofuran derivatives, toxol (2S-isopropenyl-3S-hydroxy-5-acetyl-2,3-dihydrobenzofuran), dehydrotremetone (2-isopropenyl-5-acetylbenzofuran), tremetone (2S-isopropenyl-5-acetyl-2,3-dihydrobenzofuran) and 2,5-diacetylbenzofuran. Dehydrotremetone and tremetone have also been found in white snakeroot, a plant which produces a similar syndrome in cattle and sheep; toxol and 2,5-diacetylbenzofuran have not been previously reported. Toxol and dehydrotremetone were found to be bacteriostatic, the other benzofuran derivatives have not yet been tested.

The absolute configuration of toxol was established by degradation to D(+) dimethyltartrate. Toxol and tremetone were interrelated via (-) dihydrotremetone. Toxol and tremetone have also been interrelated with rotenone and methyl D(+) malate.

2,5-Diacetylbenzofuran and cis and trans dihydrotoxol have been synthesized. Synthetic cis-dihydrotoxol was identical in all respects with that obtained from natural toxol.

5α-Androstane-3β, 16α, 17α-triol and 24-ethylcholesta 8(14), 22-dien-3β-ol have also been isolated from rayless goldenrod. The former has been

synthesized.

The following terpenes have been identified in the plant extract: d-limonene, l-carvone, l-borneol, caryophyllene and friedelene. The following acids have been shown to be present in the extract: caprylic, capric, lauric, myristic, palmitic and linoleic.

Although the plant extract has been shown to be toxic to sheep, no single compound has been found to be responsible for this toxicity at this time.

B. Detailed Report

(1) Description of research accomplished

The following description of research accomplished includes the four reprints:

"Toxic Constituents of Rayless Goldenrod," L. H. Zalkow, N. Burke, G. Cabat and E. A. Grula, J. Med. Chem., 5, 1342 (1962).

"Constitution of Toxol: A Toxic Constituent of Aplopappus heterophyllus," L. H. Zalkow and N. Burke, Chem. and Ind., 1963, 292.

"The Occurrence of 5α-Androstane-3β, 16α, 17α-triol in Rayless Goldenrod," L. H. Zalkow, N. I. Burke and G. Keen, Tetrahedron Letters, 1964, 217.

"The Absolute Configurations of Tremetone and Toxol," W. A. Bonner, N. I. Burke, W. E. Fleck, R. K. Hill, J. A. Joule, B. Sjoberg and L. H. Zalkow, Tetrahedron, 20, 1419 (1964).

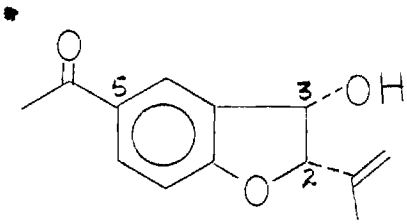
And in addition a report of progress which has not yet been published is enclosed.

The Synthesis of cis and trans Dihydrotoxol

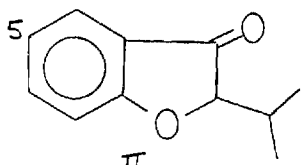
L. H. Zalkow and M. Ghosal

Schools of Chemistry, Georgia Institute of Technology
and
Oklahoma State University

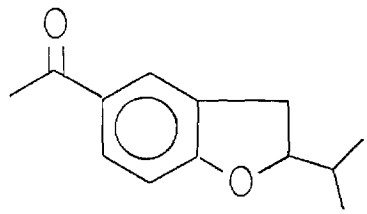
Toxol has been shown to possess the absolute configuration depicted



I



II



III

in I.[1] We wish to describe here an efficient synthesis of racemic dihydrotoxol (cis-2-isopropyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran or 3-hydroxy-5-acetylcoumaran) and its trans isomer after a number of unsuccessful attempts.

Previous work[2] had shown that it was not possible to acetylate at C-5 in 2-isopropyl-3-coumarone, II, and reduction of II gave 2-isopropyl-3-hydroxycoumaran, now known on the basis of this work to be the trans isomer, which on Friedel-Crafts acylation gave 2-isopropyl-5-acetylbenzofuran. Reduction of 2-isopropylidine-3-coumarone with sodium borohydride also gave trans-2-isopropyl-3-hydroxycoumaran. Likewise acylation of 2-isopropyl-3-acetoxycoumaran gave 2-isopropyl-5-acetylbenzofuran.[2]

When 2-isopropylcoumaran was treated with N-bromosuccinimide, the bromine entered the aromatic ring; similarly on reaction with Fenton's reagent, the hydroxyl group was found in the aromatic ring.[2] No reaction was observed in the reaction of 2-isopropylbenzofuran with diborane and the reaction of the ketal of 2-isopropyl-5-acetylcoumaran with N-bromosuccinimide

[Reprinted from the Journal of Medicinal and Pharmaceutical Chemistry, **5**, 1312 (1962).]

Toxic Constituents of Rayless Goldenrod[1]

L. H. Zalkow,[2] N. Burke, G. Cabat, and E. A. Grula

Department of Chemistry, and Department of Microbiology, Oklahoma State University, Stillwater, Oklahoma

Received July 14, 1962

Bacillus cereus has been found to be an excellent assay organism in the study of the toxin isolated from rayless goldenrod. Two compounds which inhibit the growth of *Bacillus cereus* have been isolated from the crude toxin. One of these, dehydrotremetone (2-isopropenyl-5-acetylbenzofuran) recently has been reported to occur in white snakeroot, a poisonous plant that produces symptoms in higher animals similar to those produced by rayless goldenrod. The second, more abundant and more toxic compound, toxol, has not been reported previously. Toxol is shown to be optically active 2-isopropenyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran.

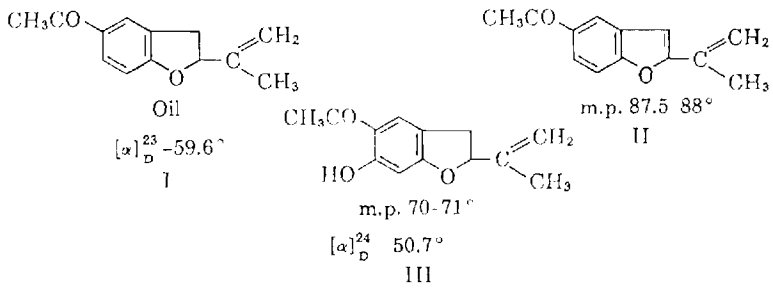
"Milksickness" or "trembles," a disease that attacks both humans and animals, has been known in this country since colonial times. It was early suspected that animals contracted the disease by foraging on a poisonous plant, and that it was passed on to humans through the milk of an affected cow.[3] White snakeroot (*Eupatorium urticaefolium*) was shown to be the plant responsible for the disease in the central states.[4] Couch,[1,5] after extensive studies, concluded that the toxin in white snakeroot was an unsaturated alcohol, tremetal ($C_{16}H_{32}O_3$, straw-yellow oil, $[\alpha]^{30}D$ − 33.82°), of unknown structure. Tremetol was found to produce "trembles" and the other characteristic symptoms of white snakeroot poisoning in test animals.

In the early part of this century a disease of animals and humans clinically identical with "milksickness" appeared in the southwestern part of this country, in a vicinity devoid of white snakeroot. It was eventually established that southwestern "milksickness" arose from rayless goldenrod (*Aplopappus heterophyllus*).[6] Couch[5,7] reported that tremetol was also the toxin present in rayless goldenrod.

(1) This investigation was generously supported by the National Institutes of Health through research grant RG-8346.
(2) To whom inquiries should be sent.
(3) J. F. Couch, U. S. Department Agr. Cir. No. 306, November, 1933.
(4) J. F. Couch, *J. Agric. Research*, **36**, 517 (1927).
(5) J. F. Couch, *J. Am. Chem. Soc.*, **51**, 3617 (1929).
(6) C. D. Marsh, C. G. Roe, and A. B. Clawson, U. S. Dept. Agr. Bull. 1391 (1926).
(7) J. F. Couch, *J. Agric. Research*, **40**, 649 (1930).

Dermer[8,9] and his students found that tremetol was not a pure compound as reported by Couch but rather a complex mixture. However, these workers were unable, with the methods available at that time, to isolate the pure toxin of rayless goldenrod. Further work on these poisonous plants apparently ceased during the next twenty-year period, and fortunately "milksickness" became less of a problem with time for two reasons. First, farmers were educated to recognize and eradicate the poisonous plants, and, secondly, milk was consumed on a local level less frequently and went into large dairy pools where toxin-containing milk became diluted. Actually, cattle eat these poisonous plants only when other forage is unavailable.[3]

Recently, Bonner and co-workers[10] reported the results of their reinvestigation of white snakeroot. By the use of modern methods of chromatography, these workers found that "white snakeroot tremetol" could be separated into a number of components. Three closely related ketones, tremetone, I, dehydrotremetone, II, and hydroxytremetone, III, proved to be toxic to goldfish, and tremetone, the most abundant constituent, was suspected of being the active toxin in white snakeroot.



In June of 1961 we began a reinvestigation of rayless goldenrod, and isolated "rayless goldenrod tremetol" by a procedure similar to that reported by the earlier workers.[5,8,9] Our first objective was to find a simple, rapid method of assaying the toxic compound(s) present in the crude toxin (the "rayless goldenrod tremetol" of Couch). Since we wished to test the many fractions obtained from a chromatographic separation, a bacteriological test seemed most appropriate. After a rather detailed study, described in the next section, *Bacillus cereus*

(8) C. A. Lathrop, Master's Thesis, Oklahoma State University, "Isolation and Fractionation of Tremetol from Rayless Goldenrod," 1939.

(9) R. Cleveldon, Master's Thesis, Oklahoma State University, "The Chemical Constituents of Rayless Goldenrod," 1939.

(10) W. A. Bonner, J. L. O. Craw, D. M. Bowen, and V. R. Shah, *Tetrahedron Letters*, **12**, 317 (1961).

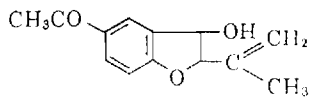
was selected as the test organism, and all toxicities reported in this paper refer to inhibition of growth of this organism using the solid agar assay method.

Our next objective was to determine whether the toxic constituents in rayless goldenrod were identical with those from white snakeroot. In order to do this we attempted to use the partition chromatographic separation used by Bonner,[11] *et al.* Our crude toxin was found to be only partially soluble (65%) in the mobile phase (ligroin). This portion was found to be toxic whereas the fraction insoluble in ligroin was non-toxic. Chromatography on Celite according to the procedure of Bonner, *et al.*[12] gave a rapidly eluted toxic fraction which after purification was found to be identical in all respects with dehydrotremetone (II). A second more polar toxic fraction was eluted only very slowly from the column. When the ligroin soluble oil was chromatographed on alumina (adsorption chromatography) two distinct toxic fractions were obtained. The less polar one again was identified as dehydrotremetone, whereas the more polar toxic component, toxol, obtained as a viscous dark yellow oil, appeared to be a new compound. Several other unidentified but non-toxic compounds also were obtained from the chromatography. Toxol could be obtained as a pure substance only after repeated chromatography on alumina, with subsequent preparative thin layer chromatography on silica gel. However, a more efficient means for obtaining pure toxol in good yield was found by chromatographing the crude toxin on Florisil. Elution with the mobile phase (ligroin) gave dehydrotremetone as the only toxic compound. When the chromatography column was cleared with methanol, the highly toxic fraction obtained was found to contain toxol. Toxol was obtained as a colorless viscous liquid by a further partition chromatography on Florisil, then an adsorption chromatography on alumina and finally distillation at reduced pressure. On standing, toxol crystallized in needles. The yield of pure crystalline toxol starting from the crude toxin (tremetol of the earlier workers) was 10%.

Toxol ($C_{13}H_{14}O_3$) is isomeric with hydroxytremetone, III, isolated by Bonner,[10] *et al.* from white snakeroot. Structure IV is suggested for toxol on the basis of the experimental evidence. The infrared spectrum of toxol shows the presence of an OH group (2.96 μ), and the presence of a conjugated carbonyl group (5.95 μ). The bands at 6.05 μ and 6.21 μ (shoulder at 6.26 μ) indicated that toxol is aro-

(11) Professor Bonner was kind enough to supply us with details of their work prior to publication.

(12) J. J. DeGraw, Jr., Ph.D. Dissertation, Stanford University, "Neutral Constituents of the White Snakeroot Plant," 1961.



m.p. 52–53°
$[\alpha]_D^{27}$ 25.1°

IV

matic and contains an isolated double bond. The ultraviolet spectrum of toxol (λ_{max} 223 mμ, log E 4.02; λ_{max} 273 mμ, log E 4.13, ethanol) was similar to that reported[9] for tremetone (I). Toxol readily gives a monoacetate ($C_{15}H_{16}O_4$), and the nuclear magnetic resonance spectra of toxol and its acetate clearly show that toxol contains a secondary hydroxyl group by the characteristic downfield shift ($\sim$ 100 cps.) of *one* proton in going from the alcohol to its acetate.[13] The proton showing this shift is attached to the carbon atom containing the hydroxyl group. This proton appears as a doublet ($J \sim 3$ cps.) centered at δ 5.93 in the acetate, indicating that it is flanked by only one adjacent proton.

Both toxol and its acetate give iodoform on treatment with sodium hypoiodite, indicating the presence of a methyl ketone. This was confirmed by the n.m.r. spectra of toxol and its acetate. Toxol also readily forms a 2,4-dinitrophenylhydrazone ($C_{19}H_{18}N_4O_6$, m.p. 176–177°).

Hydrogenation of toxol using palladium or platinum catalysts resulted in hydrogenolysis and gave inconclusive results, whereas the use of a 5% rhodium on alumina catalyst led to an uptake of one mole of hydrogen per mole of toxol, and the infrared spectrum of the product showed that the hydroxyl and carbonyl groups were retained. Ozonolysis of toxol gave a 38% yield of formaldehyde isolated as the dimethone derivative. The infrared spectrum of the larger fragment from the ozonolysis showed a conjugated carbonyl band (5.94 μ) and an unconjugated carbonyl band (5.80 μ). No aldehyde C–H band appeared to be present in the spectrum.

The positions of substitution on the aromatic ring were shown by oxidation of toxol with dilute permanganate to 5-acetylsalicylic acid and by the n.m.r. spectra of toxol and its acetate which showed two adjacent aromatic protons and a further single aromatic proton at slightly higher field.

During this study it was observed that acids readily converted both toxol (IV) and dehydrotremetone (II) into the same substance. However, the structure of this new substance has not been elucidated. Even so weak an acid as iodine converted toxol to this new substance,

(13) L. H. Zalkow, E. J. Eisenbraun, and J. N. Shoolery, *J. Org. Chem.*, **26**, 981 (1961).

but in addition a very small amount of dehydrotremetone also was isolated from this reaction.

Dehydrotremetone was degraded to 2,5-diacetylbenzofuran by treatment with a limited amount of osmium tetroxide followed by lead tetracetate. 2,5-Diacetylbenzofuran was also obtained from both toxol and toxol acetate by ozonolysis followed by pyrolysis.

Toxol apparently does not occur in white snakeroot[10,12] and since it occurs to a large extent in rayless goldenrod and is more toxic than dehydrotremetone (see experimental), it probably is the active toxin of the plant. The toxicity of toxol to sheep will be tested.

Bacteriological Testing

Seventeen bacterial cultures were selected at random from a stock collection for initial screening with the crude toxin and 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 hr. at 30°. Organisms were agitated and washed from the slants with sterile physiological saline and one drop of this solution added to sterile tubes of nutrient broth. After mixing, two drops of crude toxin were added, 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 hr. of incubation and the results are given in Table I.

When incubation was continued to 66 hr. 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 was 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

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 hoagii	−
Aerobacter aerogenes	+
Micrococcus lysodeikticus	+
Proteus vulgaris	+
Sarcina lutea	−
Serratia marcescens	+
Staphylococcus albus	−

recorded at 19 and 43 hr. Using this procedure, three of the organisms exhibited excellent growth inhibition (*B. cereus*, *Staph. albus* and *Coryneb. hoagii*). *B. subtilis*, *Strep. lactis* and *Chromo. violaceum* were less susceptible whereas *S. lutea* was resistant. Although three organisms were inhibited by the crude toxin both in the 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*.

Pure toxol could be identified readily 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 at R_f 0.81 which exhibited quenching of ultraviolet light (Woods lamp, 2537 A). In addition, quenching areas and inhibition zones on plates of *B. cereus* coincided exactly. Under the same conditions toxol acetate gave R_f 0.84; dehydrotremetone gave R_f 0.60 but with extensive trailing. Toxol acetate quenched ultraviolet light similarly to toxol (blue-black color) whereas dehydrotremetone showed a brownish color. It was found that 200 μg. of each 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 became increasingly obvious with increasing concentrations. Because of the low water solubility of the three compounds, growth inhibition was

restricted to the area of compound deposition, with little diffusion of the toxic compounds through the water base of the medium. Judging from the completeness of growth inhibition, toxol is more toxic than either toxol acetate or dehydrotremetone at equal concentrations.

Experimental

Melting points were taken on a Fisher–Johns apparatus and are uncorrected. Analyses were performed by Dr. A. Bernhardt (Mülheim, Germany). Infrared spectra were recorded on a Beckman IR-5 spectrophotometer. The n.m.r. spectra were run in carbon tetrachloride using tetramethylsilane as an internal standard ($\delta = 0$) using the Varian A-60 NMR spectrometer.

Isolation of "Tremetol."—Rayless goldenrod was collected while in full bloom (August, 1960 and 1961) just east of Roswell, New Mexico, on U. S. Highway 70. The plant was allowed to air dry and just prior to extraction the entire plant (except for roots) was ground in a Wiley mill with a 20-mesh screen. The ground meal was continuously extracted with methanol in a large Soxhlet extractor. In a typical run 2.5 kg. of plant was extracted for 75 hr. with approximately 12 l. of methanol. After standing at 4° overnight the precipitated waxes were removed from the extract by filtration through glass wool and the methanol solution concentrated on the steam bath with a water aspirator to give about 0.5 l. of viscous, dark green residue. This residue, after washing several times with warm water, was dissolved in hot 50% aqueous ethanol (1.5 l.) and the solution filtered while hot. To the filtrate was added 105 g. of potassium hydroxide and 0.5 l. of ethanol and the solution refluxed for 8 hr. Later it was found that the above procedure, which is essentially the same as that used by earlier workers,[5,8] could be shortened (giving the same results) by simply adding methanolic potassium hydroxide directly to the extract to give about 1 l. of a 5% potassium hydroxide solution and then heating as above. The alkaline solution after cooling was filtered and concentrated under reduced pressure (water aspirator) to a viscous gum which was partitioned between water and ethyl ether. The ether layer after drying over sodium sulfate was concentrated under reduced pressure to give 15 g. of a viscous red oil—the "tremetol" of the earlier workers.

Isolation of Dehydrotremetone. Partition Chromatography on Celite.[12]—Ligroin (20 ml., b.p. 60–75°) saturated with 95% methanol, the mobile phase, was added to 8.5 g. of "tremetol." The mobile phase soluble portion of "tremetol" (5.4 g.) in ligroin, was added to a column of Celite prepared by intimately mixing 200 ml. of 95% methanol saturated with ligroin, the stationary phase, with 200 g. of Celite (Johns-Manville). The column was eluted continuously with the mobile phase. The first 350 ml. of eluent removed 2 g. of non-toxic material from the column. The next 450 ml. of eluent gave 2.2 g. of a mixture of a viscous oil and solid. The solid proved to be toxic and was found to be identical in melting point and infrared and ultraviolet spectra with that reported for dehydrotremetone.[10,12] The next liter of eluent removed only a negligible amount of material from the column but toxicity tests showed that a new toxic compound was being eluted very slowly from the column.

Adsorption Chromatography on Alumina.—The ligroin soluble fraction of tremetol (30 g.) was chromatographed on a column of alumina (508 g., Merck acid-washed). Benzene (1500 ml.) eluted 5.4 g. of a viscous oil containing a white

solid. The solid was found to be toxic and again was identified as dehydrotremetone. Another 8.5 g. of non-toxic liquid and solids was eluted with benzene–ether and ether (total 2 l.). A second toxic fraction (4.2 g. of viscous orange oil) was eluted with 2–6% methanol in ether. Higher concentrations of methanol in ether continued to elute material from the column but none of these fractions was toxic. Rechromatography of the second toxic fraction on alumina removed the yellow color and gave a viscous, colorless oil as the toxic component. However, gas chromatography of this oil using a 0.3 cm., 1.67 m., 5% SE-30 on Chromosorb W column at 190° showed two major peaks at retention times of 0.75 and 7.0 min., respectively. Thin layer chromatography on silica gel G (250 μ) using chloroform–methanol (95:5) and spraying with methanolic 2,4-dinitrophenylhydrazine solution showed one major spot (*Rf* 0.75) and several smaller spots. Similar results were obtained by spraying with 5% nitric acid in sulfuric acid. By using repeated preparative thin layer chromatography (silica gel G, 500 μ) the pure toxic compound, toxol (properties described in the next section), was obtained. However, a more efficient, rapid means of obtaining toxol is given.

Isolation and Properties of Toxol.—Florisil (300 g., Floridin Company) was poured into a chromatography column containing the stationary phase (95% methanol saturated with ligroin). After standing overnight, the excess stationary phase was withdrawn and the column rinsed several times with the mobile phase (ligroin). Tremetol (20 g.) was added directly to the top of the column and elution continued until no further material was eluted. Approximately 80% of the material introduced on the column was eluted but dehydrotremetone was the only toxic compound obtained. The column was then cleaned with stationary phase and the material thus removed proved to be quite toxic. The material removed from the column was rechromatographed on Florisil as follows. In a typical run 16 g. of the oil was placed on a column prepared by intimately mixing 185 ml. of stationary phase with 300 g. of Florisil. The first 2.5 l. of mobile phase eluted no toxic material and then toxol was eluted in the next 3.5 l. as a pale yellow viscous liquid. Toxol was further purified by chromatography on neutral alumina where it was eluted in 1:1 ether–chloroform, and finally it was distilled at 110° (0.05 mm.). The toxol thus obtained crystallized on standing and was recrystallized from ether–ligroin, m.p. 52–53°. Pure crystalline toxol was obtained in a 10% yield from "tremetol."

Anal. Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.64; H, 6.51. $[\alpha]_D^{27} - 25.1$ (*c* 0.44, methanol).

Treatment of toxol in 10% sodium hydroxide, with a solution of 1 g. of iodine and 2 g. of potassium iodide in 10 ml. of water, gave an immediate precipitate of iodoform identified by melting point and mixed melting point with an authentic sample.

Hydrogenation of toxol (534 mg.) in absolute ethanol at atmospheric pressure using a 5% rhodium on alumina catalyst (62 mg.) ceased with the absorption of 1 *M*-equivalent of hydrogen. Removal of the catalyst and solvent gave dihydrotoxol (412 mg.) as a viscous oil, the infared spectrum of which no longer contained the C=C band at 6.05 μ. Dihydrotoxol readily gave a 2,4-dinitrophenylhydrazone with an acidic methanolic solution of the reagent which after chromatography on acid-washed alumina and recrystallization from benzene–ligroin melted at 215–216°. The analysis of the dinitrophenylhydrazone indicated that 1 mole of water was lost in its formation. This is consistent with the observation (see below) that toxol also loses water when treated with acids.

Anal. Calcd. for $C_{19}H_{18}N_4O_6$: C, 59.67; H, 5.03. Found: C, 59.76; H, 5.35.

A stream of approximately 3% ozone in oxygen was passed into a solution containing 294 mg. of toxol in 5 ml. of methylene chloride at −70° for 5 hr. The solution after warming to room temperature, was added to water containing zinc dust, stirred for several hr., the water layer separated and the methylene chloride layer further extracted with water. The combined water layers were added to a saturated solution of dimethone in methanol, from which the dimethone derivative of formaldehyde precipitated in 38% yield. The derivative was identical (m.p. and mixture m.p.) with an authentic sample.

Preparation of Toxol Acetate and Toxol Dinitrophenylhydrazone.—Toxol acetate was prepared by dissolving 100 mg. of toxol in 10 ml. of pyridine, adding 2 ml. of acetic anhydride and refluxing the solution overnight. The solution then was poured into water, and extracted with ether. After washing with water and 5% hydrochloric acid, the ether layer was dried over sodium sulfate and then concentrated to give a viscous yellow oil. Distillation of the oil gave the acetate as a colorless oil, b.p. 70–75° (0.05 mm.); infrared spectrum, λ_{max} 5.75, 5.95, 6.21 μ.

Anal. Calcd. for $C_{15}H_{16}O_4$: C, 69.17; H, 6.19; O, 24.34. Found: C, 69.41; H, 6.47; O, 24.31.

The 2,4-dinitrophenylhydrazone of toxol was prepared by adding 50 mg. of toxol to 3 ml. of a solution prepared by dissolving 3 g. of 2,4-dinitrophenylhydrazine in 270 ml. of methanol and 30 ml. of concd. hydrochloric acid. The precipitated derivative was chromatographed on neutral alumina and was recrystallized from ethanol–water to m.p. 176–177°. The infrared spectrum showed no carbonyl band.

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.

Degradation of Toxol to 5-Acetylsalicylic Acid.—To a solution of 180 mg. of toxol in 10 ml. of acetone was added 50 ml. of a 5% potassium permanganate solution. After heating on the steam bath for 30 min., 100 ml. of 5% hydrochloric acid and then an aqueous sodium bisulfite solution was added until the reaction solution was colorless. Acetone was removed by distillation and the residue extracted with ether. After drying over anhydrous sodium sulfate, evaporation of the ether gave 74 mg. (65%) of 5-acetylsalicylic acid (m.p. 209–210°) identified by m.p. and infrared spectral comparisons with an authentic sample. Treatment of the 5-acetylsalicylic acid obtained from toxol with sodium hypoiodite gave iodoform and 4-hydroxyisophthalic acid (m.p. found, 304–307°; reported 306° and 310°). Chromium trioxide in acetic acid likewise oxidized toxol to 5-acetylsalicylic acid, whereas chromium trioxide in pyridine at room temperature had no effect.

Treatment of Toxol and Dehydrotremetone with Acid.—Hydrochloric acid (5%, 10 ml.) was added to a solution containing 216 mg. of toxol in 10 ml. of dioxane and the mixture refluxed for 24 hr. Dilution with water, extraction with ether and the usual work-up gave 213 mg. of a yellow oil, which after distillation b.p. 100° (0.05 mm.) solidified. Recrystallization from ether-petroleum ether gave m.p. 105–108°, λ_{max}. 5.95 μ (very similar to infrared spectrum of dehydrotremetone); mixture m.p. with dehydrotremetone was depressed. This same rearrangement product was obtained by treatment of toxol or dehydrotremetone with acetic acid containing a few drops of sulfuric acid

Anal. Calcd. for $C_{13}H_{12}O_3 \cdot 1/6H_2O$: C, 76.83; H, 6.12; O, 17.06. Found: C, 77.19; H, 5.92; O, 16.89

Alumina chromatography of the crude product obtained by treating toxol, at its melting point, with a crystal of iodine gave a small amount of dehydrotremetone in the benzene fraction but the major product, eluted with chloroform, was the rearrangement product mentioned above.

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 of the previously unreported 2,5-diacetylbenzofuran was very simple. The four aromatic protons appeared 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 δ 2.62 and δ 2.65. For comparison, 2-acetylbenzofuran was prepared by a known procedure[10] and its n.m.r. curve run. This curve was very similar to the one mentioned above. Besides the aromatic protons (5) at low field a single sharp line (3 protons) at δ 2.50 was present due to the protons of the 2-acetyl group.

2,5-Diacetylbenzofuran from Toxol.—Ozone was passed through a solution containing 400 mg. of toxol in 20 ml. of methylene chloride at −70° until the solution turned blue. This solution then was poured into 15 ml. of acetic acid to which was added 3 g. of zinc. After stirring for 2 hr. the solution was diluted with 100 ml. of water and the aqueous solution extracted with ether. The ether solution was washed with 5% sodium carbonate, dried over sodium sulfate and evaporated to give 320 mg. of a yellow oil. Distillation at 140° (0.04 mm.) resulted in dehydration and gave 2,5-diacetylbenzofuran (m.p. 139–140°) identical in all respects with that obtained from dehydrotremetone.

A solution of 273 mg. of toxol acetate in 40 ml. dry tetrahydrofuran was ozonized at −70° for 6 hr. The solution was diluted with 50 ml. of water and stirred for 1 hr. after which it was extracted with ether. After drying, the combined ether extracts were evaporated and the residue was heated to 240° at reduced pressure. Recrystallization of this product from methanol–water again gave diacetylbenzofuran (m.p. 139–140°).

Acknowledgments.—We wish to express our appreciation to Dr. O. C. Dermer for first bringing this problem to our attention, and to Mr. J. P. Sherle for plant collecting and preliminary experimental work. We thank Sister M. Roger Brennan for running the n.m.r. spectra and for numerous helpful discussions.

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Constitution of Toxol: A Toxic Constituent of *Aplopappus heterophyllus*

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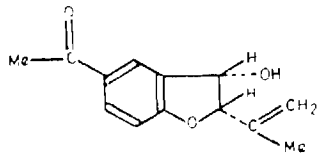
Aplopappus heterophyllus (rayless goldenrod) a plant indigenous to the Southwestern United States, has been known for many years to be responsible for "milk sickness" in higher animals and humans.[1-3] Previous work established that the saponified methanol plant extract was toxic, but the constitution of the toxic principle was not determined.[4]

Using repeated adsorption and partition chromatography and following the separation with a bacterial assay (*Bacillus cereus*), two toxic compounds have been isolated from the saponified methanol extract. The less toxic compound was identified as 2-isopropenyl-5-acetylbenzofuran (dehydrotremetone). This compound has recently been found to be present in *Eupatorium urticaefolium*,[5] a plant which appears to be identical to *Aplopappus heterophyllus* in its effects on higher animals. The second and more toxic constituent, toxol, has not been previously reported, and has been found to have the constitution (I).



(I)

Toxol, ($C_{13}H_{14}O_3$) m.p. 52-53°, $[\alpha]_D^{27}$ -25·1° (*c.*, 0·44 in MeOH), $\lambda_{max.}^{KBr}$ 2·96, 5·95, 6·05, 6·21μ, $\lambda_{max.}$ 223 mμ (log ε 4·02), 273 mμ (log ε 4·13) readily formed an oily acetate and a crystalline 2,4-dinitrophenylhydrazone, m.p. 176-177°.

In the presence of 5% rhodium on alumina, toxol absorbed one mole of hydrogen to give the dihydroderivative, which lost a mole of water in the formation of its 2,4-dinitrophenylhydrazone. Oxidation of toxol with potassium permanganate in acetone gave 5-acetylsalicylic acid (65%). Ozonolysis in methylene chloride at -70° gave formaldehyde (38%) and 2,5-diacetylbenzofuran (86%). This product results from ozonolysis followed by dehydration. An authentic sample of 2,5-diacetylbenzofuran was prepared from 2-isopropenyl-5-acetylbenzofuran(dehydrotremetone), isolated from the plant, by treatment with one molar equivalent of osmium tetroxide followed by cleavage of the resulting diol with lead tetracetate.

Treatment of toxol at its melting point with a crystal of iodine gave a small amount of dehydrotremetone and an unidentified product resulting from rearrangement. This latter product could also be obtained from dehydrotremetone in the presence of acids. The nuclear magnetic resonance spectra of toxol and its acetate showed that toxol contained a secondary hydroxyl group; this was evident by the characteristic downfield shift (~100 c.p.s.) of one proton in going from the alcohol to its acetate.[6]

The absolute configuration of toxol was determined by its degradation to *d*-dimethyltartrate of known absolute configuration.[7,8] Ozonolysis in acetic acid at room temperature for 30 hours gave a keto-acid which was esterified with diazomethane. This material was then treated with an alkaline iodine solution; esterification of the resulting product followed by chromatography on silica gel gave *d*-dimethyltartrate which gave a plain negative rotatory dispersion curve[9] (dioxan, *c.*, 0·056), [α] 589 mμ + 15·59°, [α] 450mμ + 8·66°, [α] 400mμ -8·66°, [α] 260mμ -806°) as previously described.[10] The configuration at *C*(2) in toxol is therefore the same as at *C*(5[1]), the corresponding centre, in rotenone.[11,12]

We thank Dr. O. C. Dermer for first bringing this problem to our attention, Dr. E. A. Grula for the bacteriological assay, Dr. P. Crabbé, for rotatory dispersion measurements, and the National Institutes of Health for a grant (RG-8346).

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THE OCCURRENCE OF 5α-ANDROSTANE-3β,16α,17α-TRIOL IN "RAYLESS GOLDENROD" (APLOPAPPUS HETEROPHYLLUS BLAKE)

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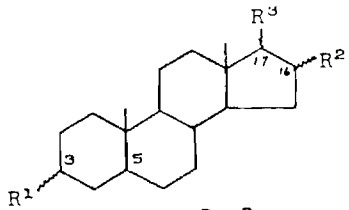
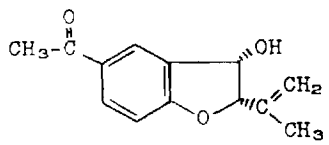
(Received 20 November 1963)

The naturally occurring male hormones or androgens have been isolated from urine and from testicular extracts and are based on the androstane skeleton I (1). We wish to report here the



I $R^1=R^2=R^3=H$
II $R^1=\beta$-OH, $R^2=R^3=\alpha$-OH



III

rather surprising occurrence of 5α-androstane-3β,16α,17α-triol, II, in the plant "rayless goldenrod." "Rayless goldenrod", indigenous to the southwestern United States, has been known for many years to be responsible for a disease of higher animals known as "trembles" or "milksickness" (2,3,4). Recent work has led to the isolation and structure elucidation of a dihydrobenzofuran III, toxol, from the crude plant toxin (5,6). Although toxol was found to inhibit the growth of several bacteria, it has not been shown to be responsible for the plant's toxicity to higher animals.

The sterol II was isolated as follows. Saponification of the methanolic extract of the whole dried plant (10% of weight of plant) with 5% potassium hydroxide gave the non-saponifiable crude toxin ("tremetol", "red-oil") which comprised approximately 1% of the plant. After steam distillation the residual crude toxin was separated into a ketone fraction (25%) and a non-ketone fraction (65%) by the use of Girard's T reagent. Chromatography of the non-ketone fraction on alumina gave II ($C_{19}H_{32}O_3$, m.p. 288° with previous melting at 265°, $[\alpha]_D$ - 16.5° $CHCl_3$) in 0.2% yield based on the crude toxin. The infrared spectrum of II showed strong hydroxyl absorption; unsaturation was not indicated in the infrared spectrum or by the tetranitromethane test.

II readily gave an acetate ($C_{19}H_{32}O_3$, m.p. 168-169°, $[\alpha]_D$ + 8.1 $CHCl_3$) giving an n.m.r. spectrum that indicated a ratio of three acetate groups to two bridgehead methyl groups. That II was an androstane derivative was shown by its conversion to 5α-androstane, I (R=H) by preparation of the tritosylate followed by hydrogenolysis with lithium aluminum hydride. An authentic sample of 5α-androstane was prepared by Huang-Minlon (7) reduction of 5α-androstane-3,17-dione. The two samples of 5α-androstane gave identical melting points (47-49°) alone and on admixture, and identical gas chromatograms (using a 5% SE-30 column) were obtained for each and on admixture. Ruzicka, Prelog and Wieland (8) had previously reported the preparation of 5α-androstane-3β,16α,17α-triol (m.p. 265-266°, $[\alpha]_D$ - 19 $\pm$ 4° C_2H_5OH) from 5α-androst-16-en-3β-ol and their triol likewise gave a triacetate (m.p. 165°, $[\alpha]_D$ + 10 $\pm$ 4° C_2H_5OH). 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 (8) was repeated. 17α-Hydroxy-5α-androstan-3-one benzoate was hydrogenated and reoxidized to give 17α-hydroxy-5α-androstan-3-one hexahydrobenzoate (m.p. 138-139°, $[\alpha]_D$ + 25° $CHCl_3$; reported (9): m.p. 137.5-138°) which on pyrolysis gave 5α-androst-16-en-3-one (m.p. 140-141°, $[\alpha]_D$ + 35° $CHCl_3$; reported (10): m.p. 140-141°, $[\alpha]_D$ + 38° $CHCl_3$). Reduction of the latter compound with lithium aluminum hydride gave 5α-androst-16-en-3β-ol (m.p. 126-127°, $[\alpha]_D$ + 16.1° $CHCl_3$; reported (10): m.p. 125-127°, $[\alpha]_D$ + 11.2 ± 2.5° $CHCl_3$) which on treatment with osmium tetroxide gave 5α-androstane-3β,16α,17α-triol ($[\alpha]_D$ - 17.1 $CHCl_3$) identical in infrared spectrum and melting point with that isolated from "rayless goldenrod". It was found that 5α-androstane-3β,16α,17α-triol exhibits two melting points, one at 265-270° and another at 280° if the material is allowed to resolidify after first melting.

Huffman and Lott (11) suggested that the product was obtained by hydroxylation of 5α-andros-16-en-3β-ol with osmium tetroxide was 5α-androstane-3β,16β,17β-triol since it was not identical with the product (m.p. 251-253°, $[\alpha]_D$ + 18°) they obtained on reduction of Butenandt's triol (12) (androst-5-ene-3β,16α, 17α-triol ?). This conclusion is most probably incorrect in view of the recent studies of Brutcher and Bauer (13) on the conformations of the D rings in steroidal 16,17-cis glycols. The product of hydroxylation of andros-16-en-3β-ol acetate with osmium tetroxide was found to possess a D ring half-chain conformation containing 16α,17α hydroxyl groups. In addition, the closely related estra-1,3,5 (10)-triene-3, 16α,17α-triol is prepared in an analogous manner from the corresponding C_{16} olefin.

5α-Androstane-3β,16α,17α-triol may have been isolated previously by Butler (14) 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. We have isolated a second sterol ($C_{29}H_{48}O$, m.p. 152-156, a_D - 9 $CHCl_3$) which appears to be isomeric with "α" spinasterol (15) from the non-ketone fraction of the crude toxin. "White snakeroot", a plant which produces a syndrome in higher animals similar to that produced by "rayless goldenrod", contains several benzofurans related to toxol and, in addition, has been reported to contain two unidentified sterols: Sterol I ($C_{30}H_{50}O$, m.p. 184.5-185.5°, $[\alpha]_D$ + 57.2° $CHCl_3$) and sterol II ($C_{21}H_{34}O$, m.p. 147-148°, $[\alpha]_D$ - 32.8 $CHCl_3$) (16).

This is believed to be the first report of the isolation of 5α-androstane-3β,16α,17α-triol from any natural source and its presence in the plant kingdom is particularly intriguing because of its close relationship to the urinary sterols such as 5α-androstane-3α,16α,17β-triol, 5β-androstane-3α,16α,17β-triol and andros-5-ene-3β, 16α,17β-triol.

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THE ABSOLUTE CONFIGURATIONS OF TREMETONE AND TOXOL[1]

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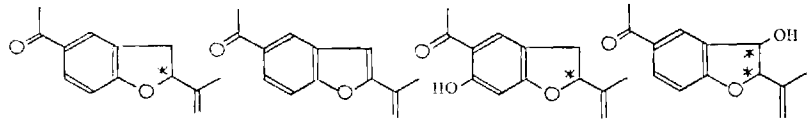
(*Received* 17 *January* 1964)

"TREMBLES" in cattle and "milksickness" in higher animals and humans are diseases which have been traced to the consumption by livestock of the white snakeroot plant (*Eupatorium urticaefolium*)[3] of the middle states and the rayless goldenrod plant (*Aploppapus heteroplyllus*)[4] of the southwestern portions of the United States. In the late 1920's Couch[3,5] isolated from these plants a dark tar, "tremetol", which he showed to be the toxin responsible for cattle "trembles," and in 1939 Dermer and his students[6,7] found that rayless goldenrod tremetol was not a homogeneous substance as reported by Couch, but rather a complex mixture. In 1961 Bonner *et al.*[8,9] re-investigated white snakeroot tremetol and succeeded in isolating from the crude toxin three ketones, namely, tremetone, (−)-2-isopropenyl-5-acetyl-2,3-dihydrobenzofuran (I); dehydrotremetone, 2-isopropenyl-5-acetylbenzofuran (II) and hydroxytremetone,(−)-2-isopropenyl-5-acetyl-6-hydroxy-2,3-dihydrobenzofuran (III). The structures of these ketones were established by chemical degradations[9,10] and



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were confirmed by the synthesis of dihydrotremetone (2-isopropyl-5-acetyl-2,3-dihydrobenzofuran)[11] and of tremetone itself, both in the racemic[12] and optically active[13] forms. These ketones proved to be toxic to goldfish,[9,13] and (−)-tremetone, the most abundant constituent, showed some insecticidal properties.[13] In contrast to crude white snakeroot[13] and rayless goldenrod[14] tremetol, however, (−)-tremetone proved non-toxic to chickens, and is presumably not the responsible toxin in this plant.[13] More recently Zalkow *et al.*[15] have established the presence of both dehydrotremetone (II) and toxol, (−)-2-isopropenyl-3-hydroxy-5-acetyl-2,3-dihydrobenzofuran (IV) in crude rayless-goldenrod tremetol. Toxol was shown[15] to be bacteriostatic towards *Bacillus cereus*, *Staphylococcus albus* and *Corynebacterium hoagii*, but its toxicity towards higher animals has not yet been confirmed. The absolute configurations of toxol has been established by its conversion *via* ozonization, hypoiodite degradation and esterification into methyl (+)-tartarate of known absolute configuration.[16] We now wish to present further details of the absolute configurational establishment of both toxol from rayless goldenrod and tremetone from white snakeroot.

Natural (−)-tremetone (I) has been synthesized from (+)-dihydrocoumarilic acid (V) (Chart 1) by a series of reactions which did not affect the single asymmetric center in the latter.[13] Accordingly, establishment of the absolute configuration of V was deemed the simplest approach to determining the absolute configuration of I. (+)-Dihydrocoumarilic acid (V) was esterified with diazomethane, and the resulting methyl dihydrocoumarilate was ozonized at 0° in a mixture of acetic acid and ethyl acetate. The ozonide was decomposed with hydrogen peroxide and the by-product oxalic acid was removed as its calcium salt. The remaining acidic material was esterified with diazomethane and distilled, affording an ester whose IR spectrum was almost identical with that of methyl D-(+)-malate (VI) prepared according to the procedure of Shoppee and Reichstein.[17] Its specific rotation, $[\alpha]^{20}$ +12·8° (*c*, 1·1; acetone), however, was somewhat higher than that anticipated[18] for methyl D-(+)-malate, and vapor-liquid partition chromatography revealed the presence of about 5% of an extraneous component, both in the present ester product and in that prepared similarly from malic acid. Chromatography on alumina readily separated the two compounds, and the fraction eluted with ether proved to be methyl methoxysuccinate. The purified methyl D-(+)-malate had a specific rotation of +11·5° in good agreement with the literature.[18] (+)-Dihydrocoumarilic acid itself afforded methyl D-(+)-malate on similar ozonization, oxidation and esterification. The overall yield was somewhat lower, however, presumably because of partial oxidation of the malic acid intermediate by hydrogen peroxide.[19] The conversion of (+)-dihydrocoumarilic acid (V) into methyl D-(+)-malate (VI) indicates that the former acid has the absolute configuration V in Chart I, in contrast to the previous tentative configurational

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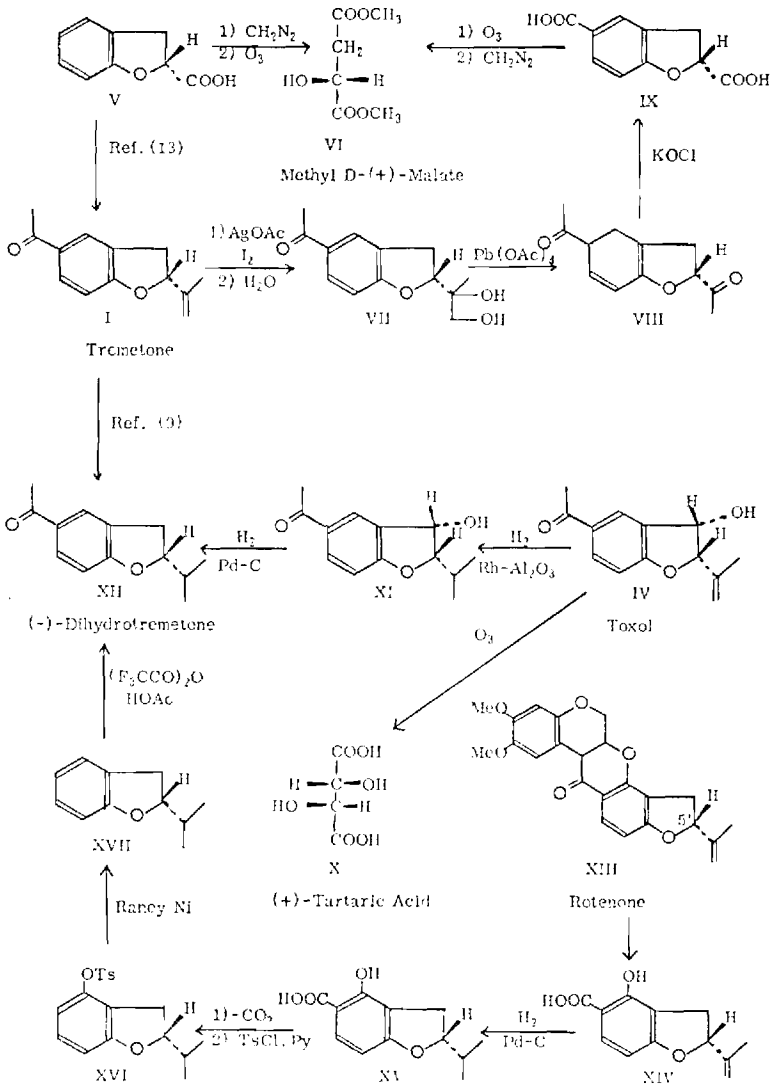
prediction based on plant physiological tests[20] and optical rotatory dispersion.[21] Tremetone accordingly has the configuration I shown in Chart I.

Confirmation of these conclusions was next undertaken by the direct degradation

CHART I



of (−)-tremetone (I) itself. Preliminary ozonization, as applied to V above, proved inapplicable, but the sequence of steps, I → VII → VIII → IX → VI in Chart I, again demonstrated that the single asymmetric center in (−)-tremetone was configurationally related to that of D-(+)-malic acid.

The configurational relationship of the two asymmetric centers in toxol (IV) with

[20] A. Fredga and C. V. de Castro y Sarmiento, *Arkiv. Kemi* 7, 387 (1954).
[21] B. Sjöberg, *Arkiv. Kemi* 15, 461 (1960).

those of (+)-tartaric acid (X) has been previously reported in a preliminary communication.[16] The sequence of reactions, involving ozonization of IV followed by oxidation with 30% hydrogen peroxide, is described in detail below. In addition, the configuration of the C2 asymmetric center of IV (bearing the isopropenyl function) has now been directly related to the corresponding asymmetric center in (−)-tremetone (I). Although the hydrogenation of toxol using palladium or platinum catalysts resulted in extensive hydrogenolysis and a complex product mixture,[15] hydrogenation with the very selective catalyst, rhodium on alumina, smoothly afforded dihydrotoxol (XI). Hydrogenolysis of the latter using 10% palladium on charcoal as catalyst yielded a sample of (−)-dihydrotremetone (XII) identical with that obtained[9] by the catalytic hydrogenation of natural tremetone (I). This series of interconversions establishes the configurational identity at C2 of toxol and tremetone, and directly relates the configuration of the latter ketone with (+)-tartaric acid as well.

Two groups independently have established the configuration at the C-5′ asymmetric center of rotenone (XIII), by direct interrelations with D-glyceraldehyde[22] and L-valine,[23] respectively. Consequently it appeared feasible to confirm these assignments of the configuration of tremetone by directly interrelating rotenone (XIII) with (−)-tremetone (I). Vigorous alkaline hydrolysis of rotenone, according to literature procedures,[24,25] gave the degradation product, tubaic acid (XIV), which was hydrogenated to the known dihydrotubaic acid (XV). Takei and Koide[24] have reported (with no experimental details or elementary analyses) the formation of a tosylate from XV, which we hoped might be reduced using Raney nickel according to the method of Kenner and Murray.[26] We were unable, however, to obtain tosylates from either XV or its methyl ester. An alternative method of dehydroxylating phenols, namely, the sodium–ammonia reduction of aryl diethyl phosphate esters,[27] was similarly precluded by our inability to prepare the requisite phenolic phosphate from the methyl ester of XV.

Since the adjacent carboxyl group at C5 appeared likely to be responsible for the difficulty in forming phenolic esters of XV, this group was removed by thermal decarboxylation. The decarboxylation product, dihydrotubanol, readily formed a crystalline tosylate (XVI), which underwent smooth hydrogenolysis by Raney nickel in refluxing ethanol to yield (−)-2-isopropyl-2,3-dihydrobenzofuran (XVII). The latter product was readily acetylated by the mild procedure employing acetic acid and trifluoroacetic anhydride,[11,28,29] giving (−)-dehydrotremetone (XII), m.p. 47–47·5°. Identity with the levorotatory hydrogenation product from natural (−)-tremetone[9] was confirmed by IR spectra of the ketones and their 2,4-dinitrophenylhydrazones, and by mixture melting point comparison with these latter derivatives. Since the asymmetric center at C2 in tubaic acid is unaffected during the series of transformation (XIV → XV → XVI → XVII → XII) in Chart I, the single C2 asymmetric center in

[22] G. Büchi, L. Crombie, P. J. Godin, J. S. Kaltenbronn, K. S. Siddalingaiah and D. A. Whiting, *J. Chem. Soc.* 2843 (1961).
[23] M. Nakazaki and H. Arakawa, *Bull. Chem. Soc., Japan* **34**, 1246 (1961).
[24] S. Takei and M. Koide, *Ber. Dtsch. Chem. Ges.* **62**, 3030 (1929).
[25] H. L. Haller and F. B. LaForge, *J. Amer. Chem. Soc.* **52**, 3207 (1930).
[26] G. W. Kenner and M. A. Murray, *J. Chem. Soc.* 178 (1949).
[27] G. W. Kenner and N. R. Williams, *J. Chem. Soc.* 522 (1955).
[28] E. Bourne, M. Stacey, J. Tatlow and J. Tedder, *J. Chem. Soc.* 718 (1951).
[29] J. Tedder, *Chem. Rev.* **55**, 787 (1955).

tremetone, the C-5′ center in rotenone and the C2 asymmetric center in toxol may be assigned the same R-configuration. This configurational identity suggests the possibility that tremetone, toxol and rotenone might have a common biosynthetic precursor.[30]

EXPERIMENTAL

Ethyl(−)-dihydrocoumarilate. (+)-Dihydrocoumarilic acid (V) (450 mg; $[\alpha]_D^{20}$ +22·7°, *c*, 2·08, ethanol) was dissolved in a mixture of anhydrous ethanol (20 ml) and benzene (30 ml) containing a drop of conc. H_2SO_4. The mixture was refluxed 1 hr while the water was continuously withdrawn, then cooled and poured into water. The product was extracted with ether and the ether solution washed with $NaHCO_3$ aq, water and then dried. Solvent removal and distillation under vacuum yielded 470 mg of levorotatory ester, n_D^{25} 1·5195, $[\alpha]_D^{20}$ −18·4° (*c*, 0·935; hexane) and +0·9° (*c*, 0·716, ethanol).

(+)-Dihydrocoumarilamide. The above ethyl(−)-dihydrocoumarilate (450 mg) was dissolved in methanol (25 ml) and ammonia was passed through the solution for 5 hr at 0°. After standing in the refrigerator overnight, the precipitated amide was filtered off and recrystallized from methanol, yield 360 mg, m.p. 182–183°, $[\alpha]_D^{20}$ +58·5° (*c*, 0·961; acetone). (Found: C, 66·2; H, 5·45; N, 8·78. $C_9H_9NO_2$ requires: C, 66·25; H, 5·56; N, 8·58%).

Ozonolysis of (+)-dihydrocoumarilic acid (V). The above (+)-dihydrocoumarilic acid (500 mg) was dissolved in a mixture of acetic acid (10 ml) and ethyl acetate (10 ml), and the solution was ozonized for 12 hr at 0°. The ethyl acetate was evaporated under vacuum, 30% H_2O_2 (3 ml) was added, and the solution kept at room temp for 12 hr, whereupon water (10 ml) and some Pd-C catalyst was added, followed by addition of calcium acetate (200 mg). The mixture was filtered and the filtrate was percolated through a column of Dowex 50 in the hydrogen form. The ion exchange column was washed with acetic acid–water (1:1, 50 ml) and the combined effluent was evaporated under vacuum. The resulting syrup was dissolved in a small amount of methanol and treated with excess diazomethane in ether. After 2 hr the solution was dried (Na_2SO_4), the solvent evaporated and the residue distilled under vacuum to yield 340 mg of oil, $[\alpha]_D^{20}$ +12·8° (*c*, 1·10, acetone), whose IR spectrum was identical with that of a sample of methyl malate obtained by treatment of malic acid with diazomethane in methanol–ether.[17] Vapor–liquid partition chromatography of the crude oil on a polyglycol column (179°) showed two peaks for both ester samples (retention time 10·0 and 6·6 min; area 20:1, respectively). The minor product had the same retention time as methyl methoxysuccinate prepared according to Lardon and Reichstein.[31] The crude ester from the ozonolysis was dissolved in ether and chromatographed on alumina (30 g). The methyl methoxysuccinate component was eluted with ether and the methyl malate with ether–methanol (1:1). Distillation of the latter fraction under vacuum gave pure methyl D-(+)-malate (VI), $[\alpha]_D^{20}$ +11·4° (*c*, 1·01; acetone).

Ozonolysis of methyl dihydrocoumarilate. (+)-Dihydrocoumarilic acid (500 mg, $[\alpha]_D^{20}$ +22·7°, ethanol) was dissolved in ether and treated with excess diazomethane. The ether was evaporated and the residue was ozonized and processed as described above, with the exception that treatment with calcium acetate and percolation through the ion exchange column was performed in more concentrated acetic acid (70%), due to different solubility properties. Vacuum distillation afforded 450 mg of the crude methyl D-(+)-malate (VI), $[\alpha]_D^{20}$ +13·0° (*c*, 1·01; acetone). Chromatography on alumina gave the pure ester $[\alpha]_D^{20}$ +11·5° (*c*, 1·01; acetone). Walden[18] has reported $[\alpha]_D^{20}$ −11·58 (*c*, 4·23; acetone) for the enantiomeric methyl L-(−)-malate.

(−)-2-(5′-Acetyl-2′,3′-dihydro-2′-benzofuryl)-1,2-propanediol (VII). A mixture of slightly impure (−)-tremetone (I; 4·04 g), silver acetate (7·4 g) and iodine (5·1 g) in acetic acid (200 ml) was shaken at room temp for 1 hr, then treated with water (0·4 ml) in acetic acid (10 ml), heated under reflux for 30 min, cooled and filtered free of silver salts. The filtrate was evaporated nearly to dryness (red. press.), the residue dissolved in ether (300 ml), the solution washed (sat. K_2CO_3 aq.), dried over $MgSO_4$ and filtered, and the filtrate evaporated to dryness. The residue was dissolved in methanol (400 ml), treated with a solution of KOH (15 g) in water (400 ml) and stirred at 40–50° for 1 hr,

[30] For discussions of the biosynthesis of rotenone, see H. Grisebach and W. D. Ollis, *Experentia* **17**, 4 (1961); H. Grisebach, in *Recent Progress in the Chemistry of Natural and Synthetic Colouring Matters* (Edited by T. S. Gore, B. S. Joshi, S. V. Sunthankar and B. D. Tilak) p. 301. Academic Press, New York, N.Y. (1962).

[31] A. Lardon and T. Reichstein, *Helv. Chim. Acta* **32**, 2003 (1949).

whereupon the mixture was concentrated to 400 ml at red. press. and extracted with ether. The extract was dried and stripped of solvent to yield 2·80 g of amber oil which crystallized from acetone, 1·80 g (39%), m.p. 125–130°. Recrystallization from acetone afforded the pure, colorless diol VII, m.p. 137–138°, $[\alpha]_D^{26}$ – 105° (*c*, 1·0; methanol). Its IR spectrum showed an OH band at 3400 cm^{-1} and carbonyl band at 1660 cm^{-1}. (Found: C, 66·19; H, 6·91. $C_{13}H_{16}O_4$ requires: C, 66·08; H, 6·83%).

(–)-2,5-*Diacetyl*-2,3-*dihydrobenzofuran* (VIII). A mixture of the above diol VII (1·60 g) and lead tetraacetate (4·00 g) in benzene (100 ml) and acetic acid (20 ml) was stirred for 1 hr at 25°, then diluted with ether (200 ml). The mixture was then treated dropwise with stirring (30 min) with sat. K_2CO_3 aq, until gas evolution ceased. The ether layer was separated and the aqueous layer was extracted twice with ether. The combined ether extracts were dried ($MgSO_4$), filtered and evaporated, yielding 1·10 g (75%) of white solid, m.p. 72–74°. The pure diketone VIII was obtained on recrystallization from ligroin, m.p. 73–74°, $[\alpha]_D^{27}$ – 41° (*c*, 1·0; methanol), carbonyl absorption bands at 1720 and 1670 cm^{-1}. (Nujol mull). (Found: C, 70·30; H, 5·77. $C_{12}H_{12}O_3$ requires: C, 70·57; H, 5·92%).

Racemic 2,5-diacetyl-2,3-dihydropyran was also prepared for comparison with the above sample and to provide starting material for developing the degradative technique described below. A solution of 2-acetyl-2,3-dihydrobenzofuran (3·2 g) and acetic anhydride (4 g) in benzene (30 ml) was cooled to 0° and treated dropwise with stirring over 10 min with a solution of $SnCl_4$ (13 g) in benzene (20 ml). The purple solution was stirred for 2 hr at 25°, then poured onto ice and extracted with ether. The extract was shaken for several min with 30% KOH aq, then dried, filtered and evaporated to yield 3·8 g of purple oil. This was dissolved in acetone, and the solution decolorized (Norit) and evaporated. The residue was crystallized with ligroin, giving 3·1 g (76%) of solid, m.p. 77–80°. The product was recrystallized twice by extraction with a Soxhlet extractor into ligroin. The pure racemic diketone VIII had m.p. 81–82° and displayed an IR spectrum (chloroform) identical with that of the above (–)-isomer. (Found: C, 70·61; H, 6·07. $C_{12}H_{12}O_3$ requires: C, 70·57; H, 5·92%).

Degradation of (–)-2,5-*diacetyl*-2,3-*dihydrobenzofuran* (VIII). The above levorotatory diketone VIII (1·0 g) was dissolved in methanol (100 ml) and the solution treated dropwise at 60° with KOH (3·0 g) in water (100 ml) which had been saturated with chlorine, maintaining the reaction mixture at about pH 9·0 by occasional addition of a few drops of 20% KOH aq. The mixture was kept at 60° for 2 min after the addition, then treated with a few drops sat. Na_2SO_3 aq and finally distilled at red. press. (40°) to ⅓ volume. The residue was extracted with ether (discard), acidified with HCl aq and extracted again with ether 4 times. The extract was dried ($MgSO_4$), filtered and evaporated to yield 0·90 g of amber oil which crystallized on rubbing with ether, 0·51 g, m.p. 220–232°, $[\alpha]_D^{27}$ –5·2° (*c*, 5·0; methanol). As no suitable solvent for purifying the crude IX could be found, it was degraded directly. Thin layer chromatographic examination showed the crude product to consist of one principal component and 3 minor constituents.

The crude acid (0·50 g) was dissolved in acetic acid (10 ml) and ozonized for 8 hr at room temp. The solution was then treated with 30% H_2O_2 (3 ml) and allowed to stand for 12 hr, whereupon it was treated with 10% Pd–C and water (5 ml) and stirred for 3 hr, after which excess peroxide proved absent (KI-starch paper). The catalyst was filtered and the filtrate concentrated to ½ volume, then treated with water (10 ml) containing small amounts of Ca and Ba acetates. The precipitated salts were filtered and the filtrate percolated through an ion exchange column (Amberlite IR-120 in the acid form), which was then washed thoroughly with water (200 ml). The combined eluates were evaporated to dryness at red. press. and the residual sirup esterified using diazomethane in ether, yielding 0·360 g yellow oil. This was purified chromatographically on neutral alumina (3% H_2O), eluting with ether and then ether–methanol (1:1). The latter eluate was evaporated to yield an oil. This was dissolved in a small amount of ether, and the solution dried ($MgSO_4$), filtered and evaporated to yield 74 mg methyl D-(+)-malate (VI), $[\alpha]_D^{28}$ +12·1° (*c*, 3·2; acetone). Its IR spectrum (neat) was superimposable on that of a sample of racemic methyl malate prepared by the action of diazomethane on malic acid.

Ozonization of toxol (IV). A stream of oxygen containing ozone (~4%) was passed through a solution of toxol (0·1 g; m.p. 52–53°, $[\alpha]_D^{27}$ –25·1°; *c*, 0·44; methanol) in acetic acid (25 ml) at room temp for 25 hr. Hydrogen peroxide 30% (8 ml) was then added and the solution stirred for 18 hr, whereupon Pd–C was added and stirring continued for an additional 2 hr. After filtration, the acetic acid was removed by rotary evaporation, and a viscous residue obtained. Oxalic acid was removed from the residue by sublimation on heating at 90° (0·5 mm) for 12 hr. The remaining residue was

decolorized by dissolving in water (2 ml) and heating with charcoal, after which filtration and concentration afforded 110 mg (+)-tartaric acid (X). After one recrystallization from water the sample had m.p. 171° (reported[32] 170°), and $[\alpha]_D^{27}$ +8·40°, (*c*, 0·032; water), reported:[32] $[\alpha]_D^{20}$ +12° (20% aqueous solution).

The above (+)-tartaric acid was esterified by treatment with ethereal diazomethane. After distillation, b.p. 65° (1·2 mm), pure methyl (+)-tartarate was obtained, $[\alpha]_D^{25}$ +10·81° (*c*, 0·021; methanol), reported:[33] $[\alpha]_D^{16}$ +13·82 (methanol). The IR spectrum of the methyl (+)-tartarate was identical with that of an authentic sample of racemic methyl tartarate.

Conversion of toxol (IV) *into dihydrotremetone* (XII). Toxol (0·5 g) was hydrogenated at atm. press. using 50 mg 5% Rh–Al catalyst in 20 cc of 95% ethanol. Hydrogen uptake ceased after the absorption of one molar equiv. H_2. The catalyst was filtered and hydrogenation was continued after the addition of 50 mg 10% Pd–C catalyst. Again, hydrogen uptake ceased after the absorption of approximately one molar equiv. H_2. After removed of the catalyst by filtration, the solvent was removed by a rotary evaporation, the residue dissolved in ether, and the solution poured through a 0·5 cm × 2 cm column of acid washed alumina (Merck). Evaporation of the solvent and distillation of the residue, b.p. 65° (0·04 mm), yielded 97 mg dihydrotremetone (XII), $[\alpha]_D^{24}$ −43°, (*c*, 2·71; ethanol), reported:[9] $[\alpha]_D^{25}$ −47·0°, (*c*, 1·78; ethanol). The IR spectrum of the dihydrotremetone was identical with that of a sample of racemic dihydrotremetone prepared by hydrogenation of dehydrotremetone (II) using a 5% Rh–Al catalyst.

Tubaic acid (XIV). This was prepared from XIII by the procedure of Haller and LaForge,[25] on 5 times the scale reported. The yield of crude product averaged 6–7 g from 50 g rotenone. Recrystallization from aqueous ethanol gave material m.p. 128–129° (reported[25] m.p. 129°).

Methyl dihydrotubate. Dihydrotubaic acid (XV) was prepared by hydrogenation of XIV in ethyl acetate over 10% Pd–C catalyst, m.p. 167·5–168·5° (reported[24] m.p. 166°). Methyl dihydrotubate resulted in turn by treatment of XV with ethereal diazomethane. The ester was recrystallized from ether, m.p. 78–79°. (Found: C, 66·28; H, 6·82. $C_{13}H_{16}O_4$ requires: C, 66·08; H, 6·83%).

The ester was soluble in dil. alkali, showed a phenolic hydroxyl band in the IR at 3·17 μ, and gave a deep red color with alcoholic $FeCl_3$. Similar unreactivity of the phenolic hydroxyl of tubaic acid towards diazomethane has been reported.[24]

Dihydrotubanol p-toluenesulfonate (XVI). A solution of 5·79 g dihydrotubanol[24] in pyridine (20 ml) was treated at 0° with *p*-toluenesulfonyl chloride (11·58 g; 2 equivs) in portions, with swirling until all the chloride had dissolved. After standing 3 days in the refrigerator, the mixture was poured onto ice water and extracted with ether. The ether layer was washed with dil. HCl aq, dried, and concentrated, affording the crystalline ester XVI. Recrystallized from 60–70° pet. ether, it showed m.p. 69–70° and $[\alpha]_D^{20}$ −25·76° (*c*, 1·25; chloroform); 6·65 g (56%) of recrystallized material was obtained. (Found: C, 65·01; H, 6·39. $C_{15}H_{20}O_4S$ requires: C, 65·06; H, 6·02%).

(−)*Dihydrotremetone* (XII). Freshly prepared Raney nickel catalyst (30 g) was added to a solution of 7·86 g of the above tosylate XVI in ethanol (250 ml), and the mixture refluxed for 24 hr, then filtered (Celite) and the filtrate poured into water (2 l.). The solution was extracted 4 times with 250-ml portions of ether, and the extracts washed with water and dil. NaOH aq, then dried and distilled, yielding 3·23 g crude XVII, b.p. 216–221°, $[\alpha]_D^{25}$ −26·5° (*c*, 1·41; chloroform), which was employed directly below.

A mixture of trifluoroacetic anhydride (6·80 g) and acetic acid (1·94 g) was cooled in ice, while 2·20 g of the above XVII was added dropwise. The resulting purple solution was kept for 4 hr at room temp, then poured into ice water and neutralized with excess Na_2CO_3. The solution was steam-distilled until about 1·5 l. of distillate had been collected, and the colorless crystals in the distillate filtered and dried; yield 1·56 g, m.p. 44–46°. Another 0·48 g of yellow solid was obtained by ether extraction of the filtrate, bringing the total yield to 2·04 g (73%). Recrystallization from 30–60° pet. ether, in which the ketone proved quite soluble, gave beautiful, long needles of XII, m.p. 47–47·5°, $[\alpha]_D^{22}$ −71·8° (*c*, 2·56; ethanol), −72·7° (*c*, 4·37; CCl_4). The IR spectrum was identical with that of an authentic specimen of XII. Preparation of the 2,4-dinitrophenylhydrazone of the above XII, recrystallized from chloroform–ethanol, gave scarlet needles, m.p. 184–186° (reported[9] m.p. 181–184°). These showed no m.p. depression on admixture with an authentic sample, and IR spectra ($CHCl_3$) of the two specimens were identical.

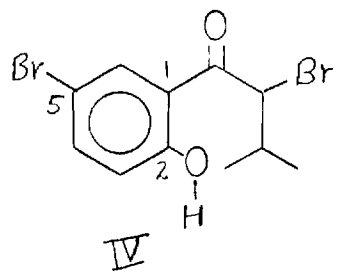
[32] *The Merck Index* (7th Edition) p. 1012. Merck and Co., Rahway, N.J. (1960).

[33] C. Weygand, A. Weissberger and H. Baumgartel, *Ber. Dtsch. Chem. Ges.* **65**, 698 (1932).

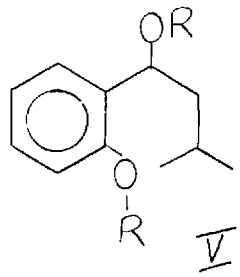
gave 2-isopropyl-5-acetylbenzofuran.[2] Treatment of the latter compound with lead tetraacetate gave 2-(5'-acetyl-2'-benzofuryl-2-propanol.

Other unsuccessful approaches to toxol or dihydrotoxol involved mild oxidation of 2-(5'-acetyl-2'-dihydrobenzofuryl)-2-propyl acetate which gave 5-acetylsalicylic acid and the synthesis of methyl 3-hydroxy-5-acetyl-coumarilate which, however, could not be acetylated under any conditions and hence was not further useful. This unsuccessful acetylation is particularly surprising since 3-hydroxycoumarilate is readily acetylated.

In view of the above described work it was felt that a worthwhile approach would involve a precursor in which the furan ring was not preformed and which contained a functional group which could eventually be converted into the 5-acetyl group of toxol or dihydrotoxol. Such a compound would be 2-hydroxy-5-bromo-(α-bromo-β-methyl)butyrophenone, IV. This material was readily prepared from phenol by treatment with isovaleryl chloride to give



IV

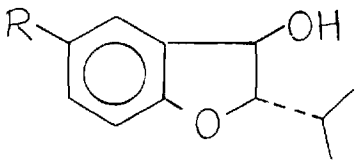


V

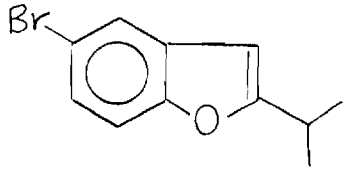
phenyl isovalerate, which was immediately converted into O-hydroxy isovalerophenone [b.p. 73°/0.4 mm; λ_{max}^{film} 6.11 μ; δ 0.96 (6H, doublet, J=6cps), 2.76 (2H), 6.81-7.77 (4H] by the Fries rearrangement. Bromination in acetic acid gave IV [m.p. 88.5-90°; λ_{max}^{KBr} 6.13 μ; δ 1.05 (3H, doublet, J=6cps); 1.25 (3H, doublet, J=6 cps); 4.83 (1H, doublet, J=9cps); 6.87-7.9 (3H)].

Substitution of a bromine atom at C-5 was particularly advantageous since it made possible the introduction of an acetyl group at this position

under non-acidic conditions. Thus, attempts to acetylate V (R=H, tetrahydropyranyl or acetyl) lead to resinification; o-hydroxy isovalerophenone could, of course, not be acylated at C-3. Reduction of IV with sodium hydroxide in an aqueous-ethanolic solution of potassium hydroxide gave in 54% yield the 2-isopropyl-3-hydroxy-5-bromocoumaran VI [m.p. 112-113°; λ_{max}^{KBr} 3.0μ; δ 1.09 (3H, doublet, J=6 cps); 1.15 (3H, doublet, J=6 cps); 3.99 (1H, quartet, J=5,10 cps); 5.08 (1H, doublet, J=5 cps)]. The mother



VI R = Br
VIII R = CO_2H
IX R = $COCH_3$



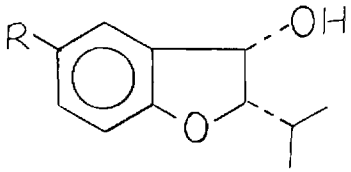
VII

liquor remaining after the removal of VI yielded a viscous oil which was distilled and identified as 2-isopropyl-5-bromobenzofuran, VII [b.p. 75°/0.075 mm; δ 1.25 (6H, doublet, J=6 cps); 2.98 (1H, septet); 6.15 (1H); 7.21-7.5 (3H)] by the similarity of its NMR spectrum to that of 2-isopropylbenzofuran.[2]

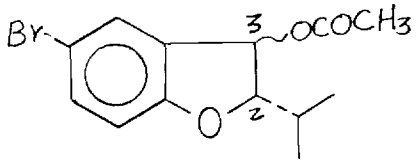
Treatment of VI with butyl lithium and then carbon dioxide gave the acid VIII [m.p. 196-197°; λ_{max}^{nujol} 3.01, 5.93 μ]. Acid VIII on treatment with methyl lithium gave IX [m.p. 115°; λ_{max}^{nujol} 2.98, 6.03 μ; δ 0.47 (3H, doublet, J=6.5 cps); 0.51 (3H, doublet, J=6.5 cps); 1.85 (3H), 3.44 (1H, quartet, J=5 and 10 cps); 4.51 (1H, doublet, J=5 cps)]. Product IX was not identical with dihydrotoxol prepared from natural toxol and hence a trans configuration can be assigned to the hydroxyl and isopropyl groups in

IX and therefore also in VI and VIII. However, a slight variation in the experimental conditions did lead to dihydrotoxol as described below.

When the reduction of 2-hydroxy-3-bromo-(α-bromo-β-methyl) butyrophenone, V, with sodium borohydride was conducted in ethanol in the absence of potassium hydroxide and then the crude product treated with ethanolic potassium hydroxide, cis-2-isopropyl-3-hydroxy-5-bromocoumaran, X [m.p.45°; λ_{max}^{nujol} 3.02 μ; δ 0.88 (6H, doublet, J=6 cps); 3.95 (1H, quartet, J=4 and 6 cps); 4.65 (1H, doublet, J=4 cps)] was obtained in good yield. Alcohol X was acetylated with acetic anhydride and pyridine to give XI [b.p. 95°/0.3 mm;



X R=Br
XIII R=CO_2H
XIV R=$COCH_3$



XI 2,3 cis
XII 2,3 trans

λ_{max}^{film} 5.75, 8.11 μ; δ 0.94 (3H, doublet, J=7 cps); 0.99 (3H, doublet, J=7 cps); 2.02 (3H); 4.33 (1H, quartet, J=3 and 6 cps); 6.01 (1H, doublet, J=3 cps)]. For comparison purposes VI was converted into its acetate XII [m.p. 90°; λ_{max}^{nujol} 5.78, 8.06 μ; δ 0.97 (3H, doublet, J=6 cps); 1.15 (3H, doublet, J=6 cps); 1.97 (3H), 4.0 (1H, quartet, J=6 and 9.5 cps); 6.01 (1H, doublet, J=6 cps)].

Compound X was converted into acid XIII [m.p. 157.5-159°; λ_{max}^{nujol} 3.08,

5.98 μ; δ 1.0 (6H, doublet, J=6 cps); 4.33 (1H, quartet, J=4 and 6 cps); 5.22 (1H, doublet, J=4 cps)] with butyl lithium followed by carbon dioxide. Finally acid XIII was converted into dihydrotoxol XIV [b.p. 86-91°/0.01 mm.; λ_{max}^{film} 2.96, 6.01, 6.22 μ; δ 0.98 (6H, doublet, J=6 cps); 2.35 (3H); 4.21 (1H, quartet, J=4 and 6 cps); 4.97 (1H, doublet, J=4 cps] identical in all respects with that obtained from natural toxol.[2]

In the two step conversion of IV into X the carbonyl group is reduced in the first step and ring closure occurs in the second step. An examination of Dreiding models clearly shows that by application of Cram's rule[3] in the borohydride reduction of IV, one would expect to get predominantly the erythro alcohol. Backside displacement of the bromine atom by the phenolic O-H group would then lead to the cis isomer as observed. Isolation of the trans isomer in the borohydride reduction under alkaline conditions might occur as follows. If ring closure precedes reduction in this case, then reduction of the coumarone could precede from both steric directions (thermodynamic and kinetic control) to give the cis and trans alcohols. The cis alcohol was not isolated in this case, but a rather large amount of benzofuran VII was obtained which could conceivably have arisen by elimination of water from the cis alcohol (trans OH and H).

A comparison of the NMR spectra of the two series of cis and trans dihydrobenzofuran derivatives provides some interesting information. Thus, the coupling between the C-2 and C-3 protons in the cis series is always smaller (J=3 to 4 cps) than in the trans series (J=5 to 6 cps). This is exactly opposite to what would be expected on the basis of the Karplus equation[4] assuming a planar furan ring. The furan ring is, however, probably not planar and in the cis isomers repulsion between the O-H and

isopropyl groups at C-2 and C-3 would result in rotation around the C-C bond increasing the dihedral angle (between hydrogen atoms) and decreasing the coupling constants. To avoid eclipsing in the trans isomers rotation around the C-C bond (O-H and isopropyl groups moving away from each other) would result in a decrease in dihedral angle and an increase in coupling constants. Another interesting but yet unexplained observation is the large up field shift of the isopropyl methyl groups in trans isomer IX.

Correct elemental analyses were obtained for all compounds mentioned in this study.

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Isolation and Synthesis of 2,5-Diacetylbenzofuran[1]

L. H. Zalkow and C. T. Ramming

The residue from the methanol extract of rayless goldenrod was distributed between water and chloroform. The chloroform soluble residue was extracted with 50% ethanol and the 50% ethanol soluble residue was extracted with 30% ethanol. The 30% ethanol residue was extracted with benzene to give a viscous gum which after chromatography on alumina gave 2,5-diacetylbenzofuran (0.01% based on dried plant) in the ether-benzene (3:2) eluent.

2,5-Diacetylbenzofuran has been previously reported as a degradation product of both dehydrotremetone and toxol[2] and was synthesized from synthetic racemic tremetone[3] by conversion to 2,5-diacetyl-2,3-dihydrobenzofuran as previously described[4] followed by dehydrogenation with Pd-C. A number of unsuccessful synthetic approaches were investigated.[1] The I.R., U.V. and N.M.R. spectra of 2,5-diacetylbenzofuran have been recorded and the biosynthesis of this compound has been discussed.[1]

The Fatty Acid and Terpene Constituents of Rayless Goldenrod[5]

L. H. Zalkow and N. I. Burke

The concentrated methanolic extract of rayless goldenrod was saponified and extracted with ether. The alkaline aqueous solution was acidified with carbon dioxide to give an ether soluble fraction which on treatment with lead acetate gave a solid precipitate. The solid, on treatment with hydrogen sulfide gave stearic acid, while the filtrate from above, after concentration and treatment with diazomethane, gave compounds identical by g.l.c. with methyl myristate and methyl laurate.

The ether insoluble fraction, from the carbon dioxide treatment was acidified (pH2) with aq. hydrochloric acid and the resulting ether soluble material was esterified with boron trifluoride in methanol or diazomethane. The following esters were identified by g.l.c.: methyl hexanoate, methyl octanoate, methyl laurate, methyl myristate and methyl palmitate.

Steam distillation of the ether fraction from the alkaline saponification of the methanol extract of the plant gave an essential oil which on distillation and chromatography on alumina gave d-limonene, l-carvone, l-borneol and caryophylene.

The non-volatile residue from the steam distillation was separated into a ketone fraction and a non-ketone fraction. Chromatography of the ketone fraction gave dehydrotremetone, toxol and the triterpene friedelin, while chromatography of the non-ketone fraction gave 5α-androstan-3β, 16α, 17α-triol and 24-ethyl-5α-cholesta-8(14), 22-dien-3β-yl.

On the Presence of Pyridine and Alkaloids In Rayless Goldenrod

L. H. Zalkow and M. Ghosal

The rayless goldenrod alcoholic extract has been reported to contain pyridine and a "good amount" of alkaloids.[6] We were unable to detect pyridine or any other basic compound, except ammonia, using the same conditions used by the earlier workers and a number of other conditions designed to free basic compounds. The earlier workers may have mistook ammonia for pyridine and friedelin and dehydrotremetone for alkaloids. The latter two compounds crystallized readily during our search for basic compounds.

By a modification of the previous isolation procedure, trametone

has now been isolated from the saponified methanol extract of the plant. The modified procedure involves the following steps: the saponified residue was extracted with ether and the concentrated ether extract was extracted with 85% ethanol. Evaporation of the clear alcoholic solution, after filtration, gave a red oil which was chromatographed on silica gel. The benzene eluent gave first a dehydrotremetone rich fraction and then a tremetone rich fraction. These fractions were rechromatographed over alumina to give the pure compounds.

(1) Abstracted from the Masters thesis of C. T. Ramming, Oklahoma State University, August 1965.

(2) L. H. Zalkow, N. Burke, G. Cabat and E. A. Grula, J. Med. Chem., 5, 1342 (1962).

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(5) Abstracted from the Ph.D. dissertation of N. I. Burke, Oklahoma State University, August, 1965.

(6) T. H. Buchrer, C. M. Mason and J. A. Crowder, Am. J. Pharm., 111, 105 (1939).

B. Detailed Report

(2) List of Publications

"Toxic Constituents of Rayless Goldenrod," L. H. Zalkow, N. Burke, G. Cabat and E. A. Grula, J. Med. Chem., 5, 1342 (1962).

"Constitution of Toxol: A Toxic Constituent of Aplopappus heterophyllus," L. H. Zalkow and N. Burke, Chem. and Ind., 1963, 292

"The Occurrence of 5α-Androstane-3β, 16α, 17α-triol in Rayless Goldenrod," L. H. Zalkow, N. I. Burke and G. Keen, Tetrahedron Letters, 1964, 217.

"The Absolute Configurations of Tremetone and Toxol," W. A. Bonner, N. I. Burke, W. E. Fleck, R. K. Hill, J. A. Joule, B. Sjoberg and L. H. Zalkow, Tetrahedron, 20, 1419 (1964).

"Chemical Investigations of Aplpappus heterophyllus V. Naturally Occurring Benzofuran Derivatives," L. H. Zalkow, M. Ghosal, C. Ramming and N. Burke, in preparation.

(3) Staffing

G. Cabat, teaching assistant and research assistant, September 1961 - December 1963, full time summers, 20% time (average) academic year.

N. I. Burke, research assistant, September 1961 - September 1964, 50% time.

G. Keen, teaching assistant and research assistant, June 1963 - June 1965, 20% time (average).

C. Ramming, teaching assistant and research assistant, May 1964 - August 1965, 25% time (average).

Dr. M. Ghosal, research associate, January 1964 - to present, full time.

Dr. L. H. Zalkow, principal investigator, September 1961 - to present, 20% time.