Toxic Constituents of Rayless Goldenrod[1]

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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,[4,5] 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*, **35**, 547 (1927).

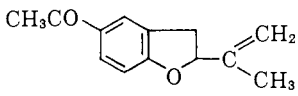
(5) J. F. Couch, *J. Am. Chem. Soc.*, **51**, 3617 (1929).

(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]

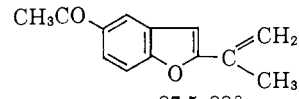
Recently, Bonner and co-workers[10] reported the results of their reinvestigation of white snakeroot. By the use of modern methods of chromatography, these workers found that "white snakeroot tremetol" could be separated into a number of components. Three closely related ketones, tremetone, I, dehydrotremetone, 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.



Oil

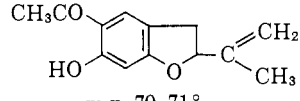
$[\alpha]_D^{23}$ −59.6°

I



m.p. 87.5–88°

II



m.p. 70–71°

$[\alpha]_D^{24}$ −50.7°

III

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. Cleverdon, Master's Thesis, Oklahoma State University, "The Chemical Constituents of Rayless Goldenrod," 1939.

(10) W. A. Bonner, J. I. DeGraw, D. M. Bowen, and V. R. Shah, *Tetrahedron Letters*, **12**, 417 (1961).

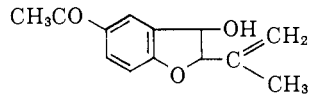
was selected as the test organism, and all toxicities reported in this paper refer to inhibition of growth of this organism using the solid agar assay method.

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. I. 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. $[\alpha]_D^{25}$ − 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 $C_{16}H_{16}O_4$: 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 $C_{13}H_{12}O_2 \cdot 1/6H_2O$: 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.