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THE ABSOLUTE CONFIGURATIONS OF TREMETONE AND TOXOL[1]

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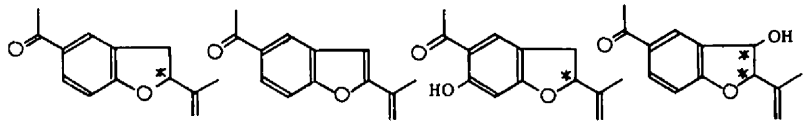
"TREMBLES" in cattle and "milksickness" in higher animals and humans are diseases which have been traced to the consumption by livestock of the white snakeroot plant (*Eupatorium urticaefolium*)[3] of the middle states and the rayless goldenrod plant (*Aploppapus heterophyllus*)[4] of the southwestern 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



I II III IV

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[3] J. F. Couch, *J. Agr. Res.* **35,** 547 (1927); *J. Amer. Med. Assoc.* **91,** 234 (1928); *U.S. Dept. Agr. Circular* No. 306, Nov. (1933).

[4] C. D. Marsh, C. G. Roe and A. B. Clawson, *U.S. Dept. Agr. Bull.* 1391 (1926).

[5] J. F. Couch, *J. Amer. Chem. Soc.* **51,** 3617 (1929); *J. Agr. Res.* **40,** 649 (1930).

[6] C. A. Lathrop, *Isolation and Fractionation of Tremetol from Rayless Goldenrod* M. S. Thesis, Oklahoma State Univ. (1939).

[7] R. Cleverdon, *The Chemical Constituents of Rayless Goldenrod* M. S. Thesis, Oklahoma State Univ. (1939).

[8] W. A. Bonner, J. I. DeGraw, Jr., D. M. Bowen and V. R. Shah, *Tetrahedron Letters* No 12, 417 (1961).

[9] W. A. Bonner and J. I. DeGraw, Jr., *Tetrahedron* **18,** 1295 (1962).

[10] J. I. DeGraw, Jr. and W. A. Bonner, *J. Org. Chem.* **27,** 3917 (1962).

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.

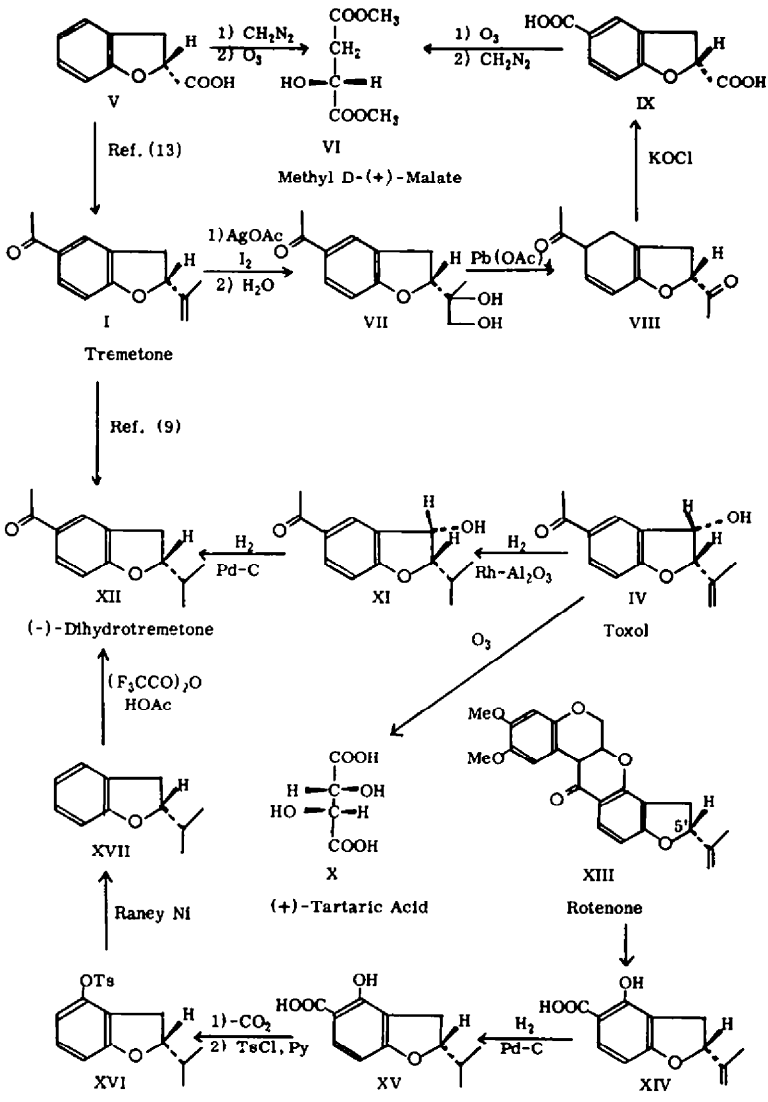
Natural (−)-tremetone (I) has been synthesized from (+)-dihydrocoumarilic acid (V) (Chart I) by a series of reactions which did not affect the single asymmetric center in the latter.[13] Accordingly, establishment of the absolute configuration of V was deemed the simplest approach to determining the 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

[11] J. I. DeGraw, Jr. and W. A. Bonner, *Tetrahedron* **18**, 1311 (1962).
[12] J. I. DeGraw, Jr. and W. A. Bonner, *Tetrahedron* **19**, 19 (1963).
[13] D. M. Bowen, J. I. DeGraw, Jr., V. R. Shah and W. A. Bonner, *J. Med. Chem.* **6**, 315 (1963).
[14] S. O. Butler, *Fractions of Tremetol and their Toxicities* M.S. Thesis, Oklahoma State University (1945).
[15] L. H. Zalkow, N. Burke, G. Cabat and E. A. Grula, *J. Med. Pharm. Chem.* **5**, 1342 (1962).
[16] L. H. Zalkow and N. Burke, *Chem. & Ind.* 292 (1963).
[17] C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta* **25**, 1620 (1942).
[18] P. Walden, *Ber. Dtsch. Chem. Ges.* **39**, 671 (1906).
[19] W. H. Hatcher and W. H. Mueller, *Canad. J. Res.* **3**, 291 (1930).

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 $[\alpha]_D^{30}$ −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 ½ 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), $[\alpha]_D^{26}$ +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 (2·1 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[22] 170°), and $[\alpha]_D^{27}$ +8·40°, (*c*, 0·032; water), reported:[22] $[\alpha]_D^{20}$ +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:[23] $[\alpha]_D^{15}$ +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. $C_{18}H_{20}O_4S$ 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.

[22] *The Merck Index* (7th Edition) p. 1012. Merck and Co., Rahway, N.J. (1960).
[23] C. Weygand, A. Weissberger and H. Baumgartel, *Ber. Dtsch. Chem. Ges.* **65**, 698 (1932).