The Structures of Dehydrotremetone and Hydroxytremetone

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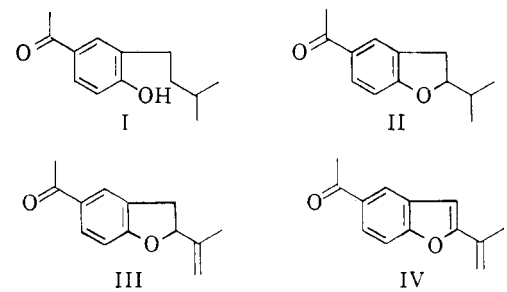
The two minor components, dehydrotremetone and hydroxytremetone, of the ketone fraction from "tremetol," the crude toxin of *Eupatorium urticaefolium*, have been investigated from a structural viewpoint. Ozonization of dehydrotremetone ($C_{13}H_{12}O_2$) yielded formaldehyde and catalytic hydrogenation resulted in the uptake of two moles of hydrogen and the formation of a sample of dihydrotremetone structurally identical with the product isolated by hydrogenation of tremetone [$C_{13}H_{14}O_2$; 2-isopropenyl-2,3-dihydro-5-acetylbenzofuran (III)]. These facts require that dehydrotremetone have the constitution 2-isopropenyl-5-acetylbenzofuran (IV). Hydroxytremetone ($C_{13}H_{14}O_3$) yielded an *O*-acetate which gave iodoform with hypoiodite. Its catalytic reduction proceeded with hydrogenolysis, affording a sample of 4-isoamyl-6-acetylresorcinol (VII) which was identical in all respects with an authentic synthetic sample. These observations establish the structure of hydroxytremetone as the 6-hydroxy analog of tremetone (III), namely, 2-isopropenyl-2,3-dihydro-5-acetyl-6-hydroxybenzofuran (VI).

Recently we have undertaken an investigation[2,3] of "tremetol," the crude toxin of white snakeroot (*Eupatorium urticaefolium*) responsible for trembles in cattle and milk sickness in humans,[4,5] extending the earlier chemical investigation of this toxin reported by Couch.[6] Crude tremetol was separated by partition chromatography into a sterol fraction and a ketone fraction. The sterol fraction was further separated by column chromatography into three pure components: a sesquiterpene hydrocarbon ($C_{15}H_{24}$), and two sterols ($C_{27}H_{46}O$ and $C_{30}H_{50}O$). These components have not yet been extensively investigated. The ketone fraction was separated into four components, three of which were characterized: tremetone (48%; $C_{13}H_{14}O_2$), dehydrotremetone (17%; $C_{13}H_{12}O_2$), and hydroxytremetone (2.6%; $C_{13}H_{14}O_3$). Since these ketones proved toxic to goldfish and showed the red color test with sulfuric acid which Couch found characteristic of trembles-producing fractions,[4,5] they were suspected of being the active toxins of tremetol, and tremetone, the most abundant ketone, has been investigated by degradative techniques.[2,3] Hydrogenation yielded dihydrotremetone ($C_{13}H_{16}O_2$) and a phenolic ketone hydrogenolysis product ($C_{13}H_{18}O_2$) which proved to be 2-isoamyl-4-acetylphenol (I).[2,3] The latter conclusion required that dihydrotremetone have the constitution 2-isopropyl-2,3-dihydro-5-acetylbenzofuran (II) and that tremetone itself must be 2-isopropenyl-2,3-dihydro-5-acetylbenzofuran (III). These carbon-skeleton assignments have recently been confirmed by the synthesis of racemic dihydrotremetone from salicylaldehyde.[7] We now wish to report experiments bearing on the structures of dehydrotremetone and hydroxytremetone, the two minor ketone constituents isolated from white snakeroot.

The molecular formulas of dehydrotremetone ($C_{13}H_{12}O_2$) and its oxime,[3] as well as their two C—CH_3 groups and optical inactivity, immediately suggested this ketone to be the completely unsaturated analog of tremetone (III), namely, 2-isopropenyl-5-acetylbenzofuran (IV). As with tremetone, the ozonization of dehydrotremetone afforded formaldehyde, and the infrared spectra of the two ketones were generally quite similar. For confirmation of the structure of dehydrotremetone as IV, this ketone was hydrogenated over 10% palladium–carbon. It absorbed exactly two moles of hydrogen, the first in five minutes and the second after six hours. The ultimate hydrogenation product was a liquid whose infrared spectrum was identical in all respects with that of dihydrotremetone (II), and the oximes of the two samples were also identical both by infrared and mixed melting point criteria. The two-step hydrogenation of the 2-isopropenylbenzofuran system has been observed previously during the hydrogenation of euparin (V) by Robertson and Kamthong,[8] and we have recently demonstrated unequivocally[7] that the initial hydrogenation product of 2-isopropenylbenzofuran is 2-isopropylbenzofuran. These observations place the structure of dehydrotremetone as 2-isopropenyl-5-acetylbenzofuran (IV) on a firm basis.



I II III IV

Hydroxytremetone[2] contained two C—CH_3 groups. Its formula ($C_{13}H_{14}O_3$) was suggestive of an oxygenated derivative of tremetone, possibly a hydroxylated derivative. The infrared spectrum,

(1) The authors are indebted to the National Institutes of Health for a research grant (RG-6232) which supported this investigation.

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

(3) W. A. Bonner and J. I. DeGraw, Jr., *Tetrahedron*, in press.

(4) J. F. Couch, *J. Agr. Res.*, **35**, 547 (1927).

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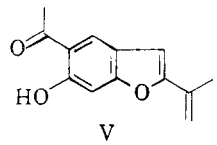
(8) A. Robertson and B. Kamthong, *J. Chem. Soc.*, 925 (1939).

however, failed to show hydroxyl adsorption, though the shift of its carbonyl band to 6.15 μ suggested the possibility of chelation between an *ortho*-hydroxy group and an acetyl or hetero-oxygen function. A ferric chloride test with hydroxytremetone gave a purple-black color, indicating a phenolic hydroxyl function. The presence of the latter was demonstrated by the acetylation of hydroxytremetone[2] to produce a mono-*O*-acetate, $C_{15}H_{16}O_4$, whose infrared spectrum showed a strong phenolic ester band at 5.70 μ and a return of the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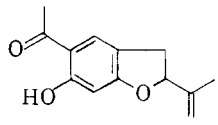
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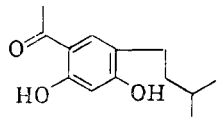
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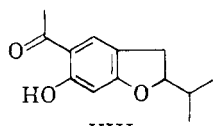
VIII

carbonyl band to 5.98 μ. Hydroxytremetone acetate readily gave a positive iodoform test in methanol.

Since very little hydroxytremetone was available it was felt that catalytic hydrogenation would afford the most revealing degradative information, since the anticipated hydrogenolysis product from such a reaction could be compared with alternative synthetic samples. When hydroxytremetone was hydrogenated using palladium–carbon the reduction product, $C_{13}H_{18}O_3$, possessed a strong hydroxyl band in the infrared indicating, along with the molecular formula, that hydrogenolysis had, indeed, occurred. By analogy with the structure of euparin (V), a skeletally similar ketone isolated by Robertson and Kamthong[8] from a plant of the same family, *Eupatorium purpureum*, it was suspected that hydroxytremetone might be the 6-hydroxy derivative (VI) of tremetone. To test this hypothesis a synthesis of the unique[3] hydrogenolysis product of VI, namely, 4-isoamyl-6-acetylresorcinol (VII), was undertaken.

To synthesize VII, resorcinol was treated with isovaleryl chloride at 95°, affording crude 4-isovalerylresorcinol. The latter was reduced by the Clemmensen method to 4-isoamylresorcinol, which was converted directly to its di-*O*-acetate by means of acetyl chloride. It has been previously found that di-*O*-acetates of this type yield 4,6-diacetyl compounds when rearranged by the Fries method using aluminum chloride.[9] To prepare 6-acetyl compounds, Rosenmund and co-workers have rearranged an equimolar mixture of the di-*O*-acetate and the unacetylated 4-alkylresorcinol, using aluminum chloride in nitrobenzene at room temperature.[10]

(9) E. Klarmann, *J. Am. Chem. Soc.*, **48**, 2358 (1926).

(10) K. Rosenmund, R. Buchwald, and T. Deligiannis, *Arch. Pharm.*, **271**, 342 (1933).

We have employed the latter procedure, using an equimolar mixture of the above 4-isoamylresorcinol and its di-*O*-acetate to prepare the desired 4-isoamyl-6-acetylresorcinol (VII). The synthetic VII was identical by both infrared and mixed melting point criteria with the above hydrogenolysis product of hydroxytremetone, thus establishing unequivocally the structure of the latter as 2-isopropenyl - 2,3 - dihydro - 5 - acetyl - 6 - hydroxybenzofuran (dihydroeuparin) (VI).

An attempt was made to isolate the expected simple hydrogenation product accompanying VII, namely, dihydrohydroxytremetone (VIII), the optically active form of tetrahydrouperin,[8] m.p. 71°. The above hydrogenation was conducted with only 115 milligrams of hydroxytremetone. Fluorisil chromatography of the mother liquors from the isolation of the above hydrogenolysis product VII yielded eight milligrams of white solid, m.p. 55–69°. Attempts to purify and characterize this material were abandoned for lack of material.

Experimental

Hydrogenation of Dehydrotremetone.—Dehydrotremetone (200 mg.) in ethyl acetate (8 ml.) containing prereduced 10% palladized charcoal (20 mg.) was hydrogenated in a volumetric apparatus at room temperature and atmospheric pressure. After 5 min. 1 mole of hydrogen was absorbed, and after 6 hr. the second mole had been absorbed. The catalyst was filtered and the filtrate was evaporated *in vacuo* to give 170 mg. of oil whose infrared spectrum was identical with that of dihydrotremetone.[3] The oxime of this product was prepared in the same manner as that previously described[3] for dihydrotremetone oxime. It had m.p. 89.5–91°, and a mixed m.p. with the oxime of dihydrotremetone (m.p. 91.5–93.5°) was undepressed, 89.5–92.5°.

Ozonization of Dehydrotremetone.—A solution of dehydrotremetone (126 mg.) in methylene chloride (10 ml.) was ozonized at 0° for 3 hr., then treated with a solution of ferrous sulfate (2 g.) in water (10 ml.) and stirred vigorously for 20 min. The methylene chloride layer was washed with water, and the combined water layers were distilled into an ice-cooled receiver, collecting 25 ml. of distillate. A solution of dimedon (300 mg.) in ethanol (5 ml.) was added to the distillate, and the mixture was allowed to stand overnight, yielding 19 mg. of white solid, m.p. 191–193°, mixed m.p. with authentic formaldehyde dimedon undepressed.

Hydrogenation of Hydroxytremetone.—A solution of hydroxytremetone (115 mg.) in absolute ethanol (5 ml.) containing 10% palladized charcoal (10 mg.) was hydrogenated as above. Hydrogen absorption was complete after 2 hr., 1.75 moles of hydrogen being consumed. The mixture was filtered and the filtrate was evaporated *in vacuo* to dryness yielding 115 mg. of syrupy residue which was dissolved in hexane–benzene (9:1; 10 ml.). The resulting crystalline material, 72 mg., m.p. 93–95°, was recrystallized in the same manner giving a sample having m.p. 92.5–94°. The infrared spectrum (Nujol mull) showed bands at 3.23 and 6.15 μ. This material was shown by the synthetic procedure below to be 4-isoamyl-6-acetylresorcinol.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.18; H, 8.12.

An attempt was made to isolate the simple hydrogenation product, 2-isopropyl-2,3-dihydro-5-acetyl-6-hydroxybenzofuran (VIII), from the mother liquors of the above hydrogenolysis product. A small amount (8 mg.) of material, m.p. 55–69°, was isolated, but the quantity was deemed too small for convenient purification and characterization.

Synthesis of 4-Isoamyl-6-acetylresorcinol.—Molten resorcinol (9.3 g.) was treated with 1 ml. of isovaleryl chloride, which was sufficient to keep the mixture liquid at 95°. Additional isovaleryl chloride (total 10.2 g.) was added over a period of 10 min. and the mixture was heated at 95° for another 20 min. The crude 4-isovalerylresorcinol was used directly in the next step.

A mixture of zinc amalgam (prepared from 28 g. of zinc[11]) and 6 *N* hydrochloric acid (45 ml.) was treated with a solution of the above 4-isovalerylresorcinol (7.0 g.) in absolute ethanol (35 ml.), and the mixture was heated under reflux for 6 hr., at which time a negative ferric chloride test was noted. The mixture was diluted with an equal volume of water and extracted with three 60-ml. portions of methylene chloride. The extracts were washed with water, dried over anhydrous magnesium sulfate, filtered, and evaporated at reduced pressure to yield 6.0 g. of oil. This was distilled, b.p. 128–131° (0.5 mm.), to give 1.7 g. of distillate which solidified. This was recrystallized from ligroin–benzene (9:1), 1.5 g., m.p. 64–67°. A second recrystallization gave 0.9 g., m.p. 70–71.5°, and a final recrystallization gave m.p. 72.5–73.5°. Cox and co-workers[12] reported m.p. 61–62.5° for this compound, prepared in the same manner. The infrared spectrum of our material left no doubt as to its identity as 4-isoamylresorcinol. It is possible that polymorphism may explain the indicated melting point discrepancy.

A mixture of the above 4-isoamylresorcinol (0.50 g.) and acetyl chloride (0.50 g.) was warmed for 2 hr. on the steam bath, then evaporated to dryness at reduced pressure. The residue was dissolved in ether (10 ml.) and the solution was washed with saturated aqueous sodium bicarbonate (5 ml.). The bicarbonate layer was re-extracted with ether and the ether extracts were dried, filtered, and stripped of solvent *in vacuo* to yield 0.63 g. (85%) of oil, crude di-*O*-acetyl-4-isoamylresorcinol, whose infrared spectrum indicated that acetylation was complete.

A solution of the latter di-*O*-acetate (0.56 g.) and the above 4-isoamylresorcinol (0.38 g.) in nitrobenzene (7 ml.) was stirred vigorously and treated with anhydrous aluminum chloride (0.56 g.). The mixture was allowed to stand at room temperature overnight, then was warmed to 50–55° for 4 hr. and finally cooled and treated with ice and water. The mixture was acidified with concentrated hydrochloric acid (2 ml.) and stirred until solution was complete. The aqueous layer was separated and extracted with chloroform, and the combined organic phases were steam distilled until the distillate was clear. The cooled residue was extracted with ether and the extracts were dried, filtered, and stripped of solvent to yield a dark sirup. The latter was dissolved in benzene (5 ml.) and diluted with hexane (10 ml.); the dark tar depositing was removed by filtration and the filtrate was set aside to crystallize. The resulting product (0.60 g.) was collected and recrystallized four times from hexane–benzene, yielding 0.09 g. of 4-isoamyl-6-acetylresorcinol, m.p. 92.5–94.5°. The infrared spectrum of this material was identical with that of the above hydrogenolysis product of dehydrotremetone, and admixture of the two samples led to no melting point depression.

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Oxidations of Substituted Azo- and Hydrazobenzenes with Peracetic Acid

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A series of substituted azobenzenes was oxidized with peracetic acid. The two isomers α- and β-3-nitroazoxybenzene were obtained from *trans*-3-nitroazobenzene by this method. Oxidation of 4,4'-dichloro-2-nitroazobenzene on the other hand gave only the *alpha* isomer of the azoxybenzene. By refluxiug with 30% hydrogen peroxide and glacial acetic acid or treatment with the former in acetic anhydride, some chlorinated azoxybenzenes were prepared from the stable azobenzenes. In this work, a series of dihalogenated and tetrasubstituted hydrazobenzenes was also oxidized by means of peracetic acid. Very good yields of the dihalogenated azoxybenzenes were generally achieved, the hydrazobenzenes were quite stable in the oxidizing medium, and several tetra-substituted azoxybenzenes were also obtained in improved yield.

The oxidation of azobenzenes to the corresponding azoxybenzenes has been a subject of some interest, particularly since peracetic acid was first employed as an oxidizing agent for this purpose. Swern has summarized the literature concerning this topic up to 1949 in a review,[1] and the peracetic acid oxidation of a series of halogenated azobenzenes was recently reported.[2] One of the objects of the present work was to study the oxidation of a variety of substituted azobenzenes by peracetic acid as a method of preparing the corresponding azoxybenzenes.

The oxidation of hydrazobenzenes to azobenzenes has been achieved with a wide variety of oxidizing agents. For instance, hydrazobenzene itself has been oxidized to azobenzene by mercuric oxide,[3] potassium dichromate and acetic acid,[4] lead peroxide in acetone,[5] and iodine.[6] Grammaticakis[7] reported the oxidation of a series of dichlorohydrazobenzenes to the corresponding azobenzenes by means of heating in air or potassium ferricyanide. The literature however, does not contain, as far as we know, any reference to the preparation of azoxybenzenes from the hydrazobenzenes by direct oxidation. Another object of this work was to prepare a series of azoxybenzenes from the hydrazobenzenes by means of oxidation with peracetic acid and to study the stability of the hydrazobenzenes in this oxidizing agent.

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