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TOXIC CONSTITUENTS OF "WHITE SNAKEROOT"

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"Tremetol", the crude toxin responsible for "trembles" in cattle[1] and "milk sickness" in human beings[2] has been isolated from the "White Snakeroot" plant (Eupatorium urticaefolium) in 0.14-0.32% yield by the alcohol extraction procedure of Couch.[1] Partition chromatography (ligroin-95% MeOH) separated "tremetol" into a Sterol fraction (39%) and a Ketone fraction (61%). Alumina chromatography of the Sterol fraction yielded Terpene-I (15%, oil, $[\alpha]^{24}D$ +44.7° ($CHCl_3$), $C_{15}H_{24}$, Found: C, 88.35; H, 11.60; M.W. 205; Microhydrog., 1.84, 1.93 moles for M.W. 204), Sterol-I (32%, m.p. 184.5-185.5°, $[\alpha]^{24}D$ +57.2° ($CHCl_3$), $C_{30}H_{50}O$, Found: C, 83.8; H, 11.70; O, 4.43; M.W., 427), and Sterol-II (9%, m.p. 147-148°, $[\alpha]^{24}D$ -32.8° ($CHCl_3$), $C_{21}H_{34}O$, Found: C, 82.95; H, 11.52; O, 5.39; M.W. 314). In view of their non-toxicity to goldfish[3] these constituents have not yet been investigated structurally. The Ketone fraction was separated by alumina chromatography into "dehydrotremetone" (17%, m.p. 87.5-88.5°, opt. inactive, $C_{13}H_{12}O_2$, Found: C, 77.64; H, 6.30; O, 16.11; M.W., 207, C-Me, 13.40; oxime, m.p. 131-132°, $C_{13}H_{13}O_2N$, Found: C, 72.16; H, 6.13; N, 6.43), "tremetone" (48%, oil, $[\alpha]^{28}D$ -59.6° (EtOH), d^{28}_4 1.080, $n^{25}D$ 1.5658, $C_{13}H_{14}O_2$; semicarbazone, m.p. 222°,

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[1] J. F. Couch, J. Agric. Research 35, 547-576 (1927).

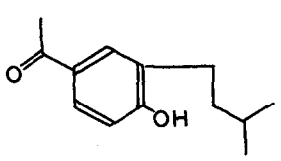
[2] J. F. Couch, J. Am. Med. Assoc. 91, 234 (1928).

[3] W. A. Gersdorff, J. Am. Chem. Soc. 52, 3440 (1930).

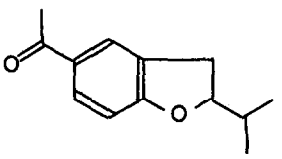
$[\alpha]^{28}D$ -56.2° ($CHCl_3$), $C_{14}H_{17}O_2N_3$, Found: C, 65.01; H, 6.61; N, 16.49; 2,4-dinitrophenylhydrazone, m.p. 183.8-184.2°, $C_{19}H_{18}O_5N_4$, Found: C, 59.50; H, 4.61; N, 14.57), and "hydroxytremetone" (3%, m.p. 70-71°, $[\alpha]^{24}D$ -50.7° (EtOH), $C_{13}H_{14}O_3$, Found: C, 71.29; H, 6.37; O, 21.77; M.W., 212; C-Me, 13.74; acetate, m.p. 89-89.5°, $C_{15}H_{16}O_4$, Found: C, 68.49; H, 6.20; O, 24.76). Since each of the ketones proved toxic to goldfish, tremetone, the most abundant constituent, was suspected of being the active toxin in "White Snakeroot" and was investigated structurally.

Reaction of tremetone with ozone yielded formaldehyde and with sodium hypoiodite iodoform, indicating the presence of methylene and acetyl functions. Catalytic hydrogenation of tremetone (Pd/C) yielded hydrotremetone (oil, $[\alpha]^{25}D$ -47.0° (EtOH), $C_{13}H_{16}O_2$; oxime, m.p. 91.5-93.5°, $C_{13}H_{17}O_2N$, Found: C, 71.20; H, 7.64; O, 14.39; N, 6.55; 2,4-dinitrophenylhydrazone, m.p. 181-184°; $C_{19}H_{20}O_5N_4$, Found: C, 59.25; H, 5.11; N, 14.64) and a phenolic ketone hydrogenolysis product whose oxime (m.p. 104.5-105°, $C_{13}H_{19}O_2N$, Found: C, 70.32; H, 8.70; O, 14.55; N, 6.32, C-Me, 11.8) and 2,4-dinitrophenylhydrazone (m.p. 202.5-203.5°, $C_{19}H_{22}O_5N_4$, Found: C, 58.80; H, 4.70; N, 14.66) indicated it to be $C_{13}H_{18}O_2$.

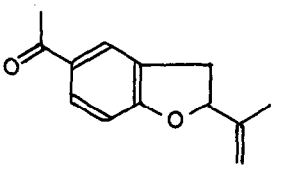
O-Methylation of the above phenolic ketone, followed by permanganate oxidation and esterification afforded methyl 4-methoxyisophthalate, m.p. 97.5-98.5° alone and on admixture with an authentic sample. Beckmann rearrangement of the oxime of the phenolic ketone, followed by hydrolysis of the resulting amide yielded acetic acid and 2-isoamyl-4-aminophenol (m.p. 118-119.5°, $C_{11}H_{17}ON$, Found: C, 72.52; H, 9.34; N, 7.71); diacetate, m.p. 90.5-91.5°, $C_{15}H_{21}O_3N$, Found: C, 68.21; H, 7.86; O, 18.39; N, 5.28), indicating the phenolic ketone hydrogenolysis product to be 2-isoamyl-4-acetylphenol (I). This conclusion plus the above evidence requires hydrotremetone to possess structure II and tremetone III.



I



II

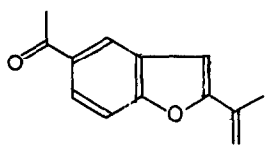


III

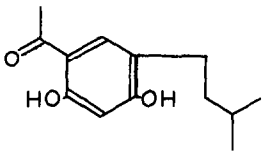
The structure I was confirmed by independent synthesis employing the sequence: phenol ⟶ phenyl isovalerate —(Fries)⟶ 2-isovalerylphenol (2,4-dinitrophenylhydrazone, m.p. 187-189°, $C_{17}H_{18}O_5N_4$, Found: C, 57.14; H, 4.91; N, 15.31) —(Clemmenson)⟶ 2-isoamylphenol (3,5-dinitrobenzoate, m.p. 86.5-87.5°, $C_{18}H_{18}O_6N_2$, Found: C, 60.03; N, 5.01; N, 7.80)⟶ 2-isoamylphenyl acetate —(Fries)⟶ I.

The structure II was confirmed by the synthetic sequence: salicaldehyde —($ClCH_2COCH_3$)⟶ 2-acetylbenzofuran —(1. CH_3MgI, 2. $-H_2O$)⟶ 2-isopropenylbenzofuran —(H_2-Pd/C)⟶ 2-isopropyl-2,3-dihydrobenzofuran —($(F_3CCO)_2O$-HOAc)⟶ racemic II (infrared spectra of oxime and 2,4-dinitrophenylhydrazone identical with those of hydrotremetone derivatives).

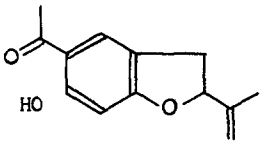
Structure III for tremetone was confirmed by several independent syntheses, the most convenient of which utilized the sequence: benzofuran-2-carboxylic acid —(Na-Hg)⟶ 2,3-dihydrobenzofuran-2-carboxylic acid ⟶ ethyl ester —(CH_3MgBr)⟶ 2-(2,3-dihydro-2-benzofuryl)-2-propanol —(Ac_2O-$SnCl_4$)⟶ 2-(5-acetyl-2,3-dihydro-2-benzofuryl)-2-propyl acetate —(pyrolysis)⟶ racemic III (separated chromatographically from other double bond isomers). After preliminary resolution of the intermediate 2,3-dihydrobenzofuran-2-carboxylic acid with (+)-amphetamine, the above sequence afforded a sample of tremetone which was identical in all respects with the natural product and which yielded crystalline derivatives similarly identical.



IV



V



VI

The structure of dehydrotremetone as IV was deduced from the observations that this ketone yielded formaldehyde on ozonization and produced on catalytic hydrogenation a sample of racemic hydrotremetone (II) whose infrared spectrum (and that of its oxime) was identical with that of the hydrotremetone (and oxime) obtained by the above reduction of tremetone.

Hydroxytremetone 1) yielded on O-acetate (m.p. 89-89.5°, Cf. above) which gave iodoform with hypoiodite and 2) produced a phenolic ketone (m.p. 92.5-94°, $C_{13}H_{18}O_3$, Found: C, 70.18; H, 8.12) on hydrogenolysis (Pd/C). The identity of this phenolic ketone as 2-isoamyl-4-acetylresorcinol (V) was established by comparison with an authentic sample produced by the sequence: resorcinol ⟶ 2-isovalerylresorcinol ⟶ 2-isoamylresorcinol ⟶ 2-isoamylresorcinol diacetate —(Fries)⟶ V. These data require that hydroxytremetone possess structure VI. This conclusion has not yet been confirmed synthetically.

While quite lethal to goldfish, as might be anticipated from the similarities of their structures to that of the 2-isopropenyl-2,3-dihydrobenzofuran moiety of the rotenone molecule, the above ketones have not yet been established as toxic for higher animals.

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