ness and dilute hydrochloric acid added. The crude solid product was collected by filtration, washed with water and recrystallized from methanol–chloroform (charcoal) giving white crystals (2.0 g.), m.p. 248–250°. Further recrystallization from 50% ethanol gave the compound, m.p. 255°; $\lambda_{max}$ 229 mμ (ε 23,500); 236 mμ (ε 23,000); 257 mμ (ε 5,400); 263 mμ (ε 4,600); 318 mμ (ε 7,200).

*Anal.* Calcd. for $C_6H_7Cl_2N_3O_4S_2$: Cl, 22.16; N, 13.13. Found: Cl, 21.96; N, 12.99.

Catalytic reduction of the compound in alkaline solution under conditions similar to those employed for the reduction of 2-amino-5-chloro-*m*-benzenedisulfonamide yielded 2-amino-*m*-benzenedisulfonamide as shown by comparison with the sample previously obtained.

**4,5-Dichloro-6-nitro-*m*-benzenedisulfonamide (X).**—4-Amino-5,6-dichloro-*m*-benzenedisulfonamide (1.0 g.) was dissolved in sulfuric acid (5 ml.) and the solution added to a mixture of 20% fuming sulfuric acid (20 ml.) and 30% hydrogen peroxide solution (10 ml.), cooled in an ice-bath. After 2 hr. at ice-bath temperature and 4 hr. at room temperature, the reaction mixture was diluted with water (200 ml.) and then allowed to stand overnight. The crude product was collected by filtration, washed with water and air dried; yield, 0.58 g. m.p. 237–242°. Recrystallization was effected from dilute ethanol yielding the pure product, m.p. 248–249°; $\lambda_{max}$ 214 mμ (ε 41,400); plateau 286–290 mμ (ε 1,900); 295 mμ (ε 2,200); $\lambda_{max}$ 6.50 μ (s).

*Anal.* Calcd. for $C_6H_5Cl_2N_3O_4S_2$: Cl, 20.25; N, 12.00. Found: Cl, 20.56; N, 11.96.

**4,5-Dichloro-6-methoxy-*m*-benzenedisulfonamide (XI).**—A mixture of 4,5-dichloro-6-nitro-*m*-benzenedisulfonamide (1.0 g.), sodium methoxide (2.0 g.) and methanol (20 ml.) was refluxed for 16 hr. The cooled reaction mixture was diluted with water, acidified with dilute hydrochloric acid, and the crude product collected by filtration, washed with water and air dried. Recrystallization was effected from water yielding product of m.p. 240–242°; $\lambda_{max}$ 222 mμ (ε 17,500); sh. 245 mμ (ε 8,600); 269 mμ (ε 4,400); 299 mμ (ε 3,200).

*Anal.* Calcd. for $C_7H_8Cl_2N_2O_6S_2$: Cl, 21.15. Found: Cl, 21.08.

**Monosulfonamide of 2,3-Dichlorophenol.**—2,3-Dichloroanisole was chlorosulfonated and the resulting product treated with ammonia according to the procedure of Lustig and Katscher.[1] The product obtained had m.p. 252–253°; $\lambda_{max}$ 241 mμ (ε 8,600); 272 mμ (ε 3,200); sh. 279 mμ (ε 2,800); 291 mμ (ε 2,500).

*Anal.* Calcd. for $C_6H_5Cl_2NO_3S$: N, 5.79; Cl, 29.29. Found: N, 5.90; Cl, 29.08.

The same product was obtained when 2,3-dichlorophenol was used instead of 2,3-dichloroanisole.

**Acknowledgments.**—The authors are indebted to Mr. R. Wayne for the infrared spectral data, Mr. E. Connor for the microanalytical results, and Mr. W. Boraczek for the preparation of certain of the compounds described.

# The Synthesis and Pharmacological Action of Tremetone

DOUGLAS M. BOWEN,[1] JOSEPH I. DEGRAW, JR., VINOD R. SHAH, AND WILLIAM A. BONNER[2]

*Department of Chemistry, Stanford University, Stanford, California*

*Received November 2, 1962*

Tremetone (I), the principal levorotatory ketonic constituent of "tremetol," the crude toxin of *Eupatorium urticaefolium*, has been synthesized. The synthetic sequence involved: coumarilic acid—(NaHg)→ hydrocoumarilic acid —(EtOH, $H^+$)→ ethyl hydrocoumarilate —(MeMgBr)→ 2-(2,3-dihydro-2-benzofuryl)-2-propanol —($Ac_2O$, $SnCl_4$)→ 2-(2,3-dihydro-5-acetyl-2-benzofuryl)-2-propyl acetate —(pyrolysis)→ I. Optical resolution was accomplished at the hydrocoumarilic acid stage, and the synthetic enantiomer (−)-I proved identical in all respects with natural levorotatory tremetone. Preliminary toxicity data for tremetone and tremetol are discussed, as applied to goldfish, several species of insects, mice, rabbits, sheep and chickens. Crude tremetol proved toxic to chickens, whereas tremetone was harmless, suggesting that the ketone may not be the active toxin in tremetol responsible for "trembles" in cattle and "milk sickness" in humans.

White snakeroot (*Eupatorium urticaefolium*) is a toxic weed which grows extensively in damp areas of the central United States. Its ingestion by cattle causes the veterinary disease "trembles,"[3,4] while human consumption of dairy products from infected cattle engenders the fatal malady known as "milk sickness,"[5] an incurable illness which periodically ravished early pioneer communities of the central west. In 1929 J. F. Couch isolated from white snakeroot a straw-colored oil, "tremetol," which produced the symptoms of trembles in sheep and which was considered to be the homogeneous active toxin of this weed.[3,6] No subsequent confirmation of Couch's observations has been reported, and indeed this challenging problem has lain dormant until only recently, when we became interested in its pursuit.[7]

Application of column chromatography to the crude tremetol described by Couch afforded six individual components: a sesquiterpene hydrocarbon ($C_{15}H_{24}$), two steroids ($C_{27}H_{46}O$ and $C_{30}H_{48}O$) and three ketones, namely, tremetone ($C_{13}H_{14}O_2$), dehydrotremetone ($C_{13}H_{12}O_2$) and hydroxytremetone ($C_{13}H_{14}O_3$).[8] These ketones proved toxic to goldfish (*vide infra*) and produced a red color test with sulfuric acid, a test which Couch found characteristic of trembles-producing fractions.[3,6] Accordingly, one or more of these ketones was suspected of being the active toxin of white snakeroot, and their structural investigations were therefore undertaken.

Degradative reactions and catalytic hydrogenation revealed that tremetone, the principal ketone con-

(1) National Science Foundation Faculty Fellow, 1961–1962.

(2) The authors are indebted to the National Institutes of Health for a research grant (RG-6232) which supported this and its preceding investigations.

(3) J. F. Couch, *J. Agr. Res.*, **35**, 547 (1927); see this article for an extensive review of the earlier literature.

(4) L. R. Tehon, C. C. Morrill, and R. Graham, "Illinois Plants Poisonous to Livestock," Circular 599, University of Illinois, College of Agriculture, Extensions Service in Agriculture and Home Economics, 1946, p. 43 ff.

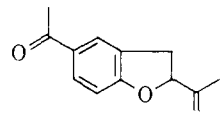
(5) J. F. Couch, *J. Am. Med. Assoc.*, **91**, 234 (1928).

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

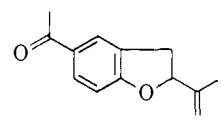
(7) W. A. Bonner, J. I. DeGraw, Jr., D. M. Bowen, and V. R. Shah, *Tetrahedron Letters*, 417 (1961); W. A. Bonner, Phytochemistry Symposium, Golden Jubilee Celebration, University of Hong Kong, Sept., 1961.

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

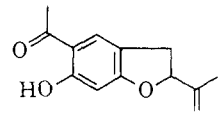
stituent of tremetol, was (−)5-acetyl-2,3-dihydro-2-isopropenyl-benzofuran (I),[8] while the two minor ketones, dehydrotremetone and hydroxytremetone, respectively, were 5-acetyl-2-isopropenylbenzofuran (II) and (−)5-acetyl-2,3-dihydro-2-isopropenyl-6-hydroxy-benzofuran (III).[9] These degradative conclusions more recently have been confirmed by the synthesis of dihydrotremetone (IV), one of the hydrogenation products of tremetone,[10] and of racemic tremetone itself.[11] The latter synthesis involved the following



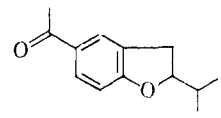
I



II



III



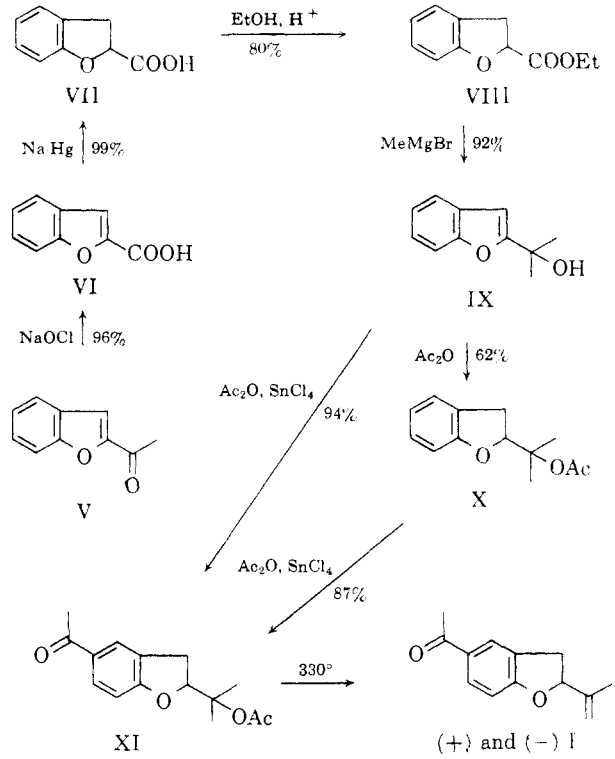
IV

reaction sequence: 2-acetyl-2,3-dihydrobenzofuran—(MeMgI) → (2,3-dihydro-2-benzofuryl)-2-propanol—($(F_3CCO)_2O$, HOAc) → 2-(2,3-dihydro-5-acetyl-2-benzofuryl)-2-propyl acetate—(hydrolysis, dehydration) → racemic I. Although it confirmed our degradative conclusions as to the structure of tremetone, this synthesis suffered the disadvantage that none of its intermediates provided a suitable structure to permit convenient optical resolution, and accordingly the natural levorotatory antipode of I could not be prepared. We now wish to report an alternative synthesis which afforded natural tremetone and a number of observations on the biological activity of this ketone. Our improved synthetic sequence is shown in Chart I.

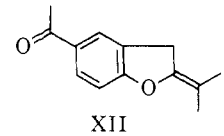
2-Acetylbenzofuran (V) was oxidized to coumarilic acid (VI) by an improved procedure involving hypochlorite, and VI was reduced quantitatively to hydrocoumarilic acid (VII) by means of sodium amalgam. This acid was resolved into its two enantiomers, (+) and (−) VII, by means of the enantiomers of amphetamine, modifying the reported procedure of Fredga.[12] Conversion of each antipode of VII to ethyl hydrocoumarilate (VIII) was accomplished by Fisher esterification, the ethyl esters being selected to avoid solubility difficulties in the succeeding Grignard reactions. Treatment of each ethyl ester with methylmagnesium bromide afforded the enantiomers of 2-(2,3-dihydro-2-benzofuryl)-2-propanol (IX). Conversion of these alcohols into the enantiomers of 2-(2,3-dihydro-5-acetyl-2-benzofuryl)-2-propyl acetate (XI) was accomplished smoothly by action of acetic anhydride and stannic chloride, although our initial preparation of XI proceeded through the acetate ester X. Pyrolysis of racemic XI at 330° eliminated acetic acid, liberation of which commenced at 280°. The crude pyrolysis product was subjected to column and vapor–liquid partition chromatography, affording three ketonic products in a ratio of 1:2:10. The most abundant constituent had

Chart I

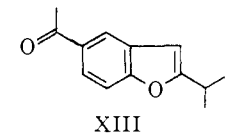
Synthesis of Enantiomers of Tremetone




an infrared spectrum identical with that of natural tremetone. The minor constituents have not yet been fully characterized, but appear on preliminary examination to be the isomeric ketones XII and XIII. Pyrolysis of the levorotatory enantiomer of the acetate XI in a similar fashion afforded a sample of (−)-tremetone which, with its semicarbazone and 2,4-dinitrophenylhydrazone, proved identical in all measured respects with natural tremetone and its corresponding derivatives. Pyrolysis of the dextrorotatory acetate XI led to (+)-tremetone, the enantiomer of the natural product.



XII



XIII

While the benzofuran ring system is found in a wide variety of natural products, it usually is present as part of a more extensive fused ring system, and only rarely occurs by itself in a state of simple substitution.[13] In 1939 Robertson isolated[14] the skeletally similar ketone, "euparin" [2-isopropenyl-5-acetyl-6-hydroxybenzofuran (XIV)] from a plant of the same family, *Eupatorium purpureum* (gravel root). Since that time euparin also has been observed to be a constituent of the European *Eupatorium cannabinum*[15] as well as the Japanese *Eupatorium japonicum*.[16] Chinese *eupator-*

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

(10) J. I. DeGraw, Jr., and W. A. Bonner, *Tetrahedron*, **18**, 1311 (1962).

(11) J. I. DeGraw, Jr., D. M. Brown, and W. A. Bonner, *ibid.*, **19**, 19 (1963).

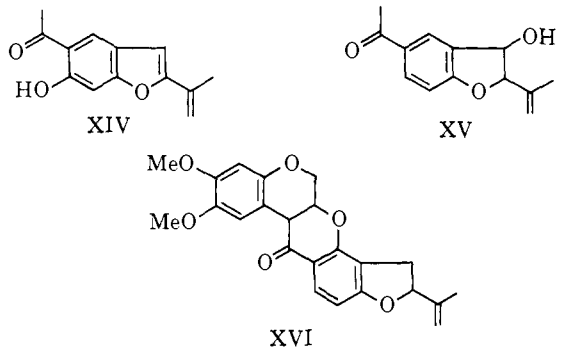
(12) A. Fredga and C. Vazquez de Castro y Sarmiento, *Arkiv. Kemi*, **7**, 387 (1954); *C. A.*, **49**, 14746a (1955).

(13) W. Karrer, "Konstitution und Vorkommen der organischen Pflanzenstoffe," Birkhaüser Verlag, Basel, 1958, pp. 551–561; 700–715.

(14) B. Kamthong and A. Robertson, *J. Chem. Soc.*, 921, 933 (1939).

(15) J. Sykulski, *Acta Polon. Pharm.*, **15**, 361 (1958); *C. A.*, **53**, 6536 (1959); J. Grzybowska and Z. Jerzmanowska, *Roczniki Chem.*, **28**, 213 (1954); *C. A.*, **48**, 12378 (1954); Z. Jerzmanowska, *Polska Akad. Umiez., Prace Kom. Farm., Dissertationes Pharm.*, **3**, 165 (1951); *C. A.*, **48**, 5848 (1954).

(16) T. Nakaoki, N. Morita, and S. Nishino, *Yakugaku Zasshi*, **78**, 557 (1958); *C. A.*, **52**, 13190 (1958).



XIV XV



XVI

*ium* is reported to contain a toxic principle, m.p. 67°, which, like that of white snakeroot, is capable of passage through milk into suckling cattle.[17] Although close in melting point to our hydroxytremetone (m.p. 70–71°),[8] this toxin is said to resemble coumarin and not to give reactions characteristic of crude tremetol. More recently Zalkow and co-workers[18] have established the presence of both dehydrotremetone (II) and "toxol" [(−)-2-isopropenyl-2,3-dihydro-3-hydroxy-5-acetylbenzofuran (XV)] in the crude tremetol of the rayless goldenrod (*Aplopappus heterophyllus*) of New Mexico. Originally discovered in 1870, euparin has no reported physiological action,[19] while toxol has been shown to be bacteriostatic toward *Bacillus cereus*, *Staphylococcus albus* and *Corynebacterium hoagii*.[18]

Comparison of the structure of the well known fish poison rotenone (XVI) with that of tremetone (I) indicates an almost exact similarity in the substituted benzofuran nuclei of these toxins. It was on the basis of this similarity that we originally employed goldfish as test organisms for our tremetol fractions.[7,8] Semiquantitative fish toxicity tests, employing goldfish in the procedure of Gersdorff,[20] suggested that on a molar basis tremetone (I), dehydrotremetone (II) and hydroxytremetone (III) had toxicities toward goldfish approximately half that of rotenone.

Derris preparations have also enjoyed widespread use as insecticides against a wide variety of pests, and the toxicities of rotenone and other derris components toward higher animals have been the subject of a number of investigations.[21] Accordingly, we have also investigated the insecticidal properties of tremetone, and wish to report the following preliminary observations. Against mosquito larvae, the common housefly and the German roach, tremetone had approximately 7, 55 and 2%, respectively, of the insecticidal potency of pyrethrins. Against the boll weevil, cotton aphid, two-spotted spider mite and southern army worm, neither tremetone nor crude tremetol showed significant toxicity.

Toxicity tests on both the rabbit and the mouse indicated that these were not suitable hosts for the evaluation of trembles-producing activity. With both oral and intraperitoneal doses of 50–300 mg./kg. in the mouse, only a slight lethargy was noted when either tremetone or crude tremetol was administered. In toxic intraperitoneal doses of tremetone (500 mg./kg. and above), jerks and clonic convulsions were observed prior to death. Neither oral nor intraperitoneal administrations (550 mg./kg. and 150 mg./kg., respectively) of crude tremetol to rabbits produced tremor symptoms, although the latter administration elicited muscular weakness and increased respiration.

In the hope of establishing in a single experiment whether tremetone was the responsible toxin of *Eupatorium urticaefolium* toward cattle, a single feeding of pure tremetone (130 mg./kg., administered with alfalfa feed) was given to 22.6 kg. sheep. No untoward symptoms were observed during 7 days of subsequent observation. In view of the results of Couch,[3] however, such a single negative result could not be considered definitive, and further assays were deemed necessary. Unfortunately, our limited supply of pure tremetone precluded further tests with animals as large as sheep.

In 1945, after preliminary experiments with a variety of hosts, Butler[22] achieved promising results using chickens in toxicity assays of tremetol and other fractions obtained after extraction of the rayless goldenrod (*Aplopappus heterophyllus*) of the Southwest. With the hope of obtaining critical data, therefore, we have repeated Butler's assays on our samples of tremetone and crude tremetol, with the following tentatively definitive results. Whether administered orally or by injection (in sesame oil) into the breast muscle, pure tremetone elicited no symptoms of distress in white leghorn cockerels during a 7-day period. Crude tremetol at the same dose level, on the other hand, killed our test chickens in 3–7 days by either mode of administration, and produced extreme lethargy and breathing difficulties within 24-36 hours prior to death.

If therefore appears tenatively valid to conclude that, while it is a potent fish poison and shows the sulfuric acid color test which Couch associated with trembles-producing activity,[3,6] tremetone (I) is probably not the toxin in tremetol which is responsible for trembles in cattle and milk sickness in humans. The responsible toxin, which must clearly be a more potent minor component of crude tremetol, is now being actively sought after.

## Experimental

**Coumarilic Acid (VI).**—The following improved method for the oxidation of 2-acetylbenzofuran to coumarilic acid was developed after preliminary experimentation with previously reported procedures.[23,24] "Sanichlor" household bleach (1500 ml.) was warmed to 60°, then treated over 20 min. with stirring and mild cooling with a solution of 2-acetylbenzofuran (64.0 g.) in methanol (400 ml.) at such a rate that gentle reflux was maintained. Toward the end of the addition the solution darkened, but could be restored to its original yellow color by intermittent addition of more "Sanichlor" (100–200 ml.). The small excess of hypochlorite remaining was destroyed by the addition of sufficient sodium bisulfite. The clear, yellow solution was cooled, washed with ether and acidified to precipitate 62.1 g. (96%) of crude coumarilic acid as nearly colorless granules. The sample was

(17) C. Pak and B. E. Read, *Chinese J. Physiol.*, **12**, 263 (1937); *C. A.*, **32**, 2221 (1938); H. L. Hi and C. Pak, *Chinese J. Physiol.*, **12**, 275 (1937); *C. A.*, **32**, 2222 (1938).

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

(19) J. U. Lloyd, as reported by H. Trimble, *Am. J. Pharm.*, **62**, 73 (1890); C. C. Manger, *ibid.*, **66**, 120 (1894).

(20) W. A. Gersdorff, *J. Am. Chem. Soc.*, **52**, 3440, 5051 (1930).

(21) *Cf.* A. M. Ambrose, F. DeEds, and J. B. McNaught, *Ind. Eng. Chem.*, **34**, 684 (1942), for bibliography of earlier studies.

(22) S. O. Butler, "Fractions of Tremetol and their Toxicities," M. S. Thesis, Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma, 1945.

(23) M. W. Farrar and R. Levine, *J. Am. Chem. Soc.*, **72**, 4436 (1950).

(24) R. C. Fuson, J. W. Kneisley, and E. W. Kaiser, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, New York, N. Y., p. 209.

recrystallized from 50% ethanol to yield sufficiently pure product, m.p. 192.5–194° (reported[24] m.p. 195–196°), in 94% over-all yield.

**Hydrocoumarilic Acid (VII).**—Coumarilic acid (15.3 g.) was suspended in water (150 ml.) and neutralized to the phenolphthalein end point with 6 *N* sodium hydroxide. A small amount of solid remained undissolved until reduction began. The solution was shaken vigorously in a stoppered vessel with 2% sodium amalgam[25] (330 g.), resulting in spontaneous warming. After 5 min. the mercury was separated, the aqueous solution was filtered (Filter-cel) and the filtrate was acidified, cooled and extracted with ether. Solvent removal afforded 15.4 g. (99%) of crude hydrocoumarilic acid, m.p. 115–117.5° (reported,[26,27] 116.5°), which was used directly in subsequent work. Less pure samples of coumarilic acid gave samples of hydrocoumarilic acid which were barely solid at room temperature and required crystallization from benzene before they were acceptably pure.

**Resolution of Hydrocoumarilic Acid.**—Our initial attempts to employ brucine as a resolving agent for this acid after the procedure of Fredga[12] were unsuccessful. In our experience racemic brucine hydrocoumarilate failed to crystallize, as did also the brucine salts of the resolved samples obtained below. We subsequently found that amphetamine served conveniently to resolve hydrocoumarilic acid and, since it is commercially available in both enantiomeric forms, had the additional advantage of permitting procurement of both enantiomers of this acid. Fredga has used amphetamine only on the mother liquors from his brucine resolution.[12]

In a typical experiment hydrocoumarilic acid (4.30 g.) was treated with the amine liberated from (+) amphetamine sulfate (5.0 g.) and the mixture was dissolved in water (60 ml.) at 80°. Cooling deposited 3.75 g. of salt, m.p. 150–181°. Recrystallization of the salt from water (50 ml.) afforded 1.73 g. of salt, m.p. 185–187°, the acid recovered from which had m.p. 92–96°, $[\alpha]^{25}D$ +20.0° (*c*, 6.1, ethanol). A second recrystallization gave 0.54 g. of salt having m.p. 186–187°, the acid (0.22 g.) from which had m.p. 100–104° and $[\alpha]^{25}D$ 22.6° (*c*, 7.3, ethanol), in agreement with previously reported values.[12] By conversion of the free acid obtained from the mother liquors of this resolution into its (−) amphetamine salt, the enantiomeric (−) hydrocoumarilic acid could similarly be obtained.[12] Subsequently it was found that recrystallization of such salts from 95% ethanol permitted somewhat more rapid resolution. In larger scale resolutions full purification of the amphetamine salts was not undertaken, since complete resolution was attended by heavy losses and it was further found that the succeeding crystalline acetate XI could be rendered optically pure with less over-all loss by simple recrystallization.

**Ethyl Hydrocoumarilate (VIII).**—The enantiomers of ethyl hydrocoumarilate were prepared by applying the esterification procedure of von Auwers[28] directly to the enantiomeric amphetamine salts of hydrocoumarilic acid. A mixture of the (−) amphetamine salt of (−)hydrocoumarilic acid (68.8 g.), absolute ethanol (150 ml.) and sulfuric acid (25 ml.) was heated under reflux for 1.5 hr., then was cooled, diluted with water and processed as usual. The crude ester was distilled, b.p. 130° (11 mm.), to give 30.4 g. (69%) of pure ethyl (+) hydrocoumarilate, $n^{28}D$ 1.5172, $[\alpha]^{25}D$ +17.9° (*c*, 3.98; hexane); +12.6° (neat).

*Anal.* Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 69.10; H, 6.18.

A sample of the (+)-amphetamine salt of (+)-hydrocoumarilic acid was converted to ethyl (−)hydrocoumarilate (77%) in the same manner, b.p. 130° (9 mm.), $n^{28}D$ 1.5190,] $\alpha]^{25}D$ −11.6° (neat).

*Anal.* Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.42; H, 6.57.

**2-(2,3-Dihydro-2-benzofuryl)-2-propanol (IX).**—Methylmagnesium bromide was prepared from magnesium (4.86 g.) in ether (130 ml.) and sufficient methyl bromide. Addition of a solution of racemic ethyl hydrocoumarilate (14.4 g.) in ether (50 ml.) over 10 min., and customary processing afforded 12.3 g. (92%) of distilled product, b.p. 107–107.5° (2 mm.), the infrared spectrum of which was identical with that of our previously reported sample.[10] The enantiomeric forms of this compound were prepared in an identical manner. The above ethyl (+) hydrocoumarilate yielded (+)2-(2,3-dihydro-2-benzofuryl)-2-propanol, b.p. 120–121° (4 mm.), $[\alpha]^{25}D$ +36.2° (*c*, 6.7; ethanol). Ethyl (−)hydrocoumarilate afforded a sample of (−)-2-(2,3-dihydrobenzofuryl)-2-propanol having b.p. 118° (2.5 mm.), $[\alpha]^{25}D$ −34.1° (*c*, 1.6, ethanol). Combustion analysis revealed that these samples were not completely pure, but they gave satisfactory results in the reactions described below.

**2-(2,3-Dihydro-2-benzofuryl)-2-propyl Acetate (X).**—A mixture of the racemic alcohol IX (8.90 g.) and acetic anhydride (19 ml.) was heated under reflux for 5 hr., then cooled and poured into water. The product was extracted with ether and the extract was washed with sodium hydroxide solution until neutral, then was dried and freed of solvent. The residue was distilled into two fractions (a) 2.75 g., b.p. 102–105° (1 mm.) and (b) 6.85 g. (62% c, b.p. 116–116.5° (1 mm.). The infrared spectrum of fraction (b) was consistent with that expected for the desired acetate X, while that of fraction a suggested the presence of some dehydration product. Fraction (b) was employed below without further purification.

**2-(2,3-Dihydro-5-acetyl-2-benzofuryl)-2-propyl Acetate (XI).**—Racemic acetate (X) (2.20 g.) in benzene (10 ml.) containing acetic anhydride (1.5 g.) was stirred in an ice bath while a solution of stannic chloride (7.8 g.) in benzene (5 ml.) was added dropwise over a 15-min. period. The purple solution was stirred for an additional 15 min., then was poured onto ice. The product was extracted into ether and the extract was washed until neutral. Solvent evaporation afforded 2.78 g. of off-white solid which was recrystallized from hexane. The pure racemic acetate XI, 2.27 g. (87%), had m.p. 95.5–96.5° in agreement with our previously observed value.[11]

The free racemic tertiary alcohol (IX) could be converted more conveniently directly into the acetate XI by a similar procedure. Thus a mixture of alcohol (1.78 g.), acetic anhydride (3.1 g.) and benzene (10 ml.), when treated as above with stannic chloride (7.81 g.) in benzene (5 ml.) and processed as before, yielded 2.42 g. of crude product, recrystallization of which gave 1.65 g. of racemic XI, m.p. 95–96°, having an infrared spectrum identical with that of the above sample.

The enantiomers of the acetate XI were also prepared in this fashion. Crude (−)2-(2,3-dihydro-2-benzofuryl)-2-propanol (22.4 g.) with acetic anhydride (36.3 ml.) in benzene (125 ml.), treated at 0° with stannic chloride (45 ml.) in benzene (65 ml.), afforded 31.0 g. (94%) of crude product. A single recrystallization from cyclohexane yielded 25.8 g. of (−)2-(2,3-dihydro-5-acetyl-2-benzofuryl)-2-propyl acetate having m.p. 100–102°, $[\alpha]^{25}D$ −104.2° (*c*, 2.0, ethanol). Further recrystallization from cyclohexane and from ethanol gave a pure product, colorless needles, m.p. 102.5–103°, $[\alpha]^{25}D$ −103.2° (*c*, 4.0, ethanol), −101.6° (*c*, 4.2, chloroform).

*Anal.* Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.92; H, 6.84.

Similar acetylation of (+)IX yielded a sample of (+)2-(2,3-dihydro-5-acetyl-2-benzofuryl)-2-propyl acetate, m.p. 102–103°, $[\alpha]^{25}D$ +102.2° (*c*, 4.6, ethanol), +99.3° (*c*, 0.43; chloroform).

*Anal.* Calcd. for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92. Found: C, 68.89; H, 6.87.

**Tremetone.**—Pyrolysis of the racemic acetate XI commenced with evolution of acetic acid at about 280°, when XI (2.27 was placed in a test tube containing an indented wall to prevent the return of condensed volatiles, and heated in a metal bath at 330° for 10 min. The tube was cooled, the contents were dissolved in ether and the solution was washed with water and sodium hydroxide solution, then concentrated to yield an oily residue. A portion of the latter was examined by vapor–liquid partition chromatography (Apiezon column, 225°). Three components were noted: (1) 15 min. retention time, *ca.* 5–10%; (2) 18 min., 10–15%; (3) 21 min., 75–80%. Component (3) had an infrared spectrum identical in all respect with that of natural tremetone.[11] Components (1) and (2) have not yet been identified. The remainder of the crude pyrolysis mixture could be conveniently separated into these components by column chromatography on alumina, using as eluents solvent mixtures of increasing polarity from hexane through methanol.

The levorotatory acetate (−)XI was subjected to a similar pyrolysis and the crude product was separated and purified by column chromatography as above. The (−)tremetone fraction was shown to be identical with natural tremetone by these criteria: $[\alpha]^{28}D$: synthetic, −61.4° (*c*, 4.5, abs. ethanol); natural, −59.6° (*c*, 5.5, abs. ethanol); $d^{28}_4$: synthetic, 1.079; natural,

(25) L. F. Fieser, "Experiments in Organic Chemistry," 2nd Edition, D. C. Heath and Company, New York, N. Y., 1941, p. 418.

(26) R. Fittig and G. Ebert, *Ann.*, **216**, 166 (1883).

(27) R. Stoermer and W. König, *Ber.*, **39**, 493 (1906).

(28) K. von Auwers, Ann., **415**, 152 (1918).

1.080; $R_f$ in silica thin layer chromatography: synthetic, 0.20 (benzene), 0.83 (ethyl acetate); natural, 0.19 (benzene), 0.84 (ethyl acetate); infrared spectra (neat), superimposable.

**Semicarbazones:** synthetic, m.p. 222°, $[\alpha]^{28}D$ −56.6° (*c*, 0.74, chloroform); natural, m.p. 222°, mixture m.p. 222°, $[\alpha]^{28}D$ −56.2° (*c*, 0.84; chloroform); $R_f$ on silica plates: synthetic, 0.27; natural, 0.26 (ethyl acetate); identical infrared spectra in chloroform solution.

*Anal.* Calcd. for $C_{14}H_{17}N_3O_2$: C, 64.84; H, 6.61; N, 16.21. Found: (synthetic), C, 64.80; H, 6.91; N, 16.35; (natural), C, 65.01; H, 6.61; N, 16.49.

**2,4-Dinitrophenylhydrazones:** synthetic, m.p. 184.2–184.7°; natural, m.p. 183.8–184.2°, mixture m.p. 183.8–184.2°. $R_f$ on silica plates: synthetic, 0.96; natural, 0.96 (ethyl acetate); identical infrared spectra in chloroform solution.

*Anal.* Calcd. for $C_{19}H_{18}N_4O_5$: C, 59.68; H, 4.74; N, 14.65. Found: (synthetic), C, 59.87; H, 4.99; N, 14.63; (natural), C, 59.50; H, 4.61; N, 14.57.

A sample of unnatural (+)tremetone was prepared by pyrolysis of the dextrorotatory acetate (+)XI. Vapor phase chromatography of the crude pyrolysis product showed the usual mixture of components. The tremetone fraction was isolated as before, $[\alpha]^{25}D$ +55.3° (*c*, 4.5, ethanol).

**Goldfish Toxicity Tests.**—In general these were conducted as follows[20]: The goldfish (1.5–2 g.) was placed in a large beaker of water at room temperature, the toxin in ethanol solution (20 mg./ml.) was added in such quantity as to give the indicated final concentration, and the time required for death of the goldfish was noted. The criterion for death was cessation of gill motion. Complete paralysis of the fish, other than gill motion, was generally noted in approximately 33% of the time required for gill motion to cease. Crude tremetol was administered to goldfish at concentrations of 50, 100 and 200 mg./l. Death times were, respectively, about 10, 5 and 3 min. Several of the pure compounds previously isolated[1,3] from white snakeroot were administered to goldfish at a dose level of 30 mg./l. The approximate death time observed for each of these compounds was: Terpene I, non-toxic; dehydrotremeton, 20 min.; tremetone, 24 min.; hydroxytremetone, 21 min. A rough comparison of the fish toxicity of tremetone with that of rotenone was made in this fashion. At $10^{-5}$ *M* rotenone gave a death time of about 45 min., in agreement with the previous literature.[20] At $10^{-5}$ *M* tremetone required 110 min., while at $2 \times 10^{-5}$ *M* it required about 53 min.

**Mouse and Rabbit Toxicity Tests.**—Tremetone was given orally and intraperitoneally to groups of white mice (3 per dose level). The doses were 50, 100, 200, 300, 500, 750 and 1000 mg./kg. Tremor was not observed at any dose level. The only symptoms observed in doses of 100–300 mg./kg. was a slight decrease in activity. In toxic doses (500 mg./kg. and above, intraperitoneally only) jerks and clonic convulsions were present. These data suggest that this compound is not a tremor-producing agent in the mouse. The substance was also run in a standard battery of anticonvulsant tests in mice, but showed no anticonvulsive activity. Essentially similar results were obtained in mouse tests employing crude tremetol. In addition, crude tremetol was given to rabbits with the following observations: oral administration at 550 mg./kg. produced no tremors; intraperitoneal administration at 150 mg./kg. likewise produced no tremors, but did appear to elicit general muscular weakness and increased respiration rate. It is apparent that neither the mouse nor the rabbit is a suitable animal for screening these types of compounds. We are indebted to Dr. Eugene L. Worach and Dr. Guy Everett of the Abbott Laboratories, North Chicago Illinois, for conducting and interpreting these experiments.

**Lamb Toxicity Tests.**—A 250-g. portion of alfalfa hay was mixed thoroughly with a solution of tremetone (3 g.) in chloroform and the solvent was allowed to evaporate. In a control, the alfalfa was mixed with chloroform only and allowed to dry. Both diets were prepared immediately before feeding to the test animals, which were two female lambs, 22–25 kg., fasted for 24 hours prior to the test. Each animal consumed the test meal and was observed subsequently during 7 days. No untoward symptoms were noted in either animal. This test was performed by the Diablo Laboratories, Berkeley, California.

**Chicken Toxicity Tests.**—Butler[22] has studied the toxicities of tremetol preparations from rayless goldenrod in a number of animals including the cat, dog, guinea pig, rabbit, rat, duck and chicken. Only the chicken proved sufficiently sensitive to merit detailed study. We have accordingly modified Butler's technique for our own purposes, employing 1-month old white leghorn cockerels (approximately 90 g. each) and using both parenteral and oral administration. Parenteral injections were made directly into the breast muscle using 200 mg. of toxin dissolved in 1 ml. sesame oil, uncontaminated sesame oil (1 ml.) serving as a control. Oral administration involved 200-mg. samples of toxin placed in No. 5 gelatin capsules which were forced down the subject's gullet with a glass stirring rod, sesame oil in a capsule being used as the control. Samples were administered by each method once every 24 hr. In either parenteral or oral administrations, no effects were noted using tremetone after 5 or 6 doses. When crude tremetol was administered parenterally the chickens died between the 5th and 7th dosage. When tremetol was administered orally death occurred after the 3rd or 4th administration, and the chickens showed a typical crouched position, great lethargy and difficult breathing after the 2nd or 3rd dosage.

**Insecticide Tests.**—A series of insecticide tests was performed on tremetone by the procedures described and with these results. Mosquito larvae (*Culex quinquefasciatus*) in batches of 25 were placed in aqueous tremetone solutions at a series of concentrations, and after 24 hr. the dead and surviving were counted. $LC_{50}$ proved to be 0.7 ppm. The common housefly (*Musca domestica*) was exposed to a series of concentrations of tremetone dissolved in oil and placed on the interiors of shell vials (2 $mm.^3$/vial). Female flies, 4–5 days old, were placed in the vials in groups of 25 and exposed for 1 hr., then removed to holding cages, given sugar and water, and examined for mortality 24 hr. later. $LD_{50}$ proved to be 20 μg./vial. In the case of German roaches (*Blatella germanica*), tremetone in acetone solutions of varying strength was applied topically to the thorax of males, and the mortality was read after 48 hr.; $LD_{50}$ proved to be 50 μg. Typical data in these tests for pyrethrin insecticides are: mosquito larvae, $LC_{50}$, 0.05 ppm.; common housefly, $LD_{50}$, 11 μg./vial; German roach, $LD_{50}$, 1 μg. These tests, interpretations and data were generously furnished by Prof. W. M. Hoskins, Department of Entomology and Parasitology, University of California, Berkeley. In addition, both tremetone and crude tremetol were subjected to standard laboratory tests involving toxic behavior toward the boll weevil, cotton aphid, two-spotted spider mite and southern army worm. Neither sample proved toxic to any of the above insects in these tests. We are indebted to Mr. B. A. Butt, Pesticide Chemicals Research Branch, Entomology Research Division, U. S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, for generously conducting and interpreting the latter tests.