kind to read through our manuscript and revise it linguistically, and we also express our gratitude to him. The elementary analyses were carried out in the Laboratories of Sankyo Co., Ltd., and Daiichi Seiyaku Co., Ltd., for which we are very grateful.

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[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE]

5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-barbituric Acid and its Thio Analog. Metabolites from Pentobarbital and Thiopental

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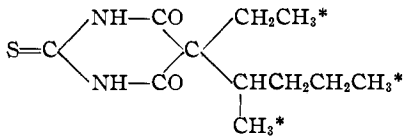
5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-thiobarbituric acid has been synthesized and has been found identical with a metabolic product isolated from human urine after the administration of thiopental. The preparation of 5-ethyl-5-(1-methylpentenyl-4)-2-thiobarbituric acid and 5-ethyl-5-(1-methylbutenyl-3)-2-thiobarbituric acid is also described; these thiobarbiturates were prepared for metabolism studies. 5-Ethyl-5-(1-methyl-3-carboxypropyl)-barbituric acid has been synthesized and shown to be identical with a metabolite isolated from the urine of dogs receiving pentobarbital.

5-Ethyl-5-(1-methylbutyl)-2-thiobarbituric acid[1] is widely used in intravenous anesthesia. It is usually classified as an ultra-short-acting barbiturate; the studies of Brodie[2] on the physiological disposition and chemical transformation of thiopental in the body show that this thiobarbiturate is almost completely metabolized in man at a relatively slow rate following extensive localization in fat. From the urine of humans after intravenous administration of thiopental it was possible to isolate a metabolic product of the drug. This compound was evidently a thiobarbiturate, since its ultraviolet absorption spectrum was qualitatively identical with that of thiopental. The analysis and infrared spectrum indicated that a terminal methyl group had been converted to a carboxylic acid group; this was supported by the electrometric titration data (pK_a 5.2 and 8.2). There are three possible positions for the carboxylic acid group, corresponding to the three terminal methyl groups of thiopental, as indicated by asterisks in the structure. Of these three possibilities, it was considered



most likely that oxidation occurred at the terminal position most distant from the quaternary carbon atom. This view[2] was dictated in part by the fact that 5,5-diethylbarbituric acid is largely excreted in unchanged form, and by the observations of Maynert[3] on the metabolic transformation of 5-ethyl-5-(1-methylbutyl)-barbituric acid (pentobarbital or Nembutal) into an oxidized product containing an hydroxyl group in the 3-position of the 1-methylbutyl chain.

5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-thiobarbituric acid was synthesized by the method shown in Chart I. Diethyl (1-methyl-3-carboxypropyl)-ethylmalonate (VII) was obtained by ozonolysis of diethyl (1-methylpentenyl-4)-ethylmalonate (III). The initial product of the ozonolysis was the corresponding aldehyde, and this was oxidized by permanganate to the acid. The condensation of the acid ester VII with thiourea occurred under normal conditions although in low yield. The desired product VIII was isolated by countercurrent distribution. It was a colorless crystalline solid whose ultraviolet and infrared spectra were identical with those of the thiopental metabolite previously isolated from human urine.[2] A mixed melting point was not depressed. Paper chromatography experiments indicated that both substances (isolated and synthetic) were homogeneous and had the same R_f value.[4]

This synthesis therefore establishes the structure of a metabolic product of thiopental.

The oxidation of a terminal methyl group of a hydrocarbon chain, which occurs in the body in this instance, may be related to the analogous reaction of ω-oxidation of fatty acids.[5] The intermediate steps in a transformation of this kind are not known, but one possible route from a methyl group to a carboxylic acid group involves dehydrogenation to a terminal methylene group, followed by hydration to a primary alcohol and subsequent conversion of the alcohol to an acid.[6] Other possibilities include the direct introduction of an hydroxyl group, followed by further oxidation. To test the dehydrogenation hypothesis, and to determine the metabolic fate of the compounds, two thiobarbiturates containing a chain with a terminal methylene group were prepared. These were 5-ethyl-5-(1-methylpentenyl-4)-2-thiobarbituric acid and 5-ethyl-5-(1-methylbutenyl-3)-2-thiobarbituric acid. The latter compound corresponds to a hypothetical intermediate in the oxidation of thiopental.

In order to study the generality of ω-oxidation in barbiturate metabolism in related compounds, Titus and co-workers[7] have studied the fate of pentobarbital in the dog. A barbituric acid with a terminal methyl group oxidized to a carboxyl group was iso-

(1) Thiopental or Pentothal.

(2) B. B. Brodie, L. C. Mark, E. M. Papper, P. A. Lief, E. Bernstein and E. A. Rovenstine, *J. Pharmacol. Exper. Therap.*, **98**, 85 (1950).

(3) E. W. Maynert and H. B. Van Dyke, *Science*, **110**, 661 (1949); E. W. Maynert and J. M. Watson, *J. Biol. Chem.*, **195**, 389 (1952).

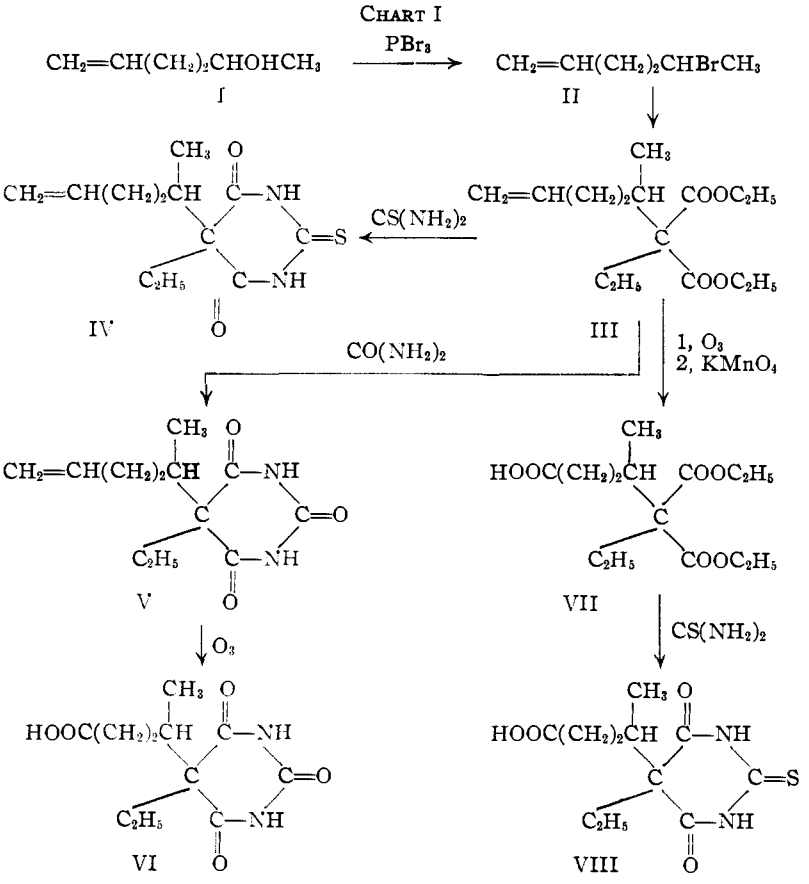
(4) The results of these chromatography experiments were very kindly communicated to us by Dr. Elwood Titus of the Laboratory of Chemical Pharmacology, National Heart Institute.

(5) K. Bernhard and N. Lincke, *Fortschr. Chem. org. Naturstoffe*, **4**, 251 (1945).

(6) B. B. Brodie, *Federation Proc.*, **11**, 632 (1952).

(7) E. O. Titus and H. Weiss, private communication.

CHART I



lated and characterized as one of the products. We have synthesized 5-ethyl-5-(1-methyl-3-carboxypropyl)-barbituric acid (VI) by ozonization and oxidation of 5-ethyl-5-(1-methylpentenyl-4)-barbituric acid (V), which was prepared by condensing urea with diethyl (1-methylpentenyl-4)-ethyl-malonate (III). The synthetic 5-ethyl-5-(1-methyl-3-carboxypropyl)-barbituric acid and the acid isolated from the urine of dogs receiving pentobarbital had the same melting point, and a mixed melting point was not depressed. The ultraviolet and infrared absorption spectra were identical, as were the X-ray powder diffraction diagrams. This synthesis establishes the structure of the metabolite, and it may be concluded that both thiopental and pentobarbital undergo ω-oxidation of the 1-methylbutyl chain in the course of metabolic transformation. A second metabolic pathway in the case of pentobarbital leads to the hydroxylated product described by Maynert.[3]

Acknowledgment.—We are indebted to Dr. William Alford and his staff for the analytical data, and to Mrs. Iris Siewers and Miss Alice Bernardi of our Instrumental Laboratory for the spectra.

Experimental

Allylacetone.—A mixture of ethyl allylacetoacetate (111 g., 0.65 mole) and barium hydroxide (132 g., 0.77 mole) in 2.6 l. of water was heated under reflux for eight hours. Using steam distillation, about 500 ml. of distillate was collected and extracted with ether. The product[8] was secured by distillation at atmospheric pressure, b.p. 128–129°, yield 43.8 g. (69%).

(8) Merling, *Ann.*, **264**, 323 (1891).

The yellow 2,4-dinitrophenylhydrazone, m.p. 108–109°, has not been reported previously; it was recrystallized from aqueous ethanol.

Anal. Calcd. for $C_{12}H_{19}O_4N_4$: C, 51.78; H, 5.07. Found: C, 51.95; H, 5.31.

1-Hexen-5-ol.—A solution of 43.7 g. (0.45 mole) of allylacetone in 100 ml. of dry ether was added dropwise to a stirred mixture containing 4.6 g. (0.45 mole) of finely ground lithium aluminum hydride in 500 ml. of dry ether. After heating under reflux with stirring for two hours, the mixture was treated with a little alcohol and then with a saturated aqueous solution of potassium sodium tartrate. The product was extracted with ether, and the combined ether extracts were dried and distilled. The product was collected at 138–139°, yield 39.8 g. (89%). It was a colorless oil with a disagreeable odor.

Anal. Calcd. for $C_6H_{12}O$: C, 71.94; H, 12.08. Found: C, 72.17; H, 12.01.

5-Bromohexene-1.—The general method of Newman and Wotiz[9] was employed. A solution of 38 g. (0.38 mole) of hexen-1-ol-5 and 2 g. of pyridine in 100 ml. of dry ether was added dropwise with good stirring to a solution of 35 g. (0.13 mole) of phosphorus tribromide in 100 ml. of dry ether, at a rate which maintained the system under steady reflux. The mixture was heated under reflux for an additional three hours, cooled and poured on crushed ice. The organic phase was separated and the aqueous phase was extracted with ether. The combined ether layers were washed with sodium chloride–sodium carbonate solution, dried and distilled. The product was collected at 138–144°, in 35.4 g. (58%) yield. An analytical sample was obtained by redistillation, b.p. 141–143°.

Anal. Calcd. for $C_6H_{11}Br$: C, 44.18; H, 6.79. Found: C, 44.05; H, 6.59.

Diethyl (1-Methylpentenyl-4)-ethylmalonate.—This ester was made through use of the general alkylation method of Wallingford, Thorp and Homeyer.[10] To sodium ethoxide prepared from 3.8 g. (0.17 mole) of sodium there were added 80 ml. of redistilled ethyl carbonate and 36 g. (0.19 mole) of diethyl ethylmalonate. The mixture was distilled under 150 mm. pressure until the ethanol was removed, and 27.4 g. (0.17 mole) of 5-bromohexene-1 was added dropwise. The mixture was heated under reflux for 12 hours, and the major portion of the solvent was removed by distillation under reduced pressure. The residue was treated with water (200 ml.) and extracted with isopropyl ether. The organic extracts were combined, washed with a saturated salt solution, dried, and distilled *in vacuo*. The product was collected at 130–133° (5 mm.).

The yield was 23.3 g. (52%). The infrared spectrum was in agreement with the expected structure.

Anal. Calcd. for $C_{15}H_{26}O_4$: C, 66.63; H, 9.67. Found: C, 66.47; H, 9.53.

5-Ethyl-5-(1-methylpentenyl-4)-2-thiobarbituric Acid.—A sodium ethoxide solution was prepared from 3.1 g. (0.13 mole) of sodium and 70 ml. of anhydrous ethanol. After the addition of 10.0 g. (0.13 mole) of thiourea and 11.9 g. (0.044 mole) of diethyl (1-methylpentenyl-4)-ethylmalonate the mixture was heated under reflux for 12 hours. The ethanol was removed by distillation under reduced pressure, using a bath kept at 50°. The residue was dissolved in ice-water (50 ml.), and the chilled solution was extracted with ether. It was found essential to maintain the solution at 0–5° during these operations. The solution was acidified (congo red) with 6 *N* sulfuric acid, and the crystalline prod-

(9) M. S. Newman and J. H. Wotiz, THIS JOURNAL, **71**, 1294 (1949).

(10) V. H. Wallingford, M. A. Thorp and A. H. Homeyer, *ibid.*, **64**, 580 (1942).

uct was separated. The solid material was treated with boiling ethanol. The hot ethanol solution was diluted to turbidity and chilled. The yield was 5.5 g. of crystalline 5-ethyl-5-(1-methylpentenyl-4)-2-thiobarbituric acid, m.p. 136–139°. Recrystallization from aqueous ethanol provided an analytical sample, m.p. 138–140°. The ultraviolet and infrared spectra of this material were in agreement with the expected structure.

Anal. Calcd. for $C_{12}H_{18}O_2N_2S$: C, 56.66; H, 7.13. Found: C, 56.91; H, 7.07.

Diethyl (1-Methyl-3-carboxypropyl)-ethylmalonate.—A solution of 5.8 g. of diethyl (1-methylpentenyl-4)-ethylmalonate in 300 ml. of methylene chloride was chilled in an acetone–Dry Ice-bath, and treated with an ozone–oxygen flow at the rate of 0.087 millimole of ozone per minute for six hours. The solution was poured into 50 ml. of water containing 20 ml. of 30% hydrogen peroxide, and was heated on a steam-bath. After evaporation of the methylene chloride, the solution was cooled, diluted and extracted with ether. The ether extracts were washed with sodium bicarbonate solution, the peroxides were destroyed and the ether solution was dried and evaporated to yield 5.4 g. of nearly colorless sirup. The infrared spectrum of this material indicated that it was an aldehyde; from the bicarbonate wash there was obtained 0.6 g. of the desired acid.

The aldehyde was oxidized to the corresponding acid with potassium permanganate. A solution of 3.24 g. of potassium permanganate in 250 ml. of acetone was added dropwise to a stirred solution of the aldehyde (5.4 g.) in acetone (25 ml.) at room temperature. The mixture was stirred for one hour after the addition was completed, and was then diluted with water. The solution was decolorized with sodium hydrosulfite, filtered and heated gently under reduced pressure to remove the acetone. The aqueous solution was extracted with ether; the combined extracts were dried and evaporated to give 1.9 g. of clear, colorless sirup. This material was soluble in sodium bicarbonate solution and it gave an infrared spectrum identical with that obtained for the bicarbonate-soluble material resulting directly from the ozonolysis. The material did not crystallize and was not subjected to analysis.

5-Ethyl-5-(1-methyl-3-carboxypropyl)-2-thiobarbituric Acid.—The condensation of 2.02 g. of thiourea and 2.55 g. of diethyl (1-methyl-3-carboxypropyl)-ethylmalonate was carried out in the usual way with sodium ethoxide from 0.81 g. of sodium in 15 ml. of dry ethanol. After heating under reflux for ten hours, the ethanol was removed and the residue was treated with ice-water. The chilled solution was washed with benzene, acidified (congo red) with 6 *N* sulfuric acid, and extracted with ether. The product was removed from the ether solution with sodium bicarbonate solution, and again separated by acidification and ether extraction. After drying and evaporation of the ether, there remained 1.57 g. of semi-solid material; an ultraviolet spectral analysis indicated that this material contained approximately 200 mg. of a thiobarbituric acid. The product was isolated using countercurrent distribution with 15 transfers, following Craig,[11] with an ether–*p*H 6.0 phosphate buffer pair. The separation was followed by measuring ultraviolet absorption at 305 mμ. The material in the product-containing aqueous phases was transferred to the ether phase in each case by acidification to *p*H 1, and the combined ether phases were washed well with a salt solution, with water and dried. Removal of the ether gave a sirup which was crystallized from aqueous ethanol. Recrystallization from the same medium gave 99 mg. of colorless crystalline thiobarbiturate, m.p. 184–188°, with softening at 175°. A mixed melting point (Kofler stage) with a sample of the metabolic product derived from thiopental and isolated from human urine showed no depression. The infrared and ultraviolet spectra of the synthetic and isolated products were identical.

Anal. Calcd. for $C_{11}H_{16}O_4N_2S$: C, 48.51; H, 5.92. Found: C, 48.64; H, 5.97.

4-Pentene-2-ol.—This alcohol was prepared according to the procedure of Stöhn[12] in 63% yield; b.p. 115–116° (reported b.p. 115–116°).

4-Bromopentene-1.—The preparation of the halide followed the procedure described for 5-bromohexene-1. The materials used were 45.6 g. of 4-penten-2-ol, 48 g. of phosphorus tribromide and 3 ml. of dry pyridine, with a total volume of 300 ml. of dry ether. A 34.1 g. (43%) yield of 4-bromopentene-1, b.p. 114–116°, resulted; this material was used without further purification.

Diethyl (1-Methylbutenyl-3)-ethylmalonate.—This alkylation followed the procedure described previously. From 46.5 g. of diethyl ethylmalonate and 33.2 g. of 4-bromopentene-1, with sodium ethoxide from 5.0 g. of sodium, there was obtained 11.1 g. (58%) of the expected substituted malonic ester, b.p. 116–117° (6 mm.).

Anal. Calcd. for $C_{14}H_{24}O_4$: C, 65.59; H, 9.44. Found: C, 65.35; H, 9.63.

5-Ethyl-5-(1-methylbutenyl-3)-2-thiobarbituric Acid.—A condensation was carried out using 3.5 g. of the substituted malonic ester, 3.1 g. of thiourea and sodium ethoxide from 0.93 g. of sodium in 15 ml. of dry ethanol (15 hour reflux). There was obtained, after the usual isolation procedure, 0.60 g. (25%) of the thiobarbiturate as colorless needles (from aqueous alcohol), m.p. 155–156°. The infrared and ultraviolet spectra were in agreement with the expected structure.

Anal. Calcd. for $C_{11}H_{16}O_2N_2S$: C, 54.97; H, 6.71; N, 11.66. Found: C, 54.91; H, 6.64; N, 11.68.

5-Ethyl-5-(1-methylpentenyl-4)-barbituric Acid.—This compound was prepared as described for the corresponding thiobarbituric acid. A solution of sodium ethoxide was prepared from 2.8 g. (0.12 mole) of sodium and 100 ml. of anhydrous ethanol. After the addition of 7.3 g. (0.12 mole) of dry, finely ground urea and 11 g. (0.041 mole) of diethyl (1-methylpentenyl-4)-ethylmalonate the mixture was heated under reflux for 12 hours. The ethanol was removed by evaporation under reduced pressure using a bath maintained at 50°. The residue was dissolved with 100 ml. of ice and water and extracted with ether. At no time was the alkaline solution of the product above 5°. The solution was acidified (congo red) with 6 *N* sulfuric acid, and the crystalline barbituric acid was separated. The yield was 3.4 g. of 5-ethyl-5-(1-methylpentenyl-4)-barbituric acid, m.p. 76–84°. Purification by recrystallization from aqueous ethanol provided an analytical sample, m.p. 102–104°. The ultraviolet and infrared spectra of this acid were in agreement with the expected structure.

Anal. Calcd. for $C_{12}H_{18}O_3N_2$: C, 60.47; H, 7.16. Found: C, 60.67; H, 7.57.

5-Ethyl-5-(1-methyl-3-carboxypropyl)-barbituric Acid.—A solution of 508 mg. of 5-ethyl-5-(1-methylpentenyl-4)-barbituric acid in 30 ml. of methylene chloride was cooled to 0° and subjected to a stream of ozonized oxygen (0.073 mmole of ozone per minute) for 29 minutes. The methylene chloride solution was added to 50 ml. of water, the solution was placed on a steam-cone and 2 ml. of hydrogen peroxide (30%) was added. After evaporation of the methylene chloride, the solution was cooled and extracted with ether. The ether solution containing the reaction products was washed with ferrous ammonium sulfate in 0.1 *N* HCl until free of peroxides.

Isolation and purification[13] was achieved by the countercurrent method. The reaction product was subjected to 20-plate countercurrent distribution in 10 ml. layers of ether and a *p*H 4.56 buffer prepared by mixing 46 volumes of 0.2 *M* Na_2HPO_4 and 54 volumes of 0.1 *M* citric acid.

Tubes 8, 9 and 10 (calculated to contain 35 mg. by ultraviolet absorption, o.d. 255 mμ) were combined, acidified to *p*H 1 and the lower phase exhaustively extracted with ether. The combined ether solution after washing with water and evaporation to dryness left a residue of 29 mg. of amorphous solid m.p. 177–187°.

Several recrystallizations from 65% ethanol yielded 17.7 mg., m.p. 192–194°. This m.p. was unchanged on mixing with a sample of the carboxylic acid isolated[7] from the urine of dogs receiving pentobarbital and melting at 194–196°. The infrared and ultraviolet absorption spectra for these two compounds were identical and were in agreement with the expected structure. The X-ray powder diffraction diagrams were identical.

Anal. Calcd. for $C_{11}H_{16}O_5N_2$: C, 51.55; H, 6.29. Found: C, 51.39; H, 6.33.

BETHESDA 14, MD.

(11) L. C. Craig, *J. Biol. Chem.*, **150**, 33 (1943).

(12) L. Stöhn, *Ber.*, **72**, 1138 (1939).

(13) We are indebted to Dr. Elwood O. Titus, of the Laboratory of Chemical Pharmacology, National Heart Institute, for the final isolation and purification by countercurrent techniques.