were not analyzed: *o*-methoxalylamino-acetophenone, *o*-oxamamino-acetophenone, benzoyl-isatinic acid, phthaloyl-isatinic acid, 2-methyl-quinazoline-4-carboxylic acid, 4-methylquinazoline-2-carboxylic acid and 2-phenylquinazoline-4,2′-dicarboxylic acid.

6. The following compounds, already known, were prepared by new methods: *o*-benzoylamino-acetophenone and acetyl-isatinic acid.

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ETHYL-NORMAL-HEXYLBARBITURIC ACID AND OTHER DERIVATIVES OF NORMAL-HEXYLMALONIC ACID

BY ARTHUR W. DOX

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5,5-Dialkylbarbituric acids, in which one alkyl is ethyl and the other methyl, ethyl, propyl, butyl, *iso*butyl, *iso*-amyl and heptyl, have been compared with respect to their hypnotic activity by Carnot and Tiffeneau.[1] The maximum activity was manifested by the butyl, *iso*-butyl and *iso*-amyl derivatives, where the hypnotic effect was thrice that of veronal (diethylbarbituric acid). With the heptyl derivative there was a slight falling off in activity to 2.5 times that of veronal. The *iso*-butyl and *iso*-amyl derivatives, with their branched carbon chains, do not, strictly speaking, fit into the series. Unfortunately, the *n*-amyl and *n*-hexyl derivatives were not included in the tests. Until these are prepared and tested we are still uncertain as to the place in the series where the maximum hypnotic activity is attained.

The present work was undertaken primarily for the purpose of preparing one of the missing homologs, ethyl-*n*-hexylbarbituric acid. With *n*-hexyl alcohol now available as a by-product in the commercial manufacture of *n*-butyl alcohol, hexyl halides may be easily prepared and employed in the various malonic ester syntheses. The successive alkylation of ethyl malonate with hexyl bromide and ethyl bromide, or with these alkyl halides in the reverse order, gives ethyl ethylhexylmalonate which condenses with urea under the conditions of the Fischer and Dilthey synthesis to form ethylhexylbarbituric acid. The initial product, ethyl hexylmalonate, being easily obtained, a portion of this was used in the preparation of certain other derivatives of incidental interest which are included in this paper.

Experimental Part

Hexyl Bromide.—This substance has been previously prepared by Lieben and Janecek[2] from hexyl alcohol and hydrogen bromide in a sealed tube at 100°. The writer

[1] Carnot and Tiffeneau, *Compt. rend.*, **175**, 241 (1922).

[2] Lieben and Janecek, *Ann.*, **187**, 137 (1877).

used the simpler and more recent method of Kamm and Marvel for homologous alkyl bromides.[3]

A mixture of 200 g. of hexyl alcohol, 375 cc. of hydrobromic acid (equivalent to 200 g. of bromine) and 100 g. of sulfuric acid was refluxed for one and a half hours, then distilled during one and a half hours. The first half of the distillate consisted of an upper layer of water and a lower layer of hexyl bromide, while the second half contained hexyl bromide in the upper layer and aqueous hydrobromic acid in the lower. The hexyl bromide was separated in each case and the combined product washed thrice with water, then with 25 cc. of sulfuric acid, 100 cc. of 10% sodium carbonate solution and finally with water, and dried with 5 g. of calcium chloride. Distillation at atmospheric pressure gave 272 g., or 82%, of a product boiling at 154–156°.

Ethyl *n*-Hexylmalonate.—To a solution containing 23 g. of sodium in 400 cc. of absolute alcohol, 160 g. of ethyl malonate was added while the liquid was mechanically stirred. The solution was then warmed on a steam-bath and 165 g. of hexyl bromide slowly added through a dropping funnel. Sodium bromide separated rapidly. After four hours on the steam-bath the mixture was neutral to litmus. The greater part of the alcohol was then distilled, while the liquid was vigorously stirred to prevent bumping. The residue was dissolved in water and the oily layer separated. This was washed with water and dried over calcium chloride. The oil was purified by distillation under reduced pressure. A small amount of hexyl bromide and ethyl malonate came over in the first fraction; then the temperature rose suddenly to 140° at a pressure of 15 mm. Between 140° and 147°, a yield of 170 g. was obtained, leaving practically no residue in the flask. Redistillation gave a colorless, oily liquid with a faint fruity odor; b. p., 268–270° (749 mm.); d_{25}, 0.9556; yield, 73%.

Analysis. Subs., 0.2276: CO_2, 0.5365; H_2O, 0.1948. Calc. for $C_{13}H_{24}O_4$: C, 63.93; H, 9.84. Found: C, 64.33; H, 9.52.

***n*-Hexylmalonic Acid.**—The ester described above was saponified by boiling 30 g. for six hours with 15 g. of potassium hydroxide dissolved in 100 cc. of water and 50 cc. of alcohol. The alcohol was evaporated on the steam-bath, and the residue dissolved in water and shaken with ether to remove any unsaponified ester. The aqueous solution was again evaporated, then cooled and acidified with hydrochloric acid. The free acid separated as an oil which soon solidified. It was purified by dissolving in benzene, filtering while hot and allowing to cool. Colorless, transparent crystals were obtained; yield, 18.3 g., or 79%. Hexylmalonic acid is soluble in benzene, alcohol and ether, difficultly soluble in water, and insoluble in petroleum ether. It has a sour taste with a slightly bitter and astringent after-taste. It melts at 105–106° and decomposes at about 130° with evolution of carbon dioxide.

Analysis. Subs., 0.200: CO_2, 0.4175; H_2O, 0.1485. Calc. for $C_9H_{16}O_4$: C, 57.45; H, 8.51. Found: C, 56.93; H, 8.21.

***n*-Hexylmalonamide.**—Fifteen g. of ethyl hexylmalonate and 150 cc. of concd. ammonium hydroxide were shaken at frequent intervals in a stoppered bottle during 13 days. Crystals of the amide began to appear on the second day. At the end of 13 days the reaction was still incomplete as shown by the presence of oily drops of the ester. The amide was filtered off, washed with water and then with ether, to remove unchanged ester. Recrystallization from dil. alcohol gave fine, silky needles that matted together into a felt-like mass. The amide is soluble in alcohol, difficultly soluble in water, insoluble in benzene and ether. It melts at 208°.

Analyses (Kjeldahl). Subs., 0.2548, 0.400: 27.18, 42.64 cc. of 0.1 *N* acid. Calc. for $C_9H_{18}N_2O_2$: N, 15.05. Found: 14.93, 14.94.

[3] Kamm and Marvel, THIS JOURNAL, **42**, 307 (1920).

n-**Hexylmalonanilide.**—Ten g. of the ester and 8.5 g. of aniline (2 molecular equivalents) were refluxed for three hours under an air condenser. The mixture solidified as it cooled. It was extracted with alcohol and a small amount of insoluble, white residue removed by filtration. Evaporation of the solvent gave 11 g. of microscopic crystals which after recrystallization melted at 156°. The substance is insoluble in water and petroleum ether, and is soluble in alcohol, ether and benzene.

Analysis. Subs., 0.250: 17.16 cc. of 0.1 *N* acid. Calc. for $C_{21}H_{26}N_2O_2$: N, 8.28. Found: 8.34.

n-**Hexylbarbituric Acid.**—To a solution of 3 g. of sodium in 40 cc. of absolute alcohol (99.8%), 10 g. of ethyl hexylmalonate and 4 g. of urea were added. The mixture was heated for seven hours in an autoclave at an average temperature of 104°. The alcohol was then evaporated on a steam-bath, and the residue dissolved in water and acidified with hydrochloric acid. The product separated at first as a white gummy mass that rapidly became crystalline. Recrystallization from alcohol gave 8 g., or 92%, of long, white needles melting at 216°. The substance is readily soluble in alcohol, difficultly soluble in water, benzene and ether, and insoluble in petroleum ether. It is practically tasteless.

Analyses. Subs., 0.3948, 0.4381: 37.47, 41.12 cc. of 0.1 *N* acid. Calc. for $C_{10}H_{16}N_2O_3$: N, 13.21. Found: 13.28, 13.14.

5-Hexyl-1-methylbarbituric Acid.—The reaction mixture consisted of 4 g. of sodium in 55 cc. of absolute alcohol, 10 g. of ethyl hexylmalonate and 6 g. of methylurea nitrate. It was autoclaved for seven hours at 104°. The product was treated as described in the preceding preparation. At first a yellow oil separated which solidified as it was cooled with ice. Recrystallization gave 8.0 g. of white needles; yield, 86%; m. p., 68°. It is soluble in alcohol, ether and benzene, insoluble in water and petroleum ether. When a solution in dil. alkali is acidified, the acid separates in oily drops which gradually become crystalline. The substance has a bitter taste.

Analysis. Subs., 0.200: N_2, 23.2 cc. (746 mm., 25°). Calc. for $C_{11}H_{18}N_2O_3$: N, 12.39. Found: 12.69.

5-Hexyl-1-phenylbarbituric Acid.—Three g. of sodium dissolved in 40 cc. of absolute alcohol, 10 g. of ethyl hexylmalonate and 9 g. of phenylurea were autoclaved for seven hours at 104°. After the alcohol was evaporated and the residue dissolved in water, the solution was shaken with ether to remove unchanged ester. The excess of phenylurea was filtered off and the filtrate acidified with hydrochloric acid. An oil separated which immediately solidified; yield, 10.4 g., or 88%. Recrystallization from alcohol gave white lustrous scales. The substance melts at 141° and has a bitter taste. It is soluble in alcohol, benzene and ether, but insoluble in water.

Analysis. Subs., 0.252: 24.46 cc. of 0.1 *N* acid. Calc. for $C_{16}H_{20}N_2O_3$: N, 9.93. Found: 9.97.

5-Hexyl-1-*o*-tolylbarbituric Acid.—This was prepared in the same manner as the compound just described, using *o*-tolylurea in place of phenylurea. The yield was 9.9 g., or 80%, of fine, hair-like needles; m. p., 118°. The solubility was the same as that of the corresponding phenyl derivative.

Analysis. Subs., 0.200: N_2, 17.1 cc. (746 mm., 25°). Calc. for $C_{17}H_{22}N_2O_3$: N, 9.27. Found: 9.35.

Ethyl Ethylhexylmalonate.—The ethyl group was introduced first for the reason that a greater difference in boiling points occurs between the mono-alkylated ester and the dialkylated product, thus facilitating purification of the product. To a solution of 37.7 g. of sodium in 500 cc. of absolute alcohol was added 308 g. of ethyl ethylmalonate

while the liquid was mechanically stirred. The mixture was heated on a steam-bath and stirred while 272 g. of hexyl bromide was added slowly through a dropping funnel. The heating was continued for one and a half hours during which time a copious separation of sodium bromide occurred, and the mixture became neutral to litmus. The greater part of the alcohol was distilled during continued stirring and the residue dissolved in water. The oily layer was separated and treated in the usual way. Fractionation at atmospheric pressure gave 284 g. of a colorless oil boiling at 277–283°; yield, 64%. The product was purified further by distillation under reduced pressure; at 6 mm., 246 g. distilled at 133–134°; b. p., 280–282° (755 mm.); d_{25}, 0.9473.

Analyses. Subs., 0.2055: CO_2, 0.4864; H_2O, 0.1820. Calc. for $C_{15}H_{28}O_4$: C, 66.16, H, 10.29. Found: C, 66.57; H, 9.94.

Ethylhexylmalonic Acid.—Saponification of the ester was effected by refluxing for five hours a homogeneous mixture of 13 g. of ester, 25 cc. of water and 50 cc. of alcohol with 10 g. of potassium hydroxide. The solvent was then evaporated and the residue cooled and acidified with 20 cc. of concd. hydrochloric acid. The free acid separated as an oil. This was extracted with ether and the ether evaporated. Crystallization did not occur until the sirupy residue had been allowed to stand for nearly two weeks in the cold. The crystals were then dried on a porous plate and washed with a little petroleum ether. The product consisted of very small, white needles melting at 75° and decomposing at about 140° with evolution of carbon dioxide. It is soluble in water and most organic solvents including carbon tetrachloride, but insoluble in aliphatic hydrocarbons. It has a sour taste and a very bitter and astringent after-taste.

Analyses. Subs., 0.200: CO_2, 0.445; H_2O, 0.1655. Calc. for $C_{11}H_{20}O_4$: C, 61.11; H, 9.26. Found: C, 60.68; H, 9.19.

Ethylhexylbarbituric Acid.—A mixture consisting of 25 g. of ethyl ethylhexylmalonate, 6.9 g. of sodium dissolved in 100 cc. of absolute alcohol, and 9 g. of urea was autoclaved for six hours at 102°. Most of the alcohol was then evaporated and the residue dissolved in ice water. Hydrochloric acid was added until the liquid was distinctly acid. A colorless oil separated, which solidified almost instantly. The solid cake was broken up, filtered and washed with water and finally with petroleum ether. The yield was 20 g. or 91%, of small white crystals; m. p., 112–113°. The substance is soluble in alcohol, ether and benzene, but difficultly soluble in water. From hot water it separates first as a fine emulsion which later clears with the formation of long, slender needles. It has a strongly bitter taste.

Analysis. Subs., 0.1060: N_2, 11.4 cc. (744 mm., 25°). Calc. for $C_{12}H_{20}N_2O_3$: N, 11.67. Found: 11.72.

Physiological Tests

In the first series of tests a solution of the sodium salt of ethylhexylbarbituric acid was used intraperitoneally on white mice. The control tests were made in the same manner with luminal (phenylethylbarbituric acid) which has a potency of 2.5 in terms of veronal. The new substance administered in this way was considerably more effective than luminal. It was much more rapid in its action and somewhat less toxic, but the effect was of shorter duration. The substance is now being subjected to a more elaborate physiological study by oral administration to dogs. It is expected that a detailed report of this work will be published elsewhere. Of the other derivatives described in this paper, 5-hexyl-1-methyl- and

5-hexyl-1-phenyl-barbituric acid were tested with mice. The former was inert and the latter toxic without preliminary hypnotic effect.

Summary

A number of derivatives of *n*-hexylmalonic acid are described. Chief among these in point of interest is ethylhexylbarbituric acid, which is a powerful hypnotic and may prove to be of therapeutic value.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, No. 450]

THE ISO-ELECTRIC POINT OF MALT AMYLASE[1]

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The experiments reported in this paper originated in attempts to standardize further the methods which have been developed here for obtaining purified and highly active malt amylase preparations. Work in this Laboratory has repeatedly pointed either to the protein nature of this amylase or to protein as an essential constituent of it. Since proteins are least soluble at their iso-electric points, it was thought, provided the enzyme is protein or intimately associated with protein, that it might be separated from inactive proteins found in crude extracts by precipitating at the iso-electric point of the enzyme. We have, therefore, studied the iso-electric point by electrophoresis experiments with solutions of malt amylase adjusted to a wide range of hydrogen-ion concentrations by means of buffer solutions.

Extensive preliminary experiments with commercially concentrated malt extracts adjusted to different hydrogen-ion concentrations showed that while there was some evidence of electrophoresis of the active substance, consistent results could not be obtained with the crude material. This was probably due to the presence in the extracts of relatively large amounts of inert proteins of varying iso-electric points which may have masked the electrophoresis of the amylase.

Entirely consistent evidence of electrophoresis was, however, obtained from experiments upon solutions of purified malt amylase which had been made in this Laboratory by our modification of the Osborne method as described in previous papers.[2,3]

Experimental Part

The Apparatus and Technique finally adopted as satisfactory may be described briefly as follows. The U-tube apparatus of Michaelis[4] was

[1] The expenses of this investigation were shared by the Carnegie Institution of Washington and the Department of Chemistry of Columbia University.

[2] Osborne, THIS JOURNAL, **17,** 587 (1895); **18,** 536 (1896).

[3] Sherman and Schlesinger, *ibid.*, **35,** 1617 (1913); **37,** 643, 1305 (1915).

[4] Michaelis, *Biochem. Z.*, **16,** 81 (1909).