[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF DELAWARE]

Glycyl Derivatives of Aminobarbituric Acids

BY GLENN S. SKINNER AND DONALD J. LYMAN

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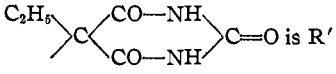
Seventeen new barbituric acid derivatives have been prepared in connection with the synthesis of simulated dipeptides. Selected compounds have been subjected to pharmacological tests.

A series of compounds including types A and B together with intermediates and derivatives has been prepared for pharmacological examination. The structural design of A was of special interest to us in that it provided the pattern of a dipeptide in which one of the amino acid residues is replaced by an aminobarbituric acid residue. The value of n was varied from zero to two by starting with the appropriate halide.

$$R'-(CH_2)_nX \longrightarrow R'-(CH_2)_nNHCH_2CO_2C_2H_5$$
B

$$\downarrow$$

$$R'-(CH_2)_nNH_2 \longrightarrow R'-(CH_2)_nNHCOCH_2Cl \longrightarrow R'-(CH_2)_nNHCOCH_2NH_2$$ where
A



Where n is zero, 5-bromo-5-ethylbarbituric acid reacted easily with ammonia to give the amine leading to A or with glycine ester to yield B. If n is 1, the needed halogen derivative was prepared by treating chloromethyl bromide with diethyl sodioethylmalonate in benzene. The resulting diethyl chloromethylethylmalonate was then condensed with urea under mild conditions to yield the desired chloromethylethylbarbituric acid (I) without serious loss due to the formation of the ethyl ether. Where n is 2, 5-ethyl-5-β-hydroxyethylbarbituric acid reacted with fuming hydrobromic acid[1] to yield the desired bromo acid.

With respect to the above systematic variation in structure it may be stated that 5-chloromethyl-5-ethylbarbituric acid reacted sluggishly with glycine ester under the usual conditions to yield the desired amination product XIV and the amination with ammonia to yield II required elevated temperature in the presence of sodium iodide. The action of fuming hydrobromic acid on 5-ethyl-5-methoxymethylbarbituric acid gave 5-ethyl-5-hydroxymethylbarbituric acid (XV) and none of the corresponding bromomethyl compound. 5-(β-Bromoethyl)-5-ethylbarbituric acid, however, reacted readily with ammonia and so easily with glycine ester that both hydrogen atoms of the amino group were replaced to give XVI. This yielded the anilide XVII.

$$C_2H_5O_2CCH_2N(CH_2CH_2R')_2$$
XVI

$$C_6H_5NHCOCH_2N(CH_2CH_2R')_2$$
XVII

Although 5-aminomethyl-5-ethylbarbituric and 5-(β-aminoethyl)-5-ethylbarbituric acids condensed normally to IV and V with chloroacetyl chloride in the presence of sodium hydroxide solution, the 5-amino-5-ethylbarbituric acid did not. In this case it was found that trimethylamine was suitable for effecting the introduction of the chloroacetyl group to give III.

The amination of all three chloroacetyl derivatives proceeded normally to yield VI, VII and VIII. These simulated dipeptides in turn were acetylated to IX, X and XI for the purpose of blocking the basic function.

When orally administered to rats[2] 100 mg./kg. of I gave 20% protection and 250 mg./kg. gave 40% protection by the electroshock method. By the metrazol method E.D.$_{50}$ ± S.E. = 84.0 ± 6.6. Hypnosis resulted at 160 mg./kg., anesthesia at 250 mg./kg. and death at 620 mg./kg. It is long acting but not active enough to justify clinical tests. At 400 mg./kg. VI gave no protection by either the electroshock or metrazol methods, XII at 620 mg./kg. gave no hypnosis, at 400 mg./kg. no protection by the electroshock method and 40% protection by the metrazol method. Other selected compounds were subjected to the Sloan–Kettering sarcoma mouse test. These results will be reported elsewhere.

Experimental

Diethyl Chloromethylethylmalonate.—Diethyl ethylmalonate (235.3 g., 1.25 moles) was added dropwise to a stirred suspension of 23 g. of finely divided sodium in 800 cc. of dry benzene kept at 20° by a bath of water. After standing overnight 258.8 g. (2 moles) of cooled bromochloromethane was added rapidly with stirring to the ice-cold solution. After standing until the next day there was little evidence of reaction, so the mixture was heated 12 hours at 70°. After neutralization with 3.5 cc. of hydrochloric acid (1.19) in enough water to dissolve the salt the benzene layer was distilled under diminished pressure. The yield of diethyl chloromethylethylmalonate was 131.4 g. (56%). It was obtained as a colorless liquid; b.p. 125–126.5° (18 mm.), $n^{25}D$ 1.4346, d^{25} 1.0921.

Anal. Calcd. for $C_{10}H_{17}O_4Cl$: M_r, 56.56; Cl, 14.98. Found: M_r, 56.51; Cl, 14.90.

Halogen Derivatives of the Barbituric Acids.—5-Bromo-5-ethylbarbituric acid was prepared as previously described,[3] yield of pure product 40%.

To a stirred solution of sodium ethoxide prepared from 21.5 g. (0.93 mole) of sodium and 350 cc. of absolute alcohol were added 42.0 g. (0.70 mole) of fused urea and 82 cc. (0.38 mole) of ethyl chloromethylethylmalonate. A precipitate started to form in about 15 minutes. The mixture was heated at 45° for 5 hours and then allowed to stand at 30–35° for 8 days. The alcohol was removed by distillation under diminished pressure. The 5-chloromethyl-5-ethylbarbituric acid was obtained by dissolving the residue in a minimum amount of water, and acidification of the ice cold solution to congo red. After crystallization from hot water it underwent transition at 210–212° from small crystals to long needles having m.p. 243–245° (m.p. block). Crystallization from alcohol followed by recrystallization

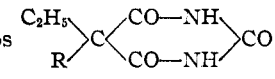
(1) G. S. Skinner, THIS JOURNAL, 59, 822 (1937).

(2) Tests by Eli Lilly and Company.

(3) A. B. Cox, A. K. MacBeth and S. W. Pennycuik, *J. Chem. Soc.*, 1870 (1931).

TABLE I

BARBITURIC ACIDS



No.	R-	Yield, %	M.p., °C.[c]	Formula	Nitrogen, % Calcd.	Found
I	$ClCH_2$-	60	247–247.5	$C_7H_9O_3N_2Cl$	13.70	13.75
II	H_2NCH_2-	81[b]	233–235	$C_7H_{11}O_3N_3$	22.69	22.57
III	$ClCH_2$—CO—NH-	95[b]	196.5–198	$C_6H_{10}O_4N_3Cl$	16.97	16.71
IV	$ClCH_2$—CO—NH—CH_2-	92[b]	221–222	$C_9H_{12}O_4N_3Cl$	16.06	15.81
V	$ClCH_2$—CO—NH—CH_2CH_2-	70	202–203	$C_{10}H_{14}O_4N_3Cl$	15.24	15.13
VI	H_2N—CH_2—CO—NH-	51	210–212[a]	$C_6H_{12}O_4N_4$	24.56	24.42
VII	H_2N—CH_2—CO—NH—CH_2-	70	228–230[a]	$C_9H_{14}O_4N_4$	23.13	22.93
VIII	H_2N—CH_2—CO—NH—CH_2CH_2-	62	210–212[a]	$C_{10}H_{16}O_4N_4$	21.87	21.71
IX	$CH_3CONHCH_2CONH$-	96	200–201.5[a]	$C_{10}H_{14}O_5N_4$	20.74	20.58
X	$CH_3CONHCH_2CONHCH_2$-	97	225–227[a]	$C_{11}H_{16}O_5N_4$	19.70	19.51
XI	$CH_3CONHCH_2CONHCH_2CH_2$-	95	203–204[a]	$C_{12}H_{18}O_5N_4$	18.78	18.81
XII	$C_2H_5O_2CCH_2NH$-	28	185–187	$C_{10}H_{15}O_5N_3$	16.33	16.78[a]
XIII	$C_6H_5NHCOCH_2NH$-		213–214	$C_{14}H_{16}O_4N_4$	18.41	18.30
XIV	$C_2H_5O_2CCH_2NHCH_2$-	22[b]	180–182	$C_{11}H_{17}O_5N_3$	15.49	15.38[b]
XV	$HOCH_2$-		170–171	$C_7H_{10}O_4N_2$	15.05	15.08

[a] Dec. [b] Based on the portion of the barbituric acid derivative reacting. Mol. wt. calcd. and found (Rast): a, 257, 282; b, 271, 292. [c] Capillary tube method. Melting points corrected.

from water gave the pure substance I. When the reaction mixture was allowed to stand 15 days the yield of I was 31% and the yield of ethoxymethyl derivative (m.p. 167–168°)[4] was 11.5%, m.p. 167–168°. The chloro compound was crystallized from water and the dry residue from the filtrate was crystallized from alcohol to obtain the ethoxy compound.

5-β-Bromoethyl-5-ethylbarbituric acid[5] was prepared as previously reported. To avoid rupture of the barbituric ring it is desirable to get the hydroxy acid into solution at the lowest possible temperature and then let the mixture stand at room temperature, yield 92%.

5-Ethyl-5-hydroxymethylbarbituric Acid.—An ice-cold mixture of 6.0 g. (0.030 mole) of 5-ethyl-5-methoxymethylbarbituric acid and 65 g. of fuming hydrobromic acid (60%) was heated to 45° in a pressure bottle and then allowed to stand at room temperature for two days. Concentration under diminished pressure to a small volume gave 2.7 g. of crystalline product XV which was crystallized from alcohol. It contained no halogen and gave the Dille–Koppanyi test[6] for barbiturates.

Aminobarbituric Acids.—A pressure bottle containing 10.2 g. (0.050 mole) of 5-chloromethyl-5-ethylbarbituric acid and 8.0 g. (0.050 mole) of sodium iodide was cooled in a salt–ice-bath. A solution prepared by saturation of 80 cc. of absolute alcohol with ammonia at −10° was added and the bottle was immediately capped (rubber disc). The bottle was heated slowly in a water-bath to 70° and kept at that temperature for 5 days. After cooling, the precipitate of II was filtered and recrystallized from hot alcohol. From the filtrate 5.3 g. of the unchanged chloro acid was recovered.

From 23.5 g. (0.10 mole) of 5-bromo-5-ethylbarbituric acid and 70 cc. of a saturated solution of ammonia in alcohol heated at 60° for 3 days there was obtained 9.5 g. (56%) of 5-amino-5-ethylbarbituric acid,[7] m.p. 214–216°. The yields were lower for lower temperatures and shorter times of heating.

The yield of 5-β-aminoethyl-5-ethylbarbituric acid by the previously described procedure[5] was 83%.

Chloroacetyl Derivatives of the Aminobarbituric Acids.—To a stirred solution of 3.42 g. (0.020 mole) of 5-amino-5-ethylbarbituric acid in 12 cc. of 2 *N* trimethylamine at 0° was slowly added 3.49 g. (0.030 mole) of chloroacetyl chloride and enough trimethylamine solution to keep the mixture basic. After standing 10 minutes 0.81 g. of the reaction product III precipitated upon acidification with hydrochloric acid. Concentration of the filtrate gave 0.9 g. more. From the mother liquor 2.2 g. of the amino acid was recovered.

To a stirred and cooled solution of 1.85 g. (0.010 mole) of 5-aminomethyl-5-ethylbarbituric acid in 10 cc. of 1 *N* sodium hydroxide at 0° was slowly added 2.26 g. (0.020 mole) of chloroacetyl chloride and enough alkali to keep the solution basic. In a few minutes the odor of chloroacetyl chloride had disappeared. The product IV was then precipitated by acidification with hydrochloric acid and recrystallized from hot water. From the mother liquor 1.0 g. of the amino acid was recovered.

Similarly, using sodium hydroxide, 3.98 g. of 5-β-aminoethyl-5-ethylbarbituric acid gave 3.85 g. of the chloroacetyl derivative V.

Glycyl Derivatives.—In a typical preparation 2.76 g. (0.010 mole) of 5-(chloroacetyl-β-aminoethyl)-5-ethylbarbituric acid was mixed in a pressure bottle at 0° with 25 cc. of alcohol saturated with ammonia at 0°. The mixture was allowed to stand under pressure overnight at room temperature. The product VIII was filtered after cooling in ice and purified by crystallization from alcohol. Melting was accompanied by gaseous decomposition and upon further heating the sealed tube exploded. 5-Ethyl-5-glycylaminobarbituric acid (VI) and 5-ethyl-5-glycylaminomethylbarbituric acid (VII) were obtained similarly.

Acetylglycyl Derivatives.—In a typical experiment a mixture of 50 cc. of dry benzene, 1.14 g. (0.0050 mole) of 5-ethyl-5-glycylaminobarbituric acid and 10 cc. of acetic anhydride was refluxed for 1.5 hours and then cooled in ice. The crude product was filtered, washed with benzene and recrystallized from hot water IX. 5-Acetylglycylaminomethyl-5-ethylbarbituric acid (X) and 5-(β-acetylglycylamino)-ethyl-5-ethylbarbituric acid (XI) were obtained similarly.

Condensations with Glycine Ester.—To a cooled (0°) and stirred solution of sodium ethoxide prepared from 2.53 g. (0.105 mole) of sodium and 50 cc. of absolute alcohol were added 7.67 g. (0.055 mole) of glycine ester hydrochloride and 11.75 g. (0.050 mole) of 5-bromo-5-ethylbarbituric acid. The mixture was kept in an ice–salt-bath for 64 hours. The filtrate from the inorganic salts was concentrated under diminished pressure to crystallization of (XII). When the reactants were kept in an ice-bath for 12 hours and then allowed to stand for 2 days the yield was 21%.

The anilide XIII was prepared by heating the ester with aniline and crystallizing of the product from alcohol.

Similarly, the reaction mixture using 10.23 g. (0.050 mole) of 5-chloromethyl-5-ethylbarbituric acid was kept in an ice–salt-bath for 65 hours and then allowed to stand 30 hours longer at room temperature. Careful fractional crystallization of the product from alcohol gave 8.30 g. of the unchanged chloro compound and 0.42 g. of the desired condensation product XIV.

To a solution of sodium ethoxide at −10° prepared from

(4) A. J. Hill and D. T. Keach, THIS JOURNAL, **48**, 257 (1926).

(5) G. S. Skinner, Arthur Stokes and George Spiller, *ibid.*, **69**, 3083 (1947).

(6) J. M. Dille and T. Koppanyi, *J. Am. Pharm. Assoc.*, **23**, 1079 (1934).

(7) E. Fischer and A. Dilthey, *Ann.*, **335**, 361 (1904).

1.38 g. (0.060 mole) of sodium and 50 cc. of absolute alcohol were added 8.38 g. (0.060 mole) of glycine ester hydrochloride and then 7.89 g. (0.030 mole) of 5-β-bromoethyl-5-ethylbarbituric acid. The mixture was allowed to stand in an ice–salt-bath 12 hours and then to come to room temperature overnight. Concentration of the filtrate followed by recrystallization of the product from alcohol gave 1.8 g. of the product XVI, m.p. 163–165°.

Anal. Calcd. for $C_{26}H_{29}O_8N_5$: mol. wt., 467; N, 14.98. Found: mol. wt. (Rast), 453; N, 14.87.

The anilide XVII was prepared by heating the above ester with freshly distilled aniline, m.p. 197–199°.

Anal. Calcd. for $C_{24}H_{30}O_7N_6$: N, 16.30. Found: N, 16.08.

NEWARK, DELAWARE

[CONTRIBUTION FROM THE STARCH AND DEXTROSE DIVISION, NORTHERN REGIONAL RESEARCH LABORATORY][1]

Isomaltose and Isomaltotriose from Enzymic Hydrolyzates of Dextran

BY ALLENE JEANES, C. A. WILHAM, R. W. JONES, H. M. TSUCHIYA AND C. E. RIST

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Isomaltose (6-[α-D-glucopyranosyl]-D-glucose), and a new sugar which appears to be isomaltotriose (6-α-isomaltopyranosyl-D-glucose), were prepared in yields of 50 and 20%, respectively, from enzymic hydrolyzates of the dextran from *Leuconostoc mesenteroides* NRRL B-512. The dextran was hydrolyzed by culture filtrates from *Penicillium funiculosum*, NRRL strains 1768 and 1132 which had been cultured on a medium containing B-512 dextran. Characterizing data are given for the amorphous sugars and their acetates. Reaction of the sugars with sodium metaperiodate showed that they were composed of aldopyranosidic units linked 1,6. Reaction of the sugar acetates with hydrogen bromide–acetic acid–acetyl bromide was typical of glucopyranosidic units linked α-1,6.

During the past eighty years the name isomaltose has been applied to numerous poorly defined substances or mixtures derived mainly from the action of strong acid on glucose and from enzymic action on starch.[2] In recent years isomaltose has been established definitely as 6-[α-D-glucopyranosyl]-D-glucose through study of the pure sugar and some of its crystalline derivatives. The pure sugar has been obtained in about 5% yield by the action of taka-amylase on amylopectin.[3] Crystalline octaacetyl-β-isomaltose has been obtained from acid hydrolyzates of a bacterial dextran in about 5% yield,[2,4] from acid hydrolyzates of glycogen[5] and of amylopectin[6] in yields of 1 to 2%, and from hydrol in 5% yield.[7] It has also been synthesized from octaacetyl-β-gentiobiose.[8]

Reported here is the direct isolation, for the first time in high yields, of isomaltose and in addition its homolog, the new sugar isomaltotriose.

The present report of the preparation of isomaltose and isomaltotriose in high yields from the dextran from *Leuconostoc mesenteroides* NRRL B-512, is the fruition of a previously stated objective.[9] The respective yields of 50 and 20% from dextran make these two sugars accessible on a preparative basis. The dextran used, having about 95% of its constituent glucopyranosidic units linked α-1,6-,[10,11] is peculiarly suited to the preparation of isomaltose and its homologs. It was degraded by mold enzyme preparations developed especially for this purpose,[12] for at the initiation of this work in 1945, enzymes capable of degrading dextran were not known.

Results and Discussion

Products of Enzyme Action.—Numerous species and strains of *Penicillium* and other molds, when cultured on B-512 dextran, were found to produce exocellular enzymes[12] which degraded this dextran with resulting high yields of isomaltose. Chosen from among these for our present work were the two strains of *Penicillium funiculosum* NRRL 1768 and 1132. Culture filtrates from these molds acted on B-512 dextran to give different distributions of mono-, di- and trisaccharides (Table I). In addition, each culture filtrate also produced a continuous series of higher homologs. As shown in Table I, the amounts of mono-, di- and trisaccharides isolated by carbon column chromatography were in close agreement with the amounts indicated by quantitative paper chromatography.

TABLE I

PROPORTION OF SUGARS IN DEXTRAN HYDROLYZATES

Saccharide	Isolated from columns, %[b] Strain[a] 1768	Strain 1132	By paper chromatography, % Strain 1768	Strain 1132
Mono	3.7	11	..	9
Di	45	53	42	52
Tri	22	7	25	7

[a] NRRL strains of *Penicillium funiculosum*. [b] Per cent. by weight of carbohydrate applied to column.

Identity and Characterization of the Di- and Trisaccharides.—The identity of the disaccharide as isomaltose has been established by the corre-

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

(2) The history of isomaltose has been given by M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **71**, 125 (1949).

(3) (a) Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, *ibid.*, **69**, 2249 (1947); (b) **71**, 1682 (1949).

(4) L. W. Georges, I. L. Miller and M. L. Wolfrom, *ibid.*, **69**, 473 (1947).

(5) M. L. Wolfrom, E. N. Lassettre and A. N. O'Neill, *ibid.*, **73**, 595 (1951).

(6) M. L. Wolfrom, J. T. Tyree, T. T. Galkowski and A. N. O'Neill, *ibid.*, **73**, 4927 (1951).

(7) M. L. Wolfrom, A. Thompson, A. N. O'Neill and T. T. Galkowski, *ibid.*, **74**, 1062 (1952).

(8) B. Lindberg, *Acta Chem. Scand.*, **3**, 1355 (1949); *Nature*, **164**, 706 (1949).

(9) Allene Jeanes, C. A. Wilham and J. C. Miers, *J. Biol. Chem.*, **176**, 603 (1948).

(10) Allene Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(11) M. Abdel-Akher, J. K. Hamilton, R. Montgomery and F. Smith, *ibid.*, **74**, 4970 (1952).

(12) H. M. Tsuchiya, Allene Jeanes, Helen M. Bricker and C. A. Wilham, *J. Bact.*, **64**, 513 (1952).