several minutes to coagulate the gelatinous precipitate. The latter was removed by filtration, washed with water, dried, and recrystallized from the appropriate solvent. Yields were nearly quantitative. Refluxing with phosphorus oxychloride or heating with phosphorus pentachloride for forty-eight hours in an open vessel gave a product in only one case, and then in poor yield, indicating that a closed system was essential.

5-Triarylpyridylmethanes.—The 5-triarylmethyl-2-chloropyridines were reduced to the corresponding pyridines by the same general method, using Raney nickel catalyst in ethanolic potassium hydroxide solution. The reductions were run in very dilute solution and at 70° to effect solution of the reactants and products. A typical run is described.

One gram of 5-triphenylmethyl-2-chloropyridine and 0.50 g. of potassium hydroxide were dissolved in 200 ml. of hot absolute ethanol. One-half gram of Raney nickel was added and the mixture shaken for six hours at 70° under 45 lb. hydrogen pressure. After removing the catalyst by filtration the solution was evaporated until it became cloudy. Upon cooling, white crystals separated. Two recrystallizations from benzene–ethanol mixture (1:1) gave a product melting at 269–270° (cor.).

N-Carboxymethyl-5-triphenylmethyl-2-pyridone.—A suspension of 0.80 g. of 5-triphenylmethyl-2-hydroxypyridine, 0.67 g. of chloroacetic acid and 0.85 g. of potassium hydroxide in 25 ml. of absolute ethanol was refluxed for six hours. After removal of precipitated potassium chloride by filtration, the resulting solution was diluted with 25 ml. of water and acidified with hydrochloric acid. The bulky white precipitate which formed was removed by filtration, washed well on the filter with water and dried; yield, 0.84 g. (92%). Two recrystallizations from dioxane gave microscopic white crystals, m.p. 264–266° (cor.) with decomposition. The product forms an emulsion with aqueous 10% sodium hydroxide but dissolves upon the addition of an equal volume of ethanol.

Anal. Calcd. for $C_{26}H_{21}O_3N$: C, 78.97; H, 5.35. Found: C, 79.04; H, 5.04.

Summary

1. The Fries rearrangement has been applied to the O-benzoate of 2-hydroxypyridine. A very small yield of 5-benzoyl-2-hydroxypyridine resulted. The structure of this latter product was determined by an unequivocal synthesis; methyl coumalinate was converted by ammonia followed by alkali to 2-hydroxypyridine-5-carboxylic acid which was transformed into the acid chloride and condensed with benzene in the presence of aluminum chloride.

2. Triphenylchloromethane and diphenylxenylchloromethane condense with 2-hydroxypyridine and 3-methyl-2-hydroxypyridine to give the corresponding 5-triarylmethyl-2-hydroxy- or 5-triarylmethyl - 2 - hydroxy - 3 - methylpyridines. Similar condensations occur with the triarylcarbinols and the 2-hydroxypyridines in presence of a few drops of sulfuric acid. With phosphorus oxychloride the triarylmethyl hydroxypyridines are converted to the chloropyridines which upon reduction yield triarylpyridylmethanes. Triphenylpyridylmethane resembles tetraphenylmethane in physical properties and in its phosphorescence after illumination with ultraviolet light.

3. 6-Methyl-2-hydroxypyridine condenses with triphenylchloromethane or the carbinol in low yields to give a product, presumably 3-triphenylmethyl-6-methyl-2-hydroxypyridine since it is alkali-insoluble and does not react with phosphorus oxychloride.

URBANA, ILLINOIS RECEIVED AUGUST 2, 1948

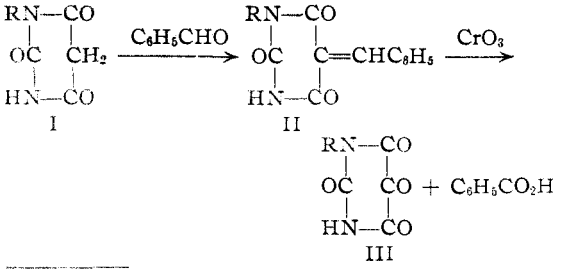
[CONTRIBUTION FROM THE SECTION OF PHARMACOLOGY, THE HEBREW UNIVERSITY AND HADASSAH]

Preparation and Properties of New Derivatives of Alloxan

BY G. BRÜCKMANN[1] AND S. D. ISAACS

The discovery by Shaw-Dunn and co-workers[2] that alloxan causes diabetes in experimental animals by selective destruction of the insulin-producing pancreatic islet cells has evoked considerable interest. An investigation undertaken in this Laboratory of the structural specificity of the effect required the preparation of a number of new members of the series, since the only previously described N-substituted alloxans are the 1-methyl,[3] 1-ethyl,[4] 1-phenyl,[5] 1,3-dimethyl,[6] 1,3-diethyl[7] and 1-methyl-3-ethyl[8] derivatives.

For preparation of several additional derivatives of interest for pharmacological study, an N-substituted urea was first condensed with malonic acid in the presence of acetic anhydride,[9] or with malonic ester in the presence of sodium methoxide (advantageous in the preparation of 1-phenylbarbituric acid). In accordance with the method of Biilman and Berg,[10] the resulting N-substituted barbituric acid (I) was then converted into the benzal derivative (II) and this was oxidized with chromic acid to the alloxan (III) and benzoic



(1) Dr. Brückmann was killed during the War in Israel. The revised version of the paper was prepared by L. F. Fieser.—*The Editor.*

(2) Shaw-Dunn, Sheehan and McLetchie, *Lancet*, **1**, 484 (1943).

(3) Fischer and Clemm, *Ber.*, **30**, 3090 (1897).

(4) Biltz and Sedlatschek, *ibid.*, **57**, 175 (1924).

(5) Winslow, THIS JOURNAL, **61**, 2089 (1939).

(6) Biltz, *Ber.*, **45**, 3659 (1912).

(7) Sembritzki, *ibid.*, **30**, 1821 (1897).

(8) Biltz and Max, *Ann.*, **414**, 95 (1917).

(9) Biltz and Wittek, *Ber.*, **54**, 1035 (1921).

(10) Biilman and Berg, *ibid.*, **63**, 2188 (1930).

acid. The benzal derivative can be prepared from the reaction mixture containing the barbituric acid without isolation of this intermediate. The alloxans are not very soluble and can be isolated from the oxidation reaction mixture, but in most cases it is preferable to reduce the crude product with stannous chloride and to isolate the resulting alloxantin, which subsequently can be reoxidized to the alloxan with nitric acid. A number of new N-alkyl and N-aryl alloxans and alloxantins were prepared by this process. When dried *in vacuo* over sulfuric acid, the alloxans invariably retained one molecule of water, whereas the alloxantins were obtained anhydrous or as mono- or dihydrates.

Attempts to prepare 2-thioalloxan by the method of Biilman and Berg were no more successful than the attempts of Robinson and Tomlinson[11] to prepare the compound by other methods. The substance apparently is unstable, possibly because of tautomerism to the sulfhydryl derivative and subsequent oxidation.

Aqueous solutions of the new alloxans all exhibited the following typical reactions in varying degree of intensity: (a) murexide formation in contact with the skin or when heated with α-amino acids; (b) reduction by ascorbic acid to a dialuric acid, as indicated by the formation of a violet precipitate on addition of barium hydroxide; (c) transient blue color on addition of traces of ferrous sulfate and alkali; (d) reaction with hydroxylamine hydrochloride to give a violuric acid recognizable from the intense, stable blue color resulting on the addition of ferrous sulfate.

Since a reaction analogous to the known ready decomposition of alloxan by alkali to alloxanic acid may possibly play a role in the inactivation of alloxan in the body, we compared various alloxan derivatives with respect to their stability to alkali. Known amounts of the compounds were added to 5-cc. portions of Krebs–Ringer phosphate buffer (*p*H 7.3) and the alloxan content of the solution was determined at two-minute intervals by the colorimetric method recently described.[12] The decomposition curves for the various alloxans studied were of similar shape and the percentages of unaltered material remaining after two minutes were as follows: alloxan, 68%; butylalloxan, 65%; dimethylalloxan, 34%; benzylalloxan, 29%; phenylalloxan, 12%. Similar figures were obtained when sodium hydroxide was used for alkalinization instead of phosphate buffer. Comparative determinations of the rate of α-amino acid oxidation by the same compounds have indicated that the order of decreasing activity is as follows: alloxan, butyl-, propyl-, methyl-, ethyl-, benzyl-, dimethyl- and phenylalloxan.[13] It is thus evident that high stability toward alkali corresponds to high potency in amino acid oxidation.

(11) R. Robinson and Tomlinson, *J. Chem. Soc.*, 467 (1935).

(12) Brückmann, *J. Biol. Chem.*, **165**, 103 (1946).

(13) Brückmann and Wertheimer, *ibid.*, **168**, 241 (1947).

The toxicity of the N-alkylalloxans increases sharply with increasing length of the aliphatic side chain. The disubstituted alloxans are particularly toxic, whereas phenylalloxan is practically non-toxic. Methyl-, ethyl- and propylalloxan possess diabetogenic activity.[13]

Experimental[14]

1-*n*-Propylbarbituric Acid.—A mixture of carefully dried malonic acid (15 g.) and N-propylurea (13 g.) in glacial acetic acid (34 cc.) was heated in an oil-bath maintained at 70–80° and acetic anhydride (34 cc.) was added in the course of ninety minutes. The temperature was then raised to 90° for three hours, the red-brown mixture was cooled, treated with a little water (6 cc.), and concentrated in vacuum at 60° to a sirup. This was dissolved in hot ethanol, and the solution on cooling deposited 7.7 g. of crude yellow crystals. Repeated crystallization from water gave white needles of product, m. p. 104° (sparingly soluble in water).

Anal. Calcd. for $C_7H_{10}O_3N_2$: N, 16.48. Found (Kjeldahl): N, 16.08.

Treatment of the mother liquors with benzaldehyde (5 cc.) afforded 8 g. of the benzal compound.

1-*n*-Propyl-5-benzalbarbituric Acid.—Benzaldehyde (1.5 cc.) was added slowly to a refluxing solution of crude propylbarbituric acid (2.1 g.) in ethanol (12 cc.) and after one-half hour the mixture was cooled and the crystalline product collected and washed with alcohol and ether. The first crop of 2.2 g. of yellow plates, m. p. 168°, was directly pure; a further 0.3 g. was obtained from the mother liquor.

Anal. Calcd. for $C_{14}H_{14}O_3N_2$: N, 10.85. Found (Kjeldahl): N, 10.54.

Di-(1-*n*-propyl)-alloxantin.—1-*n*-Propyl-5-benzalbarbituric acid (2.6 g.) was added in small portions in the course of thirty minutes to a solution of chromic anhydride (2 g.) in water (0.7 cc.)–acetic acid (7 cc.) at 50–60°. The solution was cooled, treated with water (5 cc.) and hydrochloric acid (3.5 cc.), left for one hour at 0°. The benzoic acid that separated was removed by filtration and the filtrate was treated with stannous chloride (2 g.) dissolved in hydrochloric acid (2 cc.). The alloxantin that separated when washed with warm water until colorless weighed 1.4 g. Recrystallization from water (sparingly soluble) gave hexagonal plates, m. p. 214°, dec.

Anal. Calcd. for $C_{14}H_{18}O_8N_4 \cdot H_2O$: N, 14.42. Found (Kjeldahl): N, 14.36.

1-*n*-Propylalloxan.—A suspension of the above alloxantin (1 g.) in water (5 cc.) was treated at about 50° with concentrated nitric acid, added by drops, until the solid had dissolved. The solution, filtered if necessary, was kept in a desiccator over sulfuric acid until the product crystallized. The alloxan separated in small plates, m. p. 122° dec. The first crop amounted to 0.15 g.; a further crop of 0.16 g. was obtained from the mother liquor.

Anal. Calcd. for $C_7H_{10}O_5N_2$: N, 13.86. Found (Kjeldahl): N, 14.22.

1-*n*-Butylbarbituric Acid.—N-Butylurea (4.4 g.) was condensed with malonic acid (4.4 g.) in acetic acid (10 cc.) –acetic anhydride (10 cc.) by the procedure described above. Crystallization of the reaction product from ethanol gave yellowish needles, m. p. 136–140°, very sparingly soluble in water.

(14) In the alloxan series melting points are not very characteristic and in most cases represent temperatures of decomposition that depend upon such factors as the rate of heating and the nature of the capillary tube. Kjeldahl nitrogen determinations gave satisfactory results for all compounds studied except 1-phenylalloxan, for which the figures found for nitrogen were consistently low, even when copper sulfate was replaced by mercuric oxide, as recommended for the analysis of lysine by Miller and Houghton, *ibid.*, **165**, 103 (1946). Satisfactory results were obtainable only by the micro-Dumas method.

Anal. Calcd. for $C_8H_{12}O_3N_2 \cdot 2H_2O$: N, 12.72. Found (Kjeldahl): N, 12.31.

1-*n*-Butyl-5-benzalbarbituric acid was prepared without isolation of the butylbarbituric acid by the procedure described for the propyl homolog. The total yield of satisfactory product (two crops) from 4.4 g. of N-butylurea was 3.9 g. The substance crystallized in yellow plates, m. p. 154–156°.

Anal. Calcd. for $C_{15}H_{16}O_3N_2$: N, 10.28. Found (Kjeldahl): N, 9.99.

Di-(1-*n*-Butyl)-alloxantin.—Oxidation of 3.8 g. of the benzal compound and reduction of the crude product by the procedure described above afforded 1.8 g. of crude alloxantin. Crystallization from water afforded small white plates, m. p. 204°, dec.

Anal. Calcd. for $C_{16}H_{22}O_8N_4$: N, 14.13. Found (Kjeldahl): N, 13.89.

1-*n*-Butylalloxan.—Nitric acid oxidation of the alloxantin (1 g.) gave a first crop of 0.7 g. of product and 0.1 g. from the mother liquor. The substance crystallized in thin plates, m. p. 106–109°.

Anal. Calcd. for $C_8H_{12}O_5N_2$: N, 12.96. Found (Kjeldahl): N, 13.00.

1-Isobutylbarbituric Acid.—The sirup resulting from evaporation of the reaction mixture from isobutylurea (3 g.), malonic acid (3 g.), acetic acid (6.5 cc.) and acetic anhydride (6.5 cc.) when mixed with hot ethanol (7 cc.) afforded 1.8 g. of crystalline product. Crystallization from alcohol gave slightly pink plates, m. p. 138–140°, insoluble in water.

Anal. Calcd. for $C_8H_{12}O_3N_2$: N, 15.21. Found (Kjeldahl): N, 15.90.

1-Isobutyl-5-benzalalloxan.—Condensation of 1.5 g. of the barbituric acid with benzaldehyde (1.5 cc.) in ethanol (15 cc.) yielded 1.3 g. of yellow plates, m. p. 177–178°.

Anal. Calcd. for $C_{15}H_{16}O_3N_2$: N, 10.28. Found (Kjeldahl): N, 10.36.

Di-(1-isobutyl)-alloxantin.—The benzal compound (2.3 g.) was oxidized with chromic anhydride (1.7 g.) in acetic acid (5 cc.), and the alloxantin was obtained by addition of water (12 cc.) and stannous chloride (1 g.)-hydrochloric acid (1 cc.). The product separated as white plates (0.3 g.), m. p. 214–216°, moderately soluble in water.

Anal. Calcd. for $C_{16}H_{22}O_8N_4 \cdot 2H_2O$: N, 12.96. Found (Kjeldahl): N, 12.61.

1-Isobutylalloxan was obtained directly by cooling the above-described oxidation mixture; yield 0.5 g. (crude). Crystallization afforded white plates, m. p. 145–147°, dec.

Anal. Calcd. for $C_8H_{12}O_5N_2$: N, 12.96. Found (Kjeldahl): N, 12.70.

1-Phenylbarbituric Acid.—The method used was similar to that of Aspelund and Lindh,[15] but ethanol was replaced by methanol, as follows. To a cooled solution of sodium (4.2 g.) in absolute methanol (50 cc.), a solution of diethyl malonate (35 g.) and phenylurea (29 g.) in hot methanol (50 cc.) was added. The mixture was heated under reflux at 70–80° for six hours and poured into hydrochloric acid (25 cc.) and water (120 cc.) and cooled. The yield of the barbituric acid, washed with ethanol and ether, was 25 g.; recrystallized material melted at 262°.

1-Phenyl-5-benzalbarbituric Acid.—The condensation of crude phenylbarbituric acid (24 g.) with benzaldehyde (16 cc.) in boiling ethanol (180 cc.) afforded 31 g. of yellow, sharp-edged needles, m. p. 237–238°.

1-Phenylalloxan.[16]—The benzal compound (2.5 g.) was added in the course of twenty minutes to a mixture of chromic anhydride (3 g.), water (2.5 cc.) and acetic acid (13 cc.) at a temperature of 50–60°. The alloxan separated in a stiff paste and was collected and washed on the filter with acetic acid until the washings were nearly colorless. The product was dissolved in a little hot water and the solution treated with acetic acid and cooled, when 1.2 g. of silky white needles separated. The substance, m. p. 158°, is very soluble in water, ethanol or hot acetic acid and is sparingly soluble in chloroform.

Anal. Calcd. for $C_{10}H_8O_5N_2$: N, 11.87. Found (Dumas): N, 11.66.

Reduction with stannous chloride afforded the alloxantin (white needles from water, m. p. 255° dec.) described by Winslow.[16]

1-Benzylbarbituric acid was prepared by condensing benzylurea (2.9 g.) with malonic acid (2.2 g.) in acetic acid (5 cc.)-acetic anhydride (5 cc.). The crude product (2 g.) on crystallization from ethanol gave white prisms, m. p. 146–150°.

Anal. Calcd. for $C_{11}H_{10}O_3N_2$: N, 12.84. Found (Kjeldahl): N, 13.30.

1-Benzyl-5-benzalbarbituric acid was prepared from the barbituric acid (1.8 g.) by the usual process, yield 2.2 g. The substance formed thin yellow prisms, m. p. 200–204°.

Anal. Calcd. for $C_{18}H_{14}O_3N_2$: N, 9.15. Found (Kjeldahl): N, 9.08.

Di-(1-benzyl)-alloxantin was prepared by the general procedure described; yield 1 g. from 2 g. of benzal compound. Crystallization from hot water gave small needles, m. p. 199°.

Anal. Calcd. for $C_{22}H_{18}O_8N_4 \cdot H_2O$: N, 11.58. Found (Kjeldahl): N, 11.73.

1-Benzylalloxan.—The alloxantin (1.2 g.) was warmed with nitric acid until dissolved, and the white crystals that separated on cooling were washed with water and with chloroform; yield 0.7 g. The product, m. p. 140–143°, is soluble in acetone, ethanol, or ether, and sparingly soluble in water.

Anal. Calcd. for $C_{11}H_{10}O_5N_2$: N, 11.20. Found (Kjeldahl): N, 11.20.

Summary

A number of new derivatives of alloxan and alloxantin have been synthesized. In the alloxan series, ability to oxidize α-amino acids runs parallel to the stability of the compounds to decomposition by alkali.

JERUSALEM, PALESTINE RECEIVED JULY 31, 1948

(15) Aspelund and Lindh, *Acta Acad. Aboensis Math. et Phys.*, **11**, No. 10 (1938).

(16) In preparing this paper for publication we found that this compound has been prepared by a different method by Winslow, THIS JOURNAL, **61**, 2089 (1939).