erlenmeyer flask which was placed for two minutes in an oil-bath at 125°. The melt was taken up in aqueous sodium carbonate. From the filtrate on gradual acidification was obtained the half amide, 0.62 g. A reprecipitation through the sodium salt gave crystals, m.p. 147–149°.

Anal. Calcd. for $C_{14}H_{16}O_3N_2$: C, 64.58; H, 6.20. Found: C, 64.26; H, 6.26.

Longer heating favored formation of the succinimide derivative, isolated as a carbonate insoluble fraction, m.p. 198–199° (from ethanol).

Anal. Calcd. for $C_{14}H_{14}O_2N_2$: C, 69.38; H, 5.82. Found: C, 69.22; H, 6.05.

NEW YORK, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

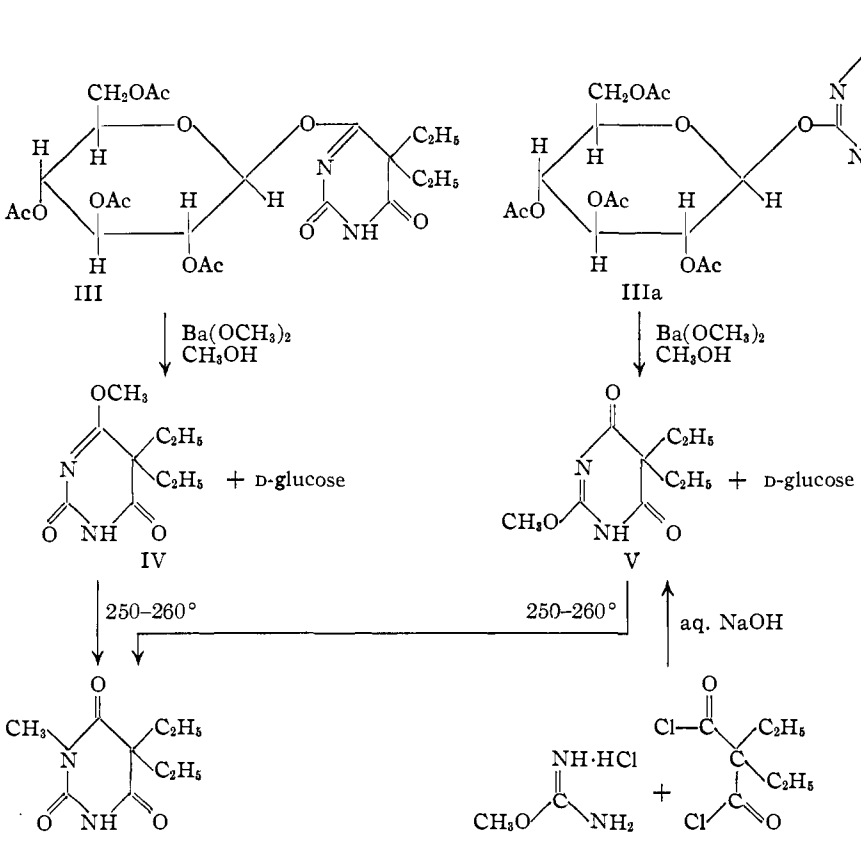
Preparation and Characterization by Alkaline Methanolysis of 5,5-Diethyl-4-(tetraacetyl-β-D-glucosyloxy)-2,6(1,5)-pyrimidinedione[1]

BY JACK A. SNYDER AND KARL PAUL LINK

RECEIVED NOVEMBER 15, 1952

The synthesis and alkaline methanolysis of 5,5-diethyl-4-(tetraacetyl-β-D-glucosyloxy)-2,6-(1,5)pyrimidinedione (III) is reported. The position of the glucosidic linkage was determined by comparison of the product of alkaline methanolysis, 5,5-diethyl-4-methoxy-2,6(1,5)-pyrimidinedione (IV), with 5,5-diethyl-2-methoxy-4,6(1,5)-pyrimidinedione (V) synthesized from diethylmalonyl chloride and O-methylisourea hydrochloride. The ultraviolet absorption spectra of IV and III are similar, but differ from that of V, indicating that rearrangement does not occur during the alkaline methanolysis of III.

Ballou and Link[2] found the ultraviolet absorption spectra of theobromine β-D-glucoside tetraacetate (I)[3] and the methoxy-3,7-dimethylpurine (II) formed by alkaline methanolysis of I to be similar (maxima at 245 and 295 mμ in methanol), but distinctly different from the spectra of theobromine and caffeine (maxima at 275 mμ). From this they concluded that the methoxyl group in II occupied the position at which the sugar molecule was attached, and because it was not known whether the glucosidic linkage in I was to position 2 or 6 of the aglucon, they suggested determination of the structure of II as a means of indicating the correct position of the linkage.



This method was applied to the characterization of 5,5-diethyl-4-(tetraacetyl-β-D-glycosyloxy)-2,6-(1,5)-pyrimidinedione (III), in which the glucosidic linkage might be to either the 2 (IIIa) or 4 (III) position. Because of the method of its preparation, III is assumed to be a β-glucoside. It is alkali-sensitive, and undergoes alkaline methanolysis in a manner similar to I.

Attempts to prepare III from the silver salt of barbital (5,5-diethylbarbituric acid) and tetraacetyl-D-glucosyl bromide in the method for the preparation of I, and from IV and barbital in the usual Koenigs–Knorr procedure were unsuccessful. The use of a catalytic amount of quinoline in the Koenigs–Knorr reaction, a modification of the Robertson procedure[4] introduced by Huebner, *et al.*,[5] gave the desired product. This modification has been used to prepare enol-glycosides of 4-hydroxycoumarins, β-keto esters, β-diketones and β-keto anil-

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(2) C. E. Ballou and K. P. Link, THIS JOURNAL, **71**, 3743 (1949).

(3) E. Fischer and B. Helferich, *Ber.*, **47**, 210 (1914).

(4) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 2729 (1930).

(5) C. F. Huebner, S. A. Karjala, W. R. Sullivan and K. P. Link, THIS JOURNAL, **66**, 906 (1944).

ides,[5-8] but it has not previously been used for synthesis of glycosides of enolic pyrimidines. In the synthesis of III it results in the formation of an enol-type lactim ether glucosidic linkage.

This is a new demonstration of the wide applicability of this method to enol-glycoside synthesis. It is successful in many condensations where the Michael synthesis and the silver salt method fail. Although it characteristically affords low yields of the desired product, it should be a valuable addition to the limited number of methods available.

The product of the alkaline methanolysis of III, 5,5-diethyl-4-methoxy-2,6(1,5)-pyrimidinedione (IV), was isolated and compared with 5,5-diethyl-2-methoxy-4,6(1,5)-pyrimidinedione (V) synthesized from O-methylisourea hydrochloride and diethylmalonyl chloride by the method of Baeyer and Co.[9] Under identical conditions IV and V were converted to N-methylbarbital (VI) by an imidoester rearrangement,[10] and were hydrolyzed to barbital with 15% hydrochloric acid. However, the melting point of a mixture of IV and V was greatly depressed, and IV (no absorption maximum above 220 mμ) and V ($\epsilon_{273\ m\mu}$ 0.898 × 10^4) differed widely in their absorption spectra in methanol.

Only two methyl ethers of the aglucon are possible, and on the above evidence of the non-identity of IV and V it was concluded that the methoxyl group in IV occupied position 4. Assuming that no rearrangement occurs during methanolysis, the glucosidic linkage in III is likewise to position 4. This assumption is supported by the similarity of the ultraviolet absorption spectra of III and IV.

Experimental

Preparation of 5,5-Diethyl-4-(tetraacetyl-β-D-glucosyloxy)-2,6(1,5)-pyrimidinedione (III).—A mixture of 41.1 g. (0.10 mole) of tetraacetyl-D-glucosyl bromide, 18.5 g. (0.10 mole) of barbital (U.S.P.) and 41 g. of Drierite was shaken in 400 ml. of dry ether with 17.4 g. (0.15 equiv.) of silver oxide and 0.9 ml. of quinoline. The bromide ion test with alcoholic silver nitrate became negative in 20 hours.

The suspension was filtered through a layer of carbon on Celite 545, the solids were washed with three 100-ml. portions of chloroform, and the filtrate and washings were concentrated *in vacuo* (50° bath). The light yellow sirup obtained was taken up in 150 ml. of warm ether, set aside at 5° overnight and the gummy, crystalline mass which separated was filtered and washed with two 50-ml. portions of ether. The yield was 6.57 g., m.p. 143–147°. This was taken up in 100 ml. of warm methanol, decolorized with carbon, and 100 ml. of water was added. Fine, bunched needles began to separate immediately on cooling to room temperature. The mixture was placed at 5° overnight, and was then filtered and washed with 100 ml. of water. The yield was 4.07 g., m.p. 158–164°, $[\alpha]^{25}D$ +8.18° (*c* 1.223, chloroform). Five additional recrystallizations[11] from 1:1 methanol–water gave 1.35 g. of long needles, m.p. 178–178.3°, $[\alpha]^{27}D$ +8.33° (*c* 1.020, chloroform), $[\alpha]^{27}D$ −2.40° (*c* 1.042, pyridine), $[\alpha]^{30}D$ −7.65° (*c* 1.502, methanol).

Anal. Calcd. for $C_{22}H_{30}O_{12}N_2$: C, 51.35; H, 5.88; N, 5.45. Found: C, 51.33; H, 6.05; N, 5.77.

Methanolysis of III. Isolation of 5,5-Diethyl-4-methoxy-2,6(1,5)-pyrimidinedione (IV).—A 3.729-g. sample of III was dissolved in 200.0 ml. of dry methanol. The optical rotation of this solution was −0.28° (22°, 2 dcm.). To this solution was added 1.50 ml. of 0.462 *N* barium methoxide in methanol, and the rotation was observed until it stopped changing after 5.5 days, at the value +0.71° (25°, 2 dcm.). Complete conversion of the glucoside to free glucose would give a value of +0.86° (25°, 2 dcm.). To this was added 6.00 ml. of 0.115 *N* sulfuric acid, the barium sulfate was removed by filtering through Celite 545 and the filtrate was concentrated *in vacuo* (35° bath) to a colorless oil. This was taken up in 10 ml. of water, extracted with three 50-ml. portions of ether, and the ether extracts were combined and extracted with two 10-ml. portions of water. The water extracts were combined and saved for subsequent isolation of D-glucose. The ether extract was dried with anhydrous sodium sulfate and concentrated to dryness *in vacuo* (35° bath). After drying at 10 mm. over calcium chloride for 18 hours, 1.12 g. of crystals was obtained, m.p. 112–116°.

This was taken up in 10 ml. of chloroform, 20 ml. of Skellysolve B was added, and the mixture was placed at 5° overnight. On filtration, 0.071 g. of fine needles was obtained, m.p. 180–184°. The melting point of a mixture of this product with authentic barbital showed no depression. The filtrate was concentrated to dryness *in vacuo* (35° bath), the crystalline material was taken up in 12 ml. of absolute ether, 24 ml. of Skellysolve B was added, and the mixture was placed at 5° overnight. The long needles were filtered and washed with 5 ml. of Skellysolve B. The yield was 0.375 g., m.p. 128–130°. Another recrystallization from 2:1 Skellysolve B–ether gave 0.276 g. of IV, m.p. 130–131°.

Anal. Calcd. for $C_9H_{14}O_3N_2$: C, 54.6; H, 7.12; N, 14.14. Found: C, 54.7; H, 7.05; N, 13.94.

The melting points of mixtures of IV with authentic samples of barbital, N-methylbarbital, and 5,5-diethyl-2-methoxy-4,6(1,5)-pyrimidinedione (V) were greatly depressed.

Isolation of D-Glucose from the Alkaline Methanolysis of III.—The combined water extracts from the above preparation were concentrated to 1 ml. in an air stream and 20 ml. of absolute ethanol was added. The mixture was kept at room temperature until crystallization began (36 hours) and then placed at 5° for 4 weeks. The crystals were filtered, washed with two 5-ml. portions of absolute ethanol, and dried 12 hours at 10 mm. and 56° over phosphorus pentoxide. The yield was 0.702 g., m.p. 140–142°, $[\alpha]^{22}D$ +51.6° (*c* 4.139, water). The melting point of a mixture with authentic α-D-glucose was not depressed.

Preparation of 5,5-Diethyl-2-methoxy-4,6(1,5)-pyrimidinedione (V).—The procedure of Baeyer and Co.[9] for synthesis of V (reported m.p. 130–131°) was employed without success. However, long shaking with subsequent neutralization of the primary reaction product with carbonic acid gave the desired product, m.p. 136–137.5°.

To a cold (5°) solution of 5.0 g. of diethylmalonyl chloride in 30 ml. of dry benzene, was added a cold solution of 3.5 g. of O-methylisourea hydrochloride[12] in 5 ml. of water. The mixture was placed in an ice-bath, and 11.25 g. of 27% sodium hydroxide was added dropwise with continual shaking over the course of 10 minutes. Shaking was continued for 18 hours. The mixture was then concentrated *in vacuo* (35° bath) until crystals appeared. These were filtered (yield 2.63 g.), dissolved in 20 ml. of water, and carbon dioxide was passed into the solution for 4 hours. The mixture was extracted with four 50-ml. portions of ether. The ether extracts were combined, washed with two 10-ml. portions of water, dried with anhydrous sodium sulfate, and concentrated *in vacuo* (35° bath) to a colorless sirup. This was taken up in 10 ml. of ether, 20 ml. of Skellysolve B was added, and the mixture was left at room temperature for 4 hours until crystallization began. It was then placed at 5° overnight, filtered and dried *in vacuo* 18 hours over phosphorus pentoxide. The yield was 0.550 g., m.p. 131–134°.

(6) L. Spero, C. E. Ballou and K. P. Link, THIS JOURNAL, **71**, 3740 (1949).

(7) C. E. Ballou and K. P. Link, *ibid.*, **72**, 3147 (1950).

(8) C. E. Ballou and K. P. Link, *ibid.*, **73**, 1134 (1951).

(9) Baeyer and Co., *Frdl.*, **11**, 929 (1912–1914); German Patent 249,907, F 32,009.

(10) G. E. Hilbert and T. B. Johnson, THIS JOURNAL, **52**, 2001 (1930).

(11) It was noted that agitation of the massecuite caused the separation of microcrystals of an impure material with the long needles. If the massecuite was protected from agitation during crystallization and the supernatant liquid was quickly decanted through a buchner funnel, the crystalline form could be collected on the filter, and the microcrystalline material separated rapidly from the filtrate. This could be recrystallized to give a product identical with III, so it is assumed that agitation favors coprecipitation of unreacted barbital with the desired product.

(12) J. Stieglitz and R. H. McKee, *Ber.*, **33**, 807 (1900).

Another recrystallization from 2:1 Skellysolve B–ether gave 0.251 g. of long needles, m.p. 136–137.5°.

Anal. Calcd. for $C_9H_{14}O_3N_2$: C, 54.6; H, 7.12; N, 14.14. Found: C, 54.6; H, 7.10; N, 13.93.

The melting points of mixtures of V with authentic samples of barbital and N-methylbarbital were greatly depressed.

Rearrangement of IV and V to N-Methylbarbital.—Samples of IV (0.116 g.) and V (0.110 g.) were heated at 250–260° for 6 hours in sealed tubes evacuated to 1 mm. The nearly colorless liquids obtained crystallized on cooling. These were sublimed at 155° and 6 mm., and recrystallized from water. The yield from rearrangement of IV was 0.044 g., m.p. 148–150°, and from the rearrangement of V was 0.046 g., m.p. 150–151°.

Anal. Calcd. for $C_9H_{14}O_3N_2$: C, 54.6; H, 7.12; N, 14.14. Found for N-methylbarbital from IV: C, 54.6; H, 7.12; N, 14.42. Found for N-methylbarbital from V: C, 54.6; H, 7.10; N, 13.86.

The melting points of mixtures of the products from IV and V, and of each with authentic N-methylbarbital showed no depression.

Conversion of IV and V to Barbital.—Samples of IV (0.071 g.) and V (0.067 g.) were dissolved in 2 ml. of water with gentle heating. Two ml. of 30% hydrochloric acid was added, the mixtures were cooled to room temperature and then placed at 5° overnight. The crystals were filtered, washed with two 1-ml. portions of water, dried 12 hours *in vacuo* over potassium hydroxide and sublimed at 150° and 7 mm. The yield from IV was 0.048 g., m.p. 185–186°. The yield from V was 0.047 g., m.p. 184–185°.

Anal. Calcd. for $C_8H_{12}O_3N_2$: C, 52.2; H, 6.57; N, 15.19. Found for barbital from IV: C, 52.4; H, 6.54; N, 14.94. Found for barbital from V: C, 52.4; H, 6.59; N, 15.19.

The melting points of mixtures of the products from IV and V, and of each with authentic barbital showed no depression.

Acknowledgment.—We wish to thank our colleague, Dr. C. E. Ballou, for his kindness in reviewing the manuscript prior to its submission. We are also indebted to Mr. Tien-Hui Lin for his preparation of III in the quantity necessary for this study, and to Mr. Collin H. Schroeder for all carbon, hydrogen and nitrogen microanalyses.

MADISON 6, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Studies on 4-Hydroxycoumarins. XII. 3-Substituted-aminomethyl-4-hydroxycoumarin Derivatives by the Mannich Reaction[1]

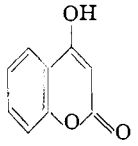
BY DALE N. ROBERTSON AND KARL PAUL LINK

RECEIVED NOVEMBER 22, 1952

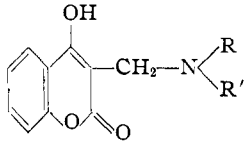
A series of 3-substituted-aminomethyl-4-hydroxycoumarins has been synthesized by an application of the Mannich reaction. A number of secondary amines gave the amine salts of 3,3′-methylenebis-4-hydroxycoumarin (III) (Dicumarol ®) instead of the expected Mannich products. Regeneration of 3,3′-methylenebis-4-hydroxycoumarin from the amine salts was effected by acidification with dilute hydrochloric acid at 25°. By heating an aqueous acid solution of 3-piperidinomethyl-4-hydroxycoumarin (IV) at 100° for 24 hours, III was obtained in essentially quantitative yield (97%). A mechanism for the conversion of IV to III is proposed.

Previous papers in this series have demonstrated the reactivity of the 3-position of 4-hydroxycoumarin (I) in the aldol[2] and Michael[3] addition reactions.

We have employed the Mannich reaction to obtain the 3-aminomethyl derivatives (II) of I.



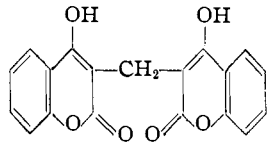
I



II

where R is hydrogen or alkyl and R′ is alkyl or aralkyl, or where –NRR′ makes up a heterocyclic ring

Attempts to prepare the desired compounds from paraformaldehyde and the amine hydrochlorides did not give the Mannich base, but exclusively the bis product, 3,3′-methylenebis-4-hydroxycoumarin (III) (Dicumarol®). Since the reaction between I and formaldehyde to form III is very rapid, the order in which the reactants are added is important. In this work, a solution of the amine (1.25 moles)



III

and formaldehyde[4] (1.00 mole) in absolute ethanol was poured at room temperature into a solution of I (1.00 mole) in absolute ethanol. The procedure of Leffler and Hathaway[5] for the preparation of 2-hydroxy-3-substituted-aminomethyl derivatives of naphthoquinone in which the amine and lawsone (2-hydroxy-1,4-naphthoquinone) are mixed prior to addition of the formaldehyde also gave satisfactory results.

The reaction of 4-hydroxycoumarin (I) with primary amines and formaldehyde proceeded very rapidly. The 3-substituted-aminomethyl-4-hydroxycoumarins separated from the reaction mixture in an analytically pure crystalline state. An additional 10 to 15% of the products could be realized by diluting the mother liquors with absolute ether.

In contrast to the other products, 3-isobutylaminomethyl-4-hydroxycoumarin and 3-benzylaminomethyl-4-hydroxycoumarin underwent slight

(1) Published with the approval of the Director of the Wisconsin Agricultural Experimental Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

(2) W. R. Sullivan, C. F. Huebner, M. A. Stahmann and K. P. Link, THIS JOURNAL, **65**, 2288 (1943).

(3) M. Ikawa, M. A. Stahmann and K. P. Link, *ibid.*, **66**, 902 (1944).

(4) Formalin—37% formaldehyde.

(5) M. T. Leffler and R. J. Hathaway, THIS JOURNAL, **70**, 3222 (1948).