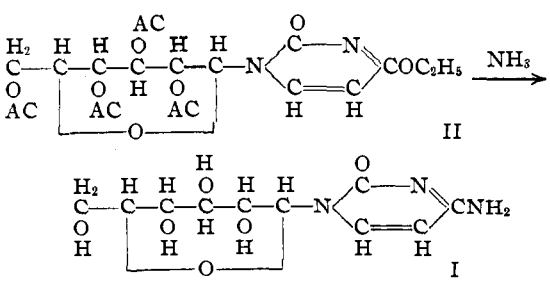
[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

Synthesis of 1-*d*-Glucosidocytosine

BY GUIDO E. HILBERT AND EUGENE F. JANSEN

Previous attempts to prepare analogs of cytidine, as the 1-glycosidocytosines, have been unsuccessful. Fischer[1] in an investigation of the reaction between acetobromoglucose and the silver salt of cytosine isolated a relatively unstable amorphous product that was not characterized. Levene and Sobotka[2] studied the interaction of acetobromoxylose and the silver salt of cytosine but they did not secure a condensation product containing nitrogen. More recently Hilbert[3] failed to attach glucose to the 1-position of cytosine by the interaction of acetobromoglucose and 4-amino-2-methoxypyrimidine.

The observation[4] that alkoxy groups in the 2- and 4-positions of the pyrimidine ring are labile and may be hydrolyzed with alkali to give the lactam or ammonolyzed with ammonia to give the corresponding amino compound has led to the following simple method for the preparation of 1-*d*-glucosidocytosine (I). The intermediate, 1,2-dihydro - 2 - keto - 4 - ethoxy - 1 - tetraacetyl - *d* - glucosidopyrimidine (II), was prepared by the interaction of acetobromoglucose and 2,4-diethoxypyrimidine and was utilized for this work in preference to the known 4-methoxy analog because the ethoxy group is more easily attacked by ammonia than the methoxy group. On treatment with ammoniacal alcohol at 80° for several hours (II) was simultaneously deacetylated and ammonolyzed to yield (I) practically quantitatively.



II

I

1-*d*-Glucosidocytosine separates from absolute alcohol with alcohol of crystallization and from aqueous alcohol with alcohol and water of crystallization and the properties of these solvates are so unusual that they seem worthy of mention. The major portion of the solvent in these two products cannot be removed by heating at 140° in a high vacuum but on standing for several weeks in an atmosphere of relatively high humidity the alcohol of crystallization is gradually displaced by water with the concomitant lowering of the melting point. This hydrate loses its water of crystallization easily.

The chemical properties of 1-*d*-glucosidocytosine are in general quite similar to those of cytidine. It behaves as a base and readily yields a nitrate and picrate. Upon acetylation a pentaacetyl derivative was formed, one of the acetyl groups presumably being attached to the amino group. It did not respond to the Wheeler–Johnson color test nor did it reduce Fehling's solution before or after treatment with hydrochloric acid. In fact, even treatment with 25% sulfuric acid at 150° did not affect a scission of the glucose–nitrogen linkage, indicating that the glucose is more firmly attached to nitrogen than is the ribose in cytidine. Experiments such as nitric acid oxidation and pyrolysis in a high vacuum which were designed to adduce evidence confirming the structure of 1-*d*-glucosidocytosine were unsuccessful.

We wish to express our appreciation to Dr. R. T. Milner and Mrs. M. S. Sherman for determining the microanalyses recorded.

Experimental

The 1-glycosidopyrimidines are in general more conveniently prepared by the use of 2,4-diethoxypyrimidine instead of 2,4-dimethoxypyrimidine in the interaction with the acetobromoglycosides. When the former is used, the sugar pyrimidine combinations are more easily isolated from the reaction mixture as the by-products formed are in solution, are very soluble in ether and can be separated readily from the crystallized nucleoside, which has only slight solubility in ether. If 2,4-dimethoxypyrimidine is the intermediate, the major by-product, namely, the high melting 1-methyluracil, separates from the reaction mixture and masks the rate of precipitation of the desired sugar derivative; the separation of the latter from the former although not difficult is more troublesome.

Preparation of 1,2-Dihydro-2-keto-4-ethoxy-1-tetraacetyl-*d*-glucosidopyrimidine.— Fifty grams of 2,4-diethoxypyrimidine[5] was added to 50 g. of acetobromoglucose and heated at 65° overnight. The acetobromoglucose dissolved quite rapidly and this was followed by

(1) Fischer, *Ber.*, **47**, 1377 (1914).
(2) Levene and Sobotka, *J. Biol. Chem.*, **65**, 475 (1925).
(3) Hilbert, THIS JOURNAL, **56**, 190 (1934).
(4) Hilbert and Jansen, *ibid.*, **56**, 134 (1934); **57**, 552 (1935).
(5) Hilbert and Jansen, *ibid.*, **57**, 553 (1935).

the gradual evolution of considerable amounts of ethyl bromide and the separation of a mass of colorless needles. After the reaction was complete the mixture was cooled, treated with 100 cc. of dry ether and the crystals (m. p. 200°) collected; yield 28 g. It was crystallized twice from ethyl alcohol and melted at 206° (corr.) (resolidified at 201°) $[\alpha]^{26}_D$ +36.1° (*C* = 12.0 in c. p. chloroform); the product is slightly soluble in hot water and ether, soluble in hot alcohol and very soluble in chloroform.

Anal. Calcd. for $C_{20}H_{26}O_{11}N_2$: C, 51.04; H, 5.57; N, 5.96; OC_2H_5, 9.58. Found: C, 51.22; H, 5.77; N, 6.07; OC_2H_5, 9.52.

An alcoholic solution of the glucoside when treated with alcoholic hydrochloric acid deposited within twenty-four hours a colorless crystalline product, which proved to be identical with 1-*d*-glucosidouracil.[6]

Preparation of 1-*d*-Glucosidocytosine.—A mixture of 3.9 g. of 1,2-dihydro-2-keto-4-ethoxy-1-tetraacetyl-*d*-glucosidopyrimidine and 20 cc. of absolute ethyl alcohol saturated at 0° with dry ammonia was heated in a sealed glass tube at 80° for ninety-six hours. The reaction mixture was cooled very slowly and star-like clusters of long, narrow prisms separated; the tube was finally chilled in an ice-bath, opened and the cytosine derivative collected; sintering point 192°, m. p. 197–199° with effervescence; yield, 2.06 g. The crystals were air-dried as rapidly as possible and immediately analyzed.

Anal. Calcd. for $C_{10}H_{15}O_6N_3 \cdot {}^1/_3C_2H_5OH$: OC_2H_5, 5.20; N, 14.57. Found: OC_2H_5, 5.13; N, 14.64.

An attempt to desolvate the material was made by heating it at a pressure of 1 mm. in an Abderhalden dryer using xylene as the refluxing liquid until the weight was constant; loss 2.31%. The product dried in this manner was analyzed for ethoxy; found: 2.71%. Specimens of the heated and the unheated material were allowed to stand in the atmosphere (high humidity) from one to two months and the displacement of the alcohol by water was followed analytically. The hydrate melts at about 128° (rapid heating) with effervescence and readily gives up its water when heated at 100° at a pressure of 1 mm.; loss, 7.7%. Anhydrous 1-*d*-glucosidocytosine is extremely hygroscopic and in twenty-four hours gains 6.6% of its weight in water. The hydrated material was analyzed and the results corrected for the water content; ethoxy was found to be absent.

Anal. Calcd. for $C_{10}H_{15}O_6N_3$: C, 43.93; H, 5.54; N, 15.39. Found: C, 43.87; H, 5.58; N, 15.28.

When the above preparation or the sirup, in which form the cytosine derivative usually separates from the reaction, was crystallized from about 90–95% ethyl alcohol the product deposited had a different composition than the crystals isolated directly from the reaction mixture. Large colorless prisms separated on cooling the alcoholic solution very slowly; m. p. 194–195° (effervescence).[7]

Anal. (substance air-dried). Found: N, 14.12; OC_2H_5, 6.93; $[\alpha]^{21}_D$ +23.7 (*C* = 2.8 in distilled water).

From the analysis it is evident that the substance has both alcohol and water of crystallization. It was dried to constant weight at the temperature of boiling xylene at a pressure of 1 mm., loss 3.74%. An attempt to remove the remainder of the solvent of crystallization by heating to 160° was unsuccessful owing to decomposition; drying in a high vacuum (10^{-5} mm.) was no more effective than drying at 1 mm. pressure. The material could be desolvated easily, after dissolving in water and concentrating to dryness. The anhydrous material showed $[\alpha]^{21}_D$ +25.6° (*C* = 1.8 in distilled water).

An attempt to degrade the glucoside was made; 0.5 g. was heated in a bomb tube at 150° for four hours with 25% sulfuric acid (1.5 cc.). The brown colored reaction mixture was filtered from a small amount of flocculent matter, diluted with water and the sulfuric acid removed quantitatively with an equivalent amount of barium hydroxide. As a small fraction of the clarified liquid did not form a precipitate when treated with an aqueous solution of phosphotungstic acid, indicating the absence of cytosine, the remainder was concentrated to a sirup which crystallized on standing. The product was recrystallized from aqueous alcohol and dried for analysis, at 100°; loss: 9.26%; m. p. 180–185°; $[\alpha]^{23}_D$ +22.3° (*C* = 1.21 in distilled water).

Anal. Found: C, 44.09; H, 5.33; N, 12.65; 12.67; ash, 1.34.

The analytical results and the optical rotation are compatible with those of a mixture of 1-*d*-glucosidouracil and 1-*d*-glucosidocytosine. The latter compound was isolated and characterized as the picrate.

The picrate was prepared by adding a solution of picric acid (5 g.) in boiling methanol (50 cc.) to a solution of 1-glucosidocytosine (6 g. alcoholate-hydrate) in water (4 cc.) and hot methanol (enough so that the solution was not quite cloudy). On cooling the picrate separated in clusters of needles; it was recrystallized twice from 60% ethyl alcohol and then melted at 216–218° (dec.); yield, 6.3 g. The picrate was anhydrous.

Anal. Calcd. for $C_{16}H_{18}O_{13}N_6$: C, 38.23; H, 3.61; N, 16.74. Found: C, 38.36; H, 3.64; N, 16.58.

1-*d*-Glucosidocytosine was recovered from the picrate by acidifying an aqueous solution with sulfuric acid and extracting the picric acid with ether. Sulfuric acid was removed by the addition of an equivalent amount of barium hydroxide. The aqueous solution of the nucleoside was concentrated under diminished pressure and the residue crystallized from 90% alcohol. The properties and analysis of the product agreed with those of the original alcoholate hydrate.

The nitrate was prepared by adding 0.5 cc. of concentrated nitric acid to a solution consisting of 0.1 g. of 1-*d*-glucosidocytosine, three drops of water and 10 cc. of hot absolute ethyl alcohol. On cooling colorless irregular massive crystals separated; m. p. 143° (effervescence); $[\alpha]^{23}_D$ +21.3° (*C* = 2.3 in distilled water). This product was dried to constant weight at 100° at a pressure of 1 mm. The analytical results agree best with the nitrate containing one molecule of water of crystallization. Attempts to remove this by drying at higher temperatures were unsuccessful owing to decomposition. Apparently the

(6) Hilbert and Johnson, THIS JOURNAL, **52**, 4489 (1930).

(7) The melting points and analyses of specimens obtained from different crystallizations varied somewhat; apparently the amount of the solvent of crystallization is rather sensitive to slight differences in the conditions of crystallization.

water of crystallization in this compound is held as tenaciously as the alcohol in 1-*d*-glucosidocytosine alcoholate.

Anal. Calcd. for $C_{10}H_{15}O_6N_3 \cdot HNO_3H_2O$: C, 33.88; H, 5.12; N, 15.82; HNO_3, 17.79. Found: C, 34.13; H, 5.15; N, 14.94, 15.18;[8] HNO_3 (by titration), 17.36.

1-Tetraacetyl-*d*-glucosido-7-acetylcytosine.—To a solution of 1.9 cc. of acetic anhydride and 2.5 cc. of pyridine was added 0.5 g. of 1-*d*-glucosidocytosine hydrate. The nucleoside gradually dissolved and after standing for three days at room temperature the solution was added to 10 g. of ice. The crystalline material that deposited was collected and dried; yield 0.7 g. It was crystallized twice from 2 cc. of alcohol and separated as clusters of elongated plates; m. p. 225°; $[\alpha]^{23}D$ +38.1° (C = 1.7 in C. P. chloroform).

Anal. Calcd. for $C_{20}H_{25}O_{11}N_3$: C, 49.67; H, 5.21; N, 8.70; $COCH_3$, 44.52. Found: C, 49.94; H, 5.29; N, 8.73; $COCH_3$, 43.85.

(8) The Dumas method has been found to give low results for a number of pyrimidines.

Summary

1-*d*-Glucosidocytosine was prepared by the interaction of ammonia and 1,2-dihydro-2-keto-4-ethoxy - 1 - tetraacetyl - *d* - glucosidopyrimidine. A curious physical property of this synthetic nucleoside is the tenacity with which it attaches itself to alcohol. In general, its chemical properties are quite similar to those of cytidine.

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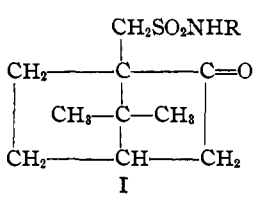
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

Anomalous Mutarotation of Salts of Reychler's Acid. IV. Comparison of 2-(N-phenylketimine)-*d*-camphane-10-sulfonic Acid with *d*-Camphor-10-sulfonanilide

BY HARRY SUTHERLAND[1] AND R. L. SHRINER

It has been pointed out that the dehydration product obtained from primary amine salts of Reychler's acid could possess the structure of a substituted sulfonamide (I), as well as that of a substituted ketimine (II).



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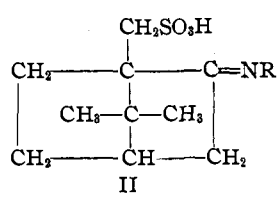

II

Since sulfonamides cannot ordinarily be obtained by direct dehydration of a salt of a sulfonic acid, the ketimine structure was preferred. However, it must be admitted that most of the evidence thus far cited[2] could be explained on the basis of either formula. Hence, it was desirable to prepare a substituted sulfonamide of Reychler's acid and compare it with the corresponding product to which the ketimine structure had been assigned.

d-Camphor-10-sulfonanilide has been prepared by Reychler,[3] who merely recorded its melting point, and by Armstrong and Lowry,[4] who recorded its optical rotation, but no information was available concerning its stability, ease of hydrolysis or possible mutarotation in solvents containing water. Hence, the preparation of this sulfonanilide was repeated, its properties determined and compared with those of the compound obtained by dehydration of the aniline salt of *d*-camphor-10-sulfonic acid. The information obtained has been collected in Table I.

TABLE I

COMPARISON OF ANILINE SALT AND ANILIDE OF REYCHLER'S ACID WITH N-PHENYLKETIMINE-*d*-CAMPHANE-10-SULFONIC ACID

Property	Aniline salt	Anilide	N-Phenyl-ketimine
Melting point, °C.	184–186	120.5–121	294–295
Specific rotation[5] in chloroform	+37.5°	+76.0°	−170.5°
Mutarotation			
(a) 95% alcohol	Yes	No	Yes
(b) Chloroform	Yes	No	No
Neutral equivalent (by titration)	325	None	306.3
Hydrolysis	.....	Difficultly	Readily

Examination of the data in Table I shows that the *dextro*-rotatory anilide differs markedly from the *levo*-rotatory dehydration product. It behaved like a typical substituted sulfonanilide in that it gave no neutral equivalent and was hydrolyzed with difficulty. It did not mutarotate in a solvent containing water—a property characteristic of the ketimine. These results show definitely that the dehydration product of an amine

(1) Chemical Foundation Fellow in Organic Chemistry.

(2) Schreiber and Shriner, THIS JOURNAL, **57**, 1306, 1445, 1896 (1935).

(3) Reychler, *Bull. soc. chim.*, [III] **19**, 124 (1898).

(4) Armstrong and Lowry, *J. Chem. Soc.*, **81**, 1447 (1902).

(5) All specific rotations were determined at 25° with sodium D light at a concentration of 1 g. per 100 cc. of solvent.