of 12 *N* hydrochloric acid were added to a 2-liter stainless steel autoclave, and digested at 160° for three hours. Two hydrolyses were carried out and the liquors combined before analysis.

Fractionation of Products.—Efficient columns were developed for this and similar studies; one of these has been described in detail.[5] An extremely satisfactory method of fractionation involved the preliminary concentration of the reaction mixture by removal of the solvent. Two distillations in a column having an efficiency equivalent to 30 theoretical plates sufficed for concentration. The entire reaction mixture was then distilled through a 30-plate column, and the distillate added to the pot of another 30-plate column. The distillate from the second column was placed in the pot of a 60-plate column.[5] These three columns were run simultaneously in this cascade system, keeping the volume of liquid in the higher pots at a minimum to increase the efficiency of separation. The technique of fractionation was generally similar to that already described.[5]

Yields were determined by the weight of distillate and the elimination curve.[5]

Identification of Products

(1) **Acetone.**—The 2,4-dinitrophenylhydrazone had a melting point of 125°. The semicarbazone melted at 185–186°, and the mixed melting point determination with acetone semicarbazone was 185°.

(2) ***n*-Butyraldehyde.**—The 2,4-dinitrophenylhydrazone melted at 120–121°, and the semicarbazone at 105°.

(3) **Methanol.**—The *p*-nitrobenzoate melted at 107°, and the 3,5-dinitrobenzoate at 107°.

(4) **Allyl Alcohol.**—The 3,5-dinitrobenzoate melted at 49°, and the *p*-nitrobenzoate at 28°.

(5) **Propyl Alcohol.**—The 3,5-dinitrobenzoate melted at 73–74° and the *p*-nitrobenzoate at 35°.

(6) **Formic Acid.**—The formyl radical occurred in the distillate as *n*-butyl formate, and the weight obtained is reported as formic acid. Actually, the *n*-butyl formate was isolated as the binary azeotrope, *n*-butyl formate–*n*-butanol, boiling at 105.8°. The butanol was removed from the mixture as the addition product of calcium chloride, and the residual butyl formate hydrolyzed by refluxing with 25% sodium hydroxide for one-half hour. The *n*-butanol was then distilled off as the binary azeotrope with water boiling at 92.7°, and salted out with potassium carbonate. The residual contents of the distillation flask were cooled, acidified and distilled. The formic acid was recovered as the binary water azeotrope; b. p. 107°.

The *n*-butanol was identified by preparing the 3,5-dinitrobenzoate, m. p. 62–63°, and *p*-nitrobenzoate, m. p. 35°.

The formic acid was identified by preparing the *p*-nitrobenzyl and the *p*-bromophenacyl esters. The *p*-nitrobenzyl ester had a melting point of 30–31°; the *p*-bromophenacyl ester, 138°.

(7) **β-Ethyl-α-methylacrolein.**—The 2,4-dinitrophenylhydrazone melted at 160°, the semicarbazone at 205°.

All of the above products were checked by identification with literature citation, melting points, or by mixed melting point determination.

Summary

Six aliphatic compounds, representing a yield of 25.7%, were identified in the acid hydrolyzate of butanol lignin.

SEATTLE, WASHINGTON RECEIVED SEPTEMBER 4, 1941

(5) A. Bailey, *Ind. Eng. Chem., Anal. Ed.*, **13**, 487 (1941).

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND OF PHYSIOLOGICAL CHEMISTRY, THE OHIO STATE UNIVERSITY]

The β-Form of the Cori Ester (*d*-Glucopyranose 1-Phosphate)

BY M. L. WOLFROM, C. S. SMITH, D. E. PLETCHER AND A. E. BROWN

Of all the biologically important sugar phosphates occurring in nature, the Cori ester (*d*-glucopyranose 1-phosphate) is perhaps of prime importance because of its role in the initial reactions of carbohydrate metabolism. The enzymatic synthesis of starch[1] and of glycogen[2] from this compound is indicative of its further significance. In a rigorous proof of structure of this substance reported from this Laboratory,[3] no definitive α,β-assignment could be made since the corresponding α,β-isomer was unknown. The rather high dextrorotation (+78.5°) exhibited by the crystalline dipotassium salt of the Cori ester does not in itself allow an α-assignment to be made. For example, β-*d*-galactose (+52°) and β-maltose (+118°) show high dextrorotations, but their corresponding α-isomers possess still greater rotations in the dextro direction. Accordingly, we sought a synthetic method of preparation for the second α,β-form.

Cori, Colowick and Cori[4] state in a footnote that an attempt was made to prepare the α,β-isomer of the Cori ester by the interaction of β-acetochloroglucose and silver phosphate. Using an alkaline deacetylation procedure, they state that in one case they obtained an acid-labile bar-

(1) C. S. Hanes, *Proc. Roy. Soc.* (London), **B129**, 174 (1940).

(2) R. S. Bear and C. F. Cori, *J. Biol. Chem.*, **140**, 111 (1941); *cf.* ref. 3 for previous citations.

(3) M. L. Wolfrom and D. E. Pletcher, THIS JOURNAL, **63**, 1050 (1941).

(4) C. F. Cori, S. P. Colowick and Gerty T. Cori, *J. Biol. Chem.*, **121**, 470 (1937).

ium salt with a specific rotation of +9.7° (5892.5 Å.) but that on using the mild acid deacetylation procedure which was successfully employed in their important synthesis of the Cori ester, they obtained a product with a specific rotation of +69° (5892.5 Å.).

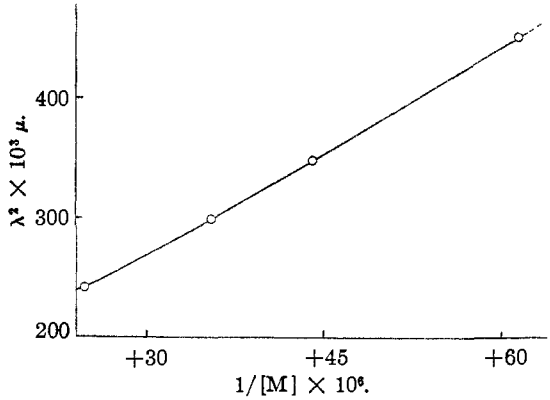

Fig. 1.—Rotatory dispersion of β-*d*-glucopyranose dibrucine 1-phosphate.

Zervas,[5] in introducing an important new phosphorylation method, interacted α-acetobromoglucose with silver dibenzyl phosphate and synthesized crystalline glucose tetraacetate dibenzyl 1-phosphate which was sensitive to both acid and alkali and thus possessed reducing properties. He stated that this alkali sensitivity was still present after hydrogenolysis of the benzyl groups but no further characterization of the hydrogenolysis product was given.

Since the Zervas procedure establishes the structure of the product as a glucopyranose 1-phosphate by method of synthesis, we have further investigated the reaction as a possible method of preparing the α,β-isomer of the Cori ester. The glucopyranose 1-phosphate obtained on hydrogenolysis and alkaline saponification of the crystalline glucopyranose tetraacetate dibenzyl 1-phosphate of Zervas was characterized as its crystalline dibrucine salt. This salt, and also the inorganic salts (amorphous) investigated, were stable to alkali and reduced Fehling solution only after acid hydrolysis.

To maintain our work on a crystalline basis, the above dibrucine salt (I) was compared with the crystalline dibrucine salt of the Cori ester (II). Cori and co-workers frequently mention the latter compound but to our knowledge, no characterization of it has appeared in the literature. Compound I has a specific rotation of −20° (29°, water, 5892.5 Å.) and exhibits a normal rotatory dispersion in the visible region of the spectrum (Fig. 1). Compound II has a specific rotation of +0.5° (29°, water, 5892.5 Å.) and exhibits an anomalous rotatory dispersion in the visible region (Fig. 2). Both substances are non-reducing

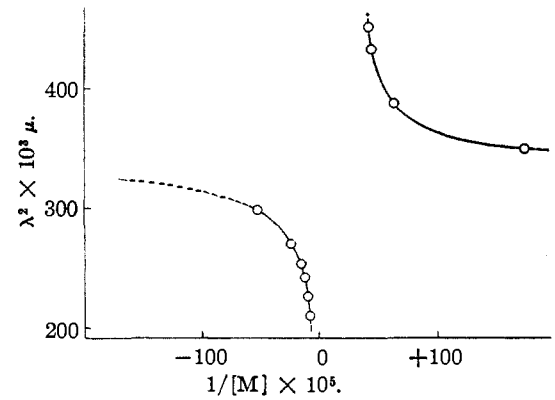

Fig. 2.—Rotatory dispersion of α-*d*-glucopyranose dibrucine 1-phosphate.

toward Fehling solution. On hydrolysis by *N* hydrochloric acid at room temperature, I exhibits an upward (becoming more dextrorotatory) mutarotation and II exhibits a downward mutarotation (Fig. 3), both reaching the same final value which agrees with that calculated for the expected mixture of *d*-glucose and levorotatory brucine. The specific reaction constant of this hydrolysis for I is *ca.* 0.015 and that for II is *ca.* 0.005 (33°, minutes and decimal logarithms). Compound I is therefore appreciably more acid-labile than II.

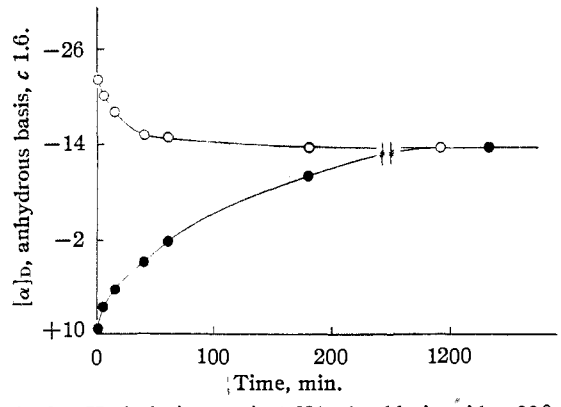

Fig. 3.—Hydrolysis rates in 1 *N* hydrochloric acid at 33° of *d*-glucopyranose 1-phosphates: ●, α-form; ○, β-form.

The above data lead to a definitive assignment of the α-configuration to the natural Cori ester and of the β-configuration for the new synthetic *d*-glucopyranose 1-phosphate. It is of interest

(5) L. Zervas, *Naturwissenschaften*, **27**, 317 (1939).

to note that we have here an example of an α-form occurring naturally, and the β-form only synthetically, the opposite of what is usually found with glycosides. However, should cellulose be formed in the plant by the "dephosphorolysis" of β-*d*-glucopyranose 1-phosphate, as starch and glycogen are formed by the "dephosphorolysis" of α-*d*-glucopyranose 1-phosphate, perhaps it is merely a matter of time before the β-form will be found in nature.

Experimental

Preparation of β-*d*-Glucopyranose Tetraacetate Dibenzyl 1-Phosphate.—The general phosphorylation procedure indicated by Zervas[5] was followed. To our knowledge, no details of this procedure have appeared in the literature. The silver dibenzyl phosphate used was prepared according to the method of Lossen and Köhler[6] (calcd. for $C_{14}H_{14}O_4PAg$: Ag, 28.0; found: Ag, 27.3). In a flask equipped with a condenser and drying tube, 10 g. (1 mol) of dry α-acetobromoglucose, 14 g. (1.5 mols) of dry silver dibenzyl phosphate, 5 g. of powdered Drierite (anhydrous calcium sulfate) and 55 cc. of dry benzene were warmed at 50° for thirty minutes. The mixture was then refluxed for ninety minutes, vigorous mechanical agitation being maintained throughout. After cooling, the mixture was filtered through a precoat of decolorizing charcoal and Super-cel (Johns-Manville), and the sirup obtained on solvent removal under reduced pressure (30–40°) was crystallized from ether by the addition of petroleum ether; yield 10.8 g. (73%), m. p. 74–76°. Pure material was obtained on two further crystallizations from ether (containing a few drops of pyridine) by the addition of petroleum ether; m. p. 78–79°, spec. rot. −9° (25°, *c* 3, 5892.5 Å., abs. $CHCl_3$). Zervas[5] reported the following constants for this substance: m. p. 79°, spec. rot. −9° (20°, $CHCl_3$, 5892.5 Å.).

The substance crystallized in colorless, rhombic crystals. It gave a strong Fehling reduction after heating for three minutes at 80° with *N* sulfuric acid. It was also rather unstable to alkali and slowly reduced boiling Fehling solution. This instability to both acidity and alkalinity is in agreement with the report of Zervas.[5]

Anal. Calcd. for $C_{20}H_{21}O_9P(CH_3CO)_4$: C, 55.25; H, 5.46; CH_3CO, 6.57 cc. 0.1 *N* NaOH per 100 mg.; saponification value, 8.23 cc. 0.1 *N* NaOH per 100 mg. Found: C, 54.98; H, 5.51; CH_3CO, 6.49 cc.; saponification value, 8.28 cc.

β-*d*-Glucopyranose Dibrucine 1-Phosphate.—β-*d*-Glucopyranose tetraacetate dibenzyl 1-phosphate (1 g.) was reduced in absolute ethanol (15 cc.) solution with hydrogen at atmospheric pressure and palladous oxide (0.2 g.) catalyst. At the end of the hydrogenation (thirty minutes), the odor of toluene was marked. The catalyst was removed by filtration and 9 cc. of 10% potassium hydroxide was added to the filtrate. A sirup precipitated which was removed by decantation, dissolved in 20 cc. of water, and 1 cc. of 10% potassium hydroxide added. Upon the addition of a solution of 1 g. of neutral lead acetate in 15 cc. of water, a white precipitate formed that was collected by filtration and washed well with water; yield 1.25 g. of a white powder which reduced Fehling solution only after acid hydrolysis. This basic lead salt was decomposed in water suspension with hydrogen sulfide at 1°, filtered and aerated rapidly and then made slightly alkaline with a solution of brucine (1.25 g.) in 6 cc. of methanol. Crystallization was initiated on concentration (40°) of the solution under reduced pressure, and was completed by the addition of acetone and standing overnight in the ice chest; yield 1.25 g. Pure material (a decahydrate) was obtained on recrystallization from water; colorless prisms, m. p. 160–165° (dec.) with sintering at 120–122°, spec. rot. −20° (29°, *c* 1.7, H_2O, 5892.5 Å.), normal rotatory dispersion in the visible region (Fig. 1). The substance did not reduce hot Fehling solution but readily gave a reduction after treatment at room temperature for three minutes with *N* hydrochloric acid.

Anal. Calcd. for $C_6H_{13}O_9P(C_{23}H_{26}N_2O_4)_2 \cdot 10H_2O$: H_2O, 14.65. Found: H_2O, 14.48.

The anhydrous form was likewise crystalline and was obtained by heating the decahydrate at 110° at 4 mm. over phosphorus pentoxide; m. p. 162–166° (dec.).

Anal. Calcd. for $C_6H_{13}O_9P(C_{23}H_{26}N_2O_4)_2$: C, 59.51; H, 6.25; N, 5.34; P, 2.96. Found: C, 59.3; H, 6.18; N, 5.15; P, 2.92.

The crystalline brucine salt was converted to the potassium and barium salts. These salts were amorphous, the barium salt being gelatinous, but all were reducing toward Fehling solution only after acid hydrolysis.

α-*d*-Glucopyranose Dibrucine 1-Phosphate.—Although Cori and co-workers refer to the crystalline brucine salt of the Cori ester, to our knowledge no characterization or analysis of this substance has appeared in the literature. To a solution of 2 g. of the crystalline dipotassium salt of the Cori ester in 16 cc. of water was added a solution of 2 g. of barium acetate dissolved in a like amount of water. Two volumes of ethanol were added. Following cooling, the barium salt was collected on a precoat and extracted with 75 cc. of hot water after which the extract was cleared by filtration through a precoat. The extract was cooled to 0° and the barium removed by the addition of 85% of the calculated amount of *N* sulfuric acid, followed by the rapid addition of more acid until only a faint barium test was given. The barium sulfate was removed by filtration and a methanol solution of brucine was added until the filtrate was alkaline to litmus. About 5 g. of brucine was needed.

After concentration of the above solution under reduced pressure, 30 cc. of acetone was added and crystallization was initiated by cooling. Pure material (an octahydrate) was obtained on recrystallization from methanol–water or acetone–water; yield 3.3 g., m. p. 173–178° with sintering at 165°; spec. rot. +0.5° (27°, *c* 3, H_2O, 5892.5 Å.), anomalous rotatory dispersion in the visible region (Fig. 2). The substance crystallized in colorless needles and was appreciably more soluble in water and methanol than the above-described β-isomer. It showed no Fehling reduction on prolonged boiling but readily gave a reduction after treatment at room temperature for three minutes with *N* hydrochloric acid.

(6) W. Lossen and A. Köhler, *Ann.*, **262**, 212 (1891).

Anal. Calcd. for $C_6H_{13}O_9P(C_{23}H_{26}N_2O_4)_2 \cdot 8H_2O$: H_2O, 12.1; C, 52.33; H, 6.85; N, 4.70; P, 2.60. Found: H_2O, 12.2; C, 52.7; H, 6.88; N, 4.57; P, 2.66.

The anhydrous form was likewise crystalline and was obtained by heating the octahydrate at 110° under reduced pressure over phosphorus pentoxide; m. p. 182–184° (dec.). The same dibrucine salt was obtained on starting with a dipotassium salt from either the synthetic or natural[1] source.

The crystalline dipotassium salt was regenerated from the above dibrucine salt. To 0.5 g. of the dibrucine salt dissolved in 5 cc. of water was added 0.8 cc. of 10% potassium hydroxide to bring the *p*H to about 8.4 (thymol blue). The brucine was removed by filtration and ethanol was added to the filtrate to incipient turbidity. The dipotassium salt crystallized on scratching and cooling; yield 0.14 g. (93%), spec. rot. +78° (33°, *c* 1, 5892.5 Å., H_2O). This rotation is in exact agreement with the previously published value[3] (+78°).

Hydrolysis of the α- and β-Forms of *d*-Glucopyranose Dibrucine 1-Phosphate.—The two previously described dibrucine salts of *d*-glucopyranose 1-phosphate were hydrolyzed at room temperature with *N* hydrochloric acid, and the course of the reactions followed polarimetrically (Fig. 3). The rotation change (anhydrous basis) of the α-form was +9° (extrapolated) → −14° while that of the β-form was −21.5° (extrapolated) → −14°. The specific reaction constant at 33° of the α-form was *ca.* 0.005 while that of the β-form was *ca.* 0.015 (minutes and decimal logarithms) when calculated according to the equation $k = 1/t \log (r_0 - r_\infty / r_t - r_\infty)$ wherein t is time, r_0 is initial rotation, r_∞ is final rotation and r_t is rotation at time t. Thus the β-form is appreciably more sensitive to acidity than is the α-form. Using the value of +53° for the specific rotation of *d*-glucose in *N* hydrochloric acid[7] and a determined value of −26.4° for the specific rotation of levorotatory brucine under the same conditions, the calculated specific rotation for the final hydrolyzate may be obtained from the equation: [M]/1048 = (+53)(180) + 2(−26.4)(466) = −14.4°, wherein [M] is molecular rotation. The value of the specific rotation thus calculated is in agreement with the determined value of −14.2° (anhydrous basis).

We are indebted to Mr. Walter M. Anderson for assistance in the laboratory.

Summary

1. It is shown that the Cori ester is the α-form of *d*-glucopyranose 1-phosphate, by the synthesis of its α,β-isomer, characterized as its crystalline dibrucine salt.

2. The crystalline dibrucine salt of the Cori ester has been characterized.

3. It is shown that both of the above dibrucine salts are stable to alkali but sensitive to acid, with the β-form being even more acid-labile than the α-form.

(7) M. L. Wolfrom and L. W. Georges, THIS JOURNAL, **59**, 282 (1937).

COLUMBUS, OHIO RECEIVED OCTOBER 6, 1941

[CONTRIBUTION FROM THE U. S. BUREAU OF NARCOTICS LABORATORY]

Isolation of a Physiologically Active Tetrahydrocannabinol from Cannabis Sativa Resin

BY H. J. WOLLNER, JOHN R. MATCHETT,[1] JOSEPH LEVINE AND S. LOEWE[2]

Greatly increased interest has developed in recent years in the chemistry and pharmacology of the oil derived from *Cannabis sativa*. The researches initiated have greatly extended the previous knowledge of the complex mixture, which has been ably reviewed by Blatt.[3]

Adams and co-workers, in a series of brilliant researches, have rigidly proven the structure of cannabinol,[4] isolated cannabidiol,[5] isomerized it to two isomeric, physiologically active tetrahydrocannabinols,[6] and proven its structure except for final placement of one double linkage.[7] The same workers have prepared synthetic tetrahydrocannabinols as well as a number of similarly constituted substances,[8] which have been shown to possess "marihuana-like" physiological activity in dogs, but in smaller degree than those prepared from the naturally-occurring cannabidiol.

Todd and his co-workers have isolated cannabol,[9] and in synthetic studies have recorded results similar to those reported by Adams.[10] The Geyer test was used to measure physiological activity of their synthetic material.

(1) Present address: Western Regional Research Laboratory, U. S. Department of Agriculture, Albany, California.

(2) Department of Pharmacology, Cornell University Medical College.

(3) Blatt, *J. Wash. Acad. Sci.*, **28**, 465 (1938).

(4) Adams, Baker and Wearn, THIS JOURNAL, **62**, 2204 (1940).

(5) Adams, Hunt and Clark, *ibid.*, **62**, 196 (1940).

(6) (a) Adams, Pease, Cain and Clark, *ibid.*, **62**, 2402 (1940). (b) Adams, Cain, McPhee and Wearn, *ibid.*, **63**, 2209 (1941).

(7) Most recent paper of series, Adams, Loewe, Pease, Cain, Wearn, Baker and Wolff, *ibid.*, **62**, 2566 (1940).

(8) Most recent paper of series, Adams, Cain and Loewe, *ibid.*, **63**, 1977 (1941).

(9) Jacob and Todd, *Nature*, **145**, 350 (1940).

(10) (a) Ghosh, Todd and Wilkinson, *J. Chem. Soc.*, 1121 (1940). (b) Ghosh, Todd and Wright, *ibid.*, 137 (1941). (c) Russell, Todd, Wilkinson, Macdonald and Woolfe, *ibid.*, 169 (1941).