[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF UTAH]

Selective Benzoylation of Methyl D-Glucopyranosides Using Boric Acid

BY JAMES M. SUGIHARA AND J. CLAINE PETERSEN

RECEIVED OCTOBER 13, 1955

Benzoylation of the complex formed by the reaction of boron oxide or metaboric acid-III with methyl β-D-glucopyranoside, followed by methanolysis of the borate linkages and acetylation, gave the 4,6-diacetate 2,3-dibenzoate (IV), the 3,4-diacetate 2,6-dibenzoate (V) and the 4-acetate 2,3,6-tribenzoate (VI) of the β-glucoside as the major products. The 2,3,4-triacetate 6-benzoate, the tetraacetate and the tetrabenzoate were obtained in smaller amounts. Recovery of crystalline compounds under different experimental conditions varied between 59–73%. Methyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside was prepared in a similar fashion from the α-glucoside. An interpretation of these findings is presented.

The reaction of boric acid with glycols has received much attention.[1] The use of the complex esters derived in an anhydrous medium to prepare partially esterified, etherified or acetonated derivatives has been studied to a lesser extent. Partial benzoylation of D-mannitol, D-glucose and D-glucose diethyl thioacetal[2] and partial acetonation of D-mannitol[3] and D-glucose[4] are described. The blocking exhibited by boric acid in these reactions may be rationalized, in most instances, by assuming the formation of one or more 5-membered ring borate complexes with the glycols.

Methyl α- and β-D-glucopyranosides did not increase the conductivity of aqueous boric acid solutions.[5] In these cyclic systems with no adjacent hydroxyl groups in a *cis* relation in the ring, complexing was assumed to be impossible. However, filter paper ionophoresis of methyl α- and β-D-glucopyranosides in a borate buffer at *p*H 10 indicated interaction involving C-4 and C-6 hydroxyl groups; in a solution of boric acid this interaction was not observed.[6] In an anhydrous system, the degree and type of interaction could be different. Indeed, these glucosides were found to react with metaboric acid.[7] Methylation of the product derived from the β-glucoside yielded 50% of a mixture of dimethyl ethers, of which 25% was the 3,6-di-*O*-methyl derivative. The 2,6-di-*O*-methyl derivative was also present, but apparently only a small amount was isolated as methyl 2,6-di-*O*-methyl-3,4-di-*O*-tosyl-β-D-glucopyranoside. The incomplete characterization of products formed provides little information concerning the role played by boric acid in this reaction. In the work described herein, the nature of the products formed by reaction of methyl β-D-glucopyranoside, and to a more limited extent methyl α-D-glucopyranoside, with metaboric acid and boron oxide were studied by subsequent benzoylation. The mixed products were methanolized to cleave borate–ester linkages, and then acetylated. Chromatography[8] enabled the separation of crystalline compounds in amounts sufficient to account for the major portion of the methyl β-D-glucopyranoside applied.

The borate esters were prepared in the usual manner[2,7] by refluxing in anhydrous acetone. The rate of reaction was followed by noting the rate of disappearance of the acetone-insoluble glucosides and boron oxide. The length of the refluxing period, after complete solution had been attained, did not influence amounts or types of derivatives obtained. Both boron oxide and a special form of metaboric acid (metaboric acid-III)[9] were used in the preparation of these esters. No appreciable differences were found in the application of either 0.5 mole of boron oxide or one mole of metaboric acid-III. Kracek and co-workers[9] report that metaboric acid-III reacts with acetone, suggesting that acetone may play some subtle role in the formation of the borate complex with the glucosides. In order to provide evidence for this possibility, anhydrous, alcohol-free 1,2-dimethoxyethane was applied instead of acetone. The metaboric acid was soluble in this solvent also, and the amounts and types of products obtained were indistinguishable from those obtained with acetone as the solvent. Ordinary metaboric acid, obtained by the slow dehydration of boric acid at 135° in a partially closed vessel, was found to be much less reactive in the formation of borates of methyl α- and β-D-glucosides. With the use of a one-to-one molar ratio of reactants, only a little over one-half of the glucosides could be dissolved by refluxing in acetone for 24 hours, whereas, using metaboric acid-III all of the glucosides reacted in a matter of minutes.

The use of chloroform as a solvent for the benzoylation reaction prevented the precipitation of solids, obtained upon addition of benzoyl chloride to the pyridine solution of the borate complex. This appeared desirable since the rate of benzoylation would be enhanced. (Compare experiments G and L, Table I. Times indicated are the periods sufficient to recover high yields.) However, the rate of cleavage of the borate ester linkages was also greatly increased, as shown by comparing experiments E and F, in which chloroform was used, with experiment K in which the solvent was omitted. In the former cases, cleavage to form methyl tetra-*O*-benzoyl-β-D-glucopyranoside (VII) accounted for 50% of the starting material. In the latter case only 12% of VII was isolated although the period of benzoylation was four times as long. The use of 3 or 4 moles of benzoyl chloride per mole of methyl β-D-glucopyranoside (I) (experiments A, B, C and H) instead of 6 resulted in a slightly higher recovery of compounds benzoylated to a smaller extent.

Following benzoylation, surviving borate ester

(1) A review of much of this literature is provided by J. Böeseken, *Advances in Carbohydrate Chem.*, **4**, 189 (1949).

(2) P. Brigl and H. Grüner, *Ann.*, **495**, 60 (1932).

(3) L. v. Vargha, *Ber.*, **66B**, 1394 (1933); P. Brigl and H. Grüner, *ibid.*, **67B**, 1969 (1934).

(4) L. v. Vargha, *ibid.*, **66B**, 704 (1933).

(5) J. Böeseken and H. Couvert, *Rec. trav. chim.*, **40**, 354 (1921).

(6) A. B. Foster and M. Stacey, *J. Chem. Soc.*, 1778 (1955).

(7) D. J. Bell, *ibid.*, 175 (1935).

(8) W. H. McNeely, W. W. Pinkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(9) A crystalline form of metaboric acid, m.p. 176°, prepared by the method of F. C. Kracek, G. W. Morey and H. E. Merwin, *Am. J. Sci.*, **35A**, 143 (1938).

TABLE I

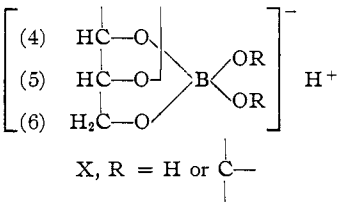
RECOVERY OF THE SEVERAL DERIVATIVES OF METHYL β-D-GLUCOSIDE UNDER VARYING EXPERIMENTAL CONDITIONS

Expt.	Moles reagent/mole glucoside B_2O_3 or HBO_2-III	C_6H_5COCl	Benzoylation conditions	Yield of glucoside derivatives, % Tetra-acetate II	2,3,4-Tri-acetate 6-benzoate (III)	4,6-Diacetate 2,3-dibenzo-ate (IV)	3,4-Diacetate 2,6-dibenzo-ate (V)	4-Acetate 2,3,6-tri-benzoate (VI)	Tetra-benzoate VII	Total
A	0.5 B_2O_3	4	0°, 1 hr.	4	4	13	19	18	4	62
B	0.5 B_2O_3	3	0°, 1 hr.	4	4	12	23	16	5	64
C	1.0 HBO_2-III	4	0°, 1 hr.	3	3	12	18	14	9	59
D	1.0 HBO_2-III	6	30°, 1 hr.	Not determined		14	15	12	17	58
E	1.0 HBO_2-III	6	30°, 24 hr.	0	0	7	6	4	48	65
F	1.0 B_2O_3	6	30°, 24 hr.	0	0	7	4	3	53	67
G	None	6	0°, 1 hr.						85	85
H	0.5 B_2O_3	4	60°, 3.5 hr., no $CHCl_3$	7	8	24	17	9	1	66
I	0.5 B_2O_3	6	30°, 20 hr., no $CHCl_3$	0	2	13	20	14	10	59
J	0.5 HBO_2-III	6	30°, 20 hr., no $CHCl_3$	0	Trace	16	18	16	23	73
K	0.5 B_2O_3	6	30°, 4 days, no $CHCl_3$	0	2	12	20	13	12	59
L	None	6	30°, 20 hr., no $CHCl_3$						86	86

groups were methanolized, and the hydroxyl groups freed were acetylated to produce completely esterified derivatives of the glucoside. Such compounds could be recovered in crystalline form in higher yields than the partially benzoylated derivatives. The crude sirups obtained by this general procedure were found to crystallize from methanol as a mixture of the more methanol-insoluble derivatives. The crystals were separated from the mother liquor, and the two fractions were analyzed by chromatographic methods.

A summary of the types and amounts of derivatives of methyl β-D-glucopyranoside (I) isolated is given in Table I. All compounds formed were positively identified by comparison with compounds made again by established methods. No new compounds not previously reported in the literature were found. When the amount of the tetrabenzoate recovered was small (cleavage of borate ester linkages during benzoylation minimized), the major products were the 4,6-diacetate 2,3-dibenzoate (IV), the 3,4-diacetate 2,6-dibenzoate (V) and the 4-acetate 2,3,6-tribenzoate (VI), indicating 4,6-, 3,4- and 4-blocking, respectively. The minor products include the 2,3,4-triacetate 6-benzoate (III) and the tetraacetate II, indicating 2,3,4- and complete blocking. The tetrabenzoate VII could only be formed as a result of total cleavage of borate groups during the benzoylation reaction. The crystalline compounds isolated accounted for 58–73% of the original glucoside. Methyl 2,4-di-*O*-acetyl-3,6-di-*O*-benzoyl-β-D-glucopyranoside, which should be obtained as a result of 2,4-blocking, was not found, although a small amount could have been present in the sirupy fractions not identified. This compound would correspond to methyl 3,6-di-*O*-methyl-β-D-glucopyranoside, obtained by Bell[7] by the methylation of the borate–glucoside complex. However, the benzoylation procedure could lead to the selective cleavage of different borate linkages.

Apparently the important boron ring systems involved are the 6-membered ring involving the hydroxyl groups at C-4 and C-6 and the 5-membered ring involving the hydroxyl groups at C-3 and C-4 of the glucoside. These types of blocking would give rise to the 4,6-diacetate 2,3-dibenzoate (IV) and the 3,4-diacetate 2,6-dibenzoate (V), respectively. A part of the 4-acetate 2,3,6-tribenzoate (VI) may arise from cleavage of the 4,6-ring at C-6, possibly from a structure of the type X, which may be cleaved easily at the primary position. This



is indicated by experiment H in which the amount of the 4,6-diacetate 2,3-dibenzoate (IV) is higher and the 4-acetate 2,3,6-tribenzoate (VI) lower than in the other experiments. When formation of the tetrabenzoate VII was limited, the sum of the amounts of IV and VI was nearly constant. The assumption of greater lability toward cleavage of the primary function receives support from the observations[10] that primary alkyl borates hydrolyze more readily than secondary. Certain tri-*t*-alkyl borates have high hydrolytic stability. *t*-Alkyl borates may even be prepared by alcoholysis of primary alkyl borates.[11]

The results of experiments I and K, in which the time of benzoylation differed by a factor of nearly five, present an interesting comparison. Almost identical amounts of products were obtained, which suggests that the conditions imposed brought about the cleavage of the more labile borate linkages but preserved the remainder in both instances. Added support for this supposition was obtained in experiment J. In this case only one mole of metaboric acid-III was used per 2 moles of the glucoside. The glucoside dissolved to the extent of 88%, indicating that a reaction of one metaboric acid molecule with two molecules of the glucoside had taken place to a considerable extent, since the glucoside itself has little acetone solubility. The acetone-soluble portion was treated in the same fashion as in the other experiments to yield 23% of tetrabenzoate. However, the 4,6-diacetate 2,3-dibenzoate (IV), the

(10) A. Scattergood, W. H. Miller and J. Gammon, Jr., THIS JOURNAL, **67**, 2150 (1945); H. Steinberg and D. L. Hunter, *Abstracts Papers Am. Chem. Soc.*, **128**, 36-O (1955).

(11) P. D. George and J. R. Ladd, THIS JOURNAL, **77**, 1900 (1955).

3,4-diacetate 2,6-dibenzoate (V) and the 4-acetate 2,3,6-tribenzoate (VI) were recovered in amounts of the same order of magnitude as obtained in experiments I and K, again suggesting the preservation of the more stable bonds and cleavage of the more labile to form the tetrabenzoate. The tetraacetate II and the 2,3,4-triacetate 6-benzoate(III), isolated in small amounts under milder benzoylation conditions (experiments A, B, C and particularly H), were recoverable in trace or negligible amounts. These compounds probably arise because of the added protection afforded by the more labile borate linkages, preserved under these conditions, and less likely because of incomplete benzoylation.

Methyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside was obtained from the α-glucoside under comparable conditions as applied in the β-series. The recovery of this compound was of the same order of magnitude as the comparable compound (methyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-glucoside (V)) in the β-glucoside series. Studies on methyl α-D-glucoside were not extended because many of the mixed acetate-benzoate esters either are not crystalline or are not reported.

Experimental[12]

A general procedure is described. Variations are mentioned where applicable. Amounts of products indicated are those which were obtained in experiment A.

Borate Ester Complex of Methyl β-D-Glucoside.—A finely powdered mixture of methyl β-D-glucoside (1.00 g., 1 mole) and boron oxide (0.18 g., 0.5 mole) or metaboric acid-III[13] (0.23 g., 1 mole) was added to 10 ml. of anhydrous, reagent-grade acetone (prepared by drying over Drierite[13] and followed by distillation) or to 10 ml. of anhydrous, alcohol-free 1,2-dimethoxyethane. The suspension was refluxed in a water-free system. After a few minutes, all of the solids dissolved. Refluxing was continued for 2 hours. There was no change in rotation observed after solution had been effected. The acetone was removed under reduced pressure on a water-bath, the last few ml. being removed at room temperature, leaving either a clear glassy sirup or a white amorphous solid. In the case of experiment J, one-eighth of the glucoside did not react after 14 hours of refluxing and was filtered off before evaporation of the acetone. When metaboric acid was used instead of boron oxide or metaboric acid-III, the rate of solution was slow and the glucoside failed to dissolve completely.

Benzoylation of the Borate Ester Complex.—The solid residue obtained as described in the above was dissolved in 8 ml. of anhydrous pyridine (obtained by distillation over barium oxide) to which was added slowly at 0°, with agitation, 2.4 ml. of reagent-grade benzoyl chloride mixed with 8 ml. of freshly prepared, dry, alcohol-free chloroform. (In the experiments in which the chloroform was omitted, and salts precipitated during the addition of the benzoyl chloride, a mechanical stirrer was used during the course of the reaction.) After one hour at 0°, 10 ml. of methanol was added to react with the excess benzoyl chloride and to cleave the borate–glucoside complex. The reaction mixture was placed on a steam-bath and most of the solvents were removed under reduced pressure.

Acetylation of the Benzoylation Mixture.—To the residue obtained from benzoylation was added 8 ml. of acetic anhydride in 8 ml. of anhydrous pyridine. The mixture was allowed to remain at room temperature for 24 hours and then was poured over ice and water. After 15 minutes, the acetylation mixture was extracted with 75 ml. of chloroform. The chloroform solution was washed twice each with 25-ml. portions of 2 *N* hydrochloric acid and 2 *N* sodium hydroxide and once with 25 ml. of water. The solution was dried over anhydrous sodium sulfate and evaporated to a sirup on a steam-bath. While on the steam-bath, most of the methyl benzoate was removed by allowing a jet of air to blow over the sirup for about an hour. Upon cooling, 2.28 g. of a hard, glass-like sirup was obtained, which was dissolved in 8 ml. of hot methanol. When the methanol solution was cooled, 0.82 g. of a crystalline mixture was deposited. The crystalline mixture and the remaining sirup, obtained by evaporation of the methanol, were analyzed by chromatographic methods.

Chromatography of the Crystalline Mixture.—The crystalline mixture (0.15 g.), dissolved in 20 ml. of benzene, was placed on a column (190 × 33 mm., diam.) of acid-washed[14] Magnesol[15]–Celite[16] (5:1 by wt.) and developed with 700 ml. of dry, sulfuric acid-washed benzene containing 2.6 ml. of *t*-butyl alcohol. The column was extruded and separated into three sections. Elution was effected with acetone. Section A-1 (25–90 mm. from the top of the column), upon evaporation of the acetone, contained 0.09 g. of a sirup which crystallized readily. Addition of a few drops of methanol yielded 0.085 g. of methyl 3,4-di-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-glucoside (V), m.p. 160–165°. Recrystallization from methanol gave needles, m.p. 165–166°, $[\alpha]^{26}D$ +53° (*c* 2.5, in acetone). The melting point was not depressed when admixed with an authentic sample (reported[2] m.p. 166°, $[\alpha]^{23}D$ +54.8° (*c* 1.34, acetone)) prepared by acetylation of the 2,6-dibenzoate obtained by the method of Lieser and Schweizer.[17] Section A-2 (90–120 mm. from the top), upon evaporation of the acetone, contained 0.06 g. of sirup which crystallized upon cooling. Addition of a few drops of methanol yielded 0.055 g. of methyl 4-*O*-acetyl-2,3,6-tri-*O*-benzoyl-β-D-glucoside (VI), m.p. 153–156°. Recrystallization from methanol yielded a product, m.p. 156–157°, $[\alpha]^{20}D$ +91° (*c* 2.1, chloroform). The melting point of a mixture of VI and an authentic sample of m.p. 156–157° and $[\alpha]^{26}D$ +92° (*c* 2.1, chloroform), prepared by the method of Levene and Raymond,[18] who report m.p. 156–157°, $[\alpha]^{20}D$ +96.0° (*c* 2, chloroform), was not depressed. Section A-3 (120–160 mm. from the top), which in most experiments contained most of the tetrabenzoate present, upon evaporation of the acetone was shown to contain nothing. Knowledge as to the position of the various compounds on the column was determined arbitrarily by the cutting of trial columns. At times a permanganate streak reagent[8] (1:10:100 parts by wt., respectively, of potassium permanganate, sodium hydroxide and water) would give a faint indication of the zones, but in general this was unreliable. Since those compounds containing a larger number of acetyl groups are found higher up on the column, it was soon possible to predict, with some degree of accuracy, the position on the column in which any of the mixed benzoate-acetate esters would be found. At times some of the 4,6-diacetate 2,3-dibenzoate (IV) was found in the crystalline mixture as well as in the mother liquor, in which case it appeared on the column just above the 3,4-diacetate 2,6-dibenzoate (V) (20–35 mm. from the top of the column), the zones of the two compounds overlapping only slightly. In general, most of the tetrabenzoate VII and the 3,4-diacetate 2,6-dibenzoate (V), with less of the 4-acetate 2,3,6-tribenzoate (VI), and occasionally a small amount of the 4,6-diacetate 2,3-dibenzoate (IV) were found in these crystals. Results of the above series of reactions for the various experiments are summarized in Table I.

Chromatography of the Sirup.—The sirup (0.30 g.) was chromatographed in the same manner as described for the crystalline mixture. The extruded column was separated into five sections, eluted with acetone, and the solvent evaporated. The zones were located in a similar manner as described for the crystalline mixture. The sirups from each zone were crystallized by the addition of a few drops of methanol. Section A-4 (5–20 mm. from the top of the column) contained 0.04 g. of sirup which yielded 0.015 g. of methyl tetra-*O*-acetyl-β-D-glucoside (II), m.p. 103–105°, $[\alpha]^{28}D$ −17° (*c* 1.4, chloroform). This compound did not

(12) All melting points are corrected.

(13) A form of anhydrous calcium sulfate supplied by the W. A. Hammond Drierite Co., Xenia, Ohio.

(14) J. M. Sugihara and M. L. Wolfrom, THIS JOURNAL, **71**, 3509 (1949).

(15) A product of Westvaco Chlorine Products Co., South Charleston, West Virginia.

(16) A siliceous filter-aid produced by the Johns-Manville Co., New York, N. Y.

(17) T. Lieser and R. Schweizer, *Ann.*, **519**, 271 (1935).

(18) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **97**, 763 (1932).

depress the melting point of an authentic sample (reported[19] m.p. 104–105°, $[\alpha]^{20}D$ −18.2° (*c* 3.81, chloroform)) prepared by acetylating the glucoside with acetic anhydride in pyridine. Section A-5 (20–45 mm. from the top) contained 0.05 g. of sirup which yielded 0.02 g. of crystals melting at 124–128°. Recrystallization from benzene–petroleum ether (b.p. 30–60°) raised the melting point to 129–130°, $[\alpha]^{25}D$ +18° (*c* 1.2, chloroform). The melting point of a mixture with an authentic sample of 2,3,4-tri-*O*-acetyl-6-*O*-benzoyl-β-D-glucoside, prepared by the method of Ohle and Spencker,[20] who report m.p. 127°, $[\alpha]^{19}D$ +15.15° (*c* 1.65, chloroform), was not depressed. Section A-6 (45–80 mm. from the top) contained 0.09 g. of sirup which crystallized easily giving 0.07 g. of methyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-glucoside (IV), m.p. 126–130°. Recrystallization from benzene–petroleum ether (b.p. 30–60°) increased the melting point to 130–132°, $[\alpha]^{28}D$ +76° (*c* 3.6, chloroform). The melting point was not depressed when a sample was admixed with authentic IV, prepared by the method of Levene and Raymond,[18] who report m.p. 131–132°, $[\alpha]^{20}D$ +79.8° (*c* 2, chloroform). Section A-7 (80–125 mm. from the top) contained 0.08 g. of a sirup which yielded 0.04 g. of crystals melting at 154–156°. Recrystallization from methanol increased the melting point to 156–157°. This compound was identical with that obtained from section A-2 of the crystalline mixture, a mixed melting point with an authentic sample of methyl 4-*O*-acetyl-2,3,6-tri-*O*-benzoyl-β-D-glucoside (VI) showing no depression. Section A-8 (125–160 mm. from the top) contained 0.05 g. of a sirup which yielded 0.025 g. of methyl tetra-*O*-benzoyl-β-D-glucoside (VII), m.p. 158–160°. Recrystallization from methanol gave a product with a melting point of 161–162°, $[\alpha]^{26}D$ +31° (*c* 2.6, chloroform). A mixed melting point with an authentic sample of VII (reported[21] m.p. 160–162°, $[\alpha]^{19}D$ +30.79° (*c* 10.5, chloroform)) prepared by treating the glucoside with benzoyl chloride in pyridine, showed no depression. The recovery of the various compounds for the several experiments are summarized in Table I.

Preparation and Benzoylation of the Borate Ester Complex of Methyl α-D-Glucoside.—Methyl α-D-glucoside (2.00 g., 1 mole) and metaboric acid-III (0.45 g., 1 mole) were mixed with 20 ml. of anhydrous reagent-grade acetone and processed in the same manner as described for the β-glucoside. The solid borate-ester complex was dissolved in 15 ml. of anhydrous pyridine to which was added slowly, with cooling, 6.0 ml. (5 moles) of reagent-grade benzoyl chloride. The reaction mixture was left at room temperature for 18 hours after which a few milliliters of water was added. After 30 minutes the reaction mixture was poured onto 100 g. of ice and water. The resulting suspension was extracted with 100 ml. of ether. The ether solution was washed twice each with 25-ml. portions of 2 *N* hydrochloric acid and 2 *N* sodium hydroxide, and once with 25 ml. of water. The solution was dried over anhydrous sodium sulfate and concentrated to a sirup on a steam-bath. The sirup was dissolved in 20 ml. of benzene, and petroleum ether (60–120°) was added to produce a slight turbidity at about 50°. Upon cooling slowly, flocculent crystals were deposited. Recrystallization from benzene–petroleum ether in a similar manner yielded 1.0 g. (24%) of methyl 2,6-di-*O*-benzoyl-α-D-glucoside, m.p. 130–136°. Several recrystallizations from the same solvent raised the melting point to 141–142°, $[\alpha]^{30}D$ +75° (*c* 3.1, chloroform). An admixture of this compound with an authentic sample prepared by the method of Lieser and Schweizer,[17] who report m.p. 144°, $[\alpha]^{23}D$ +80.37° (*c* 4.0, chloroform), showed no depression of melting point.

Acknowledgment.—J. C. P. gratefully acknowledges support from a University Research Committee Fellowship.

SALT LAKE CITY, UTAH

(19) C. S. Hudson and J. K. Dale, THIS JOURNAL, **37**, 1264 (1915).
(20) H. Ohle and K. Spencker, *Ber.*, **59B**, 1836 (1926).
(21) E. Fischer and B. Helferich, *Ann.*, **383**, 68 (1911).

[CONTRIBUTION FROM THE DEPARTMENT OF BACTERIOLOGY OF THE UNIVERSITY OF ILLINOIS]

Biosynthesis and Structure of Lipoic Acid Derivatives[1]

BY I. C. GUNSALUS, LOIS S. BARTON AND W. GRUBER

RECEIVED AUGUST 24, 1955

A thioester transacetylase, prepared from *E. coli*, transports one acetyl group from an acetyl donor (lithium acetyl phosphate) to reduced lipoic (dihydrolipoic[2]) acid in the presence of phosphotransacetylase and Coenzyme A. This transacetylation is stereospecific for (−)-dihydrolipoic acid[3] (II) obtained by $NaBH_4$ reduction of (+)-lipoic acid (I). By chemical acetylation a monoacetyl-dihydrolipoic acid has been prepared, and shown to be 8-S-acetyl-6,8-dithioloctanoic acid (IVb). The enzymatically prepared compound is different from the synthetic one and contains a free primary SH-group; therefore, it is 6-S-acetyl-6,8-dithioloctanoic acid (IIIa). The basic carbodiimide (VI) was used for the characterization of the acids producing crystalline and well defined ureas; N-propionyldiphenylketimine (VII) was found to be a useful reagent for the differentiation of primary and secondary thiol groups.

Lipoic acid, an essential cofactor for keto acid oxidation,[4] has been isolated by following its catalytic activity in a pyruvate oxidation assay employing cells of *Streptococcus faecalis* grown in a medium deficient in this substance.[5] Chemically, lipoic acid has been identified as the dextrorotatory isomer of 5-(dithiolane-3)-pentanoic acid.[6,7] Subsequent synthesis of (±)-lipoic acid yielded a racemate with biological activity only 50% of that shown by the dextrorotatory acid.[8,9] Synthesis of the (+)- and (−)-isomers[10] has further confirmed the optical specificity of the catalyst, *i.e.*, synthetic (+)-lipoic acid possesses activity equal to the isolated material whereas the (−)-isomer is inactive. Hypotheses of the mechanism of lipoic acid function in the keto acid oxidation have been summarized as[11] in Fig. 1.

As indicated, three derivatives of lipoic acid are visualized as playing catalytic roles. Two of the

(1) We wish to express our appreciation to the United States Public Health Service, Department of Health, Education, and Welfare, which have supported in part this research.

(2) The pyruvate oxidation factor (POF) first isolated as a yellow crystalline solid, m.p. 49–50°, $[\alpha]^{20}D$ +100°, was termed α-lipoic acid. This catalyst, identified as 5-(dithiolane-3)-pentanoic acid, has since been shown to react enzymatically in the disulfide and dithiol forms. For simplicity, the term lipoic is suggested for the disulfide and the term dihydrolipoic acid is used to designate the reduced or dithiol derivative (6,8-dithioloctanoic acid).

(3) All reference here is to the sign of rotation, the biologically active disulfide (+)-lipoic acid is reduced to a biologically active (−)-dihydrolipoic acid, and enzymatically acetylated to the (+)-6-S-acetyl-dihydrolipoic acid. Chemical and biological evidence indicates these acids to be of the same configuration.

(4) D. J. O'Kane and I. C. Gunsalus, *J. Bact.*, **56**, 499 (1948).
(5) L. J. Reed, *et al.*, *Science*, **114**, 93 (1951).
(6) L. J. Reed, *et al.*, THIS JOURNAL, **75**, 1267 (1953).
(7) J. A. Brockman, *et al.*, *ibid.*, **74**, 1868 (1952).
(8) C. S. Hornberger, Jr., *et al.*, *ibid.*, **75**, 1273 (1953).
(9) M. W. Bullock, *et al.*, *ibid.*, **74**, 1868 (1952).
(10) E. Walton, *et al.*, *ibid.*, **76**, 4748 (1954).
(11) I. C. Gunsalus, in "The Mechanism of Enzyme Action," The Johns Hopkins Press, Baltimore, Md., 1954, pp. 545.