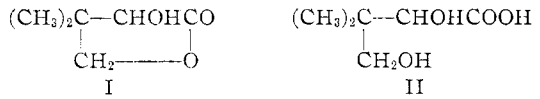
[CONTRIBUTION FROM THE MERCK RESEARCH LABORATORIES]

Resolution of Racemic α-Hydroxy-β,β-dimethyl-γ-butyrolactone

BY RALPH BEUTEL AND MAX TISHLER

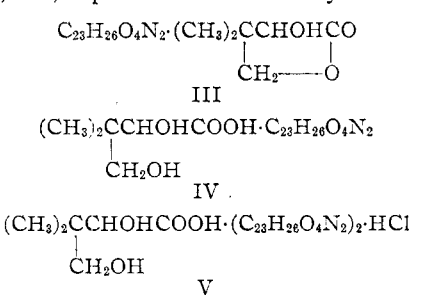
Levorotatory α-hydroxy-β,β-dimethyl-γ-butyrolactone, I, an essential component in the synthesis of pantothenic acid, has been obtained by converting the racemic lactone into alkaloidal salts of the corresponding α,γ-dihydroxy-β,β-dimethylbutyric acid, II, separating the required diastereoisomer, and isolating the optically active lactone from the resolved alkaloidal salt.



I II

The above method of resolution has been carried out successfully using quinine[1a,b,c,d] and other alkaloids[1d] as well as the methohydroxides of quinine, cinchonine and quinidine.[2] Although the use of quinine is excellently suited for the resolution, the restricted use of this alkaloid from the outset of hostilities prompted us to study the use of other alkaloids. Of a number investigated, brucine proved to be best. It also proved to be the most interesting, as three distinctly different brucine complexes can be prepared depending on the procedure employed.

When warm solutions of brucine and of the racemic lactone, I, are mixed, a molecular complex of brucine and *l*-α-hydroxy-β,β-dimethylbutyrolactone, III, separates in excellent yields.



III

IV

V

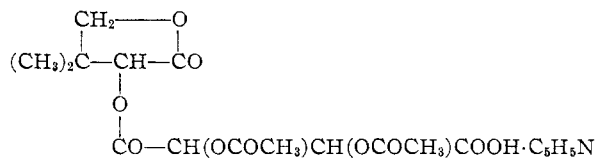
The physical constants of the complex, optical rotation, solubility and melting point, are distinctly different from those of the brucine salt of the butyric acid IV, prepared by treating the barium salt of II with brucine sulfate. It is noteworthy that brucine differs from quinine in its behavior toward the lactone, I. When an ethanol solution of the latter and of quinine are heated, the quinine salt of the butyric acid, II, is formed.[1b]

The brucine complexes of the *d* and *l*-forms of the butyrolactone differ considerably in their solubilities in most solvents, the brucine complex of the biologically active, levorotatory isomer being the less soluble. The solubility difference is sufficiently favorable to permit an excellent resolution when one half mole of brucine per mole of *r*-lactone is employed. The solubility effect is illustrated also by the fact that the brucine salt of the *l*-lactone separates upon addition of the *l*-lactone to an alcohol solution of the brucine complex of the *d*-lactone.

When the resolution method of Stiller and coworkers,[1a] consisting of treating the sodium salt of the racemic lactone with quinine hydrochloride, was carried out with brucine hydrochloride, a precipitate was formed which by analysis and synthesis was established as a complex corresponding to V. The new complex on decomposition and lactonization of the dihydroxy acid gave almost pure levorotatory lactone, I. The complex, V, also was formed as a precipitate by adding one equivalent of brucine hydrochloride to an aqueous solution of the brucine salt of *d*-α,γ-dihydroxy-β,β-dimethylbutyric acid.[3]

The brucine salt of *d*-α,γ-dihydroxy-β,β-dimethylbutyric acid was prepared by interaction of the barium salt of the optically active acid and of brucine sulfate. Under the same conditions the racemic acid yields a mixture of brucine salts in which the levo- and not the dextrorotatory dihydroxy acid predominates. This method is not practical for the preparation of the natural lactone.

The racemic lactone was resolved also by acylation with diacetyl-*d*-tartaric anhydride.[4] The reaction was carried out in benzene solution containing pyridine as a catalyst. The pyridine salt of the tartrate ester of the (−) lactone, VI, separates preferentially from the mixture providing a facile method of resolution.



VI

The pyridine salt on treatment with acid is con-

(1) (a) Stiller, Harris, Finkelstein, Keresztesy and Folkers, THIS JOURNAL, **62**, 1785 (1940); (b) Reichstein and Grüssner, *Helv. Chim. Acta*, **23**, 655 (1940); Grüssner, Gatzi-Fichter and Reichstein, *ibid.*, 1276 (1940); (c) Kuhn and Wieland, *Ber.*, **73**, 971 (1940); (d) Harris and Folkers, U. S. Patent 2,319,545 (1943) [*C. A.*, **37**, 6280 (1943)].

(2) Major and Finkelstein, THIS JOURNAL, **63**, 1368 (1941).

(3) As pointed out by previous investigators, ref. 1a, the optical rotation of the biologically active lactone, I, is levorotatory, whereas the rotation of the corresponding dihydroxy acid is dextrorotatory.

(4) It is surprising that the diacyl-*d*-tartaric anhydrides have not been used for the resolution of alcohols. The acylated anhydrides are readily prepared [Wohl and Oesterlim, *Ber.*, **34**, 1144 (1901); Lucas and Baumgarten, THIS JOURNAL, **63**, 1655 (1941)] and are excellent acylating agents. As the acylated product has a free carboxyl group, the opportunity for salt formation in facilitating resolution is present. We believe that the use of the acylated tartaric anhydrides in many instances would be superior to the classical methods.

verted to the free tartrate acid ester of the lactone, whereas hydrolysis with stronger acid regenerates the optically active lactone.

Experimental

Brucine Complex of α-Hydroxy-β,β-dimethyl-γ-butyrolactone, III. (A) Brucine Complex of *l*-Lactone, III.—To a warm solution of 46.6 g. of brucine (0.1 mole) in 93 cc. ethanol was added 26 g. (0.2 mole) of *r*-α-hydroxy-β,β-dimethyl-γ-butyrolactone dissolved in 30 cc. of ethanol. Crystallization of the product occurred within a few minutes. After cooling at 5° for several hours, the product was separated by filtration and washed with ethanol. The complex was recrystallized twice from hot 95% ethanol, using 540 cc. in the first recrystallization and 440 cc. in the second. The dried brucine complex weighed 46.5 g. (78%) and melted at 211–212°; $[\alpha]^{25}D$ +87.5° (*c*, 0.1 g. in 5 cc. chloroform).

Anal. Calcd. for $C_{29}H_{36}O_7N_2$: C, 66.39; H, 6.92; N, 5.34. Found: C, 66.52; H, 6.96; N, 5.51.

When the above brucine complex (46.5 g.) was decomposed in the manner described below, pure *l*-lactone was obtained; wt. 8.4 g.

The same complex also separated when the reaction was carried out in water, ethyl acetate or dioxane. The complex is sparingly soluble in cold water, but very soluble in hot ethyl acetate. The brucine complex of the *l*-lactone was prepared also by mixing methanol solutions of equivalent quantities of brucine and of the pure *l*-lactone. The physical properties of the complex corresponded to the purified complex described above.

(B) Brucine Complex of *d*-Lactone.—The brucine complex was prepared by heating a mixture of the pure *d*-lactone (0.65 g.) and brucine (2.3 g.) in ethanol. The complex crystallized on storing the mixture at 5° for several hours. After recrystallization from ethanol the product melted at 165–168°; $[\alpha]^{25}D$ +80.0° (*c*, 0.1 g. in 5 cc. chloroform). The brucine complex is soluble in water and in hot ethyl acetate.

When an ethanol solution of pure *l*-lactone (0.65 g.) was added to a solution of the brucine complex of the *d*-lactone (m. p. 165–168°) in ethanol (3 g. in *ca.* 60 cc. ethanol) essentially pure brucine complex of the *l*-lactone separated; m. p. 208–211°. After recrystallization from ethanol, the product melted at 211–212°.

Brucine Salts of α,γ-Dihydroxy-β,β-dimethylbutyric Acid.—The brucine salt of the dextrorotatory acid was prepared from the corresponding pure *l*-lactone. A solution of 3.25 g. of the lactone and 2.5 g. of barium hydroxide monohydrate dissolved in 10 cc. water was heated at 90° for fifteen minutes. The excess alkali was neutralized with sulfuric acid and the barium sulfate was removed by filtration. To the filtrate was added a solution of 11.7 g. of brucine in 25 cc. of 1 *N* sulfuric acid and 125 cc. of water. As the filtered mixture showed no tendency to crystallize, it was concentrated to dryness under reduced pressure. The oily residue was dissolved in 25 cc. of warm isopropanol and the solution was diluted with an equal volume of ether. The solid product was separated and recrystallized from ethanol. The brucine salt melted at 212–214° (mixed m. p. with brucine complex, III, gave no depression); $[\alpha]^{26}D$ −3.25° (*c*, 0.1 g. in 5 cc. chloroform).

Anal. Calcd. for $C_{29}H_{38}O_8N_2$: C, 64.19; H, 7.06; N, 5.16. Found: C, 64.14; H, 6.68; N, 4.98.

The above brucine salt in contrast to the brucine complex, III, is very soluble in water and slightly soluble in ethyl acetate.

Under the same conditions, starting with the racemic lactone, a mixture of brucine salts consisting predominantly of the levo hydroxy acid was obtained. The barium salt of the *r*-hydroxy acid prepared from 13.0 g. of the lactone in 50 cc. water was treated with a solution of 23.3 g. brucine in 50 cc. water containing one equivalent of sulfuric acid. The resulting filtered mixture was stored at 5° for fourteen hours. The separated solid was washed with 50% acetone and weighed 7.7 g. When the brucine salt was decomposed and worked up in the manner described below, the isolated lactone fraction was dextrorotatory; $[\alpha]^{25}D$ +15° (*c*, 2% H_2O). Recrystallizations of the above brucine salt brought about further concentration of the *d*-lactone.

Brucine Complex, V.—A mixture of 2.60 g. of the *r*-lactone and 20 cc. of 1.04 *N* sodium hydroxide was heated on the steam-bath for fifteen minutes. The excess caustic was neutralized with dilute hydrochloric acid. To the solution was added 9.32 g. of brucine dissolved in 24 cc. of hot water containing 8 cc. of 2.5 *N* hydrochloric acid. The resulting mixture was cooled and after several hours the solid was filtered and washed with ice water; wt. 8.6 g. A sample recrystallized from isopropanol had a wide melting point (205–250°); $[\alpha]^{25}D$ −11.8° (*c*, 0.1 g. in 5 cc. chloroform). The complex is fairly soluble in water at room temperature and only slightly soluble in hot ethyl acetate.

Anal. Calcd. for $(C_{23}H_{26}O_4N_2)_2 \cdot C_6H_{12}O_4 \cdot HCl \cdot H_2O$: C, 62.98; H, 6.81; N, 5.65. Found: C, 62.49; H, 7.26; N, 5.79.

Chemical analyses for brucine, the lactone and chlorine established the respective ratios of 1.04 brucine:0.44 lactone:0.50 chlorine.

When the complex isolated above (8.6 g.) was decomposed and worked up for the lactone in the manner described below, 0.94 g. of the *l*-lactone, I, was isolated (72.3% over-all yield); $[\alpha]^{25}D$ −47.0° (*c*, 2% water).

The same complex, V, was prepared by the method described above starting with pure *l*-lactone. It was also prepared from the brucine salt of the *d*-dihydroxy acid, IV, and brucine hydrochloride. In the latter instance, a solution of 0.54 g. of the brucine salt in 1.63 cc. of water was added to a solution of 0.46 g. of brucine in 2 cc. of water and 0.40 cc. of 2.5 *N* hydrochloric acid. Within a few minutes, a heavy precipitate formed which was filtered and washed with acetone. In both experiments the products, after recrystallization from isopropanol, were identical with that obtained in the resolution experiment.

Isolation of *l*-α-Hydroxy-β,β-dimethyl-γ-butyrolactone from Brucine Complexes.—The following procedure was applied to the brucine derivatives mentioned above. A solution or mixture of the brucine complex (about 40 g.) in 200 cc. of chloroform was stirred with 80 cc. of 2.5 *N* sodium hydroxide for about one hour. The water layer was extracted with small amounts of chloroform to remove traces of the alkaloid. It was then acidified with hydrochloric acid to *p*H 3 and heated on the steam-bath for fifteen minutes. The solution was saturated with ammonium sulfate and extracted several times with chloroform. The chloroform extracts were concentrated to dryness under reduced pressure whereupon the product crystallized.

Resolution of *r*-Lactone using Diacetyl *d*-Tartaric Anhydride.—To a mixture of 64.5 g. of the *r*-lactone and 108 g. of diacetyl-*d*-tartaric anhydride in 250 cc. of dry benzene was added 79 g. of anhydrous pyridine. The temperature was maintained at 50° for one hour. After the completed reaction mixture was stored at room temperature for ten hours, the pyridine salt was filtered and washed well with benzene. The crude product weighing 82.5 g. and melting at 157–160° was recrystallized twice by dissolving in 400 cc. of hot isopropanol and adding an equal volume of ether to the cooled solution. The pure pyridine salt weighed 52.9 g. and melted at 164–165°.

The pyridine salt was decomposed by refluxing a solution of 32.1 g. in 150 cc. of 2 *N* sulfuric acid for two hours. The solution was cooled, saturated with ammonium sulfate and extracted several times with chloroform. The combined extracts were concentrated to dryness under reduced pressure and the residue was washed with petroleum ether. The yield of *l*-lactone was 9.2 g.; m. p. 88–89°; $[\alpha]^{25}D$ −49.1° (*c*, 2% water). The over-all yield of pure *l*-lactone from the *r*-lactone was 45%.

The diacetyl-*d*-hydrogen tartrate ester of *l*-α-hydroxy-β,β-dimethyl-γ-butyrolactone was prepared from the pyridine salt by dissolving the latter in water and acidifying with hydrochloric acid to *p*H 3. The precipitate

was filtered and recrystallized from ethyl acetate; m. p. 188°; $[\alpha]^{25}D$ −1.2° (*c*, 0.1 g. in 5 cc. ethanol).

Anal. Calcd. for $C_{14}H_{18}O_{10}$: C, 48.54; H, 5.24. Found: C, 48.56; H, 5.35.

Summary

Racemic α-hydroxy-β,β-dimethyl-γ-butyrolactone was readily resolved by the use of brucine and of diacetyl-*d*-tartaric anhydride. With brucine two different complexes have been prepared, both yielding the biologically active levorotatory lactone.

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Isolation and Structure of an Enzymatically Synthesized Crystalline Disaccharide D-Glucosido-D-ketoxyloside

BY W. Z. HASSID, M. DOUDOROFF, H. A. BARKER AND W. H. DORE

In a preliminary report[1] evidence was presented indicating that preparations from the bacterium *Pseudomonas saccharophila*, capable of synthesizing sucrose[2] from glucose-1-phosphate and fructose, can also combine glucose-1-phosphate with L-sorbose or D-ketoxylose to form the corresponding disaccharides. One of these disaccharides has already been isolated in crystalline form and its structure appears to be α-D-glucopyranosido-α-L-sorbofuranoside.[3] The present work deals with the preparation and the molecular constitution of the other crystalline disaccharide, D-glucosido-D-ketoxyloside, formed from α-D-glucose-1-phosphate and D-ketoxylose by the phosphorylase from *Pseudomonas saccharophila*.

This disaccharide does not reduce Fehling solution or alkaline ferricyanide. Its empirical formula obtained by elementary analysis is $C_{11}H_{20}O_{10}$. The compound is practically unaffected by invertase, but is easily hydrolyzed with acid. When the disaccharide is hydrolyzed with acid and the glucose fermented out, an osazone is obtained which is identical with that of xylose. The specific rotation of the disaccharide is $[\alpha]D$ +43°. Hydrolysis with 1 *N* hydrochloric acid changes the rotation to +16.2°. Taking Schmidt and Treiber's[4] value for the specific rotation of ketoxylose as −33.2°, the calculated rotation of an equimolar mixture of glucose and D-ketoxylose in water is +14.3°. The melting point of the disaccharide is 156–157°. Its rate of hydrolysis with acid is approximately 30% greater than that of sucrose. The acetylated derivative has a rotation in chloroform, $[\alpha]D$ +22° and a melting point of 180–181°.

Since the disaccharide is non-reducing, the glucose and D-ketoxylose units are obviously linked through the carbonyl groups. Inasmuch as the carbonyl group in ketoxylose occurs on the second carbon atom, the largest possible semiacetal ring for the ketose component is the 2,5-furanose ring and the possibility of a pyranose ring is definitely excluded. Smaller rings such as the 2,3 or 2,4 ring are sterically improbable. The furanose structure of the ketoxylose was definitely confirmed experimentally by oxidation of the disaccharide with sodium periodate. A disaccharide consisting of glucopyranose and ketoxylofuranose glycosidically united through positions 1 and 2 of the aldose and ketose monosaccharides, would possess three adjacent free hydroxyls on carbon atoms 2, 3 and 4 in the glucose residue and two free hydroxyls on carbon atoms 3 and 4 in the ketoxylose residue. When subjected to oxidation, a disaccharide of this structure should consume two moles of periodate and form one mole of formic acid due to the glucose residue and consume one mole of periodate due to the ketoxylose residue. A total of 3 moles of periodate would thus be consumed and one mole of formic acid should be formed per mole of disaccharide. Actually, on oxidation of the carbohydrate with periodate, 2.96 moles of periodate are consumed and 0.95 mole of formic acid is formed.

Like the previously synthesized D-glucosido-L-sorboside,[3] this disaccharide gives a blue-green color with diazouracil, a reaction shown by Raybin[5] to be specific for compound sugars containing the same type of glycosidic glucosefructose linkage that exists in sucrose.[6] The fact that phosphorylase from *Pseudomonas saccharophila*, capable of synthesizing sucrose from glucose-1-phosphate and fructose,[2] can also effect the synthesis of a disaccharide from glucose-1-phosphate and D-ketoxylose, indicates that the linkage joining the two monosaccharide units in the glucosido-ketoxyloside is probably the same as that existing in sucrose. The formation of the disaccharide is a product of "de-phosphorolytic" condensation involving α-D-glucose-1-phosphate. This is good evidence that glucose exists in the disaccharide as the α-form.

(1) M. Doudoroff, W. Z. Hassid and H. A. Barker, *Science*, **100**, 315 (1944).

(2) W. Z. Hassid, M. Doudoroff and H. A. Barker, THIS JOURNAL, **66**, 1416 (1944).

(3) W. Z. Hassid, M. Doudoroff, H. A. Barker and W. H. Dore, *ibid.*, **67**, 1394 (1945).

(4) O. T. Schmidt and R. Treiber, *Ber.*, **66**, 1765 (1933).

(5) H. W. Raybin, THIS JOURNAL, **55**, 2603 (1933); **59**, 1402 (1937).

(6) C. B. Purves and C. S. Hudson, *ibid.*, **59**, 1170 (1937).