therein was the first mixture on which this analytical procedure was tried. The difference of 2.7% between observed and calculated percentage of glucose is higher than that encountered in subsequent determinations, and it was usually less than 2%. Errors in the di- and trisaccharide fractions tended to be a little higher (about 2–4%). If one of these fractions was small the error was larger than usual, as in the fourth mixture of Table I. Such an error may be counteracted in part by taking a larger sample (see last mixture, Table I). The disadvantage of a large sample ordinarily is the greater time required for the distillation.

Mixture Containing Fructose.—The mixture was composed of fructose hydrate (5.06 g.), glucose (5.00 g.), sucrose (4.99 g.), raffinose pentahydrate (5.35 g.). On an anhydrous basis the weights would be 4.60, 5.00, 4.99, 4.54 g.; theoretical percentages 24.1, 26.1, 26.1, 23.7, respectively. Very mild conditions were used to avoid decomposition of the fructose propionate. The degassing operation and the pressure lowering were done at 140–150°. Distillation started at the bath temperature of 165° (0.003 mm.) which was unusually low for mixtures. Ebullition was violent and some fumes were carried away to the trap. The material darkened considerably but the distillate was pale yellow in color. Rapid distillation occurred between 168–175°, after which the temperature was raised gradually to 195° (0.002 mm.); yield, 11.02 g. in this first fraction. It was believed to contain the fructose pentapropionate and some of the glucose analog. At 195° the distillate became more nearly colorless and the remainder of the glucose pentapropionate was collected separately (8.63 g.). After this, there was 10.57 g. of a disaccharide fraction, and 8.37 g. of a weighed residue of trisaccharide. The theoretical residue should have been 10.17 g.

From the appearance of the fructose fraction as it distilled, some decomposition evidently was occurring. It was estimated that 1.3 g. of the 1.8 g. of total loss was from the fructose portion. This figure is about 12% of the fructose fraction. It was arrived at otherwise, however, by assuming that the loss of about 0.06 g. for each 1 g. of weighed trisaccharide residue, which was noticed in runs without fructose, should maintain itself as the approximate trisaccharide loss if fructose was present. In this case, it would be 8.4 × 0.06 or 0.5 g.

Thus, the combined weight of monosaccharide propionates was 20.95 g. (11.02 + 8.63 + 1.3); disaccharide propionate 10.57 g.; and trisaccharide propionate 8.87 g. by difference (10.17 − 1.3). From these data these percentages follow: monosaccharide 48.9, sucrose 27.3, raffinose 23.8, compared with 50.2, 26.1, 23.7, respectively. If the trisaccharide fraction is taken as 8.37 g. (the weighed residue) then these percentages follow: monosaccharides 49.6, sucrose 27.6, raffinose 22.8.

The sample taken contained 24.1% fructose and 26.1% glucose. The first fraction of distillate (11.02 g. or 12.32 g., corrected) contained glucose as well as fructose. If 1.58 g. is subtracted from the 11.02 g., or if 2.27 g. is taken from the 12.32 g., and added to the weight (8.63 g.) of the second fraction then the correct ratios for fructose and glucose would appear. The correction of 1.58 g. is 18% of the weight of the second fraction.

Summary

A method of analysis of sugar mixtures is presented which is applicable for mixtures of mono-, di- and trisaccharides. In this method the carbohydrates are converted to propionic esters and distilled under controlled conditions in a special apparatus. The method includes most sugars without the necessity of correction factors and will even include fructose if appropriate corrections are introduced. The accuracy which has been attained for monosaccharides is about 1–2%, and for di- and trisaccharides about 2–4%.

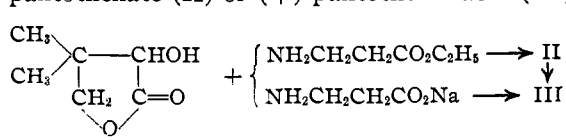
EVANSTON, ILLINOIS RECEIVED JULY 5, 1941

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

On the Synthesis of Pantothenic Acid and Derivatives*

BY STANTON A. HARRIS, GERALD A. BOYACK AND KARL FOLKERS

Subsequent to the announcement of the structure and synthesis of pantothenic acid,[1] details of the structure[2,3] and synthesis[4,5] were published. The last step in the synthesis involved the reaction between (−)α-hydroxy-β,β-dimethyl-γ-butyrolactone (I) and β-alanine ethyl ester or the sodium salt of β-alanine to give either ethyl (+)-pantothenate (II) or (+)-pantothenic acid (III).



I

$HOCH_2C(CH_3)_2CHORCONHCH_2CH_2CO_2R'$

II. R = H, R′ = C_2H_5
III. R and R′ = H (pantothenic acid)
IV. R = CH_3CO—, R′ = C_2H_5—
V. R = p-$NO_2C_6H_4CO$—, R′ = H
VI. R = $C_6H_5CH_2OCO$—, R′ = C_2H_5—

* This paper was presented before the Organic Division of the American Chemical Society at St. Louis, Missouri on April 8, 1941.

(1) Williams and Major, *Science*, **91**, 246 (1940).

(2) Mitchell, Weinstock, Jr., Snell, Stanberry and Williams, THIS JOURNAL, **62**, 1776 (1940).

(3) Stiller, Keresztesy and Finkelstein, *ibid.*, **62**, 1779 (1940).

(4) Williams, Mitchell, Weinstock and Snell, *ibid.*, **62**, 1784 (1940).

(5) Stiller, Harris, Finkelstein, Keresztesy and Folkers, *ibid.*, **62**, 1785 (1940).

In the interim, experiments on the reaction of the *dl*-lactone (I) with β-alanine[6] and the *dl*-lactone with the sodium salt of β-alanine[7] have been described as yielding pantothenic acid in solution. The reactions between the (−) and *dl*-lactone and β-alanine methyl ester[8] and β-alanine benzyl ester[8a] also have been described. All these investigators have combined the lactone with either a β-alanine ester or sodium salt.

This reaction has now been applied successfully to the three diverse acyl derivatives of the lactone to give the corresponding pantothenic acid derivatives. The acetyl derivative of the (−)lactone[3] (I) and *p*-nitrobenzoyl derivative[3] were combined with β-alanine ethyl ester and the sodium salt of β-alanine, respectively, to give ethyl monoacetylpantothenate (IV) and mono-*p*-nitrobenzoylpantothenic acid (V). Of these derivatives, IV was an oil distillable in high vacuum whereas V was crystalline.

It was of interest to test mono-*p*-nitrobenzoylpantothenic acid microbiologically, because nothing is known about derivatives of pantothenic acid having the α-hydroxy group protected. Its activity was kindly determined by Dr. John C. Keresztesy in this Laboratory, who reported that it was inactive (or had less than 1% of the activity of pantothenic acid) for the growth stimulation of *Lactobacillus casei*.[9]

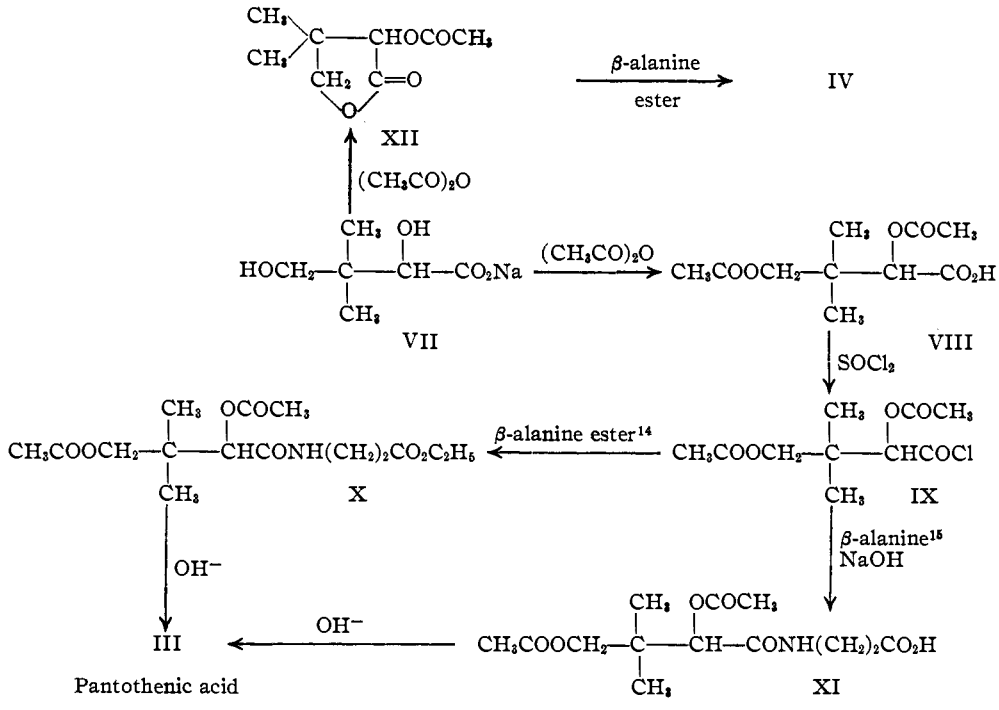
The carbobenzoxy group introduced[10] for the protection of amino groups in peptide syntheses has been utilized in these pantothenic acid studies. The carbobenzoxy derivative of the lactone (I) was made and combined with β-alanine ester to give ethyl carbobenzoxypantothenate (VI) which was an oil distillable in a high vacuum. When the carbobenzoxy lactone was treated with the sodium salt of β-alanine in aqueous solution, a group interchange took place and N-carbobenzoxy-β-alanine[11] was isolated.

A classical method for synthesis of pantothenic acid, which is a substituted N-acyl-β-aminopropionic acid, would be to treat the suitable acyl chloride with a β-alanine ester or salt. As soon as the amide character of pantothenic acid was realized,[12,13] Woolley, Waisman and Elvehjem[13] were successful in applying this method for partial synthesis. Since the acyl group of pantothenic acid was known to possess free hydroxy groups, acetylation was introduced to protect the hydroxy groups prior to the thionyl chloride treatment for preparation of the acyl chloride. After the announcement of the pantothenic acid structure[1] showing that the lactone moiety was a gamma lactone, the same fundamental acetic anhydride–thionyl chloride steps were used by Woolley[14,15] for syntheses, and since active pantothenic acid was obtained, it might be assumed that the reaction proceeded by the steps VII → VIII → IX → X or XI → III.

When synthetic (−)lactone (I) became available, it was converted to the sodium salt (VII) and then carried through the above steps.[13] Since the final pantothenic acid derivative was apparently a mixture of mono and diacetyl ethyl pantothenate, we investigated the intermediate products of the synthesis with the larger quantities of material which were available to us. These experiments demonstrated that the sodium salt (VII) with acetic anhydride gave the known acetyl lactone (XII) together with the diacetyl acid. Another experiment was made to determine the relative proportion of these two products. The ether extract from the acetylation of the sodium salt was fractionally distilled and it was found to consist of 73.5% of the acetyl lactone and 17.7% of diacetyl acid. The 73.5% yield of the acetyl lactone is nearly a true value, whereas the 17.7% of diacetyl acid is low because of the distillation loss of this higher boiling fraction. Others[16] have observed the recovery of lactones on attempting to acylate sodium salts of the hydroxy acids corresponding to lactones.

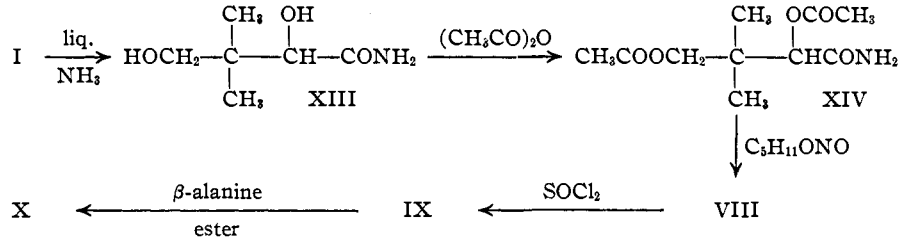
Therefore, we believe that the reactions used by Woolley, *et al.*,[13,14,15] for the synthesis of pantothenic acid proceeded only to a small extent in the direction involving interaction of the acid chloride with the β-alanine ester or salt and that the major course of the reaction involved the interaction of

(6) Weinstock, Jr., Arnold, May and Price, *Science*, **91**, 411 (1940). The manner in which β-alanine was used is not described.
(7) Babcock, Jr., and Jukes, THIS JOURNAL, **62**, 1628 (1940).
(8) Reichstein and Grüssner, *Helv. Chim. Acta*, **23**, 650 (1940).
(8a) Kuhn and Wieland, *Ber.*, **73**, 971 (1940).
(9) Snell, Strong and Peterson, *J. Bact.*, **38**, 293 (1939).
(10) Bergmann and Zervas, *Ber.*, **65**, 1192 (1932).
(11) Sifferd and du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
(12) Williams, Weinstock, Jr., and Mitchell, Abstracts, Division of Organic Chemistry, Amer. Chem. Soc., Milwaukee, Wis., 1938.
(13) Woolley, Waisman and Elvehjem, THIS JOURNAL, **61**, 977 (1939); *J. Biol. Chem.*, **129**, 673 (1939).
(14) Woolley, *J. Biol. Chem.*, **134**, 461 (1940).
(15) Woolley, THIS JOURNAL, **62**, 2251 (1940).
(16) For example, Ohle and Wolter [*Ber.*, **63**, 843 (1930)] obtained triacetyl-2-ketogluconic acid lactone from the sodium salt in pyridine with acetic anhydride. Dr. E. T. Stiller (unpublished data) in this Laboratory observed a similar reaction for other γ-hydroxy aliphatic acids.



the acetyl lactone with the β-alanine ester or salt. When the sodium salt of the acid was treated with acetic anhydride, freed of excess reagent and then treated with thionyl chloride (the excess of which was afterward removed by distillation), a 60% yield of acetyl lactone was obtained by distillation of the reaction product. In another experiment, after the removal of the excess thionyl chloride, the residue contained 4.16% chlorine, whereas the theoretical amount of chlorine in the diacetyl acid chloride is 14.15%. This chlorine content indicated that the product consisted of about 29% of the diacetyl acid chloride and about 71% of acetyl lactone. This content of acetyl lactone is in good agreement with 73.5% shown to be present in the distillate from the ether extract described above. The agreement between the acetyl lactone content of the two preparations indicates that reaction between the acetyl lactone and thionyl chloride to give an acid chloride must have been negligible.

Because of our experience with low yields in attempts to acylate the sodium salt of (+)α,γ-dihydroxy-β,β-dimethylbutyric acid (VII), we prepared the diacetyl acid derivative (VIII) indirectly through the following reactions, I → XIII → XIV → VIII. The diacetyl acid was then treated with thionyl chloride to give the acid chloride (IX) which in turn was treated with β-alanine ethyl ester to give pure ethyl diacetylpantothenate (X) which on saponification yielded pantothenic acid.



Reichstein and Grüssner[8] obtained the racemic amide by treating the optically active lactones with alcoholic ammonia. Our amide was prepared with liquid ammonia and was optically active.

It was of interest to test our samples of ethyl pantothenate and ethyl monoacetyl pantothenate microbiologically and in rats and chicks because of previous reports.[17] This investigation was done

(17) Mitchell, Weinstock, Snell, Stanberry and Williams [THIS JOURNAL, **62**, 1776 (1940)] found it necessary to hydrolyze methyl acetylpantothenate for microbiological assay. Snell, Strong and Peterson [*Biochem. J.*, **31**, 1789 (1937)] found that acetylation inactivated their growth factor for lactic acid bacteria. Woolley, Waisman, Mickelsen and Elvehjem [*J. Biol. Chem.*, **125**, 715 (1938)]

in the Merck Institute for Therapeutic Research by Dr. Unna and Mr. Mushett who found that these esters of pantothenic acid were active in rats and chicks, but were practically inactive microbiologically. Their studies on the biological utilization of these esters will be described elsewhere.

Experimental Part

Ethyl N-(α-Acetoxy-β,β-dimethyl-γ-hydroxybutyryl)-β-aminopropionate (Ethyl Monoacetylpantothenate) IV.—The sodium salt (VII) (2 g.) was acetylated with an excess of acetic anhydride as described.[13] A crystalline precipitate separated which was filtered, washed with ether and found to be sodium acetate. The acetic anhydride was distilled *in vacuo* and the residual sodium acetate removed by treatment with ether. The oily residue from the ether solution was treated with an excess of thionyl chloride which was removed later by vacuum distillation. The product was condensed with a 20% excess of β-alanine ester in pyridine as described.[13] There was no visible evidence of the formation of pyridine hydrochloride. The solution was warmed and the excess of pyridine was removed *in vacuo*. The residue was treated with ice water and made acid to congo red paper with hydrochloric acid. The oily droplets were extracted by chloroform which was washed, dried and concentrated. The oily concentrate was distilled at 10^{-5}–10^{-6} mm. at a bath temperature of 100–110°. After redistillation, the following analysis was obtained.

Anal. Calcd. for $C_{13}H_{23}O_6N$: C, 53.96; H, 8.01; N, 4.84; acetyl, 14.88. Found: C, 54.22, 53.96; H, 8.30, 8.30; N, 4.58; acetyl, 19.75.

Anal. Calcd. for $C_{15}H_{25}O_7N$: C, 54.37; H, 7.61; N, 4.23; acetyl, 25.98.

The analyses indicated that the substance was a mixture of the mono- and diacetates of ethyl pantothenate. This was confirmed by boiling the substance for four hours with 0.1 *N* sodium hydroxide solution when only 2.3 moles was used for the saponification.

Pure monoacetyl ethyl pantothenate was made by heating acetyl lactone and β-alanine ester together without solvent. The resulting oil was distilled at 10^{-6} mm. A non-viscous oil was obtained below 100°, and a viscous oil was obtained at about 120° bath temperature. This oil was hygroscopic.

Anal. Calcd. for $C_{13}H_{23}O_6N$: C, 53.96; H, 8.01; N, 4.84; acetyl, 14.88. Found: C, 53.83; H, 7.86; N, 4.63; acetyl, 13.2.

Acetylation of Sodium α,γ-Dihydroxy-β,β-dimethylbutyrate.—The sodium salt (28.2 g.) was refluxed for one-half hour with acetic anhydride and the solution concentrated to dryness. After adding 20 cc. each of 2.5 *N* hydrochloric acid and water, an oily, ether-soluble layer separated. The mixture was extracted for six hours with ether; the extract was concentrated and the sirupy residue was distilled at 10^{-3} mm. The yield of monoacetyl lactone was 19.06 g. (67%) and the yield of the diacetyl acid was 4.56 g. (12%). The main loss in yield occurred in the extraction process. Addition of sodium acetate to the acetylation mixture did not increase the yield of the diacetyl acid. The lactone fraction crystallized when seeded with a known sample of α-acetoxy-β,β-dimethyl-γ-butyrolactone.[3] After three recrystallizations of the compound from ether, the melting point was raised from 40–41° to 44–45°. It showed no depression of melting point with the above compound; $[\alpha]^{29}D$ −13.1° in 95% alcohol (*C* = 2.72%).

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.81; H, 7.03. Found: C, 55.90; H, 7.05.

Another experiment was made with 4.7 g. of sodium salt for the above acetylation step. The residual oil was treated with a 100% excess of thionyl chloride, heated at 100° for fifteen minutes, evaporated to remove thionyl chloride and distilled at 2 mm. pressure; b. p. 95°. Crystallization took place on seeding with acetyl lactone. After two crystallizations of the compound from ether, the melting point was 41–42°, and the mixed melting point was 41–43°; yield, 2.83 g. The mother liquor from the recrystallization gave a negative test for inorganic chlorine, indicating the absence of an acid chloride.

N-(α-*p*-Nitrobenzoxy-β,β-dimethyl-γ-hydroxybutyryl)-β-aminopropionic Acid (Mononitrobenzoate of Pantothenic Acid) V.—An attempt was made to prepare the dinitrobenzoate of α-γ-dihydroxy-β,β-dimethylbutyric acid by esterifying the sodium salt in pyridine with *p*-nitrobenzoyl chloride. However, the product was the mononitrobenzoate described in a previous paper.[5] This ester (0.62 g.) was heated with 0.233 g. of the sodium salt of β-alanine for one hour at 100°. After a few minutes, the mixture became gummy and when cooled, solidified to a brittle mass. After the material was dissolved in 10 cc. of water, a few crystals of starting material separated. When the filtrate was acidified with hydrochloric acid, a gummy precipitate separated. It readily dissolved in alcohol and reprecipitated as an oil on the addition of water. On standing overnight, the oil crystallized, and it was recrystallized from acetone by the addition of water, m. p. 137–138°. It was the mononitrobenzoate of pantothenic acid; $[\alpha]^{29}D$ +4.5° in 95% alcohol (*C* = 0.78%).

Anal. Calcd. for $C_{16}H_{20}N_2O_8$: C, 52.17; H, 5.47; N, 7.61. Found: C, 52.23; H, 5.64; N, 7.38.

α-Carbobenzdioxy-β,β-dimethyl-γ-butyrolactone.—An attempt was made to prepare the dicarbobenzoxy ester of α,γ-dihydroxy-β,β-dimethylbutyric acid by treating an alkaline solution of the acid with carbobenzoxy chloride. Little or no reaction was observable. On acidification of the alkaline solution, no insoluble acid was obtained.

The monocarbobenzoxy lactone was made as follows. A benzene solution containing 6 g. of the lactone and 8.65 g. (1 equivalent) of antipyrine was added to a benzene solution containing 4.6 g. (1 equivalent) of phosgene. Crystalline antipyrine hydrochloride separated. After fifteen minutes, a benzene solution containing equivalent quantities of benzyl alcohol (5 g.) and antipyrine (8.65 g.) was added. Heat was evolved and additional antipyrine hydrochloride separated. The mixture was heated at 100° for fifteen minutes and filtered. The filtrate was washed three times with water, and then dried over

reported that acetylation inactivated their chick antidermatitis factor and that the activity was recovered on hydrolysis. Grüssner, Gätzi-Fichter and Reichstein [*Helv. Chim. Acta*, **23**, 1276 (1940)] reported that ethyl pantothenate was active in rats.

calcium chloride. After distillation of solvent, the sirup crystallized. Recrystallization was done by solution in alcohol and addition of water to turbidity. On cooling, the ester crystallized; m. p. 78°; yield, 5.05 g., 41.8%; $[\alpha]^{29}D$ +12.3° in 95% alcohol (C = 2.1%).

Anal. Calcd. for $C_{14}H_{16}O_6$: C, 63.63; H, 6.09. Found: C, 63.48; H, 6.32.

N-Carbobenzoxy-β-alanine from the Condensation of the α-Carbobenzdioxy-β,β-dimethyl-γ-butyrolactone with the Sodium Salt of β-Alanine.—A mixture of 1.32 g. (0.005 mole) of the carbobenzoxylactone and 0.005 mole of the sodium salt of β-alanine was heated for two hours at 100° with frequent stirring. On the addition of water, 0.78 g. of unchanged lactone crystallized. When the water solution was acidified, shiny crystals separated which were recrystallized from alcohol and water; m. p. 103°. Judged by analysis and melting point, the compound was the carbobenzoxy derivative of β-alanine which was originally prepared by Sifferd and du Vigneaud.[10]

Anal. Calcd. for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.83; N, 6.28. Found: C, 58.81; H, 5.79; N, 6.20.

Ethyl N-(α-Carbobenzdioxy-β,β-dimethyl-γ-hydroxybutyryl)-β-aminopropionate.—After 1.9 g. of freshly distilled β-alanine ester was mixed with 4.2 g. of carbobenzoxy lactone and heated at 100° for one and one-half hours, it was shaken with water and extracted with ether. The ether solution was washed with dilute hydrochloric acid and water, dried over calcium chloride, filtered with charcoal and distilled. The oil was distilled between 140–150° bath temperature at 4 × 10^{-6} mm. pressure. It was the carbobenzoxy ester of ethyl pantothenate (VI).

Anal. Calcd. for $C_{19}H_{27}NO_7$: C, 59.83; H, 7.14; N, 3.67. Found: C, 59.57; H, 6.86; N, 3.89.

α-γ-Dihydroxy-β,β-dimethylbutyramide (XIII).—The lactone, on treatment with either aqueous ammonia or alcoholic ammonia, followed by evaporation of the solvent, yielded a substance which was crystallized from alcohol; m. p. 135–136°. Analyses indicated that it was mainly the ammonium salt of α,γ-dihydroxy-β,β-dimethylbutyric acid. Sublimation under vacuum yielded the lactone.

Anal. Calcd. for $C_6H_{13}O_4N$: C, 43.62; H, 9.15; N, 8.48. Found: C, 43.96; H, 8.73; N, 7.64.

The amide (XII) was made by enclosing 13.6 g. of the (−) lactone and 50 cc. of liquid ammonia in a bomb tube, allowing the mixture to warm to 25° (the lactone dissolved) and to stand overnight. After opening the cooled tube and allowing the ammonia to evaporate, the amide crystallized. The last of the ammonia was removed under vacuum and the residue recrystallized from ethyl acetate; yield, 15.13 g., 98.5%; m. p. 92–94°; $[\alpha]^{29}D$ +30.9° in H_2O (C = 2.09%).

Anal. Calcd. for $C_6H_{13}O_3N$: C, 48.97; H, 8.90; N, 9.52. Found: C, 48.93; H, 8.90; N, 9.52.

α,γ-Diacetoxy-β,β-dimethylbutyramide (XIV).—The amide was acetylated with acetic anhydride both with and without pyridine. The main fraction came over at 125° bath temperature at 10^{-6} mm. It analyzed for α,γ-diacetoxy-β,β-dimethylbutyramide. The specific rotation varied considerably depending on the solvent: $[\alpha]^{25}D$ +6.8°, C = 5.3% in ether; −0.7°, C = 2.8% in chloroform; −10.3°, C = 3.7% in water; −3.2°, C = 2.2% in absolute ethanol; +5.7°, C = 2.3% in ethyl acetate; −5.4°, C = 1.5% in dioxane.

Anal. Calcd. for $C_{10}H_{17}O_5N$: C, 51.93; H, 7.41; N, 6.06. Found: C, 51.66; H, 7.34; N, 5.90.

α,γ-Diacetoxy-β,β-dimethylbutyric Acid (VIII).—A solution of 11.54 g. of the diacetoxyamide (XIV) in about 50 cc. of glacial acetic acid was treated with 20 cc. of amyl nitrite and heated at 100° for eighty minutes. The solution was concentrated *in vacuo* and the residue taken up in water in which it dissolved very slowly. After making just alkaline with sodium hydroxide and extracting with chloroform, the solution was acidified to *p*H 2 and extracted with chloroform. This solution was concentrated to a sirup under 2 mm. pressure. The residue (7.52 g.) titrated for 90% acid, and was distilled at 10^{-3} mm. at 100° bath temperature. Three fractions were taken, each of which contained nitrogen by qualitative test. The third fraction was repurified as described above and then redistilled. It was free of nitrogen; $[\alpha]^{25}D$ −2.6°, C = 1.512 in methanol; 0 in ether.

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95; acetyl, 37.06. Found: C, 51.60; H, 7.20; acetyl, 33.93.

Ethyl N-(α-γ-Diacetoxy-β,β-dimethylbutyryl)-β-aminopropionate. (Ethyl Diacetylpantothenate) (X.)—A mixture of 4.4 g. of the diacetyl acid and 2.77 cc. of thionyl chloride was heated at 100° for one-half hour and the excess of thionyl chloride was removed under vacuum. The residue was dissolved in 5 cc. of pyridine containing 2.23 g. of β-alanine ethyl ester. Heat was evolved and the solution became slightly colored. After standing for seventy-two hours, the excess pyridine was removed by distillation under vacuum, the sirup was dissolved in chloroform which was extracted first with dilute hydrochloric acid, then with water and sodium bicarbonate solution, and finally dried over calcium chloride. After solvent removal, the sirup was distilled at 10^{-6} mm. and 110–120° bath temperature. The viscous sirup was hygroscopic and care was taken not to expose it to moist air before analysis; yield, 3.06 g. (48.5%); $[\alpha]^{25}D$ +24.2°, C = 2.08% in ether.

Anal. Calcd. for $C_{15}H_{25}O_7N$: C, 54.37; H, 7.61; N, 4.23; acetyl, 25.98. Found: C, 54.18; H, 7.35; N, 4.04; acetyl, 24.04.

A solution of 0.11 g. of the ester was dissolved in 10 cc. of 0.5 *N* barium hydroxide and allowed to stand at 20–25° for one hour. The barium ion was removed as the sulfate and the solution brought to *p*H 7.5 with sodium hydroxide. Aliquots were assayed microbiologically[9] and were found to contain 50% of the theoretical amount of pantothenic acid.

Acknowledgment.—We greatly appreciate the coöperation of Messrs. D. F. Hayman, W. Reiss and H. S. Clark in performing the microanalyses.

Summary

The acetyl and carbobenzoxy derivatives of (−)α - hydroxy - β,β - dimethyl - γ - butyrolactone were treated with β-alanine ethyl ester, and the *p*-nitrobenzoyl lactone with the sodium

salt of β-alanine, to give the corresponding ethyl acetylpantothenate, ethyl carbobenzoxypantothenate and *p*-nitrobenzoylpantothenic acid. *p*-Nitrobenzoylpantothenic acid was inactive in microbiological tests. Ethyl pantothenate and ethyl acetylpantothenate were practically inactive in microbiological tests but were active in rats and chicks.

Evidence is described which shows that when sodium α,γ-dihydroxy-β,β-dimethylbutyrate is acetylated, then treated with thionyl chloride, then treated with β-alanine or its ester, the major mechanism of the formation of the pantothenic acid derivative involves the condensation of α-acetoxy-β,β-dimethylbutyrolactone with β-alanine, while only a minor part of the synthesis is accomplished through the condensation of the diacetyl acid chloride with β-alanine.

Pure α,γ-diacetoxy-β,β-dimethylbutyric acid was made by the treatment of α,γ-diacetoxy-β,β-dimethylbutyramide with amyl nitrite in acetic acid. Subsequent treatment with thionyl chloride and β-alanine ester gave pure ethyl diacetylpantothenate which was saponified to pantothenic acid.

RAHWAY, NEW JERSEY RECEIVED JUNE 17, 1941

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING OF THE UNIVERSITY OF PENNSYLVANIA]

The Dissociation Constant of Brom Cresol Green in Water[1]

BY MARTIN KILPATRICK

Several determinations have been made of the equilibrium constant for the reaction

$$\underset{\text{(yellow)}}{\text{Brom cresol green}} + H_2O \rightleftharpoons H_3O^+ + \underset{\text{(blue)}}{\text{Brom cresol green}} \quad (1)$$

and the dissociation constant

$$K_C = C_{H_2O}K_{(A_i,H_2O)} = C_{H_3O^+}C_{B_i}/C_{A_i} \quad (2)$$

at zero ion concentration has been reported by various workers.[2,3,4,5] Since the values vary from 1.03 to 1.20 × 10^{-5}, it has seemed worthwhile to present a summary of a number of determinations made in this Laboratory. The first series involves the colorimetric determination of the equilibrium constant, K_{A_iB}, of the reaction

$$\underset{\text{(yellow)}}{\text{Brom cresol green}} + \text{Acetate} \rightleftharpoons \text{Acetic acid} + \underset{\text{(blue)}}{\text{Brom cresol green}} \quad (3)$$

where

$$K_{A_iB} = \frac{C_A}{C_B}\frac{C_{B_i}}{C_{A_i}} \quad (4)$$

The constant K_{A_iB} may be related to the thermodynamic equilibrium constant $[K_{A_iB}]^0$ by the equation

$$\log K_{A_iB} = \log [K_{A_iB}]^0 + A(Z_A - Z_{A_i})\sqrt{\mu} + B\mu \quad (5)$$

where $\mu = {}^1/_2 \sum CZ^2$ and Z_A and Z_{A_i} are the charges on the buffer acid and indicator acid, re-

(1) Aided by a grant from the Penrose Fund of the American Philosophical Society.

(2) Sendroy and Hastings, *J. Biol. Chem.*, **82**, 197 (1929).

(3) Guggenheim and Schindler, *J. Phys. Chem.*, **38**, 543 (1934).

(4) Kilpatrick, *Chem. Rev.*, **16**, 57 (1935).

(5) Minnick and Kilpatrick, *J. Phys. Chem.*, **43**, 259 (1939).

TABLE I

THE EQUILIBRIUM CONSTANT AT 25°

$$\underset{\text{(yellow)}}{\text{Brom cresol green}} + \text{Acetate} \rightleftharpoons \text{Acetic acid} + \underset{\text{(blue)}}{\text{Brom cresol green}}$$

Ionic strength, μ	log K_{A_iB}, obsd.
0.00079	$\bar{1}$.827
.00082	$\bar{1}$.826
.00129	$\bar{1}$.833
.00162	$\bar{1}$.837
.00188	$\bar{1}$.844
.00202	$\bar{1}$.847
.00208	$\bar{1}$.851
.00216	$\bar{1}$.851
.00233	$\bar{1}$.847
.00246	$\bar{1}$.849
.00256	$\bar{1}$.859
.00302	$\bar{1}$.849
.00375	$\bar{1}$.855
.00506	$\bar{1}$.860
.00516	$\bar{1}$.861
.00602	$\bar{1}$.866
.00622	$\bar{1}$.855
.00740	$\bar{1}$.868
.00784	$\bar{1}$.867
.00950	$\bar{1}$.873
.01000	$\bar{1}$.886
.01000	$\bar{1}$.877
.01035	$\bar{1}$.886
.01152	$\bar{1}$.887
.01286	$\bar{1}$.897
.01477	$\bar{1}$.881
.01842	$\bar{1}$.889
.02000	$\bar{1}$.895
.02020	$\bar{1}$.897