99.9 mg. of the substance III in 20 ml. of acetic acid with 500 mg. of palladium-on-charcoal (5% Pd) under slightly more than one atmosphere of hydrogen. When 2 moles of hydrogen had been absorbed the reaction was stopped, the solution filtered, and evaporated to dryness. The residue crystallized and after recrystallization three times from dilute alcohol and once from xylene yielded 14 mg. of dibenzoyl-1,4-mannitan of m. p. 145–146°.

Rebenzoylation of IV to III was accomplished by dissolving 20 mg. of Hockett's benzylidenemannitan (IV) in 0.3 ml. of dry pyridine, adding 50 mg. of benzoyl chloride and allowing to stand overnight at room temperature. A small amount of water was then added and the crystalline precipitate was filtered off and recrystallized from alcohol; yield 32 mg. of dibenzoylbenzylidene-1,4-mannitan (III), m. p. 161–2°, $[\alpha]^{25}D$ +42° (chloroform).

Oxidation of the two benzylideneanhydromannitols with lead tetraacetate was performed under the standard conditions of Hockett, Dienes and Ramsden,[7] in specially purified, anhydrous acetic acid. The results are shown in Fig. 1.

(7) R. C. Hockett, M. T. Dienes and H. E. Ramsden, THIS JOURNAL, **65**, 1474 (1943).

Acknowledgment.—The writer is indebted to Dr. L. A. Goldblatt and Miss D. M. Oldroyd of the Naval Stores Division for performing the catalytic reduction and to Mr. Lawrence E. Brown for the micro carbon and hydrogen analyses. The authentic samples of 1,4-mannitan and benzylidene-1,4-mannitan were kindly supplied by Dr. Morris Zief of the Sugar Research Foundation.

Summary

A new anhydro-D-mannitol derivative, 2,3-benzylidene-1,4-anhydro-D-mannitol is described and doubt is cast on the structures 5,6-benzylidene-1,4-anhydro-D-mannitol and 2,3-dibenzoyl-5,6-benzylidene-1,4-anhydro-D-mannitol previously assigned to two other substances.

NEW ORLEANS, LOUISIANA RECEIVED JANUARY 10, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

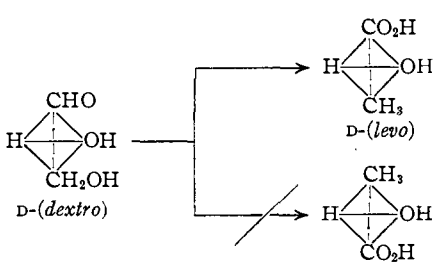
Configurational Correlation of L-(*levo*)-Glyceraldehyde with Natural (*dextro*)-Alanine by a Direct Chemical Method[1]

BY M. L. WOLFROM, R. U. LEMIEUX[2] AND S. M. OLIN[2]

In order to establish a system of relative configurations among a group of optically active compounds it is absolutely essential that a compound be selected as a standard of reference. Such a standard must be a compound containing only one asymmetric center. The parameters of optical activity are numerous and embrace wave length, temperature, solvent, and concentration. When possible it is convenient to select such parameters that specific rotation is defined as $[\alpha]^{20-25}D$ ($c<5$, water). The form of the standard that is dextrorotatory under these conditions is then arbitrarily assigned the D-configuration and its stereoformula is written in a definitive form. In the sugar series this standard is glyceraldehyde written by agreed convention as shown.[3,4]

D-(*dextro*)-Glyceraldehyde

The simplified tetrahedral representation is that suggested by Hudson[5] and this has its counterpart in the Fischer[6] projection formula shown above, the conventions for writing which are little understood by present-day organic chemists.[7] Having established such a standard, it is then feasible to relate it chemically to other compounds containing a like asymmetric center by operations which do not break the bonds attached to the reference carbon. In representing these relations it is essential that the reference center be oriented according to agreed conventions. Thus, in relating D-(*dextro*)-glyceraldehyde to lactic acid, it is agreed that the carboxyl group of the latter compound arises from the aldehyde group of the former and not from its primary alcohol group.



Furthermore, in a compound containing more than one asymmetric carbon, it is necessary to decide which of the centers present represents the standard of reference. In the sugar series, the highest numbered asymmetric center, or the bottom one in the conventional representation, is selected as that standard. In compounds containing more than one asymmetric center and with like terminal groups, an agreed orientation is particularly essential. Some of these substances may have a

(1) A complete report of this work appeared in *Abstracts Papers Am. Chem. Soc.*, **112**, 12Q (1947).

(2) Bristol Laboratories Research Associate (R. U. L.) and Research Fellow (S. M. O.) of The Ohio State University Research Foundation (Project 224).

(3) M. A. Rosanoff, THIS JOURNAL, **28**, 114 (1906).

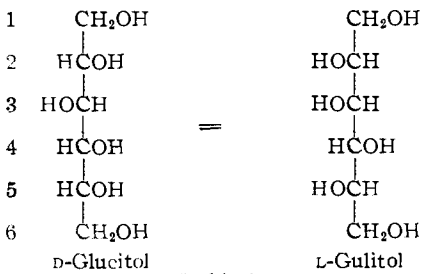
(4) A. Wohl and K. Freudenberg, *Ber.*, **56**, 809 (1923).

(5) C. S. Hudson, *J. Chem. Education*, **18**, 353 (1941).

(6) E. Fischer, *Ber.*, **24**, 2683 (1891).

(7) See C. S. Hudson, *Advances in Carbohydrate Chem.*, **3**, 7 (1948).

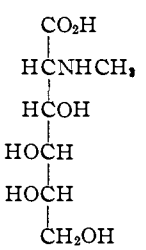
D-configuration when oriented in one manner and an L-configuration when oriented with the ends reversed. Such substances were designated as *amphi*-compounds by Rosanoff[3] and in the sugar series are best treated by employing suitable Fischer trivial names which now represent definitive configurations. A case in point is sorbitol.



Sorbitol

In the case of malic acid it is not so clear whether this substance should be treated as an α-hydroxy acid or as a 2-desoxytartaric acid; the orientations are enantiomorphous.

It is a striking fact that in the amino acid series no standard of reference has been explicitly agreed upon by the investigators concerned albeit a recently published nomenclature report[8] does suggest that serine would be suitable for this role. Most of the amino acids possess but one asymmetric center. Those with more than one are in the main the hydroxy amino acids in which the serine reference carbon atom (herein designated by the subscript s[8]) will be at the top, in the customary orientation, and the glyceraldehyde reference carbon (herein designated by the subscript g[8]) will be at the bottom; a seemingly irreconcilable situation.



N-Methyl-L_g-D_s-mannosaminic acid[9]

A few of the amino acids may be considered to have been related directly to serine by chemical operations not involving the possibility of Walden inversions.[10] Others have been purportedly related by indirect physical methods,[10] among the best of which are probably those involving the change of rotation with *p*H[11] and the measurement of rotatory dispersion.[12]

The primary amino group of natural glucosamine has been configurationally related to glyceraldehyde[13] by methods involving the established occurrence of Walden inversions in the opening and closing of epoxy rings fixed in cyclic structures; natural glucosamine (from chitosamine) was found to be 2-amino-2-desoxy-D-glucosamine, the amino group having the same relative configuration as the C-2 hydroxyl of D-glucose.

We wish to report herein the degradation of D-glucosamine to an α-amino acid by methods not involving chemical operations on the bonds of the carbon bearing the amino group. Unsuccessful attempts to effect such a transformation have been recorded.[14,15] N-Acetyl-D-glucosamine[16] was converted to the diethyl thioacetal and the pentaacetyl derivative of the latter (I) was reductively desulfurized[17,18] to yield pentaacetyl-2-amino-1,2-didesoxy-D-glucitol (II); synonym, pentaacetyl-5-amino-5,6-didesoxy-L-gulitol. Partial deacetylation of II produced 2-acetamido-1,2-didesoxy-D-glucitol (III) which on glycol cleavage with lead tetraacetate and subsequent oxidation yielded an N-acetylalanine (IV) [m. p. 122–123°, $[\alpha]^{23}D$ − 62° (*c* 3, water)] identical with that obtained from natural (*dextro*)-alanine (V).

These transformations effect a configurational correlation of (*levo*)-glyceraldehyde with (*dextro*)-alanine. Since the latter has been related to (*levo*)-serine by similar methods,[19] this effects a direct correlation between the two configurational standards, glyceraldehyde and serine. The configuration of the carbon bearing the amino group in D-glucosamine is configurationally D_g but it becomes L_s because the aldehyde group of D-glucosamine (C-1) is reduced to the hydrocarbon stage and thus becomes C-3 in the approved orientation of N-acetylalanine. This effectively amounts to an interchange of groups on the standard of reference with a consequent reversal of configuration.

Experimental[20]

Pentaacetyl-D-glucosamine Diethyl Thioacetal (I).—N-Acetyl-D-glucosamine (29 g.) prepared according to

(8) E. J. Crane, *Chem. Eng. News*, **25**, 1364 (1947).

(9) M. L. Wolfrom and A. Thompson, THIS JOURNAL, **69**, 1847 (1947).

(10) A. Neuberger, *Advances in Protein Chem.*, **4**, 297 (1948).

(11) O. Lutz, *Ber.*, **62**, 1879, 1916 (1929); O. Lutz and B. Jirgensons, *ibid.*, **63**, 448 (1930); **64**, 1221 (1931).

(12) J. W. Patterson and W. R. Brode, *Arch. Biochem.*, **2**, 247 (1943).

(13) W. N. Haworth, W. H. G. Lake and S. Peat, *J. Chem. Soc.*, 271 (1939); *cf.* W. O. Cutler and S. Peat, *ibid.*, 782.

(14) P. Karrer and J. Mayer, *Helv. Chim. Acta*, **20**, 407 (1937).

(15) A. Neuberger, *J. Chem. Soc.*, 47 (1941).

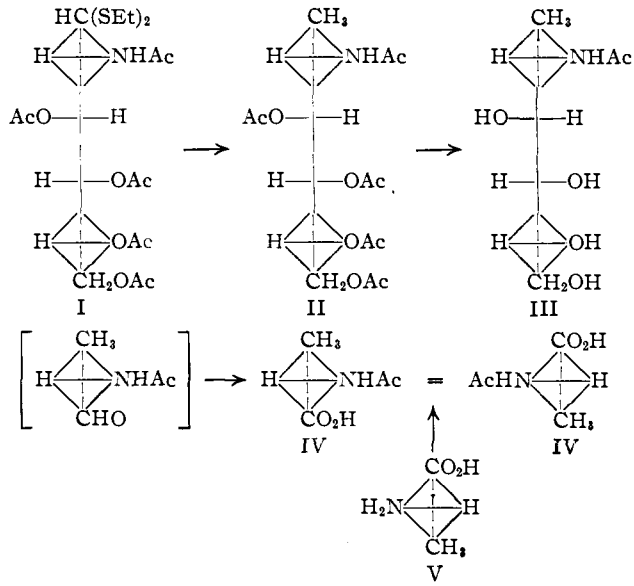
(16) R. Breuer, *Ber.*, **31**, 2193 (1898); T. White, *J. Chem. Soc.*, 428 (1940).

(17) J. Bougault, E. Cattelain and P. Chabrier, *Bull. soc. chim.*, [5] **5**, 1699 (1938); [5] **7**, 780, 781 (1940).

(18) M. L. Wolfrom and J. V. Karabinos, THIS JOURNAL, **66**, 909 (1944).

(19) E. Fischer and K. Raske, *Ber.*, **40**, 3717 (1907).

(20) Unless otherwise noted, all experimental work was performed by Mr. S. M. Olin.



the procedure of White,[16] was dissolved in 120 ml. of concentrated hydrochloric acid (*ca.* 12 *N*) at 0° and 120 ml. of ethyl mercaptan was added. The reaction mixture was stirred vigorously at 0° for twenty hours and was then neutralized in the cold with concentrated ammonium hydroxide (*ca.* 15 *N*). The separated aqueous layer was concentrated under reduced pressure and the resultant solid was dried by distilling absolute ethanol from it under reduced pressure. The dried solid was acetylated at room temperature for eighteen hours with 150 ml. of a mixture of acetic anhydride (2 vols.) and pyridine (1 vol.). The acetylation mixture was poured into 700 ml. of ice and water and the resultant mixture was extracted with four 75-ml. portions of chloroform. The chloroform extract was washed with water, saturated aqueous sodium bicarbonate and again with water. The dried chloroform solution was concentrated under reduced pressure to a sirup. This sirup was purified in 0.50 g. lots by dissolving in 10 ml. of benzene (thiophene-free) and adding this solution to the top of a 180 × 45 mm. (diam.)[21] column of a mixture of Magnesol[22]–Celite[22] (5:1 by wt.). The chromatogram was developed with 350 ml. of benzene (thiophene-free)–ethanol (100:1 by vol.). Three zones located 20–25 mm., 35–40 mm. and 80–85 mm. from the column top were detected by the alkaline permanganate streak reagent[22] on the extruded column. The sectioned zones were eluted with acetone. The top zone yielded pentaacetyl-β-D-glucosamine of m. p. 184.5–186° and $[\alpha]^{23}D$ +3° (*c* 4, chloroform); the middle zone gave pentaacetyl-α-D-glucosamine of m. p. 128–130° and $[\alpha]^{26}D$ +93° (*c* 4, chloroform), and the bottom zone yielded crystalline pentaacetyl-D-glucosamine diethyl thioacetal in 40% total yield and exhibiting the m. p. 123–126° and $[\alpha]^{20}D$ −26° (*c* 5, chloroform). The thioacetal was obtained pure from methanol–water; m. p. 126–127°, $[\alpha]^{22}D$ −32° (*c* 4, chloroform).

Anal. Calcd. for $C_{20}H_{33}O_9NS_2$: C, 48.47; H, 6.71; N, 2.83; S, 12.93. Found: C, 47.95; H, 6.83; N, 2.98; S, 12.50.

This substance exhibited poor crystallizing properties and was difficult to analyze in the combustion train. Reacetylation of N-acetyl-D-glucosamine diethyl thioacetal described below yielded a crystalline product with the same constants and exhibiting analyses of the same order of accuracy. The low yield was due to the poor crystallizing properties of the acetate and higher yields (80%) were obtainable by deacetylating the sirup and isolating the substance (without chromatography) as the deacetylated compound by the procedure described below.

N-Acetyl-D-glucosamine Diethyl Thioacetal.—Pentaacetyl-D-glucosamine diethyl thioacetal (2.8 g.) was dissolved in 75 ml. of absolute methanol and anhydrous ammonia was passed into the solution, previously cooled to 0°, for fifteen minutes at a rate that maintained the temperature at 0 to 5°. The solution was allowed to stand at room temperature for two hours and was then concentrated to dryness under reduced pressure. The residue was dissolved in 3 ml. of warm methanol and an equal volume of chloroform was added. The solution was treated with activated carbon and filtered. Anhydrous ether was added to the filtrate to incipient turbidity and the mixture was allowed to crystallize overnight in the icebox; yield 1.5 g., *m.* p. 128–129°, $[\alpha]^{23}D$ −23° (*c* 4, methanol). Pure material was obtained on further crystallization effected in the same manner; m. p. 130–131°, $[\alpha]^{23}D$ −35° (*c* 4, water).

Anal. Calcd. for $C_{12}H_{25}O_5NS_2$: C, 44.02; H, 7.70; N, 4.28; S, 19.58. Found: C, 44.03; H, 7.60; N, 4.26; S, 19.89.

As mentioned above, this substance is best obtained by direct isolation from the deacetylated mercaptalation mixture.

Pentaacetyl-2-amino-1,2-didesoxy-D-glucitol (II).—Pentaacetyl-D-glucosamine diethyl thioacetal (2.8 g.) and Raney nickel (25 g.) were refluxed in 70% aqueous ethanol (100 ml.). After five hours no mercaptan odor could be detected by acidification of a test portion of the solution and the reaction was assumed to be complete. The catalyst was removed by centrifugation and washed with 95% ethanol. The centrifugate and washings were combined, treated with decolorizing charcoal and filtered through Celite.[22] The filtrate was concentrated under reduced pressure to a sirup; yield 2.0 g.

An amount of 0.5 g. of the above sirup was dissolved in 25 ml. of benzene (thiophene-free) and added to the top of a 200 × 45 mm. (diam.)[21] column of a mixture (110 g.) of Magnesol[22]–Celite[22] (5:1 by wt.). The chromatogram was developed with 2000 ml. of benzene (thiophene-free)–ethanol (100:1 by vol.). A zone 9 to 30 mm. from the column top was detected by the streak indicator[22] on the extruded column. Crystalline material was obtained on solvent removal from the acetone eluate of the sectioned zone; yield 0.25 g., m. p. 55–58°, $[\alpha]^{28}D$ +7.5° (*c* 3, chloroform). Pure material was obtained on recrystallization from ether–petroleum ether (b. p. 85–100°); m. p. 65–67°, $[\alpha]^{27}D$ +8.5° (*c* 3, chloroform).

Anal. Calcd. for $C_{16}H_{25}O_9N$: C, 51.19; H, 6.71; N, 3.73. Found: C, 50.59; H, 6.98; N, 3.83.

2-Acetamido-1,2-didesoxy-D-glucitol (III).—Pentaacetyl-2-amino-1,2-didesoxy-D-glucitol (1.0 g.) was dissolved in methanol (50 ml.) and cooled to 0°. A stream of anhydrous ammonia was passed into the solution for ten minutes and then the reaction mixture was allowed to stand at room temperature for two hours. The solvent was removed under reduced pressure and the resulting solid was recrystallized by dissolving in methanol, adding an equal volume of chloroform and then ether to incipient opalescence; yield 0.35 g. (63%), m. p. 162–164°, $[\alpha]^{23}D$ −9° (*c* 2, water). Further recrystallization effected in the same manner did not alter these constants.

Anal. Calcd. for $C_8H_{17}O_5N$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.62; H, 8.13; N, 6.26.

N-Acetyl-L-alanine (IV) from 2-Acetamido-1,2-didesoxy-D-glucitol (III).—An aqueous solution of 2-acetamido-1,2-didesoxy-D-glucitol (1.65 g. in 70 ml.) was stirred vigorously and a solution of lead tetraacetate (11.0 g.) in glacial acetic acid (70 ml.) was introduced dropwise. At the end of the addition, *M* sulfuric acid

(21) Adsorbent dimensions.

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

(25 ml.) was added. The separated lead sulfate was removed by filtration and washed with water. The filtrate and washings were combined and bromine (4.0 g.) was added. The oxidation mixture was stirred for three hours. The excess bromine was removed by aeration. The bromide ion was removed by the addition of an excess of silver acetate with subsequent filtration. Hydrogen sulfide was passed into the filtrate to remove the silver ion. The filtered solution was extracted with five 75-ml. portions of ethyl acetate and the dried extract was concentrated to a sirup which crystallized on standing over phosphorus pentoxide in a vacuum desiccator. Pure material was obtained on further crystallization effected by dissolving in ethyl acetate, adding an equal volume of benzene and allowing to stand at icebox temperature; yield 0.40 g. (40%), m. p. 120–122° unchanged on admixture with the N-acetyl derivative of the natural amino acid prepared as described below, $[\alpha]^{23}D$ −62° (*c* 3, water).

Anal. Calcd. for $C_5H_9O_3N$: C, 45.79; H, 6.92; N, 10.68. Found: C, 45.69; H, 7.00; N, 10.57.

N-Acetyl-L-alanine (IV) from (*dextro*)-Alanine (V).—An aqueous solution (2 g. in 20 ml.) of a sample of natural (*dextro*)-alanine [m. p. 290–292° (dec.), $[\alpha]^{27}D$ +10° (*c* 3, *N* hydrochloric acid)] was treated with aqueous sodium hydroxide (33%, 7 ml.) and acetic anhydride (5.3 g.). After one hour the reaction mixture was neutralized with sulfuric acid and extracted with four 25-ml. portions of ethyl acetate. The solvent was removed in a stream of dry air and the resultant sirup crystallized on standing overnight in a vacuum desiccator. This preparation [m. p. 112–128°, $[\alpha]^{23}D$ −36° (*c* 2, water)] contained racemic N-acetylalanine and was purified in the following manner. Recrystallization from ethyl acetate–benzene yielded a substance of m. p. 118–123° and $[\alpha]^{23}D$ −43° (*c* 3, water). The racemic substance was less soluble in ethyl acetate than the optically active isomer. By incompletely dissolving the recrystallized product in ethyl acetate, a solution richer in the optically active component was obtained and from this the compound was crystallized by the addition of benzene. Repeating this procedure three times yielded pure material; m. p. 122–123°, $[\alpha]^{23}D$ −62° (*c* 3, water). For the N-acetyl derivative of (*dextro*)-alanine Karrer, Escher and Widmer[23] report: m. p. 116°, $[\alpha]^{16}D$ −46° (*c* 4.8, water). Bloch and Rittenberg[24] report for the enantiomorphous forms of deuterio-acetylalanine: m. p. 130–132° (L) and 131–132° (D), $[\alpha]D$ −60° (L) and +63° (D) (*c* 1, water). It is apparent that the preparation of Karrer and co-workers was partially racemized.

Anal. Calcd. for $C_5H_9O_3N$: C, 45.79; H, 6.92; N, 10.68. Found: C, 45.70; H, 6.95; N, 10.72.

Acknowledgment.—We are indebted to Dr. H. B. Vickery of the Connecticut Agricultural Experiment Station, New Haven, for a sample of natural (*dextro*)-alanine originally prepared by Dr. T. B. Osborne.

Summary

N-Acetyl- and pentaacetyl-D-glucosamine diethyl thioacetal are described. Reductive desulfurization of the latter (I) yielded pentaacetyl-2-amino-1,2-didesoxy-D-glucitol (II). This on partial deacetylation gave 2-acetamido-1,2-didesoxy-D-glucitol (III), which on glycol cleavage, effected with lead tetraacetate, and subsequent oxidation produced N-acetylalanine [IV, m. p. 122–123°, $[\alpha]^{23}D$ −62° (*c* 3, water)] identical with that obtained from natural (*dextro*)-alanine (V).

Accepting the correlation of C-2 of D-glucosamine with D-(*dextro*)-glyceraldehyde established by Haworth, Lake and Peat,[13] the above series of reactions gives a direct chemical correlation of L-(*levo*)-glyceraldehyde with a natural amino acid and effects a configurational correlation between the standard reference compounds, glyceraldehyde and serine.

(23) P. Karrer, K. Escher and Rose Widmer, *Helv. Chim. Acta*, **9**, 301 (1926).

(24) K. Bloch and R. Rittenberg, *J. Biol. Chem.*, **169**, 467 (1947).

COLUMBUS, OHIO RECEIVED MARCH 24, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Enzymic Hydrolysis of Amylopectin. Isolation of a Crystalline Trisaccharide Hendecaacetate[1]

BY M. L. WOLFROM, L. W. GEORGES,[2] ALVA THOMPSON[2] AND I. L. MILLER[3]

The isolation of low molecular weight, sirupy oligosaccharides by the enzymic or acid hydrolysis of starch has been often reported and has been studied in recent years especially by Myrbäck and co-workers.[4] The action of Takadiastase on whole corn starch followed by fermentation of the hydrolyzate with yeast yielded material which was repeatedly fractionated by ethanol and from which a subfraction was isolated having properties indicative of a trisaccharide.[5] A similar procedure applied to the hydrolyzate obtained by the action of malt amylases upon whole corn starch led to the isolation of a sirupy trisaccharide fraction.[6] Evidence was recorded for the presence of an α-D-1,6 linkage in this fraction.[7] Sirupy tri- and tetrasaccharides, containing α-D-1,6 linkages, were believed to have been obtained by using malt α-amylases upon starches.[7,8] By enzymic hydrolysis with Takadiastase a fraction was isolated that was considered to be a trisaccharide on the basis of its reducing value.[9] "Maltotriose" was obtained as the principal product of hydrolysis of a starch degradation

(1) A preliminary communication by M. L. Wolfrom, L. W. Georges and I. L. Miller describing the crystalline trisaccharide hendecaacetate appeared in THIS JOURNAL, **69**, 473 (1947).

(2) Corn Industries Research Foundation Associate of The Ohio State University Research Foundation (Project 203).

(3) Corn Industries Research Foundation Fellow of The Ohio State University Research Foundation (Project 203).

(4) K. Myrbäck, *Advances in Carbohydrate Chem.*, **3**, 251 (1948).

(5) K. Myrbäck, *Biochem. Z.*, **297**, 179 (1938).

(6) B. Örtenblad and K. Myrbäck, *ibid.*, **303**, 335 (1940).

(7) K. Myrbäck and K. Ahlborg, *ibid.*, **307**, 69 (1940).

(8) K. Myrbäck, *J. prakt. Chem.*, **162**, 29 (1943); *Biochem. Z.*, **304**, 147 (1940).

(9) K. Ahlborg and K. Myrbäck, *Biochem. Z.*, **308**, 187 (1941).