(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).

product believed to be a maltohexose.[10] About one-fourth of a normal α-dextrin ("hexasaccharide") was converted into "maltotriose" by malt α-amylase, as based on fermentation data.[11] The digestion of corn and potato starch with malt α-amylase gave a fraction believed to be maltotriose. With the β-amylase of malt this fraction was not obtained.[12] Unfortunately all the above characterization work was based upon the manipulation of sirupy products and can be definitive only when placed upon a crystalline basis. Furthermore, evidence for the presence of a trisaccharide fraction often rested merely upon analytical data such as reducing values or fermentation tests.

In the work herein described waxy maize starch,[13] which contains practically 100% of the branched-chain fraction, amylopectin, was employed as the substrate for enzymic hydrolysis. This hydrolysis was effected with a commercial, diastatic enzyme preparation, derived from barley malt, containing a mixture of α- and β-amylases. The lengthy incubation period of three months described by Örtenblad and Myrbäck[6] was successfully reduced to five days by increasing the temperature of the enzymic hydrolysis from 30 to 55°. The subsequent subjection of the hydrolyzate to yeast fermentation[1] was found to be undesirable. The crude solids in the hydrolyzate were acetylated with hot acetic anhydride and sodium acetate and the resultant mixture of β-D-acetates was subjected to chromatographic analysis by the methods developed in this Laboratory.[14] The main product was β-maltose octaacetate but from a zone above this there was isolated a new crystalline substance which was characterized as a trisaccharide hendecaacetate; m. p. 134–136°, $[\alpha]^{25}D$ +86° (c 1.6, chloroform). Structural work on this substance is in progress and will be reported subsequently.

Other acetate zone material eluted from the chromatographic column has failed to crystallize. No β-isomaltose octaacetate (6-α-D-glucopyranosyl-β-D-glucopyranose octaacetate[1,15]) was found. The chromatographic techniques employed would have unfailingly revealed this compound should it have been present. Appropriate blank experiments demonstrated that the crystalline β-D-acetate of the trisaccharide was not preformed in the enzyme preparation employed, nor was it formed by the interaction of the enzyme with β-D-glucose or maltose.

(10) K. Myrbäck and Elsa Leissner, *Arkiv. Kemi, Mineral. Geol.*, **17A**, No. 18, 22 pp. (1943).

(11) K. Myrbäck, *Arch. Biochem.*, **14**, 53 (1947).

(12) K. Myrbäck and R. Lundén, *Arkiv. Kemi, Mineral. Geol.*, **A23**, No. 7, 10 pp. (1946).

(13) H. H. Schopmeyer, G. E. Felton and C. L. Ford, *Ind. Eng. Chem.*, **35**, 1169 (1943).

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

(15) M. L. Wolfrom, L. W. Georges and I. L. Miller, *ibid.*, **71**, 125 (1949).

Experimental

Enzymic Hydrolysis of Amylopectin.—Amylopectin in the form of waxy maize starch[13] (500 g.) was suspended in 2500 ml. of water and 800 ml. of a phosphate buffer solution (40 g. of ammonium diacid phosphate, 8 g. of potassium monoacid phosphate trihydrate and 28 g. of potassium diacid phosphate per liter of solution). Exsize TX-1[16] (5 g.) was added, and the suspension was heated in a water-bath to 75° for fifteen minutes with manual stirring. At this temperature the starch began to paste. The temperature of the bath was raised to the boiling point of water and maintained there until the starch was completely pasted (*ca.* thirty minutes). The paste was cooled to 50–55°, and 10 g. more of Exsize TX-1[16] was added. After a few minutes the paste had liquefied. This solution, contained in a 5-liter flask fitted with a cotton stopper, was placed in an oven maintained at 55° for a period of five days. The solution was then boiled to inactivate the enzymes, activated carbon was added, and the solution was filtered. Inorganic compounds were removed by passage of the solution successively through Amberlite[17] resins IR-100 and IR-4 and the solution was concentrated to a sirup under reduced pressure. Water remaining was removed as the ethanol–water azeotrope under reduced pressure; yield of sirup, *ca.* 500 g.

Acetylation and Separation of the Hydrolytic Products.—An amount of 25 g. of the above sirup was subjected to a hot acetylation with 15 g. of anhydrous sodium acetate and 150–200 ml. of acetic anhydride. The reaction proceeded rapidly at 120–130° bath temperature. As soon as the material was completely in solution and the reaction had subsided, the reaction mixture was cooled and poured into *ca.* 2500 g. of ice and water. After the acetic anhydride had been hydrolyzed, the solids were placed on a filter and thoroughly washed with water; yield of air-dried product, *ca.* 45 g.

An amount of 5 g. of the crude acetate was dissolved in 75 ml. of benzene and placed on a column of Magnesol[18]-Celite[19] (5:1 by wt.) (240 × 80 mm. in diam.[20]) and developed with 3000–3500 ml. of benzene (technical grade)–ethanol (100:1 by vol.). The first and major zone near the bottom of the extruded column, as located by means of a streak indicator (1 part of potassium permanganate, 10 parts of sodium hydroxide and 100 parts of water), contained β-maltose octaacetate, m. p. 159–160°, mixed melting point unchanged, $[\alpha]^{25}D$ +62° (c 1.1, chloroform); accepted values[21]: m. p. 159–160°, $[\alpha]^{20}D$ +63° (c <5, chloroform). A second zone near the middle of the column was sectioned and eluted with acetone. Solvent removal left a sirup which crystallized from ethanol after standing overnight. The crude product was recrystallized from ethanol; yield, 0.03–0.4 g., m. p. 134–136°, $[\alpha]^{25}D$ +86° (c 1.5, chloroform).

Anal. Calcd. for $C_{40}H_{54}O_{27}$: C, 49.68; H, 5.63; mol. wt., 967. Found: C, 49.45; H, 5.57; mol. wt. (Rast), 971.

Two other zones above the trisaccharide zone, sometimes poorly separated, were sectioned and eluted with acetone. Evaporation of the solvent yielded sirups which have failed to crystallize upon long standing.

The above is the procedure of choice for isolating the acetylated trisaccharide. Many other variations were studied including the long enzymic digestion at room temperature with subsequent yeast fermentation as recommended by Örtenblad and Myrbäck[6] and outlined in our preliminary communication.[1] Removal by ethanol crys-

(16) A diastatic (barley malt) product of Pabst Brewing Co., Peoria, Ill.; contains α- and β-amylases.

(17) A product of the Resinous Products and Chemicals Co., Philadelphia, Pennsylvania.

(18) A product of Westvaco Chlorine Products Corp., South Charleston, West Virginia.

(19) No. 535, a product of Johns-Manville Co., New York, N. Y.

(20) Dimensions of the adsorbent.

(21) C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 1276 (1915).

tallization of most of the β-maltose octaacetate preliminary to chromatography was also found to be undesirable.

No β-isomaltose octaacetate was found although nuclei of this substance were at hand and its isolation by chromatographic methods was an established and familiar procedure in this Laboratory.[15]

A blank on the enzyme preparation employed showed that it contained no trisaccharide isolable as the β-D-acetate by the chromatographic procedure described; neither was such isolable when the experimental procedure was repeated employing D-glucose or maltose as substrate.

Acknowledgment.—The assistance of Dr. J. M. Sugihara in preparing this work for publication is acknowledged. Mr. T. T. Galkowski assisted in a portion of the experimental work.

Summary

The digestion of waxy maize starch with malt amylases followed by acetylation of the hydrolyzate gave a crude product, which was essentially β-maltose octaacetate. By utilizing a chromatographic technique a second crystalline compound was obtained. By analysis, this substance was shown to be a trisaccharide hendecaacetate, m. p. 134–136°, $[\alpha]^{25}D + 86°$ (chloroform). No β-isomaltose octaacetate was found.

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

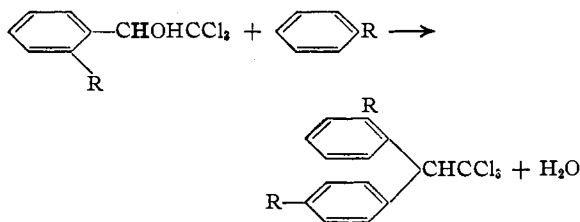
Some Positional Isomers of DDT Analogs

BY STANLEY J. CRISTOL AND DONALD L. HARMS[1]

Previous work with DDT isomers has shown that the position of the ring chlorine atoms is of utmost importance in insecticidal activity. For example, Cristol, Haller and Lindquist[2] have shown that when one of the *para* chlorine atoms in DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane] was moved to an *ortho* position to give *o,p'*-DDT [1,1,1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane], the dosage for equivalent toxicity against larvae of the common malaria mosquito (*Anopheles quadrimaculatus* Say) was increased approximately five- to ten-fold and the toxicity toward houseflies (*Musca domestica* L.) was almost eliminated. When the second chlorine atom was also moved to an *ortho* position, *o,o'*-DDT [1,1,1-trichloro-2,2-bis-(*o*-chlorophenyl)-ethane] was effective against mosquito larvae only at dosages approximately 1000 times greater than *p,p'*-DDT. Similar effects were noted in the dichloroethane series, 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethane being equally effective against mosquito larvae as approximately ten-fold dosages of its *o,p'* isomer, 1,1-dichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane.[3]

As the analogs of *p,p'*-DDT wherein the two *para* chlorine atoms are replaced by bromine, methyl and methoxy groups, are fairly effective insecticidally,[3,4] it seemed worthwhile to prepare the *o,p'* isomers of these compounds to test whether loss of insecticidal activity was general for such changes.

The preparations of 1,1,1-trichloro-2-*o*-bromophenyl-2-*p*-bromophenylethane, 1,1,1-trichloro-2-*o*-tolyl-2-*p*-tolylethane and 1,1,1-trichloro-2-*o*-anisyl-2-*p*-anisylethane are described in the experimental section. The general method involved the condensation of the *ortho*-substituted aryltrichloromethylcarbinol with the appropriately substituted benzene in the presence of sulfuric acid according to the equation, where R is Br, CH_3 or CH_3O.



A dinitro derivative was prepared by nitration of the bromine analog, and tetranitro derivatives were prepared of the methyl and methoxy analogs to further characterize these compounds.

Each of these compounds has been compared with its *p,p'* isomer in tests against *A. quadrimaculatus* larvae, and each required approximately ten-fold dosages for equivalent insecticidal activity. None of the *o,p'* compounds was effective against adult houseflies.[5]

Experimental

1,1,1-Trichloro-2-*o*-bromophenyl-2-*p*-bromophenylethane.—The carbinol required for this synthesis was prepared by a method analogous to that described[6] for the preparation of trichloromethyl-*o*-chlorophenylcarbinol, by the base-catalyzed condensation of *o*-bromobenzaldehyde and chloroform. The product, trichloromethyl-*o*-chlorophenylcarbinol, boiled at 134.5–135° (1.5 mm.) and was obtained in 11–18% yield. It crystallized slowly, and, after crystallization from Skellysolve B (petroleum ether, b. p. 60–70°), melted at 50°.

Anal. Calcd. for $C_8H_6OCl_3Br$: C, 31.50; H, 1.99. Found: C, 31.72; H, 2.11.

(1) Present address: Chemical Department, General Electric Company, Pittsfield, Mass.

(2) Cristol, Haller and Lindquist, *Science*, **104**, 343 (1946).

(3) Deonier, Jones, Haller, Hinchey and Incho, *Soap, Sanit. Chemicals*, **22** [11], 118 (1946).

(4) Haller, *Ind. Eng. Chem.*, **39**, 467 (1947).

(5) We are indebted to Dr. W. V. King, Dr. C. C. Deonier, and Mr. I. H. Gilbert, of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Orlando, Florida, for the biological data, which will be published in detail elsewhere.

(6) Howard and Castles, THIS JOURNAL, **57**, 376 (1935).