pine, *Pinus densiflora*, Sieb. and Zucc., and supplied to us by the Arakawa Forest Products Company of Osaka, Japan. The sesquiterpene was distilled from sodium before use and had the following constants: b.p. 144–146° (30 mm.), $n^{25}\text{D}$ 1.5000, $\alpha^{25}\text{D}$ +38.14°; **hydrochloride**, m.p. 58.5–59.5°, $[\alpha]^{25}\text{D}$ +9.8° (in $CHCl_3$); hydrobromide, m.p 69°. Simonsen[8] reports these constants for longifolene obtained from Indian terpentine oil: b.p. 150–151° (36 mm.), $n^{30}\text{D}$ 1.495, αD +42.73°; hydrochloride, m.p. 59–60°, $[\alpha]\text{D}$ +7.1° (in $CHCl_3$); hydrobromide, m.p. 69–70°.

Longifolene hydrobromide was dehydrobrominated in 10% alcoholic potassium hydroxide at reflux for 11 hr. to longifolene. The hydrochloride was considerably more resistant to this treatment.

Anal. Calcd. for $C_{15}H_{24}$: C, 88.16; H, 11.84. Found: C, 88.12; H, 11.51.

The Longifolic Acids.—Longifolene was oxidized and ozonized according to the procedures of Simonsen.[5,6] Discrepant values are given: longifolic acid, $[\alpha]\text{D}$ +5.6° (opt. inact.[9]); neut. equiv., 233, 234 ($C_{15}H_{24}O_2$ requires 236); isolongifolic acid, neut. equiv., 236, 236; α-longifolic acid, neut. equiv., 239, 239.5.

The Longifolols.—Methyl isolongifolate (3.2 g.), m.p. 54–55° (54–55°),[9] was reduced in ether with excess lithium aluminum hydride. After working up in the usual manner, the crude alcohol was crystallized from petroleum ether, yielding 2.8 g. of pure isolongifolol, m.p. 111–112°, $[\alpha]^{25}\text{D}$ −46.9°. Similar reduction of isolongifolic acid gave the same product.

Anal. Calcd. for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found: C, 80.98; H, 11.38.

Reduction of longifolic acid (3.0 g.) yielded 1.6 g. of crude alcohol, m.p. 50–90°. Crystallization of this material from petroleum ether gave as fraction 1, isolongifolol (0.46 g.), m.p. 111–112°, $[\alpha]\text{D}$ −46.4°; as fraction 2, crude longifolol, m.p. 75–80°, whose m.p. became constant at 78.5–80.5° after two recrystallizations from the same solvent: 0.21 g., $[\alpha]\text{D}$ +23.5°.

Anal. Calcd. for $C_{15}H_{26}O$: C, 81.02; H, 11.79. Found: C, 81.03; H, 11.52.

The reduction of α-longifolic acid (1.4 g.) gave 1.1 g. of crude alcohol, m.p. 57–90°. Fraction 1 from petroleum ether proved to be slightly impure isolongifolol (0.3 g.) which further crystallization failed to improve: m.p. 107–109°, $[\alpha]\text{D}$ −45.0°. fraction 2 (0.2 g.), m.p. 63–66°, $[\alpha]\text{D}$ −23.5°, and fraction 3 (0.3 g.), m.p. 63–70°, $[\alpha]\text{D}$ −29.2°, also were not improved by recrystallization. The infrared spectra of the three first fraction isolongifolols were identical, and those of fractions 2 and 3 from the reduction of α-longifolic acid were very similar to that spectrum of isolongifolol.

Longifolene from Isolongifolol.—Pure isolongifolol (2.0 g.) was dissolved in 50 ml. of anhydrous ether and 2.0 g. of phosphorus pentachloride was added. While the mixture stood for 36 hours at room temperature the solid pentachloride lying initially at the bottom of the flask slowly dissolved. The clear solution was poured into water, and the ether layer was removed. This ether layer was washed with water, then with dilute sodium hydroxide solution, again with water and finally dried over sodium sulfate. Evaporative distillation of the residue, remaining after evaporation of ether, at 100° (1 mm.) gave 0.2 g. of longifolene, $[\alpha]\text{D}$ +36.2°, whose infrared spectrum was superimposable upon that of the sesquiterpene described above. If the ether solution of the reaction product is concentrated, a white crystalline substance is deposited, m.p. 121–123°, as major product, and this material, which gave a positive phosphate test, is believed to be the phosphate ester of isolongifolol.

Isomerization of Longifolene.—Longifolene (10 g.), $[\alpha]\text{D}$ +33.7°, was added to 20 ml. of sulfuric acid, 50 ml. of acetic acid and 70 ml. of water. This mixture was maintained at *ca.* 80° on the steam-bath for 14 days, during which time aliquots were withdrawn for rotation and infrared measurements. Specific rotation changes were: +33.7° (0 days), +21.1° (1), +10.1° (2), +1.8° (3), −3.9° (4), −7.2° (5), −9.6° (6), −15.4° (8), −15.7° (9), −15.0° (11), −16.5° (14).

The isomerized product, β-longifolene, decolorized permanganate solution and bromine in carbon tetrachloride.

Anal. Calcd. for $C_{15}H_{24}$: C, 88.16; H, 11.84. Found: C, 87.72; H, 11.80.

Dehydration of Isolongifolol.—Isolongifolol (1.3 g.) was heated with 0.9 g. of freshly fused potassium bisulfate between 220–230° for 1.5 hours. The product was extracted with ether, washed and dried. Evaporative distillation at 100° (1 mm.) yielded 0.5 g. of colorless, liquid hydrocarbon, $[\alpha]\text{D}$ −62.6°.

New Haven, Connecticut

---

[8] J. L. Simonsen, *J. Chem. Soc.*, **117**, 570 (1920).

[9] Value reported by J. L. Simonsen.

[Contribution from the Department of Chemistry, University of Toronto]

Cyclitols. VI. A New Tetrol and Enediol from Cyclohexadiene-1,4 by the Prevost Reaction[1]

By G. E. McCasland and E. Clyde Horswill[2]

Received November 5, 1953

Cyclohexadiene-1,4 in excess reacts with silver iodine dibenzoate to give *trans*-cyclohexenediol-4,5 dibenzoate, which can be saponified to *trans*-cyclohexenediol-4,5. The diene in deficiency reacts to give cyclohexanetetrol-1,2,4,5 tetrabenzoate (m.p. 181°), which on ammonolysis gives a new cyclohexanetetrol-1,2,4,5 of m.p. 208°. Acetylation of the tetrol gives a tetraacetate of m.p. 148° The benzoylation of cyclohexenediol-3,4 is described. New Prevost reaction products of cyclohexene are reported.

Continuing our investigations on cyclitol chemistry,[1] we wish to report the synthesis of a new cyclohexanetetrol III and a new cyclohexenediol V. The cyclohexanetetrols are of interest because of their close relationship to the naturally occurring betitol,[3] quercitols and inositols. Cyclohexenediol-4,5 and -3,4 were needed for use in a configurational determination which is still in progress.

It previously had been shown that tetrols or enediols can be prepared by the hydroxylation (or acyloxylation) of cyclohexadienes with suitable reagents. Products of *cis* configuration are obtained with potassium permanganate[4] or osmium tetroxide,[5] while the perbenzoic acid method[6] gives *trans* prod-

---

[1] For previous (unnumbered) papers in this series, see G. E. McCasland and E. Clyde Horswill, This Journal, **75**, 4020 (1953), and references there cited.

[2] Fellow of the National Research Council, 1952–1953.

[3] Betitol, a dextrorotatory cyclohexanetetrol, was isolated in 1901 from beet sugar molasses; see *Ber.*, **34**, 1159 (1901). If it has a 1,2,4,5-structure it could conceivably be an active form of our inactive tetrol now reported.

[4] (a) N. D. Zelinski and A. N. Titowa, *ibid.*, **64**, 1399 (1931); (b) N. D. Zelinski, J. I. Denisenko and M. S. Eventova, *Chem. Zentr.*, **106**, II, 3765 (1935).

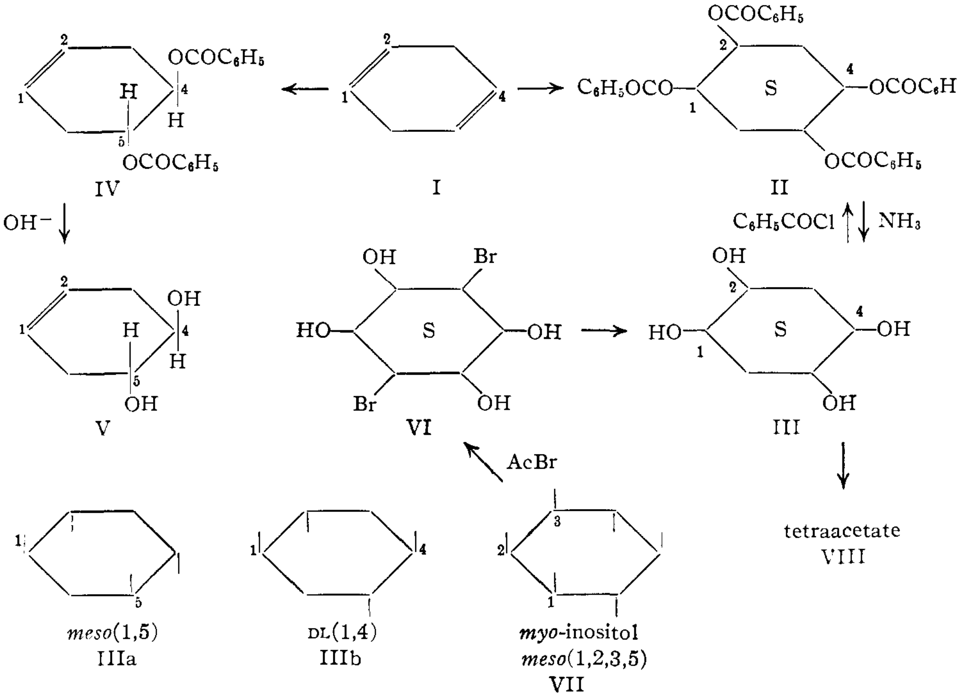
[5] T. Posternak, *Helv. Chim. Acta*, **36**, 251 (1953).

[6] (a) P. Bedos and A. Ruyer, *Compt. rend.*, **196**, 625 (1933); (b) **204**, 1350 (1937); (c) **195**, 802 (1932).

ucts. Lead tetraacetate[7] gives a mixture of *cis* and *trans* isomers.

We now find that the Prevost glycol synthesis[8–11] is useful for the synthesis of *trans* products and more convenient than previous methods.

CHART I. PREVOST REACTION WITH CYCLOHEXADIENE-1,4



Products from 1,4-Cyclohexadiene.—Apparently only one diene (butadiene[8c]) has previously been used in the Prevost synthesis. When we used excess cyclohexadiene-1,4 (I) the predominant product was the enediol dibenzoate IV, while with excess iodine and silver salt the tetrol tetrabenzoate II was obtained.

The enediol dibenzoate IV, m.p. 100°, on saponification gave the free enediol V, m.p. 96°. This is the first reported cyclohexenediol with a "4,5" structure; the hydroxy groups undoubtedly have a *trans* configuration. It is interesting to note that the enediol dibenzoates tend to melt at the same temperature as the enediols themselves. The free enediols are easily recognized by their much greater solubility in water. Cyclohexenediol-4,5 (or its dibenzoate) is inert to bromine in carbon tetrachloride (but does decolorize bromine water); this is surprising since cyclohexenediol-3,4 reacts instantly with bromine–carbon tetrachloride.

The tetrol tetrabenzoate II, m.p. 181°, on ammonolysis with methanolic ammonia gave a new 1,2,4,5-cyclohexanetetrol III of m.p. 208°. On acetylation of this tetrol a tetraacetate of m.p. 148° was obtained.

This same tetrol can be prepared from the dibromohydrin VI of m.p. 190° derived from *myo*-inositol VII; details will be given in a subsequent communication.[12] The tetrol III must have the "para" or 1,2,4,5-structure, and since *trans*-addition no doubt occurs at each double bond, only the diastereomeric configurations *meso*(1,5) and DL(1,4)[13] need be considered (see formulas IIIa and IIIb). For reasons which will be discussed elsewhere the DL(1,4) configuration seems more probable.[14] Five diastereomeric "para" cyclohexenetetrols are possible; it appears that at least three of these are now known (see Table I).

The situation is confused because Zelinski and Titowa by the permanganate method (which should yield at most only two diastereomers) reportedly[4a] obtained three tetraacetates and also one free tetrol. The free tetrol (m.p. 241°) undoubtedly corresponds to one of the three tetraacetates (m.p. 54°, 101° and 168°), but unfortunately the relationship was not determined. None of these permanganate products can be the same as our new tetrol (or tetraacetate).

Zelinski and Titowa[4a] also reported preparation by the perbenzoic method of a tetrol monohydrate, m.p. 195°. This undoubtedly differs from our anhydrous tetrol of m.p. 208° which shows no tend-

---

[7] (a) R. Criegee, *et al.*, *Ann.*, **481**, 285, 292 (1930); (b) **550**, 128 (1942).

[8] (a) For a recent brief review of the Prevost reaction see the chapter on "Oxidation Processes" by W. A. Waters, in H. Gilman's "Organic Chemistry: An Advanced Treatise," Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1953; (b) C. Prevost, *et al.*, *Compt. rend.*, **196**, 1129 (1933); (c) **198**, 2264 (1934); (d) **204**, 989 (1937); (e) **197**, 1661 (1933). Note errors in the *C. A.* summary of ref. (d).

[9] (a) *Cf.* M. I. Uschakov and O. Tchistov, *Ber.*, **68**, 829 (1935); (b) *cf.* B. I. Halperin, *et al.*, *J. Org. Chem.*, **17**, 623 (1952).

[10] A. Simonini, *Monatsh.*, **13**, 320 (1892).

[11] H. Wieland and F. G. Fischer, *Ann.*, **446**, 49 (1926).

[12] G. E. McCasland and E. Clyde Horswill, THIS JOURNAL, in press.

[13] For an explanation of the symbols used in this article to designate stereoisomers, see the "Report of the Advisory Committee on Configurational Nomenclature," a pamphlet available from "Chemical Abstracts," Ohio State University, Columbus 10, Ohio (in press). The symbol "R" stands for "relative" configuration.

[14] The configuration proposed for this compound is tentative, and the basis for assigning it will be discussed in a subsequent communication (ref. 12).

Table I

The Known "Para" or 1,2,4,5-Cyclohexanetetrols[3] (1954)[a]

M.p., °C.	Probable configuration[13] (tentative)	M.p. of tetrabenzoate, °C.	M.p. of tetraacetates, °C.	Prepared from	Ref.
195(H_2O)	*meso*(1,5)	...	...	Diene + PBA	4a
208	DL(1,4)	181	148	Diene (Prevost); dibromotetrol	See text 12
241	*meso*(1,2) or *meso*(1,2,4,5)	...	...	Diene, $KMnO_4$	4a
...[b]	*meso*(1,2) or *meso*(1,2,4,5)	...	54	Diene, $KMnO_4$	4a
...[b]	*meso*(1,2) or *meso*(1,2,4,5)	...	101	Diene, $KMnO_4$	4a
...[b]	*meso*(1,2) or *meso*(1,2,4,5)	...	168	Diene, $KMnO_4$	4a

[a] Three *meso* and two active diastereomers possible. Free tetrols not made.

ency to form a hydrate. We have isolated a very small amount of a second product, m.p. 265°, which appears to be the tetrabenzoate of the remaining member of the pair of tetrols IIIa–IIIb. This compound may be a derivative of Zelinski's 195° isomer, but we have not yet obtained a quantity sufficient for definite characterization.

Enediol Dibenzoate from 1,3-Cyclohexadiene.—Attempts to convert 1,3-cyclohexadiene to a benzoylated enediol or tetrol by the Prevost method failed to yield any pure product at all. The conjugation of double bonds in this isomer no doubt permits both 1,2- and 1,4-addition, giving a complex mixture of products.

Since the enediol-3,4 dibenzoate was needed for a configurational proof still in progress, we prepared it by benzoylating the enediol. This enediol can be prepared from the 1,3-diene *via* 3,6-dibromocyclohexene. Posternak's recent article[5] gives the only detailed and satisfactory procedure for this preparation. Although a dibenzoate melting at 77° is mentioned in previous literature,[6a] the only detailed description of it is that which appears below (see Experimental section).

Prevost Reaction with Cyclohexene.—In order to facilitate the reactions using cyclohexadiene, preliminary experiments with cyclohexene were carried out. Under the usual Prevost conditions cyclohexene was converted to the *trans*-diol dibenzoate and the *trans*-diol bis-3,5-dinitrobenzoate. This is apparently the first preparation of these esters directly from cyclohexene.

By using conditions favoring halohydrin ester, we also converted cyclohexene to the previously unreported DL-*trans*-2-bromocyclohexyl 3,5-dinitrobenzoate, m.p. 160°.

Experimental

All melting and boiling points have been corrected. Melting points taken on Köfler block unless otherwise noted. Microanalyses by Micro-Tech Laboratories, Skokie, Ill. Calculated analytic figures taken from Gysel's tables.[15] Benzene was dried with commercial sodium shot. Chloroform was dried with Drierite.

Silver 3,5-Dinitrobenzoate.—The procedure was similar to that of Halperin, *et al.*[9b] The product (like most organic silver salts) retains water stubbornly, and it is essential to dry it with heat (about 100°) until the weight is constant (about 15 hours).

Silver Benzoate.—The procedure was similar to that for the 3,5-dinitrobenzoate, except that the aqueous ammonia used for dissolving benzoic acid need not be warmed.

Products from 1,4-Cyclohexadiene

DL-*trans*-**Cyclohexenediol-4,5 Dibenzoate.**—Commercial[16a] 1,4-cyclohexadiene was redistilled, b.p. 88.5–89.0° (752 mm.), n^{20}_D 1.4731 (reported[16b,c] 88.7–88.9° (760 mm.), n^{20}_D 1.4725). To 4.8 g. (21 millimoles) of dry, finely pulverized silver benzoate was added in portions with shaking a solution of 2.54 g. (10 millimoles) of iodine in 50 ml. of dry benzene. To the resulting suspension was added all at once 2.4 g. (30 millimoles) of the redistilled diene. After shaking well, the mixture was boiled under reflux for one hour (anhydrous conditions), and allowed to cool overnight.

The light yellow precipitate was filtered off (dry weight 4.9 g.). The deep yellow benzene filtrate was washed successively with water, 1 *M* sodium carbonate, and again with water, and dried over sodium sulfate. The dried solution was evaporated, giving 2.3 g. of nearly colorless crystalline residue, m.p. about 80°.

The sodium carbonate wash on acidification gave a precipitate of about 0.3 g. of benzoic acid.

The above product (2.3 g.) when recrystallized from 95% ethanol (3 ml.) gave 1.8 g. of crystals, m.p. (cap.) about 95°, after softening at 80°. After three more crystallizations from ethanol, 1.4 g. of colorless needles melting sharply at 99–100° (cap.) were obtained. The yield (based on iodine) is 37%.

One gram of the compound is soluble in less than 2 ml. of boiling ethanol, but in order to speed up purification it would be preferable to use 5 or 10 ml. of ethanol per gram in the early crystallizations. For analysis a sample was recrystallized again from ethanol.

Anal. Calcd. for $C_{20}H_{18}O_4$ (322.34): C, 74.52; H, 5.63. Found: C, 74.30; H, 5.71.

On ignition, the compound melted and burned with a smoky flame, leaving no residue. It gave a negative Beilstein test for halogen. It failed to react with bromine–carbon tetrachloride, even when heated, and did not decolorize an acetone solution of potassium permanganate. However, it gave a positive bromine water test for unsaturation.

DL-*trans*-**Cyclohexenediol-4,5.**—Potassium hydroxide (5.6 g.) was dissolved in 5 ml. of water, and absolute ethanol added q.s. 100 ml. A 7.5-ml. (7.5 millimoles) portion of the resulting solution was added to 600 mg. (1.9 millimoles) of the enediol dibenzoate, and the mixture boiled for one hour. Within a few minutes (before the dibenzoate had completely dissolved) glistening leaflets of potassium benzoate began to separate.

After cooling, 0.62 ml. (7.5 millimoles) of 12 *M* hydrochloric acid was added with stirring, and the resulting potassium chloride and benzoic acid partly removed by filtration. The filtrate was distilled to near-dryness and the residue extracted twice with boiling benzene (10 ml., 5 ml.). The benzene extract was boiled down to half volume and when allowed to stand overnight it deposited 79 mg. of the desired enediol, colorless crystals, m.p. 95–96° (cap.) after softening at 93°.

The crystals (79 mg.) were recrystallized from 1.0 ml. (soluble in 0.5 ml.) of boiling benzene, giving 72 mg. of slender, colorless, bipyramidal prisms of unchanged m.p.

A further 62 mg. of product was obtained by extracting the original benzene filtrate with water, and evaporating the water extract to dryness. The total yield was thus 141 mg. (66%).

A 72-mg. portion was recrystallized for analysis from about 2 ml. of benzene, and dried at 1 mm. (25°). (A sample dried at 1 mm. (60°) sublimed completely away.)

Anal. Calcd. for $C_6H_{10}O_2$ (114.14): C, 63.13; H, 8.83. Found: C, 62.98; H, 9.04.

The product (unlike starting material) is very soluble in water or ethanol. It gives a positive bromine water test for unsaturation.

---

[15] H. Gysel, "Tables of Percentage Composition of Organic Compounds," Verlag Birkhauser, Basel, Switzerland, 1951.

[16] (a) Farchan Laboratories, Cleveland, Ohio. (b) The first assuredly pure sample of 1,4-cyclohexadiene was prepared by J. A. Wibaut and F. A. Haak, *Rec. trav. chim.*, **67**, 85 (1948). (c) It is noteworthy that neither 1,3- nor 1,4-cyclohexadiene shows an exaltation of the molar refraction, probably because non-coplanarity inhibits resonance. From the examination of Hirschfelder models it appears that the 1,4-diene has a strained "boat" conformation, while in the 1,3-diene only five of the six ring atoms are coplanar.

A saponification of the enediol dibenzoate with sodium hydroxide gave similar results

DL(1,4)R-Cyclohexanetetrol-1,2,4,5 Tetrabenzoate.[13,14]—To 9.2 g. (40 millimoles) of silver benzoate in 10 ml. of dry benzene was added in portions with shaking 5.1 g. (20 millimoles) of iodine in 40 ml. of benzene, giving a yellow precipitate suspended in a red solution. A 0.46-ml. (0.40 g., 5 millimoles portion of redistilled commercial cyclohexadiene-1,4 (b.p. 88.5–89.0°) was added all at once and the mixture shaken vigorously for several minutes (red color disappears, giving light yellow precipitate in a deep yellow solution). The solution was boiled under reflux for one hour, and left overnight (on cooling, excess silver iodine dibenzoate crystallizes out).

After filtration (dry weight of residue 12.0 g.), the solution was washed successively with water, 1 *M* sodium carbonate, and again with water. This washing (to destroy any remaining silver iodine benzoate) is essential for good results. The benzene solution (dried with sodium sulfate) on evaporation left a viscous reddish oil, which turned crystalline on digestion with 20 ml. of boiling ethanol.

Upon filtration and drying, 0.9 g. of a colorless powder, m.p. 160–200° (cap.), was obtained. The powder was recrystallized from 5 ml. of *n*-hexyl alcohol (residue at b.p. not removed), giving 0.75 g. of crystals, m.p. 170–200°.

The product was next recrystallized from 5.0 ml. of *n*-butyl alcohol. An insoluble residue (73 mg.—see below) was filtered off at the b.p. The filtrate on cooling deposited 524 mg. of colorless prisms, m.p. 175–180° (cap.).

The prisms (524 mg.) were recrystallized from 3 ml. of *n*-butyl alcohol. Filtration at the b.p. this time showed almost no residue. The filtrate on cooling deposited an oil. Additional butanol (2 ml.) was therefore added, and the mixture reheated to the b.p., and cooled with seeding. The product then separated as colorless prisms (438 mg.), m.p. 176–180° (cap.).

The product (438 mg.) was next recrystallized from boiling absolute ethanol (about 15 ml. needed). Hot filtration showed a negligible residue. On cooling, the filtrate yielded 308 mg. (11%) of 1,2,4,5-cyclohexanetetrol tetrabenzoate melting sharply at 179–180° (cap.).

For analysis a sample was recrystallized again from absolute ethanol, m.p. 180–181°.

Anal. Calcd. for $C_{34}H_{28}O_8$ (564.56): C, 72.33; H, 5.00. Found: C, 72.07; H, 5.36.

The compound is freely soluble in cold acetone, and moderately soluble in cold benzene, but insoluble in water.

This tetrabenzoate was shown by mixed m.p. and by X-ray powder spectra to be identical with an isomer derived from inositol dibromohydrin.[12]

Product of M.p. 265°.—The insoluble residue (73 mg.) from the first *n*-butyl alcohol crystallization (m.p. 230–260°), was recrystallized from 5–6 ml. of *n*-hexyl alcohol. A small residue at the b.p. was removed by filtration. The filtrate on cooling gave colorless needles, m.p. about 265°, shown by ignition test to be an organic compound. This is presumably the tetrabenzoate of the other "*trans–trans*" diastereomer of 1,2,4,5-cyclohexanetetrol, but cannot be characterized definitely until larger quantities are available.

Attempts to obtain further product were made by extracting the original silver iodide–silver benzoate residue with 200 ml. of boiling benzene. The benzene extract on cooling deposited about 1.2 g. of colorless needles, which turned out to be merely unreacted silver iodine dibenzoate, $IAg(RCOO)_2$. After removal of this complex by filtration, the benzene filtrate was worked up, but yielded less than 200 mg. of solid product, consisting mainly of the tetrol tetrabenzoate of m.p. 180°.

The ethanolic filtrate from the original 0.9 g. of reaction product was evaporated to dryness, but gave only a discolored, viscous, glassy residue which we have not been able to purify.

DL(1,4)R-Cyclohexanetetrol-1,2,4,5.[13,14]—A 101-mg. (0.18 millimole) portion of the tetrabenzoate (m.p. 180°) was stirred with 2 ml. of saturated methanolic ammonia at 25° for one day. The resulting clear solution was vacuum distilled to dryness. The residue was extracted with three 3-ml. portions of hot dry benzene. The residue then remaining was recrystallized from methanol–benzene, giving 7.2 mg. (27%) of the tetrol, m.p. 203–205°. The m.p. of the tetrol is 208° when completely pure.

This tetrol was shown by mixed m.p. and X-ray powder spectra to be identical with a tetrol of similar m.p.[12] obtained by catalytic debromination of the dibromocyclohexanetetrol of m.p. 190°.

When a solution of the tetrol in water was evaporated at 25° and one atmosphere, no evidence for formation of a hydrate was observed.

DL(1,4)R-Cyclohexanediol-1,2,4,5 Tetraacetate.[13,14]—A mixture of 1.48 g. of the tetrol, 10 ml. of acetic anhydride and 0.2 g. of freshly fused zinc chloride was boiled for 15 minutes, then vacuum distilled down to a sirup. On stirring with 20 ml. of warm water the sirup solidified, giving 2.96 g. (94%) of colorless crystals, m.p. 145.5–146°. On recrystallization from aqueous methanol, 2.50 g. of colorless prisms, m.p. 146–147°, was obtained. When crystallized again from methanol for analysis the compound melted very sharply at 148°.

Anal. Calcd. for $C_{14}H_{20}O_8$: C, 53.16; H, 6.37. Found: C, 53.00; H, 6.48.

Products from 1,3-Cyclohexadiene

DL-*trans*-Cyclohexenediol-3,4 Dibenzoate.—To 458 mg. (4 millimoles) of *trans*-cyclohexenediol-3,4[5] in 2.0 ml. (1.96 g., 25 millimoles) of dry pyridine was added 1.38 ml. (1.69 g., 12 millimoles) of benzoyl chloride with stirring and cooling. A large amount of colorless precipitate formed at once. The mixture was allowed to stand 16 hours.

Water (0.2 ml., 11 millimoles)[17] was added with stirring and the mixture allowed to stand for one hour. Ether (15 ml.), 10 ml. of water and 2 ml. of 6 *M* hydrochloric acid were then added, and the entire mixture transferred to a separatory funnel. The ether phase was washed again with 1 *M* hydrochloric acid, then with 1 *M* sodium carbonate and with water, and finally dried over sodium sulfate.

The dry ether solution on evaporation gave 893 mg. of sticky colorless crystals. These were recrystallized from about 1 ml. of absolute ethanol, giving 696 mg. (54%) of the enediol dibenzoate, large colorless prisms, m.p. 74–76° (cap.), reported[6] m.p. 77°. The dibenzoate, unlike the diol, is insoluble in water.

Attempts to carry out the benzoylation in aqueous sodium hydroxide or in hot pyridine were unsuccessful.

Bromine Test.—A few mg. of the enediol dibenzoate was suspended in carbon tetrachloride. Bromine–carbon tetrachloride added dropwise with stirring at room temperature was rapidly decolorized. Evaporation of the solution gave an oil which after a few hours turned crystalline; this product is no doubt the dibromodiol dibenzoate but has not been further investigated. The free enediol decolorized bromine–carbon tetrachloride even more rapidly; it dissolved as the reaction proceeded and crystals of the resulting dibromodiol[6] separated almost immediately.

Attempted Conversion of Cyclohexadiene-1,3 to an Enediol or Tetrol Derivative.—The reaction of cyclohexadiene-1,3 with iodine (or bromine) and silver benzoate (or 3,5-dinitrobenzoate), using procedures similar to those above, failed to give any of the expected benzoylated tetrols, enediols or halohydrins. The products were oils or oily solids, apparently complex mixtures, from which no pure compounds could be isolated.

Products from Cyclohexene

DL-*trans*-2-Bromocyclohexyl 3,5-Dinitrobenzoate.—To 0.95 g. (3 millimoles) of silver 3,5-dinitrobenzoate in 7.5 ml. of dry chloroform at −10° was added 0.077 ml. of bromine in 2.0 ml. of dry chloroform. After stirring several minutes, 0.15 ml. of cyclohexene was added.[9a,b] A few minutes later a second equal portion of bromine–chloroform was added, and shortly afterward 0.35 ml. more cyclohexene. The total amount of bromine was thus 0.48 g. (3 millimoles), and the total amount of cyclohexene 0.405 g. (5 millimoles). After a few minutes more stirring at −10° the mixture was allowed to warm up to 25°.

The dark mixture was filtered, and the filtrate washed with 1 *M* sodium carbonate and with water, and then dried with sodium sulfate. On evaporation the dried solution left 0.55 g. of light tan solid residue.

The residue was dissolved in about 2 ml. of boiling benzene, and 5 ml. of hot cyclohexane was added. Crystals

---

[17] After benzoylation of a polyol in pyridine it is important to destroy excess benzoyl chloride by adding only a small amount of water at first. This prevents separation of a second liquid phase, which would slow down the hydrolysis. When the benzoyl chloride is gone, the product may be precipitated with a large amount of water.

separated almost at once. These were collected and dried, giving 0.30 g. (27%) of colorless needles, m.p. 156–160°.

The crystals (0.30 g.) were recrystallized from 12 ml. of cyclohexane–benzene (5:1), giving 0.25 g. of colorless needle clusters, m.p. 158–159.5°.

For analysis a 150-mg. sample was recrystallized from about 7 ml. of absolute ethanol, m.p. 159–160°.

Anal. Calcd. for $C_{13}H_{13}BrN_2O_6$ (373.17): C, 41.84; H, 3.51; Br, 21.42; N, 7.51. Found: C, 41.83; H, 3.66; Br, 21.50; N, 7.51.

The compound gave a positive Beilstein halogen test, but was inert to alcoholic silver nitrate even after one minute boiling.

The compound was not attacked on prolonged stirring with 15 *M* aqueous ammonia or 4 *M* aqueous sodium hydroxide at 25°. It was attacked by boiling sodium hydroxide, giving a red solution. When heated with strong ethanolic ammonia in a sealed tube for several hours at 100°, the compound gradually dissolved, giving a solid product which we have not yet been able to purify.

DL-*trans*-Cyclohexanediol-1,2 Bis-3,5-dinitrobenzoate.—To 893 mg. (2.8 millimoles) of dry, finely pulverized silver 3,5-dinitrobenzoate in 3.6 ml. of dry benzene was added dropwise 254 mg. (1.0 millimole) of iodine in 2.5 ml. of benzene. To the resulting light tan suspension was added dropwise 0.112 ml. (90.3 mg., 1.1 millimoles) of cyclohexene in 1.2 ml. of benzene. After a few minutes stirring, the mixture was boiled under reflux for one hour. The hot mixture was filtered at once.

On cooling, the hot filtrate gave a small amount of crystals, presumably of the complex $AgI(C_6H_5COO)_2$. The solution was again filtered, and evaporated down to an oily residue.

The original residue (consisting mainly of silver iodide) was washed with 20 ml. of chloroform. The chloroform solution was used to dissolve the above oily residue. The resulting solution was concentrated to about 10 ml. and 10 ml. of cyclohexane was added at the b.p. On cooling, 218 mg. of crystals, m.p. 160–188°, was obtained.

The product was recrystallized from acetic acid, giving 52 mg. (10%) of the diol bis-dinitrobenzoate, yellow prisms, m.p. 181–182°, reported[18] m.p. 179°. (The corresponding iodohydrin ester reportedly[9b] melts at 159°.)

DL-*trans*-Cyclohexanediol-1,2 Dibenzoate.—The procedure was similar to that for the bis-dinitrobenzoate. From 11 millimoles of cyclohexene, 10 millimoles of iodine and 21 millimoles of silver benzoate (reflux period 3 hours) there was obtained an oily, yellowish crude product. This was recrystallized from absolute ethanol, giving 1.4 g. (44%) of the diol dibenzoate as colorless prisms, m.p. 92.5–93.5° (block), reported[18] m.p. 93°.

Acknowledgment.—We are indebted to Tennyson Campbell and Daniel Glick for assistance in some of the preparative work. Financial support by the National Research Council, the Research Council of Ontario and the Advisory Committee on Scientific Research is gratefully acknowledged.

(18) N. A. B. Wilson and J. Reed, *J. Chem. Soc.*, 1269 (1935).

TORONTO, CANADA

[CONTRIBUTION FROM THE HARRIS RESEARCH LABORATORIES]

Biosynthesis of C^{14}-Specifically Labeled Cellulose by *Acetobacter xylinum*. I. From D-Glucose-1-C^{14} with and without Ethanol[1]

BY FRANCIS W. MINOR, GLENN A. GREATHOUSE, HAROLD G. SHIRK, ANTHONY M. SCHWARTZ AND MILTON HARRIS

RECEIVED OCTOBER 24, 1953

C^{14}-Specifically labeled cellulose was biosynthesized by *Acetobacter xylinum* from D-glucose-1-C^{14} as the sole labeled carbon source. The presence of ethanol in the medium increased the yield and the quantity of C^{14} found in the cellulose. The distribution of C^{14} in D-glucose from the bacterial cellulose hydrolysate indicated that approximately 82% of the activity was in position 1 for the cellulose produced from D-glucose-1-C^{14} in the ethanol-free medium compared to 70% in that position for the cellulose produced with ethanol present. Positions 3 and 4 contained the remainder of the activity in approximately equal amounts. The cellulose had a lower specific radioactivity than the D-glucose-1-C^{14} that was supplied. This result shows that some of the original hexose units are cleaved prior to cellulose formation. Thus, polymerization of the D-glucose as such, without prior chain cleavage, is not the sole mechanism of cellulose biosynthesis by this bacterium.

Introduction

This study was initiated to determine the ability of *Acetobacter xylinum* to produce labeled cellulose from various C^{14}-specifically labeled carbohydrates and derivatives used as substrates for the growth of the organism, and at the same time to provide some information regarding the involved mechanism in cellulose formation.[2] It is the purpose of this paper to report experiments concerned with the biosynthesis of C^{14}-specifically labeled cellulose by this bacterium when cultured in suitable media containing D-glucose-1-C^{14}. Since the inclusion of ethanol in the culture medium has been observed to increase the yield of cellulose from a given quantity of D-glucose,[3] experiments were performed to determine what advantage, if any, such addition might have with respect to the distribution and specific activity of C^{14} in the cellulose.

The biosynthesis of cellulose by *A. xylinum* at the surface of appropriate media was first noted by Brown[4] in 1886. Corroboration that this membraneous bacterial product, synthesized from either D-glucose or other suitable carbohydrate substrates, is chemically identical to cellulose produced by higher plants was obtained by Hibbert and Barsha[5-7] employing chemical analytical procedures in conjunction with X-ray techniques. Others[8-10] have also compared the X-ray patterns of bacterial cellulose with those of cotton cellulose. More recently electron microscopic techniques

---

(1) This series of papers is based on work supported by the Atomic Energy Commission under contract AT(30-1)-915 with the Harris Research Laboratories.

(2) G. A. Greathouse, *Science*, **117**, 553 (1953).

(3) H. L. A. Tarr and H. Hibbert, *Can. J. Research*, **4**, 372 (1930).

(4) A. J. Brown, *J. Chem. Soc.*, **49**, 172 (1886).

(5) H. Hibbert and J. Barsha, *Can. J. Research*, **5**, 580 (1931).

(6) H. Hibbert and J. Barsha, THIS JOURNAL, **53**, 3907 (1931).

(7) J. Barsha and H. Hibbert, *Can. J. Research*, **10**, 170 (1934).

(8) H. Mark and G. V. Susich, *Z. physik. Chem.*, **B4**, 431 (1929).

(9) J. Eggert and F. Luft, *ibid.*, **B7**, 468 (1930).

(10) V. Khouvine, G. Champetier and R. Sutra, *Compt. rend.*, **194**, 208 (1932).