[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

The Catalytic Hydrogenation of Polyhydric Phenols. I. The Synthesis of *meso*-Inositol, Scyllitol and a New Isomeric Cyclitol

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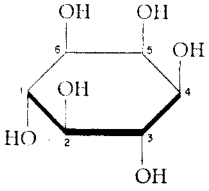
The stereochemistry of cyclohexane and cyclohexane derivatives has been involved in a number of recent investigations.[1] In this paper we wish to describe certain results we have obtained on the catalytic hydrogenation of hexahydroxybenzene. Our ultimate purpose was to obtain, if possible, some new isomeric cyclitols, and in so doing to elucidate further the stereochemistry of this important class of compounds. Aside from certain attempts to prepare these compounds by cyclization of naturally occurring sugars and their derivatives,[2] and the preparation of certain cyclitols by the interconversions of their naturally occurring isomers,[3] only one chemical approach to the synthesis of these compounds has claimed success.[4] Wieland and Wishart have claimed to have hydrogenated catalytically hexahydroxybenzene to *meso*-inositol in a yield of 80%. There are, however, a number of points in their procedure which are obscure, and it has been reported[5] that the synthesis has failed in the hands of other investigators. We are compelled also to report that all attempts by us to repeat this work have failed. In addition, we have been unable to reduce either the hexamethoxy or the hexaacetoxy derivatives under the conditions described by Wieland. Even varying the method of preparation of hexahydroxybenzene to eliminate any doubt concerning purity of product led to negative results. It was found possible to reduce triquinoyl octahydrate smoothly to hexahydroxybenzene but further reduction could not be made to take place using platinum as a catalyst. Indeed, in view of the present concept of the structure of *meso*-inositol[6] as being 2,4,5,6/1,3, the production of this single isomer in good yields by hydrogenation with platinum, as Wieland claims, would constitute a remarkable instance of catalytic specificity.[7] This result would be reasonable only if the structure of *meso*-inositol had proved to be the "all-*cis*" form, as Wieland tentatively postulated, in consideration of the evidence for *cis* addition of hydrogen over platinum catalysts.



A solution of this problem has been achieved by the employment of higher pressures, and by the use of Raney nickel as the hydrogenation catalyst. Under these conditions this polyphenol can be hydrogenated. When either an ethanol–water mixture, or water alone was used as the solvent the uptake of hydrogen was observed to progress smoothly at temperatures varying from 125–150° and at a pressure of one hundred atmospheres. The use of nickel on kieselguhr was found to give essentially the same results. As was to be expected in this latter case a temperature of 175–190° was required. It was observed that reduction did not occur when the polyphenol was suspended in ethanol. Also, when pure aqueous solutions of the phenol were used more hydrogenolysis took place than when ethanol–water mixtures were used as the solvent. The products of such a reaction ranged all the way from isomeric dihydroxy cyclohexanes to compounds analyzing correctly for the hexahydroxy cyclitols. In all of these experiments it was found that the separation of the cyclitol mixture itself into its components was tedious. To date five crystalline cyclitols have been isolated, three of which are definitely characterized. They are listed in Table I.

TABLE I

Name	Configuration	M. p., °C.	M. p. of hexa-acetate, °C.
Scyllitol	1, 3, 5/2, 4, 6	353–355	299–300
meso-Inositol	1, 3/2, 3, 5, 6	224–225	213–214
213° melting isomer	....	213–214	205–206
epi-Inositol "140"	1, 2, 4, 5, 6/3	285	189–190
isomer	....		139–140

We have made certain other observations which are of interest. The experimental results indicate that scyllitol and *meso*-inositol are obtained in approximately equal amounts. The percentages based upon the cyclitol fraction are relatively

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[1] K. C. Kauer, R. B. Duvall and F. N. Alquist, *Ind. Eng. Chem.*, **39**, 1335 (1947); N. G. Brink, F. A. Kuehl, Jr., and K. Folkers, *Science*, **102**, 506–507 (1945); H. E. Carter, *et al.*, *ibid.*, **103**, 53–54 (1946); R. C. Peck, *et al.*, THIS JOURNAL, **68**, 776 (1946); C. W. Beckett, *et al.*, *ibid.*, **69**, 2488 (1947).

[2] J. Needham, *Biochem. J.*, **18**, 891–904 (1924); F. Micheel and H. Rukopf, *Ber.*, **68**, 1523 (1935); **70B**, 850 (1937); F. Micheel, *Ann.*, **496**, 77 (1932); H. O. L. Fischer, *Harvey Lectures*, Ser. **40**, 156 (1944–1945).

[3] Th. Posternak, *Helv. chim. acta*, **29**, 1991 (1946).

[4] H. Wieland and R. S. Wishart, *Ber.*, **45**, 2082 (1914).

[5] M. R. Stetten and DeWitt Stetten, Jr., *J. Biol. Chem.*, **164**, 85 (1946).

[6] G. Dangschat and H. O. L. Fischer, *Naturwiss.*, **30**, 146 (1942); Th. Posternak, *Helv. chim. acta*, **25**, 746 (1942).

[7] The numbering of the positions would appear to be arbitrary, especially as the flat ring formulation (see Gilman's "Organic Chemistry," Vol. I, 337, 2nd ed.) is employed. However, it is suggested that in the future, as is done here, the numbering of those cyclitols attacked by *A. suboxydans* be arranged so that the carbon oxidized by this organism is labelled *5*, corresponding to the position attacked in gluconic acid.

small, approximately 6%. It is to be pointed out that this same qualitative relationship of the two isomers has been observed in the isomeric hexachlorocyclohexanes. In this latter case the "β" isomer of hexachlorocyclohexane corresponds to scyllitol, and the physiologically active "γ" isomer of the chloro series corresponds to *meso*-inositol with which it has been linked structurally on biological evidence.[8] Because of this relationship and particularly because Slade[9] has postulated that the "α" isomer in the chloro series is the racemic modification special efforts were made to isolate *d,l*-inositol from the reaction mixture. Maquenne and Tanret[10] produced artificially this racemic substance and characterized their product as a compound melting at 253°, the corresponding hexaacetate melting at 111°. The surprisingly low melting point of the hexaacetate led us to believe that we would be able to separate this isomer by fractional distillation provided of course any of the material was present in our mixture. To date, however, no crystalline fraction corresponding to *d,l*-inositol has been obtained.

Exhaustive acetonation of the reduction mixture was considered as a method for grouping the possible isomers according to the number of isopropylidene residues introduced. The preliminary note[6] of Dangschat and Fischer does not give the exact conditions by which they acetonated *meso*-inositol, and no further communication has become available. We were unable to reproduce the results as outlined and under other conditions typical for acetonation. The striking resistance of "suitable" polyhydroxy cyclohexane compounds to acetonation has been noted by Karrer[11] and by Posternak.[12] Indeed the latter investigators have recorded that even the proper pyrogallitols, where the solubility is much more favorable, do not condense with acetone. The models clearly show that in this cyclohexane series a typical "vicinal-*cis*" system does not exist, based on the "chair form," for which much evidence has already been presented.[13] Evidence for a boric acid complex with any of the known cyclitols is also lacking. Whether tautomerism of "boat–chair" or of "polar–equatorial" types applies to the problem above is still unanswered. It is interesting to note that conduritol,[14] a tetrahydroxycyclohexene acetonates readily. Here, however, a model reveals that the double bond forces the 3,4- carbon atoms into a truly "*cis*" relationship fully capable of forming a strainless five-membered ring.

Chromatography proved of value for the isolation of only one of the compounds obtained. By partition of a mixture of cyclitols (from which scyllitol and *meso*-inositol had been removed) on a column of alumina crystals of the 205 melting isomer were obtained. These seed crystals made chromatography unnecessary in further work on the separation of this particular isomer.

meso-Inositol, when subjected to conditions strictly comparable to those used for the reduction of hexahydroxybenzene, was recovered quantitatively without change. This would seem to indicate that the production of the various isomers is due to the catalytic process and not to some sort of isomerization. Likewise, the hydrogenolysis observed does not result from the breakdown of the cyclitols formed in the reaction. In another paper we plan to discuss results on the determination of the stereochemical structure of the new isomers described above. From a knowledge of these structures we hope to be able to elucidate partly the mechanism of the hydrogenation over nickel.

Experimental

Preparation of Hexahydroxybenzene. A. From Hydroquinones.—The procedure of Nietzki and Benckiser[14a] as modified by Henle[15] and Backer[16] was first employed. We found that the over-all yield from this time-consuming five-step procedure was approximately 20% based on hydroquinone. The nitration and oxidation of the diacetylhydroquinone was carried out in a three-necked flask immersed in a large crock containing the ice–salt mixture. Although filtration was used to separate the resulting slurry centrifugation would have been better since uncontrollable decomposition often sets in.

We found that reduction of the di-potassium salt of nitranilic acid was best carried out on 50-g. lots, since larger runs are more difficult to handle with ordinary equipment.

The literature value of a 60% yield for the oxidation of diaminotetrahydroxybenzene to the triquinoyl octahydrate could not be duplicated. Our yields of this material were approximately 40%. The catalytic reduction of this substance, however, was quantitative (see below) and on large batches the tin–hydrochloric acid procedure resulted in 80–85% yields of hexahydroxybenzene.

Preparation of Hexahydroxybenzene. B. From Glyoxal.—The procedure of Homolka[17] for the trimerization of glyoxal was repeated. The product, isolated as beautiful deep green glistening prisms, was the rather insoluble disodium salt of tetra-hydroxybenzoquinone. The yields of 5–10% reported by Homolka could not be improved either by varying widely the concentrations or by changing the catalyst employed. We found that it was possible to use a stabilized solution of free glyoxal,[18] if the bisulfite, sulfite or cyanide ion is present in 0.1 molar quantities in addition to the sodium carbonate. It is also necessary that prior to the addition of the glyoxal to the alkaline solution oxygen be supplied to the latter by either aeration or addition of hydrogen peroxide to obtain the quinone salt.

This salt was reduced as follows. Sixty grams (0.28 mole) was dissolved in a solution of 35 ml. of concentrated hydrochloric acid in 400 ml. of water. To this deep red solution was added a filtered solution of 85 g. (0.38 mole) of stannous chloride dihydrate in 300 ml. of 50% concentrated hydrochloric acid in water. The mixture was swirled by hand for a few seconds. Very soon clumps of needles appeared, the color of the solution disappeared,

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(8) H. W. Burton, S. E. Jacobs and A. Goldstein, *Nature*, **158**, 22 (1946).

(9) R. E. Slade, *J. Chem. Ind.*, 314 (1945).

(10) Maquenne and Tanret, *Compt. rend.*, **145**, 1196 (1890).

(11) P. Karrer, *Helv. chim. acta*, **9**, 116 (1926).

(12) Posternak, *ibid.*, **30**, 441 (1947).

(13) C. W. Beckett, *et al.*, THIS JOURNAL, **69**, 2488 (1947).

(14) Dangschat and Fischer, *Naturwiss.*, **27**, 756 (1939).

(14a) Nietzki and Benckiser, *Ber.*, **18**, 499 (1885).

(15) Henle, *Ann.*, **350**, 334 (1906).

(16) Backer and van der Baan, *Rec. trav. chem.*, **56**, 1161 (1937).

(17) Homolka, *Ber.*, **54**, 1393 (1921).

(18) A generous sample of a 30% aqueous solution of glyoxal furnished by Carbide and Carbon Chemicals Corporation is gratefully acknowledged.

and finally, the mixture set to a crystalline mass. The mixture was allowed to stand under carbon dioxide until cooled to room temperature, and then filtered under this gas. The crystals were washed twice with 10-ml. portions of water, twice with 75 cc. of air-free methanol, and finally with ether through which carbon dioxide had been passed. The light tan crystals, which took on a slight purple color on standing, were dried *in vacuo*. This first crop of material was ash-free and weighed 25 g. (52% yield). Further crops of material were obtained by two methods. The first method involved saturating the mother liquor with hydrogen chloride gas at 5°. The second method employed the sirup made by melting calcium chloride octahydrate. The combined yield of material from these treatments amounted to 10 g. (20% yield), but this latter fraction contained ash. However, only ash-free hexahydroxybenzene could be smoothly hydrogenated. The impure material was recrystallized by dissolving in three times its weight of water by warming and then passing in hydrogen chloride gas until crystallization began.

Preparation of Hexahydroxybenzene. C. From *meso*-Inositol.—The literature[19] records that di-sodium tetrahydroxybenzoquinone can be obtained by the controlled oxidation of *meso*-inositol. In our hands the yield of the green quinone salt obtained by this method was 55% and since *meso*-inositol is fairly readily available, this procedure is to be preferred. It is to be noted that no *meso*-inositol, as such, was found to withstand the oxidation to the quinone. It was also observed that the yield of *meso*-inositol found after the catalytic reduction was independent of the *source* of the hexahydroxybenzene.

Preparation of Hexamethoxybenzene.—This new derivative was prepared by treating 500 mg. of hexahydroxybenzene, suspended in 5 ml. of methanol, with 100% excess of diazomethane in ether. The compound dissolved to give a clear yellow solution as nitrogen was evolved. The excess diazomethane and the ether were removed by warming. The residual methanolic solution was filtered and evaporated to dryness, wt. 700 mg. (theory 740 mg.). The residue was dissolved in methanol and the solution treated with water to incipient cloudiness. Fine needles appeared which, when filtered and dried, melted at 81–82°. This material remained unchanged when treated with an acetylating mixture of acetic anhydride and zinc chloride. For analysis it was recrystallized from aqueous methanol.

Anal. Calcd. for $C_{12}H_{18}O_6$: C, 55.80; H, 7.02. Found: C, 56.01; H, 6.80.

Attempted Reductions of Hexadroxybenzene with Platinum and Palladium.—The usual Parr-type shaking outfit was used. The Pyrex reaction flask was fitted with a resistance wire heater calibrated on blank runs while shaking within ±3°. A glass baffle was inserted in the rubber stopper closure for the flask to prevent splashing onto the rubber surface. Prior to each new set of runs 0.1 mole of phenol was reduced with the platinum oxide or palladium catalyst to be employed to be sure of the cleanliness of the apparatus. All the catalysts used were active in the reduction of phenol but proved completely ineffective in reducing the hexahydroxybenzene. Water and various aqueous–ethanolic solutions up to 30% ethanol were tried, as was the *addition* of small amounts of hydrochloric acid.[20] Equally ineffective was the use of glacial acetic acid as the hydrogenation medium up to temperatures of 70°. In our experiments using water as the solvent temperatures up to 75° were employed and a pressure of four atmospheres of hydrogen was used.

Hydrogenation of Triquinoyl.—Four grams of white, ash-free triquinoyl octahydrate was suspended in 100 ml. of redistilled water and one ml. of 0.1 molar ferrous sulfate added along with 100 mg. of platinum oxide. The flask[21] was flushed and filled with hydrogen to 40 pounds pressure. On shaking a smooth uptake of hydrogen was noted and one-half the theoretical amount of hydrogen for the conversion of triquinoyl to cyclitol was used in twenty-five minutes. No further uptake occurred in two hours, however, and the flask was opened. An immediate appearance of the red color typical of solutions of hexahydroxybenzene occurred at the surface of the solution. A second portion of 100 mg. of platinum oxide was added and hydrogenation was again attempted but to no avail. On removing the bottle from the shaker and cooling in the refrigerator overnight, long white needles of hexahydroxybenzene appeared. The mixture was warmed to effect solution and filtered under carbon dioxide. A fresh batch of 100 mg. of platinum oxide was added and the bottle returned to the shaker. However even at elevated temperatures and with repeated additions of fresh catalyst no appreciable hydrogen uptake was observed. On filtering and cooling the solutions, the white crystals again appeared: weight of first fraction, 1.3 g. (60%). Addition of dry hydrogen chloride gas to the solution gave an addition crop of crystals with a purplish tinge, weight 250 mg. A small sample of crystals was treated with acetic anhydride and a chip of zinc chloride to give an acetate melting at 211°. No melting point depression with an authentic sample of hexaacetylhexahydroxybenzene was observed.

Reduction of Hexahydroxybenzene with Raney Nickel.—In a typical run 15 g. of hexahydroxybenzene obtained from triquinoyl by a stannous chloride reduction, was added to a mixture of 150 ml. of ethanol (previously distilled from Raney nickel) and 100 ml. of redistilled water in a glass liner. A teaspoonful of Raney nickel[22] which had been stored under ethyl alcohol was added and the liner sealed in the bomb in the usual manner. The initial pressure was 1910 pounds. Upon heating the first break in the pressure occurred at about 100° and hydrogenation proceeded rapidly at 125° in this solvent. The theoretical amount of hydrogen was taken up in fifty minutes. The shaking and heating were stopped. Although under these conditions there was usually left a portion of the starting material the amount of hydrogenolysis was held to a minimum. The cooled liner was removed and the contents were filtered. A transient red color sometimes appeared. The clear colorless filtrate was then evaporated to dryness *in vacuo*. Lyophilizing did not work with this mixture of cyclitols and complete drying was difficult. The total sirup weighing 12.9 g., was treated with 300 ml. of methanol. After an hour most of the sirup had dissolved and crystals started to form. The crystalline precipitate "A" was filtered. A second crop of crystals "B" was obtained on further dilution of the filtrate with methanol. The combined weight of fractions A and B amounted to 1.2 g.

Isolation of Scyllitol.—Fractions A and B were dissolved in 15–20 ml. of water. A small amount of inorganic residue was filtered with a celite filter aid. The clear colorless aqueous solution was then treated with methanol to bring the concentration to 50%. On standing crystals appeared which melted at 350–355°. One further recrystallization from water–methanol gave a product which melted at 350–354°, weight 500 mg. The analytical sample was dried for one hour at 100° and one mm. pressure.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.72. Found: C, 40.10; H, 6.94.

The hexaacetate was prepared by refluxing the scyllitol with acetic anhydride and a catalytic amount of sulfuric acid for twenty-five minutes. After working up in the usual manner, the crude crystalline material was sublimed at 200° under one mm. pressure and this sublimate re-

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[19] Gelormini and Artz, THIS JOURNAL, **52**, 2483 (1930); Hoglan and Bartow, *ibid.*, **62**, 2397 (1940).

[20] In contrast to Wieland's claim that small traces of hydrogen chloride hindered the reduction of his polyphenol, C. S. Marvel (THIS JOURNAL, **58**, 1594 (1936)) found that addition of hydrochloric acid or hydrobromic acid activated ring hydrogenation of phenol using platinum

[21] Carothers and Adams, THIS JOURNAL, **45**, 1071 (1923); **47**, 1047 (1925). The catalyst was prepared by fusion at 560–590° for six minutes.

[22] R. Mozingo, "Organic Synthesis," **21**, 15 (1941).

crystallized from boiling methanol, needles of m. p. 300–301° were obtained. It was necessary to sublime the hexaacetate to free it of firmly held acetic acid.

Anal. Calcd. for $C_{18}H_{24}O_{12}$: C, 50.00; H, 5.59. Found: C, 50.18; H, 5.68.

Isolation of *meso*-Inositol.—The mother liquor from the crystallization of scyllitol was diluted with methanol to an 80% mixture. Crystals formed which melted at 219–220°. Recrystallization from water–methanol did not appreciably raise the melting point. On sublimation at 200° and at a pressure of 1×10^{-4} mm., material was obtained which melted at 224–225°. A mixed melting point with an authentic sample of *meso*-inositol from a natural source showed no depression. A positive Scherer test was also obtained.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.72. Found: C, 40.28; H, 6.49.

The material in the methanol solution from which scyllitol and *meso*-inositol were removed (fractions A and B), was concentrated to dryness. The resultant viscous sirup did not yield any crystalline material and was consequently completely acetylated. The sirup weighing 11.2 g. was covered with 200 ml. of redistilled acetic anhydride in which 3 g. of dry hydrogen chloride gas had been dissolved. The mixture was slowly warmed to form a clear solution, and then was heated under reflux for one hour. The excess acetic anhydride and acetic acid was partially removed at the pump. The reaction product was poured into a beaker containing crushed ice and allowed to stand with stirring until the temperature of the mixture arose to that of the room. The sirup so formed was dissolved in 500 ml. of ether and washed five times with 25-ml. portions of water. Washing was continued with dilute sodium carbonate solution until the wash water became definitely alkaline to litmus. It was then transferred to a flask, the solvent removed and the residue dried by use of the benzene azeotrope. The material was transferred to a vacuum alembic and fractionally distilled at 3.5×10^{-5} mm.

Fraction I.—Approximately a fifth of the total weight of the acetate mixture distilled at 60–85° (vapor) from a bath at 125–130° to give a mobile colorless liquid. This liquid was redistilled. Four fractions were collected. Analysis showed that these fractions contained compounds representing different degrees of hydrogenolysis. The composition varied all the way from a mixture of diacetates to the hexaacetate mixture. The second of these four fractions came over at a bath temperature of 120° and 1×10^{-5} mm. It analyzed as follows.

Anal. Calcd. for $C_{10}H_{16}O_4$: C, 59.98; H, 8.06. Found: C, 60.07, 59.86; H, 7.42, 7.62.

The third fraction showed a larger oxygen content. It was collected at 150–160° at 1×10^{-5} mm.

Anal. Found: C, 56.90, 56.92; H, 6.77, 6.58.

Fraction II.—Two-thirds of the acetate mixture distilled at 165–200° at 1×10^{-5} mm. By dissolving this fraction in solvent mixtures such as ethyl acetate and petroleum ether (30–60°) or ethanol and petroleum ether (30–60°) a small amount of a semi-crystalline mass was obtained. This material melted at 130–140°. A portion of this fraction was sublimed at 1×10^{-5} mm. for twelve hours, the bath temperature being kept at 90–100°. The clear amorphous sublimate so obtained although analyzing for a hexaacetate admixed with small amounts of products of hydrogenolysis could not be induced to crystallize.

The remainder of the semicrystalline material (130–140°) was fractionally crystallized from ethanol-petroleum ether (60–70°). Two products were obtained. One compound melted at 188–189° and in all probability is the hexaacetate *epi*-inositol contaminated with small amounts of hydrogenolysis products.

Anal. Calcd. for $C_{18}H_{14}O_{12}$: C, 50.00; H, 5.59. Found: C, 51.84, 51.62; H, 5.83, 5.72.

The other fraction melted at 139–141° and contained with persistence some hydrogenolysis products. This material presumably contains a new cyclitol since it does not correspond to any known isomer.

Anal. Calcd. for $C_{18}H_{24}O_{12}$: C, 50.00; H, 5.59. Found: C, 51.27, 51.06; H, 5.11, 5.27.

The Isolation of a New Cyclitol (213° isomer). Method A.—The residue left in the alembic after the distillation described above was dissolved with boiling methanol. Crystals appeared on concentration of the filtered solution to a small volume. They were filtered and combined with a second drop obtained on further concentration. They melted at 201–203°. After several recrystallizations from boiling methanol a new cyclitol hexaacetate was obtained which had a constant melting point of 205–206°.

Anal. Calcd. for $C_{18}H_{24}O_{12}$: C, 50.00; H, 5.59; mol. wt., 432. Found: C, 50.18; H, 5.85; mol. wt. (Rast), 434.

The above acetate was hydrolyzed with catalytic amount of sodium methylate.[23] On working up the product a crude crystalline material was obtained which on recrystallization from water and methanol containing a trace of hydrochloric acid melted at 212–213°. Scherer test was positive.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40,00; H, 6.72. Found: C, 40.25; 6.85.

Isolation by Method B, Chromatography.—A portion (670 mg.) of the residual sirup, obtained from a small pilot reduction of hexahydroxybenzene over Raney nickel after the removal of scyllitol and *meso*-inositol, was dissolved in 15 ml. of water. The solution was diluted with 45 ml. of acetone and chromatographed over aluminuma (20 g. alumina, bore of column 75 mm.). The following fractions were obtained:

40 ml. of	15 water/	45 acetone gave	22 mg.	A
30	15	40	30	B
30	15	35	44	C
30	20	30	26	D
30	25	25	14	E
30	30	20	59	F
30	35	15	123	G
30	40	10	88	H
30	45	5	172	I

From fraction C crystals were obtained which melted at 198–200° with sintering at 190°. Upon recrystallization from water and ethanol a product was obtained which melted at 212–213°.

Attempted Acetonation of *meso*-Inositol.—Fifty grams of freshly fused zinc chloride and 50 ml. of glacial acetic acid were dissolved in 500 ml. of dry reagent acetone.[6] When the solution was clear, 6.47 g. of *meso*-inositol was added and the suspension heated to reflux for five days. After this time the undissolved solid was filtered from the dark brown opaque liquid, washed once with acetone, and dried at 95°. The undissolved material weighed 6.22 g., melted at 225.5–226.5° and proved to be starting material. The conditions and length of time of heating were varied without success. Copper sulfate, sulfuric acid, 20% zinc chloride by weight in the acetone, were equally ineffective in promoting the condensation.

Control Reaction of *meso*-Inositol with Raney Nickel.—A sample of natural *meso*-inositol melting at 225–225° and weighing 2.098 g. was dissolved in 20 ml. of water and treated in a micro-hydrogenation bomb with approximately 1.0 g. of Raney nickel. Another sample of the same Raney nickel sample employed here was used the same day for a successful hydrogenation of a sample of hexahydroxybenzene. The material was treated for four hours at 180–190° with hydrogen under 1900 lb. per sq. in. The contents and washing from the bomb were filtered through kieselguhr. The clear, colorless filtrate was evaporated to dryness *in vacuo*. The crude white crystalline residue weighed 2.094 g. and melted at 219–220. It contained a minute amount of ash. On recrystallization the material melted at 222–223°. No other crystalline

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[23] G. Zemplén and E. Pacsu, *Ber.*, **62**, 1613 (1929).

material was observed to be present, although about 50 mg. of *meso*-inositol containing more ash was left in the mother liquor.

We wish to take this opportunity to express our thanks to Standard Brands, Inc., for a fellowship which made possible this work. Also we wish to thank Merck and Co., Inc., for the microanalyses reported in this paper.

Summary

A new method for the preparation of hexahydroxybenzene is described.

Hexahydroxybenzene has been found not to reduce with platinum catalysts as previously reported but has been reduced using Raney nickel to give two known cyclitols; scyllitol, m. p. 353–355°; *meso*-inositol, m. p. 224–225°; and one new cyclitol, m. p. 213–214° (hexaacetate 205–206°). Evidence is also given for the presence of *epi*-inositol and another new cyclitol (hexaacetate, m. p. 139–140°) in the reduction mixture.

A discussion of these results is given.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Binding of Organic Ions by Proteins. Competition Phenomena and Denaturation Effects

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Competition between organic anions for loci of attachment on protein molecules has been demonstrated recently by spectral methods.[2] From changes in optical absorption of a given reference dye, it has been possible to arrange a series of noncolored anions in the order of their relative abilities to displace the dye and hence to combine with the protein. These investigations have now been extended to several series of isomeric compounds to elucidate certain structural effects and have been placed on a more quantitative basis for evaluating molecular weight and substituent influences.

Since the change in absorption of the dye is also a reflection of the state of the protein molecule, the spectral method has been used also to examine the effects of various denaturants on anion-protein complexes.

Experimental

Reagents.—All experiments involving protein have utilized crystallized bovine serum albumin from Armour and Co. The azo dyes which served as reference ions have been described previously.[2]

Salicylic acid, p-toluenesulfonic acid, naphthalene β-sulfonic acid, urea and urethan were Eastman "highest purity" grade. The sodium dodecyl sulfate was a specially purified sample generously supplied by the du Pont Co. Acetyl-*l*-tryptophan was prepared for us by Dr. A. H. Schlesinger following the procedure of du Vigneaud and Sealock.[3] The product had a m. p. of 189–190°. Acetyl-*d*,*l*-leucine was also prepared by Dr. Schlesinger, in an analogous fashion to the tryptophan derivative, and exhibited a m. p. of 160.5–161.5°.

Procedure.—All buffers were made from reagent grade phosphate salts. In the measurement of displacement effects, the following order of additions was generally used. Weighed quantities of albumin and of dye, respectively, were added to separate flasks containing known quantities of the buffer solution. A weighed quantity of the competing substance was then placed in a third dry volumetric flask to which were added 10 cc. of the dye solution, measured with a pipet, and enough protein solution to bring the volume to the 50 cc. mark on the flask. In every case two controls were also prepared, one of which contained 10 cc. of dye diluted to 50 cc. with buffer, and the second of which consisted of 10 cc. of dye diluted to 50 cc. with protein-buffer solution.

In the case of urea and urethan, however, this procedure was not convenient because large quantities of these substances were required. The required quantity of urea, or urethan, was added, therefore, to the initial buffer solution, in separate portions of which the dye and protein were subsequently dissolved. In experiments in which the order of addition was to be varied, the following procedure was adopted. Methyl orange was dissolved in a sample of buffer containing no urea. Practically equal quantities of albumin were added to each of two dry volumetric flasks. In one of these was placed 10 cc. of the dye solution, whereas the other was given 10 cc. of urea-buffer solution. Each flask was permitted to stand 10 minutes. The first was then filled with urea–buffer solution, the second with 10 cc. of dye solution and additional urea–buffer solution, to the 50 cc. mark. Spectra were taken immediately after complete mixing in each case.

Spectra were obtained in 1-cm. silica cells with the Beckman spectrophotometer.

The dialysis-equilibrium experiments have been described adequately in previous publications.[4,5]

Results and Discussion

As has been demonstrated previously,[2] a dye ion bound by a protein molecule shows a spectral absorption curve distinctly different from that of the free ion (Fig. 1). In the presence of a competing ion, some of the bound dye may be displaced from the protein, and hence the spectrum will be shifted back toward that of the free ion. The extent of the reversal is a measure of the displacing ability of the competitor ion and therefore of its affinity for the protein. On this basis it has

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[2] I. M. Klotz, THIS JOURNAL, **68**, 2299 (1946).

[3] V. du Vigneaud and R. R. Sealock, *J. Biol. Chem.*, **96**, 514 (1932).

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[5] I. M. Klotz and F. M. Walker, *ibid.*, **69**, 1609 (1947).