[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Inositol Methyl Ethers. II. The Formula of Sequoyitol[1-3]

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Sequoyitol was converted to a mono-*O*-isopropylidenetri-*O*-acetyl-*O*-methyl-*myo*-inositol, and this compound was deacetylated to a monoacetate and to the free isopropylidene derivative. Cleavage of the ring of the monoacetate failed to produce definitive evidence as to the position of the methyl group in sequoyitol. Sequoyitol was then synthesized from natural pinitol (5-*O*-methyl-D-inositol). This synthesis establishes the 5-*O*-methyl-*myo*-inositol formula for sequoyitol. On the basis of this formula, structures are assigned to the isopropylidene derivative and its acetates. Procedures for the cellulose column chromatography of the cyclitols are described.

Sequoyitol is one of the six possible *myo*-inositol monomethyl ethers, which differ from each other in the position occupied by the methyl group.[4] Sherrard and Kurth[5] first found it in aqueous extracts of California redwood (*Sequoia sempervirens*); since then it has been found in two Australian cycads,[6] and in sugar pine (*Pinus lambertiana*).[7] Although 29 years have elapsed since its discovery, and 15 since the configuration of the parent cyclitol was elucidated,[8] no evidence has so far been obtained which locates the methyl group in sequoyitol with certainty.

Only the two *meso* formulas II (methyl at position 5) and IX (methyl at position 2) have been seriously considered for sequoyitol, since it is optically inactive. Substitution of the *myo*-inositol structure at any position other than 2 or 5 renders it asymmetric,[9] and thus any other formula would be possible only if sequoyitol were a DL-mixture. This restriction of the possibilities recently has been vindicated. For a long time, only one optically active mono-*O*-methyl-*myo*-inositol, (+)-bornesitol,[10] was known. With the discovery of (+)-ononitol,[11] at least one of the enantiomorphs of each of the asymmetric possibilities was accounted for, and it could be shown by infrared spectrometry[12] that sequoyitol is not the DL-modification of either. In addition, DL-bornesitol is known,[2] and it is clearly different from sequoyitol.

Various attempts have been made to choose between formulas II and IX. Riggs[6] proposed formula IX on the basis of periodate oxidation studies, but Foster[13] concluded that the behavior of the compound on ionophoresis in borate buffer indicated formula II. Anderson and Landel[2] tried to synthesize 2-*O*-methyl-*myo*-inositol (IX), but obtained DL-bornesitol instead. The apparent oxidation of sequoyitol by *Acetobacter suboxydans* observed in this Laboratory would suggest formula II, but this oxidation turned out to be spurious.[14] Further work was therefore required.

The present communication describes the results of both degradative and synthetic attacks on the sequoyitol problem. Degradation through an isopropylidene derivative gave equivocal results, but it was found possible to synthesize sequoyitol from pinitol (V). This synthesis conclusively establishes formula II for sequoyitol.

Acetone Derivatives of Sequoyitol.—In the course of their work on the cyclitols of sugar pine, Ballou and Anderson[7] showed that sequoyitol could be acetonated less readily than D-inositol and pinitol, but more readily than *myo*-inositol. The Californians, who used zinc chloride–acetic acid as their catalyst, did not isolate their acetone sequoyitol.

Several variations of the zinc chloride–acetic acid technique were tried by the present authors. That of Angyal and Macdonald,[15] in which the product is acetylated before isolation, was most successful in our hands. The mono-*O*-isopropylidenesequoyitol triacetate which is obtained may be crystallized, or the crude sirupy product may be deacetylated as such. When the deacetylation was carried out with ethanolic ammonia in the usual way, the product was a crystalline substance which we supposed was *O*-isopropylidenesequoyitol.[3] It consumed the expected one molar equiva-

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(2) Paper I of this series, L. Anderson and Aurora M. Landel, THIS JOURNAL, **76**, 6130 (1954).

(3) Part of this work was presented orally to the American Chemical Society: L. Anderson, Aurora M. Landel and Emily B. Swan, Abst. 126th Meeting A.C.S., New York, September, 1954, p. 27D. The compound there described as *O*-isopropylidenesequoyitol has now been found to be the monoacetate VI.

(4) Strictly speaking, the *myo*-inositol methyl ethers differ from each other in *configuration*, since all inositol monomethyl ethers have the same *structure*. The situation is thus different from that in the open-chain series, where the position-isomeric methyl ethers of a given sugar or sugar alcohol have different structures. It is convenient, however, to deal (mentally) with all of these compounds in the same way, *i.e.*, to group all the *O*-methyl derivatives of a parent sugar or alcohol together, and distinguish the individuals according to the positions of their methyl groups.

(5) E. C. Sherrard and E. F. Kurth, *Ind. Eng. Chem.*, **20**, 722 (1928); THIS JOURNAL, **51**, 3139 (1929).

(6) N. V. Riggs, *J. Chem. Soc.*, 3199 (1949); private communication.

(7) C. E. Ballou and A. B. Anderson, THIS JOURNAL, **75**, 648 (1953).

(8) Gerda Dangschat, *Naturwissenschaften*, **30**, 146 (1942); T. Posternak, *Helv. Chim. Acta*, **25**, 746 (1942).

(9) The cyclitols discussed here are named and numbered according to H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951). In this system, numbering may be clockwise or counterclockwise as the occasion demands. The numbering shown in formulas I, II and IX, is a *composite* of clockwise and counterclockwise numbering. It readily shows the six ethers to be D-1-, 2-, L-1-, D-4-, 5- and L-4-*O*-methyl-*myo*-inositol, *i.e.*, two *meso* forms and two DL-pairs.

(10) (+)-Bornesitol, m.p. 200°, A. Girard, *Compt. rend.*, **77**, 995 (1873); L. Maquenne, *Ann. chim. phys.*, [6] **12**, 566 (1887).

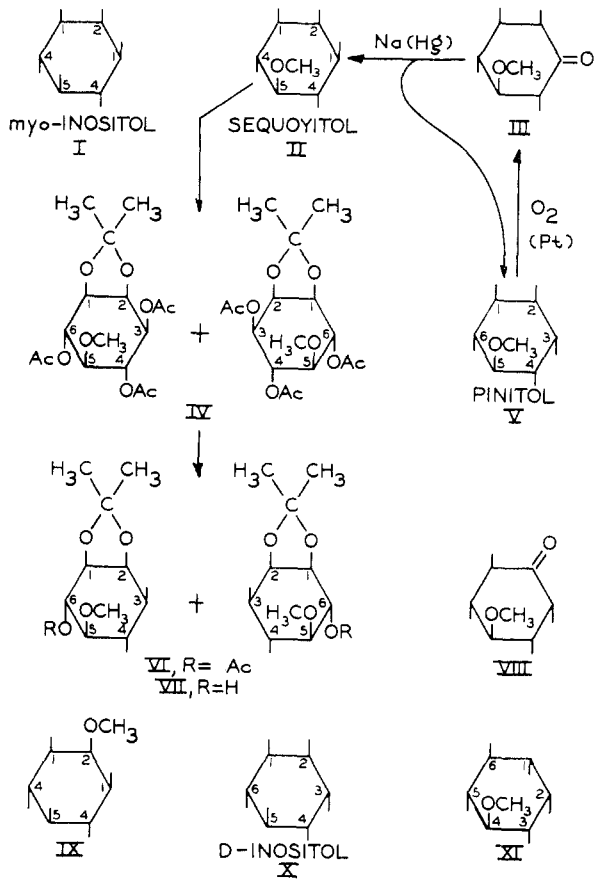
(11) (+)-Ononitol, m.p. 172°; V. Plouvier, *Compt. rend.*, **241**, 983 (1955).

(12) S. J. Angyal, P. T. Gilham and C. G. Macdonald, *J. Chem. Soc.*, in press. These workers have prepared 2-*O*-methyl-*myo*-inositol, m.p. 212°.

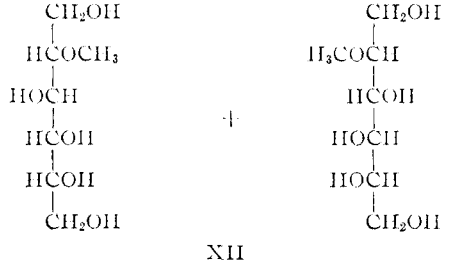
(13) A. B. Foster, *Chemistry & Industry*, 591 (1953).

(14) L. Anderson, *et al.*, *J. Biol. Chem.*, **204**, 769 (1953). The le work on the *Acetobacter* oxidation of the inositol methyl ethers wi the subject of a future communication.

(15) S. J. Angyal and C. G. Macdonald, *J. Chem. Soc.*, 686 (1



lent of periodate, and on cleavage with lead tetraacetate, reduction and hydrolysis, it gave what we may now say with certainty is 2-*O*-methyl-DL-glucitol (XII). However, the C, H and $-OCH_3$ analyses of the supposed *O*-isopropylidenesequoyitol did



XII

not quite fit the theoretical values. They did fit the values for an *O*-isopropylidenesequoyitol monoacetate, and the presence of one acetyl group was verified by direct determination. Further deacetylation with sodium ethoxide gave the free isopropylidenesequoyitol.

The fact that sequoyitol forms a monoacetone derivative is strong evidence for formula II as against formula IX. Only in formula II are there adjacent *cis* hydroxyl groups, and no case is known in which acetone condenses with a cyclitol at a *trans*-hydroxyl pair unless two acetone ketal groups are already present in the molecule.[15] It would follow that the acetone derivative and its triacetate have structures VII and IV, respectively.

Unequivocal evidence for formula II would have been provided by the rigorous identification of the product of the cleavage, reduction and hydrolysis of *O*-isopropylidenesequoyitol as 2-*O*-methyl-DL-glucitol (XII). In attempting this identification, one is faced with the unusual situation of having derived a racemic mixture from a natural product and of being able to synthesize only an optically active product (2-*O*-methyl-D-glucitol) for comparison. Both paper chromatography and the determination of infrared spectra in solution were investigated as methods of comparison, since the results are independent of the crystal structure differences between racemate and active modification. It was found, however, that these techniques do not distinguish between 2-*O*-methylglucitol and 5-*O*-methylglucitol. Inasmuch as 5-*O*-methyl-DL-glucitol could conceivably arise from a compound of formula IX by acetonation, cleavage, etc., no firm conclusion as to the identity of the cleavage product could be drawn. The assignment of the 2-*O*-methyl-DL-glucitol structure to this product, and the assignment of structures VII and IV to the acetone derivatives actually rest largely on the independent evidence presented below for formula II for sequoyitol. Periodate analysis gave confirmatory information, and showed that the single acetyl group in VI is at position 6. The synthesis of 2-*O*-methyl-D-glucitol and the preparation of the DL-modification from *O*-isopropylidenesequoyitol monoacetate form the subject of a separate communication.[16]

Synthesis of Sequoyitol from Pinitol.—Pinitol (V) has long been known as a methyl ether of D-inositol (X),[17] and the work of Anderson, MacDonald and Fischer[18] and of Angyal and coworkers[15,19] conclusively located the methyl group in the 5-position. Its configuration is thus such that the inversion of a single hydroxyl (no. 3) would convert it to 5-*O*-methyl-*myo*-inositol.

When pinitol was subjected to catalytic air oxidation according to Heyns and Paulsen,[20] the solution developed the strong reducing power characteristic of inososes. On reduction with sodium amalgam, a component with the paper chromatographic mobility of sequoyitol appeared in the solution. This substance was separated from the accompanying pinitol by chromatography on a cellulose column. Its melting point, alone and mixed with an authentic sample, was that of sequoyitol and, like sequoyitol, it gave *myo*-inositol on demethylation with hydriodic acid. These facts alone are sufficient to identify it as sequoyitol, for sequoyitol has a higher melting point (see Experimental) than any of the other *myo*-inositol methyl ethers.[10-12] The identification was checked, however, by paper chromatography, and by preparing the pentaacetate and comparing its melting point and infrared spectrum with that of the pentaacetate of natural sequoyitol.

(16) L. Anderson, A. Bieder and Emily S. DeLuca, forthcoming paper.
(17) L. Maquenne, *Ann. chim. phys.*, [6] **22**, 264 (1891).
(18) A. B. Anderson, D. L. MacDonald and H. O. L. Fischer, THIS JOURNAL, **74**, 1479 (1952).
(19) S. J. Angyal, C. G. Macdonald and N. K. Matheson, *J. Chem. Soc.*, 3321 (1953).
(20) K. Heyns and H. Paulsen, *Chem. Ber.*, **86**, 833 (1953).

A study of formula V reveals that the only *myo*-inositol derivatives which could arise from pinitol by the operations performed[21] are 5-*O*-methyl-*myo*-inositol (II, from the ketose III) and L-4-*O*-methyl-*myo*-inositol (XI, from the ketose VIII). Inasmuch as XI is asymmetric, it must correspond to one of the ononitols or one of the bornesitols (see above), and it cannot therefore be sequoyitol. It follows that sequoyitol is 5-*O*-methyl-*myo*-inositol (II), and the optically inactive *O*-methyl-*myo*-inositol described by Angyal, Gilham and Macdonald[12] is the 2-isomer IX as proposed by them. Since both *O*-isopropylidenesequoyitol and its monoacetate consume one molar equivalent of periodate, they must both contain a single vicinal glycol grouping. They thus have the structures VII and VI, respectively, and the triacetate must be IV. Since it gives sequoyitol as well as pinitol on reduction, the ketose from pinitol must be III. The phenylhydrazone of this ketose has been isolated from an oxidized pinitol solution, and the inosose itself, so far a sirup, has been obtained. It will be more fully described in a future communication on the air oxidation of the inositol methyl ethers.

Cellulose Column Chromatography.—The chromatography of cyclitols on columns of cellulose powder, with aqueous acetone as the developing solvent, has been mentioned by Ballou and Anderson[7] and by Angyal and McHugh.[22] We have found this method to be an excellent one for the resolution of a variety of cyclitol mixtures. The technique as practiced in our laboratory is briefly described in the experimental section.

Acknowledgment.—The authors are grateful to Dr. Ray E. Schreck of the Union Lumber Co., San Francisco, for arranging shipment of redwood heartwood plank; to Dr. Edward G. Locke, U. S. Forest Products Laboratory, Madison, Wis., for making pilot plant extraction facilities available; to Dr. Aurora M. Landel of our own group for some of the early isolations and analyses; to Mr. W. A. Carleton of the Winton Lumber Co., Martell, Calif., for a gift of sugar pine sawdust; and to Dr. V. W. Meloche of the Analytical Division, Department of Chemistry, for the infrared spectra.

Experimental[23]

Sequoyitol (II).—Redwood heartwood planks (see Acknowledgment) were reduced to sawdust or shavings in the carpenter shop. Three batches of the lumber were worked up in one or more portions. Almost no sequoyitol was obtained from a kiln-dried batch. One batch of uncured wood gave a product containing much *myo*-inositol, which is difficult to separate from sequoyitol by crystallization. The third batch readily furnished pure sequoyitol. This batch, which consisted of about 70 kg. of shavings from uncured plank, was extracted twice for 24 hours in a 450-gallon digester. The combined extracts, totalling several hundred gallons, were concentrated in three stages, first in a large (120 sq. ft.) tube evaporator, then in a laboratory size one, and finally on the steam-bath in an open dish. About 1.5 l. of very thick dark sirup resulted. This sirup was diluted to 2.8 l. and poured into an equal volume of ethanol. The solid (1365 g.) which deposited during several days in the cold room was filtered off, washed with ethanol, and dried.

Further purification was accomplished with barium hydroxide as recommended by A. B. Anderson[24] for pinitol. The solution of the crude solids in 1.5 l. of water was subjected to four treatments with a total of 1.65 l. of saturated $Ba(OH)_2$. After removal of the excess barium with H_2SO_4 and decolorization, the solution was concentrated to 300 ml. *in vacuo*. On the addition of an equal volume of absolute alcohol, crude sequoyitol precipitated.

For recrystallization, the material was dissolved in about four volumes of boiling 50% ethanol and treated with charcoal. The twice recrystallized product weighed 50 g. (*ca.* 0.07% of the original wood) and melted at 241–242°; and a sample chromatographed on cellulose (see below) melted at 243°. Sherrard and Kurth[5] reported yields up to 0.06%, and m.p. 234–235°. The collected mother liquors, on evaporation, left a considerable residue. Paper chromatography showed that the principal cyclitol in this residue was pinitol.

Anal. Calcd. for $C_7H_{14}O_6$ (194.18): C, 43.29; H, 7.27; $-OCH_3$, 15.98. Found: C, 43.22; H, 7.37; $-OCH_3$, 15.76; ash, 0.16.

***O*-Isopropylidenesequoyitol Triacetate (DL-1,2-*O*-Isopropylidene-3,4,6-tri-*O*-acetyl-5-*O*-methyl-*myo*-inositol, IV).** Preparatory to acetonation the sequoyitol was powdered and dried overnight in an oven at 100°. The acetone was likewise dried, and the manner of drying seemed to have a great deal of influence on the yield of acetonated product. The best yields were obtained when the acetone had been kept for 8 to 16 hours over each of two batches of anhydrous K_2CO_3, then either used immediately or stored over Drierite. Acetone stored for a long time over K_2CO_3 is inferior.

The directions given by Angyal and Macdonald[15] for the acetonation and acetylation of *proto*-quercitol were slightly modified to give optimal results with sequoyitol. For each gram of sequoyitol, 40 ml. of acetone containing 6 g. of freshly fused zinc chloride and 5 ml. of glacial acetic acid was used. A reflux time of 2.5 hours was adopted. Twenty ml. of pyridine was calculated to be sufficient for complexing the zinc chloride and neutralizing the acetic acid involved in the process. In a 5-g. run made with good acetone, 4.4 g. of the sequoyitol dissolved and 3.7 g. (45% based on sequoyitol disappearing) of crude product was obtained. The first batch was slow to crystallize, but no difficulty was experienced once seed crystals were available.

O-Isopropylidenesequoyitol triacetate is very soluble in hot ethanol, benzene, and ethyl acetate, and sparingly soluble in the Skellysolves. It may be recrystallized from absolute ethanol alone (4 vols.) or from any of the following mixtures: absolute ethanol, 3 vols., Skellysolve B, 10 vols.; benzene, 1 vol., Skellysolve B, 25 vols.; ethyl acetate, 4 vols., Skellysolve B, 15 vols. The compound is dimorphic. Rapid crystallization from any of the solvents mentioned tends to give fine needles melting at 100–101°. Slower crystallization, with appropriate seeding, gives hexagonal prisms melting at 107–108°.

Anal. Calcd. for $C_{16}H_{24}O_9$ (360.35): C, 53.33; H, 6.71; $-OCH_3$, 8.61; acetyl, 35.84. Found: C, 53.57; H, 6.73; $-OCH_3$, 8.69; acetyl, 35.41.

***O*-Isopropylidenesequoyitol Monoacetate (DL-1,2-*O*-Isopropylidene-5-*O*-methyl-6-*O*-acetyl-*myo*-inositol, VI).**—The sirupy triacetate from 8.65 g. of sequoyitol was taken up in absolute ethanol and the solution cooled in ice. Ammonia gas was bubbled in for 30 minutes, then the solution was kept at 3° for 24 hours, and finally evaporated under reduced pressure to give a sirupy residue. The complete removal of the acetamide formed in the reaction was found to be a critical factor in obtaining a good yield of recrystallized product. This was accomplished by heating the sirup in a *boiling* water-bath at <0.1 mm. pressure for 30

(21) It is assumed that the configurations at the positions other than the one oxidized are not altered by enolization or similar processes. The only such rearrangements which have been observed in inososes subjected to the mild conditions used here have been shown to be enzymatically catalyzed.

(22) S. J. Angyal and D. J. McHugh, *Chemistry & Industry*, 947 (1955).

(23) All compounds were recrystallized to constant melting point. Melting points were taken in capillary tubes. The thermometer was standardized against an N.B.S. calibrated Anschütz set. C and H analyses by the Micro-Tech Laboratories, Skokie, Ill., and by the Huffman Microanalytical Laboratories, Wheatridge, Colo. The latter laboratory did some of the methoxyl analyses.

(24) A. B. Anderson, *Tappi*, **35**, 198 (1952).

minutes. On cooling and scratching, the product crystallized.

Either absolute or 60% ethanol served as the recrystallization solvent. The yield of once-recrystallized compound melting at 165–169° was 4.65 g., or 38% for the two steps. The melting point was raised to 171–172° by three more recrystallizations. To establish the presence of the isopropylidene group, a sample (7 mg.) was heated in a sealed tube with *p*-nitrophenylhydrazine and acetic acid. On cooling, there deposited a 60% yield of acetone *p*-nitrophenylhydrazone, identified by melting point and mixed melting point. When treated with excess sodium metaperiodate, *O*-isopropylidenesequoyitol monoacetate consumed the following amounts (molar equivalents) of the reagent: at 3.25 hr., 0.87; at 17 hr., 0.94; at 48 hr., 1.24.

Anal. Calcd. for $C_{12}H_{20}O_7$ (276.28): C, 52.16; H, 7.30; $-OCH_3$, 11.23; acetyl, 15.58. Found: C, 52.48; H, 7.65; $-OCH_3$, 11.10; acetyl, 15.63.

***O*-Isopropylidenesequoyitol** (DL-1,2-*O*-**Isopropylidene-5-*O*-methyl-*myo*-inositol, VII**).—The well-dried monoacetate (1.42 g.) was refluxed for 40 min. with 8 ml. of absolute ethanol containing just less than 1 molar equivalent of sodium ethoxide. As efforts to get the product to crystallize at this point failed, the solution was taken to dryness, and the residue dissolved in 4 ml. of water. The aqueous solution was deionized in two portions on a column of Amberlite IRC-50 (0.5 g. of resin in a 10 mm. o.d. tube) buffered at *p*H 11 with NH_4OH. In this way the exposure of the sensitive acetone-ketal linkage to an acid environment was avoided. On evaporation of the eluates *in vacuo*, the product was obtained as a clear glass, soluble in the cold in 4 vols. of absolute ethanol.

Seed crystals were obtained by evaporating a portion of the ethanol solution under an air jet, and boiling the resulting sirup with benzene. With the aid of these, the *O*-isopropylidenesequoyitol was crystallized from *ca.* 6 vols. of boiling ethyl acetate. The crystals (1 g., 85% of theory) were long and fibrous, with the appearance of spun glass. The melting point, 115–116°, was not altered by further recrystallizations. The crystalline product was free of acetyl groups. When tested for acetone groups, it gave, like its precursor, a 60% yield of acetone *p*-nitrophenylhydrazone. Its periodate consumption was 1.0 molar equivalent at one hour; no further reagent was used when the time was extended to 26 hours.

Anal. Calcd. for $C_{10}H_{18}O_6$ (234.24): C, 51.27; H, 7.74; $-OCH_3$, 13.25. Found: C, 51.40; H, 7.95; $-OCH_3$, 13.56.

Pinitol (V) was isolated from sugar pine (*Pinus lambertiana*) sawdust (see Acknowledgment) by the procedure of A. B. Anderson.[24] The product contained small amounts of D-inositol and sequoyitol. In order to obtain a sequoyitol-free preparation, a 2-g. batch was chromatographed on a cellulose column with acetone–water 4:1 as described below. The pinitol was the first component to come off the column, and it was cleanly separated from the sequoyitol.

Sequoyitol from Pinitol.—One gram of the chromatographed pinitol was dissolved in 100 ml. of water, and 1 g. of 10% Pt-on-charcoal (Darco G-60) was suspended in the solution.[25] The mixture was held at 85–90° while air was drawn vigorously through it for four hours, then filtered and the filtrate concentrated to 50 ml. *in vacuo*. This filtrate was strongly reducing to hot Benedict solution. Paper chromatograms, run with acetone–water[7] 9:1 (v./v.) showed a pinitol spot and a new spot with $R_{pinitol} = 0.73$, corresponding to the inosose III.

The concentrated filtrate from above was treated with 3% sodium amalgam (six 20-g. batches) until the reducing power disappeared. The *p*H was held at 6 during the reduction by the addition of glacial acetic acid. Mercury was removed by filtration, and the filtrate and washings, after dilution to 150 ml., were deionized on a Dowex 50 column. The effluent was concentrated *in vacuo* to a sirup, and the sirup was dissolved in water and reconcentrated several times to remove acetic acid. Paper chromatography showed a strong pinitol spot and a spot with the mobility of sequoyitol ($R_{pinitol} = 0.66$).

An attempt to resolve the mixture on cellulose powder (see below) with acetone–water 4:1 was not successful. Clean separation was obtained with acetone–water 8:1. On the addition of ethanol to the decolorized concentrate of the second band eluted from the column, 80 mg. of crystalline substance precipitated. After recrystallization from ethanol–water, this substance melted at 239–240°. A mixture with authentic sequoyitol of m.p. 243° melted at 241–242°. When chromatographed on paper with acetone–water 9:1, the synthetic material travelled as a single spot with a mobility identical with that of natural sequoyitol run on the same paper.

A portion (10 mg.) of the compound was heated for one-half hour with 0.1 ml. of 47% HI. The material recovered by ethanol precipitation was chromatographed on paper with acetone–water 9:1. Two spots appeared, one with the mobility of the *myo*-inositol control ($R_{pinitol} = 0.33$) and one with the mobility of the sequoyitol control.

Synthetic Sequoyitol Pentaacetate.—Twenty-one mg. of the synthetic material was acetylated with acetic anhydride and sodium acetate. The crystalline acetate melted at 202° and did not depress the m.p. of the pentaacetate of natural sequoyitol. Sherrard and Kurth[5] give 198°, and we have observed 203–203.5°, for the acetate of the natural material.

Angyal, Gilham and Macdonald[12] have found that the acetates of the isomeric *myo*-inositol methyl ethers show characteristic differences in their infrared spectra. The spectrum of our synthetic acetate in chloroform solution was taken with the Baird model B instrument. It was essentially identical with the spectrum of the pentaacetate of natural sequoyitol.

Resolution of Cyclitol Mixtures on Cellulose Columns.—For total weights of material ranging from 200 mg. to 2 g. or more, we routinely use a 4.5-cm. diameter column packed to a depth of 40 cm. Smaller columns doubtless would suffice for the smaller amounts. The developing solvent is usually acetone–water 4:1 (v./v.), but on occasion mixtures containing 90% (v./v.) or more of acetone are employed. The adsorbent is Whatman ashless cellulose powder, standard grade. It may be packed into the column dry, as recommended by Hough, Jones and Wadman,[26] or slurried in the solvent to be used. A Waring blendor is convenient for making the slurries. In either case, the cellulose is added in small portions, and tamped before the next portion is put in. As noted by Hough, Jones and Wadman, columns should be conditioned with the solvent to be used. We can confirm the English authors' statement that these columns can be re-used repeatedly without repacking. One dry-packed column served us for over a year, but the wet-packed ones do not seem to be so durable.

In use, the columns are mounted on an automatic fraction collector and solvent is fed in from a constant level reservoir. A very small head of solvent will maintain a good flow rate (2.5–4.0 ml. per min.) in a new column; with the older columns, slight air pressure is necessary. Twenty-five to forty ml. is a convenient fraction volume.

To detect the peaks, each fraction is transferred to an erlenmeyer flask, and the acetone is boiled off. Strips of Whatman no. 1 paper are ruled into squares, and an aliquot from a selected fraction is spotted in each square. The paper is then sprayed with ammoniacal silver nitrate (6% w./v. $AgNO_3$ treated with concd. NH_4OH until the precipitated Ag_2O dissolves) and heated at 100–110° for three to five minutes. Black spots indicate cyclitol containing fractions. The lower limit of detection is about 1 μg. When 40-ml. fractions are taken, a fast moving cyclitol like pinitol will begin to appear at about fraction 25. *myo*-Inositol, which is one of the slowest moving cyclitols in the acetone–water system, shows up at fraction 85–100.

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(25) The catalyst was prepared as described by K. Heyns, *Ann.*, **558**, 177 (1947).

(26) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).