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CHEMISTRY OF THE GENUS *SEQUOIA*—V.

CYCLITOLS FROM THE HEARTWOOD OF *SEQUOIA GIGANTEA*

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Abstract—The heartwood of giant redwood, *Sequoia gigantea*, has been shown to contain three cyclitols. The principal cyclitol was sequoyitol, followed by *myo*-inositol and pinitol. The possible taxonomic relevance of cyclitol composition is discussed.

INTRODUCTION

INVESTIGATIONS into the chemistry of heartwood constituents of two species of the *Sequoia* have been largely confined to California coast redwood, *Sequoia sempervirens*.[1] In their study of the heartwood extractives in this species, Sherrard and Kurth found the cyclitols pinitol[2] and sequoyitol.[3] Yield of the latter reportedly varied from traces to 0·06 per cent (dry wood basis), while no yield was reported for pinitol. A subsequent report indicated that the combined yield of these two cyclitols was 1·2 per cent in the heartwood and 1·9 per cent in old redwood stumps.[4] Relative percentages of these two cyclitols was not given.

In our continuing investigations into the chemistry of this genus, we undertook to examine the composition of the cyclitol fraction in the heartwood of giant redwood, *S. gigantea*, which is reported here.

RESULTS

In conjunction with extraction of cyclitols from *Sequoia gigantea* sawdust, we found it more difficult to recover cyclitols from fresh, green sawdust or hot-water extractions, because of gums and other components accompanying the crude isolated cyclitol fraction which interfered with purification of the cyclitols. The best method appeared to be extraction of air-dried sawdust with water at room temperature.

Even with water extractions, several pre-treatments were necessary to expedite recovery of cyclitols. The total aqueous extract was concentrated to about 8 to 10 per cent solids and then allowed to stand overnight, during which time insolubles formed. After removal of insolubles, a saturated solution of barium hydroxide was added to the extract to precipitate gums, which were then removed. Acidification of the filtrate with dilute sulfuric acid precipitated excess barium. After barium sulfate removal, the filtrate was concentrated to about

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[2] E. C. SHERRARD and E. F. KURTH, *Ind. Engng Chem.* **20**, 722 (1928).
[3] E. C. SHERRARD and E. F. KURTH, *J. Am. Chem. Soc.* **51**, 3139 (1929).
[4] H. F. LEWIS, *Tappi* **34**, 388 (1951).

15-20 per cent solids, after which an equal volume of ethanol was added to precipitate polysaccharides. Following removal of this component, the treated extract was concentrated to a syrup, to which was added dry ethanol–acetone (1–1) to precipitate the cyclitols. Although yield of cyclitols varied among various samples, average yield was around 0·6 per cent (dry-wood basis).

Examination of total cyclitol fraction by paper chromatography led to detection and subsequent isolation of the cyclitols-sequoyitol, *myo*-inositol, and pinitol. We found that the solvent system *n*-propanol–ethyl acetate–water (7:1:2) gave better resolution than did previously used acetone–water (95:5).[5]

Isolation of individual cyclitols was effected by a procedure of fractional acetonation.[5] With sulfuric acid as the catalyst, pinitol was acetonated to the acetone-soluble isopropylidene derivative, while the unreacted cyclitols, sequoyitol and *myo*-inositol, remained undissolved. Sequoyitol was separated from *myo*-inositol by cellulose column chromatography. The acetone-soluble isopropylidene compound was hydrolyzed and the parent compound, pinitol, recovered.

The average composition of the cyclitols indicated 78 per cent sequoyitol, 14 per cent *myo*-inositol and 8 per cent pinitol. (+)-Inositol was not indicated within the limit of detectability employed.

A sample of the pith area taken from 15-ft dia. tree sections which was estimated to be about 2000 years old was examined for its cyclitol composition.[6] The cyclitol yield amounted to 0·58 per cent (dry-wood basis). Paper chromatography examination of this material clearly indicated sequoyitol and *myo*-inositol, but pinitol was not detectable.

The heartwood of an 85-year-old giant sequoia was similarly examined for cyclitols. Cyclitols recovered amounted to 0·34 per cent, and with paper chromatography only *myo*-inositol could be detected.

DISCUSSION

Cyclitols are widely spread throughout the plant world.[7] Referring to coniferous species, Lindstedt, Erdtman and others have commented on the general occurrence of pinitol in the heartwood of *Haploxylon* pines (five-needled), while the apparent absence of this and other cyclitols was noted in heartwood of Diploxylon pines.[8]

Sherrard and Kurth discovered sequoyitol in the heartwood of *Sequoia sempervirens* in addition to pinitol.[2, 3] Subsequent investigations of the Haploxylon pine, *Pinus lambertiana*, indicated that while pinitol was by far the principal cyclitol it also contained sequoyitol, together with *myo*-inositol and traces of (+)-inositol.[5] There are a number of examples in which the monomethyl cyclitols, pinitol and sequoyitol, are found together.[9, 10]

In the biogenesis of cyclitols in plants, Hoffmann-Ostenhof and co-workers described work on sequoyitol, (+)-pinitol and (+)-inositol.[11] Glucose ^{14}C, *myo*-inositol ^{14}C and various

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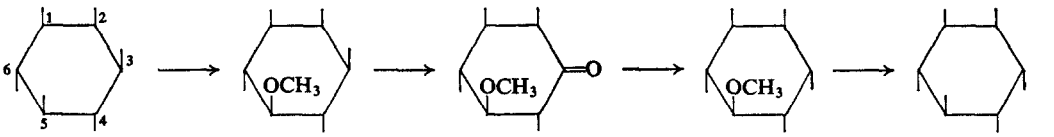
[9] S. J. ANGYAL and L. ANDERSON, *Advances in Carbohydrate Chemistry*, 14, p. 169. Academic Press New York (1959).
[10] Th. POSTERNAK, *The Cyclitols*, p. 136. Holden-Day, San Francisco (1965).
[11] R. SCHOLDA, G. BILLEK and O. HOFFMANN-OSTENHOF, *Z. Phys. Chem.* **335**, 180 (1964).

of the product cyclitols themselves were used as precursors. These were fed to detached leaves of *Trifolium incarnation* or crimson clover from water solutions for several days, and the cyclitols were then isolated.

From this the authors conclude that *myo*-inositol, or an intermediate easily formed from it, is the normal metabolic precursor of the other cyclitols. Much information on the interconversion of cyclitols was also obtained; presumably these interconversions are part of the normal biogenetic process. In the transformation of *myo*-inositol to other cyclitols, an inversion of configuration at one position or a methylation, or both, is involved.

The cyclitols of *T. incarnation* (sequoyitol, (+)-pinitol, (+)-inositol) appear to be formed as depicted in Fig. 1.



myo-inositol (I) | sequoyitol (II) | keto-inositol (III) | (+)-pinitol (IV) | (+)-inositol (V)

FIG. 1. FORMATION OF CYCLITOLS IN *Trifolium incarnation.*

The order of events is inferred from labeling experiments, showing that each of the cyclitols, including the keto-inositol (III), gives rise only to the compounds shown subsequent to it in the scheme. (+)-Inositol is apparently not a precursor of any of the others; the methylation step thus comes first, and (+)-pinitol is formed from sequoyitol by inversion of configuration at C_3. Interestingly, each of the four cyclitols, except the keto-inositol, has been found together in *P. lambertiana* heartwood[5] and in the wood of *Phyllocladus trichomanoides.*[12]

From the taxonomic standpoint it appears that relative amounts of sequoyitol to pinitol may have some relevance. When pinitol predominates, genus *Pinus* (Haploxylon)—i.e. *P. lambertiana*[5]—inversion is quite active; in species such as *Sequoia gigantea* (in which sequoyitol is more abundant) less inversion apparently takes place. This pattern appears to be consistent, irrespective of tree age, as both *myo*-inositol and sequoyitol were found in the 2000-year-old pith areas of giant sequoia trees, while in the pith area of mature old-growth sugar pine (up to 600 years old) cyclitols consisted largely of pinitol.[13]

In examining cyclitols present in the juvenile heartwood of an 85-year-old giant redwood, we found it to contain mostly *myo*-inositol in yields of 0·34 per cent. The heartwood of a 13-year-old *Metasequoia glyptostroboides* was reported to contain sequoyitol (0·14 per cent).[14] Thus it would appear that methyl transferase is very active in the latter species.

As pointed out by Plouvier, it appears that occurrence of a hydroxyl or a methyl group in this or that position is a sufficiently precise character to be of use in taxonomy.[7] This suggests that while sequoyitol and pinitol may appear together in the same plant, the ratio of each of these may have taxonomic and biogenetic significance.

EXPERIMENTAL

Microanalyses were made by Alfred Bernhardt in the Max-Planck-Institut für Kohlenforschung, Mülheim, Germany. Melting points are corrected.

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Preparation of Wood Extracts

Cross sections of the butt area above stump swell from four giant sequoia trees were made available through the courtesy of the State of California Resource Agency, Division of Forestry. These cross sections measured 11 ft 4 in. to 15 ft 4 in. in dia., respectively. It is estimated that the larger trees were about 2000 years old.[6] Composite samples, representing each of the four trees, were prepared in determining average yield and composition of the cyclitol fraction.

Air-dried *Sequoia gigantea* sawdust (500 g) was introduced into a 4 l. percolator, and water was added to cover the sawdust; water was circulated by the airlift extraction method for 24 hr and the solution was then drained.[5] Aqueous extraction was repeated three times, and the residue was washed with 3 l. of water after final extraction. Solutions were combined and concentrated to about 400 ml in a flash evaporator under reduced pressure. The concentrated solution was allowed to set overnight and then centrifuged to remove water-insoluble material.

35 ml of a saturated solution of $Ba(OH)_2$ was added to the clear, aqueous extract, this precipitated gums. Barium precipitate was removed by centrifuging, and the resulting solution was acidified to Congo red with dil H_2SO_4 to remove excess barium. $BaSO_4$ was removed by filtration, and the solution was then concentrated to about 100 ml under reduced pressure in a rotary evaporator. The concentrate was poured into an equal volume of 95 per cent ethanol with stirring, and the resulting precipitate was removed by centrifugation. The supernatant liquid was concentrated to heavy syrup under reduced pressure, and the cyclitols were precipitated by addition of 150 ml of absolute ethanol–acetone (2:1). This was permitted to set overnight and then filtered, and the crude product washed with absolute ethanol and dry acetone. Additional quantities of cyclitols were recovered from the mother liquors, resulting in an over-all recovery of 3 0 g of cyclitols or 0 6 per cent yield (oven-dry basis).

Chromatography of Cyclitols

The cyclitol mixture was examined by descending paper chromatography using Whatman No. 3 paper and the solvent system *n*-propanol–ethyl acetate–water (7:1:2). This solvent system proved superior to previously-used acetone–water (95:5).[5] Development was allowed to go overnight. Cyclitols were located on the dry-paper strips by spraying with 5 per cent ammoniacal $AgNO_3$ solution followed by heating at 100° for 2 to 3 min. R_f values when pinitol = 1·00, were: sequoyitol 0·74, (+)-inositol 0·47, and *myo*-inositol 0·33. The chromatogram revealed three components in order of intensity: sequoyitol, *myo*-inositol and pinitol. Crystals recovered from the second and in particular the third mother liquor proved to be largely pinitol.

Fractionation of the Heartwood Cyclitol Mixture

The following steps in the fractionation are based on the fact that the cyclitols differ in ease with which they are acetonated.[5] Pinitol and (+)-inositol present are acetonated in acetone containing 1·5 per cent conc. H_2SO_4. Sequoyitol and *myo*-inositol are unaffected by these conditions.

Sequoyitol–myo-inositol. 2 g of the cyclitol mixture was stirred for 7 hr at room temperature with 150 ml of acetone containing 3 ml of conc. H_2SO_4. The undesolved residue, amounting to 1·8 g (or 90 per cent), was collected and washed with dry acetone. Paper chromatography indicated that the residue was a mixture of sequoyitol and *myo*-inositol.

Pinitol. The above acetone filtrate was neutralized with Na_2CO_3 and filtered. Acetone was removed by distillation and 100 ml of 1 N HCl added to the residue, and the mixture was refluxed for 4 hr. The solution was cooled and filtered, and then concentrated to a syrup under reduced pressure in a rotary evaporator. Absolute ethanol was added and the mixture was set aside for several days to crystallize. The crystals, amounting to 0·12 g, were collected and washed with dry acetone. This was shown by paper chromatography to be mainly pinitol.

Sequoyitol. A mixture (1·0 g) of sequoyitol–*myo*-inositol was resolved, using column chromatography and an automatic fraction collector. Cellulose powder (500 g) of thin-layer quality was used with the solvent acetone–water (4:1). Fractions were examined by paper chromatography. Sequoyitol fractions were combined and concentrated to a syrup under vacuum. Absolute ethanol–dry acetone (1–1) was added and the mixture was permitted to set overnight. The crystals 0·6 g (60 per cent) were washed with dry acetone. Several recrystallizations from ethanol–water (2.1) produced optically inactive crystals which melted at 238 to 240°, and the melting point (m.p.) was unchanged when mixed with authentic sequoyitol. (Anal. Calcd. for $C_7H_{14}O_6$: C, 43·3; H, 7·2; OCH_3, 15 9. Found: C, 43·13; H, 7·2; OCH_3, 16·38 per cent.)

The acetate was prepared and the m p. remained unchanged when mixed with authentic sequoyitol pentacetate, m p. 200–202°

Myo-*Inositol.* The *myo*-inositol fractions from the cellulose column were combined and concentrated to a syrup. Absolute ethanol-dry acetone (1–1) was added and the mixture was permitted to set overnight. The crystals (0 2 g) were recovered and recrystallized several times from ethanol–water (8:1). The optically inactive crystals melted at 225–227° and when mixed with authentic *myo*-inositol, the m p. was unchanged. (Anal. Calcd. for $C_6H_{12}O_6$: C, 39·9; H, 6·6. Found: C, 39·86; H, 6 67 per cent.)

The acetate melted at 210–212° and the m.p. was unchanged when mixed with *myo*-inositol hexacetate, m.p. 210–212°.

Pinitol. A mixture totaling 0·6 g recovered from a number of mother liquors during the preparation of crude cyclitols from a number of extractions proved chromatographically to be largely pinitol. Several recrystallizations from 9–1 ethanol–water produced 0·3 g of crystals which melted at 184–186° $[\alpha]_D^{22}+66{\cdot}1$ (*c*, 1 per cent in H_2O). The m.p. remained unchanged when mixed with authentic pinitol. (Anal. Calcd. for $C_7H_{14}O_6$: C, 43·3; H, 7·2; OCH_3, 15·9. Found: C, 43·28; H, 7·32; OCH_3, 15·28 per cent.)

The acetate was prepared and its m.p. remained unchanged when mixed with pentacetyl pinitol, m.p. 98–99°.

Quantitative Estimation of Cyclitols

The resolution of cyclitols on the cellulose column indicated that the approximate composition of the cyclitols in the heartwood of giant redwood is as shown in Table 1.

TABLE 1. QUANTITATIVE ESTIMATION OF CYCLITOLS IN GIANT REDWOOD (500 g SAWDUST)

	Sequoyitol	*myo*-Inositol	Pinitol
Per cent of total cyclitols	78	14	8
Per cent of dry wood	0·47	0·08	0·05

Distribution of cyclitols. A composite sample of 400 g (dry basis) of *S. gigantea* sawdust taken from the pith area of the two 15 ft dia. trees was extracted with water. This wood was laid down in approximately 100 A.D. The aqueous extract was processed as previously described and 2·31 g or 0·58 per cent yield of cyclitols were recovered. This is comparable to the yield of cyclitols recovered from composite samples of sawdust from the four trees, and paper chromatography indicated only sequoyitol and *myo*-inositol to be present.

The heartwood taken from an 85-year-old giant sequoia was similarly processed for cyclitols. The heartwood region consisted of the first twenty annual rings, the rest being sapwood; cyclitol yield amounted to 0·34 per cent. Paper chromatography indicated only *myo*-inositol to be present.