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CHEMISTRY OF THE GENUS *SEQUOIA*—VI.

ON THE CYCLITOLS PRESENT IN HEARTWOOD OF *SEQUOIA SEMPERVIRENS*

ARTHUR B. ANDERSON, RICHARD RIFFER and ADDIE WONG

University of California Forest Products Laboratory, Richmond, California

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Abstract—The heartwood of California coast redwood, *Sequoia sempervirens*, has been shown to contain *myo*-inositol and (+)-inositol in addition to the previously reported sequoyitol and pinitol. The possible taxonomic relevance of cyclitol composition is discussed.

INTRODUCTION

IN THEIR study of the heartwood extractives in California coast redwood, *Sequoia sempervirens*, Sherrard and Kurth found the cyclitols pinitol[1] and sequoyitol.[2] The yield of the latter was reported to vary 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.[3] The relative percentages of these two cyclitols were not given.

In our continuing investigation into the chemistry of this genus, the work reported below was undertaken to clarify the facts concerning the composition of the cyclitol fraction in California coast redwood heartwood.

RESULTS AND DISCUSSION

Re-examination of the water-soluble constituents led to the detection and subsequent isolation of the cyclitols *myo*-inositol and (+)-inositol, in addition to sequoyitol and pinitol. The average cyclitol yield was approximately 1·0 per cent (dry wood basis).

In conjunction with the chemical separations, we have applied paper-strip chromatography for the detection of the cyclitols and cellulose-column chromatography for the separation of the individual cyclitols.

Isolation of the individual cyclitols from the heartwood extract was effected by a procedure of fractional acetonation.[4] With sulfuric acid as the catalyst, pinitol and (+)-inositol were acetonated to acetone-soluble isopropylidene compounds; while the unreacted cyclitols, sequoyitol and *myo*-inositol, remained undissolved. The sequoyitol-*myo*-inositol fraction was separated on a cellulose column. The acetone-soluble isopropylidene mixture was hydrolyzed with dilute hydrochloric acid and the resulting cyclitol mixture on several recrystallizations produced pure pinitol. The resulting mother liquor residues were combined and put through the cellulose column, and a small amount of (+)-inositol was recovered.

[1] E. C. SHERRARD and E. F. KURTH, *Ind. Engng Chem.* **20**, 722 (1928).
[2] E. C. SHERRARD and E. F. KURTH, *J. Am. Chem. Soc.* **51**, 3139 (1929).
[3] H. F. LEWIS, *Tappi*, **34**, 388 (1951).
[4] C. E. BALLOU and A. B. ANDERSON, *J. Am. Chem. Soc.* **75**, 648 (1953).

The average composition of the cyclitol fraction indicated 61 per cent pinitol, 31 per cent sequoyitol, 7 per cent *myo*-inositol and less than 1 per cent (+)-inositol.

Cyclitols are widely spread throughout the plant world.[5] Referring to coniferous species, Lindstedt, Erdtman and others have commented on the general occurrence of pinitol in the heartwood of *Haploxylon* pine (generally five-needled), while noting the apparent absence of this and other cyclitols in the heartwood of *Diploxylon* pine.[6] Subsequent investigation of the wood of the haploxylon pine *Pinus lambertiana* indicated that although pinitol was by far the principal cyclitol, it also contained some sequoyitol, together with *myo*-inositol and traces of (+)-inositol.[4] There are a number of examples in which the monomethyl cyclitols, pinitol and sequoyitol are found together.[7,8]

In the biogenesis of cyclitols in plants, Hoffmann-Ostenhof and co-workers described work on sequoyitol, pinitol and (+)-inositol, using carbon-labelled glucose and *myo*-inositol.[9] From this, the authors concluded that *myo*-inositol, or an intermediate easily formed from it, is the normal metabolic precursor of the other cyclitols. The first step is the methylation of *myo*-inositol to sequoyitol, followed by the epimerization of this compound to pinitol via 5-*O*-methyl-meso-inosose-3. (+)-Inositol arises from demethylation of pinitol only; obviously, no direct epimerization of *myo*-inositol to (+)-inositol is possible. In this connexion it is of interest that each of the four cyclitols has been found together in *P. lambertiana* heartwood[4] and in the wood of *Phylloclidus trichomenoldes*,[10] as well as in the presently investigated *Sequoia sempervirens*.

From the taxonomic standpoint, it would appear that the relative amounts of sequoyitol to pinitol may have some relevance. In those cases in which pinitol predominates, i.e. more than 90 per cent (e.g. genus *Pinus* (Haploxylon), *Pinus lambertiana*[4]), inversion is quite active; in the genus *Sequoia*, in which larger amounts of sequoyitol occur, less inversion apparently takes place. Further, while *S. sempervirens* cyclitols consist of approximately 31 per cent sequoyitol, *S. gigantea* cyclitols were recently found to contain 72 per cent sequoyitol.[11]

Although samples of the latter were too limited to ascertain whether this is a relatively consistent pattern of percentage composition, it would suggest that inversion to pinitol is more active in *S. sempervirens* than in *S. gigantea*.

EXPERIMENTAL

Preparation of Wood Extract

A composite sample of fresh green wood was prepared from redwood heartwood obtained from several areas in the redwood region. 500 g (oven dry basis) was introduced into a 4 l. percolator and water added to cover the sawdust. The water was circulated through use of the airlift extraction method for 24 hr, and the solution was drained.[4] The aqueous extraction was repeated for a total of three times and the residue washed with 3 l. of water after the final extraction. The solutions were combined and concentrated to about 400 ml in a flash evaporator. The concentrated solution was allowed to set overnight, then centrifuged to remove water-insoluble material.

25 ml of a 10 per cent solution of $Pb(OAc)_2$ was added to the clear aqueous extract to precipitate the tannins and other polyphenolics. The Pb precipitate was removed by centrifuging and the resulting solution treated

[5] V. PLOUVIER, *Chemical Plant Taxonomy*, p. 313, Academic Press, New York (1963).
[6] H. ERDTMAN, *Perspectives in Organic Chemistry*, p. 465, Interscience (1956); G. LINDSTEDT, *Acta Chem. Scand.* **5**, 129 (1951).
[7] S. J. ANGYAL and L. ANDERSON, *Advances in Carbohydrate Chemistry*, p. 169, Academic Press, New York (1959).
[8] TH. PASTERNAK, *The Cyclitols*, p. 136, Holden-Day, San Francisco, California (1965).
[9] R. SCHOLDA, G. BILLEK and O. HOFFMANN-OSTENHOF, *Z. Physiol. Chem.* **335**, 180 (1964).
[10] S. K. ADHIKARI, R. A. BELL and W. E. HARVEY, *J. Chem. Soc.* 2829 (1962).
[11] A. B. ANDERSON, R. RIFFER and ADDIE WONG, in press.

with H_2S to remove excess Pb. The PbS was removed by filtration and the clear filtrate was concentrated to about 100 ml using a rotary evaporator. The concentrated solution was poured into an equal volume of 95 per cent ethanol with stirring, and the resulting amorphous precipitate removed by centrifugation. The supernatant liquid was concentrated to a heavy syrup under reduced pressure and the cyclitols precipitated by the addition of 150 ml of absolute ethanol. This was permitted to set overnight and then filtered, and the crude product was washed with absolute ethanol and dry acetone. Additional quantities of cyclitols were recovered from the mother liquors, resulting in an overall recovery of 5·0 g of cyclitols, a 1·0 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 v/v/v). This solvent system proved to be superior to previously used acetone–water (95:5).[4] The development was allowed to go overnight. The cyclitols were located on the dry paper strips by spraying with 5% ammonical $AgNO_3$ solution followed by heating at 100° for 2–3 min. R_f values, when pinitol = 1·00, were: sequoyitol 0·74, (+)-inositol 0·47 and *myo*-inositol 0·33. The chromatogram revealed four components in order of intensity, namely pinitol, sequoyitol, *myo*-inositol and (+)-inositol.

Sequoyitol-myo-*Inositol*

2 g of the cyclitol mixture was stirred for 7 hr at room temperature with 100 ml of acetone containing 4 ml of conc. H_2SO_4. The undissolved residue, amounting to 0·76 g or 38%, was collected and washed with dry acetone. Paper chromatography indicated that the residue was a mixture of sequoyitol and *myo*-inositol.

Pinitol-(+)-*Inositol*

The above acetone filtrate was neutralized with Na_2CO_3 and filtered. The acetone was removed by distillation and 100 ml of 1-N HCl added to the residue and refluxed for 4 hr. The solution was cooled and filtered and then concentrated to a heavy syrup in a rotary evaporator; absolute ethanol was added and the mixture set aside for several days to crystallize. The crystals amounting to 0·58 g were collected and washed with dry acetone. This was shown by paper chromatography to be mainly pinitol and a trace of (+)-inositol.

Sequoyitol

A mixture (0·5 g) of sequoyitol–*myo*-inositol was resolved using column chromatography and an automatic fraction collector. Cellulose powder (500 g) of the thin-layer quality was used with the solvent acetone–water (4-1 v/v). The fractions were examined by paper chromatography. The sequoyitol fractions were combined and concentrated to dryness under vacuum. The crude crystals, 0·4 g, were recrystallized several times from ethanol–water (2:1) producing optically inactive crystals which melted at 238–240°, and the melting point was unchanged when mixed with authentic sequoyitol.

The acetate was prepared, and the melting point remained unchanged when mixed with authentic sequoyitol pentaacetate, melting point 200–202°.

myo-*Inositol*

The *myo*-inositol fractions from the cellulose column were combined and concentrated to dryness, wt. 90 mg, and recrystallized from ethanol–water (8:1). The optically inactive crystals melted at 225–227° and when mixed with authentic *myo*-inositol, the melting point was unchanged.

The acetate melted at 210–211° and the melting point was unchanged when mixed with *myo*-inositol hexacetate, melting point 210–211°.

Pinitol

The crude cyclitols (0·58 g) recovered from the acetonation reaction proved to be largely pinitol with trace of (+)-inositol. Several recrystallizations from ethanol–water (9:1) produced 0·3 g of crystals which melted at 184–186° $[\alpha]_D^{22}$ +66° (*c* 1 in H_2O). The melting point remained unchanged when mixed with authentic pinitol.

The acetate was prepared and the melting point remained unchanged when mixed with pentacetyl pinitol, melting point 98–99°.

(+)-*Inositol*

A number of the mother liquors from the crystallization of pinitol were combined and concentrated to dryness. The residue showed two spots on the paper chromatogram. About 0·3 g of the mixture was separated on a cellulose column. The (+)-inositol fraction was concentrated to dryness and yielded crystals (100 mg)

from ethanol–water. The material was chromatographically homogeneous, migrating with authentic (+)-inositol. It had a melting point of 246–247° which remained unchanged when mixed with authentic (+)-inositol. The optical rotation was +63° (*c* 1·0 in water) which is in fair agreement with the value +65° (water) recorded for pure (+)-inositol.

Quantitative Estimation of Cyclitols

The resolution of the cyclitols on the cellulose column indicated the approximate composition of the cyclitols in the heartwood of California coast redwood was (as a percentage of dry wood) pinitol, 0·61; sequoyitol, 0·31; *myo*-inositol, 0·07; and (+)-inositol, <0·01.