crystalline precipitate formed; m.p. 85°, lit. m.p. benzenesulfinic acid 83–84° (Beilstein, ref. 4), 86–87° (Thomas[6]).

Reaction with Hydroxylamine.—An 8% soln. (approx.) of hydroxylamine was made by treating the hydrochloride dissolved in the smallest possible quantity of water with a soln. of the equivalent of sodium dissolved in abs. ethanol. Five cc. of this soln. was added to 0.02 g. of the sirup and the mixture was evaporated on the boiling water-bath. When dry, the beaker was covered with an inverted watch-glass and left on the water-bath. In a short time the watch-glass became clouded and on examination under the microscope, was seen to be covered with the characteristic crystals of sublimed glyoxime. The residue in the beaker gave the ferric chloride test for benzenesulfinic acid.

Disproportionation of Benzenesulfinic Acid in Alkaline Solution.—Benzenesulfinic acid was prepared by the method of Thomas.[6] The sample had m.p. 83–84°. An aqueous solution was made alkaline with caustic soda and left aside. After a few days the solution had a smell of thiophenol, which increased considerably on warming.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE FOREST PRODUCTS LABORATORY, UNIVERSITY OF CALIFORNIA]

On the Cyclitols Present in Sugar Pine (*Pinus lambertiana* Dougl.)

BY CLINTON E. BALLOU[1] AND ARTHUR B. ANDERSON[2]

A water extract of sugar pine heartwood has been shown to contain myoinositol, *d*-inositol and sequoyitol in addition to the previously reported pinitol. Myo-inositol was found to be present in the aqueous sapwood extract along with pinitol. A procedure of fractional acetonation was employed to separate the cyclitol mixture and led to isolation of the pure compounds. Filter paper chromatography was applied to the separation and identification of four cyclitols, and three cyclitol derivatives.

The constituents of the heartwood of sugar pine and other members of the genus *Pinus* have been investigated systematically by Erdtman, Lindstedt and others in an effort to obtain evidence for a classification of these plants on the basis of the chemical composition of the heartwood extractives.[3] In addition to the large number of phenolic substances identified, these workers have commented on the general occurrence of pinitol[4] in the heartwood of *Haploxylon* pines (five needled). Although chromatographic techniques were employed, no other cyclitols were identified.[5] Erdtman, who was unable to isolate pinitol from the sapwood of the Haploxylon pine *Pinus strobus*, suggested that pinitol is a true heartwood substance.[6] One of us,[7] however, has recently shown pinitol to be present in the sapwood of the sugar pine.

The work reported below was undertaken to clarify the facts concerning the composition and distribution of the cyclitol fraction in sugar pine heartwood and sapwood. Re-examination of the water-soluble constituents of the heartwood of the sugar pine led to the detection and subsequent isolation of the cyclitols myoinositol, *d*-inositol and sequoyitol,[8] in addition to pinitol. The presence of pinitol and myo-inositol in the sapwood of sugar pine has been confirmed by paper chromatography, and a method for their quantitative estimation in wood extracts has been devised.

In conjunction with the chemical separations, we have applied the paper-strip chromatographic technique to the separation of the four cyclitols. myo-inositol, *d*-inositol, allo-inositol and muco-inositol, and the cyclitol derivatives, pinitol, sequoyitol and quebrachitol. That they may be readily separated by chromatography is in contrast to the difficulty encountered in separating the hexitols by this method.[9]

Isolation of the individual cyclitols from the heartwood extract was effected by a procedure of fractional acetonation. With sulfuric acid as the catalyst, pinitol and *d*-inositol were acetonated to acetone-soluble isopropylidene compounds, while the unreacted cyclitols, sequoyitol and myo-inositol, remained undissolved. Part of the sequoyitol was then isolated from this insoluble residue by fractional crystallization from water.[8] The sequoyitol remaining in the mixture with myo-inositol was removed by acetonation with zinc chloride–glacial acetic acid catalyst,[10] which left most of the myo-inositol undissolved[11] and in a form easily purified. *d*-Inositol was isolated by column chromatography from the mother liquors left after crystallization of the diisopropylidene pinitol.

Experimental

Preparation of Wood Extracts.—Air-dried sugar pine sawdust (500 g.) was introduced into a 4-liter glass percolator and extracted three times with water using the airlift extraction method.[12] The extracts were combined and concentrated to 100 ml. *in vacuo*. This concentrate was cooled and centrifuged to remove water insolubles that had precipitated during concentration. The supernatant was then poured slowly, with vigorous stirring in 300 ml. of 95% ethanol to precipitate the gums that were present. The precipitate was removed by centrifugation, and the resulting clear solution was used in the following studies. It will be called the "water extract."

Chromatography of Cyclitols and Sugar Pine Extracts.—Various procedures have been described for the chroma-

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(3) For reviews see: H. Erdtman, *Svensk. Chem. Tid.*, **63**, 43 (1951); G. Lindstedt, *Acta Chem. Scand.*, **5**, 129 (1951).

(4) A monomethyl ether of *d*-inositol first discovered by M. Berthelot, *Compt. rend.*, **41**, 392 (1855), in the exudate of sugar pine.

(5) G. Lindstedt and A. Misiorny, *Acta Chem. Scand.*, **5**, 121 (1951).

(6) H. Erdtman, *Svensk. Chem. Tid.*, **56**, 2 (1944).

(7) A. B. Anderson, *TAPPI*, **35**, No. 5, 198 (1952).

(8) A monomethyl ether of myo-inositol discovered by E. C. Sherrard and E. F. Kurth, THIS JOURNAL, **51**, 3139 (1929), in redwood heartwood.

(9) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.* 1702 (1950).

(10) S. J. Angyal and C. G. MacDonald, *ibid.*, 686 (1952).

(11) G. Dangschat and H. O. L. Fischer, *Naturwissenschaften*, **30**, 146 (1942).

(12) A. A. Morton, "Laboratory Technique in Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1938, p. 208.

tography of myoinositol,[14] but none has been studied for general use in separation of the cyclitols. The following procedure describes their separation by filter paper chromatography.

The cyclitols (20% solutions in water) were applied as spots 5 mm. in diameter at the starting line of a Whatman No. 1 filter paper strip. The paper was suspended in a closed jar, and developed (descending) with a mixture of acetone and water (80:20 to 95:5 v./v.)[14] for 6–8 hours. The solvent was allowed to drip from the bottom edge of the paper which was serrated.

The cyclitols were located on the dry paper strips by spraying with 5% ammoniacal silver nitrate solution followed by heating at 100° for 2–3 minutes. R_f values in acetone–water (95:5 v./v.) were: myo-inositol 0.13, *d*-inositol 0.17, allo-inositol 0.24, muco-inositol 0.30, sequoyitol 0.27, quebrachitol 0.31 and pinitol 0.38.[15]

A chromatogram of the "water extract" of sugar pine heartwood revealed five major components when sprayed with ammoniacal silver nitrate. Only one of these (probably arabinose)[6] reacted with the aniline oxalate spray.[9] The other four components were tentatively identified with myo-inositol, *d*-inositol, sequoyitol and pinitol by a comparison of the migration rates with known standards.

A chromatogram of the "water extract" of sugar pine sapwood showed spots corresponding to myo-inositol and pinitol. The corresponding zones of an unsprayed chromatogram were eluted and the eluate tested for inositol qualitatively by the Scherer test.[16] Both gave strongly positive results.

Fractionation of the Heartwood Cyclitol Mixture.—The "water extract" obtained from 5 kg. of sugar pine heartwood sawdust was concentrated *in vacuo* to a thin sirup, which was transferred to an evaporating dish and further concentrated to a heavy sirup on a steam-bath. A volume of acetone equal to that of the sirup was added with stirring and most of the pinitol crystallized from solution. After standing one hour, this mixture was filtered and the solid was washed with a little acetone. The crystals (fraction A) have been shown to be nearly pure pinitol[7] and were not further investigated.

The acetone mother liquor was concentrated to a thick sirup which was stirred with 250 ml. of absolute ethanol. A light-tan amorphous material (fraction B) separated immediately. This was collected and dried in a vacuum oven at 60°. A chromatogram of this material indicated that it contained myo-inositol, *d*-inositol, sequoyitol and pinitol (about 50% of the latter).

The following steps in the fractionation are based on the fact that the cyclitols differ in ease with which they are acetonated.[10,11,17] Pinitol and *d*-inositol are acetonated in acetone containing 1.5% concentrated sulfuric acid. Sequoyitol and myo-inositol are unaffected by these conditions, although sequoyitol is readily acetonated by the Dangschat procedure.[10] Myo-inositol reacts only to a small extent.[11]

(A) **Diacetone Pinitol.**—About 10 g. of the heartwood cyclitol mixture (fraction B) was shaken for five hours at room temperature with 150 ml. of commercial acetone containing 3 ml. of concentrated sulfuric acid. The undissolved residue (R-1), amounting to 5 g., was collected and washed with acetone. From the combined acetone filtrate was isolated 5 g. of diisopropylidene-pinitol (I) (m.p. 105–106°), according to Anderson, MacDonald and Fischer.[17] On admixture with authentic diisopropylidene pinitol, the melting point was not depressed.

(B) **Sequoyitol.**—Analysis by a paper chromatogram indicated that the residue (R-1) from part A contained only myo-inositol and sequoyitol. This material (5 g.) was dissolved in 5 ml. of water and left at 5° for several days. Crystals which formed were collected and washed with an ethanol–water mixture (1:1). Several recrystallizations from the ethanol–water mixture and from glacial acetic acid gave 1.0 g. of shiny plates (II) which melted at 238–241° and were optically inactive (1 dm., 3% in water). When II was mixed with authentic sequoyitol (m.p. 237–240°), the m.p. was 237–240°.

Anal. Calcd. for $C_7H_{14}O_6$: C, 43.3; H, 7.2; OCH_3, 15.9. Found: C, 43.4; H, 7.2; OCH_3, 15.9.

The acetate of II melted at 200–202°. When the acetate was mixed with sequoyitol pentaacetate (m.p. 200–202°) the melting point was not depressed.

Anal. Calcd. for $C_7H_9O_6(CH_3CO)_5$: C, 50.6; H, 5.9; acetyl, 53.3. Found: C, 50.6; H, 6.0; acetyl, 54.9.

(C) **Myo-inositol.**—The mother liquor remaining from the isolation of II was concentrated to dryness, giving 3.5 g. of amorphous material (R-2) which was shown by chromatography to contain both sequoyitol and myo-inositol. The amorphous material was dried and powdered, then refluxed 4 hours with 100 ml. of dry acetone containing 10 g. of fused zinc chloride and 10 ml. of glacial acetic acid.[11] This dissolved the sequoyitol from the mixture, leaving a residue (2 g.) of nearly pure myo-inositol. The myo-inositol was collected on a funnel, washed with acetone, then recrystallized from hot glacial acid. Crystallization from ethanol–water (1:1) gave 1.0 g. of the optically inactive cyclitol (III) which melted at 225–227°. When III was mixed with authentic myo-inositol, the m.p. was 225–227°.

Anal. Calcd. for $C_6H_{12}O_6$: C, 39.9; H, 6.6. Found: C, 39.9; H, 6.7.

The growth response of myo-inositol requiring yeast[18] to III was identical with its response to authentic myo-inositol.

The acetate (IV) of III melted at 210–212° and the melting point was undepressed when IV was mixed with myo-inositol hexaacetate, m.p. 210–212°.

Anal. Calcd. for $C_6H_6O_6(CH_3CO)_6$: C, 50.0; H, 5.5. Found: C, 50.3; H, 5.8.

(D) ***d*-Inositol.**—Water and dilute acid were added to the mother liquors from the crystallization of diisopropylidene pinitol (Part A) in order to hydrolyze the acetonated compounds in solution. After a day the solvent was moved by distillation and the resulting sirup was stirred with absolute ethanol. The amorphous material obtained showed two spots on a paper chromatogram corresponding to pinitol and *d*-inositol. About 1 g. of this mixture was separated on a cellulose column ($1^1/_4 \times 30$ inches) by development with acetone–water (9:1 v./v.).[14] The eluate was collected in 8-ml. fractions and the cyclitol components were detected by analyzing the contents of every tenth tube by paper-strip chromatography. The *d*-inositol fraction was concentrated to dryness and yielded crystals (50 mg.) from ethanol–water. The material was chromatographically homogeneous, migrated with authentic *d*-inositol and gave a positive Scherer test.[16] It did not melt sharply. The optical rotation was +60° (*c* 2.5, water) which is in fair agreement with the value +65° (water) recorded for pure *d*-inositol.

Quantitative Estimation of Cyclitols in Water Extracts of Sugar Pine.—An aliquot of the "water extract" containing about 100 mg. of the cyclitol mixture was refluxed 6 hours with 6 *N* hydrochloric acid to destroy any glycosides or inositol phosphates that may have been present. To the cooled solution was added an excess of bromine to oxidize any reducing sugars to acids and the mixture was left overnight at room temperature. The solution was aerated to remove the excess bromine and all acidic substances were then removed by treatment of the solution with an exchange resin.

The amount of myo-inositol in this solution was determined by the yeast assay method[18] and the amount of sequoyitol by the increase of myo-inositol following treatment of an aliquot of the solution with 47% hydriodic acid.

Since the absolute concentration of myo-inositol was determined by the yeast assay, the absolute amount of pinitol can be calculated from their ratio. The ratio of pinitol to myo-inositol was determined as follows: Approximately 3 mg. of the cyclitol mixture was spotted across the top of a paper strip and the paper was then developed with acetone–water (9:1 v./v.). The paper was dried and the

(13) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., THIS JOURNAL, **68**, 1449 (1946); R. C. Anderson and E. S. Wallis, *ibid.*, **70**, 2931 (1948); L. Hough, *Nature*, **165**, 400 (1950).

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

(15) We thank Dr. Herman O. L. Fischer for samples of allo-inositol and muco-inositol.

(16) J. Scherer, *Ann.*, **81**, 375 (1852).

(17) A. B. Anderson, D. L. MacDonald and H. O. L. Fischer, THIS JOURNAL, **74**, 1479 (1952).

(18) L. Atkin, A. Schultz, W. L. Williams and C. N. Frey, *Ind. Eng. Chem., Anal. Ed.*, **15**, 141 (1943).

zone corresponding to each cyclitol was cut out and eluted into 100 ml. of water. The amount of cyclitol in each was estimated by periodate oxidation. The oxidation was carried out with periodic acid at room temperature for 24 hours; standard oxidations upon which the calculations are based showed an uptake for myo-inositol of 6.5 moles and for pinitol of 5.5 moles of periodate per mole of cyclitol.[19] Results of an assay on random samples of heartwood and sapwood are given in Table I.

(19) A. M. Stephens, *J. Chem. Soc.*, 738 (1952).

TABLE I

QUANTITATIVE ESTIMATION OF CYCLITOLS IN WATER EXTRACT OF SUGAR PINE SAWDUST (FROM 500 G. SAWDUST)

	Pinitol, g.	Myo-inositol, g.	Sequoyitol, g.	*d*-Inositol
Heartwood	15.0	0.58	0.40	Trace
Sapwood	0.55	.01	..	...

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[CONTRIBUTION FROM VISTER RESEARCH LABORATORIES]

An Improved Method of Preparing Testosterone, Dihydrotestosterone and Some of their Esters

BY ALBERTO ERCOLI AND PIETRO DE RUGGIERI

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The 17-monocyanohydrin of Δ^4-androstene-3,17-dione can be prepared in excellent yield by an interchange reaction between the dione and acetone cyanohydrin. This derivative can be converted in almost quantitative yield into the 3-enol ethyl ether, which on reduction with sodium and *n*-propanol is converted into the enol ethyl ether of testosterone. This substance can be converted into testosterone by acid hydrolysis; esters of testosterone are prepared conveniently by reaction with acid anhydrides in pyridine before hydrolysis. Two alternative routes through the 3-enol benzyl ether and the 3-ethylene glycol ketal of the 17-cyanohydrin derivative of Δ^4-androstenedione are described. The method has also been applied to the preparation of androstane-17β-ol-3-one and the acetyl derivative.

The usual precursor for the preparation of testosterone (Ic) is Δ^4-androstene-3,17-dione (Ia), readily available by oxidation of dehydroepiandrosterone. Direct reduction of the 17-keto group by the Meerwein–Ponndorf method without protection of the 3-keto group does furnish testosterone,[1] but in unsatisfactory yield. Serini and Köster[2] found that the 3-ketone group reacts preferentially with ethyl orthoformate to form an enol ethyl ether (IIb) and that this derivative can be converted into testosterone by sodium–propanol reduction of the 17-keto group followed by regeneration of the 3-keto group by acid hydrolysis. The same reaction sequence was also applied to androstane-3,17-dione (IIIa)[2]; in this case the intermediate is the 3-diethyl ketal (IIIe). The Serini–Köster method has been used extensively, but has certain disadvantages. If the theoretical amount of ethyl orthoformate is employed, the yield of the 3-enol ether is far from quantitative; if an excess is employed, the 17-keto group also reacts.

We have been able to eliminate these drawbacks by prior protection of the 17-carbonyl group in the form of the cyanohydrin, prepared by an improved method. The reaction of a 3,17-diketone with alcoholic hydrogen cyanide under the usual conditions is not satisfactory because some dicyanohydrin is always formed. We have found that cyanohydrins of steroid ketones are obtained readily by hydrogen cyanide interchange with acetone cyanohydrin[3] and that by this procedure the 17-monocyanohydrin of Δ^4-androstene-3,17-dione (Ib) can be obtained in practically quantitative yield. This cyanohydrin has also been prepared, but in only 43% yield, from the known cyanohydrin of dehydroepiandrosterone[4] by bromination of the double bond, chromic acid oxidation of the hydroxyl group and debromination with zinc and acetic acid. When this cyanohydrin derivative of androstenedione (Ib) is treated in hot benzene with an excess of ethyl orthoformate in the presence of alcoholic hydrogen chloride, the 3-enol ethyl ether (IIa) is formed in 90% yield. The structure is established by alkaline hydrolysis to the known 3-enol ether of Δ^4-androstene-3,17-dione (IIb). Reduction with sodium and *n*-propanol of the enol ether cyanohydrin (IIa) leads to the known enol ether of testosterone (IIc), from which testosterone is obtained by mild acidic hydrolysis. The yield in these two steps, without isolation of the intermediate enol ether, is 92%.

We have also found that the enol ethyl ether of testosterone (IIc) can be used directly for the preparation of esters of testosterone. Thus this derivative IIc can be acylated in pyridine solution by acid anhydrides[5] such as propionic or β-cyclopentylpropionic anhydride to give the corresponding esters (IId and IIe, respectively). Hydrolysis with hydrochloric acid in acetone furnishes the corresponding esters of testosterone.

Our procedure for the preparation of testosterone and its esters can be modified in various respects. The 3-keto function can be protected in other ways; in the Experimental part routes through the 3-enol benzyl ether (IIf) and the 3-glycol ketal (Ie) of androstenedione cyanohydrin are described.

This procedure is also applicable to the preparation of dihydrotestosterone (androstane-17β-ol-3-one, IIIg) and its esters from androstane-3β-ol-17-

(1) K. Miescher and W. H. Fischer, *Helv. Chim. Acta*, **22**, 158 (1939).

(2) A. Serini and H. Köster, *Ber.*, **71**, 1766 (1938).

(3) ADDED IN PROOF.—F. E. Küng (U. S. Patent 2,259,167, *C. A.*, **36**, 494 (1942)) had prepared the cyanohydrins of some non-steroid carbonyl compounds, such as CH_2O, by *trans*cyanohydrination with a cyanohydrin, such as methyl ethyl ketone cyanohydrin.

(4) S. Kuwada and M. Miyasaka, *Chem. Zentr.*, **108**, II, 1825 (1937). This cyanohydrin can also be prepared in excellent yield by our method.

(5) G. Rosenkranz, St. Kaufmann and J. Romo, THIS JOURNAL, **71**, 3689 (1949), have acylated thioenol ethers of testosterone with acyl chlorides in pyridine solution.