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CHEMISTRY OF THE GENUS *SEQUOIA*—IV.

THE STRUCTURES OF THE C_{17} PHENOLS FROM *SEQUOIA SEMPERVIRENS*

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Abstract—Further structural investigation was carried out on the recently isolated C_{17} polyphenolic materials from California redwood (*Sequoia sempervirens*) heartwood extract. Structures for these compounds are proposed on the basis of chemical and spectral evidence.

INTRODUCTION

THE NEW C_{17} phenolic compounds from California coast redwood heartwood (*Sequoia sempervirens*)[1] were subjected to extensive chemical and spectral analysis. The structure of isosequirin, a rearrangement product from sequirins -B and -C, was proposed in our recent paper.[2]

Naturally occurring C_{17} compounds of this type have been quite rare. Biosynthetic pathways involving the coupling of phenylpropane units lead to the common C_{18} compounds, but a route to the C_{17} materials could result from coupling of *p*-hydroxyphenylpyruvic acid units.[3]

We have found sequirin-A, $C_{17}H_{18}O_4$, and sequirin-B, $C_{17}H_{18}O_5$, to be identical to sugiresinol and hydroxysugiresinol, respectively, from the heartwood of *Cryptomeria japonica* (Japanese cedar).[4,5] More recently, sugiresinol has been found in *Athrotaxis selaginoides* (Tasmanian cedar),[6,7] and related C_{17} compounds have been found in *Chamaecyparis obtusa* (Japanese hinoki cypress)[8] and in *Agathis australis* (New Zealand kauri pine).[3] All of these species are members of the Pinaceae family living in Pacific coastal areas, as is *S. sempervirens*.

Structure 1 for sugiresinol was proposed by Kai.[9]‡ (To avoid confusion, sequirins-A and -B will here be designated as sugiresinol and hydroxysugiresinol, respectively, and sequirin-C will be referred to as sequirin.)

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‡ While this manuscript was in preparation, Hatam and Whiting published structures for hydroxysugiresinol and sequirin identical to those presented here (*Tetrahedron Letters*, No. 9, 781 (1967)).

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2 B. BALOGH and A. B. ANDERSON, *Phytochem.* **5**, 325 (1966).
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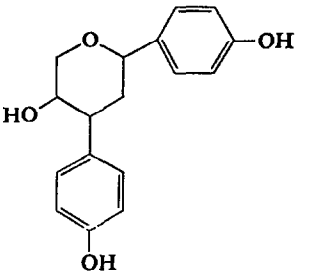
9 Y. KAI, *J. Japan Wood Res. Soc.* **11**, 23 (1965).



(I)

RESULTS AND DISCUSSION

Structure of Hydroxysugiresinol

Use of cation exchange resin in the isolation of this material was undesirable, as it was apparently leading to racemization. To retain optical activity, fresh expressed extract was evaporated to dryness under reduced pressure and the residue was triturated with anhydrous acetone. The acetone-soluble fraction was separated by column chromatography. Optically active hydroxysugiresinol, $[\alpha]_D^{25} = -19{\cdot}0^\circ$ (c, 1 in EtOH), was obtained, in agreement with the value reported by Kai.[5]

Trimethoxy-hydroxysugiresinol was subjected to potassium hydroxide fusion, and the degradation products anisic and veratric acids were identified, using thin-layer and gas chromatography. The mass spectrum confirmed these aromatic systems with important fragments at P-57, P-77, P-79, P-91, and P-105 (parent ion M^+, m/e 320).[10] The spectrum also indicated absence of side chains in the heterocyclic ring.

The oxygen atom in the heterocyclic ring appeared to be part of a benzyl-ether system as it was readily cleaved by dilute mineral acid in the hydrolysis to isosequirin. To obtain the required glycol upon rearrangement, hydroxysugiresinol could have only the structure II (Fig. 1).

The NMR spectrum of trimethoxy-hydroxysugiresinol is in complete agreement with the assigned structure (Fig. 2). The coupling constants for the signals H-4 ($\delta = 3{\cdot}06$ ppm) and H-5 ($\delta = 5{\cdot}25$ ppm), $J_{4,5} = 11$ c/s, indicates that these two protons are axial. The configuration at C-2 cannot be definitely established because the H-2 signal ($\delta = 3{\cdot}5$ ppm) is not clearly separated from other signals. However, the broad pattern indicates a large coupling constant with the axial proton at C-3 and suggests that H-2 is axial. Thus all substituents in the tetrahydropyran ring appear to be in the conformationally more favourable equatorial positions.

Structure of Sequirin

Sequirin is a highly-tinctorial substance which is rapidly oxidized in alkali and by various reagents (Fe^{+3}, Ag^+, $K_3Fe(CN)_6$, horseradish peroxidase). Optically active sequirin, $[\alpha]_D^{25} = 17{\cdot}5^\circ$ (c, 1 in EtOH), was obtained using column chromatography. The aromatic systems are identical to those in hydroxysugiresinol, as shown by alkaline fusion of the trimethyl ether.

A positive test for unsaturation was obtained with tetranitromethane, and the NMR spectrum showed a signal group centered at about 6·5 ppm ($J = 15{\cdot}5$ c/s) of the AB-type, probably because of olefinic protons of a *trans* conjugated system; this indicated that sequirin was a substituted styrene. Hydrogenation of sequirin using a finely-divided platinum catalyst yielded dihydrosequirin, $C_{17}H_{20}O_5$, which exhibited a shift in the u.v. from $\lambda_{max}^{EtOH} = 263$ nm

[10] H. E. LUMPKIN, *Anal. Chem.* **32**, 1819 (1960).

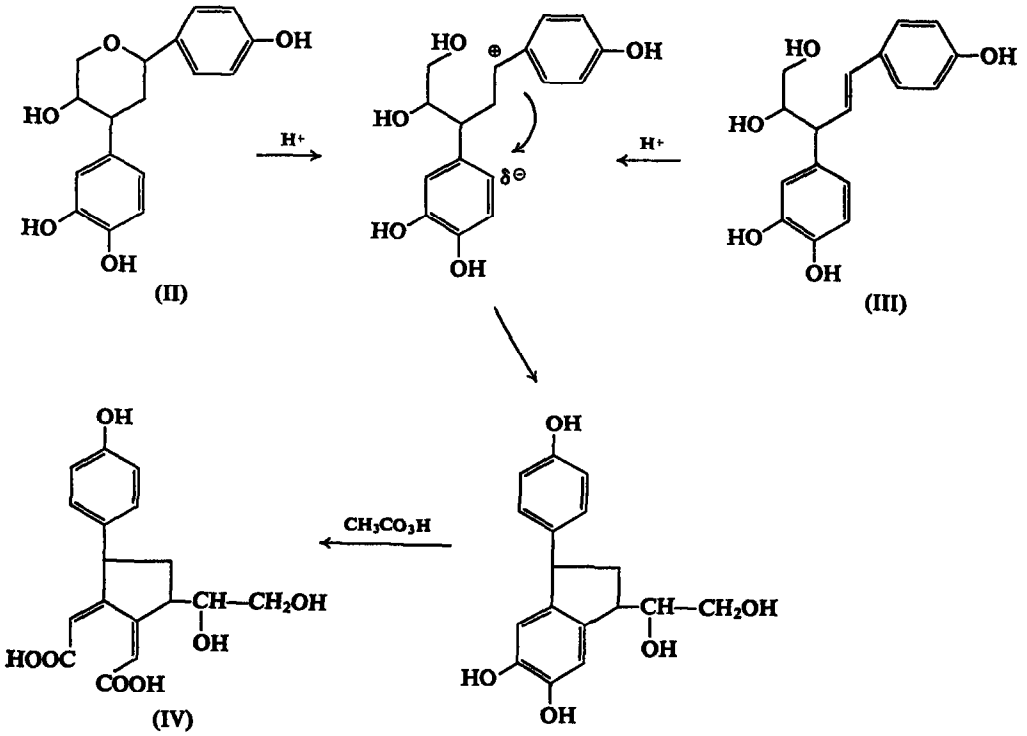


FIG. 1. ISOSEQUIRIN REARRANGEMENT AND OXIDATION TO ISOSEQUIRIC ACID.

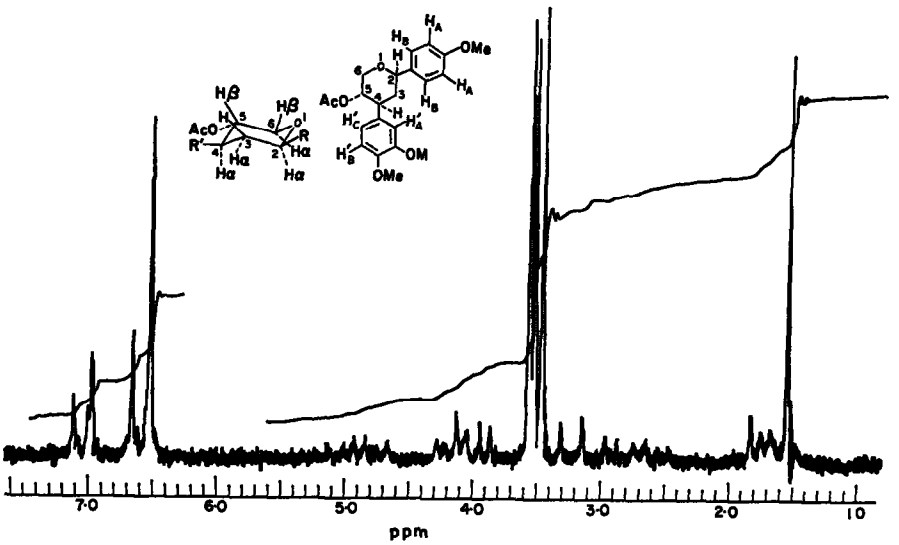


FIG. 2. NMR SPECTRUM OF TRIMETHOXY-ACETYL-HYDROXYSUGIRESINOL.

(ϵ 16,000) in sequirin to λ_{max}^{EtOH} = 285 nm (ϵ 3280) in the reduced compound. Dihydrosequirin forms a pentaacetate and is oxidized by periodic acid with the formation of formaldehyde, indicating a terminal vicinal glycol. Thus the structure of sequirin must be III (Fig. 1).

The positions of the two aromatic rings follow from the rearrangement to isosequirin. This structure is confirmed by the NMR spectrum (Fig. 3). Proton H-1 (δ = 6·50 ppm) is a doublet, $J_{1,2}$ = 15·5 c/s, and H-2 (δ = 6·26 ppm) is a quartet, with $J_{2,3}$ = 7·0 c/s. Racemization of sequirin in its isolation using cation-exchange resin probably resulted after loss of the benzylic proton upon elution with dilute alkali.

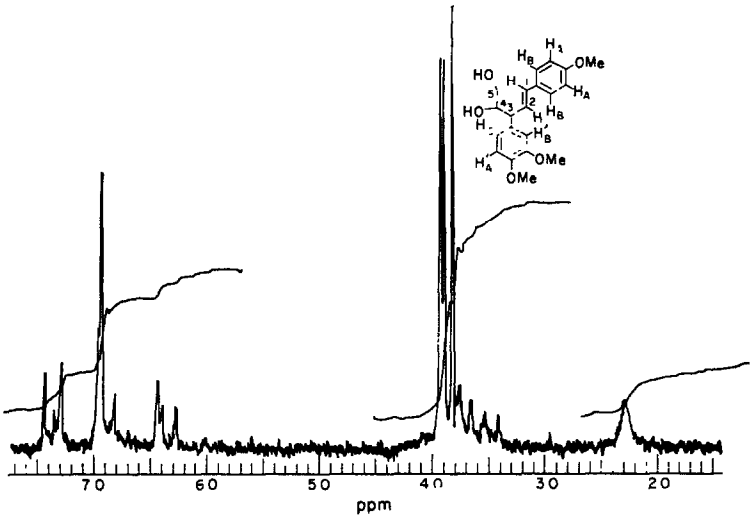


FIG. 3. NMR SPECTRUM OF TRIMETHOXY-SEQUIRIN.

Isosequirin Rearrangement

The rearrangement of both hydroxysugiresinol and sequirin to isosequirin in mineral acid is explained by the observation that cleavage of the benzyl ether in hydroxysugiresinol would furnish the same carbonium ion as that formed in protonation of sequirin. Ring closure then apparently occurs by electrophilic attack in the catecholic ring, at the one available position *para* to a hydroxyl group (Fig. 1). To prove the presence of the carbonium ion the reaction was carried out in the presence of excess phenol or dimedone to "trap" the intermediate. However, all attempts to divert the reaction were unsuccessful; apparently the carbonium ion is so short-lived that proximity to a nucleophilic site is more important than the ease of attack at such a site. Reformation of the six-membered tetrahydropyran ring would be expected to occur readily.

Trimethoxy-hydroxysugiresinol and trimethoxy-sequirin do not rearrange. The free hydroxyl groups apparently increase the electron density in the catecholic ring above the threshold value required for effective substitution; the dimethyl ether is less attractive for electrophilic attack in the ring-closing step. The reason for the resistance of sugiresinol to rearrangement is clear; ring closure *ortho* to the hydroxyl group is precluded by the rigidity of the aromatic nucleus. Sequirin readily undergoes ring closure to hydroxysugiresinol in the presence of mineral acid, or with cupric sulfate in anhydrous methanol.

The kinetics of the isosequirin rearrangement have been studied in detail. [11] At the physiological pH of fresh redwood heartwood, 3·5, the rate of isosequirin formation is extremely slow. We have not found any evidence of isosequirin in the redwood samples we have examined.

Structure of Isosequiric Acid

Isosequiric acid, $C_{17}H_{18}O_7$, is a dibasic acid formed upon mild oxidation of isosequirin with peracetic acid.[2] Hydrogenation using a finely-divided platinum catalyst indicated the presence of two double bonds. Pyrolysis of isosequiric acid in a nitrogen stream yielded phenol and *p*-cresol, but no catechol—although catechol is a pyrolysis product of isosequirin. This suggested that the catecholic ring in isosequirin had been cleaved in oxidation. The infrared spectrum indicated that the carboxyl groups belonged to α,β-unsaturated systems and the NMR spectrum supported structure IV.

The absorption maximum in the u.v. region, $\lambda_{max}^{EtOH} = 226$ nm (ϵ 7400), is in agreement with the proposed structure, using Woodward's rules.[12, 13]

Biogenetic Pathways in Redwood

Early in the study of the redwood C_{17} phenolics there was evidence that these materials were related to redwood tannin and phlobaphene and were probably their precursors. Closely corresponding elemental analyses were observed, and the pyrolysis products of the "monomers" were the same as those of tannin, *viz.* *p*-cresol, *p*-ethyl phenol, catechol, and homocatechol. Polymerization of sequirin under acidic conditions resulted in a material having an i.r. spectrum nearly identical to that of the water-soluble polymers from redwood.

Only trace amounts of the C_{17} phenolics have been found in redwood sapwood. If tannins are formed in this region, polymerization is probably enzyme controlled. However, if polymerization occurs in the heartwood (where enzymes are thought to be absent) the reaction is probably a purely acid-catalyzed post-mortem process, occurring over a long period.

Our study of the chemistry of the genus *Sequoia* is continuing. Recently we have found in *S. gigantea* evidence of additional polyphenolic materials which may also be C_{17} compounds.

EXPERIMENTAL

U.v. spectra were determined on a Beckman Model DK-2 spectrophotometer, and i.r. spectra on a Perkin-Elmer Model 21, using samples imbedded in KBr pellets. Microanalyses were made by Alfred Bernhard in the Max-Planck Institut für Kohlenforschung, Mülheim, Germany. M.ps are corrected.

Separation of the Polyphenols

4 G. of acetone solubles from fresh expressed redwood extract were separated on each of three columns containing 500 g of silica gel G (Merck, Darmstadt), using the solvent system chloroform: acetone: acetic acid (6:5:1). Fractions were examined by TLC on silica gel using the same solvent system. Each column ultimately provided about 1·0 g of sequirin and about 1·5 g of hydroxysugiresinol. Only very small amounts of sugiresinol were usually obtained. These three components comprise about 0·7% of the weight of the dry wood—sugiresinol, 0·1%; hydroxysugiresinol, 0·4%; and sequirin, 0·2%.

Dihydrosequirin. Sequirin (100 mg, $3{\cdot}31 \times 10^{-4}$ mole) and 50 mg of chloroplatinic acid were dissolved in 5 ml of ethanol. The solution was flushed with H_2, and a few drops of a concentrated slurry of $NaBH_4$ in water was added to generate the Pt catalyst. The mixture was hydrogenated in a mechanical shaker (H_2 3 ats.). After 1 hr, the Pt was filtered off and carefully washed with ethanol. The filtrate was evaporated to dryness

[11] R. RIFFER, *The Chemistry of New Phenolic Compounds in Sequoia sempervirens.* Doctoral dissertation, University of California at Berkeley (1967).
[12] R. B. WOODWARD, *J. Am. Chem. Soc.* **64**, 72 (1942).
[13] L. F. FIESER and M. FIESER, *Steroids*, p. 15. Reinhold, New York (1959).

under reduced pressure, and the residue was taken up in absolute ethanol. Evaporation of the alcohol furnished dihydrosequirin (92 mg, 91% of the theoretical amount, m.p. 185°). Recrystallization from water furnished colorless platelets, m.p. 194°. (Calc. for $C_{17}H_{20}O_5$ (304·3): C, 67·2; H, 6·6. Found: C, 67·33; H, 6·39%).

Dihydrosequirin pentaacetate. The compound was obtained via acetic anhydride and sodium acetate, furnishing colorless light needles from methylene chloride, m.p. 119°. (Calc. for $C_{17}H_{15}O_{15}(CH_3CO)_5$ (514·5): C, 63·0; H, 5·9; acetyl, 41·9. Found: C, 62·90; H, 5·70; acetyl, 42·36%).

Hydrogenation of Isosequiric Acid

Isosequiric acid (9·9 mg, $2·96 \times 10^{-5}$ mole) in isopropanol was hydrogenated using a Brown Micro Hydro-Analyzer. The catalyst is generated *in situ* by reduction of chloroplatinic acid with $NaBH_4$. The hydrogen consumed (2·09) corresponded to two double bonds.

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