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CHEMISTRY OF THE GENUS *SEQUOIA*—II

ISOLATION OF SEQUIRINS, NEW PHENOLIC COMPOUNDS FROM THE COAST REDWOOD (*SEQUOIA SEMPERVIRENS*)*

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Abstract—New phenolic compounds, sequirins A, B, and C were isolated from the extractives of redwood heartwood. These compounds account for the durability and staining of redwood, and they are probably the precursors of polymers present in redwood.

THE surface of redwood (*Sequoia sempervirens*) is subject to chemical staining which appears as brown-black discoloration of randomly distributed areas of various sizes, usually elongated in grain direction. Previous investigations have shown that the stain is associated with the migration of certain extractive components to the wood surface during drying and subsequent chemical changes resulting in their darkening.[1] The rate of formation of this characteristic discoloration is dependent on the partial pressure of oxygen, pH, temperature and on the formation of dark-colored complexes with oxides of heavy metals.[2] This paper describes the isolation and chemical properties of new phenolic compounds named "sequirins" which are mainly responsible for the staining of redwood.

The sequirins were isolated from an extract expressed from fresh, green redwood chips in a hydraulic press. The fresh redwood extract is light yellow in color, tastes bitter, and is sticky to the touch. It contains the sensitive coloring substances chemically unaltered. The original pH of the expressed extract is 3·4–3·6 at which it is completely stable; at higher pH, rapid oxidation takes place. Even 1 ml of the extract, made alkaline, turns 1000 ml of tap water (with high oxygen content) to ink-like violet. If acidified, the solution becomes bright yellow and the color change is freely reversible. Upon longer standing in alkali the initial violet color turns light brown as a result of polycondensation of the oxidized coloring materials. No oxidation and subsequent polymerization occurs in the absence of oxygen.

In the course of examination of the extract it was observed that cation-exchange resins readily adsorb the coloring substances. The alcoholic eluate of the cation-exchange resin was examined by thin-layer chromatography, and six spots (three large and three minor), were detected. The three main components of the mixture were successfully separated in larger quantities by using a new preparative thin-layer chromatography technique developed

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[1] A. B. ANDERSON, E. L. ELLWOOD and E. ZAVARIN, *Forest Prod. J.* **10**, 212 (1960) and E. L. ELLWOOD A. B. ANDERSON, E. ZAVARIN and R. ERICKSON, *Forest Sci.* **6**, 315 (1960).
[2] E. ZAVARIN and L. V. SMITH, *Holzforschung* **16**, 11 (1962).

in our laboratory.[3] The three components were purified and named sequirin-A, sequirin-B, and sequirin-C.

Sequirin-A, colorless, m.p. 245°, has the formula $C_{17}H_{18}O_4$. It gives a green color with ferric chloride in alcohol and couples with diazotized sulfanilic acid. It is soluble in both polar and non-polar solvents, slightly soluble in hot water, and soluble in dilute alkaline solutions from which it precipitates with acids. No precipitation occurs with lead acetate in boiling aqueous solution. It is a very stable compound and resists attack of strong mineral acids or alkaline solutions. It contains three hydroxyl groups (forms a triacetate) but fails to give a crystalline methoxy derivative with dimethyl sulfate. No olefinic double bond could be detected in the molecule. It shows an u.v. absorption maximum at 274 mμ (ε 3250).

Sequirin-B, colorless, m.p. 218°, has the formula $C_{17}H_{18}O_5$, is insoluble in non-polar solvents, soluble in hot water and polar solvents and exhibits phenolic properties similar to sequirin-A. It couples with diazotized sulfanilic acid (brown color) and gives a green color in alcoholic ferric chloride. Lead acetate solution precipitates sequirin-B; concentrated sulfuric acid produces a dark red color. It is stable in hot, dilute alkaline solutions: longer heating results in a brown discoloration however. On acetylation it yields a tetraacetyl derivative; acetylation of its trimethoxy derivative prepared with dimethyl sulfate affords a monoacetate, showing the presence of three phenolic and one alcoholic hydroxyl groups in the molecule. It has a maximum at 276 mμ (ε 3250).

Sequirin-C, colorless, m.p. 193°, has the formula $C_{17}H_{18}O_5$, and is soluble in hot water and in polar solvents. It reduces Tollen's ammoniacal silver nitrate reagent but gives no precipitate with Fehling's solution. In alkaline solution, sequirin-C undergoes rapid oxidation forming a violet color similar to that observed in examination of the expressed extract. The oxidation product is an acid-base indicator changing from violet to yellow at pH 3·6. Upon standing longer in alkali, a brown precipitate of polymeric character appears. The presence of inorganic reducing agents such as sodium sulfite does not entirely prevent oxidation. Strong mineral acids act upon sequirin-C as do alkalies: after longer standing (a few days) in strong mineral acidic solution, brown polymeric materials precipitate. Neither of the other two sequirins give this reaction.

A trimethoxy derivative was prepared with dimethyl sulfate, but no derivatives could be obtained with acidic reagents. Immediate polymerization took place with acid halides, acid anhydrides, Lewis acids, bromine, etc. The compound contains at least four hydroxyl groups. There is a single maximum at 263 mμ (ε 7570) in alcohol.

Sequirin-B and sequirin-C are closely related. When heated in dilute mineral acid solution, both give the same compound: isosequirin, colorless, m.p. 195°, formula $C_{17}H_{18}O_5$, in good yields. This compound has similar chemical properties to sequirin-C. The freshly recrystallized colorless compound turns brown if exposed to the light. It is a strong reducing agent: when dissolved in alkali, rapid oxidation takes place resulting first in an acid-base indicator with a bright red to yellow color change at pH 5·6; upon longer standing brown polymers precipitate.

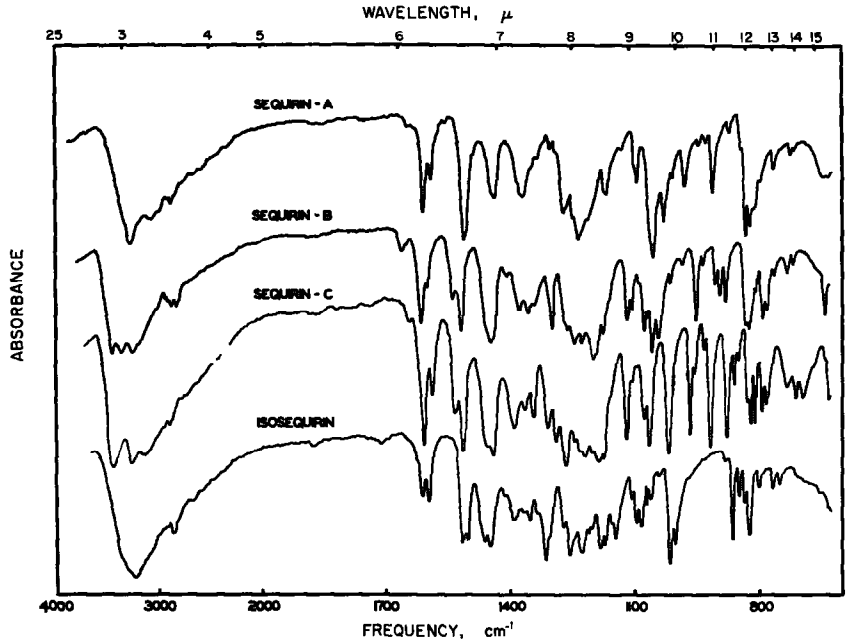
The treatment of its suspension in non-polar solvents (benzene, ether) with mineral acids results in a violet syrup, implying a positive Lucas test, the exchange of secondary or tertiary alcoholic hydroxyl groups. Isosequirin can be recovered by addition of water. There are five hydroxyl groups in the molecule (pentaacetate). It shows a single u.v. absorption maximum at 284–5 mμ (ε 5500) at pH 5. The maximum shows a bathocromic shift to 300 mμ (ε 7950) in 0·1 N sodium hydroxide solution containing 1% sodium sulfite. The extreme ease of oxidation

[3] B. BALOGH, *Anal. Chem.* **36**, 2498 (1964).

required the use of sodium sulfite; even so, oxidation occurred and a wide long-wave band appears at about 490 mμ due to some quinoid anion.

It should be mentioned that a mixture of sequirins was probably isolated by Sherrard and Kurth in 1933.[4] They called their crystalline material "sequoyin" which was believed to afford two compounds upon hydrolysis: "sequein" and "sequeinol". The reported properties of these materials suggest that sequein is identical with isosequirin and sequeinol with sequirin-A, since isosequirin and sequirin-A must appear in the hydrolysate of the mixture of sequirin-A, -B, and -C. Interpreted thus there is no contradiction with the former findings.

The sequirins are optically inactive, contain no C-methyl or methoxy groups (NMR); their i.r. spectra (Fig. 1) show strong O–H stretching absorptions as very broad bands at



3200–3400 cm^{-1} and C–O stretching absorptions at 1200–1300 cm^{-1} frequencies. There are no absorption bands arising from carbonyl stretching vibrations in the spectra. Upon pyrolysis in a nitrogen stream, they gave the same fragments: phenol, *p*-cresol, *p*-ethylphenol, catechol, homocatechol, and two unidentified disubstituted catechols.

The chemical properties and spectral behavior of the three compounds are consistent with those possessing one disubstituted catechol and probably one p-hydroxyphenyl ring. This belief is supported by NMR evidence showing the presence of six aromatic protons in each sequirin. The rings are probably connected with a saturated system containing alcoholic hydroxyl groups. Sequirin-B is probably a cyclic hemi-acetal which opens upon acidic treatment, and since no water uptake could be demonstrated a subsequent recyclization takes place producing isosequirin, which contains only five hydroxyl groups. Sequirin-C is the most interesting compound of the group with regard to the durability and staining of redwood.

[4] E. C. SHERRARD and E. F. KURTH, *J. Am. Chem. Soc.* **55**, 1728 (1933).

On acid treatment, it undergoes rearrangement forming isosequirin, a more stable compound, but similar in chemical character, since isosequirin results from acidic treatment from both sequirin-B and -C. The structural studies of isosequirin and the sequirins are continuing.

EXPERIMENTAL

Ultraviolet spectra were determined on a Beckmann Model DK-2 spectrophotometer, i.r. spectra on a Perkin-Elmer Model 21 (the compounds were imbedded in KBr pellets), and NMR spectra on a Varian A-60 using tetramethylsilane (TMS) ($\delta = 0$) as internal standard. Microanalyses were made by Alfred Bernhard, in the Max-Planck-Institut fuer Kohlenforschung, Mülheim, Germany. Melting points are corrected.

Isolation of sequirins

Eighty kg of freshly cut, green heartwood of redwood were sawn to 30 × 5 × 3 cm pieces. Six to nine of these were piled between the plates of a hydraulic press, the lower plate being equipped with a ledge around its rim, and squeezed at approximately 60 atm. at room temperature. Forty-six kg (58 per cent) of expressed extract was collected, and after being kept at 5° for 5 days it was filtered from coagulated proteins, sawdust, and cell materials. The extract thus obtained has a light-yellow color, tastes bitter and is quite stable when stored in a refrigerator.

For the isolation of sequirins a battery of ten chromatographic columns filled with Amberlite IR 112 or Amberlite IR 120 resin (40 ml) were used. The expressed extract (300 ml) was passed through the columns with an average flow rate of 5 ml/min. The eluate was checked for residual monomer using the oxidation test (4 ml of eluate and 1 ml of 1 % NaOH in 1 l. of water did not produce a visible violet color). A rinse with 150–200 ml of distilled water proved sufficient to remove weakly adsorbed materials. The sequirins were then eluted with 100 ml of 80 % (v/v) alcohol and the column finally rinsed with 300 ml distilled water. This cycle was repeated until all the extract had been treated. The alcoholic eluates were pooled, and after removal of the solvent under a vacuum at 40°, the brown syrup was collected. It was dried and dissolved in 100 ml of absolute alcohol; upon addition of 800 ml of benzene, a brown crystalline material precipitated slowly. The complete crystallization required 2 weeks. After filtration and drying in vacuum, a brown, solid material was obtained, weight 112 g, m.p. 167°. This material was examined by thin layer chromatography on silica gel G (Merck, Darmstadt) developed with chloroform:acetone:acetic acid (12:10:2). The chromatogram sprayed with diazotized sulfanilic acid showed three strong spots (R_f values: 0·68, 0·54, and 0·33) and three hardly visible spots.

Separation of sequirin-A, -B, and -C

The crude mixture was further purified before the separation of its main components. It was repeatedly dissolved in methanol and precipitated by dilution with distilled water. After three precipitations, the mixture was almost white and melted at 182°. The separation was carried out on "chromatosticks"[3] prepared from silica gel. Twenty-five grams of silica gel G (Marck, Darmstadt) slurried with 50 ml of water were poured into a filter paper roll. The formed wet column was rolled out after 15 min, dried overnight at 70° and activated at 115° for 2 hr. Forty to fifty mg of the prepurified mixture dissolved in 0·3 ml of methanol were applied on the planed end of a chromatostick in concentric circles with a micro-pipette until a layer about 0·5 mm thick was formed. The chromatostick was developed standing on a

cotton-wool pad with the chloroform:acetone:acetic acid solvent. Since partial oxidation resulted in dark rings, two of the zones could be easily detected; they were centred at R_f 0·54 and 0·33 after the solvent had reached the top of the chromatostick. The R_f zone of the third component was found to be located at R_f 0·65–0·70.

The zones from twenty chromatosticks were separated by dissection and the collected sections treated individually.

Sequirin-A. Sections at R_f 0·65–0·70 were crushed and eluted with 200 ml of methanol. The filtrate was evaporated to 50 ml in vacuum, then any dissolved silica gel precipitated by addition of 50 ml of acetone. After removal of the solvent by evaporation in vacuum, the residue was taken up in 2 ml of hot water. Upon cooling, 0·08 g of thin, colorless needles, m.p. 235–240° was formed. The material was purified by dissolution in sodium carbonate solution and precipitation with hydrochloric acid. A second recrystallization furnished needles, m.p. 245°.

Anal. Found: C, 71·35, 71·20; H, 6·31, 6·27; mol. wt. (in camphor, acc. to Rast) 278. Calc. for $C_{17}H_{18}O_4$ (286·2): C, 71·35; H, 6·33.

Sequirin-A triacetate. The compound was obtained via acetic anhydride and sodium acetate, as colorless, fluffy needles, m.p. 183° from EtOH.

Anal. Found: C, 67·02, 66·86; H, 6·07, 6·02; Acetyl, 29·9; mol. wt. 401 (in camphor, acc. to Rast). Calc. for $C_{17}H_{15}O_4(CH_3CO)_3$ (412·4): C, 67·00; H, 5·86; Acetyl, 31·3.

Sequirin-B. This was extracted as above from the sections of the chromatosticks at R_f 0·54. The residue was taken up in 2 ml of alcohol and poured into 40 ml of benzene. Violet-colored crystals appeared upon standing overnight. Weight 0·27 g, m.p. 207°. Recrystallization three times from water containing 0·05% sodium bisulfite furnished colorless short needles, m.p. 218°. It crystallized with 1 mole of water.

Anal. Found: C, 63·78, 63·92; H, 6·32, 6·21. Calc. for $C_{17}H_{18}O_5 \cdot H_2O$ (320·3): C, 63·75; H, 6·30.

Sequirin-B tetracetate. Colorless, glittering needles, (from benzene), m.p. 114°.

Anal. Found: C, 63·65, 64·06; H, 5·58, 5·46; Acetyl, 36·1. Calc. for $C_{17}H_{14}O_5(CH_3CO)_4$ (470·4): C, 63.80; H, 5·57; Acetyl, 36·6.

The NMR spectrum in carbon tetrachloride exhibited aromatic proton absorption (six protons), centred at δ 7·1; CH_3CO methyl peak (three protons) at δ 1·82 (acetylated alcoholic hydroxyl); and three CH_3CO (9 protons) at δ 2·2 (three acetylated phenolic hydroxyls). The other protons absorb from 2–5 ppm with several overlaps.

Sequirin-B tetrabenzoate. Colorless needles, m.p. 125°, from ethanol.

Anal. Found: C, 74·96, 74·76; H, 4·65, 4·77; mol. wt. 682 (in camphor acc. to Rast). Calc. for $C_{17}H_{14}O_5(C_6H_5CO)_4$ (718·6): C, 75·05; H, 4·76.

Sequirin-B tetramesylate (tetramethanesulfonic acid ester). Using methanesulfonyl chloride[5] in pyridine gave the ester which after recrystallization from 50% acetic acid furnished long, colorless, silky needles; m.p. 168°.

Anal. Found: C, 41·00, 41·06; H, 4·36, 4·54; S, 20·1. Calc. for $C_{17}H_{14}O_5(CH_3SO_2)_4$ (614·6): C, 41·02; H, 4·27; S, 20·88.

Trimethoxy sequirin-B (using dimethyl sulfate and 45% KOH). Colorless, small needles, from benzene m.p. 117°.

Anal. Found: C, 70·12, 70·20; H, 7·11, 6·98; CH_3O 27·01. Calc. for $C_{17}H_{15}O_2(OCH_3)_3$ (344·4): C, 69·75; H, 7·27; CH_3O 27·06.

[5] A. C. KOSLOVA. Zhur. Obshchi Khm. *J. Genet. Chem.* **18**, 729 (1948).

The i.r. spectrum shows a sharp absorption maximum at 3480 cm $^{-1}$, suggesting the presence of one alcoholic hydroxyl group.

Acetyl-trimethoxy sequirin-B. Colorless glittering plates (EtOH), m.p. 156 .

Anal. Found: C, 68·41, 68·48; H, 6·99, 6·80; CH_3O, 23·84; Acetyl, 11·33; mol. wt. 425 (in camphor, acc. to Rast). Calc. for $C_{17}H_{14}O_2(CH_3O)_3CH_3CO$ (458·5): C, 68·30; H, 6·75; CH_3O 24·1; Acetyl 11·14.

The NMR spectrum (in $CDCl_3$) confirmed the presence of three methoxy groups: absorption maxima (CH_3O) at 3·91, 3·97 and 4·0 ppm (nine protons) and a sharp singlet (CH_3CO) at 2·0 ppm (three protons); therefore one acetyl group is present.

Sequirin-C. The sections obtained at R_f 0·33 were processed as described for sequirin-B. A dark-violet crude material (0·19 g) was obtained which upon recrystallization from hot water gave 0·17 g of glittering colorless, long needles, m.p. 193 .

Anal. Found: C, 67·62; 67·71; H, 6·12, 6·05; mol. wt. 299, 314 (in camphor acc. to Rast), 304 (thermoosmometric vapor pressure measurement in acetone). Calc. for C_1-$H_{18}O_5$ (302·3): C, 67·55; H, 6·00.

Trimethoxy sequirin-C (dimethyl sulfate and 45 % KOH under hydrogen). Recrystallization first from chloroform/n-hexane, then from benzene, furnished opaque, light needles, m.p. 121°.

Anal. Found: C, 69·60, 60·62; H, 7·07, 7·18; CH_3O, 26·83; mol wt. 355 (in camphor, acc. to Rast). Calc. for $C_{17}H_{15}O_2(OCH_3)_3$ (344·4): C, 69·75; H, 7·27; CH_3O, 27·06.

The i.r. spectrum exhibits a rather sharp absorption maximum at 3500 cm^{-1} frequency. All attempts to acetylate the free hydroxyl group failed.

Isosequirin from sequirin-B and sequirin-C. Isosequirin can be obtained upon refluxing sequirin-B or -C with dilute sulfuric or hydrochloric acid. The reaction is faster with hydrochloric acid, but the mixture has to be neutralized, otherwise no crystallization of isosequirin will occur.

A 0·1 g portion of sequirin-B was dissolved in 1 ml of 3 % sulfuric acid and refluxed for 8 hr. Upon cooling 0·0515 g of thin, very long crystals appeared. The mother liquor was adjusted to pH 3, whereupon further crystals were deposited. The mother liquor was finally extracted with ether. A small amount of crystalline material was obtained after removal of ether from the etheral extract. The combined crystals weighed 0·07 g, m.p. 148 . After repeated recrystallization from water and precipitation from alcohol with benzene, colorless, long, soft crystals, m.p. 195° were obtained.

Anal. Found: C, 67·39, 67·49; 67·55; H, 5·96, 6·00, 5·95; mol. wt. 297, 307, 315 (in camphor, acc. to Rast) and 300 (thermoosmometric vapor-pressure measurement in acetone). Calc. for $C_{17}H_{18}O_5$ (302·3): C, 67·55; H, 6·00.

The yield from sequirin-C was considerably smaller (34 per cent) due to polymer formation. The acidic mother liquor had to be extracted to remove isosequirin.

Isosequirin pentaacetate. Colorless light needles from carbon tetrachloride, m.p. 121 .

Anal. Found: C, 63·14, 63·33; H, 5·62, 5·50; Acetyl, 42·3. Calc. for $C_{17}H_{13}O_5(CH_3CO)_5$ (512·5): C, 63·25; H, 5·51; Acetyl, 41·9. The NMR spectrum in $CDCl_3$ shows 5 $CH_3(CH_3CO)$ absorption maxima centered at 2·25 ppm (15 protons).

Pyrolysis of sequirins. 0·1 g of each of the sequirins in 0·5 ml of EtOH were dried on glass wool. The glass wool was then loosely placed in a 5 cm portion of a glass tube (30 × 0·5 cm) 5 cm from one end. Nitrogen was passed through the tube (10 ml/min) and the tube heated with a micro burner. After pyrolysis was complete, the tube was dissected, and the condensed pyrolysis products were dissolved in methanol. This solution was chromatographed on

Whatman No. 1 paper impregnated with dimethylsulfoxide with system pyridine: isooctane (1:25) (for monohydroxy phenols)[6]. All sequirins gave identical pyrolysis products: Phenol (R_f 0·1) *p*-cresol (R_f 0·17), *p*-ethylphenol (R_f 0·32). Dihydroxy phenolic compounds were identified on Whatman No. 1, paper using benzene:acetic acid:water (2:2:1). Catechol (R_f 0·3), homocatechol (R_f 0·39), and unidentified disubstituted catechols (R_f 0·54, 0·03) were found in the pyrolysate of each sequirin.

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[6] J. FRANC, *Chem. listy* **52**, 55 (1958).