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

CHEMISTRY OF THE GENUS *Sequoia*. I. THE CONE SOLID OF COAST REDWOOD (*Sequoia sempervirens*) AND GIANT SEQUOIA (*Sequoia gigantea*)

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The domestic trees of the *Sequoia* group consist of two species, namely, California or coast redwood (*Sequoia sempervirens*) and the giant sequoia, Sierra redwood or bigtree (*Sequoia gigantea*). As their common names indicate, except for a small area in the southwestern tip of Oregon, these trees are indigenous to California. The coast redwood is found along the upper half of the California coastal regions, while the giant sequoia grows in small scattered restricted areas in the south central Sierra. Because of the relative scarcity of the latter, very few trees are harvested for commercial use. The red-brown wood of the coast redwood is light, soft, and straight grained. It is prized for fence posts, rails, and other exterior uses requiring resistance to decay. Its reputation for durability, the ease with which it is split and otherwise worked, have long made it desirable for many commercial purposes.

An excellent review of the physical properties, together with the chemistry of the coast redwood is presented by the Institute of Paper Chemistry (1). The investigation into the chemistry of the giant sequoia has largely been neglected. One of the objects of the present studies is to make a comparative analysis of the extractives from each of the *Sequoia* species and to examine further the nature of the natural fungicides, insecticides, and coloring matter present in this genus. Interestingly enough, bird life is relatively limited in the redwood forests because of the lack of insects. The rather high tannin content in redwood is reported to aid in protecting the tree from insect and fungal attack (2).

The *Sequoia* cones are egg-shaped bodies of closely packed, woody, persistent thick scales, and are from about an inch to 3½ inches long, the smaller cones being from the coast redwood. Five to seven seeds—minute, brown, stiff, wing-margined, flat bodies are closely packed beneath each scale. When the cones are dried and shaken, a reddish-violet amorphous solid, the so-called "cone crystals" or cone solid accompanies the seed. The cones may contain from 1.2 to 2.6 per cent of this highly tinctorial material.

The cone solid of the coast redwood is largely water-soluble (82–92 per cent), of which 95% or more is reported to be tannin, free of carbohydrate material (1). In 1901, Heyl investigated the cone solid from the giant sequoia and reported that the hydrolytic and thermal products included gallic acid, pyrogallol, and a sugar which yielded a crystalline phenylosazone (3). No melting point of the products or their derivatives was given and the state of purity of the crude cone solids used is a matter of conjecture. The present investigation is a preliminary report on the employment of chromatography in determining the nature of some of the constituents present and derived from *Sequoia* cone solids.

The sample of coast redwood cone solid used here was found to contain 4.4%

ethyl ether-soluble material and a total of 74.5% was soluble in water. The giant sequoia cone solid contained 66% water-soluble material and 4.2% was soluble in ethyl ether.

The ether extracts from both cone solids were submitted to descending paper partition chromatography. Several runs were made using different developing solvents and chromogenic agents. The R_f values obtained for each of the extracts corresponded to the reference compound values for pyrogallol, phloroglucinol, gallic acid, and protocatechuic acid. This indicates that the gallic acid and pyrogallol reported by Heyl as hydrolytic products of giant sequoia cone solid are likewise present in the free state (3).

It was of interest to ascertain whether any of these phenolic compounds is present in the heartwood. For this purpose, a sample of ether extract was obtained from a fresh sample of the coast redwood heartwood. None of the four compounds identified in the cone solids appeared to be present in the wood extract.

The total water solubles from the cone solids were taken up in alcohol and the extract largely was recovered in the form of a precipitate by the addition of several volumes of ethyl ether. This precipitate was washed thoroughly with ethyl ether to remove all traces of the above mentioned four free phenolic compounds. This solid material was submitted to acid hydrolysis according to Heyl's procedure and the resulting phlobaphenes which formed, were removed by filtration (3). The recovered water-soluble hydrolysate proved to contain catechol, in addition to all the aforementioned free phenolic compounds, upon chromatographic analysis. This clearly indicated that the water solubles also consist of hydrolyzable products containing units of the five identified phenolic compounds.

Another quantity of water-soluble cone material was acid hydrolyzed directly. After this reaction mixture was freed of phlobaphenes, the resulting solution, after treatment with lead acetate, was free of carbohydrates. The absence of carbohydrates in coast redwood cone solids confirms the results reported by the Institute of Paper Chemistry (1). However, contrary to Heyl's report, no tests for carbohydrate material could be detected in giant sequoia cone solid (3).

After the filtered phlobaphenes had been thoroughly freed of all traces of the reported phenolic compounds, they were submitted to alkali fusion. Here, again, all five phenolic compounds were found to be present among the fusion products. This demonstrates that the phlobaphenes formed during acid treatment are derived, in part, from less complex water-soluble phenolic constituents, whose units are made up of a number of simple polyhydroxy phenolic compounds.

In all chromatograms, spot size and intensity indicated that phloroglucinol seemed to be the major constituent. A small amount of this compound was isolated and identified from the alkali fusion of the phlobaphenes from giant sequoia. A number of unidentified spots appeared in the various chromatograms and will be considered in future studies on redwood extractives.

EXPERIMENTAL

In the experiments to follow, each was run in duplicate, using the cone solid from coast redwood and from the giant sequoia.

Paper partition chromatography. The descending chromatography procedure used in the experiments to follow was similar to that described by Lindstedt (4). Three developing solvents were employed. These consisted of:

1. *m*-Cresol (48 vol.), acetic acid (2 vol.), and water (50 vol.) (5).
2. *n*-Butanol (4 vol.), acetic acid (1 vol.), and water (5 vol.) (5).
3. Isobutyric acid (4 vol.) and water (1 vol.) (6).

The appropriate amount of the various cone solid extract preparations, to be described, was brought to the paper (57.0 x 15.0 cm., Whatman No. 1) along with the reference compounds. Five reference substances were used; these consisted of alcoholic solutions of pyrogallol, phloroglucinol, gallic acid, protocatechuic acid, and catechol. The chromatograms were allowed to run until the solvent front had reached within 10 cm. of the bottom of the paper. The paper was removed from the chromatographic chamber, air-dried, and then was sprayed with the chromogenic agent. Two such spraying agents were employed, (a) ammoniacal silver nitrate (6) used with developing solvent 1 and (b) phosphomolybdic acid spray followed by exposure to ammonia vapor (7) for the remaining developing solvents.

Ether extract of cone solids. The finely ground cone solid (5 g.) carefully separated from foreign matter, was extracted 8 hours in a Soxhlet extractor with ethyl ether. The solutions containing the extracts (4.2–4.4%) were concentrated to 3 ml. and then were submitted to chromatographic analysis. The results are summarized in Table I. As indicated, each of the extracts revealed four R_f values corresponding to the reference compound spots, identified as gallic acid, pyrogallol, phloroglucinol, and protocatechuic acid.

Ether extract of coast redwood heartwood. A fresh sample of coast redwood heartwood sawdust was air-dried for 48 hours. The sawdust was extracted 8 hours in a Soxhlet extractor. The concentrated ether extract, upon chromatographic analysis, exhibited no R_f values corresponding to any of the phenolic compounds identified in the cone solid ether extracts.

Hydrolysis of the reprecipitated cone solids. The powdered cone solid (15 g.) was shaken one hour with 250 ml. of water. The combined filtrate and washings (400 ml.) were evaporated to dryness at 40° and 25 mm. The water-soluble residue (66% for giant sequoia and 74.5% for coast redwood) was dissolved in absolute alcohol and filtered. The slow addition of several volumes of dry ethyl ether caused a precipitate to form, which was filtered and washed well with ether until no test could be detected for the five reference compounds employed. The insoluble material was redissolved in 400 ml. of water, 10 ml. of conc'd sulfuric acid was added, and the mixture was refluxed for 22 hours. The cooled mixture was filtered to remove the precipitated phlobaphenes. The filtrate was extracted with ethyl ether, the ether was removed by evaporation, and the residue was taken up in 3 ml. of ethanol. This alcoholic solution, upon being chromatographed, indicated the presence of each of the above mentioned four phenolic compounds plus catechol (See Table I).

The absence of catechol from several of the chromatograms developed by the *m*-cresol solvent mixture was probably due to evaporation during the air drying period. However, catechol, when present, was readily identified upon being chromatographed with the other developing solvents as indicated in Table I.

Test for simple sugars. Finely powdered cone solid (15 g.) was shaken one hour with 250 ml. of water. The combined filtrates and washings (400 ml.) were refluxed for 22 hours with 10 ml. of conc'd sulfuric acid according to Heyl's procedure (3). The cooled reaction mixture was filtered to remove the precipitated phlobaphenes. The clear filtrate was treated with a slight excess of lead acetate. The mixture was filtered and the pale yellow filtrate was saturated with hydrogen sulfide. Following the removal of lead sulfide, the filtrate was concentrated to 10 ml. at 40° and 18 mm. Benedict's test for reducing sugars was negative. No indication of sugars could be detected when the filtrate was submitted to various chromatographic tests.

Alkali fusion of phlobaphenes. The phlobaphene, which had precipitated during the

TABLE I

R_f VALUES

Preparation	Developing Solvent	Volumes	Chromogenic Spray	Gallic acid	Pyrogallol	Phloroglucinol	Protocatechuic acid	Catechol
Reference....	1.[c] *m*-Cresol	48	[a]	0.09–0.10	0.39	0.16–0.17	0.35–0.36	0.73–0.74
Preps. 5 & 6..	Acetic acid	2		.09	.39	.16	.36	.73
Preps. 1, 2, 3, & 4.....	Water	50		.09–.10	.39	.17	.35	—
Reference.....	2.[c] *n*-Butanol	4	[b]	.67–.68	.76	.75	.85	.90
Preps. 2, 3, 4, & 6.....	Acetic acid	1		.67	.76	.75	.85	.90
Prep. 1.....	Water	5		.67	.76	.75	.84	—
Reference.....	3.[d] Isobutyric acid	4	[b]	.22–.23	.38–.39	.24–.25	.48	.72–.73
Preps. 2, 3, 5, & 6.....	Water	1		.22–.23	.39	.24	.49	.72–.73
Prep. 4.....				.22	.39	.24	.49	—

Preparations: 1. *Sequoia gigantea*, ether extract of cone solid. 2. *Sequoia gigantea*, hydrolysate of reprecipitated cone solid. 3. *Sequoia gigantea*, alkali fusion product of phlobaphene. 4. *Sequoia sempervirens*, ether extract of cone solid. 5. *Sequoia sempervirens*, hydrolysate of reprecipitated cone solid. 6. *Sequoia sempervirens*, alkali fusion product of phlobaphene.

[a] Ammoniacal silver nitrate (6). [b] Phosphomolybdic acid, followed by exposure to ammonia vapor (7). [c] Ref 5. [d] Ref. 6.

above acid hydrolysis, was dried, finely ground and extracted with ethyl ether for four hours in a Soxhlet extractor to remove any of the above phenolic compounds, if present. During a 25-minute period, 2.0 g. of the ether-extracted phlobaphene was added to a 10 g. melt of potassium hydroxide-sodium hydroxide (1–1). Heating was continued for an additional 8 minutes, after which the cooled mixture was taken up in 350 ml. of water. The aqueous extract was acidified to Congo Red with dilute sulfuric acid, filtered, and the filtrate was evaporated to dryness at 40° and 20 mm. The residue was extracted with a little ethyl ether and the solution was submitted to chromatographic analysis as before. The results are summarized in Table I. The same five phenols present in the acid hydrolysate were found again among the fusion products of the phlobaphenes.

Phloroglucinol. Following chromatographic analysis, the remaining ether solution of alkali fusion products from giant sequoia was evaporated to dryness. The residue, after two sublimations, yielded crystals with m.p. 211–212°. No change in melting point resulted when admixed with an authentic sample of phloroglucinol. This product was further identified by preparing the acetylated derivative with acetic anhydride in pyridine. After two sublimations, the derivative melted at 105.2–105.8°. The mixture melting point with an authentic sample of phloroglucinol triacetate remained unchanged.

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SUMMARY

Pyrogallol, catechol, phloroglucinol, gallic acid, and protocatechuic acid appear to be among the degradation products of the cone solids from coast redwood and giant sequoia. These compounds, with the possible exception of catechol, also occur in the free state in the cone solids. No sugars were detected among the hydrolysis products.

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