Preliminary Phytochemical Investigation of the Stem Wood of *Suriana maritima* Linn (Simarubaceae)

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Abstract □ A study of the stem wood of *Suriana maritima* L. (Simarubaceae) has indicated the presence of a high molecular weight alkane fraction, β-sitosterol, and a soluble form of lignin, a Brauns' native lignin. No previously reported investigation of this species has been reported in the literature.

Keyphrases □ *Suriana maritima* stem wood—phytochemical investigation □ Alkane hydrocarbons, *S. maritima*—extracted □ β-Sitosterol—isolation, identification □ Lignin, Brauns'—isolation, identification □ IR spectrophotometry—identity □ UV spectrophotometry—identity

The family Simarubaceae comprises approximately 32 genera and 200 species of tropical and subtropical woody plants closely related to the Rutaceae. The range of variation within the Simarubaceae has caused certain taxonomists to split the family into six subfamilies (1). *Suriana* is a monotypic genus and some authors have placed it in the family Surianaceae (2). However, the present authors have followed the system of Engler (3) and consider the genus *Suriana* as a member of the Simarubaceae.

Suriana maritima (bay-cedar) is a much branched shrub or small tree with simple, alternate, and pubescent leaves. It is found growing abundantly along the coasts of the New and Old World tropics. The stems are quite pubescent and exude a reddish-brown resinous material on their cut surfaces. The wood of this plant is extremely hard and dense with considerable secondary xylem development. The flowers are yellow and occur in short clusters and the achene-like fruits are pubescent on all parts.

The native inhabitants of several West Indian islands have used the flowers and stems of this plant for medicinal purposes. The flowers have been used to treat blood disorders (4) and the stems have been employed in treating high fevers. Infusions of these parts are made with water and taken by mouth.

Extraction and fractionation of a large sample of the ground wood devoid of any bark tissue yielded a petroleum ether fraction from which a mixture of alkane hydrocarbons and β-sitosterol was isolated. The petroleum ether-exhausted marc was reextracted with 95% ethanol and this fraction yielded a type of lignin identified as a Brauns' native lignin. This was subsequently degraded in alkali. The degradation mixture was subjected to TLC and was found to consist of vanillin, syringaldehyde, and several aromatic acids.

EXPERIMENTAL

Materials and Methods—The plant material used in this investigation was collected in the Rock Sound area of Eleuthera, Bahamas, during March 1966, and consisted of the above-ground stem from which the bark was later removed. Voucher specimens were prepared at the time of collection and were authenticated at the Gray Herbarium of Harvard University, Cambridge, Mass. TLC was performed using Silica Gel G plates of 250μ thickness.

Preparation of Fractions (see Table I)—Two kilograms of the ground stem devoid of bark tissue was placed in a large percolator after being moistened with a sufficient quantity of petroleum ether. The extract was collected over a period of 72 hr. to yield a total volume of 6 l. This extract was allowed to evaporate spontaneously to yield 4.3 g. of a yellowish-brown residue, which was labeled Fraction I. The petroleum ether-exhausted marc was removed from the percolator for extraction with 95% ethanol. Extraction was carried out as above and a total of 6 l. was collected. This extract was evaporated *in vacuo* using a water aspirator to yield 215.6 g. of a dark red powder. This extract was labeled Fraction II.

Isolation of Alkane Fraction—Four grams of Fraction I was refluxed on a steam bath for 2 hr. with 70 ml. of a 10% alcoholic solution of potassium hydroxide. The alcohol was removed by evaporation on a steam bath and the residue was transferred to a separatory funnel with the aid of hot, distilled water and extracted with three 50-ml. portions of ether. The ether extracts were combined and washed with three 75-ml. portions of 10% sodium hydroxide and finally with distilled water until the washings exhibited a neutral pH. The purified ether extract was allowed to evaporate spontaneously and, after drying over calcium sulfate for 2 days, yielded 0.5 g. of a residue melting at 60–95°.[1] This unsaponified residue was recrystallized from 95% ethanol to yield 0.275 g. of a white, waxy substance; m.p. 64–66°. A negative Leibermann-Burchard test (5) indicated that the isolate was not steroidal in nature. The IR spectrum[2] was that of a typical hydrocarbon with no functional groups present. An elemental analysis gave a result[3]: C, 84.8; H, 14.5, which is indicative of high molecular weight alkanes. Compounds of this type have been previously isolated from other plant sources (6). A separation of the components of this fraction has not yet been attempted.

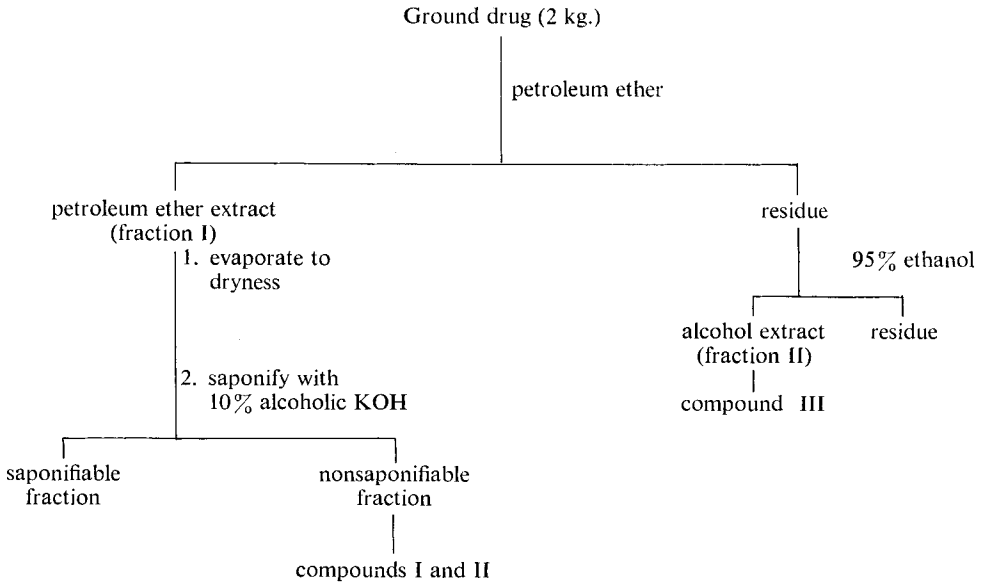
Isolation of β-Sitosterol—This compound was isolated from the ethanol filtrate from the above recrystallization of the alkane fraction. Upon slow evaporation of the ethanol filtrate at room temperature during several days gray crystals were deposited. These crystals were collected, and, after drying over calcium sulfate for 2 days, 0.175 g. of off-white crystals, m.p. 115–122°, were obtained. Several recrystallizations from an ethanol–methanol mixture (50:50) yielded 0.105 g. of a white, crystalline substance, m.p. 134–136°. The literature value for β-sitosterol is 137.5–138.5° (7). This compound gave a positive Liebermann-Burchard test indicating this material to be steroidal. TLC of this compound, and of a reference sample of β-sitosterol, was run in two systems, chloroform–isobutanol, 148:1.5, and cyclohexane–ethyl acetate–water, 60:40:1. The results indicated the presence of only one sterol. The IR spectrum of the isolate was identical with that of reference β-sitosterol. The optical rotation determined in chloroform was $[\alpha]_D^{25}$ −40°.[4]

Anal.—Calcd. for β-sitosterol ($C_{29}H_{50}O.0.5\ H_2O$): C, 82.2; H, 12.1; O, 5.7. Found: C, 81.6; H, 12.5; O, 6.1.

Isolation of Lignin Fraction—This compound was isolated from Fraction II (ethanolic). Twenty grams of this fraction was dissolved in 400 ml. of 95% ethanol and 200 ml. of acetone with slight warming. After cooling, a sufficient amount of chloroform was added to the solution to cause the precipitation of a light brown amorphous material which was collected and found to weigh 19 g. The elemental analysis showed C, 66.94; H, 4.91; and O, 27.87, and the molecular weight determined by the freezing point depression of

[1] Determined using a Mel-Temp apparatus, uncorrected.
[2] Determined in KBr using a Perkin-Elmer Infracord spectrometer, model 137 B.
[3] Analysis performed by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y.
[4] Determined utilizing a Bendix Ericsson ETL-NPL Automatic Polarimeter Control Unit, Type 143-A.

Table I—Preparation of Fractions



benzene was approximately 2000. UV absorption[5]: λ ethanol maxima at 275 and 310 mμ. The IR spectrum showed a broad band at 3400 cm.$^{-1}$ (hydroxyl); other peaks were noted at 1725 cm.$^{-1}$ (ester or acid carbonyl); 1660 cm.$^{-1}$ (aldehyde or ketone carbonyl); 1510 cm.$^{-1}$ (aromatic); and 1440 cm.$^{-1}$ (aliphatic). The UV and IR data compared favorably with similar data reported by Hess *et al.* (8) and tentatively identified the isolate as a lignin-like material. According to Varner (9) there are several types of lignin based upon their solubility characteristics. Lignin extracted by or soluble in alcohol is referred to as Brauns' lignin, native lignin, or soluble lignin.

Degradation Studies—Three hundred milligrams of the above fraction and 1.3 g. of copper hydroxide were placed in a Parr stainless steel bomb (15-ml. capacity) along with 10 ml. of 2 *N* sodium hydroxide solution. The bomb was placed in an oil bath which had been preheated to 175°. After 3 hr. the bomb was removed from the bath and cooled to room temperature. The reaction mixture was filtered and the dark brown filtrate was acidified to pH 2.0 with concentrated hydrochloric acid, placed in a separatory funnel, and was extracted with chloroform. The chloroformic extract was evaporated spontaneously and the residue was dissolved in 95% ethanol and subjected to TLC. The eluant was benzene–glacial acetic acid 9:1 (10). The chromatogram was air-dried and sprayed with 2,4-dinitrophenylhydrazine yielding two burnt-orange spots at R_f 0.44 and 0.29, respectively. Reference samples of vanillin (m.p. 79–81°) and syringaldehyde (m.p. 110–112°) were subjected to TLC as above and similar R_f's were obtained; vanillin, 0.45 and syringaldehyde 0.29.

Hydrolysis of the lignin fraction was accomplished by treating 10 g. of the compound with 50 ml. of 5 *N* sodium hydroxide at 35° for 2 hr. The reaction mixture was cooled to room temperature and was acidified to pH 2.0 with concentrated hydrochloric acid. The solution was extracted with several portions of ether and the combined ethereal extracts were evaporated spontaneously to dryness. Twenty milligrams of the ethereal extract was subjected to TLC using an eluant of benzene–glacial acetic acid–hydrochloric acid–water, 5:7:2:3. The chromatogram was viewed under longwave UV light and then sprayed with 1% ferric chloride. A duplicate run was made and the chromatogram was sprayed with Millon's reagent. In both instances, six aromatic acids were detected. In order to ascertain the identity of the unknown acids, R_f's of thirteen reference aromatic acids[6] were determined in several different solvent systems and were compared to the R_f's of the unknown aromatic acids run in the same systems. The six aromatic acids were identified as syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid), *p*-hydroxybenzoic acid, gentisic acid (2,5-dihydroxybenzoic acid), cinnamic acid, and 3,4-dihydroxycinnamic acid.

[5] Determined on a Beckmann-DB spectrophotometer with 1-cm. silica cells.

[6] Syringic, vanillic, gentisic, *p*-hydroxybenzoic, 2,3-dihydroxybenzoic, 2,4-dihydroxybenzoic, *o*-melilotic, cinnamic, 3,4-dihydroxycinnamic, ferulic, *o*-coumaric, *o*-acetoxycinnamic acid, and *o*-hydroxyphenylacetic acids.

SUMMARY

A phytochemical investigation of the stem wood of *Suriana maritima* has resulted in the isolation of an alkane hydrocarbon fraction, β-sitosterol, and a soluble lignin fraction or a Brauns' native lignin.

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