

STUDIES IN THE FIXED OIL FROM THUJA ORIENTALIS SEEDS

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Oil from the seeds of *Thuja orientalis* has been examined by chemical and physical means, including gas chromatography. The saturated acids have been identified as palmitic and stearic acids. The unsaturated acids have been hydrogenated and shown to be of the C₁₈ and C₂₀ series. The unsaturated acids have also been identified by the preparation of their bromo-derivatives.

The unsaponifiable matter has been found to consist of β -sitosterol, yellow liquid and an amorphous substance, the last two being unidentified.

Introduction

Thuja orientalis is widely grown in the sub-continent of Indo-Pakistan as a decorative plant. It bears fruit in early March which ripens by the end of August. The seeds are enclosed in a covering which contains essential oil.¹ The covering opens when the fruit matures. The seeds are dark brown in colour and contain 5.6% of a yellow coloured oil. This oil does not appear to have been studied so far. Investigations were, therefore, carried out on the chemical nature of the oil by chemical and physical methods which include spectroscopic and gas chromatographic studies.

Experimental

(a) *Recovery of the Oil.*—The seeds were separated from the coverings by hand, crushed in an iron mortar and the meal extracted with petroleum-ether (40–60°) in a Soxhlet apparatus. The solvent was removed after drying over anhydrous sodium sulphate.

(b) *Properties of the Oil.*—The Physico-chemical characteristics of the oil, determined by the usual methods² are as follows :

Refractive index, 1.485 at 15.5°C.; Specific gravity, 0.8163 at 21°C.; Tint, 4.324 yellow 0.2 red (yellow orange); Acid value, 1.87; Saponification value, 187.10; Iodine value (Wij's 1 hr.), 178.01; Acetyl value, 9.5; Thiocyanogen value, 100.18; Maleic anhydride value, 28.736; R. M. value, 1.1; Polensky value, 0.3; Kirschner value, 0.06; Saturated acids (Bertram), 12.6%; and Unsaponifiable matter, 1.47%.

(c) *Saponification of the Oil.*—The oil was saponified with alcoholic potassium hydroxide (0.5 N)

under reflux for five hours. The alcohol was then distilled under reduced pressure from the soap solution. The concentrated solution was diluted with water and extracted with diethyl ether. The unsaponifiable matter amounted to 1.47%.

(i) *Saponifiable Portion:* The soap left after the separation of the unsaponifiable matter was neutralized with sulphuric acid (4 N) and extracted with diethyl ether. The ethereal solution was dried over anhydrous sodium sulphate, and the solvent distilled off. The infra-red spectrum of the total acids showed a broad peak at 1712 cm.⁻¹ (free acids).

(ii) *Resolution of Total Acids:*³ The total acids were taken in hot ethanol (95%) containing a little acetic acid, and, while still hot, a solution of lead acetate in the same solvent was added. The clear solution was allowed to cool over-night, when lead salts of saturated acids separated out, while those of unsaturated acids remained in solution. They were filtered and decomposed with Hydrochloric acid (4 N). The saturated acids (m. p. 59–63°C.) amounted to (12.4%).

(d) *Methylation of Acid Fraction.*—Saturated Acids: The methyl esters³ of the saturated acids (m. p. 59–63°C.) were prepared by refluxing them with methanol containing a little concentrated sulphuric acid for six hours. The solution was then diluted with water and extracted with diethyl ether. The ethereal extract was washed successively with dilute sodium bicarbonate solution and water to remove any free acid. The solvent was removed after drying the solution over anhydrous sodium sulphate. The ester (m. p. 31–32°C.) so obtained showed strong infra-red absorption at 1720–1740 cm.⁻¹ thus confirming the methylation of the acid. The same results were reproducible when the acids were esterified with diazomethane.

(e) *Examination of Saturated and Unsaturated Acids by Vapour-phase Chromatography.*—(i) Saturated acids: The methyl esters of the saturated acids were injected into a column (5% silicon elastomer E-301 on celite 30—80 mesh having column temp. 246°; bridge current, 145 mA; Nitrogen flow rate, 1.2 L/hour; column inlet pressure, 46 cm.; column outlet pressure, 4 cm.; chart speed, 12"/hour, and recorder sensitivity $\times 3$) and palmitic and stearic acids were detected by the method of "the addition of supposed constituents".⁴ The percentage composition of the two acids, palmitic acid (41.9%) and stearic acid (58.1%) was calculated from their respective areas (3.86/sq. cm. and 5.4 sq./cm.).

The identification of these acids was further confirmed by taking melting and mixed melting points in the same proportion as calculated from the chromatogram.

(ii) Unsaturated Acids: The unsaturated acids were hydrogenated in a Parr Fischer hydrogenator using active palladium generated in situ by Brown's method.⁵ The hydrogenated acids (m. p. 62–63°C.) were esterified with diazomethane. The ester showed strong infra-red absorption at 1720–1740 cm^{-1} . These esters were injected in the vapour phase chromatographic column under conditions similar to those used for identifying saturated acids and found to be stearic and arachidic esters. The respective areas were 11.40/sq. cm. and 0.85/sq. cm. From these results, indirectly, the percentage of the unsaturated acids was calculated to be: C_{18} acids, 93.00% and C_{20} acids, 6.9%.

(f) *Bromo-derivatives of Unsaturated Acids.*—6 A portion of liquid acids (4.8 g.) was taken in diethyl ether and brominated using ice-salt bath. The solid separated (1.5 g., 30.7%) was identified as hexabromostearic acid (m. p. 180–181°C.). The filtrate was evaporated to dryness and petroleum ether (40–60°C.) was added. This precipitated more of the bromo-derivatives, (m. p. 154–162°C.) which did not melt at a sharp temperature indicating the presence of dienoic acids of varying nature.

(g) *Special Characteristics of Total Fatty Acids.*—The total fatty acids did not show any absorption at 10.34μ in the infra-red showing the absence of trans acid.⁷ In the ultra-violet, the absorption maxima fell at 230μ confirming the presence of diene conjugation.⁸

(h) *Unsaponifiable Portion.*—The unsaponifiable matter was taken in methanol when a white amorphous portion (m. p. 70–71°, 0.21%) remained

undissolved. Its infra-red spectrum showed a broad peak at 3300–3400 cm^{-1} . The compound did not give Liebermann—Burchard test.

The methanol soluble portion yielded on concentration, white leaflets. (m. p. 140–141°C.) giving positive Liebermann-Burchard test. Its acetate (m. p. 127–128°C.) and benzoate (m. p. 146–147°C.) were prepared by the usual methods. These results indicated it to be β -sitosterol. The I. R. Spectrum was identical with that of an authentic sample of sterol. A yellow oil (0.25%) was left behind after the crystallization of sterol which has not yet been studied.

Results and Discussion

The fixed oil of *Thuja orientalis* has been shown to contain palmitic and stearic as the main saturated acids. The unsaturated acids are of C_{18} and C_{20} series. The only unsaturated acid which has been clearly identified is the linolenic. Assuming that liquid and solid hexabromostearic acids are produced in the ratio of 75:25,⁹ the total amount of Linolenic acid comes to 44.6%. Findings of ultra-violet spectra corroborate the maleic anhydride value. However, it cannot be said with certainty whether the conjugated acids are of C_{18} or C_{20} chain length. The overall composition of the oil is as follows: Palmitic acid, 5.28%; Stearic acid, 7.30%; C_{18} acids, 81.29% (44.6% linolenic acid); and C_{20} acids, 6.10%.

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