

STUDIES IN WAXES

Part I.—Khavi Grass (*Cymbopogon iwarncusa*) Wax

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(Received August 3, 1962)

Khavi grass (*Cymbopogon iwarncusa*) wax has been examined by chemical and physical methods including gas chromatography. The wax, after saponification, has been shown to consist of 48.5% of unsaponifiable matter and 25.93% of simple and hydroxy acids. The unsaponifiable fraction was found to be composed of 86.6% of hydrocarbons, 13.02% of alcohols and traces of ketonic bodies.

A feature of the wax is the presence of very large amounts of hydrocarbons separable into two distinct fractions by column chromatography, having melting ranges (25—28°) and (66—67°) respectively. In the acid fraction, behenic acid has been shown to be the major constituent (more than 80%) while other acids which could not be identified have also been traced in small quantities. In alcohol fraction, 81.8% of tetradecanol was detected while two other alcohols, however, could not be identified.

Introduction

Khavi grass (*Cymbopogon iwarncusa*) is widely distributed in the Himalayan regions, upto 8,000 ft. from Kashmir to Assam. It also grows abundantly in the arid zones of West Pakistan, especially in Mianwali, Muzaffargarh, Jhang, Campbellpur, Jhelum, Lyallpur and Lahore districts.¹ Another variety of the grass, *Cymbopogon martini*, grows in the Khyber Hills and the former Frontier province.

The grass has been found to contain about 2% of a yellow pleasant smelling volatile oil which is composed of about 80% of piperitone and three other components as detected by vapour phase chromatography. The predominance of piperitone in the oil has been further established by chemical means.² The leaves and stem of the grass also contain a waxy material which can easily be extracted from the crushed grass by organic solvents such as various petroleum fractions. No chemical examination of the wax has been reported before, although some work pertaining mainly to physico-chemical values, has now been carried out on the oil.³ An investigation has now been carried out on the chemical nature of the wax by the usual methods which include infra-red and gas chromatographic studies.

Examination of the Wax and its Hydrolysed Products

The sample of wax was obtained by solvent extraction (petroleum ether, 62—82°) from the stems and leaves of the grass. The concomitant essential oil was removed by steam distillation. The steam distilled wax had an acid value of 9.1, saponification value of 59.8 and hydroxyl value of 74.74, showing the presence of considerable proportion of free alcohols and esters. Saponi-

fication of the sample yielded 25.9% by weight of acids and 48.5% of an unsaponifiable material. The various constituents of the wax are discussed as follows :

(a) *Acids*.—Examination of the wax after steam distillation shows the presence of a little amount of free fatty acids (acid number 9.1). The hydroxyl value (104.7) of the acid fraction, obtained after saponification of the steam distilled wax, indicates the presence of hydroxy acids also, while the acid number of the acid fraction has been found to be 13.1. The low acid number of the acid fraction led to determination of lactone number, which was found to be 68.8.

(b) *Hydrocarbons*.—The unsaponifiable matter consisted of hydrocarbons, 86.81% separable into two fractions (14.4% ; melting point 25—28°; iodine value 24.7) and (72—85°; melting point 66—67°; iodine value 84.8) (Wij's three hours).

(c) *Alcohols*.—Separation of alcohols from the unsaponifiable material by column chromatography reveals the presence of 13.02% of free and combined alcohols. An examination of these alcohols by vapour phase chromatography shows the presence of large amounts (more than 80%) of tetradecanol and small amounts of higher alcohols (higher than octadecanol).

Experimental

All IR spectra were run on a Leitz double beam instrument in CCl₄ solution

(a) *Recovery of the Wax*.—The main sample of the wax used in the present work was obtained from the grass from Thal area. The wax was recovered from the grass (1 kg.) after cutting into

small pieces and by dipping the pieces in petroleum ether (10 litres) (62–82°) for two to three days in a revolving drum. The petroleum ether extract was freed from the grass by filtering it through Buchner funnel and finally through a sintered glass funnel (1-G₄). The petroleum ether was removed from the clear yellow extract by distillation on a water-bath and the waxy material recovered. It was then steam distilled in order to free it from the accompanying essential oil. After cooling the whole mass, the steam distilled wax was separated from water by filtering it through sintered glass crucible and was finally dried by keeping it in a desiccator. The removal of the solvent and essential oil yielded 12g. (1.2%) of a hard dark yellowish-green waxy material.

(b) *Properties of the Wax.*—The following are the physico-chemical characteristics of the essential oil-free wax.⁴

Colour of the wax, dark yellowish-green; Melting point, 67°C.; Specific gravity, 0.9289 at 26°C.; Refractive index, 1.58 at 87°C.; Acid number, 9.1; Ester number after acetylation, 74.34; Carbonyl value, 40.16; Iodine value, 129.74 (Wijs three hours); Ester number, 59.85; Lactone number, nil; Solubility of the wax; 23.18% of the wax is clearly soluble in 95% of hot ethanol; Ash content, 0.42%.

The infra-red spectrum showed a broad band at 3300–3400 cm⁻¹ (alcohols) and strong band at 1712 cm⁻¹ (free acids) with a shoulder at 1740 cm⁻¹ (esters). The wax did not give any Liebermann-Burchard test.

(c) *Saponification of the Wax.*—Six grams of the wax was weighed into a one-litre, two-necked flask and 120 ml. of 1.N methanolic potassium hydroxide and 180 ml. of petroleum ether (100–140°C.), were added. The reactants were refluxed at 80°C. for nine hours in nitrogen atmosphere.⁵ After the completion of the reaction, the alcohol and water were removed under reduced pressure. The unsaponifiable matter was separated from the saponifiable mass (the potassium salts of the acids) in a Soxhlet extractor using diethyl ether as solvent.⁶ The extraction was carried out for nine hours.

(d) *Unsaponifiable Matter.*—The Wax after saponification yielded 48.5% of unsaponifiable matter melting range (48–50°C.) and hydroxyl value 108.95. Its infra-red spectrum showed a medium broad band at 3200–3500 cm⁻¹

Determination of the alcohol and hydrocarbon contents was carried out by chromatography

of unsaponifiable fraction on alumina column. This procedure yielded three distinct fractions by the procedure described below.

(i) *Hydrocarbons Eluted by Hot n-Hexane;* A sample of the unsaponifiable fraction (0.434 g.) was dissolved in hot *n*-hexane, passed through alumina column at 45°C. and the fraction rapidly eluted by 100 ml. of hot *n*-hexane. The yield from five similar chromatograms gave 0.062 g. or 14.4% of unsaponifiable fraction. This was a colourless crystalline semi-solid mass, m.p. 25–28°C., shown by its infra-red spectrum to consist solely of hydrocarbons.

(ii) *Hydrocarbons Eluted by Hot Chloroform-methanol mixture:* The alumina column was further washed with 500 ml. of methanol and chloroform mixture (50:50) maintaining column temperature at 45°C. The combined chromatograms yielded 0.315 g. or 72.58% of unsaponifiable fraction. This fraction was pale in colour (m.p. 66–67°) and had infra-red spectrum similar to those of hydrocarbons.

(iii) *Alcohol Fraction:* The strongly adsorbed part of unsaponifiable fraction was recovered from the alumina column by washing it with 400 ml. of hot chloroform-ethyl acetate mixture (50:50). This fraction was brown in colour and semi-solid in texture. Its infra-red spectrum showed strong band at 3400 cm⁻¹ (hydroxyl) and weak band at 1710 cm⁻¹ (carbonyl).

(e) *Acids.*—After the removal of the unsaponifiable matter, the residual dry mass of the potassium salts of fatty acids was then reacted at 60°C. with 3 N hydrochloric acid for one hour. The liberated acids which were solid, were dried and extracted with diethyl ether in a Soxhlet apparatus. On the removal of the solvent pure acids (melting range 80–81°C.) forming 25.93% by weight of the wax were obtained.

The infra-red spectrum showed absorption over the range of 2500–3700 cm⁻¹. The hydroxyl value, acid number and lactone number of the acid fraction were found to be 104.7, 34.3 and 68.8 respectively.

(f) *Examination of Free and Combined Alcohols by Vapour Phase Chromatography.*—Free and combined alcohols after the separation of hydrocarbons from the unsaponifiable material by column chromatography were dissolved into *o*-decanol and 0.01 ml. of the sample was injected with a micrometer syringe into a column (30% silicon elastomer E. 301 on sodium chloride 30–40 mesh), having column temperature 238°C.; bridge

current, 150 mA; nitrogen flow rate, 1.2 L/hour; column inlet pressure, 16 cm; column outlet pressure, 2 cm.; chart speed, 12"/hour and recorder sensitivity ($\times 1$) and the tetradecanol was detected by the method of the addition of supposed constituents,⁸ while two other higher alcohols were also traced in rather small quantities.

Methylation of the Acid Fraction and its Examination by Vapour Phase Chromatography

Free and combined fatty acids obtained after saponification of the wax were converted into methyl esters in the usual manner by refluxing the calcium salts of the acids with methanol, benzene and small amount of concentrated sulphuric acid.⁹ This method avoids the formation of estolidic material from the hydroxy acids which always occurs when the free acids are isolated.

Six grams of the calcium salts of the fatty acids were refluxed for six hours with methanol (100 ml.), benzene (50 ml.) and concentrated sulphuric acid (6 ml.; 25 N, 78-79%). The mixture was then washed freely with sodium bicarbonate and sodium chloride solution. The solution of methyl esters in benzene was dried over anhydrous sodium sulphate, filtered and the solvent removed. The residual solid methyl esters had an acid value nil and hydroxyl value 71.5. The esters showed strong infra-red absorption at 1720-1740 cm^{-1} confirming the methylation of the acids.

The ester fraction was dissolved in methyl laurate as solvent and injected into column filled with celite (30-80 mesh) coated with 15% by weight of silicon elastomer E. 301¹⁰ (with column temp. 244°C.; bridge current, 145 mA; Attenuator, $\times 1$; nitrogen flow rate, 1.2 L./hour; chart speed, 12"/hour; column inlet pressure, 41 cm.; column outlet pressure, 5 cm.). Behenic acid was detected by the method of "the addition of supposed constituents". Besides two other acids showed up in traces on the chromatograph. They could not, however, be identified.

Conclusion

The main constituents of the wax are hydrocarbons amounting to 86.6% of unsaponifiable fraction. These hydrocarbons are found in two main fractions (14.4%), melting range, 25-28°, and (72-85%) melting range 66.67°, respectively. The alcohol fraction constitutes 13.02% of unsaponifiable matter, and was found by gas chro-

matography to contain about 81.8% of tetradecanol; the rest of the alcohols, however, could not be identified.

The acids are the next abundant components (25.9%) of the wax. Determination of the lactone number (68.8) and low acid number (13.1) of the acid fraction clearly showed the presence of γ -hydroxy acid which formed inert lactone with the carboxyl group of the same acid. Another hydroxy acid, other than γ -hydroxy acid, was also suspected, because the acid fraction showed high hydroxyl number (104.7). However, no attempt has been made to determine the nature of these acids to separate them from the non-hydroxy acids. Further, it has been shown by vapour phase chromatographic analysis that the acid fraction consists of three acids only; behenic acid (more than 80%) and two other acids, which are most likely to be hydroxy acids.

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