Analysis of the Seeds of *Ventilago calyculata* Tul.

G.S. GROVER and J.T. RAO[1], Chemistry Department,
University of Saugar, Sagar (M.P.), India

ABSTRACT

Seeds of *Ventilago calyculata* contained 3.4% moisture, 1.8% ash, 32.9% fiber, 3.5% protein, 1.3% reducing sugars (as glucose), and 40% fixed oil, sterols, coloring matter, glycosides and free acids. The fixed oil of the seeds has been investigated in detail. Oleic acid was the major constituent at 63.1%; others were 15.9% palmitic, 13.6% linolenic, 4.5% linoleic, 1.2% lauric, 1.1% stearic and small amounts of caprylic acids. The unsaponfiable matter contained β-amyrin and lupeol along with traces of two unidentified hydrocarbons.

INTRODUCTION

Ventilago calyculata Tul. Syn. *V. denticulata* Willd., *V. madraspatana* Roxb., or *V. macrantha silhetiana* belongs to the natural order Rhamnaceae. This plant is a well known drug in the Ayurvedic system (1-2). In view of the medicinal importance of the plant, we thought it desirable to do a systematic chemical investigation of the plant seeds. This study is aimed at analyzing the fixed oil content of the seeds.

MATERIALS AND METHODS

Seeds were collected around Sagar (M.P., India). Proximate analysis of the dried, powdered seeds showed 3.4% moisture, 1.8% ash, 32.9% fiber, 3.5% crude protein and 1.3% reducing sugars.

Fixed oil was extracted from the seed powder with petroleum ether (60-80) in a Soxhlet apparatus (40% yield). Physicochemical constants of the oil were: specific gravity^{30}, 0.9332; n_d^{30}, 1.521; acid value, 1.5; saponification value, 166.4; iodine value, 79.2; acetyl value, 4.7, and unsaponifiable matter, 1.6%.

Fatty acids and unsaponifiable matter were separated from the oil by saponifying with alcoholic potassium hydroxide. The mixed fatty acids were further resolved into 18.7% saturated and 81.3% unsaturated acids using the lead salt method (3).

The saturated and unsaturated acids were methylated separately (methanol/H_2SO_4) and subjected to gas liquid chromatography (GLC) (F&M unit; column 8 ft.; EGSS on 15% Chromosorb W; column temperature, 200 C; detector, FID; IPT and DBT, 300 C). The individual fatty acids present in the oil were identified by coinjection with authentic samples (Fig. 1).

Thin layer chromatography (TLC) of the unsaponifiable matter showed the presence of four constituents. They were separated by column chromatography over a column of grade II alumina (125 x 1 cm) by eluting with petroleum ether (60-80), benzene, chloroform and ethanol. Fractions (20-ml) were collected and each fraction was subjected to TLC. The fractions having the same R_f values were mixed.

RESULTS

Petroleum ether fractions of the chromatographed unsaponifiable matter produced a white, crystalline compound of mp 64-65 C. The compound was a saturated hydrocarbon with bands at 2,900, 2,825, 1,463, 1,375, 780 and 720 cm^{-1} in infrared (IR) spectra. The hydrocarbon could not be further identified because of the paucity of the compound.

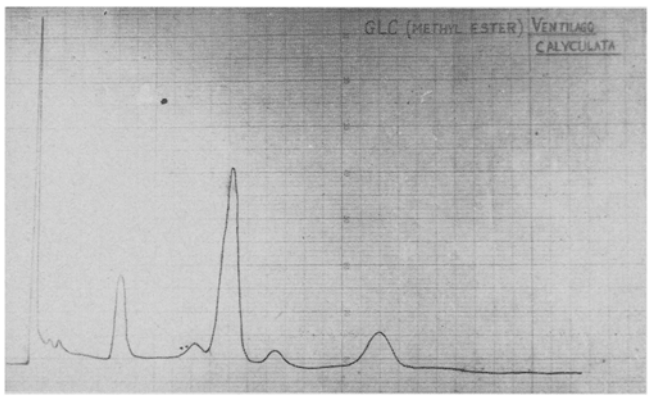

FIG. 1.

The first five fractions of benzene contained the same saturated hydrocarbon along with another hydrocarbon. The 6-15 fractions of benzene produced a colorless substance of mp 67-68 C, with bands at 2,960, 2,920, 2,850, 1,470, 1,460, 1,375, 725 and 716 cm^{-1}. This hydrocarbon also could not be identified because of the lack of compound.

The 16-19 benzene fractions contained the same saturated hydrocarbon and lupeol (by co-TLC). The remaining benzene fractions yielded colorless needles of mp 214-215 C having $(\alpha)_d^{28}$, 26.4 C. With sulfuric acid, it gave a violet color, and with Noller's reagent (4), it gave a pink color, changing finally to orange-green overnight. The IR spectrum had bands similar to lupeol. Identity of the compound as lupeol was confirmed by preparation of the derivative, benzoate (mp 264-265 C).

The first six fractions of chloroform contained lupeol and β-amyrin by co-TLC. The remaining chloroform and ethanol fractions gave a white, waxy solid which, upon recrystallization from alcohol, gave long needles of mp 197-198 C and $(\alpha)_d^{20}$, 85 C. It has responded to β-amyrin color tests, i.e., the Liebermann-Burchard test (5), the Kahlenberg test (6) and the Salkowski test (7). Its identity as β-amyrin was further confirmed by the IR spectrum and preparation of derivatives: acetate (mp 236-237 C) and benzoate (mp 232-233 C).

The percentage of fatty acids calculated from GLC of fatty acid esters was: 0.5% caprylic acid, 1.2% lauric acid, 15.9% palmitic acid, 1.1% stearic acid, 63.1% oleic acid, 4.5% linoleic acid and 13.6% linolenic acid.

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[1]Correspondence address: TH-8, Saugar University, Sagar (m.P.) 470 003, India.

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Triterpene Alcohols and Sterols of Spanish Olive Oil

T. ITOH, K. YOSHIDA, T. YATSU, T. TAMURA and **T. MATSUMOTO**, College of Science and Technology, Nihon University 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101, Japan, and **G.F. SPENCER**, Northern Regional Research Center, Agricultural Research, Science and Education Administration, USDA, Peoria, IL 61604

ABSTRACT

Nine Spanish olive oils, including three each of virgin (pressed oil), refined virgin, and B-residue (solvent-extracted pomace oil) oils from different commercial sources, have been analyzed for their unsaponifiable matter (USM). Four sterolic fractions separated from the oils have been analyzed by preparative thin-layer chromatography (TLC); these fractions are triterpene alcohols, 4-methylsterols, sterols and triterpene dialcohols. The compositions of the four sterolic fractions were determined as their acetates by gas-liquid chromatography (GLC) on an OV-17 glass capillary column. Identification of each component was carried out by argentation TLC, GLC and combined gas chromatography-mass spectrometry (GC-MS); 44 components were identified, of which four: 24-methylene-31-nor-9(11)-lanostenol, 24-methyl-31-nor-E-23-dehydrocycloartanol, 24-ethyl-E-23-dehydrolophenol and 5,E-23-stigmastadienol, were considered to be new sterols from natural sources. Several characteristics, including the content of triterpene dialcohols in the USM and that of C-24(28) unsaturated sterols in each of the four sterolic fractions, which can be used to distinguish between virgin and B-residue olive oils, were observed.

INTRODUCTION

Olive oils of different qualities from various origins have been much investigated for their sterolic constituents: sterols (4-desmethylsterols) (1-16); triterpene alcohols (4,4-dimethylsterols) (2,4-6,10,16-21); 4-methyl sterols (4α-monomethylsterols) (16,18,20,22-24); and triterpene dialcohols (9,10,13,16,25). Because of a high complexity of the sterolic compositions, however, especially those of triterpene alcohols and 4-methylsterols, a number of components remained unidentified. It has been proposed or established that the triterpene alcohol composition (2,21) or the content of triterpene dialcohols (26) could be used as tools for the detection and identification of B-residue oil in commercial olive oils. An extensive investigation of the sterolic constituents of virgin, refined virgin and B-residue olive oils coming from Spanish commercial sources was therefore undertaken in this study by the combined use of thin layer chromatography (TLC), gas liquid chromatography (GLC) on an OV-17 glass capillary column, argentation TLC, and gas chromatography-mass spectrometry (GC-MS) as the analysis and identification aids.

EXPERIMENTAL

Saponification of the oils, fractionation by preparative TLC on silica gel (20 × 20 cm, 0.5 mm thick) of the unsaponifiable matter (USM) with three developments using *n*-hexane/ethyl ether (7:3, v/v) as developer, and combined GC-MS (70 eV, >m/e 100; 2% OV-17, 2 m × 3 mm id glass column) were performed. These methods are similar to methods described in earlier reports (8). Preparative argentation (silver nitrate/silica gel, 1:4, w/w) TLC (0.5-mm-thick) was developed 3-5 times with methylene chloride/carbon tetrachloride (1:5, v/v). Upon TLC and argentation TLC, the separated bands and the fractions recovered therefrom were numbered in the order of polarity, beginning with the least polar. GLC on an OV-17 SCOT glass capillary column (30 m × 0.3 mm id, column 260 C) was carried out as previously described (27), and relative retention time (RRT) was given relative to cholesterol acetate. ^{13}C FT NMR spectrum was recorded on a JNM FX-100 spectrometer (Japan Electron Optics Lab. Co., Tokyo) operating at 25.05 MHz in deuteriochloroform. Other techniques used in this work have also been described previously (27). The configuration at C-24 of the sterols possessing an asymmetric carbon atom at the 24 position was undetermined in this study, with the exception of sitosterol.

The USM was separated by preparative TLC into six fractions: fraction 1 (R_f ca. 0.4-1.0), less polar compounds (e.g., hydrocarbons); fraction 2 (R_f 0.37), triterpene alcohols (containing aliphatic alcohols); fraction 3 (R_f 0.29), 4-methylsterols (containing aliphatic alcohols); fraction 4 (R_f 0.20), sterols; fraction 5 (R_f 0.12), triterpene dialcohols; and fraction 6 (R_f 0.0-ca. 0.1), highly polar compounds. The percentage yield of the six fractions was determined from the recovery figure. To determine the approximate relative contents of the four sterolic constituents—triterpene alcohols, 4-methylsterols, sterols and triterpene dialcohols—the acetylated USM was analyzed by GLC (a number of GLC peaks that eluted with small RRT and were attributable to hydrocarbons and aliphatic alcohols were excluded from the determination of sterolic compositions) and the percentages of the component peaks with RRT 2.07 (mainly 24-methylenecycloartanol acetate), 1.49 (obtusifoliol acetate), 1.63 (mainly sitosterol acetate) and 4.46 (erythrodiol diacetate) (which apparently represent characteristic components for each of the four sterolic constituents in the USM) were determined. Since the percentages of these components in each of the fractions were separately determined, calculation of ratios between the percentages in the USM and those in each fraction for the characteristic components enabled the determination of the relative contents of the four sterolic fractions.

Each of the four sterolic fractions was rechromatographed separately on TLC in order to obtain purified