# Analysis of *Paeonia emodi* Root Oil

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## ABSTRACT

The root oil of *Paeonia emodi* was analyzed. Unsaponifiable lipid was found to contain a mixture of *n*-alkanes C14–C33; β-amyrin, butyrospermol, cycloartenol, lupeol, 24-methylenecycloartanol; two unidentified triterpene diols; cholesterol (?), campesterol, and sitosterol. Saponifiable lipid contains octanoic, decanoic, lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic and linoleic acids.

## INTRODUCTION

*Paeonia emodi* wall (Ranunculaceae; Paeoniaceae) having a cluster of fleshy tuber roots is a native of Himalaya (1). According to the Unani System of Medicine, its roots are effective in a number of ailments including uterine and nervous disorders, epilepsy and dropsy; and as a repellant for poisonous insects (1,2). The roots of *P. emodi* are reported to contain sugar, essential oil, fixed oil, sitosterol and gallic acid ethyl ester (1,3). No information is available as to the content and composition of its root oil; the present communication reports a detailed compositional study of the oil based on spectral and chromatographic techniques.

## EXPERIMENTAL

### Oil Recovery and Methyl Ester Formation

The roots of *Paeonia emodi* were procured from Dawakhana (Unani Medical Store) A.K. Tibbiya College A.M.U., Aligarh. The powdered dry roots (500 g) were extracted in a soxhlet with petroleum ether (40–60) for 24 hr. The concentrated extract (4 g), obtained after the removal of solvent, was neutralized by passing through a column of alumina. The oil was characterized by the procedures recommended by AOCS (4). The oil (3.5 g) was saponified by refluxing for 2 hr with 1N KOH in ethanol. The excess of ethanol was removed under reduced pressure. The solution was then cooled, diluted with water and extracted with ether which on evaporation yielded an unsaponifiable fraction (0.3 g). The alkaline liquor was acidified with dilute HCl and extracted with ether. The extract was demineralized by washing with water, and the mixed fatty acids (MFA) (2.7 g) were obtained by evaporating the solvent at elevated temperature.

Methyl esters of MFA were prepared by refluxing the mixed acids for 1 hr in large excess of anhydrous methanol containing a catalytic amount of conc $H_2SO_4$. The resulting mixture was diluted to the cloud point with water, chilled in an ice bath, and then extracted repeatedly with ether. The combined ethereal extract was washed, dried over anhydrous sodium sulphate, and evaporated in vacuo. The total methyl esters (2.0 g) were examined for the component acids by spectroscopic and chromatographic techniques.

### Thin Layer Chromatography (TLC)

Analytical TLC was performed on plates layered with Silica Gel G (1.0 mm). A mixture of petroleum ether/ether (4:1, v/v) was used as developing solvent. Separation of saturated ester was accomplished on the siliconized layer of silica gel with acetonitrile/acetic acid/water (7:1:2, v/v/v) used as developing solvent (5). Plates were sprayed with perchloric acid (20%) and heated in an oven (110 C) for 10 min for visualization.

### Preparative Thin Layer Chromatography (PLC)

PLC was performed on plates coated with silica gel, Wakogel B-10 (0.5 mm). Three developments were carried out with the solvent *n*-hexane/ethyl ether (7:3, v/v) and the bands were detected under ultraviolet (UV) light after spraying with Rhodamine 6G (0.1% ethanol solution). Each band was scraped and extracted with ether. PLC of the unsaponifiable lipid (75 mg) yielded four fractions, FI (17.5 mg), FII (4.2 mg), FIII (2.0 mg) and FIV (1.0 mg), after repeated refining.

### Infrared Spectroscopy

Infrared (IR) analysis was performed with a Perkin-Elmer 621 spectrophotometer in $CCl_4$ and as liquid film.

### Gas Liquid Chromatography and Mass Spectroscopy (GC-MS)

GC-MS was done on a Simadzu LKB-9000 gas chromatograph-mass spectrometer. A glass column of 2 m × 3 mm id packed with 2% OV-17 on Gas Chrom-Q (80/100 mesh), temperature 280 C, carrier gas He, 25 mL/min, was used. Mass spectra were taken over m/e 100 with 70 eV ionizing voltage.

### Gas Liquid Chromatography (GLC)

The GLC of FI was carried out on OV-17 SCOT glass capillary column 30 m × 0.3 id; column temperature 280 C; injection temperature 300 C; carrier gas He, 0.60 mL/min; split ratio 120:1; detector, FID; chart speed 5 mm/min. Standard *n*-alkanes were used for establishing the identity of isolated *n*-alkanes.

Fractions FII, FIII and FIV, were analyzed as acetates. These fractions were acetylated separately in acetic anhydride/pyridine overnight at room temperature. Cholesteryl acetate was used as internal standard to express the relative retention time (RRT). The conditions for GLC were the same as mentioned except the column temperature which was 260 C, and the injection temperature was 280 C. Authentic samples were used for identification. The relative amount of each component was measured by peak area measurement.

### GLC (Fatty Acids)

Acid concentrations were determined by GLC in a Perkin

Elmer 154 gas chromatograph, equipped with a thermal conductivity detector and a stainless steel column, 2 m x 1/8 in. id, packed with 60/80 mesh chromosorb-W (AW-DMCS) coated with 13% diethyleneglycol succinate (DEGS). The column temperature was 200 C; and the chart speed, 30 in./hr with a hydrogen flow of 70 mL/cm. Methyl esters of lipid standards were used for establishing equivalent chain lengths (ECL) and identifying the component fatty acids. The relative amount of each acid was determined by peak area measurement.

## RESULTS AND DISCUSSION

The unsaponifiable lipid was positive to Liebermann-Burchard and tetranitromethane tests; its IR spectra showed absorption peaks at 3350 $cm^{-1}$ (OH), 1655 $cm^{-1}$ (C=C) and 1050 $cm^{-1}$ (C-O). The PLC of the unsaponifiable lipid led to the isolation of four fractions (FI, FII, FIII and FIV) and each fraction was analyzed by GLC and/or by GC-MS.

The GLC fraction FI revealed that it contained a mixture of C14-C33 *n*-alkanes (Table I). The odd carbon alkanes (61%) predominated over even carbon alkanes (39%) as usual. The major alkane was C19 (12.58%), and the general pattern of C29 or C31 alkanes predominating in higher plants was not observed. Moreover, the ratio of odd- to even-numbered alkanes (3:2) is much less than that found in higher plants (6).

Fraction FII acetate when analyzed by GLC showed that it consisted of β-amyrin, butyrospermol, cycloartenol, lupeol and 24-methylenecycloartanol (Table I). Butyrospermol and 24-methylenecycloartanol were also identified by GC-MS. Butyrospermol acetate gave a molecular ion peak ($M^+$) at m/z 468 (22%), corresponding to the molecular formula $C_{32}H_{52}O_2$, and other important peaks were found at m/z 453 (31%) M-15, 408(6%)M-60, 394 (25%) M-59-15, 393(6%)M-60-15, 355(12%)M-113, 301(10%) M-152-15. 24-Methylenecycloartanol acetate gave a molecular ion peak ($M^+$) at m/z 482(6%), corresponding to the molecular formula $C_{33}H_{54}C_2$, and other important peaks at m/z 467(8%)M-15, 422(33%)M-60, 407(32%)M-60-15, 379(18%)M-60-43, 357(10%)M-125, 297(20%)M-125-60.

GC-MS analysis of fraction FIII acetate gave two peaks with large relative retention time (RRT), 3.29 and 3.43 (Table I), both having the same molecular ion peaks ($M^+$) at m/z 512(1%) and (5%), respectively. The component with RRT 3.29 gave other peaks at m/z 497(0.2%)M-15, 452(5.9%)M-60, 437(2%)M-60-15, 393(1.8%), 315(3.6%), 262(45%), 249(9%), 203(100%) corresponding to $C_{15}H_{23}$, 189(36%) corresponding to $C_{14}H_{21}$. The other component with RRT 3.43 furnished peaks at m/z 497(1%)M-15, 452(24%)M-60, 437(7.8%)M-60-15, 409(5%), 393(3.2%), 383(1.8%), 370(2%), 262(27%), 249(24%), 203(37%) corresponding to $C_{15}H_{23}$, 189(100%) corresponding to $C_{14}H_{21}$. Identification of the two was not done due to paucity of material. However, on the basis of PLC and GC-MS pattern, the two may be triterpene dialcohols, since triterpenoid-3-acetates exhibit small loss of acetic acid from the molecular ion in contrast to steroidal-3-acetates (7). Further, the peak at m/z 203 is characteristic of pentacyclic triterpenes having a double bond between C12 and C13 as in α or β-amyrin type compounds (8). If the fragment ion m/z 203 is assumed to be formed from the ion m/z 262 by the loss of $CH_3COO$, then the second -OH group may be attached in the ring D or E replacing any one of the methyl groups.

GC-MS analysis of fraction FIV acetate revealed it to be a mixture of cholesterol (?), campesterol and sitosterol (Table I). Recently, on the basis of GLC, cholesterol has been reported (9) from the roots of *Delphinium denudatum*, a member of family Ranunculaceae.

Analytical TLC showed that the oil consisted primarily of lipids, mainly triglycerides. The spectrometric analysis of MFA and their methyl esters gave no evidence of any unusual functional group in the acids. The methyl esters revealed the presence of octanoic, decanoic, lauric, myristic, palmitic and stearic acids, when chromatographed on a siliconized layer of silica (5). Examination of the methyl

TABLE I

**Percentage Composition of Different Fractions of Unsaponifiable Lipid of *Paeonia emodi* Root oil**

| FI | | FII | FIII | FIV |
|---|---|---|---|---|
| C14(1.53) | C15(5.99) | β-amyrin (3.2) RRT: 1.63 | Unidentified (25) RRT: 3.29 | Cholesterol (?) (0.21) |
| C16(9.59) | C17(10.21) | | | |
| C18(6.64) | C19(12.58) | Butyrospermol (82) RRT: 1.68 | Unidentified (75) RRT: 3.43 | Campesterol (6.04) RRT: 1.30 |
| C20(8.99) | C21(10.65) | Cycloartenol (2.4) RRT: 1.86 | | Sitosterol (93.75) RRT: 1.61 |
| C22(5.04) | C23(7.40) | | | |
| C24(4.22) | C25(7.30) | Lupeol (6.2) RRT: 1.92 | | |
| C26(1.47) | C27(2.40) | 24-methylenecycloartanol (6.2) RRT: 2.08 | | |
| C28(0.68) | C29(1.50) | | | |
| C30(0.54) | C31(2.59) | | | |
| C32(0.27) | C33(0.41) | | | |

The (%) values are given in parenthesis.

esters by TLC on silver nitrate-impregnated silica showed distinct spots attributable to saturates, monoene, and diene. The GLC analysis of the methyl esters gave ten peaks which were tentatively identified by comparison of their retention times with those of known methyl esters of fatty acids analyzed under the same conditions. Peak areas calculated from peak height and from the width at half-height are quoted as composition (% wt) without correction (Table II). From these experimental results it is concluded that *P. emodi* root oil contains octanoic, decanoic, lauric, myristic, myristoleic, palmitic, palmitoleic, stearic, oleic, and linoleic acids. The theoretical iodine and saponification values (Table III) and the values determined by AOCS methods (4) were found in fair conformity.

**TABLE II**

**Fatty Acid Composition of *P. emodi* Root Oil (Percent by Weight)**

| | |
|---|---|
| Octanoic | 0.93 |
| Decanoic | 0.56 |
| Lauric | 2.42 |
| Myristic | 4.08 |
| Myristoleic | 3.53 |
| Palmitic | 23.16 |
| Palmitoleic | 4.46 |
| Stearic | 0.76 |
| Oleic | 7.98 |
| Linoleic | 52.12 |

**TABLE III**

**Analytical Data on *P. emodi* Root Oil**

| | |
|---|---|
| Oil content (% DB) | 0.8 |
| Iodine value | 118 |
| Saponification value | 267 |
| Refractive index $n_D^{30}$ | 1.4896 |
| Unsaponifiable (%) | 8.5 |

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