Short Communication
Pak. j. sci. ind. res., vol. 35, no. 7-8, July-August 1992

Lipid Components from the Roots of *Myrsine africana* L.

MUHAMMAD NAZIR, WAQAR AHMAD AND MUSHTAQ AHMAD
PCSIR Laboratories Complex, Lahore-54600, Pakistan

(Received March 18, 1990; revised August 2, 1992)

Myrsine africana L. (Family: Myrsinaceae) a small evergreen shrub growing in the salt range and the outer Himalyas has got medicinal importance [1,2]. Previously embelin and quercitol had been isolated from the berries [3,4]. Very recently emodin and 2- hydroxychrysophanol as cytotoxic components have been reported [5]. The hexane extracts of various parts of the plant exhibited antibacterial activity [6]. The lipid components of the hexane extract from the roots are being reported here. The roots of the plant, collected from Murre Hills, were soaked in *n*-hexane for 48 hrs. The extract was evaporated on rotary evaporator to yield an orange coloured semi-solid material (3.39g).

After removal of the coloured material by the method described earlier [7], the remaining solution was stirred with aqueous potassium hydroxide (2.5%, 25 ml) for 10 mins at room temperature with diethyl ether and acidified with HCl (2M). It yielded semi-solid acids (400 mg, 12.1%) which were esterified by methanol-hydrochloric acid.

The organic phase was evaporated and a neutral fraction (2.4 gm) so obtained was chromatographed on silica gel (120 gm) using hexane, hexane-ether, ether and methanol. The fractions were monitored by TLC and their purification was supplemented by crystallisation or by PLC.

A mixture of the wax esters (0.20 g) benzene (10 ml) and 2M ethanolic potassium hydroxide (10 ml) was refluxed for 4 hrs, diluted with distilled water and extracted with ether to give the unsaponifiable (100 mg, 50%) on evaporation. The soap solution was acidified with H_2SO_4 (2N) and was extracted with ether to give semi-solid acids (100 mg, 50%) which were converted to methyl esters (95 mg, 95%).

The GLC of methyl esters was carried out on a Pye-Unicam series 104 instrument equipped with an FID. The esters were separated on a WCOT CW 20M (25 m) column. The initial T° was held at 120° for 7.5 min. and then increased by 5°/min., to 200°. This T° was held until no more peaks were recorded. The signals were recorded and integrated with a Hitachi M 833 Data Processor. The component peaks were identified by comparing the retention times with the respective standards as well as by co-injection. HPLC analysis of the acetates was carried out using an Hitachi LC controller 638-30 operated in an isocratic mode. A RP C-18 column (Lichrocart 250-4, Merck) was used for the separations. Methanol: tetrahydrofuran (87.5: 12.5) was used as the eluent. The eluate was monitored with an RI detector (ERC-7510, Erma Optical Works Ltd. Japan). The peaks were registered and processed with an Hitachi M 833 Data Processor. The components were identified by comparison of the elution times and by coinjecting the standard alkyl acetates.

The free fatty acids of neutral component were converted to methyl esters and analysed by GLC (Table 1). Ninety percent of the area under this chromatogram could be assigned to saturated (65.32%) and unsaturated fatty acids (24.33%). These acids ranged from capric acid (C_{10}) to lignoceric acid (C_{24}) with the highest percentage of palmitic acid and very small percentages of odd numbered fatty acids.

The fraction R_f 0.85, (4.8%) hydrocarbon in nature was separated by PLC into the polar thick liquid (20.0%) and the less polar semi-solid (80.0%). The GLC of the latter fraction showed 96 peaks. The n-alkanes constituted 88.4% of the total area and ranged from tridecane (C_{13}) to tritriacontane (C_{33}, Table 1). The distribution showed two maxima at octadecane (C_{18}) and henetriacontane (C_{31}). The even to odd ratio was higher around the first maxima. In analogy to higher plant hydrocarbons, the odd to even ratio was higher in the second portion of the chromatogram.

The free alcoholic fraction R_f 0.16 (11.6%) was a mixture of triterpenols and fatty alcohols. Crystallisation from boiling hexane gave shining crystals m.p. 280° and the acetate 303. IR and NMR spectra were overlapping with that of taraxerol obtained from another source [8]. The mother liquor after acetylation separated into four fractions with R_f values 0.50, 0.38, 0.25 and 0.13 in argentation TLC. Taraxerol acetate R_f 0.25 thus raised the yield of this triterpenol to 41.6%. The fraction with R_f 0.38 (25%) was identified as bauerenol acetate on the basis of its m.p. (285-8°), mixed m.p. 285°-8 with bauerenol acetate isolated from *Ardisia solanace* [9] and comparison of the infrared spectrum. The least polar amorphous fraction (16.0%) was a mixture of saturated alkyl acetates. HPLC analysis showed at least 20 peaks out of which eight peaks covering an area of 44.2% could be assigned to even numbered n-alkyl acetates ranging from C_{18}–C_{32} (Table 1).

The sterol fraction R_f 0.08 (4.8%) was repeatedly crystallised from boiling ethanol to give flakes m.p. 165-8° acetate m.p. 185° (lit. 187-10°) and was identified as spinasterol which has also been isolated from *Aegiceras majus* [11] (N.O. Myrsinaceae). The identity was further confirmed by IR spectrum of this sterol with the reported one [12].

The fraction R_f 0.75 was obtained as a solid in 7.8% yield and was saponified with alkali. The unsaponifiable (50% yield) comprised of two spots with R_f values of free triterpenols and free sterols which were separated by PLC. The triterpenol fraction (25%) was acetylated and separated on silver nitrate impregnated plates, into four zones, bauerenol acetate (10.8%) alkyl acetate (3.6%), alkenyl acetates (10.8%), and polar components (3.0%). The alkyl acetate in HPLC analysis showed a similar composition as that of free alcohols (Table 1). The alkaline portion was acidified and the acids converted to their methyl esters. GLC analysis showed that these acids ranged from lauric acid (C_{12}) to behenic acid (C_{22}) (Table 1). The two unsaturated fatty acids, oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) were also detected (35.35% and 4.14% respectively).

The fraction having, R_f 0.37, (17.2% of the extract) showed in the IR spectrum both the keto and ester functions.

TABLE 1. DISTRIBUTION OF HYDROCARBONS, FATTY ALCOHOLS, AND FATTY ACIDS IN THE HEXANE EXTRACT OF *MYRSINE AFRICANA* L. (RELATIVE PEAK AREAS FROM GLC AND HPLC).

Carbon chain length	Hydrocarbons	Fatty alcohols free from esters			Fatty acids free from esters	
	R_f 0.85	R_f 0.16	R_f 0.75	Free	R_f 0.75	R_f 0.37
10	-	-	-	0.83	-	-
11	-	-	-	-	-	-
12	-	-	-	1.84	2.62	+
13	-	-	-	0.42	-	-
14	2.78	-	-	3.10	7.52	+
15	4.19	-	-	1.13	-	-
16	8.57	-	-	39.00	27.15	5.02
17	7.14	-	-	1.52	-	-
18:0	9.02	0.62	0.03	12.42	10.66	28.60
18:1	-	-	-	3.58	35.35	0.60
18:2	-	-	-	19.30	4.14	14.29
18:3	-	-	-	1.45	-	-
19	3.02	-	-	0.49	-	-
20	4.66	1.20	1.76	2.12	2.37	39.38
21	2.00	-	-	-	-	-
22	2.80	4.51	2.35	1.53	0.73	2.81
23	2.61	-	-	-	-	-
24	2.29	5.77	3.09	0.92	-	-
25	3.20	-	-	-	-	-
26	2.24	7.02	4.07	-	-	-
27	4.34	-	-	-	-	-
28	2.25	9.74	5.61	-	-	-
29	11.62	-	-	-	-	-
30	2.04	21.93	13.47	-	-	-
31	12.04	-	-	-	-	-
32	-	0.46	7.67	-	-	-
33	1.30	-	-	-	-	-

On crystallisation from boiling ethyl acetate needle-like crystals of taraxerone (m.p. 243-245°) separated out (13.8%). The identity was further confirmed by reduction with sodium borohydride to tarexerol, acetylation of the triterpenol, determination of m.p., mixed m.p. and comparison of the spectral data with authentic sample [8].

The mother liquor of the fraction (R_f 0.37) after removel of taraxerone and the solvent gave slightly coloured thick liquid (3.45%) which indicated all the functions of an ester. It was saponified when the alcholic semi-solid fraction 44.2% and the acidic fraction 53.5% were obtained. The acidic fraction (Table 1) differed from the above combined acid fraction quantitatively in composition. Arachidic acid (C_{20}) was the largest component among these fatty acids.

Key words: Lipid components, *Myrsine africana* L.

References

1. R. N. Chopra, I. C. Chopra, K. L. Handa and L. D. Kapur, *Chopra's Indigenous Drugs of India* (Academic Publishers, New Delhi, 1958), 2nd ed., pp. 367.
2. K. M. Nadkarni, *Indian Material Medica, I* (Bombay Popular Prakashan, 1982), Revised and England Edition, pp. 835.
3. S. Krishna and B. S. Varma, J. Ind. Chem. Soc., **13,** 115 (1936).
4. R. Merian and E. Schlittler, Helv. Chim. Acta., **31,** 2237 (1948).
5. Xiao-Haua Li and J. L. McLaughlin, J. Nat. Prod., **52,** 660 (1989).
6. M. T. J. Khan, M. Ashraf, M. Ahmad and M. K. Bhatty, Fitoterapia, **59** (5), 399 (1989).
7. M. Nazir and M. T. J. Khan, Pak. J. Pharmacy, **3,** 91 (1990).
8. M. Nazir and Waqar Ahmad, Taraxerol Isolated from *Euphorbia caducifolia*, (PCSIR Labs. Complex, Lahore).
9. S. A. Ahmad, S. Catalano, A. Marsili, I. Morelli and V. Scartoni, Planta Medica, **32,** 162 (1977).
10. I. Heibron, H. M. Bunbury, A. H. Cook and E. R. Jones (Eyre and Spottiswoode Publishers, Ltd., 1953), Vol.4, pp. 369.
11. K. V. Rao and P. K. Bose, Ann. Biochem. Exptl. Med. (Calcutta), **21,** 354 (1961); C. A. **57,** 16670 g (1962).
12. K. Dobriner, E. R. Katzenellenbogen and R. N. Jones, *Infrared Absorption Spectra of Steroids* (Interscience Publishers Inc., N. York, 1953), Spectrum No.61.