Chemistry of Natural Compounds, Vol. 45, No. 5, 2009

FATTY ACID COMPOSITION OF *Prosopis cineraria* SEEDS

S. Gangal, S. Sharma, and A. Rauf*

UDC 547.915

The genus *Prosopis* belongs to the family Fabaceae, subfamily Mimosaceae and comprises 44 species distributed mainly in arid and semi-arid, tropical, and subtropical countries [1]. In many desert regions of the world the seeds of *Prosopis* species have been proposed as a source for agro-industrial development of economic foods for humans and animals [2, 3]. Furthermore, the seeds and pods have been used in traditional medicine for treating a broad range of diseases and illness [4, 5]. Sugars, flavonones, fatty acids, tannins from the heartwood [1], flavones, glycoside patulitrin from flowers [6], and piperidine-3-ol alkaloid [7] were previously reported.

In continuation of our previous studies on various seed oils and the identification of hydroxy fatty acids [8], a study of seed oils of *P. cineraria* has been carried out for their component fatty acids and to confirm the presence or absence of hydroxy fatty acid in this seed oil.

The analytical values of oil and seeds (Table 1) were determined according to the procedure recommended by the AOCS [9]. The seed oil did not respond to the Halphen test [10], the 2,4-dinitrophenyl hydrazine thin layer chromatography test [11], and the picric acid TLC test [12], thereby indicating the absence of cyclopropenoid, keto and epoxy fatty acids. The IR spectra of the oil, as well as its methyl ester, showed the presence of the hydroxy group. The infrared spectrum also showed characteristic absorption bands at 756 and 1600 cm^{-1}, indicating the presence of a *cis*-double bond. However, IR and UV spectra of oils showed no evidence for *trans*-unsaturation or the presence of conjugation. TLC analysis of the methyl esters showed two spots that correspond to standard usual and monohydroxy methyl esters.

Quantitation of component acids was made by GLC analysis of the methyl esters as their trimethylsilyl (TMS) derivatives and data are given in Table 2. The *P. cineraria* seed oil has been found to contain total saturated fatty esters (28.6%), total unsaturated fatty esters (68.3%), and methyl hydroxy fatty ester (3.1%). The seed oil is rich in linoleic acid (32.1%) along with oleic acid (31.3%).

To determine the absolute identity of hydroxy ester, freshly prepared mixed fatty acid methyl esters were chromatographed over a column of silica-gel. Elution with petroleum ether–diethyl ether (70:30 v/v) gave pure hydroxy fatty ester. IR of the isolated ester again showed the hydroxyl band at 3425 cm^{-1} and the absence of *trans*-unsaturation. The elemental analysis of the ethyl ester corresponded to the molecular formula $C_{19}H_{36}O_3$, suggesting an 18-carbon fatty acid.

The ^{1}H NMR spectrum exhibited signals at δ 5.43 (2H, m, -C<u>H</u>=C<u>H</u>-), 3.75 (1H, br.s, -C<u>H</u>-OH), 3.61 (3H, s, -COOC<u>H</u>$_3$), 2.71 (1H, br.s, -CH-O<u>H</u>, disappearing upon addition of D_2O), 2.32 (2H, m, α to -C<u>H</u>$_2$-CO-), 2.04 (4H, m, -<u>CH</u>$_2$-CH=CH-C<u>H</u>$_2$), 1.27 (20H, br.s, chain C<u>H</u>$_2$), and 0.88 (3H, dist.t , terminal C<u>H</u>$_3$). The ^{13}C NMR spectrum of the methyl ester of hydroxy fatty acid was also helpful in assigning the structure. It showed a characteristic signal at δ 172.2 for carbonyl carbon, 130.1 attributed to C-9 and C-10, 29.4 (C-8), 31.5 (C-11), 71.5 (C-12), 33.8 (C-13), and 51.4 (OCH_3).

The acetate derivative of the pure hydroxy ester showed a strong band at 1235 cm^{-1} and no hydroxyl absorption band in its IR spectrum. The ^{1}H NMR spectrum (acetate derivative) showed no unusual features apart from two expected but significant signals at δ 4.7 as multiplet for one methine proton (C<u>H</u>-OAc) and at δ 1.9 as singlet for methyl protons (-OCOC<u>H</u>$_3$). The disappearance of the signal for the hydroxyl group confirmed the original acid as a hydroxy acid.

Catalytic hydrogenation [13] of the hydroxy-olefinic ester gave a saturated hydroxy ester melting at 54–55°C, which was identified as methyl 12-hydroxystearate with an authentic sample.

Department of Chemistry, Aligarh Muslim University, Aligarh 202001, India, e-mail: abduloafchem@gmail.com.
Published in Khimiya Prirodnykh Soedinenii, No. 5, p. 592–593, September–October, 2009. Original article submitted January 14, 2008.

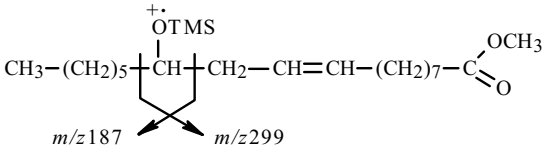
0009-3130/09/4505-0705

TABLE 1. Analytical Values of *P. cineraria* Seed Oil

Oil content in seeds, %	10.6	Refractive index, n_D^{30}	1.4885
Protein Content, %	16.5	Halphen test	–ve
Iodine value, %	87.3	Picric-acid TLC test	–ve
Saponification value	195.4	2,4-DNPH TLC test	–ve

TABLE 2. Composition of Silylated Fatty Acid Methyl Esters (Percentage of Total Silylated Fatty Acid Methyl Esters) of *Prosopis cineraria* Seed Oil

Component	Area, % by GLC	Component	Area, % by GLC
14:0	1.5	18:3	2.9
16:0	15.2	20:0	4.4
16:1	0.8	20:1	1.2
18:0	2.2	22:0	4.1
18:1	31.3	24:0	1.2
18:2	32.1	18:1-OH	3.1



The oxidative degradation of the original hydroxy acid obtained from *P. cineraria* seed oil was performed [14]. Formation of the azelaic acid showed that the double bond was at C-9 and that the hydroxyl function was not located between the double bond and the carbonyl group. The other product, 3-hydroxypelargonic acid, placed the hydroxyl position at C-12. The MS of the TMS derivative of the hydroxy-olefinic ester was identical to the TMS derivative of the authentic methyl ricinoleate. Structure-revealing ions were observed at *m/z* 187 and 299, and a TMS rearrangement ion [15] at *m/z* 270 unequivocally established the position of hydroxyl at C-12 and indicated the double bond at C-9.

On the basis of these physical and chemical evidences, the hydroxy acid was characterized as (*Z*)-12-hydroxyoctadec-9-enoic acid, commercially known as ricinoleic acid.

Plant Material and Extraction of Seed Oil. The seed were purchased from Pratap nursery, Dehradun (U.P.). The air-dried seeds were powdered and extracted thoroughly with light petroleum ether (bp 40–60°C) in a Soxhlet extractor for 8 h to yield 10.6% of oil.

Isolation of Hydroxy Fatty Ester. Saponification of the seed oil was carried out by refluxing it with 0.5 N alcoholic KOH. The unsaponifiable material was removed by diethyl ether extraction. The methyl esters of fatty acids were prepared by Fischer esterification.

Preparation of Trimethylsilyl Derivatives of Methyl Ester. Silylation of the methyl ester from *P. cineraria* was done by treating it with hexamethyldisilazane and trimethylchlorosilane [16]. The silylated methyl esters were subjected to GLC analysis using silylated methyl esters of castor oil (*Ricinus communis*) as a reference standard.

Acetylation of Hydroxy Methyl Ester. The hydroxy methyl ester (50 mg) was dissolved in acetic anhydride in pyridine (2 mL, 5:1, v/v) and left at room temperature overnight. The solvent was removed in a stream of nitrogen with gentle warming, and the acetylated ester was purified by TLC on silica gel G layers.

Instrumentation. IR spectra were obtained on Shimadzu 8201 PC FT-IR apparatus as liquid films. The ultraviolet (UV) measurements were made on a methanolic solution with a Cintra 5 UV-vis. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance II-400 instrument using $CDCl_3$ as a solvent. Chemical shifts were measured in ppm downfield from internal tetramethylsilane ($\delta = 0$). Mass spectra were obtained in a Shimadzu QP-2000 spectrometer. The GLC analysis were carried out using a Varian Vista 6000 instrument equipped with an FID (290°C) detector using a stainless steel column packed with 15% OV-275 on chromosorb-W (80–100 mesh). Separations were carried out at programmed temperature of 140–200°C (10°C min^{-1}). The machine recorded directly the area% of individual peaks. The peaks were identified by comparing their retention times with those of standard reference samples under similar conditions.

REFERENCES

1. A. Burkat, *J. Arn. Arb*., **57**, 219, 450 (1976).
2. R. M. Beri, P. C. Dodhal, and K. S. Ayyar, *Indian Forester*, **108**, 669 (1982).
3. M. A. Mella and S. Masson, *Grasas Aceites*, **35**, 240 (1984).
4. H. D. Neuwinger, *Afrikanische Arzneipflanzen und Jagdgifte*, WVG, Stuttgart, 1993, p. 600.
5. J. A. Duke, *CRC Handbook of Medicinal Herbs*, Library of Congress/CRC Press: Boca Raton, FL, 1986.
6. C. S. I. R. (Council of Scientific and Industrial Research) New Delhi, *The Wealth of India.* Vol. **VIII**, 1969, p. 247–249.
7. K. Jewers, M. J. Nagler, K. A. Nirvi, and F. Amir, *Phytochemistry*, **15**, 238 (1976).
8. M. U. Ahmad, S. K. Husain, and S. M. Osman, *J. Am. Oil Chem. Soc.*, **58**, 673 (1981); M. S. Ahmad, A. Rauf, J. Mustafa, and S. M. Osman, *Phytochemistry*, **23**, 2247 (1984); A. Rauf, M. S. Ahmad, and S. M. Osman, *Indian J.Chem.*, **26B**, 283 (1987); H. Parveen and A. Rauf, *Ind. Crops Prod*., **27**, 118 (2008).
9. W. E. Link, *Official and Tentative Methods of the American Oil Chemist's Society*, Third Ed., AOCS, Champaigh, IL, USA (Methods DA 15–48 and DA 16–48).
10. G. Halphen, *J. Pharm. Chim.*, **6**, 390 (1897).
11. E. N. Davis, L. L. Wallen, J. C. Rohwedder, and R. A. Rhodes, *Lipids*, **4**, 356 (1969).
12. J. A. Fioriti and R. J. Sims, *J. Chromatogr.*, **32**, 761 (1968).
13. A. I. Vogel, *A Text Book of Practical Organic Chemistry*, Third Ed., Longmanns, Green and Co, London, 1956, p. 866.
14. E. Von Rudloff, *Can. J. Chem.,* **34**, 1413 (1956).
15. R. Kleiman and G. F. Spencer, *J. Am. Oil Chem. Soc.*, **50**, 28 (1973).
16. W. W. Christie, *Lipid Analysis*, Pergamon Press, New York, 1973, p. 137.