Pakistan J.Sci.Ind.Res., Vol.26, No.1, February 1983

NEUTRAL LIPIDS FROM SEEDS OF *TRIGONELLA CORNICULATA*

Muhammad Nazir, Muhammad Riaz, Jamilur Rehman, Muhammad Akbar Saeed and Muhammad Khurshid Bhatty

PCSIR Laboratories, Lahore, Pakistan

(Received September 3, 1981)

The fatty acids of the oil of the seeds of *Trigonella corniculata,* were identified as myristic (0.90%), palmitic (16.78%), stearic (3.48%), oleic (12.65%) linoleic (50.54%), linolenic (15.71%) and arachidic acid (1.13%). The oil gave unsaponifiable matter (5.92%) which was resolved into hydrocarbons, alcohols, sterols etc. The distribution of *n*-alkanes ranged from C_{15} to C_{35}, with two maxima at C_{21} (34.67%) and C_{29} (14.26%). The *n*-alkanols ranged from dodecanol to triacontanol with maxima at octadecanol (5.04%) and hexacosanal (62.53%). Cholesterol and β-sito-sterol have also been confirmed.

INTRODUCTION

Trigonella corniculata is a pot herb, belonging to family Leguminosae, sub family Papilionaceae. The plant, locally known as Kasuri Methi cultivated all over the Punjab and Sind as a winter crop. Its leaves are used in spices, spinach and curry. The seeds are bitter astringent and styptic, and are sparingly used in local medicine [1]. The seeds have been a subject of investigation by Indian workers. Triacontane, β-sitosterol, choline, and betaine have been isolated [2], and the presence of diosgenin and yuccagenin in the seeds has been confirmed [3,4]. Later on 6.8-Di-C-β-D glucopyranosylacacetin and its mono acetate have also been isolated [5] besides isolation of ethyl galactoside from the acetone extract of the seeds [6].

The present studies aim at exploring the industrial potential of this crop. This communication therefore deals with the hexane extract of the locally available seeds of *Trigonella corniculata.*

EXPERIMENTAL

IR spectra were recorded on Perkin Elmer Spectrophotometer model 621 as a 5% solution in carbon tetrachloride. GLC of the alcohol acetates and sterol acetates was carried out on Hewlett Packard Gas Chromatograph model 5700 A, equipped with flame ionisation detector. A stainless steel column 3 m x 0.3 cm packed with 3% OV-1 coated on chromosorb AWHMDS 80-100 mesh was used for the separations. For the analysis of *n*-alkyl acetates the column temperature was programmed from 100-250° at an increment of 4° min and hold at 250° until no more peaks emerged. For the analysis of sterol acetates, the column was maintained isothermally at 250°. Helium 30 ml/min was used as the carrier gas. The signals were registered by Soltec Rikadenki Recorder B 281, and integrated by a Hewlatt Packard Integrator 3373 B. The indentification of peaks was carried out by comparison of the retention times and coinjection of standard samples. The percentage of each component was calculated as the per cent area under each peak. Silica gel grade 62 from Davison Chemicals was activated in a microwave oven for 30 mints before use. Thin layer plates were from Kodak and the spots were visualised by UV light or placing in iodine chamber. Molecular sieves (50 nm, 1.6 mm pellats from Alltech Associates) were activated in a vacuum oven at 250° for six hours and then stored in a desiccator.

Volatile Matter of the Seeds. The seeds (3.5014 g) were taken in a tarred petri dish and heated in an oven maintained at 100-105°. The constant weight (3.2618 g) was obtained after 15 hours, showing the volatile matter as 6.85%.

Extraction of the Seeds. The seeds (500g) were ground to a fine powder and extracted with freshly distilled hexane in a Soxhlet extractor for 48 hours. The hexane extract was dried over anhydrous sodium sulphate and the solvent was removed, giving a dark coloured liquid. The yield of the oil was 28.70 g, (5.74%).

Saponification of the Oil. The oil (6.00g), was refluxed with 0.5N ethanolic potassium hydroxide (150 ml) for 4 hours. The solvent was removed under reduced pressure.

The residue was dissolved in distilled water (300 ml) and extracted with ether. The combined ether extracts were washed with water and dried over anhydrous sodium sulphate. Removal of the solvent gave reddish brown resinous semi-solid matter (0.296 g, 5.92%).

Liberation of Fatty Acids. The soap solution and the washings were combined and acidified with 2N sulphuric acid. The liberated fatty acids were extracted thrice with ether. The combined extracts were dried over anhydrous sodium sulphate and evaporated to give a pale oily mass (4.44 g).

Esterification of Acids. A solution of the acids (2.15g) in methanol containing 5% HCl (30 ml) was refluxed on a water bath for 2 hours. After diluting the reaction mixture with water, the esters were extracted with ether and washed with sodium bicarbonate solution to remove any free acids. The pale oily mass, thus obtained after drying and evaporating, was again dissolved in hexane and passed through silica gel to give pure colourless esters (1.94 g, 90.2%).

Hydrogenation of Methyl Esters. Purified methyl esters (70 mg) in methanol (100 ml) containing 5% palladium charcoal were hydrogenated at a pressure of 40 lbs/sq. inch in a Parr Hydrogenator. After four hrs the catalyst was filtered and washed with methanol, the solvent was removed under reduced pressure giving saturated methyl esters (70 mg).

Gas Liquid Chromatography (GLC)of Methyl Esters. GLC analysis of methyl esters was carried out on a Pye Unicam 104 gas chromatograph equipped with a flame ionisation detector. A glass column (1.5 m. long, 0.6 mm OD) filled with chromosorb A.W. 60–70 mesh coated with 10% DEGS and maintained at 180° was used for the separation of the esters. Nitrogen 50 ml/min was used as the carrier gas. A solution of the esters in hexane (0.2 µl) was injected on the column. Identification of the individual fatty acids was carried out by comparison of retention times and coinjection of standard samples. As no standard sample of linolenic acid was available, its presence was inferred by extrapolation of the retention times and was confirmed by hydrogenation of the methyl esters and subsequent GLC analysis. The distribution of individual esters was calculated as percentage area under each peak.

Resolution of the Unsaponifiable. The unsaponifiable (800 mg) dissolved in benzene was charged to a column of silia gel (40 g). The elution was carried out with hexane, benzene, benzene-ether, and methanol-chloroform and was monitored by TLC. Elution with hexane (50 ml) furnished hydrocarbons (30 mg, 3.75 %) while benzene (250 ml) gave a mixture of three components (38 mg, 4.38%). Elution with benzene – ether (98:2, 100 ml) gave alcoholic fraction (62 mg, 7.75%). Further elution with benzene ether (95:5, 400 ml) gave the sterol fraction (312 mg, 38.75%). Metahanol-chloroform (1:3,200 ml) eluted the brownish resinous mass (320 mg, 40%).

Gas Liquid Chromatography (GLC) of Hydrocarbons. GLC of the hydrocarbons was carried out on a Hewlett Packard Gas Chromatograph 5700 equipped with a flame ionisation detector and Hewlett Packard Recorder. SP 2100 fused silica capillary column (25 m x 0.2 mm) was used for the separation. The column temperature was programmed from 100 to 275° with an increment of 3°/min and a hold at final temperature for 50 mints. The neat warm sample 0.1 µl was injected on to the column. A split ratio of 50:1 was employed to optimise the separation. Temperature of injection port and the detector were maintained at 80° and 300° respectively. The content of individual hydrocarbons was calculated as the percentage of total area under the chromatogram.

Isolation of n-Alkanes. The hydrocarbon fraction (23 mg) was dissolved in isooctane (10 ml) and refluxed with molecular seives (0.46 g) for 16 hrs. The sieves were filtered, washed with the same solvent and then soxhlet extracted with isopropyl alcohol-benzene (2:1, 150 ml) for four hrs to desorb any non normal hydrocarbons adhering to the surface of the sieves. The filtrate, washing and the extract were combined and evaporated to give the unabsorbed material (6.4 mg, 27.8%). The sieves were ground to a fine powder, placed in a teflon beaker and covered with cyclohexane (50 ml). The cooled stirred slurry was digested by the dropwise addition of (23 ml) 24% hydrofluoric acid over a period of four hrs. The grey coloured solution was diluted with water and extracted with cyclohexane. The organic layer was washed neutral with water, then passed over alumina (3 g). Evaporation of the solvent gave white semi solid mixture of hydrocarbons (15.9 mg, 69.0%).

Acetylation of Alcoholic Fraction. The alcoholic fraction (60 mg) was acetylated with pyridine-acetic anhydride mixture (1:1, 2 ml) at room temperature for 24 hrs and then worked out as usual to give an oily mass (60 mg).

Separation of Normal Alcohols and Iso/Cyclic Alcohols. The acetylated alcoholic fraction (50 mg) was dissolved in methanol chloroform (5:2, 7 ml), and powdered urea (500 mg) was added to this solution. The mixture was stirred for 2 hrs at room temperature and allowed to stand overnight at 5°. The deposited clathrate was filtered and washed with hexane. The filtrate and washings were combined together and shaken with water to remove any dissolved urea. The organic layer was dried over anhydrous sodium sulphate and evaporated to give the non-

normal alcohol acetates (35.5 mg, 71.00%). The clathrate was decomposed with warm water and the n-alkyl acetates extracted with ether, dried over anhydrous sodium sulphate and evaporated to give a semi solid mass (14 mg, 28.0%).

Isolation of β-Sitosterol. The sterol fraction (50 mg) was crystallised thrice from methanol to give shining plates m.p. 139-41° (14 mg, 28.0%) acetate m.p. 127–9°. Mixed m.p. of the sterol with an authentic sample remained undepressed (139–40°).

RESULTS AND DISCUSSION

The seeds of *Trigonella corniculata* are much smaller in size than the seeds of *Trigonelia foenum* Graecum. The average weights of 100 seeds of *Trigonella corniculata* and *Trigonella foenum* Graecum are 0.1275 gms and 1.27 gms respectively.

The seeds although appear dry, they still lose weight on heating (6.89%), which may comprise water and other organic compounds volatile at this temperature (100-105°).

The ash content of seeds as determined by muffling the seeds amounts to 3.53%. This content is slightly higher than already reported (3.0%) for fenugreek [8].

The hexane extract of the ground seeds amounts to 5.74% on seed basis and 6.16% on moisture free basis. This fig is comparable to other Pakistani species, i.e. *Trigonella foenum* Graecum (6.5%) [9]. The coloured odorous residue obtained as hexane extract appears to be fixed oil but may contain other non polar or slightly polar components. As no precipitation could be observed on standing, the whole liquid was taken as an oil.

Thin layer chromato-graphic analysis of this oil showed triglycerides, sterols, straight chain primary alcohols, hydrocarbons and traces of components polar than triglycerides but less polar than straight chain primary alcohols. The latter components may be other alcohols, esters, ketones etc.

The physical constants of the oil are shown in Table 1. The colour, odour, density and refractive index of the oil are in agreement with the values reported previously for other species [9, 10]. The acid value (6.75) of this oil is higher than that already recorded for other species [10]. This may be due to enzymatic activities. The higher acid value of oils such as rice bran oil, olive oil [11] have been attributed to the presence of lypolytic enzymes. The iodine value (147.0) of the oil proves the oil to be semi drying.

The oil was saponified and the alkali salts were separated. The liberated acids were esterified. The resulting purified methylesters were analysed by gas liquid chromatography. The composition of fatty acids of the oil is given in Table 2. The content of unsaturated acids (78.9%) is close to that of linseed oil (ca-82%). However, this oil is higher in linoleic acid and lower in linolenic acid than the corresponding acids in linseed oil. The oil contains predominently C_{18} acids (82.38%). The other acids i.e. myristic, palmitic and arachidic are present in 0.90%, 16.78% and 1.13% respectively. After a partial hydrogenation, the composition may be close to cotton seed or other non drying oils. The partially hydrogenated oil, therefore, may be suitable for human consumption, provided necessary refining of the oil has also been done.

The unsaponifiable matter was obtained as 5.92% on the basis of oil and is resinous semi solid in nature. This quantity is much higher than those reported for other species [12] but is comparable to Pakistani fenugreek [9]. The presence of hydrocarbons, unsaponified esters, alcohols, sterols and other polar substances in this unsaponifiable were confirmed by IR and TLC analysis. However, it did not show any spot corresponding to diosgenin even at high concentration on TLC plate, thereby, indicating the absence of free diosgenin in the seeds.

The column chromatography separation of unsaponi-

Table 1. Physico-chemical characteristics of the oil of the seeds of *Trigonella corniculata.*

Yield (on moisture-free basis)	6.16%
Colour (Lovibond Tintometer)	
Pink	8.9
Blue	9.0
Yellow	27.7
Density d_4^{16}	0.9322
Refractive index n_D^{32}	1.4740
Acid value	6.76
Saponification value	147.0
Iodine value (Wij's) method, one hour)	147.0
Unsaponifiable	5.92%

fiable matter furnished hydrocarbons (3.75%) unidentified mixture (4.38%), alcohols (7.75%), sterols (38.95%), and resinous material (40.00%) . Each fraction was studied separately.

Gas liquid chromatography of the hydrocarbon fraction using a Dexsil 300 column resolved it into 35 peaks and a small envelope of the unresolved components. The retention times of the resolved components correspond to n-alkanes range C_{15} to C_{35}. Therefore, the remaining 14 peaks belong to hydrocarbons other than n-alkanes. The presence of squalene type hydrocarbons has been reported in Pakistani fenugreek [9]. It was attempted to trace the presence of squalene in our hydrocarbon fraction. Addition of squalene to the hydrocarbon fraction resulted in enhancing the area under the peak corresponding to n-octacosane. A mixture of squalene and *n*-actacosane showed the same retention times on Dexsil 300 packed or capillary columns. However under the conditions described in experimental, a difference in retention times of the *n*-octacosane (55.11 min) and squalene (55.31 min) was observed. The hydrocarbon fraction did not show any signal corresponding to squalene. On the other hand squalene containing hydrocarbon fraction when chromatographed under the conditions detailed in experimental showed an additional well resolved peak for squalene, which confirmed the absence of squalene in this hydrocarbon fraction.

. For the estimation of the n-alkanes of the hydrocarbon fraction, these were isolated by absorption on molecular sieves. The n-alkanes formed 72.18% of the hydrocarbon fraction. They were analysed by gas liquid chromatography. Percentage composition of the individual *n*-alkanes is given in Table 2. An important feature of these *n*-alkanes was their precentage distribution. Their content goes on increasing and is maximum at C_{21}, after which it tapers down to the minimum at C_{24} without any discrimination between odd and even number hydrocarbons. The percentage distribution starts increasing again and C_{29} forms the maximum and then drops to C_{35}; this is in agreement with the odd even number concept in plant kingdom. Bastic *et-al* [13]. have analysed a number of unsaponifiables from olive sun-flower oils etc, but none of them seemed to behave like this and hence it may be correlated with the presence of wax as well as oil in the seeds of *Trigonella corniculata.*

The fraction having an R_f of primary alcohols by TLC analysis amounts to 7.75% of the unsaponifiable. IR spectrum of this fraction showed a sharp band at 3615 cm^{-1} (free OH), a broad band centering at 3400 cm^{-1} (H-bonded OH), and a peak at 1040 cm^{-1} (C-O stretching in cyclic alcohols). In addition to the usual C-H stretchings between 2960-2850, 1470-1450, 13.80-13.65 cm^{-1} a small peak at 715 cm^{-1} was also observed. The unsaturation of the fraction was indicated as a shoulder at 3060 cm^{-1} and a medium sized peak at 885 cm^{-1}. This leads to conclusion that the fraction may be a mixture of saturated straight chain as well as branched/cyclic alchols where the latter due to its added polarity (secondary OH and the cyclic nature) gets eluted with primary alcohols. In order to obtain less complex mixtures, this fraction was

Table 2. Distribution of hydrocarbons, fatty alcohols and fatty acids in the oil of *Trigonella corniculata* seeds.

Homologues	Hydrocarbons %	Alcohols %	Fatty acids %
12:0		0.22	–*
13:0		0.55	–
14:0		1.04	0.90
14:1			Traces
15:0	.03	1.25	–
16:0	.065	1.34	16.78
16:1			Traces
17:0	.48	1.21	–
18:0	1.64	5.04	3.48
18:1			12.65
18:2			50.54
18:3			15.71
19:0	2.66	1.50	–
20:0	2.53	1.72	1.13
21:0	34.53	1.72	–
22:0	5.43	1.18	–
23:0	5.09	0.73	–
24:0	2.61	3.04	–
25:0	4.33	1.65	–
26:0	3.04	62.53	–
27:0	5.99	1.89	–
28:0	3.67	9.66	–
29:0	14.26	0.67	–
30:0	2.64	2.21	–
31:0	6.01		–
32:0	1.39		–
33:0	1.74		–
34:0	0.64		–
35:0	0.51		–

* Not detected.

acetylated and the *n*-alkyl acetates recovered by urea complexing. Gas liquid chromatographic analysis of the *n*-alkyl acetates shows that the alcohols range from C_{12} to C_{30} (Table 2). The percentage distribution curve of alcohols shows behaviour similar to the *n*-alkane fraction (Fig.1). The two maxima in this curve occur at C_{18} and C_{26}. The content of each constituent increases from lauryl acetate to stearyl acetate and then drops down with practically no odd or even number characteristic. But after nonadecanyl acetate the odd even distribution becomes very conspicuous. Hexacosanol-1 (Ceryl alcohol) which occurs free as well as in combined form in so many plant waxes occurs in predominence (26.5%). The occurence of stearyl alcohol to triacontanol −1 can be explained from the proposed biogenesis of even numbered acids.

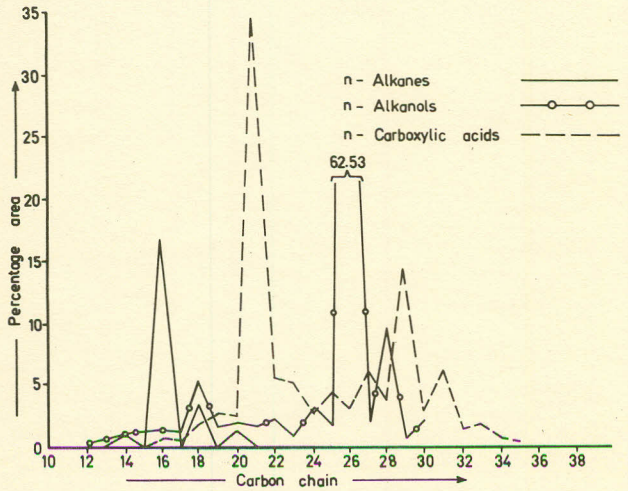

Fig. 1. The carbon NO-Distribution of *n*-alkanes, *n*-alkanols and *n*-carbozlic acids present in the oil of *Trigonella corniculata* seeds.

The sterol fraction (TLC and Liebermann Burchard test) constitutes a high percentage of the unsaponifiable (38.75%). It is white crystalline material but with no sharp melting point (90-138°) indicating it as a mixture. A part of the fraction was acetylated and analysed by gas liquid chromatography when it resolved into five components with retention times ranging from 33.75 to 55.79 mints (Table 3). These limiting values correspond to standard cholestryl acetate and β sitesterol acetate. The presence of these compounds in the acetylated sterol fraction was further confirmed by the coinjection of standard samples.

Repeated crystallisation of sterol fraction resulted in deposition of β-sitosterol, a common characteristic in plant kingdom. Isolation of any other constituent could not be attained due to the formation of mixed crystals, a phenomenon common in steroids.

Table 3. The retention times and percentage distribution of the components in sterol fraction of the unsaponifiable.

Retention time in minutes	% age areas under peak	Identified as
33.35	6.69	Cholesterol
36.00	6.08	Unidentified
43.07	12.59	"
46.40	10.81	"
55.79	63.83	β-sitosterol

Among the sterols, β-sitosterol forms major portion (63.83%). This phenomenon is common in many oils and does not disqualify an oil for human consumption. However cholestrol occuring in the plant kingdom [14] and so also in *Trigonella foenum* Graecum [9] is not much desirable for human consumption.

The material eluted by methanolchloroform is highly oxygenated as indicated by its IR spectrum. Further work on the separation and identification of this fraction is in progress.

Acknowledgement. The authors are grateful to Dr. F.M. Chaudhry of PCSIR Laboratories, Lahore for carrying out the GLC of fatty acids and Mrs. Dr. Chuang Ling Chong of the Laramie Energy Technology Centre, Laramie, Wyo. U.S.A. for the analysis of hydrocarbon fraction.

REFERENCES

1. I.C. Chopra, S.L. Nayar & K.L. Handa, Glossary of Indian Medicinal Plants (Council of Sci. Ind. Res., New Delhi, 1956) p.81.
2. C.K. Atal and S.P. Sood, J. Pharm. & Pharmacol., **16**, 627 (1964).
3. I.P. Varshney and A.R. Sood, J.Ind.Chem.Soc., **46**, 391 (1969).
4. I.P. Varshney and A.R. Sood, Ind.J.Appl.Chem.,**34**, 208 (1971).
5. T.R. Seshadri, A.R. Sood and I.P. Varshney, Ind. J. Chemistry, **10**, 26 (1972).
6. I.P. Varshney, A.R.Sood, H.C. Srivastava & S.N. Harshe, Planta.Medica, **26**, 26 (1974).

7. F.R.Y. Fazli & R. R. Hardman, Tropical Sci., **10**, 66 (1968).
8. Wunsghendorff, J. Pharm. Chim., **9** 345 (C.A. **8**, 2601).
9. F.R.Y. Fazli and R. Hardman, Phytochemistry, **10**, 2497 (1974).
10. M. Shahat, Proc. 11th Intern. Congr. Pure and Appl. Chem. (London), **3**, 569 (1947), (C.A. **45** 9896 a.)
11. A.M. Leone, E.L. Nottee and M. Vitagilane, Ital. Sostance Grasse, **54**, 310 (1977); (C.A. **88** 36035 W.)
12. B. Flaschentrager and M.H. Aboulwafa, Fette Seifen Anstrichmittel, **60**, 184 (1958); (C.A. **52** 14198 d.)
13. M. Bastic, L. J. Bastic, J. A. Jovanovic and G spitller, J. Am Oil Chem Soc., **65**, 886 (1978).
14. A.A. Ismail, W.M. Shawki and S.A. Hamza, Egypt. J. Hortic., **5**, 83 (1978).