

## NOTES

**Analysis of the Fixed Oil from the Stem of  
*Crotalaria Verrucosa***

R. N. YADAVA\* AND SUNU. R. MATHEWS

*Department of Chemistry  
Dr. H. S. Gour University  
Sagar-470 003, India,*

In the present work, the authors have analysed the fixed oil extracted from the stem of *Crotalaria Verrucosa*.

*Crotalaria Verrucosa*<sup>1-2</sup> (*N.O. Leguminosae*) is commonly known as *Banshana* in Hindi. It is found in the tropical regions of India from Himalayas to Ceylon. According to the Ayurvedic system of medicine, the leaves of the plant are used in the treatment of Kapha biliousness, dyspepsia, fever, blood impurities, throat and mouth diseases and heart ailments. Juice of the leaves is also used in scabies and impetigo both internally and externally.

About 3 kgm of air dried and finely powdered stem of *Crotalaria Verrucosa* was extracted with petroleum ether in a soxhlet extractor for 4 days. After removal of the solvent under reduced pressure, the petroleum ether extract was left in a refrigerator overnight. A light yellow coloured deposit was observed at the bottom of the flask. It was removed by filtration (yield ca 3.2%) and was found to have the following physico-chemical constants, colour-light yellow, sp. gr. at 30°C-0.845, Ref. index.  $n_D^{28} = 1.5760$ , acid value-4.60; iodine value-70.0; saponification value-170.42; unsaponifiable matter 2.4%.

The fat thus obtained from the petroleum ether extract was saponified with alcoholic potash for about 4 hrs. The excess of alcohol was distilled off and the soap formed was cooled and dissolved in water. The soap solution along with ether was transferred to a separating funnel and shaken thoroughly in order to remove the unsaponifiable matter.

**Study of the Unsaponifiable Matter**

The unsaponifiable matter was studied by TLC, when two spots were obtained, indicating the presence of two compounds A and B. They were separated by column chromatography and their purity were confirmed by TLC.

**Compound A-( $\beta$ -sitosterol)**

It crystallises from a mixture of chloroform : methanol (1 : 1). It was analysed for mol. formula  $C_{29}H_{50}O$ , Calcd C = 83.98%, H = 12.14%, found C = 83.68%, H = 12.07%, m. pt. 135-6°C, molecular ion peak at  $m/e = 412$ .

It gave positive salkowski reaction and positive Liebermann-Burchard reaction. IR peaks were obtained at  $\nu_{\max}^{\text{KBR}}$  1380, 1465, 1639, 2855, 2964, 3333, 1020, 957, 845 and 800  $\text{cm}^{-1}$ . Its identity was confirmed by mixed m.pt. determination with authentic sample.

### Compound B (Taraxerol)

It crystallises from ether as rhomboidal crystals; on analysis, it gave C = 84.39%, H = 11.80%; calculated for mol. formula  $\text{C}_{30}\text{H}_{50}\text{O}$ : C=84.50%, H=11.13%, m.pt. 280°C. On treatment with acetic anhydride and pyridine, it formed an acetate derivative, m.pt. and mixed m.pt. 304–305°C. Its identity was further confirmed by Co. TLC with authentic sample.

### Study of the fatty acids

The fatty acids were liberated from soap solution by the addition of conc.  $\text{H}_2\text{SO}_4$  and extracted with ether. The excess of acid was removed by washing the ethereal extract with water and then dried over anhydrous sodium sulphate. The ethereal extract was distilled to obtain the mixed fatty acids, which were separated into solid and liquid fatty acids by Twitchells lead salt alcohol<sup>3</sup> process as modified by Hilditch and co-workers<sup>4</sup>.

The observations and results are tabulated below :

TABLE 1

S. No.	Fraction	%	Acid value	Saponification value	Iodine value
1	Solid	72.3	4.3	223.52	—
2	Liquid	26.7	5.2	192.14	185.7

The solid and liquid fatty acids were studied by paper chromatography<sup>5</sup> on Whatman No. 1 filter paper using the following solvent system:

- (1) Methanol : petroleum ether (4 : 1)
- (2) 75% ethanol
- (3) Methanol : acetic acid : Petroleum ether (30 : 1 : 7)

The observations and results are given below:

TABLE 2

S. No.	Liquid fatty acid	Solvent System I	
		$R_f$ calculated	$R_f$ observed
1.	Ricinoleic acid	0.85	0.83
2.	Linoleic acid	0.55	0.56
3.	Oleic acid	0.52	0.51
4.	Linolenic acid	0.65	0.64

TABLE 3

S. No.	Solid fatty acid	Solvent System II		Solvent System III	
		R <sub>f</sub> Calculated	R <sub>f</sub> Observed	R <sub>f</sub> Calculated	R <sub>f</sub> Observed
1.	Stearic acid	0.28	0.26	0.25	0.24
2.	Arachidic acid	0.29	0.27	0.26	0.25
3.	Palmitic acid	0.39	0.38	0.35	0.36
4.	Myristic acid	0.58	0.56	0.48	0.47
5.	Lauric acid	0.80	0.79	0.63	0.62

The mixed fatty acids were converted to their methyl esters by refluxing with anhydrous methanol containing conc. H<sub>2</sub>SO<sub>4</sub>.<sup>6</sup> A quantitative examination of the fatty acids was done by gas chromatography using their methyl esters on AMIL NUCON Gas Chromatograph equipped with stainless steel column (4 length and 1/8 diameter) of 36%, SE-30° on chromosob W (80 mesh). The other conditions are mentioned below:

1. Column temperature — 80–180°C
2. Injection temperature — 250°C
3. Detector — FID
4. Detector temperature — 280°C
5. Carrier gas — Nitrogen
6. Rate of flow — 36 ml/min.
7. Chart speed — 10 mm/min.

The peaks were identified by comparing the retention values of peaks with those of pure components and their quantitative studies was done by calculating the various signal areas and their results are recorded as follows:

TABLE 4

S. No.	Fatty acid	% composition of the fatty acid
1.	Linoleic acid	5.5
2.	Palmitic acid	10.7
3.	Stearic acid	13.1
4.	Lauric acid	14.5
5.	Oleic acid	11.8
6.	Linolenic acid	8.2
7.	Arachidic acid	18.1
8.	Myristic acid	18.9
9.	Ricinoleic acid	12.2

**REFERENCES**

1. R. N. Chopra, S. L. Nayar and I. C. Chopra, *Glossary of Indian Medicinal Plants* CSIR Publication, New Delhi, p. 81 (1956).
2. *Wealth of India (A Dictionary of Raw Materials and Industrial Products)*, Vol. II, CSIR Publication, New Delhi, p. 372 (1950).
3. E. Twitchells, *Ind. Eng. Chem.*, **13**, 806 (1921).
4. T. P. Hilditch, *Chemical Constituents of Natural Fat*, 3rd Ed., Chapman and Hall, p. 577 (1956).
5. J. Block Richard, L. Durram Emmeld and Z. Funier, *Paper Chromatography and Paper Electrophoresis*, 2nd Ed., Academic Press Inc., New York, pp. 240-245 (1958).
6. A. Chalvardgian, *Biochem. J.*, **90**, 518 (1964).

[Received: 1 January 1992; Accepted: 19 March 1992]

AJC-422