Pakistan J. Sci. Ind. Res., Vol. 13 No. 4, December 1970

STUDIES ON THE SEED OILS OF WITHANIA COAGULANS AND THEVETIA NERIFOLIA

Part I.—Fatty Acid Composition

ABDUL SALAM and M.A. WAHID

P.C.S.I.R. Laboratories, Karachi 39

S.S. ALI

Department of Biochemistry, University of Karachi, Karachi 32

(Received September 1, 1969; revised January 7, 1970)

The seed oils of *Withania coagulans* Dunal and *Thevetia nerifolia* Juss on GLC analysis, have been shown to contain 16:0, 11.64 and 31.25; 18:0, 2.66 and 4.99; 20:0, 3.49 and 4.18; 18:1, 12.56 and 28.76 and 18:2, 69.65 and 29.25% respectively. Infrared spectra of the two oils indicated the absence of any unusual characteristics.

The seed oils of the two commonly occurring plants viz. *W. coagulans* and *T. nerifolia* which belong to the Solanaceae and Apocyanaceae families respectively were studied for their fatty acid composition.

The fruit of *W. coagulans* has been used in the Indo-Pakistan subcontinent for quite a long time for making cheese.[1–4] Due to religious bias cheese made with animal rennet is generally not acceptable to the people of this region. Therefore, attempts have been made in these Laboratories for the commercial preparation of a vegetable rennet.[5] While making the rennet, it has been found that the berries of this plant contain about 35% hard seeds which go waste. In order to economise this enzymatic preparation and in view of the importance of vegetable oils it was considered worthwhile to study the nature and composition of the oil from this source.

The seeds of *Thevetia nerifolia*, which is commonly cultivated as an ornamental shrub in the gardens in plains, were also studied for their fatty acid composition. The seeds of this plant have been used for suicidal and homicidal purposes and as an abortifaceant by women in Bengal and the neighbouring provinces. The oil has also been used externally in skin disease. De Vry Tijdschr[6] has obtained from the kernel of the seeds 57% of an oil with a density of 0.9148 at 25°C. Jean Gattefosse has reported 58% yield of the oil. In the present investigation 55% yield of the oil has been recorded. Whereas the oil has been used as a purgative, alcoholic extracts of the seed have been reported as effective contact insecticides.[7]

Experimental and Results

Material.—The berries of *W. coagulans* were purchased from the local market. They were soaked in water for ½ hr and the swollen berries were then crushed by hand and filtered through muslin cloth. The seeds were washed several times with water to free them from adhering matter, dried and ground into a coarse powder. The seeds of *T. nerifolia* were handpicked from a plant in a local garden in December and the outer coat was removed mechanically. The kernal was crushed and used for oil extraction.

Extraction of Oil.—The coarsely ground seeds of *W. coagulans and T. nerifolia* (200 g and 60 g respectively) were extracted separately with petroleum ether (b.p. 60–80°C) using a Soxhlet extractor till no oil was being extracted. The extracts were dried over anhydrous sodium sulphate and then filtered. The solvent was removed under reduced pressure and the residue was dried at 75–80°C for ½ hr and weighed (30 g; 33 g; i.e. 15% and 55% respectively).

Transmethylation of Oils.—The transmethylation was carried out by the method of Hammond and Lundberg with some modification.[10] The oils were weighed (0.0372 g and 0.0296 g respectively) and applied separately in the form of a band using petroleum ether (b.p. 40–60°C) as solvent on activated silica thin layer glass plates. The plates were prepared by spreading 30 g silica gel for TLC in 60 ml H_2O for five plates, and activated for ½ hr at 80°C. The bands were scraped off and transferred to two different transmethylation tubes with Teflon stoppers. Methanolic sulphuric acid (20%, 1 ml) was added in each tube which were placed in an oven at 80°C for 2 hr. The tubes were taken out and allowed to cool to room temperature. Petroleum ether Analar (b.p. 40–60°C, 2–3 ml) was added to each tube followed by 1 ml water. The contents of each tube were shaken vigorously to extract the transmethylated

product with petroleum ether. The etherial layer was drawn from above by a dropper into a small tube. Three such extractions were made and combined and the solvent was finally removed. The transmethylated samples were transferred into small fusion tubes and stored in a refrigerator.

Examination of Saturated and Unsaturated Acids by GLC.—The analysis was made on a Varian Aerograph model GLC apparatus equipped with a flame ionization detector. The methyl esters of the acids were injected into a stainless steel column (5 ft 10 in×0.8 in; 20% DEGS coated on 60–100 mesh with chromosorb W having column temperature 187°C, nitrogen flow rate 25 ml/min, hydrogen flow rate 20 ml/min, chart speed 0.5 in/min, flash heater 250°C. The fatty acid compositions of both the oils obtained by this method are listed in Table 1.

TABLE 1.—FATTY ACID COMPOSITION OF OILS.

Acid		*W. coagulans* %	*T. nerifolia* %
Palmitic	16:0	11.64	31.25
Palmitoleic	16:1	Trace	Trace
Stearic	18:0	2.66	4.99
Oleic	18:1	12.56	28.76
Linoleic	18:2	69.65	29.25
Linolenic	18:3	—	1.71
Arachidic	20:0	3.49	4.18

Properties of the Oils.—The physicochemical characteristics of the oils were determined by the usual methods[11] and are given in Table 2.

TABLE 2.—PHYSICOCHEMICAL CHARACTERISTICS OF THE SEED OILS.

	W. coagulans	*T. nerifolia*
Yield	15%	55%
Density at 33°	0.9062	0.8983
Sp gr at 33°	0.907	0.899
Viscosity at 33°	31.06 cs	36.02 cs
Ref index at 31°	1.4740	1.4635
Iodine No.	140	1982
Saponification No.	194.63	99–201
Acid No	19	
Freezing point	−16°C	+0.5°C

Special Characteristics of the Oils.—The thin film IR spectra of both the oils were more or less identical and showed strong carbonyl absorption band at about 1725 cm^{-1} and another strong band at 1150 cm^{-1} or in the region 3300–3500 cm^{-1}. They did not exhibit any absorption at 960 cm^{-1}.

The oils are also being studied for their nutritional value and toxicity, the details of which will be reported later. Bland oils were fed separately to albino male rats weighing 160–200 g. Untoward side effects were not noticed in a dosage of 3 ml/kg body weight. However, a little sedation was observed which wore off in about 15 min.

Discussion

The seed oils were analysed for their fatty acid composition by using gas liquid phase chromatographic technique, as this procedure is comparatively more simple and accurate than other traditional methods. The identity and percentages of the various fatty acids were determined from the retention time and peak areas of their respective methyl esters. The results show (Tables 1 and 2) that both the oils are highly unsaturated. The high percentage of unsaturated acids (*W. coagulans*, 82%; *T. nerifolia*, 59.5%) in the oils suggests that they could be a good substitute for an unsaturated oil. The high iodine value, 140 and low freezing point −16°C (Table 2) of the former oil is also in favour of this suggestion. It is interesting to note that the former seed oil possesses a very high percentage of linoleic acid (69.6%), only traces of palmitoleic acid and no linolenic acid while the oil from the latter source contains 29.2% linoleic acid, 1.71% linolenic acid, and only traces of palmitoleic acid. De Vry Tijdschr[6] has reported 57% seed oil from *T. nerifolia* with a solidification point 13°C while in the present investigation the yield is 55% and the freezing point is 0.5°C only. This change in the yield of the oil and its freezing point may be attributed to seasonal and climatic variation as has also been noticed by Quilichini and Bertucat.[8]

The presence of strong carbonyl absorption band at about 1725 cm^{-1} in the thin film IR spectra of both the oils indicates that the fatty acids of the oils do not contain any unusual substituents. Moreover, the number of carbon atoms of both saturated and unsaturated fatty acids is between C_{14}–C_{21}. [12–14] The existence of another strong band at 1150 cm^{-1} reveals the presence of a large number of even numbered carbon atom fatty acids. This observation is in agreement with the findings of O'Conner *et al.*[15] The absence of –OH group absorption in the region 3300–3500 cm^{-1} is in agreement with the GLC data (Table 1). The oils did not show any absorption at about 970 cm^{-1} in the IR spectra indicating the absence of *trans* acids.[16]

The oil from the seeds of *W. coagulans* seems to be quite useful due to the following reasons. The high content of linoleic acid, low amount of sa-

turated acids, high iodine value and absence of linolenic acid constitute an oil which forms drying, non-yellowing films that have a very good through dry and low wrinkling characteristics.[17] Since the oil is non-toxic and rich in polyunsaturated acids like linoleic acid, it can be classified as an oil containing essential fatty acids which prevent atherosclerosis and high cholesterol accumulation or synthesis.[18–19]

Acknowledgement.—The authors wish to thank Dr. A. Q. S. Bukhari and Mr. Qamar Khalid for their assistance. Thanks are also due to the Director, P.C.S.I.R. Laboratories, Karachi, for his permission to publish this paper.

References

1. Z.R. Kothavalla and P.G. Khubchandani, Indian J. Vet. Sci., **10,** 284 (1940).
2. K.M. Yeshoda, Current Sci., **10,** 23 (1941).
3. R. Narain and A. Singh, D.S.A., **5,** 65 (1943)
4. N.D. Dastur, K.N. Sham Sastry and D. Venkatappiah, Indian J. Vet. Sci., **18,** 233 (1948).
5. R.B. Qadri, M.A. Wahid, A.H. Khan, A.F.M. Ehteshamuddin and A.K. Qureshi, Sci. Ind. (In Press).
6. De Vry Tijdschr, in *A Dictionary of the Economic Products of India* (W.H. Allen, London, 1893), p. 47.
7. Jean Gattefosse, Bull. Soc. Sci. Nat. Morac., 25/26/27, **66** (1945-46-47, Pub. 1949).
8. R. Quilichini and M. Bertucat, Bull. Soc. Pharm. Boardeaux, **95,** 61 (1956).
9. F.R. Earle, *et al.*, J. Am. Oil Chem. Soc., **37,** 440 (1960).
10. E.G. Hammond and W.O. Lundberg, J. Am. Oil Chem. Soc., 438-441 (1953).
11. K.A. Williams, *Oils, Fats and Fatty Acids* (Churchill, London, 1950), pp. 107.
12. M. Flett, C. St. J. Chem. Soc., **962** (1951).
13. J.P. Corisch, and W.H.T. Davison, J. Chem. Soc., 6005 (1955).
14. P. J. Corish, and D.J. Champan, Chem. Soc., 1746 (1957).
15. R.T. O'Conner, E.T. Field, and W.S. Singleton, J. Am. Oil Chem. Soc., **28,** 154 (1951).
16. J. Devine, and P.N. Williams, *The Chemistry and Technology of Edible Oils and Fats* (Pergman, Oxford, 1961), p. 145.
17. J.A. Kneeland, J. Am. Oil Chem. Soc., **43,** 403 (1966).
18. J.F. Mead, D.R. Howton, *Essential Fatty Acids* (Academic Press, New York, 1958).
19. H. Thomson, Intern. Rev. Vitamins Res., **25,** 62 (1953).