Studies on Some Chemical Aspects of Kusum Oil

M. K. KUNDU and C. BANDYOPADHYAY,
Department of Applied Chemistry, Calcutta University, Calcutta, India

Abstract

Chemical methods, chromatography and infrared spectroscopy have been applied to ascertain the location and nature of the cyanogenic compounds in kusum oil. Observations indicate the cyanogenic compounds to be a part of glyceride molecules in which one of the hydroxyl groups of the latter is bonded to the cyanogenic compound through an ether linkage. Chromatographic behavior of the isolated cyanogenic compounds further indicates that at least two glyceride molecules are involved. These glycerides are predominantly esterified with saturated fatty acids.

Introduction

Kusum oil is obtained from the kernel of the species *Schleichera trijuga* belonging to the family Sapindaceae. The species is a large tree growing widely in the sub-Himalayan region, throughout central and southern India, Burma, Ceylon, Java and Timor. Prior to the development of new synthetic products the tree was noted as the host tree of the best grade of lac. Preliminary studies have been made on the oils and oilseeds of *S. trijuga* in our laboratory (1).

The oil is essentially an oleic-rich oil containing about 56–62% oleic acid (2,3,7). The component fatty acids of the oil differ widely, ranging from acetic (2,5,6) to arachidic. Notable features of the oil are relatively high content of arachidic acid (ca. 25%) (2–4,7), remarkably low content of linoleic acid (within 5%) and the characteristic presence of a cyanogenic compound in a concentration of 0.03–0.05% as HCN (6,8). The exact location of the cyanogenic compound in the oil or its exact nature has not been reported in the literature. The compound has been suspected to be in the form of a cyanogenic glucoside (7) or an acid amide (9). The present investigation has been undertaken to study on the location and nature of the cyanogenic compound in kusum oil.

Chemical Investigations

Studies on the Location and Nature of the Cyanogenic Compound

Steam Distillation. It was carried out with oil-water (1:30–40 w/v) with and without hydrochloric acid and mercuric chloride (10,11,13).

Refining. (a) The oil was refined with ca. 20 Bé sodium hydroxide in *n*-hexane as solvent; (b) treated with adsorbents known to have affinity for cyanogenic compounds such as freshly prepared ferrous and ferric hydroxide and filtered; (c) also treated with acetic anhydride (14) and with H_3PO_4 + NaOH (12) and filtered in each case; and (d) the crushed kusum seeds were first extracted with methanol and then with petroleum ether (40–60 C). The solvents were removed from both extracts.

Acid Digestion. The oil was treated with hydriodic acid of sp gr 1.7 (oil-HI, 1:19 w/v) in the presence of nitrogen at ca. 150 C for 5 hr.

The oil was also digested with hydrochloric acid at temperature ranging from 80 to 190 C using oil-HCl ratios varying from 2:1 to 3:10 (w/v). The reaction was carried out in a round bottom flask fitted with an air condenser and oil bath.

The residual oil after treatment by steam distillation and refining responded to Prussian blue test for cyanide ion. The residue on filtration of the treated oil in methods a, b and c of refining did not give positive test for cyanide ion. Also it was petroleum ether extract and not methanol extract (as obtained in d) that responded to test for cyanide ion. The digestion with hydriodic acid and with hydrochloric acid up to certain concentrations completely destroyed the cyanogenic linkage. The results of hydrochloric acid digestion at varying concentration, time and temperature are recorded in Table I.

Several interesting observations were made from the hydrochloric acid digestion procedure. The hydrochloric acid treated oil was extracted with petroleum ether, the ether extract washed with water and the clear wash water thus obtained was nearly neutralized with sodium carbonate. The aqueous portion, (a) with iodine and caustic soda produced a fine yellow precipitate with characteristic odor of iodoform, melting at 119 C (with decomposition); this indicates the presence of CH_3CO- or a group such as $CH_3CH(OH)-$ convertible to CH_3CO- grouping (15), (b) developed a reddish-violet coloration with freshly prepared ferric chloride solution; this might be due to the presence of the grouping $-C=C<$ (15), (c) formed a brown precipitate with Nessler's reagent indicative of the presence of NH_4^+ ion, (d) responded to test for oxalic acid (16). (e) immediately decolorized a cold, very dilute

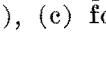

TABLE I

Effect of Digestion of Crude Kusum Oil with Hydrochloric Acid

Sample	Oil: HCl(12N) (w/v)	Temp. of digestion °C	Hr of digestion	Test for cyanide ion	Iodine values	Acid values	Slip points °C
Crude kusum oil	1:0	.....	.....	Positive	63	35.5	32
HCl-treated kusum oil							
I	2:1	90–110	6–8	Positive (faint)	61.5	104.6	43.5
II	1:1	80–100	7	Positive (faint)	57	118.2	43.5
III	1:1 (6N HCl)	100	8–10	Positive (faint)	59	131.3	45.0
IV	1:2	100	8–10	Positive (faint)	62	100.5	44.5
V	1:2	100–140	8–10	Positive (faint)	63.4	128.2	.....
VI	2:5	120–160	7	Negative	58	125	43
VII	2:5	150–155	6	Negative	63	132	44
VIII	1:3	170–190	6	Negative	61	147	46
IX	3:10	110–130	7	Negative	61	130	43
X	3:10	130–150	6	Positive (very faint)	63	138	44.5

solution of ethanolic bromine. Decolorization is probably attributable to carbon to carbon unsaturation, (f) did not respond to test for –CN ion. This might be due to –CN group being eliminated during hydrochloric acid digestion of the oil.

Chromatographic Investigations

Location of the Cyanogenic Compound by TLC

The crude oil was fractionated into classes by chromatography on silica gel G plates (0.8 mm) using the solvent system *n*-hexane-diethyl ether-acetic acid (17) but differing in ratio by 75:25:1 (v/v). The bands were located by exposure to iodine vapor, scraped off into 100 ml stoppered Erlenmeyer flasks, extracted with warm chloroform and filtered. The solvent was then removed and the residual mass from each fraction treated with 1N aq NaOH solution to convert the cyanogenic part to sodium cyanide, acidified with $9NH_2SO_4$ in the cold and the vapor evolved allowed to come in contact with a piece of filter paper soaked with copper acetate-benzidine acetate reagent (18). The vapor from the triglyceride fraction of the chromatogram alone turned the exposed zone of the filter paper blue, indicating the presence of the cyanogenic compound in the triglyceride fraction. The chromatogram is shown in Figure 1.

Thin-layer chromatography of crude kusum oil on silica gel G was also carried out using the solvent system $CHCl_3$:MeOH:H_2O (65:25:4 v/v) according to Wagner's procedure (19). Again it is the triglyceride fraction alone that showed the presence of cyanide ion.

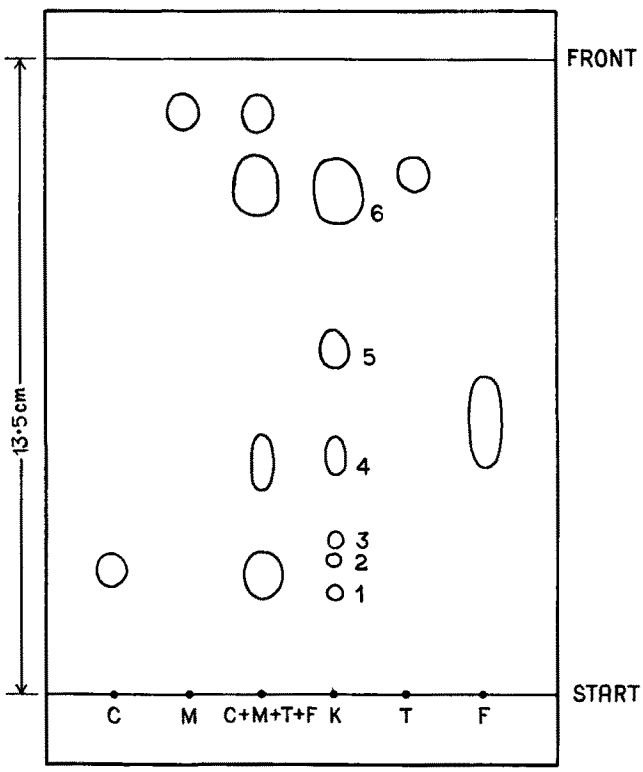

FIG. 1. TLC on silica gel G of crude kusum oil (K), cholesterol (C), methyl oleate (M), tristearin (T), and fatty acids (F). Developing solvent: *n*-hexane-diethyl ether-acetic acid (75:25:1 v/v). Spots were visualized by spraying with 50% H_2SO_4 and charring and were reproduced by tracing. The 6th component (the triglyceride fraction) gave positive test for cyanide ion by copper acetate-benzidine acetate reagent.

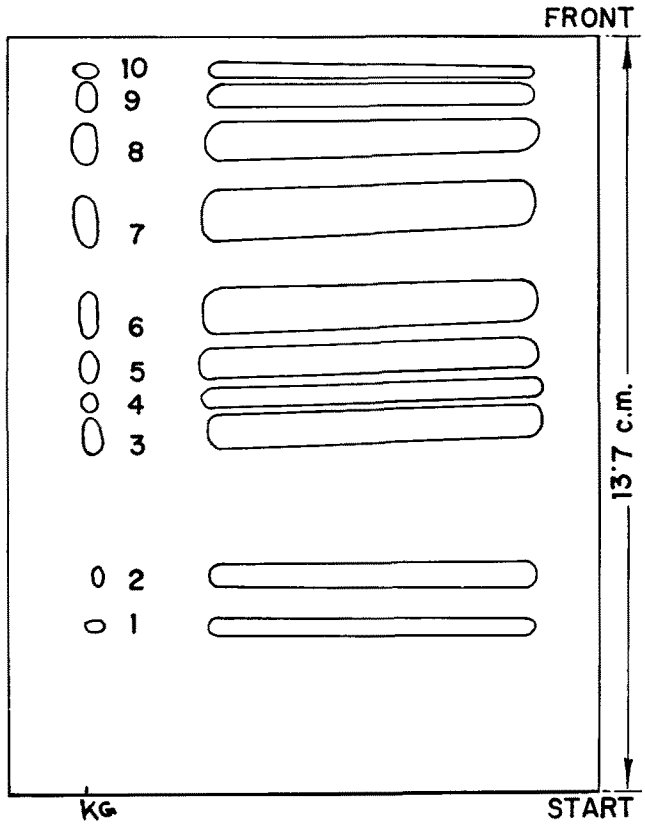

FIG. 2. TLC of the glyceride fraction (the 6th component, KG, from Fig. 1) on silica gel G impregnated with silver nitrate. Developing solvent: chloroform-acetic acid (100:0.5 v/v). Spots were visualized by spraying with 0.2% solution of 2′,7′ dichlorofluorescein in 95% ethanol, followed by exposure under UV light and were reproduced by tracing.

Isolation of the Cyanogenic Compound by Reversed Phase Column Chromatography

The procedure adopted was essentially that of Silk and Hahn (20) with minor variations in the solvent system employed. The stationary phase was Hyflo Supercel impregnated with liquid paraffin (BP) and the eluting solvents were varying proportions of acetone (CP) in methanol (AR), saturated with liquid paraffin. The ratios (v/v) of acetone-methanol used were in the order 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 100:0.

At the end of the operation, each of the fractions was monitored with the help of reversed phase TLC on kieselguhr G plate (21) and compared with the spot position of the kusum oil run side by side. Fractions having identical spot positions were combined and tested for cyanide ion by copper acetate-benzidine acetate reagent. Two fractions eluted with the solvent ratios of acetone-methanol (30:70 and 40:60) and corresponding to the spot numbers 9 and 10 of kusum oil responded to test for cyanide ion. The chromatogram is presented in Figure 3.

Removal of Liquid Paraffin From Eluates (Fractions 9 and 10) Containing Cyanogenic Compound(s)

Acetone and methanol were removed from the isolated cyanogenic compound(s) by distillation under vacuum. The liquid paraffin was next removed by adding a concentrated solution of the sample in petroleum ether to a small column (1.2 × 8.8 cm) of silica gel (BDH), followed by eluting with 50 ml petroleum ether (40–60 C). Finally the paraffin-free cyanogenic compounds were eluted with 50 ml dry, distilled diethyl ether (CP). The ether was removed under vacuum.

Tests With Isolated Cyanogenic Compound(s)

TLC on Silica Gel G as Previously Described. The spot position of the compound coincided with that for triglycerides.

Reversed Phase TLC on Kieselguhr G. As already stated, the corresponding spot numbers of kusum oil were 9 and 10.

TLC on silver nitrate impregnated silica gel G. (22). 100 γ each of 1% solution of the fractions 9 and 10 in chloroform was applied on silica gel G plate (0.5 mm) impregnated with silver nitrate and then developed using the solvent system chloroform-acetic acid (100:0.5 v/v). An equal amount of the triglyceride fraction of kusum oil separated by TLC on silica gel plate as described earlier was run side by side. The spots were visualized by spraying with a 0.2% solution of 2′, 7′ dichlorofluorescein in 95% ethanol, followed by exposure under UV light. The cyanogenic fractions were found to coincide with the spot position 8 and 9 of kusum glycerides. The chromatogram is shown in Figure 2.

Test for Glycerol. The compound developed the characteristic coloration for glycerol by the chromotropic acid color reaction of Van Handel and Zilversmit (23). The presence of glycerol was proved by TLC of the compound on air-dried silica gel plate using the solvent system, chloroform-acetone-5N ammonia (10:80:10 v/v) (24). The cyanogenic compound was applied as follows: The compound(s), fractions 9 and 10, was microsaponified with 2N methanolic sodium hydroxide solution till a clear solution was obtained. The solution was then cooled and applied in the form of spots on the plate. One per cent ethanolic solution each of sodium palmitate, ethylene glycol, glycerol, and 1% aqueous ethanolic solution of glucose were run side by side for comparison. The chromatogram is shown in Figure 5. The chromatogram further indicates the absence of glucose in the compound(s), clearly proving that the cyanogenic compound is not a glucoside.

Fatty Acid Composition. The fatty acids were liberated from the fractions 9 and 10 by microsaponification followed by acidification and then identified by partition chromatography on kieselguhr G plate coated with 5% liquid paraffin in petroleum ether, using 90% paraffin-saturated acetic acid as the developing solvent (25). Spots were located by exposure to iodine vapor and subsequent spraying with 2% solution of starch in 20% ethanol.

The fractions were found to contain predominantly saturated fatty acids composed mainly of arachidic, stearic and palmitic and a minor amount of oleic acid. Oleic acid was resolved by running a two dimensional chromatogram according to the procedure of Kaufmann et al. (26).

Infrared Analysis. The IR spectra of the compound were determined as thin film of liquid, using sodium chloride cell. The spectra indicate the presence of the following groups: nitrile (–CN), ether (C–O–C), ester (–COOR) and olefinic (>C = C<). The presence of sulfur or phosphorus is not indicated. The spectra are shown in Figure 4.

Elemental Analysis. The nitrogen content of the compound determined by micro Dumas method was found to be 1.9%.

Discussion

Steam distillation procedure forms the basis of a standard method (10) for estimating cyanogenic compound (as HCN) from cyanogenic glucoside in oils.

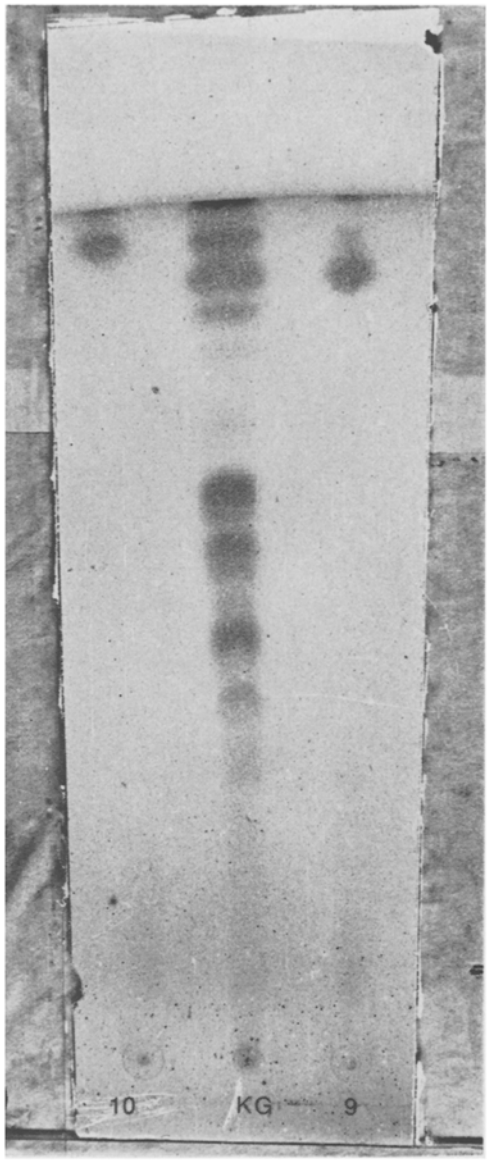

FIG. 3. TLC on liquid paraffin impregnated kieselguhr G layer of kusum oil (KG) and isolated pure cyanogenic compounds (9 and 10). Developing solvents:acetone-methanol (80:20 v/v), saturated with liquid paraffin. Spots were visualized by exposure to iodine vapor, followed by spraying with 2% solution of starch in 20% ethanol and were reproduced by direct photocopy. The spots have been numbered serially from base line upwards, base line being assumed zero.

This means that the procedure quantitatively removes the cyanide part from the cyanogenic glucoside. Again the refined oil but not the residue on filtration obtained in methods a, b and c of Refining gives positive test for cyanide ion. Also it is petroleum ether extract and not the methanol extract (as obtained in method d) that responded to test for cyanide ion. Thus the failure of steam distillation and refining to decyanide the oil precludes the possibility of the cyanogenic compound being present as free HCN or cyanogenic glucoside. Absence of glucoside in the isolated cyanogenic compound has been clearly demonstrated by TLC (Fig. 5). The isolated compound: (a) is chromatographically homogeneous (Fig. 3); (b) appears in the triglyceride region as shown by TLC on silica plate (Fig. 1); (c) indicates the presence of glycerol as confirmed by TLC on air dried silica gel plate (Fig. 5) as well as by response to test for glycerol by chromotropic acid color reaction

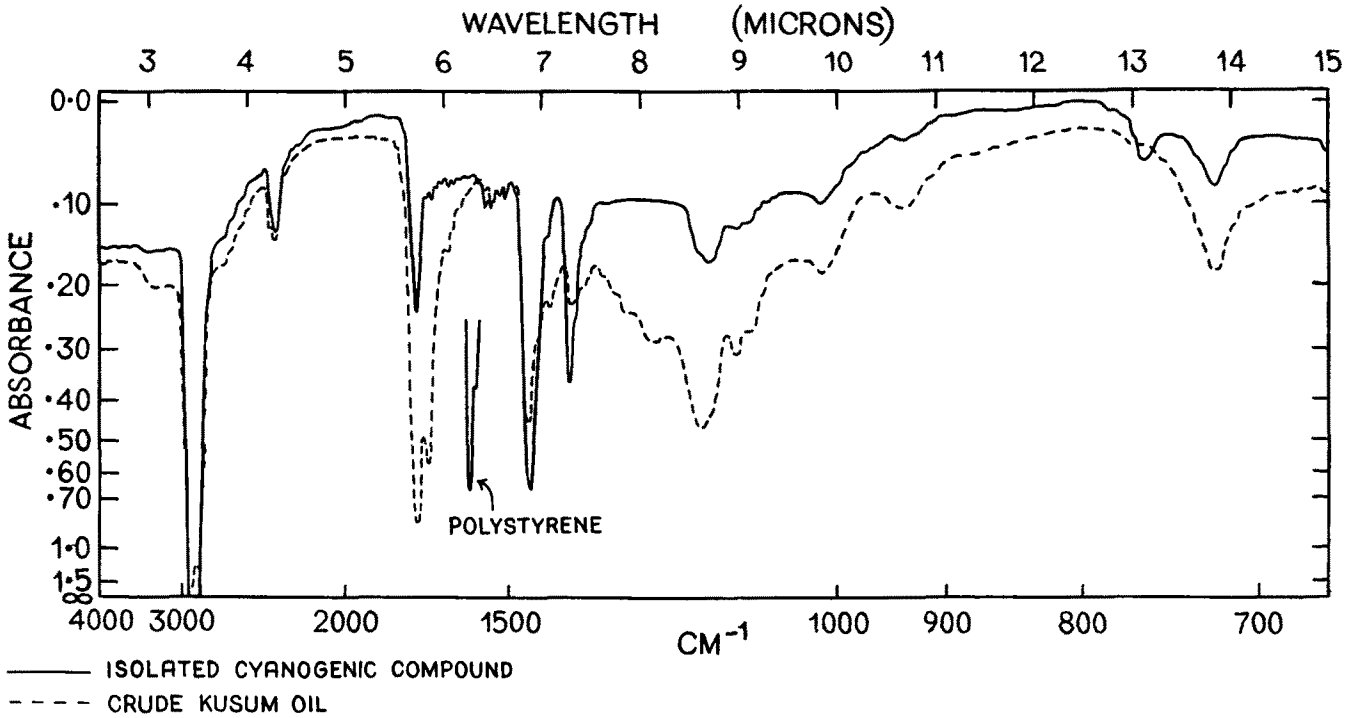

FIG. 4. IR spectra of isolated cyanogenic compound and kusum oil, determined as thin film of liquid using sodium chloride cell.

of Van Handel and Zilversmit; and (d) shows the presence of fatty acids.

These observations clearly indicate that the compound is a part of a glyceride molecule. The infrared spectra of the compound (Fig. 4) also support the conclusion that the cyano group is present in the glyceride. Qualitative tests further indicate that the compound does not contain sulfur or phosphorus.

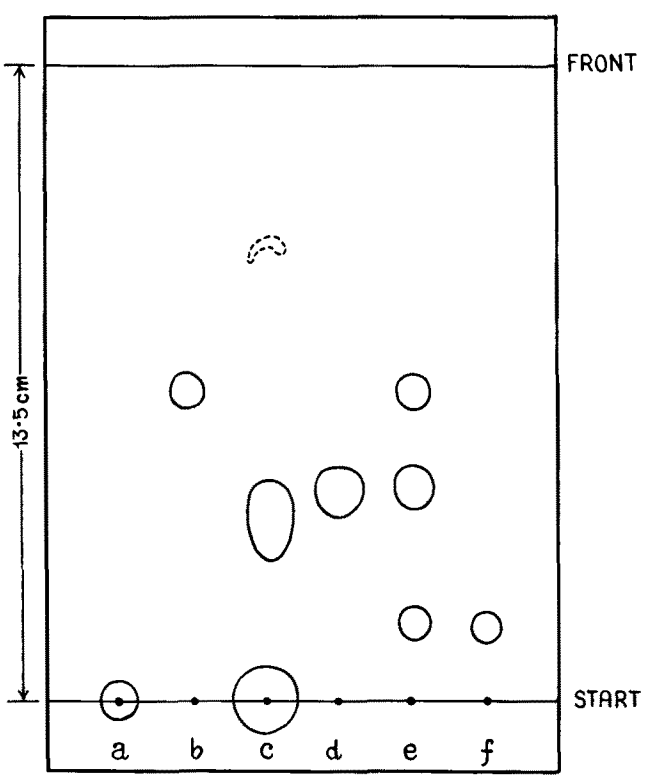

FIG. 5. TLC on air dried silica gel G plate of sodium palmitate (a), ethylene glycol (b), isolated cyanogenic glyceride (c), glycerol (d), glucose (f) and *b* + *d* + *f* (e). Developing solvent : chloroform-acetone-5N ammonia (10:80:10 v/v). Spots were visualized by spraying with 5% solution of potassium dichromate in 40% sulfuric acid.

The cyanogenic linkage in kusum glyceride is completely destroyed by hydriodic acid treatment carried out according to the Zeisel method for estimation of alkoxy group. This appears to indicate that the cyanogenic compound may have an ether linkage with glycerol, but the removal of this group by digestion with 12N hydrochloric acid implies that the linkage is not so stable as pure alkyl-oxygen-alkyl bonding. The IR analysis of the compound confirms the presence of the following groups: –CN, –C–O–C, $>C=C<$ and –COOR. The reduced intensity of the nitrile peak points to the possibility of some oxygenated group being linked to the same carbon atom as the nitrile (27). The cyanogenic moiety may indeed be visualized to be a small molecule of the type of pyruvonitrile and bonded to glyceride through etheric linkage. Such type of compound explains the observations made, namely, response to test for acetic acid, NH_4^+, CN^-, acid amide, development of reddish violet coloration with ferric chloride etc. It is also consistent with the experimentally determined value for the nitrogen content of the cyanogenic compound. Thus, for example, the experimental value for the nitrogen content of the cyanogenic compound is 1.9% as against the theoretical value of 2.07% nitrogen calculated on the basis that only one of the three hydroxyl groups of the compound is bonded to the cyanogenic moiety through etheric linkage, and the remaining two hydroxyl groups are esterified with C_{18} saturated fatty acid taken to be the mean fatty acid present in the molecule.

The presence of more than two fatty acids in the cyanogenic compound henceforth termed cyanatide indicates that it is linked to at least two glyceride molecules. This is also corroborated by reversed phase TLC on kieselguhr G plate when two spots having R_f's 0.90 and 0.95 corresponding to spot number 9 and 10, respectively, of kusum oil showed the presence of cyanide ion. The high R_f's of the cyanatides may

be ascribed to polarity of the cyanogenic part of the cyanatide molecule and to its reduced molecular weight which together more than counterbalance the dragging down effect of the predominantly saturated fatty acid constituents.

ACKNOWLEDGMENT

This research has been financed in part by University Grants Commission, New Delhi, India. The authors acknowledge the help and encouragement received from M. M. Chakrabarty, and D. Bhattacharyya and T. Guha for helpful suggestions.

REFERENCES

1. Koley, S. N., M. K. Kundu and A. N. Saha, Indian Oil Soap J. *30*, 321 (1965).
2. Dhingra, D. R., T. P. Hilditch and J. R. Vickery, J. Soc. Chem. Ind. *48*, 281T (1929).
3. Patel, S. M., Thesis, University of Bombay, 1930.
4. Weerakoon, A. H., Thesis, University of Liverpool, 1952.
5. Poleck, G., Pharma Centre *32*, 396 (1891).
6. Eckey, E. W., "Vegetable Fats and Oils" Reinhold Publishing Corp., New York, 1954, pp. 627–628.
7. Sengupta, N. N., J. Soc. Chem. Ind. *39*, 88T (1920).
8. Lewkowitsch, J., "Chemical Technology and Analysis of Oils, Fats and Waxes" Vol. 2, Macmillan & Co. Ltd., London, 1914, p. 553.
9. Datta, R. L., T. Basu and P. K. Ghosh, Indian Soap J. *16*, 71–73 (1950).
10. Fisher, H. J., A. H. Robertson and H. Reynolds, "Official Methods of Analysis of the Association of Official Agricultural Chemists" W. Horwitz, ed., 8th ed., Association of Official Agricultural Chemists, Washington, D.C., 1955, p. 380.
11. Winkler, W. O., J. Assoc. Offic. Agr. Chemists *34*, 541–548 (1951).
12. De Laval Separator Company, India 49006 (1953).
13. Williams, K. A., "Oils, Fats and Fatty Foods," 3rd ed. J. A. Churchill Ltd., London, 1950, p. 30.
14. Hayes, L. P., and H. Wolff, JAOCS *33*, 440 (1956).
15. Finar, I. L., "Organic Chemistry," 3rd ed., Vol. 1, Longmans, Green and Co. Ltd., London, 1959, pp. 151,202.
16. Rosenthaler, L., Bern Schwar Apoth. Ztg. *58*, 17–20 (1960); Chem. Abstr. *14*, 556 (1920).
17. Malins, D. C. and H. K. Mangold, JAOCS *37*, 576–578 (1960).
18. Vogel, A. I., "A Text Book of Macro and Seminicro Qualitative Inorganic Analysis," 4th ed., Longmans, Green & Co. Ltd., London W1, 1959, p. 343.
19. Wagner, H., Fette, Seifen, Anstrichmittel *62*, 1115 (1960).
20. Silk, M. H., and H. H. Hahn, Biochem J. *56*, 406 (1954).
21. Chakrabarty, M. M., D. Bhattacharyya and B. Mondal, Indian J. Technol. *1*, 473–474 (1963).
22. Barrett, C. B., M. S. J. Dallas and F. B. Padley, JAOCS *40*, 580 (1963).
23. Van Handel, E., and D. B. Zilversmit, J. Lab. Clin. Med. *50*, 152 (1957).
24. Bolliger, H. R., M. Brenner, H. Ganshirt, H. K. Mangold, H. Seiler, E. Stahl and D. Waldi, "Thin Layer Chromatography: A Laboratory Handbook," E. Stahl, ed., Academic Press Inc., New York, 1965, p. 357.
25. Kaufmann, H. P., Z. Makus and T. H. Khoe, Fette, Seifen, Anstrichmittel *63*, 689 (1961).
26. Kaufmann, H. P., Z. Makus and T. H. Khoe, Ibid. *64*, 1–5 (1962).
27. Bellamy, L. J., "The Infrared Spectra of Complex Molecules," 2nd ed., Methuen and Co. Ltd, London, 1958, p. 266.

[Received January 3, 1968]