Cupania anacardioides: A Rich Source of Cyanolipids

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Seed oil of *Cupania anacardioides* (Sapindaceae) was found to contain cyanolipid I and cyanolipid III at 41.3% and 11.7%, respectively. Structures of these cyanolipid fractions were confirmed by spectroscopic methods.

The growing use of modern methods of lipid analysis is expected to reveal new glyceride type compounds from natural sources. It is being realized that among the glyceride compounds, the occurrence of cyanolipids is more interesting in the seed oils. Cyanolipids are a class of natural lipids which are found, often in copious amounts, in the Sapindaceae seed oils (with few exceptions) and play an important role in the biochemistry of these plants. Keeping in view the unique structural features of nitrile-containing lipids, it was considered desirable to search for the cyanolipids in seed oil of *Cupania anacardioides* (Sapindaceae). The co-occurrence of cyanogenic non-glycerol esters with acyl triglycerides in the seed oils of Sapindaceae is well documented (1-9). Presence of $C_{20:1}$ (*cis*-11-eicosenoic) acid as a common component of the oils of Sapindaceae has been reported by Hopkins and Swingle (10). Earlier, Seigler and Kawahara (11) reported four other *Cupania* species which do not contain any cyanolipids. This difference may be attributed to the difference in ecological/genetic factors of these samples.

EXPERIMENTAL PROCEDURES

Infrared (IR) and Nuclear Magnetic Resonance (NMR) spectra were obtained with a Perkin-Elmer 621 spectrophotometer and a Varian A60 D spectrometer, respectively. Chemical shifts are expressed in ppm (δ).

Seeds of *Cupania anacardioides* were powdered with anhydrous sodium sulfate, and an exhaustive extraction with petroleum ether (40-60 C) yielded 24% of *Cupania anacardioides* seed oil. The mixed methyl esters were prepared either by refluxing the oil with sodium methoxide in anhydrous methanol or by treating the free fatty acids (derived by saponification of the oil) with diazomethane. Sodium picrate (12) and Prussian Blue (13) tests were used to detect the presence of cyanide moiety in the oil. Preparative thin layer chromatography (TLC) (layer 20 × 40 cm and 1 mm thick) of the oil on silica gel G may be run in one of two solvent systems, either ether:hexane (1:3) or benzene. Nitrogen-containing lipid fraction (NCLF) I had an Rf of 0.8 in the ether:hexane system and 0.7 in benzene, and the corresponding values for NCLF III were 0.7 and 0.5. NCLF I showed 3.0% nitrogen by its elemental analysis. NCLF I responded to both sodium picrate and Prussian Blue tests. Because NCLF III is not based on a cyanohydrin grouping, it did not give a positive test for HCN. Argentation TLC of the methyl esters of cyanolipids I and III on 10% silver nitrate-impregnated silica gel G plates was done using benzene as a solvent. Methyl esters derived from the oil were separated on polyester (DEGS) and silicone (SE 30) stationary phases.

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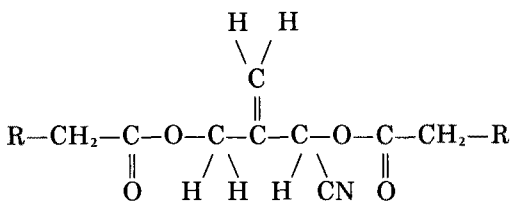
RESULTS AND DISCUSSION

NCLF I (41.3%) and NCLF III (11.7%) were isolated from the oil by preparative TLC using benzene as the developing solvent. IR and NMR spectra of the isolated cyanolipids supported structures I and III.

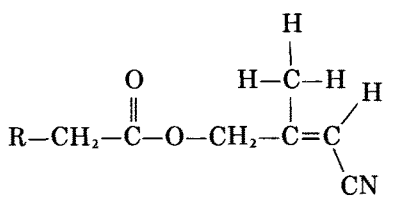
NCLF I displayed IR bands of medium intensity at 940 and 1010 cm^{-1}. There was no —C≡N absorption band at ~2250 cm^{-1}, which is attributed to the quenching effect of the oxygen function present on the same carbon atom to which the cyano group is attached. The NMR spectrum played an important role in establishing the structure of cyanolipid I. NMR of NCLF I showed a multiplet at δ 2.35 which was assigned to protons α to the acyl carbonyl groups. The $C\underline{H}_2$—O protons gave an apparent singlet at δ 4.7.

The remaining dihydroxynitrile protons of I produced three apparent singlets at δ 5.51 (1H), 5.64 (1H) and 5.96 (1H). Terminal methylene protons are often nonequivalent and show weak coupling. Therefore, the broadened singlets at δ 5.64 and 5.51 are assigned to these protons.



Nitrogen-containing Lipid Fraction I

The IR spectrum of NCLF III showed a prominent structure-revealing band at 2235 cm^{-1}. The band intensity is enhanced a great deal due to conjugation of the carbon-nitrogen triple bond with the olefinic bond and to the absence of an α-oxygen atom. The NMR spectrum of NCLF III showed a singlet for vinylic methyl group protons at δ 1.94. A shoulder appearing on this signal is due to splitting caused by allylic coupling (J = 1-1.4 Hz) with the vinyl protons. Methylene protons α to the oxygen atom are observed as a singlet at δ 4.9. The cyanohydrin proton signal was partially obscured by the acyl group vinyl proton signal at δ 5.4.



Nitrogen-containing Lipid Fraction III

GLC analysis of the methyl esters of the total oil showed 79% monoenoic acids, based on the total fatty acids present in oil. A high yield (46.0%) of C_{20} monoene was pres-

ent, which is an interesting feature of cyanolipid-containing sapindaceous seed oils. Argentation TLC of the methyl esters prepared from cyanolipid I showed a high percentage of C_{20} monoene. Such an observation was not made in the case of cyanolipid III.

GLC analyses of methyl esters derived from *C. anacardioides* show the following fatty acid composition: 11.7 (16:0), 8.2 (16:1), 6.2 (18:0), 9.6 (18:1), 15.6 (18:2), 2.0 (20:0) and 46.0 (20:1).

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Study on the Polymorphism of Normal Triglycerides of Sal *(Shorea robusta)* Fat by DSC. I. Effect of Diglycerides

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The effect of diglycerides (DG) on the phase transition of various polymorphic forms of normal triglycerides (TG) of sal fat was investigated by differential scanning calorimetry. Three levels of DG, 5, 10 and 15%, were used. DG delayed the phase transition of lower melting crystal forms to higher forms of TG when the samples were brought to a congealed state by rapid cooling (20 C/min) and heated at rates ranging from 1.25 to 10 C/min; the extent depended on the level of DG and the rate of heating. As the level of DG and the rate of heating increased, the delay in phase transition of crystal forms I → II → III was more pronounced. The phase transition of crystal forms I, II and III to form IV was delayed at 5 and 10% levels of DG, while at the 15% level the phase transition of form I to higher forms was completely stopped when the samples were tempered at 0 C for 18 hr and heated at 10 C/min. DG at 10 and 15% levels retarded the phase transition of form IV to the most stable (V) form of TG when the samples were tempered at 0 C for 1 hr followed by 3 hr at 26 C.

Sal *(Shorea robusta)* belongs to the family Dipterocarpaceae as does *Shorea stenoptera*, the source of the commonly known borneo tallow. It is found mostly in North-east and Central India. The winged seeds contain dark green kernels (72%) which yield 13–15% of dark greenish hard fat (1). Sal fat occupies an important position in its contribution to the total potential of fats of tree origin with an estimated potential of about 700,000 tons (2). Two-thirds of sal fat triglycerides comprise the symmetrical, monounsaturated, disaturated (GS_2U) type, mostly (about 50%) 2-oleodistearin (3). For this reason, and due to its attractive price, the demand for sal fat has been increasing steadily for use in cocoa butter extenders and confectionery fat formulations.

One major problem with commercial refined and bleached sal fats is inconsistency in their solidification properties. This presents problems in obtaining consistently uniform fat fractions from different lots of sal fats using a set of fractionation conditions (unpublished data). Certain minor components (Table 1) like diglycerides (DG) and triglycerides containing 9,10-dihydroxystearic acid (DHS-TG) have been found to be responsible for this inconsistent behavior of sal fat. The DHS-TG have been shown to affect the solidification properties of sal fat by accelerating the onset of crystallization and reducing the supercooling capacity (4). The present paper describes the effect of the minor component-diglycerides on the polymorphic transition of sal fat as measured by differential scanning calorimetry (DSC).

TABLE 1

Minor Components and Normal Triglycerides of Two Sal Fat Samples[a]

Sample	DHS-TG	DG	FFA	Unidentified[b]	TG
Sal fat I	1.4	7.2	1.5	1.4	89.0
Sal fat II	3.3	2.2	0.3	2.0	92.0

[a]Values are relative percentages.
[b]Probably triglycerides containing epoxystearic acid (11).

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