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Cyanolipids in Sapindaceous Seed Oils

DINESH KUMAR[1,*] and S.Q. HASAN[2]

[1]Department of Chemistry, Bipin Bihari College, Jhansi-284 001, India
[2]Department of Chemistry, Pandit J.N. Post Graduate College, Banda-210 001, India

*Corresponding author: E-mail: gautamdinesh_123@yahoo.co.in

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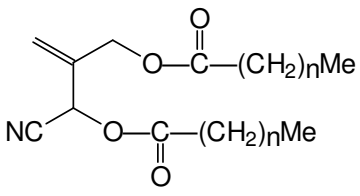
A number of sapindaceous seed oils have been investigated with respect to their cyanolipid constituents. All but one of the oils have this new class of lipids in amounts 37 %. These cyanolipids are of four different types, but all consist of long-chain fatty acids esterfied with an unsaturated isoprenoid hydroxyl or with dihydroxynitrile. The large amount of C_{20}-carbon fatty acids usually found in the oil indicate an appreciable cyanolipid content because such acids are preferentially incorporated in nitrile-containing fractions. The fatty acid composition of the cyanolipid component has also been compared with that of triglycerides. *S. obavatus* seed oil was shown to contain a diester having two fatty acid moieties esterified with 1-cyano-2-hydroxymethylprop-1-ene-3-ol.

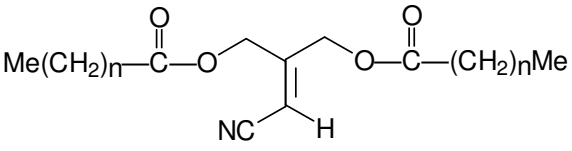
Key Words: Cyanolipids, Sapindaceae, *S. obavatus*, TLC.

INTRODUCTION

Cyanolipids are comparatively a new class of plant lipids, which are found, often in copious amounts, only in the seed oils of sapindaceous plants (with one remotely possible exception) and probably play an important role in the biochemistry of these plants. Cyanolipids are not glycerides but instead are derivatives of five-carbon mono or dihydroxynitrile moiety (I-IV) esterified with long chain fatty acids[1,2]. 1-Cyano-2-hydroxymethylprop-2-ene-1-ol (I) and 1-cyano-2-hydroxymethylprop-1-ene-3-ol (II); the other class of cyanolipids comprises mono-esters of 1-cyano-2-methylprop-1-ene-3-ol (III) and 1-cyano-2-methylprop-2-ene-1-ol (IV).



(I)



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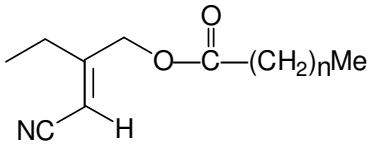
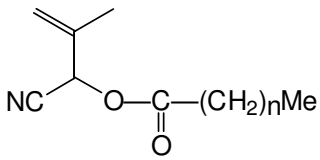


(III)



(IV)

Each cyanolipid fraction in a mixture in which the constituents differ only in the attached fatty acids; because this mixture was difficult to separate and appeared to be based on single aglycone, it was treated as a single entity during the course of investigation.

Sporadic reports[3-5] have appeared the occurrence of cyanogenetic non-glycerol esters with seed oil triglycerides. Progress in cyanolipid identification start with reports on Kusum oil (*Schleichera trijuga*) by many workers in India. Way back 1920, cyanolipids were first observed in *Schleichera trijuga* (Sapindaceae) seed oil by Sengupta[5] and Rosenthaler. But the exact location of the cyanogenic compound in the oil or its exact nature was not reported. The compound has been

suspected to be in form of cyanogenic glycoside or an acid amide. Latter by Kundu and Bandopadhyay re-investigated the same seed oil to as certain the location and nature of cyanogenic compound by applying chemical method, chromatography and infrared specetroscopy. Observation indicated the cyanogenic compounds to be a part of glycoside molecules in which one of the hydroxyl groups of the latter is bonded to the cyanogenic compound through an ether linkage. Chromatographic behaviour of the cyanogenic compounds further indicated that at least two glycoside molecules are involved. At the same time Kasbekar and Bringi working on the same seed oil found with the help of TLC that the oil is composed of *ca.* 63 % of glyceride, the rest being non-glycerol esters of fatty acids. Recently[6-14] have their structures been determined and the existence of this class of lipid acknowledged. The unifying structural feature of these cyanolipids is that they are all based on the same branched, five-carbon nitrile skeleton, although the double bond position and the number and location of hydroxyl groups are not the same (**I-IV**).

Being a relatively new development, the cyanolipid field does not possess a large body of literature as do other lipid subdivision. In order to build a firm basis for further discussion, particularly as it relates to results of kusum oil research obtained prior to the actual structure proof of cyanolipids. Cyanolipids appear to occur only in Sapindaceae plants; therefore, a sampling of pre-cyanolipid literature describing research on some of the more important plants of this family has also been made.

EXPERIMENTAL

Infrared (IR) spectra were determined with a Perkin-Elmer model 137 spectrophotometer on 1 % solution in $CHCl_3$. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian HA-100 spectrometer; the solvent used were $CDCl_3$. Chemical shifts were measured from internal tetramethylsilane (TMS) = τ10.0. A Beckman DK-2A spectrophotometer was used to determine the ultraviolet (UV) spectra.

Oil recovery and methyl ester formation: About 80 g oil were recovered from finely ground seeds (400 g) of *S. obavatus* was extracted with petroleum ether (b.p. 60-80 ºC) in a soxhlet apparatus for minimum 12 h. Methyl esters were prepared from the oil and from nitrogen containing lipid fraction by refluxing them for 3 h with 3 % conc. H_2SO_4 in methanol. The esters were recovered by ether extraction.

Thin layer and gas liquid chromatography: Analytical thin layer chromatography (TLC) was done on 0.25 mm layer of silica gel G developed with solvent of ether:hexan:acitic acid (1:3:1 drop). Spots were detected by charring the plates after they had been sprayed with a standard solution of CrO_3 in 50 % aqueous H_2SO_4. Now spots due to cyanolipid II are obliterated by the triglyceride spot, but cyanolipid II are well resolved from each other from triglycerides. Analyzing *S. obavatus* oil on slica gel with ether-hexane (1:3) gave two spots, one is triglyceride spot) (R_f 0.86, second is cyanolipid spot ((R_f 0.60) and only single spot with benzene. GLC analysis of methyl ester were performed. Direct GLC of triglycerides indicate the evidence for cyanolipids and nitrogen containing lipid fraction was achieved with an F and M model 5750 chromatographed equipped with hydrogen flame detector.

Liberation and detection of HCN: Various methods exist for qualitative detection of HCN released by cyanogenic plant material and the method of choice is often a matter of personal preference. Two tests are particularly suitable for use with oils containing cyanolipids. One is the picrate test, which depends on the ability of HCN to react with alkaline picrate-saturated filter paper strips to produce isopurpuric acid. About 70-90 mg of lipid material was placed in a test tube with 1 mL of dilute NaOH. A strip of filter paper dipped in an alkaline solution of sodium picrate (05 %) was partially dried and suspended over the mixture in the stoppered test tube. Test tube and contents were warmed at 35-50 ºC for 0.5-1.0 h. A positive test involves a colour change of filter paper form yellow to brick red.

The second test is based on formation of prussian blue and was carried out as described by Seigler[3].

RESULTS AND DISCUSSION

In present investigation, a new class of lipid in *Sapindus obavatus* seed oil is isolated and characterized. Cyanolipid was found in *S. obavatus* as a fatty acid diester of 1-cyano-2-hydroxymethylporp-1-ene-3-ol (Fig. 2).

On silica gel TLC the oil of *S. obavatus* gave two spots (triglyceride, R_f 0.84 and cyanolipid, R_f 0.62) with ether-hexane (1:3) and only a single spot with benzene.

Nitrogen containing lipid fraction was separated from triglyceride fractions in a pure state by preparative TLC. For nitrogen containing lipid fraction the plate was developed with ether-hexane (1:3).

The *Obavatus* seed oil was separated in to the triglyceride and cyanolipid fractions with the help of preparative TLC. The fatty acid composition studies (Table-1) of the triglyceride fraction of the *S. obavatus* shows that the total saturated acid content is 29.1 % and the unsaturated content is 70.9 %. The total C_{18} fatty acids are 64 % out of which 61.4 % is an unsaturated C_{18} acids. The total content of C_{20} acids is 25.7 % out of which the unsaturated is present to the extent of 9.5 %. The cyanolipid fraction of *S. obavatus* seed oil shows the presence of saturated fatty acids up to 35.5 % and the unsaturated fatty acids 64.5 %. The C_{18} saturated acid is only 1.7 % whereas unsaturated C_{18} acids are 52.4 %. The C_{20} acids of the cyanolipid fraction are present to 28.4 % out of which C_{20} mono-ene is 12.1 %.

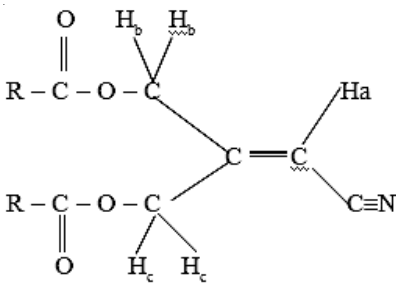
The fatty acids compositional studies show that in cyanolipid fraction the C_{20} acids are more than the triglyceride fraction which is the common phenomenon in the cyanolipid containing seed oil due to the preferential incorporation of C_{20} fatty acids the nitrile-moiety.

TABLE-1
FATTY ACID CONTENTS OF CYANOLIPID AND TRIGLYCERIDE FRACTIONS of *S. obavatus* SEED OIL

Species	Lipid fraction	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0
S. obavatus	Triglyceride	10.3	2.6	51.8	7.4	2.2	16.2	9.5	Tr
	Cyaniolipid	5.4	1.7	49.6	1.6	1.2	28.4	12.1	Tr

Analysis of nitrogen containing lipid fraction (II): The IR (1 % solution in CS_2) analysis of nitrogen containing lipid fraction (II) revealed a nitrile band of medium intensity at 2200 cm^{-1} (-C≡N) and it was superimposiable on the spectrum of corresponding cyanolipid isolated form *S. emarginatus* seed oil.

The NMR exhibited signals characteristic for long chain lipid group τ9.12 (rough t, 6H, terminal methyl) 8.75 (br s, shielded methylene), 7.97-8.05 (m, protons to the double band) 7.67 (t, protons to the carbonyl function) and 4.7 (rough t, olefinic protons). The two of methylene protone H_b and H_c (II) which are adjacent to the oxygen atoms of the dihydroxynitrile moiety gave the signals at 5.3 (singlet) and 5.33 (doublet). This difference in shielding and splitting is caused by the stereochemsitry of the methylene groups; one of them is cis to nitrile grouping and other is *trans*. As a result of the stereo-chemical difference between the two methylene groups, the protons of one group couple more strongly with vinyl proton than to the protons of the other methylene groups. The cyanohydrin proton (H_a) appeared as a slightly broadened signal at τ4.45.



Cyanolipid (II)

The comparative TLC and IR characteristice coupled with NMR data established that the cyanolipid present as a fatty acid diester of 1-cyano-2-hydroxy-methylprop-1-ene-3-ol identical to the NCLF of *S. obavatus* methyl esters of all the triglycosides and accompanying nitrogen containing lipid fraction had the composition as shown in table.

The structures of the hydroxynitrile portions of cyanolipids **I-IV** suggested that they might be derived from leucine. Two of these (**II** and **III**), which occur in the seed of *Koelreuteria paniculata*, have been shown to be derived from leucine. The aglycones of several cyanogenic glycoside have been demonstrated to obtained from amino acids.

Because of its basically isoprenoid structure, the dihydroxynitrile moiety of (II) has many biogenetic possibilities, it may be related, perhaps somewhat remotely, to biological compounds such as cordycepose or mevaldic acid. However, the studies on the biosynthesis of other cyanogenetic materials indicate that most of them are derived from amino acids or their precursors.

Conclusion

Cyanolipid is present in various medicinal plants. In the present investigation the *S. obavatus* seeds are found to contain cyanolipids (II).

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