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Study of cyanolipids in *S. laurifolia*

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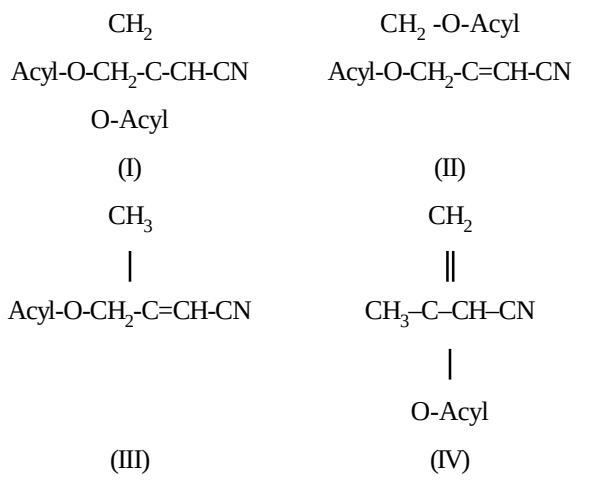
ABSTRACT : We have isolated and characterized cyanolipid (II) from *S. laurifolia*. Seeds of *S. laurifolia*. (1/2 kg) were extracted with petroleum ether (BP. 60-80°c). The fatty acid composition of the cyanolipid component has also been compared with that of triglycerides. The cyanolipids from seven sapindus species (viz. *S. drommondii, S. utilis, S. mukorossi, S. emerginatus, S. saponaria, S. trifoliatus and S. obavatus*) have been previously investigated for their cyanolipid content and all of them contain cyanolipid (II). In our present investigation *S. laurifolia* seeds were also found to contain cyanolipid (II)

Keywords : Cyanolipids, sapindaceous, TLC, NCLF,

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 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.



Sporadic reports have appeared recently regarding the cooccurrence of cyanogenetic nonglycerol esters with seed oil triglycerides. Progress in cyanolipid identification began with reports [1-4] on Kusum oil (*Schleichera trijuga*) by many workers in India. Way back 1920; Cyanolipids were first observed in *Schleichera trijuga* (Sapindaceae) seed oil by Sen-gupta1 and Rosenthaler [2]. 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 glucoside or an acid amide [3]. Latter by Kundu and Bandyupadhyay [4] 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 comounds further indicated that at least two glycoside molecules are involved. At the same time kasbekar and Bringi [3] working on the same seed oil found with the help of TLC that the oil is composed of approximately 37% of glyceride, the rest being non glycerol esters of fatty acids. Only recently [5-25] has their structures been determined and the existence of this class of lipid acknowledged [26-27] l-cyano-2-hydroxymethylprop-2-ene-1-ol (I) and l-cyano-2-hydroxymethylprop-l-ene-3-ol (II). The other class of Cyanolipids comprises mono-esters of l-Cyano-2-methylprop-l-ene-3-ol (III) and l-cyano-2-methylprop-2-ene-1-ol (IV).

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

MATERIAL AND METHODS

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 95g oil were recovered from finely ground seeds (1/2 kg) of *S. laurifolia* was extracted with petroleum ether (Bp. 60-80°C) in a soxhlet apparatus for minimum 12-hr. Methyl esters were prepared from the oil and from Nitrogen containing lipid fraction (NCLF) by refluxing them for 3 hr

with 3% conc. H_2SO_4 in methanol. The esters were recovered by ether extraction.

Thin layer chromatography

Analytical thin layer chromatography (TLC) was 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.

Liberation and detection of HCN

Two tests were used to detect HCN derived from seed oil. One of these picrate tests, depending on the reaction of HCN with alkaline picrate solution to produce isopurpuric acid [28]. About 75–100 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°C for 0.5–1 hr. A positive test involves a color change of filter paper form yellow to brick red [29].

The second test involved formation of Prussian blue [30] and was carried out as described by Seigler et. al[9].

RESULTS AND DISCUSSION

In present investigation we have isolated and characterized this new class of lipid in *Sapindus laurifolia* seed oil. Cyanolipid was found in *S. laurifolia* as a fatty acid diester of 1–cyano–2–hydroxymethylporp–1–ene–3–ol

On silica gel TLC the oil of *S. laurifolia* 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 (NCLF) was separated from triglyceride fractions in a pure state by preparative TLC. For NCLF the plate was developed with ether – hexane (1:3).

Analysis of NCLF (II)

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

The NMR spectrum of the cyanolipid revealed proton counts, chemical shifts and multiplicities identical with those displayed by the reference sample. 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) [31] 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.

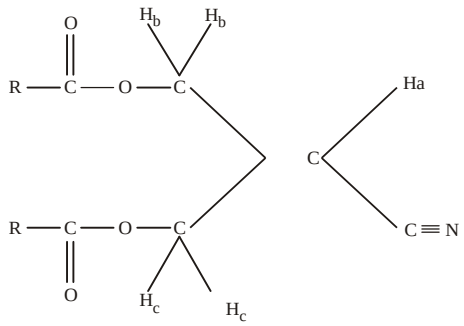


Table 1 : Fatty acid content of cyanolipid and triglyceride fractions.

Species	Lipid fraction	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0
S.laurifolia	Triglyceride	10.01	2.8	50.8	8.4	2.2	17.2	8.5	Tr
S.laurifolia	Cyanolipid	5.6	1.6	45.8	4.4	1.1	18.5	23.0	Tr

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. laurifolia.* Methyl esters of all the triglycosides and accompanying NCLF had the composition as shown in Table 1.

Only a meager amount of research has thus far been reported concerning how these strange cyanolipids are produced in plants. Mikolajczak et. al [9], first pointed out that the structures of the hydroxynitrile portions of cyanolipids I–IV suggested that they might be derived from leucine. Two of these (II & III), which occur in the seed of *koelreuteria paniculata*, have recently been shown to be derived from Leucine [7]. The aglycones of several cyanogenic glycoside have been demonstrated to come from amino acids.

Because of its basically isoprenoid structure, the dihydroxynitrile moiety of (I) has many biogenetic

possibilities, it may be related, perhaps somewhat remotely, to biological compounds such as cordycepose [32] or mevaldic acid [33]. However, rather extensive studies made on the biosynthesis of other cyanogenetic materials indicate that most of them are derived from amino acids or their precursors [34-36].

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