International Journal of Chemical Studies

P-ISSN2349–8528
E-ISSN 2321–4902
IJCS 2016; 4(4): 216-217

Received: 15-05-2016
Accepted: 16-06-2016

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Chemical studies of cyanolipids in *Koelreuteria apiculata*

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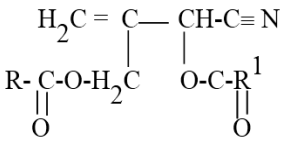
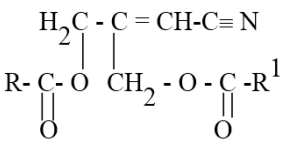
Abstract
Many species of genus Sapindus (*Sapindus saponaria* [1], *S. trifoliatus* [2], *Lepisanthes tetraphylla* [3], *S. obovatus* [4, 9]) have been previously investigated for their cyanolipid content and all were found to contain only one type of cyanolipid, i.e. fatty acid diester of 1-cyano-2 hydroxymethylprop-1-ene-3- ol (II). *Koelreuteria apiculata* seed kernel oil has now been investigated and the oil is also found to contained the same type of cyanolipid.

Keywords: *Koelreuteria apiculata,* cyanolipid, triglyceride, NCLF and 1- cyano-2-hydroxymethylprop-1-ene-3-ol

Introduction
The presence of cyanolipids which co-occur with triglycerides in the seed oils of the family Sapindaceae has been reported. Cyanolipids were first observed in *Schleichera trijuga* (Sapindaceae). Studies have been shown the presence of four types of cyanolipid. Fig (I) and all consists of long chain fatty acids preferentially C_{20} acids, esterified with an unsaturated isoprenoid mono or dihydroxynitrile moiety. A curious feature of these cyanolipid containing seed oil is their high percentage of C_{20} acid and the preferential incorporation of these acids into cyanolipids rather than into the accompanying triglycerides.



(I)



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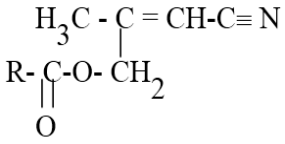

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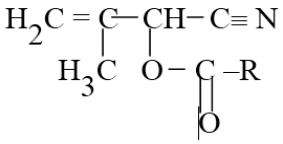


(IV)

Methods and Materials
Oil recovery and ester formation: *Koelreuteria apiculata* seeds were procured from commercial supplier and oil was recovered from finely ground seeds by petroleum ether (b.p. 40-60^0) extraction in a Soxhlet apparatus for 16 hr. The methyl esters were prepared using 10% NaOMe in MeOH or acid catalyzed methylation.

Thin layer chromatography
TLC analysis of the oil was carried out on silica gel G plates using ether-hexane (1:3) as the developing solvent.
Spot were detected by charring the plates after they had been sprayed with a saturated solution of CrO_3 in 50% aqueous H_2SO_4.

Gas liquid chromatography: GLC analysis of methyl esters were performed essentially as described by Miwa and coworker [10] by using stainless steel packed column (2m x 3mm) coated with diethylene glycol succinate (DEGS). A Perkin-Elmer Model 154 vapour Fractometer was employed in these analysis and the separations were carried out isothermally at 200 ^{0}C, with a hydrogen flow rate of 70ml/min.

Formation and detection of HCN: The picrate test, depends on the reaction of HCN with alkaline picrate solution to produce isopurpuric acid [6]. About 75-100 mg of lipid material was placed in a test tube with 1 ml of dilute NaOH or H_2SO_4. A strip of filter paper dipped in an alkaline solution of sodium picrate (0.5%) was partially dried and was then suspended over the mixture in the stoppered test tube. Test tube and contents were warmed at 35-50^0 for 0.5-1 hr. A positive test involves a colour change of the filter paper from yellow to brick red [5].

Isolation and characterisation of NCLF: The oil was fractionated by preparative TLC plates (1mm thickness) with ether hexane (1:3)

The cyanolipid (33.4%) was isolated from the oil by preparative TLC on silica gel G plate (20 x 40 cm and 1 mm thick) with ether hexane (1:3) as the developing solvent.

The seed oil of Cardiospermum halicacabum and its derived NCLF were used as reference samples for the purpose of comparison. In benzene the nitrogen containing lipid fraction (NCLF) moved with R_f 0.48 whereas in ether: hexane (1:3) it migrated at R_f 0.69. The elemental analysis of this isolated cyanolipid constituent gave a nitrogen content of 1.7% of nitrogen.

Spectroscopy

IR spectra were determined with Perkin-Elmer Model 621 Spectrophotometers on 1% solutions in $CHCl_3$. NMR spectra were obtained with a Varian A -60D spectrometer, the solvent used was CCl_4. All reported chemical shifts are measured from internal tetramethyl silane (TMS). A Beckman DK-2A instrument was used to determine UV spectra.

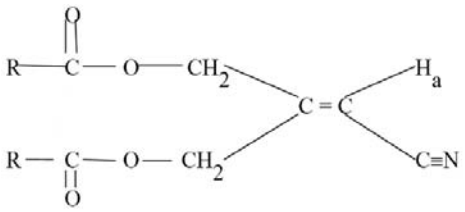
Result and Discussion

The present investigation deals with isolation and characterisation of one such cyanolipid, namely the fatty acid diester of 1- cyano-2-hydroxymethylprop-1-ene-3-ol (II) (33.4%) in the seed oil of *Koelreuteria apiculata*. The fatty acid composition of the cyanolipid component has also been compared with that of triglycerides. This oil also contains about 45% of a C_{20} monoenoic acid. The ground seeds were extracted with light petroleum ether (b.p.-40-60 ^{0}C) in a Soxhlet apparatus to afford 34.5% of oil (RI: n_D^{30-} 1.4817; IV=93.3, SV=175.5). Methyl esters of the oil were prepared by treating with sodium methoxide (1%) in dry methanol. The oil of *K. apiculata* gave positive picrate [7] and prussian blue [5] test for the presence of hydrogen cyanide.

Elemental analysis of the oil indicated that it contained 2.2% of Nitrogen. The IR spectrum of the oil exhibited V_{MAX} 940, 1010 cm^{-1} attributed to the terminal methylene and allylic ester functions of the cyanogenic moiety.

The structure of the TLC pure cyanolipid was proved by comparison of its IR and NMR spectral characteristics with those of the pure minor cyanolipid fraction. This led to the assignment of structure as the diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol (II).

NCLF had a characteristic IR band at 2235 cm^{-1} for the nitrile group. The ^{1}H NMR spectrum (60 MHz) of this fraction in carbon tetrachloride exhibited few typical signals besides the common signals of long chain lipid groups δ0.92 (terminal methyl), 1.24 (chain methylenes) 1.60 (Proton β to carbonyl), 1.96 (Proton α to the double bond) 2.29 (Proton α to the carbonyl group) and 5.28 (vinyl proton). Proton of the two methylene groups of the dihydroxynitrile moiety of compound (I) gave two signals, one a single at δ.4.82 and the other a doublet at δ4.63. A broad singlet furthest downfield at δ5.50 is due to H_a.



The lipid groups of the triglyceride as well as cyanolipid constituent of the oil (Table No. 1) were identified by converting them to their methyl ester by transesterification and comparing the methyl esters by GLC with authentic standards. On comparing it was found that a higher proportion of C: 20 acid occurs in the cyanolipid than in the triglycerides.

Table 1: Lipid groups of the triglyceride and cyanolipid constituent of the oil

Lipid fraction	Fatty acid composition (wt. %)										
	Conc. Wt.%	16:0	18:0	18:1	18:2	20:0	20:1	20:2	22:0	22:1	**Reference**
Triglyceride	68.0	5.3	0.7	19.5	8.2	3.0	44.0	5.1	6.2	7.9	
	66.6*	5.5*	0.5*	19.2*	8.0*	3.1*	44.6*	4.9*	5.7*	8.4*	8
Cyanolipid	32.0										
	33.4*										

* Previously reported

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