ON THE OCCURRENCE OF CYANOLIPIDS IN *Paullinia carpopodea* CAMBESS AND *P. cupana* KUNTH SEED OILS

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ABSTRACT - *Paullinia carpopodea* seed oil contains 70% type I cyanolipids with cyanogenetic properties, as proven by chemical and spectrometric techniques. *P. cupana* seed oil also contains cyanogenic type I cyanolipids, according to its ^{1}H-NMR spectrum. The existing controversy in the literature about the presence and/or type of cyanolipids in *P. cupana* seed oil is probably due to the low amount of these compounds (0.2%) in the seeds.

Key-words: Sapindaceae, cyanolipids, cyanogenesis, *Paullinia carpopodea*, *Paullinia cupana*.

Ocorrência de Cianolipídios em Óleos das Sementes de *Paullinia carpopodea* Cambess e *P. cupana* Kunth

RESUMO - O óleo das sementes de *Paullinia carpopodea* contém 70% de cianolipídios cianogenéticos, do tipo I, como mostrado através de métodos químicos e espectrométricos. O óleo de *P. cupana* contém o mesmo tipo de cianolipídios, como evidenciado através de seu espectro de ^{1}H-NMR. A controvérsia existente na literatura sobre a presença e/ou tipo de cianolipídios no óleo de *P. cupana* foi, provavelmente, causada pelo baixo teor com que estes componentes ocorrem nas suas sementes (0,2%).

Palavras-chave: Sapindaceae, cianolipídios, cianogênese, *Paullinia carpopodea*, *Paullinia cupana*.

INTRODUCTION

Paullinia carpopodea is known in Brazil as "timbó" and grows wild in the states of Minas Gerais, São Paulo and Paraná. The plant is used for its ictiotoxic properties (Reitz, 1980).

P. cupana is native to Amazonas and Pará states and is commonly known as "guaraná". Its seeds are used for preparing a powder used in refreshments, recommended as an energy reconstituent and for alleviating stomach disorders. "Guaraná" also has a reputation as a cardiotonic and arteriosclerosis prevention agent (Reitz, 1980). Angelucci *et al.* (1978) mentioned ca. 4% of caffeine in its seeds.

Cyanolipids are a unique class of plant lipids that seem to occur almost exclusively in the seed oils of the Sapindaceae (Mikolajczak *et al.* 1970; Gowrikumar *et al.* 1976; Nishizawa *et al.* 1983), although cyanolipids were identified for the first time in Boraginaceae, *Cordia verbenacea* (Mikolajczak, 1969). Seigler (1976) showed that, in fact, the plant studied belonged to the Sapindaceae family. According to Mikolajczak (1977), not all species of Sapindaceae contain cyanolipids.

The four known cyanolipid structures (types) present the same branched five-carbon nitrile skeleton. Types I and II are long chain fatty diesters of 1-cyano-2-hydroxymethyl prop-2-en-1-ol (I) and of 1-cyano-2-hydroxymethyl prop-1-en-3-ol (II). Types III and IV are mono-esters of 1-cyano-2-hydroxymethyl prop-1-en (III) and of 1-cyano-2-methylprop-2-

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en-1-ol (IV).

Cyanogenesis is defined as the capacity shown by certain plants of yielding HCN through the hydrolysis of chemical compounds produced by them. For a long time this process was associated with the presence of cyanogenic glycosides in plant tissues. The first and conclusive evidence that cyanogenicity in kusum seed oil (*Schleichera trijuga*) could not be attributed to a glycoside but rather to a lipid-based material was provided by Kundu & Bandyopadhyay (1969). Cyanolipids I and IV are cyanogenic.

Different seeds may yield conflicting results, as in the case of *P. cupana*; for some authors, its seeds contain type **I** cyanolipids while for others the material is devoid of such compounds (Seigler & Kawahara, 1976). This study was undertaken in order to study *P. carpopodea* lipids and to confirm whether *P. cupana* contains or not cyanogenetic compounds.

EXPERIMENTAL

P. carpopodea fruits were collected in Ouro Preto, Minas Gerais (voucher is deposited at the herbarium of the Pharmacy and Biochemistry School, Federal University of Ouro Preto, under the register number 1031 (flowers) and 1949 (fruits)). *P. cupana* fruits were from Pará state. The seeds were separated from the fruits, ground in a lab mill and their oil extracted in a Butt type apparatus, with light petroleum, b.p. 40-60°C.

The alkaline qualitative picrate test (Feigl, 1954) was conducted to detect the presence of the -CN group.

Thin-Layer Chromatography (TLC) was carried out using silica gel G plates and benzene as eluent (no other solvent gave better or equivalent separation). Triacylglycerols and cyanolipids bands were scrapped out from preparative plates (0.5mm), extracted with CH_2Cl_2 and weighed, after evaporation of the solvent. Purification was carried out through HPLC, in a Waters 403 system, equipped with Refractive Index Detector, using two Altex ODS columns (5x150mm;5mm) in series, and acetone: acetonitrile: dichloromethane 3:2:1 as eluent, at flow rate of 1ml/min.

The infrared spectrum of *P. carpopodea* cyanolipids showed carbonyl (1740cm^{-1}) and $=CH_2$ absorptions (1157cm^{-1} and 920-965 cm^{-1}).

^{1}H-NMR spectra were recorded at 200MHz in $CDCl_3$ with TMS as internal standard. ^{13}C-NMR spectrum was taken at 100MHz in $CDCl_3$.

P. carpopodea cyanolipids. ^{1}H-NMR: δ 2.33 (m, H_a and H_b); 4.70 (s, H_c, H_d), 5.52 (s, H_g), 5.67 (s, H_f), and 5.96 (s, H_e). ^{13}C-NMR: δ 120.8 (-CN), 130.0 ($=CH_2$), 135.3 (C=), 171.5 (CO) and 173.0 (CO).

P. cupana cyanolipids. ^{1}H-NMR: δ 2.34 (m, H_a, H_b), 4.63 (s, H_c, H_d), 5.50 (s, H_g), 5.66 (s, H_f) and 5.94 (s, H_e).

RESULTS AND DISCUSSION

P. carpopodea and *P. cupana* seeds yielded 40.5% and 1.2% oil, respectively. The seed oil from *P. carpopodea* was clearly positive to the picrate test which, nevertheless, failed

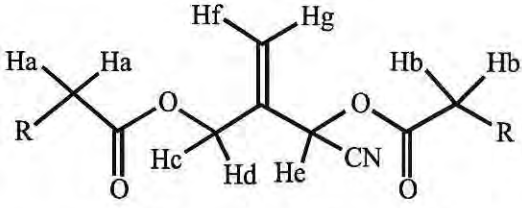

Figure 1. Protons assignment in ^{1}H-NMR spectrum of *P. Carpopodea* cyanolipids.

to prove the cyanogenicity of the *P. cupana* oil or the presence of cyanogenic cyanolipids. A band which appeared, for both oils, above the triacylglycerols on the TLC plate could only be related to either cyanolipid I or IV, since cyanolipids II and III are more polar than the triacylglycerols.

The UV spectrum exhibited a very weak nitrile absorption band at 208nm. The positive Feigl test and the absence of a -CN absorption at 2230cm^{-1} in the IR region (Bellamy, 1956) indicated the presence of a non-conjugated cyano group, corresponding either to type I or IV cyanolipids.

Through preparative TLC, cyanolipids in the oils were quantified. While *P. carpopodea* oil contained 70.7% of cyanolipids and 21.3% of other lipids, mostly triacylglycerols (Lago *et al.*, 1995), *P. cupana* oil exhibited only 12% of cyanolipids (or 0.2% in the seeds).

Lago et al. (1995) observed that eicosenoic acids are the main constituents (52.8%) in *P. carpopodea* seed oil. They are the major components in the cyanolipid fraction (64.95%) but the third in the triacylglycerol fraction (16%). In *P. elegans*, Spitzer (1995) identified *cis*-13-eicosenoic acid as the main component of total lipids (44.4%), which the author named Paullinic acid, and small amounts of *cis*-11-eicosenoic acid (gadoleic acid, 3.7%) and *cis*-15-eicosenoic acid (0.7%).

The ^{1}H-NMR spectrum of the cyanolipid fraction showed a multiplet (probably a double triplet) at δ 2.33 which was assigned to protons of methylene group a to carbonyl group (H_a, H_b) shown in Figure 1. The -CH_2- protons (H_c, H_d) gave an apparent singlet at δ 4.70. The remaining protons of the dihydroxynitrile moiety of cyanolipids produced apparent singlets at δ 5.52 (H_g), 5.67 (H_f) and 5.96 (H_e). Equivalent data have been presented by Seigler *et al.* (1970) and Mikolacjzak (1977) for type I cyanolipids.

^{1}H-NMR spectrum of *P. cupana* seed oil showed also equivalent data with a multiplet at δ 2.34 (H_a, H_b), a singlet at δ 4.63 (H_c, H_d) and singlets centered at δ 5.10, δ 5.66 and δ 5.94 (terminal -CH_2- protons, H_e, H_f, and proton bonded to α-carbon to the nitrile group, H_e, respectively).

In the ^{13}C-NMR spectrum of the cyanolipid fraction assignment for some carbon atoms are as follows: -

CN group- 120.8ppm; $=CH_2$, 130.0ppm; =C, 135.3ppm and CO groups, 171.5 and 173.0ppm. The possibility of having cyanolipids II and/or III had been discarded previously. Differences in chemical shifts between carbon atoms of types I and IV cyanolipids (Mikolajczak & Weisleder, 1978), such as the presence of two signals corresponding to distinct CO carbon atoms in the spectrum of cyanolipids I instead of only one signal, as would be the case for type IV cyanolipids, are sufficient for distinguishing one from the other.

CONCLUSIONS

The oil content and its composition make *P. carpopodea* seeds a source of type I cyanolipids, while due to its low content in *P. cupana* seed oil its presence is only detectable after concentration. The existing controversy in the literature is attributed to the low content (0.2%) of these compounds in the seeds.

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