Feeding experiments. Fruits (2 weeks old) were harvested and halved longitudinally. The halved pericarps were cut transversely into slices (0.2–0.5 mm thick) and rinsed twice into 2×10^{-4} M $CaSO_4$ soln. The slices (1.5 g) were put into a test tube (1.5 × 3.0 cm), dipped into 3 ml of 10^{-2} M Na Pi buffer (pH 6.8) containing 10^{-3} M Na ascorbate and then the radioactive compound (2 μCi) was added. CO_2 generated from the slices was trapped with 10 ml of N NaOH. After incubation for 24 hr in the light the radioactive stizolamine was extracted and isolated by the column of the Amberlite IRA 410 (OH^- form) described previously [2]. Carrier (10 mg) was added to the isolated samples and the amine was recrystallized from boiling H_2O.

Analyses. Stizolamine was measured spectrophotometrically (330 nm in MeOH; $\varepsilon = 13600$) and fluorometrically (Exc., 350 nm, Anal., 390 nm; pH 7). Radioactivities were measured using a 2,5-diphenyl oxazole (PPO)-dioxane scintillator.

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BIOSYNTHESIS OF CYANOGENIC LIPID IN *CARDIOSPERMUM GRANDIFLORUM* FORMA *HIRSUTUM* SEEDS

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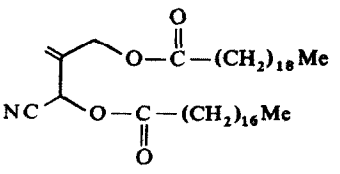
Key Word Index—*Cardiospermum grandiflorum* forma *hirsutum*; Sapindaceae; cyanolipids; biosynthesis; origin from leucine.

Cyanolipid **1** has been reported from a number of Sapindaceous plants [1–8].



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In addition, 3 other cyanolipids have been isolated, all from the seed oils of members of the Sapindaceae. Two of these, which occur in the seeds of *Koelreuteria paniculata*, have recently been shown to be derived from leucine [9]. The aglycones of several cyanogenic glycosides have also been shown to the synthesised from amino acids [10]. One of these glycosides, acacipetalin, is found in several species of *Acacia* [11]. It is a glucoside of the same α-hydroxynitrile found in the cyanolipid of *Ungnadia speciosa* [4]. The aglycone portion of acaci petalin has been shown to be derived from leucine in *Acacia sieberiana* [12, 13]. Another cyanogenic glucoside, cardiospermin has also been isolated from *Cardiospermum grandiflorum* forma *hirsutum* [14] which possesses an identical aglycone portion to that of cyanolipid **1**, but its biosynthetic origin has not been examined. Because the structure of **1** suggests that leucine is a logical biosynthetic precursor and all compounds of this type possess similar structures and occur in related plants, we initiated the present study.

We have not made comparisons of the effectiveness of labelling by other possible precursors as the fruiting period of the plant limits the number of experiments possible as well as the availability of materials.

Labelling data for samples 1, 2 and 3 are presented in Table 1. The samples were obtained by feeding 20, 20, and 50 μCi respectively of L-leucine-[U-^{14}C] as previously described. Percentage incorporation was calculated assuming a MW of 782 based on the relative percentages of the cyanogenic compound and the fatty acid composition of the whole oil. As previously observed in *Koelreuteria paniculata* [9], the radioactive label does not appear to 'turn over' rapidly in seeds of *C. grandiflorum*.

Cyanolipid (**1**) and the co-occurring glycerides were labelled but label is predominatly found in **1** (Table 2). A lesser degree of incorporation into the fatty acid portions of the molecules suggests that a certain amount of leucine is converted to acetyl CoA and this compound is subsequently incorporated into the fatty acids of both types of compounds. This hypothesis was confirmed by transesterification of the cyanolipid and of glycerides followed by measurement of label in the derived Me

Table 1. Labelling data for *Cardiospermum grandiflorum* forma *hirsutum* seed oil. Sample 1 (138 mg), Sample 2 (83.4 mg) and Sample 3 (181 mg) were fed 20,20, and 50 μCi of L-leucine-[U-^{14}C] respectively

Sample	Mg counted	dpm/mg	dpm/μmol	%Incorporation
1	26.4	199	155	0.1
2	20.9	288	225	0.1
3	30.4	9870	7700	1.6

Table 2. Labelling data for major components of the oil from *Cardiospermum grandiflorum* forma *hirsutum* seeds and the corresponding methyl esters derived from these components

Sample	Band	Wt (mg)	cpm	cpm/mg
Oil				
1	cyanolipid **1**	24	4000	167
2	cyanolipid **1**	20	4660	233
3	cyanolipid **1**	38	31800	8370
Me Esters				
1	cyanolipid **1**	16	944	59
2	cyanolipid **1**	10	1500	150
3	cyanolipid **1**	27	105000	3900
transesterified glycerides		5	7350	1470

esters. Significant loss of label from the cyanolipids indicates that the label resides largely in the 'aglycone' portion of the molecule. This confirms our predictions based on structural considerations and suggests that leucine is indeed the precursor of cyanolipids such as **1** in Sapindaceous plants.

EXPERIMENTAL

The plant used was a large specimen of *Cardiospermum grandiflorum* Swartz forma *hirsutum* (Willd. Radlk.) [16] cultivated in the University of Calfornia Arboretum, Davis (Accession #A67.623) and grown from seed originally from the Instituto de Botánica Agrícola (INTA) Castelar, Pcia Buenos Aires, Argentina. This is the same plant from which the cyanogenic glycoside cardiospermin was previously isolated [14].

Administration of radioactivity. L-Leucine-[U-^{14}C] (sp. act. 282 mCi/mmol) was supplied to the developing fruit in the amount of 20 to 50 μCi per inflorescence. The compound was dissolved in 0.2 M Pi buffer (pH 6.2, 1 ml) containing streptomycin sulfate (20 μg/ml) to prevent bacterial contamination and introduced into the stem with a capillary and wick by the system of ref. [15] as used for *Koelreuteria paniculata* [9]. Inflorescences were at about the same stage of development; the seeds were about one half their full size but did not show any signs of darkening. The seeds were harvested 3 weeks after introduction of label and stored under dry conditions until analysis.

Separation and identification of seed lipids. The seeds were ground, extracted with $CHCl_3$, the mixture filtered and concd to yield a light yellow oil. The seed oil was chromatographed on Si gel G with hexane–Et_2O–HOAc (95:5:1). After spraying with 0.2% 2′,7′-dichlorofluoroscein and viewing under UV light, two major bands were observed (R_f 0.3 and 0.1). These bands were subsequently identified as glycerides and cyanolipid **1** respectively by their IR and PMR spectra which were identical to those previously reported [2]. Oil samples were spotted on preparative Si gel G Plates (*ca* 50 mg per 20 × 20 cm plate) the bands scraped off and the lipid materials eluted with $CHCl_3$. The $CHCl_3$ soln was filtered through a small column of Si gel to remove 2′,7′-dicholorofluoroscein and subsequently concd. The samples were counted on a Packard 3350 Scintillation Spectrometer using the soln described ref. [17].

Transesterification. Glycerides and **1** were refluxed with MeOH containing 2% H_2SO_4 (1 ml) for 8 hr. The samples were then concd under vacuum and H_2O (25 ml) and Et_2O (25 ml) added. The Et_2O phase was dried, filtered and the Et_2O removed to yield a light yellow oil. Me esters from both cyanolipids and glycerides were then purified by prep-TLC and the radioactivity determined as before.

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