Table 1. Incorporation of acetate-2-^{14}C into the fatty acids of isolated spinach chloroplasts in thiocarbamate bathing solution

Compound Name	Conc.	Incorporation of Ac* into fatty acids: Saturated		Monoenoic		Dienoic		Total	
	(μM)	(CPM) (X10^2)	(%)	(CPM) (X10^2)	(%)	(CPM) (X10^2)	(%)	(CPM) (X10^2)	(%)
Butylate	0	49a*	(100)	47a	(100)	22a	(100)	118a	(100)
	0.8	30b	(62)	26b	(56)	21a	(97)	78b	(66)
	8	29b	(59)	24bc	(51)	18a	(84)	72b	(61)
	80	10c	(21)	5c	(12)	4b	(20)	20c	(17)
Pebulate	0	50a	(100)	47a	(100)	22a	(100)	118a	(100)
	0.8	46a	(92)	45a	(95)	32a	(146)	122a	(103)
	8	40a	(80	38a	(81)	21a	(96)	99a	(83)
	80	15b	(29)	8b	(16)	7a	(31)	29b	(24)
Vernolate	0	50a	(100)	47a	(100)	22a	(100)	118a	(100)
	0.8	44ab	(88)	49a	(104)	23a	(105)	115b	(98)
	8	37b	(76)	40a	(85)	26a	(121)	104c	(87)
	80	12c	(24)	7b	(16)	9b	(22)	24d	(20)

* Values in a column in a box followed by the same letter or letters are not significantly different at the 5% level. Each value is the average of ten determinations.

lation of dithiothreitol by acyl coenzyme A that was ether-soluble and destroyed by saponification [2]. The saponification prior to extraction and esterification conducted in these experiments precluded the measurement of such dithiothreitol artifacts. Although trienes compose the majority of spinach leaf fatty acids [3], demonstration of the formation of α-linolenic acid by subcellular spinach chloroplast fractions was reported in 1973 [4–6].

EXPERIMENTAL

Chloroplast extraction and experimental conditions were described previously [1]. 2-^{14}C-acetate (0.1 μCi) (6.11 mCi/mM) per treatment was applied. Lipids were saponified with 1 ml 40% NaOH at 85° for 1 hr. After acidification with 2 ml conc HCl, total fatty acids were extracted with petrol (bp 30 to 60°). Fatty acids were methylated in 14% BF_3-MeOH, separated into saturation subclasses by argentation TLC [7], and counted by liquid scintillation [1]. Each treatment was repeated ten times, and the data were analyzed by analysis of variance on a randomized block design.

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THE ORIGIN OF CYANOLIPIDS IN *KOELREUTERIA PANICULATA*

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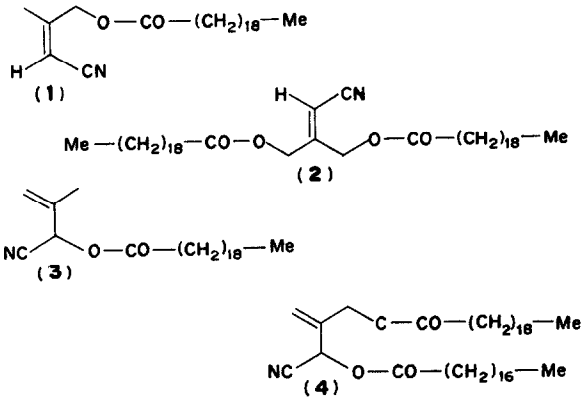
Key Word Index—*Koelreuteria paniculata*; Sapindaceae; cyanolipids; biosynthesis; origin from leucine.

Cyanolipids (**1** and **2**) were first discovered in *Koelreuteria paniculata* (Sapindaceae) by Mikolajczak [1,2] and coworkers in 1970. Unlike those of several other Sapindaceous seed oils, the compounds isolated from *Koelreuteria* do not possess the ability to liberate HCN upon hydrolysis. However, they are related to such compounds from *Ungnadia speciosa* (**3**) and *Cardiospermum halicacabum* [2] (**4**). The latter are derivatives of α-hydroxynitriles which possess the ability to liberate hydrogen cyanide under mild or enzymatic conditions. Compounds **1** and **2** appear to be structurally related to the cyanogenic compounds **3** and **4** respectively by an allylic rearrangement.

The aglycones of several cyanogenic glycosides have

been demonstrated to come from amino acids [4]; one of these, acacipetalin, found in *Acacia sieberiana* var. *woodii* and *A. hebeclada* is a derivative (glucoside) of these same α-hydroxynitriles found in the cyanolipid of *Ungnadia speciosa* (**3**) [5,6]. Recent work has shown that the aglycone of acacipetalin is derived from leucine in *A. sieberiana*. The similarity of this compound to all the cyanolipids described to date would suggest that leucine could be a logical precursor, but no work related to the biosynthesis of cyanolipids has been published, nor has the biosynthetic intermediacy of **3** and **4** as yet been tested. In an attempt to establish the pathways leading to these compounds we have conducted preliminary feeding experiments in which we have shown that leucine (U-^{14}C) is incorporated into cyanolipids (**1** and **2**) of *Koelreuteria paniculata*.



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. Each experiment was repeated at least twice.

One inflorescence in which the fruits were already fully expanded but still green did not incorporate significant amounts of label, nor did fruits at this stage of development contain the compounds of interest (as indicated by the NMR spectrum of a chloroform extract of the seed oil). However, immature inflorescences did develop **1** and **2** after harvesting and allowing them to stand for several weeks. It would appear that only a small amount of the necessary precursors were transported into the fruits at this stage of development.

Labelled U-^{14}C-leucine was incorporated into *Koelreuteria paniculata* seeds that were not fully formed; but as stated above was not significantly incorporated into fully formed but still immature seeds. If the seeds are at the proper stage of development when the radioactive precursors are introduced so that incorporation occurs, label does not appear to "turn over" rapidly and seeds that were allowed to remain on the tree from two to six weeks all retained approximately the same levels of labelled cyanolipids.

The data for incorporation of label into seed oils of *Koelreuteria paniculata* for samples harvested 18 and 38 days respectively are presented in Table 1. Each sample was obtained by feeding 18 μCi of U-^{14}C-leucine as previously described. Percentage incorporation was calculated by assuming a MW of 718 based on the relative percentages of the three components and the fatty acid composition of the whole oil. No significant differences were noted between these samples or several other labelling experiments which are not reported herein.

Table 1. Labelling data for *Koelreuteria paniculata* seed oil. Sample 1 (1.94 g, 18 days) and sample 2 (2.98 g, 38 days) were both fed 18 μCi of U-^{14}C-leucine

Sample	g counted	dpm	dpm/μmol	% incorporation
1	0.105	2216	15.1	0.1
2	0.128	1550	8.7	0.1

Two cyanolipids and the co-occurring glycerides were labelled but label is predominantly found in **1** and to a lesser extent in **2** (Table 2). A lesser degree of incorporation into glycerides suggests that a certain amount of the leucine is converted to acetyl CoA and this compound was subsequently incorporated into the fatty acid moieties of all three compounds. This hypothesis is confirmed by transesterification of the cyanolipids and glycerides followed by measurement of label in the derived methyl esters. Significant loss of label from the two cyanolipids but not from the co-occurring glycerides indicates that the label of **1** and **2** resides largely in the "aglycone" portion of the molecule (data based on two replications of three oil samples). This confirms our predictions based on structural considerations and suggests that leucine is indeed the precursor of cyanolipids such as **1** & **2** in Sapindaceous plants.

EXPERIMENTAL

Administration of radioactivity. L[U-^{14}C]-leucine (sp. act. of 282 mCi/mM, Malinckrodt Chemical Co.) was supplied to the developing fruits in the amount of 18 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 Fowden and Mazelis [8]. Inflorescences at two differing stages of development were used; in one of these (full, green), the fruits were fully expanded but still green. In the other stage, the fruits were not fully formed at the time of feeding (underdeveloped fruit). Samples 1 and 2 (see Tables 1 and 2) were obtained by labelling "underdeveloped inflorescences" on August 12 and harvesting on August 30 (Sample 1) and September 21 (Sample 2). The samples were worked up one week after harvest. The seeds were removed, frozen with liquid N_2 and ground. The resulting meal was

Table 2. Labelling data for the major components of the oil from *Koelreuteria paniculata* seeds and the corresponding methyl esters derived from these components

Sample Oil	Band	Weight (mg)	cpm	cpm/mg
1	glycerides	75	672	9
	2	34	562	17
	1	42	1111	26
2	glycerides	63	299	5
	2	42	288	7
	1	24	735	31
Methyl Esters				
1	glycerides	42	309	7
	2	22	211	10
	1	22	110	5
2	glycerides	38	107	3
	2	28	128	5
	1	9	49	5

extracted overnight with $CHCl_3$ and filtered to yield a light yellow oil.

Separation and identification of the cyanolipids. Seed oil was subjected to TLC on Si gel G plates using Et_2O–hexane, 1:3 [1,2]. After spraying with 0.2% 2',7'-dichlorofluoroscein and viewing under UV light, 3 major bands were observed (R_f 0.65, 0.53 and 0.45). These bands were subsequently identified as glycerides and compounds **2** & **1** respectively, by their IR, NMR and MS, which were identical to those previously reported [1,9]. Oil samples were spotted on preparative Si gel G plates (10–20 mg per 20 × 20 cm plate), the bands scraped off and the lipid materials desorbed with $CHCl_3$. The $CHCl_3$ solution was filtered through a small column of silica gel to remove 2',7'-dichlorofluoroscein and subsequently concentrated. The samples were counted on a Packard 3350 Scintillation spectrometer using the counting solution described by Bray [10].

Transesterification of glycerides and compounds I & II. Glycerides (R_f 0.65) and **1** and **2** (R_f 0.45 and 0.53) were transesterified by refluxing with MeOH containing 2% H_2SO_4 (1 ml) for 8 hrs. The samples were then concd under vacuum and H_2O (25 ml) and ether (25 ml) added. The ethereal phase was dried, filtered, and the Et_2O removed to yield a light yellow oil. Methyl esters from both cyanolipids and glycosides were then purified by preparative TLC and radioactivity determined as described above.

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METHYLPHENANTHRENES FROM *SAGOTIA RACEMOSA**

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Key Word Index—*Sagotia racemosa*; Euphorbiaceae; 6-hydroxy-7-methoxy-1,2-dimethylphenanthrene; 6-hydroxy-7-methoxy-1,2-dimethyl-9,10-dihydrophenanthrene; micrandrol-E; micrandrol-F.

Abstract—The trunk wood of *Sagotia racemosa* Baill. (Euphorbiaceae) contains two previously unknown micrandrols E (6-hydroxy-7-methoxy-1,2-dimethylphenanthrene) and F (6-hydroxy-7-methoxy-1,2-dimethyl-9,10-dihydrophenanthrene).

The micrandrols A (**1a**), B (**2a**) and C, considered to be diterpenoids [1], were located originally in *Micrandropsis scleroxylon* W.Rodr. [2]. Two additional compounds of this series, micrandrols E (**1b**) and F (**2b**), occur in *Sagotia racemosa* Baill., a further arboreous Amazonian species of the Euphorbiaceae.

UV spectroscopy showed micrandrol-E (**1b**), $C_{17}H_{16}O_2$, to be a hydroxylated phenanthrene. Additional substitution by two methyls and one methoxyl became evident upon inspection of the ^{1}HMR spectrum and led to the formula $C_{14}H_6.OH.OMe.Me_2$. The compound is, nevertheless, not simply a monomethyl ether of **1a**, since *O*-methylmicrandrol-E (**1d**) is not identical with di-*O*-methyl **1a** (**1c**) [1]. In spite of this fact, the substitution pattern of **1a** must prevail in micrandrol-E. The ^{1}HMR spectra of both compounds in $(CD_3)_2CO$ contain, in addition to the AB signal typical of protons at C-9 and 10 of a phenanthrene nucleus, two pairs of signals, one for *ortho*- and one for *para*-related protons, both encompassing the relatively unprotected C-4 (**1a**: τ 1.74; **1b**: τ 1.71; both *d*, *J* 9.0 Hz) and C-5 (**1a**: τ 2.02, **1b**: τ 1.98; both *s*) positions. While thus the chemical shifts of H-4 and H-5 for micrandrols A and E are closely comparable, the difference for H-3 (**1a**: τ 2.80, **1b**: τ 2.63, both *d*, *J* 9.0 Hz) and H-8 (**1a**: τ 2.40, **1b**: τ 2.73 both *s*) can be rationalized by the allo-

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