

*sativa* (Leguminosae) [5]. The occurrence of hydroxyarginines within the Leguminosae has been reviewed by Bell [6].

#### EXPERIMENTAL

Pea seedlings were grown in the dark in Petri dishes for 4 days and 200 g homogenised in 5 vol of 5% trichloroacetic acid. After standing for at least 7 days, the extract was centrifuged and the supernatant passed through a column ( $2 \times 3$  cm) of Dowex-50 W  $\times$  2, 200–400 mesh ( $H^+$  form). The column was washed with  $H_2O$  and most of the amino acids were eluted with 1.5 M  $NH_4OH$  (50 ml) and discarded. The column was washed with  $H_2O$  (75 ml) until free of  $NH_4OH$  and the inorganic cations eluted with 0.4 M HCl (40 ml). The amine/lactone fraction was then eluted with 6 M HCl (40 ml) and dried. On dissolving in 0.1 M HCl (2 ml), 10  $\mu$ l was subjected to TLC on cellulose CC-41 buffered at pH 2 with KCl–HCl, using phenol:pH 2 buffer (5:1) as solvent [7]. A yellow-brown spot suggesting a lactone was detected at  $R_f$  0.56 with ninhydrin as chromogenic reagent. The spot was also Sakaguchi positive, indicating the presence of the guanidino group. On drying the amine fraction, 2 ml of 18 M  $NH_4OH$  was added to hydrolyse the lactone. After 18 hr, the soln was diluted with 8 vol  $H_2O$  and re-applied to the column ( $H^+$  form). The unknown was eluted in 1.5 M  $NH_4OH$ , dried, dissolved in 0.1 M HCl (5 ml) and left for 24 hr to relaxonize. By this means the unknown was obtained relatively free of contamination. The properties of the unknown ( $R_f$ , guanidino group, lactonization), in agreement with the properties of authentic material, suggested that it is  $\gamma$ -hydroxyhomoarginine. The yield was ca 20 mg. Attempts to crystallize the syrupy product obtained by concentrating the final fraction

failed, as found previously for  $\gamma$ -hydroxyarginine [4]. This may be due to admixture of di and monohydrochlorides (E. A. Bell, personal communication). MS of the lactone at 220° gave peaks at  $m/e$  374, 373, 313, 297 (all less than 1%), 275 (6), 259 (77), 168 (39), 165 (46), 151 (66), 96 (100), 59 (80), indicating dimerization.  $IR_{\nu_{max}}^{KBr}$  showed 1780 ( $\gamma$ -lactone) 1650, 1410, 1350, 1310, 1220, 1200, 1080, 1000, 970, 780  $cm^{-1}$ . MS and IR of authentic (2 HCl) and extracted material were identical.

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#### CYANOLIPIDS IN *SAPINDUS EMARGINATUS* SEED OIL

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**Key Word Index**—*Sapindus emarginatus*: Sapindaceae; cyanolipids; triglycerides; argentation TLC; GLC; MS; fatty acid composition; double bond position.

Four types of cyanolipids, present individually or in pairs, have been identified in the seed lipids of the Boraginaceae [1,2] and some Sapindaceae species [3–8]. In this paper we report the isolation, identification and composition of one such cyanolipid, namely, the fatty acid diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol, in the oil of *Sapindus emarginatus* Vahl. (a soapnut). The fatty acid composition of the cyanolipid components is also compared with that of triglycerides.

*S. emarginatus* seed kernels contained 41% oil. On Si gel G TLC, the oil gave two spots (triglyceride,  $R_f$  0.77 and cyanolipid,  $R_f$  0.52) with  $Et_2O$ –petrol (40–60°) (PE) but only a single spot with  $C_6H_6$ . This TLC behaviour suggested that the cyanolipid is likely to be a diester of 1-cyano-2-hydroxymethylprop-1-ene-3-ol [5]. The oil was resolved into a triglyceride fraction (82%) and a cyanolipid fraction (16%) by preparative-TLC. The cyanolipid fraction gave  $\nu_{max}$  2220  $cm^{-1}$  and  $\epsilon$  18845 at  $\lambda_{max}$  208 nm. The PMR showed peaks at  $\tau$  9.14, 8.75, 8.05, 7.97, 7.67, 5.33, 5.13, 4.7 and 4.45 as also shown by the reference sample of fatty acid diesters of 1-cyano-2-hy-

roxymethyl-prop-1-ene-3-ol, isolated from *Cardiospermum halicacabum* seed oil [5]. The major  $M^+$  peaks in the MS of the cyanolipids were  $m/e$  671 and 669 followed by low intensity peaks corresponding to  $M^+$  of 699, 697, 645, 641 and 615. Prominent peaks belonging to  $R-C=O^+$ ,  $R-C-O^+$ , were also obtained. The picrate test



was negative, as is the case with the similar cyanolipid of *Koeleruteria paniculata* seed oil [4]. GLC of the Me esters gave the composition reported in Table 1.

The total cyanolipid was separated into three fractions (26.8, 67.7 and 5.5%) by  $AgNO_3$  TLC, based on the degree of unsaturation of the fatty acid moieties as shown by the magnitude of peak at  $\tau$  4.7. In the MS of the three fractions, the  $M^+$  ions of decreasing intensity were  $m/e$  669, 641 and 697; 671, 643, 615 and 699; and 645, 673, 617, 589 and 701. These fractions gave  $\epsilon$  16235, 18730 and 20507 at  $\lambda_{max}$  208 nm, corresponding to the major  $M^+$  peaks in the MS. The fatty acid compositions are recorded in Table 1. A higher proportion of eico-

Table 1. Fatty acid composition of triglycerides, cyanolipids and the cyanolipid fractions of *Sapindus emarginatus* seed oil

Lipid	Concn wt %	Fatty acid, (mol %)						
		16:0	18:0	18:1	18:2	20:0	20:1	22:0
Triglycerides	82.0	8.8	3.0	64.7	2.9	11.8	8.8	—
Cyanolipids	16.0	3.9	1.2	49.8	Trace	33.2	11.9	Trace
Argentation TLC fractions								
First	26.8	—	—	55.0	—	—	45.0	—
Second	67.7	2.8	—	49.2	—	46.5	—	1.5
Third	5.5	46.8	3.3	—	—	49.9	—	—

sanoic acid occurs in the cyanolipids than in the triglycerides.

MS of the methoxy derivatives [9] gave peaks at *m/e* 157, 171, 201, 215, 229 and 243. These showed the presence of a double bond at the 9 position from the terminal Me group in both the 18:1 and 20:1 acids.

#### EXPERIMENTAL

Oil was Soxhlet extracted from *S. emarginatus* kernels with PE. TLC was carried out on 0.25 mm Si gel G layers using both C<sub>6</sub>H<sub>6</sub> and Et<sub>2</sub>O-PE-HOAc (15:85:1). Cyanolipids were isolated on 1 mm Si gel G layers using 15% Et<sub>2</sub>O in PE. Cyanolipids were fractionated on 0.5 mm layers of 5% AgNO<sub>3</sub>-impregnated Si gel G using CCl<sub>4</sub>-CHCl<sub>3</sub>-EtOH (50:50:1), and detected with ethanolic 2', 7'-dichlorofluorescein and estimated gravimetrically. The Me esters were prepared using 1% NaOMe in MeOH. Total Me esters from the triglycerides and the monoene Me esters from the cyanolipids, isolated by AgNO<sub>3</sub> TLC were converted to methoxy derivatives and purified [9]. The PMR spectra were measured in CCl<sub>4</sub> at 60 MHz.

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#### ASATONE IN ASARUM (ARISTOLOCHIACEAE)

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**Key Word Index**—*Asarum*; Heterotropa; Aristolochiaceae; *Asarum hexalobum*, *A. asperum*, *A. oblongum*, *A. tamaense*, *A. tamaense* var. *muramatsui*, *A. nipponicum*; *n*-hexane extractives; asatone.

*Plants and source.* *Asarum hexalobum* F. Maekawa and *Asarum asperum* F. Maekawa collected in May at Hiroshima Prefecture. *Asarum oblongum* F. Maekawa collected in July at Shimane and Tottori. *Asarum tamaense* Makino collected in December at Tokyo. *Asarum tamaense* var. *muramatsui* (Makino) Sugimoto and *Asarum nipponicum* F. Maekawa var. *nipponicum* collected in December at Shizuoka. *Asarum hexalobum* var. *perfectum* F. Maekawa and *Asarum satsumense* F. Maekawa collected in January at Kagoshima. *Asarum unzen*

(F. Maekawa) Kitamura collected in December at Saga. *Asarum asaroides* (Morr. et Decne) Makino collected in May. *Asarum curvistigma* F. Maekawa collected at Yamanashi in December. *Uses.* Medicinal. *Previous work.* Recently Yamamura *et al.* [1,2] isolated a new neolignan, Asatone from *n*-hexane extractives of *A. taitonense* Hayata growing in Formosa and from *A. nipponicum* var. *brachypodion*, *A. takaoi* F. Maekawa var. *takaoi*, *A. hexalobum* var. *perfectum*, *A. curvistigma* F. Maekawa of Japan.