The basic portion (citric acid soluble) of the chloroform extract on chromatographic resolution over alumina afforded a yellow solid from benzene–chloroform (1:1) eluate. The solid was crystallized from benzene, m.p. 203° and on analysis was found to contain nitrogen. This compound was identified as haplopine[11] (**2**) from UV, IR, NMR and MS data. On methylation with diazomethane haplopine yielded skimmianine.

Other constituents of the root-bark isolated and characterised are skimmianine, γ-fagarine, marmesin, marmin, xanthotoxin, umbelliferone and lupeol.

Voucher specimen of the root bark of *Aegle marmelos* Correâ has been preserved in our laboratory. The plant was collected from the Indian Botanic Gardens, Shibpur, Calcutta and identified by botanist Dr. P. C. Dutta, Department of Botany, Calcutta University.

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A NEW CYANOGENIC GLYCOSIDE FROM *CARDIOSPERMUM HIRSUTUM*

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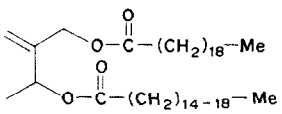
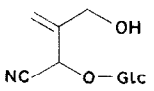
Key Word Index—*Cardiospermum hirsutum*; Sapindaceae; cardiospermin; cyanogenic glucoside.

Cardiospermum, or balloon-vine, is commonly cultivated in warmer areas of the world. The genus consists of about 12 species, two of which are cultivated in the United States, *C. halicacabum* in the southeastern portion of the country and *C. hirsutum* Willd. in California.[1] The plant is a woody vine with inflated capsular fruits, hence the name. The seed oil of both species has been shown to contain cyanolipid of structure **1** below.[2,3]



(**1**)



Cardiospermin (**2**)

We have recently isolated and characterized a new glucoside (**2**) from the vegetative portion of *C. hirsutum* for which we propose the name cardiospermin. The compound is similar in structure to the cyanolipid.

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[1] BAILEY, L. H. (1942) *The Standard Cyclopedia of Horticulture*, Vol. I, Macmillan, London.
[2] MIKOLAJCZAK, K. L., SMITH, C. R. JR. and TJARKS, L. W. (1970) *Lipids* **5**, 812.
[3] SEIGLER, D. (1974) *Phytochemistry* **13**, 841.

The IR spectrum (KBr disc) has absorptions at 3400–3200 (*s*, OH stretch), 2980 (*m*, C–H stretch), 1643 (*m*) and 1390 (*s*), terminal vinyl group, 1565 (*m*) and 1060 (*m*, C–O stretch) cm^{-1}. The absence of any nitrile absorption (*ca* 2250 cm^{-1}) is to be expected.[5] The NMR spectrum of the trimethylsilyl ether of the new cyanogenic glucoside in CCl_4 has a pair of singlets at 5·51 and 5·39 δ (2 protons, vinyl), a singlet at 5·11 δ (1 proton, cyanohydrin proton), a doublet centered at 4·44 δ (*J* 7·0 cps, 1 proton, anomeric proton of the sugar), a doublet centered at 4·13 δ (*J* 5·0 cps, 2 protons, AB multiplet of the aglycone), a doublet centered at 3·72 δ ($-CH_2-$OTMS of sugar) overlapping a broad multiplet from 3·1 to 3·8 δ (sugar protons, total of 5). This spectrum is similar in many respects to that of cyanolipid (**1**),[4,5] and by comparison of these spectra and those cyanogenic glycosides, assignment of protons was fairly straightforward.

The NMR spectrum of the TMS ether in deuterobenzene has singlets at 5·64, 5·41 and 5·29 δ (corresponding to 1 proton each), a doublet centered at 4·65 δ (*J* 7·5 cps, 1 proton), a quartet centered at 4·24 δ (*J* 14 cps, 2 protons) and a complex multiplet from 3·0 to 3·88 δ (5 protons). The change of the two central peaks of the AB quartet in CCl_4 to a more distinct quartet in C_6D_6 demonstrates that the methylene protons are nonequivalent likely because of the proximity of the sugar and the nitrile moieties.

However, it was not possible to determine the number of OH groups in the original compound from the TMS ether. To resolve this problem we prepared the acetate and determined its NMR spectrum. The NMR spectrum of the acetate ($CDCl_3$) had 4 singlets at 2·00, 2·03, 2·06 and 2·08 δ (acetate, 15 protons in ratios of 1:1:1:2 respectively), a pair of singlets at 5·54 and 5·66 δ (vinyl, 2 protons), a singlet at 5·19 δ (cyanohydrin proton, 1 proton), a multiplet at 4·2 δ ($-CH_2OAc$, 2 protons), a singlet at 4·64 δ (methylene of the aglycone, 2 protons), a doublet at 4·83 δ (*J* 7 cps, 1 proton, anomeric proton of sugar), and other multiplets corresponding to 4 protons of the sugar (from 4·0 to 5·2 δ). Thus, the original compound has one OH group of the aglycone portion of the molecule.

EXPERIMENTAL

NMR spectra were obtained on a Varian HA 100 Spectrometer (100 MHZ in CCl_4 and C_6D_6) and IR Spectra were measured as KBr discs on a Beckman IR-33 Spectrophotometer. GLC analyses were conducted on a Packard Becker Model 409 gas chromatograph fitted with FID. on a 3% OVl column (10 ft × 1/8 in., stainless steel) programmed from 150° to 190° at 5°/min.

Isolation of the cyanogenic glucoside. Cardiospermum hirsutum plant material (10 kg) (D. Seigler 6150, University of California Arboretum, Davis, voucher specimen deposited in the University of Illinois herbarium) was ground in a blender under ethanol, liquid N_2 being added before grinding. The mixture was then heated to boiling for 30 min. The ethanol extracts (4 l.) were conc. and extracted with $CHCl_3$ (500 ml) $CHCl_3$ to remove coloring matter. The aq. phases were centrifuged and solids removed. The soln was then conc. to a red–brown syrup under vacuum. This syrup was strongly cyanogenic when β-glucosidase was added but negative without it. The syrup was added to rapidly stirred, boiling ethanol, whereupon a large amount of fine precipitate formed. This ppt. was removed by filtration; it gave a negative test for cyanide. The ethanol soln was again conc. under vacuum. The syrup, mixed with a small amount of H_2O, was chromatographed on silica gel (300 g, Grace, Davison-Chemical, Grade 12) using *n*-PrOH as eluent. 100 ml fractions were collected and most of the glucoside was eluted in fractions 3–18.

Fractions 5–9 were particularly cyanogenic and were combined and further purified by PC on Whatman 3 MM paper with MeCOEt–Me_2CO–H_2O (15:5:3). After drying, a 1 cm strip was removed from the chromatograms, cut into 1 cm serial sections and each tested for the presence of cyanogenic compounds, by placing each filter

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paper section in a separate small vial, moistening the paper with a soln of β-glucosidase, placing a strip of picrate paper in the top of the vial and closing with parafilm and a cork. After several hr at 37°, the presence of glycoside was indicated by the characteristic maroon discoloration of the picrate paper.[6] Two active bands were obtained at R_fs 0·51 and 0·26 respectively. These bands were cut out and the cyanogenic compounds desorbed with methanol, to yield cardiospermin (R_f 0·51) as a solid which we were not able to crystallize and a polar cyanogenic compound (R_f 0·26). We are currently investigating the structure of the second compound.

Identification of the sugar of cardiospermin. Cardiospermin (5 mg) was dissolved in H_2O (1 ml) and β-glucosidase (1 mg) added. The mixture was allowed to stand overnight at 40°. Comparison of the hydrolysate with glucose by both PC (Whatman 3MM, EtOAc–pyridine–H_2O (12:5:4)[7] and TLC on silica gel (Silica Gel G Merck, PrOH) indicated that the sugar produced on hydrolysis was glucose. Integral data from the NMR spectrum indicate cardiospermin is a monoglycoside.

Acetylation of cardiospermin with Ac_2O/NaOAc yielded a brown syrup. The NMR spectrum of this compound was measured in $CDCl_3$.

Preparation of TMS ether of cardiospermin. Samples of this glucoside were derivativized in a similar manner to that which Mabry *et al.*[7] used for flavonoids.

Measurements of total cyanide in Cardiospermum hirsutum. Because of the method of analysis and separation it was not possible to obtain an accurate yield of the glycoside. However, vegetative plant material of *C. hirsutum* liberates 18·5 μmol hydrocyanic acid per g fr. wt when analyzed for cyanogenic glycoside content[8] by colorimetric determination of the cyanide released following enzymatic hydrolysis for 48 hr.[9] This represents the cyanide contained in both the glycosides present.

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DAPHNEOLONE IN ROOTS OF *DAPHNE ODORA*

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Key Word Index—*Daphne odora*; Thymeleaceae; δ-phenylvalerophenone; daphneolone.

In the course of the isolation of the nematocidal substances from the roots of *Daphne odora*,* we obtained a new phenolic compound, for which we proposed the name, daphneolone. In this communication we report the isolation and the structure of this compound.

* The isolation of nematocidal components will be reported in detail elsewhere.