Phytochemistry, 1973, Vol. 12, pp. 2461 to 2468. Pergamon Press. Printed in England.

IPOLEAROSIDE: A NEW GLYCOSIDE FROM *IPOMOEA LEARI* WITH ANTI-CANCER ACTIVITY*

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(*Received* 19 *March* 1973. *Accepted* 17 *April* 1973)

Key Word Index—*Ipomoea leari*; Convolvulaceae; hydroxy fatty acid glycoside; ipolearoside; methyl ipolearate; 3,11-dihydroxyhexadecanoic acid methyl ester; structural analysis; NMR; MS.

Abstract—Ipolearoside, a new glycoside with anticancer activity, has been isolated from *Ipomoea leari* Paxt. On acid hydrolysis in methanol, it gave the aglycone methyl ester, characterised as methyl 3,11-dihydroxyhexadecanoate. Ipolearoside is a complex glycoside of 3,11-dihydroxyhexadecanoic acid and glucose, rhamnose and fucose.

INTRODUCTION

A PROGRAMME aimed at screening Indian plants over a wide range of biological activities[1] led to the observation of significant activity against Walker carcinosarcoma 256 in rats, given by aqueous ethanol extracts of the whole plants of *Ipomoea leari* Paxt., an ornamental American plant which has been successfully cultivated around Darjeeling.

Chemical investigations of *Ipomoea* glycosides reported in the literature[2–8] indicate that the glycosidic constituents of *Pharbitis nil* Choisy and of *I. parasitica* (HBK) Don., *I. muricata*, *I. purga* Hayne, *I. orizabensis* Ledanois, *I. operculata* Martin and *I. turpethum* Brown possess mono or dihydroxy C_{14-16} fatty acids as aglycones with glucose, rhamnose, quinovose and fucose as the sugar components. This communication describes the isolation and structure elucidation of the anti-cancer active glycoside contained in this plant.

RESULTS AND DISCUSSION

The concentrate of the EtOH extract of the air dried plant was subjected to solvent fractionation with organic solvents to localise the biological activity. The insoluble fraction (see Experimental) containing the crude glycoside was purified by column chromatography (silica gel, using $CHCl_3$–MeOH (3:1) as eluents). The light brown material obtained, on repeated MeOH–Et_2O precipitation, gave a TLC pure amorphous powder, designated ipolearoside. It was insoluble in $CHCl_3$, C_6H_6 and C_6H_{14} but soluble in MeOH and $CHCl_3$–MeOH (3:1) mixture. Ipolearoside analysed for $C_{40}H_{72}O_{21}$. 1·5 H_2O; m.p. 184–187°,

* Communication No. 1818 from the Central Drug Research Institute, Lucknow.

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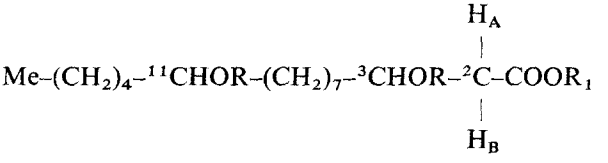
$[\alpha]_D^{20}$ −50° (*c* 1·0, MeOH). Its IR spectra showed OH and C=O absorption at 3400–3300 cm^{-1} and at 1725 cm^{-1} respectively. Hydrolysis with 10% HCl in MeOH gave the aglycone methyl ester, designated methyl ipolearate.

Characterization of Methyl Ipolearate

Methyl ipolearate (II), m.p. 66–67°, analysed for $C_{17}H_{34}O_4$.* Its IR spectrum indicated the presence of free hydroxyl (absorption at 3350 cm^{-1}) and an ester carbonyl (ν_{max} 1735 cm^{-1}). The presence of the ester group was also supported by its NMR spectrum which showed a sharp singlet of three protons at δ 3·75 ppm (–COOMe). The other characteristic features of the NMR spectrum of methyl ipolearate (II) indicating it to be a straight chain compound, were the presence of an end C–Me group signal at δ 0·9 ppm; a two-proton signal centred at δ 2·50 ppm (*J* 5·5, 7·5 Hz) corresponding to the methylene protons adjacent to the ester carbonyl and a two-proton broad signal centred at δ 2·20 ppm. The latter signal disappeared on deuterium exchange indicating the presence of two hydroxyl groups in the molecule. The protons alpha to the hydroxyl groups resonated at δ 3·60 and 4·05 ppm (1H each) as unresolved multiplets indicating that both the hydroxyl groups were secondary in nature. The presence of two-OH groups was confirmed by acetylation of II to give a diacetate (III), $C_{21}H_{38}O_6$ which was a viscous oil. The NMR spectra of III showed the presence of two acetyl methyl protons (6H) at δ 2·10 ppm. The signal of the two protons alpha to the hydroxyl groups was now shifted to δ 5·0 and 5·3 ppm; the shift of more than 1 ppm suggesting that the hydroxyl groups were secondary. Signals of the methylene protons adjacent to the carbomethoxyl now appeared at δ 2·60 ppm, as a sharp doublet (*J* 7 Hz). In the spectrum of the methyl ipolearate these two protons appeared as a three-peak signal (one of the peaks of the doublet being further split into another doublet by 2 Hz). This indicated the presence of an asymmetric centre adjacent to the C-2 methylene group. One of the hydroxyl groups could, therefore, be placed at C-3 in order that the C-2 methylene protons become non-equivalent. The chemical shift difference between them was small as compared with the geminal coupling constant. The *AB* pattern of the geminal coupling was distorted. Each methylene proton being further split by the vicinal C-3 proton with a coupling constant of 7·5 and 5·5 Hz respectively. Two of the peaks overlapped; the end peaks being lost in the base line noise. The net result, therefore, was three peaks. Such splitting is also seen in the spectrum of aspartic acid. After acetylation of methyl ipolearate, the C-2 methylene protons become deshielded and the shift of the vicinal C-3 proton made the C-2 protons equivalent. This resulted in the appearance of a doublet with a coupling constant of 7·0 Hz.



(II) R = H; R_1 = Me
(IIa) R = R_1 = H
(III) R = Ac; R_1 = Me
(V) R = H; R_1 = Et

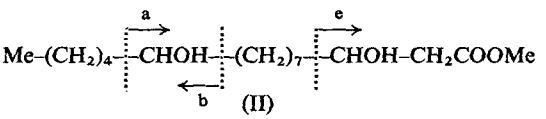
* In the MS the parent peak appeared at *m/e* 284 corresponding to (M^+-H_2O) and the molecular ion peak was not observed due to easy loss of water molecule.

The presence of C-3 hydroxyl group *vis-à-vis* the splitting of C-2 methylene by the proton alpha to the hydroxyl group, was confirmed by decoupling at δ 2·35 ppm; the C-2 methylene protons collapsed to a broad singlet at δ 2·4 ppm. This finding was in agreement with the presence of a β-hydroxyl group in methyl ipolearate (II). The position of the other hydroxyl group was fixed at C-11 on the basis of MS studies with methyl ipolearate (II) and its oxidation product, diketo methyl ipolearate (IV), $C_{17}H_{30}O_4$ (M^+ 298), m.p. 49–50°, produced by reaction with Jones' reagent. The IR spectrum of this compound showed the absence of hydroxyl absorption and presence of additional carbonyl absorption at 1710 cm^{-1} along with the 1730 cm^{-1} maxima of the ester carbonyl. The NMR spectrum of the methyl diketoipolearate (IV) showed a shift of the C-2 methylene proton signal to δ 3·45 ppm. This signal was now seen as a singlet due to the generation of an additional carbonyl group at C-3. The ester methyl resonated at δ 3·75 ppm. The signals for the protons alpha to the two hydroxyl groups were absent and instead a broad triplet appeared at δ 2·4 ppm (6H), assignable to the C-4 methylene and the pair of methylene groups adjacent to the other carbonyl on either side.

The MS of the methyl diketoipolearate (IV) as well as that of methyl ipolearate (II) showed the loss of C_5H_{11} (*m/e* 71) giving rise to a fragment (a) of *m/e* 227 and 231 respectively, showing the fission of C_{11}–C_{12} bond and indicating the presence of a functional group at C-11. The presence of an ion *m/e* 99 in the spectrum of IV may be assigned to fragment (b) arising from the alpha cleavage of the straight chain carbonyl compounds. The corresponding fragment in methyl ipolearate appeared at *m/e* 101 in agreement with the location of the second hydroxyl group at C-11 in methyl ipolearate. The other cracking pattern showing the α- and β-cleavage of the straight chain carbonyl skeleton was also evident from the mass spectrum of IV (Table 1).

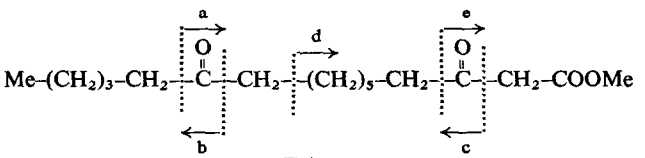
TABLE 1. MS OF METHYL IPOLEARATE AND THE CORRESPONDING DIKETONE

Compound	Mass fragments (*m/e*)							
	M^+	M^+-H_2O	M^+-OMe	(a)	(b)	(c)	(d)	(e)
II	302 (absent)	284 (parent peak)	—	231	101	—	—	103
IV	298	—	267	227	99	225	185 (base peak)	101



(II)

$\overset{+}{H}\ddot{O}$=CH$(CH_2)_7$–CHOH–CH_2COOMe (IIa) *m/e* 231; Me–$(CH_2)_4$–CH=$\overset{+}{\ddot{O}}$H (IIb) *m/e* 101; $\overset{+}{H}\ddot{O}$=CH–CH_2–COOMe (IIe) *m/e* 103.



(IV)

$\overset{+}{O}$≡C–$(CH_2)_7$–$\overset{O}{\overset{\|}{C}}$–$CH_2$–COOMe (IVa) *m/e* 227; Me–$(CH_2)_4$–C≡$\overset{+}{O}$ (IVb) *m/e* 99; Me–$(CH_2)_4$–CO–$(CH_2)_7$–C≡$\overset{+}{O}$ (IVc) *m/e* 225; $\overset{+}{H}_2$C–$(CH_2)_5$–CO–CH_2–COOMe (IVd) *m/e* 185; $\overset{+}{O}$≡C–CH_2–COOMe (IVe) *m/e* 101.

The structure of methyl ipolearate was, therefore, established as 3,11-dihydroxyhexadecanoic acid methyl ester. 3,11-Dihydroxytetradecanoic acid, 11-hydroxyhexadecanoic acid and 3,12-dihydroxyhexadecanoic acid are the other hydroxy fatty acids reported so far from higher plants.

Characterization of Ipolearoside

Ipolearoside (I) could be titrated with N/10 NaOH, showing that the carboxyl group in ipolearoside was not esterified. Hydrolysis of ipolearoside with EtOH–HCl gave the corresponding ethyl ester (V) $C_{18}H_{36}O_4$, m.p. 63–64°. The aglycone of ipolearoside is, therefore, 3,11-dihydroxyhexadecanoic acid (IIa).

Methylation of ipolearoside with Me I–NaH yielded a fully methylated glycoside (VI), $C_{52}H_{96}O_{21}$, m.p. 86–88°, which on acid hydrolysis ($MeOH–H_2SO_4$) gave aglycone-*B* (VII), $C_{18}H_{36}O_4$ as a viscous oil. VII proved to be 3-OMe-11-OH-hexadecanoic acid methyl ester. The NMR spectrum of VII showed signals of the carbomethoxymethyl protons at δ 3·75 ppm and of one *O*-methoxymethyl proton at δ 3·35 ppm, indicating that one of the hydroxyl groups of the aglycone IIa was free, and that the sugar linkage in ipolearoside was, therefore, through one of the two hydroxyl groups. Further, the C-2 methylene protons now resonated as a doublet at δ 2·5 ppm (J 5 Hz) and not as a three-peak signal as in the case of both methyl and ethylipolearates. Methylation of the free C-3 hydroxyl in aglycone-*B*, established the linking of the sugar units through C-11 hydroxyl in ipolearoside.

TABLE 2. CHROMATOGRAPHY OF SUGARS FROM IPOLEAROSIDE

Solvent system* / Reference sugar	R_f value I	R_f value II	R_{Rha} value† III
D-Glucose	0·18	0·24	0·25
D-Fucose	0·38	0·74	0·52
L-Rhamnose	0·49	0·86	1·00
D-Quinovose	0·48	0·84	0·88
Sugar portion of ipolearoside (I)	0·18	0·24	0·25
	0·38	0·74	0·52
	0·48	0·86	1·00

* Solvent system: I, *n*-BuOH–HOAc–H_2O (4:1:5). II, EtOAc–C_5H_5N–H_2O (2:1:2). III, BuOH–MeCOEt (1:1) saturated with boric acid–borax buffer[9] (for 72 hr).

† Relative to movement of L-rhamnose (28 cm).

Aqueous hydrolysis of ipolearoside followed by PC, showed the presence of glucose rhamnose and fucose (Table 2). On periodate oxidation, ipolearoside consumed 5 mol of $NaIO_4$ and yielded 2 mol HCO_2H. The periodate oxidation product after borohydride reduction followed by hydrolysis showed the presence of rhamnose as the only sugar. This suggested that there was branching of sugar units at rhamnose which was also the first sugar attached to the aglycone. The ratio of hexose to methyl pentose was found[10] to be 1:3 while that of fucose to rhamnose was 1:2. This indicated that for 1 mol of glucose there were 2 mol of rhamnose and 1 mol of fucose.

[9] KRAUSS, M. T., JAGER, H., SCHINDLER, O. and REICHSTEIN, T. (1960) *J. Chromatog.* **3**, 63.
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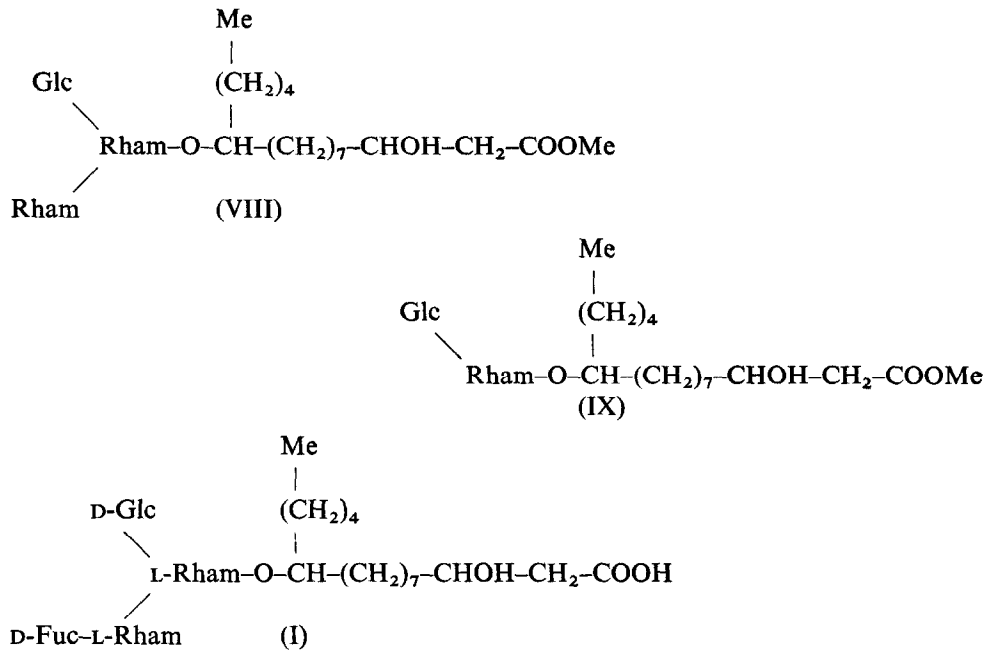

Hydrolysis of ipoleroside with takadiastase, liberated glucose, showing glucose to be one of the end sugars in the molecule. Partial hydrolysis of ipolearoside with 70–80% HCO_2H followed by methylation of the resultant product by diazomethane showed three spots on TLC which on chromatography over silica gel yielded two new degraded glycosides-*B* and -*C* as amorphous products. Both the glycosides-*B* and -*C* on acid hydrolysis yielded glucose and rhamnose. The presence of fucose and rhamnose as the sugars in the aqueous hydrolysate of the HCO_2H hydrolysis of ipolearoside and the fact that glucose and rhamnose still remained attached to glycosides-*B* and -*C*, indicated that their structures must be VIII and IX. The branching of sugar units was evident from HCO_2H generation on periodate oxidation and loss of glucose on enzymatic hydrolysis.

TABLE 3. RETENTION TIMES IN GLC OF METHYLATED SUGARS

Component sugars of methyl	R_t (min)
Ipolearoside (VI)	2·5,* 5·9, 7·8, 18·2
Methyl pyranoside of 2,3,4,6-*tetra*-*O*-Methyl-D-glucose	18·2
2,3,4-tri-*O*-Methyl-D-fucose†	7·8
2,3-di-*O*-Methyl-α-L-rhamnose	5·9
2,4-di-*O*-Methyl-α-L-rhamnose	5·0

* Could not be identified for want of mono-methoxy sugars.
† Synthesized by Hakomori method[11] from methyl-D-fucosepyranoside followed by acid hydrolysis.

Based on these observations a possible structure for ipolearoside could be I. This was confirmed since the methylated sugars obtained on acid hydrolysis of methylated ipolearoside (VI) were identified as 2,3,4,6-tetramethoxyglucose, 2,3-di-methoxyrhamnose, 2,3,4-trimethoxyfucose and a monomethoxyrhamnose by GLC (Table 3).

[11] HAKOMORI, S. (1964) *J. Biochem. (Tokyo)* **55**, 205.

EXPERIMENTAL

M.ps are uncorrected. IR spectra were taken in KBr, 60 Mcs NMR in $CDCl_3$ with tetramethylsilane as internal reference. GLC were run on a Varian aerograph 1868-4 (Model) equipped with FID: column SE30, 1·5 m × 3 mm, Inj. temp. 225°, column temp. 120°. TLC was carried out on silica gel plates and descending PC on Whatman No. 1 paper at room temp.

Isolation of ipolearoside (I). The air dried, powdered whole plant (6 kg) was percolated with 95% EtOH (5 × 15 l.) and the extract concentrated under reduced pressure below 50°. The viscous mass obtained was triturated and extracted successively with C_6H_{14} (5 × 1·5 l.), $CHCl_3$ (1 × 3 l.) and Me_2CO (1 × 2 l.). The insoluble substance obtained, was dissolved in EtOH and filtered. The filtrate on concentration *in vacuo* below 50° yielded a dark brown solid which was chromatographed over silica gel. Elution with $CHCl_3$–MeOH (3:1) yielded a light brown hygroscopic solid which was dissolved in a small amount of dry MeOH and precipitated with Et_2O (process repeated 3×). The pale amorphous powder (5·92 g) obtained was designated as ipolearoside. It gave a single spot on TLC (R_f 0·33, *n*-BuOH–HOAc–H_2O, 4:1:5, upper) m.p. 184–187°, $[\alpha]_D^{20}$ −50·0° (*c* 1·0, MeOH), ν_{max} 3400–3300 (OH), 2900, 2860, 1725 (COOH), 1385, 1265, 1075–1060, 890 cm^{-1} (Found: C, 52·6; H, 7·9; equivalent wt 928. $C_{40}H_{72}O_{21}$. 1·5 H_2O requires: C, 52·46; H, 8·19%; equivalent wt 915).

Periodate oxidation of ipolearoside (I). Ipolearoside (100 mg) was added to 0·25 M $NaIO_4$ (20 ml), stirred in dark at room temp. for 4 hr and the vol. made up to 100 ml with H_2O. (a) 10 ml aliquots (in duplicate) after the addition of $NaHCO_3$ and KI (3 g each) was titrated with $NaAsO_3$ (0·1 N) and showed the consumption of 5·2 mol of HIO_4 per mol of ipolearoside. (b) To 20 ml aliquots (in duplicate) ethylene glycol (1 ml) was added and the solutions were steam distilled. The distillate containing of HCO_2H was titrated with 0·01 N NaOH solution (methyl red indicator) and showed the generation of 1·8 mol of HCO_2H per mol of ipolearoside. There was no appreciable change in the HCO_2H content even after prolonged periodate oxidation.

Alkaline hydrolysis of ipolearoside (I). Ipolearoside (20 mg) was refluxed with alcoholic NaOH (2 ml, 2 N) and after intervals of 1 and 2 hr the hydrolysate was subjected to PC in *n*-BuOH–HOAc–H_2O (4:1:5), using aniline hydrogen phthalate as a spray reagent. No spots for sugars were obtained.

Enzymatic hydrolysis of ipolearoside (I). *Ipolearoside*-A. Ipolearoside (400 mg) was dissolved in EtOH–H_2O (1:4) (75 ml) and takadiastase (300 mg) added. After shaking well (1 hr), toluene (6 drops) was added, and the mixture left at 37° for 60 hr. The precipitate was filtered off and the filtrate dried *in vacuo*. The residue was dissolved in H_2O saturated with *n*-BuOH (20 ml) and repeatedly extracted with *n*-BuOH saturated with H_2O. The aqueous layer was concentrated and examined by PC for sugars (solvent system: *n*-BuOH–HOAc–H_2O, 4:1:5, spraying reagent: aniline hydrogen phthalate, R_f 0·18, glucose, R_f 0·18). The *n*-BuOH layer was evaporated *in vacuo* to give a brown mass which was chromatographed over silica gel. Elution with $CHCl_3$–MeOH (3:1) yielded a pale micro crystalline powder (210 mg) TLC single spot (R_f 0·40, solvent system; *n*-BuOH–HOAc–H_2O, 4:1:5, upper) m.p. 159–161°, $[\alpha]_D^{20}$ −28·6° (*c*, 1·0, MeOH), designated as ipolearoside-*A*. ν_{max}: 3400–3300 (OH), 2900, 1725 (COOH), 1380, 1265, 1072–1062 cm^{-1} (Found: C, 52·45; H, 8·1, equivalent wt 792· $C_{34}H_{62}O_{16}$. $3H_2O$ requires: C, 52·31; H, 8·71%, equivalent wt 780).

Acid hydrolysis of ipolearoside (I). Ipolearoside (2·5 g) was refluxed with 10% HCl–MeOH (25 ml) at 100° for 3 hr. The hydrolysate was diluted with H_2O (25 ml) and MeOH distilled off under reduced pressure. The remaining solution was cooled and extracted with Et_2O (4 × 30 ml). The Et_2O layers were combined, washed with H_2O, dried (Na_2SO_4) and evaporated to give a brown waxy residue (0·82 g) (*A*). The aqueous layer was neutralized with dil NaOH solution and evaporated under reduced pressure to give a syrup (*S*).

Aglycone: *methyl ipolearate* (II). The brown waxy residue (*A*) (0·82 g) was macerated with hot C_6H_{14} (5 × 20 ml), filtered while hot and recrystallized from light petrol. (40–50°) 3× giving colourless silky needles (0·59 g), m.p. 66–67°, $[\alpha]_D^{20}$ +8·0° (*c*, 1·0, MeOH), TLC R_f 0·53 ($CHCl_3$–MeOH, 93:7) designated as methyl ipolearate (II) (Found: C, 67·50; H, 11·28. $C_{17}H_{34}O_4$ requires: C, 67·55; H, 11·26%). ν_{max}: 3350 (OH), 2900, 2850, 1735 (COOMe), 1525, 1477, 1450, 1375, 1348, 1310, 1287, 1250, 1175, 1143, 1082, 1050, 985, 870, 735 cm^{-1}. NMR: δ 0·9, 1·35, 2·20 (OH), 2·50 (*J* 5·5, 7·5 Hz), 3·60, 3·75 (OMe) and 4·05 ppm. MS *m/e*: 284 (M^+-H_2O), 266, 231, 213, 202, 184, 181, 163, 152, 135, 103, 101, 97, 95, 83, 81, 74, 71.

Examination of the sugar fraction (identification). The syrup (*S*) obtained from the aqueous layer of the hydrolysate was dissolved in MeOH and examined by paper chromatography in three solvent systems given in Table 2.

Ethylipolearate (V). Ipolearoside (300 mg) was refluxed with 10% HCl—EtOH (10 ml) on a water bath for 3 hr. The hydrolysate on working up as given above (in A) yielded Et_2O and aqueous acidic phases. The aglycone from the Et_2O phase was recrystallized from light petrol. (40–50°) as colourless silky needles (78 mg) m.p. 63–64°, $[\alpha]_D^{20}$ +9·58° (*c*, 1·1, MeOH) single spot on TLC (R_f 0·54, solvent system: $CHCl_3$–MeOH, 93:7) (Found: C, 67·86; H, 11·34. $C_{18}H_{36}O_4$ requires: C, 68·35, H, 11·39%). ν_{max}: 3350 (OH), 2915, 2865, 1730 ($COOC_2H_5$), 1470, 1375, 1345, 1310, 1280, 1170, 1140, 865, 720 cm^{-1}. NMR: δ 0·95, 1·38, 2·35 (*d*, *J* 11 Hz), 2·45 (*J* 7·5 Hz), 4·2 (*q*, *J* 7 Hz) ppm.

Acetylation of methyl ipolearate (II): *methyl ipolearate diacetate* (III). To a solution of methyl ipolearate (80 mg) in dry pyridine (1 ml) Ac_2O (0·5 ml) was added and the reaction mixture allowed to stand at room

temp. overnight. It was then refluxed at 110° for 1 hr, cooled, diluted with H_2O and extracted with $CHCl_3$ (3 × 20 ml). The $CHCl_3$ extract was washed (H_2O), dried and chromatographed over silica gel. The $CHCl_3$ eluate yielded acetyl methyl ipolearate (III) (59 mg) as a colourless viscous oil (Found: C, 65·42; H, 9·38. $C_{21}H_{38}O_6$ requires: C, 65·28; H, 9·84%). ν_{max}^{neat}: 2900, 1735 (COOMe), 1482, 1379, 1250 cm^{-1}. NMR: δ 0·95, 1·35, 2·1, 2·6 (*d*, *J* 7 Hz), 3·75, 5·0 and 5·3 ppm.

Oxidation of methyl ipolearate (II): *methyldiketo ipolearate* (IV). Methyl ipolearate (60 mg) was treated in cold with 0·3 ml Jones' reagent with constant stirring and the reaction mixture left for 1 hr at room temp. It was then poured on to crushed ice and the residue filtered, washed with H_2O and taken up in Et_2O. The Et_2O layer was dried, solvent distilled off and the residue crystallized from C_6H_{14} as colourless needles (50 mg), m.p. 49–50° (Found: C, 68·60; H, 10·09. $C_{17}H_{30}O_4$ requires: C, 68·45; H, 10·07%). ν_{max}: 2910, 2830, 1730, (COOMe), 1710 (> CO), 1330, 1270, 1250, 1170, 1080, 1015, 845, and 715 cm^{-1}. NMR: δ 0·9, 1·3, 2·4 (*t*, *J* 7 Hz), 3·45, 3·75 ppm. MS *m/e*: 298 (M^+), 267, 266, 242, 227, 225, 210, 195, 185, 183, 167, 153, 116, 101, 99 and 71.

Alkaline hydrolysis of methyl ipolearate (II): *ipolearic acid* (IIa). Methyl ipolearate (100 mg) was refluxed with *N* NaOH–MeOH (5 ml) for 1 hr. Solvent was removed under reduced pressure and the gelatinous residue taken up in H_2O (5 ml) and acidified (dil. H_2SO_4) in presence of Et_2O with constant stirring. The Et_2O layer was washed, dried and solvent distilled off. The residue was chromatographed over silica gel. C_6H_{14}–C_6H_6 (1:2) eluate was crystallized (MeOH) to yield ipolearic acid (IIa) (70 mg) m.p. 82–83° (Found: C, 66·1; H, 10·98. $C_{16}H_{32}O_4$ requires: C, 66·67; H, 11·11%). ν_{max}: 3350–3200 (OH), 2900, 2837, 1720 (COOH), 1470, 1240, 1170, 1130, 1065, 1040, 1020, 965, 865 and 715 cm^{-1}.

Methylation of ipolearoside (I): *methyl ipolearoside* (VI). To a solution of ipolearoside (500 mg) in dry DMSO (10 ml) under stirring was added at intervals, NaH (1 g) during 1 hr. CH_3I (4 ml) was then added dropwise and the stirring continued overnight in the dark. The reaction mixture was then filtered and the filtrate concentrated *in vacuo*, diluted with H_2O (20 ml) and extracted with $CHCl_3$ (4 × 20 ml), washed, dried and the residue chromatographed over silica gel. $CHCl_3$–MeOH (9:1) eluate yielded methyl ipolearoside (300 mg), single spot TLC (R_f 0·9, solvent system $CHCl_3$–MeOH (9:1), m.p. 86–88°, $[\alpha]_D^{20}$ −25·3° (*c* 1·0, MeOH) (Found: C, 58·6; H, 9·15. $C_{52}H_{96}O_{21}$ requires: C, 59·09; H, 9·09%). ν_{max}: 2910, 1730 (COOMe), 1450, 1375, 1120–1080 and 880 cm^{-1}.

Acid hydrolysis of methyl ipolearoside (VI). Methyl ipolearoside (200 mg) was refluxed with 10% H_2SO_4 in 50% MeOH (10 ml) for 4 hr. The hydrolysate was diluted with H_2O (10 ml) and MeOH distilled off. The aqueous soln. was then cooled and extracted with Et_2O (4 × 20 ml). The Et_2O extract was washed (H_2O), dried (anhyd. Na_2SO_4) and evaporated to give a light brown oily residue (80 mg) which was chromatographed over silica gel. C_6H_{14}–C_6H_6 (1:1) eluate yielded the methyl aglycone (VII) as a viscous oil (60 mg) single spot TLC (R_f 0·91, C_6H_6–MeOH, 9:1) (Found: C, 68·32; H, 11·35. $C_{18}H_{36}O_4$ requires: C, 68·35; H, 11·39%). ν_{max}^{neat}: 3450 (OH), 2900, 2850, 1736 (COOMe), 1480, 1266, 1170 and 1099 cm^{-1}. NMR: δ 0·9, 1·27, 2·5 (*d*, 5 Hz), 3·35, 3·75 ppm.

Methyl sugars (*identification*). The aqueous layer from the acid hydrolysis was neutralized with $BaCO_3$, filtered and evaporated *in vacuo* to give a syrup. This was dissolved in MeOH and chromatographed over a cellulose column (10 g) using MeOH as eluent. The product obtained after removal of solvent under vacuum were examined by GLC.

Partial hydrolysis of ipolearoside (I). Ipolearoside (500 mg) in 70–80% HCO_2H (10 ml) was heated at 90° for 1 hr and the reaction mixture evaporated *in vacuo* to give a resinous mass. This was dissolved in MeOH (5 ml) and methylated with CH_2N_2 in Et_2O overnight. Solvent was then removed, the residue dissolved in H_2O sat with *n*-BuOH (20 ml) and extracted with *n*-BuOH sat with H_2O (4 × 15 ml). The BuOH layer was evaporated *in vacuo* to a brown mass (254 mg) (TLC: R_f 0·71, 0·32, 0·11; solvent system: $CHCl_3$–MeOH, 4:1). The aqueous layer was evaporated under reduced pressure to give a syrup which was dissolved in MeOH and examined by PC (two aniline phthalate-positive spots, R_f 0·38 and 0·48 in solvent system-I, identified as fucose and rhamnose respectively). The brown mass from *n*-BuOH layer was chromatographed on silica gel (10 g) column (eluent, $CHCl_3$, $CHCl_3$–MeOH (9:1, 7:2, 1:1) giving four fractions (fr. *A*–*D*). Fr. *B* and *C* on rechromatography over silica gel (eluent, $CHCl_3$–MeOH, 3:1 and 1:1) yielded partial glycoside-B (40 mg, single spot on TLC, R_f 0·70; $CHCl_3$–MeOH, 4:1) and partial glycoside-*C* (30 mg; single spot on TLC, R_f 0·33, $CHCl_3$–MeOH, 4:1). Fractions *A* and *D* gave negligible amounts of any products.

Identification of sugar components of partial glycosides-B and -C. Ipolearoside-*B* and -*C* (30 mg each) were respectively hydrolysed with 2 N HCl (1 ml) in a boiling H_2O bath (1 hr) and worked up as usual. Aqueous layer was examined by PC. Glucose and rhamnose were detected in the aqueous layer in both the cases by PC (R_f, 0·18 and 0·48 in solvent system I).

Hydrolysis of periodate oxidation product of ipolearoside (I). Ipolearoside (50 mg) was added to 2·7% $NaIO_4$ (4 ml) and stirred in cold for 3 hr in dark. Excess $NaIO_4$ was quenched with glycerin (6–8 drops), EtOH (20 ml) added and the mixture was evaporated *in vacuo* to yield a white solid. This material was reduced in dry MeOH with $NaBH_4$ (20 mg) under stirring for 15 min at room temp. Excess $NaBH_4$ was decomposed with HOAc; the mixture was evaporated *in vacuo* and the residue was hydrolysed with 2 N

HCl (4 ml) on a H_2O bath for 2 hr. The sugar portion obtained in the usual manner was examined by PC (aniline hydrogen phthalate positive spot, R_f 0·49, solvent system-I) and identified as rhamnose (R_f 0·48).

Determination of the total sugar amounts and molar ratios of D-*glucose,* D-*fucose and* L-*rhamnose in the complete hydrolysate of ipolearoside* (I). Yield of total sugar (as glucose) was determined gravimetrically by standard Fehlings method and molar ratios by Misra, Mohan Rao method.[10] (Total sugar yield: Found (as glucose): 68·0%, Calc. for $C_{40}H_{72}O_{21}$. 1·5 H_2O: 70·3%.) The molar ratio of D-glucose, D-fucose and L-rhamnose found 1·0: 0·9:1·9.

Acknowledgements—We thank Dr. T. Kawasaki, Kyushu University, Japan, for authentic samples of D-quinovose and partially methylated sugars. The collection of plant material and the anticancer tests were undertaken by units of this Institute receiving support from the National Institute of Health, U.S.A. (Grant No. PL 480–134304).