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SAPONINS FROM ROOTS OF *MOMORDICA COCHINCHINENSIS*

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Key Word Index—*Momordica cochinchinensis*; Cucurbitaceae, saponin; momordin, oleanolic acid, monodesmoside, bisdesmoside, ^{13}C NMR

Abstract—Ten minor saponins, momordins Ia–Ie and IIa–IIe, were isolated from the root of *Momordica cochinchinensis*, along with the known major saponins, momordins I and II. Their structures were established on the basis of chemical and spectroscopic analysis. The structures of the two new saponins were assigned as 3*β*-{([*O*-*β*-D-xylopyranosyl-(1→2)-*O*-*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranuronosyl)oxy}-olean-12-ene-28-oic acid for momordin Id and momordin Id-28-*β*-D-glucopyranosyl ester for momordin IId. It was confirmed that the genuine saponin present in the root was not the monodesmoside but the bisdesmoside, though the former is the major component in the dry root

INTRODUCTION

Momordica cochinchinensis (Cucurbitaceae) is a perennial plant indigenous to tropical regions and distributed throughout China, Taiwan and southeast Asia The dry root of this plant, 'Mubie-gen', is used as an expectorant and an anti-inflammatory drug in Chinese medicine [1]. Kuwada and Fuwa were the first to isolate saponins from the root of which the aglycone was oleanolic acid They named this component momordin, but they could not elucidate the structure [2] Recently, Okabe *et al.* reported that 'momordin' is composed of at least three saponins, momordins **I** (**1**), **II** (**2**), and **III** (**3**) [3], and that **2** is identical to hemsloside-Mal which was isolated from *Hemsleya macrosperma* by Tanaka *et al.* [4]. This paper deals with the detailed investigation of 'momordin' and the discussion on the genuine saponin present in the root of *M. cochinchinensis*.

RESULTS AND DISCUSSION

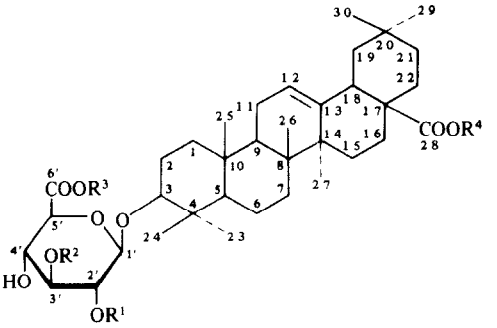
Ten minor saponins were isolated from aqueous methanol extracts of the dry root of *M. cochinchinensis*, momordins I a (**4**), Ib (**5**), Ic (**6**), Id (**7**), Ie (**8**), IIa (**9**), IIb (**10**), IIc (**11**), IId (**12**), and IIe (**13**), along with the two known saponins, momordins I (**1**) and II (**2**) by the procedure described in the Experimental

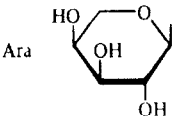
Every saponin isolated gave oleanolic acid (**14**) on acidic hydrolysis Moreover, they were separable into two classes, the monodesmosides (e.g. momordin I) and the bisdesmosides (e.g. momordin II); momordins IIa–IIe (**9**–**13**) affording momordins I, Ib–Ie, respectively, and glucose on saponification. In the ^{1}H NMR spectra of the bisdesmosides, the signals of the anomeric protons at *δ*6 33 (*d*, *J* = 8.1 Hz) showed that the esteric glucose had the *β*-configuration. In fact, the signals of momordins Ia–Ie (**4**–**8**) were very similar to those of **1** and those of momordins IIa–IIe (**9**–**13**) were close to those of **2** except for the signals for the sugar moieties at C-3 in the ^{13}C NMR spectra (Table 1).

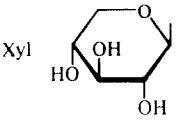
Momordin Ia (**4**) yielded glucuronolactone and arabinose together with **14** on acidic hydrolysis. In the ^{13}C NMR spectrum, the signal of the carboxyl carbon at C-6′ was shifted upfield from that of **1** by 2 ppm and a new signal ascribable to a Me ester was observed at *δ*52 1 The structure of **4**, therefore, was determined as 6′-methylmomordin I. As the ^{13}C NMR spectrum of momordin IIa (**9**) resembled that of **4** except for the glucosyl ester moiety, it was assumed that **9** was identical with 6′-methylmomordin II This was confirmed by direct comparison with a sample produced by methylation of **2** with diazomethane [3].

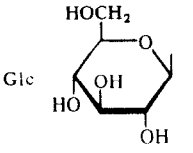
Momordin Ib (**5**) afforded glucuronolactone as the only sugar moiety on acidic hydrolysis. The ^{13}C NMR spectrum suggested that **5** was the C-3 glucuronide of **14** The signal of the anomeric proton of the glucuronide appeared at *δ*5 06 with the coupling constant (7.8 Hz) showing the *β*-configuration in the ^{1}H NMR spectrum. Consequently, the structure of **5** was elucidated as 3*β*-([*O*-*β*-*D*-glucopyranuronosyl]oxy)-olean-12-ene-28-oic acid, and **5** is identical with a saponin isolated from *Lonicera nigra* (Caprifoliaceae) [5] Additionally, the structure of momordin IIb (**10**) was determined as the 28-*β*-glucopyranosyl ester of **5** by comparison of the ^{13}C NMR spectra of **5** and **10** Momordin IIb (**10**) is identical with chikusetsusaponin IVa previously isolated from the rhizome of *Panax japonicum* [6]

.Momordin Ic (**6**) yielded **14**, glucuronolactone, and xylose on acidic hydrolysis. The glycosylation shift [7, 8] in the ^{13}C NMR spectrum of **6** suggested that xylose was linked to C-3′ of the glucuronide moiety, the signal for C-3′ of the glucuronide moiety was shifted downfield by 8.4 ppm from that of **5** and conversely, the signals for C-2′ and C-4′ were shifted upfield by 1 1 ppm and 2 1 ppm, respectively. Methanolysis of momordin Ic permethylate (**15**), prepared from **6** by Hakomori's method [9] with hydrochloric acid–methanol, afforded a methylated glucuronic acid of which the hydroxyl group at C-3 alone was not methylated and which is identical with the one derived from momordin I permethylate (**16**) The

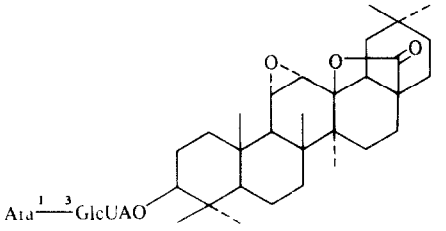








		R^1	R^2	R^3	R^4
Momordin I	(1)	H	Ara	H	H
Momordin II	(2)	H	Ara	H	Glc
Momordin Ia	(4)	H	Ara	Me	H
Momordin Ib	(5)	H	H	H	H
Momordin Ic	(6)	H	Xyl	H	H
Momordin Id	(7)	Xyl	Xyl	H	H
Momordin Ie	(8)	Xyl	Ara	H	H
Momordin IIa	(9)	H	Ara	Me	Glc
Momordin IIb	(10)	H	H	H	Glc
Momordin IIc	(11)	H	Xyl	H	Glc
Momordin IId	(12)	Xyl	Xyl	H	Glc
Momordin IIe	(13)	Xyl	Ara	H	Glc



Momordin III (3)

^{1}H NMR spectrum of **6** displayed the signals of two anomeric protons at δ5 03 (1H, *d*, J=7 3 Hz) and δ5 38 (1H, *d*, J=7 6 Hz). These coupling constants mean that each configuration is β From these results, the structure of **6** can be ascribed as 3β-([*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-glucopyranuronosyl]oxy)-olean-12-ene-28-oic acid Thus, momordin IIc (**11**) was determined as the 28-β-glucopyranosyl ester of **6** Momordins Ic (**6**) and IIc (**11**) have been reported in *Talinum tenuissimum* (Portulacaceae) [10]

Momordin Id (**7**) afforded **14**, glucuronolactone and xylose on acidic hydrolysis The ^{13}C NMR spectrum showed that the ratio was 1 1 2 In the ^{1}H NMR spectrum of **7**, the signals of three anomeric protons were observed at δ5.0 (1H, *d*, J=7 3 Hz), δ5 42 (1H, *d*, J=7 8 Hz), and δ5 60 (1H, *d*, J=7 6 Hz) This suggested that all sugars have the β configuration To determine the sequence of sugars, **7** was submitted to mild acidic hydrolysis to give three prosapogenins (**5**, **6** and an unidentified product, **17**) The ^{13}C NMR spectrum showed that the C-2′ signal of **5** at δ75.4 was shifted downfield in **17** by 8 1 ppm. This indicated that glycosylation occurred at C-2′ of **5**, thus, the xylose of **17** is linked to C-2′ of **5** The ^{13}C NMR spectrum of **17** was identical with that of pseudo-ginsenoside-RP$_1$ isolated from *Panax pseudo-ginseng* subsp *himalaicus* (Araliaceae) [11] Therefore, the structure of **7** was determined as 3β-{([*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranuronosyl)oxy}-olean-12-ene-28-oic acid. Thus, momordin IId (**12**) was determined as the 28-β-glucopyranosyl ester of **7**

Momordin Ie (**8**) gave equal proportions of **14**, glucuronolactone, xylose and arabinose on acidic hydrolysis Since **8** afforded three prosapogenins, **1**, **5**, and **17** by

Table 1. ^{13}C NMR chemical shifts of momordins in pyridine-d_5

C	1[Lit 4]	4	5	6	7	8	17	2[Lit 4]	9	10	11	12	13
1	38.4	38.5	38.6	38.6	38.6	38.6	38.7	38.6	38.7	38.6	38.6	38.7	38.7
2	26.4	26.4	26.5	26.4	26.5	26.4	26.6	26.3	26.4	26.5	26.5	26.5	26.5
3	89.1	89.2	89.0	89.2	89.7	89.7	89.3	89.1	89.4	89.0	89.2	89.8	89.8
4	39.4	39.4	39.5	39.4	39.6	39.5	39.6	39.3	39.4	39.4	39.4	39.6	39.5
5	55.6	55.7	55.7	55.7	55.8	55.8	55.9	55.6	55.8	55.7	55.7	55.9	55.8
6	18.4	18.4	18.4	18.4	18.4	18.4	18.5	18.3	18.5	18.4	18.4	18.5	18.5
7	33.2	33.1	33.1	33.1	33.1	33.1	33.2	33.1	33.1	33.0	33.0	33.1	33.1
8	39.6	39.6	39.7	39.7	39.7	39.6	39.7	39.7	39.9	39.8	39.8	39.9	39.9
9	47.9	47.9	47.9	47.9	47.9	47.9	48.0	47.9	48.0	47.9	47.9	48.0	48.0
10	36.9	36.9	36.9	36.9	36.9	36.9	37.0	36.8	36.9	36.9	36.9	36.9	36.9
11	23.7	23.7[a]	23.7[a]	23.7[a]	23.7	23.6	23.7	23.6	23.7	23.7	23.7	23.7	23.7
12	122.7	122.4	122.5	122.4	122.5	122.4	122.5	122.5	122.8	122.8	122.8	122.8	122.8
13	144.7	144.7	144.7	144.7	144.7	144.7	144.8	144.0	144.1	144.0	144.0	144.0	144.0
14	42.0	42.0	42.1	42.1	42.1	42.0	42.1	41.9	42.1	42.0	42.1	42.1	42.1
15	28.1	28.2	28.3	28.2	28.3	28.2	28.3	28.0	28.2	28.1	28.2	28.2	28.2
16	23.7	23.6[a]	23.6[a]	23.6[a]	23.7	23.6	23.7	23.6	23.4	23.3	23.3	23.4	23.4
17	46.6	46.5	46.6	46.5	46.6	46.5	46.6	46.8	47.0	46.9	46.9	46.9	47.0
18	42.0	41.9	41.9	41.9	41.9	41.8	41.9	41.6	41.7	41.6	41.9	41.7	41.7
19	46.6	46.4	46.2	46.4	46.4	46.4	46.5	46.2	46.2	46.1	46.1	46.2	46.2
20	30.9	30.9	30.9	30.9	30.9	30.9	30.9	30.7	30.7	30.7	30.7	30.7	30.7
21	34.2	34.2	34.2	34.2	34.2	34.2	34.3	33.7	34.0	33.9	34.0	34.0	34.0
22	33.2	33.1	33.1	33.1	33.1	33.1	33.2	32.5	32.5	32.4	32.4	32.5	32.5
23	28.1	28.0	28.2	28.0	27.7	27.6	27.8	28.0	28.1	28.1	28.0	27.7	27.7
24	16.9	16.8	16.9	16.9	16.3	16.3	16.3	16.8	16.8	16.9	16.9	16.3	16.3
25	15.3	15.4	15.4	15.4	15.4	15.4	15.5	15.4	15.5	15.5	15.5	15.5	15.5
26	17.4	17.3	17.3	17.3	17.3	17.2	17.3	17.3	17.4	17.3	17.4	17.4	17.4
27	26.1	26.1	26.2	26.1	26.2	26.1	26.2	26.0	26.1	26.0	26.1	26.1	26.1
28	180.2	180.0	180.1	179.9	180.0	179.9	180.0	176.3	176.3	176.6	176.3	176.3	176.4
29	33.2	33.3	33.3	33.3	33.3	33.3	33.3	33.1	33.1	33.1	33.1	33.1	33.1
30	23.7	23.8	23.7	23.8	23.8	23.7	23.8	23.6	23.7	23.6	23.6	23.6	23.7
1′	105.7	105.7	107.1	105.9	104.9[a]	104.9[a]	105.2	105.1	105.7	107.0	106.0	104.9[a]	105.0[a]
2′	74.4	74.3[b]	75.4	74.3	79.2	79.1	83.5	74.2[a]	74.3[a]	75.3	74.5	79.2	79.2
3′	85.7	85.6	78.0[b]	86.4	86.2	86.2	77.6[a]	85.7	85.8	77.9[a]	86.4	86.3	86.2
4′	72.7	72.7	73.3	71.2[b]	71.4[b]	72.6	73.0	72.5	72.7	73.2	71.3[a]	71.4[b]	72.7
5′	77.4	76.5	77.7[b]	77.8[c]	78.7[c]	78.6	78.0[a]	77.2	76.6	77.5[a]	77.9[b]	78.7[c]	78.6
6′	172.3	170.0	172.6	172.1	171.7	171.6	172.4	172.0	170.0	172.7	172.0	171.7	171.8
Me		52.1							52.1				
1″	106.7	106.7		106.6	105.3[a]	105.2[a]	106.9	106.5	106.6		106.7	105.2[a]	105.2[a]
2″	71.3	71.0		74.9	75.0	71.3[b]	76.4	71.3	71.2[b]		75.1	75.0	71.4[b]
3″	74.4	74.2[b]		77.2[c]	77.1	74.5	77.2[a]	74.5[a]	74.4[a]		77.4[b]	77.1	74.5
4″	69.2	69.1		70.8[b]	70.7[b]	69.4	71.0	69.0	69.1		70.8[a]	70.6[b]	69.4
5″	67.1	67.0		67.1	67.1[d]	66.9	67.4	67.0	67.0		67.2	67.0[d]	67.0
1‴					104.5[a]	104.4[a]						104.5[a]	104.4[a]
2‴					76.0	75.9						75.9	76.0
3‴					78.4[c]	77.0						78.3[c]	77.0
4‴					71.2[b]	71.1[b]						71.2[b]	71.2[b]
5‴					67.2[d]	67.5						67.1[d]	67.6
glc 1								95.5	95.6	95.5	95.6	95.6	95.6
2								73.9	74.0	73.9	74.0	73.9	73.9
3								78.5[b]	78.7[c]	78.6[b]	78.7	78.7[e]	78.8[c]
4								71.0	71.1[b]	71.0	71.0	71.1[b]	71.1[b]
5								78.9[b]	79.0[c]	79.0[b]	79.1	78.9[e]	79.0[c]
6								62.0	62.3	62.1	62.1	62.2	62.2

[a–e] Assignments may be interchangeable in the same column

partial hydrolysis with acid, the xylose and arabinose residues must be on C-2′ and C-3′ of **5**, respectively. In the ^1H NMR spectrum of **8**, three signals of anomeric protons were observed at δ4.98 (1H, *d*, $J = 7.1$ Hz), δ5.32 (1H, *d*, $J = 7.6$ Hz), and δ5.57 (1H, *d*, $J = 7.6$ Hz). Although each signal was not exactly assigned, it was concluded from the coupling constants that the configurations were β for glucuronic acid and xylose, and α for

arabinose Therefore, the structure of **8** was confirmed as 3β-{([*O*-β-D-xylopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-(1→3)]-*O*-β-D-glucopyranuronosyl)oxy}-olean-12-ene-28-oic acid Thus, momordin IIe (**13**) was determined as the 28-β-glucopyranosyl ester of **8** Momordin IIe (**13**) is identical with hemsloside-Ma2 isolated from *Hemsleya macrosperma* [4]

We isolated 12 saponins from the dry root of *M cochinchinensis*, but did not find momordin III (**3**) as reported by Okabe *et al* [3]

Both 'Mubie-gen' and momordin I (**1**), the major saponin, severely irritate the mucous membrane of the throat, while the fresh root and momordin II (**2**) have little effect This suggests that the major component in the fresh root might be momordin II (**2**) and momordin I (**1**) could be an artifact formed during the drying process Quantitative analyses of **1** and **2** were carried out in order to examine the differences in the saponin components of the dry and fresh roots (Table 2) Although the total amounts of **1** and **2**, given as total momordin II in Table 2, were comparable, the ratio of the contents of **1** and **2** were remarkably different and the major saponin in the fresh root was **2**

It was assumed that the major genuine saponin in the root of *M cochinchinensis* is not **1** but **2** and that some intracellular enzymes (esterases) contained in the root convert **2** to **1** during the drying process When the dry root was homogenized in water or aqueous methanol solutions and the homogenate was allowed to stand at room temperature, the conversion of **2** to **1** occurred in the homogenates in water, and 30 and 60% methanol solns, but not in the 80 and 100% MeOH solns (Fig. 1). Thus, active esterases are still present in the dry root

Although monodesmoside is always isolated in greater quantities than bisdesmoside from the dry root, it was postulated that the latter might be present in greater amounts than the former in the fresh root To prevent the decomposition of bisdesmoside to monodesmoside, it is necessary to inactivate the esterases before the fresh root dies Unlike the dry root, homogenates of the fresh root in methanol retained the enzyme activity. The enzymes were inactivated effectively by soaking the fresh root in hydrochloric acid soln, an homogenate of the treated root no longer showed enzyme activity It was shown that the treated root contained bisdesmoside as the major component (Table 3) Therefore, it was demonstrated that the genuine saponin present in the root of *M cochinchinensis* is not the monodesmoside but the bisdesmoside and the former is an artifact

Domon and Hostettmann reported that the monodesmoside of oleanolic acid was isolated as the major component from the aqueous extract of the dry berry of *Phytolacca dodecandra* (Phytolaccaceae), but the corresponding bisdesmoside was obtained as the only isolable component from the methanol extract [12]. It seems that the monodesmoside may be produced from the bisdesmoside by the action of intracellular enzymes as demonstrated here

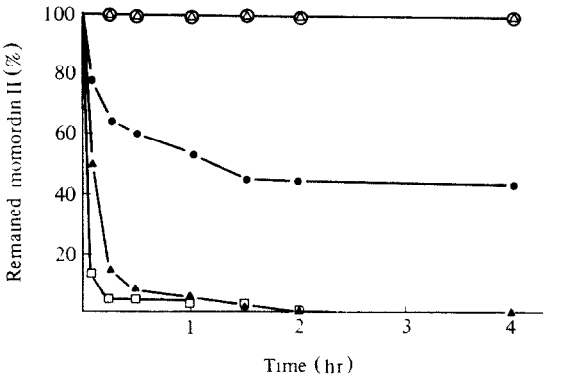


Fig 1 Enzymatic hydrolysis of momordin II (**2**) to momordin I(**1**) in aqueous MeOH solutions □, H_2O, ▲, 30% MeOH, ●, 60% MeOH, △, 80% MeOH, ○, MeOH

Table 3 Contents of momordins in dry and acid-treated roots of *M cochinchinensis*

Momordins	Dry root* (%)	Treatments† (%)
Momordin I (**1**)	7 64	0 45
Momordin Ib (**5**)	0 02	N D ‡
Momordin Ic (**6**)	0 42	0 03
Momordin Id (**7**)	0 17	0 02
Momordin Ie (**8**)	0 47	0 03
Momordin II (**2**)	0 48	7 64
Momordin IIb (**10**)	0 03	0 02
Momordin IIc (**11**)	0 05	0 31
Momordin IId (**12**)	0 05	0 13
Momordin IIe (**13**)	0 07	0 25

*Dried at 70° for 19 hr
†Treated with 4% HCl and 3% $NaHCO_3$ before drying
‡Not detected

EXPERIMENTAL

Mps uncorr NMR spectra were measured in pyridine-d_5 and were recorded at 400 MHz for ^{1}H NMR and 100 MHz for

Table 2 Difference of saponin components in dry and fresh roots of *M cochinchinensis*

	Total M II* (%/fr wt)	Momordin II/total M II
Fresh root	1 34±0 35	66 4± 6 1
Dried root (40°)	1 25±0 39	16 5±10 8
Dried root (70°)	1 21±0 45	6 2± 4 1

Each figure represents the mean ± s d ($n=5$)
*Total M II (%) = momordin II (%) + momordin I (%) × (926/764) [926. M_r of momordin II (**2**) 764 M_r of momordin I (**1**)]

^{13}C NMR Chemical shifts are given in δ (ppm) with TMS as int std GC analyses were done using a FS-WCOT silicone OV-101 capillary column (10 m × 0 25 mm) and an FID detector HPLC analyses were performed on an ODS column (100 × 6 mm) and a UV detector at 205 nm

Plant material M cochinchinensis was cultivated at Fukuchiyama Experimental Farm of Takeda Chemical Industries in Kyoto prefecture The root of this plant had been supplied to Prof H Okabe of Fukuoka University and the same plant was propagated for this work

Isolation of saponins Ground dry root (10 5 kg) of *M cochinchinensis* was extd with 60% MeOH (50 l), the pH of the suspension being maintained at 9 by adding dil NH_4OH occasionally After filtration, the extract was acidified to pH 1.5 with HCl to give a spongy ppt The ppt was collected by filtration and dissolved in MeOH After the MeOH soln was made alkaline to pH 9 5 with dil NH_4OH, the soln was concd to *ca* 3 l and dil with Me_2CO (12 l) to give a white ppt (272 g) This ppt (240 g) was recrystallized twice from *n*-BuOH satd with H_2O to give colourless needles of momordin I ammonium salt (136 g, yield 1 47%) The mother liquid was chromatographed on silica gel with $CHCl_3$–MeOH–3% HOAc (4 1 0 to 30 10 1, and finally to 14 6 1) as the eluent to yield 4 fractions (1–4)

Fraction 1 (740 mg) was repeatedly chromatographed on silica gel with *n*-BuOH–EtOAc–H_2O (8 2 1) as eluent to give momordin Ia (**4**) (150 mg, yield 0 001%) White powder (Me_2CO), $[\alpha]_D^{22}$ +28 2° (MeOH, *c* 0 99) SIMS *m/z* 817 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1740 (CO_2Me), 1695 (CO_2H) ^1H NMR δ 0 80 (3H, *s*, Me), 0 96 (3H, *s*, Me), 0 97 (3H, *s*, Me), 0 99 (3H, *s*, Me), 1 02 (3H, *s*, Me), 1 30 (3H, *s*, Me), 1 32 (3H, *s*, Me), 3 75 (3H, *s*, CO_2Me), 4 97 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 34 (1H, *d*, *J* = 7 1 Hz, anomeric H), 5 48 (1H, *br s*, olefinic H) (Found C, 62 74, H, 8 92 Calc for $C_{42}H_{66}O_{13}$ 1 5 H_2O C, 62 59, H, 8 63%)

Fraction 2 (1 96 g) was chromatographed on silica gel with *n*-BuOH–EtOAc–H_2O (4 1 5, upper layer) as eluent The eluate was concd to dryness to give a white residue which was dissolved in H_2O and then acidified to pH 1 with HCl The acidic soln was extd with *n*-BuOH The *n*-BuOH soln was concd *in vacuo* and the residue recrystallized from MeOH to afford momordin Ib (**5**) (472 mg, yield 0 005%) as colourless needles, mp 218–220° $[\alpha]_D^{21}$ +18 1° (MeOH, *c* 1 06) SIMS *m/z* 655 $[M+Na]^+$ with NaCl IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1725 (COOH), 1695 (COOH) ^1H NMR δ 0 81 (3H, *s*, Me), 0 96 (3H, *s*, Me), 0 99 (6H, *s*, Me × 2), 1 01 (3H, *s*, Me), 1 32 (6H, *s*, Me × 2), 5 06 (1H, *d*, *J* = 7.8 Hz, anomeric H), 5 47 (1H, *br s*, olefinic H) (Found C, 65 65, H, 9 02 Calc for $C_{30}H_{50}O_9$ 1 5 H_2O C, 65 52, H, 9 01%)

Fraction 3 (28 7 g) was repeatedly chromatographed on silica gel with *n*-BuOH–EtOAc–H_2O (4 1 2, upper layer) as eluent. The eluate was concd to dryness and the residue processed as described above to give momordin Ic (**6**) (1 26 g, yield 0 014%) White powder (MeOH), $[\alpha]_D^{21}$ + 15 4° (MeOH, *c* 1 03) SIMS *m/z* 803 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1725 (CO_2H), 1695 (CO_2H) ^1H NMR δ 0 80 (3H, *s*, Me), 0 97 (3H, *s*, Me), 0 99 (6H, *s*, Me × 2), 1 01 (3H, *s*, Me), 1 31 (3H, *s*, Me), 1 34 (3H, *s*, Me), 5 03 (1H, *d*, *J* = 7 3 Hz, anomeric H), 5 38 (1H, *d*, *J* = 7 6 Hz, anomeric H), 5 47 (1H, *br s*, olefinic H) (Found C, 61 59, H, 8 33. Calc for $C_{41}H_{64}O_{13}$ 2 H_2O C, 61 48, H, 8 55%)

Fraction 4 (5 63 g) was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–3% HOAc (32 8 1) and *n*-BuOH–EtOAc–H_2O (4 1 2, upper layer) as eluents The eluate was concd to dryness and the residue treated with acid as described above to give momordin Id (**7**) (699 mg, yield 0 008%) and momordin Ie (**8**) (1 56 g, yield 0 017%) **7** Colourless needles (MeOH), mp 233–235° $[\alpha]_D^{21}$ + 12 7° (MeOH, *c* 1 20). SIMS *m/z*: 935 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1725 (COOH), 1695 (COOH). ^1H NMR δ 0 80 (3H, *s*, Me), 0 96 (3H, *s*, Me), 0 98 (3H, *s*, Me), 1 01 (3H, *s*, Me), 1 06 (3H, *s*, Me), 1.27 (3H, *s*, Me), 1 32 (3H, *s*, Me), 5.00 (1H, *d*, *J* = 7 3 Hz, anomeric H), 5 42 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 45 (1H, *br s*, olefinic H), 5 60 (1H, *d*, *J* = 7 6 Hz, anomeric H) (Found C, 59 40, H, 8 31. $C_{46}H_{72}O_{17}$ 2 H_2O requires C, 59 21; H, 8 20%) **8** White powder (MeOH), $[\alpha]_D^{21}$ +20 5° (MeOH, *c* 1 03) SIMS *m/z* 935 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1}. 3400 (OH), 1720 (COOH), 1695 (COOH) ^1H NMR δ 0 80 (3H, *s*, Me), 0 96 (3H, *s*, Me), 0 98 (3H, *s*, Me), 1 01 (3H, *s*, Me), 1 06 (3H, *s*, Me), 1 27 (3H, *s*, Me), 1 32 (3H, *s*, Me), 4 98 (1H, *d*, *J* = 7.1 Hz, anomeric H), 5 32 (1H, *d*, *J* = 7 6 Hz, anomeric H), 5 45 (1H, *br s*, olefinic H), 5 57 (1H, *d*, *J* = 7 6 Hz, anomeric H) (Found C, 60 44; H, 8 29. Calc for $C_{46}H_{72}O_{17}$ H_2O: C, 60.37, H, 8.15%).

The dry root (2 5 kg) was extracted with 60% MeOH (2 × 18 l) The pH of the suspension was maintained at 9 by adding dil NH_4OH The ext was acidified to pH 1 5 with HCl to afford a white ppt (70 g). The ppt was treated as described above to give colourless needles of momordin I ammonium salt (43 7 g, yield 1 75%) The filtrate was made alkaline to pH 9 with dil NH_4OH and concd to give an aq soln (*ca* 14 l) Amberlite XAD-II (6 l) was added to the soln and the suspension stirred for 6 hr The soln was filtered off, the resin washed with H_2O (2 × 6 l) and then treated with MeOH (3 × 8 l) The MeOH soln was concd and the residue (62 3 g) chromatographed on silica gel with $CHCl_3$–MeOH–3% HOAc (14 6 1) as eluent to give four fractions (1–4)

Fraction 1 (3 4 g) was repeatedly chromatographed on silica gel and ODS with *n*-BuOH–EtOAc–H_2O (4 1 5, upper layer) and 70% MeOH as eluents, respectively, to give momordin IIa (**9**) (1 23 g, yield 0 049%). Colourless needles (MeOH), mp 247–249° $[\alpha]_D^{21}$ + 14 1° (MeOH, *c* 1 24). SIMS *m/z* 979 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1740 (CO_2R). ^1H NMR δ 0 83 (3H, *s*, Me), 0 90 (3H, *s*, Me), 0 92 (3H, *s*, Me), 0 97 (3H, *s*, Me), 1 09 (3H, *s*, Me), 1 28 (3H, *s*, Me), 1 29 (3H, *s*, Me), 3 75 (3H, *s*, CO_2Me), 4 97 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 33 (1H, *d*, *J* = 7 1 Hz, anomeric H), 5 43 (1H, *br s*, olefinic H), 6 33 (1H, *d*, *J* = 8 1 Hz, anomeric H) (Found C, 60 65, H, 8.13 Calc. for $C_{48}H_{76}O_{18}$ 0 5 H_2O C, 60 68, H, 8 17%)

Fraction 2 (31 3 g) was recrystallized from MeOH to afford a white powder of momordin II ammonium salt (20 8 g, yield 0 83%) The mother liquid was subjected to CC on silica gel with *n*-BuOH–EtOAc–H_2O (4 1 5, upper layer) as eluent The eluate was concd to dryness and the residue treated with acid as described above to give momordin IIb (**10**) (256 mg, yield 0.010%). Colourless needles (MeOH), mp 220–223° $[\alpha]_D^{21}$ +4 73° (MeOH, *c* 0 97). SIMS *m/z* 833 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1725 (COOR, COOH) ^1H NMR δ 0 83 (3H, *s*, Me), 0 89 (3H, *s*, Me), 0.92 (3H, *s*, Me), 1 00 (3H, *s*, Me), 1 09 (3H, *s*, Me), 1.28 (3H, *s*, Me), 1 31 (3H, *s*, Me), 5 04 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 42 (1H, *br s*, olefinic H), 6 33 (1H, *d*, *J* = 8 1 Hz, anomeric H) (Found: C, 61 37, H, 8 52. Calc for $C_{42}H_{66}O_{14}$ 1 5 H_2O C, 61 37, H, 8 46%)

Fraction 3 (11 4 g) was repeatedly chromatographed on silica gel and ODS with *n*-BuOH–EtOAc–H_2O (4 1 5, upper layer) and 70% MeOH as eluents, respectively, and the eluate concd to dryness The residue was treated with acid to afford momordin IIc (**11**) (469 mg, yield 0.019%) White powder (MeOH–EtOAc), $[\alpha]_D^{21}$ + 5 67° (MeOH, *c* 1 07) SIMS *m/z*: 965 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (CO_2R, CO_2H). ^1H NMR δ 0 82 (3H, *s*, Me), 0.89 (3H, *s*, Me), 0 92 (3H, *s*, Me), 0.99 (3H, *s*, Me), 1 09 (3H, *s*, Me), 1 29 (3H, *s*, Me), 1 30 (3H, *s*, Me), 5.02 (1H, *d*, *J* = 7 8 Hz, anomeric H), 5 37 (1H, *d*, *J* = 7.3 Hz, anomeric H), 5 42 (1H, *br s*, olefinic H), 6 33 (1H, *d*, *J* = 8 1 Hz, anomeric H) (Found C, 59 26, H, 8 26. Calc for $C_{47}H_{74}O_{18}$ 1 5 H_2O C, 59 17, H, 8 13%).

Fraction 4 (9 57 g) was repeatedly chromatographed on silica gel with *n*-BuOH–EtOAc–H_2O (4 1 5, upper layer) as eluent The eluate was concd to dryness and the residue treated with acid as described above to afford momordin IId (**12**) (443 mg, yield 0 018%) and momordin IIe (**13**) (989 mg, yield 0 040%) **12** White powder (MeOH–EtOAc), $[\alpha]_D^{23}$ + 1 98° (MeOH, *c* 1 06) SIMS *m/z* 1097 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1725 (CO_2R, CO_2H) ^1H NMR δ 0 83 (3H, *s*, Me), 0 89 (3H, *s*, Me), 0 91 (3H, *s*, Me), 1 07 (3H, *s*, Me), 1 08 (3H, *s*, Me), 1 26 (3H, *s*, Me), 1 28 (3H, *s*, Me), 4 99 (1H, *d*, *J* = 7 3 Hz, anomeric H), 5 40 (1H, *br s*, olefinic H), 5 41 (1H, *d*, *J* = 7 3 Hz, anomeric H), 5 60 (1H, *d*, *J* = 7 8 Hz, anomeric H), 6 33 (1H, *d*, *J* = 8 1 Hz, anomeric H) (Found C, 58 11, H, 8 06 $C_{52}H_{82}O_{22}$ H_2O requires C, 57 98, H, 7 86%) **13** White powder (MeOH–EtOAc), $[\alpha]_D^{23}$ + 11 8 (MeOH, *c* 1 10) SIMS *m/z* 1097 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1730 (CO_2R, CO_2H) ^1H NMR δ 0 82 (3H, *s*, Me), 0 89 (3H, *s*, Me), 0 91 (3H, *s*, Me), 1 07 (3H, *s*, Me), 1 08 (3H, *s*, Me), 1 25 (3H, *s*, Me), 1 28 (3H, *s*, Me), 4 97 (1H, *d*, *J* = 7 1 Hz, anomeric H), 5 31 (1H, *d*, *J* = 7 6 Hz, anomeric H), 5 41 (1H, *br s*, olefinic H), 5 55 (1H, *d*, *J* = 7 8 Hz, anomeric H), 6 33 (1H, *d*, *J* = 8 1 Hz, anomeric H) (Found C, 56 62, H, 8 24 Calc for $C_{52}H_{82}O_{22}$ 2 5 H_2O C, 56 56, H, 7 94%)

Acidic hydrolysis of saponins Saponins (*ca* 10 mg) were refluxed in 10% H_2SO_4–dioxane (1 1) (2 ml) for 3 hr and H_2O was then added to the reaction mixt The ppt was collected by filtration and recrystallized from MeOH to afford oleanolic acid (**14**) as colourless needles, mp 305–307 EIMS *m/z* 456 $[M]^+$, 248 (100) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1695 (COOH). The filtrate was neutralized with aq $NaHCO_3$ soln and concd to dryness The residue was treated with dil HCl in MeOH and concd to dryness Aliquots of the residue were dissolved in MeOH and analysed by TLC in comparison with authentic sugars The rest of the residue was dissolved in pyridine (0 2 ml) and treated with trimethylsilyl chloride-*N,O*-bis(trimethylsilyl)acetamide (1 5) (0 2 ml) at room temp for 30 min The products were analysed by GC and their R_t values compared with those of the TMSi derivatives of authentic sugars TLC silica gel, solvent A $CHCl_3$–MeOH–H_2O (6 4 1), R_f, glucose 0 40, arabinose 0 47, xylose 0 52, glucuronolactone 0 61 Solvent B *n*-BuOH–HOAc–H_2O (4 1 5, upper layer) R_f, glucose 0 25, arabinose 0 29, xylose 0 38, glucuronolactone 0 46 GC inj temp 250 Column temp prog 150 to 180° at 2 /min Carrier gas, He 0 8 kg/cm^2 R_t (min), arabinose 2 20, 2 46, xylose 3 16, 3 88, glucuronolactone 4 17, 4 46, glucose 6 79, 9 30.

Saponification of saponins A soln of each bisdesmoside (*ca* 40 mg) in 2 N KOH (10 ml) was refluxed for 3 5 hr The reaction mixt was acidified to pH 1 5 with dil HCl and then it was extd with *n*-BuOH The *n*-BuOH soln was concd to dryness and the residue compared with the authentic sample directly to identify the prosapogenin The aq soln was heated with conc H_2SO_4 (1 ml) for 2 hr at 80° The reaction mixt was neutralized with aq $NaHCO_3$ soln and concd to dryness Glucose was identified in the residue by GC as its TMSi derivative and TLC GC and TLC were performed under the same conditions as those described for the acidic hydrolysis of the saponins

Permethylation of **6** A suspension of NaH (60% in oil) (500 mg) in DMSO (20 ml) was heated for 45 min at 75° The reaction mixt was cooled to room temp and a soln of **6** (170 mg) in DMSO (3 ml) added This mixt was stirred for 10 min at room temp After dropwise addition of MeI (20 ml), the mixt was stirred for a further 1 hr The reaction mixt was then poured into ice-H_2O and extracted with $CHCl_3$ The $CHCl_3$ soln was concd to dryness and the residue chromatographed on silica gel with hexane–Me_2CO (1 7 0 3) as eluent to give momordin Ic permethylate (**15**) (120 mg, yield 63%) White powder (MeOH–H_2O) $[\alpha]_D^{23}$ + 1 65 (MeOH, *c* 1 02) EIMS *m/z* 862 $[M]^+$, 453 (100) IR ν_{max}^{KBr} cm^{-1} 1755 (COOMe), 1725 (COOMe) (Found C, 65 07, H, 9 05 $C_{48}H_{78}O_{13}$ 1 5 H_2O requires C, 64 77, H, 9 17%)

Permethylation of **1** Momordin I (**1**) (170 mg) was methylated in the same way as that described for **6** to give the permethylate (**16**) (132 mg, yield 69%) Colourless needles (MeOH), mp 162–164° $[\alpha]_D^{23}$ + 22 0° (MeOH, *c* 1 03) EIMS *m/z* 862 $[M]^+$, 453 (100) IR ν_{max}^{KBr} cm^{-1} 1750 (COOMe), 1730 (COOMe) (Found C, 66 70, H, 9 16 $C_{48}H_{78}O_{13}$ requires C, 66 79, H, 9 11%)

Methanolysis of **15** Momordin Ic permethylate (**15**) (20 mg) was dissolved in 2 N HCl–MeOH (6 ml) and the soln refluxed for 3 hr The soln was neutralized with Ag_2CO_3 and the ppt filtered off The filtrate was concd to dryness and the residue recrystallized from MeOH to give oleanolic acid Me ester as colourless needles, mp 202–204° EIMS *m/z* 470 $[M]^+$, 262 (100) IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1725 (COOMe) The mother liquid was examined by TLC and GC TLC silica gel, hexane–Me_2CO (3 1) R_f, methylated glucuronic acid 0 16, 0 21, methylated xylose 0 32, 0 34 GC inj temp 250° Column temp, prog 100° to 150° at 5°/min. Carrier gas, He 0 8 kg/cm^2 R_t (min), methylated xylose 1 49, 1 69, methylated glucuronic acid 4 79, 5 52

Methanolysis of **16** Momordin I permethylate (**16**) (20 mg) was treated in the same way as **15** to give oleanolic acid Me ester The mother liquor was examined by TLC and GC This was carried out under the same conditions as those used for methanolysis of **15** R_f, methylated glucuronic acid 0 16, 0 21, methylated arabinose 0 34 GC R_t (min), methylated arabinose 2 04, methylated glucuronic acid 4 79, 5 52

Mild acid hydrolysis of **7** Momordin Id (**7**) (154 mg) was heated in 1 5% H_2SO_4 (40 ml) for 20 hr at 80° The reaction mixt was extd with *n*-BuOH and the *n*-BuOH soln was concd to dryness The residue obtained was chromatographed on silica gel with *n*-BuOH–EtOAc–H_2O (4 1 5, upper layer) as eluent to give oleanolic acid (**14**) (24 mg, yield 31%), and prosapogenins, **5** (40 mg, yield 37%), **6** (7 mg, yield 5%), and **17** (24 mg, yield 18%). **17** colourless needles (MeOH), mp 230–232° $[\alpha]_D^{22}$ + 11 9° (MeOH, *c* 0 93) SIMS *m/z* 803 $[M+K]^+$ with KI IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1720 (CO_2H), 1695 (CO_2H) ^1H NMR δ 0 83 (3H, *s*, Me), 0 96 (3H, *s*, Me), 0 99 (3H, *s*, Me), 1 01 (3H, *s*, Me), 1 11 (3H, *s*, Me), 1 32 (3H, *s*, Me), 1 33 (3H, *s*, Me), 5 04 (1H, *d*, *J* = 7 6 Hz, anomeric H), 5 31 (1H, *d*, *J* = 7 1 Hz, anomeric H), 5 46 (1H, *br s*, olefinic H) (Found C, 63 14, H, 8 53 Calc for $C_{41}H_{64}O_{13}$ H_2O C, 62 90, H, 8 50%)

Mild acid hydrolysis of **8** Momordin Ie (**8**) (226 mg) was hydrolysed with 1 5% H_2SO_4 and treated in the same manner as **7** to give oleanolic acid (**14**) (23 mg, yield 20%) and prosapogenins, **1** (18 mg, yield 9%), **5** (42 mg, yield 26%), and **17** (45 mg, yield 23%).

Difference of saponin components in dry and fresh roots Fr root (length *ca* 4 cm) was divided into three pieces One was homogenized in hot MeOH (60 ml) and the homogenate made alkaline to pH 9 5 with dil NH_4OH The suspension was accurately dil to 100 ml with H_2O After ultrasonication of the suspension for 30 min, 10 μl of the supernatant was subjected to HPLC analysis The other pieces of root were then dried at 40 or 70° for 3 days or 1 day, respectively The ground dry root (*ca* 100 mg) was extracted with 60% MeOH (10 ml) soln made alkaline to pH 9 5 with dil NH_4OH under ultrasonication for 30 min and then 10 μl of the supernatant subjected to HPLC analysis Solvent MeOH–MeCN–H_2O (58 15 27) containing pentane sulphonic acid (4 25 mmol/l) Flow rate 1 ml/min R_t (min) momordin I (**1**) 12 3, momordin II (**2**) 3 6 The total contents of momordin II (T.M II) were calculated using the following equation T M II (%) = M II (%) + M I (%) × (926/764), where M II and M I are

the contents of **2** and **1**, respectively The figures 926 and 764 are the M_rs of **2** and **1**

Enzymatic hydrolysis of **2** *to* **1** *in aqueous* MeOH. Ground dry root (*ca* 150 mg) was accurately weighed, placed in H_2O, 30% MeOH, 60% MeOH, 80% MeOH or MeOH (each 10 ml) and allowed to stand at room temp for a definite period The suspension was made alkaline to pH 9 5 with dil NH_4OH and then H_2O or MeOH was added to the suspension to prepare a 60% MeOH soln The suspension was accurately dil to 25 ml with 60% MeOH and then sonicated for 30 min The supernatant (10 μl) was analysed by HPLC to calculate the content of momordin II (**2**)

Quantitative analysis of momordins in dry root One fr root was sliced (each slice *ca* 1 cm) and one slice (*ca* 1 g) was dried at 70° for 19 hr Other slices (1 3 kg) were soaked in 4% HCl (4 l) for 3 hr, washed with H_2O and then soaked in 3% $NaHCO_3$ soln (4 l) for 17 hr The root was then dried at 70° for 19 hr The dry root was ground and analysed quantitatively by HPLC Since the minor components could not be sepd from each other under the conditions described above, the HPLC conditions were changed as follows Column ODS (200 × 6 mm) Solvent MeCN–0.02 M Na_2HPO_4–0 05 M KH_2PO_4 (29 53.18). Flow rate 1 2 ml/min. R_t (min) **1**, 14 5, **6**, 13 2, **8**, 9 6, **2**, 7 7, **10**, 8 4, **11**, 7 0, **12**, 5 5, **13**, 5 8 Quantitative analysis of **5** and **7** was carried out using MeOH–MeCN–0.025 M NaH_2PO_4 (28 5·17) as solvent R_t (min) **5**, 15 7, **7**, 11 4

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