CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 33, No. 1 January 1985

Regular Articles

[Chem. Pharm. Bull. 33(1) 1–7 (1985)]

Studies on the Constituents of *Momordica cochinchinensis* SPRENG. II.[1] Isolation and Characterization of the Root Saponins, Momordins I, II and III[2]

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(Received March 8, 1984)

From the root of *Momordica cochinchinensis* SPRENG.(Cucurbitaceae), three saponins named momordins I, II and III have been isolated. Their structures were determined on the basis of chemical and spectral evidence as oleanolic acid 3-*O*-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranoside (momordin I), 28-*O*-β-D-glucopyranoside of momordin I (momordin II) and 3β-hydroxy-11α,12α-epoxy-olean-28,13-olide 3-*O*-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranoside (momordin III). Momordin II was proved to be identical with hemsloside Ma_1 isolated from the tubers of *Hemsleya macrosperma* and *H. chinensis*.

Keywords——*Momordica cochinchinensis*; Cucurbitaceae; pentacyclic triterpene glycoside; glucuronide saponin; bisdesmoside; oleanolic acid; 3β-hydroxy-11α,12α-epoxy-olean-28,13-olide

The root of *Momordica cochinchinensis* SPRENG. (Cucurbitaceae) is traditionally used in China as an expectorant and an antiphlogistic resolvent,[3] and it is known to contain a saponin named momordin. This saponin, isolated from the root collected in Taiwan, was once therapeutically used in Japan as an effective antitussive expectorant.[4] A chemical investigation of the structure of this saponin was carried out by Kuwada and Fuwa,[4] and the aglycone was reported to be oleanolic acid. Later, Hatta *et al.*,[5] and Nagao *et al.*[6] investigated the cultivation of this plant and the distribution of momordin in the whole plant. However, the structure of momordin has not been determined.

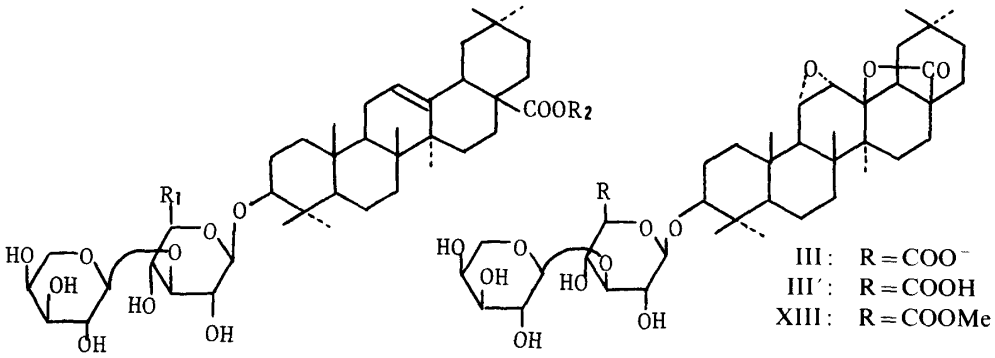
As a continuation of the studies on the constituents of this plant, the constituents of the root were investigated and one major and two minor saponins were isolated. They were designated as momordins I (I), II (II) and III (III). This paper deals with the elucidation of their structures.

The dried root was extracted first with MeOH and then with 50% MeOH. The extracts were treated as described in the experimental section, and I (yield: 10%), II (0.2%) and III (0.008%) were obtained in addition to sucrose (1.7%), fructose (0.6%) and glucose (0.6%). Momordins are neutral to litmus, but they gave acidic saponins (I′, II′ and III′, respectively) on treatment with a cation-exchange resin, indicating that they are all the salts of acidic saponins as in the case of the seed saponins.[1]

I was obtained as a white power, and its infrared (IR) spectrum showed a broad

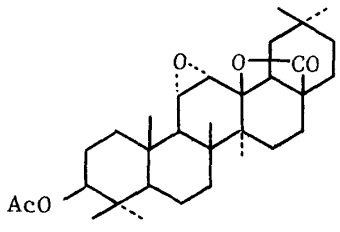
absorption band due to hydroxyl gorups and bands due to a carboxylic group (1690 cm^{-1}) and a carboxylate group (1610 cm^{-1}). I gave a monomethyl ester (IV) on treatment with diazomethane. When I was refluxed in 2 N HCl–MeOH, it gave an aglycone (V) and methyl pyranosides of α-L-arabinose and α-D-glucuronic acid methyl ester. The aglycone was identified as oleanolic acid after conversion to the methyl ester acetate (VI). On treatment of I with 0.5 N HCl–MeOH at room temperature in an attempt to obtain the prosapogenin, I gave, contrary to expectation, another kind of monomethyl ester (VII). The IR spectra of IV (1730 cm^{-1} and 1610 cm^{-1}), V (1690 cm^{-1}) and VII (1740 and 1690 cm^{-1}) indicated that IV is the methyl ester of the aglycone moiety, and VII is that of the glucuronic acid moiety. I was converted into a dimethyl ester (VIII) by treatment with a cation-exchange resin followed by methylation with diazomethane. In the field desorption mass spectrum (FD-MS) of VIII, the molecular ion peak was observed at m/z 793 $[M+1]^+$ as a base peak. This result and the elemental analysis data indicated the molecular formula $C_{43}H_{68}O_{13}\cdot 2H_2O$. The proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra exhibited signals of two anomeric protons (δ 4.97, d, $J=8$ Hz; 5.32, d, $J=7$ Hz) and anomeric carbons (δ 105.8 and 106.8). On reduction with $NaBH_4$, VIII gave a monomethyl ester (IX) (FD-MS m/z: 765 $[M+1]^+$), which gave methyl pyranosides of α-D-glucose and α-L-arabinose, and oleanolic acid methyl ester on complete methanolysis. IX was methylated by a modification of Hakomori's method to give a permethylate (X), which afforded methyl pyranosides of 2,3,4-tri-*O*-methyl-α-L-arabinose and 2,4,6-tri-*O*-methyl-α-D-glucose on methanolysis followed by thin layer chromatographic (TLC) and gas chromatographic analyses. From these data, it is apparent that the sugar moiety of I′ is L-arabinopyranosyl(1→3)-D-glucuronopyranoside. The configurations of the linkages of L-arabinopyranose and D-glucuronopyranose were deduced as α for the former and β for the latter from the coupling constants (7 and 8 Hz) of the two anomeric protons of VIII.

Consequently, I′ is oleanolic acid 3-*O*-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranoside. The large J-values of the anomeric protons indicate that both sugars take the Cl conformation in solution.



III: R = COO^-
III′: R = COOH
XIII: R = COOMe

I: $R_1 = COO^-$, $R_2 = H$
I′: R_1 = COOH, $R_2 = H$
II: $R_1 = COO^-$, R_2 = β-D-glu.pyr.
II′: R_1 = COOH, R_2 = β-D-glu.pyr.
IV: $R_1 = COO^-$, R_2 = Me
VII: R_1 = COOMe, $R_2 = H$
VIII = XII: R_1 = COOMe, R_2 = Me
IX: $R_1 = CH_2OH$, R_2 = Me
XI: R_1 = COOMe, R_2 = β-D-glu.pyr.



3β-acetoxy-11α,12α-epoxy-olean-28,13-olide

II was obtained as colorless needles. It gave oleanolic acid and methyl pyranosides of α-glucuronic acid methyl ester, α-arabinose and α-glucose on complete methanolysis. II showed in its IR spectrum absorptions due to an ester group (1725 cm^{-1}) and a carboxylate group (1610 cm^{-1}), but not that of a free carboxylic acid at 1690 cm^{-1} (due to the carboxyl group of oleanolic acid). This IR spectrum indicated that the carboxyl group of the aglycone is esterified and that of glucuronic acid is in the carboxylate form. The methyl ester (XI) of II′ showed in its FD-MS spectrum a molecular ion peak at *m/z* 963 $[M+Na]^+$. This result and the elemental analysis data indicated the molecular formula $C_{48}H_{76}O_{18}\cdot 2H_2O$. The ^{1}H-NMR and ^{13}C-NMR spectra exhibited the signals of three anomeric protons (δ 4.95, d, $J=7$ Hz; 5.28, d, $J=7$ Hz; 6.28, d, $J=7$ Hz) and carbons (δ 95.6, 105.8 and 106.7) indicating that II is composed of 1 mol each of arabinose, glucuronic acid and glucose, and the presence of the ^{1}H-NMR signal at lower field (δ 6.28) and the ^{13}C-NMR signal at higher field (δ 95.6) indicated that one of the three sugars is linked to a carboxyl group by the ester linkage. The ^{13}C-NMR spectrum of XI was similar to that of VIII except that it showed six additional signals attributable to an ester-linked β-glucopyranosyl group, and this spectrum suggested that II is the 28-*O*-β-glucopyranoside of I. II was hydrolyzed with alkali and the product was methylated with diazomethane. The dimethyl ester (XII) was identical with VIII. The absolute configuration of the β-glucopyranosyl group was determined as D because the difference of molecular rotation of XI (+125.0°) from that of VII (+157.2°) showed a negative value (−32.2°) ($[M]_D$ of methyl β-D-glucopyranoside: −66.3°).

Consequently, II′ is 3-*O*-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosido-28-*O*-β-D-glucopyranoside of oleanolic acid. II′ was proved to be identical with hemsloside Ma_1 isolated by Nie *et al.*[7] from the tubers of *Hemsleya macrosperma* and *H. chinensis*.

III was isolated as the methyl ester (XIII) (acetate: $C_{52}H_{74}O_{19}\cdot H_2O$). The IR spectrum showed an ester absorption band at 1740 cm^{-1} (shoulder) and a characteristic γ-lactone absorption at 1770 cm^{-1}. In the FD-MS spectrum, XIII gave an $[M+Na]^+$ ion peak at *m/z* 815 as a base peak. Methanolysis of III gave methyl pyranosides of α-glucuronic acid methyl ester and α-arabinose, but the aglycone fraction gave two spots on TLC, indicating that the aglycone is labile under acidic conditions. Attempts to obtain the genuine aglycone by enzymatic hydrolysis with cellulase or crude hesperidinase were not successful. The ^{1}H-NMR spectrum of XIII exhibited two anomeric proton signals at δ 4.92 (d, $J=7$ Hz) and 5.27 (d, $J=7$ Hz). The ^{13}C-NMR spectrum showed two anomeric carbon signals at δ 105.7 and 106.6. The chemical shifts of the ^{13}C-NMR signals due to the sugar moiety of XIII are almost identical with those of VIII. These results indicated that III has the same sugar moiety, *viz.* the α-L-arbinopyranosyl(1→3)-β-D-glucuronopyranosyl group, as I. The ^{1}H-NMR spectrum of XIII showed signals of seven methyl groups on quaternary carbons (δ 0.83, 0.89, 0.92, 0.94, 1.13 and 1.26(×2)), protons on carbons bearing an epoxy oxygen (δ 3.06, br d, $J=4$ Hz; 3.24, d, $J=4$ Hz), a carbomethoxyl group (δ 3.79) and anomeric protons (δ 4.92, d, $J=8$ Hz; 5.27, d, $J=7$ Hz). The ^{13}C-NMR spectrum of the aglycone moiety showed six C–C bonded quaternary carbon signals (δ 39.5, 41.0, 36.5, 41.7, 44.1 and 31.5), one lactone carbon signal (δ 178.5) and one C–O bonded quaternary carbon signal (δ 87.5), but no olefinic carbon signal. From these spectral data coupled with the fact that the other momordins are oleanolic acid glycosides, the aglycone of III was presumed to be 3β-hydroxy-11,12-epoxy-olean-28,13-olide. Katai *et al.*[8] reported the ^{13}C-NMR signal assignment of 3β-acetoxy-olean-28,13-olide (XIV). Comparison of the chemical shifts of the C–C bonded quaternary carbons of XIV [δ 37.9 (C_4)[9], 42.3 (C_8), 36.8 (C_{10}), 42.3 (C_{14}), 44.1 (C_{17}) and 31.5 (C_{20})] with those of XIII supported the presumed structure. The slight upfield shifts of the signals assigned to C_8 and C_{14} (δ 41.0 and 41.7) of XIII as compared to those of XIV are considered to be caused by the introduction of the epoxy group at $C_{11,12}$.[11] In the region from δ 49 to 60 in the ^{13}C-NMR spectrum, six carbon signals (δ 49.9 d, 51.2 d, 52.0 q, 52.6 d, 55.1 d and 57.3 d) were observed.

Taking into consideration that the C_5, C_9, C_{18} and methoxyl carbon (of methyl glucuronate) signals appear in this region,[8] the other two which collapsed into doublets in the off-resonance decoupling spectrum should be those of the epoxidic carbons.

Kitagawa *et al.*[12] synthesized 11α,12α-epoxy-oleanolic lactone by photooxidation of oleanolic acid, and in their paper, the ^{1}H-NMR signals of the epoxide protons measured in $CDCl_3$ were reported to appear at δ 2.95 as a two-proton singlet. Later in 1983, Majumder and Bagghi[13] performed the oxidative transformation of acetyl oleanolic acid to the corresponding 11α,12α-epoxy-lactone. In their case, too, the ^{1}H-NMR ($CDCl_3$) signals of the epoxide protons appeared at δ 3.00 as a singlet, confirming Kitagawa's result. On the other hand, in our case (measured in pyridine-d_5), XIII showed the epoxide proton signals as two one-proton doublets (δ 3.06, 3.24, J=4 Hz). Since the difference of the signal patterns of the epoxide protons of XIII from the reported patterns might be due to the "solvent effect," 3β-acetoxy-11α,12α-epoxy-oleanolic lactone was synthesized according to the method reported by Majumder and Bagghi,[13] and the ^{1}H-NMR spectra both in $CDCl_3$ and pyridine-d_5 were compared. In $CDCl_3$ solution, the epoxidic protons appeared as a two-proton singlet, in accordance with the data reported by Majumder and Bagghi. In pyridine-d_5, the epoxidic protons appeared at δ 3.25 (H, d, J=4 Hz) and 3.07 (H, dd, J=4, 2 Hz) and this signal pattern was similar to that of XIII. The ^{13}C-NMR spectrum measured in pyridine-d_5 was almost identical with that of the aglycone moiety of XIII, although some deviation of the chemical shifts caused by glycosidation was noted.

From these chemical and spectral data, III′ is concluded to be 3β-hydroxy-11α,12α-epoxy-olean-28,13-olide 3-*O*-α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranoside.

Experimental[12]

Extraction and Fractionation——The dried roots (1 kg) of *Momordica cochinchinensis* SPRENG. (cultivated for three years and harvested in 1982 at Kyoto Herbal Garden, Pharmacognosy Laboratory, Central Research Division, Takeda Chemical Industries, Ltd.) were crushed and percolated with MeOH (5 l) and then with 50% MeOH (8 l). The solutions were evaporated *in vacuo* to give the MeOH extract (73 g) and the 50% MeOH extract (100 g). When the extracts were triturated with MeOH, almost homogeneous I was obtained as a white powder (18 g from the MeOH extract, and 65 g from the 50% MeOH extract). The filtrates were combined and evaporated *in vacuo* to give a light brown residue (90 g), which was dissolved in 50% MeOH and chromatographed on an MCI Gel column (200 ml). Elution with 50% MeOH gave the sugar fraction (59 g), and the further elution with MeOH gave the saponin fraction (26 g). The saponin fraction was repeatedly chromatographed on silica gel (100 times the weight of materials) using $CHCl_3$–MeOH–H_2O (7:3:0.5 and 32:8:1) to give fractions A and B containing thin-layer-chromatographically homogeneous I (17 g) and II (2 g), respectively, and fraction C (0.1 g), which contained III with a slight impurity. The MeOH solution of fraction C was passed through Amberlite IRC-84 (20 g) and the effluent (III′) was methylated with diazomethane. The product was purified by chromatography on LiChroprep RP-18 (solvent: 80% MeOH) to give the methyl ester (XIII) of III′.

I: A white powder from MeOH. mp 235—240 C (dec.). $[\alpha]_D^{16}$ +28.0° (c=0.4, $CHCl_3$–MeOH–H_2O (2:7:1)). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (–COOH), 1610 (–COO^-). *Anal.* Found: C, 57.40; H, 8.11.

II: Colorless needles from MeOH. mp 245—250 C (dec.). $[\alpha]_D^{16}$ +5.7° (c=0.7, MeOH–H_2O (2:1)). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (–COO-sugar), 1610 (–COO^-). *Anal.* Found: C, 50.91; H, 7.34.

XIII: Colorless needles from MeOH. mp 232—235 C. IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1770 (γ-lactone), 1740 (shoulder, –COOR), 870 (epoxide), FD-MS m/z: 815 $[M+Na]^+$. ^{1}H-NMR δ: 0.83, 0.89, 0.92, 0.94, 1.13, 1.26 (×2) (all s, >C–CH_3), 3.06 (H, br d, J=4 Hz, C_{11}-H_β), 3.24 (H, d, J=4 Hz, C_{12}-H_β), 3.79 (3H, s, –$COOCH_3$), 4.92 (H, d, J=7 Hz, anomeric H of methyl glucuronate), 5.27 (H, d, J=7 Hz, anomeric H of arabinose). ^{13}C-NMR δ: 31.5 s (C_{20}), 36.5 s (C_{10}), 39.5 s (C_4), 41.0 s ($C_{8/14}$), 41.7 s ($C_{14/8}$), 44.1 s (C_{17}), 52.0 q (–$COOCH_3$), 52.6 d, 57.3 d (C_{11} and C_{12}), 87.5 s (C_{13}), 89.1 d (C_3), 105.7 d, 106.6 d (anomeric carbons), 169.9 s (–$COOCH_3$), 178.5 s (C_{28}).

Acetate (XV): XIII (35 mg) was acetylated with Ac_2O–pyridine mixture at room temperature. The product was purified by silica gel chromatography with hexane–AcOEt (3:1) to give XV (42 mg): colorless needles from MeOH. mp 184—185 C. FD-MS m/z: 1025 $[M+Na]^+$, 1002 $[M]^+$. *Anal.* Calcd for $C_{52}H_{74}O_{19}\cdot H_2O$: C, 61.16; H, 7.50. Found: C, 61.36; H, 7.52. ^{1}H-NMR ($CDCl_3$) δ: 2.0—2.1 (5 singlets, OAc), 3.01 (2H, s, C_{11}-H_β and C_{12}-H_β), 3.73 (3H, s, –$COOCH_3$).

The sugar fraction was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (7:3:0.5 and

32 : 8 : 1), and three sugars were obtained. Two were obtained as syrups, and they were identified as glucose and fructose after conversion to the peracetates followed by GC comparison with authentic samples. The third sugar gave colorless needles from dil. MeOH: mp 185—188 °C. $[\alpha]_D^{26}$ +67.0° (c=1.0, MeOH–H_2O (1 : 2)). Its IR spectrum was identical with that of sucrose.

Momordin I Monomethyl Ester (IV)——I (100 mg) was suspended in dil. MeOH and an ether solution of diazomethane was added. The product was purified by silica gel column chromatography (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)) to give IV (36 mg): a white powder. IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1730 (–COOR), 1610 (–COO^-).

Methanolysis of I and Identification of the Aglycone and the Component Sugars——I (500 mg) was dissolved in 2 N HCl (MeOH) (5 ml) and the solution was refluxed for 3 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated and the residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (32 : 8 : 1) to separate the aglycone fraction (297 mg) and the sugar fraction (175 mg). The aglycone fraction was further chromatographed on silica gel with 10% acetone in benzene to give V (207 mg): colorless needles from MeOH. mp 306—308 °C. $[\alpha]_D^{26}$ +72.5° (c=1.0, $CHCl_3$). EI-MS m/z: 456.361 $[M]^+$ (Calcd for $C_{30}H_{48}O_3$: 456.360), 438, 248 (base peak), 203. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1690 (–COOH). ^{1}H-NMR δ: 0.91, 0.97, 1.02(×2), 1.24, 1.30 (all s, >C–CH_3), 3.44 (H, t, J=8 Hz, C_3-H_α), 5.50 (H, br s, C_{12}-H). ^{13}C-NMR δ: 30.9 s (C_{20}), 37.4 s (C_{10}), 39.4 s (C_4), 39.8 s (C_8), 42.2 s (C_{14}), 46.7 s (C_{17}), 78.1 d (C_3), 122.5 d (C_{12}), 144.8 s (C_{13}), 180.1 s (C_{28}). V (100 mg) was acetylated with Ac_2O–pyridine at room temperature and then methylated with diazomethane to give VI (110 mg): colorless needles from $CHCl_3$–MeOH. mp 223—224 °C. $[\alpha]_D^{27}$ +66.9° (c=1.0, $CHCl_3$). EI-MS m/z: 512.386 $[M]^+$ (Calcd for $C_{33}H_{52}O_4$: 512.386), 452, 262, 248, 203 (base peak). ^{1}H-NMR ($CDCl_3$) δ: 0.73, 0.86(×2), 0.90, 0.93(×2), 1.12 (all s, >C–CH_3), 2.04 (3H, s, –Ac), 2.7—3.0 (H, m, C_{18}-H_β), 3.62 (3H, s, –$COOCH_3$), 4.50 (H, t, J=8 Hz, C_3-H_α), 5.28 (H, br t, J=4 Hz, C_{12}-H). ^{13}C-NMR ($CDCl_3$) δ: 30.6 s (C_{20}), 36.9 s (C_{10}), 37.6 s (C_4), 39.3 s (C_8), 41.6 s (C_{14}), 46.7 s (C_{17}), 51.5 q (–$COO\underline{C}H_3$), 80.9 d (C_3), 122.2 d (C_{12}), 143.7 s (C_{13}), 170.2 s (Ac), 178.2 s (C_{28}).

The methyl glycoside fraction was repeatedly chromatographed on silica gel with 10% MeOH in $CHCl_3$ to give four methyl glycosides in a thin-layer-chromatographically homogeneous state. Each glycoside was acetylated in a usual manner. Among four acetylated methyl glycosides, methyl 2,3,4-tri-*O*-acetyl-6-*O*-methyl-α-D-glucuronopyranoside ($[\alpha]_D^{12}$ +109.6° (c=1.2, $CHCl_3$)) and methyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranoside ($[\alpha]_D^{12}$ +10.7° (c=0.4, $CHCl_3$)) were identified by comparing their ^{1}H-NMR spectra and specific rotations with those of authentic samples. The other two acetylated methyl glycosides were proved to be the same as the acetylated methyl arabinosides which were obtained by methanolysis of L-arabinose followed by acetylation, by comparing the ^{1}H-NMR spectra.

Treatment of I with 0.2 N HCl–MeOH Leading to the Monomethyl Ester (VII)——I (200 mg) was dissolved in 0.2 N HCl (MeOH) (5 ml) and the solution was stirred at room temperature for 5 h. After neutralization with Ag_2CO_3 and removal of the precipitates by filtration, the filtrate was concentrated and the residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5). The effluent was again chromatographed and elution with AcOEt–MeOH (9 : 1) gave VII (180 mg): colorless needles from dil. MeOH. mp 225—227 °C. $[\alpha]_D^{15}$ +20.2°. FD-MS m/z: 801 $[M+Na]^+$ (base peak). IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1740 (–COOR), 1690 (–COOH). ^{1}H-NMR δ: 0.81, 0.97(×2), 1.02, 1.22, 1.29(×2) (all s, >C–CH_3), 3.1—3.5 (H, m, C_{18}-H_β), 3.75 (3H, s, –$COOCH_3$), 4.94 (H, d, J=8 Hz, anomeric H of methyl glucuronate), 5.28 (H, d, J=7 Hz, anomeric H of arabinose), 5.47 (H, br s, C_{12}-H). ^{13}C-NMR δ: 30.9 s (C_{20}), 37.0 s (C_{10}), 39.5 s (C_4), 39.7 s (C_8), 42.2 s (C_{14}), 46.7 s (C_{17}), 52.1 q (–$COO\underline{C}H_3$), 89.3 d (C_3), 105.9 d, 106.7 d (anomeric carbons), 123.8 d (C_{12}), 144.8 s (C_{13}), 170.2 s (–$\underline{C}OOCH_3$), 180.1 s (C_{28}).

Momordin I Dimethyl Ester (VIII)——I (1 g) was dissolved in MeOH–H_2O–BuOH mixture, and the solution was passed through a column of Amberlite IRC-84 (50 g). The column was washed with 50% MeOH. The acidic effluents were concentrated *in vacuo* to give I′ as a white powder (IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1730 (–COOH), 1690 (–COOH)). I′ (800 mg) was dissolved in MeOH (40 ml), an ethereal solution of diazomethane was added, and the mixture was stirred. After evaporation of the solvent, the residue was chromatographed on silica gel using 5% MeOH in $CHCl_3$ as the eluting solvent to give VIII (700 mg): colorless needles from MeOH. mp 205—207 °C. $[\alpha]_D^{28}$ +25.1° (c=1, MeOH). FD-MS m/z: 793 $[M+1]^+$. *Anal.* Calcd for $C_{43}H_{68}O_{13}\cdot 2H_2O$: C, 62.30; H, 8.75. Found: C, 62.15; H, 8.67. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (–COOR). ^{1}H-NMR δ: 0.83(×2), 0.94(×2), 0.97, 1.26, 1.31 (all s, >C–CH_3), 3.72 (3H, s, –$COOCH_3$), 3.76 (3H, s, –$COOCH_3$), 4.97 (H, d, J=8 Hz, anomeric H of methyl glucuronate), 5.32 (H, d, J=7 Hz, anomeric H of arabinose), 5.39 (H, br s, C_{12}-H). ^{13}C-NMR δ: 30.8 s (C_{20}), 36.9 s (C_{10}), 39.5 s (C_4), 39.7 s (C_8), 41.9 s (C_{14}), 46.9 s (C_{17}), 51.6 q, 52.1 q (–$COO\underline{C}H_3$), 89.3 d (C_3), 105.8 d, 106.8 d (anomeric carbons), 122.7 d (C_{12}), 144.1 s (C_{13}), 170.2 s (–$\underline{C}OOCH_3$), 177.9 s (C_{28}).

$NaBH_4$ Reduction of VIII——$NaBH_4$ (200 mg) was added to a solution of VIII (200 mg) in MeOH (10 ml) and the whole was stirred at room temperature for 1 h. The reaction mixture was treated with Dowex 50W×8 (15 g) and then with Amberlite IRA-45 (15 g). The product was chromatographed on silica gel with 5% MeOH in $CHCl_3$ to give IX (160 mg): colorless needles from MeOH. mp 198—201 °C. $[\alpha]_D^{28}$ +38.5° (c=1.0, MeOH). FD-MS m/z: 765 $[M+1]^+$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (–COOR). ^{1}H-NMR δ: 0.83, 0.85, 0.94(×2), 1.01, 1.25, 1.32 (all s, >C–CH_3), 3.72 (3H, s, –$COOCH_3$), 4.90 (H, d, J=7 Hz, anomeric H of glucose), 5.24 (H, d, J=7 Hz, anomeric H of arabinose), 5.41 (H, br s, C_{12}-H). ^{13}C-NMR δ: 30.8 s (C_{20}), 37.0 s (C_{10}), 39.5 s (C_4), 39.7 s (C_8), 41.9 s (C_{14}), 47.0 s (C_{17}), 51.6 q (–$COO\underline{C}H_3$), 89.0 d (C_3), 106.0 d, 106.4 d (anomeric carbons), 122.8 d (C_{12}), 144.1 s (C_{13}), 177.9 s (C_{28}).

Methanolysis of IX and Identification of the Aglycone and the Component Sugars——IX (5 mg) was dissolved in

2 N HCl (MeOH) (1 ml) and the solution was refluxed for 3 h. The solution was then neutralized with Ag_2CO_3, the precipitates were filtered off, and the filtrate was concentrated *in vacuo*. The aglycone was identified as oleanolic acid methyl ester by TLC (benzene–acetone (7 : 1)). The methanolysate was acetylated and the product was checked by gas chromatography (2% ECNSS-M on Chromosorb WAW DMCS (60—80 mesh), 1.4 m × 3 mmϕ glass column; column temp., 150 °C; carrier gas, He 52 ml/min). The t_R values and identifications of the peaks were as follows: 1.03, 1.34 (peracetates of methyl L-arabinosides), 5.53 (methyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside), 7.13 (methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside).

Methylation of IX——IX (20 mg) and NaH (100 mg) were added to anhydrous tetrahydrofuran (3 ml), and the whole was stirred for 10 min. CH_3I (2 ml) was then added and the mixture was stirred for 2 h at room temperature. After dilution with water, the mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried and concentrated. The residue was chromatographed on the silica gel column with 15% acetone in benzene to give X as a solid. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1730 (–COOR), no hydroxyl group absorption was observed. ^{1}H-NMR ($CDCl_3$) δ: 0.73, 0.84, 0.93(×3), 1.02, 1.12 (all s, >C–CH_3), 3.4—3.6 (–OCH_3 ×6), 3.62 (3H, s, –$COOCH_3$), 4.29 (H, d, J=8 Hz, anomeric H of the methylated glucose moiety), 4.79 (H, d, J=5.5 Hz, anomeric H of the methylated arabinose moiety), 5.28 (H, br t, J=4 Hz, C_{12}-H).

Methanolysis of X and Identification of the Component Methylated Sugars by Gas Chromatography——X (3 mg) was dissolved in 2 N HCl (MeOH) (1 ml) and the solution was refluxed for 3 h, then worked up in a usual manner to give the methanolysate. The product was subjected to GC (the same column as mentioned above; column temp., 130 °C; carrier gas, He 52 ml/min). The t_R values and identifications of the peaks were as follows: 1.34 (methyl 2,3,4-tri-*O*-methyl-L-arabinopyranosides), 4.00 (methyl 2,4,6-tri-*O*-methyl-α-D-glucopyranoside), 6.74 (methyl 2,4,6-tri-*O*-methyl-β-D-glucopyranoside).

Methanolysis of II, and Identification of the Aglycone and the Component Sugars——II (40 mg) was dissolved in 2 N HCl (MeOH) (2 ml) and the solution was refluxed for 3 h. After neutralization with Ag_2CO_3, the methanolysate was diluted with water. The precipitates (12 mg) were collected by filtration and crystallized from MeOH to give colorless needles. mp 305—308 °C. The IR spectrum was identical with that of V. The filtrate was concentrated and the residue was acetylated. The acetylation product was checked by GC. The acetates of methyl glycosides of arabinose, methyl glucuronate and glucose were identified.

Methyl Ester (XI) of II′——II (300 mg) was dissolved in 50% MeOH and passed through a column of Amberlite IRC-84 (30 g). The effluent was concentrated *in vacuo* to give a white powder (II′). IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1730 (–COOH and –COO–sugar). II′ (250 mg) was dissolved in MeOH (3 ml) and ethereal diazomethane was added to the solution. After evaporation of the solvent, the residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (32 : 8 : 1) as the eluting solvent to give XI (220 mg): colorless needles from dil. EtOH. mp 240—242 °C. $[\alpha]_D^{16}$ +13.3° (c=1.0, MeOH). FD-MS m/z: 963 $[M+Na]^+$. *Anal.* Calcd for $C_{48}H_{76}O_{18}\cdot 2H_2O$: C, 59.00; H, 8.25. Found: C, 58.76; H, 8.25. IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1740 (–$COOCH_3$ and –COO–sugar). ^{1}H-NMR δ: 0.86, 0.90, 0.92, 0.96, 1.08, 1.28, 1.29 (all s, >C–CH_3), 3.0—3.4 (H, m, C_{18}-H_β), 3.74 (3H, s, –$COOCH_3$), 4.95 (H, d, J= 7 Hz, anomeric H of methyl glucuronate), 5.28 (H, d, J=7 Hz, anomeric H of arabinose), 5.42 (H, br s, C_{12}-H), 6.28 (H, d, J=7 Hz, anomeric H of glucose). ^{13}C-NMR δ: 30.7 s (C_{20}), 36.9 s (C_{10}), 39.4 s (C_4), 39.8 s (C_8), 42.0 s (C_{14}), 46.9 s (C_{17}), 52.1 q (–$COOCH_3$), 89.2 d (C_3), 95.6 d, 105.8 d, 106.7 d (anomeric carbons), 122.7 d (C_{12}), 144.0 s (C_{13}), 170.1 s (–$COOCH_3$), 176.3 s (C_{28}).

Alkaline Hydrolysis of II and Methylation with Diazomethane——II (100 mg) was suspended in 2 N KOH (2 ml) and the suspension was heated on a boiling water bath for 3 h, then treated with Dowex 50W × 8. The alkaline hydrolysis product was dissolved in MeOH (3 ml) and methylated with diazomethane. The product was chromatographed on silica gel with 5% MeOH in $CHCl_3$ to give XII (70 mg): colorless needles from MeOH. mp 206—209 °C. $[\alpha]_D^{26}$ +24.7° (c=1.0, MeOH). The ^{1}H-NMR and ^{13}C-NMR spectra were identical with those of VIII.

Methanolysis of III——III (5 mg) was dissolved in 2 N HCl (MeOH) (1 ml) and refluxed for 3 h. The reaction mixture was neutralized and acetylated in a usual manner. The product was checked by GC to identify the derivatives of arabinose and glucuronic acid.

Oxidative Transformation of Acetyl Oleanolic Acid to 3-*O*-Acetyl-11α,12α-epoxy-olean-28,13-olide——A mixture (6 ml) of 35% H_2O_2 and AcOH (1 : 1) was added to a hot solution of acetyl oleanolic acid (800 mg) in AcOH (25 ml). The whole was heated for 2 h on a boiling water bath, another 3 ml of the mixed reagent was added, and the reaction mixture was heated for a further 1 h, then diluted and extracted with $CHCl_3$. The $CHCl_3$ extract was washed, dried and evaporated. The residue was chromatographed on silica gel with hexane–AcOEt (9 : 1). The effluent was repeatedly chromatographed on silica gel using hexane–AcOEt (9 : 1) and hexane–acetone (9 : 1) as the eluting solvents to give 3β-*O*-acetyl-11α,12α-epoxy-olean-28,13-olide: colorless needles from $CHCl_3$–MeOH. mp 295—296 °C (reported: mp 295 °C). EI-MS m/z: 512.351 $[M]^+$ (calcd for $C_{32}H_{48}O_5$: 512.350). IR ν_{max}^{KBr} cm^{-1}: 1770 (γ-lactone), 1730, 1250 (OAc), 870 (epoxide). ^{1}H-NMR δ: 2.07 (3H, s, OAc), 3.07 (H, dd, J=4, 2 Hz, C_{11}-H_β), 3.25 (H, d, J= 4 Hz, C_{12}-H_β), 4.5—4.8 (H, m, C_3-H_β). ^{1}H-NMR ($CDCl_3$) δ: 2.05 (3H, s, OAc), 3.01 (2H, s, C_{11}-H_β and C_{12}-H_β), 4.50 (H, t, J=8 Hz, C_3-H_α). ^{13}C-NMR δ: 31.5 s (C_{20}), 36.6 s (C_{10}), 37.9 s (C_4), 40.9 s, 41.6 s (C_8 and C_{14}), 44.1 s (C_{17}), 52.6 d, 57.3 d (C_{11} and C_{12}), 80.4 d (C_3), 87.5 s (C_{13}), 170.4 s (CH_3COO–), 178.8 s (C_{28}).

Acknowledgement The authors wish to express their gratitude to Dr. M. Goto and Dr. Y. Nagao of Takeda Chemical Industries Ltd., for their geneous supply of the roots of *M. cochinchinensis*. Thanks are also due to Prof. Tanaka of Hiroshima University for his help in demonstrating the identity of momordin II with hemsloside Ma_1. An authentic sample of methyl 2,3,4-tri-*O*-methyl-α-L-arabinopyranoside was kindly provided by Assoc. Prof. Mihashi of this University, and mass, ^{1}H-NMR and ^{13}C-NMR spectra were measured by Mr. T. Fujioka, Miss K. Sato and Mrs. Y. Iwase of this Faculty.

This work was supported in part by a grant from the Central Research Institute of this University.

References and Notes

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