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## Studies on the Constituents of *Momordica charantia* L. II.<sup>1)</sup> Isolation and Characterization of Minor Seed Glycosides, Momordicosides C, D and E<sup>2)</sup>

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Three new triterpene glycosides, named momordicosides C, D and E, were isolated from the seeds of *Momordica charantia* L. (Cucurbitaceae). Their structures were determined on the basis of spectral and chemical evidence as 3-O- $\beta$ -gentiobiosides of cucurbit-5-ene-3 $\beta$ ,23,24,25-tetraol, cucurbita-5,24-diene-3 $\beta$ ,22,23-triol and 3 $\beta$ -hydroxy-23,24,25,26,27-pentanor-20( $\xi$ )-cucurbit-5-en-22-al, respectively.

**Keywords**—*Momordica charantia* L.; Cucurbitaceae; momordicosides C, D and E; triterpene glycosides; cucurbit-5-ene-3 $\beta$ ,23,24,25-tetraol; cucurbita-5,24-diene-3 $\beta$ ,22,23-triol; 3 $\beta$ -hydroxy-23,24,25,26,27-pentanor-20( $\xi$ )-cucurbit-5-en-22-al; cucurbitane; pentanorcucurbitacin

In the preceding paper<sup>1)</sup> of this series, we reported the isolation and structure elucidation of momordicosides A (I) and B (II), which are glycosides of cucurbit-5-ene-3 $\beta$ ,22(*S*),23(*R*),-24(*R*), 25-pentaol (III), isolated from the seeds of *Momordica charantia* L.

As a continuation of our examination of the less polar fraction, we have isolated  $\beta$ -gentiobiosides of three kinds of cucurbit-5-ene derivatives which differ only in the structures of the side chains. They were designated as momordicosides C (IV), D (V) and E (VI) in order of decreasing polarity, and this paper deals with their structures.

The less polar glycoside fraction after removal of I and II was repeatedly chromatographed on silica gel, and the thin-layer-chromatographically homogeneous momordicosides C, D and E were obtained. The latter two momordicosides could not be crystallized and were purified by acetylation followed by chromatography.

Momordicoside C (IV) was formulated as C<sub>42</sub>H<sub>72</sub>O<sub>14</sub> based on elementary analysis and the (M+Na)<sup>+</sup> ion peak observed at *m/z* 823 in the field desorption mass spectrum (FD-MS).

On methanolysis under mild conditions, IV gave an aglycone (VII), which was analyzed as C<sub>30</sub>H<sub>52</sub>O<sub>4</sub>. The proton nuclear magnetic resonance (PMR) spectrum of VII showed signals of seven tertiary methyl groups ( $\delta$  0.86, 0.88, 0.96, 1.15, 1.42, 1.73 and 1.77), one secondary methyl group ( $\delta$  1.18, d, *J*=6 Hz), one olefinic proton ( $\delta$  5.65, d, *J*=5 Hz) and three carbinyl protons ( $\delta$  3.77, bs; 3.81, d, *J*=8 Hz; 4.45, m). The C-13 nuclear magnetic resonance (CMR) spectrum exhibited the signals of tri-substituted olefinic carbons ( $\delta$  143.2s and 119.3d), four oxygen-bearing carbons ( $\delta$  79.7d, 76.0d, 74.4s and 71.0d) and four quaternary carbons ( $\delta$  49.6, 46.6, 41.7 and 34.7).

On treatment with Ac<sub>2</sub>O-pyridine at room temperature, VII gave a triacetate in which the tertiary hydroxyl group remained unacetylated. When VII was treated with periodic acid in methanol, it provided a dimethylacetal (VIII) having one hydroxyl group.

VIII showed the PMR signal of one proton on the dimethylacetal carbon next to a methylene group ( $\delta$  4.65, dd, *J*=4, 7 Hz) in addition to those of five tertiary methyl groups, one secondary methyl group, one carbinyl proton ( $\delta$  3.72, bs), one olefinic proton ( $\delta$  5.64, d, *J*=6 Hz) and protons of two methoxyl groups ( $\delta$  3.36).

The EI-MS showed the molecular ion peak at *m/z* 432 and fragment ion peaks at *m/z* 280 (M<sup>+</sup> -152), 163 and 152, the last of which was furnished by the retro-Diels-Alder fragmentation of a typical cucurbit-5-ene derivative having one hydroxyl group in ring A.<sup>3)</sup> The frag-

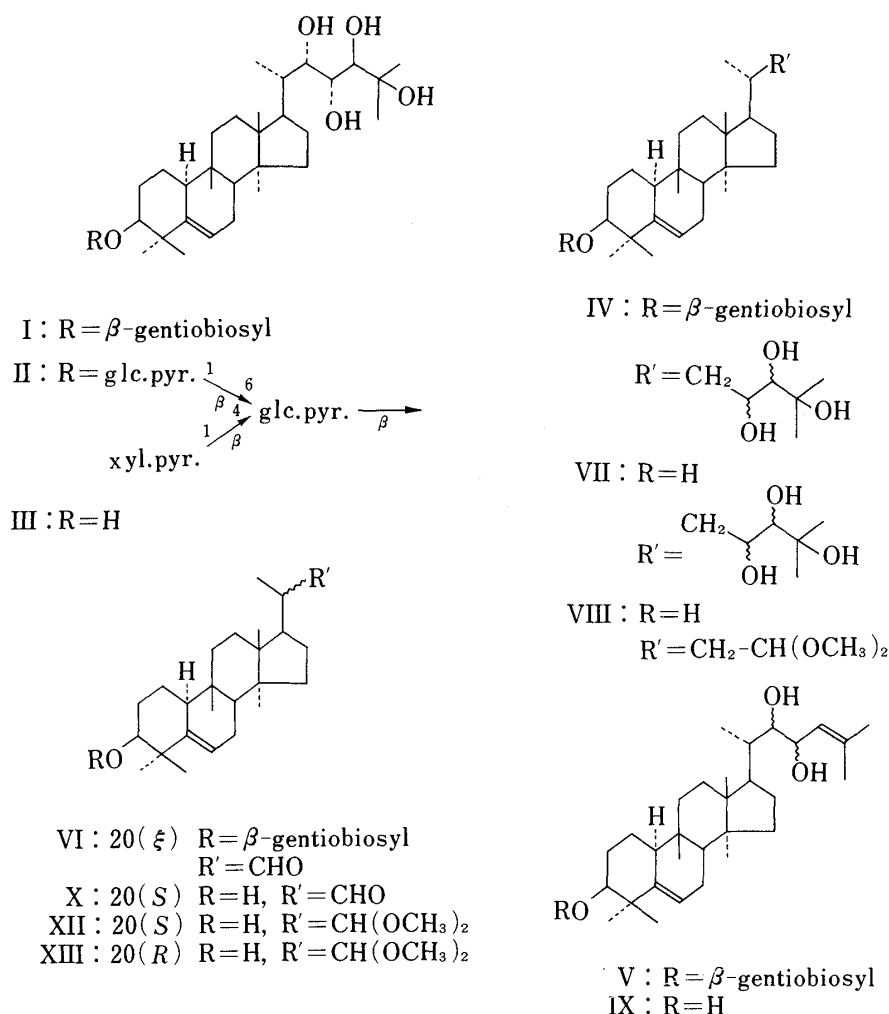


Fig. 1

ment ion peak at  $m/z$  163 appeared to have originated from the higher mass fragment ion ( $m/z$  280) by splitting of the side chain ( $m/z$  117).

These PMR and mass spectral data suggested that VIII has the monohydroxylated cucurbit-5-ene nucleus with a  $\text{CH}_3\text{-CH-CH}_2\text{-CH}(\text{OCH}_3)_2$  group as the side chain.

The CMR spectrum of VIII showed the signals of two olefinic carbons ( $\delta$  143.3s and 119.3d), four oxygen-bearing carbons ( $\delta$  103.6d, a dimethylacetal carbon; 76.0d, a carbinyl carbon; 53.0q and 52.0q, methoxyl carbons) and four quaternary carbons.

The CMR chemical shifts of the carbinyl carbon and the quaternary carbons were quite similar to those of 22,22-di-O-methyl-23,24,25,26,27-pentanorcucurbit-5-en-3 $\beta$ -ol which was obtained by periodic acid oxidation of III.<sup>1)</sup>

From both chemical and spectral evidence, VIII was considered to be 23,23-di-O-methyl-24,25,26,27-tetanorcucurbit-5-en-3 $\beta$ -ol, and consequently, VII was concluded to be cucurbit-5-ene-3 $\beta$ ,23,24,25-tetraol. The absolute configurations at C-23 and -24 could not be determined.

Momordicoside D (V) was isolated in homogeneous form as its acetate. V was formulated as  $\text{C}_{42}\text{H}_{70}\text{O}_{13}$  by elementary analysis and by the FD-MS showing a single peak at  $m/z$  805, which was ascribable to the  $(\text{M} + \text{Na})^+$  ion.

On enzymatic hydrolysis using crude hesperidinase,<sup>4)</sup> V gave an aglycone (IX). The CMR spectrum of IX exhibited signals of four olefinic carbons ( $\delta$  143.3s, 133.0s, 128.1d and 119.3d) and three oxygen-bearing carbons ( $\delta$  76.3d, 76.0d and 68.1d).

The PMR spectrum showed signals of two methyl groups on an olefinic carbon ( $\delta$  1.76 and 1.75), one carbinyl proton ( $\delta$  3.74, bs), two protons on the glycolic carbons ( $\delta$  3.95, dd,  $J=6$ ,

2 Hz; 4.70, dd,  $J=6, 10$  Hz) and two olefinic protons ( $\delta$  5.68, 2H, m). The CMR signals of four quaternary carbons and fragment ion peaks ( $m/z$  152 and 163) in the EI-MS of IX were similar to those of VII.

These data indicated IX to be also a cucurbit-5-ene derivative having one hydroxyl group in ring A, and one glycol unit and one tri-substituted double bond in the side chain.

When IX was treated with sodium periodate, an aldehyde (X) was formed. Its EI-MS was identical with that of 3 $\beta$ -hydroxy-23,24,25,26,27-pentanorcucurbit-5-en-22-al, which was obtained by periodate oxidation of III,<sup>1)</sup> and there was no depression of the melting points on admixture.

Thus, IX is cucurbita-5,24-diene-3 $\beta$ ,22,23-triol. The absolute configurations at C-22 and -23 could not be determined.

Momordicoside E (VI) was also isolated as an acetate (XI), which was analyzed as  $C_{51}H_{74}O_{19} \cdot 1/2H_2O$ . The PMR spectrum showed the signal of an aldehydic proton ( $\delta$  9.65, d,  $J=3$  Hz) together with five tertiary methyl, one secondary methyl and acetyl methyl signals. The CMR spectrum exhibited signals of an aldehydic carbon ( $\delta$  205.0d), two olefinic carbons ( $\delta$  142.4s and 119.1d), four quaternary carbons and thirteen oxygen-bearing carbons including two anomeric carbons ( $\delta$  102.2d and 101.2d).

XI was treated with sodium methoxide at room temperature to give a deacetylation product (VI') which gave a single spot on TLC, but it could not be crystallized. The FD-MS of VI' showed a peak at  $m/z$  719 assignable to the  $(M+Na)^+$  ion, but the PMR and CMR spectra showed two aldehydic protons ( $\delta$  9.66, d,  $J=2.5$  Hz; 9.67, d,  $J=4$  Hz) and carbon signals ( $\delta$  205.7d and 204.9d), respectively, which indicated VI' to be a mixture of two aldehydes.

VI' was treated with methanolic hydrochloride at room temperature to give two dimethylacetals (XII and XIII). The EI-MS of the less polar one (XII) exhibited a molecular ion peak at  $m/z$  418 and fragment ion peaks at  $m/z$  266 ( $M^+ - 152$ ), 163 and 152. The PMR spectrum<sup>5)</sup> of XII showed signals due to five tertiary methyl groups, a secondary methyl group, two methoxyl groups ( $\delta$  3.41 and 3.48), a dimethylacetal proton ( $\delta$  4.27, d,  $J=1.5$  Hz), a carbinyll proton ( $\delta$  3.76, bs) and one olefinic proton ( $\delta$  5.65, d,  $J=5$  Hz). These data were identical with those of 22,22-di-O-methyl-23,24,25,26,27-pentanorcucurbit-5-en-3 $\beta$ -ol,<sup>1)</sup> and no depression of the melting point was observed on admixture.

The polar dimethylacetal (XIII) gave the same EI-MS as XII, but the PMR spectrum was slightly different from that of XII, especially at the chemical shifts and coupling constant of the dimethylacetal proton ( $\delta$  4.46, d,  $J=2$  Hz) and methoxyl proton signals ( $\delta$  3.39 and 3.44), indicating XIII to be a 20(R)-epimer of XII.

Therefore, the aglycone of VI' is a mixture of 3 $\beta$ -hydroxy-23,24,25,26,27-pentanorcucurbit-5-en-22-al and its 20(R)-epimer.

Since the acetate (XI) is a pure compound, the aglycone of the original glycoside (VI) should be one of the two epimers formed by epimerization at C-20 when XI was treated with alkali,<sup>6)</sup> but no definite data to decide the absolute configuration at C-20 could be obtained.

Momordicosides C, D and E gave, in all cases, a mixture of methyl  $\alpha$ - and  $\beta$ -D-glucopyranosides on methanolysis. The CMR signals due to the sugar moieties and the glycosidated carbons ( $\delta$  87.4d in all cases) were identical with those of momordicoside A (I) suggesting that they are all 3-O- $\beta$ -gentiobiosides. The structures of the sugar moieties were confirmed as usual by converting the glycosides to the corresponding permethylates, followed by identification of their component methylated sugars (methyl glycosides of 2,3,4,6-tetra-O-methyl  $\alpha$ -D-glucopyranose and 2,3,4-tri-O-methyl  $\alpha$ -D-glucopyranose) by gas chromatography after methanolysis.

Thus, momordicosides C, D and E are 3-O- $\beta$ -gentiobiosides of cucurbit-5-ene-3 $\beta$ ,23,24,25-tetraol, cucurbita-5,24-diene-3 $\beta$ ,22,23-triol and 3 $\beta$ -hydroxy-23,24,25,26,27-pentanor-20( $\xi$ )-cucurbit-5-en-22-al, respectively.

Several hexanorcucurbitacins have been isolated from *Begonia tuberhybrida* Voss var.

*alba*<sup>7)</sup> and *Ecballium elaterium* L.,<sup>8)</sup> and they have been considered to be degradation products of normal cucurbitacins. Momordicoside E is the first glycoside of pentanorcucurbitacin to be found in nature.

### Experimental<sup>9)</sup>

**Isolation of Momordicosides C (IV), D (V) and E (VI)**—The less polar glycoside fraction (*ca.* 900 mg) which was obtained by column chromatography of the BuOH extract of the seeds (4.5 kg) was repeatedly chromatographed on silica gel (50—100 times the weight of material) using CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (70:30:5) to give Fr. 1 (a light brown powder; 250 mg), Fr. 2 (a light brown powder; 120 mg) and Fr. 3 (a light yellow powder; 550 mg), which contained VI, V and IV, respectively. Fr. 1 and 2 could not be crystallized.

Fr. 3 was crystallized from MeOH–H<sub>2</sub>O to give colorless needles (IV) (381 mg): mp 224—227°,  $[\alpha]_D^{20} + 13.9^\circ$  ( $c=1.15$ , MeOH), FD-MS  $m/z$ : 823 (M+Na)<sup>+</sup>. *Anal.* Calcd for C<sub>42</sub>H<sub>72</sub>O<sub>14</sub>·H<sub>2</sub>O: C, 61.59; H, 9.11. Found: C, 61.79; H, 9.12. PMR: tertiary methyl groups; 0.80, 0.86 ( $\times 2$ ), 1.05, 1.48, 1.69, 1.73. CMR: olefinic carbons; 143.3s, 118.7d, oxygen-bearing carbons; sugar moiety: 107.0d, 105.3d, 78.6d, 77.4d, 75.2d, 71.7d, 70.3t, 62.8t, aglycone moiety: 87.4d (C<sub>3</sub>), 79.8d (C<sub>24</sub>), 74.5s (C<sub>25</sub>), 71.0d (C<sub>23</sub>), quaternary carbons; 49.5, 46.6, 41.8, 34.7.

Fr. 1 was dissolved in a mixture (1 ml) of acetic anhydride and pyridine (1:1) and the whole was stirred at room temperature. After 2 days the solvent was removed and the residue was chromatographed on silica gel (30 g) (AcOEt–hexane (1:1)) to give a thin-layer-chromatographically homogeneous powder (192 mg), which was crystallized from MeOH to give colorless needles (XI) (122 mg): mp 177—183°,  $[\alpha]_D^{20} + 0.83^\circ$  ( $c=2.00$ , CHCl<sub>3</sub>). *Anal.* Calcd for C<sub>51</sub>H<sub>74</sub>O<sub>19</sub>·1/2H<sub>2</sub>O: C, 61.26; H, 7.51. Found: C, 60.95; H, 7.35. PMR: tertiary methyl groups; 0.81 ( $\times 2$ ), 0.97, 1.12, 1.26, secondary methyl group; 1.08 (d,  $J=8$  Hz), acetyl methyl groups; 1.97, 2.02, 2.09, 2.10, 2.25, aldehydic proton; 9.65 (H, d,  $J=3$  Hz). CMR: aldehydic carbon; 205.0d, olefinic carbons; 142.4s, 119.1d, oxygen-bearing carbons; 102.2d, 101.2d, 86.7d, 74.0, 73.5, 73.3, 72.5, 72.2, 71.9, 69.8, 69.1, 68.3t, 62.4t, quaternary carbons; 48.9, 47.0, 41.4, 34.9. EI-MS  $m/z$ : acetylated sugar moiety; 619, 331. aglycone moiety; 354, 220, 163, 134, 57, 43 (base).

Fr. 2 was acetylated as mentioned above, and the product (130 mg) was chromatographed on silica gel (20 g) (10% (v/v) acetone in benzene) to give 76 mg of a thin-layer-chromatographically homogeneous resin, which could not be crystallized. PMR: tertiary methyl groups; 0.82, 0.85, 0.96, 1.06, 1.24, 1.70 ( $\times 2$ ), secondary methyl group; 1.13 (d,  $J=6$  Hz), acetyl methyl groups; 1.90, 1.94, 2.00 ( $\times 2$ ), 2.04, 2.08, 2.10, 2.12, 2.22, olefinic proton; 6.12 (H, m). CMR: olefinic carbons; 142.3s, 138.5s, 121.2d, 119.1d, oxygen-bearing carbons; 102.1d, 101.2d, 86.7d, 76.6d, 73.9, 73.5, 73.3, 72.2, 71.9, 69.7, 69.0, 68.3t, 62.3t, quaternary carbons; 49.2, 46.7, 41.3, 34.8.

The acetate (76 mg) was dissolved in MeOH (2 ml) and 2.2 N MeONa solution (50  $\mu$ l) was added. The reaction mixture was stirred for 1 hr, neutralized by adding 2 N AcOH–MeOH and then evaporated to dryness. The residue was dissolved in CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (70:30:5) and passed through a silica gel column. The eluate was evaporated to dryness and the residue was crystallized from CH<sub>3</sub>CN–H<sub>2</sub>O mixture to give colorless needles (V) (35 mg): mp 199—203°.  $[\alpha]_D^{20} - 1.26^\circ$  ( $c=1.59$ , MeOH–CHCl<sub>3</sub> (2:1)), FD-MS  $m/z$ : 805 (M+Na)<sup>+</sup>. *Anal.* Calcd for C<sub>42</sub>H<sub>70</sub>O<sub>13</sub>·H<sub>2</sub>O: C, 62.97; H, 9.06. Found: C, 62.94; H, 8.87. PMR: tertiary methyl groups; 0.85 ( $\times 2$ ), 0.90, 1.05, 1.51, 1.73, 1.76, secondary methyl group; 1.28 (d,  $J=5$  Hz). CMR: olefinic carbons; 143.2s, 133.0s, 128.1d, 118.7d, oxygen-bearing carbons; sugar moiety: 107.0d, 105.3d, 78.5d, 77.3d, 75.2d, 71.6d, 70.2t, 62.7t, aglycone moiety: 87.4d (C<sub>3</sub>), 76.3d (C<sub>22</sub>), 68.0d (C<sub>23</sub>), quaternary carbons; 49.1, 46.7, 41.7, 34.7.

**Methanolysis of IV**—IV (157 mg) was suspended in 1 N HCl–MeOH (2 ml) and the whole was stirred at room temperature for 2 days. The clear reaction solution was neutralized with Ag<sub>2</sub>CO<sub>3</sub> and filtered. The filtrate was bubbled through with H<sub>2</sub>S and evaporated to dryness, and the residue was extracted with hot water. The water-insoluble material (77 mg) was chromatographed on silica gel (15 g) (5% (v/v) MeOH in CHCl<sub>3</sub>) and crystallized from MeOH to give colorless plates (VII): mp 223—225°,  $[\alpha]_D^{20} + 55.8^\circ$  ( $c=1.96$ , MeOH). EI-MS  $m/z$ : 476.3887 (M<sup>+</sup>) (Calcd for C<sub>30</sub>H<sub>52</sub>O<sub>4</sub>: 476.3865), 324 (M<sup>+</sup>–152), 234, 163, 152, 134 (base). PMR: tertiary methyl groups; 0.86, 0.88, 0.96, 1.15, 1.42, 1.73, 1.77, secondary methyl group; 1.18 (d,  $J=6$  Hz), carbinylic protons; 3.77 (H, bs), 3.81 (H, d,  $J=8$  Hz), 4.45 (H, m), olefinic proton; 5.65 (H, d,  $J=5$  Hz). CMR: olefinic carbons; 143.2s, 119.3d, oxygen-bearing carbons;<sup>10)</sup> 79.7d (C<sub>24</sub>), 76.0d (C<sub>3</sub>), 74.4s (C<sub>25</sub>), 71.0d (C<sub>23</sub>), quaternary carbons; 49.6, 46.6, 41.7, 34.7.

**Acetylation of VII**—VII (22 mg) was dissolved in 0.1 ml of Ac<sub>2</sub>O–pyridine (1:1) mixture and the whole was stirred at room temperature for 15 hr. The solvent was evaporated off and the residue was chromatographed on silica gel (10 g) (hexane–AcOEt (4:1)). The thin-layer-chromatographically homogeneous fraction was crystallized from hexane to give an acetate (12 mg): colorless needles; mp 153—156°. PMR: tertiary methyl groups; 0.76, 0.88, 0.92, 1.08, 1.15, 1.46, 1.58, secondary methyl group; 1.14 (d,  $J=5$  Hz), acetyl methyl groups; 2.03; 2.13, 2.15, acetylated carbinylic protons; 4.98 (overlapped by the water signal), 5.55 (d,  $J=2$  Hz), olefinic proton; 5.89 (H, m). EI-MS  $m/z$ : 602.4199 (M<sup>+</sup>) (Calcd for C<sub>36</sub>H<sub>58</sub>O<sub>7</sub>: 602.4182), 542 (M<sup>+</sup>–AcOH), 408 (M<sup>+</sup>–194), 134 (194–AcOH), 43 (base).

**Periodic Acid Oxidation of VII**—A solution of VII (36 mg) in MeOH (2 ml) was treated with periodic acid (25 mg in 0.1 ml of H<sub>2</sub>O) as described in the preceding paper.<sup>1)</sup> The product (VIII) (colorless resin 35 mg) could not be crystallized.  $[\alpha]_D^{20} + 68.0^\circ$  ( $c = 0.73$  mg/ml, dioxane). PMR: tertiary methyl groups; 0.86 ( $\times 2$ ), 0.95, 1.13, 1.40, secondary methyl group; 1.05 (d,  $J = 6$  Hz), methoxyl groups; 3.36 ( $\times 2$ ), carbinyl proton; 3.72 (H, bs), dimethylacetal proton; 4.65 (H, dd,  $J = 4, 7$  Hz), olefinic proton; 5.64 (H, d,  $J = 6$  Hz). CMR: olefinic carbons; 143.3s, 119.3d, oxygen-bearing carbons; 103.6d, 76.0d, 53.0q, 52.0q, quaternary carbons; 49.6, 46.5, 41.7, 34.7. EI-MS  $m/z$ : 432 ( $M^+$ ), 400 ( $M^+ - \text{MeOH}$ ), 280 ( $M^+ - 152$ ), 248 ( $280 - \text{MeOH}$ ), 163, 152, 134 ( $152 - \text{H}_2\text{O}$ ), 75 (base).

**Enzymatic Hydrolysis of V**—V (82 mg) obtained from another lot of the BuOH extract was suspended in 20% EtOH (4 ml) and crude hesperidinase (80 mg) was added. The suspension was incubated at 38° for 66 hr. After being diluted with water (10 ml), the reaction mixture was extracted with CHCl<sub>3</sub> (10 ml) five times. The CHCl<sub>3</sub> layer was washed with water and evaporated to dryness. The residue (54 mg) was purified by column chromatography (silica gel, CHCl<sub>3</sub>-MeOH, acetone-benzene) and crystallized from MeOH to give colorless prisms (IX) (23 mg): mp 189–192.5°,  $[\alpha]_D^{20} + 37.4^\circ$  ( $c = 1.01$ , CHCl<sub>3</sub>-MeOH (2:1)). PMR: tertiary methyl groups; 0.88 ( $\times 2$ ), 0.98, 1.14, 1.43, 1.75, 1.76, secondary methyl group; 1.31 (d,  $J = 6$  Hz), carbinyl protons; 3.74 (H, bs), 3.95 (H, dd,  $J = 6, 2$  Hz), 4.70 (H, dd,  $J = 6, 10$  Hz), olefinic protons; 5.68 (2H, m). CMR: olefinic carbons; 143.3s, 133.0s, 128.1d, 119.3d, oxygen-bearing carbons;<sup>10)</sup> 76.3d ( $C_{22}$ ), 76.0d ( $C_3$ ), 68.1d ( $C_{23}$ ), quaternary carbons; 49.2, 46.7, 41.7, 34.8. EI-MS  $m/z$ : 458 ( $M^+$ ), 440 ( $M^+ - \text{H}_2\text{O}$ ), 372, 355, 220, 163, 152, 134 (base), 85.

The aqueous layer was evaporated to dryness *in vacuo* and extracted with MeOH.

**Periodate Oxidation of IX**—A solution of IX (8 mg) in 0.5 ml of MeOH-tetrahydrofuran (3:1) was treated with 20  $\mu$ l of 30% NaIO<sub>4</sub> solution. The mixture was stirred in the dark at room temperature. After 8 hr, water (10 ml) was added and the solution was extracted with CHCl<sub>3</sub> (5 ml) four times. The combined CHCl<sub>3</sub> layer was washed with water and concentrated. The residue (6 mg) was applied to a silica gel column and eluted with 15% (v/v) AcOEt in hexane. The residue obtained from the eluate was crystallized from MeOH to give colorless needles (X) (1.4 mg): mp 153–159°. It gave the same *R<sub>f</sub>* value on TLC (hexane-AcOEt (3:1)) as  $\beta$ -hydroxy-23,24,25,26,27-pentanorcucurbit-5-en-22-al and no depression of the melting point was observed on admixture.

**Treatment of XI with Sodium Methoxide**—XI (58 mg) was dissolved in MeOH (2 ml) containing 80  $\mu$ l of 1 *N* MeONa and stirred at room temperature. After 3 hr, the solution was neutralized with 1 *N* AcOH in MeOH and evaporated to dryness. The residue (48 mg) was purified by column chromatography (silica gel, 12–40% (v/v) MeOH in CHCl<sub>3</sub>) to give a thick syrup (VI') (40 mg):  $[\alpha]_D^{20} + 1.82^\circ$  ( $c = 1.10$ , MeOH). FD-MS  $m/z$ : 719 ( $M + \text{Na}$ )<sup>+</sup>. PMR: aldehydic protons; 9.66 (d,  $J = 2.5$  Hz), 9.67 (d,  $J = 4$  Hz). CMR: aldehydic carbons; 205.7d, 204.9d, olefinic carbons; 143.3s, 118.5d, oxygen-bearing carbons; sugar moiety: 107.0d, 105.3d, 78.5d, 77.4d, 75.4, 75.2, 71.7d, 70.2t, 62.8t, aglycone moiety: 87.4d ( $C_3$ ), quaternary carbons; 49.0, 47.0, 41.7, 34.8.

**Methanolysis of VI'**—VI' (100 mg) was dissolved in 1.34 *N* HCl-MeOH (2 ml) and warmed at 38° for 17 hr. The reaction mixture was neutralized with Ag<sub>2</sub>CO<sub>3</sub>, filtered and evaporated to dryness. The residue (97 mg) was chromatographed on silica gel (12 g). The aglycone fraction (51 mg) was eluted with 5% (v/v) MeOH in CHCl<sub>3</sub> and the methyl glycoside was eluted with 20% (v/v) MeOH in CHCl<sub>3</sub>. The aglycone fraction was chromatographed on silica gel (200 times the weight of the material). Elution with 7% (v/v) AcOEt in hexane gave XII (13.6 mg) and XIII (15 mg). XII: colorless plates from MeOH, mp 160–162°,  $[\alpha]_D^{20} + 42.0^\circ$  ( $c = 0.707$  mg/ml, dioxane). PMR: tertiary methyl groups; 0.87 ( $\times 2$ ), 0.97, 1.15, 1.42, secondary methyl group; 1.18 (d,  $J = 6$  Hz), methoxyl groups; 3.41, 3.48, carbinyl proton; 3.76 (H, bs), dimethylacetal proton; 4.27 (H, d,  $J = 1.5$  Hz), olefinic proton; 5.65 (H, d,  $J = 5$  Hz). EI-MS  $m/z$ : 418 ( $M^+$ ), 386 ( $M^+ - \text{MeOH}$ ), 266 ( $M^+ - 152$ ), 234, 163, 152, 134 ( $152 - \text{H}_2\text{O}$ ), 75 (base).

XII showed a single spot on co-chromatography with 22,22-di-O-methyl-23,24,25,26,27-pentanorcucurbit-5-en-3 $\beta$ -ol, and the melting point of XII was not depressed on admixture.

XIII: colorless needles from hexane, mp 153–155°,  $[\alpha]_D^{20} + 56^\circ$  ( $c = 0.71$  mg/ml, dioxane). PMR: tertiary methyl groups; 0.93 ( $\times 2$ ), 1.00, 1.09, 1.42, secondary methyl group; 1.12 (d,  $J = 6$  Hz), methoxyl groups; 3.39, 3.44, carbinyl proton; 3.74 (H, bs), dimethylacetal proton; 4.46 (H, d,  $J = 2$  Hz), olefinic proton; 5.66 (H, d,  $J = 6$  Hz). EI-MS  $m/z$ : 418 ( $M^+$ ), 386 ( $M^+ - \text{MeOH}$ ), 266 ( $M^+ - 152$ ), 234, 163, 152, 134 ( $152 - \text{H}_2\text{O}$ ), 75 (base).

**Identification of Component Sugars of IV, V and VI'**—The methyl glycoside fractions obtained by methanolysis of IV and VI' were purified by column chromatography. The sugar fraction of V was converted to the methyl glycoside by refluxing in 1 *N* HCl-MeOH and purified in the same manner. The identification of the products as mixtures of methyl  $\alpha$ - and  $\beta$ -D-glucopyranosides was performed essentially as described in the preceding paper,<sup>1)</sup> *viz.* by comparison of their *R<sub>f</sub>* values on TLC, chemical shifts and coupling constants of anomeric protons in the PMR spectra, and optical rotations with those of authentic samples of methyl  $\alpha$ - and  $\beta$ -D-glucopyranosides, and by comparison of GLC *t<sub>R</sub>* values of their acetates with those of authentic acetate samples.

**Permethylation of IV, V and VI', and Identification of Component Methylated Sugars**—IV (5 mg) and NaH (4 mg) were dissolved in 60  $\mu$ l of tetrahydrofuran and sonicated for a few minutes. Methyl iodide

(40  $\mu$ l) was added to the mixture and heated in a sealed tube for 1 hr. The reaction mixture was diluted with  $\text{CHCl}_3$  (5 ml) and washed with water. The  $\text{CHCl}_3$  layer was concentrated *in vacuo* and the residue was chromatographed on silica gel (5 g) (10% (v/v) acetone in benzene) to give two products (IV-M-1, 2.5 mg; IV-M-2, 2 mg) as syrups. V (10 mg) and NaH (20 mg) were dissolved in tetrahydrofuran (0.1 ml) and sonicated for a few minutes. Methyl iodide (0.2 ml) was added and the mixture was stirred at room temperature overnight. The reaction mixture was worked up as described above to give a permethylate (V-M) as a syrup (7.6 mg). The same treatment of VI' (8 mg) gave a syrupy permethylate (VI'-M) (4.4 mg).

The methylation products were each dissolved in 0.2 ml of 1 N HCl-MeOH and each solution was heated at 90° for 1 hr. The reaction mixture was neutralized with  $\text{Ag}_2\text{CO}_3$ , filtered, treated with  $\text{H}_2\text{S}$  and evaporated to dryness. The residue was checked by GLC (column, 5% 1,4-butanediol succinate on Shimalite W, 2 m  $\times$  0.3 cm  $\phi$ ; column temperature, 172°; carrier gas,  $\text{N}_2$  at 1 kg/cm<sup>2</sup>). All methanolysates gave peaks at  $t_R$  1.70, 2.20, 3.85 and 5.20 min (authentic samples ( $t_R$ ): methyl pyranosides of 2,3,4,6-tetra-O-methyl  $\alpha$ -D-glucose (2.20),  $\beta$ -anomer (1.70), 2,3,4-tri-O-methyl  $\alpha$ - (5.20), 2,4,6-tri-O-methyl  $\alpha$ - (6.70), 3,4,6-tri-O-methyl  $\alpha$ - (4.45), 2,3,6-tri-O-methyl  $\alpha$ - (4.90)).

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### References and Notes

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- 2) This work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1980.
- 3) P. Tunmann, W. Gerner, and G. Stapel, *Ann.*, **694**, 162 (1966).
- 4) This enzyme was provided by Dr. T. Nohara of Tokushima University, to whom we are grateful.
- 5) These data were obtained on a 100 MHz FT NMR spectrometer (JEOL JNM FX-100). The PMR spectrum of 22,22-di-O-methyl-23,24,25,26,27-pentanorcucurbit-5-en-3 $\beta$ -ol was taken on the same instrument for comparison. Differences between the chemical shifts of corresponding signals were less than 1 Hz.
- 6) When 3 $\beta$ -acetoxy-23,24,25,26,27-pentanorcucurbit-5-en-22-al was treated with sodium methoxide under the same conditions, a mixture of epimers was obtained. The PMR and CMR spectra were identical with those of VI' except for signals due to the sugar moiety.
- 7) R.W. Doskotch and C.D. Hufford, *Can. J. Chem.*, **48**, 1787 (1970).
- 8) M.M. Rao, H. Meshulam, and D. Lavie, *J. Chem. Soc. Perkin Transactions I*, **1974**, 2552.
- 9) Instruments and materials employed in this work were the same as those described in the preceding paper,<sup>1)</sup> unless otherwise stated. Melting points are uncorrected. PMR and CMR spectra were measured in pyridine- $d_5$  and chemical shifts are expressed in the  $\delta$ -scale with tetramethylsilane as an internal standard (s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet).
- 10) Assignment of the carbon signals was carried out by the selective decoupling technique.