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BASIC STEROID SAPONINS FROM AERIAL PARTS OF *FRITILLARIA THUNBERGII**

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Key Word Index—*Fritillaria thunbergii*; Liliaceae; structural elucidation; field desorption mass spectrometry; basic steroid saponins; solanidine- and hapepunine-oligoglycosides.

Abstract—From the aerial parts of *Fritillaria thunbergii*, three glycosidal *Solanum* alkaloids (basic steroid saponins) were isolated together with minor amounts of the two ceveratrum alkaloids verticine and verticinone. The saponins were identified as solanidine 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (β_1-chaconine), solanidine 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)-]β-D-glucopyranoside and hapepunine 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside. The latter two are newly discovered natural compounds. Field desorption MS was used to provide information on the purity and MW of the complex oligoglycosides, and, to some extent, the nature and sequence of their sugar moieties. The direct examination of yet unknown polar glycosides prior to derivatization and application of conventional techniques proves to be of great diagnostic value and aids the structural elucidation considerably.

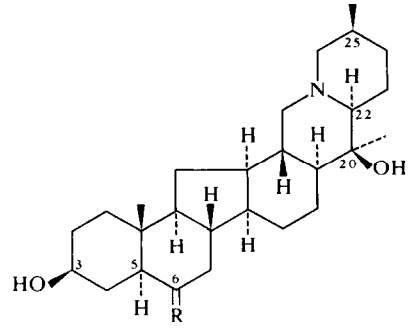
INTRODUCTION

The processed bulbs of *Fritillaria thunbergii* Miq. [*F. verticillata* Willd. var. *thunbergii* (Miq.) Baker] have long been known as one of the principal Chinese crude drugs [1]. The alkaloids of unprocessed fresh bulbs of the plant cultivated in Japan were studied by Fukuda [2], Morimoto *et al.* [3], Itô *et al.* [4, 5], and Kaneko *et al.* [6], and three ceveratrum alkaloids, verticine (**1**), verticinone (**2**) and isoverticine were isolated in a free state and characterized. A study on the alkaloids [7] and other constituents of the crude drug in comparison with those of fresh bulbs has been carried out in this laboratory. With regard to the constituents of the fresh aerial parts of this plant, Kaneko *et al.* have surveyed the alkamine obtained by acid hydrolysis of the extracts in the course of biogenetic studies on *Fritillaria* alkaloids. They obtained, in addition to solanidine (**3**) [8, 9] and hapepunine (**4**) [10], five new steroid alkamines and identified them as 5α-cevanine-3α,6β,20β-triol (epimeric at C-3 and C-6 to **1**) (baimonidine) [6], the 3-epimer of **1** (isobaimonidine) [11], (22*S*, 25*S*)-cev-5-enine-3α,20β-diol (fritillarizine) [12] and (22*S*, 25*R*)-5α-cevanine-3α,6α-diol (petilinine) and its 6β-epimer [13].

We now report on the isolation and characterization from unhydrolysed MeOH extracts of the aerial parts of *F. thunbergii* of three glycosidal *Solanum* alkaloids (basic steroid saponins) [14], **5**, **6** and **7**, along with small amounts of the cervatum alkaloids **1** and **2**. In view of the established capacity of field desorption (FD) mass spectrometry (MS) for the investigation of micrograms of free oligoglycosides [15–19], in order to know the purity, to determine the MW and to evaluate the structural features of each glycoside, FD mass spectra were taken directly after chromatographic separation and purification. The foreknowledge at this early stage of the study facilitated the subsequent unequivocal identification made on the basis of the conventional chemical and spectral data.

RESULTS AND DISCUSSION

The isolation and separation of compounds **1**, **2**, **5**, **6** and **7**, were carried out by combination of CC on Amberlite XAD-2, Sephadex LH-20, Al_2O_3 and Si gel, and droplet countercurrent chromatography (DCCC) [20] followed by crystallization. Compounds **1** and **2** were identified as verticine (**1**) and verticinone (**2**) [4, 5].



1 R = (verticine)

2 R = =O (verticinone)

* Part 9 in the series 'Field Desorption Mass Spectrometry of Natural Products'. For Part 8 see Komori, T., Maetani, I., Okamura, N., Kawasaki, T., Nohara, T. and Schulten, H.-R. (1981) *Justus Liebigs Ann. Chem.* 683.

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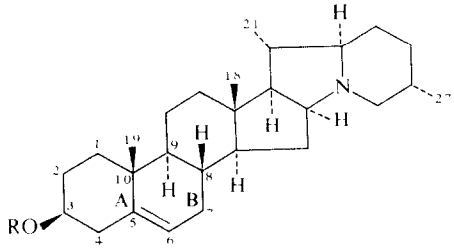
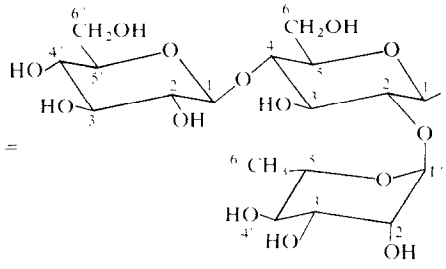
Compound **5** was first subjected to FDMS. The spectrum showed $[M + Na]^+$, $[M + H]^+$ and the fragment ions $[(M + Na) - 146]^+$, $[(M + H) - 146]^+$, $[(M + Na) - 308]^+$ and $[(M + H) - 308]^+$, suggesting that **5** was a homogeneous oligoglycoside having a methylpentosyl-hexose moiety attached to an aglycone of MW 397. Based on these results and elemental analytical data **5** was given the molecular formula $C_{39}H_{63}NO_{10}$. On treatment with crude hesperidinase **5** gave an aglycone and two sugars, which were identified as solanidine (**3**) [8, 9], D-glucose and L-rhamnose, respectively. The ^{13}C NMR spectrum [21, 22] of **5** exhibited signals due to the carbon atoms of the sugar moiety and of the A and B rings of the aglycone (Table 1), the δ values of which were in good agreement with those of diosgenin 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside [23]. Permethylation of **5** followed by methanolysis afforded the methyl pyranosides of 2,3,4-tri-*O*-methyl-α-L-rhamnose and 3,4,6-tri-*O*-methyl-α-D-glucose. Two anomeric proton signals in the ^1H NMR spectrum of permethyl **5** and the coupling constant (J = 170 Hz, at 101.8 ppm) of C_1–H_1 of the rhamnose unit observed in the ^{13}C NMR spectrum of **5** indicated a β-configuration for the D-glucose unit and an α configuration for the L-rhamnose. From this, it followed that **5** was solanidine 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (β_1-chaconine) [24].

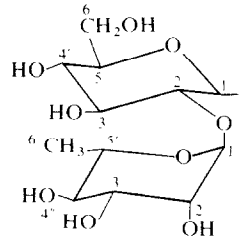
The FDMS of compound **6** (Fig. 1) showed a cationized cluster ion $[M + Na]^+$ and the fragment ion peaks $[(M + Na) - 146]^+$, $[(M + Na) - 162]^+$ and $[(M + Na) - 308]^+$. These data suggested that **6** was in a pure state and had a branched-chain trisaccharide, methylpentosyl (hexosyl)-hexose, combined with a compound of MW 397. From the elemental analytical data, the molecular formula of **6** was $C_{45}H_{73}NO_{15}$. The fact that the relative abundance [31; $[M + Na]^+$ (100)] of the fragment ion $[(M + Na) - 146]^+$ was larger than that (12) of $[(M + Na) - 162]^+$ in the FDMS of **6** suggested, according to an established analogy between fragmentation in FDMS and sugar cleavage in acidic solvolysis [19], that on mild acid hydrolysis, removal of a methylpentose unit would be easier than that of the hexose unit. Subsequently, **6** was treated with 1 N H_2SO_4 to yield a dihexoside (**8**). $C_{39}H_{63}NO_{11}$ (FDMS: m/z 722 $[M - H]^+$). When **6** and **8** were hydrolysed with hesperidinase, **6** gave the same products, **3**, D-glucose and L-rhamnose, as **5**, while **8** provided **3** and D-glucose. The signals due to sugar carbons (Table 1) in the ^{13}C NMR spectrum [21, 22] of **8** were in good agreement with those of methyl β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside (β-cellobioside) [15]. Taking the coexistence of **5** and **6** into account, **6** was presumed to be the 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 4)]-β-D-glucopyranoside of **3**. Unequivocal evidence for this structure was provided by the methanolysis of permethyl **6**, yielding methyl pyranosides of 2,3,4-tri-*O*-methyl-α-L-rhamnose, 2,3,4,6-tetra-*O*-methyl- and 3,6-di-*O*-methyl-α-D-glucoses, by the ^1H NMR spectrum of permethyl **6** showing three anomeric proton signals, and by the molecular rotation difference, −233.5°, between **6** and **11** ($[M]_D$: methyl α-L-rhamnopyranoside, −111°; β-anomer, +170°) [25].



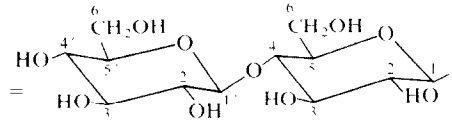
3 R = H (solanidine)



6 R =



5 R =

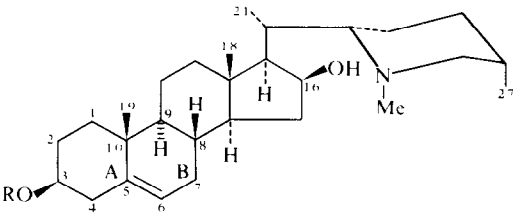


8 R =

The FDMS of compound **7** (Fig. 2) showed ion peaks of $[M + Na]^+$, $[M + H]^+$, $[(M + Na) - 146]^+$, $[(M + H) - 146]^+$, and $[(M + H) - 308]^+$, indicating that **7** was homogeneous and had a methylpentosyl-hexose moiety linked to an aglycone of MW 429. Elemental analysis and the above data gave the molecular formula $C_{40}H_{67}NO_{11}$. Incubation of **7** with hesperidinase afforded D-glucose and L-rhamnose, together with an aglycone, which was identified as hapepunine (**4**) [10]. Therefore, **7** was a rhamnosyl-glucoside of **4**. The ^{13}C NMR spectrum of **7** was the same as that of **5** with respect to the signals due to carbon atoms of the sugar moiety and of the A and B rings of the aglycone (Table 1). Consequently, **7** was characterized as the 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside of **4**.

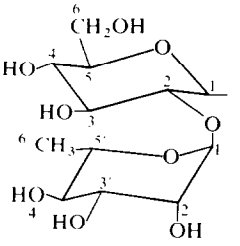
is noteworthy that the glycosidal *Solanum* alkaloids of the aerial parts of *F. thunbergii* are different from those (predominantly ceveratrum and jerveratrum alkaloids in a free state) in the bulbs [6, 7, 12]. **5**, **6** and **7** correspond to the parent glycosides of **3** and **4**, respectively, reported by Kaneko *et al.* [6].

FDMS, requiring a very minute amount (in the order of micrograms or less) of underivatized sample, has proved to be of great diagnostic value prior to conventional chemical and spectral studies in purity control, MW determination, and structure elucidation of unknown oligoglycosides. It should be noted that the essential processes of ion formation are due to field-promoted ion-molecule reactions such as proton transfer and cationization. In particular, the analogy between acidic



4 R = H (hapepunine)

7 R =



5 is one of the glycosidal *Solanum* alkaloids (basic steroid saponins) [14] previously isolated from the leaves of *Solanum chacoense* Bitt. (Solanaceae) and characterized by Kuhn *et al.* [24], and might be regarded as a prosapogenin of **6** with which it coexists in *F. thunbergii*. It

solvolysis and FD fragmentation [19] is a very helpful heuristic principle and can be taken as a guideline for straightforward interpretation of the FDMS and for the choice and design of appropriate analytical techniques for more subtle structural work.

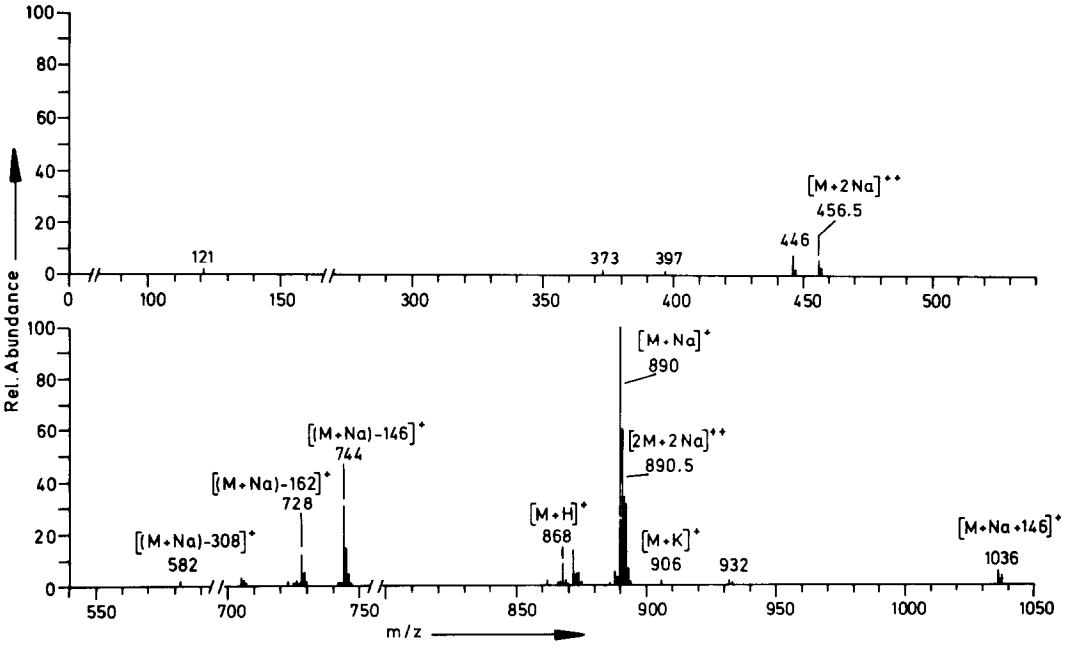


Fig. 1. FDMS of **6** (MW, 867, 498).

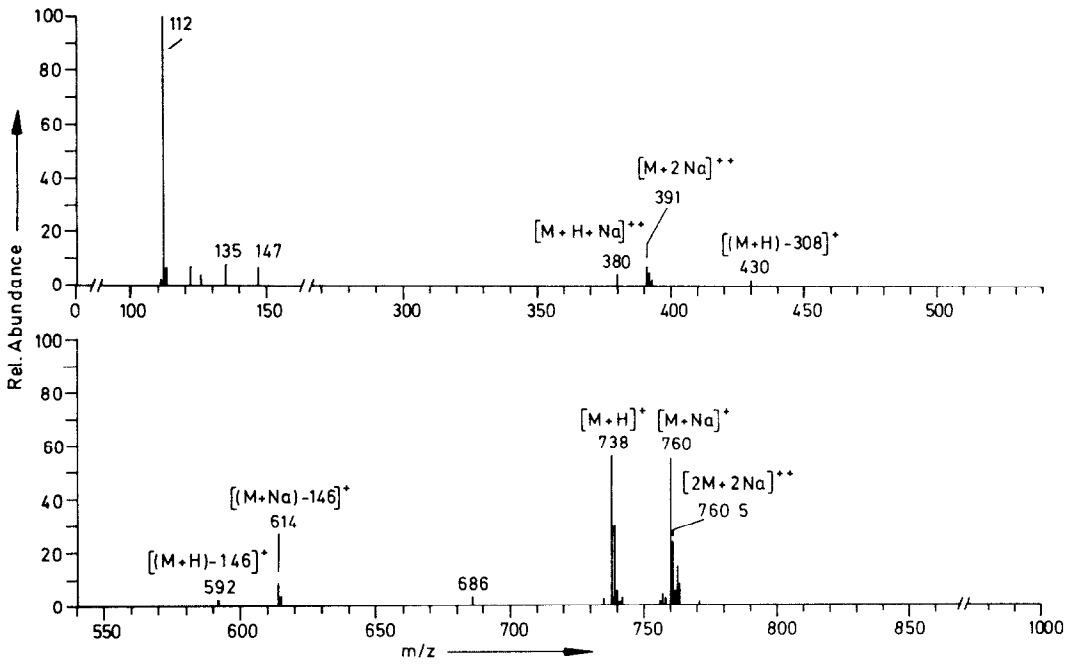


Fig. 2. FDMS of **7** (MW, 737, 471).

Table 1. ^{13}C NMR chemical shifts of compounds **5**–**8** (25.05 MHz, C_5D_5N, TMS as int. standard, FT mode)

Carbon	5	8	6	7
1	37.5			37.5
2	30.2			30.2
3	77.6			77.5
4	39.0			38.8
5	140.5			140.5
6	121.6			121.7
7	31.9			32.0
8	31.5			31.8
9	50.4			50.2
10	37.0			36.9
19	19.4			19.4
1′	100.1	102.1	99.7	100.1
2′	79.4	74.6	78.0	79.4
3′	77.8	76.6	75.9	77.7
4′	71.6	81.1	81.7	71.5
5′	78.0	76.2	77.4	78.0
6′	62.4	62.3	61.8	62.4
1″	101.8		101.5	101.8
2″	72.6		72.5	72.6
3″	72.3		72.1	72.3
4″	73.9		73.9	73.9
5″	69.3		69.1	69.3
6″	18.6		18.5	18.6
1‴		104.7	104.9	
2‴		74.6	74.7	
3‴		78.2	78.0	
4‴		71.3	70.9	
5‴		78.2	78.0	
6‴		62.3	61.8	

EXPERIMENTAL

All mps were uncorr. Optical rotations were taken at 15–20° using a 1-dm cell. GLC (FID mode): glass column (1.5 m × 3 mm) packed with 10% SE-30, column temp. 150°, injection temp. 170°, carrier gases N_2 (0.8 kg/cm^2) and H_2 (0.7 kg/cm^2). CC and TLC (Si gel and Avicel): solvent systems: (A) $CHCl_3$–MeOH–H_2O (14:6:1); (B) upper layer of *n*-BuOH–pyridine–H_2O (6:2:3) + equal vol. of pyridine. The FDMS [15–19] were produced on a double-focusing MS (in Bonn) and recorded electrically with scan-times between 4 and 8 sec/decade and at a mass resolution of better than 3000 (10% valley definition). All spectra were produced at ion source potentials of 8 kV for the field anode and −3 kV for the slotted cathode plate, an ion source pressure of *ca* 10^{-6} Torr and an ion source temp. between 50 and 60°. The samples were desorbed by direct heating using the supplied emitter heating current (0–70 mA) and by controlling the emission of FD ions roughly by the total ion monitor. MeOH was used as solvent and an estimated amount of *ca* 1 μg was transferred to the emitter via the syringe technique.

Plant material. The aerial parts (excluding flowers) were collected in April from plants cultivated in the Nara Prefecture and in the Medicinal Botanical Garden of Kyushu University, Fukuoka Prefecture, Japan.

Isolation of constituents. Cut material (8.7 kg) was extracted with MeOH (25 l.) and the solvent removed *in vacuo*. The extract (610 g) was shaken with Et_2O and H_2O, then the H_2O layer was extracted with *n*-BuOH and the extracts evapd *in vacuo* to dryness. The residue (67 g) dissolved in 5% HOAc (1 l.) was shaken with *n*-BuOH, the aq. layer was then adjusted to pH 9 with aq. 28% NH_3 (200 ml) and extracted with *n*-BuOH. The extracts (21 g) were passed through an Amberlite XAD-2 (1 l.) column using H_2O and 80% MeOH as eluants. The latter eluate (9 g) was chromatographed on Si gel (500 g) and elution with solvent A gave fraction (Fr.) 1–Fr. 6. Further chromatography of Fr. 2 on Al_2O_3 using $CHCl_3$–MeOH (9:1 to 4:1) and then on Si gel ($CHCl_3$–MeOH–H_2O, 7:12:8, lower layer) afforded **1** (13.1 mg) and **2** (6.7 mg). Fr. 4 was separated on a Si gel column (solvent A) into two fractions each of which was passed through a

Sephadex LH-20 column (eluant, MeOH) to give **7** (50 mg) and **5** (74 mg) respectively. Fr. 5 was purified in the same way as above to give **5** (40 mg). Fr. 6 was subjected to DCCC [400 glass tubes (60 cm × 1.65 mm); stationary phase, upper layer of $CHCl_3$–MeOH–aq. 1% NH_3 (7:12:8); moving phase, bottom layer of the above mixt.], and the first eluate was chromatographed on Si gel (EtOAc–$CHCl_3$–MeOH–H_2O, 2:4:4:1) to give **6** (580 mg). Compound **1**, needles (from EtOAc–hexane), mp 220–222°, and **2**, needles (from EtOAc–hexane), mp 212–214°, were identified [mmp, co-TLC (Si gel, solvent A), IR] as verticine and verticinone, respectively.

Compound **5**. Needles (from di. MeOH), mp 287–292° (dec.), $[\alpha]_D$ −52.5° (pyridine; *c* 0.9). FDMS [*m/z* (rel. abundance (cf. Figs. 1 and 2)]: 744 $[M+K]^+$ (7), 728 $[M+Na]^+$ (100), 706 $[M+H]^+$ (86), 582 $[(M+Na)-146]^+$ (30), 560 $[(M+H)-146]^+$ (8), 420 $[(M+Na)-308]^+$ (4), 398 $[(M+H)-308]^+$ (12), 375.5 $[M+2Na]^{2+}$ (1), 364.5 $[M+Na+H]^{2+}$ (16); ^{1}H NMR (100 MHz, C_5D_5N. TMS as int. standard): δ 0.84 (3 H, *d*, *J* = 7 Hz, H-27), 0.95 (3 H, *s*, H-18), 0.97 (3 H, *d*, *J* = 7 Hz, H-21), 1.07 (3 H, *s*, H-19), 1.78 (3 H, *d*, *J* = 6 Hz, H-6″), 5.04 (1 H, *d*, *J* = 7 Hz, H-1′), 5.36 (1 H, *m*, H-6), 6.37 (1 H, *s*, H-1″). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH). ^{13}C NMR: Table 1. (Found: C, 63.3; H, 8.9; N, 1.9. Calc. for $C_{39}H_{63}NO_{10}\cdot 2H_2O$: C, 63.1; H 9.1; N, 1.9%).

Enzymatic hydrolysis of **5**. A mixture of **5** (25 mg), a crude hesperidinase (5 mg) and aq. 1% HOAc (5 ml) was left to stand at 37° for 24 hr. The mixture was evaporated *in vacuo* to dryness and the residue was chromatographed on Si gel (10 g) using $CHCl_3$–MeOH–H_2O (40:10:1) and solvent A has eluants to give an aglycone, needles (from MeOH), mp 217–218° (dec.), $[\alpha]_D$ −29.0° ($CHCl_3$; *c* 0.9), and two sugars: sugar 1, syrup, $[\alpha]_D$ +54.0° (MeOH; *c*, 0.3), Avicel TLC (R_f 0.25, solvent B); sugar 2, syrup, $[\alpha]_D$ +10.0° (MeOH; *c* 0.3), Avicel TLC (R_f 0.50, solvent B). The aglycone was identified (mmp, co-TLC, ^{1}H NMR) as solanidine, while sugars 1 and 2 were identified (Avicel TLC) as D-glucose and L-rhamnose, respectively.

Methylation of **5** [26]. NaH (100 mg) in DMF (3 ml) was stirred for 10 min, then **5** (25 mg) and MeI (1 ml) were added, the mixture was stirred for 5 hr and then poured into ice–H_2O. Extraction with EtOAc and the usual work-up gave a crude product, which was chromatographed on Si gel (5 g) ($CHCl_3$–MeOH–H_2O (95:5:0.5)) to provide permethyl **5** as an oil (23 mg). IR: no OH; ^{1}H NMR (100 MHz, $CDCl_3$ TMS as int. standard): δ 4.26 (1 H, *d*, *J* = 7 Hz, H-1′), 5.21 (1 H, *d*, *J* = 1.5 Hz, H-1″).

Methanolysis of permethyl **5**. A soln of permethyl **5** (20 mg) in 8% HCl–MeOH (5 ml) was refluxed for 2.5 hr. After cooling the mixture was neutralized with $NaHCO_3$, filtered, and the filtrate was passed through Sephadex LH-20 (eluant, MeOH) and ion-exchange resin IR-4B (eluent, MeOH) columns. The methylated sugars in the eluate were identified as the methyl pyranosides of 2,3,4-tri-*O*-methyl-α-L-rhamnose and 3,4,6-tri-*O*-methyl-α-D-glucose by direct comparison [GLC, Si gel TLC (EtOAc–MeOH, 49:1)] with authentic samples [27].

Compound **6**. Needles (from dil. MeOH), mp 278–283° (dec.), $[\alpha]_D$ −58.4° (pyridine; *c* 1.0). MS: Fig. 1; ^{1}H NMR (100 MHz, C_5D_5N, TMS as int. standard): δ 0.84, 0.95 (each 3 H, *d*, *J* = 7 Hz, H-27, H-21), 0.93, 1.04 (each 3 H, *s*, H-18, H-19), 1.75 (3 H, *d*, *J* = 6 Hz, H-6″), 5.34 (1 H, *m*, H-6), 6.21 (1 H, *d*, *J* = 1.5 Hz, H-1″); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). ^{13}C NMR: Table 1. (Found: C, 59.6; H, 8.4; N, 1.6. $C_{45}H_{73}NO_{15}\cdot 2H_2O$ requires: C, 59.8; H, 8.6; N, 1.6%.)

Partial hydrolysis of **6**. A soln of **6** (70 mg) in 1 N H_2SO_4 (10 ml) was heated on a boiling water-bath for 2 hr. The mixture was made alkaline with 10% aq. NH_3, extracted with *n*-BuOH, and the organic layer was evapd *in vacuo* to dryness. The residue was passed through Sephadex LH-20 column (eluent, MeOH) and the eluate was further chromatographed on Si gel (solvent A) to afford a crude product. It was crystd from dil. MeOH to give a glycoside (**8**) as needles (from dil. MeOH) (38 mg), mp 260–261° (dec.), $[\alpha]_D$ −38.0° (Py; *c* 1.2). FDMS: *m/z* 722 $[M(C_{39}H_{63}NO_{11})+H]^+$; ^{13}C NMR: Table 1.

Enzymatic hydrolysis of **6** *and* **8**. **6** (150 mg) and a crude hesperidinase (20 mg) in aq. 1% HOAc (10 ml) were left to stand at 37° for 24 hr and worked up in the same way as for **5** to yield **3**, D-glucose and L-rhamnose, which were identified by direct comparison with authentic samples. **8** (15 mg) treated in the same manner gave **3** and D-glucose.

Methylation of **6**. This was carried out in the same way as for **5** (NaH, 100 mg; DMF, 4 ml; **6**, 40 mg; MeI, 2 ml) to provide permethyl **6** as an oil (37 mg). IR: no OH; ^{1}H (100 MHz, C_5D_5N, TMS as int. standard) NMR: δ 4.26, 4.36 (each 1 H, *d*, *J* = 7 Hz, H-1′, H-1‴), 5.25 (1 H, *d*, *J* = 2 Hz, H-1″).

Methanolysis of permethyl **6**. This was carried out in the same way as for permethyl **5** (permethyl **6**, 37 mg; 8% HCl–MeOH, 5 ml). The methylated sugars were identified as methyl pyranosides of 2,3,4-tri-*O*-methyl-α-L-rhamnose, 2,3,4,6-tetra-*O*-methyl- and 3,6-di-*O*-methyl-α-D-glucoses by comparison (GLC, TLC) with authentic samples [27] run in parallel.

Compound **7**. Needles (from dil. MeOH). mp 269–274° (dec.), $[\alpha]_D$ −67.2° (Py; *c* 1.5). FDMS: Fig. 2; ^{1}H NMR (100 MHz, C_5D_5N, TMS as int. standard): δ 1.07 (6 H, *s*, H-18, H-19), 1.07 (6 H, *d*, *J* = 7 Hz, H-21, H-27), 1.85 (3 H, *d*, *J* = 6 Hz, H-6″), 2.21 (3 H, *s*, N-Me), 5.00 (1 H, *d*, *J* = 7 Hz, H-1′), 5.25 (1 H, *m*, H-6), 6.32 (1 H, *s*, H-1″); ^{13}C NMR: Table 1. (Found: C, 64.1; H, 9.3; N, 1.6. $C_{40}H_{67}NO_{11}\cdot H_2O$ requires: C, 63.6; H, 9.2; N, 1.6%.)

Enzymatic hydrolysis of **7**. This was carried out in the same way as for **5** (**7**, 40 mg; hesperidinase, 5 mg; aq. 1% HOAc, 5 ml) providing D-glucose and L-rhamnose together with an aglycone, needles (from Me_2CO–hexane), mp 201–202°, $[\alpha]_D$ −71.2° (Py; *c* 1.0). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH); ^{1}H NMR [100 MHz, $CDCl_3$–CD_3OD (9:1), TMS as int. standard]: δ 0.94, 1.01 (each 3 H, *s*, H-18, H-19), 1.07 (6 H, *d*, *J* = 7 Hz, H-21, H-27), 2.40 (3 H, *s*, N-Me), 3.52 (1 H, *m*, H-3α), 4.45 (1 H, *m*, H-16α), 5.35 (1 H, *m*, H-6). (Found: C, 76.0; H, 11.0; N, 3.0. Calc. for $C_{28}H_{47}NO_2\cdot 1/2H_2O$: C, 76.7; H, 11.0; N, 3.2%.) It was identified as hapepunine by direct comparison (mmp, TLC, ^{1}H NMR) with an authentic sample.

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