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STEROIDAL ALKALOIDS OF *FRITILLARIA MAXIMOWICZII*

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Key Word Index—*Fritillaria maximowiczii*; Liliaceae; steroidal alkaloids; 23-isokuroyurinidine; 15,16-*seco*-22α*H*,25β*H*-solanida-5,14-dien-3β-ol *O*-D-glucopyranosyl-(1 → 4)-β-D-xylopyranoside; hapepunine 3-*O*-β-cellobioside.

Abstract—From the bulbs of *Fritillaria maximowiczii*, in addition to the known jerveratrin alkaloid, kuroyurinidine, three new steroidal alkaloids, 23-isokuroyurinidine, 15,16-*seco*-22α*H*,25β*H*-solanida-5,14-dien-3β-ol *O*-β-D-glucopyranosyl-(1 → 4)-β-D-xylopyranoside and hapepunine 3-*O*-β-cellobioside have been isolated and structurally elucidated.

INTRODUCTION

Fritillaria species are one of the important drug sources often used for depressing coughs, especially in the area of Chain and south-east Asia. In extensive chemical studies, some steroidal alkaloids of the ceveratrum and jerveratrum alkaloid groups [1–5] had been isolated, together with some diterpenes [6]. The bulbs of *F. maximowiczii* (Rinyou-Baimo) grown in north-east China is expected to be a substitute for the bulbs of other *Fritillaria* species, *F. thunbergii* (Setu-Baimo), *cirrhosa*, *unibracteata* and *taipaiensis* (Sen-Baimo), since pharmacological tests of an ethanol extract of this species provided evidence that it also could be used to treat coughs [7]. Therefore, the chemical constituents of this species were investigated.

RESULTS AND DISCUSSION

Fresh bulbs of *F. maximowiczii* were extracted with ethanol to give an extractive, which was partitioned between *n*-butanol and water. The organic layer was shaken with *n*-hexane and 40% methanol, and the aqueous layer was concentrated to give a residue. The residue was subjected to column chromatography on Diaion HP-20 with aqueous methanol to afford several fractions, one of which was subsequently chromatographed on silica gel using chloroform–methanol–water as solvent to provide the four steroidal alkaloids, **1**–**4**.

Compound **1** showed in its mass spectrum a $[M]^+$ at *m/z* 445 and fragment peaks at *m/z* 125 ($C_8H_{15}N$: base peak) and 114 ($C_6H_{12}O$: 99%) characteristic of the side-chain ring of C-*nor*-D-homo steroids of the jerveratrum group. The ^1H and ^{13}C NMR spectra were coincident with those of kuroyurinidine, 2β,3α,6β-trihydroxy-5α-jerv-12-enine, obtained from *F. camtschatcensis* by Mimaki and Sashida [8].

Compound **2** showed a ^1H NMR spectrum analogous to that of **1**. Therefore, this compound was thought to be a stereoisomer of **1**. The ^1H–^1H 2D COSY spectrum and the coupling constants revealed the connectivities of the respective protons at 2α-*eq.* (*br d*, J = 2.6 Hz, at δ4.58), 3β-*eq* (*br d*, J = 2.9 Hz at δ4.66) and 6α-*eq.* (*br d*, J = 2.9 Hz, at δ4.25). The steric situation at C-16, -20, -22, -23 and -25 was solved by assignment of the respective signals and coupling constants of the sequence of H_3-21 (*d*, J = 7.3 Hz, at δ1.42), H-20 (*dd*, J = 7.3, 8.8 Hz, at δ2.90), H-22 (*dd*, J = 8.8, 8.8 Hz, at δ3.33), H-23 (*ddd*, J = 3.7, 8.8, 8.8 Hz, at δ3.96), H-26α-*eq.* (*dd*, J = 4.0, 12.6 Hz, at δ3.57), H-26β-*ax.* (*dd*, J = 12.6, 12.6 Hz, at δ2.84) and the occurrence of NOEs between H_3-18 and H-20, H-20 and H-22 as well as H-22 and H-23. Therefore, the structure of **2** was determined to be 23-isokuroyurinidine. Assignment of the ^{13}C NMR signals for **2** were also attributed without inconsistence.

The mass spectrum of compound **3** showed a $[M]^+$ at *m/z* 692 (base peak) and fragment ions at *m/z* 530 [692-hexose] and 398 [530-pentose]. In the ^1H NMR spectrum, signals due to two tertiary methyl groups at δ0.96 and 0.97, two secondary methyl groups at δ1.09 and 1.18 (each *d*, J = 6.6 Hz), one exomethylene group at δ4.89, 5.06 (each *br s*), one olefinic proton at δ5.45 (*br d*, J = 1.1 Hz) and two anomeric proton signals (each *d*, J = 7.7 Hz) were observed. The ^{13}C NMR signals suggested the occurrence of a glucopyranosyl-(1 → 4)-xylopyranosyl moiety. Therefore, **3** was hydrolysed with β-glucosidase to give an aglycone, whose electron impact (EI) mass sepctrum indicated a $[M + H]^+$ at *m/z* 398 and a prominent fragment ion at *m/z* 150 (base peak). The ^1H NMR signals were assigned to two tertiary methyl groups (H_3-18 and -19 at δ0.98 and 1.08), two secondary

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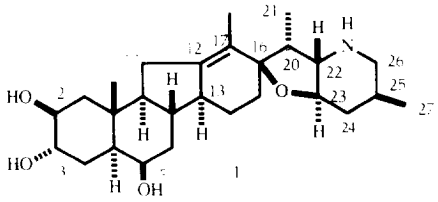

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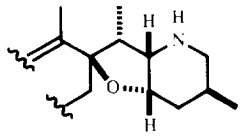

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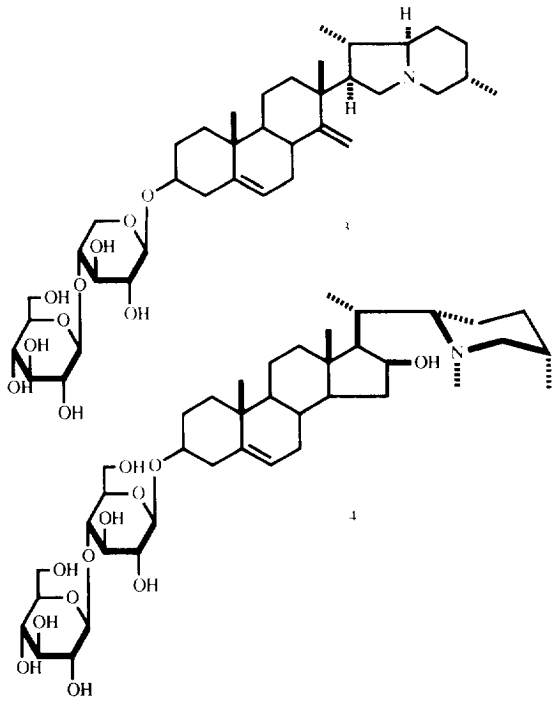

3

4

methyl groups (H_3-21 and -27, each *d*, J = 6.6 Hz, at δ1.12 and 1.20), one exomethylene group (each 1H, *br s*, at δ4.85 and 5.00) and one olefinic proton (H-5, *br d*, J = 1.1 Hz at δ5.42). The position of the exomethylene group was referred to the ^{13}C NMR data, in which the signals at δ135.0 (*s*) and 106.4 (*t*) could be assigned to C-14 and C-15, respectively, by comparison with those of solanidine. The five proton signals assignable to H_2-16, H-23 and H_2-26 adjacent to the nitrogen appeared at δ2.30–3.35, suggesting that C-16 is not bonded to C-15. On the other hand, the cleaved sugar configurations were determined by GC analysis [9]. Thus, the deduced structure of **3** is 15,16-*seco*-22α*H*,25β*H*-solanida-5,14-dien-3β-ol *O*-β-D-glucopyranosyl-(1 → 4)-β-D-xylopyranoside.

Compound **4** showed in its positive FAB-mass spectrum a $[M + H]^+$ at *m/z* 754 (75%) and fragment ions at *m/z* 738 $[M - Me]^+$ (21%) and 722 $[738 - OH]^+$. The ^{1}H NMR spectrum displayed two steroidal angular methyl groups (δ0.89 and 1.08), two secondary methyl groups (δ0.94 and 1.10) and one *N*-methyl group at δ2.28, as well as two anomeric protons at δ5.01 and 5.23 (each 1H, *d*, J = 7.7 Hz). Compound **4** was hydrolysed by β-glucosidase. The EI-mass spectrum of the obtained aglycone showed a $[M]^+$ at *m/z* 429 and a fragment ion at *m/z* 112 (base peak) characteristic of an *N*-methyl-22,26-epiminocholestane side-chain. The ^{1}H NMR spectrum exhibited signals due to H-3 (*m*, δ3.87), H-16α (*m*, δ4.59) and H-6 (*d*, J = 5.1 Hz, δ5.43), which were identical to those in hapepunine (22S,25S)-*N*-methyl-22,26-epiminocholest-5-ene-3β,16β-diol [10]. These results and the ^{13}C NMR signals indicated that **4** was hapepunine β-cellobioside.

In contrast to other *Fritillaria* species, *F. maximowczii* does not contain ceveratrum-type alkaloids, such as peimine and peiminine.

EXPERIMENTAL

^{1}H and ^{13}C NMR were measured with a JEOL JUM-GX 400 NMR spectrometer and chemical shifts are given in δ (ppm) values with TMS as int. standard. FAB-MS were recorded in a glycerol matrix containing NaI. CC was carried out on Diaion HP-20, Sephadex LH-20 (Pharmacia) and Kieselgel 60 (70–230 and 230–400 mesh, Merck).

Extraction and isolation. Fresh bulbs of *F. maximovizii* Freyn. (10 kg), collected in Heilongjiang province, China, near Mt. Da-Xing-An-Ling in June, were exhaustively extracted with EtOH. The EtOH extract was concd under red. pres. The viscous concentrate was partitioned between H_2O and 1-BuOH, and then the 1-BuOH portion was shaken with *n*-hexane and MeOH. The MeOH concentrate was chromatographed on Diaion HP-20, eluting with H_2O to MeOH. The 40% MeOH eluate was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH (50:1–5:1) and on Sephadex LH-20 with 80% MeOH to give compounds **1** (850 mg), **2** (570 mg), **3** (260 mg) and **4** (440 mg).

Kuroyurinidine (**1**). Amorphous. $[\alpha]_D^{25}$ − 10.1° (MeOH; *c* 0.40). EI-MS *m/z* (rel. int.): 445 (13) $[M]^+$, 430 (18), 332 (72), 125 (100), 124 (89), 114 (99), 110 (56), ^{1}H NMR (pyridine-d_5) δ: 0.82 (3H, *d*, J = 6.6 Hz, H_3-27), 1.08 (3H, *d*, J = 7.4 Hz, H_3-21), 1.26 (1H, *ddd*, J = 11.3, 11.3, 9.1 Hz, H*ax*-24), 1.72 (3H, *s*, H_3-18), 1.86 (3H, *s*, H_3-19), 2.50 (1H, *dq*, J = 9.1, 7.4 Hz, H-20), 2.79 (1H, *dd*, J = 9.1, 9.1 Hz, H-22), 3.09 (1H, *ddd*, J = 13.3, 13.3, 2.2 Hz, H*ax*-4), 3.14 (1H, *dd*, J = 12.3, 3.6 Hz, H*eq*-26), 3.40 (1H, *ddd*, J = 9.1, 9.1, 3.7 Hz, H-23), 4.25 (1H, *br d*, J = 2.9 Hz, H-6), 4.58 (1H, *br d*, J = 2.6 Hz, H-2), 4.66 (1H, *br d*, J = 2.9 Hz, H-3). ^{13}C NMR (pyridine-d_5) δ: 43.5 (C-1), 72.0 (C-2), 72.0 (C-3), 30.5 (C-4), 43.7 (C-5), 72.3 (C-6), 39.6 (C-7), 40.3 (C-8), 57.2 (C-9), 36.9 (C-10), 29.3 (C-11), 126.7 (C-12), 142.6 (C-13), 48.8 (C-14), 25.1 (C-15), 32.4 (C-16), 85.5 (C-17), 13.5 (C-18), 17.6 (C-19), 40.8 (C-20), 11.3 (C-21), 66.9 (C-22), 75.5 (C-23), 40.0 (C-24), 31.4 (C-25), 55.0 (C-26), 19.0 (C-27).

23-*Isokuroyurinidine* (**2**). Amorphous. $[\alpha]_D^{25}$ − 14.2° (MeOH, *c* 0.40). EI-MS *m/z* (rel. int.): 445 (12) $[M]^+$, 430

(28), 332 (48), 125 (100), 124 (76), 114 (55), 110 (46). ^{1}H NMR (pyridine-d_5) δ: 0.84 (3H, J = 6.6 Hz, H_3-27), 1.42 (3H, d, J = 7.3 Hz, H_3-21), 1.64 (3H, s, H_3-18), 1.86 (3H, s, H_3-19), 2.84 (1H, dd, J = 12.6, 12.6 Hz, H*ax*-26), 2.90 (1H, dq, J = 8.8, 7.3 Hz, H-20), 3.10 (1H, ddd, J = 13.3, 13.3, 2.2 Hz, H*ax*-4), 3.33 (1H, dd, J = 8.8, 8.8 Hz, H-22), 3.57 (1H, dd, J = 12.6, 4.0 Hz, H*eq*-26), 3.96 (1H, ddd, J = 8.8, 8.8, 3.7 Hz, H-23), 4.25 (1H, br d, J = 2.9 Hz, H-6), 4.58 (1H, br d, J = 2.6 Hz, H-2), 4.66 (1H, br d, J = 2.9 Hz, H-3). NOEs were observed between H_3-18 and H-20; H-20 and H-22; H-22 and H-23. ^{13}C NMR (pyridine-d_5) δ: 43.4 (C-1), 71.8 (C-2), 71.9 (C-3), 30.4 (C-4), 43.6 (C-5), 72.2 (C-6), 39.9 (C-7), 40.7 (C-8), 57.0 (C-9), 36.8 (C-10), 29.3 (C-11), 126.2 (C-12), 144.0 (C-13), 48.6 (C-14), 24.8 (C-15), 31.8 (C-16), 86.4 (C-17), 13.4 (C-18), 17.5 (C-19), 38.6 (C-20), 12.2 (C-21), 64.3 (C-22), 72.9 (C-23), 37.9 (C-24), 28.4 (C-25), 51.5 (C-26), 18.2 (C-27).

15,16-seco-22αH,25βH-*Solanida*-5,14-*dien*-3β-ol *O*-β-D-*glucopyranosyl*-(1 → 4)-β-D-*xylopyranoside* (**3**). Amorphous. $[\alpha]_D^{25}$ − 20.5 (MeOH, c 0.40). Pos. FAB-MS m/z (rel. int.): 692 (100) $[M + H]^+$, 530 (10) $[692 - glc]^+$, 398 (16) $[530 - xyl]^+$, 380 (23) $[530 - 150]^+$. ^{1}H NMR (pyridine-d_5) δ: 0.96, 0.97 (each, 3H, s, H-18, 19), 1.09, 1.18 (each, 3H, d, J = 6.6 Hz, H_3-21, -27), 4.89, 5.06 (each, br s, H_2-15), 4.91, 5.16 (each, 1H, d, J = 7.7 Hz, xyl, glc H-1), 5.45 (1H, br d, J = 1.1 Hz, H-6). ^{13}C NMR (pyridine-d_5) δ: 37.1 (C-1), 30.2 (C-2), 78.2 (C-3), 39.0 (C-4), 141.0 (C-5), 121.0 (C-6), 35.6 (C-7), 33.7 (C-8), 50.9 (C-9), 36.3 (C-10), 20.5 (C-11), 39.0 (C-12), 42.3 (C-13), 135.0 (C-14), 106.7 (C-15), 56.3 (C-16), 51.5 (C-17), 17.6 (C-18), 19.1 (C-19), 38.2 (C-20), 19.0 (C-21), 69.3 (C-22), 30.2 (C-23), 30.8 (C-24), 27.7 (C-25), 58.4 (C-26), 19.9 (C-27). xyl: 103.2 (C-1), 74.7 (C-2), 76.5 (C-3), 78.9 (C-4), 64.7 (C-5). glc: 103.7 (C-1), 74.4 (C-2), 78.2 (C-3), 71.7 (C-4), 78.2 (C-5), 62.7 (C-6).

15,16-*seco*-22αH,25βH-*Solanida*-5,14-*dien*-3β-*ol*. A suspension of **3** (28 mg) and crude hesperidinase in NaOAc–HOAc was incubated for 2 days at 37°. The reaction mixt. was evapd under red. pres. to give a residue, which was chromatographed over silica gel with n-hexane–Me_2CO (4:1) to afford the aglycone (12 mg). EI-MS m/z (rel. int.): 398 (2) $[M + H]^+$, 397 (3) $[M]^+$, 396 (4) $[M - H]^+$, 246 (1) $[396-150]^+$, 204 (2), 150 (100), 136 (26), 98 (5). ^{1}H NMR (pyridine-d_5) δ: 0.98, 1.08 (3H, each, s, H_3-18, 19), 1.12, 1.20 (each, 3H, d, J = 6.6 Hz, H_3-21, -27), 4.85, 5.00 (1H, each, br s, H_2-15), 5.42 (1H, br d, J = 1.1 Hz, H-6). ^{13}C NMR (pyridine-d_5) δ: 37.4 (C-1), 32.3 (C-2), 71.1 (C-3), 43.1 (C-4), 141.0 (C-5), 121.2 (C-6), 35.6 (C-7), 34.0 (C-8), 50.8 (C-9), 36.3 (C-10), 20.7 (C-11), 38.2 (C-12), 42.5 (C-13), 135.0 (C-14), 106.4 (C-15), 56.9 (C-16), 51.2 (C-17), 18.4 (C-18), 19.1 (C-19), 37.1 (C-20), 18.7 (C-21), 68.3 (C-22), 29.9 (C-23), 31.0 (C-24), 28.0 (C-25), 58.7 (C-26), 19.9 (C-27). The insol. part from the above solvent was converted into the corresponding TMSi ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(R)-carboxylates followed by GC analysis. The sugar moiety was composed of D-glucose and D-xylose.

Hapepunine 3-*O*-β-*cellobioside* (**4**). Amorphous. $[\alpha]_D^{27}$ − 29.1 (MeOH, c 0.43). Pos. FAB-MS m/z (rel. int.): 754 (75) $[M + H]^+$, 738 (21) $[M - Me]^+$, 722 (100) $[738 - OH]^+$. ^{1}H NMR (pyridine-d_5) δ: 0.89, 1.08 (3H each, H_3-18, -19), 0.94, 1.10 (each, 3H, d, J = 7.0 Hz, H_3-21, -27), 2.28 (3H, s, N-Me), 5.01, 5.23 (each, 1H, d, J = 7.7 Hz, 2 × glc H-1). ^{13}C NMR (pyridine-d_5) δ: 37.3 (C-1), 30.7 (C-2), 78.4 (C-3), 38.8 (C-4), 141.0 (C-5), 121.7 (C-6), 32.0 (C-7), 31.6 (C-8), 50.2 (C-9), 36.8 (C-10), 21.1 (C-11), 40.5 (C-12), 43.1 (C-13), 54.3 (C-14), 29.9 (C-15), 71.4 (C-16), 50.2 (C-17), 13.3 (C-18), 17.5 (C-19), 40.4 (C-20), 19.0 (C-21), 58.8 (C-22), 30.1 (C-23), 37.2 (C-24), 31.6 (C-25), 62.4 (C-26), 19.3 (C-27), 43.9 (N-Me), glc: 102.2 (C-1), 74.8 (C-2), 76.8 (C-3), 81.3 (C-4), 76.4 (C-5), 62.4 (C-6), glc: 104.9 (C-1), 74.8 (C-2), 78.2 (C-3), 71.4 (C-4), 78.2 (C-5), 62.1 (C-6).

Hapepunine. Compound **4** (35 mg) was dissolved in a small amount of DMSO (0.8 ml) and suspended in NaOAc–HOAc buffer and β-glucosidase (from almonds). The mixt. was incubated for 3 days at 37° and was then evapd under red. pres. The residue was chromatographed over silica gel with n-hexane–Me_2CO (3:1) to provide the aglycone (8 mg). EI-MS m/z (rel. int.): 429 (1) $[M]^+$, 428 (2) $[M - H]^+$, 414 (2) $[M - Me]^+$ 397 (5) $[414 - OH]^+$, 112 (100). ^{1}H NMR (pyridine-d_5): δ1.07, 1.10 (each, 3H, s, H_3-18, -19), 1.07, 1.08 (each, 3H, d, J = 7.0 Hz, H_3-21, -27), 2.28 (3H, s, N-Me), 3.87 (1H, m, H-3α), 4.59 (1H, m, H-16α), 5.43 (1H, d, J = 5.1 Hz, H-6). The sugar was identified using the method described above.

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