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Scillasaponins A, B, and C, New Triterpenoid Oligosaccharides from the Plants of the Subfamily Scilloideae

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Three new triterpenoid oligosaccharides, scillasaponins A, B, and C, were isolated from the fresh bulbs of *Eucomis bicolor*, *Scilla peruviana*, and *Chionodoxa gigantea*, respectively. Their structures were established by extensive NMR studies. Scillasaponins were active as a cyclic AMP phosphodiesterase inhibitor.

The genera *Eucomis*, *Scilla*, and *Chionodoxa* belong to the subfamily Scilloideae in the Liliaceae. Some plants of the subfamily Scilloideae are known to be poisonous plants,[1] and several cardenolide glycosides were isolated and identified. As part of a systematic study on the bioactive constituents of the bulbs of Liliaceae plants, we investigated the MeOH extract of the *Eucomis bicolor*, *Scilla peruviana*, and *Chionodoxa gigantea* bulbs, resulting in the isolation of three new triterpenoid oligosaccharides, named scillasaponins A (**1**), B (**2**), and C (**3**). This paper briefly reports the structural elucidation of the new compounds by extensive NMR studies.

Scillasaponin A (**1**) (53.9 mg) was isolated from the *n*-BuOH-soluble phase of the methanolic bulb extract of *Eucomis bicolor* (6.5 kg), scillasaponin B (**2**) (963 mg) from *Scilla peruviana* (4.0 kg), and scillasaponin C (**3**) (49.8 mg) from *Chionodoxa gigantea* (5.8 kg) after a series of chromatographic separations.

Scillasaponin A (**1**), $C_{58}H_{92}O_{27}$, was obtained as an amorphous powder, $[\alpha]_D$ -45.6° (MeOH).[2] Acid hydrolysis of **1** with 1M HCl in dioxane - H_2O (1 : 1) gave D-glucose, L-rhamnose, L-arabinose, and D-xylose,[3] together with unidentified artifactual sapogenols. The IR and ^1H NMR spectral data, and comparison of the ^{13}C NMR signals of the aglycon moiety of **1** with those of the known lanosterols,[4] indicated **1** to be a lanost-8-ene-3β,31-diol pentasaccharide with the side-chain being modified. The ^1H-^1H COSY spectrum combined with the 2D HOHAHA sepctrum allowed us to deduce the three structural units formed of the D-ring and side-chain of the lanostane skeleton (Fig. 1).

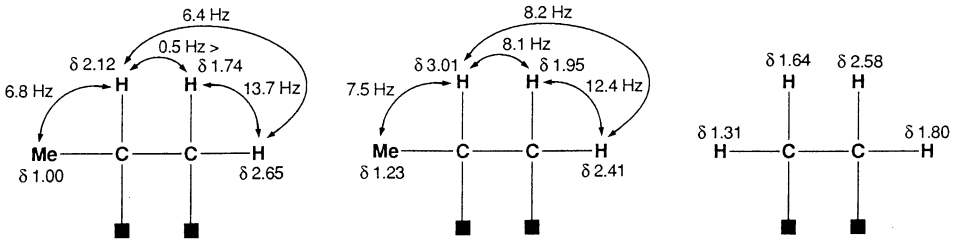

Fig. 1. Partial structures of **1**.

The connectivity of each unit through the quaternary carbons was shown by interpretation of the HMBC spectrum (Fig. 2). The configurations at the C-17, C-20, C-23, and C-25 were confirmed by the NOE

correlations observed in the phase-sensitive NOESY spectrum (Fig 3).

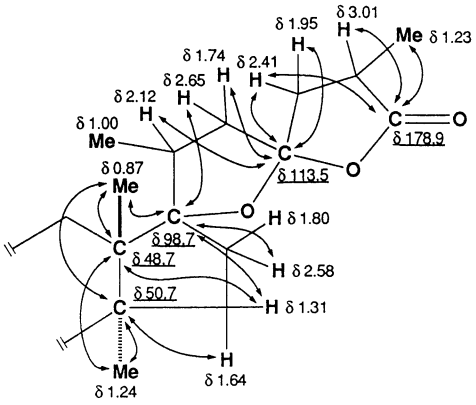

Fig. 2. ^{1}H -^{13}C long-range correlations of the aglycon moiety of **1** in pyridine-d_5.

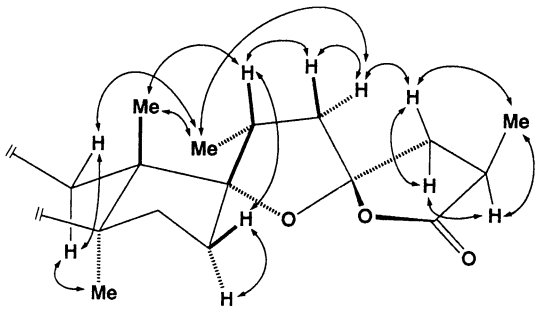

Fig. 3. NOE correlations of the aglycon moiety of **1** in pyridine-d_5.

The sequence of the saccharide moiety was determined by the following NMR analysis without such chemical degradation studies as per-methylation followed by hydrolysis and/or partial hydrolysis, which often consume relatively large amount of material. All proton signals of the carbohydrate groups of **1** could be assigned by a combined use of the ^{1}H-^{1}H COSY and 2D HOHAHA spectra. Assignments of the ^{13}C signals of the each monosaccharide were achieved by tracing out the one-bond ^{1}H-^{13}C connectivities through the use of the ^{1}H-^{13}C COSY spectrum. Comparison of the ^{13}C assignments with those of reference methyl glycosides[5] indicated the presence of a terminal β-D-xylopyranosyl unit, a terminal α-L-rhamnopyranosyl unit, 2,3-disubstituted β-D-glucopyranosyl unit, 2-substituted α-L-arabinopyranosyl unit, and 6-substituted β-D-glucopyranosyl unit in the molecule. The arabinopyranosyl unit was shown to exist in a $^{1}C_4$ conformer by the $^{3}J_{H\text{-}1,H\text{-}2}$ value of the anomeric proton and the ^{13}C NMR shifts.[6] The ^{1}H-^{13}C long-range correlation from each anomeric proton traversing the glycosidic linkage to the carbon of another substituted monosaccharide or the aglycon confirmed the sugar sequence, which was well supported by the FABMS fragments (Fig. 4). Thus, the structure of **1** was elucidated.

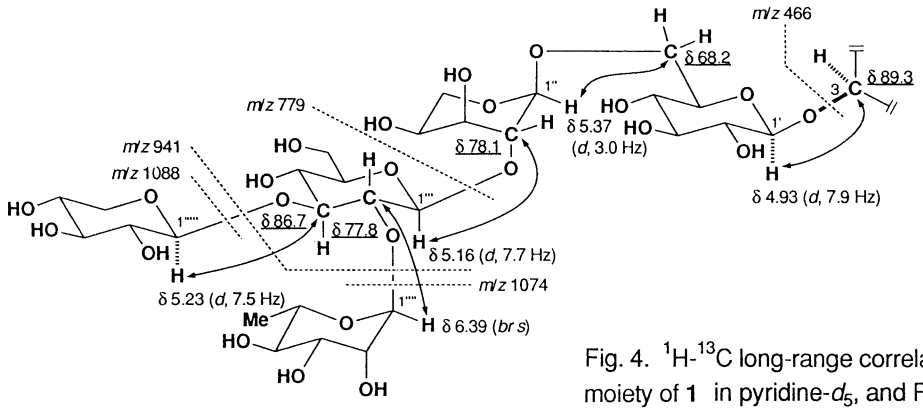

Fig. 4. ^{1}H-^{13}C long-range correlations of the saccharide moiety of **1** in pyridine-d_5, and FABMS fragments.

The structure of the aglycon of scillasaponin B (**2**), $C_{59}H_{94}O_{29}$,[7] was identified by spectral comparison to scillasaponin A (**1**). The ^{1}H and ^{13}C NMR spectra of **2** indicated that the C-30 which was present as a methyl in **1** was modified to hydroxymethyl in **2**. Acid hydrolysis of **2** gave D-glucose, L-rhamnose, and L-arabinose. The structure of the saccharide moiety of **2** was determined by the same procedures as for **1** (Fig. 5).

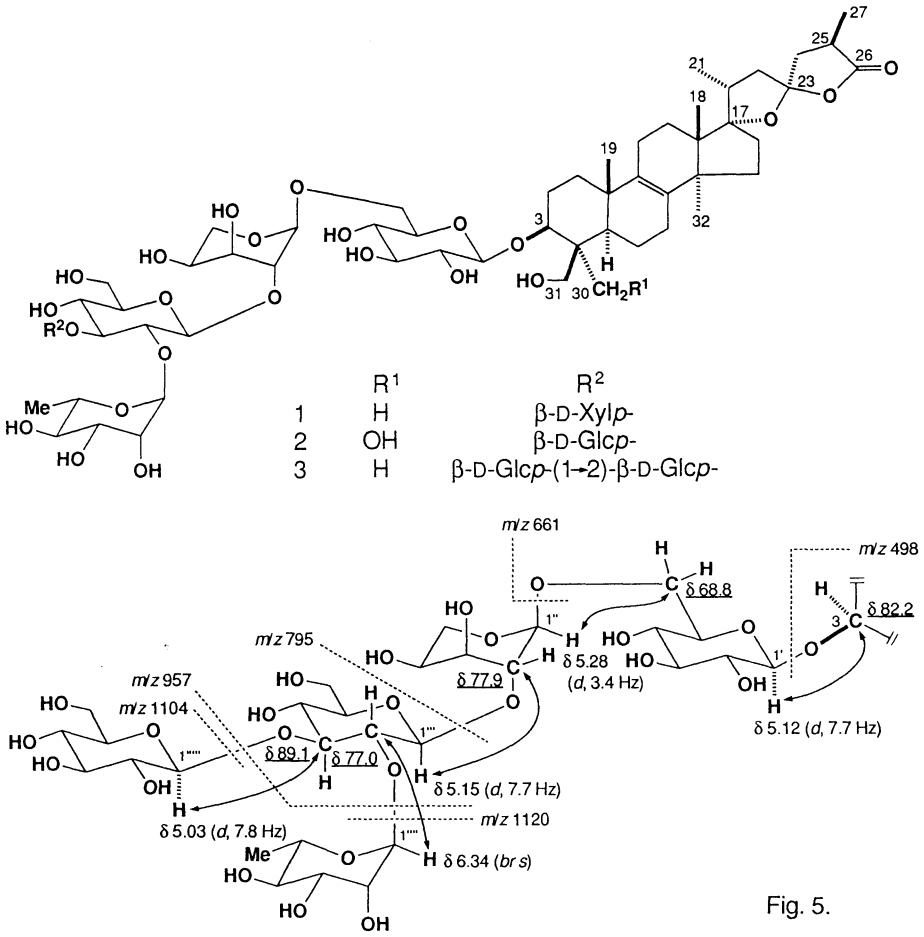

Fig. 5.

Scillasaponin C (**3**), $C_{65}H_{104}O_{33}$,[8] has the same aglycon structure as **1**. Acid hydrolysis and spectral analysis verified the oligosaccharide structure of **3** (Fig. 6).

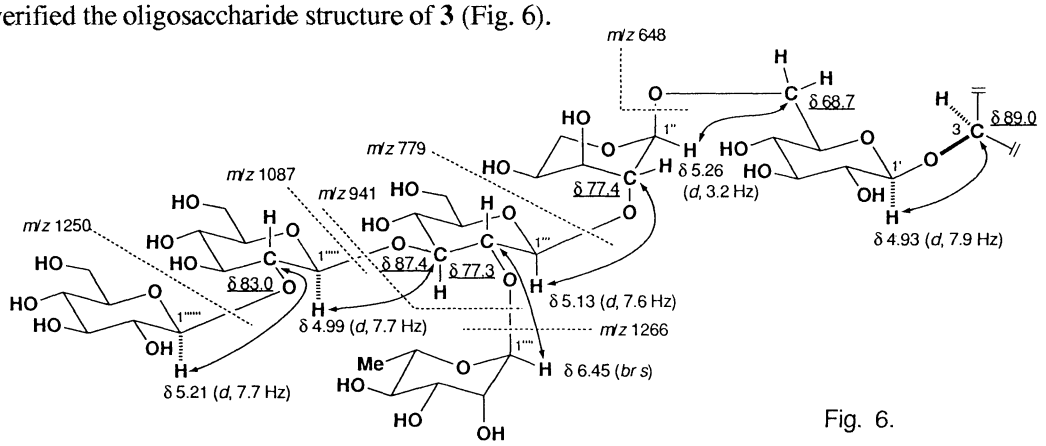

Fig. 6.

Scillasaponins A (**1**), B (**2**), and C (**3**) are the representatives of the rare types of the lanosterol penta- and hexasaccharides with the side-chain of the aglycon being modified, and exhibited inhibitory activity on cyclic AMP phosphodiesterase (**1**: IC_{50} 11.5 x 10^{-5} M; **2**: 14.0 x 10^{-5} M; **3**: 11.2 x 10^{-5} M).

References

1) J. E. Bryan, "Bulbs," Timber Press Inc., Portland and Oregon (1989), Vols. 1 and 2.

2) Negative-ion FABMS *m/z* 1219 [M - H]$^-$; IR ν_{max} (KBr) cm^{-1}: 3440 (OH) and 1760 (C=O); ^{1}H NMR

(pyridine-d_5) δ = 6.39 (1H, br s, H-1""), 5.37 (1H, d, J = 3.0 Hz, H-1"), 5.23 (1H, d, J = 7.5 Hz, H-1""), 5.16 (1H, d, J = 7.7 Hz, H-1"'), 4.93 (1H, d, J = 7.9 Hz, H-1'), 1.76 (3H, d, J = 6.2 Hz, H-6""), 1.52 (3H, s, H-30), 1.24 (3H, s, H-32), 1.23 (3H, d, J = 7.5 Hz, H-27), 1.00 (3H, d, J = 6.8 Hz, H-21), 0.93 (3H, s, H-19), and 0.87 (3H, s, H-18); ^{13}C NMR (pyridine-d_5) δ = 35.8, 27.4, 89.3, 44.4, 51.8, 18.7, 26.9, 134.8, 135.1, 36.9, 21.0, 25.0, 48.7, 50.7, 31.9, 37.5, 98.7, 18.8, 19.5, 44.1, 18.6, 45.0, 113.5, 44.8, 35.8, 178.9, 15.1, 23.0, 63.2,and 26.0 (C-1 - C-32), 105.6, 75.4, 78.1, 70.3, 74.6,and 68.2 (C-1' - C-6'), 101.0, 78.1, 71.3, 66.2,and 62.4 (C-1" - C-5"), 103.0, 77.8, 86.7, 71.6, 78.1,and 61.9 (C-1"' - C-6"'), 101.9, 72.3, 72.6, 74.3, 69.7,and 18.7 (C-1"" - C-6""), and 105.9, 75.0, 79.3, 70.9,and 67.4 (C-1""' - C-5""').

3) The identification of the monosaccharides including their absolute configurations was established by converting them to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives followed by HPLC analysis; R. Oshima, Y. Yamauchi, and J. Kumanotani, *Carbohydr. Res.*, **107**, 169 (1982).

4) J. -F. Cheng, J. Kobayashi, H. Nakamura, Y. Ohizumi, Y. Hirata, and T. Sasaki, *J. Chem. Soc., Perkin Trans. 1*, **1988**, 2403; M. Adinolfi, G. Barone, R. Lanzetta, G. Laonigro, L. Mangoni, and M. Parrilli, *J. Nat. Prod.*, **47**, 100 (1984).

5) P. K. Agrawal, D. C. Jain, R. K. Gupta, and R. S. Thakur, *Phytochemistry*, **24**, 2479 (1985).

6) O. Tanaka, *Yakugaku Zasshi*, **105**, 323 (1985).

7) $[\alpha]_D$ -44.0° (MeOH); Negative-ion FABMS *m/z* 1265 $[M - H]^-$; IR ν_{max} (KBr) cm^{-1}: 3400 (OH) and 1760 (C=O); ^{1}H NMR (pyridine-d_5) δ = 6.34 (1H, br s, H-1""), 5.28 (1H, d, J = 3.4 Hz, H-1"), 5.15 (1H, d, J = 7.7 Hz, H-1"'), 5.12 (1H, d, J = 7.7 Hz, H-1'), 5.03 (1H, d, J = 7.8 Hz, H-1""'), 1.75 (3H, d, J = 6.2 Hz, H-6""), 1.21 (3H, d, J = 7.1 Hz, H-27), 1.19 (3H, s, H-32), 1.05 (3H, s, H-19), 1.01 (3H, d, J = 6.8 Hz, H-21), and 0.89 (3H, s, H-18); ^{13}C NMR (pyridine-d_5) δ = 35.8, 27.2, 82.2, 48.2, 43.6, 18.7, 26.6, 135.0, 135.8, 36.8, 21.1, 25.0, 48.8, 50.7, 31.9, 37.5, 98.7, 18.7, 19.6, 44.1, 18.6, 45.0, 113.5, 44.8, 35.8, 178.8, 15.1, 61.2, 62.6,and 25.9 (C-1 - C-32), 105.5, 75.4, 78.2, 71.5, 75.3,and 68.8 (C-1' - C-6'), 101.0, 77.9, 72.7, 66.7,and 62.4 (C-1" - C-5"), 102.6, 77.0, 89.1, 69.1, 77.7,and 61.9 (C-1"' - C-6"'), 102.1, 72.2, 72.6, 74.1, 69.8,and 18.8 (C-1"" - C-6""), and 104.3, 75.0, 78.5, 71.5, 78.6,and 62.6 (C-1""' - C-6""').

8) $[\alpha]_D$ -32.1° (MeOH); Negative-ion FABMS *m/z* 1412 $[M]^-$; IR ν_{max} (KBr) cm^{-1}: 3425 (OH) and 1770 (C=O); ^{1}H NMR (pyridine-d_5) δ = 6.45 (1H, br s, H-1""), 5.26 (1H, d, J = 3.2 Hz, H-1"), 5.21 (1H, d, = 7.7 Hz, H-1""'), 5.13 (1H, d, J = 7.6 Hz, H-1"'), 4.99 (1H, d, J = 7.7 Hz, H-1""'), 4.93 (1H, d, J = 7.9 Hz, H-1'), 1.82 (3H, d, J = 6.1 Hz, H-6""), 1.53 (3H, s, H-30), 1.24 (3H, s, H-32), 1.23 (3H, d, J = 6.5 Hz, H-27), 1.02 (3H, d, J = 6.7 Hz, H-21), 0.94 (3H, s, H-19), and 0.88 (3H, s, H-18); ^{13}C NMR (pyridine-d_5) δ = 35.9, 27.5, 89.0, 44.5, 51.8, 18.6, 26.9, 134.8, 135.1, 36.9, 21.0, 25.0, 48.8, 50.7, 31.9, 37.4, 98.7, 18.8, 19.6, 44.1, 18.5, 45.0, 113.5, 44.9, 35.9, 178.9, 15.2, 23.1, 63.2,and 26.0 (C-1 - C-32), 106.1, 75.4, 78.2, 72.5, 75.6,and 68.7 (C-1' - C-6'), 101.3, 77.4, 69.6, 66.7,and 62.8 (C-1" - C-5"), 102.3, 77.3, 87.4, 68.7, 77.9,and 61.8 (C-1"' - C-6"'), 101.3, 72.1, 72.5, 74.1, 69.9,and 18.5 (C-1"" - C-6""), 102.0, 83.0, 76.0, 71.3, 77.1,and 62.0 (C-1""' - C-6""'), and 106.9, 75.2, 78.3, 70.2, 78.7,and 61.8 (C-1""" - C-6""").

9) T. Nikaido, T. Ohmoto, H. Noguchi, T. Kinoshita, H. Saitoh, and U. Sankawa, *Planta Med.*, **43**, 18 (1981).

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