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Peculiar steroidal saponins with opened E-ring from *Solanum* genera plants

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Abstract—Peculiar novel steroidal saponins with E-ring opened skeleton, a cholesten-3β,16,22,26-tetraol 3,26-di-*O*-glycoside corresponding to the precursor of the spirostanol and furostanol, a 16,22-dicarbonyl type steroid and a 20–22 *seco*-type steroid have been obtained from some *Solanum* genera plants. The occurrence of their unusual compounds would propose new important biogenetic routes for steroidal glycosides. © 2001 Elsevier Science Ltd. All rights reserved.

Our studies on the constituents of so far above 40 species of *Solanum* genera plants resulted in the isolation of steroidal glycosides potent cytotoxicity,[1] antifeedant,[2] anti HSV-1[3] activities and of the key intermediate[4] on the biosynthesis of spirosolane. On the way of this *Solanum* plants investigation, peculiar unusual steroidal saponins with opened E-ring type, a cholesten-3β,16,22,26-tetrol 3,26-di-*O*-glycoside, named anguivioside A (**1**) corresponding to the precursor of the spirostanol and furostanol, a 16,22-dicarbonyl type steroid, named anguiviosides B_1 (**2a**) and B_2 (**2b**), a 20-22 *seco*-type steroid, named nigrumoside A (**3**) have been obtained together with usual spirostanol glycosides from some *Solanum* genera, three formers[5] of which were from the fruits of *Solanum anguivi*, and the latter[5] from the aerial parts of *Solanum nigrum*, respectively.

Anguivioside A (**1**) has a molecular formula of $C_{51}H_{86}O_{22}$ based on the HR-FAB-MS.[6] The methyl signals at δ 0.71 (s), 1.03 (s), 1.15 (d, $J=6.7$ Hz) and 1.32 (d, $J=6.7$ Hz) and a lot of glycosidic signals (anomeric protons: δ 4.81, d, $J=6.7$ Hz, 4.92, br d, $J=7.2$ Hz; 5.83, br s; 6.37, br s) on the ^{1}H NMR spectrum suggested **1** to be a steroidal glycoside. Twenty-seven carbon signals originated from the sapogenol part were observed along with the sugar part of terminal β-chacotriosyl moiety (δ 100.3, 102.0, 102.9) and a terminal β-D-glucopyranosyl moiety (δ 105.2) in the ^{13}C NMR spectrum.[7] The presence of one primary hydroxyl (δ 75.5) and three secondary hydroxyl groups (δ 75.2, 77.2, 78.5) could be estimated. Their locations were substantiated to be at C-3, -16, -22 and -26 based on the ^{1}H–^{1}H COSY, HMQC and HMBC techniques. Namely, the correlations between H-17 (δ 1.88, m) and H-16 (δ 3.84, m), H_3-21 (δ 1.32) and C-22 (δ 77.2), and H_3-27 (δ 1.15) and C-26 (δ 75.5) were observed. The presence of the oxygen-bearing carbon at C-3 (δ 78.5) was supported by comparing with other 3β-spirostanol 3-*O*-glycoside. Furthermore, the presence of a double bond (δ_H 5.31, m; δ_C 122.0, 140.8) was suggested at C-5 by the HMBC (between H_3-19 at δ 1.03 and C-5 at δ 140.8). Meanwhile, the glycosidic linkages were also determined that the β-chacotriosyl moiety is connected to C-3-OH (HMBC between H-1 at δ 4.92 of inner glucosyl moiety and C-3 at δ 78.5), and the terminal glucosyl moiety to C-26 (HMBC between H-1 of glucosyl moiety at δ 4.81 and C-26 at δ 75.5) by the HMBC. Therefore, the chemical structure was represented as 3-*O*-β-chacotriosyl 3β,16β,22,26-tetrahydroxycholest-5-ene 26-*O*-β-D-glucopyranoside.[8] The configuration at C-22 was not defined, however, this compound is regarded as an important precursor which would produce furostanol and spirostanol showing a variety of bio-activities.

Anguiviosides B_1 (**2a**) and B_2 (**2b**) exhibited the same formula, $C_{45}H_{70}O_{18}$ in the HR-FAB-MS.[9] The ^{1}H and ^{13}C NMR spectra[10] of both compounds were resembled to each other, so they are assumed to be isomer. Both compounds were obtained as respective homogenous

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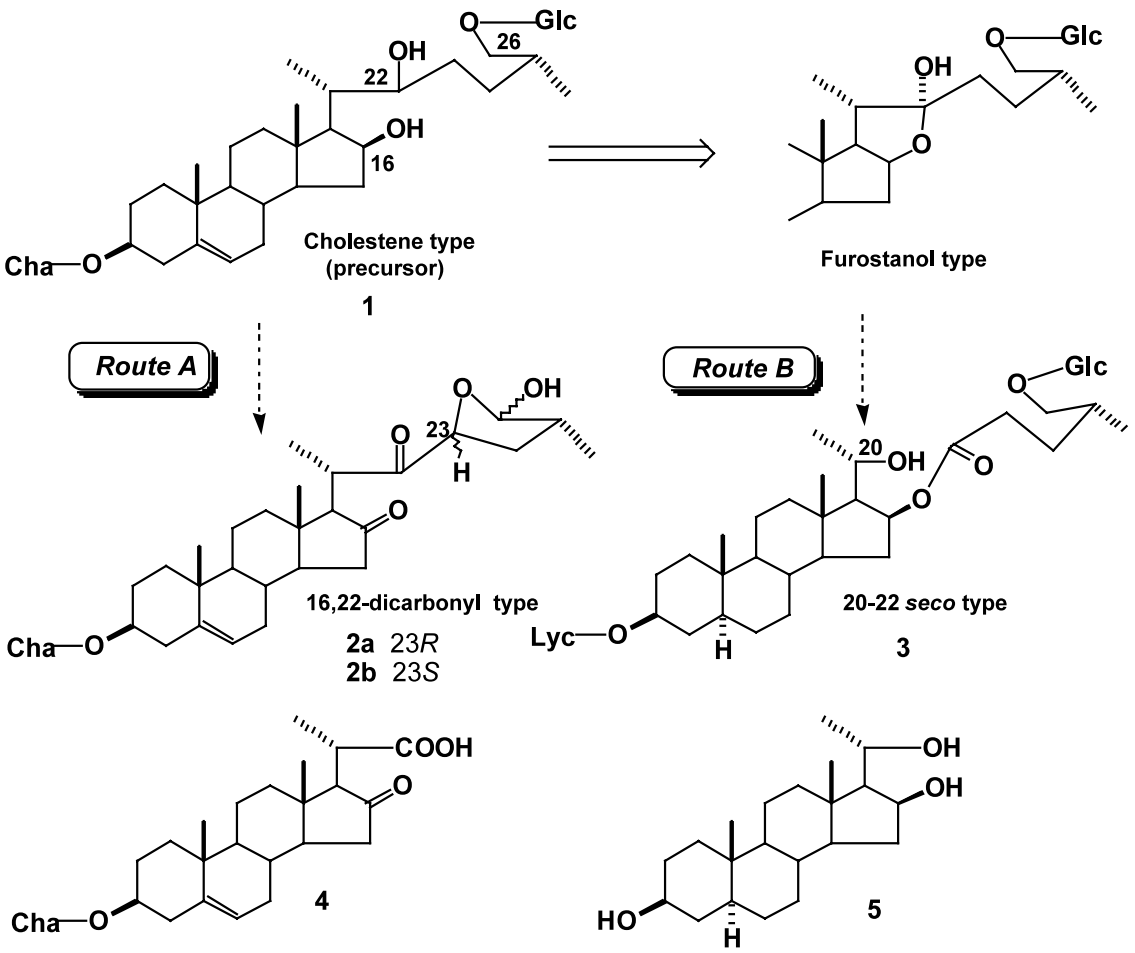
peak by HPLC, but the ^{1}H and ^{13}C NMR spectra indicated duplicate signals suggesting them to be inseparable. Various 2D NMR measurements of **2a** led to the assignments of two carbonyl groups at C-16 and -22 (δ 217.2; 217.4, 215.1; 215.5, respectively) by the correlations between C-16 and H-17 (δ 2.83, d, $J=10.4$ Hz, δ 2.85, d, $J=10.4$ Hz), and H_3-21 (δ 1.20, d, $J=7.3$ Hz; 1.26, d, $J=7.3$ Hz) and C-22, an acetal center at C-26 (δ_H 5.55, d, $J=1.9$ Hz; 5.91, d, $J=4.9$ Hz, δ_C 101.0; 106.1) by the correlation with H_3-27 (δ 1.20, d, $J=7.3$ Hz; 1.26, d, $J=6.7$ Hz), an epoxy ring between C-23 (δ_H 5.17, br d, $J=9.7$ Hz: another signal was overlapped with water, δ_C 82.0; 83.3) and C-26 by the correlations between H-23 and C-22;-26, and a β-chacotriosyl moiety linked to C-3-OH. These inseparable compounds were conceivable to be arisen from the occurrence of the equilibrium mixture at C-26 acetal center. J values at H-23 and H-26 (coupled with H-25R) in **2a** were suited to a stable conformational model with 23R configuration calculated by CAChe CONFLEX.[11] Consequently, the structure of **2a** was characterized as 3-*O*-β-chacotriosyl (23*S*,26ψ)-23,26-epoxy-3β,26-dihydroxycholest-5-ene-16,22-dione. On the other hand, signals due to H-26 appeared at δ 5.67 (d, $J=4.3$ Hz) and 5.73 (d, $J=4.3$ Hz), and signal due to H-23 at δ 5.19 (t-like, $J=7.9$ Hz). Therefore, **2b** was regarded as a stereoisomer of **2a** at C-23. On alkaline treatment, both **2a** and **2b** afforded the identical product, **4**,[12] of which ^{13}C signals gave a fine, not duplicate, spectrum. This compound was shown to be 3-*O*-β-chacotriosyl 20-carboxypregna-5-ene 16-one. As a 16,22-dicarbonyl steroid, kryptogenin[13] obtained by artificially acid treatment of pennogenin is known, however, the occurrence[14] of **2a** and **2b** suggest naturally occurring constituents as genuine form probably produced directly from **1** on the biogenetic route.

Nigroside A (**3**) gave a molecular formula, $C_{56}H_{94}O_{29}$ in the HR-FAB-MS.[15] The proton signals due to H_3-19 at δ 0.67, H_3-18 at δ 0.88, H_3-27 at δ 0.92 and H_3-21 at δ



1.46 together with five anomeric proton signals at δ 4.78 (d, $J=7.9$ Hz), 4.89 (d, $J=7.3$ Hz), 5.18 (d, $J=7.9$ Hz), 5.22 (d, $J=7.9$ Hz) and 5.56 (d, $J=7.3$ Hz) also indicated **3** to be a steroidal glycoside. The ^{13}C signals[16] constituted of 56 carbons contained a β-lycotetraosyl and a terminal glucosyl moieties. When excludes them, twenty seven signals of the sapogenol part were disclosed. As regards with the assignments for signals around D-ring, the ^1H–^1H COSY were observed starting from H-14 (δ 0.80, m), H_2-15 (δ 2.40, m), H-16 (δ 5.77, m), H-17 (δ 1.60, t-like, $J=7.3$ Hz), H-20 (δ 4.37, m), to H_3-21 (δ 1.46, d, $J=6.1$ Hz), successively, also between H_2-23 (δ 2.50, m) and H_2-24 (δ 2.54, m). The HMBC revealed the connectivities between H_3-21 and C-20 (δ 63.3);C-17 (δ 64.5), C-22 (δ 173.3) and H_2-23, and H_3-27 and C-25 (δ 33.5); C-24 (δ 29.2);C-26 (δ 74.9). Taking into consideration chemical shifts of C-16 and C-26, it was deduced that the ester group was connected to C-16 OH and the glucosyl moiety is linked to C-26 OH. Acid treatment with 1N HCl–dioxane provided 3β,16,20-trihydroxy-5α-pregnane (**5**),[17] which was further derived into the benzylidene acetal (**5′**).[18] In the NOESY of **5′**, NOEs were observed between H_3-18 and H-20, and acetal H and H-16;H_3-21. Since the hydroxyl group at C-16 is β, the stereo-configuration at C-20 was decided to be *S*. Therefore, the structure of **3** was characterized as 3-*O*-β-lycotetraosyl 3β,16β,20(*S*)-trihydroxy-5α-pregnane 16-*O*-(5-*O*-β-D-glucopyranosyl-4(*S*)-methyl-5-hydroxy-pentanoic acid)-ester. This compound was supposed that it would be produced via furostanol glycoside as if Marker degradation[19] took place in the plant body.

These unusual novel compounds obtained in this time would provide additional important new pathways (Route A and Route B) for steroidal biogenesis.

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5. Compounds **1**, **2a**, **2b** and **3** were obtained in yields of 0.082, 0.104, 0.0753 and 0.082%, respectively, from the MeOH extract by the extraction and successive various column chromatographies using highly porous copolymer resin of styrene and dibinylbenzene, reversed silica gel and silica gel, and HPLC using reversed silica gel.
6. Positive HR-FAB-MS (*m*/*z*) of **1**: 1073.5498 $[M+Na]^+$ ($C_{51}H_{86}O_{22}Na$, calcd for 1073.5484).
7. ^{13}C NMR (in Py-d_5) of **1**, aglycone moiety C-1-27 δ: 37.5, 30.2, 78.5, 39.0, 140.8, 122.2, 32.2, 32.2, 50.5, 36.9, 21.3, 40.2, 42.5, 57.1, 28.2, 75.2, 53.0, 12.1, 19.5, 37.1, 12.7, 77.2, 24.6, 40.9, 31.1, 75.5, 19.4, chacotriosyl moiety glc 1-6: 100.3, 78.1, 76.9, 77.9, 77.8, 61.3, (glc 2-1)-rha 1-6: 102.0, 72.5, 72.7, 74.1, 69.5, 18.5, (glc 4-1)-rha 1-6; 102.9, 72.5, 72.8, 73.9, 70.4, 18.6, 26-*O*-glucosyl moiety C-1-6: 105.0, 75.3, 78.4, 71.6, 78.6, 62.8.
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9. Positive HR-FAB-MS (*m*/*z*) of **2a**: 921.4473 $[M+Na]^+$ ($C_{45}H_{70}O_{18}Na$, calcd for 921.4460), **2b**: 921.4481 $[M+Na]^+$ ($C_{45}H_{70}O_{18}Na$, calcd for 921.4460).
10. ^{13}C NMR (in Py-d_5) of **2a**: aglycone moiety C-1-27 δ: 37.2, 30.1, 78.0, 38.8, 140.9, 121.4, 31.9, 31.0, 50.0, 37.0, 20.7, 38.9, 41.8; 41.9, 51.0; 51.1, 37.4; 37.6 217.2; 217.4, 65.2; 65.6, 12.8, 19.3; 19.4, 38.7; 38.8, 13.5; 15.5, 215.1; 215.5, 82.1; 83.3, 35.5; 35.6, 40.6, 101.0; 106.1, 15.7; 16.4. Chacotriosyl moiety superimposed on that of **1**. ^{13}C NMR (in Py-d_5) of **2b**: aglycone moiety C-1-27 δ: 37.2, 30.1, 78.0, 38.8, 140.9, 121.4, 31.9, 31.0, 50.0, 37.0, 20.7, 38.9, 41.8; 41.9, 51.0; 51.1, 37.4; 37.6, 217.0, 65.3; 65.4, 12.8, 19.3; 19.4, 38.6; 38.7, 13.1; 15.5, 214.2, 81.8; 84.8, 35.2; 36.5, 41.3, 100.9; 106.0, 15.8; 17.1.
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12. **Compound 4**. Positive FAB-MS (*m*/*z*): 815 $[M+H]^+$, ^{13}C NMR (in Py-d_5), aglycone moiety C-1-22 δ: 37.2, 30.1, 78.7, 39.0, 140.9, 121.5, 32.0, 31.0, 50.1, 37.1, 20.8, 38.9, 41.9, 50.9, 37.9, 216.8, 65.4, 12.9, 19.4, 38.6, 17.2, 178.3. Chacotriosyl moiety superimposed on that of **1**.
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14. These sorts of five dicarbonyl-type glycosides were also obtained from *S. anguivi* and *S. nodiflorum* (unpublished data).
15. Positive HR-FAB-MS (*m*/*z*) of **3**: 1253.5753 $[M+Na]^+$ ($C_{56}H_{94}O_{29}Na$, calcd for 1253.5751).
16. ^{13}C NMR (in Py-d_5) of **3**, aglycone moiety C-1-27 δ: 37.1, 29.9, 77.5, 35.0, 44.7, 28.9, 32.6, 34.8, 54.5, 35.7, 21.0, 39.3, 41.7, 54.3, 35.0, 75.1, 64.5, 13.4, 12.3, 63.3, 24.1, 173.3, 32.6, 29.2, 33.5, 74.9, 17.0, lycotetraosyl moiety, gal 1-6: 102.5, 73.1, 75.0, 79.9, 76.2, 60.6, inner glc 1-6: 104.9, 81.3, 86.8, 70.7, 78.7, 62.4, terminal glc 1-6: 105.0, 75.3, 78.6, 71.0, 77.7, 63.0, xyl 1-5: 105.1, 75.6, 77.7, 70.4, 67.3, 26-*O*-glc 1-6: 104.8, 75.1, 78.5, 71.7, 78.5, 62.8.
17. **Compound 5**. ^1H NMR (in Py-d_5) δ: 0.60 (1H, m, H-9), 0.84 (3H, s, H_3-19), 1.08 (3H, s, H_3-18), 1.51 (3H, d, $J=6.7$ Hz, H_3-21), 2.37 (1H, m, H_2-15), 3.87 (1H, m, H-3), 4.50 (1H, m, H-20), 4.84 (1H, m, H-16). ^{13}C NMR (in Py-d_5) sapogenol C-1-21 δ: 37.5, 32.4, 70.6, 39.3, 45.2, 29.1, 32.5, 35.0, 54.7, 35.9, 21.1, 39.9, 40.6, 54.4, 36.5, 73.0, 63.9, 14.5, 12.5, 66.0, 24.1.

18. **Compound 5′**. ^{1}H NMR (in Py-d_5) δ: 0.84 (3H, s, H_3-19), 1.03 (3H, s, H_3-18), 1.11 (1H, m, H-17), 1.40 (3H, d, $J=6.7$ Hz, H_3-21), 2.23 (1H, m, H_2-15), 4.18 (1H, m, H-20), 4.47 (1H, m, H-16). ^{13}C NMR (in Py-d_6) sapogenol C-1-21 δ: 37.0, 32.2, 71.3, 38.2, 45.0, 28.6, 31.5, 34.8, 54.8, 35.7, 20.7, 38.4, 41.8, 53.2, 33.6, 75.4, 55.5, 14.6, 12.4, 66.6, 20.9, benzylidene moiety δ: 94.5, 126.2×2, 128.2×2, 128.5, 139.7.
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