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FOUR NEW 3α-HYDROXY SPIROST-5-ENE DERIVATIVES FROM *GYNURA JAPONICA* MAKINO

Masayuki Takahira, Yoshikazu Kondo, Genjiro Kusano, and Shigeo Nozoe*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, Japan

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Four new steroidal compounds were isolated as the major constituents from the roots of *Gynura japonica* Makino (Compositae), which have been applied for the purpose of the coagulation of blood in Chinese Medicine.[1] They were determined to be 3-*epi*-diosgenin 3-β-D-glucopyranoside (I), 3-*epi*-sceptrumgenin 3-β-D-glucopyranoside (II), 3-*epi*-ruscogenin (III), and 3-*epi*-neoruscogenin (IV) on the basis of the chemical and physicochemical evidences. These compounds are the first examples of naturally occurring 3α-hydroxy spirost-5-enes.

The roots of *G. japonica* were extracted with 70% ethanol under reflux and the solution was concentrated under reduced pressure. The concentrate was extracted with n-butanol, and chromatographed on silica gel eluting with chloroform-methanol (20:1).

The compound I, mp 218 -221°, $[\alpha]_D$-122°, $C_{33}H_{52}O_8$, M^+: m/e 576, was isolated in pure form as the acetate, mp 212 -215°, $[\alpha]_D$-180°, $C_{41}H_{60}O_{12}$, in silver nitrate loaded silica gel chromatography of the acetates of the mixture of I and II. After saponification of the isolated acetate, the original compound I was recovered. Hydrolysis of I with emulsin gave glucose and the genin Ia, mp 244 -246°, $[\alpha]_D$-145°, $C_{27}H_{42}O_3$, M^+: m/e 414. The mass spectrum of Ia displayed the prominent fragment peaks at m/e 342, 300, 282, 271, 253 and 139 and the NMR spectrum indicated the presence of two tertiary methyl groups (0.81 and 1.04 ppm: 13-Me and 10-Me) and two secondary methyl groups (0.97 and 0.79 ppm: 20-Me and 25-Me). These properties are very reminescent of those of diosgenin.[2) 3)] An additional signal at 4.06 ppm (1H, $W_{1/2}$: 8 Hz) was assigned to an equatorial carbinyl hydrogen at C_3 as in the case of cordylagenin.[4)] These physicochemical data suggested for Ia 25R-spirost-5-en-3α-ol (3-*epi*-diosgenin).

The compound II, mp 236 - 238°, $[\alpha]_D$-109°, $C_{33}H_{50}O_8$, M^+: m/e 574, eluted with I, was isolated in pure form as the acetate, mp 174 - 177°, $[\alpha]_D$-168°, $C_{41}H_{58}O_{12}$. After saponification of the isolated acetate, the original compound II was obtained. Hydrolysis of II with emulsin gave glucose and the genin IIa, mp 197 - 198°, $[\alpha]_D$-136°, $C_{27}H_{40}O_3$, M^+: m/e 412. The bands at 1650 and 875 cm^{-1} in the IR spectrum of IIa and a broad singlet at 4.82 ppm (2H, $W_{1/2}$= 6 Hz) in the NMR spectrum indicated the presence of an exocyclic methylene group in IIa. A predominant fragment ion at m/e 137 in the mass spectrum rationalized the exocyclic methylene group should be located in ring F of a spirostane skeleton.[2)] The NMR spectrum lacked a doublet for 25-Me, which was recognized in those of I and Ia, while displaying a pair of doublets of two hydrogens on C_{26} (3.90 and 4.37 ppm, J= 12 Hz). These properties suggested that IIa has an exocyclic methylene group on C_{25} in the place of the secondary methyl group in Ia. Besides of the signal of the exocyclic methylene group, the NMR spectrum

containing the signal for 3β-hydrogen at 4.07 ppm was similar to that of Ia. Therefore, IIa was supposed to be 25R-spirosta-5,25-dien-3α-ol (3-*epi*-sceptumgenin).[5)]

The above assignments for the genins Ia and IIa were confirmed chemically as follows. Diosgenin was submitted to Jones oxidation, followed by reduction with $NaBH_4$ to yield diosgenin (main product) and 3α-hydroxyl devivative, mp 240 - 242°, $C_{27}H_{42}O_3$, M^+: m/e 414, and the latter was found to be identical with Ia. Catalytic hydrogenation of IIa over palladium on charcoal afforded two isomeric products, one of which was identified to Ia and the other product was identified to 3-*epi*-yamogenin by comparison of Rf values on TLC with an authentic specimen.

The NMR spectrum of I-acetate and $[M]_D$ values of I and Ia were instructive for the stereostructural elucidation of the original glucoside I. The NMR spectrum displayed a doublet (4.56 ppm, 1H, J= 7 Hz, 1'-H), a pair of triplets (5.01 and 5.16 ppm, 1H each, 2'-H and 3'-H), a pair of quartets (4.30 and 3.92 ppm, 1H each, 6'-2H) and two multiplets (5.2 and 4.2 ppm, 1H each, 4'-H and 5'-H). The difference of $[M]_D$ values between I and Ia was -45°.[6)] These data lead us to a conclusion that I should be 3-*epi*-diosgenin 3-β-D-glucopyranoside. The same examination of the NMR spectrum of II-acetate and the difference between $[M]_D$ values of II and IIa (-70°) resulted in the conclusion that II should be 3-*epi*-sceptumgenin 3-β-D-glucopyranoside.

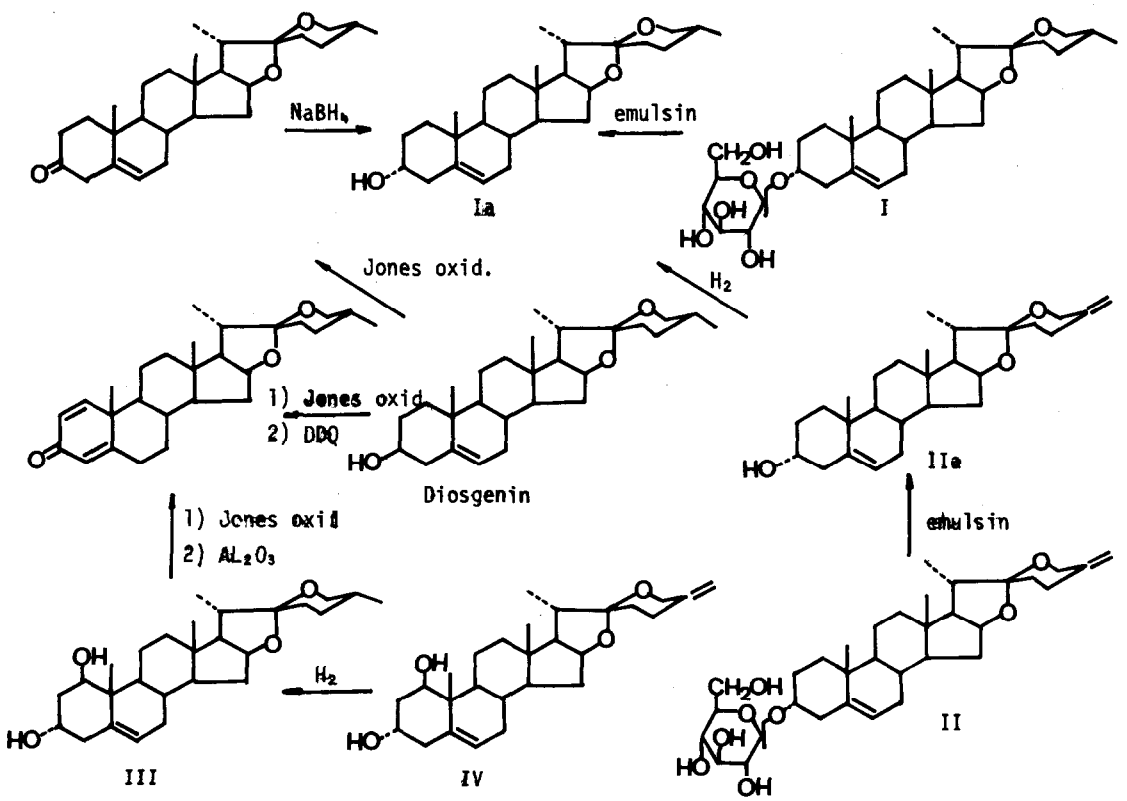
The compound III, mp 196 - 197°, $C_{27}H_{42}O_4$, M^+: m/e 430, eluted along with IV next to phytosterols in the silica gel chromatography, was isolated as the acetate. The IR spectrum of III showed the bands characteristic of the spirostane sapogenins in the region of 850-1000 cm^{-1} together with a hydroxyl band in 3400 cm^{-1}.[7)] The NMR spectrum indicated the presence of an equatorial carbinyl hydrogen (4.08 ppm, 1H, m, $W_{1/2}$= 8 Hz) and an axial carbinyl hydrogen (3.84, 1H, J_1= 10.5, J_2= 5.5 Hz). The similar assignment has been reported in the structural elucidation of 1β, 3α-dihydroxy spirostane.[4)] The signal of the equatorial hydrogen (4.08 ppm) was so similar to those of 3β-hydrogen in Ia and IIa that III was supposed to have 3α-hydroxyl group on a spirostane skeleton. Acetylation of III gave diacetate IIIa, mp 195 - 196°, $[\alpha]_D$-74°, $C_{31}H_{46}O_6$, M^+: m/e 514, the mass spectrum of which showed the prominent fragment ions at m/e 139 and 280, indicating no hydroxyl groups on ring E and F.[8)] An examination of these properties comparing with those of ruscogenin and its derivatives suggested for III 25R-spirost-5-ene-1β, 3α-diol (3-*epi*-ruscogenin).

The compound IV, mp 167 -169°, $C_{27}H_{40}O_4$, M^+: m/e 428, provided the diacetate IVa, mp 188 - 190°, $[\alpha]_D$-70°, $C_{31}H_{44}O_6$, M^+: 512. The bands at 1651 and 880 cm^{-1} in the IR spectrum of IVa and a broad singlet at 4.80 ppm (2H, m, $W_{1/2}$ 7 Hz) in the NMR spectrum indicated the presence of an exocyclic methylene group. The predominent fragment ions at m/e 137 and 280 in the mass spectrum of IV and lack of a secondary methyl group and appearance of a pair of doublets (3.88 and 4.34 ppm, J= 12 Hz) in the NMR spectrum comparing with that of III suggested that the exocyclic methylene group should be located on C_{25} of a spirostane skeleton. These properties indicated that IV should be spirosta-5,25(27)-diene-1β, 3α-diol (3-*epi*-neoruscogenin).[9)]

The above assignments for III and IV were comfirmed chemically as follows. Mild hydrolysis of IIIa afforded the monoacetate IIIb, mp 218 - 221°, $[\alpha]_D$-106°, $C_{29}H_{44}O_5$,

M^+: m/e 472, NMR ($CDCl_3$)δ 5.05 ppm (1H, dd, J_1= 11 Hz, J_2= 5.5 Hz; 1α-H), 4.10 (1H, m, 3β-H), 2.05 (3H, s, $OCOCH_3$), 1.16 (3H, s, 10-Me). Jones oxidation of IIIb, followed by reflux in methanol containing alumina provided colorless needles, mp 199 - 202°, UV λ_{max}^{MeOH} nm: 245, $C_{27}H_{38}O_3$, M^+: 410, IR (KBr) cm^{-1}: 1665. NMR ($CDCl_3$)δ: 7.03 ppm (1H, d, J= 10 Hz, 1-H), 6.21 (1H, dd, J_1= 10 Hz, J_2= 2 Hz, 2-H), 6.06 (1H, bs, 4-H), 1.25 (3H, s, 10-Me), 0.95 (3H, d, J= 6.5 Hz, 20-Me), 0.78 (3H, d, J= 5.5, 25-Me). After comparison of the physicochemical data with an authentic specimen of 25R-spirost-1,4-dien-3-one, which was prepared from diosgenin according to the known process, [10] both products were proved to be identical. Catalytic hydrogenation of IVa over palladium on charcoal gave two products which were identified to IIIa and 25S-spirost-5-ene-1β,3α-di-O-acetate, mp 162 - 163°, $[\alpha]_D$-43°, $C_{31}H_{46}O_6$, M^+: m/e 514, NMR ($CDCl_3$)δ: 3.34 ppm (1H, d, J= 12 Hz, 26-H), 3.99 (1H, dd, J_1= 12, J_2= 3.5 Hz, 26-H). The pattern and chemical shifts of two hydrogens on C_{26} in the NMR spectrum of the latter product appear to be characteristic of an axial (25S) 25-Me spirostane system. Similar results have been observed on catalytic hydrogenation of neoruscogenin.[9]

Although many kinds of 3α-hydroxy-5α- and -5β-spirostane derivatives are known,[11] 3α-hydroxy spirost-5-ene derivatives such as I-IV are the first examples of naturally occurring spirostanols, according to our knowledge.



The roots of this plant did not provided traces of the corresponding 3β-hydroxy spirost-5-ene derivatives, which are rather widly spread in Nature. Several other steroidal polyalcohols, which are also expected to be 3α-hydroxyl derivatives, have been isolated in the course of this reseach and a study for their structural elucidation is going on.

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