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SPERGULATRIOL, A NEW BISNOR-TRITERPENOID SAPOGENOL FROM THE ROOT OF MOLLUGO SPERGULA L.

Isao Kitagawa*, Hideaki Yamanaka, Tsutomu Nakanishi, and Itiro Yosioka[1)]

Faculty of Pharmaceutical Sciences, Osaka University

133-1, Yamada-kami, Suita, Osaka 565, Japan

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Recently, we have elucidated the structure of a new migrated hopane-type sapogenol named spergulagenin A (1) which was obtained by acid hydrolysis of the saponin mixture isolated from the root of *Mollugo spergula* L.[2)] In order to ascertain the genuineness of spergulagenin A (1), the soil bacterial[3)] and enzymatic hydrolysis of the above saponin mixture has been undertaken. In addition to accomplishing this objective, the enzymatic hydrolysis has led to the isolation of another genuine sapogenol which is a new bisnor-triterpenoid and is now designated as spergulatriol. This paper deals with evidence which is consistent with the structure 2 for spergulatriol.

Hydrolysis of the total saponin mixture with naringinase[4)] furnished spergulagenin A (1), oleanolic acid (3), methyl spergulagenate (4),[5)] and spergulatriol (2), while acid hydrolysis (10% H_2SO_4-MeOH= 1:1, under reflux) of the same saponin mixture gave 1, 3, and 4. 2 was not detected in the acid hydrolysate but instead a dienic compound (5, now named isoanhydrospergulatriol, *vide infra*) was obtained.

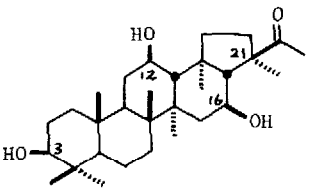
Spergulatriol (2), $C_{28}H_{46}O_3$[6)], mp 224-226°, $[\alpha]_D^{18}$ +60.9° ($CHCl_3$), possesses three hydroxyls [IR (KBr): 3370 cm^{-1}; PMR (d_5-pyridine-D_2O, δ): 3.38 (1H, t-like), 4.0-4.4 (2H, m)(>CH-OH x 3)], a terminal methylene [IR: 1665, 882 cm^{-1}; PMR (δ): 5.02, 5.86 (1H each, br.s)] and six tertiary methyls [PMR (δ): 0.80 (3H), 0.96 (3H), 1.00 (3H), 1.06 (6H), 1.15 (3H)(all s)]. A characteristic PMR signal pattern observed at δ3.38 due to a carbinyl proton is suggestive that one of the three hydroxyls is 3β-OH.[2)] The mass spectrum of spergulatriol (2) shows a base peak of $C_{14}H_{23}O$[7)] (m/e 207, i) and prominent fragment ions of $C_{14}H_{21}$ (m/e 189, i-H_2O, 47%) and $C_{14}H_{20}O$ (m/e 204, ii, 47%).

Acetylation of 2 gave a triacetate (2a), $C_{34}H_{52}O_6$, mp 243-244°, $[\alpha]_D^{16}$ +64.4° ($CHCl_3$); IR (KBr, cm^{-1}):1737, 1249 (OAc), 1665, 884 ($>C=CH_2$). The PMR spectrum of 2a shows signals due to three acetoxyls [δ2.02 (6H, s), 2.04 (3H, s)], a terminal methylene [δ4.78 (2H, br. s)], and three methine protons which are geminal to acetoxyls [δ4.47 (1H, t-like, 3α-H), 4.9-5.3 (2H,m)]

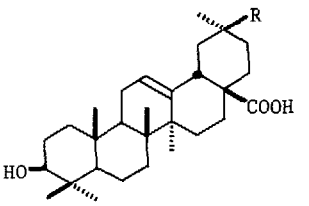
Comparison of the above described physical data with those[2] of the coexisting spergulagenin A (1) and its acetyl derivatives has led us to assign the structure 2 to spergulatriol, and the deshielded signal position of one of the terminal methylene protons (δ5.86) is probably attributable to the anisotropic effect of the nearby 16β-OH. This assumption has been verified by the following conversion of 1 to 2. Thus, irradiation of 1 in dry dioxane with a high pressure mercury lamp (500 W) followed by chromatographic separation (SiO_2 and SiO_2-$AgNO_3$) and acetylation (Ac_2O-pyridine) furnished two products (8% and 9% yields), of which one (8%) has been found to be identical with spergulatriol triacetate (2a) in all respects (mixed mp, IR, $[\alpha]_D$, mass, and TLC). The derivation along with the mechanistic considerations (Norrish Type I cleavage between C-21 and 21-$COCH_3$ in 1) has assured the correctness of the structure 2 (including the 17β-H configuration) for spergulatriol.

Since the other irradiation product was suspected to be a mixture (ca. 7:2) of two triacetates $C_{34}H_{54}O_6$ (6) and $C_{34}H_{52}O_6$ (7) on the basis of the mass spectrum and mechanistic considerations and since the separation of both could not be effected at this stage, the product was subjected to 5% HCl-EtOH treatment under reflux and reacetylation[8] (to convert only 7 to 5a)[9] and the resulting mixture was separated by preparative TLC (SiO_2) to furnish an unaffected saturated triacetate (6)(PMR: no olefinic proton) and a newly formed dienic diacetate (5a). The former, $C_{34}H_{54}O_6$ (high mass), mp 235.5-237°; IR (KBr, cm^{-1}): 1732 (OAc); PMR ($CDCl_3$, δ): 0.85 (12H, s), 1.03 (3H, s), 1.09 (3H, s), 0.88 (3H, d, J= ca. 6 Hz)(totally seven methyls), 2.01 (9H, s)(AcO x 3), 4.46 (1H, t-like, 3α-H), 4.9-5.3 (2H, m, 12α-H, 16α-H), has been assigned 6 (the configuration at C-21 being undefined), and the latter has been found to be identical with the diacetate (5a) of isoanhydrospergulatriol (5) which, as mentioned above, was obtained as a minor sapogenol by acid hydrolysis of the total saponin. Therefore, the minor triacetate of $C_{34}H_{52}O_6$ is assumed to be 7 although its isolation could not be achieved.

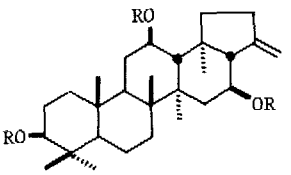
Isoanhydrospergulatriol (5), $C_{28}H_{44}O_2$ (high mass)(amorphous) possesses a heteroannular diene chromophore as shown by IR (KBr, cm^{-1}): 1657, 787, 779; UV (EtOH, nm, ε): 244 (17500), 252 (19700), 261 (14200),[9] and PMR ($CDCl_3$, δ): 5.53, 6.17 (1H each, ABq, J= 9.8 Hz). It also possesses two hydroxyls [IR: 3365 cm^{-1}; δ: 3.20 (1H, t-like, 3α-H), 3.95 (1H, m, $W_{h/2}$= 14



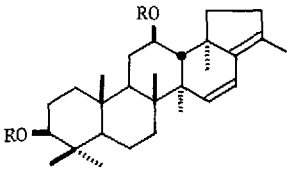
1 spergulagenin A



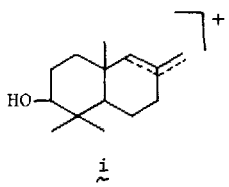
3 R= CH_3; 4 R= $COOCH_3$



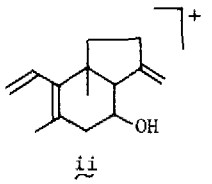
2 R= H spergulatriol
2a R= Ac



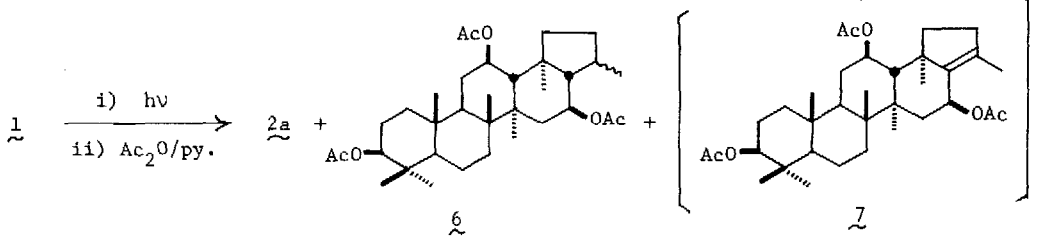
5 R= H isoanhydrospergulatriol
5a R= Ac



i



ii



6 7

Hz, 12α-H)],six tertiary methyls [δ0.78, 0.83, 0.92, 0.99, 1.05, 1.14 (3H each, all s)], and one vinyl methyl (δ1.73, 3H, br.s). The mass spectrum of 5 shows fragment ion peaks at m/e 207 (i, 11%),[7] 189 ($C_{14}H_{21}$, i-H_2O, 12%), and 171 ($C_{13}H_{15}$, ii-H_2O-CH_3, 18%) along with a base peak at m/e 159.

Ordinary acetylation of 5 gave the diacetate 5a, $C_{32}H_{48}O_4$, mp 215-216°, $[\alpha]_D^{18}$ +97.4° ($CHCl_3$); IR (KBr, cm^{-1}): 1725 (OAc), 1656, 785, 779 (diene); PMR ($CDCl_3$, δ): 0.84 (6H), 0.86 (3H), 0.93 (6H), 1.19 (3H)(all s, tert. CH_3 x 6), 1.71 (3H, br.s, vinyl CH_3), 2.02 (6H, s, AcO x 2), 4.48

(1H, t-like, 3α-H), 5.27 (1H, m, 12α-H), 5.55, 6.17 (1H each, ABq, J= 11 Hz, 15,16-H_2); UV (EtOH, nm, ε): 244 (19100), 252 (21600), 261 (15300). The diacetate prepared here has been identified as the one obtained by acid treatment of 7 as described above.

Finally, a direct correlation of isoanhydrospergulatriol (5) with spergulatriol (2) has been made. Treatment of spergulatriol triacetate (2a) with 5% HCl-EtOH under reflux followed by reacetylation[8] furnished a diacetate which was identical to 5a by mixed mp, IR (KBr), and TLC. Thus, the structure of isoanhydrospergulatriol is assigned as 5.

Since isoanhydrospergulatriol (5) was obtained only by acid hydrolysis of the total saponin mixture and was not detected in the enzymatic hydrolysate and since 5a has been readily prepared from spergulatriol triacetate (2a) by acid treatment, 5 has been concluded to be an artifact sapogenol secondarily formed from 2. Spergulatriol (2) seems to be the first naturally occurring bisnor-triterpenoid of the hopane-analog.

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References and Footnotes

1) Present address: Tsumura Laboratory, 1421 Izumi, Komae-shi, Tokyo, Japan. 2) a) I. Kitagawa, H. Suzuki, I. Yosioka, T. Akiyama, and J. V. Silverton, Tetrahedron Letters, 1974, 1173; b) I. Kitagawa, H. Suzuki, K. Kitazawa, N. Yamao, and I. Yosioka, Chem. Pharm. Bull.(Tokyo), 23, 355 (1975); c) I. Kitagawa, H. Suzuki, and I. Yosioka, Chem. Pharm. Bull.(Tokyo), 23, 2087 (1975). 3) a) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, Tetrahedron Letters, 1966, 6303; b) I. Yosioka, K. Imai, Y. Morii, and I. Kitagawa, Tetrahedron, 30, 2283 (1974), and the preceding papers of the series. 4) Crude naringinase kindly supplied by Dr. M. Suzuki of Tanabe Pharm. Co. (Lot no. 1-22). 5) a) P. Chakrabarti, D. K. Mukherjee, A. K. Barua, and B. C. Das, Tetrahedron, 24, 1107 (1968); b) I. Kitagawa, K. Kitazawa, K. Aoyama, M. Asanuma, and I. Yosioka, Tetrahedron, 28, 923 (1972). 6) The elemental compositions of the compounds given with the chemical formulae were confirmed by high resolution mass spectrometry. 7) The elemental compositions of the ions given with the chemical formulae were confirmed by high resolution mass spectrometry. 8) The TLC examinations of the initial reaction products disclosed that the acetyl group was partly removed during the acid treatment. 9) a) I. Yosioka, T. Nakanishi, and I. Kitagawa, Chem. Pharm. Bull.(Tokyo), 17, 279 (1969); b) I. Yosioka, T. Nakanishi, M. Yamaki, and I. Kitagawa, Chem. Pharm. Bull.(Tokyo), 20, 487 (1972).