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Saponin and Sapogenol. XXII.[1] Structure of Spergulatriol, A New Bisnorhopane-type Genuine Sapogenol of Isoanhydrospergulatriol, from the Root of *Mollugo spergula* L.

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By using the soil bacterial hydrolysis method and by enzymatic hydrolysis, spergulagenin A (**1**), which was previously obtained along with two oleanene-type sapogenols (oleanolic acid (**2**) and methyl spergulagenate (**3**)) by acid hydrolysis of the total saponin mixture extracted from the root of *Mollugo spergula* L. (Molluginaceae), has been shown to be a genuine sapogenol. In addition, enzymatic hydrolysis using crude naringinase of the same saponin mixture has led to the isolation of a new bisnorhopane-type sapogenol named spergulatriol (**4**). Based on the chemical and spectral evidence, the structure of spergulatriol has been elucidated to be **4** and it has been demonstrated that spergulatriol (**4**) is the genuine sapogenol of isoanhydrospergulatriol (**5**), which is a dienic bisnorhopane-type sapogenol and is isolated as a minor sapogenol from the acid hydrolysate of total saponin mixture.

Keywords——*Mollugo spergula* L.; spergulatriol; isoanhydrospergulatriol; soil bacterial hydrolysis; enzymatic hydrolysis; crude naringinase; Norrish type I photolysis

Recently, we have established the structure of spergulagenin A (**1**)[3] which was obtained as the second major sapogenol by acid hydrolysis of the saponin mixture extracted from the root of *Mollugo spergula* L. (Molluginaceae). Spergulagenin A (**1**) was isolated along with two oleanene-type sapogenols, oleanolic acid (**2**) and methyl spergulagenate (**3**)(major), and was characterized by having a new migrated hopane-type carbon skeleton, *i.e.* a methyl at C–22 in hopane-skeleton being migrated to C–21α.

In order to ascertain the genuineness of spergulagenin A (**1**), we have examined the soil bacterial hydrolysis[4] and enzymatic hydrolysis of the above saponin mixture. As shown in our preliminary report,[5] spergulagenin A (**1**) has been demonstrated to be one of genuine sapogenols of the root and in addition, the enzymatic hydrolysis has led us to isolate a new bisnorhopane-type sapogenol named spergulatriol (**4**) which is also a genuine sapogenol and is readily converted to an artifact sapogenol named isoanhydrospergulatriol (**5**) during the acid hydrolysis of saponin mixture. The present paper provides the full account on the structure elucidation of spergulatriol (**4**) and isoanhydrospergulatriol (**5**).

Application of the soil bacterial hydrolysis method[4] to the total saponin mixture followed by chromatographic separation of the hydrolysate resulted in the isolation of spergulagenin A (**1**), oleanolic acid (**2**), and methyl spergulagenate (**3**),[6] thus the genuineness of these

1) Part XXI: I. Kitagawa, K.S. Im, and Y. Fujimoto, *Chem. Pharm. Bull.* (Tokyo), **25**, 800 (1977).
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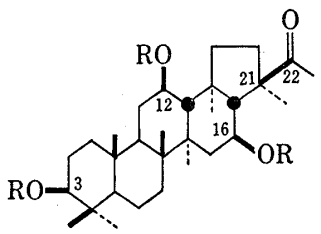
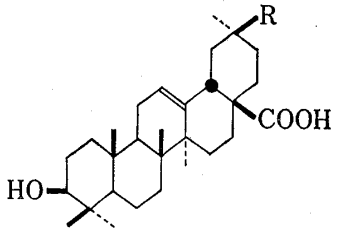
4) *a*) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Lett.*, **1966**, 6303; *b*) I. Kitagawa, T. Nakanishi, Y. Morii, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2343 (1977), and the preceding papers of the series.
5) I. Kitagawa, H. Yamanaka, T. Nakanishi, and I. Yosioka, *Tetrahedron Lett.*, **1976**, 2327.
6) I. Kitagawa, K. Kitazawa, K. Aoyama, M. Asanuma, and I. Yosioka, *Tetrahedron*, **28**, 923 (1972).

sapogenols being verified respectively. However, as described in the experimental section, the soil bacterial hydrolysis in the present case was inefficient. Although we have made every effort for finding a suitable strain of microorganism from many soil samples, only a strain being able to insufficiently hydrolyze the saponin could be selected. Therefore, we have altered our approach for hydrolysis of the saponin mixture to use of various kinds of enzymes, since the increasing number of successful examples for enzymatic hydrolysis of saponins have been reported in these years,[7] and crude naringinase[7d] has been found to be suitable for the present purpose.

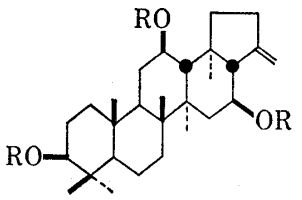
Hydrolysis of the saponin mixture using crude naringinase[8] followed by chromatographic separation furnished spergulagenin A (**1**), oleanolic acid (**2**), methyl spergulagenate (**3**), and a hitherto unknown sapogenol named spergulatriol (**4**). Here again, free spergulagenic acid (**6**) was not detected in the total hydrolysate as experienced in the above soil bacterial hydrolysis and it has become evident that the acid is contained in saponin as its monomethyl ester (**3**).[6] Furthermore, **2**, **3**, and **4** have been also ascertained to be genuine sapogenols of the root.



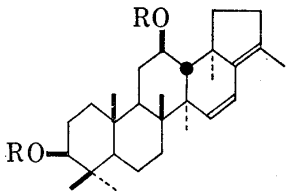
1 : R=H spergulagenin A
1a : R=Ac



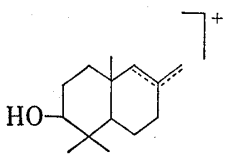
2 : R=CH_3 oleanolic acid
3 : R=$COOCH_3$ methyl spergulagenate
6 : R=COOH spergulagenic acid



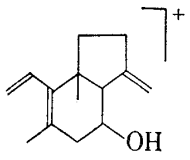
4 : R=H spergulatriol
4a : R=Ac



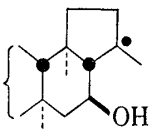
5 : R=H isoanhydrospergulatriol
5a : R=Ac



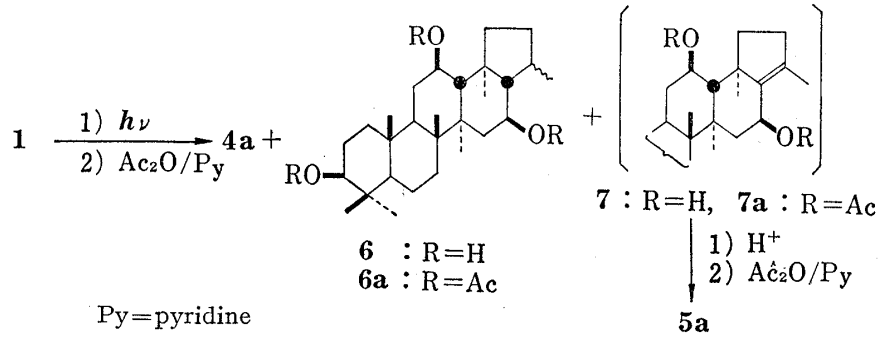
i *m/e* 207
$-H_2O$
ii *m/e* 189



iii *m/e* 204
$-H_2O$, $-CH_3$
v *m/e* 171



iv



Py=pyridine

Chart 1

7) *a*) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975); *b*) I. Kitagawa, A. Inada, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 2268 (1975); *c*) I. Kitagawa, M. Kobayashi, T. Sugawara, and I. Yosioka, *Tetrahedron Lett.*, **1975**, 967; *d*) I. Kitagawa, T. Sugawara, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **24**, 275 (1976).

8) A generous gift of Drs. T. Okuda and Y. Egawa of Tanabe Pharm. Co., to whom the authors deepest thanks are due.

On the other hand, for the purpose of comparison, the same saponin mixture was subjected to acid hydrolysis using a 10% sulfuric acid–methanol (1:1) mixture. After chromatographic separation, liberated sapogenols were elucidated to comprise spergulagenin A (**1**), oleanolic acid (**2**), methyl spergulagenate (**3**), and a new dienic sapogenol isoanhydrospergulatriol (**5**). Spergulatriol (**4**) obtained above by enzymatic hydrolysis was not found in the total acid hydrolysate, and as mentioned below, **4** has been shown to be the genuine sapogenol of **5**.

High resolution mass spectrometry has shown that spergulatriol (**4**) is a bisnor-triterpenoid having a molecular composition $C_{28}H_{46}O_3$. The infrared (IR) (KBr) and proton magnetic resonance (PMR) (in pentadeutero (d_5)-pyridine–deuterium oxide (D_2O)) spectra of **4** show the presence of three secondary hydroxyls (3370 (br) cm^{-1}; three carbinyl protons at δ 3.38 (1H, t-like) and δ 4.0—4.4 (2H, m)), a terminal methylene (1665, 882 cm^{-1}; 1H each, br.s at δ 5.02 and 5.86),[9] and six tertiary methyls. Among three carbinyl proton signals, a triplet-like signal observed at δ 3.38 is assignable to 3α-H of a triterpenoid,[3b] so that the location of one secondary hydroxyl is presumed to be C-3β.

Comparison of the PMR spectrum of spergulatriol (**4**) with that of spergulagenin A (**1**)[3] has revealed that signal patterns of both spectra including three carbinyl proton signals are quite resembled each other (Table I). Distinguished difference is that signals due to a methylcarbonyl (δ 2.31) and 21α-methyl (δ 1.61) observed in the spectrum of **1** are lost in that of **4** while signals due to a terminal methylene (δ 5.02, 5.86) are newly observed in the latter. In the mass spectrum of **4**, is observed a base peak of $C_{14}H_{23}O$ (*m/e* 207), which is presumably expressed as **i**, along with fragment ion peaks at *m/e* 189 (**ii**, 47%) and at *m/e* 204 (**iii**, 47%) which are respectively derivable by dehydration of **i** and through retro-Diels-Alder type fragmentation at ring C.

On acetylation of spergulatriol (**4**) with acetic anhydride and pyridine, was obtained a triacetate (**4a**), the IR (KBr) and PMR (deuterochloroform) spectra of which give support for its structure by absorption bands at 1737, 1249 cm^{-1}, by signals due to three acetoxyls at δ 2.02 (6H, s) and 2.04 (3H, s), and three methine protons at δ 4.47 (1H, t-like, 3α-H) and δ 4.9—5.3 (2H, m), and by absence of free hydroxyl in **4a**. The IR and PMR spectra of **4a** also exhibit the presence of a terminal methylene (1665, 884 cm^{-1}; δ 4.78 (2H, br,s)) and six tertiary methyls, and both similarity and difference in the PMR spectra of **4a** and spergulagenin A triacetate (**1a**)[3] are as observed between **4** and **1** (Table I).

Table I. Comparison of PMR Data of **1**, **1a**, **4**, and **4a**[a]

	3α-H	12α-H, 16α-H	*tert.* CH_3	Others
1[b,3b]	3.42 (t-like)	3.8—4.4[c] (2H, m)	0.87, 0.99, 1.04, 1.09 (3H each), 1.19 (6H), 1.61 (3H) (all s)	2.31 (3H, s, CH_3CO)
1a[d,3b]	4.45 (t-like)	4.7—5.3[e] (2H, m)	0.84 (9H), 0.99, 1.05, 1.10, 1.16 (3H each) (all s)	2.16 (3H, s, CH_3CO) 1.89, 1.99, 2.02 (3H each, all s, AcO×3)
4[f]	3.38 (t-like)	4.0—4.4[c] (2H, m)	0.80, 0.96, 1.00 (3H each), 1.06 (6H), 1.15 (3H) (all s)	5.02, 5.86 (1H each, br.s, >C=CH_2)
4a[d]	4.47 (t-like)	4.9—5.3[e] (2H, m)	0.72 (3H), 0.85 (9H), 1.04, 1.10 (3H each) (all s)	4.78 (2H, br.s, >C=CH_2) 2.02 (6H), 2.04 (3H) (both s, AcO×3)

a) Abbreviations: br. s=broad singlet, m=multiplet, s=singlet, t=triplet.
b) In d_5-pyridine.
c), e) The signal patterns are almost superimposable respectively.
d) In $CDCl_3$.
f) In d_5-pyridine-D_2O.

9) The deshielded chemical shift of one of methylene protons is presumably ascribable to an anisotropic effect of 16β-OH (*vide infra*).

Based on the accumulated spectral evidence of spergulatriol and its triacetate and based on the co-occurrence of spergulatriol with spergulagenin A (**1**), the structure **4** (the configuration at C–17 being still uncertain) has been proposed for spergulatriol. A deshielded signal (δ 5.86) due to one of terminal methylene protons in the PMR spectrum of spergulatriol (**4**) (*vide supra*) is now ascribable to an anisotropic effect of 16β-hydroxyl in the vicinity, since this deshielding is not observed in the case of triacetate (**4a**) (Table I). Next, the presumption for the structure **4** has been verified by direct conversion of spergulagenin A (**1**) to spergulatriol as described below.

Thus, irradiation of spergulagenin A (**1**) in dry dioxane using a 500 W high pressure mercury lamp[10] afforded a reaction mixture, which ,through repeated chromatographic purification using silica gel and silver nitrate impregnated silica gel and acetylation, was purified to give two products in 8% and 9% yields, respectively. The one obtained in 8% yield was found to be identical to spergulatriol triacetate (**4a**) in all respects (mixed mp, IR (KBr), $[\alpha]_D$, mass spectra(MS), and thin-layer chromatography (TLC)). Therefore, based on mechanistic considerations for the Norrish Type I cleavage[10] of the 21,22-bond in **1** (*via* a probable intermediate having a partial structure **iv**), the bisnorhopane-type structure **4** including the 17β-H configuration has been substantiated for spergulatriol.

The other product obtained in 9% yield was shown by mass spectrometry to be a mixture (7: 2) of two triacetates of $C_{34}H_{54}O_6$ (*m/e* 558, saturated) and $C_{34}H_{52}O_6$ (*m/e* 556, unsaturated), whose structures have been respectively assumed to be **6a** and **7a** on the basis of mechanistic considerations for the present photolysis (intermediate **iv**). The absence of signal due to any olefinic proton in the PMR spectrum of this mixture has suggested that the unsaturated triacetate would possess a tetrasubstituted double bond as shown in **7a** which presumably results from **iv** by loss of hydrogen. Due to difficulty in direct separation of **6a** and **7a**, acid treatment of the mixture was carried out for converting only **7a** to a dienic compound (**5a**) so that the separation could be readily effected. Thus, on treatment with 5% ethanolic hydrogen chloride under reflux followed by reacetylation (due to partial deacetylation during the acid treatment) and chromatographic separation, two compounds (**5a** and **6a**) were isolated.

The saturated triacetate, which suffered only partial deacetylation on acid treatment, possesses three secondary acetoxyls (IR (KBr): 1732 cm^{-1}; PMR ($CDCl_3$): δ 2.01 (9H, s), δ 4.46 (1H, t-like, 3α-H), δ 4.9—5.3 (2H, m, 12α-H, 16α-H)), six tertiary methyls (PMR), and one secondary methyl (δ 0.88, 3H, d, J=*ca.* 6 Hz), but it does not possess free hydroxyl and olefinic proton, thus the structure **6a**, which presumably results from **iv** through hydrogen abstraction from solvent, being suggested. However, the configuration at C–21 has not yet been defined. On the other hand, the unsaturated product was found to be identical to isoanhydrospergulatriol diacetate (**5a**) which is described below, thus the corresponding initial photolysis product from **1** being presumed to be **7**.

As mentioned above, direct acid hydrolysis of the total saponin mixture extracted from the root liberated a new dienic bisnor-triterpenoid ($C_{28}H_{44}O_2$: high mass) named isoanhydrospergulatriol whose structure has now been determined to be **5** on the following basis. Thus, the ultraviolet (UV) spectrum of isoanhydrospergulatriol (**5**) reveals the presence of a heteroannular diene chromophore by a characteristic triplet-like maxima at 244, 252, and 261 nm (ε=17500, 19700, and 14200).[11] This dienic system is also shown up by the IR (KBr: 1657, 787, 779 cm^{-1}) and PMR ($CDCl_3$: δ 5.53, 6.17, 1H each, AB q, J= 9.8 Hz) spectra of **5**, and has been presumably assigned as $\Delta^{15,17(21)}$ which is contained in dienic derivatives of hopane-type lichen triterpenoids: leucotylin and leucotylic acid.[11] The IR and PMR spectra of **5**

10) B.J. Clarke, J.L. Courtney, and W. Stern, *Aust. J. Chem.*, **23**, 1651 (1970).
11) *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, *ibid.*, **20**, 487 (1972).

also show the presence of two secondary hydroxyls (IR: 3365 cm^{-1}; PMR: δ 3.20 (1H, t-like, 3α-H) and δ 3.95 (1H, m, 12α-H), six tertiary methyls, and one olefinic methyl (δ 1.73, 3H, br.s). In the mass spectrum of **5**, as experienced in the case of spergulatriol (**4**), are observed three fragment ion peaks: **i** (11%), **ii** (12%)(both derivable from rings A and B), and **v** (18%) (from rings C, D, and E).

On ordinary acetylation, isoanhydrospergulatriol (**5**) was converted to a diacetate (**5a**), which also holds a heteroannular diene chromophore as suggested by its spectral data (UV, IR, and PMR). The PMR spectrum of **5a** shows the presence of six tertiary methyls, one olefinic methyl (δ 1.71, 3H, br.s), and two secondary acetoxyls. Finally, the diacetate obtained here was found to be identical (by mixed mp, UV, and TLC) to one of the photochemical products (**5a**) which was prepared above from spergulagenin A (**1**) through **7a**. Furthermore, the diacetate was directly prepared from spergulatriol triacetate (**4a**) by treatment with 5% ethanolic hydrogen chloride under reflux and acetylation, thus the structure of isoanhydrospergulatriol being established as **5**.

Since isoanhydrospergulatriol (**5**) has been obtained as a minor sapogenol only by acid hydrolysis of the total saponin mixture and has not been found in the total hydrolysate which was obtained by soil bacterial and enzymatic hydrolysis of the same saponin mixture as described above, and furthermore, since **5** has been readily formed by acid treatment from spergulatriol (**4**) which was obtained by enzymatic hydrolysis of the total saponin mixture, isoanhydrospergulatriol (**5**) has been concluded to be an artifact sapogenol originated from spergulatriol (**4**).

Spergulatriol (**4**) seems to be the first example of naturally occurring bisnorhopane-type sapogenol.

Experimental[12]

Isolation of Saponin Mixture——The methanolic extractive (656 g) obtained from the air-dried root (cut, 9 kg) of the titled plant was partitioned into *n*-BuOH–water mixture and the *n*-BuOH soluble portion (300 g) was treated with MeOH–ether as usual to give a crude saponin mixture (100 g). A solution of the crude saponin mixture (50 g) in MeOH was passed through a column of active charcoal (125 g, Seisei-shirasagi, Takeda Chem. Ind.) and Celite 535 (125 g, Wako Pure Chem.) and successive elution with MeOH furnished a saponin mixture (24 g).

Soil Bacterial Hydrolysis of Saponin Mixture——A selected strain of microorganism (YSB-27, unidentified yet) was cultivated at 31° for 30 days as reported previously[4] in a synthetic medium containing the saponin mixture isolated above as a sole carbon source. Successive extraction of the total culture with ether and *n*-BuOH gave an oily yellow extractive (4.6 g) and recovered saponin mixture (1.54 g), respectively. The ether extractive (4.5 g) was subjected to column chromatography (silica gel, 100 g, 2×70 cm) eluting with $CHCl_3$–acetone (50: 7) to afford a fraction (103 mg) containing oleanolic acid (**2**) and methyl spergulagenate (**3**) and a fraction (210 mg) of spergulagenin A (**1**). Successive elution of the column with MeOH recovered a saponin mixture (1.52 g). Purification of each fraction by preparative TLC (Kieselgel G, $CHCl_3$–acetone=3: 1) furnished pure samples of oleanolic acid (**2**, 3 mg), methyl spergulagenate (**3**, 6 mg), and spergulagenin A (**1**, 60 mg). **2** and **3** were respectively identified with authentic samples by TLC ($CHCl_3$–acetone=3: 1, benzene–acetone=2: 1, ether–acetone=3: 1) and **1**, after recrystallization from MeOH, was identified by mixed mp, IR (KBr), $[\alpha]_D$, and TLC. Detailed TLC examinations showed the absence of spergulagenic acid (**6**) in the total hydrolysate.

Hydrolysis of Saponin Mixture with Crude Naringinase——A suspension of the saponin mixture (2.0 g) in acetic acid–sodium acetate buffer (pH 5.0, 500 ml) was treated with crude naringinase (Lot No. 1—22, 2.0 g)[8]

12) The following instruments were used for obtaining the physical data: mp (Yanagimoto Micro-melting-point Apparatus, recorded uncorrected); Specific rotations (Rex Photoelectric Polarimeter NEP-2 (1=one dm); IR spectra (Hitachi IR Spectrometer EPI-G3); MS (Hitachi RMU-6D Mass Spectrometer, at 75 eV, direct inlet); High resolution MS (JEOL JMS-01SG Mass Spectrometer); PMR spectra (Hitachi R-22 (90 MHz) or R-20A (60 MHz) NMR Spectrometer, tetramethylsilane as an internal standard); UV spectra (Shimadzu MPS-50L Spectrophotometer). Silica gel (Merck Kieselgel 60, 0.05—0.2 mm) was used for column chromatography, and silica gel (Camag D-5 or Merck Kieselgel G) was used for TLC and detection by 1% $Ce(SO_4)_2$–10% H_2SO_4 with heating. For preparative TLC, detection was made by UV (for Kieselgel PF_{254}) and by spraying water or by I_2 vapor (for silica gel Camag D-5 or Merck Kieselgel G).

and was kept stirring at 37—40° for 6 days. After treatment with small amount of EtOH and heating, the total mixture was treated with *n*-BuOH (100 ml), heated for a while with stirring, and filtered with an aid of Celite 535. The filtrate (*n*-BuOH–water mixture) was added with additional amount of *n*-BuOH, shaken well, and *n*-BuOH layer was separated. The Celite 535 was washed with *n*-BuOH. The combined *n*-BuOH layer and washings were evaporated under reduced pressure to give a product (1.65 g) which was submitted to column chromatography (silica gel, 160 g, 3×50 cm) eluting with $CHCl_3$-acetone (20: 3) and to preparative TLC (Kieselgel G) developing with $CHCl_3$–acetone (3: 1) to afford pure samples of oleanolic acid (**2**, 8 mg), methyl spergulagenate (**3**, 10 mg), spergulagenin A (**1**, 85 mg), and spergulatriol (**4**, 8 mg). Identification with respective authentic samples were made by mixed mp, IR (KBr), and $[\alpha]_D$ for **1**, and by TLC (solvents as above) for **2** and **3**. Detailed TLC examinations showed also the absence of spergulagenic acid (**6**) in the total hydrolysate.

Recrystallization from MeOH–acetone gave an analytical sample of spergulatriol (**4**) as colorless needles of mp 224—226°, $[\alpha]_D^{16}$ +60.9° (c=0.29, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1665, 882 (>C=CH_2). PMR (90 MHz): as given Table I. MS *m/e* (%): 430 (M^+, 2), 412 (M^+-H_2O, 8), 375 (22), 207 (**i**, 100), 204 (**iii**, 47), 189 (**ii**, 47). High resolution MS: Found 430.345, 412.336, 207.174, 204.153, 189.164; Calcd. for $C_{28}H_{46}O_3$ (M^+) 430.345, $C_{28}H_{44}O_2$ 412.334, $C_{14}H_{23}O$ (**i**) 207.175, $C_{14}H_{20}O$ (**iii**) 204.151, $C_{14}H_{21}$ (**ii**) 189.164.

Acetylation of Spergulatriol (4) giving Triacetate (4a)——A solution of **4** (24 mg) in acetic anhydride (0.3 ml) and pyridine (1.0 ml) was left standing at room temperature overnight and poured into ice–water. The precipitated product was collected by filtration and worked up in the usual manner to give triacetate (**4a**, 30 mg), which was crystallized from MeOH. Colorless needles of **4a**, mp 243—244°, $[\alpha]_D^{16}$ +64.4° (c=0.76, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1737, 1249 (OAc), 1665, 884 (>C=CH_2). PMR (90 MHz): as given in Table I. MS *m/e* (%): 556 (M^+, <1), 496 (M^+—AcOH, 26), 436 (M^+—2AcOH, 44), 376 (M^+—3AcOH, 6), 249 (8), 189 (**ii**, 36), 186 (47), 159 (100). High resolution MS: Found 556.376; Calcd. for $C_{34}H_{52}O_6$ (M^+) 556.376.

Photolysis of 1 followed by Acetylation and Acid Treatment giving 4a, 6a, and 5a——A solution of spergulagenin A (**1**, 300 mg) in dry dioxane (120 ml) was bubbled with a stream of dry nitrogen for one hour, put in a Pyrex tube (7 ml in each tube), and irradiated under cooling (water bath temp.: 14—24°) externally (distance: 2 cm) with a 500 W high pressure mercury lamp (Eikosha PIH-500). The product (330 mg) obtained by evaporation of the solvent under reduced pressure was submitted to column chromatography (silica gel, 15 g, 1×27 cm) eluting with benzene–acetone (20: 3) to afford fraction I (120 mg) and II (111 mg). Preparative TLC purification of fraction II recovered spergulagenin A (**1**, 93 mg). Column chromatography of fraction I with 10% silver nitrate impregnated silica gel (8.4 g, 1.5×10 cm) and eluting with benzene–acetone (20: 3) furnished a mixture of **6** and **7** (94 mg) and spergulatriol (**4**, 22 mg). Spergulatriol (19 mg) thus obtained was acetylated with acetic anhydride (0.3 ml) and pyridine (1 ml) at room temperature overnight and resulting triacetate (21 mg) of mp 242—243° (recryst. from MeOH) was identified with authentic spergulatriol triacetate (**4a**) by mixed mp, IR (KBr), $[\alpha]_D$, MS, and TLC (using three different solvent systems).

The mixture of **6** and **7** was further purified by preparative TLC (Camag D-5, benzene–acetone=3: 1, giving 70 mg of a mixture), acetylated with acetic anhydride (1 ml) and pyridine (3 ml) as above, and purified by preparative TLC again (Camag D-5, $CHCl_3$–acetone=40: 1, developing twice) to furnish a mixture of **6a** and **7a** (27 mg). A solution of the mixture (24 mg) in 5% HCl–EtOH (10 ml) was refluxed for 30 min, neutralized with $NaHCO_3$, diluted with water, and concentrated under reduced pressure to remove EtOH. The product was then collected by filtration, washed with water, dried *in vacuo* (giving 18 mg of the product), acetylated with acetic anhydride (0.5 ml) and pyridine (1.5 ml) as above, and submitted to preparative TLC (Kieselgel PF_{254}, 0.25 mm, $CHCl_3$–acetone=30: 1) to furnish **6a** (15 mg) and **5a** (4 mg). Recrystallization from MeOH yielded an analytical sample of **6a** as colorless needles of mp 235.5—237°. IR ν_{max}^{KBr} cm^{-1}: 1732 (OAc). PMR (90 MHz, $CDCl_3$) δ: 0.85 (12H, s), 1.03 (3H, s), 1.09 (3H, s) (*tert.* CH_3×6), 0.88 (3H, d, J=*ca.* 6 Hz, *sec.* CH_3), 2.01 (9H, s, OAc×3), 4.46 (1H, t-like, 3α-H), 4.9—5.3 (2H, m, 12α-H, 16α-H). High resolution MS: Found 558.392; Calcd. for $C_{34}H_{54}O_6$ (M^+) 558.392. **5a** was identified with isoanhydrospergulatriol diacetate prepared below.

The ratio of **6a** and **7a** in the above described initial mixture was examined by the following method. Thus, the mixture was dissolved in 5% HCl–EtOH, refluxed for 15 min, diluted with EtOH, and subjected to UV determination (measuring ε values at 252 and 261 nm). On the other hand, the standard was prepared by treating **5a** with 5% HCl–EtOH as a blank test. Comparison of ε values of both showed the acid treated product of the mixture contained *ca.* 25% of **5a** (*i.e.* **7a** in the initial mixture).

Isolation of Isoanhydrospergulatriol (5) from Acid Hydrolysate of Saponin——A mixture of crude saponin mixture (45 g) in 10% H_2SO_4 (500 ml) and MeOH (500 ml) was heated under reflux for 7 hr and diluted with water. The precipitate was collected by filtration, washed with water, and dried *in vacuo* to give a sapogenol mixture (20 g). Column chromatography (silica gel, 1 kg) of the mixture (17 g) eluting with benzene–EtOAc (5: 1) afforded a fraction containing oleanolic acid (**2**) and isoanhydrospergulatriol (**5**). The fraction was then treated with ethereal diazomethane and submitted to column chromatography again eluting with benzene–EtOAc (20: 1) to furnish pure isoanhydrospergulatriol (**5**, 210 mg) and crude **5** (140 mg). Crystallization of **5** was unsuccessful and further purified by precipitation with MeOH. UV λ_{max}^{EtOH} nm (ε): 244 (17500), 252 (19700), 261 (14200). IR ν_{max}^{KBr} cm^{-1}: 3365 (OH), 1657, 787, 779 (diene). PMR (60 MHz, $CDCl_3$) δ: 0.78, 0.83, 0.92, 0.99, 1.05, 1.14 (3H each, all s, *tert.* CH_3×6), 1.73 (3H, br.s, olefinic CH_3), 3.20 (1H, t-like, 3α-H),

3.95 (1H, m, $W_{h/2}$=14 Hz, 12α-H), 5.53, 6.17 (2H, ABq, J=9.8 Hz, 15-H, 16-H). MS *m/e* (%): 412 (M^+, 8), 397 (M^+-CH_3, 14), 379 ($M^+-CH_3-H_2O$, 4), 361 ($M^+-CH_3-2H_2O$, 3), 207 (**i**, 11), 189 (**ii**, 12), 171 (**v**, 18). High resolution MS: Found 412.335; Calcd. for $C_{28}H_{44}O_2$ (M^+) 412.334.

Acetylation of **5** in the usual manner gave diacetate (**5a**), colorless needles of mp 215—216° (recryst. from MeOH), $[\alpha]_D^{20}$ +97.4° (c=0.61, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 244 (19100), 252 (21600), 261 (15300). IR ν_{max}^{KBr} cm^{-1}: 1725 (OAc), 1656, 785, 779 (diene). PMR (90 MHz, $CDCl_3$) δ: 0.84 (6H), 0.86 (3H), 0.93 (6H), 1.19 (3H) (all s, *tert.* $CH_3\times6$), 1.71 (3H, br.s, olefinic CH_3), 2.02 (6H, s, OAc×2), 4.48 (1H, t-like, 3α-H), 5.27 (1H, m, 12α-H), 5.55, 6.17 (2H, ABq, J=11 Hz, 15-H, 16-H). MS *m/e* (%): 496 (M^+, 1), 481 (M^+-CH_3, 3), 436 (M^+-AcOH, 3), 421 (M^+-AcOH$-CH_3$, 4), 376 (M^+-2AcOH, 3), 361 (M^+-2AcOH$-CH_3$, 8), 186 (**iii**$-H_2O$, 16), 171 (**v**, 25). High resolution MS: Found 496.355; Calcd. for $C_{32}H_{48}O_4$ (M^+) 496.356. The diacetate (**5a**) obtained here was identified with **5a** prepared above from **1** through photolysis by mixed mp, UV, and TLC.

Acid Treatment of 4a giving 5a——A solution of **4a** (12 mg) in 5% HCl–EtOH (10 ml) was heated under reflux for 30 min, diluted with water (90 ml), and filtered. The collected product was washed with water, dried *in vacuo*, and acetylated with acetic anhydride (0.3 ml) and pyridine (1 ml) in the usual manner. Recrystallization of the product (10 mg) from MeOH gave colorless needles of mp 210—211°, being identical to isoanhydrospergulatriol diacetate (**5a**) obtained above by mixed mp, IR (KBr), and TLC.

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