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Studies on the Constituents of Asclepiadaceae Plants. XLVI.[1] Aglycones from *Stephanotis japonica* MAKINO[2]

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In addition to stephanthraniline A and stephanthraniline C, whose structures have been reported previously, five new pregnane derivatives, stephanthraniline B (12β-*O*,N-methyl-anthraniloyl-20-*O*-acetyldihydrosarcostin), SG-A (12β-*O*-(2-methylbutyryl)-20-*O*-acetylsarcostin), SG-B (20-*O*-(2-methylbutyryl)sarcostin), SG-C (20-*O*-tigloylsarcostin), and dihydrogagaminin (12β-*O*-cinnamoyl-20-*O*-nicotinoyl-dihydrosarcostin) were isolated from the aerial parts of *Stephanotis japonica* MAKINO (Asclepiadaceae) and their structures were determined. Penupogenin, kidjoranin, and gagaminin were also isolated and identified.

Keywords——*Stephanotis japonica* MAKINO; Asclepiadaceae; aglycones; C/D-*cis* polyoxypregnane; ester derivatives; stephanthraniline B; SG-A; SG-B; SG-C; dihydrogagaminin

Stephanotis japonica MAKINO (Japanese name, Shitakiso) is a plant of the Asclepiadaceae family, which is indigenous to the warm forest zone along the seashore of Japan. The components of this plant were studied as part of a systematic investigation on constituents of Asclepiadaceae plants.

The aerial part of *S. japonica* was extracted with chloroform and the extract was treated to afford an aglycone mixture by the usual methods.[4] By a combination of column chromatography and preparative thin-layer chromatography (TLC), ten compounds were isolated (Table I). Of these ten compounds, three known compounds, penupogenin[5] (IV), kidjoranin[6] (V), and gagaminin[7] (IX) were identified by direct comparison with authentic samples. Of

TABLE I. Chromatographic Results with Aglycone Mixture

Fraction No.	Solvent	Weight	Compound
1	10% acetone in benzene	Trace	Not identified
2	15% acetone in benzene	350 mg	I, II
3	15% acetone in benzene	1.12 g	I, II, III
4	15% acetone in benzene	710 mg	I
5	15% acetone in benzene	300 mg	IV, V
6	15% acetone in benzene	900 mg	IV, V
7	20% acetone in benzene	570 mg	VI, VII
8	25% acetone in benzene	340 mg	VIII
9	30% acetone in benzene	1.05 g	IX
10	30% acetone in benzene	550 mg	X

1) Part XLV: T. Nomura, S. Yamada, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **27**, 516 (1979).
2) A part of this work was presented at the Annual Meeting of the Pharmacognostical Society of Japan, November 1978.
3) Location: *Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo, 060, Japan.*
4) H. Mitsuhashi, I. Takemori, Y. Shimizu, T. Nomura, and E. Yamada, *Chem. Pharm. Bull.* (Tokyo), **10**, 904 (1962).
5) H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **10**, 725 (1962).
6) T. Sasaki, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 628 (1972).
7) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2289 (1972).

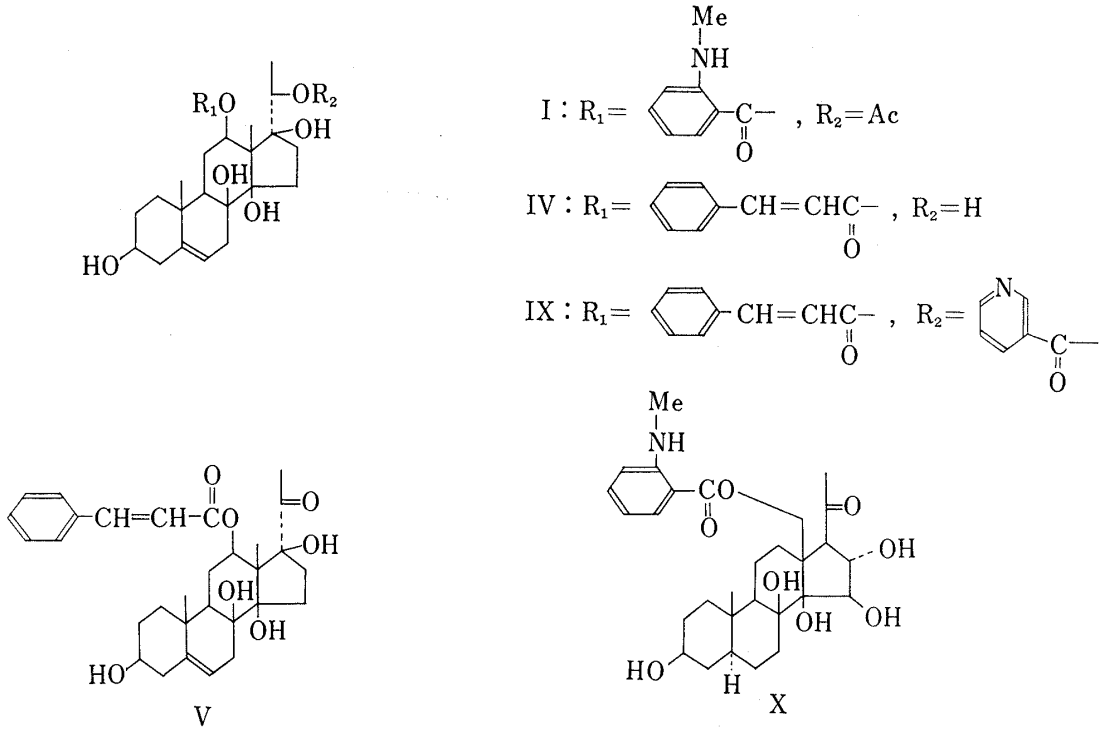


Chart 1

the seven new compounds, the structures of stephanthraniline A[8] (I) and stephanthraniline C[9] (X) have been reported previously (Chart 1).

This paper deals with the structures of five minor components named stephanthraniline B (II), SG-A (III), SG-B (VI), SG-C (VII), and dihydrogagaminin (VIII).

Stephanthraniline B (II) showed the following properties: mp 165—168°, $[\alpha]_D$ −24.6° (c=1.14, $CHCl_3$); molecular formula of $C_{31}H_{45}NO_8$ from elemental analysis and mass spectrum (MS) (M^+ at m/e 559). The ultraviolet (UV) spectrum of II showed absorption maxima at 222 (ε 35000) and 253 nm (11200), indicating the presence of an N-methylanthraniloyl group.[8]

Hydrolysis of II with 5% methanolic potassium hydroxide gave dihydrosarcostin[10] (XI); the mother liquor fraction contained N-methylanthranilic acid as determined by gas chromatographic analysis. A prominent mass spectral peak indicative of the acetate functional group was observed at m/e 43.

The ^{1}H-nuclear magnetic resonance (PMR) spectrum of II showed signals of two tertiary methyl groups at δ 0.94 and 1.48, two secondary methyl groups at δ 1.28 (d, J=6 Hz) and 2.90 (d, J=6 Hz), one acetyl group at δ 1.87, one hydroxy-methine at δ 3.64 (m), and two acyloxy-methines at δ 4.61 (d.d, J=4, 10 Hz) and 4.75 (q, J=6 Hz).

These findings suggest that II is a diester of XI with N-methylanthranilic acid and acetic acid linked at the C-12 and/or C-20 hydroxyl groups. The biogenetic analogy with stephanthraniline A (I) suggests that the structure of stephanthraniline B can be formulated as II (Chart 2). SG-A (III), mp 235—240°, has a molecular formula of $C_{28}H_{44}O_8$, as determined by elemental analysis and from the mass spectrum. Hydrolysis of III with 5% methanolic potassium hydroxide afforded sarcostin[10] (XII), and 2-methylbutyric acid was detected in the mother liquor by gas chromatography.

The PMR spectrum of III showed signals of one acetyl group at δ 2.02, one hydroxy-methine at δ 3.52 (m), and two acyloxy-methines at δ 4.66 (q, J=6 Hz) and 4.78 (d.d, J=4,

8) S. Terada, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **25**, 2802 (1977).
9) S. Terada, K. Hayashi, and H. Mitsuhashi, *Tetrahedron Lett.*, **1978**, 1995.
10) M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2248 (1969).

10 Hz). The ^{13}C-nuclear magnetic resonance (CMR) spectrum of III showed signals of two ester carbonyl carbons at δ 171.38 and 175.08. Prominent mass spectral peaks indicative of 2-methylbutyrate and acetate were observed at *m/e* 85 (2-methylbutyryl cation) and 43 (acetyl cation), which suggested that SG–A (III) is a diester of sarcostin (XII) with 2-methylbutyric acid and acetic acid.

In order to confirm the position of the ester linkages of III, the following experiments were carried out (Chart 3). SG–A (III) was treated with acetic anhydride to afford the 3β-acetate (XIII), and 3β,12β-di-*O*-acetylsarcostin (XIV)[11] was acylated with *dl*-2-methylbutyryl chloride to yield 3β,12β-di-*O*-acetyl-20-*O*-(2-methylbutyryl)sarcostin (XV). In the PMR spectrum of XV, the hydroxy-methines of C-20 appeared at δ 4.80 (q, J=6 Hz) and 5.08 (q, J=6 Hz). Comparison of the PMR spectrum of XIII with that of XV clearly distinguished the two compounds.

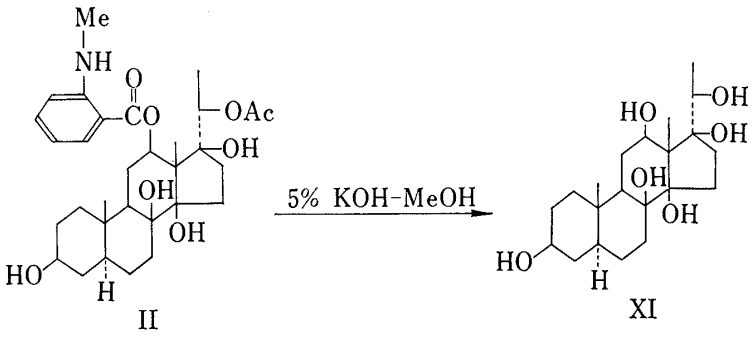
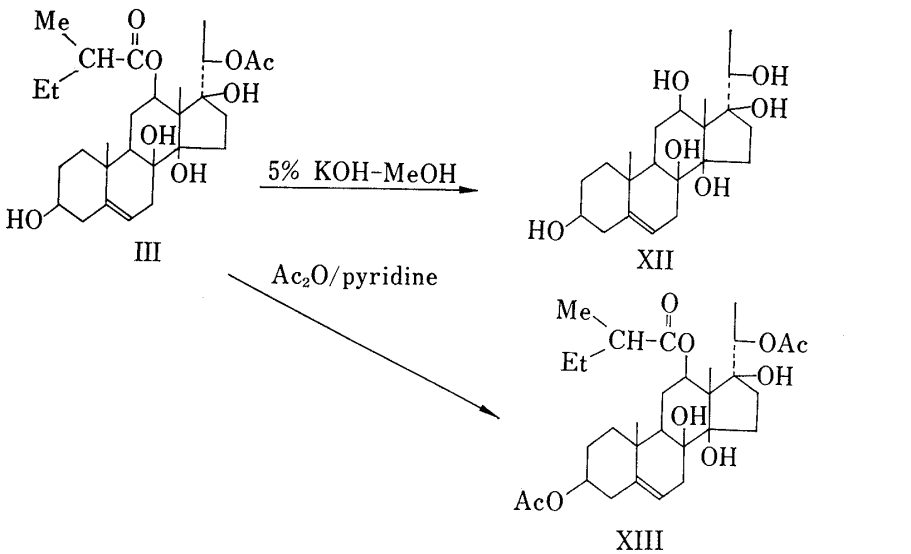


Chart 2



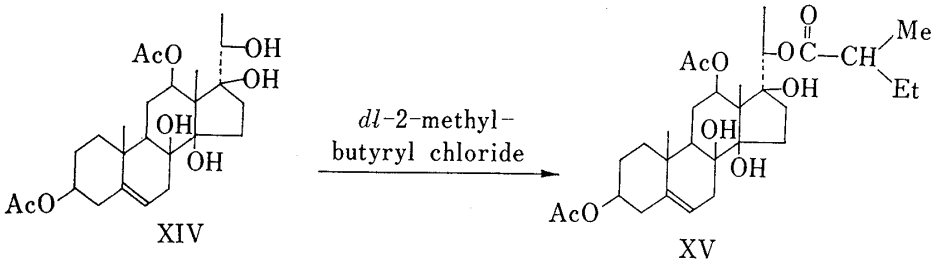


Chart 3

11) K. Hayashi and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), 23, 1845 (1975).

Thus, SG–A (III) was identified as 12β-*O*-(2-methylbutyryl)-20-*O*-acetylsarcostin, though the absolute stereochemistry of 2-methylbutyric acid was not defined. SG–B (VI) and SG–C (VII) have very similar structures. On alkaline hydrolysis, both VI and VII yielded a compound whose *Rf* value and color reaction (with $SbCl_3$) were identical with those of sarcostin (XII) on TLC. From the results of MS and elemental analysis, the molecular formulae of SG–B and SG–C were determined to be $C_{26}H_{42}O_7$ and $C_{26}H_{40}O_7$, respectively.

The PMR spectrum of VI exhibited two hydroxy-methines at δ 3.48 (2H, m+d.d, J=5, 11 Hz) and one acyloxy-methine at δ 5.20 (q, J=6 Hz). The MS of VI showed a 2-methylbutyryl cation peak at *m/e* 85. Gas chromatographic examination revealed the presence of 2-methylbutyric acid in the reaction mixture on alkaline hydrolysis of VI.

Thus, SG–B was identified as 20-*O*-(2-methylbutyryl)sarcostin, though the absolute stereochemistry of the acyl group was not defined (Chart 4).

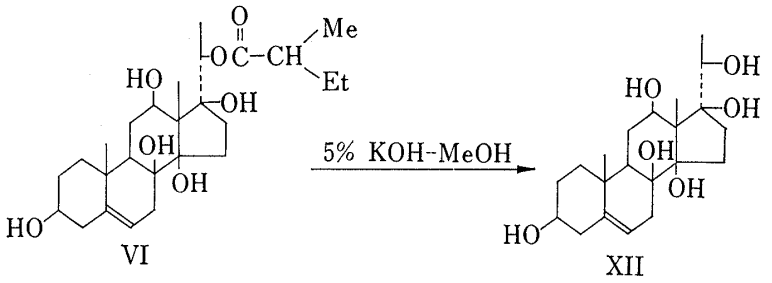


Chart 4

The PMR spectrum of SG–C showed signals for two hydroxy-methines at δ 3.50—3.60 (2H, m), one acyloxy-methine at δ 5.87 (1H, q, J=6 Hz), and two olefinic protons at δ 5.42 (m) and 6.99 (q, J=7 Hz). In the MS of VII, a tigloyl cation peak was observed at *m/e* 83. On alkaline hydrolysis of VII, tiglic acid was detected by gas chromatographic analysis. Therefore, SG–C is 20-*O*-tigloylsarcostin, formulated as VII (Chart 5).

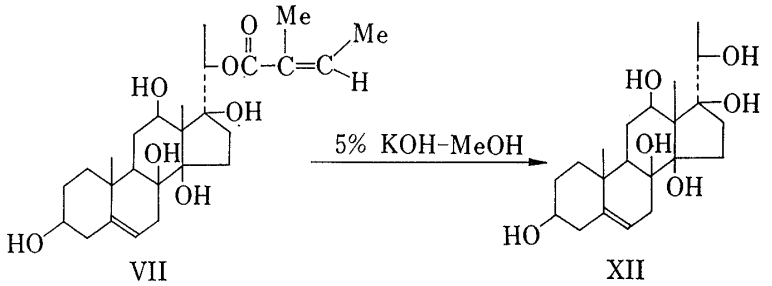
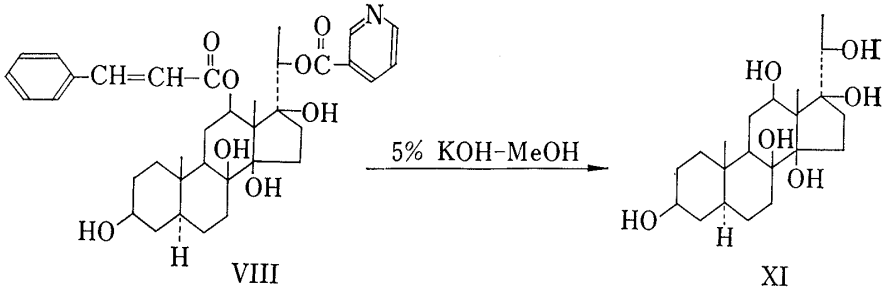


Chart 5

Dihydrogagaminin (VIII), $C_{36}H_{45}NO_8$, was obtained as an amorphous substance. The UV spectrum of VIII was essentially identical with that of gagaminin[7] (IX), which indicated the presence of cinnamic acid and nicotinic acid as acid moieties of VIII. The MS of VIII showed peaks of cinnamoyl cation (*m/e* 131) and nicotinoyl cation (*m/e* 106). The PMR spectrum of VIII showed signals of one hydroxy-methine at δ 3.58 (m), and two acyloxy-methines at δ 4.80 (d.d, J=4, 11 Hz) and 4.91 (q, J=6 Hz). Alkaline hydrolysis of VIII afforded a compound whose *Rf* value and color reaction (with $SbCl_3$) were identical with those of dihydrosarcostin (XI) on TLC. Accordingly, VIII is a diester of XI with cinnamic acid and nicotinic acid. However, we could not determine the positions of the ester linkages of VIII from the spectral data. From the biogenetic analogy with gagaminin (IX), VIII is tentatively proposed to be 12β-*O*-cinnamoyl-20-*O*-nicotinoylsarcostin (Chart 6).



VIII XI

Chart 6

Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in $CHCl_3$ solution on a JASCO DIP-4 digital polarimeter. PMR spectra were determined at 100 MHz with a JEOL JNM-FX 100 spectrometer, using tetramethylsilane as an internal standard (s, singlet; d, doublet; q, quartet; m, multiplet). CMR was measured at 25.0 MHz using a JNM-FX 100 spectrometer. Mass spectra (MS) were determined on a JEOL NMS D-300 mass spectrometer. IR spectra were taken as Nujol mulls on a Hitachi EPS-3T spectrometer, Gas chromatography was carried out with a Shimadzu GC-4BPF machine. TLC was performed on silica gel HF_{254} (Merck, Type 60), and silica gel (Merck, 70—325 mesh ASTM) was also used for column chromatography.

Isolation of Aglycone Mixture——The aerial part (3.2 kg) of *S. japonica*, collected at Owase, Mie Prefecture, in November 1976, was extracted with $CHCl_3$ to yield a crude extract (110 g). The crude extract was redissolved in $CHCl_3$, and addition of hexane to the solution precipitated a glucoside mixture. The same procedures were repeated seven times, and the precipitate was collected by filtration. The crude glycoside mixture (56 g) was dissolved in MeOH (350 ml), then the solution was brought to reflux, and a preheated solution of 0.1 N H_2SO_4 (350 ml) was poured into it. This solution was refluxed for 30 min. After addition of 350 ml of H_2O, MeOH was evaporated off *in vacuo*, and the residual aqueous solution was heated at 60° for 30 min. The reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed successively with H_2O, 5% $NaHCO_3$ solution, and H_2O, then dried over Na_2SO_4. Removal of $CHCl_3$ afforded an aglycone mixture (14 g). This aglycone mixture was chromatographed over silica gel (450 g), eluting with solvents of increasing polarity. The results of the chromatography are summarized in Table I.

Separation of Stephanthraniline (B) (II) and SG-A (III)——Fraction 2 was subjected to preparative TLC. On development with hexane–acetone–$CHCl_3$ (3: 1: 1) solution (15 times), the mixture was separated into stephanthraniline A (I) and stephanthraniline B (II). The same procedures for fr. 3 afforded I, II, and SG-A (III).

Separation of SG-B (VI) and SG-C (VII)——Fr. 7 exhibited about 15 spots on TLC, of which two spots were major. In order to remove the minor components, fr. 7 was subjected to preparative TLC under the same conditions as fr. 2 and fr. 3, and SG-B fraction and SG-C fraction were obtained. Further preparative TLC of the two fractions with 10% MeOH in $CHCl_3$ solution afforded pure SG-B and SG-C.

Separation of Dihydrogagaminin (VIII)——Preparative TLC of fr. 8, developing with a solution of MeOH–$CHCl_3$–hexane (1: 14: 4), gave a fraction containing dihydrogagaminin. An impurity was removed by repeated preparative TLC (MeOH–$CHCl_3$–hexane = 1: 28: 4).

Separation of Penupogenin (IV), Kidjoranin (V), and Gagaminin (IX)——Fr. 5 and fr. 6 gave similar chromatograms on TLC. Repeated preparative TLC (acetone–$CHCl_3$–hexane = 1: 1: 3) of fr. 5 and fr. 6 afforded IV (mp 147—150°) and V (mp 148—151°), respectively. Mass and PMR spectra of IV were identical with those of an authentic sample of penupogenin, and no depression of the melting point occurred on admixture with the authentic sample. The mobility on TLC, and mass and PMR spectra of V were identical with those of an authentic sample of kidjoranin. The mixed melting point did not show depression. Preparative TLC (10% MeOH in $CHCl_3$) of fr. 9 yielded a compound whose mass and PMR spectra were identical with those of an authentic sample of gagaminin.

Identification of Deacylated Aglycone——As SG-A, SG-B, SG-C, and dihydrogagaminin were very minor components, identification of the deacylgenins was carried out by the following procedures: an aglycone (1 mg) was dissolved in 5% MeOH–KOH, and the solution was subjected to TLC (10% MeOH in $CHCl_3$, acetone–$CHCl_3$–hexane = 1: 1: 1; spray reagent, saturated solution of $SbCl_3$ in $CHCl_3$).

Stephanthraniline B (II)——Colorless needles (45 mg), mp 165—168°, from acetone/(iso-Pr)$_2$O, $[\alpha]_D$ −24.6° (c = 1.14, $CHCl_3$). UV λ_{max}^{EtOH} nm (ε): 222 (35000), 253 (112000). MS m/e: 559 (M^+), 541 ($M^+ - H_2O$), 499 (M^+—AcOH), 151 (N-methylanthranilic acid, base peak). PMR ($CDCl_3$): 0.94 (3H, s), 1.28 (3H, d, J = 6 Hz), 1.48 (3H, s), 1.87 (3H, s), 2.90 (3H, d, J = 6 Hz), 3.64 (1H, m), 4.61 (1H, d.d, J = 4, 10 Hz), 4.75 (1H, q, J = 6 Hz). *Anal.* Calcd. for $C_{31}H_{45}NO_8$: C, 66.52; H, 8.10; N, 2.50. Found: C, 66.76; H, 8.35; N, 2.37.

Alkaline Hydrolysis of II——Stephanthraniline B (30 mg) was hydrolyzed in 5% MeOH–KOH (4 ml) at room temperature for 24 hr. After addition of H_2O (5 ml), MeOH was removed *in vacuo*. The resulting aqueous solution was extracted with CH_2Cl_2 using a continuous liquid–liquid extractor. Removal of CH_2Cl_2 and recrystallization of the residue from acetone gave XI as needles, mp 239—245°; the material was found to be identical with dihydrosarcostin by mixed fusion with an authentic sample.

SG-A (III)——Colorless prisms (30 mg), mp 235—240° (from acetone), $[\alpha]_D$ +15.3° (c=0.59, $CHCl_3$). PMR δ ($CDCl_3$): 0.92 (3H, t, J=7 Hz), 1.17 (3H, d, J=7 Hz), 1.17 (3H, s), 1.21 (3H, d, J=6 Hz), 1.43 (3H, s), 2.02 (3H, s), 3.52 (1H, m), 4.66 (1H, q, J=6 Hz), 4.78 (1H, d.d, J=4, 10 Hz), 5.38 (1H, m). MS *m/e*: 472 (M^+-2H_2O), 448 (M^+—AcOH), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation, base peak). CMR δ ($CDCl_3$): 10.28, 11.69, 15.20, 18.47, and 22.02 (methyl carbons), 72.02 (d), 73.87 (d and d), 74.21 (s), and 87.85 (s and s) (carbinyl carbons), 171.38 and 175.08 (ester carbonyl carbons). *Anal.* Calcd. for $C_{28}H_{44}O_8$: C, 66.11; H, 8.72. Found: C, 66.16; H, 8.78.

Alkaline Hydrolysis of SG-A (III)——SG-A (1 mg) was dissolved in 5% MeOH–KOH (1 ml), and the solution was examined by TLC and GLC. Sarcostin was detected on TLC. The same solution was subjected to GLC examination (column; neopentylglycol succinate, 2.1 m glass column, 100°); the retention time of 8.6 min was identical with that of 2-methylbutyric acid.

Acetylation of III——SG-A (10 mg) was dissolved in a mixture of 1 ml of pyridine and 1 ml of Ac_2O, and the solution was kept at room temperature for 3 hr. The solution was poured into ice-water and extracted with $CHCl_3$. After the usual work-up, the residue was crystallized from acetone/hexane to give needles, mp 218—223°. PMR δ ($CDCl_3$): 0.93 (3H, t, J=7 Hz), 1.17 (3H, d, J=7 Hz), 1.18 (3H, s), 1.23 (3H, d, J=6 Hz), 1.23 (3H, s), 2.00 (3H, s), 2.03 (3H, s), 4.63 (1H, m), 4.66—4.70 (2H, m), 5.40 (1H, m). MS *m/e*: 412 (M^+—2AcOH–H_2O), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation), 43 (acetyl cation).

***dl*-2-Methylbutyrylation of 3β,12β-Di-*O*-acetylsarcostin (XIV)**——A solution of 5 ml of *dl*-2-methylbutyric acid and 6 ml of $SOCl_2$ was heated under reflux for 1 hr. After cooling, the flask was fitted with an apparatus for atmospheric pressure distillation. The excess $SOCl_2$ was removed and 2-methylbutyryl chloride was obtained as the fraction boiling at 95—100°. Next, *dl*-2-methylbutyryl chloride (0.5 ml) was added to a solution of XIV (30 mg) in pyridine (2 ml) at 0°, and the mixture was stirred at room temperature for 10 min. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ layer was worked up as usual. Removal of $CHCl_3$ gave a light yellow residue, which was purified by preparative TLC (acetone–$CHCl_3$–hexane=1:1:3). 3β,12β-Di-*O*-acetyl-20-*O*-(2-methylbutyryl)sarcostin (12 mg), needles from acetone/hexane, mp 143—150°. PMR δ ($CDCl_3$): 1.19 (3H, s), 1.26 (3H, t, J=7 Hz), 1.29 (3H, d, J=6 Hz), 1.34 (3H, s), 1.45 (3H, d, J=6 Hz), 2.04 (3H, s), 2.08 (3H, s), 4.30—4.68 (2H, m), 4.80+5.08 (1H, q+q, J=6 Hz), 5.39 (1H, m). MS *m/e*: 412 (M^+—2AcOH–H_2O), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation), 43 (acetyl cation).

SG-B (VI)——Colorless needles (25 mg) from acetone, mp 183—186°, $[\alpha]_D$ −50.6° (c=0.67, MeOH). MS *m/e*: 466 (M^+), 448 (M^+-H_2O), 364 (M^+—2-methyl-butyric acid), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation). PMR δ ($CDCl_3$): 0.90 (3H, t, J=7 Hz), 1.13 (3H, d, J=7 Hz), 1.19 (3H, s), 1.25 (3H, d, J=6 Hz), 1.34 (3H, s), 3.48 (2H, m+d.d, J=5, 11 Hz), 5.20 (1H, q, J=6 Hz), 5.38 (1H, m). IR ν_{max}^{Nujol} cm^{-1}: 3475, 3320, 1710. *Anal.* Calcd. for $C_{26}H_{42}O_7$: C, 66.92; H, 9.07. Found: C, 66.70; H, 9.12. Sarcostin was detected in a solution of VI and 5% MeOH–KOH in TLC, and 2-methylbutyric acid was detected by GLC as a peak having a retention time of 8.6 min (column; neopentylglycol succinate, 2.1 m glass column, 100°).

SG-C (VII)——Colorless needles (6 mg) from acetone mp 217—222°, $[\alpha]_D$ +89.0° (c=0.60, MeOH). MS *m/e*: 464 (M^+), 446 (M^+-H_2O), 364 (M^+—tiglic acid), 83 (tigloyl cation), 55 ((tigloyl-co) cation). PMR δ (pyridine-d_5): 1.49 (3H, s), 1.58 (3H, s), 1.61 (3H, d, J=6 Hz), 2.02 (6H, broad singlet), 3.50—3.60 (2H, m), 5.42 (1H, m), 5.87 (1H, q, J=6 Hz), 6.99 (1H, q, J=7 Hz). UV λ_{max}^{EtOH} nm (log ε): 216 (4.14). *Anal.* Calcd. for $C_{26}H_{40}O_7$: C, 67.21; H, 8.68. Found: C, 67.05; H, 8.56. TLC analysis of the alkaline hydrolysate of VII showed a spot of sarcostin. Tiglic acid (retention time, 7.3 min) was detected in the same solution by GLC analysis (column; diethylene glycol succinate, 3.1 m glass column, 170°).

Dihydrogagaminin (VIII)——Amorphous (10 mg), $[\alpha]_D$ +105° (c=0.5, $CHCl_3$). PMR δ ($CDCl_3$): 0.94 (3H, s), 1.35 (3H, d, J=6 Hz), 1.59 (3H, s), 3.58 (1H, m), 4.80 (1H, d.d, J=4, 11 Hz), 4.91 (1H, q, J=6 Hz), 6.07+7.35 (2H, AB q, J=16 Hz), 8.05 (2H, m), 8.71 (1H, m), 9.13 (1H, broad singlet). MS *m/e*: 496 (M^+—nicotinic acid), 471 (M^+—cinnamic acid), 348 (M^+—nicotinic acid—cinnamic acid), 131 (cinnamoyl cation), 123 (nicotinic acid, base peak), 106 (nicotinoyl cation). UV λ_{max}^{EtOH} nm (ε): 218 (18000), 282 (17100), *Anal.* Calcd. for $C_{36}H_{45}NO_8$: C, 69.77; H, 7.32; N, 2.26. Found: C, 69.68; H, 7.45; N, 2.31. TLC analysis of a solution of VIII and 5% MeOH–KOH showed a spot whose mobility and color reaction were identical with those of dihydrosarcostin.