Studies on the Constituents of Asclepiadaceae Plants. XLVI. 1)  
Glycone from *Stephanotis japonica Makino* 2)

SUMIO TERADA and HIROSHI MITSUHASHI

Faculty of Pharmaceutical Sciences, Hokkaido University 3)

(Received March 14, 1979)

In addition to stephantraniline A and stephantraniline C, whose structures have been reported previously, five new pregnane derivatives, stephantraniline B (12β-O,N-methyl-anthraniloyl-20-O-acetyldihydroarosocin), SG-A (12β-O-(2-methylbutyryl)-20-O-acetylarsocin), SG-B (20-O-(2-methylbutyryl)arsocin), SG-C (20-O-tigloylsarocin), and dihydrogagaminin (12β-O-cinnamoyl-20-O-nicotinoyl-dihydroarsocin) 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; glycone; C/D-cis polyoxy pregnane; ester derivatives; stephantraniline 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**

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

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.*
the seven new compounds, the structures of stephanthraniline A\textsuperscript{8)} (I) and stephanthraniline C\textsuperscript{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 dihydroagamin (VIII).

Stephanthraniline B (II) showed the following properties: mp 165–168°, $[\alpha]_D = -24.6^\circ$ ($c = 1.14$, CHCl\textsubscript{3}); molecular formula of C\textsubscript{23}H\textsubscript{46}NO\textsubscript{8} from elemental analysis and mass spectrum (MS) (M\textsuperscript{+} at m/e 559). The ultraviolet (UV) spectrum of II showed absorption maxima at 222 (ε 35000) and 255 nm (11200), indicating the presence of an N-methylanthraniloyl group.\textsuperscript{8)}

Hydrolysis of II with 5\% methanolic potassium hydroxide gave dihydroscarcostin\textsuperscript{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 \textsuperscript{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\textsubscript{20}H\textsubscript{44}O\textsubscript{8}, as determined by elemental analysis and from the mass spectrum. Hydrolysis of III with 5\% methanolic potassium hydroxide afforded sarcostin\textsuperscript{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 hydroxymethine at δ 3.52 (m), and two acyloxy-methines at δ 4.66 (q, $J = 6$ Hz) and 4.78 (d,d, $J = 4$,

10 Hz). The $^{13}$C-nuclear magnetic resonance (CMR) spectrum of III showed signals of two ester carbonyl carbons at $\delta$ 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 $3\beta$-acetate (XIII), and $3\beta,12\beta$-di-O-acetyl sarcostin (XIV) was acylated with $dl$-2-methylbutyryl chloride to yield $3\beta,12\beta$-di-O-acetyl-20-$O$-(2-methylbutyryl)sarcostin (XV). In the PMR spectrum of XV, the hydroxy-methines of C-20 appeared at $\delta$ 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

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 SbCl₅) 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 C₂₅H₄₇O₇ and C₂₆H₅₀O₇, 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/z* 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).

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/z* 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).

Dihydrogagaminin (VIII), C₃₉H₅₉NO₈, was obtained as an amorphous substance. The UV spectrum of VIII was essentially identical with that of gagaminin⁹ (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/z* 131) and nicotinoyl cation (*m/z* 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 SbCl₅) were identical with those of dihydroaspersarin (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).
Experimental

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in CHCl₃ 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-4BP machine. TLC was performed on silica gel HFX₂₅₄ (Merk, Type 60), and silica gel (Merk, 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 CHCl₃ to yield a crude extract (110 g). The crude extract was redissolved in CHCl₃, 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 glucoside mixture (56 g) was dissolved in MeOH (350 ml), then the solution was brought to reflux, and a preheated solution of 0.1 N H₂SO₄ (350 ml) was poured into it. This solution was refluxed for 30 min. After addition of 350 ml of H₂O, MeOH was evaporated off in vacuo, and the residual aqueous solution was heated at 60° for 30 min. The reaction mixture was extracted with CHCl₃, and the CHCl₃ layer was washed successively with H₂O, 5% NaHCO₃ solution, and H₂O, then dried over Na₂SO₄. Removal of CHCl₃ 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 Stephananthrinine (B) (II) and SG-A (III) — Fraction 2 was subjected to preparative TLC. On development with hexane—acetone—CHCl₃ (5:1:1) solution (15 times), the mixture was separated into stephananthrinine A (I) and stephananthrinine 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 CHCl₃ solution afforded pure SG-B and SG-C.

Separation of Dihydrogagaminin (VIII) — Preparative TLC of fr. 8, developing with a solution of MeOH—CHCl₃—hexane (1:14:4), gave a fraction containing dihydrogagaminin. An impurity was removed by repeated preparative TLC (MeOH—CHCl₃—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—CHCl₃—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 CHCl₃) of fr. 9 yielded a compound whose mass and PMR spectra were identical with those of an authentic sample of gagaminin.

Identification of Decaoylated Aglycone — As SG-A, SG-B, SG-C, and dihydrogagaminin were very minor components, the 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 CHCl₃, acetone—CHCl₃—hexane=1:1:1; spray reagent, saturated solution of SbCl₅ in CHCl₃).

Stephananthrinine B (II) — Colorless needles (45 mg), mp 165—168°, from acetone/(iso-Pr)₂O, [α]D _24.6° ε=1.14, CHCl₃. UV λ_max nm (ε) 222 (35000), 253 (112000). MS m/z: 559 (M⁺), 541 (M⁺—H₂O), 499 (M⁺—AcOH), 151 (N-methylanthranilic acid, base peak). PMR (CDCl₃) 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 C₂₁H₂₄NO₄: C, 66.52; H, 8.10; N, 2.50. Found: C, 66.76; H, 8.35; N, 2.37.
Alkaline Hydrolysis of II—Stephanantraniline B (30 mg) was hydrolyzed in 5% MeOH–KOH (4 ml) at room temperature for 24 hr. After addition of H₂O (5 ml), MeOH was removed in vacuo. The resulting aqueous solution was extracted with CH₂Cl₂ using a continuous liquid–liquid extractor. Removal of CH₂Cl₂ and recrystallization of the residue from acetone gave XI as needles, mp 239–245°; the material was found to be identical with dihydrosarcosin by mixed fusion with an authentic sample.

SG-A (III)—Colorless prisms (30 mg), mp 235–240° (from acetone), [α]D +15.3° (c = 0.59, CHCl₃).

PMR δ (CDCl₃): 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, J = 4, 10 Hz), 5.38 (1H, m). MS m/z: 472 (M+ − 2H₂O), 448 (M+ − AcOH), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation, base peak). CMR δ (CDCl₃): 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) (carbonyl carbons), 171.38 and 175.08 (ester carbonyl carbons). Anal. Calcd. for C₂₃H₄₂O₃: 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. Sarcosin 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 Ac₂O, and the solution was kept at room temperature for 3 hr. The solution was poured into ice-water and extracted with CHCl₃. After the usual work-up, the residue was crystallized from acetone/hexane to give needles, mp 218–223°. PMR δ (CDCl₃): 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/z: 412 (M+ − 2AcOH−H₂O), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation), 43 (acetyl cation).

dl-2-Methylbutyrylation of 3β,12β-Di-O-acetyl-20-O-(2-methylbutyryl)sarcosin (XIV)—A solution of 5 ml of dl-2-methylbutyric acid and 6 ml of SOCl₂ was heated under reflux for 1 hr. After cooling, the flask was fitted with an apparatus for atmospheric pressure distillation. The excess SOCl₂ 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 1 hr. The reaction mixture was poured into ice-water and extracted with CHCl₃. The CHCl₃ layer was worked up as usual. Removal of CHCl₃ gave a light yellow residue, which was purified by preparative TLC (acetone–CHCl₃–hexane = 1:1:3). 3β,12β-Di-O-acetyl-20-O-(2-methylbutyryl)sarcosin (12 mg), needles from acetone/hexane, mp 143–150°. PMR δ (CDCl₃): 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, J = 6 Hz), 5.39 (1H, m). MS m/z: 412 (M+ − 2AcOH−H₂O), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation), 43 (acetyl cation).

SG-B (VI)—Colorless needles (25 mg) from acetone, mp 183–186°, [α]D +50.6° (c = 0.67, MeOH). MS m/z: 468 (M+), 448 (M+ − H₂O), 364 (M+ − 2-methylbutyric acid), 85 (2-methylbutyryl cation), 57 (2-methylbutyl cation). PMR δ (CDCl₃): 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 cm⁻¹: 3475, 3320, 1710. Anal. Calcd. for C₂₃H₄₄O₄: C, 66.92; H, 9.07. Found: C, 66.70; H, 9.12. Sarcosin 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°, [α]D +89.0° (c = 0.60, MeOH). MS m/z: 464 (M+), 446 (M+ − H₂O), 364 (M+ − tiglic acid), 85 (tigloyl co-cation), 55 (tigloyl co-cation). PMR δ (pyrroline-d₅): 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 nm (log ε): 216 (4.14). Anal. Calcd. for C₂₃H₄₄O₄: C, 67.21; H, 8.68. Found: C, 67.05; H, 8.56. TLC analysis of the alkaline hydrolysat of VII showed a spot of sarcosin. Tiglic acid (retention time, 7.3 min) was detected in the same solution by GLC analysis (column; neopentylglycol succinate, 3.1 m glass column, 170°).

Dihydromagamin (VIII)—Amorphous (10 mg), [α]D +105° (c = 0.5, CHCl₃). PMR δ (CDCl₃): 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), 0.13 (1H, broad singlet). MS m/z: 496 (M+ − nicotinic acid), 471 (M+ − nissamic acid), 348 (M+ − nicotinic acid − nissamic acid), 131 (tigloyl co-cation), 123 (nicotinic acid, base peak), 106 (nicotinoyl cation). UV λ max nm (log ε): 215 (18000), 282 (17100). Anal. Calcd. for C₂₃H₄₄N₂O₄: C, 69.77; H, 7.32; N, 2.26. Found: C, 69.88; 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 dihydrosarcosin.