

Studies on the Constituents of Asclepiadaceae Plants. XXIV.¹⁾ Structure of Stephanol

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A new pregnane, stephanol, was isolated from *Stephanotis japonica* (Asclepiadaceae), and its chemical and physicochemical studies suggest the allocation of its structure.

From taxonomic considerations, we were interested in examining the dried whole plants of *Stephanotis japonica* MAKINO (Asclepiadaceae) from which a constituent named stephanol was reported in our earlier communication.³⁾ The present paper describes in full detail the evidence which leads to the proposal of structure (VI) for this substance.

By the extraction of dried whole plants, we isolated a mixture of aglycones by the usual isolation procedure.⁴⁾ Chromatography of this mixture gave lineolon⁵⁾ (I), deacylmetaplexigenin⁶⁾ (II), sarcostin⁷⁾ (III), 5 α -dihydrosarcostin⁸⁾ (IV), and stephanol (VI).

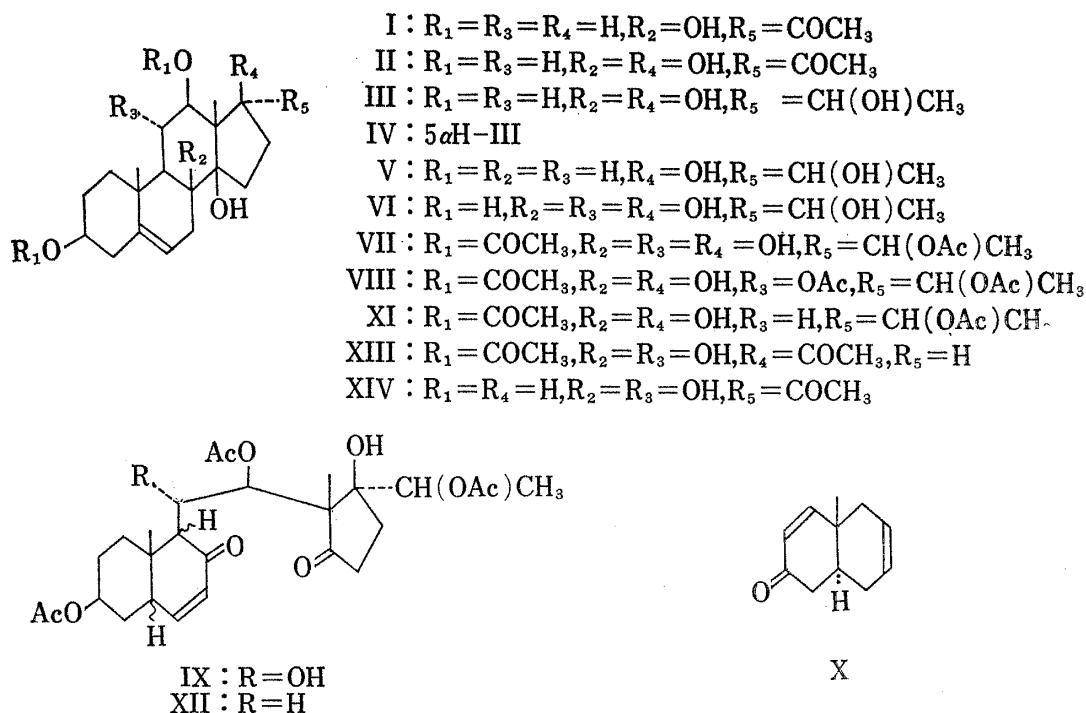


Chart 1

- 1) Part XXIII: *Shoyakugaku Zasshi*, **22**, 86 (1968).
- 2) Location: Kita-12-jo, Nishi-7-chome, Sapporo.
- 3) M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 553 (1968).
- 4) J.V. Euw, H. Hess, P. Speiser, and T. Reichstein, *Helv. Chim. Acta*, **34**, 1821 (1951).
- 5) E. Abisch, Ch. Tamm, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1014 (1959); H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **8**, 318 (1960).
- 6) H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **11**, 1198 (1963).
- 7) K.A. Jaeggi, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 694 (1963).
- 8) K.A. Jaeggi, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **50**, 1201 (1967).

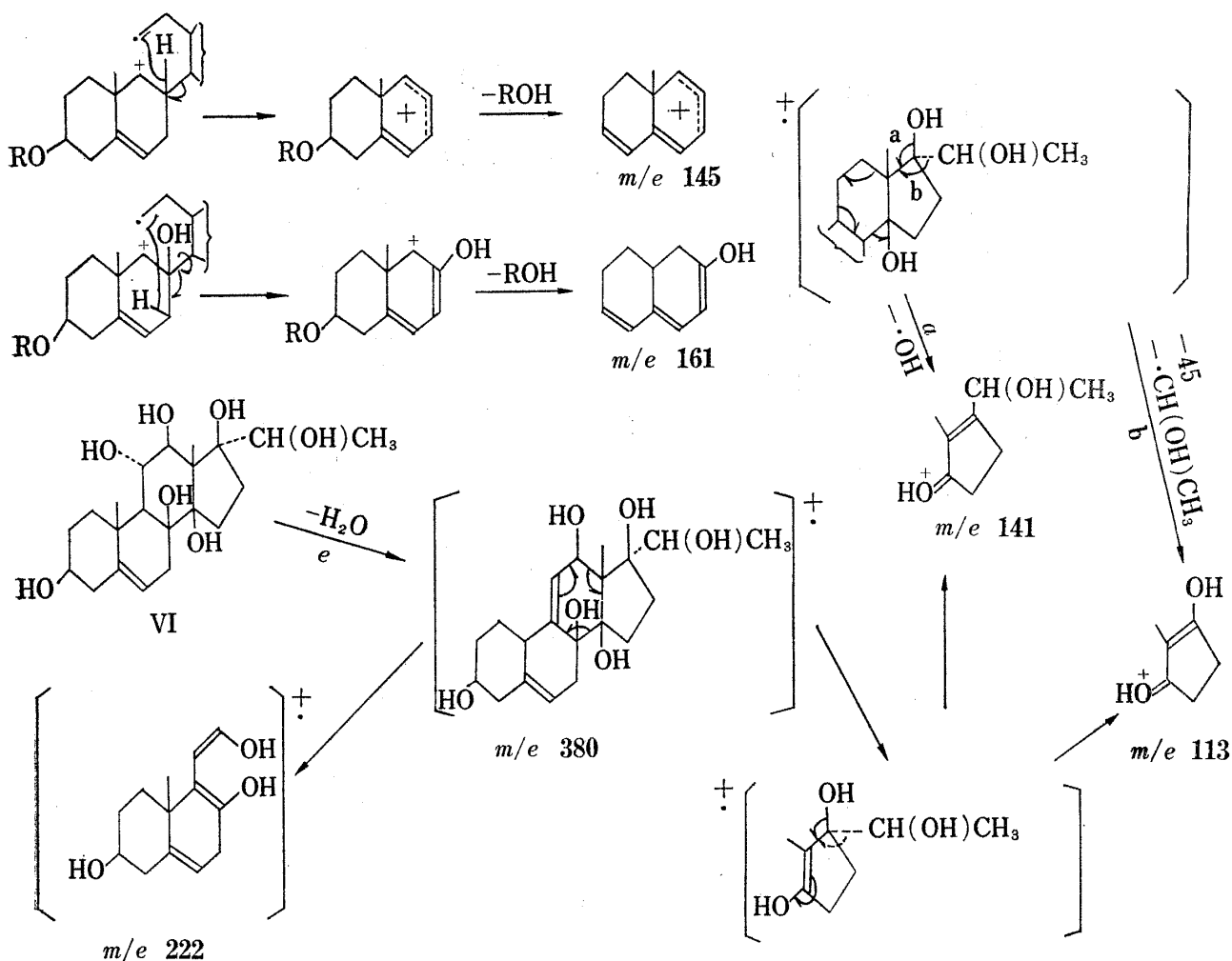


Chart 2

Stephanol, mp 222.5–225.5°, has the molecular formula of $C_{21}H_{34}O_7$ as shown by elemental analysis and mass spectrometry. The mass data (Fig. 1) were compared with that of utendin⁹⁾ (V), sarcostin (III), and our synthetic C/D-*cis* polyhydroxypregnanes, and the fragmentation was strongly suggestive of a steroid containing seven oxygen functions in the pregnane system (*cf.* Fig. 2). Assuming the abundant fragment ion ($M^+ - 45$) to originate by simple cleavage of the C-17-C-20 bond, the steroid ring would have to contain a glycol at C-17 and C-20 (evidences for the configurational assignments at the C-17 position will be discussed later).

The unsaturated character of stephanol (VI) was indicated by its infrared spectral absorptions at 865 and 805 cm^{-1} , ultraviolet spectrum which exhibited an endabsorption at 210 $m\mu$, and by the nuclear magnetic resonance (NMR) spectrum of stephanol triacetate (VII) which exhibited a vinyl proton adjacent to a CH_2 group at 5.5 ppm. The nature of the oxygen atoms in the molecule was clarified by subsequent observations. Stephanol shows the infrared bands at 3550, 3480, and 3350 cm^{-1} , and a positive for a metaperiodate-benzidine test,¹⁰⁾ and therefore contains at least one vicinal glycol.

9) The structure of utendin was synthetically established by us *cf.* M. Fukuoka and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1634 (1968).

10) a) J.A. Cifouelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954); b) M.R. Ardenne, R. Tümmeler, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **47**, 1032 (1964).

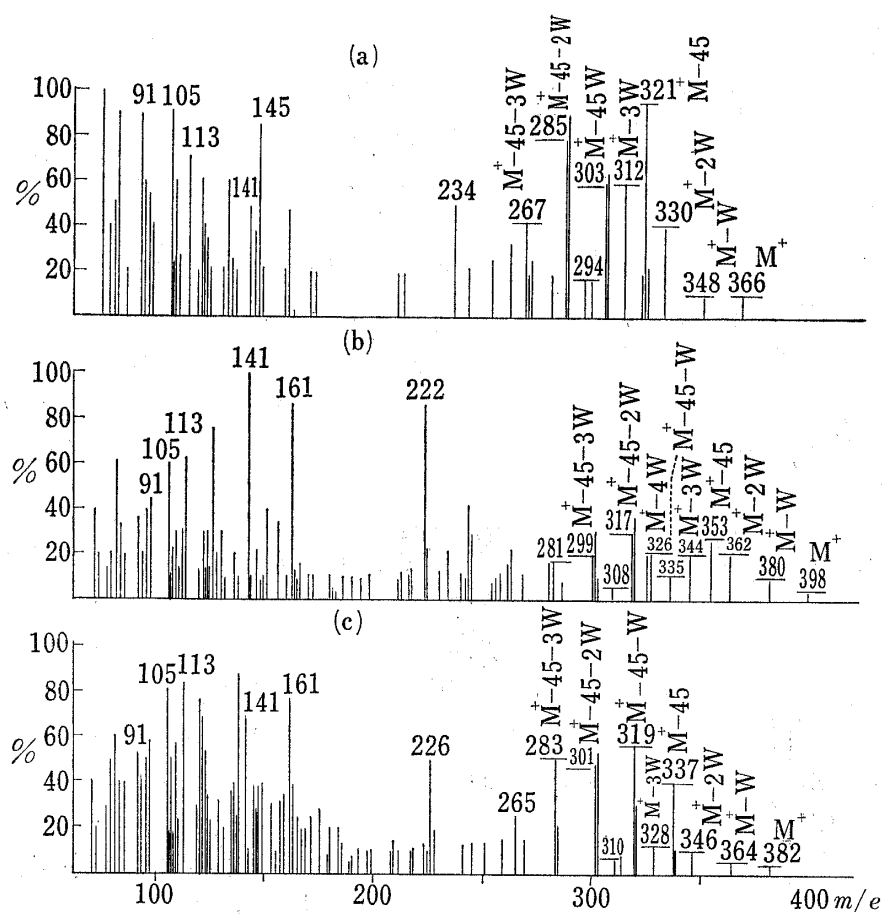


Fig. 1. Mass Spectra of (a) Utendin (V), (b) Stephanol (VI) and (c) Sarcostin

W=H₂O

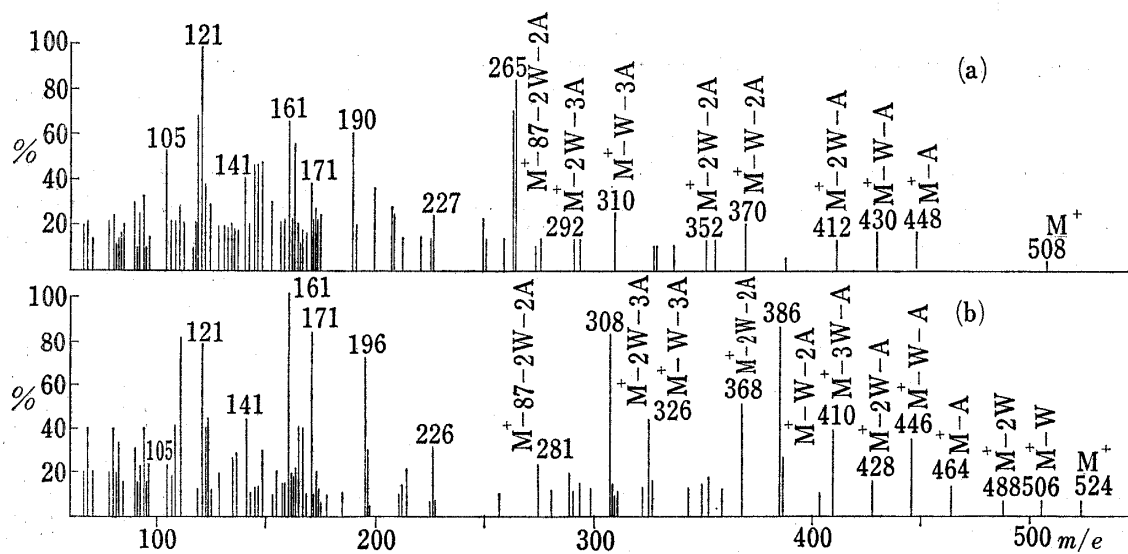
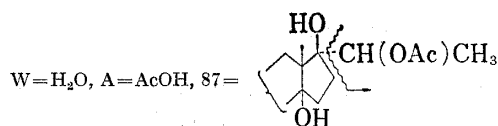


Fig. 2. Mass Spectra of (a) Sarcostin Triacetate (XI) and (b) Stephanol Triacetate (VII)



On acetylation with pyridine-acetic anhydride at room temperature, VI gave a corresponding triacetate (VII), and the drastic treatment of VII with pyridine-acetic anhydride at 150° for 10 hr in the sealed tube afforded a corresponding tetraacetate (VIII) whose infrared spectrum exhibited the hydroxyl absorption. The NMR spectrum of VII had signals due to one vinyl proton adjacent to a CH₂ group at 5.50 ppm, protons adjacent to a hydroxyl or acetoxy group [at 5.82 ppm (triplet, $J=9$ cps), 5.2 ppm (multiplet), 4.6 ppm (doublet, $J=9$ cps), and 4.6 ppm (half band width 15 cps)], acetate-methyl (at 2.20, 2.11, and 2.02 ppm), a secondary methyl (a doublet at 1.15 ppm $J=6$ cps), and two tertiary methyl groups (at 1.44 and 1.29 ppm), and a broad signal due to four hydroxyl protons disappeared by addition of deuterium oxide. The signal with a half-band width, 15 cps at 4.6 ppm, was inferred axially to be at C-3 from its chemical shift and coupling constant,¹¹⁾ and the behavior (coupling constants) of the signals at 5.82 and 4.60 ppm was due to the *trans*-diaxial protons in the RO-CH-CH-CH system.

Of the two possibilities (1 β ,2 α -diol or 11 α ,12 β -diol) for the position of this diol ($R=R'=H$) on the pregnane ring, the 11 α ,12 β -diol was favored by the following observation. The signal of an axial proton at C-12 α was in accordance with that observed for the derivatives of drevogenin^{10b)} and condurangogenin¹²⁾ which had a *trans* diol at C-11 α and C-12 β , and the signal of the proton at C-11 β appeared in the lower field than that of the proton observed in these derivatives. It was considered from this phenomenon that one of the remaining hydroxyl groups should be located at the position C-8 β which had been the 1:3-diaxial interrelation with the proton at C-11 β .

At this stage the only remaining feature of the structural elucidation of stephanol (VI) was the location of the remaining tertiary hydroxyl group. This group was assumed to be a hydroxyl group at C-14 from the absence of a signal of a proton adjacent to oxygen and from the fragment ions 113, 141, 161 and M^+-45 (Chart 2).

Having thus pointed out the positions (8 β , 14, 17) as the most probable site for three tertiary hydroxyl groups, it was our plan to bring about conclusive proof by making use of the oxidative cleavage reaction of a glycol and of Serini reaction of the C-17 side chain.

On oxidation with chromium trioxide in acetone, stephanol triacetate (VII) gave a compound (IX) which had an α,β -unsaturated and five-membered ring ketone, and hydroxyl groups from UV and IR spectral data. This product showed the negative Cotton effect (Fig. 3) due to an α,β -unsaturated ketone compound similar to 5 α -cholest-1-en-3-one and the compound (X),¹³⁾ and after solution in dilute alkali followed by acidification, showed a violet color with ferric chloride.¹⁴⁾ It is suggested that IX could be formed by oxidative cleavage of the C-8-14 bond, and migration of the double bond at C-5 to C-6, since we found that the same system could origi-

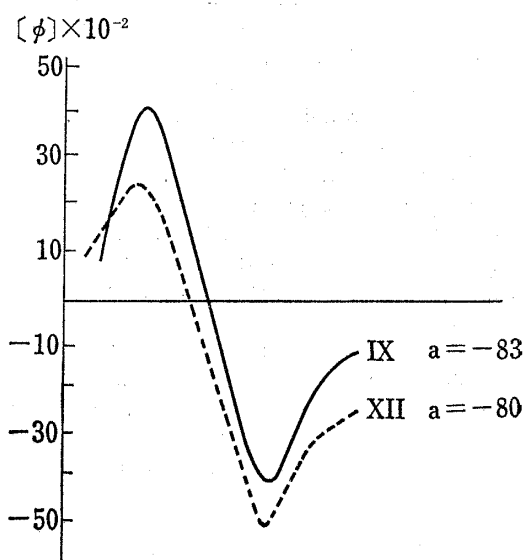


Fig. 3. Optical Rotatory Dispersion Spectra of the Compound IX and XII in MeOH Solution

11) From our NMR data of 35 C/D-*cis* pregnane compounds.

12) R. Tschesche, M. Baumgarth, and D. Welzel, *Tetrahedron*, **23**, 249 (1967).

13) C. Djerassi, R. Riniker, and B. Riniker, *J. Am. Chem. Soc.*, **78**, 6377 (1956).

14) J.W. Cornforth, *Chem. Ind. (London)*, **1959**, 602; H. Mitsuhashi and Y. Shimizu, *Chem. Pharm. Bull. (Tokyo)*, **8**, 719 (1962).

nate from sarcostin triacetate (XI) having a diol at C-8 β and C-14 β , and a double bond at C-5 (6), by the same oxidation and gave a compound (XII). From this result, stephanol (VI) would have a glycol at C-8 β and 14.

The configuration of two hydroxyl groups at C-14 and C-17 was considered to be 14 β - and 17 β -type since Serini reaction of VII gave a product (XIII) which showed a positive CD Cotton effect, and furthermore, the latter compound, after treatment with alkali, was identified with marsdenin¹⁵⁾ (XIV) by the multiple developed thin-layer chromatography using the methylene chloride:methanol or benzene:AcOEt solvent system.

From the considerations discussed above, we propose the tentative structure of formula VI for stephanol.

Experimental¹⁶⁾

Isolation of Glycosides—Glycosides (30 g) were isolated from the dried whole plant (2.7 kg), of *Stephanotis japonica* MAKINO by the procedure reported in an earlier paper.⁴⁾

Hydrolysis of Glycosides—A solution of the crude glycosides (15 g) in MeOH (500 ml) and 0.2N H₂SO₄ (167 ml) was refluxed for 25 min. After adding H₂O (501 ml), MeOH was evaporated *in vacuo* and the residue was extracted with CHCl₃. The CHCl₃ layer was washed with 5% NaHCO₃ solution and H₂O, and dried over Na₂SO₄. Removal of the solvent gave a crystalline powder (7.5 g) which was shown negative to the Keller–Kiliani reaction. The H₂O layer was neutralized with Ba(OH)₂ solution was extracted with BuOH–CHCl₃ (2:1). The organic solvent was removed *in vacuo* and the residue (2 g), after separation by the preparative thin-layer chromatography on Kiesel gel HF₂₅₄ Nach Stahl in the methanol:methylene chloride=1:10 solvent system, gave lineolon (96 mg), deacylmetaplexigenin (190.2 mg), and sarcostin (106 mg).

The above crystalline powder was hydrolysed with 5% methanolic KOH (200 ml) in N₂ atmosphere at 37° for 72 hr. After adding H₂O (200 ml), MeOH was removed *in vacuo*. The aqueous solution was extracted with BuOH, the BuOH layer was washed with H₂O (saturated with BuOH), and the solvent was evaporated to give a mixture of aglycones (4 g). The aqueous layer was acidified with H₃PO₄ and extracted with ether. Removal of the solvent gave an acidic substance which was identified with cinnamic acid by the mixed melting point and IR spectra. A mixture of aglycones, obtained twice by the above hydrolysis, was recrystallized from acetone to give sarcostin (3.5 g). The mother liquor was evaporated to dryness and the residue (4.5 g) was passed through a column of Al₂O₃ (100 g, 12 × 810 mm) to give the results shown in Table I.

Lineolon—The aglycone was recrystallized from acetone, mp 240–243°, ORD: (*c*=0.15) [α]_D²⁵ (peak) +1400°, [α]₃₀₄ (trough) –680°, [α]₃₈₉¹⁹ –30°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3450 (broad), 1680. Anal. Calcd. for C₂₁H₃₂O₅: C, 69.20; H, 8.85. Found: C, 69.12; H, 8.86. This compound was identified with an authentic sample⁵⁾ by the mixed mp and IR spectra.

Deacylmetaplexigenin (II)—The aglycone was recrystallized from AcOEt, mp 193–195°. ORD: (*c*=0.158), [α]_D²⁷⁰ (peak) 433.1°, [α]₃₁₆ (trough) –343.9°, [α]₃₈₉¹⁹ +38.2°. IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3380 (broad), 1685. Anal. Calcd. for C₂₁H₃₂O₆: C, 66.30; H, 8.48. Found: C, 66.09; H, 8.51. This compound was identified with an authentic sample⁶⁾ by the mixed mp and IR spectra.

Sarcostin (III)—The aglycone was recrystallized from MeOH, mp 150°/250–255°, [α]_D²³ +67° (*c*=1, MeOH). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3500. Anal. Calcd. for C₂₁H₃₄O₆: C, 65.94; H, 8.96. Found: C, 65.90; H, 8.87. This compound was identified with an authentic sample⁷⁾ by the mixed mp and IR spectra.

Sarcostin Triacetate (XI)—Sarcostin (99.3 mg) was acetylated with pyridine (1 ml) and Ac₂O (1 ml) at 32° for 3 days. The mixture was treated as usual. Recrystallization from ether–hexane gave XI (106 mg), mp 210–212°, [α]_D²³ +27° (*c*=1.0, CHCl₃). Anal. Calcd. for C₂₇H₄₀O₉: C, 63.76; H, 7.93. Found: C, 63.76; H, 7.87. This acetate was identified with an authentic sample⁷⁾ by the mixed mp and IR spectra.

5 α -Dihydrosarcostin (IV)—Fractions No. 31–38 were separated into eight compounds by preparative thin-layer chromatography (Kiesel gel-HF₂₅₄, developing solvent: CH₂Cl₂:MeOH=10:1). The most polar compound was recrystallized from AcOEt to 126 mg of 5 α -dihydrosarcostin (IV), mp 240–245°. [α]_D²⁰ +41.2° (*c*=1.03, MeOH). IR $\nu_{\max}^{\text{Nujol}}$ cm⁻¹: 3470. Anal. Calcd. for C₂₁H₃₆O₆: C, 65.59; H, 9.44. Found: C, 65.26; H, 9.25. The aglycone was identified with an authentic sample⁷⁾ by the mixed mp and IR spectra.

15) H. Mitsuhashi and K. Hayashi, Abstr. of Papers Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968, p. 262; *Chem. Pharm. Bull.* (Tokyo), **16**, 2522 (1968).

16) All melting points were measured with a Kofler Hot Stage Microscope and are uncorrected. NMR spectra were run on a Hitachi H-60 and Varian A-60 spectrometers using CDCl₃ as solvent and tetramethylsilane as an internal reference. ORD curves were run in MeOH with a JASCO Model ORD/UV-5.

TABLE I

Fraction No.	Solvent	Eluted product (mg)	Note
1—20	benzene	47.4	
21—30	CH ₂ Cl ₂	1186.0	11 compds
31—38	0.5—1 % MeOH-CH ₂ Cl ₂	440.0	III+IV+6 compds
39—47	1 —1.5% MeOH-CH ₂ Cl ₂	1000.0	III
48—59	1.5—3 % MeOH-CH ₂ Cl ₂	257.6	III+VI+unknown compd

5 α -Dihydrosarcostin (30.5 mg) was acetylated with pyridine (0.75 ml) and Ac₂O (0.5 ml) at room temperature for 3 days. The mixture was treated as usual. Recrystallization from acetone-hexane gave 5 α -dihydrosarcostin triacetate (25.25 mg), mp 238—241°, $[\alpha]_D^{25} +20.0^\circ$ ($c=1.0$, MeOH). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3560, 1735, 1250. Anal. Calcd. for C₂₇H₄₂O₉: C, 63.51; H, 8.29. Found: C, 63.35; H, 8.22. This acetate was identified with an authentic sample by the mixed mp and IR spectra.

The next polar compound was identified with sarcostin (96.6 mg).

Stephanol (VI)—Fractions No. 48—59 were combined and chromatographed on silicagel to separate sarcostin (VI) (111.3 mg). The purification of VI was carried on by the multiple development preparative thin-layer chromatography¹⁷⁾ (alumina G, developing solvent: 5% MeOH in CH₂Cl₂) and by recrystallization from acetone to 31.5 mg of (VI) mp 222.5—225.2°, $[\alpha]_D^{25} +37.5^\circ$ ($c=0.65$, MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ 210 m μ end-absorption (ϵ ca. 5000). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3550, 3480, 3350, 1110, 865, 805. Anal. Calcd. for C₂₁H₃₄O₇: C, 63.29; H, 8.60. Found: C, 63.30; H, 8.58.

Stephanol Triacetate (VII)—Stephanol (25 mg) was acetylated with pyridine (1 ml) and Ac₂O (1 ml) at room temperature for 3 days. The mixture was treated as usual. Recrystallization from acetone-hexane gave VII (24.8 mg), mp 138—140°, $[\alpha]_D^{25} +20.3^\circ$ ($c=0.89$, CHCl₃). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3500, 3450, 1735, 1250, 1235. NMR (ppm) 1.15 (3H, doublet, $J=6.0$ cps), 1.29 (3H), 1.44 (3H), 2.02, 2.11, 2.20 (acetate-CH₃), 4.60 (1H), 4.60 (1H doublet, $J=9$ cps), 5.20 (1H, multiplet), 5.50 (1H), 5.82 (1H, triplet, $J=9$ cps). Anal. Calcd. for C₂₇H₄₀O₁₀: C, 61.81; H, 7.69. Found: C, 61.80; H, 7.82.

Stephanol Tetraacetate (VIII)—VII (10mg) was treated with pyridine (0.3 ml) and Ac₂O (0.2 ml), in a tube sealed with N₂ at 150° for 10 hr. The mixture was treated as usual. The product was repeatedly purified by the multiple development preparative thin-layer chromatography¹⁷⁾ but could not be crystallized. Amorphous, 4 mg. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3550, 1730 (broad), 1240 (broad). NMR (ppm) 1.27 (3H, doublet, $J=6$ cps), 1.28 (3H), 1.34 (3H), 2.06, 2.11, 2.13, 2.14 (acetate-CH₃). Mass spectrum (MS): 566 (M⁺) 488, 470, 446, 428, 410, 386, 368, 350, 326, 308, 290, etc.

Hydrolysis of the Tetraacetate (VIII)—A solution of the tetraacetate (3.5 mg) in 5% MeOH-KOH (0.1 ml) was allowed to stand at 32° for 3 days. The reaction mixture was not treated as usual but was spotted on a paper for chromatography. The product was only one and was identified with stephanol (VI) from the comparison of R_f value and the green color with SbCl₅-CHCl₃ solution. Paper: Toyo Roshi No. 51, static phase: 20% HCONH₂-acetone, mobile phase:CHCl₃ saturated with HCONH₂. Developing time: 16 hr.

Oxidation of Stephanol Triacetate (VII)—To a solution of VII (5 mg) in acetone (1 ml), CrO₃-mixture¹⁸⁾ (0.02 ml) was added, and the whole was allowed to stand for 15 min at 0°. After destroying of the excess oxidant with MeOH and adding H₂O, the organic solvent was removed *in vacuo*, and the residue was extracted with CH₂Cl₂. The CH₂Cl₂ layer consisted of two compounds on thin-layer chromatography, and the main product was isolated by preparative thin-layer chromatography (Kiesel gel-HF₂₅₄, CH₂Cl₂:MeOH=200:1). Recrystallization from ether-hexane gave an amorphous crystals, mp 94—97° ORD: ($c=0.12$), $[\alpha]_{312}$ (peak) +812.5°, $[\alpha]_{352}$ (trough) -781.3°, $[\alpha]_{369}^{19} -41.6^\circ$. IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm⁻¹: 3500, 1745, 1735, 1725 (shoulder) 1680, 1250, and 795 cm⁻¹ was appeared in the KBr tablet). UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (log ϵ), 220 (4.05). NMR (ppm) 1.85 (3H), 1.30 (3H), 1.35 (3H, doublet, $J=6$ cps), 2.09, 2.14, 2.17 (acetate-CH₃). MS 522 (M⁺), 504, 462, 444, 426, 406, 384, 369, 366, etc. Anal. Calcd. for C₂₇H₃₈O₁₀: C, 62.05; H, 7.33. Found: C, 62.25; H, 7.34.

Oxidation of Sarcostin Triacetate (XI)—Sarcostin triacetate (XI) was oxidized with acetone (2 ml) and the CrO₃-mixture¹⁸⁾ (0.04 ml) under the same condition as described above. After separating the main product¹⁴⁾ by multiple preparative thin-layer chromatography,¹⁷⁾ purification of the minor product was carried out by multiple preparative thin-layer chromatograph¹⁷⁾ but the product was amorphous, ORD: ($c=0.075$), $[\alpha]_{309}$ (peak) +500°, $[\alpha]_{353}$ (trough) -1080°. IR $\nu_{\text{max}}^{\text{EtOH}}$ cm⁻¹: 3550, 1745, 1735, 1725 (shoulder), 1680, 1250. UV $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (log ϵ): 221 (4.0). MS: 506 (M⁺), 488, 446, 428, 386, 326, etc. Anal. Calcd. for C₂₇H₃₈O₉: C, 64.01; H, 7.56. Found: C, 63.98; H, 7.53.

17) The thin-layer plate was repeatedly developed and dried.

18) A solution of CrO₃ (5.3 g), H₂SO₄ (8.0 g), and H₂O (40 ml).

Serini Reaction of Stephanol Triacetate (VII)—To a solution of VII (2.1 mg) in absolute xylene (2 ml), activated Zn (456 mg) was added. The reaction mixture was refluxed in N_2 atmosphere for 24 hr. Zn was separated by filtration and removal of the solvent gave a mixture with a positive ORD Cotton effect (300/270 $m\mu$, peak/trough). The mixture consisted of several compounds and the starting material, from the result of thin-layer chromatography. The mixture was allowed to stand in 5% MeOH-KOH (1 ml) for 24 hr at room temperature and separated into stephanol and an unknown compound by the multiple development preparative thin-layer chromatography¹⁷⁾ (Kiesel gel-HF₂₅₄, CH_2Cl_2 :MeOH=20:1). The unknown compound was identified with marsdenin by the multiple development thin-layer chromatography (Kiesel gel-HF₂₅₄ developed 8 times, with CH_2Cl_2 :MeOH=20:1 and benzene:AcOEt=1:1).

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