[Chem. Pharm. Bull. 23(8)1845—1851(1975)]

UDC 547.92.02 : 581.192

Studies on the Constituents of Asclepiadaceae Plants. XXXIV.[1] Chemistry of Sarcostin

Koji Hayashi and Hiroshi Mitsuhashi

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received January 16, 1975)

(a) Partial acetylation of sarcostin (**1**) gave two diacetates; 3,12-O-acetate (**4**) and 3,20-di-O-acetate (**3**) in 3 : 1 ratio, besides 3-mono-O-acetate (**5**) and 3,12,20-tri-O-acetate (**2**). On the other hand, benzoylation of **5** with equimolar amount of benzoyl chloride gave its 20-O-benzoate (**6**). (b) Treatment of **1** with paraldehyde under acid catalytic conditions afforded 14,17-12,20-di-O-ethylidene-sarcostin (**14**). (c) The configuration at C-20 position of **1** is *S* on the basis of the X-ray analysis of 3-O-*p*-bromobenzoate (**16**). The *o*-nitrobenzoate (**17**) showed a negative Cotton effect contrary to the expectation from Nagai's data but the 14,17-O-ethylidene derivative (**18**) of **17** showed a positive Cotton effect. (d) The configuration of the C-20 carbinol derived from sodium borohydride reduction of the corresponding ketone is ascribable to the stereochemistry at C-17 of the ketone. The 20-O-*o*-nitrobenzoate (**24**) derived from the sodium borohydride reduction product of lineolon diacetate (**25**) shows a positive Cotton effect while the *o*-nitrobenzoate (**26**) from isolineolon diacetate (**27**) shows a negative effect.

Sarcostin (**1**), 3β,8β,12β,14β,20α-hexahydroxypregn-5-ene, is one of the commonest C/D-*cis* polyoxypregnane derivatives in Asclepiadaceae plants. The structure of **1** has been proposed by Reichstein's group[3] and by Mitsuhashi and Shimizu[4] except for the configuration at the 20 position. In 1967, Reichstein suggested S configuration at C-20, although no detailed information was given.[5] The diesters of **1** at 12 and 20 positions with various carboxylic acids have been isolated, but the determination of the position of each linkage in the diesters with different carboxylic acids is relatively difficult and has not been achieved by means of spectroscopy. C-20 configuration of most of polyoxypregnanes with C-20 carbinol has not been determined.

From these facts, the difference in reactivity of the hydroxyl groups of **1**, and the optical rotatory dispersion (ORD) examination of C-20-O-*o*-nitro-benzoyl derivatives of **1** were made.

Acylation of Sarcostin (1)

In order to compare the reactivity to acylations among secondary hydroxyl groups at 3, 12, and 20 positions in **1**, acetylation was carried out first with a suitable amount of acetic anhydride in pyridine at room temperature and stopped when the starting material disappeared, resulting in the formation of four different acetates; 3,12,20-triacetate (**2**), 3,20-diacetate (**3**), 3,12-diacetate (**4**), and 3-monoacetate (**5**). The nuclear magnetic resonance (NMR) spectrum of **3** showed signals assignable for C-12α proton at δ 3.52 ppm as a double doublet (J=3.6 and 10.2 Hz) and of C-20 proton at 5.26 ppm as a quartet (J=6.6 Hz), whereas that of **4** showed a signals for 12 proton at 4.60 ppm as a double doublet (J=5.4 and 9.6 Hz) and for C-20 proton at 3.57 as a quartet (J=6.6 Hz). Yields of **2** to **5** are given in Table I, which shows the ratio of **3** to **4** formed to be 1 : 3. These facts indicate that the acetylation of C-12 hydroxyl group occurred more easily than that of C-20 hydroxyl group, in spite of relatively

1) Part XXXIII: H. Seto, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 1552 (1975).
2) Location: *Nishi-6-chome, Kita-12-jo, Kita-ku, Sapporo, 060, Japan.*
3) K.A. Jaeggi, E.K. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 694 (1963).
4) Y. Shimizu and H. Mitsuhashi, *Tetrahedron*, **24**, 4143 (1968).
5) T. Reichstein, *Naturwissenschaften*, **54**, 53 (1967).

less hindered position of the latter compared to the former. Actually, benzoylation of **5** afforded its C-20 benzoate (**6**). Reichstein, *et al.* also reported the benzoylation of **1** to give only 3,20-dibenzoate (**7**) other than tribenzoate[6] (**8**). There was a difference in the reactivity between 12 and 20 hydroxyl groups on acetylation and benzoylation. Since C-20 position is less hindered than C-12 position, results of acetylation of **1** were unexplainable enough. It appeares that the hydroxyl group at 17 has little influence on acetylation of **1** since the acetylation of 20-dihydrolineolon (**9**) corresponding to 17-deoxy-**1** gave similar results; 3,12,20-triacetate in 21% yield, 3,20-diacetate (**11**) in 10.9%, 3,12-diacetate (**12**) in 20.6%, and 3-monoacetate (**13**) in 47.4%.

Table I. Yields of Sarcostin Acetates

Acetic anhydride	2	3	4	5 (%)
1.2 mole	—	—	—	98
2.5 mole	4	10	30	25
10.0 mole	33	9	29	4

Reaction of 1 with Paraldehyde

The next project was focussed on the masking of hydroxyl groups except that at C-12 for the introduction of a suitable substituent at 11 position. The suspension of **1** in paraldehyde with a catalytic amount of *p*-toluenesulfonic acid afforded a bis (ethylidene) derivative (**14**), $C_{25}H_{38}O_6$, in 68% yield, whose NMR spectrum showed signals due to the two ethylidene groups at δ 1.35 ppm (two doublet methyls, J=5 Hz), and at 4.66 and 5.16 ppm (each quartet methine, J=5 Hz). Benzoylation of **14** gave a monobenzoate (**15**) which showed hydroxyl band at 3580 cm^{-1} in its infrared (IR) spectrum. The benzoyl group at C-3 was confirmed from the chemical shift of C-3α proton at δ 4.78 (broad multiplet).

The benzoate (**15**) resisted Saret's and Jones' oxidation, and dehydration with thionyl chloride and/or phosphoryl chloride in pyridine. These facts indicate that the hydroxyl group remaining in **15** is a very hindered tertiary hydroxyl group which probably corresponds to 8β-hydroxyl group. Therefore, C-12 hydroxyl group takes part in the formation of an ethylidene group with C-20 hydroxyl since the distance between the two oxygens in these hydroxyl groups is about 2.4—2.6Å from examination of the Dreiding model. Another ethylidene group is located at C-14 and 17 hydroxyl groups which protrude over the D-ring with a 1—3 diaxial relationship. Attempts to mask hydroxyl groups except that at 12 position in **1** failed, but the formation of a seven-membered cyclic ethylidene ring is an interesting fact, together with the acyl migration between 12- and 20-hydroxyl groups.[7]

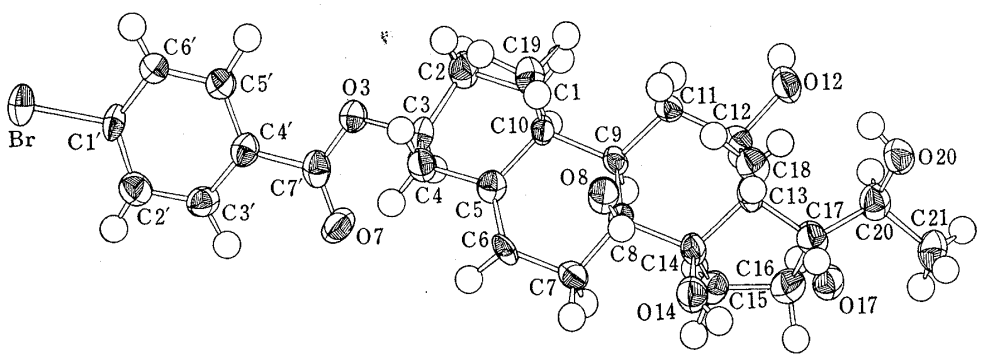


Fig. 1. Atomic Model of 3-O-*p*-Bromobenzoylsarcostin

6) F. Schaub, H. Kaufmann, W. Stocklin, and T. Reichstein, *Helv. Chim. Acta*, **51**, 738 (1968).
7) T. Yamagishi, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 2289 (1972).

Stereochemistry at 20 Position and the Optical Rotatory Dispersion of Its *o*-Nitrobenzoate

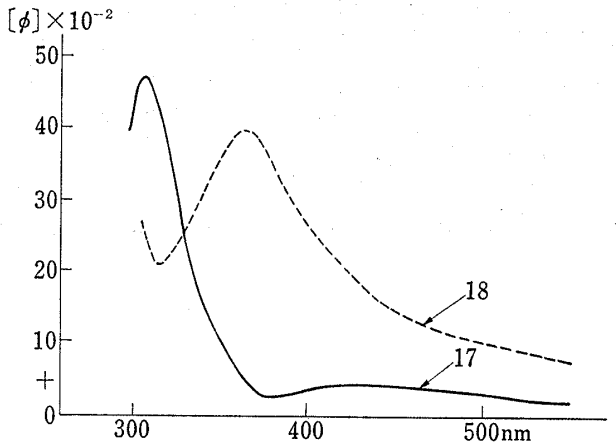


Fig. 2. ORD Curves of **17** and **18**

A number of C/D-*cis*-polyoxypregnane derivatives possessing a carbinol at C-20 has been isolated from Asclepiadaceae plants. The configuration at C-20 was confirmed in a few of these compounds.

It is known that xanthate or nitrite is used for the determination of the configuration of assymmetric secondary alcohol by means of ORD measurement in addition to the benzoate sector rule. On the other hand, Nagai[8] reported that the *o*-nitrobenzoate of assymmetric secondary alcohol shows a Cotton effect on the basis of the $n\rightarrow\pi^*$ transition of aromatic nitro group at *ca.* 330 nm in methanol solution, and that the *o*-nitro-benzoate with *R*-configuration shows a negative Cotton effect, while the others with *S* shows a positive effect. This method was selected to determine the configuration at C-20 in C/D-*cis* polyoxypregnane because of mild conditions of *o*-nitrobenzoylation. Before using this method, the absolute configuration of **1** was confirmed by the X-ray analysis of its 3-O-*p*-bromobenzoate (**16**) which showed *S* configuration at C-20.[9] Contrary to the expectation from Nagai's data, however, the *o*-nitrobenzoate (**17**) from **4** showed a negative Cotton effect. It is considered that 17β-hydroxyl group has influence on the Cotton effect. In order to mask the hydroxyl group, **17** was treated in paraldehyde with boron trifluoride ethereate as a catalyst to give a cyclic *O*-ethylidene derivative (**18**), which showed a positive Cotton effect. These facts indicate that, although the polar functional group around the *o*-nitrobenzoyl group has a strong effect on the Cotton effect, configuration of the secondary carbinol group can be determined by a suitable protection of the neighbouring polar group.

Configuration of the Sodium Borohydride Reduction Product of 20-Ketosteroid

It has been well known that sodium borohydride reduction of the 20-ketone gives the 20β-alcohol (*R* configuration) as the major product[10] and also that the Cotton effect of the

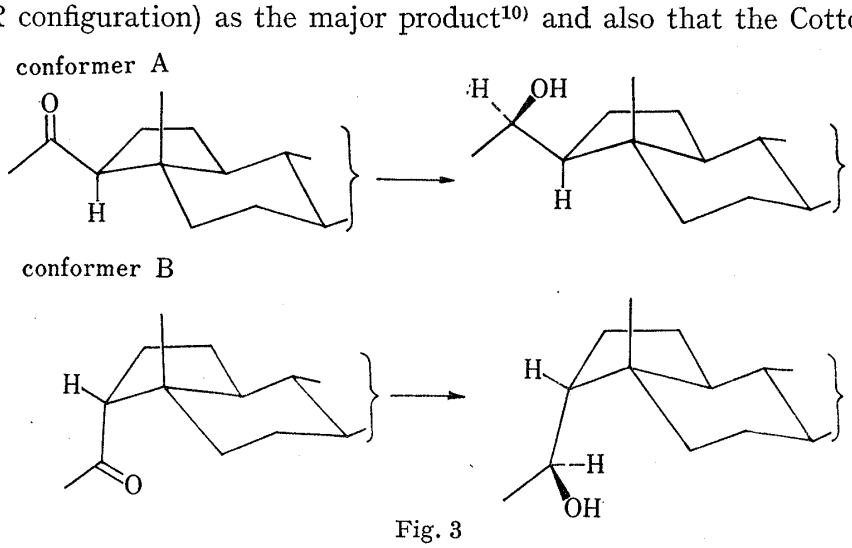
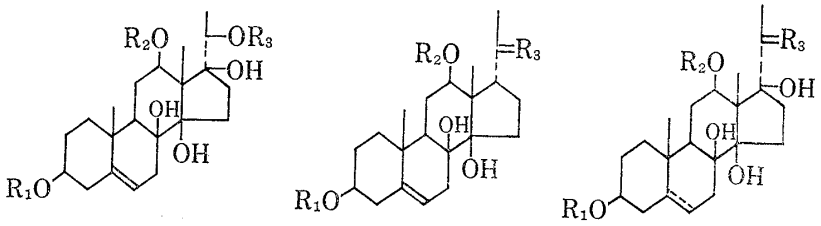


Fig. 3

8) U. Nagai and F. Iga, *Tetrahedron*, **26**, 725 (1971).
9) H. Mitsuhashi, T. Yamagishi, K. Hayashi, and H. Koyama, unpublished data.
10) D.M.S. Wheeler and M.M. Wheeler, "Organic Reactions in Steroid Chemistry," Vol. 1, ed. by J. Fried and J.A. Edwards, Van Nostrand-Reinhold Co., New York, 1972, p. 61.

ketone is positive to the 17β-side chain but negative to the 17α-side chain.[11] From the octant rule, it is considered that the ketone with a 17β-side chain has preferential conformer A whereas another with a 17α-side chain has B shown in Fig. 3. In the former case, the reducing agent attacks from less hindered side of the ketone (under side of the paper) to give a β-alcohol (R configuration). Kirk described a similar interpretation from the product analysis.[12]

In contrast with this, the latter ketone probably gives α-alcohol (S configuration). Sarcostin (**1**), possessing S configuration at 20, is indeed obtained from sodium borohydride reduc-



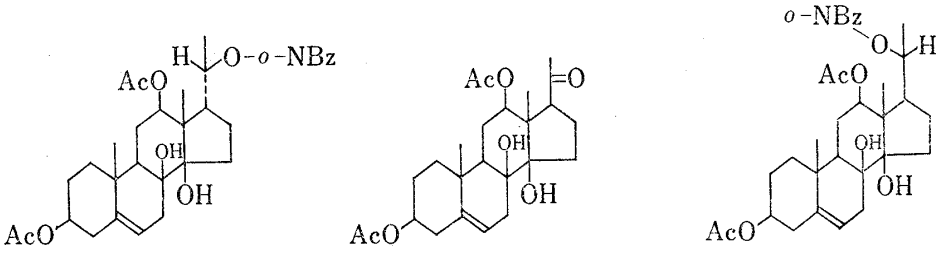
1: $R_1=R_2=R_3=H$
2: $R_1=R_2=R_3=Ac$
3: $R_1=R_3=Ac$, $R_2=H$
4: $R_1=R_2=Ac$, $R_3=H$
5: $R_1=Ac$, $R_2=R_3=H$
6: $R_1=Ac$, $R_2=H$, $R_3=Bz$
7: $R_1=R_3=Bz$, $R_2=H$
8: $R_1=R_2=R_3=Bz$
16: $R_1=p$.Br.Bz, $R_2=R_3=H$
17: $R_1=R_2=Ac$, $R_3=o$-NO_2Bz
20: $R_1=R_3=H$, $R_2=$Cin.

9: $R_1=R_2=H$, $R_3=\langle^{H}_{OH}$
10: $R_1=R_2=Ac$, $R_3=\langle^{H}_{OAc}$
11: $R_1=Ac$, $R_2=H$, $R_3=\langle^{H}_{OAc}$
12: $R_1=R_2=Ac$, $R_3=\langle^{H}_{OAc}$
13: $R_1=Ac$, $R_2=H$, $R_3=\langle^{H}_{OH}$
25: $R_1=R_2=Ac$, $R_3=O$

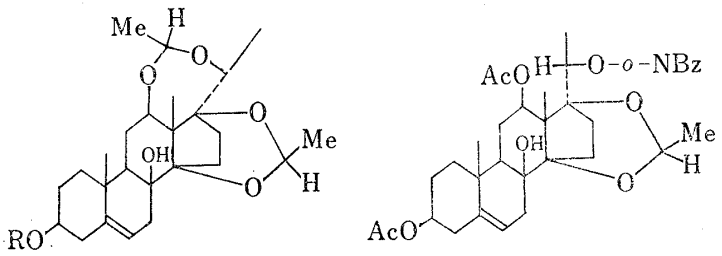
19: $R_1=R_2=H$, $R_3=O$, Δ^5
21: $R_1=R_2=H$, $R_3=\langle^{H}_{OH}$, 5αH
22: $R_1=H$, $R_2=$Cin, $R_3=O$, Δ^5
23: $R_1=R_2=H$, $R_3=O$, 5αH

Bz=benzoyl
o-NO_2Bz=o-nitrobenzoyl
Cin=cinnamoyl
p.Br.Bz=p.bromobenzoyl

Chart 1



24 **27** **26**



14: R=H
15: R=Bz

18

Bz=benzoyl
o-NBz=o-nitrobenzoyl

Chart 2

11) C. Djerassi, "Optical Rotatory Dispersion to Organic Chemistry," MacGraw-Hill Book Co., New York, 1960.
12) Kirk, "Steroid Reaction Mechanisms," Chapt. 4, Elsevier Publishing Co., Amsterdam, 1968.

tion of deacyl-metaplexigenin[13] (**19**). Penupogenin (**20**) and dihydrosarcostin (**21**) were also obtained from Kidjoranin[14] (**22**) and tayloron[3] (**23**) respectively. These facts support our deduction. Therefore, stereochemistry of the C-20 carbinol obtained by sodium borohydride reduction of the corresponding ketone is ascribed to the configuration at C-17 of the ketone regardless of C/D ring juncture. To clarify this point, the *o*-nitrobenzoate (**24**) from sodium borohydride reduction of lineolon diacetate (**25**) followed by treatment with *o*-nitrobenzoyl chloride in pyridine showed a positive Cotton effect indicating that C-20 configuration is *S*. On the other hand, the other *o*-nitrobenzoate (**26**) formed in a similar manner from isolineolon diacetate (**27**) showed a negative Cotton effect indicating *R* configuration at C-20. These results agree with our expectation.

Experimental

Melting points were determined using a Kofler hot stage and are uncorrected. IR spectra were recorded on a Hitachi 215 grating photometer using Nujol mull. NMR spectra were recorded using a JEOL-PS-100 spectrometer with tetramethylsilane as the internal standard. ORD curves were obtained on a JASCO Model ORD/UV-5. Sarcostin and lineolon used were obtained from *Cynanchum caudatum* and from *C. wilfordi*.

Acetylation of Sarcostin (I)——General Procedures: To a solution of sarcostin (**1**) in pyridine, acetic anhydride was added and kept at room temperature. When 1 disappeared from the mixture, the mixture was poured into ice-water and extracted with chloroform. The aqueous layer was extracted again by means of continuous extraction with methylene chloride. The organic layers were combined and concentrated to dryness. The residue was recrystallized or column chromatographed over silica gel eluted with chloroform. These results are given in Table I.

3-Monoacetate (**5**): From methanol as needles, mp 230—234°. $[\alpha]_D$ +62.6° (c=0.242, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3530, 3470, 3380, 3330, 1722, 1270. NMR ($CDCl_3$): δ 1.18 (d, J=7.5 Hz, 3H), 1.22 (s, 3H), 1.36 (s, 3H), 2.05 (s, 3H), 3.59 (d.d, J=9 and 5 Hz), 4.05 (q, J=7.5 Hz, 1H), 4.58 (m, 1H), 5.39 (br. s, 1H). *Anal.* Calcd. for $C_{23}H_{36}O_7$: C, 65.07; H, 8.55. Found: C, 64.93; H, 8.60.

3,20-Diacetate (**3**): From acetone-hexane as prisms, mp 222—229°. $[\alpha]_D$ +8.3° (c=0.22, MeOH). RI ν_{max}^{Nujol} cm^{-1}: 3540, 1745, 1716, 1260, 1240. NMR (Py-d_5: $CDCl_3$=1: 5): δ 1.23 (s, 3H), 1.31 (d, J=6.6 Hz, 3H), 1.50 (s, 3H), 1.94 (s, 3H), 2.00 (s, 3H), 3.52 (d.d, J=3.6 and 10.2 Hz, 1H), 5.26 (q, J=6.6 Hz, 1H), 5.36 (m, 1H). *Anal.* Calcd. for $C_{25}H_{38}O_8$: C, 64.43; H, 8.19. Found: C, 64.36; H, 8.21.

3,12-Diacetate (**4**): From methanol–chloroform–hexane as prisms, mp 152—156°. $[\alpha]_D$ +44.1° (c= 0.23, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3520, 3420, 1740, 1715, 1245. NMR ($CDCl_3$): δ 1.12 (d, J=6.6 Hz, 3H), 1.17 (s, 3H), 1.45 (s, 3H), 2.01 (s, 3H), 2.10 (s, 3H), 3.57 (q, J=6.6 Hz, 1H), 4.60 (d.d, J=5.4 and 9.6 Hz, 1H), 5.38 (m, 1H). *Anal.* Calcd. for $C_{25}H_{38}O_8$–CH_3OH: C, 62.63; H, 8.49. Found: C, 62.69; H, 8.57.

Triacetate (**2**) from acetone-hexane as prisms, mp 204—205°, $[\alpha]_D$ +20.1° was confirmed by comparison with authentic samples.

Benzoylation of 5——To a solution of 182 mg of 5 in 2 ml of pyridine, 0.5 ml of benzoyl chloride was added. The mixture was kept to stand for 5 hr, poured into water, and extracted with ether. The organic layer was treated in a usual manner, dried over sodium sulfate, and the solvent was evaporated. The residue was purified by preparative thin-layer chromatography (TLC) to give 54 mg of 6 as colorless needles from hexane-ethyl acetate, mp 198—204°. IR ν_{max}^{Nujol} cm^{-1}: 3550, 1720, 1600, 1500, 1260. NMR ($CDCl_3$): δ 1.15 (s, 3H), 1.37 (d, J=6 Hz, 3H), 1.40 (s, 3H), 2.04 (s, 3H), 3.57 (d.d, J=11 and 5.5 Hz, 1H), 4.57 (m, 1H), 5.41 (m, 1H), 5.50 (q, J=6 Hz, 1H), 7.5—8.0 (m, 5H). *Anal.* Calcd. for $C_{30}H_{40}O_8$: C, 65.65; H, 11.02. Found: C, 65.49; H, 10.97.

Acetylation of 20-Dihydrolineolon (**9**)——To a solution of 239.5 mg of 20-dihydrolineolon (**9**) in 1.5 ml of pyridine, 0.29 ml (5 molar equivalent) of acetic anhydride was added and the mixture was kept to stand over night at room temperature. The solution was poured into water and extracted continuously with chloroform. After removal of the solvent *in vacuo* from the extract, the residue was submitted to preparative TLC to give in order of the mobility; **10** from methanol as prisms, mp 107—113°. $[\alpha]_D$ +29.4° (c=0.381, MeOH). *Anal.* Calcd. for $C_{27}H_{40}O_8$: C, 75.83; H, 8.19. Found: C, 65.97; H, 8.11. 3,20-Diacetate (**11**) from acetone–hexane as prisms, mp 147—150°. NMR ($CDCl_3$): δ 1.18 (s, 3H), 1.22 (d, J=6 Hz, 3H), 1.27 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 3.62 (d.d, J=4 and 12 Hz, 1H), 4.64 (br, m, 1H), 5.13 (d.q, J=10 and 6 Hz, 1H), 5.39 (m, 1H). *Anal.* Calcd. for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.39; H, 8.77. 3,12-Diacetate (**12**) from acetone–hexane as prisms, mp 219—224°. $[\alpha]_D$ +31.8° (c=0.418, MeOH). NMR ($CDCl_3$): δ 1.11

13) H. Mitsuhashi and T. Nomura, *Chem. Pharm. Bull.* (Tokyo), **13**, 274 (1965).
14) T. Sasaki, K. Hayashi, and H. Mitsuhashi, *Chem. Pharm. Bull.* (Tokyo), **20**, 628 (1972).

(d, J=6 Hz, 3H), 1.17 (s, 3H), 1.44 (s, 3H), 2.04 (s, 3H), 2.12 (s, 3H), 3.50 (d.q, J=10 and 6 Hz, 1H), 4.61 (m, 1H), 4.75 (d.d, J=4 and 11 Hz, 1H), 5.37 (m, 1H), and 3-monoacetate (**13**) from methanol as needles, mp 245—248°. *Anal.* Calcd. for $C_{23}H_{36}O_6$: C, 67.62; H, 8.88. Found: C, 67.74; H, 8.92.

Reaction of Sarcostin (1) with Paraldehyde——Sarcostin (**1**) (111 mg) was suspended in 6 ml of paraldehyde and 9 mg of *p*-toluenesulfonic acid was added to the mixture. After stirring of the mixture for 24 hr, 0.5 g of anhydrous potassium carbonate was added and the mixture was stirred again for 5 min. Excess potassium carbonate was filtered off and the filtrate was concentrated. The residual syrup was purified by preparative TLC to give 75.9 mg of **14** as colorless fine needles (from methanol), mp 165°/198—205°. $[\alpha]_D$ +43.8° (c=0.737, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3550, 3350, 1240, 1155, 1130, 1100. NMR ($CDCl_3$): δ 1.16 (d, J=7 Hz, 3H), 1.21 (s, 3H), 1.35 (d, J=5 Hz, 6H), 1.47 (s, 3H), 3.47 (m, 1H), 3.54 (d.d, J=5 and 11 Hz, 1H), 4.07 (q, J=7 Hz, 1H), 4.66 (q, J=5 Hz, 1H), 5.16 (q, J=5 Hz, 1H), 5.33 (m, 1H). *Anal.* Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 69.10; H, 8.77.

Benzoylation of 14——To a solution of 100 mg of **14** in 1.5 ml of pyridine, 0.9 ml of benzoyl chloride was added. The mixture was stirred for 20 min, poured into ice-water, and extracted with ether. The ether solution was washed successively, with 2N hydrochloric acid, 5% sodium bicarbonate solution and water, and dried over sodium sulfate. After evaporation of the solvent, the residue was purified by preparative TLC to give 65 mg of **15** as colorless needles (from hexane–acetone), mp 239—246°. IR ν_{max}^{Nujol} cm^{-1}: 3580, 1710, 1600, 1580, 1285, 1120. NMR ($CDCl_3$): δ 1.13 (d, J=7.2 Hz, 3H), 1.23 (s, 3H), 1.26 (s, 3H), 1.40 (d, J=5 Hz, 6H), 3.52 (d.d, J=5 and 10 Hz, 1H), 4.03 (q, J=7.2 Hz, 1H), 4.62 (q, J=5 Hz, 1H), 4.71 (m, 1H), 5.11 (q, J=5 Hz, 1H), 5.37 (m, 1H), 7.4—8.0 (m, 5H). *Anal.* Calcd. for $C_{32}H_{42}O_7$: C, 71.35; H, 7.86. Found: C, 71.63; H, 7.93.

***o*-Nitrobenzoylation of Sarcostin 3,12-Diacetate** (4)——To a solution of 148 mg of **4** in 2 ml of pyridine, 50 mg of *o*-nitrobenzoyl chloride was added and the mixture was stirred for 16 hr at room temperature. The mixture was poured into ice-water and extracted with ether. The ether solution was washed successively with 2N hydrochloric acid, 5% sodium bicarbonate solution and water, and dried over sodium sulfate. After removal of the solvent, the residue was recrystallized from methanol to give 56.4 mg of **17** as fine needles and, in addition, further purification of the mother liquor by preparative TLC gave 47.2 mg of **17**. Compound (**17**): mp 257—259°. IR ν_{max}^{Nujol} cm^{-1}: 3600, 3580, 1730, 1600, 1580, 1553 (strong), 1270, 1260, 1250. ORD (c=0.187, MeOH) [ϕ] (nm): 329° (400), 263° (380 trough), 953° (350), 2433° (330), 4734° (305, peak), 3484° (295), molecular amplitude −44.71. *Anal.* Calcd. for $C_{32}H_{41}O_{11}N$: C, 62.33; H, 6.71; N, 2.27. Found: C, 61.98; H, 6.81; N, 2.17.

Reaction of 17 with Paraldehyde——The compound (**17**) (260 mg) was suspended in 6 ml of paraldehyde and to the mixture, three drops of boron trifluoride ethereate was added. After stirring of the mixture for 1.5 hr, 0.3 g of potassium carbonate was added. Excess potassium carbonate was filtered off, the filtrate was concentrated, and purified by preparative TLC to give 135.5 mg of an ethylidene compound (**18**). NMR ($CDCl_3$): δ 1.19 (s, 3H), 1.33 (d, J=7 Hz, 3H), 1.43 (d, J=5 Hz, 3H), 1.51 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 4.4—4.9 (m, 2H), 5.02 (q, J=7 Hz, 1H), 5.29 (q, J=5 Hz, 1H), 5.38 (m, 1H), 7.6—7.9 (m, 4H). ORD (c= 0.424, MeOH), [ϕ] (nm): 4279° (400), 6566° (366, peak), 5769° (350), 5115° (335, trough), 5721° (320), molecular amplitude +18.90. *Anal.* Calcd. for $C_{34}H_{43}O_{11}N$: C, 63.63; H, 6.75; N, 2.18. Found: C, 63.55; H, 6.95; N, 1.97.

***o*-Nitrobenzoate (24) from Lineolon Diacetate** (25)——Lineolon diacetate (**25**) was dissolved in 5 ml of methanol and 72 mg of sodium borohydride was added to the mixture, which was stirred for 15 min. After decomposition of the excess reagents with acetic acid, the mixture was diluted with 70 ml of water and extracted with ether. The ether soln was washed five times with water, and dried over magnesium sulfate. Removal of the solvent afforded 154.2 mg of foamy substance identified with **12** by TLC analysis. To a solution of 128.5 mg of this substance in 2.5 ml of pyridine, *o*-nitrobenzoyl chloride (*ca.* 120 mg) was added. The mixture was stirred for 26 hr at room temp. then poured into ice-water, and extracted with ether. After usual work and removal of the solvent, the residue obtained was purified by preparative TLC to give 87.5 mg of **24** from methanol as needles, mp 180.5—182.5°. IR ν_{max}^{Nujol} cm^{-1}: 3550, 1730, 1600, 1553 (strong), 1290, 1270, 1255. NMR ($CDCl_3$): δ 1.15 (s, 3H), 1.30 (d, J=6 Hz, 3H), 1.32 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 4.60 (m, 1H), 4.79 (d.d, J=4 and 10 Hz, 1H), 5.03 (d.q, J=10 and 6 Hz, 1H), 5.38 (m, 1H), 7.6—7.94 (m, 4H). ORD (c=0.328, MeOH) [ϕ] (nm): 3175° (400), 4960° (362, peak), 4517° (350), 1014° (309, trough), 1604° (300), molecular amplitude +38.97. *Anal.* Calcd. for $C_{32}H_{41}O_{10}N$: C, 64.09; H, 6.89; N, 2.34. Found: C, 63.98; H, 6.69; N, 2.41.

***o*-Nitrobenzoate (26) from Isolineolon Diacetate** (27)——To a solution of 273 mg of isolineolon diacetate (**27**) in 5 ml of methanol, 98 mg of sodium borohydride was added, the mixture was stirred for 15 min, poured into water, and extracted with ether. The ether solution was worked up as usual and then the solvent was removed. The residue was dissolved in 4 ml of pyridine and 220 mg of *o*-nitrobenzoyl chloride was added to the solution, which was stirred for 16 hr. The mixture was poured into water and extracted with ether. After usual work up and removal of the solvent, the residue was purified by preparative TLC to give 57 mg of **26** as a foamy matter. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 1720, 1600, 1530 (strong). NMR ($CDCl_3$): δ 1.15 (3H), 1.24 (s, 3H), 1.33 (d, J=6 Hz, 3H), 1.80 (s, 3H), 4.58 (d.d, J=5 and 11 Hz, and m, 2H), 5.23 (d.q, J=10 and 6 Hz,

1H), 5.40 (m, 1H), 7.5—8.0 (m, 4H). ORD (c=0.57, MeOH) [ϕ] (nm): −2556° (400), −3494° (370, trough), −2723° (350), −2151° (330, peak), −2520° (320), molecular amplitude −13.43.

Acknowledgement The authors thank Dr. U. Nagai for valuable discussion on ORD analyses of *o*-nitrobenzoate and Dr. H. Koyama for X-ray analysis. They also thank Misses H. Kakizaki and T. Obara for microanalyses and Miss T. Araki for NMR measurements.