[Chem. Pharm. Bull. 20(7)1424—1427(1972)]

UDC 547.92'466.057

# Synthesis of 3-Suberoylamino Acid Esters of Digitoxigenin[1]

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(Received December 3, 1971)

In order to examine the physiological activity the suberoylamino acid esters of digitoxigenin (VIIa—h) as the bufotoxin analogs have been synthesized. Digitoxigenin 3-(hydrogen suberate) (Ib) was transformed into the *p*-nitrophenyl ester (IVb), which in turn was condensed with a variety of amino acids to yield the desired compounds. The results of the model experiment employing digitoxigenin 3-(hydrogen succinate) (Ia) were also described.

In 1922 Wieland, *et al.* separated from the toad venom drug, *Ch'an Su*, the so-called "bufotoxin",[3] whose structure was subsequently assigned to 14-suberoylarginine ester of bufotalin.[4] Recently Kamano, *et al.* isolated 3-suberoylbufogenins from *Ch'an Su*,[5] and Linde-Tempel revised the structure of "bufotoxin" to be the 3-suberoylarginine ester from the evidences of enzymatic degradation.[6] In addition the occurrence of the 3-(hydrogen suberate) as well as the cardenolide itself in *Ch'an Su* has recently been reported.[7] As a series of studies we have investigated the structure-activity relationship with the cardiotonic steroid analogs.[8] A particular interest in the physiological activity of the cardiac steroids conjugated with other amino acids than arginine prompted us to prepare the titled compounds from digitoxigenin.

As a model experiment an initial attempt was focused to the synthesis of the 3-succinoylglycine ester. Treatment of digitoxigenin 3-(hydrogen succinate) (Ia)[9] with ethyl chlorocarbonate in tetrahydrofuran provided the carbonic-carboxylic acid anhydride (II). Subsequent condensation with *tert*-butyl glycinate proceeded with ease to give the butyl ester of 3-succinoyl glycine (III) in the reasonable yield. However, the difficulties were encountered with the elaboration in the final step. Removal of the *tert*-butyl group with hydrogen bromide in acetic acid[10] was accompanied with undesirable dehydration of the C-14 hydroxyl

1) This paper constitutes Part XII of the series entitled "Studies on Cardiotonic Steroid Analogs"; Part XI: T. Nambara and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **19**, 1937 (1971); A part of this work has been presented as a preliminary report (K. Shimada and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **19**, 1073 (1971).

2) Location: *Aobayama, Sendai.*

3) H. Wieland and R. Alles, *Ber.*, **55**, 1789 (1922); H. Wieland, G. Hesse and R. Hüttel, *Ann.*, **524**, 203 (1936); H. Wieland and H. Behringer, *ibid.*, **549**, 209 (1941).

4) K. Meyer, *Helv. Chim. Acta*, **32**, 1993 (1949); H.R. Urscheler, Ch. Tamm and T. Reichstein, *ibid.*, **38**, 883 (1955).

5) Y. Kamano, H. Yamamoto, Y. Tanaka and M. Komatsu, *Tetrahedron Letters*, **1968**, 5673.

6) H.O. Linde-Tempel, *Helv. Chim. Acta*, **53**, 2188 (1970).

7) *a*) N. Höriger, H.H.A. Linde and K. Meyer, *Helv. Chim. Acta*, **53**, 1503 (1970); *b*) N. Höriger, D. Živanov, H.H.A. Linde and K. Meyer, *ibid.*, **53**, 1993 (1970); *c*) *idem*, *ibid.*, **53**, 2051 (1970).

8) T. Nambara, K. Shimada, S. Goya, and J. Goto, *Chem. Pharm. Bull.* (Tokyo), **16**, 2236 (1968); T. Nambara, K. Shimada and S. Goya, *ibid.*, **18**, 453 (1970); S. Goya, K. Shimada, J. Goto, S. Usuda and T. Nambara, *Yakugaku Zasshi*, **90**, 537 (1970); T. Nambara, K. Shimada, T. Nemoto and S. Goya, *Chem. Pharm. Bull.* (Tokyo), **18**, 1658 (1970); T. Nambara and K. Shimada, *ibid.*, **19**, 16 (1971); T. Nambara, K. Shimada, J. Goto and S. Goya, *ibid.*, **19**, 21 (1971); T. Nambara, T. Shibata, M. Mimura and H. Hosoda, *ibid.*, **19**, 954 (1971).

9) M. Zingg and K. Meyer, *Pharm. Acta Helv.*, **32**, 393 (1957); A. Yamada, *Yakugaku Zasshi*, **79**, 1440 (1959).

10) G.W. Anderson and F.M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960).

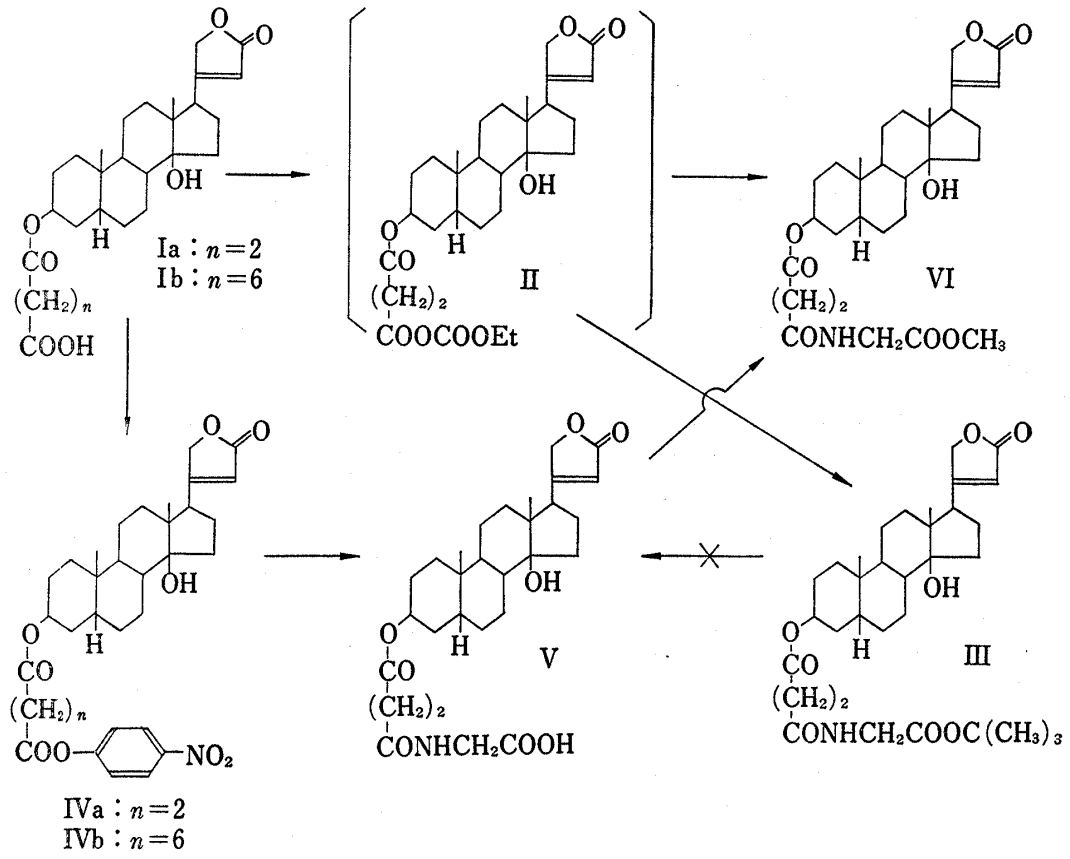
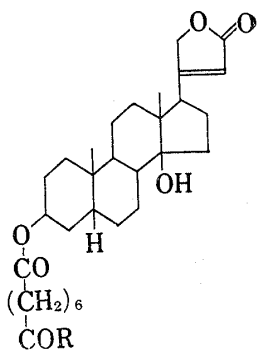



Chart 1

group to a considerable extent. Several other attempts to hydrolyze the butyl ester without affecting the steroidal moiety also resulted in failure. Accordingly the development of an alternative way, that is the *p*-nitrophenyl ester method,[11] was then undertaken. Being stirred with *p*-nitrophenol in the presence of N,N-dicyclohexylcarbodiimide, Ia was transformed into the *p*-nitrophenyl ester (IVa) in the satisfactory yield. Subsequent treatment with glycine in aqueous pyridine furnished the desired 3-succinoylglycine ester of digitoxigenin (V). Usual methylation with diazomethane gave the methyl ester (VI), which proved to be identical with the product obtained from Ia and methyl glycinate by the mixed anhydride method mentioned above.



VIIa : R=Gly·OH
VIIb : R=L-Ala·OH
VIIc : R=L-Try·OH
VIId : R=L-Pro·OH
VIIe : R=L-Asp·OH
VIIf : R=L-Asp·(OMe)$_2$
VIIg : R=D-Ala·OH
VIIh : R=D-Try·OH

Chart 2

On the basis of these results the next project was directed to the synthesis of a variety of the suberoylamino acid esters starting from 3-(hydrogen suberate) of digitoxigenin (Ib).[7b] The *p*-nitrophenyl ester (IVb) was prepared as a key intermediate from Ib in the same manner as described above. Condensation with the neutral and acidic amino acids such as glycine, L-alanine, L-tryptophan, L-proline, and L-aspartic acid followed by chromatographic purification on silica gel afforded the desired 3-suberoyl-L-amino acid esters (VIIa—e), respectively.

For the purpose of comparing the physiological activity the analogous compounds conjugated with D-amino acid were also synthesized. Reaction of the *p*-nitrophenyl ester (IVb)

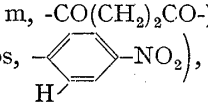
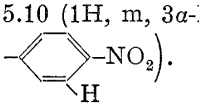
11) M.A. Ondetti, *J. Med. Chem.*, **6**, 10 (1963).

with D-alanine and D-tryptophan proceeded similarly yielding the desired 3-suberoylamino acid esters (VIIg,h). It is to be noted that the L-amino acid conjugate is somewhat more hydrophillic than the corresponding isomer in the D-series, although no plausible explanation is now available.

The results of the biological test on these 3-suberoylamino acid esters thus prepared will be reported elsewhere in the near future.

## Experimental[12]

***tert*-Butyl Ester of V (III)**——To a stirred solution of digitoxigenin 3-(hydrogen succinate) (Ia) (60 mg) and triethylamine (0.06 ml) in anhydrous tetrahydrofuran (4 ml) was added ethyl chlorocarbonate (0.03 ml) at −5° and the resulting solution was kept at this temperature for 15 min. Then a solution of *tert*-butyl glycinate (50 mg) in tetrahydrofuran (1 ml) was added under ice-cooling over a period of 30 min and allowed to stand at room temperature for 1 hr. The resulting solution was extracted with ether, washed with 5% HCl, $H_2O$ and dried over anhydrous $Na_2SO_4$. After usual work-up the residue obtained was submitted to the preparative thin-layer chromatography (TLC) using benzene–AcOEt (1:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.24) was eluted with AcOEt and recrystallization of the eluate from AcOEt gave III (40 mg) as colorless leaflets. mp 185—187°. $[\alpha]_D^{22}$ +29.5° ($c$=0.22). *Anal.* Calcd. for $C_{33}H_{49}O_8N$: C, 67.43; H, 8.40; N, 2.38. Found: C, 67.47; H, 8.51; N, 2.52. NMR (4% solution in $CDCl_3$) $\delta$: 0.88 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 1.45 (9H, s, -C($CH_3$)$_3$), 2.60 (4H, m, -CO($CH_2$)$_2$CO-), 3.90 (2H, d, $J$=6 cps, -NHC$\underline{H}_2$CO-), 4.85 (2H, m, 21-$CH_2$), 5.10 (1H, m, 3$\alpha$-H), 5.85 (1H, m, 22-H), 6.15 (1H, m, N-H).

**3-Succinoyl-*p*-nitrophenol Ester of Digitoxigenin (IVa)**——To a solution of Ia (50 mg) and *p*-nitrophenol (20 mg) in AcOEt (3 ml) was added N,N-dicyclohexylcarbodiimide (20 mg) under cooling and allowed to stand at room temperature for 5 hr. The precipitated dicyclohexylurea was filtered off and washed with AcOEt. The filtrate and washings were combined and concentrated *in vacuo*. The crude product obtained was submitted to the preparative TLC using benzene–AcOEt (4:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.50) was eluted with AcOEt and recrystallization of the eluate from MeOH gave IVa (25 mg) as colorless prisms. mp 172—174°. $[\alpha]_D^{17}$ +3.1° ($c$=0.16). *Anal.* Calcd. for $C_{33}H_{41}O_9N$: C, 66.54; H, 6.94; N, 2.35. Found: C, 66.53; H, 7.15; N, 2.35. NMR (4% solution in $CDCl_3$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 2.80 (4H, m, -CO($CH_2$)$_2$CO-), 4.85 (2H, m, 21-$CH_2$), 5.10 (1H, m, 3$\alpha$-H), 5.80 (1H, m, 22-H), 7.20 (2H, d, $J$=10 cps,  ), 8.18 (2H, d, $J$=10 cps,  ).

**3-Succinoylglycine Ester of Digitoxigenin (V)**——To a solution of IVa (50 mg) in pyridine (3 ml) was added an aq. solution of glycine (20 mg in 2 ml) and allowed to stand at room temperature for 16 hr. The resulting solution was evaporated *in vacuo* below 40° and the residue obtained was chromatographed on silica gel (100 mg). Elution with AcOEt gave V (25 mg) as colorless oil. NMR (4% solution in $CDCl_3$) $\delta$: 0.86 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 2.60 (4H, m, -CO($CH_2$)$_2$CO-), 4.10 (2H, d, $J$=6 cps, -NHC$\underline{H}_2$CO-), 4.90 (2H, m, 21-$CH_2$), 5.10 (1H, m, 3$\alpha$-H), 5.85 (2H, m, 22-H, -COOH), 6.50 (1H, m, N-H).

**Methyl Ester of V (VI)**——i) To a solution of V (20 mg) in MeOH (2 ml) was added an ethereal solution of $CH_2N_2$ and allowed to stand at room temperature for 1 hr. On usual work-up a crystalline product was obtained. Recrystallization from MeOH–ether gave VI (20 mg) as colorless needles. mp 156—157°. $[\alpha]_D^{17}$ +9.5° ($c$=0.11). *Anal.* Calcd. for $C_{30}H_{43}O_8N$: C, 66.03; H, 7.94; N, 2.57. Found: C, 66.38; H, 8.06; N, 2.35. NMR (4% solution in $CDCl_3$) $\delta$: 0.86 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 2.65 (4H, m, -CO($CH_2$)$_2$-CO-), 3.75 (3H, s, -COOC$H_3$), 4.05 (2H, d, $J$=6 cps, -NHC$\underline{H}_2$CO-), 4.90 (2H, m, 21-$CH_2$), 5.15 (1H, m, 3$\alpha$-H), 5.90 (1H, m, 22-H), 6.25 (1H, m, N-H).

ii) A solution of Ia (50 mg) and triethylamine (0.05 ml) in anhydrous tetrahydrofuran (2 ml) was treated with ethyl chlorocarbonate (0.025 ml) and methyl glycinate hydrochloride (40 mg) in the same manner as described in III. After usual work-up the residue obtained was submitted to the preparative TLC using benzene–AcOEt (1:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.10) was eluted with AcOEt and recrystallization of the eluate from MeOH–ether gave VI (20 mg) as cololess needles. mp 156—157°. Mixed melting point on admixture with the sample obtained in i) showed no depression.

**3-Suberoyl-*p*-nitrophenol Ester of Digitoxigenin (IVb)**——To a solution of digitoxigenin 3-(hydrogen suberate) (Ib) (20 mg) and *p*-nitrophenol (10 mg) in AcOEt (2 ml) was added N,N-dicyclohexylcarbodiimide (10 mg) and treated in the same manner as described in IVa. The crude product obtained was submitted to

12) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ unless otherwise specified. Nuclear magnetic resonance spectra (NMR) were obtained on Hitachi Model H-60 spectrometer at 60 Mc; the chemical shifts are quoted as ppm downfield from tetramethylsilane used as an internal standard. Abbreviation used s=singlet, d=doublet, and m=multiplet.

the preparative TLC using benzene–AcOEt (4:1) as developing solvent. The adsorbent corresponding to the spot (*Rf* 0.60) was eluted with AcOEt and recrystallization of the eluate from MeOH gave IVb (15 mg) as colorless leaflets. mp 165—166°. $[\alpha]_D^{18}$ +12.8° ($c$=0.23). *Anal.* Calcd. for $C_{37}H_{49}O_9N$: C, 68.18; H, 7.58; N, 2.15. Found: C, 68.10; H, 7.64; N, 2.09.

**3-Suberoylglycine Ester of Digitoxigenin (VIIa)**——A solution of IVb (40 mg) in pyridine (2 ml) was treated with an aq. solution of glycine (15 mg in 1 ml) in the same manner as described in V. The crude product obtained was chromatographed on silica gel (100 mg). Elution with AcOEt and recrystallization of the eluate from acetone gave VIIa (15 mg) as colorless prims. mp 189—192°. $[\alpha]_D^{23}$ +40.0° ($c$=0.10). *Anal.* Calcd. for $C_{33}H_{49}O_8N$: C, 67.43; H, 8.40; N, 2.38. Found: C, 67.27; H, 8.42; N, 2.87. NMR (4% solution in $CDCl_3$) $\delta$: 0.88 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 4.05 (2H, d, $J$=6 cps, -NHC$\underline{H}_2$CO-), 4.90 (2H, m, 21-$CH_2$), 5.05 (1H, m, 3$\alpha$-H), 5.60 (1H, m, -COOH), 5.88 (1H, m, 22-H), 6.45 (1H, m, N-H).

**3-Suberoyl-L-alanine Ester of Digitoxigenin (VIIb)**——A solution of IVb (30 mg) in pyridine (2 ml) was treated with an aq. solution of L-alanine (10 mg in 1 ml) in the same manner as described in V. The crude product obtained was chromatographed on silica gel (100 mg). Elution with AcOEt and recrystallization of the eluate from AcOEt–ether gave VIIb (15 mg) as colorless amorphous substance. mp 148—150°. $[\alpha]_D^{15}$ −11.4° ($c$=0.10). *Anal.* Calcd. for $C_{34}H_{51}O_8N$: C, 67.86; H, 8.54; N, 2.33. Found: C, 67.75; H, 8.44; N, 2.42. NMR (4% solution in $CDCl_3$) $\delta$: 0.86 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 4.90 (2H, m, 21-$CH_2$), 5.10 (1H, m, 3$\alpha$-H), 5.55 (1H, m, -COOH), 5.85 (1H, m, 22-H), 6.40 (1H, m, N-H).

**3-Suberoyl-L-tryptophan Ester of Digitoxigenin (VIIc)**——A solution of IVb (30 mg) in pyridine (2 ml) was treated with an aq. solution of L-tryptophan (10 mg in 1 ml) in the same manner as described in V. The crude product obtained was chromatographed on silica gel (100 mg). Elution with AcOEt and recrystallization of the eluate from AcOEt–ether–hexane gave VIIc (15 mg) as colorless amorphous substance. mp 115—118°. $[\alpha]_D^{18}$ +18.7° ($c$=0.11). *Anal.* Calcd. for $C_{42}H_{56}O_8N_2$: C, 70.36; H, 7.87; N, 3.91. Found: C, 70.36; H, 8.26; N, 3.77. NMR (4% solution in $CH_3OD$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 5.80 (1H, m, 22-H), 6.80—7.50 (5H, m, tryptophan-2-H, -arylprotons).

**3-Suberoyl-L-proline Ester of Digitoxigenin (VIId)**——A solution of IVb (25 mg) in pyridine (2 ml) was treated with an aq. solution of L-proline (20 mg in 1 ml) in the same manner as described in V. The crude product obtained was chromatographed on silica gel (100 mg). Elution with AcOEt and recrystallization of the eluate from AcOEt–ether gave VIId (15 mg) as colorless amorphous substance. mp 167—169°. $[\alpha]_D^{18}$ −49.5° ($c$=0.09). *Anal.* Calcd. for $C_{36}H_{53}O_8N$: C, 68.87; H, 8.51; N, 2.23. Found: C, 68.70; H, 8.87; N, 2.36. NMR (4% solution in $CDCl_3$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 3.50 (2H, m, proline-5-H), 4.50 (1H, m, proline-2-H), 4.80 (2H, m, 21-$CH_2$), 5.00 (1H, m, 3$\alpha$-H), 5.80 (1H, m, 22-H).

**3-Suberoyl-L-aspartic Acid Ester of Digitoxigenin (VIIe)**——A solution of IVb (40 mg) in pyridine (2 ml) was treated with an aq. solution of L-aspartic acid (15 mg in 2 ml) in the same manner as described in V. The crude product obtained was chromatographed on silica gel (100 mg). Elution with AcOEt gave VIIe (15 mg) as colorless amorphous substance. mp 85—87°. The analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

**Dimethyl Ester of VIIe (VIIf)**——A solution of VIIe (15 mg) in MeOH (2 ml) was treated with an ethereal solution of $CH_2N_2$ in the same manner as described in VI. After usual work-up the crude product obtained was recrystallized from AcOEt–ether to give VIIf (10 mg) as colosless prirms. mp 110—111°. $[\alpha]_D^{22}$ +18.2° ($c$=0.11). *Anal.* Calcd. for $C_{37}H_{55}O_{10}N$: C, 65.95; H, 8.22; N, 2.08. Found: C, 66.30; H, 8.11; N, 1.93. NMR (4% solution in $CDCl_3$) $\delta$: 0.85 (3H, s, 18-$CH_3$), 0.95 (3H, s, 19-$CH_3$), 3.62 (3H, s, -COOC$\underline{H}_3$), 3.70 (3H, s, -COOC$\underline{H}_3$), 4.85 (2H, m, 21-$CH_2$), 5.00 (1H, m, 3$\alpha$-H), 5.80 (1H, m, 22-H), 6.40 (1H, m, N-H).

**3-Suberoyl-D-alanine Ester of Digitoxigenin (VIIg)**——A solution of IVb (70 mg) in pyridine (9 ml) was treated with an aq. solution of D-alanine (30 mg in 6 ml) in the same manner as described in V. The crude product obtained was chromatographed on silica gel (800 mg). Elution with $CH_2Cl_2$–AcOEt (1:3) and recrystallization of the eluate from AcOEt gave VIIg (24 mg) as colorless prisms. mp 173—175°. $[\alpha]_D^{25}$ +17.9° ($c$=0.11). *Anal.* Calcd. for $C_{34}H_{51}O_8N$: C, 67.86; H, 8.54; N, 2.33. Found: C, 67.77; H, 8.70; N, 2.05. NMR (5% solution in $CDCl_3$) $\delta$: 0.88 (3H, s, 18-$CH_3$), 0.97 (3H, s, 19-$CH_3$), 4.00 (1H, m, -COOH), 4.90 (2H, m, 21-$CH_2$), 5.08 (1H, m, 3$\alpha$-H), 5.90 (1H, m, 22-H), 6.35 (1H, m, N-H).

**3-Suberoyl-D-tryptophan Ester of Digitoxigenin (VIIh)**——A solution of IVb (57 mg) in pyridine (4 ml) was treated with an aq. solution of D-tryptophan (20 mg in 2 ml) in the same manner as described in V. The crude product obtained was chromatographed on silica gel (500 mg). Elution with $CH_2Cl_2$–AcOEt (1:3) and trituration of the eluate with hexane gave VIIh (25 mg) as colorless amorphous substance. mp 120—128°. $[\alpha]_D^{20}$ −10.8° ($c$=0.09). *Anal.* Calcd. for $C_{42}H_{56}O_8N_2$: C, 70.36; H, 7.87; N, 3.91. Found: C, 69.93; H, 7.92; N, 3.71. NMR (5% solution in $CDCl_3$) $\delta$: 0.84 (3H, s, 18-$CH_3$), 0.93 (3H, s, 19-$CH_3$), 3.82 (1H, m, -COOH), 4.86 (2H, m, 21-$CH_2$), 5.08 (1H, m, 3$\alpha$-H), 6.15 (1H, m, -CONH-), 7.0—7.5 (5H, m, tryptophan -2-H, -arylprotons), 8.73 (1H, m, tryphtophan-NH-).

**Acknowledgement** The authors are indebted to all the staffs of the central analytical laboratory of this Institute for elemental analyses and spectral measurements. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged.