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Syntheses of Digitoxigenin 3-Suberoylpeptides[1]

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In order to obtain the more potent cardiotonic drug digitoxigenin 3-suberoylpeptides whose peptide moieties are derived from the partial structure of kinin, have been synthesized. Some of the desired compounds were prepared from digitoxigenin 3-suberoyl-*p*-nitrophenol (I) by direct condensation with the pertinent di- or tri-peptide. As for the steroidal peptides having the readily unavailable amino acid sequence the stepwise method involving the repeated *p*-nitrophenyl ester formation was carried out. The condensation reaction of *p*-nitrophenyl ester with amino acid or peptide was considerably improved in respect with the yield, when the amino group was activated by leading to the carbamate.

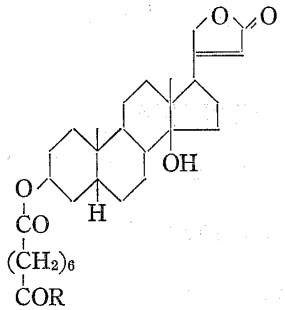
In a previous paper of this series the syntheses of digitoxigenin 3-suberoylamino acids as bufotoxin analog have been reported.[3] A particular interest in the physiologically active peptides which occur in the skin of amphibian animals[4] prompted us to prepare the steroidal peptides to obtain the more potent cardiotonic drug. The present paper deals with the syntheses of digitoxigenin 3-suberoylpeptides whose peptide moieties are derived from the partial structure of kinin.

An initial project was focused to the preparation of the desired compounds by direct condensation of the pertinent peptides with digitoxigenin 3-suberoyl-*p*-nitrophenol (I).[3] The activated ester and di- or tri-peptide being stirred in aqueous pyridine, the glycyl-glycine (IVa), glycyl-L-phenylalanine (Va), glycyl-L-proline (VIa), L-prolyl-glycine (VII), and glycyl-glycyl-glycine (Xa) derivatives were obtained in a satisfactory yield. On treatment with diazomethane these peptide conjugates were transformed into their methyl esters (IVb, Vb, VIb, Xb).

With regard to the peptides having the commercially unavailable amino acid sequence the stepwise method involving the repeated *p*-nitrophenyl ester formation was carried out. Digitoxigenin 3-suberoyl-glycine (IIa) and -L-proline (IIIa)[3] were led to the *p*-nitrophenyl esters (IIb, IIIb) with use of N,N'-dicyclohexylcarbodiimide in a reasonable yield. These activated esters were condensed with glycine to yield the glycyl-glycine and L-prolyl-glycine derivatives (IVa, VII), which proved to be identical with the product obtained by the direct method described above. In a similar manner digitoxigenin 3-suberoyl-L-prolyl-L-proline (VIIIa) and -L-prolyl-glycyl-L-phenylalanine (XII) were prepared from IIIb by treatment with L-proline and glycyl-L-phenylalanine, respectively.

The stepwise method, however, was unfavorable in respect with the overall yield, since the *p*-nitrophenyl ester linkage was suffered from the preferential hydrolysis. Recently a novel method to activate the amino group by the formation of the carbamate of amino acids and peptides has been reported.[5] Therefore examination was made on the applicability of

1) This paper constitutes Part XIV of the series entitled "Studies on Cardiotonic Steroid Analogs"; Part XIII: T. Nambara, J. Goto, A. Sasaki, and K. Sudo, *Chem. Pharm. Bull.* (Tokyo), **21**, 565 (1973).
2) Location: *Aobayama, Sendai.*
3) K. Shimada and T. Nambara, *Chem. Pharm. Bull.* (Tokyo), **19**, 1937 (1971); T. Nambara, K. Shimada, and Y. Fujii, *ibid.*, **20**, 1424 (1972).
4) V. Erspamer and A. Anastasi, "Hypotensive Peptides," ed. by E.G. Erdös, N. Back, and F. Sicuteri, Springer-Verlag, Inc., New York, 1966, pp. 63—75.
5) M. Itoh, *Chem. Pharm. Bull.* (Tokyo), **20**, 664 (1972).



I : R=ONP
IIa : R=Gly·OH
IIb : R=Gly·ONP
IIIa : R=L-Pro·OH
IIIb : R=L-Pro·ONP
IVa : R=$(Gly)_2$·OH
IVb : R=$(Gly)_2$·OMe

Va : R=Gly-L-Phe·OH
Vb : R=Gly-L-Phe·OMe
Vc : R=Gly-L-Phe·ONP
VIa : R=Gly-L-Pro·OH
VIb : R=Gly-L-Pro·OMe
VII : R=L-Pro-Gly·OH
VIIIa : R=L-Pro-L-Pro·OH
VIIIb : R=L-Pro-L-Pro·ONP
IXa : R=L-Ser-L-Pro·OH
IXb : R=L-Ser-L-Pro·OMe
Xa : R=$(Gly)_3$·OH
Xb : R=$(Gly)_3$·OMe
XIa : R=Gly-L-Phe-L-Ser·OH
XIb : R=Gly-L-Phe-L Ser·OMe
XII : R=L-Pro-Gly-L-Phe·OH
XIII : R=L-Pro-L-Pro-Gly-L-Phe·OH
XIVa : R=$(Gly)_4$·OH
XIVb : R=$(Gly)_4$·OMe

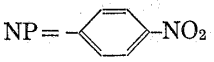


Chart 1

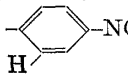
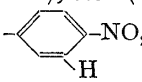
the carbamate method to the synthesis of the L-prolyl-L-proline derivative (VIII). In actuality the carbamate formation improved considerably the yield of the desired compound by supressing the hydrolysis prior to condensation as compared with the usual method. In addition the glycyl–glycyl–glycyl–glycine derivative (XIVa) could be prepared with success, when the carbamate formation method was used. The dipeptide conjugates, digitoxigenin 3-suberoyl–glycyl-L-phenylalanine (Va) and -L-prolyl-L-proline (VIIIa) were similarly converted into the corresponding *p*-nitrophenyl esters (Vc, VIIIb) employing N,N′-dicyclohexylcarbodiimide as a catalyst for condensation.[6] Applying the carbamate formation method to I, Vc and VIIIb, digitoxigenin 3-suberoyl-L-seryl-L-proline (IXa), -glycyl-L-phenylalanyl-L-serine (XIa) and -L-prolyl-L-prolyl–glycyl-L-phenylalanine (XIII) could be synthesized in a satisfactory yield. As for the formation of IXa and XIa the occurrence of selective condensation with L-serine at the α-amino group was justified by the negative result of the product with ninhydrin test. Of these peptide conjugates IXa and XIa were further led to the methyl esters (IXb, XIb) by the usual treatment with diazomethane.

The results on the pharmacological test will be reported elsewhere in the near future.

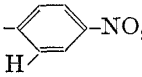
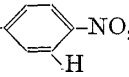
Experimental[8]

Digitoxigenin 3-Suberoylglycine *p*-Nitrophenyl Ester (IIb)——To a solution of digitoxigenin 3-suberoylglycine (IIa)[3] (84 mg) and *p*-nitrophenol (75 mg) in AcOEt (15 ml) was added N,N′-dicyclohexylcarbodiimide (75 mg) under ice–cooling and allowed to stand at room temperature for 8 hr. Precipitated dicyclohexylurea was filtered off and washed with AcOEt. The filtrate and washings were combined and concentrated *in*

6) Formation of *p*-nitrophenyl ester under these conditions might possibly be accompanied with racemization of L-phenylalanine but its extent would be slight.[7]
7) K. Lübke and E. Schröder, *Z. Naturforsch.*, **16b**, 765 (1961); F. Weygand, A. Prox, L. Schmidhammer, and W. König, *Angew. Chem.*, **75**, 282 (1963).
8) 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 run on Hitachi Model R-20A spectrometer at 60 MHz; the chemical shifts are quoted as ppm downfield from tetramethylsilane used as an internal standard. Abbreviation used s=singlet, d=doublet, and m=multiplet. Infrared (IR) spectra were obtained by JASCO Model IR-S spectrometer. Thin-layer chromatography (TLC) was carried out on silica gel G (E. Merck AG) by the following systems: TL-I=benzene–AcOEt (1: 1); TL-II=benzene–AcOEt (1: 4); TL-III=AcOEt; TL-IV=AcOEt–MeOH (9: 1), and *Rf* values are given.

vacuo. The crude product obtained was submitted to the preparative TLC using benzene–AcOEt (1: 1) as developing solvent. The adsorbent corresponding to the spot was eluted with AcOEt to give IIb (14 mg) as a yellow oil. NMR (5% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 4.35 (2H, d, J=6 Hz, -NHC$\underline{H}_2$CO-), 4.92 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.91 (1H, m, 22-H), 6.40 (1H, m, N-H), 6.95 (2H, d, J=9 Hz, ), 8.17 (2H, d, J=9 Hz, ). TL-III: 0.65.

Digitoxigenin 3-Suberoyl-L-proline (IIIa)——A solution of L-Pro·OH (40 mg) and triethylamine (0.6 ml) in H_2O (5.4 ml)–dioxane (8.6 ml) was adjusted to pH 8 by the addition of dry ice with stirring. Digitoxigenin 3-suberoyl-*p*-nitrophenol (I)[3] (135 mg) was added to the resulting solution and stirred at room temperature for 4 hr. After acidification with 1N HCl the reaction mixture was extracted with AcOEt. The organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. After usual work-up the crude product obtained was chromatographed on silica gel (500 mg). Elution with AcOEt and recrystallization of the eluate from AcOEt–ether gave IIIa (87 mg) as colorless prisms. mp 165—167°. Mixed melting point on admixture with the authentic sample[3] showed no depression and IR spectra of two samples were entirely identical in every respect.

Digitoxigenin 3-Suberoyl-L-proline *p*-Nitrophenyl Ester (IIIb)——To a solution of IIIa (50 mg) and *p*-nitrophenol (20 mg) in AcOEt (2 ml) was added N,N'-dicyclohexylcarbodiimide (20 mg) and treated in the same manner as described in IIb. The crude product obtained was submitted to the preparative TLC using benzene–AcOEt (1: 1) as developing solvent. Elution of the adsorbent corresponding to the spot with AcOEt gave IIIb (30 mg) as a yellow oil. NMR (4% solution in $CDCl_3$) δ: 0.87 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.65 (2H, m, Pro-5-CH_2), 4.60 (1H, m, Pro-2-H), 4.90 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.90 (1H, m, 22-H), 7.35 (2H, d, J=9 Hz, ), 8.25 (2H, d, J=9 Hz, ). TL-I: 0.25.

Digitoxigenin 3-Suberoylglycyl-glycine (IVa)——To a solution of I (30 mg) in pyridine (2 ml) was added an aq. solution of $(Gly)_2$–OH (10 mg in 1 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 (300 mg). Elution with AcOEt and recrystallization of the eluate from AcOEt–ether gave IVa (15 mg) as colorless amorphous substance. mp 81—83°. Analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

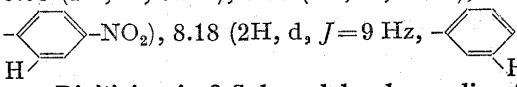
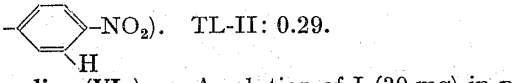
Digitoxigenin 3-Suberoylglycyl-glycine Methyl Ester (IVb)——i) To a solution of IVa (20 mg) in MeOH (2 ml) was added an ethereal solution of CH_2N_2 and allowed to stand at room temperature for 1 hr. On usual work-up a crystalline product was obtained. Recrystallization from acetone gave IVb (15 mg) as colorless needles. mp 166—167°. $[\alpha]_D^{24}$ −5.7° (c=0.09). *Anal.* Calcd. for $C_{36}H_{54}O_9N_2$: C, 65.63; H, 8.26; N, 4.25. Found: C, 65.45; H, 8.41; N, 4.36. NMR (4% solution in $CDCl_3$) δ: 0.87 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.72 (3H, s, -$COOCH_3$), 4.00 (4H m, (-NHC$\underline{H}_2$CO-)$_2$), 4.85 (2H, m, 21-CH_2), 5.05 (1H, m, 3α-H), 5.85 (1H, m, 22-H), 6.35 (1H, m, N-H), 6.70 (1H, m, N-H).

ii) A solution of IIb (14 mg) in pyridine (2 ml) was treated with an aq. solution of Gly·OH (7 mg in 1 ml) in the same manner as described in IVa. The crude product obtained was chromatographed on silica gel (300 mg). The eluate (6.3 mg) with AcOEt was methylated with CH_2N_2 in the usual manner. Recrystallization of the product from acetone gave IVb (4 mg) as colorless needles. mp 165.5—167°. Mixed melting point on admixture with the sample obtained in i) showed no depression.

Digitoxigenin 3-Suberoylglycyl-L-phenylalanine (Va)——A solution of I (40 mg) in pyridine (2 ml) was treated with an aq. solution of Gly-L-Phe·OH (15 mg in 1 ml) in the same manner as described in IVa. The crude product obtained was chromatographed on silica gel (300 mg). Elution with AcOEt and recrystallization of the eluate from AcOEt–hexane gave Va (15 mg) as colorless amorphous substance. mp 89—91°. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.10 (2H, m, $C_6H_5C\underline{H}_2$-), 3.85 (2H, m, -NHC$\underline{H}_2$CO-), 4.85 (2H, m, 21-CH_2), 5.05 (1H, m, 3α-H), 5.85 (1H, m, 22-H), 6.75 (2H, m, N-H), 7.19 (5H, m, C_6H_5-). The analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

Digitoxigenin 3-Suberoylglycyl-L-phenylalanine Methyl Ester (Vb)——Va (20 mg) was methylated with CH_2N_2 in the usual manner. Recrystallization of the product from MeOH–ether gave Vb (15 mg) as colorless prisms. mp 110—112°. $[\alpha]_D^{23}$ +50.0° (c=0.12). *Anal.* Calcd. for $C_{43}H_{60}O_9N_2$: C, 68.96; H, 8.08; N, 3.74. Found: C, 68.57; H, 8.11; N, 3.86. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.10 (2H, d, J=6 Hz, $C_6H_5C\underline{H}_2$-), 3.70 (3H, s, -$COOCH_3$), 3.87 (2H, m, -NHC$\underline{H}_2$CO-), 4.90 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.85 (1H, m, 22-H), 6.40 (1H, m, N-H), 6.70 (1H, m, N-H), 7.20 (5H, m, C_6H_5-).

Digitoxigenin 3-Suberoylglycyl-L-phenylalanine *p*-Nitrophenyl Ester (Vc)——To a solution of Va (130 mg) and *p*-nitrophenol (50 mg) in AcOEt (20 ml) was added N,N'-dicyclohexylcarbodiimide (50 mg) and treated in the same manner as described in IIb. The crude product obtained was submitted to the preparative TLC using benzene–AcOEt (3: 7) as developing solvent. The adsorbent corresponding to the spot was eluted with AcOEt to give Vc (95 mg) as a yellow oil. NMR (5% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.21 (2H, d, J=6 Hz, $C_6H_5C\underline{H}_2$-), 3.92 (2H, m, -NHC$\underline{H}_2$CO-), 4.90 (2H, m, 21-CH_2),

5.08 (1H, m, 3α-H), 5.88 (1H, m, 22-H), 6.55 (1H, m, N–H), 6.80 (1H, m, N–H), 7.25 (7H, m, C_6H_5– and ), 8.18 (2H, d, J=9 Hz, ). TL-II: 0.29.

Digitixigenin 3-Suberoylglycyl-L-proline (VIa)——A solution of I (30 mg) in pyridine (2 ml) was treated with an aq. solution of Gly-L-Pro·OH (10 mg in 1 ml) in the same manner as described in IVa. The crude product obtained was chromatographed on silica gel (300 mg). Elution with AcOEt and recrystallization of the eluate from AcOEt–ether gave VIa (15 mg) as colorless amorphous substance. mp 104—106°. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.50 (2H, m, Pro-5-CH_2), 4.00 (2H, m, –NHC$\underline{H}_2$CO–), 4.50 (1H, m, Pro-2-H), 4.85 (2H, m, 21-CH_2), 5.00 (1H, m, 3α-H), 5.30 (1H, m, –COOH), 5.80 (1H, m, 22-H), 6.60 (1H, m, N–H). The analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

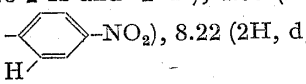
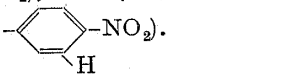
Digitoxigenin 3-Suberoylglycyl-L-proline Methyl Ester (VIb)——VIa (20 mg) was methylated with CH_2N_2 in the usual manner. Recrystallization of the product from AcOEt–ether gave VIb (20 mg) as colorless prisms. mp 139—141°. $[\alpha]_D^{22}$ −13.3° (c=0.15). *Anal.* Calcd. for $C_{39}H_{58}O_9N_2$: C, 67.02; H, 8.37; N, 4.01. Found: C, 66.85; H, 8.37; N, 4.07. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.45 (2H, m, Pro-5-CH_2), 3.70 (3H, s, –$COOCH_3$), 4.00 (2H, d, J=4 Hz, –NHC$\underline{H}_2$CO–), 4.50 (1H, m, Pro-2-H), 4.85 (2H, m, 21-CH_2), 5.05 (1H, m, 3α-H), 5.82 (1H, m, 22-H), 6.55 (1H, m, N–H).

Digitixigenin 3-Suberoyl-L-prolyl-glycine (VII)——i) A solution of IIIb (61 mg) in pyridine (6 ml) was treated with an aq. solution of Gly·OH (30 mg in 1 ml) in the same manner as described in IVa. The crude product obtained was chromatographed on silica gel (800 mg). Elution with AcOEt and trituration of the eluate with hexane gave VII (30 mg) as colorless amorphous substance. mp 108—114°. $[\alpha]_D^{21}$ −33.9° (c=0.12). *Anal.* Calcd. for $C_{38}H_{56}O_9N_2$: C, 66.64; H, 8.24; N, 4.09. Found: C, 66.45; H, 8.71; N, 4.18. NMR (5% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.55 (2H, m, Pro-5-CH_2), 4.05 (2H, m, –NHC$\underline{H}_2$CO–), 4.60 (1H, m, Pro-2-H), 4.90 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.55 (1H, m, N–H or –COOH), 5.88 (1H, m, 22-H), 7.44 (1H, m, N–H or –COOH).

ii) A solution of I (30 mg) in pyridine (2 ml) was treated with an aq. solution of L-Pro-Gly·OH[9] (10 mg in 1 ml) in the same manner as described in IVa. The crude product obtained was chromatographed on silica gel (200 mg). Elution with AcOEt and trituration of the eluate with hexane gave VII (20 mg) as colorless amorphous substance. IR spectra of two samples obtained in i) and ii) were entirely identical in every respect.

Digitoxigenin 3-Suberoyl-L-prolyl-L-proline (VIIIa)——i) A solution of IIIb (39 mg) in pyridine (6 ml) was treated with a methanolic solution of L-Pro–OH (30 mg in 2 ml) in the same manner as described in IVa. The crude product obtained was chromatographed on silica gel (500 mg). Elution with AcOEt and trituration of the eluate with hexane gave VIIIa (18 mg) as colorless amorphous substance. mp 110—117°. $[\alpha]_D^{19}$ −45.6° (c=0.08). *Anal.* Calcd. for $C_{41}H_{60}O_9N_2$: C, 67.93; H, 8.34; N, 3.86. Found: C, 67.29; H, 8.78; N, 3.85. NMR (4% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.60 (4H, m, Pro-5-CH_2 and –5'-CH_2), 4.60 (2H, m, Pro-2-H and –2'–H), 4.90 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.88 (1H, m, 22-H).

ii) A solution of L-Pro–OH (107 mg) and triethylamine (0.24 ml) in H_2O (2.2 ml)–dioxane (3.5 ml) was adjusted to pH 8 by the addition of dry ice with stirring. To the resulting solution was added IIIb (79 mg) and treated in the same manner as described in IIIa. The crude product obtained was chromatographed on silica gel (500 mg). Elution with AcOEt and trituration of the eluate with hexane gave VIIIa (43 mg) as colorless amorphous substance.

Digitoxigenin 3-Suberoyl-L-prolyl-L-proline *p*-Nitrophenyl Ester (VIIIb)——To a solution of VIIIa (43 mg) and *p*-nitrophenol (28 mg) in AcOEt (4 ml) was added N,N'-dicyclohexylcarbodiimide (25 mg) and treated in the same manner as described in IIb. The crude product obtained was submitted to the preparative TLC using AcOEt as developing solvent. The adsorbent corresponding to the spot was eluted with AcOEt to give VIIIb (36 mg) as a yellow oil. NMR (5% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.60 (4H, m, Pro-5-CH_2 and –5'-CH_2), 4.65 (2H, m, Pro-2-H and -2'-H), 4.90 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.88 (1H, m, 22-H), 7.27 (2H, d, J=9 Hz, ), 8.22 (2H, d, J=9 Hz, ). TL-II: 0.10.

Digitoxigenin 3-Suberoyl-L-seryl-L-proline (IXa)——A solution of L-Ser-L-Pro·OH[10] (15 mg) and triethylamine (0.02 ml) in H_2O (0.5 ml)–dioxane (0.5 ml) was adjusted to pH 8 by the addtion of dry ice with stirring. To the resulting solution was added I (40 mg) and treated in the same manner as described in IIIa. The crude product obtained was chromatographed on silica gel (300 mg). Elution with AcOEt–MeOH (10: 1) and trituration of the eluate with CH_2Cl_2–ether gave IXa (30 mg) as colorless amorphous substance. mp 180—182°. NMR (4% solution in $CDCl_3$) δ: 0.87 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 3.75 (4H, m, Pro-5-

9) F. Weygand, A. Prox, M.A. Tilak, D. Hoffter, and H. Fritz, *Chem. Ber.*, 97, 1024 (1964).
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CH_2 and Ser–C<u>H</u>$_2$OH), 4.50 (1H, m, Pro-2-H), 4.70 (2H, m, 21-CH_2), 5.05 (1H, m, 3α-H), 5.85 (1H, m, 22-H). The analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

Digitoxigenin 3-Suberoyl-L-seryl-L-proline Methyl Ester (IXb)——IXa (30 mg) was methylated with CH_2N_2 in the usual manner. Recrystallization of the product from MeOH–ether gave IXb (14 mg) as colorless prisms. mp 116—117°. $[\alpha]_D^{25}$ −4.5° (c=0.11). *Anal.* Calcd. for $C_{40}H_{60}O_{10}N_2 \cdot H_2O$: C, 64.32; H, 8.37; N, 3.75. Found: C, 64.20; H, 8.48; N, 3.73. NMR (4% solution in $CDCl_3$) δ: 0.87 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.72 (3H, s, –$COOCH_3$), 3.72 (2H, m, Pro-5-CH_2), 3.80 (2H, m, Ser–C<u>H</u>$_2$OH), 4.55 (1H, m, Pro-2-H), 4.90 (2H, m, 21-CH_2), 5.05 (1H, m, 3α-H), 5.85 (1H, m, 22-H), 6.60 (1H, m, N–H).

Digitoxigenin 3-Suberoylglycyl-glycyl-glycine (Xa)——A solution of I (30 mg) in pyridine (2 ml) was treated with an aq. solution of $(Gly)_3 \cdot OH$ (10 mg in 1 ml) in the same manner as described in IVa. The crude product obtained was submitted to further elaboration without purification.

Digitoxigenin 3-Suberoylglycyl-glycyl-glycine Methyl Ester (Xb)——Xa (30 mg) was methylated with CH_2N_2 in the usual manner. The crude product obtained was submitted to the preparative TLC using AcOEt–MeOH (9: 1) as developing solvent. The adsorbent corresponding to the spot was eluted with AcOEt. Recrystallization of the eluate from MeOH–acetone gave Xb (20 mg) as colorless leaflets. mp 206—208°. $[\alpha]_D^{22}$ +15.3° (c=0.07, MeOH). *Anal.* Calcd. for $C_{38}H_{57}O_{10}N_3$: C, 63.75; H, 8.03; N, 5.87. Found: C, 63.78; H, 8.30; N, 5.62. TL-IV: 0.08.

Digitoxigenin 3-Suberoylglycyl-L-phenylalanyl-L-serine (XIa)——A solution of L-Ser·OH (20 mg) and triethylamine (0.03 ml) in H_2O (0.3 ml)-dioxane (0.5 ml) was adjusted to pH 8 by the addition of dry ice with stirring. To the resulting solution was added Vc (53 mg) and treated in the same manner as described in IIIa. The crude product obtained was chromatographed on silica gel (400 mg). Elution with acetone–MeOH (10: 1) and trituration of the eluate with hexane gave XIa (26 mg) as colorless amorphous substance. mp 85—89°. The analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

Digitoxigenin 3-Suberoylglycyl-L-phenylalanyl-L-serine Methyl Ester (XIb)——XIa (17 mg) was methylated with CH_2N_2 in the usual manner. The crude product obtained was submitted to the preparative TLC using AcOEt as developing solvent. The adsorbent corresponding to the spot was eluted with AcOEt to give XIb (12 mg) as colorless amorphous substance. mp 83—90°. $[\alpha]_D^{21}$ +40.5° (c=0.07). *Anal.* Calcd. for $C_{46}H_{65}O_{11}N_3 \cdot H_2O$: C, 64.69; H, 7.91; N, 4.92. Found: C, 64.36; H, 7.77; N, 4.40. NMR (6% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 3.15 (2H, m, C_6H_5C<u>H</u>$_2$–), 3.75 (3H, s, –$COOCH_3$), 3.80 (4H, m, –NHC<u>H</u>$_2$CO– and Ser–C<u>H</u>$_2$OH), 4.90 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.88 (1H, m, 22-H), 7.25 (5H, m, C_6H_5–). TL-III: 0.15.

Digitoxigenin 3-Suberoyl-L-prolyl-glycyl-L-phenylalanine (XII)——A solution of IIIb (80 mg) in pyridine (2 ml) was treated with an aq. solution of Gly-L-Phe·OH (40 mg in 2 ml) in the same manner as described in IVa. The crude product obtained was chromatographed on silica gel (500 mg). Elution with AcOEt–MeOH (4: 1) and trituration of the eluate with AcOEt gave XII (40 mg) as colorless amorphous substance. mp 125—127°. $[\alpha]_D^{18}$ +28.6° (c=0.07). *Anal.* Calcd. for $C_{47}H_{65}O_{10}N_3 \cdot 2H_2O$: C, 65.02; H, 8.01; N, 4.84. Found: C, 64.75; H, 7.73; N, 4.96. NMR (4% solution in $CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 3.10 (2H, m, C_6H_5C<u>H</u>$_2$–), 3.50 (2H, m, Pro-5-CH_2), 4.35 (1H, m, Pro-2-H), 4.85 (2H, m, 21-CH_2), 5.05 (1H, m, 3α-H), 5.50 (2H, m, N–H or –COOH), 5.85 (1H, m, 22-H), 7.15 (5H, m, C_6H_5–), 7.60 (1H, m, N–H or –COOH).

Digitoxigenin 3-Suberoyl-L-prolyl-L-prolyl-glycyl-L-phenylalanine (XIII)——A solution of Gly-L-Phe·OH (50 mg) and triethylamine (0.11 ml) in H_2O (1 ml)–dioxane (1.6 ml) was adjusted to pH 8 by the addition of dry ice with stirring. To the resulting solution was added VIIIb (36 mg) and treated in the same manner as described in IIIa. The crude product obtained was chromatographed on silica gel (400 mg). The eluate with acetone–MeOH (1: 1) was submitted to gel filtration on Sephadex LH-20 using MeOH as eluent. Trituration of the eluate with hexane gave XIII (10 mg) as colorless amorphous substance. mp 131—138°. $[\alpha]_D^{23}$ −31.1° (c=0.08). *Anal.* Calcd. for $C_{52}H_{72}O_{11}N_4 \cdot H_2O$: C, 65.94; H, 7.88; N, 5.92. Found: C, 66.32; H, 7.89; N, 5.64. NMR (5% solution in $CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 0.96 (3H, s, 19-CH_3), 3.50 (4H, m, Pro-5-CH_2 and -5'-CH_2), 4.50 (2H, m, Pro-2-H and -2'-H), 4.90 (2H, m, 21-CH_2), 5.07 (1H, m, 3α-H), 5.85 (1H, m, 22-H), 7.15 (5H, m, C_6H_5–).

Digitoxigenin 3-Suberoylglycyl-glycyl-glycyl-glycine (XIVa)——A solution of $(Gly)_4 \cdot OH$ (60 mg) and triethylamine (0.06 ml) in H_2O (0.6 ml)–dioxane (0.9 ml) was adjusted to pH 8 by the addition of dry ice with stirring. To the resulting solution was added I (120 mg) and stirred at room temperature for 20 hr. After acidification with 1N HCl the precipitated product was collected by filtration and washed with H_2O. XIVa (88 mg) was obtained as colorless amorphous substance. mp 220—225° (decomp.). The analytical sample could not be obtained and therefore the crude product was submitted to further elaboration without purification.

Digitoxigenin 3-Suberoylglycyl-glycyl-glycyl-glycine Methyl Ester (XIVb)——To a solution of XIVa (40 mg) in CH_2Cl_2–MeOH (2: 5) (35 ml) was added an ethereal solution of CH_2N_2 and stirred for 5 hr. The resulting solution was evaporated *in vacuo* and the residue obtained was chromatographed on silica gel (400

mg). Eluate with $CHCl_3$–MeOH (1: 1) was further purified by gel filtration on Sephadex LH-20 using $CHCl_3$–MeOH (1: 1) as eluent to give XIVb (30 mg) as colorless amorphous substance. mp 223—226° (decomp.). $[\alpha]_D^{26}$ +29.4° (c=0.14, $CHCl_3$–MeOH (1: 1)). *Anal.* Calcd. for $C_{40}H_{60}O_{11}N_4$: C, 62.15; H, 7.82; N, 7.25. Found: C, 61.84; H, 8.05; N, 7.24. NMR (5% solution in $CDCl_3$–CD_3OD (3: 1)) δ: 0.88 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 3.72 (3H, s, -$COOCH_3$), 3.90 (8H, m, (-$NHCH_2CO$-)$_4$), 4.94 (2H, m, 21-CH_2) 5.08 (1H, m, 3α-H), 5.88 (1H, m, 22-H).

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