by Santesson[1] in some *Caloplaca* species on account of its significant molecular ion peak at 402 *m/e* might be identical with the present caloploicin (I).

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Synthesis of 3-Suberoylalanine Ester of Digitoxigenin

Since the first discovery by Wieland, *et al.*,[1] the so-called "bufotoxin" isolated from the toad venom drug, *Ch'an Su*, has been assigned to the structure 14-suberoylarginine ester of bufotalin.[2] Kamano, *et al.*[3] isolated 3-suberoylbufogenins from *Ch'an Su*, and Linde-Tempel[4] revised the structure of "bufotoxin" to be the 3-suberoylarginine ester from the evidence of enzymatic degradation. In addition the isolation of the 3-(hydrogen suberate) as well as cardenolide itself from *Ch'an Su* has recently been reported.[5] These findings together led to the assumption that the suberoylamino acid ester of cardenolide may possibly occur as the natural product. The physiological activity of the potential cardenolide conjugate also appeared to be of interest to us. In this paper we wish to report the synthesis of the titled compound starting from digitoxigenin (Ia).

As a preliminary experiment an attempt was initiated on the preparation of the 3-succinoylglycine ester. Reaction of digitoxigenin 3-(hydrogen succinate) (Ib)[6] with ethyl chlorocarbonate in tetrahydrofuran gave the carbonic-carboxylic acid anhydride, which in turn was condensed with *tert*-butyl glycinate to give the 3-succinoylglycine ester (IIa), mp 185—187°, as colorless leaflets (from ethyl acetate). Upon brief exposure to hydrogen bromide in acetic acid[7] elimination of the *tert*-butyl group was readily attained, but undesirable dehydration of the 14-hydroxyl function was accompanied to a considerable extent. Some other attempts for the selective hydrolysis without affecting any disturbance on the steroidal moiety also resulted in failure. Accordingly an alternative synthetic route, that is the *p*-nitrophenyl ester method,[8] was undertaken. Being treated with *p*-nitrophenol in the presence of N,N-dicyclohexylcarbodiimide, Ib was transformed into the *p*-nitrophenyl ester (IIIa), mp 172—174°, colorless prisms (from MeOH). When stirred with glycine in aqueous pyridine, IIIa

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6) M. Zingg and K. Meyer, *Pharm. Acta Helv.*, **32**, 393 (1957); A. Yamada, *Yakugaku Zasshi*, **79**, 1440 (1959).
7) G.W. Anderson and F.M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960).
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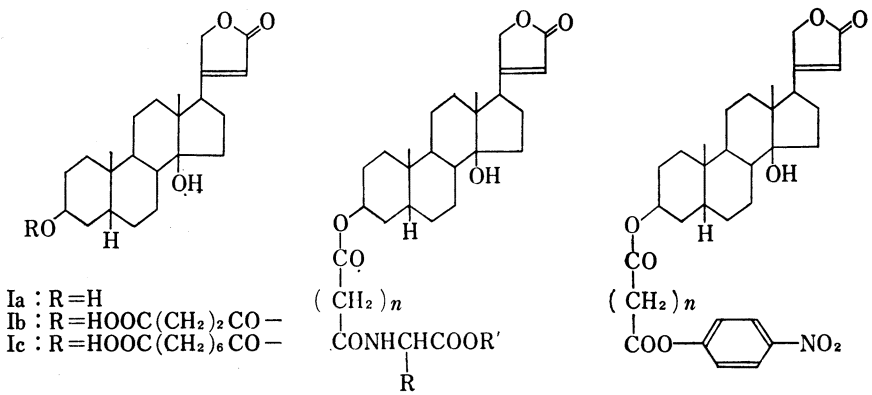
was led to 3-succinoylglycine ester of digitoxigenin (IIc) in satisfactory yield. Unfortunately this substance could not be obtained in the crystalline state, but the structure was rationalized by the spectral data. NMR (in $CDCl_3$) δ: 0.86 (3H, s, 18-CH_3), 0.95 (3H, s, 19-CH_3), 2.60 (4H, m, $-CO(CH_2)_2CO-$), 4.10 (2H, d, J=6 cps, $-NHCH_2CO-$), 4.90 (2H, m, 21-CH_2), 5.10 (1H, m, 3α-H), 5.85 (2H, m, 22-H, -COOH), 6.50 (1H, m, N-H). Treatment with diazomethane in the usual manner afforded a methyl ester (IIb), mp 156—157°, as colorless needles (from MeOH–ether), which proved to be identical with the specimen prepared from Ib by the mixed anhydride method.

On the basis of these model experiments the next project was directed to the synthesis of the titled compound employing 3-(hydrogen suberate) of digitoxigenin (Ic).[5b] The *p*-nitrophenyl ester (IIIb) was prepared from Ic in the same manner as described above, mp 165—166°, $[\alpha]_D^{18}$ +12.8° (c=0.23, $CHCl_3$). *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. Condensation with alanine followed by chromatographic purification on silica gel gave the desired 3-suberoylalanine ester (IId) as colorless amorphous substance, mp 148—150°, $[\alpha]_D^{15}$ −11.4° (c=0.10, $CHCl_3$). *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 (in $CDCl_3$) δ: 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, 3α-H), 5.55 (1H, m, -COOH), 5.85 (1H, m, 22-H), 6.40 (1H, m, N-H).



Ia : R=H
Ib : R=HOOC$(CH_2)_2$CO−
Ic : R=HOOC$(CH_2)_6$CO−

IIa : n=2, R=H, R'=$(CH_3)_3C-$
IIb : n=2, R=H, R'=CH_3-
IIc : n=2, R=R'=H,
IId : n=6, R=CH_3-, R'=H

IIIa : n=2
IIIb : n=6

Chart 1

The preparation of the various 3-suberoylamino acid esters of cardenolide is being conducted in this laboratory and the details will be reported in the near future.

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