Seeds of *Thevetia* Species as an Alternative Source of Digitoxigenin[1,2]

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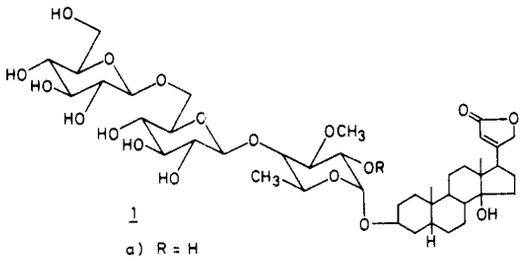
Received April 29, 1977

The Collins oxidation of neriifolin (**2a**) resulted in the selective formation of the β-ketol **3a**. This substance, after acetylation and pyridine-induced elimination of the elements of acetic acid, gave the enone **5**, which underwent hydrolysis to digitoxigenin (**2e**) under very mild acidic conditions.

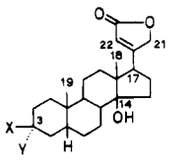
Several species of *Thevetia* (Apocinacae), for example, *Th. thevetoides* Schum., and *Th. neriifolia*, Juss., grow wild in Mexico,[3] and the latter species in particular is also found in many other areas of the world.[4] The seeds of these plants have a high content of cardenolide triglycosides, mainly thevetin (**1a**) and acetylated or oxidized derivatives thereof.[5,6] Hydrolytic cleavage of the triglycosides by the endogenous enzyme(s) of the plant is known to give[5-7] a mixture of monoglycosides which consists mainly of neriifolin (**2a**), as well as



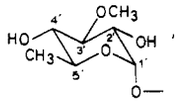
1

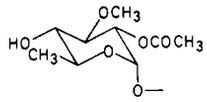
a) R = H

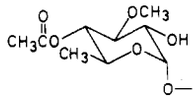
b) R = CH_3CO

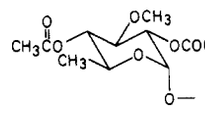


2

a) X =  Y = H

b) X =  Y = H

c) X =  Y = H

d) X =  Y = H

e) X = OH, Y = H

f) X, Y = O

g) X = OCHO, Y = H

lesser amounts of neriifolin monoacetate (**2b**) and other minor components. In principle, neriifolin and neriifolin monoacetate might serve as practical sources of digitoxigenin (**2e**), but the cleavage of the glycosidic linkages of these α-L-thevetosides[8] has to date not been accomplished in satisfactory yield either by chemical[7] or enzymatic[11] methods. Digitoxigenin is of importance in that it can serve as a useful point of embarcation for the synthesis of modified cardenolides.[12]

This paper describes a method whereby neriifolin and neriifolin monoacetate can be chemically degraded, under mild conditions and in practical yield, to digitoxigenin.

In connection with the determination of the structure of neriifolin, Helfenberger and Reichstein[7a] showed that acidic hydrolysis (0.35 N hydrochloric acid in acetone at room temperature) of this substance could not be effected without prior (or concomitant) elimination of the hydroxyl group at C-14 of the steroidal residue. These authors[7b] did, however, demonstrate that the glycosidic linkage could be cleaved, without loss of the 14-hydroxyl group, by the combined oxidative–hydrolytic process shown in eq 1 and 2. Digitoxigenin must have been liberated during the second phase of the process, at least, because oxidation (step 3) of the crude hydrolysate gave digitoxigenone (**2f**) in about 20% overall yield.

$$\text{Neriifolin} \xrightarrow[\text{2. 0.05 N } H_2SO_4]{\text{1. } CrO_3/CH_3COOH/\text{R.T.}} \text{Digitoxigenin?} \quad (1)$$

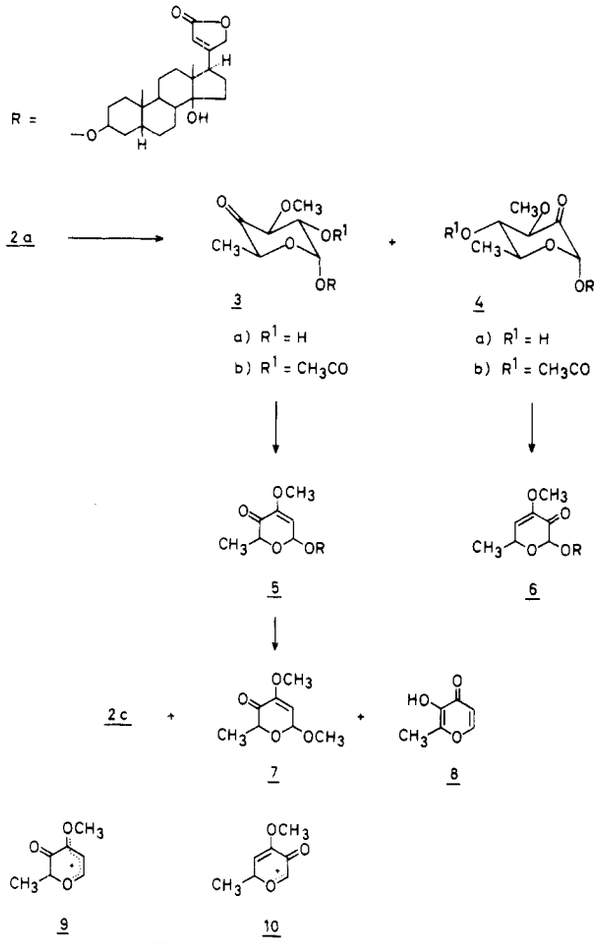
$$\text{Digitoxigenin} \xrightarrow[CH_3OH\text{-}H_2O/\text{reflux}]{\text{3. } CrO_3/CH_3COOH/\text{R.T.}} \text{Digitoxigenone} \quad (2)$$

Repetition of the first two steps of the above process gave a mixture in which the presence of digitoxigenin was confirmed (17% isolated yield), but this substance was accompanied by an equal amount of digitoxigenone. Indeed, careful examination of the oxidation mixture before acidic hydrolysis showed that digitoxigenone[13] was already present at this stage. Two glycosidic α-ketols (**3a** and **4a**, see below), digitoxigenin formate (**2g**), and an acid-soluble degradation product still containing the butenolide moiety were also isolated from this mixture. Digitoxigenin formate was rapidly converted into digitoxigenin under the conditions of step 2.

The early formation of digitoxigenone in the above process suggested that perhaps a part of the degradation of neriifolin was occurring via a glycosidic intermediate which fragmented to digitoxigenin under the acidic oxidation conditions. The synthesis of such an acid-labile intermediate, the hypothetical structure of which was based on speculation concerning the mechanistic nature of the oxidative degradation, was therefore investigated.

Collins oxidation[15] of neriifolin gave a 5.2–6.0:1 mixture of two β-ketols, **3a** and **4a** (Scheme I), which were easily distinguished by means of the multiplicity of the NMR absorptions (see Table I) of the anomeric hydrogens. The anomeric proton of the major product **3a** appeared as a doublet at δ 5.06 ($J_{1',2'}$ = 4 Hz), whereas this proton resonated as a singlet at δ 4.77 for the less abundant ketone. Both ketols were stable to the hydrolysis conditions shown in eq 1, but, as expected, chromic acid oxidation of either ketol gave digitoxigenin formate and digitoxigenone in a 1:2 ratio. The 4′-ketone **4a** was, however, oxidized at least five times as rapidly as **3a**. Acetylation of **3a** and **4a** with acetic anhydride in pyridine solution gave the acetate **3b** and the enone **6**, respectively. The acetate **4b**, obviously, had lost the elements of acetic acid under the acetylation conditions. This substance was preparable, albeit in an impure state, by the chromic acid oxidation of neriifolin 4′-acetate (**2c**, see below), but attempted purification of this substance by chromatography on alumina or silica gel resulted in the formation of the enone **6**. The acetate **3b** required heating in pyridine solution at 80 °C to effect the elimination

Scheme I



of acetic acid. The enone **5**, thus obtained, was readily differentiated from **6** by means of its NMR spectrum. In particular, for **5**, the anomeric hydrogen and the adjacent olefinic proton appeared as a pair of doublets (J = 4.4 Hz) at δ 5.41 and 5.68, while for **6**, H-1′ and H-4′ showed singlet and doublet resonances at δ 4.95 and 5.71 (J = 1.5 Hz), respectively. In addition to the NMR spectral differences, the enones possessed markedly dissimilar stabilities toward 0.05 N sulfuric acid in 50% aqueous methanol. Whereas **5** was rapidly hydrolyzed to an easily separable mixture of digitoxigenin (45% yield from neriifolin), the methyl glycoside **7**, and 2-methyl-3-hydroxy-4-pyrone (**8**), even at room temperature, **6** was largely recovered from the hydrolytic medium after 1 h at reflux temperature. The ease of hydrolysis of **5** is presumably a reflection of the enhanced stability of the carbonium ion **9**, while the resistance to cleavage of **6** must derive from the destabilized nature of the α-oxocarbonium ion **10**, which would be generated if the hydrolysis of **6** was to occur.

The formation of the ketol **4a** was obviously deleterious to the yield of digitoxigenin, and therefore, various oxidative methods were investigated in order to reduce the amount of this substance in the mixture, or to eliminate it entirely. None of the methods studied was, however, as effective as the Collins oxidation, since a lower ratio of **3a/4a** was produced in every case.[16]

As mentioned previously, neriifolin monoacetate represents a considerable portion of the glycosidic material obtainable from *Thevetia* species, and consequently the conversion of this substance into digitoxigenin is also of importance. Oxidation of this acetate by the Collins method gave the ketoacetate **3b** in high yield, the degradation of which to digitoxigenin has already been described.

The formation of **3b** from neriifolin monoacetate conclusively establishes the structure of this substance as **2b**. Furthermore, it unambiguously demonstrates that, in both thevetin (**1a**) and thevetin monoacetate (**1b**), the gentobiose residue is attached to the C-4 oxygen of the thevetose moiety. This corrects a previous,[6] admittedly[21] uncertain, assignment of this structural point.

Neriifolin isolated from Mexican sources, as mentioned previously, contains neriifolin monoacetate and a glycoside (as well as the monoacetate thereof), the structure of the aglycone portion of which is not yet known.[22] The separation of these substances could be achieved by a combination of crystallization and column chromatography on silica gel. A more convenient procedure was to acetylate the glycosidic mixture and then separate the diacetates by column chromatography on silica gel. Neriifolin diacetate (**2d**) was then converted back into neriifolin by the zinc acetate or 1,5-diazabicyclo[4.3.0]non-5-ene promoted transesterification in methanol.

The experiments described above show that neriifolin is an attractive alternative source of digitoxigenin, especially in those parts of the world where *Thevetia* species are abundant.

Experimental Section

The melting points were determined in a Mel-Temp melting point apparatus and are not corrected. The infrared spectra were measured with a Perkin-Elmer Model 237 grating infrared spectrophotometer. The ultraviolet spectra were recorded on a Perkin-Elmer Model 402 ultraviolet visible spectrophotometer. The NMR spectra were measured with a Varian HA-100 spectrometer and are expressed as parts per million (δ) from internal tetramethylsilane.

Isolation of Neriifolin and Monoacetylneriifolin from *Thevetia thevetoides* Schum. The defatted (hexane), powdered meal (1.06 kg) from the seeds was incubated in water in the manner described by Helfenberger and Reichstein (see ref 7a, p 1479). The crude methanol extract (52 g) obtained therefrom crystallized spontaneously. Crystallization of this material from methanol–water gave crude neriifolin (25 g): mp 198–206 °C; $[\alpha]_D$ −46° (MeOH); UV (MeOH) 218 nm (ϵ 15 800). After recrystallization from the same solvent system, material with mp 209–214 °C was obtained: $[\alpha]_D$ −49° (c 0.39, MeOH); UV (MeOH) 218 nm (ϵ 17 300) [lit.[7a] mp 218–225 °C; $[\alpha]_D$ −50.2 ± 2° (MeOH); UV (EtOH) 217 nm (ϵ 12 500)]. Chromatography of the mother liquors on a column of silica gel gave monoacetylneriifolin (9 g, eluted with ethyl acetate–hexane, 2:3), which after crystallization from methanol had: mp 203–205 °C; $[\alpha]_D$ −88° (MeOH); UV (MeOH) 218 nm (ϵ 14 700). Recrystallization from aqueous methanol gave material with: mp 218–220 °C; $[\alpha]_D$ −91° ($CHCl_3$); UV (MeOH) 218 nm (ϵ 23 000) [lit.[7a] mp 240 °C; $[\alpha]_D$ −72.5° ($CHCl_3$)].

There was also obtained from the above chromatographic separation a mixture of glycosides (3.5 g) of unknown[22] structures.

Isolation of Neriifolin via Neriifolin Diacetate. The crude mixture of glycosides (100 g), from which some of the neriifolin had been removed (see above), pyridine (300 mL), and acetic anhydride (100 mL) were left to react at room temperature for 18 h. The solution was diluted with water and the products were extracted into ethyl acetate. The extract was washed successively with dilute hydrochloric acid, dilute sodium bicarbonate solution, and water, and then dried over sodium sulfate. Removal of the solvent in vacuo gave a residue (113 g) which was subjected to chromatography on neutral alumina (7 kg, Fluka, activity III). The column was eluted with hexane and then hexane–ethyl acetate mixtures, the hexane content of which was decreased gradually from 80 to 65%. Neriifolin diacetate (57.1 g, 95% pure by TLC), of a purity sufficient to be used in the transesterification reaction, was removed from the column with the 65:35 hexane–ethyl acetate mixture. One crystallization of this material from hexane–dichloromethane gave material: mp 126–128 °C; $[\alpha]_D$ −83° (c 0.41, $CHCl_3$); UV (MeOH) 219 nm (ϵ 13 800) [lit.[7a] mp 136–138 °C; $[\alpha]_D$ −79 ± 2° ($CHCl_3$)].

Elution of the column with pure ethyl acetate gave a mixture (28 g) of more polar acetates.

A solution of neriifolin diacetate (32 g, 0.052 mol) and zinc acetate (64 g, 0.42 mol) in anhydrous methanol (400 mL) was boiled under reflux for 48 h. Most of the methanol was removed in vacuo and water was added to the residue. The mixture was extracted with a large volume of ethyl acetate, and the extract was washed with dilute hydrochloric acid and then with water. The extract was dried over so-

Table I. NMR Data[a] for Neriifolin and Related Compounds

Compd	Registry no.	1′-H	2′-H	3′-H	3′-OCH_3	4′-H	5′-H	5′-CH_3	3α-H	17α-H	18-CH_3	19-CH_3	H-21	H-21′	H-22
2a[b]	466-07-9	4.63 d J = 3.3			3.49	2.81 t J = 8.6		1.06 d J = 6.2	3.79 m	2.73 m	0.76	0.88	4.93	4.93	5.90
2b	25633-34-5	5.04 d J = 3.7	4.61 q J = 3.7 J = 10		3.55	3.16 t J = 9		1.24 d[c] J = 6	3.84 m	2.74 m	0.86	0.94	4.77 q[d,e]	5.02 q	5.85 t
2c	25633-33-4	4.91 d J = 3.7		3.36 t J = 9.1	3.50	4.65 t J = 9.1	3.79 m J = 6 J = 9.1	1.09 d[f] J = 6		2.73 m	0.87	0.96	4.74 q	4.97 q	5.82 t
2d	1065-34-5	5.06 d J = 3.4	4.65 q J = 3.4 J = 9.5	3.65 t J = 9.7	3.42	4.70 t J = 9.1	3.87 m J = 6 J = 9.1	1.10 d[g] J = 6	3.86 m	2.73 m	0.87	0.95	4.76 q	5.01 q	5.84 t
2e	143-62-4								4.11 m	2.76 m	0.87	0.97	4.75 q	5.01 q	5.86 t
2f	1102-88-1									2.75 m	0.90	1.01	4.75 q	5.00 q	5.85 t
2g	1250-96-0								5.22 m[h]	2.78 m	0.87	0.96	4.76 q	5.02 q	5.84 t
3a	58924-92-8	5.06 d J = 4.0		4.00 d J = 10	3.60		4.29 q J = 6.5	1.24 d J = 6.5	4.05 m	2.74 m	0.87	0.97	4.75 q	5.00 q	5.85 t
3b	63493-66-3	5.29 d J = 4.0	4.93 q J = 4.0 J = 10.7	4.20 d J = 10.7	3.58		4.36 q J = 6.5	1.28 d[i] J = 6.5	4.00 m	2.76 m	0.87	0.97	4.78 q	5.03 q	5.90 t
4a	63511-68-2	4.77		4.11 d J = 9	3.67			1.32 d J = 6.2	4.03 m	2.78 m	0.88	0.94	4.73 q	5.01 q	5.86 t
4b	63493-67-4	4.80		4.21 d J = 10.4	3.49	4.93 t J = 9.7		1.20 d[j] J = 6.2	4.03 m	2.74 m	0.87	0.94	4.76 q	4.94 q	5.87 t
5	63527-41-3	5.41 d J = 4.4	5.68 d J = 4.4		3.63		4.63 q J = 7	1.37 d J = 7	4.03 m	2.76 m	0.88	0.94	4.74 q	5.00 q	5.88 t
6	63493-68-5	4.95			3.62	5.71 d J = 1.5	4.84 m J = 1.5 J = 7	1.37 d J = 7	4.04 m	2.77 m	0.86	0.90	4.80 q	5.02 q	5.84 t

[a] Unless indicated otherwise, the spectra were recorded in $CDCl_3$. [b] Measured in Me_2SO-d_6. [c] Acetate methyl at δ 2.04. [d] H-21 and H-21′ assigned arbitrarily. [e] $J_{21,21'}$ = 18.0 ± 0.5 Hz, $J_{21,22}$ = 1.5 ± 0.2 Hz = $J_{21',22}$ for this and all subsequent compounds. [f] Acetate methyl at δ 2.08. [g] Acetate methyls at δ 2.03, 2.07. [h] Formyl hydrogen at δ 8.03. [i] Acetate methyl at δ 2.08. [j] Acetate methyl at δ 2.10.

dium sulfate and evaporated in vacuo, giving a residue which on crystallization from ethyl acetate gave neriifolin (20 g) with mp 216–218 °C. The mother liquor from the crystallization was subjected to column chromatography on neutral alumina (800 g, Fluka, Activity III). Elution with hexane–ethyl acetate (60:40) gave a mixture of monoacetates which could be transesterified in the manner described above to give more neriifolin. Elution with hexane–ethyl acetate (1:1) gave a further quantity (6 g) of neriifolin (total of 26 g or 94% based on diacetate taken).

The transesterification could also be effected with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in methanol at room temperature (23 h). The reaction was worked up as described above to give a mixture which was separated by column chromatography on alumina. Neriifolin was isolated in 64% yield together with neriifolin 4′-monoacetate (**2c**, 30%). If the transesterification was allowed to proceed for 6 h the principal product was **2c** (see below).

Neriifolin 4′-Monoacetate (2c). A solution of neriifolin diacetate (6.0 g, 0.0097 mol) in dry methanol (250 mL) containing DBN (15 drops) was left at room temperature for 6 h. The solution was diluted with water, the product was extracted into ethyl acetate, and the extract was washed with water and dried over sodium sulfate. Evaporation of the solvent gave a residue which was chromatographed on neutral alumina (1.1 kg, Fluka, activity III). Elution with hexane–ethyl acetate (65:35) gave the starting diacetate (0.61 g, 10%). Neriifolin 4′-monoacetate (3.6 g, 64%) was eluted with hexane–ethyl acetate (3:2) and after crystallization from ether–pentane it had: mp 221–222 °C; $[\alpha]_D$ −55° (*c* 0.34, $CHCl_3$); UV (MeOH) 220.5 nm (ε 13 200); IR ($CHCl_3$) 3590, 3560, 1785, 1745, 1625 cm^{-1}.

Anal. Calcd for $C_{32}H_{48}O_9$: C, 66.64; H, 8.39. Found: C, 66.44; H, 8.41.

Finally, elution with hexane–ethyl acetate (9:11) gave neriifolin (1.2 g, 23%, mp 212–215 °C).

Oxidation of Neriifolin. (A) Chromium Trioxide in Acetic Acid. A solution of chromium trioxide in acetic acid (10 mg CrO_3/mL) was added, in a dropwise manner at room temperature, to a stirred solution of neriifolin (1.30 g, 0.00243 mol) in acetic acid (10 mL). The consumption of the starting material was followed by TLC, and the addition of the oxidant was continued until the starting material had almost disappeared. A total of 89 mL of the chromium trioxide solution was added during 28 h. The mixture was poured into ice–water and the resultant was exhaustively extracted with chloroform. The extract was washed with saturated sodium bicarbonate solution, then with water, and finally, it was dried over sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel (100 g) to give the following products in succession.

1. Digitoxigen 3-formate (**2g**, 0.061 g; 6%) eluted with hexane–ethyl acetate (7:3). After crystallization from chloroform–ether it had: mp 191–193 °C; $[\alpha]_D$ +18° (*c* 0.276, $CHCl_3$); UV (MeOH) 218.5 nm (ε 17 000) [lit.[23] mp 198–201 °C; $[\alpha]_D$ +18 ± 3° ($CHCl_3$)].

2. Digitoxigenone (**2f**, 0.137 g, 15% eluted with hexane–ethyl acetate (3:2). After crystallization from acetone–ether it had: mp 194–197 °C; $[\alpha]_D$ +25° (*c* 0.313, $CHCl_3$); UV (MeOH) 217 nm (ε 16 200) [lit.[7b] mp 204–205 °C; $[\alpha]_D$ +32.3 ± 2° ($CHCl_3$)]. This was identical with an authentic specimen prepared by the oxidation of digitoxigenin.

3. Neriifolin-2′-one (**4a**, 0.087 g, 7%) eluted with hexane–ethyl acetate (3:2). The physical constants of this substance are recorded below.

4. Neriifolin-4′-one (**3a**, 0.234 g, 18%) eluted with hexane–ethyl acetate (3:2 and 1:1). The physical constants for this substance are recorded below.

5. Neriifolin (0.038 g, 3%) eluted with ethyl acetate.

6. A polar acidic material (0.415 g) eluted with methanol. After dissolution in aqueous sodium bicarbonate solution and reprecipitation with dilute hydrochloric acid, it had: mp 250–260 °C dec; $[\alpha]_D$ 0° (MeOH); UV (MeOH) 219 nm (ε 6600); IR (KBr) 3450, 1745, 1625 cm^{-1}.

Repetition of the above oxidation on a larger scale, using neriifolin (4.2 g) and chromium trioxide (6.0 g) in acetic acid (total volume of 185 mL) over a 48-h period, gave a crude product which was subjected to acidic hydrolysis. This was effected by heating a solution of the above mixture in methanol (160 mL) and 0.1 N sulfuric acid (120 mL) for 0.5 h at reflux temperature. The solvent was removed in vacuo and the residue was extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate, and then evaporated in vacuo. The residue was subjected to thin-layer chromatography on silica gel using hexane–ethyl acetate (7:3) as the developing solvent. From this mixture was isolated digitoxigenone (0.50 g, 17%, mp 194–197 °C after crystallization from acetone–ether) and digitoxigenin (0.50 g, 17%). This latter substance had mp 233–236 °C after crystallization from acetone–ether: $[\alpha]_D$ +22° (*c* 0.382, $CHCl_3$); UV (MeOH) 217 nm (ε 15 500) [lit.[24] mp 243–246 °C; $[\alpha]_D$ +23° ($CHCl_3$); UV (MeOH) 217 nm (ε 16 200)].

(B) Collins Oxidation. To a vigorously stirred suspension of dry Celite (60 g) and pyridinium chromate (60 g) in dry dichloromethane (800 mL) at 0 °C was added a solution of neriifolin (10 g, 0.0187 mol) in anhydrous dichloromethane (100 mL). The mixture was stirred at 0 °C for 2.5 h and then at room temperature for 2 h. Sodium bisulfate monohydrate (100 g) was then added and agitation was continued for an additional 0.5 h. The mixture was filtered, the filter cake was exhaustively extracted with dichloromethane (total of 12 L), and the combined dichloromethane filtrate and extracts were washed with water and then dried over sodium sulfate. Evaporation of the solvent in vacuo gave a residue which was chromatographed on neutral alumina (1 kg, Fluka, activity III). Elution with hexane–ethyl acetate (65:35) removed a small amount of nonpolar material, which was followed by neriifolin-2′-one (1.0 g, 10%). This substance had mp 145–148 °C after crystallization from aqueous methanol: $[\alpha]_D$ 0° (*c* 0.108, $CHCl_3$); UV (MeOH) 217 nm (ε 11 750); IR (KBr) 3400, 1780, 1740 cm^{-1}.

Anal. Calcd for $C_{30}H_{44}O_8 \cdot 2H_2O$: C, 63.36; H, 8.51. Found: C, 63.66; H, 8.21.

Elution with hexane–ethyl acetate (60:40) gave neriifolin-4′-one (5.80 g, 58%), which after crystallization from aqueous methanol had: mp 163–166 °C; $[\alpha]_D$ −84° (*c* 0.274, MeOH); UV (MeOH) 216 nm (ε 13 500); IR (KBr) 3400, 1780, 1740, 1620 cm^{-1}.

Anal. Calcd for $C_{30}H_{44}O_8$: C, 67.64; H, 8.33. Found: C, 66.86; H, 8.42.

(C) Brown Oxidation.[18] A solution of neriifolin (5.14 g, 0.096 mol) in dichloromethane (500 mL) was vigorously stirred at room temperature with a chromic acid solution prepared from sodium dichromate dihydrate (7.63 g), water (48 mL), and concentrated sulfuric acid (4 mL). The reaction was followed by TLC on silica gel using a hexane–ethyl acetate (3:1) solvent system. After 48 h the chromic acid solution was replaced by an equivalent amount of fresh reagent and agitation was continued for 128 h. The organic phase was separated and combined with a dichloromethane extract of the aqueous phase. The dichloromethane solution was washed with water, dried over sodium sulfate, and evaporated in vacuo. The complex mixture thus obtained was resolved by column chromatography on silica gel (500 g). Elution with hexane–ethyl acetate (3:2) gave, in succession, digitoxigenin 3-formate (0.43 g, 11%), the enone **6** (0.072 g, 1.5%; the physical constants of this substance are recorded below), and the enone **5** (0.025 g, 0.5%; the physical constants of this substance are given below). Elution with hexane–ethyl acetate (9:1) gave digitoxigenone (0.13 g, 4%), followed by neriifolin-2′-one (**4a**, 0.74 g, 14%). Elution with ethyl acetate–hexane (1:1, 3:2, and 3:1) gave neriifolin-4′-one (**3a**, 2.62 g, 51%). Finally, neriifolin (0.94 g, 18%) was removed from the column by elution with ethyl acetate and then with methanol.

Acetylation of Neriifolin-4′-one (3a). A solution of **3a** (0.887 g, 0.00167 mol) in pyridine (25 mL), containing acetic anhydride (5 mL), was left at room temperature for 1 h. The solution was evaporated to dryness in vacuo and the residue (0.884 g) was crystallized from dichloromethane–ether to give **3b**: mp 193–194 °C; $[\alpha]_D$ −114° (*c* 0.302, $CHCl_3$); UV (MeOH) 218 nm (ε 11 500); IR (KBr) 3560, 1790, 1745, 1635 cm^{-1}.

Anal. Calcd for $C_{32}H_{46}O_9$: C, 66.87; H, 8.07. Found: C, 66.88; H, 8.17.

The acetate **3b**, synthesized in this way, was identical with a sample prepared by the Collins oxidation of neriifolin monoacetate (**2b**).

Synthesis of the Enone 5. A solution of the acetate **3b** (5.00 g, 0.0087 mol) in pyridine (100 mL) was heated at 80 °C for 48 h. The solvent was removed in vacuo and the residue was chromatographed on silica gel (500 g). The solvents used for the development of the column contained a small amount of pyridine to minimize the acid hydrolysis of the enone to digitoxigenin. Elution with ethyl acetate–hexane (35:65) removed a small amount of a less polar impurity. The enone **5** [3.16 g, 71%; UV (MeOH) 221, 257 nm (ε 14 000, 5720)] was obtained as an amorphous solid, which after crystallization from methanol–water had mp 119–123 °C. Several recrystallizations from the same solvent system gave material: mp 175–178 °C; UV (identical with above); IR (KBr) 3500, 1790, 1755, 1715, 1650 cm^{-1}.

Anal. Calcd for $C_{30}H_{42}O_7 \cdot 0.5\ H_2O$: C, 68.88; H, 8.28. Found: C, 69.01; H, 8.10.

Elution with ethyl acetate–hexane (3:2) gave digitoxigenin (0.89 g, 27%), which was spectroscopically indistinguishable from that prepared in the manner described below.

Synthesis of Acetate 4b and Enone 6. Acetylation of **4a** in the manner described above for **3a** gave the enone **6** directly in high yield. After crystallization from acetone–ether it had: mp 217–221 °C; $[\alpha]_D$

−11° (c 0.227, $CHCl_3$); UV (MeOH) 220, 263 nm (ε 15 900, 6600); IR (KBr) 3530, 1795, 1750, 1720, 1640 cm^{-1}.

Anal. Calcd for $C_{30}H_{42}O_7$: C, 70.01; H, 8.23. Found: C, 69.71; H, 8.25.

The acetate **4b** could, however, be obtained as the major component of a mixture in the following manner.

A solution of chromium trioxide (0.260 g) in acetic acid (20 mL) was added slowly (144 h) to a solution of neriifolin 4′-acetate (**2c**, 0.500 g). The reaction mixture was worked up in the manner described above for neriifolin to give a crude product (0.380 g), which was mainly the acetate **4b** (see Table I for NMR spectrum) contaminated with a small amount of digitoxigenin formate **2g**. The mixture was subjected to column chromatography on neutral alumina (80 g, Fluka, activity I). The enone **6** (0.350 g, 78%) was eluted with hexane–ethyl acetate (9:1 and 17:3).

Digitoxigenin (2e). (A) Hydrolysis of Digitoxigenin Formate (2g). A solution of the formate (2.28 g, 0.00564 mol) in methanol (250 mL) and 0.1 N sulfuric acid (125 mL) was boiled under reflux for 1 h. The solution was neutralized by the addition of dilute sodium bicarbonate solution, the solvent was then removed in vacuo, and the residue was extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate, and evaporated in vacuo, giving a residue which after crystallization from aqueous methanol and then ethyl acetate–ether gave digitoxigenin (1.56 g): mp 237–238 °C; $[\alpha]_D$ +18° (c 0.348, $CHCl_3$); UV (MeOH) 218 nm (ε 14 500) [lit.[25] mp 249–255 °C; $[\alpha]_D$ +14.6 ± 2° (MeOH)]. Chromatography of the mother liquor on silica gel (50 g) gave digitoxigenin formate [0.326 g, 14% recovery, eluted with hexane–ethyl acetate (2:1)] and a small amount (0.194 g) of digitoxigenin (total yield 1.75 g, 83%).

(B) Hydrolysis of the Enone 5. A solution of the enone (1.2 g, 0.00233 mol) in methanol (60 mL) and 0.1 N sulfuric acid (30 mL) was left at room temperature for 18 h. The reaction was worked up as described above to provide a solid, which was crystallized from ethyl acetate–ether to give digitoxigenin (0.61 g), mp 242–245 °C. The residue obtained on evaporation of the mother liquor was separated by TLC on silica gel using hexane–ethyl acetate as the developing solvent. In this way a further quantity (0.17 g, total yield 89%) of digitoxigenin was isolated as well as the nonpolar methyl glycoside **7** and the γ-pyrone **8**. After crystallization from dichloromethane–ether compound **7**, obtained in 8% yield, had: mp 97–98 °C; $[\alpha]_D$ −64° (c 0.45, $CHCl_3$); UV (MeOH) 255.5 nm (ε 5410); IR (KBr) 1710, 1645 cm^{-1}; NMR ($CDCl_3$) δ 1.39 (d, 3 H, J = 6.8 Hz), 3.48 (s, 3 H), 3.62 (s, 3 H), 4.61 (q, 1 H, J = 6.8 Hz), 5.24 (d, 1 H, J = 4.2 Hz), 5.74 (d, 1 H, J = 4.2 Hz).

Anal. Calcd for $C_8H_{12}O_4$: C, 55.80; H, 7.03. Found: C, 56.06; H, 7.06.

The γ-pyrone **8**, obtained in 23% yield, had mp 155–157 °C [lit.[26] mp 160–162 °C] after crystallization from dichloromethane–ether: $[\alpha]_D$ 0° (c 0.252, $CHCl_3$); UV (MeOH) 277 nm (ε 11 000); IR (KBr) 3265, 1655, 1620, 1563 cm^{-1}.

Anal. Calcd for $C_6H_6O_3$: C, 57.14; H, 4.80. Found: C, 57.16; H, 4.69.

Acknowledgment. We thank Dr. M. Maddox for recording the NMR spectra.

Registry No.—**7**, 63493-69-6; **8**, 118-71-8.

References and Notes

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Studies Directed toward Synthesis of Quassinoids. 5.[1] Conversion of D-Ring Seco Derivatives of Cholic Acid to δ-Lactones

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Received May 3, 1977

Various δ-lactones, 5,14-*epi*-28,30-dinorquassinoids, were synthesized from D-ring seco derivatives of cholic acid. Chemical and spectral evidence suggests that the δ-lactone ring in these compounds exists in a strained boat conformation.

In pursuit of our goal to convert cholic acid into analogues of quassin (**1**), we had the opportunity to synthesize a number of unique δ-lactones that may be regarded as 5,14-*epi*-28,30-dinorquassinoids. Herein, we describe our results in the lactonization of D-ring seco derivatives of cholic acid.[3]

Results

Conversion of the various ketones **2a** to **2f** to 16-en-20-ones for subsequent ozonolysis to give D-ring seco derivatives was explored. The ester ketone **2d** was converted to **2a** by sapon-