Cardiotonic Steroids II: 3-Deoxycardenolides and 3-Deoxycardanolides

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Abstract □ A series of compounds related to 3-deoxydigitoxigenin was prepared and assayed for inhibition of myocardial Na^+,K^+-adenosine triphosphatase. Although the relatively high activity of 3-deoxydigitoxin was confirmed, the corresponding 3β,4β-epoxide and a mixture of 2,3-olefins and 3,4-olefins were less active. 3-Deoxy compounds with variations at the 14-position and the butenolide ring were much less active than the corresponding 3β-hydroxy analogs. Thus the activity of 3-deoxydigitoxigenin appears to be particularly susceptible to structural changes elsewhere in the molecule.

Keyphrases □ 3-Deoxydigitoxigenin analogs—synthesis and inhibition of Na^+,K^+-adenosine triphosphatase □ Cardenolide and cardanolide derivatives—synthesis of 3-deoxydigitoxigenin analogs, inhibition of Na^+,K^+-adenosine triphosphatase □ Cardiotonic steroids—synthesis of 3-deoxydigitoxigenin analogs, inhibition of Na^+,K^+-adenosine triphosphatase □ Structure–activity relationships—3-deoxydigitoxigenin analog inhibition of Na^+,K^+-adenosine triphosphatase

For many years it was accepted that a 3β-oxygen function was indispensable to the activity of cardiotonic steroids (1). Useful oxygen functions were exemplified by glycosides, ketals, esters, and the free hydroxy group. However, recent reports that 3-deoxydigitoxigenin (Ia) shows cardiotonic activity comparable to that of digitoxigenin (IIa) in the isolated frog heart (2) and in a Na^+,K^+-adenosine triphosphatase inhibition assay (3) appeared to refute this concept. These reports prompted a more extensive investigation on 3-deoxydigitoxigenin analogs, including compounds where the 14-substituent and the butenolide ring were varied (4).

One purpose of this investigation was to determine if the 3-deoxydigitoxigenin structure was more sensitive in biological activity to changes elsewhere in the molecule than was digitoxigenin. Another purpose was to generate some parent cardenolide and cardanolide structures (*e.g.*, IVa and IVb) since these compounds, although unlikely to have potent cardiotonic activity, might serve as starting points for structure–activity relationships.

RESULTS AND DISCUSSION

Synthesis—3-Deoxydigitoxigenin (Ia) was prepared from a mixture of 2,3- and 3,4-dehydrodigitoxigen isomers as described previously (2). This olefin mixture also was converted by *m*-chloroperbenzoic acid into two compounds, but only one could be purified. It is considered to be the 3β,4β-epoxide (V) for the following reasons. The NMR spectrum showed a broad, unsymmetrical pattern of peaks for the protons attached to the epoxide ring. This pattern is consistent with a 3,4-epoxide but not a 2,3-epoxide. Since the α-side of the 3,4-olefin from which V is derived has significant steric crowding (as determined from Dreiding models), *m*-chloroperbenzoic acid should form preferentially the β-epoxide. Literature precedent also exists for the selective formation of β-epoxides from ring A olefins in 5β-steroids (5).

Treatment of Ia with thionyl chloride gave the 14-olefin (IIIa), which was converted into 14β,15β-epoxide (VI) by *N*-bromoacetamide (Scheme I). Catalytic hydrogenation of IIIa gave 5β,14α-cardenolide (IVa) when palladium-on-barium sulfate was the catalyst, but the corresponding cardanolide [IVb, mixture of 20(*R*)- and 20(*S*)-epimers] was obtained when platinum was the catalyst. The pure 20(*R*)-epimer was reported previously (6). Hydrogenation of Ia in the presence of platinum gave 14β-hydroxycardanolide [Ib, mixture of 20(*R*)- and 20(*S*)-epimers], which afforded the 14-olefin (IIIb) upon treatment with thionyl chloride.

Table I—K_I Values for the Inhibition of Na^+,K^+-Adenosine Triphosphatase

Compound	K_I, μM
IIa (digitoxigenin)	0.043
Mixture of 2- and 3-dehydro-3-deoxydigitoxigenin	0.16
Ia (3-deoxydigitoxigenin)	0.076
V	0.65
IIIa	17.0
VI	100.0
IVb	>100.0
Ib	25.0
IIb (20,22-dihydrodigitoxigenin)	2.3

Biological Testing—Certain cardenolides and cardanolides were tested for their ability to inhibit a Na^+,K^+-stimulated, Mg^{+2}-dependent adenosine triphosphatase isolated from calf or canine myocardium (7). The ability of a large number of cardenolides to inhibit specifically this adenosine triphosphatase has been shown to correlate well with their positive inotropic activity (8, 9).

As recorded in Table I, the relatively high potency of Ia was confirmed. In this assay, it had 0.57 times the potency of digitoxigenin. No other compound had comparable activity, although the olefin mixture (2) was 0.22 times as potent as digitoxigenin. The 3β,4β-epoxide (V) had 0.066 times the activity of digitoxigenin, whereas the 14β,15β-epoxide (VI) had very poor activity (0.0004 times digitoxigenin). The 14-olefin (IIIa) was somewhat more active than VI, in contrast to the relative order of activity of 14-ene and 14β,15β-epoxide in the digitoxigenin-3-acetate series (4).

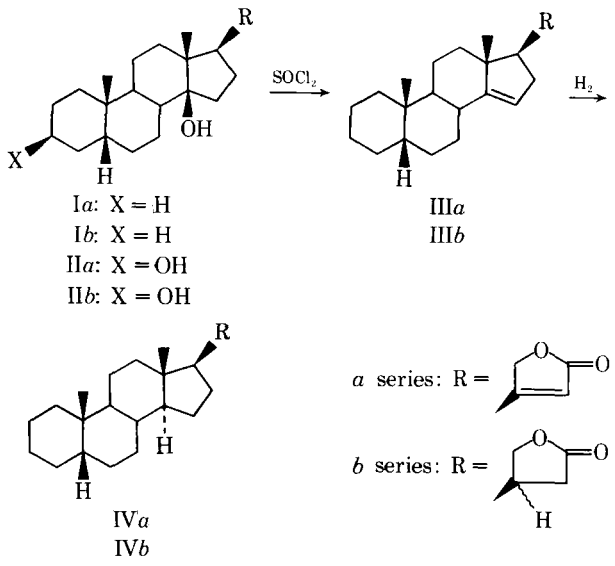
Of the saturated lactone compounds, 20,22-dihydrodigitoxigenin (IIb) (10) had 0.019 times the activity of digitoxigenin, but the 3-deoxy analog (Ib) had only 0.0017 times its activity. Thus, it appears that changes in the structure of 3-deoxydigitoxigenin result in much greater loss of activity than those occurring when corresponding changes are made in the digitoxigenin structure.

The parent 5β,14α-cardanolide (IVb) was too inactive to give a 50% inhibition value in the assay. Compounds IVa and IIIb lacked sufficient solubility for evaluation of their activities.

EXPERIMENTAL[1]

14-Hydroxy-3β,4β-oxido-5β,14β-card-20(22)-enolide (V)—A solution of 356 mg of the mixture of isomers, 14-hydroxy-5β,14β-carda-2,20(22)-dienolide and 14-hydroxy-5β,14β-carda-3,20(22)-dienolide (2), in 2.5 ml of methylene chloride was treated with 173 mg of *m*-chloroperbenzoic acid. After 2 hr, a small amount of sodi-

[1] Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR absorption spectra were recorded on a Perkin-Elmer 237B spectrophotometer using $CHCl_3$ solutions or KBr disks. NMR spectra were determined in $CDCl_3$ using tetramethylsilane as the reference on a Varian XL-100 spectrometer. Mass spectra were determined on a Consolidated Electronics Corp. 21-110B mass spectrometer. Microanalyses were carried out by the Microanalytical Laboratory, Department of Chemistry, Purdue University. A Guilford recording spectrophotometer with a Hoake constant-temperature bath was used for the biological assay.



Scheme I

um bisulfite was added followed by 5% sodium bicarbonate solution. The organic layer was washed with water, dried (magnesium sulfate), and concentrated.

Chromatography of the crude product on silica gel, with chloroform–acetone (6:1) as the solvent, gave starting material followed by two new components. The first of these compounds, after two recrystallizations from acetone–petroleum ether, gave 70 mg of needles, mp 206–209°; IR (KBr): 5.75 (butenolide) μm; NMR: δ 5.84 (m, 1, vinyl of butenolide), 5.02 and 4.76 (each m, 1 each, methylene of butenolide), complicated asymmetrical multiplet with main peaks at 3.22, 3.19, 3.12, 3.07, 3.00, and 2.96 (2, protons on epoxide ring), 2.78 (m, 1, allylic 17-proton), and 0.89 (6, angular methyls) ppm.

Anal.—Calc. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.22; H, 8.73.

The second component gave, upon concentration, a small amount of glassy solid, mp 75–87°, after recrystallization from acetone–hexane. It was not investigated further.

5β-Carda-14,20(22)-dienolide (IIIa)—A solution of 360 mg of 3-deoxydigitoxigenin (Ia) (2) in 24 ml of pyridine was cooled to −20° and treated dropwise with 1.2 ml of thionyl chloride in 12 ml of pyridine. After the mixture was stirred for 1 hr at 0°, it was worked up in the usual way (4) to give 270 mg (79%) of white crystals, mp 164–164.5°, after recrystallization from ethyl acetate–methanol.

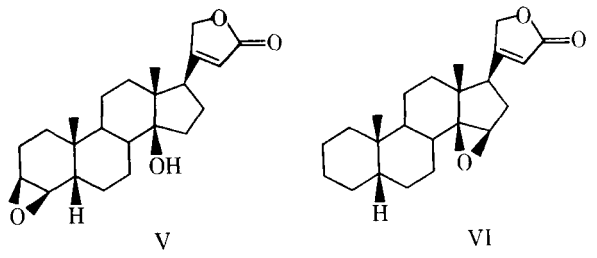
Anal.—Calc. for $C_{23}H_{32}O_2$: C, 81.13; H, 9.47. Found: C, 81.06; H, 9.52.

14β,15β-Oxido-5β,14β-card-20(22)-enolide (VI)—A solution of 200 mg of IIIa in 8 ml of acetone containing 0.8 ml of water was treated with 200 mg of *N*-bromoacetamide. The mixture was kept in the dark, and 60-mg portions of *N*-bromoacetamide were added at 24 and 48 hr. After 84 hr, the mixture was worked up in the usual way (4) and the product was purified by chromatography on silica gel with chloroform–acetone (6:1) as the solvent. Recrystallization of the product from ether gave colorless needles, mp 220–222°; the mass spectrum showed a molecular ion at M^+ 356.

Anal.—Calc. for $C_{23}H_{32}O_3$: C, 77.49; H, 9.05. Found: C, 77.24; H, 8.82.

5β,14α-Card-20(22)-enolide (IVa)—A mixture of 100 mg of 5β-carda-14,20(22)-dienolide (IIa), 20 ml of acetic acid, and 100 mg of 5% palladium-on-barium sulfate was shaken with hydrogen at an initial pressure of 1.1 kg/cm² (15 psi) for 5 hr. It then was filtered and concentrated to a solid, which was recrystallized from methylene chloride–methanol and then from methanol to give 33 mg of colorless needles, mp 154–155°; IR (KBr): 5.75 (butenolide) μm; NMR: δ 5.85 (m, 1, butenolide vinyl proton) ppm. The analytical sample was recrystallized once more from methanol, mp 166–166.5°.

Anal.—Calc. for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.40; H, 9.72.



5β,14α-Cardanolide [20(*R,S*)] (IVb)—A mixture of 150 mg of IVa, 35 ml of ethanol, and 100 mg of platinum oxide was shaken with hydrogen at an initial pressure of 0.7 kg/cm² (10 psi) for 1 hr. It then was filtered and concentrated to give 91 mg of a solid, mp 174–175°, after two recrystallizations from ethyl acetate–methanol; IR (KBr): 5.62 (saturated γ-lactone) μm. The NMR spectrum showed an angular methyl group at δ 0.66 ppm, which confirms the saturated lactone (11).

Anal.—Calc. for $C_{23}H_{36}O_2$: C, 80.18; H, 10.53. Found: C, 80.34; H, 10.70.

14-Hydroxy-5β,14β-cardanolide [20(*R,S*)] (Ib)—A mixture of 100 mg of Ia, 25 ml of ethanol, and 100 mg of platinum oxide was shaken with hydrogen at an initial pressure of 0.7 kg/cm² (10 psi) for 1 hr. It then was filtered and concentrated to a solid (101 mg), mp 170–172°, after recrystallization from ethyl acetate–methanol. Repeated recrystallizations from methanol failed to separate the mixture of 20(*R,S*)-epimers. The analytical sample had a melting point of 185–190° with softening at 175°; IR (KBr): 5.62 (saturated γ-lactone) μm. The NMR spectrum showed approximately equal peaks at δ 0.94 and 0.92 ppm, the 18-methyl groups for the epimers.

Anal.—Calc. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.52; H, 9.98.

5β-Card-14-enolide [20(*R,S*)] (IIIb)—A solution of 90 mg of Ib in 3 ml of pyridine was treated at −20° with 0.3 ml of thionyl chloride. The solution was stirred for 1 hr at 0° and then worked up in the usual way (4). The product (26 mg) had a melting point of 166–167° after two recrystallizations from methylene chloride–methanol; IR (KBr): 5.62 (saturated γ-lactone) μm; NMR: δ 5.15 (m, 1, 15-vinyl proton) and 0.89 (s, 6, 18-methyl and 19-methyl) ppm.

Anal.—Calc. for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.63; H, 9.88.

Biological Assay—The Na^+,K^+-adenosine triphosphatase activity was determined by continuously monitoring at 340 nm the oxidation of NADH linked *via* a series of enzymatic reactions to the hydrolysis of ATP (12). This assay was described in detail previously (4).

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Synthesis and Pharmacological Activity of 5-Substituted 2-(*N,N*-Dialkylaminoethyl)amino- and 2-*N*-Methylpiperazinyl-1,3,4-thiadiazoles

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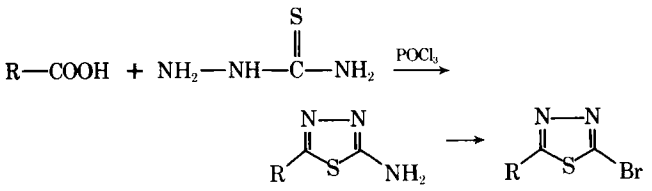
Abstract □ 5-Substituted 2-amino-1,3,4-thiadiazoles were transformed to their corresponding 2-bromo derivatives. The reaction of the 5-substituted 2-bromo-1,3,4-thiadiazoles with *N,N*-dialkylaminoethylamines or *N*-methylpiperazine afforded the corresponding amino-1,3,4-thiadiazole derivatives. All prepared compounds displayed antihistaminic, anticholinergic, and norepinephrine-potentiating activities.

Keyphrases □ 1,3,4-Thiadiazoles, 5-substituted 2-(*N,N*-dialkylaminoethyl)amino and 2-*N*-methylpiperazinyl—synthesis, antihistaminic, anticholinergic, and norepinephrine-potentiating activities □ 2-(*N,N*-Dialkylaminoethyl)amino-1,3,4-thiadiazoles, 5-substituted—synthesis and pharmacological activity □ 2-*N*-Methylpiperazinyl-1,3,4-thiadiazoles, 5-substituted—synthesis and pharmacological activity □ Antihistaminic, anticholinergic, and norepinephrine-potentiating activities—synthesis and screening of 5-substituted 2-(*N,N*-dialkylaminoethyl)amino- and 2-*N*-methylpiperazinyl-1,3,4-thiadiazoles

In continuing studies on the chemistry and pharmacological activity of 1,3,4-thiadiazoles and their derivatives, it was of interest to introduce the =N—CH_2CH_2—N= moiety, the important group in known compounds with antihistaminic activity, to the 1,3,4-thiadiazole ring system. A previously reported method was used for the preparation of 2-amino-5-substituted-1,3,4-thiadiazoles (1, 2); the 2-amino compounds were then transformed to their corresponding 2-bromo compounds by a modified Sandmeyer reaction (3) (Scheme I).

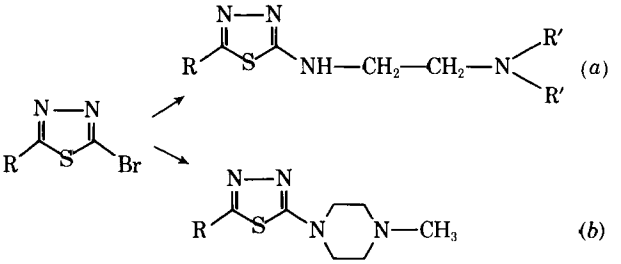


Scheme I

The 2-bromo-5-substituted-1,3,4-thiadiazoles thus obtained were subjected to a nucleophilic substitution reaction with *N*-methylpiperazine or asymmetrical dialkylaminoethylamine (Scheme II).

EXPERIMENTAL

Synthesis of 5-Substituted 2-*N*-Methylpiperazinyl- and 2-*N,N*-Dialkylaminoethylamino-1,3,4-thiadiazole Hydrochlorides—5-Substituted 2-bromo-1,3,4-thiadiazole (0.01 mole) and 2 *M* portions of *N*-methylpiperazine or asymmetric dialkylethylenediamine were refluxed for 8 hr in 40 ml of dry benzene. After cooling, the solid hydrobromide of the starting amine was separated by



Scheme II

1*a*: R = *o*-$CH_3C_6H_4$, R′ = CH_3
2*a*: R = *m*-$CH_3C_6H_4$, R′ = CH_3
3*a*: R = *p*-$CH_3C_6H_4$, R′ = CH_3
4*a*: R = *m*-$CH_3C_6H_4$, R′ = C_2H_5
5*a*: R = *p*-$CH_3OC_6H_4$, R′ = CH_3
6*a*: R = *m*-$NO_2C_6H_4$, R′ = CH_3
7*a*: R = *m*-$NO_2C_6H_4$, R′ = C_2H_5
8*a*: R = *p*-$NO_2C_6H_4$, R′ = CH_3
9*a*: R = *p*-$NO_2C_6H_4$, R′ = C_2H_5

1*b*: R = CH_3
2*b*: R = C_2H_5
3*b*: R = CF_3
4*b*: R = C_6H_5
5*b*: R = *o*-$CH_3C_6H_4$
6*b*: R = *m*-$CH_3C_6H_4$
7*b*: R = *p*-$CH_3C_6H_4$
8*b*: R = *m*-$CH_3OC_6H_4$
9*b*: R = *p*-$CH_3OC_6H_4$
10*b*: R = *p*-FC_6H_4
11*b*: R = *o*-ClC_6H_4
12*b*: R = *m*-ClC_6H_4
13*b*: R = *p*-ClC_6H_4
14*b*: R = *m*-BrC_6H_4
15*b*: R = *p*-BrC_6H_4
16*b*: R = *m*-$NO_2C_6H_4$
17*b*: R = *p*-$NO_2C_6H_4$