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Preparation of Ring A-Modified Cardenolides

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Preparation of several ring A-modified cardenolides from digitoxigenin is presented. 3,5-Seco-4-nor-cardenolides were prepared by oxidative cleavage of the ring A of the 3-oxo-Δ^4-derivative with ruthenium tetroxide and 4-oxa-cardenolides were derived from the 3,5-seco-4-nor-cardenolide. The structure–activity relationship in these ring A-modified cardenolides is discussed.

Keywords——3,5-seco-4-nor-cardenolide; 4-oxa-cardenolide; ring A-modified cardenolide; digitoxigenin derivatives; cardiotonic steroid; structure-activity relationship

Against the long-standing belief[2] on the structure-activity relationship of the cardenolide, it was demonstrated earlier that the oxygen function at C-3 of the steroid nucleus is not an indispensable requirement for cardiotonic activity, because 3-deoxydigitoxigenin[3] proved to be nearly equipotent with digitoxigenin (I) when tested for its effect on the isolated heart[4] or its inhibitory action on Na^+,K^+-ATPase.[5] Then, to see if the steroid skeleton of the cardenolide is an essential structural requirement for cardiotonic activity the preparation of the 3,5-seco-4-nor-cardenolide was carried out by cleaving the ring A of I, that is presented in this paper. Moreover, the 4-oxa-cardenolide was derived from the seco-cardenolide as a model of the heterocyclic cardenolide.

Cleavage of the ring A of I was performed *via* its 3-oxo-Δ^4-derivative, 3-oxo-14-hydroxy-14β-carda-4,20(22)-dienolide (anhydroperiplogenone) (II), according to the unequivocal way using ruthenium tetroxide.[6] Thus II was treated with the oxidizing agent generated from ruthenium trichloride[7] and sodium periodate to give 14-hydroxy-3,5-seco-4-nor-5-oxo-14β-card-20(22)-enolid-3-oic acid (IIIa) in about 40% yield from the acidic fraction of the reaction product. It was converted to the methyl ester (IIIb) by treatment with diazomethane.

Reduction of the ester (IIIb) with sodium borohydride afforded 5-epimeric 3,5,14-triols, 5β,14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (IVa) and 5α,14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol(IVc), the former being predominant. The more polar one (IVa) was obtainable after fractional recrystallizations of the reduction product, while the 5α-epimer (IVc) was obtained together with IVa by preparative thin–layer chromatography (TLC) from the mother liquors of the recrystallizations. The configurational assignment of the hydroxyl groups at C-5 of these epimers was made on the basis of the nuclear

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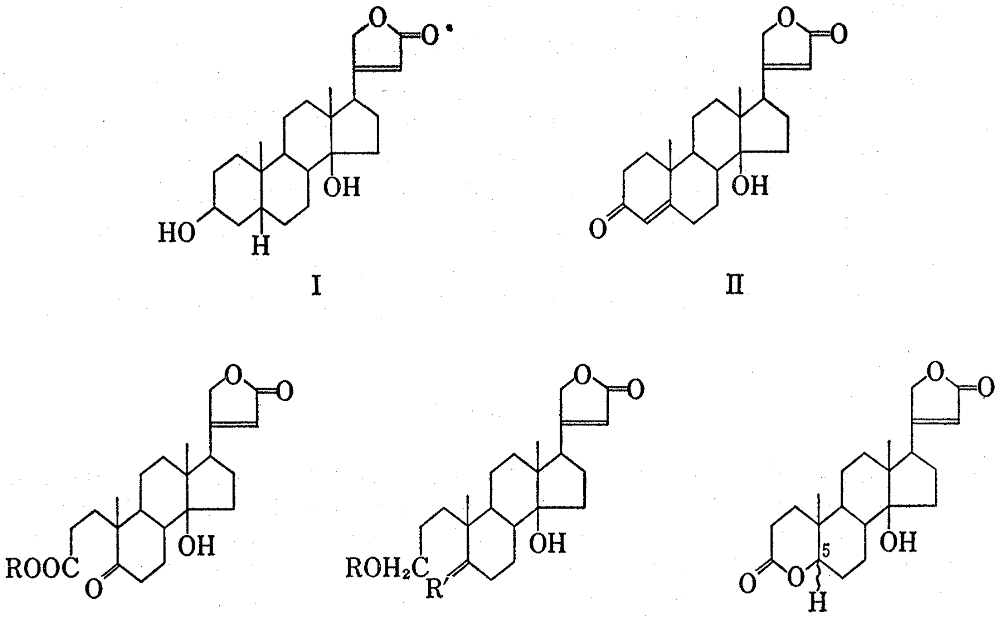

I II

IIIa: R=H
IIIb: R=CH_3

IVa: R=H, R'=β-OH, α-H
IVb: R=Ac, R'=β-OAc, α-H
IVc: R=H, R'=α-OH, β-H
IVd: R=Ac, R'=α-OAc, β-H
Ac=$COCH_3$

Va: 5α-H
Vb: 5β-H

Chart 1

magnetic resonance (NMR) spectra[8] of their acetates, IVb and IVd. The C-5 proton signals of IVb (5α: axial) and IVd (5β: equatorial) were observed at 4.75 ppm and 4.74 ppm as multiplets with the half-band width (W 1/2) of 15 Hz and 6 Hz respectively, clearly indicating[9] that IVa was the epimer with 5β-hydroxyl group and IVc was the 5α-epimer.

Treatment of IIIa with sodium borohydride, on the other hand, afforded a mixture of isomeric 4-oxa-cardenolides, 3-oxo-4-oxa-14-hydroxy-5α,14β-card-20(22)-enolide (3-dehydro-4-oxa-uzarigenin)(Va) and 3-oxo-4-oxa-14-hydroxy-5β,14β-card-20(22)-enolide(3-dehydro-4-oxa-digitoxigenin)(Vb), which were formed by lactonization from the resulting 5,14-dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-oic acids. Based on the NMR spectral data, 5α- and 5β-configuration was assigned to Va and Vb respectively, since Va exhibited a signal due to the 5α-proton (axial) at 3.98 ppm as a multiplet with W 1/2=19 Hz and the C-5 proton (equatorial) signal of Vb appeared at 4.17 ppm as a multiplet with W 1/2=5 Hz.[10]

Cardiotonic activities of the six newly synthesized ring A-modified cardenolides, four 3,5-seco-4-nor-cardenolides (IIIa, IIIb, IVa, and IVc) and two 4-oxa-cardenolides (Va and Vb), were studied by using frog hearts (Straub's preparation) and guinea-pig atria.[11] Of the four 3,5-seco-4-nor-cardenolides IIIb and IVa produced definite cardiotonic activities, thus demonstrating that steroid nucleus is not an indispensable requirement for the cardiotonic activity. The finding that IVa with 5β-hydroxyl group had a weak but definite car-

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[8] NMR spectra were measured at room temperature in deuteriochloroform ($CDCl_3$) at 60 MHz, using Hitachi Model R-20A 60 MHz spectrometer. Chemical shifts are expressed in δ (parts per million) with tetramethylsilane as internal standard and coupling constants are given in Hz: s, singlet; m, multiplet; t, triplet.

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diotonic activity while its 5α-epimer IVc was much less active, is suggestive of the previous observations[5b,12,13] that digitoxigenin with 3β-hydroxyl group is much more potent than the 3-epimer (3-epidigitoxigenin) in various bioassay methods. As for the cardiotonic activity of the 4-oxa-cardenolides, Va and Vb, which are isomeric at C-5-position, it was found that Vb (5β-H) was much more potent than Va (5α-H). This fact indicates the similar structure-activity relationship observed with the cardenolide aglycones in regard to the configuration at C-5 that the A/B *cis*-cardenolides (*e.g.*, digitoxigenin, digoxigenin) are much more potent than the A/B *trans*-cardenolides (*e.g.*, uzarigenin, syriogenin).[12] Moreover, the heterocyclic cardenolide analogs other than the 4-oxa-cardenolide may be expected to possess the cardiotonic activity.

Experimental[14]

14-Hydroxy-3,5-seco-4-nor-5-oxo-14β-card-20(22)-enolid-3-oic Acid (IIIa) and Its Methyl Ester (IIIb)——A solution of 3-oxo-14-hydroxy-14β-carda-4,20(22)-dienolide (anhydroperiplogenone) (II) (895 mg) in acetone (90 ml), which was prepared from digitoxigenin (I) according to the procedure reported earlier,[15] was added dropwise to the yellow ruthenium tetroxide solution generated by stirring ruthenium trichloride (150 mg) suspended in acetone (25 ml) with sodium periodate (800 mg) in water (10 ml). As the reaction mixture turned from yellow to black during the addition of II in acetone and during the reaction time, portions of the periodate solution were introduced. After 23 hr of stirring at room temperature the reaction was terminated by the addition of a few ml of isopropyl alcohol. The solids were collected on Celite by filtration, and the acetone in the filtrate removed *in vacuo*. The residue was then dissolved in chloroform and partitioned into neutral and acidic fractions with $NaHCO_3$. The acidic fraction was recovered by extraction of the acidified solution with chloroform. The organic layer was washed with water and dried over anhydrous Na_2SO_4. After usual work-up an oily residue obtained was chromatographed on a column of Silica gel (Wakogel C-200, 40 g) by successive elution with chloroform and chloroform–methanol mixtures. The fraction eluted with chloroform–methanol (10: 1) was recrystallized from acetone–hexane to give IIIa (385 mg) as colorless leaflets. mp 210—220°. *Anal.* Calcd. for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.76; H, 7.79. IR ν_{max} cm^{-1}: 3480, 3150 (OH), 1820 (sh), 1725, 1630 (butenolide, COOH, and 5C=O).

The acid IIIa (328 mg) was treated with diazomethane in the usual way to afford the methyl ester (IIIb) (337 mg) which was recrystallized from methanol as colorless needles. mp 210—213°. *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.27; H, 7.99. IR ν_{max} cm^{-1}: 3470 (OH), 1810, 1730 (sh), 1720, 1700 (sh), 1620 (butenolide, $COOCH_3$, and 5C=O). NMR (5% solution in $CDCl_3$) δ: 0.97, 1.10 (3H, 3H, s, s, 18- and 19-CH_3), 3.66 (3H, s, 3-$COOCH_3$), 4.91 (2H, m, 21-CH_2), 5.90 (1H, m, 22-H).

5β,14-Dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (IVa) and 5α,14-Dihydroxy-3,5-seco-4-nor-14β-card-20(22)-enolid-3-ol (IVc)——To a solution of IIIb (156 mg) in a mixture of tetrahydrofuran (10 ml) and water (1 ml) was added portionwise $NaBH_4$ (130 mg) under ice-cooling and the reaction mixture allowed to stand at 0° for 4 hr. After decomposition of the excess reagent with acetic acid the resulting solution was extracted with chloroform. The organic layer was washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a crystalline product, which was recrystallized from acetone–hexane to afford IVa (43 mg) as colorless leaflets. mp 215—218°. *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.87; H, 9.08. IR ν_{max} cm^{-1}: 3425 (OH), 1830 (sh), 1708, 1615 (butenolide).

Acetylation of IVa in the usual way with acetic anhydride and pyridine yielded IVb which could not be crystallized. NMR (5% solution in $CDCl_3$) δ: 0.89, 0.92 (3H, 3H, s, s, 18- and 19-CH_3), 2.03 (6H, s, 3-$CH_2OCOC\underline{H}_3$ and 5β-$OCOCH_3$), 3.98 (2H, t, 3-$C\underline{H}_2OCOCH_3$), 4.75 (1H, m, *W*1/2=15 Hz, 5α-H), 4.90 (2H, m, 21-CH_2), 5.89 (1H, m, 22-H).

The mother liquors of recrystallization of IVa were combined and evaporated *in vacuo* to give a crystalline residue (57 mg), which was submitted to preparative TLC using chloroform–methanol (14: 1) as solvent. A fraction corresponding to the spot (*Rf*: 0.36) gave IVc (25 mg) as colorless needles after recrystallization from acetone. mp 228—231°. *Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05. Found: C, 69.69; H, 9.02. IR ν_{max} cm^{-1}: 3440 (OH), 1810, 1740, 1623 (butenolide).

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Treatment of IVc in pyridine with acetic anhydride in the usual manner gave IVd which was unable to crystallize. NMR (5% solution in $CDCl_3$) δ: 0.89 (6H, s, 18- and 19-CH_3), 2.03, 2.06 (3H, 3H, s, s, 3-$CH_2OCOC\underline{H}_3$ and 5α-$OCOCH_3$), 4.00 (2H, m, 3-$C\underline{H}_2OCOCH_3$), 4.74 (1H, m, $W1/2=6$ Hz, 5β-H), 4.90 (2H, m, 21-CH_2), 5.90 (1H, m, 22-H).

3-Oxo-4-oxa-14-hydroxy-5α,14β-card-20(22)-enolide (Va) and 3-Oxo-4-oxa-14-hydroxy-5β,14β-card-20(22)-enolide (Vb)——To a solution of IIIa (83 mg) in a mixture of methanol (12 ml) and water (1 ml) was added portionwise $NaBH_4$ (100 mg) under stirring for 2 hr at 0°. The reaction mixture was poured into ice-water, acidified to pH 4 with 2 N HCl under continuous stirring for 1 hr at 0°. After neutralization of the reaction mixture with 5% $NaHCO_3$ the resulting solution was concentrated *in vacuo* to a small volume, and then extracted with chloroform. The organic layer was washed with water and dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded a crystalline residue which was recrystallized from acetone–hexane to afford Va (24 mg) as colorless leaflets. mp 275—282°. *Anal.* Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.70; H, 8.08. IR ν_{max} cm^{-1}: 3520 (OH), 1795, 1735, 1635 (butenolide and δ-lactone). NMR (5% solution in $CDCl_3$) δ: 0.91 (sh), 0.92 (6H, s, 18- and 19-CH_3), 3.98 (1H, m, $W1/2=19$ Hz, 5α-H), 4.92 (2H, m, 21-CH_2), 5.91 (1H, m, 22-H).

The mother liquors of recrystallization of Va were combined and evaporated *in vacuo* to give a crystalline residue (52 mg) which was submitted to preparative TLC using chloroform–methanol (49: 1) as solvent. A fraction corresponding to the spot (*Rf*: 0.41; *Rf* of Va: 0.38) afforded Vb (26 mg) as colorless leaflets after recrystallization from acetone–hexane. mp 265—271°. *Anal.* Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.71; H, 8.05. IR ν_{max} cm^{-1}: 3500 (OH), 1795, 1760, 1720, 1640 (butenolide and δ-lactone). NMR (5% solution in $CDCl_3$) δ: 0.90, 1.00 (3H, 3H, s, s, 18- and 19-CH_3), 4.17 (1H, m, $W1/2=5$ Hz, 5β-H), 4.89 (2H, m, 21-CH_2), 5.88 (1H, m, 22-H).

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Synthesis of Epimeric 6-Deuterio-4-cholesten-3-ones[1]

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In order to clarify the stereochemistry of hydrogen transfer from C-4 to C-6 during enzymatic transformation of cholesterol into cholestenone, the title compounds stereospecifically labeled with deuterium have been synthesized as an authentic specimen. The stereospecific labeling at 6β was attained by *trans*-diaxial opening of the $5\alpha,6\alpha$-oxido ring with lithium aluminum deuteride. Oxidation with chromium trioxide followed by dehydration with *p*-toluenesulfonic acid provided 6β-d_1-cholestenone (**4a**). In the similar fashion 6α-d_1-cholestenone (**4b**) was prepared from 6β-d_1-$3\alpha,5$-cyclo-5α-cholestan-6α-ol (**6**) by way of 6-d_1-cholesteryl acetate (**7**).

Keywords——$\Delta^5 \rightarrow \Delta^4$ isomerization; cholesterol oxidase; stereospecific labeling; lithium aluminum deuteride; *trans*-diaxial opening of epoxide; 6-d_1-5α-hydroxycholestan-3-ones; 6-d_1-5α-cholestane-3β,5-diols

It has previously been demonstrated that cholesterol oxidase from *Brevibacterium sterolicum* is capable of transforming cholesterol into 4-cholesten-3-one.[3] Although the properties of this enzyme have been disclosed to a certain extent,[4] the steric mechanism of migration

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