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Structures of Modified Cardenolides. III. Digoxigenin Dihydrate

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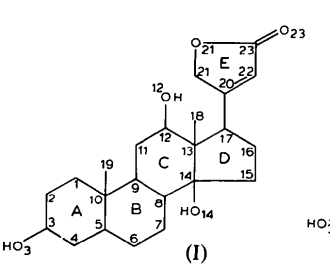
Abstract

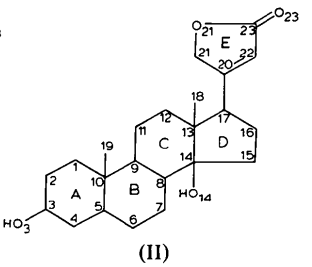
$C_{23}H_{35}O_5.2H_2O$, $M_r = 427{\cdot}56$, $P2_12_12_1$, $a = 14{\cdot}9981\ (8)$, $b = 17{\cdot}165\ (1)$, $c = 8{\cdot}7576\ (5)$ Å ($\lambda = 1{\cdot}5418$ Å, $T = 292$ K), $V = 2254{\cdot}6$ Å^3, $Z = 4$, $\rho_x = 1{\cdot}260$ Mg m^{-3}. The 17β-lactone side group is disordered, existing in two orientations related by a rotation of 175° about the C(17)–C(20) bond. These conformations represent the two energy-minimum conformations as calculated by molecular mechanics techniques.

Introduction

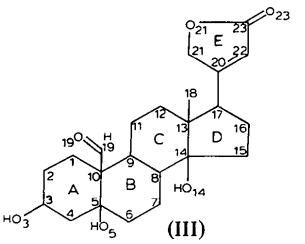
The cardiotonic effects of digitalis glycosides, such as digoxin and digitoxin, have made these drugs among the most widely used today. However, they are also among the most toxic. Since their pharmacological effects appear to be related to inhibition of Na^+,K^+–ATPase (Akera & Brody, 1978; Schwartz, Lindenmayer & Allen, 1975), an understanding of the structural basis for the digitalis Na^+,K^+–ATPase inhibition would be a significant step in eventually designing less-toxic analogues. It is also needed to continue our progress (Rohrer, Fullerton, Yoshioka, From & Ahmed, 1979; Fullerton, Yoshioka, Rohrer, From & Ahmed, 1979, 1980) in resolving conflicts in existing binding models (Thomas, Boutagy & Gelbart, 1974; Kupchan, Ognyanov & Moniot, 1971). The X-ray structures of digoxigen (I) (reported here and Rohrer & Fullerton, 1978), digitoxigenin (II) (Karle & Karle, 1969) and strophanthidin (III) (Gilardi & Flippen, 1973) provide the data needed to explore the conformational preferences of the 17β-lactone side group which are expected to play an important part in binding to the receptor. The conformational flexibility can be determined by combining these data with molecular mechanics calculations for rotation of the lactone about the C(17)–C(20) bond.



(I)



(II)



(III)

The crystal data were measured on a specimen crystal of dimensions 0·24 × 0·40 × 0·48 mm on an Enraf–Nonius CAD-4 diffractometer using Ni-filtered Cu $K\alpha$ radiation. The space group was determined to be $P2_12_12_1$ from the systematic absences along each of the axial rows. The lattice parameters are $a = 14{\cdot}9981\ (8)$, $b = 17{\cdot}165\ (1)$ and $c = 8{\cdot}7576\ (5)$ Å from a least-squares procedure using the 2θ values of 67 reflections in the interval $50 < 2\theta < 70°$. Integrated intensities for 2625 independent reflections having $2\theta < 150°$ were measured as θ–2θ scans; 2197 of these were significantly above the background level ($I > 2\sigma_I$).

Structure determination and refinement for digoxigenin

The intensities were reduced to structure factor amplitudes, and phase angles sufficient to locate the nonhydrogen atoms, including both positions for the

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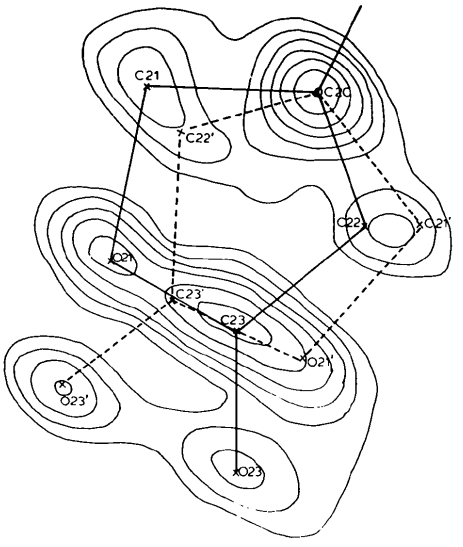

Fig. 1. Electron density section calculated for the mean plane of the two orientations of the disordered 17β-lactone.

Table 1. *Atomic coordinates for the nonhydrogen atoms of digoxigenin dihydrate*

The e.s.d.'s in the last digit are given in parentheses.

	x	*y*	*z*
C(1)	0·4747 (4)	0·0660 (3)	0·9751 (6)
C(2)	0·3868 (4)	0·1085 (3)	0·9798 (7)
C(3)	0·3694 (4)	0·1401 (3)	1·1368 (8)
C(4)	0·3741 (4)	0·0760 (3)	1·2573 (7)
C(5)	0·4616 (4)	0·0291 (3)	1·2499 (6)
C(6)	0·4593 (5)	−0·0372 (3)	1·3693 (6)
C(7)	0·3987 (4)	−0·1040 (3)	1·3231 (5)
C(8)	0·4238 (3)	−0·1367 (3)	1·1664 (5)
C(9)	0·4227 (3)	−0·0717 (2)	1·0455 (5)
C(10)	0·4845 (3)	−0·0026 (3)	1·0888 (6)
C(11)	0·4438 (4)	−0·1063 (3)	0·8844 (5)
C(12)	0·3792 (3)	−0·1701 (3)	0·8474 (5)
C(13)	0·3814 (3)	−0·2394 (2)	0·9603 (5)
C(14)	0·3664 (3)	−0·2080 (3)	1·1244 (5)
C(15)	0·2654 (3)	−0·1940 (3)	1·1304 (6)
C(16)	0·2233 (3)	−0·2596 (4)	1·0373 (8)
C(17)	0·2957 (3)	−0·2913 (3)	0·9267 (5)
C(18)	0·4689 (3)	−0·2836 (3)	0·9464 (6)
C(19)	0·5836 (4)	−0·0280 (3)	1·0842 (7)
C(20)	0·3093 (3)	−0·3767 (3)	0·9324 (6)
O(3*B*)	0·4307 (3)	0·2001 (2)	1·1775 (5)
O(12*B*)	0·4011 (3)	−0·1949 (2)	0·6934 (4)
O(14*B*)	0·3824 (2)	−0·2701 (2)	1·2313 (4)
C(21)	0·3016	−0·4179	1·0782
C(22)	0·3214	−0·4279	0·8192
C(23)	0·3271	−0·5049	0·8839
O(21)	0·3083	−0·4986	1·0329
O(23)	0·3431	−0·5656	0·8237
C(21′)	0·3357	−0·4127	0·7887
C(22′)	0·3042	−0·4302	1·0404
C(23′)	0·3170	−0·5041	0·9729
O(21′)	0·3376	−0·4967	0·8230
O(23′)	0·3109	−0·5690	1·0169
O(1*w*)	0·2049 (3)	−0·7120 (3)	1·0098 (5)
O(2*w*)	0·4261 (3)	−0·6991 (2)	0·9295 (6)

disordered O(23), were derived with the direct-methods program *MULTAN* (Germain, Main & Woolfson, 1971). Two water O atoms were located after several cycles of full-matrix least-squares refinement. Fourier maps also indicated that the lactone ring was disordered and existed in two orientations related by a rotation of 175° about the C(17)–C(20) bond. The two positions of the carbonyl oxygen were resolved, but the two sets of positions of the ring atoms were not resolvable. The geometry of the lactone ring in digitoxigenin was fitted to a Fourier section calculated through the lactone-ring plane, see Fig. 1. The resulting positions were used together with an occupancy factor of 0·5, determined from the relative size of the two O(23) peaks, in the remainder of the refinement. Hydrogen-atom positions were generated using expected geometries for the carbon atoms. The hydroxyl hydrogens and two of the four water hydrogens were located in difference-Fourier maps. The positional and anisotropic thermal parameters for all the nonhydrogen atoms, except those in the lactone ring, and the isotropic thermal parameters for the lactone-ring atoms were allowed to vary in the final cycles of least-squares refinement. The quantities $(1/\sigma_F^2)$, where σ_F is as defined by Stout & Jensen (1968), were used to weight the refinement; data determined to be unobserved were given zero weight. The final residuals, $R = \sum||F_o| - |F_c||/\sum|F_o|$, were 0·091 for the observed data and 0·104 for all data. The scattering factors for carbon, hydrogen and oxygen were calculated from the coefficients given in Table 2.2*B* of *International Tables for X-ray Crystallography* (Cromer & Waber, 1974). Tables 1 and 2 list the positional parameters together with their e.s.d.'s.* Two sets of *ORTEP* (Johnson, 1965) stereopairs depicting the two orientations of the disordered lactone rings are given in Fig. 2.

* Lists of structure factors, anisotropic thermal parameters and torsion angles have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 35104 (16 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

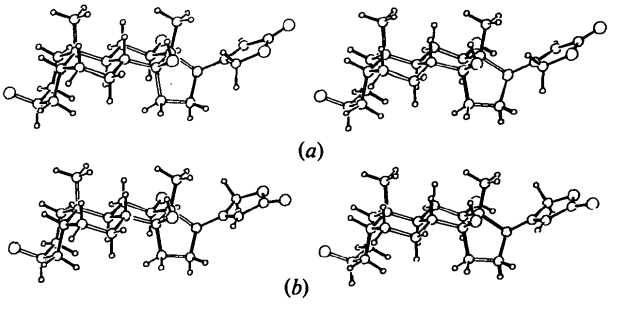

Fig. 2. *ORTEP* stereopairs for digoxigenin molecule. (*a*) The away-from-the-*D*-ring conformation and (*b*) the over-the-*D*-ring conformation.

Table 2. *Atomic coordinates for* H *atoms*

The *T*'s are H coordinates generated at theoretical locations on carbons.

	x	*y*	*z*
T(1*A*)	0·526	0·107	1·002
T(1*B*)	0·483	0·044	0·863
T(2*A*)	0·334	0·069	0·950
T(2*B*)	0·389	0·156	0·902
T(3)	0·302	0·163	1·137
T(4*A*)	0·319	0·036	1·250
T(4*B*)	0·371	0·103	1·373
T(5*B*)	0·516	0·069	1·282
T(6*A*)	0·435	−0·011	1·474
T(6*B*)	0·526	−0·057	1·385
T(7*A*)	0·331	−0·084	1·323
T(7*B*)	0·407	−0·149	1·408
T(8*B*)	0·491	−0·157	1·171
T(9*A*)	0·356	−0·048	1·039
T(11*A*)	0·511	−0·129	0·883
T(11*B*)	0·439	−0·061	0·798
T(12)	0·313	−0·146	0·845
T(15*A*)	0·247	−0·138	1·086
T(15*B*)	0·240	−0·197	1·250
T(16*A*)	0·168	−0·237	0·973
T(16*B*)	0·200	−0·305	1·113
T(17*A*)	0·274	−0·275	0·817
T(18*A*)	0·523	−0·245	0·971
T(18*B*)	0·469	−0·332	1·025
T(18*C*)	0·475	−0·306	0·830
T(19*A*)	0·627	0·018	1·113
T(19*B*)	0·593	−0·078	1·157
T(19*C*)	0·600	−0·047	0·964
T(21*A*)	0·238	−0·406	1·132
T(21*B*)	0·355	−0·402	1·155
T(22)	0·326	−0·413	0·700
T(21′*A*)	0·401	−0·392	0·753
T(21′*B*)	0·288	−0·400	0·700
T(22′)	0·292	−0·419	1·160
H(12O)	0·350	−0·224	0·643
H(14O)	0·446	−0·275	1·251
H(1*w*)	0·274	−0·706	0·985
H(2*w*)	0·483	−0·686	0·907

Discussion

The bond distances and angles for (I) are shown in Fig. 3. These values are all very similar to those observed in the structures of (II) and (III). The ranges of e.s.d.'s in the non-disordered portions of the molecule are 0·006 to 0·009 Å for the bond lengths and 0·4° for the bond angles. The *A*, *B* and *C* rings all have a chair conformation. The *C*-ring conformation is slightly distorted from the expected chair symmetry for the twofold axis bisecting the C(8)–C(9) and C(12)–C(13) bonds, ΔC_2[C(8)–C(9)] = 10·7° (Duax, Weeks & Rohrer, 1976). The *D*-ring conformation is a 14β-envelope, which is characteristic for this type of cardenolide molecule. The corresponding asymmetry parameter, ΔC_s[C(14)], is 0·5°. The lactone rings were fixed in a planar conformation. A table containing a complete list of torsion angles for the nonhydrogen atoms has been deposited.*

* See previous footnote.

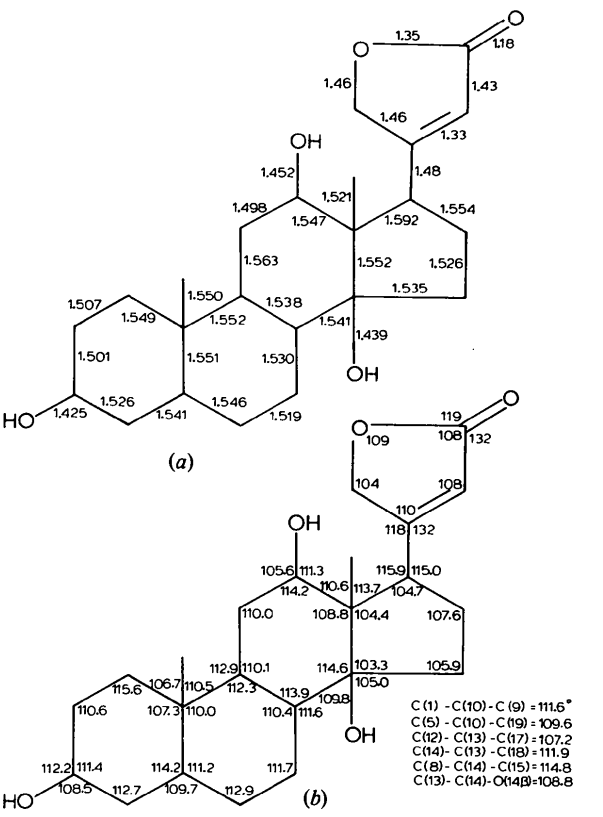

Fig. 3. Intramolecular dimensions of digoxigenin (the dimensions given for the disordered portion are the averaged values). (*a*) Bond distances (Å) (e.s.d.'s 0·006–0·009 Å). (*b*) Bond angles (°) (e.s.d.'s 0·4°).

The two orientations resulting from the disorder of the lactone ring provide additional support for the observation that there are two preferred conformations (Rohrer, Duax & Fullerton, 1976). One orientation directs the lactone carbonyl away from the *D* ring [torsion angle C(13)–C(17)–C(20)–C(22) = −99°] and the other directs the carbonyl over the *D* ring [torsion angle C(13)–C(17)–C(20)–C(22′) = 90°] (see Fig. 2*a* and *b*).

Molecular mechanics calculations, using a version of *CAMSEQ* (Weintraub & Hopfinger, 1975) modified to be used in conjunction with the modeling and graphing features of the NIH PROPHET computer system (Weeks, Cody, Pokrywiecki, Rohrer & Duax, 1974), confirmed the presence of two energy minima corresponding to the two crystallographically observed lactone orientations. The energy minima and the energy curve did not change when the effects of solvent (water) were introduced, thus providing a direct correlation with the aqueous biological environment *in vivo*. The parameters used in the molecular mechanics calculations, including those used to calculate the solvent interactions, were those provided in the *CAMSEQ* program. Fig. 4 shows the resulting energy curves for

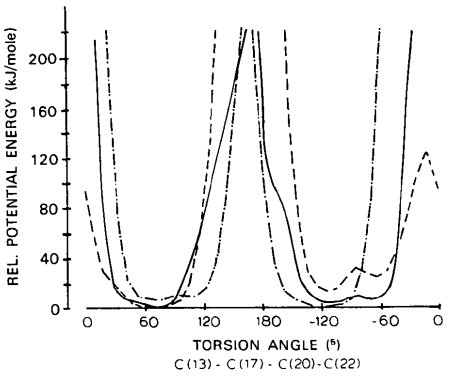

Fig. 4. Molecular mechanics potential-energy curves for rotation of the 17β-lactone substituent about the C(17)–C(20) bond of digoxigenin (——), digitoxigenin (– – –) and strophanthidin (–·–·–).

Table 3. *Hydrogen-bond distances*

Donor Acceptor	Distance (Å)	Symmetry operator on second atom
O(3)···O(2*w*)	2·778	$x, 1+y, z$
O(12)···O(1*w*)	2·770	$\frac{1}{2}-x, -1-y, -\frac{1}{2}+z$
O(14)···O(3)	2·959	$1-x, -\frac{1}{2}+y, \frac{5}{2}-z$
O(1*w*)···O(23′)	2·925	x, y, z
O(1*w*)···O(14)	2·786	$\frac{1}{2}-x, -1-y, -\frac{1}{2}+z$
O(2*w*)···O(12)	2·807	$1-x, -\frac{1}{2}+y, \frac{3}{2}-z$
O(2*w*)···O(23)	2·768	x, y, z
O(2*w*)···O(23′)	2·926	x, y, z

molecules (I), (II) and (III). Since the differences in these curves result from relatively small differences in the overall structures of the molecule, a curve representing both structural and conformational flexibility can be generated by using the minimum energy at each side-group orientation. The resulting curve still shows two energy minima with two large barriers to rotation. The very large barrier at 180° results from the steric interactions between the hydrogens on C(18) and C(21), while the lower barrier at 0° involves the hydrogen of C(22) with those on C(18).

The hydrogen bonding in this structure is also interesting. It appears that in either of the two disordered locations the carbonyl oxygen can take part in a hydrogen bond with the water molecules. The possible hydrogen-bond distances are given in Table 3.

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