BÜRGI, H. B. & DUNITZ, J. D. (1970). *Helv. Chim. Acta*, **53**, 1747–1764.
CORRADINI, P. (1973). *Chim. Ind. (Milan)*, **55**, 122–129.
COSTINES, M., PIES, W. & WEISS, A. (1981). *Z. Phys. Chem. (Frankfurt am Main)*, **128**, 73–92.
DOMENICANO, A., VACIAGO, A. & COULSON, C. A. (1975*a*). *Acta Cryst.* B**31**, 221–234.
DOMENICANO, A., VACIAGO, A. & COULSON, C. A. (1975*b*). *Acta Cryst.* B**31**, 1630–1641.
GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1972). *MULTAN. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data.* Univs. of York, England, and Louvain, Belgium.
International Tables for X-ray Crystallography (1968). Vol. III, pp. 202–206. Birmingham: Kynoch Press.
JONES, R. D. G. & WELBERRY, T. R. (1980). *Acta Cryst.* B**36**, 852–857.
KITAIGORODSKII, A. I. (1973). *Molecular Crystals and Molecules*, p. 18. New York: Academic Press.
KLUG, A. (1947). *Nature (London)*, 570–572.
PANAGIOTOPOULOS, N. C., JEFFREY, G. A., LA PLACA, S. J. & HAMILTON, W. C. (1974). *Acta Cryst.* B**30**, 1421–1430.
SHELDRICK, G. M. (1976). *SHELX* 76. A program for crystal structure determination. Univ. of Cambridge, England.
WELBERRY, T. R. & JONES, R. D. G. (1980). *J. Appl. Cryst.* **13**, 244–251.

Acta Cryst. (1983). B**39**, 272–280

Structures of Modified Cardenolides. IV. [20*S*]20(22)-Dihydrodigitoxigenin Analogues

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Abstract

Many often-conflicting models have been proposed to describe the biological roles of digitalis structure and conformation. In an effort to resolve these conflicts, the structures of two 20(22)-dihydro analogues of digitalis genins have been determined as part of a continuing study of the effects changes in the C(17)β side chain have on biological activity. A double bond in this side group has been considered important for biological activity and binding at the receptor. The structural and biological data from three analogues, [20*R*]- and [20*S*]3β,14-dihydroxy-5β,14β-cardanolide (I), [20*R*]- and [20*S*]3β,14-dihydroxy-22-methylene-5β,14β-cardanolide (II), and [20*R*]- and [20*S*]3β-hydroxy-22-methylene-5β-card-14-enolide (III) indicate that the double bond is not necessary for activity, but rather plays a geometric role in positioning the lactone carbonyl O relative to the steroid backbone. The relative position of this functional O is the major determinant in each analogue's biological activity. Crystal and molecular structures for two of these analogues, (I_S) and (II_S), are reported here. The crystals obtained for analogue (I_S)($C_{23}H_{36}O_4$, M_r = 376·54) were triclinic with space group $P\bar{1}$ and a = 7·726 (1), b = 10·224 (2), c = 6·379 (1) Å, α = 84·55 (1), β = 97·46 (1), γ = 90·92 (1)°, V = 497·33 Å^3, Z = 1 and D_x = 1·257 Mg m^{-3}. The final R factor was 0·049 for all the independent reflection data (2030) with $\theta < 75°$. Analogue (II_S) ($C_{24}H_{36}O_4$, M_r = 388·56) crystallized in the monoclinic space group $P2_1$ with a = 21·474 (2), b = 13·444 (2), c = 7·279 (1) Å, β = 94·98 (2)°, V = 2093·49 Å^3, Z = 4 and D_x = 1·233 Mg m^{-3}. The final R factor was 0·078 for all of the independent reflection data (4477) with $\theta < 75°$.

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Introduction

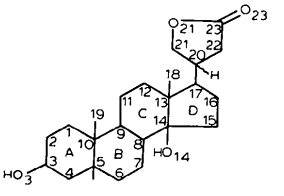
Cardio-active steroids have been used therapeutically in the management of cardiovascular disease for almost 200 years. Their ability to increase the contractility of the heart muscle (the inotropic activity) combined with the slowing of the heart rate (the chronotropic activity), resulting in a generally improved heart efficiency, keeps these drugs among the most prescribed even today. Unfortunately, these drugs are also extremely toxic, with cardenolide toxicity accounting for up to half of drug-induced in-hospital deaths. When the dose level required to attain the desired therapeutic response has been administered, 60% of the toxic dosage has also been given. Clearly, the demonstrated importance of these types of drugs combined with the need for improvement in the therapeutic–toxic ratio provides a strong incentive for their study and for reaching a better understanding of their mode of action.

The pharmacological effects of the digitalis glycosides (such as digitoxin) and their genins appear to be the result of the inihbition of the membrane-bound Na^+,K^+-ATPase. A number of often-conflicting models have been proposed to describe the chemical and structural characteristics of a genin which govern its ability to inhibit the Na^+,K^+-ATPase. None of these models has been able to explain consistently the activities of a number of modified genins. A multidisciplinary approach, including X-ray crystallography, organic synthesis and Na^+,K^+-ATPase inhibition studies, has been used to find relationships between genin structure and activity.

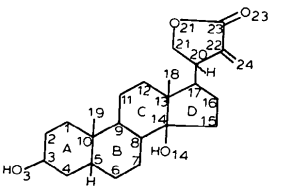
Among the structural features considered important for activity was a short C(17)β substituent containing a conjugated system which includes a hetero-atom. This is normally provided in the form of a lactone ring. In the activity model of Repke and co-workers (Repke & Portius, 1966; Repke & Dittrich, 1982), the conjugated system enhances the basicity of the carbonyl O and thus its function as a proton acceptor. These hydrogen-bond forces are thought to act over a relatively long distance to guide the lactone group to the binding site on the enzyme. The model then proposes that a loose one-point attachment is formed followed by rotation of the lactone ring about the C(17)—C(20) bond until the steroid skeleton finds an orientation which is complementary to the enzyme surface. At this point, short-range van der Waals forces stabilize the hydrogen bridge.

Another activity model proposed by Thomas and co-workers (Thomas, Boutagy & Gelbart, 1974; Thomas, Brown, Boutagy & Gelbart, 1980) calls for the same type of C(17)β substituent. In this case, the side chain is depicted as lying within a narrow cleft on the receptor surface. Binding involves two points of attachment, a hydrogen bond to the electron-rich hetero-atom and an electrostatic bond involving the electron-deficient end of the conjugated system.

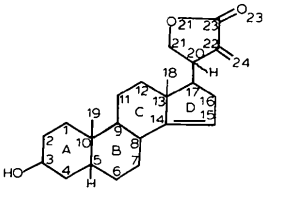
In order to determine the influence of the conjugation and conformation on activity, we reported the first separation of the 20*S* and 20*R* diastereomers of several 20(22)-dihydro digitalis analogues (Yoshioka, Fullerton & Rohrer, 1978). We found that removal of the double bond decreased the biological activity relative to digitoxigenin (IV), the digitalis genin prototype, by approximately two orders of magnitude (Fullerton, Yoshioka, Rohrer, From & Ahmed, 1979). Comparison of crystal structures of other digitalis analogues with their biological activity, inhibition of Na^+,K^+-ATPase, has shown that the relative position of the carbonyl O plays a significant role in determining biological activity of the analogue (Rohrer, Fullerton, Yoshioka, From & Ahmed, 1979; Fullerton, Rohrer, Ahmed, Kitatsuji, Deffo & From, 1982). These comparisons showed that for each 2·2 Å the relative location of the O is displaced from that of digitoxigenin, the activity drops by one order of magnitude. The crystal and molecular structures of two of these 20(22)-dihydrodigitoxigenin analogues are reported here: [20*S*]20(22)-dihydrodigitoxigenin (I_S) and [20*S*]-22-methylene-20(22)-dihydrodigitoxigenin [(II_S)*A* and *B*, two molecules in the asymmetric unit]. The structures of these molecules when compared to their activity support our earlier observation that the structure and conformation of the C(17)β side group rather than conjugation or electrostatic attraction directly control activity.



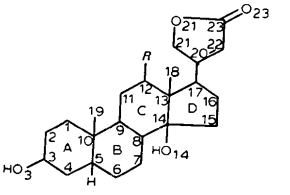
(I_S) and (I_R)
[20*R*]- or [20*S*]20(22)-Dihydrodigitoxigenin



(II_S) and (II_R)
[20*R*]- or [20*S*]22-Methylene-20(22)-dihydrodigitoxigenin



(III_S) and (III_R)
[20*R*]- or [20*S*]22-Methylene-14-didehydro-20(22)-dihydrodigitoxigenin



(IV) Digitoxigenin; R = H
(V) Digoxigenin; R = OH

Experimental

Crystal data for the specimen crystals of (I_S) and (II_S) were each measured on an Enraf–Nonius CAD-4 diffractometer using Ni-filtered Cu $K\alpha$ radiation. The dimensions of the specimen crystal of (I_S) were 0·33 ×

0·38 × 0·64 mm and the crystal size of (II_S) was 0·12 × 0·60 × 0·70 mm. The lattice parameters for each were obtained from a least-squares procedure using the 2θ values for 45 reflections in the interval $54° < 2\theta < 90°$ for (I_S) and 50 reflections in the interval $40° < 2\theta < 50°$ for (II_S). Integrated intensities for (I_S) and (II_S) were measured using θ–2θ scans to a maximum 2θ of 150°. The total number of independent reflections measured for (I_S) was 2030 and 4477 for (II_S) of which, using an $F > 4\sigma_F$ significance criterion, 2007 for (I_S) and 3739 for (II_S) were considered observed above background.

The two crystal structures which represent three independent molecular structures, one of (I_S) and two of (II_S), were solved by a straightforward application of the *MULTAN* 77 program (Main, Lessinger, Woolfson, Germain & Declercq, 1977). Each structure was refined by a full-matrix least-squares procedure using the quantities $1/\sigma_F^2$ to weight the least-squares differences for the observed data, where σ_F was as defined by Stout & Jensen (1968, p. 457, eq H14) but with an instability factor of 0·06; unobserved data were given zero weight. The H atoms in each structure were located in difference electron-density maps calculated at intermediate stages of the refinement. In the final cycles of refinement of each structure positional parameters for all atoms, anisotropic thermal parameters for the nonhydrogen atoms and isotropic thermal parameters for the H atoms were varied. The final values of the residual $R = \sum||F_o| - |F_c||/\sum|F_o|$ were 0·049 for all the unique data from (I_S) and 0·078 for (II_S). The scattering factors used were generated from coefficients given in Table 2.2B of *International Tables for X-ray Crystallography* (Cromer & Waber, 1974). Final positional parameters and isotropic thermal parameters for (I_S) and (II_S) are given in Tables 1 and 2.* The equivalent isotropic thermal parameters for the nonhydrogen atoms were calculated using equation (18) of Hamilton (1959).

Potential-energy profiles for rotation of the C(17)β side groups were calculated using the molecular-mechanics program *CAMSEQ* (Weintraub & Hopfinger, 1975) in conjunction with the modeling and graphing features of the NIH *PROPHET* computer system (Weeks, Cody, Pokrywiecki, Rohrer & Duax, 1974). The potential energies were plotted at intervals of 10° rotation of the lactone ring about the C(17)–C(20) bond, while holding the geometry fixed as observed in the crystal structure. The effects of non-bonded interactions between the lactone and the C(18) H atoms were relaxed by allowing the methyl group to be oriented in the minimum-energy direction.

Table 1. *Atomic coordinates* ($\times 10^4$, *for* H $\times 10^3$) *and isotropic thermal parameters* ($\times 10^2$, *for* H $\times 10$) *for* (I_S)

$B_{iso} = \frac{4}{3}\sum_i\sum_j\beta_{ij}(\mathbf{a}_i.\mathbf{a}_j)$ for the non-H atoms. The e.s.d.'s are given in parentheses.

	x	y	z	B_{iso} (Å^2)
C(1)	1715 (4)	4780 (2)	4403 (4)	331 (5)
C(2)	370 (5)	4890 (3)	5914 (4)	410 (7)
C(3)	−1449 (4)	5168 (3)	4725 (5)	408 (6)
C(4)	−1413 (3)	6332 (3)	3063 (4)	337 (5)
C(5)	17 (3)	6272 (2)	1603 (4)	251 (4)
C(6)	5 (4)	7531 (3)	70 (4)	302 (5)
C(7)	614 (3)	8733 (2)	1227 (4)	283 (4)
C(8)	2408 (3)	8530 (2)	2565 (3)	207 (4)
C(9)	2465 (3)	7238 (2)	4048 (3)	197 (4)
C(10)	1852 (3)	6022 (2)	2846 (4)	226 (4)
C(11)	4271 (3)	7084 (2)	5330 (4)	280 (4)
C(12)	4676 (3)	8254 (2)	6631 (4)	257 (4)
C(13)	4678 (3)	9602 (2)	5301 (3)	210 (4)
C(14)	2950 (3)	9758 (2)	3743 (3)	198 (4)
C(15)	1650 (3)	10232 (2)	5122 (4)	243 (4)
C(16)	2713 (3)	11251 (2)	6378 (4)	295 (5)
C(17)	4579 (3)	10682 (2)	6893 (3)	223 (4)
C(18)	6268 (3)	9700 (3)	4076 (4)	317 (5)
C(19)	3142 (3)	5694 (3)	1326 (4)	339 (5)
C(20)	5983 (3)	11758 (2)	6991 (4)	266 (4)
C(21)	5598 (4)	12766 (3)	8515 (5)	380 (6)
C(22)	7799 (4)	11271 (3)	7962 (6)	422 (7)
C(23)	8509 (4)	12264 (3)	9427 (4)	325 (5)
O(3)	−2197 (4)	4015 (3)	3867 (4)	587 (7)
O(14)	3248	10825	2173	282 (3)
O(21)	7281 (3)	13147 (2)	9584 (4)	402 (4)
O(23)	9957 (3)	12332 (2)	10373 (4)	456 (5)
H(1*A*)	288 (3)	461 (2)	509 (4)	26 (4)
H(1*B*)	156 (4)	398 (3)	373 (5)	43 (7)
H(2*A*)	74 (4)	567 (3)	684 (5)	36 (6)
H(2*B*)	27 (7)	418 (4)	695 (7)	76 (11)
H(3*A*)	−208 (4)	539 (3)	573 (5)	35 (6)
H(4*A*)	−120 (4)	714 (3)	388 (5)	33 (5)
H(4*B*)	−252 (6)	639 (4)	228 (7)	72 (11)
H(5*B*)	−12 (4)	553 (2)	81 (4)	25 (4)
H(6*A*)	−125 (4)	760 (3)	−57 (5)	40 (6)
H(6*B*)	78 (4)	740 (3)	−106 (5)	37 (6)
H(7*A*)	−26 (3)	890 (3)	213 (4)	27 (5)
H(7*B*)	57 (4)	944 (3)	21 (5)	33 (5)
H(8*B*)	319 (3)	845 (2)	165 (4)	22 (4)
H(9*A*)	171 (3)	734 (2)	512 (3)	14 (3)
H(11*A*)	439 (3)	631 (2)	626 (4)	26 (5)
H(11*B*)	508 (4)	701 (3)	451 (5)	36 (6)
H(12*A*)	388 (3)	824 (2)	769 (4)	19 (4)
H(12*B*)	582 (4)	811 (3)	750 (4)	29 (5)
H(15*A*)	131 (3)	958 (2)	599 (4)	19 (4)
H(15*B*)	52 (4)	1064 (2)	426 (4)	27 (5)
H(16*A*)	216 (4)	1145 (3)	766 (5)	36 (6)
H(16*B*)	269 (5)	1213 (4)	552 (6)	60 (9)
H(17*A*)	458 (3)	1026 (2)	839 (4)	26 (5)
H(18*A*)	611 (4)	906 (3)	311 (5)	33 (5)
H(18*B*)	659 (4)	1056 (3)	365 (5)	37 (6)
H(18*C*)	728 (5)	950 (3)	491 (5)	44 (7)
H(19*A*)	265 (4)	505 (3)	55 (5)	40 (6)
H(19*B*)	318 (7)	652 (4)	23 (8)	73 (11)
H(19*C*)	408 (6)	560 (4)	182 (8)	67 (10)
H(20)	626 (4)	1211 (3)	576 (5)	34 (6)
H(21*A*)	496 (4)	1239 (3)	987 (5)	37 (6)
H(21*B*)	509 (4)	1359 (3)	782 (6)	42 (6)
H(22*A*)	784 (5)	1030 (3)	873 (6)	48 (7)
H(22*B*)	857 (6)	1122 (4)	694 (6)	58 (9)
H(O3)	−159 (6)	388 (5)	304 (7)	66 (10)
H(O14)	235 (4)	1113 (3)	162 (5)	32 (5)

Discussion

The crystallographically observed structures, anisotropic thermal ellipsoids and atomic numbering for molecules (I_S), (II_S)*A* and (II_S)*B* are shown in Fig. 1. The intramolecular dimensions involving the non-H

* Lists of structure factors and anisotropic thermal parameters for (I_S) and (II_S) have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 38226 (35 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Atomic coordinates* ($\times 10^4$, *for* H $\times 10^3$) *and isotropic thermal parameters* ($\times 10^2$, *for* H $\times 10$) *for* (II_S)

$B_{iso} = \frac{4}{3}\sum_i\sum_j \beta_{ij}(\mathbf{a}_i.\mathbf{a}_j)$ for the non-H atoms. The e.s.d.'s are given in parentheses.

	x	y	z	B_{iso}(Å^2)		x	y	z	B_{iso} (Å^2)
Molecule *A*					Molecule *B*				
C(1)*A*	16 (2)	5030 (4)	4409 (6)	479 (12)	C(1)*B*	7720 (2)	3034 (4)	5865 (5)	356 (10)
C(2)*A*	157 (2)	5482 (4)	2571 (7)	513 (13)	C(2)*B*	7445 (2)	3979 (3)	6540 (6)	386 (11)
C(3)*A*	−281 (2)	5019 (3)	1065 (6)	401 (11)	C(3)*B*	7781 (2)	4348 (3)	8328 (6)	374 (10)
C(4)*A*	−197 (2)	3909 (3)	1053 (5)	345 (10)	C(4)*B*	7835 (2)	3520 (3)	9755 (5)	367 (10)
C(5)*A*	−295 (2)	3413 (3)	2906 (5)	294 (9)	C(5)*B*	8101 (2)	2554 (3)	9079 (5)	328 (9)
C(6)*A*	−185 (2)	2296 (3)	2817 (6)	371 (10)	C(6)*B*	8122 (2)	1754 (4)	10605 (6)	434 (11)
C(7)*A*	508 (2)	2020 (3)	2837 (5)	302 (9)	C(7)*B*	7473 (2)	1397 (3)	10953 (6)	424 (11)
C(8)*A*	888 (2)	2464 (3)	4534 (5)	274 (8)	C(8)*B*	7113 (2)	992 (3)	9169 (5)	337 (10)
C(9)*A*	802 (2)	3603 (3)	4586 (5)	295 (9)	C(9)*B*	7088 (2)	1791 (3)	7639 (5)	305 (9)
C(10)*A*	105 (2)	3914 (3)	4558 (5)	351 (10)	C(10)*B*	7755 (2)	2160 (3)	7257 (5)	311 (9)
C(11)*A*	1225 (2)	4067 (3)	6163 (6)	392 (11)	C(11)*B*	6704 (2)	1393 (4)	5907 (6)	402 (11)
C(12)*A*	1892 (2)	3757 (3)	6099 (5)	356 (10)	C(12)*B*	6056 (2)	1067 (4)	6373 (5)	396 (11)
C(13)*A*	2010 (2)	2636 (3)	6207 (5)	276 (9)	C(13)*B*	6051 (2)	247 (3)	7826 (5)	340 (10)
C(14)*A*	1568 (2)	2108	4668 (5)	267 (8)	C(14)*B*	6470 (2)	585 (3)	9566 (6)	354 (10)
C(15)*A*	1909 (2)	2269 (3)	2941 (5)	334 (10)	C(15)*B*	6039 (2)	1288 (4)	10532 (6)	477 (13)
C(16)*A*	2590 (2)	2101 (4)	3573 (5)	384 (10)	C(16)*B*	5414 (2)	784 (4)	10324 (6)	542 (14)
C(17)*A*	2687 (2)	2453 (3)	5603 (5)	305 (9)	C(17)*B*	5373 (2)	201 (3)	8496 (6)	374 (11)
C(18)*A*	1886 (2)	2264 (4)	8117 (5)	370 (10)	C(18)*B*	6286 (2)	−728 (4)	7056 (7)	563 (14)
C(19)*A*	−163 (2)	3600 (5)	6357 (6)	563 (15)	C(19)*B*	8134 (2)	1337 (3)	6439 (6)	421 (11)
C(20)*A*	3120 (2)	1710 (3)	6770 (6)	381 (11)	C(20)*B*	5065 (2)	−825 (4)	8634 (6)	416 (11)
C(21)*A*	3319 (2)	2101 (4)	8702 (6)	520 (14)	C(21)*B*	4875 (2)	−1304 (5)	6740 (8)	662 (16)
C(22)*A*	3742 (2)	1636 (4)	5985 (6)	450 (12)	C(22)*B*	4438 (2)	−778 (4)	9415 (7)	498 (13)
C(23)*A*	4180 (2)	2277 (4)	7096 (8)	505 (13)	C(23)*B*	3946 (2)	−875 (5)	7856 (8)	675 (17)
C(24)*A*	3936 (2)	1088 (5)	4669 (7)	661 (16)	C(24)*B*	4290 (2)	−787 (5)	11131 (8)	711 (18)
O(3)*A*	−899 (1)	5332 (2)	1327 (4)	489 (8)	O(3)*B*	8404 (1)	4673 (2)	8057 (4)	449 (8)
O(14)*A*	1589 (1)	1044 (2)	4992 (3)	328 (6)	O(14)*B*	6594 (1)	−263 (3)	10761 (4)	556 (9)
O(21)*A*	3918 (2)	2554 (3)	8645 (5)	632 (10)	O(21)*B*	4221 (2)	−1155 (3)	6361 (5)	725 (12)
O(23)*A*	4689 (2)	2557 (3)	6789 (6)	754 (12)	O(23)*B*	3399 (2)	−736 (5)	7865 (6)	933 (15)
H(1*A*)*A*	31 (2)	540 (4)	549 (6)	55 (11)	H(1*A*)*B*	750 (2)	275 (3)	474 (5)	39 (9)
H(1*B*)*A*	−44 (2)	517 (3)	470 (5)	30 (9)	H(1*B*)*B*	815 (1)	323 (3)	560 (4)	22 (7)
H(2*A*)*A*	61 (2)	528 (3)	222 (4)	26 (8)	H(2*A*)*B*	699 (2)	390 (3)	682 (5)	33 (8)
H(2*B*)*A*	2 (2)	608 (4)	265 (6)	57 (12)	H(2*B*)*B*	738 (2)	456 (4)	567 (6)	55 (11)
H(3*A*)*A*	−19 (1)	529 (3)	5 (5)	28 (8)	H(3*A*)*B*	758 (2)	488 (3)	882 (5)	32 (9)
H(4*A*)*A*	24 (1)	377 (3)	67 (4)	13 (8)	H(4*A*)*B*	745 (1)	337 (3)	1023 (4)	15 (7)
H(4*B*)*A*	−54 (1)	355 (3)	6 (4)	30 (7)	H(4*B*)*B*	807 (2)	383 (4)	1097 (6)	49 (12)
H(5*B*)*A*	−75 (1)	356 (2)	307 (4)	21 (7)	H(5*B*)*B*	852 (1)	276 (3)	890 (4)	1 (8)
H(6*A*)*A*	−48 (2)	193 (3)	180 (5)	29 (8)	H(6*A*)*B*	837 (2)	196 (3)	1149 (5)	51 (10)
H(6*B*)*A*	−39 (2)	193 (4)	384 (6)	87 (13)	H(6*B*)*B*	838 (2)	113 (3)	1026 (5)	41 (9)
H(7*A*)*A*	72 (2)	222 (3)	170 (5)	37 (9)	H(7*A*)*B*	720 (2)	198 (4)	1122 (5)	52 (11)
H(7*B*)*A*	64 (2)	131 (4)	307 (6)	50 (11)	H(7*B*)*B*	755 (2)	79 (3)	1174 (5)	50 (10)
H(8*B*)*A*	74 (2)	215 (4)	573 (6)	54 (11)	H(8*B*)*B*	741 (2)	48 (4)	884 (5)	48 (10)
H(9*A*)*A*	95 (2)	391 (4)	326 (6)	55 (11)	H(9*A*)*B*	684 (1)	241 (3)	796 (4)	19 (7)
H(11*A*)*A*	114 (2)	483 (4)	611 (7)	71 (15)	H(11*A*)*B*	657 (2)	202 (4)	522 (6)	49 (12)
H(11*B*)*A*	107 (2)	393 (3)	710 (5)	34 (9)	H(11*B*)*B*	697 (2)	67 (4)	537 (6)	91 (14)
H(12*A*)*A*	207 (2)	422 (4)	502 (6)	51 (11)	H(12*A*)*B*	590 (2)	159 (4)	671 (6)	71 (13)
H(12*B*)*A*	216 (2)	408 (3)	712 (5)	43 (10)	H(12*B*)*B*	585 (1)	76 (3)	541 (4)	24 (7)
H(15*A*)*A*	169 (2)	171 (3)	199 (5)	31 (8)	H(15*A*)*B*	606 (2)	174 (4)	985 (6)	51 (11)
H(15*B*)*A*	181 (2)	297 (4)	242 (6)	42 (11)	H(15*B*)*B*	618 (2)	154 (5)	1172 (7)	95 (15)
H(16*A*)*A*	256 (2)	150 (4)	320 (6)	59 (13)	H(16*A*)*B*	506 (2)	115 (4)	1056 (5)	58 (11)
H(16*B*)*A*	293 (2)	249 (3)	288 (5)	38 (10)	H(16*B*)*B*	543 (1)	34 (3)	1136 (4)	21 (8)
H(17*A*)*A*	296 (1)	305 (3)	579 (4)	19 (7)	H(17*A*)*B*	508 (1)	69 (3)	755 (4)	21 (8)
H(18*A*)*A*	148 (2)	228 (3)	822 (5)	33 (8)	H(18*A*)*B*	668 (2)	−75 (4)	673 (6)	79 (12)
H(18*B*)*A*	207 (2)	281 (4)	885 (6)	105 (14)	H(18*B*)*B*	618 (2)	−151 (4)	771 (7)	88 (13)
H(18*C*)*A*	207 (2)	153 (4)	847 (6)	64 (12)	H(18*C*)*B*	601 (2)	−101 (4)	599 (6)	74 (12)
H(19*A*)*A*	−64 (2)	384 (3)	628 (5)	33 (8)	H(19*A*)*B*	859 (2)	164 (3)	624 (5)	40 (9)
H(19*B*)*A*	2 (2)	400 (4)	725 (6)	82 (14)	H(19*B*)*B*	816 (2)	77 (3)	715 (5)	38 (9)
H(19*C*)*A*	−24 (2)	304 (5)	652 (7)	107 (16)	H(19*C*)*B*	797 (2)	97 (3)	523 (5)	39 (10)
H(20)*A*	288 (2)	113 (3)	672 (5)	39 (9)	H(20)*B*	537 (1)	−128 (3)	924 (4)	26 (8)
H(21*A*)*A*	310 (2)	254 (3)	924 (5)	36 (10)	H(21*A*)*B*	516 (2)	−113 (4)	567 (6)	62 (12)
H(21*B*)*A*	343 (2)	151 (5)	959 (6)	80 (15)	H(21*B*)*B*	486 (2)	−209 (5)	706 (7)	138 (17)
H(24*A*)*A*	369 (2)	53 (4)	387 (5)	51 (10)	H(24*A*)*B*	464 (2)	−84 (4)	1205 (6)	68 (13)
H(24*B*)*A*	440 (2)	101 (3)	428 (5)	49 (11)	H(24*B*)*B*	383 (2)	−74 (4)	1096 (6)	75 (13)
H(O3)*A*	−110 (2)	499 (4)	65 (7)	58 (14)	H(O3)*B*	841 (2)	507 (3)	736 (5)	33 (9)
H(O14)*A*	143 (3)	72 (6)	472 (8)	184 (18)	H(O14)*B*	626 (2)	−51 (5)	1098 (7)	119 (16)

atoms are given in Figs. 2 and 3. The C—H bond distances in the structure of (I_S) average 0·96 ± 0·07 Å and in the structures of (II_S)*A* and *B* the averages are both 0·99 ± 0·09 Å. The structural parameters for these structures agree well with the corresponding parts of other cardenolide steroids like digitoxigenin (IV) (Karle & Karle, 1969) and digoxigenin (V) (Rohrer & Fullerton, 1980).

The crystal structure of (I_S) was also recently published by another group (Messerschmidt, Höhme & Lindig, 1981). The space group and lattice parameters are essentially the same as reported here. A half-normal probability plot (Abrahams & Keve, 1971) comparing all of the corresponding interatomic distances for the non-H atoms in the two molecules (Fig. 4) shows no significant difference. The crystal structure reported here has greater resolution with a maximum sin θ/λ of 0·626 Å^{-1} *versus* 0·572 Å^{-1} and greater precision with average estimated standard deviations of 0·004 Å in the non-H bond lengths and 0·2° in the angles *versus*

0·015 Å and 0·9°. The structure reported here will be used for all subsequent comparisons.

The *A*, *B* and *C* rings in the steroid backbone in (I_S) and (II_S) all have chair conformations similar to the conformations in (IV) and (V). However, the *D*-ring conformations are different from that of the standard cardenolides. The *D*-ring conformation of (I_S) is intermediate between a C(14)/C(15) β/α-half-chair conformation and a C(15) α-envelope conformation as indicated by the nearly equal values of the corresponding asymmetry parameters (Duax, Weeks & Rohrer, 1976): ΔC_2[C(17)] = 8·5° and ΔC_s[C(15)] = 9·9°. The *D*-ring conformations for (II_S)*A* and *B* were both slightly distorted C(14)/C(15) β/α-half-chairs as indicated by the relatively small ΔC_2[C(17)] values: 3·3 and 5·0°. The *D* rings in (IV) and (V) both have a C(14) β-envelope conformation with ΔC_s[C(14)] values of 1·5 and 0·5°. The *cis–trans–cis* configurations at the ring junctions in the steroid backbone are a characteristic feature of cardenolides and give them a semicircular shape rather than the flat shape of the steroid hormones.

All of the hydroxyl groups in the two crystal structures are used to form hydrogen bonds. Table 3 gives the data for the hydrogen bonding in each crystal structure. The only possible exception is O(14)*B* in (II_S) where the distances to O(21)*A* are much longer than are normally accepted for hydrogen bonding. The location of the hydroxyl H obtained from a difference electron-density map is, however, oriented toward O(21)*A*.

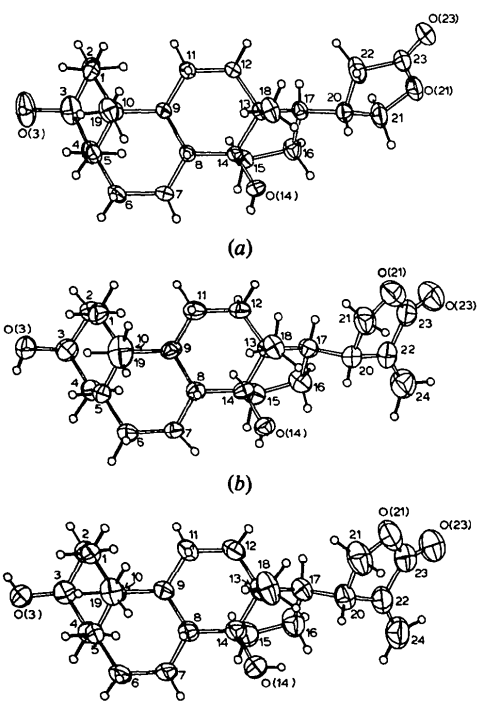


Fig. 1. *ORTEP* (Johnson, 1965) drawings with atomic numbering for (*a*) (I_S), (*b*) (II_S)*A* and (*c*) (II_S)*B*. The thermal ellipsoids for the non-H atoms are drawn at the 60% probability level.

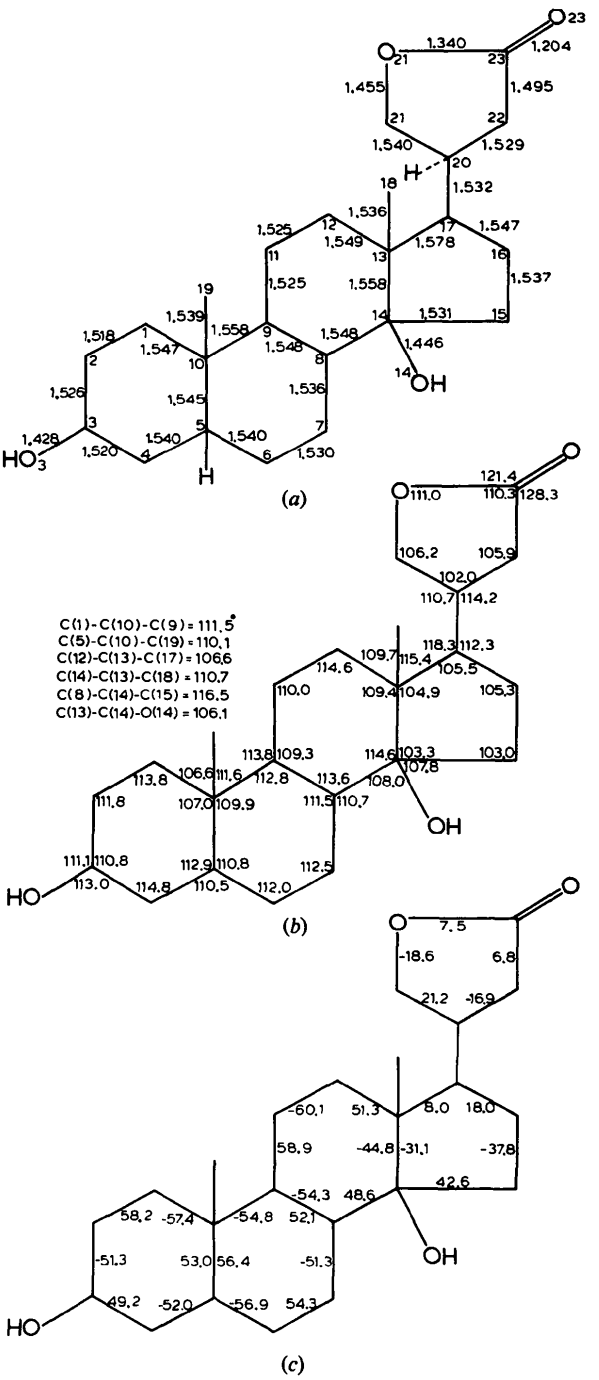


Fig. 2. Intramolecular dimensions for (I_S). (*a*) Bond distances (Å); σ range = 0·003 to 0·005 Å. (*b*) Bond angles (°); σ range = 0·2 to 0·3°. (*c*) Endocyclic torsion angles (°); σ range = 0·2 to 0·3°.

The structures of four cardenolide analogues containing saturated C(17)β side-chain lactone rings have been reported. In addition to the two analogues reported here (I_S and II_S), the structures of two other analogues containing a saturated lactone ring have been reported: (III_R) (Rohrer, Duax & Fullerton, 1976) and (III_S) (Rohrer & Fullerton, 1983). The crystal structures of analogues (II_S) and (III_S) both have two independent molecules, *A* and *B*, in the asymmetric unit which with (I_S) and (III_R) provide a total of six molecular structures for comparison. In the case of these molecules, the *R*/*S* nomenclature is misleading. The 20*S* forms of (II) and (III) both have the same configurations, while the 20*S* form of (I) has the opposite configuration, see Fig. 1. With this in mind, it is interesting that the lactone rings in (I_S) and (III_R) both have C(20)/C(21)-half-chair conformations, ΔC_2[C(23)] = 1·3 and 1·2°, while the lactones in (II_S)*A* and *B* both have C(20)-envelope conformations, ΔC_s[C(20)] = 2·5 and 2·3°. This is in contrast to the lactone rings of (IV) and (V) which are planar.

The orientations of the lactone rings relative to the steroid *D* rings are all in a staggered conformation in the six saturated lactone structures, see Fig. 5. The three molecule structures reported here and the (III_S)*A*

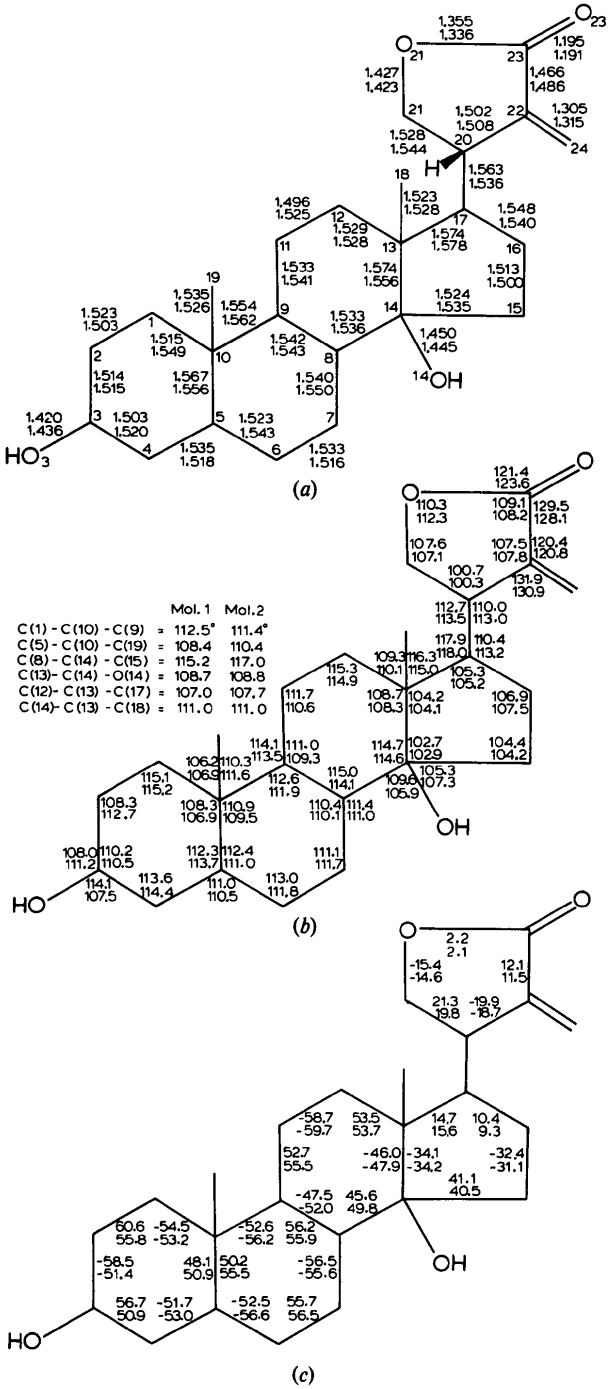


Fig. 3. Intramolecular dimensions for (II_S)*A*, the upper values, and *B* the lower values. (*a*) Bond distances (Å); σ range = 0·003 to 0·007 Å. (*b*) Bond angles (°); σ range = 0·2 to 0·4°. (*c*) Endocyclic torsion angles (°); σ range = 0·3 to 0·6°.

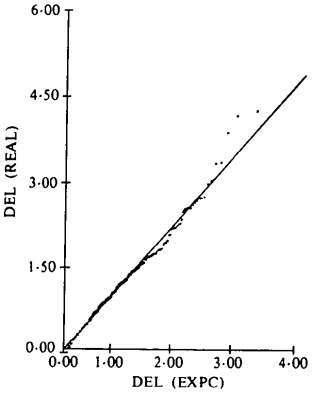


Fig. 4. A half-normal probability plot of all the intramolecular distances for the structure of (I_S) reported here and the earlier reported structure (Messerschmidt, Hölme & Lindig, 1981). The equation of the least-squares fit line is DEL(REAL) = 1·192 × DEL(EXPC) + 0·039.

Table 3. *Hydrogen-bond distances* (Å) *and angles* (°) *for* (I_S) *and* (II_S)

Donor—H···Acceptor	O···O	H···O	∠O—H···O	Symmetry operator to acceptor
(*a*) Compound (I_S)				
O(3)—H···O(21)	2·929 (4)	2·44 (5)	121 (3)	(i)
O(14)—H···O(23)	3·031	2·25 (3)	169	(ii)
(*b*) Compound (II_S)				
O(3)*A*—H···O(3)*B*	2·842 (4)	2·13 (5)	152 (4)	(ii)
O(14)*A*—H···O(3)*B*	2·886 (4)	1·52 (6)	125 (5)	(iii)
O(3)*B*—H···O(14)*A*	2·886 (4)	2·16 (4)	172 (3)	(iii)
O(14)*B*—H···O(21)*A**	3·176 (5)	2·64 (6)	124 (4)	(iv)

Symmetry operators: (i) $-1+x, -1+y, -1+z$; (ii) $-1+x, y, -1+z$; (iii) $1-x, -\frac{1}{2}+y, 1-z$; (iv) $1-x, -\frac{1}{2}+y, 2-z$.

* The distances for this close contact are rather long for a hydrogen bond, but the hydroxyl H is oriented in the direction of O(21)*A*.

and *B* structures all orient the C(20) H atom *gauche* to both C(13) and C(16) positioning it over the *D* ring. The only exception to this positioning is the (III_R) structure (Fig. 5*b*), where the C(20) H atom has a *gauche–trans* orientation relative to C(13) and C(16).

The potential-energy profile for rotation about the C(17)–C(20) bond clearly shows that each molecule is oriented in the lowest and widest minimum-energy conformation, see Fig. 6. It is clear from the energy profiles that each of these molecules has only one preferred conformation and that removal of the methylene substituent on the lactone, as in (I_S), actually decreases the orientational flexibility of the side group. This is the result of interactions between the sp^3 C(22) H atom with the C(16)β H atom. While the sp^2 C(22) methylene substituent is much bulkier, the directional aspects more than compensate for the difference in size.

Direct comparison of the structures of (I_S) and (II_S) to digitoxigenin (IV) shows that the steroid backbones are nearly identical. Fig. 7 shows the results of superimposing (I_S) on (IV) and (II_S) on (IV) using a least-squares procedure (Rohrer & Smith, 1980) to minimize the distances between the corresponding atoms C(1) to C(19), O(3) and O(14). The average separation in these atoms for (I_S) with (IV) was 0·14 Å and for (II_S)*A* and *B* with (IV) the average separations were 0·06 Å. The larger average separation of (I_S) with (IV) results primarily from the conformation difference in the *D* ring. It is also apparent from these superpositions that the lactone carbonyl O is markedly displaced from its location in prototype (IV).

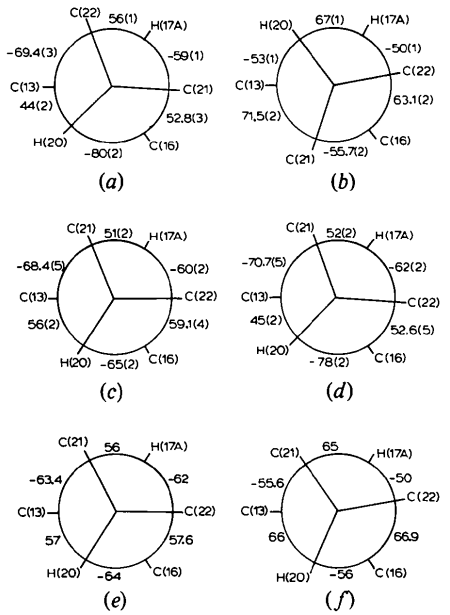


Fig. 5. Newman projections down the C(20)–C(17) bond for (*a*) (I_S), (*b*) (III_R), (*c*) (II_S)*A*, (*d*) (II_S)*B*, (*e*) (III_S)*A* and (*f*) (III_S)*B*.

As reported earlier (Rohrer, Fullerton, Yoshioka, From & Ahmed, 1979; Fullerton, Rohrer, Ahmed, Kitatsuji, Deffo & From, 1982), this type of displacement of the carbonyl O has a direct effect on the

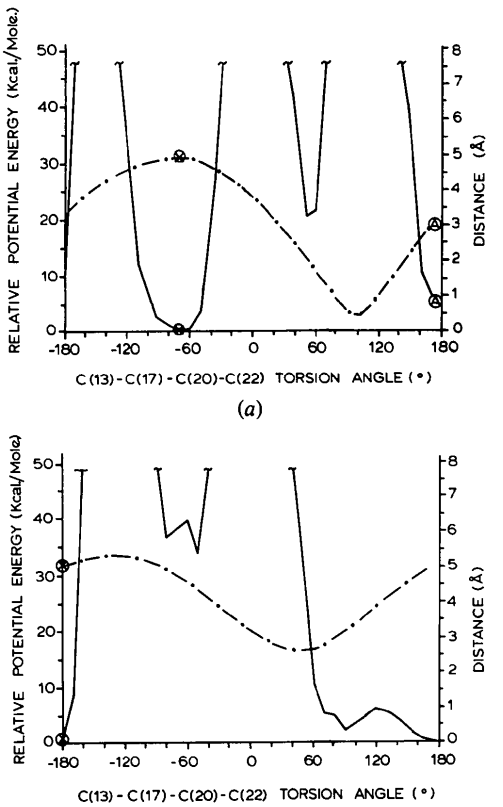


Fig. 6. Calculated potential-energy profiles (solid line) for rotation of the C(17)β lactone substituent about the C(17)–C(20) bond. The dashed curve indicates how the separation between the analogue's O(23) position and the O(23) on the prototype, (IV), changes as the analogue lactone is rotated. (*a*) Compound (I_S). (*b*) Compound (II_S).

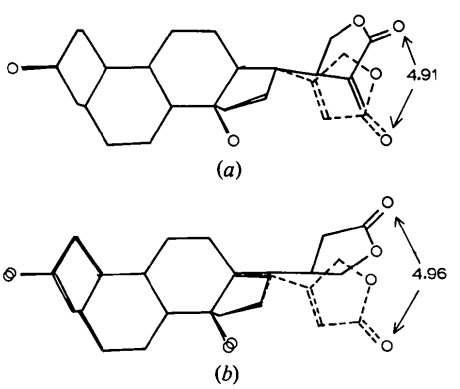


Fig. 7. Superpositions of atoms C(1) to C(19), O(3) and O(14) of (*a*) (I_S) and (*b*) (II_S)*A* [the superposition of (II_S)*B* is nearly identical to that of (II_S)*A* but the distance is 5·06 Å] on the prototype molecule (IV) (---).

Table 4. *Measured and theoretical* Na^+,K^+-ATPase 50% *inhibition,* I_{50}, *data* (*M*) *and* O(23) *displacement distances* (Å)

Compound	Configuration [at C(20)]	O(23) displacement distance, observed	O(23) displacement distance, calculated[b]	I_{50}[c], measured	I_{50}[d], calculated
(IV)	—	0·0	0·03	$3·5 \times 10^{-7}$	$3·4 \times 10^{-7}$
(V)	—	0·64	0·69	$7·0 \times 10^{-7}$	$6·6 \times 10^{-7}$
(I)	[20*R*]	—	3·29	$1·1 \times 10^{-5}$	—
	[20*S*]	4·96	3·38	$1·2 \times 10^{-5}$	$6·4 \times 10^{-5}$
		2·94[a]			$0·7 \times 10^{-5}$
(II)	[20*R*]	—	5·05	$7·0 \times 10^{-5}$	—
	[20*S*]	5·06	4·90	$6·0 \times 10^{-5}$	$7·1 \times 10^{-5}$
		4·90			$6·0 \times 10^{-5}$
(III)	[20*R*]	4·08	3·86	$2·0 \times 10^{-5}$	$2·5 \times 10^{-5}$
	[20*S*]	5·77	6·04	$1–3 \times 10^{-4}$	$1·5 \times 10^{-4}$
		5·45			$1·1 \times 10^{-4}$

Notes: (*a*) Distance for second energy-minimum conformation, see Fig. 6(*a*). (*b*) Distance calculated from equation of least-squares line: $d = (\log I_{50} + 6·471)/0·459$. (*c*) Preparation from rat brain. (*d*) Inhibition calculated from equation of line: $I_{50} = \log^{-1}(0·459d - 6·471)$.

relative rat brain Na^+,K^+-ATPase inhibition strength of these cardenolide analogues. A linear relationship between the $\log(I_{50})$ data and O displacement was obtained which indicated that for each 2·2 Å of O displacement away from the digitoxigenin position, the inhibition activity changed tenfold. Table 4 gives the O separation and activity data for the analogues compared here. Fig. 8 shows a graph of the $\log(I_{50})$ and O separations together with the line representing the linear relationship obtained from the earlier analysis. Clearly, these data and graph show that the only significant deviation from the direct relationship between carbonyl O separation obtained by comparison of the crystal structures and N^+,K^+-ATPase inhibition activity is (I_S). The energy profile (Fig. 6*a*), however, shows an alternate minimum-energy conformation for (I_S) at a C(13)–C(17)–C(20)–C(22) torsion angle of 170° where the O separation is reduced to 2·94 Å. This distance corresponds to an activity in good agreement with structure–activity relationship.

Comparing the activity models to these data clearly shows several areas of disagreement. First the degree of conjugation of the C(17)β side-group carbonyl O and corresponding basicity does not seem to play a direct

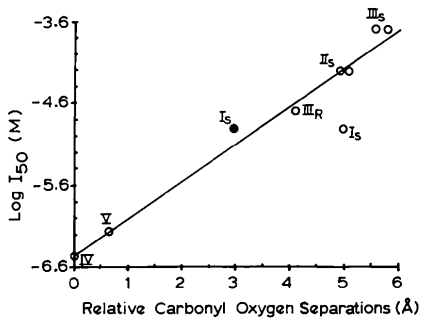


Fig. 8. Correlation between carbonyl O positions relative to digitoxigenin and Na^+,K^+-ATPase inhibition activity. (○) Distances from X-ray conformation; ● distance from alternate minimum-energy conformation.

role. If this were the case, the relative activity of analogues (II) and (III) should be much greater than that of (I). This is especially true for (II), which has a conjugated carbonyl O and would present the same geometric structure to the enzyme surface as (I) during the rotation and formation of short-range van der Waals bonds between the steroid nucleus and the enzyme. Yet (I) is more than five times more active than (II). The excellent agreement between the data for these analogues and the structural relationship indicates that the conjugated system plays a geometric role influencing the position of the carbonyl O, rather than electronic as proposed by the activity model. Second, the relationship presented here does rely on complementarity between the steroid nucleus and the enzyme surface. This is the justification for superimposing these portions of the analogue's structure to that of digitoxigenin (IV). However, the degree of complementarity of the steroid nucleus to the enzyme surface does not seem to have as great an influence on relative activity as the model proposes. The C(17)β side chains of analogues (II) and (III) are the same, but the structures of the steroid backbones are quite different. Since (II) has a steroid backbone structure identical to that of digitoxigenin (I), the model would predict that it should be more active than (III). The activity data, however, show that on average these analogues have the same activity. The structural relationship indicates that only a portion of the steroid need be complementary to the enzyme surface. Third, the analysis presented for the first comparison also shows that a two-point binding model for the C(17)β side chain is not consistent with the activity data.

Until the structure of the Na^+,K^+-ATPase receptor is known, it will not be possible to determine accurately what the structural model for genin interaction with the enzyme and activity is, but any proposed model must be at least consistent with the structural and activity data. The relationship between genin analogue structure and relative activity presented here provides a tool

for evaluating proposed models and perhaps a basis for proposing new ones. But it does not directly provide data about the types of interactions and bonding at the enzyme receptor site which are important for activity.

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References

ABRAHAMS, S. C. & KEVE, E. T. (1971). *Acta Cryst.* A**27**, 157–165.

CROMER, D. T. & WABER, J. T. (1974). *International Tables for X-ray Crystallography*, Vol. IV, edited by J. A. IBERS & W. C. HAMILTON, Table 2.2B. Birmingham: Kynoch Press.

DUAX, W. L., WEEKS, C. M. & ROHRER, D. C. (1976). *Topics in Stereochemistry*, Vol. 9, edited by E. L. ELIEL & N. ALLINGER, pp. 271–383. New York: John Wiley.

FULLERTON, D. S., ROHRER, D. C., AHMED, K., KITATSUJI, E., DEFFO, T. & FROM, A. (1982). In *Current Topics in Membranes and Transport*, edited by F. BONNER & A. KLINZELLER. New York: Academic Press.

FULLERTON, D. S., YOSHIOKA, Y., ROHRER, D. C., FROM A. H. L. & AHMED, K. (1979). *J. Med. Chem.* **22**, 529–533.

HAMILTON, W. C. (1959). *Acta Cryst.* **12**, 609–610.

JOHNSON, C. K. (1965). *ORTEP*. Report ORNL-3794. Oak Ridge National Laboratory, Tennessee.

KARLE, I. L. & KARLE, J. (1969). *Acta Cryst.* B**25**, 434–442.

MAIN, P., LESSINGER, L., WOOLFSON, M. M., GERMAIN, G. & DECLERCQ, J. P. (1977). *MULTAN 77. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray diffraction Data*. Univs. of York, England, and Louvain, Belgium.

MESSERSCHMIDT, A., HÖHME, E. & LINDIG, C. (1981). *Cryst. Struct. Commun.* **10**, 629–635.

REPKE, K. R. & DITTRICH, F. (1982). In *Current Topics in Membranes and Transport*, edited by F. BONNER & A. KLINZELLER. New York: Academic Press.

REPKE, K. R. & PORTIUS, H. J. (1966). *Sci. Pharm. Proc.* **1**, 39–57.

ROHRER, D. C., DUAX, W. L. & FULLERTON, D. S. (1976). *Acta Cryst.* B**32**, 2893–2895.

ROHRER, D. C. & FULLERTON, D. S. (1980). *Acta Cryst.* B**36**, 1565–1568.

ROHRER, D. C. & FULLERTON, D. S. (1983). *Acta Cryst.* C**39**. To be published.

ROHRER, D. C., FULLERTON, D. S., YOSHIOKA, K., FROM, A. H. L. & AHMED, K. (1979). *Advances in Chemistry: Computer Assisted Drug Design*. Vol. 112, edited by R. E. CHRISTOFFERSON & E. C. OLSON, pp. 257–279. Washington, DC: American Chemical Society.

ROHRER, D. C. & SMITH, G. D. (1980). *'FITMOL' in PROPHET Molecules*, edited by W. P. RINDONE & T. KUSH. Cambridge, MA: Bolt Beranek and Newman, Inc.

STOUT, G. H. & JENSEN, L. H. (1968). *X-ray Structure Determination*. New York: Macmillan.

THOMAS, R., BOUTAGY, J. & GELBART, A. (1974). *J. Pharmacol. Exp. Ther.* **191**, 219–231.

THOMAS, R., BROWN, L., BOUTAGY, J. & GELBART, A. (1980). *Circ. Res. Suppl.* I, **46**, 167–172.

WEEKS, C. M., CODY, V., POKRYWIECKI, S., ROHRER, D. C. & DUAX, W. L. (1974). *AFIPS Natl Comput. Conf. Expo. Conf. Proc.* **43**, 469–472.

WEINTRAUB, H. J. R. & HOPFINGER, A. J. (1975). *Int. J. Quantum Chem. Quantum Biol. Symp.* **2**, 203–208.

YOSHIOKA, K., FULLERTON, D. S. & ROHRER, D. C. (1978). *Steroids*, **32**, 511–517.

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Some Additional Changes in Space Groups of Published Crystal Structures*

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Abstract

Revised structures are reported for ten crystalline compounds, based on space groups of higher symmetry than originally deduced. For two of them the Laue symmetry is changed, from $2/m$ to $\bar{3}m$. For the remaining eight a center of symmetry has been added; for six of these we have been able to obtain F values and carry out least-squares refinements in the higher-symmetry space groups, with more satisfactory results than originally reported.

One of the most essential results of a successful crystal-structure analysis is the determination of the space group, for it is the space group that defines the

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