Novel Observations on the 'Main-Part' Isostructuralism Exhibited by Various Steroid Molecules: Structures of 5-Androstene-3β,17β-diol Monohydrate and a 1:2 Adduct of Digitoxigenin and Digirezigenin

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Abstract

An X-ray study of the 'main-part' isostructuralism observed among cardiotonic steroids has been continued on binary systems of co-crystallized isostructural pairs and extended to a few pairs of the structurally simpler androstane derivatives. The 1:1 adducts of arenobufagin–gamabufotalin and cinobufagin–cinobufotalin are solid solutions which remained isostructural with their components. Consequently, in these binary systems the component molecules are distributed at random. In contrast, the adduct of digitoxigenin–digirezigenin is partially ordered at the expense of the symmetry which is decreased from $P2_12_12_1$ to $P112_1$. The size of the unit cell remains, however, quite close to that of the components. This phenomenon seems to be governed by a mutual recognition of the component molecules. The 'main-part' isostructuralism of the androstane derivatives offers new possibilities for investigating the role of the solvent (water) and epimerization (also observed among the cardenolides: digitoxigenin, 3-epidigitoxigenin and uzarigenin) at the C(3) and C(5) atoms. Altogether they help to shed further light on the conditions and limits of isostructuralism in general. Digitoxigenin–digirezigenin (1/2), $0.36C_{23}H_{34}O_4.0.64C_{23}H_{32}O_4$, $M_r = 746.47$, monoclinic, $P2_1$ ($P112_1$), $a = 7.290$ (3), $b = 14.817$ (7), $c = 18.520$ (4) Å, $\gamma = 90.35$ (5)°, $V = 2000.5$ (21) Å³, $Z = 2$, $F(000) = 812$, $D_x = 1.240$ Mg m⁻³, λ(Cu $K\alpha$) = 1.54184 Å, $\mu = 0.626$ mm⁻¹, $R = 0.062$ for 1709 unique observed reflections, $T = 296$ (1) K. 5-Androstene-3β,17β-diol monohydrate, $C_{19}H_{30}O_2.H_2O$, $M_r = 308.46$, orthorhombic, $P2_12_12_1$, $a = 6.250$ (1), $b = 12.143$ (3), $c = 23.440$ (2) Å, $V = 1779.0$ (9) Å³, $Z = 4$, $F(000) = 680$, $D_x = 1.152$ Mg m⁻³, λ(Cu $K\alpha$) = 1.54184 Å, $\mu = 0.562$ mm⁻¹, $R = 0.055$ for 1491 unique observed reflections, $T = 296$ (1) K. Beyond these two fully reported structure determinations, three others have also been performed. However, the structures of the 1:1 disordered adducts of arenobufagin–gamabufotalin and cinobufagin–cinobufotalin are not worthy of publication as that of 5α-androstane-3β,17β-diol.H_2O has already been reported with similar accuracy. Instead, π and I_D descriptors of isostructuralism were applied in order to check the internal consistency of the old and novel structure determinations of 5α-androstane-3β,17β-diol.H_2O.

Introduction

In a previous paper (Kálmán, Argay, Scharfenberg-Pfeiffer, Höhne & Ribár, 1991) we reported on a phenomenon termed as 'main-part' isostructuralism shown by numerous cardenolides and related bufadienolides, and some descriptors of isostructuralism were also defined.

The present work investigates two further aspects of isostructuralism, namely: (a) how does the co-crystallization of related structures depend on the isostructurality index (I_D), and (b) how does isostructuralism depend on epimerization at the skeletal atoms C(3) and C(5).

In order to answer the first question a few binary systems were co-crystallized and subjected to X-ray diffraction, whereas the second question was investigated by studying the isostructuralism of simpler androstane derivatives as revealed by structure determinations and a survey of the literature.

Equivalent amounts of arenobufagin and gamabufotalin (Argay, Kálmán, Ribár, Vladimirov & Živanov-Stakić, 1987) or of cinobufagin and cinobufotalin (Kálmán, Fülöp, Argay, Ribár, Lazar, Živanov-Stakić & Vladimirov, 1988) formed binary systems which retained approximately the lattice parameters (Table 1) of the components with identical space group $P2_12_12_1$. In these structures [(1), (2)] the components occupy symmetry-equivalent positions at random, forming perfectly disordered arrays. In contrast, the adduct of digitoxigenin (Karle & Karle, 1969) and digirezigenin (Kálmán, Argay, Ribár, Vladimirov & Živanov-Stakić, 1984) (3) shows a surprising phenomenon. The crystals obtained from a solution of equivalent amounts of the two components have nearly the same unit cell as those of the component crystals, but the space-group symmetry is reduced from $P2_12_12_1$ to $P112_1$. This suggested at least a partially ordered structure. Therefore the structure determination of the binary system (3) is fully reported. The others are only summarized in Table 1. Finally, it should be mentioned that bufalin (Rohrer, Fullerton, Kitatsuji, Nambara & Yoshii, 1982) and scillarenin [originally called dihydrohelleborogenon by Ribár, Argay, Kálmán, Vladimirov & Živanov-Stakić (1983)] could not be co-crystallized hitherto (but see *Note added in proof*).

Recently, dealing with the structure–activity relationship of androgen metabolites, the crystal structures of 5-androstene-3β,17β-diol.H_2O (4) and its saturated form 5α-androstane-3β,17β-diol.H_2O (5) were solved simultaneously, and it was found that they are isostructural even in the presence of solvent (water) molecules. A check of the January 1991 release of the Cambridge Structural Database (CSD) revealed that the crystal structure of (5) had already been reported by Precigoux & Fornies-Marquina (1973), and the consistency of the two structure determinations can be checked by I_D calculation (Kálmán *et al.*, 1991) using the independent coordinate sets. A further search of the CSD showed that 5α-androstane-3α,17β-diol (Precigoux, Busetta, Courseille & Hospital, 1972) and 5β-androstane-3α,17β-diol (Weeks, Cooper, Norton, Hauptman & Fisher, 1971) are also isostructural. They are related by epimerization at C(5), and they are also related to (5) by epimerization at C(3). This prompted us to survey the effects of epimerization on isostructuralism.

Experimental

1:2 adduct of digitoxigenin and digirezigenin, (3). Data collected on a crystal of dimensions 0.30 × 0.25 × 0.45 mm obtained from methanol and mounted on a CAD-4 diffractometer using graphite-mono-

Table 1. *Crystal data of isostructural steroid pairs (bufadienolides, cardenolides, androstanes) together with their co-crystallized mixtures*

In addition to the present work, (3) and (4), which is reported in detail, the following results may be summarized: (1): $R = 0.047$, $wR = 0.053$, $S = 1.30$ for 1315 reflections with $I > 3\sigma(I)$ collected on a Philips diffractometer (Zagreb) using monochromated Mo $K\alpha$ ($\lambda = 0.71073$ Å) radiation. The 1:1 adduct as established by the occupancy factor for O(6) of arenobufagin converged to 0.5 (final $\Delta\rho_{max} = 0.19$ e Å^{-3}). (2): $R = 0.038$, $wR = 0.039$, $S = 1.14$ for 1058 reflections with $I > 3\sigma(I)$ collected on a Philips diffractometer (Zagreb) using monochromated Mo $K\alpha$ ($\lambda = 0.71073$ Å) radiation. The 1:1 adduct as established by the occupancy factor of O(7) of cinobufotalin converged to 0.5 (final $\Delta\rho_{max} = 0.12$ e Å^{-3}). (5): $R = 0.042$, $wR = 0.041$, $S = 0.65$ for 1900 reflections with $I > 3\sigma(I)$ collected on a CAD-4 diffractometer (Budapest) using monochromated Cu $K\alpha$ ($\lambda = 1.54184$ Å) radiation, final $\Delta\rho_{max} = 0.16$ e Å^{-3} [for the full structure see Precigoux & Fornies-Marquina (1973); their lattice parameters were $a = 12.143$ (6), $b = 6.433$ (4), $c = 23.100$ (9) Å, $Z = 4$].

	a (Å)	b (Å)	c (Å)	Space group	Reference
Gamabufotalin	7.850 (1)	14.766 (1)	17.836 (1)	$P2_12_12_1$	(a)
Arenobufagin	7.826 (1)	14.864 (2)	17.841 (2)		(a)
1:1 mixture (1)	7.844 (4)	14.807 (5)	17.849 (6)		
Cinobufagin	7.663 (2)	15.900 (5)	19.291 (5)	$P2_12_12_1$	(b)
Cinobufotalin	7.631 (1)	15.727 (5)	19.557 (2)		(b)
1:1 mixture (2)	7.645 (1)	15.789 (8)	19.479 (2)		
Digitoxigenin	7.250 (3)	15.015 (3)	18.464 (3)	$P2_12_12_1$	(c)
Digirezigenin	7.288 (2)	14.686 (5)	18.480 (3)		(d)
1:2 mixture (3)	7.290 (3)	14.817 (4)	18.520 (7) $\gamma = 90.35°$	$P112_1$	
5-Androstane-3β,17β-diol monohydrate (4)	6.250 (1)	12.143 (3)	23.044 (2)	$P2_12_12_1$	
5α-Androstane-3β,17β-diol monohydrate (5)	6.444 (1)	12.150 (1)	23.024 (1)		
1:1 mixture	6.352 (1)	12.159 (4)	23.234 (4)		(e)
5α-Androstane-3α,17β-diol (6)	12.327 (5)	7.191 (4)	10.347 (5) $\beta = 114.1$ (1)°	$P12_11$	(f)
5β-Androstane-3α,17β-diol (7)	11.875 (2)	7.157 (1)	10.960 (2) $\beta = 114.70$ (2)°		(g)

References: (a) Argay *et al.* (1987); (b) Kálmán *et al.* (1988); (c) Karle & Karle (1969); (d) Kálmán *et al.* (1984); (e) no structure determination was performed; (f) Precigoux *et al.* (1972); (g) Weeks *et al.* (1971).

chromated Cu $K\alpha$ radiation. Cell constants were refined by least-squares fit for 25 reflections with $25 \le \theta \le 32°$. Systematic absences $00l$: $l = 2n + 1$ if the choice of the lattice parameters follows those of the component crystals (Table 1). Thus the space group is $P112_1$ (No. 4) with $\gamma = 90.35$ (5)° deviating only slightly from 90°. Consequently, $Z = 2$ implies, at least initially, a 1:1 adduct of the components with a supposed $M_r = 747.03$. Data were collected by $\omega/2\theta$ scan in the range $0.034 \le (\sin\theta)/\lambda \le 0.531$ Å^{-1} using scan width = $(0.60 + 0.14\tan\theta)°$ with h 0 to 7, k −15 to 15, l 0 to 19. Standard reflections (400, $\overline{4}00$) were monitored every 60 min but no intensity variations were recorded. The phase problem was solved by *SHELX76* (Sheldrick, 1976). A total of 54 non-H atoms were located in an E map computed from the phase set with the best combined figure of merit of 0.104. Full-matrix least-squares refinement minimized $\Sigma w(F)^2$ for 379 variables with $w = 4F_o^2/\sigma^2(F_o)^2$. At the end of the isotropic refinement an empirical absorption correction was performed with the pro-

gram *DIFABS* (Walker & Stuart, 1983); minimum and maximum transmission coefficients were 0.588 and 1.231. Final $R = 0.062$, $wR = 0.072$, $R_{tot} = 0.093$, $S = 2.62$, $(\Delta/\sigma)_{max} = 0.232$. Maximum and minimum heights in final difference map $= \pm 0.22$ (5) e Å^{-3}. It is worth noting that in the course of the refinement no statistically disordered positions of C atoms forming ring *D* could be revealed in either of the symmetry-independent [(3*A*), (3*B*)] molecules. Only one spurious peak around C(14) of molecule *A* could be assigned as an oxygen pertaining to the digitoxigenin units mismatching their positions. Further cycles of anisotropic refinement in which the occupancy factors of atoms O(14) and O(14*) were refined resulted in 0.89 and 0.11, respectively, improving the residuals given above by a few percent. No extinction correction was applied.

Positions of H atoms bound to C atoms were generated from assumed geometries, while those linked to O atoms and C(15) were located in a difference Fourier map; their positions were taken into account without refinement in structure-factor calculations with isotropic temperature factors [$B_{iH} = (B_{ix} + 1)$ Å^2 where $x = $ C or O].

5-Androstene-3β,17β-diol.H$_2$O, (4). Data collected on a crystal of dimensions $0.05 \times 0.30 \times 0.30$ mm obtained from methanol (m.p. 453–454 K) and mounted on the same diffractometer described above. Cell constants were refined by least-squares fit for 25 centred reflections with $19 \le \theta \le 24°$. Systematic absences *h*00: $h = 2n + 1$, 0*k*0: $k = 2n + 1$, 00*l*: $l = 2n + 1$. Data were collected by $\omega/2\theta$ scan in the range $0.016 \le (\sin\theta)/\lambda \le 0.626$ Å^{-1} with *h* 0 to 7, *k* 0 to 15 and *l* 0 to 29. Of 2127 unique and non-systematically absent reflections, 1491 with $I > 3\sigma(I)$ were taken as observed. Three standard reflections (242, 313, 0,0,10) were monitored every hour but no intensity variations were recorded. The phase problem was solved by *MULTAN*82 (Main, Fiske, Hull, Lessinger, Germain, Declercq & Woolfson, 1982). All non-H atoms, including the water oxygen, were located in the *E* map computed from the phase set with the best figure of merit. Full-matrix least-squares refinement minimized $\Sigma w(\Delta F)^2$ for 199 parameters with $w = 4F_o^2/\sigma^2(F_o)^2$. At the end of the isotropic refinement an empirical absorption correction was performed with the program *DIFABS* (Walker & Stuart, 1983); relative transmission coefficients ranged from 0.679 to 1.154. Final $R = 0.055$, $wR = 0.058$, $R_{tot} = 0.105$, $S = 1.00$. Maximum and minimum peak heights in final $\Delta\rho$ map $= 0.17$ (4) e Å^{-3}. Data were not corrected for extinction. $(\Delta/\sigma)_{max} = 0.32$. H-atom treatment was as for (3). Scattering factors for both structures were taken from the program system *SDP-Plus* (Enraf–Nonius, 1982) adapted on a PDP-11/34 minicomputer (Budapest) with local modifications.

Discussion

Atomic coordinates of non-H atoms for (3) and (4) are listed in Table 2.* The bond lengths and angles for non-H atoms are given in Tables 3 and 4.

5-Androstene-3β,17β-diol monohydrate (4)

Since only the investigation of (4) provides novel structural data it will be discussed first. A perspective view of the molecule is depicted in Fig. 1. Apart from the double bond [1.322 (7) Å] between C(5)—C(6) the other C(sp^3)—C(sp^3) single bonds average 1.529 (14) Å. Ring *A* has the chair conformation, the torsion angles average 53 (3)°. Ring *B* assumes an almost perfect half-chair [puckering parameters: $Q = 0.303$ (6) Å, $\theta = 50.9$ (7)°, $\varphi = 209.2$ (9)°, if the calculation starts from C(5) to C(10) and proceeds in a clockwise direction]: the twofold axis bisects the C(5)—C(6) double bond with a low asymmetry factor (Kálmán, Czugler & Simon, 1982) $fC_2 = 0.4$ pm. The five-membered ring *D* exhibits an E^{13}-envelope conformation and is distorted somewhat towards the $_{14}T^{13}$ twist chair. The 17β-OH moiety is oriented pseudo-equatorially. It forms a hydrogen bond acting as a donor to the water molecule, and acts as an acceptor of the hydrogen bond donated by the 3β-OH group, which is also oriented equatorially (Table 5).

The water molecule plays an important role in the molecular packing. Besides the above-mentioned acceptor role it acts as a donor to two O(3) atoms which are related by a screw axis located at $(X, \frac{1}{4}, \frac{1}{2})$. This seems to explain why the isostructural 5α-androstane-3β,17β-diol (5) is also a monohydrate. Even in the presence of the water molecule the descriptor

$$\pi = \left| \frac{a + b + c}{a' + b' + c'} - 1 \right| = 0.004$$

(where a, b, c and a', b' and c' are the orthogonalized lattice parameters of the related crystals) is very low and the degree of isostructurality [defined by us (Kálmán *et al.*, 1991) as follows

$$I_D^n = \left[1 - \left(\frac{\Sigma \Delta R_i^2}{n} \right)^{1/2} \right] \times 100$$

where ΔR_i is the distance between the absolute coordinates of groups of identical atoms within the

Table 2. *Fractional coordinates for non-*H *atoms with e.s.d.'s in parentheses*

$B_{eq} = \frac{4}{3}$ trace (BG), where G is the metric tensor.

	x	y	z	B_{eq} (Å²)
1:2 Adduct of digitoxigenin–digirezigenin (3)				
C(1)	0.7302 (11)	0.8933 (5)	0.1257	4.7 (4)
C(2)	0.5431 (14)	0.9336 (5)	0.1133 (4)	5.7 (4)
C(3)	0.4490 (12)	0.9591 (5)	0.1835 (5)	5.2 (4)
C(4)	0.4459 (12)	0.8802 (5)	0.2331 (5)	5.4 (4)
C(5)	0.6305 (11)	0.8326 (5)	0.2449 (4)	4.5 (4)
C(6)	0.6102 (14)	0.7526 (6)	0.2964 (5)	6.3 (5)
C(7)	0.5119 (13)	0.6734 (5)	0.2560 (4)	5.2 (4)
C(8)	0.6050 (10)	0.6475 (5)	0.1856 (4)	3.6 (3)
C(9)	0.6281 (9)	0.7290 (5)	0.1350 (4)	3.4 (3)
C(10)	0.7266 (10)	0.8084 (5)	0.1741 (4)	3.9 (3)
C(11)	0.7136 (11)	0.7010 (5)	0.0631 (4)	4.3 (4)
C(12)	0.6027 (10)	0.6255 (5)	0.0270 (4)	4.1 (4)
C(13)	0.5865 (9)	0.5407 (4)	0.0749 (4)	2.9 (3)
C(14)	0.5123 (9)	0.5699 (4)	0.1468 (4)	3.1 (3)
C(15)	0.3101 (10)	0.5570 (6)	0.1457 (5)	5.4 (4)
C(16)	0.2529 (10)	0.5104 (5)	0.0782 (5)	4.5 (4)
C(17)	0.4326 (9)	0.4801 (5)	0.0401 (4)	3.4 (3)
C(18)	0.7732 (11)	0.4922 (5)	0.0799 (5)	5.2 (4)
C(19)	0.9321 (12)	0.7846 (6)	0.1882 (5)	6.1 (5)
C(20)	0.4646 (10)	0.3811 (5)	0.0442 (5)	4.0 (3)
C(21)	0.5168 (15)	0.3308 (6)	−0.0205 (6)	7.6 (6)
C(22)	0.4467 (12)	0.3210 (5)	0.0979 (5)	5.4 (4)
C(23)	0.4863 (14)	0.2322 (6)	0.0719 (5)	6.5 (5)
O(3)	0.5433 (9)	1.0316 (3)	0.2202 (3)	7.4 (3)
O(145)	0.4598 (8)	0.4933 (3)	0.1916 (3)	6.0 (3)
O(21)	0.5338 (11)	0.2363 (4)	0.0024 (5)	9.4 (4)
O(23)	0.4973 (11)	0.1605 (4)	0.1002 (4)	10.5 (4)
C(1')	0.7765 (11)	0.4007 (5)	0.3722 (5)	4.6 (4)
C(2')	0.9710 (11)	0.4398 (5)	0.3887 (5)	5.0 (4)
C(3')	1.0608 (11)	0.4636 (5)	0.3180 (5)	5.0 (4)
C(4')	1.0657 (12)	0.3848 (5)	0.2673 (5)	5.1 (4)
C(5')	0.8785 (11)	0.3399 (5)	0.2556 (5)	4.5 (4)
C(6')	0.8974 (13)	0.2598 (6)	0.2039 (4)	5.5 (5)
C(7')	0.9900 (12)	0.1800 (5)	0.2403 (4)	5.0 (4)
C(8')	0.8944 (9)	0.1541 (5)	0.3105 (4)	3.8 (3)
C(9')	0.8741 (9)	0.2350 (5)	0.3615 (4)	3.4 (3)
C(10')	0.7812 (9)	0.3174 (5)	0.3243 (4)	3.6 (3)
C(11')	0.7889 (10)	0.2080 (5)	0.4335 (4)	3.9 (4)
C(12')	0.8927 (10)	0.1316 (5)	0.4684 (4)	3.8 (3)
C(13')	0.9103 (8)	0.0462 (5)	0.4208 (4)	3.3 (3)
C(14')	0.9833 (9)	0.0751 (4)	0.3486 (4)	3.3 (3)
C(15')	1.1909 (12)	0.0688 (7)	0.3565 (6)	6.8 (5)
C(16')	1.2435 (10)	0.0117 (5)	0.4170 (4)	4.1 (3)
C(17')	1.0615 (9)	−0.0158 (5)	0.4558 (5)	4.2 (3)
C(18')	0.7197 (9)	−0.0001 (5)	0.4148 (4)	4.1 (4)
C(19')	0.5743 (11)	0.2956 (6)	0.3080 (5)	5.4 (4)
C(20')	1.0297 (9)	−0.1175 (5)	0.4536 (4)	3.7 (3)
C(21')	0.9793 (16)	−0.1633 (6)	0.5217 (6)	8.0 (6)
C(22')	1.0437 (13)	−0.1755 (5)	0.4000 (5)	5.2 (4)
C(23')	1.0042 (13)	−0.2656 (5)	0.4295 (6)	7.5 (5)
O(3')	0.9725 (8)	0.5378 (3)	0.2828 (3)	6.4 (3)
O(14')	1.0139 (12)	−0.0029 (4)	0.3028 (3)	8.6 (3)
O(21')	0.9707 (10)	−0.2571 (4)	0.5023 (5)	9.0 (4)
O(23')	1.0064 (12)	−0.3379 (4)	0.4020 (5)	11.8 (5)
5-Androstene-3β,17β-diol monohydrate (4)				
O(w)	0.5496 (5)	0.1632 (2)	0.5177 (1)	5.0 (1)
O(3)	0.9609 (5)	0.1637 (2)	0.5606 (1)	3.8 (1)
O(17)	1.2578 (5)	−0.0096 (2)	1.0241 (1)	4.0 (1)
C(1)	0.8836 (7)	0.0644 (4)	0.7114 (2)	3.7 (2)
C(2)	0.8703 (8)	0.0620 (4)	0.6465 (2)	3.9 (2)
C(3)	0.9697 (8)	0.1655 (3)	0.6215 (1)	3.4 (1)
C(4)	1.1990 (8)	0.1794 (3)	0.6424 (2)	3.7 (2)
C(5)	1.2185 (7)	0.1740 (3)	0.7063 (1)	3.0 (1)
C(6)	1.3241 (8)	0.2499 (3)	0.7354 (2)	3.8 (2)
C(7)	1.3613 (9)	0.2487 (3)	0.7985 (2)	4.1 (2)
C(8)	1.3147 (7)	0.1374 (3)	0.8252 (1)	2.9 (1)
C(9)	1.1033 (7)	0.0903 (3)	0.8002 (1)	3.0 (1)
C(10)	1.1140 (7)	0.0748 (3)	0.7350 (1)	2.8 (1)
C(11)	1.0259 (9)	−0.0122 (4)	0.8321 (2)	4.3 (2)
C(12)	1.0262 (7)	−0.0016 (4)	0.8968 (2)	3.8 (2)
C(13)	1.2443 (6)	0.0357 (3)	0.9193 (1)	2.8 (1)
C(14)	1.2961 (8)	0.1454 (3)	0.8898 (1)	3.0 (1)
C(15)	1.4847 (9)	0.1913 (3)	0.9245 (2)	4.3 (2)
C(16)	1.446 (1)	0.1486 (4)	0.9857 (2)	5.1 (2)
C(17)	1.2490 (8)	0.0734 (3)	0.9812 (1)	3.5 (1)
C(18)	1.4138 (8)	−0.0533 (3)	0.9097 (2)	4.0 (2)
C(19)	1.2423 (8)	−0.0296 (3)	0.7194 (2)	4.0 (2)

Table 3. *Bond lengths* (Å) *with e.s.d.'s in parentheses*

1:2 Adduct of digitoxigenin–digirezigenin (3)

C(1)—C(2)	1.510 (12)	C(1')—C(2')	1.559 (12)
C(1)—C(10)	1.545 (11)	C(1')—C(10')	1.521 (12)
C(2)—C(3)	1.519 (13)	C(2')—C(3')	1.505 (14)
C(3)—C(4)	1.487 (13)	C(3')—C(4')	1.499 (13)
C(3)—O(3)	1.442 (11)	C(3')—O(3')	1.435 (11)
C(4)—C(5)	1.538 (12)	C(4')—C(5')	1.530 (12)
C(5)—C(6)	1.529 (13)	C(5')—C(6')	1.531 (13)
C(5)—C(10)	1.531 (12)	C(5')—C(10')	1.493 (12)
C(6)—C(7)	1.561 (13)	C(6')—C(7')	1.523 (13)
C(7)—C(8)	1.521 (12)	C(7')—C(8')	1.523 (12)
C(8)—C(9)	1.537 (11)	C(8')—C(9')	1.533 (11)
C(8)—C(14)	1.511 (11)	C(8')—C(14')	1.516 (11)
C(9)—C(10)	1.553 (11)	C(9')—C(10')	1.560 (11)
C(9)—C(11)	1.530 (12)	C(9')—C(11')	1.523 (12)
C(10) C(19)	1.563 (11)	C(10')—C(19')	1.569 (11)
C(11)—C(12)	1.530 (11)	C(11')—C(12')	1.511 (11)
C(12)—C(13)	1.542 (11)	C(12')—C(13')	1.549 (11)
C(13)—C(14)	1.501 (11)	C(13')—C(14')	1.501 (12)
C(13)—C(17)	1.571 (10)	C(13')—C(17')	1.578 (11)
C(13)—C(18)	1.546 (11)	C(13')—C(18')	1.549 (10)
C(14)—C(15)	1.486 (10)	C(14')—C(15')	1.524 (11)
C(14)—O(145)	1.454 (10)	C(14')—O(14')	1.450 (10)
C(15)—C(16)	1.486 (13)	C(15')—C(16')	1.458 (14)
C(15)—O(145)	1.678 (11)	C(16')—C(17')	1.561 (11)
C(16)—C(17)	1.557 (11)	C(17')—C(20')	1.524 (11)
C(17)—C(20)	1.489 (11)	C(20')—C(21')	1.477 (15)
C(20)—C(21)	1.462 (14)	C(20')—C(22')	1.318 (12)
C(20)—C(22)	1.341 (13)	C(21')—O(21')	1.437 (13)
C(21)—O(21)	1.469 (12)	C(22')—C(23')	1.469 (13)
C(22)—C(23)	1.433 (13)	C(23')—O(21')	1.375 (16)
C(23)—O(21)	1.333 (15)	C(23')—O(23')	1.186 (12)
C(23)—O(23)	1.189 (12)		

5-Androstene-3β,17β-diol monohydrate (4)

O(3)—C(3)	1.429 (5)	C(8)—C(14)	1.521 (6)
O(17)—C(17)	1.425 (6)	C(9)—C(10)	1.539 (6)
C(1)—C(2)	1.524 (7)	C(9)—C(11)	1.531 (7)
C(1)—C(10)	1.548 (7)	C(10)—C(19)	1.544 (6)
C(2)—C(3)	1.520 (7)	C(11)—C(12)	1.522 (7)
C(3)—C(4)	1.524 (7)	C(12)—C(13)	1.530 (7)
C(4)—C(5)	1.505 (7)	C(13)—C(14)	1.535 (6)
C(5)—C(6)	1.322 (7)	C(13)—C(17)	1.523 (6)
C(5)—C(10)	1.526 (6)	C(13)—C(18)	1.530 (7)
C(6)—C(7)	1.498 (7)	C(14)—C(15)	1.537 (7)
C(7)—C(8)	1.519 (6)	C(15)—C(16)	1.546 (7)
C(8)—C(9)	1.555 (6)	C(16)—C(17)	1.534 (8)

Table 4. *Bond angles* (°) *with e.s.d.'s in parentheses*

Bond angles for the 1:2 adduct of digitoxigenin–digirezigenin (3) have been deposited.

5-Androstene-3β,17β-diol monohydrate (4)

C(2)—C(1)—C(10)	114.2 (7)	C(1)—C(10)—C(19)	109.3 (6)
C(1)—C(2)—C(3)	110.3 (7)	C(5)—C(10)—C(9)	111.1 (6)
O(3)—C(3)—C(2)	110.9 (7)	C(5)—C(10)—C(19)	108.7 (6)
O(3)—C(3)—C(4)	111.0 (7)	C(9)—C(10)—C(19)	111.0 (6)
C(2)—C(3)—C(4)	110.6 (7)	C(9)—C(11)—C(12)	114.8 (7)
C(3)—C(4)—C(5)	113.1 (7)	C(11)—C(12)—C(13)	111.7 (7)
C(4)—C(5)—C(6)	121.5 (7)	C(12)—C(13)—C(14)	106.8 (6)
C(4)—C(5)—C(10)	116.0 (7)	C(12)—C(13)—C(17)	115.8 (6)
C(6)—C(5)—C(10)	122.5 (7)	C(12)—C(13)—C(18)	110.9 (6)
C(5)—C(6)—C(7)	125.4 (8)	C(14)—C(13)—C(17)	99.5 (6)
C(6)—C(7)—C(8)	112.7 (7)	C(14)—C(13)—C(18)	113.6 (6)
C(7)—C(8)—C(9)	109.5 (6)	C(17)—C(13)—C(18)	109.9 (6)
C(7)—C(8)—C(14)	111.6 (6)	C(8)—C(14)—C(13)	114.1 (6)
C(9)—C(8)—C(14)	109.6 (6)	C(8)—C(14)—C(15)	119.4 (7)
C(8)—C(9)—C(10)	112.5 (6)	C(13)—C(14)—C(15)	103.8 (6)
C(8)—C(9)—C(11)	112.5 (6)	C(14)—C(15)—C(16)	104.4 (7)
C(10)—C(9)—C(11)	113.6 (7)	C(15)—C(16)—C(17)	105.2 (7)
C(1)—C(10)—C(5)	107.8 (6)	O(17)—C(17)—C(13)	117.4 (7)
C(1)—C(10)—C(9)	108.9 (6)	O(17)—C(17)—C(16)	110.0 (7)
		C(13)—C(17)—C(16)	105.1 (7)

asymmetric unit (whose number is n)] $I_D^{19} = I_D^{21} = 78\%$ decreases by only 2% if the positions of the water molecules are taken into account. I_D values are the same if either the structure (5) given by Precigoux & Fornies-Marquina (1973) or the reinvestigated (5)

is compared to (4); using I_D to compare the two structure determinations one gets $I_D^{22} = 98.5\%$, and $\pi = 0.0014$, which indicates good internal consistency between them.

A comparison of $I_D^{19} = I_D^{21} = 78\%$ with the values for scillarenin and bufalin (Kálmán et al., 1991) ($I_D^{19} = 51$, $I_D^{21} = 50\%$) indicates that the double bond maintained by C(5) in ring B has a smaller distortion effect upon the steroid skeleton than if it is in ring A.

Correlation between isostructuralism and co-crystallization

The significant difference ($\Delta I_D = 27\%$) observed between the I_D values for the androstane and the bufalin pairs seems to explain why (4) and (5) could be co-crystallized easily, also resulting in a solid solution, whereas bufalin and scillarenin could not. The 1:1 mixture of (4) and (5) (Table 1) with $\pi = 0.007$ for (4) and 0.003 for (5) – similar to (1) and (2) – is perfectly isostructural with the component crystals. From this it follows that the components are distributed at random in the four molecular sites.

In contrast to these solid solutions (Table 1) the asymmetric unit of the monoclinic adduct of digitoxigenin and digirezigenin (3) contains two molecules. Therefore, the four symmetry-related positions of $P2_12_12_1$ split into 2×2 positions in the monoclinic unit cell. Consequently, the adduct remains almost isostructural with both component crystals (Fig. 2). Molecule (3A) is characterized by the 14,15β-oxirane ring, which, however, is distorted in comparison with that in digirezigenin and cinobufotalin (Kálmán et al., 1988). This is in agreement with the final result of refinement of this molecular position, where a 10% disordered O atom was found. In contrast, molecule 3B resembles digitoxigenin with a rather short C(15')···O(14') = 1.94 (1) Å distance (Table 6) indicating the presence of mismatching digirezigenin molecules. No spurious peaks were found around this molecular position. The puckering parameters (Cremer & Pople, 1975) for
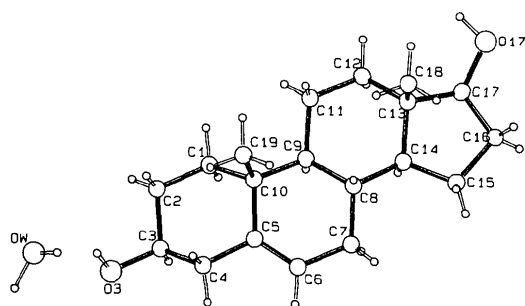
Table 5. *Intermolecular hydrogen bonds for* (3) *and* (4)

Com- No.	pound	Donor–acceptor	Symmetry relation	H···O (Å)	O···O (Å)	OH···O (°)
1	(3)	O(3)—H···O(23)	$x, y+1, z$	1.86 (3)	2.950 (8)	167 (1)
2	(3)	O(3')—H···O(23')	$x, y+1, z$	1.96 (3)	2.885 (9)	137 (1)
3	(4)	O(3)—H···O(17)	$\frac{1}{2}-x, -y, z-\frac{1}{2}$	1.61 (1)	2.706 (4)	154.0 (3)
4	(4)	O(17)—H···O(W)	$\frac{1}{2}-x, -y, z+\frac{1}{2}$	1.71 (1)	2.681 (3)	158.7 (3)
5	(4)	O(W)—H(W1)···O(3)	$x-\frac{1}{2}, \frac{1}{2}-y, 1-z$	1.83 (1)	2.845 (4)	162.9 (3)
6	(4)	O(W)—H(W2)···O(3)	x, y, z	1.77 (1)	2.761 (4)	154.3 (3)

ring D found in the symmetry-independent (3A) and (3B) molecules revealed substantial differences to both digitoxigenin and digirezigenin (Fig. 3). Assuming that the observed ring puckerings are the averaged superpositions of different amounts of digitoxigenin (E^{14}-envelope) and digirezigenin (E^{17}-envelope), in order to estimate their relative amounts the five puckering coordinates of both D rings have been compared with the r.m.s. values of 11 cardenolides/bufadienolides possessing the 14β-OH moiety and those of four crystal structures in which there is an 14,15β-oxirane ring (the corresponding

(a)

(b)
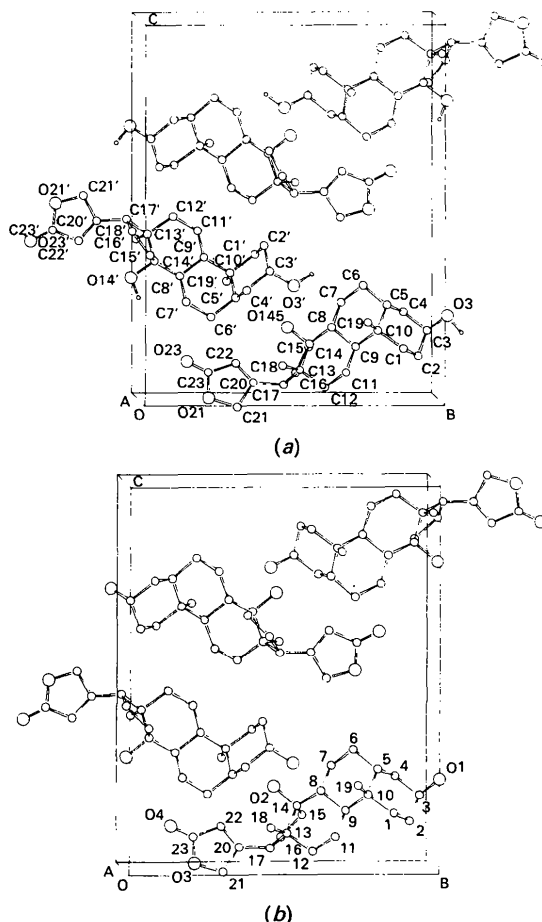
Fig. 2. Molecular packing of (a) the 1:2 adduct of digitoxigenin/ digirezigenin (3) and (b) digitoxigenin showing their isostructuralism.

Fig. 1. Perspective view of 5-androstene-3β,17β-diol monohydrate (4) with atomic numbering. The H atoms are shown but not labelled.

puckering coordinates are deposited). Accordingly, in position A approximately 20% of digirezigenin is substituted by mismatching digitoxigenin molecules, whereas in position B digitoxigenin (53%) somewhat exceeds digirezigenin (47%). From this it follows that the adduct crystallized from a solution of equivalent amounts of the components contains 64 (6)% digirezigenin and 36 (6)% digitoxigenin thus forming a partially ordered crystal lattice.

The two symmetry-independent molecular sites are linked together separately by infinite head-to-tail hydrogen-bonded chains (Table 5).

The epimerization effect on isostructuralism

In a previous paper (Kálmán *et al.*, 1991) two special isostructural pairs were recognized. Within the group of five isostructural digitoxigenin derivatives the chemical formulae of digitoxigenin and 3-epidigitoxigenin $(C_{23}H_{34}O_4)$ and, moreover, those of $(21R)$- and $(21S)$-methyldigitoxigenin $(C_{24}H_{36}O_4)$ are the same. Consequently, these pairs differ only by epimerization either at C(3) of ring A or C(21) of the γ-lactone ring.

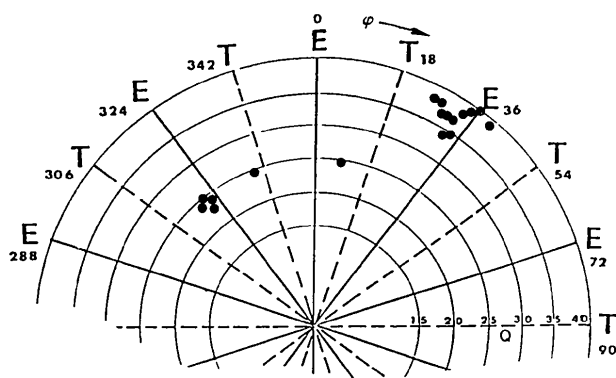The study of androstane-3,17-diols enabled us to shed more light on the effect of epimerization at C(3) and C(5), respectively. The 5α- and 5β-epimers of

Fig. 3. Scattergram of the puckering parameters (Q, φ) for the five-membered D ring observed for the molecular superpositions $(3A)$ $(Q = 0.25$ Å, $\varphi = 338°)$ and $(3B)$ $(Q = 0.25$ Å, $\varphi = 9°)$ in comparison with those of 11 cardenolides and bufadienolides (group *a*: $Q = 0.34$–0.40 Å, $\varphi = 27$–$40°)$ possessing the 14β-OH moiety and the four antipodean derivatives (group *b*: $Q = 0.23$–0.25 Å, $\varphi = 317$–$323°)$ having the $14,15\beta$-oxirane ring. Group *a*: (1) digitoxigenin (Karle & Karle, 1969); (2) 3-epidigitoxigenin (Messerschmidt, Höhne & Megges, 1981); (3) $(21R)$-methyldigitoxigenin (Prasad & Gabe, 1983); (4) $(21S)$-methyldigitoxigenin and (5) uzarigenin (Kálmán *et al.*, 1991); (6) 19-nordigitoxigenin (Scharfenberg-Pfeiffer, Höhne & Wunderwald, 1987); (7) bufalin (Rohrer *et al.*, 1982); (8) scillarenin (Ribár *et al.*, 1983); (9) bufotalin (Kálmán *et al.*, 1988); (10) arenobufagin and (11) gamabufotalin (Árgay *et al.*, 1987). Group *b*: (1) digirezigenin (Kálmán *et al.*, 1984); (2) cinobufagin and (3) cinobufotalin (Kálmán *et al.*, 1988); (4) 1:1 mixture of cinobufagin and cinobufotalin (see Table 1).

Table 6. *Relevant bond distances* (Å) *and angles* (°) *pertaining to the* O(14) *atom in* $(3A)$, $(3B)$ *and selected molecules*

For references see the caption of Fig. 3.

	C(14)— C(15)	C(15)— O(14)	C(14)— O(14)— C(15)	O(14)— C(14)— C(15)	O(14)— C(15)— C(14)
Molecule (3A)	1.486 (10)	1.678 (11)	56.1 (8)	69.6 (9)	54.3 (8)
Digirezigenin	1.469 (5)	1.452 (4)	60.8 (3)	59.6 (3)	59.6 (3)
Cinobufotalin	1.471 (7)	1.443 (6)	61.0 (5)	59.1 (5)	59.9 (5)
Molecule (3B)	1.524 (11)	1.940 (10)		81.4 (11)	
Digitoxigenin	1.508	2.363		105.4	
(21S)-Methyldigitoxigenin	1.535 (6)	2.358 (4)		104.8 (5)	

androstane-3α,17β-diol [(6), (7)] had been suspected to be isostructural ('isomorphous') by Norton, Lu & Campbell (1962) who reported only their lattice parameters. The structure determinations undertaken by Weeks *et al.* (1971) and Precigoux *et al.* (1972) corroborated this conclusion although it was left unnoticed. In both structures close contacts are formed between the molecules translated by the 2_1 operator at $(\frac{1}{2}, y, \frac{1}{2})$ *via* two head-to-tail OH···O hydrogen bonds arranged helically by a second 2_1 operator at $(0, y, \frac{1}{2})$. However, close inspection of these molecular packings (Fig. 4) reveals that the different A/B ring junctions (*cis* for the 5β- and *trans* for the 5α-epimer) permit a low degree of isostructuralism $(I_D^{21} = 41\%)$. The 5β-epimer is bent along the main molecular axis, towards the 2_1 operator at $(\frac{1}{2}, y, \frac{1}{2})$. This results in a slight increase in the unit-cell volume $(\Delta V = 9$ Å$^3)$. Partitioning I_D for the rigid part of the steroid skeleton it can be seen that the C and D rings exhibit a higher degree of isostructurality, $\Delta I_D^{11} = 76\%$ [for atoms C(7)—C(9) and C(11)—C(18)]; the remaining deviation can be attributed to a mandatory balance of the A-ring displacement, thus maintaining a similar packing mode. From these results it follows that a similar packing motif in these systems can be maintained even with considerable changes in the molecular conformation caused by epimerization.

In contrast, epimerization about C(3) of (6) resulting in (5) forbids their isostructuralism. (5) crystallizes in $P2_12_12_1$ with one water molecule. The rigid steroid skeleton without a flexible substituent (such as lactone rings) cannot counterbalance the large O(3) displacement (*cf.* 2.07 Å for 3-epidigitoxigenin and digitoxigenin) thus the packing mode must be altered. Nevertheless, as discussed above, a new close packing is developed by the use of water molecules, and (5) is isostructural with the hydrated 5-androstene-3β,17β-diol (4). The relationship between these androstanes is depicted in Fig. 5. Since saturation of 5-androstene-3β,17β-diol results in both 5α- and 5β-epimers, the missing 5β-androstane-3β,17β-diol may help provide more information concerning the relationship between epimerization and isostructuralism. Preparation of this epimer is in progress.

Concluding remarks

Isostructuralism reflects the inclination of rigid and semirigid molecules (*e.g.* steroids) with related structures to crystallize in a similar packing mode. The 'isostructural' molecules have slightly different or identical chemical formulae, and differ only by the epimerization which has been observed in different forms. We have quantified the degree of isostructurality, and the full and partitioned I_D values enabled us to scrutinize a few characteristics of isostructuralism as summarized below:

(1) It seems that the degree of isostructurality really does have an impact on the co-crystallization of the pairs investigated. The components related by high I_D (1:1 mixtures of gamabufotalin–arenobufagin: $I_D^{19} = I_D^{28} = 95\%$, and cinobufagin–cino-

bufotalin: $I_D^{19} = 94$, $I_D^{28} = 89\%$) co-crystallize readily forming disordered solid solutions, while those which possess low value(s) (bufalin and scillarenin: $I_D^{19} = 51$, $I_D^{28} = 48\%$) cannot be co-crystallized (but see *Note added in proof*). The 'related' molecules cannot mutually substitute each other at the symmetry-equivalent sites. Digitoxigenin and digirezigenin which are related by lower $I_D^{19} = 85$ and $I_D^{27} = 79\%$ values in the first attempt formed a 1:2 mixture controlled by a trend of limited recognition of the molecules. Presumably, depending on the conditions of crystallization, various kinetically and/or thermodynamically preferred adducts of digitoxigenin and digirezigenin may be obtained. Finally, although $I_D^{22} = 76\%$ for (4) and (5) the easy crystallization of the ternary adduct must be helped by the presence of water molecules. The low $\Delta R_i = 0.32$ Å for the water oxygen in (4) and (5) supports this conjecture.

(2) Isostructuralism is not stereospecific. Epimerization, depending on its site, permits or forbids isostructuralism between the steroids investigated depending on whether the displacement of the active sites (*e.g.* hydroxy moieties) is efficiently counterbalanced by proper motion of the molecules within the closely related (shape, volume) asymmetric units.

Note added in proof: After several fruitless attempts a 1:1 adduct of bufalin and scillarenin has recently been co-crystallized from a mixture of methanol and *n*-butyl acetate.
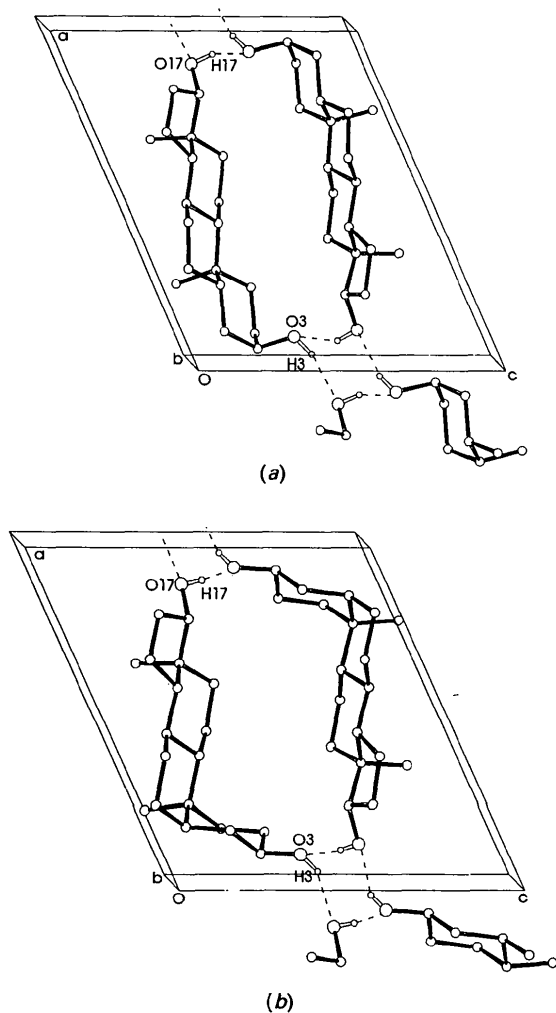

(*a*)

(*b*)

Fig. 4. Molecular packing of (*a*) 5α-androstane-3α,17β-diol (6) and (*b*) 5β-androstane-3α,17β-diol (7) showing their isostructuralism with the dominant similarities and slight differences.

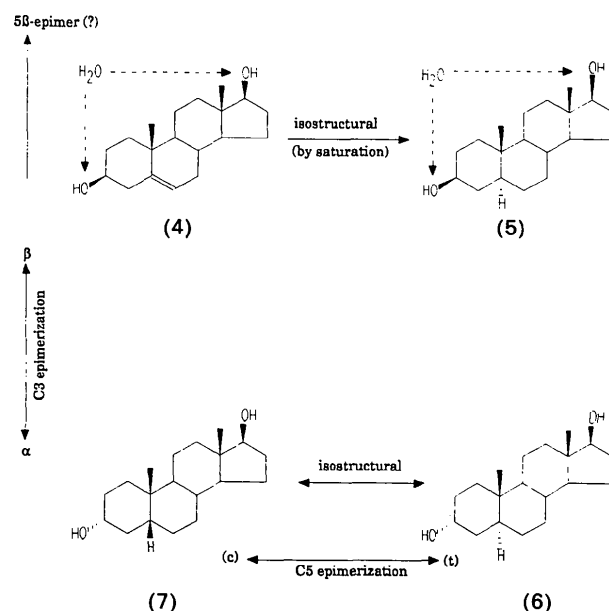Fig. 5. Structures of some androstane-3,17β-diols related by saturation and epimerization at the C(3) and C(5) atoms. The 5β-epimer (?) refers to the unknown product which may also be obtained from (4) by saturation.

References

ARGAY, GY., KÁLMÁN, A., RIBÁR, B., VLADIMIROV, S. & ŽIVANOV-STAKIĆ, D. (1987). *Acta Cryst.* C43, 922–926.
CREMER, D. & POPLE, J. A. (1975). *J. Am. Chem. Soc.* 97, 1354–1358.
Enraf–Nonius (1982). *Structure Determination Package.* Enraf–Nonius, Delft, The Netherlands.
KÁLMÁN, A., ARGAY, GY., RIBÁR, B., VLADIMIROV, S. & ŽIVANOV-STAKIĆ, D. (1984). *Croat. Chem. Acta,* 57, 519–528.
KÁLMÁN, A., ARGAY, GY., SCHARFENBERG-PFEIFFER, D., HÖHNE, E. & RIBÁR, B. (1991). *Acta Cryst.* B47, 68–77.
KÁLMÁN, A., CZUGLER, M. & SIMON, K. (1982). *Molecular Structure and Biological Activity,* edited by J. F. GRIFFIN & W. L. DUAX, pp. 367–376. New York: Elsevier Biomedical.
KÁLMÁN, A., FÜLÖP, V., ARGAY, GY., RIBÁR, B., LAZAR, D., ŽIVANOV-STAKIĆ, D. & VLADIMIROV, S. (1988). *Acta Cryst.* C44, 1634–1638.

KARLE, I. L. & KARLE, J. (1969). *Acta Cryst.* B25, 434–442.
MAIN, P., FISKE, S. J., HULL, S. E., LESSINGER, L., GERMAIN, G., DECLERCQ, J.-P. & WOOLFSON, M. M. (1982). *MULTAN82. 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., HÖHNE, E. & MEGGES, R. (1981). *Cryst. Struct. Commun.* 10, 149–156.
NORTON, D. A., LU, C. T. & CAMPBELL, A. E. (1962). *Acta Cryst.* 15, 1189.
PRASAD, L. & GABE, E. J. (1983). *Acta Cryst.* C39, 273–275.
PRECIGOUX, G., BUSETTA, B., COURSEILLE, C. & HOSPITAL, M. (1972). *Cryst. Struct. Commun.* 1, 265–268.
PRECIGOUX, G. & FORNIES-MARQUINA, F. (1973). *Cryst. Struct. Commun.* 2, 287–290.
RIBÁR, B., ARGAY, GY., KÁLMÁN, A., VLADIMIROV, S. & ŽIVANOV-STAKIĆ, D. (1983). *J. Chem. Res. (M),* pp. 1001–1042.
ROHRER, D. C., FULLERTON, D. S., KITATSUJI, E., NAMBARA, T. & YOSHII, E. (1982). *Acta Cryst.* B38, 1865–1868.
SCHARFENBERG-PFEIFFER, D., HÖHNE, E. & WUNDERWALD, M. (1987). *Cryst. Res. Technol.* 22, 1403–1408.
SHELDRICK, G. M. (1976). *SHELX76. Program for crystal structure determination.* Univ. of Cambridge, England.
WALKER, N. & STUART, D. (1983). *Acta Cryst.* A39, 158–166.
WEEKS, C. M., COOPER, A., NORTON, D. A., HAUPTMAN, H. & FISHER, J. (1971). *Acta Cryst.* B27, 1562–1572.

Geometric Analysis of Non-Ionic O—H···O Hydrogen Bonds and Non-Bonding Arrangements in Neutron Diffraction Studies of Carbohydrates

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Abstract

The geometry of chemically related O—H···O hydrogen bonds in strictly non-ionic surroundings is analyzed using neutron diffraction data derived from carbohydrates. Correlations between the hydrogen-bond parameters $d_{H···O}$, $d_{O···O}$ and $\alpha_{OH···O}$ are studied in scatterplots, which are extended far beyond the normally accepted hydrogen-bonded region to the limits $d_{H···O} < 5.0$ Å and $0 < \alpha_{OH···O} < 180°$. The restriction to non-ionic arrangements produces scatterplots that differ considerably from those obtained from chemically heterogeneous data samples. Minor components of three-center and four-center hydrogen bonds frequently differ with respect to the major components and produce significant outliers compared with correlation plots obtained from major components alone. The hydrogen-bonded regions merge with regions of non-bonding arrangements, as $\alpha_{OH···O}$ decreases below 90° and $d_{H···O}$ increases above ~3.0 Å, and no clear separation between these regions can be observed. It is shown that the scatterplots of $\alpha_{OH···O}$ against $d_{H···O}$ and $d_{O···O}$ are equivalent if variations of the covalent bond length d_{OH} are neglected.

Introduction

The geometry of O—H···O hydrogen bonds has repeatedly been analyzed based on accurate neutron crystallographic data, see Olovsson & Jönsson (1976); Ceccarelli, Jeffrey & Taylor (1981); Taylor & Kennard (1984); Savage (1986); Jeffrey & Saenger (1991). The hydrogen bonds are usually described by the distances O—H, H···O, O···O (d_{OH}, $d_{H···O}$, $d_{O···O}$) and the angle O—H···O ($\alpha_{OH···O}$), Fig. 1. As a rule of thumb, an O—H···O interaction was formerly called a hydrogen bond, if the H···O separation was significantly shorter than the sum of the van der Waals