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Structure of 14β-Hydroxyprogesterone

BY CONNIE K. CHO AND ANTHONY S. SECCO*

Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

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Abstract

14β-Hydroxy-4-pregnene-3,20-dione, $C_{21}H_{30}O_3$, $M_r = 330{\cdot}47$, monoclinic, $P2_1$, $a = 11{\cdot}831\ (3)$, $b = 8{\cdot}096\ (2)$, $c = 18{\cdot}696\ (6)$ Å, $\beta = 91{\cdot}38\ (2)°$, $V = 1790{\cdot}3\ (8)$ Å^3, $Z = 4$, D_m(flotation) = 1·225, $D_x = 1{\cdot}226$ g cm^{-3}, λ(Mo $K\alpha$) = 0·71069 Å, $\mu = 0{\cdot}86$ cm^{-1}, $F(000) = 720$, $T = 294$ K, $R = 0{\cdot}036$ for 1588 reflections with $I \geq 3\sigma(I)$. The structures of both conformers (I) and (II) in the asymmetric unit resemble the typical cardiac glycoside digitoxigenin, with *cis C/D* ring junctions. *B* and *C* rings are in chair conformations. Both *A* rings are between sofa and half-chair conformations, with the 3-carbonyl O atom bent out of the ring plane. The *D* ring of (I) exists primarily as a half-chair stabilized by *intra*-molecular hydrogen bonding between O(14) and O(20), whereas the *D* ring of (II) is a deep envelope stabilized by *inter*molecular hydrogen bonding between O(14) and O(14)′. The C(16)—C(17)—C(20)—O(20) torsion angle is equal to −46·8°, similar to the majority of other progestins, while C(16)′—C(17)′—C(20)′—O(20)′ has an unusual torsion angle of 168·8° which is a probable result of crystal packing forces. The relative spatial displacements of O(20) and O(20)′ from O(4) of digitoxigenin are 2·88 and 2·87 Å, respectively, which are shorter than expected based on receptor affinity.

Introduction

14β-Hydroxyprogesterone is the first semisynthetic analog of hydroxyprogesterone which induces

* To whom all correspondence should be addressed.

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cardiac glycoside (CG)-like activity in isolated heart muscle (Templeton, Sashi Kumar, Cote, Bose, Elliot, Kim & LaBella, 1987). CGs such as digitalis, of plant origin, are foreign to mammalian systems, and many steroid derivatives have been assayed in order to categorize the endogenous digitalis-like hormone which is the putative natural target molecule for the digitalis receptor (Chow, Kim, LaBella & Queen, 1979; Duax, Cody, Griffin, Hazel & Weeks, 1978; Duax, Cody & Hazel, 1977; Hnatowich & LaBella, 1984; Kim, LaBella, Zunza, Zunza & Templeton, 1980; Tanz, 1963). The steroid nature of the CG skeleton suggests that the endogenous hormone is likely to be a steroid as well. Mammalian steroids, which are planar and have no CG activity, may be enzymatically modified *in vivo* to approximate the characteristic globular CG structure in which *cis A/B* and *C/D* ring junctions cause the *A* and *D* rings to lie almost perpendicular to the *B* and *C* rings (LaBella, Bihler, Templeton, Kim, Hnatowich & Rohrer, 1985). The crystal structures of a number of compounds which show CG-like activity have been compared and a close relationship between the activity of the compounds and several structural features has been observed (Fullerton, Kitatsuji, Deffo, Rohrer, Ahmed & From, 1983). The crystal and molecular structures of 14β-hydroxyprogesterone were determined in an effort to provide some explanation for its activity and to furnish information in identifying more effective endogenous digitalis-like hormones.

Experimental

A colorless prismatic crystal of dimensions 0·4 × 0·2 × 0·2 mm was used for data collection. Data were collected on a Nicolet *R3m* diffractometer by an $\omega/2\theta$ scan mode, and unit-cell parameters determined by least-squares refinement of 25 reflections. 1914 reflections ($-12 \le h \le 12$, $0 \le k \le 8$, $0 \le l \le 18$) collected; crystal quality limited the 2θ range to 2–40°; data corrected for Lorentz–polarization effects, resulting in 1838 unique reflections; $R_{int} = 0{\cdot}023$. Three standard reflections monitored during data collection showed no significant intensity variation after 48 h. The structure was solved by the direct methods program *MULTAN*87 (Debaerdemaeker, Germain, Main, Tate & Woolfson, 1987) by increasing the number of *E*'s used to 600 from the default 350 and using a randomly oriented fragment. Two independent molecules in the asymmetric unit were revealed. All non-H atoms appeared in the solution and were initially refined isotropically by full-matrix least-squares refinement using the 1588 reflections with $I \ge 3\sigma(I)$. Further refinement with anisotropic temperature factors and subsequent difference Fourier maps revealed positions for the H atoms, but their coordinates would not refine, so all H atoms except those on the hydroxyl groups were placed in calculated positions for the final two cycles without refinement. The two hydroxyl H atoms were located on a difference Fourier map, and inserted without refinement. All H atoms were assigned isotropic temperature factors twice those of their attached atoms. After the final cycle of refinement, $R = 0{\cdot}047$ and $wR = 0{\cdot}044$ for all 1838 reflections while $R = 0{\cdot}036$ and $wR = 0{\cdot}044$ for the 1588 observed reflections used [where $R = \sum||F_o| - k|F_c||/\sum|F_o|$; $wR = [\sum w(|F_o| - k|F_c|)^2/\sum w|F_o|^2]^{1/2}$: $w = 1$ for $F < 22{\cdot}8$, $w = 22{\cdot}8/F$ for $F \ge 22{\cdot}8$]; the function minimized $w(|F_o| - k|F_c|)^2$, where k is a scale factor; the goodness of fit $S = 0{\cdot}623$; $\Delta/\sigma = 0{\cdot}049$; the isotropic extinction coefficient $g = 2{\cdot}1\ (6) \times 10^4$ (Coppens & Hamilton, 1970). The final difference map was essentially featureless, with maximum and minimum residuals of 0·171 and $-0{\cdot}156$ e Å^{-3}, respectively. The refinement results of the other enantiomer were not significantly different. Atomic scattering factors for non-H atoms were from Cromer & Mann (1968) and from Stewart, Davidson & Simpson (1965) for H atoms.* Final atomic coordinates and thermal factors of non-H atoms are given in Table 1.† The two molecules in the asymmetric unit are shown in Fig. 1, and entire unit-cell contents are shown in Fig. 2.

Discussion

In the crystalline state, 14β-hydroxyprogesterone adopts two unique conformations, (I) and (II) (Fig. 1), both of which resemble the crescent or globular shape of a typical CG more than the essentially planar steroid skeleton of progesterone (Campsteyn, Dupont & Dideberg, 1972; Serantoni, Krajewski, Mongiorgi, Riva di Sanseverino & Cameroni, 1975). The crescent shape was expected in view of the compound's CG-like effects on cardiac tissue (Templeton *et al.*, 1987). Structural elements required for the drug to bind to the receptor are strictly defined (Guntert & Linde, 1981) and primarily involve the *A* and *D* rings, specifically the functional groups attached to these rings. Concerted

* Computation was carried out on the University of Manitoba Computer Services Department's Amdahl 5870 main-frame computer using locally written programs for processing and modified versions of the following programs for structure solution, refinement and calculations: *FORDAP* (A. Zalkin, unpublished), *ORFLS* (Busing, Martin & Levy, 1962), *ORFFE* (Busing, Martin & Levy, 1964), *ORTEP*II (Johnson, 1976).

† Lists of structure factors, anisotropic thermal parameters, bond distances and angles involving H atoms, torsion angles, and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 53829 (35 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

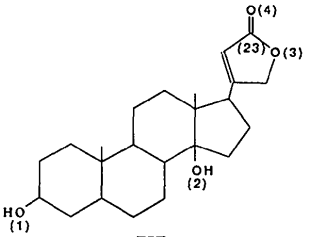
Table 1. *Final positional parameters (fractional* $\times 10^4$*) and equivalent isotropic thermal parameters* ($Å^2 \times 10^3$) *with e.s.d.'s in parentheses*

U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

	x	*y*	*z*	U_{eq}
C(1)	4620 (5)	1009	8566 (4)	62
C(2)	4917 (6)	2257 (16)	9148 (4)	57
C(3)	4186 (7)	3726 (15)	9092 (4)	54
O(3)	4517 (6)	5111 (14)	9249 (4)	96
C(4)	2990 (6)	3417 (14)	8892 (4)	50
C(5)	2614 (5)	1941 (14)	8662 (3)	39
C(6)	1375 (5)	1677 (13)	8523 (4)	48
C(7)	1154 (5)	1023 (14)	7767 (4)	47
C(8)	1831 (5)	−541 (13)	7624 (3)	34
C(9)	3092 (5)	−194 (14)	7771 (3)	40
C(10)	3367 (5)	479 (14)	8533 (3)	40
C(11)	3797 (6)	−1702 (15)	7576 (4)	58
C(12)	3566 (5)	−2284 (15)	6807 (4)	59
C(13)	2314 (5)	−2732 (14)	6667 (3)	41
C(14)	1564 (5)	−1253 (13)	6877 (3)	38
O(14)	393 (3)	−1734 (12)	6897 (2)	48
C(15)	1707 (6)	−32 (14)	6260 (4)	49
C(16)	1868 (7)	−1086 (15)	5590 (4)	65
C(17)	2136 (5)	−2863 (15)	5838 (4)	48
C(18)	2034 (7)	−4342 (14)	7061 (4)	58
C(19)	3160 (6)	−819 (14)	9117 (4)	57
C(20)	1229 (7)	−4071 (15)	5601 (4)	55
O(20)	232 (5)	−3736 (13)	5683 (3)	74
C(21)	1555 (7)	−5657 (16)	5272 (5)	79
C(1)′	6483 (7)	−8018 (14)	6896 (4)	58
C(2)′	5898 (7)	−8759 (17)	6241 (4)	71
C(3)′	5193 (7)	−7500 (16)	5867 (4)	65
O(3)′	4265 (5)	−7804 (14)	5593 (3)	104
C(4)′	5703 (6)	−5863 (14)	5808 (4)	52
C(5)′	6653 (5)	−5416 (14)	6168 (3)	41
C(6)′	7182 (6)	−3747 (14)	6053 (3)	49
C(7)′	7273 (5)	−2803 (13)	6753 (3)	41
C(8)′	7903 (5)	−3770 (13)	7337 (3)	32
C(9)′	7381 (5)	−5471 (13)	7433 (3)	36
C(10)′	7227 (5)	−6503 (13)	6729 (3)	39
C(11)′	8026 (6)	−6418 (13)	8029 (3)	50
C(12)′	8030 (6)	−5437 (14)	8726 (3)	46
C(13)′	8568 (5)	−3727 (13)	8665 (3)	33
C(14)′	7995 (5)	−2792 (13)	8034 (3)	29
O(14)′	8603 (3)	−1280 (12)	7904 (2)	39
C(15)′	6857 (5)	−2219 (14)	8326 (3)	43
C(16)′	6968 (5)	−2265 (14)	9152 (3)	47
C(17)′	8210 (5)	−2706 (13)	9330 (3)	39
C(18)′	9845 (5)	−3887 (14)	8592 (3)	49
C(19)′	8371 (6)	−7105 (14)	6438 (4)	54
C(20)′	8958 (6)	−1252 (14)	9524 (3)	45
O(20)′	9902 (5)	−1494 (12)	9777 (3)	75
C(21)′	8566 (7)	493 (14)	9438 (4)	58

motions appear to be needed, as appropriate parameters in one region alone are not sufficient to account for all activity; both (I) and (II) in the crystalline state exhibit the conformational requirements, but both also have regions that do not conform. These structural deviations may provide a partial explanation for the compound's relatively weak affinity (about one-tenth that of ouabagenin) for the digitalis receptor, Na^+,K^+-ATPase, inferred from inhibition of [^{3}H]ouabain binding to cardiac tissue (Templeton *et al.*, 1987).

In Fig. 3, both structures of 14β-hydroxyprogesterone are superimposed on the crystal structure of digitoxigenin, (III) (Karle & Karle, 1969), a CG derived from a component of digitalis. A least-squares fit of the *B* and *C* rings to the corresponding atoms in digitoxigenin shows good correlation for both molecules, with the r.m.s. deviation of 0·029 Å for the C(5)···C(14) atoms, slightly less than the 0·055 Å for the C(5)′···C(14)′ atoms. Bond lengths and angles are generally normal in (I) and (II); the largest deviations occurring between the two structures are found in the *B* ring (Table 2). All *B* and *C* rings are in the chair conformation.



(III)

The *A*/*B* ring junctions are quasi-*trans* in both (I) and (II), as in other progestins, and have similar endocyclic angles. Asymmetry parameters of the *A* ring (Duax & Norton, 1975) are: $\Delta C_s(1) = 20{\cdot}2$, $\Delta C_2(1,2) = 0{\cdot}9$; $\Delta C_s(1)' = 24{\cdot}1$, $\Delta C_2(1,2)' = 2{\cdot}6°$, *indicating that both A* rings tend toward the 1α,2β-half-chair instead of the 1α-sofa. Both rings are in the normal conformation, with the 2β H atom axial. The C(2)—C(3) and C(2)′—C(3)′ bonds, 1·47 (1) and 1·48 (1) Å respectively, are close to the observed average of 1·49 Å. The torsion angles O(3)—C(3)—C(4)—C(5) of 174·6 and O(3)′—C(3)′—C(4)′—C(5)′ of 171·9° (Table 3) indicate less than perfect conjugation. The deviation from planarity has been found

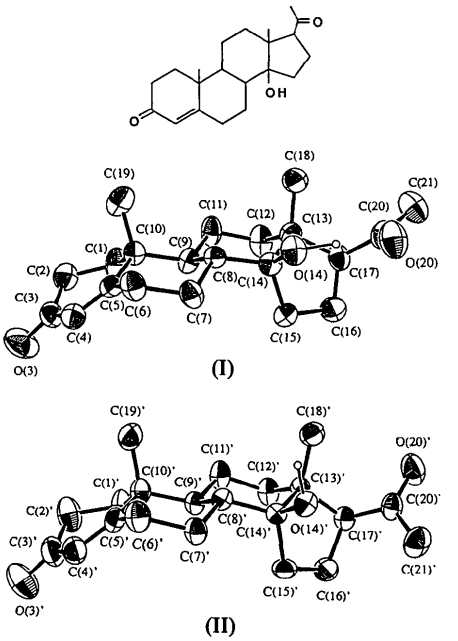


Fig. 1. Schematic drawing of 14β-hydroxyprogesterone and *ORTEP* (Johnson, 1976) plots of molecules (I) and (II) with ellipsoids at 50% probability level. H atoms other than those on hydroxyl groups are omitted for clarity.

to be generally small in a number of steroids (Duax & Norton, 1975) compared with the values observed in the title compound, which lie at the extreme of the previously observed range. While neither the *A* ring of (I) nor (II) approaches the chair form with a *cis A/B* junction (as in the hydroxylated *A* ring of digitoxigenin), both show increased downward displacement [*i.e.* in the α direction, opposite to the orientation of the C(18) and C(19) methyl groups], compared to either form of progesterone. The 3-carbonyl O atom is considerably bent away from the plane of the ring, with O(3)′ bent more than O(3). This out-of-plane bending is also demonstrated, to a lesser extent, by chlormadinone acetate (Chandross & Bordner, 1975), the most potent semisynthetic analog of digitoxigenin discovered so far (LaBella *et al.*, 1985). The extrusion of the 3-carbonyl group may be of importance in determining the compound's biological activity, by bringing it into a more favorable position for hydrogen bonding to the digitalis receptor. It is not entirely clear what is responsible for this pucker of the *A* ring, or why the two asymmetric molecules should show different degrees of bending. The only intermolecular contact of note for either molecule is the O(14)···H—O(14)′ hydrogen bond, which appears to have a significant effect on the *D* ring (see below).

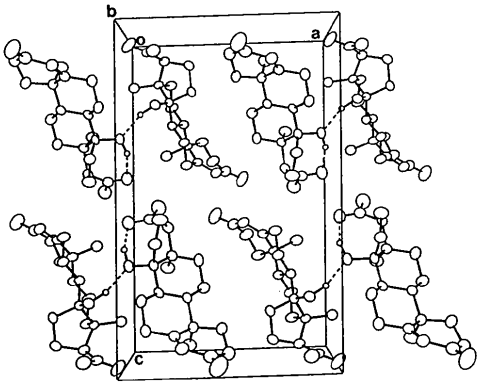


Fig. 2. Packing diagram of unit cell viewed down *b* axis (into page), showing hydrogen bonding.

Long-range intramolecular effects may be responsible for the conformation of the *A* ring. 17α-Acetoxy substitution restricts the freedom of the C(17) side chain and induces a 1α-sofa conformation (Duax *et al.*, 1977), while *B*-ring substitution in the 6α position induces formation of a 1α,2β-half-chair (Duax *et al.*, 1978), but it is not clear whether 14β-hydroxylation has similar effects. The overall downward displacement of (II) is greater than that of (I), both as a result of increased *A*-ring pucker and out-of-plane distortion of O(3)′. The actual shape of the ring may not be significant provided that the functional groups are aligned properly. Despite the radically different conformations of the *A* rings of 14β-hydroxyprogesterone and digitoxigenin, O(3) and O(3)′ are positioned very similarly to O(1) of (III), with separations between O atoms of 0·62 and 0·73 Å for O(3)···O(1)$_{III}$ and O(3)′···O(1)$_{III}$, respectively. However, the orientations of the particular groups are quite different, which may limit binding to highly specific enzymes such as Na^+,K^+-ATPase.

The conformational differences between (I) and (II) may be due to the different hydrogen bonding experienced by the two molecules. O(14)(x,y,z) of

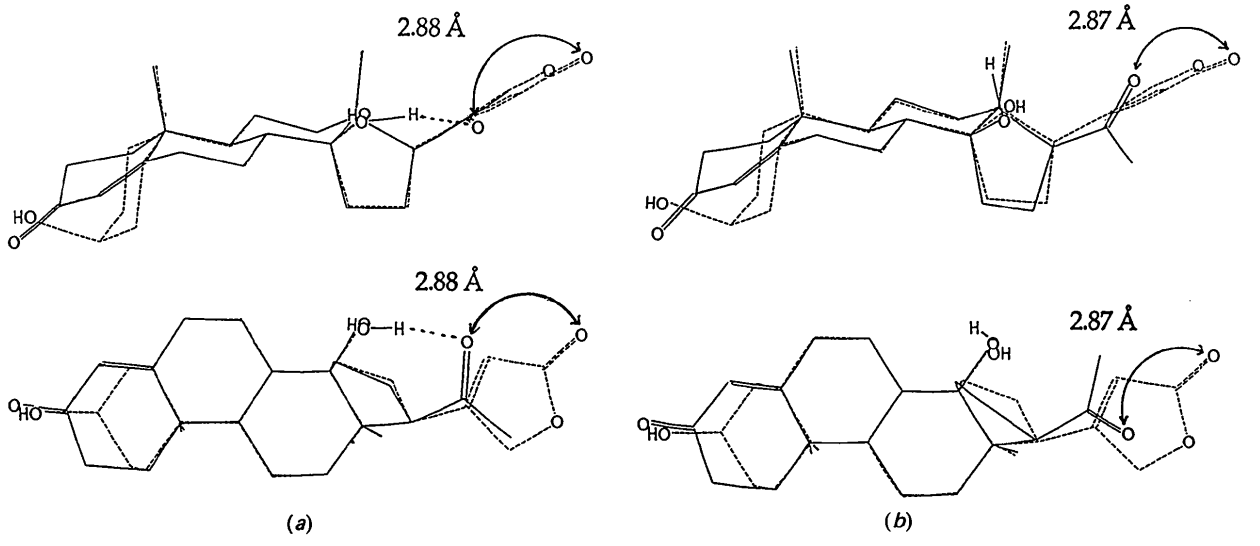


Fig. 3. Orthogonal projections showing superposition of *B* and *C* ring atoms of digitoxigenin and (*a*) conformer (I) and (*b*) conformer (II) of 14β-hydroxyprogesterone [least-squares fit through atoms C(5)···C(14)]. Solid line: 14β-hydroxyprogesterone. Dashed line: digitoxigenin.

Table 2. *Bond lengths* (Å) *and angles* (°) *with e.s.d.'s in parentheses*

C(1)—C(2)	1·519 (10)	C(1)′—C(2)′	1·517 (10)
C(1)—C(10)	1·543 (9)	C(1)′—C(10)′	1·546 (10)
C(2)—C(3)	1·473 (12)	C(2)′—C(3)′	1·481 (12)
C(3)—O(3)	1·220 (10)	C(3)′—O(3)′	1·226 (9)
C(3)—C(4)	1·476 (11)	C(3)′—C(4)′	1·461 (11)
C(4)—C(5)	1·342 (10)	C(4)′—C(5)′	1·345 (9)
C(5)—C(6)	1·499 (9)	C(5)′—C(6)′	1·506 (10)
C(5)—C(10)	1·504 (10)	C(5)′—C(10)′	1·517 (9)
C(6)—C(7)	1·527 (9)	C(6)′—C(7)′	1·519 (10)
C(7)—C(8)	1·525 (9)	C(7)′—C(8)′	1·524 (9)
C(8)—C(9)	1·537 (8)	C(8)′—C(9)′	1·522 (9)
C(9)—C(10)	1·553 (9)	C(9)′—C(10)′	1·566 (9)
C(9)—C(11)	1·527 (10)	C(9)′—C(11)′	1·540 (9)
C(10)—C(19)	1·539 (10)	C(10)′—C(19)′	1·549 (10)
C(11)—C(12)	1·530 (10)	C(11)′—C(12)′	1·526 (10)
C(12)—C(13)	1·541 (9)	C(12)′—C(13)′	1·529 (10)
C(13)—C(14)	1·547 (9)	C(13)′—C(14)′	1·544 (8)
C(13)—C(17)	1·564 (9)	C(13)′—C(17)′	1·560 (9)
C(13)—C(18)	1·537 (10)	C(13)′—C(18)′	1·526 (8)
C(14)—O(14)	1·440 (7)	C(14)′—O(14)′	1·444 (8)
C(14)—C(15)	1·532 (9)	C(14)′—C(15)′	1·537 (8)
C(15)—C(16)	1·531 (11)	C(15)′—C(16)′	1·548 (9)
C(16)—C(17)	1·542 (12)	C(16)′—C(17)′	1·541 (9)
C(17)—C(20)	1·510 (10)	C(17)′—C(20)′	1·512 (10)
C(20)—O(20)	1·223 (8)	C(20)′—O(20)′	1·219 (8)
C(20)—C(21)	1·480 (12)	C(20)′—C(21)′	1·494 (11)
O(14)—H[O(14)]	1·102*	O(14)′—H[O(14)′]	1·074*
C(2)—C(1)—C(10)	114·7 (6)	C(2)′—C(1)′—C(10)′	113·6 (6)
C(1)—C(2)—C(3)	111·2 (6)	C(1)′—C(2)′—C(3)′	110·5 (8)
C(2)—C(3)—O(3)	122·8 (7)	C(2)′—C(3)′—O(3)′	123·2 (9)
C(2)—C(3)—C(4)	116·0 (7)	C(2)′—C(3)′—C(4)′	115·5 (7)
O(3)—C(3)—C(4)	121·1 (8)	O(3)′—C(3)′—C(4)′	121·2 (9)
C(3)—C(4)—C(5)	122·7 (7)	C(3)′—C(4)′—C(5)′	123·2 (7)
C(4)—C(5)—C(6)	119·9 (6)	C(4)′—C(5)′—C(6)′	120·9 (6)
C(4)—C(5)—C(10)	124·0 (6)	C(4)′—C(5)′—C(10)′	123·0 (7)
C(6)—C(5)—C(10)	116·1 (6)	C(6)′—C(5)′—C(10)′	116·0 (5)
C(5)—C(6)—C(7)	110·8 (5)	C(5)′—C(6)′—C(7)′	110·4 (5)
C(6)—C(7)—C(8)	111·7 (6)	C(6)′—C(7)′—C(8)′	112·5 (6)
C(7)—C(8)—C(9)	109·2 (5)	C(7)′—C(8)′—C(9)′	110·9 (5)
C(8)—C(9)—C(10)	114·1 (5)	C(8)′—C(9)′—C(10)′	115·0 (5)
C(8)—C(9)—C(11)	110·2 (6)	C(8)′—C(9)′—C(11)′	109·9 (5)
C(10)—C(9)—C(11)	113·3 (5)	C(10)′—C(9)′—C(11)′	112·9 (6)
C(1)—C(10)—C(5)	110·3 (6)	C(1)′—C(10)′—C(5)′	110·6 (5)
C(1)—C(10)—C(9)	108·3 (5)	C(1)′—C(10)′—C(9)′	107·9 (5)
C(1)—C(10)—C(19)	109·3 (6)	C(1)′—C(10)′—C(19)′	109·1 (6)
C(5)—C(10)—C(9)	108·2 (5)	C(5)′—C(10)′—C(9)′	108·2 (5)
C(5)—C(10)—C(19)	108·6 (5)	C(5)′—C(10)′—C(19)′	108·7 (5)
C(9)—C(10)—C(19)	112·2 (6)	C(9)′—C(10)′—C(19)′	112·2 (5)
C(9)—C(11)—C(12)	112·6 (6)	C(9)′—C(11)′—C(12)′	110·5 (6)
C(11)—C(12)—C(13)	112·4 (6)	C(11)′—C(12)′—C(13)′	113·6 (5)
C(12)—C(13)—C(14)	109·2 (6)	C(12)′—C(13)′—C(14)′	109·0 (5)
C(12)—C(13)—C(17)	106·9 (5)	C(12)′—C(13)′—C(17)′	107·4 (5)
C(12)—C(13)—C(18)	109·6 (6)	C(12)′—C(13)′—C(18)′	110·1 (6)
C(14)—C(13)—C(17)	103·9 (6)	C(14)′—C(13)′—C(17)′	103·1 (5)
C(14)—C(13)—C(18)	113·8 (5)	C(14)′—C(13)′—C(18)′	113·0 (5)
C(17)—C(13)—C(18)	113·1 (6)	C(17)′—C(13)′—C(18)′	113·8 (5)
C(13)—C(14)—O(14)	110·8 (5)	C(13)′—C(14)′—O(14)′	109·4 (5)
C(13)—C(14)—C(15)	103·5 (5)	C(13)′—C(14)′—C(15)′	104·4 (5)
O(14)—C(14)—C(15)	108·6 (5)	O(14)′—C(14)′—C(15)′	104·3 (5)
C(14)—C(15)—C(16)	105·9 (6)	C(14)′—C(15)′—C(16)′	107·1 (5)
C(15)—C(16)—C(17)	107·7 (6)	C(15)′—C(16)′—C(17)′	106·1 (5)
C(13)—C(17)—C(16)	104·8 (6)	C(13)′—C(17)′—C(16)′	103·1 (5)
C(13)—C(17)—C(20)	114·4 (6)	C(13)′—C(17)′—C(20)′	115·8 (5)
C(16)—C(17)—C(20)	112·1 (6)	C(16)′—C(17)′—C(20)′	114·9 (6)
C(17)—C(20)—O(20)	120·0 (7)	C(17)′—C(20)′—O(20)′	119·6 (7)
C(17)—C(20)—C(21)	119·6 (7)	C(17)′—C(20)′—C(21)′	122·1 (6)
O(20)—C(20)—C(21)	120·4 (8)	O(20)′—C(20)′—C(21)′	118·3 (7)
C(14)—O(14)—H[O(14)]	113·9*	C(14)′—O(14)′—H[O(14)′]	109·9*

* Calculated from unrefined hydrogen coordinates.

Table 3. *Selected torsion angles* (°) *with e.s.d.'s in parentheses*

O(3)—C(3)—C(4)—C(5)	174·6 (7)	O(3)′—C(3)′—C(4)′—C(5)′	171·9 (7)
C(17)—C(13)—C(14)—C(15)	−37·9 (6)	C(17)′—C(13)′—C(14)′—C(15)′	−36·7 (6)
C(15)—C(16)—C(17)—C(13)	−8·8 (8)	C(15)′—C(16)′—C(17)′—C(13)′	−27·8 (7)
C(14)—C(15)—C(16)—C(17)	−14·8 (8)	C(14)′—C(15)′—C(16)′—C(17)′	5·3 (8)
C(14)—C(13)—C(17)—C(16)	28·8 (7)	C(14)′—C(13)′—C(17)′—C(16)′	39·9 (6)
C(13)—C(14)—C(15)—C(16)	32·7 (6)	C(13)′—C(14)′—C(15)′—C(16)′	19·6 (7)
C(13)—C(17)—C(20)—O(20)	72·4 (9)	C(13)′—C(17)′—C(20)′—O(20)′	−71·1 (8)
C(13)—C(17)—C(20)—C(21)	−107·5 (8)	C(13)′—C(17)′—C(20)′—C(21)′	111·0 (7)
C(16)—C(17)—C(20)—O(20)	−46·8 (9)	C(16)′—C(17)′—C(20)′—O(20)′	168·8 (6)
C(16)—C(17)—C(20)—C(21)	133·4 (8)	C(16)′—C(17)′—C(20)′—C(21)′	−9·2 (9)

conformer (I) is involved in intramolecular hydrogen bonding to O(20) and intermolecular hydrogen bonding to O(14)′($x-1,y,z$) of conformer (II), whereas O(14)′ experiences only the intermolecular hydrogen bond (Fig. 2). H[O(14)]···O(20) and O(14)···H[O(14)′] have distances of 1·74 and 1·83 Å, respectively. The O(14)···H[O(14)′]—O(14)′ angle is 161·5 while the O(14)—H[O(14)]···O(20) angle is 158·1°.

Although angles involving C(14) of the *C/D* ring junction differ by up to 4°, C(14)—O(14) and C(14)′—O(14)′ are similarly oriented with respect to each other and C(14)—O(2) of digitoxigenin. The *C/D* ring junctions in (I) and (II) are fully *cis*, as in digitoxigenin, illustrating the observed importance of 14β-hydroxyl substitution in CG activity (LaBella *et al.*, 1985). The rest of the *D* ring is very different, however, in (I) and (II) as demonstrated by the appropriate asymmetry parameters: $\Delta C_s(14) = 10{\cdot}6$, $\Delta C_2(16) = 5{\cdot}1$; $\Delta C_s(13)' = 6{\cdot}2$, $\Delta C_2(16)' = 27{\cdot}5°$. The pseudorotation parameter Δ (Altona, Geise & Romers, 1968), which indicates overall asymmetry of the ring, has values Δ(I) = −9·7, Δ(II) = 50·6°. The *D* ring of (I) exists primarily in the half-chair conformation with a slight tendency towards the 14-envelope; as shown in Fig. 3 the *D* ring of (I) is superimposed with almost perfect registry onto the *D* ring of digitoxigenin. In (II), however, the 13-envelope conformation is pronounced. The torsion angles C(14)—C(15)—C(16)—C(17) and C(14)′—C(15)′—C(16)′—C(17)′, −14·8 (8) and 5·3 (8)° respectively, differ most in that they switch from −*syn* to +*syn* between the two structures; other corresponding torsion angles in the *D* rings also differ considerably but do not demonstrate this inversion from −*syn* to +*syn*. Endocyclic bond angles are similar in the two structures and are generally close to the average values for progestins except at the *C/D* ring junction – C(14)—C(13)—C(17) and C(14)′—C(13)′—C(17)′ angles of 103·9 (6) and 103·1 (5)°, respectively, are 4° larger than average, as are C(17)—C(13)—C(18) at 113·1 (6) and C(17)′—C(13)′—C(18)′ at 113·8 (5)°.

The existence in the crystalline state of two minimum energy conformations does not appear to be consistent with solution data. ^{1}H NMR studies of the title structure show a strong peak arising from a proton involved in hydrogen bonding (Templeton *et al.*, 1987). The existence of a single hydrogen bond in solution would suggest that only molecule (I) is present in the solution, stabilized by the intramolecular hydrogen bond, while the intermolecular O(14)···H[O(14)′] bond is an artifact of crystal pack-

ing which induces an alternative conformation of the molecule, *i.e.* (II). Static energy calculations* reveal that, while (I) is indeed a lower energy conformation than (II), the difference is minimal.

Since there is no bulky 17α substituent, such as an acetoxy group, one would expect the 17β side chain to have almost free rotation, as suggested by an examination of Dreiding models of the title compound. This expectation is contradicted by crystallographic data on other unhindered progestins, which reveal that the side chains are generally restricted to a common range (Duax & Norton, 1975). Energy calculations of rotation about C(17)—C(20) (*PCMODEL*; rigid-rotor approximation) on (I) support the previous crystallographic results, demonstrating that two energy barriers exist to discourage free rotation about the C(17)—C(20) bond of (I). The energy profile is similar to that seen for 16β-methylprogesterone (Schmit & Rousseau, 1978). A large barrier of over 962 kJ mol^{-1} (230 kcal mol^{-1}) appears to block the counterclockwise [as viewed along C(20)—C(17)] rotation of the side chain of (I) to the orientation in (II); in this pathway, repulsion occurs between the H atoms of C(21) and those of C(18) and O(14). The barrier in the clockwise rotation is much smaller, at approximately 146 kJ mol^{-1} (35 kcal mol^{-1}). Even this latter energy barrier requires that the pathway between the two observed orientations include a concomitant change in the *D* ring [although not to the same extent exhibited in (II)]. Calculations of energy barriers to free rotation in the 'unconstrained' molecule yield values of 33·5 and 18·8 kJ mol^{-1} (8·0 and 4·5 kcal mol^{-1}) for the counterclockwise and clockwise rotations of the side chain, respectively. The C(16)—C(17)—C(20)—O(20) torsion angle, τ, of $-46{\cdot}8°$ [molecule (I)] is not exceptional, but a τ value of 168·8° [molecule (II)] is very unusual. It would appear that while in solution 14β-hydroxyprogesterone is stabilized by hydrogen bonding of the more common side chain, crystal packing forces cause the side chain of conformer (II) to adopt the uncommon orientation as the intramolecular hydrogen bond is disrupted and the intermolecular hydrogen bond is formed. This finding is quite contrary to earlier conclusions regarding crystal packing forces on side-chain orientation in 17β-pregnanes (Duax, Griffin & Rohrer, 1981). None of those steroids studied had the 14β-OH group, whose effects appear to be as influential as those attributed to 16β-substitution (Duax *et al.*, 1981). Other steric effects may also have an influence on stabilization of the C(17)′ side chain. The C(18) methyl group is 3·35 (1) Å from O(20), so it is unlikely that any contact has a significant effect. C(18)′, however, is only 2·94 (1) Å from O(20)′, close enough for a possible C—H···O interaction (Berkovitch-Yellin & Leiserowitz, 1984). Unfortunately, attempts to refine H-atom coordinates from the difference maps were unsuccessful, probably owing to an insufficient observation to variable ratio.

The 14β-OH group's influence on the side chain plays a dominant role in the activity of the title compound. There has been a close relationship observed between the Na^+,K^+-ATPase binding of a number of digitalis-like hormones and the relative separation of the 20-carbonyl O atom of the hormone from O(4) of digitoxigenin (Fullerton *et al.*, 1983; LaBella *et al.*, 1985). Activity also appears to be linked to a preferred orientation of the carbonyl O atom, which presumably causes correct alignment with the receptor binding site. In Fig. 3 it can be seen that neither C(20)—O(20) nor C(20)′—O(20)′ lie in favorable (*i.e.* parallel) orientations with respect to C(23)—O(4) of digitoxigenin. In fact, they appear almost symmetrically distributed around C(23)—$O(4)_{III}$. The relative displacements are also very similar, with an O(3)···$O(4)_{III}$ distance of 2·88 and an O(3)′···$O(4)_{III}$ distance of 2·87 Å. This quasi-symmetry may conceivably be exploited by the cognate enzyme, which might interconvert the structures, depending on which conformation is actually responsible for the activity. The displacements are surprisingly short in view of the compound's fairly low measured activity. The observed trend has been that the shorter the separation relative to digitoxigenin, the greater the inhibition. The unfavorable orientations of the carbonyl groups account, in part, for the unexpectedly low affinity; there may be a direct relationship between the angle offsetting the 17β side chain from the digitoxigenin group and the observed activity.

In conclusion, the crystal structure, which shows an intriguing combination of favorable and unfavorable aspects, cannot entirely account for the activity of the title compound. Interesting observations and questions arise from the structural effect of 14β-hydroxyl substitution; while necessary for CG activity, such substitution also induces a unique orientation in the 17β side chain that may inhibit binding. It is also not clear which minimum energy conformation is the biologically active conformation. However, 14β-hydroxyprogesterone is but the first in the series of CG-like progesterone congeners to be characterized, and further work toward characterizing an endogenous digitalis-like hormone, in a variety of similarly substituted progestins, is

* All energy calculations were carried out on a Silicon Graphics IRIS 4D70/GT workstation using the program *PCMODEL* (Serena Software, 1989) with default parameters. Calculations with the rigid-rotor approximation involved rotating τ, the C(16)—C(17)—C(20)—O(20) torsion angle, through 360° in steps of 8°; the unconstrained energies were obtained by fixing τ in 10° increments and minimizing the energy of the remainder of the molecule at each step (*i.e.* allowing the molecule to relax).

suggested by this initial study. Possible structures to be considered might be those with either the O(20) or O(14) position protected from intramolecular hydrogen bonding, or converted into more complex substituents with additional carbonyl or hydroxyl groups which could also serve as critical functional groups. Any negative effects of poor orientation of the 17β side chain might perhaps be mitigated by increasing the outward displacement (relative to the main body of the steroid) of the keto group, the effect of which has been seen to be increased enzyme inhibition (Fullerton *et al.*, 1983).

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Dihydropyran Ring Conformations. I. Structures of 2-Methoxy- and 2-Hydroxy-2,4-dimethyl-3,4-dihydro-2*H*,5*H*-pyrano[3,2-*c*][1]benzopyran-5-ones

BY EDWARD J. VALENTE

Department of Chemistry, Mississippi College, Clinton, MS 39058, *USA*

AND DRAKE S. EGGLESTON

Department of Physical and Structural Chemistry, Smith, Kline and French Laboratories, King of Prussia, PA 19406, *USA*

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Abstract

Conformations of embedded 3,4-dihydro-2*H*-pyrans (DHP's) are studied in a closely related series of nine molecular structures, including the *cis* and *trans* isomers of both racemic and resolved homologs of 2,4-dimethyl-3,4-dihydro-2*H*,5*H*-pyrano[3,2-*c*][1]-benzopyran-5-one. DHP rings in these structures

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