Crystal and Molecular Structure of 17α-Hydroxyandrost-4-en-3-one (Epitestosterone)

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Crystals of the title steroid are orthorhombic, $a = 11{\cdot}423(1)$, $b = 20{\cdot}151(1)$, $c = 7{\cdot}003(1)$ Å, $Z = 4$, space group $P2_12_12_1$. The structure has been determined by direct methods from diffractometer data and refined by full-matrix least-squares to a final R of 0·058 for 1646 reflections. All the ring junctions are *trans/trans*. The conformation of ring A is half-chair while that of rings B and C is chair. The cyclopentane ring D is a slightly distorted half-chair. The molecules are linked in the crystal structure ' head-to-tail ' by a hydrogen bond between the C(17) hydroxy-group and the C(3) keto-group.

KORENMAN *et al.*[1] have identified epitestosterone in human urine and have shown that it is not derived from the peripheral metabolism of androstenolone, androstenedione, or testosterone. The biological activity of epitestosterone is *ca.* 25 times less than that of testosterone which acts as a powerful androgen and is responsible for the male sex characteristics. The preference for the β-orientation of the 17-hydroxy-group is a common feature of the biologically active estrogens and androgens.[2] To investigate this characteristic we have undertaken the determination of the structures of unsubstituted testosterone and epitestosterone. We report here the structure of epitesterone.

EXPERIMENTAL

Crystal Data.—$C_{19}H_{28}O_2$, $M = 288$. Orthorhombic, $a = 11{\cdot}423(1)$, $b = 20{\cdot}151(1)$, $c = 7{\cdot}003(1)$ Å, $U = 611{\cdot}9(1)$ Å^3, $D_m = 1{\cdot}14$ (gradient column), $Z = 4$, $D_c = 1{\cdot}19$, $F(000) = 632$. Cu-K_α radiation, $\lambda = 1{\cdot}5418$ Å; $\mu(\text{Cu-}K_\alpha) = 5{\cdot}89$ cm^{-1}. Space group $P2_12_12_1$ from systematic absences.

Epitestosterone was obtained from Steraloids Limited, Croydon, Surrey. The crystal used for data collection was a rectangular prism (0·48 × 0·36 × 0·10 mm) mounted

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[1] S. G. Korenman, H. Wilson, and M. B. Lipsett, *J. Biol. Chem.*, 1964, **239**, 1004.

[2] L. F. Fieser and M. Fieser, ' Steroids,' Reinhold, New York, 1959, p. 518.

with the *c* axis parallel to the ϕ axis of a Picker four-circle automatic diffractometer. Cell dimensions were obtained from a least-squares refinement of the 2θ values of 15 axial reflections measured on the diffractometer. Intensity data were collected with nickel-filtered Cu radiation, by use of the θ—2θ scanning mode at a speed of 1° min^{-1} in 2θ. Background measurements were made for 40 s on either side of the peak. Of the 2717 reflections measured with $\sin\theta < 0{\cdot}93$, 1734 were unique and 88 of these were considered to be unobserved [$I < \sqrt{2}\,\sigma(I)$ where the $\sigma(I)$ were obtained from counting statistics]. The agreement between measurements of equivalent reflections was 1·7% (based on $|F|$). Four monitor reflections, measured *ca.* every 6 h, showed no signs of decay over the period of the data collection. Data were corrected for Lorentz and polarisation effects, but not for absorption.

Determination of the Structure.—The structure was determined by direct methods. A full description of the method used with a definition of symbols and major references has been given elsewhere.[3] Normalised structure factors $|E|$ were obtained in the usual way after the structure amplitudes had been corrected for thermal motion using an overall temperature factor derived from a Wilson plot. The data were then scaled so that the average value of $|E|^2$ was 1·00; the $|E|$ statistics obtained are compared with theoretical values[4] in Table 1. Table 2 lists the starting set of reflections used in the multisolution tangent formula calculations. These were chosen on the basis of the number and strength of their interactions with other reflections of large $|E|$ value. The origin-defining reflections were selected according to the limitations imposed by the space group.[5] The enantiomorph was defined by restricting the initial phase value of one of the symbols (*b*) to lie in the range 0—π. Σ_1 relationships indicated a phase of 0 for the 200 reflection which was therefore included in the starting set with a fixed-phase value. In the tangent formula calculations, the 50 reflections with highest $|E|$ values were refined for 5 cycles, then the 100 highest for 5 cycles, and finally all the reflections for 15 cycles. The values of $E_{\text{min.}}$, $\alpha_{\text{min.}}$, and $t_{\text{min.}}$[3] were 2·00, 2·50, and 0·25. Of the 8 phase sets calculated one had a significantly low R_K value (20·4%) with 173 of the 178 data phased. The positions of all non-hydrogen atoms were located from a Karle map computed with these phased E values as coefficients. Three cycles of full-matrix least-squares refinement reduced the conventional R factor from 0·22 to 0·14. Two further cycles of refinement with anisotropic temperature factors reduced R to 0·13.

TABLE 1

$|E|$ statistics

		Calc.*	
	Obs.	Centro	Non-centro
$\langle\|E\|\rangle$	0·841	0·798	0·886
$\langle\|E^2\|\rangle$	1·000	1·000	1·000
$\langle\|E^2 - 1\|\rangle$	0·858	0·968	0·736
$\|E\| \geq 1{\cdot}0$ (%)	32·4	32·00	36·80
$\|E\| \geq 2{\cdot}0$ (%)	3·3	5·00	1·80
$\|E\| \geq 3{\cdot}0$ (%)	0·2	0·30	0·01

* From ref. 4.

TABLE 2

Starting set of reflections together with final refined values for symbolic phases

h	*k*	*l*	Φ initial	Φ final
3	15	0	$\pi/2$	Fixed for origin definition
2	9	0	0	
0	8	5	0	
2	0	0	0	0
7	11	2	a ($\pm\pi/4$, $\pm3\pi/4$)	138°
2	16	6	b ($\pi/4$), $3\pi/4$)	356°

It was not possible to locate unequivocally any hydrogen atoms from a difference electron-density synthesis computed at this stage of the refinement. Hydrogen atom positions for all but the hydroxy- and methyl groups were calculated assuming C–H 1·0 Å. The hydrogen atoms were assigned isotropic temperature factors equal to the B values of the atoms to which they are bonded. A further cycle of least-squares calculations, keeping the hydrogen parameters fixed, reduced R to 0·085. A subsequent difference electron-density map showed the positions of the methyl hydrogens. Another round of refinement and a difference electron-density synthesis indicated the position of the hydroxy-hydrogen. A final cycle of refinement gave R 0·058 and R' 0·038 {where $R' = [(\Sigma_w(|F_o| - |F_c|)^2/\Sigma_w|F_o|^2]^{\frac{1}{2}}$}. The final difference electron-density map showed no peaks >0·3 eÅ^{-3}.

The quantity minimised throughout the refinement was $\Sigma_w(F_o - F_c)^2$ where the weights were assigned to the various observations according to counting statistics. The scattering factors used were taken from ref. 6. The final atomic co-ordinates of the non-hydrogen atoms are listed in Table 3 and the thermal parameters in Table 4. The hydrogen atom parameters are given in Table 5. A list of observed calculated structure amplitudes appears

TABLE 3

Final fractional co-ordinates for the non-hydrogen atoms

Atom	*x*	*y*	*z*
C(1)	0·5883(4)	0·1257(2)	0·7150(6)
C(2)	0·5857(4)	0·0543(2)	0·7891(6)
C(3)	0·4663(4)	0·0250(2)	0·7562(7)
C(4)	0·4065(4)	0·0438(2)	0·5842(6)
C(5)	0·4505(4)	0·0887(2)	0·4615(6)
C(6)	0·3965(3)	0·0977(2)	0·2666(6)
C(7)	0·3731(3)	0·1699(2)	0·2133(6)
C(8)	0·4784(3)	0·2149(2)	0·2534(5)
C(9)	0·5223(3)	0·2051(2)	0·4592(5)
C(10)	0·5570(4)	0·1316(2)	0·5034(6)
C(11)	0·6183(3)	0·2550(2)	0·5146(7)
C(12)	0·5818(4)	0·3280(2)	0·4729(6)
C(13)	0·5455(3)	0·3364(2)	0·2679(6)
C(14)	0·4461(3)	0·2876(2)	0·2248(5)
C(15)	0·3988(4)	0·3101(2)	0·0269(6)
C(16)	0·4155(4)	0·3868(2)	0·0345(6)
C(17)	0·4896(4)	0·4033(2)	0·2130(6)
C(18)	0·6481(4)	0·3277(2)	0·1335(7)
C(19)	0·6607(4)	0·1085(2)	0·3785(6)
O(20)	0·4262(3)	−0·0163(1)	0·8674(5)
O(21)	0·4123(3)	0·4283(1)	0·3558(5)

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in Supplementary Publication No. SUP 20444 (10 pp., 1 microfiche).*

TABLE 4

Anisotropic thermal parameters ($\beta_{ij} \times 10^4$)

Atom	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	116(5)	22(1)	158(11)	0(4)	−36(12)	−2(6)
C(2)	122(5)	23(1)	241(14)	2(5)	−37(14)	14(6)
C(3)	133(6)	19(1)	198(13)	3(4)	25(16)	−4(6)
C(4)	94(4)	20(1)	200(13)	−1(4)	5(12)	−1(6)
C(5)	73(4)	15(1)	210(13)	3(3)	−2(11)	−12(5)
C(6)	89(4)	23(1)	197(13)	−9(4)	−28(12)	−14(6)
C(7)	67(4)	20(1)	284(13)	−2(4)	−44(12)	−4(6)
C(8)	61(3)	19(1)	151(10)	−3(3)	−3(10)	−4(5)
C(9)	61(3)	18(1)	180(11)	0(3)	−2(10)	−4(5)
C(10)	75(4)	18(1)	162(11)	0(3)	−15(11)	−7(5)
C(11)	89(4)	20(1)	313(13)	−6(4)	−69(13)	−2(6)
C(12)	94(4)	19(1)	225(13)	−6(4)	−20(13)	−7(6)
C(13)	68(4)	21(1)	198(13)	−2(3)	23(12)	−4(6)
C(14)	57(3)	20(1)	163(11)	−2(3)	5(10)	−2(5)
C(15)	116(5)	26(1)	175(12)	−4(4)	−7(13)	18(6)
C(16)	141(6)	24(1)	252(14)	3(5)	−15(15)	17(7)
C(17)	96(4)	21(1)	168(13)	1(4)	18(12)	7(6)
C(18)	76(4)	30(1)	442(17)	−2(4)	118(14)	−5(9)
C(19)	74(4)	23(1)	305(14)	12(4)	24(12)	−9(7)
O(20)	168(4)	30(1)	308(10)	−11(4)	27(11)	33(5)
O(21)	109(3)	25(1)	340(10)	10(3)	32(10)	−8(5)

* In the form: $\exp -(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl)$.

TABLE 5

Final fractional co-ordinates and isotropic thermal parameters (B) for the hydrogen atoms

Atom	x	y	z	B/Å^2
H(1C1)	0·540	0·155	0·798	4·2
H(2C1)	0·669	0·147	0·741	4·2
H(1C2)	0·647	0·028	0·725	5·5
H(2C2)	0·609	0·051	0·925	5·5
H(C4)	0·327	0·022	0·552	3·9
H(1C6)	0·447	0·077	0·168	4·2
H(2C6)	0·326	0·072	0·255	4·2
H(1C7)	0·351	0·175	0·079	4·2
H(2C7)	0·307	0·188	0·282	4·2
H(C8)	0·556	0·203	0·162	3·1
H(C9)	0·455	0·217	0·544	3·5
H(1C11)	0·645	0·248	0·646	4·4
H(2C11)	0·692	0·244	0·439	4·4
H(1C12)	0·517	0·340	0·563	4·4
H(2C12)	0·645	0·359	0·509	4·4
H(C14)	0·383	0·297	0·323	3·3
H(1C15)	0·442	0·287	−0·080	4·7
H(2C15)	0·314	0·297	0·085	4·7
H(1C16)	0·455	0·402	−0·089	5·1
H(2C16)	0·341	0·410	0·031	5·1
H(C17)	0·555	0·437	0·178	4·2
H(1C18)	0·693	0·284	0·123	5·3
H(2C18)	0·679	0·338	0·012	5·3
H(3C18)	0·726	0·351	0·201	5·3
H(1C19)	0·652	0·094	0·240	4·3
H(2C19)	0·685	0·064	0·405	4·3
H(3C19)	0·731	0·136	0·402	4·3
H(O21)	0·477	0·445	0·462	5·7

DISCUSSION

Description of the Molecule.—Figure 1 shows a view of the molecule. The bond distances and angles are summarised in Figure 2. The program-estimated standard deviations for non-hydrogen atoms are 0·006 Å for bond lengths and 0·3° for angles. The C–H bond distances range from 0·96—1·11 Å and the (tetrahedral) angles involving hydrogen from 85—127°. The mean $C(sp^3)$–$C(sp^3)$ bond length is 1·537 Å with the individual distances in good agreement with those found in other steroids of this type (*e.g.* the 2 : 1 complex between testosterone and mercuric chloride[7]). The valence angles are generally larger than expected for tetrahedral carbon atoms (mean values: ring B 111·5, and ring C 111·3°), but are in accord with similar angles found in other steroids.[8]

Rings B, C, and D are *trans*-fused. The results of torsion angle and least-squares planes calculations are given in Tables 6 and 7. Ring A contains a conjugated 4-en-3-one system, which in epitestosterone is rigorously planar. Normally this would require that ring A adopts a sofa-conformation with C(1) out of the plane. However, the torsion angle at the A/B junction (see Figure 3) would in this case be around 27° and thus impose a considerable flattening on ring B since the sum of the torsion angles at a ring junction with a quaternary carbon atom is of the order of 60° (δ = 120°).[8] In epitestosterone this situation is avoided by ring A adopting an unsymmetrically distorted half-chair configuration (Table 6). In most other steroids so far examined by *X*-ray diffraction methods it appears to be more favourable to allow some puckering of the

TABLE 6

Intra-annular torsion angles (ω) for epitestosterone compared with theoretical values from minimum-energy calculations. The standard deviations were computed by use of the formula of ref. 9.

Ring A

Bond	$\omega_{obs.}$	$\omega_{calc.}$ [a]
1–2	−57·6(5)	−62
2–3	36·2(5)	45
3–4	−4·0(6)	−15
4–5	−9·5(7)	0
5–10	−11·4(6)	−15
10–1	44·7(5)	45

‘ Half-chair ’

Ring B

Bond	$\omega_{obs.}$	$\omega_{calc.}$ [b]
9–10	−56·6(4)	−46
10–5	52·4(5)	42·5
5–6	−49·8(5)	−48
6–7	47·4(4)	59
7–8	−51·1(4)	−62
8–9	57·6(4)	56

‘ Chair ’

Ring C

Bond	$\omega_{obs.}$	$\omega_{calc.}$ [c]
12–11	−54·6(4)	−56
11–9	51·6(4)	56
9–8	−51·1(4)	−58
8–14	56·3(4)	58
14–13	−59·6(4)	−59
13–12	56·6(4)	58

‘ Chair ’

Ring D

Bond	$\omega_{obs.}$	$\omega_{calc.}$ [d]
13–14	44·9(4)	43·8
14–15	−33·7(4)	−33·3
15–16	10·0(4)	10·0
16–17	17·0(4)	17·2
17–13	−37·6(4)	−37·7

‘ Half-chair ’

[a] Ref. 10. [b] From Table 12, col. 7 of ref. 8. [c] From Table 4, col. 7, of ref. 8. [d] Ref. 11.

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* For details see Notice to Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

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[11] J. B. Hendrickson, *J. Amer. Chem. Soc.*, 1961, **83**, 4537; 1963, **85**, 4059.

4-en-3-one system. A survey of the literature indicates that the root-mean-square displacements range from 0·05 Å, in cortisone,[12] to 0·02 Å, in androstendione.[13] The only other reported case of a planar 4-en-3-one system we have been able to find is 17α-hydroxyprogesterone[14] where the root-mean-square displacement calculated from the authors' co-ordinate is 0·002 Å.

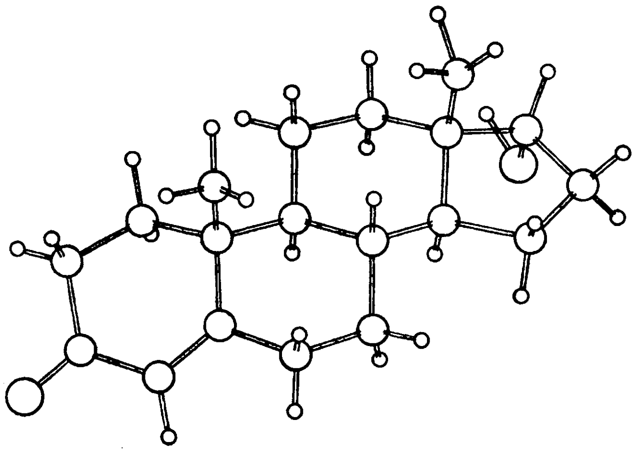

FIGURE 1 A perspective view of the molecule

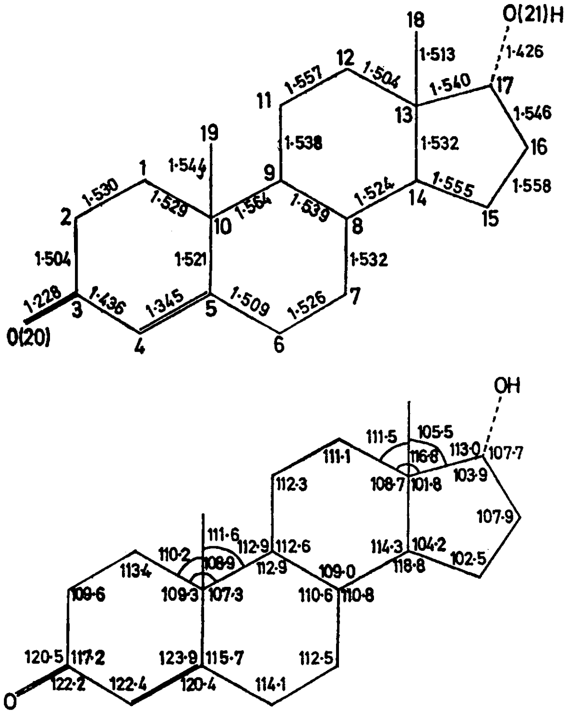

FIGURE 2 The observed bond distances and valency angles, showing the numbering of the atoms used in the discussion; C(14)–C(13)–C(18) is 112·2 and C(5)–C(10)–C(19) is 109·4°

Distortion of ring B from the normal chair-configuration is indicated in Table 6 where a comparison of the observed and theoretical torsion angles shows that the ring has become flattened around C(7) and raised around C(9) (see also Table 7). Ring C has a chair conformation with reasonable agreement between the observed and theoretical torsion angles. Ring D is a distorted half-chair with a phase angle[15] Δ of 9·0°.

TABLE 7

Least-squares planes calculations. *d*, the perpendicular distance (Å) of an atom from the plane, is given followed in parentheses by d/σ where σ is the mean standard deviation of the atomic position obtained from least-squares refinement. Atoms marked (*) were not included in the mean plane calculation

Plane (1): Ring A

C(2) −0·008 (1·0), C(3) 0·017 (2·1), C(4) −0·018 (2·3), C(5) 0·009 (1·1), C(1) * 0·794 (99·3), C(10*) 0·285 (35·6), O(20)* −0·016 (3·2)

Plane (2): Ring A

C(3) 0·002 (0·3), C(4) 0·002 (0·3), C(5) −0·002 (0·3), O(20) −0·002 (0·4), C(1)* 0·682 (85·3), C(2)* −0·096 (12·0), C(10)* 0·201 (25·1)

Plane (3): Ring B

C(5) −0·005 (0·6), C(10) 0·005 (0·6), C(7) 0·005 (0·8), C(8) −0·005 (0·8), C(6)* 0·565 (94·2), C(9)* −0·681 (113·5)

Plane (4): Ring C

C(9) 0·023 (3·8), C(11) −0·023 (3·3), C(13) 0·023 (3·8), C(14) −0·023 (3·8), C(8)* 0·657 (109·5), C(12)* −0·669 (83·6)

Plane (5): Ring D

C(14) −0·038 (6·3), C(15) 0·059 (7·4), C(16) −0·060 (7·5), C(17) 0·040 (5·0), C(13)* 0·643 (107·2)

Equations of planes X, Y, Z are co-ordinates in Å.

Plane (1): $-0{\cdot}4276X + 0{\cdot}7487Y + 0{\cdot}5065Z = 0{\cdot}7655$
Plane (2): $-0{\cdot}4711X + 0{\cdot}7290Y + 0{\cdot}4966Z = 0{\cdot}4856$
Plane (3): $0{\cdot}5162X - 0{\cdot}4736Y - 0{\cdot}7137Z = -0{\cdot}4922$
Plane (4): $0{\cdot}5838X - 0{\cdot}4126Y - 0{\cdot}6992Z = -0{\cdot}4937$
Plane (5): $0{\cdot}8696X - 0{\cdot}1685Y - 0{\cdot}4641Z = 2{\cdot}7615$

The best four-atom least-squares plane contains C(14), C(15), C(16), C(17), which are planar to within 0·06 Å (Table 7). The hydroxy-group on C(17) is in the α-position with a torsion angle C(18)–C(13)–C(17)–O(21) of −163·9°. The steroid skeleton is convex towards the methyl groups C(18) and C(19). For an undistorted skeleton the vectors C(10) · · · C(19) and C(13) · · · C(18) should be parallel. In this compound the angle between these vectors is 11·1° and the bending of the molecule is caused by interactions between the methyl groups and the axial hydrogens on C(8) and C(11).

Newman projections down the ring junctions A/B, B/C, and C/D are shown in Figure 3. The A/B junction has already been discussed. The sum of the two intra-annular torsion angles about the B/C junction

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[12] J. P. Declercq, G. Germain, and M. Van Meerssche, *Cryst. Struct. Comm.*, 1972, **1**, 13.

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[14] J. P. Declercq, G. Germain, and M. Van Meerssche, *Cryst. Struct. Comm.*, 1972, **1**, 9.

[15] C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, 1968, **24**, 13.

(108·7°) is less than the expected value[8] of 115°. This decrease is probably a result of the steric interactions between the methyl groups and axial hydrogens on C(8) and C(11). The sum of the two intra-annular torsion angles around the C/D junction is 104·5°. Although the hydroxy-group on C(17) is in the α-position, this value is close to the empirical value of 109° ± 3° quoted[8] for cases where an axial group is present on C(13) and the substituent on C(17) is in the β-position.

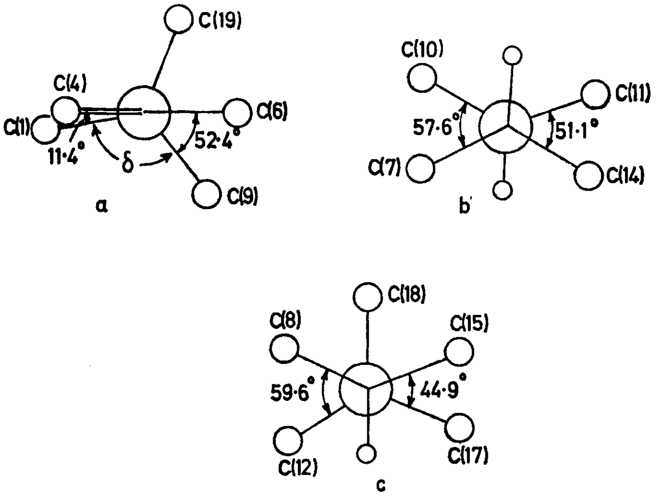

FIGURE 3 Newman projections down the three ring-junctions; a, A/B junction C(5)–C(10); b, B/C junction C(8)–C(9); and c, C/D junction C(14)–C(13)

TABLE 8

Some interatomic distances <4·0 Å

C(1) · · · O(21^{I})	3·889	C(11) · · · C(7^{I})	3·794
C(2) · · · O(21^{I})	3·882	C(12) · · · C(7^{I})	3·988
C(19) · · · O(21^{I})	3·503	C(18) · · · C(7^{II})	3·536

Roman numerals as superscripts refer to the following equivalent positions relative to the reference molecule at x, y, z:

I $1/2 + x, 1/2 - y, z + 1$ II $1/2 + x, 1/2 - y, z$

The Packing of the Molecules.—The molecules are hydrogen bonded 'head-to-tail' through the 3-keto- and 17-hydroxy-groups; the OH · · · O distance and the C(17)–OH · · · O angle being 2·899 Å and 102·2° respectively. The ribbons of hydrogen-bonded molecules extend through the crystal parallel to the *b* axis (see Figure 4). The steroid nuclei lie almost parallel to the (100) plane of the crystal and are stacked 'top-to-bottom'. Apart from the hydrogen bond there are only sixteeen intermolecular distances <4·0 Å. Ten of these are between rings A and D of hydrogen-bonded molecules and the remainder (see Table 8) are between pairs of almost parallel molecules separated by *ca.* *a*/2.

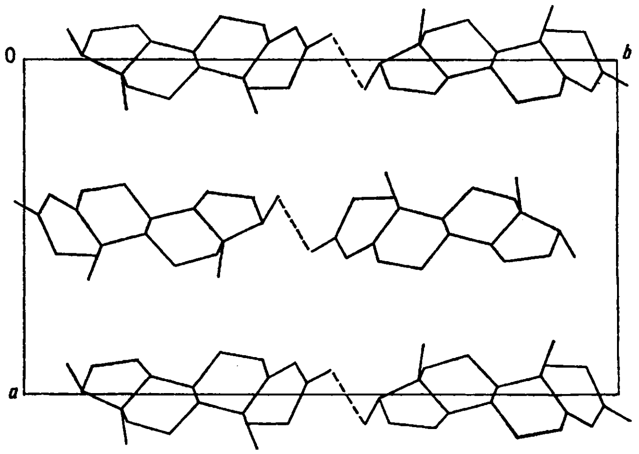

FIGURE 4 A packing diagram projected down the *z* axis. The hydrogen bond is shown by dotted lines

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