

The Molecular Structure of [1,2,5]Oxadiazolo[3',4':3,4]-5 α -pregn-16-en-20-one (HS974). Crystal Structure and NMR Investigations

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The molecular structure of the steroid [1,2,5]oxadiazolo[3',4':3,4]-5 α -pregn-16-en-20-one has been determined by single-crystal X-ray diffraction analysis in the solid state and by ¹H and ¹³C, 1D and 2D homo- and proton-detected-hetero-nuclear NMR spectroscopy in the solution state. The proton-proton distances in solution were derived from a 2D-NOESY build-up experiment and found to be in good agreement with those in the solid-state structure, obtained from X-ray diffraction data.

Medicinal chemists have modified the structure of testosterone [Fig. 1(a)] in various ways with the aim of increasing its anabolic (nitrogen retention) propensity and decreasing its effect as a male hormone.¹ This assumes that the target receptors associated with these two effects are sufficiently different to be sensitive to small changes in the structure of the drug molecule and to react accordingly. One successful approach has been to introduce different A-ring fused heterocycles.²⁻⁴ The compound [1,2,5]oxadiazolo[3',4':3,4]-5 α -pregn-16-en-20-one [Fig. 1(b)], hereafter compound **1**, was prepared during the course of work on the synthesis of steroid oxadiazoles.⁵ We determined the structure of compound **1** in order to study the effect of the 5-en-oxadiazole system on the steroid skeleton and to clarify some conformational aspects for future structure-activity studies.

The solid-state structure and conformations were determined by X-ray diffraction. The solution-state conformation was studied by 1D and 2D ¹H and ¹³C NMR spectroscopy. Several assignment strategies for steroids using a combination of hetero- and homo-nuclear correlation spectroscopy have been applied⁶ in the past. The heteronuclear correlation experiments include ¹³C-detected heteronuclear RELAY spectroscopy applied to the elucidation of a steroid⁷ and several other natural products (terpenoids,⁸ alkaloids,⁹ erythromycin).¹⁰ The strategy used in the present report for compound **1** was proposed¹¹ recently for another steroidal compound. It is based on inverse detected HMQC spectroscopy (HMQC-RELAY) for the skeleton assignment combined with 2D-NOESY spectroscopy for the determination of interatomic distances in the solution state. However, compound **1** exhibits four low-field quaternary carbon-13 resonances, the assignment of which cannot be made either by the above mentioned 2D-NMR techniques or by the earlier proposed¹² approach using selective high-field proton decoupling. Therefore an additional inverse detected heteronuclear multiple bond correlation experiment (HMBC-COSY) allowed us both to complete and to check upon self-consistency the NMR spectral, structural and conformational characterization of compound **1** in solution.

Results and Discussion

Solid-state Structure Conformation from X-Ray Diffraction.—Bond lengths and angles. The crystal structure of compound **1** was determined. The refined atomic coordinates are given in Table 1. Bond distances and angles are listed in

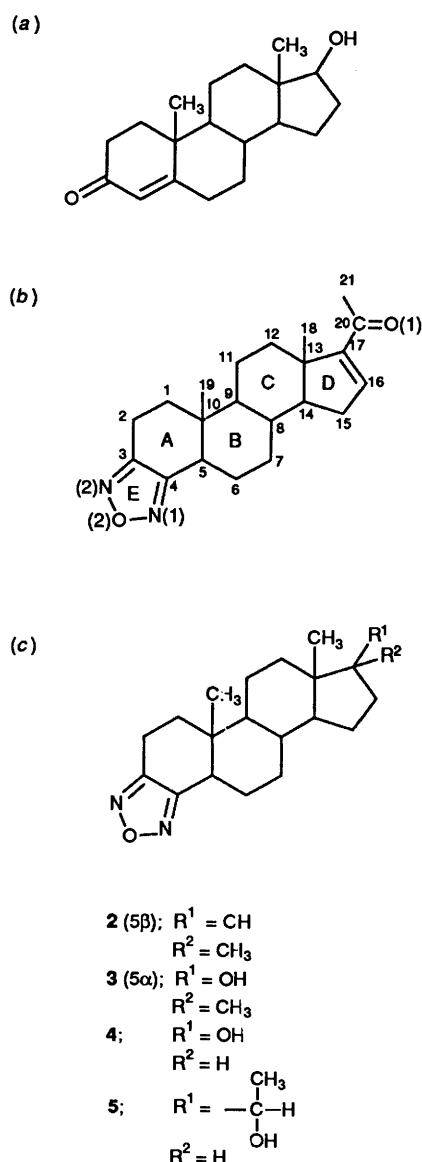


Fig. 1 (a) Structural formula of testosterone. (b) Structural formula and numbering scheme of compound **1**. The numbers refer to carbon atoms unless otherwise indicated. (c) Structural formula of selected steroids oxadiazoles.

Table 1 Refined positional parameters with esds in parentheses

Atom	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>
C(1)	0.3858(2)	0.6340(2)	0.9278(5)
C(2)	0.3806(2)	0.7424(3)	0.8609(5)
C(3)	0.4172(2)	0.7600(3)	0.6640(5)
C(4)	0.4455(2)	0.6851(3)	0.5438(6)
C(5)	0.4443(2)	0.5782(3)	0.6076(5)
C(6)	0.4551(2)	0.5064(3)	0.4273(5)
C(7)	0.4537(2)	0.4011(3)	0.5100(6)
C(8)	0.3947(1)	0.3793(2)	0.6367(5)
C(9)	0.3873(1)	0.4535(2)	0.8225(4)
C(10)	0.3845(1)	0.5607(2)	0.7396(4)
C(11)	0.3331(2)	0.4268(2)	0.9727(5)
C(12)	0.3322(2)	0.3193(2)	1.0447(5)
C(13)	0.3343(1)	0.2514(2)	0.8527(5)
C(14)	0.3949(1)	0.2760(2)	0.7297(5)
C(15)	0.4063(2)	0.1846(3)	0.5920(6)
C(16)	0.3828(2)	0.1063(3)	0.7403(7)
C(17)	0.3449(2)	0.1428(3)	0.8919(6)
C(18)	0.2758(2)	0.2611(3)	0.7153(6)
C(19)	0.3264(2)	0.5780(3)	0.6028(6)
C(20)	0.3109(2)	0.0854(3)	1.0549(8)
C(21)	0.3289(3)	-0.0195(4)	1.0922(11)
N(1)	0.4722(2)	0.7220(3)	0.3739(5)
N(2)	0.4266(2)	0.8447(3)	0.5707(6)
O(1)	0.2692(2)	0.1235(3)	1.1563(7)
O(2)	0.4614(2)	0.8229(2)	0.3886(5)

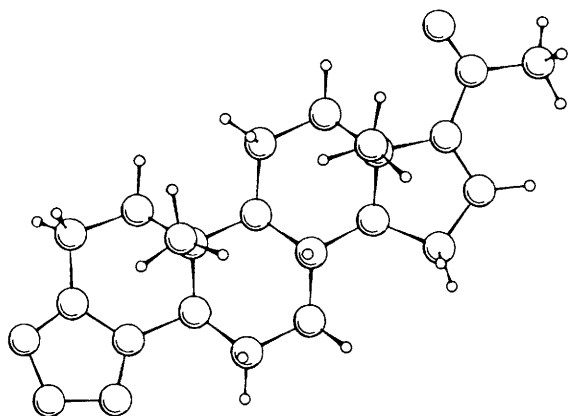
**Fig. 2** View of the molecule

Table 2.* The chemical formula with the atomic labelling is depicted in Fig. 1(b). Fig. 2 shows the molecular conformation. The diagrams and tables correspond to the absolute configuration assumed for steroids.

Most of the bond lengths in compound **1** are close to the expected values. The average C–C single bond lengths in rings A, B, C and D are 1.505(5), 1.536(5), 1.536(4) and 1.526(5) Å, respectively. The average value of all the C–C single bond lengths in the molecule is 1.528(3) Å. This is in agreement with the values found in similar steroid structures [see Fig. 1(c)], e.g., 17 α -methyl[1,2,5]oxadiazolo[3',4':3,4]-5 β -androstan-17 β -ol (HS804, hereafter compound **2**),¹³ 17 α -methyl[1,2,5]oxadiazolo[3',4':3,4]-5 α -androstan-17 β -ol (HS805, hereafter compound **3**),¹³ [1,2,5]oxadiazolo[3',4':3,4]-androstan-17 α -ol (HS963, hereafter compound **4**)¹⁴ and (20*R*)-[1,2,5]oxadiazolo[3',4':3,4]-5 α -pregnan-20-ol (HS1011, hereafter compound **5**).¹⁵ The shortening in bond length C(2)–C(3) [= 1.483(5) Å]

is associated with the fusion of the oxadiazole ring with ring A; C(3) and C(4) both have sp² character. The bond length C(3)–C(4) [= 1.406(5) Å] is in agreement with those found in compounds **2**, **3** and **4**^{13,14} the values of which are 1.425(4), 1.429(4) and 1.429(3) Å, respectively. The C(3)=N(2) bond length is 1.309(5) Å which agrees well with those quoted for compounds **2**, **3** and **4**. The bond length C(4)=N(1) [= 1.310(5) Å] agrees with compound **4**, but is significantly larger than the value of 1.273(5) Å in compound **3**. The shortening of bond lengths C(3)–C(4) probably originates from the π -delocalization in the system N(2)=C(3)–C(4)=N(1), as found for other heterocyclic oxadiazoles.^{13,16–18} The N–O bond lengths N(2)–O(2) of 1.396(5) and N(1)–O(2) of 1.395(5) Å in compound **1** are comparable to those of 3-amino-4-methylfuran¹⁸ ranging from 1.380(3) to 1.406(3) Å. Likewise, compounds **2**, **3** and **4** have N–O bond lengths ranging from 1.367(7) to 1.393(5) Å, whereas average N–O bond lengths of 1.380(3) Å were found in furazan.¹⁶

Apart from bond angles involving C(3) and C(4), the average C–C–C bond angle within the steroid skeleton is 109.4°. The internal angle C(17)–C(13)–C(14) [99.4(3)°] of ring D is the same as in compound **4**, but is significantly smaller than the same angle in compounds **2** and **3** [100.8(3) and 101.1(3)°, respectively]. All of the large bond angles have central either tertiary (CH) or secondary (CH₂) carbon atoms, while the small bond angles are associated with the central quaternary carbon atoms, C(10) or C(13), both bearing CH₃ groups. The average values of the bond angles in these three categories are 110.2, 110.7 and 106.2° for secondary, tertiary and quaternary carbon atoms, respectively. The data for the steroid oxadiazoles **2** and **3** show a similar effect with average bond angles in these three categories of 110.5° (CH₂), 111.8° (CH) and 107.8° (C bearing a methyl group).

Ring conformations. The conformational features of compound **1** are represented in terms of (i) torsion angles in Table 2(C) and (ii) asymmetry parameters in Table 3. The pseudo torsion angle C(19)–C(10)⋯C(13)–C(18), giving a quantitative measure of the twist about the length of the molecule has a value of 2.3° in compound **1**.¹⁹ Confirmation and symmetry in the six-membered rings A, B and C depart, as expected, from the ideal ones. Following Duax *et al.*,¹⁹ the magnitude of the asymmetry parameters ΔC_s and ΔC_2 indicate the deviation (about bond directions and bond angle bisectors) from mirror and twofold symmetry; a true *m* plane corresponds to $\Delta C_s = 0^\circ$, and a twofold axis to $\Delta C_2 = 0^\circ$. These parameters have been calculated and are tabulated in Table 3. Ring A is a moderately strained 10 β -sofa/10 β ,1 α -half-chair. Rings B and C have low values for both ΔC_s and ΔC_2 showing good approximation to the ideal chair conformation. Ring D is intermediate between a 13 β envelope and a 13 β ,14 α half-chair. The oxadiazole ring E is planar (RMS deviations 0.1173 Å). Ring connections are as follows: A/B *trans*, B/C *trans* and C/D *quasi trans*. There are no intermolecular close contacts. The packing of the molecules along the *a*-axis is shown in Fig. 3.

NMR Studies of the Solution Structure.—¹H and ¹³C resonance assignments. The final ¹H and ¹³C assignments are represented in Table 4. The resonance of the olefinic proton C(16) is easily identified at 6.62 ppm. The ¹³C DEPT-135 spectrum²⁰ identifies the corresponding low-field tertiary carbon. From this starting point all the ¹H and ¹³C chemical shifts of the protonated carbons can be assigned using the information provided by the HMQC-COSY²¹ and the HMQC-RELAY²² spectra. The HMQC-COSY spectrum exhibits the correlations *via* one-bond ¹J_{H-¹³C} couplings. These are suppressed or attenuated in the HMQC-RELAY with a long initial evolution delay (20 ms). The HMQC-RELAY allowed the easiest correlation between each ¹H–¹³C pair and its

* Tables of hydrogen atom coordinates and isotropic and anisotropic thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. For details of the CCDC deposition scheme, see 'Instructions for Authors (1992)', *J. Chem. Soc., Perkin Trans. 2*, 1992, issue 1.

Table 2 Bond lengths (Å), bond angles (°) and selected torsion angles (°) with esds in parentheses

Bond lengths		Bond angles		Torsion angles	
C(1)–C(2)	1.538(5)	C(1)–C(2)–C(3)	110.0(3)	Ring A	
C(1)–C(10)	1.544(5)	C(2)–C(3)–C(4)	124.0(3)	C(1)–C(2)–C(3)–C(4)	7.49
C(2)–C(3)	1.483(5)	C(3)–C(4)–C(5)	123.3(3)	C(2)–C(3)–C(4)–C(5)	–2.95
C(3)–C(4)	1.406(5)	C(4)–C(5)–C(10)	107.6(3)	C(3)–C(4)–C(5)–C(10)	28.14
C(4)–C(5)	1.509(5)	C(5)–C(10)–C(1)	107.0(2)	C(4)–C(5)–C(10)–C(1)	–55.99
C(5)–C(6)	1.511(5)	C(10)–C(1)–C(2)	114.2(2)	C(5)–C(10)–C(1)–C(2)	66.19
C(5)–C(10)	1.552(4)	C(5)–C(6)–C(7)	110.5(3)	C(10)–C(1)–C(2)–C(3)	–39.51
C(6)–C(7)	1.524(5)	C(6)–C(7)–C(8)	112.0(3)	Ring B	
C(7)–C(8)	1.529(4)	C(7)–C(8)–C(9)	110.5(3)	C(5)–C(6)–C(7)–C(8)	53.00
C(8)–C(9)	1.548(4)	C(8)–C(9)–C(10)	111.5(2)	C(6)–C(7)–C(8)–C(9)	–55.93
C(8)–C(14)	1.523(4)	C(9)–C(10)–C(5)	106.8(2)	C(7)–C(8)–C(9)–C(10)	58.79
C(9)–C(10)	1.550(4)	C(10)–C(5)–C(6)	115.3(3)	C(8)–C(9)–C(10)–C(5)	–56.41
C(9)–C(11)	1.544(4)	C(8)–C(9)–C(11)	112.5(2)	C(9)–C(10)–C(5)–C(6)	55.62
C(10)–C(19)	1.534(4)	C(9)–C(11)–C(12)	114.4(3)	C(10)–C(5)–C(6)–C(7)	–54.47
C(11)–C(12)	1.530(4)	C(11)–C(12)–C(13)	110.3(2)	Ring C	
C(12)–C(13)	1.518(4)	C(12)–C(13)–C(14)	106.7(2)	C(8)–C(9)–C(11)–C(12)	48.39
C(13)–C(14)	1.554(4)	C(13)–C(14)–C(8)	112.7(2)	C(9)–C(11)–C(12)–C(13)	–53.61
C(13)–C(17)	1.516(4)	C(14)–C(8)–C(9)	108.3(2)	C(11)–C(12)–C(13)–C(14)	59.01
C(13)–C(18)	1.534(5)	C(13)–C(14)–C(15)	103.8(3)	C(12)–C(13)–C(14)–C(8)	–65.24
C(14)–C(15)	1.533(4)	C(14)–C(15)–C(16)	100.0(3)	C(13)–C(14)–C(8)–C(9)	59.79
C(15)–C(16)	1.501(6)	C(15)–C(16)–C(17)	112.3(3)	C(14)–C(8)–C(9)–C(11)	–49.56
C(16)–C(17)	1.348(6)	C(16)–C(17)–C(13)	109.6(3)	Ring D	
C(17)–C(20)	1.479(6)	C(17)–C(13)–C(14)	99.4(3)	C(13)–C(14)–C(15)–C(16)	–34.83
C(20)–C(21)	1.498(6)	C(3)–C(4)–N(1)	110.4(3)	C(14)–C(15)–C(16)–C(17)	19.67
C(20)–O(1)	1.217(6)	C(4)–N(1)–O(2)	104.5(3)	C(15)–C(16)–C(17)–C(13)	4.33
C(3)–N(2)	1.309(5)	N(1)–O(2)–N(2)	110.7(3)	C(16)–C(17)–C(13)–C(14)	–25.95
C(4)–N(1)	1.310(5)	O(2)–N(2)–C(3)	105.1(3)	C(17)–C(13)–C(14)–C(15)	37.19
N(2)–O(2)	1.396(5)	N(2)–C(3)–C(4)	109.4(3)		
N(1)–O(2)	1.395(5)	C(2)–C(3)–N(2)	126.6(4)		
		N(1)–C(4)–C(5)	126.3(4)		
		C(4)–C(5)–C(6)	115.0(3)		
		C(1)–C(10)–C(9)	110.5(2)		
		C(19)–C(10)–C(1)	110.1(3)		
		C(19)–C(10)–C(5)	111.0(2)		
		C(19)–C(10)–C(9)	111.3(2)		
		C(7)–C(8)–C(14)	112.0(2)		
		C(10)–C(9)–C(11)	113.3(2)		
		C(12)–C(13)–C(17)	118.0(3)		
		C(18)–C(13)–C(12)	111.5(3)		
		C(18)–C(13)–C(14)	113.3(3)		
		C(18)–C(13)–C(17)	107.4(3)		
		C(15)–C(14)–C(8)	122.3(3)		
		C(13)–C(17)–C(20)	123.5(3)		
		C(16)–C(17)–C(20)	126.4(3)		
		C(17)–C(20)–C(21)	118.8(5)		
		C(17)–C(20)–O(1)	120.1(4)		
		C(21)–C(20)–O(1)	121.1(5)		

Table 3 Asymmetry parameters (°)

Ring A	
$\Delta C_s^1 = 35.1$	$\Delta C_2^{1,2} = 55.9$
$\Delta C_s^2 = 44.0$	$\Delta C_2^{2,3} = 37.3$
$\Delta C_s^3 = 9.2$	$\Delta C_2^{3,4} = 18.7$
Ring B	
$\Delta C_s^9 = 1.6$	$\Delta C_2^{9,10} = 2.5$
$\Delta C_s^{10} = 1.7$	$\Delta C_2^{5,10} = 4.1$
$\Delta C_s^8 = 2.6$	$\Delta C_2^{5,6} = 1.9$
Ring C	
$\Delta C_s^{12} = 11.8$	$\Delta C_2^{11,12} = 13.4$
$\Delta C_s^{11} = 7.0$	$\Delta C_2^{11,9} = 2.9$
$\Delta C_s^9 = 5.1$	$\Delta C_2^{9,8} = 11.5$
Ring D	
$\Delta C_s^{13} = 23.0$	$\Delta C_2^{16} = 12.5$
$\Delta C_s^{14} = 4.7$	

neighbour pairs. At least one unambiguous relayed cross-peak was found between all neighbour pairs.

However, the HMQC-RELAY technique is irrelevant to the identification of non-protonated carbon atoms. C(20) is easily identified by its chemical shift. C(3), C(4) and C(17) only differ slightly in their chemical shifts as do C(10) and C(13). Therefore an inverse detected heteronuclear multiple bond correlation spectrum (HMBC-COSY)²³ was recorded correlating these quaternary carbon atoms to protons by $^{2,3}J_{\text{H}-^{13}\text{C}}$ long-range couplings. Fig. 4 shows a F1 low-field expansion of the HMBC-COSY spectrum that enabled the assignment of these quaternary carbon atoms. Some doubt remained about the carbon atoms C(4) and C(17) because of resonance overlap beyond the F1- ^{13}C spectral resolution. In principle this kind of experiment gives somewhat similar information as the HMQC-RELAY, but the presence of more than 100 HMBC-COSY cross-peaks in the high-field region results in a very crowded spectrum. Nevertheless the HMBC-COSY confirmed the assignments made by the HMQC-RELAY.

Proton–proton distances in solution. Two-dimensional nuclear Overhauser enhancement spectroscopy²⁴ (2D-NOESY) was used to assign the α or β position of the methylene protons. The proton–proton distances that could be determined from the 2D NOESY build-up experiments in the initial rate approximation

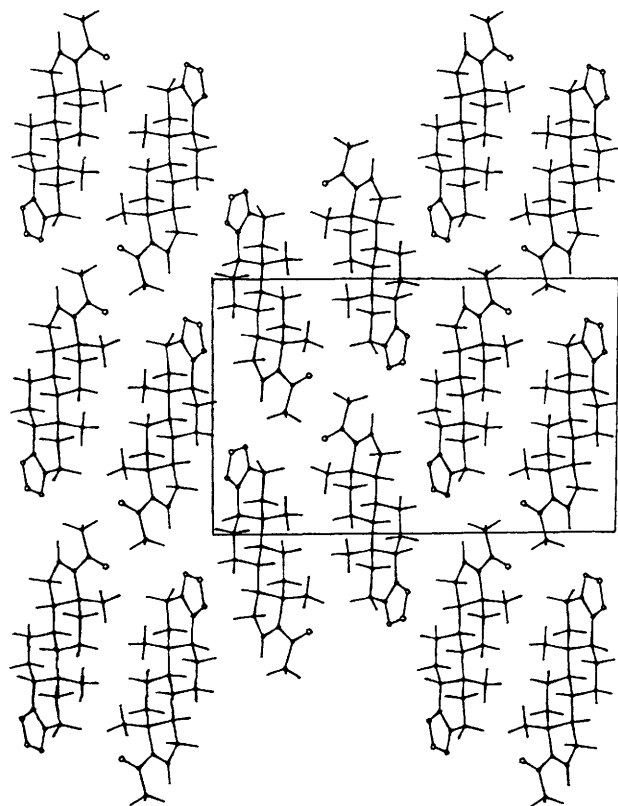


Fig. 3 View illustrating the molecular packing as seen along the *a* axis

Table 4 Chemical shift data for compound 1. ^{13}C chemical shifts were obtained from a proton-decoupled spectrum. ^1H shifts were obtained from 1D-proton and/or inverse-detected HMQC-COSY spectra. Ax and eq refer to axial and equatorial hydrogens; α and β refer to the position of the hydrogen atoms with respect to the average plane of the molecule.

Carbon	δ_{C}	Proton	δ^{a}
C(1)	32.80	H(1 ax)	α 1.35
		H(1 eq)	β 2.13
C(2)	16.51	H(2 ax)	β 2.68
		H(2 eq)	α 3.00
C(3)	150.43		
C(4)	155.00 ^b		
C(5)	43.90	H(5)	α 2.45
C(6)	23.03	H(6 ax)	β 1.59
		H(6 eq)	α 2.31
C(7)	30.68	H(7 ax)	α 1.14
		H(7 eq)	β 1.85
C(8)	33.32	H(8)	β 1.68
C(9)	52.77	H(9)	α 1.02
C(10)	36.65		
C(11)	21.58	H(11 ax)	β 1.43
		H(11 eq)	α 1.68
C(12)	34.64	H(12 ax)	α 1.35
		H(12 eq)	β 2.38
C(13)	46.15		
C(14)	55.92	H(14)	α 1.47
C(15)	32.04	H(15 ax)	β 2.04
		H(15 eq)	α 2.31
C(16)	144.19	H(16)	6.62(s)
C(17)	155.17 ^b		
C(18)	15.85	H(18)	β 0.88(s)
C(19)	12.17	H(19)	β 0.72(s)
C(20)	196.61		
C(21)	27.03	H(21)	2.23(s)

^a Multiplicity for proton resonances: s, singlet; all other protons exhibit complex and/or overlapping coupling patterns. ^b These assignments are interchangeable.

Table 5 Comparative overview of proton-proton distances as obtained in solution from 2D NOESY build-up NMR data and in the solid state from hydrogen position calculations from X-ray diffraction data. * Indicates values obtained from correlation <0.99 .

Proton pairs	<i>d</i> /Å		
	In solution from NMR data	In the solid state from X-ray data	% Difference
15eq-16	2.08	2.74	32
14ax-16	2.64	2.84	10
2ax-2eq	1.77*	1.76	<1
1eq-2eq	2.48*	2.59	5
1ax-2eq	2.25	2.34	4
1eq-2ax	2.10	2.35	12
2ax-19Me	2.33	—	—
5-6eq	2.66*	2.27	17
1ax-5	2.14	2.42	6
5-7ax	2.20	2.36	7
5-9	2.03	2.31	14
5-19Me	3.29*	3.76	14
11eq-12eq	2.26	2.48	10
11ax-12eq	2.01	2.41	20
12ax-12eq	1.78	1.76	2
12eq-18Me	2.57	2.53	2
15ax-15eq	1.73	1.76	2
6eq-7eq	1.96	2.50	22
6ax-6eq	1.77	1.76	<1
14-15eq	2.39	2.30	4
6eq-7ax	2.11	2.42	15
1eq-11eq	1.86	2.03	9
1ax-1eq	1.73	1.76	2
1eq-19Me	2.52	2.48	2
7eq-15ax	2.52	2.81	12
8-15ax	2.61*	2.79	7
15ax-18Me	2.37	2.32	3
7eq-8	2.32*	2.40	4
6ax-7eq	2.34	2.41	2
6ax-8	2.64	2.61	2
7ax-7eq (ref.)	1.76	1.76	0.000
11eq-12ax	2.29	2.41	11
9-11eq	2.45	2.35	5
8-18Me	2.27	2.22	3
8-19Me	2.23	1.97	3
6ax-19Me	2.35	2.37	<1
12ax-14ax	2.76*	2.47	12
7ax-14	2.21	2.29	0
9-14	2.07	2.31	6
11ax-18Me	2.40	2.01	20
11ax-19Me	2.34	2.15	9
1ax-9	2.20	2.48	4
1ax-19Me	3.17	3.63	15
7ax-9	2.56*	2.52	2

are reviewed in Table 5. Two examples of build-up straight lines from which these distances were determined are displayed in Fig. 5. All the build-up curves were obtained from negative cross-peaks, being of opposite sign with respect to the positive auto-peaks, indicating conditions of extreme narrowing.²⁵ The corresponding distances determined from the X-ray diffraction data are also given for comparison.

The differences in solution and solid-state distances exceed 15% in only five of the 42 distances determined. In 21 cases they even differ by less than 5%. These results indicate the solid- and solution-state conformations to be very similar. Significant differences appear in distances involving the H(15) atom, indicating slightly different conformations at the level of ring D. In addition, the distances found for protons of the $\text{CH}_2\text{-CH}_2$ chain fragments involving carbon atoms C(1) and C(2), C(6) and C(7), and C(11) and C(12) are in general shorter in solution than in the solid state. This reflects the consequence of distance averaging in solution, as outlined by Neuhaus and Williamson;²⁵ averaging distances through inverse sixth powers puts greater weight on smaller distances.

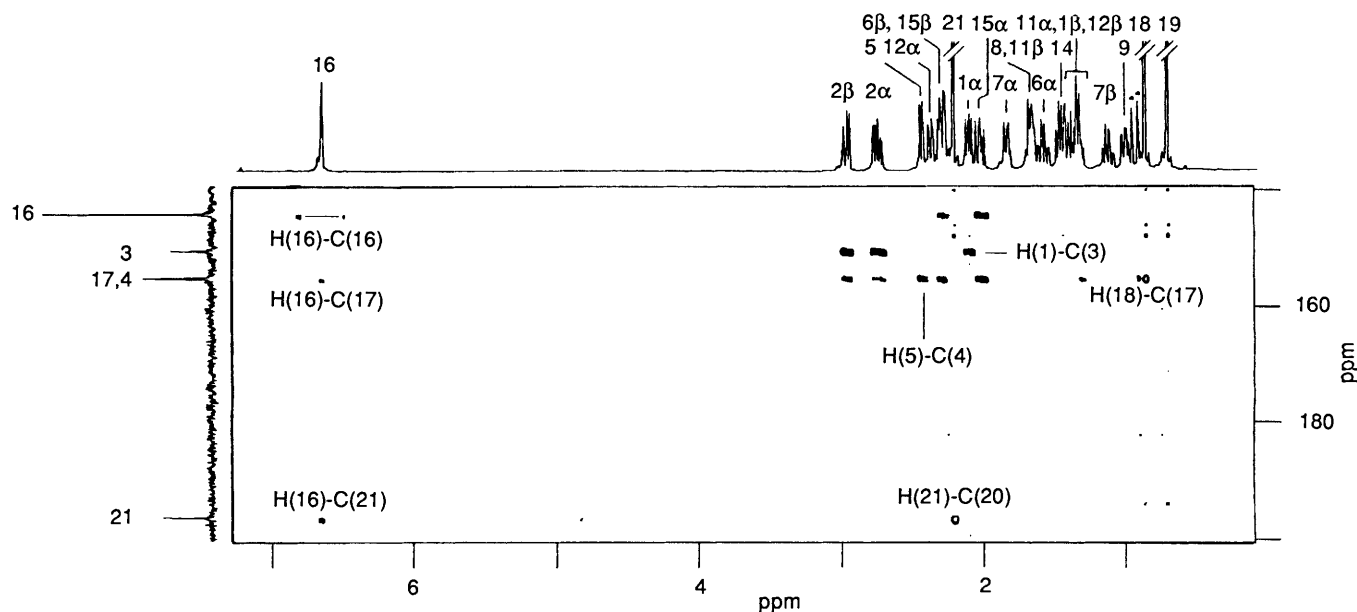


Fig. 4 F_1 -low field expansion of the HMBC-COSY spectrum. F_1 -axis (vertical) carbon-13 chemical shifts; F_2 -axis (horizontal) proton chemical shifts. Some important cross-peaks are indicated. * Denote impurities.

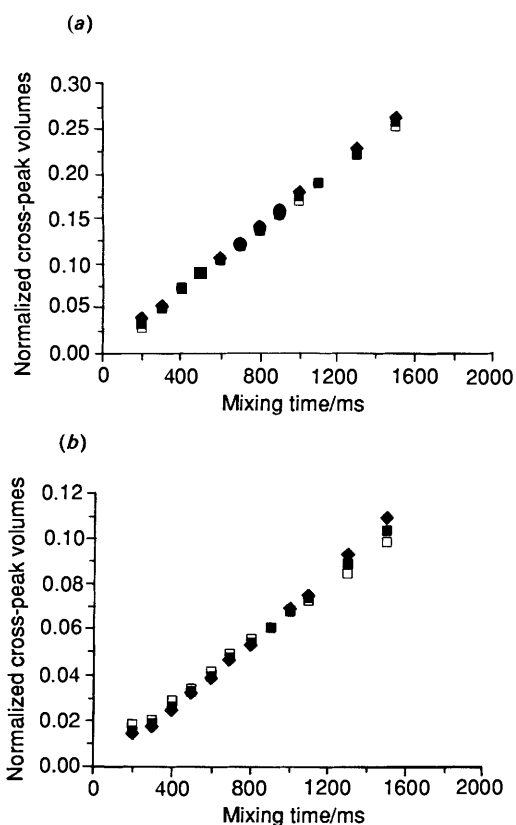


Fig. 5 Two examples of 2D-NOESY build-up curves: (a) for the cross peak arising from the H-atoms H(7eq) and H(7ax) used as reference for the determination of the solution-state distances: □, 7eq to 7ax; ◆, 7ax to 7eq; ■, mean of 7eq to 7ax and 7ax to 7eq; (b) for a typical cross-peak pair without resonance overlapping, from the H-atoms H(5) and H(9): □, 5 to 9; ◆, 9 to 5; ■, mean of 5 to 9 and 9 to 5. Means of cross-peaks refer to average values as defined in the experimental section.

Experimental

NMR Experiments.—The samples were prepared by dissolving the appropriate amount of the steroid in 0.5 cm³ CDCl₃, ca. 5 mg for ¹H NMR (degassed and sealed sample) and 30 mg for ¹³C, DEPT-135 and for proton detected heteronuclear

experiments. All spectra were recorded on a Bruker AMX500 spectrometer interfaced with an X32 computer. Chemical shifts were referenced to the residual solvent peak and converted to the standard Me₄Si scale by adding 7.24 ppm and 77.0 ppm for ¹H and ¹³C nuclei, respectively.

¹³C BB proton decoupled and DEPT-135 spectra were recorded using standard pulse sequences and delays.²⁰

The proton-detected ¹H-¹³C correlation spectrum (HMBC-COSY) was recorded using the pulse sequence and phase cycling proposed by Bax and Subramanian²¹ using ¹³C decoupling (GARP).²⁶ 256 free induction decays (FID) were recorded and zero-filled to 512 W in the F_1 dimension with a spectral width of 8300 Hz. 1K data points, no zero-filling, spectral width 2008 Hz (acquisition time 255 ms), 16 scans, relaxation delay 1.5 s were used in the F_2 dimension.

The proton-detected ¹H-¹³C RELAY spectrum (HMBC-RELAY) was recorded using the pulse sequence and phase cycling proposed by Lerner and Bax²² using ¹³C decoupling (GARP).²⁶ The correlations *via* one-bond couplings were not suppressed by delayed decoupling, *i.e.* decoupling was started at the same time as the acquisition after the final 90° ¹H pulse. The experiment was optimized to the ¹J_{H-¹³C} coupling value of 132 Hz. 512 free induction decays (FID) were recorded and zero-filled to 1K in the F_1 dimension with a spectral width of 20 877 Hz and an initial evolution delay of 20 ms was used. 2K data points, no zero-filling, a spectral width of 3759 Hz (acquisition time 272 ms), 32 scans, and a relaxation delay of 2.5 s, were used in the F_2 dimension. The delay after the BIRD sequence in the HMBC-RELAY and HMBC-COSY experiments was optimized to 650 ms.

A proton-detected heteronuclear multiple bond correlation spectrum (HMBC-COSY) was recorded using the pulse sequence and phase cycling proposed by Bax and Summers.²³ The experiment was optimized to ^{2,3}J_{H-¹³C} average values of 8.3 Hz (delay = 60 ms). The lowpass *J*-filter was optimized to the ¹J_{H-¹³C} value of 132 Hz. 512 FIDs were recorded and zero-filled to 1K in the F_1 dimension with a spectral width of 30 184 Hz. 2K data points, no zero-filling, a spectral width of 3759 Hz (acquisition time 272 ms), 48 scans, and a relaxation delay of 1.9 s, were used in the F_2 dimension.

Twelve 2D NOESY²⁴ spectra were recorded using TPPI. 256 FIDs were recorded and zero-filled to 512 W in the F_1 dimension with a spectral width of 3759 Hz. 2K data points, no

zero-filling, a spectral width of 3759 Hz (acquisition time 272 ms), 16 scans, and a relaxation delay of 3.7 s, were used in the F_2 dimension. The mixing times had values from 200 to 1100 ms in steps of 100 ms as well as 1300 and 1500 ms.

All the 2D-NMR spectra were obtained after multiplication with a shifted sine bell, $F(t) = \sin\{\pi - \varphi(t/AT)\} + \varphi$ with $\varphi = \pi/2$ or $\varphi = \pi/3$ in both dimensions prior to Fourier transformation.

Proton-proton distances from 2D NOESY build-up experiments. Proton-proton distances were determined from the formula $r_{kl} = r_{\text{ref}} (S_{\text{ref}}/S_{kl})^{1/6, 25}$ where r_{kl} represents the distance of interest between the protons k and l ; r_{ref} represents the reference distance separating two protons, the mutual distance of which is unambiguously established from geometrical considerations; S_{kl} is the slope of the build-up straight line, as defined below, in the initial rate approximation, associated with the cross-peaks correlating the pairs of protons k and l ; these build-up straight lines represent the growth of the cross-peak V_{kl} , normalized to the auto-peak V_{ll} of the same F_2 -coordinate, as a function of the mixing time. S_{ref} is the corresponding slope associated with the reference pair of protons.

The reference distance r_{ref} chosen is the standard distance 1.76 Å separating the two hydrogen atoms of a methylene group as calculated from standard C-H bond lengths and H-C-H bond angles of sp^3 carbon atoms. The pair of reference protons chosen was that of the methylene group 7, because the two cross-peaks associated with this pair exhibited identical build-up slopes, to within 1.5% as determined from straight lines displaying correlation coefficients of 0.999 with 12 build-up points.

When the slopes associated with a pair of cross peaks arising from the interacting protons k and l differed by less than 20%, S_{kl} was defined according to Andersen *et al.*²⁷ as $S_{kl} = 1/2[(V_{kl}/V_{ll}) + (V_{lk}/V_{kk})]$, where V_{kl} and V_{ll} represent cross- and auto-peak volumes, respectively. When the slopes differed by more than 20%, the cross-peak and associated auto-peak subject to no or least overlapping and/or isochrony was selected.

X-Ray Crystallography.—Colourless needle-shaped crystals were obtained from ethanol.

Crystal data. $C_{21}H_{28}N_2O_2$, $M_r = 340.45$, orthorhombic, $P2_12_12_1$, $a = 21.571(6)$, $b = 13.606(4)$, $c = 6.263(3)$ Å, $V = 1838.16$ Å³, $Z = 4$, $D_x = 1.23$ g cm⁻³, $\lambda = 1.5418$ Å, $\mu = 5.88$ cm⁻¹, $F(000) = 736$, room temperature, $R = 0.085$ for 2471 observed reflections.

Data collection and processing. A crystal specimen of $0.8 \times 0.2 \times 0.1$ mm³ was used for data collection. Preliminary Weissenberg photographs yielded approximate cell dimensions and showed orthorhombic (*mmm*) symmetry. The space group $P2_12_12_1$ was determined unambiguously from systematic absences ($h00$, $h = 2n + 1$; $0k0$, $k = 2n + 1$; $00l$, $l = 2n + 1$). Data were collected on a Enraf-Nonius CAD-4 automated diffractometer with graphite monochromator, operating with $CuK\alpha$ radiation. 25 High angle reflections ($25 < 2\theta < 28$) were used to obtain accurate cell dimensions by least-squares refinement. 7608 reflections $3 < \theta < 69$ were measured ($-25 < h < 25$; $-1 < k < 16$; $-1 < l < 8$) using the ω - 2θ scan technique with scan width $(0.85 + 0.15 \tan \theta)^\circ$ and a vertical aperture of 4 mm. Three intensity standards (220, 200, 008) were monitored at intervals of 100 measurements. They showed no significant variation during data collection. These intensity data were corrected for Lorentz-polarization factors. No absorption correction was carried out. Merging of the reflection files and averaging of the unique reflections resulted in a unique data set of 3489 reflections ($R_{\text{int}} = 0.046$).

Structure analysis and refinement. The structure was solved by direct methods with SHELX76.²⁸ Refinement (SHELX76)

was carried out by full-matrix least-squares with anisotropic thermal factors for non-H atoms and isotropic for H-atoms. The hydrogen atoms were generated geometrically and included as fixed isotropic contribution to F_o , (C-H = 1.08 Å). Methyl hydrogens were refined as rigid groups possessing a local C_3 symmetry. The function minimized was $w = (F_o - F_c)^2$ with a weighting factor $w = [\sigma^2(F_o) + 0.012855 F_o^2]^{-1}$; $R = 0.085$, $w_R = 0.078$ for 2471 unique reflections with $\theta < 60^\circ$, R (all data) = 0.11. The max (shift/esd) = 0.267. No residual density was found outside the range -0.42 to 0.40 e Å⁻³. The atomic scattering factors were taken from the International Tables for X-ray Crystallography.²⁹

Calculations were carried out on VAX and AMDAHL 470V/8 computers. Geometrical calculations were performed with XANADU³⁰ and molecular illustrations were drawn with PLUTO.³¹

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