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Conformational comparison of five follicular fluid meiosis-activating sterol-related active and inactive compounds

D. Roeland Boer,[a] Huub Kooijman,[a]* Marinus Groen,[b] Jaap van der Louw,[b] Jan Kelder[c] and Jan Kroon[a]

[a]Bijvoet Center for Biomolecular Research, Department of Crystal and Structural Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands, [b]Department of Medicinal Chemistry, NV Organon, Oss, The Netherlands, and [c]Department of Molecular Design and Informatics, NV Organon, Oss, The Netherlands
Correspondence e-mail: h.kooijman@chem.uu.nl

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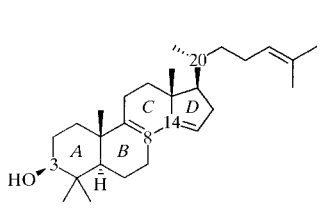
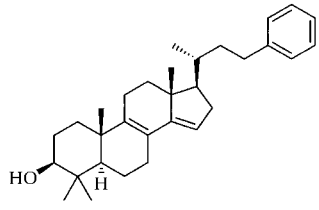
The crystal structures of five follicular fluid meiosis-activating sterol-related $\Delta^{8,14}$-sterol compounds are presented. These are 4,4-dimethyl-23-phenyl-24-nor-5α-chola-8,14-dien-3β-ol, $C_{31}H_{44}O$, 4,4-dimethyl-22-phenyl-23,24-dinor-5α-chola-8,14-dien-3β-ol, $C_{30}H_{42}O$, (20*R*)-4,4-dimethyl-22-oxa-5α,20-cholesta-8,14,24-trien-3β-ol, $C_{28}H_{44}O_2$, 4,4-dimethyl-23-phenyl-22-oxa-24-nor-5α-chola-8,14-dien-3β-ol–water (4/1), $4C_{30}H_{42}O_2\cdot H_2O$, and 4,4-dimethyl-5$\alpha$-cholesta-8,14-dien-3-one, $C_{29}H_{46}O$. Two of the derivatives are inactive and three are active as agonists. Preliminary structure–activity relationship studies showed that the positions of the double bonds in the skeleton and the structures of the side chains are important determinants for activity. The conformations of the skeletons were compared with double-bond isomers retrieved from the Cambridge Structural Database [Allen & Kennard (1993). *Chem. Des. Autom. News*, **8**, 1, 31–37]; no significant differences were found. Thus, conformational changes induced by the double bonds are not discriminative with respect to the activity of the compounds. Comparisons of the side-chain conformations of active and inactive structures revealed that the crystal structures were not conclusive as far as correlation of conformation and activity of the side chains were concerned.

Comment

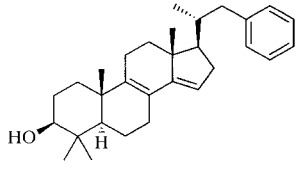
Sterol compounds are intermediates in the biosynthetic pathway of cholesterol. One of these compounds, called follicular fluid meiosis-activating sterol [FF–MAS, (I)], was found to initiate the resumption of prophase I-arrested human oocytes (Byskov *et al.*, 1995). For this reason, FF–MAS may serve as a lead compound in the development of agonists and antagonists to either stimulate or block the production of functional egg cells. A number of FF–MAS-related sterols have been synthesized and tested with a varying number of double bonds at different positions in the steroid skeleton. Very few double-bond configurations were found to yield active compounds. Those that are active have double bonds at positions 8 and 14 or an aromatic *C* ring (Grønvald *et al.*, 1997). Sterols with Δ^5, $\Delta^{5,7}$, $\Delta^{7,9(11)}$, Δ^8, $\Delta^{8(14)}$ *etc.* double bond(s) gave inactive compounds. Thus, the position and number of double bonds appear to be an important prerequisite for activity, which leads to the question whether there is a correlation between activity and the conformation of the skeleton as induced by the double-bond configuration.



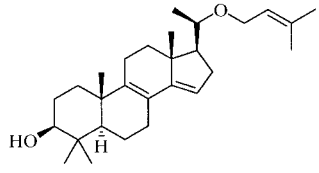
(I)



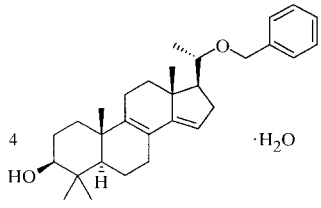
(II)



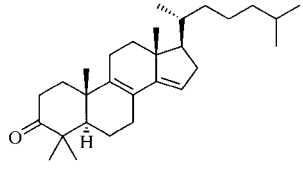
(III)



(IV)



(V)



(VI)

The side-chain structure appears to have an impact on activity as well. FF–MAS-related sterols show a similar structure–activity relationship with respect to the stereochemistry of C-20 as 1α,25-dihydroxy–vitamin D_3, 1α,25-$(OH)_2$-D_3. It was found that a derivative of 1α,25-$(OH)_2$-D_3 with an inverted stereochemistry at C-20 ('unnatural configuration') has a similar affinity for the vitamin D receptor (VDR) as the endogenous ligand 1α,25-$(OH)_2$-D_3 with a 'natural configuration' at C-20, and even has an increased transcriptional activity (Liu *et al.*, 1997; Väisänen *et al.*, 1999; Boullion *et al.*, 1995). Similarly, analogs of FF–MAS with unnatural C-20 stereochemistry are able to induce meiosis, provided that C-22 is replaced by an O atom. This is exemplified by compound (IV), *i.e.* (20*R*)-4,4-dimethyl-22-oxa-5α,20-cholesta-8,14,24-trien-3β-ol. In contrast, 22-oxa-sterols with the C-20 configuration, as in FF–MAS, are in general inactive, as is the case for compound (V), *i.e.* 4,4-dimethyl-23-phenyl-22-oxa-24-nor-5α-chola-8,14-dien-3β-ol–water (4/1). Examination of the crystal structures may provide an understanding of the underlying structure–activity relationships.

In this paper, we present the crystal structures of five FF–MAS-derived sterols (II)–(VI), where (II) is 4,4-dimethyl-23-phenyl-24-nor-5α-chola-8,14-dien-3β-ol, (III) is 4,4-dimethyl-22-phenyl-23,24-dinor-5α-chola-8,14-dien-3β-ol and (VI) is 4,4-dimethyl-5α-cholesta-8,14-dien-3-one. All five compounds have a $\Delta^{8,14}$ double-bond configuration and all but (VI) have a 3β-OH moiety. Compounds (II)–(IV) are active and (V) and (VI) are inactive in *in vitro* tests. The inactivity of compound (VI) is most likely caused by the absence of the mandatory 3β-OH group. Ellipsoid plots of the major components of one of the molecules in each structure are given in Fig. 1 as representative of that compound.

In the crystal structures of compounds (II)–(V), the hydroxyl groups form hydrogen bonds to other hydroxyl groups present in the structure. Different types of hydrogen-bonding schemes are found. The two independent molecules in the crystal structure of (II) form hydrogen bonds in a pairwise fashion, denoted by a *D* by the graph-set notation (Etter, 1990; Bernstein *et al.*, 1995). The hydroxyl H atoms are disordered over two positions in which one component donates a hydrogen bond to the other molecule. The second component does not participate in a hydrogen bond, as no hydrogen-bond acceptor was available within 3.6 Å. In the crystal structure of (III), hydrogen bonds are found in a cyclic arrangement of co-operative hydrogen bonds involving the two independent molecules and two of their symmetry-related copies [$R_4^4(8)$ in graph-set notation], with disordered directionality. The hydroxyl H atoms of compound (IV) form spiralling hydrogen-bond chains of which the central axis coincides with the 6_2 axis [$C(2)$ in graph-set notation], each H atom donating to the molecule on the α-side of the molecule. The O$\cdots$O hydrogen-bond distance is rather long, implying a weaker hydrogen bond. The long donor–acceptor distance is most likely a consequence of packing optimization of the bulky steroid moiety. The water molecule of the monohydrate structure of compound (V) is located at a special position, *i.e.* on the twofold rotation axis. The structure contains one potential hydrogen-bond donor more than there are potential hydrogen-bond acceptors. The free H atom was placed at electron density found on the twofold axis near the water O atom, taking into account a reasonable geometry. The hydroxyl groups of compound (V) and the other H atom of the water molecule form infinite co-operative hydrogen-bonded chains with disordered directionality. The chains consisted of three different motifs, which can be described by $DD_3^3(6)$ in unitary graph-set notation. The binary notation is $C_5^5(10)$.

To determine whether differences in the conformation of the skeleton can explain the differences in activity of double-bond isomers, the skeletal conformations of compounds (II)–(V) and of compound (VI) were compared with those of double-bond sterol isomers in the Cambridge Structural Database (CSD Version of October 2000, 224 400 entries; Allen & Kennard, 1993). The CSD was searched for compounds containing the four-ring sterol topology with androstane stereochemistry, having a varying number of double bonds at different positions. For the $\Delta^{8,14}$ structures presented here and that of an aromatic *C*-ring structure

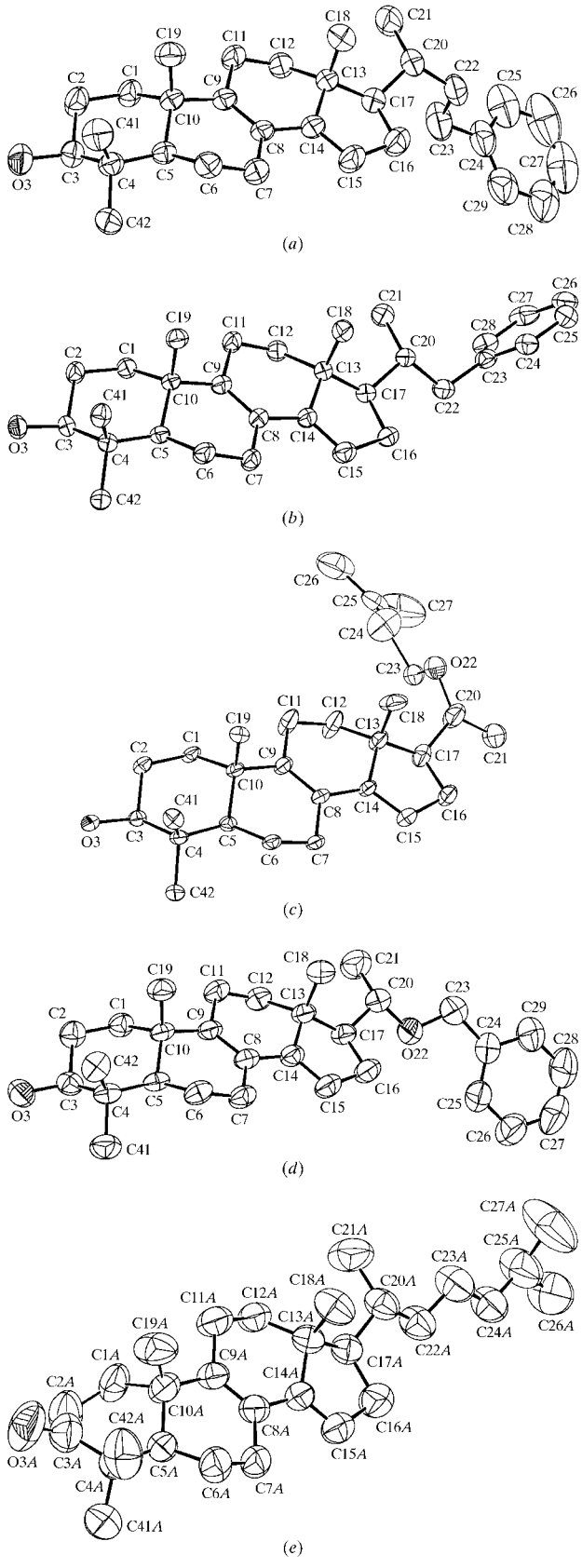


Figure 1
Displacement ellipsoid plots at the 50% probability level for (*a*) (II), (*b*) (III), (*c*) (IV), (*d*) (V) and (*e*) (VI). The disordered systems and the other independent molecules in each of the structures are similar. H atoms have been omitted for clarity.

(Ferguson *et al.*, 1982), a type of skeleton that can yield active compounds, it is found that the atomic positions of the superimposed skeletons are very similar. However, differences between the atomic positions of these classes of skeletons with skeleton classes which are known to give inactive compounds (*i.e.* the Δ^5 and fully saturated structures) are of the same magnitude. This finding indicates that, although the positions of the double bonds in the skeletons are important, the conformation of the skeleton is not the determining factor for the activity of double-bond FF–MAS isomers.

Although the skeletal structure of (V) satisfies the conditions required for activity discussed in the first paragraph, the compound is not active. Its lack of activity is therefore probably related to the structure of the side chain. In order to compare the side-chain conformations of the molecules in all five crystal structures, the skeletons of all the independent molecules were superimposed. A stereoview of the superposition of the skeleton atoms of crystal structures (II)–(V) is given in Fig. 2. Rings *A*, *B* and *C* were included in the fit but have been omitted from the figure for clarity. The independent molecules of compound (V) are shown in black, those of the other compounds in grey. Table 5 gives successive torsion angles in the side chains of the five structures for each of the independent molecules and for disordered side-chain conformations. The orientations of the side chains correspond to those found for other sterols with both an *R* and *S* C-20 stereochemistry (Nes *et al.*, 1984). Compounds (II), (III) and

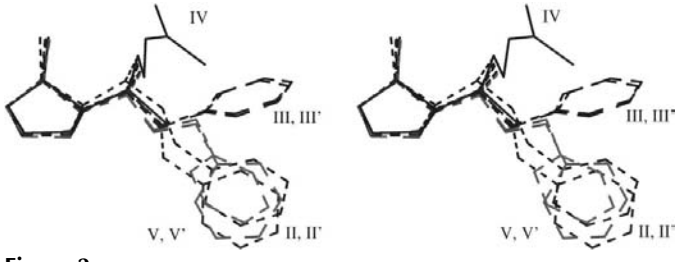


Figure 2
Stereoview of the side chains in the crystal structures of compounds (II)–(V) after superposition of the skeletons. Compound (V) is shown in grey, while compounds (II)–(IV) are shown in black. The primes indicate the independent molecules in the structures.

(V), with a natural configuration (see above) at C-20, crystallize with the side chain extended pointing away from ring *D* (the so-called 'north-east' orientation). The orientation of the side chain in the structure of (IV), which has un unnatural C-20 stereochemistry, is called the 'north-west' orientation and points upwards from the plane of the paper in the Scheme. It can be seen from Fig. 2 that the phenyl rings of the side chains of compound (II) occupy the same region in space as those of inactive compound (V). The side chains of the active compounds (II), (III) and (IV) occupy different regions in space. The active conformation(s) can therefore not be distilled from the crystal structure conformations. Either the active conformation of compound (II) is a conformation present in solution but not in the crystal structure, or compound (V) is mainly present in another (inactive) conformation in solution.

An interesting aspect of the aromatic *C*-ring structure mentioned above is the orientation of the side chain with respect to the skeleton. The C17—C20 bond of the aromatic *C*-ring structure points upwards with respect to the plane through the skeleton rings (Fig. 3). This is also found for 1α,25-

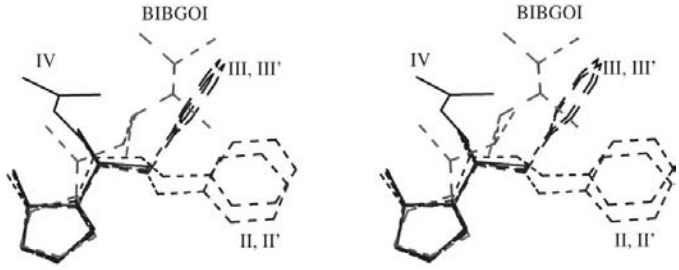


Figure 3
Stereoview of the side chains of in the crystal structure of compounds (II)–(IV) (grey) and the structure of the aromatic *C*-ring structure 22α,23α-dibromo-18-nor-12-methyl-5α,17β-ergosta-8,11,13(14)-triene-3β-*tert*-butyldimethylsylyl ether (Ferguson *et al.*, 1982) (black).

$(OH)_2$-D_3 bound to its receptor (Rochel *et al.*, 2000). By rotation of the C17—C20 torsion angle, the conformation of the side chains of compounds (I)–(VI) can change such that the direction is similar to the side chain of the aromatic *C*-ring structure. NMR experiments are planned to gain insight into the conformational behaviour of the compounds in solution.

Experimental

Colourless single crystals of compounds (II), (III) and (V) suitable for X-ray analysis were obtained by vapour diffusion of a pyridine solution into acetonitrile. Crystals formed after several days. Colourless single crystals of compounds (IV) and (VI) were obtained by slow evaporation of ethanol solutions.

Compound (II)

Crystal data

$C_{31}H_{44}O$	D_x = 1.130 Mg m^{-3}
M_r = 432.66	Mo $K\alpha$ radiation
Monoclinic, $P2_1$	Cell parameters from 114 reflections
a = 7.271 (3) Å	θ = 3.1–16.8°
b = 9.733 (5) Å	μ = 0.07 mm^{-1}
c = 35.98 (2) Å	T = 150 K
β = 93.28 (4)°	Block, colourless
V = 2542 (2) Å^3	0.3 × 0.3 × 0.3 mm
Z = 4	

Data collection

Enraf–Nonius κ-CCD diffractometer	R_{int} = 0.048
φ and ω scans with κ offsets	θ_{max} = 25.3°
15 221 measured reflections	$h = -8 \rightarrow 8$
4842 independent reflections	$k = -11 \rightarrow 9$
4455 reflections with $I > 2\sigma(I)$	$l = -43 \rightarrow 41$

2Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.0516P)^2 + 0.49P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R(F) = 0.044$	$(\Delta/\sigma)_{max} = 0.001$
$wR(F^2) = 0.116$	$\Delta\rho_{max} = 0.18$ e Å^{-3}
$S = 1.10$	$\Delta\rho_{min} = -0.14$ e Å^{-3}
4842 reflections	
590 parameters	
H-atom parameters constrained	

Table 1
Hydrogen-bonding geometry (Å, °) for (II).

D—H···A	D—H	H···A	D···A	D—H···A
O3—H31C···O3A^{i}	0.96	2.06	2.890 (3)	143
O3A—H32A···O3^{ii}	0.96	1.96	2.890 (3)	164

Symmetry codes: (i) $x, y-1, z$; (ii) $x, 1+y, z$.

Compound (III)

Crystal data

$C_{30}H_{42}O$	$D_x = 1.061$ Mg m^{-3}
$M_r = 418.64$	Mo $K\alpha$ radiation
Monoclinic, $C2$	Cell parameters from 32 980 reflections
$a = 23.785$ (9) Å	$\theta = 2.0$–$28.4°$
$b = 13.610$ (3) Å	$\mu = 0.06$ mm^{-1}
$c = 19.080$ (7) Å	$T = 150$ K
$\beta = 121.927$ (12)°	Block, colourless
$V = 5242$ (3) Å^3	0.2 × 0.2 × 0.1 mm
$Z = 8$	

Data collection

Enraf–Nonius κ-CCD diffractometer	$R_{int} = 0.049$
φ and ω scans with κ offsets	$\theta_{max} = 25.3°$
29 708 measured reflections	$h = -28 \rightarrow 28$
4917 independent reflections	$k = -15 \rightarrow 15$
4763 reflections with $I > 2\sigma(I)$	$l = -22 \rightarrow 22$

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.0671P)^2 + 2.59P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R(F) = 0.046$	$(\Delta/\sigma)_{max} = 0.034$
$wR(F^2) = 0.119$	$\Delta\rho_{max} = 0.27$ e Å^{-3}
$S = 1.09$	$\Delta\rho_{min} = -0.29$ e Å^{-3}
4917 reflections	
559 parameters	
H-atom parameters constrained	

Table 2
Hydrogen-bonding geometry (Å, °) for (III).

D—H···A	D—H	H···A	D···A	D—H···A
O3—H3B···O3A^{i}	0.96	2.01	2.855 (3)	146
O3—H3C···O3A^{ii}	0.96	1.81	2.749 (4)	167
O3A—H32A···O3^{iii}	0.96	1.96	2.855 (3)	155
O3A—H33A···O3^{iv}	0.96	1.85	2.749 (4)	154

Symmetry codes: (i) $-x, y-1, -z$; (ii) $x, y-1, z$; (iii) $-x, 1+y, -z$; (iv) $x, 1+y, z$.

Compound (IV)

Crystal data

$C_{28}H_{44}O_2$	Mo $K\alpha$ radiation
$M_r = 412.63$	Cell parameters from 45 reflections
Hexagonal, $P6_2$	$\theta = 3.8$–$11.5°$
$a = 24.661$ (10) Å	$\mu = 0.07$ mm^{-1}
$c = 7.232$ (10) Å	$T = 150$ K
$V = 3809$ (6) Å^3	Block, colourless
$Z = 6$	0.6 × 0.2 × 0.2 mm
$D_x = 1.079$ Mg m^{-3}	

Data collection

Enraf–Nonius κ-CCD diffractometer	$R_{int} = 0.045$
φ and ω scans	$\theta_{max} = 24.8°$
13 233 measured reflections	$h = -28 \rightarrow 18$
2343 independent reflections	$k = -28 \rightarrow 28$
2161 reflections with $I > 2\sigma(I)$	$l = -8 \rightarrow 6$

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.001P)^2 + 8.00P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R(F) = 0.075$	$(\Delta/\sigma)_{max} < 0.001$
$wR(F^2) = 0.140$	$\Delta\rho_{max} = 0.52$ e Å^{-3}
$S = 1.05$	$\Delta\rho_{min} = -0.36$ e Å^{-3}
2343 reflections	
275 parameters	
H-atom parameters constrained	

Table 3
Hydrogen-bonding geometry (Å, °) for (IV).

D—H···A	D—H	H···A	D···A	D—H···A
O3—H3O···O3^{i}	0.82	2.18	2.979 (8)	165

Symmetry code: (i) $-x+y, 1-x, \frac{1}{3}+z$.

Compound (V)

Crystal data

$4C_{30}H_{42}O_2{\cdot}H_2O$	$D_x = 1.112$ Mg m^{-3}
$M_r = 1756.56$	Mo $K\alpha$ radiation
Monoclinic, $C2$	Cell parameters from 47 reflections
$a = 64.902$ (4) Å	$\theta = 3.3$–$12.7°$
$b = 7.2926$ (14) Å	$\mu = 0.07$ mm^{-1}
$c = 11.1447$ (11) Å	$T = 293$ K
$\beta = 96.055$ (13)°	Block, colourless
$V = 5245.4$ (12) Å^3	0.20 × 0.20 × 0.15 mm
$Z = 2$	

Data collection

Enraf–Nonius κ-CCD diffractometer	$R_{int} = 0.048$
φ and ω scans	$\theta_{max} = 25.3°$
17 152 measured reflections	$h = -64 \rightarrow 77$
5110 independent reflections	$k = -8 \rightarrow 7$
3899 reflections with $I > 2\sigma(I)$	$l = -13 \rightarrow 13$

Refinement

Refinement on F^2	$w = 1/[\sigma^2(F_o^2) + (0.0324P)^2 + 2.90P]$ where $P = (F_o^2 + 2F_c^2)/3$
$R(F) = 0.045$	$(\Delta/\sigma)_{max} = 0.001$
$wR(F^2) = 0.106$	$\Delta\rho_{max} = 0.12$ e Å^{-3}
$S = 1.03$	$\Delta\rho_{min} = -0.12$ e Å^{-3}
5110 reflections	
596 parameters	
H-atom parameters constrained	

Table 4
Hydrogen-bonding geometry (Å, °) for (V).

D—H···A	D—H	H···A	D···A	D—H···A
O3A—H3C···O3A^{i}	0.96	2.01	2.882 (3)	150
O3A—H3D···O3^{ii}	0.96	2.22	2.816 (3)	119
O3—H31O···O40^{iii}	0.96	2.01	2.831 (3)	143
O3—H31O···O40^{iv}	0.96	2.01	2.831 (3)	143
O3—H32O···O3A^{ii}	0.96	2.00	2.816 (3)	142
O40—H40B···O3^{v}	0.89	2.15	2.831 (3)	133

Symmetry codes: (i) $2-x, y, 2-z$; (ii) $2-x, y, 1-z$; (iii) $x, y-1, z$; (iv) $2-x, y-1, 1-z$; (v) $2-x, 1+y, 1-z$.

Compound (VI)

Crystal data

$C_{29}H_{46}O$
M_r = 410.66
Orthorhombic, $P2_12_12_1$
a = 10.5743 (7) Å
b = 20.941 (17) Å
c = 23.70 (2) Å
V = 5247 (6) Å^3
Z = 8
D_x = 1.040 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 42 635 reflections
θ = 3.0–26.5°
μ = 0.06 mm^{-1}
T = 293 K
Block, colourless
0.20 × 0.20 × 0.15 mm

Data collection

Enraf–Nonius κ-CCD diffractometer
φ and ω scans (κ = 0°)
37 782 measured reflections
5260 independent reflections
3231 reflections with $I > 2\sigma(I)$
R_{int} = 0.099
θ_{max} = 25.3°
$h = -12 \rightarrow 12$
$k = -25 \rightarrow 25$
$l = -28 \rightarrow 28$

Refinement

Refinement on F^2
$R(F)$ = 0.051
$wR(F^2)$ = 0.112
S = 1.01
5260 reflections
574 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.01P)^2 + 1.50P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.15 e Å^{-3}
$\Delta\rho_{min}$ = −0.12 e Å^{-3}
Extinction correction: *SHELXL*97
Extinction coefficient: 0.0144 (10)

Table 5
Side-chain torsion angles of the independent and disordered molecules in the crystal structures of (II)–(VI).

Primes (′) indicate a second independent molecule if present, while an asterisk indicates the less populated conformation of a disordered side chain.

Molecule	C17—C20	C20—*X*22	*X*22—C23	C23—C24
(II)	179.2 (3)	59.0 (4)	168.7 (3)	−80.7 (5)
(II′)	172.7 (2)	63.7 (3)	−179.9 (3)	87.5 (4)
(III)	179.4 (3)	−177.7 (3)	79.2 (4)	
(III′)	174.3 (3)	−173.9 (3)	−100.3 (4)	
(IV)	−32.7 (10)	−79.4 (8)	169.8 (6)	147.2 (10)
(IV)*	−72.1 (7)	163.0 (8)	−154.2 (8)	−132.2 (11)
(V)	175.4 (3)	−161.7 (4)	156.2 (4)	127.5 (4)
(V′)	179.7 (3)	−159.5 (3)	168.3 (3)	−9.4 (5)
(VI)	−178.5 (3)	−179.5 (3)	179.0 (4)	171.0 (4)
(VI′)	−178.9 (3)	−174.8 (3)	179.2 (4)	169.2 (5)
(VI′)*	−178.9 (3)	−174.8 (3)	179.2 (4)	−160.4 (18)

Due to the absence of significant anomalous dispersion, the absolute structure could not be determined reliably. The configurations were chosen such that for each compound the stereochemistry of the skeleton corresponds to those of related androstanes. Friedel pairs were merged for each structure. H atoms were introduced at calculated positions, riding on their carrier atoms. The methyl groups were refined as rigid groups, allowing for rotation around the C—C bonds. The hydroxyl H atoms were also treated as rigid groups. Their initial positions were determined from a circular residual-density map. H atoms were refined with a fixed isotropic displacement parameter related to the value of the equivalent isotropic displacement parameter of the carrier atom by a factor of 1.5 for the methyl and hydroxyl H atoms and 1.2 for all other H atoms. The structure of (III) contains a disordered solvent region (15 e, 170 Å^3) that could not be interpreted. A new set of structure factors was calculated using the *SQUEEZE* algorithm in *PLATON* (van der Sluis & Spek, 1990). Atoms C25*A*, C26*A* and C27*A* show large anisotropy, $(U^3/U^1)_{max}$ = 16.7 for C26*A*. Schomaker & Trueblood (1968) **TLS** analysis on C22*A* and the phenyl ring resulted in a maximum libration amplitude $<\pi^2>$ = 152. Since the electron-density map did not show separate peaks, a disorder model was not thought appropriate. The two independent molecules in the crystal structure of (VI) are both disordered and are related by intermolecular contacts. A flip of ring *A* from a chair to a twist-boat conformation of one of the molecules causes a 30° rotation over the C-24 to C-25 dihedral in the other. The occupancy factors of the disorder components in both molecules were linked.

For all compounds, data collection: *COLLECT* (Nonius, 1998); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *PLATON* (Spek, 2001); software used to prepare material for publication: *PLATON*. For compounds (II), (IV) and (V), cell refinement: *DiRaX* (Duisenberg, 1992); data reduction: *EVAL*14 (Duisenberg, 1998). For compounds (III) and (VI), cell refinement: *DENZO* (Otwinowski & Minor, 1997); data reduction: *DENZO*.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: GG1052). Services for accessing these data are described at the back of the journal.

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