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NMR and X-ray studies of isomeric 22,23-dihydroxy stigmastanes

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ABSTRACT

A comparative conformational study of steroidal side chain of (22*R*,23*R*)- and (22*S*,23*S*)-dihydroxy stigmastane derivatives was performed using single crystal X-ray diffraction and NMR spectroscopy. The preferred conformation in solution was shown to be close to that in the crystal. (22*R*,23*R*)-Isomers typical for natural plant steroid hormones brassinosteroids adopt a conformation in which both hydroxyl groups are pointed toward unhindered α-side of the steroidal plane and can thus participate in biochemical processes. Unnatural (22*S*,23*S*)-counterparts exhibit a conformation with the two hydroxyl groups oriented in the opposite direction and sterically hindered by 21-methyl group and terminal side chain fragment.

1. Introduction

Oxygenated sterols form a large group of naturally occurring compounds, among which an important place belongs to steroidal plant hormones brassinosteroids (BS). These are a group of about 60 different steroids that are widely distributed in plants affecting many aspects of their growth and development [1]. Most BS have a dihydroxylated side chain as an almost obligatory structural element having a significant influence on their biological activity and many of them have a C-29 carbon skeleton. Only BS possessing a (22*R*,23*R*)-diol function reveal high activity whereas the corresponding (22*S*,23*S*)-counterparts are 2–3 magnitudes less active [2–4]. The underlying reason of this differential activity is evidently associated with the difference in spatial orientation of substituents and carbon backbone conformation of the side chain.

The preferred conformations of BS side chain were subject of numerous studies, especially those involving molecular modeling approaches [5–11]. The obtained conclusions can be based on some speculations and should be verified experimentally. Probably the most accurate way to get insight into spatial aspects of this part of BS molecule is a combination of NMR and X-ray diffraction. A comparative study of (22*R*,23*R*)- and (22*S*,23*S*)-BS side chain conformations in the present work was carried out by the example of natural phytohormone homocastasterone **1** and its (22*S*,23*S*)-analogue **2** as well as model compounds **3**–**6** (Fig. 1). The absence of hydroxyl groups in the cycle A in molecules of **3**–**6** enabled easier analysis and assignment of the corresponding NMR spectra. Position and form of signals of H-22 and H-23 protons for (22*R*,23*R*)-isomers **1**,**3**,**5** and (22*S*,23*S*)-isomers **2**,**4**,**6** was independent of substitution pattern of A-ring. This was evidence that data on side chain conformations obtained for model compounds could be extrapolated for homocastasterone **1** and its (22*S*,23*S*)-analogue **2**.

2. Experimental

2.1. Materials and preparation of crystals

The compounds **1**–**6** were synthesized according to the published procedures [12,13]. Attempts to prepare crystals suitable for X-ray diffraction analysis were carried out with all compounds **1**–**6**, but for (22*S*,23*S*)-diol **4** and (22*R*,23*R*)-diol **5** colorless single crystals suitable for X-ray analysis were obtained by slow solvent evaporation from a solution in EtOAc-hexane (for diol **4**) and in benzene (for diol **5**) at room temperature.

2.2. Crystal structure determination and refinement

X-ray data for **4** and **5** were collected at room temperature on a Nicolet R3 m diffractometer (graphite-monochromated Mo Kα radiation, ω-2θ scans). The structures were solved by direct methods using program SIR2004 [14]. Refinement on F^2 was carried out by full matrix least-squares techniques (SHELXL-97 [15]). Anisotropic displacement parameters were used for all non-hydrogen

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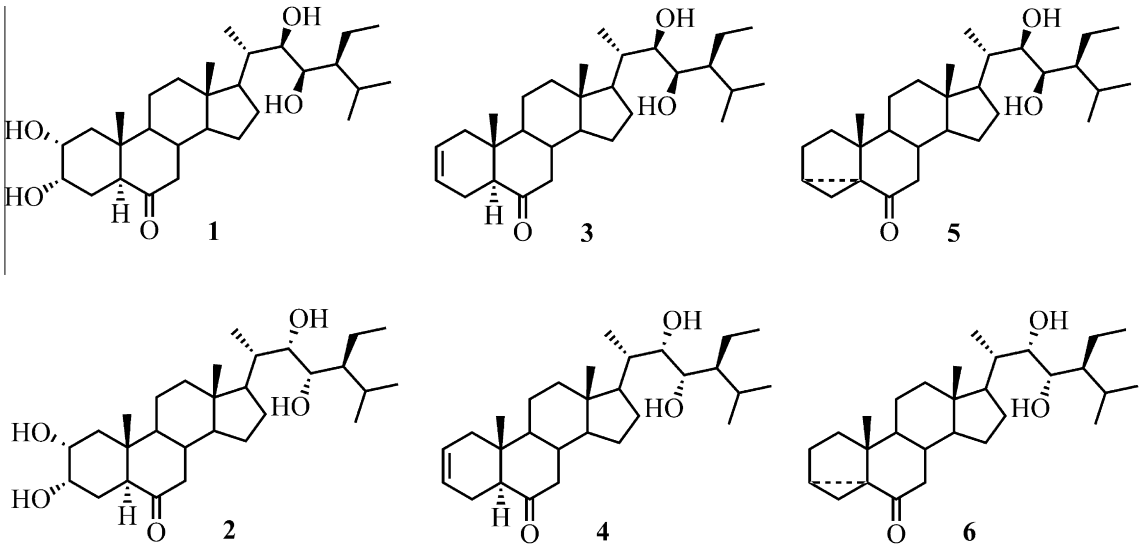

Fig. 1. The chemical structures of homocastasterone (**1**), (22*S*,23*S*)-homocastasterone (**2**) and model compounds (**3**–**6**).

atoms. For **4** and **5**, all hydrogen atoms were introduced in calculated positions (excluding hydroxyl H atoms in **5**, determined from a difference map) and refined using "riding" model, with U_{iso}(H) equal to $1.5U_{eq}$ of carrier atoms for the methyl and hydroxyl groups, and $1.2U_{eq}$(C) for other H atoms. Because of the absence of any significant anomalous scatterers, the absolute configurations of the molecules were not determined from the X-ray data. Therefore, the Friedel pairs were merged before the final refinement and the absolute configuration was assigned to correspond to the known chiral centres in a precursor molecule, which remained unchanged during the synthesis of **4** and **5**. Main crystal data and refinement details for **4** and **5** are given in Table 1.

2.3. NMR spectroscopy

NMR spectra were recorded in $CDCl_3$ and C_6D_6 at room temperature using a Bruker Avance DRX-500 spectrometer. Chemical shifts are given on the δ-scale with TMS as internal standard. In the one-dimensional (1D) measurements (^1H, ^{13}C, DEPT) 64 K data points were used for the FID. The pulse programs of the two-dimensional (2D) experiments ((gs)-COSY, TOCSY, gs-HMQC, 13C-coupled gs-HMQC, ROESY, gs-HMBC and 2D-INAPT) were obtained from the Bruker software library.

3. Results and discussion

3.1. Crystal structure

Compound **4** crystallizes in the orthorhombic $P2_12_12_1$ space group, with one molecule in the asymmetric unit. Fig. 2 shows molecular structure of **4**. The ring A adopts a half-chair conformation, rings B, C have a chair form, and ring D reveals C13–C14 twist conformation. Geometry of the rings C and D is typical of steroids. Hydroxyl group at C22 and C17 atom are in a *trans* position to each other, with a dihedral angle C17–C20–C22–O22 of −161.0(3)°. The conformation of the side chain is stabilized by intramolecular hydrogen bond 23(OH)···O22 (Table 2). Intermolecular hydrogen bonds 22(OH)···O6 link molecules together forming polymeric chains running along the *c* axis (Fig. 3). Only van der Waals interactions are between the chains.

Compound **5** crystallizes in the triclinic space group *P*1. There are four molecules in the asymmetric unit (atom numbering of corresponding atoms of the molecules differs in the first digit indicating a number of an independent molecule). Fig. 4 shows the molecular structure of one of them. Five-membered rings A (C*n*01, C*n*02, C*n*03, C*n*05, C*n*10 where $n = 1, 2, 3, 4$) have C*n*01 envelope form. Rings B in all molecules have similar conformation being intermediate between the chair and half-chair ones. All C rings are close to chair conformation. As to the rings D, the ring for $n = 3$ is C313 envelop, whereas all other rings are twisted on C*n*13–C*n*14. Dihedral angle analysis in the side chains showed that their conformations are similar showing only slight differences in all independent molecules.

Table 1
Crystal data and structure refinement details for **4** and **5**.

Compound	**4**	**5**
Empirical formula	$C_{29}H_{48}O_3$	$C_{29}H_{48}O_3$
Formula weight	444.67	444.67
Crystal system	Orthorhombic	Triclinic
Space group	$P2_12_12_1$	$P\bar{1}$
Unit cell dimensions		
a, Å	12.533(3)	13.945(3)
b, Å	13.054(3)	14.668(4)
c, Å	16.521(4)	16.886(4)
α, °	90	65.562(18)
β, °	90	66.400(17)
γ, °	90	64.229(17)
V, Å^3	2702.9(11)	2727.4(11)
Z	4	4
Density (calculated), Mg m^{-3}	1.093	1.083
Absorption coefficient, mm^{-1}	0.068	0.068
F(0 0 0)	984	984
Crystal size, mm^3	0.54 × 0.53 × 0.38	0.52 × 0.44 × 0.42
θ Range for data collection	1.99–27.57°	1.38–26.56°
Reflections collected	7471	11,881
Independent reflections	3504 [R_{int} = 0.0296]	11,393 [R_{int} = 0.0358]
Data/restraints/parameters	3504/0/298	11,393/3/1201
Goodness-of-fit on F^2	1.000	1.013
Final *R* indices [$I > 2\sigma(I)$]	*R*1 = 0.0559, w*R*2 = 0.1372	*R*1 = 0.0502, w*R*2 = 0.1166
R indices (all data)	*R*1 = 0.1292, w*R*2 = 0.1730	*R*1 = 0.0722, w*R*2 = 0.1318
Extinction coefficient	0.015(2)	–
Largest diff. peak and hole e, Å^{-3}	0.127 and −0.100	0.141 and −0.213

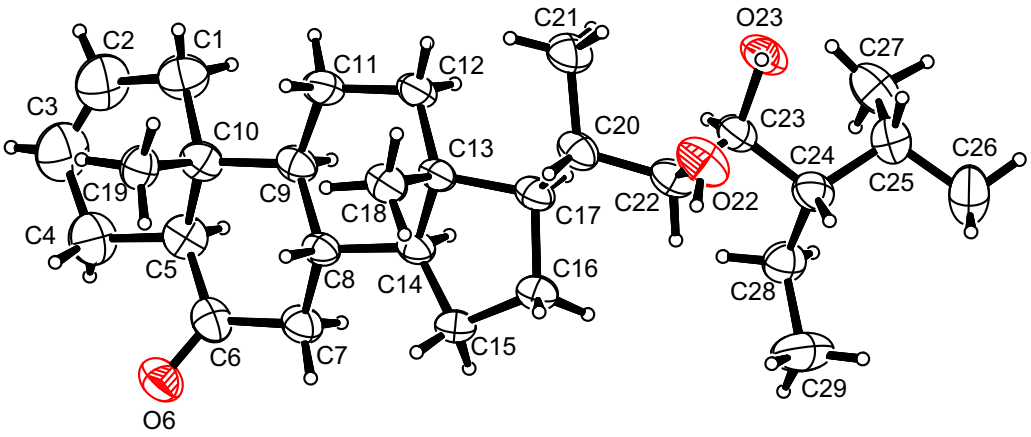

Fig. 2. The molecular structure of compound (**4**), showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

Table 2
Hydrogen bonding geometry for (**4**) [Å and °][a].

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
O22–H22A···O6#1	0.82	1.93	2.742(5)	171
O23–H23C···O22	0.82	2.02	2.539(4)	120

[a] Symmetry transformations used to generate equivalent atoms: #1 $-x+3/2, -y, z+1/2$.

Numerous hydroxyl–carbonyl and hydroxyl–hydroxyl hydrogen bonds (Table 3) are responsible for formation of three-dimensional network. Analysis of possible voids shows that there are potential solvent areas of ca 5% (vol.) in the crystal structure of **5**. However no solvent molecules are inside them.

3.2. Spectroscopy

The ^{1}H and ^{13}C chemical shifts of compounds **1**–**4** in $CDCl_3$ are collected in Table 4. For correct assignment of NOESY cross peaks, spectra in C_6D_6 were also recorded (not shown). NMR assignments for compounds **5** and **6** were reported by us earlier [12]. It appeared that NMR spectra of isomeric pairs of (22*R*,23*R*)- and (22*S*,23*S*)-diols differed noticeably in signals attributable to the side chain and cycle D only. A characteristic difference was found in ^{13}C NMR between signals of C-20 (more than 5 ppm) and C-24 (more than 3 ppm) being downshifted for (22*R*,23*R*)-isomers. Detailed analysis of coupling constants and NOE correlations of the side chain protons showed that these NMR data were essentially the same within compounds belonging to each of the two isomeric groups. Thus, the (22*R*,23*R*)-diols revealed coupling constants $J_{22,23} = 8.7$ Hz, $J_{20,22} \leqslant 1.5$ Hz, $J_{23,24} \leqslant 1.5$ Hz, whereas for the (22*S*,23*S*)-diols the corresponding values were 1.5, 6.0, and 7.0 Hz, respectively. The results of NOESY experiments for **1**–**6** are summarized in Table 5. As follows from these data, orientation of the steroidal side chain part adjacent to the cycle D is not dependent on the stereochemistry at C-22. In both cases C-22 hydrogen is located near C-16 and C-20 hydrogens and opposite to C-21, because no NOESY correlation was observed between H-22 and H-21. On the contrary, H-23 is located near C-21 methyl group based on the NOESY data.

It should be noted that similar findings were observed for brassinolide analogues having a trans-22,23-diol group [7]. NOESY experiment showed a through space correlation of H-22 with H-16 and H-20 independently on the C-22 stereochemistry. Probably steric and electronic factors affecting the interaction of C-22 hydroxy group with the steroidal skeleton and 21-methyl group have a decisive influence on the orientation of substituents around C-20–C-22 bond.

The situation is different with conformation around the C22–C23 bond which to a great extent is defined by the stereochemistry. In this case for (22*R*,23*R*)-isomers the most stable is conformer,

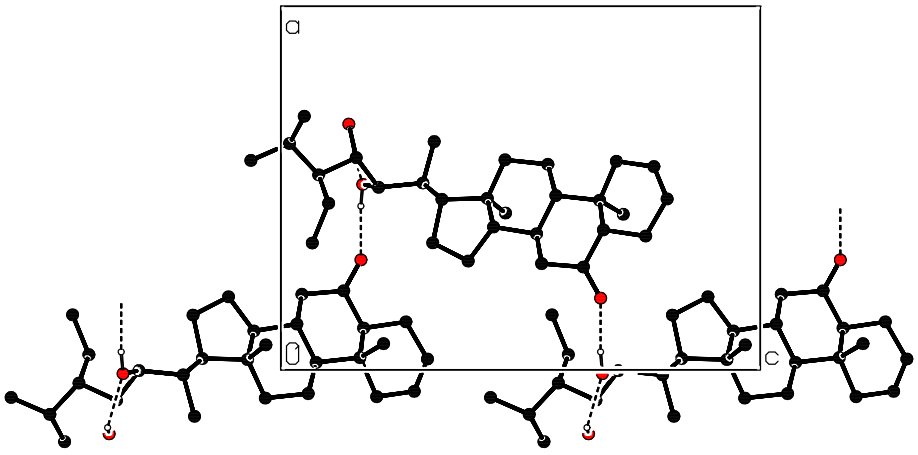

Fig. 3. A hydrogen bonded polymeric chain viewed along the *b* axes. Hydrogen bonds are shown by dashed lines (only H atoms participating in hydrogen bonds are shown).

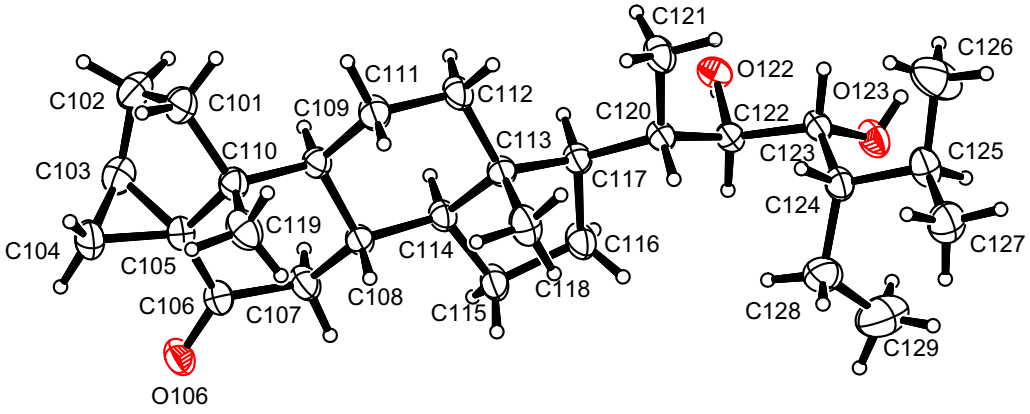

Fig. 4. One of four independent molecules in the crystal structure of (**5**). Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

Table 3
Hydrogen bonding geometry for (**5**) [Å and °][a].

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
O122–H22V···O306	0.82	2.06	2.849(4)	161
O123–H23V···O206	0.82	2.12	2.921(4)	167
O222–H22X···O406#1	0.82	1.98	2.796(4)	175
O223–H23X···O322#2	0.81	2.02	2.811(3)	167
O322–H22Y···O106#3	0.84	1.95	2.782(4)	169
O323–H23Y···O222#4	0.83	2.00	2.814(4)	164
O422–H22Z···O206#5	0.83	2.19	2.972(4)	158
O423–H23Z···O122#5	0.81	2.06	2.852(3)	164

[a] Symmetry transformations used to generate equivalent atoms: #1 *x*, *y*, *z* + 1; #2 *x* − 1, *y*, *z* + 1; #3 *x*, *y*, *z* − 1; #4 *x* + 1, *y*, *z* − 1; #5 *x*, *y* − 1, *z*.

in which H22 and H23 are trans-oriented (based on $J_{22,23}$ = 8.7 Hz and H22–H24 and H22–H20 NOESY correlations).

The two hydroxy groups have a gauche orientation that serves as a stabilizing factor of the side chain conformation. An additional stabilizing interaction is formation of 22(OH)···23(O) hydrogen bond. Molecular visualizing based on the experimental results showed that part of the steroid side chain starting with C23 for (22*R*,23*R*)-isomer is β-oriented. The corresponding part of the side chain of (22*S*,23*S*)-isomer is oriented in the opposite direction to the α-surface of the steroidal plane.

A comparison of conformation in solid state and in solution revealed that the molecules of 22,23-dihydroxy stigmastanes have similar conformation in both cases. Natural (22*R*,23*R*)-isomers adopt a conformation in which both hydroxy groups are pointed toward unhindered α-side of the steroidal plane. Such a conformation is of biological significance, since it enables participation of unhindered α-oriented 22 and 23 hydroxy groups in biochemical processes in plants. Unnatural (22*S*,23*S*)-counterparts exhibit a conformation with the two hydroxy groups oriented in the oppo-

Table 4
The ^{1}H and ^{13}C chemical shifts for (**1**–**4**).

Atom	**1**		**2**		**3**		**4**	
	C	H (α/β)	C	H (α/β)	C	H (α/β)	C	H (α/β)
1	40.19	1.56/1.76	40.18	1.55/1.76	39.46	2	39.31	2.0
2	68.30	3.77	68.27	3.77	124.48	5.57	124.48	5.57
3	68.39	4.05	68.37	4.05	124.9	5.61	124.90	5.68
4	26.29	1.92/1.73	26.24	1.93/1.72	21.68	2.03/2.24	21.68	2.08/2.25
5	50.69	2.70	50.71	2.68	53.79	2.36	53.81	2.34
6	212.11		212.00		212.08		211.98	
7	46.71	2.01/2.31	46.71	2.00/2.31	46.91	1.99/2.36	46.91	1.99/2.36
8	37.73	1.78	37.63	1.79	37.75	1.74	37.63	1.74
9	53.64	1.41	53.64	1.40	53.28	1.31	53.31	1.32
10	42.60		42.55		40.02		39.97	
11	21.22	1.67/1.33	21.18	1.67/1.36	21.11	1.63/1.44	21.07	1.62/1.44
12	39.40	1.30/2.03	39.33	1.26/2.08	39.29	1.3/2.03	39.4	1.28/2.09
13	42.78		43.57		42.63		43.3	
14	56.55	1.33	56.26	1.25	56.59	1.3	56.32	1.24
15	23.82	1.58/1.11	24.19	1.54/1.12	23.8	1.6/1.1	24.16	1.59/1.14
16	27.64	1.98/1.28	27.79	1.79/1.40	27.6	1.97/1.3	27.76	1.78/1.40
17	52.44	1.60	52.56	1.25	52.48	1.58	52.56	1.25
18	11.88	0.68	11.92	0.70	11.77	0.69	11.8	0.71
19	13.58	0.76	13.56	0.76	13.44	0.72	13.47	0.72
20	36.92	1.52	42.30	1.76	36.9	1.53	42.29	1.77
21	11.93	0.91	14.12	1.03	11.92	0.92	14.07	1.04
22	74.54	3.59	72.10	3.62	74.52	3.59	72.07	3.63
23	72.73	3.72	70.60	3.60	72.7	3.72	70.53	3.6
24	46.23	1.09	49.58	1.21	46.25	1.1	49.58	1.21
25	28.78	1.85	26.89	2.10	28.8	1.85	26.85	2.09
26	21.28	0.97	21.73	0.945	21.23	0.97	21.72	0.94
27	19.41	0.96	17.67	0.88	19.52	0.96	17.66	0.87
28	18.84	1.46, 1.35	18.53	1.37, 1.09	18.82	1.36, 1.47	18.49	1.38, 1.1
29	13.46	0.95	14.54	0.96	13.43	0.95	14.48	0.96

Table 5
Selected NOESY correlations observed for (**1**–**6**) in $CDCl_3$[a].

Atom H	Correlated atoms										
	16	20	21	22	23	24	25	26	27	28	29
22	s (s)[b]	s (s)	n (n)	–	n (s)	w (s)	n (s)	n (n)	s (n)	s (s)	s (n)
23	n (n)	n (n)	s (s)	n (s)	–	s (s)	w (n)	s (n)	n (s)	n (s)	n (n)
24	n (n)	s (n)	n (n)	w (n)	s (n)	–	s (s)	n (n)	n (n)	n (n)	s (n)

[a] Results shown without parentheses are for (22*R*,23*R*)-isomers (**1**,**3**,**5**), while those in parentheses are for (22*S*,23*S*)-diols (**2**,**4**,**6**).
[b] s – strong, w – weak, n – none.

site direction and sterically hindered by 21-methyl group and terminal side chain fragment. An approach to both hydroxy groups in this conformation is hindered by 21-methyl group and terminal side chain fragment.

4. Supplementary material

Further details of crystal structure determination can be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk) on request. Deposition No. CCDC 765794 and CCDC 765795 for **4** and **5**, respectively.

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