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Absolute configuration of 14β-hydroxy-3β-*O*-(L-thevetosyl)-5β-card-20(22)-enolide chloroform disolvate

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Key indicators

Single-crystal X-ray study
T = 293 K
Mean σ(C–C) = 0.009 Å
Disorder in solvent or counterion
R factor = 0.077
wR factor = 0.227
Data-to-parameter ratio = 15.7

For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

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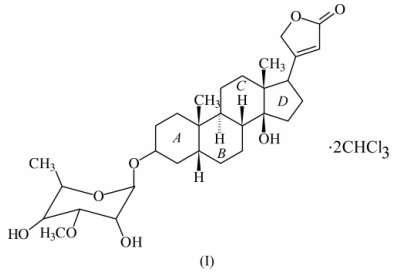
The structure of 14β-hydroxy-3β-*O*-(L-thevetosyl)-5β-card-20(22)-enolide, $C_{30}H_{46}O_8$, (I), has been reported previously [Chantrapromma *et al.* (2003). *Acta Cryst.* C**59**, o68–o70] in the assumed L form. In order to establish the absolute configuration of this important naturally occurring triterpenoidal glycoside, we have successfully incorporated chloroform molecules in the crystal structure by using chloroform as a solvent during crystallization, *viz*. $C_{30}H_{46}O_8{\cdot}2CHCl_3$, (I). The present X-ray study confirms that the previously assumed stereochemistry, *viz*. the L form, is correct. As previously reported, the steroid nucleus has a *cis*/*trans*/*cis* configuration. However, the present structure differs from that reported earlier in the orientations of the lactone ring and glycosidic linkages.

Comment

Medicinally important *Cerbera odollam Gearnth* (*Apocynaceae*) is widely distributed in the South-East Asian and Indian Ocean regions. The leaves and fruit of this plant possess cardiotonic properties, antibacterial activity, anticancer activity and have effects on the central nervous system (Chen & Steldt, 1942; Hien *et al.*, 1991; Lasserre *et al.*, 1992; Laphookhieo *et al.*, 2001). The title compound, (I), a cardenolide glycoside was previously isolated by us from the seeds of *Cerbera odollam* and it showed potent activity against human breast-cancer cell lines, human lung-cancer cell lines and human oral epidermoid carcinoma. The X-ray structure determination showed that it crystallized together with one methanol and one water molecule, in space group *P*1 (Chantrapromma *et al.*, 2003). However, the absolute configuration could not be determined as the structure did not contain heavy atoms. In order to establish the absolute stereochemistry of this important naturally occurring triterpenoidal glycoside, we have successfully incorporated chloroform molecules in compound (I) by using chloroform as a solvent during crystallization. We report here the results of the X-ray structure determination.



(I)

The asymmetric unit contains one cardenolide molecule, (I) (Fig. 1) and two chloroform molecules. The geometries of the

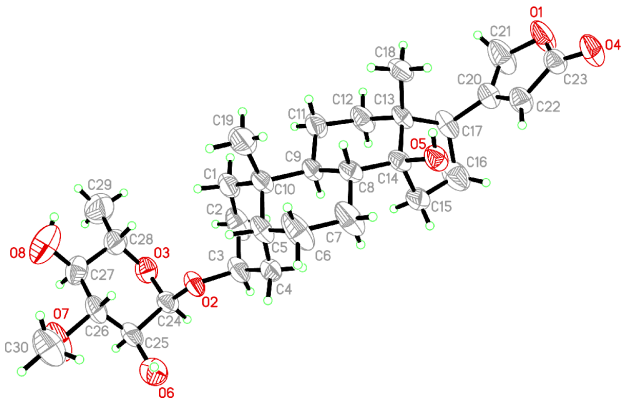


Figure 1
The structure of (I), showing 50% probability displacement ellipsoids and the atom-numbering scheme. The chloroform solvent molecules have been omitted for clarity.

steroid nucleus, lactone ring and glycoside are in agreement with those reported earlier for the unsolvated structure (Chantrapromma *et al.*, 2003). As previously reported, the steroid nucleus has a *cis*/*trans*/*cis* configuration for the *A*–*B*/*B*–*C*/*C*–*D* rings and the cyclohexane *A*, *B* and *C* rings have standard chair conformations; the cyclopentane ring *D* has an envelope conformation. The lactone ring (O1/C20–C23) attached at atom C17 is essentially planar, mainly due to conjugation of the C═C and C═O bonds.

The present structure differs from the earlier structure (Chantrapromma *et al.*, 2003) in the orientations of the lactone ring and glycosidic linkages. The orientation of the lactone ring is determined by the C13—C17—C20—C22 torsion angle, which is 78.3 (8)° in the present structure compared with −101.7 (7)° (molecule *A*) and −107.8 (7)° (molecule *B*) in the earlier structure. The relative orientations of the glycosidic linkages (O3/C24–C28) are determined by the C2—C3—O2—C24 (φ_1) and C3—O2—C24—C25 (φ_2) torsion angles; φ_1 and φ_2 are 82.0 (6) and 154.2 (5)°, respectively, in the present structure. These values significantly differ from those reported earlier [$\varphi1$ = 159.6 (4)° and $\varphi2$ = 173.1(4)° for molecule *A*, $\varphi1$ = 156.6(4)° and $\varphi2$ = 173.2(4)° for molecule *B*; Chantrapromma *et al.*, 2003].

The molecular structure is stabilized by C—H···O interactions. The solvent molecules are linked to the carenolide through C—H···O hydrogen bonds (Table 2). Screw-related molecules are linked together by O—H···O hydrogen bonds (Fig. 2).

Although the strict and demanding criteria of Flack & Bernardinelli (2000) are not met in this study, it confirms with a reasonable confidence that the previously assumed stereochemistry, *viz.* the L form, is correct. This novel method, consisting of the introduction of chloroform as solvent and refinement of the Flack parameter (Flack & Bernardinelli, 1999), is an extremely useful and easy method to determine absolute stereochemistry of natural products.

Experimental

The title compound was isolated from the seeds of *Cerbera Odollam* as described in our earlier work (Chantrapromma *et al.*, 2003).

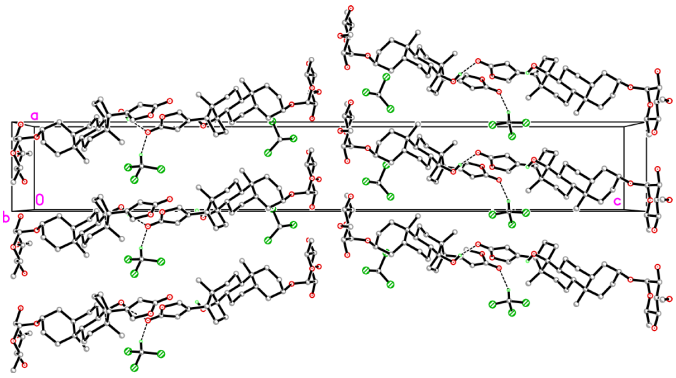


Figure 2
Packing diagram for (I), showing the molecular ribbons. H atoms have been omitted, except for those involved in hydrogen-bond interactions (dashed lines).

Colourless single crystals suitable for X-ray diffraction were obtained by slow evaporation of a solution of the title compound in chloroform–methanol (3:0.05 *v*/*v*) over a period of 3–4 d.

Crystal data

$C_{30}H_{46}O_8\cdot 2CHCl_3$
M_r = 773.40
Orthorhombic, $P2_12_12_1$
a = 7.6426 (4) Å
b = 9.1932 (5) Å
c = 54.362 (3) Å
V = 3819.5 (4) Å^3
Z = 4
D_x = 1.345 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 6197 reflections
θ = 2.3–25.4°
μ = 0.50 mm^{-1}
T = 293 (2) K
Block, colourless
0.36 × 0.34 × 0.24 mm

Data collection

Siemens SMART CCD area-detector diffractometer
ω scans
Absorption correction: multi-scan (*SADABS*; Sheldrick, 1996)
T_{min} = 0.798, T_{max} = 0.891
18659 measured reflections
6626 independent reflections
4427 reflections with $I > 2\sigma(I)$
R_{int} = 0.044
θ_{max} = 25.0°
$h = -9 \rightarrow 9$
$k = -10 \rightarrow 8$
$l = -62 \rightarrow 64$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.077
$wR(F^2)$ = 0.227
S = 1.04
6626 reflections
422 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.1044P)^2 + 4.0909P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max}$ = 0.33 e Å^{-3}
$\Delta\rho_{min}$ = −0.40 e Å^{-3}
Absolute structure: Flack (1983), 2780 Friedel pairs
Flack parameter = 0.14 (14)

Table 1
Selected geometric parameters (Å, °).

O1—C23	1.329 (7)	O6—C25	1.405 (8)
O1—C21	1.439 (7)	O7—C30	1.394 (8)
O2—C24	1.398 (6)	O7—C26	1.423 (7)
O2—C3	1.426 (6)	O8—C27	1.399 (8)
O3—C24	1.403 (7)	C20—C22	1.313 (7)
O3—C28	1.433 (7)	C20—C21	1.453 (9)
O4—C23	1.213 (6)	C22—C23	1.444 (7)
O5—C14	1.430 (5)		
C24—O2—C3—C4	−156.0 (4)	C16—C17—C20—C21	133.9 (7)
C24—O2—C3—C2	82.0 (6)	C13—C17—C20—C21	−104.9 (7)
C16—C17—C20—C22	−42.9 (9)	C3—O2—C24—O3	−84.3 (6)
C13—C17—C20—C22	78.3 (8)	C3—O2—C24—C25	154.2 (5)

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

D—H···A	D—H	H···A	D···A	D—H···A
O5—H5O···O4^{i}	0.82	2.22	2.967 (5)	151
O5—H5O···O1^{i}	0.82	2.71	3.294 (5)	129
O8—H8O···O6^{ii}	0.82	2.62	2.896 (8)	101
C2—H2*A*···O3	0.97	2.52	3.176 (7)	125
C7—H7*B*···O5	0.97	2.58	2.912 (7)	100
C18—H18*C*···O5	0.96	2.56	2.887 (7)	100
C32—H32···O4^{ii}	0.98	2.19	3.133 (8)	160

Symmetry codes: (i) $-x, y-\frac{1}{2}, \frac{1}{2}-z$; (ii) $1+x, y, z$.

H atoms were placed in calculated positions, with an O—H distance of 0.82 Å and C—H distances in the range 0.96–0.98 Å. The U_{iso} values were constrained to be $1.5U_{eq}$ of the carrier atom for hydroxyl and methyl H atoms and $1.2U_{eq}$ for the remaining H atoms. One of the chloroform solvent molecules is disordered. No satisfactory rotational disorder model was found. However, the difference map showed two split positions for atom Cl2 and the occupancies of the disordered positions Cl2*A* and Cl2*B* were refined to 0.491 (8) and 0.509 (8), respectively. The U_{ij} values of these atoms were restrained to be equal. Owing to a large fraction of weak data at higher angles, the 2θ maximum was limited to 50°.

Data collection: *SMART* (Siemens, 1996); cell refinement: *SAINT* (Siemens, 1996); data reduction: *SAINT*; program(s) used to solve structure: *SHELXTL* (Sheldrick, 1997); program(s) used to refine structure: *SHELXTL*; molecular graphics: *SHELXTL*; software used to prepare material for publication: *SHELXTL, PARST* (Nardelli, 1995) and *PLATON* (Spek, 2003).

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