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Absolute configuration of chloro-bisabolene sesquiterpene

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The crystal structure of a cholor–bisabolene sesquiterpene has been determined for the absolute configuration. Its structure was elucidated as (–)-(1*R*,2*R*,3*R*,4*R*,6*S*,8*S*,10*S*)-chloro-2,10-diacetoxy-1,8-diangeloyloxy-3-hydroxy-11-methoxy-bisabol-7(14)-ene. The chlorine atom at C4 is axial in the cyclohexane ring. The molecule shows a stable chair conformation, and crystallizes in the monoclinic space group $P2_1$ with one molecule in the asymmetric unit.

1. Introduction

Bisabolene sesquiterpenes are widely distributed in nature, especially in higher plants. They exhibit a wide range of biological activities, such as antibacterial, antiflammatory, antiaging, antitumour, antimalaria, insect sex hormone and insect antifeedant activities, which have evoked wide interest of chemists and biochemists (Marui 1992; Spring et al. 1992; Stadler et al. 1994 and Carman et al. 1989). So far the structures of the natural bisabolene sesquiterpenes have been assigned by spectroscopic means, but only few of them have been established by X-ray crystallography (Carman et al. 1989; Burk 1992; Chen et al. 1996). Especially, the absolute configuration of these type compounds has only assigned on the basics of their chemical correlation, and CD or ORD measurements so far.

We investigated bisabolene sesquiterpenes and their relative stereochemistry from the genus *Cremanthodium* in China (Chen et al. 1996; Zhu et al. 1999, 2000; Su et al. 2000), which are Tibetan-traditional medicinal plants used for the treatment of apoplexy, anti-inflammation, detoxification and pain-relief (North-Western Plateau Institute of Biology Chinese Academy of Sciences 1991), but their absolute configuration are still not assigned. This paper reports the crystal structure of compound **A1**, a cholorbisabolene sesquiterpene, by X-ray analysis in order to provide the first direct evidence for the absolute configuration and the chiral identities of the bisabolenes from *C. discoideum*. The presence of polychiral centers in this compound together with the biological activity studies will trigger the investigation of synthesis of enantiomers and relationship between structure and activity.

2. Investigations, results and discussion

A polysubstituent natural sesquiterpene **1**, 4α-chloro-2β-acetoxy-1β,8-diangeloyloxy-3β,10-dihydroxy-11-methoxy-bisabol-7(14)-ene, was isolated from *Cremanthodium discoideum.* Acetylation of **1** afforded the crystalline compound **A1**. As Fig. 1 shows, compound **A1** is a bisabolene sesquiterpene with a terminal double bond (C7–C14 1.321 (3) Å) and is substituted with a chlorine, a hydroxyl, a methoxy, two acetoxy and two angeloyloxy groups. The chlorine atom at C4 is axial at the cyclohexane ring. The bond distance between carbon and chlorine is 1.815 (3) Å due to the effect of an adjacent to hydroxyl group at C3, which has been reported a similar bond distance (1.803 Å) of C(sp^3)–Cl type (Allen et al. 1987). The cyclohexane ring is in the expected chair conformation, which was confirmed by the endocyclic torsion angles $\omega_{i,j}$ around about the bond between atom i and j: $\omega_{1,2}$ −50.9(3), $\omega_{2,3}$ 52.0 (3), $\omega_{3,4}$ −54.2 (3), $\omega_{4,5}$ 58.3 (3), $\omega_{5,6}$ −54.7 (2), $\omega_{1,6}$ 49.8 (2)°. Compound **A1** has seven chrial carbons and showed a rotatory value $[\alpha]_D^{20}$ of −60° (c 1.0, $CHCl_3$).

In order to give the correct enantiomer, 8070 of all reflections were collected, including 7125 refined reflections and 3357 reflections of Fridel pairs which were collected in the range of two theta below 54°. At the final refinement, structure factors and absolute configuration flack parameters (Flack 1983) were calculated for both enantiomers, the reported configuration gave R 0.067 and Flack −0.03(7), while the inverse configuration enantio-

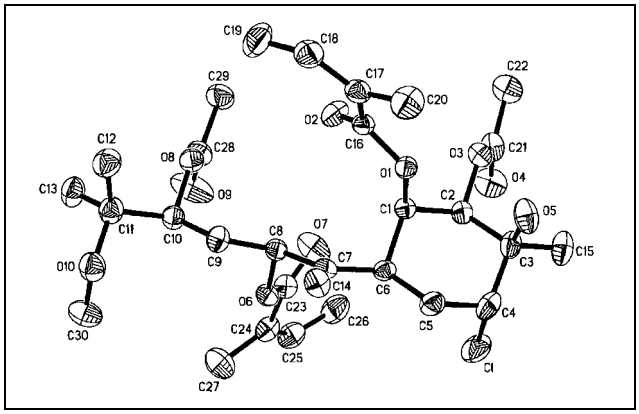

Fig. 1: The structure of **A1** showing 50% probability thermal ellipsoids

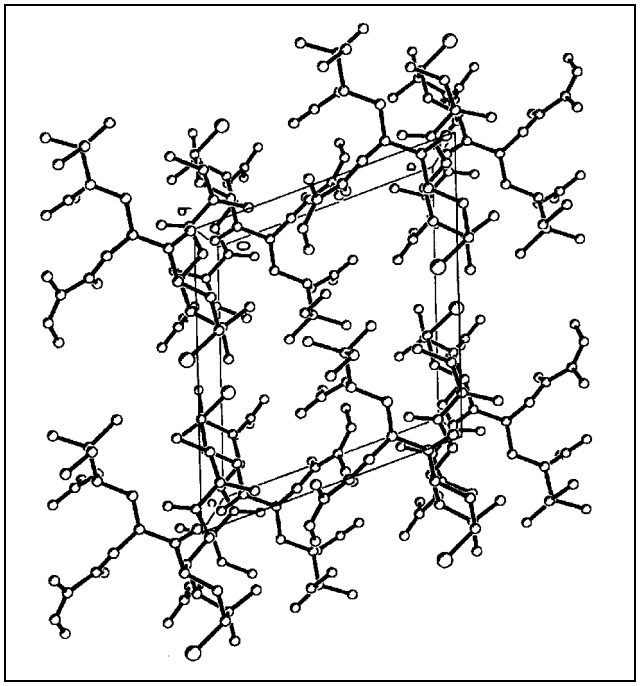

Fig. 2: Crystal packing for **A1** in the cell

mer have R 0.068 and Flack 1.02(7), respectively. Thus the absolute configuration of compound **A1** is as (−)-(1*R*,2*R*,3*R*,4*R*,6*S*,8*S*,10*S*)-4-chloro-2,10-diacetoxy-1,8-diangeloyloxy-3-hydroxy-11-methoxy-bisabol-7(14)-ene. The molecules are associated in the crystal state by a O−H - - - O hydrogen bond, in which the hydroxy O5 at C3 is an intermolecular hydrogen bound to O10 at methyloxy group (O5 - - - O10 2.960(3) Å.

Compound **A1** showed an activity level equal to that of chloramphenicol against *Bacillus subtillis*, reaching an inhibition zone of 13–15 mm at a concentration of 100 μg · ml^{-1}, but exhibited only moderate antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* with inhibition zones of, 10–12 mm. Aditionally, the cytotoxic activities of compound **A1** for three tumour cell lines were tested. The compound showed cytotoxicity against melanoma cell B16, human cervix uteri tumor cell HeLa and human hapatoma cell SMMC-7721 with IC_{50} values ranging from 119 to 141 μg ml^{-1}, which were compared with that of vincristine (IC_{50} values ranging from 63 to 71 μg ml^{-1}).

3. Experimental

3.1. Isolation

Compound **A1** was prepared from a mixture of compound **1** (20 mg) and Ac_2O in pyridine (1 : 1) for 24 h, then purified by preparative thin layer chromotography (PTLC). The sample used was recrystallised with hexane-Me_2CO (1 : 1) as colorless tubular plates, m.p. 86–89°C; $[\alpha]_D^{20}$ −60° (c 1.0, $CHCl_3$)

3.2. Antibacterial and cytotoxicity assay

Antibacterial and cytotoxicity assay of compound **A1** were carried out in the School of Life Sciences, Lanzhou University. The antibacterial tests were performed employing the cup-plate method (Xu et al. 1982). The cytotoxicity assay on B16, HeLa and SMMC-7721 tumor cells *in vitro* was carried out by the MMT method according to procedures described by Han (Han 1997). Half inhibition concentration (IC_{50}) was calculated by linear regression (Price et al. 1990).

3.3. Crystal data and structure determination

(−)(1*R*,2*R*,3*R*,4*R*,6*S*,8*S*,10*S*)-chloro-2,10-diacetoxy-1,8-diangeloyloxy-3-hydroxy-11-methoxy-bisabol-7(14)-ene: $C_{30}H_{45}ClO_{10}$, M = 601.11, monoclinic, space group $P2_1$, a = 10.556 (1), b = 13.723 (2), c = 11.809 (1) Å, β = 109.710 (1)°, V = 1610.4 (3) Å^3, Z = 2; Dc = 1.240 g · cm^{-3}, λ(Mo Kα) = 0.71073 Å, μ = 0.171 cm^{-1}, F(000) = 644.

A colorless block crystal with dimensions 0.48 mm × 0.38 mm × 0.20mm was mounted on a Siemens P4 four circle X-ray diffractometer with graphite–monochromatized Mo Kα radiation. The intensities were collected at 296 K with the ω/2θ scan technique by the Siemens XSCANS program. The intensities of the three standards were monitored during the data collection and indicated no crystal decomposition. A total of 8070 reflections were collected in the range of 1.83° ≤ θ ≤ 27.24°, h from −13 to 13, k from −17 to 17, l from −15 to 14. Of these, 4893 reflections with I > 2σ (I) were used in the structure determination and refinement. Rint = 0.0161 after absorption correction maximum and minimum transmission factors are 0.9602 and 0.9262, respectively. The intensities were corrected for Lorentz-polarization effects, and for empirical absorption.

Table: Selected bond lengths (Å) and bond angles (°)

Cl−C(4)	1.815(3)	O(10)−C(30)	1.417(3)	C(7)−C(8)	1.515(3)
O(1)−C(16)	1.352(3)	O(10)−C(11)	1.420(3)	C(8)−C(9)	1.516(3)
O(1)−C(1)	1.453(2)	C(1)−C(2)	1.528(3)	C(9)−C(10)	1.517(3)
O(2)−C(16)	1.202(3)	C(1)−C(6)	1.530(3)	C(10)−C(11)	1.552(3)
O(3)−C(21)	1.365(3)	C(2)−C(3)	1.540(3)	C(11)−C(13)	1.517(3)
O(3)−C(2)	1.444(3)	C(3)−C(15)	1.515(4)	C(11)−C(12)	1.559(4)
O(4)−C(21)	1.195(3)	C(3)−C(4)	1.528(4)	C(16)−C(17)	1.492(3)
O(5)−C(3)	1.430(3)	C(4)−C(5)	1.524(4)	C(17)−C(18)	1.342(4)
O(6)−C(8)	1.458(2)	C(5)−C(6)	1.531(3)	C(17)−C(20)	1.485(4)
O(8)−C(28)	1.341(3)	C(6)−C(7)	1.519(3)	C(18)−C(19)	1.482(4)
O(8)−C(10)	1.444(3)	C(7)−C(14)	1.321(3)	C(21)−C(22)	1.474(4)
C(16)−O(1)−C(1)	116.73(16)	C(4)−C(5)−C(6)	112.1(2)	O(2)−C(16)−O(1)	123.3(2)
C(21)−O(3)−C(2)	117.55(18)	C(7)−C(6)−C(1)	112.18(17)	O(2)−C(16)−C(17)	125.7(2)
C(23)−O(6)−C(8)	118.68(18)	C(7)−C(6)−C(5)	112.75(19)	O(1)−C(16)−C(17)	111.0(2)
C(28)−O(8)−C(10)	119.7(2)	C(1)−C(6)−C(5)	111.36(19)	C(18)−C(17)−C(20)	122.0(2)
C(30)−O(10)−C(11)	116.3(2)	C(14)−C(7)−C(6)	121.9(2)	C(13)−C(11)−C(10)	109.8(2)
O(1)−C(1)−C(2)	111.40(16)	C(8)−C(7)−C(6)	116.88(17)	O(10)−C(11)−C(12)	103.2(2)
C(2)−C(1)−C(6)	111.07(17)	O(6)−C(8)−C(7)	108.97(16)	C(13)−C(11)−C(12)	111.1(2)
O(3)−C(2)−C(3)	109.70(19)	O(6)−C(8)−C(9)	106.77(17)	C(20)−C(17)−C(16)	118.1(2)
O(5)−C(3)−C(15)	110.1(2)	C(7)−C(8)−C(9)	114.84(18)	C(18)−C(17)−C(16)	119.9(2)
C(1)−C(2)−C(3)	116.68(18)	C(8)−C(9)−C(10)	114.02(18)	C(20)−C(17)−C(16)	118.1(2)
C(4)−C(3)−C(2)	107.8(2)	O(8)−C(10)−C(9)	107.13(19)	C(17)−C(18)−C(19)	130.8(3)
C(5)−C(4)−C(3)	112.7(2)	C(9)−C(10)−C(11)	113.90(19)	O(4)−C(21)−O(3)	123.1(3)
C(5)−C(4)−Cl	109.49(19)	O(10)−C(11)−C(13)	111.8(2)	O(4)−C(21)−C(22)	125.4(3)
C(3)−C(4)−Cl	111.4(2)	O(10)−C(11)−C(10)	108.73(19)	O(3)−C(21)−C(22)	111.5(2)

The structure was solved by direct methods with subsequent difference-Fourier synthesis and refined by full-matrix least-squares methods with anisotropic thermal factors using the SHELXL-97 programs. All the hydrogen atoms were placed in calculated positions and not refined. The final cycle of full-matrix least-squares refinement (F^2) was based on 7125 reflections with R_{all} value of 0.0656, and converged to $R = 0.043$, $R_w = 0.1097$, $S = 0.001$ for 382 parameters and 4893 observed reflections with $I > 2\sigma(I)$. The weighting scheme was $\omega = 1/[(\sigma^2(Fo)^2 + (0.0593P)^2 + 0.0000P)$, where $P = (Fo^2 + 2Fc^2)/3$. The maximum and minimum peaks on the final difference-Fourier map corresponded to 0.325 and -0.142 e $\cdot$ Å^{-3}. All of calculations were performed using the Siemens SHELXTL-PC program (Sheldrik 1994).

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