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(+)-Geodin from *Aspergillus terreus*

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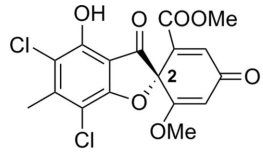
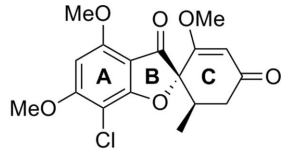
The fungal metabolite (+)-geodin [systematic name: (2*R*)-methyl 5,7-dichloro-4-hydroxy-6′-methoxy-6-methyl-3,4′-dioxospiro[benzofuran-2,1′-cyclohexa-2′,5′-diene]-2′-carboxylate], $C_{17}H_{12}Cl_2O_7$, was isolated from *Aspergillus terreus*. The crystal structure contains two independent molecules in the asymmetric unit. Molecules denoted 1 interact through O—H···O hydrogen bonds creating chains of molecules parallel to the crystallographic 2_1 screw axis. Molecules denoted 2 interact through an O···Cl halogen bond, also creating chains of molecules parallel to the crystallographic 2_1 screw axis. Molecules 1 and 2 interact through another O···Cl halogen bond. The two molecules are similar but molecules 2 have a slightly more planar cyclohexadiene ring than molecules 1. The absolute structure of (+)-geodin has been unequivocally assigned with the spiro centre having the *R* configuration in both molecules. The structurally related (+)-griseofulvin has an *S* configuration at the spiro centre, a difference of potential biological and biosynthetic relevance.

Comment

(+)-Geodin, (I), was originally isolated from *Aspergillus terreus* (Raistrick & Smith, 1936) and elucidation of its structure was initiated (Clutterbuck *et al.*, 1937; Calam *et al.*, 1939, 1947), eventually resulting in the correct relative structure (Barton & Scott, 1958). A number of biological activities have been reported for (I), including antiviral (Takatsuki, Suzuki *et al.*, 1969; Takatsuki, Yamaguchi *et al.*, 1969), antimicrobial (Rinderknecht *et al.*, 1947), enhancement of fibrinolytic activity (Shinohara *et al.*, 2000) and stimulation of glucose uptake for rat adipocytes (Sato *et al.*, 2005). Furthermore, (I) is a subunit of the compound Sch 202596, an antagonist of the galanin receptor subtype GALR1 (Chu *et al.*, 1997). In an effort to synthesize Sch 202596, the total synthesis of racemic geodin was completed (Katoh *et al.*, 2002; Katoh & Ohmori, 2000). Geodin, (I), shares the same grisan backbone as (+)-griseofulvin, (II), consisting of ring systems *A*, *B* and *C*, as shown for (II) in the Scheme below (Grove *et al.*, 1952). Additionally, both compounds (I) and (II) are dextrorotatory and this general similarity prompted our interest in (I) since (II) has anticancer properties (Ho *et al.*, 2001; Panda *et al.*, 2005). (I) was isolated from *A. terreus* and tested in our cellular anticancer assay (Rebacz *et al.*, 2007) but did not exhibit any activity (data not shown).



(I)



(II)

(I) crystallizes with two independent molecules in the asymmetric unit (Fig. 1). Although the two molecules are quite similar, there are small differences in their geometries. Cyclohexadienone ring *C* in (I) is almost planar, with an r.m.s. deviation of the least-square planes of 0.045 and 0.016 Å for molecules 1 and 2, respectively. The largest deviation from this plane is 0.070 (2)/0.024 (2) Å found for atoms C12/C22. The distances O15···O13/O25···O23 are 4.999 (3)/5.245 (4) Å, reflecting the fact that the *C* ring in (I) for molecule 2 is slightly more planar than that of molecule 1. In comparison, cyclohexenone ring *C* in griseofulvin, (II) (Puttaraja *et al.*, 1982), has a half-chair conformation, with atoms C2 and C6′ on the opposite sides of the plane. This means that the distance O5···O3 is only 4.06 Å, *i.e.* much shorter than the equivalents O15···O13 and O25···O23 in (I). The ester groups in the two molecules in the asymmetric unit of (I) are rotated 21.4 (5)/16.9 (4)° with respect to the planes of the respective cyclohexadienone rings so that atoms O17/O27 are located more or less above the centre of the respective five-membered ring with short interatomic O17···O11/O27···O21 distances of 2.916 (4)/2.954 (3) Å. The five-membered *B* rings in the two molecules in (I) are rotated 89.89 (9)/88.13 (10)° with respect to the *C* rings. They are almost planar, with an

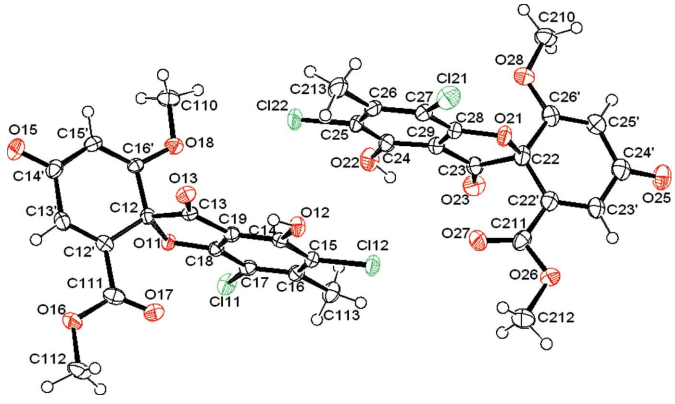


Figure 1
A perspective view of the two independent molecules of geodin, (I), showing the atom-numbering scheme and with displacement ellipsoids drawn at the 50% probability level.

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r.m.s. deviation of the five atoms of 0.028/0.034 Å for both molecules. However, atoms O11(O21) and C13(C23) are below the plane and C12(C22) above the plane. In both molecules, there is an intramolecular hydrogen bond from atom O12/O22 to O13/O23 (see Table 2).

The crystal packing down the *a* axis is shown in Figs. 2 and 3, with the view showing the different packing of molecules 1 (Fig. 3*a*) and 2 (Fig. 3*b*) down the *c* axis. Molecules 1 are hydrogen bonded with a hydrogen bond from atom O12 to atom O15 in a neighbouring molecule (see Table 2, and Figs. 2 and 3*a*). This creates a chain of molecules along the crystallographic 2_1 screw axis. A similar hydrogen bond is not found in molecules 2. They are tilted slightly and the distance between atoms O22 and O25($-x+2$, $y+\frac{1}{2}$, $-z+2$) is 4.521 (4) Å. There are, however, halogen bonds between atoms O23 and Cl21 from neighbouring molecules (see Table 1, and Figs. 2 and 3*b*) creating chains of molecules along the crystallographic 2_1 screw axis. Furthermore, molecules of type 2 are oriented so that the *AB* ring system stacks with the *C* ring from the next molecule in the helix. Molecules 1 and 2 interact *via* halogen bonds between atoms O27 and Cl12 (see Table 1).

To increase knowledge of the structure–activity relationship of these related compounds (Rønnest *et al.*, 2009) the absolute structure of (I) presented here was determined using anomalous signal from all reflections (Flack, 1983). This showed an *R* configuration at the spiro centre of both crystallographically independent molecules. In contrast, for (II) the absolute configuration was determined based on alcoholytic reactions (MacMillan, 1959) and later verified by Brown & Sim (1963) by crystal structure determination of a brominated derivative using film data to be *S* at the spiro centre and *R* at atom C6′. This structural difference between (I) and (II) could potentially contribute to the observed absence of anticancer activity (Rebacz *et al.*, 2007) for (I).

Based on enzymatic studies of the biosynthesis of geodin, (I) (Fujii *et al.*, 1983), the spirocyclization reaction joining the

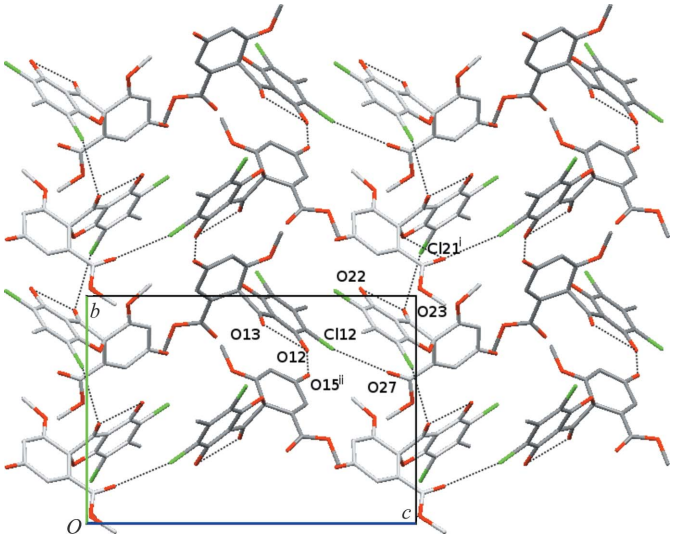

Figure 2
The molecular packing of (I), showing the hydrogen- and halogen-bond architecture; the view direction is down [100]. Hydrogen and halogen bonds are drawn as dashed lines. [Symmetry codes: (i) $-x+1, y+\frac{1}{2}, -z+2$; (ii) $-x, y-\frac{1}{2}, -z+1$.]

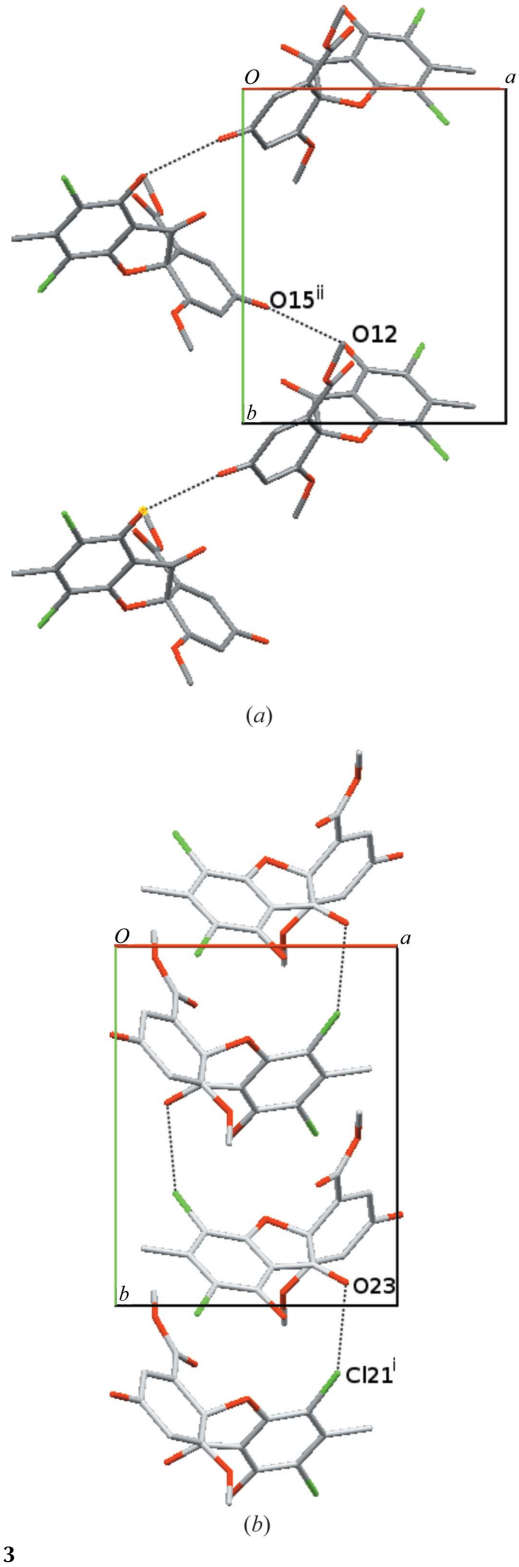

Figure 3
Helical chains in (I), viewed in the [001] direction, showing (*a*) molecules of type 1 and (*b*) molecules of type 2. [Symmetry codes: (i) $-x+1, y+\frac{1}{2}, -z+2$; (ii) $-x, y-\frac{1}{2}, -z+1$.]

B and *C* rings is believed to be catalysed by an enzyme of the multicopper protein class. The same reaction in the griseofulvin, (II), biosynthesis, on the other hand, is presumed to be mediated by a cytochrome P450 enzyme. The latter assumption is founded on the identification of the griseofulvin, (II), biosynthesis gene cluster (Chooi *et al.*, 2010). These observations could explain the different configuration of the spiro centres of (I) and (II) since it is reasonable that enzymes of different classes lead to a disparate outcome in a similar reaction with comparable substrates.

Experimental

A. terreus [IBT 28226, culture collection at Department of Systems Biology, Technical University of Denmark (Lyngby, Denmark)] was cultured on 50 plates of yeast extract sucrose agar at 298 K for 7 d, extracted with ethyl acetate (2 l) and then concentrated to afford 1.2 g of raw extract. The raw extract was dissolved in 10% H_2O in MeOH (50 ml) and the aqueous phase was extracted with heptane (50 ml). The water content was increased to 50% by adding H_2O (40 ml) and the resulting mixture was shaken with CH_2Cl_2 (90 ml). The CH_2Cl_2 phase was concentrated (0.86 g) and further purification was performed on a Phenomenex Luna(2) HPLC column (250 × 10 mm, 5 µm, C-18) using 5 ml min^{-1} H_2O/CH_3CN (isocratic run at 50/50 for 15 min) as the mobile phase to yield (I) (11.6 mg as a yellow oil). Geodin, (I), was crystallized using sitting-drop vapour diffusion, the drop consisting of EtOAc–heptane (4:1 *v*/*v*) and the reservoir containing heptane, to afford yellow crystals.

Crystal data

$C_{17}H_{12}Cl_2O_7$
M_r = 399.17
Monoclinic, $P2_1$
a = 8.9276 (3) Å
b = 11.3625 (4) Å
c = 16.5006 (6) Å
β = 94.456 (1)°
V = 1668.76 (10) Å^3
Z = 4
Mo $K\alpha$ radiation
μ = 0.43 mm^{-1}
T = 120 K
0.25 × 0.15 × 0.08 mm

Data collection

Bruker SMART platform CCD diffractometer
Absorption correction: multi-scan (*SADABS*; Bruker, 2000)
T_{min} = 0.82, T_{max} = 0.97
22828 measured reflections
8165 independent reflections
7496 reflections with $I > 2\sigma(I)$
R_{int} = 0.029

Refinement

$R[F^2 > 2\sigma(F^2)]$ = 0.053
$wR(F^2)$ = 0.145
S = 1.06
8165 reflections
480 parameters
1 restraint
H-atom parameters constrained
$\Delta\rho_{max}$ = 0.81 e Å^{-3}
$\Delta\rho_{min}$ = −0.36 e Å^{-3}
Absolute structure: Flack (1983), 3819 Friedel pairs
Flack parameter: 0.04 (6)

H atoms were observed in a difference synthesis and subsequently placed in idealized positions. They were refined using a riding model, with aryl C—H = 0.95 Å and U_{iso}(H) = 1.2U_{eq}(C), methyl C—H = 0.98 Å and U_{iso}(H) = 1.5U_{eq}(C), and hydroxy O—H = 0.84 Å and U_{iso}(H) = 1.2U_{eq}(O).

Data collection: *SMART* and *SAINT* (Bruker, 1998); cell refinement: *SMART* and *SAINT*; data reduction: *SMART* and *SAINT*; program(s) used to solve structure: *SHELXS97* (Sheldrick, 2008); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *ORTEP-3* (Farrugia, 1997) and *Mercury* (Macrae *et al.*, 2006); software used to prepare material for publication: *enCIFer* (Allen *et al.*, 2004).

Table 1
Selected interatomic distances (Å).

O27···Cl12	3.070 (3)	O23···Cl21^{i}	3.006 (3)

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

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

D—H···A	D—H	H···A	D···A	D—H···A
O12—H12···O15^{ii}	0.84	2.33	2.916 (3)	128
O12—H12···O13	0.84	2.50	3.149 (3)	135
O22—H22···O23	0.84	2.36	3.021 (4)	136

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

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: LG3050). Services for accessing these data are described at the back of the journal.

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