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# Azadirachtol, a tetranortriterpenoid from neem kernels

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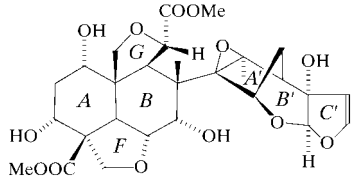
The title compound, dimethyl (−)-(2a*R*,3*R*,4*R*,4a*S*,5*R*,-7a*S*,8*R*,10*S*,10a*R*)-3,8,10-trihydroxy-4-[(2*R*,6*R*)-2-hydroxy-11-methyl-5,7,10-trioxatetracyclo[6.3.1.0$^{2,6}$0$^{9,11}$]dodec-3-en-9-yl]-4-methylperhydroisobenzofurano[5,4,3a-*cd*]isobenzofuran-5,10a-diacetate, $C_{28}H_{36}O_{13}$, which exhibits higher antifeedant activity than azadirachtin-A, a known potent antifeedant, was isolated from neem kernels. The asymmetric unit of the structure contains two independent molecules, which differ in the conformations of their functional groups and also in the conformations of some of the rings. The relative orientation between the decalin and furanyl moieties is similar to that observed in the majority of azadirachtin structures, but is different from that in azadirachtin-A. The two symmetry-independent molecules are linked into dimeric units by intermolecular O—H···O hydrogen bonds.

## Comment

At present, due to the harmful effects of synthetic insecticides on humans and the environment, ecofriendly bio-insecticides from *Azadirachta indica*, widely known as neem, are preferred in pest control. Chemical investigations of different parts of the neem tree have yielded many interesting and structurally varied secondary metabolites which are potent antifeedant and ecdysis inhibitors, and their structures have been studied with a view to understanding structure–activity relationships. We report here the isolation from neem kernels and the crystal structure of an azadirachtin, azadirachtol, (I), a minor tetranortriterpenoid, which shows higher antifeedant activity than azadirachtin-A, a known potent antifeedant (Suresh *et al.*, 2002). This is the first report of the isolation of this compound from neem kernels, although an earlier report described the synthesis of this compound through the chemical modification of azadirachtin-B (Ley *et al.*, 1989).




(I)

There are two independent molecules in the asymmetric unit of (I), and packing of these molecules within the crystal is stabilized by intra- and intermolecular hydrogen bonds (Table 1). In both molecules, *A* and *B*, intramolecular O1···O3 and O7···O6 hydrogen bonds are observed. In molecule *B*, there is an additional intramolecular hydrogen bond, *viz.* O20*B*···O7*B*. The two molecules in the asymmetric unit exist as dimers connected *via* parallel O7*A*···O6*B* and O7*B*···O6*A* hydrogen bonds, which generate a graph-set motif (Bernstein *et al.*, 1995) of $R_2^2(10)$ (Fig. 1). The dimeric unit is linked into infinite chains along all three axes (Fig. 2).

Atom O3*A* acts as a hydrogen-bond donor *via* H3*A* to O12*A* at ($1-x, y-\frac{1}{2}, \frac{3}{2}-z$), producing a $C(10)$ chain parallel to [010], generated by the $2_1$ screw axis along ($1,y,\frac{3}{4}$). Atom O1*A* acts as a donor *via* H1*A* to O13*B* at

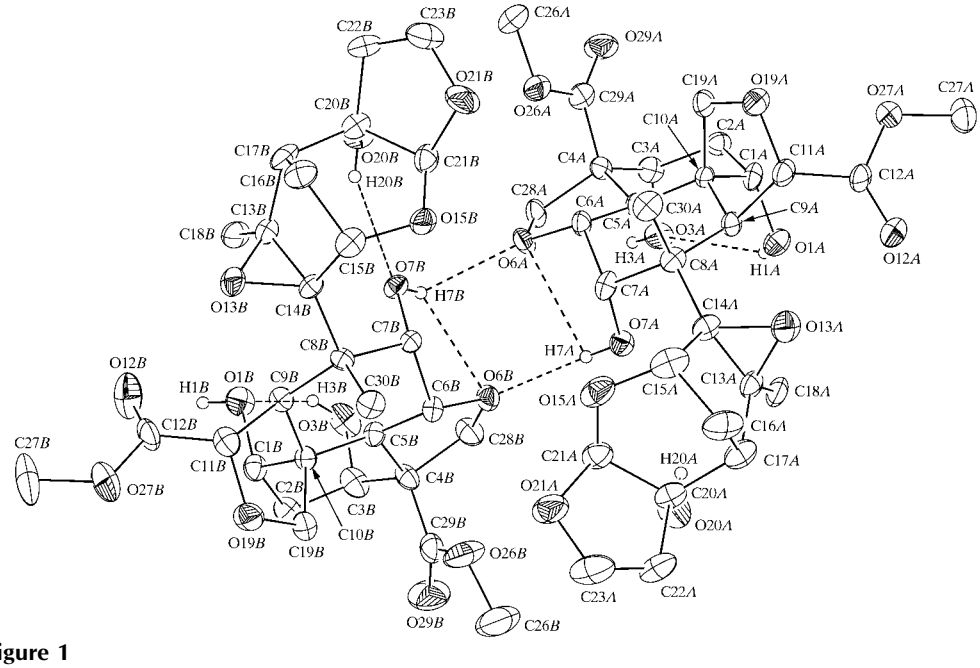



**Figure 1**
A view of the two independent molecules of (I), shown with 30% probability displacement ellipsoids and the atomic numbering scheme. Hydrogen bonds are shown as dotted lines; H atoms not involved in these contacts have been omitted for clarity.

$(\frac{3}{2}-x, 1-y, z-\frac{1}{2})$. Finally, atom O1*B* acts as a donor *via* H1*B* to O20*B* at $(x+\frac{1}{2}, \frac{1}{2}-y, 2-z)$, producing a $C(11)$ chain parallel to [100], generated along $(x,\frac{1}{4},1)$ by a $2_1$ screw axis. The combination of these chains generates a ring with an $R_5^5(26)$ motif. The interior of this ring is occupied by the methoxycarbonyl group at C11 of the molecule at $(x-\frac{1}{2}, \frac{3}{2}-y, z)$ and at C4 of the molecule at $(x, y, z)$ (Fig. 3). All the hydroxy O atoms, except for O20*A*, take part in hydrogen bonds. This exception may be due to steric hindrance in the crystalline state. Other azadirachtins extracted from the same source show different hydrogen-bonding graph-set motifs.

Among the azadirachtins studied previously, it was reported that azadirachtin-A possesses maximum antifeedant activity and azadirachtin-I and -H have medium activities (60–80% of azadirachtin-A; Govindachari, Narasimhan *et al.*, 1996). Compound (I) has higher antifeedant activity than azadirachtin-A (Suresh *et al.*, 2002). The overall conformation of the molecule of (I) defined by the dihedral angle about the C8—C14 bond, *viz*. C9—C8—C14—O13, shows that the values in the present structure [26.5 (7) and 26.2 (7)° for molecules *A* and *B*, respectively] are closer to those of azadirachtin-H [25.6 (3)°; Govindachari, Geetha Gopalakrishnan *et al.*, 1996] and azadirachtin-I [23.5 (8)°; Malathi *et al.*, 2002] than that of azadirachtin-A [−162.1 (6)°; Kabaleeswaran *et al.*, 1994], which indicates that this may not be a factor contributing to the activity of the compound.

The two molecules in the asymmetric unit of (I) differ from one another in the orientations of their functional groups. The C3—C4—C29—O26 dihedral angle, defining the orientation of the methoxycarbonyl group at C4, is −179.4 (5)° in molecule *A* and −162.8 (7)° in molecule *B*. These are close to the corresponding value observed in azadirachtin-H [C3—C4—C29—O26 = 176.8 (3)°], but differ from that observed in azadirachtin-A [8.0 (11)°]. Another difference observed between the two molecules is in the conformation of the methoxycarbonyl group at C11, defined by the C9—C11—C12—O27 torsion angle, which is 128.2 (7)° in molecule *A* and 170.9 (6)° in molecule *B*. The corresponding value in azadirachtin-A (Kabaleeswaran *et al.*, 1994) is 28.4 (11)°. These variations in the orientations of functional groups within chemically equivalent but crystallographically non-equivalent molecules suggest a high flexibility of the functional groups in azadirachtins.

The ring-puckering amplitudes (Cremer & Pople, 1975; values are given in the order molecule *A* followed by molecule *B*) show that rings *A* [$Q_T$ = 0.556 (7) and 0.573 (6) Å, $\theta$ = 4.6 (6) and 3.2 (7)°, and $\varphi_2$ = −135 (8) and 114 (13)°], *B* [$Q_T$ = 0.538 (6) and 0.526 (6) Å, $\theta$ = 19.8 (7) and 16.1 (6)°, and $\varphi_2$ = 78 (2) and 70 (2)°] and *B*′ [$Q_T$ = 0.662 (8) and 0.706 (7) Å, $\theta$ = 129.4 (6) and 118.0 (5)°, and $\varphi_2$ = −115.2 (9) and −117.8 (6)°] are in chair, distorted chair and sofa conformations, respectively, in both independent molecules of (I). Rings *A*′ [$q_2$ = 0.446 (8) and 0.450 (7) Å, and $\varphi_2$ = 110.8 (9) and 113.3 (8)°] and *C*′ are in envelope and planar conformations, respectively, in molecules *A* and *B*. The envelope is puckered (Nardelli, 1995) at C16 in both molecules. However, ring *F* adopts a distorted envelope conformation in molecule *A* [$q_2$ = 0.390 (6) Å and $\varphi_2$ = 26.3 (9)°], with the apex at C5, and a half-chair conformation in molecule *B* [$q_2$ = 0.392 (6) Å and $\varphi_2$ = 21.8 (9)°], with atoms C4 and C5 deviating from the least-squares plane defined by atoms C6, O6 and C28 by 0.257 (7) and −0.382 (6) Å, respectively. Similarly, ring *G* of molecule *B* is in a half-chair conformation [$q_2$ = 0.349 (6) Å and $\varphi_2$ = 165 (1)°], with atoms C9*B* and C10*B* deviating from the least-squares plane defined by atoms C11*B*, C19*B* and O19*B* by −0.235 (6) and 0.346 (6) Å, respectively, while in molecule *A*, the ring conformation lies between that of an envelope and a half-chair [$q_2$ = 0.342 (6) Å and $\varphi_2$ = 154 (1)°]. The ring-pair fusion configurations are similar to those observed for other azadirachtins (Kabaleeswaran *et al.*, 1994; Govindachari, Geetha Gopalakrishnan *et al.*, 1996; Malathi *et al.*, 2002).

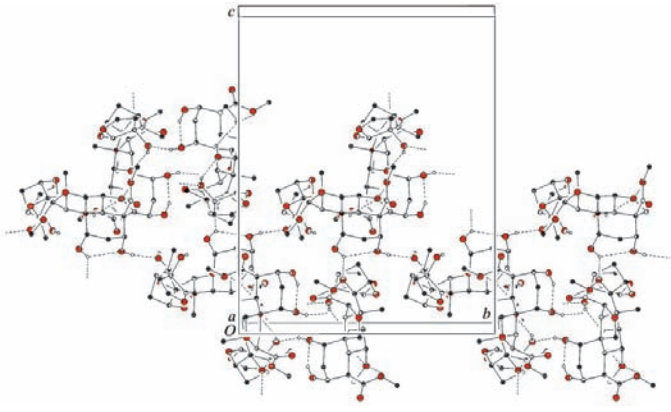



**Figure 2**
Part of the crystal structure of (I), showing the repeat units of the $R_5^5(26)$ rings. For the sake of clarity, H atoms bonded to C atoms have been omitted.

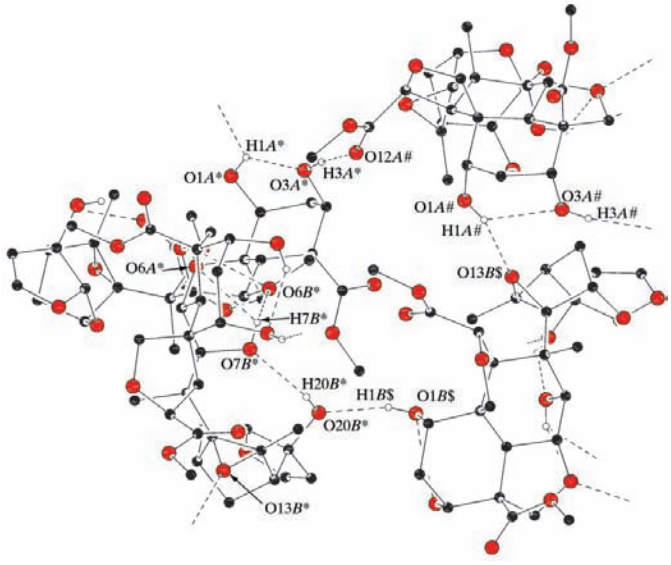



**Figure 3**
A view of the supramolecular structure of (I), showing the $R_5^5(26)$ ring. The molecules labelled with an asterisk (*), dollar sign ($) or hash (#) are at the symmetry positions $(x, y, z)$, $(x-\frac{1}{2}, \frac{3}{2}-y, z)$ and $(1-x, y-\frac{1}{2}, \frac{3}{2}-z)$, respectively.

## Experimental

Powdered neem kernels (500 g) were defatted with hexane (1.5 l) and extracted twice with 95% methanol (1 l and 500 ml) under reflux. On removing the solvent under reduced pressure, a brown residue (25 g) was obtained. This enriched azadirachtin fraction was subjected to preparative high-performance liquid chromatography (HPLC) on a Shimpack RP-18 (25 × 5 cm) ODS (octadecylsilane) column, using methanol–water (60:40) as eluant at a flow rate of 20 ml $min^{-1}$ and a UV detector (215 nm), for preliminary separation of the azadirachtins. The peak with a retention time of 20.4 min (1.2 g) was subjected to reseparation using a semi-preparative HPLC column [endcapped, Merck, 10 mm, acetonitrile–water (25:75), 2 ml $min^{-1}$, UV detector (215 nm)]. On solvent evaporation, this separation yielded an unknown compound (13 mg), which was identified as azadirachtol, based on one- and two-dimensional NMR data. Compound (I) was crystallized by the hanging-drop vapour-diffusion technique; a stock solution of 1 mg of (I) in methanol (100 µl) was prepared and a single crystal of dimensions 3 × 1 × 0.15 mm was obtained from a drop containing 10 µl of this solution saturated with 30% methanol–water.

*Crystal data*

$C_{28}H_{36}O_{13}$
$M_r$ = 580.57
Orthorhombic, $P2_12_12_1$
$a$ = 14.142 (6) Å
$b$ = 17.287 (3) Å
$c$ = 21.505 (4) Å
$V$ = 5257 (3) Å$^3$
$Z$ = 8
$D_x$ = 1.467 Mg $m^{-3}$
Cu $K\alpha$ radiation
Cell parameters from 25 reflections
$\theta$ = 15–30°
$\mu$ = 0.99 $mm^{-1}$
$T$ = 293 (2) K
Rod, colourless
0.35 × 0.23 × 0.15 mm

*Data collection*

Enraf–Nonius CAD-4 diffractometer
Non-profiled $\omega/2\theta$ scans
6108 measured reflections
5699 independent reflections
3087 reflections with $I > 2\sigma(I)$
$R_{int}$ = 0.035
$\theta_{max}$ = 72.1°
$h = -1 \rightarrow 17$
$k = -15 \rightarrow 21$
$l = -26 \rightarrow 26$
3 standard reflections
every 200 reflections
intensity decay: 7%

*Refinement*

Refinement on $F^2$
$R[F^2 > 2\sigma(F^2)]$ = 0.060
$wR(F^2)$ = 0.173
$S$ = 1.04
5699 reflections
748 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0808P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.26 e Å$^{-3}$
$\Delta\rho_{min}$ = −0.31 e Å$^{-3}$
Extinction correction: *SHELXL*97
Extinction coefficient: 0.00108 (13)

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

| $D$—H···$A$ | $D$—H | H···$A$ | $D$···$A$ | $D$—H···$A$ |
|---|---|---|---|---|
| O1*A*—H1*A*···O3*A* | 0.82 | 2.45 | 2.964 (7) | 121 |
| O1*A*—H1*A*···O13*B*$^{i}$ | 0.82 | 2.42 | 2.943 (5) | 122 |
| O3*A*—H3*A*···O12*A*$^{ii}$ | 0.82 | 2.05 | 2.850 (7) | 166 |
| O7*A*—H7*A*···O6*A* | 0.82 | 2.59 | 2.865 (6) | 101 |
| O7*A*—H7*A*···O6*B* | 0.82 | 2.05 | 2.813 (5) | 156 |
| O1*B*—H1*B*···O20*B*$^{iii}$ | 0.82 | 2.07 | 2.846 (7) | 157 |
| O3*B*—H3*B*···O1*B* | 0.82 | 2.05 | 2.760 (8) | 145 |
| O7*B*—H7*B*···O6*A* | 0.82 | 2.30 | 2.864 (5) | 127 |
| O7*B*—H7*B*···O6*B* | 0.82 | 2.41 | 2.860 (6) | 116 |
| O20*B*—H20*B*···O7*B* | 0.82 | 2.24 | 2.977 (6) | 150 |

Symmetry codes: (i) $\frac{3}{2}-x, 1-y, z-\frac{1}{2}$; (ii) $1-x, y-\frac{1}{2}, \frac{3}{2}-z$; (iii) $\frac{1}{2}+x, \frac{1}{2}-y, 2-z$.

In the absence of suitable anomalous scatters, Friedel equivalents could not be used to determine the absolute structure. Refinement of the Flack (1983) parameter led to an inconclusive value (Flack & Bernadinelli, 2000) of −0.3 (4). Therefore, the 434 Friedel equivalents were merged before the final refinement. The enantiomer employed in the refined model was chosen to agree with the accepted configuration of limonoids (Ley *et al.*, 1989). The methyl and hydroxy H atoms were constrained to an ideal geometry (C—H = 0.96 Å and O—H = 0.82 Å), with $U_{iso}$(H) values equal to $1.5U_{eq}$(C,O), but were allowed to rotate freely about the C—C and C—O bonds, respectively. All remaining H atoms were placed in geometrically idealized positions (C—H distances in the range 0.97–0.98 Å) and constrained to ride on their parent atoms, with $U_{iso}$(H) values equal to $1.2U_{eq}$(C). The atomic coordinates and displacement parameters of the two molecules were refined individually, blocking each molecule in alternate cycles.

Data collection: *CAD*-4 *EXPRESS* (Enraf–Nonius, 1994); cell refinement: *CAD*-4 *EXPRESS*; data reduction: *XCAD*4 (Harms & Wocadlo, 1995); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *ORTEP*III (Burnett & Johnson, 1996); software used to prepare material for publication: *SHELXL*97 and *PARST* (Nardelli, 1995).

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: LN1147). Services for accessing these data are described at the back of the journal.

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