Acta Crystallographica Section C
Crystal Structure Communications
ISSN 0108-2701

7-Nitro-1*H*-indazole, an inhibitor of nitric oxide synthase

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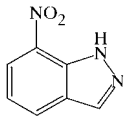
Received 6 April 2000
Accepted 20 September 2000

The crystal structure of 7-nitro-1*H*-indazole, $C_7H_5N_3O_2$, an inhibitor of nitric oxide synthase, shows the existence of an intramolecular hydrogen bond between an O atom of the nitro group and the NH group of the indazole ring. The crystal packing consists of intermolecular hydrogen bonding and indazole···indazole interactions.

Comment

Nitric oxide (NO) formation by the enzyme nitric oxide synthase (NOS) has been implicated in many neuronal processes, such as regulation of local cerebral blood flow, long-term potentiation, nociception and NMDA receptor-mediated excitotoxicity (Iadecola, 1993; Meller & Gebhart, 1993; Chabrier *et al.*, 1999). Nitric oxide synthase occurs in three isoforms, an inductible form (iNOS) and two constitutive forms, neuronal (nNOS) and endothelial (eNOS) (Hemmens & Mayer, 1998). All three isoforms catalyse the formation of nitric oxide from L-arginine, O_2 and NADPH, with the production of citrulline, nitric oxide and $NADP^+$ (Marletta, 1994). In view of the potential role of NO in various neurological processes, selective inhibitors of nNOS have been used as experimental tools to examine the effect of NO in these systems. Among these compounds, 7-nitro-1*H*-indazole, (I), has been identified as a potential selective inhibitor of neuronal NOS activity and is now considered as a very important tool in pharmacological studies.



(I)

The affinity of (I) is about 0.9 μ*M* for nNOS, about 0.7 μ*M* for eNOS and 57 μ*M* for iNOS (Moore, Babbedge *et al.*, 1993; Babbedge *et al.*, 1993). However, the precise molecular mechanism of the inhibitory action of (I) remains very unclear. It probably binds to the prosthetic heme moiety, competing with the substrate L-arginine (Moore, Wallace *et al.*, 1993). Therefore, it is most likely that (I) will be bound in the L-arginine binding site, which was identified in the X-ray structure of the complex of iNOS with the L-arginine substrate (Crane *et al.*, 1998). From this structure, the important role of Glu 371, close to the heme group, was identified.

In the crystal structure of (I), the O atoms of the nitro group are coplanar with the indazole ring, the angle between the planes of the ring and the nitro group being 3.6 (2)°. This result was expected, but a search of the structures deposited in the Cambridge Structural Database (CSD, Version 5.18; Allen & Kennard, 1993) indicated that the nitro group attached to the phenyl ring can deviate somewhat from this coplanar arrangement (by as much as 70°; Zinner *et al.*, 1994). Thus, one O atom of the nitro group (O11) lies in the proximity of atom H2, which is bound to atom N2 of the indazole ring (Fig. 1). The intramolecular contact distance between O11 and H2 is

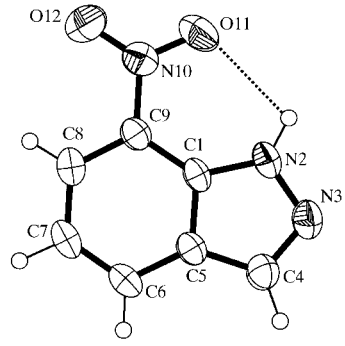


Figure 1
The molecular view of (I) showing the atom-labelling scheme. Displacement ellipsoids are shown at 50% probability levels and H atoms are drawn as small circles of arbitrary radii. The dashed line indicates the intramolecular hydrogen bond.

2.35 (2) Å, indicating formation of an intramolecular hydrogen bond (Table 1). With this hydrogen bond, the six atoms O11, N10, C9, C1, N2 and H2 form a pseudo-six-membered ring (the r.m.s. deviation of a least-squares plane through these atoms is about 0.021 Å). The observed length of the C9—N10 single bond [1.4427 (16) Å] is shorter than the theoretical length for a C_{arom}—NO_2 bond of 1.47 Å (Glusker *et al.*, 1994), which indicates the formation of a weak conjugated π-electron system along this bond.

The crystal packing in (I) consists of indazole base pairs made up of two symmetry-equivalent hydrogen bonds, N2—H2···N3, that form across inversion centres (Fig. 2 and Table 1). The H2 atom is therefore implicated in two hydrogen bonds. The coplanar base-pair dimers stack in parallel planes which form columns, with an interplanar spacing of 3.3 Å between the dimers. Two kinds of these columns of stacked dimers are formed by the crystal packing and the difference between them is given by the dimer orientation. The planes of the dimer indazole rings in neighbouring columns are approximately perpendicular to each other, with the shortest C4—H4···*Cg*1 distance of 3.12 Å (*Cg*1 is the centroid of the phenyl ring of the indazole at $-\frac{1}{2}-x, \frac{1}{2}+y, \frac{3}{2}-z$). Theoretical simulations show that such an arrangement results in favourable intermolecular attractive forces (Koch & Egert, 1995).

Modelling studies based on the Protein Data Bank (PDB; Berman *et al.*, 2000) structure of iNOS complexed with imidazole (Crane *et al.*, 1997), in which we calculated the probable position of (I) in the heme cavity of iNOS, showed that the nitro group can lie in the proximity of Glu 371 and can interact with it through a solvent molecule. In this model, the N3 atom of the indazole partially covers the Fe ion of the heme.

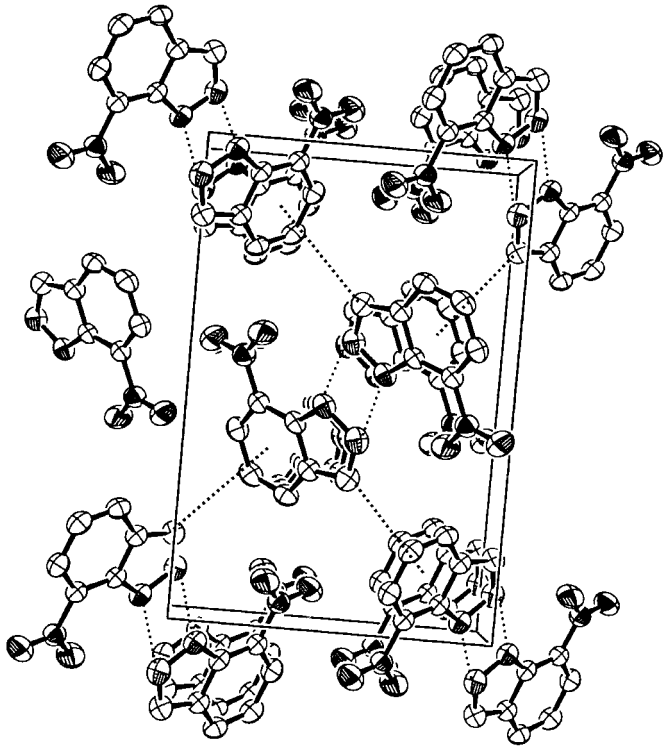


Figure 2
The crystal packing of the molecules of (I) projected onto the *bc* plane. H atoms have been omitted for clarity. Dashed lines indicate the hydrogen bonds and the distances of the centres of mass of the interacting aromatic rings.

Experimental

Compound (I) was synthesized according to the procedure of Bartsch & Yang (1984). The compound is poorly soluble. Suitable crystals were obtained by slow evaporation from methanol at room temperature. The diameter of the collimator used for the data collection was 1.3 mm.

Crystal data

$C_7H_5N_3O_2$
M_r = 163.14
Monoclinic, $P2_1/n$
a = 5.0186 (5) Å
b = 9.6408 (9) Å
c = 14.5082 (14) Å
β = 98.210 (10)°
V = 694.76 (12) Å^3
Z = 4
D_x = 1.560 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 25 reflections
θ = 18–25°
μ = 0.119 mm^{-1}
T = 293 (2) K
Prism, dark yellow
1.0 × 0.4 × 0.1 mm

Data collection

Enraf–Nonius CAD-4 diffractometer
$\theta/2\theta$ scans
2088 measured reflections
2022 independent reflections
1625 reflections with $I > 2\sigma(I)$
R_{int} = 0.019
θ_{max} = 29.98°
$h = -7 \rightarrow 6$
$k = 0 \rightarrow 13$
$l = 0 \rightarrow 20$
3 standard reflections
frequency: 60 min
intensity decay: 1.8%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.046
$wR(F^2)$ = 0.135
S = 1.056
2022 reflections
129 parameters
All H-atom parameters refined
$w = 1/[\sigma^2(F_o^2) + (0.0757P)^2 + 0.1137P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.003
$\Delta\rho_{max}$ = 0.38 e Å^{-3}
$\Delta\rho_{min}$ = −0.16 e Å^{-3}

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

D—H···A	D—H	H···A	D···A	D—H···A
N2—H2···N3^{i}	0.902 (18)	2.190 (18)	2.9369 (14)	139.8 (16)
N2—H2···O11	0.902 (18)	2.351 (19)	2.7465 (16)	106.5 (13)

Symmetry code: (i) $-1-x, 2-y, 2-z$.

The refined aromatic C—H distances are in the range 0.918 (19)–0.993 (17) Å.

Data collection: *CAD*-4-*PC Software* (Enraf–Nonius, 1992); cell refinement: *CAD*-4-*PC Software*; data reduction: *JANA*98 (Petříček & Dušek, 1998); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1990); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *ORTEP*-3 (Farrugia, 1997); software used to prepare material for publication: *SHELXL*97.

Supplementary data for this paper are available from the IUCr electronic archives (Reference: SX1105). Services for accessing these data are described at the back of the journal.

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