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7-Methoxy-1*H*-indazole, a new inhibitor of neuronal nitric oxide synthase

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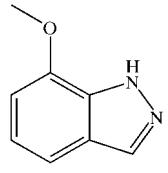
The crystal structure of 7-methoxy-1*H*-indazole, $C_8H_8N_2O$, an inhibitor of nitric oxide synthase, shows that the methoxy group lies in the plane of the indazole system with its methyl group located *trans* to the indazole N—H group. The crystal packing consists principally of hydrogen-bonded trimers. Intermolecular hydrogen-bonding interactions are formed between the indazole N atoms, with the N—H group as a hydrogen-bond donor and the remaining N atom as an acceptor.

Comment

Nitric oxide (NO) is an important biological messenger involved in numerous physiological processes, including neurotransmission, blood-pressure and blood-flow regulation, platelet aggregation, and inflammation (Bredt, 1999). On the other hand, overproduction of NO plays a role in a variety of disorders, such as septic shock, pain, ischaemia and several neurodegenerative diseases (Dawson & Dawson, 1996). NO is synthesized in several cell types from L-arginine by different isoforms of nitric oxide synthase (NOS). Its substrate binding site (heme cavity) is located in the catalytic heme domain. The guanidinium side chain of the substrate interacts with a glutamic acid residue in the binding site and its terminal guanidinium N atom is therefore located approximately 4.0 Å from the heme Fe atom and can be hydroxylated by the Fe-bound O atom. To date, three isoforms of NOS have been cloned, namely neuronal (nNOS) and endothelial (eNOS), which are both constitutive and calcium-dependent, and an inducible calcium-independent form (iNOS) (Stuehr, 1997). Development of inhibitors selective for one of these isoforms is of considerable interest, both for therapeutical purposes and for their use as specific pharmacological tools. Among the synthetic inhibitors, 7-nitroindazole (7-NI) has been identified as a potential selective inhibitor of nNOS activity and is now considered as a very important tool in pharmacological studies (Moore *et al.*, 1991; Babbedge *et al.*, 1993).

The recently determined X-ray structure of the catalytic heme domain of eNOS, complexed with a derivative of the 7-NI inhibitor (3-bromo-7-NI; Raman *et al.*, 2001), showed that 3-bromo-7-NI does not interact directly either with the glutamic acid residue or with the heme Fe atom, but stacks parallel to the heme plane within the van der Waals contact distance. Its position in the substrate-binding site is ensured by two hydrogen bonds, one between the indazole N—H group and the carbonyl O atom of Trp 358, and a second between one nitro O atom and the peptide N—H group of Met 360. The fixation of 3-bromo-7-NI causes a displacement of the glutamic acid side chain in the substrate-binding cavity from its original (potential substrate-binding position) at the edge of the cavity.

The synthesis and pharmacological evaluation of several substituted indazoles and potential analogues of 7-NI has recently been reported by our group (Schumann *et al.*, 2001). Among them, 7-methoxyindazole (7-MI), (I), the most active compound of the series in an *in vitro* enzymatic assay of nNOS activity (6.3 n*M*, *cf.* 0.9 n*M* for 7-NI), is considered as a novel lead in the field of NOS-inhibitory drugs development. The aim of the present study was to determine both the three-dimensional arrangement of (I) and whether the new inhibition mechanism described for 7-NI can be considered as a potential mechanism for (I) as well. In order to find an answer to this question, we have studied the X-ray structure of (I).



(I)

There are 18 molecules in the unit cell in the crystal structure, *i.e.* nine molecules of (I) per asymmetric unit, denoted *A*–*I*. In all these nine structures, the methoxy group lies in the plane of the indazole system with its methyl group located *trans* to N2—H2 (Fig. 1), which is to be expected considering that atom H2 of the N2—H2 group constitutes a steric hindrance for the opposite conformation. The C8—C9—O10—C11 torsion angle, characterizing the conformation of the methoxy group with respect to the indazole ring, is close to 0° for all nine molecules, but varies slightly (Table 1), with extremes from −9.6° in molecule *I* to 4.8° in molecule *A*.

The observed C9—O10 (mean 1.37 Å) and O10—C11 (mean 1.42 Å) bond lengths in the methoxy group are comparable with values found for aromatic methoxy groups deposited in the Cambridge Structural Database (CSD, Version 5.18; Allen & Kennard, 1993). However, greater differences are observed between the methoxy group bond angles (C1—C9—O10 and C8—C9—C10) in the structures of (I). Generally, in the CSD structures, the bond angle toward which the methyl group of the methoxy group is rotated (hereinafter the first bond angle) is about 124° and the second bond angle is about 115°. In the structure of (I), the first bond angle (C8—C9—C10) has an average value of about 128.4° and the second (C1—C9—O10) of about 115.0°. The more

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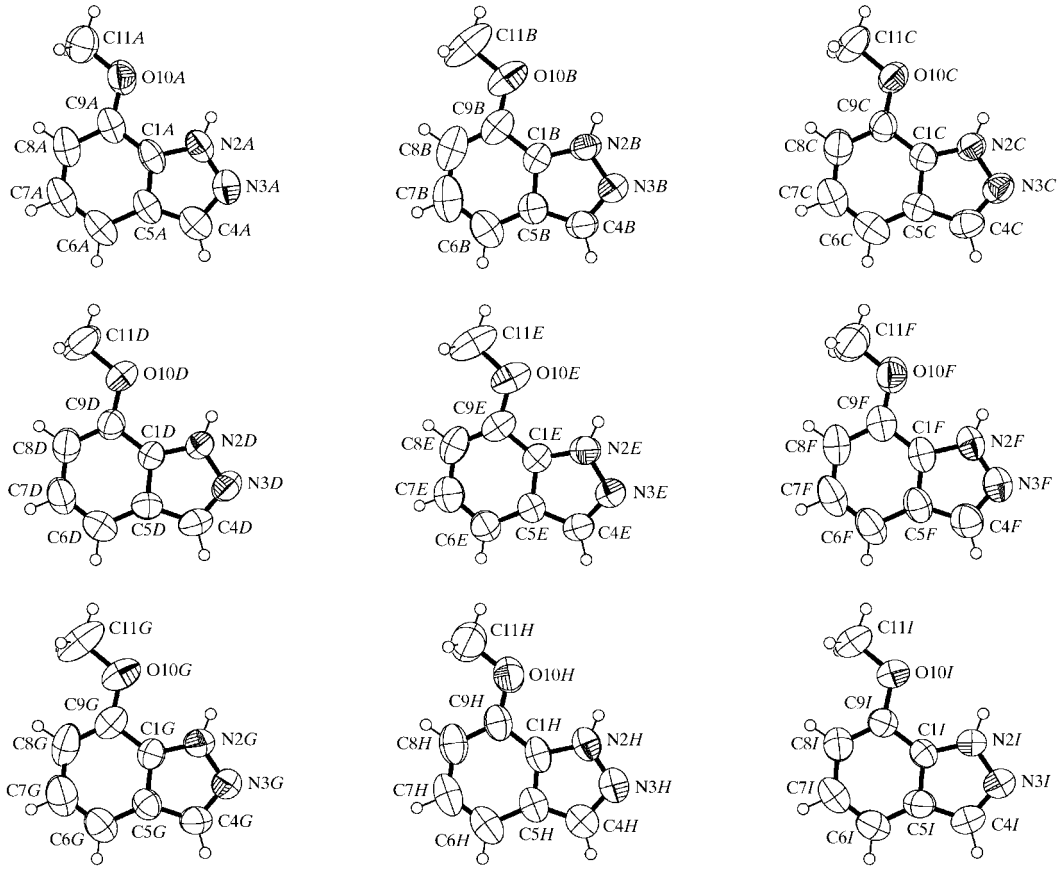

Figure 1
Views of all nine molecules of (I), showing the atom-numbering schemes. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small circles of arbitrary radii.

significant deviation of the methoxy groups in (I) is probably a result of the electrostatic attraction between atom O10 of the methoxy group and indazole atom H2. If the N2—H2 group in (I) is replaced by a C_{ar}—H group, the modelled three-dimensional structure [*MOPAC*, method Austin Model 1 (AM1); Stewart, 1990] gives C8—C9—C10 and C1—C9—O10 bond angles of 125 and 116°, respectively.

The crystal packing consists of trimers of (I), connected by symmetry-equivalent hydrogen bonds from each indazole system, N2—H2···N3 (Fig. 2 and Table 2). One molecule of a trimer interacts through N2—H2 with the second molecule and through N3 with the third, and *vice versa*. The nine molecules are arranged in three trimers: 1st trimer, molecules *A* + *B* + *D*($x+1, y, z+1$); 2nd trimer, molecules *C* + *E* + *F*($x+1, y, z+1$); 3rd trimer, molecules *H* + *G* + *I*($x+1, y, z+1$). The molecules in the trimers are not strictly coplanar, deviating slightly from a planar arrangement (1st trimer: $A/B \simeq 14°$, $A/D \simeq 7°$, $B/D \simeq 6°$; 2nd trimer: $E/F \simeq 5°$, $C/F \simeq 10°$, $C/E \simeq 9°$; 3rd trimer: $I/G \simeq 13°$, $I/H \simeq 15°$, $G/H \simeq 6°$). In the crystal packing, the coplanar trimers stack in parallel planes, with an interplanar spacing of 3.5 Å between the trimers. Two kinds of these trimer strips are observed in the crystal in projection along the *a* axis (Fig. 3), without any direct interaction between them. The difference between the strips is given by the initial trimer orientation. The planes of the trimer indazole rings in neighbouring strips are approximately perpendicular to each other (80°).

Superposition of the X-ray structure of (I) on that of 7-NI (Sopková-de Oliveira Santos *et al.*, 2000) shows that the substitutions at the 7-position are of approximately the same size. Therefore, (I) could be positioned in the substrate-binding site in a similar way to 7-NI, without any motion of the side chains in the substrate-binding cavity. However, replacement of a nitro by a methoxy group will suppress the potentially strong hydrogen acceptor, *viz*. NO_2. This could be a reason for the weaker affinity of (I) compared with 7-NI.

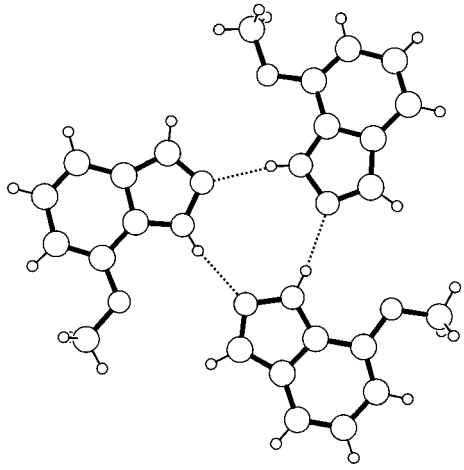

Figure 2
A view of a trimer in the crystal packing of (I). Dashed lines indicate hydrogen bonds.

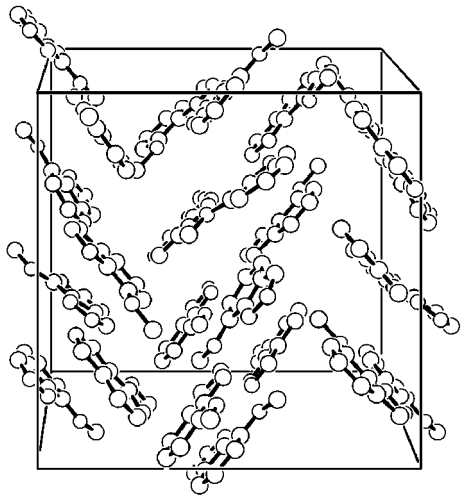

Figure 3
A general view of the crystal packing of (I), projected along the *a* axis.

Experimental

The title compound was synthesized from 2-methoxy-6-methylaniline according to the method of Bartsh & Yang (1984). Crystals of (I) suitable for single-crystal X-ray diffraction were obtained by slow evaporation of a solution in cyclohexane at room temperature.

Crystal data

$C_8H_8N_2O$
M_r = 148.16
Monoclinic, $P2_1$
a = 14.1297 (5) Å
b = 16.0074 (7) Å
c = 15.699 (1) Å
β = 102.533 (4)°
V = 3466.2 (3) Å^3
Z = 18
D_x = 1.278 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 25 reflections
θ = 18–25°
μ = 0.09 mm^{-1}
T = 293 (2) K
Prism, translucent dark yellow
0.5 × 0.4 × 0.3 mm

Data collection

Enraf–Nonius CAD-4 diffractometer
$\theta/2\theta$ scans
10 744 measured reflections
10 405 independent reflections
4332 reflections with $I > 2\sigma(I)$
R_{int} = 0.026
θ_{max} = 30°
$h = -19 \rightarrow 19$
$k = 0 \rightarrow 22$
$l = 0 \rightarrow 22$
3 standard reflections
frequency: 60 min
intensity decay: 5.8%

Refinement

Refinement on F^2
$R(F)$ = 0.053
$wR(F^2)$ = 0.128
S = 0.98
10 405 reflections
901 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0529P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.001
$\Delta\rho_{max}$ = 0.12 e Å^{-3}
$\Delta\rho_{min}$ = −0.10 e Å^{-3}

Table 1
Selected torsion angles (°).

C8*A*—C9*A*—O10*A*—C11*A*	2.7 (6)	C8*F*—C9*F*—O10*F*—C11*F*	−1.8 (6)
C8*B*—C9*B*—O10*B*—C11*B*	−0.8 (6)	C8*G*—C9*G*—O10*G*—C11*G*	1.2 (6)
C8*C*—C9*C*—O10*C*—C11*C*	5.4 (6)	C8*H*—C9*H*—O10*H*—C11*H*	5.5 (6)
C8*D*—C9*D*—O10*D*—C11*D*	2.7 (6)	C8*I*—C9*I*—O10*I*—C11*I*	−8.8 (5)
C8*E*—C9*E*—O10*E*—C11*E*	−5.7 (6)		

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

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
N2*A*—H2*A*···N3*D*^{i}	0.86	2.03	2.879 (4)	170
N2*B*—H2*B*···N3*A*^{ii}	0.86	2.06	2.907 (4)	168
N2*C*—H2*C*···N3*E*^{iii}	0.86	2.04	2.891 (4)	172
N2*D*—H2*D*···N3*B*^{iv}	0.86	2.06	2.909 (4)	167
N2*E*—H2*E*···N3*F*^{i}	0.86	2.02	2.871 (4)	168
N2*F*—H2*F*···N3*C*^{v}	0.86	2.08	2.914 (4)	163
N2*G*—H2*G*···N3*H*	0.86	2.06	2.911 (4)	169
N2*H*—H2*H*···N3*I*^{i}	0.86	2.06	2.906 (5)	169
N2*I*—H2*I*···N3*G*^{vi}	0.86	2.04	2.890 (4)	168

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

The result of the Flack (1983) test was not significant as the number of Friedel pairs measured was low and thus not sufficient for a meaningful Flack parameter. However, (I) does not contain any asymmetric centres, so knowledge of its absolute configuration would not reveal any new information on its structure. H atoms were treated as riding, with C—H distances in the range 0.93–0.96 Å and N—H distances of 0.86 Å.

Data collection: *CAD*-4-*PC Software* (Enraf–Nonius, 1996); cell refinement: *CAD*-4-*PC Software*; data reduction: *JANA*98 (Petříček & Dušek, 1998); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); 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: GD1218). Services for accessing these data are described at the back of the journal.

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