examination could be obtained with tropicamide concentrations much lower (10–20 times) than those currently used in the clinical practice is also noteworthy. The use of dilute (0·05–0·1 % w/v) tropicamide solutions in suitable vehicles should be recommended in order to prevent potential risks of side effects and of systemic toxicity (cf. also Wang & Hammarlund 1970, Brown & Hanna 1978).

March 5, 1980

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LETTERS TO THE EDITOR

2-Amino-6,7-dihydroxytetrahydronaphthalene and the receptor-site preferred conformation of dopamine—a commentary

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The study of the preferred conformation of neurotransmitters, hormones, peptides and drugs is an important one because of the possible usefulness of such information as an aid to the understanding of their molecular modes of action and at a practical level in the design of new agonists or antagonists (Richards 1977). The problem of preferred conformation is, however, twofold. It is possible to refer to the crystal, solution or vacuum preferred conformation of a molecule, depending on the method of conformational analysis, but with a very flexible molecule that this corresponds to the receptor-preferred conformation is not a certainty. Indeed, there is direct evidence that often these two preferred conformations are different (Burgen et al 1975; Williams 1977). One approach that avoids some of these difficulties is that of the rigid or semi-rigid analogue method (Portoghese 1970; Horn & Rodgers 1977). Here attempts are made to constrain the molecule into a hypothesized receptor-preferred conformation by, for example, the addition of an extra ring system to replace a flexible side chain. If activity is still retained, then depending on the rigidity of the analogue, it is possible to make inferences about the actual receptor site preferred conformation of the original non-rigid molecule.

Recently various attempts have been made to determine the receptor site preferred conformation of the neurotransmitter dopamine (DA) (Fig. 1a) (Dandiya et al 1975; Grol & Rollema 1977; Miller 1978). This process has been aided by the fact that various catechol derivatives of 2-aminotetrahydronaphthalene (ATN), which can be considered as DA analogues with a restricted conformation, have been shown to be very potent DA receptor agonists (Woodruff et al 1974, 1977; Miller et al 1974; McDermed et al 1975; Costall et al 1977). We now report details of the conformation in the crystal of one of these analogues namely 2-amino-6,7-dihydroxytetrahydronaphthalene (6,7-diOHATN) (ADTN or 6,7-dihydroxy-2-aminotetralin) (Figs. 1b and 3b) which enables us for the first time to suggest, with some degree of molecular detail (Table 1) and certainty, the most probable receptor conformation of DA.

The conformational analysis of the receptor preferred conformation of DA is a three-fold problem due to the following possibilities:

1. DA can exist in a *trans* or two *gauche* forms (Fig. 2a).
2. There are two possibilities for the *trans* form of DA (Fig. 2b) i.e. the catechol ring is perpendicular to the $-CH_2-NH_2$ bond (*trans* α) or coplanar with it (*trans* β).
3. If the catechol ring is coplanar to the side chain (*trans* β) there are two further possibilities depending on the orientation of the catechol ring i.e. the α and β rotamers (Fig. 2c).

Regarding the possibilities under section 1, some theoretical studies indicate a preference for the *trans* and some for the *gauche* form (Bustard & Egan 1971; Rekker et al 1972; Kier 1973; Pullman et al 1972,

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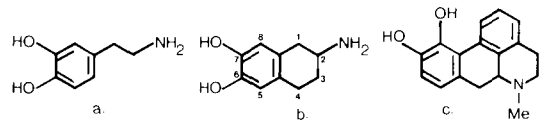
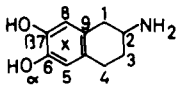


FIG. 1. a. Dopamine. b. 2-amino-6,7-dihydroxytetrahydronaphthalane (6,7-diOHATN). c. Apomorphine.

1974; Katz et al 1973; Grol & Rollema 1977) the general conclusion is that the energy difference between these forms is only of the order of a 4–8 kJ mol^{-1}. However, all the pharmacological evidence using rigid and semi-rigid DA analogues shows convincingly that the receptor preferred conformation is a form of the *trans* species (Miller et al 1974; Costall et al 1974; Komiskey et al 1978; Miller 1978). The second question of whether, at the receptor, the catechol ring is perpendicular (*trans* α) or coplanar (*trans* β) to the $-CH_2-NH_2$ bond is more difficult to answer. In the crystal the preferred form is *trans* α (Fig. 3a, Table 2) (Bergin & Carlstrom 1968). Molecular orbital studies of n.m.r. coupling constants also indicate that the preferred form is *trans* α (Giessner-Prettre & Pullman 1975). It is known, however, that the potential energy difference between these two forms is quite small. The most direct evidence in favour of the hypothesis that the *trans* β form is the active one is the fact that such pharmacologically active analogues as apomorphine (Figs 1c, 3c), 5,6-diOHATN and 6,7-diOHATN (Figs 2c, 3b) are all allied to the *trans* β rather than the *trans* α form, i.e. they are fairly 'planar' molecules. Indeed, the inactivity of a cyclopropane analogue of DA on peripheral (Borgman et al 1978; Erhardt et al 1979) and central dopaminergic receptors (Watling et al 1979) has been explained by the fact that its preferred conformation may correspond more closely to the *trans* α than the *trans* β form of DA (Borgman et al 1978). This conclusion is also supported by the X-ray analysis of the conformation of *NN*-dimethylphenylcyclopropylamine (Carlström 1975).

If it is accepted that the *trans* coplanar form of DA is the receptor-site preferred one, the final question to be answered is what is the preferred orientation of the catechol ring i.e. the α or β rotamer (Fig. 2c)? The 5,6- and 6,7-diOHATNs (Fig. 2c) readily provide an answer to this question. In various in vitro and in vivo tests the 6,7-isomer is consistently much more active than its 5,6 positional isomer iso-ADTN, (Woodruff et al 1977; Seeman et al 1978; Schorderet et al 1978; Sheppard et al 1978; Cannon et al 1978; Westerink et al 1979; Rick et al 1979). Certain authors, however, are of the opinion that in some behavioural test systems the 5,6-isomer is more potent than the 6,7-analogue (Costall et al 1977; Cannon et al 1977). Such behavioural experiments are always complicated by the large number of factors affecting drug action such as distribution, metabolism, uptake and actions at

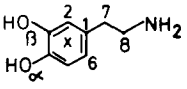
Table 1. 6,7-diOHATN.HBr Torsion angles and interatomic distances. The values are derived from atomic coordinates obtained by X-ray analysis.



Torsion angles		Interatomic distances	
$C_8C_9C_1C_2$	165·2°	N–x	5·15 Å
$C_9C_1C_2N$	168·1°	N–Oα	7·88 Å
$C_1C_2C_3C_4$	−59·9°	N–Oβ	7·32 Å
$NC_2C_3C_4$	177·6°	N above plane of	
$C_9C_1C_2C_3$	44·7°	benzene ring	0·001 Å

multiple DA receptors (Horn et al 1978a, b). We have recently shown that some of the in vivo differences between the 6,7-ADTN and 5,6-isomers are due to the fact that the former compound is readily metabolized by COMT (Rollema et al 1980; Horn et al 1980). The consistent in vitro data are thus probably a much better guide to the receptor-site preferred conformation of DA than are the behavioural results. The third possible catechol isomer of ATN i.e. the 7,8-diOH form, has been shown to be inactive (Costall & Naylor, personal communication). It is also of interest that although the 6,7-isomer may be regarded as an α-substituted DA analogue, unlike its ring-opened lower homologue α-Me-DA (Miller et al 1974; Costall et al 1974; Seeman et al 1978; Cannon et al 1979), it is very potent. The loss of dopaminergic activity in α-Me-DA is probably due to the fact that the preferred conformation of the side chain does not correspond to that occurring in 6,7-diOHATN (Cannon et al 1979), thus the presence of an α-substituent, itself does not preclude strong dopaminergic activity if the overall conformation of the molecule is correct. In addition, it has recently been shown that the (+)-enantiomer of 6,7-diOHATN is about 100 times more potent than the (−)-form and about 4 times more active than DA itself in stimulating the DA sensitive adenylate cyclase system (Andrews et al 1978). Thus, with regard to its pharmacological activity, 6,7-diOHATN seems to be almost an ideal semi-rigid analogue of DA.

Table 2. DA.HCl torsion angles and interatomic distances. The values are derived from published atomic coordinates (Bergin & Carlström 1968)



Torsion angles		Interatomic distances	
$C_6C_1C_7C_8$ =	−99°	N–x	= 5·14 Å
$C_2C_1C_7C_8$ =	79°	N–Oα	7·83 Å
$C_1C_7C_8N$ =	174°	N–Oβ	= 6·83 Å
		N above plane of ring	= 1·61 Å

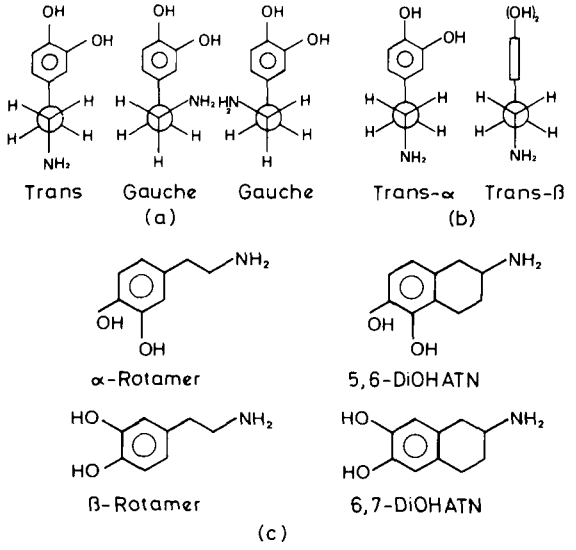


FIG. 2. Conformational analysis of dopamine, see text for a clarification of the terminology.

The amino group in 6,7-diOHATN can, of course, adopt an equatorial or an axial conformation. In the crystal structure, the equatorial form was found to be the preferred one. Theoretical calculations have also shown that this form is 36·4 kJ mol^{-1} more stable than the axial one (Grol & Rollema 1977). Thus although 6,7-diOHATN is not a 'completely' rigid molecule (this is, of course, an idealized concept) its conformational mobility is sufficiently restricted to allow the suggestion that the non-bonded distances and torsion angles of the DA skeleton in it (Table 1) probably correspond closely to those of DA at its receptor. For comparison the corresponding values found in the crystal structure of DAHBr are shown in Table 2. Full crystallographic details of the structure of 6,7-diOHATN.HBr will be reported elsewhere.

The results presented are of interest in several respects. They illustrate that great caution must be excercised in drawing conclusions about the receptor-site preferred conformation of a molecule when this is based on results from a single method. The potential usefulness of the rigid-analogue approach, in combination with other methods, is clearly evident. Sometimes, however, the modification of the parent molecule leads to much less active compounds which in turn complicates any possible interpretation of the receptor preferred conformation. The fact that this is not the case with 6,7-diOHATN clearly shows that if the analogue is carefully designed then a loss of activity is not inevitable.

March 4, 1980

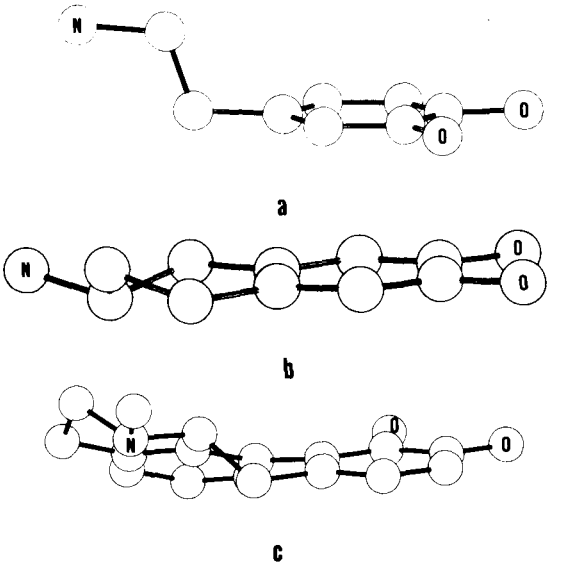


FIG. 3. Solid-state conformations as determined by X-ray crystallographic analysis for a. Dopamine HCl (Bergin & Carlström 1968). b. 6,7-diOHATN.HBr. c. Apomorphine HCl (Giesecke 1976). The above molecules are depicted in a 'side-on' format across the ring system. The large circles are carbon atoms whilst nitrogen and oxygen atoms are designated by N and O, respectively.

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Oxidation of chlorprothixene with potassium permanganate

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Psychotropic thioxanthene derivatives can be analysed fluorimetrically after oxidation with potassium permanganate (Mellinger & Keeler 1964; Mjörndal & Oreland 1971); the structure of the derivative of chlorprothixene thus formed and the influence of pH on the oxidation have been examined.

The oxidation product was prepared as follows: to chlorprothixene hydrochloride (300 mg) in distilled water (10 ml), alkaline potassium permanganate solution (3%, pH 12·4, 30 ml) was added slowly with constant stirring. The precipitated oxidation product, together with manganese dioxide, was filtered off and the reaction product was separated from the MnO_2 by washing the precipitate with acetone. The organic solvent was evaporated and the residue crystallized from ethanol, m.p. 150–151 °C. Found; C, 63·3; H, 2·99; calc. for $C_{13}H_7ClOS$: C, 63·3; H, 2·9.

The u.v., i.r. and p.m.r. spectra were identical with an authentic specimen of 2-chloro-10-thioxanthone (Agarwal & Blake 1969). The melting point and the thin layer chromatographic behaviour were likewise identical with this substance.

The pure thioxanthone shows intense fluorescence in aqueous solutions. With excess of potassium permanganate, when oxidation is carried out in solutions of pH < 3, complete disappearance of fluorescence results.

The new reaction product, isolated in a similar way to the thioxanthone above, crystallized from ethanol, m.p.222–224 °C. Found: C,56·2; H, 2·7; calc. for $C_{13}H_7ClO_3S$: C, 56·0; H, 2·53.

The appearance in the i.r. spectra of this new compound of intense bands at 1300 cm^{-1} and 1160–1140 cm^{-1} suggests that the sulphur atom in the thioxanthone has been further oxidized to a sulphone group. The carbonyl absorption is also shifted to higher frequencies (from 1640 to 1675 cm^{-1}). Since the thioxanthone-sulphone has no fluorescent properties, the importance of controlling the pH of the reaction solution during the oxidation step of the fluorimetric analysis is underlined.

October 23, 1979

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