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Structural Studies of Metabolic Products of Dopamine IV.* Crystal and Molecular Structure of (—)-Noradrenaline

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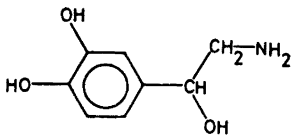
The crystal structure of (−)-noradrenaline has been determined by X-ray methods, using 1000 observed reflections collected by counter diffractometer techniques. The crystals are orthorhombic, space group $P2_12_12_1$, with $a = 8.611(2)$, $b = 6.138(2)$, and $c = 14.912(4)$ Å. Least-squares refinements yielded a conventional R-factor of 0.036. Standard deviations in bond lengths are 0.003 Å and in bond angles 0.2°. The crystal structure of (−)-noradrenaline corresponds closely to that of (−)-adrenaline in molecular geometry, conformation, and arrangement in the crystals The results of the structure investigations are discussed in relation to the biological activity of these biogenic amines.

Noradrenaline (norepinephrine) is an adrenal medullary hormone as well as a neurotransmitter at most sympathetic postganglionic nerve endings and in certain synapses in the central nervous system. In the catecholamine biosynthesis noradrenaline is the immediate precursor of adrenaline, and differs from the latter by lacking a methyl substituent in the amino group. Both drugs act directly on α- and β-adrenergic receptors, but with noradrenaline the α-receptor stimulation generally dominates. Of each compound the naturally occurring $R(-)$-form is the more active isomer.

The crystal structure of (−)-noradrenaline hydrochloride and also the salts of several other sympathomimetic catecholamines have been determined by X-ray diffraction methods.[1] The present study forms part of a structure investigation of the free base form of these biogenic amines, as information



concerning their molecular characteristics is of fundamental importance for structure-activity relationship (SAR) studies.

EXPERIMENTAL

The crystallization procedure employed in the structure investigation of (−)-adrenaline [2] also yielded single crystals of (−)-noradrenaline. Three-dimensional intensity data were obtained from a crystal of dimensions $0.08 \times 0.12 \times 0.43$ mm³ on a SYNTEX $P\bar{1}$ automatic diffractometer with graphite crystal monochromated Mo$K\alpha$ radiation.

Oscillation and Weissenberg photographs indicated orthorhombic symmetry; systematically absent reflections determined the space group to be $P2_12_12_1$. Unit cell parameters were obtained from a least-squares treatment of diffractometer measurements on 15 general reflections.

Intensities of 1063 reflections were recorded using the $\omega - 2\theta$ scan technique with scan range 1.6° and scan speed 1° min^{-1} ($2\theta \leq 50°$), and 1 − 1.5 ° min^{-1} ($50° < 2\theta \leq 60°$). The ratio of total background couting time to scan time was 0.7. 1000 reflections having net intensities greater than $2.5\sigma(I)$ were considered to be observed, the remaining reflections were excluded from the further calculations. The intensity data were corrected for Lorentz and polarization effects and scaled to an absolute level by Wilson's statistical method.

Atomic form factors used were those of Doyle and Turner [3] for oxygen, nitrogen, and carbon atoms, and of Stewart *et al.* [4] for hydrogen atoms.

* The previous publication in this series: *Acta Chem. Scand. B 29* (1975) 239.

Table 1. Fractional atomic coordinates and thermal parameters with estimated standard deviations for non-hydrogen atoms ($\times 10^4$). The temperature factor is given by exp $-(B_{11}h^2+B_{22}k^2+B_{33}l^2+B_{12}hk+B_{13}hl+B_{23}kl)$.

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
O1	1627(2)	3005(3)	7898(1)	451(18)	1646(50)	342(8)	− 62(56)	−69(21)	−332(34)
O2	1188(2)	6486(3)	8997(1)	568(21)	1907(56)	440(10)	456(61)	−73(24)	−772(42)
O3	8388(2)	5068(3)	8380(1)	464(18)	1650(47)	392(9)	−301(56)	−93(24)	417(36)
N	9279(2)	537(4)	8580(2)	588(25)	1400(55)	344(11)	272(68)	−86(29)	151(45)
C1	5648(2)	4123(4)	8585(1)	495(24)	1263(59)	233(10)	7(71)	−37(26)	146(44)
C2	4383(2)	3104(4)	8188(2)	594(26)	1118(59)	286(11)	202(74)	27(29)	−226(46)
C3	2864(2)	3850(4)	8315(1)	494(24)	1205(60)	220(10)	− 86(68)	−36(27)	− 34(42)
C4	2661(2)	5675(4)	8871(1)	516(26)	1365(62)	231(10)	241(75)	45(26)	12(44)
C5	3908(2)	6683(4)	9278(1)	705(28)	1406(65)	239(10)	55(82)	−15(29)	−401(46)
C6	5407(3)	5904(4)	9138(2)	559(27)	1438(66)	245(11)	−243(71)	−99(28)	−214(46)
C7	7274(2)	3352(4)	8344(2)	485(24)	1402(61)	263(11)	− 31(71)	3(28)	54(49)
C8	7851(3)	1572(4)	8959(2)	697(31)	1576(69)	367(13)	245(83)	134(33)	348(55)

Table 2. Fractional atomic coordinates ($\times 10^3$) and isotropic thermal parameters with estimated standard deviations for hydrogen atoms.

Atom	x	y	z	B
HC2	455(3)	191(4)	782(1)	2.4(5)
HC5	371(3)	800(4)	965(1)	2.9(5)
HC6	622(3)	660(4)	941(1)	2.4(5)
HC7	719(3)	269(4)	768(2)	2.7(5)
H1C8	706(3)	44(5)	902(2)	3.5(6)
H2C8	813(3)	220(4)	955(2)	3.9(6)
H1N	975(3)	− 43(6)	897(2)	4.5(7)
H2N	1012(3)	148(5)	840(2)	4.1(7)
H3N	908(3)	− 21(5)	803(2)	3.5(6)
HO2	67(4)	573(6)	875(2)	5.9(11)
HO3	819(3)	607(5)	790(2)	4.8(7)

CRYSTAL DATA

Noradrenaline, $C_8H_{11}O_3N$
$a=8.611(2)$ Å; $b=6.138(2)$ Å; $c=14.912(4)$ Å. $V=788.1$ Å^3; $M=169.20$; $F_{(000)}=360$; $Z=4$; $D_{calc}=1.427$ g cm^{-3}.

STRUCTURE DETERMINATION

The structure was solved by direct methods [5] and refined by full-matrix least-squares technique. All computer programs employed are desribed in Refs. 5 and 6.

Calculated coordinates of hydrogen atoms bonded to carbon atoms were introduced, and a few cycles of anisotropic refinement of non-hydrogen atoms yielded an R-factor of 0.054. A difference Fourier map calculated at this stage revealed the positions of the remaining five hydrogen atoms at peaks ranging from 0.46 to 0.26 e Å^{-3}, compared with background at 0.20 e Å^{-3}. The hydrogen atoms were located in positions showing a zwitterionic structure of the noradrenaline molecule. Subsequent least-squares refinements including positional and isotropic thermal parameters of the hydrogen atoms yielded a conventional R-factor of 0.036 and a weighted R-factor of 0.034. A total difference Fourier map demonstrated irregular electron density with no peaks larger than 0.22 e Å^{-3}.

The final parameters for non-hydrogen atoms are given in Table 1 and for hydrogen atoms in Table 2. An analysis of the anisotropic vibration parameters indicated that the thermal motion in the catechol part of the molecule could be described in terms of a rigid body model. Bond lengths in this part of the molecule were accordingly corrected. Bond lengths and angles are listed in Table 3. The ellipsoids of thermal motion are illustrated in Fig. 1, which also presents corrected bond lengths.

The structure factor list may be obtained from this institute upon request.

DESCRIPTION OF THE STRUCTURE

The noradrenaline molecule is found to have a zwitterionic structure, formed by a proton transfer from the *meta* phenolic hydroxyl group to the nitrogen atom. This was also

Table 3. Bond lengths (Å) and bond angles (°) with estimated standard deviations in parantheses.

Bond length		Corrected	Bond angle	
C1 – C2	1.389(3)	1.391	C1 – C2 – C3	121.8(2)
C2 – C3	1.399(3)	1.401	C2 – C3 – C4	117.3(2)
C3 – C4	1.404(3)	1.409	C3 – C4 – C5	121.3(2)
C4 – C5	1.379(3)	1.381	C4 – C5 – C6	120.1(2)
C5 – C6	1.393(3)	1.395	C5 – C6 – C1	119.9(2)
C1 – C6	1.385(3)	1.390	C2 – C1 – C6	119.5(2)
C1 – C7	1.522(3)	1.524	C6 – C1 – C7	121.6(2)
C3 – O1	1.338(3)	1.340	C2 – C1 – C7	118.7(2)
C4 – O2	1.376(3)	1.378	C2 – C3 – O1	123.7(2)
C7 – C8	1.511(3)		O1 – C3 – C4	118.9(2)
C7 – O3	1.424(3)		C3 – C4 – O2	119.0(2)
C8 – N	1.496(3)		O2 – C4 – C5	119.7(2)
C2 – HC2	0.93(3)		C1 – C7 – C8	112.6(2)
C5 – HC5	0.99(3)		C1 – C7 – O3	112.4(2)
C6 – HC6	0.91(3)		O3 – C7 – C8	106.9(2)
C7 – HC7	1.08(3)		C7 – C8 – N	110.5(2)
C8 – H1C8	0.98(3)			
C8 – H2C8	0.99(3)			
N – H1N	0.93(3)			
N – H2N	0.96(3)			
N – H3N	0.96(3)			
O2 – HO2	0.74(3)			
O3 – HO3	0.96(3)			

observed for the adrenaline molecule in a previous structure investigation.[2] Both crystal structures are characterized by chains of zwitterions extending in the direction of a crystallographic axis. The ions are connected by hydrogen bonds to build double molecular layers, which are held together by van der Waals interactions. This arrangement of the noradrenaline molecules is illustrated in Fig. 2. Van der Waals contacts and hydrogen bond parameters are listed in Table 4. The charged oxygen atom is acting as an acceptor

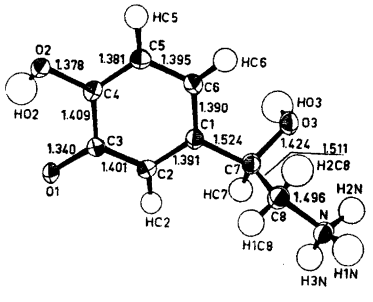


Fig. 1. Bond lengths (corrected) and 50 % probability ellipsoids. (The drawing was prepared using the computer program ORTEP[16]).

in three fairly short and approximately linear hydrogen bonds from two nitrogen atoms and one hydroxyl oxygen atom in neighbouring molecules. The hydrogen bonds of lengths 2.722 Å (N···O1), 2.808 Å (N···O1), and 2.624 Å (O3···O1) are directed nearly tetrahedrally about O1. The nitrogen atom is also acting as a hydrogen donor in a relatively weak hydrogen bond of length 3.046 Å to O2 in an adjacent molecule. The HO2 hydrogen atom is situated in the plane of the benzene ring at a distance of 2.26 Å from the O1 oxygen atom and 2.08 Å from O3 in a neighbouring molecule. The O2···O3 separation of 2.724 Å represents a normal hydrogen bond. The presence of an intramolecular hydrogen bond between O2 and O1 seems thus unlikely, particularly in view of the intermolecular hydrogen bond system already involving O1. In the crystals of adrenaline [2] the arrangement around the charged oxygen atom is almost indentical, only the phenolic hydrogen atom is situated out of the ring plane by −0.123 Å, and thus at larger distance from O1.

The C – O$^-$ bonds of noradrenaline and adrenaline [2] (1.340 and 1.348 Å) are significantly shorter than the phenolic C – O bonds.

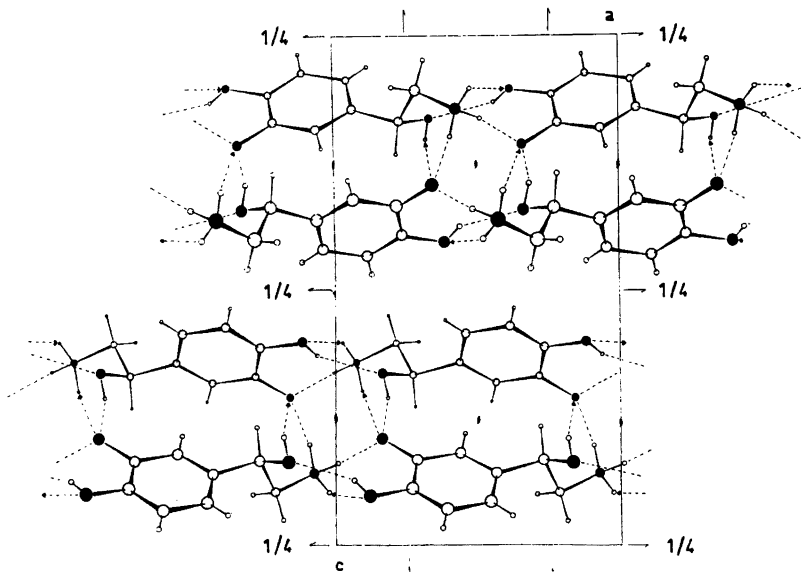


Fig. 2. The structure as seen down the *b*-axis. Hydrogen bonds are indicated by broken lines and the arrow-heads indicate atoms displaced by one unit cell up the *b*-axis.

Table 4.
a. Hydrogen bonded interactions X−H · · ·Y.

X	Y	X···Y (Å)	H···Y (Å)	X−H···Y (°)
N	O1(*a*)	2.722	1.77	170
N	O1(*b*)	2.808	1.87	168
N	O2(*c*)	3.046	2.26	142
O3(*b*)	O1	2.624	1.69	164
O2(*a*)	O3	2.724	2.08	145

b. Other short contacts (Å).

N	O3	2.902
O3	O1(*a*)	3.147
C2	C1(*b*)	3.600
C7	C4(*b*)	3.690
C5	C8(*d*)	3.425
C6	O2(*e*)	3.279

$a = x+1, y, z$
$b = -x+1, y-1/2, -z+3/2$
$c = x+1, y-1, z$
$d = x-1/2, -y+1/2, -z+2$
$e = x+1/2, -y+3/2, -z+2$

No structure determinations of comparable phenolates have hitherto been reported. However, the C−O⁻ distances observed in the two amines are larger than might be expected for this type of bond. This relative lengthening may be an effect of the extensive hydrogen bond system involving the charged oxygen atom in both crystal structures. The bond lengths in the phenolic hydroxyl group are normal; the values found in noradrenaline are 1.378 Å (C4−O2) and 0.74 Å (O2−HO2). The nitrogen atom is tetrahedrally bonded, and the conformation about the C−N bond is staggered. The bond distances C−N (1.496 Å) and N−H (0.93−0.96 Å) are all in the range commonly reported for a C−NH_3^+ group. Altogether, the molecular geometry of noradrenaline corresponds closely to that of adrenaline,[2] and except for the zwitterionic character, also to that reported for the salts of these biogenic amines.[7,8]

The catechol part of the molecule is nearly planar. The deviations from a least-squares plane through the ring carbon atoms are less than 0.007 Å for the atoms defining the plane, and for the exocyclic atoms 0.077 (O1), 0.037 (O2), and 0.137 Å (C7). The deviation of the HO2 hydrogen atom from the ring plane is 0.003 Å.

The ethylamine side chain is fully extended and approximately perpendicular to the ring

system. The dihedral angle C1 – C7 – C8 – N is 167.5° and C6 – C1 – C7 – C8 is 94.2°. This is the conformation usually encountered among the salts of the sympathomimetic amines in the crystals,[1] and is also the one observed in the crystal structure of (–)-adrenaline.[2] The dihedral angle C6 – C1 – C7 – O3 is – 26.6°. Thus, the hydroxyl group of the side chain is situated in a *trans* position relative to the *meta* oxygen atom of the benzene ring. An identical arrangement is found in the crystal structure of (–)-adrenaline,[2] but in the salts of the *R*(–)-form of these biogenic amines a *cis* position of the corresponding hydroxyl groups is observed.[1]

DISCUSSION WITH RELEVANCE TO BIOLOGICAL ACTIVITY

The conformational requirements of the sympathomimetic amines for adrenergic activity have been a central topic in SAR studies in recent years.[1,9–12] In a series of X-ray analyses [1] performed on the salts of these biogenic amines, the ethylamine side chain is with no exception found in an extended conformation. This is therefore regarded as the prefered conformation of the amines in the solid state.[1] The structure investigations of the free base form of adrenaline [2] and noradrenaline (present study) are consistent with this assumption, as both molecules are found with the extended conformation in the crystals. Whether this is also the more stable conformation in solution and of the isolated molecules has been extensively discussed.[1,9,10]

The crystal structures of noradrenaline and adrenaline [2] have revealed another interesting quality of these amines. Both amines have demonstrated the ability to form zwitterions with a proton transfered from the *meta* phenolic group to the amino group. This indicates that the former group is more acidic than the protonated amino group, which has been a subject of some discussion.[13] As the aromatic moiety is assumed to be particularly important for β-adrenergic activity,[9] the zwitterionic character might be involved in the receptor stimulation. In theories concerning drug-receptor interactions, the ability of the catechol moiety to engage in hydrogen bonding and complex formation have been discussed.[9,14,15] A negatively charged oxygen atom could also be engaged in other types of reactions; for example acting as a proton acceptor, or being involved in an ion-pair formation with a cationic receptor site corresponding to the type of interaction proposed for the protonated amino group.

The assumption that a zwitterionic form may be involved in β-receptor interactions is consistent with the increase in β-potency generally observed when going from primary to secondary catecholamines,[15] as the base strengths of the amines normally increase in this series, and hence the ability of zwitterion formation. It is furthermore reported that the adrenaline activity of the tertiary amine *N*-methyladrenaline is markedly reduced, and that the quaternary ammonium salt *N*,*N*-dimethyladrenaline is practically free from adrenergic effects.[15]

A catechol monoanion as participant in biological reactions has been mentioned in connection with the catalytic conversion of ATP to cyclic 3′5′-AMP, and also in the enzymatic *O*-methylation of the catecholamines.[14] The latter reaction is assumed to constitute the principal metabolic pathway of this class of amines, and is regarded as a nucleophilic reaction of a phenolic group with the electron-deficient carbon of *S*-adenosylmethionine.[13,14] This *O*-methylation occurs *in vivo* predominantly on the *meta* phenolic oxygen atom [13], the one found to be negatively charged in the crystals of both noradrenaline and adrenaline.[2]

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