MOLECULAR CONFORMATION OF L-DOPA

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SUMMARY: The structure of L-DOPA, a biological precursor of melanins has been determined by a new systematic analysis of the Patterson function. Its molecular conformation is different from that so far reported for any aromatic aminoacid, peptide or related compound. Its crystal structure is characterized by a three dimensional network of hydrogen bonds in which all the oxygens, the nitrogen and all the hydrogens of the NH_3 and OH groups participate.

INTRODUCTION: The specificity of biochemical reactions whereby one molecule recognizes another causing the particular reaction is believed to be related to the structure and shape of the molecules involved. There has been considerable interest therefore in the study of the conformation of biopolymers, their component units and related structures. We undertook the determination of the molecular structure of 3,4 dihydroxyphenylalanine (L-DOPA) as part of a study of the structure and role of melanins and their precursors. The enzymic oxidation of L-tyrosine to L-DOPA is the first step in the biosynthesis of melanin from L-tyrosine, its physiological precursor (1). The compound is one of additional interest as it has been recently shown to reduce the symptoms of Parkinson's disease. Here the conversion of L-DOPA to dopamine by decarboxylation seems to be involved (2).

METHODS: X-ray diffraction data were collected for a single crystal of L-DOPA with a Hilger & Watts Y 290 computer controlled four circle

diffractometer using Cu Kα_1 radiation reflected from a doubly bent Li F monochromator. 668 unique reflections with Bragg angles less than 57° were used after correcting for Lorentz and polarization effects. No absorption correction was made and the scale and temperature factors were obtained by the method of Wilson (3). The structure was solved by the 'reliable image' method, a new procedure for crystal structure determination based on Patterson function analysis using a minimum of noncrystallographic information (4). From a set of trial atoms chosen from the analysis of the Harker region, trial images were derived that provided the best fit with the observed Patterson function. The hydrogen positions were located from difference Fourier maps. The structure was refined by least squares technique. The 'reliability factor' was .09.

RESULTS

Unit Cell: The unit cell parameters are: a = 6.044 ± .003 A; b = 13.607 ± .003 A; c = 5.311 ± .003 A and γ = 97.55 ± .02 °. Spacegroup: Monoclinic P 2_1. The calculated density based on these data and assuming two molecules per unit cell was 1.512 g. cm $^{-3}$ in agreement with the value 1.51 ± .02 obtained by flotation.

Molecular Conformation: It is seen from Fig. 1 that the conformation of L-DOPA is quite different from that of L-tyrosine (5), dopamine (6) or L-phenylalanine (7). While in L-DOPA the nitrogen is close to the phenyl group and the carboxyl is farther away the reverse is true for L-tyrosine. In L-phenylalanine both the carboxyl and nitrogen are close to the phenyl group. Dopamine and noradrenaline (8) have the same conformation as L-tyrosine. These three conformations correspond to the three staggered positions with angles of rotation $\chi 1$=300° , 180° and 60° around the single bond CαCβ. The eclipsed position with Cγ in the plane N CαCβ has $\chi 1$ = 0°. The numbering of

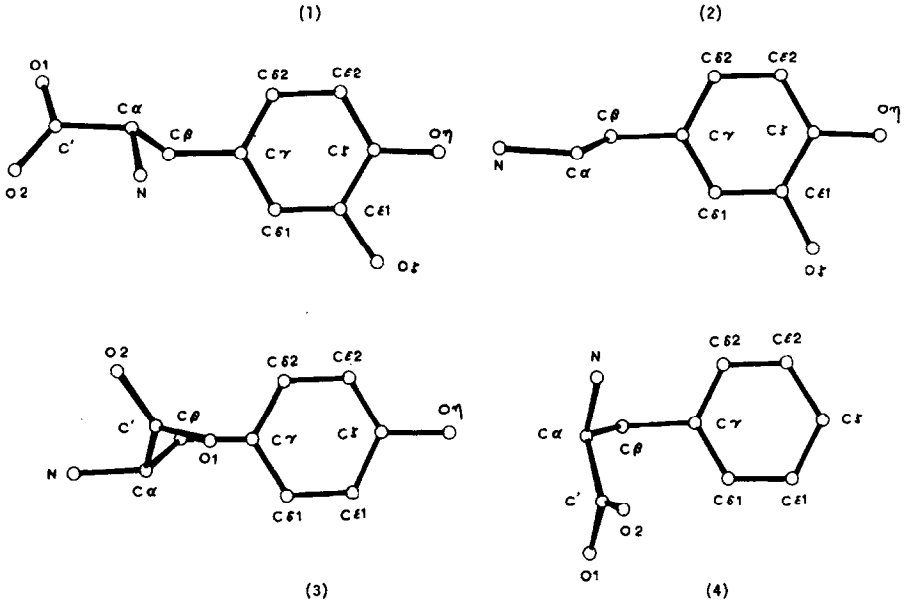

Fig. 1. Molecular Conformations of 1. L-DOPA; 2. dopamine; 3. L-tyrosine; and 4. L-phenylalanine. The phenyl group is in the plane of the paper.

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the atoms and the designation of the rotation angles follow the conventions suggested by Edsall et al (9). Values of $\chi 1$ close to 300° place the bulky aromatic group between N and H (bonded to Cα) and are expected to be sterically favored (10). The conformations also differ by rotations about the bonds CαC' and CβCγ the corresponding angles being denoted by ψ and $\chi 2$. There are 2 ψ's, $\psi 1$, $\psi 2$ nearly 180° apart corresponding to the two oxygens in the carboxyl (when present). They relate to the deviation of the N Cα bond from the plane of the carboxyl. In L-DOPA this deviation is about 14°. The two $\chi 2$'s, $\chi 21$ and $\chi 22$ are also 180° apart and relate to the deviation of the Cβ from the planar phenyl group. ψ and $\chi 2$ seem to follow the choice of $\chi 1$. The conformation parameters of a number of aromatic aminoacids and related structures are given in Table 1. The bond lengths and angles of L-DOPA are not different

Table 1

Conformation parameters for L-DOPA and other aromatic aminoacids and related compounds. The angles ψ are about Cα - C', χ_1 about Cα-Cβ and χ_2 about Cβ - Cγ.

Aminoacid or related compound	ψ_1	ψ_2	χ_1	χ_{21}	χ_{22}
L-DOPA	166	344	294	113	291
L-DOPA H Cl(11)	168	346	298	111	289
N acetyltyrosinamide(12)	---	335	306	109	293
L-tyrosine H Cl(5)	150	324	185	64	244
" H Br(5)	155	322	187	65	250
Glycyl L-tyrosine H Cl(13)	149	327	190	65	242
Dopamine H Cl(6)	---	---	174	77	259
Noradrenaline H Cl(8)	---	---	176	82	263
Glycylphenylalanylglycine(14)	---	314	185	102	278
L-threonyl L-phenylalanine p nitrobenzyl ester H Br (15)	117	299	176	87	268
L-phenylalanine H Cl(7)	178	358	62	84	262

from those of other compounds.

HYDROGEN BONDS: The crystal structure of L-DOPA is characterized by a three dimensional network of hydrogen bonds. All the oxygens, the nitrogen and all the hydrogens in the NH_3 and OH groups are involved in the hydrogen bonding. The nitrogen is bonded to O_1 of an adjacent molecule and O_2 of two adjacent molecules; Oζ to O_1 of an adjacent molecule and Oη to Oη of two adjacent molecules. There is a twofold disorder in the hydrogen bound to Oη.

DISCUSSION: In its crystalline form L-DOPA has the zwitterion structure: $(NH_3)^+$ - (CHR) - $(COO)^-$ found also in neutral solutions. It is not unlikely that the conformation of L-DOPA found by us is its preferred conformation. However the reported conformations of the related compounds L-tyrosine, dopamine, noradrenaline and L-phenylalanine correspond to the H Cl or H Br derivatives. In all these compounds the heavy atom is linked by hydrogen bonds to the nitrogen and some of the oxygens. One might ask if the hydrogen bond requirements might force conformational changes. Just as this report was being prepared the result of L-DOPA H Cl was made available to us (11). The conformation parameters computed from these data are included in Table 1. It is readily seen that the conformation of L-DOPA in crystals of the zwitterion as well as its H Cl derivative is the same. In L-DOPA H Cl as in other H Cl derivatives Cl is linked by hydrogen bond to N and O_2. However the different hydrogen bond requirements as compared to the zwitterion crystal are accommodated by altering the dispositions of adjacent molecules. They result in slight changes in the size and shape of the unit cell and the position and orientation of the molecules in the unit cell. The conformation of the molecule however remains unchanged. This would probably indicate that the L-DOPA conformation we report is its natural conformation which differ from that of the closely related compound: L-tyrosine, dopamine, L-phenylalanine or noradrenaline. We do not yet know the significance of these conformational changes and if they have any relation to the biochemical reactions involving these compounds. After the structure of L-DOPA was determined we found the conformation of the tyrosyl group in N acetyltyrosinamide (12) was different from that of L-tyrosine and close to that of L-DOPA. This conformational change may be related to the presence of the other groups.

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