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The Structure of Harman, a Comutagen

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Harman, a comutagen, has been crystallized and studied by X-ray diffraction techniques. The molecule is shown to be flat, apart from the hydrogen atoms of the methyl group. Crystals are orthorhombic, space group $P2_12_12_1$. The unit cell dimensions are a = 13.368(4), b = 15.537(3), c = 9.535(3) Å, V = 1980.4(9) Å^3 with two molecules in the asymmetric unit. Diffraction data were measured at room temperature with CuKα radiation. The structure was solved by direct methods and refined by a full-matrix least-squares procedure (nonhydrogen atoms anisotropic, hydrogen atoms isotropic) to a final R = 0.046 for 1961 independent observed reflections. The hydrogen bonding between molecules leads to a hypothesis on the relationship to mutagens such as proflavine, particularly when harman is complexed with an aromatic amine so that the pair of molecules can then interact with biological macromolecules in a manner similar to that of certain known mutagens.

INTRODUCTION

When tryptophan-rich foods are pyrolyzed, as in cooking, several carboline derivatives are formed. The γ-carbolines, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1, **I**), and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2, **II**), which are strongly mutagenic, have been characterized in such pyrolysates (*1*). It was noted that the mutagenicities of these two compounds were greatly increased by two other compounds, β-carbolines, that were also found to be present in the tryptophan pyrolysate; these compounds, harman, 1-methyl-9*H*-pyrido[3,4-*b*]indole (**III**), and norharman, 9*H*-pyrido[3,4-*b*]indole (**IV**), are themselves not mutagenic; however, they appear to be able to enhance or cause the mutagenicities of certain other compounds, particularly nonmutagenic aromatic amines such as aniline or *o*-toluidine. The term "comutagenicity" was coined to describe this phenomenon (*2*). Harman and norharman have been identified as components of cooked foods (*3*) and cigarette smoke condensate (*4*).

The biological activities of these two compounds are complicated; generally norharman is more active than harman. They can be considered to have modifying effects on the activities of five different groups of chemicals. These effects are generally observed only in the presence of metabolizing enzymes unless the compound is already an activated metabolite (*5*). Harman and norharman may cause certain aromatic amines (that are not themselves mutagenic) to become mutagenic

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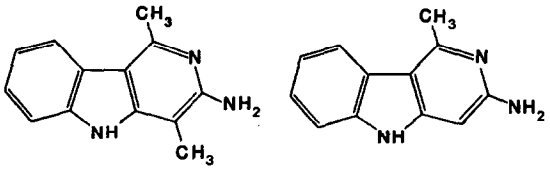

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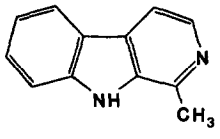

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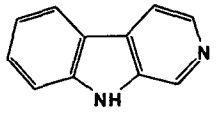

IV

(*5–8*). They may enhance the mutagenicity of certain mildly mutagenic compounds (*9*). The mutagenicities of certain polycyclic hydrocarbons such as benzo[*a*]pyrene may be enhanced by comutagens if large amounts of microsomal enzymes are present (*10–12*); if small amounts of enzyme are present, the opposite effect may be seen. On the other hand, the mutagenicities of certain other compounds, such as dimethylbenz[*a*]anthracene, are not affected by these comutagens. Some other compounds, such as the flavonoid kaempferol, are less mutagenic in the presence of norharman, regardless of the concentration of microsomal enzymes (*5*). In each case norharman is more effective than harman.

The mechanism of this comutagenic effect has been studied extensively. Both harman and norharman interact with DNA, presumably by intercalation, with an unwinding of 17 ± 3° per base pair (*13*). Harman binds more efficiently in this case. It appears that when these comutagens interact with DNA they do not affect the affinity of proven mutagens for DNA (*14*); however, they do inhibit covalent binding of the mutagens to DNA (*14*).

One site of activity of harman and norharman with polycyclic aromatic hydrocarbons appears to be cytochrome *P*-450 (*15, 16*). Both compounds influence the distribution of metabolites obtained by the action of this enzyme; the formation of polar (less mutagenic) metabolites is inhibited by norharman (*12, 17*). It has been concluded (*17*) that norharman binds to cytochrome *P*-450 on the sixth ligand position of iron and near the active site area where polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene, bind and are activated (*18*). Thus norharman is more effective as a comutagen than harman, but binds less tightly to DNA than does harman (*19*).

The detailed three-dimensional structure of harman was determined in order to shed more light on the types of intermolecular interactions formed by this comutagen.

EXPERIMENTAL PROCEDURES

Harman was purchased from Aldrich Chemical Co. and recrystallized from ethanol. Three-dimensional X-ray diffraction data were collected on a Nicolet $P2_1$

diffractometer; a crystal of dimensions 0.4 × 0.2 × 0.4 mm was used for the intensity data collection. The space group is $P2_12_12_1$. Unit cell dimensions were obtained from a least-squares fit of nine centered reflections. Three-dimensional X-ray intensity data were collected with the $\theta - 2\theta$ scan technique, using graphite-monochromated CuKα radiation. Of 2140 reflections scanned in the range $(\sin\theta)/\lambda = 0.05$ to 0.61 Å^{-1} ($2\theta = 138°$), 1961 had intensity (I) greater than the threshold of $2\sigma(I)$ [with $\sigma(I)$ derived from counting statistics]. These were considered "observed" and used in the structure solution and refinement. Values of $\sigma(F) = (F/2)\{[\sigma^2(I)/I^2] + \delta^2\}^{1/2}$, where δ is the instrumental uncertainty ($\delta = 0.0254$) determined from the variation in measured intensities in four periodically scanned standard reflections, were computed. There was no fall-off in intensity as a function of time. The 1961 reflections and their associated standard deviations were converted to structure amplitudes by application of Lorentz and polarization factors and placed on an absolute scale with a Wilson plot; an empirical absorption correction, obtained from a ψ scan, was made.

The structural solution was obtained by use of the computer program MULTAN 80 (*20*). An E map revealed the positions of all of the nonhydrogen atoms which were then refined, first with isotropic and then anisotropic temperature factors, using a full-matrix least-squares procedure. All hydrogen atoms were then located from a difference electron density map. Their inclusion and subsequent refinement with isotropic temperature factors led to final residuals $R = 0.046$ and $wR = 0.055$. Largest ratio of shift to esd = 0.15; $\Sigma w\Delta F^2$ minimized in the least-squares calculations; $w = 1/[\sigma^2(F)]$, with zero weight for the reflections below the threshold value. The final difference Fourier map had no peak higher than 0.21 e/Å^3.

The computer programs used were a locally modified version of UCLALS4 (*21*, *22*) and other programs written at the Institute for Cancer Research laboratory. The atomic scattering factors used are from a compilation of published values (*23*). The numbering of atoms is shown in Fig. 1. Refined coordinates of atoms in one asymmetric unit and average equivalent isotropic temperature factors are listed in Table 1. Anisotropic temperature factors and observed and calculated

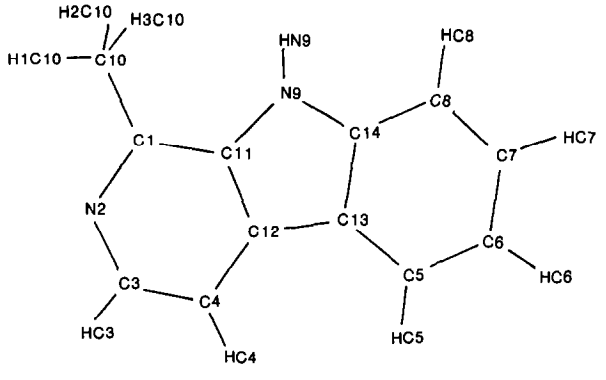

FIG. 1. Numbering system used for harman.

TABLE 1

ATOMIC COORDINATES(esd) AND AVERAGE B(esd)

Atom	x	y	z	B_{av}, B_{iso}
C(1)	0.0981(2)	0.0656(2)	0.9176(3)	4.7(1)
N(2)	0.0826(2)	0.0138(1)	1.0265(2)	4.03(9)
C(3)	0.1555(2)	0.0037(2)	1.1214(3)	5.8(1)
C(4)	0.2478(2)	0.0432(2)	1.1169(3)	5.3(1)
C(5)	0.4415(2)	0.1697(2)	1.0181(3)	5.4(1)
C(6)	0.5011(2)	0.2291(2)	0.9511(4)	6.3(1)
C(7)	0.4693(2)	0.2694(2)	0.8299(4)	6.6(1)
C(8)	0.3767(2)	0.2532(2)	0.7696(3)	5.7(1)
N(9)	0.2201(1)	0.1673(1)	0.8047(2)	4.47(8)
C(10)	0.0160(2)	0.0774(2)	0.8131(3)	6.2(1)
C(11)	0.1895(2)	0.1087(1)	0.9046(2)	3.92(9)
C(12)	0.2652(2)	0.0979(1)	1.0040(2)	4.23(9)
C(13)	0.3464(2)	0.1519(2)	0.9618(3)	4.47(9)
C(14)	0.3154(2)	0.1940(2)	0.8386(3)	4.44(9)
C(1′)	0.1144(2)	0.2975(2)	0.5095(3)	5.2(1)
N(2′)	0.1663(2)	0.2254(2)	0.5300(2)	6.2(1)
C(3′)	0.2341(2)	0.2007(2)	0.4317(3)	6.8(1)
C(4′)	0.2527(2)	0.2456(2)	0.3106(3)	5.9(1)
C(5′)	0.2402(2)	0.3909(2)	0.0460(3)	6.4(1)
C(6′)	0.2139(3)	0.4591(2)	−0.0375(3)	7.7(2)
C(7′)	0.1420(3)	0.5180(2)	0.0035(4)	7.7(2)
C(8′)	0.0932(2)	0.5112(2)	0.1307(3)	6.5(1)
N(9′)	0.0813(2)	0.4194(1)	0.3460(2)	4.86(9)
C(10′)	0.0401(3)	0.3238(2)	0.6210(4)	8.3(2)
C(11′)	0.1283(2)	0.3455(2)	0.3883(2)	4.29(9)
C(12′)	0.1981(2)	0.3205(2)	0.2873(2)	4.49(9)
C(13′)	0.1927(2)	0.3824(2)	0.1764(2)	4.7(1)
C(14′)	0.1190(2)	0.4424(2)	0.2167(3)	5.0(1)
H(C3)	0.137(2)	−0.029(2)	1.201(3)	8.1(8)
H(C4)	0.297(2)	0.035(2)	1.186(3)	5.9(6)
H(C5)	0.463(2)	0.136(2)	1.110(3)	7.3(7)
H(C6)	0.572(2)	0.244(2)	0.984(3)	5.8(6)
H(C7)	0.523(2)	0.316(2)	0.787(3)	7.0(7)
H(C8)	0.355(2)	0.284(2)	0.684(3)	6.1(6)
H(N9)	0.198(2)	0.173(2)	0.720(3)	6.1(6)
H(1C10)	0.004(3)	0.132(2)	0.790(4)	12.(1)
H(2C10)	−0.038(3)	0.052(2)	0.835(4)	10.0(9)
H(3C10)	0.041(4)	0.043(3)	0.707(5)	14.(1)
H(C3′)	0.265(2)	0.139(2)	0.443(4)	8.6(8)
H(C4′)	0.297(3)	0.212(2)	0.244(3)	8.2(8)
H(C5′)	0.297(3)	0.343(2)	0.030(4)	8.6(8)
H(C6′)	0.251(2)	0.466(2)	−0.123(4)	7.5(8)
H(C7′)	0.119(3)	0.568(2)	−0.051(4)	10.2(9)
H(C8′)	0.041(3)	0.549(2)	0.163(4)	8.4(8)
H(N9′)	0.035(2)	0.449(2)	0.403(3)	7.2(7)
H(1C10′)	−0.037(5)	0.334(4)	0.576(7)	18.(2)
H(2C10′)	0.036(5)	0.391(4)	0.661(6)	17.(2)
H(3C10′)	0.049(4)	0.295(3)	0.689(6)	13.(1)

$B_{av} = \frac{1}{3}$ (trace orthogonalized B_{ij} matrix).

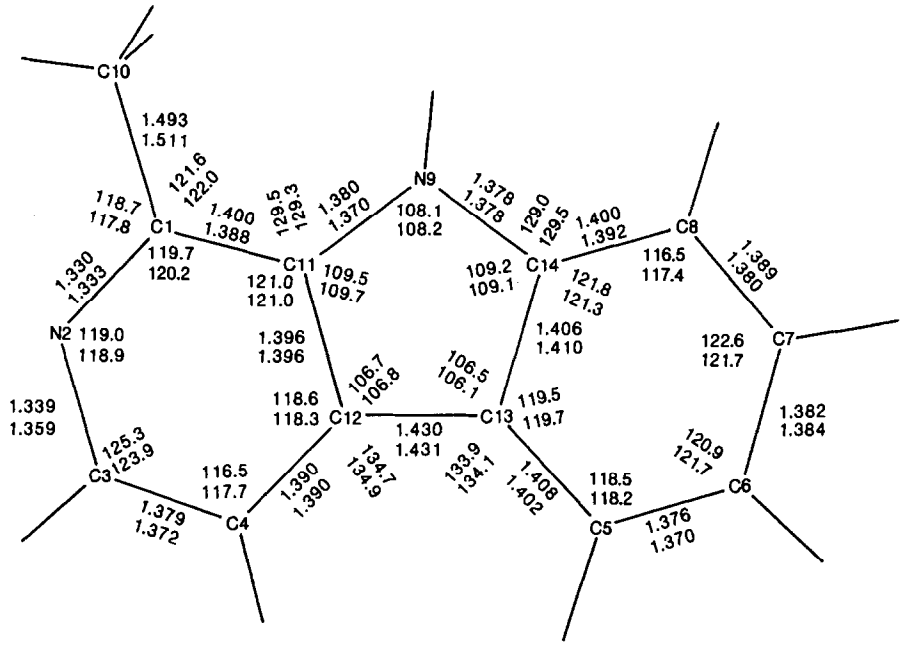

FIG. 2. Bond distances and interbond angles.

structure factors have been deposited.[1] Bond distances and interbond angles are shown in Fig. 2.

RESULTS

This analysis has provided structural data on two independent molecules of harman. Both are shown to be extremely flat; deviations from the molecular plane are listed in Table 2. Only the hydrogen atoms of the methyl group project appreciably from the molecular plane. This planarity is surprising in view of the fact that two planar six-membered rings (with bond angles 117–122°) are fused onto a five-membered ring (with bond angles 106–110°). The bond distances and interbond angles, illustrated in Fig. 2, show that this fusion results in large external angles (at C(11), C(12), C(13), and C(14)). The aromatic characters of the two six-membered rings (one of which is heterocyclic) must promote planarity even though the angles at which substituents are bound are larger than usual (129–135° instead of 120°).

[1] See NAPS document No. 04449 for 16 pages of supplementary material. Order from ASIS/NAPS, Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163. Remit in advance $4.00 for microfiche copy or for photocopy, $7.75 up to 20 pages plus $.30 for each additional page. All orders must be prepaid. Institutions and organizations may order by purchase order. However, there is a billing and handling charge for this service of $15. Foreign orders add $4.50 for postage and handling, for the first 20 pages, and $1.00 for additional 10 pages of material. Remit $1.50 for postage of any microfiche orders.

TABLE 2

DEVIATIONS IN Å OF ATOMS FROM THE LEAST-SQUARES BEST PLANE THROUGH EACH OF THE TWO MOLECULES

Atom	x	esd	Atom	x	esd
C(1)	−0.003	0.002	C(1′)	−0.004	0.002
N(2)	0.030	0.002	N(2′)	−0.037	0.002
C(3)	0.029	0.003	C(3′)	−0.023	0.003
C(4)	−0.002	0.003	C(4′)	0.016	0.003
C(5)	−0.019	0.003	C(5′)	0.001	0.003
C(6)	0.028	0.003	C(6′)	−0.026	0.003
C(7)	0.038	0.003	C(7′)	−0.032	0.004
C(8)	0.011	0.003	C(8′)	−0.009	0.003
N(9)	−0.016	0.002	N(9′)	0.018	0.002
C(10)	0.016	0.003	C(10′)	−0.009	0.004
C(11)	−0.032	0.002	C(11′)	0.018	0.002
C(12)	−0.032	0.002	C(12′)	0.035	0.002
C(13)	−0.031	0.002	C(13′)	0.036	0.002
C(14)	−0.015	0.002	C(14′)	0.018	0.003
rms deviation from the plane = 0.024 Å			rms deviation from the plane = 0.023 Å		
H(C3)	0.15	0.02	H(C)	−0.21	0.02
H(C4)	0.01		H(C4′)	−0.12	
H(C5)	−0.06		H(C5′)	0.06	
H(C6)	0.02		H(C6′)	0.02	
H(C7)	0.09		H(C7′)	−0.06	
H(C8)	0.04		H(C8′)	−0.05	
H(N9)	−0.27		H(N9′)	0.07	
H(1C10)	0.59		H(1C10′)	−0.85	
H(2C10)	0.11		H(2C10′)	0.69	
H(3C10)	−1.05		H(3C10′)	0.12	

Two molecules, comprising the asymmetric unit, have an N–H . . . N hydrogen bond between them, as shown in Fig. 3; in this hydrogen bond (in the two cases in the asymmetric unit) N(9)–H(N9) is 0.86, 0.94 Å, H(N9) . . . N(2) is 2.03, 1.99 Å, and N . . . N is 2.862, 2.903 Å. These two types of hydrogen bonds are from N(9) of a molecule at x, y, z to N(2′) of a primed molecule at x, y, z and from N(2) in the molecule at x, y, z to N(9′) of a primed molecule at $(-x, -\frac{1}{2} + y, \frac{3}{2} - z)$. This results in infinite chains of hydrogen-bonded molecules in the sequence *A–B–A–B* (where *A* and *B* are the two different molecules in the asymmetric unit). These chains run along the *b* direction as shown in the crystal packing diagram in Fig. 4. This hydrogen bonding shows the effect of the methyl group; the N–H . . . N hydrogen bond is not linear. Not only does the methyl group interfere with access to the N–H group but it prevents the two hydrogen-bonded molecules from being coplanar—their methyl groups would "bump" into ring C–H groups; only in the absence of methyl groups, that is, in the case of norharman, can the two molecules form a hydrogen bond and remain coplanar. The ring receiving the

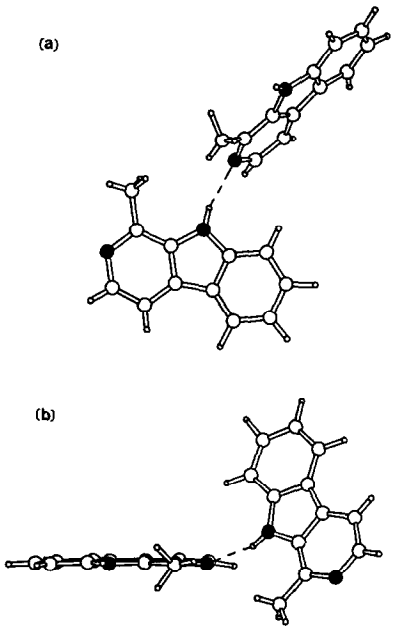

FIG. 3. Hydrogen bonding of harman viewed (a) onto the molecular plane, and (b) along the molecular plane.

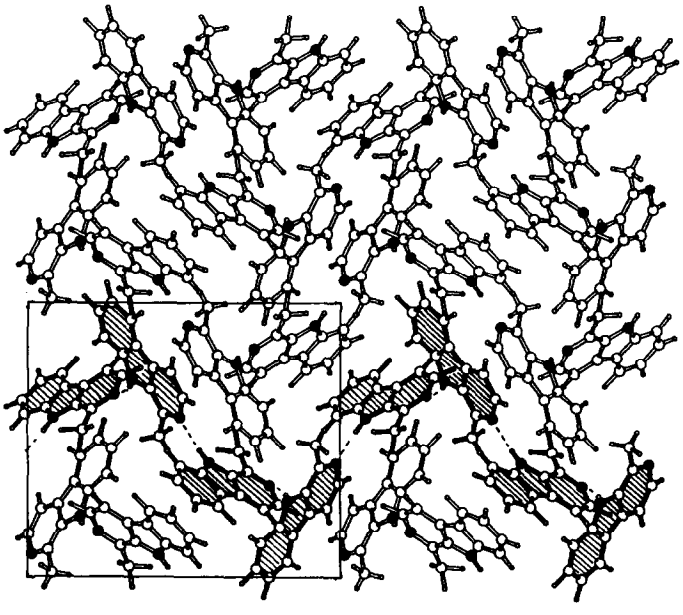

FIG. 4. Packing of harman molecules. View down the *c*-axis. One unit cell is outlined. One spiral of harman molecules, hydrogen bonded to each other in the *b*-direction, is emphasized by shaded rings.

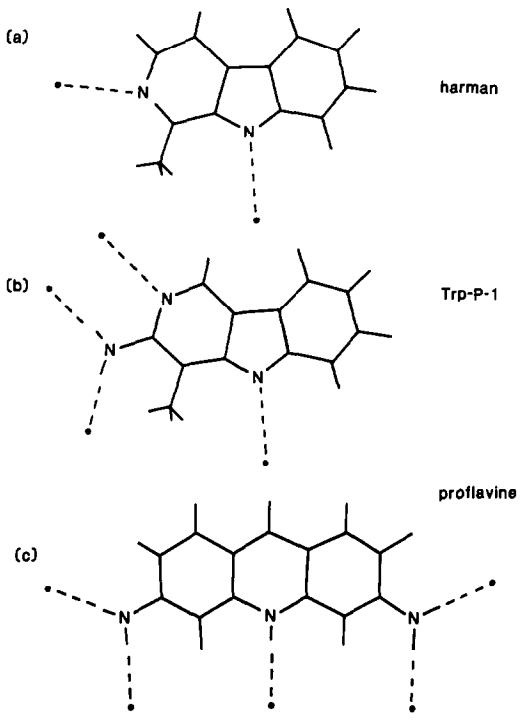

FIG. 5. Comparison of harman, Trp-P-1, and proflavine. (a) Harman, (b) Trp-P-1, and (c) proflavine.

hydrogen bond is not coplanar with the ring donating it. This implies that the planarity of harman does not impose constraints on the geometry of hydrogen bonding. In fact there is considerable flexibility in this geometry.

DISCUSSION

The crystal structure of the tryptophan pyrolysate (Trp-P-1) has been determined (*24*). The nitrogen atom in the six-membering ring is in a different position from that in harman and there is an additional amino group. As a result the resemblance of Trp-P-1 to a portion of proflavine, a proven mutagen, is readily seen. This is illustrated in Fig. 5 which shows a comparison of the hydrogen bonding in harman, Trp-P-1, and proflavine. As can be seen, two hydrogen bonds for Trp-P-1 are similarly oriented to two such hydrogen bonds in proflavin, although the methyl group in Trp-P-1 may cause steric problems.

In a similar way, when harman (or norharman) is hydrogen bonded to an aromatic amine such as *o*-toluidine, a relationship to the structure of proflavine, illustrated in Fig. 6, can be postulated. Two different orientations of *o*-toluidine, hydrogen-bonded to harman, are shown. The jagged line in Fig. 6b indicates steric hindrance between two hydrogen atoms so that the two molecules cannot be

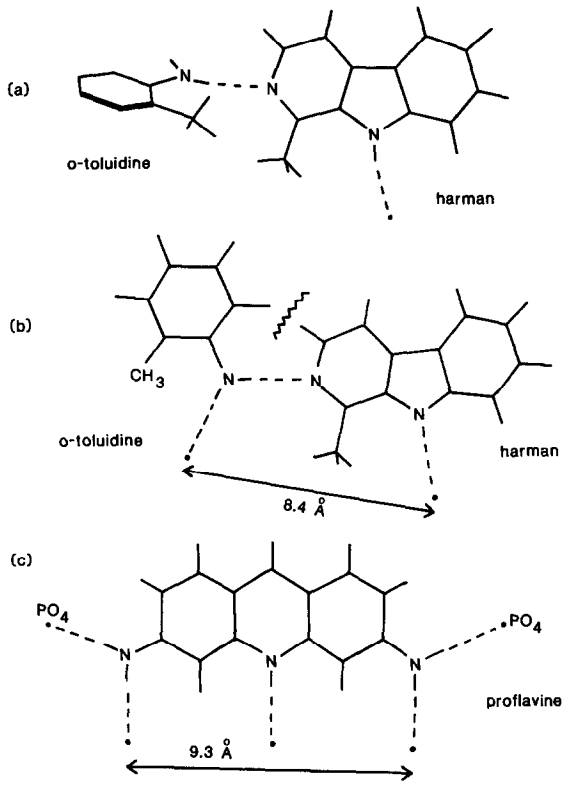

FIG. 6. (a) Model of harman complexed with *o*-toluidine in the orientation found in the crystal; (b) model with the two ring systems in the same plane, and (c) proflavine on the same scale.

exactly coplanar; nevertheless the nitrogen atoms in this complex can hydrogen-bond to two groups approximately 8.4 Å apart. This may enable the complex to interact with a macromolecule in a similar manner to proflavine, provided the 1-Å difference in distances between interacting groups can be accommodated. In this way the comutagenicity with aromatic amines may possibly be explained.

Since cytochrome *P*-450 is a possible site of interaction of harman we also constructed models of harman bound axially at each of the two nitrogen atoms. In each case there is some steric overcrowding (indicated by a jagged line in Fig. 7) from the methyl group; however, by analogy with corrins which bind dimethylbenzimidazole with similar steric problems, the porphyrin skeleton may accommodate this overcrowding by some buckling or flexing. Norharman would not have this problem and is known to interact more strongly with cytochrome *P*-450. Proofs of these ideas must await further experimental evidence.

In summary, we have shown the molecular dimensions of harman which is an almost planar molecule, and the manner in which it forms hydrogen bonds to other harman molecules. This has led to a hypothesis on the way that harman can act as a comutagen, possibly by forming a hydrogen bond to the compound it renders

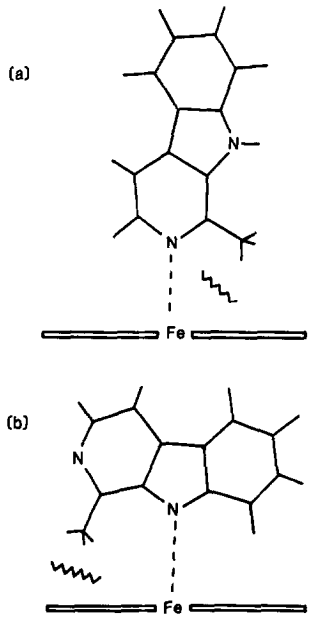

FIG. 7. Axial coordination of norharman to an iron porphyrin. (a) Coordination to the 6-ring, and (b) coordination to the 5-ring.

mutagenic and thereby making a complex that resembles certain known mutagens.

ACKNOWLEDGMENTS

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Note added in proof. The X-ray structure determination of harman recently was independently reported (*25*).

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