this group is shifted to a value higher than the highest pH examined in the profiles. In contrast, in the CPA-catalyzed hydrolysis of *O*-acetyl-L-mandelate[35] or *O*-(*p*-nitrobenzoyl)-L-mandelate[33] an ionizing group with a pK near 8 is reflected in the descending limbs of the $k_{cat.}$ profiles in the alkaline range, suggesting strongly the influence of the ionization of the Tyr-248 hydroxyl function.

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References and Notes

(1) E. T. Kaiser, T. W. Chan, and J. Suh in "Protein–Metal Interactions", M. Friedman, Ed., Plenum Press, New York, N.Y., 1974, p 59.
(2) W. N. Lipscomb, *Tetrahedron*, **30**, 1725 (1974).
(3) W. N. Lipscomb, *Acc. Chem. Res.*, **3**, 81 (1970).
(4) J. P. Felber, T. L. Coombs, and B. L. Vallee, *Biochemistry*, **1**, 231 (1962), and references cited therein.
(5) P. H. Petra, *Biochemistry*, **10**, 3163 (1971).
(6) P. H. Petra and H. Neurath, *Biochemistry*, **10**, 3171 (1971).
(7) G. M. Hass and H. Neurath, *Biochemistry*, **10**, 3535, 3541 (1971).
(8) R. T. Simpson, J. F. Riordan, and B. L. Vallee, *Biochemistry*, **2**, 616 (1963).
(9) J. R. Whitaker, F. Menger, and M. L. Bender, *Biochemistry*, **5**, 386 (1966).
(10) J. F. Riordan and B. L. Vallee, *Biochemistry*, **2**, 1460 (1963).
(11) R. T. Simpson and B. L. Vallee, *Biochemistry*, **5**, 1760 (1966).
(12) M. Sokolovsky and B. L. Vallee, *Biochemistry*, **6**, 700 (1967).
(13) J. F. Riordan, M. Sokolovsky, and B. L. Vallee, *Biochemistry*, **6**, 358 (1967).
(14) J. T. Johansen and B. L. Vallee, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 2532 (1971), and references cited therein.
(15) E. T. Kaiser and B. L. Kaiser, *Acc. Chem. Res.*, **5**, 219 (1972).
(16) J. Glovsky, P. L. Hall, and E. T. Kaiser, *Biochem. Biophys. Res. Commun.*, **47**, 244 (1972).
(17) J. T. Johansen and B. L. Vallee, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2006 (1973), and references cited therein.
(18) W. N. Lipscomb, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 3797 (1973), and references cited therein.
(19) G. Tomalin, B. L. Kaiser, and E. T. Kaiser, *J. Am. Chem. Soc.*, **92**, 6046 (1970).
(20) M. L. Anson, *J. Gen. Physiol.*, **20**, 663 (1937).
(21) F. W. Putnam and H. Neurath, *J. Biol. Chem.*, **166**, 603 (1946).
(22) G. Muszynska and J. F. Riordan, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 466 (1973).
(23) M. Sokolovsky, J. F. Riordan, and B. L. Vallee, *Biochemistry*, **5**, 3582 (1966).
(24) H. M. Kagan and B. L. Vallee, *Biochem. Biophys. Res. Commun.*, **34**, 654 (1969).
(25) J. T. Johansen, D. M. Livingston, and B. L. Vallee, *Biochemistry*, **11**, 2584 (1972).
(26) H. M. Kagan and B. L. Vallee, *Biochemistry*, **8**, 4223 (1969).
(27) O. H. Lowry, N. J. Roseborough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(28) P. L. Hall, B. L. Kaiser, and E. T. Kaiser, *J. Am. Chem. Soc.*, **91**, 485 (1969).
(29) F. A. Quiocho, C. H. McMurray, and W. N. Lipscomb, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2850 (1972).
(30) The spectral titration of nitroCPA has not revealed any evidence for the coordination of the phenolate residue of nitroTyr-248 to the active site zinc ion. This result does not necessarily exclude the possibility of such coordination, however.
(31) D. S. Auld and B. L. Vallee, *Biochemistry*, **9**, 4352 (1970), and earlier references cited therein.
(32) R. S. Stephens, J. E. Jentoft, and R. C. Bryant, *J. Am. Chem. Soc.*, **96**, 8041 (1974).
(33) J. W. Bunting, J. Murphy, C. D. Myers, and G. G. Cross, *Can. J. Chem.*, **52**, 2648 (1974).
(34) D. S. Auld and B. Holmquist, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **31**, 435 (1972).
(35) F. W. Carson and E. T. Kaiser, *J. Am. Chem. Soc.*, **88**, 1212 (1966).
(36) F. A. Quiocho and W. N. Lipscomb, *Adv. Protein Chem.*, **25**, 1 (1971).
(37) P. Valenzuela and M. L. Bender, *Proc. Natl. Acad. Sci. U.S.A.*, **63**, 1214 (1969); *Biochemistry*, **9**, 2440 (1970).
(38) S. F. Bosen and E. T. Kaiser, *J. Am. Chem. Soc.*, **93**, 1038 (1971).
(39) W. P. Jencks, "Catalysis in Chemistry and Enzymology", McGraw-Hill, New York, N.Y., 1969, pp 508–517.

Conformation and Reactivity of the Macrocyclic Tumor-Inhibitory Alkaloid Tetrandrine[1]

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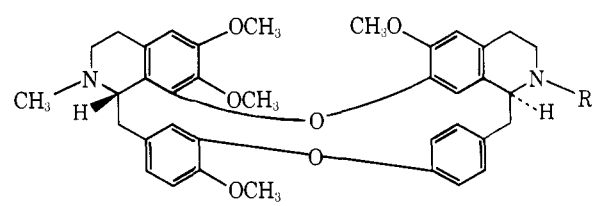
Abstract: The crystal structure of the tumor-inhibitory bisbenzylisoquinoline alkaloid tetrandrine has been determined by direct x-ray analysis. Crystals are orthorhombic, space group $P2_12_12_1$, with a = 38.368 (6), b = 7.230 (2), c = 12.046 (2) Å, and Z = 4. All atoms, including hydrogen, were located and least-squares refinement gave R = 0.041 for 2633 nonzero reflections measured by diffractometry. The absolute configuration determined by taking account of anomalous dispersion agrees with that assigned chemically. The molecule is shaped as a rough equilateral triangle. The base is defined by one benzylisoquinoline residue in an extended conformation, the other two sides by the second benzylisoquinoline residue in a folded conformation. The marked difference in reactivity of the two chemically equivalent tertiary nitrogen atoms is explained in terms of the observed conformation. One N-methyl group occupies a pseudoequatorial site with unrestricted access to the lone pair, the other is in a pseudoaxial site with access to the lone pair sterically restricted at medium and long range.

The bisbenzylisoquinoline alkaloid *dl*-tetrandrine was found to have a significant inhibitory activity against the Walker intramuscular carcinosarcoma 256 in rats, over a wide dosage range.[3,4] Subsequent studies revealed that the dextrorotatory enantiomer, tetrandrine (**1**), was equally active.[5] Tetrandrine has undergone extensive preclinical toxicological studies and is now in clinical trial, under the auspices of the National Cancer Institute.

During studies directed toward interrelation of tetrandrine with co-occurring alkaloids from *Cyclea peltata*, a markedly greater reactivity of N(2′) over N(2) was observed.[5] Thus, treatment of tetrandrine with an excess of



1, R = CH_3
2, R = $COOCH_3$

methyl chloroformate led to selective demethylation at N(2′) and formation of the monocarbamate **2**. Further-

Table I. Starting Set of Phases and Symbols

h	k	l	E	ϕ	
0	3	3	3.33	$\pi/2$	Origin
0	1	2	2.88	$\pi/2$	
11	0	1	2.52	$\pi/2$	
1	3	3	2.23	$\pi/4, 3\pi/4$	Enantiomer
0	6	0	1.78	π	Double tangent and Σ_1 (p = 0.89)
0	2	4	1.58	π	Σ_1 (p = 0.85)
7	0	2	2.24		Symbols
32	0	2	2.63		
31	1	2	2.60		

more, methylation of tetrandrine with methyl iodide yielded the monomethiodide resulting from selective methylation at N(2′). The electronic environments of the respective nitrogen atoms in tetrandrine are quite similar and differences in reactivity, therefore, were deemed to be most probably attributable to steric factors. A direct x-ray crystallographic analysis was undertaken to determine conformational effects in the solid state which might parallel steric factors affecting reactivity in solution.

Experimental Section

Crystal Data. Recrystallization of tetrandrine from aqueous ethyl acetate yielded needles elongated along c. A fragment, 0.2 × 0.2 × 0.3 mm^3, cut from a needle and mounted with c parallel to the goniometer axis was used. Precession photographs taken with Mo Kα radiation showed the crystal to have orthorhombic symmetry. Systematic absences only in the axial reflections of odd order unambiguously indicated space group $P2_12_12_1$.

The unit cell dimensions, found by a least-squares fit to the diffractometer values of $\pm 2\theta$ for 20 strong general reflections, are a = 38.368 (6), b = 7.230 (2), and c = 12.046 (2) Å (λ = 1.5418 Å) giving a unit cell volume of 3342 Å^3. The crystal density, by flotation, is 1.23 g cm^{-3} in agreement with 1.24 g cm^{-3} calculated for four molecules in the unit cell.

Intensity Data. Measurements of intensity were made from the same crystal by standard diffractometer methods with a Picker four-circle instrument controlled by an XDS Sigma 2 computer. With Cu Kα radiation made monochromatic by Bragg reflection from a highly oriented graphite crystal one octant of reciprocal space was surveyed to $2\theta \leq 121°$. Intensity significantly above background [$I > 3\sigma(I)$] was found at 2633 of the 3050 independent reflections examined. The θ–2θ scan method was used with scintillation counting and pulse-height analysis. Background corrections were made from a carefully predetermined curve of scattered intensity vs. diffractometer angle. For the radiation used μ is small, 5 cm^{-1}, and no absorption corrections were made. Structure amplitudes and $E(hkl)$ values were obtained in the usual ways.

Structure Determination. Solution of the phase problem was unusually difficult and was only achieved when a version of MULTAN[6] became available which incorporated fast Fourier transform, peak search, molecular geometry, and molecular drawing routines. Many different starting sets of reflections had to be considered and in the ultimately successful set given in Table I two weak phase indications for smaller $E(hkl)$ had to be included to give a satisfactory convergence map.

With all $E(hkl) > 1.46$ and with 2000 Σ_2 interactions, 32 phase sets were generated. That of highest figure of merit, 1.17, and lowest Karle residual, 30.8, led to an E map with geometrically acceptable sites among the 55 largest peaks for 35 of the 46 nonhydrogen atoms. The remaining 11 atoms were located from an electron-density synthesis.

Refinement. This was carried out by blocked full-matrix least-squares methods first with isotropic (R = 0.129) and then with anisotropic thermal parameters (R = 0.097). Throughout the refinement the content of the blocks was varied to take account of as many correlations as possible. The positions of all hydrogen atoms were found from a three-dimensional ($\rho_o - \rho_c$) synthesis. Inclusion of these atoms in fixed positions and with B = 5.0 Å^2 gave R = 0.073. Refinement with the positional parameters of the hydrogen atoms allowed to vary gave R = 0.069. Corrections for extinction errors[7] were made for 21 reflections and all parameters allowed to vary giving R = 0.043 and R_w = 0.066 at convergence.

Determination of Absolute Configuration. This was done by applying Hamilton's R ratio test[8] to the results of separate structure factor calculations carried out for each enantiomer and taking into account the anomalous dispersion terms for oxygen.[9] For one enantiomer R and R_w were 0.04333 and 0.06623, for the other 0.04345 and 0.06643. The ratio R_2/R_1 for both the normal and weighted residuals is 1.003, indicating a significant discrimination at the 99.5% confidence level. The assignment of absolute configuration has not been confirmed by measurement of intensity differences in pairs of Bijvoet reflections, but the preferred enantiomer indicated by the Hamilton test has the chemically assigned absolute configuration.

Final Refinement. The parameters for the preferred enantiomer were refined to convergence in six cycles of blocked full-matrix least-squares treatment. Scattering factors were taken for the neutral atoms[10] and the anomalous dispersion terms for oxygen included. Weights, w, were assigned as $1/\sigma^2(|F_o|)$ with σ^2 given by the analytically derived polynomial: $4.905 - 0.1909|F_o| + 0.0082|F_o|^2$. The quantity minimized was $\Sigma w(|F_o| - |F_c|)^2$. In the final cycle the largest shift in any parameter was 0.6σ and the mean 0.1σ. The standard deviation of an observation of unit weight was 0.82 and the final ratio of observation to parameters 4.5:1.

Results

The x-ray study has yielded the solid-state conformation of tetrandrine, illustrated in Figure 1, and has confirmed the chemically based assignment of absolute configuration for this molecule. Furthermore, the observed conformation allows a rationalization of the marked difference in reactivity in solution of the two chemically equivalent nitrogen atoms, with access to the lone pair on one of them restricted by medium and long-range steric effects.

Atomic parameters for the crystal structure are given in the microfilm edition. Bond lengths and angles have normal values and for those not involving hydrogen are given in Figure 2 which also shows the numbering scheme. C-H bond lengths are tabulated in the microfilm edition; the mean value is 1.01 Å. Estimated standard deviations for the quantities in Figure 2 lie in the ranges 0.004–0.006 Å and 0.2–0.4°. From the agreement between chemically equivalent bond lengths and angles in the two isoquinoline residues these estimates seem reliable.

Selected torsion angles are given in Figure 3 and show the nitrogen-containing ring of each isoquinoline residue to have a conformation close to the minimum energy monoplanar (half-chair) form for substituted cyclohexene.[11] In each ring the endocyclic torsion angle at the phenyl ring junction is significantly nonzero and transmission of strain results in substantial nonplanarity of the two phenyl moieties of the isoquinolines.

The C(16) methyl group at N(2) occupies a pseudoaxial position whereas that at N(2′) is in a pseudoequatorial position.

Figure 4 shows the stereochemistry of the methylene linkages between the isoquinoline and phenyl residues. In each case the conformation about the C-C bond is close to completely staggered and, inferentially, nearly strain free. Importantly, whereas the N(2)-C(1)-C(15)-C(9) torsion angle is −69°, that for N(2′)-C(1′)-C(15′)-C(9′) is −175°. The implications of this difference in stereochemistry on the reactivities of the two nitrogen atoms are considered in the discussion.

The overall molecular shape may be described in terms of the four labeled near planar units A, B, C, and D outlined in Figure 3. Detailed information on these and other least-squares mean planes is given in the microfilm edition. For the atoms making up unit A, the mean deviation from planarity is 0.04 Å, the maximum 0.10 Å, and for unit B these values are 0.06 and 0.15 Å. The phenyl rings of units C and D are rigorously planar, but for the units as a whole the

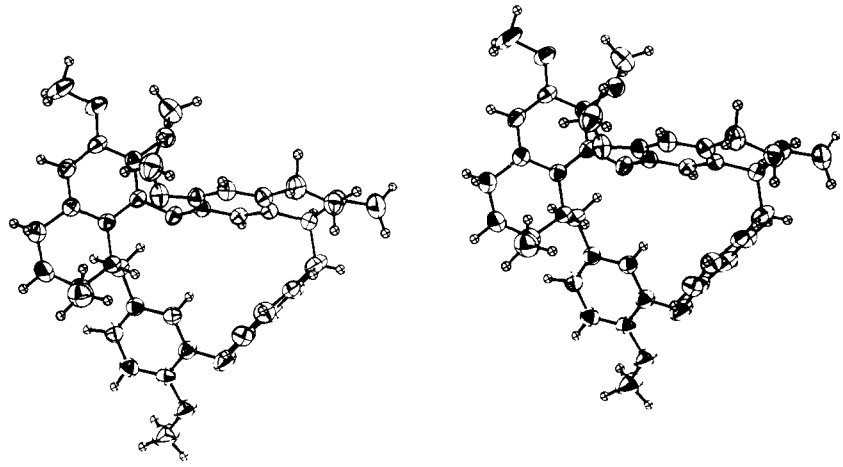


Figure 1. Stereoscopic view of the crystal structure. Thermal ellipsoids for C, N, and O are drawn with the 50% probability level as boundary surface and hydrogen atoms are represented by spheres of arbitrary radius.

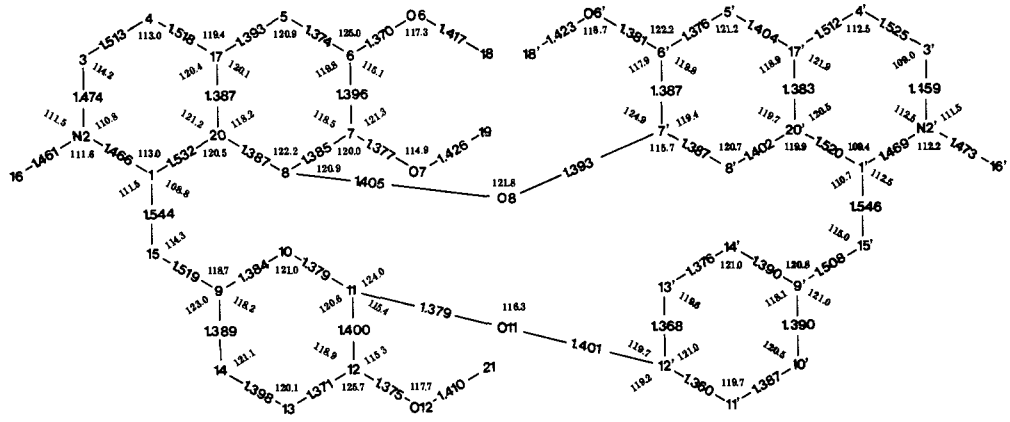


Figure 2. Bond lengths (Å) and bond angles (degrees) in the molecule with the numbering scheme adopted. Atoms are carbon unless otherwise labeled.

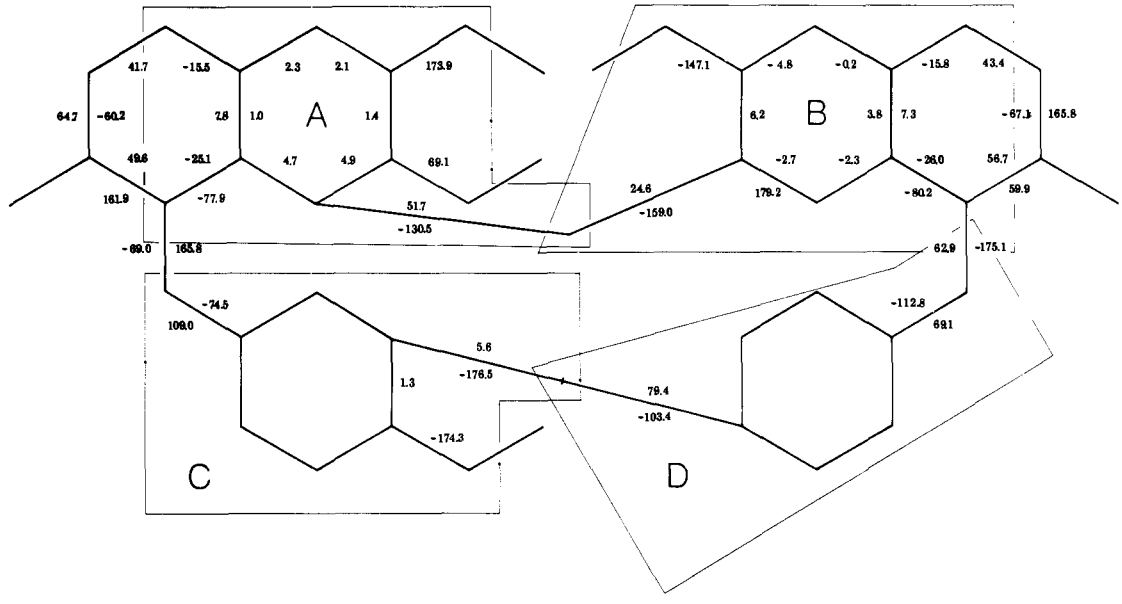


Figure 3. Selected torsion angles (degrees) in the molecule and the labeled near-planar units used in describing the molecular conformation.

mean and maximum deviations from planarity are 0.01 and 0.02 Å for C, 0.02 and 0.04 Å for D.

The molecule is shaped as a rough equilateral triangle with units A and C constituting the base. Planes A and C make an angle of 26° with one another and the axis O(12) ···C(15)of C is almost parallel to the axis O(6)···C(1) of A. The base line of the triangle is then roughly defined by the axis O(6)···O(12), the benzylisoquinoline moiety being in an extended conformation. The axis C(5)···O(8) of A and the axis O(6′)···C(1′) of B are almost parallel and together define the second side of the triangle. The dihedral angle between A and B is 62°. The third side is completed by unit

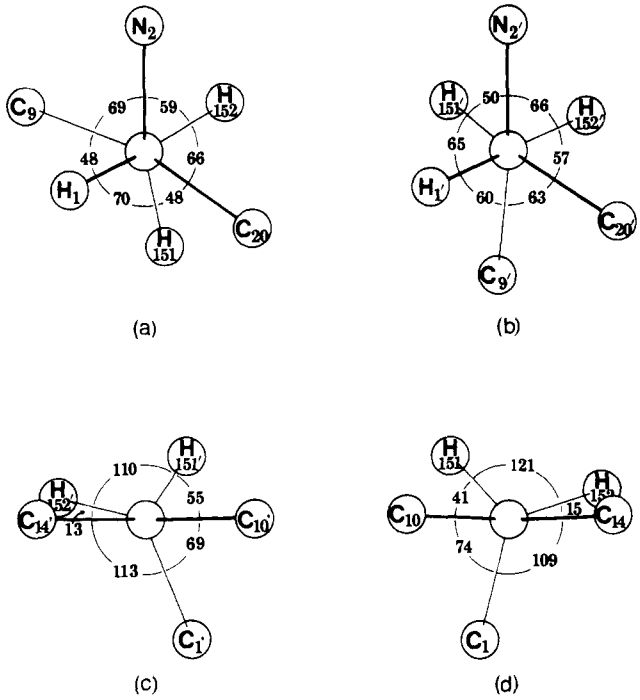


Figure 4. Newman projections showing the stereochemistry of the ring linkages: (a) projection down C(1)-C(15); (b) C(1′)-C(15′); (c) C(9)-C(15); (d) C(9′)-C(15′).

Table II. Selected Intramolecular Contact Distances (Å)

Contact	d	Contact	d
H(1) . . . H(10)	2.68	C(8) . . . O(6′)	2.69
H(1) . . . O(8)	2.49	O(7) . . . O(6′)	3.09
H(1) . . .H(163)	2.27	O(7) . . . C(18′)	3.48
H(152) . . . H(31)	1.94	O(7) . . . C(6′)	3.02
H(151) . . . O(8)	2.63	H(31′) . . . H(151′)	2.90
H(10) . . . O(8)	3.31	H(31′) . . . H(152′)	2.27
H(10) . . . H(8′)	3.38	H(151′) . . . H(162′)	2.12
H(10) . . . C(12′)	2.46	H(151′) . . . H(163′)	2.74
H(10) . . . C(13′)	2.83	H(10′) . . . H(1′)	2.68
H(10) . . . C(11′)	3.01	C(14′) . . . C(20′)	3.64
N(2) . . . N(2′)	9.75	N(2) . . . O(6)	6.46
N(2) . . . O(7)	6.13	N(2) . . . O(8)	4.18
N(2) . . . O(11)	6.12	N(2) . . . O(12)	6.65
N(2) . . . O(6′)	5.04	N(2′) . . . O(6)	9.14
N(2′) . . . O(7)	6.55	N(2′) . . . O(8)	6.04
N(2′) . . . O(11)	7.93	N(2′) . . . O(6′)	6.38
O(6) . . . O(7)	2.69	O(6) . . . O(8)	4.76
O(6) . . . O(6′)	4.32	O(7) . . . O(8)	2.80
O(7) . . . O(11)	8.51	O(8) . . . O(11)	5.82
O(8) . . . O(12)	7.65	O(8) . . . O(6′)	2.83
O(11) . . . O(12)	2.58	O(11) . . . O(6′)	7.92
O(12) . . . O(6′)	9.77		

D oriented about the axis C(15′)···O(12), with the dihedral angle between B and D being 48°, between C and D 81°, and between A and D 77°.

This arrangement leaves a small central cavity in the molecule bounded by H(10), O(8), C(7′), C(8′), H(8′), and the inner surface of the phenyl ring of D. The overall conformation seems energetically favorable with few unusually close intramolecular contacts. The endocyclic bond angles at C(15) and C(15′) are slightly opened over the extended chain value for C-C-C angles and the C-O-C angle at the ether oxygen O(8) is significantly greater than the more normal value at O(11).

Of the four methoxy groups those at O(7) and O(6′) have the carbon atom out of the plane of the aromatic residue, whereas those at O(6) and O(12) have the methyl group close to the ring plane. In these two latter groups there is

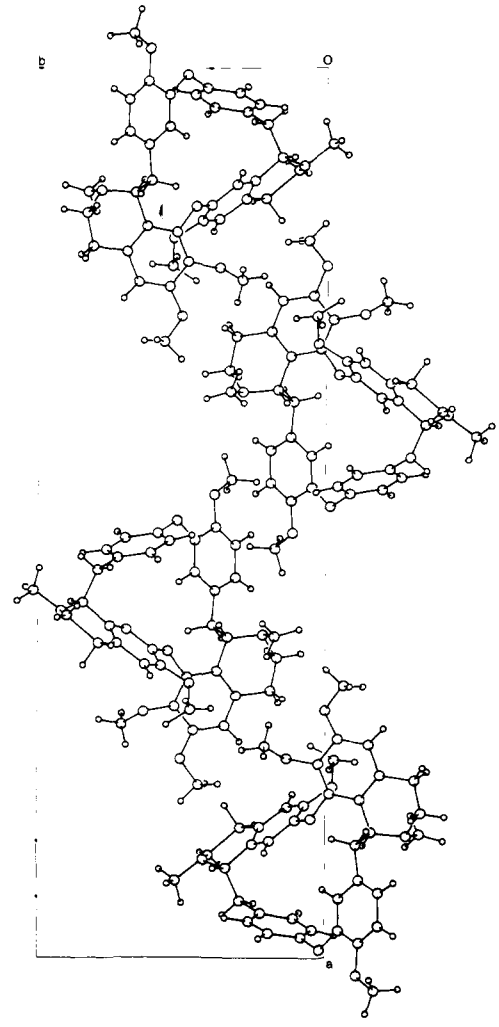


Figure 5. Packing of molecules in the unit cells viewed in *c*-axis projection.

the normal asymmetry of exocyclic C-C-O angles found for these coplanar arrangements. A similar pattern of angles is found at C(11) in consequence of the coplanarity of C(12′) with ring C.

The more important intramolecular interatomic distances are given in Table II. The molecular packing in the crystal is shown in Figure 5. All contacts between molecules are of normal van der Waals type and there are no unusually close approaches. Shorter intermolecular contacts are given in the microfilm edition.

Discussion

Selective Reactivity of N(2′). Both isoquinoline residues have the same half-chair conformation for the nitrogen-containing ring. However, the C(16) methyl group occupies a pseudoaxial position on N(2) with the lone pair equatorial, whereas C(16′) is in a pseudoequatorial position at N(2′) with the lone pair axial. This difference is not, in itself, sufficient to account for the preferred reactivity. The similarity of the immediate environments of N(2) and N(2′) is clear from Table III, and there is very little steric hindrance from the *immediate* neighbors of the lone pair on either atom. We must therefore consider medium and long-range steric effects.

The phenyl ring defined by atoms C(9′)-C(14′) folds back upon the isoquinoline to which it is attached, generating an angle of 46° between the two ring planes and leaving the lone pair on N(2′) pointing out from the molecular surface and sterically unhindered. However, the phenyl ring

Table III. Immediate Coordination Sphere of the N Lone Pairs[a]

N(2)		N(2′)	
C(1)	1.88 Å	C(1′)	1.88 Å
C(3)	1.89	C(3′)	1.88
H(31)	2.20	C(4′)	2.50
H(32)	2.22	H(32′)	2.21
H(1)	2.24	H(1′)	2.13
C(16)	1.83	C(16′)	1.82
H(161)	2.14	H(161′)	2.11
H(163)	2.36	H(163′)	2.27

[a] The lone pair is assumed to be centered on an axis passing through the nitrogen atom and the centroid of the triangle defined by the three attached carbon atoms, with $r_{\text{lone-pair}-N}$ 0.8 Å.[12]

C(9)-C(14), whose plane makes an angle of only 26° with that of the attached isoquinoline, extends outward from the isoquinoline with its major axis C(15)···O(12) almost parallel to the lone-pair axis and with a perpendicular separation between the two of about 2.3 Å. This places atoms C(14), C(13), O(12), and C(21), with their associated hydrogen atoms, in positions where they sterically hinder direct attack along the direction of the lone pair axis, with van der Waals repulsions between these atoms and any incoming reagent deflecting the electrophile from its optimum approach route. The situation is illustrated by Figure 6.

This argument may be placed upon a semiquantitative basis by applying the concepts of accessibility and congestion developed by Wipke and Gund[13] for stereoselective addition to unsaturated carbon. In this technique a cone of preferred approach is generated for each sterically hindering atom. For reaction at nitrogen each cone is centered on the nitrogen lone-pair axis and tangentially to the hindering atom. The latter is given a radius equal to its van der Waals radius.[14] Intersection of this cone with a sphere of unit radius centered on the N atom defines a spherical cross-section of preferred approach. The solid angle thus generated is equated with the accessibility of the lone pair with respect to the hindering atom. The inverse of accessibility is defined as congestion, and the total congestion is computed as the sum of the individual congestion terms. The larger the congestion, the more sterically hindered is the site. The results of the calculations for N(2) and N(2′) are given in Table IV. For substitution at N(2′) only one atom has a congestion term greater than unity, whereas there are 13 hindering atoms around N(2) with congestions ranging from 1.97 to 31.43. The total congestion at N(2′) is 1.06, that at N(2) is 107.64. This difference is sufficiently large to account for the observed differential reactivity of the two atoms. Clearly, the largest term, that for H(21), and the term for C(21) need not occur in the solution conformation, but the largest remaining term is for medium range effects at C(13). This strongly suggests that the solution conformation of tetrandrine retains an extended arrangement of this benzylisoquinoline group which, in turn, would call for retention of the folded arrangement of the second benzylisoquinoline group. The implication then is that the overall molecular conformation in solution is not too different from that observed in the solid.

Nature of the Molecular Surface. The mode of action by which tetrandrine exercises its tumor-inhibitory effect is unknown at this time. It is reasonable to assume that if tetrandrine acts directly upon some receptor, interactions of either a covalent, hydrogen-bonded, or nonbonded variety take place. With this in mind it is of interest to note some features of the molecular surface, always bearing in mind that unforeseen and significant changes may take place in the molecular conformation in solution.

If the molecule is viewed in the orientation shown in Figure 1, a predominantly hydrophobic aspect is presented by both the upper and lower surfaces and by the left-hand side of the molecule, as well as by the topmost region of the right-hand side. Points of polarity are found, however, on the outside of the molecule at O(11) and O(12) at the lower left-hand corner and, less accessibly, at O(6) and O(7) in the upper left-hand corner. For an orientation of the molecule in the conformation found which would place the reactive lone pair of N(2′) pointing vertically downward, the methoxy oxygen O(7) might act as a point of polarity directed toward a receptor in van der Waals contact with the surface of the otherwise hydrophobic isoquinoline residue B. In such an orientation the surfaces of units C and D form a hydrophobic plane inclined at about 60° to the vertical.

The potential importance of the selective accessibility of the N(2′) lone pair for interaction with a receptor is self-evident.

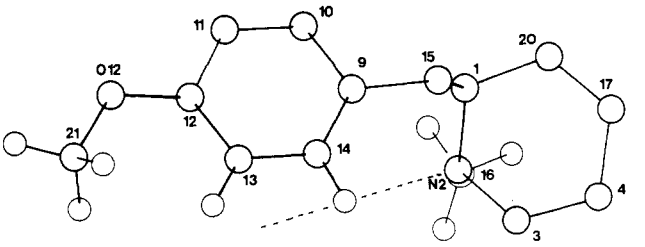


Figure 6. Local environment of the sterically restricted lone pair on N(2). The lone-pair axis is indicated by the broken line.

Table IV. Congestion Factors > 1.0 for Electrophilic Substitution at N(2) and N(2′)

Lone pair on N(2)			
Atom	Congestion	Atom	Congestion
C(9)	2.66	O(12)	3.39
C(10)	1.06	H(13)	6.56
C(11)	1.49	H(14)	1.97
C(12)	4.87	H(211)	31.43
C(13)	21.64	H(212)	4.21
C(14)	12.41	H(213)	3.91
C(21)	12.04		
Lone pair on N(2′)			
Atom	Congestion		
H(191)	1.06		

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Supplementary Material Available: A listing of the observed and calculated structure amplitudes, the positional and thermal parameters, information on least-squares mean planes, and on intermolecular contacts (12 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Tumor Inhibitors. 110. Part 109: L.-L. Liao, S. M. Kupchan, and S. B. Horwitz, submitted for publication.
(2) Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, Scotland.
(3) S. M. Kupchan, 7th Pan American Congress of Pharmacy and Biochemistry, Buenos Aires, Argentina, Dec 1966; *Chem. Eng. News*, **44**, 64 (1966).
(4) J. L. Hartwell and B. J. Abbott, *Adv. Pharmacol. Chemother.*, **7**, 117 (1969).
(5) S. M. Kupchan, A. J. Liepa, R. L. Baxter, and H. P. J. Hintz, *J. Org. Chem.*, **38**, 1846 (1973).
(6) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).
(7) W. H. Zachariasen, *Acta Crystallogr.*, **16**, 1139 (1963).

(8) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).
(9) D. T. Cromer and D. Liberman, *J. Chem. Phys.*, **53**, 1891 (1970).
(10) D. T. Cromer and J. B. Mann, *Acta Crystallogr., Sect. A*, **24**, 321 (1968); R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).
(11) R. Bucourt and D. Hainaut, *Bull. Soc. Chim. Fr.*, 1366 (1965).
(12) M. A. Robb, W. J. Haines, and I. G. Csizmadia, *J. Am. Chem. Soc.*, **95**, 42 (1973).
(13) W. T. Wipke and P. Gund, *J. Am. Chem. Soc.*, **96**, 299 (1974).
(14) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).

Enzymatic Hydration of [^{18}O]Epoxides. Role of Nucleophilic Mechanisms

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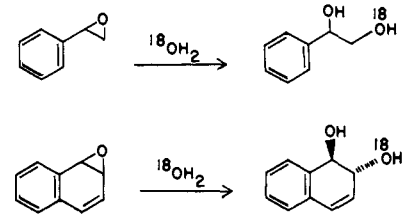
Abstract: The hydration of five mono- and 1,1-disubstituted oxiranes by enzymes in rat liver microsomes has been shown, by means of ^{18}O-tracer studies, to be extremely regiospecific, involving cleavage of the C(2)–O bond of the oxirane ring. Base-catalyzed hydration of these epoxides also involved C(2)–O bond cleavage but was much less regiospecific, whereas acid-catalyzed hydration involved mainly C(1)–O bond cleavage. Both the enzymatic and metal ion catalyzed hydration of [^{18}O]-2-pyridyloxirane were found to involve ≥95% C(2)–O bond cleavage. In the presence of large concentrations of added nucleophiles and metal binding agents the enzymatic hydration of 1,2-epoxytetradecane was not inhibited nor were any products other than diol formed. These results preclude the involvement of (1) a metal ion at the active site of epoxide hydrase and (2) a simple enzyme–acid-catalyzed hydration in which water or hydroxide from solution attacks a protonated epoxide and suggest that epoxide hydrase activates a water molecule per se for nucleophilic attack at the less hindered oxirane carbon. A *hypothetical* active site mechanism for epoxide hydrase is discussed.

The metabolism of aromatic or olefinic compounds by mammals often involves the epoxide-diol pathway, the enzymes of which are localized in the microsomal fraction of liver and other tissues.[1] The first step of this pathway, epoxide formation, is catalyzed by various nonspecific cytochrome P_{450}-dependent mixed function oxygenases. Epoxides produced by these enzymes, especially the arene oxides, have been shown to have various toxic and deleterious effects upon the organism. These effects are presumably associated with the alkylation of critical cellular constituents by the chemically reactive epoxides. The second enzyme of the pathway, epoxide hydrase, is thus thought to play a protective role by converting reactive epoxides into less toxic diol products which may be metabolized further and excreted.

Oesch and co-workers have shown that epoxide hydrase is a relatively nonspecific enzyme, both *d* and *l* enantiomers of monosubstituted and 1,1-disubstituted oxiranes bearing at least one large lipophilic substituent being among the best substrates, while highly substituted oxiranes do not serve as substrates and fail to inhibit the hydration of styrene oxide.[1c,2,3] Stereochemical studies have shown that enzymatic hydration of epoxides of cyclic olefins, including arene oxides, produces *trans*-1,2-diols.[4,5]

The direction of epoxide opening has been determined in two cases using $^{18}OH_2$ and unlabeled epoxides. Acid dehydration of the dihydrodiol obtained by incubation of naphthalene with microsomes in buffers containing $^{18}OH_2$ gave a mixture of 1- and 2-naphthol; only the 2-naphthol contained ^{18}O.[4] Similarly, diol obtained from the enzymatic hydration of styrene epoxide in $^{18}OH_2$ media contained ^{18}O, 90% of which was in the β position.[5] The result obtained with styrene oxide suggests the possible importance of nucleophilic as opposed to acid-catalyzed mechanisms. Unfortunately, it was difficult to evaluate the relative importance of steric hindrance and carbonium ion stability in the case of the naphthalene oxide. Therefore we decided to examine the question of carbonium ion vs. nucleophilic pathways and to test the *generality* of the styrene oxide result with a group of epoxides representing the *range of structural types* known to be good substrates for epoxide hydrase. For reasons of economy, and to complement the labeling method used in the above cases, we decided to study the enzymatic hydration of [^{18}O]epoxides in ordinary buffers. During our study, Jeffrey et al. reported that C, N, and S nucleophiles attack preferentially at C(2) of naphthalene 1,2-dioxide.[6]



Results and Discussion

The epoxide substrates **1a–5a** were chosen for this study for several reasons. First, they represent close structural analogs of compounds previously shown[3] to be good substrates for epoxide hydrase, i.e., lipophilic mono- and 1,1-disubstituted oxiranes.[7] Second, they were chosen to allow unambiguous evaluation of the relative importance of carbonium ion formation vs. nucleophilic attack in the hydration process. 2-Pyridyloxirane (**2a**) is apparently the first example of an oxirane with a heterocyclic substituent to be studied but was included to allow comparison of the enzymatic results to those obtained with a regiospecific "chemical model" for epoxide hydrase.[8] Epoxides **1a–5a** were all found to be excellent substrates for epoxide hydrase.

Mass spectral data indicating the ^{18}O content of epoxides **1a–5a**, as well as the content and distribution of ^{18}O in the corresponding diols **1b–5b**, are given in Tables I and II. Differences in ^{18}O content in the starting epoxides are attributed to the presence of small and variable amounts of extraneous ordinary water in solvents and/or reagents used in their synthesis. Results obtained from multiple mass spec-