Registry No. 1, 27407-00-7; 2, 6326-01-8; 3, 85925-91-3; 4, 6319-54-6; 5, 6331-77-7; 6, 85925-92-4; 7, 26465-72-5; 8, 85925-93-5; 9, 35707-43-8; 10, 85925-94-6; 11, 85925-95-7; 12, 85925-96-8; 13, 35598-63-1; 13 acetate, 51065-28-2; 14, 85925-97-9; 15, 85925-98-0; 16, 85925-99-1; 17, 85926-00-7; 18, 85926-01-8; 19, 85926-02-9; 20, 35594-98-0; 21, 1242-27-9; 22, 85926-03-0; 23, 85926-04-1; 24, 85926-05-2; 25, 85926-06-3; 26, 59543-80-5; 27, 85926-07-4; 28, 62813-00-7; 29, 62812-99-1; 30, 85926-08-5; 31, 13667-49-7; 32, 19178-84-8; 33, 85926-09-6; 34, 60717-48-8; 35, 60717-47-7; 36, 60717-52-4; 37, 85926-10-9; 38, 59543-79-2; 39, 85926-11-0; 40, 85926-12-1; 41, 85926-13-2; 42, 62019-09-4; 43, 85926-14-3; 44, 85926-15-4; 45, 85926-16-5; 46, 62019-10-7; 47, 85926-18-7; 48, 85926-19-8; 49, 85926-17-6; 50, 85926-20-1; *N*-ethyl-3-(methylamino)piperidine, 42389-64-0; *N*-ethyl-3-piperidinone hydrochloride, 41361-28-8; *N*-ethyl-3-piperidinone, 43152-93-8; 9-hydroxy-9*H*-xanthenyl acetate, 35598-76-6; 3-[(methylamino)methyl]pyridine, 20173-04-0; formic acid hydrazide, 624-84-0; 9*H*-xanthen-9-ol, 90-46-0; 9-hydroxy-9*H*-xanthenyl *N*-methylcarbamate, 30190-26-2; 2-pyridylhydrazine, 4930-98-7; 3,5-dimethyl-1-formamidinopyrazole nitrate, 38184-47-3; ethyl acetimidate fluoroborate, 372-08-7; xanthene, 92-83-1; *N*-bromosuccinimide, 128-08-5.

Structure–Activity Relationships of Convulsant and Anticonvulsant Barbiturates: A Computer-Graphic-Based Pattern-Recognition Analysis[1]

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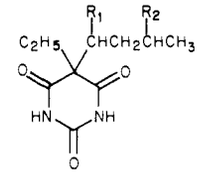
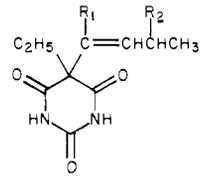
A computer-graphic-based pattern-recognition study of two series of 5-ethyl-5-substituted barbiturates has been undertaken in an attempt to find a correlation between molecular conformation and convulsant and anticonvulsant activity. Studies of a first (trial) set of barbiturates related to pentobarbital revealed a region of space in which at least one low-energy conformation of the hydrocarbon side chain of each of the anticonvulsant barbiturates resides. Another region was occupied by a low-energy conformation of each of the convulsant barbiturates. These regions of space are, thus, possible pharmacophores for convulsant and anticonvulsant activity. Analysis of a second (test) set of barbiturates related to phenobarbital has shown that the activities and structures of these molecules are consistent with the above model. These pharmacophores thus provide a basis for the design of rigid, new analogues with potent convulsant or anticonvulsant activities.

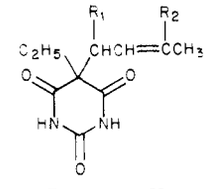
Studies on barbiturates[2,3] and related drugs[4-6] indicate that variations in molecular conformation rather than electronic or other physicochemical properties are responsible for the qualitative activity differences between structurally related convulsant and anticonvulsant drugs. In the series of barbiturates **1–3**, for example, compounds



1a, $R_1 = R_2 = H$ (butethal)
b, $R_1 = CH_3$; $R_2 = H$ (pentobarbital)
c, $R_1 = H$; $R_2 = CH_3$ (amytal)
d, $R_1 = R_2 = CH_3$



2a, $R_1 = R_2 = H$
b, $R_1 = CH_3$; $R_2 = H$ (vinbarbital)
c, $R_1 = H$; $R_2 = CH_3$
d, $R_1 = R_2 = CH_3$



3a, $R_1 = R_2 = H$
b, $R_1 = CH_3$; $R_2 = H$
c, $R_1 = H$; $R_2 = CH_3$
d, $R_1 = R_2 = CH_3$

1a, **2d**, **3c**, and **3d** are convulsant, while the remainder, although closely related, are anticonvulsant.[2,3] The importance of conformation in determining the qualitative activity differences is particularly evident in **1d**, for which the *S*(−) isomer is anticonvulsant, while the *R*(+) isomer and the racemic compound are potent convulsants.[7] In an effort to explain the structure–activity relationships of these barbiturates, we have studied their molecular conformations using classical[8] and molecular orbital[9] potential energy calculations, ^1H and ^{13}C NMR,[10] and X-ray crystallography.[11] However, while the preceding data provide details of the solution, crystal, and gas phase conformations, they do not directly identify the convulsant and anticonvulsant pharmacophores responsible for biological activity. In the present paper, we report a computer-graphic-based pattern-recognition study of barbiturates **1–3** that has enabled us to identify the distinct conformational regions likely to be responsible for convulsant and anticonvulsant activity in this series. We also report potential energy calculations on two more series of barbiturates in which small structural changes convert the anticonvulsant **4**[12] to the convulsants **5** and **6**[13] and the anticonvulsants **7** and **8** to the convulsants **9** and **10**.[12,14]

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(1) Supported in part by a Fulbright award from the Australian–American Educational Foundation to L.C.M.
(2) P. R. Andrews, G. P. Jones, and D. Lodge, *Eur. J. Pharmacol.*, **55**, 115 (1979).
(3) P. R. Andrews, G. P. Jones, and D. B. Poulton, *Eur. J. Pharmacol.*, **79**, 61 (1982).
(4) P. R. Andrews, *J. Med. Chem.*, **12**, 761 (1969).
(5) P. R. Andrews and A. S. Buchanan, *Biochem. Pharmacol.*, **20**, 1599 (1971).
(6) T. B. Patrick and R. R. Bresee, *J. Pharm. Sci.*, **65**, 1066 (1976).
(7) H. Downes, R. S. Perry, R. E. Ostlund, and R. Karler, *J. Pharmacol. Exp. Ther.*, **175**, 692 (1970).
(8) G. P. Jones and P. R. Andrews, *J. Med. Chem.*, **23**, 444 (1980).
(9) P. R. Andrews and G. P. Jones, *Eur. J. Med. Chem.*, **16**, 139 (1980).
(10) P. R. Andrews, A. J. Jones, G. P. Jones, A. Marker, and E. A. Owen, *Eur. J. Med. Chem.*, **16**, 145 (1980).
(11) G. J. Jones and P. R. Andrews, submitted to *Mol. Pharmacol.*
(12) F. F. Blicke and M. F. Zienty, *J. Am. Chem. Soc.*, **63**, 2991 (1941).
(13) L. Velluz, J. Mathieu, and R. Jequier, *Ann. Pharm. Fr.*, **9**, 271 (1951).
(14) P. K. Knoefel, *J. Pharmacol. Exp. Ther.*, **84**, 26 (1945).

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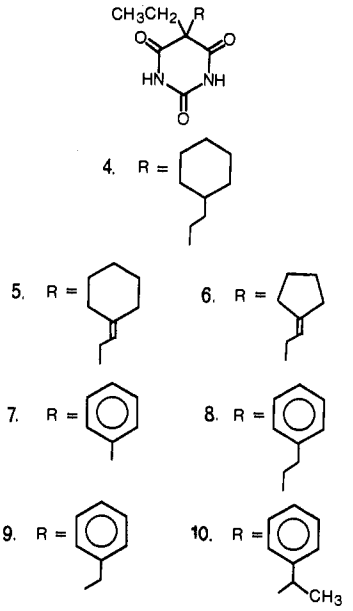
Analysis of these data confirms the conclusions drawn with barbiturates 1–3.



Experimental Section

The calculations were done on the neutral triketo form of the barbiturates. The biologically active ionization state of the barbiturates in the central nervous system is not known with certainty, but the pK_a values of the barbiturates and structurally related drugs suggest that they probably act in the nonionized form.[10] Fortunately, from the conformational viewpoint, calculations and NMR data for related barbiturates show that they adopt the same conformations in the anionic and neutral species.[10] The molecular geometries were based on the crystal structures of related barbiturates[11] and bond lengths and angles from standard compilations.[15]

The calculations were carried out at fixed values of all bond lengths and bond angles; previous studies on related barbiturates showed that relaxation of this condition did not affect the qualitative nature of the potential energy surfaces, although the energy differences between alternative conformations were reduced by relaxation of nontorsional degrees of freedom.[9]

The calculations were performed with a Cyber 73 computer with the program COMOL.[16] The program performs classical conformation calculations by pairwise summation of the van der Waals interactions between nonbonded atoms, together with electrostatic and torsional potentials. The paramerization, which was developed by Giglio on the basis of a series of hydrocarbon and amide structures,[17] has been used to study a number of systems of biological interest;[8,18,19] the results obtained are consistent with those obtained from semiempirical molecular orbital calculations.[9]

Four torsion angles are required to describe the conformations of the barbiturates, and these are defined in Figure 1. Because the 5-ethyl group is common to all the barbiturates and because previous studies[8,9,11] have established that the conformation with the methyl group of the 5-ethyl substituent located immediately below the barbiturate ring ($\tau_0 = 180°$) is always lowest in energy, this conformation has been assumed throughout the series. The other torsion angles were varied two at a time, and approximate potential energy surfaces were computed for each barbiturate by using rotational intervals of 5°. The calculations for each pair of variables were then repeated for alternative values of the other

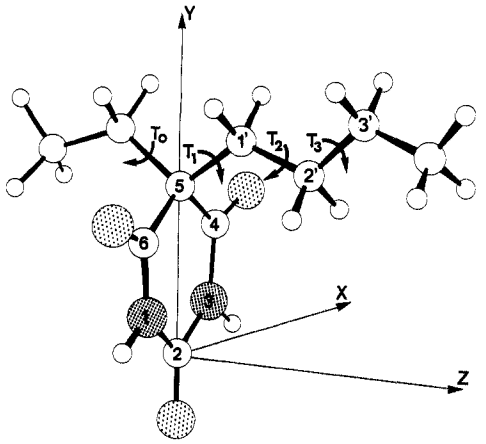


Figure 1. Coordinate systems, conformational variables, and atomic numbering for barbiturates under study. Torsion angles are defined by clockwise rotations around the appropriate bonds; in this illustration, $\tau_0 = \tau_1 = \tau_2 = \tau_3 = 180°$. Light and dark shadings represent oxygen and nitrogen atoms, respectively.

conformational parameters until a complete picture of the conformational surface was built up. Conformational energy maps were prepared by using a modification of the contouring program KONTOR.[20]

To quantify stereochemical comparisons between conformations of different barbiturates, we have assumed that the barbiturate ring in closely related structures will bind to any given receptor site(s) in the same orientation. The problem then is to determine which of the possible conformations place the hydrocarbon groups in the appropriate positions for interaction with the convulsant or anticonvulsant binding sites. Of particular importance is the location of the methyl group or groups attached to $C_{3'}$, since the presence of two methyl (or equivalent) groups at this position appears to be essential for convulsant activity.[2,3] We have therefore taken the positions and orientations of the barbiturate ring and the methyl (or equivalent) groups attached to $C_{3'}$ as the quantitative descriptors for conformational comparisons. For this purpose the coordinate system is defined as in Figure 1, where the barbiturate ring lies in the x–y plane, with C_2 at the origin and C_5 on the $+y$ axis. The 5-ethyl group lies below the barbiturate plane ($-z$), while the variable side chain falls in the two octants with positive y and z coordinates.

Results

Trial Set (1–3). Although only a single conformation is generally observed for each of these barbiturates in solution,[10] the range of conformations that is energetically accessible under physiological conditions is relatively large. Thus, if we assume that any conformation that the classical potential energy calculations[8] place within 10 kcal/mol of the global minimum is potentially biologically active, then the possible conformations for **1a** are τ_1 = 150–210 or ±40°, τ_2 = 90–270°, and τ_3 = 30–330°. Figure 2 shows the position of the terminal methyl group of the butyl side chain in the 385 accessible conformations of **1a** obtained by varying these torsion angles in increments of 30° over this range of conformations.

We have applied this same treatment to each of the barbiturates **1–3** and, thus, obtained a series of maps similar to those given in Figure 2. These are summarized in Figure 3, in which we have used a single point to represent each low-energy conformation of any given barbiturate. For this purpose, each distinct valley in the potential energy map is defined as a single low-energy conformation: individual conformations may thus span a range of 60° or more in any one torsion angle. It is apparent from Figure 3 that there are low-energy conformations of both con-

(15) L. E. Sutton, Ed., "Tables of Interatomic Distances", Special Publication No. 11 and 18, The Chemical Society, Burlington House, London, 1958 and 1965.
(16) M. H. J. Koch, *Acta Crystallogr., Sect. B*, **29**, 379 (1973).
(17) E. Giglio, *Nature (London)*, **222**, 339 (1969).
(18) M. D'Alagni, E. Giglio, and N. V. Pavel, *Polymer*, 17, 257 (1976).
(19) M. L. de Winter, T. Bultsma, and W. T. Nauta, *Eur. J. Med. Chem.*, **12**, 137 (1977).
(20) J. A. B. Palmer, *Aust. Comp. J.*, **2**, 27 (1970).

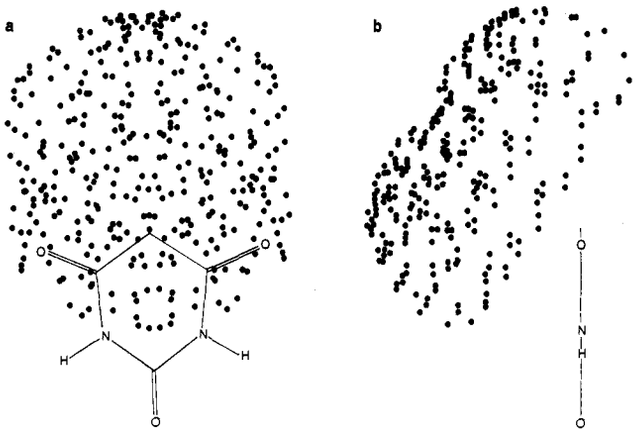


Figure 2. Range of positions available to the terminal methyl group (●) of **1a** in its physiologically accessible conformations: (a) viewed from the $+z$ conformations; (b) viewed from the $+x$ direction; only those conformations with $-ve$ x coordinates are illustrated.

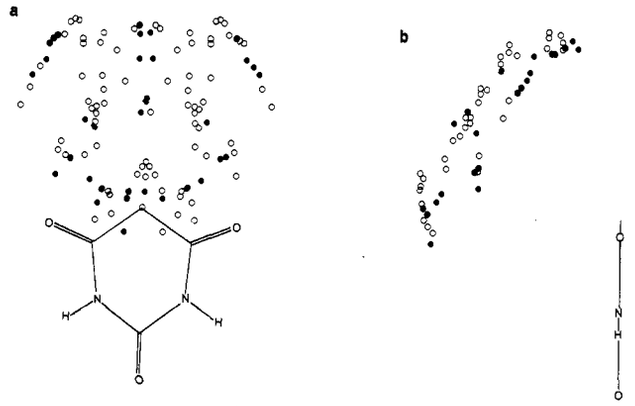


Figure 3. Range of positions available to the terminal methyl groups of the anticonvulsant (○) and convulsant (●) barbiturates 1–3. In the optically active derivatives **1b**, **1d**, **3b**, and **3d**, the low-energy conformations of the R isomers all have $-x$ coordinates, and the S isomers, $+x$ values. The regions on the left and right of the y axis in Figure 3a are therefore designated the R and S lobes, respectively: (a) viewed from the $+z$ direction; (b) viewed from the $+x$ direction; only the R lobe is shown.

vulsant and anticonvulsant barbiturates scattered over the entire accessible space. To identify the biologically active pharmacophores within this space, we therefore need to determine two things. First, which regions within the overall conformational space contain at least one low-energy conformation of *each* of the anticonvulsants or each of the convulsants? Second, which of these regions are accessible to all the anticonvulsants but not the convulsants, or vice versa?

To identify conformational regions common to all of the anticonvulsants, we compared maps like those in Figure 2 for each anticonvulsant member of the series. This procedure generated only one conformational region which contains a low-energy conformation of each anticonvulsant. Occupation of this region, which is illustrated in Figure 4, appears to be an important component of the anticonvulsant pharmacophore for the present series of barbiturates.

The torsion angles corresponding to this placement of the terminal methyl group do not vary significantly among the anticonvulsant barbiturates. They are $\tau_1 \simeq 180°$, $\tau_2 \simeq 180°$, and $\tau_3 \simeq 180°$.

It is evident from Figure 4 that several of the convulsant barbiturates can also adopt the common anticonvulsant conformation without an undue increase in energy. This

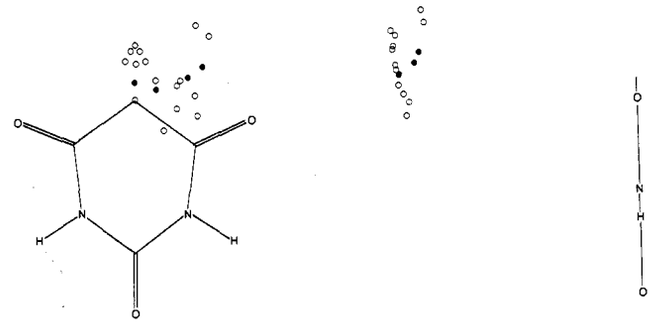


Figure 4. Positions of the terminal methyl groups of representative conformations of convulsants (●) and anticonvulsants (○) in the conformational region common to all of the anticonvulsant barbiturates.

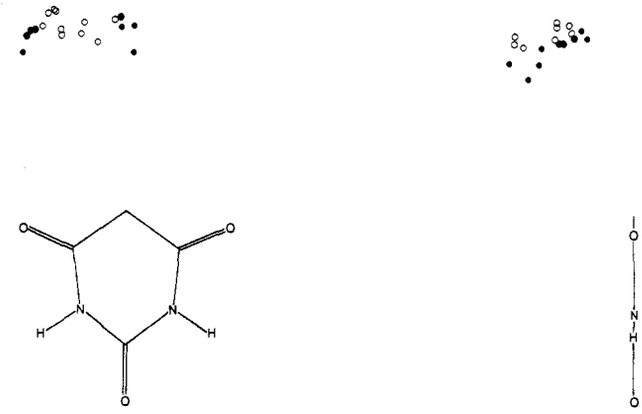


Figure 5. Positions of terminal methyl groups of representative conformations of convulsants (●) and anticonvulsants (○) in the conformational region common to all the convulsant barbiturates.

finding is supported by experimental observations on the convulsant barbiturates in this series, which have been shown to inhibit pentylenetetrazole-induced seizures when administered at doses below their own convulsant thresholds.[3] It thus appears that the convulsant barbiturates may have underlying anticonvulsant activity.

Application of a similar procedure to the convulsant barbiturates also gives rise to a single common conformational region, illustrated in Figure 5. There are several anticonvulsants with low-energy conformations within this convulsant region. One of these is the $R(+)$ enantiomer of **1b**, which is consistent with the greater excitatory activity observed for this enantiomer in vivo,[21] but the others show no sign of convulsant activity other than preanesthetic excitation.

In order to obtain a unique convulsant pharmacophore it is necessary to invoke the apparent requirement for two methyl (or equivalent) groups attached to C_3' in each of the convulsant barbiturates.[2,3] We have therefore included both groups in the analysis by using the vector connecting the two terminal methyl groups as the descriptor for further conformational comparisons, the results of which are given in Figure 6. It is clear that the location of this vector is relatively constant in the four convulsant barbiturates and that none of the conformations accessible to 3′-methyl barbiturates with anticonvulsant activity occupies the same conformational region. This region therefore defines the probable hydrocarbon-binding component of the convulsant pharmacophore. The torsion angles giving rise to this convulsant pharmacophore do not vary significantly between the convulsant barbiturates.

(21) H. D. Christensen and I. S. Lee, *Toxicol. Appl. Pharmacol.*, **26**, 495 (1973).

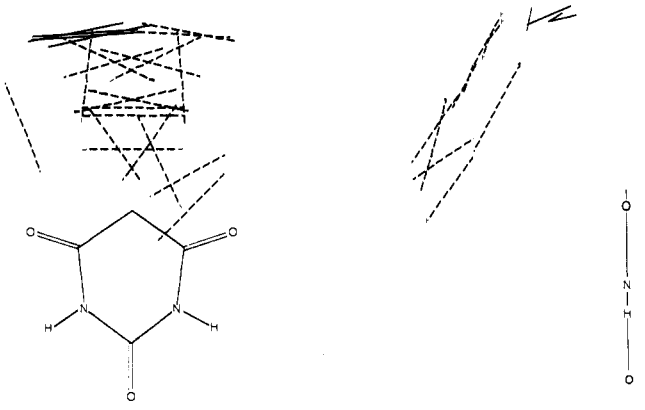


Figure 6. Locations of vectors (—) connecting the terminal methyl groups of convulsant conformations in the common convulsant region. The corresponding vectors for low-energy conformations of the nonconvulsant 3′-methyl barbiturates (---) are included for comparison.

Table I. Accessible Conformations (<10 kcal/mol) of Barbiturates 4–10[a]

	torsion angle,[b] deg		
no.	τ_1	τ_2	τ_3
4	180	180	180
	±60 (6)	±30	±30
5	180	180	
	±60 (2)	±90	
6	180	180	
	±60 (2)	±90	
7	40		
	140 (0)		
8	180	180	±90
	±60 (8)	±30	
9	180	±90	
	±60 (5)		
10[c]	60	±90	
	180 (1)		
	−60 (6)		

[a] Values in parentheses are approximate energies, in kilocalories per mole, of secondary minima. [b] In each case, torsion angles, other than that being varied, are fixed at these optimum values. [c] Values given are for the *R* isomer; the corresponding torsion angles for the *S* isomer are minus those for the *R* isomer.

They are $\tau_1 = 60°$, $\tau_2 = -140°$, and $\tau_3 = 180°$.

The preceding analyses of the conformational data for barbiturates 1–3 identify the conformational regions likely to be responsible for convulsant and anticonvulsant activity in this series. The following calculations on two further series of barbiturates have been carried out in order to test the proposed pharmacophores.

Test Set (4–10). The results of the potential energy calculations for barbiturates 4–10 are summarized in Table I, which lists the conformations accessible to each of these barbiturates, and the contour maps showing the effects of varying τ_1 and τ_2 in **5** and **10** are given in Figure 7 and 8, respectively.

The saturated cyclic derivatives 4–6 favor the extended conformation ($\tau_1 = 180°$) over those with $\tau_1 = \pm 60°$, although the latter conformations are only slightly less stable in the convulsant compounds **5** and **6**. The barriers to rotation around τ_1 in the convulsants are relatively low, approximately 10 kcal/mol, but the corresponding barrier in **4** is 20 kcal/mol. These barriers are consistent with those observed in the analogous barbiturates **3** and **1**, respectively.[8] Rotation τ_2 is considerably more restricted, with insurmountable barriers between 0 and ±60°. For the convulsant compounds, low-energy conformations are

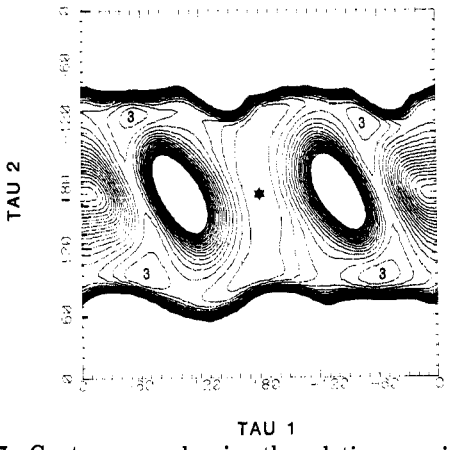


Figure 7. Contour map showing the relative energies of conformations defined by rotations τ_1 and τ_2 in CHEB (**5**). The contour interval is 2.5 kcal/mol and the first 20 contour lines are shown. The global minimum is marked by a star, and energies (in kilocalories per mole) of key secondary minima are as indicated.

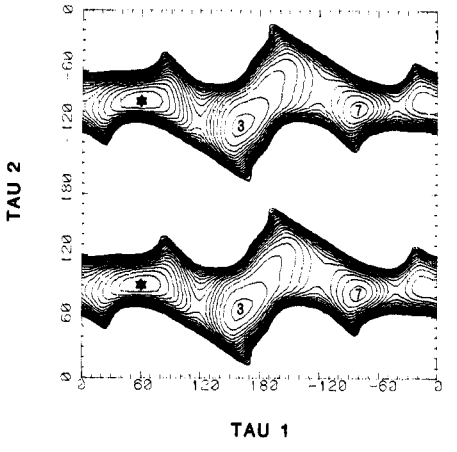


Figure 8. Contour map obtained for rotations τ_1 and τ_2 in the *R* isomer of the convulsant barbiturate **10**. The first 20 2.5-kcal/mol contour lines are shown, and the global minimum is marked by a star. Energies (in kilocalories per mole) of key secondary minima are as indicated.

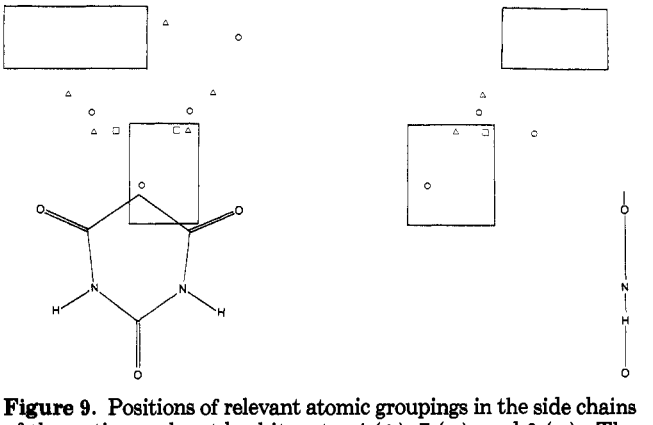


Figure 9. Positions of relevant atomic groupings in the side chains of the anticonvulsant barbiturates 4 (○), 7 (□), and 8 (△). The groups illustrated are topographically equivalent to the terminal methyl groups of barbiturates 1–3.

found in the region $\tau_2 = 180 \pm 90°$, but the corresponding region in **4** is much more restricted. Rotation τ_3 in the latter compound is virtually free, with a barrier of 8 kcal/mol.

The contour maps for the aromatic derivatives vary considerably, depending on the length of the alkyl chain. In **7** the only conformational variable, excluding the ethyl group, is rotation of the phenyl ring, which strongly favors

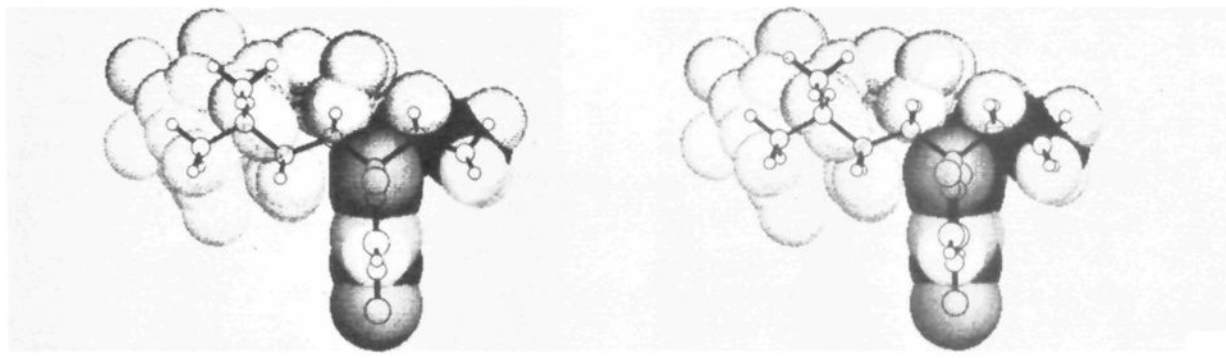


Figure 10. Stereoscopic view of the proposed anticonvulsant pharmacophore obtained by superimposing the barbiturate rings and 5-ethyl substituents of all the anticonvulsant barbiturates while holding the side chains in the proposed anticonvulsant conformation. The surface shown is calculated from the van der Waals radii of the atoms, and the lightly shaded regions are hydrogens. The ball and stick model lying within the surface is the anticonvulsant barbiturate **1c** in its anticonvulsant conformation.

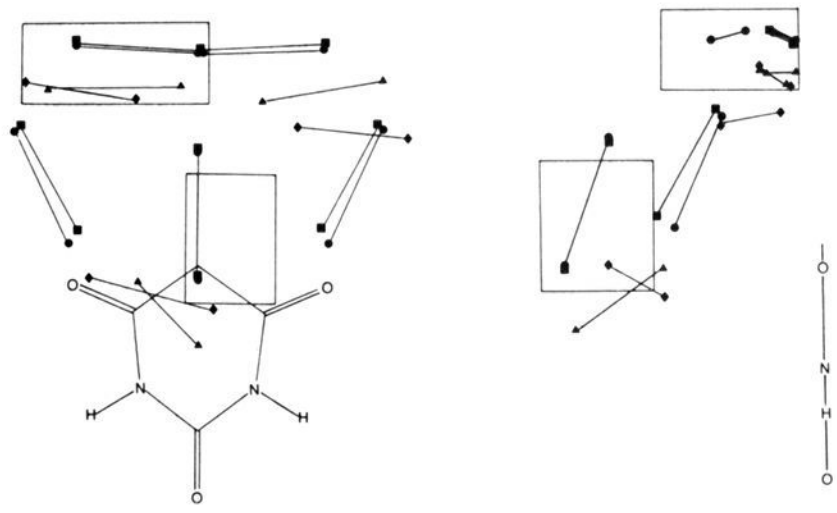


Figure 11. Locations of vectors connecting the two carbon atoms distal to C_3' in the side chains of the convulsant barbiturates **5** (●–●), **6** (■–■), **9** (▲–▲), and **10** (♦–♦). These vectors are topographically equivalent to those connecting the terminal methyl groups in barbiturates **1–3**.

the skew conformation (τ_1 = 40 or 140°) over intermediate conformations (τ_1 = ±90°, 25 kcal/mol). In **8–10** there are three low-energy conformations for rotation around τ_1, with τ_1 = 180° being favored in **8** and **9** but τ_1 = 60° being marginally more stable in **10** (*R* isomer; the corresponding value for the *S* isomer is –60°). The major differences are in τ_2, where the convulsants **9** and **10** favor τ_2 = ±90°, and conformations with τ_2 = 0 or 180° are totally prohibited, whereas in the anticonvulsant **8**, the only accessible region is τ_2 = 180 ± 30°. Rotation around τ_3 in the latter compound favors τ_3 = ±90°, but the barrier is only 4 kcal/mol.

The preceding data define a limited range of conformations within which the biologically active conformation(s) of barbiturates **4–10** should fall. These can now be used to test the proposed pharmacophores derived from barbiturates **1–3**. Figure 9 shows how the three anticonvulsants in the test series, compounds **4**, **7**, and **8**, fit the proposed anticonvulsant pharmacophore. In each case, one of the low-energy conformations falls within the proposed anticonvulsant region, but none of these conformations allows the equivalent of two methyl groups on C_3, to fall within the convulsant region. The proposed pharmacophore thus provides a satisfactory "explanation" for activity in barbiturates **1–10**. It is graphically illustrated in Figure 10, which is a stereoscopic view of the space occupied by a composite of all the anticonvulsant barbiturates in the proposed anticonvulsant conformation. This model, by defining the regions of space occupied by the anticonvulsant barbiturates, provides a first indication of the stereochemical properties of the binding site for anticonvulsant barbiturates.

Figure 11 shows how the convulsants in the test series, compounds **5**, **6**, **9**, and **10**, fit the proposed convulsant pharmacophore. Once again there is at least one low-energy conformation of each convulsant in the proposed convulsant region, which thus provides a satisfactory "explanation" for convulsant activity in barbiturates **1–10**. Of particular importance is the presence of the terminal methyl groups in **1–3** or the topographically equivalent carbon atoms (the α-methylene groups in **5** and **6**, and the meta aromatic carbons in **9** and **10**) in the other convulsant barbiturates. The consistent placement of this grouping throughout all the convulsant barbiturates studied strongly supports its role in interacting with the convulsant binding site, which does not appear to be affected by the additional ring carbons in compounds **5**, **6**, **9**, and **10**. An indication of the stereochemistry of this site is thus provided by the proposed convulsant pharmacophore, which is illustrated in Figure 12. As for the anticonvulsants, this model has been derived by superimposing all of the convulsant barbiturates in their proposed convulsant conformation. Despite the range of structures found in the side chains of the convulsant barbiturates studied, the spatial region

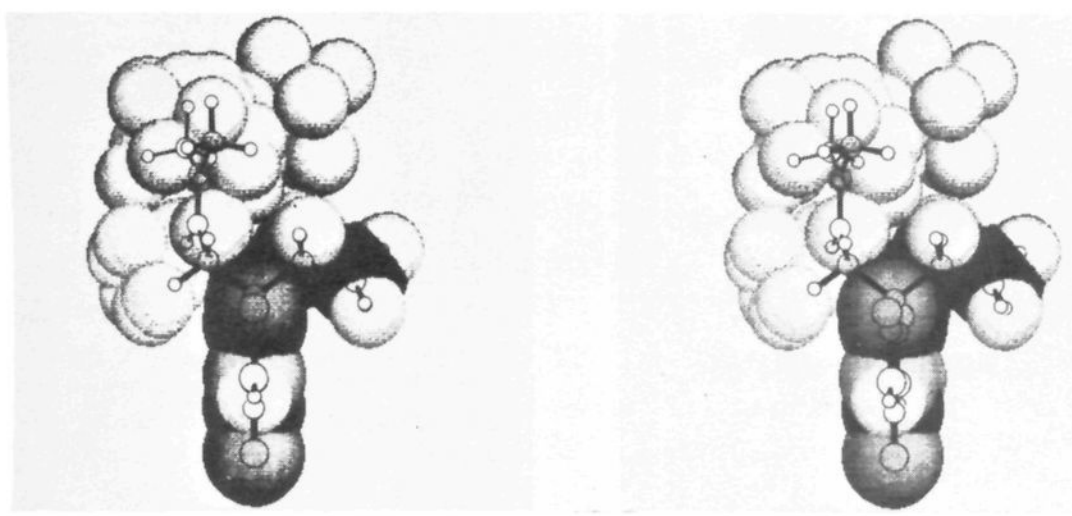


Figure 12. Stereoscopic view of the proposed convulsant pharmacophore obtained by superimposing the barbiturate rings and the 5-ethyl substituents of all the convulsant barbiturates while holding the side chains in the proposed convulsant conformation. The surface shown is calculated from the van der Waals' radii of the atoms, and the lightly shaded regions are hydrogens. The ball and stick model lying within the surface is the convulsant barbiturate **3c** in its convulsant conformation.

required to accommodate the entire series is remarkably small and may thus account for the fleeting nature of the convulsant property in barbiturates and related drugs.

Discussion

The identification of the biologically active conformations of compounds with several degrees of torsional freedom gives rise to a number of problems. For example, although it seems reasonable to assume that the same conformation will be the biologically active form throughout a series of closely related drugs, it is difficult to define which conformations of two different compounds are the "same". Must all the torsion angles be identical, or merely similar? Or can identical locations of key binding groups be achieved with quite different backbone conformations? In the present case, there are data to suggest that the terminal methyl groups of barbiturates **1–3** are important determinants of qualitative activity, and we have therefore chosen to restrict our attention to the location of these groups relative to the barbiturate ring as the basis for conformational comparisons. In so doing, however, we have derived convulsant and anticonvulsant pharmacophores for which the torsion angles defining the biologically active conformations turn out to be relatively constant throughout this series of barbiturates. For the anticonvulsant barbiturates, the biologically active conformations are near $\tau_1 = 180°$ and $\tau_2 = 180°$, while for the convulsants, $\tau_1 \simeq 60°$ and $\tau_2 \simeq -140°$. These values may thus be used to define the convulsant and anticonvulsant pharmacophores for barbiturates **1–10**, and possibly for other barbiturates, but will not apply to topologically distinct species acting at the same receptor.

In view of the qualitatively opposed activities observed in barbiturate stereoisomers, the proposed pharmacophores should discriminate between convulsant and anticonvulsant activity solely on the grounds of conformational energies. The quality of this discrimination can be seen in Table II, where we given the potential energies (relative to the global minimum) of each barbiturate in the conformations that most closely match the convulsant and anticonvulsant pharmacophores, respectively. It is clear that the energies of the anticonvulsants in conformations that match the anticonvulsant pharmacophore and of the convulsants in conformations that match the convulsant pharmacophore are readily accessible. On the other hand, for those anticonvulsants that contain two 3′-methyl groups (or equivalent), the energies of the conformations that fit the convulsant pharmacophore are invariably prohibitive. There is also a considerable energy penalty in matching most of the convulsants to the anticonvulsant pharmacophore. As noted above, the exceptions to the latter statement are not too disquieting, since underlying anticonvulsant activity has been observed experimentally in several convulsant barbiturates[3] and thiobarbiturates.[22] It is noteworthy that the anticonvulsants with the lowest energy "convulsant conformations" are (*R*)-(+)-pentobarbital, which is considerably more excitatory than the *S*(−) isomer,[21] and **8**, of which the 2-thio analogue is known to be convulsant.[14]

Having now obtained pharmacophores for convulsant and anticonvulsant activity, what should we do with them? Clearly, they can be used to predict the activity of closely related barbiturates (e.g., the *R* isomers of the convulsant barbiturates **3d** and **10** should be convulsant, and the *S* isomers should be anticonvulsants), but a more valuable application may be in the design of rigid new analogues with potent convulsant or anticonvulsant activity. Of particular value would be the design of anticonvulsant and anesthetic barbiturates that lack the excitatory side effect frequently seen prior to and during barbiturate anesthesia. If, as seems likely, this is a manifestation of residual convulsant activity, then it should be eliminated by confining the barbiturate side chains to the regions above and below the barbiturate ring ($\tau_1 = 180°$), thus excluding the $\tau_1 = 60°$ region favored by the convulsants. Examples of this type would include the bicyclic imides originally proposed by Smissman et al.[23] as potential anticonvulsant agents, certain spirobarbiturates substituted α to the barbiturate ring, and barbiturates with a bulky group in the 5-position, thus forcing the second side chain into the region above the barbiturate ring.

It is also of interest to ask whether these pharmacophores could be extended to explain the convulsant and anticonvulsant activities of a broader range of structures than the barbiturates considered here. If so, the definition of the essential components in the biologically active pharmacophores should become considerably more precise as the number of common structural features in the series is reduced. Such an approach has been used by Camerman and Camerman,[24] whose X-ray studies of a series of structurally dissimilar anticonvulsants led to the proposal of a common stereochemical basis for anticonvulsant activity that relies solely on the proper spatial arrangement of two hydrophobic substituents and two electronegative H-bonding groups. Recent calculations in our laboratory on these and other clinically useful anticonvulsants confirm and extend the Camermans' conclusions, and the pharmacophore thus derived is entirely consistent with that based on the present series of barbiturates. We are now

Table II. Energies of Barbiturates in Convulsant and Anticonvulsant Conformations

	energy, kcal/mol	
anticonvulsant	anticonvulsant conformation	convulsant conformation
1a	0	NA
1b	0	NA
1c	0	>10
1d (*S*)	0	>10
2a	2	NA
2b	2	NA
2c	0	>10
3a	0	NA
3b	0	NA
3d (*S*)	1	>10
4	0	>10
7	0	>10
8	0	>10
	energy, kcal/mol	
convulsant	anticonvulsant conformation	convulsant conformation
1d (*R*)	>10	3
2d	0	5
3c	0	6
3d (*R*)	>10	0
5	0	4
6	0	3
9	>10	6
10	>10	0

[a] NA = not applicable because molecule does not contain terminal isopropyl or equivalent moiety.

(22) R. K. Richards, *Anesth. Analg. (Paris)*, **30**, 348 (1951).

(23) E. E. Smissman, A. J. Matuszak, and C. N. Corder, *J. Pharm. Sci.*, **53**, 1541 (1964).

(24) A. Camerman and N. Camerman, in "Antiepileptic Drugs: Mechanism of Action", G. H. Glaser, J. K. Penry, and D. M. Woodbury, Eds., Raven, New York, 1980, p P223.

undertaking a similar study in a series of convulsant drugs that appear to share physiological sites of action despite markedly different chemical structures.

Registry No. 1a, 77-28-1; 1b, 76-74-4; 1c, 57-43-2; (*S*)-1d, 24016-64-6; (*R*)-1d, 24016-63-5; 2a, 2237-92-5; 2b, 125-42-8; 2c, 66968-52-3; 2d, 72961-79-6; 3a, 1952-67-6; 3b, 17013-35-3; 3c, 21149-88-2; (*S*)-3d, 86195-90-6; (*R*)-3d, 86195-91-7; 4, 86162-59-6; 5, 22173-64-4; 6, 66940-72-5; 7, 50-06-6; 8, 17013-38-6; 9, 36226-64-9; 10, 68996-50-9.

Importance of C-6 Chirality in Conferring Irreversible Opioid Antagonism to Naltrexone-Derived Affinity Labels[1]

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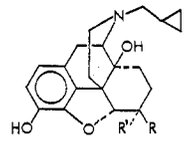
A series of five epimeric pairs of naltrexone derivatives that contain an electrophilic substituent at the 6α- or 6β-position was synthesized and tested on the guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD) preparations in order to determine if the orientation of the electrophile is important for covalent bonding to opioid receptors. In the GPI all compounds were pharmacologically active as reversible agonists, but only the 6β-isomers of the fumaramate ester **2b** (β-FNA) and isothiocyanate **6b** exhibited covalent reactivity, involving a selective irreversible antagonism of the μ agonist, morphine, without affecting κ agonists. The 6α-isomer **2a** (α-FNA) was itself nonalkylating but was able to protect the GPI against alkylation by its epimer, β-FNA, indicating that the two epimers bind to the same receptor. These results suggest that the proper orientation of the electrophilic substituent is required for covalent bonding with a proximal nucleophile in the case of μ receptor blockade. Moreover, the lack of covalent bonding to κ receptors by these or other ligands in this series indicates the possible absence of sufficiently reactive nucleophiles on this recognitition site. In the MVD, **2b**, but not **2a**, irreversibly antagonized morphine (as in GPI), whereas neither epimer exhibited irreversible antagonism toward the δ agonist, [D-Ala^2,D-Leu^5]enkephalin (DADLE). In contrast, both of the isothiocyanate epimers (**6a**,**b**) irreversibly blocked μ and δ receptors. Evidence suggesting differences between μ receptors in the MVD and GPI was obtained with the β-iodoacetamide **5b**, which was an irreversible blocker of morphine only in the MVD. When analyzed together with those of previous studies with the nitrogen mustard analogues, α- and β-chlornaltrexamine, the data suggest that the receptor-alkylating ability of each isomer in an epimeric pair differs most when the electrophile possesses a narrow spectrum of reactivity.

Selective affinity-labeling agents are useful tools for investigating opioid receptors. The affinity labels that have been employed extensively for this purpose are β-chlornaltrexamine[2–5] (β-CNA, **1b**) and β-funaltrexamine[6–10] (β-FNA, **2b**). β-CNA irreversibly blocks at least three opioid receptor types (μ, κ, and δ), while β-FNA is a μ-specific, irreversible antagonist.[11]

Implicit in the approach that we have employed in the design of affinity labels is the assumption that covalent bond formation with opioid receptors is dependent on (1) a primary recognition step of forming a reversible ligand–receptor complex, followed by (2) a secondary recognition step involving proper alignment of the electrophile with a proximal nucleophile on the receptor.

A recently reported[1] study of α-CNA [**1a**, the C-6 epimer of β-CNA (**1b**)] has suggested that each of the epimeric CNA ligands alkylates different receptor nucleophiles by virtue of the different orientation of the electrophile attached to the C-6 epimeric center. Presumably, the high reactivity of the aziridinium ion generated from α- and β-CNA has facilitated secondary recognition but has made this step less selective because of the array of nucleophiles with which this electrophile can react.

In order to provide a more rigorous test for the second recognition process, we have synthesized epimeric pairs of ligands (**2–6**) that contain less reactive electrophiles at the C-6 position and have evaluated them for irreversible opioid antagonist activity.[12] Since secondary recognition should be more sensitive to stereochemical factors when



	R	R^1
1a (α-CNA)	H	$N(CH_2CH_2Cl)_2$
b (β-CNA)	$N(CH_2CH_2Cl)_2$	H
2a (α-FNA)	H	NHCOC(H)=C(H)COOMe
b (β-FNA)	NHCOC(H)=C(H)COOMe	H
3a	H	NHCOC(H,H)=CCOOMe
b	NHCOC(H,H)=CCOOMe	H
4a	H	$NHCOCH=CH_2$
b	$NHCOCH=CH_2$	H
5a	H	$NHCOCH_2I$
b	$NHCOCH_2I$	H
6a	H	N=C=S
b	N=C=S	H
7a	H	$NHCOCH_2CH_3$
b	$NHCOCH_2CH_3$	H
8a	H	NH_2
b	NH_2	H
9	NHCOC(H,H)=CCOOH	H

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