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# Solution Conformations of Ethyl-1′-Methylbutylbarbituric Acids: Implications for Drug–Receptor Site Interactions

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SUMMARY

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Solution conformations of 5-ethyl-5-(1′-methylbutyl)-, 5-ethyl-5-(1′,3′-dimethylbutyl)-, and 5-ethyl-5-(1′,3′,3′-trimethylbutyl)barbituric acids in a variety of solvents have been studied by nuclear magnetic resonance techniques. Implications of the results obtained in this study for discrimination between the enantiomers of 5-ethyl-5-(1′,3′-dimethylbutyl)barbituric acid [*R*-(+), excitatory, and *S*-(−), depressant] at the excitatory receptor site are discussed. It is suggested that discrimination between the enantiomers is based primarily on a required binding orientation of the pyrimidinetrione ring and differing aliphatic side chain geometries resulting from a strong conformational preference of the isopropyl termini.

## INTRODUCTION

The relationship between chemical structure of barbituric acid derivatives and drug action has been a subject of active investigation for many years (1). While the mechanism of barbiturate action at the molecular level remains obscure, selective intermolecular hydrogen bonding between a barbiturate molecule and one or more receptor sites within the biological system is probably required. In addition, lipophilic substitution at C-5 of the pyrimidine ring is necessary.

Hundreds of barbiturates have been studied pharmacologically, and many diverse effects have been observed (1). The vast majority of barbiturates contain, in unaltered form, the identical array of polar functional groups which are capable of hydrogen bonding and differ solely with respect to the C-5 substituents, which can bind to receptors only through weak physical interactions, i.e., van der Waals forces. As a result, attempts to rationalize the different drug effects exhibited by barbiturates have focused on the roles of the C-5 substituents in receptor site binding; of particular interest has been drug conformation.

A number of crystal structures of barbiturates (2–9), metal complexes of barbiturates (10–12), and intermolecular complexes of barbiturates (13–15) have been reported. Of particular interest are intermolecular complexes of barbiturates with

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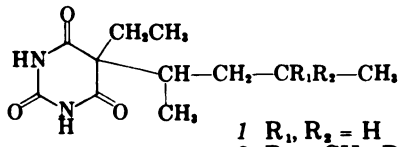
adenine (16) and adenine derivatives (17). The affinity of barbiturates for hydrogen bonding to adenine led to the suggestion (16–18) that barbiturates may exert their biological activity by binding to and thereby inactivating adenine-containing molecules such as adenosine cyclic monophosphate and adenosine 5'-triphosphate.

Studies of barbiturates in solution have utilized a variety of techniques, including infrared (19), optical rotatory dispersion (20), circular dichroism (21), Raman (22), fluorescence and phosphorescence (23), electron spin resonance (24), and proton (25–27) and carbon (28–30) magnetic resonance spectrometries, and Pullman *et al.* (31) have considered barbiturate conformation via molecular orbital calculations.

It is well known that small changes in structure may alter the activity of barbiturates (32), and even optical isomers may show marked differences in pharmacological effect (33–36). The optical isomers of 5 - (1',3'-dimethylbutyl) - 5 - ethylbarbituric acid (*2*) show such differences both *in vivo* and *in vitro*. In the intact animal, Downes *et al.* (34) found that the (+) isomer produced lethal tonic extensor seizures whereas the (−) isomer caused anesthesia. The $LD_{50}$ of the (−) isomer (anesthesia) was over 20 times greater than the $LD_{50}$ of the (+) isomer (convulsions). These differences in behavioral effect have been confirmed and extended by Sitsen and Fresen (37). *In vitro*, Hupka *et al.* (38) have shown that the (+) isomer contracts isolated aortic strips; the (−) isomer lacks this effect and blocks contractions induced by the (+) isomer. Contractor effects *in vitro* are also characteristic of other convulsant barbiturates that produced maximal seizures (38, 39).

In view of this striking dependence on steric factors (for recent review, see ref. 40) remote from the presumed binding sites (the nitrogen and oxygen atoms via hydrogen bonding), we were led to a study of the proton magnetic resonance spectra of 5-ethyl-5-(1'-methylbutyl)barbituric acid (pentobarbital, *1*), 5-(1',3'-dimethylbutyl)-5-ethylbarbituric acid (*2*), and 5-ethyl-5-(1',3',3'-trimethylbutyl)barbituric acid (*3*) as an approach to understanding solution conformations of these interesting compounds. Compounds *1* and *3*, although structurally very similar to *2*, do not cause convulsions in animals or contraction in isolated aortic strips.



*1* $R_1, R_2 = H$
*2* $R_1 = CH_3, R_2 = H$
*3* $R_1, R_2 = CH_3$

## EXPERIMENTAL PROCEDURE

Proton magnetic resonance spectra were obtained with a Varian HA-100 spectrometer with a probe temperature of 27° ± 2°. Chemical shifts were measured in parts per million downfield from an external capillary containing 100% tetramethylsilane and are considered accurate to ±0.01 ppm. The concentration of solutions was approximately 20 mg/ml unless otherwise stated.

For comparisons with literature data referenced to internal TMS,[3] correction for solvent and bulk susceptibility effects was necessary. This was accomplished by measuring the chemical shift difference between 100% TMS contained in a capillary and a 1% solution of TMS in the solvent of interest placed in the annular space around the capillary. The $\Delta^{1\%}_{100\%}$ TMS obtained in this way was subtracted from chemical shifts referenced to external TMS to obtain shifts referenced to internal TMS (1%).

Barbiturate *1* (pentobarbital) was obtained from Sigma Chemical Company, barbiturate *2* was synthesized by Dr. J. H. Block at the School of Pharmacy of Oregon State University, and barbiturate *3* was synthesized by the late J. K. Williams, Department of Pharmacology, University of Utah College of Medicine.

## RESULTS

Frequencies for methyl resonances for barbiturates *1–3* in methanol, dimethyl sulfoxide, trifluoroacetic acid, and aqueous base (pH 12) are given in Tables 1 and 2. Aromatic solvent-induced shifts for reso-

[3] The abbreviation used is: TMS, tetramethylsilane.

nances of *2* are shown in Table 3. In Table 4 are data obtained in a study of the effect of concentration of 5-(1',3'-dimethylbutyl)-5-ethylbarbituric acid (*2*) in chloroform solution on proton resonances.

## DISCUSSION

Crystallographic structure studies of a number of barbiturates (2–18) have revealed that (a) the pyrimidinetrione ring is usually distorted slightly from planarity, and (b) the 5,5-dialkyl substituents are disposed in such a way that they form a chain essentially perpendicular to the best plane through the ring atoms. This results, in the case of 5,5-diethylbarbituric acid, in placing the methyl substituents well over the ring. In this conformation the methyl groups should experience diamagnetic shielding owing to the $\pi$-electron system of the ring and/or that of the amide carbonyls. Indeed, Neville and Cook (27) estimated that the methyl groups of 5,5-diethylbarbituric acid are shielded by 0.11 ppm as compared with similar methyl groups of a conformationally more flexible acyclic model.

*Resonance frequencies.* The data included in Table 1 show that the methyl (Et) resonances for 5-ethyl-5-isopropylbarbituric acid and compounds *1–3* are similarly shielded, indicating that in these compounds, too, the methyl (Et) substituent is situated over the ring. Indeed, when the second alkyl group is larger than ethyl, the methyl (Et) protons are even more effectively shielded. This may be due to a

TABLE 1

*Proton magnetic resonance frequencies[a] for 5-ethyl-5-alkylbarbituric acids in dimethyl sulfoxide and trifluoroacetic acid*

| Alkyl substituent | Resonance | Dimethyl sulfoxide | Trifluoroacetic acid |
|---|---|---|---|
| | | *ppm* | *ppm* |
| Ethyl[b] | $CH_2$ | 1.87 | 2.23 |
| | $CH_3$ | 0.80 | 1.02 |
| Isopropyl[b] | $CH_2$(Et) | 1.90 | 2.42 |
| | $CH_3$(Et) | 0.73 | 0.97 |
| | $CH(CH_3)_2$ | 0.93 | 1.17 |
| 1'-Methylbutyl (*1*) | $CH_2$ (Et) | 1.90 | 2.32 |
| | $CH_3$ (Et) | 0.72 | 1.00 |
| | 1'-$CH_3$ | 0.93 | 1.20 |
| | 3'-$CH_3$ | 0.85 | 0.97 |
| 1',3'-Dimethylbutyl (*2*) | $CH_2$ (Et) | 1.87 | 2.30 |
| | $CH_3$ (Et) | 0.71 | 0.99 |
| | 1'-$CH_3$ | 0.91 | 1.18 |
| | 3'-$CH_3$ | 0.75 | 0.90 |
| | | 0.85 | 0.96 |
| 1',3'3'-Trimethylbutyl (*3*) | $CH_2$ (Et) | 1.85 | 2.28 |
| | $CH_3$ (Et) | 0.70 | 0.98 |
| | 1'-$CH_3$ | 0.97 | 1.26 |
| | 3'$CH_3$ | 0.84 | 0.97 |

[a] Downfield from internal (1%) TMS.
[b] Data taken from Neville and Cook (27).

TABLE 3

*Aromatic solvent-induced shifts [reference-independent (41) for proton resonances of 5-(1',3'-dimethyl)-5-ethylbarbituric acid (compound 2)*

| Resonance | $\bar{\Delta}$ $CCl_4$ $C_6H_6$ | $\bar{\Delta}$ $CCl_4$ $C_5H_5N$ |
|---|---|---|
| | *ppm* | *ppm* |
| $CH_2$ (Et) | 0.65 | 0.41 |
| $CH_3$ (Et) | 0.78 | 0.53 |
| 1'-$CH_3$ | 0.68 | 0.48 |
| 3'-$CH_3$ | 0.68, 0.67 | 0.72, 0.67 |

TABLE 2

*Methyl proton magnetic resonance frequencies of barbiturates 1–3 upfield from corresponding $CH_2$ (Et) resonances*

| Solvent | $CH_3$ (Et) | | | 1'-$CH_3$ | | | 3'-$CH_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *1* | *2* | *3* | *1* | *2* | *3* |
| | *ppm* | | | *ppm* | | | *ppm* | | |
| Methanol | 1.20 | 1.21 | | 0.97 | 1.01 | | 1.08 | 1.12, 1.20 | |
| Dimethyl sulfoxide | 1.18 | 1.16 | 1.15 | 0.97 | 0.96 | 0.88 | 1.05 | 1.02, 1.12 | 1.01 |
| Trifluoroacetic acid | 1.32 | 1.31 | 1.30 | 1.12 | 1.12 | 1.02 | 1.35 | 1.34, 1.40 | 1.31 |
| Aqueous NaOH (pH 12) | 1.22 | 1.20 | 1.18 | 0.99 | 0.96 | 0.90 | 1.11 | 1.00, 1.07 | 0.96 |

TABLE 4

*Effect of concentration on proton resonance frequencies[a] of 5-(1',3'-dimethylbutyl)-5-ethylbarbituric acid (compound 2) in deuterochloroform*

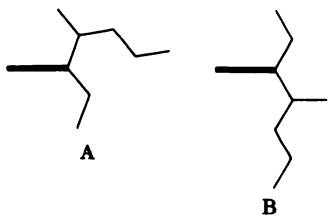
| Concentration | $CH_3$ (Et) | $CH_3$ (Et) | 1'-$CH_3$ | 1'-CH | 3'-$CH_3$[b] |
|---|---|---|---|---|---|
| *mg/ml* | *ppm* | *ppm* | *ppm* | *ppm* | *ppm* |
| 1.0 | 2.085 | 0.857 | 1.032 | 2.313 | 0.837 |
| 13 | 2.092 | 0.854 | 1.035 | 2.318 | 0.835 |
| 25 | 2.096 | 0.855 | 1.038 | 2.321 | 0.835 |
| 40 | 2.104 | 0.852 | 1.038 | 2.324 | 0.833 |
| 67 | 2.104 | 0.853 | 1.040 | 2.324 | 0.833 |
| Δ (67 - 1.0 mg/ml) | +0.019 | -0.004 | +0.008 | +0.011 | -0.004 |

[a] Downfield from internal (1%) TMS.

[b] Average value for two methyl signals.

slight change in ring conformation such that the larger substituent tends toward equatorial orientation and the smaller ethyl substituent is then in a pseudoaxial position, with the methyl group situated more directly over the ring or carbonyl $\pi$-electron system.

5-Ethyl-5-isopropylbarbituric acid and compounds *1–3* possess branched chains in which a methyl group is present $\beta$ to C-5 of the ring. As a result, these molecules could assume a conformation in which this methyl group is oriented over the ring in a manner similar to the methyl (Et) substituent (conformation A illustrated in Scheme 1 for compound *1*, with the ring shown as planar for simplicity); alternatively the larger alkyl substituent could be oriented perpendicular to the ring with the $\beta$-methyl group oriented away from the ring (compound *1*, conformation B).




SCHEME 1

The data in Table 1 allow a clear choice in favor of conformation B. That the 1'-methyl group is oriented away from the ring is shown by the fact that for the isopropyl compound and compounds *1–3* the 1-methyl groups of the larger alkyl substituents at C-5 are deshielded at least 20 Hz compared with the corresponding methyl (Et) resonances. These protons therefore must be situated conformationally such that they are not exposed to the shielding environment experienced by the methyl (Et) protons and, in fact, are probably exposed to the deshielding regions of the C-4 and C-6 carbonyl groups and/or the deshielding region which might arise as a result of a partial ring current in the barbiturate ring. Recently Smit and Kantars (2) and Sitsen and Fresen (42) have reported that in the crystalline state 5-(1',3'-dimethylbutyl)-5-ethylbarbituric acid (*2*) possesses a conformation much like A; all other barbiturates for which crystal structures are known (2–9) possess solid-state conformations with extended alkyl chains (B). The reasons for the unusual solid-state conformation of *2* and the differences between solid and solution conformations are unknown.

In order to facilitate comparisons of the effects of various solvents on the barbiturate methyl proton resonances it is necessary to correct for bulk magnetic susceptibility differences between the solvents used. Since solvent shift data using two different spectrometer sample geometries (41) are not available for the solvents studied, we chose to remove the bulk susceptibility effects by referencing the resonance frequencies intramolecularly (43) to the $CH_3$ (Et) frequency. This resonance was chosen for use as a reference because it is easily assigned and conforma-

tional effects are expected to be minimal for these protons. In Table 2 are methyl resonance frequencies in four polar, hydrogen-bonding solvents. From these data, changes in ring conformation are seen to have little effect, since the resonance frequencies in aqueous solution at pH 12 in which the barbiturate ring is planar owing to dianion formation (23) differ little from those in methanol or dimethyl sulfoxide, in which the potentially nonplanar triketo tautomer exists.

That the alkyl side chain has strong conformational preferences is indicated by the fact that, in all solvents studied, the isopropyl methyl groups of 2 are magnetically nonequivalent (44, 45). In methanol, dimethyl sulfoxide, and aqueous base solutions, the resonance frequencies for the chain terminal (3′) methyl groups for compounds *1–3* are intermediate between those for the methyl (Et) and 1′-methyl substituents (Tables 1 and 2). This would seem to rule out "folded" conformations, in which the chain ends are brought into close proximity to the ring. Rather, the resonance frequencies are consistent with an extended conformation, in which the terminal methyl groups are remote from both shielding and deshielding regions resulting from ring and/or carbonyl unsaturation. On the other hand, in trifluoroacetic acid (Tables 1 and 2) and chloroform (Table 4) the 3′-methyl protons are shielded to about the same extent as the methyl (Et) protons. In these solvents, therefore, it is possible that the longer alkyl chains "fold" so that the terminal methyl groups lie over the plane of the ring.

*Aromatic solvent-induced shifts.* In the case of 5-(1′,3′-dimethyl)-5-ethylbarbituric acid (2) aromatic solvent-induced shifts were determined. The aromatic solvent-induced shifts observed in benzene solution ($\bar{\Delta}_{C_6H_6}$) (41) corroborate the assigned conformation (B). Williams and Wilson (46) showed empirically that the magnitudes of benzene solvent effects for proton resonances in carbonyl-containing compounds depend on their orientation with respect to a plane through the carbonyl carbon and perpendicular to the carbonyl bond. Because of symmetry the predictions for the C-4 and C-6 carbonyls of 2 are identical and show that the $CH_2$ (Et) and 1′-$CH_3$ groups (conformation B) lie on the plane and therefore experience no specific solvent shift [for these correlations the solvent shift, $\bar{\Delta}(\perp)^{CCl_4}_{C_6H_6}$, experienced by internal TMS, +0.67 ppm, is used to approximate the nonspecific effect] (41). In contrast, in conformation B the $CH_3$ (Et) group clearly lies behind the planes through carbonyls C-4 and C-6 and the observed excess, i.e., specific, benzene shift (+0.78 minus +0.67 ppm) (41) clearly reflects this and serves to confirm the proposed orientation. The 3′-methyl resonances are remote from the molecular dipoles which might serve to orient the benzene solvent molecules (47) and therefore experience no differential aromatic solvent-induced shifts.

The pyridine shifts, $\bar{\Delta}^{CCl_4}_{C_5H_5N}$, are less informative; all the shifts (Table 3) are seen to be specific and negative [with respect to $\bar{\Delta}(\perp)^{CCl_4}_{C_5H_5N} = +0.80$ for internal TMS] (41). According to Williams (48) this requires that all the protons in question lie on the same side as the carbonyl (C-4 or C-6) of corresponding planes through C-5 and either N-1 or N-3. The aromatic solvent-induced shifts observed for $CH_2$ (Et) and 1′-$CH_3$ are consistent; however, $CH_3$ (Et) would appear to lie slightly on the other (positive specific solvent shift) side of the plane even though the observed specific shift is not consistent with this orientation. In pyridine strong dipole-dipole interactions between pyridine nitrogen atoms and the electropositive carbonyl carbon atoms (47, 48) may cause sufficient steric crowding above and below the plane of the barbiturate ring to exclude the aliphatic chains from these regions.

*Concentration effects.* It appeared possible that, at high barbiturate concentrations, barbiturate-barbiturate interactions might affect the alkyl chain conformations. To examine this possibility a saturated solution of 2 in chloroform (67 mg/ml) was prepared and its NMR spectrum was recorded. Spectra were similarly obtained using more dilute solutions. The results (Table 4) show only very small changes in proton resonance frequencies, indicating that, in the concentration range studied, no important conformation changes occur.

*Coupling constants.* Use of the Karplus

relationship, which relates the magnitude of proton-proton coupling constants with the dihedral angle between interacting nuclei, is a powerful technique for investigation of solution conformations (49, 50). Unfortunately, in the present investigation the complexity of the spectra caused by the small differences between resonance frequencies for the various alkyl protons of interest precluded accurate measurement of coupling constants.

## CONCLUSIONS

Barbiturates *1–3* possess conformations in both polar and nonpolar solvents in which the 5,5-dialkyl substituents form a chain which is essentially perpendicular to the plane of the pyrimidinetrione ring (conformation B). The enantiomers of *2* differ sterically in two ways if, in each case, the molecule in conformation B (see above) is viewed such that the 6 carbons which comprise the alkyl chain perpendicular to the plane of the pyrimidinetrione ring are identically diposed (shown in Scheme 2). First, in the enantiomers of *2* the 1′-methyl substituent lies, respectively, on opposite sides of a plane through the alkyl chain and through C-2 and C-5 of the pyrimidinetrione ring. Second, the 3′-methyl substituent, shown by our studies to have restricted rotational freedom, is expected to assume a preferred conformation such that interactions with the 1′-methyl substituent are minimal; i.e., the 3′-methyl substituent is expected to lie on the side of the C-2,C-5 plane opposite that occupied by the 1′-methyl group.

*2*, *R*-(+), convulsant  *2*, *S*-(−), depressant

SCHEME 2

The knowledge that the solution conformational preferences of barbiturates *1–3* are similar and the known differentiation between the enantiomers of *2* at the receptor binding level *in vivo* (34, 37) and *in vitro* (38, 39) allow several inferences to be drawn concerning steric parameters associated with the excitatory effect of barbiturate *2*. First, there is an inherent dissymmetry at the receptor site such that, although the pyrimidinetrione ring is symmetrical with respect to 180° rotation, the differing steric bulks of the two alkyl substituents are distinguished and only a single orientation allows for adequate binding of barbiturate to receptor. Receptor binding could involve hydrogen bonds and/or van der Waals forces. If the mode of binding of barbiturate to excitatory receptor is via hydrogen bonds to pyrimidinetrione ring nitrogen and carbonyls, such binding is specific and asymmetrical, involving, for example, the N-1 hydrogen and C-6 carbonyl and not the chemically indistinguishable N-3 and C-4 functions. We infer this because only in this case can we envisage a critical role for the 1′-methyl group in determining barbiturate-receptor binding. In a given enantiomer of *2* the 1′-methyl group lies relatively close to one of the carbonyls (either C-4 or C-6, depending on the enantiomer in question) and, through steric interactions with structural components of the receptor molecule, may not allow the proper orientation for strong hydrogen bonds to form. In contrast, the other enantiomer of *2* would experience no such steric inhibition toward binding with this same receptor site, since the 1′-methyl group in this isomer is sterically remote from the clinical area.

These characteristics which distinguish the receptor binding ability of the enantiomers of *2* are equally applicable to enantiomers of *1* and *3*, barbiturates which cause only anesthesia (34). The only structural feature which distinguishes barbiturate *2* from *1* and *3*, and, therefore, could provide a basis for differential binding to an excitatory receptor, is the substituent at the terminus of the longer alkyl side chain. Perhaps significantly, the chain termini of compounds *1* (methyl) and *3* (*tert*-butyl) are symmetrical whereas the isopropyl termini of the enantiomers of *2* are asymmetrical and expected (44, 45) to exhibit strong (and opposite, see above) conformational preferences.

A great many barbiturates incorporating widely varying substituents at C-5 exhibit

some efficacy as depressants (1), indicating that binding to the receptor site(s) associated with anesthesia has minimal structural specificity. In contrast, excitatory receptor binding, as evident from this study, is dependent on meeting very subtle structural parameters. To emphasize further this high selectivity of the excitatory receptor, it is noted that barbiturates in which the shorter alkyl chain at C-5 is contracted or extended, i.e., 5-methyl- or 5-propyl-5-(1',3'-dimethylbutyl)barbituric acid (51),[4] and barbiturates in which an additional carbon is added in the longer alkyl chain, i.e., 5-ethyl-5-(1',3'-dimethylpentyl or 1',4'-dimethylpentyl)barbituric acid, are devoid of excitatory activity (52-54).

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