pressure, 83 ± 2 bars; chart speed, 0.5 cm/min, detector sensitivity, 0.04–0.1 aufs; and oven temperature, 20°. The chromatograms were recorded at 237 nm.

RESULTS AND DISCUSSION

The maximum absorbance wavelengths in the mobile phase were 267 (I) and 237 (II–IV). A weak absorbance of V was observed at 267 nm; 237 nm was most suitable for the simultaneous determination of I–V. In injections, lidocaine did not absorb at 237 and 267 nm at the concentration used (125 μg/ml) for the determination of II–IV.

A chromatogram of a standard solution is shown in Fig. 1 and Table I lists the retention times of each compound.

Figure 1 and Table I show a good resolution of each compound. The resolution obtained was always better than 1 for I–V.

A calibration graph was plotted of peak area ratio of the solute to the internal standard against solute concentration. The calibration graph was linear in the concentration range studied and went through the origin, using peak areas as well as peak heights. The correlation coefficients of the linear regression analysis were always better than 0.999 using peak areas as well as peak heights.

The minimum amount detectable, defined as the amount (in micrograms) that gives a peak height equal to twice the background, and the sensitivity, defined as the change in area value (measured at the maximum detector sensitivity) resulting from a concentration change of one unit (milligrams per liter) are given in Table II.

The repeatability tested by five replicates and evaluated by the coefficient of variation was 0.978 (I), 1.064 (II), 1.210 (III), and 1.510% (IV). The average data of duplicate assays for recovery studies on laboratory prepared injections with and without added amounts of II–IV are given in Table III.

A comparative separation of I–IV was carried out on a silica column[8]. With hexane–chloroform–methanol–formic acid (400:200:20:1) as a mobile phase, retention times were ~4 (I), 8 (II), 12 (III), 17 (IV), and 25 min for benzenesulfonamide used as an internal standard. Reversed-phase HPLC was faster (14 min) and more convenient than normal HPLC. The insignificant amounts of II–IV (probably present in the reference drug) detected by injecting 10 μl of a concentrated solution of I (2.5 g/liter) and the accuracy obtained for the determination of trace II–IV reveal that reversed-phase HPLC allows the determination of I–IV without decomposition during analysis.

With GLC, artifact peaks were observed when degradation products were present, and it is suggested (3) that the breakdown of the degradates occurs at the injection port maintained at 230°. HPLC is achieved at ambient temperature and prevents this drawback.

With TLC, a significant oxidation of I on the plate was observed during analysis (1). The iron present in the silica coating was responsible (1) for the accelerated air oxidation of I. The absence of degradation with the HPLC procedure is consistent with this hypothesis. The analysis time (13 min), the absence of air exposure, and the presence of citric acid (chelating agent for iron) in the mobile phase (used as an eluting agent and as a solvent in the preparation of solutions) contribute to prevent the degradation of I.

A commercial injection formulation was analyzed by the proposed HPLC procedure. Compound I was 1000.2 mg (100.0% of the labeled strength), III was 14.92 mg (1.49% with respect to theoretical I content), IV was 21.8 mg (2.18% with respect to theoretical I content), and II was not detected. The results are in agreement with other data (4) that shows II is so readily hydrolyzed into IV that it cannot be found in alkaline solution, and the major products of I degradation in injections are III and IV.

Reversed-phase HPLC is a sensitive (20 times more than TLC) and convenient procedure that can be used to evaluate the purity of phenylbutazone and to monitor its stability in pharmaceutical formulations. Because the metabolites of I have been identified as similar to the degradation products *in vitro*, the method is suggested to be applicable to pharmacokinetic studies.

[8] 30 μm Zorbax Sil, 30-cm length.

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COMMUNICATIONS

Active Conformation of Polycyclic Antidepressants

Keyphrases □ Antidepressants, polycyclic—active conformation □ Conformation, active—of polycyclic antidepressants

To the Editor:

A 300-MHz ^{1}H-NMR study of a series of polycyclic (tetracyclic and pentacyclic) antidepressant agents of the amitriptyline type indicated a preferred conformation in solution in which the alkylamino side chain is folded toward the polycyclic skeleton and its positively charged dimethylammonium ion is oriented above the adjacent aromatic ring.

Amitriptyline (I) and imipramine, prototype tricyclic antidepressant drugs, are potent inhibitors of active reuptake of biogenic amines in nerve endings. The therapeutic effects of these drugs may be related to this activity[1] (1–5). Reuptake inhibition may lead to an accumulation of neurotransmitters, *e.g.*, norepinephrine and serotonin, at the receptor site and to a subsequent increase in activity. The preferred conformation of the tricyclic antidepressant at the biogenic amine uptake jump may be crucial to their antidepressant activity.

Of particular significance is the conformation of the alkylamino side chain *vis-à-vis* the tricyclic skeleton (6, 7). X-ray structure determinations (8–13) indicated that in the crystalline state the side chain is almost fully extended, away from the tricyclic skeleton. However, it was suggested (6, 14) that the active conformation of the alkylamino side chain in polycyclic antidepressants is folded toward the aromatic ring. We present evidence for the amitriptyline-type series in favor of this hypothesis and

[1] For the state of the art of the mode of action of tricyclic antidepressants, see Horn (5).

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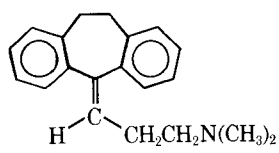
Table I—^{1}H-NMR $N(CH_3)_2$ Chemical Shifts [a] of I–VI Hydrochlorides in Deuterochloroform (2.4×10^{-5} mole/ml) at 23° and 300 MHz [b]

Compound	$\delta(Z)$	$\delta(E)$	$\Delta\delta = \delta(E) - \delta(Z)$
II	2.633		0
III	2.635	2.693	0.058
IV	2.610	2.706	0.096
V	2.657	2.691	0.034
VI	2.596	2.635	0.039
I	2.680		0

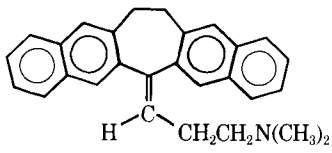
[a] In parts per million relative to tetramethylsilane. [b] Spectra of isomeric mixtures of III–VI were recorded on a Bruker WH-300 spectrometer, FT mode.

establish that the alkylammonium terminus of the side chain is oriented toward and above the plane of the aromatic ring.

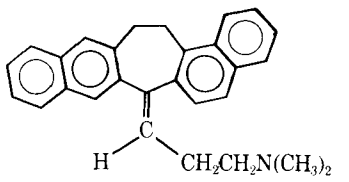
The following polycyclic antidepressants were synthesized and evaluated as antidepressants according to their ability to inhibit serotonin uptake into human blood platelets: 6-(3-dimethylaminopropylidene)-13,14-dihydro-6*H*-cyclohepta[1,2-*b*:4,5-*b*′]dinaphthalene (II), 7-(3-dimethylaminopropylidene)-14,15-dihydro-7*H*- cyclohepta[1,2-*a*:4,5-*b*′]dinaphthalene (III), 5-(3-dimethylaminopropylidene)-12,13-dihydro- 5*H* -benzo[4,5]cyclohepta[1,2-*b*]naphthalene (IV), 7-(3-dimethylaminopropylidene) - 12,13-dihydro-7*H*-benzo[4,5]cyclohepta-[1,2-*a*]naphthalene (V), and 7-(3-dimethylaminopropylidene) - 7*H*-benzo[4,5]cyclohepta[1,2 - *a*]naphthalene (VI). These naphthalene variations of the amitriptyline theme that showed comparable activity to the parent drug[2] offered a favorable opportunity to study the conformation of the alkylamino side chain *vis-à-vis* the aromatic ring in solution.



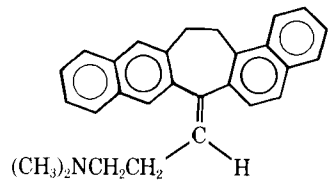
I



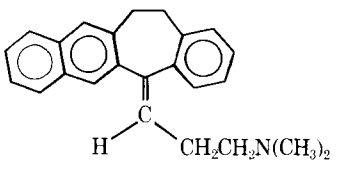
II



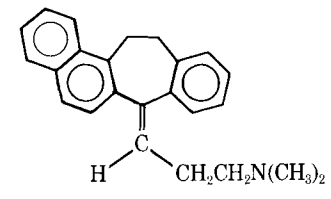
E-III



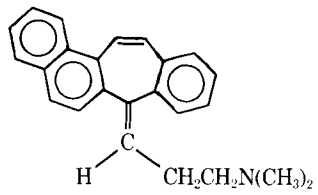
Z-III



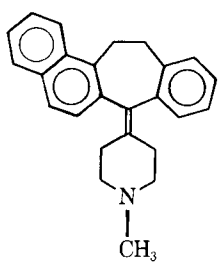
E-IV



E-V



E-VI



VII

The 300-MHz ^{1}H-NMR chemical shifts of the dimethylamino groups [$\delta N(CH_3)_2$] of I–VI hydrochloride salts in deuterochloroform solution are recorded in Table I. Under the conditions of a superconducting magnet, small differences of chemical shifts (0.001 ppm) in very dilute solutions are detectable and significant. Consider the two symmetric systems, I and II. The long range effect of the naphthalene nuclei in II (as compared with the benzene rings of I) is manifested in the shielding of 0.047 ppm. The unsymmetrical structure of III–VI may exhibit π diastereoisomerism (geometrical isomerism). The presence in each case of the *E* and *Z* isomers is reflected in the ^{1}H-NMR spectrum, which exhibits two vinylic triplets [*e.g.*, δ_1(III) 6.028 (J = 5.9 Hz), δ_2(III) 5.884 (J = 5.9 Hz), $\Delta\delta$ 0.144]. The expected nonequivalence is probably due to an inductive effect and to the proximity of the vinylic proton to the corresponding aromatic nucleus. More significantly, in each pair of *E*, *Z* isomers, the remote dimethylamino protons are also magnetically nonequivalent, leading to two singlets representing the *E* and *Z* isomers.

The barriers for conformational inversion of the seven-membered ring in amitriptyline-type drugs are low. A dynamic NMR study of II revealed a $\Delta G_c^{\neq}$ value of 15.6 kcal/mole (in deuteroacetonitrile). In the more rigid 5-(3-dimethylaminopropylidene)-5*H*-dibenzo[*a*,*d*]cycloheptene, $\Delta G_c^{\neq}$ equals 22.6 kcal/mole (15). Thus, it is unlikely that the two $N(CH_3)_2$ absorptions in each of compounds III–VI are due to two different conformations of the same geometrical isomer. The magnetic nonequivalence of the *N*-methyl groups cannot be ascribed to an inductive effect in view of the number of bonds separating the *N*-methyl protons from the structural perturbation (*E versus Z*) in the polycyclic skeleton. The spatial magnetic environment of the *E*-$N(CH_3)_2$ and *Z*-$N(CH_3)_2$ protons apparently are not identical, thus leading to the observed shifts. The *E*,*Z* assignments (Table I) are based on comparison with the symmetrical drugs I and II. The data indicate that whenever the alkylamino side chain is *Z* to the naphthalene nucleus, δ-$N(CH_3)_2$ is shielded relative to an alkylamino side chain *Z* to the benzene nucleus (II *versus* I, *E*-IV *versus* *Z*-IV, and *E*-V *versus* *Z*-V). In the linearly annelated derivative (*Z*-IV), the long range diamagnetic shielding effect of the naphthalene nucleus is more pronounced than in the angularly annelated derivative *Z*-V, indicating a variation in the proximity of the side chain to the naphthalene (also note *Z*-III *versus* *E*-III).

The observed diamagnetic effect as revealed in the shielding of the *N*-methyl protons is attributed to a preferred conformer of the alkylamino side chain *vis-à-vis* the polycyclic skeleton. The side chain is folded toward the polycyclic system, and the dimethylamino group is oriented above and not in the plane of the aromatic ring (Fig. 1). Such geometry permits interaction between the ammonium-ion terminus and the aromatic π-electron cloud so that the *N*-methyl groups are exposed to the diamagnetic ring current of the aromatic ring. Consistently with

[2] The synthesis of II–VI (hydrochlorides) and their structure–activity relationships will be reported elsewhere.

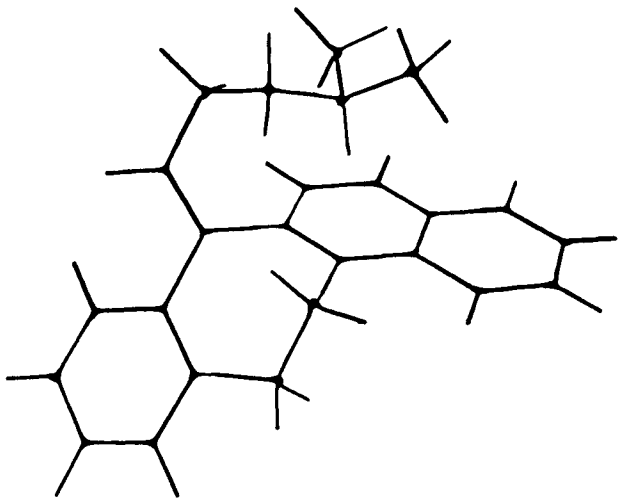


Figure 1—*Preferred conformation of Z-V.*

this picture, the *N*-methylpiperidinylidene analogs of III–VI [*e.g.*, 7-(1-methyl-4-piperidinylidene)-12,13-dihydro-7*H*-benzo[4,5]cyclohepta[1,2-*a*]naphthalene (VII)], in which the nitrogen atoms are rigidly immobilized and positioned away from the polycyclic skeletons, were found to be inactive as inhibitors of serotonin uptake into human blood platelets. The rather considerable difference in $\Delta\delta$ values between IV [$\delta(E\text{-IV}) - \delta(Z\text{-IV}) = 0.096$ ppm] and I–II [$\delta(\text{I}) - \delta(\text{II}) = 0.047$ ppm] should be noted. This difference may be rationalized by considering the conjugation effect of the remote aromatic cycle (*trans* to the side chain) on the diamagnetic ring current of the adjacent aromatic cycle (*cis* to the side chain). The delocalization of the π-electron cloud into the *trans*-aromatic cycle (*via* the exocyclic double bond) weakens the diamagnetic effect of the *cis*-aromatic cycle. This diminution is more pronounced in II (*trans*-naphthalene) than in *Z*-IV (*trans*-benzene) and in *E*-IV than in I. In fact, the order of the net diamagnetic ring current effect is *Z*-IV > II > I > *E*-IV, with the strongest shielding produced by *Z*-IV with its *cis*-naphthalene, *trans*-benzene configuration.

The higher shielding effect in VI relative to V [$\delta(Z\text{-V}) - \delta(Z\text{-VI}) = 0.061$ ppm; $\delta(E\text{-V}) - \delta(E\text{-VI}) = 0.056$ ppm] may be due to the different geometries of the two polycyclic systems. The CH=CH bridge in VI flattens the ring system somewhat, drawing the aromatic surface nearer to the alkylamino side chain. However, the difference in $\Delta\delta$ values between V and VI is very small. It may be concluded that the impact of the *E*,*Z* parameter on the net diamagnetic shielding (due to spatial orientation and conjugation) does not vary significantly from V to VI. A potential energy map for the antidepressant iprindole (as a free base) shows a conformer in which the side chain is folded back toward the plane of the tricyclic system (16). Likewise, in the potent antidepressant *N*,*N*-dimethylspiro[5*H*-dibenzo[*a*,*d*]cyclohepten-5,1′-cyclohexane]-4′ amine, the cyclohexane ring may adopt a boat conformation in which the *N*-methyl group is oriented above the aromatic ring (12, 17).

In a recent conformational structure–activity relationship study of compounds related to tricyclic antidepressants, the projected height of the nitrogen atom over the plane of the nearest aromatic ring seemed to be the most important structural feature (7). The $C^{\gamma}H_2C^{\beta}H_2C^{\alpha}H_2N(CH_3)_2$ side chain of imipramine hydrochloride in a nonaqueous medium (such as deuterochloroform) was shown to exist almost entirely in one fixed conformation with a *gauche* C^{α}–C^{β} and a *trans* C^{β}–C^{γ} fragment (18). The less flexible amitriptyline-type ring system with its rigid exocyclic double bond may be responsible for the preferred active conformation in which the alkylamino side chain is not only folded toward the polycyclic skeleton but its positively charged dimethylammonium ion is oriented above the most adjacent aromatic ring. This effect is manifested even in deuteromethanol solution, which resembles the aqueous physiological environment in which the polycyclic antidepressant agents function: $\Delta\delta(CD_3OD) = \delta(E\text{-III}) - \delta(Z\text{-III}) = 0.045$ ppm. Thus, the preferred conformation of the polycyclic antidepressants in solution is at variance with the conformation in the crystalline state.

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