Proton Nuclear Magnetic Resonance Markers as Probes for the Study of Side-Chain Interactions and Conformations of Polypeptides in Solution*

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ABSTRACT: The concept of proton nuclear magnetic resonance markers for the study of side-chain interactions and, hence, conformations of polypeptides in solution is introduced.

Model compounds containing phthalimide or benzamide plus phenyl groups were studied. Mutual ring-current shielding between the aromatic acyl and the phenyl groups is demonstrated for certain types of close-range interaction. The results indicate that the phthalimide group (which can be introduced into natural and synthetic polypeptides containing amino groups) is an especially promising nuclear magnetic resonance marker. It can signalize the proximity of the phenyl groups of phenylalanine side chains. Conclusions as to the relative positions in space of the aromatic rings can be drawn. Ring-current influences of the side chains of tyrosine, tryptophan, and histidine will be the subject of further investigations.

Proton nuclear magnetic resonance is a very sensitive tool for the study of conformations in solution. However, the spectra of even moderately complex polypeptides are rather difficult to interpret in terms of three-dimensional structure. This is due to the great number of all kinds of protons giving rise to an almost contingent field of signals between approximately 0 (tetramethylsilane) and 7.5 ppm (aromatic protons).

There are two "windows" in the spectrum in which a nuclear magnetic resonance marker could be seen and in which close-range interactions of other parts of the polypeptide molecule on the protons of the marker could be measured. One lies at high fields (in the region of tetramethylsilane), the other one at low fields (adjacent to the signals of the aromatic protons of phenylalanine, tryptophan, and histidine). The low-field "window" contains signals of some of the amide protons, but, as we shall see (Schwyzer and Ludescher, 1968), this is not very disturbing.

In the high-field range, ring-current shielding of aromatic nuclei on the protons of methyl groups of amino acid side chains has been observed (lysozyme: Sternlicht and Wilson, 1967).

The possible use of the low-field window for placing a conformational probe is the subject of this paper. Application of the resulting method to gramicidin S is treated separately (Schwyzer and Ludescher, 1968).

The reporter groups (markers) studied here are the benzoyl and the phthaloyl groups. They are easily introduced into natural and synthetic polypeptides, and their proton signals appear between ~7.5 and 8.0 ppm. Their protons are subject to ring-current shielding by phenyl groups (of polypeptide side chains). The benzoyl and the phthaloyl rings, conversely, cause shielding of the protons of the phenyl rings with which they are in close contact. Both effects can be used to analyze the relative positions in space of the marker and the side-chain aromatic group. This is facilitated by the fact that the C3,6 and the C4,5 protons of the phthaloyl group, as well as the *ortho* and *meta,para* protons of the benzoyl group, give rise to distinct signals, and the shielding effect on these various groups of proton can be measured independently.

Simple model compounds used in this work demonstrate the magnitude of the proximity effects and their dependence on the spatial relationship of the interacting ring system. It is concluded that the phthaloyl group is better from a practical point of view than the benzoyl group. Certainly, other groups could also be used (see R. Schwyzer, B. Donzel, E. Fischer, and U. Ludescher, in preparation).

Nuclear magnetic resonance markers promise to be a valuable new tool for the recognition of specific side-chain interactions involving phenylalanine and other aromatic amino acids in polypeptides. We have already discussed the significance of defining side-chain interactions to the study of conformations in solution (Carrión *et al.*, 1967, 1968; see also Schwyzer, 1968).

Materials and Methods

N-Benzoyl-*trans*-2-phenylcyclopentylamine (I, racemic, mp 154°), *N*-benzoyl-*cis*-2-phenylcyclopentylamine (II, racemic, mp 112°), *N*-phthaloyl-L-phenylalanine methyl ester (III), and *N*-phthaloyl-*cis*-2-phenylcyclopentylamine (IV, racemic, mp 101°) were prepared according to R. Schwyzer, B. Donzel, E. Fischer, and U. Ludescher (in preparation).

Proton nuclear magnetic resonance spectra were de-

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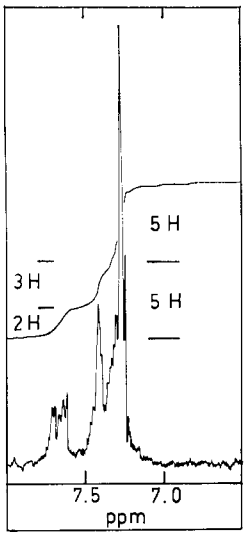
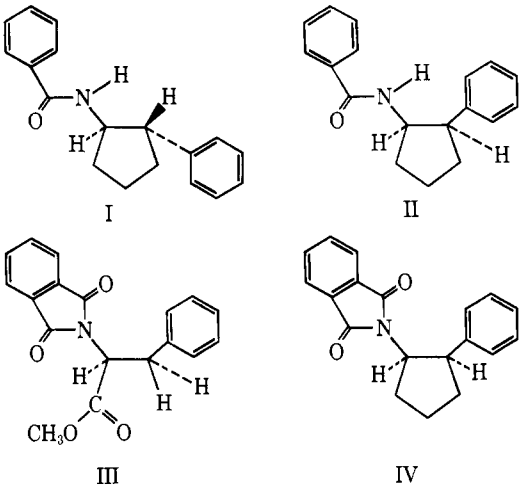


FIGURE 1: Proton nuclear magnetic resonance spectrum (between 6.5 and 8.0 ppm at 100 Mc) of *N*-benzoyl-*trans*-2-phenylcyclopentylamine (I) in $CDCl_3$. Integration tracing included.



termined at either 100 or 60 MHz on Varian instruments with deuterated chloroform ($CDCl_3$) as solvent and tetramethylsilane as zero reference. Chemical shifts of various compounds (benzene, benzamide, and *N*-phthaloyl-2-bromoethylamine) were taken from the literature (Bhacca *et al.*, 1962; Mathieson, 1965).

Results

N-Benzoyl-trans-2-phenylcyclopentylamine (I). The proton nuclear magnetic resonance spectrum in the region of the aromatic protons is shown in Figure 1 (the amide proton causes a peak outside of this area at 6.08 ppm). Two protons of the benzoyl group (*ortho*) appear in a composite signal centered at 7.67 ppm. Whether the apparent splitting of the signal into two groups centered at 7.71 and 7.63 ppm is due to preferential aromatic shielding or to coupling was not investigated. The three remaining protons (*meta, para*) of the benzoyl group show up at 7.42 ppm. This represents upfield shifts of the two *ortho* protons of 0.10 and of the three

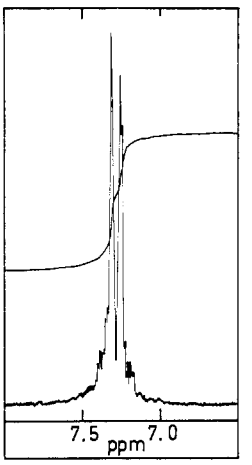


FIGURE 2: Proton nuclear magnetic resonance spectrum (between 6.5 and 8.0 ppm at 100 Mc) of *N*-benzoyl-*cis*-2-phenylcyclopentylamine (II) in $CDCl_3$. Integration tracing included.

meta,para protons of 0.05 ppm against the standard position in benzamide. The five protons of the phenyl group signalize at 7.28 ppm, approximately at the same place as benzene protons.

N-Benzoyl-cis-2-phenylcyclopentylamine (II). Figure 2 shows the region of the aromatic protons in the nuclear magnetic resonance spectrum of this compound (the amide proton appears at 5.62 ppm, an upfield shift of 0.46 ppm against the *trans* isomer). All five protons of the benzoyl group now show up in a single peak at 7.31 ppm. This represents an upfield shift of the *ortho* protons of 0.46 ppm and of the *meta,para* protons of 0.16 ppm. The phenyl protons appear at 7.26 ppm, only very slightly shifted against benzene and the *trans* isomer (Figure 3).

N-Phthaloyl-L-phenylalanine Methyl Ester (III). Figure 4 shows the region of the four phthaloyl protons, the five phenyl protons, and the one α-carbon proton. The peak of the α-carbon proton at $\sim$5.17 ppm is split into four

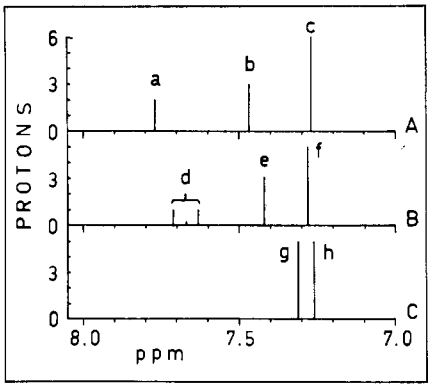


FIGURE 3: Comparison of line positions and number of protons in the proton nuclear magnetic resonance spectra. (A) Of benzamide (two *ortho* protons, a; three *meta,para* protons, b) and benzene (c). (B) Of *N*-benzoyl-*trans*-2-phenylcyclopentylamine (two *ortho*, d, and three *meta,para*, e, protons of the benzoyl group; five protons, f, of the phenyl group). (C) Of *N*-benzoyl-*cis*-2-phenylcyclopentylamine (five protons, g, of the benzoyl and five protons, h, of the phenyl groups).

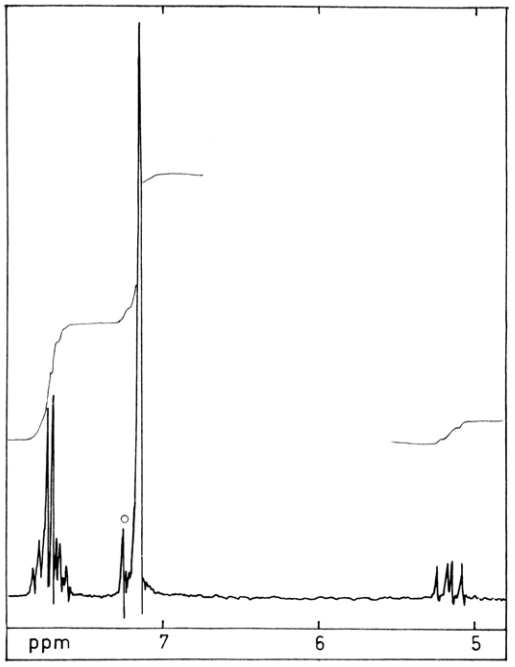


FIGURE 4: Proton nuclear magnetic resonance spectrum (between ~5 and 8 ppm at 100 Mc) of *N*-phthaloyl-L-phenylalanine methyl ester (III) in $CDCl_3$ (signal marked O is $CHCl_3$). Integration tracing included.

peaks with coupling constants of $J_{12} \simeq 6.2$ cps and J_{13} of ~9.0 cps. The phenyl protons appear at 7.16 ppm (the small peak of ~0.5 proton at 7.27 ppm is due to traces of $CHCl_3$ in the solvent, $CDCl_3$), and the phthaloyl protons in two peaks at 7.74 ppm (two "a" protons at carbon atoms 3 and 6) and at 7.70 ppm (two "b" protons at carbon atoms 4 and 5). This represents upfield shifts of the phenyl protons of 0.11 ppm and of the phthaloyl protons of 0.13 ppm (equal for the a and b protons) against the standards 2-bromoethylphthalimide and benzene.

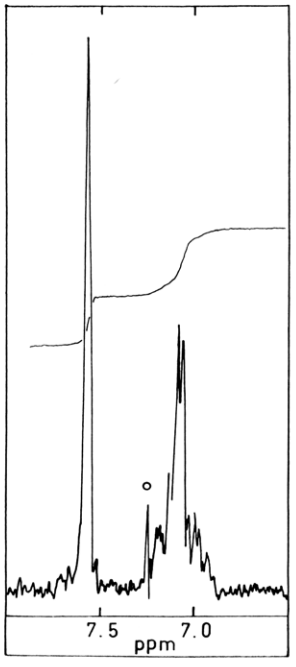


FIGURE 5: Proton nuclear magnetic resonance spectrum (between 6.5 and 8.0 ppm at 60 Mc) of *N*-phthaloyl-*cis*-2-phenylcyclopentylamine (IV) in $CDCl_3$ (signal marked O is $CHCl_3$). Integration tracing included.

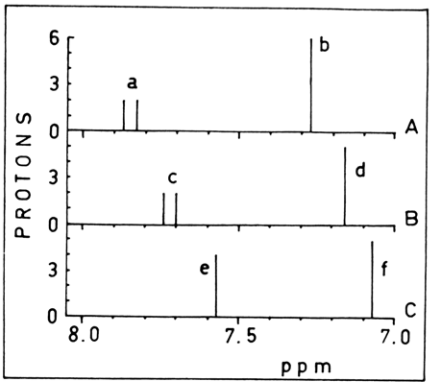


FIGURE 6: Comparison of line positions and number of protons. (A) In phthalimide (a) and benzene (b). (B) In *N*-phthaloyl-L-phenylalanine methyl ester (III; phthaloyl protons, c, phenyl protons, d). (C) In *N*-phthaloyl-*cis*-2-phenylcyclopentylamine (IV: phthaloyl protons, e, and phenyl protons, f).

N-Phthaloyl-cis-2-phenylcyclopentylamine (*IV*). Figure 5 shows the region of the five phenyl protons and the four phthaloyl protons. The phenyl proton peak is rather broad and split into a multitude of signals (7.25 ppm is $CHCl_3$). It is centered at 7.07 ppm. The separation of the two main peaks is 0.03 ppm; we did not investigate whether this is due to coupling or to preferential aromatic shielding of certain protons over others. The phthaloyl protons appear in a single peak at 7.57 ppm. The upfield shifts are 0.30 and 0.26 ppm for the phthaloyl a and b protons, respectively, and on the average 0.20 ppm for the phenyl protons (Figure 6).

Discussion

The Benzoyl System. Proximity of the phenyl ring to the benzamide group in I and II causes upfield shifts of the benzoyl protons. The shifts are not very conspicuous in the *trans* I, but are dramatic in the *cis* II where the signals of the *ortho*, *meta*, and *para* protons coalesce to one very sharp line (Figures 1–3).

These upfield shifts can be explained by shielding due to ring currents in the phenyl ring at position 2 of the cyclopentyl system. Because of the relative flexibility and rotability of the phenyl and benzamide groups in I and

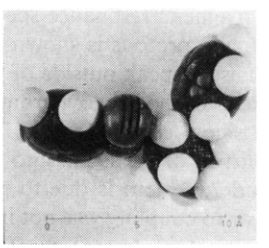


FIGURE 7: Ealing CPK model of *N*-benzoyl-*trans*-2-phenylcyclopentylamine (I).

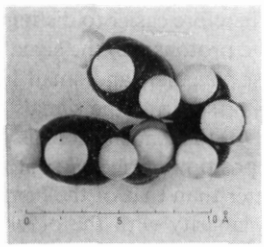


FIGURE 8: Ealing CPK model of *N*-benzoyl-*cis*-2-phenylcyclopentylamine (II).

II, calculations of the magnitude of ring-current shielding (according to Johnson and Bovey, 1958; Appendix B in Emsley *et al.*, 1965) are subject to great uncertainties in the choice of parameters and, subsequently, to large errors. In the Ealing model of I, shown in Figure 7, slight deformation of the amide bond from planarity brings the *ortho* protons to a distance from the phenyl ring of $z \simeq 3.5$ ring radii and $p \simeq 3.0$ ring radii (the z axis is normal to the plane of the phenyl ring at its center; p is the distance of the protons from the z axis). These parameters give a calculated shielding effect on the *ortho* protons of -0.077 ppm (found 0.10 ppm). The effect on the *meta* and *para* protons ($z \simeq 5.0$ and 5.5, $p \simeq 2.4$ and 1.5 ring radii, respectively) was not calculated in detail, but must be very small (found 0.05 ppm). Semiquantitatively, we can say that the stronger shielding of the *ortho* protons compared with that of the *meta* and *para* protons is to be expected from the model, and that it is in the right order of magnitude. As ring-current shielding is an average effect, taking into account the relative lifetimes of all populated conformers, it seems as though the one shown in Figure 7 would represent a kind of an "average" conformer.

In the *cis* II (Figure 8), the *ortho* protons of the benzoyl ring are again closer to the center of the phenyl ring ($z \simeq 2.5$, $p \simeq 1.5$ ring radii) than the *meta* and *para* protons (mean $z \simeq 3.1$, mean $p \simeq 2.3$ ring radii). Attaching great caution to the upfield shifts calculated from these values ($\sim$0.50 ppm for the *ortho*, $\sim$0.17 ppm for the *meta,para* protons) we can again say that the model accounts semiquantitatively for the order of magnitude of the ring-current effects (found 0.46 and 0.16 ppm, respectively), and, consequently, for the fact that all benzoyl protons appear in the same peak (Figures 2 and 3).

In the *cis* II, we would also expect an influence of the phenyl ring current on the amide proton. In the model (Figure 8), we can measure the parameters, $z \simeq 1.5$, and $p \simeq 1.5$–2.0 ring radii. At small values of z, the shielding is very sensitive to slight variations in p, hence, the calculated effects range from -0.85 to -0.065 ppm (found 0.46 ppm).

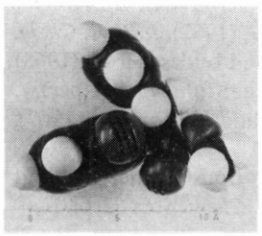


FIGURE 9: Ealing CPK model of the eclipsed, "interacting" conformation of *N*-phthaloyl-L-phenylalanine methyl ester (III).

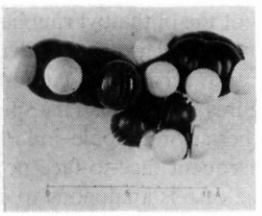


FIGURE 10: Ealing CPK model of the staggered, noninteracting conformation of *N*-phthaloyl-L-phenylalanine methyl ester (III).

The influence of the ring current of the benzoyl ring on the protons of the phenyl ring seems to be negligible, when compared with the phthaloyl system. We are at loss to offer an explanation, although one could argue that the conformers with greatest lifetime are not those depicted in Figures 7 and 8, but those in which the benzoyl group is rotated to positions perpendicular to the ones shown. If the rotation were rapid and the stabilities of the various "perpendicular" rotamers not too different from one another, the shielding effects on the benzoyl protons would not be expected to differ much from those calculated from Figures 7 and 8 (this is another way of saying that the conformers of Figures 7 and 8 represent "average" conformations). However, the induced magnetic field emanating from the benzoyl rings would be pointing away from the phenyl protons most of the time, and, hence, its influence on these protons would be very small.

The Phthaloyl System. In contrast to the benzoyl system, we can observe upfield shifts of both the phthaloyl *and* the phenyl protons in *N*-phthaloyl-L-phenylalanine methyl ester (III) and in *N*-phthaloyl-*cis*-phenylcyclopentylamine (IV). As would be expected, the effect is especially great in the compound with fixed *cis* configuration (IV), where the originally separated peaks of the two pairs of phthaloyl protons, a and b, coalesce to one sharp signal (Figures 4–6).

Upfield shifts of *both* the phthaloyl and the phenyl protons suggest a preferred face-to-face orientation of the two ring systems in III and IV (Figures 9–11). This would allow for the mutual interaction of ring-current effects due to the phthaloyl and the phenyl rings. The situation is somewhat similar to the one in paracyclophanes (Wilson *et al.*, 1960), although the planes of the rings, in our cases, are not arranged parallel, but at an angle ($\sim$40°) to one another. The opposed dipoles of the two

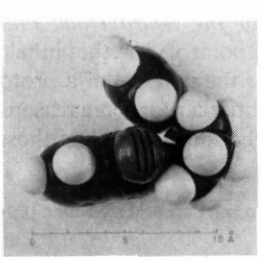


FIGURE 11: Ealing CPK model of *N*-phthaloyl-*cis*-2-phenylcyclopentylamine (IV).

carbonyl groups of the phthaloyl ring lie almost parallel to the plane of the phenyl group, and their negative ends point outward and across the circumference of the phenyl ring (Figures 9 and 11). This special situation is favorable to rather strong dipole–induced dipole interactions and consequent face-to-face positioning of the ring systems. The case is analogous to certain intermolecular and other intramolecular ones (Schneider, 1962; Hatton and Schneider, 1962; Kopple and Marr, 1967).

Although the observed splitting of the signal of the proton on the α carbon in *N*-phthaloyl-L-phenylalanine methyl ester (III, Figure 4) allows a rough calculation of the residence times of the molecules in the three most stable conformations to be carried out according to Pachler (1964), we have not attempted to correlate ring-current effects with the dynamic model obtained in this way. We wish to point out, however, that the (rather improbable) eclipsed conformer (Figure 9) and the two adjacent staggered ones (torsion around the C^{α}–C^{β} bond of $\sim\pm 60°$) constitute a family of conformers which allow for mutual ring-current and charge-transfer effects. The third staggered conformer (Figure 10) is not expected to give (intramolecular) interactions between the aromatic groups. With coupling constants obtained in this work, the standardized residence times (Pachler, 1964) of the interacting and the noninteracting species turn out to be $\sim$0.67 and $\sim$0.33, respectively. Similar results are obtained for nitrophthaloylamino acids; they are confirmed by the charge-transfer behavior of such compounds (R. Schwyzer, B. Donzel, E. Fischer, and U. Ludescher, in preparation). Whatever the situation, the ring-current shielding should be smaller than in the fixed molecule, IV, as is confirmed (Figure 6).

The model of IV (Figure 11) is probably better related to reality than the other models, because the dipole–induced dipole effect should be quite strong (the rather broad signal of the phenyl protons (Figure 5) might even be indicative of restricted rotation of the phenyl group). Calculations of shielding effects on the phthaloyl protons were carried out using parameters obtained from Ealing CPK[1] models ($z \simeq 3.55$ and 4.2, $p \simeq 1.5$ and 0.7 ring radii for the a and b protons, respectively) and from Dreiding models ($z \simeq 2.7$ and 3.4, $p \simeq 1.8$ and 1.8 ring radii for the same). The calculated shielding effects on the a and b protons are thus ~ -0.28 and ~ -0.24 ppm (Ealing), and ~ -0.33 and ~ -0.26 ppm (Dreiding). These values are very close to the observed ones: $-$ 0.30 ppm for the a and $-$ 0.26 ppm for the b protons. The relative positions in space of the phthalimide and phenyl rings are thus obviously responsible for the coalescence of proton signals a and b.

Comparison of the Benzoyl and Phthaloyl Systems. From a practical point of view, the phthaloyl system seems better suited for the purpose of a proton nuclear magnetic resonance marker for three reasons: (1) the signals are located further downfield than those of the benzoyl group, and are therefore easier to distinguish from those of other aromatic protons; (2) the signals of the a and b protons are close together, and small influences which tend to coalesce or separate the signals are more easily recognized; and (3) the tendency of the phthaloyl group to influence the position of phenyl proton signals seems to be much greater than that of the benzoyl group (due to preferred coplanarity with the other phenyl ring?). Points two and three are especially important for analyzing special situations of side-chain interaction. Thus, in diphthaloylgramicidin S, the signals of the a and b protons are further separated than usual, indicating that, contrary to the case studied in this piece of work, the b protons are closer to the shielding phenyl ring than the a protons. Probably because of this situation, two of the five phenyl protons are shielded more than the remaining three by the "off-center" phthaloyl group and appear at higher fields (Schwyzer and Ludescher, 1968).

[1] The Ealing Corp., Cambridge, Mass. 02140.

Acknowledgment

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