# A CONFORMATIONAL STUDY OF THE OPIOID PEPTIDE DERMORPHIN BY ONE-DIMENSIONAL AND TWO-DIMENSIONAL NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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ABSTRACT Dermorphin, a natural peptide opioid containing a D-Ala$^2$ residue, has been studied in dimethyl sulfoxide (DMSO) solution by means of several one-dimensional and two-dimensional $^1$H nuclear magnetic resonance (NMR) methods at various fields from 80 to 600 MHz. The combined use of conventional NMR parameters and of nuclear Overhauser effect effects points to an essentially extended structure. This conformation may be, in part, the result of strong interaction of the amide groups with DMSO molecules.

## INTRODUCTION

Since the discovery of the enkephalins in 1975 (1), many different endogenous peptides with opioid properties have been found in living organisms. These compounds interact with several different receptor sites ($\mu$, $\delta$, $\chi$, and $\sigma$) (2) and are often many times more active than morphine and other exogenous opioids. Moreover, in spite of the serious difficulty posed by their conformational flexibility in the study of substrate-receptor interactions, they can be quite a useful source of complementary information with respect to their alkaloid counterparts. In 1981 a heptapeptide opioid, dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-$NH_2$), was isolated from the skin of South American frogs (3, 4). Its preferential receptor site is the $\mu$ site, like morphine, but its activity is by far much higher, ~2,000 times more than morphine. The presence of a D-amino acid, quite unusual for a biological substance of nonbacterial origin, seems to be fundamental for its activity because its L-Ala$^2$ analogue is virtually devoid of analgesic activity (5). Here we wish to extend our preliminary studies (6, 7) with a major emphasis on the dynamic behavior in solution of DMSO. In fact, although this medium is far from the likely biological environment in which the substrate-receptor interaction takes place, it is one of the best solvents for a preliminary delimitation of the conformational space and its flexibility in it. Therefore this paper moves in two different but complementary directions: (*a*) a comparison between dermorphin and its less active, synthetic, L-analogue; (*b*) a careful study of the conformation in solution of the former.

## METHODS

The hydrochloride salt of dermorphin and the trifluoroacetic salt of its L-analogue were prepared by conventional methods in solution as previously described (8). All solutions were obtained by dissolving the appropriate amount in ~0.500 ml of dimethyl sulfoxide (DMSO-$d_6$). Concentrations ~10 mM and 0.300 ml of solution were used for nuclear magnetic resonance (NMR) measurements in a 5 mm OD tube. NMR spectra were recorded at different fields on a 200 Nicolet spectrometer (Nicolet Instrument Corp., Madison, WI), on 80, 270, 400, and 500 Bruker spectrometer (Bruker Instruments, Inc., Billerica, MA) and on a 600 MHz homebuilt spectrometer of the Carnegie-Mellon Institute (Pittsburgh, PA). The use of different higher fields was suggested from considerable overlap of peaks. Moreover, as previously described (9) on the dodecapeptide valinomycin for molecules of appropriate size, dipolar and scalar mechanisms may be distinguished by the frequency dependence of the nuclear Overhauser effect (NOE). All peaks were referred to the DMSO resonance arbitrarily located at 2.5 ppm and all spectra were run at a temperature of 298°K. For one-dimensional experiments 16 or 32 K data points were used for acquisition and 32 or 64 K, respectively, for transformation. NOE difference spectra were determined by applying a 1.5-s low-power saturating pulse at appropriate peak position, followed immediately by a high power 90° observing pulse (10). Classical sequences for COSY and NOESY experiments were used (11, 12).

## RESULTS AND DISCUSSION

The previous assigments (6, 7) were confirmed by two-dimensional-COSY and one-dimensional and two-dimensional NOE's experiments at high fields. The chemical shifts and coupling constants are shown in Table I. The 400 MHz spectra of native and L-Ala$^2$ dermorphins are shown in Fig. 1. A representative two-dimensional spectrum is shown in Fig. 2.

### Conformational Information from Chemical Shifts

Ring current shifts were suspected in dermorphin owing to the presence of three aromatic residues in the sequence. Indeed, a comparison of the chemical shifts of the alanine methyl group in the two dermorphins with each other and

TABLE I
COMPARISON BETWEEN DATA IN THE LITERATURE (13) OF MODEL PEPTIDES AND CHEMICAL SHIFTS AT 400 MHz OF THE DEMORPHIN AND ITS L-Ala$^2$ ANALOGUE*

| | Tyr | Ala | Phe | Gly | Tyr | Ser |
|---|---|---|---|---|---|---|
| N-H | | | | | | |
| Standard | — | 8.04 | 8.14 | 8.15 | 8.04 | 7.98 |
| D-Epta | 8.11 | 8.38 | 8.39 | 8.29 | 8.31 | 7.76 |
| L-Epta | 7.95 | 8.58 | 8.18 | 8.21 | 8.58 | 7.72 |
| $C_\alpha H$ | | | | | | |
| Standard | — | 4.34 | 4.56 | 3.76<br>3.76 | 4.46 | — |
| D-Epta | 3.96 | 4.30 | 4.56 | 3.57<br>3.81 | 4.60 | — |
| L-Epta | 3.93 | 4.36 | 4.62 | 3.60<br>3.76 | 4.54 | — |
| $C_\beta H_2$ | | | | | | |
| Standard | — | 1.22 | 2.75<br>3.03 | — | 2.64<br>2.91 | — |
| D-Epta | 2.86 | 0.74 | 2.72<br>3.02 | — | 2.65<br>2.97 | — |
| L-Epta | 2.82<br>2.92 | 1.20 | 3.71<br>3.03 | — | 2.62<br>2.92 | — |

*The L-Ala$^2$ analogue refers to the DMSO-$d_6$ resonance arbitrarily located at 2.50 ppm.

with standard alanine methyl chemical shifts (13) reveals an upfield shift of the D-Ala$^2$ methyl protons by 0.5 ppm. The corresponding L-Ala derivative does not exhibit any relevant shift. Proof that this shift is due to the Phe$^3$ ring current and to the Tyr$^1$ ring current was obtained from specific NOE between the methyl and aromatic protons. Further confirmation came from the rather high temperature coefficient of the D-Ala$^2$-$CH_3$ chemical shift (0.0035 ppm/°C). The downfield shift observed with increasing temperature indicates that the $\chi$ rotamers causing the anomalous shift have higher than average populations. These data are therefore consistent with the hypothesis that the D-Ala$^2$ methyl group is located between the two aromatic rings and with its hydrogens placed <3 Å from each ring (14), thus imposing severe restrictions on the backbone ($\phi$,$\psi$) angles and on the $\chi$ rotamer populations of residues 1 and 3. A comparative analysis of all chemical shift data (Table I) of the two dermorphins reveals that, although the dramatic difference observed for the alanine methyl groups is a singular data, there are many small but significant differences that point to the existence of different conformational states in solution for the two compounds. For instance the chemical shift of the D-Ala NH is shifted by 0.2 ppm with respect to the corresponding L-Ala

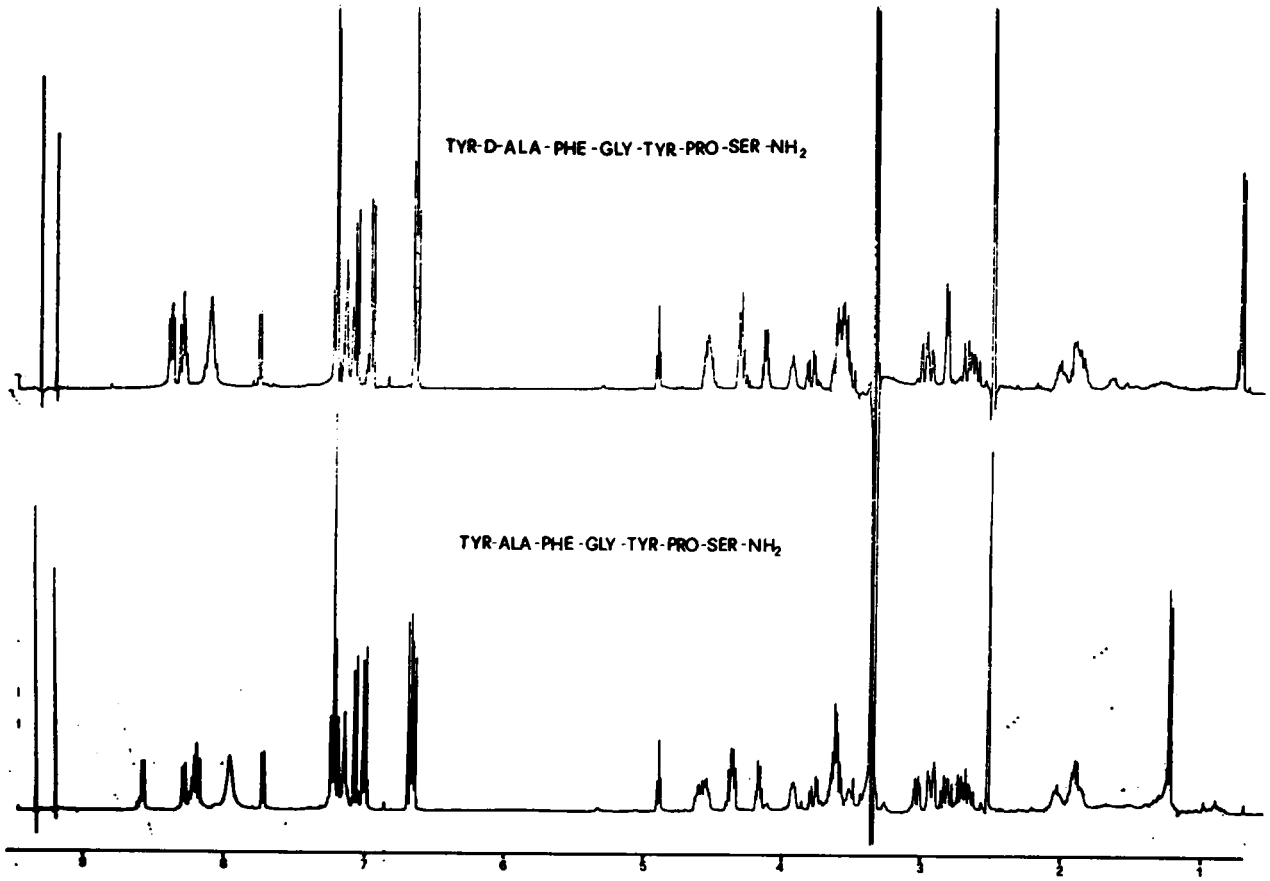



FIGURE 1 $^1$H NMR spectra of dermorphin and its L-Ala$^2$ analogue (*top*) in DMSO-$d_6$ at 400 MHz.

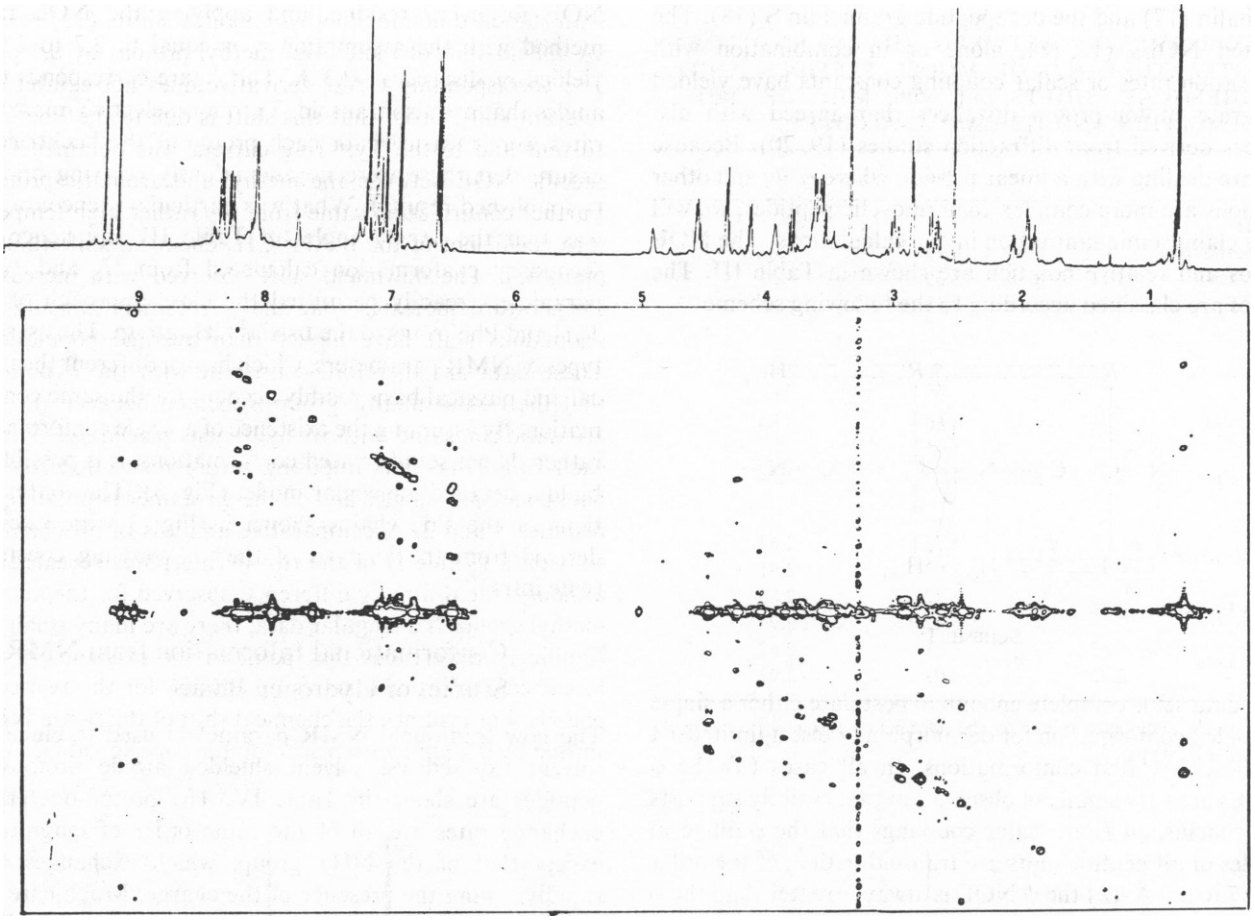



FIGURE 2 600 MHz NOESY spectrum of dermorphin (10 mM) at 298°K.

value and by 0.54 ppm with respect to values given in the literature for random coil conformations. A similar behavior is also exhibited by the $\delta_{NH}$ of Phe (3). In view of the extreme similarity of constitution of the two peptides (the only difference being in the chirality of the second residue), any experimental data that may account for their different biological activity is of potential diagnostic value.

## Conformational Information from Scalar $^3J_\phi$ Values

All $^3J_\phi$ values reported in Table II fall in the range 7.9 to 8.8 Hz (corresponding to 8.7–9.6 Hz if the correction for electronegativity is considered). At this stage it is not yet possible to discriminate between single $\phi$ angles and appropriate averages over all accessible $\phi$ angles. At any rate, the very high values found experimentally automatically exclude fourfold degeneracies essentially yielding pairs of likely angles that correspond to a rather narrow range of $r_\phi$ distances (averaged or single), i.e., to values comprised between 2.7 and 3.0 Å (15). All possible pairs of $\phi$ values derived from the Karplus relationship are shown in Table II.

## Conformational Information from $\phi$ and $\psi$ NOEs

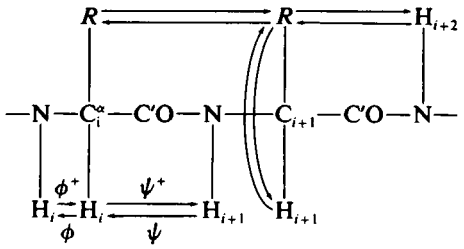
No significant NOEs were detected at 80 MHz but they became negative and of progressively increasing magnitude at 270, 400, 500, and 600 MHz. This implies that the correlation time in DMSO-$d_6$ at 25°C is $\sim 10^{-9}$ s (16), a value, as expected, that is intermediate between those observed, in the same solvent, for the pentapeptide meten-

TABLE II
$^3J_{HNCH}$ COUPLING CONSTANTS OF DERMORPHIN AND THE CORRELATED Θ AND Φ ANGLES*

| | $^3J_{HNCH}$ | $^3J^{corr}_{HNCH}$ | Θ | Φ |
|---|---|---|---|---|
| | *Hz* | *Hz* | | |
| D-Ala | 7.9 | 8.7 | 152° | 92°, 148° |
| Phe | 8.8 | 8.9 | 153° | −93°, −147° |
| Gly | 5.7 | 5.9 | ±60° | — |
| | 6.0 | 6.2 | ±128 | — |
| Tyr | 8.1 | 9.6 | 159° | −141°, −99° |
| Ser | 7.9 | 8.5 | 150° | −150°, −90° |
| | — | — | 9° | 69°, 51° |

*$^3J^{corr}_{HNCH}$ are corrected by the electronegativity factor (26).

kephalin (17) and the decapeptide gramicidin S (18). The quoted NOEs (17, 18), alone or in combination with relaxation rates or scalar coupling constants have yielded accurate proton-proton distances that agreed with distances derived from diffraction studies (19, 20). Because we are dealing with a linear peptide where $\phi$, $\psi$, and other motions are more complex than in cyclic peptides, we will only claim semiquantitation in our calculations. The NOE ratios and relative notation are shown in Table III. The ratios are classified according to the following scheme:



SCHEME I

The data set is complete enough to postulate either a single extended conformation for dermorphin or else a limited set of closely related conformations. In all cases (*a*) the $\phi$ NOEs are very small, an observation that entirely supports the conclusions from scalar couplings that the $\phi$ dihedral angles of all peptide units are transoid with $r_\phi$ of the order of 2.7 to 2.9 Å; (*b*) the $\psi$ NOE is always greater than the $\phi$ NOE for every residue, and applying the NOE ratio method with the assumption $r_\phi$ is equal to 2.7 to 2.9 Å yielded $r\psi$ distances ~2.3 Å. This figure corresponds to $\psi$ angles that are also transoid. The monoselective relaxation rates were measured for each proton of the backbone to ensure that there were no anomalously relaxing protons (unpublished results). What was particularly encouraging was that the ($\phi$, $\psi$) angles of Table III and hence the secondary conformation calculated from $^3J_\phi$ and NOE parameters readily permitted the close approach of the Tyr[1] and Phe[3] rings to the D-Ala[2] $CH_3$ group. Thus several types of NMR parameters, which have a different theoretical and physical basis readily account for the same conformation. By assuming the existence of a single conformation rather than a set of related conformations, it is possible to build a detailed molecular model (Fig. 3). The conformations of the side chains shown in Fig. 3 were likewise derived from the analysis of the $^3J_x$ coupling constants (vide infra).

TABLE III
PROTON-PROTON NUCLEAR OVERHAUSER EFFECT MEASUREMENTS AT 500 MHz

| Irradiated proton | Observed proton | Actual NOE* |
|---|---|---|
| | | % |
| Tyr[1] $NH_3^+$ | Tyr[1] $C_\alpha H$ | 3 |
| | Tyr[1] $C_\beta H_2$ | 4 |
| | Ala[2] $C_\alpha H$ | 19 |
| D-Ala[2] NH and Phe[3] NH | Ala[2] $CH_3$ | 2 |
| | Phe[3] $C_\alpha H$ | 5 |
| | Tyr[1] $C_\alpha H$ | 9 |
| | Tyr[5] $C_\alpha H$ | 13 |
| Gly[4] and Tyr[5] NH | Gly[4] $C_\alpha H_u$ | 4 |
| | Gly[4] $C_\alpha H_d$ | 5 |
| Ser[7] NH | Ser[7] $C_\alpha H$ | 10 |
| | Pro[6] $C_\alpha H$ | 12 |
| Tyr[1] $C_\alpha H$ | Ala[2] NH | 22 |
| | Tyr[1] NH | 7 |
| | Phe[3] NH | 18 |
| D-Ala[2] $C_\alpha H$ and Pro[6] $C_\alpha H$ | Ala[2] NH | 0 |
| | Tyr[1] $NH_3$ | 4 |
| | Ser[7] NH | 6 |
| | Pro[6] $C_\beta H_2$ | 2 |
| Tyr[5] $C_\alpha H$ and Phe[3] $C_\alpha H$ | Tyr[5] NH + Gly[4] NH | 19 |
| | Phe[3] NH + Ala[2] NH | 15 |
| Ser[7] $C_\alpha H$ | Ser NH | 4 |

*The NOE effects were calculated as indicated in reference 27 and are classified according to Scheme I given in the text.

## Conformational Information from NMR Studies of Hydrogen Bonds

The now traditional NMR parameters used to elucidate solvent exposed vs. solvent shielded amide protons in peptides are shown in Table IV. The proton-deuterium exchange rates are all of the same order of magnitude, except that of the $NH_3^+$ group, which exchanges very rapidly, owing the presence of the charge. Much care has to be taken to interpret these measurements because little is known about the effect of DMSO on the amide-water hydrogen exchange (21, 22). Furthermore, absolute rates of exchange depend critically on the pH and on the amount of water present; also the maximum of care must be taken to exclude traces of metal ions, which can greatly affect proton exchange rates. Our data seem to indicate that all amide protons have similar local conformations. The $\Delta\delta/\Delta T$ coefficients are neither very small nor very large and are again consistent with a similar environment for each.

TABLE IV
TEMPERATURE COEFFICIENTS ($\partial\Delta/\partial T$) OF THE AMIDE PROTONS OF DERMORPHIN AND ITS L-Ala[2] ANALOGUE IN THE RANGE 297–325°K AND PROTON-DEUTERIUM EXCHANGE RATES OF DERMORPHIN ADDING 2% $D_2O$ IN DMSO-$d_6$

| | L-Epta $\Delta\partial/\Delta T$ | D-Epta $\Delta\partial/\Delta T$ | $\tau^1/_2$ |
|---|---|---|---|
| | *ppb/c** | | *h* |
| Tyr[1] | −2.3 | −2.3 | 5[m] |
| Ala[2] | −5.7 | −5.2 | 3.0 |
| Phe[3] | (−7.7) | −8.1 | 3.3 |
| Gly[4] | (−7.7) | (−10) | 4.0 |
| Tyr[5] | −8.8 | (−10) | 4.2 |
| Ser[7] | −6.0 | −6.2 | 2.2 |

Temperature was also varied from 297 to 325°K during the exchange in order to increase the rates.

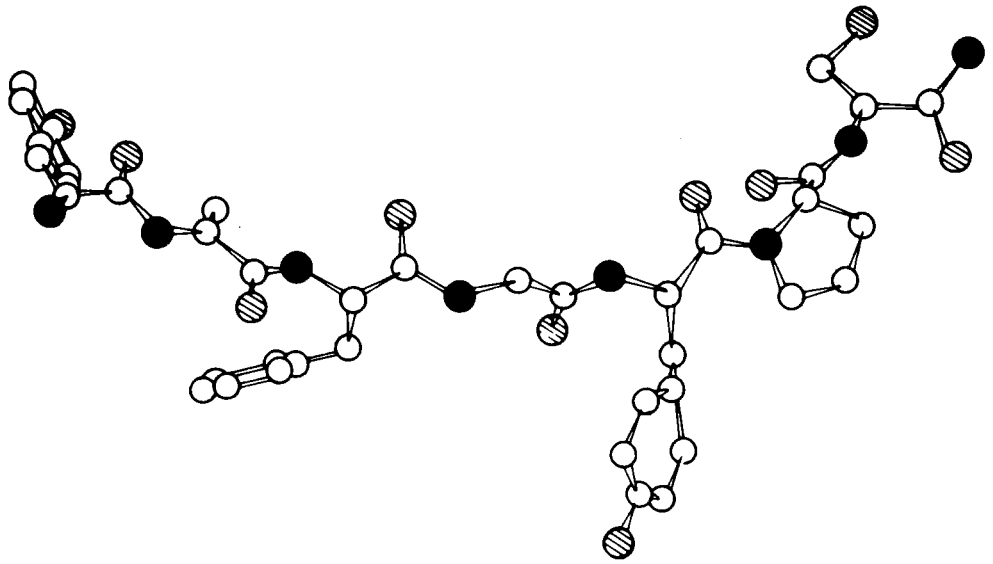


FIGURE 3 One of several conformations for dermorphin that fits all the NMR data. The $\psi$ angles are transoid, the $\phi$ angles are about $-150°$ ($+150°$ for D-Ala), and the $\chi$ angles are consistent with the value of the higher population (*trans*). ○, C; ●, N; ○, O.

The vast majority of model peptides used to establish relationships between $\Delta\delta/\Delta T$ and solvent shielding were cyclic and contained $\beta$ sheet, $\beta$ turn, and $\gamma$ turn moieties (23, 24). An insufficient number of model linear peptides of defined conformation have been studied. It is therefore premature at this stage to speculate that this data for dermorphin can distinguish between the many different types of helix or extended structures that fit all our data. What is clear is that it supports a regular structure that is not random coil. The other NMR data clearly delineate that the conformation is linear and extended with all $\phi$ and $\psi$ angles transoid.

## Conformational Information from Side-Chain Populations

The populations of some of the side chains of both D- and L-Ala dermorphin were calculated by means of the well-known equations of Pachler (25) and are reported in Table V. Conformers I, II, and III correspond to conformations in which the R group of a $-CH_2R$ side chain is *trans* to the following carbonyl, *gauche* to the same carbonyl or *gauche* to both the following carbonyl and to the preceding NH, respectively. The figures for Tyr[1] of the D-Ala[2] peptide are missing since the ABX system of this side chain yields a deceptively simple spectrum. Conformer I is always the most populated one in all residues but note that the distribution among the three conformers is more uniform for the side chains of the L-Ala[2] analogue, whereas the distribution for the side chains of the natural peptide is suggestive of the prevalence of a single conformer. These data support once again the hypothesis of a smaller number of global conformations of the D-analogue with respect to the L.

TABLE V
FRACTIONAL POPULATIONS OF SIDE CHAINS FOR SOME RESIDUES OF DERMORPHIN AND ITS L-Ala[2] ANALOGUE

| Conformer | [1]Tyr | | [3]Phe | | [5]Tyr | |
|---|---|---|---|---|---|---|
| | D-Epta | L-Epta | D-Epta | L-Epta | D-Epta | L-Epta |
| I | — | 56.6 | 68.1 | 54.0 | 68.5 | 56.9 |
| II | — | 24.6 | 15.2 | 19.4 | 14.7 | 19.4 |
| III | — | 18.8 | 16.7 | 26.6 | 16.8 | 23.7 |

The values are calculated according to Pachler (25).

## CONCLUSION

The solution structure of dermorphin has been studied in great detail with the aid of all possible $^1H$ NMR methods. Although it may be difficult to describe accurately the conformation of a linear heptapeptide in solution, this attempt was justified by several reasons. For instance, the clear $\mu$ activity of this opioid makes a comparison of its conformation(s) with the structure of rigid alkaloid opiates potentially more useful than in the case of enkephalins, whose selectivity is rather toward the $\delta$ receptor. Most of all, observation of clear NOE effects at several fields can substantiate all other NMR data, whereas in the case of all linear peptide opioids previously studied these effects were barely detectable owing to the extreme narrowing limit condition imposed by the lower molecular weight. The combined use of conventional NMR parameters and of NOE effects delineates, for dermorphin in DMSO, an essentially extended structure that might be the result of the average among a limited number of structures in a small portion of conformational space. It is likely that this kind of global shape of dermorphin in solution be, in part, a

consequence of strong interactions of the NH groups with DMSO molecules. This doubt was not clarified by the $^2H$ exchange data and by the temperature dependence of the NH chemical shifts, but such a situation emphasizes once more the need for acquiring better reference data, in this solvent, with model peptides. Nonetheless it is fair to say that our structural data do give a very accurate description of local preferences, at least for single residues and for pairs of adjacent residues. Of particular importance for the structure-activity relationship is the arrangement of the first three residues, also considering the fact that the [L-Ala$^2$] analogue showed clearly different NMR parameters for these residues. In general, the comparative study of the two dermorphins, i.e., the natural (D-Ala$^2$) one and the synthetic (L-Ala$^2$) analogue suggests that the role of (D-Ala$^2$) in directing the conformational preferences of the natural peptide is central. In fact, all measured NMR parameters do indicate that the two dermorphins occupy distinct regions of the conformational space. It is not far fetching to suppose that it will be possible, in the very near future, to correlate these differences to the large difference of analgesic activity.

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