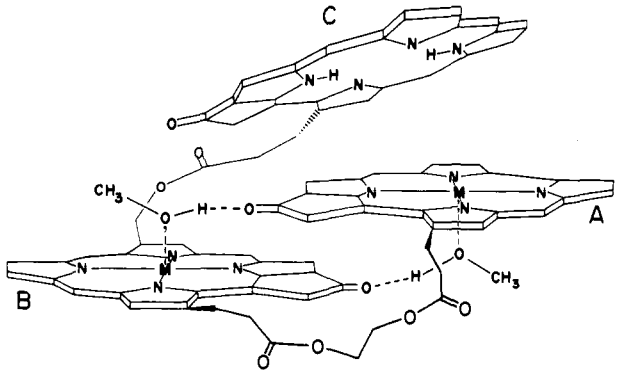

Figure 3. Schematic of average solution structure proposed for Mg_2 and Zn_2 model compounds in the presence of hydroxylic ligands which is consistent with all NMR data.

4a methylene protons are diastereotopic (AB part of an ABX_3 multiplet), with the largest chemical-shift difference in ring A,[19] consistent with their close proximity to two chiral centers in the reduced pyrrole ring of ring C. The distance from a point defined by the intersection of a line drawn between the metals and the C_2 axis of the A–B fragment and the center of ring C is estimated to be ~7–8 Å.[20] Though qualitatively similar the Mg and Zn compounds exhibit a substantial difference in the ease with which the folded form yields to displacement by pyridine. Whereas a 10^2-fold molar excess of pyridine fully disrupts the folded Zn model, a (2×10^3)-fold molar excess is required for the Mg model. We believe that the specificity of the observed interaction between ring C and the A–B fragment is stabilized by the transient substitution of a hydrogen bond between the carbonyl oxygen in ring C and the hydroxyl ligand for the hydrogen bond to the ring-B carbonyl, in concert with π–π interactions.

In contrast to all other porphyrin–protein interactions, where strong axial ligation to the central metal or direct covalent linkages position the macrocycle in combination with nonpolar interactions, there is no evidence for a covalent linkage between chlorophyll or pheophytin and reaction center proteins. Presumably, nonpolar interactions and weak coordination to the central magnesium atom (for the chlorophylls) play crucial roles in determining reaction center chromophore structure.[21] Therefore an analysis of ligand-mediated spontaneous self-assembly among components which are crucial to the primary photochemistry can provide a useful working model for their relationship in vivo. These molecules provide a model of defined structure which is amenable to study, and we are encouraged by the remarkable strength and specificity of the interactions. In a forthcoming paper, we will report a detailed analysis of the photochemistry and photophysics of these molecules[22] with particular emphasis on time-resolved optical absorption, emission, and ESR spectroscopy, which have been widely applied to in vivo systems, and extensions of the synthesis to bacterial chlorophylls and secondary electron acceptors.

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(10) Reduction of the conjugated aldehyde renders the absorption spectrum of ring B indistinguishable from that of ring A. As a consequence, all rings are chlorophyll a macrocycles, with minor differences in proton chemical shifts which are very useful for assigning individual protons.

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(13) (a) Purification: Zn, preparative silica TLC, 250 μ, 2:1 CCl_4–acetone, r_f = 0.28; Mg, preparative Florisil TLC, 1000 μ, 2:1 ethyl acetate–cyclohexane, r_f = 0.40. (b) Purification: same as for precursor; Zn, r_f = 0.52; Mg, r_f = 0.32.

(14) The methylene proton chemical shift in the linkage between the B and C rings is very sensitive both to esterification and the presence of metal in ring B, and comparison with simple models confirms that both conditions are met.

(15) Merck Isotopes Ltd.

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(17) In wet solvents, somewhat different chemical shifts are observed. Assignment of the spectrum and structure awaits further experimentation.

(18) The pattern of chemical-shift changes is independent of concentration, which rules out a significant contribution to the shifts from intermolecular aggregation.

(19) 4a methylene proton chemical-shift differences (parts per million): A-4a (0.12) > C-4a (0.09) > B-4a (0.07). Also it is noted that the B–C link methylene protons (position 3, ring B) become measureably diastereotopic (shift difference 0.20 ppm).

(20) To date there has been no direct experimental determination of the distance between the pair of chlorophylls and the primary acceptor in either bacterial or green plant photosynthesis. In bacterial reaction centers, the observed temperature independence of the upper limit for the formation time of the radical ion pair can be related to distance in the framework of the theory of electron tunneling, assuming a thermally relaxed precursor excited singlet state; cf. K. Peters, P. Avouris, and P. M. Rentzepis, *Biophys. J.*, **23**, 207 (1978). Though no distance is specified, the authors cautiously suggest that it is shorter than the distance between bacteriopheophytin and ubiquinone, estimated at 9–13 Å. Also, the apparently negligible electron–electron exchange coupling in the primary radical ion pair can be taken to indicate a substantial separation between donor and acceptor; cf. H.-J. Werner, K. Schulten, and A. Weller, *Biochim. Biophys. Acta*, **502**, 255 (1978).

(21) The existence of a high level of structure among bacterial reaction center chromophores has been elegantly demonstrated optically by A. Vermeglio and R. K. Clayton, *Biochim. Biophys. Acta*, **449**, 500 (1976), and by high resolution photoselection experiments in our laboratory, S. G. Boxer and M. G. Roelofs, submitted for publication.

(22) The absorption spectrum of the folded form of the model is a superposition of the spectra of the metal-free ring and folded dimer components (red absorption maxima at 673 and 692 nm). Fluorescence emission for the folded form of the model occurs exclusively from the folded dimer portion (maximum at 717 nm) irrespective of excitation wavelength, indicating efficient intramolecular energy transfer. The fluorescence quantum yield following excitation at 420 nm is reduced by a factor of two for the folded Mg_2 model relative to an identically absorbing 1:1 mixture of metal-free monomer and folded dimer.

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On the Ease of Base-Catalyzed Epimerization of N-Methylated Peptides and Diketopiperazines

Sir:

N-Methylation of peptides promotes base-catalyzed epimerization at the adjacent C_α position,[1] complicating the

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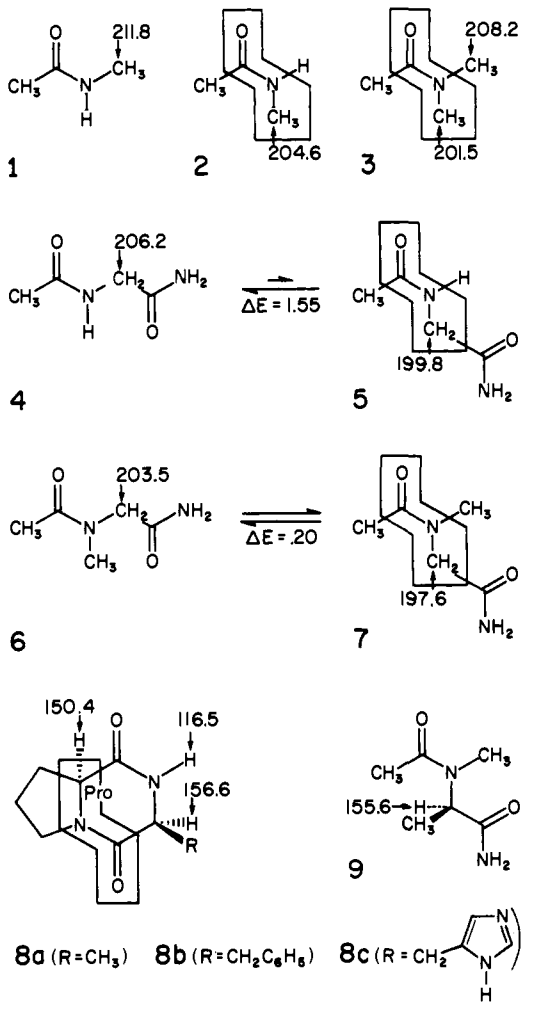
Chart I. CNDO/2 Calculated Deprotonation Energies of Peptide Model Systems (Kilocalories)



stepwise stereospecific synthesis of N-alkylated polypeptides. Furthermore, cyclic dipeptides (diketopiperazines), especially when N-alkylated, undergo extremely fast epimerization.[2] For example, cyclo-L-Phe-L-Pro[3] (**8b**) and cyclo-L-Pro-L-His[4] (**8c**) epimerize selectively at the proline α carbon in dilute alkali in a matter of minutes. Proposed rationalizations of these observations are not convincing.[5] We report here CNDO/2 calculations[6] on model systems which offer a possible explanation of the observed behavior and which illuminate several of the factors which may govern these epimerizations.

Cisoid and transoid conformers of the substituted acetamides listed in Chart I and their conjugate bases were examined. The carbanions were studied with sp^3 and sp^2 hybridization. In all cases, the sp^3-hybridized form was computed to be more stable by 3 kcal (for **8a** and **9** carbanions) to 24 kcal (for **2**). The predicted small preference for sp^3 hybridization for carbanions from **8** and **9** was unexpected and is not supported by experiment;[7] similarly the calculated difference favoring sp^3 hybridization for nonconjugated carbanions (e.g., that from **2**), while in the expected direction,[8] is too large.[9] Inversion barriers for carbanions are generally overestimated by self-consistent field calculations owing to electron correlation difficulties.[9]

Whatever the relative energies of these two hybridization states, we were primarily interested in estimating the transition state for epimerization, which must lie somewhere between sp^2 and sp^3. Since we find the calculated relative energies for these systems to fall in the same order for both sp^2- and sp^3-hybridized carbanions, it is reasonable to assume that the transition states should also fall in the same order. The deprotonation energies listed in Chart I represent the difference in binding energy between the neutral molecule and the sp^3 carbanion.[10]

Three carbanion stabilizing effects have been identified and related to relative rates of peptide racemization.

(a) One factor affecting epimerization of peptides appears to be the conformation of the amido carbanion grouping (O=C—N—C$^-$), which is isoelectronic with the butadienyl dianion. Our calculations suggest that the transoid arrangement of this grouping is more stable (by 6-7 kcal) than the cisoid conformation. Therefore, a cis peptide (e.g., **2**), which gives rise to a transoid amido carbanion, should epimerize more rapidly than a trans peptide (e.g., **1**). In Chart I, each deprotonation which leads to a favored transoid amido carbanion is outlined.

Thus, proton abstraction from *N,N*-dimethylacetamide (**3**) is calculated to occur from the methyl trans to the carbonyl oxygen more easily (by 6.7 kcal/mol) than from the *cis*-methyl group. Similarly, deprotonation of the corresponding *cis*-*N*-methylacetamide (**2**) requires less energy (7.2 kcal) than that of the trans conformer (**1**). For a more typical model of a polypeptide, *N*-acetylglycinamide, the cis isomer (**5**) has a more stable conjugate base (by 6.4 kcal) than the trans form (**4**), while for the N-alkylated analogues the conjugate base of **7** is more stable (by 5.8 kcal) than that of **6**.

The destabilization of *cis*-butadienes and -heterobutadienes relative to the trans conformations has been attributed to unfavorable orbital interactions.[11] For these amido carbanions, electrostatic effects may also be important. As for 1,2 diketones,[11,12] bond dipoles are unfavorably aligned for the cis and favorably opposed for the trans isomer. This is confirmed by the calculated dipole moments for the carbanions derived from **1** (18.0 D) and **2** (8.6 D).

(b) N-Alkylation of a linear peptide increases the proportion of cis peptide present.[13] Our calculations concur with this observation. Thus, **6** and **7** are computed to be about equal in energy, while the unalkylated cis peptide **5** is less stable than trans (**4**) by 1.6 kcal.[14] If more cis peptide is present, deprotonation to the more stable transoid amido carbanion is favored.

(c) An additional effect of N-alkylation is to stabilize hyperconjugatively[15-18] the amido carbanion by 2 to 6 kcal/mol. This occurs for all of the model systems. Thus, it is easier to deprotonate the *trans*-methyl of **3** than the methyl of **1** (by 3.6 kcal), and deprotonating the *cis*-methyl of **3** (outlined) is easier than the methyl of **2** (by 3.1 kcal). Similarly, C_α deprotonation is favored for **6** over **4** (by 2.7 kcal), for **7** over **5** (by 2.2 kcal), and Pro (outlined) over Ala of **8a** (by 6.2 kcal). Our calculations suggest that, not only may the "versatile methyl group" [16] support anionic and cationic hyperconjugation, but it also may stabilize hyperconjugatively a negative charge formally located two atoms away.

The major part of this hyperconjugative stabilization appears to be due to energy lowering of the highest occupied molecular orbital (HOMO) for the N-methylated carbanion. For example, the energy difference between HOMO's for transoid carbanions derived from **2** and **3** is 5.1 kcal, favoring **3**, and the HOMO energies for the Pro carbanion and Ala carbanion of **8a** favor the former by 5.0 kcal.[19] A similar orbital mixing was invoked, based on ab initio studies, for hyperconjugative stabilization of a carbanion by methyl.[16]

The conclusions of this study are pertinent in spite of the fact that we have not been able to include solvent effects in our calculations. Since the calculated trends agree with the observed solution chemistry, the effects found to be important in vacuo must be considered as possibly important in solution. Thus, CNDO/2 calculations of conformation, rotational barriers, dipole moments, and other phenomena,[20] without

correction for solvent effect, have often given useful insights into molecular behavior in solution.

Although the calculated energy differences are small (2–7 kcal), we note that the actual energy differences are also small. Furthermore we make the usual assumption[21] that, when only small perturbations (such as addition of a methyl group) are made to the system, errors of the CNDO/2 method will tend to cancel.

For linear polypeptides, then, N-alkylation enhances epimerization because of a higher concentration of cis peptide (effect b), which is more easily deprotonated (effect a), and because of hyperconjugative stabilization of the β carbanion (effect c).[22] For diketopiperazines, the incipient carbanion is held in the more favorable transoid conformation (effect a), as shown by the outline in **8**. N-Alkylation of diketopiperazine speeds epimerization further by hyperconjugation (effect c).

Diketopiperazine carbanions are calculated to be substantially more easily formed than those from the peptide models **1–7**. This is primarily due to carbanion stabilization by the C_α substituent, as shown by the comparable deprotonation energy computed for a representative conformation of the N-methylalanine derivative (**9**).

In conclusion, it appears that CNDO/2 calculations not only reproduce the observed relative ease of epimerization of N-alkylated polypeptides and cyclic dipeptides, but also help identify some effects which govern this reaction.

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(7) If the sp^3 carbanion (which is the intermediate for deuterium exchange) were actually lower in energy than the sp^2 carbanion (which is the intermediate for epimerization), then for cyclo-L-Pro-L-His (**8c**) the exchange rate should be greater than the racemization rate. However, an NMR experiment failed to find any rate difference (D. Veber and B. Arison, unpublished work).

(8) Adjacent electronegative heteroatom substituents raise the inversion barrier of CH_3^- and NH_3; see review by A. Veillard in "Quantum Mechanics of Molecular Conformations", B. Pullman, Ed., Wiley, London, 1976, p 1 (see especially p 69).

(9) For example, CNDO/2 gives 16.2 kcal for inversion of CH_3^- vs. actual value of ~1 kcal (ref 8, p 60).

(10) The carbanion conformation chosen for each of the linear models was that formed by deprotonation of a planar polypeptide—i.e., the carbanionic lone pair of electrons formed an angle of 60° with a plane containing the C_α–N–CO grouping. The minimum energy carbanion conformation in all cases had the lone pair in that plane (angle of 0°), but this is unlikely to resemble the transition state for deprotonation. In any case, the conformation chosen was consistently 2–3 kcal above the minimum energy conformation, and the arguments presented are not affected by this choice.

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(19) Interestingly, for carbanions derived from the better polypeptide models, **5** and **7**, N-methylation actually slightly destabilizes the HOMO (of π character, centered on the carbanion) by 1.0 kcal, but stabilizes the third highest occupied MO (which is the next highest orbital of π character, but has a small amplitude at the carbanionic center) by 3.3 kcal. This ordering of energy levels is subject to the usual uncertainties of the CNDO method.

(20) For reviews, see ref 8 and J. I. Fernández-Alonso in "Quantum Mechanics of Molecular Conformations", B. Pullman, Ed., Wiley, London, 1976, p 117.

(21) For example, see the discussion of approximations in the calculations of molecular conformations: ref 8, p 14.

(22) It should not be overlooked that, as demonstrated for **8** in Chart I, deprotonation at nitrogen is always easier than at carbon. However, these compounds should mostly be un-ionized in dilute aqueous base; so it is unlikely, as proposed by McDermott and Benoiton,[1b] that the major effect of N-methylation is to promote epimerization by preventing formation of the N anion.

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Conformations of Cyclododecyne. Evidence from Dynamic Nuclear Magnetic Resonance Spectroscopy and Iterative Force-Field Calculations

Sir:

The structural and conformational information available on cyclic acetylenes and their derivatives is rather limited,[1-3] and in the homologous cycloalkynes only the structure of cyclooctyne is known.[2] We now show that the major conformational features of cyclododecyne (I) can be determined by dynamic nuclear magnetic resonance spectroscopy and iterative force-field calculations.

The α-CH_2 resonance in the 251-MHz ^1H NMR spectrum[4,5] of I broadens strongly below about −100 °C and is observed as a complex pattern spread over at least 160 Hz below −140 °C. Since all proton–proton coupling constants should be <20 Hz, this pattern must represent more than two chemical shifts. The "coalescence temperature" is about −107 °C and thus some conformational process with a $\Delta G^{\ddagger}$ of ~7.8 ± 0.3 kcal/mol must be present.[6]

In the ^{13}C NMR spectra[4] of I, the acetylenic carbon resonance, which is a sharp single line above about −60 °C, broadens as the temperature is lowered, reaches a maximum broadening at about −95 °C, and finally gives rise to three sharp lines with intensity ratios of ~1:4:1.2 at −133 °C.[7] These results can be rationalized in terms of two conformations, one symmetrical and the other unsymmetrical (idealized intensity ratio for the acetylenic carbons of 1:4:1 for a conformational ratio of 2:1; i.e., $\Delta G° \simeq 0.2$ kcal/mol at −133 °C). The major conformation is symmetrical and is immediately consistent with a $[3_{yne}333]$[8] structure (Figure 1), which can be thought of as derived from the lowest energy conformation of cyclododecane, i.e., the [3333] or "square" conformation.[9] The minor conformation of I lacks symmetry and is difficult to identify without further information such as that provided by force-field calculations.

Boyd's iterative force-field program,[10] with modified parameters,[3b] has been used to calculate the strain energies and geometries of the conformations of I shown in Table I. The initial geometries required for the calculations have been obtained by replacing CH_2CH_2 by C≡C groups in molecular models of the known low-energy conformations of cyclododecane.[9] Because of its linear (or nearly linear) geometry, the acetylenic unit (-C≡C-) cannot reside at corner positions, and this greatly limits the number of available conformations. Vibrational frequencies have been calculated in all cases to

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