Geometrical Correspondence between Phenazocine and the Enkephalins

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Calculations have been performed on phenazocine using Allinger's MM2 (molecular mechanics II) program with full energy minimization. The *N*-phenethyl group was found to have considerable flexibility with a number of low-energy conformers. The best *N*-phenethyl axial conformer was 1.6 kcal/mol higher in energy than the best equatorial one. Calculations were also performed on the β isomer of phenazocine with the result that the energy difference between the best equatorial and axial conformers rose to a substantial 4.6 kcal/mol. The hypothesis that opiate agonism requires an N substituent in the axial position does not appear to be consistent with the increased potency of β isomers in which axial N substituents are thermodynamically more unstable. Comparisons have also been made between the low-energy conformers of phenazocine and those that have been observed or proposed for the enkephalins. One conformation of the tyrosine portion of the enkephalins that was observed by X-ray crystallography by Karle et al. was found to be a good fit to morphine-like opiates. The backbone conformer suggested by Gorin et al. was found to be the best fit to the two phenyl rings of phenazocine.

(-)-Phenazocine (Figure 1a) is a 6,7-benzomorphan opiate in which an *N*-phenethyl group increases potency significantly relative to the *N*-methyl derivative on in vivo and in vitro assays of analgesic activity.[1,2] The potency effect of the *N*-phenethyl appears to be due to a favorable specific interaction at the receptor site rather than just increased hydrophobicity since lengthening the alkyl chain to phenpropyl results in a 100-fold decrease of in vivo potency.[1] The *N*-phenethyl effect appears to be a general rule for relatively rigid multicyclic phenyl axial opiates since it also increases potency in morphine[3] and is optimal in the morphinan series with shortening or lengthening of the chain resulting in decreased activity as does replacing the *N*-phenethyl by its saturated equivalent.[4]

With the isolation and identification of Leu-enkephalin (Figure 1b) and Met-enkephalin, pentapeptides with morphine-like activity,[5] a number of authors have proposed that the phenol ring in opiates corresponds to the tyrosine phenyl in the enkephalins since the presence of the phenyl hydroxyl is required for maximum potency in the former.[6,7] It has also been proposed that the phenyl of the *N*-phenethyl group of phenazocine corresponds to the phenyl of the phenylalanine portion of the enkephalins.[8] However, to superimpose the nonphenolic phenyl of phenazocine with that of other opiates in which a second phenyl is attached to a different portion of the molecule, it was necessary to propose that the *N*-phenethyl group in phenazocine be in the generally unfavorable axial position of the piperidine ring.[8] The same authors also proposed that the agonist activity of opiates is mediated through conformers in which the N substituent is in the axial position whereas antagonist activity requires that the N substituent be in the equatorial position.

In this work, MM2 calculations are performed on phenazocine to determine the conformational preferences of the *N*-phenethyl group in a relatively quantitative fashion. Attempts will also be made to relate the preferred conformers to those of the enkephalins since there should be corresponding conformers. However, there is a great deal of flexibility possible in a pentapeptide and a variety of conformers have been proposed as will be detailed below. These will be examined to see whether they correspond geometrically to any of the low-energy conformers of phenazocine by using a molecular superposition program to determine the best fit between the conformers in a least-squares (root-mean-square) sense.

Using *X-ray crystallography*, it is possible to obtain a detailed picture of the actual conformation that a molecule assumes in the solid state. Smith et al. were the first to analyze a crystal of Leu-enkephalin, which was found to contain four virtually identical conformers that were stabilized by an intramolecular hydrogen bond (Table I).[9,10] However, there were some problems in the refinement of the crystal structure that may explain why the tyrosine phenyls in conformers 1 and 3 do not have $\chi 2$ dihedral angles near the expected 90° and 270° (Table I). More recently, a second crystal, which was kept in continuous contact with the mother liquor and incorporated a great deal of solvent, was studied by Karle et al.[11,12] This crystal was found to contain four distinctly different conformers all of which had more extended conformations (Table I). However, conformers B and C are closely related, differing significantly only in the conformation of the terminal leucine. Similarly, conformers A and D are closely related. Since the conformation of the first four peptide units is very similar in A and D and in B and C, the phenyl–phenyl distances are similar in each pair. It was noted that conformers B and C are the only ones that are consistent with the feasible conformations of [D-Ala^2]enkephalin, which has enhanced activity over its parent compound. Finally, it was suggested that in the presence of solvent, which can form intermolecular hydrogen bonds, that more extended conformers would be preferred.

A second approach to determining the preferred conformations of the enkephalins has been to use *computational methods*. As is generally done, the computations

(1) Eddy, N. B.; May, E. L. In "Synthetic Analgesics", Part IIB; Pergamon Press: New York, 1966; pp 115–182.
(2) Pert, C. B.; Snyder, S. H.; May, E. L. *J. Pharmacol. Exp. Ther.* **1976**, *196*, 316–322.
(3) Winter, C. A.; Orahovats, P. D.; Lehman, E. G. *Arch. Int. Pharmacodyn.* **1957**, *110*, 186–202.
(4) Eddy, N. B.; Besendorf, H.; Pellmont, B. *Bull. Narc.* **1958**, *10*, 23–41.
(5) Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature (London)* **1975**, *258*, 577–579.
(6) Bradbury, A. F.; Smyth, D. G.; Snell, C. R. *Nature (London)* **1976**, *260*, 165–166.
(7) Horn, A. S.; Rodgers, J. R. *Nature (London)* **1976**, *260*, 795–797.
(8) Feinberg, A. P.; Creese, I.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4215–4219.
(9) Smith, G. D.; Griffin, J. F. *Science (Washington, D.C.)* **1978**, *199*, 1214–1216.
(10) Blundell, T. L.; Hearn, L.; Tickle, I. J.; Palmer, R. A.; Morgan, B. A.; Smith, G. D.; Griffin, J. F. *Science (Washington, D.C.)* **1979**, *205*, 220.
(11) Karle, I. L.; Karle, J.; Mastropaola, D.; Camerman, A.; Camerman, N. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1983**, *B39*, 625–637.
(12) Camerman, A.; Mastropaolo, D.; Karle, I.; Karle, J.; Camerman, N. *Nature (London)* **1983**, *306*, 447–450.

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Table I. Observed and Computed Dihedral Angles for the Enkephalins[a]

	1	2	3	4	A	B	C	D	Mom	Gorin	Maig
$\psi 1$	129	123	126	121	135	154	155	137	82	129	−176
$\omega 1$	−179	−179	175	180	172	177	177	173	−178	180	180
$\varphi 2$	52	52	62	57	−144	151	141	−131	69	160	109
$\psi 2$	31	35	21	26	114	−155	−157	142	−126	−87	37
$\omega 2$	179	178	179	178	−177	180	−178	179	180	180	180
$\varphi 3$	94	91	99	98	−122	154	174	−144	−65	−118	133
$\psi 3$	−3	−2	−9	−9	132	−151	−170	131	−55	98	−99
$\omega 3$	−177	−179	−178	−174	−179	−170	179	178	177	180	180
$\varphi 4$	−133	−128	−129	−128	−122	−128	−119	−147	−64	−87	−33
$\psi 4$	149	145	152	144	139	130	149	152	114		
$\omega 4$	177	178	180	178	168	174	179	171	−174		
$\varphi 5$	−107	−108	−107	−106	−79	−72	−141	−141	49		
$\psi 5$	158	174	161	173	−9	−17	−48	−30	56		
$\chi 1(1)$	−80	−53	−87	−52	177	70	53	169	−61	−106	−162
$\chi 2(1)$	138	93	157	99	93	99	101	71	−75	−163	−140
$\chi 1(4)$	−59	−61	−60	−62	−63	−55	−71	−68	177	−87	−33
$\chi 2(4)$	90	88	89	89	87	87	98	93	−109	−56	56
$\chi 1(5)$	−66	−71	−70	−64	−64	−62	−171	−80	−62		
$\chi 21(5)$	165	170	169	166	173	165	68	179	−176		
ring–ring, Å	11.3	10.8	11.4	10.7	9.4	13.3	13.9	8.9			

[a] The dihedral angles for conformers 1–4 were computed from the fractional coordinates of the Smith crystal[9,10] while those for conformers A–D were computed from those of the Karle crystal.[11,12] The remaining conformers are those proposed by Momany,[14] Gorin et al.,[19,20] and Maigret et al.[18]

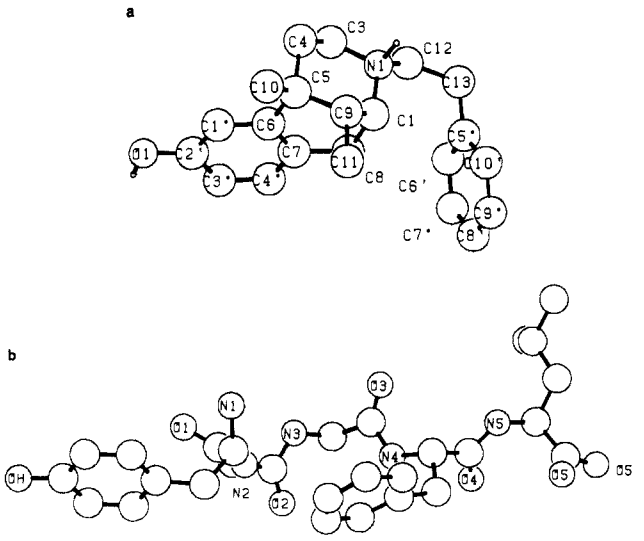

Figure 1. Phenazocine and enkephalin conformers that superimpose with the lowest RMS distances. The phenazocine conformer is the [176,82,59] one listed in Table I while the enkephalin conformer has $\chi 1(1) = 70°$, $\chi 1(4) = 300°$ with the backbone dihedral angles suggested by Gorin et al.[19,20] Both figures have been drawn with identical perspectives with respect to the phenol ring to the left.

were carried out in the absence of solvent and conformers with β bends were found to be preferred.[13–18] This result is not surprising since there are no competitive intermolecular hydrogen bonds possible in the absence of solvent that might otherwise stabilize other conformers. The most interesting and perhaps most relevant calculations are those on enkephalin analogues that have retained analgesic activity despite conformational restriction. Thus, using the empirical potential function ECEPP method, Momany found that [D-Ala^2]enkephalin prefers a very different conformer from the endogenous compound.[14] More recently, Manavalan and Momany found three families of low-energy conformers in a series of enkephalin analogues.[15] Using both the empirical ECEPP and semiempirical quantum mechanical PCILO methods, Loew et al. have attempted to fit enkephalin conformers to the tyramine moiety of morphine and to the two phenyl rings of a very potent oripavine derivative and have proposed candidate active and inactive conformers though they did not fully specify the dihedral angles that they obtained.[16,17] Using an empirical potential function method, Maigret et al. have proposed an energy-minimized conformer that was constrained to be morphine-like.[18] This approach has been taken furthest by Gorin et al. who deduced the possible conformations of active analogues that would have severe conformational restrictions, and they have proposed a highly specific "receptor-bound" conformer on the basis of their work.[19,20] These conformers are also listed in Table I.

Finally, a number of groups have used *NMR techniques* to determine the conformation of the enkephalins in solution.[21–25] However, the solution conformation remains unclear since there is disagreement among the various groups. While Stimson et al. have proposed a highly specific conformer in solution,[25] it does not appear to be biologically relevant since it is not consistent with the high activity of the [D-Ala^2] analogue that could not easily as-

(13) Isogai, Y.; Nemethy, G.; Scheraga, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 414–418.
(14) Momany, F. A. *Biochem. Biophys. Res. Commun.* **1977**, *75*, 1098–1103.
(15) Manavalan, P.; Momany, F. A. *Int. J. Peptide Protein Res.* **1981**, *18*, 256–275.
(16) Loew, G. H.; Burt, S. K. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 7–11.
(17) Loew, G.; Hashimoto, G.; Williamson, L.; Burt, S.; Anderson, W. *Mol. Pharmacol.* **1982**, *22*, 667–677.
(18) Maigret, B.; Premilat, S.; Fournie-Zaluski, M.-C.; Roques, B. P. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 267–274.
(19) Gorin, F. A.; Marshall, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5179–5183.
(20) Gorin, F. A.; Balasubramanian, T. M.; Barry, C. D.; Marshall, G. R. *J. Supramol. Struct.* **1978**, *9*, 27–39.
(21) Roques, B. P.; Garbay-Jaureguiberry, C.; Oberlin, R.; Anteunis, M.; Lala, A. K. *Nature (London)* **1976**, *262*, 778–779.
(22) Jones, C. R.; Gibbons, W. A.; Garsky, V. *Nature (London)* **1976**, *262*, 779–782.
(23) Bleich, H. E.; Cutnell, J. D.; Day, A. R.; Freer, R. J.; Glasel, J. A.; McKelvy, J. F. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 2589–2593.
(24) Khaled, M. A.; Long, M. M.; Thompson, W. D.; Bradley, R. J.; Brown, G. B.; Urry, D. W. *Biochem. Biophys. Res. Commun.* **1977**, *76*, 224–231.
(25) Stimson, E. R.; Meinwald, Y. C.; Scheraga, H. A. *Biochemistry* **1979**, *18*, 1661–1671.

Table II. Conformational Energies for the α and β Isomers of Phenazocine[a]

conformer	N2–ring 1 ctr, Å	N2–ring 1 plane, Å	N2–ring 2 ctr, Å	N2–ring 2 plane, Å	ring 1–ring 2 ctr, Å	energy, kcal/mol α	energy, kcal/mol β
			Phenethyl Equatorial				
[69,55,74]	4.5	1.6	3.9	2.5	8.4	6.1	7.3
[69,173,88]	4.5	1.6	5.2	1.5	8.7	6.7	8.0
[76,–73,119]	4.5	1.7	4.3	2.5	7.1	7.8	9.1
[176,82,59]	4.5	1.7	4.4	2.5	6.5	6.7	8.1
[178,–172,97]	4.5	1.7	5.2	1.5	8.5	6.2	7.6
[176,–53,117]	4.5	1.7	4.0	2.5	8.2	5.8	6.7
[–56,59,62]	4.5	1.7	4.3	2.6	5.2	10.9	12.4
[–39,179,80]	4.4	1.8	5.2	1.7	7.9	10.5	11.8
[–39,–87,126]	4.5	1.7	4.6	2.5	5.4	12.6	13.9
			Phenethyl Axial				
[67,77,37]	4.4	1.7	4.6	2.3	7.5	14.6	
[46,172,95]	4.4	1.7	5.2	1.7	9.3	13.5	
[56,–75,142]	4.4	1.7	4.6	2.3	7.5	15.6	21.6
[–172,59,77]	4.5	1.6	4.0	2.5	8.0	7.4	11.3
[–176,179,83]	4.5	1.5	5.2	1.6	9.4	7.9	11.5
[–168,–76,123]	4.5	1.6	4.4	2.5	7.5	8.0	13.6
[–66,80,56]	4.5	1.6	4.4	2.5	7.5	8.8	13.0
[–67,–179,94]	4.5	1.6	5.2	1.6	9.5	8.1	12.0
[–67,–59,105]	4.5	1.6	4.0	2.5	8.0	7.6	11.4

[a] The specific dihedral angles and geometries, however, only refer to the α isomer. The conformers are described by [τ1, τ2, τ3] = [τ(C13–C12–N2–C3), τ(C5′–C13–C12–N2), τ(C6′–C5′–C13–C12)].

sume this conformer. Using fluorescence spectroscopy, Schiller et al. have suggested that the average phenyl–phenyl distance for the enkephalins in solution is 10.0 ± 1.1 Å.[26]

Methods

Energy minimizations of the phenazocine conformers were performed with the MM2 program developed by Allinger and Yuh.[27] Special parameters for the phenyl ring were used,[28] and the equilibrium bond length of a C–N bond was increased by 0.03 Å since this bond length is consistently increased when the nitrogen is protonated.[29] Electrostatic dipole moments were computed with the corrected dipole moment for an N–H bond.[30] However, intramolecular electrostatic interactions play an insignificant role in phenazocine, suggesting that solvent effects will not significantly affect the conformational equilibrium of the molecule.[31]

The MM2 program has been shown to produce quantitatively correct thermodynamic results for diverse hydrocarbons[32,33] and amines.[34] It is expected that the results obtained here would be relatively accurate since they consist almost entirely of hydrocarbon with polar groups that are distant from each other. We have previously found that the conformational and geometric results of the MM2 program and its predecessor to be in excellent agreement with those of crystallography despite the very different molecular environments of the two methods.[35-40]

A molecular superposition program (SUPER) was written to determine the relative fit between different portions of phenazocine and enkephalin in the least-squares (root-mean-square) sense. This is an improvement over monitoring such quantities as interatomic distances since it allows one to examine whether the molecular groups are located in similar regions of three-dimensional space when the molecules were optimally aligned by rotation and translation. In order to simplify the input and to remove ambiguities with regard to the ordering of the atoms of the phenyl ring, the six atoms of the phenyl rings were replaced by two parameters: (1) the center of the phenyl ring and (2) a dummy atom that was placed perpendicular to the phenyl ring center at a height identical with the average distance of the ring edge (~1.4 Å). Since a phenyl ring has two sides, the dummy atom was always placed on the side where it makes an acute angle with the line from the phenyl center to the nitrogen atom. The dummy atom allows one to determine whether the tilts of the two rings are similar relative to other axes. Superposition was achieved in two steps. In the first, the phenolic oxygen, the center of the phenol ring, and its dummy atom were superimposed as closely as possible. Because of the rigidity of the phenol group, the RMS fit was always quite good, being on the order of 0.05 Å. After this molecular anchor was fixed, the goodness of fit of the ammonium nitrogen, the nonphenolic ring center, and its dummy atom were calculated. The mathematical method used for the best-fit superposition is detailed in the Appendix.

Results and Discussion

The geometries and thermodynamic results of the calculations are presented in Table II. The *N*-phenethyl group can be seen to have considerable flexibility, with five of the nine possible equatorial conformers having energies

(26) Schiller, P. W.; Yam, C. F.; Lis, M. *Biochemistry* **1977**, *16*, 1831–1838.
(27) Allinger, N. L.; Yuh, Y. H. *QCPE* **1980**, No. 395.
(28) Allinger, N. L. *QCPE Bull.* **1983**, *3*, 32–33.
(29) Profeta, S., Jr., unpublished data.
(30) *QCPE Bull.* **1983**, *3*, 36.
(31) Burkert, U.; Allinger, N. L. "Molecular Mechanics"; American Chemical Society: Washington, DC 1982; ACS Monogr. No. 177.
(32) Engler, E. M.; Andose, J. D.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1973**, *95*, 8005–8025.
(33) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.
(34) Profeta, S., Jr.; Allinger, N. L. *J. Am. Chem. Soc.* **1985**, *107*, 1907–1918.
(35) Froimowitz, M. *J. Med. Chem.* **1982**, *25*, 689–696.
(36) Froimowitz, M. *J. Med. Chem.* **1982**, *25*, 1127–1133.
(37) Froimowitz, M.; Matthysse, S. *Mol. Pharmacol.* **1983**, *24*, 243–250.
(38) Froimowitz, M.; Salva, P.; Hite, G. J.; Gianutsos, G.; Suzdak, P.; Heyman, R. *J. Comput. Chem.* **1984**, *5*, 291–298.
(39) Froimowitz, M. *J. Med. Chem.* **1984**, *27*, 1234–1237.
(40) Froimowitz, M.; Kollman, P. *J. Comput. Chem.* **1984**, *5*, 507–516.

Table III. Dihedral Angles That Describe the Lowest Energy Conformer Found by MM2 Calculations for Phenazocine (Observed Dihedral Angles for the 6,7-Benzomorphan Structure in Six Crystal Conformers Included for Comparison)

	MM2	1[a]	2[a]	3[b]	4[c]	5[d]	6[e]
C5–C6–C7–C8	1	5	3	−3	4	10	6
C6–C7–C8–C1	−8	−9	−7	3	−15	−12	−12
C7–C8–C1–C9	39	38	36	30	45	38	41
C8–C1–C9–C5	−65	−64	−62	−62	−66	−62	−64
C1–C9–C5–C6	58	58	57	60	55	57	55
C9–C5–C6–C7	−26	−30	−27	−29	−24	−32	−27
C5–C9–C1–N2	63	62	63	65	62	65	64
C9–C1–N2–C3	−61	−57	−59	−63	−56	−60	−60
C1–N2–C3–C4	55	52	55	57	51	55	56
N2–C3–C4–C5	−54	−53	−55	−56	−53	−52	−56
C3–C4–C5–C9	56	57	58	59	58	54	61
C4–C5–C9–C1	−60	−60	−62	−61	−62	−62	−65
C10–C5–C6–C1′	33	30	30	25	33	28	39
C10–C5–C6–C7	−149	−153	−152	−152	−150	−156	−150
C10–C5–C9–C1	−177	−177	−178	−175	−179	−177	179
C10–C5–C9–C11	57	59	60	62	57	61	54
C10–C5–C4–C3	174	175	177	175	177	173	178
C11–C9–C5–C10	57	59	60	62	57	61	54
C11–C9–C5–C6	−68	−66	−66	−63	−70	−65	−71
C11–C9–C5–C4	175	176	176	177	174	176	170
C11–C9–C1–C8	62	63	64	63	60	62	60
C11–C9–C1–N2	−170	−171	−171	−171	−172	−171	−172
C1′–C6–C5–C9	156	153	154	148	159	152	162
C1′–C6–C5–C4	−87	−91	−89	−95	−86	−91	−81
C7–C6–C5–C4	91	87	89	88	91	84	89
C4′–C7–C8–C1	172	174	175	−177	166	171	174
C12–N2–C1–C9	174	174	175	170	179	174	176
C12–N2–C1–C8	−62	−61	−62	−64	−57	−61	−58
C12–N2–C3–C4	−176	180	−175	−176	177	178	−177
C13–C12–N2–C1	−58	−158	−54	−167	−166	−157	−50
C13–C12–N2–C3	176	74	177	66	67	78	−177
C5′–C13–C12–N2	−53						
C6′–C5–C13–C12	117						
C10′–C5′–C13–C12	−63						

[a] Molecules 1 and 2.[42] [b] Cylazocine.[43] [c] MR 1526.[44] [d] MR 2034.[44] [e] Reference 45.

within 0.9 kcal/mol of the global minimum. Similarly, six of nine axial conformers have energies within 1.4 kcal/mol of the lowest energy axial conformer. Many more stable energy minima are found here than in a previous PCILO study of the conformational preferences of the *N*-phenethyl group in morphine derivatives.[41] We attribute those results to a lack of energy minimization with respect to the internal coordinates of the molecule. There also appears to be considerable latitude about the τ(C6′–C5′–C13–C12) dihedral angle with values between 59 and 117° for conformers that are within 0.9 kcal/mol of the global minimum.

The 6,7-benzomorphan structure is relatively rigid, and the distance of the ammonium nitrogen to the center of the phenolic ring ranges from 4.4 to 4.5 Å while its distance from the phenol plane ranges from 1.5 to 1.8 Å over all possible conformers. The distances between the centers of the two phenyl rings ranges from 6.5 to 8.7 Å for the six best equatorial conformers and from 7.5 to 9.5 Å for the six best axial conformers. It should be noted that the second (phenethyl) phenyl ring has *N*2–phenyl distances that are comparable to those of the phenolic ring (Table II). Thus, the second phenyl might take the place of the phenolic phenyl in the receptor site under certain conditions.

The detailed dihedral angles that describe the lowest energy conformer are presented in Table III along with the dihedral angles that have been observed in the crystal structures of various 6,7-benzomorphans.[42-45] There is good agreement between the energy-minimized and observed dihedral angles that describe the 6,7-benzomorphan portion of the molecule.

Axial N Substituents. With regard to the hypothesis that agonist action is promoted by N substituents being in the axial position of the piperidine ring, the best axial conformer is 1.6 kcal/mol above the global minimum. The small energy difference between the equatorial and axial conformers is due to an equatorial N substituent making two trans interactions and one gauche interaction whereas an axial group makes two gauche interactions and one trans interaction. Thus, the difference between the two consists of a single unfavorable gauche interaction. The computed energy differences between equatorial and axial N substituents are considerably less than was found previously by PCILO calculations on equivalent compounds (e.g., 5.7 kcal/mol for morphine).[41] We believe that the present values are more realistic since full energy minimization, which was not done in the PCILO calculations, can significantly reduce computed energy differences.[40,46]

An energy difference of 1.6 kcal/mol does not appear to be decisive in determining the validity of the axial N substituent hypothesis. However, calculations have also

(41) Loew, G. H.; Berkowitz, D. S. *J. Med. Chem.* **1975**, *18*, 656–662.
(42) Peeters, O. M.; De Ranter, C. J.; Blaton, N. M. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1982**, *B38*, 3168–3171.
(43) Karle, I. L.; Gilardi, R. D.; Fratini, A. V.; Karle, J. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1969**, *B25*, 1469–1479.
(44) Peeters, O. M.; De Ranter, C. J.; Blaton, N. M. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1982**, *B38*, 3055–3059.
(45) Fedeli, W.; Giacomello, G.; Cerrini, S.; Vaciago, A. *J. Chem. Soc. B* **1970**, 1190–1195.
(46) Gelin, B. R.; Karplus, M. *J. Am. Chem. Soc.* **1975**, *97*, 6996–7006.

been performed on the β isomer of phenazocine in which the C11 methyl group is axial rather than equatorial (Table I). In general, β isomers of 6,7-benzomorphans are significantly more potent on assays of agonist activity.[1] While an axial C11 methyl group has only a minimal effect on the *N*-phenethyl equatorial conformers, it sterically interferes with an axial N substituent with the energy difference between the best phenethyl equatorial and axial conformers rising to a more substantial 4.6 kcal/mol. While this is still less than the expected binding energy of a ligand–receptor complex, the necessity of putting that amount of energy into the ligand to get it into the right conformation should result in a significant weakening of the complex. It would appear to be extremely unlikely that the increased destabilization of axial N substituent conformers should be associated with the increased potency of β isomers if the former were required for agonist activity. Thus, the hypothesis is not consistent with the relative potencies of α- and β-6,7-benzomorphans. A similar conclusion regarding this hypothesis was reached on the basis of PCILO calculations on oxymorphone derivatives.[47] In any case, a better explanation for the differences between agonists and antagonists may be that they bind to different sites in the receptor.[48]

If the active conformer of phenazocine has the *N*-phenethyl group in the equatorial position, it would appear that the nonphenolic phenyls in phenazocine and in the oripavine derivatives[8] will not occupy the same position in space when bound to opiate receptors. If one continues to make the assumption that the second phenyl group in both compounds increases potency through specific receptor interactions and also corresponds to the phenylalanine phenyl of the enkephalins, this suggests that there may be two distinct enkephalin conformers that may be biologically relevant with one of these binding to δ receptors whereas the second binds to μ receptors. The native enkephalins and some of their analogues,[49-52] most notably [D-Pen^2,D-Pen^5]enkephalin,[53] which prefer δ receptors may have a greater concentration of the first conformer. Other analogues such as morphiceptin,[54] DAGO,[55] and a cyclic analogue[56] that interact primarily with μ receptors may have a preference for the second conformer. Phenazocine appears to have a greater preference for the δ receptor relative to morphine and to other benzomorphans such as pentazocine[51] though the preference is not substantial. This suggests that the phenazocine conformers may be associated with δ receptors rather than μ receptors. A number of authors have suggested that conformation may be the key to the discrimination of μ and δ receptors.[15,53,56,57]

Geometric Correspondence between Phenazocine and the Enkephalins. As indicated in the introduction, one goal of this work is to relate the geometry of phenazocine to that of the enkephalins. The geometrical relationship of the ammonium nitrogen to the tyrosine phenyl in the enkephalins is primarily a function of a single dihedral angle χ1(1) since χ2(1) should have values in the vicinity of 90 and 270°, which would result in indistinguishable conformers. On the basis of a statistical analysis of protein crystal structures, this dihedral angle can have one trans and two gauche orientations.[58] All three of these have been observed in the crystal structures of Leu-enkephalin. Values in the vicinity of −60° are observed in the four Smith conformers, values in the vicinity of 180° are observed in conformers A and D of the Karle crystal, and values in the vicinity of 60° appear in conformers B and C of the Karle crystal (Table I). Using the SUPER program to superimpose the phenolic rings in phenazocine and the crystal conformers of Leu-enkephalin and looking at the relative geometry of the nitrogen, conformers B and C of the Karle crystal provide the best correspondence with a distance of 0.4–0.5 Å while the others have distances of 2.0–2.5 Å. Thus, it would appear that χ1(1) in the vicinity of 70° (the actual value in conformer B) provides an optimal fit to that portion of the phenazocine structure and that flexibility in both the enkephalins and in the receptor site should accommodate its small RMS. This result differs from the χ1(1) ≈ −90° that was suggested by Loew and Burt,[16] the −106° suggested by Gorin et al.,[19,20] and the −162° suggested by Maigret et al.[18]

The relative geometrical orientations of the two phenyl rings in the enkephalins is a more complex problem since there are a large number of intervening dihedral angles in the backbone (ψ1, ω1, φ2, ψ2, ω2, φ3, ψ3, ω3, φ4) as well as the χ1(1) and χ1(4) sidechain dihedral angles. As indicated above, the χ2(1) and χ2(4) dihedral angles are not important since the expected values are 90 and 270°, which would produce indistinguishable conformers. Assuming that χ1(1) = 70° is the correct conformer, as in conformer B of the Karle crystal, we have examined a number of possible backbone conformations that have previously been observed or suggested. In addition, χ1(4) has been set to 60, 180, and 300° since those are the possible low-energy conformations of the phenyl ring.[58] For example, we examined conformer A of the Karle crystal with χ1(1) = 70° and χ1(4) = 60, 180, and 300°. Similarly, conformer B, which already has the correct value of χ1(1), the latter three were examined. However, all of these conformers had phenyl–phenyl distances (10.8–16.0 Å) that were too great to the compatible with those for phenazocine. A similar procedure was followed for conformer 2, which was chosen to be representative of the four Smith conformers. With χ1(1) = 70°, the phenyl–phenyl distances were 6.1 and 6.8 Å when χ1(4) was 180° and 300°, respectively. With χ1(4) = 60°, the distance was too short at 2.0 Å and there were serious steric overlaps.

A similar procedure was followed for the backbone conformers proposed by Momany, Maigret et al., and Gorin et al. again with χ1(1) = 70°. For the Momany conformers, the phenyl–phenyl distances were too short ranging from 1.5 to 4.7 Å. Moreover, these short distances resulted is significant atomic overlaps that clearly would preclude their viability. For the Maigret conformers, the one with χ1(4) = 60° had a phenyl–phenyl distance of 7.7

(47) Loew, G. H.; Berkowitz, D. S. *J. Med. Chem.* **1978**, *21*, 101–106.
(48) Portoghese, P. S.; Takemori, A. E. *J. Med. Chem.* **1983**, *26*, 1341–1343.
(49) Lord, J. A. H.; Waterfield, A. A.; Hughes, J.; Kosterlitz, H. W. *Nature (London)* **1977**, *267*, 495–499.
(50) Chang, K.-J.; Cuatrecasas, P. *J. Biol. Chem.* **1979**, *254*, 2610–2618.
(51) Childers, S. R.; Creese, I.; Snowman, A. M.; Snyder, S. H. *Eur. J. Pharmacol.* **1979**, *55*, 11–18.
(52) Kosterlitz, H. W.; Lord, J. A. H.; Paterson, S. J.; Waterfield, A. A. *Br. J. Pharmacol.* **1980**, *68*, 333–342.
(53) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871–5874.
(54) Chang, K.-J.; Killian, A.; Hazum, E.; Cuatrecasas, P.; Chang, J.-K. *Science (Washington, D.C.)* **1981**, *212*, 75–77.
(55) Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. *Eur. J. Pharmacol.* **1981**, *70*, 531–540.
(56) Schiller, P. W.; DiMaio, J. *Nature (London)* **1982**, *297*, 74–76.
(57) Fournie-Zaluski, M.-C.; Gacel, G.; Maigret, B.; Premilat, S.; Roques, B. P. *Mol. Pharmacol.* **1981**, *20*, 484–491.
(58) Janin, J.; Wodak, S.; Levitt, M.; Maigret, B. *J. Mol. Biol.* **1978**, *125*, 357–386.

Å, which was comparable while the remaining two had distances of 12.1 and 11.5 Å. For the Gorin et al. "receptor-bound" backbone conformer, the one with $\chi 1(4) = 300°$ was found to have a phenyl–phenyl distance of 7.9 Å that was compatible with that distance in phenazocine while the other two conformers had distances of 11.5 and 12.5 Å.

At this point, the only enkephalin conformers that were possibilities were two Smith conformers, one Maigret conformer, and one Gorin conformer. The SUPER program was then used to test whether the two nonphenolic phenyl orientations in these enkephalin conformers were compatible with any of the six lowest energy equatorial and six lowest energy axial phenazocine conformers. The two Smith conformers were found not to be compatible since the RMS distances for the phenyls ranged from 6.8 to 12.0 Å. Similarly, the RMS for the superposition of the phenyl rings ranged from 5.7 to 8.3 Å for the remaining Maigret conformers. However, the Gorin conformer had a reasonably good fit with the [176,82,59] conformer of phenazocine. As before, the ammonium nitrogens superimposed with a RMS of 0.4 Å, and the nonphenolic phenyl superimposed to 1.6 Å and the dummy atom for the phenyl as 3.3 Å. Although the latter two RMS distances are greater, it may be unreasonable to expect very good superimposibility for compounds in which there are a large number of intervening dihedral angles. It would be expected that small adjustments to the dihedral angles that were used should bring the compounds into better register. Moreover, one would expect the receptor itself to be flexible to a certain extent and, therefore, to tolerate some deviation from perfect superimposibility.

It should be noted that the superimposibility method used here differs from previous methods. Both Loew et al. and Gorin et al. also tried to superimpose the enkephalins onto morphine-like compounds though without regard to the energy cost, and thus their phenyl conformations do not have experimentally observed dihedral angles. It was noted by Loew that the morphine-like conformers had very high energies.[16,17] Our approach has been to superimpose probable enkephalins conformers onto energy-minimized phenazocine conformers. Of course, this means that the superimposibility is not as good. However, as noted above, both ligands and receptors would be expected to have a certain amount of flexibility. This would be particularly true for a ligand in which the critical geometries are functions of a large number of intervening dihedral angles. Thus, trying to achieve perfect superimposibility may be too rigorous an approach.

In any case, a previously proposed enkephalin conformer has been identified that has a reasonable fit to the two phenyl rings of phenazocine. Of course, there may be other conformers of enkephalin that may fit as well or possibly even better. However, these results do provide some limited evidence for the conformer proposed by Gorin et al. Previous work with a semirigid analogue of Leu-enkephalin has also suggested that the Gorin conformer may be the biologically active one.[59]

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Appendix

Consider two rigid bodies K and K′, each composed of N points internally connected. Let $\mathbf{X}_i$ ($i = 1, ..., N$) and $\mathbf{X}_i'$ ($i = 1, ..., N$) be 3-vectors denoting the positions of the points of K and K′; assume

$$\sum_{i=1}^{N} \mathbf{X}_i = \sum_{i=1}^{N} \mathbf{X}_i' = 0 \tag{1}$$

Let K′ be subjected to a rotation $\mathbf{R}$ followed by a translation $\mathbf{T}$; the problem is to find $\mathbf{R}$ and $\mathbf{T}$ such that, after they are applied, K′ is as closely as possible superimposed upon K. We use as a criterion of closeness the sum of the squares of the distances between corresponding pairs of points. Thus, the quantity

$$F = \sum_{i=1}^{N} \|\mathbf{X}_i - (\mathbf{T} + \mathbf{R}\mathbf{X}_i')\|^2 \tag{2}$$

($\|$ denoting the Euclidean norm) is to be minimized. Because of (1), (2) reduces to $\sum_{i=1}^{N}\|\mathbf{X}_i - \mathbf{R}\mathbf{X}_i'\|^2 + N\|\mathbf{T}\|^2$; evidently $\mathbf{T} = 0$ is the optimal choice. $\sum_{i=1}^{N}\|\mathbf{X}_i - \mathbf{R}\mathbf{X}_i'\|^2$ can be expressed as

$$\sum_{i=1}^{N} \|\mathbf{X}_i\|^2 + \sum_{i=1}^{N} \|\mathbf{X}_i'\|^2 - 2\sum_{i=1}^{N} \mathbf{X}_i \cdot \mathbf{R}\mathbf{X}_i' \tag{3}$$

($\cdot$ denoting the scalar product), so our task is to maximize

$$U = \sum_{i=1}^{N} \mathbf{X}_i \cdot \mathbf{R}\mathbf{X}_i' \tag{4}$$

According to a theorem of Cayley,[60] every rotation matrix can be expressed as eq 5 where $\sum_{i=1}^{4} q_i^2 = 1$. Since $\mathbf{R}$

$$\mathbf{R} = \begin{bmatrix} q_4^2 + q_1^2 - q_2^2 - q_3^2 & 2(q_1q_2 + q_3q_4) & 2(q_1q_3 - q_2q_4) \\ 2(q_1q_2 - q_3q_4) & q_4^2 + q_2^2 - q_1^2 - q_3^2 & 2(q_2q_3 + q_1q_4) \\ 2(q_1q_3 + q_2q_4) & 2(q_2q_3 - q_1q_4) & q_4^2 + q_3^2 - q_1^2 - q_2^2 \end{bmatrix} \tag{5}$$

is a quadratic form in $q_1, ..., q_4$, U is also, which we will denote by

$$U = \sum_{s,t=1}^{4} H_{st} q_s q_t \qquad H_{st} = H_{ts} \tag{6}$$

where the H_{st} depend on $\mathbf{X}_i$ and $\mathbf{X}_i'$ and can readily be calculated as functions of those variables. As $q_1, ..., q_4$ are varied subject to the constraint $\sum_{i=1}^{4} q_i^2 = 1$, the maximum value of U is achieved by setting the q's equal to that eigenvector of $\mathbf{H}$ which corresponds to the largest eigenvalue λ_m; the constrained maximum of U is, indeed, λ_m.

Applying this theory to molecular superposition, the N points of K and K′ are the atoms that are superimposed on each other. The largest eigenvalue and corresponding eigenvector of $\mathbf{H}$ are computed, and the rotation matrix $\mathbf{R}$ is determined by (5). The final position of every atom in K′ (not included among the N points) is found by applying $\mathbf{R}$. As a measure of goodness of fit, we use $\sqrt{F}$ (from (2)), the root-mean-square distance between corresponding atoms. Applying (3) and (4)

$$\text{min RMS distance} = \left(\sum_{i=1}^{N} \|\mathbf{X}_i\|^2 + \sum_{i=1}^{N} \|\mathbf{X}_i'\|^2 - 2\lambda_m\right)^{1/2} \tag{7}$$

Registry No. β-Phenazocine, 58073-76-0; α-phenazocine, 100100-58-1.

(59) Siemion, I. Z.; Szewczuk, Z.; Herman, Z. S.; Stachura, Z. *Mol. Cell. Biochem.* **1981**, *34*, 23–29.

(60) Courant, R.; Hilbert, D. "Methods of Mathematical Physics"; Interscience: New York, 1953; Vol. 1, pp 536–537.