resulting crude product (148 g) was dissolved in boiling EtOH (800 mL); the solution was clarified with charcoal and allowed to stand overnight at 5 °C. The resulting white suspension was diluted with Et_2O (400 mL) and left to stand overnight at 5 °C. The product was filtered off and washed with an ice-cold mixture of EtOH/Et_2O (2:1) to give 1 as a white, crystalline substance: yield, 122.2 g (86%); mp 214–216 °C; $[\alpha]^{25}_D$ −80.2° (c 1, AcOH); R_f (B) 0.39; R_f (E) 0.43; ^1H NMR ($CDCl_3$) δ 0.92, 0.94 (dd, 6, 2 CH_3), 2.35 (m, 2, $COCH_2$), 3.65 (m, 1, Leu NCH), 3.88 (m, 2, NCH_2), 4.52 (m, 1, Pro NCH), 4.75 (m, 1, pAad NCH), 5.35, 7.67 (2 s, 2, NH_2), 8.00 (s, 1, Leu NH), 8.40 (d, 1, pAad NH). Amino acid analysis: Aad, 0.99 (1); Leu, 1.00 (1); Pro, 0.98 (1); NH_3, 1.02 (1).

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Registry No. 1, 78664-73-0; 2, 78664-74-1; 3, 78664-71-8; 6a, 102922-73-6; 4, 102922-69-0; 5, 78685-15-1; 5a, 78664-66-1; 6, 65126-64-9; 6a, 102922-74-7; 7, 102922-70-3; 8, 78664-32-1; 8a, 78674-39-2; 9, 78664-37-6; 9a, 78664-36-5; 10, 78664-44-5; 10a, 78664-43-4; 11, 78664-65-0; 11a, 78664-64-9; 12, 39705-61-8; 12a, 39705-62-9; 13, 78664-49-0; 13a, 78664-48-9; 14, 78664-53-6; 14a, 78664-52-5; 15, 78664-59-2; 15a, 102940-32-9; 16, 78664-82-1; 17, 102922-71-4; 18, 6033-32-5; 19, 102922-72-5; 20, 24325-14-2; 21, 1118-90-7; 22, 34622-39-4; 23, 1155-64-2; 24, 83793-27-5; 25, 78664-72-9; Z-Ica-OPfp, 102922-75-8; Blc-OPfp, 102922-76-9; BOC-Tca-OPfp, 102922-77-0; PfpOH, 771-61-9; H-Leu-Pro-NH_2·HCl, 78664-34-3; benzyl chloroformate, 501-53-1.

Topological Similarities between a Cyclic Enkephalin Analogue and a Potent Opiate Alkaloid: A Computer-Modeling Approach

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The cyclic enkephalin analogue H-Tyr-*cyclo*[-D-N^δ-Orn-Gly-Phe-Leu-] (**1-c**) and the rigid narcotic alkaloid 7α-[(1*R*)-1-hydroxy-1-methyl-3-phenylpropyl]-6,14-*endo*-ethenotetrahydrooripavine (PEO) (**2**) were studied by using computer graphics methods to investigate potential geometrical congruencies of their respective pharmacophoric elements. Particular emphasis was placed on the relative spatial disposition of the tyramine moiety and the additional aromatic ring that occurs in both molecules. A three-dimensional vector map was generated defining the locus of the C_{21} aromatic ring for all those conformers of PEO having up to 10 kcal above the minimum energy conformer. A systematic conformational search on the cyclic peptide afforded four allowable sets of conformers whose side chain of the phenylalanine residue coincided with the vector map of PEO. Local energy minima for the peptide within the revised mutual vector space were found and subjected to bimolecular energy refinement with correspondingly local energy minima for the opiate alkaloid. Several low-energy conformers of the cyclic peptide were identified that permitted a good fit with the alkaloid provided that the tyramine moiety of the respective molecules does not coincide. In the designated conformations the basic nitrogen of the former occupies a distinct geometrical locus, and the side chain of the leucine residue has no structural correlate in the alkaloid.

Intensive studies with synthetic analogues have established the relative importance and contribution to opiate activity of the structural elements in the peptide backbone and side chains of enkephalins.[1] The molecular conformation of these opioid peptides have also been investigated extensively[2] and several theories relating to the native and receptor-bound[3,4] conformation have been proposed. Furthermore, since enkephalins and rigid and semirigid opiate alkaloids bind, albeit with different degree of affinity and selectivity, to the same receptors, several authors have alluded to the possibility of chemical and spatial equivalence of key functional groups which could serve homologous roles at the receptor level. Among the plethora of structural analogies drawn, the *p*-hydroxyphenethylamine (tyramine) moiety common to opioid peptides and narcotics of the morphine, morphinan, and oripavine class has been proposed to serve a similar pharmacophoric role in receptor recognition and activation.[5-8,11,12] However, the spatial organization of the atoms comprising the tyramine pharmacophore may be different at the receptor level for these two classes of opioids[7,10] and this may be related to differences in regional flexibility and respective chirality of the α-carbon in the tyrosyl residue in the former and corresponding C_9 in the latter category. Several authors have also suggested that the secondary structure of enkephalins permits the additional aromatic ring present in the side chain of the phenylalanine residue to serve a functional role equivalent to the C_{21} ring in the potent

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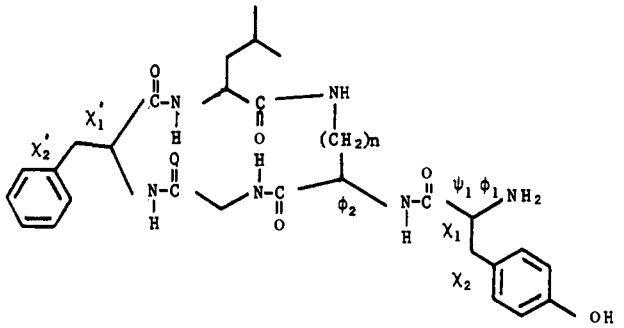
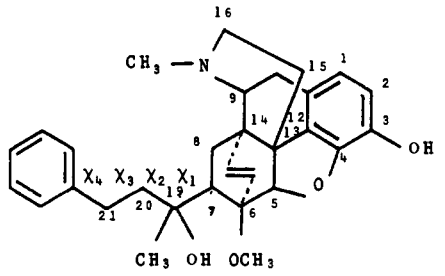


1-a, $n = 1$
b, $n = 2$
c, $n = 3$
d, $n = 4$



PEO (2)

Figure 1. Molecular structure of cyclic enkephalin analogues and PEO with conformational variables under study.

narcotic alkaloid 7α-[(1*R*)-1-methyl-1-hydroxy-3-phenylpropyl]-6,14-*endo*-ethenotetrahydrooripavine (PEO) (**2**) (Figure 1).[12,13] Presumably these analogous aromatic rings would occupy a common secondary lipophilic receptor site.[14]

Noteworthy among studies aimed at correlating biological activity of opioid peptides with molecular conformation are those dealing with conformationally constrained cyclic analogues of enkephalin prototyped by **1** (Figure 1).[15,16] It has been shown previously that these derivatives bind to rat brain opiate receptors with high affinity and that in vitro opiate activity is subject to similar structural and stereochemical factors as linear analogues.[17] Furthermore this type of cyclization confers μ-receptor selectivity, enhanced enzymatic stability, and increased in vitro potency up to several orders of magnitude.[18] These properties, together with enhanced conformational rigidity inherent in cyclic peptides,[19] render this series of opioid peptide derivatives attractive for computer modeling studies to investigate the geometrical and energetic parameters governing topological congruencies of pharmacophoric groups reported to correspond to those of model rigid opiates. In this study we report the results of our investigations by using molecular mechanics with particular emphasis on those energetically, favorable conformations of the tyramine function and side chain of the tyrosine and phenylalanine residues of the cyclic opioid peptide H-Tyr-*cyclo*[-D-N^{δ}-Orn-Gly-Phe-Leu-] (**1-c**) that permit maximum congruency with corresponding groups in PEO.

Experimental Section

Torsional angles (ϕ_i,ψ_i,χ_i) used in this study are defined by clockwise rotations around the appropriate bonds according to the convention of Klyne and Prelog.[20] Energy calculations were based on molecular mechanics allowing full bond stretching and bending modes and were executed on a Gould-SEL 32/30A host computer using SYBYL software.[21]

Conformation of PEO. The fused ring structure of PEO was constructed based on crystal data of its congener 7α-[(1*R*)-1-hydroxy-1-methylbutyl]-6,14-*endo*-ethenotetetrahydrothebaine hydrobromide (THT).[22] C_7 substituents were assumed to be tetrahedral and with standard bond lengths. Since the flexibility of the 7α-(1*R*)-1-hydroxy-1-methyl-3-phenylpropyl side chain precludes an accurate designation of the conformational preference(s) of the C_{21} aromatic ring of PEO,[23] a systematic conformational search was conducted varying the relevant torsional angles (χ_2,χ_3,χ_4) (Figure 1) by 30-deg increments. The rotation of the C_7–C_{19} bond was restricted by implementing a distance constraint of 2.5–3.2 Å between the oxygen atoms of the C_6–CH_3O and C_{19}–OH groups to simulate a hydrogen bond reported to occur between the two groups in orvinols with bulky C_{19} tertiary alkyl or arylalkyl carbinols.[24,25] A vector defined by the normal to the centroid of the C_{21} aromatic ring was constructed and a three-dimensional vector map was generated corresponding to those sterically allowed conformers whose energy was 10 kcal/mol (or lower) above the minimum energy conformation. One arbitrary PEO conformer (displayed within the resulting vector map) is shown schematically in Figure 2a.

Conformation of the Cyclic Peptide. For the opioid analogue H-Tyr-cyclo[-D-N^{δ}-Orn-Gly-Phe-Leu-] (**1-c**), the peptide backbone conformation reported by Kessler et al.[27] was invoked. The conformation of the cyclic region of the peptide is characterized by a strong hydrogen bond between the amide hydrogen of Leu^5 and the carbon oxygen of Gly^3 (Gly^3 O←H–N Leu^5, 2.99 Å) and a second between the carbonyl of Orn^2 and the δ amide hydrogen of the same residue (Orn^2 O←H–N^{δ} Orn^2, 2.93 Å). The cyclic peptide was energy minimized by using the MAXIMIN program,[28]

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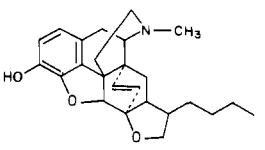

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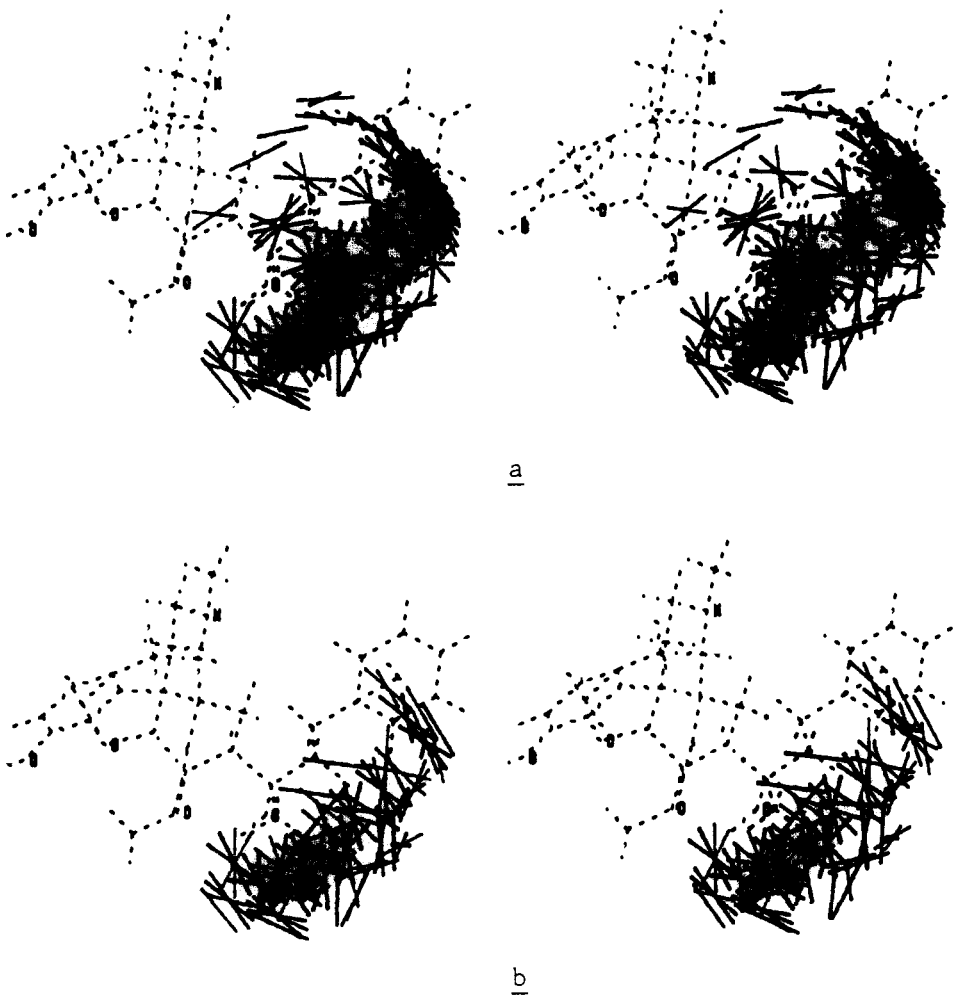

Figure 2. (a) Stereoscopic diagram of a PEO conformer displayed within a 10-kcal vector map for the C_{21} aromatic ring. (b) Same conformer shown within the reduced vector map representing the mutual region accessible to the secondary aromatic rings of peptide **1-c** and PEO.

but no major rearrangement from the starting conformation was observed. Subsequently the phenolic moieties of the peptide and PEO were superimposed and anchored while the remaining rotable bonds of the tyrosine (ψ_1,ϕ_2,χ_1,χ_2) and the phenylalanine (χ_1',χ_2') residues in the peptide were allowed to vary. In this way a similar normal to the aromatic ring of the latter was used to probe the original PEO vector map to an accuracy of 1 Å affording a considerably reduced vector map corresponding to the mutual space accessible to the second aromatic ring of both molecules.

Bimolecular Energy Refinement. The flexible fit procedure is an option of the MAXIMIN program[28] that permits the minimization of a bimolecular system such that the molecules will relax and converge to a mutual low-energy state. Two atoms (one from each molecule) comprising the congruent pharmacophoric element(s) are attached by a constant of assignable arbitrary value according to Hooke's law relationship. The separation between the two functions may be equated to a "spring", and the strength of the spring is reflected by the "spring constant" such that the two atoms relax toward superposition until the increasing the energy of distortion counterbalances the decreasing energy of the "spring". The final rms deviation of the molecular pairs with respect to the matching functionalities was 0.10 ± 0.02 Å.

In this study, for each PEO-peptide local minimum pair submitted to the flexible fit, the phenolic oxygen and the tyramine nitrogen of the cyclic peptide were attached to the corresponding chemical functions in PEO. Similarly each end of the normals to the centroid of the phenyl and phenol rings of the former were paired with the ends of the normals of the analogous aromatic rings in the latter. The "spring constants" associated with the three-point attachment were assigned an arbitrary strength of 20 except for the nitrogen which was assigned a value of 5. The choice for the points of attachment was in accordance with the receptor model of Becket and Casey[29] and refined by Lewis et al.[14]

Results and Discussion

Previous attempts to delineate important chemical functions in enkephalins have identified the phenolic moiety of the tyrosine residue as a determinant parameter for biologic activity. Furthermore, the effects of chemical modification of the phenolic group parallel closely those resulting from similar changes in rigid opiates,[6,8,11] implying similar steric and electronic roles for this functionality. Consequently, in this study the phenolic groups of **1-c** and PEO were superimposed and anchored while a systematic conformational search varying the relevant torsional angles (χ_1,χ_2,χ_1',χ_2',ψ_1,ϕ_2) of the peptide by 10–15-deg increments afforded four independent sets of stable conformers whose phenylalanyl aromatic plane coincided with the C_{21} vector space of PEO in Figure 2. The four families of peptide conformers could be most clearly differentiated on the basis of variable orientations of the ethylamine moiety (χ_1,χ_2) and different ψ_1 and ϕ_2 angles. The four sets were not interconvertible since intermediate conformers afforded structures whose phenylalanine side chain fell outside the region of the PEO vector map. The confor-

(28) MAXIMIN is a program from Tripos Associates that minimizes the potential energy of the molecule by the molecular mechanics method (force field method) and reflects the weighted sum of bond stretching, angle bending, torsional energies, and electrostatic and 1-3 and 1-4 van der Waals interactions.

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Table I. Energy Parameters of the Conformations Resulting from the Flexible Fit of Each Local Minimum of Cyclic Peptide 1-c onto Each of Five Local Minima of PEO

ref fit	molecule[b]	FLEXIBLE E[a]	MXMN E[c] PEO	MXMN E[c] peptide
1	Orn1-P1	10.99	18.78	-7.79
2	-P2	10.22	18.86	-8.64
3	-P3	8.19	17.16	-8.97
4	-P4	16.55	17.83	-1.29
5	-P5	12.75	16.89	-4.16
6	Orn2-P1	19.58	21.39	-1.82
7	-P2	14.45	20.07	-5.63
8	-P3	9.81	19.65	-9.85
9	-P4	21.92	19.50	2.40
10	-P5	18.00	18.47	-0.48
11	Orn3-P1	14.03	20.02	-6.00
12	-P2	14.41	21.55	-7.15
13	-P3	14.67	19.88	-5.22
14	-P4	27.63	25.69	1.93
15	-P5	12.29	19.07	-6.78
16	Orn4-P1	13.46	17.77	-4.32
17	-P2	14.12	20.86	-6.75
18	-P3	17.56	22.99	-5.43
19	-P4	11.73	18.14	-6.41
20	-P5	11.94	17.47	-5.54

[a] Multimolecule fitting term (kcal/mol) reflecting the quality of the fit. [b] The designation (Orn1-P1) is such that the local minimum from the first family of peptide conformers of 1-c (Orn1) is flexibly fitted on the local minimum P1 found in the reduced vector map of PEO in Figure 3. [c] MXMNE is the energy (kcal/mol) of the cyclic peptide and alkaloid in the relaxed conformation.

mational search significantly reduced the geometrical space available to the C_{21} aromatic ring of PEO and afforded the revised vector map in Figure 2b corresponding to that region mutually accessible to the secondary aromatic rings of both molecules. Five local energy minima for PEO and one local minimum for each set of cyclic peptide conformers were found within the revised vector map with energies up to 4.0 and 3.5 kcal/mol, respectively.

Table I shows the data for the bimolecular refinements resulting from the fit of each local minimum of the cyclic peptide with each correspondly local minimum of PEO using the MAXIMIN program in the multimolecule-fitting mode as described above. The quality of the fit is best represented by the FLEXIBLE E parameter while the MXMNE E designates the energies of PEO and the cyclic peptide in the resulting conformation. Table II lists some molecular parameters of the cyclic peptide in the fitted conformation designated and shows that the Gly^3 O←H–N Leu^5 hydrogen bond is severely strained during the flexible fit process especially in the case of 3, 8, and 19. Interestingly those peptide conformations with greater Gly^3 O←H–N Leu^5 distortion have correspondly lower Maximin energy and represent a better fit with respect to PEO. Therefore, the stabilization energy that may be lost by breaking the Gly^3 O←H–N Leu^5 hydrogen bond may be compensated by hydrophobic interactions of the phenylalanine aromatic ring with the hypothetical lipophilic receptor site.

Intramolecular distances between the respective aromatic rings of PEO and the cyclic peptide in the designated conformation are also shown in Table II and were obtained by using the normal to the centroid of the respective aromatic planes as reference points. The values range between 8.3 and 10.6 Å well in agreement with intramolecular distances observed in Trp^4 linear enkephalin analogues using fluorescence energy transfer measurements.[30]

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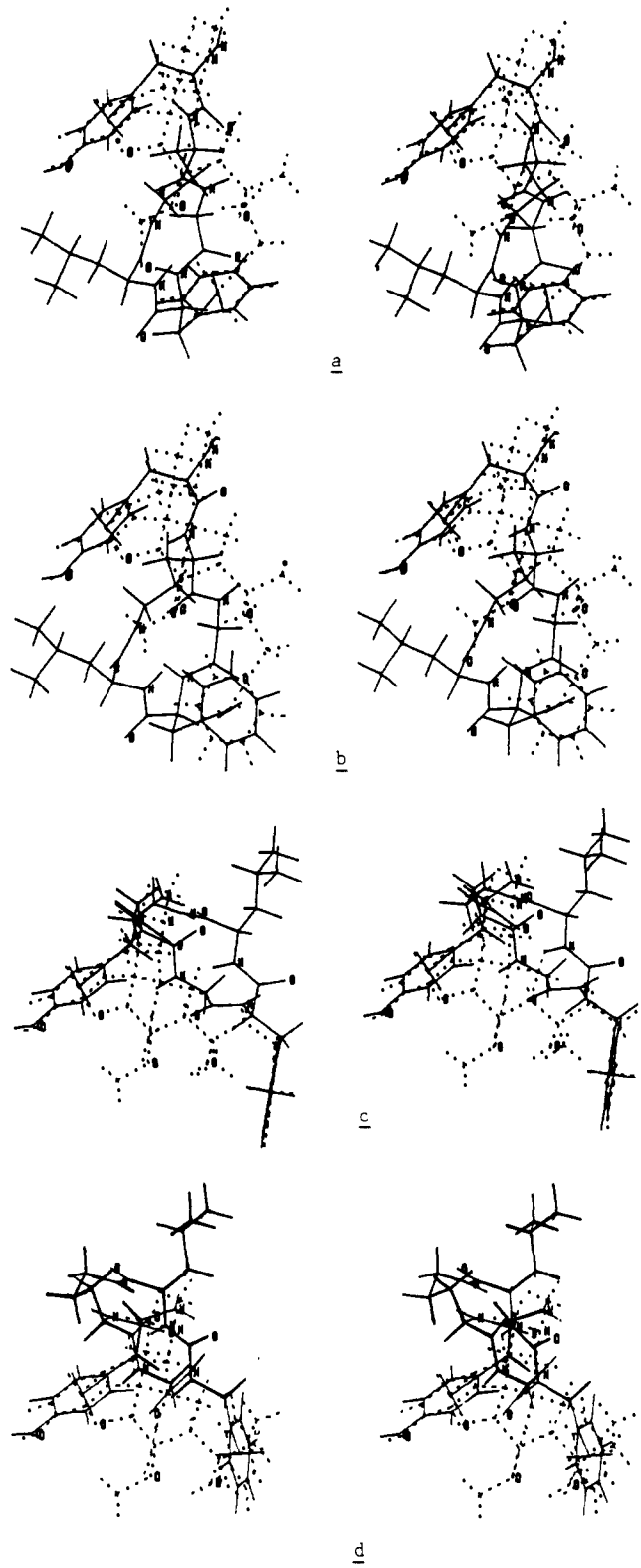

Figure 3. Stereoscopic views of four representative conformers of cyclic peptide 1-c fitted on PEO by using the MAXIMIN program in the bimolecular mode: (a) Orn1-P3; (b) Orn2-P3; (c) Orn3-P5; (d) Orn4-P4.

Figure 3 shows computer-generated stereoscopic views of four representative low-energy conformers of 1-c fitted on PEO, and a complete list of dihedral angles corresponding to the represented conformations is shown in Table III. Conformers a and b in Figure 3 are similar in that the cyclic portion of the peptide occupies the region corresponding to the α face of PEO whereas in c and d it sweeps above the β plane of the alkaloid. It is also interesting to note that in none of the peptide conformations

Table II. Geometrical Parameters of the Cyclic Opioid Peptide 1-c Resulting from the Bimolecular Energy Refinements

fit no.	tyrosine residue χ_1	χ_2	ψ_1	ϕ_2	phenylalanine χ_1'	χ_2'	distance, Å: ring → ring[a] peptide alkaloid		distance, Å: hydrogen bonding Orn O←H-N Orn[5]	Gly[3] O←H-N Leu
1	53.1	23.4	15.2	68.1	-56.1	0	9.8	9.8	2.91	3.08
2	59.8	15.3	17.3	65.6	-54.6	167.5	10.5	10.6	2.97	3.20
3	53.4	49.4	24.9	62.4	-44.0	106.4	8.3	8.3	3.04	3.75
4	67.4	10.8	-5.9	72.2	-41.2	-50.6	9.0	9.0	2.82	3.35
5	58.7	22.0	33.4	48.9	-41.3	-25.0	10.4	10.3	2.96	3.12
6	57.2	44.3	-142.4	-116.9	-46.4	-11.3	10.0	9.9	2.76	3.15
7	56.9	45.2	-162.7	-110.5	-47.9	161.9	10.7	10.6	2.78	3.27
8	75.8	56.5	-74.9	148.9	-50.3	-103.4	8.9	8.9	2.70	4.06
9	56.2	45.3	-172.6	-98.2	-37.2	-55.8	9.2	9.1	2.67	3.56
10	59.2	50.4	-174.8	-106.1	-38.0	-33.0	10.5	10.3	2.76	3.16
11	67.7	-73.8	67.6	130.1	-62.6	-120.3	9.5	9.6	2.69	3.72
12	74.4	-67.9	67.0	131.5	-56.7	76.1	10.4	10.5	2.73	3.49
13	73.7	-44.6	57.8	126.0	-46.6	130.8	8.4	8.4	2.72	3.50
14	81.2	-76.3	86.8	100.6	-52.8	-17.8	9.4	9.4	2.73	3.18
15	66.3	-76.5	93.9	123.0	-21.9	-99.1	10.1	10.3	2.60	3.71
16	62.5	-63.4	-128.2	10.9	-70.4	-107.6	9.4	9.6	2.95	3.47
17	73.3	-53.7	-125.2	14.3	-47.4	-86.7	9.9	10.3	2.88	3.09
18	68.5	-55.7	-122.6	9.8	-52.3	-100.9	9.8	9.9	2.90	3.28
19	46.4	-74.4	-96.8	0.0	-48.0	-109.2	9.2	9.3	2.83	3.98
20	68.0	-60.8	-126.0	19.6	-56.0	-105.2	10.3	10.4	2.89	3.36

[a] The centroids of the phenolic and secondary aromatic ring were used as reference points.

Table III. Dihedral Angles of Individual Residues for the Representative Conformers of the Cyclic Peptide 1-c Depicted in Figure 3

residue	angle, deg	Orn1-P3	Orn2-P3	Orn3-P5	Orn4-P4
Tyr[1]	ψ	-96.9	163.1	-22.0	147.7
	ω	-175.1	-178.5	176.7	175.5
	χ_1	175.8	-160.6	-177.4	163.4
	χ_2	-136.3	-126.6	106.7	107.8
Orn[2]	ϕ	62.4	148.9	123.0	0
	ψ	-87.0	-78.2	-69.7	-73.3
	ω	178.7	167.8	172.3	167.9
	χ_1	161.9	178.7	180.0	161.0
	χ_2	135.2	133.3	132.6	133.1
	χ_3	-77.7	-70.9	-71.0	-73.7
	χ_4	-145.3	-152.2	-146.1	-148.1
Gly[3]	ϕ	85.6	63.2	55.5	80.1
	ψ	-127.2	-103.3	-127.9	-114.9
	ω	-175.3	-164.7	180.0	-176.0
Phe[4]	ϕ	-123.1	-135.3	-117.0	-131.5
	ψ	46.8	35.5	52.1	42.6
	ω	-169.3	-165.0	-173.8	-170.4
	χ_1	-44.0	-50.3	-51.9	-48.0
	χ_2	106.4	-103.4	-99.1	-109.2
Leu[5]	ϕ	-159.5	-163.8	-157.2	-157.1
	ψ	-58.8	-55.2	-57.7	-57.2
	ω	177.4	177.6	176.0	178.7
	χ_1	172.8	171.8	172.1	171.7
	χ_2	63.2	64.5	63.4	64.1

depicted does the tyramine moiety assume an identical geometrical arrangement as the same fragment in the alkaloid. In fact the basic nitrogens of the four peptide and PEO conformers shown are located in different geometrical loci approximately 1.1 Å apart. Similarly, the distance between the basic nitrogen and the center of the phenolic ring was found to be 5.1 Å without significant variation and contrasts with the value of 4.6 and 4.3 Å for morphine and *N*-allylnormetazocine, respectively.[5] Accordingly the tyramine moiety of the cyclic peptide may be found in a more extended conformation. If a perfect fit of the tyramine functions is imposed on the two molecules, the aromatic ring of the phenylalanine residue in the peptide is redirected away from the region of the PEO vector map, and only high-energy conformers emerge. This finding is in agreement with the observations of Ramakrishnan and Portoghese[10] showing that a synthetic hybrid of enkephalinamide and metazocine, sharing a common tyramine moiety, was inactive. Similarly, other reports demonstrate that N-positional isomers of benzomorphans show retention and, in some cases, enhancement of in vivo analgesic activity,[31,32] suggesting that the nitrogen locus for opiate receptor activation is variable. Because the basic nitrogens are located in different stereochemical environments, it is tempting to rationalize the absence of antagonist properties by simple N-allylation of the peptide on the basis of different stereoelectronic properties directed in different receptor regions. However, it is now well-known that N-diallylation of certain linear enkephalin derivatives does confer antagonist properties[33] while antagonist activity is anomalous in the 6,14-*endo*-ethenotetrahydrooripavine series of analgesics.

We have shown previously that there exists significant variation in receptor selectivity within the series of cyclic enkephalin analogues shown in Figure 1, and we concluded that μ and δ receptors may be distinguished on the basis of different conformational requirements.[34] Examination of the stereodiagrams in Figure 3 reveals that the Leu[5] residue has no structural correlate in the opiate alkaloid contrary to previous reports.[12,37] As a corallary, μ and δ receptor discrimination may be modulated by the structure and stereochemistry of this residue in combination with the relative position of the ethylamine moiety in the tyrosine unit. For example in the D-Lys[2] homologue **1-d**, a different intramolecular hydrogen-bonding arrangement has been reported[35] involving the carbonyl oxygen of Gly[2] and the amide hydrogen of Leu[5] (Gly[2] O←H-N Leu[5]). Conceivably this property, together with chain lengthening

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in position two, may perturb the geometry of the ethylamine fragment and realign the orientation of the Leu[5] side chain differently from **1-c** once the phenolic and phenyl rings bind to their respective sites affording a conformer with enhanced μ-receptor preference. In accordance with this hypothesis is the observation that the D-Leu[5] diastereomer of **1-d** has considerably reduced selectivity (by a factor of 4 based on the IC_{50} ratio between the MVD and GPI assays).[16]

Conclusion

In the series of 7α-(1-hydroxy-1-methylphenalkyl)oripavines maximal analgesic activity has been reported to be associated with a 19-phenylpropyl chain as in PEO. The fact that analgesic potency is drastically reduced by shortening or lengthening the phenylalkyl chain implicates an additional well-defined binding site. We found that the conformation reported for the cyclic opioid peptide H-Tyr-*cyclo*[-D-N^5-Orn-Gly-Phe-Leu-] provides the possibility of topographical congruency between the C_{21} aromatic ring in PEO and that of the phenylalanyl side chain of the peptide. Accordingly, the two may interact with a common lipophilic receptor site. Low-energy peptide conformers were obtained by a bimolecular energy minimization procedure with PEO, and it was found that the ethylamine fragment of the tyramine moiety in the former assumes a different conformation than its counterpart in the latter such that the basic nitrogens occupy distinct spatial loci approximately 1.1 Å apart. The variation in receptor selectivity among the members of the homologous series shown in Figure 1 may arise from subtle differences in backbone conformation which governs the direction of the leucine side chain and the geometrical orientation of the ethylamine fragment once the phenolic and phenylalanyl rings are bound to the receptor.

Previous conformational comparisons of flexible opioid peptides with rigid opiates resulted in intermediate- to high-energy conformers for those peptides having maximum resemblence to rigid alkaloids.[36] Since the rigidity of the cyclic opioid derivative **1-c** precludes any major backbone rearrangement, we found that an alternate approach is to anchor the minimal common element (the phenolic group) and attach the basic nitrogens and the secondary aromatic rings of the respective molecules by "spring constants" followed by the relaxation. Since the procedure balances mutual spatial orientations of pharmacophoric elements, it may be regarded analogous to an induced fit.

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In Vitro Labeling of Serotonin-S_2 Receptors: Synthesis and Binding Characteristics of [^{3}H]-7-Aminoketanserin

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[^{3}H]-7-Aminoketanserin (7-amino-3-[2-[4-(2-tritio-4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4-(1*H*,3*H*)-quinazolinedione), an amino derivative of the selective serotonin-S_2 antagonist ketanserin, was synthesized and tested for in vitro labeling of serotonin-S_2 receptors. The compound showed a very high affinity for both membrane-bound and detergent-solubilized serotonin-S_2 receptors with K_D values of 0.35 and 2.03 nM, respectively. At nanomolar concentrations, binding to serotonin-S_1 sites was totally absent. Serotonin-S_2 receptor binding was characterized by a slow dissociation and a very low nonspecific binding. In rat frontal cortex preparations, binding could be displaced by nanomolar concentrations of different serotonin antagonists and micromolar concentrations of serotonin agonists. Compounds with other pharmacological profiles were poorly or not active. Introduction of an amino function in this new radioligand led to a decreased lipophilicity. Therefore, besides being a valuable radioligand for routine binding studies, [^{3}H]-7-aminoketanserin will probably be a good ligand for labeling serotonin-S_2 receptors on intact cells.

Serotonin-S_2 receptors have been identified in the central nervous system as well as in the periphery. These receptor sites were shown to mediate the antagonism of various serotonergic effects, measured both in vivo and in vitro, such as behavioral excitation in rodents induced by serotonin mimetic agents ("the serotonin syndrome"), serotonin-induced contractions in isolated arteries, and serotonin-induced shape changes and aggregation of blood platelets.[1]

Several radioligands have been used for the in vitro biochemical characterization of serotonin-S_2 receptors, including [^{3}H]spiperone,[2] [^{3}H]LSD,[3] [^{125}I]LSD,[4] [^{3}H]mianserin,[5] and [^{3}H]metergoline.[6] However, most of these ligands were either not selective for serotonin-S_2 sites or showed a too high nonspecific binding. Recently [^{3}H]-ketanserin was introduced as a high-affinity, selective

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