Articles

Conformation of 2,9-Dimethyl-3′-hydroxy-5-phenyl-6,7-benzomorphan and Its Relation to Other Analgetics and Enkephalin

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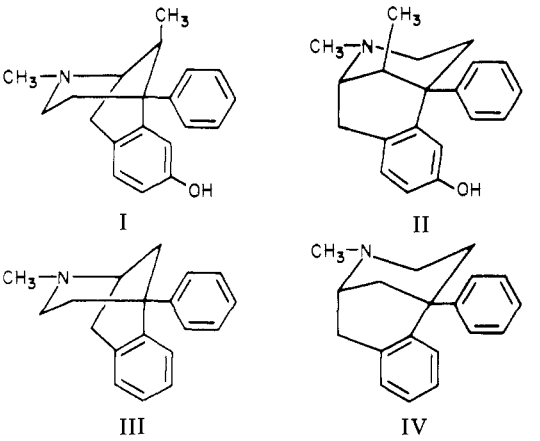
X-ray crystallographic data for 2,9-dimethyl-3′-hydroxy-5-phenyl-6,7-benzomorphan (I) as its *p*-bromobenzoyl ester are presented. The structure of I is compared with that of morphine, meperidine, α-allylprodine, methadone, and moramide as well as with a proposed structure of the enkephalins. A quantitative relationship is found between in vitro opiate receptor binding potency and in vivo analgesia for analgesics of diverse structure, including I. A new view of the analgetic pharmacophore is presented. Programs for the TI Programmable 59 calculator are described for conversion of X-ray crystallographic data to rectangular coordinates with reorientation of the molecule and for the calculation of torsion angles.

Elucidation of the conformation of the analgetic pharmacophore is one of the most challenging problems in medicinal chemistry. The original three-center proposal of Beckett and Casey[2] applied to what was then known about the conformations of the rigid morphine analgetics, the phenylpiperidines, and the diarylpropylamines. Later Portoghese[3] postulated that phenolic and nonphenolic analgetics may bind in two different modes, respectively, to the same receptor or to multiple receptors. More recently Feinberg et al.[4] used molecular models as a basis for suggesting additional analgetic binding sites and proposed that the agonist receptor has different structural requirements for binding than the antagonist receptor. In a very recent paper Galt[5] discussed some of the difficulties in these and other proposals and suggested explanations of the anomalies.

We have examined the X-ray crystallographic data for a number of synthetic analgetics in the light of similar data for 2,9-dimethyl-3′-hydroxy-5-phenyl-6,7-benzomorphan[6] (GPA 1657, I) (Chart I). Our findings indicate that, in addition to the basic nitrogen and the two phenyl substituents of many synthetic analgetics, a carbonyl group is an important structural feature. We have related the compounds we studied to the enkephalins and propose a conformation that seems consistent with the published data. This conformation provides a model for the analgetic pharmacophore. The phenolic and nonphenolic analgetics would fit this pharmacophore in different modes.[3a] Our proposal seems to apply to the explanation of Galt[5] and, in addition, provides a reason for the requirement of a nitro group in etonitazine.[7] Although our proposal seems to be satisfying as far as it goes, it must still be tested against all analgetics and with new data that is rapidly becoming available. It does not provide an explanation for the structural requirements for binding to the analgetic antagonist receptor.

We first examined the conformation of I (Figure 1, b). We have already noted that the 5-phenylbenzomorphan analgetics, such as I, provide a structural link between morphine and meperidine.[8] Gorin and Marshall[9,10] and, more recently, Galt[5] have pointed to the conceptional link between I and the more flexible analgetics. We noted that two of our nonphenolic 5-phenylbenzomorphans, namely III and IV (Chart I), are also potent analgetics,[11] and, since they have the same rigid skeleton as I, they should be even more informative concerning the conformation of nonphenolic analgetics.

Chart I



For the determination of its X-ray crystallographic structure I was converted to its *p*-bromobenzoyl ester (VI).[6] The benzomorphan skeleton is rigid so that the conformation of the ester will apply as well to the conformation of I. Since I was shown[6] to be of the same absolute configuration as morphine,[12] these two compounds are representative of the rigid analgetics. We selected the salts of meperidine[13] and (+)-α-allylprodine[14] to represent the piperidine analgetics and methadone[15] and dextromoramide[16] as examples of flexible analgetics. X-ray crystallographic data for these compounds should provide definitive information for at least one stable conformation that is relevant in solution at physiological pH. Shefter has noted that even for the acyclic analgetics the degree of conformational flexibility is very limited.[17]

The X-ray crystallographic data were first transformed to orthogonal coordinates,[18] expressed in ångströms with the quaternary carbon atom at the origin. Then each molecule was rotated about the Z and Y axes, respectively, until the nitrogen atom lay on the $+X$ axis. A final rotation about the X axis placed the carbonyl oxygen in the $X-Y$ plane. For I the 9-methyl group was placed in the $X-Y$ plane. Morphine was oriented so that its phenolic benzene ring was in the same orientation about the X axis as that of I. The coordinates of each nonhydrogen atom were then calculated and expressed in centimeters using the scale of 1.25 cm/Å. In the course of these studies we developed calculator programs which simplify the construction of molecular models from X-ray coordinate data.

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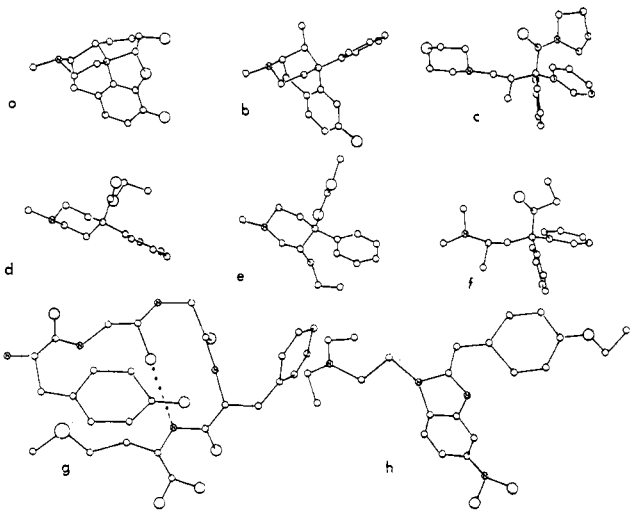


Figure 1. Comparison of analgetic conformations: nonhydrogen atoms of analgetic molecules drawn from X-ray crystallographic data for their salts except for enkephalin and etonitazine; nitrogen atoms indicated by N; oxygen atoms by larger circles; sulfur atoms by largest circle. (a) morphine, (b) I, (c) *d*-moramide, (d) meperidine, (e) α-allylprodine, (f) methadone, (g) Met-enkephalin, (h) etonitazine.

These are described below. Drawings were prepared from the $X-Y$ coordinates and were used for the construction of color-coded skeletal (CCS) models.[19-21] The CCS models are especially convenient for our purpose because they retain a rigid conformation and because they are readily converted to the corresponding space-filling models without hiding the skeletal bonds. The torsion angles provided by Shefter[17] were helpful in constructing models of the diphenylpropylamine analgetics. Perspective drawings prepared from the graphs of the compounds studied are shown in Figure 1, a–f.

The coordinate transformations were performed on an SR-52 calculator (Texas Instruments Inc.). With the introduction of the TI Programmable 59 calculator,[22] the original programs have been revised and condensed. They are found as Tables V and VI in the microfilm edition of this Journal (see paragraph at end of paper regarding supplementary material). Table V provides a program for coordinate transformations from original X-ray crystallographic data to orthogonal coordinates. In the new coordinate system the molecule is placed in any desired position by appropriate rotation about the orthogonal axes. The program may be used for orthorhombic or monoclinic crystals and the output may be adapted for any scale. Table VI facilitates model building by providing a program for the calculation of torsion angles from orthogonal coordinates. The program is convenient to use because it accommodates branches and because the coordinates of each atom are entered only once when successive angles along a chain of atoms are calculated.

It is apparent from inspection of the models and the drawings of Figure 1, c–f, that the carbonyl oxygen of the flexible analgesics could correspond to the ester carbonyl oxygen of meperidine and allylprodine if the piperidine ring of these molecules were oriented "sideways" to its orientation in the benzomorphans. The drawings of Figure 1 illustrate this and other features that could interact with a common analgetic receptor. Each of the analgetics has a basic nitrogen atom separated from one or two phenyl groups which are held in a particular orientation by a central atom. For the less rigid piperidine and acyclic analgetics there is a carbonyl oxygen atom in a particular orientation relative to the other groups. The positions of the carbonyl oxygen atoms are noted in Table I. The centers of these oxygen atoms all lie within an area of radius 1.0 Å (the radius of a hydrogen atom), suggesting that they could accept a hydrogen bond donated by the receptor. The position of the basic nitrogen varies from 2.8 to 3.9 Å from the origin of the coordinate system. Perhaps the nitrogen cation interacts with a carboxylate anion on the receptor. Since the oxygen atom is large (2.7 Å in diameter) the receptor could accommodate the variation in the distance of the nitrogen atom from the quaternary carbon atom that is seen in Table I. This idea is similar to that proposed by Berkowitz and Loew.[23] The two phenyl groups appear to have variable positions, although they are always in the same general area and both are not required for analgesia as long as there is a carbonyl group or a rigid framework to hold one phenyl group in position, as in the 5-phenylmorphans.[8]

Table I. Molecular Parameters[a] of Analgesics

Compd	N-(X)	O-(X)	O-(Y)	N-O
Morphine	2.81			
GPA 1657	2.90	(1.07)	(−2.37)	(3.01)
Meperidine	2.92	−0.75	−2.32	4.34
α-Allylprodine	2.95	−0.88	−2.56	4.61
Methadone	3.84	0.78	−2.27	3.81
Moramide	3.90	0.80	−2.29	3.85
Enkephalin	2.80	0.00	−1.60	3.22

[a] Molecular parameters are the X and Y coordinates in ångströms of the nitrogen and oxygen atoms in the $X-Y$ plane of the compounds in Figure 1. (For I the 9β-CH_3 replaces the oxygen.) The N-O distances were calculated from these coordinates.

One point is of special interest. The cationic hydrogen atom on the piperidine analgetics of Figure 1 faces in the Y direction, perpendicular to that of the salts of morphine and the benzomorphans. Opheim and Cox[24] have suggested that only the cation and not the cationic hydrogen atom is required for analgesia. In support of this suggestion, they found that the quaternary salt, *N*-methyllevorphanol, interacts with opiate receptors in vitro although it would not be expected to penetrate the central nervous system in vivo. Following their example we prepared the quaternary *N*-methyl derivative (V) of I and showed that it too binds to the opiate receptor (Table II), although with diminished potency relative to I. In the crystalline state the cationic hydrogen atoms of methadone and moramide salts also face in directions different from that of the morphine salt. Although these molecules are flexible and could have a different orientation at the receptor site, we support the concept that only the cationic nitrogen is required for analgesia.

In the proposal described above for the relationship between these analgetics it is assumed that metabolites are not responsible for the analgetic activity. This assumption is supported by the demonstration that there is a quantitative correlation between in vitro binding to the opiate receptor from brain tissue and in vivo analgetic activity. Jacobson et al.[25] correlated interactions with the opiate receptor in vitro with analgetic potency in vivo for a diverse series of analgetics of rigid and flexible structures. There was a wide variation in the lipophilicity of the compounds they selected so that it was necessary to consider partition coefficients to obtain a reasonable correlation. We used their method and compared a number of the compounds they studied with I–IV. Our results are summarized in Table II.

We found a good correlation between in vivo analgetic potency and in vitro opiate receptor binding capacity for our series of analgetics without considering partition

Table II. Correlation[a] of Analgesia[b] with Opiate Receptor Binding[c]

No.	Compd[d]	Mol wt	IC_{50}, μM	ED_{50}, mg/kg	Log $1/IC_{50}$	Log $1/ED_{50}$ obsd	Log $1/ED_{50}$ calcd	Δ
1	Levorphanol	443.44	0.0045	0.24	2.35	3.27	3.07	+0.20
2	Morphine	379.41	0.022	1.0	1.66	2.58	2.77	−0.19
3	Methadone	345.90	0.051	0.9	1.29	2.58	2.61	−0.03
4	Meperidine	283.79	10.0	7.0	−1.00	1.61	1.64	−0.03
5	I	329.91	0.019	0.5	1.72	2.82	2.80	−0.02
6	II	329.91	10.0	4.2	−1.00	1.90	1.64	+0.26
7	III	413.47	0.6	5.5	−0.22	1.88	1.97	−0.09
8	IV	413.47	3.0	8.0	−0.48	1.71	1.86	−0.15
9	V	435.33	0.32					
10	Met-enkephalin	573.74	0.09					
11	Leu-enkephalin	592.16	1.0					

[a] Log $1/ED_{50}$ = 2.06 (0.16) + 0.43 (0.12) log $1/IC_{50}$; r = 0.964, s = 0.173, n = 8. [b] Analgetic potency in mice determined by the phenylquinone writhing test following subcutaneous administration of the test compound. [c] IC_{50} is the concentration of the test compound which reduces specific [^{3}H]naloxone binding by 50% in the absence of sodium. [d] Standard compounds: levorphanol tartrate dihydrate; morphine hemisulfate pentahydrate; *dl*-methadone hydrochloride; meperidine hydrochloride. I and II are hydrochloride salts; III and IV are tartrate salts; and V is the methiodide quaternary ammonium salt of I. See Chart I for structures.

coefficients. Data for the binding of Leu-enkephalin and Met-enkephalin are included in Table II. Although Leu- and Met-enkephalin have poor analgetic activity on parenteral administration because of rapid degradation by metabolizing enzymes,[26] Roemer and his associates[27] have shown that enkephalin analogues that resist such degradation are potent analgetics, both parenterally and orally. We conclude that the representatives of the rigid analgetics as well as the piperidine and acyclic analgetics we examined all bind to the opiate receptor without structural alteration and that this is the same receptor which accepts the enkephalins. It is therefore of great interest to try to discern what features these synthetic analgetics have in common with the enkephalins.

The conformation of the enkephalins has been the subject of a number of investigations involving spectroscopic measurements in solution[28-36] as well as model building and theoretical calculations,[37-41] including those based on structure–activity relationships of enkephalin analogues.[42] The conformation deduced by Isogai et al. on theoretical considerations[41] involves a βII′ bend[43] with a hydrogen bond between the NH of residue 5 and the C=O of Gly^2. These authors have noted that this conclusion is consistent with the NMR data of Anteunis et al.[32] and of Jones et al.[29] In a later paper Jones et al.[34] reported that their data are applicable only to the zwitterionic form of the enkephalins which is the physiological form. A very recent report by Beddell and his associates[42] shows that from theoretical considerations the βII′ bend is consistent with structure–activity relationships (although they report that other bends would also be consistent). We noted that the illustration of Lewis et al.[44] for the βII′ bend involves the residues of Tyr-Gly-Phe-Leu at positions 277–280 of carboxypeptidase A. The last three of these residues would be involved at the same positions in the βII′ bend for Leu-enkephalin. Furthermore, Chou and Fasman[45] have shown recently that a tyrosine residue often occurs immediately before a β turn in the enzymes of established conformation. Thus, we conclude that a βII′ bend with an N–H(5) C=O(2) hydrogen bond is possible in the physiologically active conformation of Met-enkephalin. In building a model of Met-enkephalin we selected a conformation in which the orientation of the side chains of Tyr^1, Phe^4, and Met^5 offered an explanation for the structure–activity relationships of the synthetic analgetics. Isogai et al.[41] placed the tyrosine residue "behind" the rest of the molecule to form a hydrogen bond between the phenolic hydroxyl and the Gly^3 carbonyl. Khaled et al.[35] placed the tyrosine "in front" with a similar hydrogen bond to the Gly^3 carbonyl but they chose a βII turn. The βII turn can be excluded on theoretical grounds[44] and there is a low probability of its occurrence on the basis of an analysis of the conformation of proteins of known structure.[45] It is well known that a free phenolic hydroxyl group is an important feature of many analgetics and it is also required for activity in the enkephalins.[26] A phenolic hydrogen that was bonded to a carbonyl group would not be free to bind to the receptor. Accordingly, we have chosen a βII′ conformation of enkephalin in which the tyrosine is "in front" of the molecule. It is tempting to place the oxygen of the phenol in a position such that one of its free electron pairs could accept a hydrogen bond from the NH of Phe^4. A weak hydrogen bond of this nature occurs, for instance, between the peptide NH of Gly^{155} and the phenolic oxygen of Tyr^{248} in carboxypeptidase A.[46] However, Roemer and his associates[27] report that an enkephalin analogue in which there is a methyl substituent on the N of Phe^4 is a very potent analgetic. Furthermore, in our view, the 5-phenyl of I and the bulk of 7-(1-phenyl-3-hydroxybutyl-3)endoethenotetrahydrothebaine, "PET",[4] would fit more within the analgetic pharmacophore if the tyrosine of enkephalin were below the position required for hydrogen bonding to the NH of Phe^4. Since PET is one of the most potent analgetics,[47] we have placed the phenol of Tyr^1 in front of the plane of the βII′ turn and midway between Gly^2 and the Met^5 (see Figure 1, g). Our model would place the sulfur atom of the methionine residue beneath the NH of Gly^2 and close enough to form a hydrogen bond. One explanation of the observed low potency ratio (0.01) of [des-Met^5]enkephalin is that the methionine residue is necessary for stabilizing the biologically active conformation.[36] One of the phenyl groups of the nonphenolic analgetics would fall in this position when it occupied the analgetic receptor (compare Figure 2, a–c, with Figure 2, d). Data based on fluorescence spectroscopy[36] indicate that the Phe^4 phenyl is 10.0 ± 1.1 Å from the aromatic ring of Tyr^1. Thus, we support a representation in which the Phe^4 phenyl extends outward away from the rest of the molecule[41] (Figure 2, d and e). The carboxylate anion in our model would be at the "bottom right-hand corner" of the molecule and would be an important feature for receptor binding. Our model of Met-enkephalin is illustrated in Figure 1, g, and Figure 2, d and e. The drawings of Figure 2 depict a skeletal model and a CPK space-filling model, viewed from "above". The molecule is shown as

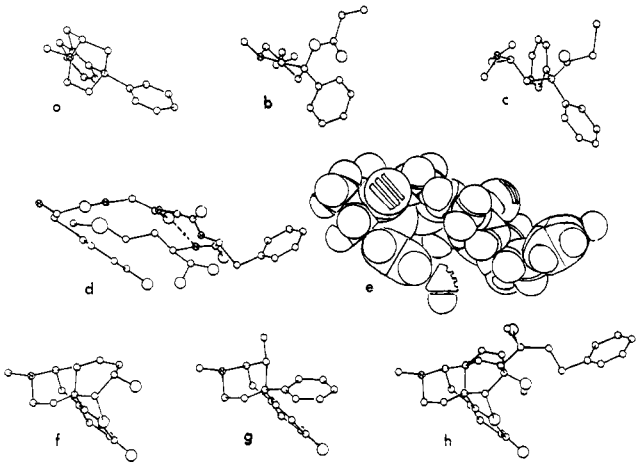


Figure 2. Comparison of analgetic conformations: (a) III, (b) α-allylprodine, (c) methadone, (d) Met-enkephalin from CCS model, (e) Met-enkephalin from CPK model, (f) morphine, (g) I, (h) PET. f, g, and h are drawn with the phenolic moiety in the orientation of enkephalin. Nitrogen atoms indicated by N, oxygen atoms by larger circles, and sulfur atoms by largest circle.

a box-like shape with the tyrosine in front, the βII′ hydrogen bond in the back, and the methionine at the bottom. The tyrosine NH_3^+ is at the extreme left and the Phe[4] phenyl at the extreme right of the molecule. Although many features of this model of enkephalin are speculative, it does offer a rational interpretation for the structure–activity relationships of many series of analgetics of diverse structure.

The phenyl group in the *X*–*Y* plane of Figure 2, a–c, of III (Chart I), α-allylprodine, and methadone, respectively, falls within the space occupied by enkephalin, Figure 2, d. Models show that the comparable phenyl group of meperidine and moramide falls in the same area. As shown in Figure 2, a–c, the second phenyl group of III and of the acyclic analgetics, such as methadone, and the allyl substituent of α-allylprodine are oriented in the direction of the *–Z* axis. When viewed along the *Z* axis, this position is within the space occupied by enkephalin, Figure 2, d. Models show that this position, when viewed along the *Y* axis, could correspond to the location of the sulfur atom of Met-enkephalin or the isopropyl group of Leu-enkephalin.

In this concept of the analgetic pharmacophore the nitrogen cation and the carbonyl oxygen are involved in strong interactions with the receptor while the phenyl groups supply bulk in the right places and hold the molecules semirigid. The *d* and *l* isomers of the nonphenolic benzomorphans, III and IV, respectively (Chart I), both bind to the analgetic receptor and are analgetics of comparable potency in vivo (see Table II) because both of their phenyl groups fall within the analgetic pharmacophore.[5] As Portoghese and Shefter have noted,[14] the allyl group of β-allylprodine may not fall entirely within the pharmacophoric space and the compound is much less active as an analgetic.

Horn and Rodgers have pointed out[37] that the phenol of morphine probably corresponds to the tyrosine of the enkephalins. To view the molecule so that this feature is more apparent, the morphine coordinates were calculated with the nitrogen atom at the origin, the phenolic oxygen along the *X* axis, and C-2 in the *X*–*Z* plane to correspond to the tyrosine moiety of Figure 2, d. The *X*–*Y* coordinates were then plotted and drawn as in Figure 2, f. As seen from the drawings morphine fits very well into the same space as enkephalin occupies. Its oxygen atoms and the 7,8 double bond are located in the same areas as the electron-rich groups of enkephalin.

I fits into the analgetic pharmacophore in much the same manner as morphine, with its 5-phenyl group within the area occupied by the electron-rich groups of enkephalin (Figure 2, g). It is interesting to see that these rigid molecules can be made to fit in the space occupied by enkephalin because both morphine and I have the opposite absolute configuration to tyrosine at its α-carbon atom.[48] They could be regarded as being derived from D-tyrosine instead of the natural isomer.[37,49]

The optical isomer of I, compound II (Chart I), is less potent as an analgetic,[6,8] probably because its 9β-methyl substituent falls outside the analgetic pharmacophore.[5] Our concept of the analgetic pharmacophore, in contrast to that of Gorin and Marshall,[9,10] does not envisage that Phe[4] of the enkephalins corresponds to the 5-phenyl of I. In fact, fluorescence emission spectral data[36] and models show that Phe[4] of the enkephalins could correspond to the phenyl of the extremely potent 6,14-endoethenotetrahydrothebaine derivative, "PET", discussed by Feinberg et al.[4] However, if so, this is not the "F position" described by these authors. The entire complex molecule of PET can conform to a shape that nearly fills the same volume of space as our model of Met-enkephalin with the basic nitrogen at one end and the phenyl in the corresponding position at the other end of the molecule. This is shown clearly in Figure 2, h.

In summary, the phenolic benzomorphan and morphinan analgesics fit very well into the space occupied by the first three amino acids of the enkephalins in the conformation depicted in Figure 1, g, and Figure 2, d and e. The phenolic moiety of the benzomorphans and morphinans corresponds to the tyramine portion of the enkephalins.[38] Nonphenolic benzomorphans, the piperidine analgetics, as well as the nonrigid analgetics of the diphenylpropylamine type could fit this analgetic pharmacophore in a somewhat different manner. For those analgesics with a carbonyl oxygen atom the C=O may correspond to the C=O of Tyr[1] of the enkephalins. This concept might provide a molecular basis for the differing modes of analgetic receptor association discussed by Portoghese in 1965.[3a]

Much of the recent discussion of Galt[5] regarding the conformation of the many classes of synthetic analgetics applies to our concept of the analgetic pharmacophore. One interesting aspect, however, that has never been explained is why the nitro group of etonitazine is essential for its potent analgetic[7] and opiate receptor binding[50] activity or why replacement of the chlorine atom in methopholine with a nitro group should produce a more potent analgetic.[51] A CPK model of the nitro group looks very much like a carboxylate anion which must be present in the zwitterionic (physiological) form[34] of enkephalin. When nitro and carboxylate groups of models of etonitazine and enkephalin, respectively, are placed in corresponding positions, the basic nitrogen atoms can be made to correspond exactly and in this position the ethoxy group of etonitazine just reaches the space occupied by the phenyl of enkephalin. The entire molecule of etonitazine falls within the "envelope" of the enkephalin pharmacophore (Figure 1, h). The structure–activity relationships of analogues of etonitazine discussed by deStevens[7] seem to find explanation in this model. The nitro analogue of methopholine is a more potent analgetic than methopholine itself.[49] Although it is a smaller molecule than etonitazine and does not completely fill the envelope of the enkephalin pharmacophore, its dimethoxyisoquinoline moiety falls within the electron-rich region of enkephalin

when the basic nitrogens and the nitro and carboxyl groups, respectively, are placed in corresponding positions.

We realize that the model proposed in this paper is largely speculative. However, it offers a basis for further experiments and it will be interesting to see whether it will survive as additional data are obtained.

Experimental Section

(1) (a) **Preparation of V (*l*-3,11-β-Dimethyl-8-hydroxy-6-phenyl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocine Methiodide).** GPA 1657 as the free base generated from the hydrochloride salt (2.93 g) was refluxed in 30 mL of methanol and 35.5 g of methyl iodide for 1 h and then brought to dryness under reduced pressure to leave a crystalline residue. The residue was recrystallized from about 75 mL of methanol to obtain 2.40 g of off-white prisms: mp 280–281 °C dec; $[\alpha]^{24}_D$ −76° (*c* 0.84, MeOH). Anal. Calcd for $C_{21}H_{26}INO$: C, 57.94; H, 6.02; N, 3.22; I, 29.15. Found: C, 57.96; H, 6.05; N, 3.41; I, 29.13.

(b) **Met-enkephalin** was obtained from Penninsula Laboratories, San Carlos, Calif.

(c) **Leu-enkephalin** was prepared as the hydrochloride by W. Rittel, CIBA-GEIGY Ltd., Basel.

(2) (a) **Mouse Phenylquinone Writhing Test.** A short version of the procedure of Tabor et al.[52] was used. At various time intervals after subcutaneous administration of the test compound the animals (male mice, 14–25 g) received 0.1 mL/10-g body weight of a solution[53] (0.25 mg/mL) of phenyl-*p*-quinone (Eastman) made in 5% aqueous ethanol, intraperitoneally (25 mg/kg). Five minutes later the animals were placed in observation cages and the number of animals which did not perform a characteristic writhe during the next 10 min was recorded. Under these conditions, this dose of phenylquinone has been demonstrated to induce one or more writhes in 95% of the mice in a large separate control study. Test compounds were administered subcutaneously in a volume of 0.1 mL/10-g body weight in an appropriate vehicle. Mice received food and water ad libitum up until the time of testing. Ten mice were used for each dose of each agent at each time. Statistical analyses on the quantal data (number of mice not writhing/total number of mice tested) were performed by the logit method of Berkson.[54]

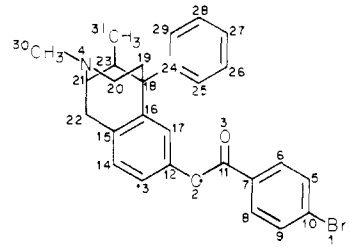
(b) **Opiate Receptor Binding Procedure.**[55,56] Membrane suspensions were prepared from rat brain and incubated with [^{3}H]naloxone. Male Sprague–Dawley rats (200–300 g) were decapitated and their brains were rapidly removed. The cerebellum was dissected and discarded; the remainder of the brain was rapidly weighed and homogenized immediately in 19 vol of ice-cold 50 mM Tris–HCl (pH 7.4) with a motor-driven Teflon-pestle homogenizer. The homogenate was centrifuged at 105 000g for 30 min at 0–5 °C; the pellet was resuspended in Tris–HCl, incubated at 37 °C for 30 min, and recentrifuged. It was then suspended in 200 vol of cold Tris–HCl (based on the original tissue weight), placed in an ice bath, and used promptly for binding assays.

Sets of triplicate 2-mL aliquots of the membrane suspension were preincubated at 35 °C for 5 min: one set of tubes contained membrane suspension alone, one contained 10 μM levorphanol, and the others contained varying concentrations of test compound. The tubes were then cooled in an ice bath for 5 min, [^{3}H]naloxone (New England Nuclear, approximately 10 Ci/mM) was added to a final concentration of 8 nM, and incubation at 35 °C was resumed for 15 min. The test suspensions were returned to an ice bath for 15 min and then filtered under vacuum through Whatman glass fiber filters (GF/B). The filters were washed three times with 6-mL portions of cold Tris–HCl, transferred to scintillation vials, and counted for radioactivity in 10 mL of Hydromix.

Saturable, specific binding was calculated by subtracting the value obtained in the presence of 10 μM levorphanol (nonsaturable, nonspecific binding) from that obtained with membrane suspension alone (total binding) and membrane suspension plus test compounds. IC_{50} values (concentrations of test compounds required for 50% inhibition of the specific binding of 8 nM [^{3}H]naloxone) were determined graphically on log–probit paper.

(3) **X-ray Crystallography.** For the X-ray crystallographic study GPA 1657 was converted to its *p*-bromobenzoyl ester,[6] VI ($C_{27}H_{26}BrNO_2$). The colorless needles obtained by recrystallization from 2-propanol are orthorhombic, space group $P2_12_12_1$, with a = 9.83 Å, b = 8.87 Å, and c = 26.30 Å; Z = 4; density (calcd) = 1.38 g cm^{-3}.

Chart II. Numbering for VI



Intensity data was collected by the multiple film method on an integrating Nonius–Weissenberg camera for the layer lines *h*0*l* to *h*8*l* as well as 0*kl* and 1*kl* using nickel-filtered copper radiation (λ = 1.5418 Å). For 1449 of the theoretically accessible 2440 reflections the intensity could be estimated visually by comparison with a series of calibrated exposures. The other 991 reflections were treated as unobserved. Corrections were applied for α_1/α_2 splitting and Lorentz–polarization effects but not for absorption. The structure was solved by the heavy-atom method. The electron density map calculated with the phases obtained from the bromine position yielded 26 plausible positions for 30 C, N, and O atoms. A few cycles of block-diagonal least-squares refinement on 26 carbon atoms at these positions reduced the R index to 26%. A difference Fourier synthesis confirmed 23 of the previous atoms and showed plausible positions for the other seven atoms. Some additional cycles of block-diagonal least-squares refinement let the R index drop to 17%. At this stage the temperature factor for bromine was made anisotropic and provision was made for the anomalous part of the bromine scattering factor. Some more cycles of least-squares refinement with all 1449 observed reflections reduced the R index to 9.7%. Exclusion of all reflections with $|F_{obsd} - F_{calcd}| > 0.35F_{obsd}$ from the least-squares refinement involved 43 reflections generally affected by extinction and reduced the R index to 9.0%. A final difference Fourier map gave no indication of misplaced atoms. At the end of the block-diagonal least-squares refinement, all shifts of coordinates or temperature factors were not more than half the respective estimated standard errors.

The fractional atomic coordinates and the orthogonal coordinates in ångströms are given in Table III (supplementary material). They correspond with the atoms of one molecule. The standard deviations as calculated by the least-squares program are printed in units of the last given digit of the corresponding value. To determine the absolute configuration, structure factors were calculated for the two enantiomorphs. The pairs of reflections with the greatest relative differences were directly compared with the recorded film intensities and were all found to correspond with the chirality reflected in the coordinates in Table III. Table IV (supplementary material) provides the interatomic distances in ångströms and the interatomic bonding angles. The estimated standard errors are ±0.02 Å and ±2°, respectively. Chart II shows the numbering system used for VI. The following are torsion angles for the rotatable bonds: C_{16}–C_{18}–C_{24}–C_{25}, 53°; C_{17}–C_{12}–O_2–C_{11}, 66°; C_{12}–O_2–C_{11}–C_7, 180°; O_2–C_{11}–C_7–C_6, −20°. The torsion angles were computed using a TI 59 programmable calculator (Texas Instruments Inc.) and Program 2 (Table VI). All other calculations were made on an IBM 360/40 computer using a set of programs based on the programs by F. R. Ahmed et al. (National Research Council, Ottawa) and locally adapted in cooperation with W. Oberhäensli (Hoffmann-La Roche). Tables III and IV are found in the microfilm edition.

4. **X-ray Coordinate Transformations.** The original calculations were performed on an SR-52 programmable slide-rule calculator (Texas Instruments Inc.) for which programs were designed. The programs have been redesigned for use with the TI 59 programmable calculator which has greatly increased capacity. The microfilm edition of this Journal provides the programs in Tables V and VI, respectively. The use of the new program is described below.

(a) **Program 1 (Table V). Coordinate Transformations. A. Fractional Coordinates to Rectangular Coordinates.**[18] The program converts fractional coordinates X/a, Y/b, and Z/c along the crystal axes to rectangular coordinates X_R, Y_R, and Z_R in ångströms using the equations $X_R = a \cdot X/a + c \cdot Z/c \cos \beta$, $Y_R = b \cdot Y/c$, and $Z_R = c \cdot Z/c \sin \beta$, where β is the inclination angle (90° for orthorhombic crystals), X/a, Y/b, and Z/c are unit cell fractions, a, b, and c are unit cell dimensions in ångströms, and X_R, Y_R, and Z_R are rectangular coordinates in ångströms.

B. Change of Origin. The program can place a selected atom, B, at the origin of the new coordinate system by subtracting the coordinates of B from those of each atom entered.

C. Reorientation of the Molecule. Three atoms, A, B, and C, of the molecule are selected by the program user to define the final (standard) orientation of the molecule. The standard orientation is defined by standard angles θ_{XS}, θ_{YS}, and θ_{ZS} which are entered into the program as constants. The program calculates the rotation angles θ_X, θ_Y, and θ_Z (with limits of ±180°) through which the molecule must be rotated about each respective coordinate axis from its original position to reach the standard orientation. In the standard orientation A will lie along the X axis in the $+X$ or $-X$ direction (see Chart III). When A is in the $+X$ direction, the standard angle, θ_{ZS} (looking down the Z axis toward the origin), is 0°. With A in the $-X$ direction, θ_{ZS} = 180°. With A in the $+X$ direction, θ_{YS} = 90°; with A in the $-X$ direction, θ_{YS} = −90°. Finally, the molecule is rotated about B–A (the X axis) until the angle θ_{XS} (looking down the X axis) (YBC, Chart III) is the one desired. If the molecule remains in its original orientation, $\theta_{XS} = \theta_{YS} = \theta_{ZS} = 0$.

D. Scale. A scale of 1.00 provides the final coordinates in ångströms. The value 1.25 is used for CCS or CPK models.

E. Transformed Coordinates. Once the raw data are entered into the program, the key B is pressed to calculate the intermediate data base (about 50 s is required). This data base includes the final coordinates of the atoms A and C. The entire register constants, including the raw data constants and the intermediate data base, may be displayed sequentially by pressing the key A'. It may also be stored on a card for future calculations. Once the intermediate data base has been compiled, the original X-ray coordinates of each atom i may be entered. The final coordinates in the new coordinate system are then calculated and displayed by pressing the appropriate keys.

Procedure

Step 1: Insert preprogrammed card, sides 1 and 2 (partitioning 459.59).

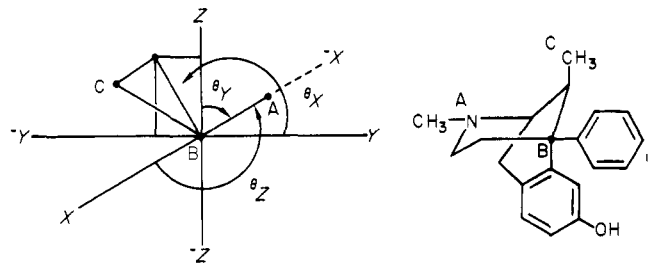
Step 2: Press E' to initialize, 11 is displayed.

Step 3: Enter each constant in sequence, pressing A after each entry. The constants are stored in the registers, the numbers of which are displayed before each entry. When all constants are entered the display reads 28. The sequence of constants is

R_{11} β (90° for orthorhombic crystal)
R_{12} a, R_{13} b, R_{14} c: unit cell dimensions; 1.0 if original coordinates are in ångströms
R_{15} θ_{XS}, R_{16} θ_{YS}, R_{17} θ_{ZS}: 0 if there is no rotation
R_{18} scale: 1.25 for CCS models, 1.0 for ångströms
R_{19} X/a for A
R_{20} Y/b for A
R_{21} Z/c for A
R_{22} X/a for B
R_{23} Y/b for B
R_{24} Z/c for B
R_{25} X/a for C
R_{26} Y/b for C
R_{27} Z/c for C
(fractional coordinates; omit if not used)

Step 4: Press B to calculate θ_X, θ_Y, and θ_Z, to provide rectangular coordinates of A and C, and to transform these to the final coordinates x, y, and z in the desired scale. The new values constitute the intermediate data base and are stored in the registers as follows.

Chart III



rectangular coordinates	rotation angles	final coordinates
R_{28} X_R for A	R_{34} θ_X	R_{37} x for A
R_{29} Y_R for A	R_{35} θ_Y	R_{38} y for A
R_{30} Z_R for A	R_{36} θ_Z	R_{39} z for A
R_{31} X_R for C		R_{40} x for C
R_{32} Y_R for C		R_{41} y for C
R_{33} Z_R for C		R_{42} z for C

Step 5: Press A' to display the constants R_{11}–R_{42} sequentially. Recall each one individually if desired.

Step 6: To calculate new coordinates of any atom i: enter X/a for i, press C; enter Y/b for i, press D; enter Z/c for i, press E (z_i is displayed); press D' to recall y_i; press C' to recall x_i.

Program 1 has 479 steps. Flag 1 is set when there is no rotation ($R_{16} = 0$). θ_X, θ_Y, and θ_Z are ≤180°. B is not pressed if R_{19}–R_{27} are not used.

(b) **Program 2 (Table VI). Torsion Angles from Orthogonal Coordinates.** This program provides the torsion angle θ between the plane ABC and the plane BCD from the orthogonal coordinates of four atoms A, B, C, and D. The X, Y, and Z coordinates of A, B, C, and D, respectively, are entered sequentially. With the last entry, θ is calculated and displayed. The program is written so that when the next torsion angle to be calculated is ABCE (branch) or BCDE (continuation) it is only necessary to press key B or key C, respectively, and then to enter the X, Y, and Z coordinates of the atom E. Thus, the program accommodates branches in a chain of atoms or each successive torsion angle in a linear chain of atoms may be determined easily.

Program 2 uses the polar/rectangular system conversion of the calculator and performs coordinate transformations in the same manner as program 1. In the linear sequence of atoms A, B, C, and D, B is placed at the origin of the coordinate system. When a new angle ABCE is calculated, pressing the key B first sets a flag so that the coordinates of B are subtracted from those of the atom E before the torsion angle is determined. When the new angle is BCDE, pressing the key C first derives the original coordinates of B, C, and D. Then, when the coordinates of E are entered, C becomes the origin of the coordinate system before the torsion angle is calculated. Program 2 has 290 steps.

Procedure

Step 1: Insert preprogrammed card, sides 1 and 2 (partitioning 459.59).

Step 2: Press E to initialize, 1 appears in the display.

Step 3: Enter the X, Y, and Z coordinates of A, B, C, and D, respectively, in sequence by pressing A after each entry. The numbers 1–12 in turn appear in the display. The angle θ is calculated and displayed when A is pressed after entering Z for the atom D.

Step 4: Press B when the next angle to be calculated is ABCE. The number 10 appears in the display. Enter the X, Y, and Z coordinates of E, pressing A after each entry. After the final entry, θ is calculated and displayed. The limits of θ are ±180°.

Step 5: Press C when the next angle to be calculated is BCDE. The number 10 appears in the display. Enter the X, Y, and Z coordinates of E, pressing A after each entry. With the final entry, θ is calculated and displayed.

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Supplementary Material Available: Tables III–VI listing fractional coordinates, thermal parameters, and interatomic distances and angles for 2,9-dimethyl-3′-hydroxy-5-phenyl-6,7-benzomorphan and programs for coordinate transformations from original x-ray crystallographic data to orthogonal coordinates and for calculation of torsion angles from orthogonal coordinates (4 pages). Ordering information is given on any current masthead page.

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