undertaking a similar study in a series of convulsant drugs that appear to share physiological sites of action despite markedly different chemical structures.

Registry No. **1a**, 77-28-1; **1b**, 76-74-4; **1c**, 57-43-2; (*S*)-**1d**, 24016-64-6; (*R*)-**1d**, 24016-63-5; **2a**, 2237-92-5; **2b**, 125-42-8; **2c**, 66968-52-3; **2d**, 72961-79-6; **3a**, 1952-67-6; **3b**, 17013-35-3; **3c**, 21149-88-2; (*S*)-**3d**, 86195-90-6; (*R*)-**3d**, 86195-91-7; **4**, 86162-59-6; **5**, 22173-64-4; **6**, 66940-72-5; **7**, 50-06-6; **8**, 17013-38-6; **9**, 36226-64-9; **10**, 68996-50-9.

Importance of C-6 Chirality in Conferring Irreversible Opioid Antagonism to Naltrexone-Derived Affinity Labels[1]

L. M. Sayre,[†,§] D. L. Larson,[†] D. S. Fries,[†,⊥] A. E. Takemori,[‡] and P. S. Portoghese*[,†]

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455. *Received January 27, 1983*

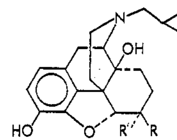
A series of five epimeric pairs of naltrexone derivatives that contain an electrophilic substituent at the 6α- or 6β-position was synthesized and tested on the guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD) preparations in order to determine if the orientation of the electrophile is important for covalent bonding to opioid receptors. In the GPI all compounds were pharmacologically active as reversible agonists, but only the 6β-isomers of the fumaramate ester **2b** (β-FNA) and isothiocyanate **6b** exhibited covalent reactivity, involving a selective irreversible antagonism of the μ agonist, morphine, without affecting κ agonists. The 6α-isomer **2a** (α-FNA) was itself nonalkylating but was able to protect the GPI against alkylation by its epimer, β-FNA, indicating that the two epimers bind to the same receptor. These results suggest that the proper orientation of the electrophilic substituent is required for covalent bonding with a proximal nucleophile in the case of μ receptor blockade. Moreover, the lack of covalent bonding to κ receptors by these or other ligands in this series indicates the possible absence of sufficiently reactive nucleophiles on this recognitition site. In the MVD, **2b**, but not **2a**, irreversibly antagonized morphine (as in GPI), whereas neither epimer exhibited irreversible antagonism toward the δ agonist, [D-Ala[2],D-Leu[5]]enkephalin (DADLE). In contrast, both of the isothiocyanate epimers (**6a,b**) irreversibly blocked μ and δ receptors. Evidence suggesting differences between μ receptors in the MVD and GPI was obtained with the β-iodoacetamide **5b**, which was an irreversible blocker of morphine only in the MVD. When analyzed together with those of previous studies with the nitrogen mustard analogues, α- and β-chlornaltrexamine, the data suggest that the receptor-alkylating ability of each isomer in an epimeric pair differs most when the electrophile possesses a narrow spectrum of reactivity.

Selective affinity-labeling agents are useful tools for investigating opioid receptors. The affinity labels that have been employed extensively for this purpose are β-chlornaltrexamine[2–5] (β-CNA, **1b**) and β-funaltrexamine[6–10] (β-FNA, **2b**). β-CNA irreversibly blocks at least three opioid receptor types (μ, κ, and δ), while β-FNA is a μ-specific, irreversible antagonist.[11]

Implicit in the approach that we have employed in the design of affinity labels is the assumption that covalent bond formation with opioid receptors is dependent on (1) a primary recognition step of forming a reversible ligand–receptor complex, followed by (2) a secondary recognition step involving proper alignment of the electrophile with a proximal nucleophile on the receptor.

A recently reported[1] study of α-CNA [**1a**, the C-6 epimer of β-CNA (**1b**)] has suggested that each of the epimeric CNA ligands alkylates different receptor nucleophiles by virtue of the different orientation of the electrophile attached to the C-6 epimeric center. Presumably, the high reactivity of the aziridinium ion generated from α- and β-CNA has facilitated secondary recognition but has made this step less selective because of the array of nucleophiles with which this electrophile can react.

In order to provide a more rigorous test for the second recognition process, we have synthesized epimeric pairs of ligands (**2–6**) that contain less reactive electrophiles at the C-6 position and have evaluated them for irreversible



	R	R[1]
1a (α-CNA)	H	$N(CH_2CH_2Cl)_2$
b (β-CNA)	$N(CH_2CH_2Cl)_2$	H
2a (α-FNA)	H	NHCOC(H)=C(H)COOMe
b (β-FNA)	NHCOC(H)=C(H)COOMe	H
3a	H	NHCOC(H)=C(H)COOMe
b	NHCOC(H)=C(H)COOMe	H
4a	H	$NHCOCH=CH_2$
b	$NHCOCH=CH_2$	H
5a	H	$NHCOCH_2I$
b	$NHCOCH_2I$	H
6a	H	N=C=S
b	N=C=S	H
7a	H	$NHCOCH_2CH_3$
b	$NHCOCH_2CH_3$	H
8a	H	NH_2
b	NH_2	H
9	NHCOC(H)=C(H)COOH	H

opioid antagonist activity.[12] Since secondary recognition should be more sensitive to stereochemical factors when

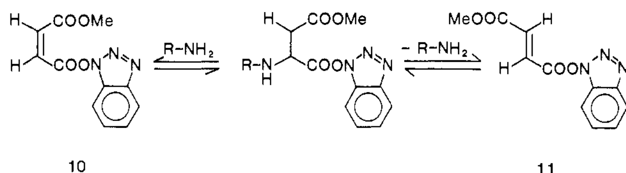
† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Present Address: Department of Chemistry, Case Western Reserve University, Cleveland, OH 44106.
⊥ Visiting Professor, Unit of Medicinal and Biological Chemistry, School of Pharmacy, University of the Pacific, Stockton, CA 95211.

0022-2623/83/1826-1229$01.50/0 © 1983 American Chemical Society

less reactive electrophiles are involved, it seemed possible that only one epimer (of an epimeric pair) might engage in covalent bond formation with opioid receptors. This report describes studies that demonstrate that this is indeed the case and that other types of stereodiscrimination also are important in covalent bonding to opioid receptors.

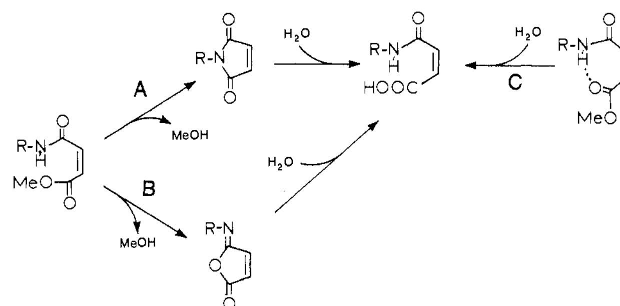
Chemistry. All of the target compounds were prepared from the corresponding epimers of naltrexamine (8), which in turn were obtained stereospecifically from naltrexone.[13] Compounds **2**, **4**, and **7** were prepared by treatment of the amines with the corresponding acid chlorides in the presence of triethylamine. In these reactions, acylation of the phenolic hydroxyl competed to a minor extent with N-acylation so that complete conversion of the starting amine was not achieved without the formation of a small amount of N,O-diacylated material. However, selective solvolysis of the phenolic ester with methanolic triethylamine prior to workup permitted isolation of the desired amides in excellent yields.

The α-maleamate **3a** was obtained by reaction of the amine 8a with the 1-hydroxybenzotriazole (HOBt) active ester (**10**) of methyl hydrogen maleate. Since some isomerization to the trans isomer **2a** occurred during this coupling, chromatographic separation was required. In this connection, it was demonstrated that **2a** did not form by isomerization of **3a** under either neutral, basic (dilute NH_4OH), or acidic (dilute HCl) conditions, indicating that **2a** was a direct product of the reaction. Thus, it is possible that **2a** was produced from the isomerized trans active ester **11**, which arose as a consequence of reversible conjugate addition of amine **8a** across the double bond of **10**.



The attempted synthesis of the β-maleamate **3b** from **8b** and **10** afforded predominantly the isomerized trans product **2b**, and consequently, an alternative route to **3b** was employed. Reaction of **8b** with maleic anhydride, conversion of the resulting maleamic acid **9** to its cesium salt, and alkylation with methyl iodide in DMF[14] afforded pure *cis*-**3b**.

Scheme I



It was found that the maleamate **3b** was unstable in aqueous buffer, ultimately being transformed to the maleamic acid **9**. Under the conditions of biological evaluation (pH 7.4), the half-life of **6b** was determined to be 18 min. In contrast, the fumaramates **2** (α- and β-FNA) were entirely stable under these same conditions.

The enhanced hydrolytic lability of the maleamate esters (**3a,b**) is obviously related to intramolecular catalysis due to the cis geometry of the double bond. Possible pathways leading to hydrolysis are depicted in Scheme I. Pathway A, which involves the maleimide intermediate, does not appear to be a primary contributor to the accelerated hydrolysis because the synthetically prepared maleimide was found to yield **9** more slowly than did the ester **3b**. More likely possibilities are pathways involving an isomaleimide intermediate (B)[15] or intramolecular general-acid catalysis by the amide NH (C). With regard to the latter mechanism, we have reported[16] evidence for an internally H-bonded species.

The iodoacetamides **5** were prepared by reaction of the epimeric amines **8** with the *N*-hydroxysuccinimide active ester[17] of iodoacetic acid. The isothiocyanates **6** were obtained by treatment of the amines **8** with thiophosgene in a two-phase organic–aqueous bicarbonate solvent mixture.

In order to establish that there is no substantial difference in reactivity between 6α- and 6β-epimers, we measured the relative rates of reaction of **2a** and **2b** using cysteine as a model nucleophile. The half-lives for the pseudo-first-order disappearance of these agents in the presence of a 13-fold excess of cysteine at pH 7.4 were found to be nearly identical (approximately 0.5 min). The maleamate **3b** was found to have a half-life similar to β-FNA (**2b**) under the same conditions.

Pharmacology. Guinea Pig Ileal Longitudinal Muscle (GPI). The GPI preparation was prepared by the method of Rang,[18] as described previously.[1] All of the compounds (**2**–**7**) possessed agonist potencies in the range of 0.5–10 times that of morphine (Table I). Most of the α-epimers displayed full concentration–response relationships with maxima equal to that of morphine. In contrast, the β-epimers were generally less potent and displayed partial agonism with shallow concentration–response

(1) Stereochemical Studies on Medicinal Agents. 28. For paper 27, see Sayre, L. M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* 1983, *26*, 503.
(2) Portoghese, P. S.; Larson, D. L.; Jiang, J. B.; Takemori, A. E.; Caruso, T. P. *J. Med. Chem.* 1978, *21*, 598.
(3) Portoghese, P. S.; Larson, D. L.; Jiang, J. B.; Caruso, T. P.; Takemori, A. E. *J. Med. Chem.* 1979, *22*, 168.
(4) Caruso, T. P.; Takemori, A. E.; Larson, D. L.; Portoghese, P. S. *Science* 1979, *204*, 316.
(5) Caruso, T. P.; Larson, D. L.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* 1980, *213*, 539.
(6) Portoghese, P. S.; Larson, D. L.; Sayre, L. M.; Fries, D. S.; Takemori, A. E. *J. Med. Chem.* 1980, *23*, 233.
(7) Takemori, A. E.; Larson, D. L.; Portoghese, P. S. *Eur. J. Pharmacol.* 1981, *70*, 445.
(8) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* 1982, *220*, 494.
(9) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* 1982, *80*, 377.
(10) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* 1982, *85*, 163.
(11) For a recent review of this subject, see Portoghese, P. S.; Takemori, A. E. In "The Chemical Regulation of Biological Mechanisms"; Creighton, A. M.; Turner, S., Eds.; Whitstable Litho LTD: Whitstable, England, 1982; p 181.
(12) Some preliminary studies have been reported: Sayre, L. M.; Portoghese, P. S.; Takemori, A. E. In "Abstracts of Papers", 181st National Meeting of the American Chemical Society, Atlanta, GA, Mar 29–Apr 3, 1981; American Chemical Society: Washington, DC, 1981; Abstr MEDI 47.
(13) Sayre, L. M.; Portoghese, P. S. *J. Org. Chem.* 1980, *45*, 3366.
(14) Wang, S.-S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* 1977, *42*, 1286.
(15) Kirby, A. J.; Fersht, A. R. *Progr. Bioorg. Chem.* 1971, *1*, 1.
(16) Portoghese, P. S.; Telang, V. G.; Takemori, A. E.; Hayashi, G. *J. Med. Chem.* 1971, *14*, 144.
(17) Wilchek, M.; Givol, D. *Methods Enzymol.* 1977, *46*, 153.
(18) Rang, H. B. *Br. J. Pharmacol.* 1964, *22*, 356.

Table I. Activities of Epimeric Naltrexamine Derivatives on the GPI

		irreversible antagonist potency		
no.	agonist potency[a] (n)	concn, nM	morphine IC_{50} ratio[b] ± SE (n)	nalorphine (N) or EK (E) IC_{50} ratio[b] ± SE (n)
2a	7.3 (4)	20	1.2 ± 0.01 (4)	(N) 0.92 ± 0.13 (4)
		200	0.87 ± 0.13 (3)	(E) 0.86 ± 0.08 (3)
2b[c]	5.0 (5)	20	6.0 ± 0.6 (6)	(E) 1.0 ± 0.1 (5)
		200		(E) 1.2 ± 0.1 (4)
		1000	18.8 ± 4.3 (3)	(E) 1.3 ± 0.1 (3)
3a	0.8 (3)	20	0.95 ± 0.13 (4)	(N) 0.49 ± 0.1 (4)
		200	1.1 ± 0.1 (4)	(N) 0.98 ± 0.33 (4)
3b	1.1 (3)	20	1.5 ± 0.6 (5)	(N) 1.0 ± 0.1 (4)
		200	0.63 ± 0.02 (4)	(N) 0.60 ± 0.19 (4)
4a	1.5 (2)	20	1.2, 0.80	(N) 0.78, 0.61
		200	1.2 ± 0.2 (3)	(N) 0.8 ± 0.06 (3)
4b	40% max inhibn at 1 μM (3)	20	1.9 (1)	(N) 1.1 (1)
		200	4.2 ± 0.9 (5)	(N) 1.4 ± 0.2 (4)
		1000	2.9 ± 0.7 (3)	(E) 1.5 ± 0.3 (3)
5a	0.49 (3)	20	0.97 (1)	(N) 1.2 (1)
		200	1.1 ± 0.2 (3)	(N) 0.87 ± 0.15 (3)
5b	55% max inhibn at 1 μM (3)	20	2.4 (1)	(N) 1.2 (1)
		200	2.1 ± 0.4 (3)	(N) 1.2 ± 0.2 (3)
		1000	3.4 ± 1.2 (3)	(E) 0.7 ± 0.1 (3)
6a	9.6 (4)	20	2.1 ± 0.4 (3)	(E) 0.8 (1)
		200	2.4 ± 0.3 (3)	(N) 1.2 ± 0.2 (3)
		500	2.3 ± 0.7 (3)	(E) 1.4 ± 0.4 (4)
6b	1.9 (2)	20	6.9 ± 3.2 (3)	(N) 1.5 (1)
		200	14.9 ± 3.3 (3)	(N) 1.4 ± 0.3 (4)
7a	2.9 (3)	20	1.1 (1)	(N) 0.7 (1)
		200	1.0 ± 0.1 (3)	(N) 0.90 ± 0.18 (3)
7b	0.81 (3)	20	1.3 ± 0.2 (4)	(N) 1.0 ± 0.1 (4)
		200	1.5 ± 0.3 (4)	(N) 0.85 ± 0.17 (3)

[a] Relative to morphine (=1) (IC_{50} = 2.92 ± 0.71 × 10^{-7} M; n = 17) on the same ileum preparation; inhibition of contraction is greater than 80%. Values in parentheses are the number of determinations. [b] Agonist IC_{50} [after 30-min incubation of the GPI with the test compound followed by washing (20 times)] divided by control agonist IC_{50} in same preparation. Values in parentheses are the number of determinations. Nalorphine: IC_{50} = 5.13 ± 0.93 × 10^{-8} M, n = 20. EK: IC_{50} = 9.73 ± 0.82 × 10^{-10} M, n = 21. [c] Taken in part from data from ref 7 and 9.

curves. The agonistic effect was *reversible* with washing in all cases.

The possibility that the compounds **2–7** would exhibit irreversible antagonism was evaluated by incubating the tissue at various concentration levels for 30 min, followed by washing (20 times). Any antagonism to morphine and ethylketazocine (EK) or nalorphine that remained was indicative of irreversible blockage of μ and κ receptors, respectively, and was expressed quantitatively as an IC_{50} ratio (Table I). Only β-FNA (**2b**) and the β-isothiocyanate **6b** produced a marked concentration-dependent, irreversible antagonism to the agonist effect of morphine. None of the compounds blocked the effect of the κ-selective agonists, as indicated by the fact that the IC_{50} ratios for nalorphine or EK were close to unity and concentration independent. A nonelectrophilic epimeric pair (**7a**,**b**) was included as a control for the closely related electrophiles.

Although α-FNA (**2a**) did not exhibit an irreversible antagonist effect itself, its ability to inhibit the irreversible antagonist effect of β-FNA (**2b**) (for morphine) was evaluated in order to determine whether or not both epimers bind to common receptors. For a 20 nM, 30-min treatment with β-FNA, the morphine IC_{50} ratio in the unprotected preparation was 6.0 ± 0.6, whereas in the presence of 200 nM α-FNA, the IC_{50} ratio was 1.3 ± 0.02. A similar experiment using 200 nM morphine as the protector afforded an IC_{50} ratio of 2.5 ± 0.2. These data indicate that α-FNA efficiently protects against the irreversible antagonist effect of β-FNA and is more effective in this regard than is morphine itself.

After the ileum had been pretreated with β-FNA (20 or 100 nM, 30–45 min) and washed (20 times), the *reversible* agonist activities of both α-FNA and β-FNA were not significantly different from those exhibited in the untreated preparation. This same behavior was observed for nalorphine and EK and suggests that the reversible agonist activities of both epimeric series are mediated through κ receptors.

Mouse Vas Deferens (MVD). This assay was performed by the method of Henderson et al.[19] as described previously.[1] Selected pairs of epimers (**2**, **5**, and **6**) were evaluated for agonist activity. With the exception of **5b**, which produced no agonist effect up to 1 μM, the compounds behaved as partial agonists (Table II). The agonism was reversible in all cases.

The effects of pretreatment of the MVD with the electrophiles (**2**, **5**, and **6**) on the IC_{50} values of morphine and [D-Ala[2],D-Leu[5]]enkephalin (DADLE) (μ- and δ-selective agonists, respectively) are listed in Table II. Pretreatment with α-FNA (**2a**) produced no significant changes in the IC_{50} of either morphine or DADLE. A maximal 10.6-fold rightward shift in the morphine concentration–response curve resulted when the MVD was pretreated with 200 nM β-FNA (**2b**) for 30 min, whereas no change was noted with DADLE. These results indicate that in the MVD, μ receptors were blocked in the presence of δ receptors, which are inert to this treatment.

The epimeric iodoacetamides (**5a**,**b**) afforded an activity profile on the MVD that was qualitatively similar to α- and β-FNA. These data (Table II) suggested that **5b** irreversibly blocked μ receptors in the MVD but had no significant effect on δ receptors. It is noteworthy that no blockage of μ receptors was observed by **5b** in the GPI.

Both isothiocyanate epimers (**6a**,**b**) produced concentration-dependent increases in the IC_{50} ratios of morphine

(19) Henderson, G.; Hughes, J.; Kosterlitz, H. N. *Br. J. Pharmacol.* 1972, *46*, 764.

Table II. Activities of Epimeric Naltrexamine Derivatives on the MVD

		antagonist potency		
no.	agonist act.[a] (n)	concn, nM	morphine IC_{50} ratio[b] ± SE (n)	DADLE IC_{50} ratio[b] ± SE (n)
2a	60% max; IC_{30} = 80 nM (3)	200	0.52 ± 0.25 (4)	0.37 ± 0.11 (3)
2b	IC_{50} = 82 ± 35 nM (4)	100	3.9 ± 0.8 (3)	1.8 ± 0.4 (3)
		200	10.6 ± 0.4 (3)	1.1 ± 0.2 (3)
5a	60% max; IC_{30} = 200 nM (6)	200	2.5 ± 0.9 (4)	1.2 ± 0.3 (4)
5b	IC_{50} > 1 μM (2)	200	20[c] (5)	1.8[c] (4)
6a	45% max; IC_{30} = 100 nM (4)	20	3.1 ± 1.0 (6)	1.2 ± 0.2 (3)
		200	10.7 ± 5.0 (5)	6.6 ± 0.4 (3)
6b	43% max; IC_{30} = 100 nM (3)	20	13.8[c] (4)	1.8, 2.5
		200	120[c] (4)	6.9 ± 3.6 (5)

[a] Where the maximum inhibition of contraction was less than that required (70%) for an IC_{50} determination, an IC_{30} was approximated. [b] See footnote *b*, Table I, for definition of IC_{50} ratio. Morphine IC_{50} = 1.42 ± 0.32 × 10^{-6} M (n = 30); DADLE IC_{50} = 3.57 ± 0.47 × 10^{-10} (n = 30). [c] Where the standard agonist did not produce a full response (>70%) following compound treatment, an estimate of an IC_{30} ratio was made from the plotted dose-response curves.

and DADLE. The unusually large shift of the morphine concentration–response curve (~135) by **6b** indicates that other receptor populations in the MVD in addition to μ receptors are irreversibly blocked, since the selective μ-blocker β-FNA effected only an 11-fold shift to morphine.

The increased IC_{50} ratios of DADLE after treatment with **6a** or **6b** are suggestive of irreversible blockade of δ receptors but could result partly from a very high degree of blockade of μ receptors, which mediate a component of DADLE agonism in MVD. In order to address this question, an MVD preparation[10] possessing only δ receptors was prepared by alkylating the tissue with β-CNA (**1b**, 100 nM, 20 min) in the presence of a protecting concentration (2 μM) of the δ-selective ligand Tyr-D-Ser-Gly-Phe-Leu-Thr.[20] The IC_{50} ratios of DADLE obtained on this δ preparation for a 200 nM, 30-min treatment with **6a** and **6b** were 2.45 ± 0.33 and 4.56 ± 0.72, respectively, suggesting that both epimers (especially the β) block δ receptors. A comparison of these values with those obtained on the naive tissue (Table II) indicates that almost the entire shift of DADLE exerted by **6b** is due to δ receptor blockage.

Discussion

The results of the present study are consistent with the notion that two recognition processes are involved in the affinity labeling of opioid receptors: a reversible, high-affinity binding (primary recognition) of the ligand, followed by covalent association with a proximal receptor nucleophile.[11,12] This second step has been termed secondary recognition because it involves proper alignment between complementary reactive centers of sufficient reactivity to engage in covalent binding.

Because two consecutive recognition steps are operative, this, in principle, should afford highly selective agents. This is exemplified by β-FNA (**2b**), which specifically and irreversibly blocks the effects of morphine at μ opioid receptors without affecting κ or δ receptors. Presumably, the specificity of β-FNA is due to the narrow spectrum of reactivity of its Michael acceptor group. The fact that β-FNA (**2b**) is recognized at κ opioid receptors (as a reversible agonist) without covalent binding underscores the importance of the second recognition step in discriminating between different receptor types.

Additional evidence for the importance of secondary recognition was obtained from experiments with α-FNA (**2a**). This epimer behaved as a reversible κ agonist [as does β-FNA (**2b**)], but it also afforded efficient protection of

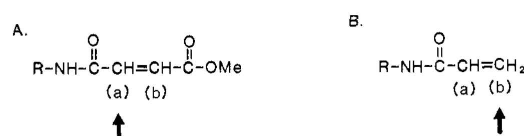


Figure 1. The more reactive electrophilic carbon (*a*) in the fumaramate ester A and the reactive center (*b*) in the acrylamide B.

μ receptors against irreversible blockade by β-FNA (**2b**). This indicates that both epimeric fumaramates bind to common sites, and despite possessing nearly identical intrinsic reactivities as Michael acceptors, only β-FNA is observed to effect receptor alkylation. The most likely explanation is that the Michael acceptor group of β-FNA (**2b**), but not of α-FNA (**2a**), is within covalent binding distance of a sufficiently reactive nucleophile when complexed to the receptor.

The importance of secondary recognition also is suggested from the ineffectiveness of the maleamates (**3a,b**) as irreversibly acting agents. Since maleamate and fumaramate esters are equally good Michael acceptors, it appears that other factors are responsible for the lack of irreversible activity. While the facile hydrolysis of **3b** at pH 7.4 may contribute to an underestimate of the IC_{50} ratio, it seems unlikely that this alone can account for the apparent absence of covalent association. The most likely explanation is that the different geometry of the maleamate group places its electrophilic center in an environment that is distant from a sufficiently reactive nucleophile on the opioid receptor.

Similarly, the inability of the epimeric acrylamides (**4a,b**) to react covalently with opioid receptors may be a consequence of poor secondary recognition. Two possible explanations for this relate to their lower reactivity (relative to **2**) and to a different location of the electrophilic center. The greater resonance stabilization of amides compared to esters results in a reduced electrophilicity of the vinyl group conjugated to the former, relative to the latter. In this connection, it is known that conjugate addition to methyl acrylate occurs 24–29 times faster than to acrylamide.[21] The consequences of this difference are, firstly, that the acrylamides **4** possess overall lower reactivity than the fumaramates **2** and, secondly, that the preferred reactive center (Figure 1) in the fumaramate (A) is site *a* rather than site *b*. On the other hand, nucleophilic addition to the acrylamide (B) can occur only to site *b*.

(20) Gacel, G.; Fournie-Zaluski, M.-C.; Roques, B. P. *FEBS Lett.* **1980**, *118*, 245.

(21) Friedman, M.; Wall, J. S. *J. Org. Chem.* **1966**, *31*, 2888. Friedman, M.; Cavins, J. F.; Wall, J. S. *J. Am. Chem. Soc.* **1965**, *87*, 3672.

Thus, if carbon *a* were closer to the receptor nucleophile than carbon *b*, it is conceivable that the mode of conjugate addition (site *b*) in the acrylamide (B) together with its lower reactivity might reduce the likelihood of covalent bond formation.

It is noteworthy that the epimeric iodoacetamides (**5a,b**) exhibited no significant irreversible blockade of μ or κ receptors in the GPI. Once again, lack of adequate secondary recognition may be in part responsible. The intrinsic reactivity of this electrophile relative to that of the fumaramate is not known.

Of significance is the ability of the β-iodoacetamide **5b** to irreversibly block the μ receptor-mediated response to morphine in the MVD despite the fact that no such blockage was observed in the GPI. This raises the possibility that the organization of the μ receptor in the GPI may differ from that in the MVD.[22] The existence of differences between μ receptors in different organs is not wholly unexpected in view of the similar situation found with other receptor classes.

The isothiocyanates (**6a,b**) have a stereostructure–activity profile similar to that of the fumaramates (**2a,b**) in the GPI. Thus, of the two epimers, only the β-isomer **6b** irreversibly blocks the effects of morphine at μ receptors. Also, κ receptors are not affected by exposure to the isothiocyanates. These results may reflect the importance of the second recognition step that leads to the blockage of μ opioid receptors in the GPI.

However, on the MVD preparation, the isothiocyanates behave quite differently from the fumaramates in that both epimers are irreversible blockers of the agonist effects mediated by both μ and δ receptors. This decreased selectivity for these receptor types may be related to a greater reactivity of the isothiocyanate group relative to the fumaramate ester. The irreversible antagonism of μ-mediated effects by both **6a** and **6b** in the MVD again suggests possible differences between the μ receptor systems in the GPI and MVD.

Conclusions

The results of the present study provide evidence for stereocontrolled covalent binding (secondary recognition) of β-FNA (**2b**) after the formation of a reversible ligand–receptor complex (primary recognition). Ligands with electrophiles of intermediate reactivity and high group selectivity have greater ability to distinguish between opioid receptor types (recognition amplification), as exemplified by β-FNA (**2b**), which specifically and irreversibly blocks μ receptors. Its epimer, α-FNA (**2a**), does not react with opioid receptors because of inefficient secondary recognition.

A schematic illustration of the primary and secondary recognition processes in the action of β-FNA (**2b**) is shown in Figure 2A. Although an array of nucleophiles are present (G^1–G^4), some within covalent bonding distance, the fumaramate moiety reacts only with one of these. Due to the epimeric relationship of the Michael acceptor group in α-FNA (**2a**), the electrophilic moiety projects into a receptor locus where the nucleophiles either are not in proper juxtaposition or are incapable of conjugate addition (Figure 2B). Similarly, β-FNA does not alkylate other receptor types (Figure 2C) because the proper nucleophile (G^1) is distal to the electrophilic center of the Michael acceptor.

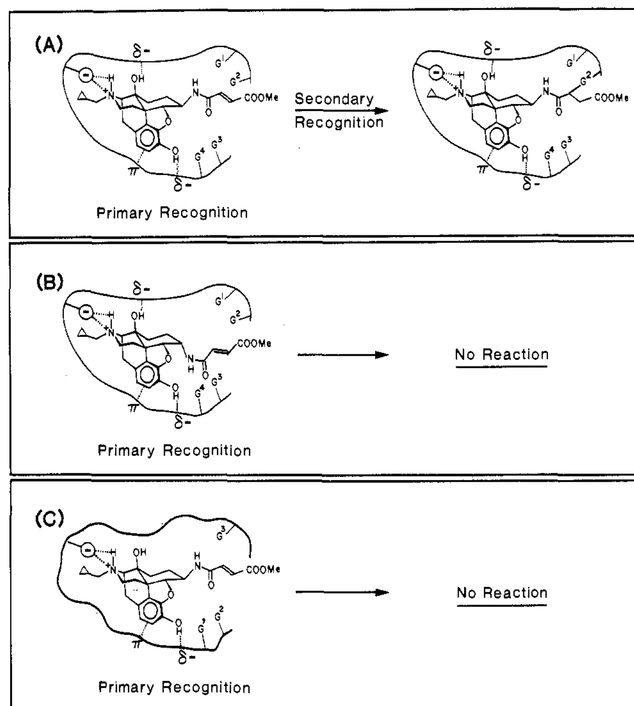


Figure 2. A schematic illustration of recognition between (A) the fumaramate moiety of β-FNA (**2b**) and a proximal nucleophile G^2. No reaction (no secondary recognition) takes place with α-FNA (**2a**) at the same receptor type (B) or with β-FNA at a different receptor type (C) because an appropriate receptor nucleophile is not properly aligned with the electrophilic center.

A more reactive electrophile is less efficient in its ability to distinguish between different types of nucleophiles. Consequently, the chirality at C-6 becomes less important in the secondary recognition process. In the present study, the epimeric isothiocyanates (**6a,b**), which are presumably more reactive and less selective than the fumaramates, exhibit a pharmacological profile that is consistent with this view.

With an extremely reactive electrophile, the difference between the receptor covalent-binding capacity of the two epimers is still less pronounced. This has been demonstrated in previous studies with α-CNA (**1a**) and β-CNA (**1b**), both of which generate the aziridinium ion as the electrophilic species. It is likely that this highly reactive electrophile reacts with multiple nucleophiles within covalent bonding distance of either the β-epimer **1b** (Figure 3A) or the α-epimer **1a** (Figure 3B).

The model we have presented assumes that the C-6 stereochemistry does not alter the mode of binding at the receptor. Although this is a reasonable expectation, differences in the receptor-alkylating capacities of C-6 epimers could be interpreted alternatively in terms of substituent-induced alteration of binding modes.[23] It should be noted, however, that in terms of the definition of secondary recognition we have presented, the consequence of different binding modes is equivalent to the model shown in Figures 2 and 3.

Experimental Section

General. Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and were within ±0.4% of the theoretical values. IR spectra were obtained from KBr pellets with a Perkin-Elmer 281 instrument. NMR spectra were recorded at

(22) Other evidence consistent with μ isoreceptors has been reported: Wood, P. L.; Richard, J. W.; Thakur, M. *Life Sci.* **1982**, *31*, 2313. Schulz, R.; Wüster, M. *Eur. J. Pharmacol.* **1981**, *76*, 61.

(23) Portoghese, P. S. *J. Med. Chem.* **1965**, *8*, 609. Portoghese, P. S. *J. Pharm. Sci.* **1966**, *55*, 865.

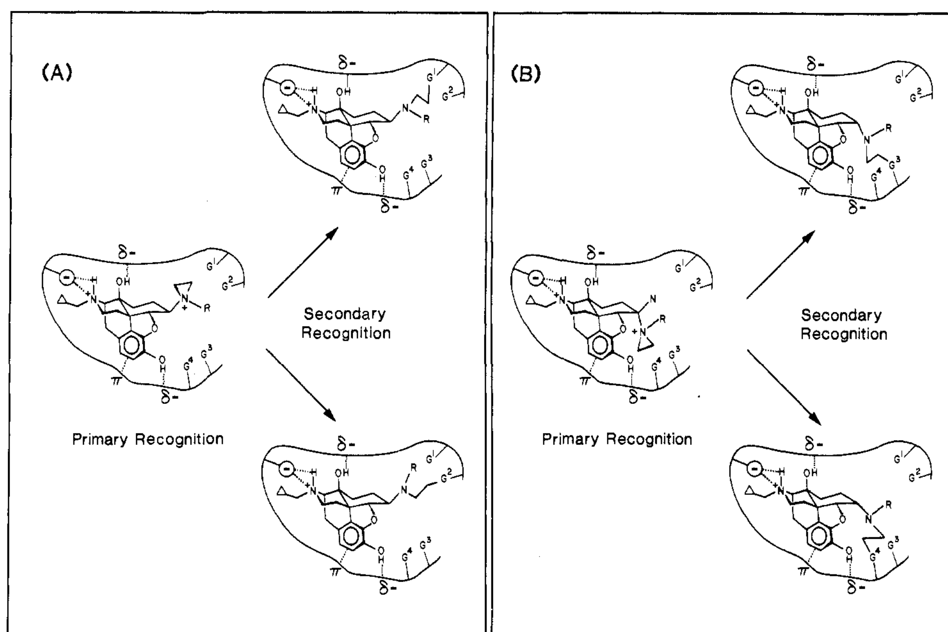


Figure 3. A schematic illustration of the interaction of β-CNA (**1b**) (A) and α-CNA (**1a**) (B) at identical receptors. Note that different nucleophiles are alkylated (secondary recognition) due to the high reactivity of the aziridinium electrophile.

ambient temperature on Varian A-60D and T-60 instruments, with tetramethylsilane and DSS as internal standard in nonaqueous media and D_2O, respectively. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Mass spectra were obtained on an AEI MS-30 instrument. All TLC data were determined with Eastman "Chromogram" 13181 plastic-backed sheets (silica gel), and the eluant EMA refers to EtOAc–MeOH–NH_4OH. Unless otherwise stated, all reagents and solvents used were reagent grade, without subsequent purification.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6-[*trans*-3-(methoxycarbonyl)acrylamido]morphinan Hydrochloride (2a·HCl and 2b·HCl). Both epimers were synthesized by a modification of that originally reported for **2b**·HCl. A THF–CH_3CN (9:1, 10 mL) solution containing either **8a**·2HCl or **8b**·2HCl (1 mmol) was stirred over 3Å molecular sieves for 1 h and cooled to −25 °C. To this a solution of methyl 2-(chloroformyl)-2-butenoate (1:1 mmol) in THF (3 mL) was added dropwise with stirring over 30 min, and the mixture then was stirred for 3 h to room temperature. The reaction mixture was concentrated to 2 mL, and to this residue was added MeOH (4 mL) and 1 drop of Et_3N. After 20 h at 25 °C, the solvent was removed, and the solids were extracted with $CHCl_3$–MeOH (9:1, 10 mL) and washed with aqueous $NaHCO_3$. Back-extraction of the aqueous wash with $CHCl_3$ (2 times), evaporation of the combined organic extracts, and acidification either with HCl–MeOH or with HCl–Et_2O afforded the desired product in yields of 70–80%. **2a**·HCl: mp 220 °C dec; $[\alpha]^{25}_D$ −166.2° (*c* 0.5, MeOH); R_f 0.58 (EMA, 95:5:1); EIMS (70 eV), *m/e* 454 (M^+); NMR (D_2O) δ 6.70 and 7.05 (2 d, *J* = 15.4 Hz, 2 H, vinyl). Anal. ($C_{25}H_{31}ClN_2O_6$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-[*cis*-3-(methoxycarbonyl)acrylamido]morphinan Hydrochloride (3a·HCl). Methyl hydrogen maleate (0.195 g, 1.5 mmol) was stirred with HOBt (0.141 g, 1.5 mmol) in THF (20 mL) for 90 min. To this mixture was added rapidly (30 S) **8a** (0.492 g, 1.2 mmol) in THF (40 mL) and the solution was stirred for 2 h. After the solution was concentrated to 20 mL and filtered, the contents of the filtrate were subjected to two chromatographic purifications (silica gel; EtOAc–MeOH–NEt_3, 100:2:0.2) to separate the desired product **3a** from its isomer **2a**. The free base **3a** (0.120 g) was crystallized from Et_2O–hexane: mp 192–193 °C; EIMS (70 eV), *m/e* 454 (M^+). A $CHCl_3$ (4 mL) solution of **3a** (0.075 g) was treated with Et_2O–HCl to afford **3a**·HCl: mp 175–180 °C dec; $[\alpha]^{25}_D$ −90.4° (*c* 0.5, MeOH); NMR (D_2O) δ 6.25 and 6.53 (2 d, *J* = 12 Hz, 2 H, vinyl); R_f 0.39 (EMA, 95:5:1). Anal. ($C_{25}H_{30}N_2O_6$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-[*cis*-3-(methoxycarbonyl)acrylamido]morphinan Hydrochloride (3b·HCl). The maleamic acid **9** (0.617 g, 1.4 mmol) and Cs_2CO_3 (0.228 g, 0.7 mmol) were dissolved in MeOH (20 mL) and DMF (30 mL). The solution was concentrated slowly to a volume of 25 mL at 30 °C (reduced pressure), diluted with DMF (10 mL), and then concentrated to a volume of 30 mL. To the resulting anhydrous solution cooled to 0 °C was added a solution of MeI (0.241 g, 1.7 mmol) in DMF (3 mL), and the reaction was refrigerated for 10 h. The solution was diluted with aqueous $NaHCO_3$ (120 mL) and extracted with $CHCl_3$ (3 × 80 mL). The $CHCl_3$ was evaporated to leave a DMF concentrate, MeOH–HCl was added to pH 1.5, and the solution was again concentrated in vacuo. Dilution with 3 vol of THF and then 1 vol of Et_2O caused precipitation of 0.594 g (86%) of nearly pure **3b**·HCl. Recrystallization from MeOH–acetone afforded analytically pure material: mp 257 °C dec; $[\alpha]^{25}_D$ −150° (*c* 1.3, CH_3OH); EIMS (70 eV), *m/e* 454 (M^+); NMR (D_2O) δ 6.58 and 6.27 (2 d, *J* = 12.0 Hz, 2 H, vinyl); R_f 0.66 (EMA, 80:20:2), 0.22 (EMA, 98:2:2). Anal. ($C_{25}H_{31}N_2O_6Cl\cdot H_2O$) C, H, N.

6α-Acrylamido-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (4a). A solution of **8a**·2HOAc (0.37 g, 0.8 mmol) in 50% aqueous MeOH (25 mL) was brought to pH 10.5 with 2 N NaOH. A solution of CH_2=CHCOCl (0.145 g, 1.6 mmol) in THF (10 mL) was added dropwise with stirring at 10 °C, keeping the pH between 10 and 11 by frequent addition of 2 N NaOH. After the addition was complete, the solution was brought to pH 11.5 for 20 h and then down to pH 10 with HOAc, concentrated, and extracted ($CHCl_3$). The combined organic extracts were evaporated, and the residue was chromatographed on silica gel (EtOAc–MeOH, 9:1). Fractions containing the fastest moving material were combined and evaporated. Dissolution of the residue in aqueous MeOH, concentrating until cloudy, and cooling yielded 0.246 g (75%) of **4a** in two crops: decomposes without melting at 140 °C; EIMS (70 eV), *m/e* 396 (M^+); R_f 0.62 (EMA, 80:20:2); $[\alpha]^{25}_D$ −266° (*c* 1.2, CH_3CN). Anal. ($C_{23}H_{28}N_2O_4\cdot{}^2/_3H_2O$) C, H, N.

6β-Acrylamido-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (4b). According to the procedure given above for **4a**, 0.37 g (0.8 mmol) of **8b**·2HOAc was converted to 0.266 g (79%) of **4b** in two crops: decomposes without melting at 150 °C; EIMS (70 eV), *m/e* 396 (M^+); R_f 0.42 (EMA, 95:5:2); $[\alpha]^{25}_D$ −171° (*c* 1.3, CH_3CN). Anal. ($C_{23}H_{28}N_2O_4\cdot{}^4/_3H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-(2-iodoacetamido)morphinan Methanesulfonate (5a·CH_3SO_3H). According to the procedure given below for **5b**, **8a** free base (0.44 g, 1.3 mmol) was converted to 0.50 g (63%) of **5a**·CH_3SO_3H in two crops: decomposes without melting at >200 °C, EIMS (40 eV), *m/e* 510 (M^+); R_f 0.65 (EMA, 80:20:2); $[\alpha]^{25}_D$ −166° (*c* 1.3,

CH_3OH). Anal. ($C_{23}H_{31}N_2O_7SI$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-(2-iodoacetamido)morphinan Methanesulfonate (5b·CH_3SO_3H). A solution of **8b** free base (0.38 g, 1.1 mmol) in 2-propanol (20 mL) and THF (40 mL) and a solution of *N*-(2-iodoacetoxy)-succinimide (0.34 g, 1.2 mmol) in THF (20 mL) were each cooled to −20 °C and then mixed. The resulting solution was stored at −10 °C for 16 h and then at room temperature for 40 h. A solution of CH_3SO_3H in MeOH was added to pH 1.2, toluene (5 mL) was added, and all solvent was removed at reduced pressure. The residue was stirred for 40 h with acetone, and the solid obtained upon filtration was recrystallized from EtOH–toluene: yield of two crops of **5b**·CH_3SO_3H was 0.49 g (73%); decomposes without melting at >200 °C; EIMS (70 eV), *m/e* 510 (M^+); R_f 0.65 (EMA, 80:20:2); $[\alpha]^{25}_D$ −101° (*c* 1.0, CH_3OH). Anal. ($C_{23}H_{31}N_2O_7SI$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-isothiocyanatomorphinan Hydrochloride (6a·HCl). A mixture of **8a**·2HOAc (0.37 g, 0.8 mmol) and $NaHCO_3$ (0.42 g, 5 mmol) was dissolved in 15 mL H_2O–THF (2:1), and a solution of thiophosgene (0.15 g of an 85% solution in CCl_4, 1.1 mmol) in THF (10 mL) was added dropwise with stirring at 0 °C. The resulting two-phase solution was stirred to 25 °C overnight, and Et_2O was added. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic extracts were evaporated, and the residue was taken up in MeOH–CH_3CN and acidified to pH 1.8 with HCl. Concentration of the solution yielded 0.29 g (85%) of **6a**·HCl, which was crystallized from MeOH–*i*-PrOH: mp 230 °C dec; $[\alpha]^{25}_D$ −265° (*c* 1.0, CH_3OH); R_f 0.72 (EMA, 95:5:2); EIMS (CH_3SO_3H salt, 70 eV), *m/e* 384 (M^+); IR 2080 (−N=C=S stretch) cm^{-1}. Anal. ($C_{21}H_{25}N_2O_3SCl\cdot{}^2/_3H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-isothiocyanatomorphinan Hydrochloride (6b·HCl). A mixture of **8b**·2HCl (1.25 g, 3.0 mmol) and $NaHCO_3$ (1.68 g, 20 mmol) was dissolved in H_2O (20 mL) and THF (8 mL). A solution of thiophosgene (0.49 g of an 85% solution in CCl_4, 3.6 mmol) in THF (20 mL) was added dropwise with rapid stirring at 0 °C. The resulting two-layer solution was stirred to 25 °C overnight, Et_2O was added, and the organic layer was separated. The aqueous layer was then extracted twice with $CHCl_3$. The combined organic extracts were evaporated, and the residue was chromatographed rapidly on silica gel ($CHCl_3$–acetone–Et_3N, 90:10:1). All fast-moving material was converted into its HCl salt, yielding 0.93 g (74%) of **6b**·HCl. Recrystallization from CH_3CN afforded analytically pure material: decomposes without melting at 300 °C; $[\alpha]^{25}_D$ −289° (*c* 1.2, MeOH); R_f 0.75 (EMA, 80:20:2), 0.32 ($CHCl_3$–Et_3N, 100:1); EIMS (CH_3SO_3H salt, 70 eV) 384 (M^+); IR 2065 (−N=C=S stretch) cm^{-1}. Anal. ($C_{21}H_{25}N_2O_3SCl$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-propanamidomorphinan (7a). A solution of **8a**·2HOAc (0.46 g, 1 mmol) in 50% aqueous THF (30 mL) was brought to pH 10 with 2 N NaOH. A solution of CH_3CH_2COCl (0.32 g, 3.5 mmol) in THF (10 mL) was added dropwise with stirring at 10 °C, keeping the pH between 10 and 11 by frequent addition of 2 N NaOH. After the addition was complete, the solution was maintained at pH 10.5 for 40 h, diluted with water, concentrated, and extracted with $CHCl_3$. The residue obtained upon evaporation of the combined organic extracts was taken up in MeOH, and HCl was added to pH 1. Ethanol and toluene were added, and all solvent was evaporated at reduced pressure. The residue was triturated with acetone to yield 0.33 g (76%) of the HCl salt. The HCl salt was converted to the free base and crystallized from aqueous MeOH: mp 220–221 °C; EIMS (70 eV), *m/e* 398 (M^+); R_f 0.56 (EMA, 90:10:2); $[\alpha]^{25}_D$ −225° (*c* 0.5 CH_3CN–$CHCl_3$, 1:1). Anal. ($C_{23}H_{30}N_2O_4$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-propanamidomorphinan (7b). The propionylation was conducted with **8b**·2HOAc by using the above conditions for **7a**. The salt **7b**·HCl (0.312 g, 72%) was converted to the free base and crystallized from 75% aqueous EtOH: mp 233–234 °C; EIMS (70 eV), *m/e* 398 (M^+); R_f 0.30 (EMA, 95:5:2); $[\alpha]^{25}_D$ −168° (*c* 0.5, CH_3CN–$CHCl_3$, 1:1). Anal. ($C_{23}H_{30}N_2O_4$) C, H, N.

6β-(*cis*-3-Carboxyacrylamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (9). (a) A solution of **8b** base (1.4 g, 4.1 mmol) in 20 mL of CH_2Cl_2–THF (3:2) was mixed with a solution of maleic anydride (0.417 g, 4.25 mmol) in THF (12 mL) at 0 °C and then stirred to room temperature overnight. The resulting slurry was filtered, and the product was washed with Et_2O, yielding 1.82 g (100%) of **9**.

(b) To a solution of **8a**·2HCl·0.75H_2O (2.0 g, 4.66 mmol) and Et_3N (0.980 g, 9.7 mmol) in CH_2Cl_2 (30 mL), stirred over 3Å sieves for 1 h, was added a solution of maleic anhydride (0.476 g, 4.85 mmol) in THF (30 mL) at 0 °C. The resulting mixture was allowed to stir at room temperature overnight, coarse filtered to remove the sieves, and then fine filtered. The solid (3.2 g) was taken up in 80 mL of MeOH–EtOH (8:2), heated to 60 °C with stirring, cooled, and filtered to yield **9** (19.8 g, 96%): mp >270 °C; EIMS (70 eV), *m/e* 440 (M^+). Anal. ($C_{24}H_{28}N_2O_6\cdot H_2O$) C, H, N.

Acknowledgment. This research was supported by the National Institute on Drug Abuse (DA 01533). We thank Masako Ikeda, Victoria Darrow and Mary Schwartz for the capable technical assistance in performing the biological testing.

Ion-Sensitive Electrode Potentiometry of Organic Anions: Application to Quantitative Structure–Activity Relationships

Roland F. Hirsch* and Elliot M. Rachlin

Department of Chemistry, Seton Hall University, South Orange, New Jersey 07079. *Received August 9, 1982*

Ion-sensitive electrode potentiometry is proposed for determination of substituent constants for structural modifications of organic acids. A liquid membrane anion-sensitive electrode responds reproducibly to a wide range of carboxylate and sulfonate ions. Fragment constants for the addition of a methylene group to aromatic and aliphatic acids are −2.4 and −3.3 ± 0.15 kJ/mol, respectively. Agreement is observed between these constants and those determined by other techniques, including partitioning studies in biphasic systems, suggesting the use of potentiometry for quantitative structure–activity relationship studies. Furthermore, the electrode measurements correlate with biological effects resulting from hydrophobic interactions.

Quantitative structure–activity relationships (QSAR) have become increasingly useful in interpreting data in the fields of physical organic chemistry, biological chemistry, and new drug development. Several examples can be found for the use of these QSAR's in the study of the activity of currently available drugs.[1,2] In some cases modification of a drug may be desired to increase or decrease its potency. Where lipophilicity is the determining factor, the effect may be adjusted through the addition of one or more groups.[3] Substituent constants such as those

(1) Quinn, F. R.; Beisler, J. A. *J. Med. Chem.* 1981, *24*, 251–256.

(2) Hansch, C.; Leo, A.; Schmidt, C.; Jow, P. Y. C. *J. Med. Chem.* 1980, *23*, 1095–1101.

(3) James, R.; Glen, J. B. *J. Med. Chem.* 1981, *23*, 1350–1357.

0022-2623/83/1826-1235$01.50/0 © 1983 American Chemical Society