Finally, the possible presence of separate recognition sites provides a physiological mechanism for the fine tuning of responses to endogenous opioid ligands. It also has implications regarding the structure-activity relationship of μ opioid agonists because such agonism should reflect the relative affinity at two sites rather than one.

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Articles

Role of Substrate Lipophilicity on the N-Demethylation and Type I Binding of 3-*O*-Alkylmorphine Analogues

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A series of 3-*O*-alkylmorphine analogues was synthesized to determine if there was a good correlation between the rate of metabolism, type I binding affinity, and lipid solubility. The data indicate that the K_m for the N-demethylation declines with increasing chain length from C_1 to C_9, while for increasing chain length the V_{max} for the N-demethylation increases to a maximum of 15.20 nmol min^{-1} (mg of protein)$^{-1}$ for the butyl analogue (C_4) and then slowly declines with analogues with chain lengths greater than butyl (C_4). The decyl (C_{10}) and dodecyl (C_{12}) analogues showed no activity. There was a good correlation between the lipophilicity and K_m values, except for codeine and the C_{10} and C_{12} analogues. The type I binding dissociation constants (K_s) also decreased with increasing alkyl chain length with an excellent correlation between the K_s and log P. The OD_{max} did not change with increasing the chain length of the analogues. Our data suggest that in male rat hepatic microsomes the catalytic site for N-demethylation and the site for type I binding in this series of compounds are similar but distinct.

Imai and Sato[1] and Remmer et al.[2] simultaneously reported that in the presence of various drugs, hepatic microsomes exhibited characteristic absorbance changes in the Soret spectrum of cytochrome P-450. Schenkman and co-workers[3-7] classified these interactions into three types termed type I, type II, and reverse type I. A number of studies suggest that type I, but not the other two types, could be related to the metabolism of drugs.

On the basis of the concentration-dependent kinetics of the binding spectrum and metabolism, Schenkman et al.[5] proposed that the type I binding of some substrates is related to the binding of the drug to the catalytic site of the cytochrome P-450. They observed that for many substrates there was a similarity between the type I spectral dissociation constant, K_s, and the Michaelis constant, K_m.[5] However, Davies et al.,[8] in investigating the effect of sex and species differences on drug metabolism, did not find a direct relationship between metabolism and type I binding. Similarly, Hedwick and Fouts[9] reported no correlation between the type I binding and metabolism of a series of sympathominetics. Further, Al-Gailany et al.[10] reported that for a series of *p*-nitrophenyl alkyl ethers, the K_m's for the dealkylation were generally one order of magnitude lower than the corresponding K_s. In line with these observations, Anders et al.[11] found no correlation between the type I binding parameters and the rates of N-demethylation for several enantiomeric drugs. Finally, studies from our laboratory have suggested that the type I binding site and the catalytic site are probably separate sites.[12]

The effect of substrate lipophilicity on type I binding and metabolism has also been examined. Al-Gailany et al.[13] found a very good correlation between the binding

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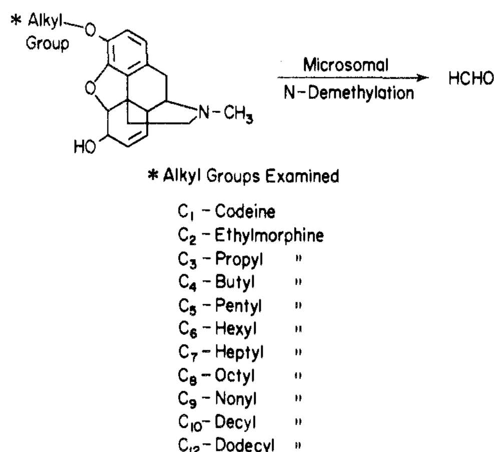
Scheme I. Metabolic *N*-Demethylation of a Series of 3-*O*-Alkylmorphine Analogues by Male Rat Hepatic Microsomes



affinity and substrate lipophilicity for a series of aliphatic, alicyclic, and aromatic compounds. On the other hand, in studies on a series of barbituric acid derivatives, Jansson et al.[14] found that all of the compounds produced typical type I spectra but there was only a poor correlation between the binding affinities and the partition coefficients. Likewise, there was no statistical correlation between the partition coefficient and the rate of metabolism, even though the more lipid-soluble substrates were more rapidly metabolized.

As has been well reviewed by Backes and Canady,[15] the effect of lipophilicity on the kinetic and binding parameters of a drug will depend on the structural characteristics of the enzyme complex. Since this complex contains both lipid and highly hydrophobic proteins, the metabolism of highly lipophilic substrates could be markedly affected by the solubility of the substrate in the lipids. Firstly, the substrate may nonspecifically dissolve in the lipid, which may significantly decrease the concentration of substrate in equilibrium with the catalytic site. Alternatively, if the catalytic site is buried in the lipid membrane, increasing lipophilicity would increase the chemical activity at the catalytic site.

In the current study, we have examined a series of morphine analogues having alkyl ether groups on the 3-position to determine what effect the increase in chain length would have on the parameters of type I binding and N-demethylation in order to determine what effect increasing lipophilicity has on these actions. These agents are all quite water soluble so that the added concentration is the free concentration. Hence, any effect that we observe can be attributed to the nature of the catalytic site itself rather than to changes in partitioning into the membrane lipids.

Our data indicate that there is no simple relationship between the maximal rate of metabolism and the lipophilicity of the drug. Further, the maximal type I binding spectrum is the same for all drugs and, finally, the K_s and K_m decrease in a constant ratio with increasing lipophilicity of the substrates. These studies would suggest that the catalytic site for the N-demethylation of the 3-*O*-alkyl substituted morphines is either buried in a crevice in the enzyme protein or a hydrophobic binding site 5.0 Å from the heme of the cytochrome P-450.

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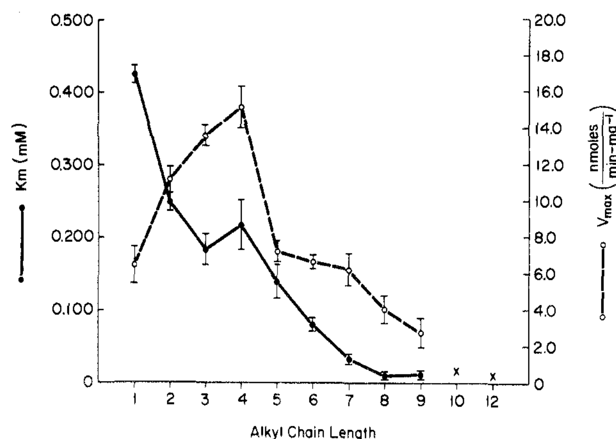


Figure 1. Michaelis–Menten kinetic parameters for 3-*O*-alkylmorphine analogues. The X denotes that the decyl (C_{10}) and dodecyl (C_{12}) analogues were not metabolized at detectable levels. K_m and V_{max} (±SEM) were calculated by Wilkerson regression for triplicate determination of $N = 11$ substrate concentrations for each analogue.

Table I. Rate of Ethylmorphine *N*-Demethylase in the Presence of Various Concentrations of Decyl and Dodecyl Analogues[a]

analogue	concn, μM	rate,[b] nmol min⁻¹ (mg of protein)⁻¹
3-*O*-decyl	0	9.11 ± 0.34
	20	8.92 ± 0.35
	200	9.12 ± 0.41
	2000	9.22 ± 0.38
3-*O*-dodecyl	0	8.92 ± 0.48
	20	8.81 ± 0.46
	200[c]	8.98 ± 0.51
	2000	9.06 ± 0.36

[a] Incubations were run with 2 mM 3-*O*-ethylmorphine for 10 min. [b] Values of N-demethylation are averages plus or minus standard errors of triplicate incubations. [c] 3-*O*-Dodecyl analogue (200 μM concentration) had no effect upon the K_s value of 3-*O*-ethylmorphine; basal = 33 μM ± 2.1; 3-*O*-dodecyl analogue = 38 μM ± 3.5.

Results

Synthesis of Substrates. A series of 3-*O*-alkylmorphine analogues was synthesized in which the alkyl substitutent was lengthened from codeine (C_1) to dodecylmorphine (C_{12}) (Scheme I). The pure *n*-undecyl derivative (C_{11}) could not be synthesized with the commercial *n*-undecyl bromide, since instrumental analysis indicated the bromide was a mixture of structural isomers.

N-Demethylase Activities. Metabolic data indicated that the K_m for the N-demethylation progressively declined with increasing chain length from C_1 to C_9 (Figure 1). However, the effect of increasing chain length on the V_{max} for the N-demethylation is quite different. The maximal activity increased from 5.60 nmol min^{-1} (mg of protein)$^{-1}$ for codeine (C_1) to 15.20 nmol min^{-1} (mg of protein)$^{-1}$ for the butyl (C_4) analogue. For alkyl groups greater than butyl (C_4), the N-demethylation slowly declines with increasing chain length. The decyl (C_{10}) and dodecyl (C_{12}) showed no activity.

It is interesting that the activity did not monotonically decrease from C_4 but rather there was a marked decline between C_4 and C_5 and followed a much slower decline to C_9. Since these analogues are soluble at 2 mM, the fact that the C_{10} and C_{12} analogues did not show activity could be due to either the side chain holding the N-17 too far

Table II. Comparison of the Kinetic Parameters for N- and O-Dealkylation of 3-O-Ethylmorphine and 3-O-Octylmorphine[a]

substrate	N-demethylation[b] K_m, mM	N-demethylation[b] V_{max}, nmol min^{-1} (mg of protein)$^{-1}$	O-dealkylation[b] K_m, mM	O-dealkylation[b] V_{max}, nmol min^{-1} (mg of protein)$^{-1}$
3-O-ethylmorphine (C_2)	0.245 ± 0.016	9.31 ± 0.21	0.055 ± 0.006	1.86 ± 0.06
3-O-octylmorphine (C_8)	0.067 ± 0.010	1.98 ± 0.09	0.125 ± 0.017	3.57 ± 0.11

[a] Incubations were run in triplicate for 20 min with eight concentrations of 3-O-octylmorphine ranging from 0.1 to 1.0 mM. [b] Values for the N- and O-dealkylation are averages plus or minus standard errors of three separate studies. Data were derived from Lineweaver-Burk plots with 1 mg/mL of microsomal protein.

Table III. Apparent Type I Spectral Interactions of 3-O-Alkylmorphines[a]

compound	formula	OD_{max}[b] (OD × 10^{-2}/3 mg)	$K_s \times 10^{-2}$,[b] mM
codeine sulfate	$(C_{18}H_{21}NO_3)_2 \cdot H_2SO_4 \cdot 5H_2O$	1.85 ± 0.04	c
3-O-ethylmorphine hydrochloride	$C_{19}H_{23}NO_3 \cdot HCl \cdot 2H_2O$	1.90 ± 0.05	2.91 ± 0.08
3-O-propylmorphine hydrochloride	$C_{20}H_{25}NO_3 \cdot HCl \cdot H_2O$	1.80 ± 0.08	2.84 ± 0.06
3-O-butylmorphine hydrochloride	$C_{21}H_{27}NO_3 \cdot HCl \cdot 2H_2O$	2.06 ± 0.13	1.81 ± 0.09
3-O-pentylmorphine hydrochloride	$C_{22}H_{29}NO_3 \cdot HCl \cdot H_2O$	2.00 ± 0.09	1.46 ± 0.04
3-O-hexylmorphine hydrochloride	$C_{23}H_{31}NO_3 \cdot HCl \cdot H_2O$	1.90 ± 0.08	1.28 ± 0.09
3-O-heptylmorphine hydrochloride	$C_{24}H_{33}NO_3 \cdot HCl \cdot 2H_2O$	1.80 ± 0.09	1.14 ± 0.11
3-O-octylmorphine hydrochloride	$C_{25}H_{35}NO_3 \cdot HCl \cdot H_2O$	2.10 ± 0.04	0.88 ± 0.77
3-O-nonylmorphine hydrochloride	$C_{26}H_{37}NO_3 \cdot HCl \cdot H_2O$	2.15 ± 0.09	0.76 ± 0.09
3-O-decylmorphine hydrochloride	$C_{27}H_{39}NO_3 \cdot HCl \cdot H_2O$	ND	ND
3-O-dodecylmorphine hydrochloride	$C_{29}H_{43}NO_3 \cdot HCl \cdot H_2O$	ND	ND

[a] Difference spectra and spectral dissociation constants were determined as described under Experimental Section. [b] Values are averages plus or minus standard errors of triplicate incubations. [c] Codeine produced a type I spectral change up to 1.90 mM; a K_s could not be determined. [d] ND, no detectable binding spectra.

from the active oxygen or the analogues acting as detergents, which causes a loss of total mixed function oxidase activity. The former would appear to be the case, since the kinetics for the N-demethylation of 3-O-ethylmorphine (C_2) were unaffected by the presence of the decyl (C_{10}) or dodecyl (C_{12}) analogues at concentrations of 20 μM, 200 μM, and 2 mM (Table I).

It is also possible that the decrease in the rate of N-demethylation of the analogues with increasing chain length could be due to a shift in metabolism to O-dealkylation and not due to the orientation of the N-17-methyl group. Yet, when kinetics for both the N- and O-dealkylation of the 3-O-octyl[6-^{3}H]morphine were determined, the V_{max} for N-demethylation was markedly less than for 3-O-ethylmorphine, but that for O-dealkylation was comparable (Table II). Even though the rate of O-dealkylation was twice the rate of N-demethylation, it still does not account for the decrease in the rate of N-demethylation for the long-chain analogues, since the sum of the two rates [5.55 nmol min^{-1} (mg of protein)$^{-1}$] is considerably less than the rate of 8 to 15 nmol min^{-1} (mg of protein)$^{-1}$ seen with 3-O-ethylmorphine. These data suggest that the chain length does not cause a change in pathways but only has an effect upon the orientation of the N-17 methyl group with respect to the catalytic site and subsequent metabolism.

The relationship between the K_m and the log P indicates that the increasing affinity of the longer chain analogues for the catalytic site is paralleled by an increase in the 1-octanol–water partition (Figure 2). Except for codeine and the C_{10} and C_{12} analogues, a good correlation was observed between the lipophilicity of the 3-O-alkylmorphine analogues and their K_m values (eq 1).

$$\log K_m = -0.552 \log P + 2.668 \quad (1)$$

$$n = 8, r = 0.922, \text{SD of the slope} = 0.0468$$

The lack of a good correlation for codeine may be due to the inability of codeine to bind to the hydrophobic region of the catalytic site of the enzyme. The possibility exists that the hydrophobic region at the catalytic site will not bind any of the 3-O-alkyl analogues that possess less

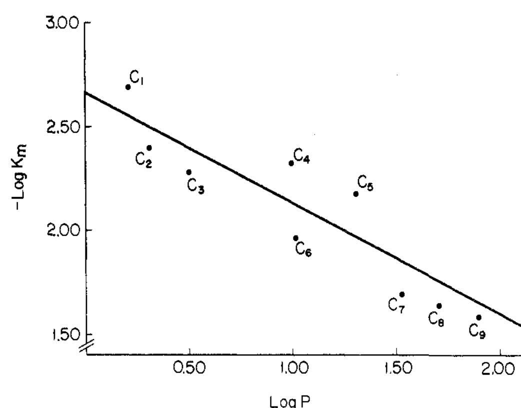


Figure 2. The relationship between −log K_m and log P of 3-O-alkylmorphine analogues. K_m was determined by a double-reciprocal plot of the rate against the substrate concentration. Partition coefficients and the correlation coefficients for the structure–activity relationships were determined as described in the text.

than a two-carbon chain on the 3-O-position. Codeine, which possesses a single methyl group on the phenolic hydroxyl group, may lack enough lipophilicity to tightly bind at the catalytic site, as indicated by the higher K_m.

Nearly all of the 3-O-alkylmorphine analogues studied interact with hepatic microsomes to produce a type I spectral change, the exceptions being morphine, codeine, and the C_{10} and C_{12} derivatives (Table III). Morphine exhibits a reverse type I spectra with a trough at 390 nm and a peak at 420 nm, while codeine produces a different spectral profile. At concentrations from 0.120 to 1.90 mM, it gives a type I spectrum; however, at higher concentrations it exhibits a reverse type I spectral change similar to that seen with morphine. Schenkman et al.[7] have reported a similar phenomenon with phenacetin.

Schenkman et al.[7] have suggested that the type I spectral change appears to be related to the metabolism of some substrates. In line with this, all the analogues that are metabolized give a type I spectrum. Similarly, the decyl (C_{10}) and the dodecyl (C_{12}) analogues failed to produce any

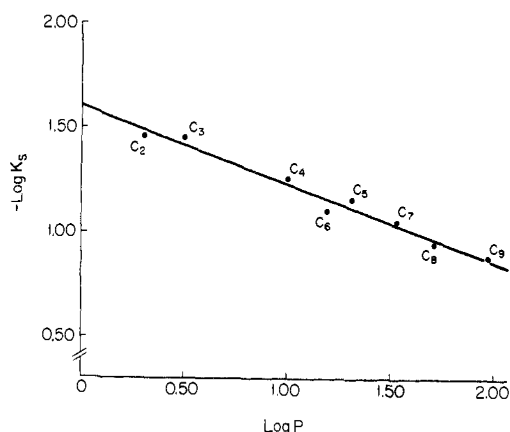


Figure 3. The relationship between $-\log K_s$ and the log of P of 3-*O*-alkylmorphine analogues. The spectral dissociation constants (K_s) were determined by double-reciprocal plots of the absorbance difference between 390 and 420 nm. Partition coefficients and correlation coefficients for the structure–activity relationships were determined as described in Figure 2.

binding spectra, suggesting that a nine-carbon chain may be the maximal length that will allow the substrate to bind to the type I binding site (Table III). Again, the failure of C_{10} and C_{12} derivatives to give a type I spectrum is not due to a detergent effect, since the C_{12} derivative did not affect the binding of 3-*O*-ethylmorphine (C_2) (Table I).

Binding Studies. The type I binding dissociation constants (K_s) also showed significant changes with increasing alkyl chain length (Figure 3). As previously reported, codeine did not give a typical type I spectrum and, thus, was eliminated from the following analysis. Even though the differences in the K_s values were small from C_2 to the C_9 analogues, there was an excellent correlation (eq 2) between the K_s value and log P.

$$\log K_s = -0.370 \log P + 1.606 \quad (2)$$

$$n = 8,\ r = 0.986,\ \text{SD of the slope} = 0.038$$

A second interesting observation is that the OD_{max} is unchanged by increasing the chain length (Table III). This would be expected, since the type I difference spectrum is thought to result from an increase in the proportion of the high spin form of cytochrome P-450. If the analogues do not directly bind to the heme of the cytochrome, then there should be no change in the extinction coefficient for either the high or low spin forms. Hence, the maximal difference in absorbance will represent only a maximal ratio of the high to low spin forms.

Discussion

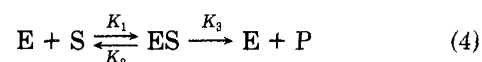
Early work on the relationship between the K_m and K_s values and the rate of metabolism[5] has suggested that because of the similarity between the K_m and K_s values for some substrates, the type I binding site and the catalytic site were the same. In the present study, there are significant differences between the K_m's and K_s's, indicating that the type I binding site and the catalytic site for this series of compounds are distinct sites. The correlation between the K_m and the rate of metabolism indicates that as the chain length increases, the K_m decreases, suggesting a tighter binding to the catalytic site. However, for the short-chain analogues, as the K_m decreases, the rate of N-demethylation increases up to the butyl (C_4) analogue, after which there is a decrease in the N-demethylation. This would indicate that an analogue that possesses up to four carbons can readily bind to the catalytic site. If we assume that the bond length from each methylene group is 1.5 Å, then these data would suggest that the catalytic site is 6 Å from the heme-active oxygen complex or that the catalytic site is buried in a protein cleft with an opening of 6 Å. As the chain length increases beyond this value, the N-17-methyl group is no longer held close to the active oxygen on the heme of the cytochrome P-450, resulting in a decrease in the rate of N-demethylation.

Like the K_m, there is a decrease in K_s with increasing alkyl chain length, supporting the generally held assumption that type I binding is dependent on the lipophilicity of the compound. The correlation for both activities between the lipophilicity and the affinity constants would suggest that although the binding sites are distinct, they do share the same basic structure of an hydrophobic characteristic. In line with this, the percentage change in the K_s and K_m for each log unit of change in P is the same (% Δ/log P for K_m = 53%; % Δ/log P for K_s = 44%).

Finally, the marked differences in K_m and K_s could be an artifact of the system. Clearly, K_m is not a true dissociation constant but rather a "pseudo dissociation constant":

$$K_m = \frac{K_2 + K_3}{K_1} \quad (3)$$

where K_1, K_2, and K_3 are the rate constants for the following model:



$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_3} E + P \quad (4)$$

where E is the enzyme and S and P are the substrate and product, respectively. One may question whether a complex system as the mixed function oxidases fit this simplistic model, but data from our laboratory and many others have consistently found that these pathways fit the simple kinetics.[25] Our current data would further suggest that the activated oxygen–cytochrome P-450 complex is formed and fits standard Michaelis-Menton kinetics.[25] Yet, the K_m is only a true dissociation constant as K_3 goes to zero. If $K_3 \gg K_2$ and both change in the same direction with changes in log P, then the K_m would change in a constant proprotion with K_s. Against this model is that the decrease in V_m, which is proportional to K_3, between C_4 and C_5 is much greater than the decrease in K_m.

We recognize that our conclusions concerning the characteristics of the catalytic site for N-demethylation of morphine analogues must be tentative, since some of the effects we are observing might be due to changes in the particular isozyme of cytochrome P-450 catalyzing the N-demethylation of the various analogues. We feel that the current data are still of importance for three reasons. Firstly, these isozymes have yet to be purified, so that studies with the pure enzymes may not be feasible in the foreseeable future. Secondly, irrespective of whether different isozymes catalyze the N-demethylation, these studies still place constraints on the nature of the catalytic site(s).

Finally, a major deficiency in reconstitution studies is that there is the possibility that the purified isozyme may exhibit activities that they lack when studied in situ. Hence, to verify that a reconstituted system contains the isozymes that act in situ, these isozymes must show the same structure–activity relationships as we have observed here. Hence, our current data represent a reference point for purification studies of the microsomal enzymes as are now in progress in our laboratory and in others.

In summary, our data suggest that the catalytic site for the N-demethylation of 3-*O*-alkyl analogues of morphine and for the type I binding sites are similar but distinct sites in hepatic microsomes from male rats.

Table IV. Analysis of 3-O-Alkylmorphines

compound	mp of HCl salts, °C (hydrous uncorrected)	major peak by EI MS [(M + 1)+]	R_f[a]
morphine (C_0)[b]			0.19
codeine (C_1)[b]			0.46
3-O-ethylmorphine (C_2)[b]			0.48
3-O-propylmorphine (C_3)	108-110[c]	328	0.49
3-O-butylmorphine (C_4)	100-103[c]	342	0.51
3-O-pentylmorphine (C_5)	117-119	356	0.50
3-O-hexylmorphine (C_6)	115-117	370	0.52
3-O-heptylmorphine (C_7)	110-112	384	0.53
3-O-octylmorphine (C_8)	121-123	398	0.54
3-O-nonylmorphine (C_9)	116-118	412	0.56
3-O-decylmorphine (C_{10})	128-130 dec	426	0.59
3-O-dodecylmorphine (C_{12})	135-137 dec	454	0.60

[a] Determined for free bases on silica gel GF with $CHCl_3$/methanol (90:10). [b] Commercially available. [c] Chichibabin, A. E.; Kirsandy, A. *Bull. Soc. Chim. Fr.* 1927, 41, 1649-1652.

Experimental Section

Materials. Codeine sulfate (Mallinckrodt, St. Louis, MO), 3-O-ethylmorphine (Dionin, Merck and Co., Rahway, NJ), and morphine sulfate (Merck and Co., Rahway, NJ) were purchased and converted to the corresponding free bases. The higher 3-O-alkylmorphine derivatives were synthesized by the method of Baizer and Ellner[16] in which the morphine base was coupled to the appropriate *n*-alkyl bromides in the presence of zinc powder. All of the alkyl bromides were purified by distillation before use, and the products were purified by preparative thin-layer chromatography on silica gel GF with $CHCl_3$–methanol (90:10). The purity of the analogues was confirmed by infrared spectroscopy, which indicated the disappearance of the phenolic hydroxyl group. The NMR spectra were consistent with the presumed structures. All melting points showed narrow ranges when determined on a Fischer-Johns melting point apparatus (Table IV) (values uncorrected). Elemental analysis (C, H, and N) were performed by Schwarzkopf Microlabs, Woodside, NY, and were within 0.4% of theoretical values.

Enzyme Preparation. Hepatic microsomes were prepared from fed, untreated, 180–250-g male CD rats obtained from Charles River Laboratories (Wilmington, MA). The animals were killed by cervical fracture, and the livers were removed, chilled on ice, and then homogenized in 0.15 M KCl–0.05 M Tris, pH 7.4 (3 mL/g of liver). The microsomes were prepared by standard centrifugation techniques and washed in the same buffer.[17] The microsomal protein was determined by the method of Sutherland et al.[18]

Incubation Conditions. Triplicate incubations were performed as previously described unless otherwise stated.[19] In this method the appropriate amount of each analogue was added to 20-mL serum vials with a Hamilton Pb 600 dispenser, and the reaction vials were equilibrated at 37 °C for 5 min in a shaking water bath. Three milliliters of an incubation mixture containing nicotinamide adenine dinucleotide phosphate (0.33 mM), glucose 6-phosphate (5.5 mM), and glucose-6-phosphate dehydrogenase (0.67 unit/mL) in 0.15 M KCl–0.05 M Tris buffer with 5 mM $MgCl_2$ and 1 mg of microsomal protein/mL were added to each vial to initiate the reaction. The samples were incubated in air for 10 min at 37 °C.

Assay of N-Demethylation. The N-demethylation of the 3-O-alkylmorphine analogues was determined colorimetrically by measuring formaldehyde production.[20,21] Incubations were terminated by the addition of 1 mL of 5% $ZnSO_4$. This was followed by a 3:1 mixture of saturated $Ba(OH)_2$ (1.5 mL) and saturated $Na_2B_4O_7$ (0.5 mL). After centrifugation at 2000 rpm in a PR-2 centrifuge (IEC-Damon) for 10 min, the formaldehyde was determined in 3 mL of the clear supernatant after addition of 1.5 mL of a mixture of acetyl acetone in 4 M ammonium acetate (0.1 mL of acetyl acetone per 25 mL of solution). The color was developed for 10 min at 60 °C, and the absorbance was determined at 412 nm in a Gilford spectrophotometer. The millimolar extinction coefficient was taken as 7.74 for the complex. The endogeneous microsomal formaldehyde was determined in incubations without the substrate in the incubation vial.

Radiometric Assays. The N-demethylation of [6-^{3}H]codeine and the N- and O-dealkylation of octyl[6-^{3}H]morphine were determined by a radiometric assay.[17] In this assay the incubations were terminated with 5 mL of acetone and saturated with potassium acetate.[22] The aqueous incubation medium was extracted 3 times with 5 mL of acetone and combined with the original extract. The acetone was removed, dried over Na_2SO_4, and evaporated under a stream of nitrogen. Unlabeled morphine and the appropriate nor metabolites were added, and the samples were plated onto silica gel GF (LK 5DF, Whatman Inc., Clifton, NJ). The plates were developed in chloroform–methanol–concentrated aqueous NH_4OH (18.0:0.5:1.5). The metabolites were identified by shortwave UV spectroscopy, scraped, and counted in a Beckman LS-100C liquid scintillation counter as previously described.[17]

Determination of Type I Binding. The difference spectra for the type I binding site were determined by placing a cuvette containing 3 mL of a washed microsomal suspension (3 mg of protein/mL in 0.15 M KCl–0.05 M Tris) into each side of an Aminco-DW-2 spectrophotometer in the split-beam mode.[12] The cells were allowed to warm up to 37 °C for 5 min, after which a solution of the substrate was added stepwise to the sample cuvette, and an equal volume of buffer was added to the reference cuvette. Difference spectra were recorded between 350 to 500 nm after each addition. The spectral dissociation constants were determined by using double-reciprocal plots of the difference in absorbance between the peak (390 nm) and the trough (420 nm) as previously reported.[3]

Determination of Partition Coefficient. The partition coefficient, *P*, for each substrate was determined between 1-octanol and 0.15 M KCl–0.05 M Tris–0.005 M $MgCl_2$ buffer, pH 7.4. Each phase was presaturated with the other before use. After 1 h of agitation, the samples were centrifuged. The concentrations of the compounds in both of the phases were assayed spectrophotometrically by using the wavelengths at which the derivative exhibited absorption maxima in each of the solvents.[10,23]

Regression Analysis. The equation used in these structure–activity relationship studies, the correlation coefficient (*r*), and the residual standard deviation (*s*) were calculated by the "Hansch regression technique".[24] The results have been expressed

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as the mean ± SD and tests for statistical significance were performed by using the appropriate Student's t test.

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Registry No. Codeine, 76-57-3; 3-*O*-ethylmorphine, 76-58-4; 3-*O*-propylmorphine, 74886-10-5; 3-*O*-propylmorphine hydrochloride, 86261-14-5; 3-*O*-butylmorphine, 74886-09-2; 3-*O*-butylmorphine hydrochloride, 86261-15-6; 3-*O*-pentylmorphine, 74886-08-1; 3-*O*-pentylmorphine hydrochloride, 86261-16-7; 3-*O*-hexylmorphine, 74886-11-6; 3-*O*-hexylmorphine hydrochloride, 86261-17-8; 3-*O*-heptylmorphine, 74886-12-7; 3-*O*-heptylmorphine hydrochloride, 86272-04-0; 3-*O*-octylmorphine, 74886-13-8; 3-*O*-octylmorphine hydrochloride, 86261-18-9; 3-*O*-nonylmorphine, 74886-14-9; 3-*O*-nonylmorphine hydrochloride, 86261-19-0; 3-*O*-decylmorphine, 74886-15-0; 3-*O*-decylmorphine hydrochloride, 86261-20-3; 3-*O*-dodecylmorphine, 74886-07-0; 3-*O*-dodecylmorphine hydrochloride, 86261-21-4.

Aporphines. 50.[1] Kinetics of Solvolysis of *N*-(2-Chloroethyl)norapomorphine, an Irreversible Dopamine Receptor Antagonist[2]

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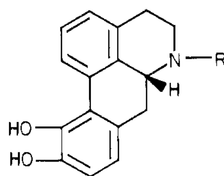
The rates and mechanism of solvolysis of (-)-*N*-(2-chloroethyl)norapomorphine (NCA, **1c**) in aqueous solution have been examined by reversed-phase liquid chromatography (HPLC) to follow the levels of starting material and products. The first-order rate constants for aziridinium ion formation at 25 and 37 °C at pH 7.0 are 0.024 and 0.096 min^{-1}, respectively. Determination of the first-order rate constant for the disappearance of NCA as a function of pH has allowed the calculation of an approximate pK_a of 6.3 for the tertiary amine, while the influence of reaction conditions (e.g., pH, buffer salt and concentration, and added nucleophiles) on product distribution support the view that NCA solvolysis proceeds through an intermediate aziridinium ion. Application of the HPLC procedure allowed us to observe simultaneously the loss of NCA and the appearance of an intermediate and multiple products at trace levels; it also permitted the facile isolation and subsequent identification of small amounts of hydrolysis products. At pH 7, maximum aziridinium concentration is reached only after 10 min at 37 °C and at 25 °C after 1 h. Increased temperatures and pH facilitate the rate of aziridinium ion formation, as well as of non-dopamine antagonist solvolysis products. The significance of these findings, including the ease with which buffer ions add to the intermediate ion, are discussed in relation to the use of NCA and its tritiated isomer, [^{3}H]NCA, in dopamine receptor studies.

The role of dopamine (DA) as a neurotransmitter at receptors in the central nervous system (CNS) and at several sites in the peripheral nervous system has stimulated intense interest in the pharmacology of DA receptors and sites of probable interactions of DA and its agonists and antagonists. Since the discovery of therapeutically useful DA agonist activity in aporphine derivatives, apomorphine (APO, **1a**) and its *N*-alkyl congener *N*-*n*-



1a, R = CH_3 [(−)-APO]
b, R = $CH_2CH_2CH_3$ [(−)-NPA]
c, R = CH_2CH_2Cl [(−)-NCA]
d, R = $CH_2CH_2OPO_3H_2$
e, R = CH_2CH_2OH [(−)-NHA]
f, R = $CH_2CH_2OCOCH_3$

propylnorapomorphine (NPA, **1b**), considerable interest has developed in the study of the interactions with DA receptors.[3-6] Such studies have been facilitated by the direct radioligand binding using high specific activity, tritiated, dopaminergic agonists and antagonists.[1,7]

Substitution of the *N*-alkyl side chain on apomorphine has a profound effect on the dopamine receptor binding and on the pharmacological effects of such aporphines.[4] Increasing the size of the *N*-alkyl substituent increases the selectivity for [^{3}H]spiperone ([^{3}H]SPR) binding sites, a property which may reflect greater lipophilicity or altered selectivity for D-2 receptors.[6]

It has been suggested that compounds containing an alkylating moiety, such as the chlorethyl group, might be useful to label membrane DNA receptor components irreversibly.[7-9] Baldessarini and his group[8] have shown that phenoxybenzamine (PBZ) can bind to DA (D-1) receptor sites in the CNS, as evidenced by its ability to block DA-sensitive adenylate cyclase activity in rat striatal homogenates. This observation has been followed up by Hamblin and Creese,[9] who reported that PBZ has a noncom-

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