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STRUCTURAL INFLUENCES ON THE AMPEROMETRIC DETECTION OF OPIATES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The oxidation reactions of a series of opiates occuring at a glassy-carbon electrode in amperometric high-performance liquid chromatographic detection has been investigated. A structure–reactivity correlation has been drawn for morphone, morphinan and benzomorphan derivatives. Polarography and hydrodynamic voltammography were used to show the importance of phenolic groups to this reaction. Acyl substitution on the phenol did not prevent amperometric detection.

INTRODUCTION

Many intriguing problems surround the oxidation reactions of opiates. Among these are questions of reaction pathway as related to oxidation conditions and whether reaction products measured chromatographically are end products of oxidation reactions or intermediates. Previous studies on substrate structural requirements for various oxidizing conditions have also led to conflicting results. It has been claimed for example that benzomorphans are not detected amperometrically because of the lack of the furan ether bridge[1]. Yet other authors have detected cyclazocine and pentazocine electrochemically[2,3]. While a free phenolic hydroxyl is apparently required for electrochemical oxidation of morphine[1,2,4–7] it is also needed for opiates' oxidation with basic potassium ferricyanide[8–10]. These reactions give rise to dimerized products as morphine gives rise to the fluorescent pseudomorphine. This phenol does not however guarantee a fluorescent product even if dimerization does occur. For example, naloxone, naltrexone, hydromorphone and oxycodone do not give fluorescent products under these conditions presumably because of internal quenching by the 6-keto group. Similarly the benzomorphans cyclazocine and pentazocine do not fluoresce under these conditions according to some authors because of the absent furan link[10], while a fluorescent dimer of pentazocine has been measured by others[8].

The present investigation centers on the structure–reactivity relation for the oxidation reactions of opiates occurring in high-performance liquid chromatography (HPLC) amperometric detectors. Comparisons will be made between these reactions as supported by polarographic oxidation studies and structural features present in a series of opiates.

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EXPERIMENTAL

Reagents

Methanol was HPLC grade MCB Omnisolve, EM Scientific. Water was distilled and filtered (Millipore 0.45 μm). Phosphoric acid, 85%, was from Mallinckrodt and 1-octanesulfonic acid, sodium salt, was from Eastman. Acetonitrile was HPLC grade and disodium EDTA was reagent grade, both from Fisher Scientific. Sodium phosphate, dibasic, anhydrous was from J. T. Baker.

Compounds studied

The compounds studied are shown in Fig. 1. These include: (I) naloxone hydrochloride (Endo), (II) naltrexone hydrochloride (Endo), (III) hydromorphone hydrochloride (Knoll), (IV) oxycodone hydrochloride (Endo), (V) levorphanol tartrate (Hoffman-La Roche), (VI) dextromethorphan hydrobromide (Hoffman-La Roche), (VII) cyclazocine hydrochloride (Sterling-Winthrop), (VIII) 8-acetylpentazocine hydrochloride (Sterling-Winthrop) and (IX) N-1-propylnorapomorphine hydrochloride (Sterling-Winthrop).

Apparatus

Modular HPLC systems were used including Varian 5000, Waters M6000 and Beckman 110A pumps, Micromeritics 725 and Varian 5000 autosamplers and a Rheodyne 7125 manual injection valve. An electrochemical detector (Bioanalytical Systems LC-4) with a TL-5 glassy-carbon thin-layer transducer *versus* an Ag/AgCl reference electrode, a 5-sec time constant in the oxidation mode, a UV detector (Waters 440 set at 280 nm) and a Fisher Recordall Series 5000 recorder were used. All columns used were stainless-steel Whatman Partisil PXS 10/25 ODS-3.

Mobile phase

The mobile phase consisted of water–methanol–acetonitrile–85% phosphoric acid–0.1 *M* disodium EDTA (600:200:200:1:1) and was 0.0028 *M* overall in 1-octanesulfonic acid, sodium salt.

Chromatographic conditions

The flow-rate was 1.0 ml/min at ambient temperature. The injection volume was 20 μl. Solutions were prepared at about 0.1 mg/ml in methanol–water (1:1).

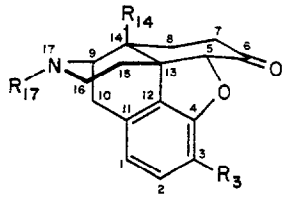
Polarographic analysis

Polarographic analysis was done on a Princeton Applied Research Model 174A polarographic analyzer using a 1/4-in. diameter glassy-carbon working electrode *versus* SCE at a scan-rate of 10 mV/sec.

The Supporting electrolytes were (1) the mobile phase and (2) 0.048 *M* ionic strength phosphate buffer prepared by adjusting the pH of a 0.01 *M* disodium hydrogen phosphate (Na_2HPO_4) solution in distilled water to 2.00 with phosphoric acid. Solutions were prepared in both supporting electrolytes at about 0.1 mg/ml.

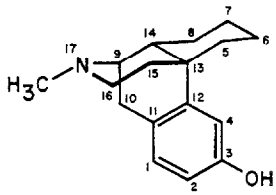
The recorder was a Fisher Recordall Series 5000.

MORPHONES



COMPOUND	R_{17}	R_3	R_{14}
I	$-CH_2-CH=CH_2$	-OH	-OH
II	$-CH_2-$ 	-OH	-OH
III	$-CH_3$	-OH	-H
IV	$-CH_3$	$-OCH_3$	-OH

MORPHINANS



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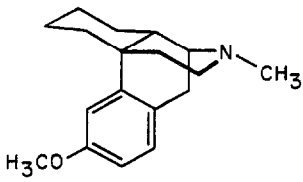

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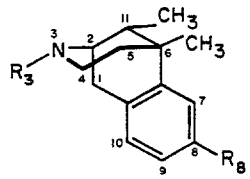
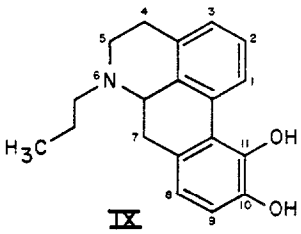
BENZOMORPHANS



COMPOUND	R_3	R_8
VII	$-CH_2-$ 	-OH
VIII	$-CH_2-CH=C(CH_3)-CH_3$	$-O-C(=O)-CH_3$

APOMORPHINES



IX

Fig. 1. Structures of compounds investigated.

RESULTS AND DISCUSSION

Hydrodynamic voltammograms of the compounds studied made under the paired-ion reversed-phase conditions are shown in Fig. 2. Extrapolation of the linear segments of these curves to zero gave the hydrodynamic potential values listed in Table I. A typical chromatogram of naloxone run at 0.95 V is shown in Fig. 3.

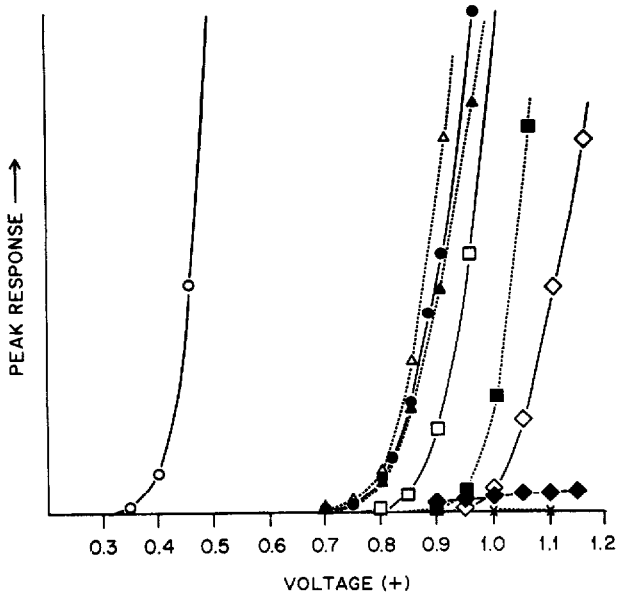


Fig. 2. Hydrodynamic voltammograms of compounds detected with a glassy-carbon electrode. Chromatographic conditions as described in text. ○—○ = IX; △---△ = II; ●—● = III; ▲---▲ = I, □—□ = VII; ■---■ = V; ◇—◇ = VIII; ◆—◆ = IV; ×---× = VI.

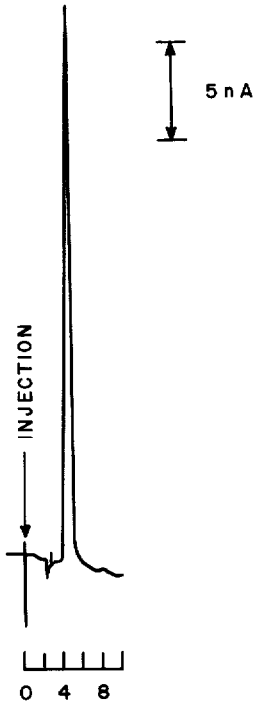


Fig. 3. Chromatogram of naloxone (0.1 mg/ml) with amperometric detection at 0.95 V and 50 nA/V.

TABLE I

HALF-WAVE POTENTIALS ($E_{\frac{1}{2}}$) MEASURED POLAROGRAPHICALLY AND EXTRAPOLATED HYDRODYNAMIC POTENTIALS FOR OPIATES

N.O. = none observed.

	Compound	$E_{\frac{1}{2}}$		*Hydrodynamic potential*
		Mobile phase	*Phosphate buffer*	
IX	N-1-propyl norapomerphine	0.50	0.45	0.43
I	Naloxone	0.85	0.70	0.84
III	Hydromorphone	0.88	–	0.82
II	Naltrexone	0.91	0.73	0.82
V	Levorphanol	0.95	–	0.98
VII	Cyclazocine	0.95	0.86	0.88
VIII	8-Acetylpentazocine	1.26	0.82	1.02
IV	Oxycodone	N.O.	1.25	N.O.
VI	Dextromethorphan	N.O.	1.35	N.O.
Ionic Strength (M)		0.092	0.048	
Apparent pH		2.39	2.00	

TABLE II

k' VALUES FOR OPIATES INVESTIGATED

Chromatographic conditions as in text.

	Compound	k'
I	Naloxone	0.65
III	Hydromorphone	0.65
IV	Oxycodone	0.95
II	Naltrexone	1.60
V	Levorphanol	1.92
VIII	8-Acetylpentazocine	3.22
IX	N-1-propylnorapomorphine	3.40
VII	Cyclozocine	3.80
VI	Dextromethorphan	6.46

Table II gives capacity factors (k') for the compounds of the series. Those for dextromethorphan and oxycodone were determined from HPLC–UV chromatograms when this detector was run in series with the electrochemical detector. Structural effects on reversed-phase retention are evident here. The fastest eluting compounds, I–IV, are all morphones containing the 6-keto group plus the furan ether giving increased polarity. Although the 14-hydroxy group has no effect on retention (compound I *versus* III), methylation of the 3-hydroxy group causes a large increase in retention (levorphanol *versus* dextromethorphan).

Polarographic waves for four of the compounds of the series in phosphate buffer (pH 2.00) are shown in Fig. 4. Half-wave potentials ($E_{\frac{1}{2}}$) for the series measured in this electrolyte and in the mobile phase at 0.1 mg/ml are listed in Table I. It can be observed that $E_{\frac{1}{2}}$ values of compounds measured in 0.048 M ionic strength phosphate buffer were 0.05 to 0.44 V more negative than in the 0.092 M ionic strength

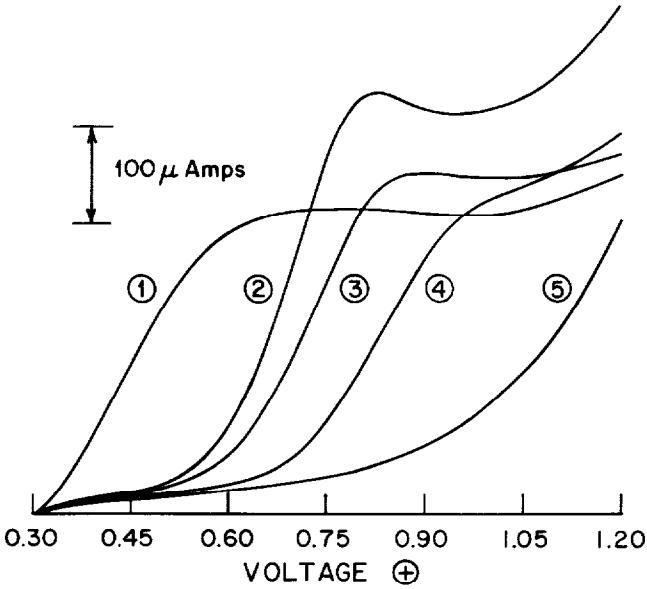


Fig. 4. Polarograms of four opiates in 0.048 *M* ionic strength phosphate buffer (pH 2.0). A glassy-carbon electrode *vs.* SCE were used at ambient temperature. Waves: 1 = IX; 2 = I; 3 = II; 4 = VII; 5 = phosphate buffer (ionic strength 0.048 *M*, pH 2.0).

mobile phase. This is consistent with an activity coefficient effect of ionic strength[11], as well as with a decreased dielectric constant effect caused by the increase in organic modifier content of the mobile phase[12].

A linear correlation is clearly seen between the polarographic $E_{\frac{1}{2}}$ values and the extrapolated oxidation potentials obtained from the hydrodynamic voltammograms. Thus, while at this relatively high concentration the same oxidative process occurs in both static and dynamic systems, the solvent effect must be included for a full explanation.

The structural features investigated here with respect to this oxidation reaction include the effect of alkyl or acyl substitution on the 3-position of the morphone or morphinan ring systems or the corresponding 8-position of the benzomorphan system. The effect of the 4-5 furan oxygen link, the C ring and the 14-hydroxyl group were also studied.

While a free phenolic 3-hydroxyl group is apparently necessary for oxidative dimerization of morphine and surrogates to pseudomorphine-like structures, this restriction is not as stringent for the electrochemical oxidation reaction. Dextromethorphan (VI) was not detected amperometrically as would be expected from the 3-methoxy substitution. A polarographic wave was observed however for this compound in the phosphate buffer with an $E_{\frac{1}{2}}$ of 1.35 V at the limit of usefulness of the glassy-carbon electrode[13]. Similarly oxycodone showed little electrochemical reactivity except at high oxidation potentials although it displayed a polarographic wave with an $E_{\frac{1}{2}}$ of 1.25 V in the more hospitable phosphate buffer.

All compounds possessing one free 3-hydroxy group were oxidized and detected electrochemically with $E_{\frac{1}{2}}$ between 0.85 and 0.95 V in the mobile phase. These

could undergo several potential reaction pathways such as the well-documented oxidative dimerization[14,15]. Other possibilities involving a radical cation intermediate include reaction with a nucleophile, disproportionation, cleavage reactions (to a catechol for example), oxidation to a dication or benzylic oxidation giving 10-hydroxy products[16–18].

The *ortho* dihydroxy substituted N-1-propylnorapomorphine (IX) was much more easily oxidized with an $E_{\frac{1}{2}}$ of 0.50 V. A mechanism such as that shown in Fig. 5 could be implicated here. This is similar to the electrochemical oxidation of the catechol levodopa leading to the stable conjugated orthoquinone[19].

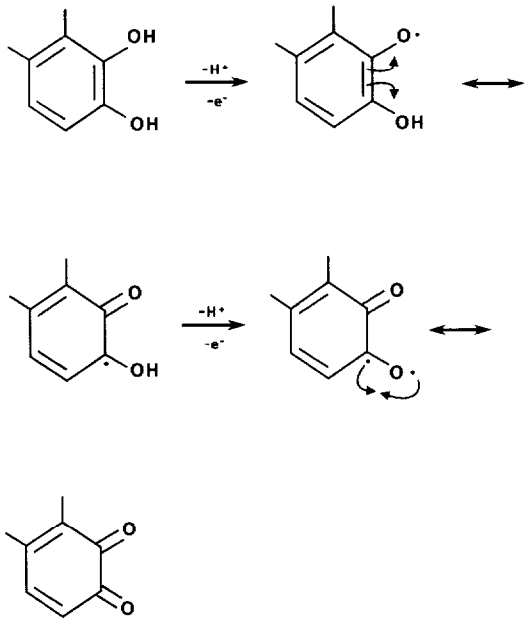


Fig. 5. Possible oxidation mechanism for N-1-propylnorapomorphine in amperometric detection.

A polarographic wave and electrochemical detection (ED) were observed for 8-acetylpentazocine (VIII) with an $E_{\frac{1}{2}}$ of 1.26 V. This could be an example of an anodic electrochemical substitution reaction (hydrolysis) at an easily cleavable ester linkage as was seen in the thermal oxidation of diacetylmorphine by gaseous ammonia[20]. The resulting free phenol would be subject to the reactions for compounds with that functionality.

The effect of the 4-5 furan ether link can be seen in comparing levorphanol (V) and cyclazocine (VII) to hydromorphone (III) which contains this linkage. The $E_{\frac{1}{2}}$ value for this latter compound is only 0.07 V lower than the non-furan containing molecules, indicating its lack of importance in ED.

A similar lack of effect of the 14-hydroxyl group on susceptability of opiates to amperometric detection is demonstrated by the $E_{\frac{1}{2}}$ values of hydromorphone compared to naloxone (I) and naltrexone (II). The 0.88 V of hydromorphone is midway between the 0.85 V of naloxone and 0.91 V of naltrexone.

The effect of the C ring on amperometric detection of opiates can be seen by comparing levorphanol (V) to cyclazocine (VII). The presence of this ring in the former has no effect on its oxidation $E_{\frac{1}{4}}$ as compared to the latter, both being 0.95 V.

The present results agree with the findings of Peterson *et al.*[1] on the lack of effect of the 14-hydroxy group on ED of opiates. Agreement, however, is found with results described by White[2] and Wallace *et al.*[3] on detectability of benzomorphans such as cyclazocine containing no furan ring. While the 3-hydroxy group is apparently the active center for the initiation of ED reactions, a reaction can also occur if this position has been acetylated. A more detailed elucidation of the various mechanisms discussed here awaits further work in each specific reaction including kinetics studies and product isolation and structural determination.

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