

Polarography of Narcotine-*N*-oxide*

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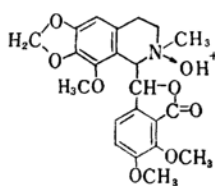
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It has been reported by Kirkpatrick¹⁾ that narcotine, an opium alkaloid, shows a catalytic hydrogen wave as do the other alkaloids, and according to Holubek²⁾, narcotine and hydrastin can be determined quantitatively with the the reduction wave of their oxidation product, opianic acid. It is known that *N*-oxides such as pyridine-*N*-oxide³⁾ and nitrogen mustard *N*-oxide⁴⁾ are reduced polarographically to the corresponding amines, but the polarography of narcotine-*N*-oxide has not been reported yet.

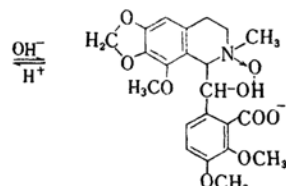
In this paper the polarographic determination of narcotine-*N*-oxide in the oxidation products of narcotine is attempted and further-more, the electrode reaction and behaviors of narcotine-*N*-oxide in aqueous solutions are examined by means of DC- and AC-polarography, electrocapillarity, potentiometric titration and ultra-violet absorption spectrometry. Narcotine-*N*-oxide shows 2-electron reduction waves of *N*-oxide, which are complicated by the adsorption on the electrode, the kinetic current of protonation and the reversible cleavage of lactone.

Experimental

The Yanagimoto pen-recording polarograph model PA 2, the Beckman pH meter model G and Beckman spectrophotometer model DU or DK 2 were used. The dropping mercury electrode has such characteristics as $m=0.817$ mg. sec⁻¹, $t=3.94$ sec., $m^{2/3}t^{1/6}=1.097$ and $h=70$ cm. Polarograms were taken in buffer solutions of acetate, phosphate,



(I)



(II)

borate, hydrochloric acid and sodium hydroxide at $25 \pm 0.1^\circ\text{C}$ after bubbling nitrogen gas, using N calomel electrode as the external reference electrode.

Narcotine hydrochloride and its *N*-oxide were given by Dr. H. Matsumaru of the Tokyo factory of our company.

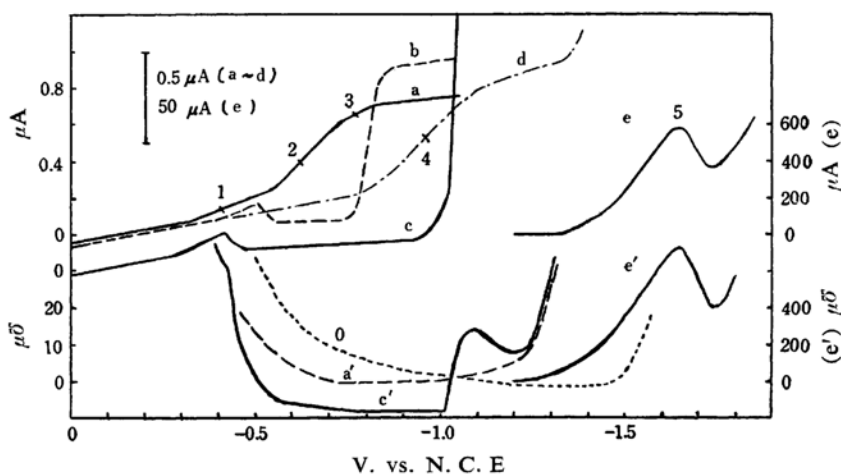


Fig. 1. D. C. (upper) and A. C. (lower) Polarograms of narcotine-*N*-oxide in pH 4. a, a, e, e', d: 0.2 mM, b: 0.3 mM, c, c': 1 mM, O: blank, d: hydroxycarboxylic acid form. 1~5: see Fig. 2.

* Polarographic studies of Pharmaceuticals. XXV. XXIV: *J. Pharm. Soc. Japan (Yakugaku Zasshi)*, **80**, 1226 (1960).

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2) J. Holubek, *Pharmazie*, **11**, 577 (1956).

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4) I. Aiko, *Pharm. Bull.*, **1**, 335 (1953).

Results

Catalytic Hydrogen Wave.—Narcotine-*N*-oxide shows a great increase of current from about -1.2 V. and generally a maximum wave in a buffer solution of pH 1~9. (Fig. 1e) The maximum wave height (i_m) is greatest at about pH 7 (Fig. 2 (5)) and tends to reach a limiting value at higher concentrations. The height (i_m) in a 0.2 mM solution of the *N*-oxide is suppressed from 56 μ amp. to 40 and 20 μ amp. in the presence of 0.01 and 0.05% gelatin, respectively. The maximum potential (E_m), $-1.4 \sim -2$ V., becomes more negative in higher wave heights and higher concentrations: $E_m -1.44$ V., i_m 0.09 μ amp. c 0.05 mM; $E_m -1.55$ V., i_m 7.6 μ amp., c 0.2 mM; $E_m -1.70$ V., i_m

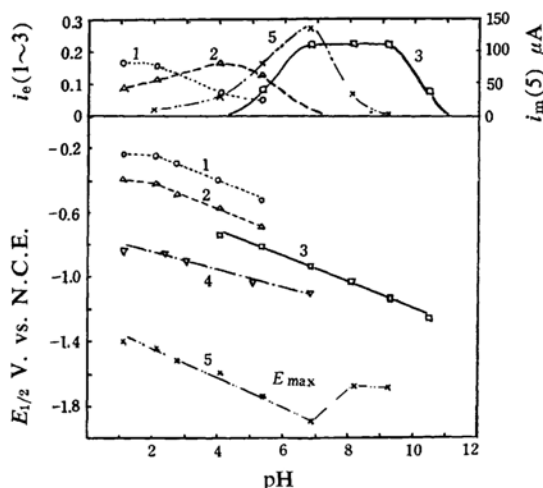


Fig. 2. $E_{1/2}$ -pH curves (lower) and wave height-pH curves of narcotine-*N*-oxide (0.1 mM).

1) Absorption wave, 2) Reduction wave of cation, 3) Reduction wave of free base, 4) Reduction wave of hydroxy-carboxylic acid form, 5) Catalytic hydrogen wave.

38.2 μ amp., c 0.6 mM at pH 9.2. The temperature coefficient of i_m , 8% deg. $^{-1}$, is very high. The height decreases with the increase of mercury head (h): i_m in a 0.1 mM solution of pH 9.2 is 1.62, 1.19 and 0.89 μ amp. at 48, 68 and 88 cm. of h respectively.

Reduction Wave of Lactone type Narcotine-*N*-oxide (I).—0.1 mM narcotine-*N*-oxide hydrochloride in a buffer solution of pH 1~10 shows 1~3 steps of reduction waves at the potential more positive than that of the catalytic hydrogen wave (Fig. 1a). The total wave height is independent of pH in a range of 1~9, but decreases at above pH 9 (Fig. 2 (1~3)). In 0.1 N hydrochloric acid, the total wave height (i_a) of 3 steps is proportional to

the concentration (c) in a range of 0.1~1 mM and the diffusion current constant (k_D) is calculated as 2.3 (μ amp. mM $^{-1}$ mg $^{-2/3}$ sec $^{1/2}$).

Their 1st step is considered as an adsorption wave since the relationships between the wave height (i_1) and the concentration (c) fulfil the equation $i_1 = a \cdot b \cdot c / (1 + b \cdot c)$ with $a = 1.25$ $b = 2.67$ mM $^{-1}$.

In acetate buffer of pH 4, 3 steps of reduction waves are also obtained in low concentration (0.05~0.23 mM), but in higher concentration (0.24~1 mM) an anomalous minimum wave in some potential range is obtained (Fig. 1, b, c). Thus the current is strongly suppressed in the potential range of $-0.42 \sim -1.04$ V. at 1 mM and $-0.6 \sim -0.7$ V. at 0.24 mM. The limiting current at -1.1 V. corrected for the residual current is, however, proportional to the concentration.

At pH 6.8~9.2, one reduction wave is observed near a catalytic hydrogen wave. Since the wave height tends to a limiting value (0.3 μ amp.) at higher concentration (0.05~0.6 mM) and its temperature coefficient is small (0.4% deg. $^{-1}$), this wave is assumed to be an adsorption wave.

When the pH value increases from 4 to 7, the wave heights of the 1st and the 2nd steps decrease and that of the 3rd step increases (Fig. 2). The apparent pK of I is assumed to be 5.6 from the pH in which the 1st and 2nd wave height is equal to the 3rd one.

$E_{1/2}$ -pH curves can be shown by the equation $E_{1/2} = E_0 + a$ pH, where E_0 are -0.08 , -0.26 and -0.30 V. for 1st, 2nd and 3rd waves, respectively and a is about -0.08 V. (Fig. 2).

The slope of the reduction wave of narcotine-*N*-oxide at $-0.2 \sim -0.9$ V. in 0.25 mM solution of pH 3.7, becomes steep and the foot of the wave shifts toward the negative side in the presence of 0.1~2 mM narcotine. The potential range of the anomalous wave observed at $-0.47 \sim -0.89$ V. in 0.5 mM at pH 4.1 becomes more negative and less wide ($-0.63 \sim -0.92$ V.) in the presence of 2 mM narcotine. In both cases the total wave height is also proportional to the concentration of the *N*-oxide and is not altered with narcotine. Similar phenomena are observed in the coexistence of 0.01~0.02% gelatin and 0.2~1 mM the *N*-oxide.

Change of Polarograms of Narcotine-*N*-oxide by Alkali and Acid.—The reduction wave of narcotine-*N*-oxide hydrochloride (I) disappears gradually in alkali. From the current-time curve at a fixed potential, the reaction is estimated to be of the 1st order. Its velocity constant (k_1) is proportional to $[\text{OH}^-]$ ion concentration. The reaction product II gives no wave above pH 9 but a reduction wave at more negative potential than that of I at pH

1~7 (Fig. 1d) and reverse to the original one I by acid. The velocity constant (k_2) of the reverse reaction is proportional to $[H^+]$ ion concentration (Fig. 3).

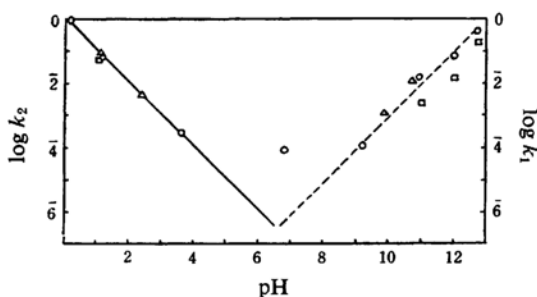


Fig. 3. Relationships between pH and reaction velocity constant of cleavage of the lactone (k_1) and of its formation (k_2) of narcotine (\square) or its *N*-oxide (\circ , \triangle) observed by reduction wave height (\triangle) or $\epsilon_{313m\mu}$ (\circ , \square) at 25°C.

Change of Ultraviolet Absorption Spectra of Narcotine and its *N*-oxide by Alkali and Acid.—Narcotine hydrochloride gives a peak with λ_{max} 313 $m\mu$ (ϵ 4000) and a shoulder at 290 $m\mu$ in 20% ethanol solution (pH 5). The spectra change gradually to λ_{max} 282 $m\mu$ (ϵ 3200) in alkali.

Narcotine-*N*-oxide gives λ_{max} 313 $m\mu$ (ϵ 4000) and shoulders at 290 and 240 $m\mu$ in acid, and λ_{max} 278 $m\mu$ (ϵ 2800) in alkali. These changes occur reversibly and in measurable speed. The velocity constant of the 1st order reaction observed by absorbance at 313 $m\mu$ is proportional to $[OH^-]$ in alkali and $[H^+]$ in acid, and is in good agreement with that obtained by polarography (Fig. 3).

A. C. Polarography.—0.2 mm Narcotine-*N*-oxide in a buffer solution of pH 4 gives a large A. C. Polarogram (i_p 710 $\mu\bar{v}$) at the potential E_p -1.67 V. corresponding to the catalytic hydrogen wave but no wave at the potential of reduction of *N*-oxide. At higher concentration (1 mm) in the same buffer, the A. C. polarogram is suppressed in the potential range -0.5~-1 V. as D. C. polarogram.

Electrocapillary Curve.—The relationships between the applied potential and drop time of the mercury electrode in an acetate buffer of pH 4 is shown in the electrocapillary curve with a maximum at -0.6 V. (Fig. 4 —). In the presence of 0.2 mm narcotine-*N*-oxide, the surface tension of the electrode is suppressed at -0.2~-1.4 V. and the curve extends to negative at -1.5~-1.7 V. because of the ohmic drop with the large catalytic current (Fig. 4-----).

Potentiometric Titration.—Titration of 1 cc. of 10 mm narcotine-*N*-oxide·HCl with 0.1 cc. of

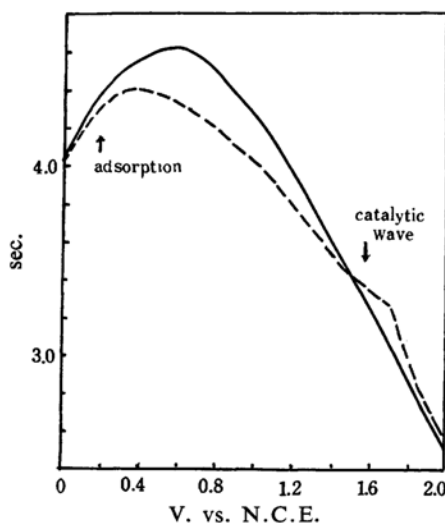


Fig. 4. Electrocapillary curves of acetate buffer (pH 4, —) and with 0.2 mm narcotine-*N*-oxide (-----).

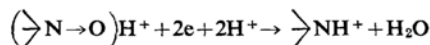
0.1 N sodium hydroxide at 25°C reveals it to be an acid of pK 3.75. After an alkaline- (5 mm the *N*-oxide, 0.02 N sodium hydroxide, 10 cc.) is heated at 100°C for 5 min., two acid groups of pK 6.0 and below 3 are observed by titration with 0.1 N hydrochloric acid or sodium hydroxide in nitrogen atmosphere and the product shows an absorption maximum at 280 $m\mu$. When the solution of pH 2.7 is heated at 100°C for 30 min., an acid group of pK 3.7 and an absorption maximum at 313 $m\mu$ is obtained again. This alternation is ascribed to the reversible cleavage of the lactone. A similar phenomenon is also observed in narcotine, where the lactone type gives pK 6.1 and the hydroxycarboxyl type gives pK 9.4 and below 3.5 in 50% ethanol solution.

Discussion

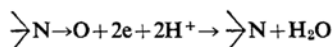
The maximum wave of narcotine-*N*-oxide at about -1.4~-2 V. is considered to be a catalytic hydrogen wave as narcotine and other alkaloids. This is useful for a microdetermination of pure alkaloid because of its high sensitivity, but can not be used for the specific determination of each alkaloid.

The reduction wave of narcotine-*N*-oxide must be due to the *N*-oxide group as this wave is not observed in narcotine. The total wave height of the *N*-oxide in acetate buffer solution of pH about 4 can be utilized for its determination in the mixture of alkaloids. Applying Ilkovič's equation, $k_D = 607 n D^{1/2} = i_a \cdot c^{-1} m^{-2/3} t^{-1/6}$, with $k_D = 2.3$ at pH 1~9 and $D = 4.1 \cdot 10^{-6}$ ($cm^2 \text{ sec}^{-1}$) which is estimated from the molecular weight, $n = 1.88 \approx 2$ is obtained.

As the wave is very flat and no A. C. polarogram is obtained at the corresponding potential, the electrode process may be irreversible. In strong acid solutions, narcotine (pK 6.1) and its *N*-oxide (pK 3.75) exist as proton adducts with lactone moiety I. Then the 1st and 2nd steps at pH 1~5 are assumed to be due to the following reaction.



The fact that the apparent pK 5.6 obtained from the wave height-pH curves is greater than pK 3.75, is explained by the kinetic current due to the protonation of the base on the electrode. The 3rd wave at pH 5~10 may be ascribed to the reduction of the *N*-oxide base to narcotine.



It is clear from the hysteresis of potentiometric titration curve and ultraviolet absorption spectra in acid and alkali that narcotine and its *N*-oxide change reversibly from lactone types I in acid to hydroxy-carboxylate types II in alkali. Because of this reaction, the wave height of the 3rd step decreases at pH above 10. The *N*-oxide with a hydroxy-carboxylate group II give more negative $E_{1/2}$ than that of lactone type I, perhaps because the *N*-oxide is stabilized by the formation of hydrogen bond ($\text{>N}\rightarrow\text{O}\cdots\text{HO}-$).

The 1st step of the *N*-oxide with lactone ring has the character of an adsorption wave and disappears in the presence of narcotine or gelatin. Moreover the fact that the surface tension of the mercury electrode is suppressed in more negative potential than the 1st wave, confirms the adsorption of the reduction product, narcotine, on the electrode surface. Applying Brdicka's equation⁵⁾, $z = i_a t / nF \Delta q$, with $i_a = 0.3 \mu\text{amp}$. (pH 9.2), $t = 3.94 \text{ sec}$. and

$\Delta q = 0.85 (mt)^{2/3} = 0.0185 \text{ cm}^2$, the maximum number of adsorbed molecule (z) is estimated to be $3.3 \times 10^{-10} \text{ mol. cm}^{-2}$ and the cross-sectional area of a narcotine molecule on the electrode surface (A) is calculated as 50 \AA^2 . From $i_a = 1.25 \mu\text{amp}$. in 0.1 N hydrochloric acid, A is estimated to be 12 \AA^2 . These values are comparable to the minimum cross-sectional area of a molecule packing closely on the mercury surface.

The curious polarograms in higher concentration at pH about 4 are ascribed to the inactivation of the mercury electrode by the adsorption layer.

Summary

Narcotine-*N*-oxide shows a catalytic hydrogen wave as narcotine and other alkaloids. The reduction waves of narcotine-*N*-oxide are ascribed to the 2-electron reduction of the *N*-oxide to narcotine. Their 1st step is an adsorption wave of the reductant. The adsorbed film disturbs the further electrode reaction, an anomalous minimum wave is observed in higher concentration. The 2nd step in pH 1~5 corresponds to the proton adduct of the *N*-oxide with lactone and the 3rd step in pH 5~10 to the base of the *N*-oxide.

Narcotine and its *N*-oxide change reversibly to hydroxycarboxylate type in alkali, which gives more negative reduction potential of the *N*-oxide and an absorption maximum in shorter wavelength. The velocity constants of cleavage of the lactone and of the reverse reaction are proportional to $[\text{OH}^-]$ and $[\text{H}^+]$, respectively.

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