Communications to the Editor

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Codeine-7,8-oxide (4,5α-Epoxy-7β,8β-epoxy-3-methoxy-17-methylmorphinan-6α-ol): Identification as a Metabolite of Codeine[1,2]

The metabolism of codeine (**1**) by rat liver microsomal suspension was investigated. Codeine-7,8-oxide (**2**) was identified as a new metabolite of **1** by gas chromatography-mass fragmentography and high-pressure liquid chromatography.

Keywords——codeine; codeine-7,8-oxide; codeine[6β-^{3}H]; oxidative metabolism; epoxydation; hepatic microsomes; gas chromatography-mass fragmentography; high-pressure liquid chromatography

Epoxides are known as the electrophilically active intermediates formed during the metabolism of aromatic and olefinic compounds in the presence of microsomal mono-oxygenase. However, the instability of these epoxides has made their identification in biological fluids difficult.

As to the metabolism of morphine alkaloids, several oxidative reactions have been studied, such as N-oxidation, N- and O-dealkylation, *etc.*,[3] but the epoxydation of 7,8-double bond has not been reported yet.

To elucidate this metabolism, we first attempted the chemical synthesis of 7,8-epoxides, and the synthesis of codeine-7,8-oxide (**2**) was described previously.[1] In this paper, we report the formation of **2** as a new metabolite by the incubation of codeine (**1**) with microsomal suspension.

Livers were obtained from Wistar strain rats which had been treated with phenobarbital for 3 days and subsequently fasted for 1 day. Preparation of microsomes was carried out according to the method of Watabe *et al.*[4] Microsomal suspension (4.5 mg protein/ml)[5] in 0.2 M phosphate buffer (pH 7.5) was used for the experiments.

Codeine (1 mM) was incubated with microsomal suspension in the presence of NADPH-generating system and epoxide hydratase inhibitor (phenanthrene-9,10-oxide, 10 mM). After 1 hr incubation, the incubation mixture (3 ml) was adjusted to pH 10 with ammonium buffer, saturated with NaCl, and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, evaporated to dryness, and the residue was dissolved in methanol, which was subjected to gas chromatography-mass fragmentography (GC-MF).

Fig. 1 shows the gas chromatography-mass spectrum of **2**. A molecular ion peak at *m/e* 315 and fragment peaks at *m/e* 286 and 230 were detected as characteristic peaks. The ion peaks at *m/e* 286 and 230 were used for GC-MF.[6] The mass fragmentogram (Fig. 2) showed a peak with a retention time of 7.2 min corresponding to that of **2**. When **1** was incubated

1) This forms part II of a series entitled "Chemical Studies on Drug Metabolism." Part I: K. Uba, N. Miyata, K. Watanabe, and M. Hirobe, *Chem. Pharm. Bull.*, **27**, 2257 (1979).
2) A part of this work was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1979.
3) J.D. Phillipson, W. El-Dabbas, and J.W. Gorrod, "Biological Oxidation of Nitrogen," ed. by J.W. Gorrod, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, p. 125; A. Klutch, *Drug Metab. Dispos.*, **2**, 23 (1974); S.Y. Yeh, R.L. McQuinn, and C.W. Gorrodetzky, *ibid.*, **5**, 335 (1977); A.L. Misra, N.L. Vadlamani, R.B. Pontani, and S.J. Mulé, *Biochem. Pharmacol.*, **22**, 2129 (1973).
4) T. Watabe, M. Isobe, K. Yoshikawa, and E. Takabatake, *J. Pharm. Dyn.*, **1**, 98 (1978).
5) Protein content of the suspension was determined by the method of Lowry *et al.*, O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
6) The ion peak at *m/e* 315 could not be used for the large background peak derived from microsomes.

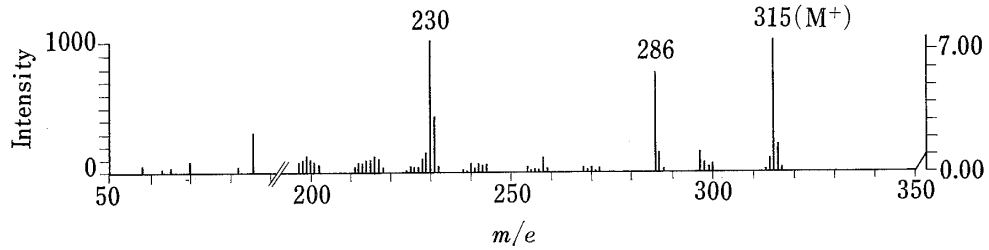


Fig. 1. Mass Spectrum of Authentic Codeine-7,8-oxide (2)

A gas chromatography-mass spectrometer (JMS-D300 with JMA-2000) was used under following conditions: Column, 2% OV-1 (3 m); column temperature, 250°; energy of ionization beam, 70 eV.

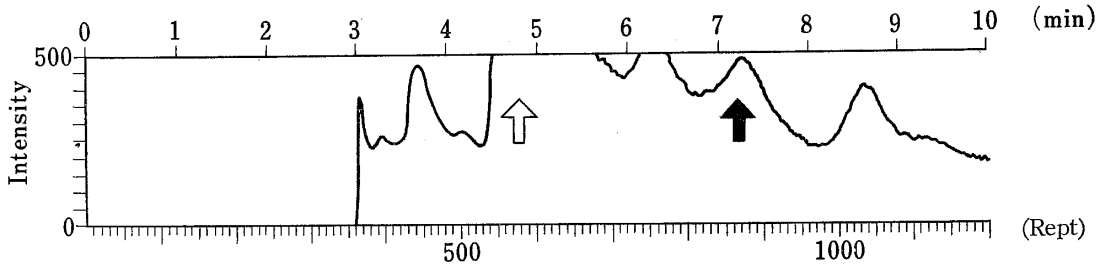


Fig. 2. Mass Fragmentogram, Used *m/e* 230, of Codeine Incubation Mixture

The retention time of **1** and **2** was 4.7 and 7.2 min, respectively. The peak corresponding to **2** was also detected with *m/e* 286.
⇧, codeine (**1**); ⬆, codeine-7,8-oxide (**2**).

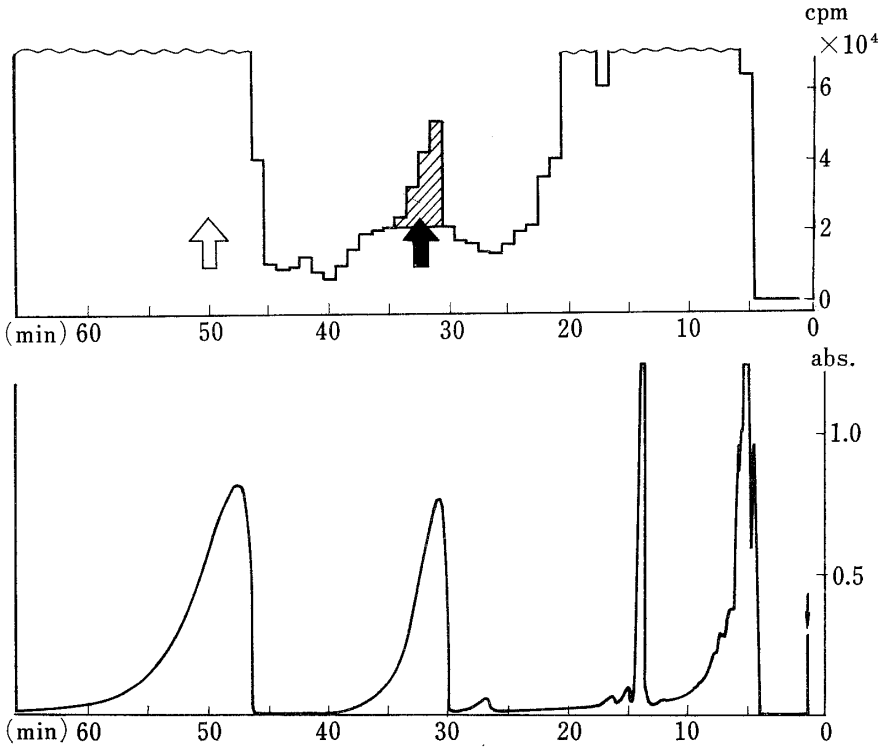


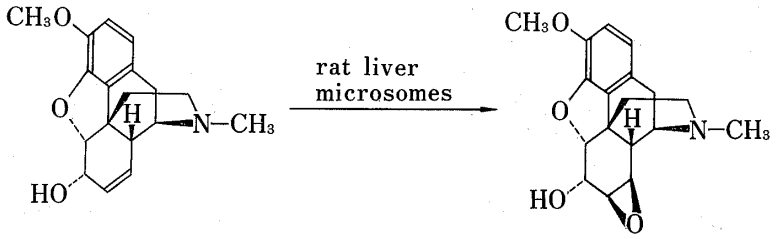
Fig. 3. High-pressure Liquid Chromatogram of the Incubation Mixture

Instruments: Waters 6000 A pump, Waters U6K injector, Waters M-440 detector (254 nm).
Conditions: Column (8×300 mm), LiChrosorb SI-60 (5 μm); mobile phase, methylene chloride–acetonitrile–methanol–ammonium hydroxide (35: 60: 5: 0.5); flow rate, 3.0 ml/min.
The curve in the lower figure shows the optical density and the histogram in the upper one ³H distribution.
⇧, codeine (**1**); ⬆, codeine-7,8-oxide (**2**).

without microsomes, we could not observe the formation of **2**. These results indicate that the epoxide formation is enzymatic.

The rate of biotransformation was examined with labeled compound. Codeine [6β-^{3}H][7] (102 mCi/mmol) was incubated and post-treated as before. The residue was subjected to high-pressure liquid chromatography after the addition of non-labeled **1** and **2** as carriers.

As shown in Fig. 3, the elution patterns of **1** and **2** were continuously detected with ultraviolet absorption detector, and the eluant was collected in 1 min fractions. The fractions were evaporated and their radioactivities were counted with a liquid scintillation spectrometer. The biologically formed codeine-7,8-oxide[6β-^{3}H] was clearly detected at the retention time corresponding to the non-labeled **2**. It was confirmed that **1** was metabolized to **2** with microsomes at the conversion rate of 0.13%.



1 **2**

Chart 1

Though the conversion rate is not so good, these results suggest that the epoxydation of 7,8-double bond may be one of the common oxidative metabolism of morphine alkaloids.[8] The metabolic fates and the biological activities of 7,8-oxides[9] will be reported in the subsequent papers.

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7) Codeine[6β-^{3}H] was synthesized from codeinone with NaB^3H_4 modifying the method reported by M. Gates, *J. Am. Chem. Soc.*, **75**, 4340 (1953).
8) Recently, Yeh *et al.* suggested that the formation of morphine-7,8-oxide was one of the possible pathways to β- or γ-isomorphine. S.Y. Yeh, H.A. Krebs, and C.W. Gorrodetzky, *J. Pharm. Sci.*, **68**, 133 (1979).
9) Preliminary rat tail stimulus assay showed that **2** had analgesic activity three times as potent as **1**.