[Chem. Pharm. Bull. 32(12)5055—5058(1984)]

Aporphine Alkaloids from *Parabenzoin praecox* (SIEB. *et* ZUCC.) NAKAI[1)]

MUTSUO KOZUKA,* AKIRA INADA,[2)] TAKAO KONOSHIMA, and TOKUNOSUKE SAWADA

Kyoto Pharmaceutical University, Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan

(Received April 11, 1984)

A new aporphine alkaloid, praecoxine (**1**), was isolated from the root of *Parabenzoin praecox* (SIEB. *et* ZUCC.) NAKAI. The alkaloid was shown to be identical with *N*-methylhernagine, (*S*)-(+)-1,2,11-trimethoxy-10-hydroxyaporphine (**1**), on the basis of chemical transformations and spectral evidence. The occurrence of nandigerine (**10**) in the root and the stem wood was also proved.

Keywords——*Parabenzoin praecox*; Lauraceae; aporphine alkaloid; praecoxine; nandigerine; ^{1}H-NMR; chemical transformation; (*S*)-(+)-1,2,11-trimethoxy-10-hydroxyaporphine

Parabenzoin praecox (SIEB. *et* ZUCC.) NAKAI (Lauraceae) is a tree occurring in Kyushu, Shikoku, and Honshu. The insect antifeedant substances,[3)] and the essential oil, especially mono- and sesquiterpenoids,[4)] from this plant have been investigated. However, no study on alkaloidal constituents of the plant seems to have been published. As a part of our continuing work on alkaloids of Lauraceous plants,[5)] we report here the isolation and characterization of a new aporphine alkaloid, that has been named praecoxine[1)] (**1**), from the root of *P. praecox*.

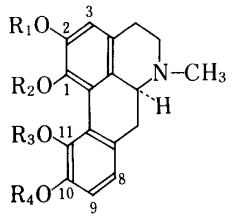
Praecoxine (**1**) was isolated as a colorless crystalline compound, which decomposes at 230 °C, $[\alpha]_D$ +270° (methanol), infrared (IR) spectrum ($CHCl_3$) cm^{-1}: 3500 (OH). The molecular formula $C_{20}H_{23}NO_4$ was derived by high resolution mass spectroscopy. The ultraviolet (UV) spectrum of the base was clearly of a 1,2,10,11-tetrasubstituted aporphine[6,7)] and exhibited a bathochromic shift in alkaline ethanol solution. Its ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum showed an *N*-methyl group, three methoxyl groups, a hydroxyl group, and three aromatic protons. Two of the three aromatic protons appeared as overlapped singlets at δ 6.87 [C(8)– and C(9)–H].[7)]

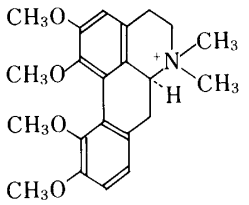
Methylation of praecoxine (**1**) with diazomethane afforded amorphous *O*-methylpraecoxine (**7**). Its ^{1}H-NMR spectrum showed an *N*-methyl group, four methoxyl groups, and three aromatic protons.

Praecoxine (**1**) reacts with methyl iodide and KOH in methanol to give *O*-methylpraecoxine methiodide (**8**), mp 249 °C (dec.), $[\alpha]_D$ +147° (ethanol), which was identical with authentic *O*,*O*-dimethylcorytuberine methiodide[8)] (**8**) on the basis of mixed melting point determination and IR spectral comparison.

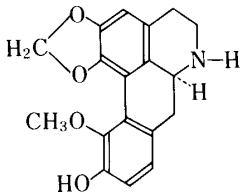
The position of the phenolic hydroxyl group in praecoxine (**1**) was proven in the following manner. Praecoxine (**1**) was heated in deuterium oxide containing sodium hydroxide to give a monodeuterio derivative (M^+, *m/z* 342). The ^{1}H-NMR spectrum of the monodeuterated base (**3**) differed from that of the non-deuterated compound (**1**) only in the intensity of the δ 6.87 aromatic proton signal, which was found to be decreased to one-half by the deuteration, while the δ 6.68 signal due to 1H at C-3 was unchanged. Since the formula **4** was excluded,[9)] the site of deuterium exchange must be C-9, and the position of the phenolic hydroxyl in praecoxine (**1**) at C-10 was established.

On the basis of the foregoing and other results, it was concluded that praecoxine is (*S*)-(+)-1,2,11-trimethoxy-10-hydroxyaporphine (**1**).



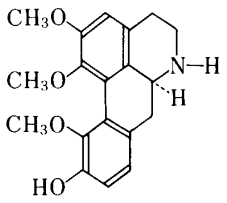


8



10

1 : $R_1=R_2=R_3=CH_3$, $R_4=H$
2 : racemic form of **1**
3 : **1** [C(9)-D instead of C(9)-H]
4 : $R_1=H$, $R_2=R_3=R_4=CH_3$
5 : $R_1=R_3=R_4=CH_3$, $R_2=H$
6 : $R_1=R_2=R_4=CH_3$, $R_3=H$
7 : $R_1=R_2=R_3=R_4=CH_3$



9

Chart 1

Syntheses of the racemic form (**2**) have already been reported.[10,11b] Further, a new aporphine alkaloid which has the *N*-norpraecoxine structure, named hernagine (**9**), was isolated from *Hernandia nymphaefolia*[11a] and *H. cordigera*.[11b] *N*-Methylhernagine was obtained from hernagine (**9**), and the structure **1** was confirmed by the spectral data.[11a] The IR and ^{1}H-NMR spectra of the racemic base[10b] (**2**), and *N*-methylhernagine[11a] (**1**) were compared with those of praecoxine (**1**), and they were found to be identical.

The isolation and characterization of praecoxine (**1**) thus provide further support for the structures of hernagine (**9**) and its *N*-methyl derivative (**1**). This paper is the first report of isolation of *N*-methylhernagine (=praecoxine) from a natural source.

Further, a major alkaloidal constituent of the root and the stem wood of *P. praecox* was found to be nandigerine (**10**), which was identified by direct comparison with an authentic specimen.[12b]

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were obtained on a Hitachi EPS-2 spectrophotometer and IR spectra were recorded on a Shimadzu IR-27C spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian A-60A spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were taken with Hitachi RMU-7 and RMU-6E machines. Optical rotations were measured on a Rex photoelectric polarimeter. Column chromatography was carried out with silica gel (Wakogel C-200, 100—200 mesh) or with alumina (Aluminiumoxid nach Brockmann, Merck). Thin-layer chromatography (TLC) was performed on Aluminiumoxid G (Merck) in $CHCl_3$–acetone (1 : 1, v/v) unless otherwise stated, and the developed spots were detected with iodine vapor and Dragendorff's reagent.

Material and Extraction——Root and stem wood of *Parabenzoin praecox* (SIEB. *et* ZUCC.) NAKAI were collected in Sakyo-ku, Kyoto, and in Hyogo Prefecture in November 1968. Air-dried and cut materials (root: 28.3 kg; stem wood: 21.0 kg) were extracted with hot MeOH and the MeOH was evaporated off under reduced pressure to leave a residue (root, *ca.* 600 g; stem wood, *ca.* 530 g). The residue was dissolved in 3% aqueous citric acid, and the solution was filtered, and washed with Et_2O to remove the non-basic substances. The acidic solution was made alkaline with conc. NH_4OH and extracted with Et_2O. The Et_2O solution was shaken with 4% NaOH solution, and the NaOH solution was made ammoniacal with NH_4Cl. The NH_4OH-alkaline solution was further extracted with Et_2O, and the Et_2O solution was washed with H_2O, then dried over anhyd. $MgSO_4$. Evaporation of the Et_2O gave the crude phenolic alkaloid mixture (root, 6.5 g; stem wood, 6.1 g). The Et_2O solution freed of phenolic alkaloids was washed with H_2O, dried over anhyd. K_2CO_3, and evaporated to leave a small amount of crude non-phenolic

bases (root, 0.562 g; stem wood, 0.310 g).

Isolation of Praecoxine (1) and Nandigerine (10) from the Root——The crude phenolic alkaloid mixture from the root (6.5 g) was dissolved in a small amount of $CHCl_3$ and a saturated $CHCl_3$ solution of picrolonic acid was added to yield crystalline nandigerine (**10**) picrolonate (0.613 g). The $CHCl_3$ was removed from the mother liquor of this picrolonate. The residue was dissolved in acetone, and poured into 3% aqueous HCl solution. The resulting suspension was washed with Et_2O, then made alkaline with conc. NH_4OH and extracted with Et_2O. The Et_2O was evaporated off and the residue was chromatographed on silica gel. The fraction eluted with acetone–$CHCl_3$ (5 : 95, v/v) gave crude praecoxine (**1**) (110.8 mg), while the fraction eluted with acetone–$CHCl_3$ (1 : 9) contained crude nandigerine (**10**) (1.4 g).

Praecoxine (1)——Colorless crystals (from acetone), decomposes at 230 °C, $[\alpha]_D^{27}$ +270° (c=0.1, MeOH). IR ($CHCl_3$) cm^{-1}: 3500 (OH). UV $\lambda_{max}^{95\%EtOH}$ nm (ε): 271 (12380), 302 (5000). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.55 (3H, s, NCH_3), 3.50 (3H, s, OCH_3 at C-1), 3.54 (3H, s, OCH_3 at C-11), 3.87 (3H, s, OCH_3 at C-2), 4.87—5.60 (1H, br, exchanged by D_2O, OH), 6.68 (1H, s, C-3 aromatic H), 6.87 (2H, overlapped s, C-8 and C-9 aromatic H).[7] MS m/z: 341 (M^+), 310 (base peak). $C_{20}H_{23}NO_4$ (M^+, Calcd m/z 341.1627; Found m/z 341.1628).

***O*-Methylpraecoxine (7)**——An excess of Et_2O solution of diazomethane was added to a solution of praecoxine (**1**) (20 mg) in MeOH, and the mixture was allowed to stand for 2 d at room temperature. The solvent was evaporated off and the residue was dissolved in 3% aqueous citric acid. The acidic solution was washed with Et_2O, made alkaline with NH_4OH and extracted with Et_2O. The combined Et_2O extract was washed successively with 5% NaOH solution and with H_2O, then dried over anhyd. K_2CO_3 and the solvent was evaporated off. The oily product (21 mg) was chromatographed on alumina with benzene to give **7**. TLC: 1 spot. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.53 (3H, s, NCH_3), 3.64, 3.72, 3.87, and 3.88 (3H × 4, each s, four OCH_3), 6.68 (1H, s, aromatic H), 6.81 and 6.96 (each 1H, a pair of d, J=9 Hz, aromatic H).

***O*-Methylpraecoxine Methiodide (*O,O*-Dimethylcorytuberine Methiodide) (8)**——A methanolic solution of KOH (0.04 g) and an excess (0.5 ml) of MeI were added to a solution of praecoxine (**1**) (38.7 mg) in MeOH. After this mixture had been heated under reflux for 2 h, other portions of the methanolic KOH (0.04 g) and MeI (0.5 ml) were added, and the heating was continued for 2.5 h. This process was repeated again and the mixture was further refluxed for 4 h. The solution was evaporated *in vacuo*, and the residue was extracted with a large amount of $CHCl_3$. The $CHCl_3$ solution was dried over anhyd. $MgSO_4$ and the solvent was evaporated off. Recrystallization of the residue from MeOH–acetone furnished 19.2 mg of colorless needles, mp 249 °C (dec.), $[\alpha]_D^{29}$ +147° (c=0.16, EtOH). This compound was identified as *O,O*-dimethylcorytuberine methiodide[8] (**8**) by mixed melting point determination and by comparison of the IR spectra and behavior on TLC.[13]

9-Monodeuteriopraecoxine (3)——A solution of **1** (64.6 mg) and NaOH (25 mg) in D_2O (3.5 ml) was heated in a sealed tube at 100 °C for 50 h. The alkaline solution was acidified with 3% aqueous HCl, washed with Et_2O, made alkaline with NH_4OH, and extracted with Et_2O. The Et_2O extract was dried over anhyd. $MgSO_4$ and the solvent was evaporated off to give 51 mg of **3** as an oily mass. MS m/z: 342 (M^+), 311 (base peak). ^{1}H-NMR ($CDCl_3$) δ ppm: 2.53 (3H, s, NCH_3), 3.49, 3.53, 3.86 (3H × 3, each s, three OCH_3), 5.00—5.83 (1H, br, exchanged by D_2O, OH), 6.68 and 6.87 (1H × 2, each s, two aromatic H).

Nandigerine (10)——Light green crystals, mp 122—124 °C (from MeOH) [lit.,[12a] solvent-free base: mp 176—177 °C (from MeOH); MeOH solvate: mp 99—100 °C]. $[\alpha]_D^{27}$ +380° (c=0.25, EtOH). The UV, IR, and ^{1}H-NMR spectra were indistinguishable from those of an authentic sample of nandigerine[12b] (**10**). Picrolonate: decomposes at 218—219 °C (from MeOH). *Anal.* Calcd for $C_{18}H_{17}NO_4 \cdot C_{10}H_8N_4O_5$: C, 58.43; H, 4.38; N, 12.17. Found: C, 58.66; H, 4.46; N, 12.22.

Isolation of Nandigerine (10) from the Stem Wood——The crude phenolic bases (6.1 g) from the stem wood were treated with picrolonic acid in $CHCl_3$, but the picrolonate was not obtained in a crystalline form. The oily picrolonate and the mother liquor were combined and free bases were obtained by the procedure described above. The free bases were separated by differential pH extraction[14] (McIlvaine buffer solution, double strength, pH 5.8 and 4.8) and by the use of 0.2 M citric acid to yield 3.255, 0.437 and 0.113 g, respectively, of crude bases. The crude alkaloids (2.578 g) from the pH 5.8 extract were dissolved in a small amount of acetone and an excess of saturated solution of *p*-nitrobenzoic acid in acetone ws added to yield crystalline nandigerine (**10**) *p*-nitrobenzoate (2.213 g). The salt was recrystallized from MeOH to give pale yellow crystals, mp 193—194 °C. Nandigerine (**10**) (0.3606 g) was liberated from the salt. The UV, IR, and ^{1}H-NMR spectra were superimposable on those of an authentic sample of nandigerine[12b] (**10**).

Acknowledgement The authors are grateful to Dr. M. Tomita, Professor Emeritus, Kyoto University, for his interest and encouragement throughout this work. The authors wish to thank Professsor A. Tatematsu and Dr. H. Yoshizumi, Meijo University, for measuring high resolution mass spectra; Professor M. Shamma, The Pennsylvania State University, for providing the IR and ^{1}H-NMR spectra of (±)-1,2,11-trimethoxy-10-hydroxyaporphine; Professor J. Kunitomo, Mukogawa Women's University, for an authentic sample of *O,O*-dimethylcorytuberine methiodide; Professor K. Hozumi, Mr. Y. Fujiwara, and Miss M. Fujioka of this university, for elemental analyses, ^{1}H-NMR, and MS measurements, respectively. The authors are also particularly indebted to Professor H. Furukawa,

Meijo University, for providing samples and spectra of corydine, nandigerine, and *N*-methylhernagine.

References and Notes

1) A part of this work was presented at The 20th Annual Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Kobe, November 1970 (M. Kozuka, A. Inada, T. Konoshima, and T. Sawada, Abstracts of Papers, p. 27).
2) Present address: *Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagao Tohge-cho, Hirakata 573-01, Japan.*
3) K. Matsui, K. Wada, and K. Munakata, *Agric. Biol. Chem.*, **40**, 1045 (1976).
4) K. Ohara, Y. Ohta, and Y. Hirose, *Bull. Chem. Soc. Jpn.*, **46**, 641 (1973); and references cited therein.
5) M. Tomita and M. Kozuka, *Yakugaku Zasshi*, **84**, 362 (1964); M. Tomita, T. Sawada, M. Kozuka, D. Hamano, and K. Yoshimura, *ibid.*, **89**, 737 (1969); and references cited therein.
6) A. W. Sangster and K. L. Stuart, *Chem. Rev.*, **65**, 69 (1965).
7) M. Shamma, "The Isoquinoline Alkaloids: Chemistry and Pharmacology," Academic Press, Inc., New York and London, 1972, Chapter 10.
8) The sample was kindly donated by Professor J. Kunitomo.
9) Praecoxine was not identical with corydine (**5**) or isocorydine (**6**).
10) *a*) B. Frydman, R. Bendisch, J. Comin, and V. Deulofeu, *J. Org. Chem.*, **25**, 100 (1960); *b*) M. Shamma and A. Slusarchyk, *Chem. Commun.*, **1965**, 528.
11) *a*) K. Yakushijin, S. Sugiyama, Y. Mori, H. Murata, and H. Furukawa, *Phytochemistry*, **19**, 161 (1980); *b*) J. Bruneton, *C. R. Acad. Sci.*, Paris, Serie *C*, **291**, 187 (1980).
12) *a*) M. P. Cava, K. Bessho, B. Douglas, S. Markey, R. F. Raffauf, and J. A. Weisbach, *Tetrahedron Lett.*, **1966**, 1577; *b*) H. Furukawa and S.-T. Lu, *Yakugaku Zasshi*, **86**, 1143 (1966).
13) Kieselgel G (Merck) in acetone–MeOH (1 : 1, v/v).
14) Y. Watanabe, M. Uchiyama, and K. Yasuda, *Yakugaku Zasshi*, **77**, 807 (1957).