and recrystallization of the eluate from benzene gave IId (10 mg) as colorless needles. mp 171—173°. $[\alpha]_D^{19}$ +50° (c=0.11). *Anal.* Calcd. for $C_{19}H_{23}O_3Cl \cdot C_6H_6$: C, 72.71; H, 7.08. Found: C, 72.82; H, 6.93. IR ν_{max}^{KBr} cm^{-1}: 1758 (C=O). NMR (1.6% solution in $CDCl_3$) δ: 0.95 (3H, s, 18-CH_3), 3.82 (3H, s, 3-OCH_3), 4.38 (1H, t, J=3.9 Hz, 16β-H), 5.31 (1H, s, 3-OH), 6.49 (1H, s, 4-H), 6.78 (1H, s, 1-H).

2-Hydroxy-3-methoxy-16β-chloroestra-1,3,5(10)-trien-17-one (IIId)——IIc (50 mg) was dissolved in benzene, adsorbed on Al_2O_3 (100 mg), and allowed to stand overnight. Elution with benzene gave a mixture of two C-16 epimers as colorless oil. A solution of this crude product was shaken with 10% Pd/C (40 mg) under a stream of H_2 gas for 2 hr. After usual work-up the crude product obtained was submitted to preparative TLC by multiple runs using hexane–$CHCl_3$ (1:1) as developing solvent. Elution of the adsorbent corresponding to the spot with AcOEt and recrystallization of the eluate from MeOH gave IIId (20 mg) as colorless needles. mp 175—177°. $[\alpha]_D^{16}$ +120° (c=0.10). *Anal.* Calcd. for $C_{19}H_{23}O_3Cl \cdot 1/2H_2O$: C, 66.37; H, 7.04. Found: C, 66.21, 66.12; H, 6.86, 6.91. IR ν_{max}^{KBr} cm^{-1}: 1753 (C=O). NMR (5% solution in CCl_4) δ: 1.02 (3H, s, 18-CH_3), 3.80 (3H, s, 3-OCH_3), 5.17 (1H, s, 2-OH), 6.39 (1H, s, 4-H), 6.66 (1H, s, 1-H).

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Studies on Berberine Derivatives and Related Alkaloids. VII.[1] On the Biosynthesis of Protopine[2]

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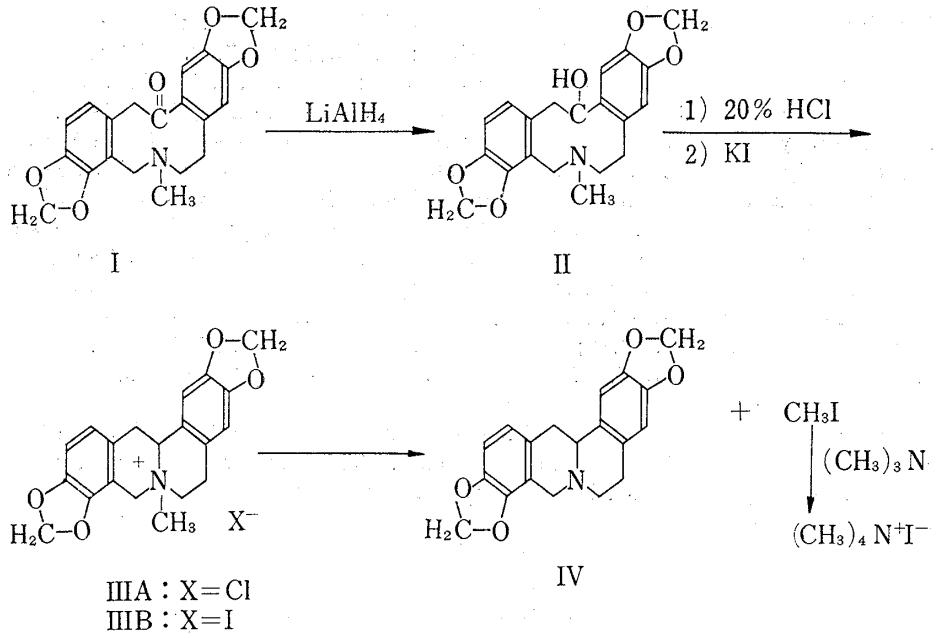
Contrary to our expectations, tetrahydrocoptisine methochloride (III A) was not incorporated into corynoline.[4] We further examined the fate of III A and found that this alkaloid was actually incorporated into protopine (I) on which we describe in this paper.

Incorporation of (+)-reticuline, (—)-scoulerine, stylopine and cheilanthifoline besides S-methyl group of L-methionine into protopine (I) have already been reported.[5] However, there was no evidence of the incorporation of tetrahydrocoptisine N-methyl derivative (III) into protopine (I).

(N-$^{14}CH_3$)-tetrahydrocoptisine methochloride (III A) was administered to *Corydalis incisa* in May by the cotton wick method and radioactive protopine (I) was obtained through the usual work up, 0.19% incorporation.

In order to prove the labelled position of protopine (I), the alkaloid was reduced with $LiAlH_4$ to give dihydroprotopine (II), which was heated with 20% HCl yielding tetrahydrocoptisine methochloride (III A).

1) Part VI: C. Tani, S. Takao and K. Tagahara, *Yakugaku Zasshi*, 93, 197 (1973).
2) This work was presented at the 21st Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, November, 1971, Abstracts of Papers, p. 25.
3) Location: *Motoyamakita-cho, Higashinada-ku, Kobe.*
4) This work was presented at the 91th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April, 1971, Abstracts of Papers, p. 727.
5) M. Sribney and S. Kirkwood, *Nature*, 71, 931 (1953); D.H.R. Barton, R.H. Hesse and G.W. Kirby, *J. Chem. Soc.*, 1965, 6379; A.R. Battersby, R.J. Francis, M. Hirst, R. Southgate and J. Staunton, *Chem. Commun.*, 1967(12), 602; N. Takao and K. Iwasa (This work was presented at the 91th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April, 1971, Abstracts of Papers, p. 727.



I II IIIA : X=Cl IIIB : X=I IV

Chart 1

The iodide (III B) derived from III A was decomposed by heating at 270° under nitrogen atmosphere and the resulting methyl iodide was converted into $(CH_3)_4NI$ which was found to be radioactive. Purification of the reaction mixture also gave non-radioactive tetrahydrocoptisine (IV).

Thus it was substantiated that tetrahydrocoptisine N-methyl derivative (III) served as a precursor of protopine (I) maintaining its N-methyl group at the original position.

Experimental[6]

Administration of (N-$^{14}CH_3$)-Tetrahydrocoptisine Methochloride (III A) to *Corydalis incisa* and Isolation of Protopine (I)——Tetrahydrocoptisine methochloride (III A) (18 mg) was dissolved in water (10 ml) and administered by the cotton wick method to ten *Corydalis incisa* plants at their flowering stage in May. Seven days after the beginning of the administration, the plants (dry weight 64 g) were cut into pieces and extracted with MeOH (500 ml). The solvent was removed *in vacuo* and the residue was extracted with 2% tartaric acid (2×100 ml). After washing with ether (100 ml), the extracts were made alkaline with aq. ammonia and extracted with $CHCl_3$ (3×80 ml). The $CHCl_3$ extracts were combined and dried over anhyd. Na_2SO_4. After removal of the solvent, the resulting crude tertiary bases were diluted with carrier protopine (8 mg) and chromatographed on a column of silica gel with benzene: ether (1 :1) and then with $CHCl_3$: MeOH (9: 1) as eluents.

The crystals obtained from $CHCl_3$: MeOH fractions were colored violet with conc. H_2SO_4 and were identified with an authentic sample of protopine (I) by TLC (silica gel, $CHCl_3$: MeOH, 9: 1). The radioactive protopine was diluted with carrier (67.6 mg) and repeatedly recrystallized from $CHCl_3$–MeOH to give colorless needles having a constant specific activity, 57 mg, mp 207°, spec. activity 2.35×10^6 dpm/mmole.

Degradation of Protopine (I), Formation of Dihydroprotopine (II)——To a solution of radioactive protopine (54.2 mg) in absolute benzene was added $LiAlH_4$ (134.9 mg) dissolved in absolute ether and the mixture was stirred under reflux for 3 hr. After addition of 20% H_2SO_4 to the reaction mixture cooled in an ice-bath, the acidic solution was made alkaline with 30% NaOH and extracted with benzene. After drying over K_2CO_3, the solvent was removed *in vacuo* to give a brown viscous residue (37.5 mg). Recrystallization of the residue from MeOH gave colorless needles, mp 152—153°, which were identified with an authentic

6) Unless otherwise noted, Silica gel G acc. to Stahl (E. Merck) was used for the thin-layer chromatography (TLC) and silica gel plates PF_{254} (E. Merck) were employed for the radio TLC. The spots were detected by exposing the plates to iodine vapour. Silica gel (Mallinckrodt) were used for column chromatography. The solvent ratio was expressed by volume. The radioactivity was measured by a Ten Liquid Scintillation Counter Model GSL-260 with samples dissolved in a scintillation mixture consisting of dioxane (10 ml), naphthalene (1 g), 2,5-diphenyloxazole (40 mg), 2,2′-*p*-phenylene-bis-(5-phenyl-oxazole) (0.6 mg).

sample of dihydroprotopine (II) by admixture and TLC (silica gel, $CHCl_3$: MeOH, 4: 1). spec. activity 2.48×10^6 dpm/mmole.

Tetrahydrocoptisine Methiodide (III B)——Dihydroprotopine (II) (37 mg) was dissolved in 20% HCl (5 ml) and refluxed over a free flame for 10 min. After addition of potassium iodide to the reaction mixture, the resulting precipitates were collected by centrifugation. Recrystallization of the precipitates (67 mg) from MeOH gave colorless needles, mp 260° (decomp.), spec. activity 2.37×10^6 dpm/mmole.

Pyrolysis of Tetrahydrocoptisine Methiodide (III B)——The iodide (III B) (60 mg) was heated on an oil bath at 270—280° in a stream of N_2 for 1 hr and the resulting methyl iodide was introduced into a solution of 30% trimethylamine. After removal of the solvent, the resulting white residue (12.5 mg) was recrystallized from MeOH, which was identified with an authentic sample of tetramethylammonium iodide by TLC (silica gel, $CHCl_3$: MeOH, 4: 1), spec. activity 2.44×10^6 dpm/mmole.

The residue in the reaction flask was chromatographed on silica gel with benzene: ether (1: 1) as eluent to give a non-radioactive light yellow residue (3.1 mg), which was identified with an authentic sample of tetrahydrocoptisine (IV) by TLC (silica gel, benzene: ether, 1: 1).

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Effect of Gastric Emptying on Absorption of Aminopyrine in Rat[1a,b]

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In the previous paper,[3] it was described that the initial plasma concentration of aminopyrine increased significantly by the simultaneous oral administration with barbital in rabbits as compared to the single administration. Furthermore, it was also reported[4] that the rate of gastric emptying of aminopyrine increased by barbital in spite of the inhibition of the gastric emptying by aminopyrine. As for gastrointestinal absorption of aminopyrine, it has been known that the delay of the time at which the peak level of plasma concentration of aminopyrine becomes the highest is proportional to the increasing dose.[5] It is not clear, however, why such a delay of it occurs. In consideration of the reason of the delay, the gastric emptying might be one of the important factors.

The present study was performed to clarify the relationship between the gastric emptying and the absorption rate of aminopyrine in rat.

Experimental

Procedure——Male Donryu rats weighing between 190—210 g were fasted for 14—16 hr. However, drinking water was permitted *ad libitum* until 2 hr before the experiment. One ml of 0.07% phenol red solution containing aminopyrine (20 mg/kg or 50 mg/kg) was introduced into the stomach by intubation.

1) *a*) This paper forms Part VI of a series entitled "Effect of Combination of Pharmaceuticals on Gastrointestinal Absorption"; *b*) Part V: S. Goto, T. Yamagata, O. Tsuzuki, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **21**, 2495 (1973).
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