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BIOSYNTHETIC RELATIONS BETWEEN PROTOBERBERINE-, BENZO(C)PHENANTHRIDINE- AND B-SECOPROTOBERBERINE TYPE ALKALOIDS IN *CORYDALIS INCISA*

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Key Word Index—*Corydalis incisa*; Papaveraceae; (±)-tetrahydrocoptisine; (±)-tetrahydrocorysamine; corynoline; B-secoprotoberberine; biosynthesis.

Abstract—The biosynthetic relations between protoberberine-, benzo[C]phenanthridine- and B-secoprotoberberine type alkaloids were demonstrated by use of (±)-tetrahydrocoptisine-[8,14-^{3}H HCl, (±)-tetrahydrocorysamine-[8,14-^{3}H]HCl and corynoline-[6-^{3}H]HCl in *Corydalis incisa*, and the following results were presented. (±)-Tetrahydrocoptisine was converted to corynoline, corydalic acid methyl ester and corydamine hydrochloride. (±)-Tetrahydrocorysamine was converted to corynoline and corydalic acid methyl ester. Evidence that *N*-methyl-3-[6′-(3′,4′-methylenedioxyphenethylalcohol)]-4-methyl-7,8-methylenedioxy-1,2,3,4-tetrahydroisoquinoline-[α-^{3}H] HCl was incorporated into corynoline-[11-^{3}H] indicates the occurrence of the ring fission at C_6-N followed by linking of the C_6 and C_{13} positions in (±)-tetrahydrocoptisine and (±)-tetrahydrocorysamine, and suggests the participation of one of two possible intermediates in the biosynthesis of these alkaloids.

INTRODUCTION

Recently, Leete [1], Battersby [2], Takao [3] and Nonaka [4] reported that an iminoaldehyde, such as **5** or **8** might be a possible biosynthetic intermediate between protoberberine type and benzo[C]phenanthridine type alkaloids. The occurrence of B-secoprotoberberine alkaloids, corydalic acid methyl ester [5], corydamine hydrochloride and *N*-formylcorydamine [6] in *Corydalis incisa* is important for biosynthetic studies of protoberberine- and benzo[C]phenanthridine type alkaloids. This paper deals with the biosynthetic relations between (±)-tetrahydrocoptisine, (±)-tetrahydrocorysamine, corynoline and B-secoprotoberberine alkaloids in this plant.

RESULTS AND DISCUSSION

Conversion of (±)-*tetrahydrocorysamine-[8,14-^{3}H]* (**1**) *into corynoline-[6-^{3}H]* (**2**) *and corydalic acid methyl ester-[1-^{3}H]* (**4**).

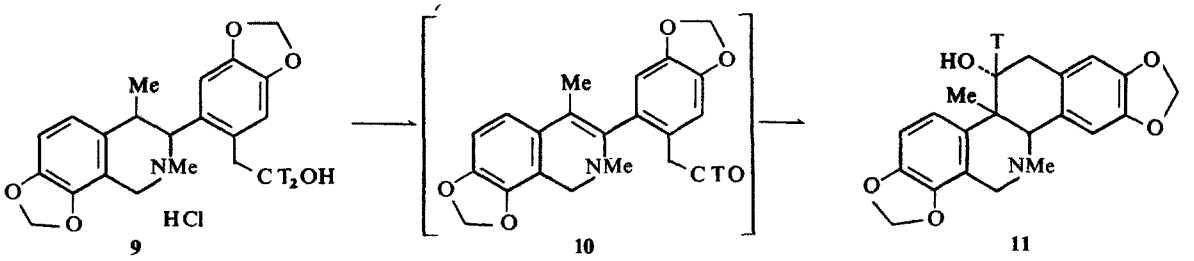
(±)-Tetrahydrocorysamine-[8,14-^{3}H]HCl(3.05×10^7 dpm, 1.34×10^{10} dpm/mM) (**1**) was administered to the cuttings for 7 days. The alkaloid fraction was separated by preparative TLC to give coynoline (2.68×10^5 dpm, 6.27×10^7 dpm/mM) (**2**), acetylcorynoline (3.84×10^4 dpm, 1.57×10^7 dpm/mM) (**3**) and corydalic acid methyl ester (5.23×10^3 dpm, 3.56×10^6 dpm/mM) (**4**). The location of tritium in radioactive corynoline (**2**) was verified to be at C_6 position as follows. Radioactive acetylcorynoline (1.10×10^4 dpm) (**3**) dissolved in pyridine was oxidized with 0.5% $KMnO_4$ to give 6-oxocorynoline which showed no radioactivity. The findings that (±)-tetrahydrocorysamine is incorporated into corydalic acid methyl ester and corynoline, and tritium in corynoline obtained is located at the C-6 position led to the conclusion that (±)-tetrahydrocorysamine is transformed to corynoline by oxidative bond fission at C_6—N followed by linking of the C_6 to C_{13} positions and a presence of a possible intermediate **5** is suggested; and that (±)-tetrahydrocorysamine is converted to corydalic acid methyl ester via **5**.

Conversion of (±)-*tetrahydrocoptisine-[8,14-^{3}H]HCl* (**6**) *into corynoline-[6-^{3}H]*(**2**), *corydamine hydrochloride-[1-^{3}H]*(**7**) *and corydalic acid methyl ester-[1-^{3}H]* (**4**).

(±)-Tetrahydrocoptisine-[8,14-^{3}H]HCl (1.88×10^7 dpm, 7.10×10^9 dpm/mM) (**6**) was administered to cuttings for 7 days. The alkaloid fraction was separated by preparative TLC to give corynoline (2.54×10^4 dpm, 5.32×10^6 dpm/mM) (**2**), acetylcorynoline (1.08×10^4 dpm, 4.31×10^6 dpm/mM) (**3**), corydamine HCl (6.31×10^4 dpm, 7.10×10^7 dpm/mM) (**7**) and corydalic acid methyl ester (3.24×10^3 dpm, 1.81×10^6 dpm/mM) (**4**). The location of tritium in radioactive corynoline (**2**) was verified to be at the C_6 position as follows. Radioactive acetylcorynoline (5.31×10^3 dpm) (**3**) was oxidized with 0.5% $KMnO_4$ to give 6-oxocorynoline which showed no radioactivity. The findings that (±)-tetrahydrocoptisine is incorporated into corydalic acid methyl ester, corydamine HCl and corynoline, and tritium in corynoline obtained is located at the C_6 position proved that (±)-tetrahydrocoptisine is transformed to corynoline via **1** by oxidative bond fission at C_6-N followed by the linking of the C_6 to C_{13} positions (±)-tetrahydrocoptisine is converted into corydamine HCl and corydalic acid methyl ester via **8** and **5**, respectively.

Conversion of N-*methyl-3-[6′-(3′,4′-methylenedioxyphenethylalcohol)]-4-methyl-7,8-methylenedioxy-1,2,3,4-tetrahydroisoquinoline-[α-^{3}H]* (**9**) *into corynoline-[11-^{3}H]* (**11**)

N-Methyl-3-[6′-(3′,4′-methylenedioxyphenethyl-



Scheme 1. Biosynthetic conversion of *N*-methyl-3-[6'-(3',4'-methylenedioxyphenethylalcohol)]-4-methyl-7,8-methylenedioxy-1,2,3,4-tetrahydroisoquinoline-[α-^{3}H] HCl (**9**).

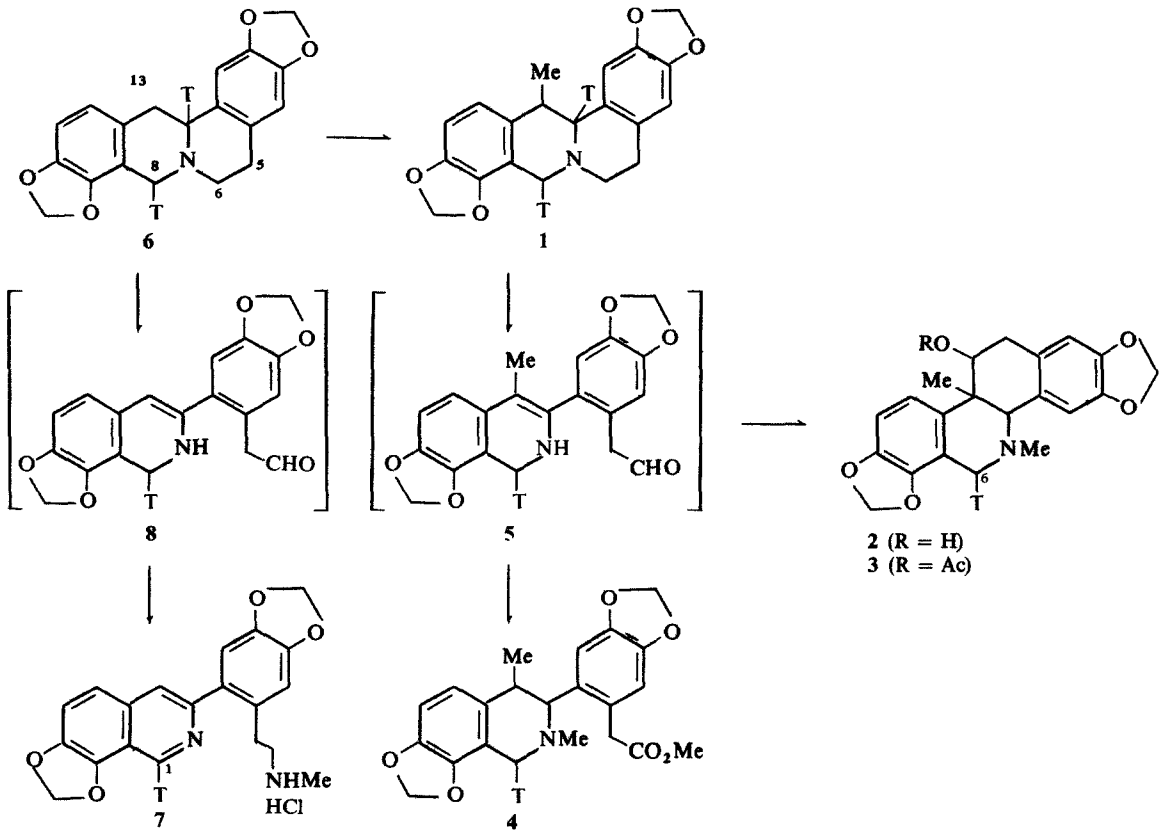
alcohol)] - 4 - methyl - 7,8 - methylenedioxy - 1,2,3,4 - tetrahydroisoquinoline - [α - ^{3}H] HCl (3.45 × 10^8 dpm, 2.24 × 10^{11} dpm/mM) (**9**) was administered to the cuttings for 7 days, and the alkaloids were separated and identified as above to give corynoline (7.74 × 10^3 dpm, 3.20 × 10^6 dpm/mM) (**2**) and acetylcorynoline (3.57 × 10^3 dpm, 1.34 × 10^6 dpm/mM) (**3**). The location of tritium in radioactive corynoline was proved to be at C_{11} position as following way. Radioactive corynoline (**11**) was oxidized by Oppenauer oxidation to give inactive 11-oxocorynoline. This shows that the tritium in corynoline is at C_{11} and that **9** is incorporated intact into corynoline in this plant. Furthermore, it suggests that **9** is converted to an active precursor such as **10** in this plant.

Table 1. Incorporation of labelled precursors into *Corydalis* alkaloids

Precursors metabolites	1		6		9	
	Incorpn (%)	Dilution	Incorpn (%)	Dilution	Incorpn (%)	Dilution
Corynoline	0.88	210	0.12	1340	0.002	70000
Acetylcorynoline	0.13	850	0.06	1650	0.001	164000
Corydalic acid methyl ester	0.02	3760	0.02	3920		
Corydamine HCl			0.33	100		

Feeding of corynoline-[6-^{3}H] (2).

Corynoline-[6-^{3}H]HCl (8.42 × 10^7 dpm, 1.34 × 10^{10} dpm/mM) (**2**) was administered to the cuttings for 7 days. The alkaloids were separated and identified as above to



Scheme 2. Biosynthetic relations between protoberberine-, benzo[C]phenanthridine- and B-secoprotoberberine type alkaloids.

give inactive corydalic acid methyl ester. Thus corydalic acid methyl ester is derived only from (±)-tetrahydrocorysamine and not from corynoline. Accordingly, the biosynthetic relations between protoberberine-, benzo[C]phenanthridine- and B-secoprotoberberine type alkaloids in this plant are established.

EXPERIMENTAL

General procedure. Mps are uncorr. TLC was performed on Si gel (Merck) developing with hexane–EtOAc (2:1), TLC-1; C_6H_6–Et_2O (10:1), TLC-2; $CHCl_3$–MeOH–H_2O (7:3:1, lower layer), TLC-3; $CHCl_3$–MeOH (10:1), TLC-4; $CHCl_3$–MeOH (20:1), TLC-5. The spots were detected with Dragendorff reagent. Radioactivity measurements were made on Packard 3375/80 Tri-Carb liquid scintillation spectrometer and radioscantgrams were taken on Aloka model TRM-1B. The scintillator soln used was made up of POPOP (0.01%), POP (0.4%) in toluene. All radioactive products were recrystallized to constant sp. act.

Preparation of (±)-tetrahydrocorysamine-[8,14-^3H]HCl (**1**). Corysamine HCl (14.8 mg) dissolved in MeOH was hydrogenated with NaB^3H_4 at room temp. for 2 min. **1** (11 mg, mp 203–204°, recrystallization from $CHCl_3$–MeOH, 1.50×10^{10} dpm/mM). (Hydrochloride 3.05×10^7 dpm, 1.34×10^{10} dpm/mM) was identified with an authentic sample on TLC (TLC-1).

Preparation of (±)-tetrahydrocoptisine-[8,14-^3H]HCl (**6**). Coptisine HCl (13.9 mg) dissolved in MeOH was hydrogenated with NaB^3H_4 at room temp. to give **6** (11.2 mg, mp 217–218°, recrystallization from $CHCl_3$–MeOH, 7.56×10^9 dpm/mM) (Hydrochloride 1.88×10^7 dpm, 7.10×10^9 dpm/mM) which was identified with an authentic sample of TLC (TLC-1).

Preparation of N-*methyl-3-[6'-(3',4'-methylenedioxyphenethylalcohol)]-4-methyl-7,8-methylenedioxy-1,2,3,4-tetrahydroisoquinoline-[α-^3H]HCl* (**9**). To a soln of corydalic acid methyl ester (12.5 mg) suspended in anhydrous tetrahydrofuran (10 ml) $LiAl^3H_4$ (1.9 mg) was added and the reaction mixture was refluxed for 30 min. After the usual work up, **9** (12.2 mg) was identified with an authentic sample on TLC (TLC-1). Hydrochloride; mp 216–218°, recrystallized from MeOH, $3,45 \times 10^8$ dpm, 2.24×10^{11} dpm/mM.

Preparation of corynoline-[6-^3H] HCl (**2**). Corynoloxine HCl (28 mg) was hydrogenated with NaB^3H_4 (0.7 mg) followed by the same way as that of **1**. **2** (23 mg, mp 223–224°, recrystallization from $CHCl_3$–MeOH). (Hydrochloride, 8.42×10^7 dpm, 1.34×10^{10} dpm/mM) was identified with an authentic sample on TLC (TLC-1).

Feeding experiments. Each radioactive precursor was fed to the cuttings at vegetative phase of this young plant for 7 days [4]. After the cultivation the cuttings were extracted with MeOH and 5% tartaric acid. The acidic solution was alkalized with dil. NH_4OH and was extracted with Et_2O. The Et_2O extract was separated by preparative TLC to give metabolites. Each metabolite was purified and identified by the dilution method.

Feeding of (±)-tetrahydrocorysamine-[8,14-^3H]HCl (3.05 × 10^7 dpm, 1.34×10^{10} dpm/mM (**1**). **1** dissolved in H_2O was administered to the cuttings (23 g) for 7 days. After feeding, the alkaloid fraction in Et_2O extract was separated by preparative TLC to give radioactive corynoline, acetylcorynoline and corydalic acid methyl ester. Acetylcorynoline (1.10×10^4 dpm) diluted with carrier (16.8 mg) was dissolved by Py (10 ml) and oxidized with 0.5% $KMnO_4$ until continuing purple colouration appears in the soln. The reaction mixture was stirred overnight and after the filtration of MnO_2 the soln was evapd to dryness. The residue was extracted with $CHCl_3$ and recrystallized from MeOH to give 6-oxocorynoline (6 mg, mp > 300°) which was identified with an authentic sample on TLC (TLC-4) and showed no radioactivity.

Feeding of (±)-tetrahydrocoptisine-[8,14-^3H]HCl (1.88 × 10^7 dpm, 7.10×10^9 dpm/mM) (**6**). **6** dissolved in H_2O was fed as above to give radioactive corynoline, acetylcorynoline, corydamine HCl and corydalic acid methyl ester. Corydamine HCl (6.31×10^4 dpm) diluted with carrier (29.8 mg) was acetylated. *N*-Acetylcorydamine was purified by preparative TLC (TLC-5) and was recrystallized from $CHCl_3$–MeOH to give *N*-acetylcorydamine (mp 177–178°, 7.55×10^5 dpm/mM). Acetylcorynoline (5.31×10^3 dpm) diluted with carrier (17.1 mg) was oxidized with 5% $KMnO_4$ to afford 6-oxocorynoline (mp > 300°) which was identified with an authentic sample on TLC (TLC-4) and showed no radioactivity.

Feeding of N-*methyl-3-[6'-(3',4'-methylenedioxyphenethylalcohol)] - 4 - methyl - 7,8-methylenedioxy - 1,2,3,4 - tetrahydroiso - quinoline-[α-^3H]HCl (3.45 × 10^8 dpm, 2.24 × 10^{11} dpm/mM)* (**9**). Followed by the same way to that of **1**, **9** was fed to give radioactive corynoline and acetylcorynoline. By Oppenauer oxidation by use of fluorenone (200 mg) and *ter* BuOK (2 g) suspended in anhydrous C_6H_6 (10 ml), radioactive corynoline (3.45×10^3 dpm) diluted with carrier (10.2 mg) was oxidized for 2 hr at room temp. in N_2. The C_6H_6 layer was washed and evapd to dryness. The residue was washed with hexane. Recrystallization from $CHCl_3$–MeOH gave 11-oxocorynoline, mp 236–237°, which was identified with an authentic sample on TLC (TLC-2) and showed no radioactivity.

Feeding of corynoline-[6-^3H]HCl (8.42 × 10^7 dpm, 1.34 × 10^{10} dpm/mM) (**2**). Followed by the same way to that of **1**. **2** was fed to give acetylcorynoline (4.26×10^6 dpm, 1.72×10^9 dpm/mM) identified with an authentic sample on TLC(TLC-1) and no radioactive corydalic acid methyl ester was obtained.

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