FIVE NEW DITERPENOIDS FROM ACONITUM DOLICHORHYNCHUM

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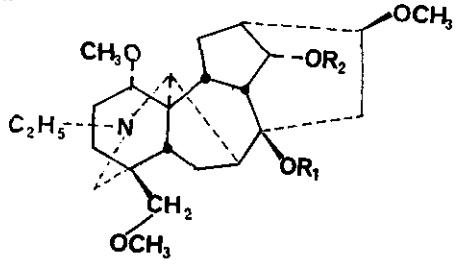
Abstract - From the root extracts of Aconitum dolichorhynchum Wang var. subglabratum T. L. Ming, we have isolated five new minor alkaloids: dolichotine A (I), dolichotine B (II), dolichotine C (III), dolichotine D (IV) and dolichotine E (V), besides yunaconitine (VI), 8-deacetylyunaconitine (VII), crassicauline A (VIII), talatisamine (IX), columbidine (X) and cammaconine (XI). The structures of these alkaloids were determined with the aid of spectral data and correlation with compounds of established structures. The structure of dolichotine C was confirmed by synthesis from talatisamine and partial hydrolysis. All the new alkaloids are characteristic of aromatic acid ester or palmitic acid ester substituted at C_8 of C_{19}-diterpenoid alkaloids.

The root of Aconitum dolichorhynchum Wang var. subglabratum T. L. Ming was collected in Zhongdian, the northwest of Yunnan. Being strong poisonous, it has been used to smear on an arrowhead to kill animal. After our investigating this plant material, it showed that the major component is yunaconitine in 0.13 % yield. The toxicity of yunaconotine was reported.[1] From the root of A. dolichorhynchum, five new minor alkaloids - dolichotine A (I), dolichotine B (II), dolichotine C (III), dolichotine D (IV) and dolichotine E (V), as well as six known alkaloids - yunaconitine (VI), 8-deacetylyunaconitine (VII), crassicauline A (VIII), talatisamine (IX), columbidine (X) and cammaconine (XI), have been isolated. In this report we wish to describe the separation and structure determination of these compounds. The crude alkaloid mixture was obtained by acidification with 2% H_2SO_4, basification with 30% NH_4OH to pH 8-9 and extraction with $CHCl_3$. Column chromatography and preparative tlc afforded eleven alkaloids.



(I) Dolichotine A R^1=As; R^2=Ac

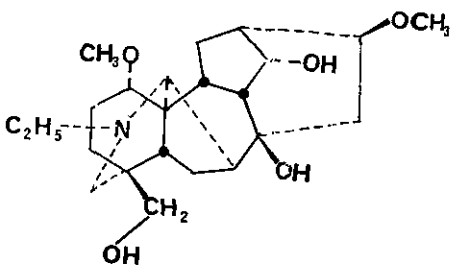
(II) Dolichotine B R^1=Vr; R^2=Ac

(III) Dolichotine C R^1=Cn; R^2=Ac

(IX) Talatisamine R^1=R^2=H

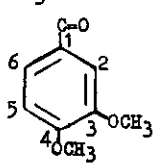
(IXa) 14-Acetyltalatisamine R^1=H; R^2=Ac

(X) Clumbidine R^1=C_2H_5; R^2=H



(XI) Cammaconine

Table 1. ^{13}C nmr chemical shifts and assignments for talatisamine(IX), 14-acetyltalatisamine(IXa), cammaconine(XI), columbidine(X), dolichotine A(I), dolichotine B(II), dolichotine D(IV), dolichotine E(V), crassicauline A(VIII) and vilmorrianine C(XII). ($CDCl_3$)

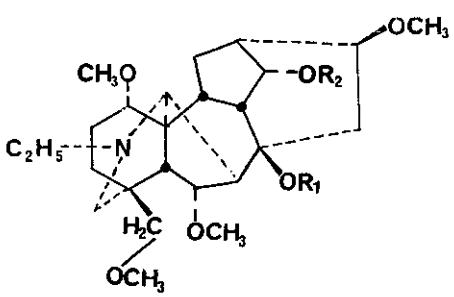
Carbons	IX	IXa	XI	X	I	II	IV	XII	V	VIII
C(1)	86.3	85.5	86.0	85.6	85.1	85.2	85.1	85.1	84.2	84.8
C(2)	25.9	26.0	25.6	26.0	26.1	26.3	26.4	26.4	25.8	25.9
C(3)	32.8	34.2	32.2	32.0	32.2	32.3	34.8	34.9	35.3	35.4
C(4)	38.6	38.5	38.7	38.5	38.1	38.4	39.1	39.1	39.6	39.1
C(5)	38.9	40.9	38.7	38.5	41.4	41.8	49.1	49.2	48.8	48.9
C(6)	24.8	24.9	24.7	23.9	24.7	25.1	83.4	82.6	83.8	83.3
C(7)	46.0	46.3	45.9	40.0	45.6	45.6	44.9	44.9	49.1	50.4
C(8)	72.8	73.6	73.4	78.2	85.9	86.5	85.8	85.9	85.4	85.3
C(9)	47.1	45.3	47.3	45.4	42.1	42.1	49.2	49.3	40.7	40.9
C(10)	46.0	40.6	45.7	45.7	38.6	39.2	43.9	43.9	44.6	44.9
C(11)	48.8	48.8	48.9	49.1	48.5	49.0	50.6	50.3	50.0	49.8
C(12)	27.9	28.4	28.0	28.9	28.4	28.8	29.2	29.0	34.5	34.5
C(13)	45.1	45.8	37.8	39.1	44.8	45.2	39.8	39.1	75.8	75.1
C(14)	75.6	76.9	75.7	75.1	75.3	75.8	75.3	75.4	79.0	78.9
C(15)	38.8	37.8	39.1	35.2	37.5	37.8	38.1	37.9	38.4	38.9
C(16)	82.4	81.7	82.4	82.6	82.7	83.1	82.9	83.5	84.0	83.9
C(17)	62.9	62.1	62.9	62.4	61.6	61.7	61.3	61.7	62.3	61.6
C(18)	79.5	80.0	68.9	79.2	79.1	79.4	80.4	80.6	80.4	80.4
C(19)	53.3	53.2	53.5	53.2	52.7	53.2	54.0	53.8	54.2	53.8
$N-CH_2$	49.5	49.3	49.5	49.4	49.0	49.3	49.3	49.0	49.4	49.1
CH_3	13.7	13.4	13.4	13.6	13.1	13.3	13.0	13.4	13.6	13.1
C(1)'	56.3	56.0	56.1	56.1	55.1	56.0	56.6	56.6	55.7	56.1
C(6)'	-	-	-	-	-	-	58.1	57.8	58.0	57.8
C(16)'	56.4	56.0	55.8	56.4	55.8	56.0	55.9	56.0	58.9	58.7
C(18)'	59.5	59.4		59.5	59.1	59.4	59.1	59.1	59.4	59.1
$C(8)-OCH_2$		-		55.9	-	-	-	-	-	-
CH_3		-		16.2	-	-	-	-	-	-
C=O		170.6			171.1	171.5	-	169.8	-	169.8
CH_3		21.2			21.1	21.6	-	21.8	-	21.3
C=O					-	-	172.5	-	172.6	-
CH_2					-	-	22.6	-	22.8	-
$(CH_2)_{13}$					-	-	24.0-30.0	-	24.0-30.0	-
CH_3					-	-	11.4	-	11.6	-
					164.3	164.7	166.0	166.2	166.3	166.3
					1 123.8	124.0	123.0	123.0	123.2	123.1
					2 131.1	112.0	131.8	131.8	132.1	131.9
					3 113.2	149.8	113.8	113.7	114.2	114.1
					4 162.9	153.0	163.5	163.5	163.9	163.8
					5 113.2	110.4	113.8	113.7	114.2	114.1
					6 131.1	123.4	131.8	131.8	132.1	131.9
					3' -	56.1	-	-	-	-
					4' 56.2	56.6	55.4	55.4	55.5	55.4

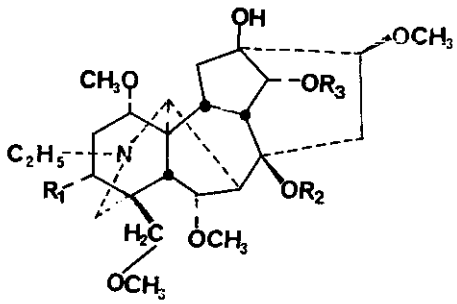
Dolichotine A (I) was obtained as an amorphous compound, $[\alpha]_D$ +15.2° ($CHCl_3$), $C_{34}H_{47}NO_8$ (deduced from ms, ^1H- and ^{13}C-nmr spectral data). The ^{13}C-nmr spectrum exhibited 34 lines corresponding to 34 carbon atoms of the molecule (see Table 1). The ir spectrum showed no absorption above 3200 cm^{-1}, indicating the absence of hydroxyl groups in dolichotine A. The ^1H-nmr spectrum gave signals at δ 1.09 (3H, t, J = 7 Hz, $NCH_2\underline{CH_3}$), 1.79 (3H, s, $OCO\underline{CH_3}$), 3.24, 3.30, 3.33 (each 3H, s, $O\underline{CH_3}$), 3.85 (3H, s, Ar-$O\underline{CH_3}$), 4.83 (1H, t, J = 4.5 Hz, C_{14}-β-H), 6.91, 7.94 (each 2H, d, J = 9 Hz, A_2B_2 type, Ar-H), showing that dolichotine A is a C_{19}-diterpenoid alkaloid having an N-ethyl, three methoxyls, an acetyl and one anisoyl group. Comparison of ^1H-nmr spectral data of dolichotine A (I) with those of anisoezochasmaconitine[2] which appeared a signal at δ 4.10 (1H, dd, J_1 = 6 Hz, J_2 = 1 Hz, C_6-β-H) in its spectrum, showed that dolichotine A has no methoxyl group at C_6. The loss of 31 mass unit from the molecular ion to give an intense peak suggested a methoxyl group at C_1 of a C_{19}-diterpenoid alkaloid and the methoxyl group at C_1 being α-orient,[3] which is also supported by the chemical shifts of the ^{13}C-nmr spectrum at δ 85.1 (d), 26.1 (t) and 32.3 (t) corresponding to C_1, C_2 and C_3 of dolichotine A, respectively.[4] The presence of C_{18}-OCH_3 in I was supported by the carbon signals at δ 59.3 (q) and 79.7 (t) of the ^{13}C-nmr spectrum.[4] According to the biogenesis of C_{19}-diterpenoid alkaloids,[5,6] there is usually β-OCH_3 substituent at C_{16}. Alkaloids without an oxygen substituent at C_{13}, but bearing C_8-OBz or C_8-OAs and C_{14}-OAc, show a 3H singlet for the acetate methyl group between δ 1.76-1.79 and a 1H triplet for C_{14}-H between δ 4.80-4.82.[2] However, alkaloids with the reverse arrangement, viz. C_8-OAc and C_{14}-OBz or C_{14}-OAs, such as 8-acetyl-14-benzoylneoline, show a 3H singlet for the acetate methyl between δ 1.34-1.46 and a 1H triplet for C_{14}-H between δ 5.00-5.11.[7] Because dolichotine A revealed a 3H singlet at δ 1.79 and a 1H triplet at δ 4.83, it was proposed for structure I. Hydrolysis of dolichotine A with 2% KOH in MeOH gave talatisamine and anisic acid. So dolichotine A is 8-anisoyl-14-acetyltalatisamine.

Dolichotine B (II) was obtained as an amorphous compound, $C_{35}H_{49}NO_9$ (deduced from ms, ^1H- and ^{13}C-nmr spectral data), $[\alpha]_D$ 0° ($CHCl_3$). The ^1H- and ^{13}C-nmr spectral data of dolichotine B (II) are very similar to those of dolichotine A (I), except for differences of signals in the aromatic region. Comparison of aromatic signals of II with those of I revealed that dolichotine B possesses a veratroyl group instead of an anisoyl group as in dolichotine A. In addition II is 30 mass unit more than I, showing that II has one methoxyl group more than I. The ^{13}C-nmr data for dolichotine B are given in Table 1. Hydrolysis of dolichotine B with 2% KOH in MeOH gave talatisamine and veratric acid. So dolichotine B is 8-veratroyl-14-acetyltalatisamine.

Dolichotine C (III) was obtained as an amorphous compound, $C_{35}H_{47}NO_7$ (derived from ms, ^1H- and ^{13}C-nmr spectral data). The ir and ^1H-nmr spectral data showed that dolichotine C possesses a cinnamoyl group[8] instead of an anisoyl group as in dolichotine A and a veratroyl group as in dolichotine B. The signal for the acetoxyl protons in the ^1H-nmr spectrum of dolichotine C occurs at δ 1.95, which is at a little higher field than the normal signal[9] but is at lower field than the corresponding signal in dolichotine A (δ 1.79) and dolichotine B (δ 1.74). In dolichotine C which was esterified with cinnamic acid, the plane of the benzene ring is further away from the acetate methyl group which, however, is close to the double bond. Hence the smaller shift (δ 1.95) in the acetoxyl protons signal must be due to the anisotropic action of the double bond, which is not as strong as that of the aromatic ring. In addition, chasmanthinnine[8] manifests a 3H singlet for the acetoxyl protons at δ 1.77 and a 1H signal for C_{14}-H at δ 4.80. But dolichotine C shows a

3H singlet (δ 1.95) and a 1H signal at δ 4.82. So dolichotine C was designated as 8-trans-cinnamoyl-14-acetyltalatisamine. It was confirmed by synthesis from talatisamine (IX). Acetylation[10] of IX with Ac_2O and pyridine gave 14-acetyltalatisamine (IXa). The ^{13}C-nmr spectral data for IXa are given in Table 1. Cinnamoylation of IXa with cinnamic anhydride and p-TsOH in toluene afforded 8-trans-cinnamoyl-14-acetyltalatisamine (IXb), which is identical in every respect with III. Furthermore, partial hydrolysis of IXb and III with dioxane-H_2O (1:1)[11] gave IXa showing one spot on tlc. Therefore, dolichotine C has structure III.





(IV) Dolichotine D R^1=$COC_{15}H_{31}$; R^2=As

(XII) Vilmorrianine C R^1=Ac; R^2=As

(XIII) Chasmanine R^1=R^2=H

(V) Dolichotine E R^1=H; R^2=$COC_{15}H_{31}$; R^3=As

(VI) Yunaconitine R^1=OH; R^2=Ac; R^3=As

(VII) 8-Deacetylyunaconitine R^1=OH; R^2=H; R^3=As

(VIII) Crassicauline A R^1=H; R^2=Ac; R^3=As

(XIV) Bikhaconine R^1=R^2=R^3=H

Dolichotine D (IV) was an amorphous compound, $C_{49}H_{77}NO_9$ (derived from the ms, ^1H- and ^{13}C-nmr spectral data). The ^{13}C-nmr spectral data are given in Table 1. The ^1H-nmr spectrum showed that dolichotine D has the functional formula of C_{19}-diterpenoid alkaloids - $C_{19}H_{22}(NCH_2CH_3)(OCH_3)_4(CH_3OC_6H_4COO)(C_{15}H_{31}COO)$.[12] The mass spectrum exhibited two fragments at m/z 256 and m/z 135 corresponding to palmitic acid and anisoyl group, respectively. According to the ms fragmentation pattern[3,13] of C_{19}-diterpenoid alkaloids substituted with ester, molecular ion preferred losing C_8-ester to losing C_1-methoxy when the ester group attaching at C_8 is large; whether first losing C_8-ester or C_1-methoxy the intense characteristic peak is always corresponding to the fragment ion which has just lost C_1-OCH_3. The mass spectrum of dolichotine D exhibited an intense peak at m/z 536 (M^+- $C_{15}H_{31}COOH$ - OCH_3, 90) showing a α-methoxyl group at C_1, and a fragment peak at m/z 567 (M^+- $C_{15}H_{31}COOH$, 27) but no fragment peak at m/z 671 (M^+- 152) or m/z 688 (M^+- 135), so this revealed that dolichotine D possesses a palmityl group at C_8. Comparison of ^{13}C-nmr spectral data of dolichotine D with those of vilmorrianine C (XII) indicated that dolichotine D possesses a palmityl group instead of an acetyl group as in vilmorrianine C. Hydrolysis of dolichotine D with 2% KOH in MeOH gave chasmanine, palmitic acid and anisic acid. Dolichotine D was designated as structure IV.

Dolichotine E (V) was obtained as an amorphous compound, $C_{49}H_{77}NO_{10}$ (deduced from the ms, ^1H- and ^{13}C-nmr spectral data). The ^{13}C-nmr spectral data for dolichotine E are given in Table 1. Compari-

son of ir, ^1H- and ^{13}C-nmr spectra of dolichotine E with those of dolichotine D revealed that there is a hydroxyl substituent in dolichotine E. Alkaloids with a hydroxyl group at C_{13}, bearing C_{14}-OAs and C_{16}-OCH_3, show a 1H doublet for the C_{14}-H between δ 5.00-5.11 and a 3H singlet for the C_{16}-OCH_3 between δ 3.50-3.65. Examples are crassicauline A, crassicausine and forestine.[14] But alkaloids without a hydroxyl group at C_{13}, still bearing C_{14}-OAs and C_{16}-OCH_3, show a 1H triplet for the C_{14}-H between δ 5.00-5.10 and a 3H singlet for the C_{16}-OCH_3 between δ 3.30-3.40, such as dolichotine D, fotesaconitine and crassicaudine.[14] Because dolichotine E shows a 1H doublet at δ 5.10 and a 3H singlet at δ 3.54, it indicated that V possesses a hydroxyl group at C_{13}. Hydrolysis of V with 2% KOH in MeOH gave bikhaconine, palmitic acid and anisic acid. So dolichotine E was assigned as structure V.

EXPERIMENTAL

Melting point was determined on a Thomas-Kofler hot stage equipped with a microscope. Ir spectra were taken on Perkin-Elmer model 577 spectrophotometer. ^1H-Nmr spectra were run on BRUCKER WH-90 spectrometer with TMS as an internal reference. ^{13}C-Nmr spectra were operated on BRUCKER AM-400 spectrometer in $CDCl_3$; chemical shifts are reported in ppm downfield from TMS. Mass spectra were measured on a Finnigan-4510 instrument.

Plant material. The root of Aconitum dolichorhynchum Wang var. subglabratum T. L. Ming was collected in Zhongdian, Yunnan, China. This plant was identified by Professor Ming Tianlu, Kunming Institute of Botany.

Extraction and fractionation. Powdered roots of A. dolichorhynchum (10.3 Kg) were extracted with 85% ethanol (4 × 4 l) at room temperature for one week. After evaporation of the solvent, the residue (304 g) was acidified with 2% H_2SO_4 (2 l) and extracted with $CHCl_3$ (5 × 1.5 l) to give a crude alkaloid mixture - base A (160 g), which is due to unperfect acidification. The acidic water phase was basified with 30% NH_4OH and extracted with $CHCl_3$ (5 × 1.5 l) to give a crude alkaloid mixture - base B (100 g).

Isolation of dolichotine A (I), dolichotine B (II), dolichotine C (III), dolichotine D (IV), dolichotine E (V), crassicauline A (VIII) and yunaconitine (VI). A solution of base A (160 g) in $CHCl_3$ (250 ml) was evaporated with 400 g of silica gel. The mixture was placed on the top of a column filled with 4.5 Kg of silica gel and eluted with petroleum ether-acetone (9:5) to afford fraction A_1 (91 g), and with acetone to afford fraction A_2 (4.5 g). A_1 was chromatographed on a column containing 2 Kg of Al_2O_3 (neutral, activity II) and eluted with petroleum ether, petroleum ether-EtOAc (98:2) to afford dolichotine D (IV, 31 mg), petroleum ether-EtOAc (98:6) to give fraction A_3 (1.5 g), petroleum ether-EtOAc (98:10) to afford fraction A_4 (2.1 g), petroleum ether-EtOAc (98:20) to give dolichotine E (V, 30 mg), petroleum ether-EtOAc (98:30) to give crassicauline A (VIII, 1.5 g) and EtOAc. By preparative tlc over silica gel (GF_{254}, cyclohexane-20% Et_2NH), A_3 and A_4 afforded dolichotine A (I, 120 mg), dolichotine B (II, 140 mg) and dolichotine C (III, 20 mg). Crystallization of A_2 from ether gave yunaconitine (VI, 3.5 g).

Isolation of yunaconitine (VI), 8-deacetylyunaconitine (VII), talatisamine (IX), columbidine (X) and cammaconine (XI). A solution of base B (100 g) in $CHCl_3$ (200 ml) was evaporated with 300 g of Al_2O_3 (neutral, activity II). The mixture was placed on the top of a column filled with 2.5 Kg of Al_2O_3 and eluted with ether to give columbidine (X, 30 mg), hexane-EtOAc (90:1) to afford talatisamine (IX, 2.4 g), hexane-EtOAc (1:2) to give yunaconitine (VI, 10 g), hexane-EtOAc (1:50) to give 8-deacetylyunaconitine (VII, 15 mg) and EtOAc to afford cammaconine (XI, 32 mg).

Identification of dolichotine A (I). Dolichotine A is an amorphous compound, $[\alpha]_D$ +15.2° ($CHCl_3$); ^1H nmr ($CDCl_3$) δ 1.09 (3H, t, J = 7 Hz, NCH_2CH_3), 1.79 (3H, s, $OCOCH_3$), 3.24, 3.30, 3.33 (each 3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.83 (1H, t, J = 4.5 Hz, C_{14}-β-H), 6.91, 7.94 (each 2H, d, J = 9 Hz, A_2B_2 type, Ar-H); ir (KBr) 1735, 1700 (ester), 1600, 1510, 850, 770 (Ar); ms m/z 597 (M^+, 1.6), 566 (M^+- OCH_3, 47.1), 445 (M^+- $CH_3OC_6H_4COOH$, 68.1), 414 (M^+- OCH_3 - $CH_3OC_6H_4COOH$, 80), 152 ($CH_3OC_6H_4COOH$, 100); the ^{13}C-NMR spectral data are given in Table 1.

Hydrolysis of dolichotine A. Dolichotine A (50 mg) was dissolved in 5 ml of 2% KOH in MeOH and allowed to stand at room temperature for 6 h. Removal of solvent under reduced pressure gave a residue which was mixed with a small amount of H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated to give a pale yellow residue which was crystallized from acetone to give colorless needles (25 mg) being identical with those of talatisamine in its co-tlc, ir spectrum and ^1H-NMR spectrum. The water phase was acidified with 2% H_2SO_4 and extracted with $CHCl_3$ to give anisic acid (10 mg), mp 183-184 °C; ms m/z 152 (M^+, 100), 135 (M^+- OH, 95); ir (KBr) 2720 (br, COOH), 1116, 830, 770 (Ar), which was identical with those reported.[15,16]

Identification of dolichotine B (II). Dolichotine B is an amorphous compound, $[\alpha]_D$ 0° ($CHCl_3$); ^1H nmr ($CDCl_3$) δ 1.10 (3H, t, J = 7 Hz, NCH_2CH_3), 1.74 (3H, s, $OCOCH_3$), 3.26, 3.30, 3.38 (each 3H, s, OCH_3), 3.90, 3.98 (each 3H, s, OCH_3), 4.79 (1H, t, J = 4.5 Hz, C_{14}-β-H), 7.02 (1H, d, J = 9 Hz, Ar-5'-H), 7.60 (1H, dd, J_1 = 9 Hz, J_2 = 3 Hz, Ar-6'-H), 7.73 (1H, d, J = 3 Hz, Ar-2'-H); ir (KBr) 1730, 1700 (ester), 1650, 1510, 910, 765 (Ar); ms m/z 627 (M^+, 1), 596 (M^+- OCH_3, 41), 445 (M^+- $C_2H_6O_2C_6H_3COOH$, 40), 414 (M^+- $C_2H_6O_2C_6H_3COOH$ - OCH_3, 100), 386 (M^+- $C_2H_6O_2C_6H_3COOH$ - CH_3COO, 20), 182 ($C_2H_6O_2C_6H_3COOH$, 76); the ^{13}C-NMR spectral data are given in Table 1.

Hydrolysis of dolichotine B. Dolichotine B (50 mg) was hydrolized by the same method to dolichotine A as colorless needles (26 mg) which was identical as talatisamine (IX) by comparison of the mp 145-146 °C, co-tlc, ir spectrum and ^1H-NMR spectrum with those of talatisamine, and veratric acid, mp 180-181 °C; ms m/z 182 (M^+, 60), 135 (100); ir (KBr) 2710 (br, COOH), 910, 765 (Ar), which were identical with those reported.[17,18]

Identification of dolichotine C (III). Dolichotine C is an amorphous compound, ^1H nmr ($CDCl_3$) δ 1.08 (3H, t, J = 7 Hz, NCH_2CH_3), 1.95 (3H, s, $OCOCH_3$), 3.22, 3.25, 3.36 (each 3H, s, OCH_3), 4.82 (1H, t, J = 4.5 Hz, C_{14}-β-H), 6.32, 7.60 (each 1H, d, J = 16 Hz, trans-HC=CH), 6.90 - 7.50 (5H, m, Ar-H); ir (KBr) 1730, 1705 (ester), 1680, 970 (trans-double bond), 1600, 1460, 770, 710 (Ar); ms m/z 593 (M^+, 0.1), 562 (M^+- OCH_3, 31), 445 (M^+- $C_6H_5C_2H_2COOH$, 100), 414 (M^+- $C_6H_5C_2H_2COOH$ - OCH_3, 82), 386 (M^+- $C_6H_5C_2H_2COOH$ - $OCOCH_3$, 42), 148 ($C_6H_5C_2H_2COOH$, 70).

Preparation of 14-acetyltalatisamine (IXa). A solution of talatisamine (100 mg) in 5 ml of acetic anhydride and 5 ml of pyridine was allowed to stand at room temperature for 2 days. To the residue obtained on evaporation of solvent was added 10 ml of H_2O; the mixture was basified with 30% NH_4OH to pH 8 and then extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4 and evaporated to give a brownish foam (100 mg) containing 14-acetyltalatisamine (IXa) as major component in 91 % yield. ^1H Nmr ($CDCl_3$) δ 1.09 (3H, t, J = 7 Hz, $NCH_2\underline{CH_3}$), 2.09 (3H, s, $OCO\underline{CH_3}$), 3.28, 3.30, 3.31 (each 3H, s, $O\underline{CH_3}$), 5.09 (1H, t, J = 4.5 Hz, C_{14}-β-H); ms m/z 463 (M^+, 1), 432 (M^+- OCH_3, 100); the ^{13}C-NMR spectral data are given in Table 1. Structure IXa was assigned on the basis of ^1H- and ^{13}C-nmr spectra, and was identical with an authentic sample.[19]

Synthesis of dolichotine C. IXa (100 mg) and cinnamic anhydride (1.5 g) were heated at 110 °C with toluene (50 ml) and p-TsOH (10 mg) for 12 h. This reaction gave 8-cinnamyl-14-acetyltalatisamine (IXb, 20 mg) in 12.8 % yield. IXb was identical with dolichotine C on the basis of their ^1H-nmr spectrum, ir spectrum, ms and co-tlc.

Partial hydrolysis of dolichotine C and IXb.[11] Dolichotine C (10 mg) and IXb (15 mg), dissolved in 10 ml of dioxane-H_2O (1:1), were heated on an oil bath at 120° C with stirring for 1 h and extracted with $CHCl_3$. The $CHCl_3$ extract was purified by preparative tlc over silica gel (GF_{254}, cyclohexane-20% Et_2NH) and gave IXa (15 mg, in 78 % yield) which was identical with the IXa prepared above on the basis of their co-tlc, ir spectra, ms and ^1H-nmr spectra.

Identification of dolichotine D (IV). Dolichotine D is an amorphous compound, ^1H nmr ($CDCl_3$) δ 1.05 (3H, t, J = 7 Hz, $NCH_2\underline{CH_3}$), 3.17, 3.28, 3.39 (each 3H, s, $O\underline{CH_3}$), 3.84 (3H, s, Ar-$O\underline{CH_3}$), 4.05 (1H, dd, J_1 = 6 Hz, J_2 = 1 Hz, C_6-β-H), 5.03 (1H, t, J = 4.5 Hz, C_{14}-β-H), 6.89, 7.99 (each 2H, d, J = 9 Hz, A_2B_2 type, Ar-H); ir (KBr) 2920, 2850, 2820 (-CH_3, -CH_2-), 1715, 1710 (ester), 1600, 1510, 1460, 850, 770 (Ar); ms m/z 823 (M^+, 0.01), 792 (M^+- OCH_3, 16), 567 (M^+- $C_{15}H_{31}COOH$, 27), 536 (M^+- OCH_3 - $C_{15}H_{31}COOH$, 90), 416 (M^+- $C_{15}H_{31}COOH$ - $OCH_3C_6H_4COO$, 100), 384 (M^+- OCH_3 - $C_{15}H_{31}COOH$ - $OCH_3C_6H_4COOH$, 66), 256 ($C_{15}H_{31}COOH$, 25), 238 ($C_{15}H_{30}CO$, 60), 135 ($OCH_3C_6H_4CO$, 78); the ^{13}C-nmr spectral data are given in Table 1.

Hydrolysis of dolichotine D. With the same method to dolichotine A, dolichotine D (15 mg) was hydrolized and gave a compound (6.5 mg) which was identical with chasmanine (XIII) on the basis of their co-tlc, ir spectra and mass spectra.[20] The water phase was acidified with 2% H_2SO_4 and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated to give a residue which was further esterified with 1% H_2SO_4 in MeOH (10 ml) and then operated on gc-ms to show molecular ion peaks at m/z 166 and m/z 270 for methyl anisic ester and methyl palmitic ester, respectively.

Identification of dolichotine E (V). Dolichotine E is an amorphous compound, ir (KBr) 3500 (OH), 2920, 2850, 2820 (-CH_3, -CH_2-), 1715, 1710 (ester), 1604, 1576, 1455, 850, 770 (Ar); ^1H nmr ($CDCl_3$) δ 1.05 (3H, t, J = 7 Hz, $NCH_2\underline{CH_3}$), 3.18, 3.25, 3.33, 3.54 (each 3H, s, $O\underline{CH_3}$), 3.77 (3H, s, Ar-$O\underline{CH_3}$), 4.09 (1H, dd, J_1 = 6 Hz, J_2 = 1 Hz, C_6-β-H), 5.10 (1H, d, J = 4.5 Hz, C_{14}-β-H), 5.70 - 5.90 (1H, br, disappearing after exchanging with D_2O, O$\underline{H}$), 6.90, 8.10 (each 2H, d, J = 9 Hz, Ar-H); ms m/z 839 (M^+, 0.02), 808 (M^+- OCH_3, 6), 583 (M^+- $C_{15}H_{31}COOH$, 35), 552 (M^+- OCH_3 -

$C_{15}H_{31}COOH$, 50), 432 (M^+- $C_{15}H_{31}COOH$ - $CH_3OC_6H_4CO$, 20), 256 ($C_{15}H_{31}COOH$, 20), 238 ($C_{15}H_{30}O$, 60), 135 ($CH_3OC_6H_4CO$, 100); the ^{13}C-nmr spectral data are given in Table 1.

Hydrolysis of dolichotine E. With the same method to dolichotine D, dolichotine E (15 mg) was hydrolized and gave a compound (6 mg) which was identical with bikhaconine (XIV) on the basis of their co-tlc, ir spectra and mass spectra.[21] The water layer was acidified with 2% H_2SO_4 and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated to give a residue which was esterified with 1% H_2SO_4 in MeOH (10 ml) and then operated on go-ms to show molecular ion peaks at m/z 166 and m/z 270 for methyl anisic ester and methyl palmitic ester, respectively.

Identification of yunaconitine (VI). VI was crystallized as rhombus from ether, mp 140-141 °C; ms m/z 659 (M^+, 2), 628 (M^+- OCH_3, 50), 135 ($CH_3OC_6H_4CO$, 65), 43 (CH_3CO, 75); ir (KBr) 3450 (OH), 1715, 1708, 1251 (ester), 1608, 1510, 1480, 850 (Ar); ^1H nmr ($CDCl_3$) δ 1.11 (3H, t, J = 7 Hz, NCH_2CH_3), 1.34 (3H, s, $OCOCH_3$), 3.16, 3.26, 3.30, 3.55 (each 3H, s, OCH_3), 3.87 (3H, s, Ar-OCH_3), 4.06 (1H, dd, J_1 = 6 Hz, J_2 = 1 Hz, C_6-β-H), 5.02 (1H, d, J = 4.5 Hz, C_{14}-β-H), 6.93, 8.01 (each 2H, d, J = 9 Hz, AB q, Ar-H). It was identified as yunaconitine by direct comparison of its spectral data and co-tlc with those of an authentic sample.[1]

Identification of 8-deacetylyunaconitine (VII). VII was obtained as an amorphous compound, ms m/z 617 (M^+, 0.1), 586 (M^+- OCH_3, 45), 135 ($CH_3OC_6H_4CO$, 50); ir (KBr) 3530, 3420 (OH), 1705, 1250 (ester), 1602, 1520, 840, 770 (Ar); ^1H nmr δ 1.12 (3H, t, J = 7 Hz, NCH_2CH_3), 3.25, 3.29, 3.31, 3.41 (each 3H, s, OCH_3), 3.86 (3H, s, Ar-OCH_3), 4.08 (1H, dd, J_1 = 6 Hz, J_2 = 1 Hz, C_6-β-H), 5.02 (1H, d, J = 4.5 Hz, C_{14}-β-H), 6.80, 8.03 (each 2H, d, J = 9 Hz, AB q, Ar-H). It was identified as 8-deacetylyunaconitine by comparison of its spectral data with those reported.[22]

Identification of crassicauline A (VIII). VIII was crystallized as prism from acetone, mp 162-163 °C; ms m/z 643 (M^+, 1), 612 (M^+- OCH_3, 100), 583 (M^+- CH_3COOH, 50), 552 (M^+- OCH_3 - CH_3COOH, 80), 135 ($CH_3OC_6H_4CO$, 50); ir (KBr) 3500 (OH), 1725, 1710, 1256 (ester), 1600, 1508, 849, 770 (Ar); ^1H nmr ($CDCl_3$) δ 1.06 (3H, t, J = 7 Hz, NCH_2CH_3), 1.33 (3H, s, $OCOCH_3$), 3.20, 3.27, 3.50 (each 3H, s, OCH_3), 3.91 (3H, s, Ar-OCH_3), 4.04 (1H, dd, J_1 = 6 Hz, J_2 = 1 Hz, C_6-β-H), 4.84 (1H, d, J = 4.5 Hz, C_{14}-β-H), 7.07, 8.07 (each 2H, d, J = 9 Hz, AB q, Ar-H). The ^{13}C-nmr spectral data are given in Table 1. Hydrolysis of VIII (20 mg) with 2% KOH in MeOH (15 ml) gave bikhaconine (9 mg) which was identical with an authentic sample[21] on the basis of ms, ^1H-nmr and ir spectral analysis. So VIII was identified as crassicauline A by comparison of its spectral data and co-tlc with those of an authentic sample.[23]

Identification of talatisamine (IX). IX was crystallized as needles from acetone, mp 141-142 °C; ms m/z 421 (M^+, 2), 390 (M^+- OCH_3, 100); ir (KBr) 3520, 3410 (OH), 1100, 1080 (C-O); ^1H nmr ($CDCl_3$) δ 1.07 (3H, t, J = 7 Hz, NCH_2CH_3), 3.26, 3.31, 3.40 (each 3H, s, OCH_3), 4.20 (1H, t, J = 4.5 Hz, C_{14}-β-H); ^{13}C-nmr spectral data (see Table 1). It was proved to be talatisamine by comparison of its spectral data and co-tlc with those reported.[24]

Identification of columbidine (X). X was isolated as an amorphous compound, $C_{26}H_{43}NO_5$ (deduced from the ms, ^1H- and ^{13}C-nmr spectral data); ms m/z 449 (M^+, 2), 418 (M^+- OCH_3, 100), 404 (M^+- OC_2H_5, 5), 386 (M^+- OC_2H_5 - H_2O, 2), 373 (M^+- OCH_3 - OC_2H_5, 4); ir (KBr) 3540 (OH), 2965, 2910, 2870, 2810, 1493, 1382, 1260 (-CH_3, -CH_2-); ^1H nmr ($CDCl_3$) δ 1.04, 1.07 (each 3H, t, J = 7 Hz, NCH_2CH_3), 3.20, 3.23, 3.29 (each 3H, s, OCH_3); ^{13}C-nmr spectral data (see Table 1). It was identified as columbidine by comparison of its spectral data with those reported.[4]

Identification of cammaconine (XI). XI was crystallized as prism from $CHCl_3$-MeOH (1:1), mp 135-136 °C; ms m/z 407 (M^+, 1), 376 (M^+- OCH_3, 100); ir (KBr) 3530, 3460 (OH), 2930, 2860, 1382, 1100 (-CH_3, -CH_2-); ^1H nmr ($CDCl_3$) δ 1.06 (3H, t, J = 7 Hz, NCH_2CH_3), 3.21, 3.29 (each 3H, s, OCH_3), 4.20 (1H, t, J = 4.5 Hz, C_{14}-β-H); ^{13}C-nmr spectral data (see Table 1). It was proved to be cammaconine by direct comparison of its ir spectrum, ^1H- and ^{13}C-nmr spectra with those reported.[4]

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