STRUCTURE OF HOKBUSINE A AND B, DITERPENIC ALKALOIDS OF *ACONITUM CARMICHAELI* ROOTS FROM JAPAN[1]

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ABSTRACT.—The study of *Aconitum carmichaeli* roots from Hokkaido, Japan, has resulted in the isolation of eight alkaloids. Four of them have been identified as mesaconitine, hypaconitine, aconitine and senbusine C, which are known components of this plant. Among the remaining four alkaloids, two have been found identical with neoline (**1**) and ignavine (**2**) which have been obtained from other *Aconitum* species. The two new alkaloids, hokbusine A and B, are represented by formulas **3** and **4**, respectively.

The crude drug "bushi", prepared from the roots of certain species of *Aconitum* plants (Ranunculaceae), is an important material in Oriental medicine. One of the main sources, *A. carmichaeli* Debeaux native to China, is cultivated in China and Japan. Because we have recently clarified the alkaloid composition of the crude drug "sen-bushi", produced from this species in Sichuan, China (1), we became interested in the alkaloidal constituents of the crude drug prepared from the same species but cultivated in Hokkaido, Japan, and utilized as raw material for a processed aconite used for therapeutic purposes.

The basic portion of the methanol extract of the crude drug was chromatographed over alumina and silica gel to afford eight alkaloids. Identification of the known alkaloids, mesaconitine, hypaconitine, aconitine and senbusine C, was readily carried out by comparison of their physico-chemical properties.

This paper deals with the identification of two of the remaining four alkaloids as neoline (**1**) and ignavine (**2**), which had not been previously reported in this plant, as well as the structure elucidation of the two new alkaloids hokbusine A and B represented by formulas **3** and **4**, respectively.

Alkaloid **1**, $C_{24}H_{39}NO_6$ (ms *m/e* 437, M^+), exhibited an ir band at 3300 cm^{-1} (hydroxyls). The ^1H nmr spectrum disclosed the presence of an *N*-ethyl (3H t, δ 1.12, *J* 7 Hz) and three methoxyls (3H s each, δ 3.32, 3.33, 3.33). These data indicated that the alkaloid had a C_{19} carbon skeleton which was most likely to be the aconitine skeleton. Comparison of the ^{13}C nmr spectrum with those of the aconitine alkaloids (2) revealed that the alkaloid was identical with neoline (**1**) (3), a fact which was confirmed by direct comparison.

Alkaloid **2**, $C_{27}H_{31}NO_5$ (ms *m/e* 449, M^+), showed ir bands at 3350 (hydroxyls), 1725 and 1260 cm^{-1} (aromatic ester). In the ^1H nmr spectrum, the presence of a benzoyl was demonstrated (3H m, δ 7.54; 2H dd, δ 7.99, *J* 8, 2 Hz). These findings led to the assumption that this alkaloid possessed a C_{20} carbon skeleton which was considered to be most probably the atisine skeleton. When its ^1H and ^{13}C nmr spectra were compared with those of the atisine alkaloids (4), this alkaloid was suspected of being ignavine (**2**) (5); and indeed, the identity was substantiated by direct comparison.

Hokbusine A displayed, in the high resolution mass spectrum, the molecular ion peak at *m/e* 603.3069, showing it to have the composition $C_{32}H_{45}NO_{10}$. In the ir spectrum, bands for hydroxyls (3450 cm^{-1}) and an aromatic ester (1720, 1270 cm^{-1}) were visible. The ^1H nmr spectrum revealed the presence of an *N*-methyl (3H s, δ 2.36) and five methoxyls (3H s, δ 3.13, 3.28, 3.28, 3.31, 3.72) as well as a benzoyl group (3H m, δ 7.50; 2H dd, δ 8.04, *J* 8, 2 Hz). Based on these data, it was suspected that this alkaloid had a C_{19} carbon skeleton. Assuming

[1]Pharmaceutical studies on *Aconitum* roots—Part 13. This paper also forms Part 39 in the series on the validity of the Oriental medicines. For the preceding paper, see H. Hikino, H. Takata, M. Fujiwara, C. Konno and K. Ohuchi, *European J. Pharmacol.*, **82**, 65 (1982).

that this alkaloid was an aconitine congener, an ^1H nmr signal at δ 4.84 (1H d, J 5 Hz) was attributable to the hydrogen on C–14 bearing a benzoyloxy group; while the signals at δ 4.04 (1H broad d, J 7 Hz) and 4.53 (1H broad) were attributable to the hydrogens on C–16 and C–15 bearing oxygen functions. Comparison of the ^{13}C nmr spectrum of this alkaloid with that of benzoylmesaconine (**5**) showed that, in the former, the signals for C–7 and C–15 were shifted upfield by 3.7 and 4.8 ppm, respectively, and those for C–8 and C–16 displayed displacements toward lower field by 4.4 and 2.2 ppm, respectively. This observation together with the fact that hokbusine A (**3**) on acetylation with acetic anhydride in pyridine yielded the diacetate (**6**) indicated that hokbusine A is the 8-*O*-methyl derivative of benzoylmesaconine. In order to verify this hypothesis, the alkaloid and benzoylmesaconine (**5**) were methylated by means of the Hakomori method (6), and both deacyl permethylates (**7**) were found to be identical. Hokbusine A thus corresponds to 8-*O*-methylbenzoylmesaconine (**3**).

TABLE 1. Carbon-13 shieldings in the *Aconitum* alkaloids.

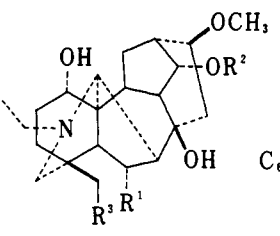
	3 (CD_3OD)	**5** (CD_3OD)	**4** (C_5D_5N)	**8** (C_5D_5N)	**9** ($CDCl_3$)
C–1	83.9 d	83.8 d	72.7 d	72.8 d	72.2 d
C–2	35.8 t	35.6 t	30.8 t	30.7 t*	29.1 t*
C–3	69.9 d	70.2 d	32.1 t	32.2 t	29.7 t*
C–4	44.7 s	44.8 s	33.3 s	33.1 s	37.3 s
C–5	46.7 d	46.7 d	47.0 d	46.9 d	41.4 d
C–6	84.7 d	84.5 d	25.8 t	26.0 t	25.1 t
C–7	43.1 d	46.7 d	55.0 d	45.8 d	45.8 d
C–8	83.7 s	79.3 s	74.0 s	74.7 s	74.5 s
C–9	49.9 d	49.3 d	44.4 d	47.4 d	44.6 d
C–10	42.4 d	42.8 d	38.3 d	41.4 d	37.0 d
C–11	51.3 s	51.2 s	49.1 s	49.4 s	49.0 s
C–12	38.2 t	38.0 t	29.6 t	30.4 t*	26.7 t
C–13	74.2 s	75.0 s	43.6 d	44.8 d	43.5 d
C–14	80.9 d	81.0 d	77.0 d	75.9 d	76.9 d
C–15	77.6 d	82.4 d	41.8 t	43.1 t	42.4 t
C–16	95.3 d	93.1 d	83.1 d	83.6 d	82.2 d
C–17	63.2 d	63.5 d	57.7 d	63.1 d	63.5 d
C–18	76.1 t	76.3 t	27.6 q	27.5 q	79.0 t
C–19	50.3 t	49.6 t	52.4 t	60.3 t	56.6 t
NCH_3	42.7 q	42.8 q			
NCH_2CH_3				48.4 t	48.4 t
NCH_2CH_3				13.2 q	13.0 q
1–OCH_3	56.3 q	56.2 q			
6–OCH_3	59.0 q	58.1 q			
8–OCH_3	59.0 q				
16–OCH_3	62.2 q	61.8 q	55.8 q	55.9 q	55.9 q
18–OCH_3	59.0 q	59.1 q			59.3 q
$COCH_3$			171.0 s		170.3 s
$COCH_3$			21.4 q		21.2 q
COC_6H_5	167.8 s	167.8 s			
COC_6H_5	131.4 s	131.5 s			
	130.6 d x 2	130.9 d x 2			
	129.2 d x 2	129.2 d x 2			
	133.9 d	133.9 d			

The assignments of the asterisked signals are ambiguous and might have to be reversed.

Turning now to hokbusine B, this alkaloid showed in the mass spectrum the molecular ion peak at m/e 391, suggesting it to possess the composition $C_{22}H_{33}NO_5$. The ir spectrum revealed an intense band at 3460 cm^{-1} attributable to hydroxyl or amino group and bands at 1730 and 1237 cm^{-1} due to an ester. In the ^1H nmr spectrum, a 3H singlet at δ 1.94 could be assigned to an acetoxyl, while a 3H singlet at δ 3.16 was clearly due to a methoxyl. Accumulated data demonstrated that hokbusine B was constructed from a C_{19} carbon skeleton, most likely the aconitine skeleton. The ^1H nmr spectrum disclosed a 1H multiplet at δ 3.69

and a 1H triplet at δ 4.69 (J 5 Hz) whose chemical shifts and splitting patterns indicated them to be attributable to carbinyl hydrogens at C-1 which also bears a hydroxyl function, and C-14 which carries an acetoxyl group. When the ^{13}C nmr spectrum was compared with those of karacoline (**8**) (7) and condelphine (**9**) (2,8), it was found that the signals for $C_{(1)}$-$C_{(6)}$ and those for $C_{(7)}$-$C_{(16)}$ in hokbusine B were in good agreement with those for the corresponding carbons in karacoline (**8**) and condelphine (**9**), respectively, revealing that the partial structures and stereochemistry at $C_{(1)}$-$C_{(6)}$ and $C_{(7)}$-$C_{(16)}$ in hokbusine B were the same as those in karacoline and condelphine, respectively. The ^{13}C nmr signals for $C_{(7)}$, $C_{(17)}$ and $C_{(19)}$ in hokbusine B, when compared with those for karacoline (**8**), exhibited displacements (-9.2, $+5.4$ and $+7.9$ ppm, respectively) which could be explained by the replacement of the tertiary amino group ($>N-C_2H_5$) in the latter by a secondary amino group ($>N-H$) in the former. The observation that no apparent differences were found in the ^{13}C nmr signals for $C_{(4)}$ and $C_{(11)}$ may be due to the fact that these two carbons are quaternary, and are insensitive to substitution effects. The combined evidence thus led to the assumption that hokbusine B is *N*-desethyl-14-*O*-acetylkaracoline (**4**). Ethylation of hokbusine B with ethyl iodide in acetone (9) afforded the *N*-ethyl derivative (**10**) which was acetylated with acetic anhydride in pyridine to yield *N*-ethylhokbusine B monoacetate (**11**). Because this *N*-ethylhokbusine B monoacetate (**11**) was revealed to be identical with karacoline 1,14-diacetate, hokbusine B was established as being *N*-desethyl-14-*O*-acetylkaracoline (**4**).

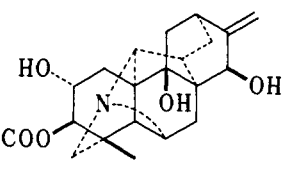
The alkaloidal composition of a plant belonging to the *Aconitum* genus is known to vary remarkably, which may be due to underdevelopment of this genus from an evolutionary standpoint. Although we have recently indicated that the contents of the aconitines and the benzoylaconines in *A. carmichaeli* show great variation depending on seasons (10), mesaconitine, hypaconitine and



1 : $R^1=R^3=OCH_3$, $R^2=H$

8 : $R^1=R^2=R^3=H$

9 : $R^1=H$, $R^2=COCH_3$, $R^3=OCH_3$



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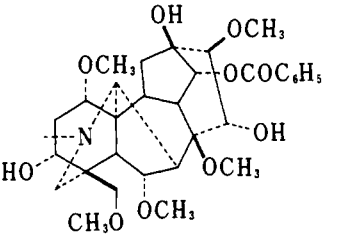

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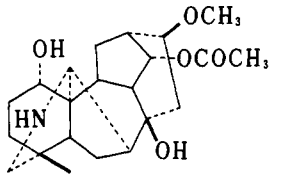

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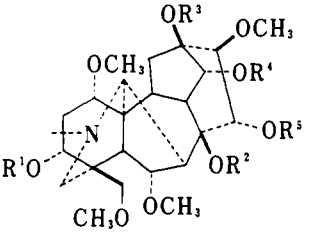
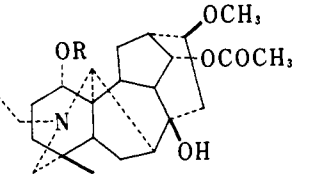


5 : $R^1=R^2=R^3=R^5=H$, $R^4=COC_6H_5$

6 : $R^1=R^5=COCH_3$, $R^2=CH_3$, $R^3=H$, $R^4=COC_6H_5$

7 : $R^1=R^2=R^3=R^4=R^5=CH_3$



10 : $R=H$

11 : $R=COCH_3$

aconitine, which take considerable part in therapeutic efficacy, inevitably exist in the roots of this species. Minor alkaloids, on the other hand, seem to reveal significant alteration in quality, owing to difference of lot as judged from the results of the present work and the literature (1, 11, 12).

EXPERIMENTAL[2]

ISOLATION OF THE DITERPENOID ALKALOIDS, HYPACONITINE, ACONITINE, MESACONITINE, NEOLINE, HOKBUSINE A, IGNAVINE, SENBUSINE C AND HOKBUSINE B FROM *Aconitum carmichaeli*.—The crude drug "bushi" (28 kg), the dried roots of *Aconitum carmichaeli* from Hokkaido, Japan, was extracted with methanol (40 liters x 3) for 4 days (each extraction) at room temperature to give the extract (1.8 kg). The extract was fractionated in the customary manners (1) to obtain the alkaloid portion (42 g), which on chromatography over alumina (500 g) afforded the ethyl acetate eluate, the ethyl acetate-methanol (9:1) eluate and the ethyl acetate-methanol (1:1) eluate.

The ethyl acetate eluate was rechromatographed over alumina.

Elution with ethyl acetate and crystallization from ethyl acetate yielded hypaconitine as colorless needles, mp 186–188°. The identity was confirmed by tlc and ir spectra comparison.

Successive elution with the same solvent and crystallization from ethyl acetate afforded aconitine as colorless needles, mp 195–197°. Identification was carried out by the usual criteria.

Subsequent elution with the same solvent and crystallization from ethyl acetate gave mesaconitine, mp 197–199°. Identification was performed by the customary manner.

The ethyl acetate-methanol (9:1) eluate from previous alumina chromatography was subjected to silica gel chromatography.

Elution with ethyl acetate-methanol (4:1) furnished neoline (**1**) as colorless needles (363 mg), mp 166.5–167.5°; $[\alpha]_D$+26.0° (c 0.16, methanol); ir νmax (KBr) cm^{-1} 3300 (OH); ^1H nmr δ 1.12 (3H, t, *J* 7, CH_2–CH_3), 3.32 (3H, s, OCH_3), 3.33 (6H, s, OCH_3 x 2), 4.16 (2H, m, $C_{(6)}H$, $C_{(14)}H$); ms *m/e* 437 (M^+). The identity was confirmed by comparison of mass, ir and ^{13}C nmr spectra, and tlc.

Successive elution with methanol afforded hokbusine A (**3**) as a colorless amorphous powder (180 mg), $[\alpha]_D$+11.4° (c 0.23, methanol); ir νmax (KBr) cm^{-1} 3450 (OH), 1720, 1270 (COO); ^1H nmr δ 2.36 (3H, s, N-CH_3), 3.13 (3H, s, OCH_3), 3.28 (6H, s, OCH_3 x 2), 3.31 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 4.04 (1H, broad d, *J* 7, $C_{(16)}H$), 4.53 (1H, broad, $C_{(15)}H$), 4.84 (1H, d, *J* 5, $C_{(14)}H$), 7.50 (3H, m), 8.04 (2H, dd, *J* 8, 2); ^{13}C nmr data shown in table 1; high resolution-ms *m/e* 603.3069 (M^+).

Subsequent elution with the same solvent and crystallization from chloroform gave ignavine (**2**) as colorless needles (83 mg), mp 172–173°; $[\alpha]_D$+47.0° (c 0.20, methanol); ir νmax (KBr) cm^{-1} 3350 (OH), 1725, 1260 (COO); ^1H nmr (CD_3OD) δ 1.17 (3H, s, C-CH_3), 4.99 (2H, broad s, C=CH_2), 5.39 (1H, broad s, *CH*OCO), 7.54 (3H, m), 7.99 (2H, dd, *J* 8, 2); ^{13}C nmr (CD_3OD) δ 25.7 (q), 25.7 (t), 30.0 (t), 34.3 (s), 36.3 (d), 39.7 (t), 42.2 (s), 43.2 (d), 45.4 (d), 51.5 (s), 52.1 (t), 62.4 (t), 65.7 (d), 71.3 (d), 73.0 (d), 74.7 (d), 75.8 (d), 80.1 (s), 110.2 (t), 129.8 (d, C x 2), 130.2 (d, C x 2), 130.4 (s), 134.4 (d), 155.6 (s), 166.8 (s); ms *m/e* 449 (M^+). Identification was performed by comparison of mass, ir and ^1H nmr spectra, and tlc.

The ethyl acetate-methanol (1:1) eluate from previous alumina chromatography was submitted to silica gel chromatography. Elution with ethyl acetate-methanol (4:1) and crystallization from methanol yielded senbusine C as colorless needles (11.5 mg), mp 193–194°; $[\alpha]_D$+6.7° (c 0.21, methanol); ir νmax (KBr) cm^{-1} 3250 (OH); ^1H nmr δ 1.12 (3H, t, *J* 7), 3.32 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 4.40 (1H, d, *J* 7, $C_{(15)}H$); ms *m/e* 453 (M^+). Identification was carried out by mass, ir, ^1H and ^{13}C nmr spectra, and tlc comparison.

Successive elution with methanol and crystallization from methanol gave hokbusine B (**4**) as colorless prisms (31 mg), mp 183–185°; ir νmax (KBr) cm^{-1} 3460 (OH,NH), 1730, 1237 (COO); ^1H nmr (CD_3OD) δ 0.75 (3H, s, C-CH_3), 1.94 (3H, s, CH_3CO), 3.16 (3H, s, OCH_3), 3.69 (1H, m, $C_{(1)}H$), 4.69 (1H, t, *J* 5, $C_{(14)}H$); ^{13}C nmr data shown in table 1; ms *m/e* 391 (M^+).

ACETYLATION OF HOKBUSINE A.—Hokbusine A (5 mg) in acetic anhydride (0.1 ml) and pyridine (0.3 ml) was kept at room temperature for 10 days. After usual working up, the product was submitted to silica gel chromatography (10 g). Elution with chloroform-methanol (4:1) afforded hokbusine A 3,15-diacetate (**6**) as a colorless powder (3 mg), ir νmax (KBr) cm^{-1} 3450 (OH), 1745 (CO), 1740 (CO), 1720 (CO), 1235 (COO); ^1H nmr δ 2.01 (3H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 2.37 (3H, s, N-CH_3), 2.86 (3H, s, OCH_3), 3.14 (3H, s, OCH_3), 3.21 (6H, s, OCH_3 x 2), 3.48 (3H, s, OCH_3), 4.01 (1H, d, *J* 7, $C_{(16)}H$), 4.80 (1H, d, *J* 5, $C_{(14)}H$), 4.94 (1H, t, *J* 7, $C_{(3)}H$), 5.93 (1H, d, *J* 7, $C_{(15)}H$), 7.45 (3H, m), 8.03 (2H, dd, *J* 8, 2); fd-ms *m/e* 687 (M^+).

METHYLATION OF HOKBUSINE A.—To a solution of hokbusine A (10 mg) in dimethyl sulfoxide (1 ml), methylsulfinium carbanion prepared from sodium hydride (55 mg) and dimethyl sulfoxide (1 ml) was added. After the solution was stirred at room temperature for 2 hrs, methyl iodide (1 ml) was added. The reaction mixture was further stirred at room temperature for 2 hrs. All procedures were carried out under nitrogen atmosphere. After the addition of water, the

[2]Melting points were determined on a hot stage and are uncorrected. ^1H and ^{13}C nmr spectra were measured in chloroform-d at 100 and 25 MHz, respectively, unless stated otherwise. Chemical shifts (δ) are expressed in ppm downfield from TMS as internal standard and coupling constants (*J*) in Hz. Abbreviations: s=singlet, d=doublet, t=triplet, q=quadruplet, m=multiplet, dd=doublet of doublets.

mixture was extracted 5 times with chloroform (10 ml each). The extract was washed with water and dried over anhydrous sodium sulfate. The filtrate was evaporated. The residue was methylated two more times under the same conditions. The final residue was chromatographed over silica gel (10 g). Elution with chloroform-methanol (4:1) afforded deacylhokbusine A tetrametyl ether (7) as a colorless powder (3.3 mg), ir νmax (KBr) cm^{-1} 1095 (C–O–C); ^1H nmr δ 2.32 (3H, s, N-CH_3), 3.28 (3H, s, OCH_3), 3.29 (3H, s, OCH_3), 3.33 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.37 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 3.51 (3H, s, OCH_3), 3.62 (3H, s, OCH_3), 4.03 (1H, d, *J* 6, $C_{(16)}H$), 4.12 (1H, d, *J* 6, $C_{(15)}H$); ms *m/e* 555 (M^+). Identification with mesaconine pentamethyl ether (7) prepared by methylation of benzoylmesaconine under the same conditions as hokbusine A was carried out by comparison of glc (1.5% OV-101 and 3% OV-1), mass, ir and ^1H nmr spectra.

ETHYLATION OF HOKBUSINE B.—Ethyl iodide (0.1 ml) and potassium carbonate (50 mg) were added to a solution of hokbusine B (9 mg) in anhydrous acetone (3 ml). The mixture was refluxed for 1.5 hrs. After sodium thiosulfate was added, the mixture was diluted with water and extracted with chloroform (10 ml x 5). The combined extract was washed with water, dried over anhydrous sodium sulfate and concentrated to dryness. The residue was subjected to alumina chromatography (30 g). Elution with benzene-chloroform (1:1) deposited *N*-ethylhokbusine B (**10**) as colorless needles (4.6 mg), ir νmax cm^{-1} 3500 (OH), 1740, 1240 (COO); ^1H nmr δ 0.88 (3H, s, CH_3), 1.12 (3H, t, *J* 7, CH_2CH_3), 2.05 (3H, s, CH_3CO), 3.25 (3H, s, OCH_3), 3.68 (2H, m), 4.85 (1H, t, *J* 4, $C_{(14)}H$); ms *m/e* 419 (M^+).

ACETYLATION OF *N*-ETHYLHOKBUSINE B.—*N*-Ethylhokbusine B (**10**) (1.5 mg) in acetic anhydride (0.1 ml) and pyridine (0.3 ml) was set aside at room temperature overnight. The reaction mixture was worked up in the usual ways and the product was submitted to alumina chromatography (20 g). Elution with chloroform yielded *N*-ethylhokbusine B monoacetate (**11**) as colorless needles (1.5 mg) in νmax cm^{-1} 3450 (OH), 1738, 1720, 1238 (COO); ^1H nmr δ 0.80 (3H, s, CH_3), 1.09 (3H, t, *J* 7, CH_2CH_3), 2.03 (3H, s, CH_3CO), 2.04 (3H, s, CH_3CO), 3.24 (3H, s, OCH_3), 4.85 (2H, m); ms *m/e* 461 (M^+). The identity with karacoline 1,14-diacetate prepared from karacoline was confirmed by comparison of mass, ir and ^1H nmr spectra, and tlc.

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