STRUCTURE OF SENBUSINE A, B AND C, DITERPENIC ALKALOIDS OF *ACONITUM CARMICHAELI* ROOTS FROM CHINA[1]

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ABSTRACT.—The study of *Aconitum carmichaeli* roots from China led to the isolation of ten alkaloids. Four of them have been identified as hypaconitine, aconitine, mesaconitine and talatizamine, which are known constituents of this plant. Among the remaining six alkaloids, three have been identified as isotalatizidine (**1**), karacoline (**2**) and monoacetyltalatizamine (**3**), which have been isolated from other *Aconitum* species. The stereostructures of the three new alkaloids, which have been designated as senbusine A, B and C, have been deduced as those represented by formulas, **4**, **5** and **6**, respectively, on the basis of chemical and physico-chemical evidence.

The crude drug "bushi", prepared from the roots of certain species of *Aconitum* plants (Ranunculaceae), is an indispensable material in Oriental medicine. In particular, *A. carmichaeli* DEBEAUX is native to China and is cultivated in China and Japan. Because the crude drug "sen-bushi", produced from this species in China, is utilized for medicinal purposes in a large quantity in China and Japan, we are interested in its alkaloidal constituents. Generally *Aconitum* roots contain the diterpenoid alkaloids of the strongly toxic aconitine series and of the weakly toxic atisine series (1). As for the alkaloidal constituents of *A. carmichaeli*, Chen *et al.* (2) reported the isolation of hypaconitine, aconitine, mesaconitine, talatizamine and two novel alkaloids, chuan-wu-base A ($C_{23}H_{37}NO_6$, mp 111°) and chuan-wu-base B ($C_{22}H_{35}NO_4$, mp 185°). The two novel alkaloids were not chemically characterized. Iwasa and Naruto (3) also reported the presence of carmichaeline ($C_{22}H_{35}NO_4$, mp 185–186°), whose structure was not determined, together with hypaconitine, aconitine and mesaconitine.

During the course of our chemical and pharmacological investigation of the crude drug "bushi", we have carried out a survey of alkaloids of "sen-bushi", the roots of *A. carmichaeli* from China, for the purpose of clarification.

The basic portion of the methanol extract of the crude drug was subjected to alumina and silica gel chromatography to yield ten alkaloids. Identification of the known constituents, hypaconitine, aconitine, mesaconitine and talatizamine, was easily determined by their physico-chemical properties.

This paper concerns the identification of three of the remaining six alkaloids, isotalatizidine (**1**), karacoline (**2**) and monoacetyltalizamine (**3**), which have not been reported from this plant, and the structure determination of the remaining three new alkaloids, now designated as senbusine A, B and C, as in formulas **4**–**6**.

Alkaloid **1** had the molecular formula $C_{23}H_{37}NO_5$. The ir spectrum showed a strong band at 3350 cm^{-1} due to hydroxyls. In the ^1H nmr spectrum, there were a 3H triplet at δ 1.12 for a methyl of an N-ethyl group and two 3H singlets at δ 3.32 and 3.36 for two O-methyl groups. This evidence, coupled with biogenetic considerations, led to the assumption that this alkaloid may have the aconitine skeleton. The ^1H nmr spectrum also displayed two 1H signals at δ 3.03 and 3.18 in an AB pattern attributed to an oxygenated 18-methylene, and two 1H signals at δ 3.74 and 4.19 due to two oxygenated methines which were assigned to $H_{(1\beta)}$ and $H_{(14\beta)}$, respectively, from the chemical shifts and splitting patterns (4). The remaining oxygen functions of this alkaloid were concluded to be a methoxyl and a tertiary hydroxyl group based on the ir and nmr data of its diacetate. Since alkaloids which possess the aconitine skeleton generally have a 16β-methoxyl and

[1]Pharmaceutical studies on *Aconitum* roots—Part 9. This paper is also Part 27 in the series on the validity of the Oriental medicines.

a 8-hydroxyl group, it was considered that this alkaloid also has a 16β-methoxyl and a 8-hydroxyl group. On the basis of the above evidence, this alkaloid was deduced to be isotalatizidine (**1**) (4), which has been isolated from *A. talassicum* Popov. This deduction was further supported by the fact that the ^{13}C nmr spectrum of this alkaloid (table 1) is in accord with that of isotalatizidine (5). The identification as isotalatizidine was carried out by direct comparison with an authentic sample.

Table 1. Carbon-13 shieldings in the *Aconitum* alkaloids and their derivatives.

	1	**2**	deacyl-**3**	**3**	**4**	**4** tri-acetate	**5**	**6**	**6** tri-acetate
C-1	72.2 d	72.6 d	86.1 d	85.8 d	72.1 d	73.7 d	72.1 d	72.1 d	75.1 d
C-2	29.0 t	29.1 t	25.7 t	26.2 t	29.2 t*	27.6 t	29.2 t*	29.3 t*	27.6 t*
C-3	29.6 t	31.5 t	32.6 t	32.7 t	29.8 t*	29.2 t	29.6 t*	29.9 t*	29.7 t*
C-4	37.2 s	33.1 s	38.6 s	38.6 s	37.9 s	38.7 s	37.2 s	38.1 s	38.8 s
C-5	41.5 d	47.0 d	37.7 d	35.4 d	48.2 d	48.7 d	40.9 d	44.1 d	43.9 d
C-6	24.9 t	25.4 t	24.8 t	25.0 t	72.6 d	74.3 d	24.8 t	84.1 d	85.1 d
C-7	45.2 d	45.4 d	45.7 d*	45.4 d*	55.4 d	54.4 d	46.8 d	49.5 d	49.5 d
C-8	74.3 s	74.6 s	72.7 s	73.7 s	75.6 s	75.8 s	79.0 s	79.1 s	76.4 s
C-9	46.6 d	47.0 d	46.9 d*	46.3 d*	45.6 d	46.8 d	47.9 d	46.7 d	45.0 d
C-10	40.2 d	40.5 d	45.7 d*	45.0 d*	40.6 d	37.2 d	40.7 d	40.7 d	37.7 d
C-11	48.6 s	49.1 s	48.6 s	48.8 s	48.2 s	49.3 s	48.7 s	49.3 s	49.0 s
C-12	26.7 t	29.9 t	28.6 t	28.5 t	29.9 t	29.2 t	26.4 t	29.9 t	29.7 t
C-13	44.0 d	44.3 d	45.7 d*	45.0 d*	44.2 d	44.4 d	44.0 d	43.6 d	43.9 d
C-14	75.6 d	75.9 d	75.7 d	77.0 d	75.4 d	77.3 d	75.4 d	75.6 d	77.7 d
C-15	42.3 t	42.5 t	39.2 t	41.0 t	42.2 t	39.7 t	77.6 d	78.6 d	82.8 d
C-16	82.2 d	82.5 d	82.2 d	81.7 d	82.4 d	82.4 d	90.5 d	90.5 d	87.9 d
C-17	63.9 d	63.5 d	62.8 d	62.2 d	63.5 d	61.3 d	63.2 d	62.6 d	60.1 d
C-18	78.9 t	27.8 q	79.4 t	79.7 t	80.3 t	80.2 t	79.0 t	80.0 t	80.2 t
C-19	56.4 t	60.5 t	53.1 t	53.1 t	57.1 t	55.0 t	56.5 t	56.8 t	54.1 t
C-20	48.6 t	48.6 t	49.4 t	49.4 t	49.7 t	49.6 t	48.7 t	48.5 t	48.8 t
C-21	13.0 q	13.3 q	13.6 q	13.6 q	12.9 q	13.4 q	12.8 q	13.0 q	13.3 q
1-OCH_3			56.1 q	56.1 q					
6-OCH_3								58.5 q	57.7 q
16-OCH_3	56.3 q	56.6 q	56.3 q	56.1 q	56.3 q	56.2 q	57.4 q	57.5 q	57.1 q
18-OCH_3	59.4 q		59.3 q	59.5 q	59.2 q	59.3 q	59.4 q	59.1 q	59.1 q
$COCH_3$				170.7 s		170.1 s			170.1 s
						171.0 s			170.8 s
						171.0 s			173.6 s
$COCH_3$				21.4 q		21.4 q			21.0 q
						21.7 q			21.3 q
						22.0 q			22.0 q

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

Alkaloid **2** had the molecular formula $C_{22}H_{35}NO_4$. The ^1H nmr spectrum was quite similar to that of isotalatizidine, but the 18-methylene signals at δ 3.03 and 3.18 were coupled in an AB manner, and a methoxyl signal observed in isotalatizidine at δ 3.32 was absent. Instead a 3H singlet due to a tertiary methyl appeared at δ 0.88, suggesting this alkaloid to be the 18-demethoxy-derivative of isotalatizidine. This was further supported by the fact that in the ^{13}C nmr spectrum of this alkaloid (table 1), the methoxy signal (δ 59.4) and 18-methylene signal (δ 78.9) observed in the spectrum of isotalatizidine were absent in this alkaloid; instead a methyl signal (δ 27.8) was present. Hence this alkaloid was suspected to be karacoline (**2**) (6) isolated from *A. karakolicum* Rapaics. Confirmation was provided by direct comparison with an authentic sample. Further, carmichaeline was directly compared with karacoline to reveal that both the substances were identical. It is also believed that Chen's chuan-wu-base B is the same as karacoline by data comparison.

Alkaloid **3** possessed the composition $C_{26}H_{41}NO_6$. The ir spectrum showed a band at 3410 cm^{-1} due to a hydroxyl and bands at 1722 and 1202 cm^{-1} due to an ester. The ^1H nmr spectrum exhibited the presence of an acetoxyl group (δ 2.06) and a methine bearing an acetoxyl group (δ 4.80). From the chemical shift and

coupling constants, the methine signal was assigned to $H_{(14\beta)}$. Alkaline hydrolysis of this alkaloid gave a deacyl derivative whose ^1H nmr spectrum was the same as that of talatizamine. The ^{13}C nmr spectrum (table 1) was also found to be essentially identical with that of monoacetyltalatizamine reported by Sakai *et al.* (7), although certain assignments are different. The deacyl derivative was thus identified as talatizamine by direct comparison with an authentic sample. Hence this alkaloid was deduced to be monoacetyltalatizamine (**3**), which has been isolated from *A. nemorum* Popov (8).

Senbusine A showed in the high resolution mass spectrum the molecular ion peak at *m/e* 423.2620, indicating it to have the composition $C_{23}H_{37}NO_6$. This demonstrated that senbusine A had one more oxygen atom than isotalatizidine. The ir spectrum revealed a strong band at 3380 cm^{-1} due to hydroxyls. In the ^1H nmr spectrum, a 3H triplet at δ 1.14 for a methyl of an N-ethyl, a 6H singlet at δ 3.34 for two methoxyls and a 1H triplet at δ 4.20 for a methine bearing an oxygen function were visible. The ^1H nmr spectrum was found to be very similar to that of isotalatizidine, suggesting senbusine A to be a derivative of isotalatizidine to which one hydroxyl has been introduced. In order to substantiate this deduction, the ^{13}C nmr spectrum of senbusine A was compared with that of isotalatizidine (table 1). Since the parameters of all carbon signals of senbusine A, except for $C_{(5)}$–$C_{(7)}$, were consistent with those of isotalatizidine, it was thought that the stereostructure of senbusine A was identical with that of isotalatizidine except for the $C_{(5)}$–$C_{(7)}$ environment. As for the location of the newly introduced hydroxyl, it was concluded to be at $C_{(6)}$ from the fact that in the ^{13}C nmr spectrum of senbusine A, the $C_{(6)}$ signal appeared at a distinctly lower field (δ 72.6) than that of isotalatizidine (δ 24.9). In order to confirm the location of the hydroxyl at $C_{(6)}$ as well as to determine the orientation of the hydroxyl, senbusine A was acetylated to afford the triacetate. In the ^{13}C nmr spectrum, the signals for $C_{(1)}$, $C_{(2)}$, $C_{(6)}$, $C_{(7)}$ and $C_{(14)}$ showed shifts (+1.6, −1.6, +1.7, −1.0 and +1.9 ppm, respectively) as compared with those in senbusine A (table 1), verifying the situation of acetylatable hydroxyls at $C_{(1)}$ and $C_{(14)}$ as well as at $C_{(6)}$. Comparison of the ^{13}C nmr spectrum of senbusine A with that of its triacetate revealed that the chemical shifts of the signals attributed to $C_{(10)}$, $C_{(15)}$, $C_{(17)}$ and $C_{(19)}$ were significantly displaced on acetylation of the hydroxyls at $C_{(1)}$, $C_{(6)}$ and $C_{(14)}$ (table 1). Although similar shifts were also found in the ^{13}C nmr spectra of senbusine C and its triacetate (table 1), which will be discussed below, the cause of the displacements has not been clarified. The ^1H nmr spectrum of senbusine A triacetate demonstrated the occurrence of the signals for three acetoxyl methyls at δ 2.04, 2.04 and 2.10 and for three hydrogens on carbons carrying acetoxyls at δ 4.76, 4.82 and 5.66. Comparison of the spectrum with that of isotalatizidine diacetate revealed from the chemical shifts and splitting patterns that the signals at δ 4.76 and 4.82 in the former could be attributed to the $H_{(14\beta)}$ and $H_{(1\beta)}$, respectively. The remaining carbinyl hydrogen signal at δ 5.66, therefore, was assigned to the $H_{(6)}$. This carbinyl hydrogen signal showed a pyridine-induced solvent shift by −0.41 ppm on passing from chloroform to pyridine, a fact which indicates that the $H_{(6)}$ is located close to the 8-hydroxyl (9). Hence the 6-hydrogen is β-oriented, and the 6-hydroxyl is α-oriented.

Senbusine B exhibited a molecular ion peak at *m/e* 423 in its mass spectrum, indicating that it possesses the composition $C_{23}H_{37}NO_6$. The ir spectrum displayed bands at 3350 and 3260 cm^{-1} due to hydroxyls. The parameters of the ^1H nmr signals were similar to those of isotalatizidine. Thus, there were a 3H triplet at δ 1.13 for a methyl in an N-ethyl, a 2H multiplet of δ 3.12 for the 18-methylene, two 3H singlets at δ 3.33 and 3.45 for two methoxyls, and a 1H triplet at δ 4.11 for an oxygenated methine. The signal at δ 4.11 was assigned to $H_{(14\beta)}$ from the chemical shift and coupling pattern. The ^1H nmr spectrum of senbusine B further showed a 1H doublet at δ 4.37 for a hydrogen on carbon-bearing

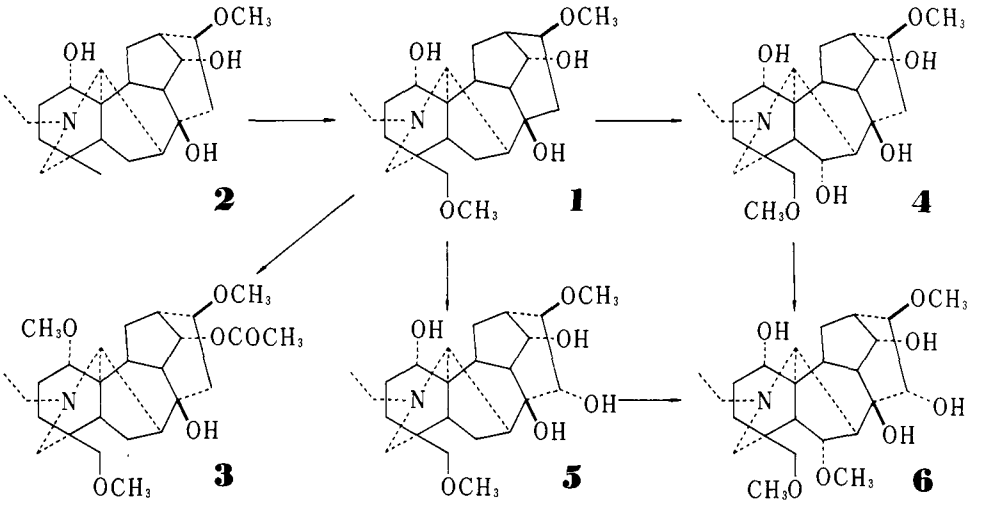
oxygen. From the above ^{1}H nmr data together with the molecular formula, it was deduced that senbusine B is a derivative of isotalatizidine to which one secondary hydroxyl group has been introduced. In the ^{13}C nmr spectrum (table 1), the parameters of the signals for $C_{(1)}$–$C_{(7)}$, $C_{(9)}$–$C_{(14)}$ and $C_{(17)}$–$C_{(21)}$ were in accord with those of the corresponding signals of isotalatizidine. However, the parameters of the signals for $C_{(8)}$, $C_{(15)}$ and $C_{(16)}$ of senbusine B differed from those of isotalatizidine, especially $C_{(15)}$ (δ 42.3 in isotalatizidine and δ 77.6 in senbusine B). On the basis of the above evidence, the stereostructure of senbusine B is concluded to be 15-hydroxy-isotalatizidine (**5**) except for the configuration of the 15-hydroxyl.

Senbusine C exhibited a molecular ion peak at m/e 453.2757 in the high resolution mass spectrum, indicating it to have the composition $C_{24}H_{39}NO_7$. The ir spectrum showed an intense band at 3400 cm^{-1} due to hydroxyls. In the ^{1}H nmr spectrum there were a triplet at δ 1.14 for a methyl of an N-ethyl, a multiplet at δ 3.17 for the 18-methylene, two singlets at δ 3.35 (6H) and 3.45 (3H) for three methoxyls, a triplet at δ 4.15 for the 14-methine, and a doublet at δ 4.42 for an oxygenated methine. The signal at δ 4.42 was assigned to the 15-methine by comparison of its chemical shift and splitting pattern with those of the ^{1}H nmr signal (δ 4.37) of the 15-methine in senbusine B. The ^{1}H nmr spectrum, on the whole, was very similar to that of senbusine B except for the signals associated with methoxyls. The above nmr data along with the molecular formula indicated that senbusine C was a derivative of senbusine B containing an additional methoxyl group. In the ^{13}C nmr spectrum (table 1), an extra methoxyl signal was observed compared with that of senbusine B. The parameters of all carbon signals except for $C_{(5)}$–$C_{(7)}$ were in good agreement with those of senbusine B, suggesting that the stereostructure of senbusine C (except for the $C_{(5)}$–$C_{(7)}$ environment) was identical with that of senbusine B. In order to substantiate this deduction, senbusine C was acetylated to give the triacetate. Inspection of the ^{13}C nmr spectra of senbusine C and its triacetate (table 1) demonstrated that the signals due to $C_{(1)}$, $C_{(2)}$, $C_{(9)}$, $C_{(14)}$, $C_{(8)}$, $C_{(15)}$ and $C_{(16)}$ exhibited shifts (+3.0, −1.7, −1.7, +2.1, −2.7, +4.2 and −2.6 ppm, respectively), indicating senbusine C to possess hydroxyls at $C_{(1)}$, $C_{(14)}$ and $C_{(15)}$. As for the location of a newly introduced methoxyl group, it was concluded that the methoxyl is situated at $C_{(6)}$ because the $C_{(6)}$ signal appeared at a distinctly lower field (δ 84.1) than that of senbusine B (δ 24.8). Regarding stereochemistry, the methoxyl group was deduced to have α-orientation from the fact that the parameters for the $C_{(5)}$–$C_{(6)}$ signals were in accord with those of aconitine (5). On the basis of the above evidence, it was concluded that senbusine C is represented by formula **6** with the exception of the configuration at $C_{(15)}$.

Next, the configurations of the 15-hydroxyls in senbusine B and senbusine C were examined. As has been mentioned above, the 15-carbinyl hydrogen signals in senbusine B and senbusine C appeared as the doublets (J 6 Hz) at approximately the same position (δ 4.37 and 4.42). In the ^{13}C nmr spectra of senbusine B and senbusine C, the chemical shifts of the signals due to $C_{(8)}$, $C_{(9)}$ and $C_{(13)}$–$C_{(16)}$ were essentially identical in both the substances. These data indicated that the configurations of the 15-hydroxyls were the same in senbusine B and senbusine C. The ^{1}H nmr spectrum of senbusine C triacetate disclosed the three carbinyl hydrogen signals at δ 4.74, 4.82 and 5.13 in which the former two were allocated to $H_{(1\beta)}$ and $H_{(14\beta)}$ by comparison of their chemical shifts and splitting patterns with those of the corresponding signals in isotalatizidine diacetate. Consequently, the remaining signal at δ 5.13 was to be assigned to $H_{(15)}$. This signal showed a pyridine-induced solvent shift by −0.76 ppm on passing from chloroform to pyridine, exhibiting this 15-hydrogen to be situated close to the 8-hydroxyl (9). Therefore, the orientation of the 15-hydrogen was concluded to be β, and the configurations of the 15-hydroxyls in senbusine B and senbusine C were deduced to be α as shown in formulas **5** and **6**.

Karacoline (**2**), isotalatizidine (**1**) and senbusine A–C (**4**–**6**), which have now

been isolated from *A. carmichaeli* containing aconitine, are considered to be the intermediates in the biosynthesis of aconitine. The finding of such successively oxygenated substances in the same plant source provides a useful suggestion for the biosynthetic pathway of the aconitine alkaloids.



Arrows denote probable biosynthetic pathways.

EXPERIMENTAL[2]

ISOLATION OF THE DITERPENIC ALKALOIDS, HYPACONITINE, ACONITINE, MESACONITINE, TALATIZAMINE, ISOTALATIZIDINE, KARACOLINE, ACETYLTALATIZAMINE AND SENBUSINE A-C FROM *Aconitum carmichaeli*.—The crude drug, "sen-bushi" (34 kg), the dried roots of *Aconitum carmichaeli* from China, was extracted with methanol (70 liters x 4) for 4 days (each extraction) at room temperature. The methanol solutions were combined and concentrated under reduced pressure to give the extract (8.3 kg) which was suspended in chloroform and extracted with 1% hydrochloric acid solution. The water layer was made alkaline with ammonia and extracted with chloroform. The chloroform solution was concentrated to afford the alkaloid fraction (125 g), which was chromatographed over alumina (500 g) to give the ethyl acetate eluate and the ethyl acetate-methanol (1:1) eluate.

The ethyl acetate eluate was subjected to chromatography over alumina.

Elution with ethyl acetate and crystallization from ethyl acetate furnished hypaconitine, mp 185–187°. Identification was based on mixed melting points and comparison of tlc and ir spectra.

Successive elution with the same solvent and crystallization from ethyl acetate yielded aconitine, mp 195–197°. The identity was confirmed by the usual criteria.

Subsequent elution with the same solvent and crystallization from ethyl acetate afforded mesaconitine, mp 197–199°. Identification was carried out in the customary manner.

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

Elution with chloroform-methanol (93:7) and crystallization from ether-*n*-hexane (1:1) gave isotalatizidine (**1**) as colorless needles (4.3 g), mp 115–118°; ir ν max (KBr) cm^{-1} 3350 (hydroxyls); ^1H nmr δ 1.12 (3H, t, *J* 7, $C_{(21)}H_3$), 3.03, 3.18 (1H each, d, *J* 9, $C_{(18)}H_2$), 3.32 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 3.74 (1H, m, $C_{(1)}H$), 4.19 (1H, t, *J* 4.5, $C_{(14)}H$); ^{13}C nmr data shown in table 1; ms *m/e* 407 (M^+).

Anal. Calc. for $C_{23}H_{37}NO_5$: C, 67.78; H, 9.15; N, 3.44. Found: C, 67.96; H, 9.07; N, 3.30.

It was identified as an authentic specimen by mixed melting points and comparison of tlc and ir spectra.

Subsequent elution with chloroform-methanol (9:1) and crystallization from ethyl acetate yielded karacoline (**2**) as colorless prisms (1.6 g), mp 188–189°; ir ν max (KBr) cm^{-1} 3560, 3270 (hydroxyls); ^1H nmr δ 0.88 (3H, s, $C_{(18)}H_3$), 1.11 (3H, t, *J* 7, $C_{(21)}H_3$), 3.35 (3H, s, OCH_3), 3.70 (1H, m, $C_{(1)}H$), 4.18 (1H, t, *J* 4.5, $C_{(14)}H$); ^{13}C nmr data shown in table 1; ms *m/e* 377 (M^+).

Anal. Calc. for $C_{22}H_{35}NO_4$: C, 69.99; H, 9.35; N, 3.71. Found: C, 69.52; H, 9.71; N, 3.87.

The identity with an authentic specimen of karacoline was confirmed by mixed melting points and tlc and ir comparison. It was also identified as carmichaeline by the usual criteria.

Successive elution with chloroform-methanol (4:1) afforded acetyltalatizamine (**3**) as

[2]Melting points were determined on a hot stage and are uncorrected. ^1H and ^{13}C nmr spectra were taken in chloroform-d_3 at 100 and 25 MHz, respectively, unless stated otherwise. Chemical shifts (δ) are expressed in ppm downfield from TMS as internal reference and coupling constants (*J*) in Hz. Abbreviations: s=singlet, d=doublet, t=triplet, m=multiplet, dd=doublet of doublets.

colorless amorphous powder (0.82 g), ir ν max ($CHCl_3$) cm^{-1} 3410 (hydroxyl), 1722, 1202 (ester); ^1H nmr δ 1.06 (3H, t, J 7, $C_{(21)}H_3$), 2.06 (3H, s, $OCOCH_3$), 3.02 (2H, m, $C_{(18)}H_2$), 3.23 (3H, s, OCH_3), 3.27 (3H, s, OCH_3), 3.30 (3H, s, OCH_3), 4.80 (1H, t, J 4.5, $C_{(14)}H$); ^{13}C nmr data shown in table 1; ms m/e 464 (M^++1).

Elution with chloroform-methanol (3:1) and crystallization from methanol gave talatizamine as colorless prisms (1.2 g), mp 144–146°. Identification was performed by the usual criteria.

Successive elution with the same solvent yielded senbusine A (**4**) as colorless amorphous powder (0.086 g), ir ν max (KBr) cm^{-1} 3380 (hydroxyls); ^1H nmr δ 1.14 (3H, t, J 7, $C_{(21)}H_3$), 3.34 (6H, s, 2 x OCH_3) 4.20 (1H, t, J 4.5, $C_{(14)}H$) 4.74 (1H, m); ^{13}C nmr data shown in table 1; ms m/e 423.2620 (M^+).

Subsequent elution with the same solvent afforded senbusine C (**6**) as colorless amorphous powder (0.052 g), ir ν max (KBr) cm^{-1} 3400 (hydroxyls); ^1H nmr δ 1.04 (3H, t, J 7, $C_{(21)}H_3$), 3.14 (2H, m, $C_{(18)}H_2$), 3.35 (6H, s, 2 x OCH_3), 3.45 (3H, s, OCH_3), 4.15 (1H, t, J 4.5, $C_{(14)}H$), 4.42 (1H, d, J 6, $C_{(15)}H$); ^{13}C nmr data shown in table 1; ms m/e 453.2757 (M^+).

Further elution with the same solvent furnished senbusine B (**5**) as colorless amorphous powder (0.032 g), ir ν max (KBr) cm^{-1} 3350, 3260 (hydroxyls); ^1H nmr δ 1.13 (3H, t, J 7, $C_{(21)}H_3$), 3.12 (2H, m, $C_{(18)}H_2$), 3.33 (3H, s, OCH_3), 3.45 (3H, s, OCH_3), 4.11 (1H, t, J 4.5, $C_{(14)}H$), 4.37 (1H, d, J 6, $C_{(15)}H$); ^{13}C nmr data shown in table 1; ms m/e 423 (M^+).

ACETYLATION OF ISOTALATIZIDINE.—Isotalatizidine (136 mg) in acetic anhydride (2 ml) and pyridine (4 ml) was set aside at room temperature overnight. After isolation in the usual manner, the product was chromatographed over alumina (30 g). Elution with benzene-chloroform (3:1) afforded isotalatizidine 1,14-diacetate as colorless amorphous powder (68 mg), ir ν max (KBr) cm^{-1} 3440 (hydroxyl), 1725, 1228 (ester); ^1H nmr δ 1.07 (3H, t, J 7, $C_{(21)}H_3$), 2.02 (6H, s, 2 x $OCOCH_3$), 3.19 (3H, s, OCH_3), 3.26 (3H, s, OCH_3), 4.74 (1H, t, J 4.5, $C_{(14)}H$), 4.88 (1H, dd, J 7, 10, $C_{(1)}H$); ms m/e 432 (M^+–CH_3COO).

ALKALINE HYDROLYSIS OF ACETYLTALATIZAMINE.—Acetyltalatizamine (120 mg) in 5% methanolic K_2CO_3 solution (2 ml) was left standing at room temperature overnight. After it was worked up in the customary way, the product was chromatographed over silica gel (30 g). Elution with chloroform-methanol (3:1) and crystallization from methanol furnished the deacyl derivative as colorless prisms (67 mg), mp 144–146°; ir ν max ($CHCl_3$) cm^{-1} 3400 (hydroxyls); ^1H nmr δ 1.08 (3H, t, J 8, $C_{(21)}H_3$), 3.00, 3.12 (1H each, d, J 9, $C_{(18)}H_2$), 3.28 (3H, s, OCH_3), 3.30 (3H, s, OCH_3), 3.36 (3H, s, OCH_3), 4.13 (1H, t, J 4.5, $C_{(14)}H$); ^{13}C nmr data shown in table 1; ms m/e 421 (M^+). The identity with talatizamine was corroborated in the usual criteria.

ACETYLATION OF SENBUSINE A.—Senbusine A (85 mg) in acetic anhydride (0.5 ml) and pyridine (1 ml) was kept at room temperature overnight. After the usual working up, the product was subjected to silica gel chromatography (15 g). Elution with chloroform-methanol (19:1) gave senbusine A 1,6,14-triacetate as colorless amorphous powder (42 mg), ir ν max (KBr) cm^{-1} 3470 (hydroxyl), 1733, 1235 (ester); ^1H nmr δ 1.12 (3H, t, J 7, $C_{(21)}H_3$), 2.04 (6H, s, 2 x $OCOCH_3$), 2.10 (3H, s, $OCOCH_3$), 3.24 (3H, s, OCH_3), 3.28 (3H, s, OCH_3), 4.76 (1H, t, J 4.5, $C_{(14)}H$), 4.82 (1H, dd, J 7, 10, $C_{(1)}H$), 5.66 (1H, d, J 7, $C_{(6)}H$); ^1H nmr (pyridine-d_5) δ 1.12 (3H, t, J 7, $C_{(21)}H_3$), 2.15 (3H, s, $OCOCH_3$), 2.17 (3H, s, $OCOCH_3$), 2.19 (3H, s, $OCOCH_3$), 3.28 (3H, s, OCH_3), 3.32 (3H, s, OCH_3), 5.01 (1H, dd, J 7, 10, $C_{(1)}H$), 5.07 (1H, t, J 4.5, $C_{(14)}H$), 6.07 (1H, d, J 7, $C_{(6)}H$); ^{13}C nmr data shown in table 1; ms m/e 548 (M^+–1).

ACETYLATION OF SENBUSINE C.—Senbusine C (40 mg) in acetic anhydride (0.5 ml) and pyridine (1 ml) was set aside at room temperature overnight. After isolation in the customary way, the product was chromatographed over silica gel (15 g). Elution with chloroform-methanol (19:1) gave senbusine C 1,14,15-triacetate as colorless amorphous powder (38 mg), ir ν max (KBr) cm^{-1} 3490 (hydroxyl), 1734, 1239 (ester); ^1H nmr δ 1.13 (3H, t, J 7, $C_{(21)}H_3$), 2.04 (3H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 2.20 (3H, s, $OCOCH_3$), 3.28 (6H, s, 2 x OCH_3), 3.36 (3H, s, OCH_3), 4.74 (1H, dd, J 7, 10, $C_{(1)}H$), 4.82 (1H, t, J 4.5, $C_{(14)}H$), 5.13 (1H, d, J 7, $C_{(15)}H$); ^1H nmr (pyridine-d_5) δ 1.20 (3H, t, J 7, $C_{(21)}H_3$), 2.04 (6H, s, 2 x $OCOCH_3$), 2.08 (3H, s, $OCOCH_3$), 3.31 (3H, s, OCH_3), 3.33 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 5.01 (1H, dd, J 7, 10, $C_{(1)}H$), 5.10 (1H, t, J 4.5, $C_{(14)}H$), 5.89 (1H, d, J 7, $C_{(15)}H$); ^{13}C nmr data shown in table 1; ms m/e 579 (M^+), 520 (M^+–CH_3COO).

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