Journal of Natural Products
Vol. 58, No. 5, pp. 804-806, May 1995

REVISED STRUCTURE OF PSEUDODISTOMIN A, A PIPERIDINE ALKALOID ISOLATED FROM THE OKINAWAN TUNICATE *PSEUDODISTOMA KANOKO*

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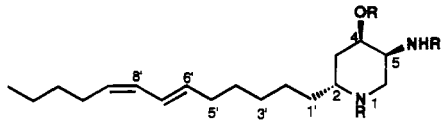
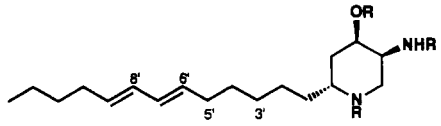
ABSTRACT.—Pseudodistomin A [**1**], a piperidine alkaloid isolated from the Okinawan tunicate *Pseudodistoma kanoko*, was previously assigned with a 3′*E*,5′*Z*-diene in the side-chain; it is now shown to possess a 6′*E*,8′*Z*-diene. Ozonolysis of the triacetate of **1** afforded the same product as that obtained from the triacetate of the 6′,8′-diene pseudodistomin B [**2**], indicating that the diene position of **1** is the same as that of **2**.

During our studies on bioactive substances from marine organisms, we previously isolated two piperidine alkaloids, pseudodistomins A [**1**] and B [**2**], from extracts of the Okinawan tunicate *Pseudodistoma kanoko* Tokioka & Nishikawa (family Polyclinidae) (1). These compounds were the first piperidine alkaloids obtained from marine sources, and exhibited cytotoxic and calmodulin-antagonistic activity. The structures of pseudodistomins A and B were initially proposed as **1** and **2**, respectively, but with a 3′,5′-diene unit in the side-chain. The structure of the piperidine nucleus portion was unambiguously established by synthesis of tetrahydroacetyl derivatives [**3**] achieved by three research groups (racemate: 2,3; optically active form: 4,5), while the structure of the diene position of pseudodistiomin B was later revised to **2**, having a 6′,8′-diene in the side-chain (6). As a result, the diene position of pseudodistomin A was also questioned, but remained undetermined because a natural specimen was unavailable. We recently reexamined the toluene-soluble fraction of extracts of this tunicate, and succeeded in reisolating pseudodistomin A as its acetate [**4**]. This report revises the structure of pseudodistomin A, which also proved to possess a 6′,8′-diene in the side-chain on the basis of an ozonolysis experiment.



1 R=H **4** R=Ac



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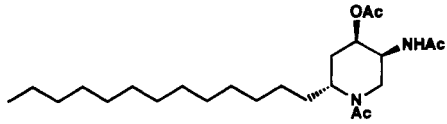

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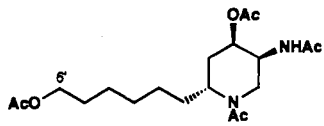

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The tunicate *P. kanoko*, collected off the Ie Islands, Okinawa, Japan, was extracted with MeOH-toluene (3:1), and the extract was partitioned between toluene and H_2O.[1] The toluene-soluble material was subjected to Si gel flash cc ($CHCl_3$-*n*-BuOH-AcOH-H_2O, 1.5:6:1:1). A part of the fraction containing the alkaloid mixture was acetylated (Ac_2O/pyridine)

[1]The H_2O-soluble fraction was further extracted with $CHCl_3$. In the previous report (1), pseudodistomins A and B were isolated from this $CHCl_3$-soluble fraction.

and the acetate mixture was purified by reversed-phase hplc (ODS, 85% MeOH) to give the acetate of pseudodistomin A [**4**] together with the acetate of pseudodistomin B [**5**] in 0.012 and 0.016% yield, respectively, based on wet wt of the tunicate.

To determine the position of its conjugated diene moiety, pseudodistomin A acetate was subjected to ozonolysis by the same procedures used for pseudodistomin B acetate [**5**] (6), namely, treatment with ozone followed by reduction with $NaBH_4$ and acetylation with Ac_2O and pyridine. The product was identical with the tetraacetate **6** previously obtained from pseudodistomin B acetate [**5**] (6), on the basis of tlc, ^{1}H-nmr, and eims analyses. From this result, the position of the diene moiety of pseudodistomin A was revised as occurring at the C-6′,C-8′ position [**1**]. In the HOHAHA nmr spectrum (mixing time: 67 msec) of pseudodistomin A acetate, the terminal methyl protons (H_3-13′) showed a clear correlation with the allylic methylene protons at δ_H 2.15 (H_2-10′), while no correlation was observed between H_3-13′ and the other allylic methylene protons at δ_H 2.07 (H_2-5′). The latter methylene (C-5′) was adjacent to an *E* olefin whereas the former methylene (C-10′) was vicinal to a *Z* olefin, which was revealed by the ^{1}H-^{1}H COSY nmr spectrum and coupling constant data of **4** (cross-peaks: H_2-5′/H-6′, H_2-5′/H-7′, H_2-10′/H-8′, and H_2-10′/H-9′; $J_{6',7'}$=15 Hz and $J_{8',9'}$=11 Hz). Pseudodistomin A [**1**] must therefore have a 6′*E*,8′*Z*-diene in the side-chain. In Table 1, the revised assignments of ^{1}H-nmr data of pseudodistomin A acetate [**4**] are described together with those of pseudodistomin B acetate [**5**].

EXPERIMENTAL

ANIMAL MATERIAL.—The tunicate *Pseudodistoma kanoko* was collected off the Ie Islands (at depths of 5 to 10 m), Okinawa, Japan, in 1985, and

TABLE 1. Revised ^{1}H-Nmr Assignments for Pseudodistomin A and B Acetates [**4** and **5**].

Position	Compound	
	4	**5**
H-2	4.93 br s	4.86 br s
H-2[a]	4.00 br s	3.97 br s
H_2-3	1.75 m	1.74 m
H-4	5.15 m	5.11 m
H-5	4.31 br s	4.34 br s
H-5[a]	4.51 br s	4.47 br s
H-6_{ax}	3.27 br d (J=14.5 Hz)	3.26 br d (J=14.5 Hz)
H-6_{ax}[a]	2.91 br d (J=14.5 Hz)	2.88 br d (J=14.5 Hz)
H-6_{eq}	3.97 br d (J=14.5 Hz)	3.87 br d (J=14.5 Hz)
H-6_{eq}[a]	4.61 br d (J=14.5 Hz)	4.58 br d (J=14.5 Hz)
H_2-1′	1.60 m	1.55 m
H_2-2′–H_2-4′	1.20–1.40 br s	1.20–1.40 br s
H_2-5′	2.07 m	2.03 m
H-6′	5.65 m	5.46–5.57 m
H-7′	6.29 dd (J=15 and 11 Hz)	5.91–5.99 m
H-8′	5.93 t (J=11 Hz)	5.91–5.99 m
H-9′	5.30 m	5.46–5.57 m
H_2-10′	2.15 m	2.03 m
H_2-11′–H_2-12′	1.20–1.40 br s	1.20–1.40 br s
H_3-13′	0.89 t (J=7 Hz)	0.86 t (J=7 Hz)
-NH-	5.60 br s	6.18 br d (J=8 Hz)
Ac	2.00, 2.04, and 2.06	2.00, 2.00, and 2.01

[a]Signals of the minor conformer.

kept frozen until used. The voucher specimen (TN-84) was deposited at the Graduate School of Human Informatics, Nagoya University.

EXTRACTION AND ISOLATION.—The MeOH-toluene (3:1, 1 liter×2) extract of this tunicate (0.4 kg) was partitioned between toluene (500 ml×4 and 1 M NaCl (1 liter). The toluene-soluble fraction was evaporated under reduced pressure to give a crude residue (1.68 g), a portion of which (0.76 g) was subjected to Si gel cc (2.4×36 cm) with $CHCl_3$-*n*-BuOH-AcOH-H_2O (1.5:6:1:1). The fraction (224 mg) eluting from 930 ml to 1100 ml was ninhydrin-positive and contained a mixture of piperidine alkaloids. A part (65 mg) of this fraction was treated with Ac_2O (0.6 ml) and pyridine (0.6 ml) at room temperature overnight, and the acetate mixture was purified by reversed-phase hplc (Develosil ODS-5, 5 μm, 10×250 mm; eluent, 85% MeOH; flow rate, 2.0 ml/min; detection, uv at 230 nm) to give pseudodistomin A acetate (**4**, 6.2 mg, R_t 19.4 min) together with pseudodistomin B acetate (**5**, 8.6 mg, R_t 21.1 min).

OZONOLYSIS OF PSEUDODISTOMIN A ACETATE [**4**].—A solution of pseudodistomin A acetate (**4**, 1.0 mg) in MeOH (0.5 ml) was bubbled with O_3 at −78° for 30 min. After the removal of excess ozone by bubbling N_2, a solution of $NaBH_4$ (10 mg) in MeOH (0.4 ml) was added and the mixture was stirred for 1 h at 0°. After addition of 1.5 ml of 1 M potassium phosphate buffer (pH 7.0), the reaction mixture was evaporated under reduced pressure. The residue was treated for Ac_2O (0.5 ml) and pyridine (1 ml) at room temperature for 12 h. Brine (0.5 ml) was added and the mixture was extracted with EtOAc (0.5 ml×3). The organic phase was dried over $MgSO_4$ followed by evaporation under reduced pressure to give a residue (0.9 mg), which was subjected to tlc, ^{1}H-nmr, and eims analysis, and proved to be identical with the tetraacetate **6** (6) [tlc, Si gel, MeOH-$CHCl_3$ (1:9), visualized with anisaldehyde, R_f 0.55; ^{1}H nmr ($CDCl_3$, 270 MHz) (6); eims (70 eV) *m/z* 325 ($M-CH_3CONH_2$)$^+$ (62), 282 (31), 181 (40), 139 (50) and 80 (100).

ACKNOWLEDGMENTS

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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Received 14 December 1994