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PYRROLIZIDINE ALKALOIDS FROM *MESSERSCHMIDIA ARGENTEA*

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Key Word Index—*Messerschmidia argentea*; Boraginaceae; twigs; pyrrolizidine alkaloids; 3′-acetylindicine; indicine; 3′-acetylindicine-*N*-oxide; indicine-*N*-oxide.

Abstract—A novel pyrrolizidine alkaloid, 3′-acetylindicine-*N*-oxide {[1*R*-[1α, 7(2*R**, 3*S**), 7$\alpha\beta$]]-3-acetoxy-2-hydroxy-2-(1-methylethyl)-butanoic acid (2,3,5,7a-tetrahydro-1-hydroxy-1*H*-pyrrolizin-7-yl)methyl ester *N*-oxide}, was isolated from the twigs of *Messerschmidia argentea*, in addition to 3′-acetylindicine, indicine and its *N*-oxide. Structures of these alkaloids were determined by spectroscopic methods and from chemical evidence. Copyright © 1997 Elsevier Science Ltd

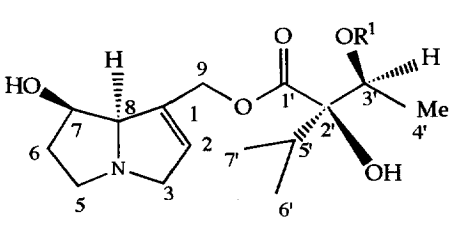
INTRODUCTION

Messerschmidia argentea (Japanese name: Monpanoki, Boraginaceae) is a small tree with large inverted-ovate leaves found on tropical shores of the Pacific and Indian Oceans. In some areas of Okinawa Islands, the plant is visited by a Danaidae butterfly, *Anosia genutia*, which sucks dew-drops on the twigs of the plant. Edgar has reported the presence of pyrrolizidine alkaloids in this species by GC–mass spectrometric analyses of butylboronate derivatives of the alkaloids [1]. In the process of a chemical investigation on the ecological relationship between *A. genutia* and *M. argentea*, we isolated a novel pyrrolizidine alkaloid, in addition to three known alkaloids from the twigs of the plant. Herein, we describe the separation and structural elucidation of these alkaloids.

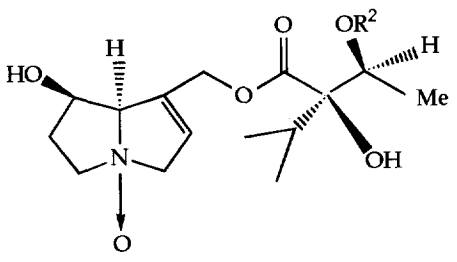
RESULTS AND DISCUSSION

A water extract of the twigs of *M. argentea* was subjected to gel filtration chromatography on Sephadex G-25 and then column chromatography on silica gel and aluminium oxide to give four alkaloids (**1**–**4**). Alkaloids **2** and **4** were identified as indicine and indicine-*N*-oxide, respectively, by comparison of their physical and spectral data with those described in the literature [2, 3].

Alkaloid **1** was obtained as a gummy solid. The EI mass spectrum showed a $[M]^+$ at *m/z* 341, which is 42 mu more than **2**, and a base peak due to a necic base ion at *m/z* 138. The ^1H and ^{13}C NMR spectra of **1** coincided with those of **2**, except for the signals due to the Me-CH(OH) moiety of a (−)-trachelantic acid ester and additional signals due to an acetyl group (Table 1). These observations suggest that **1** is a monoacetyl derivative of **2** and indicate that the acetoxy group is located on a necic acid moiety. In the ^1H NMR spectrum, the signal due to 3′-H of **1** shifted to a lower field than that due to the corresponding proton of **2**, which indicates that the acetoxy group is located on C-3′. Alkaloid **1** was hydrolysed with 0.1 M KOH to give **2**. Thus, alkaloid **1** is 3′-acetylindicine (**1**). Edgar has already reported the presence of either the 2′- or



	R^1
1	Ac
2	H



	R^2
3	Ac
4	H

Table 1. ^{1}H and ^{13}C NMR data and coupling constants* for pyrrolizidine alkaloids **1–4**

	1		**2**		**3**		**4**	
C or H	H	C	H	C	H	C	H	C
1	—	132.3	—	132.5	—	132.4	—	132.2
2	5.92, *s*	130.7	5.94, *s*	129.8	5.79, *s*	121.9	5.87, *s*	123.1
3	3.96, *d* (15.5)	62.8	3.95, *d* (15.5)	62.5	4.45, AB*q* (16)	78.0	4.45, AB*q* (16.5)	78.3
	3.45, *dd* (15.5, 4.5)		3.44, *dd* (15.5, 4.5)					
5	3.29, *t* (8)	53.8	3.28, *t* (8)	53.7	3.73–3.80, *m*	69.6	3.72–3.82, *m*	69.2
	2.76, *dq* (8, 4.5)		2.74, *dq* (8, 6.5)					
6	1.96–2.00, *m*	36.1	2.01, *m*	36.2	2.01, *m*	34.7	1.99, *m*	34.6
	2.02, *m*		2.02 *m*		2.58–2.66, *m*		2.57–2.63, *m*	
7	4.30, *br t* (3)	71.1	4.29, *s*	71.1	4.70, *t* (4.5)	69.6	4.70, *s*	69.2
8	4,21, *s*	78.5	4.19, *s*	78.6	4.66, *s*	95.8	4.64, *s*	96.1
9	4.80, AB*q* (13)	63.3	5.11, *d* (13)	62.4	4.83, AB*q* (14)	62.1	5.09, *d* (14)	61.6
			4.60, *d* (13)				4.71, *d* (14)	
1′	—	174.5	—	175.3	—	173.8	—	175.1
2′	—	81.6	—	82.8	—	81.9	—	83.7
3′	5.20, *q* (6.5)	71.8	4.05, *q* (6.5)	69.4	5.23, *q* (6.5)	72.4	4.12, *q* (6.5)	69.3
4′	1.23, *d* (6.5)	14.3	1.19, *d* (6.5)	16.7	1.24, *d* (6.5)	14.0	1.20, *d* (6.5)	17.8
5′	2.09, *sept* (7)	32.3	2.15, *sept* (7)	32.5	2.05, *sept* (7)	33.0	2.07, *sept* (7)	33.1
6′	0.95, *d* (7)	17.2	0.96, *d* (7)	17.4	0.97, *d* (7)	17.3	0.97, *d* (7)	17.2
7′	0.92, *d* (7)	16.3	0.93, *d* (7)	17.1	0.91, *d* (7)	16.8	0.94, *d* (7)	17.1
Ac	2.04, *s*	21.1	—	—	2.03, *s*	21.2	—	—
	—	170.0	—	—	—	170.3	—	—

Solvents: $CDCl_3$ for **1–3**, $CDCl_3$–CD_3OD (9:1) for **4**.
*Coupling constants (Hz) in parentheses.

3′-acetyl derivative of **2** in *M. argentea*, but did not elucidate the location of the acetoxy group [1]. We have now proved **1** to be present in this species for the first time.

The novel alkaloid **3** was also obtained as a gummy solid, $[\alpha]_D^{25} - 3.7°$ (ethanol; *c* 5.4). The IR spectrum gave two ester bands. The EI mass spectrum of **3** closely resembled that of **1**. Its molecular formula was assumed to be $C_{17}H_{27}NO_6$ on the basis of an ion peak at *m/z* 341.1828 by HR mass spectrometric analysis. In the ^{13}C NMR spectrum of **3**, the signals at δ 78.0 (C-3), 69.6 (C-5), 34.7 (C-6), 69.6 (C-7) and 95.8 (C-8) suggested the presence of a retronecine ester *N*-oxide. The ^{1}H NMR spectrum of **3** coincided with that of **4**, except for the signals due to a (−)-trachelantic acid moiety. These observations indicate that **3** possesses the same retronecine *N*-oxide skeleton as **4** and that its molecular formula is $C_{17}H_{27}NO_7$, which is one oxygen more than the assumed formula. In addition, the ^{1}H and ^{13}C NMR spectra showed signals due to a carbonyl group (δ_C 173.8), a quaternary carbon (δ_C 81.9) bonded to a hydroxyl group, an Me-CH(OAc) moiety [δ_H 5.23 (1H, *q*, J = 6.5 Hz), 2.03 (3H, *s*) and 1.24 (3H, *d*, J = 6.5 Hz) and δ_C 170.3, 77.3, 21.2 and 14.0] and an isopropyl group [δ_H 2.05 (1H, *sept*, J = 7 Hz), 0.97 (3H, *d*, J = 7 Hz) and 0.91 (3H, *d*, J = 7 Hz) and δ_C 33.0, 17.3 and 16.7], which are assigned to the same 3-acetyltrachelantic acid ester as **1** (Table 1). Alkaloid **3** was reduced with zinc in dilute H_2SO_4 to give **1**. Thus, **3** was elucidated to be 3′-acetylindicine-*N*-oxide (**3**). Although **1** has been isolated from reduced mixtures of basic constituents present in *Heliotropium indica*, no spot having the same R_f value as **3** has been detected on paper chromatograms of the basic fraction in the unreduced extract from *H. indica* [4]. Therefore, this is the first report of the isolation of **3** from a plant species.

EXPERIMENTAL

Analyt. and prep. TLC were carried out on Merck 60 F_{254} silica gel (thickness: 0.25 and 0.5 mm, respectively) and Merck aluminium oxide F_{254} (type E) (thickness: 0.25 mm). ^{1}H (90 and 500 MHz) and ^{13}C NMR (25 and 100 MHz) spectra were determined in $CDCl_3$ for **1–3** and $CDCl_3$–CD_3OD (9:1) for **4** with TMS as int. standard. EIMS were obtained on a double focusing mass spectrometer at 70 eV.

Extraction and isolation. Fresh twigs (1.5 kg) of *M. argentea* Johnst, collected at Ikei Island, Okinawa prefecture, in April, were ground in a mixer and immersed in H_2O for 3 days. The H_2O soln was concd *in vacuo* and the obtained concentrate (90 g) was subjected to gel filtration CC on Sephadex G-25 to give an alkaloidal fr. This was subjected to CC on silica gel, developed with $CHCl_3$–MeOH–NH_4OH (6:2:0.1), to

give a basic mixt. (5 g), which included alkaloids **1**–**4**. The mixt. (3 g) was rechromatographed on an aluminium oxide column with $CHCl_3$–EtOH (36:1–10:1) to give **1** (120 mg), **2** (100 mg), **3** (60 mg) and **4** (90 mg). The mixt. (0.5 g) was also subjected to prep. TLC on silica gel with $CHCl_3$–MeOH–NH_4OH (6:1:0.1) to give **1** (20 mg, R_f 0.65), **2** (17 mg, R_f 0.51), **3** (10 mg, R_f 0.47) and **4** (17 mg, R_f 0.19).

3′-Acetylindicine (**1**). Gummy solid. $[\alpha]_D^{25}$ − 13.8° (EtOH; *c* 2.2). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1740 (C=O), 1730 (C=O), 1245. ^1H (500 MHz) and ^{13}C NMR (125 MHz): Table 1. EIMS *m/z* (rel. int.): 341 $[M]^+$ (2), 255 (2), 181 (5), 138 (100), 93 (48), 43 (10). Physical data coincided with that described in ref. [4].

Indicine (**2**). Gummy solid. $[\alpha]_D^{25}$ + 20° (EtOH; *c* 0.2). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1740, 1220 (O–C=O). ^1H (500 MHz) and ^{13}C NMR (125 MHz): Table 1. EIMS *m/z* (rel. int.): 299 $[M]^+$ (12), 255 (4), 156 (11), 138 (100) and 93 (95). Physical and spectral data coincided with those described in refs [2, 3].

3′-Acetylindicine-N-oxide (**3**). Gummy solid. $[\alpha]_D^{25}$ − 3.7° (EtOH; *c* 5.4). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (C=O), 1730 (C=O), 1245. ^1H (500 MHz) and ^{13}C NMR (125 MHz): Table 1. HRMS *m/z*: 341.1828 ($[M - O]^+$, calcd for $C_{17}H_{27}NO_6$: 341.1836); EIMS *m/z* (rel. int.): 341 $[M - O]^+$ (4), 339 (1), 255 (3), 181 (6), 138 (100), 136 (50) 93 (51), 43 (46).

Indicine-N-oxide (**4**). Gummy solid. $[\alpha]_D^{25}$ + 31.2° (EtOH; *c* 0.9). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1735, 1220 (O–C=). ^1H (500 MHz) and ^{13}C NMR (125 MHz): Table 1. EIMS *m/z* (rel. int.): 299 $[M - O]^+$ (6), 279 (5), 156 (9), 138 (100), 118 (99), 103 (90), 93 (60). Physical and spectral data coincided with those described in refs [2, 3].

Hydrolysis of **1**. Alkaloid **1** (24 mg) was dissolved in 10 ml 0.1 M KOH. The soln was stirred at room temp. for 8 hr and then diluted with 10 ml H_2O. The reaction mixt. was extracted with 3 × 2 ml $CHCl_3$. The combined $CHCl_3$-soluble frs were washed with H_2O and concd to dryness. The residue was subjected to prep. TLC on silica gel with $CHCl_3$–MeOH–NH_4OH (6:1:0.1). In this way, 12 mg **2** was obtained and identified by co-TLC and direct comparison of its physical data, ^1H and ^{13}C NMR and MS with those of indicine (**2**) isolated from the plant.

Reduction **3**. Alkaloid **3** (24 mg) was dissolved in 10 ml 4 M H_2SO_4, then 10 mg Zn powder was added. The soln was stirred at room temp. for 8 hr and then diluted with 10 ml H_2O. The reaction mixt. was filtered and the Zn residue washed with a few drops dilute aq. H_2SO_4. The aq. filtrate and washings were extracted with 3 × 2 ml $CHCl_3$. The remaining aq. layer was then made basic with NH_4OH to pH *ca* 10 (indicator paper) and extracted with 5 × 2 ml $CHCl_3$. The combined $CHCl_3$-soluble frs were concd to dryness. The residue was subjected to prep. TLC on silica gel with $CHCl_3$–MeOH–NH_4OH (6:1:0.1). In this way, 5 mg **1** was obtained and identified by co-TLC and direct comparison of its physical data, ^1H and ^{13}C NMR and MS with those of **1** isolated from the plant.

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