Angustilodine, an Unusual Pentacyclic Indole Alkaloid from *Alstonia*

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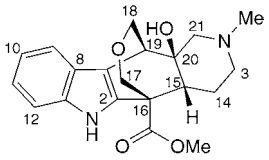
Two new indole alkaloids, angustilodine (**1**), with an unprecedented pentacyclic carbon skeleton, and angustilocine (**2**), belonging to the *seco*-angustilobine-B group of alkaloids, were obtained from the leaf extract of the Malayan species *Alstonia angustiloba*, and their structures were established by spectroscopic analysis.

Introduction. – The genus *Alstonia* is rich in indole alkaloids [1–16]. In continuation of our studies on the Malaysian members of this genus [2–5], we would like to report the structure of angustilodine (**1**), a novel pentacyclic indole isolated from *A. angustiloba* Miq. There has been no previous study of the Malayan species, although Indonesian *A. angustiloba* has been reported to contain vallesamine-type compounds [16].



1

Results and Discussion. – Angustilodine (**1**) was obtained from the leaf extract of *A. angustiloba* as a colorless oil, with $[\alpha]_D = -622$ ($c = 0.165$, $CHCl_3$). The UV spectrum was characteristic of an indole chromophore, with absorption maxima at 224 and 281 nm (log ε 4.23 and 3.60, resp.), and the IR spectrum showed bands at 3422 and 1726 cm^{-1} due to NH/OH and C=O functions, respectively. The mass spectrum of **1** showed a molecular ion at *m/z* 356, which analyzed for $C_{20}H_{24}N_2O_4$, requiring ten degrees of unsaturation. The ^{13}C-NMR spectrum (*Table*) showed a total of 20 separate C-atom resonances (two Me, five CH_2, six CH, and seven C_q groups/atoms), in agreement with the molecular formula. The ^{1}H-NMR spectrum of **1** (*Table*) showed the presence of an unsubstituted indole chromophore, (four contiguous aromatic H-atoms), an indole NH (δ_H 9.57), an NMe (δ_H 2.24), and a COOMe group (δ_H 3.84). In addition, two isolated CH_2 groups were observed, an NCH_2 group (δ_H 2.27 and 2.35; δ_C 61.1), and an OCH_2 moiety (δ_H 3.85 and 4.13; δ_C 72.8). These data, and the presence of another *AB dd*-like signal (δ_H 3.55, 4.39; $J = 11$ and 3 Hz) due to another OCH_2 group as part of a OCH_2CH fragment, suggested a vallesamine-type alkaloid [15][16]. In addition to the isolated NCH_2 group, the COSY NMR spectrum revealed another fragment branching from N(4), *i.e.*, NCH_2CH_2CH. With all the fragments identified, the structure of **1** could be derived by means of the HMBC data (*Figure,a*).

Table. *^{1}H- and ^{13}C-NMR Spectral Data for Compounds* **1** *and* **2** (400 MHz, $CDCl_3$)[a])

1				**2**			
H-Atoms	δ_H (J in Hz)	C-Atoms	δ_C	H-Atoms	δ_H (J in Hz)	C-Atoms	δ_C
CH_2(3)	2.25 (*m*),	C(2)	135.0	CH_2(3)	2.60 (*td*, J = 12, 3),	C(2)	133.8
	3.01 (*ddd*, J = 12, 11, 6)	C(3)	49.9		3.06 (*dt*, J = 12, 3)	C(3)	47.0
H–C(9)	7.48 (br. *d*, J = 8)	C(7)	111.2	H–C(7)	6.20 (*d*, J = 2)	C(7)	100.5
H–C(10)	7.12 (*td*, J = 8, 1)	C(8)	125.5	H–C(9)	7.54 (br. *d*, J = 8)	C(8)	127.6
H–C(11)	7.14 (*td*, J = 8, 1)	C(9)	117.3	H–C(10)	7.09 (*td*, J = 8, 1)	C(9)	120.3
H–C(12)	7.41 (br. *d*, J = 8)	C(10)	121.1	H–C(11)	7.18 (*td*, J = 8, 1)	C(10)	120.0
CH_2(14)	0.98 (*tdd*, J = 13, 11, 8),	C(11)	119.6	H–C(12)	7.35 (br. *d*, J = 8)	C(11)	122.3
	1.31 (*dddd*, J = 13, 6, 5, 1)	C(12)	111.6	CH_2(14)	1.23 (*dq*, J = 13, 3),	C(12)	111.0
H–C(15)	2.65 (*dd*, J = 13, 5)	C(13)	135.5		1.48 (*dtd*, J = 13, 12, 3)	C(13)	135.7
CH_2(17)	3.85 (*d*, J = 11),	C(14)	24.9	H–C(15)	3.32 (br. *d*, J = 12)	C(14)	28.8
	4.13 (*d*, J = 11)	C(15)	52.5	CH_2(17)	3.75 (*d*, J = 13),	C(15)	46.8
CH_2(18)	3.55 (*dd*, J = 11, 3),	C(16)	55.2		4.77 (*dd*, J = 13, 1)	C(16)	53.0
	4.39 (*dd*, J = 11, 3)	C(17)	72.8	CH_2(18)	3.95 (*d*, J = 14),	C(17)	70.3
H–C(19)	3.06 (*t*, J = 3)	C(18)	66.1		4.39 (*dd*, J = 14, 3)	C(18)	67.1
CH_2(21)	2.27 (*d*, J = 12),	C(19)	41.8	H–C(19)	2.93 (*d*, J = 3)	C(19)	62.5
	2.35 (*d*, J = 12)	C(20)	76.5	CH_2(21)	2.52 (*d*, J = 12),	C(20)	64.2
MeN	2.24 (*s*)	C(21)	61.1		3.20 (*d*, J = 12)	C(21)	57.0
MeO	3.84 (*s*)	MeN	45.4	MeO	3.79 (*s*)	MeO	53.3
NH	9.57 (br. *s*)	MeO	52.4	NH	8.47 (br. *s*)	CO	172.9
OH	1.99 (br. *s*)	CO	173.2				

[a]) Assignments based on COSY and HMQC (arbitrary atom numbering).

From a total of seven quaternary C-atoms (C_q), four (δ_C 135.0, 111.2, 125.5, 135.5) were readily assigned to the indole portion of the molecule, and one to the ester C=O group (δ_C 173.2). Of the remaining two, C(20) at δ_C 76.5 was adjacent to an O-atom. The observed three-bond heteronuclear correlations from H–C(21) to the methine C(15), and from H–C(14) to the quaternary C(20), indicated that the $CH_2NCH_2CH_2CH$ fragment was linked to C(20) at both ends to constitute the piperidine ring (ring E). The three-bond correlations from H–C(17) to C(15) and from H–C(15) to C(2) indicated attachment of C(15) and C(17) to the indole C(2) *via* the ester bearing the quaternary C(16), and the observed H–C(18)/C(20) and H–C(19)/C(15) correlations indicated that C(19) was linked to C(20). As C(20) is oxygenated, the remaining substituent had to be a tertiary alcohol, C(20)–OH. These observed patterns are also common to the vallesamine as well as to the related *seco*-angustilobine-B type alkaloids [15][16]. In angustilodine, however, two additional

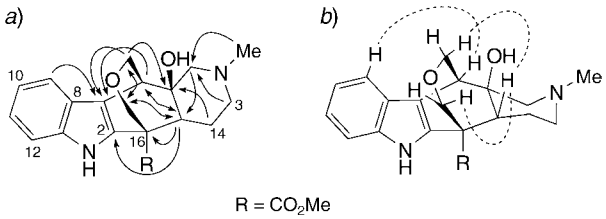
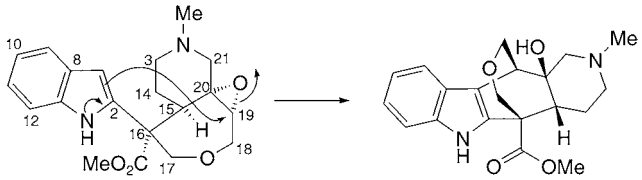


Figure. *Structure elucidation of angustilodine* (**1**) *by means of* a) *HMBC and* b) *NOE experiments*

correlations are observed, representing a crucial point of departure from the vallesamine and *seco*-angustilobine-B series, and revealing formation of a new ring system. The observed two- and three-bond correlations from H–C(19) to C(7) and from H–C(18) to C(7), respectively, indicated the connection of C(19) to C(7), thus completing the assembly of the angustilodine molecule, as shown in **1**.

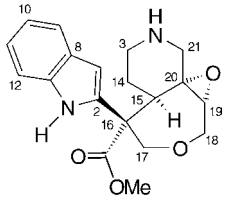
Angustilodine (**1**) represents a new, structurally-related subclass of the vallesamine and *seco*-angustilobine-B group of alkaloids. A possible biosynthetic pathway to the ring system of **1**, starting from a *seco*-angustilobine-B type precursor such as **3**, could involve an intramolecular epoxide-ring opening, resulting in bond-formation between C(7) and C(19), as shown in the *Scheme*. Such a process would be in accord with the observed stereochemistry (ring junction) of angustilodine, as indicated by the NOE interactions found for **1** (*Figure,b*).

Scheme



3 **1**

In addition to **1**, another new alkaloid was also obtained, angustilocine (**2**), the *N*(4)-demethyl derivative of the known alkaloid 6,7-*seco*-19,20α-epoxyangustilobine B (**3**) [15][16]. The molecular formula of **2**, $C_{19}H_{22}N_2O_4$, lacked 14 mass units relative to **3**, and the ^{1}H- and ^{13}C-NMR spectral data were similar in all respects to that of **3**, except for the absence of signals due to the *N*(4) Me group. In addition to the two new alkaloids, fifteen other known alkaloids, mainly of the vallesamine/*seco*-vallesamine and strychnan types were also isolated (see the *Exper. Part*).



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Experimental Part

General. Optical rotations were determined on a *JASCO DIP-370* digital polarimeter. UV Spectra were obtained on a *Shimadzu UV-3101PC* spectrophotometer; λ_{max} in nm, log ε. IR Spectra were recorded on a *Perkin-Elmer 1600* FT-IR spectrophotometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a *JEOL JNM-LA-400* spectrometer at 400 and 100 MHz, resp.; δ in ppm rel. to $SiMe_4$, *J* in Hz. ESI-MS: on a *Perkin-Elmer API-100* instrument; EI-MS, HR-EI-MS, and HR-FAB-MS: on a *JEOL JMS-AX505 H* mass spectrometer, courtesy of Dr. *K. Komiyama* of the *Kitasato Institute*, Tokyo, Japan; in *m*/*z*.

Plant Material. Plants were collected in Kuala Lumpur, Malaysia (May, 1998) and identified by Dr. *K. M. Wong*, *Institute of Biological Sciences*, University of Malaya. Herbarium voucher specimens (K 655) were deposited at the Herbarium, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation. Extraction of the well-ground leaf material was carried out in the usual manner by partitioning the conc. EtOH extract with dilute acid, as described in detail elsewhere [17]. The alkaloids were isolated by initial column chromatography (CC) on silica gel, with $CHCl_3$ containing increasing proportions of MeOH as eluants. Partially resolved fractions were rechromatographed by centrifugal TLC (thin-layer chromatography). Initial chromatography of the basic fraction from the leaves provided essentially six fractions. Angustilodine (**1**) was obtained from fractions *4* and *5* after rechromatography ($MeOH/CHCl_3$), followed by successive centrifugal TLC (NH_3-sat. $CHCl_3$; 2% MeOH/AcOEt; NH_3-sat. 3% $MeOH/CHCl_3$). Angustilocine (**2**) was obtained from fraction *5* after rechromatography ($MeOH/CHCl_3$) and centrifugal TLC (NH_3-sat. 3% $MeOH/CHCl_3$). Other solvent systems used for centrifugal TLC were Et_2O, Et_2O (sat. with NH_3),Et_2O/hexane, $CHCl_3$, $CHCl_3$ (sat. with NH_3), $CHCl_3$/MeOH (sat. with NH_3), AcOEt/hexane, and AcOEt/MeOH. The yields (mg kg^{-1}) of the alkaloids from the leaf extract were: angustilodine (2.1), angustilocine (6.3), vallesamine (33.2), angustilobine B (6.1), 6,7-*seco*-angustilobine B (19.9), losbanine (1.1), 6,7-*seco*-19,20α-epoxyangustilobine B (1.9), picrinine (0.6), picraline (12.8), echitamidine (0.2), scholaricine (7.0), and leuconoxine (0.1). The yields (mg kg^{-1}) of the alkaloids from the bark extract were: vallesamine (8.7), angustilobine B (3.1), 6,7-*seco*-19,20α-epoxyangustilobine B (2.5), *N*(4)-demethylechitamine (21.1), ajmalicine (5.6), akuammicine (3.7), venoterpine (6.0), and cantleyine (6.1).

Angustilodine (= *Methyl 1,2,3,4,4a,5,11,11a-Octahydro-11a-hydroxy-2-methyl-5,11-(oxydimethylene)-6*H-*pyrido[4,3*-b*]carbazole-5-carboxylate*; **1**). Colorless oil. $[\alpha]_D$ −622 ($c = 0.165$, $CHCl_3$). UV (EtOH): 224 (4.23), 281 (3.60). IR (dry film): 3422, 1726. ^{1}H- and ^{13}C-NMR: see *Table*. EI-MS: 356 (100, M^+), 244 (20), 214 (23), 167 (19), 149 (28), 112 (48), 82 (76), 58 (55), 44 (71). HR-EI-MS: 356.1751 (M^+, $C_{20}H_{24}N_2O_4^+$; calc.: 356.1736).

Angustilocine (= *Methyl 1a,2,4,5,5a,6,7,8,9,9a-Decahydro-5-(1*H-*indol-2-yl)oxireno[2′,3′:3,4]oxepino[4,5-c]pyridine-5-carboxylate*; **2**). Light yellowish oil. $[\alpha]_D$ −546 ($c = 0.50$, $CHCl_3$). UV (EtOH): 216 (4.36), 273 (3.79), 282 (3.77), 291 (3.62). IR (dry film): 3359, 1728. ^{1}H- and ^{13}C-NMR: see *Table*. FAB-MS: 365 (55, $[M + Na]^+$), 343 (27, $[M + H]^+$). HR-FAB-MS: 365.1498 ($[M + Na]^+$, $C_{19}H_{22}N_2NaO_4^+$; calc.: 365.1477).

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