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ALSTOGUSTINE AND 19-EPIALSTOGUSTINE, QUATERNARY INDOLE ALKALOIDS FROM *ALSTONIA ANGUSTIFOLIA*

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Key Word Index—*Alstonia angustifolia*; Apocynaceae; alstogustine; 19-epialstogustine; absolute configuration; crystal structure; spectral data.

Abstract—Two new quaternary alkaloids, alstogustine and 19-epialstogustine, have been isolated from the stem bark of *Alstonia angustifolia*. The structure of **1** was determined by means of spectroscopic methods and X-ray analysis. The structure of 19-epialstogustine could be elucidated by comparison of its spectra with those of alstogustine and confirmed by chemical correlation. The absolute configurations of both alkaloids were established by direct comparison of their CD spectra with that of the known alkaloid, akuammicine.

INTRODUCTION

In our phytochemical investigation of the plant *Alstonia angustifolia* (Apocynaceae), we have isolated two quaternary indole alkaloids. The structures of both alkaloids are similar to echitamidine methosalt. The plane structure of echitamidine (**3**), isolated from *Alstonia* species, was elucidated by Djerassi *et al.* by means of ^{1}H NMR (60 MHz) and mass spectra [1]. The stereochemistry of **3**, especially the configuration at C(19) and C(20) is until now unknown. The methosalt of echitamidine was never isolated from plants.

In this paper we will report the determination of the structures of these two new alkaloids inclusive their absolute configurations. This structural work was done independently from that of echitamidine (**3**) [1]. A direct comparison of echitamidine methosalt and the new compounds was not possible; therefore we have given independent names to the new alkaloids.

RESULTS AND DISCUSSION

From the water-soluble component of the acetic acid–methanol extract of stem bark two new quaternary alkaloids, alstogustine and 19-epialstogustine, have been isolated, they were characterized as their chlorides.

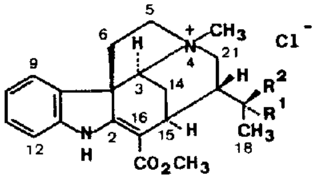
The mass spectrum of alstogustine (**1**, $C_{21}H_{27}N_2O_3Cl$, 390) measured under electron impact condition is characterized by the $[M]^{+\cdot}$ signals of its thermal decomposition products [2]: *m/z* 354 ($[M-HCl]^{+\cdot}$, corresponding to a Hofmann degradation), *m/z* 340 ($[M-MeCl]^{+\cdot}$) and signals at *m/z* 52 and 50 ($Me^{37}Cl$ and $Me^{35}Cl$). The UV spectrum (λ_{max}: 227, 292, 326) is in agreement with a β-anilinoacrylester chromophore. This is confirmed by the IR spectrum which shows at $\nu = 1664$ cm^{-1} an α,β-unsaturated ester group.

A more detailed understanding of the structure was gained from ^{1}H and ^{13}C NMR spectra. From the 400 MHz ^{1}H NMR spectral data listed in Table 1 we can see four aromatic protons in the region δ7.5–7.0 and three methyl groups in the aliphatic region, corresponding to CO_2Me [δ3.82 (*s*)], $(N^+)-Me$ [δ3.50 (*s*)] and a methyl group in the neighbourhood of a methine [δ1.34 (*d*)]. Most of the signals are well separated from each other. Using 2D ^{1}H COSY and ^{1}H decoupling experiments as well as the consideration of chemical shifts the information about the following coupling systems of protons could be accumulated: (i) $-CH_2$ ($\delta = 3.91$ and 3.80 ppm) $-CH_2$ (2.66 and 2.36)$-N^+-$, (ii) $-CH(3.38)-CH_2$ (2.46 and 1.44)$-CH(4.52)-N^+-$, and (iii) Me (1.34) $-C(OR)H(3.76)-CH(2.19)-CH_2(3.64$ and $3.45)-N^+-$. In the 2D ^{1}H COSY spectrum there was still a small coupling between two protons (δ3.38 and 2.19).

Combining the coupling systems and the chromophore established from the UV spectrum we could propose the plane structure of **1**. The ^{13}C NMR spectrum supports this interpretation. An essential point from the ^{13}C NMR spectrum is an ester carbonyl group; the chemical shifts and multiplicities of the other carbon atoms are in agreement with structure **1** (Table 2).

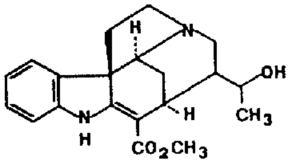
The stereochemistry of **1** was deduced by X-ray analysis, (see Fig. 1). The atom coordinates and temperature factors of alstogustine (**1**) will be sent to the Cambridge Crystallographic Data Centre. In this X-ray analysis experiment the absolute configuration, represented in structure **1**, could be established with 77% probability. Confirmation of the absolute configuration was obtained by direct comparing of the CD spectrum of **1** with that of (−)-akuammicine (**4**) (Fig. 2), of known absolute configuration [3].

The second quaternary alkaloid, 19-epialstogustine (**2**), has very similar UV, IR and mass spectra, but different ^{1}H and ^{13}C NMR spectra to those of **1** (Tables 2 and 3), indicating that the two are stereoisomers. The most probable differences could be at C-19 and C-20 according to biogenetic considerations [4]. The signals of H-21α as well as H-21β of **1** and **2** show the same shape at different chemical shifts (Table 3) which implies the same stereochemistry at C-20 in both compounds. Accordingly the only difference between the two compounds must be at C-19. This hypothesis was proved by the oxidation (DMSO–Ac_2O) of **1** and **2** to the same ketone **5** with [5]. In ^{1}H NMR spectra the chemical shifts of the C-18 methyl group of **1** [δ1.34 (*d*)] and of **2** [δ1.19 (*d*)] were both

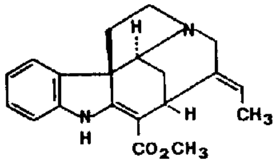


1 R^1 = OH, R^2 = H
Alstogustine

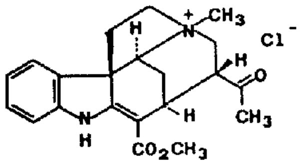
2 R^1 = H, R^2 = OH
19-Epialstogustine



3 Echitamidine



4 Akuammicine



5

Table 1. ^1H NMR spectral data of alstogustine (1) (400 MHz) in CD_3OD

H	δ (ppm)	Multiplicity	J (Hz)
3	4.52	*br s*	—
5a	3.80	*m*	—
5b	3.91	*m*	—
6a*	2.32	*m*	—
6b*	2.66	*ddd*	$J_1 = 14.3$, $J_2 = 9.8$, $J_3 = 6.9$
9	7.53	*dd*	$J_1 = 7.7$, $J_2 = 1$
10	7.02	*dt*	$J_1 = 7.7$, $J_2 = 1$
11	7.24	*dt*	$J_1 = 7.7$, $J_2 = 1$
12	6.98	*dd*	$J_1 = 7.7$, $J_2 = 1$
14a	1.44	*br d*	$J_1 = 15.0$
14b	2.46	*dt*	$J_1 = 15.0$, $J_2 = 3.3$
15	3.38	*br s*	—
18 (3H)	1.34	*d*	$J = 6.5$
19	3.76	*dq*	$J_1 = 6.5$, $J_2 = 3.0$
20	2.19	*m*	—
21a	3.45	*t*	$J = 13.1$
21b	3.64	*dd*	$J_1 = 13.4$, $J_2 = 6.3$
$CO_2\underline{Me}$	3.82	*s*	—
N^+-$\underline{Me}$	3.50	*s*	—

*Signals interchangeable, the assignment of α and β were based on NOE experiments.

Table 2. ^{13}C NMR spectral data (CD_3OD) of alstogustine (**1**) and epialstogustine (**2**)

C	**1**	**2**	C	**1**	**2**
2	133.45 *s*	133.67 *s*	14	27.10 *t*	28.27 *t*
3	73.36 *d*	72.31 *d*	15	24.96 *d*	28.91 *d*
5	64.50 *t*	64.60 *t*	16	105.18 *s*	98.63 *s*
6	43.21 *t*	40.69 *t*	18	20.68 *q*	20.12 *q*
7	58.23 *s*	56.62 *s*	19	68.67 *d*	68.18 *d*
8	164.13 *s*[a]	168.41 *s*[a]	20	44.56 *d*	42.85 *d*
9	130.31 *d*[b]	130.17 *d*[b]	21	61.75 *t*	58.22 *t*
10	122.44 *d*[a]	122.73 *d*[b]	$\underline{C}O_2Me$	167.98 *s*	170.15 *s*
11	122.16 *d*[a]	121.40 *d*[b]	$CO_2\underline{Me}$	51.79 *q*	52.61 *q*
12	111.64 *d*[a]	111.77 *d*[b]	N^+-$\underline{Me}$	56.19 *q*	49.41 *q*
13	145.17 *s*[b]	145.35 *s*[a]	—	—	—

[a, b] Signal interchangeable.

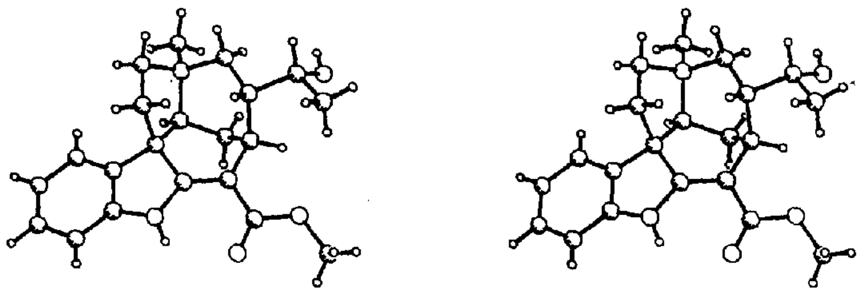


Fig. 1.

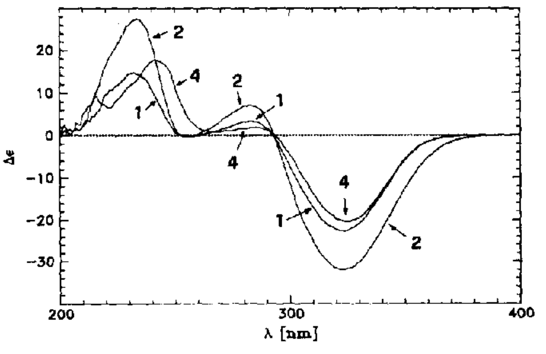


Fig. 2.

shifted to δ2.38 (*s*) in **5**, a typical chemical shift for a methyl group in the neighbourhood of a carbonyl group. Compound **2** could be determined therefore as 19-epialstogustine. The same absolute configuration at C-15 of **1** and **2** was concluded according to their CD spectra (Fig. 2).

EXPERIMENTAL

^{1}H NMR spectra were recorded at 400 MHz with MeOH (δ3.30) as int. std. ^{13}C NMR spectra at 35 MHz with MeOH (δ49.0) as int. std.

Extraction and chromatography. Dried stem bark (1.4 kg) of *A. angustifolia* Miq. was extd × 3 with 3% HOAc-MeOH at 22°. The MeOH extract was evapd *in vacuo* (< 40°) and dissolved in 0.25 M aq. HCl. After usual extn for organic sol alkaloids [6], the H_2O phase was acidified to pH 3 and deposited with Reinecke salt. The solid was dissolved in Me_2CO-MeOH-H_2O (3:1:1) and exchanged to chlorides by an ion exchange column (Amberlite® IRA-400, Cl^- form). The quaternary bases were sepd by chromatography on silica gel with different solvent systems. After crystallization we obtained 670 mg alstogustine (**1**) and 44 mg 19-epialstogustine (**2**), as chlorides.

Alstogustine (**1**). Mp: 231.2–231.7° (as chloride, crystallized from EtOH); blue colour with the $Ce(SO_4)_2$ reagent; $[\alpha]_D^{22}$ −442° (MeOH; *c* 0.90). UV λ_{max}^{EtOH} nm (log ε): 227 (4.06), 292 (4.06), 326 (4.17); λ_{min}: 255 (2.84), 304 (3.95). IR ν_{max}^{KBr} cm^{-1}: 3300 (*br*), 1664, 1608, 1487, 1475, 1432, 1296, 1250, 1235, 1218, 1180, 1136, 1110, 1088, 921, 782. ^{1}H and ^{13}C NMR see Tables. EIMS 70 eV, *m/z* (rel. int.): 354 (3, $[M]^+$ of Hofmann base), 340 (15, $[M]^+$ of demethylated base), 322 (4), 309 (4), 296 (9), 295 (9), 282 (9), 281 (6), 241 (10), 239 (12), 225 (25), 194 (18), 186 (23), 182 (18), 181 (16), 180 (23), 167 (18), 158 (22), 151 (19), 145 (20), 140 (52), 96 (36), 94 (66), 52 (28), 50 (87), 44 (100); CIMS: 355 (14), 341 (9), 297 (35), 283 (100); FABMS: 355 (100), 323 (27), 185 (32). CD see Fig. 2.

19-*Epialstogustine* (**2**). Mp 249° (as chloride, crystallized from PrOH); blue colour with the $Ce(SO_4)_2$ reagent; $[\alpha]_D^{22}$ −467° (MeOH; *c* 1.05). UV λ_{max}^{EtOH} nm (log ε): 232 (3.95), 289 (3.82), 327 (3.99); λ_{min}: 258 (2.96), 302 (3.72). IR ν_{max}^{KBr} cm^{-1}: 3380 (*br*), 1647, 1595, 1479, 1463, 1438, 1385, 1353, 1230, 1165, 1120, 1100, 1080, 1052, 1012, 942, 770, 760. ^{1}H and ^{13}C NMR see Tables. EIMS 70 eV, *m/z* (rel. int.): 354 (1), 340 (1), 336 (3), 322 (5), 296 (7), 295 (6), 282 (9), 241 (5), 239 (7), 225 (6), 221 (8), 194 (11), 182 (15), 181 (10), 180 (16), 168 (8), 167 (9), 158 (12), 151 (21), 140 (24), 96 (11), 94 (23), 70 (10), 52 (24), 50 (81), 44 (100). CD see Fig. 2.

Table 3. ^{1}H NMR spectral data of 19-epialstogustine (2) (400 MHz) in $CDCl_3$

H	δ(ppm)	Multiplicity	J(Hz)
3	4.57	*br s*	—
5a	3.70	*dd*	$J_1 = 12.1$, $J_2 = 7.6$
5b	3.83	*dt*	$J_1 = 12.1$, $J_2 = 7.9$
6a*	2.16	*m*	—
6b*	3.01	*ddd*	$J_1 = 14.5$, $J_2 = 12.1$, $J_3 = 7.6$
9	7.49	*dd*	$J_1 = 7.7$, $J_2 = 1$
10	6.97	*dt*	$J_1 = 7.7$, $J_2 = 1$
11	7.02	*dd*	$J_1 = 7.7$, $J_2 = 1$
12	7.02	*dd*	$J_1 = 7.7$, $J_2 = 1$
14a	1.58	*br d*	$J =$ *ca* 15
14b	2.35	*dt*	$J_1 = 15.0$, $J_2 = 3.0$
15	3.54	*br s*	$J_1 =$ *ca* 2
18 (3H)	1.19	*d*	$J = 6.3$
19	3.43	*dq*	$J_1 = 6.3$
20	2.13–2.06	*m*	—
21a	3.23	*t*	$J = 13.6$
21b	3.39	*dd*	$J_1 = 13.6$, $J_2 = 4.6$
$CO_2\underline{Me}$	3.88	*s*	—
N^+-$\underline{Me}$	3.47	*s*	—

*Signals interchangeable, the assignment according to comparison with that of **1**.

Oxidation of alstogustine (**1**). A sample of alstogustine (**1**) was dissolved in DMSO/Ac_2O (*ca* 2:1) and stirred at 22°. The starting material had disappeared after 3 hr. After flash CC on silica gel one product was isolated. ^{1}H NMR (400 MHz, CD_3OD): δ7.53 (*dd*, H-9), 7.21 (*dt*, H-11), 7.00 (*dt*, H-10), 6.97 (*dd*, H-12), 4.67 (*br s*, H-3), 3.86 (*m*, H-5), 3.77 (*m*, H-5), 3.73 (*m*, H-20), 3.71 (*s*, CO_2Me), 3.57 (2H, *m*, H-15, H-21), 3.46 (*s*, N^+-Me), 3.38 (*m*, H-21), 2.99 (*ddd*, H-6), 2.52 (*dt*, H-14), 2.38 (*s*, CH_3-18), 2.20 (*m*, H-6), 1.62 (*br d*, H-14). 19-Epialstogustine (**2**) was oxidized in analogous manner to give the same oxidation product. This was confirmed by means of TLC (different solvent systems) and ^{1}H NMR spectra.

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