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Mersiphyllines A and B from *Kopsia*. Determination of relative configuration at a quaternary center via formation of an alkaloid–borane complex

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ABSTRACT

Two new pentacyclic alkaloids, mersiphyllines A (**1**) and B (**2**), belonging to the novel mersinine group, and incorporating carboxylic acid functionalities on a quaternary center, were isolated from *Kopsia singapurensis*. The structures were determined by spectroscopic analysis. Determination of the relative configuration at the quaternary center bearing the acid function was facilitated by the formation of an alkaloid–borane complex. The structure of **1** was also confirmed by X-ray diffraction analysis.

1. Introduction

The alkaloids of the mersinine group represent a novel subclass of the monoterpenoid indole alkaloids.[1–3] To date these alkaloids have been found exclusively and for the first time in only one species, a variant of the Malayan *Kopsia singapurensis*, which has also provided other interesting alkaloids with unusual skeletons.[4–7] The quinolinic carbon skeleton of the mersinines is postulated to arise from an aspidofractinine precursor through ring-expansion of an aziridinium intermediate.[1] There are a total of 16 alkaloids, representing variations in aromatic substitution, functional groups, and stereochemistry. To date, the mersinine alkaloids can be divided into two broad stereochemical groups, viz., those with cis-D/E ring junction stereochemistry and a C(20)–βCO_2Me group (e.g., mersinines A and B, **3** and **4**), and those with trans-D/E ring junction stereochemistry and a C(20)–αCO_2Me group (e.g., mersinine C **5**).[1] The relative configurations at C-2, C-7 and C-21 are all (*R*), based on extensive NOE experiments[1] as well as an X-ray diffraction study of mersinine A (**3**),[8] while the relative configuration at the quaternary C-16 can be assigned from the NMR data as described previously.[1,2]

2. Results and discussion

We now report the isolation of two additional polar alkaloids, mersiphyllines A and B, from the leaf-extract. These polar alkaloids proved to be difficult to purify as they resisted resolution by conventional chromatography as well as HPLC. Eventually they were successfully separated by repeated passage through Sephadex G-75. The distinguishing feature of the ^{1}H NMR spectra of both alkaloids is the presence of a low field one-H singlet at ca. δ 16, which undergoes exchange with D_2O. Another departure in the NMR spectroscopic data when compared with the other mersinine alkaloids concerns the downfield shift of C-6 and H-6α to δ_C 40 and δ_H 2.1, respectively, and the upfield shift of H-18α and H-19α to δ 0.8.[1,2]

Mersiphylline A (**1**) was initially obtained as a light yellowish oil, and subsequently, as colorless crystals from EtOH, mp 184–186 °C, $[\alpha]_D^{25}$ −59 (*c* 0.43, $CHCl_3$). The UV spectrum (219, 245 and 287 nm) was similar to those of the other mersinine alkaloids, while the IR spectrum showed bands at 3463, 1746 and 1717 cm^{-1}, due to OH, ester/acid and carbamate functionalities, respectively. The EIMS showed an M^+ at *m*/*z* 486, which analyzed for $C_{24}H_{26}N_2O_9$, differing from mersinines A–C (**3**–**5**) by 14 mass units. The ^{13}C NMR spectrum (Table 1) accounted for all 24 carbon resonances, and confirmed the presence of carbamate (δ 154.4) and two carboxyl functions (δ 170.8 and 175.4, attributable to ester and/or acid groups), in addition to a low-field quaternary resonance (δ 87.3) due to C-16, which is α to both a nitrogen and an oxygen atom. The ^{1}H NMR spectrum (Table 1) showed signals due to two adjacent aromatic hydrogens (AB doublets

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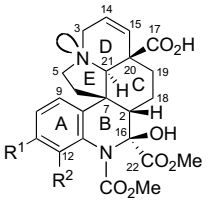
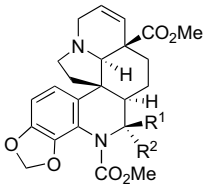
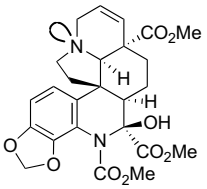


1 R^1, R^2 = OCH_2O
2 R^1 = H, R^2 = OMe



3 R^1 = CO_2Me, R^2 = OH
4 R^1 = OH, R^2 = CO_2Me



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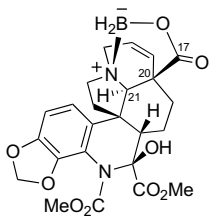

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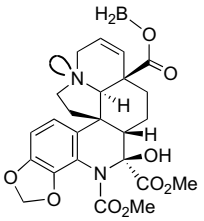

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at δ_H 6.65, 6.74), two olefinic hydrogens (δ 5.86), a methylenedioxy function (δ_H 6.01, 6.02), two singlets due to carbamate and ester methoxy groups (δ 3.77, 3.81), and two broad OH singlets, δ 5.25 and δ 16.35, which undergo exchange with D_2O. The COSY and HMQC data showed the presence of NCH_2CH_2, $NCH_2CH{=}CH$, $CHCH_2CH_2$ partial structures, as well as an isolated aminomethine corresponding to H-21. These, and the HMBC data (H-2 to C-8, C-6, C-16; H-5 to C-7; H-9 to C-7; H-15 to C-17; H-19 to C-2, C-17; H-21 to C-3, C-15, C-17, C-19) indicated that mersiphylline A has the same carbon skeleton as the mersinines (e.g., mersinines A–C, **3**–**5**).[1,2]

However, instead of the presence of the two characteristic methyl ester groups, one at C-16 and the other at C-20, as is the case in the other mersinine alkaloids, the ^{1}H NMR spectrum showed the presence of only one ester function, and two OH signals (one strongly deshielded), although two carboxyl functionalities associated with ester and/or acid functions were present (δ 170.8 and 175.4). One of the two carbonyl resonances must therefore be due to an acid group. In the HMBC spectrum, the ester methyl hydrogens at δ 3.77 showed a clear three-bond correlation to the carbonyl resonance at δ 170.8, indicating that this carbonyl (C-22) is associated with the methyl ester function. On the other hand, clear three-bond correlations were observed from H-21 and H-19 to the carbonyl carbon at δ 175.4 (C-17) indicating that this carbonyl is associated with the acid group attached to C-20. In both mersiphylline A (**1**) and mersiphylline B (**2**) (which differs from mersiphylline A (**1**) only in aromatic substitution, i.e., 12-OMe instead of 11,12-methylenedioxy, Table 1), the EI-mass spectra showed in addition to the $[M]^+$ peaks, strong fragment peaks due to $M-CO_2$ and M−COOH (*m*/*z* 442 and 441, respectively, in the case of **1**, and *m*/*z* 428 and 427, respectively, in the case of **2**), while the acid functionality in **1** can be readily esterified with $TMSCHN_2$ in MeOH/$PhCH_3$ (replacement of low field acid signal at δ 16.35 by a methyl ester signal at δ 3.71 in the methyl ester product),[9] providing additional proof for the presence of the carboxylic acid function in **1** and **2**.

The reciprocal NOEs observed for H-9/H-21 and H-19α/H-21 (Fig. 1), permitted assignment of the relative configurations at C-7 and C-21 which were similar to those in mersinines A and B.[1] The configuration at the quaternary C-16 was deduced to be similar to that in mersinine B, i.e., (*S*), from the characteristic C(16)–OH shift

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of **1**, **2** and **6**

atom	**1**[a]		**2**[a]		**6**[b]	
	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)
2	48.0	2.60 (m)	48.5	2.66 (m)	46.7	2.66 (dd, 12.6, 6.6)
3α	52.7	3.89 (m)	52.9	4.14 (br d, 16)	66.1	4.24 (ddd, 16, 4, 1)
3β		3.51 (br d, 16)		3.59 (br d, 16)		3.75 (m)
5α	50.2	3.37 (m)	51.1	3.80 (m)	59.7	3.46 (dd, 10, 6)
5β		2.60 (m)		2.72 (m)		2.85 (ddd, 13, 10, 5)
6α	39.0	2.08 (dd, 13, 4.5)	38.8	2.14 (dd, 13, 4.5)	37.3	1.99 (dd, 13, 5)
6β		2.78 (td, 13, 6)		2.84 (td, 13, 6)		3.29 (td, 13, 6)
7	44.7	—	44.5	—	43.6	—
8	129.0	—	136.3	—	129.1	—
9	117.2	6.74 (d, 8.2)	116.2	6.90 (d, 8)	115.7	6.44 (d, 8.6)
10	103.9	6.65 (d, 8.2)	125.0	7.17 (t, 8)	104.0	6.66 (d, 8.6)
11	147.8	—	111.7	6.94 (d, 8)	148.2	—
12	139.8	—	152.6	—	140.4	—
13	119.3	—	125.7	—	119.5	—
14	127.1	5.86 (m)	126.9	5.89 (m)	127.1	5.99 (m)
15	133.4	5.86 (m)	133.3	5.89 (m)	132.1	5.89 (ddd, 9.5, 2.4, 1)
16	87.3	—	87.4	—	87.4	—
17	175.4	—	175.9	—	169.4	—
18α	20.4	0.84 (m)	20.4	0.75 (s)	20.6	0.79 (m)
18β		1.45 (m)		1.44 (m)		1.43 (m)
19α	23.9	0.84 (m)	24.2	0.82 (m)	24.8	0.79 (m)
19β		2.84 (m)		2.74 (m)		2.46 (m)
20	44.1	—	44.7	—	43.2	—
21	70.7	3.35 (s)	70.4	3.49 (s)	73.4	3.85 (m)
22	170.8	—	170.6	—	170.5	—
12-OMe		—	56.6	3.88 (s)	—	—
22-OMe	53.0	3.77 (s)	53.1	3.75 (s)	53.1	3.74 (s)
NCO_2Me	53.3	3.81 (s)	53.1	3.75 (s)	53.2	3.79 (s)
NCO_2Me	154.4	—	155.9	—	154.5	—
OCH_2O	101.6	6.01 (d, 1.3), 6.02 (d, 1.3)	—	—	102.1	6.02 (d, 1.2) 6.03 (d, 1.2)
16-OH	—	5.25 (br s)	—	5.32 (br s)	—	5.36 (br s)
17-OH	—	16.35 (s)	—	15.27 (br s)	—	—

[a] Measured in $CDCl_3$.
[b] Measured in CD_2Cl_2.

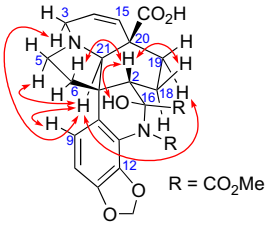

Figure 1. Selected NOEs of **1**.

of δ 5.25 and the C-2 shift of δ 48.0.[1] The presence of Wenkert–Bohlmann bands[10,11] in the IR has been previously invoked to signify the presence of a trans-D/E ring junction stereochemistry, with a β-oriented N-4 lone pair, in mersinine C, mersifoline C, mersidasine F and mersidasine G.[1] This conclusion was also supported by the NOE enhancement observed for H-3α, H-5α and H-9 on irradiation of H-21. In the case of mersiphyllines A and B, although Wenkert–Bohlmann bands were not detected (possibly due to intramolecular H-bonding involving the N-4 lone pair, vide infra), these NOEs were also observed, suggesting the presence of a trans-C/D junction. Irradiation of H-2 resulted in enhancement of H-6β, H-18β and 16-OH, while irradiation of H-18β resulted in enhancement of H-2 (Fig. 1). These observations indicated a β-orientaion for H-2 (2*S*) and represents a significant departure from the previous mersinine compounds, where the orientation of H-2 is α (2*R*) as indicated by the observed H-2/H-21 or H-6β/H-18β NOEs. This may also be reflected to some extent by the noticeable departure in the ^{1}H (H-6α, H-18α, H-19α) and ^{13}C (C-6) NMR data compared to those of the mersinines (vide supra).

The remaining issue concerns the relative configuration at the carboxyl bearing quaternary center, C-20. In this instance the observed NOEs were insufficient to unambiguously assign the configuration. An early indication that the orientation of the acid group is β was from the observation of the deshielded low-field acid-H signal at δ 16.35, suggesting intramolecular H-bonding to N-4 (with its β-oriented lone pair in view of the trans-D/E fusion mentioned earlier).

In the event, a second line of evidence was obtained which provided cogent proof of 20(*R*) configuration. In an attempt to reduce the acid group, **1** was treated with BH_3–THF which unexpectedly gave rise to an organoborane complex, as deduced from the spectral data. The mass-spectral data clearly showed boron incorporation ($[M]^+$, *m*/*z* 498), while the IR spectrum showed the characteristic B–H stretching frequencies at 2431, 2376 and 2285 cm^{-1}.[12] The ^{1}H and ^{13}C NMR spectral data (Table 1) of the complex were essentially similar to those of **1**, except for loss of the low field acid signal in ^{1}H NMR, and the distinct downfield shifts of the C-3, C-5 and C-21 signals in the ^{13}C NMR spectrum (and the corresponding H-3, H-5 and H-21 signals in the ^{1}H NMR spectrum), an effect somewhat reminiscent of that shown by alkaloid *N*-oxide derivatives, suggesting that N-4 has been rendered electron-deficient.

A likely formulation for the organoborane complex is one in which BH_2 has been incorporated into the molecule via formation of a O═CO–boron as well as a dative N(4)→B bond, linked at the carboxyl oxygen and at N-4, respectively, as shown in **6**. Such a structure would be compatible with the MS, IR and NMR data (the B–H hydrogens were not observed in the ^{1}H NMR spectrum due to broadening[13,14]). Additional confirmation was obtained by accurate mass measurements of both the $C_{24}H_{27}N_2O_9{}^{11}B$ ($[M]^+$) as well as the $C_{24}H_{26}N_2O_9{}^{10}B$ ($[M-H]^+$) peaks in HREIMS which were in excellent agreement with the proposed constitution of the complex. The formation of such a complex, presumably via intramolecular interception by N-4 of the initially formed organoborane intermediate **7**,[15,16] is only possible if the C-20 carboxylic acid function has β-orientation (20*R*) (C(20)–COOH and N-4 lone pair *syn*). The alkaloid–borane complex also showed a better resolved ^{1}H NMR spectrum with less overlap compared to that of mersiphylline A allowing for better NOE data to be obtained.

Finally, to obtain support for the above deduction, as well as to secure unambiguous proof of the structure, X-ray diffraction analysis was carried out for **1** (Fig. 2) which provided confirmation of the structure and relative configuration deduced from all the above observations.[17]

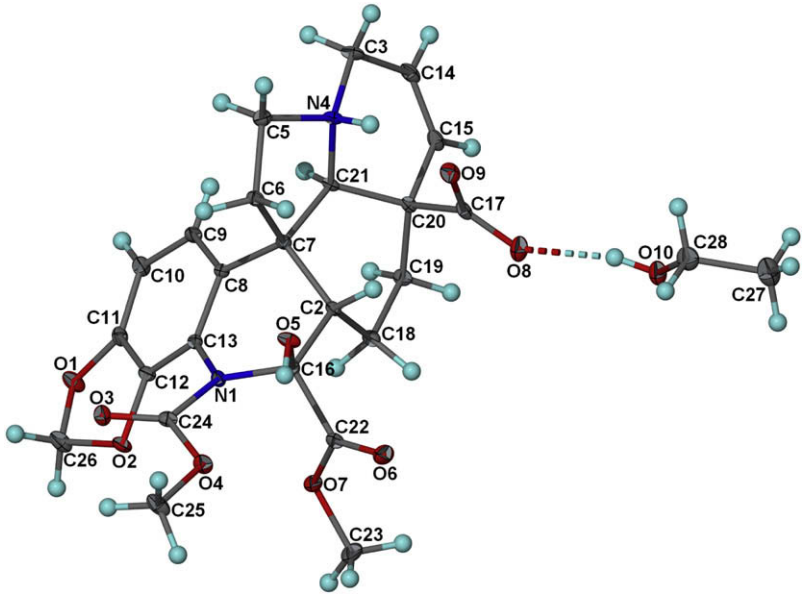

Figure 2. X-ray crystal structure of **1**. Thermal ellipsoids are shown at the 50% probability level.

With the structure of mersiphylline A thus established, the structure of mersiphylline B follows readily from the spectroscopic data. Mersiphyllines A and B represent yet another addition to the mersinine group of alkaloids, constituting a new and distinct stereochemical group, with trans D/E ring junction stereochemistry, a β-oriented carboxylic acid functionality linked to the quaternary

C-20 (20*R*), and a β-oriented hydrogen at C-2 (2*S*). Although several examples of related organoborane complexes exist in the literature, such as the condylocarpine–BH_3 adduct,[18] various simple cyclic borane derivatives of amino acids,[12] and the chiral oxazaborolidines (or CBS reagent),[13] the present example nevertheless represents the first instance where the formation of an alkaloid–borane complex has been invoked to underpin a difficult stereochemical assignment at a quaternary stereogenic center in an alkaloid.

3. Experimental

3.1. General

Optical rotations were determined on a JASCO DIP-370 digital polarimeter or an Atago Polax-D polarimeter. IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz respectively. X-ray diffraction analysis was carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube (λ=0.71073 Å). EIMS and HREIMS were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia. All air-moisture-sensitive reactions were carried out under N_2 in oven-dried glassware. THF was freshly distilled from Na/benzophenone, under N_2. All other reagents were used without further purification.

3.2. Material and extraction of alkaloids

Details of collection, identification, deposition and extraction of plant material have been reported previously.[1]

3.3. Isolation

Column chromatography (silica gel, vacuum, MeOH/$CHCl_3$) of the basic fraction derived from the EtOH extract of the leaves provided ten fractions.[1] Repeated chromatography of fraction-10 (Sephadex G-75, MeOH) gave **1** (4.4 mg kg^{-1}) and **2** (5.0 mg kg^{-1}).

3.3.1. *Mersiphylline A* (***1***)

Colorless crystals from ethanol; mp 184–186 °C; $[\alpha]_D^{25}$ −59 (*c* 0.43, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 219 (4.21), 245 (3.79), 287 (3.16) nm; IR (dry film) ν_{max} 3463, 1746, 1717 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 486 $[M]^+$ (43), 442 (100), 441 (88), 427 (37), 409 (29), 381 (48), 355 (22), 327 (20), 295 (15), 260 (10), 204 (26), 158 (42); HREIMS *m*/*z* 486.1629 (calcd for $C_{24}H_{26}N_2O_9$ $[M]^+$, 486.1638).

3.3.2. *Mersiphylline B* (***2***)

Colorless oil; $[\alpha]_D^{25}$ −58 (*c* 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 212 (3.84), 245 (3.22), 287 (2.90) nm; IR (dry film) ν_{max} 3427, 1744, 1711 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 472 $[M]^+$ (73), 441 (18), 428 $[M-CO_2]^+$ (100), 427 $[M-CO_2H]^+$ (91), 413 (98), 395 (18), 381 (59), 367 (55), 351 (13), 335 (23), 299 (21); HREIMS *m*/*z* 472.1836 (calcd for $C_{24}H_{28}N_2O_8$ $[M]^+$, 472.1846).

3.4. Mersiphylline A–borane (6)

BH_3–THF (28 μL, 1 M) was added to **1** (7 mg, 0.0014 mmol) in THF at 0 °C under N_2 and the mixture was stirred for 5 h, after which a further 28 μL (1 M) BH_3–THF solution was added, and the mixture stirred for another 1 h at 0 °C. Removal of the solvent in vacuo, followed by preparative centrifugal TLC over SiO_2 (5% MeOH–Et_2O) gave **6** (6 mg, 84%; direct workup without H_2O or MeOH quenching gave the best yield of **6**) as a colorless oil: $[\alpha]_D^{25}$ −58 (*c* 0.04, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 219 (4.78), 243 (4.24), 289 (3.67) nm; IR (dry film) ν_{max} 3480 (OH), 2432, 2376, 2285 (BH), 1748 (C=O, ester), 1704 (C=O, carbamate), 1694 (C=O, borane ester) cm^{-1}; ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; EIMS *m*/*z* (rel int) 498 $[M]^+$ (35), 496 (16), 441 (100), 409 (33), 381 (49), 323 (25), 204 (18), 158 (26), 44 (63); HREIMS *m*/*z* 498.1780 (calcd for $C_{24}H_{27}N_2O_9{}^{11}B$ $[M]^+$, 498.1804), *m*/*z* 496.1754 (calcd for $C_{24}H_{26}N_2O_9{}^{10}B$ $[M-H]^+$, 496.1762).

3.5. X-ray crystallographic analysis of 1

A single crystal of **1** was obtained from EtOH; $C_{24}H_{26}N_2O_9\cdot C_2H_5OH$, M_r=532.54, orthorhombic, space group $P2_12_12_1$, a=10.8951(5) Å, b=14.6956(7) Å, c=15.0936(7) Å; V=2416.6(2) Å^3, Z=4, D_{calcd}=1.464 g cm^{-3}. The structure was solved by direct methods and refined by the least-squares method. The final R value is 0.0426 (R_W=0.0931) for 3334 reflections [$I>2\sigma(I)$]. Further details of crystal structure including final atomic parameters have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 725732). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

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