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Tetrahedron Letters
Tetrahedron Letters 47 (2006) 8653–8655

Arbophylline, a novel heptacyclic indole with a cage skeleton incorporating an acetal moiety

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Received 1 September 2006; revised 20 September 2006; accepted 28 September 2006
Available online 20 October 2006

Abstract—A new indole alkaloid, arbophylline, possessing a novel heptacyclic cage skeleton, and incorporating an acetal function, was obtained from the Malayan *Kopsia arborea*. The structure was established by spectroscopic analysis and a possible biogenetic pathway from an akuammiline-type precursor is presented.

Plants of the genus *Kopsia* (Apocynaceae) have proven to be fertile sources of novel indole alkaloids, characterized by unusual carbon skeletons as well as useful biological activities.[1–16] We recently reported the structure of arboflorine (**1**), a minor alkaloid isolated from the stem-bark extract of *K. arborea*.[1] Arboflorine (**1**) is characterized by a novel pentacyclic structure, incorporating a third nitrogen atom embedded within a δ-lactam E ring. We proposed a possible biogenetic pathway from a preakuammicine-type precursor, featuring an initial Grob-like fragmentation, conjugate addition by ammonia to a conjugated iminium ion, a retro-vinylogous Mannich reaction, followed by an intramolecular Mannich reaction, *en route* to the novel ring system of arboflorine (**1**). Other recent examples of unusual alkaloids from *Kopsia*, which are notable for possessing novel ring systems, and which were postulated to derive from known monoterpenoid indole precursors through pathways involving deep-seated rearrangements and/or loss of key fragments include, *inter alia*, mersicarpine,[3] and mersilongine.[4] We now report the isolation and structural elucidation of yet another novel alkaloid, arbophylline (**2**), this time from the leaf extract of the same plant, characterized by an unprecendented heptacyclic cage structure, and incorporating an acetal unit. Previous examples of indole alkaloids from *Kopsia* possessing a cage structure include the kopsinitarines and mersingines.[12,14]

Arbophylline (**2**), was obtained as a minor alkaloid from the basic fraction derived from the EtOH extract of the leaf extract of *K. arborea* following repeated chromatographic fractionation (colorless oil, yield ca. 2.8 mg kg^{-1}), $[\alpha]_D$ +2 (*c* 0.16, $CHCl_3$). The UV spectrum (EtOH) showed characteristic dihydroindole absorptions at 210, 242, and 297 nm, while the IR spectrum showed bands at 3331 and 1730 cm^{-1}, suggesting the presence of *N*H and ester functionalities, respectively. The presence of the *N*H signal at δ 5.06, the ester carbonyl signal at δ_C 172.8, and a methoxy singlet at δ_H 3.87, confirmed the presence of indolic *N*H and methyl ester functions. The EIMS of **2** showed a molecular ion at *m*/*z* 366 with a base peak observed at *m*/*z* 323, and another prominent peak due to loss of carbomethoxy at *m*/*z* 307. The ^{1}H NMR spectrum (Table 1) showed, in addition to the *N*H and ester methyl functionalities mentioned previously, the presence of an unsubstituted indole ring from the occurrence of the signals of four contiguous aromatic hydrogens, an ethylidene side chain (methyl doublet of triplets at δ 1.52; one-H quartet at δ 5.30), a methine doublet, attributed to an acetal function at δ 5.52, another isolated methine, observed downfield as a singlet at δ 4.74 due to it being adjacent to both a nitrogen and an oxygen atom, and an isolated aminomethylene seen as an unresolved multiplet at δ 3.46 (δ_C 53.9).

The ^{13}C NMR spectrum (Table 1) gave a total of 21 carbon resonances (two methyls, three methylenes, nine methines, and seven quaternary carbons) in agreement with the molecular formula ($C_{21}H_{22}N_2O_4$, 12 degrees of unsaturation) obtained from HREIMS

Keywords: Alkaloids; Indoles; NMR; Plants.
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doi:10.1016/j.tetlet.2006.09.152

Table 1. ^{1}H and ^{13}C NMR spectral data of **2**[a]

Position	δ_C	δ_H	NOE/NOESY
2	104.0	—	
3	57.5	3.79 d (6.5)	14α, 21α, *N*H
5	98.5	5.52 d (2.7)	6α, 6β
6α	42.7	1.74 dd (12.1, 2.7)	5
6β		2.30 d (12.1)	5, CO_2Me
7	54.6	—	
8	131.3	—	
9	125.3	7.67 d (7.5)	10, CO_2Me
10	118.9	6.76 td (7.5, 0.9)	9, 11
11	128.9	7.13 td (7.7, 1.2)	10
12	109.4	6.65 d (7.7)	11, *N*H
13	149.2	—	
14α	22.4	1.38 dd (13.8, 6.7)	3, 15, 21α
14β		1.84 dd (13.8, 5.9)	15
15	34.6	3.20 d (5.9)	14β, 18, CO_2Me
16	53.6	—	
17	88.8	4.74 s	21β, CO_2Me
18	13.0	1.52 dt (6.8, 1.5)	15, 19, CO_2Me
19	118.1	5.30 q (6.8)	18, 21β
20	135.8	—	
21β	53.9	3.46 m	17, 19
21α		3.46 m	3, 14α
CO_2Me	52.5	3.87 s	6β, 9, 15, 17, 18
CO_2Me	172.8	—	
*N*H	—	5.06 br s	3, 12

[a] $CDCl_3$, 400 MHz; assignments based on COSY, HMQC and HMBC.

measurements.[17] After accounting for the six aromatic carbons from consideration of their characteristic shifts as well as from the HMBC spectrum, the remaining two carbons corresponding to C(7) and C(2) of the dihydroindole moiety, can be assigned from the three-bond correlations from H(9) to C(7), and from H(6) and H(14) to C(2), respectively. The observed shift of C(2) was noticeably downfield for a dihydroindole, and indicated oxygenation at this position.

The COSY and HMQC spectra revealed in addition to the aromatic and ethylidene hydrogens, as well as the isolated methine and methylene mentioned previously, the presence of two additional spin systems, namely, a $CHCH_2$, and an $NCHCH_2CH$. The former corresponds to the C(5)–C(6) fragment, from the three-bond correlations observed from H(5) to C(7) and from H(6) to C(8) in the HMBC spectrum. The observed downfield shift of C(5) at δ 98.5 is consistent with it being associated with an acetal function, for which the corresponding methine shift at δ 5.52 has been alluded to previously (vide supra). Since there are a total of four oxygens in **2**, and two are due to the ester function, one of the oxygens of the acetal function must be linked to C(2), while the other to the methine at C(17) (δ_H 4.74, δ_C 88.8), which is in turn linked to *N*(4). This assignment is also supported by the observed three-bond correlation from H(5) to C(17) and from H(17) to C(5) and C(7) in the HMBC spectrum. The second fragment branching from *N*(4) is the $CHCH_2CH$ unit, which is linked from the C(15) methine to the quaternary C(16) bearing the methyl ester function, as indicated by the correlations from H(14) to C(16), and from H(15) to the ester carbonyl in the HMBC spectrum. The other methine corresponding to C(3) is deduced to be linked to C(2) from the observed correlations from H(14) to C(2), and from H(3) to C(7). Completion of the assembly of the structure of **2** requires insertion of the remaining fragment, comprising the ethylidene side chain linked to the quaternary C(20), which is in turn linked to C(15) and the isolated aminomethylene C(21). The structure of arbophylline thus unravelled is entirely consistent with the rest of the HMBC data (Fig. 1). Determination of the structure of **2** also allowed assignment of the base peak (*m*/*z* 323) observed in the mass spectrum of **2**, which though initially unintelligible, could now be attributed to the fragment ion resulting from loss of CO_2 ($M-CO_2+H$), as a consequence of the acetal functionality residing in the molecule.

The structure is also consistent with the NOE/NOESY data, which also confirms the relative stereochemistry at all the stereogenic centers. Thus, irradiation of the indolic *N*H caused the enhancement of H(12) and H(3), while the irradiation of H(3) resulted in enhancement of H(21α) and H(14α), in addition to *N*H. Irradiation of H(15) resulted in the enhancement of H(18) and vice versa confirming the geometry of the C(19),C(20)-double bond as *E*. NOEs were also observed between H(17) and H(21β), as well as between H(9) and the ester methyl. Although there are a total of seven stereogenic centers in **2**, the rigid cage architecture of the molecule in essence restricts the number of stereochemical possibilities to one enantiomeric pair, corresponding to the relative configuration shown. In any case, the NOEs observed were in complete agreement with the structure and relative configuration of arbophylline as shown in **2**.

The structure of arbophylline constitutes a novel skeleton of the monoterpenoid indole alkaloids. Its unprecedented heptacyclic ring system incorporates a cage structure, which is bounded by pyrrolidinyl, tetrahydrofuranyl, and tetrahydropyranyl rings. Another notable feature is the presence of an acetal functionality, with the acetal carbon being shared by the tetrahydrofuranyl and tetrahydropyranyl units.

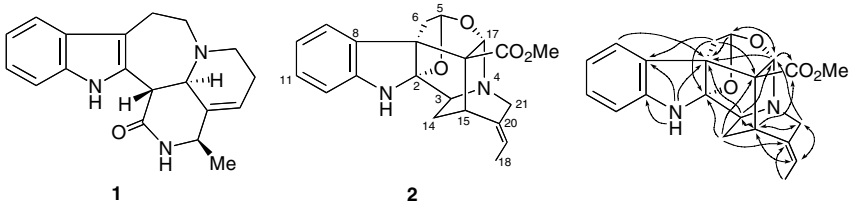
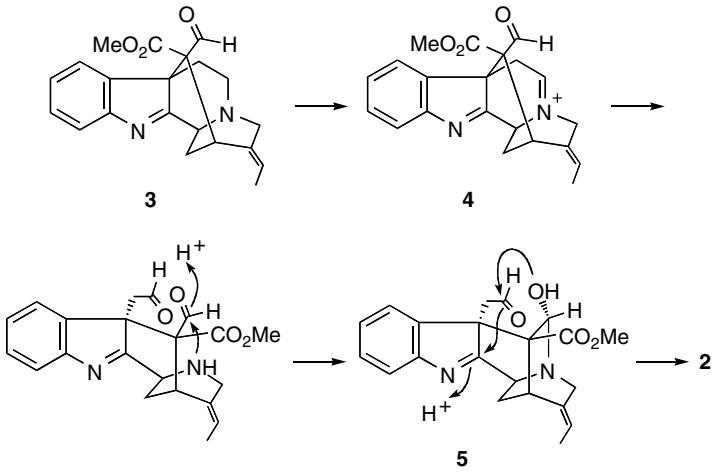


Figure 1. Selected HMBC's of **2**.



Scheme 1. Possible biogenetic pathway to **2**.

The novel ring system of arbophylline suggests that a rearrangement must have occurred from a known monoterpenoid alkaloid precursor, which in all probability involves cleavage of the C(5) to *N*(4) bond as a key step. A possible biogenetic route to **2** is shown in Scheme 1, from an akuammiline-type precursor such as rhazimal (**3**),[18–20] which was also detected as a minor alkaloid in the plant. Cleavage of the C(5)–*N*(4) bond occurs via hydrolysis of the C(5)–*N*(4) iminium ion **4**, in turn derived from the oxidation of **3**. An amine–aldehyde nucleophilic addition, involving attack of *N*(4) onto the C(16) aldehyde carbonyl leads to the pentacyclic hydroxy–aldehyde **5**. A tandem intramolecular process then ensues to effect the formation of the remaining two rings. This cascade is initiated by hemiacetal formation involving attack by the secondary alcohol function on the aldehyde carbonyl. This is then followed by hemiacetal OH attack on the imine function to complete the assembly of the heptacyclic ring system of arbophylline (**2**).

Arbophylline did not show any appreciable cytotoxicity when tested against drug-sensitive and vincristine-resistant KB cells, as well as Jurkat cells ($IC_{50} > 25$ μg/ml in all three cases).

Acknowledgements

We would like to thank the University of Malaya and IRPA for financial support, and Dr. K. Komiyama of The Kitasato Institute, Tokyo, Japan, for cytotoxicity assays. Mass spectral measurements were carried out by Dr. Noel Davies at OIC Organic Mass Spectrometry, University of Tasmania, Tasmania, Australia.

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