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Moschamide: An Unusual Alkaloid from the Seeds of *Centaurea moschata*

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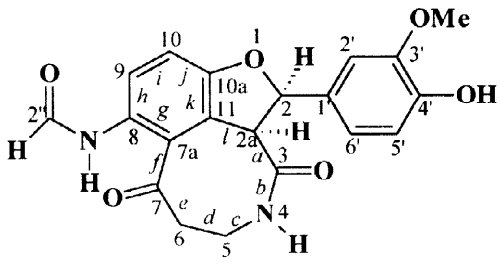
Abstract: 8-formamido-2-(4-hydroxy-3-methoxyphenyl)-2,2a,5,6-tetrahydro-3*H*-furo[2,3,4-*kl*][3]benz azocine-3,7(4*H*)-dione (named: moschamide), a novel alkaloid, has been isolated from the seeds of *Centaurea moschata* (Compositae). The structure of this compound has been determined primarily by extensive 1D and 2D NMR spectroscopic analysis, notably, ^{1}H, ^{13}C PENDANT, COSY45, NOESY, HMBC and HMQC .

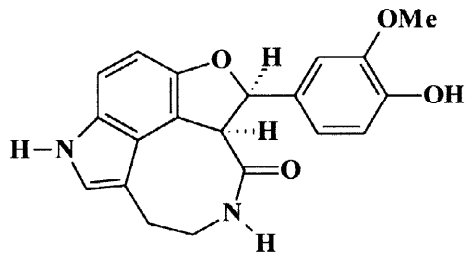
INTRODUCTION

Centaurea moschata L. (Family: Compositae) is an oriental annual from the genus *Centaurea* L. which comprises *ca.* 500 species, mainly from the Old World.[1] Many species of this genus have traditionally been used to cure various ailments, *e.g.* diabetes, diarrhoea, rheumatism, malaria, hypertension etc.[2] Several plant secondary metabolites have been reported from various species of *Centaurea*,[2, 3] and many of those compounds have also been found to have different kinds of potential pharmacological properties.[2] We have previously reported four new indole alkaloids[3], ecdysteroids[4] and a steroid glycoside[5] from the seeds of *C. moschata*. Herein, we report the isolation and structure elucidation of a novel alkaloid with an unusual structure from this plant.

RESULTS AND DISCUSSION

RP-HPLC analysis of the MeOH extract (defatted with n-hexane) of the seeds of *C. moschata* yielded a novel alkaloid which, on the basis of extensive spectroscopic analysis, was identified as 8-formamido-2-(4-hydroxy-3-methoxyphenyl)-2,2a,5,6-tetrahydro-3*H*-furo[2,3,4-*kl*][3]benzazocine-3,7(4*H*)-dione (**1**), and was named as moschamide (**1**).





Compound **1** showed a positive colour reaction with Dragendorff's reagent. The UV absorption maxima suggested the presence of substituted aromatic chromophores.[7] The IR spectrum indicated the presence

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of carbonyls (as secondary amides [1670-1650 cm^{-1}] and a ketone [1682 cm^{-1}]), a hydroxyl (3637 cm^{-1}) and aromaticity (3016, 1600-1500 cm^{-1}). An EIMS spectrum revealed the molecular ion peak at *m/z* 382 solving for $C_{20}H_{18}N_2O_6$. The ^{1}H-NMR spectrum (in CD_3OD) (Table 1), together with a ^{1}H-^{1}H COSY45 spectrum revealed signals resulting from five aromatic protons (δ_H 7.77, 7.03, 6.94, 6.83 and 6.80) from two aromatic rings (one trisubstituted and one tetrasubstituted), two methine protons including a highly deshielded oxymethine (δ_H 6.28), a formyl proton (δ_H 8.22), two methylene protons and protons from a methoxyl group (δ_H 3.83). The ^{1}H-NMR spectrum (in pyridine-d_5) (Table 1), in addition to the equivalent signals observed in the ^{1}H-NMR spectrum taken in CD_3OD, showed the presence of three broad singlets (δ_H 11.30, 10.80 and 9.09; each integrated for one proton) resulting from two NH protons and a phenolic hydroxyl proton.

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-PENDANT NMR (100 MHz) data (δ in ppm, *J* in Hz) for **1**

C/H	δ_H*	δ_H†	δ_C*	^{1}H-^{13}C long-range correlations§	
				^2J	^3J
2	6.28 d (5.8)	7.02 d (6.0)	85.9	C-2a, C-1'	C-2', C-6', C-11, C-10a, C-3
2a	4.75 d (5.8)	5.23 d (6.2)	54.6	C-2 , C-11, C-3	C-1', C-7a
3	-	-	172.8		
4-NH	-	10.80 bs‡	-		
5	3.50 m	3.62 m	37.5	C-6	C-3, C-7
	3.80 m	3.93 m			
6	3.55 m	3.80 m	43.2	C-5, C-7	
	2.89 m	3.03 m			
7	-	-	201.8		
7a	-	-	129.0		
8	-	-	127.5		
9	7.77 d (8.8)	8.72¶	125.2		C-7a, C-10a
10	7.03 d (8.7)	8.29 d (8.8)	112.6	C-10a	C-8, C-11
10a	-	-	156.9		
11	-	-	124.3		
1'	-	-	131.9		
2'	6.94 d (1.6)	7.30 s (1.6)	109.3	C-3'	C-2, C-6', C-4'
3'	-	-	147.9		
3'-MeO	3.83 s	3.69 s	55.1		C-3'
4'	-	-	146.7		
4'-OH	-	9.09 bs‡	-		
5'	6.80 d (8.1)	7.16 d (8.1)	115.1	C-4'	C-1', C-3'
6'	6.83 dd (1.7, 8.2)	7.20¶	118.4		C-4', C-2', C-2
1''-NH	-	11.30 bs‡	-		
2''	8.22 s	8.61 s	160.7		C-8

* Spectra obtained in CD_3OD referenced to CH_3OH at δ 3.31 (^{1}H) and δ 49.15 (^{13}C). † Spectrum obtained in pyridine-d_5 referenced to C_5H_5N at δ 8.74. ‡ data may be interchanged. ¶ Under solvent peak. § Direct ^{1}H-^{13}C correlations from HMQC and ^{1}H-^{13}C long-range correlations from HMBC.

The ^{13}C-PENDANT NMR[8] spectrum (Table 1) displayed 20 carbons including the 12 carbons of two aromatic rings, three carbonyl carbons (two amide and one ketonic), two methines (one oxymethine), two methylenes and a methoxyl carbon. All these ^{1}H and ^{13}C signals suggested that this compound has similar structural features as moschamindole (**2**).[3] The absence of the pyrrole ring system (present in moschamindole) in **1** was evident from the two extra carbonyls (δ_C 201.8, 160.7); the former, being part of the furobenzazocine nucleus, was linked to both the methylene protons (^3J from δ_H 3.50 and 3.80, and ^2J from 3.55 and 2.89). The formyl proton H-2'' (δ_H 8.22) was directly linked to C-2'' (δ_C 160.7), which was confirmed from HMQC direct ^{1}H-^{13}C correlation. This formyl proton showed only a ^3J correlation to the aromatic quaternary carbon (C-8, δ_C 127.5) which established the absence of the pyrrole ring and presence of a formamido group attached to C-8 . In the HMBC spectrum (Table 1) the methoxyl protons (δ_H 3.83) showed ^3J correlation to C-3' (δ_C 147.9) which

in turn showed a ^2J correlation from H-2' (δ_H 6.94) and a ^3J correlation from H-5' (δ_H 6.80), thus confirming the attachment of the methoxyl group at C-3'. Both H-2' and H-6' (δ_H 6.83) were correlated (^3J) to C-2 (δ_C 85.9), the proton (δ_H 6.28) of which in turn showed connectivities to C-1' (δ_C 131.9: ^2J), to C-2' (δ_C 109.3: ^3J) and C-6' (δ_C 118.4: ^3J). This established that the 4-hydroxy-3-methoxyphenyl moiety is connected to C-2. H-2 also showed ^3J correlation to C-11 (δ_C 124.3) and C-10a (δ_C 156.9). The latter was also connected to H-9 (δ_H 7.77: ^3J) and to H-10 (δ_H 7.03: ^2J). This confirmed that the carbons C-10a and C-2, sharing the same oxygen, formed the dihydrofuran ring fused to the benzazocine nucleus. Other key long-range ^{1}H-^{13}C HMBC correlations (Table 1) leading to the unambiguous identity of **1** were: from H-2a, ^2J to C-11 (δ_C 124.3) and ^3J to C-1' (δ_C 131.9) and C-7a (δ_C 129.0); from H_2-5 (δ_H 3.50, 3.80) ^3J to C-3 (δ_C 172.8) and C-7 (δ_C 201.8); from H_2-6 (δ_H 2.89, 3.55) ^2J to C-5 (δ_C 37.5) and C-7 (δ_C 201.8); from H-9 (δ_H 7.77), ^3J to C-7a (δ_C 129.0); from H-10 (δ_H 7.03), ^3J to C-8 (δ_C 127.5) and C-11 (δ_C 124.3). It is worth mentioning that the quaternary carbon at C-7a (δ_C 129.0), owing to the absence of any nearby protons, produced very weak signal in ^{13}C spectrum and that the expected ^3J correlation from H_2-6 to C-7a was not observed in the HMBC spectrum. The absence of H_2-6 to C-7a correlation might be because the dihedral angle approaches 90°.[9] The deshielded nature of the signal for H-9 (δ_H 7.77) suggested its close proximity to the deshielding cone of formamido carbonyl (C-2''), and a lack of nOe interaction between H-2'' and H-9 also established the spatial orientation of this formamido group which possibly also allows the 1''-NH to form a hydrogen bond with C-7 carbonyl oxygen leading to a more stable configuration. ^{1}H-^{1}H NOESY spectrum (Figure 1) determined the relative stereochemistry of this molecule and thus, the structure of this compound was confirmed as **1**. The extract as well as the HPLC fraction from which this alkaloid was purified was active in a bioassay[6] for ecdysteroid agonists. However, **1** is not responsible for this activity.

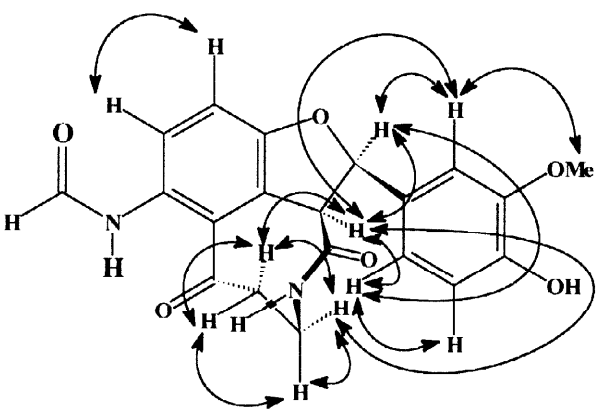

Figure 1: Relative stereochemistry of **1** based on ^{1}H-^{1}H NOESY experiment

MATERIALS AND METHODS

General experimental procedures. The UV spectrum was in MeOH. The IR spectrum was recorded in $CHCl_3$ on a Perkin-Elmer IR spectrometer (Model: 881). NMR spectra were obtained on a Bruker AVANCE DRX400 instrument using Bruker microprograms. HREIMS was recorded with a JEOL AX505HA instrument. HPLC separations were performed in a Gilson 802C HPLC coupled with Gilson UV-Visible detector, using a Technoprep $10C_8$ preparative column for initial isolation, and a Spherisorb C_6 semi-preparative column for final purification. RP stands for reversed-phase. HPLC separations were monitored at 242 nm.

Plant material. Seeds of *C. moschata* L. were purchased from Chiltern Seeds, Ulverston, UK. A voucher specimen has been retained at the Department of Biological Sciences, University of Exeter.

Extraction and isolation. Ground seeds (11 g) were extracted in a Soxhlet with, successively, n-hexane, $CHCl_3$ and MeOH, 500 mL each. The MeOH extract was concentrated using a rotary evaporator at a maximum temperature of 45°C. Preparative RP-HPLC with 55% MeOH/water at 5 mL/min yielded **1** as a broad UV-

absorbing peak eluting between 26 and 36 min. Compound **1** was further purified on C_6 semi-preparative column, eluted with 45% MeOH in water at 2 mL/min (retention time: 12 min.).

Moschamide (**1**) (2.8 mg): Yellow amorphous, UV λ_{max} nm (log ε) = 236 (3.93), 271 (3.53), 320 (2.98), 354 (3.01). IR ν_{max} cm^{-1} = 3637, 3450, 3016, 2434, 2401, 1824, 1682, 1670-1650, 1601, 1513, 1476, 1421, 1335, 1196, 1018, 927, 846, 805, 665; Found: $[M]^+$ 382.1151; $C_{20}H_{18}N_2O_6$ requires 382.1165; EIMS *m/z* (rel. int.) = 382 (37), 354 (6), 343 (12), 337 (9), 316 (14), 310 (12), 277 (100), 243 (19), 201 (21), 199 (21), 169 (60), 124 (33). ^1H and ^{13}C NMR (Table 1).

Bioassay. Ecdysteroid agonist and antagonist activities of **1** were assessed with a microplate-based bioassay using the *Drosophila melanogaster* B_{II} cell line.[6] The compound was tested at concentrations ranging from 10^{-8}M to 10^{-4}M. For the antagonist assay, a concentration of 20-hydroxyecdysone of 5 x 10^{-8}M was used.

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