

Concavine, an unusual diterpenic alkaloid produced by the fungus *Clitocybe concava*[☆]

Alberto Arnone,^a Adriana Bava,^a Giovanni Fronza,^a Gianluca Nasini^{a,*} and Enzo Ragg^b

^aCNR-ICRM, Dipartimento di Chimica, Materiali ed Ingegneria Chimica del Politecnico, Via Mancinelli 7, 20131 Milano, Italy

^bDipartimento di Scienze Molecolari Agroalimentari, Università degli Studi di Milano, via Celoria, 20133 Milano, Italy

Received 27 June 2005; revised 9 September 2005; accepted 12 September 2005

Available online 3 October 2005

Abstract—A novel alkaloid with an unprecedented ring system consisting of a dodecahydro-7-oxa-9a-aza-benzo[*a*]azulene ring (**1**), has been isolated from cultures of *Clitocybe concava* (Basidiomycetae). Its structure and relative stereochemistry were elucidated on the basis of spectroscopic data. Concavine presents a weak antibacterial activity.

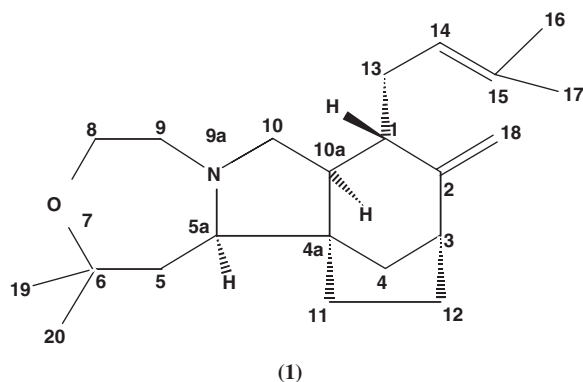
© 2005 Elsevier Ltd. All rights reserved.

In the course of a screening program for biologically active metabolites from Basidiomycetes, we have studied many strains of *Clitocybe* spp. and reported the isolation of a large number of protoilludene sesquiterpenoids from *C. elegans*, *C. candicans* and *C. illudens*, allenes from *C. eucaliptorum*, acetylenes from *C. catinus*² and clavilactones from *C. clavipes*.³ Further investigation of *Clitocybe* genus led to the isolation from a strain of *C. concava* of a novel diterpenic alkaloid; this letter deals with the isolation and the structure elucidation of concavine **1**, obtained from large-scale cultures of the fungus.

The ethyl acetate extracts of the mycelial cultures of the fungus⁴ grown on MPGA⁵ were chromatographed on silica gel and purified by repeated preparative TLC.⁶

Concavine **1** was isolated as an oil, $[\alpha]_D^{25} +8.4$ (*c* 0.14, MeOH); its molecular formula C₂₂H₃₅NO was deduced from HRMS,⁷ ¹H and ¹³C NMR spectral data (Tables 1 and 2). A first consideration on the structure was the fact that only three signals in ¹H NMR were greater than 4.1–1.0 ppm, thus diagnostic for a terpenoidic moiety. The presence in the mass spectrum of *m/z* 69/70 peaks is attributable to an isoprenoidic residue and the presence of an N atom suggests a diterpenic alkaloid

structure. The failure to acetylate the product together with the absence of significant peaks in the carbonyl region of the IR spectrum suggested that oxygen atom is present in the molecule as part of an ether moiety.



The structure and the relative stereochemistry of such a novel alkaloid were deduced from the analysis of ¹H and ¹³C spectra. The assignment of the spectra was accomplished through the proton–proton (COSY) and carbon–proton (HMQC, HMBC) chemical shift correlation techniques carried out at 600 MHz, whereas the spatial arrangement of the molecule was deduced from NOESY and ROESY spectra.

The NMR data are collected in Table 1 and confirm that **1** consists of 22 carbon and 35 hydrogen atoms in agreement with the HRMS results.

Keywords: *Clitocybe*; Basidiomycetes; In vitro culture; Isolation; Diterpenic alkaloid; NMR.

[☆]Secondary fungal metabolites: Part 65; for Part 64, see Ref. 1.

*Corresponding author. Tel.: +39 2 2399 3046; e-mail: gianluca.nasini@polimi.it

Table 1. ^{13}C and ^1H NMR data of compound **1** in acetone- d_6 and (CDCl_3)

	δ_{C}			δ_{H}		$J(\text{H,H})$ (Hz)	
1	40.84	(40.93)	D	2.19	(2.27)	1,10a = 10.5	9 α ,9 β = 12.5
2	155.63	(155.52)	S			1,13a = (7.0)	10a,10 α = 10.0
3	41.88	(41.91)	D	2.84	(2.88)	1,13b = (7.0)	10a,10 β = 4.1
4 α	29.98	(30.28)	T	0.99	(1.06)	1,18a = 1.0	10 α ,10 β = 11.5
4 β				1.86	(2.07)	1,18b = 1.0	11 α ,11 β = 12.5
4a	53.04	(52.99)	S			3,4 α = 4.5	11 α ,12 α = 8.5
5 α	38.92	(39.07)	T	1.51	(1.50)	3,4 β = 2.0	11 α ,12 β = 5.0
5 β				2.08	(2.27)	3,12 α = 1.0	11 β ,12 α = 5.0
5a	65.47	(65.90)	D	2.91	(3.13)	3,12 β = 7.0	11 β ,12 β = 9.5
6	74.78	(74.75)	S			3,18a = 1.0	12 α ,12 β = 12.5
8 α	60.35	(60.46)	T	3.55	(3.63)	3,18b = 1.0	13a,13b = (14.0)
8 β				3.95	(4.08)	4 α ,4 β = 11.5	13a,14 = 6.9
9 α	57.49	(57.82)	T	2.51	(2.63)	5a,5 α = 0.5	13b,14 = 6.9
9 β				3.28	(3.39)	5a,5 β = 8.5	14,16 = 1.5
10 α	60.06	(60.46)	T	2.63	(2.78)	5 α ,5 β = 14.8	14,17 = 1.5
10 β				3.28	(3.39)	8 α ,8 β = 14.0	18a,18b = 2.3
10a	49.08	(49.30)	D	1.66	(1.77)	8 α ,9 α = 2.5	
11 α	33.37	(33.41)	T	1.32	(1.41)	8 α ,9 β = 2.5	
11 β				1.48	(1.50)	8 β ,9 α = 10.5	
12 α	32.98	(33.12)	T	1.43	(1.50)	8 β ,9 β = 2.5	
12 β				1.92	(1.95)		
13a	30.14	(30.28)	T	2.19	(2.23)		
13b				2.19	(2.17)		
14	122.28	(122.39)	D	5.08	(5.08)		
15	132.15	(132.42)	S				
16	17.86	(17.99)	Q	1.59	(1.69)		
17	25.67	(25.80)	Q	1.67	(1.61)		
18a	107.98	(108.08)	T	4.73	(4.72)		
18b				4.86	(4.85)		
19	27.96	(27.05)	Q	1.21	(1.26)		
20	28.03	(28.37)	Q	1.15	(1.18)		

Table 2. Structural data of concavine **1** obtained by different NMR experiments

Experiment	Correlated nuclei
Proton–proton correlation (COSY)	CH ₂ (8)CH ₂ (9) CH ₂ (10)CH(10a)CH(1)CH ₂ (13)CH(14)C(15)CH ₃ (16,17) CH ₂ (5)CH(5a) CH ₂ (4)CH(3)CH ₂ (12)CH ₂ (11) CH(1)C(2)CH ₂ (18) CH(3)C(2)CH ₂ (18)
Long-range proton–carbon correlation (HMBC) ^a	C(6)OCH ₂ (8), C(6)CH ₂ (5), C(6)CH ₃ (19), C(6)CH ₃ (20) C(19)CH ₂ (5), C(20)CH ₂ (5) C(4)CH(10a), C(4)CH ₂ (11) C(11)CH(10a) C(4a)CH ₂ (5), C(4a)CH(10a), C(4a)CH ₂ (11), C(4a)CH ₂ (12), C(4a)CH(3)C(4a)CH ₂ (4) C(18)CH(3) C(2)CH(1), C(2)CH ₂ (4), C(2)CH ₂ (12), C(2)CH ₂ (13), C(2)CH(3)

^a Selected most significant data for the structural elucidation of **1**.

^{13}C spectrum shows the presence of one *gem*-disubstituted and one trisubstituted double bond and of 18 sp^3 -hybridized carbons: four methyl, eight methylene, four methine and two quaternary carbons. The structural fragments identified through the various NMR techniques are reported in Table 2 in condensed form.

The proton–proton correlation technique (COSY) identified six different fragments through the network of vicinal and long-range hydrogen coupling constants.

The link between these fragments was established by $^1\text{H}\{^{13}\text{C}\}$ HMBC spectrum which provided the correlation between carbons and protons separated by two and three bonds. In this way, it was possible to reconstruct the fragments CH₂(8)OCMe₂(6)CH₂(5), CH(10a)C(4a)CH₂(11)CH₂(12), C(10a)C(4a)CH₂(4), CH(1)C(2)CH₂(18) and CH(3)C(2)CH₂(18). Finally, the groups CH(5a), CH₂(9) and CH₂(10), whose proton signals are broad and did not consequently show any mutual correlation, must be linked to the only nitrogen atom present in the molecule.

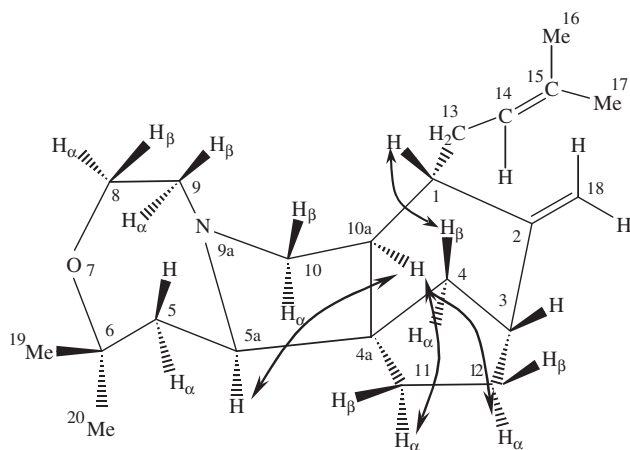
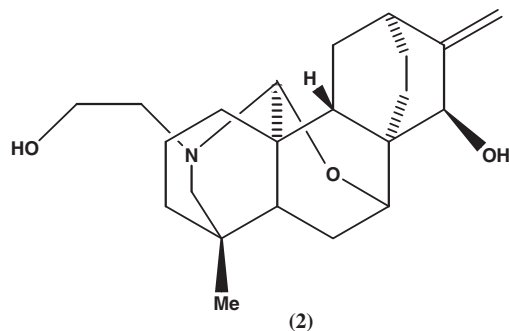


Figure 1. Selected NOESY correlations and relative stereochemistry for compound **1**.

The relative configuration of the five chiral carbons present in concavine **1** was determined from the ROESY spectrum (300 ms mixing time). As reported in Figure 1, the most significant among the observed NOE enhancements are those between the proton pairs H(5a)/H(10a), H(10a)/H(11a), H(10a)/H(12a) and H(1)/H(4b). The occurrence of these effects establishes that the relative orientation is *syn*. Therefore, assuming that the proton H(5a) is α -oriented, the five chiral carbons must have the relative configurations C(5a)(*R**), C(10a)(*S**), C(1)(*S**), C(3)(*R**) and C(4a)(*R**) (Fig. 1).

Plant species of the genera *Aconitum* and *Delphinium* are known sources of C_{20} diterpenoid alkaloids (DA) of pharmacological and economic importance.⁸ Ajaconine **2** from *Delphinium ajacis* was the first example of a C_{20} DA bearing an internal β -hydroxyethyl group attached to the N function, whereas usually the amine moiety is attached to one of the two geminal methyls in the first ring.⁹



The concavine structure represents a new skeletal variant, the two extra carbons relative to a normal DA suggesting the presence of an N(β -oxyethyl) system as in ajaconine **2** and related DA. To our knowledge, this is the first report of a natural DA from Basidiomycetes.

On biogenetic considerations, concavine **1** can be assumed to be a C_{20} DA, with an additional hydroxyethylamine group that becomes part of the aza-azulene ring. The four isoprenic moieties are evident in the formula, but their sequence is different from that common in most C_{20} – C_{22} DA (pimaradiene ring).¹⁰

Concavine **1** was tested for biological activity: it did not show any antifungal (*Cladosporium cladosporioides*, *Aspergillus niger*) and antitumor (tyrosine-kinase test) activity and had a weak antibacterial activities (50 μ g/disc) on *Bacillus cereus* (ATCC 1072) and *Bacillus subtilis* (ATCC 6633).¹¹

Acknowledgements

This work was in part supported by a Grant of MIUR-FIRB (Italy).

References and notes

- Assante, G.; Dallavalle, S.; Malpezzi, L.; Nasini, G.; Burruano, S.; Torta, L. *Tetrahedron* **2005**, *61*, 7686–7692.
- Nasini, G.; Vajna de Pava, O. In *Virtual Activity, Real Pharmacology, Different Approaches to the Search of Bioactive Natural Compounds*; Verotta, L., Ed.; Research Signpost: Trivandrum, 1997, and references cited therein.
- Merlini, L.; Nasini, G.; Scaglioni, L.; Cassinelli, G.; Lanzi, C. *Phytochemistry* **2000**, *53*, 1039–1041, and references cited therein.
- Strain CBS 473.87, obtained from the Central Bureau voor Schimmel Cultures, Baarn.
- Malt–peptone–glucose–agar 20:5:20:15 g/l, in 40 Roux Flasks 100 ml each, time of growth, four weeks at 24 °C; crude extract 0.4 g.
- Flash silica gel Merck filled with EtOAc and eluted with EtOAc–MeOH (95:5); PLC with CH_2Cl_2 –MeOH–Et₃N (9:1, 0.25% v/v); **1**, 35 mg.
- HRMS-*m/z* 329,2720; $C_{22}H_{35}NO$ requires 329,2718; EIMS, M^+ 329 (10%), 328 (12), 274 (20), 258 (100), 243 (62), 214 (15), 174 (25) and 69 (20); FABMS. MH^+ 330.
- Pelletier, S. W.; Keith, L. H. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic Press: New York, 1970; Pelletier, S. W.; Page, S. W. *Nat. Prod. Rep.* **1984**, 375–386.
- Dvornik, D.; Edwards, D. E. *Tetrahedron* **1961**, *14*, 54–57.
- Whalley, W. B. *Tetrahedron* **1962**, *18*, 43–54.
- Antibacterial and antifungal tests were performed using paper disks (6 mm diameter), soaked with metabolite **1** (100, 50 and 25 μ g dissolved in DMSO) and placed in a suitable culture medium on Petri dishes.