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Conicamin, a Novel Histamine Antagonist from the Mediterranean Tunicate *Aplidium conicum*

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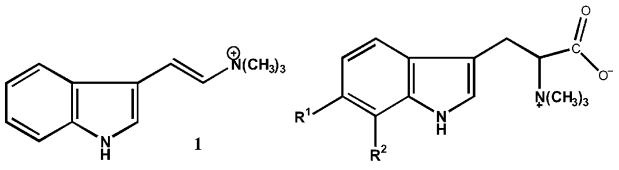
Abstract—In addition to the known 6-bromo-hypaphorine (**2**) and plakohypaphorine-A (**3**), the methanol extract of the Mediterranean tunicate *Aplidium conicum* was shown to contain conicamin, a novel indole alkaloid having histamine-antagonistic activity which structure was determined to be **1** on the basis of the spectral data.

Several reports have shown that marine ascidians remain unique among marine invertebrates in that they overwhelmingly produce nitrogen-containing secondary metabolites, almost all being derived from amino acids. Peptides, polycyclic aromatic alkaloids and tryptophan-derived metabolites have been isolated, in the last 25 years, from several species of ascidians.[1] Perhaps more significant than their interesting and sometime unique structures, the ascidian metabolites exhibit significant biological activities. Remarkable examples are didemnin B[2] and ecteinascidin 743,[3] potent antitumoral agents,[4] turbinamide, a selective cytotoxic agent against glioma cells,[5] eudistomins,[6] antiviral, eudistomidins A and C, which exhibit powerful calmodulin antagonistic activity,[7] and piclavine indolizines A–C, each exhibiting antifungal and antimicrobial activity against Gram-positive bacteria.[8]

As a part of our ongoing search for bioactive metabolites of Mediterranean ascidians, we have recently examined the species *Aplidium conicum* Olivi (= *Amoroucium conicum*; order Aplousobranchia, family Polyclinidae) which yielded an EtOAc extract that was shown to contain conicaquinones A and B; the two terpene quinones, containing a 1,1-dioxo-1,4-thiazine ring, exhibited a strong cytotoxicity in vitro against C6 (rat glioma) cells.[9] We have now examined the hydrophilic fractions obtained from the methanolic extract of the organism. This resulted in the isolation, in addition to the known 6-bromo-hypaphorine (**2**) and plakohypaphorine-A (**3**), of a novel alkaloid (**1**). Compound **1**, named conicamin, was identified as (*1E*)-[2-(1*H*-indol-3-yl)-vinyl]-trimethyl-ammonium ion by an extensive spectroscopic analysis. Conicamin was shown to be a specific histamine antagonist.

Chemistry

The orange coloured colonial tunicate *A. conicum*, collected along the coasts of Sardinia, Italy, was homogenised and exhaustively extracted twice with methanol and twice with chloroform. The combined extracts were concentrated and the resulting aqueous residue was partitioned between water and ethylacetate and, subsequently, the polar layer was re-extracted with *n*-BuOH. The latter organic phase was initially subjected to chromatography over a column packed with reverse phase silica gel (RP-18 MPLC) and eluted with five eluants:



2 R^1 = Br R^2 = H
3 R^1 = H R^2 = I

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H_2O, MeOH/H_2O 1:1, MeOH/H_2O 7:3, MeOH/$CHCl_3$ 9:1 and $CHCl_3$. Following this procedure, the MeOH/H_2O 1:1 fraction, separated by C_{18} reverse phase HPLC (Luna C_{18} 150×4.6 mm, 5 μm, MeOH/H_2O 25:75), gave 6-bromo-hypaphorine (**2**, 2.5 mg) while the fraction eluted with MeOH/H_2O 7:3 was further separated and purified by repeated C18 reverse phase HPLC (Luna C_{18} 150×4.6 mm, 3 μm, MeOH/H_2O 55:45) yielding conicamin (**1**, 10 mg) in a pure state. The aqueous phase was concentrated and separated by RP-18 MPLC. Fractions eluted with MeOH/H_2O (7:3) were pooled and purified by HPLC (Luna C_{18} 150×4.6 mm, 3 μm, MeOH/H_2O 4:6) to yield plakohypaphorine-A (**3**, 1.5 mg).

Both of the known hypaphorin-halogenated metabolites **2** and **3** were identified by comparison of their spectral data with literature values.[10,11]

Conicamin (**1**) was analysed for $C_{13}H_{17}N_2$ by FABMS (positive mode) in conjunction with ^1H and ^{13}C NMR data. A detailed analysis of ^1H and ^{13}C NMR spectra of **1**, aided by COSY, HSQC and HMBC 2D experiments, allowed the assignment of all protons and carbons signals (see Table 1).

Of the seven degrees of unsaturation in **1**, six were accounted for as an indole nucleus by the characteristic chemical shifts and coupling patterns of the five aromatic protons. In particular, in DMSO, the typical substitution pattern for a 3-substituted indole ring[12] was readily observed [δ 7.71 (s), δ 7.93 (bd, 7.4 Hz), δ 7.16 (dd, 7.4, 7.4 Hz), δ 7.21 (dd, 7.4, 7.4 Hz) and δ 7.47 (bd, 7.4 Hz)].

The two remaining mutually coupled doublets [δ 7.05 (d, 14.8 Hz) and δ 7.31 (d, 14.8 Hz)], present in the low-field region of the ^1H NMR spectrum, were assigned to the vinylic protons of a (*E*)-disubstituted conjugated olefin. The presence of this conjugated system was also substantiated by UV absorbance [λ_{max} 320 nm (ε = 4300)] typical of an indolic extended chromophore.

Table 1. ^{13}C (125 MHz) and ^1H (500 MHz) NMR spectroscopic data of compound **1** (DMSO-d_6) with ^1H–^{13}C HMBC correlations

Pos.[a]	δ_C[b]	δ_H[b] mult., int., *J* in Hz	^1H–^{13}C HMBC[c]
1-NH		11.5[d] (bs, 1H)	
2	129.6	7.71 (bs, 1H)	**3**, **3a**, **7a**
3	107.8		
3a	124.3		
4	120.2	7.93 (bd, 1H, 7.4)	**3**, **3a**, **6**, **7a**
5	120.5	7.16 (dd, 1H, 7.4, 7.4)	**3a**, **7**
6	122.7	7.21 (dd, 1H, 7.4, 7.4)	**5**, **7a**
7	112.5	7.47 (bd, 1H, 7.4)	**3a**, **5**
7a	137.3		
1′	131.4	7.05 (d, 1H, 14.8)	**2′**, **3**
2′	120.3	7.31 (d, 1H, 14.8)	**1′**, **2**, **3a**
$N(CH_3)_3$	55.0	3.41 (s, 9H)	**1′**

[a] ^1H NMR and ^{13}C NMR shifts are referenced to DMSO (^1H δ 2.49 and ^{13}C δ 39.5 ppm).
[b] Assignment are based on ^1H–^1H COSY, HMQC and HMBC experiments.
[c] ^1H correlating with ^{13}C resonance.
[d] D_2O exchangeable signal.

Additional features of the ^1H NMR spectrum were a signal at δ 3.41 (9H, s) attributable to the methyl protons of a trimethylammonium salt and a signal of an exchangeable proton at δ 11.5 long range coupled to the proton at δ 7.71, due to the NH proton of the indole ring.

The connectivities of the indole ring and the vinyltrimethylammonium moiety were fully established by long range heterocorrelation NMR methods (HMBC experiment performed at $J = 10$ Hz). In particular, the HMBC correlations of H-2′ (δ 7.31) with the indolic carbons at δ 129.6 and 124.3, and that of $-N(C\underline{H}_3)_3$ with the carbon at δ 131.4 which in turn was long range coupled with the olefin proton at δ 7.31 proved that the vinyltrimethylammonium moiety was linked at C-3.

Pharmacology

Antihistaminic activity has been studied on ileum of male guinea-pigs[13] and it is based on the antagonism to the contraction of intestinal smooth muscle produced by histamine. Animals have been killed, the ileum removed and placed in organ baths containing warm (37 °C) and aerated (5% CO_2 and 95% O_2) Krebs solution. The preparations have been connected to an isotonic transducer (load 0.5 g) in such a way as to record contractions mainly from the longitudinal axis: contractions have been recorded using a PowerLab system (Ugo Basile, Comerio, Italy). Concentration–response curves for histamine alone and in presence of conicamin have been performed. Histamine (10^{-7}–10^{-3} M) produced a concentration-dependent contraction of the isolated ileum. Conicamin at the concentration of 10^{-6}–10^{-5} M produced a significant ($p < 0.001$; $n = 8$) concentration-dependent reduction of the histamine-induced contraction (Fig. 1). The calculation of pA_2 value (negative logarithm of the molar concentrations of the antagonist

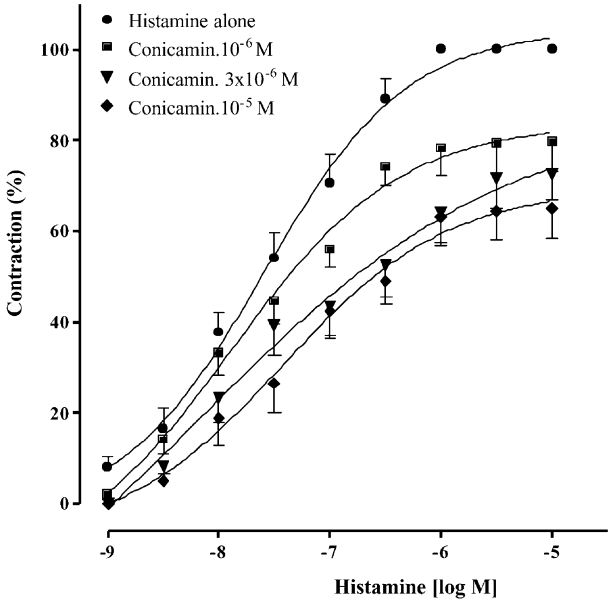

Figure 1. Effect of conicamin (10^{-6}–10^{-5} M) on histamine-induced contraction.

which induced a 50% decrease in the maximal response to the agonist) indicated a non-competitive antagonism effect. In addition, conicamin did not have affect on acetylcholine and $BaCl_2$-induced contraction suggesting a specific antagonistic effect for histamine (data not shown).

The present study shows that conicamin, an alkaloid isolated from *A. conicum*, exhibits an antagonist effect of a non-competitive type toward histamine receptors. This effect is concentration-dependent, occurs in the micromolar range and seems specific. In fact, conicamin is not able to reduce the contraction of intestinal smooth muscle induced by acetylcholine or $BaCl_2$.

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