Pergamon

Tetrahedron Letters, Vol. 35, No. 38, pp. 7023-7026, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01454-X

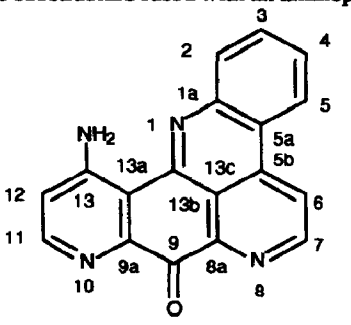
Cystodamine, a New Cytotoxic Fused Polyaromatic Alkaloid from the Mediterranean Ascidian *Cystodytes delle chiajei.*

Nataly Bontemps, Isabelle Bonnard, Bernard Banaigs, Georges Combaut and Christian Francisco*

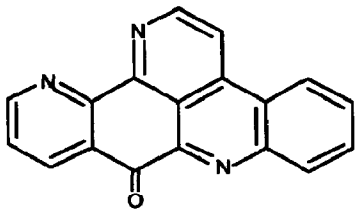
Groupe d'Etudes des Métabolites Marins d'intérêt Biologique,
Université de Perpignan, 66860 Perpignan Cedex.(France).

Abstract : a fused pentacyclic aromatic alkaloid - cystodamine - was isolated from a Mediterranean ascidian *Cystodytes delle chiajei* (Polycitoridae). The structure, determinated by extensive 2D-NMR means, is the first example of a marine product displaying a ^{1}H-^{14}N coupling during ^{1}H NMR analysis.

The prolific variety of nitrogenous natural products found in ascidians portrays these marine animals as experts in the production of unusual cyclopeptides[1] and of alkaloids.[2] Our continuing interest in the chemistry of tunicates[3] is drawn to new biological active alkaloids. However, such compounds are often biosynthetized in tiny amounts by these marine organisms, leading to the use of a simplified HPLC system for the detection of compounds capable of binding to DNA[4] to guide the chromatographic separations. In this paper, we report the structure of a new alkaloid, that we called cystodamine **1**, which was found to contain a phenanthroline substructure fused with an aminopyridine moiety.



1



2

Green morphs of the encrusting colonial ascidian *Cystodytes delle chiajei* (Polycitoridae), were collected near the Bay of Gabes, at Skhira (-7m depth), Tunisia, in September 1992 by using SCUBA. The animals were ground and extracted with $CHCl_3$ / MeOH. Solvent partition of the crude extract and extensive chromatographic purification monitored by the aforementioned bioassay allowed separation of the major alkaloid **1** (6 mg, 0.025% of dry weight). These colonies were found to harbor a green symbiotic alga (prochloron). A similar work-up was used to extract the major alkaloid[5] - ascididemin **2** (120 mg, 0.43% of

dry weight) - from grey morphs of the same ascidian devoid of symbiotic algae, collected near "Punta de la Creu", the Balearic Isles, Spain in July 1985. Asciddemin had been already isolated from a *Didemnum sp.*[6] Both extracts were found free of cystodytins,[7] the main alkaloids previously described from Japanese collections of *Cystodytes delle chiajei* .

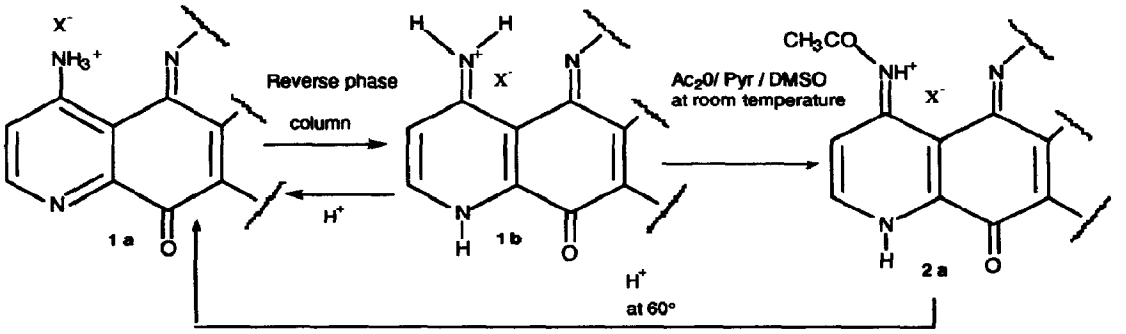
Accurate mass measurement (peak matching) was obtained for the parent ion at m/z 273.0760 $(M+H)^+$ in the positive FAB mass spectrum of **1**, and provided the formula $C_{17}H_{11}N_3O$. This formula did not fit well with the results of NMR experiments[8] (8H and 18C). The supplementary protons could be due like in cystodytins, to the reduction behavior $(M+2H/M+3H)^+$ of iminoquinone moieties in FAB solvents. So we tried to reach the molecular ion by the electrospray mass spectrometry mean. Only one fragment, corresponding to the mass-to-one charge ratio, was observed at m/z 300. This data was in agreement with the reduced form $(M+2H)^+$ of free base **1** which predominated during the whole mass spectrometry analysis of similar compounds,[6-7] and indicated the possibility of a supplementary amino group - the parent ion observed during FABMS being formed by the loss of HCN -. The infrared spectra showed conjugated double bonds ($\nu_{max.}$ 3025 and 1600 cm^{-1}) and a conjugated ketone ($\nu_{max.}$ 1680 cm^{-1}) not involved in hydrogen bonds like in meridine (a previously described alkaloid which structure was confirmed by X-ray diffraction).[9] As **1** was found to need at least one drop of acidic solvent to be dissolve, exchangeable protons were overlapped by TFA during ^1H NMR analysis. The ^{13}C NMR spectra were assigned on the basis of reverse 2D-NMR: HMQC experiments were optimized for $^1J_{CH}$ = 160 Hz and HMBC experiments with $^3J_{CH}$ = 10 Hz for dihedral angles = 180, $^3J_{CH}$ = 5 Hz for dihedral angles = 0 or for $^2J_{CH}$.[10]

The phenanthroline substructure was readily proposed to account for close similarities between ^1H and ^{13}C NMR spectra of **1** with those of asciddemin and meridine. For instance, double quantum filtered COSY verified two spin coupled networks in **1**: one spin system H - 2, 3, 4, 5 (8.65, d, J = 8Hz; 8.28, t, J = 8Hz; 8.26, t, J = 8Hz; 8.92, d, J = 8Hz) of a disubstituted benzene and a two coupled one-proton signals of a pyridine moiety - 8.98, d / 9.35, d, J = 5.6Hz.

Concerning the two other rings, the resonance at δ 175.3 in **1** was indicative of a cross-conjugated ketone by correlation with the strong IR absorption at 1680 cm^{-1}. The chemical shifts (δ_C 146.3 and δ_H 8.85) and the $^1J_{CH}$ value (192 Hz) of the carbon C-11 indicated a carbon adjacent to a nitrogen atom to account for MS results. Long range ^1H and ^{13}C 2D hetero-correlations showed couplings between H-11 / C-9a, H-12 / C-11 and H-12 / C-13a thus identifying C-9a and C-13a in agreement with a pyridine moiety. As the most shielded carbon due to three β shielding effects was identified by the $^3J_{CH}$ coupling with H-12, the amino group had to be bore by a supplementary deshielded quaternary carbon which was overlapped by the acidic solvent during this experiment. NMR analysis in DMSO-d_6 revealed the existence of an aminopyridine moiety as the pyridine ring was changed in a pyrolidine one **1b**[11] - based on the spin system H-10 (δ_{NH} 12.5, bs) / H-11 (δ_H 7.76, bt, J = 5.6 and 5.1 Hz) / H-12 (δ_H 6.46, dd, J = 5.6 and 1.6 Hz) - and as one ammonium group was also found (2H, triplet system at 7.20, 7.08 and 6.95 ppm). Structure **1b** was consistent with the behaviour of 4-aminopyridine in DMSO where the pyridylamide ion was possible.[12] The triplet system was due to the coupling ^{14}N-^1H (J = 52 Hz),[13] the NH absorption band as an ammonium ion resolving itself into a triplet fine structure from spin-spin interaction with the ^{14}N nucleus (I = 1). Here, we were lucky enough to be at the right temperature (20° C) to observe it as a triplet absorption and not as a broad singlet one. To our knowledge, this is the first time that such a rare phenomenon is reported from a marine natural product.

Conclusive evidences for structure **1** were provided by converting **1b** to monoacetate derivative **2a**. [14]

Structure **2a** was consistent with mass spectral, IR and ^1H NMR features. Two rotameric forms were discerned in **2a** by ^1H NMR analysis (δCH_3 2.143 and 2.139) and confirmed by NOE results as NOE enhancements of H-2 (7.5%) and H-12 (4.5%) were observed upon irradiation of the acetamide methyl signals. The acetate derivative displayed also the signal of an ammonium group (triplet system in the range of 7.00 ppm) during ^1H NMR analysis. Finally, treatment of **2a** with acidic medium (CF_3COOH or HCl) followed by heating led to **1a**, confidently establishing that the acetyl group was involved in an amido functionality. The NOE result between H-2 and the acetamide methyl signal was in strong support of the proposed structure for **1** rather than an ascididemin skeleton derivative.



Cystodamine showed activities during our cytotoxic test against CEM human leukemic lymphoblasts (IC_{50} at 1.0 µg / ml). *Cystodytes delle chiajei* seems to biosynthesize several cytotoxic products that share in common the same diazaphenanthroline moiety: cystodytin, ascididemin and cystodamine. The synthesis of **1** is in progress to evaluate its pharmacological potencies.

Acknowledgements: N. Bontemps and I. Bonnard are indebted to the Ligue National contre le Cancer for financial support. We thank the association ARDOUKOBA for assistance in the collection of ascidians, Dr X.Turon (Universidad de Barcelona) for identifying the ascidian and Dr J. Favre-Bonvin for HRMS analysis.

References and notes:

1 Ireland, C. M.; Roll, D. M.; Molinski, T. F.; McKee, T. C.; Zabriskie, T. M.; Swersey, J. C.; Foster, M. *Bioorganic Marine Chemistry*, Scheuer P. J. Ed.; Springer - Verlag, Inc.: Berlin; **1988**, vol. 3, pp 1- 46

2 Molinski, T.F. *Chem. Rev.* **1993**, 93, 1825-1838.

3 Banaigs, B.; Combaut, G.; Francisco, C. *Trends in Organic Chemistry* **1993**, 4, 173-183.

4 Pezzuto, J.M.; Che, C.T.; McPherson, D.D.; Zhu, J.P.; Topcu, G.; Erdelmeier, C.A.; Cordell, G.A. *J. Nat.. Prod.* **1991**, 54, 1522-1530.

5 **2**: IR ($CHCl_3$) ν_{max} cm^{-1} : 3025, 1680, 1595, 1579; HRFABMS: 286.0964 ($C_{18}H_{12}N_3O$, Δ = 2.1 mmu); EIMS m / z (%): 283 (100); 255 (55), 228 (12); CIMS m / z (%): 284 (100); ^1H and ^{13}C NMR ($CDCl_3$ / CD_3OH) : H-6 (9.23, d, J = 5.8 Hz), H-9 (9.15, dd, J = 1.5 + 4.9 Hz), H-4 (8.80, dd, J = 1.3 + 7.5 Hz), H-11 (8.76, dd, J = 1.5 + 7.9 Hz), H-5 (8.72, d, J = 5.8 Hz), H-1 (8.45, dd, J = 1.3 + 8.3Hz), H-2 (8.01, ddd, J = 1.3+8.3+7.5 Hz), H-3 (7.95, ddd, J = 1.3+8.3+7.5 Hz), H-10 (7.77,

dd, J = 4.5+7.9 Hz); 182.3, 155.9, 152.4, 150.1, 150.0, 146.8, 145.7, 139.6, 138.0, 133.4, 132.8, 132.1, 130.0, 127.4, 124.7, 124.7, 118.9, 118.8.

6 Kobayashi, J.; Cheng, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Otha, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, 29, 1177-1180.

7 Kobayashi, J.; Cheng, J.; Walchli, M.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Org. Chem.* **1988**, 53, 1800-1804.

8 Compound 1 - UV (HCl 1N - CH OH) λ_{max} nm (log e) : 250 (3.62), 278 (3.35), 385 (3.15); IR ($CHCl_3$ + TFA) ν_{max} cm^{-1}: 3025, 1680, 1601, 1462, 1207, 1141, 845; HRFABMS : 273.0884 ($C_{17}H_{11}N_3O$, Δ = 1.6 mmu); FABMS m / z (%) : 273 (25); 245 (6.5), 176 (40), 154 (100); ESMS $(M+2)^+ = 300$; ^1H(400 MHz) and ^{13}C(100MHz) NMR data for **1** in CD_2Cl_2+2 drops of CF_3-COOD : δH ppm, mult, J Hz, δC ppm, $^nJ_{CH}$=10Hz/5Hz: 1a (145.1, H-3/H-5); 2 (8.65, d, 8, 132.4, H-4); 3 (8.28, t, 8+8,134.9, H-5); 4 (8.26, t, 8+ 8, 134.7, H-2); 5 (8.92, d, 8, 124.6, H-3); 5a (125.2, H-2 / H-4 / H-6); 5b (139.1, H-6 / H-7 / H-5); 6 (8.98, d, 5.6, 122.4); 7 (9.35, d, 5.6, 150.6, H-6); 8a (140.5, H-7); 9 (175.3); 9a (148.3, H-11); 11 (8.85, d, 6.8, 146.3, H-12); 12 (7.67, d, 6.8, 118.4, H-11); 13 (158.0, overlapped by the signal of CF_3-COOD but found from **1a** in DMSO-d_6+1 drop of HCl); 13a (114.6, H-12); 13b (143.2); 13c (118.0, H-6). Compound **1a** - ^1H NMR (DMSO-d_6+one drop of HCl): H-7 (9.30, d, 5.6), H-6 (9.19, d, 5.6), H-5 (9.10, d, 7.2), H-11 (8.55, d, 6.4), H-2 (8.50, d,7.2), H-5 (8.15, t, 7.2), H-4 (8.10, t, 7.2), H-12 (7.54, d, 6.4), NH_3^+(7.47, 7.34 and 7.21, 3H, t, 52).

9 Schmitz, F. J.; DeGuzman, F.S.; Hossain, M.B.; Van der Helm, D. *J. Org. Chem.* **1991**, 56, 804 - 808.

10 Martin, G. E.; Zektzer, A. S. In *Two dimensional NMR methods for establishing molecular connectivity* VCH Publishers, Inc.: New-York **1988**, pp 162-279.

11 UV (MeOH) λ_{max} nm (log e): 231 (3.5), 277 (3.41), 374 (3.25); ^1H NMR (DMSO - d_6): H-10 (12.5, bs), H-7 (9.20, d, 5.6), H-6 (9.04, d, 5.6), H-5 (9.02, d, 7.2), H-2 (8.45, d, 7.2), H-5 (8.09, t, 7.2) H-4 (8.0 2, t, 7.2), H-11 (7.76, bt, 5.6 + 5.1), NH_2^+(7.21, 7.08 + 6.95, 2H, t, 52), H-12 (6.46, dd, 5.6+1.6).

12 Bordwell, F. G., Singer, D. L.; Satish, A. V. *J. Am. Chem. Soc.* **1993**, 115, 3543-3547.

13 Emsley, J. W.; Feeney, J.; Sutcliffe, L. H. In *High Resolution Nuclear Magnetic Resonance Spectroscopy* ; Pergamon Press: Oxford; **1968**, vol. 2, pp 818 - 821.

14 UV (MeOH) λ_{max} nm (log e) : 230 (3.85), 270 (3.6), 285 (3.6), 323 (3.20), 385 (3.25), 393 (3.15), 450 (3.1); IR ($CHCl_3$) ν_{max} cm^{-1}: 3026, 1679, 1649, 1600, 1462, 1207, 1142; ^1H NMR (DMSO-d_6): H-10 (12.5, s), H-7 (9.20, d, 5.6), H-6 (9.04, d, 5.6), H-5 (9.00, d, 7.2), H-2 (8.45, d, 7.2), H-5 (8.10, t, 7.2), H-4 (8.00, t, 7.2), H-11 (7.76, bt, 5.6+ 5.1), NH+ (7.22, 7.08 and 6.95, 1H, bt, 52) H-12 (6.46, d, 5.6), CH_3 - acetamide (2.146 and 2.139, 3H, s); FABMS m / z (%) with $(M+3H)^+$: 343 (45), 301 (5), 273 (40), 245 (20), 176 (100).

(*Received in France* 7 *July* 1994; *accepted* 28 *July* 1994)