Tetrahedron Letters, Vol.31, No.45, pp 6531-6534, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

Two new guanidine alkaloids from the Mediterranean sponge *Crambe crambe.*

R.G.S. Berlinck[1], J.C. Braekman[1], D. Daloze[1], K. Hallenga[2], R. Ottinger[3]
University of Brussels - 1050 Brussels - Belgium
[1] Laboratory of Bio-organic Chemistry , Faculty of Sciences
[2]Plant Genetic Systems, Unit of Conformation of Biological Macromolecules
[3]Organic Chemistry, Faculty of Applied Sciences

I. Bruno, R. Riccio
Dipartimento di Chimica delle Sostanze Naturali - Universita di Napoli
Federico II - Via D. Montesano 49 - 80131 Napoli - Italy

Abstract : Crambine A (**1**) and B (**2**) were isolated from the Mediterranean sponge *Crambe crambe* . Their structures were determined mainly by extensive NMR 2D experiments at 600 MHz.

Crambe crambe is a bright red encrusting sponge very common between -1 and -20 m along the rocky coasts of the Mediterranean. It is generally devoid of epizoites and it induces necrosis of the tissues of other sponges when they are kept in contact[1]. The methanol extract of *C. crambe* produces high toxicity against the fish *Lebistes reticulatus* and inhibits the reaggregation of cells of *Ephydiatia fluviatilis* [2].
Specimens of the fresh sponge (80 g dry weight) collected at Banyuls were stored in methanol and exhaustively extracted with dichloromethane / methanol 1:1. After evaporation of the dichloromethane, the residual methanol/water solution was successively extracted with hexane and tetrachloromethane. The methanol was evaporated and the resulting water suspension extracted with n-butanol. The n-butanol extract (4.1 g) possessed most of the ichthyotoxicity of the methanol extract. It was subjected to the following fractionations : 1. two successive chromatographies on Sephadex LH20 (MeOH and CH_2Cl_2/MeOH 3:2), 2. ascending mode DCCC ($CHCl_3$/MeOH/n-butanol/H_2O 10:10:1:6), 3. flash chromatography on silica gel (CH_2Cl_2/MeOH 9:1 to 7:3, + 0.5% CF_3COOH). This procedure led to two main fractions which were further purified by ion exchange chromatography (Amberlite IRA 400, Cl^-form), followed by silica gel flash chromatography (CH_2Cl_2/EtOAc/MeOH 9:9:1 to 1:1:1), to afford two Sakaguchi-positive fractions : fraction 1 (292 mg) containing crambine A (**1** ; n = 9) accompanied by three homologs (n= 8,10,11) and fraction 2 (98 mg) containing crambine B (**2** ; n = 9) also accompanied by three homologs (n = 8,10,11).

Crambine A [glassy solid, $[\alpha]_D$: +2 °(MeOH, c = 0.7); UV(MeOH): λ_{max} 205 (10,600) and 288 nm (5,500); IR (film) : 3200-3120, 1694 and 1680-1650 cm^{-1}] displays a quasi molecular ion at m/z 449.3605 [$(M+H)^+$; calculated for $C_{24}H_{45}N_6O_2$: 449.3607] in HR.FAB+MS, which corresponds to the molecular formula $C_{24}H_{44}N_6O_2$. This molecular ion was confirmed by the FAB-MS which exhibits peaks at m/z 483 and 485 [3:1 ratio, $(M-H+HCl)^-$] and 519, 521, 523 [10:6:1 ratio, $(M-H+2HCl)^-$]. The FAB+MS also shows three more peaks at m/z

435, 463 and 477 (about 5, 40 and 20% respectively of the peak at m/z 449) attributable to quasi-molecular ions of homologs of crambine A.

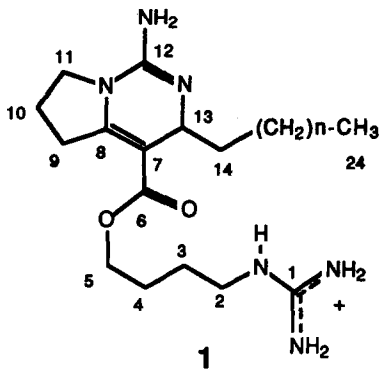
The ^{13}C NMR spectra (BBD and DEPT) confirm the presence of 24 carbon atoms in the major homolog (5 C,1 CH, 17 CH_2 and 1 CH_3). The ^1H NMR spectrum in DMSO indicates the presence of 7 exchangeable protons (δ 7.25 4H, broad ; 7.93 1H, triplet ; 8.71 2H, broad) thus suggesting that crambine A was isolated as an hydrochloride after ion exchange chromatography. Analysis of the ^1H NMR spectra (CD_3OD, 1D and $^1H/^1H$ COSY) clearly shows the presence of three separate spin systems : 1. a long alkyl chain with a terminal methyl group and a methine (δ4.42) at the other end; 2. four methylenes in an open chain linked on one side to an oxygen atom (H_2C-5 : δ_H 4.22, δ_C 65.4) and to a NH group on the other side (H_2C-2 : δ_H 3.24, δ_C 42.3). Coupling of this methylene with the exchangeable proton at δ7.93 was demonstrated by decoupling experiments in DMSO; 3. three methylene groups involved in a ring system.

The proton-carbon connectivities were deduced by HETCOR experiments. The quaternary carbon signal at δ166.4 was assigned to the carbonyl group of an ester which, according to the IR spectrum should be conjugated. The presence of 6 nitrogen atoms together with the positive reaction to Sakaguchi reagent and quaternary carbon signals at δ153.3 and 158.9 suggest that the compound contains two guanidine moieties. The two remaining sp^2 carbon signals at δ153.0 and 103.6 may be assigned to a β-enaminocarbonyl moiety [3,4]. Finally, analysis of the HMBC [5] spectrum led us to propose structure **1** for crambine A (major homolog n=9). Particularly relevant were the correlations observed between H-13 (δ4.42) and the ^{13}C signals at δ166.4 (C-6), 103.6 (C-7), 152.9 (C-8), 153.3 (C-12), 37.7 (C-14), 25.4 (C-15) and between H_2-14 and the ^{13}C signals at δ103.6 (C-7), 51.6 (C-13), 25.4 (C-15) and 30.6* (C-16), allowing *inter alia* to make a distinction between 2 and 3 bonds correlations with H-13.

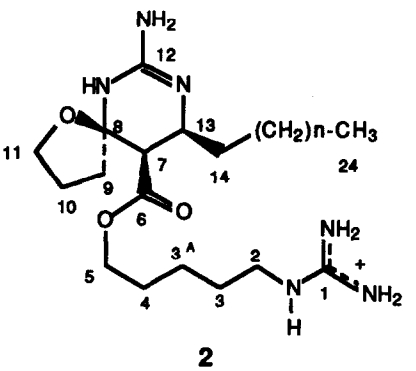
Crambine B [glassy solid; $[\alpha]_D$: +52 ° (MeOH; c = 0.9); UV(MeOH): end absorption; IR(film) : 3320-3120, 1730, 1660, 1650 and 1615 cm^{-1}] displays a quasi-molecular ion at m/z 481 in its FAB+MS. This spectrum also shows three further quasi-molecular peaks at m/z 467, 495 and 509 (about 30, 35 and 10% respectively of the peak at m/z 481) attributable to homologs of crambine B. High resolution measurements on the peak at m/z 481 (found : 481.3863; calc. for $C_{25}H_{49}N_6O_3$: 481.3869) point to the molecular formula $C_{25}H_{48}N_6O_3$, thus indicating that crambine B possess one methylene group and one molecule of water more than crambine A. Again there are three separate spin systems. The alkyl long chain is still present, but its terminal methine (δ3.85) is now coupled to another methine (δ2.95). The open chain linked to the ester group is now made up of five methylene groups as shown by the $^1H/^1H$ COSY spectrum and by the fragmentation in FAB+MS (fragment ion at m/z 308, corresponding to the loss of the ester chain, found at m/z 174). The presence of only 3 sp^2 carbon atom signals (δ170.0, 158.9 and 155.4) suggests that the carbon-carbon enamine double bond of crambine A is missing. All these data, together with the HMBC spectrum, led us to

propose structure **2** for crambine B (major homolog n=9). Among others, the HMBC spectrum shows that H-13 is correlated to the quaternary carbon signal at δ170.0 (C-6) and to the tertiary carbon signal at δ50.2 (C-7). The quaternary carbon signal at δ90.3 (C-8) may be assigned [6] to a N,O-alkylidene carbon atom originating from the presence of an additional oxygen atom between C-11 and C-8. Accordingly, C-11 now appears at δ69.1. These assignments are corroborated by the long range correlations observed between the H-11 at δ4.02 and C-8 and between H_2-9 and C-8. Moreover, the existence of NOE effects between H-13 and H-7 as well as between H-7 and H-9 strongly suggests that crambine B has the relative configuration depicted in **2**.

Crambine A and crambine B are bis-guanidine alkaloids having unprecedented skeletons. They are probably related biogenetically to the alkaloid ptilomycalin A, recently isolated from the Red Sea sponges *Ptilocaulis spiculifer* and *Hemimycale* sp.[7]. Other related guanidine compounds are present in the n-butanol extract of *Crambe crambe* . The determination of their structures is currently underway in our laboratories.



1



2

n = 8, 9 (major), 10, 11

^1H and ^{13}C NMR data of crambine A and B (Varian VXR 600 , δ, TMS, CD_3OD)

Crambine A : C-1 (158.9), H_2C-2 (3.24 t, 7Hz; 42.3), H_2C-3 (1.70 m; 26.9),H_2C-4 (1.80 m; 27.3), H_2C-5 (4.22 m; 65.4), C-6 (166.4), C-7 (103.6), C-8 (152.9), H_2C-9 (3.04 ddd, 9.5/9.5/18Hz and 3.37 ddd, 3/9/18Hz; 32.2), H_2C-10 (2.13 m and 2.25 m; 23.2), H_2C-11 (3.71 ddd, 6/9/9Hz and 3.82 ddd, 3/9/9Hz; 49.3), C-12 (153.3), HC-13 (4.42 dd, 4.5/7Hz; 51.6), H_2C-14 (1.57 m; 37.7), H_2C-15 (1.42 m; 25.4), H_2C-16 (1.29 m; 30.6*), H_2C-17 (1.29 m; 30.7*), H_2C-18 (1.29 m; 30.8*), H_2C-19 (1.29 m; 30.9*), H_2C-20 (1.29 m; 30.95*), H_2C-21 (1.29 m; 31.0*), H_2C-22 (1.29 m; 33.3), H_2C-23 (1.29 m; 23.9), H_3C-24 (0.89 t, 6Hz; 14.7). * Interchangeable signals.
$^1H/^{13}C$ long range correlations observed by HMBC optimized for J = 10 Hz: H-2 (C-1, C-3, C-4), H-3 (C-2,C-4,C-5), H-4 (C-2,C-3,C-5), H-5 (C-3,C-4,C-6),

H-9 at 3.04 ppm (C-7,C-8,C-10), H-9 at 3.37 ppm (C-7,C-8,C-10,C-11), H-10 at 2.13 ppm (C-8,C-9), H-10 at 2.25 ppm (C-8,C-9), H-11 at 3.71 ppm (C-10), H-11 at 3.82 ppm (C-8,C-9,C-10), H-13 (C-6,C-7,C-8,C-12,C-14,C-15), H-14 (C-7,C-13,C-15,C-16), H-24 (C-22,C-23).

Crambine B : C-1 (158.9), H_2C-2 (3.17 m; 42.7), H_2C-3 (1.60 m; 30.0), H_2C-3A (1.40 m; 27.3*), H_2C-4 (1.67 m; 29.9), H_2C-5 (4.15 m; 66.5), C-6 (170.0), HC-7 (2.95 d, 4Hz; 50.2), C-8 (90.1), H_2C-9 (2.08 m; 36.4), H_2C-10 (2.08 m and 2.19 m ; 25.9), H_2C-11 (3.92 m and 4.02 m; 69.1), C-12 (155.4), HC-13 (3.85 ddd, 4/7.5/7.5Hz; 50.3), H_2C-14 (1.55 m; 33.0), H_2C-15 (1.30-1.47 m; 26.7**), H_2C-16 (1.30-1.47 m; 27.8**), H_2C-17 (1.30-1.47 m; 30.6*), H_2C-18 (1.30-1.47 m; 30.6*), H_2C-19 (1.30-1.47 m; 30.7*), H_2C-20 (1.30-1.47 m; 30.8*), H_2C-21 (1.30-1.47 m; 30.1*), H_2C-22 (1.30-1.47 m; 33.2), H_2C-23 (1.30-1.47 m; 23.9), H_3C-24 (0.9 t, 6Hz; 14.7). * and ** Interchangeable signals.

$^1H/^{13}C$ long range correlations observed by HMBC optimized for J = 10 Hz : H-2 (C-1,C-3,C-3A), H-3 (C-2,C-3A,C-4), H-4 (C-3,C-3A, C-5), H-5 (C-3A,C-4,C-6), H-7 (C-6,C-8,C-13), H-9 and H-10 at 2.08 ppm (C-8,C-9,C-10,C-11), H-10 at 2.19 ppm (C-8,C-9), H-11 at 3.92 ppm (C-9,C-10), H-11 at 4.02 ppm (C-8,C-9,C-10), H-13 (C-6,C-7,C-14).

Acknowledgments: We would like to thank Drs. G. Van de Vyver and N. Boury-Esnault for sponge collection and identification, Dr. B. Moussiaux for preliminary separation work and C. Maerschalk for the NMR spectra at 600 MHz. One of us (R.G.S.B.) is indebted to the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" for financial support (proc. 200383/88.4) and to the "Centro Pluridisciplinar de Pesquisas Químicas Biológicas e Agrícolas" of UNICAMP (Campinas, Brazil). This work was supported by FRFC, Belgium (nº 2.4513.85 and 2.4554.87), by a NATO grant for collaboration research (Ref. D.210/86) and by CNR, Italy (nº 89.03744.03).

References :

1. M. Buscema, Ph.D. Thesis, University of Brussels, 1982.
2. J. Huysecom, G. Van de Vyver, J.C. Braekman and D. Daloze, Vie et Milieu, in press.
3. G.R. Bedford, P.J. Taylor, Org. Mag. Reson., 1977, **9**, 49.
4. D. Tourwé, G. Van Binst, S.A.G. De Graaf, U.K. Pandit, Org.Mag. Reson., 1975, **7**, 433
5. M.P. Summers, L.G. Marzilli, A. Bax, J.Am.Chem.Soc., 1986, **108**, 4285.
6. E. Pretsch, T. Clerc, J. Seibl, W. Simon, "Tables of Spectral Data for Structure Determination of Organic Compounds", Springer-Verlag, Second Edition,1988, Berlin.
7. Y. Kashman, S. Hirsh, O.J. McConnell, I. Ohtani, T. Kusumi, H. Kakisawa, J. Am.Chem.Soc., 1989, **111**, 8925.

(Received in France 23 July 1990)