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ON THE STRUCTURES OF CRAMBESCINS B AND C1

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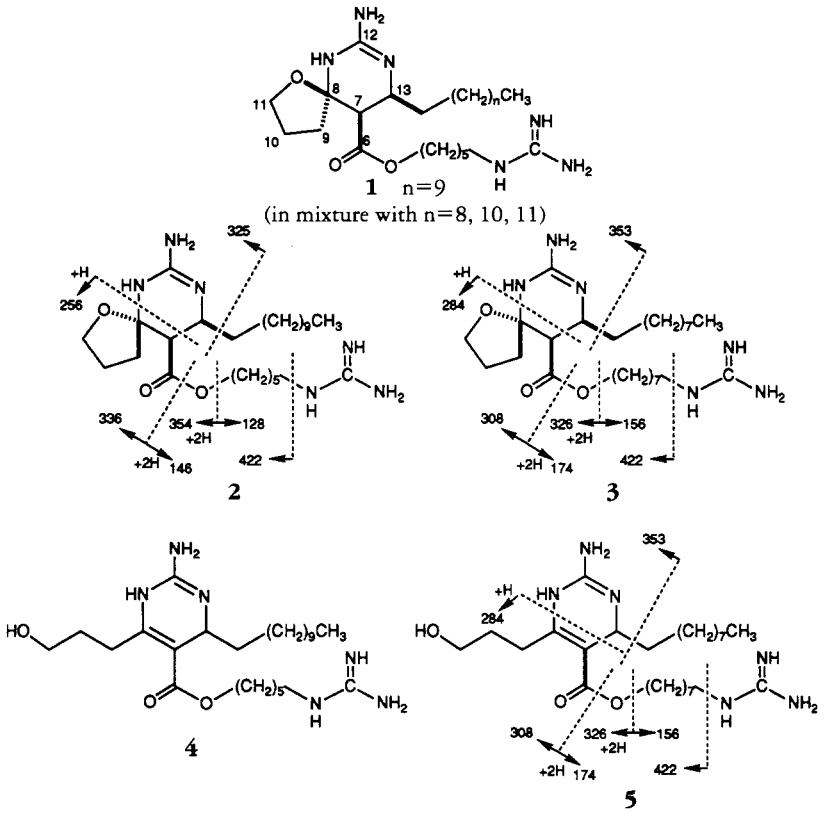
ABSTRACT.—Revised structures have been assigned to the cyclic guanidine-containing crambescins B [**3**] and C1 [**5**], from the Mediterranean sponge *Crambe crambe*. The revisions were based on the fabms/cid/ms spectra of the $[M+H]^+$ ions from crambescins B and C1 and hrfabms measurements on key fragment ions in the ms/ms spectra, which identify losses of C_9H_{19} and $C_8H_{18}N_3O$ side-chains.

We recently described the isolation of the cytotoxic crambescidins 800, 816, and 844 from the Mediterranean sponge *Crambe crambe* (1), and related, simpler compounds, "crambines A, B, C-1, and C-2" have also been reported from this sponge (2,3). Unfortunately, the name crambin had been given 25 years earlier to a protein (46 amino acids) isolated from the plant *Crambe abyssinica* (4). The name crambin (sometimes spelled crambine) has been regularly used since then in referring to this common and widely studied protein (e.g., 5). Consequently, and to avoid confusing the literature further, we recommend changing the name of the sponge-derived products to crambescins A, B, and C and shall use those names in the sequela. [Drs. Braekman and Snider agree to this nomenclature change. The name "crambine" was also applied recently to an uncharacterized compound, said to be an "aminosaccharide," from the sponge *Cramba cramba* (sic) which induced a reversible depolarization in neurons from *Helix pomatia* (gastropod) ganglia (6).]

The structure of crambescin B was first assigned as **1** (major homologue, n=9) by Berlinck *et al.* (2), who isolated the compound as a mixture of homologues. Snider and Shi (7) reassigned the stereochemistry at C-8 to **2**, the major homologue, prepared **2**, and established that the ^{13}C-nmr chemical shifts of the guanidinoalkyl side chain of **2** did not correspond to those of the natural product. At their request we isolated a comparison sample of crambescin B from *C. crambe*. Our initial characterization of crambescin B by ms indicated that a further regiochemical correction of the structure is necessary, which we describe here.

Crambescin B (major homologue) isolated in our laboratory from *C. crambe* has the expected molecular formula $C_{25}H_{48}N_6O_3$, determined by hrfabms on the $[M+H]^+$ ion (*m/z* 481.3869, $\Delta=-0.3$ mDa). The ^{1}H- and ^{13}C-nmr chemical shifts were within 0.03 and 0.3 ppm, respectively, of those reported by Berlinck *et al.* (2). Tandem mass spectrometry (fabms/cid/ms) on *m/z* 481 showed fragment ions at *m/z* 353, 326, 308, 284, and 174, and hrfabms assigned these ions as $C_{16}H_{29}N_6O_3$ ($[M-C_9H_{19}]^+$ 353.2297, $\Delta=0.4$ mDa), $C_{17}H_{32}N_3O_3$ ($[M-C_8H_{16}N_3]^+$ 326.2436, $\Delta=0.8$ mDa), $C_{17}H_{30}N_3O_2$ ($[M-C_8H_{18}N_3O]^+$ 308.2347, $\Delta=0.4$ mDa), $C_{14}H_{26}N_3O_3$ ($[M-C_{11}H_{21}N_3]^+$ $\Delta=0.6$ mDa), and $C_8H_{20}N_3O$ ($\Delta=0.5$ mDa), respectively. Berlinck *et al.* (2) reported fragment ions at *m/z* 308 and *m/z* 174 in the lrfabms spectrum but attributed them (incorrectly, lacking high resolution data) to $[M+H-CO_2(CH_2)_5NHC(NH_2)_2]^+$, and $[CO_2(CH_2)_5NHC(NH_2)_2+H]^+$, respectively. From the correct fragment ion compositions the alkyl side chain must be $-C_9H_{19}$ and the guanidinylalkyl side chain $-C_7H_{14}NHC(=NH)NH_2$ in crambescin B [**3**] instead of $-C_{11}H_{23}$ and $-C_5H_{10}NHC(=NH)NH_2$ as in the previously assigned structures **1** and **2** (8).

A sample of synthetic **2**, kindly provided by Dr. Snider, gave ions at *m/z* 325, 354, 336, 256 and 146 in its fabms/cid/



ms spectrum (corresponding to those at *m/z* 353, 326, 308, 284, and 174 for **3**), as predicted. Snider and Shi (8) have recently come to the same structure conclusions from ^{13}C-nmr comparisons.

Crambescin B had IC_{50} = 1 μg/ml vs. L1210 murine leukemia cells and is 10 to 20 times less cytotoxic than crambescidin 816 against those cells (1).

After a discussion of the above structure revision with Professor Braekman he sent us a sample of crambescin C1 (3) for reinvestigation by the same techniques. The fabms spectrum of crambescin C1 showed the $[M+H]^+$ ion at *m/z* 481.3873 and fragment ions at *m/z* 353 and 326. These were identified as $C_{16}H_{29}N_6O_3$ (353.2298, $[M-C_9H_{19}]^+$) and $C_{17}H_{32}N_3O_3$ (326.2448, $[M-C_8H_{16}N_3]^+$), in accord with the fragmentation scheme shown for **5**. This allowed us to revise the previously proposed structure **4** to structure **5**, analogous to **3** (8).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were obtained with General Electric QE-300 (300 MHz for ^{1}H, 75 MHz for ^{13}C), or GN-500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) or Varian Unity 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometers; chemical shifts (δ) are reported in ppm from TMS calibrated to the solvent peak. High- and low-resolution fab mass spectra were measured on a ZAB-SE spectrometer, and fabms/cid/ms spectra on a 70 SE4F instrument using dithiothreitol/dithioerythritol as matrix (9). A Beckman C-18 column (25×0.8 cm, 5 μm particle size) and MeOH-0.1 M NaCl (8:2) were used for hplc separation. Tlc on C-18 was developed with MeOH-0.1 M NaCl (8:2).

BIOASSAY.—L1210 murine leukemia cells were used in cytotoxicity measurements. Cell growth was estimated by microscopic examination when control wells had reached approximately 8000 cells (72 h). Percent inhibition was calculated from the difference between experimental and control.

EXTRACTION AND ISOLATION.—The specimen of *C. crambe* was collected by scuba (−9 m) in October 1988 at Isla de Formentor (Cueva), Palma

de Mallorca, Spain, and was identified by Dr. M. Uriz-Lespé (Centre d' Estudis Avançats de Blanes, Blanes, Spain). A voucher specimen is located at PharmaMar, S.A., Tres Cantos, Madrid, Spain. The frozen sample was extracted with MeOH-toluene (3:1). The extract was evaporated to give an oil (13 g), which was partitioned between $CHCl_3$ and 1 M NaCl (1:1, 100 ml×3). The bioactive organic layer (6.6 g) was partitioned between the lower phase (4.2 g) and upper phase (2.4 g) of hexane-EtOAc-MeOH-H_2O (4:7:4:3). Half of the active lower phase was purified by flash chromatography [C-18, MeOH-0.1 M NaCl (15:4)], affording 5 fractions. Separation of the active fraction 4 (R_f 0.4, ninhydrin-positive spot) by hplc afforded crambescidin 816 (Rt 30.4 min, 30 mg), crambescin A (mol wt 477, Rt 25.2 min, 12 mg) and other previously described crambescidins (800, 830, 844).

Similar separation of fraction 3 (R_f 0.45, Sakaguchi-positive spot) afforded crambescin B (Rt 18.7 min, 8 mg).

Crambescin B [**3**].—Glassy solid: $[\alpha]_D$ +48° (c=0.1, MeOH), [lit. (2) $[\alpha]_D$ +52° (c=0.9, MeOH)]; lrfabms m/z $[M]^+$ 481 (100); fabms/cid/ms 481→422, 353, 326, 308, 284, 174, 156; ^1H nmr (500 MHz, CD_3OD) ppm 0.90 (3H, t, J=6 Hz), 1.30–1.50 (18H), 1.40 (2H, m), 1.55 (2H, m), 1.60 (2H, m, J=4 Hz), 3.18 (2H, m), 3.86 (1H, dt, J=4, 7.5 Hz), 3.92 (1H, m), 4.04 (1H, m), 4.15 (2H, m), 4.42 (1H, dd, J=4.5, 7 Hz); ^{13}C nmr (100 MHz, CD_3OD) ppm 14.4 (q), 23.7 (t), 25.7 (t), 26.5 (t), 27.0 (t), 27.6 (t), 29.6 (t), 29.8 (t), 29.9 (t), 30.5 (t), 30.5 (t), 30.6 (t), 30.7 (t), 32.8 (t), 33.0 (t), 36.1 (t), 42.7 (t), 49.8 (d), 50.1 (t), 66.2 (t), 68.9 (t), 89.8 (s), 155.1 (s), 158.6 (s), 169.7 (s). *Anal.* calcd for $C_{25}H_{49}N_6O_3$: M_r $[M+H]^+$ 481.3866. Found M_r 481.3869 (hrfabms).

Crambescin C1 [**5**].—Lrfabms m/z 495 (homologue)(5%), $[M]^+$ 481 (100), 467 (homologue) (6), 422 (4), 353 (5), 326 (2), 241 (5), 174 (10); fabms/cid/ms 481→422, 353, 308, 284, 174, 156. *Anal.* calcd for $C_{25}H_{49}N_6O_3$: M_r $[M+H]^+$ 481.3866. Found M_r 481.3873.

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LITERATURE CITED

1. E.A. Jares-Erijman, R. Sakai, and K.L. Rinehart, *J. Org. Chem.*, **56**, 5712 (1991).
2. R.G.S. Berlinck, J.C. Braekman, D. Daloze, K. Hallenga, R. Ottinger, I. Bruno, and R. Riccio, *Tetrahedron Lett.*, **31**, 6531 (1990).
3. R.G.S. Berlinck, J.C. Braekman, D. Daloze, I. Bruno, R. Riccio, D. Rogeau, and P. Amade, *J. Nat. Prod.*, **55**, 528 (1992).
4. C.H. Van Etten, H.C. Nielsen, and J.E. Peters, *Phytochemistry*, **4**, 467 (1965).
5. M.M. Teeter, J.A. Manzer, and J.J. L'Italien, *Biochemistry*, **20**, 5437 (1981).
6. O. Nešić, N. Dogović, M.I. Gašić, and M. Pašić, *Period. Biol.*, **88**, 176 (1986).
7. B.B. Snider and Z. Shi, *J. Org. Chem.*, **57**, 2526 (1992).
8. B.B. Snider and Z. Shi, *J. Org. Chem.*, **58**, 3828 (1993).
9. J.L. Witten, M.H. Schaffer, M. O'Shea, J.C. Cook, M.E. Hemling, and K.L. Rinehart Jr., *Biochem. Biophys. Res. Commun.*, **124**, 350 (1984).

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