JOURNAL OF NATURAL PRODUCTS

Note

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Total Synthesis and Structural Revision of (+)-Cristatumin C

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Supporting Information

ABSTRACT: Naturally occurring (+)-cristatumin C, a bis-pyrrolidinoindoline diketopiperazine alkaloid isolated from *Eurotium cristatum* EN-220, is the 2*R*,3*R*,11*S*,15*R*,2′*R*,3′*R*,11′*S*,15′*S* enantiomer, as confirmed by total synthesis.

Fungi are rich sources of secondary metabolites derived from tryptophan.[1,2] Several members of this alkaloid class have been recently isolated from the culture extract of *Eurotium cristatum* EN-220, an endophytic fungus of the marine alga *Sargassum thunbergii*.[3] The heterodimeric (+)-cristatumin C (**6**) stands out as the most complex of the isolates since it is composed of two subunits of a diketopiperazine-fused hexahydropyrrolo[2,3-*b*]indole connected to each other through the two equally configured contiguous quaternary stereogenic centers C3 and C3′.

Although these compounds are derived in Nature from the condensation of tryptophan with other amino acids,[4,5] the biosynthetic machinery combines these simple building blocks using a variety of connection patterns, C3−C3′ being the most common, within either homodimeric or heterodimeric structures. Stereochemical diversity is also achieved through the presence of either antipode of the amino acid components. Although the C11 and C15 chiral centers of the diketopiperazine can be found in the *cis* or *trans* relative configuration, with the exception of (−)-ditryptophenaline (**3**)[6] all other members of this family of homodimeric and heterodimeric bis-pyrrolidinoindoline diketopiperazine alkaloids have opposite configuration at the C2/C3 hexahydropyrroloindole junction relative to C11 (cf. **1**, **2**, **4**, **5** in Figure 1).[4,5] After having corrected the proposed structure[7] of (+)-asperdimin to **5**,[8] we were surprised to find yet another cis relative configuration at C3′−C11′−C15′ in the purported structure of (+)-cristatumin C (**6**) (one of the three different drawings used in the article to depict the same natural product).[3] On the basis of our previous experience with this family of alkaloids,[8−10] we surmised by analogy that the natural isolate had the relative and absolute configuration shown in **7** and carried out the first total synthesis of this compound to test this hypothesis. As anticipated, the proposed structure of the natural product (+)-cristatumin C (**6**) needs to be revised to the stereoisomer **7**.[3]

Our synthesis of bis-pyrrolidinoindoline diketopiperazines is based on the final assembly of the diketopiperazine rings after deprotection of the Fmoc groups in the tetrapeptide **8**, itself obtained by peptide bond formation in homodimeric hexahydropyrroloindole **9** (Scheme 1). Compound **9** is a

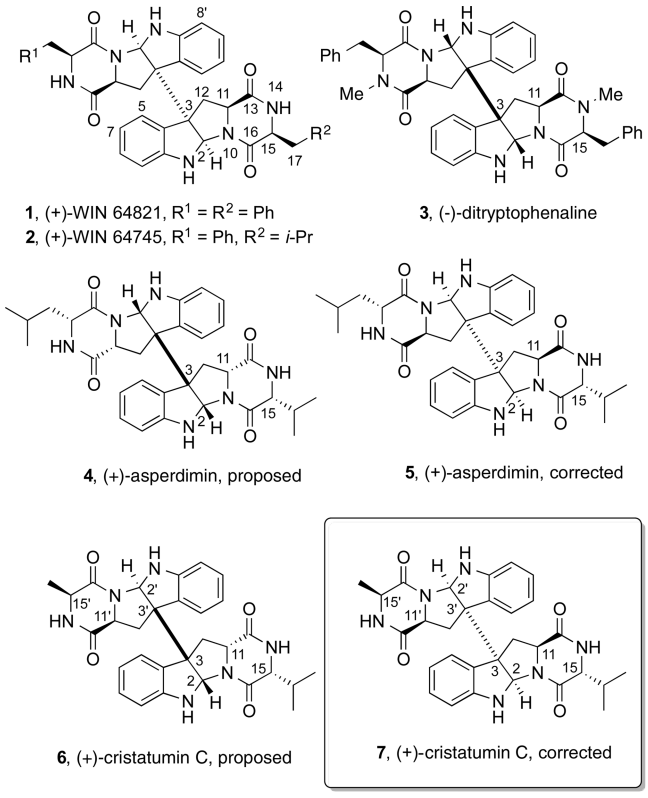
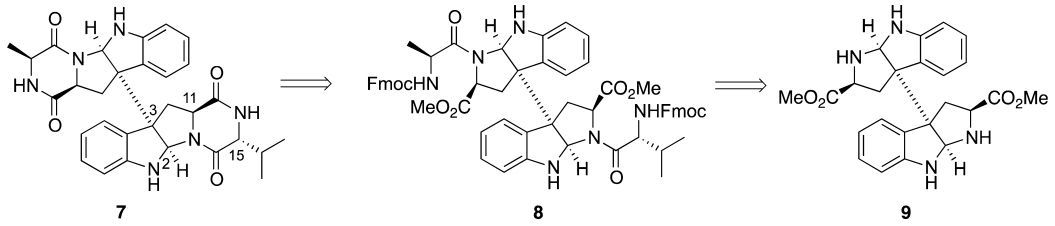


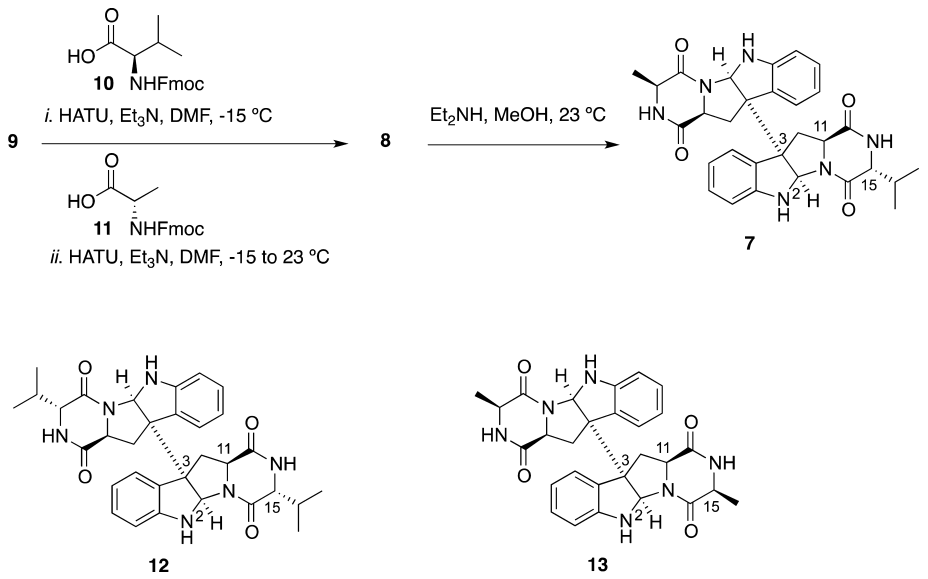
Figure 1. Representative dimeric pyrrolidinoindoline diketopiperazine alkaloids, with the numbering system indicated in structure **1**.

Received: November 20, 2013
Published: January 17, 2014

Scheme 1. Retrosynthetic Analysis of (+)-Cristatumin C



Scheme 2. Synthesis of (+)-Cristatumin C (7)



powerful synthetic platform[11] for the straightforward construction of both C3−C3′ symmetric[8,9,12] and nonsymmetric heterodimers.[8,9] The latter are acquired by the sequential peptide coupling of the tetraamine in **9**[8] with two different amino acids. In the case of (+)-cristatumin C (**6**/**7**) L-alanine and D-valine are chosen since their absolute configurations were secured by application of Marfey's method[13] based on chiral HPLC analysis of derivatives of the acid hydrolysate of natural **6**.[3]

The peptide coupling reactions of compound **9** were sequentially carried out with *N*-Fmoc-D-valine (**10**) (1.0 equiv, −15 °C) and *N*-Fmoc-L-alanine (**11**) (1.2 equiv, 25 °C) in the presence of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxide hexafluorophosphate (HATU), Et_3N, and DMF[14] to give tetrapeptide **8** (Scheme 2). This was not fully characterized due to the presence of rotamers (also of a secondary product, *vide infra*), which complicated the analysis of the spectra. Hence, Fmoc-deprotection under basic conditions (Et_2NH in MeOH at 25 °C) and spontaneous cyclization afforded heterodimeric diketopiperazine **7** in 50% combined yield. Also isolated in 19% yield was the homodimer **12**, the result of the 2-fold condensation of **9** and protected D-valine **10**. Its formation could not be suppressed even at the low temperature of the first coupling (−15 °C). This result indicates that the steric bulk of the amino acid used in the first reaction greatly influences the selectivity of the coupling.[8,9] As additional support of this assumption, if the order of amino acids is reversed, coupling **9** first with *N*-Fmoc-L-alanine (**11**) at −15 °C then with protected D-valine **10**, heterodimeric compound **7** was obtained in 43% yield and homodimer **13** in 41% yield (Scheme 2).[15]

As anticipated, the spectroscopic data (including optical rotation, $[\alpha]^{20}_D$ +347.6 (*c* 0.62, MeOH); lit.[3] $[\alpha]^{20}_D$ +315.7 (*c* 1.21, MeOH)) of **7** matched those of the natural product[3] (see the Supporting Information). The relative configuration of synthetic (+)-cristatumin C (**7**) is consistent with the analysis of one- and two-dimensional NMR experiments. Particularly revealing were the NOESY correlations of H11′ and H15′ and the lack of correlation between H11 and H15, which confirmed the relative configuration at these positions.

To summarize, the relative and absolute configuration of the purported structure of the natural product (+)-cristatumin C[3] has been corrected by total synthesis of a rationally guided stereoisomer. We have shown elsewhere that the assignment based merely on the spectroscopic data of this family of natural products can be misleading.[10] Although the configuration of the C15 stereocenter is always confirmed through Marfey's degradative amino acid analysis, the (sometimes weak) NOE correlation of H15 with its neighboring atom is insufficient evidence to establish the relative configuration at C11. In addition, the configuration of the C2−C3 pyrrolidinoindoline fusion atoms cannot be determined in the absence of X-ray diffraction data or comparison of DFT-computed and experimental CD curves.[16] Chemical synthesis remains in those cases as the ultimate structural proof.[17]

EXPERIMENTAL SECTION

General Experimental Procedures. Solvents were dried according to published methods and distilled before use. All reagents were commercial compounds of the highest purity available. Reactions were carried out under an argon atmosphere, unless indicated otherwise. Analytical TLC was performed on aluminum plates with

dx.doi.org/10.1021/np400969u | *J. Nat. Prod.* 2014, 77, 421−423

Merck Kieselgel 60F_{254} and visualized by UV irradiation (254 nm) or by staining with a ethanolic solution of phosphomolybdic acid. Flash-column chromatography was carried out using Merck Kieselgel 60 (230−400 mesh) under pressure. IR spectra were obtained on a JASCO IR 4200 spectrophotometer from a thin film deposited onto NaCl glass. Specific rotations were obtained on a JASCO P-1020 polarimeter. Mass spectra and HRMS (ESI+) were taken on an Apex III FT ICR MS (Bruker Daltonics) apparatus. ^{1}H NMR spectra were recorded in CD_3OD or DMSO-d_6 at ambient temperature on a Bruker AMX-400 or AMX-600 spectrometer at 400 or 600 MHz, respectively, with residual protic solvent as the internal reference (CD_3OD, δ_H = 3.31 ppm or DMSO-d_6, δ_H = 2.50 ppm). Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). The proton spectra are reported as follows: multiplicity, coupling constant J, number of protons, assignment. ^{13}C NMR spectra were recorded in CD_3OD or DMSO-d_6 at ambient temperature on a Bruker AMX-400 at 100 MHz, with the central peak of CD_3OD (δ_C = 49.15 ppm) or DMSO-d_6 (δ_C = 39.51 ppm) as the internal reference. DEPT135 and two-dimensional (COSY, HSQCed, HMBC, and NOESY) sequences were used where appropriate to aid in the assignment of signals.

Synthesis of Cristatumin C (7). To a cooled (−15 °C) solution of the tetraamine **9**[8] (60 mg, 0.14 mmol) and *N*-Fmoc-D-valine (**10**) (47 mg, 0.14 mmol) in DMF (2.3 mL) were added HATU (53 mg, 0.14 mmol) and Et_3N (38 μL, 0.28 mmol, 2.0 equiv). The reaction mixture was stirred at −15 °C for 5.5 h and allowed to reach 0 °C, and then *N*-Fmoc-L-alanine (**11**) (56 mg, 0.18 mmol), HATU (68 mg, 0.18 mmol), and Et_3N (50 μL, 0.47 mmol, 2.6 equiv) were added. The cooling bath was removed, and the mixture was further stirred for 19 h at 25 °C. The reaction was quenched by the addition of H_2O (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with H_2O (25 mL) and dried over Na_2SO_4, and the solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (98:2 CH_2Cl_2/MeOH) to give the corresponding tetrapeptide **8**, which was used directly in the next step.

Diethylamine (394 μL) was added to a solution of the tetrapeptide **8** obtained above (92 mg) in MeOH (6.6 mL), and the mixture was stirred for 6.5 h at 25 °C. The solvents were removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (97:3 CH_2Cl_2/MeOH) to afford 44 mg (50% combined yield over the two steps) of compound **7** as a white solid: $[\alpha]^{20}_D$ +347.6 (*c* 0.62, MeOH); IR (NaCl) ν_{max} 3268 (br, m, N−H), 2961 (m, C−H), 2928 (m, C−H), 2873 (m, C−H), 1670 (s, C═O), 1606 (m), 1424 (s), 1311 (m), 1254 (m), 1094 (w), 749 (s) cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.25 (1H, br d, J = 4.2 Hz, NH-14), 8.05 (1H, s, NH-14′), 7.39 (1H, d, J = 7.4 Hz, H-5′), 7.38 (1H, d, J = 7.4 Hz, H-5), 7.02 (2H, m, H-7/H-7′), 6.70 (1H, s, NH-1), 6.7−6.6 (5H, m) [included 6.66 (1H, d, J = 7.8 Hz, H-8) + 6.63 (1H, s, NH-1′) + 6.61 (1H, d, J = 7.7 Hz, H-8′) + 6.63−6.61 (2H, m, H-6/H-6′)], 4.96 (1H, br s, H-2′), 4.80 (1H, br s, H-2), 4.20 (1H, t, J = 8.4 Hz, H-11′), 4.08 (1H, t, J = 8.5 Hz, H-11), 4.00 (1H, q, J = 6.8 Hz, H-15′), 3.36 (1H, dd, J = 5.7, 4.6 Hz, H-15), 3.1−3.0 (2H, m, H-12a/H-12a′), 2.62 (1H, dd, J = 13.3, 8.5 Hz, H-12b′), 2.32 (1H, dd, J = 14.0, 9.5 Hz, H-12b), 1.93 (1H, app dq, J = 13.1, 6.5 Hz, H-17), 1.15 (3H, d, J = 6.9 Hz, H-17′), 0.79 (3H, d, J = 6.8 Hz, H-19), 0.69 (3H, d, J = 6.6 Hz, H-18) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.6 (s, C-13′), 169.4 (s, C-16′), 168.4 (s, C-13), 167.4 (s, C-16), 149.1 (s, C-9/C-9′), 130.7 (s, C-4′), 130.3 (s, C-4), 128.7 (d, C-7′), 128.6 (d, C-7), 124.7 (d, C-5′), 124.4 (d, C-5), 118.1 (d, C-6′), 117.9 (d, C-6), 108.9 (d, C-8′), 108.8 (d, C-8), 78.8 (d, C-2), 78.7 (d, C-2′), 62.4 (d, C-15), 60.0 (s, C-3′), 59.7 (s, C-3), 57.0 (d, C-11′), 55.6 (d, C-11), 50.6 (d, C-15′), 37.4 (t, C-12), 35.4 (t, C-12′), 31.9 (d, C-17), 18.9 (q, C-19), 18.1 (q, C-18), 14.9 (q, C-17′) ppm; HRMS (ESI$^+$) m/z 541.2569 (calcd for $C_{30}H_{33}N_6O_4$ ([M + H]$^+$), 541.2558).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Comparison of the NMR data of natural and synthetic cristatumin C, spectroscopic data for homodimers **12** and **13**, and copies of ^{1}H NMR and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by funds from the Spanish MINECO (SAF2010-17935-FEDER), Xunta de Galicia (Grant 08CSA052383PR from DXI+D+i; Consolidación 2006/15 from DXPCTSUG; INBIOMED-FEDER "Unha maneira de facer Europa").

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