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Note

NOTE

The isolation of (6*S*, 9*S*)-cyclo(prolylvalyl) from marine actinomycete, by use of high speed countercurrent chromatography

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The marine actinomycete B 1758 came from the actinomycete collection of the Alfred Wegener Institute for Polar and Marine Research in Bremerhafen, Germany. 1.079 g of raw extract was obtained by fermentation. Seven fractions were separated by column chromatography on silica gel. Fraction 4 was separated by high speed countercurrent chromatography. Fraction 4.6 yielded 5.2 mg of (6*S*, 9*S*)-cyclo(prolylvalyl).

Keywords: (6*S*, 9*S*)-cyclo(prolylvalyl), marine actinomycete B 1758, HSCCC.

INTRODUCTION

The world seas, making up 2/3 of the Earth's surface and 90 % of the biosphere, have recently been seen as a new source of natural substances.[1,2] For a long time, marine microorganisms were little investigated because they were considered hard to isolate and cultivate, which is applicable to a large number of marine microorganisms.[3]

Countercurrent chromatography (CCC) is an original tool for separating natural products, finding application in the isolation of secondary metabolites such as antibiotics.[4–6] In this work, the isolation of (6*S*, 9*S*)-cyclo(prolylvalyl) from marine actinomycete, by use of high speed countercurrent chromatography (HSCCC), is described.

EXPERIMENTAL

Materials and methods

The ^{1}H-NMR spectra were recorded on a Bruker WM 300 (300.1 MHz) spectrophotometer. The EI-MS mass spectra were recorded on a Varian MAT 731 (70 eV) instrument; high resolutions were compared with perfluorokerosine as a comparison substance. The DCI-MS mass spectra were recorded on a Finnigan MAT 95 A instrument with NH_3 as the reacting gas. Column chromatography was accomplished on silica gel 30-60 μm (J. T. Baker). Thin-layer chromatography was performed on DC-folien Polygram SIL G/UV_{254} (Macherey Nagel&Co). HSCCC was performed using a P.C. INC. High Speed Countercurrent Chromatograph with two Pharmacia LKB HPLC pumps 2150. The optical rotations were measured using a Perkin-Elmer 343 polarimeter.

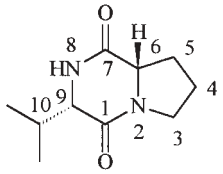
Breed B 1758

The marine actinomycete B 1758 came from the actinomycete collection of the Alfred Wegener Institute for Polar and Marine Research in Bremerhafen. 1.079 g of raw extract was obtained by fermentation. The raw extract was degreased with cyclohexane and then separated by column chromatography on silica gel, with step gradients consisting of chloroform and methanol (1.5 dm^3 $CHCl_3$, 1 dm^3 $CHCl_3$/1 % CH_3OH, 1 dm^3 $CHCl_3$/2 % CH_3OH, 0.5 dm^3 $CHCl_3$/5 % CH_3OH, 0.7 dm^3 $CHCl_3$/15 % CH_3OH). Seven fractions were separated by thin layer chromatography ($CHCl_3$/5 % CH_3OH), but still contained mixtures of substances (fraction 1, 875 cm^3, 20.3 mg; fraction 2, 375 cm^3, 189.8 mg; fraction 3, 850 cm^3, 100.5 mg; fraction 4, 875 cm^3, 82.7 mg; fraction 5, 950 cm^3, 103.7 mg; fraction 6, 325 cm^3, 71.3 mg; fraction 7, 250 cm^3, 28.8 mg). The fraction 4 was separated by HSCCC (215 cm^3 column, solvent chloroform/ methanol/ethyl acetate/water 1:1:1:1, the phase was lighter than the stationary phase, flow rate 1 cm^3 per minute). Six fractions were separated by thin layer chromatography ($CHCl_3$/5 % CH_3OH) (fraction 4.1, 35 cm^3, 21 mg; fraction 4.2, 10 cm^3, 13.3 mg; fraction 4.3, 10 cm^3, 24.8 mg; fraction 4.4, 15 cm^3, 5.6 mg; fraction 4.5, 15 cm^3, 9.4 mg; fraction 4.6, 10 cm^3, 5.2 mg). Fraction 4.6 yielded 5.2 mg of (6*S*, 9*S*)-cyclo(prolylvalyl) (**1**; $C_{10}H_{16}N_2O_2$).

RESULTS AND DISCUSSION

In ^{1}H-NMR spectrum of the isolated compound, the wide signal at $\delta = 5.67$ indicated an OH-or NH-proton. The spectrum gave two doublets at $\delta = 1.05$ and $\delta = 0.90$ with the intensity 3, indicating the presence of methyl groups. The EI and DCI mass spectra indicated a mass of 196 Da. The high resolution of this signal suggested the formula $C_{10}H_{16}N_2O_2$. Searching the AntiBase ®[7] data bank for the above formula, 6 structures were obtained. With respect to the above information, the structure corresponded with that of cyclo(prolylvalyl). A spectroscopic investigation of the corresponding diketopiperazines showed that the protons 6-H and 9-H are in *cis*-position with respect to each other, in DMSO always at $\delta = 3.8$ or lower, while in the *trans*- position they lay higher than $\delta = 3.5$.[8] This suggests that the isolated compound is *S,S*- or *R,R*-cyclo(prolylvalyl). Comparison of the cited optical rotation values[9] with those of the newly isolated compound indicated the compound was (6*S*, 9*S*)- cyclo(prolylvalyl) (**1**). Studies of diketopiperazines isolation from marine sources have been already cited.[10–13] This is the first example of diketopiperazines isolation by use of HSCCC.

($[\alpha]_D^{20}$ (reference works)[9] = –157° (*c* 1 in $CHCl_3$), $[\alpha]_D^{20}$ (investigation reading) = – (155±10)° (*c* 0.1 in $CHCl_3$).



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(6*S*, 9*S*)-Cyclo(prolylvalyl): $C_{10}H_{16}N_2O_2$ (196.2). EI-MS (70 eV): *m*/*z* (%) = 196.2 $[M]^+$ (4), 154.1 (100), 125.1 (24), 70.1 (44). DCl-MS (NH_3): *m*/*z* (%) = 214.2 $[M + NH_4]^+$ (100), 197.2 $[M+H]^+$ (30). ^{1}H-NMR ($CDCl_3$, 300 MHz): $\delta = 5.67$ (*s* (*br*), 1 H,

NH), 4.07 (*t*, ^3J = 9 Hz, 1 H, 6-H), 3.92 (*t*, ^3J = 2 Hz, 1 H, 9-H), 3.66–3.48 (*m*, 2 H, 3-CH_2), 2.62 (*dsep*, ^3J = 7.5 Hz, ^3J = 2 Hz, 1 H, 10-H), 2.42–2.32 (*m*, 1 H, 5-H), 2.09–1.83 (*m*, 3H, 5-H, 4-CH_2), 1.05 (*d*, ^3J = 7.5 Hz, 3 H, CH_3), 0.90 (*d*, ^3J = 7.5 Hz, 3 H, CH_3). ^{1}H-NMR (DMSO-d_6, 300 MHz): δ = 7.88 (*s* (*br*), 1 H, NH), 4.10 (*t*, ^3J = 7.5 Hz, 1 H, 6-H), 3.90 (*t*, ^3J = 2 Hz, 1 H, 9-H), 3.45–3.14 (*m*, ^3J = 7.5, 2 H, 3-CH_2), 2.33 (*dsep*, ^3J = 7.5 Hz, ^3J = 2 Hz, 1 H, 10-H), 2.18–2.07 (*m*, 1 H, 5-H), 1.91–1.72 (*m*, 3 H, 5-H, 4-CH_2), 1.01 (*d*, ^3J = 7.5 Hz, 3 H, CH_3), 0.85 (*d*, ^3J = 7.5 Hz, 3 H, CH_3). $C_{10}H_{16}N_2O_2$: Calculated: 196, 1208; Recorded: 196, 1211.

CONCLUSION

The isolation of (6*S*, 9*S*)-cyclo(prolylvalyl) from marine actinomycete B 1758 is a new example of a diketopiperazine from marine sources. However, this is the first example of diketopiperazines isolation by use of high speed countercurrent chromatography.

Abbreviations: *s* = singlet; *d* = doublet; *dsep* = doublet septet; *t* = triplet; *m* = multiplet; *br* = broad.

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И З В О Д

ИЗОЛОВАЊЕ (6*S*, 9*S*)-ЦИКЛО (ПРОЛИЛВАЛИЛА) ИЗ МОРСКЕ АКТИНОМИЦЕТЕ, КОРИШЋЕЊЕМ HSCCC ХРОМАТОГРАФИЈЕ

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Морска актиномицета B 1758 потиче из збирке актиномицета института Alfred Wegener за поларна и морска истраживања у Bremerhafen-у, Немачка. Ферментацијом је добијено 1,079 g сировог екстракта. Колонском хроматографијом на силикагелу је раздвојено седам фракција. Четврта фракција је раздвојена коришћењем HSCCC хроматографије. У фракцији 4.6 је добијено 5,2 mg (6*S*, 9*S*)-цикло (пролилвалила).

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REFERENCES

1. W. Fenical, *Chem. Rev.* **93** (1993) 1673
2. P. Jensen, W. Fenical, *Annu. Rev. Microbiol.* **48** (1994) 559
3. D. J. Faulkner, *Nat. Prod. Rep.* **9** (1993) 497
4. G. M. Brill, J. B. McAlpine, J. E. Hochlowski, *J. Liq. Chromatog.* **8** (1984) 2259
5. J. W. Lightbown, P. Newland, I. A. Sutherland, J. W. A. Dymond, *Proc. Analyst. Div. Chem. Soc.* **14** (1977) 34
6. I. A. Sutherland, J. S. Lee, D. J. Gaureau, *Anal. Biochem.* **89** (1978) 213
7. H. Laatsch, *Naturstoffdatenbank AntiBase®*, Chemical Concept, Weinheim, 1998
8. I. Z. Siemion, *Org. Magn. Reson.* **3** (1971) 545
9. *Dictionary of Natural Products on CD-ROM*, Chapman& Hall Chemical Database, Version 7, 1998
10. P. G. Sammes, *Fortschr. Chem. Org. Naturstoffe* **32** (1975) 51

11. G. R. Pettit, R. B. Von Dreele, G. G. Bolliger, P. M. Traxler, P. Brown, *Experientia* **29** (1973) 521
12. A. C. Stierle, J. H. Cardellina II, F. L. Singleton, *Experientia* **44** (1988) 1021
13. M. Adamczeski, E. Quiñoá, P. Crews, *J. Am. Chem. Soc.* **111** (1989) 6.