Notes

FIRST ISOLATION OF (+)-EPIPENTENOMYCIN I FROM *PEZIZA* SP. CARPOPHORES

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We report here the first isolation of (+)-epipentenomycin I (**1**) from carpophores of *Peziza* sp., collected on horse manure. Pentenomycins are cyclopentenoid antibiotics isolated, until now, from only a few *Streptomyces* species:

HATANO *et al.*[1] had isolated three compounds whose structures had first been postulated as (−)-epipentenomycins I (**1**), II (**2**) and III (**3**), but later found to be identical with pentenomycins I (**7**), II (**8**) and III (**9**)[2].

Various syntheses have been achieved for all these compounds (see ref 3).

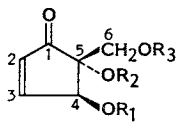
Materials and Methods

Extraction and Purification

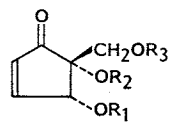
After several trials the following protocol was established: 20 g thawed fungus (dry weight 1.22 g, 6% fresh weight), were extracted 4 times with H_2O (sonication for the last extraction). The combined extracts (280 ml) contained *ca.* 240 mg of epipentenomycin (quantitated by HPLC). After evaporation under reduced pressure the residue was dissolved in a few ml of H_2O, centrifuged and purified, firstly on QAE Sephadex (anionic) column, followed by a ca-

Table 1. Pentenomycins isolated from *Streptomyces* and *Streptoverticillium* species.

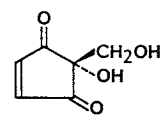
	Pentenomycins				ref
	I (7)	II (8)	III (9)	12	
Streptomyces eurythermus	+	+			7, 8
S. lavenduligriseus	+		+		1, 2
S. cattleya				+	9
Streptoverticillium eurocidicum		+	+		10



Epipentenomycin	R_1	R_2	R_3
I (1)	H	H	H
II (2)	H	H	Ac
III (3)	Ac	H	H
4	Ac	Ac	Ac
5	TMSi	TMSi	TMSi
6	H	CH_3 CH_3	



Pentenomycin	R_1	R_2	R_3
I (7)	H	H	H
II (8)	H	H	Ac
III (9)	Ac	H	H
10	Ac	Ac	Ac
11	CH_3 CH_3		H



4-Dehydropentenomycin (**12**)

tionic exchanger SPC 25, both eluted with H_2O, and, secondly, by preparative HPLC on RP 18, and eluted with H_2O containing 0.05% of AcOH.

A total of 84 mg of pure epipentenomycin was obtained. Purity was monitored by GC of the natural compound or its trimethylsilylated(TMSi) derivative on OV1 series column, raising oven temperature from 120 to 320°C at 3°C/minute.

The acetylated (**4**), TMSi (**5**) and acetonide (**6**) derivatives were carried out and purified by standard methods.

All NMR assignments were made from double irradiation and nuclear Overhauser effect (NOE) experiments; they were completed by 2D NMR (XH-CORR).

Results and Discussion

Structural Elucidation

The spectral data of the natural compound did not allow us to distinguish between epimers, pentenomycin I and epipentenomycin I. The structure of epipentenomycin was elucidated as follows; treatment with acetone and methylchloroformate, yielded an acetonide between C(5)-O and C(6)H_2-O and not between C(4)-O and C(5)-O as it is observed for pentenomycin I (**7**). Moreover, the acetylated compound exhibited the same ^{1}H NMR spectrum as the synthetic acetylated compound (**4**), and the same important deshielding of the C(4)H, was observed by comparison with the C(4)H of **10**[4]. As shown by SHONO *et al.*[4], this appears to be a characteristic of epipentenomycin and rules out the pentenomycin structure. Based on an $[\alpha]_D$ of +130°, the compound was identified as the (+)-enantiomer of epipentenomycin.

Biological Properties

The antimicrobial activities of epipentenomycin were measured by the paper-disk method. (+)-Epipentenomycin exhibited antimicrobial activity against some Gram-positive bacteria, such as *Staphylococcus aureus* ATCC 9144, *S. aureus* IAM 1241, *Staphylococcus epidermidis* IAM 1118, *Staphylococcus haemolyticus* IAM 1662, *Micrococcus luteus* IAM 1456 and *M. luteus* IAM 1300 but showed no activity against *Escherichia coli* CIP 54 127, *Candida tropicalis* S 120, *Candida albicans* ATCC 2091 and *Absidia corymbifera* CIP 1129 75.

Physical and Chemical Properties

(+)-Epipentenomycin I (**1**): ^{1}H NMR (Cameca 350 MHz, D_2O, δ ppm/TMS, J Hz) 7.73 (1H, dd, $J_{2,3}$=6 Hz, $J_{3,4}$=1.5 Hz, 3-H), 6.41 (1H, dd, $J_{2,3}$=6 Hz, $J_{2,4}$=1.5, 2-H), 4.84 (under DOH, 4-H), 3.84 (1H, d, J_{gem}=12 Hz, 6-H_a), 3.74 (1H, d, J_{gem}=12 Hz, 6-H_b); ^{13}C NMR (Bruker 50.3 MHz, D_2O, δ ppm, dioxane at 67.4 ppm) 208.12 (C-1), 164.35 (C-3), 133.06 (C-2), 82.96 (C-5), 78.11 (C-4), 64.18 (C-6); UV $\lambda_{max}^{H_2O}$ nm (ε) 212 (6,450), 320 (71); $[\alpha]_D$ +130° (c 0.51, H_2O).

(+)-Epipentenomycin Tri-TMSi (**5**): Electron impact MS (70 eV) m/z (relative intensity) 360 (M^+, 6), 345 (M−CH_3, 15), 331 (5), 257 (M−CH_2O−TMSi, 8), 204 (17), 156 (10), 147 (25), 73 (100).

Epipentenomycin Acetonide (**6**): ^{1}H NMR (Bruker 200 MHz, $CDCl_3$, δ ppm/TMS) 7.50 (1H, dd, $J_{2,3}$=6.6 Hz, $J_{3,4}$=1.7 Hz, 3-H), 6.34 (1H, dd, $J_{2,3}$=6.6 Hz, $J_{2,4}$=1.7 Hz, 2-H), 4.86 (1H, m, 4-H), 4.45 (1H, d, J_{gem}=8.8 Hz, 6-H_a), 3.84 (1H, d, J_{gem}=8.8 Hz, 6-H_b), 2.73 (1H, br d, J=4.3 Hz, 4-OH), 1.56 (3H, s, CH_3), 1.51 (3H, s, CH_3).

In our knowledge, this is the first report of (+)-epipentenomycin as natural compound; moreover, until now, this class of antibiotics had never been found outside the Streptomycetales.

The large amount of (+)-epipentenomycin in the carpophores of *Peziza* sp. (*ca.* 20% of dry weight) appears to be very noteworthy and reminds one of the large amount of mannitol found in many fungi. Curiously, the carpophores of *Peziza* sp. are entirely devoid of this hexitol which is regarded as osmoprotector[5]. A high concentration of (+)-epipentenomycin might protect the carpophore against the numerous microorganisms present in horse manure.

Therefore, unlike *Agaricus* species which accumulate mannitol and agaritine (*p*-hydroxymethylphenylhydrazine) and other related compounds regarded as antibiotics[6], *Peziza* sp. seems to be protected against dehydratation and other microorganisms by a single compound, (+)-epipentenomycin.

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