DESERTOMYCIN B, A NEW DESERTOMYCIN RELATED ANTIBIOTIC

Sir:

Desertomycin[1,2] (**1**) and a new related antibiotic termed desertomycin B (**2**) (Fig. 1) were found in the culture broth of *Streptomyces* strain SD167 (Donegani collection). The producing organism was isolated from a soil sample collected in the north of Italy in a screening program for the discovery of agrochemicals of microbial origin for use against phytopathogenic fungi.

1 and **2** were isolated and their structures were determined by NMR and MS methods.

The production, isolation, structure and biological activity of **2** are reported in this communication. The producing organism was grown on a agar slant incubated at 28°C for 5 days (PM8 medium).

The mature slant culture was used to inoculate PM8 medium which contained soluble starch 20 g, glucose 10 g, $CaCO_3$ 3 g, casein hydrolysate 2 g, cotton seed flour 2 g, yeast extract 2 g, and beef extract 2 g added per liter of deionized water. The presterilization pH was adjusted to 7 with NaOH and 100 ml of PM8 medium were dispensed into 500 ml fermentation flasks. The medium was sterilized by autoclaving for 20 min at 121°C.

The inoculated flasks were incubated at 28°C for 72 hours on a rotary shaker (180 rpm). The resulting culture was used to inoculate (5% seed rate) 10 liters of PM8 medium which was employed as described above.

After 72 hours the fermentation broth was centrifuged and the mecelial cake was treated with ethanol-water (50:50). The extract was filtered, concentrated and added to the broth. The solution obtained was ultrafiltered (polysulfonic hollow-fibers, cut-off 10,000) and adsorbed onto macroreticular neutral resin (XAD-4, Rohm & Haas Co., Philadelphia, U.S.A.), washed with water and then was eluted with acetonitrile (gradient from 20% to 80% in water).

The separation of **1** and **2** was achieved by chromatography on reverse phase silica C18 (Amicon Europe, Lausanne, CH), eluting with methanol (gradient from 20% to 80% in a 30 mM KH_2PO_4 solution adjusted to pH 3 with H_3PO_4).

1 was eluted first after a 2 column volume wash and **2** after a 4 column volume wash.

Fig. 1. Structures of desertomycin (**1**) and desertomycin B (**2**).

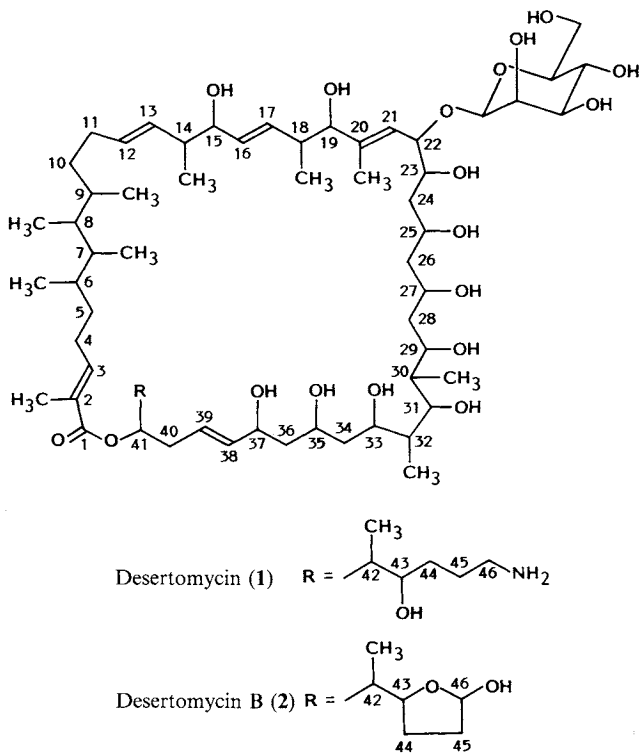
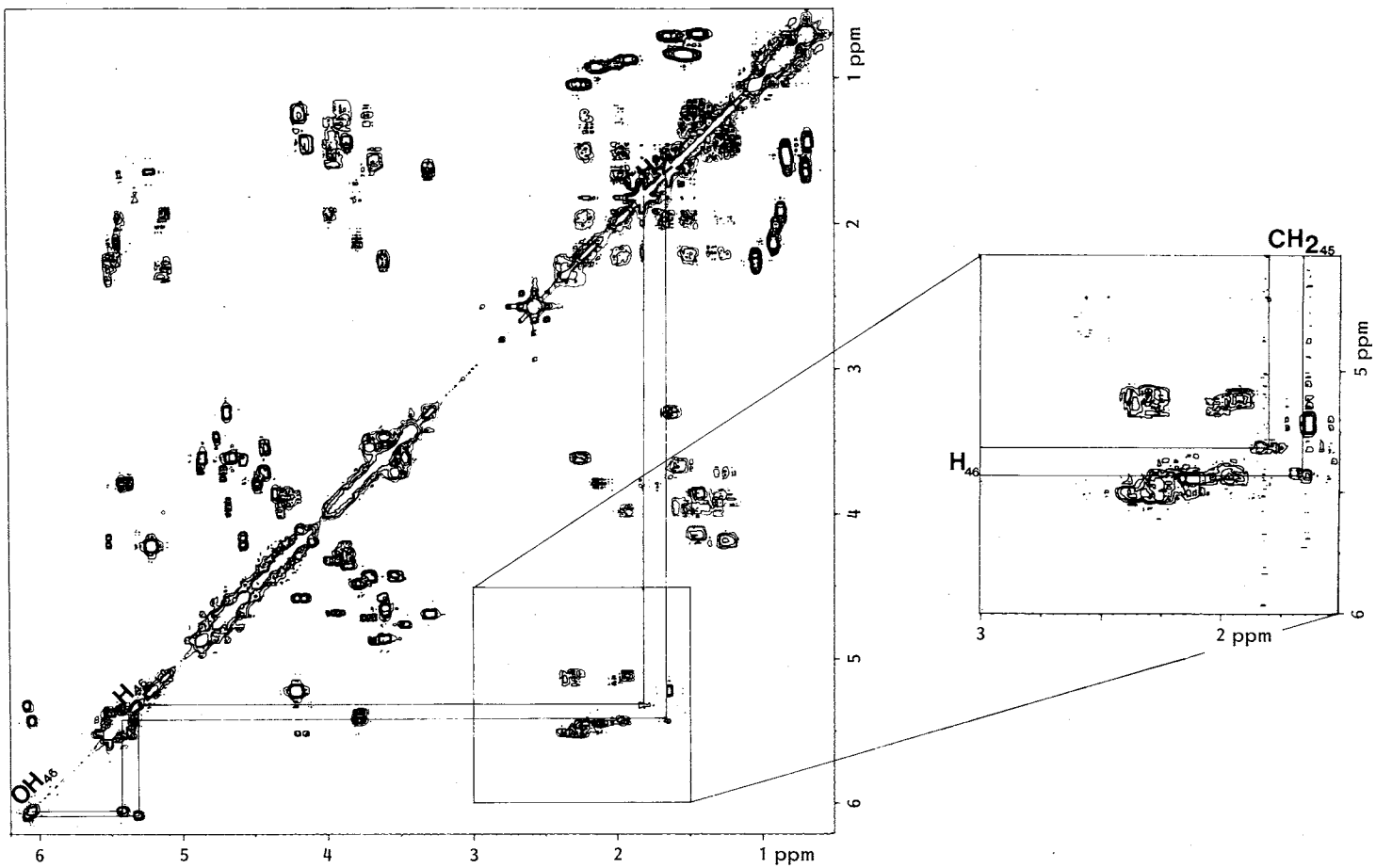


Fig. 2. 2D-COSY spectrum of desertomycin B (**2**) in DMSO-d_6.

In the enlarged area the coupling between CH-46 and CH_2-45 are emphasized.



The fractions containing each antibiotic were separately pooled and concentrated under vacuum to remove the solvent. After adjusting the pH to 7 with NaOH, the pooled fractions were adsorbed onto XAD-4 resin for salt removal and were eluted with acetonitrile - water (70 : 30).

1 and **2** were obtained as white powders by precipitation from an ethanol - water (50 : 50) solution using acetonitrile. An HPLC assay was carried out on a LiChrosorb RP18 (Merck, Darmstadt, Germany) cartridge column (7 fm, 250 × 4 mm i.d.) equipped with a guard-cartridge (μBondapak RP18 Waters/Millipore, Milford, MA). Column temperature was maintained at 30°C. The column was eluited with a mixture of 12 mM KH_2PO_4 solution (adjusted to pH 3 with H_3PO_4) and methanol (30 : 70), **1** had a retention time of 4.6 minutes, and **2** of 9.3 minutes employing a flow rate of 1 ml/minute.

1 was identified by MS and NMR analyses which were virtually identical to those reported in the literature[2,3].

2 is structurally related to **1**, but shows some definite differences.

The molecular weight of **2** is 1,190 dalton, only one mass unit lower than **1**, as determined by from FAB-MS experiments, which gave a $(M-H)^-$ ion at *m/z* 1,189 in the negative ion mode, and a $(M+Na)^+$ ion at *m/z* 1,213 or a $(M+K)^+$ ion at *m/z* 1,229 in the positive ion mode. Elemental analysis of **2** showed the absence of nitrogen in the molecule, while **1** was shown to possess a primary amino group by virtue of its positive reaction to ninhydrin[3].

The ^1H NMR, ^{13}C NMR and 2D COSY spectra, obtained for both compounds showed that the chemical structure of **2** is identical to **1** as far as the macrocyclic and sugar moieties are concerned, but some discrepancies exist in the side chain (C-42/C-46).

On the basis of detailed analysis, it was possible to demonstrate that these differences were due to the presence of a five-membered cyclic hemiacetal (R group, Fig. 1) occurring in two diastereoisomeric forms.

The ^{13}C NMR spectrum of **2** revealed the absence of the signal at 39.0 ppm which corresponded to C-46 in **1** however there were two new CH signals at 97.2 and 96.9 ppm observed in the anomeric region of **2**.

Likewise in the ^1H NMR spectrum, the signal at 2.75 ppm previously assigned to CH_2-46 was lacking while two new resonances at 5.42 and 5.31 ppm (integrating respectively as 0.4 and 0.6 protons) appeared.

Furthermore, the COSY spectrum (Fig. 2) revealed a coupling of these signals with two OH protons at 6.07 and 6.03 ppm (integrating as 0.4 and 0.6 protons as well).

These experimental data support the hypothesis of an hemiacetalic group being present in two configurations. As a consequence of the formation of the hemiacetalic ring, the involved carbons and protons of **2** underwent a significant variation in their chemical shifts in comparison with **1**:

(a) The signal corresponding to CH-43 at 70.1 ppm in **1** was lacking in **2** and there were also two new CH signals for **2** at 79.7 and 77.3 ppm. In addition the H-43 signal was shifted from 3.33 to 3.98 ppm in **2**.

(b) The ^{13}C chemical shifts for CH_2-44 and CH_2-45 which appeared in **1** at 29.6 and 23.7 ppm were shifted to 31.8 and 26.9 ppm in **2**.

The formation of the two diastereoisomeric cyclic hemiacetals can be considered a consequence of a terminal aldehydic group on CH_2-46 which reacted in a non-stereospecific way with the hydroxylic group in γ position.

The splitting of the ^1H and ^{13}C NMR signals of the groups involved in the ring are summarized in Table 1.

As expected, the splitting effects were more pronounced for the atoms adjacent to the racemic center.

Table 1. Side chain ^{13}C and ^1H NMR chemical shift differences between desertomycin and desertomycin B.

No.	Desertomycin (**1**)		Desertomycin B (**2**)	
	^{13}C	^1H	^{13}C	^1H
46	39.0 (t)	2.75 (2H)	97.2, 96.9 (d)	5.42, 5.31 (1H)
45	23.7 (t)	1.57, 1.71 (2H)	26.9 (t)	1.85, 1.65 (2H)
44	29.6 (t)	1.28, 1.47 (2H)	31.8 (t)	—
43	70.1 (d)	3.33 (1H)	79.7, 77.3 (d)	3.98 (1H)
42	41.7 (d)	1.83 (1H)	41.4 (d)	1.92, 2.02 (1H)

Table 2. Antifungal activity (MIC, μg/ml) of desertomycin and desertomycin B against some phytopatogenic fungi.

Fungus	Desertomycin (1)	Desertomycin B (2)
Botrytis cinerea	25	>100
Helminthosporium teres	15	>100
Rhizoctonia solanii	30	>100
Pythium ultimum	5	40

All strains were a laboratory collection, MICs were determined by an agar dilution method in Potato dextrose agar (Oxoid).

1 is known to posses a good activity against a number of fungi and bacteria[4], however **2** shows considerably less antimicrobial activity despite its structural similarity to **1**.

In fact, among the phytopatogenic fungi tested, the only activity observed for **2** was against *Pythium ultimum* (laboratory strain), as reported in Table 2.

2 showed no mortality in mice at doses $\leqslant$20 mg/kg ip (higher doses were not tested), while **1** was reported[4] to be rather toxic (2.6 mg/kg ip).

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