Tetrahedron Letters No. 33, pp 2977 - 2978.
0040-4039/78/0808-2977$02.00/0

ISOLATION AND CHARACTERIZATION OF DESEPOXY-4,5-DIDEHYDRO-METHYLENOMYCIN A. A PRECURSOR OF THE ANTIBIOTIC METHYLENOMYCIN A IN SCP1$^+$ STRAINS OF STREPTOMYCES COELICOLOR A3(2).

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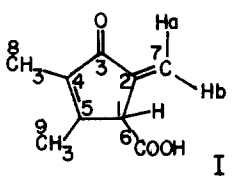
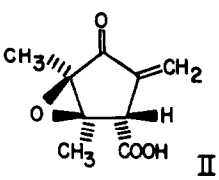
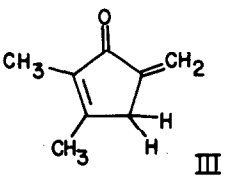
Previous studies in this laboratory have shown[2,3,4] that strains of Streptomyces coelicolor A3(2) which carry the wild-type S. coelicolor plasmid one (SCP1) produce the antibiotic methylenomycin A (MMY-A) (II)[5,6,7]. II can be detected in culture extracts of S. coelicolor as a fluorescence quenching spot (TLC on silicagel with fluorescent indicator, solvent A[3]: ethyl acetate-acetic acid-water 88:6:6, Rf 0.74; solvent B: benzene-acetic acid 100:8, run twice, Rf 0.38) along with a second fluorescence quenching spot (solvent A, Rf 0.66; solvent B, Rf 0.30). We show here that the second quenching material is desepoxy-4,5-didehydro-methylenomycin A (I) (4,5-dimethyl-2-methylene-4-cyclopenten-3-one-1-carboxylic acid) and demonstrate that it can be converted into II by two SCP1$^+$ strains of S. coelicolor A3(2).



I



II



III

S. coelicolor strains 104[3] (SCP1$^+$), R39[4] (SCP1$^+$, MMY-A$^-$, a strain acting as a converter strain in co-synthesis experiments with MMY-A$^-$ strains which themselves can act as secreters) and 1190[3] (SCP1$^-$) were grown in 50 ml or 500 ml of complete medium (CM) as described previously[2]. I was isolated from the filtered and acidified (pH 2) broth of a four day-old shake flask culture (500 ml CM, 28°C) of methylenomycin A-producing strain 104 by extraction with ethyl acetate (500 ml). The organic phase was dried with anhydrous Na_2SO_4 and evaporated in the presence of 1 g of SiO_2, and this material was added to a SiO_2/toluene column (10 g SiO_2). The column was developed with toluene-acetic acid 100:2 (5 ml fractions) to yield methylenomycin A (68 mg) in fractions 22-30 and subsequently I (97 mg) in fractions 36-60 as pure compounds (TLC in solvents A and B).

I was obtained as a colorless acidic oil, soluble in ethyl acetate, chloroform and water. It polymerizes readily unless kept in solution. MW = 166.062 ($C_6H_{10}O_3$ = 166.063) prominent ions: m/e (relative intensity) 166.0 (100), 93.0 (68.7), 121.0 (56.6), 77.0 (46.4), 79.0 (40.8), 91.0 (39.3), 39.0 (36.3), 122.0 (29.4), 43.0 (27.2). The sodium salt of I (melting range 315-320°) is relatively stable. $[\alpha]_D^{25}$ + 250.4 (c 0.585, Na salt in H_2O), the UV spectrum of the Na salt of I in water showed maxima at 252 nm (ε, 7200) and 193 nm (ε, 11300), and a shoulder at 274 nm (ε, 6000) indicating the possible existence of a cyclopentenone conjugated with an

exocyclic methylene. The IR (neat, free acid) showed absorptions at 3500-2500 and 1700 cm^{-1} due to a carboxyl group and at 1735 and 1630 cm^{-1} due to a cyclopentenone. PMR (60 MHz, δ, in $CDCl_3$, TMS) 1.82 (3H, broad s, CH_3 at C-5), 2.10 (3H, s, CH_3 at C-4), 4.08 (1H, m, H-1), 5.64 (1H, d, $J_{1,7a \text{ or } b}$ = 2, H-7a or b) 6.19 (1H, d, $J_{1,7b \text{ or } a}$ = 2, H-7b or a), 8.33 (1H, br, COOH, disappears upon addition of D_2O). Irradiation at δ 4.08 of the H-1 proton sharpens the doublets at δ 5.64 and 6.19 and increases the height of the CH_3 signal at δ 1.82 which is therefore assigned to C-9. In accordance with previous results[2,3,4] I did not show biological activity against Bacillus subtilis and it failed to inhibit the growth of strain 1190, which is inhibited by II[2,3,4].

I acts as a precursor of II in strains 104 and R39, but not in strain 1190 which lacks the SCP1 plasmid. Radioactive I (5 mg, 5 x 10^4 dpm)[10], was administered for 48 hrs to 2 day-old cultures of strains 104, R39 and 1190 grown in 50 ml of CM. The cultures were harvested after 48 hrs by filtration and extracted with ethyl acetate. The extracts were examined by autoradiography and bioautography using B. subtilis respectively. Darkening of the film at the Rf value of II (systems A and B) and a zone of inhibition at the Rf value of II (solvent B) were seen only in the extracts from strains 104[11] and R39 but not in those of strain 1190 indicating the occurence of a plasmid-determined epoxidation. The structure assigned to I is substantiated by the demonstration that it is a precursor of II and by the observation that the UV, IR and PMR spectra of I closely resemble those reported for 4,5-dimethyl-2-methylene-4-cyclopenten-1-one (III)[9,12].

Acknowledgments: We gratefully acknowledge Dr. J.L. Jernow for making information available prior to publication and we thank Mr. R. Self and Dr. D.A. Coxon, Food Research Institute, Norwich, for their help with mass and PMR spectra, respectively. U.H. acknowledges the Gustavus Pfeiffer fellowship for 1976 by the American Foundation for Pharmaceutical Education and support by grants CA 14378 and CA 18587 from the National Cancer Institute, DHEW, U.S.A.

References and Notes

1. Permanent address: Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, U.S.A.
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6. T. Haneishi, A. Terahara, M. Arai, T. Hata and C. Tamura, J. Antibiot. 27, 393 (1974).
7. Racemic II[8] and (+)II[9] were recently synthesized by different routes and the absolute configuration of II is being determined [9].
8. R.M. Scarborough, Jr. and A.B. Smith, III, J. Amer. Chem. Soc. 99, 7085 (1977).
9. J.L. Jernow, personal communication.
10. Obtained by feeding [1-^{14}C]sodium acetate (2 mg, 6 x 10^6 dpm) to a 3 day-old culture of strain 104 in 500 ml CM followed by isolation by column chromatography as described above.
11. Incorporation rate (total radioactivity recovered in II/total radioactivity administered as I x 100): 16%.
12. III was synthesized by J.L. Jernow, W. Tautz, and P. Rosen, Abstracts (and 1000 word abstract[9]), 172nd National Meeting of the American Chemical Society, San Francisco, California, Sept. 1976, No. ORGN 6, in their efforts to clarify the structure of methylenomycin B[5,6].

(Received in USA 18 April 1978; received in UK for publication 20 June 1978)