Notes

SARAMYCETIN, A THIAZOLYL PEPTIDE FROM A *STREPTOMYCES* SP.: CHEMICAL CHARACTERIZATION AND MOLECULAR WEIGHT DETERMINATION

R. COOPER†, I. TRUUMEES, T. BARRETT, M. PATEL and J. SCHWARTZ

Department of Microbial Products,

M. PUAR, P. DAS and B. PRAMANIK

Department of Analytical Spectroscopy,
Schering-Plough Research,
60 Orange Street, Bloomfield, NJ 07003, U.S.A.

(Received for publication January 10, 1990)

In the course of our continuing search for novel antifungal antibiotics of microbial origin, compound Sch 43057 was isolated in our natural products screening program using the os-1 mutants of *Neurospora crassa* described by SELITRENNIKOFF (1983)[1]. We herein report the isolation of Sch 43057, an os-1 active from a streptomycete, its identity to saramycetin[2] and the molecular formula of this compound which we have now established.

An os-1 active culture broth from *Streptomyces* sp. SCC 1943 was identified (A. HORAN, Schering Co., personal communication). A sample of previously frozen whole broth of SCC 1943 was transferred into a germination medium consisting of glucose 0.1%, trehalose 0.1%, casein 0.5%, soy flour 0.5%, yeast extract 0.5% in a volume of 1,000 ml tap water adjusted to pH 7.2 before addition of $CaCO_3$ 0.2%. After 48 hours incubation at 30°C on a rotary shaker operating at 350 rpm, a portion of the resulting cell suspension was used to inoculate a second stage germination using the same medium and conditions. After 48 hours, the cell suspension was transferred into fermentation medium consisting of the following: Soluble starch 2.5%, Proflo flour 0.1%, Marcor meat peptone SB 0.5%, Cerelose 0.5%, $NaNO_3$ 1%, $ZnSO_4 \cdot 7H_2O$ 0.0005%, $MgCl_2 \cdot 6H_2O$ 0.0005%, $FeSO_4 \cdot H_2O$ 0.00014%, in 1,000 ml H_2O adjusted to pH 7.9. Fermentation was carried out at 30°C on a rotary shaker operating at 350 rpm. Optimum activity was observed after 90 hours.

Isolation of small quantities of the active component is shown in Fig. 1. Addition of phosphate buffer during the isolation steps was necessary since the compound is unstable between pH 2~5 and 7~9. Final purification was achieved on a column packed with MCI gel CHP20P (Mitsubishi Chemical Ind. Ltd., Japan) eluting with aqueous MeOH to give an amorphous off-white solid after lyophilization.

One UV absorbing spot of Rf 0.3 was observed on silica gel TLC plates developed in $CHCl_3$-MeOH-H_2O (7:13:8, lower phase). The compound gives a positive reaction to both Rydon and Dragendorff reagents. The HPLC profile of Sch 43057 is shown in Fig. 2 using a Whatman ODS-3 (2.5 × 30 cm) column and a mixture of 70% 0.1 M sodium phosphate buffer, pH 5.5 and 30% CH_3CN as eluent at 1 ml/minute flow rate, monitoring at 254 nm. Sch 43057 eluted after 14 minutes. Sch 43057 exhibited absorption maxima at 222 nm (ε 61,000), 273 (28,000) and 300 (21,000) in the UV spectrum (Fig. 3). After 2 hours in the presence of acid, the UV spectrum had changed and the peak at 273 nm had shifted to 289 nm. Sulfur (13.7%) and nitrogen content (15.9%) was indicated by elemental analysis suggestive of a sulfur containing peptide[3].

After many unsuccessful attempts to generate a strong protonated molecular ion in the mass spectrum, the compound was solubilized in neat

Fig. 1. Isolation of Sch 43057.

Broth filtrated (10 liters)

1. adsorbed on Amberlite XAD-16 resin
   eluted 75~100% MeOH
2. chromatography on MCI gel CHP20P,
   75~150 μm
   eluted linear gradient 0 to 80%
   CH_3CN in 0.01 M phosphate buffer, pH 5.5
3. chromatography on Sephadex LH-20
   eluted MeOH-pH 5.5 phosphate buffer (7:3)
4. chromatography on MCI gel CHP20P,
   37~50 μm
   eluted stepwise gradient of H_2O to MeOH

Sch 43057 (10 mg)

† Present address: Sterling Research Group, 25 Great Valley Malvern, PA 15355, U.S.A.

Fig. 2. HPLC of Sch 43057 (=saramycetin).

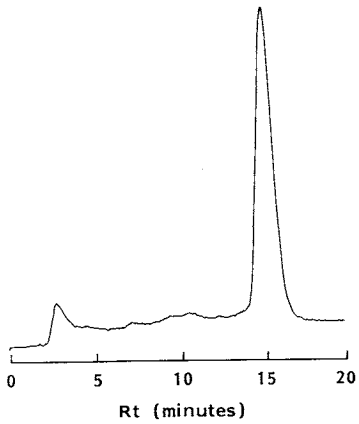


Fig. 3. UV spectra of Sch 43057 in MeOH with addition of acid and base.

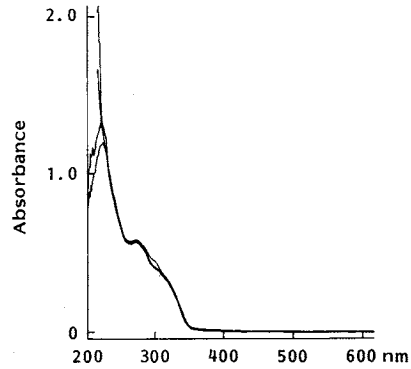
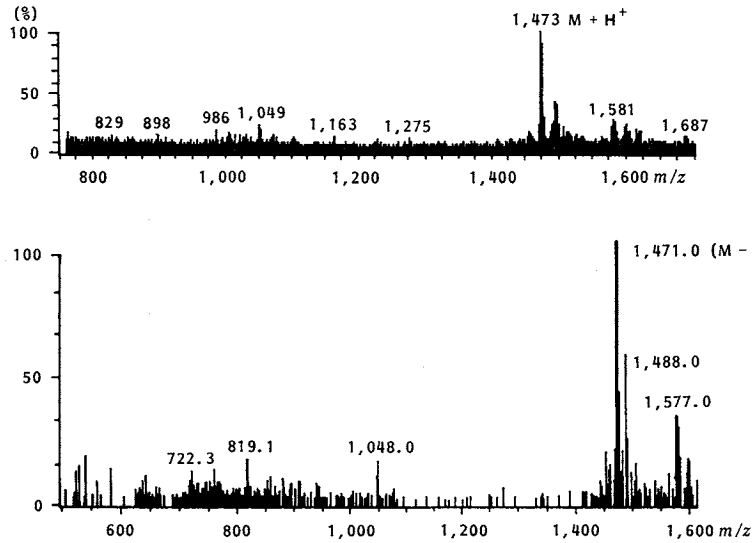


Fig. 4. FAB-MS spectra of Sch 43057 upper trace-positive ion mode lower trace-negative ion mode.



trifluoracetic acid and thioglycerol and used as the matrix for FAB analysis (Fig. 4). The protonated molecular ion, $M+H^+$, was observed at *m/z* 1,473 in an ion cluster 1,473~1,475, with fragment ions at *m/z* 1,163, 1,077, 1,049 and 849. Linked scan studies on *m/z* 1,473 confirmed some of these fragments. In the negative ion FAB mass spectrum, $(M-H)^-$ was observed at *m/z* 1,471 (in an ion cluster 1,471~1,473), and negative ion fragments were observed at *m/z* 1,162, 1,048 and 878 which were consistent with the positive ion data. These data suggest the MW of Sch 43057 is 1,472. From the ^{13}C and HR-MS data, a molecular formula $C_{60}H_{68}N_{18}O_{15}S_6$=MW 1,472 is proposed ($(M+H)^+$, 1,473.3395).

Acid hydrolysis (6N HCl) of Sch 43057 gave proline, threonine, glycine, aspartic acid and cysteine (subsequently analyzed in the form of cysteic acid) confirmed by the CI-MS data, TLC and electrophoresis with authentic standards.

At this stage, these data might have differentiated Sch 43057 from other reported thiazolyl peptides[3]. One compound belonging to this group, namely saramycetin[2], has been reported having no antibacterial activity and only a very narrow spectrum of activity against certain fungi. However, the MW of saramycetin reportedly varies[4~6] between 1,450, 2,010 and 14,000; its complete structure is unknown. Comparison of Sch 43057 with a sample of saramycetin† confirmed our suspicions that indeed the two compounds are

† A sample of saramycetin was kindly supplied by Dr. E. MEYERS, E. R. Squibb and Sons.

Fig. 5. ^{1}H NMR spectrum in DMSO-d_6 of Sch 43057.

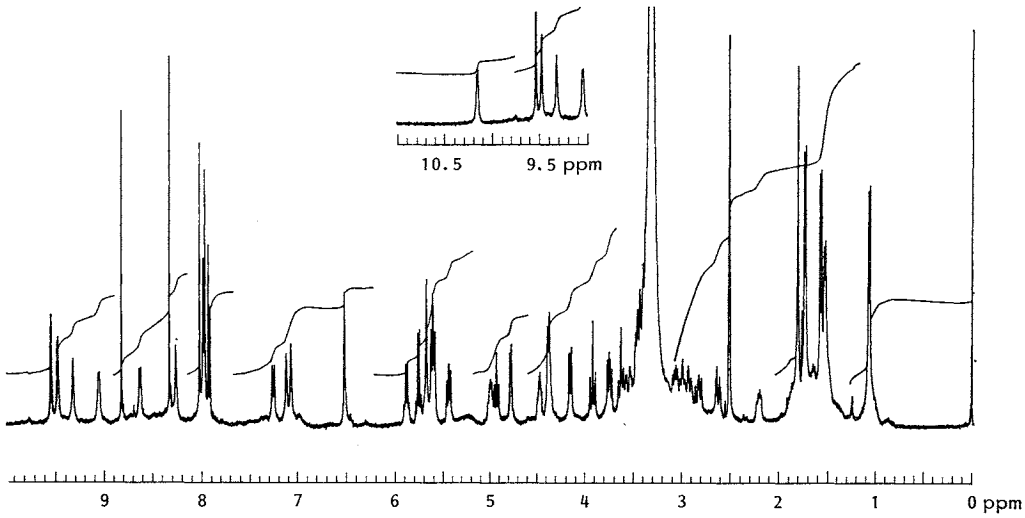
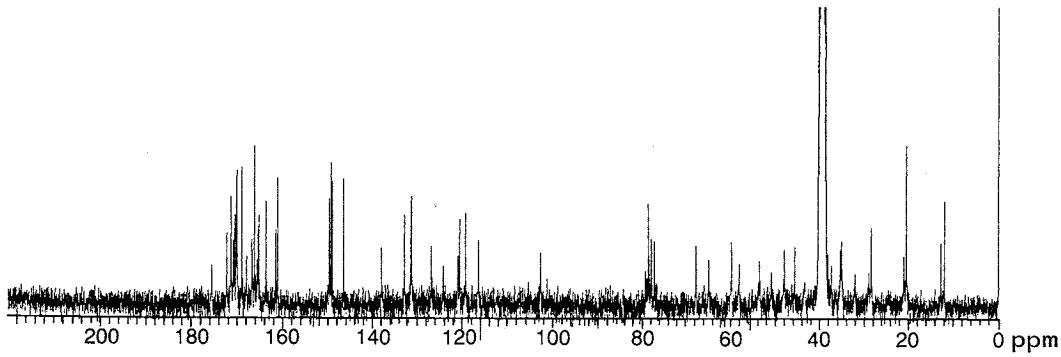


Fig. 6. ^{13}C NMR of Sch 43057 in DMSO-d_6.



identical from the following results: 1) The FAB-MS spectra in both the positive ($(M+H)^+$ m/z 1,473) and negative mode ($(M-H)^-$ m/z 1,471) for Sch 43057 and saramycetin are the same. 2) HPLC of Sch 43057 and saramycetin show the two compounds co-elute. 3) The TLC and UV data for saramycetin are identical to Sch 43057.

^{1}H and ^{13}C NMR data were obtained for Sch 43057 in DMSO-d_6 and the spectra are shown in Figs. 5 and 6, respectively. In the ^{13}C spectrum, 60 carbon signals were observed. In the ^{1}H NMR spectrum there are three sharp singlet non-exchangeable protons between δ 8~9 belonging to three thiazole rings. Three thiazoline rings account for the other three sulfur atoms in the molecule which break down during acid hydrolysis to generate cysteine. These are observed as isolated ABX systems: δ 3.5, 2H, m coupled to δ 4.93, 1H, t, J=9 Hz; δ 2.92, dd, J=3 and 12.5 Hz, δ 3.05, dd, J=6.2 and 12.5 Hz coupled to δ 4.47, 1H, m; δ 2.62, dd, J=4.5 and 15 Hz, δ 2.82, dd, J=9 and 15 Hz coupled to δ 5.02, 1H, dd, J=4.5 and 9 Hz. Dehydroalanine, (δ 6.50, 1H, s, δ 5.68, 1H, br s coupled to δ 9.54, 1H, br, N*H*) and 2 debutyrine systems (δ 1.74, 3H, d, J=7 Hz coupled to δ 5.88, 1H, br q, J=7 Hz coupled to δ 9.47, 1H, br s, N*H*) and (δ 1.56, 3H, d, J=7 Hz coupled to δ 5.74, 1H, q, J=7 Hz) were observed. These amino acids were not generated through acid hydrolysis since they are non-peptide bound in the molecule.

Extraction of the aqueous acid hydrolysate into $CHCl_3$ gave a single ninhydrin-negative, UV-positive product. A protonated molecular ion $M+H^+$ at m/z 255, and a sodiated ion $M+Na^+$ at m/z 277 were observed in the FAB-MS spectrum. Together with the UV spectrum showing a maximum at 289 nm, these data are in agreement for saramycetic acid, reportedly a hydrolysis product from saramycetin[7].

Sch 43057 is an unstable cyclic peptide belonging

to the thiazolyl peptide family, possessing a MW of 1,472, identical to saramycetin. We note that several reports of similar compounds have recently appeared including jingsimycin[8,9], globopeptin[10] and cystargin[11]. This is the first definitive evidence of the molecular formula for saramycetin (=Sch 43057).

References

1) SELITRENNIKOFF, C. P.: Use of a temperature-sensitive, protoplast-forming *Neurospora crassa* strain for the detection of antifungal antibiotics. Antimicrob. Agents Chemother. 23: 757~765, 1983
2) BERGER, J.; L. H. STERNBACH, M. MULLER, E. R. LASALA, E. GRUNBERG & M. W. GOLDBERG: X-5079C, a new polypeptide antifungal antibiotic active against systemic mycoses. Antimicrob. Agents Chemother.-1961: 436~444, 1962
3) BERDY, J.; A. ASZALOS, M. BOSTIAN & K. L. MCNITT (*Ed.*): 432. Thiapeptides. *In* CRC Handbook of Antibiotic Compounds. Volume IV. Part 1. Amino Acid and Peptide Antibiotics. p. 387, CRC Press, 1980
4) RINEHART, K. L.; M. L. MOORE, L. A. GUADIOSO, M. BARBER, R. S. BARDOLI, R. D. SEDGWICK, A. N. TYLER & B. N. GREEN: Fast Atom Bombardment Mass Spectrometry applied to peptides. Pept. Synth. Struct. Function proc., 7th Am. Peptide Symposium, pp. 757~760, 1980
5) KIRSCHBAUM, J. & A. ASZALOS: Molecular weight of the antifungal antibiotic saramycetin. J. Pharm. Sci. 56: 410, 1967
6) BAUDET, P & E. CHERBULIEZ: Sur la saramycetine (=X-5079C), polypeptide antibiotique du *Streptomyces saraceticus*. I. Premieres donnees concernant sa structure. Helv. Chim. Acta 47: 661~683, 1964
7) ASZALOS, A.; J. KIRSCHBAUM, O. KOCY, F. RUSSO-ALESI & J. ALICINO: Chemistry of saramycetin. II. Sequence studies. J. Antibiotics 22: 577~579, 1969 and refs therein
8) LU, W. Z.; M. J. ZHOU, Z. YU, Q. D. LIU, J. S. YAN & G. Y. GU: Purification and identification of jingsimycin. Acta Microbiol. Sin. 20: 191~195, 1980 [CA 94: 2894, 1980]
9) ZANG, S.; H. ZHAO & J. LIU: Studies on the agricultural antibiotics 5102. II. Isolation and characterization of antibiotic 5102-2. Acta Microbiol. Sin. 22: 145~150, 1982 [CA 97: 143076, 1983]
10) TANAKA, Y.; K. HIRATA, Y. TAKAHASHI, Y. IWAI & S. ŌMURA: Globopeptin, a new antifungal peptide antibiotic. J. Antibiotics 40: 242~244, 1987
11) URAMOTO, M.; Y. ITOH, R. SEKIGUCHI, K. SHIN-YA, H. KUSAKABE & K. ISONO: A new antifungal antibiotic, cystargin: Fermentation, isolation, and characterization. J. Antibiotics 41: 1763~1768, 1988