NOTE

MUTANTS OF *STREPTOMYCES CATTLEYA* PRODUCING N-ACETYL AND DESHYDROXY CARBAPENEMS RELATED TO THIENAMYCIN

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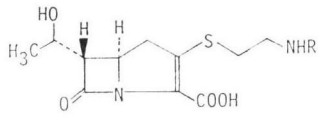
Streptomyces cattleya NRRL 8057 produces the β-lactam antibiotic thienamycin (**1a**) and traces of N-acetylthienamycin (**1b**)[1]. A closely related carbapenem, PS-5 (**2a**) is produced by *Streptomyces cremeus* subsp *auratilis*.[2,11]* The latter has recently been deacetylated with L-amino acid acylase from porcine kidney and D-amino acid acylase from *Streptomyces olivaceus* affording a desacetyl compound NS-5 (**2b**).[3] In addition, a European patent published in 1979 discloses the preparation of NS-5 (deshydroxythienamycin) *via* a lengthy chemical synthesis.[4] These compounds display very potent antimicrobial activity against Gram-positive and negative bacteria, as well as β-lactamase inhibitory properties.[5,6]

We would like to report the production of carbapenems by two new mutants of *S. cattleya* NRRL 8057. One of these mutants, S-WRI-M459 produces an N-acetyl carbapenem corresponding to N-acetylthienamycin (**1b**) while the second, S-WRI-M5301 produces a deshydroxy analog of thienamycin corresponding to NS-5 (**2b**).

This is the first report on the use of mutants of *S. cattleya* for the production of thienamycin analogues. These mutants should be useful in elucidating biosynthetic pathways leading to this interesting class of carbapenems.

S. cattleya NRRL 8057 was incubated on N-Z amine agar[7] for 7 days at 27°C. Spores were harvested in TX buffer[8] and subjected to the mutagenic action of N-methyl-N′-nitro-N-nitrosoguanidine at 1.0 mg/ml for 2 hours. The spores were collected *via* filtration, suspended in 0.1 M phosphate-buffered saline and plated on N-Z amine agar and incubated as above. Isolated colonies were transferred to N-Z amine agar slants. These stock cultures served as a source of seed for growth in flasks containing a thienamycin production medium (medium B)[9] incubated on a rotary shaker at 210 rpm at 27°C for 48 hours. Samples from each fermentation were clarified *via* centrifugation and aliquots of the crude broths were subjected to chromatography on Whatman No. 1 paper developed in ethanol-water, 70:30 (v/v) using authentic thienamycin as a reference.** The developed chromatograms were subjected to bioautography using *S. aureus* as the test organism. Using these methods we have found two mutants; S-WRI-M459 producing a compound corresponding to N-acetylthienamycin (Rf 0.59) and S-WRI-M5301 producing a compound corresponding to NS-5 (Rf 0.50). Thienamycin (Rf 0.36) was not observed in either case.

For isolation of these antibiotics, fermentations were conducted in 14 liter jars containing



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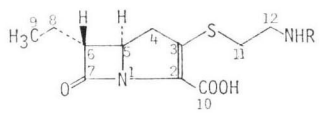
a R=H; Thienamycin
b R=$COCH_3$; N-Acetylthienamycin



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a R=$COCH_3$; PS-5
b R=H; NS-5

* Since submission of this paper, carbapenems PS-6 and PS-7 have been reported.[12]

** Thienamycin was kindly supplied by Dr. J. BIRNBAUM of Merck Institute of Therapeutic Research, Rahway, NJ, U.S.A.

10 liters of medium C[9]. These were inoculated with 500 ml of a 48-hour culture grown in the same medium. The jar fermentors were operated at 28°C with an agitation rate of 200 rpm and an air flow of 5 liters per minute for 48 hours. Antibiotic production was followed using an agar diffusion assay with *S. aureus* as the assay organism.

Antibiotic from S-WRI-M459

The culture filtrate from this organism, after adjustment to pH 7.0, was passed through Dowex 1-X2 (Cl^- form) anion exchange resin. The adsorbed antibiotic was eluted with 1.5% NaCl in 40% aqueous methanol. Fractions displaying antibiotic activity were combined, concentrated and adsorbed on Amberlite XAD-2 from which elution was conducted with 20% aqueous methanol. The material was then subjected to column chromatography *via* Bio-Gel P-2 gel filtration and Diaion HP-20. Final purification was effected *via* preparative HPLC using a Lichrosorb NH_2 (10 μ) column measuring 10× 250 mm with a solvent composed by volume of 81% acetonitrile and 19% 0.02 M phosphate buffer at pH 6.0 and a detector measuring uv absorbance at 280 nm. Antibiotic fractions were combined and lyophilized. On the basis of paper chromatography using three solvent systems and HPLC retention times, this material was indistinguishable from N-acetylthienamycin prepared chemically from pure thienamycin according to published procedures[10]. The pmr spectrum obtained in D_2O showed a sharp singlet at 2.51 ppm (relative to H_2O) attributed to the methyl of an acetyl group. This signal was also present in our chemically prepared sample of N-acetylthienamycin.

Antibiotic from S-WRI-M5301

A 48-hour seed culture of this organism incubated as above was used to inoculate jar fermentors containing 9 liters each of production medium having the following composition: distillers solubles, 1.0%; $CaCO_3$, 0.4% (pH 6.5). These fermentations were conducted as described above for 65 hours. The culture filtrate was passed over Dowex 1-X2 (Cl^- form) anion exchange resin. The antibiotic in the effluent was adsorbed on Amberlite XAD-2 and eluted with 30% aqueous methanol.

From this step, pure material was obtained *via* two methods. In one, use was made of successive column chromatography on Amberlite CG-400, Bio Gel P-2 and XAD-2 followed by HPLC purification on the Lichrosorb NH_2 column eluted with 74% acetonitrile and 26% 0.02 M ammonium acetate buffer at pH 6.5 (v/v). In the second method, successive chromatography was carried out over CM-Sephadex C-25, XAD-2 and Sephadex LH-20. Both methods afforded material with an extinguishable absorbance of 90% (λ_{max} of 297 nm) after treatment with 10 mM hydroxylamine; MH^+ (chemical ionization) 257 ($M^+ + C_2H_5$ at 285), M^+ (electron impact, direct inlet) 256; pmr (D_2O, external TMS) 1.46 (t, CH_3), 2.2 ppm (m, CH_2); ^{13}Cnmr (D_2O, 0.02 M phosphate buffer, pH 7.9, internal TMS) 11.7 (C_8), 22.7 (C_9), 30.1, 40.2, 40.4 ($C_{4,11,12}$), 56.2 (C_5), 60.9 ppm (C_6). No peaks were observed for $C_{2,3,7,10}$ since the small sample size resulted in a low signal to noise ratio. The above data are consistent with a structure corresponding to NS-5 (**2b**).

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References

1) KAHAN, J. S.; F. M. KAHAN, R. GOEGELMAN, S. A. CURRIE, M. JACKSON, E. O. STAPLEY, T. W. MILLER, A. K. MILLER, D. HENDLIN, S. MOCHALES, S. HERNANDEZ, H. B. WOODRUFF & J. BIRNBAUM: Thienamycin, a new β-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. J. Antibiotics 32: 1～12, 1979

2) OKAMURA, K.; S. HIRATA, A. KOKI, K. HORI, N. SHIBAMOTO, Y. OKUMURA, M. OKABE, M. OKAMOTO, K. KOUNO, Y. FUKAGAWA, Y. SHIMAUCHI, T. ISHIKURA & J. LEIN: PS-5, a new β-lactam antibiotic. I. Taxonomy of the producing organism, isolation and physicochemical properties. J. Antibiotics 32: 262～271, 1979

3) FUKAGAWA, Y.; K. KUBO, T. ISHIKURA & K. KOUNO: Deacetylation of PS-5, a new β-lactam compound. I. Microbial deacetylation of PS-5. J. Antibiotics 33: 543～549, 1980

4) Merck and Co. Inc.: EP Appln. 78101157.2, Pub. No. 0～001 628 A1 (1979)

5) OKAMURA, K.; M. SAKAMOTO & T. ISHIKURA: PS-5 inhibition of a β-lactamase from *Proteus vulgaris*. J. Antibiotics 33: 293~302, 1980
6) KROPP, H.; J. S. KAHAN, F. M. KAHAN, J. SUNDELOF, G. DARLAND & J. BIRNBAUM: Thienamycin, a new β-lactam antibiotic. II. *In vitro* and *in vivo* evaluation. 16th ICAAC, Abst. No. 228, 1976
7) American Type Culture Collection, Catalogue of Strains I, Thirteenth Edition, 441, 1978
8) HIRSCH, C. F. & J. C. ENSIGN: Nutritionally defined conditions for germination of *Streptomyces viridochromogenes* spores. J. Bacteriol. 126: 13~23, 1976
9) Merck and Co. Inc.: Antibiotics. U. S. Pat. 3,950,357, Apr. 13, 1976
10) ALBERS-SCHONBERG, G.; B. H. ARISON, O. D. HENSENS, J. HIRSHFIELD, K. HOOGSTEEN, E. A. KACZKA, R. E. RHODES, J. S. KAHAN, F. M. KAHAN, R. W. RATCLIFFE, E. WALTON, L. J. RUSWINKLE, R. B. MORIN & B. G. CHRISTENSEN: Structure and absolute configuration of thienamycin. J. Amer. Chem. Soc. 100: 6491~6499, 1978
11) YAMAMOTO, K.; T. YOSHIOKA, Y. KATO, N. SHIBAMOTO, K. OKAMURA, Y. SHIMAUCHI & T. ISHIKURA: Structure and stereochemistry of antibiotic PS-5. J. Antibiotics 33: 796~803, 1980
12) SHIBAMOTO, N.; A. KOKI, M. NISHINO, K. NAKAMURA, K. KIYOSHIMA, K. OKAMURA, M. OKABE, R. OKAMOTO, Y. FUKAGAWA, Y. SHIMAUCHI, T. ISHIKURA & J. LEIN: PS-6 and PS-7, new β-lactam antibiotics. Isolation, physicochemical properties and structures. J. Antibiotics 33: 1128~1137, 1980