# Sch 36605, A NOVEL ANTI-INFLAMMATORY COMPOUND

## TAXONOMY, FERMENTATION, ISOLATION AND BIOLOGICAL PROPERTIES

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A novel anti-inflammatory compound, Sch 36605, belonging to the blasticidin family of nucleoside compounds was isolated from the fermentation filtrate of a *Streptomyces* sp. Sch 36605, as well as blasticidin S, demonstrated anti-inflammatory activity in the reverse passive Arthus reaction and the adjuvant arthritic rat at doses ranging between 1~10 mg/kg and 0.3~6.0 mg/kg, respectively. A minor component, Sch 36606, co-produced in the fermentation was isolated and identified as a known compound in the blasticidin family of compounds.

In our screening program for unique microbial products with pharmacological activity, a fermentation filtrate from a *Streptomyces* sp. showed anti-inflammatory activity. The major active component, Sch 36605, was purified and identified as a novel nucleoside related to blasticidin S[1,2]. In addition, a closely related minor component, Sch 36606, was also isolated and identified. This paper describes the taxonomy and fermentation of the producing culture, and the isolation and biological properties of the active components. Details of the physico-chemical properties and structure elucidation are reported in a separate paper[3].

### Taxonomy of the Producing Organism

The producing culture, SCC 1785, was isolated from a soil sample collected in France using standard soil dilution techniques and plating the dilutions on water agar (Difco agar, 15 g; tap water, 1,000 ml) containing 10 $\mu$g/ml rifamycin SV. The organism grew abundantly on complex agar media. Vegetative mycelial pigments ranged from light pale yellow to yellow brown. A pale yellow diffusible pigment was formed in several media. The aerial mycelia, en masse, were white. The sporophores were simple, straight to flexuous (*Rectus-Flexibilis*)[4] and fragmented into chains of from 10 to greater than 50, round to ovoid non-motile spores. The spore surface by scanning electron microscopy appeared smooth.

Whole cell analysis by the procedure of LECHEVALIER[5] indicated the presence of LL-diaminopimelic acid. Diagnostic sugars were absent. Based on both whole cell analysis and morphology SCC 1785 was identified as a species of *Streptomyces*.

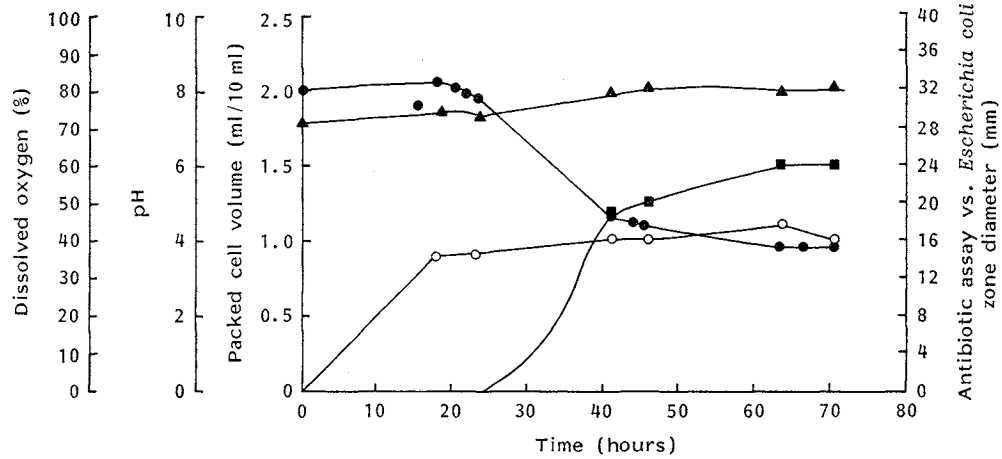
The culture was compared to the deposited *Streptomyces* sp. reported to produce blasticidins[6] (Table 1). SCC 1785 can be clearly differentiated morphologically from these strains. *Streptomyces griseochromogenes*, *Streptomyces griseoflavus* and *Streptomyces albus* subsp. *pathocidicus* all formed spiral sporophores while SCC 1785 formed straight to flexuous sporophores.

Table 1. Comparison of blasticidin producing *Streptomyces*.

| | *Streptomyces* sp. SCC 1785 | *S. griseochromogenes*[17] ATCC 14511 | *S. griseoflavus*[18] ATCC 25456 | *S. albus* subsp. *pathocidicus*[19] ATCC 14510 |
|---|---|---|---|---|
| Color of: | | | | |
| Substrate mycelium | Pale yellow to light yellow-brown | Yellow-brown to dark gray brown | Yellow to orange-yellow | Pale yellow |
| Aerial mycelium | White | Gray | Gray | White |
| Morphology: | | | | |
| Spore chain | *Rectus-Flexibilis* | *Retinaculum-Apertum Spira* | *Spira* | *Retinaculum-Apertum Spira* |
| Spores: | | | | |
| Shape | Round to ovoid | Round to ovoid | Elliptical | Elliptical |
| No./chain | 10 to >50 | 10 to >50 | 10 to >50 | 10 to >50 |
| Surface | Smooth | Spiny | Spiny | Smooth |
| Melanin production | Negative | Positive | Negative | Negative |
| DAP isomer | LL-DAP | LL-DAP | Not reported | Not reported |

DAP: Diaminopimelic acid.

Fig. 1. Fermentation profile.
● Dissolved oxygen, ▲ pH, ○ packed cell volume, ■ antibiotic assay.




## Fermentation

Stock cultures were maintained as frozen whole broths at −20°C in a final concentration of 7% glycerol. A 250-ml Erlenmeyer flask containing 70 ml of seed medium was inoculated with 3.5 ml of the stock culture. The flask was incubated at 30°C on a rotary shaker at 300 rpm for 48 hours. The seed medium consisted of beef extract 0.3%, Tryptone 0.5%, yeast extract 0.5%, Cerelose 0.1%, potato starch 2.4%, $CaCO_3$ 0.2% and Dow-Corning antifoam B 1 ml per liter of tap water. Twenty-five ml of this seed culture was used to inoculate a 2-liter Erlenmeyer flask containing 500 ml of the same seed medium and incubated as above. The entire contents of this second stage seed inoculum were used to inoculate a 14-liter fermentor containing 10 liters of a production medium consisting of Hy-soy 0.3%, Nutritone 0.5%, soluble starch 2%, Cerelose 0.5% and $CaCO_3$ 0.2% in tap water. The pH was adjusted to 6.5 before addition of $CaCO_3$. The fermentation was carried out for 65 hours at 30°C with an air flow of 3.5 liters per minute and an agitation rate of 350 rpm (Fig. 1). Antimicrobial activity against *Escherichia coli* was observed which corresponded to the anti-inflammatory activity.

Fermentations were also conducted in 500-ml Erlenmeyer flasks containing 100 ml production medium, inoculated with a 5%-second stage seed inoculum. These were incubated at 30°C on a gyratory shaker at 300 rpm for 65 hours.

## Isolation

The steps leading to the isolation and purification of Sch 36605 and Sch 36606 are outlined in Fig. 2. After fermentation, 20 liters of the cultured broth was filtered to remove the cells. The anti-inflammatory activity in the filtrate was adsorbed onto 500 ml of BioRad AG 50X8 ($H^+$) resin and the inactive spent filtrate decanted off. The charged resin was eluted with 2 liters of 2 N $NH_4OH$. Further purification steps included ion-exchange and reverse phase chromatography. Final separation of the two active components was achieved by HPLC on a reverse phase $C_{18}$ column eluting with 0.1 M phosphate buffer (pH 6) - methanol (9 : 1). The samples were desalted on MCI gel (Mitsubishi Chemical Industries Limited, Japan) and after concentration and lyophilization, Sch 36605 and Sch 36606 were obtained as white amorphous solids.

## Biological Properties

Sch 36605 demonstrated anti-inflammatory activity in two assay procedures, the reverse passive Arthus reaction (RPAR)[7] and the adjuvant arthritic rat (AAR)[8]. In the RPAR (Table 2), Sch 36605 caused a dose related inhibition of the inflammation, with the highest dose, 10 mg/kg, reducing the paw volume by 47%. Piroxicam, the standard, (obtained from Farmore Co., Italy) had similar activity, reducing the inflammation 61% at a dose of 3 mg/kg.

In the AAR (Table 3), Sch 36605 reduced the swelling of the immune induced paw inflammation at oral doses ranging from 0.3 to 0.6 mg/kg. At the higher dose the paw volume

Fig. 2. Isolation of Sch 36605 and Sch 36606.

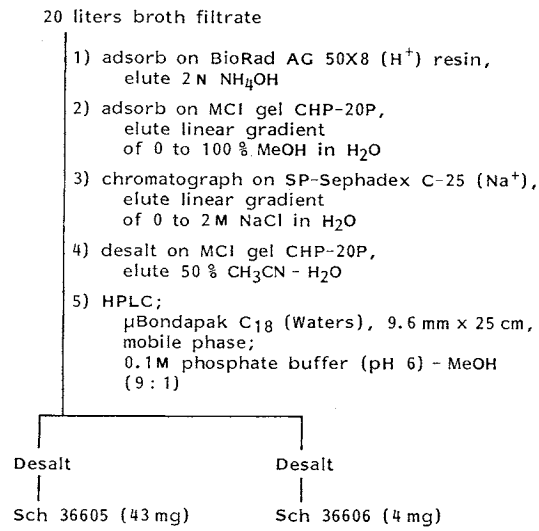



Table 2. Activity of Sch 36605 in the reverse passive Arthus reaction.

| Compound | Oral dose (mg/kg) | Inhibition (%)[a] paw edema |
|---|---|---|
| Sch 36605 | 1.0 | 27 |
| | 5.0 | 36 |
| | 10.0 | 47 |
| Piroxicam | 0.3 | 49 |
| | 1.0 | 57 |
| | 3.0 | 61 |

[a] Measured 2 hours after challenge.

$$\text{Inhibition (\%)} = \frac{\Delta\text{Paw vol control} - \Delta\text{Paw vol drug treated}}{\Delta\text{Paw vol control}} \times 100$$

Table 3. Activity of Sch 36605 in the adjuvant arthritic rat.

| Compound | Daily oral dose (mg/kg) | Inhibition (%)[a] paw edema |
|---|---|---|
| Sch 36605 | 0.3 | 27 |
| | 0.6 | 58 |
| Piroxicam | 0.1 | 52 |
| | 0.3 | 79 |
| | 1.0 | 98 |

[a] Measured on day 21 of assay.

$$\text{Inhibition (\%)} = \frac{\Delta\text{Paw vol control} - \Delta\text{Paw vol drug treated}}{\Delta\text{Paw vol control}} \times 100$$

Table 4. Activity profile of blasticidin S.

| Reverse passive Arthus reaction | | Adjuvant arthritic rat | |
|---|---|---|---|
| Oral dose (mg/kg) | Inhibition (%) paw edema | Daily oral dose (mg/kg) | Inhibition (%) paw edema |
| 1.25 | 22 | 1.1 | 30 |
| 2.5 | 52 | 3.1 | 27 |
| 5.0 | 57 | 6.3 | 27 |

Table 5. Antimicrobial properties.

| Organism | MIC range (μg/ml, MHA, 24 hours) | |
|---|---|---|
| | Sch 36605 | Blasticidin S |
| *Staphylococcus*[a] | >64 | 32~>64 |
| *Escherichia*[b] | ≥64 | ≥64 |

[a] Twenty-six strains of *Staphylococcus*.
[b] Thirteen strains of *Escherichia coli*.
MHA: Mueller-Hinton agar.

was reduced 58%. Piroxicam was also active in the AAR. At the highest dose tested, 1.0 mg/kg, piroxicam inhibits the swelling by 98%.

Structural analysis showed Sch 36605 is similar to blasticidin S. Further biological studies demonstrated that blasticidin S (obtained from Kaken Pharmaceutical Co., Ltd., Japan) was qualitatively similar to Sch 36605 in the RPAR and AAR (Table 4). Blasticidin S in the RPAR, at an oral dose of 2.5 mg/kg, inhibited the swelling by 52%. In the AAR, blasticidin S caused a flat dose response achieving approximately the same level of inhibition, 30%, at doses ranging from 1.2 to 6.3 mg/kg.

Sch 36605 and blasticidin S have weak activity against Gram-positive and Gram-negative organisms as shown in Table 5.

The iv $LD_{50}$ of Sch 36605 is 3.75 mg/kg in mice which is comparable to the reported value of 2.82 mg/kg for blasticidin S[9].

## Discussion

The search for unique pharmacological agents from microbial sources has been increasing dramatically[9]. In this paper, we report on a novel compound with anti-inflammatory activity. Other microbial products have been identified which have anti-inflammatory activity; amicoumacin-A[10], xerosin[11], zygosporin[12] and 6-MFA[13]. Recently, AI-77-B-1[14], structurally related to amicoumacin, was modified synthetically to compounds which demonstrated oral anti-inflammatory and anti-ulcerogenic activity.

Sch 36605 is a novel nucleoside structurally related to blasticidin S, an antibiotic produced by *S. griseochromogenes*. Blasticidin S has been used commercially in Japan to control rice blast disease, caused by *Piricularia oryzae*. Compounds of this type, cytosine glycosides, such as gougerotin[15] and amicetin[16], show weak antibacterial activity, antitumor and antiviral activity. The ability of these compounds to inhibit peptide chain elongation has been demonstrated[16]. The results of our work show that compounds of this type can modulate the inflammatory response as demonstrated in both the RPAR and AAR models.

## References

1) FUKUNAGA, K.; T. MISATO, I. ISHIDA & M. ASAKAWA: Blasticidin, a new antiphytopathogenic fungal substance. Bull. Agr. Chem. Soc. Jpn. 19: 181~188, 1955
2) TAKEUCHI, S.; K. HIRAYAMA, K. UEDA, H. SAKAI & H. YONEHARA: Blasticidin S, a new antibiotic. J. Antibiotics, Ser. A 11: 1~5, 1958
3) COOPER, R.; M. CONOVER & M. PATEL: Sch 36605, structure of a novel nucleoside. J. Antibiotics 41: 123~125, 1988
4) SHIRLING, E. B. & D. GOTTLIEB: Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. 16: 313~340, 1966
5) LECHEVALIER, M. P.: Identification of aerobic actinomycetes of clinical importance. J. Lab. Clin. Med. 71: 934~944, 1968
6) TDS Ltd.: The Actinomycetes Antibiotics Database. USACO Corporation, Tokyo, 1985

7) CARTER, G. W. & R. KRAUSE: The reverse passive Arthus reaction in the rat: A potential screen for novel anti-inflammatory agents. Fed. Proc. 35: 774, 1976
8) WATNICK, A. S.: The adjuvant arthritic rat — a tool for discovering antiarthritic drugs. Mongr. Physiol. Soc. Phila. 1: 155~171, 1975
9) NISBET, L. J. & J. W. WESTLEY: Developments in microbial products screening. Ann. Rep. Med. Chem. 21: 149~157, 1986
10) ITOH, J.; S. OMOTO, T. SHOMURA, N. NISHIZAWA, S. MIYADO, Y. YUDA, U. SHIBATA & S. INOUYE: Amicoumacin-A, a new antibiotic with strong antiinflammatory and antiulcer activity. J. Antibiotics 34: 611~613, 1981
11) GROUPE, V. & R. DONOVICK: Experiences in the search for anti-inflammatory agents of microbial origin. J. Antibiotics 30: 1080~1086, 1977
12) HAYAKAWA, S.; T. MATSUSHIMA, H. MINATO & K. HIROSE (Shionogi): Anti-inflammatory substance, zygosporin A, and production thereof. U.S. 3,660,568, May 2, 1972
13) NAIK, S. R.; V. K. DESHMUKH & P. H. RAMAN: Anti-inflammatory activity of 6-MFA, a compound obtained from fungus *Aspergillus ochraceus*. Jpn. J. Med. Sci. Biol. 39: 139~149, 1986
14) SHIMOJIMA, Y.; T. SHIRAI, T. BABA & H. HAYASHI: 1*H*-2-Benzopyran-1-one derivatives, microbial products with pharmacological activity. Conversion into orally active derivatives with antiinflammatory and antiulcer activities. J. Med. Chem. 28: 3~9, 1985
15) KANZAKI, T.; E. HIGASHIDE, H. YAMAMOTO, M. SHIBATA, K. NAKAZAWA, H. IWASAKI, T. TAKEWAKA & A. MIYAKE: Gougerotin, a new antibacterial antibiotic. J. Antibiotics, Ser. A 15: 93~97, 1962
16) HINUMA, Y.; M. KUROYA, T. YAJIMA, K. ISHIHARA, S. HAMADA, K. WATANABE & K. KIKUCHI: An amicetin (sacromycin) from *Streptomyces* sp. No. 5223. Studies on *Streptomyces* antibiotics, XXXVI. J. Antibiotics, Ser. A 8: 148~152, 1955
17) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. Int. J. Syst. Bacteriol. 22: 265~394, 1972
18) SHIRLING, E. B. & D. GOTTLIEB: Cooperative description of type cultures of *Streptomyces*. IV. Species descriptions from the second, third and fourth studies. Int. J. Syst. Bacteriol. 19: 391~512, 1969
19) NAGATSU, J.; K. ANZAI & S. SUZUKI: Pathocidin, a new antifungal antibiotic. II. Taxonomic studies on the pathocidin-producing organism *Streptomyces albus* var. *pathocidicus*. J. Antibiotics, Ser. A 15: 103~106, 1962