NOTES

CLAZAMYCIN B IS ANTIBIOTIC 354

L. A. DOLAK and C. DEBOER

The Upjohn Company, Research Laboratories
Kalamazoo, Michigan 49001, U.S.A.

(Received for publication September 21, 1979)

The recent report in this journal[1] of the isolation and structure[2] of clazamycin B has prompted us to describe our work on this antibiotic. We described the isolation of antibiotic 354 or U-54,702D in 1977[3] and obtained a U.S. Patent in 1978[4]. The published UV, CMR, and PMR spectra, molecular formula and base instability reported for clazamycin B[1] are in excellent agreement with our reports[3,4].

We isolated antibiotic 354 from *Streptomyces puniceus* subsp. *doliceus* NRRL 11160 coproduced with gougerotin. Seed flasks were inoculated with spores prepared from a suspension of the culture maintained in the gas phase of a liquid nitrogen freezer. The culture was inoculated and grown in shake flasks for 48~72 hours at 26°C. The seed medium consisted of 10 g glucose monohydrate, 2.5 g yeast extract and 10 g Bactopeptone per liter of deionized water. A 5% vegetative seed used to inoculate the fermentation medium which consisted of 15 g glucose monohydrate, 10 g protease peptone, 2 g yeast extract, 10 g dextrin, 20 g Brer Rabbit molasses, and 5 g peanut meal per liter of deionized H_2O. The pH was adjusted to 7.0 prior to sterilization. The isolation process consisted of sequential chromatography on charcoal, cellulose (MeOH), and Dowex 50 (NH_4^+). Pure clazamycin B was obtained after preparative HPLC on a CG-120 (NH_4^+) column eluted with 1 M $(NH_4)_2SO_4$ solution. The eluate was monitored with a UV detector and appropriate fractions were pooled and desalted with granular charcoal. Antibiotic 354 (clazamycin B) was eluted from charcoal with 25% acetone in water (v/v).

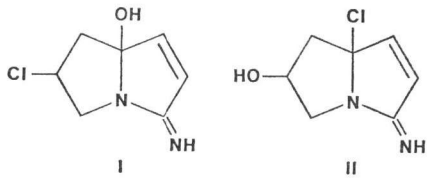
Decomposition was rapid unless concentration steps were done at the lowest practical temperatures. For example, water was removed by lyophilization and methanol at 10~20°C using efficient pumps and condensers.

A convenient assay for antibiotic 354 consists of GC-MS of the N-trifluoroacetyl-O-trimethylsilyl derivative. A dry preparation of antibiotic 354 was treated with trimethylsilyl imidazole to protect the hydroxyl group. The nitrogen function was protected by then heating with (a) *bis*-trimethylsilyltrifluoroacetamide to give a mixture of monotrimethylsilyl clazamycin B ($M^+=244$) and *bis*-trimethylsilyl clazamycin B ($M^+=316$); (b) trifluoroacetic anhydride to give N-trifluoroacetyl-O-trimethylsilyl clazamycin B ($M^+=340$). This work was done on a HP-5992A desk-top spectrometer using a 0.46×94 cm glass column packed with 3% UCW-982 on High Efficiency Chromosorb W. At a column temperature of 150°C the retention times for the above derivatives were less than 10 minutes.

Antibiotic 354 gave a negative ninhydrin test, a positive nitroprusside test and a negative SAKAGUCHI test.

We used Computer Assisted Structure Elucidation (CASE) to deduce the structure. The general approach has been described[5,6]. Our experience has been with an advanced version of CASE in cooperation with M. E. MUNK of Arizona State University. The process has been described in detail at the 1979 ICAAC meeting (Paper 1029). The chemist enters structural fragments deduced from spectroscopic and chemical experiments. Families of structures are then generated by the machine which can be examined for appropriateness. Constraints can be added to the input to forbid or require the appearance of specified structural features in the output structures. This iterative procedure led us to working hypotheses which we tested with additional experiments. Our final attempts led to a list of nineteen structures to consider. We were able to eliminate all but two of them using criteria such as strain, hydrolytic lability, and

Fig. 1.



Antibiotic 354 Alternate structure

PMR coupling constants. The remaining structures were those determined for clazamycin B by X-ray[1] (I) and its isomer wherein the hydroxyl and chlorine atoms were reversed (II).

Theoretical calculations[7] for the α,β-unsaturated amidine system indicated that the proposed structures were consistent with the observed ultraviolet spectrum. The parent system has recently been isolated by OKUYAMA, *et al.*[8]

Antibiotic 354 gave an *in vitro* ID_{90} *vs.* L-1210 in the mouse leukemia cell system of 1.6 μg/ml. Its intraperitoneal LD_{50} was found to be 26 mg/kg. Necropsy on treated mice showed no evidence of toxic effects on the kidney, liver, or spleen.

The antiviral activity was determined by measuring the yields of infectious virus from primary rabbit kidney monolayers. The virus titers were determined by the plaque method and were significantly reduced for Herpes Simplex Types 1 and 2 at concentrations as low as 12 μg/ml. Antibiotic 354 protected mice and guinea pigs from vaginal HSV-2 infections and hamsters from HSV-1 keratitis when applied topically. However, topical application proved to be toxic to the host animals at effective doses. Topical application to intact or abraded rat skin three times daily for 7~21 days resulted in formation of a thick, dark crust over the treated skin in as little as seven days.

Acknowledgement

The authors wish to thank Dr. H. E. RENIS for the antiviral studies and Drs. J. E. GRAY and E. R. LARSEN for the topical irritation study.

References

1) HORIUCHI, Y.; S. KONDO, T. IKEDA, D. IKEDA, K. MIURA, M. HAMADA, T. TAKEUCHI & H. UMEZAWA: New antibiotics, clazamycins A and B. J. Antibiotics 32: 762~764, 1979
2) NAKAMURA, H.; Y. IITAKA & H. UMEZAWA: Crystal and molecular structure of clazamycin A. J. Antibiotics 32: 765~767, 1979
3) DOLAK, L. A. & C. DEBOER: A new antibiotic from *Streptomyces griseus*. 17th Intersci. Conf. on Antimicr. Agents & Chemoth., Abstract 74, New York, N.Y., Oct., 1977
4) DEBOER, C.; L. A. DOLAK & D. H. PETERSON: Composition of matter and process. U.S. Patent 4,113,855, Sept. 12, 1978
5) SKELLEY, C. A.; H. B. WOODRUFF, C. R. SNELLING & M. E. MUNK: Interactive structure elucidation in computer-assisted structure elucidation. ACS Symposium Series #54, D. H. SMITH, *Ed.*, American Chemical Society, Washington, D.C., 1977
6) DJERASSI, C.; D. H. SMITH & T. H. VARKONY: A novel role of computers in the natural products field. Naturewiss. 66: 9~21, 1979
7) KORNILOV, M. YU & V. P. MAKOVETSKII: Electronic structure and spectra of 1,3-diaminoisoindoline, 1-imino-3-aminoisoindole, their derivatives and analogs. Ukr. Khim. Zh. 41: 933~939, 1975 (CA 84: 4251q)
8) OKUYAMA, A.; S. KONDO, T. IKEDA, K. MIURA, M. HAMADA & H. UMEZAWA: A new antibiotic, 2-hydroxy-5-iminoazacyclopent-3-ene. J. Antibiotics 32: 768~770, 1979