PEPLEOMYCIN, THE SECOND GENERATION BLEOMYCIN CHEMICALLY DERIVED FROM BLEOMYCIN A2

Wataru Tanaka
Research Laboratories, Pharmaceutical Division, Nippon-Kayaku Co. Ltd., Shimo, Kita-ku, Tokyo 115, Japan
Tomohisa Takita
Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

Dedicated to Professor Hamao Umezawa on the occasion of his sixty-fifth birthday

Abstract — Pepleomycin, 3-(S-1-phenylethylamino)-propylamino-bleomycin, has been synthesized from bleomycin A2, the main component of natural bleomycins, by a series of the following reactions: demethylation, cyclic iminoether formation, hydration and aminolysis with 3-(S-1-phenylethylamino)-propylamine. Pepleomycin has stronger activity and less pulmonary toxicity than natural bleomycin mixture clinically used today in the treatment of cancer and will become the second generation bleomycin in its cancer therapy.

Bleomycins (abbreviated as BLMs) are anti-tumor antibiotics discovered by Prof. H. Umezawa and his collaborators.[1] They are a group of structurally related glycopeptidases produced by *Streptomyces verticillus*. The total structures were conclusively determined recently (Fig. 1).[2] They are different from each other in their terminal amine moieties (R in Fig. 1).[3] From viewpoint of biosynthesis, the peptide part of BLM containing pyrimidine and bithiazole nuclei is formed by modification of a single peptide chain. The amino acid containing pyrimidine nucleus appears to be derived from asparagylasparagine *via* dehydrative cyclization, dehydrogenation, amination and methylation, and the amino acid containing bithiazole

chromophore is found to be derived from β-alanylcysteinylcysteine.[4]
The biological action of BLM has been recently found to be shown by reactive oxygen radicals which are formed at the sixth coordination site of BLM-Fe(II) complex.[5-7] BLM is now clinically used for the treatment of squamous cell caricnoma, testicular carcinoma and malignant lymphoma. BLM clinically used today is a mixture of natural BLMs, of which the major component is BLM A2 (see Fig. 1). The most serious adverse effect of the present BLM is pulmonary fibrosis. Distribution of BLM parenterally administered is affected by the terminal amine, and the affinity between BLM and DNA is also dependent on the electric charge of the terminal amine.[8] We have been devoting our efforts to develop new more effective and less toxic BLMs with unnatural terminal amine under Prof. Umezawa's guidance, and have already prepared more than 300 new BLMs by microbial, enzymatic and chemical

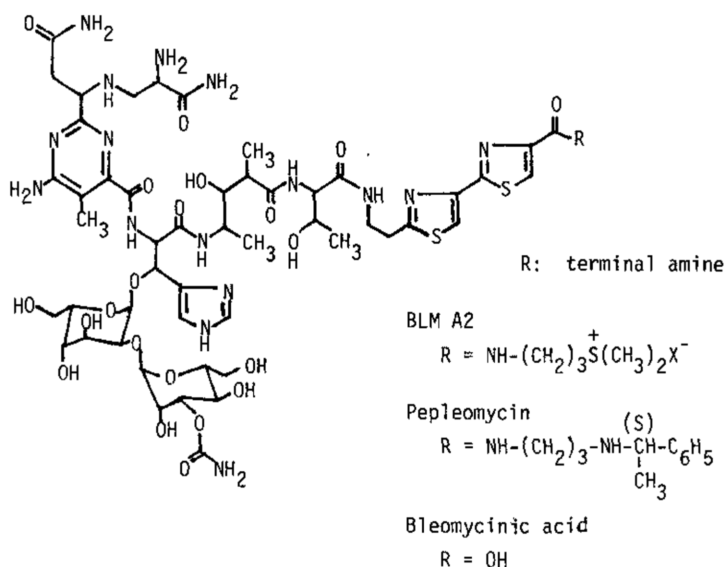


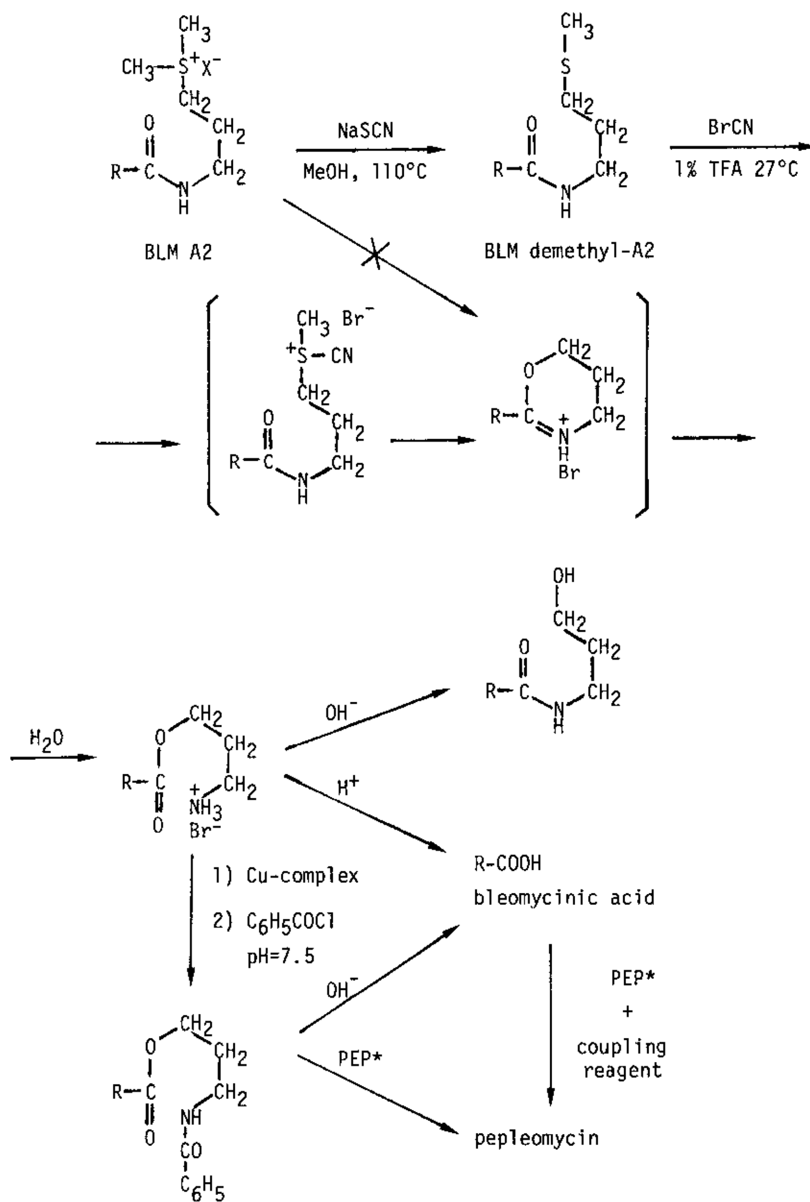
Fig. 1. Structures of bleomycin A2, pepleomycin and bleomycinic acid

procedures. Pepleomycin (see Fig. 1) has been selected among them as the second generation BLM. In this short review, chemical transformation of BLM A2 into pepleomycin is described. New artificial BLMs with unnatural terminal amine were first prepared by fermentation method.[9] When a BLM-producing microorganisms is cultured in a medium containing a special amine, which is not present in nature, the amine is incorporated into BLM, and a new BLM, which has the added amine in its terminal amine moiety, is produced. The structural requirement for the amine

in the incorporation is to have a primary amino group at the end of a short alkyl chain, which forms the amide bond with bleomycinic acid (see Fig. 1), and at least one more basic functional group such as amino, guanidium, imidazole etc.

The second method for preparation of new BLMs is the semi-synthetic method starting from bleomycinic acid, which was first obtained by enzymatic hydrolysis of BLM B2, the second major component of natural BLMs, with acylagmatine amidohydrolase produced by a Fusarium.[10] Bleomycinic acid is a starting material suitable for preparation of new semi-synthetic BLMs, because it has only one free carboxyl group, which will be connected with the terminal amine, and the free primary amino group present in bleomycinic acid can be readily protected by copper-chelation.[6] The following is concerned with the chemical selective cleavage of BLM to obtain bleomycinic acid.[11]

In the course of the structural study of BLM, it was found that the amide bond connecting between the bithiazole-containing amino acid and the terminal amine is most resistant to acid hydrolysis among seven amide bonds present in BLM. Therefore, in order to cleave this amide bond selectively without any change in the other part of the molecule, some modification of this amide bond by participation of a neighboring functional group was necessary prior to the hydrolysis. It was first noted that BLM A2 has the reactive dimethylsulfonium group at the terminal. Attempt to form the cyclic iminoether directly from BLM A2 driven by liberation of dimethyl sulfide was not successful (see Fig. 2). However in this study, it was found that under a mild pyrolytic condition the methyl group, but not dimethyl sulfide, can be easily removed from the dimethylsulfonium group to give BLM demethyl-A2. BLM demethyl-A2 is contained in natural BLM mixture as a minor component. However, it is not a direct fermentation product, but is a spontaneous degradation product of BLM A2 during isolation and storage. If BLM demethyl-A2 were available as the starting material, it would be expected that cyclic iminoether would be formed by treatment with cyanogen bromide since leaving of methyl thiocyanate is much easier than dimethyl sulfide. This idea was suggested by the selective cleavage of methionyl peptide bond by cyanogen bromide,[12] though this reaction involves the carboxy side of methionine while the present purpose is scission at the amino side of decarboxymethionine. Pyrolytic degradation of the copper complex of BLM A2 hydrochloride at 100°C for 24 hr in an evacuated vessel gave BLM demethyl-A2 in a maximum yield of about 70%. However this pyrolysis on a large scale was not satisfactory. Finally, transformation of BLM A2 into BLM



*PEP: 3-(S-1-phenylethylamino)-propylamine

Fig. 2. Chemical transformation of bleomycin A2 into pepleomycin

demethyl-A2 was achieved in over 85% yield by treatment with sodium thiocyanate at 120°C for 2 hr in methanol solution in a pressure vessel with good reproducibility. Thus, it became feasible to use BLM demethyl-A2 as the starting material for chemical transformation.

Bleomycin demethyl-A2 was dissolved in 1% trifluoroacetic acid and was reacted with excess cyanogen bromide at room temperature overnight. The main product was isolated by CM-Sephadex C-25 column chromatography in 75% yield. The product was not the expected cyclic iminoether, but the 3-aminopropyl ester of bleomycinic acid,[10] the hydration product of the former (see Fig. 2).

Under mild alkaline hydrolysis conditions, the carbonyl group of the ester bond, which was formed by the treatment of cyanogen bromide, shifted readily to the newly formed terminal amino group to form a stable amide. Under mild acid hydrolysis conditions, bleomycinic acid (see Fig. 1) was obtained in about 50% yield, but formation of an appreciable amount of by-products, which were formed by acid hydrolysis at the other weak bonds and were not easily separable from bleomycinic acid, could not be avoided. To block the O to N acyl migration in alkali, selective acylation of the terminal amino group was studied. The primary amino group present originally in BLM was protected by copper-chelation[6] and N-benzoylation was carried out with benzoyl chloride at pH 7.5. The desired mono-N-benzoyl derivative was obtained in over 90% yield. Mild alkaline hydrolysis (0.025N KOH at 0°C) of it afforded bleomycinic acid in over 90% yield. Thus, selective chemical cleavage of the terminal amide bond of BLM A2 to yield bleomycinic acid was achieved by participation of the terminal dimethylsulfonium group through successive elimination of methyl thiocyanate.

Copper-complex of bleomycinic acid was reacted with 3-(S-1-phenylethylamino)-propylamine in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, a coupling reagent for peptide synthesis, to afford pepleomycin in a good yield. Pepleomycin was derived more easily and more efficiently by direct aminolysis of benzoylaminopropyl ester of bleomycinic acid (see Fig. 2) with 3-(S-1-phenylethyl-amino)propylamine in a methanol solution in over 85% yield. The study on chemical transformation of BLM was motivated by establishment of an enzymatic method for preparation of bleomycinic acid. However, the chemical transformation method became much superior to the enzymatic method after examination of reaction conditions in each reaction step and by finding a new direct route to semi-synthetic BLM from the intermediate.

Peleomycin has been selected as the second generation BLM because of its less lung toxicity, higher antitumor effect and effectiveness against chemically induced stomach adeno-carcinoma in rats, which is not sensitive to the present BLM.[13-15] The first and the second qualities were confirmed clinically, but the third one was not successfully evaluated clinically.[16,17] However, prostatic cancer, which is a kind of adeno-carcinoma and resistant to the present BLM, was found to respond well to pepleomycin.[18]

We are now partially successful to predict the clinical qualities of new BLMs from the experimental qualities found in laboratory animals, and are searching for new BLMs with less toxicity and broader antitumor spectrum as the third generation BLMs, which are modified in their nucleus moiety. The leading principle is that of Prof. Umezawa, that is, selectivity in distribution, inactivation and activation.[19]

It seems the nature of Prof. Umezawa to find succesively the weapons to fight against cancer and other intractable diseases among the secondary metabolites of microbes. Sarkomycin[20], BLMs, anthracyclines such as aclacinomycin[21] and baumycin[22], macromomycin[23], a high molecular weight antitumor antibiotic which denatures membrane of tumor cell, Glyo-II[24], which restores the lost regulation mechanism of tumor cell-division, and coriolins[25], which react to microtubuli, were found as new antitumor substances in the Institute of Microbial Chemistry under the leadership of Prof. Umezawa. There were also found, by their enzyme inhibitor research among the secondary metabolites of microbes, bestatin[26], amastatins[27] and forphenicine[28], which are inhibitors of membrane-bound enzymes, and were found to be potential immunomodulators for the treatment of cancer, viral infectious disease and autoimmune diseases. And another enzyme inhibitor, leupeptin[29], which inhibits proteases, has become a potential drug against muscular dystrophy.

The reasons why so many important substances were found under direction of one person, Prof. Umezawa, are his diligence with his hard work and his mathematical logic in research planning. And the determinants in these two qualities are his inherent love for the research work and his respect to human lives, which has been cultivated since his childhood born in a medical family.

Celebrating the 65th birthday of Prof. Hamao Umezawa, we are glad to have an opportunity to introduce some parts of our works under his guidance and cordially wish the readers of this journal, Heterocycles, to be more interested in his works and attitude and further improvement of the substances found by him with the knowledge

of heterocyclic chemistry.

ACKNOWLEDGEMENT

We are indebted to Mr. Hideo Chimura of Nippon Kayaku Co. for his useful discussion to prepare this article.

REFERENCES

1. H. Umezawa, K. Maeda, T. Takeuchi, and Y. Okami, J. Antibiot., 1966, 19A, 200.
2. T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Umezawa, H. Naganawa, and H. Umezawa, J. Antibiot., 1978, 31, 801.
3. A. Fujii, T. Takita, K. Maeda, and H. Umezawa, J. Antibiot., 1973, 26, 396.
4. A. Fujii, 'Bleomycin: Chemical, Biochemical and Biological Aspects', ed. by S. M. Hecht, Springer-Verlag, New York, in press
5. E. A. Sausville, J. Peisach, and S. B. Horwitz, Biochem., 1978, 17, 2724.
6. T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Iitaka, and H. Umezawa, J. Antibiot., 1978, 31, 1073.
7. Y. Sugiura, and T. Kikuchi, J. Antibiot., 1978, 31, 1310.
8. H. Kasai, H. Naganawa, T. Takita, and H. Umezawa, J. Antibiot., 1978, 31, 1316.
9. A. Fujii, T. Takita, N. Shimada, and H. Umezawa, J. Antibiot., 1974, 27, 73.
10. H. Umezawa, Y. Takahashi, A. Fujii, T. Saino, T. Shirai, and T. Takita, J. Antibiot., 1973, 26, 117.
11. T. Takita, A. Fujii, T. Fukuoka, and H. Umezawa, J. Antibiot., 1973, 26, 252.
12. E. Gross, and B. Witkop, J. Amer. Chem. Soc., 1961, 83, 1510.
13. H. Umezawa, 'GANN Monograph on Cancer Research, No. 19', eds. by S. K. Carter, T. Ichikawa, G. Mathé, and H. Umezawa, University of Tokyo Press, 1976, pp. 3-36.
14. W. Tanaka, Jap. J. Antibiot., 1977, 30, 41.
15. A. Matsuda, O. Yoshioka, T. Yamashita, K. Ebihara, H. Umezawa, T. Miura, K. Katayama, M. Yokoyama, and S. Nagai, 'Recent Results in Cancer Research, Vol. 63', eds. by S. K. Carter, H. Umezawa, J. Douros, and Y. Sakurai, Springer-Verlag, 1978, pp. 191-210.
16. S. Ikeda, H. Miyazato, H. Nakayama, Y. Kobayashi, and K. Tajima, Jap. J. Cancer Clinics (in Japanese), 1979, 25, 677.
17. T. Sekiya, Y. Kawabe, A. Ito, T. Kaneda, T. Suzuki, and T. Shiraishi, Cancer and Chemoth. (in Japanese), 1979, 6, 777.

18. T. Ichikawa, Private communication

19. H. Umezawa, T. Takeuchi, S. Hori, T. Sawa, and M. Ishizuka, J. Antibiot., 1972, **25**, 409.

20. H. Umezawa, T. Takeuchi, K. Nitta, T. Yamamoto, and S. Yamaoka, J. Antibiot., 1953, **6A**, 101.

21. T. Oki, Y. Matsuzawa, A. Yoshimoto, K. Numata, I. Kitamura, S. Hori, A. Takamatsu, H. Umezawa, M. Ishizuka, H. Naganawa, H. Suda, M. Hamada, and T. Takeuchi, J. Antibiot., 1975, **28**, 830.

22. K. Komiyama, Y. Matsuzawa, T. Oki, T. Inui, Y. Takahashi, H. Naganawa, T. Takeuchi, and H. Umezawa, J. Antibiot., 1977, **30**, 619.

23. H. Chimura, M. Ishizuka, M. Hamada, S. Hori, K. Kimura, J. Iwanaga, T. Takeuchi, and H. Umezawa, J. Antibiot., 1968, **21**, 44.

24. T. Takeuchi, H. Chimura, M. Hamada, H. Umezawa, O. Yoshioka, N. Oguchi, Y. Takahashi, and A. Matsuda, J. Antibiot., 1975, **28**, 737.

25. T. Takeuchi, H. Iinuma, J. Iwanaga, S. Takahashi, T. Takita, and H. Umezawa, J. Antibiot., 1969, **22**, 215.

26. H. Umezawa, T. Aoyagi, H. Suda, M. Hamada, and T. Takeuchi, J. Antibiot., 1976, **29**, 97.

27. T. Aoyagi, H. Tobe, F. Kojima, M. Hamada, T. Takeuchi, and H. Umezawa, J. Antibiot., 1978, **31**, 636.

28. T. Aoyagi, T. Yamamoto, K. Kojiri, F. Kojima, M. Hamada, T. Takeuchi, and H. Umezawa, J. Antibiot., 1978, **31**, 244.

29. T. Aoyagi, T. Takeuchi, A. Matsuzaki, K. Kawamura, S. Kondo, M. Hamada, K. Maeda, and H. Umezawa, J. Antibiot., 1969, **22**, 283.

Received, 1st November, 1979