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A SIMPLE METHOD FOR THE PURIFICATION AND ISOLATION OF BLEOMYCIN A_2

Dale L. Boger,* Royce F. Menezes, and Wenjin Yang
Department of Chemistry, The Scripps Research Institute
10666 North Torrey Pines Road, La Jolla, California, 92037 USA

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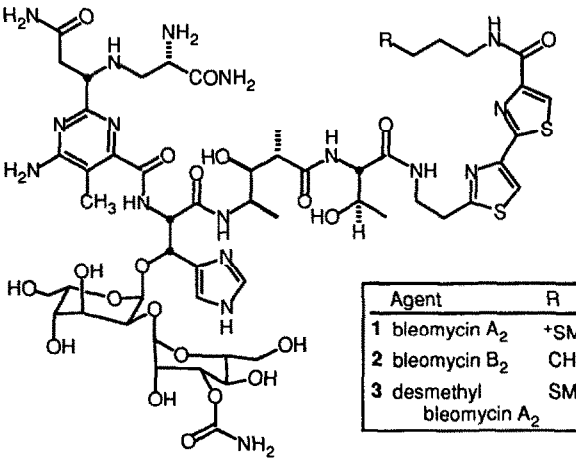
Abstract. A simple procedure for the direct purification and isolation of bleomycin A_2 from blenoxane is detailed and its use in the preparation and purification of deglycobleomycin A_2 is described.

The bleomycins are a family of glycopeptide antitumor antibiotics possessing clinically useful activity thought to be mediated through their metal-dependent oxidative cleavage of duplex DNA.[1] Bleomycin A_2 (**1**) is the major naturally occurring constituent (60-70%) of the clinical antitumor drug blenoxane which additionally contains bleomycin B_2 (20-30%) and a number of minor bleomycins including demethyl bleomycin A_2 (10%). Consequently, bleomycin A_2,[2] its naturally occurring congeners,[3] its degradation products and semisynthetic derivatives[4] as well as synthetic analogs[5] continue to be the subject of investigations in efforts to define the fundamental functional roles of the individual subunits.

In the course of recent investigations,[6-7] we required samples of pure, metal free authentic bleomycin A_2[8-10] and deglycobleomycin A_2[4] for comparison standards. Past protocols employed for the large scale purification of bleomycin A_2 have relied on an effective albeit indirect procedure requiring ion exchange chromatographic separation of the bleomycin A_2 copper(II) complex on CM-Sephadex C-25, desalting the sample to remove buffer salts by chromatography over charcoal, decomplexation of the copper(II) by reaction with Na-EDTA in conjuction with an additional ion exchange chromatography, and final reprecipitation of the natural product to secure the pure, metal free bleomycin A_2.[8-10] Most recent investigations employing bleomycin A_2 have relied on this or closely related procedures [11] for the isolation of pure material.[11-18]



Agent	R
1 bleomycin A_2	$^+SMe_2$
2 bleomycin B_2	$CH_2NHC(=NH)NH_2$
3 desmethyl bleomycin A_2	SMe

Since our studies required only modest quantities of pure bleomycin A_2 and deglycobleomycin A_2 (1 - 20 mg), we elected to examine various techniques for the direct chromatographic purification of bleomycin A_2 from blenoxane without formation of a metal complex and on immobile supports commonly accessible. Herein, we report a simple procedure for the chromatographic purification of bleomycin A_2 from blenoxane on silica gel.

Bleomycin A_2. A 5 mg sample of blenoxane containing the two major components bleomycin A_2 (**1**) and B_2 (**2**) was dissolved in 0.2 mL of H_2O and adsorbed on a column of silica gel (230-400 mesh, 7 cm x 1 cm). The components were eluted with CH_3OH-10% aq $CH_3CO_2NH_4$-10% aq NH_4OH, 30:9:1. TLC R_f 0.18 (bleomycin A_2), 0.39 (bleomycin B_2), 0.70 (minor UV active component).

The respective UV active fractions containing the desired components were concentrated in vacuo. The individual components were dissolved in 1-2 mL of H_2O and individually adsorbed on a column packed with Amberlite XAD-2 (10 cm x 1 cm). After desalting the adsorbed sample with H_2O (100 mL), the desired components were eluted with CH_3OH (50 mL). Concentration of the CH_3OH fractions gave bleomycin A_2 (2.7 mg, 54%) and bleomycin B_2 (0.9 mg, 18%) as white solids.

Deglycobleomycin A_2. The disaccharide of bleomycin A_2 was cleaved by a method described by Henichart *et al.*[4] A sample of 10 mg of bleomycin A_2, 0.4 mL of anisole and 4 mL of anhydrous HF in a Kel-F reaction vessel was stirred at 0 °C for 1 h. The reaction mixture was concentrated in vacuo. Anhydrous ether was added (20 mL) and the insoluble white solid containing the desired product was collected by filtration. Chromatography (230-400 mesh SiO_2, 3 cm x 1 cm, CH_3OH-10% aq $CH_3CO_2NH_4$-10% aq NH_4OH, 10:9:1 eluant, TLC R_f 0.1) followed by chromatography over Amberlite XAD-2 (6 cm x 2 cm), first desalting the adsorbed sample with H_2O (100 mL), then eluting the agent with CH_3OH (50 mL) gave 6.1 mg (82%) of deglycobleomycin A_2 as a white solid.

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References.

1. Ohno, M.; Otsuka, M. in *Recent Progress in the Chemical Synthesis of Antibiotics*; Lukacs, G.; Ohno, M.; Eds.; Springer-Verlag: New York, **1990**, 387. Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383. Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. Sugiura, Y.; Takita, T.; Umezawa, H. *Metal Ions in Biological Systems* **1985**, *19*, 81. Povirk, L. F. in *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S.; Waring, M. J.; Eds.; MacMillian: London, 1983. Hecht, S. M. in *Bleomycin: Chemical, Biochemical and Biolgocial Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York 1979. Umezawa, H. in *Anticancer Agents Based on Natural Product Models*; Cassady, J. M.; Douros, J. D., Eds.; Academic Press: New York, 1980; p 148. Umezawa, H. in *Bleomycin: Current Status and New Developments*; Carter, S. K.; Crooke, S. T.; Umezawa, H., Eds.; Academic Press, New York, 1978.

2. Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801.

3. Umezawa, H. *Pure Appl. Chem.* **1971**, *28*, 665.

4. *iso*-BLM: Nakayama, Y.; Kunishima, M.; Omoto, S.; Takita, T.; Umezawa, H. *J. Antibiot.* **1973**, *26*, 400. *epi*-BLM: Muraoka, Y.; Kobayashi, H.; Fujii, A.; Kunishima, M.; Fujii, T.; Nakayama, Y.; Takita, T.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 853. Deamido-BLM: Umezawa, H.; Hori, S.; Sawa, T.; Yoshioka, T.; Takeuchi, T. *J. Antibiot.* **1974**, *27*, 419. Deamido-BLM and depyruvamido-BLM: Sugiura, Y. *J. Am. Chem. Soc.* **1980**, *102*, 5208. Decarbamoyl-BLM: Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R.E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869. Deglyco-BLM: Oppenheimer, N. J.; Chang, C.; Chang, L.-H.; Ehrenfeld, G.; Rodriguez, L. O.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 1606. Sugiura, Y.; Suzuki, T.; Otsuka, M.; Kobayashi, S.; Ohno, M.; Takita, T.; Umezawa, H. *J. Biol. Chem.* **1983**, *258*, 1328. Sugiura, Y.; Kuwahara, J.; Suzuki, T. *FEBS Lett.* **1985**, *182*, 39. Kenani, A.; Lamblin, G.; Henichart, J. P. *Carbohyd. Res.* **1988**, *177*, 81. Peplomycin and liblomycin (semisynthetic BLM): Umezawa, H.; Takita, T.; Saito, S.; Muraoka, Y.; Takahashi, K.; Ekimoto, H.; Minamide, S.; Nishikawa, K.; Fukuoka, T.; Nakatani, T.; Fujii, A.; Matsuda, A. in *Bleomycin Chemotherapy*; Sikic, B. I.; Rozenweig, M.; Carter, S. K., Eds.; Academic Press: Orlando, 1985; p. 289. Takita, T.; Maeda, K. *J. Heterocyclic Chem.* **1980**, *17*, 1799. Vloon, W. J.; Kruk, C.; Pandit, U. K.; Hofs, H. P.; McVie, J. G. *J. Med. Chem.* **1987**, *30*, 20.

5. Kittaka, A.; Sugano, Y.; Otsuka M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2811, 2821. Owa, T.; Haupt, A.; Otsuka, M.; Kobayashi, S.; Tomioka, N.; Itai, A.; Ohno, M.; Shiraki, T.; Uesugi, M.; Sugiura, Y.; Maeda, K. *Tetrahedron* **1992**, *48*, 1193. Otsuka, M.; Masuda, T.; Haupt, A.; Ohno, M.: Shiraki, T.; Sugiura, Y.; Maeda, K. *J. Am. Chem. Soc.* **1990**, *112*, 838. Kilkuskie, R. E.; Suguna, H.; Yellin, B.; Murugesan, N.; Hecht, S.M. *J. Am. Chem. Soc.* **1985**, *107*, 260. Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25. Carter, B. J.; Marty, V. S.; Reddy, K. S.; Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193. Carter B. J.; Reddy, K. S.; Hecht, S. M. *Tetrahedron* **1991**, *47*, 2463. Kenani, A.; Lohez, M.; Houssin, R.; Helbecque, N.; Bernier, J. L.; Lemay, P.; Henichart, J. P. *Anti-Cancer Drug*

Design **1987**, *2*, 47. Kenani, A.; Bailly, C.; Helbecque, N.; Houssin, R.; Bernier, J. L.; Henichart, J. P. *Eur. J. Med. Chem.* **1989**, *24*, 371.

6. Boger, D. L.; Menezes, R. F.; Dang, Q.; Yang, W. *BioMed. Chem. Lett.* **1992**, *2*, 261.

7. Boger, D. L; Menezes, R. F. *J. Org. Chem.* **1992**, *57*, in press. Boger, D. L.; Menezes, R. F.; Dang, Q. *J. Org. Chem.* **1992**, *57*, in press.

8. Fujii, A. *Bleomycin, Chemical, Biochemical, and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, **1979**, 341, 345.

9. Takita, T.; Fujii, A.; Muraoka, Y.; Mizuguchi, S.; Umezawa, H. U.S. Patent 3,929,993, 1975; *Chem. Abstr.* **1973**, *79*, 149315v.

10. Muraoka, Y. *Bleomycin, Chemical, Biochemical, and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, **1979**, 92.

11. Chien, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* , **1977**, *16*, 3641.

12. Kenani, A.; Bailly, C.; Helbecque, N.; Catteau, J.-P.; Houssin, R.; Bernier, J.-L.; Henichart, J.-P. *Biochem. J.* **1988**, *253*, 497.

13. Carter, B. J.; Murty, V. S.; Reddy, K. S.;Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193. Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 3997. Huang, C.-H.; Mirabelli, C. K.; Jan, Y.; Crooke, S. T. *Biochemistry* **1981**, *20*, 233.

14. Fisher, L. M.; Kuroda, R.; Sakai, T. T. *Biochemistry* **1985**, *24*, 3199.

15. Vloon, W. J.; Kruk, C.; Pandit, U. K.; Hofs, H. P.; McVie, J. G. *J. Med. Chem.* **1987**, *30*, 20.

16. Kross, J.; Henner, W. D.; Haseltine, W. A.; Rodriguez, L.; Levin, M. D. *Biochemistry* **1982**, *21*, 3711.

17. Rabow, L. E.; Stubbe, J. ; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3196.

18. Freyder, C. P.; Zhou, W.; Doetsch, P. W.; Marzilli, L. G. *Preparative Biochemistry* **1991**, *21*, 257.