CHEMISTRY LETTERS, pp. 1889-1892, 1991.

Preparation and Enzyme-based Structure Determination of $2^{A},6^{E}$-Bis(O-disulfonyl)-β-cyclodextrin

Kahee FUJITA,* Takashi ISHIZU,*+ Noshi MINAMIURA,++ and Takehiko YAMAMOTO+++

Faculty of Pharmaceutical Sciences, Nagasaki University,
Bunkyo-machi, Nagasaki 852

+Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University,
Higashimura-cho, Fukuyama 729-02

++Faculty of Science, Osaka City University, Sugimoto, Sumiyosi-ku, Osaka 558

+++Faculty of Engineering, Fukuyama University, Higashimura-cho, Fukuyama 729-02

2,6-di-O-Disulfonates were prepared by the reaction of 2-O-(mesitylsulfonyl)-β-cyclodextrin with mesitylsulfonyl chloride in pyridine. Each regioisomer was isolated and the $2^{A},6^{E}$-isomer was structurally determined through enzymatic hydrolysis by Taka amylase A and bacterial saccharifying amylase.

Sulfonylation of two hydroxyl groups of cyclodextrins has been extensively studied since the disulfonylated cyclodextrins are starting compounds for the preparation of enzyme mimics.[1] However, most of the studies are limited to sulfonylation of two primary hydroxyl groups (6-OHs)[2] or two secondary hydroxyl groups (2-OHs[3] or 3-OHs[4]), i.e. disulfonylation on the same-side of cyclodextrins.

Recently, we reported the preparation of 3^{A}-O-(β-naphthylsulfonyl)-6^{X}-O-mesitylsulfonyl-α-cyclodextrins (X = A, B, C, D, E, and F) and their structure determination through chemical con-

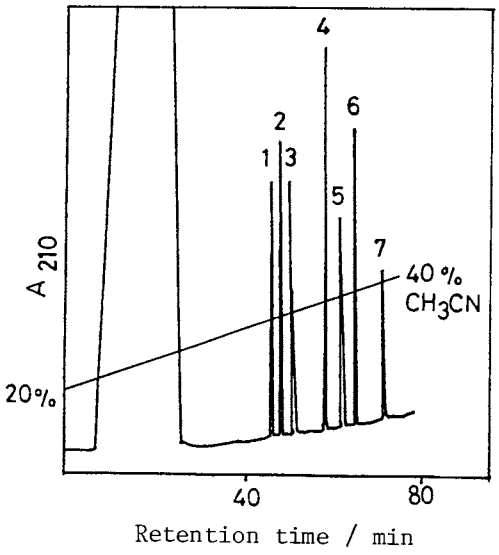

Fig. 1. Reverse-phase HPLC of the mixture of disulfonates **1-7**. An gradient elution of aqueous CH_3CN was applied.

versions to $3^A,6^A;3^X,6^X$-dianhydro-α-cyclodextrins (X = B, C, and D).[5] However, this method for structure determination cannot be applied to the case of $2^A,6^X$-disulfonates of cyclodextrins. We describe here for the first time the preparation, isolation, and structure determination of a β-cyclodextrin (**2**) which has sulfonates of 2^A-OH and 6^E-OH.

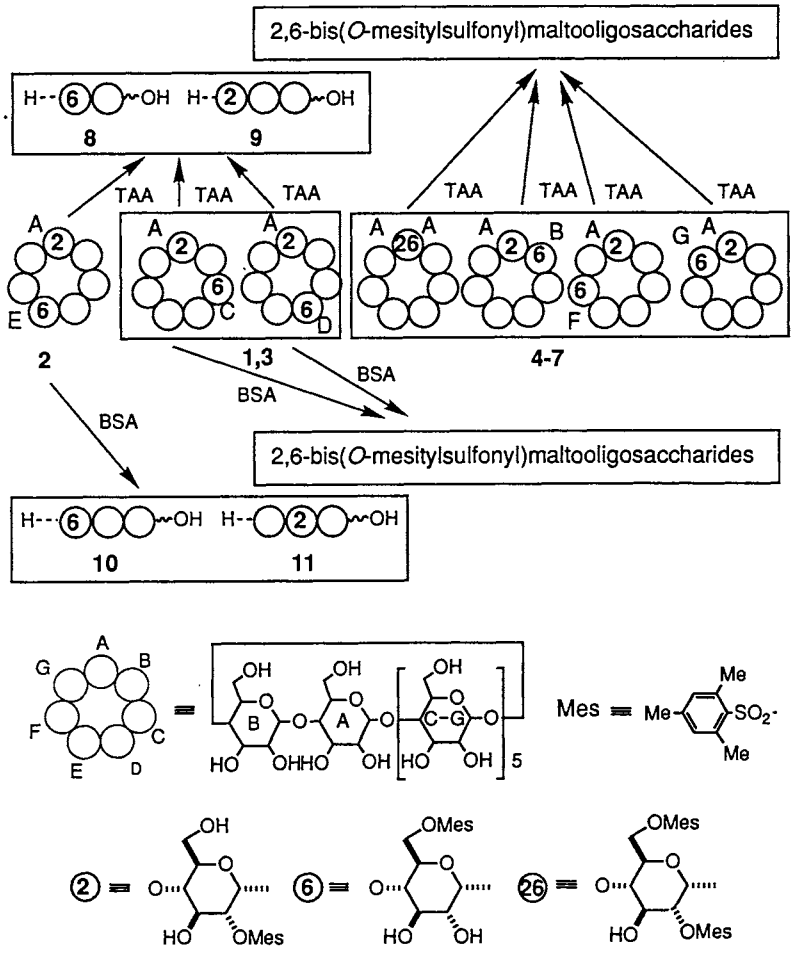

Fig. 2. Enzymatic hydrolysis of $2^A,6^X$-bis(O-mesitylsulfonyl)-β-cyclodextrin.

A reaction of 2-O-mesitylsulfonyl-β-cyclodextrin (550 mg) with mesitylsulfonyl chloride (1.37 g) in pyridine (20 mL) at room temperature for 15 min followed by usual work-up procedures gave a mixture of seven disulfonates **1-7** [the numbers of the compounds are given in the order of increasing retention time in reverse-phase HPLC (Fig. 1)]. Separation by use of the reverse-phase column gave a mixture (65 mg, 10.4%) of **1**, **2**, and **3** and each of **4-7** (2-3%). Each component (**1**; 13.0 mg, 2.1%, **2**; 9.8 mg, 1.6%, **3**; 8.2 mg, 1.3%) was isolated from the mixture by preparative HPLC with elution of 30% aqueous CH_3CN at flow rate 1.0 mL/min. The fast atom bombardment mass spectra (FABMS) and ^1H and ^{13}C NMR spectra demonstrated that they were 2,6-di-O-disulfonates of β-cyclodextrin. Therefore, each of the seven products **1-7** must be one of the possible seven isomers, $2^A,6^X$-bis(O-mesitylsulfonyl)-β-cyclodextrins (X = A, B, C, D, E, F, and G). Since the regiochemistry of the compounds cannot be determined by the spectral data mentioned above, we adopted an enzyme-based method for structure determination.

Each of enzymatic hydrolysis of **1**, **2**, and **3** by Taka amylase A (hereafter abbreviated as TAA) gave a mixture of 6'-O-(mesitylsulfonyl)maltose **8** and 2"-O-(mesitylsulfonyl)-maltotriose **9** (Fig. 2). Similar enzymatic hydrolysis of **4**-**7** by TAA gave bis(mesitylsulfonyl)maltotetraose different from each other. The former results show that one of **1**-**3** is one of the $2^A,6^D$-, $2^A,6^E$-, and $2^A,6^C$-disulfonates, but cannot discriminate among them. The latter results comfirm only that one of **4**-**7** is one of the $2^A,6^F$-, $2^A,6^B$-, $2^A,6^A$-, and $2^A,6^G$-isomers. Both results are in accord with the expectation based on the action pattern of TAA in the hydrolysis of 2-O-arylsulfonyl-β-cyclodextrin and 6-O-arylsulfonyl-β-cyclodextrin,[6] except for the case of the $2^A,6^A$- and $2^A,6^G$-isomers which are expected to give bis(mesitylsulfonyl)maltotriose.

In this situation, we disclose that an alternative enzymatic hydrolysis can differentiate the $2^A,6^E$-isomer from the $2^A,6^D$- and $2^A,6^C$-isomers effectively. Preliminarily, we already found that bacterial saccharifying amylase (hereafetr abbreviated as BSA) hydrolyzed 6-O-mesitylsulonyl-β-cyclodextrin and 2-O-mesitylsulfonyl-β-cyclodextrin to give 6"-O-(mesitylsulfonyl)maltotriose (**10**) and 2'-O-(mesitylsulfonyl)maltotriose (**11**), respectively.[7] These findings suggest that only the $2^A,6^E$-isomer gives a mixture of **10** and **11** and that each of the other two isomers affords an oligosaccharide having two mesitylsulfonyl groups.

BSA (0.1 mg) was added to an ice-cooled solution of **2** (5.0 mg) in water (1 mL). The reaction mixture was kept at room temperature for 4 d and then in boiling water for 10 min. After filtered, the solution was analyzed by reverse-phase HPLC with gradient elution from 20% aqueous CH_3CN (30 mL) to 50% aqueous CH_3CN (30 mL): the retention time (flow rate; 0.5 mL/min), **10**; 17 min, **11**; 39 min. The sulfonates were isolated by reverse-phase HPLC and analyzed by FABMS: m/z **10** 687 ($M+H^+$), **11** 687 ($M+H^+$), 709 ($M+Na^+$), 725 ($M+K^+$). By comparing their HPLC retention times of **10** and **11** with those of the corresponding authentic compounds, **10** and **11** were determined to be 6"-O-(mesitylsulfonyl)maltotriose and 2'-O-(mesitylsulfonyl)maltotriose, respectively. However, **1** and **3** gave bis(mesitylsulfonyl)oligosaccharides as expected respectively.

The finding that **2** was enzymatically hydrolyzed to **10** and **11** indicates that **2** was either the $2^A,6^E$- or the $2^A,6^F$-disulfonate. Since these two disulfonates can be differentiated by the TAA hydrolysis as mentioned above, **2** is assigned to $2^A,6^E$-bis-(O-mesitylsulfonyl)-β-cyclodextrin.

In conclusion, combination of TAA and BSA hydrolysis is a very useful and convenient method for the structure determination of cyclodextrin derivatives and the $2^A,6^E$-disulfonate is the first cyclodextrin that possesses both 2-O- and 6-O-sul-

fonates and is structurally determined unequivocally.

This work was supported by a Grant-in-Aid for Scientific Research from the ministry of Education, Science and Culture of Japan (No. 02650635). We thank Japan Maize Products Co. Ltd. (Nihon Shokuhin Kako) for a generous gift of β-cyclodextrin.

References

1) I. Tabushi, K. Shimokawa, and K. Fujita, Tetrahedron Lett., **18**, 1537 (1977); R. Breslow, J. N. Doherty, C. Guillot, and C. Lipsey, J. Am. Chem. Soc., **100**, 3277 (1978); R. Breslow, P. Bovy, and C. L. Hersh, ibid., **102**, 2115 (1980); I. Tabushi and Y. Kuroda, ibid., **106**, 4850 (1984); I. Tabushi, Y. Kuroda, M. Yamada, M. Higashimura, and R. Breslow, ibid., **107**, 5545 (1985); I. Tabushi, Y. Kuroda, and T. Mizutani, ibid., **108**, 4514 (1986); E. Anslyn and R. Bleleslow, ibid., **111**, 5972 (1989), 8931 (1989).

2) I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata, and K. Fujita, J. Am. Chem. Soc., **98**, 7855 (1976); I. Tabushi, Y. Kuroda, K. Yokota, and L. C. Yuan, ibid., **103**, 711 (1981); I. Tabushi and L. C. Yuan, ibid., **103**, 3574 (1981); I. Tabushi, T. Nabeshima, K. Fujita, A. Matsunaga, and T. Imoto, J. Org. Chem., **50**, 2638 (1985); K. Fujita, A. Matsunaga, and T. Imoto, Tetrahedron Lett., **25**, 5533 (1984); K. Fujita, A. Matsunaga, and T. Imoto, J. Am. Chem. Soc., **106**, 5740 (1984); K. Fujita, H. Yamamura, A. Matsunaga, and T. Imoto, ibid., **108**, 4509 (1986); K. Fujita, H. Yamamura, T. Imoto, T. Fujioka, and K. Mihashi, J. Org. Chem., **53**, 1943 (1988).

3) K. Fujita, S. Nagamura, T. Imoto, T. Tahara, and T. Koga, J. Am. Chem. Soc., **107**, 3233 (1985).

4) K. Fujita, T. Tahara, T. Imoto, and T. Koga, J. Am. Chem. Soc., **108**, 2030 (1986); K. Fujita, T. Ishizu, K. Oshiro, and K. Obe, Bull. Chem. Soc. Jpn., **62**, 2960 (1989).

5) K.Fujita, Y. Egashira, T. Tahara, T. Imoto, and T. Koga, Tetrahedron Lett., **30**, 1285 (1989).

6) K. Fujita, T. Tahara, S. Nagamura, T. Imoto, and T. Koga, J. Org. Chem., **52**, 636 (1987).

7) K. Fujita, et al., to be published in the near future.

(Received July 22, 1991)