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ENZYME-BASED DISCRIMINATION BETWEEN CLOCKWISE AND COUNTERCLOCKWISE ISOMERS OF UNSYMMETRICALLY DISUBSTITUTED β-CYCLODEXTRINS. 6A,6B- AND 6A,6G-DERIVATIVES

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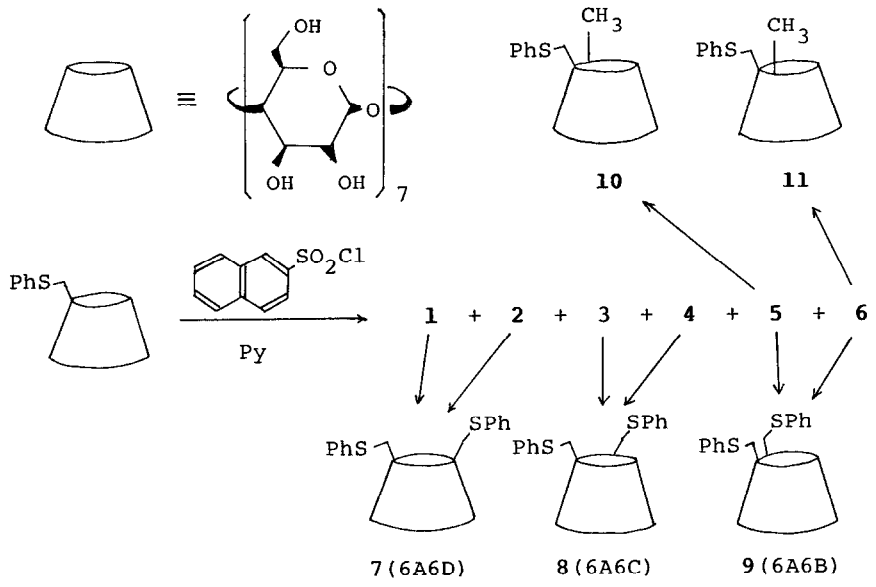
6A,6X-Dideoxy-6A-phenylthio-6X-[(β-naphthylsulfonyl)oxyl]-β-cyclodextrins (X= G and B) (**5** and **6**) were prepared together with the other isomers (X=C, D, E, and F) (**1-4**), isolated by reversed-phase column chromatography, and structurally assigned by use of Taka amylolysis.

One of the most interesting studies on preparation of enzyme (or receptor) mimics is attachment of two different groups on desirable positions of cyclodextrins.[1] There are five, six, or seven regioisomers for 6A,6X-unsymmetrically disubstituted α, β, or γ-cyclodextrins, respectively.[2] However, neither specific synthesis[3] of one isomer nor separation of one isomer from an isomeric mixture has been achieved. Accordingly, the strategy of regioisomer determination has not been developed. We describe here the complete resolution of six regioisomers **1-6** (X=B, C, D, E, F, and G) and the enzyme-based discrimination between the 6A,6G(**5**)- and the 6A,6B(**6**)-isomers.

The regioisomeric mixture (**1-6**), which was prepared by the reaction of 6-deoxy-6-phenylthio-β-cyclodextrin with β-naphthalenesulfonyl chloride in pyridine, was chromatographed by use of a reversed-phase column[4] to give pure **1** (7.5%), **2** (2.3%), **3** (3.1%), **4** (5.1%), **5** (2.8%), and **6** (2.7%).[5] By nucleophilic substitutions with thiophenol, **1** and **2**, **3** and **4**, and 5 and **6** gave 6A,6D(**7**)-, 6A,6C(**8**)-, and 6A,6B(**9**)-diphenylthio-derivatives, respectively (Scheme 1).[6] Therefore, if either **5** or **6** is assigned to the 6A,6B-isomer, the other is assigned to the 6A,6G-isomer. In order to discriminate between clockwise and counterclockwise isomer **5** and **6**, we employed Taka amylolyses of **10** and **11**[7] which were respectively derived from **5** and **6** by $NaBH_4$ reduction in DMF. We have found that Taka amylolysis of 6-deoxy-6-phenylthio-β-cyclodextrin gave 6'-deoxy-6'-phenylthio-maltose,[4b] and Melton reported that the amylolysis of 6-deoxy-α-cyclodextrin afforded 6-deoxy-glucose.[8] From these results, we expected Taka amylolysis of 6A,6G-dideoxy-6A-phenylthio-β-

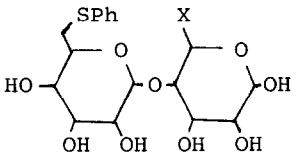
Scheme 1.



cyclodextrin or 6A,6B-dideoxy-6A-phenylthio-β-cyclodextrin to give **12** or **14**, respectively. Actually, the enzymatic hydrolysis of **10** (123 mg) or **11** (160 mg) gave two products (**12** and **13**) or one product (**14**), respectively,[9] which were reduced by 1% aqueous $NaBH_4$ to give **15** (7.4 mg) and **16** (7.4 mg), or **17** (7.7 mg), respectively. The FABMS spectrum of **17** showed M/Z 459 ($M+K^+$), 443 ($M+Na^+$), 421 ($M+H^+$), and 255 (**18**), and its ^{13}C NMR spectrum (DMSO-d_6, TMS) showed absorptions at δ62.5 [C(1)], 86.4 [C(4)], 66.2 [C(5)], 18.4 [C(6)], 100.8 [C(1')], and 34.7 [C(6')], demonstrating that its precursor **6** was 6A,6B-dideoxy-6A-phenylthio-6B-[(naphthylsulfonyl)oxyl]-β-cyclodextrin. The compound **12** was assigned to 6'-deoxy-6'-phenylthio-maltose by comparing its R_f value on silica gel TLC, its retention time in reversed-phase HPLC, and its ^{13}C NMR spectrum with those of the authentic sample.[4b] Moreover, the FABMS spectrum of **15** showed the correct molecular ion and the fragmentation ion **18**, and the ^{13}C NMR spectrum of **15** showed absorptions at δ62.5 and 62.8 [C(1) and C(6)], 83.4 [C(4)], 100.6 [C(1')], and 35.1 [C(6')], demonstrating that its precursor **5** was 6A,6G-dideoxy-6A-phenylthio-6G-[(naphthylsulfonyl)oxyl]-β-cyclodextrin. The FABMS and the ^{13}C NMR spectra[10] of **16** also confirmed the structural assignment of **5**. In conclusion, the disubstituted β-cyclodextrins, **5** and **6**, were assigned to the 6A,6G- and the 6A,6B-isomers, respectively.

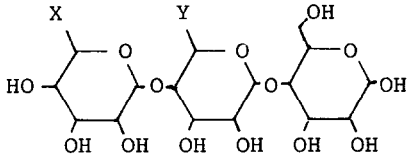
However, the present method is not applicable to the discriminations between the 6A,6C- and the 6A,6F-isomers and between the 6A,6D- and the 6A,6E-isomers, since the same products (**12** and 6-deoxy-glucose) are produced by their Taka amylolyses. Therefore, development of any novel method is necessary for such discriminations.

The assigned **5** or **6** will serve as 6A,6G- or 6A,6B-specimens for structural determinations of unsymmetrically bifunctionalized β-cyclodextrins (artificial enzymes or receptors) and also will give unique enzyme (or receptor) mimics having a hydrophobic group (phenylthio group) on the 6A carbon and a functional group, which is derived from the (naphthylsulfonyl)oxyl moiety, on the 6G or 6B carbons, respectively.



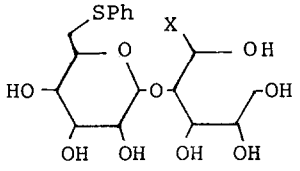
12 : X = CH_2OH

14 : X = CH_3



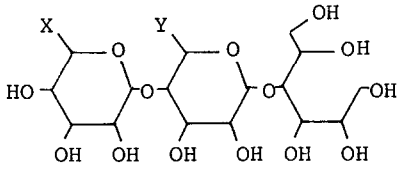
13 : X = CH_3, Y = CH_2SPh

19 : X = CH_2SPh, Y = CH_3



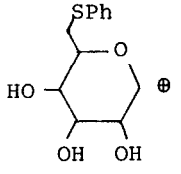
15 : X = CH_2OH

17 : X = CH_3



16 : X = CH_3, Y = CH_2SPh

20 : X = CH_2SPh, Y = CH_3



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Acknowledgement. We are indebted to Japan Maise Products Co. Ltd. for generous gift of β-cyclodextrin.

References and Notes

(1) A symmetrically 6A,6X-disubstituted β-cyclodextrin (X=B, C, or D) can be prepared through excellently effective transannular disulfonation (cap method) developed by Tabushi. (a) Tabushi, I. Tetrahedron 1984, 40, 269. (b) Tabushi, I.; Nabeshima, T.; Fujita, K.; Matsunaga, A.; Imoto, T. J. Org. Chem. 1985, 50, 2638. For the first time, attachment of two different groups on β-cyclodextrin was elegantly carried out through double nucleophilic substitution of an unsymmetrically 6A,6X-capped β-cyclodextrin by Tabushi. (c) Tabushi, I.; Nabeshima, T.; Kitaguchi, H.; Yamamura, K. J. Am. Chem. Soc. 1982, 104, 2017.

(2) 6A,6B-6A,6F isomers exist for α-cyclodextrin, 6A,6B-6A,6G isomers for β-cyclodextrin, and 6A,6B-6A,6H isomers for γ-cyclodextrin. The definition of A, B, etc. has been proposed by Tabushi [ref (1b)]. In the present report,

this definition is employed and the glucose unit of "A" indicates one having the heavier substituent.

(3) An interesting study on specific synthesis of a set of two regioisomers has been reported. See ref (1c).

(4) In order to achieve effective separation of all regioisomers, many kinds of sulfonyl chlorides were tested, where β-naphthalenesulfonyl chloride gave a mixture best resolved by the reversed-phase column (Lobar Column, RP8, Merck). Use of this column to purify substituted cyclodextrins has been reported. (a) Fujita, K.; Matsunaga, A.; Imoto, T. J. Am. Chem. Soc. 1984, 106, 5740. (b) This method was also effective to separation of some oligosaccharides. 6-Deoxy-6-phenylthio-β-cyclodextrin (100 mg) was hydrolyzed by Taka amylase at 40°C for 2 days. Analysis of the reaction mixture with reversed-phase HPLC showed that 6'-deoxy-6'-phenylthio-maltose (12) was the main product which comprised more than 96% of the all products containing a phenylthio group. The product **12** (31 mg, 88%) was isolated and reduced with $NaBH_4$ to give **15** (77%). They were easily purified by reversed-phase column chromatography. (c) Fujita, K.; Matsunaga, A.; Imoto, T. Tetrahedron Lett. 1984, 25, 5533.

(5) The isomer **1-6** showed quite similar ^1H NMR spectra. In reversed-phase HPLC (TSKgel 410 ODS SIL column, 4x300 mm, 5 μm, Toyo Soda, Japan) using a gradient elution with 20% aqueous CH_3CN-35% aqueous CH_3CN, the retention percents of CH_3CN of **1-6** were 27.2, 28.5, 29.0, 29.8, 31.5, and 31.8%, respectively.

(6) The assignments of **7-9** were made by comparing their ^1H NMR spectra and their retention times in reversed-phase HPLC with those of the authentic compounds. See (1b) and (4c).

(7) FABMS spectra demonstrated that **10** and **11** were indeed deoxy-phenylthio-β-cyclodextrins. The ^1H NMR spectrum showed doublet absorption of one methyl group at δ1.16 (J=6.0 Hz) for **10** or at δ1.32 (J=6.0 Hz) for **11**, demonstrating the sulfonation on the C(6)-hydroxyl group.

(8) Melton. D.; Slessor, K. N. Can. J. Chem. 1973, 51, 327.

(9) The progress of the enzymatic reaction (pH 5.5, 40°C) was monitored by HPLC and TLC for two weeks. Formation of both **12** and **13** was observed in the reaction of **10** during this period. On the other hand, **11** gave **14** and **19** as the minor and main products, respectively, at the early stage of the reaction. After two weeks, **14** became the main product. The intermediate **19** was isolated and reduced with aqueous $NaBH_4$ to give **20** which was structurally assigned by ^{13}C NMR and FABMS [M/Z 605 $(M+Na^+)$] spectra. ^{13}C NMR : δ62.4 and 62.7 [C(1) and C(6)], 83.1 [C(4)], 100.2 and 100.9 [C(1') and C(1")], 85.2 [C(4')], 17.7 [(6')], 66.0 [C(5')], and 35.0 [C(6")]. These spectral data also confirmed the structural assignment of **6**.

(10) Characteristic δ values are as follows. δ62.5 and 62.8 [C(1) and C(6)], 83.4 [C(4) and C(4")], 100.1 and 102.2 [C(1') and C(1")], 35.1 [C(6')], and 17.5 [C(6")].

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