of the concentrate by analytical gel electrophoresis showed it to be >90% of the monopyrrole complex[11,12] (**2a**; 16 mCi). The ^3H spectrum (Figure 1ii) of **2a** exhibits resonances[24] at 6.18 (C-2, CT), 3.28 ± 0.1 (pyrrole-CHT-X-Enz), and 2.48 ± 0.1 ppm (pyrrole-$CT_2CH_2CO_2H$) at 5.5 °C. At 23 °C (Figure 1iii) the signals at 6.18 and 3.28 ppm disappear and are replaced by new resonances at 3.58 ± 0.1 (meso methylenes (CHT) of uro'gen I (**8a**) and methylenes (CHT) of complexes **3–5**, R = ^3H), 2.75 ± 0.1 ($CT_2CH_2CO_2H$ of **3–5**, R = ^3H), 2.48 ± 0.1 ($CT_2CH_2CO_2H$ of uro'gen I (**8a**)), and 4.69 ppm (HOT, exchanged from C-2—also present in Figure 1, spectra ii and iv). The formation of uro'gen I (**8**) (whose ^3H chemical shifts are identical with the corresponding CH_2 resonances in the ^1H NMR spectrum) in absence of free PBG is ascribed to disproportionation of complex **2a** via **3–5** whose methylene (CHT) and side-chain groups (C-$T_2CH_2CO_2H$) can be observed along with those of uro'gen I in the signals at 3.58, 2.75, and 2.48 ppm. Such a disproportionation also accounts for the disappearance of the signal at 3.28 ppm, which would be expected to lose up to 90% of its original intensity, on the basis of the statistical randomization of ^3H label, from **2** → **3** → **4** → **5** → **8**.

To demonstrate the catalytic competence of the monopyrrole complex **2**, *unlabeled* PBG (**1**) was added to the ^3H complex **2a** at 3.5 °C and the formation of uro'gen I monitored by ^3H NMR. At 3.5 °C a transient low-intensity signal was observed at 4.76 ppm, a chemical shift consistent with the vinyl (R) hydrogen of the azafulvene **6a**.[25] On warming to 23 °C (Figure 1iv), sharp resonances for unbound uro'gen I (**8a**) appeared at 3.58 (20-meso CHT) and 2.48 ppm (propionate CHT) corresponding to the ^1H chemical shifts in an enzyme-free sample of uro'gen I.[22]

The large line widths of the spectrum in Figure 1 (spectra ii and iii) (~150–300 Hz) reflect an environment in which the active site of the enzyme is probably buried within the protein. The line width of the propionate side chain also suggests that it is covalently attached or ionically associated with the protein, which is consistent with literature reports on the inhibitory effects of PBG analogues.[26-30] The ^3H chemical shift (3.28 ± 0.1 ppm) of the methylene directly attached to the enzyme allows conclusions to be drawn as to the nature of the nucleophilic group "X" in Scheme I. That the methylene could be bound to the oxygen of a serine residue is ruled out, since HMB (**7**) and its methyl ether have chemical shifts of 4.4 and 4.2 ppm, respectively.[22] Model studies[31] predict δ 3.9 for methylene attached to amine while the observed value (3.28 ± 0.1 ppm) is more consistent[33] with a thioether linkage (**2a**, X = S). We therefore suggest that the active-site nucleophilic group in deaminase is a cysteine thiol residue or, less probably, an amino group (**2a**, X = NH) which on covalent binding to C-11 of PBG leads to an upfield shift of ~0.6 ppm from the anticipated value of 3.9 ppm. The former possibility is supported by the observation that deaminase is reversibly inhibited by sulfhydryl blocking reagents.[34]

Acknowledgment. We thank the National Institutes of Health (AM32034) and The Robert A. Welch Foundation for support of this work and K. Mackenzie and C. McCullar for growing the bacteria.

(24) ^3H NMR referencing was performed as follows: A ^1H NMR spectrum of [2,6,6,11,11-$^3H_5$]PBG containing internal $Me_3SiCD_2CD_2CO_2Na$ was recorded and the PBG ^1H resonances referenced. The same sample was then examined by ^3H NMR in the same probe and referenced to the ^1H spectrum ($\delta_{^3H} = \delta_{^1H}$). The absolute field frequency was then used from this to calculate the chemical shifts of subsequent spectra.

(25) Falk, H.; Schlederer, T. *Monatsh, Chem.* **1981**, *112*, 501. Interestingly, no NMR evidence for the accumulation of HMB (**7a**) was obtained under these conditions involving high concentration (1 mM) of pure deaminase, 3.5 °C, pH 8.0 (cf. ref 6–10).

(26) Frydman, R. B.; Feinstein, G. *Biochim. Biophys. Acta* **1974**, *350*, 358.

(27) Bogorad, L. *Plant Physiol.* **1975**, *32*, xli.

(28) Bogorad, L. "Comparative Biochemisty of Photoreactive Systems"; Allen, M. B., Ed.; Academic Press: New York 1960; p 227.

(29) Carpenter, A. T.; Scott, J. J. *Biochim. Biophys. Acta* **1968**, *52*, 195.

(30) Heath, H.; Hoare, D. S. *Biochem. J.* **1959**, *72*, 14. Heath, H.; Hoare, D. S. *Ibid.* **1959**, *73*, 679.

(31) PBG (**1**) displays a resonance at 4.16 ppm for the C-11 methylene protons and shifts 0.05 ppm upfield on deprotonation.[22] The (aminomethyl)bilane (**7** where $CH_2OH = CH_2NH_2$) displays a resonance at 3.92 ppm at pH 12,[32] which implies a methylene chemical shift of 3.97 ppm at pH 8.0.

(32) Dauner, H.-O.; Gunzer, G.; Heger, I.; Müller, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1976**, *357*, 149.

(33) The proton chemical shifts of α-bromotoluic acid methyl ester (δ_H-(CH_2Br) 4.94 ± 0.05), α-(ethylthio)toluic acid methyl ester ($\delta_H(CH_2SEt)$ 4.10 ± 0.05), and 3-[2-(methoxycarbonyl)ethyl]-4-[(methoxycarbonyl)methyl]-5-(bromomethyl)pyrrole (δ_H(pyrrole-CH_2Br) 4.23 ± 0.05) were used to calculate the chemical shift for 3-[2-(methoxycarbonyl)ethyl]-4-[(methoxycarbonyl)methyl]-5-[(ethylthio)methyl]pyrrole (δ_H(pyrrole-CH_2SEt) 3.39 ± 0.1).

(34) Jordan, P. M.; Shemin, D. *J. Biol. Chem.* **1973**, *248*, 1019.

6A6B-, 6A6C-, and 6A6D-Disulfonates of α-Cyclodextrin

Kahee Fujita,* Atsuo Matsunaga, and Taiji Imoto

Faculty of Pharmaceutical Sciences 62
Kyushu University, Maidashi, Higashi-ku
Fukuoka 812, Japan

Received April 18, 1984

Recently, specific preparation of primary two- (or more) substituted β-cyclodextrins has been attained to construct refined and sophisticated models of enzymes.[1] Transannular disulfonate-capping methods have been developed in order to activate 6A6C and/or 6A6D primary hydroxyls of β-cyclodextrin.[2] Although α-cyclodextrin shows different molecular recognition as an inclusion host from that of β-cyclodextrin, there have been a few studies on specific activation of two (or more) hydroxyls of α-cyclodextrin. Sulfonation on three (6A6C6E) primary hydroxyls[3] and transannular sulfonation on two primary hydroxyls,[4] whose positions were not determined, were reported.

We wish to demonstrate here a novel and absolute strategy of isomer determination through the first isolation of 6A6B-, 6A6C-, and 6A6D-disulfonates of α-cyclodextrin. A solution of α-cyclodextrin (3 g, 3.1 mmol) and mesitylenesulfonyl chloride (6 g, 27 mmol)[5] in pyridine (230 mL) was stirred for 2 h at room temperature followed by addition of water (1 mL) and concentration in vacuo. The crude concentrated mixture was applied on a reversed-phase column (Lobar column LiChroprep RP 8, Merck Ltd., 25 × 310 mm). After a stepwise elution from 900 mL of 10% aqueous MeOH to 100 mL of 20% aqueous MeOH, a gradient elution with 1 L of 40% aqueous MeOH–1 L of 60% aqueous MeOH was applied to give 6-deoxy-6-mesitylenesulfonates **1** (298 mg, 7.3%), **2** (374 mg, 9.1%), and **3** (555 mg, 13.6%) (Figure 1).[6] ^1H NMR of **1–3**[7] and **4–6**[8] obtained from $NaBH_4$ reduction of **1–3** demonstrated that **1–3** were primary dimesitylenesulfonates. Positional assignments of **1–3** were carried out

(1) (a) Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer Verlag: Berlin, 1978. (b) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66. (c) Breslow, R. *Science (Washington, D.C.* **1982**, *218*, 532. (d) Croft, A. P.; Bartsch, R. A. *Tetrahedron* **1983**, *39*, 1417.

(2) (a) Tabushi, I.; Shimokawa, K.; Shimizu, N.; Shirakata, H.; Fujita, K. *J. Am. Chem. Soc.* **1976**, *98*, 7855. (b) Breslow, R.; Bovy, P.; Hersh, C. L. *Ibid.* **1980**, *102*, 2115. (c) Tabushi, I.; Kuroda, Y.; Yokota, K.; Yuan, L. C. *Ibid.* **1981**, *103*, 711. (d) Tabushi, I.; Yuan, L. C. *Ibid.* **1981**, *103*, 3574.

(3) Boger, J.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 7631.

(4) Tabushi, I.; Shimokawa, K.; Fujita, K. *Tetrahedron Lett.* **1977**, 1524.

(5) We added mesitylenesulfonyl chloride to the pyridine solution, monitoring the formation of the desirable products. The amount of the sulfonyl chloride was dependent on the dryness of pyridine and α-cyclodextrin.

(6) These isolated compounds were shown to be pure by HPLC (TSK GEL LS-410 ODS SIL column, 4 × 300 mm, 5 μm, Toyo Soda, Japan).

(7) They differed from one another in chemical shifts of aromatic protons. **1**, δ 7.00–7.20 (4 H, m); **2**, δ 7.04 (2 H, s) and 7.08 (2 H, s); **3**, δ 7.07 (4 H, m).

(8) The deoxy derivatives **4–6** showed doublet absorptions of two methyl groups at δ 1.2 in their ^1H NMR and parent ions (M + H^+) at m/z 957 in their FABMS.

0002-7863/84/1506-5740$01.50/0

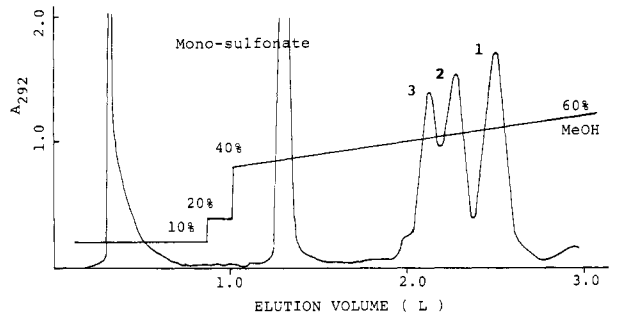
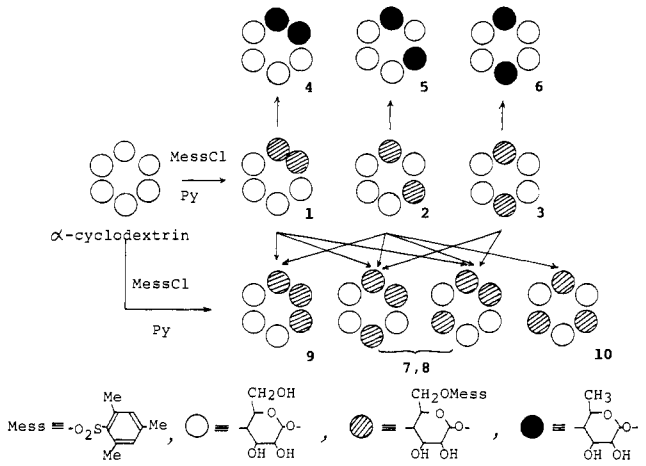


Figure 1. Reversed-phase column chromatography of the mixture obtained from the reaction of α-cyclodextrin with mesitylenesulfonyl chloride. A stepwise followed by a linear gradient elution of MeOH was applied. "Monosulfonate" represents 6-deoxy-6-[(mesitylsulfonyl)-oxyl]-α-cyclodextrin.

Scheme I



as shown in Scheme I. Trimesitylenesulfonates (**7–10**) were prepared as standard compounds for the assignments; sulfonation of α-cyclodextrin (1 g) with 27 mol excess of mesitylenesulfonyl chloride (6 g)[5] gave four trimesitylenesulfonates (**7–10**) with three dimesitylenesulfonates (**1–3**) as an overlapped pattern of those shown in Figure 2.[9] Separation by use of the reversed-phase column gave pure **7** (66 mg), **8** (96 mg), and a mixture of **9** and **10**, from which pure **9** (58 mg) and **10** (4 mg) were isolated by a preparative reversed-phase HPLC.[6] All of the isolated **7–10** were assigned to be regioisomeric trimesitylenesulfonates (6A6B6C, 6A6B6D, 6A6B6E, and 6A6C6E isomers) from their ^{1}H NMR.[10] Additional monomesitylenesulfonations of the di-mesitylenesulfonates (**1–3**) were employed as criteria of assignments of **1–3** and also **7–10**. The additional monosulfonation of 6A6B-, 6A6C-, or 6A6D-di-mesitylenesulfonates should produce three (6A6B6C, 6A6B6D, and 6A6B6E), four (6A6B6C, 6A6B6D, 6A6B6E, and 6A6C6E), or two (6A6B6D and 6A6B6E) trimesitylenesulfonates, respectively (see Scheme I). HPLC analyses (Figure 2)[9] showed that **1**, **2**, or **3** gave three (**7–9**), four (**7–10**), or two (**7** and **8**) products, respectively. Therefore, we assigned **1** as a 6A6B,[11] **2** as a 6A6C, **3** as a 6A6D, **7** as a 6A6B6D (or 6A6B6E), **8** as a 6A6B6E (or 6A6B6D), **9** as a 6A6B6C, and **10** as a 6A6C6E isomer.

Thus, the primary difunctionalized α-cyclodextrins are now easily available by the present convenient method. Also, authentic primary trisubstituted α-cyclodextrin can be derived from **7–10**.

(9) The HPLC column content is shown in ref 6.

(10) They showed quite similar NMR spectra although the spectra somewhat differed from one another in chemical shift ranges of aromatic protons (6H, m). **7**; δ 6.96–7.20, **8**; δ 6.96–7.16, **9**; δ 6.96–7.20, **10**; δ 7.00–7.16. The best differentiation method which we know is the HPLC of which column content is described in ref 6 and is commercially available.

(11) The 6A6B structure of **1** was independently determined by an enzymatic degradation of **1** by Taka-amylase to give 6',6''-dideoxy-6',6''-bis-[(mesitylsulfonyl)oxy]maltotriose. This method will be reported in near future.

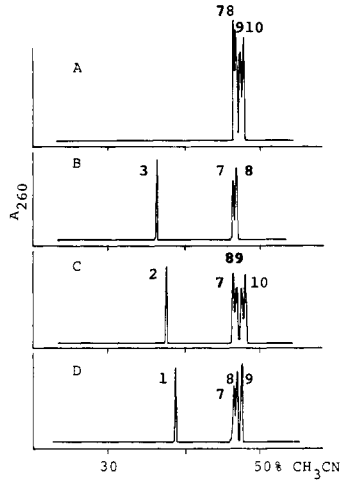


Figure 2. Reversed-phase HPLC of trimesitylenesulfonates of α-cyclodextrin prepared from α-cyclodextrin (2A) and the reaction mixture of the additional monosulfonated **3** (2B), **2** (2C), or **1** (2D). A gradient elution with water–aqueous CH_3CN was applied.

Our separation method by use of the reversed-phase column is quite suitable not only for the separation of the regioisomers but also for the elimination of the unreacted cyclodextrin and salts such as pyridinium mesitylenesulfonate. Moreover, the absolute method of isomer determination described here would be applicable for regioisomeric substitutions on the compounds (cyclophanes, crown ethers, cryptands, etc.) that are made up of several same constitutes.[12]

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

(12) A similar absolute method of isomer determination was described by Körner in assignment of disubstituted benzenes by the conversion of them to the trisubstituted benzenes. Körner, G. *Gazz. Chim. Ital.* **1874**, *4*, 305.

Electronic Control of Stereoselectivity. 23. Stereochemically Selective Course of [6 + 4] and [3 + 4] Cycloadditions to Isodicyclopentadiene[1]

Leo A. Paquette,* Susan J. Hathaway, Tina M. Kravetz, and Leh-Yeh Hsu[2]

Evans Chemical Laboratories
The Ohio State University
Columbus, Ohio 43210

Received March 19, 1984

Isodicyclopentadiene (**1**) is now recognized to favor endo-face selectivity during [4 + 2] cycloaddition to all dienophiles[3] except triazolinediones.[1,4] Guided by semiempirical calculations and photoelectron spectroscopic measurements, Gleiter has rationalized this behavior in terms of a strong σ/π interaction that causes the ψ_1 π orbital of the diene to be disrotatorily tilted toward the methano bridge.[3a,5] It follows that a less destabilizing four-

(1) Part 21. Paquette, L. A.; Green, K. E.; Hsu, L.-Y. *J. Org. Chem.*, in press.

(2) Author to whom inquiries concerning the X-ray crystal structure analysis should be directed.

(3) (a) Gleiter, R.; Paquette, L. A. *Acc. Chem. Res.* **1983**, *16*, 328. (b) Paquette, L. A. In "Stereochemistry and Reactivity of Pi systems"; Watson, W. H., Ed.; Verlag Chemie International: Deerfield Beach, FL, 1983; 41–73.

(4) Bartlett, P. D.; Wu, C. *J. Org. Chem.* **1984**, *49*, 1880.

(5) Gleiter, R.; Böhm, M. C. In "Stereochemistry and Reactivity of Pi Systems"; Watson, W. H., Ed.; Verlag Chemie International: Deerfield Beach, FL, 1983; 105–146.

0002-7863/84/1506-5741$01.50/0