The Inclusion Compounds of β-Cyclodextrin with 4-Substituted Benzoic Acid and Benzaldehyde Drugs Studied by Proton Nuclear Magnetic Resonance Spectroscopy

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Inclusion compounds of some 4-substituted benzoic acid and benzaldehyde drugs with β-cyclodextrin were prepared and characterized by IR spectroscopy, powder X-ray diffraction, thermogravimetry, and ^{1}H-NMR spectroscopy. The thermal stability and chemical stability of these drugs were strikingly improved after inclusion. The effect of inclusion on the chemical-shifts of protons H-3 and H-5 in the NMR spectroscopy is discussed. Using the relative shift theory, the preferred inclusion mode was proposed. The center of the aromatic ring of the drug molecule was considered to be located in the cavity 1.2 Å inside from the H-5 plane of β-cyclodextrin.

Key words ^{1}H-NMR; inclusion mode; β-cyclodextrin; inclusion compound; 4-substituted benzoic acid; 4-substituted benzaldehyde

Inclusion complexation of cyclodextrins (CDs) with drugs can improve certain properties of the drugs such as solubility, stability, and bioavailability.[1] The enhancement of drug activity and selective transfer or the reduction of side effects can also be achieved by inclusion compound formation.[2,3]

Some benzoic acids (BAs) and benzaldehydes (BZAs) can be used as active ingredients in carcinostatic drugs, such as those which control leukemia, adenocarcinoma, sarcoma, ascites tumors and other malignancies.[4,5] We prepared the inclusion compounds of some BA and BZA drugs with β-CD. The stabilities and probable inclusion modes of these inclusion compounds were then discussed.

The drugs used here were BZA, *p*-anisaldehyde (MOBZA), *p*-chlorobenzaldehyde (CBZA), *p*-fluorobenzaldehyde (FBZA), piperonal (PPN), cinnamaldehyde (CNA), BA, *p*-anisic acid (MOBA), *p*-toluic acid (MBA), *p*-chlorobenzoic acid (CBA) and *p*-nitrobenzoic acid (NBA).

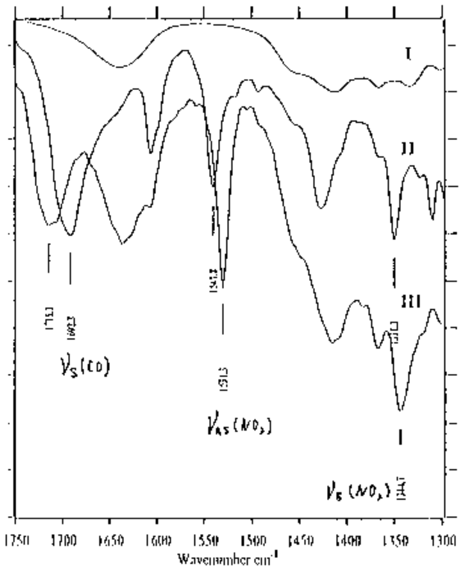

Fig. 1. IR Spectra of β-CD (I), Mixture of NBA and β-CD (II), and Inclusion Compound of NBA/β-CD (III)

Results and Discussion

IR Spectra IR spectra of β-CD (I), the physical mixture of β-CD and NBA (II), and the inclusion compound of β-CD with NBA (III), prepared as a precipitate by the procedure described in the Experimental, are shown in Fig. 1. After inclusion, the carbonyl stretching band (ν_{co}) of NBA was shifted from 1692.3 to 1715.1 cm^{-1}. The nitro asymmetric stretching band (ν_{as}) of NBA was shifted from 1541.8 to 1531.3 cm^{-1}, and the nitro symmetric stretching band (ν_s) of NBA was shifted from 1351.3 to 1344.7 cm^{-1}.

The higher frequency shift of ν_{co} observed in the IR spectra of the inclusion compounds of β-CD with BA drugs (BA, MOBA, MBA, CBA, NBA) (Table 1) can be ascribed to the destruction of strong hydrogen bonding structure in uncomplexed drugs upon the formation of inclusion complexes with β-CD. The lower frequency shift of the nitro stretching bands (*vide supra*) may be due to the formation of new hydrogen bonding between the drug and β-CD in the inclusion compound.[6] But in the case of the BZA drugs, there is not such strong hydrogen bonding existing in the pure drugs compared with the benzoic acid drugs. So, only very small shifts of ν_{co} (even negative) were observed in the IR spectra of the inclusion compounds of β-CD with BZA, MOBZA and CNA. The higher frequency shifts observed for FBZA,

Table 1. Data of IR Spectra of Drug/β-CD Systems

Drug	Frequency of carbonyl stretching band (cm^{-1})		
	In mixture	In compound	Shift[a]
BA	1686.6	1699.7	+13.1
MOBA	1686.0	1690.5	+4.5
MBA	1678.6	1699.6	+21.0
CBA	1681.6	1705.4	+23.8
NBA	1692.3	1715.1	+22.8
BZA	1700.0	1700.0	0.0
MOBZA	1696.7	1696.6	−0.1
FBZA	1684.7	1704.8	+20.1
CBZA	1699.6	1706.9	+7.3
PPN	1672.4	1689.7	+17.3
CNA	1676.5	1671.1	−5.4

a) Positive values indicate higher frequency shift upon inclusion.

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CBZA and PPN may reflect the formation of hydrogen bonds between β-CD and the 4-substituents of these drugs.

Powder X-Ray Diffraction Patterns Figure 2 shows the powder X-ray diffraction patterns of β-CD (I), the physical mixture of CBA and β-CD (II), and the inclusion compound of CBA with β-CD (III). The diffraction patterns of the inclusion compound varied significantly from the physical mixture. New peaks appeared (2θ=34.6, 35.4, 36.3, 37.6, 38.3°), and some of the peaks became very strong (2θ= 5.9,11.9°). These demonstrate that the inclusion compound of CBA with β-CD is in fact a new chemical species different from the original substances. All the other inclusion compounds showed changes similar to those observed in the CBA/β-CD system.

Differential Thermogravimetry (DTG) Results Recently, the thermal analysis method has been used satisfactorily in the investigation of inclusion complexation for its simplicity and effectiveness.[7] DTG was used here to investigate the thermal stability of the drug/β-CD systems. The DTG curves of β-CD$\cdot n$H$_2$O (I), the physical mixture of BA and β-CD$\cdot n$H$_2$O (II), and the inclusion compound of BA/β-CD$\cdot m$H$_2$O (III) are shown in Fig. 3.

Two peaks were observed in the DTG curve of the pure β-CD hydrate. The first peak between 30 and 100 °C (the maximum observed at 82 °C) corresponds to the dehydration of β-CD$\cdot n$H$_2$O. The experimental results show that the β-CD$\cdot n$H$_2$O contains 10 mol of water (Table 2) which is lost at temperatures below 100 °C. The second peak around 315 °C is related to the degradation of the β-CD structure. In the case of the physical mixture, each component behaved independently. For example, BA in the mixture decomposed at around 146 °C. The dehydration and degradation peaks of β-CD$\cdot n$H$_2$O in the mixture were clearly seen at around 80 and 315 °C, respectively. However, in that of the BA/β-CD$\cdot m$H$_2$O inclusion compound, the dehydration was completed at a much lower temperature than that of both pure β-CD$\cdot n$H$_2$O and in the mixture, and the DTG curve showed the maximum at 37 °C. The experimental water contents of the BA/β-CD$\cdot m$H$_2$O was found to be 5, which is less than that of the pure β-CD$\cdot n$H$_2$O (Table 2). The inclusion compound was stable at a temperature of 200 °C, and the decomposition peak appeared at around 271 °C. This decomposition temperature is much higher than that of the pure BA in the mixture, which decomposed at around 146 °C. The decomposition temperature differences (ΔT) between the pure

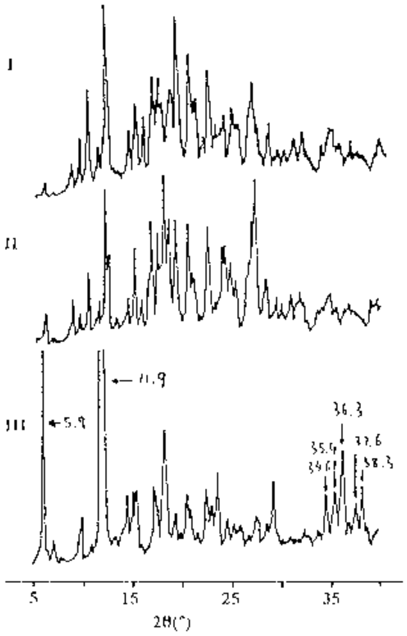

Fig. 2. Powder X-Ray Diffraction Patterns of β-CD (I), Mixture of CBA and β-CD (II), and Inclusion Compound of CBA/β-CD (III)

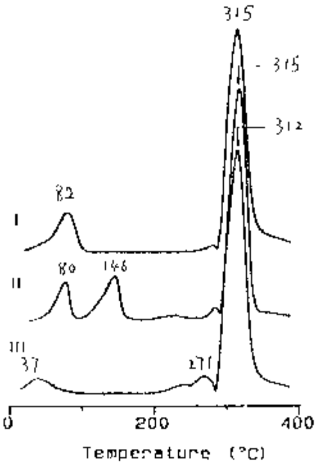

Fig. 3. DTG Curves of β-CD$\cdot n$H$_2$O (I), Mixture of BA and β-CD$\cdot n$H$_2$O (II), and Inclusion Compound of BA/β-CD$\cdot m$H$_2$O (III)

Table 2. DTG Data of Inclusion Compounds

Compound	Peak temp. of dehydration (°C)	Water contents	Peak temp. of decomposition of inclusion compound (°C)	ΔT (°C)
β-CD	82	10	—	—
BA/β-CD	37	5	271	+125
MOBA/β-CD	70	3	254	+56
MBA/β-CD	57	5	257	+79
CBA/β-CD	56	7	250	+61
NBA/β-CD	63	4	227	+15
BZA/β-CD	55	5	247	—[b]
MOBZA/β-CD	55	4	236	—[b]
FBZA/β-CD	71	6	244	—[b]
CBZA/β-CD	74	6	213	+108
PPN/β-CD	—[a]	—[a]	245	+100
CNA/β-CD	67	6	247	—[b]

a) Weak DTG signal in curve. *b*) Not determined, for the corresponding drug is a liquid at room temperature.

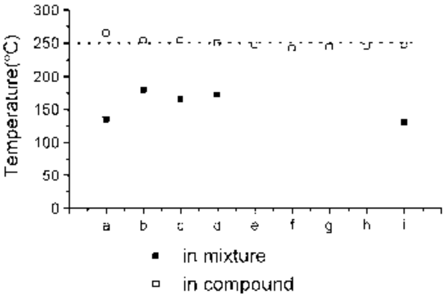

Fig. 4. Peak Temperature of Drug Decomposition

a to i refer to BA, MOBA, MBA, CBA, BZA, MOBZA, FBZA, PPN, and CNA, respectively.

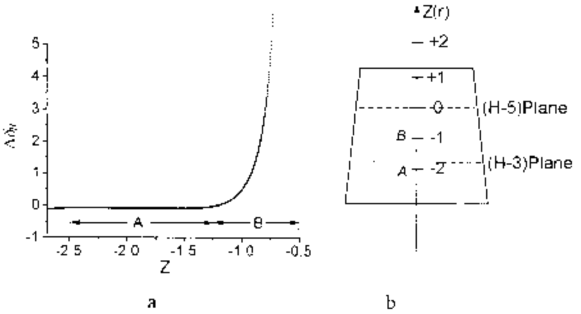

Fig. 5. The Relative Shift Curve According to Eq. 6 (a) and the Inclusion Coordinate (b)

drug and the inclusion compound containing included drug have been taken as a parameter to describe the thermal stability of the inclusion compound, and are given in Table 2.

Figure 4 shows the decomposition temperature of these drugs in inclusion compounds and in physical mixtures. Decomposition temperatures of all the drugs were greatly altered after inclusion. The most interesting is that the decomposition temperatures of these drugs in the inclusion compounds are around 250 °C. Probably, their thermal–chemical behavior near 250 °C mainly reflects the decomposition of the complexed β-CD, which seems to be affected little by the differences in the included drugs.

Analysis of the Inclusion Mode from NMR Measurements Since inclusion of aromatic substrates with CDs causes obvious shifts in the ^{1}H-NMR signals of CDs, high-resolution ^{1}H-NMR spectroscopy can be used to investigate inclusion interactions.[8] However the details about inclusion interaction still need further study.[2]

In ^{1}H-NMR measurements of the samples of inclusion compounds dissolved in D_2O, the distinct peaks of the free and the bound forms of β-CD in inclusion equilibrium exchange rapidly on the NMR time scale; therefore the observed values (δ_{obs}) can be expressed as the weighted average of the free and bound forms:

$$\delta_{obs}=\frac{C_H}{C_H+C_{HG}}\delta_H+\frac{C_{HG}}{C_H+C_{HG}}\delta_{HG} \tag{1}$$

where δ_{obs}, δ_H, and δ_{HG} represent the observed chemical shifts of β-CD protons for the sample of inclusion compound, the chemical shifts of the β-CD protons in the free form, and the theoretical chemical shifts of the β-CD protons in the bound form, respectively. C_H and C_{HG} are the concentrations of the free and bound forms of β-CD, respectively.

The difference between the observed chemical shifts (δ_{obs}) of β-CD protons for the sample of inclusion compound and the observed chemical shifts of the protons of pure β-CD, that is, the chemical shifts of the free form (δ_H), is given by:

$$\Delta\delta_{obs}=\delta_{obs}-\delta_H=\frac{C_{HG}}{C_H+C_{HG}}\left(\delta_{HG}-\delta_H\right)=\frac{C_{HG}}{C_H+C_{HG}}\Delta\delta_{HG} \tag{2}$$

where $\Delta\delta_{obs}$ and $\Delta\delta_{HG}$ are the observed and the theoretical chemical shift changes of β-CD protons upon inclusion, respectively.

When a drug molecule is included into the β-CD cavity, the H-3 and H-5 protons are more seriously affected by the included drug molecule and show more prominent changes of chemical shift in ^{1}H-NMR spectra than the other protons of β-CD. The observed H-5 and H-3 signal shifts are given by:

$$\Delta\delta_2=\Delta\delta_{obs}(\mathrm{H{-}5})=\frac{C_{HG}}{C_H+C_{HG}}\Delta\delta_{HG(H-5)} \tag{3}$$

$$\Delta\delta_1=\Delta\delta_{obs}(\mathrm{H{-}3})=\frac{C_{HG}}{C_H+C_{HG}}\Delta\delta_{HG(H-3)} \tag{4}$$

The proton signal shift ($\Delta\delta_{HG}$), when induced by inclusion of an aromatic molecule, is mainly caused by the shielding or deshielding effect. From Johnson–Bovey's work,[9] the $\Delta\delta_{HG}$ can be obtained:

$$\Delta\delta_{HG}=\frac{ne^2\cdot 10^6}{6\pi\, mc^2 a}\cdot\frac{-2\rho^2+\rho+z^2+1}{\left[(1-\rho)^2+z^2\right]\left[(1+\rho)^2+z^2\right]^{3/2}}=k\cdot f(\rho,z) \tag{5}$$

where k is a constant, and f is a function in the usual cylindrical coordinates ρ and z. The positive value of $\Delta\delta_{HG}$ means upfield shift of the proton signal in spectra.

Now by a comparison of Eqs.3 and 4, we can obtain the description of the relative shift ($\Delta\delta_R$) of H-5 and H-3:

$$\Delta\delta=\frac{\Delta\delta_2}{\Delta\delta_1}=\frac{f(\rho_2,z_2)}{f(\rho_1,z_1)}=F(\rho_1,\rho_2,z_1,z_2) \tag{6}$$

Figure 5a shows the curve derived from Eq. 6, where the H-5 plane is set as zero, the Z axis can be called the inclusion coordination and the Z value represents the distance from the phenyl-ring center of the guest molecule to the zeroplane.

The general features of how the $\Delta\delta_R$ changes as the guest molecule approaches and moves into the cavity of β-CD are illustrated in Fig. 5. At first, as the guest molecule approaches the β-CD cavity along the Z axis from the wider side of the β-CD, the $\Delta\delta_R$ experiences little change. Then the guest molecule enters the β-CD cavity. As Z is still very negative (in region A), the $\Delta\delta_R$ still does not undergo significant change. At last the $\Delta\delta_R$ rises dramatically as the guest molecule reaches the center of the cavity of β-CD, and Z is around -1.2 Å (in region B). The $\Delta\delta_R$ is extremely sensitive to the change of Z in the B region, which is close to the full inclusion position.

Figure 6 shows the ^{1}H-NMR spectra of pure β-CD (I) and the β-CD in the sample of inclusion compound with CBA (II). In pure β-CD, the proton signals of H-5 and H-3 appeared at 3.865 and 3.955 ppm, respectively. They shifted to 3.770 and 3.887 ppm in the sample of inclusion compound. The $\Delta\delta_R$ was 1.40, corresponding to the region B (Fig. 5) with the Z value of -1.2 Å. So the average position of the

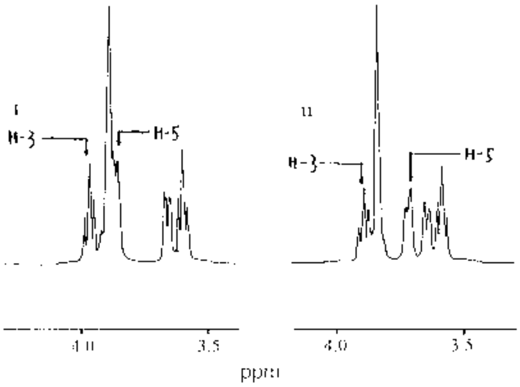

Fig. 6. Partial ^{1}H-NMR Spectra of β-CD (I, 0.8 mM), and β-CD in the Sample of Inclusion Compound with CBA (II, 0.8 mM)

Table 3. Probable Positions of the Drugs in Inclusion Compounds from Our Calculation Results

Drug	$\Delta\delta_1$(H-3)[a] (ppm)	$\Delta\delta_2$(H-5)[a] (ppm)	$\Delta\delta_2/\Delta\delta_1$	Position[b] (Å)
BA	0.061	0.078	1.28	−1.2
MOBA	0.067	0.104	1.55	−1.2
MBA	0.090	0.125	1.38	−1.2
CBA	0.068	0.095	1.40	−1.2
NBA	0.062	0.061	0.99	−1.3
BZA	0.011	0.016	1.45	−1.2
MOBZA	0.030	0.069	2.27	−1.2
FBZA	0.015	0.020	1.31	−1.2
CBZA	0.018	0.029	1.59	−1.2
PPN	0.047	0.094	2.00	−1.2
CNA	0.023	0.035	1.54	−1.2

a) Positive values indicate upfield shifts upon inclusion. *b*) Distance from the phenyl-ring center to the H-5 plane.

aromatic ring center of CBA located inside β-CD cavity is 1.2 Å away from the H-5 plane, which is very consistent with the previous work of Y. Inoue.[10]

The preferred phenyl-ring positions of these drugs in the β-CD cavity calculated by Eq. 6 are given in the Table 3. The most interesting point is that although these rings have different substituent groups, most of them are located in almost the same position in their inclusion compounds, that is, 1.2 Å inside the H-5 plane in the β-CD cavity, indicating full inclusion. These results show that a strong interaction between the aromatic ring of the drug molecule and the interior wall of β-CD cavity governs the inclusion geometry for these types of drug molecules with the preferred inclusion position of $Z=-1.2$ Å.

Generally speaking, inclusion complexation has made great improvements in the physical and chemical properties of these drugs. After inclusion, some of the drugs changed from liquids to powders that are soluble in water. Storage and delivery of the drugs become much easier and the bioavailability of certain water-insoluble drugs is increased. The thermal and chemical stability of the drugs are also significantly improved. Though some of the drugs (such as BZA, MOBZA, FBZA, CBZA, PPN, CNA) are light-sensitive, inclusion complexation retards their photo-oxidization effectively. All of these can greatly improve the pharmaceutical actions of the drugs.

Experimental

General Methods A Bruker IFS66V FT-IR spectrophotometer was used, and the measurements were made by the KBr disk method. Powder X-ray diffraction patterns were measured on a Rigaku D/Max-RA diffractometer. Differential thermogravimetric analysis curves were recorded on an American SDT-2960 thermal analyzer. All ^{1}H-NMR spectra were recorded on a Bruker-Am-500 ^{1}H-NMR spectrometer in D_2O solution at 15 °C, with 4,4-dimethyl-4-silapentane sodium sulfonate (DSS) as an external reference. Reagent grade β-CD supplied by the Suzhou Monosodium Glutamate Factory was recrystallized twice from water before use. MBA and PPN were recrystallized from 50% and 70% ethanol, respectively. MOBA, CBA, and NBA were recrystallized from ethanol. BZA, MOBZA, FBZA and CNA were distilled under reduced pressure. CBZA and BA were of analytically pure grade and were used without further purification.

Preparation of Inclusion Compound β-CD inclusion compounds were prepared by the coprecipitation method.[11] 0.53×10^{-3} mol of β-CD were dissolved in 50 ml of water, and then was added 1.06×10^{-3} mol of drug. After stirring at 70 °C for 2 h, the solution was cooled to room temperature and a white solid precipitated, which was then collected by filtration and washed twice with water and ethanol. Finally, drying of the solid in air for 4 h gave the inclusion compound as a white solid.

The inclusion compounds of BZA, MOBZA, FBZA, CBZA, PPN, and CNA with β-CD remained white in air for more than 6 months, while the corresponding pure drugs turned dark under the same conditions after only 1—2 d.

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References

1) *a*) Szejtli J., "Cyclodextrin Technology," ed. by Davis J. E. D., Kluwer Academic, Dordrecht, The Netherlands, 1988; *b*) Frömming K.-H., Szejtli J., "Cyclodextrin in Pharmacy," Kluwer Academic, Dordrecht, The Netherlands, 1994.
2) *a*) Szejtli J., Osa T. (eds.), *Compr. Supramol. Chem.*, **3** (1996); *b*) D'Souza V. T., Lipkowitz K. B. (eds.), *Chem. Rev.*, **98** (5) (1998).
3) Albers E., Miller B. W., *CRC Crit. Rev. Ther. Drug Carrier Systems*, **12**, 311—337 (1995).
4) *a*) Takeuchi S., Kochi M., Ger offen 2,710,327 (Cl. A61K31/11) 15 Sep 1977, Japan, Appl. 76/25,225, 09 Mar 1976; 31 pp.; *b*) Taetle R., Howell S. B., *Cancer Treat. Rep.*, **67**, 561—566 (1982); *c*) Ogungbamila O., Olaniyi A. A., Yisak W. Ab., *Niger. J. Pharm.*, **12**, 288—292 (1981).
5) *a*) T'Ang A., Lien E. J., *J. Clin. Hosp. Pharm.*, **6**, 245—249 (1981); *b*) Kreb H. A., Wiggins D., Stubbs M., Sols A., Redoya F., *Biochem. J.*, **214**, 657—663 (1983); *c*) Nathan D., Sakr A., Lichtin J. L., Bronaugh R. L., *Pharm. Res.*, **7**, 1147—1151 (1990); *d*) Brunner G. F., Nichols D. A., Can. Pat. Appl. CA 2,017,923 (Cl. A61K31/485), 06 Dec 1990, US Appl. 361,984 06 Jun 1989; 17 pp.; *e*) Rehn D., Nolte H., Zerling W., *Zentralbl. Bakteriol., Mikrobiol. Hyg., Abt. 1, Orig. B*, **172**, 508—519 (1981); *f*) Vincieri F. F., Mazzi G., Mulinacci N., Papini P., Gelsomini N., *Pharm. Acta Helv.*, **63**, 282—286 (1988); *g*) Ikeda Y., Matsumoto K., Kunihiro K., Fuwa T., Uekama K., *Yakugaku Zasshi*, **102**, 83—88 (1982); *h*) Uekama K., Hirayama F., Esaki K., Inoue M., *Chem. Pharm. Bull.*, **27**, 76—79 (1979).
6) Nakai Y., Yamamoto K., Terada K., and Akimoto K., *Chem. Pharm. Bull.*, **32**, 681—691 (1984).
7) Yilmaz V. T., Karadag A., Icbudak H., *Thermochim. Acta*, **261**, 107—118 (1995).
8) *a*) Nishijo J., Ushiroda Y., Ohbori H., Sugiura M., Fujii N., *Chem. Pharm. Bull.*, **45**, 899—903 (1997); *b*) Matsubara K., Irie T., and Uekama K., *ibid.*, **45**, 378—383 (1997); *c*) Schneider H.-J., Hacket F., Rüdiger V., Ikeda H., *Chem. Rev.*, **98**, 1755—1785 (1998).
9) Johnson C. E., Bovey F. A., *J. Phys. Chem.*, **29**, 1012—1015 (1958).
10) Inoue Y., Okuda T., Miyata Y., Chûjô R., *Carbohydr. Res.*, **125**, 65—76 (1984).
11) Kuroizumi M., Nambu N., and Nagai T., *Chem. Pharm. Bull.*, **23**, 3062—3068 (1975).