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Reaction of β-Cyclodextrin with N-2,3-Epoxypropylphthalimide. Preparation, Characterisation and Study of a New Substituted Cycloheptaamylose. Effects on the Water Solubility of Drugs

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Abstract. The reaction of β-cyclodextrin with N-2,3-epoxypropylphthalimide yielded a set of new amorphous host compounds with high solubility which was transmitted to the corresponding host-guest complexes. The structure was determined by comparing the ^{1}H- and ^{13}C-NMR spectra with those of the parent compound, the degree of substitution by integration of the corresponding NMR signals, and C, H, and N elemental analysis.

Key words: β-Cyclodextrin (β-CD), epoxides, amorphous.

1. Introduction

Cyclodextrins [1] are cyclic oligomers of glucose arranged in a toroidal macrocycle shape in which no hydroxyl group lies inside the circle of glucose residues and consequently the cavity has decreased polarity comparing to the external part of the molecule [2].

In the hydrated state [3] the cavity is filled with several water molecules which can be replaced by nonpolar molecules with a corresponding gain in energy [4]. The structure [5] and thermodynamics [6] of these host-guest complexes were found to determine the particular properties of solubility or accessibility. This type of compound may be used in drug delivery systems.

In order to increase the water solubility of the cyclodextrins and their complexes, a great number of derivatives have been prepared. Some randomly substituted cyclodextrins [7] can be synthesised by reaction of β-cyclodextrin with different proportions of a reagent; it was thus possible to obtain several macrocyclic com-

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pounds with different distribution of the substituents. This characteristic increases the solubility properties of the host and its complex with a nonpolar compound. In this way the solubility of cyclodextrin is increased because of the absence of crystallinity (like the host–guest complexes) and the bioavailability [8] of the active compounds is consequently increased.

The amorphous character of cycloheptaamyloses is due to the large number of hydroxyl groups in the macrocycle (18, 21 and 24 in the α, β and γ-cyclodextrins respectively). The amorphous derivatives are normally characterised by the substitution degree parameter, which shows the ratio between the number of substituents on each cyclodextrin.

As with other nucleophiles, cyclodextrins, under basic conditions can be condensed with epoxides [7]. The opening of the epoxy ring occurs by nucleophilic attack of the alkoxy anion on the least substituted carbon atom. This mechanism can involve each of the three hydroxyl groups of the cyclodextrin in an extension that depends on the stoichiometry of the reagents. The reaction of several simple epoxides with cyclodextrins has been studied, obtaining the corresponding compound in which one or more hydroxyl group is replaced by OCH_2—CHROH. For example the reaction with ethylene oxide gives a cyclodextrin ether of ethylene glycol. Hydroxypropyl-β-cyclodextrin (HPCD), the condensation product of the reaction of cyclodextrin [9] with propylene oxide, is a commercial derivative that has been widely used in the formation of host–guest complexes. In the present paper we have studied the reaction of β-cyclodextrin with N-2,3-epoxypropylphthalimide. We have isolated and purified seven (**1**–**7**) cyclodextrin derivatives with different degree of substitution and, after analysis, have tested them in the formation of host–guest complexes.

2. Experimental

2.1. Materials

β-Cyclodextrin was purchased from Janssen Biotech and the epoxide from Aldrich Chemicals and both were used as received, without further purification. NMR spectra were obtained with D_2O as solvent and referenced to the H_2O signal (4.8 ppm) using a Bruker AM-400 spectrometer operating at 400.13 MHz for ^1H. The calorimetric analyses were carried out at a heating rate of 5°/min using a Perkin-Elmer DSC-7, coupled to a Perkin-Elmer TAC7/DX thermal analysis controller. The IR spectra were recorded (film or KBr) on a Nicolet 5ZDX Fourier Transform spectrometer. The dialysis tubing was from Spectra-Por (MWCO 1000) with 28.6 mm diameter. The compounds were freeze-dried at $-45\,^{\circ}C$.

2.2. Example of preparation of substituted cyclodextrin

β-Cyclodextrin (2.5 g, 2.2 mmol) was added to a 3M solution of sodium hydroxide (7.5 mL). The suspension was stirred at room temperature until complete disso-

lution. N-2,3-Epoxypropylphthalimide (3.1 g, 15.2 mmol) was added slowly over 2.30 hours. The yellow solution was stirred at room temperature for 16 h. The reaction mixture was neutralized with concentrated HCl and, after addition of 40 mL of water, the solution was dialysed for 4 h using a 15 cm dialysis tube, and freeze-dried for 8 h. The solid obtained was treated with ethanol (250 mL) and stirred for 24 h; the operation was repeated after filtration. The precipitate was dried under vacuum, dissolved in water (5 mL) and freeze dried, yielding 3.8 g of a white solid identified as **1**.

2.3. Example of preparation of host-guest complexes

Cyclodextrin **6** (0.434 g) was added to 2 mL of distilled water and stirred at room temperature until complete dissolution of the macrocycle. Acyclovir (0.025 g) was added and stirred at room temperature overnight. After filtration, the solution was freeze-dried for 4 h yielding 0.455 g of a product containing 4.6% of acyclovir (0.8 mol acyclovir/mol **6**).

3. Results

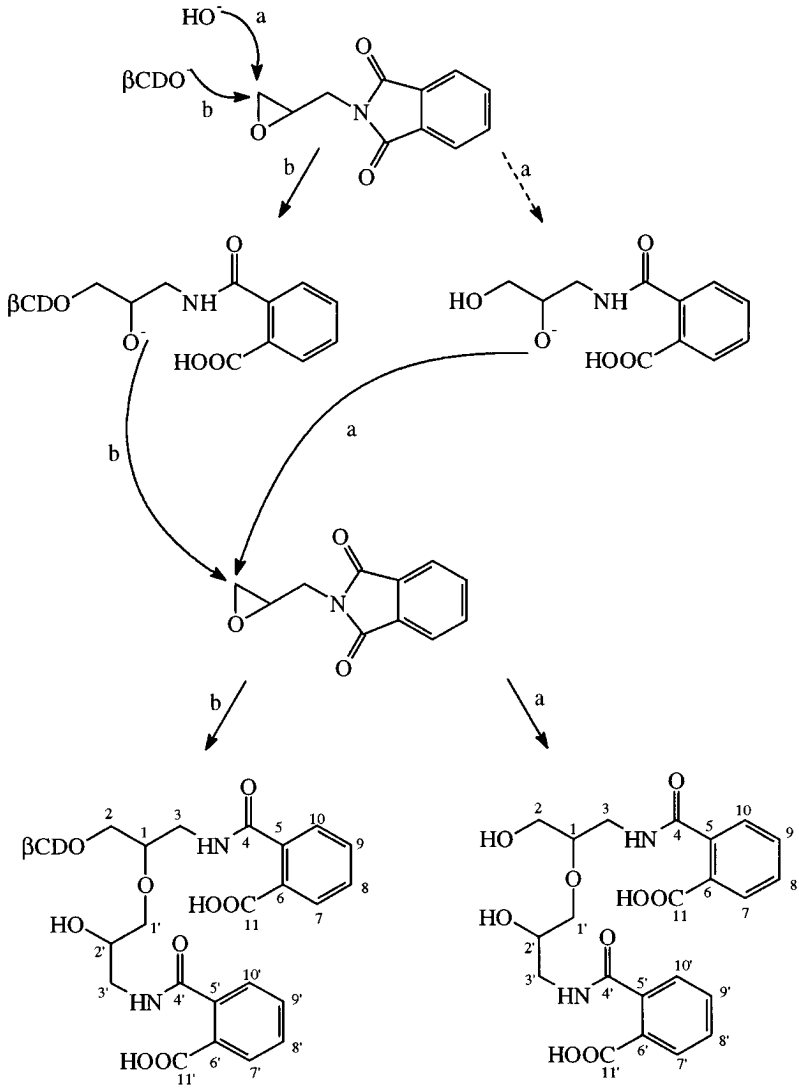
To obtain readily reproducible conditions for the condensation of β-cyclodextrin with N-2,3-epoxypropylphthalimide, a 3M aqueous solution of sodium hydroxide was used as a solvent.

Concomitantly with the condensation, the self-condensation of the epoxide also proceeded, to yield oligoglycols. This polymerisation increased with the final substitution degrees of the cyclodextrins and also with the temperature. At 0 °C no reaction occurred and the added epoxide was recovered.

Two methods were used to purify the compounds obtained: dialysis and ultrafiltration using the appropriate membranes. The first was used in two ways: total (or complete) dialysis for a long time (20 h) or a short dialysis followed by lyophilisation and solid–liquid extraction with ethanol, in which only glycol is soluble. The kinetics of elimination of NaCl was measured by potentiometric control of chloride anion. After 4 h of dialysis the concentration of chloride was less than 0.1%. The absence of epoxide derivatives (dimers or polymers) was monitored until the substitution degree revealed by the ^{1}H NMR spectrum of the lyophilised compound was constant. In the case of extraction, the ethanol was evaporated and the residue was analysed in the same way.

The structure of the cyclodextrin derivative was determined by comparison with the reaction of the same reagents without the β-cyclodextrin. Scheme 1 presents the two reactions and the results obtained.

In this way seven new derivatives of β-cyclodextrin **1**–**7** were obtained, with the structures shown and with different substitution degrees depending on the relation between the β-cyclodextrin and the oxirane. Compound **8**, obtained by the reaction of two molecules of epoxicle under the same conditions but without the partici-



Scheme 1. Reaction of *N*-2,3-epoxypropylphthalimide in basic medium with and without β-cyclodextrin.

pation of the anion of cyclodextrin, was identified as the bis-monophthalamide of (2-hydroxy-3-aminopropyl, 1-aminomethyl-2-hydroxyethyl ether). The degree of substitution was measured from the ^{1}H-NMR spectra, by comparing the integration of the seven anomeric protons of the carbohydrate with that observed in the aromatic region corresponding to the eight aromatic protons of the new function signals. A more accurate value is calculated from the C, H and N elemental analysis (Table II).

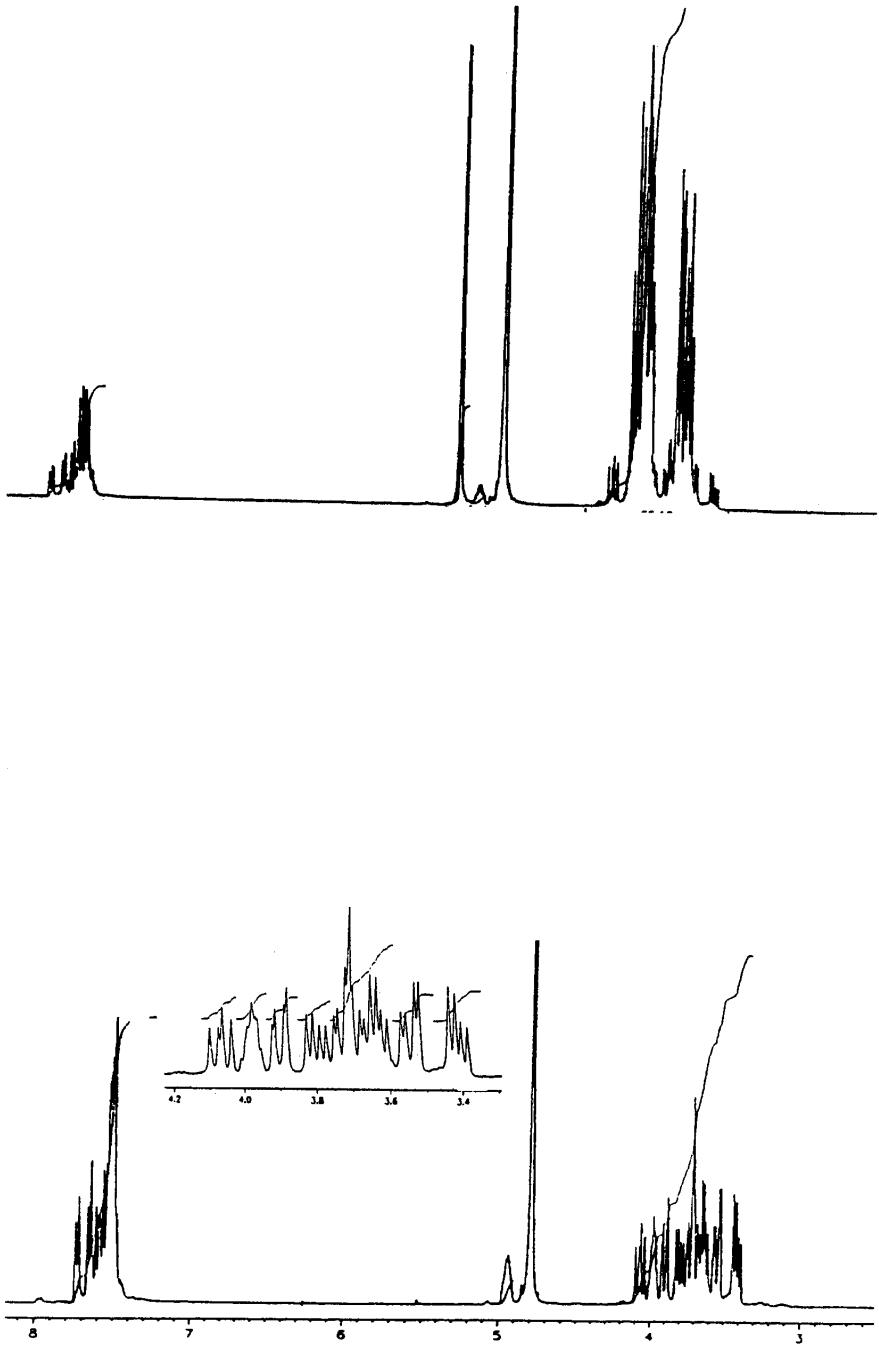


Figure 1. ^{1}H-NMR spectra of compounds **1** (upper) and **8** (lower).

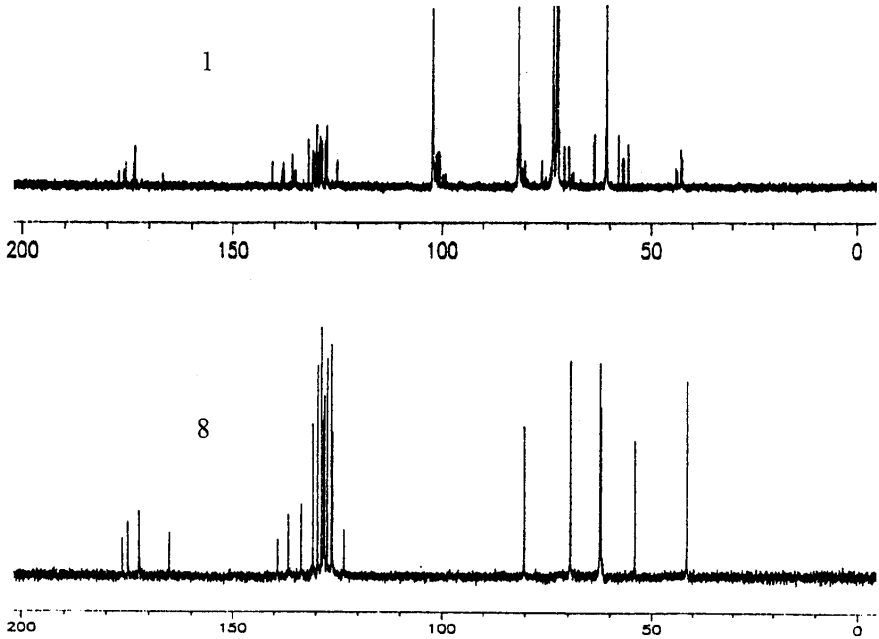


Figure 2. ^{13} C-NMR spectra of compounds **1** and **8**.

The IR analysis of cyclodextrin derivatives shows an increase of the relative intensity of the peaks corresponding to $\nu_{C=O}$ (1650, 1595 and 1560 cm^{-1}) and to ν_{C-N} (1400 cm^{-1}) from **1** to **7**.

Figure 1 shows the ^{1}H-NMR spectra of compounds **1** and **8,** which reveals the similarity of substitution. Moreover, we obtained the ^{13}C -NMR of both compounds (Figure 2) and, considering the assignment of **8** and the statistical distribution of substitution in **1**, we can assign the ^{13}C-NMR of the cyclodextrin derivatives on the basis of the distribution of signals in several regions.

Table I shows the assignment of the spectrum assuming that the absorption of the cyclodextrin derivative should be represented by a family of peaks for each carbon atom instead of only one peak, as in compound **8**.

Table II shows the properties of the compounds obtained and their solubility in water in mg/mL.

4. Formation of Complexes

The host–guest complexes were always prepared in a 1 : 1 molar ratio of modified cyclodextrin (**1**–**7**) and the corresponding compound. The insoluble compound was removed by filtration.

The products were analyzed by integration of the signals corresponding to host and guest in the ^{1}H-NMR spectra, and the absence of free guests was certified

Table I. Assignment of ^{13}C-NMR spectra of compounds **1** and **8**.

Chemical shift of **8**	Chemical shift (regions) of **1**	Assignment in compounds **1** and **8**
41.3	43–44	3
53.9	56–58	3′
–	60–61	C6-CD
62.1, 62.3	61–63	2 1′
69.4	69–71	2′
–	71–72	C5-CD C2-CD
–	73–74	C3-CD
80.1	79–81	1
–	80–81	C4-CD
–	101–102	C1-CD
126.0, 126.2, 127.2, 127.9, 128.1, 128.6, 129.5, 130.6	122–140	7, 8, 9, 10, 7′, 8′, 9′, 10′
133.5, 136.5, 139.1	122–131	5, 6, 5′, 6′
165.1, 172.2	164–172	11, 11′
174.8, 176.2	173–177	4, 4′

Table II. Analysis of cyclodextrin compounds **1**–**7**.

No.	Reactant ratio CD/oxirane/Na(OH)	Degree of substitution	%C analysed	%H analysed	%N analysed	Solubility g/l
1	1/7/10	1.09	48.87	5.85	1.89	52
2	1/7/20	1.80	50.63	5.70	2.60	64
3	1/9/10	3.25	53.13	5.54	3.55	103
4	1/9/20	4.22	53.80	5.40	3.93	214
5	1/14/14	5.22	54.73	5.35	4.25	305
6	1/14/20	6.64	55.47	5.21	4.57	378
7	1/20/20	7.82	55.94	5.08	4.77	410

(using Differential Scanning Calorimetry: DSC) by the absence of the signal corresponding to the melting point of the guest compound.

The products formed are summarised in Table III. In all cases the same complexes with β-cyclodextrin and hydroxypropyl-β-cyclodextrin were also prepared and compared.

The formation of the complex depends not on the nature or the degree of substitution of the macrocycle, but only on the nature of the host, showing that all derivatives have the same cavity in which the guest is accommodated. Compound **6** gave the best results: the encapsulation properties are better than those of β-

Table III. Host–guest molar ratio between several compounds and cyclodextrins **1**–**7**.

Compound	Cyclodextrin								
	1	**2**	**3**	**4**	**5**	**6**	**7**	β-CD	HPCD
Adamantane	0.03	0.01	–	–	–	–	–	–	–
Adamantanone	0.9	0.8	1.0	1.0	1.0	1.0	1.0	0.8	0.7
1-Adamantane carboxylic acid	1	1	1	1	1	1	1	1	1
Norcamphor	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.1	0.2
3-Bromocamphor	0.2	0.3	0.4	0.4	0.4	0.5	0.2	0.3	0.5
Irgasan	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.02	0.3
Acyclovir	0.4	0.5	0.8	1.0	0.8	0.8	0.8	0.4	0.2
L-citronelol	1.0	0.4	0.4	0.4	0.5	0.5	0.6	0.6	0.8
Phenylalanine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9
Boc-phenylalanine	0.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.6
Ibuprofen	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0

cyclodextrin and of the same order as commercial HPCD. The synthesis of the complex of acyclovir is notable.

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