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*Report*

# Amorphous Water-Soluble Cyclodextrin Derivatives: 2-Hydroxyethyl, 3-Hydroxypropyl, 2-Hydroxyisobutyl, and Carboxamidomethyl Derivatives of β-Cyclodextrin

**Tetsumi Irie,[1,2] Kazuhiro Fukunaga,[1,3] Atsuya Yoshida,[2] Kaneto Uekama,[2] Henry M. Fales,[4] and Josef Pitha[1,5]**

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The pharmaceutical usefulness of natural, crystalline cyclodextrins can be improved by chemical conversions into water-soluble, amorphous mixtures of their derivatives. Reaction of β-cyclodextrin with 2-chloroethanol, 3-chloropropanol, isobutylene oxide, or iodoacetamide yielded the title compounds. Distributions of the substitution degree were close to symmetrical and relatively narrow. The average substitution degrees increased with the amount of alkylating reagent used in the preparation. The number of components (half-width of distribution) increased with increasing average substitution degree. Further, distributions of the substitution degree were measured in glucose derivatives after hydrolysis of 2-hydroxyethyl, 2-hydroxypropyl, and 2-hydroxyisobutyl-β-cyclodextrin. The results show an uneven distribution of substitutents around the cyclodextrins, suggesting that growth of oligoglycol side chains and/or clustering of substituents on one glucose residue occurs.

KEY WORDS: amorphous water-soluble cyclodextrin derivatives.

## INTRODUCTION

Conversion of cyclodextrins, which are crystalline, into amorphous mixtures of their chemical derivatives improves their pharmaceutical potential (1–9). In the chemical modifications that have mainly been used, the hydroxy groups of cyclodextrins were alkylated in a nonselective manner. The mixtures thus produced contain many products, a feature which prevents crystallization; nevertheless, the resulting mixtures may retain the ability to form inclusion complexes similar to those of the parent cyclodextrins (2,3). Consequently, when a complex is made of drug and amorphous cyclodextrins, each molecule of drug is encapsulated in a cyclodextrin derivative which cannot crystallize; the encapsulation further prevents the drug from crystallizing on its own.

For chemical modification of cyclodextrins many different reagents and conditions can be used and the products, even when similar in principle, may vary in their specific usefulness. Preparation of pharmaceutical ingredients must be reproducible and the ingredient itself rigorously characterized. In previous work we addressed these problems for all three 2-hydroxypropylcyclodextrins (α, β, γ) and for dihydroxypropylcyclodextrin (1,3,9). These were made by reaction of cyclodextrins with epoxides. In this work we extended the former studies to the characterization of 2-hydroxyethyl, 3-hydroxypropyl, 2-hydroxyisobutyl, and carboxamidomethyl derivatives. The distribution of the substituents among the glucose residues of the cyclodextrin ring was also addressed.

## MATERIALS AND METHODS

### 2-Hydroxyisobutyl-β-Cyclodextrin

Hydrated β-cyclodextrin (33 g, 0.025 mol) was dissolved, while heating to 60°C and stirring, in a solution of sodium hydroxide (10 g, 0.25 mol) in water (75 ml). After cooling the solution to room temperature, a condenser with dry ice–acetone was attached and isobutylene oxide (19.5 g, 0.27 mol) was slowly added while stirring. After another hour at room temperature and 1 hr at 60°C, the solution was neutralized with hydrochloric acid, centrifuged, and dialized against six charges of distilled water for a total of 12 hr. Dialysis membranes from regenerated cellulose with a nominal cutoff of 6000 daltons were used; the problem of the accompanying losses was addressed previously (3). Freeze-drying of the solution yielded 18.8 g of white powder, still containing 6.6% water, which could be removed by drying for 6 hr at 80°C *in vacuo*. Thin-layer chromatography [Merck silica gel sheets, developed with 2-butanone, methanol, and water (70:15:5) and visualized by spraying with 50% sulfuric acid and charring] indicated the total absence of starting β-

[1] National Institutes of Health, NIA/GRC, Baltimore, Maryland 21224.
[2] Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862, Japan.
[3] Kaken Pharmaceutical Co., Ltd., Bunkyo-Ku, Tokyo 113, Japan.
[4] National Institutes of Health, NHLBI, Bethesda, Maryland 20892.
[5] To whom correspondence should be addressed.



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cyclodextrin ($R_F$ of 0.4); the product formed an oblong spot with a $R_F$ of 0.5–0.8.

### 2-Hydroxypropyl-γ-Cyclodextrin

γ-Cyclodextrin in a solution of aqueous strong alkali was treated with propylene oxide as described above.

### 2-Hydroxyethyl- and 3-Hydroxypropyl-β-Cyclodextrins

Postassium hydroxide (0.62 mol) was dissolved in distilled water (125 ml) and β-cyclodextrin (0.0705 mol) was added. 2-Chloroethanol or 3-chloropropanol (ranging from 0.212 to 1.41 mol) was added dropwise to the stirred solution at 70–80°C over a period of an hour and then the solution was stirred for an additional 4 hr at the same temperature. After cooling to room temperature the solution was neutralized with concentrated hydrochloric acid solution (~14 ml) and activated charcoal powder (1.6 g) was added. After filtration the clear solution was concentrated and coevaporated with ethanol (200–300 ml) *in vacuo*. The resulting solid was treated with *N,N*-dimethylformamide (250 ml) and the undissolved potassium chloride was filtered off. The filtrate was added dropwise to acetone (2000 ml) while stirring for an hour at room temperature. The precipitate was washed with acetone and dried at 70–80°C *in vacuo*.

### Carboxamidomethyl-β-Cyclodextrin

Cyclodextrin, sodium hydroxide, and alkylating agent, i.e., iodoacetamide in this case, were used in the same molar proportions as in the preparation of 2-hydroxyisobutyl-β-cyclodextrin. The reaction temperature was 80°C for 1 hr and then room temperature overnight. After dialysis and concentration of the solution by evaporation *in vacuo*, the product was precipitated by methanol. From 30.6 g of the starting β-cyclodextrin, 19.7 g of whitish powder was obtained, containing 40.2% carbon, 5.9% hydrogen, and 3.5% nitrogen. When calculated from the mass spectrum (cf. Results), the composition was 42.0, 6.1, and 2.6%, respectively (after correction for 3% humidity).

### Hydrolysis of 2-Hydroxyalkyl-β-Cyclodextrins

The cyclodextrin derivative (500 mg) was dissolved in hydrochloric acid (1 *N*, 10 ml) and kept for the length of time indicated in Table I in a stoppered test tube at 60°C. The reaction mixture was then neutralized with a solution of sodium hydroxide, filtered, and freeze-dried. The remaining white powder was used directly for mass spectrometry analysis.

### Mass Spectra

A plasma desorption mass spectrometer, designed for NHLBI by Dr. R. D. Macfarlane (Texas A&M University, College Station), was used. In this instrument radiation from Cf-252 is used for ionization of the underivatized sample; ions are formed from neutral molecules of the compound and ambient sodium or potassium ions. The average substitution degree was calculated as Σ(peak height × substitution degree)/Σpeak height. The pharmaceutical applications of the method used were recently reviewed (10).

## RESULTS

The reaction of β-cyclodextrin with various amounts of 2-chloroethanol, in strong aqueous alkali, yielded products in which the distribution of substitution was symmetrical and narrow: a representative mass spectrum is shown in Fig. 1. The average substitution degree could be easily regulated by the molar ratio of the components (Fig. 2). The half-width of distribution curves increased with the average substitution degree in a linear manner (Fig. 3).

The reaction of β-cyclodextrin with 3-chloropropanol yielded 3-hydroxypropyl-β-cyclodextrins. The representative distribution is shown in Fig. 1. Results on the dependence of the average substitution degree on the ratio of the reagents (Fig. 2) and its effects on the half-width of the distributions (Fig. 3) were similar to the above.

The reaction of β-cyclodextrin with propylene oxide, yielding 2-hydroxypropyl-β-cyclodextrin, was investigated previously (3). Evaluation of previous and present data indicates also that the average substitution degree increases with the amount of propylene oxide added (Fig. 4). The same type of substitution, when performed on γ-cyclodextrin, yielded similar products (1,7). Also, the half-width of distribution of the presently made preparations depended linearly on the average substitution degree (Fig. 3).

The reaction of β-cyclodextrin with isobutylene oxide,

**Table I.** Percentile Composition of Hydroxyalkylglucoses Obtained by Hydrolysis of Hydroxyalkyl-β-Cyclodextrins[a]

| Cyclodextrin derivative | Average degree of substitution per cyclodextrin | (2-Hydroxyalkyl)glucose | | | |
|---|---|---|---|---|---|
| | | Mono | Bis | Tris | Tetrakis |
| 2-Hydroxypropyl-β-cyclodextrin | 6.2 | 34[b] | 33 | 18 | 15 |
| | | 31[c] | 35 | 22 | 12 |
| 2-Hydroxyethyl-β-cyclodextrin | 5.0 | 60[c] | 31 | 12 | — |
| 2-Hydroxyisobutyl-β-cyclodextrin | 4.3 | 69[c] | 31 | — | — |

[a] Due to the decomposition and/or fragmentation of unsubstituted glucose, amounts of that could not be reliably quantitated.
[b] Hydrolysis for 4 days.
[c] Hydrolysis for 7 days.

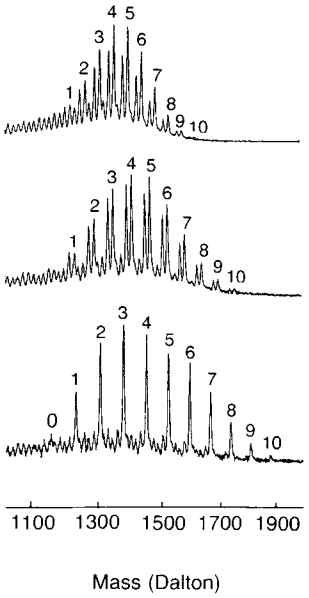


Fig. 1. Distribution of the substitution degree in preparations of (top to bottom) 2-hydroxyethyl-β-cyclodextrin, 3-hydroxypropyl-β-cyclodextrin, and 2-hydroxyisobutyl-β-cyclodextrin. The number above the peaks denotes the number of substituents per cyclodextrin; the height of the peak is a measure of the relative representation of that species. The major peaks in the doublets of the above two spectra belong to molecular ions containing potassium; the minor peaks belong to sodium species. In the bottom spectrum, only peaks of sodium species are visible.

under similar conditions, can yield principally two products, [HO–C($CH_3$)$_2$–$CH_2$–] or [HO–$CH_2$–C($CH_3$)$_2$–] ethers of cyclodextrins. On the basis of steric factors the former products, 2-hydroxyisobutyl ethers, are strongly favored (11). The molar ratios of sugar residues (anomeric protons) to the substituents (methyl protons) in the corresponding product were measured by nuclear magnetic resonance (compare Ref. 3) and gave an average substitution degree of 4.1 for the preparation (Fig. 1). By mass spectrometry (Fig. 1) the average substitution degree was 4.3.

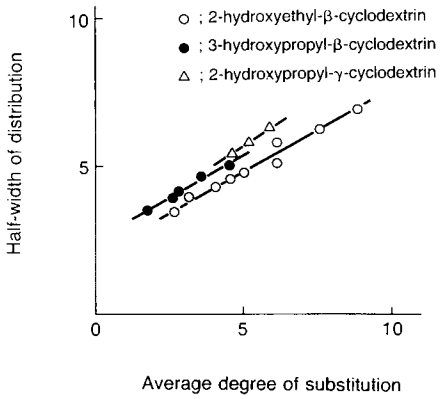


Fig. 3. Dependence of the half-width of distribution on the average substitution degree for preparations of 2-hydroxyethyl, 3-hydroxypropyl-β-cyclodextrin and 2-hydroxypropyl-γ-cyclodextrin.

Reaction of iodoacetamide with cyclodextrin was used to prepare carboxamidomethyl-β-cyclodextrin. The solubility and physical properties of that product were similar to those of the other amorphous cyclodextrins. The mass spectrum of the product had two series with an interval of 57 units. A major series of bands stemmed from the expected carboxamidomethyl derivatives of β-cyclodextrin. The average substitution degree was 2.8 and the half-width was 4.0; this series amounted to 60% of the total. The minor series (40%) are carboxamidomethyl derivatives of the sodium salt of monocarboxymethyl-β-cyclodextrin, obviously a hydrolysis product of the former series. The average substitution degree was 2.2 and the half-width was 3.3.

Mass spectrometry may also be used to study the distribution of the substituents around glucose residues of the derivatized cyclodextrins. This was achieved by acid hydrolysis of the derivatized cyclodextrin and evaluation of the substitution of the resulting hydroxyalkylglucoses by mass spectrometry. The peaks of various hydroxyalkylglucoses were distinct and could be used for quantitation. The composition found did not change upon prolongation of hydrolysis (Table I). The peaks of unsubstituted glucose and its

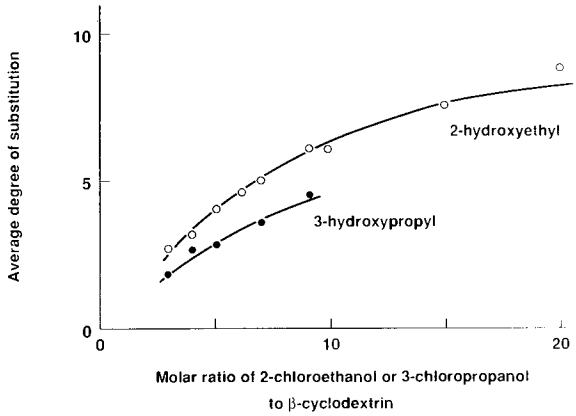


Fig. 2. Dependence of the average substitution degree on the ratio of reagent to β-cyclodextrin which was used in the preparation. Logistic curves were fitted by the least-squares method through the data points.

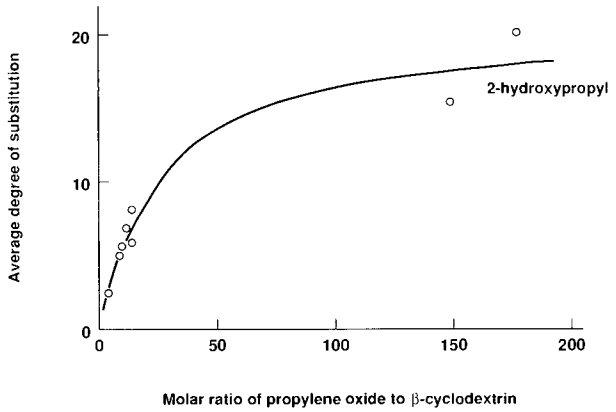


Fig. 4. Dependence of the average substitution degree on the ratio of propylene oxide to β-cyclodextrin. The logistic curve was fitted by the least-squares method through the data points.

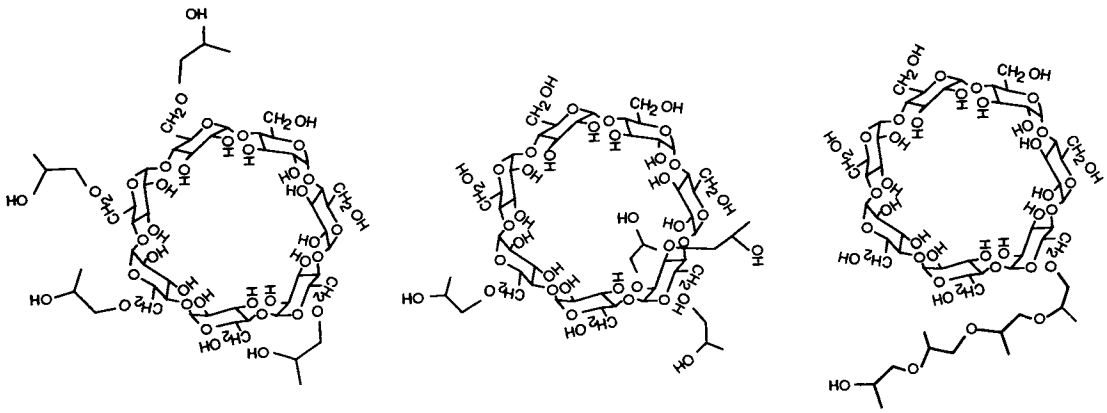

Fig. 5. Schematic representation of three types of substituent distributions in 2-hydroxypropyl-β-cyclodextrin. In this illustration three of the numerous tetrasubstituted species are depicted.

fragments which could be identified were less pronounced; obviously some decomposition of glucose occurred during the hydrolysis and/or in the measurement and thus this component was not included in the quantitation.

## DISCUSSION

Altogether, β-cyclodextrin has 21 hydroxylic groups, of three different categories, and any of these groups can react with an epoxide or chloroalcohol. This reaction introduces a hydroxyalkyl substituent into the cyclodextrin, and thus while the total number of hydroxyls in the molecule is constant, the reactivity type of the hydroxy groups changes as the reaction proceeds. Considering the complexity of the situation, it is obvious that the mixtures formed in such reactions must be nearly as rich in the number of components as polymers.

The distribution of molecular weights in products could be exactly measured using soft ionization mass spectrometry (3,10). The reliability of that approach was checked by the coincidence of the average degree of substitution, as measured by nuclear magnetic resonance or as calculated from mass spectra (3). Since in the former method no volatilization of the sample is involved, the coincidences observed assure that the results of mass spectrometry are not biased toward the more volatile compounds.

Considering the possible differences in reactivities of various hydroxy groups, it is remarkable that all the distributions measured are relatively narrow and not greatly different from symmetrical. Furthermore, there is no obvious qualitiative difference in the distributions of chemical substitutions which (a) increase the total number of hydroxyls (data in Ref. 9), (b) leave that number unchanged (present data on reaction with epoxides or chloroalcohol), and (c) decrease the total number of hydroxyls in the molecule (present data on reaction with iodoacetamide).

Mass spectrometry may be of further use to characterize the substituent distribution to the glucose units of cyclodextrin. There are three principal structural types of hydroxyalkyl cyclodextrins (Fig. 5). The substituents may be distributed about equally between the glucoses of cyclodextrin (left panel), clustered on one glucose (middle panel), or form an oligomeric chain growing from the cyclodextrin (right panel). The first of the principal types was considered the most probable since the reactivity of primary hydroxyls of cyclodextrins is so pronounced that a number of selective substitutions into those positions were achieved (12). The present results on the distribution of the substitution degree of hydroxyalkylglucoses leave no doubt that the second and/or third principal types were highly represented in the products. If all the hydroxyls in 2-hydroxypropyl-β-cyclodextrin had the same reactivity, the probability of further substitution on an already substituted glucose residue is only one in seven. Thus, the relative representation of mono-, di-, and trisubstituted derivatives should be about 85, 12, and 2%, respectively. When this compound, prepared with an average substitution degree of 6.2 (i.e., containing an average of 0.9 substituent per glucose unit), was hydrolyzed, the amount of mono- and disubstituted glucoses was about 33 and 34%, respectively. Obviously, substitution of a single glucose unit was favored also in 2-hydroxyethyl- and 2-hydroxyisobutyl-β-cyclodextrin (Table I).

Hydroxyalkyl derivatives of polysaccharides have been produced in industrial quantities and intensively studied. Only very recent data are indicative of substituent clustering toward a single saccharide residue or the growth of side chains of oligoglycols (13–18). The cause of the latter growth may be the steric hindrance to chemical reactions exerted by the chains of high molecular linear polymers (19). Perhaps the cyclodextrins, by not having end groups, resemble in reactivity the internal part of chain linear polymers.

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