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(54) Title: REGIOSELECTIVE SUBSTITUTIONS IN CYCLODEXTRINS

(57) Abstract

A process for preparing regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins wherein the substitution is either directed toward hydroxyls 2 or 2,3 of the glucose units with little substitution on hydroxyl 6 or toward hydroxyls 6 and with little substitution on the secondary hydroxyls. The regiospecificity is obtained through the proper control of basicity of the reaction mixtures which are comprised of epoxide and cyclodextrins and a suitable solvent.

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REGIOSELECTIVE SUBSTITUTIONS IN CYCLODEXTRINS

The development of procedures which would yield mixtures of cyclodextrin derivatives in which substitution at either the wide or narrow side of the toroid would be predominant was desired. Such a specific pattern of substitution has not been thought to be realizable by simple means, i.e., using cheap reagents without fractionation of the product. Nevertheless, that has been accomplished and here we disclose that by proper selection of preparative conditions mixtures of cyclodextrin derivatives with a specific pattern of substitution can be obtained. That discovery was made possible through a detailed analysis of cyclodextrin mixtures. That analysis, in conjunction with a fortuitous choice of reaction conditions, is the basis of the present invention. It should be noted that reagents and reaction conditions similar to those previously used by us and others have been employed. The novelty is the finding that there exist regions of reaction conditions which previously were not used and in which mixtures of cyclodextrin derivatives with unique substitution patterns are obtained; furthermore, these patterns are only slightly affected by the overall degree of substitution. That finding may be of importance since on its basis mixtures of cyclodextrin derivatives can be taylored for uses where recognition of a specific guest compound by a host is desired.

The usefulness of those derivatives of polysaccharides which assume random coil conformation depends primarily on their average degree of substitution and is only slightly affected by the differences in substitution patterns. Polysaccharide derivatives with an ordered conformation and derivatives of cyclic oligosaccharides (e.g. α-, β- or γ-cyclodextrins), which are *de facto* ordered by the presence of a cycle, present a different problem; there the substitution pattern may prefoundly affect their usefulness. The shape of cyclodextrins is a toroid : on the narrower side of the toroids are located all primary hydroxyls (-CH_2-OH) and on the wider sides are the secondary hydroxyls. Thus, substitution on secondary hydroxyls puts the substituents close to the wider entry of the cavity of the toroid, whereas substitutions on the primary hydroxyls close to the narrower entry. The principal use of cyclodextrins is in inclusion complexation : a guest lipophilic compound is accepted into the toroidal cavity of the host compound, i.e., of the cyclodextrin. This process is bound to be affected by specific changes at the entry sites of the host molecule. That was well demonstrated using chemically pure cyclodextrin derivatives. These compounds were prepared by multi-step synthesis

requiring multiple extensive purifications and thus are available only in small quantities and at a great price. In many applications the chemical purity (individuality) of cyclodextrin derivatives is not required or may even be of a detriment. Using mixtures of cyclodextrins is often preferred since these usually do not crystallize and thus have much higher solubilities and are also better suited as coatings.

Cyclodextrins, such as α-, β- or γ-cyclodextrins, similarly to other carbohydrates, react with epoxides yielding mixtures of oligosubstituted hydroxyalkylcyclodextrins. The latter compounds were first disclosed U.S. Patent 3,459,731. These cyclodextrins were found eminently useful for pharmaceutical purposes and this use was disclosed in U.S. Patent 4,596,795, U.S. 4,727,064, U.S. Patent 4,870,060, U.S. Patent 4,764,604, Eur. Patent No. 149,197, Int. J. Pharm. 26, 77, 1985, J. Pharm. Res. 309, 1985 and J. Pharm. Sci. 75, 571, 1986. Hydroxyalkylcyclodextrins were also prepared by reaction of cyclodextrins with ethylene or propylene carbonate catalyzed by potassium carbonate; R.B. Friedman, Modified Cyclodextrins, abstract B6 of the 4th International Symposium on Cyclodextrins, April 1988, Munich, West Germany. Furthermore, preparation of mixed alkyl and hydroxyalkylcyclodextrins was the subject of two patent applications, namely Eur. Patent Appl. EP 146,841 and EP 147,685. The multicomponent mixtures of hydroxyalkylcyclodextrins could be characterized using mass spectrometry, as far as number of substituent per cyclodextrin is concerned. Each of the peaks in such a spectrum corresponds to certain degree of substitution, but since there is a great number of possible isomeric compounds at any degree of substitution, the mixtures are only partially characterized by direct mass spectrometry. An advance in characterization was obtained by hydrolysis of hydroxypropylcyclodextrin mixtures and evaluation of the hydroxypropylglucose mixtures thus obtained by mass spectrometry (Pharmaceut. Res. 5,713-717, 1988). These results show that the substituents in hydroxypropylcyclodextrins are not evenly distributed between the glucose residues. A large number of hydroxyalkylcyclodextrins has been prepared and characterized in this manner and the average degree of substitution was found to depend primarily on the ratio of reagents used. These quite diverse reaction conditions yielded mixtures with a rather similar distribution of degree of substitution (Int. J. Pharm. 29 : 73-82, 1986; Pharmaceut. Res. 5 : 713-717, 1988). Consequently, the reaction conditions (i.e., strength of alkali added) were chosen primarily on the basis of convenience of manipulation of the mixtures. In different protocols (Int. J. Pharm. 29 : 73-82, 1986; Pharmaceut. Res. 5 : 713-717, 1988) the concentration of sodium hydroxide solution, which is used as a solvent for the other component, ranged between 5-17% w/w preferably about 11% w/w. At concentrations lower than these the reaction proceeds sluggishly; at higher

concentrations the solubility of β-cyclodextrin decreases and also the removal of sodium hydroxide after the reaction becomes tedious. Thus, in production of hydroxyalkyl-cyclodextrins the practical range of the concentrations of sodium hydroxide solution used as a solvent were 5-17% and there was no incentive to venture outside of this range.

The aim of the present invention is to provide a process enabling the attainment of a desired pattern of substitution by hydroxyalkyl groups onto α-, β- or γ-cyclodextrins through the control of basicity of the reaction mixtures which are comprised of epoxide and cyclodextrins and a suitable solvent. It was found that through the proper control of basicity the substitution may be directed either toward the wide or the narrow opening of the cavity of cyclodextrins i.e. (1) toward hydroxyls 2 or 2,3 of glucose residues with little substitution on hydroxyl 6, or (2) toward hydroxyl 6 and with little substitution on the secondary hydroxyls 2 and 3. In aqueous media the basicity of the reaction mixtures required for said regiospecificity may be obtained by a decrease or an increase of the previously used concentration range (5-17%) of sodium hydroxide solution, which is used as a reaction solvent for other components of the reaction mixtures. These concentrations represent typically less than 2.5% or more than 10.5% of sodium hydroxide content in the fully assembled reaction mixtures. In non-aqueous media the desired basicity may preferably be obtained using sodium methylsulfinylmethanide as a base and dimethyl sulfoxide as a solvent. It is however understood that other organic solvents or bases may be applied. The above method may also be applied for the preparation of mixtures of hydroxyalkylcyclodextrins which vary in their average degree of substitution, but in which the pattern of substitution is not changed.

A further aspect of the invention is to provide regiospecific hydroxyalkylated α-, β- or γ-cyclodextrins wherein the substitution is mainly on the hydroxyls 2 or 2,3 of the glucose residues with little substitution on hydroxyl 6, or wherein the substitution is mainly on the hydroxyl 6 with little substitution on the secondary hydroxyls 2 and 3, and fully or partly alkylated derivatives of these regiospecific hydroxyalkylated α-, β- or γ-cyclodextrins. Particular hydroxyalkylcyclodextrins substituted mainly on the wide side of the cavity have a relative distribution of the substitution on the 2 hydroxyl groups versus the 6 hydroxyl groups which varies from about 2:1 to about 20:1, preferably from about 5:1 to about 20:1, or from about 10:1 to about 15:1. Particular hydroxyalkylcyclodextrins substituted mainly on the narrow side of the cavity have a relative distribution of the substitution on the 6 hydroxyl groups versus the 2 hydroxyl groups from about 1.5:1 to 20:1, preferably from about 2.5:1 to 20:1, or from about 3:1 to about 15:1.

Still a further aspect of the invention is to provide mixtures comprising the above regiospecific hydroxyalkylated α-, β-, or γ-cyclodextrins.

In the foregoing definitions the term "hydroxyalkyl " defines bivalent straight or branch chained hydrocarbon radicals containing form 1 to 6 carbon atoms such as hydroxyethyl, hydroxypropyl or hydroxyisobutyl groups.

Since a hydroxy moiety of the cyclodextrin can be substituted by a hydroxyalkyl unit which itself can be substituted with yet another hydroxyalkyl unit, the average molar substitution (M.S.) is used as a measure of the average number of alkylated hydroxy functions per mole of glucose unit. Particular cyclodextrins according to the present invention have a M.S. which is in the range of 0.125 to 10, in particular of 0.3 to 3, or from 0.3 to 1.5. The average substitution degree (D.S.) expresses the average number of substituted hydroxyls per glucose unit. Particular cyclodextrins according to the present invention have a D.S. which is in the range of 0.125 to 3, in particular of 0.2 to 2, or from 0.2 to 1.5.

Hydroxyalkylated α-, β- or γ-cyclodextrins according to the present invention are prepared by an alkali catalyzed reaction of epoxides with cyclodextrins in a suitable solvent preferably at a temperature between 0 to 100°C, or between 0 to 70°C. A suitable solvent for carrying out the process of the invention is an aqueous alkali metal hydroxide solution. As the alkali metal hydroxide used may be mentioned lithium hydroxide, barium hydroxide, sodium hydroxide and potassium hydroxide. Of these, sodium hydroxide is preferable. The concentration of the sodium hydroxide solution which is used as a reaction solvent for other components of the reaction mixtures is either lower than 5% (w/w), preferably lower than 4% (w/w), or higher than 17%(w/w), preferably higher than 18%(w/w). In some instances, equinormal lithium, potassium or barium hydroxide solutions may also be applied. These concentrations represent typically less than 2.5% or more than 10.5% of alkali metal hydroxide content in the fully assembled reaction mixtures. The molar ratio of alkali metal hydroxide versus cyclodextrin should preferably be in the range of 0.5 to 3.5, more in particular less than 2.5, or should be in the range of 10 to 80, more in particular more than 13.8. The epoxide concentration in the final mixture may vary from about 1% to about 30%, more in particular from about 2% to about 20%. Particular samples of hydroxypropylated β-cyclodextrin were prepared by reacting β-cyclodextrin with propylene oxide in aqueous sodium hydroxide (Examples 1-7). The reaction conditions used in these preparations are summarized in Table I.

Table I.

Summary of preparative Conditions of Hydroxypropyl-β-cyclodextrins							
	Examples						
	1	2	3	4	5	6	7
sodium hydroxide solution used as a solvent (%w/w)	16.9%	17.5%	5.7%	1.5%	4.8%	17%	30%
Final reaction mixture (%w/w)							
sodium hydroxide	10.4%	10.5%	2.9%	1.1%	2.7%	10.3%	23.4%
cyclodextrin (anhydrous)	29.6%	21.3%	28.6%	15.1%	23.0%	21.3%	11.6%
propylene oxide	4.0%	15.4%	14.4%	10.9%	16.6%	15.4%	8.4%
Final reaction mixture (molar ratio)							
sodium hydroxide / cyclodextrin	10.0	13.9	2.9	2.1	3.4	13.8	57.2
propylene oxide / cyclodextrin	2.6	14.1	9.8	14.3	14.3	14.3	14.1

A suitable solvent for carrying out the present invention may also dimethyl sulfoxide, N,N-dimethylformamide, dioxane or mixtures thereof with water in the presence of a base. It is however understood that other organic solvents or bases may be applied. In the preparation described in Example 8 anhydrous conditions were used with sodium methylsulfinylmethanide in dimethylsulfoxide as catalyst and solvent, respectively. Pure regiospecific hydroxypropylated cyclodextrin may be isolated from the mixtures by removal of the unreacted starting material by art known procedures such as, extraction with organic solvents, adsorption chromatograpy, selective crystallization and combinations of these techniques.

In order to determine the distribution of substituents between the different positions in the α-D-glucopyranosyl residues of β-cyclodextrin each product was permethylated (Example 9), hydrolysed, and the resulting glucose ethers reduced, acetylated, and analyzed as alditol acetates, by gas liquid chromatography (Example 10).

There are several points to be clarified before the results are evaluated. Etherification with an epoxide such as propylene oxide is a complicated reaction. When racemic propylene oxide is used, diastereomeric ethers are formed, which are only partially separated by the analytical method used. In order to fully address this complication three examples (Examples 1-3) were prepared using racemic propylene oxide, whereas in Examples 4-8 (S)-propylene oxide was used, which is bound to yield a simpler pattern. Another complication is that the oxiran ring in propylene oxide can be opened either by attack on 0-1, which is the predominating reaction and gives a 2-hydroxypropyl ether, or on 0-2, giving a 2-(1-hydroxypropyl)ether. Two derivatives of the latter type were observed in the present study. The third type of complication is due to the introduction of additional hydroxyls by the substituent. Fortunately, the secondary hydroxyl of the 2-hydroxypropyl group should not be very reactive, and alkylation in this position should consequently not be very important. Nevertheless, small amounts of such derivatives were observed. The results of the analyses are summarized in Table II.

Conventional abbreviations were used, e.g., S_2 denotes mono-substitution on 0-2, S_{226} denotes bi-substitution on 0-2 (by $-CH_2-C(CH_3)H-O-CH_2-CH(OCH_3)-CH_3$ group) and mono-substitution on 0-6; glucose-derived numbering was used for alditols. In some analyses under methylation, especially in the 3-position, was observed. The products, however, were identified from their mass spectra, and the molar percentages added to those of the corresponding fully methylated components. Two 2-(1-methoxypropyl) ethers were observed with this group in the 2- and the 6-position of a glucosyl residue, respectively. The yields of these ethers were 2-4% of the corresponding 1-(2-methoxypropyl)ethers, and reflects the relative reactivities at the primary and the secondary position of propylene oxide, respectively.

Table II

Composition of Alditol Acetates in Mole % obtained from various 2-hydroxypropyl-β-cyclodextrin Preparations								
	Examples							
Substitution pattern by 2-methoxypropyl groups	1	2	3	4	5	6	7	8
S_0	77.8	43.9	39.3	74.4	40.2	42.9	53.2	65.5
S_0 non-methylated on 0-3	-	-	-	-	2.8	2.2	-	-
Total non-substituted	77.8	43.9	39.3	74.4	43.0	45.1	53.2	65.5
S_2	5.2	10.9	30.3	14.6	23.0	8.4	3.1	2.3
S_2 non-methylated on 0-3	-	-	-	-	0.6	0.2	-	-
S_2 2-(1-methoxypropyl)-	-	-	-	-	0.6	-	-	-
S_3	2.7	5.2	5.4	4.8	6.1	3.0	1.4	0.9
S_3 non-methylated on 0.6	-	-	-	-	0.5	-	-	-
S_6	12.5	23.6	3.8	2.6	7.0	26.4	33.0	23.3
S_6 non-methylated on 0-3	-	-	-	-	0.5	1.5	-	-
S_6 2-(1-methoxypropyl)-	-	-	-	-	-	0.6	-	-
Total non-substituted	20.4	39.7	39.5	22.0	38.3	40.1	37.5	36.5
S_{23}	0.6	3.9	14.3	2.2	8.9	2.8	0.7	-
S_{26}	0.9	7.5	3.7	0.9	5.2	6.4	1.9	1.8
S_{26} non-methylated on 0.3	-	-	-	-	0.7	-	-	-
S_{36}	0.3	2.3	1.4	0.5	1.6	2.2	0.9	-
S_{66}	-	-	-	-	-	0.3	4.7	6.0
Total disubstituted	1.8	13.7	19.4	3.6	16.4	11.7	8.2	7.8
S_{226}	-	-	-	-	-	0.2	0.2	-
S_{236}	0.1	2.7	1.7	-	2.4	2.3	0.7	-
S_{266}	-	-	-	-	-	0.5	0.4	-
S_{666}	-	-	-	-	-	-	0.2	-
Total trisubstituted	0.1	2.7	1.7	0	2.4	3.0	1.5	0

The relative reactivities at the three different positions in the α-D-glucopyranosyl groups may be determined from the molar percentages of the ethers. Sperlin equations (H.M. Sperlin in E. Ott, H.M. Sperlin and M.W. Grafflin (Eds.) Cellulose and Cellulose Derivatives, Part II, Interscience, New York, 1954, pp. 673-712) were used to determine the relative reactivities, k_2, k_3 and k_6, from the distribution of the substituents. The results in Table II can thus be reduced to those three parameters (Table III). The value for k_2 and k_3 there concern the relative reactivities when the other hydroxyl is not alkylated. Further calculations indicate that these reactivities are considerably enhanced when the other hydroxyl becomes alkylated, in particular the substitution on 0-3 increases the reactivity of 0-2 hydroxyls.

Table III

Relative Reactivities at the 2,3- and 6-Positions and Average Degree of Substitution Values for the Different 2-Hydroxypropyl Ethers of β-cyclodextrin					
				Average Degree of Substitution	
Example	propylene oxide	%NaOH[a]	k_2:k_3:k_6	From mole % of ethers	From m.s.
1	(RS)	16.9	1 : 0.43 : 2.1	1.7	2.5
2	(RS)	17.5	1 : 0.40 : 1.6	5.3	6.8
3	(RS)	5.7	1 : 0.15 : 0.12	5.8	6.6
4	(S)	1.5	1 : 0.36 : 0.08	2.0	3.4
5	(S)	4.8	1 : 0.27 : 0.32	5.5	6.0
6	(S)	17.0	1 : 0.28 : 2.2	5.2	5.8
7	(S)	30.0	1 : 0.41 : 7.6	4.0	5.2
8	(S)	b	1 : 0.17 : 8.3	3.0	-

a Concentration of aqueous sodium hydroxide solution (w/w) used as solvent for the other reaction components.

b Sodium methylsulfinylmethide in dimethyl sulfoxide

From the results given in Table III it is evident that the relative reactivities at 0-2 and 0-3 are rather independent of the alkali concentration during the etherification. The relative reactivity of 0-6 versus 0-2, however, varies from approximately 1:5 at low alkali concentration to 7:1 at high alkali concentration. For the reaction promoted by sodium methylsulfinylmethanide in dimethyl sulfoxide, the alkylation in the 6-position is even more favored. These drastic changes in the reactivity of 0-6 are the basis for the

regiospecificity observed at extremely low or high alkali concentrations, a phenomenon which is the subject of the present invention.

The thus prepared regiospecific hydroxyalkylated cyclodextrins may also be derivatized with an alkylating agent to obtain fully or partly substituted mixed ethers. The alkylation reaction may be carried out with appropriate alkylating agents such as alkylsulfates or alkylhalogenides in a base, liquid reaction medium containing an alkali metal hydroxide, water and, optionally, at least one organic solvent such as, for example, dimethoxy-ethane or isopropanol. In this regard, it is important to point out that if a regiospecific substitution is followed by a non-specific one even the latter acquires a measure of regiospecificity.

The following examples are intended to illustrate and not to limit the scope of the present invention in all its aspects.

Example 1

Preparation of hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (200 g of hydrate corresponding to 173.2 g anhydrous and 0.153 moles) was dissolved with stirring in warm (60°C) solution of sodium hydroxide (61.2 g or 0.53 moles in 300 ml of distilled water, i.e., 16.9% w/w). The solution was placed into round flask, cooled to ice bath temperature and after attachment of reflux condenser containing dry ice-acetone mixture, propylene oxide (25 ml, 23.2 g, 0.40 moles) was added dropwise with constant stirring. Stirring was continued for 3 hours at ice bath temperature and overnight at room temperature. Then the mixture was neutralized with concentrated hydrochloric acid and evaporated in vacuo to a consistency of thick syrup, which was added to 1 l of ethanol (190 proof). After several hours of stirring the insoluble sodium chloride was filtered off, washed with ethanol (190 proof, 200 ml). The ethanolic solutions were evaporated in vacuo, residue dissolved in distilled water (300 ml) and dialyzed for 5 hours at 0°C against several charges of distilled water. The retained fraction was freeze-dried and the resulting powder stirred with acetone (1.5 l) for one day. The acetone was decanted and residue stirred with an additional acetone (1 l) again for one day and the precipitate of hydroxypropyl-β-cyclodextrin filtered off and dried for 2 hours in vacuo. Acetone solutions upon evaporation yielded oily residue (3g) principally oligopropyleneglycols. The dried powder of hydroxypropyl-β-cyclo-dextrin was dissolved in distilled water (300 ml) and the solution freeze-dried to yield a white powder (98g).

Example 2

Preparation of hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (200 g hydrate, i.e., 173 g anhydrous, 0.153 moles) was, as above, dissolved in a solution of sodium hydroxide (85 g, 2.12 moles in 400 ml distilled water, i.e., 17.5% w/w) and in the same manner as above treated with propylene oxide (150 ml, 125 g, 2.152 moles). Using processing analogous to that above a fraction of oligopropylene glycols amounted to 38 g while altogether 193 g of hydroxypropyl-β-cyclodextrin was obtained.

Example 3

Preparation of hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (500 g hydrate, i.e., 432 g anhydrous, 0.382 moles) was, as above, dissolved in a solution of sodium hydroxide (45 g, 1.1 moles in 750 ml distilled water, i.e., 5.7% w/w) and under the same conditions as above treated with propylene oxide (260 ml, 217 g, 3.73 moles). The reaction mixture was left for five hours in an ice bath and kept at room temperature for two days. After processing similar to that described above and including extraction of oligopropylene glycols with acetone a white powder of hydroxypropyl-β-cyclodextrin (490 g) was obtained.

Example 4

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (13.3 g of hydrate, i.e., 11.5 g anhydrous, 0.010 moles) was dissolved in a solution of sodium hydroxide (0.822 g, 0.0206 mol in 54 ml distilled water, i.e., 1.5%) by stirring at 60°C. The increased amount of alkaline solution used was necessitated by the low solubility of β-cyclodextrin at very low (present case) or very high (30%) concentration of sodium hydroxide. The solution was cooled in an ice bath and in the same manner as above (S)-propylene oxide (10 ml, 8.29 g, 0.143 moles), a commercial preparation obtained from Aldrich Chemical Co., was added. Reaction mixture was kept overnight at 0-5°C and thereafter for 4 hours at room temperature. Then the mixture was neutralized with sulfuric acid (10%) to pH 7.5 and evaporated to dryness. Since the product is not well soluble either in ethanol or in water the residue, after evaporation, was suspended in distilled water (100 ml) and dialyzed against distilled water for 5 hours at room temperature. The retained suspension was evaporated to dryness, yielding a white powdery product (14.23 g).

Example 5

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (13.3 g of hydrate, i.e., 11.5 g anhydrous, 0.010 moles) was dissolved in a process as described above in a solution of sodium hydroxide (1.35 g, 0.034 moles in 27 ml distilled water, i.e., 4.8%) and treated in the manner described above with (S)-propylene oxide (10 ml, 8.29 g, 0.143 moles). The reaction mixture was kept overnight at 0-5°C and thereafter for 3 hours at room temperature. After neutralization with diluted sulfuric acid (10%) the solution was evaporated in vacuo nearly to dryness and residue stirred with ethanol (100 ml, 190 proof) for 30 minutes. After filtering off the insoluble sodium sulfate the ethanolic extracts were evaporated to dryness, dissolved in distilled water (35 ml), and dialyzed against distilled water for 3 hours at 0°C. Evaporation of the retained materials yielded a white powder of (S)-hydroxypropyl-β-cyclodextrin (17.3 g).

Example 6

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (13.3 g hydrate, i.e., 11.5 g anhydrous, 0.010 moles) was dissolved as above in the solution of sodium hydroxide (5.53 g, 0.13 moles in 27 ml distilled water, i.e., 17.0%) and treated in the manner described above with (S)-propylene oxide (10 ml, 8.29 g, 0.143 moles). The same isolation procedure as above yielded a white powder of (S)-hydroxypropyl-β-cyclodextrin (17.9 g).

Example 7

Preparation of (S)-hydroxypropyl-β-cyclodextrin

β-Cyclodextrin (8.02 g hydrate, 6.93 g anhydrous, 6.1 moles) was added to a solution of sodium hydroxide (13.955 g, 0.349 moles in water 32.6 ml, i.e., 30%) and dissolved by stirring and heating to 70°C to a clear yellowish solution. Then the mixture was cooled in an ice bath and to the solution which remained homogeneous was added, while stirring, (S)-propylene oxide (5 g, 0.086 moles). After neutralization, evaporation, ethanol extraction, and dialysis all performed as above, a white powdery product (9.22 g) was obtained.

Example 8

One pot preparation of Permethyl (S)-hydroxypropyl-β-cyclodextrin

Sodium hydride (5.51 g of 80% dispersion in mineral oil, i.e., 0.31 moles) was added to anhydrous dimethyl sulfoxide (65 ml) and left to react at 60°C with stirring under argon for 1 hour. Then anhydrous β-cyclodextrin (10 g, 0.0088 moles) dissolved

in anhydrous dimethyl sulfoxide (65 ml) was added, stirred for 3 hours at room temperature and to this solution then slowly added a solution of (S)-propylene oxide (2.05 g, 0.035 moles) in dimethyl sulfoxide (10 ml). The reaction mixture was stirred for 15 hours at room temperature. Thereafter, methyl iodide (26 ml) was added dropwise (ice bath cooling) and the mixture stirred for one day at room temperature. After decomposition with water (100 ml) the product was extracted with trichloromethane (2 x 150 ml). Trichloromethane extracts were washed with water (100 ml), saturated sodium chloride, and evaporated. The residue was partitioned between water (25 ml) and diethyl ether (2 x 100 ml). Ethereal extracts were washed with water (20 ml), dried with anhydrous sodium sulfate, filtered through aluminum oxide (8 g), and evaporated to yield a product in the form of a pale yellow syrup (10.2 g).

Example 9

Permethylation of (S)-hydroxypropyl-β-cyclodextrins

All the procedures used were similar to the following : sodium hydride (2.1 g, as above, i.e., 0.07 moles) was added to anhydrous dimethyl sulfoxide (20 ml) under argon and the mixture heated for 1 hour to about 60°C. Thereafter, well dried (3 hours, 110°C) hydroxypropyl-β-cyclodextrin (4 g) dissolved in dimethyl sulfoxide (15 ml) was added and left to react, under argon and while stirring at room temperature, for an additional 3 hours. Then the reaction mixture was cooled in an ice bath and methyl iodide (10 ml, 0.161 moles) added dropwise. After another hour at ice bath temperature the mixture was left stirring overnight. Then water (24 ml) was added while cooling and the product extracted twice by trichloromethane (total 90 ml). The trichloromethane extract was washed with water (20 ml) and evaporated. The residue was treated with water (25 ml) and three times extracted with ether (total 75 ml), ether extracts washed with water, and evaporated. The residue was dissolved in ether (100 ml), stirred for 30 minutes with neutral alumina, filtered, and evaporated yielding 3.7 g of permethylated product.

Example 10

Analysis of Permethyl Derivatives of hydroxypropyl-β-cyclodextrins

The permethylated product (3 mg) was dissolved in M aqueous trifluoroacetic acid (0.5 ml), kept in a screw-cap tube at 100°C overnight and concentrated by flushing with air. The residue and sodium borohydride (10 mg) were dissolved in M aqueous ammonia (0.5 ml) and kept at room temperature for 1 hour. The solution was acidified with 50% acetic acid (2 drops) and concentrated. Boric acid was removed by codistillation first with acetic acid-methanol (1:9, 5 ml) and then with methanol (25 ml).

The residue was treated with acetic anhydride and pyridine (2:1, 0.5 ml) at 100°C for 30 minutes, concentrated, and partitioned between trichloromethane and water (2:1, 6 ml). The trichloromethane phase was concentrated and the residue analysed by g.l.c. and g.l.c.-m.s.

G.l.c. was performed on a Hewlett Packard 5830 A instrument fitted with a flame ionization detector, with hydrogen as the carrier gas. G.l.c.-m.s. was performed on a Hewlett Packard 5790-5970 system with helium as the carrier gas. A Hewlett Packard Ultra 2 (cross-linked 5% phenyl methyl silicone) fused silica, capillary column (25 m, 0.20 mm i.d.) was used. Temperature program : 8 minutes at 185°C, → 250°C at 5° per minute, 250°C for 10 minutes.

Claims

1. A process for preparing regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins wherein the substitution is directed either toward the narrow or toward the wider opening of the cavity of the cyclodextrins in a reaction mixture comprising epoxide, cyclodextrin and a solvent characterized by controlling the basicity of the reaction mixture.

2. A process according to claim 1 wherein the reaction mixture is comprised of propylene oxide, β-cyclodextrin and a solvent.

3. A process according to claim 1 or 2 wherein the solvent is an alkali metal hydroxide solution.

4. A process according to any of claims 1 to 3 wherein the solvent is a sodium hydroxide solution having a concentration lower than 5% (w/w) or higher than 17%(w/w).

5. A process according to any of claims 1 to 3 wherein the solvent is a sodium hydroxide solution having a concentration lower than 4% (w/w) or higher than 18%(w/w).

6. A process according to claim 3 wherein the molar ratio of alkali metal hydroxide/cyclodextrin is in the range of 0.5 to 3.5 or in the range of 10 to 80.

7. A process according to claim 3 wherein the alkali metal hydroxide concentration in the fully assembled reaction mixture is less than 2.5% or more than 10.5%

8. A process according to claim 1 or 2 wherein the solvent is dimethyl sulfoxide and the desired basicity is obtained by using sodium methylsulfinylmethanide as a base.

9. A process according to any of claims 1-8 for the preparation of mixtures of α-, β- or γ-hydroxyalkylcyclodextrins which vary in their average degree of substitution but in which the pattern of substitution is not changed.

10. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins wherein the hydroxyalkyl substitution is directed either toward the narrow or the wider opening of the cavity of the cyclodextrins.

11. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 10 wherein the substitution is mainly on the hydroxyls 2 or 2,3 of the glucose residues with little substitution on hydroxyl 6, or wherein the substitution is mainly on the hydroxyl 6 with little substitution on the secondary hydroxyls 2 and 3.

12. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 11 wherein the relative distribution of the substitution on the 2 hydroxyl groups versus the 6 hydroxyl groups varies from 2:1 to 20:1.

13. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 11 wherein the relative distribution of the substitution on the 2 hydroxyl groups versus the 6 hydroxyl groups varies from 10:1 to 20:1.

14. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 11 wherein the relative distribution of the substitution on the 6 hydroxyl groups versus the 2 hydroxyl groups varies from 1.5:1 to 20:1.

15. Regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins according to claim 14 wherein the relative distribution of the substitution on the 6 hydroxyl groups versus the 2 hydroxyl groups varies from 2.5:1 to 20:1.

16. A process for preparing fully or partly alkylated derivatives of regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins defined in any of claims 10-15 characterized by reacting the latter with an alkylating agent in a basic, liquid reaction medium.

17. Fully or partly alkylated derivatives of the regiospecifically hydroxyalkylated α-, β- or γ-cyclodextrins defined in any of claims 10-15.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/00524

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 08 B 37/16

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 08 B

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	DE, A, 2260785 (MORISHITA PHARMACEUTICAL) 20 June 1974 see pages 5,6; claims	1-17
A	J. Carbohydrate Chemistry, volume 7, no. 2, 1988, Marcel Dekker, Inc., Ken'ichi Takeo et al.: "Derivatives of alpha-cyclodextrin and the synthesis of 6-O-α-D-glucopyranosyl-α-cyclodextrin", pages 293-308 see abstract	1-17

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th July 1990	26.07.90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	F.W. HECK

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9000524 SA 35772

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/07/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A- 2260785	20-06-74	None	