Functionalization of dextran with chloroacetate groups: immobilization of bioactive carboxylic acids

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Dextrans partially functionalized with chloroacetate groups were obtained by reaction of dextran with chloroacetyl chloride using pyridine as catalyst and the dimethylformamide/LiCl system as solvent. The structure of the resulting polymers was determined by means of infra-red, ^{1}H and ^{13}C nuclear magnetic resonance (n.m.r.) spectroscopy. ^{13}C n.m.r. spectra at 75.4 MHz of partially modified dextran with chloroacetate groups were studied in order to evaluate the selectivity of the reaction of dextran with chloroacetyl chloride in the homogeneous phase. Analysis of the spectra of ring carbons in the anhydroglucose units shows that the reactivity of the individual hydroxyl groups decreases in the order C2 > C3 > C4. The coupling of model bioactive carboxylic acids (α-naphthylacetic and 6-methoxy-α-2-naphthaleneacetic (naproxen)) to dextran functionalized with chloroacetate groups was carried out by reaction with their potassium salts or directly in the presence of 1,8-diazabicyclo[5.4.0]-7-undecene. High degrees of modification were obtained by both methods. However, the esterification reaction is somewhat more efficient in the case of the potassium salts.

(Keywords: dextran functionalization; chloroacetate groups; bioactive carboxylic acids)

INTRODUCTION

The advantages of dextran as a macromolecular carrier for drug immobilization are well accepted, as is apparent from the literature data[1,2]. In most cases, this polysaccharide has been previously transformed into a suitable reactive derivative, in order to achieve the attachment of bioactive compounds[1,3], as well as to introduce a suitable spacer between the carrier and the bioactive compound.

On the other hand, the reactions between alkyl halides and alkali-metal salts of carboxylic acids or carboxylic acids in the presence of 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) are very efficient methods for the grafting of low-molecular-weight compounds onto polymers[4–7].

In this paper we report the applicability of pendent chloroacetate groups previously linked to dextran in the coupling of model bioactive carboxylic acids (α-naphthylacetic and 6-methoxy-α-methyl-2-naphthaleneacetic (naproxen)) by reaction with their potassium salts or directly in the presence of DBU.

EXPERIMENTAL

Materials

The dextran was a commercial product (T-70 from Pharmacia Fine Chemicals) with a linear structure (as revealed by ^{13}C n.m.r.) and a weight-average molecular weight (from light scattering) of $M_w = 70\,000$. The polymer was dried *in vacuo* for a few days at 80°C in the presence of phosphorus pentoxide to constant weight. *N,N*-Dimethylformamide (DMF) (from Ferosa) and pyridine (from Panreac) were purified following one of the conventional methods[8,9]. Chloroacetyl chloride (from Fluka) was purified prior to use by distillation under normal pressure. LiCl (from Panreac) was dried *in vacuo* in the presence of phosphorus pentoxide. Dimethylsulfoxide (DMSO) (from Ferosa) was distilled *in vacuo* and then dried for a few days with a Merck 4 Å molecular sieve. α-Naphthylacetic acid (from Fluka) and 6-methoxy-α-methyl-2-naphthaleneacetic (naproxen) acid (from Sigma) were used without further purification. The potassium salts of α-naphthylacetic and naproxen acids were obtained by dissolving 0.1 mol of the acid in 200 ml of chloroform, then neutralizing with 0.1 mol of KOH dissolved in 80 ml of ethanol. The solution was precipitated by pouring into 1500 ml of dry acetone. After filtration, the salt was dried *in vacuo* in the presence of phosphorus pentoxide.

Reaction of dextran with chloroacetyl chloride

The dextran (20 g l^{-1}) was dissolved in DMF containing 2 g of LiCl/100 ml at 90°C using a Pyrex double-walled reactor through which thermostated water at the reaction temperature was circulated. Equimolar concentrations of pyridine and chloroacetyl chloride were added while stirring at 30°C. The polymer remained soluble throughout the process. The degree of substitution (*DS*) was controlled by the amount of chloroacetyl chloride used. Different precipitants were used to isolate the polymer, depending on the *DS*. All samples were purified by reprecipitation, using tetrahydrofuran (THF) as solvent and distilled water or

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propan-2-ol as precipitants, and then dried *in vacuo* in the presence of phosphorus pentoxide.

Characterization of chloroacetylated dextran

The i.r. spectra were obtained on a Perkin–Elmer 457 spectrometer on KBr discs. The ^{1}H n.m.r. spectra were registered in DMSO-d_6 at 40°C using a 200 MHz Bruker AM-200 spectrometer. ^{13}C n.m.r. spectra were obtained in DMSO-d_6 at 40°C using a 75.4 MHz Varian XL-300 in the proton noise-decoupled mode. Chemical shifts were measured with respect to that of the central peak of the methyl carbon of DMSO, which was taken to be at 39.7 ppm downfield from tetramethylsilane. The spectral measurement conditions were similar to those of the structural analysis of cellulose derivatives[10]. The spectra were accumulated for about 16 000 scans with a repetition time of 3 s. The degree of substitution (*DS*) was determined following a known method[11], which implies the alkaline hydrolysis at room temperature using a standard solution of 0.1 N sodium hydroxide. The excess alkali was back-titrated with 0.1 N hydrochloric acid using phenolphthalein indicator.

Reaction of chloroacetylated dextran with the potassium salts of α-naphthylacetic or naproxen acids

The chloroacetylated dextran was dissolved in DMSO at room temperature. The calculated amounts of potassium salt of α-naphthylacetic or naproxen acids were added while stirring. All the reactions were performed at constant temperature, and the polymer remained soluble throughout the process. The extent of modification was followed by sampling the reactant solution, taking aliquots after definite periods of time. The modified polymers were isolated by precipitation using propan-2-ol as precipitant. All samples were purified by reprecipitation, using THF as solvent and diethyl ether as precipitant, and then dried *in vacuo* in the presence of phosphorus pentoxide.

Reaction of chloroacetylated dextran with α-naphthylacetic or naproxen acids in the presence of DBU

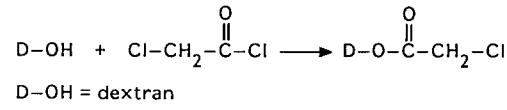
The chloroacetylated dextran was dissolved in DMSO at room temperature. Equimolar amounts of α-naphthylacetic or naproxen acids and DBU were added while stirring. All the reactions were performed at constant temperature, and the polymer remained soluble throughout the process. The isolation and purification of the modified polymers was carried out as indicated above for the reaction with the potassium salts.

Characterization of dextran–α-naphthylacetic and dextran–naproxen adducts

The ^{1}H n.m.r. spectra were registered in DMSO-d_6 at 40°C using a 200 MHz Bruker AM-200 spectrometer. ^{13}C n.m.r. spectra were obtained from a Varian XL-300 spectrometer operating at 75.4 MHz in DMSO-d_6 at 40°C. The degree of modification was determined by means of alkaline hydrolysis at 60°C using a standard solution of sodium hydroxide. The amount of released α-naphthylacetic or naproxen acid was quantitatively determined by u.v. spectroscopy (water as solvent) at the absorption wavelength of the α-naphthylacetic or naproxen acid, 281 nm ($\varepsilon = 6.32 \times 10^3$ l mol^{-1} cm^{-1}) and 271 nm ($\varepsilon = 5.12 \times 10^3$ l mol^{-1} cm^{-1}), respectively, using calibration curves previously determined.

RESULTS AND DISCUSSION

Dextrans modified with chloroacetate groups with different degrees of substitution were synthesized in a homogeneous medium by using the method followed in the bromoacetylation of poly(vinyl alcohol)[12] according to the following scheme:



The structure of the resulting polymers was confirmed by i.r. and ^{1}H and ^{13}C n.m.r. spectroscopies.

The i.r. spectra of partially modified dextran with chloroacetate groups show the characteristic bands of the pendent groups at 1740 cm^{-1} (C=O) and 690 cm^{-1} (C–Cl). The ^{1}H n.m.r. spectra of the same polymers show a peak at 4.3 ppm, which can be assigned to the methylene protons of chloroacetate groups[13]. The protons linked to the sugar carbon atoms give several bands between 3.6 and 5.4 ppm. The ^{13}C n.m.r. spectra exhibit characteristic chemical shifts at 41.6 and 166.1–166.7 ppm, which correspond to chloromethyl and carbonyl carbon atoms of chloroacetate groups, respectively[14]. The bands between 65.0 and 98.0 ppm can be assigned to the sugar carbon atoms.

On the other hand, considerable attention has recently been given to the relative reactivity of hydroxyl groups at the glucopyranosyl units of polysaccharides in view of both theoretical interest and the fact that the distribution of substituents in the anhydroglucose units may exert important effects on the behaviour and properties of partially modified polysaccharides. The relative reactivity of the hydroxyl groups at the glucopyranosyl units of dextran in the reaction with chloroacetyl chloride was evaluated by ^{13}C n.m.r., which has been suggested as an adequate method to evaluate the relative reactivity of each hydroxyl group in polysaccharides[10,15].

The ^{13}C n.m.r. spectra of the ring carbon region of dextran and several samples of partially modified dextran with chloroacetate groups (*DS* ranging from 0.60 to 2.13) are shown in *Figure 1*. The ^{13}C n.m.r. spectra of modified polymers show a decrease in the intensity of the signals assigned to C1 (98.4 ppm), C2 (71.8 ppm), C3 (73.3 ppm), C4 (70.4 ppm) and C5 (70.4 ppm) carbon atoms in the original dextran, together with the appearance of several new peaks at 95.2, 78.0, 74.9, 74.1–74.0, 69.8, 69.5, 67.8 and 67.1 ppm. The presence of these peaks is consistent with the fact that the esterification reaction of a hydroxyl group of glucopyranosic compounds causes an upfield shift of the resonance of the adjacent carbons and that the resonance of the carbon directly linked to a modified hydroxyl group is shifted downfield with respect to the chemical shift of the carbon bearing an unsubstituted hydroxyl group[16]. The assignments of these new signals were made as described previously for other esters of dextran[17]. The peak at 95.2 ppm may be ascribed to C1 carbons adjacent to C2 carbons bearing a substituted hydroxyl group. The peak at 78.0 ppm may be attributed to C3 carbons bearing a substituted hydroxyl group in monosubstituted anhydroglucose (AHG) units. The C2 carbons bearing a substituted hydroxyl group in monosubstituted AHG units are assumed to give a peak at 74.9 ppm. The peak at 74.1–74.0 ppm was attributed to C2 carbons bearing a substituted hydroxyl group in

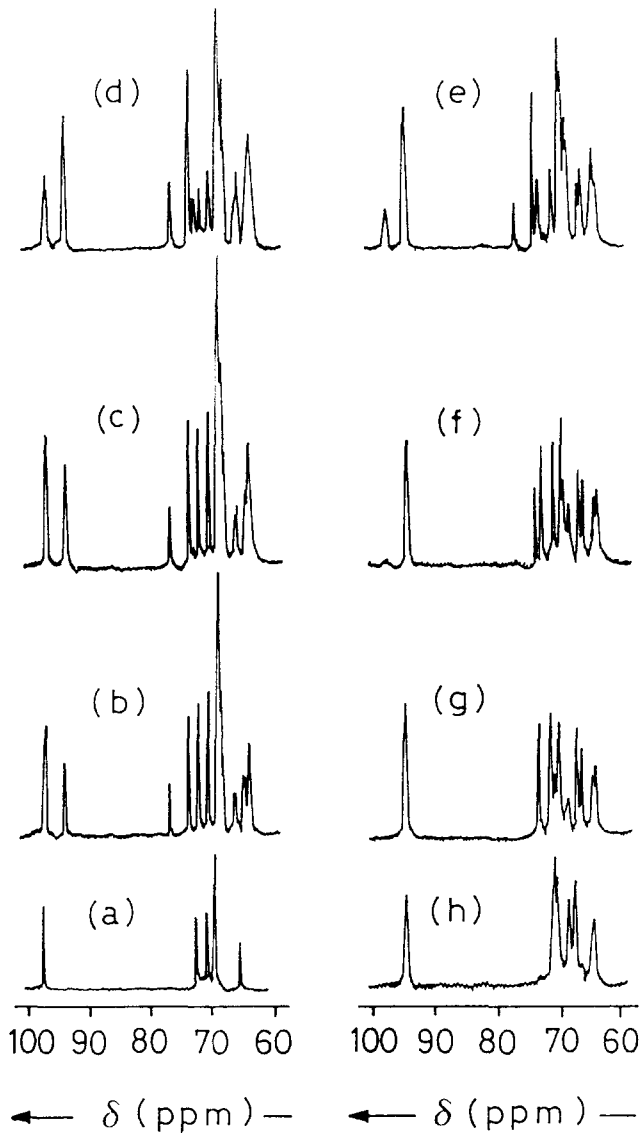


Figure 1 ^{13}C n.m.r. spectra of the ring carbon region of some partially modified dextrans with chloroacetate groups measured in DMSO-d_6: (a) $DS=0.00$, (b) $DS=0.58$, (c) $DS=0.64$, (d) $DS=0.98$, (e) $DS=1.31$, (f) $DS=1.85$, (g) $DS=2.13$, (h) $DS=2.85$

2,4-disubstituted AHG units as well as to C3 carbons bearing a substituted hydroxyl group in 2,3-disubstituted AHG units. The peak at 69.8 ppm can be assigned to C3 carbons adjacent to C2 carbons bearing a substituted hydroxyl group in monosubstituted AHG units as well as to C2 carbons adjacent to C3 carbons bearing a substituted hydroxyl group in monosubstituted AHG units. The peak at 69.5 ppm is assumed to result from the C4 carbons in trisubstituted AHG units. The peak at 67.8 ppm is considered to belong to C5 carbons adjacent to C4 carbons bearing a substituted hydroxyl groups as well as to C3 carbons in 2,4-disubstituted AHG units. The peak at 67.1 ppm may be assigned to C4 carbons adjacent to C3 carbons bearing a substituted hydroxyl group. These assignments permit us to estimate the relative reactivities of the three hydroxyl groups. In this sense, according to several authors[10,18,19] it may be considered that, under the experimental conditions used in the present work, the spin–lattice relaxation times and the nuclear Overhauser effect factors of all six carbons of the anhydroglucose units must be very similar. Therefore, peak area measurements can be used for evaluation of the relative *DS* values for individual hydroxyl groups attached to C2, C3 and C4 carbons.

In the present study, the mole fractions (A) of monosubstituted anhydroglucose units at C2 (A_2), C3 (A_3) and C4 (A_4), of disubstituted anhydroglucose units at C2, C3 ($A_{2,3}$), C2, C4 ($A_{2,4}$) and C3, C4 ($A_{3,4}$), and of trisubstituted anhydroglucose units ($A_{2,3,4}$) were calculated (*Table 1*) from the expanded forms of the spectral ranges at 100–67 ppm, using in each case an adequate combination of the relative intensities of the signals at 98.4, 95.2, 78.0, 74.9, 74.1–74.0, 73.3, 71.8 and 67.8–67.1 ppm.

The relative *DS* values (DS_i) of individual hydroxyl groups attached to the C2, C3 and C4 carbons have been estimated from the following equations:

$$DS_2 = A_2 + A_{2,3} + A_{2,4} + A_{2,3,4}$$

$$DS_3 = A_3 + A_{2,3} + A_{3,4} + A_{2,3,4}$$

$$DS_4 = A_4 + A_{2,4} + A_{3,4} + A_{2,3,4}$$

The average degree of substitution of each polymer sample was calculated from:

$$DS = DS_2 + DS_3 + DS_4$$

The values obtained from the above equations are summarized in *Table 2*. It may be noted that in all cases the magnitude of the total *DS* determined by chemical analysis and those obtained from the sum of the partial *DS* values of individual hydroxyl groups attached to C2, C3 and C4 carbons are in good agreement.

Figure 2 shows the variation of the relative DS_i of individual hydroxyl groups in the reaction of dextran with chloroacetyl chloride, as a function of the total *DS* value. The analysis of data shown in *Figure 2* clearly indicates that the relative reactivities of the three hydroxyl groups of anhydroglucose units decreased in the following

Table 1 Variation of mole fraction of mono-, di- and trisubstituted anhydroglucose units with the total *DS* in modified dextrans with chloroacetate groups

Mole fraction, A_i	*DS*[a]					
	0.59	0.69	1.00	1.35	1.77	2.16
A_2	0.31	0.32	0.43	0.42	0.24	0.07
A_3	0.14	0.14	0.15	0.12	0.04	0.01
A_4	0.04	0.05	0.04	0.03	0.00	0.00
$A_{2,3}$	0.00	0.00	0.07	0.20	0.41	0.47
$A_{2,4}$	0.05	0.07	0.08	0.09	0.17	0.18
$A_{3,4}$	0.00	0.00	0.00	0.00	0.00	0.00
$A_{2,3,4}$	0.00	0.00	0.01	0.05	0.13	0.26

[a] Determined by chemical analysis

Table 2 Quantitative structural analysis of substitution at individual hydroxyl groups in the chloroacetylation of dextran with chloroacetyl chloride

DS determined by chemical analysis	*DS* at position			Total *DS*
	C2	C3	C4	
0.59	0.36	0.14	0.09	0.59
0.69	0.39	0.14	0.12	0.65
1.00	0.59	0.23	0.13	0.95
1.35	0.76	0.37	0.17	1.30
1.77	0.95	0.58	0.30	1.83
2.16	0.98	0.74	0.44	2.16

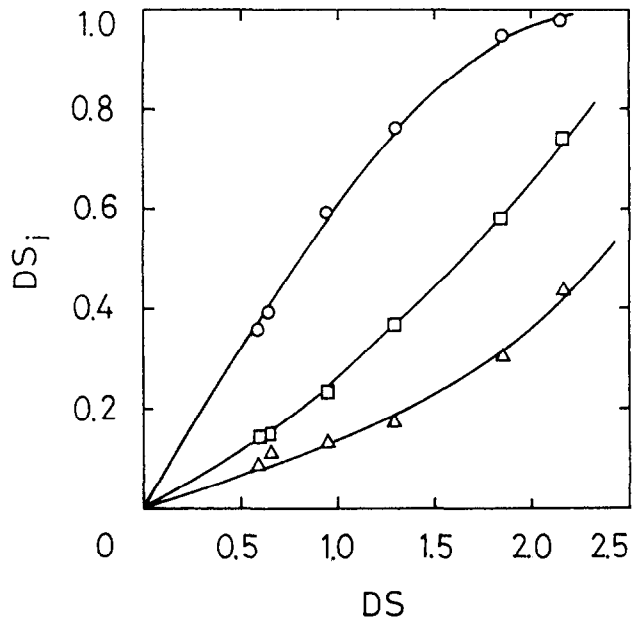
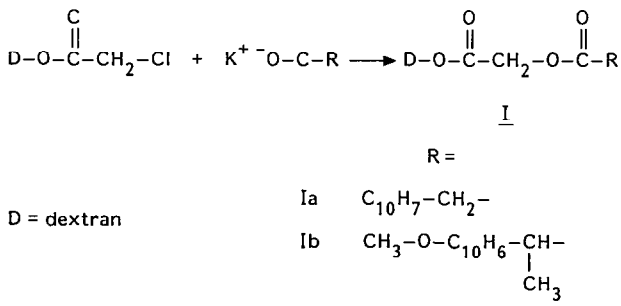


Figure 2 Variation of the degree of substitution at individual hydroxyl groups (DS_i) with the total degree of substitution in modified dextrans prepared by reaction with chloroacetyl chloride

order: C2>C3>C4. The highest reactivity of the hydroxyl groups at the C2 position may be correlated with the *cis* character of the anomeric oxygen and the hydroxyl at C2, which will be a suitable proton acceptor, as proposed in the esterification of monosaccharides with acid halides[20]. Molecular models revealed that the hydroxyl groups at C3 are less sterically hindered than the hydroxyl groups at position C4. Thus, the hydroxyl group at C3 is chloroacetylated faster than that at C4.

The coupling of bioactive carboxylic acids to dextran functionalized with chloroacetate groups was carried out by using the potassium salts of the α-naphthylacetic (**Ia**) or naproxen (**Ib**) acids according to the following scheme:



The ^1H and ^{13}C n.m.r. spectra of dextran–α-naphthylacetic and dextran–naproxen adducts show the characteristic bands of the pendent bioactive groups as can be seen in *Table 3*.

Preliminary experiments for the reaction between a partially modified dextran with chloroacetate groups ($DS=2.13$) (chloroacetate groups = 0.17 mol l^{-1}) and the potassium salts of α-naphthylacetic (0.17 mol l^{-1}) or naproxen (0.17 mol l^{-1}) acids, at 40°C using DMSO as solvent, showed that high degrees of modification (93.0 mol% and 82.0 mol%, respectively) were obtained in both cases.

Figure 3 shows the kinetic results for the reaction between a partially modified dextran with chloroacetate groups ($DS=2.13$) and potassium α-naphthylacetate at various temperatures as well as with the potassium salt of naproxen at 24°C. It is evident that the reaction rate depends on the temperature. In the case of the potassium salt of naproxen, the reaction rate is slightly lower than for the potassium α-naphthylacetate, which can be due to the presence of the methyl group in β position with respect to the carboxylate group.

Direct reaction of chloroacetylated dextran with α-naphthylacetic or naproxen acids in the presence of DBU gives modified polymers with the same structures as those obtained in the reaction with the potassium salts.

Table 3 Characteristic data of the ^1H and ^{13}C n.m.r. spectra of dextran–α-naphthylacetic and dextran–naproxen adducts

Adduct	Group	δ (ppm) ^1H n.m.r.	δ (ppm) ^{13}C n.m.r.
Ia	$-CO-\underline{CH_2}-C_{10}H_7$	4.1	60.3
	$-C_{10}H_7$	7.3–7.7	123.3–133.0
	$-\underline{CO}-CH_2-C_{10}H_7$	–	170.0
Ib	$-CO-\underline{CH}(CH_3)-C_{10}H_6-$	3.9	60.2
	$\underline{CH_3}-C-$	1.4	17.8
	$-C_{10}H_6-$	7.1–7.6	105.8–156.9
	$\underline{CH_3}-O-C_{10}H_6-$	3.8	54.7
	$-\underline{CO}-CH-$	–	172.7

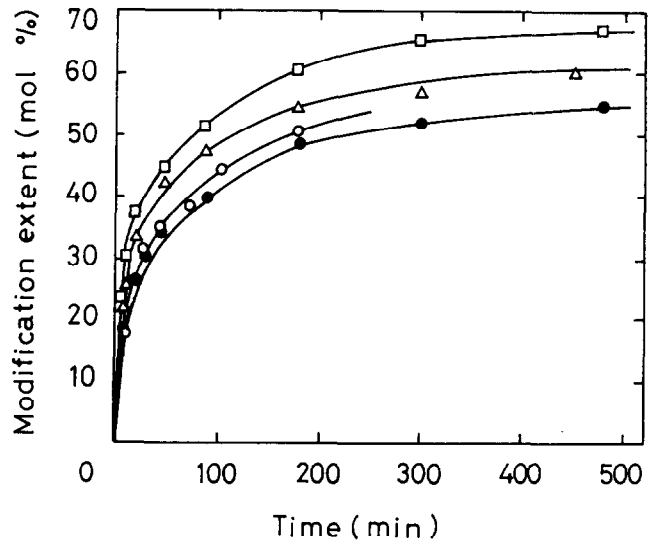


Figure 3 Plot of the extent of modification vs. time in the reaction between a partially modified dextran with chloroacetate groups ($DS=2.13$) (chloroacetate groups = 0.04 mol l^{-1}) and potassium naphthylacetate (0.04 mol l^{-1}) or the potassium salt of naproxen (0.04 mol l^{-1}) in DMSO at various temperatures. Potassium naphthylacetate: (○) 24°C, (△) 30°C, (□) 38°C. Potassium salt of naproxen: (●) 24°C

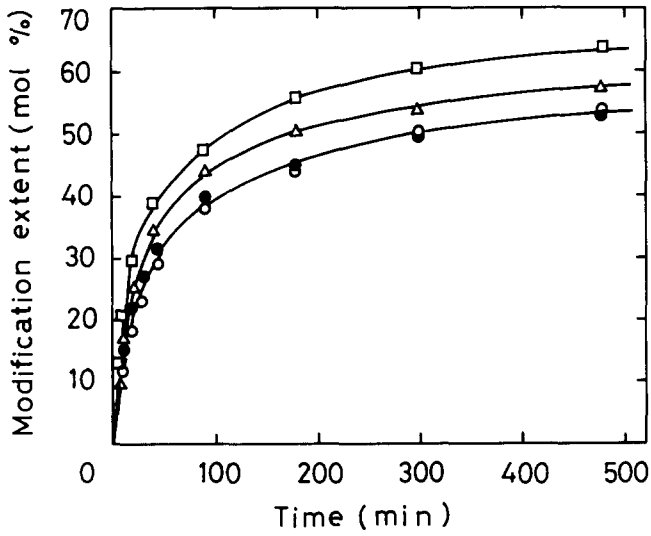
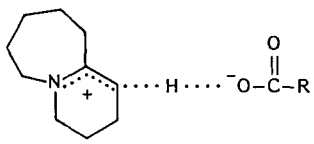


Figure 4 Plot of the extent of modification vs. time in the reaction between a partially modified dextran with chloroacetate groups ($DS = 2.13$) (chloroacetate groups = 0.04 mol l^{-1}) and α-naphthylacetic acid (0.04 mol l^{-1}) or naproxen acid (0.04 mol l^{-1}) using DBU (0.04 mol l^{-1}) as catalyst and DMSO as solvent at various temperatures. α-Naphthylacetic acid: (○) 24°C, (△) 30°C, (□) 38°C. Naproxen acid: (●) 24°C

Preliminary experiments, for the reaction between a partially modified dextran with chloroacetate groups ($DS = 2.13$) (chloroacetate groups = 0.17 mol l^{-1}) and α-naphthylacetic (0.17 mol l^{-1}) or naproxen (0.17 mol l^{-1}) acids at 40°C using DMSO as solvent and DBU (0.17 mol l^{-1}) as catalyst, showed that the esterification reaction is somewhat less efficient when it is compared with the reaction with the potassium salt of these acids. These results can be interpreted by taking into consideration that in this case the reactive intermediate may be the complex[21]:



which is more bulky in comparison to the carboxylate ion, and consequently the steric effects may be more accentuated.

Figure 4 shows the kinetic results for the reaction of a partially chloroacetylated dextran ($DS = 2.13$) and α-naphthylacetic or naproxen acids in the presence of DBU. As may be seen from *Figure 4*, the reaction rate increases as the temperature increases.

CONCLUSION

From the results quoted above, it is concluded that the two methods described are appropriate for the incorporation of bioactive compounds in partially chloroacetylated dextrans.

ACKNOWLEDGEMENT

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