Antiinflammatory Polymer-Bound Steroids for Topical Applications. I. Synthesis and Characterization

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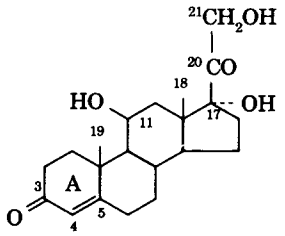
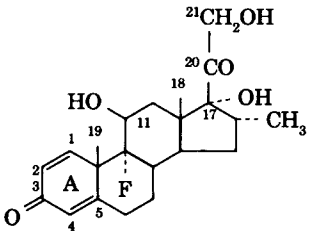
Synopsis

Covalent binding of hydrocortisone and dexamethasone to hydrophylic biocompatible macromolecular carriers through hydrolizable carbonate linkage was investigated according to two complementary strategies. (a) Radical copolymerization of hydrocortisone-^{21}C-vinylcarbonate with *N*-vinylpyrrolidone (NVP,60°C), or *N*-[tris(hydroxymethyl)methyl]acrylamide (THMMA, 50°C) in dimethylacetamide solution: In spite of a nearly zero reactivity ratio for the steroid monomer which behaves as a degradative transfer agent—$C_T \sim 5.7 \times 10^{-2}$ and 6.8×10^{-3} for NVP and THMMA, respectively—this process may afford fairly high molecular weight polymers ($\overline{M}_w \simeq 10^4$–$10^5$) with high enough hydrocortisone content (0.03–0.10 mole.fraction). (b) Condensation of the hydrocortisone or dexamethasone-^{21}C-chloroformates onto poly(oxyethylene glycol) ($\overline{M}_n = 6220$) or hydroxypropylcellulose (HPC, $\overline{M}_w = 1.35 \times 10^5$) in tetrahydrofuran solution (30°C): This straightforward process is of low efficiency (yields < 50%), and only HPC derivatives show good chemical homogeneity.

INTRODUCTION

Hydrocortisone (H-1) and dexamethasone (D-1) are among the best antiinflammatory drugs specifically used for topical applications.[1–4]





However, because of free transport through the various tissues, most of the drug is lost in the systemic circulation, and only about 2–4% of the initial dose is actually retained on its application site.[5] In spite of its great efficiency even in very low amounts, this feature implies repeated applications in order to reach the therapeutic level for a sufficient long time, with possible risk of a number of harmful secondary effects.[6] Within the now well-established framework of pharmacologically active polymers[7–10] it was thus of interest to design a steroid–polymer system showing improved properties with respect to the free drug on a number of topics such as:

—strongly restricted diffusion through the various tissues, arising from

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the high molecular weight biostable and biocompatible polymeric carrier;

—slow and progressive drug release monitored by the hydrolysis of a suitable covalent bond between the polymer and the drug: kinetics effects;

—selective or preferential drug release on the target inflammated tissues: specific effects.

Because of their wide range of biological activity, the covalent binding of steroids on various polymeric materials has received much attention during the two last decades,[11–19] and, as early as 1963, a Japanese patent claimed direct esterification of a vinylpyrrolidone–maleic anhydride copolymer by hydrocortisone and dexamethasone.[20] The choice of the bond nature between the polymer and the drug is obviously critical, and we selected the carbonate linkage—O—CO—O—for a number of reasons such as enhanced hydrolysis rate with respect to ester function, still possible enzymatic catalysis,[21] and well-developed background for analogous drug-polymer systems,[22–26] especially in the case of steroids.[14,15,17] Taking into account the necessary biostability, biocompatibility, and water solubility of the inactive polymeric carrier, two complementary strategies were used for the synthesis of the polymer bound steroids:

—preparation of the hydrocortisone-^{21}C-vinylcarbonate (H-4), and radical copolymerization with *N*-vinylpyrrolidone (NVP) or *N*-[tris(hydroxymethyl)-methyl]acrylamide (THMMA).

—preparation of the hydrocortisone and dexamethasone-^{21}C-chloroformates, and consecutive condensation on polyoxyethyleneglycol (POEG) or hydroxypropylcellulose (HPC).

EXPERIMENTAL

Starting Materials

Solvents, Monomers, and Reagents. All the solvents and liquid reagents were purified by distillation over suitable drying reagents: tetrahydrofurane (THF) over Na_2-benzophenone complex; *N,N*-dimethylacetamide (DMAC), pyridine, and triethylamine over CaH_2. Acryloyl, methacryloylchlorides, vinyl and isopropenylchloroformates (Société Nationale des Poudres et Explosifs), and *N*-vinylpyrrolidone (NVP) were vacuum-distilled over copper turnings just before use. Pure *N*-[tris(hydroxymethyl)methyl]-acrylamide (THMMA, Pharmindustrie), hydrocortisone (H-1), and dexamethasone (D-1) obtained from Vegetadrog were used as received. Azobisisobutyronitrile was recrystallized from toluene solution.

Polymers. Polyoxyethyleneglycol H-6000 (Hoechst) was purified and characterized as previously described[27]: $\overline{M}_n$ = 6220, $\overline{M}_w/\overline{M}_n$ = 1.01. Hydroxypropylcellulose KLUCEL-EF (Hercules) was used as received: dn/dc = 0.132 mL g^{-1} in CH_3OH for = 6320 Å, $\overline{M}_w = 1.35 \times 10^5$.

Analytical and Physicochemical Measurements

Melting Points (MP). Uncorrected MP (with possible sample decomposition) were determined on a heating plate microscope Mettler FP-52 fitted with a Mettler FP-5 temperature regulating system.

Thin Layer Chromatography (TLC). TLC experiments were carried out on silicagel plates (Merck DC, Kieselgel 60 F-254) using ethylacetate as eluting solvent. All polymeric materials essentially lead to $R_f = 0$, and the purity of steroid derivatives was checked by the lack of any significant spot corresponding to pure H-1 or D-1 ($R_f = 0.60$ and 0.62, respectively).

Infrared and Ultraviolet Spectoscopy. The IR and UV spectra were recorded on a Perkin-Elmer 237 (KBr pellet technique) and on a Beckman Acta C V (trifluoroethanol solution) apparatus respectively.

^{1}H-NMR Spectroscopy. ^{1}H-NMR spectra were recorded on a Hitachi-Perkin-Elmer R-24 A apparatus (60 MH_z) in dimethylsulfoxyde-d_6 (DMSO) solution, using hexamethyldisiloxane as internal reference.

Molecular Weight Measurements. Refractive index increments, *dn/dc*, were measured at room temperature on a differential refractometer Brice-Phoenix BP-10004 for $\lambda = 6320$ Å. Light scattering measurements were performed on a Fica PGD 420-OM apparatus. The chemical heterogeneity of all the polymeric materials is weak: copolymerizations stopped at low conversion (<15%), or chemical modifications of polymers limited to low extent. The experimental $\overline{M}_w$ values may be safely considered as the actual weight average molecular weights.

Monomer Synthesis, Polymerizations and Modifications of Polymers

General Experimental Procedure. All the experiments were carried out under a slight pressure of pure argon in an all Pyrex glass double wall reactor allowing the use of vacuum and argon sweeping cycles and temperature control by an external regulating device. Solvents and reagents were introduced under argon directly from Schlenk vessels, or through self-sealing rubber caps using syringue technique.

Hydrocortisone-^{21}C-Acrylate (H-2). One adds 5.04 mL (0.062 mol) of acryloylchloride dropwise to a solution of 15 g (0.041 mol) of H-1 and of 5.78 mL (0.041 mol) of Et_3N in 50 mL of DMAC previously cooled and kept at 0°C. The mixture is then stirred overnight at room temperature. After filtration of triethylamine hydrochloride, the clear solution is precipitated into 500 mL of H_2O. The insoluble monomer (15.50 g) is recovered by filtration, repeatedly washed with H_2O, and then vacuum dried at 40°C: yield = 90.0%, mp = 195.4–199.6°C, $R_f = 0.89$.

ANAL. Calcd for $C_{24}H_{32}O_6$: C, 69.20%; H, 7.75%; O,23.05%. Found: C, 68.07%; H, 7.72%; O, 24.01%.

Hydrocortisone-^{21}C-Methacrylate (H-3). Same reaction procedure as for H-2, but using methacryloylchloride: yield = 90.0%, mp = 190.4–195.2°C, $R_f = 0.90$.

ANAL. Calcd for $C_{25}H_{34}O_6$: C, 69.74%; H 17.96%; O, 22.30%. Found: C, 68.96%; H, 7.91%; O, 23.02%.

Hydrocortisone-^{21}C-Vinylcarbonate (H-4). One adds 1.63 mL (0.020 mol) of vinylchloroformate dropwise into a solution of 5 g (0.013 mol) of H-1 and of 1.66 mL (0.020 mol) of pyridine in 100 mL of THF previously cooled and kept at 0°C. The reaction mixture is then stirred overnight at room temperature. After filtration of pyridinium hydrochloride the clear solution is precipitated into 11 mL of chilled H_2O. The insoluble monomer recovered

by filtration (5.89 g) is repeatedly washed with chilled H_2O and then vacuum-dried at 40°C: yield = 99.5%, mp = 195.1–196.4°C, R_f = 0.87

ANAL. Calcd for $C_{24}H_{32}O_7$: C, 66.65%; H, 7.45%; O, 25.90%. Found: C, 66.67%, H, 7.50%; O, 25.80%.

Hydrocortisone-^{21}C-Isopropenylcarbonate (H-5). Same reaction procedure as for H-4, but using isopropenylchloroformate: yield = 95.0%, mp = 195.8–197°C, R_f = 0.90.

ANAL. Calcd for $C_{25}H_{34}O_7$: C, 67.24%; H, 7.68%; O, 25.08%. Found: C, 67.10%; H, 7.69%; O, 25.20%.

Hydrocortisone-^{21}C-Isopropylcarbonate (H-6). Same reaction procedure as for H-4 and H-5 but using isopropylchloroformate. The unreacted H-1 is separated from the crude reaction product by column chromatography on Kieselgel 60-Merck using ethylacetate as eluting solvent. The first fraction yields the isopropylcarbonate which may be further purified by precipitation from THF into H_2O; yield = 70.1%, mp = 190.9–192.7°C, R_f = 0.87.

ANAL. Calcd for $C_{25}H_{36}O_7$: C, 66.94%; H, 8.09%; O, 24.97%. Found: C, 66.91%; H, 8.13%; O, 24.92%.

Dexamethasone-^{21}C-Isopropylcarbonate (D-2). Same reaction procedure as for H-6 but using D-1 instead of H-1: yield = 69.0%, mp = 211-212.7°C, R_f = 0.90.

ANAL. Calcd for $C_{26}H_{35}OF$: C, 65.26%; H, 7.37%; F, 3.97%. Found: C, 65.26%; H, 7.38%; F, 3.57%.

Hydrocortisone-^{21}C-Chloroformate (H-7). One adds 17.22 mL of a phosgene solution in toluene (0.033 mol) dropwise into a solution of 3 g (0.008 mol) of H-1 in 60 mL of THF previously cooled and kept at 0°C. The reaction mixture is then stirred overnight at room temperature, and excess phosgene is eliminated under vacuum. After precipitation of the solution into 600 mL of dry hexane, the steroid chloroformate (3.45 g) is recovered by filtration, repeatedly washed with hexane, and vacuum-dried at 30°C: yield = 98.5%, mp = 154–154.6°C, R_f = 0.87.

ANAL. Calcd for $C_{22}H_{29}O_6Cl$: C, 62.18%; H, 6.88%; O, 22.60%; Cl, 8.34%. Found: C, 62.29%; H, 6.89%; O, 22.61%; Cl, 8.19%.

Dexamethasone-^{21}C-Chloroformate (D-3). Same experimental procedure as for H-7, but starting from D-1 instead of H-1: yield = 98.4%, mp = 150–152°C, R_f = 0.90.

ANAL. Calcd for $C_{23}H_{28}O_6FCl$: C, 60.72%; H, 6.20%; F, 4.18%; Cl, 7.80%. Found: C, 61.79%; H, 6.28%; F, 3.72%; Cl, 7.68%.

Polymerizations and Copolymerizations. The experimental conditions are given in the text. The polymers were recovered by precipitation into CH_3OH for poly(H-2) and poly(H-3) and into Et_2O for carbonate copolymers. The NVP consumption in homo- and copolymerizations was monitored by gas chromatography, using the solvent (DMAC) as internal reference: Intersmat IGC-15 apparatus fitted with a glass bead column loaded with 0.5% of polyvinylidenefluoride and regulated at 180°C.

Covalent Labeling of POEG and HPC by ^{21}C-Steroid Chloroformates. The polymers were thoroughly vacuum dried at 80°C in the reactor just before dissolution. The reactions were carried out in homogeneous THF solution at 30°C using the stoechiometric ratios [OH]:[O—CO—Cl]:[pyridine] quoted in the text. After an 18-h reaction time, an excess of isopropanol and pyridine is added to the reaction mixture, and stirring at 30°C is con-

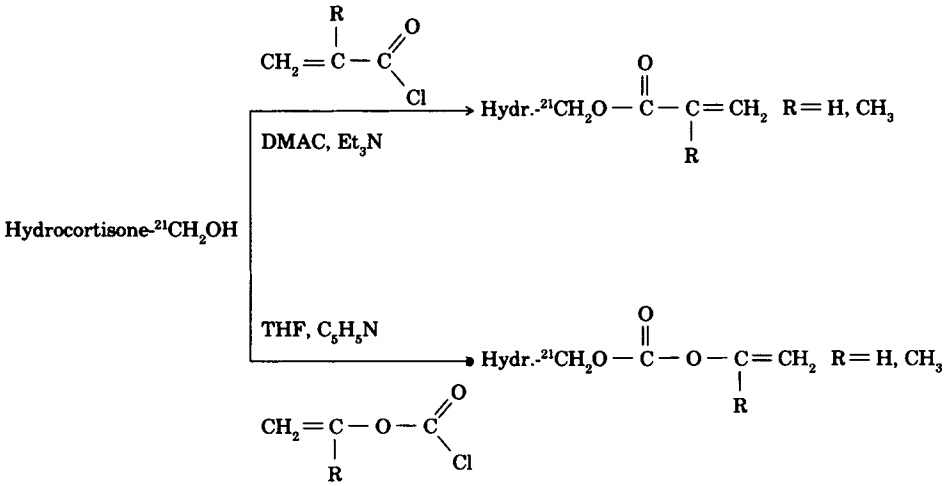
tinued overnight. After filtration of pyridinium hydrochloride, the functionnalized polymers are recovered by precipitation into a large excess of Et_2O, repeatedly washed with Et_2O, and vacuum-dried at 40°C.

RESULTS AND DISCUSSION

Hydrocortisone Vinylcarbonate Copolymers

Monomer Synthesis

Hydrocortisone acrylate (H-2), methacrylate (H-3), vinylcarbonate (H-4), and isopropenylcarbonate (H-5) are readily obtained in very high yields over 90% by condensation of hydrocortisone with the corresponding acyl halides or chloroformates in homogeneous aprotic solution (DMAC, THF) (see the Experimental Section):



Acylation is selective on the ^{21}C primary alcohol function, and all the spectrometric IR, UV, and ^1H-NMR data are in good agreement with the expected unsatured ester or carbonate structure (see Table I).

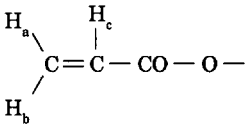
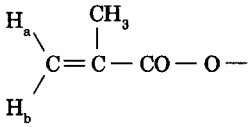
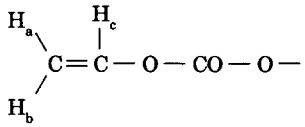
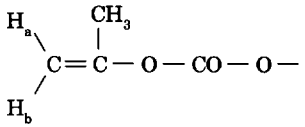
Hydrocortisone Homopolymers

Radical polymerization of the steroid monomers H-2 to H-5 was performed in homogeneous DMAC solution (0.24 mol L^{-1}) in presence of AIBN (0.24×10^{-2} mol L^{-1}) at 60°C. The acrylate (H-2) and methacrylate (H-3) readily lead to very high molecular weights with very high yields as illustrated below:

H-2: yield = 75%, $\overline{M}_w = 1.32 \times 10^6$
H-3: yield = 80%, $M_w = 1.48 \times 10^6$
for an 18-h polymerization time

The carbonates (H-4) and (H-5), however, are unable to polymerize in the same conditions. Moreover, only traces of polymeric materials are obtained

TABLE I
Characteristic Spectroscopic Data of Hydrocortisone Monomers

	H-2	H-3	H-4	H-5
				
IR λ (nm) (KBr)	1740 V(C=O)ester[a]	1740 V(C=O)ester[a]	1760 V(C=O)carbonate	1760 V(C=O)carbonate
UV (TFE) λ_{max} (nm)	248[b]	248[b]	248[b]	248[b]
UV (TFE) ϵ (L mol^{-1} cm^{-1})	14,500	14,600	15,400	16,100
^{1}H-NMR-(DMSO) δ(ppm)	H_a 5.87 d	H_a 5.55 s	H_a 4.57 d	H_a 4.65 s
	H_b 6.23 s	H_b 5.92 s	H_b 4.77 d	H_b
	H_c 6.13 d	CH_3 1.80 s	H_c 6.90 q	CH_3 1.82 s

[a] Weak shoulder on the main absorption V(C=O) at 1715 cm^{-1} of the ^{20}C ketonic function.

[b] $\pi \rightarrow \pi^*$ transition of the α—β unsaturated ketonic ring of hydrocortisone: ring A. ϵ = 16300 L mol^{-1} cm^{-1} for pure hydrocortisone (H — 1).

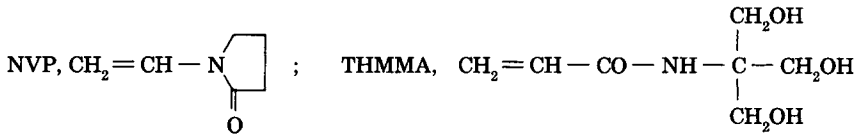
at 35°C in presence of dicyclohexylperoxydicarbonate: This specific behavior is in sharp contrast with the successful bulk polymerization of the rather sterically hindered vinylmenthylcarbonate in these optimized conditions.[28]

Hydrocortisone-^{21}C-Vinylcarbonate Copolymers: Synthesis and Characterization

All the copolymerizations were performed in homogeneous DMAC solution in the following conditions:

	Temp. (°C)	[*Monomers*] (mol. L^{-1})	[*AIBN*] (mol. L^{-1})
NVP/H-4	60	2	2×10^{-2}
THMMA/H-4	50	1.5	1.5×10^{-2}

f and F will refer to the molar fraction of a given monomer in the monomer feed and in the resulting copolymer, respectively:



Determination of the Reactivity Ratios. Copolymer composition was determined by UV spectroscopy using the $\pi \rightarrow \pi^*$ transition of the conjugated carbonyl chromophore of the hydrocortisone A ring: $\lambda = 248$ nm in TFE solution. Unfortunately, the amide absorption of NVP and THMMA units overlaps with that of the steroid, especially for copolymers of low steroid content (see Fig. 1).

In order to obtain reliable composition data, we first measured the variations of the apparent molar absorptivity of hydrocortisone-^{21}C-isopropylcarbonate H-6 (taken as a model of the steroid unit in copolymers) with its concentration in binary mixtures with poly(*N*-vinylpyrrolidone) (see Fig. 2). The UV spectra of copolymers were then analyzed optimizing the ϵ value by iteration: In most cases, three successive iterations yield F values with an accuracy better than $\pm 3\%$. The experimental results of the two copolymerization systems are collected in Tables II and III and the composition diagram is illustrated in Figure 3. Reactivity ratios were calculated according to the Kelen–Tudos method,[29] as illustrated in Figure 4:

$$\text{DMAC } 60°\text{C} \begin{cases} r(\text{NVP}) = 2.20 \pm 0.25 \\ r(\text{H-4}) = 0.029 \pm 0.086 \end{cases} \quad \text{DMAC } 50°\text{C} \begin{cases} r(\text{THMMA}) = 27.3 \pm 4.2 \\ r(\text{H-4}) = 0.743 \pm 1.368 \end{cases}$$

These values thus derived from experimental data obtained in a relatively narrow composition range [f(H-4) ≤ 0.5] cannot be considered as definitive. They point out, however, as a most interesting feature, a very low, nearly zero, reactivity ratio for the hydrocortisone vinylcarbonate monomer, in good agreement with its unability to homopolymerize: This may suggest possible penultimate effects or propagation–depropagation equilibrium in the copolymerization system. On the other hand, all steroid units are in-

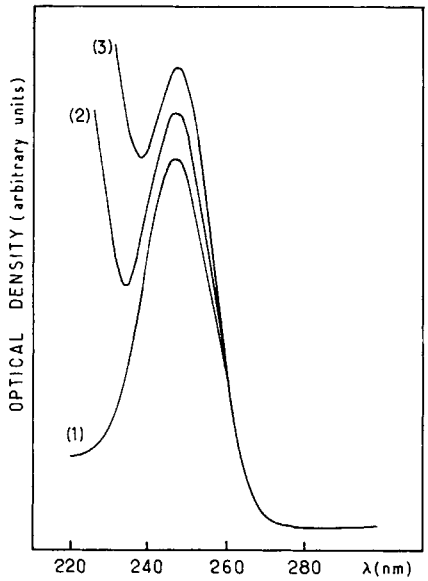


Fig. 1. UV spectra of various hydrocortisone-^{21}C-carbonates in TFE solution (spectra are arbitrarily shifted for more clearness) (1) hydrocortisone-^{21}C-isopropylcarbonate H-6 (ϵ app = 16,200 L mol^{-1} cm^{-1}); (2) and (3) NVP copolymers obtained with f(H-4) = 0.20 and 0.05, respectively (ϵ app = 15,860 and 17,530 L mol^{-1} cm^{-1}, respectively).

corporated in the polymeric chain as isolated units in NVP/H-4/NVP or THMMA/H-4/THMMA triads.

From the reactivity ratios of the NVP/H-4 monomer pair and taking into account the literature Q and e values of NVP[30] (Q = 0.088, e = −1.62), one may readily calculate Q = 0.58 and $e \sim -3.28$ for the vinyl carbonate monomer: These values, derived from only a single copolymerization system, are obviously very rough estimations, and they are to be considered with great caution, especially the very strongly negative e value. A more reliable comparison of the H-4 reactivity ratio with those of similar monomers

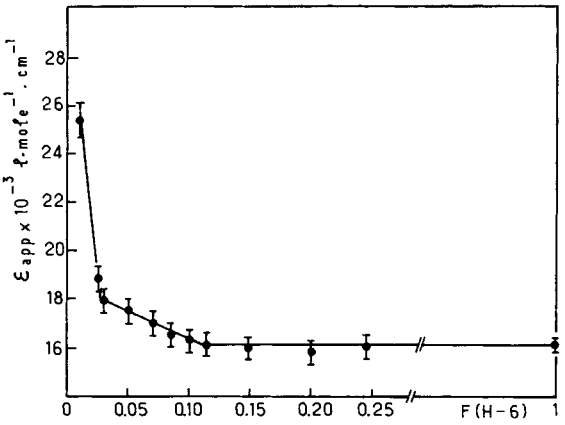


Fig. 2. Variation of ϵ app (λ = 248 nm) with composition for binary mixtures of poly(NVP) and H-6 [F(H-6) = molar fraction of H-6].

TABLE II
NVP/H-4 Radical Copolymerization in Homogeneous DMAC Solution at 60°C

H-*i*	*f*(H-*i*)	Induction period (mn)	Polym time[a] (mn)	NVP conversion (%)	NVP, $R_p \times 10^4$ ($L^{0.5}$ $mol^{-0.5}$ s^{-1})	*F*(H-4)	*dn/dc* in CH_3OH (mL g^{-1})	$\overline{M}_w \times 10^{-4}$
0	0	0	45	24.4	7.35	0	0.178	7.58
H-1	0.05	0	50	11.2	3.00	0	0.178	3.74
H-1	0.15	0	100	10.9	1.30	0	0.178	2.34
H-1	0.25	0	140	8.4	0.92	0	0.178	1.25
H-4	0.05	27	90	11.1	1.87	0.023	0.180	4.98
H-4	0.10	28	120	10.1	1.33	0.047	0.182	2.70
H-4	0.15		140	14.8		0.064	0.183	2.21
H-4	0.20	39	210	11.1	0.73	0.094	0.185	2.10

[a] Including the induction period.

TABLE III
THMMA/H-4 Radical Copolymerization in Homogeneous DMAC Solution at 50°C

f(H-4)	Polymer time (mn)	Yield[a] (%)	F(H-4)	dn/dc in CH_3OH (mL g^{-1})	$\overline{M}_w \times 10^{-5}$
0	25	17.6	0	0.103	2.33
0.05	75	17.3	0.002	0.104	1.89
0.10	100	13.9	0.004	0.104	1.44
0.15	115	10.3	0.006	0.105	
0.20	170	10.1	0.010	0.105	1.30
0.25	180	10.9	0.012	0.106	
0.30	225	6.3	0.012	0.106	1.00
0.35	280	7.1	0.013	0.106	
0.40	320	5.7	0.034	0.110	1.03
0.50	390	4.4	0.032	0.110	0.687

[a] Yield = 100 (copolymer weight/total monomer weight).

(vinylchloroformate, vinylcarbonates or carbamates) in copolymerization with NVP[31,32] is outlined in Table IV.

Hydrocortisone-[21]C-vinylcarbonate is the less reactive species, even less reactive than vinylene carbonate: The high steric hindrance of the steroid moiety may contribute to such a behavior, without excluding other unknown factors.

Kinetic Analysis of the NVP/H-4 Copolymerization System. The NVP consumption, monitored by gas chromatography (see the Experimental section), obeys first-order kinetics for conversion restricted to about 15%, as illustrated in Figure 5:

$$\ln \frac{[\text{NVP}]_0}{[\text{NVP}]_t} = R_p [\text{AIBN}]_0^{0.5} t$$

where the symbols have their usual physical meaning.

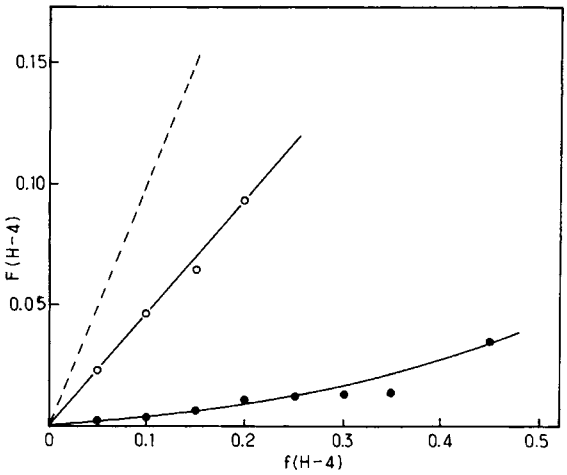


Fig. 3. Composition diagram for the two copolymerization systems NVP/H-4 (○) and THMMA/H-4 (●). Continuous lines are derived from the calculated reactivity ratios, and the dashed line corresponds to ideal pairs, r(NVP) = r(THMMA) = r(H-4) = 1.

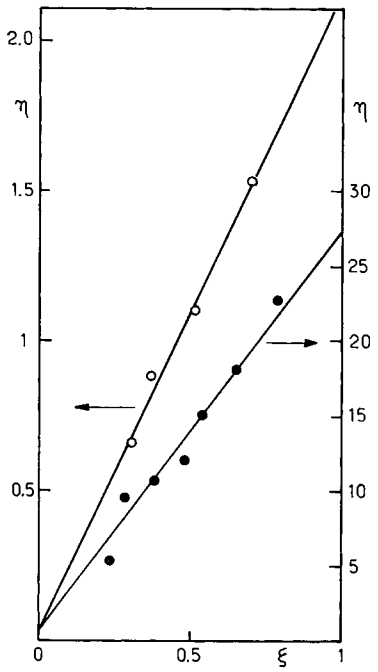


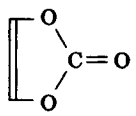
Fig. 4. Kelen–Tudos plot of the copolymerization compositional data for NVP/H-4 (○) and for THMMA/H-4 (●) systems. The symbols have the same physical meaning as in the original publication.[29]

The experimental results quoted in Table II and illustrated in Figure 5 may suggest the following comments:

—The kinetic constant R_p determined for NVP homopolymerization, $R_p = 7.35 \times 10^{-4}$ L$^{0.5}$ mol$^{-0.5}$ s^{-1}, is in excellent agreement with that measured in similar experimental conditions (dimethylformamide, 60°C) by calorimetry[33], $R_p = 7.43 \times 10^{-4}$ L$^{0.5}$ mol$^{-0.5}$ s^{-1}.

—The R_p values are a decreasing function of the molar fraction of vinylcarbonate in the monomer feed, especially for f(H-4) $\leq$ 0.05: R_p is dras-

TABLE IV
Reactivity Ratios of Some Vinyl Monomers of Similar Structure in Copolymerization with NVP

Monomer M		r(M)	r(NVP)
CH_2=CH—O—CO—CH_3	a	0.257	1.056
CH_2=CH—O—CO—Cl	b	0.677	1.425
CH_2=CH—O—CO—O—C_6H_5	b	0.445	1.97
CH_2=CH—O—CO—N(C_2H_5)$_2$	b	0.318	3.14
CH_2=CH—NH—CO—O—Et	a	0.370	1.768
H-4	a	0.029	2.20
	a	0.055	1.082

[a] Literature values derived from copolymerization data.[31]

[b] Calculated from Q and e literature values.[30,32]

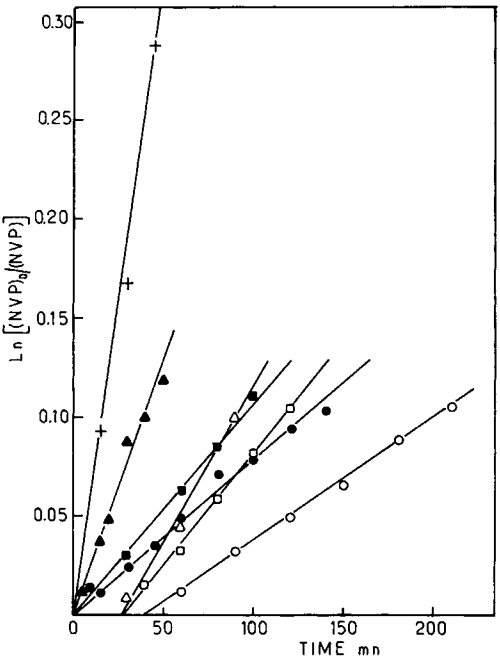


Fig. 5. Kinetics of the NVP conversion in various polymerization systems at 60°C. NVP homopolymerization + NVP homopolymerization in presence of H-1 with f(H-1) = 0.05 (▲), 0.15 (■), and 0.25 (●). NVP/H-4 copolymerization with f(H-4) = 0.05 (△), 0.10 (□), and 0.20 (○).

tically decreased by a factor of 4 when f(H-4) increases from 0 to 0.05. The presence of pure hydrocortisone in the reaction medium leads by itself to a parallel R_p decrease, showing a specific behavior very similar to that of its vinylcarbonate derivative.

—Copolymerization is characterized by the presence of an induction period which is also an increasing function of f(H-4). However, pure hydrocortisone does not lead to such an effect, even if it slows down the NVP polymerization rate.

—Weight average degrees of polymerization, $\overline{DP}_w$, were derived from experimental $\overline{M}_w$ values according to

$$\overline{M}_w = \overline{DP}_w \times \mathcal{M} \quad \text{with} \quad \mathcal{M} = \mathcal{M}(\text{H-4}) \times F(\text{H-4}) + \mathcal{M}(i) \times F(i)$$

where $\mathcal{M}_i$ is the molecular weight of the monomer i (NVP or THMMA). $\overline{DP}_w$ values are a decreasing function of the molar fraction of hydrocortisone vinyl carbonate in the monomer feed, especially for low f(H-4) values. Quite the same behavior is actually observed for NVP homopolymerization in presence of pure hydrocortisone. Two factors may contribute to the parallel characteristic R_p and $\overline{DP}_w$ decrease when increasing f(H-4) values in the copolymerization system: Low propagation with respect to high termination rate for H-4-terminated macroradicals, and possible transfer to the steroid monomer. In order to test the last assumption, and neglecting in a first approach the existence of an induction period, the $\overline{DP}_w$-f(H-4) or f(H-1)

experimental data were tentatively analyzed in terms of pure transfer, using the amended Mayo equation[34]:

$$A_w/\overline{DP}_w = B_w + C_T f(\text{H-4})$$

with

$$A_w = 2 \quad \text{for termination by disproportionation}$$

$$A_w = 1 + (1 + 3\overline{DP}_w/\overline{DP}_{w,0})^{1/2} \quad \text{for termination by combination}$$

$\overline{DP}_{w,0}$ is related to the NVP homopolymerization: f(H-1 or H-4) = 0. The experimental results are in good agreement with this linear equation, as illustrated in Figure 6, and they lead to the apparent transfer constant values (C_T) quoted in Table V.

The unknown termination mode of both polymers does not affect significantly the C_T-derived values which are rather high. The very similar behavior of H-1 and H-4 in the two NVP systems, $C_T \simeq 5.7 \times 10^{-2}$, suggests that the transfer site belongs to the steroid moiety: It cannot be obviously specified from our measurements alone, but the ^{21}C-methylenic group (—^{20}CO—^{21}CH$_2$—O) could reasonably be a potential candidate. On the other hand, H-4 shows a C_T value higher by about 1 order of magnitude for NVP than for THMMA macroradicals:

$$k_T(\text{NVP})/k_T(\text{THMMA}) \simeq 10\ k_p(\text{NVP})/k_p(\text{THMMA})$$

where k_T and k_p are the transfer and the propagation rate constants, respectively. The ratio k_p(NVP)/k_p(THMMA) is not known, and a much higher

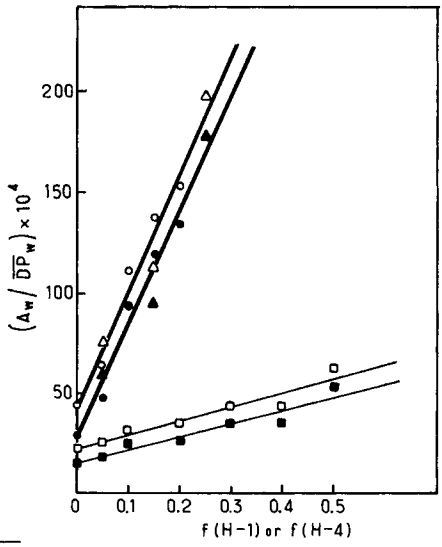


Fig. 6. Variations of $\overline{DP}_w$ with composition of the monomer feed for various polymerization systems and for the two possible termination modes: (△, ○, □) combination; (▲, ●, ■) disproportionation, black symbols. (△, ▲) NVP/H-1; (○, ●) NVP/H-4; (□, ■) THMMA/H-4.

TABLE V
Apparent Transfer Constants C_T(H-1) and C_T(H-4) in the NVP/H-4 and THMMA/H-4 Copolymerization Systems in Homogeneous DMAC Solution

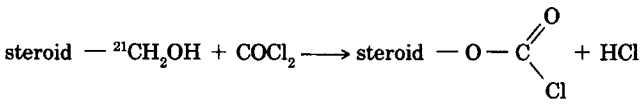
Copolymerization system	Termination mode	C_T(H-1)	C_T(H-4)
NVP, 60°C	Disproportionation	5.70×10^{-2}	5.63×10^{-2}
	Combination	5.88×10^{-2}	5.86×10^{-2}
THMMA, 50°C	Disproportionation		6.69×10^{-3}
	Combination		7.05×10^{-3}

transfer activity for the unstabilized NVP species with respect to the stabilized THMMA one cannot be definitely ascertained.

STEROID BINDING ONTO POEG AND HPC THROUGH CARBONATE LINKAGE

Synthesis and Characterization of the Steroid-^{21}C-Chloroformates

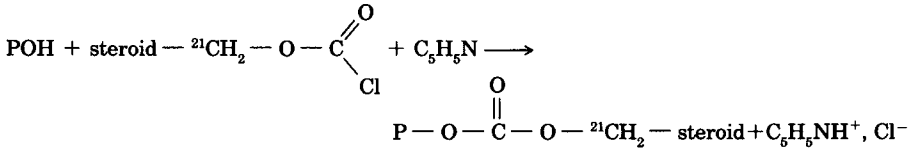
Condensation of excess phosgene ([$COCl_2$]/[steroid] = 4) onto H-1 and D-1 in homogeneous THF solution (see the Experimental section) leads quantitatively and selectively to the ^{21}C-chloroformates H-7 and D-3, respectively:



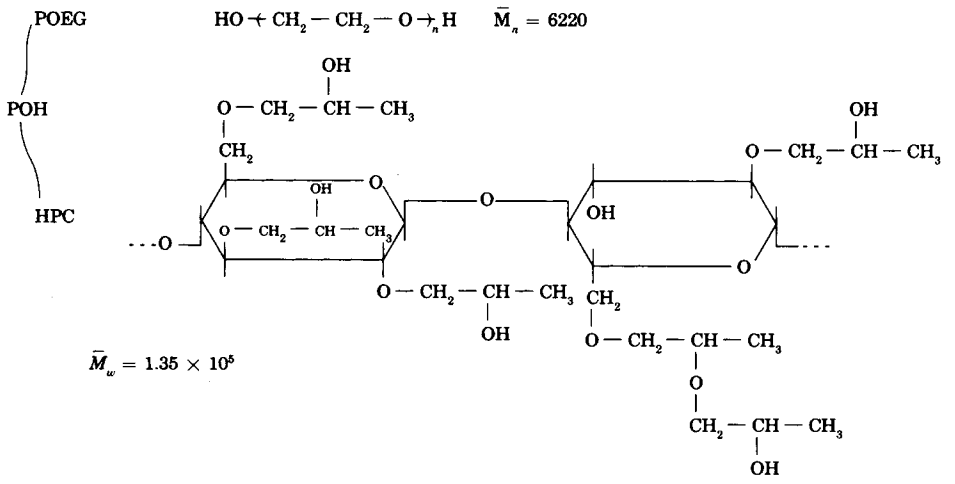
These derivatives are readily identified by IR spectroscopy: $V(C = O) = 1775\ cm^{-1}$ and $V(C—Cl) = 692\ cm^{-1}$ for the chloroformate function. Moreover, all the chlorine content (in good agreement with the calculated one) is easily removed by smooth hydrolysis: No side reaction leading to alkyl chlorides is observed in the selected experimental conditions.

Condensation of the Steroid-^{21}C-Chloroformates onto POEG and HPC

Condensation of H-7 and D-3 onto POEG and HPC was carried out in homogeneous THF solution in presence of pyridine as HCl scavenger according to the general scheme:



with



The drug binding on the macromolecular carrier through carbonate linkage is easily identified by its characteristic IR spectrum: $V(C = O)$ carbonate $= 1760\ cm^{-1}$. The composition of the modified polymers was readily derived from the analysis of their UV spectra (TFE solution) using the steroid-^{21}C-isopropylcarbonates H-6 and D-2 as reference models:

$\pi \rightarrow \pi^*$ transition of the conjugated A ring { H-6: $\epsilon = 16200\ L\ mol^{-1}\ cm^{-1}$ at $\lambda = 248$ nm; D-2: $\epsilon = 16700\ L\ mol^{-1}\ cm^{-1}$ at $\lambda = 248$ nm }

The most representative experimental results are collected in Table VI.

In spite of a greater reactivity and a lesser steric hindrance of the primary alcohol end groups of POEG, the condensation yields are similar for both polymers: They remain rather weak, within the range 20–40%, quite comparable to that recently observed for the system poly(vinyl alcohol)-*n*-butylchloroformate in analogous conditions.[35] On the other hand, D-3 appears

TABLE VI
Condensation of the Steroid-^{21}C-Chloroformates on POEG and HPC[a]

	Polymer carrier		Steroid reagent			Steroid bound polymer	
Run	Nature	[OH] (mol L^{-1})	Nature	[OCOCl]/ [OH]	Reaction yields (%)	Steroid (wt %)	Water solubility
1	POEG	0.027	H-7	1.20	34.1	3.80	+
2	POEG	0.051	H-7	1.20	38.0	4.21	+
3	POEG	0.032	D-3	1.20	41.7	4.97	+
4	HPC	0.184	H-7	0.10	27.0	7.98	–
5	HPC	0.217	H-7	0.05	20.8	3.24	+
6	HPC	0.228	D-3	0.05	38.6	6.28	–
7	HPC	0.240	D-3	0.033	41.5	4.60	+

[a] All reactions carried out at 30°C for 18 h, with a constant stoechiometric ratio [pyridine]/[H-7 or D-3] = 1.

slightly more reactive than H-7. Some attempts to improve the process were unsuccessful, as illustrated below:

—As a very disappointing result, activation of the alcohol function as sodium alcoholate (titration with the radical anion sodium naphtalenide[36]), actually lowers the yields from 34% and 27% to 17% for runs 1 and 4, respectively.

—An increase of the reaction temperature to 65°C (we have checked the lack of any decarboxylation of the chloroformates[37]) does not afford any significant improvement.

The poor efficiency of the condensation process may arise at least partly from the very low concentration of reacting functional groups implied by the poor solubility of the steroid derivatives in THF. By analogy with the polyhydroxyalkly–L-glutamine-norethyndrone–17-β-chloroformate system,[17] it may be expected that the use of a dipolar aprotic solvent such as dimethylformamide could allow a significant increase of the reagent concentration and lead to a definite improvement of the condensation yield, but this is out of the scope of the present work.

If the low substitution degree of HPC together with the high molecular weight of the polymeric precursor contribute to a very good chemical homogeneity of the functionalized HPC,[38] incomplete condensation on the POEG actually leads to a mixture involving chains with two (molar fraction F_2), only one (molar fraction F_1) and even zero (molar fraction F_0) steroid end groups. Assuming identical reactivity for the two POEG end groups, F_2, F_1, and F_0 values are easily derived from the fraction F of condensed OH functions: $F_2 = F^2$, $F_1 = 2F(1 - F)$, $F_0 = (1 - F)^2$. Experimental data of Table VI point out that F_0 is never negligible and is actually as high as 0.43 for run 1 corresponding to the least modified POEG.

Finally the good agreement observed between calculated and experimental $\overline{M}_w$ molecular weights shows the lack of any significant degradation or crosslinking reaction, as illustrated for the two representative runs 3 and 5:

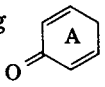
Run 3: POEG/D-3, $\overline{M}_w$ calcd = 6620, $\overline{M}_w$ exptl = 6020

Run 7: HPC/D-3, $\overline{M}_w$ calcd = 1.35×10^5, $\overline{M}_w$ exptl = 1.41×10^5

CONCLUSION

Two complementary strategies were investigated for the synthesis of biocompatible and hydrophylic steroid polymer systems, with the common requirement of a labile carbonate bond between the drug and the macromolecular carrier.

(a) Radical copolymerization of hydrocortisone-^{21}C-vinylcarbonate with *N*-vinylpyrrolidone or *N*-[tris(hydroxymethyl)methyl]acrylamide in homogeneous DMAC solution is a complex process characterized by the very low reactivity of the steroid monomer which apparently behaves as an efficient degradation transfer agent. Possible penultimate effects or propagation $\rightleftarrows$ depropagation equilibria may perturb the copolymerization and they would be compatible with the unability of the steroid vinylcarbonate to homopolymerize. However, it is still possible to obtain homogeneous high mo-

lecular weight copolymers ($\overline{M}_w \sim 10^4$–10^5) with high enough hydrocortisone content (0.03–0.10 mole fraction). Finally transposition to dexamethasone cannot be safely assumed straightforward, since its strongly conjugated A ring  may interfere with the radical chain reaction process.

(b) Condensation of the hydrocortisone or dexamethasone-^{21}C-chloroformates onto poly(oxyethyleneglycol) or hydroxypropylcellulose in homogeneous THF solution is free form side reaction but of low efficiency (yields <50%). POEG cannot be thus considered as a good precursor since its derivatives show a too high compositional heterogeneity.

In spite of an important steroid loss in both methods, the derived copolymers may be readily obtained with sufficient control of the most important structural parameters, such as molecular weight, composition, and chemical homogeneity. Because of the rather wide range of biocompatible hydroxylated polymers, the condensation of the steroid chloroformates may appear of more interest and would be worth of further optimization.

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