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Macromolecular prodrugs. III. Esters of fenoprofen and probenecid

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Fenoprofen and probenecid were covalently linked by ester bonds to α,β-poly(N-hydroxyethyl)-*DL*-aspartamide (PHEA), hydrophilic polymer, previously proposed as a drug carrier and plasma expander. In addition, two simple esters of fenoprofen and probenecid were prepared. Ester bondings were achieved via benzotriazolides synthesized by the reaction of 1-benzotriazole carboxylic acid chloride (BtcCl) with fenoprofen and probenecid, respectively. Release of the drug from PHEA-drug esters in alkaline medium was studied.

Keywords: prodrug, macromolecular carrier, α,β-poly(N-hydroxyethyl)-*DL*-aspartamide, fenoprofen ester, probenecid ester, 1-benzotriazole carboxylic acid chloride

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In recent years, there has been huge interest in the development of prodrug derivatives, biological precursors metabolized to active substances. Ester prodrugs should be considered first if the structure of the drug molecule allows such derivation since they readily undergo chemical and enzymatic hydrolysis, thus releasing active compounds. On the other hand, polymeric prodrugs in which drugs are covalently linked to polymeric matrices are presently suggested as an effective means of prolonging the pharmacological activity and minimizing unfavourable side-effects and toxicity. The use of polymers as prodrugs can decrease the required dose and increase the solubility of the drug. It can also alter the body distribution and ensure an adequate drug delivery to target cells or tissues (1).

α,β-poly(N-hydroxyethyl)-*DL*-aspartamide (PHEA) is a specially interesting and promising drug carrier since it is hydrosoluble, nontoxic, nonantigenic and biodegradable when exposed to a complex set of enzymes (2 – 4). In addition, PHEA is an easily and inexpensively prepared polymer. Several drugs from the group of hydrazide, carboxylic and amino acids have been covalently linked to PHEA (5 – 10).

In this paper, we describe the ester preparation of two well known drugs and their binding to PHEA. The first drug is fenoprofen (α-methyl-3-phenoxybenzeneacetic acid), nonsteroidal anti-inflammatory drug (NSAID), and the second is a uricosuric agent, probenecid [*p*-(dipropylsulfamoyl)benzoic acid].

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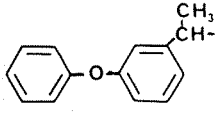
EXPERIMENTAL

Melting points are uncorrected. IR- and UV-spectra were recorded on Perkin-Elmer 457 and Pye Unicam SP-100 spectrometers, respectively. ^{1}H-NMR spectra were taken on a Varian Gemini 300. For thin layer chromatography, silica gel sheets, Kieselgel 60 F_{254} Merck, were used. The solvent system was chloroform/methanol, 9 : 1. Column chromatography was performed on silica gel 0.063 – 0.200 mm with chloroform/methanol, 9.5 : 0.5, as eluent. Fenoprofen-Ca and probenecid were purchased from Sigma (St. Louis, U.S.A.). All solvents were of analytical grade quality and were dried and distilled prior to use. 1-benzotriazole carboxylic acid chloride (BtcCl) and α,β-poly(N-hydroxyethyl)-*DL*-aspartamide (PHEA) were prepared as described earlier (11, 10). The mass-average molecular mass (M), of PHEA was 45000, according to the Mark-Houwink relationship $[\eta] = 2.32 \times 10^{-3}\ M^{0.87}$ (12). Viscosity measurements were carried out at 25 °C on an Ostwald viscosimeter with the outflow time for water from 100 to 200 s. Intrinsic viscosities [η] were determined by measuring the reduced viscosities in a concentration range from 2 to 8 mg mL^{-1} and extrapolating to zero concentration.

Benzotriazolides **2a, b.** – 1.82 g (0.01 mol) BtcCl (**1**) in 30 mL toluene was added dropwise to a solution of 0.01 mol of drug (2.42 g fenoprofen or 2.85 g probenecid) and 1.02 g (0.01 mol) triethylamine in 30 mL toluene. The reaction mixture was stirred for 2 hrs at room temperature and then extracted three times with water. The organic layer was dried over anhydrous sodium sulfate and evaporated. A mixture of ether/petrolether

*Table I. Benzotriazolides R-COBt (**2a** and **2b**)*

Compds. 2a, b	R	Yield (%)	M.p (°C)	Molecular formula	Elemental analysis C calcd./found	H calcd./found	N calcd./found	IR(KBr): ν_{max} (cm^{-1})
2a	Fen	90	64 – 66	$C_{21}H_{17}N_3O_2$ (343.39)	73.45 / 73.63	4.99 / 4.90	12.24 / 12.01	1740, 1585, 1485, 1380, 1240, 955, 755, 690
2b	Pro	70	oil	$C_{19}H_{22}N_4O_3S$ (386.47)	59.05 / 59.00	5.74 / 5.84	14.50 / 14.41	3000 – 2840, 1705, 1600, 1345, 1160, 940, 785, 760, 750

Fen = 

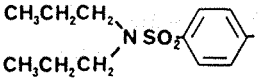
Pro = 

*Table II. ^{1}H-NMR spectra of benzotriazolides **2a** and **2b***

Compds. 2a, b	^{1}H-NMR ($CDCl_3$) (ppm)
2a	1.7323 – 1.7550 (d, 3H, –CH_3), 5.3449 – 5.4148 (k, 1H, –C**H**–CH_3) 6.8346 – 8.2935 (m, 13H, aromates)
2b	0.8827 – 0.9320 (t, 6H, –CH_3), 1.5443 – 1.6692 (m, 4H, – $CH_2CH_2CH_3$) 3.1366 – 3.1877 (t, 4H, –CH_2N), 7.5636 – 8.4123 (m, 8H, aromates)

was added to the residue and the pure product **2a, b** was filtered off. Yields and analytical data are summarized in Table I, and ^{1}H-NMR spectra in Table II.

Esters 3a, b. – A solution of 0.006 mol benzotriazolide **2a, b** and 2.43 g (0.024 mol) triethylamine in 30 mL alcohol was refluxed for 3 hrs. The solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel column. Yields and analytical data are summarized in Table III.

Table III. Esters R–COOR^1 (3a and 3b)

Compds. 3a, b	R	R^1	Yield (%)	M.p. (oC)	Lit. m.p. (°C)	IR(KBr or film): ν_{max} (cm^{-1})	UV: λ_{max} (nm)
3a	Fen	Et	82	oil	oil	2980, 1735, 1585, 1485, 1435, 1270 – 1160, 935, 760, 690	264, 273, 279*
3b	Pro	Me	80	58 – 59	59 – 60**	2980, 1730, 1345, 1285, 1160, 1115, 980, 745, 735, 695	223, 252$^+$

Et = ethyl, Me = methyl
* γ = 223.9 μg mL^{-1} (c = 8.28×10^{-4} mol L^{-1}) (EtOH)
** Ref. (13)
$^+$ γ = 16.0 μg mL^{-1} (c = 5.34×10^{-5} mol L^{-1}) (MeOH)

PHEA-drug esters (4a, b). – A solution of 1.43 g PHEA, 0.003 mol benzotriazolide **2a, b** and 2.02 g (0.02 mol) triethylamine in 45 mL DMF was left for three days at room temperature with occasional shaking. The solvent was evaporated in vacuo to a small volume. The polymeric product **4a** was precipitated by adding acetone and product **4b** by adding ether. The products were filtered off and washed several times with a small amount of acetone and ether, respectively, until benzotriazole was completely washed off (TLC control). Yields and spectral data of products **4a, b** are summarized in Table IV.

Table IV. PHEA-drug esters (4a and 4b)

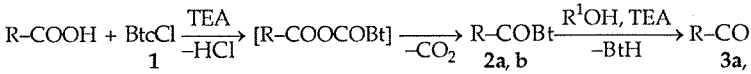
Compds. 4a, b	R	Yield (%)	Drug loading (%)	IR(KBr): ν_{max} (cm^{-1})	UV: λ_{max} (nm)
4a	Fen	76	23.3	3680 – 2700, 3300, 3060, 2930, 1735, 1650, 1525, 1235, 1050	276, 273, 280*
4b	Pro	91	18.9	3700 – 2400, 3280, 2940, 2680, 2490, 1725, 1645, 1525	252** 255$^+$

* γ = 437.1 μg mL^{-1} (H_2O); ** γ = 60.0 μg mL^{-1} (H_2O); $^+$ γ = 56.0 μg mL^{-1} (borate buffer, pH = 9.2)

Release of drugs from PHEA-Fen (4a) and PHEA-Pro (4b) in alkaline medium. – A solution of **4a** (γ = 476.9 μg mL^{-1}) or adduct **4b** (γ = 136.23 μg mL^{-1}) in 5×10^{-2} mol L^{-1} NaOH, in a well stoppered silica cell, was thermostated at 37 ± 0.1 °C. The drug release was measured by UV-spectrometry at 273 nm and 249 nm, respectively, at suitable time intervals. Rate constants were computed using a nonlinear square fitting program.

RESULTS AND DISCUSSION

In our previous paper (9), a new method for the preparation of NSAIDs esters is described. In this paper, the same method is extended to fenoprofen and probenecid. In the first step, carboxylic groups of these drugs react with BtcCl. After decarboxylation, the thus formed unstable mixed anhydrides give benzotriazolides **2**. The structures of these products are confirmed by elemental analysis and both IR- and ^{1}H-NMR spectroscopies (see Tables I and II). The benzotriazolides readily react with hydroxyl compounds: simple alcohols as well as polyhydroxyl compounds, such as PHEA, affording esters **3** and PHEA-drug esters **4**. The reactions proceed in mild conditions in the presence of triethylamine (TEA) as a catalyst. In this way, fenoprofen ethyl ester (**3a**), probenecid methyl ester (**3b**) and two PHEA-drug esters PHEA-Fen (**4a**) and PHEA-Pro (**4b**) are prepared (see Schemes 1 and 2). All data relating to these esters are given in Tables III and IV.



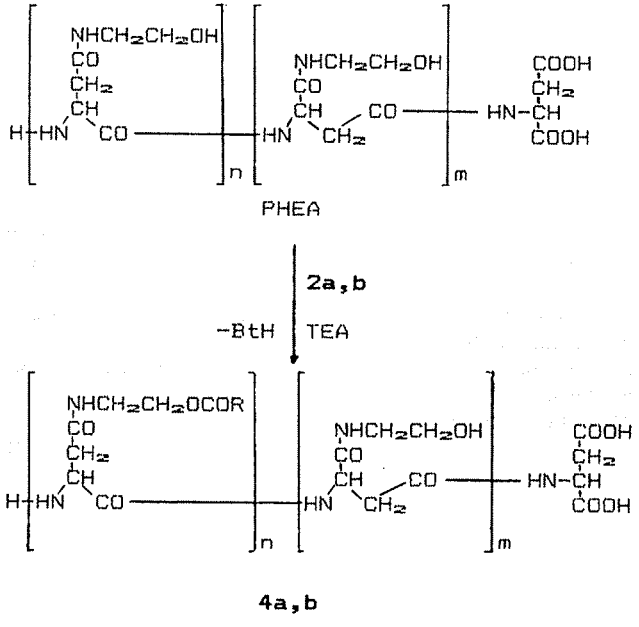
TEA = triethylamine
Btc = 1-benzotriazolylcarbonyl
Bt = 1-benzotriazolyl
BtH = benzotriazole
a R = Fen, R^1 = Et
b R = Pro, R^1 = Me

Scheme 1

The proof that the drugs are covalently bound to polymer backbone in products **4a** and **4b** can be found in IR- and UV-spectra. The IR-spectra show an ester carbonyl band centered at 1735 and 1725 cm^{-1}, respectively. Products **4a, b** absorb the UV-light in practically the same absorption ranges as the corresponding simple esters **3a, b**, whereas PHEA itself shows no UV-absorption at these wavelengths. The absence of free drugs in **4a, b** is confirmed by TLC.

One can vary the drug content in PHEA-drug esters and thus control the products solubility. The molar ratio of PHEA and **2**, allowing a substitution of approximately 35% of the available hydroxyl groups of PHEA, has been chosen, affording the water soluble products like PHEA itself.

The drug loading in PHEA-drug esters is estimated by UV-spectroscopy using the molar absorption coefficient for fenoprofen ethyl ester $\varepsilon_{273} = 1811$ in ethanol ($c = 8.28 \times 10^{-4}$ mol L^{-1}) and for probenecid methyl ester $\varepsilon_{252} = 10674$ in methanol ($c = 5.34 \times 10^{-5}$ mol L^{-1}). The load of fenoprofen in **4a** is 23.3% and the load of probenecid in **4b** is 18.9%.



Scheme 2

The release of active substances is studied based on the hydrolysis of PHEA-drug esters in alkaline medium. First-order release rate constants for fenoprofen ($k = 5.63 \times 10^{-2}$ min^{-1}) and probenecid ($k = 6.02 \times 10^{-2}$ min^{-1}) have been obtained. The results are presented in Tables V and VI.

*Table V. Release of fenoprofen from PHEA-Fen (**4a**)*

Time (min.)	1.83	3.89	6.22	8.90	12.09	15.99	21.01	28.08	40.18
% of released drug	10	20	30	40	50	60	70	80	90

*Table VI. Release of probenecid from PHEA-Pro (**4b**)*

Time (min.)	1.75	3.71	5.93	8.49	11.51	15.22	20.00	26.73	38.25
% of released drug	10	20	30	40	50	60	70	80	90

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SAŽETAK

Makromolekularni prolijekovi. III. Esteri fenoprofena i probenecida

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Fenoprofen i probenecid povezani su kovalentno esterskim vezama na α,β-poli(N-hidroksietil)-*DL*-aspartamid (PHEA), vodotopljivi polimer ranije predložen kao makromolekularni nosač lijekova i plazma ekspander. Osim toga opisana je priprava, dva jednostavna estera fenoprofena i probenecida. Esterska veza postignuta je preko benzotriazolida koji su priređeni reakcijom klorida 1-benzotriazol karboksilne kiseline (BtcCl) s fenoprofenom, odnosno probenecidom. Proučavana je kinetika otpuštanja lijeka iz PHEA-lijek konjugata u lužnatom mediju.

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