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Glycolamide Esters as Biolabile Prodrugs of Carboxylic Acid Agents: Synthesis, Stability, Bioconversion, and Physicochemical Properties

NIELS MØRK NIELSEN AND HANS BUNDGAARD[x]

Abstract ☐ Benzoic acid esters of various substituted 2-hydroxyaceta-mides (glycolamides) were found to be hydrolyzed extremely rapidly in human plasma solutions, the half-lives of hydrolysis being <5 s in 50% plasma solutions for some *N,N*-disubstituted glycolamide esters. The rapid rate of hydrolysis could be largely attributed to cholinesterase (also called pseudocholinesterase) present in plasma. From a study of a variety of substituted glycolamide esters and structurally related esters, the most prominent structural requirement needed for a rapid rate of hydrolysis was found to be the glycolamide ester structure combined with the presence of two substituents on the amide nitrogen atom. A structural similarity of such esters with benzoylcholine, a good substrate for cholinesterase, was put forward. Esters of *N,N*-disubstituted glycola-mides are suggested to be a useful biolabile prodrug type for several carboxylic acid agents. The esters combine a high susceptibility to undergo enzymatic hydrolysis in plasma with a high stability in aqueous solution. Furthermore, as demonstrated with the benzoic acid model esters, it is feasible to obtain ester derivatives with almost any desired water solubility or lipophilicity with retainment of marked lability to enzymatic hydrolysis.

In recent years much attention has been focused on the use of bioreversible derivatives (prodrugs) in order to improve the delivery characteristics of various drugs.[1-4] A basal requisite for the usefulness of the prodrug approach is the ready availability of chemical derivative types which satisfy the prodrug requirements, the most prominent of these being reconversion of the prodrug to the parent drug in vivo. Many types of bioreversible derivatives for a great number of different functional groups and chemical entities have been exploited for utilization in the design of prodrugs, and it is now feasible to obtain prodrug derivatives of many different drug molecules.[5,6]

There is, however, still a need of exploring new prodrug types, even in the field of carboxylic acid esters. Such derivatives are probably the best known prodrugs, major reasons for this being the occurrence of carboxylic or hydroxyl groups in many drug molecules, along with the ready availability of enzymes in the organism capable of hydrolyzing most esters. The distribution of esterases is ubiquitous,

and several types are found in the blood, liver, and other organs or tissues.[7-10] Several examples of ester prodrugs can be found in various reviews.[1,5,11-13]

Sometimes, however, many aliphatic or aromatic esters are not sufficiently labile in vivo to ensure a sufficiently high rate and extent of prodrug conversion. For example, simple alkyl and aryl esters of penicillins are not hydrolyzed to the active free penicillin acid in humans and therefore have no therapeutic potential.[14,15] Similarly, the much reduced anti-inflammatory activity observed for the methyl or ethyl esters of naproxen,[16] fenbufen,[17] and indomethacin,[18] relative to that of the free acids, may be ascribed to the resistance of the esters to hydrolysis in vivo.[19] In the field of angiotensin-converting enzyme inhibitors, ethyl esters have very often been developed as prodrugs for the parent active carboxylic acid drugs in order to improve the oral bioavailability of these hydrophilic agents.[20] However, the limited susceptibility of these esters to undergo enzymatic hydrolysis in vivo has been shown to result in incomplete availability of the active parent acid in a number of cases including enalapril,[21-23] pentopril,[24-26] ramipril,[27] and cilazapril.[28] Plasma enzymes do not hydrolyze enalapril, and the necessary conversion of this ethyl ester to the active enalaprilic acid takes place predominantly in the liver.[21,22] As recently suggested,[22] liver function may thus be a very important determinant for the bioactivation of enalapril and hence its therapeutic effect. In the case of pentopril, the ethyl ester prodrug is also highly stable in human plasma, and <50% of an oral dose of the prodrug ester appears to be de-esterified in vivo to the active parent acid.[24-26]

As has been demonstrated in the case of penicillins[29,30] and cephalosporins,[30-34] these shortcomings of some ester prodrugs may be overcome by preparing a double ester type, (acyloxy)alkyl or [(alkoxycarbonyl)oxy]alkyl esters, which in general shows a higher enzymatic lability than simple alkyl esters.[5] The utility of this double ester concept in prodrug design is, however, for many drugs, limited by the poor water solubility of the double esters. Also, such esters are in many cases oils,[35,36] thus creating pharmaceutical formulation problems.

In attempting to explore new generally applicable ester prodrug types possessing a high enzymatic rate of hydrolysis in plasma or blood, we recently discovered that esters of certain 2-hydroxyacetamides (glycolamides) are cleaved remarkably rapidly in human plasma. In the present work, part of which has been described in a preliminary manner,[19] a large series of glycolamide esters of benzoic acid, used as a model of a carboxylic acid drug, have been prepared and their chemical- and enzyme-mediated hydrolysis has been investigated. Human plasma was used as the enzyme source in most cases, instead of various specific enzyme preparations, in order to have conditions simulating those occurring in vivo. Water solubilities and octanol–water partition coefficients of the esters were also determined in order to test the feasibility of obtaining glycolamide ester derivatives with almost any desired hydrophilicity or lipophilicity, while still maintaining a high rate of plasma-catalyzed hydrolysis.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. The ^{1}H NMR spectra were run on a Varian 360 L instrument using tetramethylsilane as an internal standard. Measurements of pH were done at the temperature of study using a Radiometer type PHM 26 instrument. Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer, equipped with a thermostated cell compartment, using 1-cm quartz cuvettes. The HPLC analysis was generally carried out with a system consisting of a Kontron T-414 LC pump, a Kontron Uvikon 740 LC detector operated at a fixed wavelength (215 nm), and a Rheodyne 7125 injection valve with a 20-μL loop. A column, 100 × 3 mm, packed with Chromspher C8 (5-μm particles; Chrompack, Holland) and equipped with a guard column, was used. In some cases, a Spectra-Physics model 3500 B instrument equipped with a variable wavelength detector and a 10-μL loop injection valve was used. A column, 250 × 4 mm, packed with LiChrosorb RP-8 (7-μm particles; E. Merck, F.R.G.), was used with this apparatus.

Synthesis of 2-(Benzoyloxy)acetamides—The glycolamide esters were generally synthesized by reacting (benzoyloxy)acetyl chloride, prepared as outlined in Scheme I, with the appropriate amine in benzene (Method I) or alkaline aqueous solution (Method II), or by esterifying benzoic acid with the appropriate 2-chloroacetamide in dimethylformamide (Method III; Scheme I).

(Benzoyloxy)acetyl Chloride—2-Chloroacetamide (18.7 g, 0.2 mol) was added to a solution of sodium benzoate (28.8 g, 0.2 mol) and NaI (7.5 g, 0.05 mol) in 150 mL of H_2O. The mixture was stirred at 90 °C for 6 h. Upon cooling to 4 °C, 2-(benzoyloxy)acetamide (1) precipitated and was isolated by filtration. Recrystallization from EtOH:H_2O yielded 32.2 g (90%), mp 120.5–121 °C; lit. mp 121–122 °C.[37,38] Compound 1 (19.7 g, 0.11 mol) was added to 200 mL of 7.8 M HCl. The mixture was stirred at 75 °C for 10 min to complete

Scheme I

hydrolysis of the amide bond, as reported previously.[39] Upon cooling, benzoylglycolic acid precipitated. It was dried and recrystallized from benzene to give 15.8 g (80%), mp 111–112 °C; lit. mp 111–112 °C.[39] A mixture of benzoylglycolic acid (12.6 g) and SOCl$_2$ (15 mL) was refluxed for 3 h. Excess SOCl$_2$ was removed under reduced pressure and the crude (benzoyloxy)acetyl chloride obtained was purified by distillation under reduced pressure (88% yield), mp 25–26 °C; lit. mp ~22 °C.[39]

Method I—2-(Benzoyloxy)-N,N-diallylacetamide (17)—A solution of diallylamine (1.23 mL, 10 mmol) in 5 mL of benzene was added, while stirring, to a solution of (benzoyloxy)acetyl chloride (0.80 g, 4 mmol) in 4 mL of benzene. The reaction mixture was stirred at room temperature for 1.5 h and washed with H_2O (10 mL), 2 M HCl (10 mL), and H_2O (10 mL). The solution was dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure. The residue crystallized from ether:petroleum ether by standing overnight at −20 °C, giving 0.78 g (75%) of the title compound. Compounds 4–8, 13–15, 16, 18, 19, 25, 28, 29, and 31–38 were prepared according to this procedure.

2-(Benzoyloxy)-(N-methyl-N-β-hydroxyethyl) acetamide (21)—A solution of (benzoyloxy)acetyl chloride (1.6 g, 8 mmol) in 8 mL of benzene was mixed with N-methylethanolamine (1.8 g, 24 mmol). The solution was stirred at room temperature for 3 h and then concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and H_2O (10 mL). The layers were separated, and the organic phase was washed with 2 M HCl (5 mL) and H_2O (5 mL), dried over Na$_2$SO$_4$, and evaporated under reduced pressure. The residue crystallized by trituration with ether and standing overnight at −20 °C. The compound was filtered off and recrystallized from EtOAc:petroleum ether, giving 1.1 g (50%) of the title compound. Compounds 22, 23, and 24 were obtained in a similar way.

1-Methyl-4-(benzoyloxyacetyl)piperazine Hydrochloride (39)—A solution of 1-methylpiperazine (0.40 g, 4 mmol) in 5 mL of benzene was added in a dropwise manner, while stirring, to a solution of (benzoyloxy)acetyl chloride (0.80 g, 4 mmol) in 10 mL of benzene. After the addition was completed (~10 min), the reaction mixture was stirred at room temperature for 1 h. Ether (10 mL) was added and the mixture was filtered. The white crystalline compound obtained was washed with ether and finally recrystallized from EtOH, yielding 0.70 g (59%) of the title compound. Compounds 30 and 40 were prepared in a similar way.

Method II—2-(Benzoyloxy)-(N-methyl-N-ethoxycarbonylmethyl)acetamide (27)—A solution of (benzoyloxy)acetyl chloride (0.80 g, 4 mmol) in 4 mL of benzene was added to a cooled (~5 °C) solution of sarcosine ethyl ester hydrochloride (Fluka AG, Switzerland; 0.84 g, 12 mmol) in 6 mL of 2 M NaOH. The mixture was stirred vigorously at room temperature for 2 h. The layers were separated and the aqueous phase was re-extracted with EtOAc (20 mL). The combined organic extracts were washed with 2 M HCl (5 mL) and H_2O (5 mL), dried over Na$_2$SO$_4$, and evaporated under reduced pressure. The oily residue obtained crystallized upon trituration with petroleum ether at −20 °C. Recrystallization from ether:petroleum ether yielded 0.68 g (61%) of the title compound.

Compounds 2, 9–11, 20, 43, and 44 were obtained similarly with the following modifications. For 20, NaHCO$_3$ was used instead of NaOH, and in the case of 11, no NaOH was added. In the cases of 2, 9, and 10, the glycolamide esters precipitated in the reaction solutions and they were simply isolated by filtration. The sarcosinamide hydrochloride used in the synthesis of 20 was prepared as previously described.[40]

2-(Benzoyloxy)-(N-methyl-N-acetic acid)acetamide (26)—A solution of (benzoyloxy)acetyl chloride (0.80 g, 4 mmol) in 4 mL of benzene was added to a solution of sarcosine (0.71 g, 8 mmol) in 5 mL of 2 M NaOH. The mixture was stirred at room temperature for 1 h. The aqueous layer was separated and acidified with 2 M HCl to a pH of ~2.5. Upon standing at 4 °C for 3 h, a precipitate formed. The precipitate was filtered off, washed with H_2O, and recrystallized from EtOH:H_2O, affording 0.77 g (77%) of the title compound. Compounds 41 and 42 were obtained in a similar way.

Method III—2-(Benzoyloxy)-N,N-dimethylacetamide (12)—Benzoic acid (2.44 g, 20 mmol) and 2-chloro-N,N-dimethylacetamide (2.43 g, 20 mmol) were dissolved in 10 mL of N,N-dimethylformamide. Then, NaI (0.3 g, 2 mmol) and triethylamine (2.02 g, 20 mmol) were added and the mixture was stirred at room temperature overnight. After addition of 50 mL of H_2O, the reaction mixture was extracted twice with EtOAc (50 mL). The combined extracts were washed with
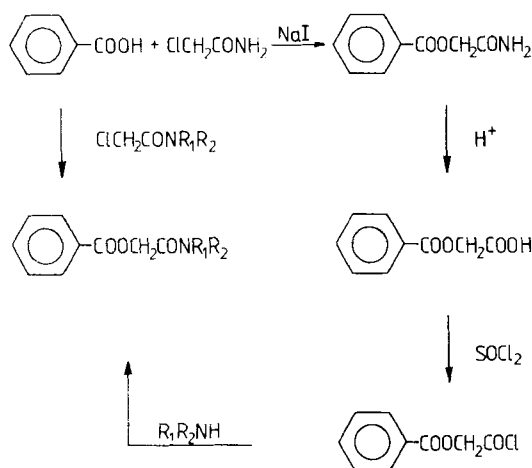
a 1% solution of sodium thiosulfate, a 2% solution of $NaHCO_3$, and H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue obtained was recrystallized from $EtOH:H_2O$ to give 3.5 g (85%) of the title compound. Compounds 3 and 14 were prepared by the same procedure.

Melting points of the glycolamide esters are given in Table I. Elemental analyses (C, H, and N) were in all cases within ±0.4% of the theoretical values. The ^1H NMR spectra of the esters were consistent with the structures.

Preparation of Other Esters—2-(Benzoyloxy)-(N-acetyl)aceta-mide (51)—A mixture of 2-(benzoyloxy)acetamide (1; 1.79 g, 10 mmol), Ac_2O (2.04 g, 20 mmol), and two drops of concentrated H_2SO_4 was heated at 135–140 °C for 3 min and then poured into 50 mL of cold H_2O. The precipitate formed was filtered off and recrystallized from $EtOH:H_2O$ to give 1.7 g (77%) of the title compound.

2-(Benzoyloxy)-(N-methyl-N-acetyl)acetamide (52)—A mixture of 2-(benzoyloxy)-N-methylacetamide (2; 0.193 g, 1 mmol), Ac_2O (1.02 g, 10 mmol), and one drop of concentrated H_2SO_4 was heated at

Table I—Physical Properties, Alkaline Hydrolysis, and Plasma Hydrolysis Data of Various O-Benzoylglycolamides
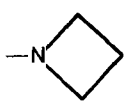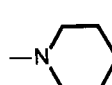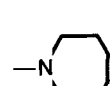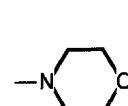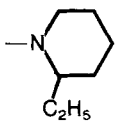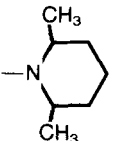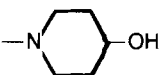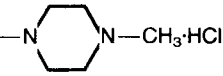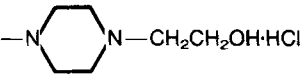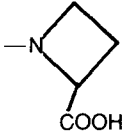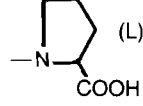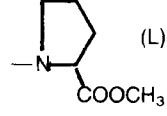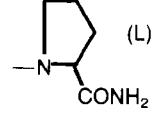

Compound	R_1	R_2	mp, °C	k_{OH}, M^{-1} min^{-1} [a]	$K_m \times 10^5$, M [b]	$V_{max} \times 10^5$, M/min [b]	$t_{1/2}$, min [c]
1	H	H	120.5–121	69.9	—	—	31
2	CH_3	H	111–112	85.0	—	—	9.3
3	C_2H_5	H	105–107	65.7	—	—	11.5
4	n-C_3H_7	H	89–90	52.1	—	—	11.2
5	i-C_3H_7	H	129–130	57.0	15.4	0.90	11.9
6	C_4H_9	H	69–70	69.1	—	—	7.1
7	t-C_4H_9	H	112–113	51.3	2.06	0.34	4.2
8	C_6H_{11}	H	130–131	63.1	—	—	9.7
9	CH_2CONH_2	H	151–152	95.9	—	—	25.0
10	$CH(CH_3)CONH_2$	H	201–202	65.0	—	—	42.5
11	$C(CH_2OH)_3$	H	126–127	100.6	—	—	69.1
12	CH_3	CH_3	81–82	19.2	11.0	51.0	0.15
13	CH_3	C_2H_5	~20	19.0	6.19	44.4	0.10
14	C_2H_5	C_2H_5	72–73	16.4	2.70	24.0	0.08
15	n-C_3H_7	n-C_3H_7	~20	14.2	1.21	5.97	0.14
16	i-C_3H_7	i-C_3H_7	105–106	10.7	1.38	11.8	0.08
17	$CH_2CH=CH_2$	$CH_2CH=CH_2$	42–43	18.4	1.40	12.0	0.08
18	C_4H_9	C_4H_9	~25	11.3	11.1	2.45	3.1
19	i-C_4H_9	i-C_4H_9	44–45	10.2	—	—	1.5
20	CH_3	CH_2CONH_2	101–102	23.5	3.35	17.3	0.13
21	CH_3	CH_2CH_2OH	78–80	16.1	2.36	8.25	0.20
22	C_2H_5	CH_2CH_2OH	79–80	16.4	2.83	12.5	0.16
23	CH_2CH_2OH	CH_2CH_2OH	80–82	13.8	—	—	0.42
24	$CH_2CHOHCH_3$	$CH_2CHOHCH_3$	105–106	13.6	—	—	0.20
25	$CH_2CH_2OCH_3$	$CH_2CH_2OCH_3$	57–58	13.4	—	—	0.25
26	CH_3	CH_2COOH	160–161	15.0	—	—	104
27	CH_3	$CH_2COOC_2H_5$	39–40	15.6	12.2	39.3	0.22
28	CH_3	C_6H_{11}	100–101	15.7	0.64	0.82	0.54
29	C_6H_{11}	C_6H_{11}	162–163	11.5	—	—	407
30	CH_3	$CH_2CH_2N(CH_3)_2$,HCl	158–159	17.8	—	—	0.12

Compound	—NR_1R_2	mp, °C	k_{OH}, M^{-1} min^{-1} [a]	$K_m \times 10^5$, M [b]	$V_{max} \times 10^5$, M/min [b]	$t_{1/2}$, min [c]
31		74–75	38.9	17.6	14.7	0.83
32		57.5–58	17.2	—	—	5.7
33		87–89	17.6	3.32	0.94	2.5
34		107–108	12.2	2.93	2.06	1.0
35		103–104	18.7	—	—	4.9

Table I—(continued)

Compound	R_1	R_2	mp, °C	k_{OH}, M^{-1} min^{-1} [a]	$K_m \times 10^5$, M [b]	$V_{max} \times 10^5$, M/min [b]	$t_{1/2}$, min [c]
36	—N(piperidine, 2-C_2H_5)		54–55	11.9	—	—	17.9
37	—N(piperidine, 2,6-di-CH_3)		118–118.5	10.1	0.83	1.43	0.40
38	—N(piperidine, 4-OH)		121–122	17.1	—	—	5.8
39	—N(piperazine, N'—CH_3·HCl)		227–228	21.4	8.55	0.47	12.7
40	—N(piperazine, N'—CH_2CH_2OH·HCl)		228–229	22.4	—	—	14.5
41	—N(azetidine, 2-COOH)		149–150	24.4	—	—	>24 h
42	—N(pyrrolidine, 2-COOH) (L)		116–117	19.3	—	—	>24 h
43	—N(pyrrolidine, 2-COOCH$_3$) (L)		72–73	16.2	6.80	2.49	1.9
44	—N(pyrrolidine, 2-CONH$_2$) (L)		194–195	25.9	9.49	2.83	2.3

[a] Second-order rate constant for hydroxide ion-catalyzed hydrolysis at 37 °C and $\mu = 0.5$. [b] Michaelis–Menten parameters for the hydrolysis of the esters in 50% human plasma solutions (pH 7.40) at 37 °C. [c] Half-lives of ester hydrolysis in 50% human plasma (pH 7.40) at 37 °C.

120 °C for 1 h and then poured into 20 mL of cold H_2O. The oily precipitate formed became crystalline after stirring for 1 h. The compound was filtered off and recrystallized from EtOH:H_2O to yield 0.17 g (72%) of white needles.
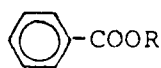
Compounds 48, 49, and 50 were prepared by reacting benzoic acid with the appropriate chloroalkyl amide according to the procedure described above for 12 (Method III). The chloroalkyl amides were prepared by reacting the appropriate chlorosubstituted acid chloride with dimethylamine or diethylamine in water or ethyl acetate.[41] Compound 53 was prepared by reacting benzoic acid with N-methyl-N-β-chloroethylacetamide according to method II, as described for 12. The acetamide derivative was prepared by treating N-methylethanolammonium hydrochloride with thionyl chloride followed by acetyl chloride, as described previously.[42]

Melting points of these esters are given in Table II. Elemental analysis (C, H, and N) were in all cases within ±0.4% of the theoretical values. The ^{1}H NMR spectra of the compounds were consistent with the structures.

Compound 54 was prepared by reacting benzoyl chloride with N,N-dimethylaminoethanol as described previously.[43] Benzoylcholine chloride (55) and the methyl (45), ethyl (46), and propyl (47) esters of benzoic acid were obtained from Fluka AG, Switzerland.

2-Hydroxy-N,N-dimethylacetamide—This compound was prepared by alkaline hydrolysis of 2-(benzoyloxy)N,N-dimethylacetamide (12). Compound 12 (20.7 g, 0.1 mol) was dissolved in 50 mL of EtOH by heating to 40–50 °C. Potassium hydroxide (2 M, 70 mL) was added and the mixture was allowed to stand at room temperature for 1 h. The pH of the solution was adjusted to 8–8.5 by the addition of 4 M

Table II—Physical and Hydrolysis Data of Various Benzoic Acid Esters

C_6H_5—COOR

Compound	R	mp, °C	k_{OH}, M^{-1} min^{-1}	$t_{1/2}$, min^a
45[b]	CH_3	—	13.6	108
46[b]	C_2H_5	—	6.6	210
47[b]	n-C_3H_7	—	5.5	46
12	$CH_2CON(CH_3)_2$	81–82	19.2	0.15
48	$CH_2CH_2CON(CH_3)_2$	oil	10.3	5.6
49	$CH_2CH_2CH_2CON(CH_3)_2$	40–41	1.8	14.1
50	$CH(CH_3)CON(C_2H_5)_2$ (D,L)	53–54	6.3	665
51	$CH_2CONHCOCH_3$	104–105	—	33.3
52	$CH_2CON(CH_3)(COCH_3)$	102–103	4.1	8.2
53	$CH_2CH_2N(CH_3)(COCH_3)$	oil	3.4	4.0
54	$CH_2CH_2N(CH_3)_2\cdot HCl$	147–148	9.8	0.04
55	$CH_2CH_2N(CH_3)_3^+\cdot Cl^-$	204–208	95.1	<0.04

[a] Half-lives of ester hydrolysis in 50% human plasma (pH 7.40) at 37 °C. [b] The data for these compounds are from a previous paper (ref 52); the half-lives shown are for 80% human plasma.

HCl, and the ethanol was removed under reduced pressure. The pH of the mixture was adjusted to 3.5–4 with 4 M HCl. Precipitated benzoic acid was filtered off, the filtrate was made alkaline (pH 8–9) with 2 M KOH, and thereafter evaporated under reduced pressure. The semisolid residue obtained was slurried in EtOAc (100 mL), and the mixture was heated to 60 °C, filtered, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give crude 2-hydroxy-N,N-dimethylacetamide. This extraction process was repeated twice. Recrystallization from ether:petroleum ether afforded 7.1 g (69%) of the title compound, mp 50–51 °C; lit. mp 51 °C.[44]

2-(Benzoyloxy)-(N-methyl-N-(N,N-dimethylglycyloxyethyl)-acetamide Monofumarate (56)—A mixture of 2-(benzoyl)-(N-methyl-N-β-hydroxyethyl)acetamide (21; 0.95 g, 4 mmol), N,N-dimethylglycine (0.41 g, 4 mmol), N,N'-dicyclohexylcarbodiimide (0.82 g, 4 mmol), and 4-toluenesulfonic acid (50 mg) in pyridine (10 mL) was stirred at room temperature for 24 h. Methylene chloride (20 mL) was added and the mixture was filtered. The residue obtained after evaporation of the filtrate under reduced pressure was extracted with 20 mL of boiling EtOAc. The extract was evaporated and the oily residue obtained was dissolved in ether (30 mL); a solution of fumaric acid (480 mg) in 8 mL of 2-propyl alcohol was added. After standing overnight at 4 °C, the title compound was isolated by filtration in a yield of 59%. Recrystallization from MeOH:ether gave an analytically pure product, mp 127–128 °C.

Anal.—Calc. for $C_{20}H_{26}N_2O_9$: C, 54.79; H, 5.98; N, 6.39. Found: C, 54.40; H, 5.99; N, 6.29.

Hydrolysis Kinetics in Human Plasma—The hydrolysis of the esters was generally studied in 0.01 M phosphate buffer (pH 7.40) containing 50% human plasma at 37 °C. Initial concentrations of the compounds were in the range 3×10^{-4}–2×10^{-5} M. The reactions were initiated by adding 50 μL of a stock solution of the compounds in acetonitrile or water to 5.00 mL of preheated plasma solutions. The solutions were kept in a water bath at 37 °C, and at appropriate intervals, 250-μL samples were withdrawn and added to 500 μL of MeOH or, in some cases, 500 μL of a 2% solution of $ZnSO_4\cdot7H_2O$ in MeOH:H_2O (1:1, v/v) in order to deproteinize the plasma. After immediate mixing and centrifugation for 3 min at 10 000 rpm, 10 or 20 μL of the clear supernatant was analyzed by HPLC for remaining ester derivative, as well as for benzoic acid.

In the HPLC method, a reversed-phase C8 column was eluted at ambient temperature with mixtures of MeOH and 0.01 M acetate buffer (pH 5.0) or MeOH and 1–2% H_3PO_4. The composition of the eluant was adjusted for each compound in order to provide an appropriate retention time and separation of ester and benzoic acid. The flow rate was 0.6–1.0 mL/min, and the column effluent was monitored at 215 or 235 nm. Quantation of the compounds was

done by measurement of the peak heights in relation to those of standards chromatographed under the same conditions.

Enzyme-Catalyzed Hydrolysis Studies—Type IV-S horse serum butyrylcholinesterase was purchased from Sigma Chemical Co., St. Louis, MO (lyophilized, essentially salt-free powder). Activity of the enzyme was reported as 13.2 units/mg solid, using butyrylcholine as substrate. α-Chymotrypsin was also purchased from Sigma Chemical Co. The reactions were studied at 37 °C in 0.01 M phosphate buffer solutions (pH 7.40) containing varying amounts of enzyme and the esters. The progress of ester hydrolysis was followed by determining remaining ester as a function of time using the HPLC methods described above. The enzymes present did not influence the chromatographic behavior of the esters.

Alkaline Hydrolysis of the Esters—The alkaline hydrolysis of the esters was studied in aqueous NaOH buffer solutions in the concentration range 0.001–0.22 M NaOH. A constant ionic strength (μ) of 0.5 was maintained for each solution by adding a calculated amount of KCl. The progress of the reactions was followed by direct UV spectrophotometry. The reactions were performed in 2.5-mL aliquot portions of buffer solutions in a thermostated quartz cuvette and were initiated by adding 25 mL of stock solutions of the derivatives in acetonitrile or H_2O to give a final concentration of ~1 $\times 10^{-4}$ M. The rate of hydrolysis of the esters was followed by monitoring the decrease in absorbance at 235 nm. Pseudo-first-order rate constants were determined from the slopes of linear plots of log $(A_t - A_\infty)$ versus time, where A_t and A_∞ are the absorbance readings at time t and infinity, respectively.

Stability Studies on Ester 12—The kinetics of the degradation of the glycolamide ester 12 was studied in aqueous buffer solutions at a constant temperature (37–70 °C). The buffers used were HCl, acetate, phosphate, borate, carbonate, and NaOH, each buffer having an ionic strength of 0.5 (maintained with KCl). The degradation was followed using an HPLC procedure capable of determining both intact ester and the products of hydrolysis, benzoic acid and benzoylglycolic acid. The chromatographic conditions used were: column: 100 × 3 mm, packed with Chrompher C8 (particle size 5 μm); solvent: MeOH:0.02 M acetate buffer (pH 5.0), (3:2, v/v); flow rate: 0.6 mL/min; detection: 215 nm. The initial concentration of ester 12 in the buffer solutions was 10^{-3} M in initial rate studies (pH 1–6) and ~10^{-4} M when the degradation course was followed to the end (at pH 1 and pH > 6).

Determination of Lipophilicity Parameters—The partition coefficients of the esters were determined in octanol–H_2O systems at 20–22 °C. The water and octanol were mutually saturated at 20–22 °C before use. The compounds were dissolved in the aqueous phase and the octanol–water mixtures were shaken for several hours to reach a

distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could readily be measured using the aforementioned HPLC procedures. The partition coefficients (P) were calculated from

$$P = \left(\frac{C_i - C_w}{C_w}\right)\left(\frac{V_w}{V_o}\right) \tag{1}$$

where C_i and C_w represent the solute concentrations in the aqueous buffer phase before and after distribution, respectively, V_w represents the volume of the aqueous phase, and V_o is the volume of the octanol phase. In some cases, the solute concentration in the octanol phase was also determined, the P values thus obtained being in good agreement with those derived on basis of eq 1.

The lipophilicity of the esters was also evaluated by means of reversed-phase HPLC. In this method, the capacity factor (k') of a solute is taken as a measure for the relative lipophilicity:

$$k' = (t_R - t_o)/t_o \tag{2}$$

where t_R is the retention time of the solute and t_o is the elution time of an unretained compound (KNO_3). The column used (100 × 3 mm, Chromspher C8) was eluted with MeOH:HNO_3 (0.01 M), (3:2, v/v; pH 2.4) at a rate of 0.7 mL/min.

Determination of Water Solubility—The solubility of the esters in water was determined at 22 °C by adding excess amounts of the compounds to water in screw-capped test tubes. The mixtures were placed in an ultrasonic water bath for ~10 min and then rotated on a mechanical spindle for 20–30 h. It was ensured that saturation equilibrium was established. Upon filtration, an aliquot of the filtrate was in most cases diluted with an appropriate amount of water and the mixture was analyzed by HPLC. The concentration of the compounds in their saturated solutions was calculated from the measured peak heights by reference to those of standards chromatographed under the same conditions.

Results and Discussion

Plasma-Catalyzed Hydrolysis of Glycolamide Esters— The rates of hydrolysis of the various benzoate esters were determined in 50% human plasma (pH 7.4) at 37 °C. In all cases, benzoic acid was found to be produced in quantitative amounts, as revealed by HPLC analysis.

At initial concentrations of 10^{-4} M, the progress of hydrolysis followed strict first-order kinetics only in some cases; examples (1, 2, and 35) are shown in Figure 1. In most cases, instead, mixed kinetics was observed, as demonstrated by the examples shown in Figure 2. As seen from this Figure, the
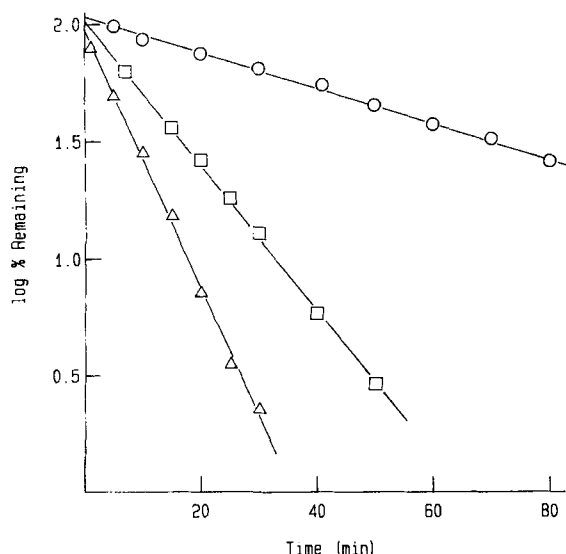

Figure 1—First-order plots for the hydrolysis of the O-benzoyl glycolamide esters 1 (O), 2 (□), and 35 (△) in 50% human plasma solutions (pH 7.40) at 37°C.
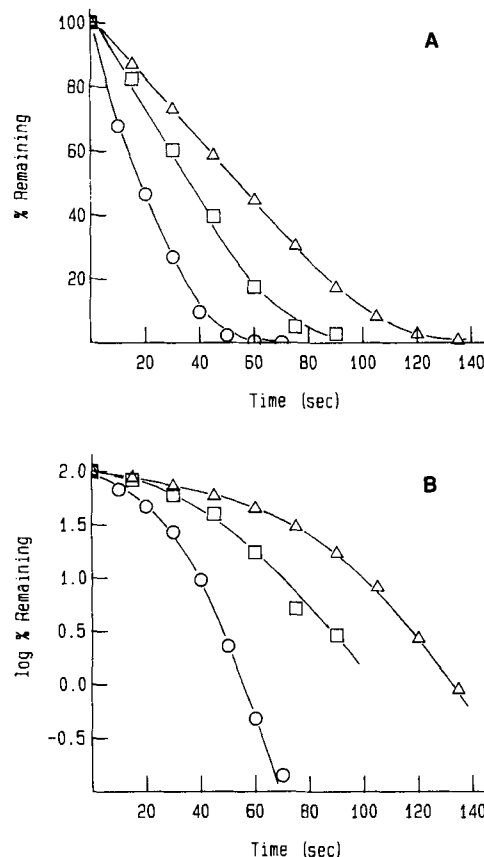

Figure 2—Plots showing the rate of hydrolysis of the O-benzoyl glycolamide esters 14 (O), 15 (△), and 17 (□) in 50% human plasma solutions (pH 7.40) at 37°C. In B the data from A are shown in the form of semilogarithmic plots. The curves in A are calculated from eq 4 and the rate parameters K_m and V_{max} given in Table I for the compounds.

rate of hydrolysis initially followed zero-order kinetics and, as the substrate depleted, it changed to follow first-order kinetics. This behavior is typical for enzyme-catalyzed reactions following Michaelis–Menten kinetics in which the initial substrate concentration is higher than the Michaelis constant K_m.[45]

The differential form of the Michaelis–Menten equation is:

$$-\frac{dS}{dt} = \frac{V_{max} S}{K_m + S} \tag{3}$$

where V_{max} is the maximum rate of substrate consumption, K_m is the Michaelis constant, and S is the substrate concentration. Integration of eq 3 gives[46]

$$V_{max}t = S_o - S + K_m \ln (S_o/S) \tag{4}$$

where S_o is the initial substrate concentration and S is the substrate concentration at time t.

By analyzing the progress curves according to eq 4, using iterative nonlinear regression analysis as described by Robinson and Characklis,[47] a reasonable fit of this equation to the curves was obtained, indicating that the enzymatic hydrolysis followed Michaelis–Menten kinetics. This also was confirmed by measuring the initial rates of hydrolysis as a function of plasma concentration and plotting the data according to the Lineweaver–Burk equation. Furthermore, it was confirmed that there was no significant product inhibition of the enzymatic hydrolysis. This was specifically shown for the hydrolysis of 12. Neither benzoic acid nor N,N-dimethylglycolamide in concentrations of 10^{-4} M changed

the progress curve for the hydrolysis of ester 12, studied at an initial concentration of 10^{-4} M.

Values of the rate parameters K_m and V_{max} for the hydrolysis of the various esters in 50% human plasma are listed in Tables I and II along with the half-lives of hydrolysis. At a low substrate concentration (i.e., $S \ll K_m$), the enzymatic reaction is first-order with the rate equal to

$$-\frac{dS}{dt} = \frac{V_{max}}{K_m} S \qquad (5)$$

The half-lives given in Tables I and II refer to this rate, that is,

$$t_{1/2} = 0.693/(V_{max}/K_m) \qquad (6)$$

For some esters, first-order kinetics was observed over the entire course of hydrolysis. In these cases, the half-lives given in the Tables were calculated from the observed pseudo-first-order rate constants.

It is generally agreed that V_{max}/K_m is the most meaningful kinetic parameter for comparing different substrates.[48–51] Good substrates will have a large V_{max} and low K_m (tight binding) and, hence, a large V_{max}/K_m value. At a low substrate concentration (i.e., similar to the conditions normally prevailing in vivo for prodrug hydrolysis), the enzymatic reaction is first-order, with the rate constant equal to V_{max}/K_m and the half-life defined as in eq 6. Hence, the half-lives listed in Tables I and II are considered to be relevant for in vivo situations. In this respect, it should be added that in undiluted human plasma, the hydrolysis rates were found to be higher than those observed for the 50% plasma solutions. The latter solutions were used in order to be able to obtain comparable data for all the esters studied, including the most reactive ones. As seen from the data in Table III, even in 1% human plasma solutions, the rate of hydrolysis of 14 was very rapid.

Structure–Enzymatic Reactivity Relationships—As can be seen from the rate data given in Table I, the O-benzoylglycolamide esters are hydrolyzed quite rapidly in 50% human plasma solutions, although with vastly different rates. The enzymatic reactivity appears predominantly to depend on the number and size of the substituents on the amide nitrogen atom. Thus, the unsubstituted glycolamide ester (1) is only relatively slowly hydrolyzed ($t_{1/2}$ = 31 min), whereas the monosubstituted glycolamide esters are generally hydrolyzed with a higher speed. However, the N,N-disubstituted glycolamide esters are hydrolyzed particularly rapidly. The N,N-diethyl- (14), N,N-diallyl- (17), and N,N-diisopropyl- (16) substituted glycolamide esters show the highest reactivity, with a half-life of hydrolysis of only 4.8 s, and are closely followed by the N,N-dimethyl-, N,N-dipropyl-, and N-methyl-N-ethyl-disubstituted esters. These rapid rates of plasma-catalyzed hydrolysis should be considered in view of the slow rates of hydrolysis in the absence of plasma. Thus, in pure buffer solutions of the same pH (7.4) and temperature (37 °C), the half-lives of hydrolysis of these esters were >1000 h (e.g., 1.2×10^3 h for 14 and 1.8×10^3 h for 16), as estimated on the

Table III—Half-lives of Hydrolysis of 2-(Benzoyloxy)-*N,N*-diethylacetamide (14) in Human Plasma Solutions[a]

Human Plasma Concentration, %	$t_{1/2}$, s
1	73
2.5	33.5
5	23
50	4.8
100	<4

[a] At pH 7.4 and 37 °C.

basis of their k_{OH} values (see below). Furthermore, the rates of hydrolysis should be viewed in the light of the much slower rates of hydrolysis of the simple methyl and ethyl esters of benzoic acid, the half-lives being 1.8 and 3.5 h, respectively, in 80% human plasma (Table II).

Inspection of the data in Table I shows further that there exists an optimal size of the amide nitrogen substituents to ensure a high enzymatic reactivity. Thus, by going from propyl (15) to butyl (18) groups, the reactivity decreases considerably. A very large decrease in reactivity is seen for the N,N-dicyclohexyl-substituted ester (29) compared with the N-methyl-N-cyclohexyl compound (28). The esters containing a cyclic amide structure (31–44) are also hydrolyzed fairly rapidly, although the reactivity is less than that of linear N,N-disubstituted esters.

The introduction of hydrophilic groups in the N-substituents does not necessarily affect the reactivity. Thus, the hydroxyl group-containing esters 21–24 possess almost the same reactivity as their nonhydroxy analogues, and the same is the case for the glycolamide ester containing a terminal amide group (20). Also, the introduction of a positive charge in the substituents does not change the reactivity much, as exemplified for 30. The pK_a of the amino group in this ester was determined by potentiometric titration to be 8.0 at 22 °C, which means that the ester is predominantly protonated at pH 7.4. The pK_a values for the piperazine esters 39 and 40 were found to be 6.8; so, these esters are largely unprotonated at pH 7.4. The introduction of a negative charge in the substituents, on the other hand, results in a marked reduction of the enzymatic reactivity, as seen from the rate data for the esters containing a carboxyl group (26, 41, and 42). As discussed before,[52] other types of esters containing a negative charge at physiological pH also show a high resistance towards enzymatic hydrolysis by plasma or blood.

In order to further delineate the structural requirements affording a high reactivity towards plasma-catalyzed hydrolysis, various esters structurally related to the glycolamide esters were prepared and investigated. The results appear in Table II. By comparing the rate data for 48 and 49 with those for 12, it is readily seen that the introduction of more methylene groups between the ester and amide moieties results in a progressive decrease of enzymatic reactivity. Thus, 49 is almost 100-fold less reactive than 12. A dramatic decrease in reactivity is seen by substituting the glycolamide moiety with a corresponding lactamide group. The lactamide ester 50 is only very slowly hydrolyzed, the reactivity being decreased by a factor of 4430 relative to the glycolamide ester 12. This decrease in reactivity observed for 50 may most likely be ascribed to the steric hindrance exhibited by the methyl group in the α-position to the ester.

Compounds 51 and 52 represent glycolamide esters in which the amide nitrogen atom has been acetylated to produce an imide structure. These compounds are hydrolyzed at about the same rate as the corresponding unacetylated analogues (esters 1 and 2), but it is noteworthy that the reactivity of 52 is somewhat reduced as compared with other N,N-disubstituted esters.

Compound 53 is some kind of a reversed-amide analogue to the N,N-disubstituted glycolamide esters, but it also shows a reduced enzymatic reactivity relative to these compounds.

Finally, the data in Table II show that the esters carrying a positive charge at pH 7.4, benzoylcholine (55) and its tertiary amine analogue (54), are, as expected, hydrolyzed very rapidly in plasma. It is more interesting, however, to note that the uncharged glycolamide esters of benzoic acid, like 14, 16, and 17, possess an enzymatic reactivity that is not very much less than that of benzoylcholine.

Hydroxide Ion-Catalyzed Hydrolysis—The intrinsic chemical reactivity of the esters was measured by hydrolysis

by hydroxide ions. The rates of hydrolysis were found to be proportional to the hydroxide ion concentration or activity in the investigated range (0.001–0.22 M NaOH) in accordance with the following rate expression:

$$K_{obs} = k_{OH}a_{OH} \quad (7)$$

where k_{obs} is the observed pseudo-first-order rate constant and k_{OH} is the second-order rate constant for hydroxide ion-catalyzed hydrolysis. The hydroxide ion activity (a_{OH}) was calculated from the hydroxide ion concentration according to Harned and Hamer.[53] The values of k_{OH} for the various esters are listed in Tables I and II. Inspection of these values shows that the intrinsic reactivity of the esters has no obvious effect on their different enzymatic reactivity. Thus, the enzymatically most reactive N,N-disubstituted glycolamide esters show even a lower intrinsic chemical reactivity than the un- or monosubstituted esters which may most likely be due to steric effects by the N-substituents. It is worthy of note that the intrinsic reactivity of the N,N-disubstituted glycolamide esters is less than that of benzoylcholine by a factor of 5–8. It is also worth noting that the chemical reactivity of these glycolamide esters is of the same order of magnitude as that of the simple methyl ester (45). As recently discussed,[52] the chemical reactivity of the esters is largely influenced by the polar effects of the alcohol part.

Cholinesterase-Catalyzed Hydrolysis—The enzyme in human plasma that is predominantly responsible for the rapid hydrolysis of the N,N-disubstituted glycolamide esters appears to be cholinesterase (E.C. 3.1.1.8; also called pseudocholinesterase or butyrylcholine esterase). Evidence for this was obtained from several findings.

1. Physostigmine, which is a specific cholinesterase inhibitor,[54,55] totally inhibited the plasma-catalyzed hydrolysis of 14 at a concentration of 10^{-3} M (Figure 3).

2. Various glycolamide esters were found to be rapidly hydrolyzed by a purified cholinesterase preparation (Table IV). The relative reactivity of the various esters towards this purified enzyme parallels the relative reactivity towards plasma-catalyzed hydrolysis (Figure 4).

3. Finally, comparison of the rates of hydrolysis of 14 by plasma and by butyrylcholinesterase, at a concentration
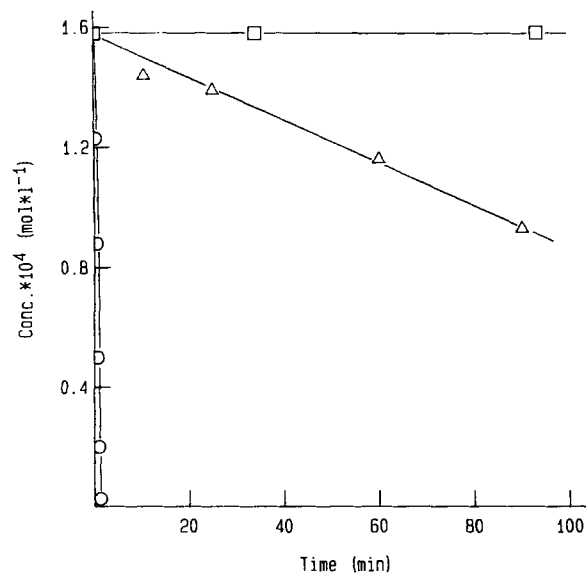
Figure 3—*Plots showing the rate of hydrolysis of the O-benzoyl glycolamide ester* **14** *in 50% human plasma solution (pH 7.40; 37°C) containing physostigmine salicylate in a concentration of 10^{-3} M (□) or 10^{-4} M (△). The open circle symbols (○) represent rate data for plasma without physostigmine.*

Table IV—Kinetic Data for the Enzymatic Hydrolysis of Various Glycolamide Esters by Type IV Cholinesterase[a]

Compound	$K_m \times 10^4$, M	$V_{max} \times 10^6$, M/min	$t_{1/2}$, min
2	8.1	2.6	213
3	6.2	2.1	201
7	0.7	1.2	42
12	5.4	122	3.1
14	0.6	115	0.38
23	5.0	35	10
27	2.2	177	0.85
31	13	23	39
32	1.9	4.1	33
33	1.5	7.2	15
35	1.5	1.9	54

[a] The hydrolysis was performed at pH 7.4 and 37 °C, the initial ester concentration being 7.8×10^{-4} M and the enzyme concentration being 0.38 mg/mL; the K_m and V_{max} values were obtained from eq 4 and the $t_{1/2}$ values from eq 6.
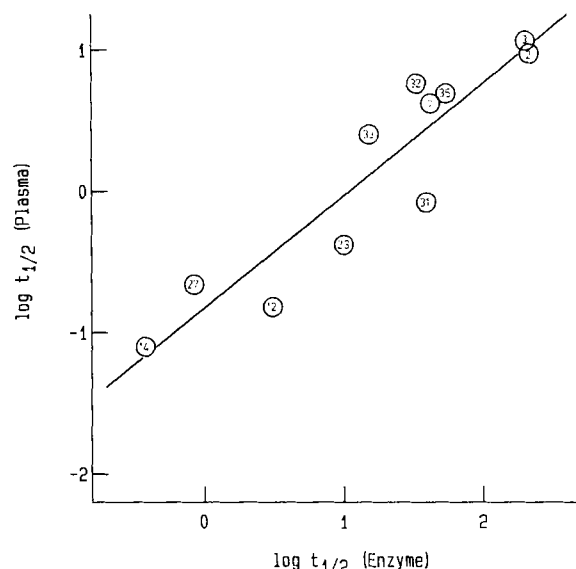
Figure 4—*Plot showing the relationship between the half-lives of hydrolysis of various O-benzoyl glycolamide esters in 50% human plasma and in buffer solution (pH 7.4) containing butyrylcholinesterase.*

identical to that present in the plasma used, provided strong evidence for the implication of cholinesterase in the plasma-catalyzed hydrolysis.

The specific activity of the purified cholinesterase preparation was found to be 0.54 µmol/min/mg when tested with benzoylcholine (10^{-4} M benzoylcholine in 0.067 M sodium phosphate buffer, pH 7.4, 37 °C) according to the method of Kalow and Lindsay.[56] Benzoylcholine is known to be rapidly hydrolyzed by human plasma or serum and no other esterase in plasma or serum contributes significantly to its hydrolysis,[57] although the latter has recently been questioned.[58] The human plasma preparation used for the glycolamide ester hydrolysis study was assayed for cholinesterase activity using benzoylcholine as a substrate. At 37 °C, the K_m value was 4×10^{-6} M and V_{max} was 6.3 M/min for benzoylcholine in 50% plasma, as determined by a spectrophotometric method.[56] These values are in the normal range for human plasma cholinesterase activity as reported by various authors.[57,59] To compare the rate of hydrolysis observed for 14 in 50% human plasma with that by pure cholinesterase, hydrolysis was measured at 37 °C with a concentration of pure cholinesterase equivalent to the concentration found in the plasma sample. The results given above show that such a concentra-

tion should be 1.17 mg/mL. At this enzyme concentration, the hydrolysis of the ester 14 was found to proceed with a half-life of 0.09 min, the K_m value being 4.2×10^{-5} M^{-1} and the V_{max} being 3.38×10^{-4} M/min. Comparing these values with the rate data for the ester listed in Table I shows that plasma cholinesterase could account for the majority of the observed enzymatic hydrolysis by plasma. This approach to determine the contribution by pseudocholinesterase to the overall plasma-catalyzed hydrolysis has previously been used in studies on cholinesterase- and plasma-catalyzed hydrolysis of various esters, including heroin, aspirin, procaine, and methyl-prednisolone acetate.[57,60,61]

The possibility that the plasma-catalyzed hydrolysis of the glycolamide esters could be due, in part, to the action of human serum albumin was tested by incubating some esters (12 and 14) in a pH 7.4 phosphate buffer solution containing 2% human serum albumin (i.e., at a concentration corresponding to that present in 50% plasma solutions). No hydrolysis was observed to take place following 8 h of incubation, thus eliminating this possible contribution.

Considering the high reactivity of the N,N-disubstituted glycolamide esters toward cholinesterase, it is of particular interest to note the structural similarity between these esters and choline esters [e.g., benzoylcholine (55); Figure 5]. It has recently been shown [62-65] that the binding subsite of acetylcholinesterase for the trimethylammonium group of acetylcholine may be better considered as an uncharged trimethyl site, as complementary to the trimethyl-substituted character of the β-substituent, rather than an "anionic" site, as commonly considered. The enzymatic reactivity appears primarily related to the volume of the β-substituent and its fit in the trimethyl site, as well as the hydrophobicity of its surface.[62,63] The different enzymatic (plasma or cholinesterase) reactivity of the glycolamide esters is seen to be in good accordance with this view, considering the similarity of the active centers in acetylcholinesterase and cholinesterase.[66] The carbonyl group in the amide moiety of the glycolamide esters may be regarded as isosteric to a methylene group in choline esters, and a good fit to the trimethyl binding site should certainly require an N,N-disubstituted amide group rather than an unsubstituted (1) or monosubstituted group (2–11; Figure 5). Among the monosubstituted compounds, the tert-butyl-substituted ester (7) is the most reactive one. This is consistent with the view about the structural similarity between glycolamide esters and choline esters and the proposed trimethyl pocket as part of the active site of cholinesterase.

There are further interesting similarities between the influence of the structure of choline esters and of glycolamide esters on the enzymatic reactivity. Thus, when the three methyl groups in choline esters are replaced by one or more ethyl groups, the enzymatic reactivity decreases, but only slightly.[67] However, a substantially decreased reactivity is observed when the methyl groups were substituted by three
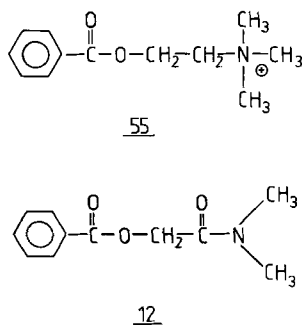
n-propyl groups,[67] emphasizing the existence of an optimal volume of the N-substituents. As noted above, the glycolamide esters behave in a similar manner. That the N,N-dimethyl-substituted ester (12) is a little less reactive than the diethyl- (14), diallyl- (17), and di-isopropyl- (16) substituted esters may be explained by the fact that whereas three methyl groups provide the optimal fit for β-substituted ethyl acetates to the enzyme, two ethyl groups in the glycolamide esters are required to give the best accommodation of the amide moiety by the trimethyl subsite (i.e., to achieve maximum reactivity).

The significantly decreased reactivity of the esters in which the amide group is separated from the ester linkage by two and three carbon atoms (48 and 49) parallels the behavior of choline derivatives, where the cholinesterase-catalyzed hydrolysis of esters of a three-carbon chain alcohol was found to be less than for esters of the two-carbon chain choline.[68,69] Finally, the very large reduction in enzymatic reactivity observed when a methyl group is introduced at the α-carbon of the glycolamide moiety (50) is paralleled by the behavior of the cholinesterase-catalyzed hydrolysis of similarly substituted choline esters.[68]

α-Chymotrypsin-Catalyzed Hydrolysis—Since esters of unsubstituted glycolamide with acetyl-L-phenylalanine,[70,71] benzoylglycine,[72] and various β-arylpropionic acids[73] have been reported to be good substrates for α-chymotrypsin, the reactivity of the N,N-diethylglycolamide ester 14 towards this enzyme was investigated and compared with that of the N-ethyl-monosubstituted (3) and the unsubstituted (1) glycolamide ester. The esters (7.8 $\times$ 10^{-4} M) were incubated at 37 °C in a 0.01 M phosphate buffer solution (pH 7.4) containing α-chymotrypsin in a concentration of 0.50 mg/mL. As can be seen from the results shown in Figure 6, the N,N-disubstituted glycolamide ester has a very low susceptibility to be hydrolyzed by α-chymotrypsin. The monosubstituted ester is only slightly more labile, whereas the unsubstituted glycolamide ester is the most reactive ester. This order of reactivity towards α-chymotrypsin is seen to be just the opposite of the behavior of the esters towards plasma or pseudocholinesterase. The observed stability of the N,N-disubstituted glycolamide ester 14 to α-chymotrypsin may be important for the applicability of the esters to increase the oral absorption or decrease local gastrointestinal side effects
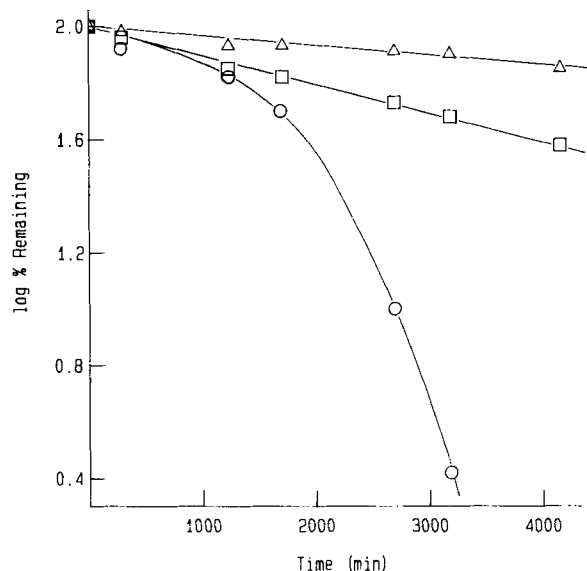

Figure 5—*Structural similarity between benzoylcholine* (55) *and the O-benzoyl glycolamide ester* (12).

Figure 6—*Plots showing the rate of hydrolysis at 37 °C of the O-benzoyl glycolamide esters* 1 (○), 3 (□), *and* 14 (△) *in 0.01 M phosphate buffer solution (pH 7.40) containing α-chymotrypsin in a concentration of 0.5 mg/mL.*

of, for example, nonsteroid anti-inflammatory carboxylic acid agents. It should be pointed out, however, that the acyl part of the esters may affect the reactivity and that the results given only concern benzoic acid.

Stability of the Glycolamide Ester 12 in Aqueous Solution—In order to predict the stability of the glycolamide esters in aqueous solution, regarding the potential application of these esters as prodrugs of carboxylic acid agents, the decomposition kinetics of the N,N-dimethyl-substituted glycolamide ester **12** in solution was examined in detail as a function of pH and temperature.

Under the experimental conditions used, the degradation of the compound followed strict first-order kinetics. Since the hydrolysis proceeded very slowly at pH < 6, the degradation rates were determined on the basis of the initial rate method at these pH values. In this method, the production of degradation products were monitored using an HPLC method as described in the *Experimental Section*. Since **12** was found to be hydrolyzed by two parallel reactions in acidic aqueous solutions, yielding benzoic acid (B) as well as benzoylglycolic acid (C; Scheme II), the initial rates of formation of both these products were followed. These rates are defined by

$$\frac{dB}{dt} = k_1 A_o \qquad (7)$$

and

$$\frac{dC}{dt} = k_2 A_o \qquad (8)$$

where A_o is the initial concentration of **12** and k_1 and k_2 are the pseudo-first-order rate constants for the two hydrolytic processes depicted in Scheme II. The degradation of **12** was followed up to an extent of <3%, so A_o can be regarded as a constant during the measurement. The pseudo-first-order rate constant (k) for the overall hydrolysis of ester **12** was obtained from k_1 and k_2 according to

$$k = k_1 + k_2 \qquad (9)$$

The utility of the initial rate method was checked by determining k directly by following the decrease in the concentration of **12** over more than one half-life at pH 1.0. The value of k thus obtained agreed within 3% with that obtained by the initial rate method.

No significant catalysis by the buffers used to maintain constant pH was observed in buffer concentrations of ≤0.05 M. The influence of pH on the rates of hydrolysis at 60 °C is shown in Figure 7 in which the logarithm of k is plotted against pH. The shape of the pH–rate profile indicates that the ester is subject to specific acid- and base-catalyzed hydrolysis, as well as a water-catalyzed reaction, according to

$$k = k_H a_H + k_o + k_{OH} a_{OH} \qquad (10)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide
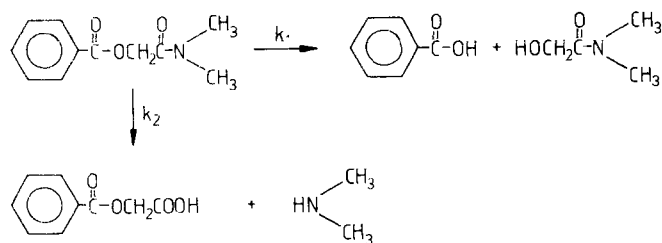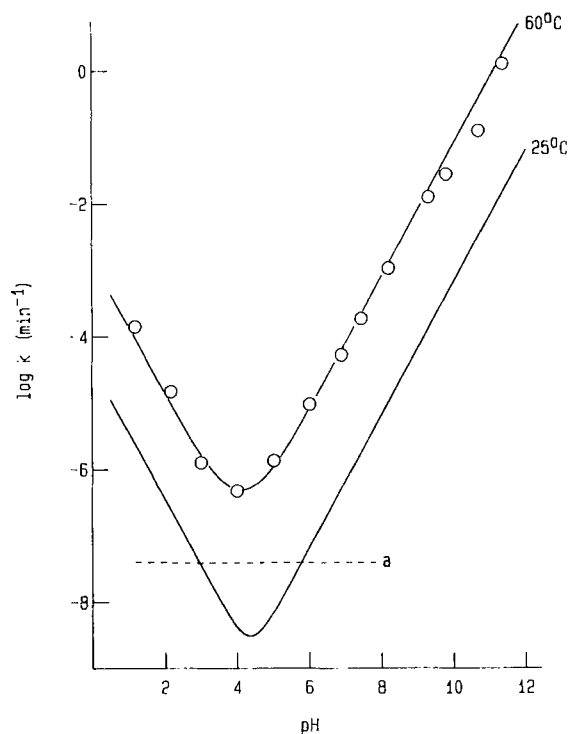
Scheme II

Figure 7—*The pH–rate profiles for the degradation of 2-(benzoyloxy)-N,N-dimethylacetamide* **(12)** *in aqueous solution (μ = 0.5) at 60 and at 25°C. Key:* (a) $t_{10\%}$ = 5 years.

ion activity, respectively. The values obtained for the specific catalytic rate constants k_H and k_{OH}, and the rate constant for water-catalyzed hydrolysis (k_o) are listed in Table V. In Figure 7, the solid curve drawn was constructed from these constants and eq 10. At 60 °C, maximum stability of **12** in aqueous solution occurs at pH 4.15. Even at this pH, the specific acid- and base-catalyzed reactions predominated over the water-catalyzed reaction.

The hydrolysis of ester **12** was further examined in 0.01 M HCl and 0.0108 M NaOH solutions (μ = 0.5) over the temperature ranges 60–90 °C and 30–60 °C, respectively. By plotting the rate constants obtained according to the Arrhenius equation (Figure 8), energies of activation of 20.3 kcal/mol for k_H and 14.3 kcal/mol for k_{OH} were obtained. From the Arrhenius-type plots, values of k_H and k_{OH} were estimated at 25 °C (Table V). On the basis of this and eq 10, the pH–rate profile for **12** was constructed at 25 °C (Figure 7). It can be seen from the figure that the maximal stability at 25 °C occurs at pH 4.5 and that a shelf-life (i.e., the time required to degrade 10% of the compound) of 96 years is predicted at this pH and temperature. To have a shelf-life ≥5 years, the pH of solution should be in the range 3–5.8.

At pH >5, the only significant degradation reaction of ester **12** was hydrolysis of the ester group to yield benzoic acid. In acidic solutions, on the other hand, the hydrolysis of the amide moiety to yield benzoylglycolic acid constituted the dominating degradation route, accounting for 67% of the

Table V—Rate Data for the Hydrolysis of the Glycolamide Ester 12 in Aqueous Solution[a]

Temperature, °C	k_H, M^{-1} min^{-1}	k_o, min^{-1}	k_{OH}, M^{-1} min^{-1}
60	1.36×10^{-3}	2.8×10^{-7}	85.1
25[b]	3.53×10^{-5}	—	6.69

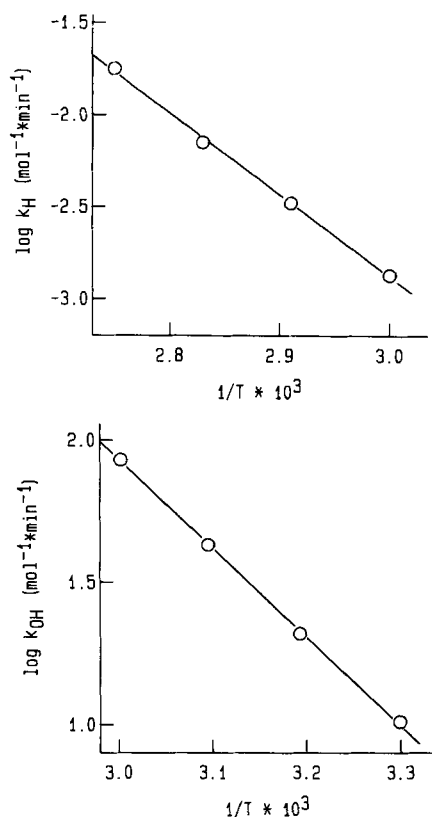[a] Ionic strength of 0.5. [b] The data at 25 °C are predicted from temperature-accelerated runs.

Figure 8—*Arrhenius-type plots for the specific acid- and base-catalyzed hydrolysis of 2-(benzoyloxy)-N,N-dimethylacetamide (12) in aqueous solution (μ = 0.5).*

overall hydrolysis, as revealed from the measurement of the amounts of benzoic and benzoylglycolic acid formed at pH 2.

Lipophilicity and Solubility of the Glycolamide Esters—As it appears from Table I, the glycolamide esters of benzoic acid are, except for a few cases, crystalline compounds with reasonable (i.e., not too high) melting points. The latter property is important in terms of solubility behavior. The aqueous solubility and octanol–water partition data for the esters are given in Table VI. The log P values are mutually in good agreement with values calculated on basis of π substituent values.[74] Thus, it may be possible to predict the partition coefficients of other glycolamide esters on the basis of the additive substituent principle.

Besides by the widely used partition coefficients between octanol and water, the lipophilicity of the ester derivatives was also evaluated by means of reversed-phase HPLC capacity factors (k'). With MeOH:0.01 M HNO₃ (3:2, v/v) as the mobile phase, the esters showed the k' values given in Table VI. As has been observed for many different types of compounds[75,76] (and references cited therein), a linear relationship was found to exist between log k' and log P for the esters (Figure 9).

It is well recognized that the relationship between aqueous solubility and octanol–water partition coefficients of crystalline compounds is dependent on the crystal lattice energy and thus the melting points.[77–79] As shown by Yalkowsky and co-workers,[77–79] the relationship between the aqueous solubility (S) in molar concentration and octanol–water partition coefficients of crystalline organic compounds contains a term for melting points:

$$\log S = -a\log P - b(\text{mp}) + c \qquad (11)$$

where a, b, and c are constants which may vary somewhat for different types of chemical structures, a usually being around

Table VI—Partition Coefficients (P), Chromatographic Capacity Factors (k'), and Water Solubility (S) of Various Glycolamide Esters and Other Esters of Benzoic Acid

Compound	log P[a]	log k'	S, mg/mL[b]
1	0.69	−0.12	4.1
2	0.99	0.02	3.7
3	1.28	0.17	1.2
4	1.88	0.34	0.64
5	1.73	0.30	0.41
6	2.36	0.57	0.41
7	2.26	0.56	0.32
8	2.81	0.78	0.033
9	0.09	−0.21	7.5
10	0.42	−0.17	0.48
11	0.25	−0.21	15.3
12	1.07	0.12	8.8
13	1.27	0.26	—
14	2.06	0.42	2.0
15	2.65	0.84	1.1
16	2.56	0.80	0.12
17	2.34	0.65	0.71
18	3.91	1.34	0.08
19	3.80	1.25	0.08
20	0.08	−0.20	30.2
21	0.58	−0.08	19.3
22	0.93	0.04	10.8
23	0.17	−0.25	720
24	0.66	—	19.6
25	1.28	—	7.89
26	0.67[c]	−0.03	1.40[d]
27	1.56	0.27	6.0
28	2.99	0.90	0.14
29	>4.5	—	0.003
30	—	—	>200
31	1.20	0.22	5.4
32	1.44	0.33	6.3
33	1.95	0.53	0.78
34	2.30	0.68	0.75
35	0.90	0.08	4.2
36	2.62	—	0.52
37	2.90	0.85	0.15
38	0.63	—	11.8
39	—	—	>200
40	—	—	>200
41	0.79[c]	0.03	1.9[d]
42	1.02[c]	0.10	7.1[d]
43	1.42	0.28	2.4
44	0.20	0.09	1.5
45	2.14	0.49	4.2
46	2.64	0.73	1.2
47	1.80	—	—
48	1.28	0.32	17.6
49	1.86	0.43	13.9
50	2.30	0.56	1.3
51	1.24	0.13	0.88
52	1.58	0.31	0.32
53	1.24	0.25	31.3
54	—	—	>200
55	—	—	>200

[a] Partition coefficients between octanol and water at 22 °C. [b] Solubility in water at 22 °C. [c] Partition coefficients between octanol and 0.01 M HCl. [d] Solubility in 0.01 M HCl.

unity and b around 0.01. Multiple-regression analysis of the present series of esters, for which S, P, and mp data are available, covering a wide variety of melting points, P values, and aqueous solubilities, yielded the relationship shown in Figure 10, the regression equation being

$$\log S = -1.03 \ (\pm \ 0.06)\log P -$$
$$0.015(\pm \ 0.002)\text{mp} + 0.80 \ (\pm \ 0.22) \qquad (12)$$
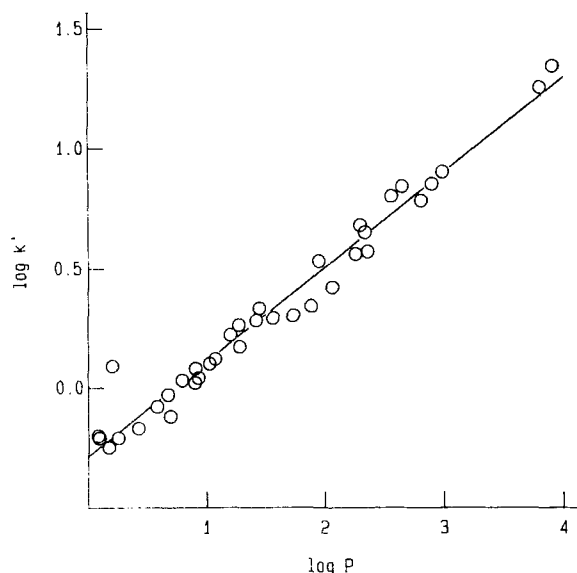
$$(n = 43, r = 0.933)$$

Figure 9—*Plot of log P versus log k' for various benzoate esters. The values are taken from Table VI.*
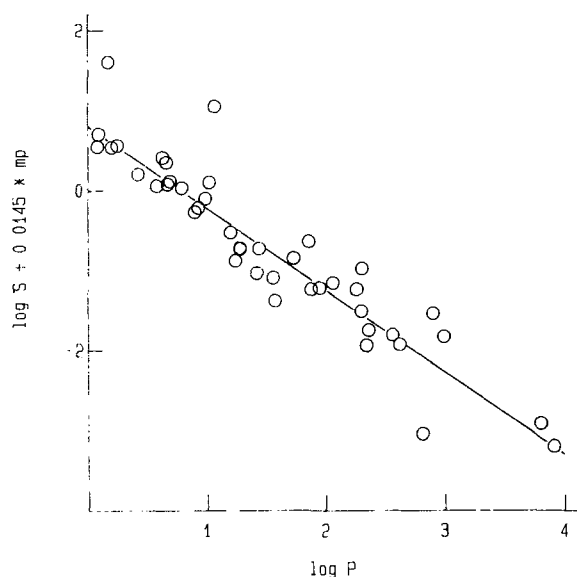

Figure 10—*Plot showing the relationship between melting point, aqueous solubility (S), and octanol–water partition coefficients (P) for the various glycolamide esters and related compounds according to eq 12.*

It is seen that the coefficient for the $\log P$ term is close to -1, and that for the melting point is close to -0.01, the theoretical values.[77]

The solubility and lipophilicity data show that it is readily feasible to select esters with greatly varying water solubilities and lipophilicities and still obtain a high rate of enzymatic conversion. Thus, 23 (derived from diethanolamine) is soluble in water to an extent of >70% (w/v), although it is a neutral molecule with a positive $\log P$ value. Other enzymatically reactive esters possessing a relatively good aqueous solubility are the sarcosinamide derivative 20, the N,N-dimethyl-substituted glycolamide ester 12, the ethanolamine derivatives 21 and 22, and the esters containing an ionizable amino side-chain function (30, 39, and 40), of which the ester 30 shows a particularly high enzymatic reactivity. For comparison, benzoic acid was determined to have a solubility of 3.1 mg/mL in 0.01 M HCl and a $\log P$ value of 1.91 (octanol–0.01 M HCl).

The availability of a free hydroxyl group in 21–23 and 38 makes it possible to further modify the physicochemical properties (e.g., by esterification with an amino acid). Thus, 21 was esterified with N,N-dimethylglycine to give the double ester derivative 56 (Scheme III). This compound has a water solubility >20% (w/v) as a fumarate salt, and it was hydrolyzed to yield benzoic acid in 50% plasma solutions even more rapidly ($t_{1/2}$ = 5 s) than the parent ester 21 ($t_{1/2}$ = 12 s; Figure 11).

Conclusions

In conclusion, esters of N,N-disubstituted glycolamides are shown to be a potentially useful biolabile prodrug type for carboxylic acid agents. As described in a preliminary communication,[19] the observed great reactivity of the disubstituted glycolamide esters of benzoic acid is valid for other acids, including several drugs and amino acids as well. Although the structure of the acyl moiety has an influence on the enzymatic reactivity, the rate of plasma-catalyzed hydrolysis of N,N-disubstituted glycolamide esters of a variety of acids has in all cases been found to be several-fold larger than the rate of hydrolysis of the corresponding simple alkyl esters.[80] As has been demonstrated above, the glycolamide esters combine a high susceptibility to undergo enzymatic hydrolysis in plasma by virtue of cholinesterase, with a high stability in aqueous solution. The new ester prodrug type is further characterized by providing ample possibilities for varying
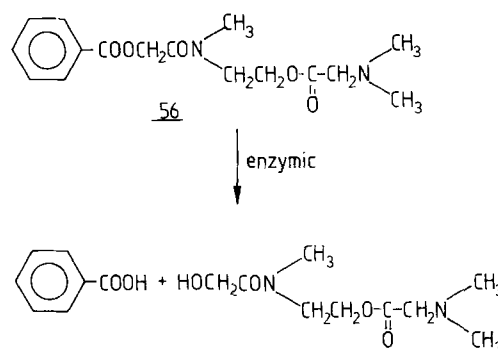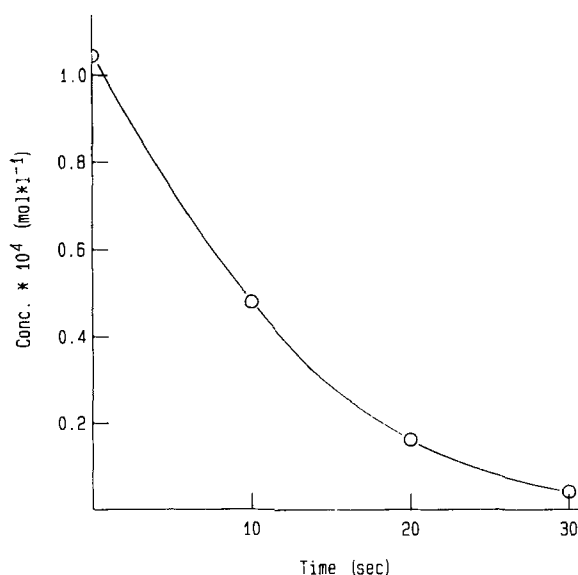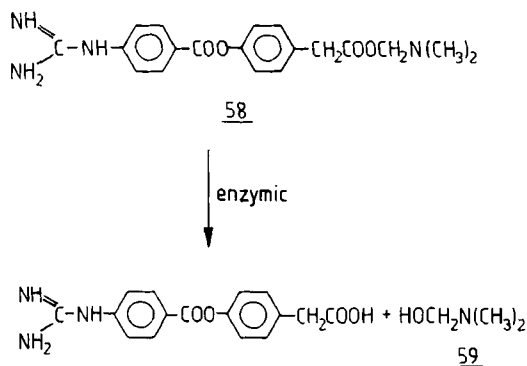

Scheme III

Figure 11—*Plot showing the rate of hydrolysis of 56 in 50% human plasma solution (pH 7.4) at 37°C.*

NH
\\
C-NH-<O>-COO-<O>-CH₂COOCH₂N(CH₃)₂
/
NH₂

58

↓ enzymic

NH
\\
C-NH-<O>-COO-<O>-CH₂COOH + HOCH₂N(CH₃)₂
/
NH₂

59

Scheme IV

the water and lipid solubilities of the derivatives, with retainment of the favorable enzymatic/nonenzymatic hydrolysis index.

Glycolic acid esters (57; R–COOCH₂COOR') have previously been proposed as prodrugs of amino acids.[81] As recently described by us elsewhere,[52] such double esters are more resistant to enzymatic hydrolysis by plasma than the corresponding glycolamide esters, and furthermore, they are not cleaved entirely to the parent acid (in the case of benzoic acid) in plasma solutions. Furthermore, glycolate esters are chemically much more unstable than glycolamide esters[52] and, in contrast to the latter, glycolic acid esters of most carboxylic acids are liquids with a slight water solubility.

In considering the potential utility of glycolamide esters as a new prodrug type for carboxylic acid agents, the possible toxicity of the pro-moiety split off during enzymatic hydrolysis (i.e., a 2-hydroxyacetamide derivative) should be taken into account. However, this does not seem to represent any problem. Glycolic acid is an endogenous substance and amides of this acid are not suspected or described to have any harmful effects. In this regard it is also interesting to note that a proteinase inhibitor, camostat mesitate (58), recently has been approved for clinical use in Japan.[82] This compound contains an N,N-dimethylglycolamide ester moiety and, in accordance with the results of the present study, this ester is in fact rapidly hydrolyzed in vivo to yield a p-guanidinobenzoate ester along with 2-hydroxy-N,N-dimethylacetamide (59; Scheme IV).[83,84]

References and Notes

1. Stella, V. In Pro-drugs as Drug Delivery Systems; Stella, V.; Higuchi, T., Eds.; American Chemical Society: Washington, D.C., 1975; pp 1–115.
2. Design of Biopharmaceutical Properties Through Prodrugs and Analogs; Roche, E. B., Ed.; American Pharmaceutical Association: Washington, D.C., 1977.
3. Design of Prodrugs; Bundgaard, H., Ed.; Elsevier Science: New York, 1985.
4. Bioreversible Carriers in Drug Design. Theory and Applications; Roche, E. B., Ed.; Pergamon: New York, 1987.
5. Bundgaard, H. In Design of Prodrugs; Bundgaard, H., Ed.; Elsevier Science: New York, 1987, pp 1–92.
6. Bundgaard, H. In Delivery Systems for Peptide Drugs; Davis, S. S.; Illum, L.; Tomlinson, E., Eds.; Plenum: New York, 1987; pp 49–68.
7. La Du, B. N.; Snady, H. Handb. Exp. Pharmacol. 1971, 28/2, 477–499.
8. Williams, F. M. Clin. Pharmacokin. 1985, 10, 392–403.
9. Walker, C. H.; Machness, M. I. Biochem. Pharmacol. 1983, 32, 3265–3269.
10. Banerjee, P. K.; Amidon, G. L. In Design of Prodrugs; Bundgaard, H., Ed.; Elsevier Science: New York, 1985; pp 93–133.
11. Sinkula, A. A.; Yalkowsky, S. H. J. Pharm. Sci. 1975, 64, 181–210.
12. Digenis, G. A.; Swintosky, J. V. Handb. Exp. Pharmacol. 1975, 28/3, 86–112.
13. Morozowich, W.; Cho, M. J.; Kezdy, F. J. In Design of Biopharmaceutical Properties Through Prodrugs and Analogs; Roche, E. B., Ed.; American Pharmaceutical Association: Washington, D.C., 1977; pp 344–391.
14. Holysz, R. P.; Stavely, H. E. J. Am. Chem. Soc. 1950, 72, 4760–4763.
15. Ferres, H. Drugs Today 1983, 19, 499–538.
16. Harrison, I. T.; Lewis, B.; Nelson, P.; Rooks, W.; Roszkowski, A.; Tomolonis, A.; Fried, J. J. Med. Chem. 1970, 13, 203–205.
17. Child, R. G.; Osterberg, A. C.; Sloboda, A. E.; Tomcufcik, A. S. J. Pharm. Sci. 1977, 66, 466–476.
18. Boltze, K.-H.; Brendler, O.; Jacobi, H.; Optiz, W.; Raddatz, S.; Seidel, P. -R.; Vollbrecht, D. Arzneim.–Forsch. 1980, 30, 1314–1325.
19. Bundgaard, H.; Nielsen, N. M. J. Med. Chem. 1987, 30, 451 454.
20. Cohen, M. L. Ann. Rev. Pharmacol. Toxicol. 1985, 25, 307–323.
21. Tocco, D. J.; De Luna, F. A.; Duncan, A. E. W.; Vassil, T. C.; Ulm, E. H. Drug Metab. Disp. 1982, 10, 15–19.
22. Larmour, I.; Jackson, B.; Cubela, R.; Johnston, C. I. Br. J. Clin. Pharmacol. 1985, 19, 701–704.
23. Todd, P. A.; Heel, R. C. Drugs 1986, 31, 198–248.
24. Rahkit, A.; Tipnis, V. Clin. Chem. 1984, 30, 1237–1239.
25. Tipnis, V.; Rakhit, A. J. Chromatogr. 1985, 345, 396–401.
26. Rakhit, A.; Kochak, G.; Dotson, R.; Tipnis, V. J. Pharm. Sci. 1985, 74, 947–952.
27. Hajdú, P.; Schmidt, D.; Bomm, M.; Hack, L.; Keller, A. Arzneim.–Forsch. 1984, 34, 1431–1435.
28. Tanaka, H.; Yoneyama, Y.; Sugawara, M.; Umeda, I.; Ohta, Y. J. Pharm. Sci. 1987, 76, 224–227.
29. Jansen, A. B. A.; Russell, T. J. J. Chem. Soc. 1965, 2127–2132.
30. For a review of penicillin and cephalosporin esters, see ref 15.
31. Kakeya, N.; Nishimura, K. -I.; Yoshimi, A.; Nakamura, S.; Nishizawa, S.; Tamaka, S.; Matsui, H.; Kawamura, T.; Kasai, M.; Kitae, K. Chem. Pharm. Bull. 1984, 32, 692–698.
32. Yoshimura, Y.; Hamaguchi, N.; Kakeya, N.; Yashiki, T. Int. J. Pharm. 1985, 26, 317–328.
33. Yoshimura, Y.; Hamaguchi, N.; Yashiki, T. Int. J. Pharm. 1985. 23, 117–129.
34. Yoshimura, Y.; Hamaguchi, N.; Yashiki, Y. J. Antibiot. 1986, 39, 1329–1342.
35. Ladkani, D.; Weiner, B. Z.; Yellin, H.; Avnir, D. Eur. Patent 112 130, 1983.
36. Uchida, K.; Masumoto, S.; Tohno, M.; Mimura, M.; Okumura, M.; Ichikawa, K.; Matsumura, M. Eur. Patent 103 265, 1983.
37. Concilio, C.; Bongini, A. Ann. Chime (Rome) 1966, 56, 417–426.
38. Boudreau, J. A.; Williams, A. J. Chem. Soc., Perkin Trans. II 1977, 1221–1225.
39. Danishefsky, S. J.; Pearson, W. H.; Segmuller, B. E. J. Am. Chem. Soc. 1985, 107, 1280–1285.
40. Marvel, C. A.; Elliott, J. R.; Boettner, F. E.; Yuska, H. J. Am. Chem. Soc. 1946, 68, 1681–1686.
41. Speziale, A. J.; Hamm, P. C. J. Am. Chem. Soc. 1956, 78, 2556–2559.
42. Nikolaev, A. F.; Rozenberg, M. E.; Daniel, N. V.; Tereshchenko, G. P. J. Gen. Chem. USSR 1963, 33, 385–387.
43. David, W.; Ross, W. C. J. J. Chem. Soc. 1950, 3056–3062.
44. Andreas, F.; Sowada, R.; Scholz, J. J. Prakt. Chem. 1962, 18, 141–149.
45. Segel, I. H. Enzyme Kinetics; Wiley-Interscience: New York, 1975; pp 18–57.
46. Orsi, B. A.; Tipton, K. F. Methods Enzymol. (Part A) 1979, 63, 159–183.
47. Robinson, J. A.; Characklis, W. G. Microb. Ecol. 1984, 10, 165–178.
48. Bender, M. L.; Kezdy, F. J. Ann. Rev. Biochem. 1965, 34, 49–76.
49. Northrop, D. B. Anal. Biochem. 1983, 132, 457–461.
50. Plesner, I. W. Biochem. J. 1986, 239, 175–178.
51. Brot, F. E.; Bender, M. L. J. Am. Chem. Soc. 1969, 91, 7187–7191.
52. Nielsen, N. M.; Bundgaard, H. Int. J. Pharm., 1987, 39, 75–85.
53. Harned, H. S.; Hamer, W. J. J. Am. Chem. Soc. 1933, 55, 2194–2206.
54. Krisch, K. In The Enzymes, Vol. 4; Boyer, P. D., Ed.; Academic: New York, 1971; pp 43–96.
55. Silk, E.; King, J.; Whittaker, M. Ann. Clin. Biochem. 1979, 16, 57–75.
56. Kalow, W.; Lindsay, H. A. Can. J. Biochem. Physiol. 1955, 33, 568–574.
57. Valentino, R. J.; Lockridge, O.; Eckerson, H. W.; La Du, B. N. Biochem. Pharmacol. 1981, 30, 1643–1649.
58. Abernethy, M. H.; George, P. M.; Herron, J. L.; Evans, R. T. Clin. Chem. 1986, 32, 194–197.
59. Davies, R. O.; Marton, A. V.; Karlow, W. Can. J. Biochem. Physiol. 1960, 38, 545–551.
60. Lockridge, O.; Mottershaw-Jackson, N.; Eckerson, H. W.; La Du, B. N. J. Pharmacol. Exp. Ther. 1980, 215, 1–8.

61. Myers, C.; Lockridge, O.; La Du, B. N. *Drug Metab. Dispos.* **1982**, *10*, 270–280.
62. Cohen, S. G.; Elkind, J. L.; Chisti, S. B.; Giner, J. -L. P.; Reese, H.: Cohen, J. B. *J. Med. Chem.* **1984**, *27*, 1643–1647.
63. Cohen, S. G.; Chisti, S. B.; Elkind, J. L.; Reese, H.; Cohen, J. B. *J. Med. Chem.* **1985**, *28*, 1309–1313.
64. Hasan, F. B.; Cohen, S. G.; Cohen, J. B. *J. Biol. Chem.* **1980**, *255*, 3898–3904.
65. Hasan, F. B.; Elkind, J. L.; Cohen, S. G.; Cohen, J. B. *J. Biol. Chem.* **1981**, *256*, 7781–7785.
66. Järv, J. *Bioorg. Chem.* **1984**, *12*, 259–278.
67. Hopff, W. H.; Riggio, G.; Hofmann, A.; Waser, P. G. *Croat. Chem. Acta* **1975**, *47*, 309–319.
68. Glick, D. *J. Biol. Chem.* **1938**, *125*, 729–739.
69. Glick, D. *J. Biol. Chem.* **1939**, *130*, 527–534.
70. Kerr, R. J.; Niemann, C. *J. Org. Chem.* **1958**, *23*, 304–305.
71. Applewhite, T. H.; Waite, H.; Niemann, C. *J. Am. Chem. Soc.* **1958**, *80*, 1465–1469.
72. Wolf, J. P.; Niemann, C. *Biochemistry* **1963**, *2*, 493–497.
73. Cohen, S. G.; Vaidya, V. M.; Schultz, R. M. *Proc. Natl. Acad. Sci.* **1970**, *66*, 249–256.
74. Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley & Sons: New York, **1979**.
75. Hakfenschied, T. L.; Tomlinson, E. *Int. J. Pharm.* **1983**, *16*, 225–239.
76. Brent, D. A.; Sabatka, J. J.; Minick, D. J.; Henry, D. W. *J. Med. Chem.* **1983**, *26*, 1014–1020.
77. Valvani, S. C.; Yalkowsky, S. K. In *Physical Chemical Properties of Drugs*; Yalkowsky, S. K.; Sinkula, A. A.; Valvani, S. C., Eds.; Marcel Dekker: New York, **1980**, pp 201–209.
78. Yalkowsky, S. H.; Valvani, S. C.; Roseman, T. J. *J. Pharm. Sci.* **1983**, *72*, 866–870.
79. Yalkowsky, S. H.; Valvani, S. C. *J. Pharm. Sci.* **1980**, *69*, 912–922.
80. Nielsen, N. M.; Bundgaard, H., unpublished observations.
81. Wermuth, C. G. *Chem. Ind.* **1980**, 433–435.
82. Hashimoto, K. *Drugs of the Future* **1985**, *10*, 897–899.
83. Saitoh, Y. In *Proteinasen-Inhibition*; Grözinger, K. H.; Schrey, A.; Wabnitz, R. W., Eds.; Dr. Wolf & Sohn: München, **1982**; pp 156–167.
84. Göke, B.; Stöckmann, F.; Müller, R.; Lankisch, P. G.; Creutzfeldt, W. *Digestion* **1984**, *30*, 171–178.