Acknowledgment. We thank Pat Loo, Dawn Malone, and Mauricio Loo for expert technical assistance.

Registry No. **1a**, 100746-45-0; **1a**·HCl, 100746-46-1; **1b**, 118715-84-7; **1b**·HCl, 118715-94-9; **1c**, 100745-90-2; **1c**·HCl, 100776-81-6; **1d**, 118715-85-8; **1d**·HCl, 118715-95-0; **1e**, 118715-86-9; **1f**, 100746-09-6; **1f**·HCl, 100746-10-9; **1g**, 100746-47-2; **1g**·HCl, 100746-48-3; **1h**, 100746-29-0; **1h**·HCl, 100746-30-3; **1i**, 118715-87-0; **1j**, 100746-23-4; **1j**·HCl, 100746-24-5; **1k**, 100745-19-5; **1k**·HCl, 100745-18-4; **1l**, 100745-52-6; **1l**·HCl, 100745-51-5; **1m**, 100745-34-4; **1m**·HCl, 100745-33-3; **2f**, 100746-17-6; **2f**·HCl, 100746-18-7; **2g**, 100746-35-8; **2g**·HCl, 100746-36-9; **2h**, 100746-37-0; **2h**·HCl, 100746-38-1; **2i**, 100746-53-0; **2i**·HCl, 100746-54-1; **2j**, 100746-27-8; **2j**·HCl, 118715-96-1; **2k**, 100745-13-9; **2k**·HCl, 100745-12-8; **2l**, 118715-88-1; **2l**·HCl, 118715-97-2; **2m**, 100745-36-6; **2m**·HCl, 100745-35-5; **2n**, 100746-39-2; **2n**·HCl, 100746-40-5; **2o**, 101392-20-5; **2o**·HCl, 101392-21-6; **2p**, 100745-46-8; **2p**·HCl, 100745-45-7; **2q**, 100745-59-3; **2q**·HCl, 100745-58-2; **2r**, 100745-48-0; **2r**·HCl, 100745-47-9; **2s**, 118715-89-2; **2s**·HCl, 118715-98-3; **2t**, 118715-90-5; **2t**·HCl, 118715-99-4; **2u**, 118715-91-6; **2u**·HCl, 118716-00-0; **2v**, 100746-42-7; **8f**, 118715-92-7; **9c**, 17061-86-8; **10c**, 100746-90-5; **11c**, 100746-91-6; **12c**, 100746-87-0; **14** (X = Y = Z = T = H; R = *n*-Pr), 118716-01-1; **14** (X = OMe; Y = Z = T = H; R = *n*-Pr), 118716-02-2; **14** (X = OMe; Y = Z = T = H; R = Me), 118716-03-3; **14** (X = OMe; Y = T = H; Z = Me; R = *n*-Pr), 118716-04-4; **14** (X = OMe; Y = T = H; Z = R = Me), 118716-05-5; **14** (X = Z = T = H; Y = OH; R = *n*-Pr), 118716-06-6; **14** (X = Z = T = H; Y = OH; R = Me), 118716-07-7; **14** (X = Y = Z = H; T = OH; R = *n*-Pr), 100745-38-8; **14** (X = Y = T = H; Z = OH; R = *n*-Pr), 118716-08-8; **14** (X = Y = Z = H; T = OMe; R = Me), 118716-09-9; chromanone, 19090-04-1; *N*-propyl-β-alanine methyl ester, 5036-62-4; 2-methoxyphenyl 2-propynyl ether, 41580-71-6; 3-methoxyphenyl 2-propynyl ether, 41580-72-7; 3-methoxy-5-methylphenyl 2-propynyl ether, 118715-93-8; 1-bromo-3-chloropropane, 109-70-6; propionyl chloride, 79-03-8.

Supplementary Material Available: Details of the structure determination of **2u**, the numbering system of the two molecules, and tables of the atomic coordinates, bond distances and angles, anisotropic displacement coefficients (10 pages); tables of observed and calculated structure factors (12 pages). Ordering information is given on any current masthead page.

Evaluation of Glycolamide Esters and Various Other Esters of Aspirin as True Aspirin Prodrugs

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A series of glycolamide, glycolate, (acyloxy)methyl, alkyl, and aryl esters of acetylsalicylic acid (aspirin) were synthesized and evaluated as potential prodrug forms of aspirin. N,N-Disubstituted glycolamide esters were found to be rapidly hydrolyzed in human plasma, resulting in the formation of aspirin as well as the corresponding salicylate esters. These in turn hydrolyzed rapidly to salicylic acid. The largest amount of aspirin formed from the esters were 50 and 55% in case of the *N,N*-dimethyl- and *N,N*-diethylglycolamide esters, respectively. Similar results were obtained in blood with the *N,N*-dimethyl- and *N,N*-diethylglycolamide esters. Unsubstituted and monosubstituted glycolamide esters as well as most other esters previously suggested to be aspirin prodrugs were shown to hydrolyze exclusively to the corresponding salicylic acid esters. Lipophilicity parameters and water solubilities of the esters were determined, and structural factors favoring ester prodrug hydrolysis at the expense of deacetylation to yield salicylate ester are discussed. The properties of some N,N-disubstituted glycolamide esters of aspirin are highlighted with respect to their use as potential aspirin prodrugs.

For several years many attempts have been made to develop bioreversible derivatives or prodrugs of aspirin (acetylsalicylic acid) in order to depress its side effects in the form of gastric irritation and bleedings.[1] Since the gastric irritation and ulcerogenicity associated with oral dosing of aspirin is largely a local phenomenon possibly involving accumulation of the acid within gastric mucosal cells,[2-4] a promising approach to minimize these side effects may be masking the acidic carboxyl group of aspirin via prodrug formation. Thus, esterification of aspirin as well as various other nonsteroidal antiinflammatory carboxylic acids to produce methyl esters has been shown to greatly suppress the gastric ulcerogenic activity.[5,6]

The aspirin prodrug derivatives developed so far can be classified in two groups according to their mechanism of conversion: derivatives that undergo enzymatic cleavage to regenerate the parent drug and derivatives being hydrolyzed nonenzymatically. The former group consists of several ester derivatives including simple alkyl or aryl esters,[5-14] triglycerides,[15-17] (acyloxy)alkyl esters,[18,19] certain

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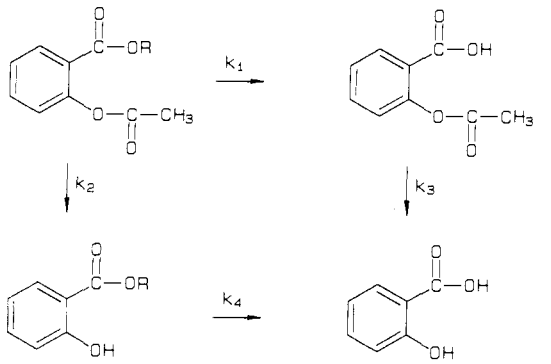

Figure 1. Scheme illustrating the bioconversion of aspirin esters. To behave as true aspirin prodrugs, the hydrolytic rate constant k_1 should be greater than the rate constant k_2 associated with deacetylation.

sulfur-containing ester types,[20,21] and amides of phenylalanine[22,23] or proline[24] derivatives. The nonenzymatically hydrolyzable derivatives include some acylal derivatives,[25] 2-substituted 2-methyl-4*H*-1,3-benzodioxin-4-one derivatives,[26] and esters of various *N*-α-(hydroxyalkyl)amides.[27]

A major problem in the design of aspirin prodrugs is, however, the great enzymatic lability of the acetyl ester functionality in aspirin derivatized at its carboxyl group. As will be shown below, blocking of the carboxylic group (i.e. neutralization of the negative charge of the aspirin molecule) by e.g. esterification renders the acetyl ester group extremely susceptibile to enzymatic cleavage. Thus, half-lives for the deacetylation of 1–3 min in human plasma were typically found for various aspirin esters whereas the hydrolysis of aspirin to salicylic acid under similar conditions proceeds with a half-life of about 2 h. Therefore, a prerequisite for any true aspirin prodrug is that the masking group cleaves faster than the acetyl ester moiety as illustrated in Figure 1. Otherwise, the derivatives will behave as prodrugs of salicylic acid and not as true aspirin prodrugs. The only derivatives that so far have been shown to release the parent aspirin in vivo or upon incubation in vitro in human plasma are (methylsulfinyl)methyl and (methylsulfonyl)methyl esters of aspirin[20,21] and esters formed with *N*-α-(hydroxyalkyl)amides,[27] the latter esters being cleaved spontaneously so fast at pH 7.4 that the plasma-catalyzed O-deacetylation cannot compete with this reaction. Since there are no proof that most or all of the other derivatives claimed as "aspirin prodrugs" fulfill this requirement of selective hydrolysis, they may essentially be regarded as prodrugs of salicylic acid rather than of aspirin.

Recently, we discovered that esters of certain N,N-disubstituted 2-hydroxyacetamides (glycolamides) are cleaved remarkably rapidly in human plasma by virtue of pseudocholinesterase present in plasma and at the same time are chemically highly stable.[28-30] This finding led us to investigate such ester type as being a potentially true prodrug form for aspirin. In this paper the synthesis and hydrolysis kinetics of a number of glycolamide esters of aspirin are reported along with physicochemical properties such as lipophilicity and water solubility. Various other esters of aspirin were also studied since data on their kinetics and mechanism of hydrolysis in plasma in most cases are absent in the literature.

Synthesis

The aspirin glycolamide esters **1–13** (Table I) were all prepared by reacting aspirin with the appropriate 2-chloroacetamide in ethyl acetate. The esters **26** and **27** (Table II) were prepared in a similar way with *N*-methyl-*N*-(β-chloroethyl)acetamide and 2-chloro-*N*,*N*-diethylpropionamide, respectively, as the alkylating agent. Aspirin phenyl ester (**15**) was obtained by acetylation of phenyl salicylate with acetic anhydride in the presence of catalytic amounts of concentrated sulfuric acid. The esters **14**, **16–24**, and **25** (Table II) were all prepared according to known procedures as referred to in Table II.

Plasma-Catalyzed Ester Hydrolysis

The rates of hydrolysis of the various aspirin esters were determined in 10% human plasma (pH 7.4) at 37 °C. At initial concentrations of about 2×10^{-4} M the progress of hydrolysis of all esters except **5–7**, **11**, and **25** followed strict first-order kinetics over several half-lives as illustrated in Figure 2. The observed pseudo-first-order rate constants (k_{obs}) for the hydrolysis were calculated from the slopes of linear plots of the logarithm of remaining ester against time; the corresponding half-lives ($t_{1/2}$) were obtained from eq 1. These data are shown in Tables I and II.

$$t_{1/2} = 0.693/k_{obs} \quad (1)$$

The rate of hydrolysis of the esters **5–7**, **11**, and the *N*,*N*-dimethylethanolamine ester **25** initially followed zero-order kinetics, and as the ester substrate depleted, the rate changed to follow first-order kinetics. Plots of the data for the hydrolysis of the esters **6** and **11** in 10% human plasma are shown in Figure 3. As previously described for similar esters of benzoic acid[28,29] and salicylic acid[30] such progress curves can be accounted for according to the integrated form of the Michaelis–Menten equation:[35]

$$V_{max}t = S_0 - S + K_m \ln (S_0/S) \quad (2)$$

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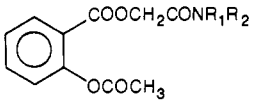
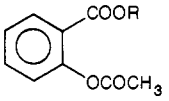
Table I. Physical Properties and Plasma Hydrolysis Data of Various Glycolamide Esters of Aspirin

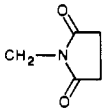


				hydrolysis data in 10% human plasma				
compd	R_1	R_2	mp, °C	10^2k_{obs}, min^{-1}	$t_{1/2}$, min	% aspirin formed[b]	10^2k_1, min^{-1}	10^2k_2, min^{-1}
1	H	H	128–129[a]	6.25	11.1	0 (0)		6.25
2	H	C_2H_5	80–81	26.0	2.7	0 (0)		26.0
3	H	CH_2CONH_2	185–187	13.6	5.1	0		13.6
4	H	$CH_2COOC_2H_5$	68–69	21.2	3.3	0		21.2
5	CH_3	CH_3	75–76	20.9	3.3	50 (49)	10.5	10.5
6	C_2H_5	C_2H_5	76–77	58.5	1.2	55 (63)	32.2	26.3
7	C_3H_7	C_3H_7	50–51	8.34	8.3	8	0.67	7.67
8	i-C_3H_7	i-C_3H_7	108–109	4.62	15.0	20 (21)	0.92	3.70
9	C_6H_{11}	C_6H_{11}	133–134	3.35	20.7	0		3.35
10	CH_3	CH_2CH_2OH	70–71	18.4	3.8	38 (40)	6.99	11.4
11	CH_3	CH_2CONH_2	132–133	9.24	7.5	37	3.42	5.82
12	CH_3	$CH_2COOC_2H_5$	47–48	34.0	2.0	0		34.0
13	morpholinediyl		97–98	12.0	5.7	2	0.24	12.0

[a] Literature[31] mp 130 °C. [b] Values given in parentheses are percent aspirin formed in undiluted plasma.

Table II. Physical Properties and Plasma Hydrolysis Data of Various Aspirin Esters



			hydrolysis data in 10% human plasma				
compd[a]	R	mp, °C	10^2k_{obs}, min^{-1}	$t_{1/2}$, min	% aspirin formed[c]	10^2k_1, min^{-1}	10^2k_2, min^{-1}
14[32]	CH_3	47–49	21.4	3.2	0 (0)		21.4
15	C_6H_5	97–98	53.0	1.3	0 (0)		53.0
16[33]	C_6H_4-2-COOH	166–167	0.94	73.7	0 (0)		0.94
17[34]	C_6H_4-4-$NHCOCH_3$	174–175	48.7	1.4	0		48.7
18[20]	CH_2SCH_3	oil	46.5	1.5	0 (0)		46.5
19[20]	CH_2SOCH_3	80–81[b]	20.7	3.3	30 (31)	6.21	14.5
20[20]	$CH_2SO_2CH_3$	149–151	43.6	1.6	20 (20)	8.72	34.9
21[19]	CH_2OOCCH_3	oil	39.5	1.8	29 (28)	11.5	28.0
22[19]	$CH_2OOCC_3H_7$	oil	72.3	1.0	0 (0)		72.3
23[31]	$CH_2COOC_2H_5$	51–52	31.5	22	0		31.5
24[19]		117–118	33.7	2.1	11 (10)	3.71	30.0
25[34]	$CH_2CH_2N(CH_3)_2$,HCl	125–126	265	0.26	6 (8)	15.9	249
26	$CH_2CH_2N(CH_3)COCH_3$	oil	20.5	3.4	0		20.5
27	$CH(CH_3)CON(C_2H_5)_2$	40–41	10.7	6.5	0		10.7

[a] The compounds were prepared according to the references given. [b] Literature[20] mp 118–122 °C for the hydrate. [c] Values given in parentheses are percent aspirin formed in undiluted plasma.

Table III. Michaelis–Menten Parameters for the Hydrolysis of Aspirin Esters in 10% Human Plasma Solutions (pH 7.40) at 37 °C

compd	10^5K_m, M	10^5V_{max}, M min^{-1}	V_{max}/K_m, min^{-1}
5	19.6	4.11	0.21
6	2.6	1.52	0.58
7	8.7	0.72	0.083
11	38.0	3.51	0.092
25	4.9	13.0	2.65

where S_0 is the initial substrate concentration and S is the substrate concentration at time t. The rate parameters K_m and V_{max} listed in Table III were obtained by analyzing the progress curves according to eq 2, using iterative nonlinear regression analysis as described by Robinson and Characklis.[36] The solid curves in Figure 3 are theoretical curves based on eq 2 and the values for K_m and V_{max} listed in Table III. At a low substrate concentration, i.e. $S \ll K_m$, the Michaelis–Menten equation

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

is simplified to

$$-\frac{dS}{dt} = \frac{V_{max}}{K_m}S \tag{4}$$

Thus, at these conditions, which are similar to those normally prevailing in vivo for prodrug hydrolysis, the enzymatic reaction becomes first-order with a rate constant equal to V_{max}/K_m. The values for k_{obs} and $t_{1/2}$ given in Tables I and II for the esters **5–7**, **11**, and **25** refer to this rate constant, i.e.

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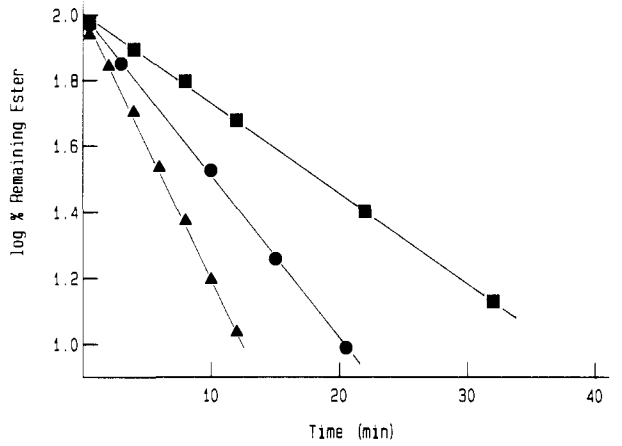

Figure 2. Plots showing the apparent first-order kinetics of degradation of the aspirin esters **1** (■), **10** (▲), and **15** (●) in 10% human plasma solutions at 37 °C.

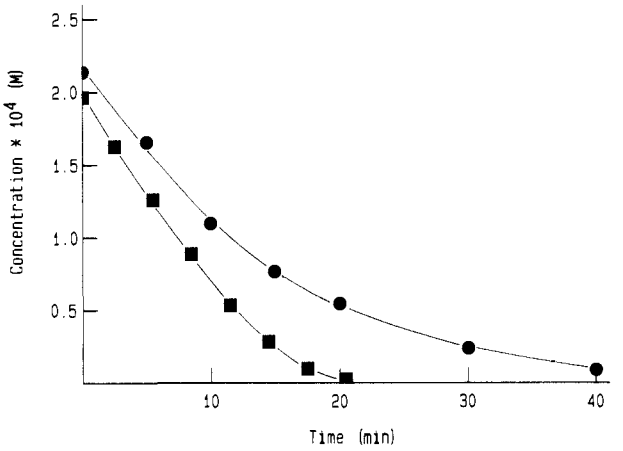

Figure 3. Plots showing the rate of hydrolysis of compound **6** (■) and compound **11** (●) in 10% human plasma solutions at 37 °C. The curves are calculated from eq 2 and the rate parameters K_m and V_{max} given in Table IV for the compounds.

$$t_{1/2} = 0.693/(V_{max}/K_m) \quad (5)$$

The products of hydrolysis of the aspirin esters were identified by HPLC. The esters **5–8**, **10**, **11**, **13**, **19–21**, **24**, and **25** were found to be cleaved via the k_1 reaction pathway depicted in Figure 1 with formation of aspirin along with a simultaneous deacetylation leading to the corresponding salicylic acid ester[37] (the k_2 reaction). All other esters produced less than 0.5% aspirin based on the initial ester concentration and they exclusively underwent deacetylation to yield the corresponding salicylate ester. An illustrative plot of the data for the aspirin methyl ester (**14**) is shown in Figure 4. The time course for the products formed upon hydrolysis of the *N,N*-dimethylglycolamide ester **5** in 10% plasma is shown in Figure 5. As can be seen from the figure, the corresponding salicylic acid ester shows only a transistory existence due to its rapid hydrolysis to yield salicylic acid as reported previously.[30]

In Figure 1 k_1–k_4 refer to pseudo-first-order rate constants for the processes depicted. Since k_3 in all cases was found to be much smaller[38] than the rate constant for the overall degradation of the esters (k_{obs}), k_1 was simply determined from the equation

$$k_1 = (\%\ \text{aspirin formed})k_{obs}/100 \quad (6)$$

and k_2 was obtained from

$$k_2 = k_{obs} - k_1 \quad (7)$$

The rate parameters obtained for the esters are shown in Tables I and II.

The use of plasma in 10% concentration in the hydrolysis studies instead of in an undiluted state was due to the desire to obtain comparable data for all the esters studied including the most reactive ones. However, the amount of aspirin formed from the esters upon plasma-catalyzed hydrolysis was found to be independent of the plasma concentration in the range 10–100%. This is shown

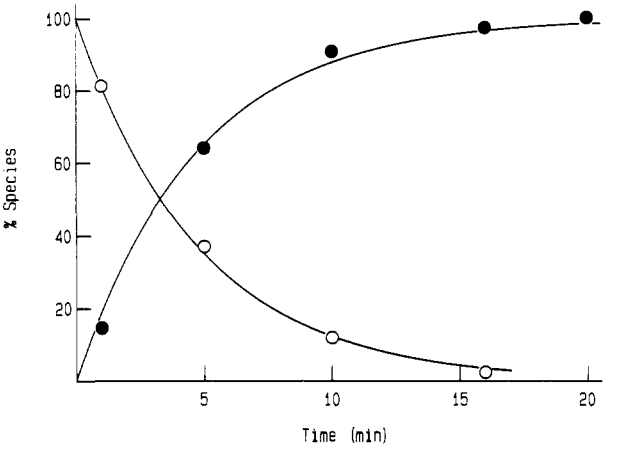

Figure 4. Time courses for the aspirin methyl ester **14** (○) and methyl salicylate (●) during the degradation of compound **14** in 10% human plasma solution at 37 °C.

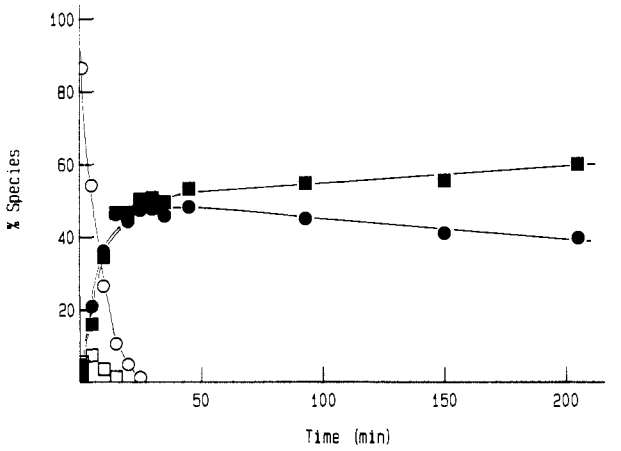

Figure 5. Time courses for the aspirin ester **5** (○) in 10% human plasma solution at 37 °C and its hydrolytic products, aspirin (●), salicyloyl-*N,N*-dimethylglycolamide (□), and salicylic acid (■).

Table IV. Half-Lives of Hydrolysis of the *N,N*-Diethylglycolamide Ester of Aspirin (**6**) and Amounts of Aspirin Formed in Human Plasma Solutions at 37 °C

human plasma concn, %	$t_{1/2}$, s	aspirin formed, %
10	72	55
20	38	63
60	15	54
80	9	60
100	4	63

[a] In buffer solution (pH 7.4) alone, the half-life of hydrolysis of ester **6** was 85.5 h at 37 °C.

(37) Various glycolamide esters of salicylic acid were described previously.[30] The salicylic acid esters corresponding to the aspirin esters **18–20** were prepared as described by Loftsson et al.[20]

(38) The hydrolysis of aspirin in 10% human plasma at 37 °C was found to proceed with a rate constant of 0.07 h^{-1} corresponding to a half-life of 9.8 h.

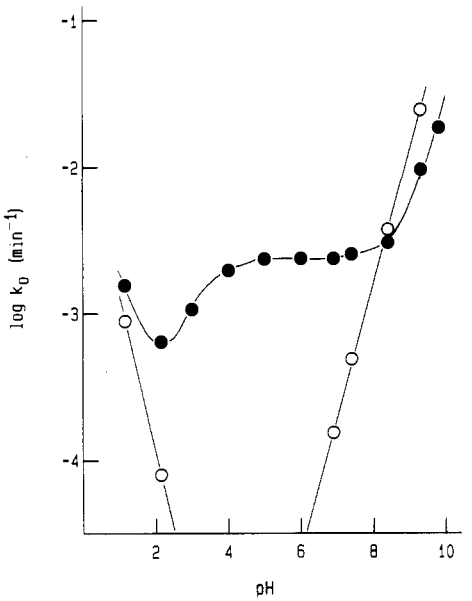

Figure 6. The pH–rate profiles for the hydrolysis of aspirin (●) and its *N*,*N*-diethylglycolamide ester **6** (○) in aqueous solution (μ = 0.5) at 50 °C; k_0 is the observed pseudo-first-order rate constant obtained at zero buffer concentration.

by the data in Table IV for the *N*,*N*-diethylglycolamide ester **6** and in Tables I and II for other esters.

The relative importance of the k_1 and k_2 routes of ester hydrolysis did not change significantly by performing the hydrolysis in human blood instead of plasma. Thus, in whole human blood and at an initial concentration of 1.1 × 10^{-4} M, the esters **5** and **6** hydrolyzed to yield aspirin in an amount of 46 and 48%, respectively, the half-life of the overall degradation being 0.5 (**5**) and 0.4 (**6**) min. The enzyme being largely responsible for the facile glycolamide ester hydrolysis appears to be pseudocholinesterase,[28,29] and its activity in human plasma and blood is almost the same.[39]

Stability of the Glycolamide Ester 6

The stability of the aspirin ester **6** was determined in aqueous solution at 50 °C and compared with that of aspirin. At constant pH the degradation of the compounds followed strict first-order kinetics over several half-lives. The influence of pH on the rates of hydrolysis (corrected for a slight buffer catalysis in some cases) is shown in Figure 6. The shape of the pH–rate profile indicates that the ester **6** is subject to specific acid and base catalyzed hydrolysis. At 50 °C the specific catalytic rate constants k_H and k_{OH} were determined to be 0.012 and 300 M^{-1} min^{-1}, respectively. It can be calculated that the ester is most stable at pH about 4.5, which is similar to the behavior of the corresponding benzoic acid ester.[29] Due to very slow degradation at pH 3–6 no rate constants were obtained at these pH values. As seen from Figure 6 the ester **6** is much more stable than aspirin in the pH range 2–7. The high reactivity of aspirin at these pH values is due to intramolecular general base catalysis by the ionized carboxyl group,[40] and blocking this group by esterification should accordingly stabilize the acetyl ester moiety. The shape of the pH–rate profile for aspirin is in accordance with that found by others[40] at 25 °C.

The nonenzymatic hydrolysis of ester **6** proceeds by the two parallel reactions (k_1 and k_2) shown in Figure 1. Product analysis studies performed at pH 9–10 showed that the main degradation route in alkaline solutions is the k_2 route, accounting for 83% of the overall hydrolysis.

Table V. Solubility and Lipophilicity Data for Various Aspirin Esters

compd	S,[a] mg/mL	log P[b]	log k'[c]
1	3.84	0.22	0.10
2	5.52	0.90	0.45
3	0.88	−0.43	0.01
4	4.32	0.99	0.59
5	7.50	0.38	0.30
6	2.28	1.16	0.76
7	0.72	2.09	1.41
8	0.18	2.03	1.41
10		0.06	0.14
11		−0.46	0.02
12	0.96	1.67	0.99
13	4.84	0.30	0.29
14	2.81	1.46	0.71
15	0.02	2.88	1.57
17	0.02	2.15	1.05
18	0.55	1.94	1.12
19	4.23	0.11	0.16
20	0.11	0.57	0.26
21	2.43	1.42	0.69
22	0.35	2.50	1.48
23	0.70	1.67	0.99
24	0.50	0.50	0.37
25	>200		
26		1.46	1.00
27	7.68	0.67	0.47

[a] Solubility in water at 21 °C. [b] P is the partition coefficient between octanol and water (at 21 °C). [c] k' is the chromatographic capacity factor.

Lipophilicity and Solubility of the Aspirin Esters

The water solubilities and octanol–water partition coefficients of the esters along with their reversed-phase HPLC capacity factors are given in Table V. As has been observed for many different types of compounds,[41] a linear relationship was found to exist between log k' and log P for the esters:

$$\log P = (1.81 \pm 0.09) \log k' - (0.14 \pm 0.08) \quad (8)$$

$$n = 24,\ r = 0.972$$

Discussion

Only a few of the aspirin esters investigated were found to undergo hydrolysis in human plasma to yield aspirin in appreciable amounts and thus to behave as true aspirin prodrugs. Contrary to previous claims[7,8,42] the simple methyl ester **14** did not release any aspirin in the presence of plasma but was exclusively hydrolyzed at the acetyl ester moiety to yield methyl salicylate (Figure 4). A similar behavior was observed for the phenolic esters **15**–**17**. Loftsson et al.[20] have previously reported that the (methylsulfinyl)methyl ester **19** and the (methylsulfonyl)methyl ester **20** were almost quantitatively hydrolyzed to aspirin in human plasma at 37 °C. In our hands, however, only 30 and 20% aspirin, respectively, were formed from these compounds in 10% plasma (Figure 7) as well as in undiluted plasma (Table II). Although no explanation for this discrepancy can be offered, our data qualitatively

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(40) St. Pierre, T.; Jencks, W. P. *J. Am. Chem. Soc.* **1968**, *90*, 3817–3827.
(41) Hafkenschied, T. L.; Tomlinson, E. *Int. J. Pharm.* **1983**, *16*, 225–239. Brent, D. A.; Sabatka, J. J.; Minick, D. J.; Henny, D. W. *J. Med. Chem.* **1983**, *26*, 1014–1020.
(42) Rainsford, K. *TIPS* **1984**, *5*, 156–159.

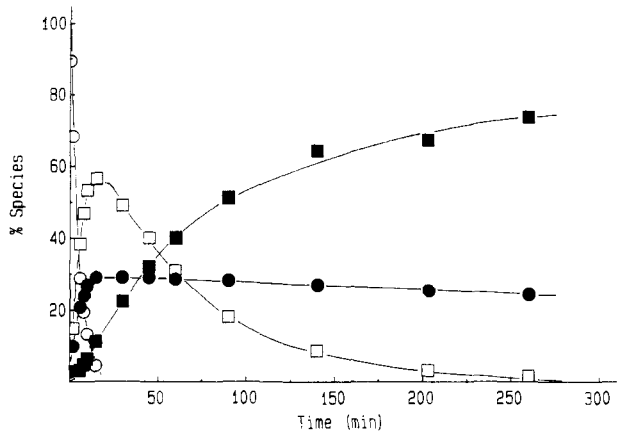

Figure 7. Time courses for the aspirin ester **20** (○) in 10% human plasma solution at 37 °C and its hydrolytic products, aspirin (●), (methylsulfonyl)methyl 2-hydroxybenzoate (□), and salicylic acid (■).

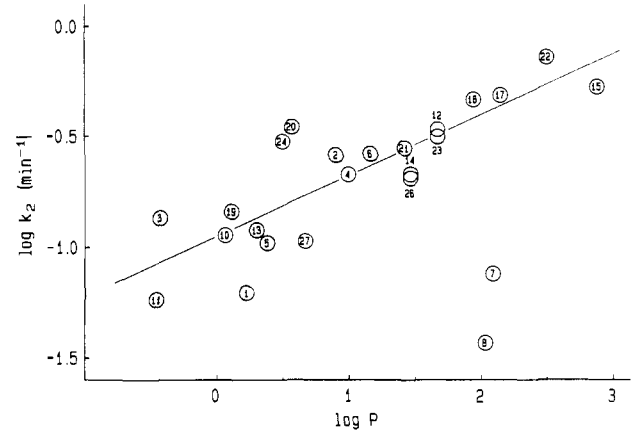

Figure 8. Plot of log k_2 versus log P for various aspirin esters. The data are taken from Tables I, II, and V. Compounds **7** and **8** were excluded from the regression plot.

corroborate the findings of Loftsson and coworkers. (Acyloxy)methyl esters are usually cleaved very fast in the presence of plasma enzymes, but also for such esters of aspirin (**21** and **22**) the deacetylation leading to the corresponding salicylate ester proceeds more rapidly. In the case of the acetoxymethyl ester **21**, however, 29% of aspirin was formed. On the basis of a previous observation[29,43] that the *N,N*-dimethylethanolamine ester of benzoic acid is hydrolyzed very fast in human plasma ($t_{1/2}$ being < 3 s in 50% plasma), the corresponding aspirin ester (**25**) was expected to form aspirin in large amounts. However, despite a very fast hydrolysis ($t_{1/2}$ being 0.26 min in 10% plasma), only 6% of aspirin was formed. Apparently, the *N,N*-dimethylaminoethyl group makes the *O*-acetyl ester moiety correspondingly more susceptible to undergo hydrolysis. As seen from the rate constants k_2 in Table II, the deacetylation occurs much faster for compound **25** than for the methyl ester **14**.

The most prominent structural requirement for glycolamide esters to be hydrolyzed rapidly by plasma is the presence of two substituents on the amide nitrogen atom as found in a study of various esters of benzoic acid.[28,29] N,N-Disubstituted glycolamide esters were much more rapidly hydrolyzed than either monosubstituted or unsubstituted glycolamide esters. This structural dependence also holds true for the aspirin esters in that only the N,N-disubstituted glycolamide esters hydrolyzed to yield aspirin (Table I). The *N,N*-diethylglycolamide ester **6** showed the highest k_1 value, and this is in accordance with the finding[29] that among various glycolamide esters of benzoic acid the *N,N*-diethyl derivative showed the highest rate of enzymatic hydrolysis.

In considering structural factors influencing the conversion of aspirin esters to the parent drug with the aim of designing true aspirin prodrugs, it is not only important to look upon the ester structure from the view of obtaining a high rate of ester cleavage per se. It is equally important to consider the possibility of depressing the rate of deacetylation (the k_2 reaction in Figure 1). It is not the absolute values of the rate constants k_1 and k_2 that determine the relative importance of the two hydrolysis pathways but the ratio of the rate constants. To obtain an aspirin prodrug the ratio k_1/k_2 should be as great as possible. Thus, if aspirin should be regenerated from an ester in a yield of 90%, the k_1/k_2 ratio should be 9. The ease of the plasma-catalyzed deacetylation of aspirin esters is expected to be dependent on the ester structure at the carboxylic group. The introduction of a negatively charged carboxy group (compound **16**) is seen to greatly slow down the rate of deacetylation, but at the same time the k_1 reaction is also greatly depressed. This finding appears to be general in that several other esters with an ionized carboxylate group are also poor substrates for hydrolytic enzymes, e.g. succinate and *O*-acylglycolate esters.[43] Similarly, the deacetylation of aspirin occurs much slower than the deacetylation of neutral aspirin esters. The introduction of sterically hindered groups in the alcohol portion of the ester moiety may presumably result in a slow deacetylation and also in a slower ester hydrolysis, and thus not give rise to a change in the k_1/k_2 ratio in a favorable direction. The *N,N*-dicyclohexylglycolamide ester **9** is seen to have a relatively low rate of deacetylation, but the k_1 reaction is so slow that no aspirin is produced.

Other factors influencing the k_2 reaction and thus the k_1/k_2 ratio could be the hydrophilicity or lipophilicity of the esters. It was indeed found that the k_2 values for the various esters generally increased with increasing lipophilicity of the esters. In Figure 8 log k_2 has been plotted against log P. By correlating the data with a linear regression equation and omitting compounds **7** and **8**, eq 9 is obtained:

$$\log k_2 = (0.28 \pm 0.04) \log P - (0.95 \pm 0.05) \quad (9)$$

$$n = 22,\ r = 0.847$$

Similar analysis of the rate constants k_1 revealed no clear dependence of these on log P. These data taken together indicate that, in order to depress the k_2 reaction and increase the k_1/k_2 ratio, an aspirin prodrug ester should not be too lipophilic. Among the esters studied, the glycolamide esters **5** and **6** show the greatest promise as aspirin prodrugs. In human plasma or blood they are rapidly cleaved with the formation of about 50% aspirin and 50% salicylic acid, the latter arising from an extremely rapid hydrolysis ($t_{1/2}$ < 5 s) of the intermediarily formed salicylate glycolamide esters.[30] Besides, by their appreciable enzymatic conversion to aspirin, these esters are chemically highly stable solid compounds with water solubilities and lipophilicities favorable for delivery. Studies are in progress to examine the peroral and dermal delivery characteristics of these prodrug derivatives.

Experimental Section

Melting points were taken on a capillary melting-point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on

(43) Nielsen, N. M.; Bundgaard, H. *Int. J. Pharm.* **1987**, *39*, 75–85.

a Varian 360 L instrument using tetramethylsilane as the internal standard. Elemental analysis (C, H, and N) were performed at the Microanalytical Laboratory, University of Copenhagen, and the results obtained were within ±0.4% of the theoretical values. High-performance liquid chromatography (HPLC) was generally done 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 Chrompack column (100 × 3 nm) packed with Chromspher C 18 (15-μm particles) was used. In some hydrolysis studies in aqueous buffer solutions and for the determination of chromatographic capacity factors, a HPLC system consisting of a Waters pump Model 510, a Waters autosampler Model 712 equipped with a thermostated sample compartment, and a variable-wavelength UV detector Waters type Lambda Max 481 operated at 230 nm was used. A Waters Radial-PAK column (100 × 8 mm) packed with NOVA-PAK, Phenyl (4-μm particles), or NOVA-PAK (C 18) for the determination of capacity factors, was used in conjunction with a Waters RCM 8 × 10 compression module.

Synthesis of Aspirin Glycolamide Esters (1–13). To a solution of aspirin (0.01 mol) in ethyl acetate (40 mL) was added triethylamine (0.011 mol), NaI (0.001 mol), and the appropriate 2-chloroacetamide (0.011 mol). The mixtures were refluxed for 3 h and filtered upon cooling. The filtrate was washed with a 2% aqueous solution of sodium thiosulfate, 2% $NaHCO_3$, and H_2O. After drying over anhydrous Na_2SO_4, the ethyl acetate was removed under reduced pressure to afford the glycolamide esters, which were purified by recrystallization from ethanol, ethyl acetate–ether, or ether–petroleum ether. Yields were in the range of 60–80%. Melting points of the glycolamide esters are given in Table I.

In most cases the appropriate 2-chloroacetamides used in ester synthesis were not commercially available. 2-Chloro-*N*-methyl-*N*-(β-hydroxyethyl)acetamide was prepared by aminolysis of methyl chloroacetate with *N*-methylethanolamine as described by Harkins.[44] α-(Chloroacetyl)sarcosinamide was prepared as previously described.[29]

In the case of esters **3**, **4**, **7–9**, **12**, and **13** the appropriate 2-chloroacetamides were prepared by reacting the appropriate amine with chloroacetyl chloride in ethyl acetate. To a stirred ice-cooled solution of the amine (0.01 mol) and triethylamine (0.011 mol) in ethyl acetate (20 mL) was added dropwise chloroacetyl chloride (0.010 mol). After stirring of the mixture at room temperature for 2 h, triethylammonium hydrochloride was filtered off and the filtrate concentrated under reduced pressure. The oily residues obtained were used for the ester synthesis without further purification.

Phenyl 2-Acetoxybenzoate (15). A solution of 10.7 g (0.05 mol) of phenyl salicylate in acetic anhydride (10 mL) and concentrated H_2SO_4 (0.1 mL) was stirred for 45 min at room temperature. The mixture was poured into cold H_2O (100 mL) and a white precipitate formed immediately. The precipitate was filtered off, dried, and recrystallized from EtOH.

***N*-Methyl-*N*-[2-[(2′-acetoxybenzoyl)oxy]ethyl]acetamide (26)** was prepared in a way similar to that for the various glycolamide esters by reacting aspirin in ethyl acetate with *N*-methyl-*N*-(β-chloroethyl)acetamide obtained as described previously.[45]

2-[(2′-Acetoxybenzoyl)oxy]-2′-methyl-*N*,*N*-diethylacetamide (27) was prepared by reacting aspirin in ethyl acetate with 2-chloro-*N*,*N*-diethylpropionamide, the latter being prepared from 2-chloropropionyl chloride and diethylamine as described above.

Preparation of Other Esters. The other esters studied were prepared according to the literature references given in Table II. The physical properties agreed with those reported.

All compounds **1–27** showed ^1H NMR spectra and elemental analyses consistent with their structures.

Hydrolysis Kinetics in Human Plasma or Blood. The hydrolysis of the esters **1–27** was generally studied in 0.01 M phosphate buffer of pH 7.40 containing 10% human plasma at 37 °C. In some cases 100% plasma and whole blood were also used. Initial concentrations of the compounds were (1–2) × 10^{-4} M. The reactions were initiated by adding 50 μL of a stock solution of the compounds in acetonitrile to 5.00 mL of preheated plasma or blood solutions. The solutions were kept in a water bath at 37 °C, and at appropriate intervals samples of 250 μL were withdrawn and added to 500 μL of a 2% solution of $ZnSO_4 \cdot 7H_2O$ in MeOH–H_2O (1:1 v/v) in order to deproteinize the plasma or blood. After immediate mixing and centrifugation for 3 min at 10000 rpm, 20 μL of the clear supernatant was analyzed by HPLC.

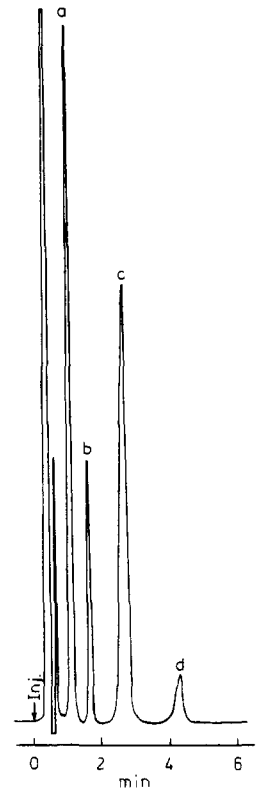

Figure 9. A chromatogram showing the separation of aspirin (a), salicylic acid (b), and the *N*,*N*-diethylglycolamide esters of aspirin (**6**) (c) and of salicylic acid (d).

The reverse-phase HPLC procedures used allowed separation and simultaneous quantitation of remaining aspirin ester and the products of hydrolysis, aspirin, salicylic acid, and the corresponding salicylic ester. An example of a chromatogram is shown in Figure 9. Mobile phase systems consisting of acetonitrile or MeOH in 1–2% aqueous solutions of H_3PO_4 were used, the composition being adjusted for each ester in order to provide appropriate retention times (1–8 min) and separation of the ester and the hydrolysis products. The flow rates were 0.6–1.5 mL/min, and the column effluent was monitored at 215 nm. Quantitation of the compounds was done by measurement of the peak heights in relation to those of standards chromatographed under the same conditions.

Kinetic Studies in Aqueous Buffer Solutions. The hydrolysis of aspirin and compound **6** was studied in aqueous buffer solutions of pH 1–11 at 50.0 ± 0.2 °C. The buffers used were hydrochloric acid, acetate, phosphate, borate, and carbonate. The total buffer concentration was 0.01–0.05 M. A constant ionic strength (μ) of 0.5 was maintained for each buffer solution by adding a calculated amount of KCl. The degradation of the compounds was followed by using an HPLC procedure capable of determining both intact ester and all products of hydrolysis. The reversed-phase column (NOVA-PAK, Phenyl) was eluted with a mobile phase consisting of CH_3CN–CH_3OH–H_2O–H_3PO_4 (40:10:50:1 v/v), the flow rate being 1.0 mL/min. The column effluent was monitored at 230 nm. The compounds were quantitated by measuring peak areas in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 50 μL of a stock solution of aspirin or ester **6** in acetonitrile to 4 mL of preheated buffer solution, the final concentrations of the compounds being about 10^{-4} M. The solutions, filled in sample vials with self-sealing septa

(44) Harkins, E. M. U.S. Patent 3,173,900, 1965.
(45) Nikolaev, A. F.; Rozenberg, M. E.; Daniel, N. V.; Tereshchenko, G. P. *J. Gen. Chem. USSR* **1963**, *33*, 385–387.

screw caps, were placed in the thermostated autosampler. At appropriate intervals 20-μL samples of the reaction solutions were injected into the chromatographic system. Pseudo-first-order rate constants for the hydrolysis were calculated from the slopes of linear plots of the logarithm of remaining ester **6** or aspirin against time.

Determination of Lipophilicity Parameters. The partition coefficients of the various aspirin esters were determined in octanol–water at 22–24 °C. The compounds were dissolved in water and the octanol–water mixtures were shaken for 30 min to reach a distribution equilibrium. The octanol and water were mutually saturated prior to use. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could readily be measured by HPLC. The partition coefficients (P) were calculated from

$$P = \left(\frac{C_i - C_w}{C_w}\right)\left(\frac{V_w}{V_0}\right) \tag{10}$$

where C_i and C_w are the solute concentrations in the aqueous phase before and after distribution, respectively, V_w is the volume of the aqueous phase, and V_0 the volume of the octanol phase.

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:

$$k' = (t_R - t_0)/t_0 \tag{11}$$

where t_R is the retention time of the solute and t_0 is the elution time for an unretained solute (KNO_3). The C18 column used was eluted at ambient temperature with MeOH–H_2O (45:55 v/v) at a rate of 1.0 mL/min.

Determination of Water Solubility. The solubility of the esters in water was determined at 23 ± 1 °C. Excess amounts of the compounds were added to water in screw-capped test tubes and the mixtures were placed in an ultrasonic water bath for 10 min and then rotated on a mechanical spindle for about 24 h. It was ensured that saturation equilibrium was established. Upon filtration, an aliquot of the filtrate was diluted with an appropriate amount of water and analyzed by HPLC.

Registry No. **1**, 50785-22-3; **2**, 118247-01-1; **3**, 118247-02-2; **4**, 118247-03-3; **5**, 118247-04-4; **6**, 116482-56-5; **7**, 116482-75-8; **8**, 116482-76-9; **9**, 116482-79-2; **10**, 118247-05-5; **11**, 116482-78-1; **12**, 116482-77-0; **13**, 116482-80-5; **14**, 580-02-9; **15**, 134-55-4; **16**, 530-75-6; **17**, 5003-48-5; **18**, 76432-30-9; **19**, 76432-33-2; **20**, 76432-35-4; **21**, 118247-06-6; **22**, 118247-07-7; **23**, 50785-24-5; **24**, 32620-72-7; **25**, 18072-98-5; **26**, 118247-08-8; **27**, 118247-09-9; $ClCH_2CONH_2$, 79-07-2; $ClCH_2CONHC_2H_5$, 105-35-1; $ClCH_2CONHCH_2CONH_2$, 41312-83-8; $ClCH_2CONHCH_2COOC_2H_5$, 41602-50-0; $ClCH_2CON(CH_3)_2$, 2675-89-0; $ClCH_2CON(C_2H_5)_2$, 2315-36-8; $ClCH_2CON(C_3H_7)_2$, 2315-37-9; $ClCH_2CON(i\text{-}C_3H_7)_2$, 7403-66-9; $ClCH_2CON(C_6H_{11})_2$, 2567-50-2; $ClCH_2CON(CH_3)CH_2CH_2OH$, 87550-50-3; $ClCH_2CON(CH_3)CH_2CONH_2$, 114665-31-5; $ClCH_2CON(CH_3)CH_2COOC_2H_5$, 24515-53-5; chloroacetylmorpholine, 1440-61-5; aspirin, 50-78-2; methyl chloroacetate, 96-34-4; *N*-methylethanolamine, 109-83-1; chloroacetyl chloride, 79-04-9; phenyl salicylate, 118-55-8; *N*-methyl-*N*-(β-chloroethyl)acetamide, 17225-69-3; 2-chloro-*N*,*N*-diethylpropionamide, 54333-75-4; 2-chloropropionyl chloride, 7623-09-8; diethylamine, 109-89-7.