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Short communication

Prodrugs – Part 1. Formylphenyl esters of aspirin

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Summary — The synthesis and study of a novel series of potential prodrugs of aspirin is reported. 2-, 3- and 4-formylphenyl aspirins, as well as a series of 4-substituted 2-formylphenyl aspirins, have been prepared. A study of their alkaline hydrolysis indicates that these compounds act as true prodrugs of aspirin which hydrolyse to aspirin and the formylphenol. The rates of hydrolysis and activation parameters indicate that hydrolysis of the 2-formylphenyl esters employs an intramolecular catalytic route.

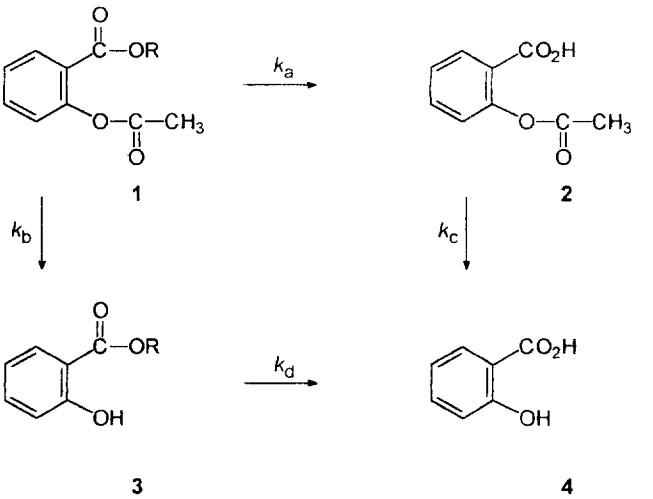
prodrugs / aspirin

Introduction

Prodrugs have been described as the chemical modification of a biologically active compound to form a new compound, which upon either in vivo enzymatic or non-enzymatic attack will liberate the parent drug [1–3]. A number of attempts have been made to develop prodrugs of aspirin in order to depress its side effects involving gastric irritation and bleeding, as well as to improve transport characteristics [4, 5]. Aspirin prodrugs have been divided into two groups, ie, derivatives that undergo enzymatic cleavage to regenerate the parent drug and that hydrolyse non-enzymatically to give the same [6].

The major problem in the design of aspirin prodrugs is the lability of the acetyl ester group in aspirin prodrugs in which the carboxyl function is itself esterified [6]. In scheme 1 is shown conversion of an aspirin ester **1** into aspirin **2**, salicylic acid ester **3** and salicylic acid **4**. The requirement for a true aspirin prodrug is that $k_a > k_b$. If $k_b > k_a$ the prodrug is a prodrug for the ester **3**. Both **2** and **3** can be considered to be prodrugs for salicylic acid **4**.

Methylthiomethyl, methylsulfinylmethyl and methylsulfonylmethyl esters of aspirin have been prepared by Bodor's group [7]. These labile esters were shown to undergo neutral hydrolysis at pH > 4 to form aspirin and in vivo studies of one of the esters showed it to be a true aspirin prodrug [7, 8]. Intramolecular catalysis for ester hydrolysis can facilitate reaction both to a marked and to a tuneable degree. Neighbouring group participation by suitably situated carbonyl groups in the alkaline hydrolysis of esters has been recently reviewed and a number of criteria have been employed to establish the occurrence of such effects [9, 10].



Scheme 1.

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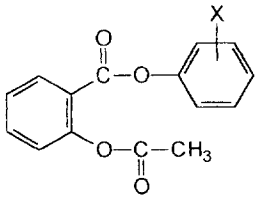
The present report details a study of the design, synthesis and hydrolysis of novel phenyl esters of aspirin having formyl substitutents, together with preliminary studies of their biological activity.

Chemistry

Design

Two series of aspirin esters have been designed in which the ester function is intended to suffer alkaline hydrolysis more rapidly than hydrolysis of the acetyl ester group. The first series is composed of 2-, 3- and 4-formylphenyl esters **5b**, **5c** and **5d**. The second series is composed of the 4-substituted 2-formylphenyl esters **5b** and **6**. The esters **5b** and **6** are expected to hydrolyse employing an intramolecular catalytic route [9, 10] (see scheme 2), while the esters **5c** and **5d** would hydrolyse by a normal $B_{AC}2$ pathway [9].

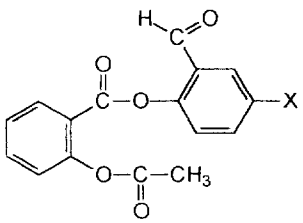


5a X = H

5b X = 2-CHO

5c X = 3-CHO

5d X = 4-CHO



6a X = CH_3

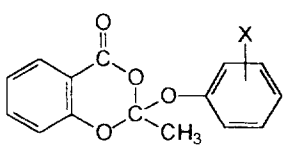
6b X = OCH_3

6c X = OPh

6d X = F

6e X = Cl

6f X = Br



7a X = H

Synthesis

The reaction of 2-acetoxybenzoyl chloride with the corresponding phenol, in the presence of triethylamine, gave the phenyl ester of aspirin. Senning and co-workers [12] have shown that this method can result in the formation of the chain (normal) esters **5/6** and/or the cyclic (pseudo) esters **7**, the 2-methyl-2-aryloxy-4*H*-1,3-benzodioxin-4-ones. All the formylphenols gave only the chain esters; **5b** to **5d** and **6**; but phenol gave both **5a** and **7a**.

An alternative synthesis which was only successful for the phenyl ester was the reaction between aspirin and phenol, using 1,3-dicyclohexylcarbodiimide (DCC) catalysed by *para*-toluenesulfonic acid [13]. The physical properties of the phenyl esters of aspirin are shown in table I.

Alkaline hydrolysis

The alkaline hydrolysis of the formylphenyl esters of aspirin resulted in the quantitative formation of aspirin **5a** and the respective formylphenol. The reactions were found to be first-order both in the ester substrate and in the hydroxide anion. However, the alkaline hydrolysis of the phenyl ester of aspirin gave the phenyl ester of salicylic acid and *not* aspirin, see [6]. The rate coefficients, k_2, for the alkaline hydrolysis of the formylphenyl aspirins in 30% (v/v) dioxane–water at 27.0, 37.0 and 47.0 °C are shown in table II. The activation parameters are shown in table III. The rates of hydrolysis of the 3- and 4-formylphenyl esters are relatively rapid as would be expected for the electron-withdrawing formyl substituent. The Hammett substituent constant, σ, is 0.35 and 0.42 for the *meta*- and *para*-formyl substituent, respectively [14]. The rate of alkaline hydrolysis of the phenyl aspirin **5a** in 30% dioxane–water at 37 °C was found to be 0.35 dm^3 mol^{-1} s^{-1} (phosphate buffers) reacting at the acetate group. The Hammett reaction constant, ρ, for the alkaline hydrolysis of phenyl acetates in 3% ethanol–water at 25 °C is 1.17 [15]. Estimates can be made for the rates of alkaline hydrolysis of the phenyl and 2-formylphenyl aspirin in 30% dioxane–water at 37 °C *if* reacting by the 'normal' pathway at the phenyl benzoate ester group as ca 2.3 and 11 dm^3 mol^{-1} s^{-1}, respectively, see [16]. Thus, the phenyl ester **5a** hydrolyses ca 6.6 times *slower* than expected and at the other ester group, which probably arises from the approximate nature of the estimate. However, the

Table I. Preparation of phenyl esters of aspirin **5** and **6**.

No	*Phenyl esters Subst*	*Mp (°C)*	*Recryst solvent*	*Anal*	*Ref*
5a	H	97[a]	Hexane	C, H	[6]
5b	2-CHO	72–74	Cyclohexane	C, H	
5c	3-CHO	86–87	Hexane	C, H	
5d	4-CHO	74–75	Hexane	C, H	
	2-Formylphenyl esters 4-Subst				
6a	CH_3	87–89	Cyclohexane	C, H	
6b	OCH_3	85–86	Hexane	C, H	
6c	OPh	Oil		C, H	
6d	F	74–75	Hexane/$CHCl_3$	C, H, F	
6e	Cl	97–98	Cyclohexane	C, H, Cl	
6f	Br	113–114	Cyclohexane	C, H, Br	

[a]Lit: mp 97–98 °C [6].

Table II. Rate coefficients (k_2) for the alkaline hydrolysis of formylphenyl esters of aspirin **5** and **6** in 30% (v/v) dioxane–water (at constant ionic strength of 1.0 mol dm^{-3})[a].

No	*Phenyl esters Subst*	*k_2/dm³ mol⁻¹ s⁻¹*			*λ/nm*[b]
		at 27.0 °C	*at 37.0 °C*	*at 47.0 °C*	
5b	2-CHO	440	498	554	389, 326
5c	3-CHO	3.64	6.32	11.0	330
5d	4-CHO	3.82	6.38	11.1	330
	2-Formylphenyl esters 4-Subst				
6a	CH_3	221	254	320	340
6b	OCH_3	370	426	486	360
6c	OPh	901	992	1040	345
6d	F	2500	2570	2380	335
6e	Cl	3580	3530	3090	340
6f	Br	3500	3530	3530	345

[a]Rate coefficients were reproducible to within ± 3%; [b]wavelength used to monitor alkaline hydrolysis.

Table III. Activation parameters for the alkaline hydrolysis of formylphenyl esters of aspirin **5** and **6** in 30% (v/v) dioxane–water at 30.0 °C.[a]

No	*Phenyl esters Subst*	$\Delta H^{\ddagger}$/kcal mol^{-1}[b]	$\Delta S^{\ddagger}$/cal mol^{-1}K^{-1}[b]
5b	2-CHO	1.6	–41
5c	3-CHO	10.0	–23
5d	4-CHO	9.6	–24
	2-Formylphenyl esters 4-Subst		
6a	CH_3	2.9	–38
6b	OCH_3	2.0	–40
6c	OPh	0.8	–42
6d	F	–1.1	–47
6e	Cl	–2.0	–49
6f	Br	–0.5	–44

[a]The uncertainties are ± 0.5 kcal mol^{-1} for $\Delta H^{\ddagger}$ and ± 2 cal mol^{-1} K^{-1} for $\Delta S^{\ddagger}$; [b]1 cal = 4.184 J.

2-formylphenyl ester hydrolyses ca 45 times faster than that expected for a 'normal' unassisted ester hydrolysis. The activation parameters for the alkaline hydrolysis of the esters are shown in table III. The values of $\Delta S^{\ddagger}$ are significantly more negative and those of $\Delta H^{\ddagger}$ smaller for *all* the 2-formylphenyl esters than for the 3- and 4-formylphenyl esters. The latter evidence clearly confirms the occurrence of intramolecular catalysis for the hydrolysis of the 2-formylphenyl esters [9, 10]. The reaction pathway for hydrolysis involving intramolecular catalysis is shown in scheme 2.

The Hammett equation (eq (1) below) has been used to study polar substituent effects for *meta*- and *para*-substituted benzene compounds [17]:

$$\log(k/k_0) = \rho\sigma. \quad (1)$$

The 4-substituted 2-formylphenyl aspirins studied here can be considered to be *para*-substituted with regard to the phenoxy link or *meta*-substituted to the formyl group. As shown in table IV, a very poor correlation was observed between log k_2 for the alkaline hydrolysis of the esters with σ_p, with only a moderate correlation with σ_m. However, application of a Jaffé-type modification [18] of the Hammett equation (2) gives an excellent correlation with ρ_m and ρ_p,

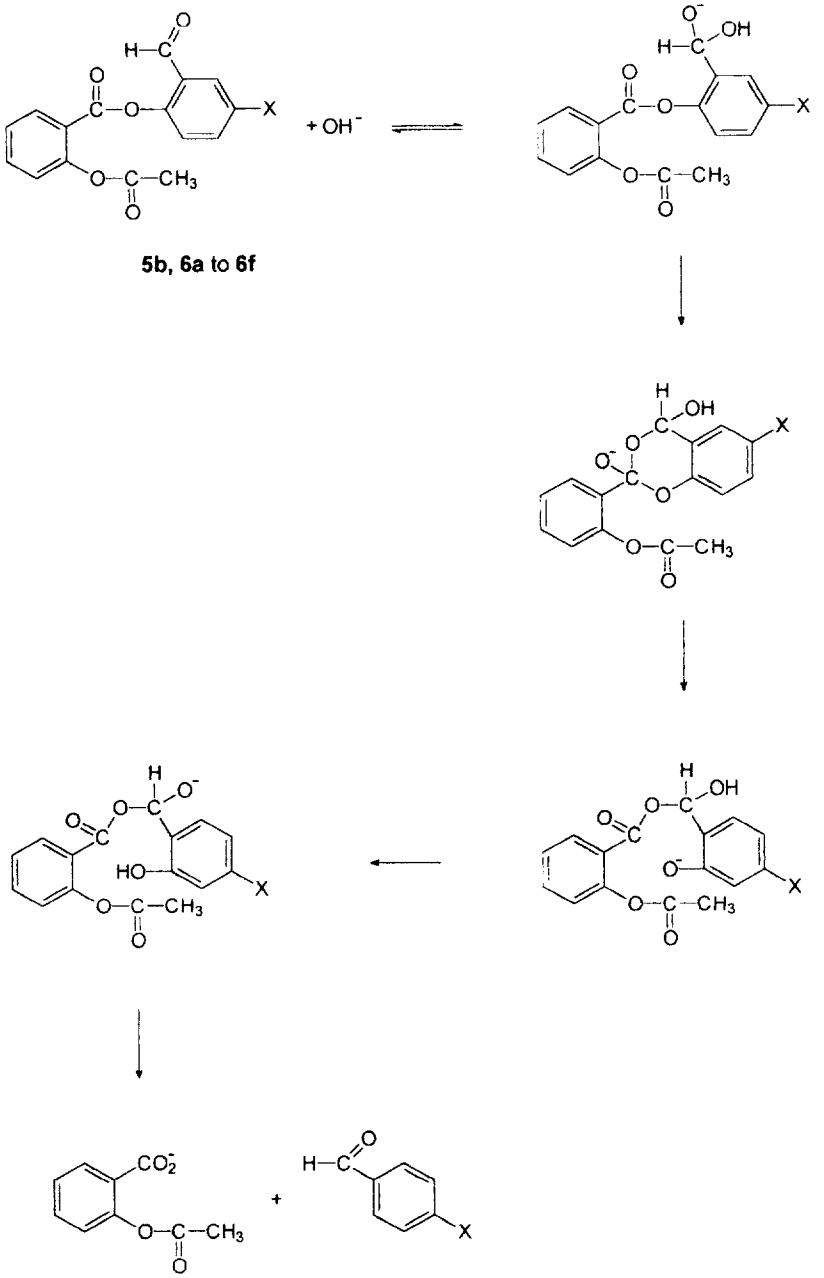
$$\log(k/k_0) = \rho_m\sigma_m + \rho_p\sigma_p \quad (2)$$

indicating the contributions to the susceptibility via the formyl group and phenoxy link, respectively. The total ρ value of ca 2.7 and the main contribution via ρ_m confirm the intramolecular pathway for the alkaline hydrolysis and the importance of the initial attack at the formyl group.

The esters are more lipophilic than aspirin. If the esters are absorbed intact (eg, in a topical application), their lability to hydrolysis at physiological pH would convert them to aspirin. The values of $t_{1/2}$ at pH 7.52 and 37 °C in 30% aqueous dioxane for the 2-, 3- and 4-formylphenyl esters are ca 18 min, 24 and 23 h, respectively, while $t_{1/2}$ under the same conditions for the 4-substituted 2-formylphenyl esters ranges from ca 2.5 to 35 min.

Pharmacology

The potential aspirin prodrugs were screened for anti-inflammatory activity in the carragheenan-induced rat paw oedema model. Aspirin, the reference compound, was observed to suppress the oedema at 3–4 h post-induction. 2-Formylphenyl aspirin **5b** showed a time-dependent response similar to aspirin and suppressed the oedema at 3–4 h. Furthermore, the compound was found to be ca twice as potent as aspirin. The latter could be due to better transport characteristics of the ester itself, as it is relatively rapidly hydrolysed to aspirin. Surprisingly, the other 2-formylphenyl esters **6a** to **6f** failed to elicit an anti-inflammatory response, whereas they are all expected to hydrolyse relatively rapidly to aspirin. The 3- and 4-formylphenyl aspirins displayed anti-inflammatory activity. The potency of the 3-formylphenyl ester **5c** was similar to that of the 2-formylphenyl ester, while the potency of the 4-formylphenyl ester **5d** was ca 1.5 times as active as aspirin. However, the esters **5c** and **5d** would not be expected to be hydrolysed rapidly non-enzymatically and the activity may arise from the anti-inflammatory activities of the esters in their own right. Further studies will be required to elucidate these points. Ester **5b** has an IC_{50} value of 1.4 x 10^{-5} mol dm^{-3} for cyclooxygenase and of > 5.0 x 10^{-4} mol dm^{-3} for lipooxygenase. The latter ester induced ulceration in rats at 30 mg kg^{-1} per os, producing haemorrhagic ulcers in the duodenium and gastrointestine with superficial mucosal lesions in the stomach. Indomethacine at the same dose level produced ulcers in the duodenum, stomach and gastrointestine.



Scheme 2.

Experimental protocols

Chemistry

The structures of all compounds prepared were confirmed by spectroscopy and microanalysis. ^{1}H- and ^{13}C-NMR spectra were recorded at ambient temperatures on deuterated chloroform solutions using a JEOL EX270 FT spectrometer with Me_4Si as internal reference; infrared spectra were obtained on a Zeiss Specord M-80 spectrophotometer. The ^{1}H-NMR spectra of **5b–d** and **6a–f** all indicated a formyl group at 9.9 to 10.2 ppm (1H, s) and ^{13}C-NMR spectra indicated three carbonyl groups at 186–191 (formyl), 162–163 (benzoate) and 169–170 ppm (acetate). Other detailed spectra were in accordance

Table IV. Hammett equation correlations of the alkaline hydrolysis of the 4–substituted 2-formylphenyl aspirins in 30% dioxane–water at 37.0 °C.[a]

	ρ	$log\ k_o$	s	r	n
With σ_p	2.25	1.13	0.50	0.893	7
With σ_m	2.45	0.66	0.32	0.959	7
σ_p and σ_m	1.70 (ρ_m), 0.99 (ρ_p)	0.80	0.21, 0.21	0.994	7

[a] s is the standard deviation, r the correlation coefficient and n the number of substituents.

with stated structures. Preparative column chromatography was performed using Still's method of flash chromatography [19]; the stationary phase used was Sorbsil C60 silica gel with hexane/ethyl acetate as eluent. All organic solutions were dried over anhydrous magnesium sulfate. 2-, 3- and 4-formylphenols were obtained commercially. The 4-substituted 2-formylphenols were prepared by the *ortho*-formylation of the appropriate phenol using the methods of Casiraghi et al [20, 21].

General method for the synthesis of phenyl esters of aspirin

2-Acetoxybenzoyl chloride (7.94 g, 0.04 mol) [22] in anhydrous diethyl ester (100 mL) was stirred and triethylamine (4.04 g, 0.04 mol) was added. After stirring for 10 min, the appropriate phenol (0.04 mol) was added. The mixture was stirred at ambient temperature for 2 h and then refluxed under a gentle stream of nitrogen for 3 h. The solution was filtered after cooling and the filtrate was washed with water, twice with 0.5 mol dm^{-3} aqueous sodium hydrogen carbonate and water. After drying and filtering, the solution was evaporated under reduced pressure. The product was usually obtained as an oil and was purified by preparative column chromatography. The solid or oil so obtained was then recrystallised to give the product described in table I, see [12].

Alkaline hydrolysis of esters

The products of the alkaline hydrolysis of the formylphenyl esters of aspirin were found to be aspirin and the formylphenol in quantitative yield, unlike the phenyl ester of aspirin [6]. The results of the HPLC study (see below) were further confirmed spectrophotometrically by comparison of the spectrum of aspirin and the respective formylphenol in buffer with that of the reaction product. Rate coefficients for the alkaline hydrolysis of the esters were determined spectrophotometrically by use of a Perkin-Elmer lambda 5 or 16 UV-VIS spectrometer. A Haake thermostatted-water circulating water bath was used to control the temperature of the cell to ± 0.05 °C. The procedure was similar to that described previously [23]. The aqueous dioxane solution was pipetted into two 1-cm cells and the cells were placed in the cell holder to enable thermal equilibration. Then ca 5 μL of a solution of the substrate in dioxane was added from a microsyringe such that the final substrate concentration was ca 5×10^{-5} mol dm^{-3}. The complete UV spectrum was recorded continuously. The rate measurements were made by monitoring at a selected fixed wavelength, normally the maximum change in absorption between substrate and product. The final optical density was assumed to be that measured after ten 'half-lives' had elapsed. The reactions were followed at the wavelengths shown in table II. A constant ionic strength of 1.0 mol dm^{-3} was maintained and buffers used were composed of acetate, phosphate, borate, carbonate and glycine. Rates were extrapolated to zero buffer concentrations. Solvents and buffer components were purified as required by literature methods [24]. The activation parameters were obtained from a least mean squares treatment of log k_2 versus $1/T$ [25].

An HPLC method was used for the analysis of hydrolysis products. These were performed using a Gilson 302 pump, holochromo UV-detector (operating at 230 nm), a Rheodine injection valve with a 20 μL loop and a Biorad RSL column (250 x 4.6 mm) with ODS (5 μm particle size). A flow rate of 1 mL min^{-1} was used and the reversed-phase column eluted with a mobile phase consisting of acetonitrile/methanol/water/phosphoric acid (40:50:10:1 (v/v)). The reactions were initiated by injecting 100 μL of a solution of the aspirin ester in dioxane into 10 mL of a buffer solution at 37 °C such that the final ester concentration was ca 6×10^{-4} mol dm^{-3}. A plot of peak heights versus the concentrations of each derivative was linear in the concentration range studied.

Biological methods

The formylphenyl esters of aspirin were screened for anti-inflammatory activity in the carrageenan-induced paw oedema test and compared with the activity of aspirin as a standard. Male Wistar rats (180–200 g) were dosed orally with either aspirin (100–200 mg kg^{-1}; $n = 6$ per group) or vehicle (1 mL of 1% (w/v in distilled water) carboxymethylcellulose). The animals were immediately gassed with 20–25% CO_2, followed by injection of carrageenan (0.1 mL of 1.3% (w/v in sterile saline)) into the subplantar of the left paw. The right non-injected paws were used as control. The animals were allowed to recover and the treated paw circumference measured at hourly intervals for each rat. The effects of the aspirin esters prepared in this study were determined using the same procedure except that the animals were dosed orally with either aspirin (100 mg kg^{-1}), vehicle (1 mL of 1% (w/v in distilled water) carboxymethyl-cellulose) or the aspirin ester (50–100 mg kg^{-1}). The results were analysed for significance using the student t-test. The cyclooxygenase assay was determined in rat whole blood after addition of the calcium ionophore A23/87 following the method of McMillan et al [26]. The ulcerogenic activity was assessed by macroscopic evaluation of stomach lesions in male rats carried out 24 h after administration of the test compounds, with ten rats per group tested.

Acknowledgment

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