ELSEVIER

International Journal of Pharmaceutics 153 (1997) 25–39

international journal of pharmaceutics

Synthesis, identification, characterization, stability, solubility, and protein binding of ester derivatives of salicylic acid and diflunisal

Daniel Y. Hung [a], George D. Mellick [a], Richard J. Prankerd [b], Michael S. Roberts [a,*]

[a] *Department of Medicine, University of Queensland, Princess Alexandra Hospital, Woolloongabba, Queensland 4102, Australia*
[b] *Department of Pharmacy, University of Queensland, Brisbane, Queensland 4072, Australia*

Received 11 December 1996; received in revised form 26 February 1997; accepted 27 February 1997

Abstract

O-Acyl esters were prepared from salicylic acid and diflunisal by esterification with the appropriate acyl anhydride (in the presence of sulfuric acid at 80°C) or acyl chloride (in the presence of pyridine at 0°C). Synthesis, identification and characterization of these compounds is described. In vitro hydrolysis, solubility and protein binding studies of these O-acyl esters were performed. For the diflunisal esters, the melting points fell as the side chain was increased from ethyl to pentyl. The melting points showed no significant difference as the length of the side chain was increased from pentyl to heptyl. The aspirin analogues showed a similar trend. The relationship between solubility and carbon chain length agreed closely with that for the melting points with carbon chain length. In vitro non-enzymatic hydrolysis studies concluded that: (1) hydrolysis rate constants generally decreased with carbon chain length; (2) the diflunisal esters have shorter half lives compared with their salicylate counterparts; and (3) the in vitro hydrolysis of these compounds was retarded by the presence of bovine serum albumin. Protein binding experiments showed that the strength of binding of the aspirin and diflunisal analogues to bovine serum albumin increased with carbon chain length. © 1997 Elsevier Science B.V.

Keywords: Aspirin analogues; Diflunisal esters; Synthesis; Melting point; Intrinsic solubility; In vitro non-enzymatic hydrolysis; Protein binding

Introduction

Aspirin is well established for secondary stroke prevention (The SALT Collaborative Group, 1991). It inhibits the synthesis of thromboxane A_2 in platelets by irreversible acetylation of a serine residue close to the active site of cyclooxygenase (Lecomte et al., 1994). Whilst such an effect is

* Corresponding author. Tel: + 61 7 32402546; fax: + 61 7 32405399; email: M.Roberts@mailbox.uq.ed.au

PII S0378-5173(97)00075-6

desirable for antiplatelet activity, aspirin has a destructive effect on the blood vessel walls, since it can inhibit the synthesis of prostacyclin. Aspirin therefore has contradictory effects as an antiplatelet drug.

It has been proposed that an optimal antithrombotic effect can be achieved by restricting aspirin to the portal circulation where selective inhibition of platelet cyclooxygenase can occur as distinct from the inhibition of vessel wall cyclooxygenase. This selective inhibition relies on aspirin being removed by the liver during the first pass (Ali et al., 1980; Siebert et al., 1983; Roberts et al., 1986; McLeod et al., 1988; Vial et al., 1990). However, previous studies have demonstrated that the extraction of aspirin by the liver is incomplete; hepatic extraction has been reported to be between 20 and 40% for man, dog, sheep and rat under physiological conditions (Harris and Riegelman, 1969; Iwamoto et al., 1982; Cossum et al., 1986; Wientjes and Levy, 1988; Mellick and Roberts, 1996). Thus, substantial quantities of aspirin bypass the liver through an inefficient hepatic extraction and aspirin is not restricted to the portal circulation.

Anti-platelet agents which work by acylation of cyclooxygenase and exhibit a higher hepatic extraction than aspirin may yield a greater selectivity in their effect on platelet inhibition relative to prostacyclin inhibition on vessel walls. These agents are more localized because their effects are more restricted to platelet cyclooxygenase in the portal circulation than aspirin. High hepatic extraction is often associated with a high lipophilicity of the agent. Potential anti-platelet agents therefore include the O-acyl esters of salicylic acid and diflunisal. This paper describes the synthesis, identification, characterization, stability, solubility and protein binding studies of these derivatives (Fig. 1 and Table 1).

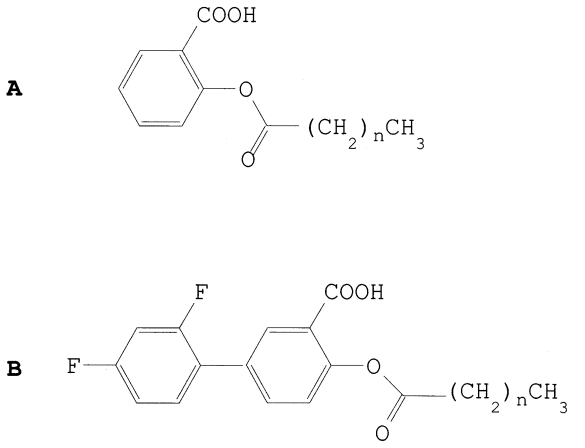


Fig. 1. Structures of O-acyl derivatives of salicylic acid (A) and diflunisal (B). The acyl derivatives differ only in the length of the side chain (i.e. the value of *n*).

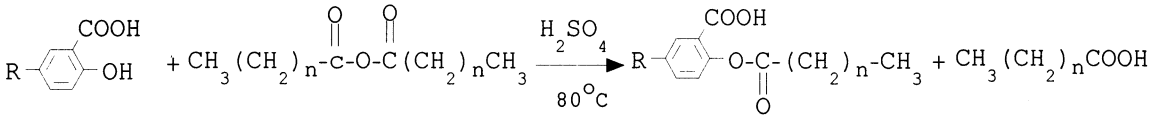
2. Materials and methods

2.1. Apparatus

Infrared (IR) spectra were recorded for solids in potassium bromide (KBr) discs on a Perkin-Elmer 599 grating spectrophotometer. ^{1}H-NMR spectra were run on a Brucker X32 500 MHz spectrometer in deuterochloroform ($CDCl_3$) with tetramethylsilane (TMS) as internal standard. Mass spectra were obtained with a Perkin-Elmer API III Biomolecular Mass Analyser. Uncorrected melting point ranges were determined in capillary

Table 1
The O-acyl derivatives of salicylic acid (SA) and diflunisal (DF)

Derivative	Abbreviation
Salicylic acid (SA) derivatives	
Acetylsalicylic acid	C2SA
n-propionylsalicylic acid	C3SA
n-butanoylsalicylic acid	C4SA
n-pentanoylsalicylic acid	C5SA
n-hexanoylsalicylic acid	C6SA
n-heptanoylsalicylic acid	C7SA
n-octanoylsalicylic acid	C8SA
Diflunisal (DF) derivatives	
Acetyldiflunisal	C2D
n-propionyldiflunisal	C3D
n-butanoyldiflunisal	C4D
n-pentanoyldiflunisal	C5D
n-hexanoyldiflunisal	C6D
n-heptanoyldiflunisal	C7D



Scheme 1. (R = H or 2,4-difluorophenyl, $n = 0$–4).

tubes with an Electrothermal melting point apparatus. High-performance liquid chromatography (HPLC) generally used a system consisting of a double piston pump (Waters, Model 510), autoinjector (Waters, Model 710b), a 5 μm Brownlee C_{18} reversed phase column (250 × 4.6 mm, Applied Biosystems) with a variable wavelength UV detector (Waters, Model 480), an interface module (Millipore-Waters), and an NEC personal computer.

2.2. Synthesis

Salicylic acid (2-hydroxybenzoic acid) and diflunisal [5-(2,4-difluorophenyl) salicylic acid] were obtained from Sigma Chemical Company (USA), and used without any further purification. Acetic anhydride, propionic anhydride, butyric anhydride, valeric anhydride (pentanoic anhydride), caproic anhydride (hexanoic anhydride), heptanoyl chloride, and octanoyl chloride were obtained from Aldrich Chemical Co. (USA). Two methods were employed for the synthesis of ester derivatives of salicylic acid and diflunisal in this work. The short chain compounds (2–6 carbon atoms in the O-acyl moiety) were prepared from acyl anhydrides in the presence of sulfuric acid as a catalyst (Scheme 1). The medium chain compounds (7–8 carbon atoms in the O-acyl moiety) were prepared from acyl chlorides in the presence of pyridine (Scheme 2).

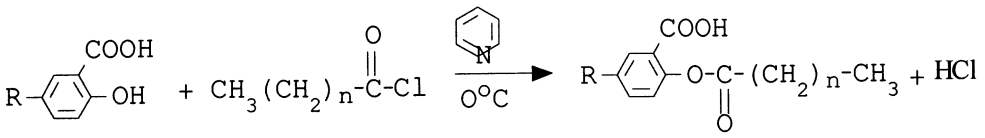
2.2.1. General procedure (Scheme 1)

Salicylic acid (2 g, 0.014 mole) or diflunisal (1 g, 0.004 mole) was placed in the reaction flask and excess of the appropriate acyl anhydride with three drops of sulfuric acid was added quickly. The mixture was heated on a water bath (80°C) for 2 h. It was then cooled and diluted with ice water (25 ml) to destroy the excess anhydride. A white to dark brown milky suspension was formed. The suspension was extracted five times with chloroform or methylene chloride. Sodium sulfate (anhydrous) was then added to the pooled extracts. The mixture was filtered and the filtrate was concentrated under reduced pressure. A crude compound was obtained as a yellowish-white to dark brown solid.

2.2.2. General procedure (Scheme 2)

Salicylic acid (2 g, 0.014 mol) or diflunisal (1 g, 0.004 mol) was placed in the reaction flask and 15 ml of pyridine was added. The flask was immersed in ice and placed on a magnetic stirrer. An appropriate acyl chloride was added dropwise over 30 min from a pressure-equalizing dropping funnel to the well-stirred mixture. The mixture was stirred at 0°C for another 60 min. Cold water (20 ml) was then added to dissolve the pyridinium salt. A greenish to orange milky suspension was formed. This was transferred to a round-bottom flask and then 50 ml of chloroform was added. After thorough mixing, the chloroform was evaporated under reduced pressure. This procedure was repeated several times until the odour of pyridine was removed. The flask was then cooled in ice and swirled continuously until a yellowish-white to light orange solid of crude compound was obtained.

(a) Synthesis of *n*-propionylsalicylic acid (C3SA). Approximately 0.036 mol (4.64 ml) of propionic anhydride was used. Crude C3SA was obtained as a yellowish-white solid (2.47 g). It was recrystallized twice from ethanol–water (2:5) and twice from petroleum spirit as white needles (2.13 g, 75.8% overall yield), m.p. 90–93°C. IR (ν_{Max} 2950 (C–H), 1740 (ester C=O), 1670 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ7.12–7.95 (m, 4H, Ar–*H*), 2.58 (q, 2H, –O(C=O)–C*H*$_2$), 1.18 (t, 3H, C*H*$_3$) ppm. M^+ 194; $C_{10}H_{10}O_4$ requires 194.



Scheme 2. (R = H or 2,4-difluorophenyl, $n = 5–6$).

(b) Synthesis of *n*-butanoylsalicylic acid (C4SA). Approximately 0.033 mol (5.46 ml) of butyric anhydride was used. Crude C4SA was obtained as a yellowish-white solid (1.15 g). It was recrystallized once from ethanol–water (2:5) and three times from petroleum spirit as white crystals (0.85 g, 28.2% overall yield), m.p. 78–79°C. IR ν_{Max} 2950 (C–H), 1740 (ester C=O), 1670 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) (6.89–7.88 (m, 4H, Ar–*H*), 2.55 (t, 2H, –O(C=O)–C*H*$_2$), 1.72 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 0.99 (t, 3H, C*H*$_3$) ppm. M^+ 208; $C_{11}H_{12}O_4$ requires 208.

(c) Synthesis of *n*-pentanoylsalicylic acid (C5SA). Approximately 0.036 mol (7.15 ml) of valeric anhydride was used. Since valeric acid is slightly soluble in water, it was difficult to separate it from C5SA by chloroform extraction (Scheme 1). A modified procedure was designed. After a brownish milky suspension was formed, it was transferred to a 25-ml conical flask and kept in a −20°C-freezer for 24 h. C5SA was converted into a solid on freezing, but valeric acid remained as a liquid (melting point −34.5°C). Therefore, valeric acid could be removed by a Pasteur pipette. Since a trace of valeric acid was still combined with the solid, a small amount of cold petroleum spirit was added to dissolve it. This petroleum spirit solution was then pipetted out. This procedure was repeated several times to eliminate the valeric acid residue. Finally, crude C5SA was obtained as a pearly white solid (1.58 g). It was recrystallized once from ethanol-water (2:5) and three times from petroleum spirit as pearly white plates (1.03 g, 32% overall yield), m.p. 81–84°C. IR ν_{Max} 2950 (C–H), 1740 (ester C=O), 1690 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ7.09–8.09 (m, 4H, Ar–*H*), 2.60 (t, 2H, –O(C=O)–C*H*$_2$), 1.74 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 1.44 (p, 2H, –O(C=O)–CH$_2$CH$_2$C*H*$_2$), 0.93 (t, 3H, C*H*$_3$) ppm. M^+ 222; $C_{12}H_{14}O_4$ requires 222.

(d) Synthesis of *n*-hexanoylsalicylic acid (C6SA). Approximately 0.036 mol (8.38 ml) of caproic anhydride was used. Crude C6SA was obtained as a white solid (2.01 g). It was recrystallized once from ethanol–water (2:5) and three times from petroleum spirit as white crystals (1.22 g, 35.7% overall yield), m.p. 73–75°C. IR ν_{Max} 2950 (C–H), 1750 (ester C=O), 1670 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ6.92–8.10 (m, 4H, Ar–*H*), 2.61 (t, 2H, –O(C=O)–C*H*$_2$), 1.78 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 1.40 (p, 4H, –O(C=O)–CH$_2$CH$_2$ C*H*$_2$ C*H*$_2$), 0.93 (t, 3H, C*H*$_3$) ppm. M^+ 236; $C_{13}H_{16}O_4$ requires 236.

(e) Synthesis of *n*-heptanoylsalicylic acid (C7SA). Approximately 0.018 mol (2.70 ml) of heptanoyl chloride was used. Crude C7SA was obtained as a yellowish-white solid (1.30 gm). It was recrystallized once from acetone-petroleum spirit (1:4) and three times from petroleum spirit as white crystals (0.46 gm, 12.7% overall yield), m.p. 72–73°C. IR ν_{Max} 2940 (C–H), 1745 (ester C=O), 1700 (acid C=O) cm^{-1}. M^+ 250; $C_{14}H_{18}O_4$ requires 250.

(f) Synthesis of *n*-octanoylsalicylic acid (C8SA). Approximately 0.018 mol (2.96 ml) of octanoyl chloride was used. Crude C8SA was obtained as a yellowish-white solid (1.46 g). It was recrystallized once from acetone–petroleum spirit (1:4) and three times from petroleum spirit as white crystals (0.78 g, 20.4% overall yield), m.p. 77–79°C. IR ν_{Max} 2940 (C–H), 1750 (ester C=O), 1700 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) (6.92–8.10 (m, 4H, Ar–*H*), 2.61 (t, 2H, –O(C=O)–C*H*$_2$), 1.77 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 1.42 (p, 2H, –O(C=O)–CH$_2$CH$_2$C*H*$_2$), 1.33 (p, 2H, –O(C=O)–CH$_2$ CH$_2$CH$_2$C*H*$_2$), 1.31 (p, 4H, –O(C=O)–CH$_2$ CH$_2$CH$_2$CH$_2$ C*H*$_2$ C*H*$_2$), 0.89 (t, 3H, C*H*$_3$) ppm. M^+ 264; $C_{15}H_{20}O_4$ requires 264.

(g) Synthesis of acetyldiflunisal (C2D). Approximately 0.03 mol (2.85 ml) of acetic anhy-

dride was used. Crude C2D was obtained as a dark brown solid (1.43 g). It was recrystallized three times from acetone–petroleum spirit (1:4) and once from ethanol–water (2:5) as white crystals (0.61 g, 52.1% overall yield), m.p. 174–176°C. IR ν_{Max} 3060 (C–H), 1760 (ester C=O), 1680 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ6.89–8.12 (m, 6H, Ar–*H*), 2.38 (s, 3H, –O(C=O)C*H*$_3$) ppm. M^+ 292; $C_{15}F_2H_{10}O_4$ requires 292.

(h) Synthesis of *n*-propionyldiflunisal (C3D). Approximately 0.024 mol (3.1 ml) of propionic anhydride was used. Crude C3D was obtained as a yellow solid (1.38 g). It was recrystallized once from ethanol–water (2:5) and three times from acetone–petroleum spirit (1:4) as yellowish-white crystals (0.83 g, 67.5% overall yield), m.p. 140–142°C. IR ν_{Max} 3000 (C–H), 1750 (ester C=O), 1690 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ8.22–6.92 (m, 6H, Ar–*H*), 2.66 (q, 2H, –O(C=O)C*H*$_2$), 1.28 (t, 3H, C*H*$_3$) ppm. M^+ 306; $C_{16}F_2H_{12}O_4$ requires 306.

(i) Synthesis of *n*-butanoyldiflunisal (C4D). Approximately 0.024 mol (3.9 ml) of butyric anhydride was used. Crude C4D was obtained as a yellowish-white solid (0.95 g). It was recrystallized once from ethanol–water (2:5) and three times from acetone–petroleum spirit (1:4) as yellowish-white needles (0.57 g, 44.5% overall yield), m.p. 116–118°C. IR ν_{Max} 2980 (C–H), 1750 (ester C=O), 1680 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ8.21–6.92 (m, 6H, Ar–*H*), 2.61 (t, 2H, –O(C=O)–C*H*$_2$), 1.80 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 1.05 (t, 3H, C*H*$_3$) ppm. M^+ 320; $C_{17}F_2H_{14}O_4$ requires 320.

(j) Synthesis of *n*-pentanoyldiflunisal (C5D). Approximately 0.02 mol (3.95 ml) of valeric anhydride was used. Since valeric acid is slightly soluble in water, it was difficult to separate it from C5D by methylene chloride extraction (Scheme 1). Therefore, a modified process was used. After reaction, the dark brown milky suspension was transferred to a 25 ml conical flask, cooled in ice and swirled continuously until yellow solid crude C5D (0.81 gm) was obtained. It was recrystallized once from ethanol–water (2:5) and three times from acetone-petroleum spirit (1:4) as white plates (0.63 gm, 47% overall yield), m.p. 101–103°C. IR ν_{Max} 2980 (C–H), 1725 (ester C=O), 1705 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ6.89–8.20 (m, 6H, Ar–*H*), 2.63 (t, 2H, –O(C=O)–C*H*$_2$), 1.75 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 1.45 (p, 2H, –O(C=O)–CH$_2$CH$_2$C*H*$_2$), 0.96 (t, 3H, C*H*$_3$) ppm. M^+ 334; $C_{18}F_2H_{16}O_4$ requires 334.

(k) Synthesis of *n*-hexanoyldiflunisal (C6D). Approximately 0.02 mol (4.63 ml) of caproic anhydride was used. Since caproic acid is also slightly soluble in water, the method used for C5D was utilized. Crude C6D was obtained after filtering and drying as a white pinkish solid (1.15 g). It was recrystallized once from ethanol–water (2:5) and three times from acetone–petroleum spirit (1:4) as white crystals (0.61 g, 43.9% overall yield), m.p. 105–106°C. IR ν_{Max} 2970 (C–H), 1725 (ester C=O), 1705 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ6.94–8.21 (m, 6H, Ar–*H*), 2.70 (t, 2H, –O(C=O)–C*H*$_2$), 2.18 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 1.79 (p, 4H, –O(C=O)–CH$_2$CH$_2$ C*H*$_2$ C*H*$_2$), 1.41 (t, 3H, C*H*$_3$) ppm. M^+ 348; $C_{19}F_2H_{18}O_4$ requires 348.

(l) Synthesis of *n*-heptanoyldiflunisal (C7D). Approximately 0.005 mol (0.74 ml) of heptanoyl chloride was used. Crude C7D was obtained as a light orange solid (0.61 gm). It was recrystallized three times from acetone–petroleum spirit (1:4) and once from ethanol–water (2:5) as white needles (0.35 g, 24.1% overall yield), m.p. 109–111°C. IR ν_{Max} 2940 (C–H), 1750 (ester C=O), 1680 (acid C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ6.94–8.37 (m, 6H, Ar–*H*), 2.64 (t, 2H, –O(C=O)–C*H*$_2$), 1.78 (p, 2H, –O(C=O)–CH$_2$C*H*$_2$), 1.44 (p, 2H, –O(C=O)–CH$_2$CH$_2$C*H*$_2$), 1.34 (p, 2H, –O(C=O)–CH$_2$ CH$_2$C*H*$_2$C*H*$_2$), 0.91 (t, 3H, C*H*$_3$) ppm. M^+ 362; $C_{20}F_2H_{20}O_4$ requires 362.

2.3. Analytical

2.3.1. HPLC analytical method

The HPLC method employed for the determination of the O-acyl salicylic acid and diflunisal derivatives was a modification of the methods of Mellick and Roberts (1996). Table 2 shows the various constituents of the mobile phase, the internal standards, and flow rates used for detecting each particular compound in these series of derivatives. The eluent was monitored at 237 nm and sensitivity 0.04 AUFs. In these solvent sys-

Table 2
Variant combination of mobile phase, internal standard, and flow rate used for detecting salicylic acid and diflunisal derivatives in HPLC assay

Composition of mobile phase	Internal standard	Flow rate	Compounds detectable
19:80:1 (acetonitrile: 0.03% (v/v) phosphoric acid: triethylamine) at pH 2.0	0.004% w/v p-toluic acid in acetonitrile	1.5 ml/min	C2SA and C3SA
49:50:1 (acetonitrile: 0.03% (v/v) phosphoric acid: triethylamine) at pH 2.0	0.004% w/v diflunisal in acetonitrile	1 ml/min	C4SA–C8SA
64:35:1 (acetonitrile: 0.03% (v/v) phosphoric acid: triethylamine) at pH 2.0	0.004% w/v salicylic acid in acetonitrile	1 ml/min	C2D–C7D (except C3D)
34:65:1 (acetonitrile: 0.03% (v/v) phosphoric acid: triethylamine) at pH 2.0	0.004% w/v salicylic acid in acetonitrile	1.5 ml/min	C3D

tems, salicylic acid and diflunisal could also be assayed, with good separation from the other solute peaks.

2.3.2. HPLC assay validation

Standard solutions of the derivatives were prepared both in Krebs–Henseleit buffer containing either no protein (for aspirin analogues) or 2% w/v BSA (for diflunisal esters) at pH 7.4. For C3SA to C5SA standard curves were prepared with concentrations ranging from 5 to 50 μg/ml. For C6SA-C8SA standard curves were prepared with concentrations ranging from 1–25 μg/ml. For C2D-C6D standard curves were prepared with concentrations ranging from 1–100 μg/ml. The standards and buffer samples (100 μl aliquots) were then prepared for HPLC analysis by addition of 20 μl of 35% perchloric acid and 200 μl of acetonitrile containing the internal standard. The solutions were thoroughly mixed (vortex mixer) and centrifuged (5 min at 9000 g), and then 20 μl of supernatant was injected onto the column. The peaks from the HPLC chromatogram were integrated and the peak area of the compound divided by the peak area of the internal standard to give the HPLC response. The within day coefficients of variation (CV) for the different derivatives were determined by establishing and running three separate standard curves on the same day. Identical unknown samples were also run and the exact concentration determined using the separated standard curves. The CV was determined by dividing the standard deviation of the determined unknown solute concentration by the mean of these concentrations. All samples and solutions were kept on ice prior to analysis to minimize hydrolysis.

For all compounds in this work, HPLC standard curves were linear within the range of concentrations studied, with linear regression analysis yielding r^2 values > 0.999. The within day coefficients of variation for all the compounds were within the range of 2.06–4.28% ($n = 3$).

2.4. Solubility

The intrinsic solubility is the concentration of the drug at equilibrium in a solvent in which the drug does not ionise appreciably. This experiment was designed to investigate the intrinsic solubility of each compound at room temperature (25°C).

These studies were carried out in aqueous phosphoric acid (pH 1.5) and at 25 ± 1°C. The pH conditions were chosen to ensure that the solubility of the unionized species was estimated. Given that the pK_a of these esters will be similar to that of aspirin (pK_a at 25°C = 3.5 (Foye, 1989)), a pH of 1.5 was chosen so that the compounds did not ionise appreciably.

Each derivative was added as an ethanol solution (100 μl) to a 15 ml siliconized screw-cap test tube (in duplicate). The solvent (9.9 ml) was then added to the test tube. The quantity of ester was chosen to be four times the predicted maximum solubility of the ester, based on aspirin solubility (0.0185 M at 25°C, The Merck Index, 1983

(Windholz, 1983). For its analogues, the solubilities are expected to decrease by about 0.5 log unit/methylene group, (Yalkowsky, 1981). The test tube was sonicated for 15 min and centrifuged for 10 min ($3000 \times g$ at 25°C). An aliquot was filtered (Minisart 0.45 μm filter, Satorius) and diluted with HPLC mobile phase, then assayed. The tube was stored carefully in a 25°C water bath after sampling and the remaining suspension was again sonicated and the process repeated seven times at four hourly intervals. In these assays, standard curves were prepared by directly dissolving the esters in mobile phase. Concentrations for standard curves ranged from 1–50 μg/ml for all compounds. Furthermore, a manual injector (Waters Model U6K) and 20 μl sample loop ensured that 20 μl was consistently injected onto the column and precluded the need for an internal standard.

2.5. In vitro non-enzymatic hydrolysis

These experiments were carried out both in Krebs-Henseleit buffer containing either no protein or 2% w/v BSA (bovine serum albumin, fraction V, Sigma) at pH 7.4 and 25 ± 1°C. The results were used to investigate the hydrolysis of these derivatives in vitro and the influence of albumin on spontaneous hydrolysis.

Solutions of the derivatives in Krebs buffer (10 ml) was prepared in siliconized glass tubes. Each solution was sonicated for 5 min and centrifuged for 5 min ($3000 \times g$) at 25°C to ensure maximum dissolution and to sediment any undissolved precipitate. About 7.5 ml of the supernatant was then transferred to a clean siliconized test tube and a 100-μl aliquot was removed and assayed. Samples were also removed from the solution at various times up to 46 h (0.25, 2, 4, 19.5 and 46 h) for C2SA and C3SA or 96 h (0.25, 2.5, 5, 25.67 and 96 h) in the case of C4SA to C8SA and the diflunisal esters. The rate of non-enzymatic hydrolysis (k) for each derivative was then estimated using the following equation:

$$C_t = C_0 \exp(-kt) \quad (1)$$

where C_t is the concentration of the ester at any time (t), and C_0 is the concentration of the ester at time zero. The half-life ($t_{1/2}$) was determined using the formula:

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

2.6. Protein binding

These experiments were carried out in Krebs-Henseleit buffer containing 2% w/v BSA (pH 7.4) and incubated at 37°C for 30 min.

A known concentration of the ethanolic ($\leq$ 1%) aspirin analogue or diflunisal ester stock solution was added to the 2% BSA Krebs solution to make final concentrations of 125 μM, 250 μM and 500 μM.

The unbound fraction (f_u) of parent drug and its hydrolysis product were investigated using ultra-filtration: a 1.0 ml aliquot sample (in triplicate) was placed in a ultrafiltration tube (MPS-1, micro-partition system, Amicon) and centrifuged at $3000 \times g$ for 10 min. The ultra-filtrate was then assayed for parent drug and its hydrolysis product by HPLC.

To eliminate possible errors caused by hydrolysis, identical samples were placed in normal tubes and subjected to the same procedure. This control group (also in triplicate) was used to adjust for the percentage of hydrolysis in the ultra-filtrate under the experimental conditions.

The f_u was determined as the ratio of the free concentration to total concentration of solutes. The association binding constant (K_a) was determined from the slope of a Scatchard plot using following equation:

$$[Db]/[D] = -K_a[Db] + vK_a[Pt] \quad (3)$$

where [Db] is the concentration of bound drug, [D] is the concentration of drug, [Pt] is the concentration of total protein, and v is the number of independent binding sites available. Eq. (3) is plotted as the ratio [Db]/[D] versus [Db] and in this way the association binding constant (K_a) is determined from the slope while vK_a[Pt] is determined from the intercept (Martin, 1983).

3. Results and discussion

3.1. Synthesis

3.1.1. Overall yield

The overall yield for ester derivatives of salicylic acid ranged from 12.7 to 75.8% whereas that for diflunisal esters ranged from 24.1 to 67.5%. The two major synthetic products, esters and aliphatic carboxylic acids, are capable of forming hydrogen bonds with other molecules and easily dissolved in most solvents. Recovery of solid esters from the reaction product mixtures after reactions is therefore difficult, accounting for the variable recoveries. Recovery is lowest when the aliphatic moiety of carboxylic acid had more than four carbons and the ester could not be easily separated from the carboxylic acid using an organic solvent extraction. Overall, the yield decreased as the carbon chain length in the ester O-acyl moiety became longer.

Diflunisal esters had higher overall yields compared to their aspirin analogue counterparts. It is suggested that the diflunisal esters' two benzene ring structure and larger molecular weight, compared to aspirin analogues, enabled them to crystallise more easily from the reaction mixture.

3.1.2. Melting point

Table 3 presents the melting points of aspirin analogues and diflunisal esters. It shows an intense fall in melting points of the diflunisal esters as the side chain was increased from ethyl to pentyl (C2D–C5D). The melting points showed no significant difference as the length of the side chain was increased from pentyl to heptyl (C5D–C7D). However, there is a trend to slightly higher melting point after the minimum value (C5D). The melting points of aspirin analogues also showed a similar pattern except for the melting point of hexyl derivative (C6SA) being the minimum value in aspirin analogues. Generally, these two homologous series followed the same trends as observed by Yalkowsky et al. (1972) for the melting points of the alkyl *p*-aminobenzoates.

The overall trend results from the side chain having increasing dominance of the crystal structure as it is lengthened over four carbon units (diflunisal esters) or five carbon units (aspirin analogues). The longer chain lengths have greater rotational freedom about C–C bonds and potentially influence the crystal packing (Forster et al., 1991). Thus, the effect per methylene group on melting point for longer chain substituents is smaller than for the shorter chain (more rigid) substituents.

Diflunisal esters have much higher melting points than those of aspirin analogues because of the increased molecular weight and aromatic stacking effects (Bailey and Bailey, 1981).

3.1.3. Spectroscopy

All spectra showed C–H stretching bands near 3000 cm^{-1}, ester functional group bands near 1745 cm^{-1} and carboxylic acid functional group bands near 1690 cm^{-1}. The spectra of aspirin analogues and diflunisal esters differ in the fingerprint regions. The IR spectra of the two starting materials, salicylic acid and diflunisal, reveal different stretching patterns from their ester derivatives. Their spectra show phenolic hydroxyl group stretching bands near 3290 cm^{-1} but lack the carbonyl stretching frequency at 1745 cm^{-1} characteristic of the ester functional group. NMR and mass spectra were consistent with the proposed structures.

Table 3
The melting points of aspirin analogues and diflunisal esters

Derivative	Melting point (°C)
Salicylic acid (SA) derivatives	
C2SA	136 ± 1.2[a]
C3SA	91 ± 1.3
C4SA	80 ± 2.3
C5SA	81 ± 3.0
C6SA	74 ± 1.3
C7SA	75 ± 0.5
C8SA	77 ± 2.1
Diflunisal (DF) derivatives	
C2D	175 ± 1.3
C3D	141 ± 0.8
C4D	116 ± 1.6
C5D	103 ± 1.6
C6D	105 ± 1.3
C7D	106 ± 1.4

[a] Data expressed as mean $\pm$ standard deviation ($n = 3$).

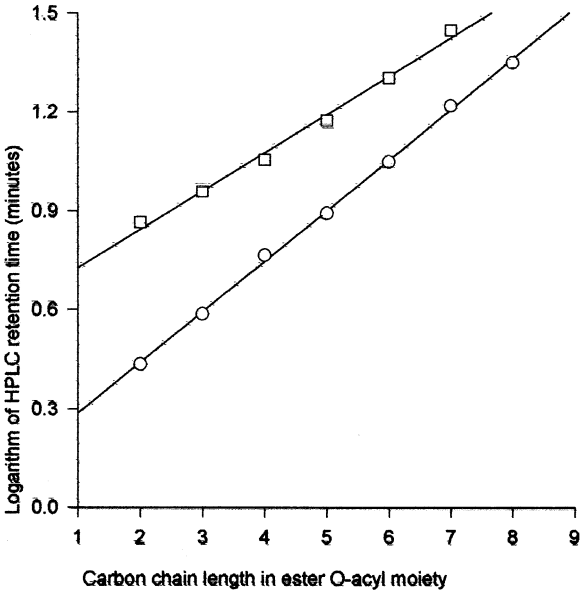


Fig. 2. The relationship between HPLC retention time and carbon chain length of the ester O-acyl moiety for (○) aspirin analogues ($y = 0.1334 + 0.1532x$, $r^2 = 0.998$) (□) diflunisal esters ($y = 0.6124 + 0.1159x$, $r^2 = 0.992$).

3.2. Analytical

Fig. 2 shows the relationship between HPLC retention time (RT) and carbon chain length in the O-acyl moiety. A linear relationship was found between the logarithm of RT and the number of carbon atoms in the ester side chain, reflecting the increase in lipophilicity as methylene groups are added to the side chain. This relationship can be expressed in terms of log PC (hydrophobic constant) by using quantitative structure analysis. Due to the efforts of Leo et al. (1975), it is possible to estimate the octanol/water partition coefficient (PC) of any organic nonelectrolyte by a variety group contribution schemes. A set of substituent constants (f values) for some common atoms and groups have been found to work well for this purpose (Yalkowsky, 1981). The log PC of the molecule is obtained by adding the f values for all of its constituents' groups since

$$\log \mathrm{PC} = \sum_{\text{all groups}} f_{\text{group}} \quad (4)$$

We can calculate the log PC values for aspirin analogues and diflunisal esters (Table 4) from f values. Fig. 3 shows the relationship between log RT and estimated log PC for aspirin analogues ($y = 0.3588 + 0.289x$, $r^2 = 0.998$) and diflunisal esters ($y = 0.2167 + 0.2186x$, $r^2 = 0.992$). A linear relationship was found between log RT and log PC.

3.3. Solubility

Table 5 lists the intrinsic solubilities for the aspirin analogues and diflunisal esters at 25 ± 1°C in water. Due to increased lipophilicity, the longer chain analogues have lower solubility than the shorter chain analogues. The diflunisal esters are more lipophilic than aspirin analogues. Consequently, their solubilities in water were very low and, except for C2D and C3D, saturation concentrations were below the limit of detection for the HPLC method used.

Since the intrinsic solubility of diflunisal esters is extremely low (Table 5), it is necessary to include 2% BSA in the perfusate to ensure adequate solubility of these diflunisal esters in the rat liver perfusion study. The solubilities of these compounds in 2% BSA Krebs–Henseleit buffer

Table 4
Hydrophobic constant (log PC) for aspirin analogues and diflunisal esters calculated from f values[a]

Derivative	log PC
Salicylic acid (SA) derivatives	
C2SA	0.28
C3SA	0.81
C4SA	1.34
C5SA	1.87
C6SA	2.40
C7SA	2.93
C8SA	3.46
Diflunisal (DF) derivatives	
C2D	2.87
C3D	3.40
C4D	3.93
C5D	4.46
C6D	4.99
C7D	5.52

[a] Nys and Rekker f values for some common atoms and groups (Yalkowsky, 1981).

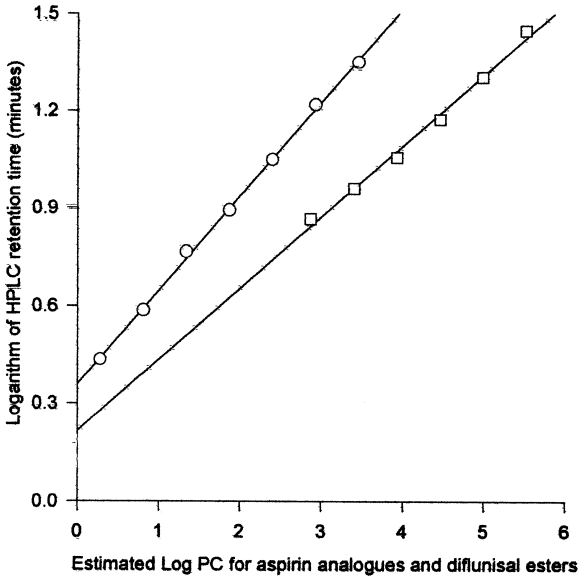


Fig. 3. The relationship between HPLC retention time (RT) and estimated octanol/water partition coefficient (PC) for (○) aspirin analogues ($y = 0.3588 + 0.2890x$, $r^2 = 0.998$) and (□) diflunisal esters ($y = 0.2167 + 0.2186x$, $r^2 = 0.992$). The figure shows that a linear relationship was found between log RT and log PC.

(pH 7.4) at 25°C were investigated and the results are presented in Table 6. The more lipophilic the analogue, the higher the solubility of solute under these conditions.

Melting point data has previously been used to adequately predict drug solubilities for solutes belonging to the *p*-hydroxybenzoate homologous series (Forster et al., 1991). Therefore, the relationship between aqueous solubilities of the aspirin analogues and their melting points was investigated. Fig. 4 shows that the relationships between solubility, melting point and carbon chain length for the aspirin analogues follow a similar trend.

The measured solubilities of the shorter chain analogues (C3SA and C4SA) agree closely with the expected values which suggest that solubilities decrease by about 0.5 log unit/methylene group (Yalkowsky, 1981). However, the solubilities of the longer chain analogues (C5SA, C6SA, and C8SA) are only about half of the expected value. An alternative semi-empirical model proposed by Yalkowsky (Yalkowsky, 1981) was employed to

Table 5
Maximum solubility of aspirin analogues and diflunisal esters at 25 ± 1°C in water at pH 1.5

Compound	MW	Solubility	
		μg/ml[a]	mM
C2SA	180	3300[b]	18.5
C3SA	194	1200 ± 120	6.3
C4SA	208	570 ± 80	2.7
C5SA	222	74 ± 31	0.33
C6SA	236	19 ± 4	0.08
C8SA	264	2 ± 0.2	0.008
C2D	292	9 ± 0.1	0.03
C3D	306	3 ± 0.2	0.01
C4D	320	U[c]	U
C5D	334	U	U
C6D	348	U	U

[a] Data expressed as mean ± standard deviation ($n = 16$).
[b] Literature value from Windholz, 1983 (The Merck Index).
[c] Undetectable.

predict the solubilities for these analogues using following equation:

$$\log X_w = -\log PC - 0.01MP - 0.69 \quad (5)$$

where X_w is the predicted solubility of a solute in water, PC is the estimated octanol/water partition coefficient of a solute, MP is the observed melting point of a solute. Table 7 and Fig. 5 show the comparison of predicted and measured solubilities for aspirin analogues using different models. The prediction for longer chain analogues using Eq. (5) is closer to the measured results than that calculated from 0.5 log unit/methylene group model. On the contrary, the prediction for shorter

Table 6
Maximum solubility of diflunisal esters in 2% BSA Krebs–Henseleit buffer (pH 7.4) at 25 ± 1°C

Compound	MW	Solubility	
		mg/ml[a]	mM
C2D	292	2.21 ± 0.07	7.57
C3D	306	3.15 ± 0.02	10.29
C4D	320	3.96 ± 0.08	12.38
C5D	334	6.98 ± 0.15	20.90
C6D	348	15.58 ± 0.3	44.77

[a] Data expressed as mean ± standard deviation ($n = 3$).

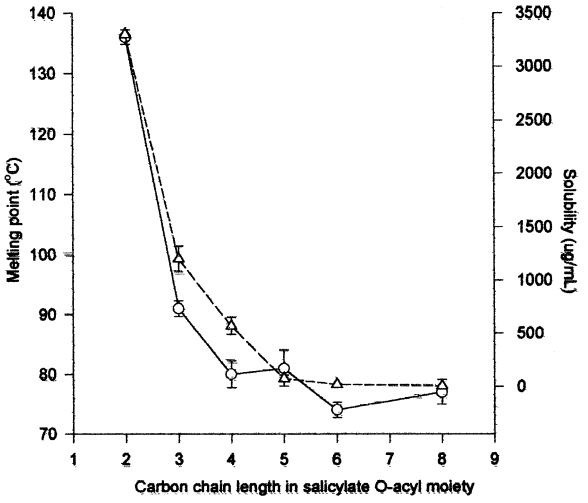


Fig. 4. The relationships between solubility (△), melting point (○) and carbon chain length for the aspirin analogues. The figure shows that the melting points and solubilities of aspirin analogues followed a similar trend.

chain analogues is worse. This phenomenon agreed with the description of methylene group contribution in solutions by Davis et al., 1972. They showed that ΔG^{E} (the free energy in excess of that for ideal mixing) changes linearly with chain length once the alkyl chain of a homologous series of compounds is greater than a few carbons in length. Thus, the combination of these two models could be the optimum model for solubility's prediction in a homologous series of compounds.

3.4. In vitro non-enzymatic hydrolysis

Fig. 6 presents the relationship between hydrolysis rate constant and carbon chain length for the aspirin analogues. The greatly diminished hydrolysis rate constants for C4SA compared to its shorter chain counterparts suggests that some degree of steric hindrance exists when four or more carbon atoms are present in the side chain. Thus, hydrolysis is much slower for the longer chain esters. This is consistent with Taft's Es (steric) parameter values (Isaacs, 1987).

Table 8 shows the hydrolysis rate constants (k) and half lives ($t_{1/2}$) of the aspirin analogues and diflunisal esters in different solvents. The diflunisal esters were found to be less stable than the aspirin analogues in Krebs–Henseleit buffer at 25°C. This can be explained by the electron withdrawing group 2,4-difluorophenyl. This reduces electron density at the ester carbonyl group and increases the leaving group ability of the phenol, thus raising the susceptibility to hydrolysis. Hence, the diflunisal esters have shorter half-lives than their salicylate counterparts. As the half-lives of these solutes are lengthy (e.g. the half-life of C8SA in 2% BSA Krebs buffer is approximately 30 days), we have investigated only one half-life in this work.

In the presence of albumin, the hydrolysis rates for the aspirin analogues and diflunisal esters were decreased. Protein binding protected these compounds against hydrolysis in vitro. In contrast, previous studies demonstrated that albumin enhanced the enzyme-catalysed hydrolysis of aspirin in whole blood by an activating effect on β-glucuronidase (Levy, 1966; Aarons et al., 1980). Hence, it is possible that in vivo, albumin may activate the enzyme-catalysed hydrolysis of these compounds.

3.5. Protein binding

It is generally accepted that the unbound concentration of drugs has consequences for the disposition and pharmacological activity of the drug (Jusko and Gretch, 1976; Yacobi et al., 1977). Therefore, there is a need to study protein binding of these compounds (including their hydrolysis products, salicylic acid and diflunisal). Table 9 presents the unbound fraction (f_u) of the aspirin analogues and diflunisal esters at various concentrations. Apparently the most lipophilic compounds have the lowest f_u values. Thus, the strength of protein binding increases in proportion to lipophilicity. Table 9 also presents the association binding constant (K_a) of the aspirin analogues and diflunisal esters. According to this table, the strength of protein binding among the aspirin analogues is in following order: C8SA > C6SA > C5SA > C2SA and the diflunisal esters is in following order: C6D > C5D > C4D > C2D. The relationship between unbound fraction and protein binding can be expressed by following Eq.

Table 7
Comparison of predicted and measured solubilities (S) in water at 25 ± 1°C for aspirin analogues using different models

Compound	MW[a]	log PC[b]	log X_w^c	Predicted S[d] (μg/ml)	Predicted S[e] (μg/ml)	Measured S[f] (μg/ml)
C3SA	194	0.81	−2.40	1200	772	1200 ± 120
C4SA	208	1.34	−2.81	420	322	570 ± 80
C5SA	222	1.87	−3.40	140	88	74 ± 31
C6SA	236	2.40	−3.84	47	34	19 ± 4
C8SA	264	3.46	−4.94	5	3	2 ± 0.2

[a] Molecular weight.
[b] PC is the estimated octanol/water partition coefficient of a solute.
[c] X_w is the predicted solubility of a solute in water.
[d] Calculated from subtraction of 0.5 log unit/methylene group based on aspirin solubility.
[e] Using Eq. (5).
[f] Data expressed as mean ± standard deviation ($n = 16$).

(6) when the solute concentration is much less than the protein concentration:

$$f_u = \frac{1}{1 + K_a[P]} \quad (6)$$

where [P] presents the concentration of protein. As f_u decreases with lipophilicity and [P] is a constant (2% BSA); it is expected that an increase in K_a will occur with increasing carbon number in the ester O-acyl group.

It is possible to predict K_a for any homologous series of compounds by using quantitative structure analysis. Fig. 7 shows the relationship between $\log K_a$ and carbon chain length in ester O-acyl moiety for aspirin analogues ($y = 1.6443 + 0.3024x$, $r^2 = 0.983$) and diflunisal esters ($y = -$

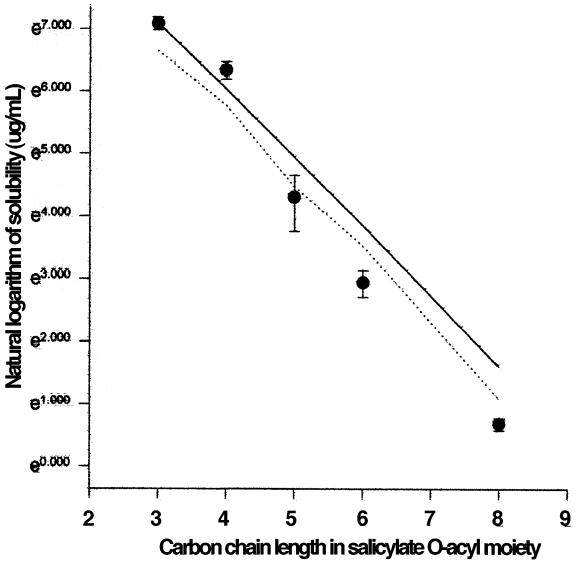


Fig. 5. The comparison of predicted and measured solubilities for aspirin analogues using different models. The solid line represents the prediction which was calculated from deduction of 0.5 log unit/methylene group from C2SA. The dotted line represents the prediction using Eq. (5). The filled circles represent the measured values.

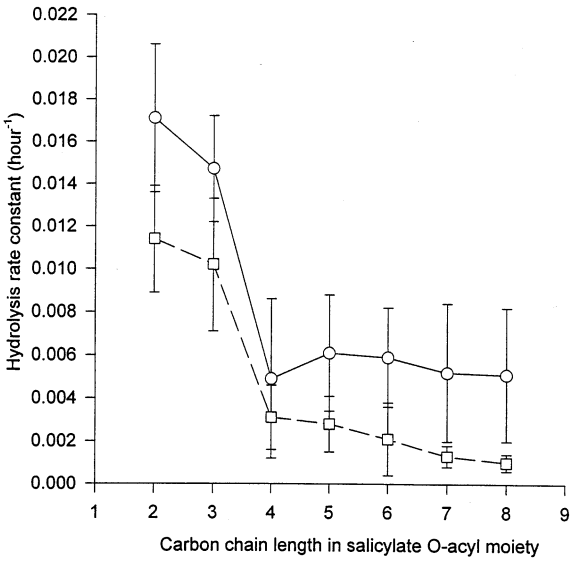


Fig. 6. The relationship between hydrolysis rate constant and carbon chain length for the aspirin analogues in Krebs buffer (○) or 2% BSA Krebs buffer (□). The figure shows that some degree of steric hindrance exists when four or more carbon atoms are present in the side chain. Thus, hydrolysis is much slower for the longer chain esters.

Table 8
The hydrolysis rate constant (k) and half life ($t_{1/2}$) of aspirin analogues and diflunisal esters in Krebs–Henseleit buffer containing 0 or 2% BSA

Compound	Hydrolysis rate constant (h^{-1})		Half life (h)[b]	
	Krebs–Henseleit buffer 0% BSA	Krebs–Henseleit buffer 2% BSA	Krebs–Henseleit buffer 0% BSA	Krebs–Henseleit buffer 2% BSA
C2SA	0.0171 ± 0.0035[a]	0.0114 ± 0.0025	40.53	60.80
C3SA	0.0147 ± 0.0025	0.0102 ± 0.0031	47.14	67.94
C4SA	0.0049 ± 0.0037	0.0031 ± 0.0015	141.43	223.55
C5SA	0.0061 ± 0.0027	0.0028 ± 0.0013	113.61	247.50
C6SA	0.0059 ± 0.0023	0.0021 ± 0.0017	117.46	330.00
C7SA	0.0052 ± 0.0032	0.0013 ± 0.0005	133.27	533.08
C8SA	0.0051 ± 0.0031	0.0010 ± 0.0004	135.88	693.00
C2D	0.0378 ± 0.0105	0.0238 ± 0.0095	18.33	29.12
C4D	U[c]	0.0138 ± 0.0058	U	50.22
C5D	U	0.0113 ± 0.0037	U	61.33
C6D	U	0.0056 ± 0.0042	U	123.75

[a] $n = 5$, $p < 0.05$.
[b] First half life.
[c] U, Undetectable.

$3.2298 + 0.0401x$, $r^2 = 0.975$). Thus, the expected K_a for any analogue can be predicted by regression analysis. The contribution of each CH_2 group is to increase K_a by adding 0.3024 or 0.0401 log unit/methylene group to the log K_a of C2SA or C2D for aspirin and diflunisal analogues, respectively. This difference in the methylene group contribution indicates the much greater importance to albumin binding of the diflunisal head group compared to the salicylate group.

4. Conclusions

This paper has described the synthesis, identification, solubility, hydrolysis and protein binding of a homologous series of O-acyl salicylic acid and O-acyl diflunisal esters. For the diflunisal esters, the melting points fell as the side chain was increased from acetyl to pentyl and rose as the length of the side chain was increased from the pentyl to the heptyl derivative. The aspirin analogues showed a similar trend. The relationship between solubility and carbon chain length agreed closely with that for the melting points with carbon chain length.

Hydrolysis rate constants generally decreased with carbon chain length and were significantly diminished as the carbon number in the O-acyl group equalled or exceeded four. For all solutes, in vitro hydrolysis was retarded by the presence of 2% BSA to the system.

Due to their greater lipophilicity, the diflunisal esters were much less water soluble than their salicylate ester counterparts.

The protein binding of the diflunisal esters was stronger compared to their salicylate ester counterparts. For both homologous series, protein binding generally increased with carbon chain length.

Acknowledgements

The authors acknowledge the support of the National Health and Medical Research Council of Australia and the Queensland and New South Wales Lions Kidney and Medical Research Foundation.

Table 9
Unbound fraction (f_u) and protein association binding constant (K_a) of aspirin analogues and diflunisal esters

Compound	Unbound fraction (f_u)			K_a (l/mol)
	125 μM	250 μM	500 μM	
C2SA	0.4499 ± 0.0007[a]	0.6589 ± 0.0053	0.7011 ± 0.0338	192
C5SA	0.3190 ± 0.0202	0.3641 ± 0.0210	0.4033 ± 0.0253	1525
C6SA	0.1747 ± 0.0419	0.2038 ± 0.0206	0.2304 ± 0.0267	2071
C8SA	0.0827 ± 0.0023	0.0840 ± 0.0235	0.0817 ± 0.0209	13978
C2D	0.2635 ± 0.0363	0.2622 ± 0.0114	0.2696 ± 0.0211	2077
C4D	0.1389 ± 0.0218	0.1422 ± 0.0417	0.1426 ± 0.0132	2365
C5D	0.1080 ± 0.0660	0.1202 ± 0.0024	0.1190 ± 0.0078	2707
C6D	U[b]	0.0784 ± 0.0038	0.0803 ± 0.0029	2995

[a] Data expressed as mean $\pm$ standard deviation ($n = 3$).
[b] U, Undetectable.

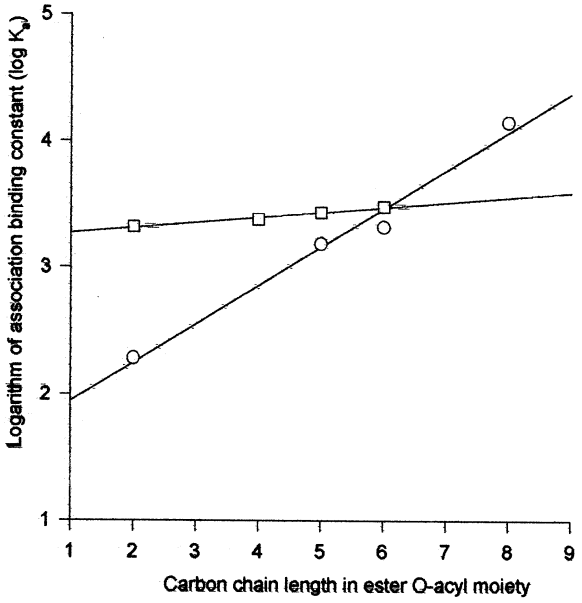


Fig. 7. The relationship between log K_a and carbon chain length in ester O-acyl moiety for (○) aspirin analogues ($y = 1.6443 + 0.3024x$, $r^2 = 0.983$) and (□) diflunisal esters ($y = 3.2298 + 0.0401x$, $r^2 = 0.975$). The expected K_a of any analogue can be predicted by regression analysis.

References

Aarons, L., Clifton, P., Rowland, M., 1980. Aspirin binding and the effect of albumin on spontaneous and enzyme-catalysed hydrolysis. J. Pharm. Pharmacol. 32, 537–543.

Ali, M., McDonald, J.W.D., Theissen, J.J., Coates, P.E., 1980. Plasma acetylsalicylate and salicylate and platelet cyclooxygenase activity following plain and enteric-coated aspirin. Stroke 11, 9–13.

Bailey, P.S., Bailey, C.A., 1981. Organic Chemistry, Allyn and Bacon, New York, pp. 57–58.

Cossum, P.A., Roberts, M.S., Kilpatrick, D., Yong, A.C., 1986. Distribution and metabolism of nitroglycerin and its metabolites in vascular beds of sheep. J. Pharm. Sci. 75, 731–737.

Davis, S.S., Higuchi, T., Rytting, J.H., 1972. Determination of thermodynamics of the methylene group in solutions of drug molecules. J. Pharm. Pharmac., 24, Suppl. 30P–46P.

Forster, S., Buckton, G., Beezer, A.E., 1991. The importance of chain length on the wettability and solubility of organic homologs. Int. J. Pharm. 72, 29–34.

Foye, W.O., 1989. Principles of Medicinal Chemistry, 3rd ed., Lea & Febiger, Philadelphia, 863.

Harris, P.A., Riegelman, S., 1969. Influence of the route of administration on the area under the plasma concentration-time curve. J. Pharm. Sci. 58, 71–75.

Isaacs, N.S., 1987. Physical Organic Chemistry, Longmans, Essex, pp. 297–301.

Iwamoto, K., Takei, M., Watanabe, J., 1982. Gastrointestinal and hepatic first-pass metabolism of aspirin in rats. J. Pharm. Pharmacol. 34, 176–180.

Jusko, W.J., Gretch, M., 1976. Plasma and tissue protein binding of drugs in pharmacokinetics. Drug Metabolism Rev. 5, 43–140.

Lecomte, M., Laneuville, O., Ji, C., Dewitt, D.L., Smith, W.L., 1994. Acetylation of human prostaglandin endoperoxide synthase-2 by aspirin. J. Biochem., 269 13 207–13 215.

Leo, A., Jow, P.Y., Silipo, C., Hansch, C., 1975. Calculation of hydrophobic constant (log P) from Pi and f constants. J. Med. Chem. 18, 865–868.

Levy, G.A., 1966. Glucuronic Acid, Academic Press, New York, p. 301.

Martin, A.N., 1983. Physical Pharmacy, 3rd ed., Lea & Febiger, Philadelphia, Ch. 13.

McLeod, L.J., Roberts, M.S., Seville, P.R., 1988. Low-dose enteric-coated aspirin as an antiplatelet preparation. Aust. NZ. J. Med. 148, 207.

Mellick, G.D., Roberts, M.S., 1996. The disposition of aspirin and salicylic acid in the isolated perfused rat liver: The effect of normal and retrograde flow on availability and mean transit times. J. Pharm. Pharmacol. 48, 738–743.

Roberts, M.S., Joyce, R.M., McLeod, L.J., Vial, J.H., Seville, P.R., 1986. Slow-release aspirin and prostaglandin inhibition. Lancet 1, 1153–1154.

Siebert, D.J., Bochner, F., Imhoff, D.M., Watts, S., Lloyd, J.V., Field, J., Gabb, B.W., 1983. Aspirin kinetics and platelet aggregation in man. Clin. Pharmacol. Ther. 33, 367–374.

The SALT Collaborative Group, Swedish Aspirin Low-dose Trial (SALT) of 75 mg aspirin as secondary prophylaxis after cerebrovascular ischaemic events. 1991. Lancet, 338, 1345–1349.

Vial, J.H., Roberts, M.S., McLeod, L.J., Seville, P.R., 1990. Selective inhibition of platelet cyclooxygenase with controlled release, low-dose aspirin. Aust. NZ. J. Med. 20, 652–656.

Wientjes, M.G., Levy, G., 1988. Nonlinear pharmacokinetics of aspirin in rats. J. Pharmacol. Exp. Ther. 245, 809–815.

Windholz, M (Ed.), 1983. The Merck Index, 10th ed., Merck, Rahway, p. 865.

Yacobi, A., Lai, C.M., Levy, G., 1977. Comparative pharmacokinetics of coumarin anticoagulants XXI: effect of plasma protein binding on distribution kinetics of warfarin in rats. J. Pharm. Sci. 66, 567–572.

Yalkowsky, S.H., Flynn, G.L., Slunick, T.G., 1972. Importance of chain length on physiochemical and crystalline properties of organic homologues. J. Pharm. Sci., 852–857.

Yalkowsky, S.H., 1981. Techniques of Solubilization of Drugs, Marcel Dekker, New York, Ch. 1.