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Synthesis and Physicochemical Properties of the Furan Dicarboxylic Acid, 3-Carboxy-4-methyl-5-propyl-2-furanpropanoic Acid, an Inhibitor of Plasma Protein Binding in Uraemia

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Abstract

The furan dicarboxylic acid, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (5-propyl FPA) accumulates in the plasma of patients with chronic renal failure and is a major contributor to the drug binding defect of uraemic plasma. This acid has also been implicated in several other aspects of the uraemic syndrome: anaemia, irregularities of thyroid function, neurological symptoms and inhibition of active tubular secretion.

The acid is not commercially available and its synthesis, starting with Meldrum's acid and methyl succinyl chloride, is described. The pK_a values were measured by titration and values of 3·2 and 3·6 respectively were assigned to the carboxylic acid groups attached directly to the ring at position 3 and at position 2 (on the side-chain). The partition coefficient (log P) between hydrochloric acid and octanol was 1·2 and the distribution coefficient (log D; octanol-phosphate buffer pH 7·4) was −0·59.

The pK_a values and the degree of hydrophobic character of 5-propyl FPA are consistent with those of other protein-bound acids which undergo active tubular secretion by the kidney and this substance may serve as an endogenous marker for the effects of drugs and disease on this process.

The furan dicarboxylic acid, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (5-propyl FPA), was first identified in human urine by Spiteller & Spiteller (1979) and subsequently found to accumulate in the plasma of patients with chronic renal failure (Liebich et al 1984) reaching concentrations of around 400 μM (Niwa et al 1990), which is close to that of plasma albumin (~600 μM). The pentyl analogue also accumulates, but probably to a lesser extent (Liebich et al 1984)

5-Propyl FPA has a high affinity for albumin (Lindup et al 1986; Mabuchi & Nakahashi 1987, 1988a; Henderson & Lindup 1990) and inhibits the binding of other ligands, particularly organic acids, to albumin (Lindup et al 1986; Mabuchi & Nakahashi 1988b; Niwa et al 1988). This furan acid is believed to be a major contributor to the drug binding defect of uraemic plasma because of its considerable affinity for albumin (apparent association constant, $K_a = 2{\cdot}5 \times 10^6\ M^{-1}$ (Henderson & Lindup 1990)), and the high concentrations reached in uraemic plasma, where it would approach a one to one molar ratio with albumin, particularly when the latter is reduced in chronic renal failure.

5-Propyl FPA is excreted unchanged in human urine and there is little evidence of further metabolism (McTigue et al 1990). It is, therefore, likely that 5-propyl FPA undergoes active tubular secretion and there is evidence for this from experiments with rat kidney slices in-vitro (Henderson & Lindup 1992; Costigan et al 1992) and in-vivo in the anaesthetized rat, in competition experiments with *p*-aminohippuric acid and probenecid (Costigan & Lindup 1996).

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Uraemic plasma contains inhibitors of active tubular secretion (Hook & Munro 1968) which share a common identity with the inhibitors of plasma protein binding and it is, therefore, likely that 5-propyl FPA is a major contributor (Depner 1981). Further insight into the inhibitors of active tubular secretion that are present in uraemic plasma would help to provide a better understanding of the effects of chronic renal failure on drug elimination.

5-Propyl FPA has also been implicated in the anaemia which occurs in chronic renal failure (Costigan et al 1995; Niwa et al 1990), in irregularities of thyroid function (Lim et al 1993) and in neurological symptoms which may be caused by inhibition of organic anion transport at the blood-brain barrier (Costigan et al 1996a). This furan acid also inhibited phase I (*O*-demethylation) and phase II (glutathione conjugation and glucuronidation) pathways of drug metabolism in rabbit liver homogenate in-vitro (Walters et al 1995). Thus, 5-propyl FPA is a uraemic toxin of pharmacological interest.

Because 5-propyl FPA was not commercially available and investigation of the biological and physical properties of the compound required greater quantities than had been generously supplied by Professor Gerhard Spiteller (Bayreuth University), it had to be synthesized. The method used was based on that of Pfordt et al (1981) who had started by synthesizing both Meldrum's acid and methyl succinyl chloride. Meldrum's acid and ethyl succinyl chloride were commercially available and relatively cheap and so the synthesis was begun at this point. The pK_a and partition coefficient of 5-propyl FPA have been measured for comparison with drugs and with model substrates for active tubular secretion, such as *p*-aminohippuric acid and probenecid.

Materials and Methods

Materials

Potassium hydroxide pellets, potassium carbonate, hydrochloric acid, zinc chloride, sodium bisulphite, sodium hydroxide, sodium sulphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, ethanol and toluene were obtained from BDH Chemicals Ltd (Lutterworth, UK). Pyridine, sodium chloride, magnesium sulphate and sulphuric acid were obtained from FSA Laboratory Supplies (Loughborough, UK). Isopropylidene malonate (Meldrum's acid), ethyl succinyl chloride, hydroxyacetone, propionic anhydride, boron trifluoride etherate, hydrazine hydrate and diethylene glycol were obtained from Lancaster Chemicals (Morecambe, UK). Octanol was obtained from Aldrich Chemical Co. (Gillingham, UK).

Chemical synthesis of 5-propyl FPA

The method followed was that of Pfordt et al (1981); we are grateful to Professor G. Spiteller for providing additional experimental details.

Synthesis of diethyl 3-oxohexanedioate

Ethyl succinyl chloride (55 mmol) was added dropwise under nitrogen at 0°C to a stirred solution of Meldrum's acid (50 mmol) in dry dichloromethane (100 mL) and dry pyridine (8 mL). The mixture was stirred for 30 min at 0°C and then for a further 1 h at room temperature.

The reaction mixture was washed with hydrochloric acid (2 M; 50 mL) followed by water (50 mL). The resulting solution was dried over anhydrous magnesium sulphate and excess dichloromethane was removed. The residue was dissolved in absolute ethanol (50 mL) and the solution heated under reflux for 2 h. Excess ethanol was removed by rotary evaporation and the resulting oil distilled under vacuum to give diethyl 3-oxohexanedioate.

Synthesis of ethyl 3-ethoxycarbonyl-4-methylfuran-2-propanoate

Diethyl 3-oxohexanedioate (0·25 mol) formed in the previous reaction, hydroxyacetone (0·23 mol), ethanol (38 mL) and zinc chloride (24·9 g) were stirred and heated together under reflux for 18 h. When the reaction mixture was cool, water (225 mL) was added. The resulting mixture was extracted with toluene (3 × 100 mL). The extracts were combined and washed in turn with water (100 mL), sodium bisulphite (30% w/v), sodium hydroxide (5% w/v), dilute hydrochloride acid (1 M) and twice more with water. After drying the organic phase, excess toluene was removed. The residue was purified by vacuum distillation.

Synthesis of ethyl 3-ethoxycarbonyl-4-methyl-5-(1-oxopropyl)-furan-2-propanoate

Boron trifluoride etherate (1·5 g) was added dropwise to a stirred solution of ethyl 3-ethoxycarbonyl-4-methylfuran-2-propanoate (0·13 mol) and propionic anhydride (0·18 mol) at 0°C. The mixture was stirred at 0°C for 45 min and then at room temperature for a further 30 min. Ice-cold water (130 mL) was added to the reaction mixture and the organic layer separated. The aqueous layer was extracted three times with diethyl ether and the combined ether extracts were washed twice with saturated potassium carbonate. The resulting solution was dried and excess ether removed. The product was purified by vacuum distillation.

Synthesis of 5-propyl FPA

Ethyl 3-ethoxycarbonyl-4-methyl-5-(1-oxopropyl)furan-2-propanoate (0·07 mol), hydrazine hydrate (0·21 mol), potassium hydroxide (0·35 mol) and diethylene glycol (140 mL) were boiled under reflux (3–4 h). Excess water and ethanol were removed by boiling and the remaining solution was heated under reflux at 170–200°C (18 h). The reaction mixture was mixed with ice (95 g) and acidified with sulphuric acid (20%, 140 mL). This caused the precipitation of potassium sulphate which was filtered off and washed with diethyl ether. The ether phase was then separated from the filtrate and the filtrate was washed a further three times with ether. The ether phases were concentrated to approximately half their original volume and were then washed twice with water to remove any remaining acid or diethylene glycol. The ether portion was dried and excess ether was removed.

The solid obtained was treated with a small amount of acetone (5 mL). After intense stirring, the significant impurities dissolved and a suspension of the desired product was obtained. This was then filtered and dried. The purity of 5-propyl FPA was analysed by HPLC (Henderson & Lindup 1990) to determine if recrystallization was necessary.

Nuclear magnetic resonance (NMR) spectra were produced on a Bruker ACE 200 (200 MHz) machine and mass spectra were obtained on a VG Micromass 7070E machine.

Determination of the pK_a of 5-propyl FPA

5-Propyl FPA (0·0018 g) was initially dissolved in absolute ethanol (0·5 mL) as the dissolution of this compound into water proved impossible. The solution was then made up to 25 mL in a volumetric flask with distilled water, giving a 5-propyl FPA concentration of 300 μM in a 2% ethanol solution. This was then titrated against dilute sodium hydroxide solution (2 mM). Throughout the titration helium was bubbled through the 5-propyl FPA solution to aid mixing of the two solutions and to degas the solution in order to avoid the effects of dissolved carbon dioxide or oxygen. The pH of the solution was measured after each addition of sodium hydroxide solution.

Results obtained from initial titrations were used to discern areas of interest. Thus, initially the pH was measured after additions of 0·2-mL vols sodium hydroxide solution (2 mM), until approximately 4 mL had been added. Measurements were then made after addition of 0·1 mL-vols sodium hydroxide solution, until there was no appreciable rise in pH. Over the same range of values, a correction curve was obtained by titration of 2% ethanol with dilute sodium hydroxide solution (2 mM).

A graph was then plotted of pH (ordinate) against volume of sodium hydroxide added (abscissa). From the graph it was possible to assess where significant changes in pH occurred. As 5-propyl FPA is a dicarboxylic acid, there would be expected to be two pK_a values, and thus two points on the graph showing a significant change in pH.

Calculation of pK_a values

To estimate accurately where the two "end-points" of the titration occurred, it was necessary to consider both areas of the titration curve where a change in slope occurred. The end-point of the titration was the volume of added titrant at which the rate of change of pH with added volume (V), i.e. d(pH)/dV, was maximum. A graph of change in pH per 0·1 mL sodium

hydroxide added was plotted either side (0·5 mL each way) of both areas of interest. The position at which a maximum occurred corresponded to the volume of sodium hydroxide required for neutralization of both carboxylic acid groups (Vo_1 and Vo_2). By considering the equations for the neutralization of a dicarboxylic acid:

$$AH_2 \rightarrow AH^- + H^+ \quad (1)$$

$$AH^- \rightarrow A^{2-} + H^+ \quad (2)$$

and the Henderson–Hasselbalch equation:

$$pH = pK_a + \log([salt]/[acid])$$

the pK_a values can be deduced.

From equation 1

$$pK_{a1} = pH - \log([AH_2]/[AH^-]) \quad (3)$$

From equation 2

$$pK_{a2} = pH - \log([AH^-]/[A^{2-}]) \quad (4)$$

At the beginning of the titration none of the 5-propyl FPA is in the salt form; at each neutralization point, however:

$$[salt] \propto V$$

and

$$[acid] \propto (Vo - V)$$

where V is the volume of alkali added and Vo is the volume of alkali required for neutralization. Therefore from equation 3:

$$pK_{a1} = pH - \log(V/(Vo_1 - V))$$

and from equation 4:

$$pK_{a2} = pH - \log(V/(Vo_2 - V))$$

Graphs can then be plotted of pH (ordinate) against log (V/(Vo − V)) (abscissa). The pK_a value will correspond to the point at which log (V/(Vo − V)) = 0.

Determination of the distribution and partition coefficients of 5-propyl FPA

The partition coefficient of 5-propyl FPA was measured between hydrochloric acid and octanol. The distribution coefficient was measured between phosphate buffer (40 mM Na_2HPO_4, 9·6 mM NaH_2PO_4 and 35·5 mM NaCl) and octanol. Before the start of the experiment, octanol (25 mL) and either hydrochloric acid (0·1 M, 25 mL) or phosphate buffer (0·05 M, pH 7·4, 25 mL) were stirred together for approximately 1 h in order to saturate one solution with the other. A known weight of 5-propyl FPA was then dissolved in the saturated solutions of octanol. The initial concentration of 5-propyl FPA in octanol saturated with hydrochloric acid was 417 μM and that in the buffer saturated with octanol 683 μM. Samples (3 mL) of the octanol-5-propyl FPA mixture were added to equivalent amounts of either octanol-saturated hydrochloric acid or octanol-saturated phosphate buffer. Each experiment was performed in quadruplicate and the solutions were left in a shaking water-bath for 18 h at 25°C.

The concentration of 5-propyl FPA in the aqueous phase at the end of the experiment was determined with a Cecil Instruments CE 506 double-beam UV spectrophotometer, using 3 mL quartz cuvettes. The final concentration of 5-propyl FPA the organic phase was determined by subtraction of the final concentration in the aqueous phase from the initial concentration in the organic phase. The distribution and partition coefficients were evaluated as follows:

Concentration of 5-propyl FPA in the organic layer/ Concentration of 5-propyl FPA in the aqueous layer

Results

Synthesis of diethyl 3-oxohexanedioate

Diethyl 3-oxohexanedioate was obtained as a straw-coloured liquid in a yield of 66%. This compared reasonably well with the literature yields of around 80% (Oikawa et al 1978; Schmidt & Klade 1988) and the 85% yield of dimethyl hexanedioate reported by Pfordt et al (1981). The nuclear magnetic resonance (NMR) spectrum compared well with that reported by Taylor & McKillop (1967):

NMR analysis: $\delta = 4{\cdot}3$ (q, 4H, $COOCH_2CH_3$), 3·5 (s, 2H, $COCH_2COOCH_2CH_3$), 2·9 (t, 2H, $CH_2CH_2CH_2COCH_2$), 2·7 (t, 2H, OCH_2CH_2), 1·3 (t, 6H, $COOCH_2CH_3$).

Synthesis of ethyl 3-ethoxycarbonyl-4-methylfuran-2-propanoate

The yield of ethyl 3-ethoxycarbonyl-4-methylfuran-2-propanoate was 69%, which again compared well with the 87% yield for a similar synthesis reported by Hanson et al (1965) and by Pfordt et al (1981) for the synthesis of the methyl derivative. The NMR spectrum of the straw-coloured liquid produced confirmed the structure of the compound.

NMR analysis: $\delta = 7{\cdot}1$ (s, 1H, $C=CH$-O), 4·3 (q, 4H, $COOCH_2CH_3$), 3·3 (t, 2H, $CCH_2CH_2COOCH_2CH_3$), 2·7 (t, 2H, $CH_2CH_2COOCH_2CH_3$), 2·6 (s, 3H, $H_3CC=C$), 1·3 (t, 6H, $COOCH_2CH_3$).

Synthesis of ethyl 3-ethoxycarbonyl-4-methyl-5-(1-oxopropyl)-furan-2-propanoate

This synthesis was based on the method described by Farrar & Levine (1950). The product was again obtained as a straw-coloured liquid; the yield of 56% was lower than the 81% reported by Pfordt et al (1981) for the synthesis of the corresponding dimethyl ester.

NMR analysis: $\delta = 4{\cdot}4$ (q, 2H, $C=CCOOCH_2CH_3$), 4·2 (q, 2H, $CH_2CH_2COOCH_2CH_3$), 3·4 (t, 2H, $C=CCH_2CH_2COOCH_2CH_3$), 2·9 (q, 2H, $CH_3CH_2C=O$), 2·8 (t, 2H, $CH_2CH_2COOCH_2CH_3$), 2·6 (s, 3H, $C=CCH_3$), 1·5 (t, 3H, $COOCH_2CH_3$), 1·2 (t, 3H, $CH_2CH_2COOCH_2CH_3$).

Mass spectral analysis: M = 310 (20%), 281 (13), 265 (27), 236 (100), 223 (16), 207 (63), 191 (10), 179 (21), 167 (6), 139 (9), 135 (6), 129 (14), 123 (6), 101 (20), 95 (5), 79 (11), 77 (14), 67 (8), 65 (8), 57 (48), 55 (18), 53 (6), 45 (5), 43 (20).

Synthesis of 5-propyl FPA

The final stage of the synthesis was based on the Huang-Minlon reaction (Huang-Minlon 1946). The yield of 5-propyl FPA was poor, approximately 25%. Pfordt et al (1981) reported a yield of 63% for synthesis via the dimethyl ester. The crystals produced were off-white in colour and the NMR spectra and mass spectra were compared with those obtained from 5-propyl FPA provided by Professor Spiteller for verification of the synthesis. Analysis of 5-propyl FPA by HPLC showed that the final product was approximately 98% pure. The complete synthesis of 5-propyl FPA is shown in Fig. 1.

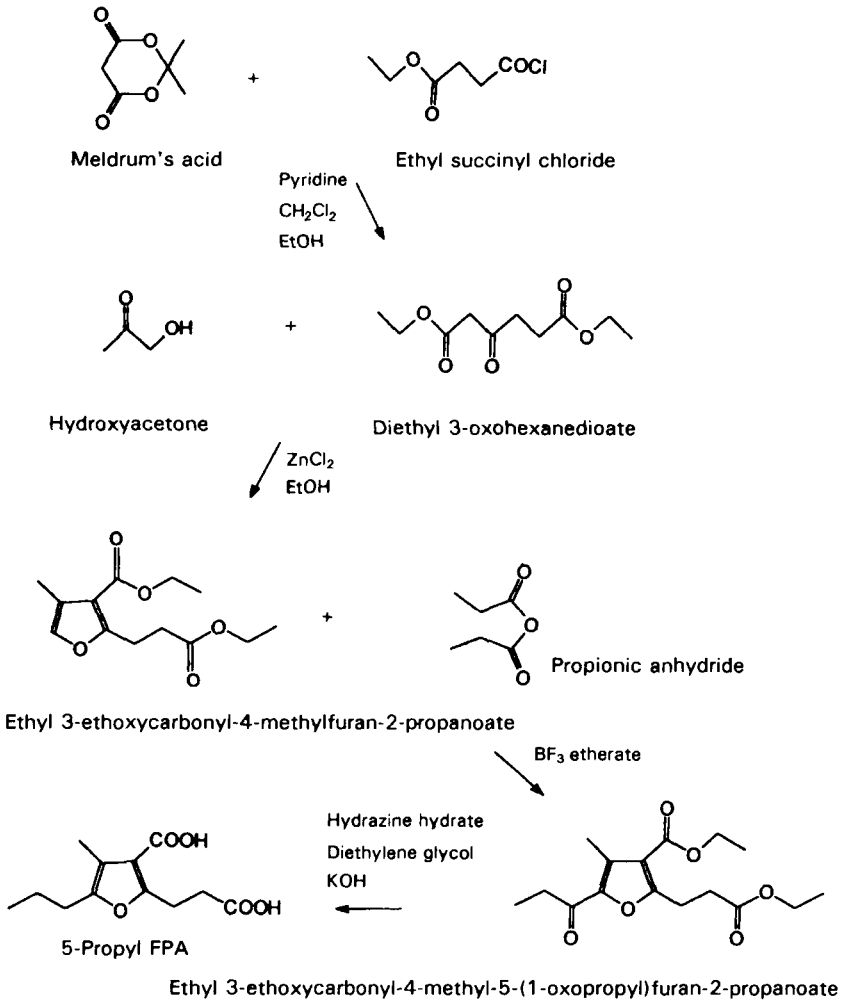

FIG. 1. Chemical synthetic pathway for 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid.

NMR analysis: $\delta = 3{\cdot}5$ (t, 2H, CH_2CH_2COOH), 2·9 (t, 2H, $CH_2CH_2CH_3$), 2·8 (t, 2H, CH_2CH_2COOH), 2·3 (s, 3H, $C=CCH_3$), 1·9 (sextet, 2H, $CH_3CH_2CH_2C=C$), 1·1 (t, 3H, $CH_2CH_2CH_3$).

Mass spectral analysis: M = 240 (21%), 222 (12), 211 (36), 194 (43), 181 (26), 165 (100), 152 (7), 149 (22), 121 (8), 77 (8), 71 (5), 57 (5), 55 (12), 43 (13).

Determination of pK_a values

The titration of a 2% ethanol solution against sodium hydroxide solution (2 mM), showed that ethanol did not interfere with the results of the titration when 5-propyl FPA was dissolved in a 2% ethanol solution. The titration curve produced (Fig. 2), showed that there were two areas of interest, the maximum changes in pH value occurring at $Vo_1 = 5{\cdot}7$ mL and $Vo_2 = 6{\cdot}7$. Analyses of the pH before these two values were reached revealed the pK_{a1} and pK_{a2} to be 3·2 and 3·6, respectively.

Distribution and partition coefficients of 5-propyl FPA

The results obtained give a partition of 16·2 (log P = 1·2) and a distribution coefficient of 0·26 (log D = − 0·59).

Discussion

Synthesis of 5-propyl FPA

The method of synthesis described here provides a straightforward route to the desired product; the final overall yield of

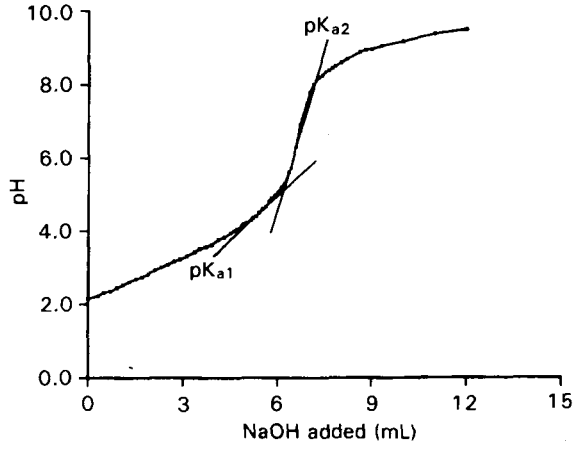

FIG. 2. Titration curve for 5-propyl FPA titrated with sodium hydroxide (2 mM).

Table 1. Comparison of the partition coefficients and pK_a values of 5-propyl FPA and other organic acids.

Organic acid	pK_a	log P
Salicylic acid	2·9	2·9
5-Propyl FPA	3·2; 3·6	1·2
Probenecid	3·4	> 100
p-Aminohippuric acid	3·8	0·01
Benzoic acid	4·2	1·87
p-Aminobenzoic acid	4·9	0·68

5-propyl FPA could, however, be improved. The synthesis of a radiolabelled form of 5-propyl FPA would be of great use in the investigation of the properties of 5-propyl FPA in-vivo, but the low yield achieved by the current method would make this an expensive process. The best way to insert an isotope would probably be during the addition of the propyl group at position 5 (Fig. 1).

Physicochemical properties of 5-propyl FPA

pK_a values. There are three main ways in which a compound can cross a membrane barrier: aqueous diffusion, lipid diffusion and carrier-facilitated transport (which includes carrier-facilitated diffusion and active transport). 5-Propyl FPA is a weak organic acid with two ionizable functional groups. Weak organic acids are considerably more lipid-soluble than water-soluble in their un-ionized form but more water-soluble in their ionized form. The absorption, distribution and excretion of 5-propyl FPA will be strongly influenced by the pH of the relevant body fluid. A measure of the ability of 5-propyl FPA to pass through lipid membranes will be given by its partition coefficient, though this also will depend on the pH of the aqueous media.

The pK_a values obtained, 3·2 and 3·6, agree well with values found for other aromatic carboxylic acids (Table 1). The pK_a value of 3·2 can be assigned to the carboxylic acid group at position 3, whereas the carboxylic acid group at position 2 can be assigned the pK_a 3·6, assignments which are based on the following arguments. The aromatic nature of the furan ring suggests that it will pull electrons towards it and thus the anions formed will be more stable e.g. propanoic acid normally has a pK_a value of 4·9, but the presence of the aromatic ring reduces the pK_a of the propyl carboxylic acid group to 3·6.

The carboxylic acid group at position 3 is likely to have the lower pK_a value because of the 2-carbon chain at position 2 which separates the carboxylic acid group from the ring. This has an electron-donating effect which, to a certain extent, will counterbalance the electron-withdrawing effect of the aromatic ring. Thus the stabilizing effect on the anion formed will be reduced and the pK_a value at position 2 will be higher than that at position 3. Further evidence in support of this assignment of pK_a values is that 3-furoic acid has a pK_a value of 3·17.

Lipophilicity. The partition coefficient (P) is a measure of the ratio of un-ionized molecules between two solvent systems, whereas the distribution coefficient (D) is defined as the ratio of the concentration of compound in the organic phase to that of both ionized and un-ionized species in the aqueous phase at a given pH (Stopher & McLean 1990). Thus the partition coefficient is a measure of the lipophilicity of the un-ionized form of the compound.

In its fully un-ionized form 5-propyl FPA shows a substantial degree of lipophilic character (Table 1) and this is in agreement with its HPLC elution characteristics (Vanholder et al 1994). 5-Propyl FPA is found at elevated concentrations in the urine and bloodstream of uraemic patients and so widespread distribution into tissues and penetration across the blood-brain barrier is likely in chronic renal failure. This could result in a large body burden of the acid which, together with the need for active tubular secretion, would account for the prolonged elevation of plasma concentrations even after a successful kidney transplant (Liebich et al 1987; Mabuchi et al 1989; Costigan et al 1996b). A degree of hydrophobic character is important for active tubular secretion by the *p*-aminohippuric acid transporter, and the pK_a values (Table 1) are similar to those for other organic acids that undergo active tubular secretion (Ullrich 1994). 5-Propyl FPA is a bivalent anion and there is evidence that the *p*-aminohippuric acid transporter accepts such anions (Ullrich 1994).

Lipid solubility and the polar groups are both important features in relation to binding to albumin (Lindup 1987), and the high affinity of 5-propyl FPA for albumin and also presumably the *p*-aminohippuric acid transporter in the kidney, is a consequence of its structure and physicochemical properties. They help to explain its inhibitory effects on transport by albumin and by the organic acid transporter in the kidney. 5-Propyl FPA could therefore serve as a model for further studies of the structure–activity relationships of transport systems for organic acids.

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References

Costigan, M. G., Lindup, W. E. (1996) Plasma clearance in the rat of a furan dicarboxylic acid which accumulates in uraemia. Kidney Int. 49: 634–638

Costigan, M. G., Gilchrist, T. L., Lindup, W. E. (1992) Inhibitors of plasma protein binding and the renal secretion of organic acids. Br. J. Clin. Pharmacol. 34: 175P

Costigan, M. G., Yaqoob, M., Lindup, W. E. (1995) Effects of haemodialysis and continuous ambulatory peritoneal dialysis on the plasma clearance of an albumin-bound furan dicarboxylic acid. Nephrol. Dial. Transplant. 10: 648–652

Costigan, M. G., O'Callaghan, C. A., Lindup, W. E. (1996a) Hypothesis: is accumulation of a furan dicarboxylic acid (3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid) related to the neurological abnormalities in patients with renal failure? Nephron In press

Costigan, M. G., Yaqoob, M., Lindup, W. E. (1996b) Retention of an albumin-bound furan dicarboxylic acid in patients with chronic renal failure or after a kidney transplant. Nephrol. Dial. Transplant. 11: 803–807

Depner, T. A. (1981) Suppression of tubular anion transport by an inhibitor of serum protein binding in uraemia. Kidney Int. 20: 511–518

Farrar, M. W., Levine, R. (1950) Condensations effected by boron fluoride complexes. III. The acylation of certain substituted thiophenes and furans. J. Am. Chem. Soc. 72: 3695–3698

Hanson, J. C., Nayler, J. H. C., Taylor, T., Gore, P. H. (1965) Derivatives of 6-aminopenicillanic acid. Part IX. 2,4-Di- and 2,4,5-tri-substituted 3-furylpenicillins. J. Chem. Soc. 5984–5988

Henderson, S. J., Lindup, W. E. (1990) Interaction of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, an inhibitor of plasma protein binding in uraemia, with human albumin. Biochem. Pharmacol. 40: 2543–2548

Henderson, S. J., Lindup, W. E. (1992) Renal organic acid transport: uptake by rat kidney slices of a furan dicarboxylic acid which inhibits plasma protein binding of acidic ligands in uraemia. J. Pharmacol. Exp. Ther. 263: 54–60

Hook, J. B., Munro, J. R. (1968) Specificity of the inhibitory effect of "uraemic" serum on *p*-aminohippurate transport. Proc. Soc. Exp. Biol. Med. 127: 289–292

Huang-Minlon, (1946) A simple modification of the Wolff-Kishner reduction. J. Am. Chem. Soc. 68: 2487

Liebich, H. M., Pickert, A., Tetschner, B. (1984) Gas chromatographic and gas chromatographic-mass spectrometric analysis of organic acids in plasma of patients with chronic renal failure. J. Chromatogr. 289: 259–266

Liebich, H. M., Risler, T., Fischer, U., Rapp, K., Tetschner, B., Eggstein, M. (1987) Gas chromatographic determination of hippuric acid and a furancarboxylic acid in plasma of patients with kidney diseases. J. Chromatogr. 399: 291–294

Lim, C.-F., Bernard, B. F., De Jong, M., Docter, R., Krenning, E. P., Hennemann, G. (1993) A furan fatty acid and indoxyl sulphate are putative inhibitors of thyroxine hepatocyte transport in uraemia. J. Clin. Endocrinol. Med. 76: 318–324

Lindup, W. E. (1987) Plasma protein binding of drugs: some basic and clinical aspects. In: Bridges, J. W., Chasseaud, L. F., Gibson, G. G. (eds) Progress in Drug Metabolism, vol. 10. Taylor & Francis, London, pp 141–185

Lindup, W. E., Bishop, K. A., Collier, R. (1986) Drug binding defect of uraemic plasma: contribution of endogenous binding inhibitors. In: Tillement, J.-P., Lindenlaub, E. (eds) Protein Binding and Drug Transport. FK Schattauer Verlag, Stuttgart-New York, pp 397–414

Mabuchi, H., Nakahashi, H. (1987) Determination of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, a major endogenous ligand substance in uraemic serum, by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. 415: 110–117

Mabuchi, H., Nakahashi, H. (1988a) A major endogenous ligand substance involved in renal failure. Nephron 49: 277–280

Mabuchi, H., Nakahashi, H. (1988b) Displacement by anionic drugs of endogenous ligands bound to albumin in uraemic serum. Ther. Drug Monit. 10: 261–264

Mabuchi, H., Nakahashi, H., Hamajima, T., Aikawa, I., Oka, T. (1989) The effect of renal transplantation on a major endogenous ligand retained in uraemic serum. Am. J. Kidney Dis. 13: 49–54

McTigue, J. J., Henderson, S. J., Lindup, W. E. (1990) Excretion of the uraemic metabolite 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid in human urine. Nephron 55: 214–215

Niwa, T., Takeda, N., Maeda, K., Shibata, M., Tatematsu, A. (1988) Accumulation of furancarboxylic acids in uraemic serum as inhibitors of drug binding. Clin. Chim. Acta 173: 127–138

Niwa, T., Yazawa, T., Kodama, T., Uehara, Y., Maeda, K., Yamada, K. (1990) Efficient removal of albumin-bound furancarboxylic acid, an inhibitor of erythropoiesis, by continuous ambulatory peritoneal dialysis. Nephron 56: 241–245

Oikawa, Y., Sugano, K., Yonemitsu, O. (1978) Meldrum's acid in organic synthesis 2. A general and versatile synthesis of β-keto esters. J. Org. Chem. 43: 2087–2088

Pfordt, J., Thoma, H., Spiteller, G. (1981) Identifizierung, Strukturableitung und Synthese bisher unbekannte Urofuransäuren im menschlichen Blut. Liebigs Annalen Chemie. 2298–2308

Schmidt, H.-W., Klade, M. (1988) A convenient synthesis of β-keto diesters. OPPI Briefs 20: 184–187

Spiteller, M., Spiteller, G. (1979) Trennung und Charakterizierung Saurer Harnbestandteile. J. Chromatogr. 164: 253–317

Stopher, D., McLean, S. (1990) An improved method for the determination of distribution coefficients. J. Pharm. Pharmacol. 42: 144

Taylor, E. C., McKillop, A. (1967) A new synthesis of diethyl β-ketoadipate. Tetrahedron 23: 897–900

Ullrich, K. J. (1994) Specificity of transporters for 'organic anions' and 'organic cations' in the kidney. Biochim. Biophys. Acta 1197: 45–62

Vanholder, R., De Smet, R., Hsu, C., Vogeleere, P., Ringoir, S. (1994) Uremic toxicity: the middle molecule hypothesis revisited. Semin. Nephrol. 14: 205–218

Walters, R. L., Nicholls, P. J., Lindup, W. E. (1995) Effect of three uraemic toxins on various pathways of hepatic drug metabolism in-vitro. J. Pharm. Pharmacol. 47: 1091