Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 3, pp. 229–240, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00
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Isolation and identification of the rearrangement products of diflunisal 1-*O*-acyl glucuronide

JENS HANSEN-MØLLER,* CLAUS CORNETT, LARS DALGAARD and STEEN HONORÉ HANSEN

Royal Danish School of Pharmacy, Department of Chemistry BC, Universitetsparken 2, DK-2100 Copenhagen, Denmark

Abstract: A preparative reversed-phase high-performance liquid chromatographic method is described for the simultaneous separation of eight different isomers formed from the 1-*O*-acyl glucuronide of diflunisal. All isomers were formed when the acyl glucuronide was incubated under mildly alkaline conditions in aqueous solution. Various forms of two-dimensional NMR studies were performed in order to identify each isomer. Seven of the isomers were identified as α- and β-forms of esters in which diflunisal forms an ester with one of the four alcohol groups in the glucupyranuronic acid. One isomer was identified as the ether glucuronide of diflunisal. To establish the exact chemical shift of the different protons, simulation of the one-dimensional NMR spectra and iterative analyses were performed.

Keywords: *Reversed-phase high-performance liquid chromatography; acyl glucuronide; acyl migration; ^{1}H-NMR; diflunisal; metabolites.*

Introduction

In recent years many examples of acidic drugs that are partly excreted as unstable ester glucuronides have been published. Among these are zomepirac [1], clofibrate [2], probenecid [3], sodium valproate [4], fenclofenac [5], isoxepac [6], WY-18,251 [3-(*p*-chlorophenyl)-thiazolo-[3,2]-benzimidazole-2-acetic acid] [7], frusemide (furosemide) [8] and diflunisal [9, 10]. Bilirubin [11] is an example of an endogenous compound that is partly excreted as an unstable ester glucuronide (conjugate). The properties of acyl glucuronides have been reviewed by Faed [12].

In neutral or mildly alkaline solutions, the aglycones of these unstable ester glucuronides migrate from the anomer OH-group at C-1 (see formula included in Fig. 1) of the glucuronic acid moiety through an 1,2-ortho ester to the OH-group at C-2. This process can then continue on to the OH-groups at C-3 and C-4 [13], thereby forming several isomers. These rearrangements have been shown to depend on temperature [14] as well as on pH of the solution [1–11].

From an analytical point of view the instability of the acyl glucuronide is interesting. The traditional approach for quantitative determination of glucuronides has been an

*To whom correspondence should be addressed.

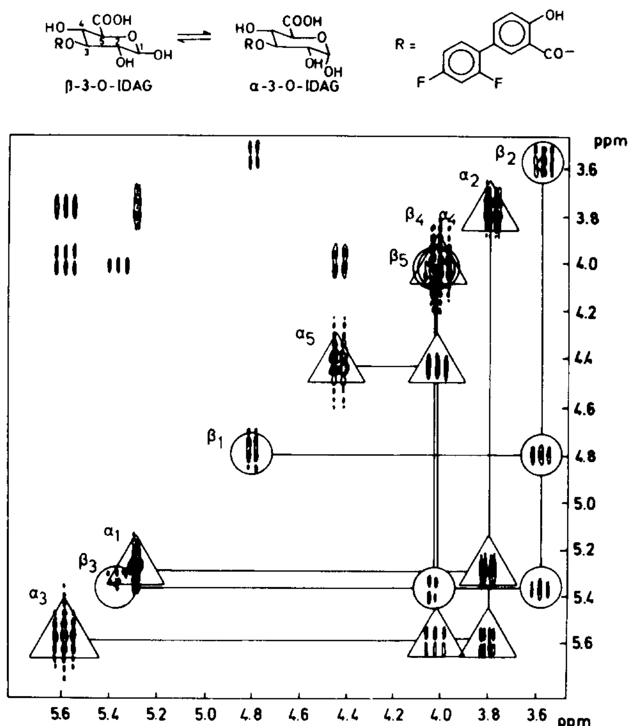

Figure 1
COSY-spectrum of the glucuronic acid moiety of 2.70 mg of α- and β-3-*O*-IDAG (acyl isomer of diflunisal acyl glucuronide) isolated as fraction 5. The spectrum was obtained at 250 MHz in 300 μl of $(CD_3)_2CO$. Concentrations in ppm are relative to that of $(CH_3)_2CO$ (2.05 ppm). At the top of the figure the structures of α- and β-3-*O*-IDAG are given.

assay of the liberated aglycone following a selective cleavage by the use of β-glucuronidase. Since the rearranged glucuronides are resistant to β-glucuronidase treatment, this assay method may give misleading results. Thus, further knowledge of the stability of these glucuronides is needed in order to achieve correct quantitative results, for example, in drug analyses.

A number of studies have proved that acyl glucuronides are capable of reacting with proteins. *In vitro* studies by van Breemen and Fenselau [15] have shown that albumin is acylated by reaction with acyl glucuronides. Studies with the acyl glucuronide of bilirubin [16–18] gave the same results. *In vitro* and *in vivo* studies by Smith *et al.* [19] with zomepirac acyl glucuronide and its isomers proved that all these compounds are capable of reacting with proteins in plasma. The products thus formed may be of toxicological interest and it has been proposed by van Breemen and Fenselau [15] that lengthy exposure to acyl glucuronides may explain the toxicity of some drugs.

Different methods have been used to identify the structures of glucuronide isomers; among these are mass spectrometry and nuclear magnetic resonance spectrometry (NMR). Compernolle *et al.* [13] converted bilirubin glucuronide isomers into partially methylated alditol acetates and determined their structures by gas chromatography–mass spectrometry. In this way they determined the size of the ring of the glucuronic acid moiety and the position of the aglycone. Eggers and Doust [3] have used ^{13}C-NMR studies to confirm the rearrangement of the glucuronide of probenecid. Electron impact

mass spectrometry combined with gas chromatography has been used by Hignit *et al.* [20] on trimethylsilyl derivatives of clofibrate acyl glucuronide isomers. They claimed the structures to be α- and β-anomers with pyranose as well as furanose ring structures. Smith and Benet [21], using proton NMR, have confirmed that the four fractions which could be separated by HPLC were four positional isomers of the 1-*O*-acyl glucuronide of zomepirac.

In the present study a preparative chromatographic method is described for simultaneous separation of the α- and β-anomers of four positional isomers of the 1-*O*-acyl glucuronide of diflunisal. The formation of the ether glucuronide from the β-1-*O*-acyl glucuronide is reported for the first time. For the identification of the different isomers, various forms of two-dimensional NMR spectrometry were used. To establish the exact chemical shift of the different proton signals, simulation of one-dimensional spectra have been performed.

Materials and Methods

Chemicals

Diflunisal (purity >99.5%) was a gift from DUMEX Ltd. (Copenhagen, DK). β-Glucuronidase from bovine liver EC 3.2.1.31, containing 1.48×10^6 Fishman units g^{-1}, was obtained from Sigma (St. Louis, MO, USA); β-glucuronidase from *Escherichia coli*, containing 7.5×10^6 Fishman units ml^{-1}, was obtained from Boehringer (Mannheim, FRG). Amberlite XAD-2 (20–50 mesh) from Serva (Heidelberg, FRG) was purified by the method described by Dieterle *et al.* [22]. Deuterated solvents for NMR were purchased from SIC (Waltham, USA). Deuterium oxide was obtained from E. Merck (Darmstadt, FRG). As column-packing material, 5-μm Spherisorb Octyl obtained from Phase Sep (Norwalk, CT, USA) was used. All other chemicals were of analytical or HPLC grade.

The ester glucuronide of diflunisal was isolated from human urine as described elsewhere [10].

Chromatography

A Waters (Milford, MA, USA) HPLC pump, model 6000A, fitted with a Rheodyne (Cotati, CA, USA) 7125 loop injector and a Waters UV absorbance detector model 440 operated at 254 and 280 nm, for the analytical and preparative system, respectively, were used.

The analytical column (250 × 4.6 mm i.d.) and the preparative column (250 × 8.0 mm i.d.) were packed with 5-μm Spherisorb Octyl and operated at ambient temperature.

The mobile phase comprised methanol–40 mM potassium citrate (pH 3.6) (48:52, v/v) with 20 mM tetramethylammonium bromide added.

The flow rates were 1.0 and 4.0 ml min^{-1} for the analytical and the preparative system, respectively.

Preparation and isolation of the isomers

To prepare the isomers, 60 mg of the glucuronide was dissolved in 10 ml of 0.1 M potassium phosphate buffer (pH 8.0). The solution was incubated at 37°C until all the parent ester glucuronide had disappeared (about 90 min). The pH of the solution was then adjusted to 4.0 by addition of acetic acid, and the solution was freeze-dried.

The freeze-dried isomers were dissolved in 4 ml of the mobile phase and 1 ml of water was added. This solution was injected into the preparative HPLC system, 500 μl at a time; all fractions eluted from the column were cooled immediately in an ice-bath. The identical fractions from several runs were pooled and diluted with two parts of ice-water, and the pH was adjusted to 3.0 with acetic acid. The different isomers were isolated by pumping each fraction at a rate of 2 ml min^{-1} through a column packed with 40 ml of Amberlite XAD-2 using a peristaltic pump. The columns had previously been washed with 100 ml of methanol and equilibrated with 100 ml of methanol–acetic acid–water (10:2:88, v/v/v). After the adsorption phase, the columns were washed with 100 ml of the solvent mixture used for equilibration. The columns were then sucked dry and eluted with 100 ml of methanol. All these manipulations were carried out at 5°C. Practically all the methanol was then removed by evaporation under reduced pressure in a water-bath at not more than 40°C; the water and acetic acid remaining were removed by freeze-drying.

Preparation of samples for NMR studies

Each of the isolated isomers was dissolved in 2 ml of dry acetone and centrifuged at 18,000 **g** for 4 min in order to remove insoluble particles. Then, 50 μl of D_2O was added and the solution was evaporated to dryness under vacuum in a rotary evaporator in a water-bath at not more than 40°C. The rotary evaporator was fitted with a cold trap on the air-inlet in order to avoid contamination of the sample with moisture from the atmosphere. A further 2 ml of acetone and 50 μl of D_2O were added; again the solvents were removed by evaporation. Finally the sample was dissolved in 300 μl of deuterated acetone and transferred to a 5-mm i.d. NMR tube.

NMR-spectroscopy

All spectra were obtained using a Bruker (Rheinstetten, FRG) AM 250 spectrometer, operated at 250 MHz. Standard microprograms from the Bruker pulse program library were used for the two-dimensional (2D) COSY-spectra. 2D-spectra were obtained using 256 points in t_1, zerofilled to 1024 points prior to 2D-fourier transformation, and 4096 points in t_2 with a relaxation delay of 1 s. Four scans were acquired for each point in t_1, yielding an excellent signal-to-noise ratio for as little as 0.5 mg of the compound in question and resulting in a total acquisition time of about 45 min. Usually 2–4 mg of the compound was employed, allowing simultaneous assignment of both the α- and β-isomer of each positional isomer.

In one case a relayed COSY spectrum was performed since the H-2 and H-3 protons of the glucuronic acid moiety (see formula included in Fig. 1) were nearly coincident. The experiment was tuned for a coupling constant of 7 Hz, allowing magnetization to be transferred from H-5 via H-4 to H-3 yielding unequivocal assignment of H-2 and H-3, all in the glucuronic acid moiety of the molecule.

Resistance to cleavage by β-glucuronidase

One milligram of ester glucuronide was dissolved in 2.0 ml of 0.05 M potassium phosphate buffer (pH 8.0) and isomerized for 1½ h at 37°C. The pH was then adjusted to 5.0 by addition of phosphoric acid. The solution was assayed in the analytical HPLC-system before and after incubation of the solution for 2 h at 37°C with 100 μl of a solution containing 0.67 mg ml^{-1} of β-glucuronidase (bovine liver).

Results and Discussion

The chromatographic system

In order to find the best conditions for separation of the isomers, the dependency of k′ as a function of pH of the buffer used in the mobile phase was investigated (Fig. 2). The best conditions for simultaneous separation of all isomers appeared to be at pH 3.66. From preliminary studies it was expected that the peak pairs 2/3, 4/5 and 6/8 (Fig. 3) were α- and β-anomers of the same positional isomers. The pH of the buffer used in the mobile phase was therefore fixed at 3.6. At this pH value the peak pairs 2/3 and 4/5 were well separated (Fig. 3). This system was used for the preparative separation of the isomerization mixture. The yields of the separations were 0.50, 2.95, 1.93, 2.76 and 2.70 mg for fractions 1–5, respectively, and 2.02 mg for fraction 8.

As shown in Fig. 3 the isomers are eluted with the 4-*O*-acyl isomer first, then the 3-*O*-acyl isomer; and the 2-*O*-acyl isomer was eluted with the original 1-*O*-acyl glucuronide between the β-2-*O*- and the α-2-*O*-acyl isomer. Furthermore, it is seen that the β-anomer is always eluted before the α-anomer of the same positional isomer. The order of elution of the positional isomers presented in the present paper is in agreement with the data of Smith and Benet [21] with only one exception; they found that the parent glucuronide of zomepirac was eluted between the third and fourth positional isomer.

Preparation of samples for NMR studies

The protons of the alcohol groups in the glucuronic acid moiety appear in the preliminary NMR studies as large broad multiplets; these interfere with signals from the H-2, H-3 and H-4 protons in the glucuronic acid moiety. To avoid this, the protons of the alcohol groups were exchanged with deuterons by twice dissolving each sample in dry acetone and D_2O and evaporating the solution to dryness *in vacuo*. By this method it was impossible to obtain spectra of the pure α- and β-anomers owing to mutarotation. By performing all manipulations at 0–4°C after collecting the fractions from the HPLC-

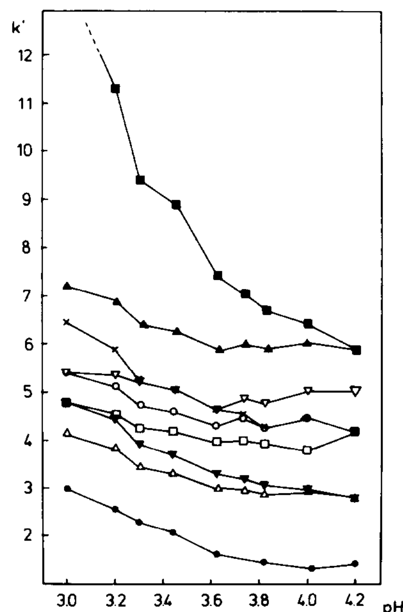

Figure 2
k′ values of the different isomers of diflunisal ester glucuronide as a function of pH. (●), Diflunisal ether glucuronide; (△), β-4-*O*-IDAG; (▼), α-4-*O*-IDAG; (□), β-3-*O*-IDAG; (○), α-3-*O*-IDAG; (▽), β-2-*O*-IDAG; (X), β-1-*O*-diflunisal acyl glucuronide (DAG); (▲), α-2-IDAG and (■), diflunisal.

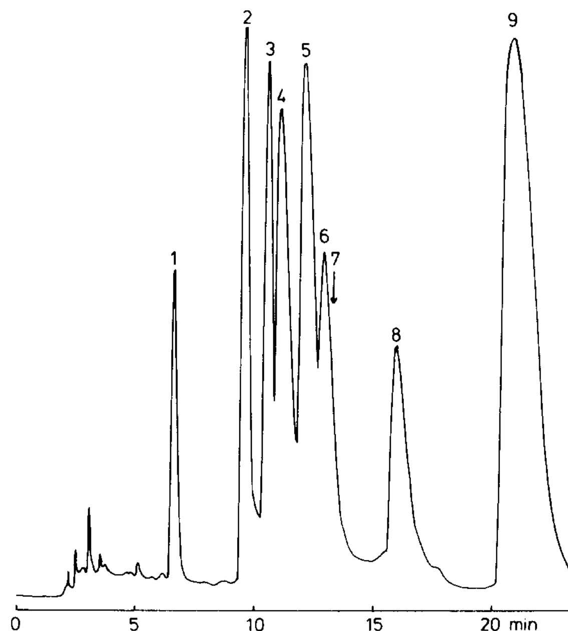

Figure 3
Chromatogram from a separation of a mixture of isomers of diflunisal ester glucuronide and diflunisal. (1) diflunisal ether glucuronide; (2) β-4-*O*-IDAG; (3) α-4-*O*-IDAG; (4) β-3-*O*-IDAG; (5) α-3-*O*-IDAG; (6) β-2-*O*-IDAG; (7) β-1-*O*-DAG; (8) α-2-IDAG and (9) diflunisal.

system, it was possible to obtain samples containing predominantly the α- or the β-anomer, present in the fraction at the time of collection; thus it was possible to assign the correct structure to each isomer.

Assignment of structures

In this study a two-dimensional COSY technique was used because it offered easily accessible information about the coupling pattern of the protons. In Fig. 1 a 2D-COSY spectrum of the protons in the glucuronic acid moiety of the isomer, isolated as fraction 5 (and partly mutarotated), is shown. In the spectrum a doublet at δ = 5.29 ppm on the diagonal from the lower left corner to the upper right corner is seen with a coupling constant of 3.5 Hz. A horizontal line through this signal shows that this proton is coupled with another proton at δ = 3.8 ppm on the diagonal. The latter signal is a double doublet with J = 3.5 and 9.6 Hz, respectively. On the vertical line through this signal, it is seen that this proton is coupled to a proton at δ = 5.59 ppm (triplet, J = 9.5 Hz) on the diagonal. This proton is coupled to another proton at δ = 4.03 ppm (triplet, J = 9.8 Hz), which is finally coupled with a proton at δ = 4.45 ppm (doublet, J = 9.6 Hz). This gives a sequence of five vicinally coupled protons. The signal at 5.29 ppm is assigned as an α-anomer proton since it is positioned at the lowest field and has a small coupling constant of 3.5 Hz which is characteristic for α-anomer protons of glucosides [23]. Table 1 shows the assignment of the chemical shift of the protons in the different isomers. The values (ppm) are given relative to internal $(CH_3)_2CO$, which was fixed at 2.05 ppm.

When comparing the different chemical shift values, it is seen that some of the signals have been shifted 1.1–1.8 ppm down-field relative to the parent glucuronide. Because

Table 1
Assignment of the chemical shift of protons in 1-, 2-, 3- and 4-*O*-acyl-glucuronic acid esters

Peak	Isomer	H-i	H-1	H-2	H-3	H-4	H-5
1	β-1-*O*-ether*	δi	5.171	3.6†	3.6†	3.6†	4.193
		J	7.7, d				9.5, d
2	β-4-*O*-acyl	δi	4.731 (1.3×10^{-3})	3.405 (1.6×10^{-3})	3.901 (1.3×10^{-3})	5.320 (1.3×10^{-3})	4.290 (1.3×10^{-3})
		$^3J_{i->i+1}$	7.7 (0.51)	9.2 (0.51)	9.7 (0.46)	11.8 (0.46)	
3	α-4-*O*-acyl	δi	5.288 (2×10^{-4})	3.607 (2×10^{-4})	4.122 (2×10^{-4})	5.319 (2×10^{-4})	4.629 (2×10^{-4})
		$^3J_{i->i+1}$	3.29 (0.08)	9.26 (0.08)	9.11 (0.08)	10.08 (0.08)	
4	β-3-*O*-acyl	δi	4.814 (1.1×10^{-3})	3.592 (1.1×10^{-3})	5.377 (1.1×10^{-3})	4.036 (1.9×10^{-3})	4.054 (1.9×10^{-3})
		$^3J_{i->i+1}$	7.7 (0.40)	9.5 (0.40)	10.9 (0.66)	6.8 (0.56)	
5	α-3-*O*-acyl	δi	5.299 (2×10^{-4})	3.809 (2×10^{-4})	5.597 (2×10^{-4})	4.030 (2×10^{-4})	4.449 (2×10^{-4})
		$^3J_{i->i+1}$	3.53 (0.06)	9.55 (0.06)	9.47 (0.06)	9.57 (0.06)	
6	β-2-*O*-acyl	δi	5.024 (9×10^{-4})	5.078 (9×10^{-4})	3.894 (9×10^{-4})	3.799 (9×10^{-4})	3.955 (9×10^{-4})
		$^3J_{i->i+1}$	7.5 (0.31)	9.7 (0.36)	9.3 (0.33)	10.3 (0.33)	
7	β-1-*O*-acyl*‡	δi	5.913	3.70	3.78	3.74	4.142
		J	7.6, d	t	t	t	8.60, d
8	α-2-*O*-acyl	δi	5.535 (4×10^{-4})	4.970 (4×10^{-4})	4.166 (4×10^{-4})	3.794 (4×10^{-4})	4.388 (4×10^{-4})
		$^3J_{i->i+1}$	3.6 (0.13)	9.6 (0.15)	9.1 (0.13)	9.8 (0.13)	

Concentrations in ppm relative to that of $(CH_3)_2CO$ (2.05 ppm). 0.5–2.0 mg isomer was dissolved in 300 μl $(CD_3)_2CO$ after removal of exchangeable protons. Numbers in parentheses: 95% confidence intervals in ppm or Hz. J = coupling constants in Hz, signal multiplicity: d, doublet; t, triplet.
*Values have not been simulated.
†Incompletely resolved multiplet.
‡The parent glucuronide.

esters produce a low field shift of the nearest proton, the aglycone in the isomer isolated as fraction 5 must be located at the third alcohol group in the glucuronic acid moiety. The other structures are assigned in the same way.

A one-dimensional proton spectrum of the protons in the glucuronic acid moiety of the α-3-*O*-acyl isomer isolated as fraction 5 and partly mutarotated to the β-3-*O*-acyl isomer is shown in Fig. 4. By performing all manipulations at a low temperature during sample clean-up, it is possible to obtain an NMR-spectrum of a partly mutarotated isomer in which either the α- or the β-form is the dominating anomer. From the integrated signals in Fig. 4 a ratio of 2.05 can be calculated between the H-2 protons at $\delta = 3.809$ ppm (the α-anomer) and $\delta = 3.592$ ppm (the β-anomer). From the peak areas in Fig. 5 a ratio of

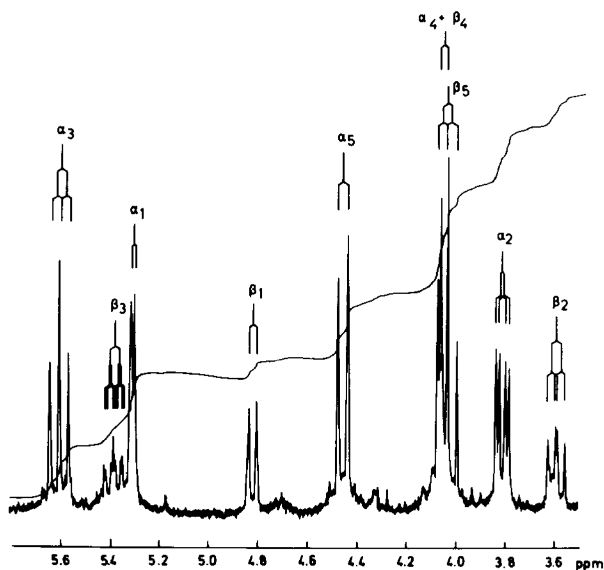

Figure 4
NMR-spectrum of the glucuronic acid moiety of 2.70 mg of the α- and β-3-*O*-IDAG isolated as fraction 5. The spectrum was obtained at 250 MHz in $(CD_3)_2CO$. Concentrations in ppm are relative to that of $(CH_3)_2CO$ (2.05 ppm).

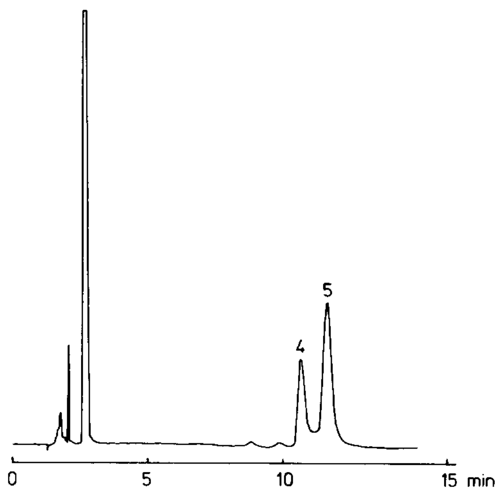

Figure 5
Chromatogram of fraction 5 prepared for NMR studies. The α-3-*O*-IDAG has partly mutarotated to the β-3-*O*-IDAG. The large peak at the front of the chromatogram is that of $(CD_3)_2CO$.

1.87 between peaks 4 and 5 can be found. Thus fraction 5 must be the α-isomer. The other α- and β-isomers were assigned in the same way.

In order to make a correct assignment of the proton signals from H-2 and H-3, in the parent glucuronide, it was necessary to use a relayed COSY technique, which made the assignment of the two proton signals possible (Table 1). Figures have been calculated using the MIMER software package [24]. There is excellent agreement between the calculated and the measured chemical shift values and coupling constants. These results agree with those of Smith and Benet [21]. In the present work, deuteroacetone has been used for all the isomers whereas Smith and Benet [21] used deuteropyridine for the fourth positional isomer and DMSO + trifluoro-acetic acid (TFA) for the other positional isomers. The effect of the different solvents was either a down-field shift of the signals in the order of 0.2–0.8 ppm when deuteropyridine was used or an up-field shift in the order of 0.1–0.5 ppm when DMSO-TFA was used, relative to the chemical shift presented in the present paper.

Time-dependent formation of isomers

The rate of the formation of the isomers has been studied. As seen in Fig. 6, at first the 2-*O*-acyl isomer (peaks 6 and 8) is formed rapidly, then the 3-*O*-acyl isomer and finally, after some delay, the 4-*O*-acyl isomer is formed. After reaching a maximum, the

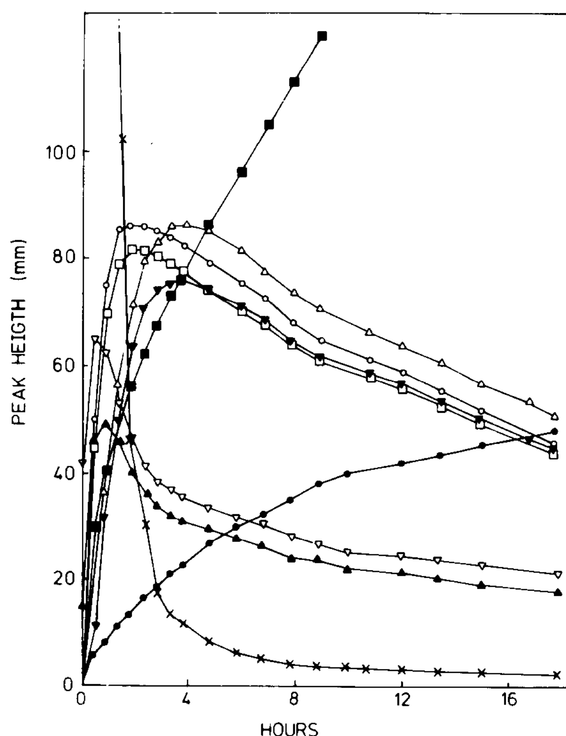

Figure 6
Time-dependent formation of the different isomers of Diflunisal ester glucuronide in potassium phosphate buffer (pH 8.0) at ambient temperature. (●), diflunisal ether glucuronide; (△), β-4-*O*-IDAG; (▼), α-4-*O*-IDAG; (□), β-3-*O*-IDAG; (O), α-3-*O*-IDAG; (▽), β-2-*O*-IDAG; (X), β-1-*O*-DAG; (▲), α-2-IDAG and (■), diflunisal.

contents of all isomers decline, except for peak 1 (the ether glucuronide). This effect is due to the higher stability of the ether glucuronide in alkaline solutions [10]. Gradually all isomers except the ether glucuronide will disappear.

Reversibility of the acyl migration

In order to confirm the reversibility of the rearrangement two different approaches were made. In the first attempt, 0.25 mg of fraction 2 (β-4-*O*-acyl isomer) was dissolved in 100 μl of 0.05 M potassium phosphate buffer (pH 8.0) and incubated at 37°C. Under these conditions the concentration of the 4-*O*-acyl isomer declined and the 3-*O*-acyl isomer was formed. After some delay the 2-*O*-acyl isomer and finally the β-1-*O*-acyl isomer were formed. In the second attempt, a solution of 1 mg ml^{-1} of the β-1-*O*-acyl glucuronide in 0.05 M potassium phosphate buffer (pH 8.0) was isomerized for 1 h (Fig. 7A). After reduction of the pH to 7.0, all the remaining ester glucuronide as well as the ether glucuronide that was formed were cleaved when incubating the solution for 1 h with 10 μl of β-glucuronidase (*Escherichia coli.*, *ca* 15,000 Fishman units) (Fig. 7B). The β-glucuronidase was then removed by ultrafiltration through a Centricon-10 filter (Amicon, Danves, MA, USA) and finally the pH was raised to 8.5. When this solution was assayed at intervals of 20 min, the concentration of the β-1-*O*-acyl glucuronide as well as that of the ether glucuronide increased slowly during 100 min to 0.32 and 0.72%, respectively, of the total amounts of the isomers in the solution (Fig. 7C) while the concentrations of the 4-, 3- and 2-*O*-acyl isomers slowly declined. After 100 min the pH was lowered to 7.0 and a further 10 μl of β-glucuronidase was added. When the solution was assayed after 1 h the formed ester and ether glucuronide had been cleaved (Fig. 7D). Thus there is strong evidence for a stepwise intramolecular rearrangement which is reversible to the original β-1-*O*-acyl glucuronide. The formation of a β-1-*O*-acyl glucuronide from its positional isomers has not been reported previously.

The formation of the ether glucuronide may be explained by the formation of a 1,2-ortho-ester of the α-2-*O*-acyl isomer (Fig. 8). This ortho-ester is then rearranged to the ether glucuronide. The formation of an ether glucuronide by transacylation from an ester glucuronide is a new and complicated factor in drug metabolism. However, the amount

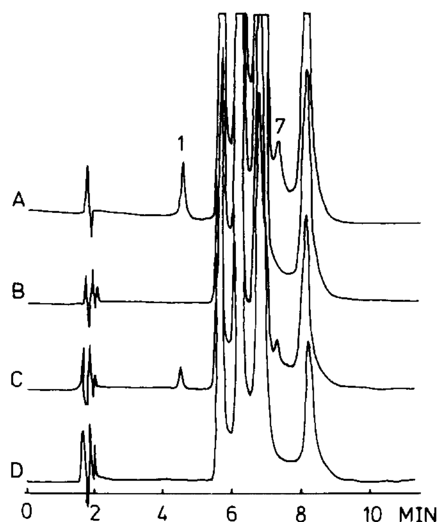

Figure 7
Formation of the ether glucuronide and the β-1-*O*-acyl glucuronide from the positional isomers of the β-1-*O*-acyl glucuronide. β-1-*O*-acyl glucuronide isomerized for 1 h at pH 8.0 (A). The solution was treated with β-glucuronidase (B). The β-glucuronidase was then removed by ultrafiltration and the solution was isomerized at pH 8.5 (C). The solution was treated with β-glucuronidase for 1 h (D). Peak assignment as in Fig. 3. pH of the mobile phase was 3.1.

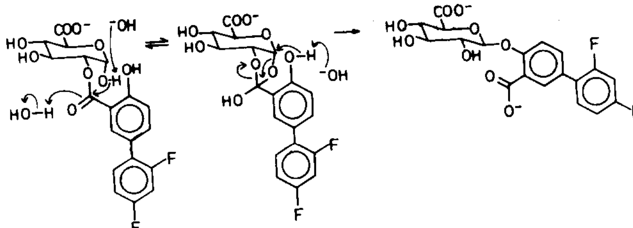

Figure 8
Possible mechanism for the formation of the ether glucuronide of diflunisal from DAG via a 1,2-ortho ester of α-2-*O*-IDAG in mildly alkaline solution.

of ether glucuronide formed is minor and is of little significance so long as the pH of the samples and the storage temperature are controlled carefully.

The significance of resistance to β-glucuronidase

The rearrangement of ester glucuronides to isomers, which is resistant to cleavage by β-glucuronidase, may be a problem in the assay of ester glucuronides because the traditional method involves determination of ester glucuronides as the liberated aglycone after cleavage with β-glucuronidase. By carefully controlling the pH of the samples at the time of collection, the problem may be reduced and purified ester glucuronides may even be used as reference compounds for a direct chromatographic assay of ester glucuronides in biological fluids [10].

The structures of the isomers found in the present study constitute a nearly complete list of the possible positional isomers and their respective anomers, in which the glucuronic acid has the pyranose structure. The α-1-*O*-acyl glucuronide and the α-1-*O*-ether glucuronide were not found. Neither was there any evidence for the furanose structure.

Acknowledgements — The NMR-facilities at the University of Copenhagen, Chemical Laboratory V, were donated by the Danish Science Research Council. The work by Claus Cornett was supported by the Danish Medical Research Council grant number 12-6049.

References

[1] J. Hasegawa, P. C. Smith and L. Z. Benet, *Drug Metab. Disp.* **10**, 469–473 (1982).
[2] K. A. Sinclair and J. Caldwell, *Biochem. Pharmacol.* **31**, 953–957 (1982).
[3] N. J. Eggers and K. Doust, *J. Pharm. Pharmacol.* **33**, 123–124 (1981).
[4] R. G. Dickinson, W. D. Hooper and M. J. Eadie, *Drug Metab. Disp.* **12**, 247–252 (1984).
[5] M. V. Marsh, J. Caldwell, T. P. Sloan, R. L. Smith, M. Horner and M. S. Moss, *Xenobiotica* **13**, 233–240 (1983).
[6] H. P. A. Illing and I. D. Wilson, *Biochem. Pharmacol.* **30**, 3381–3384 (1981).
[7] F. W. Janssen, S. K. Kirkman, C. Fenselau, M. Stogniew, B. R. Hofmann, E. M. Young and H. W. Ruelius, *Drug Metab. Disp.* **10**, 599–604 (1982).
[8] A. Rachmel, G. A. Hazelton, A. L. Yergey and D. J. Liberato, *Drug Metab. Disp.* **13**, 705–710 (1985).
[9] D. G. Musson, J. H. Lin, K. A. Lyon, D. J. Tocco and K. C. Yeh, *J. Chromatogr.* **337**, 363–378 (1985).
[10] J. Hansen-Møller, S. Honoré Hansen and L. Dalgaard, *J. Chromatogr.* **420**, 99–109 (1987).
[11] N. Blanckaert, F. Compernolle, P. Leroy, R. Van Houtte, J. Fevery and K. P. M. Heirwegh, *Biochem. J.* **171**, 203–214 (1978).
[12] E. M. Faed, *Drug Metab. Rev.* **15**, 1213–1249 (1984).
[13] F. Compernolle, G. P. Van Hees, N. Blackaert and K. P. M. Heirwegh, *Biochem. J.* **171**, 185–201 (1978).
[14] P. C. Smith, J. Hasegawa, P. N. J. Langendijk and L. Z. Benet, *Drug Metab. Disp.* **13**, 110–112 (1985).

[15] R. B. van Breemen and C. Fenselau, *Drug Metab. Disp.* **13**, 318–320 (1985).
[16] A. F. McDonagh, L. A. Palma, J. J. Lauff and T. W. Wu, *J. Clin. Invest.* **74**, 763–770 (1984).
[17] A. Gautam, H. Seligson, E. R. Gordon, D. Seligson and J. L. Boyer, *J. Clin. Invest.* **73**, 873–877 (1984).
[18] R. B. Van Breemen, C. Fenselau, W. Mogilevsky and G. B. Odell, *J. Chromatogr.* **383**, 387–392 (1986).
[19] P. C. Smith, A. F. McDonagh and L. Z. Benet, *J. Clin. Invest.* **77**, 934–939 (1986).
[20] C. E. Hignite, C. Tschanz, S. Lemons, H. Wiese, D. L. Azarnoff and D. H. Huffman, *Life Sci.* **28**, 2077–2081 (1981).
[21] P. C. Smith and L. Z. Benet, *Drug Metab. Disp.* **14**, 503–505 (1986).
[22] W. Dieterle, J. W. Faigle and H. Mory, *J. Chromatogr.* **162**, 27–34 (1979).
[23] K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa and Y. Tsuda, *Chem. Pharm. Bull.* **27**, 2661–2674 (1979).
[24] O. Manscher, K. Schaumburg and J. P. Jacobsen, *Acta Chem. Scand.* **A35**, 12–24 (1979).

[Received for review 31 July 1987]