272. C-Glucuronides, a Novel Type of Drug Metabolites

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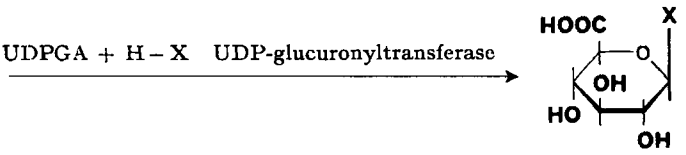
(1. IX. 75)

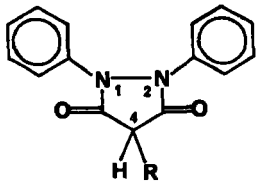
Summary. The structure elucidation of *C*-β-glucuronides of sulfinpyrazone (Anturan®) and phenylbutazone (Butazolidin®) by spectroscopic means is reported. These conjugates represent a novel type of drug metabolites.

Introduction. – Conjugation of exogenous and endogenous compounds with glucuronic acid is a dominating metabolic reaction in most mammalian species. The reaction is enzymatically catalyzed by UDP-glucuronyltransferase which transfers the glucuronic acid residue from uridine 5'-diphosphate glucuronic acid (UDPGA) to the substrate (HX). The resulting conjugates, D-glucopyranosiduronic acids, possess β-glycosidic configuration [1]:



The catalyzing enzyme is nonspecific with respect to the chemical structure of the substrate. To accomodate the new substituent within the substrate molecule, the presence of a functional group with a reactive proton, usually attached to a heteroatom, is, however, essential. The following functional groups are known to undergo glucuronidation: hydroxyl, carboxyl, amino, imino, carbamate, sulfonamide, thiol, and dithioyl. According to the nature of the linking atom the conjugates are classified as *O*-, *N*- or *S*-glucuronides [1] [2].

Recently, the existence of *C*-glucuronides as a novel additional class has become apparent in the course of drug metabolism studies performed in our laboratories [3]. It was found that the human organism converts sulfinpyrazone (I, Anturan®) and phenylbutazone (II, Butazolidin®), both derivatives of 1,2-diphenyl-3,5-dioxopyrazolidine, into conjugates in which C(1') of the glucuronic acid ligand is directly attached to C(4) of the pyrazolidine ring. This unexpected conjugation reaction taking place at a carbon rather than a heteroatom is readily rationalized in that the proton at C(4) in I and II, being part of a 1,3-dicarbonyl system, possesses fairly acidic properties. In a previous study pKa values as high as 2.8 and 4.5, respectively, had been determined for these compounds [4]. Such systems are well known to undergo *C*-alkylation in addition to, or instead of, *O*-alkylation under a variety of conditions in non-enzymatically controlled reactions.



Sulfinpyrazone (I): R = $-CH_2CH_2SOC_6H_5$
Phenylbutazone (II): R = $-CH_2CH_2CH_2CH_3$

In the following section of this paper the spectroscopic evidence relevant to the structure elucidation of these novel conjugates is presented in detail.

Results and Discussion. – The highly polar metabolites of I and II, suspected of being hydroxyl-containing carboxylates from their IR. spectra, were converted into methyl esters and methyl ester acetates to facilitate the spectroscopic analysis, initially limited to mass spectrometry and IR./UV. microtechniques by the small quantities available. These derivatives proved equally suitable for a complete structural characterization by NMR. techniques after efforts in preparation had been escalated. Structural assignments and pertinent spectroscopic data of the methyl ester acetates (III and IV, respectively) are given in the table along with structures and data of corresponding synthetic *O*-glucuronide derivatives (V and VI, respectively).

The mass spectra of the methyl esters of the metabolites gave (first) evidence for the presence of a hexuronic acid substituent in otherwise unaltered precursor molecules by exhibiting molecular ions at *m/e* 594 and 498, respectively. Conversion of these esters into the triacetates (III and IV), as had been found useful in the analysis of glucuronides [5], corroborated this finding by shifting the molecular ion peaks by 126 amu (introduction of 3 acetyl groups into the hexuronic acid residue) to *m/e* 720 and 624, respectively. Fragmentation patterns characteristic of acetylated methyl glycopyranoside uronate moieties [5] (*m/e* 317, $C_{13}H_{17}O_9$, together with secondary fragments at *m/e* 257, 215, 197, 155 and 127 due to losses of CH_3COOH and/or $CH_2{=}C{=}O$) were consistent with typical glucuronic acid conjugation, yet did not permit the exact characterization of the hexuronic acid itself. In agreement with the molecular ions, fragments at *m/e* 594 ($M^+_{III} - C_6H_5SOH$) and 278 ($594 - C_{13}H_{16}O_9$) as well as *m/e* 308 ($M^+_{IV} - C_{13}H_{16}O_9$) represented intact diphenyl-dioxo-pyrazolidine moieties in both cases, eliminating the possibility that additional biotransformations, *e.g.* aromatic hydroxylation, had occurred before conjugation. These fragments, together with *m/e* 183 ($C_6H_5\overset{+}{N}H{=}NC_6H_5$), practically rule out possible substitution at any of the aromatic rings. Fragments at *m/e* 568 ($M^+_{III} - C_6H_5SOCH{=}CH_2$) and *m/e* 265 ($M^+_{III} - C_{13}H_{16}O_9/C_3H_7$), together with pertinent metastable peaks, suggest furthermore the presence of unchanged *β*-(phenyl sulfinyl)-ethyl and *n*-butyl substituents, respectively. The spectra provide, however, no definite clue as to an attachment of the hexuronic acid substituent to C(4). In the case of glucuronides, this position represents the only remaining possibility after nonidentity with the synthetically prepared *O*-glucuronides V and VI had been established by the techniques employed (*cf.* table).

Positive evidence for substitution at C(4) was, however, deduced from the IR. and UV. spectra of III and IV. Thus, these spectra clearly show the presence of diphenyl-dioxopyrazolidine rings with intact 1,3-dicarbonyl system by exhibiting

Table. *Spectroscopic data of methyl ester acetates of metabolically formed* C-*glucuronides* (III, IV) *and synthetic* O-*glucuronides* (V, VI) *of sulfinpyrazone and phenylbutazone*

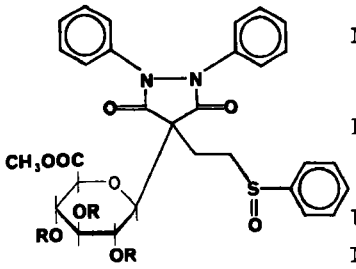
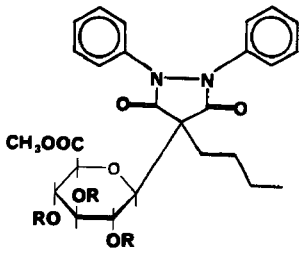
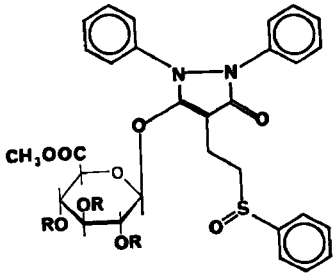
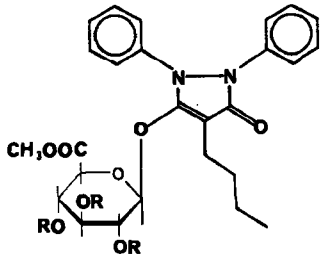
Structure	Relevant spectroscopic data
 III sulfinpyrazone metabolite, methyl ester acetate derivative (R = $COCH_3$).	MS. (*m/e* (%)): 720 (2), 660 (0,2), 594 (17), 568 (0,8), 534 (1), 317 (9), 279 (7), 278 (6), 257 (28), 215 (12), 197 (20), 183 (1), 155 (100), 127 (44), 126 (15), 78 (26), 77 (20), 43 (60). IR. (cm^{-1}, KBr): 1751/1724 (C=O; ester and 1,3-dicarbonyl-system), 1597, 1490, 752/746, 690 (phenyl), 1220 (C—O; ester), 1047/1036 (SO). UV. (MeOH): λ_{max} 237.5 nm; sh at 280 nm. NMR. ($CDCl_3$, 2%): 7,53 (*m*, C_6H_5SO); 7,1–7,4 (*m*, C_6H_5—N); 5,79 (*m*, $J_{1',2'} = 10$, $J_{2',3'} = 9$, H—C(2')); 5,0–5,35 (*m*, $J_{2',3'} = 9$, $J_{3',4'} = J_{4',5'} = 10$, H—C(3') and H—C(4')); 4,09 (*d*, $J_{1',2'} = 10$, H—C(1')); 3,88 (*m*, $J_{4',5'} = 10$, H—C(5')); 3,78 (*s*, CH_3O); 2,7–3,1 (*m*, H_2CSO); 2,1–2,4 (*m*, H_2C—C); 1,99 and 1,98 (2 *s*, $3H_3C$—C=O).
 IV phenylbutazone metabolite methyl ester acetate derivative (R = $COCH_3$).	MS. (*m/e* (%)): 624 (27), 564 (3), 403 (1), 317 (0,4), 308 (3), 265 (2), 257 (0,6), 215 (2), 197 (7), 183 (8), 155 (50), 127 (26), 77 (27), 43 (100). IR. (cm^{-1}, KBr): 1748 and 1718 (C=O; ester and 1,3-dicarbonylsystem), 1592, 1488, 755/735, 690 (phenyl), 1227 (C—O; ester). UV. (MeOH): λ_{max} 237,5 nm; sh at 280 nm. NMR. ($CDCl_3$, 2%): 7,1–7,5 (*m*, NC_6H_5); 5,87 (*q*, $J_{1',2'} = 10{,}2$, $J_{2',3'} = 9{,}0$, H—C(2')); 5,26 (*q*, $J_{2',3'} = 9{,}0$, $J_{3',4'} = 9{,}8$, H—C(3')); 5,16 (*t*, $J_{3',4'} = J_{4',5'} = 9{,}8$, H—C(4')); 4,15 (*d*, $J_{1',2'} = 10{,}2$, H—C(1')); 3,93 (*d*, $J_{4',5'} = 9{,}8$, H—C(5')); 3,59 (*s*, OCH_3); 2,00 (*s*, 3 H_3C—C=O); 1,7–2,1 (*m*, H_2C—C(4)); 1,0–1,5 (*m*, $CH_2CH_2CH_3$); 0,84 (*m*, CH_3).
 V synthetic sulfinpyrazone *O*-glucuronide, methyl ester acetate derivative (R = $COCH_3$).	MS. (*m/e* (%), measured above *m/e* 100): 720 (0,2), 594 (2), 317 (8), 278 (40), 257 (15), 252 (38), 215 (11), 197 (14), 183 (49), 155 (100), 127 (32). IR. (cm^{-1}, KBr): 1754 (C=O; ester), 1672 (C=O), 1634 (C=C), 1595, 1488, 755, 692 (phenyl), 1212 (C—O; ester), 1045/1031 (SO). UV. (MeOH): λ_{max} 242 nm; sh at 266 nm. NMR. ($CDCl_3$, 6%): 7,0–7,8 (*m*, C_6H_5); 5,75 (*d*, $J_{1',2'} = 7$, H—C(1')); 5,0–5,5 (*m*, H—C(2'), H—C(3') and H—C(4')); 4,60 (*m*, $J_{4',5'} = 10$, H—C(5')); 3,77 (*s*, OCH_3); 2,6–3,6 (*m*, CH_2CH_2); 2,05, 1,99 and 1,69 (3*s*, $3H_3C$—C=O).

Table continued

Structure	Relevant spectroscopic data
 VI synthetic phenylbutazone *O*-glucuronide, methyl ester acetate derivative (R = $COCH_3$).	MS. (*m/e* (%)): 624 (0,4), 564 (1), 403 (0,02), 317 (6), 308 (41), 257 (7), 252 (5), 215 (5), 197 (17), 183 (66), 155 (67), 127 (33), 77 (62), 43 (100). IR. (cm^{-1}, KBr): 1754 (C=O; ester), 1675 (C=O), 1634 (C=C), 1595, 1486, 757, 693 (phenyl), 1220 (C—O; ester). UV. (MeOH): λ_{max} 244 nm; sh at 265 nm. NMR. ($CDCl_3$, 5%): 7,0–7,5 (*m*, NC_6H_5); 5,0–5,4 (*m*, H—C(1′), H—C(2′), H—C(3′) and H—C(4′)); 4,10 (*m*, H—C(5′)); 3,75 (*s*, OCH_3); 2,35 (*m*, H_2C—C(4)); 2,02, 198 and 1,73 (3*s*, $3H_3C$—C=O); 1,2–1,8 (*m*, $CH_2CH_2CH_3$); 0,93 (*m*, CH_3).

pairs of carbonyl bands at 1751/1724 cm^{-1} (III) and 1748/1718 cm^{-1} (IV) in the IR., and by exhibiting UV. absorption in both cases at λ_{max}= 237,5 nm with shoulders at 280 nm. These absorptions remain unchanged upon addition of alkali or acid. Similar IR. and UV. absorptions, which were unaffected by changes in pH, had been found characteristic of derivatives of I and II in which C(4) was di- rather than monosubstituted[1]). In contrast to this, in the IR. spectra of the *O*-substituted isomers V and VI the pairs of carbonyl bands are replaced by single carbonyl absorptions at 1672 and 1675 cm^{-1}, respectively, and moreover by ν(C=C) frequencies at 1634 cm^{-1}. In the UV. spectra the maxima have shifted to 242 nm (V) and 244 nm (VI), and the shoulders, with an increase in intensity, to 266 and 265 nm, respectively. These absorptions had been found to be characteristic of *O*-substituted enolic diphenyl-dioxo-pyrazolidine derivatives[1]).

The NMR. spectra of III and IV, correlate well with the compositions arrived at by mass spectrometry, confirm the C–C linkage to C(4) between the pyrazolidine and the hexuronic acid moieties inferred from IR. and UV., and, moreover, establish the nature and configuration of the uronic acid. Hence, the latter is definitely identified as glucuronic acid, a fact of special relevance in this context, since the customary identification of this ligand as a free acid after acidic or enzymatic cleavage is precluded in this case due to the stability of the C(1′)–C(4) linkage towards hydrolysis [3]. The compositions of III and IV follow from a straightforward interpretation of the NMR. spectra, as being those of 1:1 conjugates of the pyrazolidines with glucoronic acid. The nature of their linkage can be derived by comparison of the spectra of III and IV with those of V and VI. *C*-substitution is borne out by the chemical shifts of the proton H–C(1′), being 4,09 ppm (III) and 4,15 ppm (IV), as opposed to 5,75 ppm (V) and 5,0–5,4 ppm (VI) since *O*-substitution results in absorption at lower fields. *O*-substitution at C(1′) is excluded furthermore by the chemical shift of the α-methylene group of the other C(4) substituent, since its attachment to a sp^2 carbon atom as in VI leads to a downfield shift (δ = 2,35 ppm) compared to attachment to a sp^3 carbon atom such as in IV (δ = 1,7–2,1 ppm). *C*-substitution is supported additionally by the large coupling constant of the proton H–C(1′) ($J_{1,2}$ = 10

[1]) Unpublished results from our laboratories (*K.O.A.*)

Hz), since *O*-substitution reduces the vicinal coupling constant due to substituent electronegativity [6]: $J_{1,2}$ in both *O*-glucuronides V and VI amounts to only 7 Hz. In the case of V this coupling constant is obtained directly from the spectrum since the signal of the proton H–C(1′) is well separated from those of the protons H–C(2′) to H–C(4′). In the case of VI, the signal of the proton H–C(1′) is hidden under the absorptions of the other protons, but can be separated by the addition of the shift reagent Eu(fod)$_3$. In accordance with the mass spectrometric results the integration of the aromatic part of the spectra rules out glucuronyl substitution at one of the aromatic rings, *e.g.* after preceding metabolic functionalization, since 15 (III) and 10 (IV) aromatic protons are observed. The C(1′)–C(4) linkage inferred above is, moreover, evidenced in an unusual downfield shift of the proton H–C(2′) (δ = 5,87 in IV), since only a *C*-glucuronide of this substitution type allows the close proximity of this proton to one of the carbonyl groups of the pyrazolidine ring required to explain this effect. In contrast to this, the carbonyl group in the *O*-glucuronides is far removed from this particular proton.

The remaining uncertainty as to the nature and configuration of the hexuronic acid residue can be resolved by inspection of the vicinal coupling constants of its ring protons. These coupling constants are obtained directly from the 100 MHz spectra in spite of the fact that the spectra show a certain degree of second order character. The spectrum of IV, measured at 270 MHz, was additionally available as a pure first order spectrum. The analysis of the spectra is based on the irradiation of the proton H–C(2′), yielding the assignments of the other ring protons. The vicinal coupling constants thereby obtained are all in the order of 10 Hz (*cf.* table), and thus define the configuration of the six-membered ring: all substituents occupy equatorial positions. Both metabolites, therefore, are β-glucuronides.

By combined application of IR., UV., NMR. and mass spectroscopy, a *C*-β-glucuronide structure has been established for the derivatives III and IV, and hence also for the original metabolites. To our knowledge, these metabolites represent the first examples of *C*-glucuronide formation encountered in drug metabolism. In one instance, a synthetic *C*-glucuronide has been proposed as a by-product in the preparation of steroid conjugates [7]. As natural products, the rather widespread occurrence of *C*-glucosyl derivatives in plants is of special interest in this context [8].

Experimental

The isolation and the preparation of derivatives of the metabolites have already been described elsewhere [3]. The *O*-glucuronides V and VI, which served as reference compounds, were prepared according to the *Koenigs-Knorr* method.

The spectroscopic analyses were performed using the following instrumentation. MS.: *Varian* MAT CH 5 mass spectrometer linked to a *Varian* data system SS 100 (ion source temperature 200°, ionizing energy 70 eV, direct sample insertion); IR.: *Perkin-Elmer* 221 spectrometer equipped with NaCl prism; UV.: *Beckman* DK2A and ACTA-V spectrometer; NMR.: *Varian* HA 100 (MHz) and *Bruker* HX 270 (MHz) spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane (= 0 ppm) as internal standard, coupling constants J in Hz.

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273. Umcyclopropanisierung bei der Tetrabromierung eines tricyclischen Ketons zu 3*exo*, 4*endo*, 6*exo*-Tribrom-7-brommethyl-1,5-dimethyl-tricyclo[3.2.1.$0^{2,7}$]octan-8-on.

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Transcyclopropanation during the Tetrabromination of a Tricyclic Ketone to 3*exo*, 4*endo*, 6*exo*-Tribromo-7-bromomethyl-1,5-dimethyl-tricyclo[3.2.1.$0^{2,7}$]octan-8-one. *Summary.* Bromination of the tricyclic ketone **1** with an excess of bromine at low temperature gives in approximately 30% yield the highly crystalline tricyclic tetrabromide **2** (*Scheme 1*). The structure of **2** was established by NMR.- and especially X-ray-analysis (Fig. 1).

Treatment of **1** with 1 mol-equ. of bromine gives an unstable dibromide, to which the structure **3** was assigned on the basis of its NMR.-spectrum and its further bromination to **2** (*Scheme 1*). In the course of the tetrabromination of **1** the original cyclopropane ring is opened in the first step (**1** → **3**) and another cyclopropane ring is formed in the second step (**3** → **2**) (*cf. Scheme 3*).

Bei der Umsetzung von 1,5-Dimethyl-6-methyliden-tricyclo[3.2.1.$0^{2,7}$]oct-3-en-8-on[2]) (**1**) in Methylenchlorid mit 2,2 mol Brom bei −78° resultierten nach Umkristallisation in ca. 28% Ausbeute farblose Kristalle einer Verbindung **2** vom Smp. 181,8–182,3° der Formel $C_{11}H_{12}Br_4O$. (Bei der Ausführung der Reaktion in CCl_4 bei 0 bis 20° resultierte das Tetrabromid in ca. 10% Ausbeute.) Dieser Verbindung kommt aufgrund spektraler Daten, namentlich des NMR.-Spektrums sowie einer Röntgenkristallstrukturanalyse die Struktur des 3*exo*,4*endo*,6*exo*-Tribrom-7-brommethyl-1,5-dimethyl-tricyclo[3.2.1.0.^{2,7}]octan-8-on (**2**) zu. Das IR.-Spektrum (KBr) zeigt eine 5-Ring-Keton-Bande bei 1751 cm^{-1} (Absorption von **1** bei 1745 cm^{-1} (Film)). Im Massenspektrum werden Molekularionenpike bei *m/e* 484, 482, 480, 478

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[2]) Korrekt wäre die Verbindung als 5,7-Dimethyl-8-methyliden-tricyclo[3.2.1.$0^{2,7}$]oct-3-en-6-on zu bezeichnen.