SYNTHESIS AND PROPERTIES OF $N(_7)$-β-D-GLUCOFURANOURONOSIDES OF THEOPHYLLIN AND 3-ISOBUTYL-1-METHYLXANTHINE

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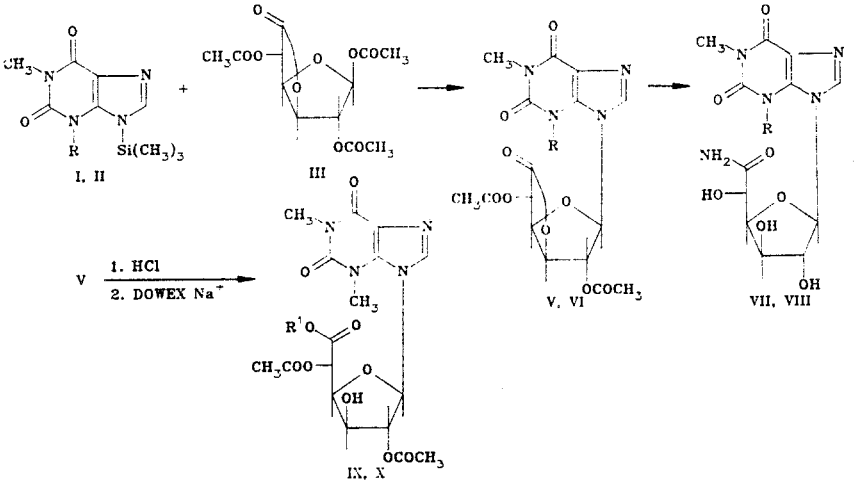
Condensation of the trimethylsilyl derivatives of theophyllin and 3-isobutyl-1-methylxanthine with β-D-glucofuranourono-6,3-lactone in the presence of trimethylsilyl trifluoromethanesulfonate has given $N(_7)$-β-D-glucofuranouronosides. Their hydrolytic stability has been examined at pH 1.4 and 7.4.

We have previously reported [1] that the regiodirectivity of the glycosylation of silylated xanthines and N^2-acetylguanine with 1,2,5-tri-O-acetyl-β-D-glucofuranourono-6,3-lactone (III) in the presence of trimethylsilyl trifluoromethanesulfonate (IV) differs from that seen in the ribosylation of these purines with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose [2].

In a further study of the positional directivity of glycosylation, it was of interest to react the lactone (III) with silylated 1,3-dialkylxanthines (1,3-dimethyl- and 3-isobutyl-1-methylxanthine). It has previously been shown repeatedly that theophyllin is glycosylated by a variety of methods at $N(_7)$ only [2-5].

Condensation of the trimethylsilyl derivative of theophyllin (I) and the lactone (III) in the presence of the condensing agent (IV) (molar ratio I:III:IV = 1.0:0.9:1.2) in 1,2-dichloroethane at 35°C for 16 h gave, as expected, the $N(_7)$-glucuronide of theophyllin (V) as the sole product. Under similar conditions, the silylated 3-isobutyl-1-methylxanthine (II) gave the lactone (VI).

Compounds (V) and (VI) were deacetylated by treatment with methanolic HCl, and treated without isolation with saturated ammonia in methanol to give the amides (VII) and (VIII).



I, V, VII, R=CH_3; II, VI, VIII R=i-C_4H_9; IX R^1=H; X R^1=Na

Treatment of the lactone (V) with 0.01 N hydrochloric acid resulted in cleavage of the lactone ring with the formation of the acetylated acid (IX), which was converted without isolation into its sodium salt (X).

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TABLE 1. PMR Spectral Parameters of (V-VIII) and (X)

Compound	Chemical shifts, δ ppm							J values, Hz			
	8-H (s)	1′-H (d)	2′-H (d)	3′-H	4′-H (d.d)	5′-H (d)	other protons	1′2′	2′3′	3′4′	4′5′
V	8,09	6,50	5,80	5,26d.d′	5,23	5,92	2,14 s (3H, OCH_3); 2,17 s (3H, OCH_3); 3,23 s (3H, CH_3); 3,45 s (3H, CH_3)	2,9	0,5	3,6	5,2
VI	8,06	6,49	5,79	5,24d.d	5,21	5,91	0,86 d (6H, $2CH_3$); 2,14 s (3H, OCH_3); 2,16 s (3H, OCH_3); 3,23 s (3H, CH_3); 3,81 d (2H, CH_2)	2,8	0,5	2,6	3,6
VII	8,26	6,07	4,20	4,03d	4,29	4,23	3,24 s (3H, CH_3); 3,44 s (3H, CH_3)	0,5	1,5	3,1	7,4
VIII	8,25	6,07	4,22	4,06d	4,26	4,31	0,88 m (6H, $2CH_3$); 2,18 m (1H, CH); 3,22 s (3H, CH_3); 2,80 d (2H, CH_2)	0,5	1,8	3,0	8,1
X	8,35	6,25	5,05	4,16d	4,40	5,18	2,04 s (3H, OCH_3); 2,08 s (3H, OCH_3); 3,22 s (3H, CH_3); 3,45 s (3H, CH_3)	1,8	1,8	4,4	1,3

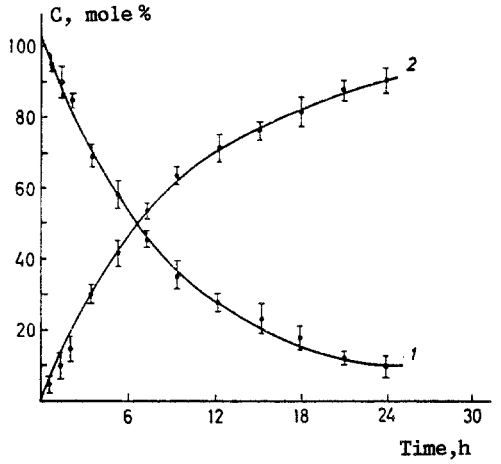

Fig. 1. Hydrolysis of the lactone (V) at pH 1.4 and 37°C. 1) Concentration of lactone (V); 2) concentration of acid (IX).

The structures of (V-VIII) and (X) were shown by their UV, IR, and PMR spectra (Table 1).

Comparison of the UV spectra of the glucuronides (V-VIII) and (X) at pH 1.7 and 13 (λ_{max} 274-275 nm) with those of $N(_7)$-glycolsylated theophyllins (λ_{max} 274 nm) [3, 4] confirmed that the carbohydrate moiety added to $N(_7)$ of the purine ring.

In the IR spectra of the lactones (V) and (VI), absorption is present at 1595-1610 (purine ring C=N), 1800-1810 (γ-lactone C=O), and 1750-1760 cm^{-1} (acetate C=O). On conversion to the amides (VII) and (VIII) the acetyl group and γ-lactone absorption disappears. Characteristic absorption is shown by the nucleoside (X) for the carboxylate anion at 1610 cm^{-1}.

The coupling constants of the protons of the carbohydrate moiety of the molecule in (V) and (VI) are virtually identical with those of the corresponding glucuronides of xanthine and guanine [1], and indicate the β-D-xylo-configuration for these nucleosides [6].

The hydrolytic stability of the glucoside bond in nucleosides, and especially their analogs, has a major effect on their physiological activity. Hydrolysis of N-glucofuranouronic acid nucleosides of the purine series has not been examined. We have studied the stability of the lactone (V) at 37°C in buffer solutions at 1.4 and 7.4, which are approximately the values found for the acidity of the stomach and blood. The progress of hydrolysis was followed by HPLC. At pH 1.4 (Fig.1), fission of the lactone ring occurs with formation of the acetylated acid (IX). After incubation for 24 h, 8% of the original lactone remained in solution. Fission of the glycoside bond and formation of the free heterocyclic base was not observed. At pH 7.4 (Fig. 2), simultaneous fission of the lactone ring and hydrolysis of the glycoside bond of the lactone (V) occurred. After 25 min, complete cleavage of the lactone ring occurred, and the percentage amounts of the acid (IX) and theophyllin in solution did not increase. This shows that the glycoside bond is cleaved only in the lactone, and is stable in the acid formed.

In its hydrolytic stability, the N-glucoside bond in the lactone (V) is very different from that in purine ribofuranosides. As compared with pyrimidine riboculeosides, the purine compounds are readily hydrolyzed in acid media. Further, replacement of the amino-group in adenosine and guanosine by the oxo-function, and introduction of alkyl groups into the purine nucleus in the 3- and 7-positions is known to result in a decrease in the stability of the glycoside bond on acid hydrolysis. For example, 50% cleavage of the glycoside bond in $N(_3)$- and $N(_7)$-methyladenosine 5'-phosphates at pH 7 and 37°C requires 1.5 h [7], but no cleavage of the glycoside bond in the acid (IX) was found even after incubation for 24 h in the buffer solutions (pH 1.4 and 7.4) at 37°C.

It is difficult to make any rational suggestions as to the reason for such a marked effect of the lactone ring on the hydrolytic stability of the lactone (V) in weakly basic media. It may only be noted that we have observed similar behavior in the hydrolysis of 1-(5-fluorouracil-1-yl)-2,5-di-O-acetyl-β-D-glucofuranourono-6,3-lactone [8].

The antitumor activity of (V) and (VI) was found to be insufficient to merit further study.

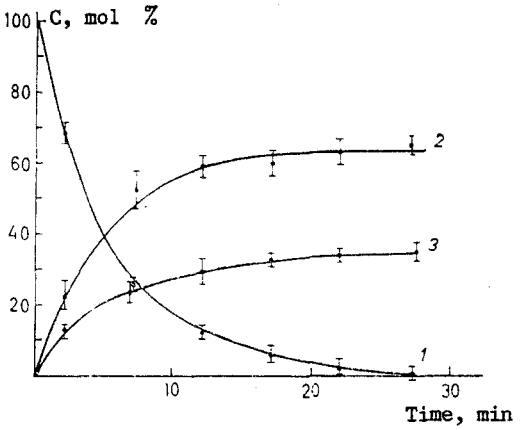

Fig. 2. Hydrolysis of lactone (V) at pH 7.4 and 37°C. 1) Concentration of lactone (V); 2) concentration of acid (IX); 3) concentration of theophyllin.

EXPERIMENTAL

IR spectra were obtained on a Perkin-Elmer 257 spectrometer (in petroleum jelly), and UV spectra on a Ultraspec 4050 in 0.1 N aqueous HCl buffer solution (pH 7.0) (Fluka), 0.1 N NaOH and methanol. PMR spectra were recorded on Bruker WM-90 (compounds (V) and (VI)) and Bruker WM-360 (VII, VIII, X) instruments in DMSO-d_6. The internal standard was TMS, and assignment of the resonance signals made by double resonance. The specific rotation was measured on a Perkin-Elmer 241 spectropolarimeter.

The reactions were followed and the homogeneity of the products checked by TLC on Silufol UV-254 plates in the systems: chloroform–methanol, 9:1 (A) and chloroform–methanol–water, 20:10:1 (B). The chromatograms were developed by spraying with a 1:1 mixture of 0.2% ethanolic solution of naphthoresorcinol and dilute (1:10) phosphoric acid, followed by heating for 15 min at 110-120°C. Column chromatography was carried out using an LKB column (2.5 × 60 cm) packed with silica gel L 100/250 (Czech SSR). HPLC was carried out on a DuPont instrument with a 4.6 × 250 mm column packed with Zorbax ODS (6 μm) as sorbent, UV detector (λ 254 nm), eluent acetone–orthophosphoric acid–water, 30:0.1:70. The eluent flow rate was 2 ml/min. The retention time of the lactone (V) was 4.0 min, acid (IX) 2.2 min, and theophyllin 1.2 min. Hydrolysis of the lactone (V) in a concentration of 10^{-3} M was carried out in the buffer solutions H_3BO_3–KCl–Na_2CO_3 (pH 7.4) and KCl–HCl (pH 1.4) heated to 37°C in a Reacti Vials thermostat (USA), with periodic withdrawal of samples for HPLC. The elemental analyses for C, H, and N were in agreement with the calculated values.

1-(Theophyllin-7-yl)-2,5-di-O-acetyl-β-D-glucofuranouron-6,3-lactone (V, $C_{17}H_{18}N_4O_9$). A mixture of 1.0 g (5.59 mmole) of theophyllin and 30 ml of hexamethyldisilazane was boiled until solution was complete, and evaporated to dryness and again evaporated with p-xylene. The residue was dissolved in 50 ml of 1,2-dichloroethane, 1.61 g (5.32 mmole) of the lactone (III) and 1.41 g (1.14 ml; 6.39 mmole) of trimethylsilyl trifluoromethane-sulfonate added with stirring, and the mixture kept for 16 h at 35°C. After cooling to 20°C, the mixture was poured with vigorous stirring into a suspension of sodium hydrogen carbonate (10 g) in 500 ml of chloroform. The suspension was stirred for 30 min, and the solid filtered off, washed with chloroform (2 × 200 ml), and the filtrates combined and evaporated under reduced pressure. The residue was dissolved in the minimum volume of chloroform, applied to a column of silica gel (100 cm^3) in chloroform, and eluted successively with chloroform (500 ml) and a mixture of chloroform and ethanol (200:1). The fractions containing the lactone (V) were evaporated to dryness under reduced pressure. The yield of analytically pure lactone (V) was 1.88 g (80%), as a foam. R_f 0.59 (system A); $[\alpha]_D^{20}$ +99.3° (c = 0.59; DMF), UV spectrum (methanol), λ_{max} (log ε): 274 nm (3.86).

1-(3-Isobutyl-1-methylxanthin-7-yl)-2,5-di-O-acetyl-β-D-glucofuranouron-6,3-lactone (VI, $C_{20}H_{24}N_4O_9$) was obtained and isolated as for (V), from 3-isobutyl-1-methylxanthine and the lactone (III). Yield 87%, mp 160-162°C, R_f 0.87 (system A); $[\alpha]_D^{20}$ +98.7° (c = 0.59; DMF). UV spectrum (methanol), λ_{max} (log ε): 275 nm (3.95).

1-(Theophyllin-1-yl)-β-D-glucofuranouronamide Hemihydrate (VII, $C_{13}H_{17}N_5O_7 \times 1/2H_2O$). To a solution of 1.0 g (2.36 mmole) of the lactone (V) in methanol (50 ml) was added 3 ml

of a 3% methanolic solution of hydrogen chloride, and the mixture kept at 20°C for 72 h. The mixture was then evaporated under reduced pressure, and the residue evaporated with methanol until all the hydrogen chloride had been removed. The residue was dissolved in a solution of ammonia in methanol, saturated at 0°C (30 ml), and the mixture kept at 20°C for 24 h and evaporated. Yield of the amide (VII) 0.61 g (72%). Mp 139-141°C (from ethanol–hexane); R_f 0.50 (system B); $[\alpha]_D^{20}$ +47.9° (c = 0.46, water). UV spectrum, λ_{max} (log ε), pH 1: 274 nm (3.81); pH 7: 274 nm (3.82); pH 13: 274 nm (3.88).

1-(3-Isobutyl-1-methylxanthin-7-yl)-β-D-glucofuranouronamide (VII, $C_{16}H_{23}N_5O_7$) was obtained as for the amide (VII), from the lactone (VI). Yield 63%, mp 110-111°C (from ethanol–hexane); R_f 0.61 (system B); $[\alpha]_D^{20}$ +26.8° (c = 0.49, water). UV spectrum, λ_{max} (log ε), pH 1: 275 nm (3.76); pH 7: 275 nm (3.78); pH 13: 275 nm (3.76).

Sodium 1-(Theophyllin-7-yl)-2,5-di-O-acetyl-β-D-glucofuranouronate Dihydrate (X, $C_{17}H_{19}N_4NaO_{10}\cdot 2H_2O$). The lactone (V) (4.22 g; 10.0 mmole) was dissolved with stirring in 0.01 N HCl, heated for 24 h at 37°C, then evaporated to dryness under reduced pressure. The residue was evaporated with ethanol until all the hydrogen chloride had been removed, then dissolved in 10 ml of water and applied to a column of Dowex AG 50 × 8 (Na^+, 150 cm^3). Elution was carried out with water, the fractions containing (X) being collected and evaporated to dryness. Yield 2.17 g (47%), mp 187-192°C (from methanol–ether); R_f 0.42 (system B); $[\alpha]_D^{20}$ +100.0° (c = 0.48, DMF). UV spectrum, λ_{max} (log ε), pH 1: 275 nm (3.92); pH 7: 275 nm (3.92; pH 13: 274 nm (3.92).

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