SYNTHESIS OF 7-O-β-D-GLUCOPYRANOSIDES OF ISOFLAVONES AND THEIR HETEROCYCLIC ANALOGUES

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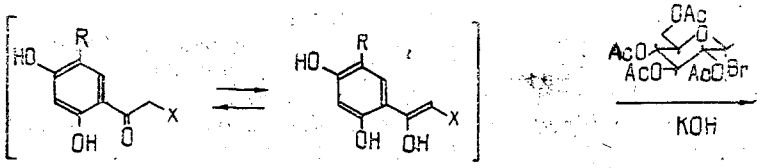
The products of the interaction of α-acetobromoglucose with 2,4,5-trihydroxyphenyl benzyl ketone and 2,4-dihydroxyphenyl 4'-hydroxybenzyl ketone and also with their heterocyclic analogues, which are present completely or partially in the enolic form, have been investigated. It has been shown that in this reaction a tetra-O-acetylglucosyl residue is added at the 4-OH hydroxy group of the phenyl residue of the ketone. The compounds obtained have been converted into isoflavone 7-O-glucosides by the action of the Vilsmeier reagent or acetoformic anhydride.

Isoflavone glycosides are natural compounds with a broad spectrum of action on human and animal organisms [3]. While possessing high hypolipidemic, analeptic, antioxidant, and hypoglycemic [4] activities, they are characterized by a low toxicity ($LD_{50} \geq 5000$ mg/kg), which makes their pharmacological study promising.

In a number of biological tests, heterocyclic analogues of the isoflavones (3-hetarylchromones) are superior to the natural isoflavones [3]. It may be surmised that with respect to the magnitude of their pharmacological action on the animal organism, O-glycosides of 3-hetarylchromones will be superior to the isoflavones and their glycosides.

The development of the chemistry and pharmacology of isoflavone glycosides and their heterocyclic analogues is being hindered by the difficulty of obtaining these substances. The set of natural compounds of this type is limited, and the biological action of the most common of them has been well studied.

The present work is a continuation of a study of methods for the directed chemical synthesis of flavonoid O-glycosides [1, 2]. The optimum scheme for the synthesis of these compounds is the glycosylation of 2,4-dihydroxyphenyl benzyl ketones with subsequent transformation of the products obtained into isoflavone 7-O-glycosides [1]. However, in the case of the 2,4,6-trihydroxyphenyl benzyl ketones this scheme proves to be unsuitable. We were interested in how, under these proposed conditions for the synthesis of O-glycosides, polyhydroxyketones of different types and, in particular, 2,4,5-trihydroxyphenyl benzyl ketone (1a) and 2,4-dihydroxyphenyl 4'-hydroxybenzyl ketone (1b), and also 2,4-dihydroxyphenyl benzyl ketones that exist completely or partially in the enolic form behave. As the latter we selected 2,4-dihydroxy-α-(2-pyridyl)acetophenone (1c), 2,4-dihydroxy-α-(4-methyl-2-thiazolyl)acetophenone (1d), and 2,4-dihydroxy-α-(2-quinolyl)acetophenone (1e). (When present in polar solvents, compound (1c) in the equilibrium state is 40% enolized [5] and (1d) 50% [6], while (1e) is more than 99% in the enolic form [7].)



1a. R=OH; 1b-e: R=H

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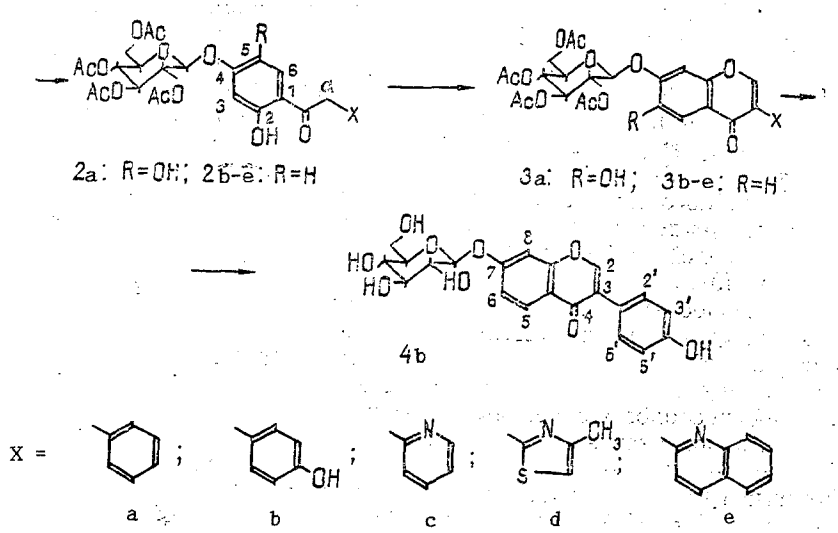

As in the preceding investigations of the glycosylation of hydroxyphenyl ketones [1, 2], the main difficulty in performing the individual experiments was the production of concentrated solutions of the potassium salts of the polyhydroxy ketones (1a-e). In the case of ketones (1a, b, and d) we used water–acetone as the solvent. For the glycosylation of ketones (1b and e), the bulk of their potassium salts were present in the reaction mixtures in the form of suspensions even in the water–acetone–dimethylformamide system.

The results obtained on the synthesis of glycosides (2a-e) (Table 1) can be interpreted in the following way. In spite of the presence in the molecules of the initial ketones of three potentially reactive hydroxy groups, for various reasons glycosylation always took place predominantly at the 4-OH hydroxy group. Most interesting, from our point of view, are the results of the reaction of acetobromoglucose with the potassium salt of the 2,4,5-trihydroxy ketone (1a). On the interaction of these two compounds, only one new compound was recorded that absorbed UV light on a chromatogram (R_f 0.7, eluent a; see the Experimental part) and gave a green coloration with iron(III) chloride. The other compounds (R_f ~ 0.4 and 0.3) present as impurities in small amounts (<10%) and giving the same coloration with $FeCl_3$ can be interpreted as partially deacetylated derivatives of glycoside (2a). Since the purification of the oily compounds (2a) from products of the degradation of acetobromoglucose (R_f ~ 0.73) proved to be difficult, it was converted into the corresponding chromone O-glucoside (3a) by the action of the Vilsmier complex in the presence of boron trifluoride etherate [2]. The PMR spectra obtained for compound (3a) and their comparison with the PMR spectra of the aglycon (6, 7-dihydroxyisoflavone – see the Experimental part) and a number of isoflavone 7-O-glucopyranoside acetates, also measured in dimethyl sulfoxide, permitted the following conclusions to be drawn.

In the reaction with acetobromoglucose, only the 4-OH hydroxy group of the 2,4,t-trihydroxy ketone (1a) underwent glycosylation. When compound (2a) was treated with a complex of the reagents dimethylformamide and PCl_5, 6-hydroxyisoflavone 7-O-glucoside (3a) was formed. This was shown by the PMR spectra of the sample contained. In the case of a mixture of isoflavone 6- and 7-O-glucosides, their NMR spectra would have had two doublets of the anomeric carbohydrate protons in the 5.6-5.9 ppm region, since the oxygen atoms in positions 6 and 7 of chromone are characterized by different electronegativities. Furthermore the spectrum of a mixture of these glucosides would have two pairs of singlets of the 5-H and 8-H protons of the chromone nucleus in the 7.0-8.2 ppm region. None of this was observed in the PMR spectra of a sample of compound (3a). The difference in the chemical shifts of the 5-H protons of the aglycon and of glycoside (3a) (–0.12 ppm) was substantially less than the difference in the chemical shifts of the 8-H protons of these compounds, which showed the presence of the carbohydrate residue in position 7 of the isoflavone (3a). An additional confirmation of the correctness of this assignment is given by the results of the interaction of 6,7-dihydroxy-4'-methoxyisoflavone with acetobromoglucose [9].

The passivity of the 2-OH group of compound (Ia) in the reaction with acetobromoglucose causes no astonishment, since this group is blocked by an intermolecular hydrogen bond. The 5-OH hydroxy group, probably possessing less pronounced acidic properties, is not glycosylated because of its smaller capacity for forming a potassium salt.

TABLE 1. Conditions of Glycosylation and Yields of Compounds (2a-e)

Initial compounds and their amounts									Compound obtained and its yield		
hydroxyphenyl ketone			acetobromoglucose		50% KOH		acetone	dimethylformamide			
	g	mmole	g	mmole	ml	mmole	ml	ml	mmole	g	%
1 a	2,4	10	3,08	7,5	0,80	11	1.5	—	2 a	—	50*
1 b	18,3	75	23,3	57	4.48	62	9	9	2 b	9.25	29
1 c	22,9	100	20,5	51	7,28	100	15	—	2 c	15,1	54
1 d	12,45	50	13,6	33	3.65	50	7	—	2 d	8,4	44
1 e	21,2	76	15.4	37,5	5,53	76	30	—	2 e	7,3	32

*From the results of the TLC analysis of the reaction mixture.

TABLE 2. Physicochemical Constants of the Glycosides Synthesized

Compound	Yield, %	mp, °C	$[\alpha]_D^{23}$, degrees (c=1; chloroform)	IR spectrum, cm^{-1}		
				$\nu_{C=C}$	$\nu_{C=O}$	$\nu_{C=O\,(OAc)}$
2 b	29	180	—41	1600	1630	1750
2 c	54	125	—32	1640	1640	1750
2 d	44	82	—26	1612	1612	1750
2 e	32	192	—41	1625	—	1750
3 a	41	197		1625	1615	1750
3 c	98	171	—42	1620	1640	1750
3 e	98	225	—38	1620	1640	1750
4 b	32	255	—30*	—	—	—

*c = 0.5 (DMSO).

On interaction with acetobromoglucose, the trihydroxyketone (1b) formed the 4-O-glucoside (2b), which was colored brown when treated with iron(III) chloride on the chromatogram. The signals of the 3-H and 5-H protons of the phenyl residue in its PMR spectrum were shifted downfield (in comparison with the signals of the same protons of the aglycon) by 0.15-0.20 ppm, while the signals of the 3-H and 5-H protons of the benzyl moiety remained in their previous positions. This showed the attachment of the carbohydrate at the 4-OH hydroxy group of the phenyl residue. Consequently, in the case of the trihydroxyketone (1b), it was the most acidic hydroxy group that underwent glycosylation. The low yield of the glycoside (2b) here is explained by the poor solubility of the potassium salt of compound (1b) in the reaction mixture.

The products of the interaction of ketones (1c-e) with acetobromoglucose were identified as 4-O-glucopyranosides. In contrast to the uncolored glucosides (2a, b) they were light yellow (2c, d) or orange (2e) compounds forming brown colorations with iron(III) chloride on a chromatogram. On the basis of their PMR spectra (Table 3), we established that in chloroform solutions there was 5-7% of the enolic form of compound (2c), while compound (2e) was present exclusively in the enolic form. We were unable to determine the amount of the enolic form of compound (2d) from its PMR spectrum. The signals of the carbohydrate proton in the PMR spectra of these glucosides coincided in form and position with those for other 4-glucosyloxy-2-hydroxyphenyl ketones [1, 2]. According to the results of the TLC analysis of the reaction mixture, neither the 2-OH hydroxy group, nor the enolic hydroxyl of any of compounds (1c-e) interacted in appreciable amounts with acetobromoglucose under the conditions studied, which is probably explained by the considerable strength of the hydrogen bonds of both the enolic and the ketonic forms of these compounds.

The hydroxyphenyl glycosides (2a-c, e) were converted into the chromone O-tetraacetylglycosides (3a-c, e). In the production of compounds (3a, b) it was convenient to use the complex of dimethylformamide with PCl_5 [2], while glycosides (3c and e) were most readily formed under the action of acetic anhydride [8]. All these compounds were obtained in the form of small colorless crystals (Table 2). They gave no coloration with iron(III) chloride. The PMR spectra and specific angles of optical rotation confirmed the structures proposed for these compounds.

It has been shown that, under the proposed conditions, acetobromoglucose interacts with

polyhydroxyphenyl ketones at the most acidic hydroxy group, forming 2-hydroxy-4-tetraacetylglycopyranosyloxyphenyl benzyl ketones. Under the action of cyclizing agents, the latter are readily converted into isoflavone glucosides.

EXPERIMENTAL

TLC analysis of the reaction mixtures was conducted in the chloroform–methanol (a – 9:1, or b – 95:5) and benzene–ethanol (9:1) systems. NMR spectra were recorded on Bruker CXP-200 and Bruker WP-100 instruments relative to TMS (internal standard). Angles of optical rotation of the compounds were measured on a Polamat A polarimeter, and melting points on a PTP instrument. Elementary analysis was carried out for carbon and hydrogen or sulfur and halogen. The values found for the amounts of these elements corresponded to the calculated figures. The initial dihydroxyphenyl benzyl ketones were synthesized from resorcinol and the corresponding (het)arylacetonitriles under the standard or modified conditions of the Hoesch reaction [1, 6-8].

2,4,5-Trihydroxyphenyl Benzyl Ketone (1a). A solution of 15.1 g (0.12 mole) of 1,2,4-trihydroxybenzene and 6.8 g (0.05 mole) of anhydrous zinc chloride in 50 ml of dry ether was added to a solution of 11.7 g (0.1 mole) of phenylacetonitrile in 75 ml of dry benzene. With stirring, a current of dry hydrogen chloride was passed through the reaction mixture at 0°C until it was completely saturated. After a day, the reaction mixture was added to 0.5 liter of water and the resulting mixture was boiled for 1 h. After cooling to room temperature, the precipitate was filtered off and was recrystallized from isopropanol. Yield 24.4 g (50.0%). Colorless prisms with mp 215°C, PMR spectrum (100 MHz) in DMSO-d_6, internal standard TMS: 12.30 s (1H, OH-2); 6.43 s (1H, H-3); 9.65 s (2H, OH-4,5); 7.43 s (1H, H-6); 7.32 m (5H, C_6H_5).

2-Hydroxy-4-(tetra-O-acetyl-β-D-glycopyranosyloxy)phenyl 4'-Hydroxybenzyl Ketone (2b). With stirring, 4.48 ml (62 mmole) of a 50% aqueous solution of potassium hydroxide was added dropwise to a mixture of 18.3 g (75 mmole) of the ketone (1b) in 9 ml of acetone and 9 ml of dimethylformamide present in a reactor in an atmosphere of nitrogen. The reaction mixture was cooled to 5-20°C, and 23.2 g (56.5 mmole) was added to it in 0.5-g portions. The resulting mixture was stirred at room temperature for 4 h and was left overnight. The viscous mass that had formed was dissolved in 100 ml of chloroform, the resulting solution was filtered, and the filtrate was cooled to 0°C, and was washed successively with 200 ml of a 0.2 N aqueous solution of sodium hydroxide and 200 ml of water. From the aqueous extract after neutralization with acetic acid 11.6 g of the initial compound (1b) was extracted. The organic layer was dried over sodium sulfate, and the solvent was evaporated off under reduced pressure. The oily residue was recrystallized from isopropanol. This gave 9.25 g (29%) of the glucoside (2b). Glucoside (2a, c-e) were obtained under the conditions given in Table 1. The physicochemical characteristics of the compounds synthesized are given in Tables 2 and 3.

6,7-Dihydroxyisoflavone. With stirring, first 0.74 ml (0.85 g, 6 mmole) of boron trifluoride etherate and then 0.22 g (1.1 mmole) of phosphorus pentachloride were added in portions to a mixture of 0.24 g (1 mmole) of ketone (1a) and 1.5 ml of dimethylformamide. The resulting solution was kept at 70°C and was then poured into 20 ml of boiling water. After the mixture had cooled, the precipitate that had deposited was filtered off and was recrystallized from ethanol. This gave 0.16 g (63%) of 6,7-dihydroxyisoflavone in the form of colorless prisms with mp 274°C. PMR spectrum (100 MHz, in DMSO-d_6, internal standard TMS): 8.38 s (1H, H-2); 7.62 s (1H, H-5); 9.95 s (1H, OH-6); 10.52 s (1H, OH-7); 7.04 s (1H, H-8); 7.57 m (5H, C_6H_5).

6,7-Dihydroxyisoflavone 7-O-(tetra-O-acetyl-β-D-glucopyranoside) (3a). With stirring, 0.8 ml (11 mmole) of a 50% aqueous solution of potassium hydroxide was added dropwise to a mixture of 2.4 g (10 mmole) of ketone (1a) and 1.5 ml of acetone present in a reactor in an atmosphere of nitrogen. The reactor was cooled to 5-20°C, and 3.08 g (7.5 mmole) of acetobromoglucose was added to it in 0.5-g portions. The mixture was stirred at room temperature for 4 h and was left overnight. The viscous mass that had formed was dissolved in 80 ml of chloroform, the solution was filtered, the filtrate was cooled to 0°C and it was washed successively with 35 ml of 0.2 N aqueous sodium hydroxide and 200 ml of water. From the aqueous extracts after neutralization with acetic acid, 0.78 g of the initial compound (1b) was extracted.

The organic layer was dried over sodium sulfate, and the solvent was evaporated off under reduced pressure. The oily residue was dissolved in 30 ml of boiling isopropanol. The

TABLE 3. Details of the PMR Spectra of Compounds (2b-e), (3a, c, e), and (4b) (solvent – deuterochloroform)

Compound	Chemical shifts of the protons of the carbohydrate moiety, ppm						Chemical shifts of the protons of the phenol residue				Chemical shifts of the protons of the $-CO-CH_2-X$ residue	
	1-H(J,Hz)	2-H	3-H	4-H		6-H; 6-H'	protons of the acetyl (hydroxy) groups	2-OH	3-H	5-R	6-H	CH_2	X
2b	5,14 (7,6)	5,23...5,37		5,15	3,90	4,28; 4,16	2,09; 2,06; 2,05; 2,04	12,20	6,52	6,49	7,47	4,15	7,11(2,6–H); 6,82(3,5–H)
2c	5,17 (7,6)	5,25...5,35		5,16	3,91	4,27; 4,17	2,09; 2,06; 2,05; 2,04	12,28	6,53	6,51	7,91	4,44	7,32(3–H); 7,68(4–H); 7,21(5–H) 8,57 (6–H)
2c*	—**							—**	—**	6,40	6,60	5,81	(H–enolic); 7,53(3–H); 6,90(6–H)
2d	5,23 (7,5)	5,23...5,41		5,22	3,96	4,33; 4,22	2,12; 2,08; 2,08; 2,07	11,80	6,52	6,50	7,71	4,66	2,46(4–CH_3); 6,81(5–H)
2d*	—**												
2e	5,07 (7,5)	5,15...5,28		5,06	3,83	4,23; 4,12	2,06; 1,99; 1,98; 1,97	13,99	6,47	6,38	6,75	5,84	(H–enolic); 14,57(OH–enolic); 7,53(3–H); 7,57(4–H 7,19(6–H); 7,45 (5-H); 7,33(7–H); 7,45(3–H)

Compound	Chemical shifts of the protons of the chromone nucleus				Chemical shifts of the protons of substituent X
	2-H	5-H	6-R	8-H	
3a	7,95	7,73	6,15	7,05	7,33...7,63(2...6-H)
3a+	8,30	7,62	8,27	7,40	7,58...7,70(2,6-H); 7,38...7,48(3...5-H)
3a++	8,47	7,50	10,42	7,31	7,63...7,50(2,6-H); 7,35...7,45(3...5-H)
3c	8,89	8,30	7,09	7,11	8,41(3-H); 7,77(4-H); 7,27(5-H); 8,62(6-H)
3e	8,88	8,30	7,09	7,11	8,39(3-H); 8,24(4-H); 7,36(5-H); 7,55(6-H); 7,73(7-H); 8,10(8-H)
4b++	8,38	8,05	7,18	7,23	7,43(2,6-H); 6,82(3,5-H); 9,53(4-OH)

*Chemical shifts of the protons of the enolic form of the compound.

**Determination impossible.

+Measured in acetone-d_6.

++Spectrum measured in DMSO-d_6.

oily precipitate that deposited after cooling (4.1 g) was separated from the solution by decantation and was dried in vacuum, and it was then dissolved in 14 ml of dimethylformamide and treated with 5.2 ml (6.0 g, 42 mmole) of boron trifluoride etherate and 1.54 g (7.7 mmole) of PCl_5 under the conditions described in the preceding procedure. The reaction mixture was poured into 100 ml of ice water and the precipitate that deposited was filtered off, washed with water, dried (dry weight 3.5 g), and recrystallized three times from isopropanol and twice from benzene. This gave 0.73 g (17.1%) of compound (3a) (colorless prisms with mp 197°C).

4',7-Dihydroxyisoflavone 7-O-(Tetra-O-acetyl-β-D-glucopyranoside) (3b) and Daidzin (4b). With stirring, in portions, first 10.4 ml (9.05 g, 84.3 mmole) of boron trifluoride etherate and then 3.1 g (14 mmole) of phosphorus pentachloride were added to a mixture of 8.05 g (14 mmole) of glucoside (2b) and 21 ml of dimethylformamide. The resulting solution was kept at 70°C for 30 min and was poured into 100 ml of ice water. The oily precipitate of compound (3b) that formed was dissolved in 100 ml of chloroform and the organic layer was separated off, dried over sodium sulfate, and evaporated to dryness under reduced pressure (15 mm). The residue was dissolved in 30 ml of methanol, and 80 ml of 1 N aqueous NaOH was carefully added to it. After 15 min, the solution was neutralized with acetic acid, and the resulting precipitate was filtered off and recrystallized from ethanol. This gave 1.88 g (32%) of compound (4b), the physicochemical constants of which agreed with those known from the literature [10].

7-Hydroxy-3-(2-pyridyl)chromone 7-O(Tetra-O-acetyl-β-D-glucopyranoside) (3c). A mixture of 5.59 g (10 mmole) of ketone (2c) was added to 10 ml of acetoformic anhydride cooled to 0°C. The reaction mixture was carefully stirred for 0.5 h and was added to 100 ml of ice-water. After the solution had been neutralized with sodium carbonate, the precipitate that had deposited was filtered off and was recrystallized from ethyl acetate. This gave 5.6 g (98%) of compound (3c) in the form of colorless needles.

7-Hydroxy-3-(2-quinolyl)chromone 7-O(Tetra-O-acetyl-β-D-glucopyranoside) (3e) was obtained from 0.61 g (1 mmole) of compound (2e) and 1 ml of acetoformic anhydride under the conditions for glucoside (3c). Yield 0.61 g (98%). Colorless needles (from ethyl acetate).

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