GLUCOSIDATION OF 2-HYDROXY- AND 5-HYDROXY-1,4-NAPHTHOQUINONE

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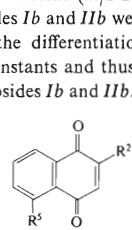
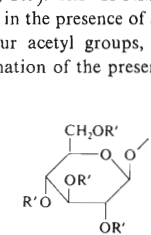
Using the Koenigs–Knorr method two isomeric monohydroxynaphthoquinones were glucosidized, *i.e.* 2-hydroxy-1,4-naphthoquinone (lawson, *Ia*) and 5-hydroxy-1,4-naphthoquinone (juglone, *IIa*). The structure of the corresponding glucosides *Ib* and *IIb* was proved by ^{1}H-NMR, mass, UV, VIS and IR spectra.

In connection with the study of chemical and microbial glucosidations of mono- and dihydroxy-9,10-anthraquinones as model substances[1–5] acetylated glucosides of hydroxy derivatives of naphthoquinone type were prepared, *i.e.* 2-hydroxy--1,4-naphthoquinone (*Ia*) and 5-hydroxy-1,4-naphthoquinone (*IIa*). For the preparation the Koenigs–Knorr[6–9] method was used, and in the case of juglone (*IIa*) calcium sulfate and iodine[10,11] were added to the reaction mixture in order to suppress the formation of by-products and to enhance the reaction.

Glucosides *Ib* and *IIb* were determined by means of spectroscopic methods. In analogy to the literature[12] we expected that both glucosides *Ib* and *IIb* (without the possibility to form hydrogen bridges with the carbonyl groups of the quinone) will display a single value for the carbonyl group frequency in their IR spectra. From a comparison of the spectra of aglycones *Ia* and *IIa* and the glucosides *Ib* and *IIb* it follows that these ideas do not apply for the glucoside of lawson *Ib*, the spectrum of which displays two bands, corresponding to the two different quinone carbonyls. For the IR spectrum of 2-methoxynaphthoquinone (*Ic*) too two[13] or one[14] value of the quinone carbonyl frequency are published. The UV and the VIS spectra were measured in the 210–500 nm range. Analogously as in the case of anthraquinone derivatives[1–3,15] the substitution of the hydroxyls of the aglycone with a glucosyl residue brings about a distinct cryptochromic shift of the bands of the $n\rightarrow\pi^*$ transition; in the case of glucoside *IIb* it is 56 nm. A similar, though smaller (26 nm) shift was also observed[16] in 5-methoxynaphthoquinone (*IIc*). The spectrum of glucoside *Ib*, similarly as *Ic*, ref.[16], does not contain any band of the $n\rightarrow\pi^*$ transition. In this region lawson (*Ia*) has a broad band at 452 nm ($\log \varepsilon_{max}$ 2·96), which is usually not mentioned[13,14]. However, its existence follows indirectly from ref.[16]. The mass spectra of lawson (*Ia*) and juglone (*IIa*) differ negligibly[17–19] from the spectrum

of unsubstituted 1,4-naphthoquinone. In addition to this for the case of *Ia* a characteristic decomposition is described[17] accompanied by a hydrogen shift under formation of the peak of *m/z* 105 which was not observed in 5-hydroxynaphthoquinone. This peak, though weak, was also observed in both acetylated glucosides *Ib* and *IIb*. The spectra of these glucosides contain further a weak molecular peak (*m/z* 504), a more intensive peak of aglycone (*m/z* 174) and peaks of differently acetylated fragments, formed from the ion of acetylated glucose (*m/z* 331) by gradual splitting off of acetic acid or ketene (*m/z* 271, 211, 169, 109). The ^{1}H-NMR spectra of both acetylated glucosides *Ib* and *IIb* were measured in the presence of a shift reagent[20,21] which permitted the differentiation of all four acetyl groups, the determination of the coupling constants and thus the confirmation of the presence of the β-glucosidic bond in glucosides *Ib* and *IIb*.





Ia, $R^2 = OH$, $R^5 = H$
Ib, $R^2 =$ *III*, $R^5 = H$
Ic, $R^2 = OCH_3$, $R^5 = H$
IIa, $R^2 = H$, $R^5 = OH$
IIb, $R^2 = H$, $R^5 =$ *III*
IIc, $R^2 = H$, $R^5 = OCH_3$

III, $R' = CH_3CO$

Aglycones *Ia* and *IIa* and glucosides *Ib* and *IIb* were tested for their immunosuppressive and immunostimulative effects on tissue cultures. The substances tested were used in concentrations 1, 10 and 100 μg in cultivations according to Marbrook[22] (10^7 of lymphatic cells of spleen and antigen, 10^7 of ram erythrocytes). Of all the hydroxyanthraquinone and hydroxynaphthoquinone derivatives tested so far, glucosides *Ib* and *IIb* were found most active. Their activity was comparable with the

TABLE I

Inhibition of the Antibody Response with Naphthoquinone Derivatives *Ia,b* and *IIa,b* (in percents of control cultivation) in Tissue Cultures

Compound		*Ia*	*Ib*	*IIa*	*IIb*	Imuran
Concentration	100	15	0	0	—	—
μg per ml	10	57	0	0	—	—
	1	54	6	54	1	5

clinically used Imuran. Both aglycones *Ia* and *IIa* were, on the contrary, inactive (Table I). When the activity against *Bacillus licheniformis* was tested, derivatives *Ia,b* and *IIa,b* displayed a different degree of inhibition[23].

EXPERIMENTAL

Material and methods. For column chromatography Kieselgel 60 (Merck) was used, while TLC was carried out on Silufol plates without an indicator (Kavalier, Czechoslovakia). Systems used (*V*/*V*): S_1 chloroform–acetone 20 : 1, S_2 chloroform–methanol 30 : 1. For spot detection the UV lamp Camag (254 nm) was used. The samples for analysis were dried over potassium hydroxide at 25°C and 15 Pa for 8 h. The melting points were measured on a Boetius HMK block and they are not corrected. Optical rotation was measured on a Bendix–Ericsson 143 A polarimeter at 25°C. The IR spectra were measured on a spectrophotometer Unicam SP 200 in KBr pellets, the UV and VIS spectra on a Cary 118 instrument in methanol. The ^{1}H-NMR spectra were recorded on a Tesla BS 487 (80 MHz)) apparatus in $CDCl_3$ using hexamethyldisiloxane (δ_H 0·06) as internal reference. As shift reagent europium(III) 2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octadionate was used; chemical shifts are expressed in δ-scale. The mass spectra were measured on a Varian MAT 311 spectrometer at 11 aJ (70 eV) energy of ionizing electrons and 1 mA current intensity and 200°C of the ion source temperature.

2-(2′,3′,4′,6′-*Tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-1,4-*naphthoquinone* (Ib): A mixture of 386 mg (2·2 mmol) of 2-hydroxynaphthoquinone (*Ia*), 1·2 g (5·2 mmol) of silver oxide and 2·5 g (6·0 mmol) of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in 6 ml of quinoline was stirred at 25°C for 30 min. The mixture was diluted with 100 ml of benzene, filtered and the filtrate washed three times with 150 ml of 5% H_2SO_4, three times with 100 ml of water and dried over calcium chloride. Benzene was distilled off and the residue crystallized from ethanol. Yield, 735 mg (71%) of yellow needles of glucoside *Ib*, m.p. 168·5—170°C, $[\alpha]_D$ —53° (*c* 0·36; $CHCl_3$), R_F 0·55 (S_1) and 0·83 (S_2), ochre colour in NH_3, brown in UV light. IR spectrum (cm^{-1}): 2955, 2870 (CH_2), 1748 (CH_3CO), 1684, 1655 (CO in quinone), 1612, 1596, 1580. UV and VIS spectrum (λ_{max}, nm; log ε_{max}): 241·5 (4·2), 247 (4·2), 269 inflexion (4·2), 273 (4·2), 331 (3·4). Mass spectrum (*m*/*z*, relat. intensity, %): 504 (0·12, M), 331 (13), 271 (14), 229 (3·5), 211 (3·5), 187 (4·7), 174 (12), 146 (7·1), 169 (66), 127 (18), 109 (36), 105 (12), 43 (100). ^{1}H-NMR spectrum: 2·06 (s, 9 H, 3 . CH_3COO), 2·10 (s, 3 H, 1 . CH_3COO), 4·00 (mt, 1 H, $H_{(5')}$), 4·15 (d, 1 H, $H_{(6'u)}$), 4·35 (q, 1 H, $H_{(6'd)}$), 5·05—5·49 (mt, 4 H, $H_{(1'-4')}$), 6·37 (s, 1 H, $H_{(3)}$), 7·62 to 7·81 (mt, 2 H, $H_{(6,7)}$), 7·89—8·14 (mt, 2 H, $H_{(5,8)}$), $J_{1',2'} = 9$ Hz, $J_{2',3'} = 8$ Hz, $J_{3',4'} = J_{4',5'} = 8·5$ Hz, $J_{5',6'u} = 0$ Hz, $J_{5',6'd} = 2$ Hz, $J_{6'u,6'd} = 12$ Hz. For $C_{24}H_{24}O_{12}$ (504·5) calculated: 57·14% C, 4·79% H; found: 57·23% C, 5·11% H.

5-(2′,3′,4′,6′-*Tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-1,4-*naphthoquinone* (IIb): A mixture of 386 mg (2·2 mmol) of 5-hydroxynaphthoquinone (*IIa*), 1·2 g (5·2 mmol) of silver oxide, 2·5 g (6·0 mmol) of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide, 5 g calcium sulfate and a crystal of iodine in 8 ml of quinoline was stirred at 25°C for 105 min, another 2·5 g of bromide, 1·2 g of silver oxide and 2·5 ml of quinoline were then added and the stirring continued for another 2 h. The mixture was diluted with 100 ml of benzene, filtered and the filtrate washed with three 100 ml portions of 5% H_2SO_4 and three 100 ml portions of water. After drying over calcium chloride benzene was evaporated and the residue freed of ballast by sorption on a silica gel column and elution with chloroform–acetone (5 : 1). Crystallization of the residue of the main fraction from ethanol gave 435 mg (43%) of a yellow glucoside *IIb*, m.p. 165·5—167°C; $[\alpha]_D$ —68° (*c* 0·41; $CHCl_3$), R_F 0·54 (S_1), in NH_3 grey-brown coloration, in UV light black. IR spectrum (cm^{-1}): 2965, 2870 (CH_2), 1754 (CH_3CO), 1668 (CO in quinone), 1612, 1588. UV and VIS spectrum (λ_{max}, nm; log ε_{max}): 237·5 (4·2), 365 (3·5). Mass spectrum (*m*/*z*; relat. intensity, %): 504 (0·43, M),

331 (5), 271 (6), 229 (7), 211 (7), 187 (8), 174 (35), 169 (57), 146 (17), 127 (27), 118 (21), 109 (63), 105 (9), 43 (100). ^{1}H-NMR spectrum: 2·11 (s, 9 H, 3 . CH_3COO), 2·17 (s, 3 H, 1 . CH_3COO), 3·91 (mt, 1 H, $H_{(5')}$), 4·15 (d, 1 H, $H_{(6'a)}$), 4·37 (q, 1 H, $H_{(6'd)}$), 5·06—5·54 (mt, 4 H, $H_{(1'-4')}$), 6·87 (s, 2 H, $H_{(2,3)}$), 7·55 (q, 1 H, $H_{(6)}$), 7·69 (t, 1 H, $H_{(7)}$), 7·90 (q, 1 H, $H_{(8)}$), $J_{2,3} = 9$ Hz, $J_{6,7} = J_{7,8} = 8$ Hz, $J_{6,8} = 2$ Hz, $J_{1',2'} = 8$ Hz, $J_{2',3'} = J_{3',4'} = 9$ Hz, $J_{4',5'} = 9{\cdot}5$ Hz, $J_{5',6'd} = 2$ Hz, $J_{6'a,6'd} = 14$ Hz. For $C_{24}H_{24}O_{12}$ (504·5) calculated: 57·14% C, 4·79% H; found: 57·09% C, 5·02% H.

Attempts at deacetylation of glucosides *Ib* and *IIb* (sodium methoxide in methanol, sodium hydroxide, ammoniacal methanol, traces of $HClO_4$ or H_2SO_4 in methanol) were unsuccessful and did not afford the required free glucosides.

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