MICROBIAL GLUCOSIDATION OF MONOHYDROXYANTHRAQUINONES

N.HOVORKOVÁ, J.CUDLÍN, J.MATĚJŮ, M.BLUMAUEROVÁ and Z.VANĚK

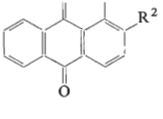
Institute of Microbiology,
Czechoslovak Academy of Sciences, 142 20 Prague 4

Received January 5th, 1974

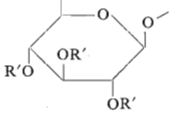
Streptomyces aureofaciens B-96 can glucosidate both isomeric monohydroxyanthraquinones, which have been added to the culture medium, to the corresponding β-D-glucosides; the degree of conversion, however, is lower than with the dihydroxyanthraquinones. The identity of the products was confirmed by a comparison with authentic samples prepared synthetically. The difference in the mechanism of glucosidation of mono- and dihydroxyanthraquinones is discussed.

We reported in the preceding communication[1] on the glucosidating ability of *Streptomyces aureofaciens* B-96 (ref.[2,3]) studied with isomeric dihydroxyanthraquinones (alizarin and anthraflavin). Since the recorded data on biological glucosidations of monofunctional phenols are very scarce and more or less hypothetical[4,5], we focused our attention on the microbial glucosidation of 1- and 2-hydroxyanthraquinone (*Ia, b*). 1-Hydroxyanthraquinone (*Ia*) being an aglycone is also interesting in view of the fact that the glucosidation of alizarin (1,2-dihydroxyanthraquinone) does not yield the corresponding 1-glucoside[1].

We prepared acetylated glucosides *IIa,b* by chemical synthesis (Koenigs–Knorr method) from the two monohydroxyanthraquinones and 2,3,4,6-tetra-O-acetyl--α-D-glucopyranosyl bromide. Compounds *IIa, b* were deacylated to glucosides *IIIa, b* by ethanolic potassium hydroxide.



Ia; $R^1 = OH$, $R^2 = H$
Ib; $R^1 = H$, $R^2 = OH$
IIa; $R^1 = IVa$, $R^2 = H$
IIb; $R^1 = H$, $R^2 = IVa$
IIIa; $R^1 = IVb$, $R^2 = H$
IIIb; $R^1 = H$, $R^2 = IVb$



IVa; $R' = CH_3CO$
IVb; $R' = H$

It follows from a comparison of the yields of microbial glucosidation of the two monohydroxyanthraquinones with the yields obtained with alizarin and anthraflavin[1] that monohydroxyanthraquinones *Ia, b* represent considerably worse substrates for glucosidation by *Streptomyces aureofaciens* than dihydroxyanthraquinones. This finding is essentially in agreement with the recorded data on glucosidation systems from wheat germ[4,5] which glucosidate di- and triphenols but not monophenols. Whereas the crude enzymatic extract showed some activity when tested even with monophenols, the pure product was completely inactive[5]. It is therefore most likely that the live cells contain a number of enzymes responsible for glucosidation of phenolic compounds.

If the glucosidation of monohydroxyanthraquinones is catalyzed by an enzyme (E_1) different from the enzyme (E_2) glucosidating di- and other oligohydroxyanthra-

TABLE I

Spectrophotometric Characteristics of Monohydroxyanthraquinones and their Glucosides

Compound	Characteristic vibrations in IR-region, cm^{-1}				
	ν(OH)	$\nu(CH_2)$(aliph.)	$\nu(CO)(CH_3CO)$	ν(CO)(quinone)	arom. backbone
Ia	3 450	—	—	1 638; 1 670	1 593
Ib	3 370	—	—	1 670	1 594
IIa	—	2 855; 2 940	1 765	1 673	1 593
IIb	—	2 860; 2 945	1 748	1 670	1 595
IIIa	3 290	2 870; 2 920	—	1 664	1 581
IIIb	3 440	2 850; 2 900	—	1 670	1 592

Compound	Absorption bands in UV and visible region (ref.[12])	
	λ_{max}, nm	log ε_{max}
Ia	252; 331; 404	4·5; 3·4 3·7
	(252; 327; 402	(4·5; 3·5; 3·7)
Ib	245; 272; 333; 371	4·2; 4·4; 3·4; 3·4
	(245; 271; 328; 368)	(4·3; 4·5; 3·6; 3·6)
IIa	253; 343	4·5; 3·5
IIb	261; 329	4·6; 3·7
IIIa	256; 381	4·4; 3·5
IIIb	265; 330	4·5; 3·6

quinones, the inability of E_2 to glucosidate monohydroxyanthraquinones is obviously a consequence of the fact that E_2 requires a special structure of the aglycone, *i.e.* of that site of the aglycone molecule which binds to the enzyme during enzymatic glucosidation. If this requirement involves the presence of a free hydroxyl group, it is understandable why E_2 is inactive toward monohydroxyanthraquinones (which do not bear any free hydroxyl group after they have bound to the enzyme). Anyhow, this conclusion is in accordance with the observation that there is no diglucoside formed during enzymatic glucosidation of dihydroxyanthraquinone bearing equivalent hydroxy groups (anthraflavin).

The antitumor activity of the compounds synthetized toward Ehrlich ascites carcinoma and HeLa cells *in vitro* is described elsewhere[6].

EXPERIMENTAL

Material and methods: *cf*[1]. UV-Lamp CAMAG (254 nm) was used for the detection of compounds on thin-layer chromatograms. The optical rotation measurements were made at 25°C in Bendix Ericsson 143A Polarimeter. The UV-spectra were measured in the mixture ethanol–dimethyl sulfoxide 95 : 5 (*v*/*v*). The spectral characteristics of the compounds are given in Table I, the R_F-values in Table II. The NMR-spectra were determined in Varian HA-100 (100 MHz) and TESLA BS 487 (80 MHz) instruments in $CDCl_3$ and $(CD_3)_2SO$; hexamethyldisiloxane was used as an internal standard.The signals were arranged with respect to their chemical shifts and multiplicity. Index u (upfield) marks in the proton pair on $C_{(6')}$ the proton whose signal lies in the higher field; the other proton is marked by index d (downfield). The shift reagent was $Eu(fod)_3$ *i.e.* europium (III) 2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octadionate.

Cultivation and isolation: *cf*[1]. The fermentation liquid (90 flasks, total volume 5·4 l) was centrifuged (10 min, 1 000 *g*) after 20 h of cultivation and the mycelium was washed twice with five-times its volume of water. The isolated mycelium was placed into 60 flasks; 50 ml of 0·2M Tris-HCl buffer (pH 7·2) and 20 mg of hydroxyanthraquinone were added to each flask. The fermentation liquid was centrifuged (10 min, 1 000 *g*) after 24 h of fermentation and extracted three-times with an equal volume of ethyl acetate. The ethyl acetate extract was dried by sodium sulfate and distilled off *in vacuo*. Chromatography on a column of silica gel (elution by chloroform–ethanol, 1 : 1) afforded 13·2 mg (0·64%) of glucoside *IIIa* from 1-hydroxyanthraquinone and 36 mg (2·1%) of

TABLE II

Thin-layer Chromatography of Monohydroxyanthraquinones and their Glucosides in System *n*-Hexane–Acetone 5 : 2 (*v*/*v*)

Compound	*Ia*	*Ib*	*IIa*	*IIb*	*IIIa*	*IIIb*
R_F	0·74	0·77	0·28	0·59	0·08	0·18
Color in NH_3	〈 orange 〉		〈 yellow 〉			
Color in UV	〈brown〉〈		black 〉			

glucoside *IVa* from 2-hydroxyanthraquinone. Analytical samples were obtained by thin-layer chromatography and crystallization from ethanol. The products obtained show the same melting points, IR-spectra, and R_F-values as glucosides *IIIa, b* prepared chemically.

Chemical Syntheses

1-(2′,3′,4′,6′-*Tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*-9,10-*anthraquinone* (IIa): A mixture of 2·38 g of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide[7], 0·59 g of 1-hydroxyanthraquinone (*Ia*), and 1·11 g of silver oxide in 5·5 ml of quinoline was stirred at 25°C for 20 min. On the subsequent day, 100 ml of chloroform was added, the solid phase was filtered off, and the filtrate was washed twice with 150 ml of 5% H_2SO_4 and 150 ml of water. The filtrate was dried with sodium sulfate, chloroform was distilled off *in vacuo*, and the dry residue recrystallized from ethanol. The yield was 910 mg (62%) of yellow needles of m.p. 212–213°C (ref.[8] 212–212·5°C, corr.), $[\alpha]_D$ −82° (*c* 0·83; chloroform). NMR (in $CDCl_3$): 2·09 (s, 9 H, 3 × CH_3COO), 2·19 (s, 3 H, 1 × CH_3COO), 3·98 (m, 1 H, $H_{(5')}$), 4·21 (d, 1 H, $H_{(6'u)}$), 4·34 (q, 1 H, $H_{(6'd)}$), 5·10–5·57 (m, 4 H), 7·53–7·84 (m, 4 H, arom.), 7·93–8·24 (m, 3 H, arom.), $J_{4',5'}$ = 9·8 Hz, $J_{5,6'u} < 0·5$ Hz, $J_{5,6'd} = 3$ Hz and $J_{6'u,6'd} = 10$ Hz. The values $J_{1',2'} = 7·6$ Hz (indicating a β-glucosidic bond), $J_{2',3'} =$ = 8·4 Hz, and $J_{3',4'} = 9·8$ Hz were read at a molar ratio of product to $Eu(fod)_3$ equal 1·2 (*cf.*[9], two-fold resonance). The acetyl groups yield four separate singlets at 2·22, 2·27, 2·37, and 2·74 p.p.m.

1-(β-D-*Glucopyranosyloxy*)-9,10-*anthraquinone* (IIIa): Acetate *IIa* (0·59 g) was deacetylated by 6% potassium hydroxide in ethanol (15 min, 25°C). Recrystallization of the product from a mixture of ethanol and acetic acid afforded 330 mg (80%) of yellow crystals, m.p. 233–235°C (ref.[8] 232·2–232·8°C, corr.), $[\alpha]_D$ −24° (*c* 0·75; pyridine). The product is identical in its physical characteristics with glucoside *IIIa* obtained by the microbial process. NMR (in $(CD_3)_2SO$): 3·19 to 3·96 (m), 4·37–5·44 (m), 7·77–8·25 (m, arom.).

2-(2′,3′,4′,6′-*Tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-9,10-*anthraquinone* (IIb): A mixture of 4·0 g of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide[7], 1·21 g of 2-hydroxyanthraquinone (*Ib*), and 2·0 g of silver oxide in 12 ml of quinoline was stirred for 2 h at 25°C. Chloroform (150 ml) was added afterwards, the solid phase was filtered off, and the filtrate was washed twice with 200 ml of 5% H_2SO_4 and with 200 ml of water. The filtrate was dried by calcium chloride, chloroform was distilled off *in vacuo*, and the dry residue was recrystallized from ethanol. The yield was 2·25 g (75%) of yellow needles, m.p. 132–134°C (ref.[10] 132°C), $[\alpha]_D$ −47° (*c* 0·71; chloroform). NMR (in $CDCl_3$): 2·06 (s, 3 H, 1 × CH_3COO), 2·09 (s, 6 H, 2 × CH_3COO), 2·14 (s, 3 H, 1 × CH_3COO), 4·04 (m, 1 H, $H_{(5')}$), 4·20 (d, 1 H, $H_{(6'u)}$), 4·32 (dd, 1 H, $H_{(6'd)}$), 5·04–5·45 (m, 4 H), 7·36 (q, 1 H, J = 2·5 and 8·5 Hz, $H_{(3)}$), 7·56–7·88 (m, 3 H), 8·15–8·41 (m, 3 H). Protons $H_{(1)}$ (7·85 d, J = 2·5 Hz) and $H_{(4)}$ (8·30 d, J = 8·5 Hz) were found by two-fold resonance. The following interaction constants can be read from the spectrum: $J_{4',5'} = 9$ Hz, $J_{5',6'd} = 3$ Hz, $J_{5',6'u} < 0·5$ Hz, and $J_{6'u,6'd} = 9·5$ Hz. At a compound to $Eu(fod)_3$ ratio of 0·9, a doublet (J = 7·5 Hz) of proton $H_{(1')}$ can be found at 6·46 p.p.m.; this finding confirms the β-configuration of the glucosidic bond and permits the magnitude of the remaining interaction constants to be estimated (8–10 Hz).

2-(β-D-*Glucopyranosyloxy*)-9,10-*anthraquinone* (IIIb): Acetate *IIb* (1·23 g) was deacetylated (40 min, 25°C) by 6% potassium hydroxide in ethanol; the product afforded after recrystallization from ethanol 530 mg (62%) of orange crystals, m.p. 249–250°C (ref.[11] 244·4–244·7°C, corr.), $[\alpha]_D$ −50° (*c* 0·74; pyridine). This product is identical according to its physical characteristics with glucoside *IIIb* obtained by the microbial procedure. NMR (in $(CD_3)_2SO$): 3·56–4·37 (m), 6·06–6·87 (m, interchangeable), 7·29–9·42 (m, arom.).

This study was supported by the International Atomic Energy Agency (contract No 845/RB). The authors thank Dr P. Sedmera for the measurement and interpretation of NMR spectra and to Dr H. Lipavská for the measurement of IR and UV spectra.

REFERENCES

1. Hovorková N., Cudlín J., Matějů J., Blumauerová M., Vaněk Z.: This Journal *39*, 662 (1974).
2. Cudlín J., Vaněk Z., Hovorková N., Blumauerová M., Vondráček M., Benda A.: Czechoslov. Pat. 144 539 (1972).
3. Cudlín J., Vaněk Z., Steinerová N., Blumauerová M.: Czechoslov. Pat. Appl. PV 8739—73 (1973).
4. Neufeld E. F., Hassid W. Z.: Advan. Carbohydrate Chem. *18*, 309 (1963).
5. Yamaha T., Cardini C. E.: Arch. Biochem. Biophys. *86*, 127 (1960).
6. Fuska J., Fusková A., Podojil M., Vaněk Z.: Neoplasma, in press.
7. Cheurer P. G., Smith F.: J. Am. Chem. Soc. *76*, 3224 (1954).
8. Gardner J. H., McDonnell T. F., Wiegand J. W.: J. Am. Chem. Soc. *57*, 1074 (1935).
9. Borén H. B., Garegg P. J., Pilotti Å., Swahn C.-G.: Acta Chem. Scand. *26*, 3261 (1972).
10. Müller A.: Ber. *62*, 2793 (1929).
11. Gardner J. H., Demaree W. H.: J. Am. Chem. Soc. *58*, 757 (1936).
12. Yukawa Y.: *Handbook of Organic Structural Analysis*, p. 138. Benjamin, New York—Amsterdam 1965.

Translated by V. Kostka.