MICROBIAL GLUCOSIDATION OF ALIZARIN AND ANTHRAFLAVIN

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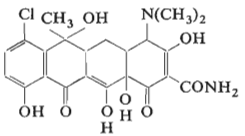
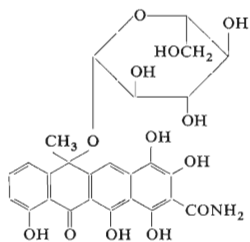
Received January 18th, 1973

Streptomyces aureofaciens B-96 (producer of aureovocin) can glucosidize isomeric dihydroxyanthraquinones added to the cultivation medium, to the corresponding mono-β-glucosides. The identity of these products was confirmed by a comparison with authentic samples prepared chemically. The dihydroxyanthraquinones were not metabolized by the microorganism (in contrast with bikaverin); resorcin is not glucosidized in this way.

In context with a study of minor compounds produced by a *Streptomyces aureofaciens* strain (producer of chlortetracycline *I*) the strain B-96 was isolated[1] and found to produce aureovocin[2] *II*. Since we are dealing here with the first described nonaketide glucoside, typical of metabolites of tetracycline type, we thought it interesting to examine the glucosidizing ability of the strain in some detail[3]. The property was



I

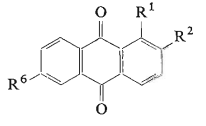


II

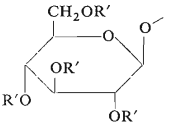
investigated with isomeric dihydroxy-9,10-anthraquinones: 1,2- (alizarin, *IIIa*) and 2,6- (anthraflavin, *IIIb*) and further with bikaverin (*VII*, ref.[4]) and resorcin. Chemical synthesis was used for preparing the corresponding glucosides from anthraquinones *IIIa,b* and from 2,3,4,6-tetra-O-acetylglucopyranosyl bromide. In the case of alizarin (*IIIa*) Michael's method was used (condensation in aqueous–alcoholic acetone[5]), in the case of anthraflavin (*IIIb*) the Koenigs–Knorr method (silver oxide in quino-

line[6]). The acetylated products *IVa,b* were then deacetylated to the corresponding glucosides *Va,b* with potassium hydroxide in methanol.

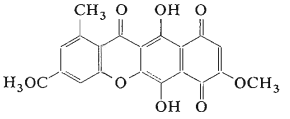
During glucosidation of anthraflavin (*IIIb*) the reaction mixture yielded besides the tetraacetate of monoglucoside *IVb*, another product identified as octaacetate of diglucoside *IVc*; its deacetylation with methanolic ammonia then gave rise to the free diglucoside *Vc*.



	R^1	R^2	R^6
IIIa,	OH	OH	H
IIIb,	H	OH	OH
IVa,	OH	*VIa*	H
IVb,	H	*VIa*	OH
IVc,	H	*VIa*	*VIa*
Va,	OH	*VIb*	H
Vb,	H	*VIb*	OH
Vc,	H	*VIb*	*VIb*



VIa; R′ = CH_3CO
VIb; R′ = H



VII

Microbial glucosidation of the two dihydroxyanthraquinones is remarkable for two reasons. *1*) The *Streptomyces* species used (producer of tetracycline antibiotics) can glucosidize fully foreign aglycones, even if to some extent they are analogous to aureovocidin. Hence the aglycones joined in the metabolic pathways of the microorganism (in contrast with resorcin) but their degradation was not completed (when the aglycone would serve as the source of carbon), unlike in the case of bikaverin. *2*) The ability to glucosidize foreign substrates may be of significance for the preparation of glucosides of compounds of general biological activity, in particular of those of poor water solubility. One must not overlook the effect of raised biological activity due to introduction of the sugar moiety into the molecule of the original compound[7,8].

The enzyme system responsible for the glucosidation is apparently nonspecific toward the aglycone; however, it is of interest that the microorganism cannot introduce two sugar moieties into the aglycone molecule even if it carries two equivalent β-hydroxyls (anthraflavin).

The antitumour activity of the compounds prepared toward the Ehrlich ascites tumour and HeLa cells *in vitro* is reported elsewhere[9].

EXPERIMENTAL

Material and methods. Column chromatography was done on Kieselgel 60 (Merck); thin-layer chromatography on Silufol without indicator (Kavalier, Czechoslovakia); detection was done under a UV lamp (254 nm, Hanovia Chromatolight). The melting points were measured in a Boetius HMK block and are not corrected. Samples for analysis were dried over potassium hydroxide at 25°C and 0·1 Torr for 8 h. The IR spectra were recorded in a Unicam SP 200 spectrophotometer in KBr pellets, UV spectra in a Specord UV VIS spectrophotometer in ethanol or ethanol–dimethyl sulfoxide (9 : 1); the spectral characteristics are shown in Table I. The NMR spectra were recorded in Varian HA-100 (100 MHz) and Tesla BS 487 (80 MHz) spectrometers in $CDCl_3$,

TABLE I

Spectrophotometric Characteristics of Compounds Prepared

Compound	Characteristic vibrations in IR region, cm^{-1}				
	OH	CH_2 (aliph.)	CO (CH_3CO)	CO (quinone)	aromatic skeleton
IIIa	3 410	—	—	1 625; 1 655	1 583
IIIb	3 300	—	—	1 655	1 560
IVa	3 520	2 910; 2 990	1 760	1 650; 1 680	1 600
IVb	3 430	2 860; 2 940	1 760	1 673	1 595
IVc	—	2 935; 2 865	1 750	1 675	1 595
Va	3 400	2 890; 2 930	—	1 636; 1 664	1 590
Vb	3 420	2 840; 2 910	—	1 665	1 590
Vc	3 400	2 845; 2 890	—	1 660	1 590

	Absorption bands in UV and visible region (ref.[14])	
	λ_{max}, nm	$\log \varepsilon_{max}$
IIIa	249; 284; 435 (251; — ; 435)	4·5; 4·2; 3·7 (4·5; — ; 3·8)
IIIb	218; 275; 302; 349 (— ; 274; 301·5; 349)	4·4; 4·5; 4·3; 3·9 (— ; 4·5; 4·3; 3·9)
IVa	226; 256; 411	4·3; 4·5; 3·8
IVb	216; 272; 295; 342	4·3; 4·4; 4·3; 3·8
IVc	270; 291; 341	4·4; 4·2; 4·0
Va	248; 256; 420	4·5; 4·4; 3·8
Vb	273; 297; 342	4·5; 4·3; 3·9
Vc	272; 295; 341	4·6; 4·3; 3·9

TABLE II

R_F Values and Chromatographic Properties of Alizarin, Anthraflavin and of Their Glucosides

Compound	R_F	System[a]	Colour in NH_3	Colour in UV
IIIa	0·81	CA 13	purple	black
IVa	0·89	CA 13	orange	orange
Va	0·32	CA 13	pink	brown-orange
IIIb	0·45	CA 51	ochre yellow	black
IVb	0·65	CA 51	orange	black
Vb	0·35	CA 25	orange-pink	black
IVc	0·75	CA 51	yellow	orange-brown
Vc	0·30	CM 54	yellow	brown-orange

[a] In TLC (all *v/v*): CA chloroform–acetone, CM chloroform–methanol; 13—1 : 3 *etc.*

$(CD_3)_2SO$ or CF_3COOD. The internal standard used was hexamethyldisiloxane, the chemical shifts are expressed in the δ-scale.

Cultivation. Streptomyces aureofaciens B-96 (from the collection of the Institute of Microbiology, Czechoslovak Academy of Sciences[1]) was used. Composition of the medium and cultivation conditions are described in detail in a patent[3].

Isolation. After 96 g of cultivation the fermentation fluid was filtered, the mycelium extracted with methanol and the extract after evaporation, like the supernatant, was extracted with two volumes of ethyl acetate. After drying the extract with sodium sulfate and evaporation *in vacuo* a residue was obtained which was chromatographed on a column of silica gel (elution with chloroform and a mixture of chloroform and methanol (5 : 1—1 : 1). Conversion of the aglycone amounted to 14% with alizarin, to 23% with anthraflavin. The analytical sample was obtained by thin-layer chromatography. The chromatograms were detected by ammonia vapour or in UV light. Zones corresponding by their R_F values to glucosides *Va,b* were eluted, evaporated *in vacuo* and crystallized; the glucoside of alizarin *Va* from ethanol, that of anthraflavin *Vb* from methanol. The products obtained had m.p., mixed m.p., IR spectra and R_F values identical with glucosides *Va,b* prepared chemically.

Cultivation in the presence of resorcin was followed under identical conditions. A similar treatment led to the isolation of practically quantitative amount of unchanged resorcin. The behaviour of bikaverin (*VII*) was investigated under analogous conditions. According to thin-layer chromatography it was not glucosidized but was metabolized by the microorganism.

Chemical Syntheses

1-*Hydroxy*-2-(2′,3′,4′,6′-*tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-9,10-*anthraquinone* (IVa): From 4·65 g 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide[10] and 2·73 g alizarin (*IIIa*), and after crystallization from ethanol a total of 0·64 g (10%) yellow crystals *IVa* was obtained, m.p. 208 to 211°C (ref.[5] gives 207°C).

1-*Hydroxy*-2-(β-D-*glucopyranosyloxy*)-9,10-*anthraquinone* (Va): From 0·64 g acetyl glucoside *IVa*, deacetylation with 5% methanolic potassium hydroxide (15 min, 25°C) and three-fold recrystallization from ethanol yielded 0·28 g (62%) yellow crystals *Va*, m.p. 241–243°C (ref.[5] 237°C). By its m.p., mixed m.p., R_F value and IR spectrum, the product is identical with the glucoside obtained from the microorganism. NMR spectrum (in $(CD_3)_2SO$ + CF_3COOD): 3·15 to 3·90 (m, O—CH), 4·87–6·21 (m, O—CH + OH), 7·09–8·27 (m, aromatic ring).

2-*Hydroxy*-6-(2′,3′,4′,6′-*tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-9,10-*anthraquinone* (IVb): Glucosidation of 2,6-dihydroxyanthraquinone (*IIIb*), was carried out by a modified procedure[6]. Silver oxide (19 g) was added in parts under stirring at 25°C to a mixture of 8·95 g *IIIb* and 28·6 g 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide[10] in 190 ml quinoline. The mixture was stirred at 60–70°C for 2 h and left to stand at 25°C. On the following day, 1 500 ml chloroform was added, the solid was filtered and the filtrate extracted three times with two volumes of 2% H_2SO_4. After drying with sodium sulfate, the chloroform was distilled *in vacuo* and the residue chromatographed on a column of silica gel (elution with chloroform); the fractions containing glucosides *IVb,c* were rechromatographed. The monoglucoside *IVb* residue was crystallized from ethanol to yield 1·77 g (8·3%) yellow crystals, melting at 219·5–221°C. NMR spectrum (in $CDCl_3$ + $(CD_3)_2SO$): 1·99 (s, 3 H), 2·01 (s, 6 H), 2·03 (s, 3 H), (4 × AcO); 3·91–4·35, 4·94–5·54 (2 m, 7 H, O—CH); 7·14 (q, $J = J_o = 8·5$ Hz, $J = J_m = 2$ Hz, 1 H, $H_{(7)}$); 7·28 (q, $J = J_o = 8·5$ Hz, $J = J_m = 2$ Hz, 1 H, $H_{(3)}$); 7·58 (d, $J = J_m = 2$ Hz, 1 H, $H_{(5)}$); 7·76 (d, $J = J_m = 2$ Hz, 1 H, $H_{(1)}$); 8·06 (d, $J = J_o = 8·5$ Hz, 1 H, $H_{(8)}$); 8·14 (d, $J = J_o = 8·5$ Hz, 1 H, $H_{(4)}$). There exist two ABC systems in the aromatic region. For $C_{28}H_{26}O_{13}$ (570·5) calculated: 59·0% C, 4·6% H; found: 59·0% C, 4·3% H.

2,6-*Bis*-(2′,3′,4′,6′-*tetra*-O-*acetyl*-β-D-*glucopyranosyloxy*)-9,10-*anthraquinone* (IVc): Residue from the preparation of monoglucoside *IVb* was rechromatographed on a column of silica gel overlayered with charcoal and then crystallized from a mixture of chloroform and ethanol. A total of 1·25 g (3·7%) yellow crystals *IVc* was obtained; m.p. 262–263·5°C (ref.[11] gives 252°C). NMR spectrum (in $CDCl_3$): 2·05 (s, 6 H), 2·07 (s, 12 H), 2·11 (s, 6 H), (8 × AcO), 3·9–4·3 (m, 6 H, O—CH), 5·05–5·45 (m, 8 H, O—CH), 7·31 (q, $J = J_o = 9$ Hz, $J = J_m = 2·5$ Hz, 2 H, $H_{(3)}$ and $H_{(7)}$), 7·81 (d, $J = J_m = 2·5$ Hz, 2 H, $H_{(1)}$ and $H_{(5)}$), 8·22 (d, $J = J_o = 9$ Hz, 2 H, $H_{(4)}$ and $H_{(8)}$).

2-*Hydroxy*-6-(β-D-*glucopyranosyloxy*)-9,10-*anthraquinone* (Vb): A solution of 320 mg acetate *IVb* in 20 ml 5% methanolic potassium hydroxide was stirred for 1 h at 25°C, acidified with acetic acid to pH 5·2 and concentrated *in vacuo*. Acetic acid was removed from the residue by freeze-drying at 0·1 Torr, potassium acetate was washed with water. The product after drying was crystallized from methanol. A total of 105 mg (46·6%) yellow crystals, melting at 248–250°C, was obtained, the substance being identical according to its physical properties with the glucoside *Vb*, obtained microbiologically. The β-configuration of the product was demonstrated by enzymic cleavage[12] with β-glucosidase. NMR spectrum (in $(CD_3)_2SO$ + CF_3COOD): 3·09–3·90 (m, 6 H, O—CH), 5·12 (d, $J = 6·5$ Hz, 1 H, O—CH—O), 7·15 (q, $J = J_o = 8·5$ Hz, $J = J_m = 2$ Hz, 1 H, $H_{(7)}$), 7·30 (q, $J = J_o = 8$ Hz, $J = J_m = 2$ Hz, 1 H, $H_{(3)}$), 7·48 (d, $J = J_m = 2$ Hz, 1 H, $H_{(5)}$), 7·64 (d, $J = J_m = 2$ Hz, 1 H, $H_{(1)}$), 8·03 (d, $J = J_o = 8·5$ Hz, 1 H, $H_{(8)}$); 8·10 (d, $J = J_o = 8$ Hz, 1 H, $H_{(4)}$). For $C_{20}H_{18}O_9$ (402·3) calculated: 59·7% C, 4·5% H; found: 59·6% C, 4·6% H.

2,6-*Bis*-(β-D-*glucopyranosyloxy*)-9,10-*anthraquinone* (Vc): A mixture of 115 mg acetate *IVc* and 40 ml ammoniacal methanol was stirred in the absence of air moisture for 1 h and then left to stand overnight at 0°C. On the following day the reaction mixture was concentrated *in vacuo* and the dried residue was twice recrystallized from methanol. A total of 45 mg (62·7%) product *Vc* was obtained, m.p. 278–280°C. (ref.[13] gives 254°C). NMR spectrum (in $(CD_3)_2SO$):

3·26—3·96 (m, 10 H, O—CH), 5·14 (m, 2 H, O—CH), 7·48 (q, $J = J_m = 2·5$ Hz, $J = J_o = 8$ Hz, 2 H, $H_{(3)}$ and $H_{(7)}$), 7·70 (d, $J = J_m = 5·5$ Hz, 2 H, $H_{(1)}$ and $H_{(5)}$), 8·14 (d, $J = J_o = 8$ Hz, 2 H, $H_{(4)}$ and $H_{(8)}$). For $C_{26}H_{28}O_{14}$ (564·5) calculated: 55·3% C, 5·0% H; found: 55·5% C, 5·3% H.

TABLE III

$[\alpha]_D$ Values of Compounds Prepared[a]

Compound	$[\alpha]_D$	*c*	Solvent
IVa	-64^0	0·68	$CHCl_3$
Va	-50^0	0·74	C_5H_5N
IVb	-32^0	0·68	$CHCl_3$
Vb	-57^0	0·72	C_5H_5N
IVc	-55^0	0·68	$CHCl_3$
Vc	-85^0	0·72	C_5H_5N

[a] Measured on automatic polarimeter Bendix Ericsson 143A at 25°C.

The authors are indebted to Dr M. Bučko, Spofa, Slovenská Lupča, for a kind gift of bikaverin. Their thanks are due to Dr P. Sedmera for recording and interpreting the NMR spectra, to Dr H. Lipavská for recording the IR and UV spectra and to Mr J. Vlna for elementary analyses.

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Translated by A. Kotyk.