9. Schmidt, K., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **25** (1971) 2476.
10. Liaaen-Jensen, S. In Isler, O. *Carotenoids*, Birkhäuser, Basel 1971, Chapter 3.
11. Hertzberg, S. and Liaaen-Jensen, S. *Phytochemistry* **8** (1969) 1281.
12. Liaaen-Jensen, S. and Jensen, A. In San Pietro, A. *Methods Enzymol.* **23** (1971) 586.
13. Kjøsen, H., Arpin, N. and Liaaen-Jensen, S. *Acta Chem. Scand. In press.*
14. Pifferi, P. G. *Anal. Chem.* **37** (1965) 925.
15. Adachi, S. *J. Chromatogr.* **17** (1965) 295.

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Bacterial Carotenoids XXXIX* C_{50}-Carotenoids 10.** Bacterioruberin Mono- and Diglucoside

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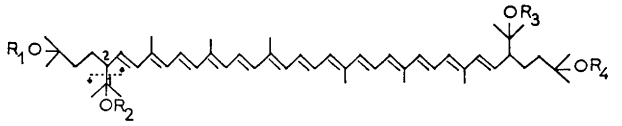
Bacterioruberin, the characteristic carotenoid of halophilic bacteria has been assigned the C_{50}-tetrol structure *1*.[2,3] We now report the first isolation of bacterioruberin monoglycoside (*2*) and diglycoside (*3*).

A moderately halophilic bacterium strain BOS 66 from glacial mud (collection Gounot) was examined. From the biological material collected from a total of 1500 Petri dishes 76 mg carotenoid was obtained, constituting 0.05 % of the extracted cell residue. Bacterioruberin (*1*) comprised 33 %, the monoglycoside *2* 30 % and the diglycoside *3* 35 % of the total carotenoid.

Bacterioruberin (*1*) was identified from its electronic spectrum (λ_{max} 370, 387, 443, 496, and 530 nm, % III/II^3 = 54 in acetone) and mass spectrum (*m/e* 740 = M corresponding to $C_{50}H_{76}O_4$, M − 18, M − 18 − 18, M − 18 − 18 − 18, M − 58, M − 18 − 18 − 18 − 18, M − 92, M − 106, M − 58 − 58, M − 106 − 18, M − 106 − 18 − 18 *etc.*) and co-chromatography tests with authentic *1*.

The monoglycoside *2* exhibited the same electronic spectrum as bacterioruberin (*1*) and was more strongly adsorbed. The IR-spectrum (KBr) suggested its glycosidic nature (ν_{max} 3400, 1075 and 1040 (broad), 955, 900 cm^{-1}; only diagnostically important bands are cited). The mass spectra of the monoglycoside (*m/e* 902 = M corresponding to $C_{50}H_{75}O_4(C_6H_{11}O_5)$, M − 58, M − 92, M − 106, M − 158, M − 162) and of the acetylation product *5* formed under standard acetylation conditions (*m/e* 1070 = M corresponding to $C_{50}H_{75}O_4C_6H_{11}O(OCOCH_3)_4$, M − 58, M − 60, M − 92, M − 106, M − 158, 331, 211, 169, 115, 109, 105, 91) and the tri(trimethylsilyl) ether *6* thereof (*m/e* 1286 = M corresponding to $C_{50}H_{75}(OSiCH_3)_3OC_6H_{11}O(OCOCH_3)_4$, M − 92, M − 106, M − 131, 331, 169, 131 (100 %), 115, 109, 105, 91) revealed the presence of four hydroxy groups accessible for acetylation and three tertiary hydroxy groups in *2*. The fragmentation pattern of the acetylated products *5* and *6* further revealed the presence of a glycosidically bound hexose (*m/e* 331, 211,



1 $R_1 = R_2 = R_3 = R_4 = H$
2 $R_2 = R_3 = R_4 = H$, R_1 = hexosyl
2a R_1 = glucosyl
2b R_1 = mannosyl
3 $R_2 = R_3 = H$, $R_1 = R_4$ = hexosyl
4 $R_1 = R_2 = R_3 = R_4 = SiMe_3$
5 $R_2 = R_3 = R_4 = H$, R_1 = tetraacetylhexosyl
6 $R_2 = R_3 = R_4 = SiMe_3$, R_1 = tetraacetylhexosyl
7 $R_2 = R_3 = H$, $R_1 = R_4$ = tetraacetylhexosyl
8 $R_2 = R_3 = SiMe_3$, $R_1 = R_4$ = tetraacetylhexosyl

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169, 115 and 109)[4] in *2*. Moreover, the chemical behaviour, electronic and mass spectra supported that the aglycone involved was bacterioruberin (*1*). Prominent peaks at M − 58 for *2* and M − 131 for *6*, compatible with cleavage of the 1-single bond,[2] are taken to support the location of the hexose unit in the extra C_5-unit of *2*. The PMR-spectrum ($CDCl_3$) of the triol tetraacetate *5* [τ 8.80 (*gem.* Me); 8.02, 8.00, 7.98, 7.96 (in-chain and acetate Me) 5.7(CH_2OAc), 6.0 − 4.5 (hexose methine H), 4.0 − 3.0 (olefinic H)] is consistent with structure *2* for the new glycoside. Glycoside hydrolysis[5] of the triol tetraacetate *5* followed by paper chromatography of the resultant hexose revealed the presence of glucose (*ca.* 80 % of total) and mannose (ca. 20 % of total). The hexoside *2* is thus a mixed glycoside, a situation previously encountered for other carotenoid glycosides, *cf.* myxoxanthophyll[6] and oscillaxanthin.[7] The glucoside *2a* could not be separated from the mannoside *2b* even as acetylated or silylated derivatives in any systems tried in agreement with previous experience.[6,7]

The diglycoside *3* exhibited the same electronic spectrum as bacterioruberin (*1*) and was considerably more strongly adsorbed that *1* and *2*. The IR-spectrum (KBr) suggested its glycosidic nature (ν_{max} 3400, 1110 − 1035 (broad) and 960 cm^{-1}) and the PMR-spectrum ($CDCl_3$) of the acetylated product *7* [τ 8.80 (8 *gem.*Me); 8.02, 8.00, 7.98 and 7.96 (14 Me, in-chain + acetate Me); 5.82(CH_2OAc); 4.8 − 6.0 (hexose methine H) and 4.0 − 3.0 (olefinic H)] was compatible with an octaacetate and revealed the dihexoside nature of *3*. The mass spectra of *7* (M_{calc} = 1400) or the silylated acetate *8* (M_{calc} = 1656) showed no molecular ions, but strong *m/e* 331 peaks. *8* exhibited fragment ions above *m/e* 1500. Sugar hydrolysis of *7* gave glucose and mannose in approximately the same proportion as for *2* above, demonstrating a mixed diglycoside. A sharp doublet at τ 5.44 (J = 9.5 cps, ax.-ax.) for the octaacetate *7* is compatible with the anomeric proton of a β-D-glucoside.[8] By analogy with the monoglycoside *2* the two hexoses are most likely symmetrically bound to the aglycone as in *3*. A disaccharide formulation is considered less likely from the PMR and mass spectrometric evidence obtained.

Both carotenoid glucosides and mannosides are previously reported.[9] Naturally occurring carotenoid glycosides have recently been reviewed[9] and further C_{50}-glycosides have subsequently been described.[10]

Experimental. Strain BOS 66 was cultivated on 1500 Petri dishes in the medium of Lochhead[11] (5 % NaCl); yield 146 g wet cells. Extraction with acetone, then with methanol and finally with dimethyl sulphoxide provided 76 mg carotenoids (0.053 % of the extracted residue). Lipids were removed by repeated precipitation from cold acetone. Additional lipids necessitated standard[12] saponification with 5 % KOH in methanol. Chromatography twice on acetylated polyamide (Machery Nagel Polyamid 6-AC) recovered 33 mg carotenoids.

Bacterioruberin (*1*), eluted with 3 % methanol in benzene; R_F = 0.43 on Schleicher & Schüll No. 287 paper, 20 % acetone in petroleum ether (S & S 287 20 % AP), was crystallized from chloroform-petroleum ether; yield *ca.* 6 mg.

The monoglycoside (*2*) required 5 − 6 % methanol in benzene for elution from acetylated polyamide, had R_F = 0.03 on TLC (silica gel G 50 % AP) and was crystallized from methanol-petroleum ether; yield *ca.* 6 mg.

The diglycoside (*3*) required 9 % methanol in benzene for elution from acetylated polyamide, exhibited R_F = 0 on TLC (silica gel G 50 % AP) and was crystallized from ethanol-petroleum ether; yield *ca.* 7 mg.

Spectra were recorded as specified elsewhere.[13] Samples for mass spectra were purified by TLC on silica gel G.

Acetylations and silylations were effected by standard procedures.[12] R_F-values of the derivatives were:

4 0.56 (S & S 287 0 % AP); *5* 0.80 (S & S 287 20 % AP) and 0.51 (TLC silica gel 50 % AP); *6* 0.46 (S & S 287 5 % AP) and 0.55 (TLC 30 % AP); *7* 0.24 (S & S 287 20 % AP) and 0.57 (TLC 50 % AP); and *8* 0.27 (S & S 287 15 % AP).

Glycoside hydrolysis was effected with *ca.* 4 mg carotenoid in methanol with 0.17 N HCl followed by hydrolysis of the resulting methyl glycoside with polystyrene sulphonic acid.[14] Paper chromatography of the reducing sugars was carried out in System 4[6] with glucose, galactose, and mannose as reference substances.

Results are given in the main text.

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1. Kelly, M. and Liaaen-Jensen, S. *Acta Chem. Scand.* **21** (1967) 2578.
2. Kelly, M., Norgård, S. and Liaaen-Jensen, S. *Acta Chem. Scand.* **24** (1970) 2169.
3. Ke, B., Imsgard, F., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta* **210** (1970) 139.
4. Bieman, K., DeJongh, D. C. and Schnoes, H. K. *J. Am. Chem. Soc.* **85** (1963) 1763.
5. Painter, T. J. *Chem. Ind. (London)* **1960** 1214.
6. Hertzberg, S. and Liaaen-Jensen, S. *Phytochemistry* **8** (1969) 1259.
7. Hertzberg, S. and Liaaen-Jensen, S. *Phytochemistry* **8** (1969) 1281.
8. a. Jackman, L. M. and Sternhell, S. *Application of Nuclear Magnetic Resonance Spectra in Organic Chemistry*, 2nd Ed., Pergamon, London 1969; b. Schmidt, K., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **25** (1971) 2476.
9. Liaaen-Jensen, S. In Isler, O. *Carotenoids*, Birkhäuser, Basel 1971.
10. Arpin, N., Liaaen-Jensen, S. and Trouilloud, M. *Acta Chem. Scand.* **26** (1972) 2524.
11. Gounot, A. M. *Ann. Spéléol.* **22** (1967) 23.
12. Liaaen-Jensen, S. and Jensen, A. *Methods Enzymol.* **23** (1971) 586.
13. Kjøsen, H., Arpin, N. and Liaaen-Jensen, S. *Acta Chem. Scand. In press.*
14. Norgård, S., Francis, G. W., Jensen, A. and Liaaen-Jensen, S. *Acta Chem. Scand.* **24** (1970) 1460.

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Bacterial Carotenoids XL.* 2′-Hydroxyflexixanthin

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The carotenoids of the gliding flexibacteria have been the subject of a number of investigations.[1-6] Flexixanthin (*2*) and the less abundant deoxyflexixanthin (*1*) are peculiar to several flexibacteria;[1-3] other flexibacteria produce structurally related carotenoids.[1,2,4-6]

We now report the isolation of flexixanthin (*2*) and the previously undescribed 2′-hydroxyflexixanthin (*3*) from a non-gliding bacterium, strain NIVA BR6-64, of uncertain taxonomic position.

Table 1. Properties of the carotenoids studied.

Carotenoid	λ_{max} in acetone in nm	R_F-values a	b
Deoxyflexixanthin (*1*)	478	0.52	0.55
Flexixanthin (*2*)	478,503	0.40	0.27
2′-Hydroxyflexixanthin (*3*)	478,504	0.19	0.10
Flexixanthin acetate (*4*)	478	0.50	0.48
4 Trimethylsilyl ether (*5*)	477	0.68	0.90
Dehydroflexixanthin (*6*)	478	0.38	0.0
2′-Ketoflexixanthin (*7*)	500	0.16	0.18
2′-Ketoflexixanthin acetate (*8*)	499	0.31	
8 Trimethylsilyl ether (*9*)	500	0.58	0.87
2′-Ketodehydroflexixanthin trimethylsilyl ether (*10*)	499	0.40	0.0

a. Schleicher & Schüll No. 287 (kieselguhr) paper; 10 % acetone in petroleum ether.
b. Schleicher & Schüll No. 288 (aluminium oxide) paper; 20 % acetone in petroleum ether.

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