Bacterial Carotenoids XXXVIII* C_{50}-Carotenoids 9.** Isolation of Decaprenoxanthin Mono- and Diglucoside from an *Arthrobacter* sp.

N. ARPIN,[a] S. LIAAEN-JENSEN[b] and M. TROUILLOUD[a]

[a] *Département de Biologie Végétale, Service de Phytochimie et Phytophysiologie, Université de Lyon, 69 Villeurbanne, France*

[b] *Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, Trondheim, Norway*

The first known naturally occurring C_{50}-carotenoid, *dehydrogenans*-P439,[1] later re-named decaprenoxanthin,[2] has been assigned structure *1*.[3,4]

Recently Weeks and Andrewes [5] assigned the decaprenoxanthin monoglucoside (*2*) structure to corynexanthin.[6] Their identification was based on electronic and mass spectra of the glucoside itself and isolation of glucose after acid hydrolysis.

We now report further characterization of decaprenoxanthin monoglucoside (*2*) isolated from an *Arthrobacter* sp. M3, and the first isolation of decaprenoxanthin diglucoside (*3*).

The carotenoids (0.03 % of the extracted residue) present in *Arthrobacter* M3 were estimated to be: decaprenoxanthin (*1*, 20 % of the total carotenoid), decaprenoxanthin monoglucoside (*2*, 30 %), P428 (1 %) and decaprenoxanthin diglucoside (*3*, 50 %). Due to a very high lipid content the isolation of the pure carotenoids was laborious.

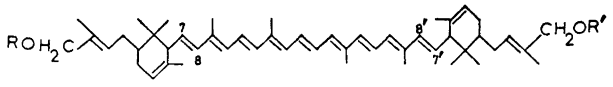
The identification of decaprenoxanthin (*1*, *ca.* 6 mg) was based on co-chromatography with authentic *1* and on spectral evidence for the free diol (λ_{max} (hexane) 413, 438 and 468 nm, % III/II[7] = 101; τ ($CDCl_3$) 9.23 (2 Me), 9.04 (2 Me), 8.48 (2 Me), 8.38 (2 Me), 8.08 (2 Me), 8.03 (2 Me), 6.00 (2 CH_2OH) and 4.7 – 3.0 (olefinic H); *m/e* 704 = M, M – 16, M – 79, M – 92, M – 106, M – 140, M – 158) and for the diacetate (*m/e* 758 = M, M – 60, M – 92, M – 106, M – 182) in agreement with previous findings.[3]

Crystalline decaprenoxanthin monoglucoside (*2*, *ca.* 3.5 mg) had m.p. 152 – 154°C; λ_{max} as for *1* above; ν_{max} (KBr) 3400, 3020, 2960, 2920, 2870, 1600, 1440, 1380, 1360, 1080, 1020 and 970 cm^{-1}; *m/e* 866 (M), M – 18, M – 79, M – 92, M – 106, M – 140, M – 162, M – 180. Acetylation afforded a pentaacetate (*4*); *m/e* 1076 (M), M – 42, M – 58, M – 60, M – 79, M – 92, M – 60 – 42, M – 106, M – 92 – 42, M – 158, M – 182, M – 92 – 60 – 42, 331 (50 %), 169 (100 %), *cf.* Refs. 3 and 8.

Crystalline decaprenoxanthin diglucoside (*3*, *ca.* 10 mg) had m.p. 192 – 195°C; λ_{max} as for *1* above; ν_{max} 3400, 3020, 2960, 2920, 2860, 1580, 1470, 1380, 1360, 1160, 1070, 1020, 970 and 895 cm^{-1}. Acetylation gave the octaacetate (*5*, 6.5 mg); m.p. 159 – 162°C, ν_{max} (KBr) 3020, 2960, 2920, 2890, 1750, 1620, 1470, 1380, 1360, 1220, 1170, 1040, 970 and 905 cm^{-1}; τ ($CDCl_3$) 9.22 (2 Me), 9.04 (2 Me), 8.40 (2 Me), 8.38 (2 Me), 8.08 (*ca.* 2 Me), 8.00 and 7.93 (*ca.* 10 Me, 2 in-chain Me and 8 acetate Me), 5.90 and 5.80 (*ca.* 4 $-CH_2O-$) and 5.7 – 3.0 (glucose methine H and olefinic H); *m/e* 1364 (M), M – 42, M – 42 – 42, M – 92, M – 60 – 42, M – 92 – 42, M – 42 – 60, M – 92 – 42 – 42, M – 42 – 42 – 42 – 60, M – 92 – 42 – 60, M – 42 – 42 – 60 – 60, M – 92 – 42 – 42 – 60, M – 42 – 42 – 42 – 60 – 60, M – 330, M – 346, 331, 169, 145, 109, 43 (100 %). On acid hydrolysis [8] *5* gave no other hexose than glucose, identified by co-chromatography. The stereochemistry of the glucose moiety (D or L) and of the glucosidic linkage was not estab-

* No. XXXVII. *Acta Chem. Scand.* **26** (1972) 2194.

** No. 8 *Acta Chem. Scand.* **25** (1971) 402.



1 R = R' = H
2 R = glucosyl, R' = H
3 R = R' = glucosyl
4 R = tetraacetylglucosyl, R' = acetyl
5 R = R' = tetraacetylglucosyl

lished. However, a doublet (*ca.* 2 H) with $J = 9$ cps (ax.-ax.) at τ 5.40 assigned to the anomeric protons, *cf.* rhodopinal-β-D-glucoside tetraacetate,[9] is suggestive of β-D or α-L configuration. Weeks and Andrewes[5] observed a positive glucose oxidase test for corynexanthin after hydrolysis supporting that corynexanthin is decaprenoxanthin mono-D-glucoside.

Finally small amounts of a new carotenoid P428, possibly representing the 7,8 or 7′,8′ dihydro derivative of *2* was obtained.

The high proportion of glucosidically bound carotenoids in *Arthrobacter* M3 is noteworthy. Several carotenoid monoglycosides are now known.[10] Beside oscillaxanthin, a dirhamnoside,[11] decaprenoxanthin diglucoside (*3*) is the only diglycoside so far reported.

Experimental. Arthrobacter sp., strain M3 collection Gounot, was cultivated in 1500 Petri dishes (solid medium containing yeast extract 7 g/l, glucose 10 g/l, glycerol 15 g/l; pH 7) at 22°C for 40 h in light; yield *ca.* 140 g wet weight after centrifugation.

For isolation and characterization of the carotenoids general methods were employed.[9,12,13] The carotenoids were extracted with acetone-methanol; yield 42 mg. Lipids were partially removed by repeated precipitation from acetone, reducing the purified carotenoids to 25 mg. Additional lipids prevented separation on acetylated polyamide (Machery Nagel Polyamid 6-AC) and standard saponification in 5 % KOH (ether-methanol) was resorted to. Usual work-up by ether extraction from an aqueous hypophase gave an ether extract containing 11 mg carotenoid. Pigments (12 mg, mainly diglucoside) insoluble in ether were extracted with water saturated butanol.

The pigments of the ether extract were chromatographed on acetylated polyamide and gave decaprenoxanthin (*1*, 6 mg) and the monoglucoside (*2*, 5 mg). *2* was further purified by preparative TLC on silica gel.

From the butanol extract additional lipids were removed by successive precipitation. Chromatography on acetylated polyamide provided the monoglucoside (*2*) and the diglucoside (*3*).

Acetylations were effected in the usual way.[12] The octaacetate (*5*, 4 mg) was hydrolyzed by the procedure previously described[8] at 100°C for 5 h. Co-chromatography of the resultant hexose with glucose, galactose, mannose, arabinose, and xylose on kieselgel G impregnated with 0.02 M sodium acetate ($CHCl_3 : CH_3OH$ 60/40)[14] and kieselgel G impregnated with 0.1 M sodium bisulfite (propanol–H_2O 85 : 15)[15] revealed identity with glucose.

Adsorptive properties were as follows: Decaprenoxanthin (*1*) was eluted from acetylated polyamide with benzene and had $R_F = 0.57$ (S & S 287 10 % AP), 0.53 (S & S 288 20 % AP), 0.25 (TLC 20 % AP), and 0.50 (TLC 30 % AP). S & S refers to Schleicher & Schüll papers, TLC to kieselgel, AP to acetone in petroleum ether and MB to methanol in benzene. *1* diacetate had $R_F = 0.73$ (S & S 287 5 % AP), 0.86 (S & S 288 10 % AP) and 0.68 (TLC 20 % AP).

The monoglucoside *2* required 2 % MB for elution from acetylated polyamide and had $R_F = 0.60$ (S & S 287 50 % MB) and 0 (TLC 30 % AP). The pentaacetate *4* had $R_F = 0.32$ (S & S 287 5 % AP), 0.80 (S & S 287 10 % AP), 0.64 (S & S 288 15 % AP), and 0.48 (TLC 30 % AP).

The diglucoside *3* was eluted with 6 % MB from acetylated polyamide. R_F-values of the octaacetate *5* were 0.18 (S & S 287 10 % AP), 0.39 (S & S 288 15 % AP), and 0.30 (TLC 30 % AP).

Other results are given in the general text.

Acknowledgements. We thank Dr. A. M. Gounot, Service d'Ecologie bacterienne, Université de Lyon, for providing a pure culture of the strain used. Siv.ing. H. Kjøsen kindly assisted in obtaining PMR and mass spectra. N. A. was supported in Trondheim by a grant from Hoffmann-La Roche, Basel, to S.L.J.

1. Liaaen-Jensen, S. and Weeks, O. B. *Nor. J. Chem., Mining, Met.* **26** (1966) 130.
2. Weeks, O. B., Andrewes, A. G., Brown, B. O. and Weedon, B. C. L. *Nature* **224** (1969) 879.
3. Liaaen-Jensen, S., Hertzberg, S., Weeks, O. B. and Schwieter, U. *Acta Chem. Scand.* **22** (1968) 1171.
4. Schwieter, U. and Liaaen-Jensen, S. *Acta Chem. Scand.* **23** (1969) 1057.
5. Weeks, O. B. and Andrewes, A. G. *Arch. Biochem. Biophys.* **137** (1970) 284.
6. Hodgkiss, W., Liston, J., Goodwin, T. W. and Jamikorn, M. *J. Gen. Microbiol.* **11** (1954) 438.
7. Ke, B., Imsgard, F., Kjøsen, H. and Liaaen-Jensen, S. *Biochim. Biophys. Acta* **210** (1970) 139.
8. Norgård, S., Francis, G. W., Jensen, A. and Liaaen-Jensen, S. *Acta Chem. Scand.* **24** (1970) 1460.

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.

Received May 12, 1972.

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

N. ARPIN,[a] J.-L. FIASSON[a] and S. LIAAEN-JENSEN[b]

[a] *Département de Biologie Végétale, Service de Phytochimie et Phytophysiologie, Université de Lyon, 69 Villeurbanne, France*

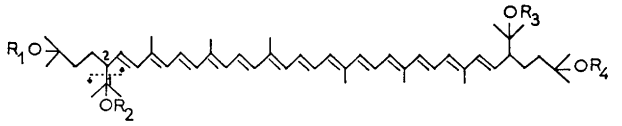
[b] *Organic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, Trondheim, Norway*

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

* No. XXXVIII *Acta Chem. Scand.* **26** (1972) 2524.

** No. 9. *Acta Chem. Scand.* **26** (1972) 2524.