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A NEW CAROTENOID GLUCOSIDE ESTER FROM *CHONDROMYCES APICULATUS*

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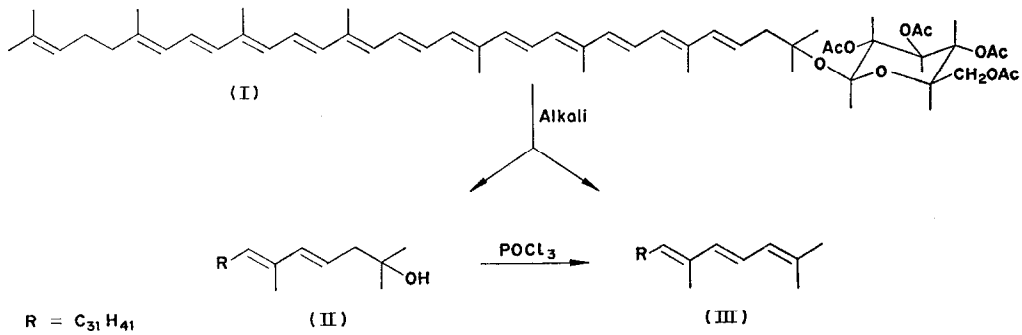
Abstract—The carotenoid composition of the myxobacterium *Chondromyces apiculatus* is reported. A new acyclic carotenoid glucoside ester was isolated and its structure determined as 1′-glucosyloxy-3′,4′-didehydro-1′,2′-dihydro-ψ,ψ-carotene monoester.

THE GLIDING myxobacteria are colored by a wide variety of carotenoid pigments, many characteristically having carotenoid glucoside esters.[1-3] The pigment composition of *Chondromyces apiculatus* Thaxter is shown in Table 1. The distribution pattern is similar to that of *Sorangium compositum* Jahn,[2] another member of the suborder of Sorangineae. *Ch. apiculatus* lacks, however, carotenoid rhamnosides and contains one new acyclic glucoside ester which has been identified as 1′-glucosyloxy-3′,4′-didehydro-1′,2′-dihydro-ψ,ψ-carotene monoester (I).



(I) (II) (III)

Spectroscopic (λ_{max} 482 nm in EtOH) and chromatographic data and saponification suggested that this new pigment was a monoacylated glucoside with 12 conjugated carbon–carbon double bonds in an acyclic polyene chain. Mild saponification of the pigment yielded only two spots when chromatographed on silica gel thin layers, the natural undegraded compound and the more polar deacylated derivative. The latter was found as the only pigment after strong saponification. Acetylation experiments followed by chromatography showed that the natural pigment yielded mono-, di-, and tri-acetates, whereas the

[1] KLEINIG, H., REICHENBACH, H. and ACHENBACH, H. (1970) *Arch. Microbiol.* **74**, 223.
[2] KLEINIG, H., REICHENBACH, H., ACHENBACH, H. and STADLER, J. (1971) *Arch. Mikrobiol.* **78**, 224.
[3] REICHENBACH, H. and KLEINIG, H. (1971) *Arch. Mikrobiol.* **76**, 364.

saponified pigment yielded an additional tetraacetate. These results show that this pigment is monoacylated, as are other carotenoid glucosides known from the myxobacteria.[1,2]

The MS of the pigment after saponification and peracetylation confirmed the calculated molecular weight of 882 and showed characteristic M-92, M-106, M-106-92, M-106-106 peaks.[4,5] A prominent ion at *m/e* 69 was indicative of an isopropylidene end group. The medium mass range was characterized by ions at *m/e* 525 and *m/e* 429 (loss of the sugar moiety including the glycosidic oxygen and additional loss of xylene, respectively). Prominent ions at *m/e* 331, *m/e* 169, *m/e* 109 and *m/e* 43 were characteristic for the sugar moiety.[6]

TABLE 1. CAROTENOID COMPOSITION OF *Chondromyces apiculatus*

Compound	% of total
Carotenes	38
γ-Carotene (β,ψ-carotene)	
Lycopene (ψ,ψ-carotene)	
Hydroxycarotenes	5
1′,2′-Dihydro-β,ψ-carotene-1′-ol	
3′,4′-Didehydro-1′,2′-dihydro-β,ψ-carotene-1′-ol	
1′,2′-Dihydro-ψ,ψ-carotene-1′-ol	
3′,4′-Didehydro-1′,2′-dihydro-ψ,ψ-carotene-1′-ol	
Carotenoid ester	2
3′,4′-Didehydro-1′,2′-dihydro-β,ψ-carotene-1′,2′-diol 2′-acylate	
3′,4′-Didehydro-1′,2′-dihydro-ψ,ψ-carotene-1′,2′-diol 2′-acylate	
Carotenoid glucoside ester	
1′-Glucosyloxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-carotene ester	31
1′-Glucosyloxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-carotene-3-ol ester	19
1′-Glycosyloxy-3′,4′-didehydro-1′,2′-dihydro-ψ,ψ-carotene ester	5

The PMR spectrum had the expected signals at δ 1·97 (in-chain methyl, theor. 5 Me) δ 1·82 (end-of-chain methyl, 1 Me), and δ 1·17 (*gem.* methyl). The isopropylidene group gave rise to signals at δ 1·68 and δ 1·62 (1 + 1 Me). The anomeric proton of the glucose gave a doublet at δ 4·68 (J 8 Hz, ax. ax.) which supported a β-D-configuration of the glucoside.[7,8]

Removal of the monoacylated glucose moiety by harsh alkaline treatment yielded 3′,4′-didehydro-lycopene (III) and 3′,4′-didehydro-1′,2′-dihydro-1′-hydroxy-lycopene (II) as expected. These pigments were identified by their electronic spectra and by cochromatography with authentic samples isolated from *Myxococcus fulvus*. II was easily silylated (not acetylated) and was converted to III by $POCl_3$ treatment.

The structure of the new glucoside was further established by nicotine inhibition experiments in another myxobacterium, *Myxococcus fulvus* Jahn. Nicotine inhibited the cyclization of carotenoids which led to the accumulation of this acyclic glucoside ester not present under normal conditions in *M. fulvus*. After removal of nicotine from the growth medium, the acyclic glucoside ester was cyclized to 1′-glucosyloxy-3′,4′-didehydro-1′,2′-dihydro-β,

[4] SCHWIETER, U., BOLLIGER, H. R., CHOPARD-DIT-JEAN, L. H., ENGLERT, G., KOFLER, M., KÖNIG, A., PLANTA, C., RÜEGG, R., VETTER, W. and ISLER, O. (1965) *Chimia* **19**, 294.
[5] ENZELL, C. R., FRANCIS, G. W. and LIAAEN JENSEN, S. (1969) *Acta Chem. Scand.* **23**, 727.
[6] BIEMAN, K., DEJONGH, D. C. and SCHNOES, H. H. (1963) *J. Am. Chem. Soc.* **85**, 1763.
[7] JACKMAN, L. M. and STERNHELL, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd Edn, Pergamon Press, Oxford.
[8] SCHMIDT, K., FRANCIS, G. W. and LIAAEN JENSEN, S. (1971) *Acta Chem. Scand.* **25**, 2476.

ψ-carotene ester under anaerobic condition and cyclized *plus* oxidized to myxobacton ester (1′-glucosyloxy-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-4-one ester) under aerobic conditions.[9] This led to the conclusion that the new pigment serves as a biosynthetic intermediate.

A similar carotenoid glucoside has been recently described from *Rhodopseudomonas acidophila* strain 7050 which lacks, however, the double bond between C3′ and C4′.[8] This double bond is very characteristic for all carotenoid glycosides hitherto isolated from myxobacteria.

EXPERIMENTAL

Organism. Chondromyces apiculatus, strain Cm a2, was isolated from rotten wood in Minneapolis, Minnesota, U.S.A., in 1966. The strain is deposited at the 'Mikrobenbank der Gesellschaft für Strahlen- und Umweltforschung' D-34 Göttingen. The organism was grown in a liquid medium consisting of a suspension of baker's yeast, 0·5% by fresh wt; Casitone (Difco), 0·4%; $CaCl_2 \times 2H_2O$, 0·15%; $MgSO_4 \times 7H_2O$, 0·15%; vitamin B_{12}, 0·1 mg/ml; pH 7·0. The cultures were kept on a rotary shaking thermostat at 30° and illuminated with 2 Phillips TLD 30W/33 fluorescent tubes. The bacteria grew under these conditions as bright orange nodules of 1–4 mm dia.

General methods. Pigments were extracted and separated by chromatography on silica gel and magnesium oxide as described.[2,10] The known pigments were identified by the spectroscopic, chromatographic, and chemical methods previously reported.[1,2,10]

1′-Glucosyloxy-3′,4′-didehydro-1′,2′-dihydro-ψ,ψ-carotene ester. Available *ca.* 7 mg, Glucose was identified after hydrolysis by TLC;[1] fatty acids were determined as their methyl esters by GLC.[1] There were several acids present (mainly 14:0, 16:0, 16:1, 18:0, 18:1; see also Ref. 1). Harsh alkaline treatment for splitting the glycosidic bond was performed in ethanolic KOH (1·3 g KOH in 10 ml 96% EtOH) for 2 hr at 70°.[2] The $POCl_3$ reaction was done as reported.[1] 5·5 mg of the saponified pigment were peracetylated in the usual manner. The PMR spectrum showed signals at δ 1·17 (*gem.* methyl), 1·62 and 1·68 (isopropylidene methyl, 1 + 1 Me), 1·82 (end-of-chain methyl, 1 Me), 1·97 (in-chain methyl, theor. 6 Me), 2·01, 2·05, 2·10 (acetate methyl, theor, 4 Me), 3·72 (H from C5 of glucose), 4·15 (2H from C6 of glucose), 4·68 (anomeric H, J 8 Hz, ax. ax.). MS were obtained from an AEI MS9 spectrometer using the direct insertion probe. The ionizing energy was 70 eV; the ion source temperature was 200°. *m/e* 882 (M), M-92, M-106, M-106-92, M-106-106, M-92/M-106 = 0·06 indicating 12 conjugated double bonds in the chain,[11] *m/e* 535, 429, 331, 211 (22%), 169 (96%), 157 (15%), 109 (62%), 69 (50%), 43 (100%).

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