# Isolation of a New Carotenoid and Two New Carotenoid Glycosides from *Curtobacterium flaccumfaciens pvar poinsettiae*

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An efficient method for the extraction of the carotenoids from *Curtobacterium flaccumfaciens pvar poinsettiae* was developed. The glucosides of *C.p. 450* (=(all-*E*,2*R*,2′*R*)-2-[4-(*β*-D-glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(4-hydroxy-3-methylbut-2-enyl)-*β*,*β*-carotene; **4**) and of *C.p. 473* (=(all-*E*,2*R*,2′*S*)-2-[4-(*β*-D-glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(3-methylbut-2-enyl)-3′,4′-didehydro-1′,2′-dihydro-*β*,*ψ*-caroten-1′-ol; **5**) were isolated for the first time. In addition, the hitherto unknown 3′,4′-dihydro derivative of *C.p. 450*, called *C.p. 460* (=(all-*E*,2*R*,2′*R*)-2-(4-hydroxy-3-methylbut-2-enyl)-2′-(3-methylbut-2-enyl)-1′,2′-dihydro-*β*,*ψ*-caroten-1′-ol; **6**), was identified. The structures were established by UV/VIS, CD, $^1$H- and $^{13}$C-NMR, and mass spectra.

**1. Introduction.** – *Curtobacterium flaccumfaciens* is a plant pathogen which exists in four pathovariations differing in their hosting plant. As the name implies, *C.f. pvar poinsettiae*, first discovered in 1942 [1][2], is a pest of the plant *Poinsettia*, which is commonly used as decorative indoor plant. Young cells exhibit the shape of short rods with a length of 0.6 to 3 μm and a diameter of *ca.* 0.5 μm, older cells are usually almost coccoid. *C. f. pvar poinsettiae* are *Gram*-positive, but with age this property is sometimes lost. The organisms owe their color to carotenoids and can be yellow, orange, or pink depending on the cultivation conditions.

The first reports on carotenoids in *C. f. pvar poinsettiae* go back to the 1950's [3–5], and the main carotenoids were described by *Norgård et al.* [6], *Andrewes* and *Liaaen-Jensen* [7], and *Britton et al.* [8]. The two major carotenoids are the $C_{50}$-diols *C.p. 450* (=(all-*E*,2*R*,2′*R*)-2,2′-bis(4-hydroxy-3-methylbut-2-enyl)-*β*,*β*-carotene; **1**) and *C.p. 473* (= (all-*E*,2*R*,2′*S*)-2-(4-hydroxy-3-methylbut-2-enyl)-2′-(3-methylbut-2-enyl)-3′,4′-didehydro-1′,2′-dihydro-*β*,*ψ*-caroten-1′-ol; **2**), named after the maxima in their UV/VIS absorption spectra. Furthermore, considerable amounts of bisanhydrobacterioruberin (=(all-*E*,2*S*,2′*S*)-3,4,3′,4′-tetradehydro-1,2,1′,2′-tetrahydro-2,2′-bis(3-methylbut-2-enyl)-*ψ*,*ψ*-carotene-1,1′-diol; *C.p. 496*; **3**) and small amounts of 3,4,3′,4′-tetrahydrobisanhydrobacterioruberin (=(all-*E*,2*R*,2′*R*)-1,2,1′,2′-tetrahydro-2,2′-bis(3-methylbut-2-enyl)-*ψ*,*ψ*-carotene-1,1′-diol), 2-isopentenyl-3,4-dehydrorhodopin ((all-*E*,2*S*)-3,4-didehydro-1,2-dihydro-2-(3-methylbut-2-enyl)-*ψ*,*ψ*-caroten-1-ol), and lycopene have been reported [6].

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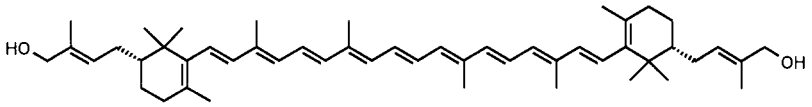
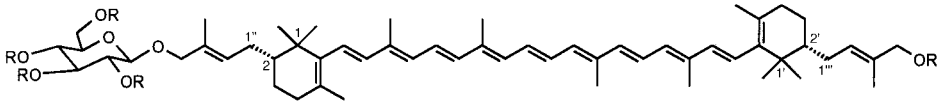
[1]) Part of the planned Ph. D. Thesis of *A. H.*

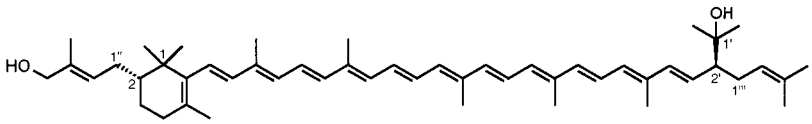
[2]) Part of the Ph. D. Thesis of *Ch. B.*



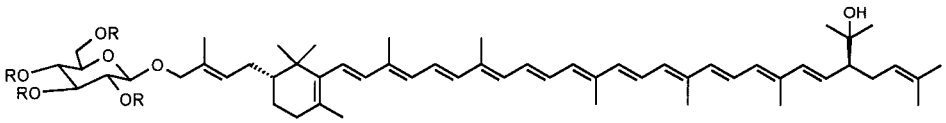
**1** *C.p. 450*



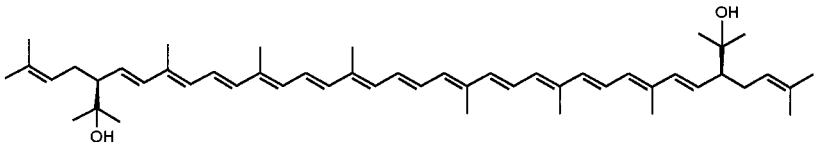
**4** R = H
**7** R = Ac



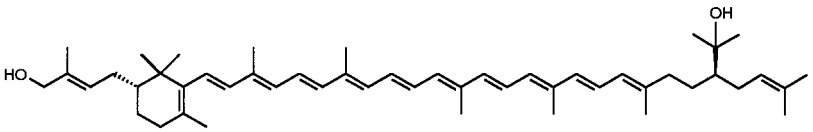
**2** *C.p. 473*



**5** R = H
**8** R = Ac



**3** bisanhydrobacterioruberin



**6** *C.p. 460*

For the determination of the absolute configuration, *C.p. 450* (**1**), *C.p. 473* (**2**), bisanhydrobacterioruberin (**3**), 3,4,3′,4′-tetrahydrobisanhydrobacterioruberin, and 2-isopentenyl-3,4-dehydrorhodopin have been synthesized in optically active form [9–11].

The present study reports the reinvestigation of the carotenoid composition of *C. f. pvar poinsettiae*, especially the isolation and structure elucidation of two new carotenoid glycosides, namely **4** ((all-*E*,2*R*,2′*R*)-2-[4-(β-D-glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(4-hydroxy-3-methylbut-2-enyl)-β,β-carotene), representing the β-D-glucoside of *C.p. 450* (**1**), and **5** ((all-*E*,2*R*,2′*S*)-2-[4-(β-D-glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(3-methylbut-2-enyl)-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-1′-ol), the β-D-glucoside of *C.p. 473* (**2**). In addition, a new carotenoid **6** was isolated and identified as (all-*E*,2*R*,2′*R*)-2-(4-hydroxy-3-methylbut-2-enyl)-2′-(3-methylbut-2-enyl)-1′,2′-dihydro-β,ψ-caroten-1′-ol. This new compound represents the 3′,4′-dihydro derivative of **2** and, in analogy to the trivial names for the compounds isolated previously from *C. f. pvar poinsettiae*, we propose for **6**, which exhibits in the UV/VIS spectrum a maximum of 460 nm, the trivial name *C.p. 460*.

**2. Results and Discussion.** – *C. f. pvar poinsettiae* was cultivated at 24° and pH 6.8 for 70 h in a medium used by *Mundy* [12]. However, to increase the biomass, the concentration of all nutrients was continuously increased tenfold during the cultivation. The cell walls were broken with lysozyme in a phosphate-buffer solution and the resulting slimy material lyophilized. After extraction with MeOH, the dry crude MeOH extract was dissolved in $CH_2Cl_2$ and further separated by washing with $H_2O$ and precipitation (see *Exper. Part*). The final extract was investigated by reversed-phase HPLC ($C_{18}$, see *Fig.*).

As shown in the *Figure*, *C.p. 450* (**1** (all-*E*); peak *15*) and *C.p. 473* (**2** (all-*E*); peak *10*) constitute, as expected, the major carotenoids of *C. f. pvar poinsettiae*, and in addition, also bisanhydrobacterioruberin (**3**; peak *8*) is identified. Furthermore, also the presence of geometrical isomers of **1** (peaks *16* and *17*) and **2** (peaks *11*, *12*, and *14*) is tentatively suggested. The question remains open whether these (*Z*)-isomers are artefacts formed during the extraction and isolation or are natural isomers. Of special interest are the highly polar compounds of the peaks *1–7* which might be glycosides, and our investigations were focused on the hitherto unknown carotenoids of peak *5* (compound **5**), peak *7* (compound **4**), and of peak *13* (compound **6**) which exhibit a medium intensity in the chromatogram. The isolation of the desired compounds was performed by repeated column chromatography and semi-prep. reversed-phase HPLC (see *Exper. Part*).

The compounds **4** and **5** exhibit the same UV/VIS spectra as *C.p. 450* (**1**) and *C.p. 473* (**2**), respectively [8]. Due to the relative instability and low volatility of **4** and **5**, the molecular mass was determined with a linear home-built MALDI-TOF-MS instrument. The molecular ion at 867.7 and 867.1 is consistent with the mass of *C.p. 450* (**1**) and *C.p. 473* (**2**), respectively, linked to a hexose. Acetylation of **4** and **5** gave the compounds **7** and **8**, respectively, which exhibit in the MALDI-TOF-MS molecular ions at *m/z* 1077.5 for **7** and 1019.4 for **8**, corresponding to the pentaacetate of **4** and to the tetraacetate of **5**, respectively. These results support our previous assumption that the polar compounds of the peaks *1–7* might be glycosides of the $C_{50}$-carotenoids formed by the increased glucose concentration in the cultivation medium. The EI-MS of **4** and **5** exhibit the highest mass peak at *m/z* 687, corresponding to the mass of *C.p. 450* (**1**) or *C.p. 473* (**2**), respectively, without the O-linked glucose moiety. The $^1$H-NMR data of the aglycon of **4** and **5** (*Table 1*) are identical with those reported by *Britton et al.* [8],

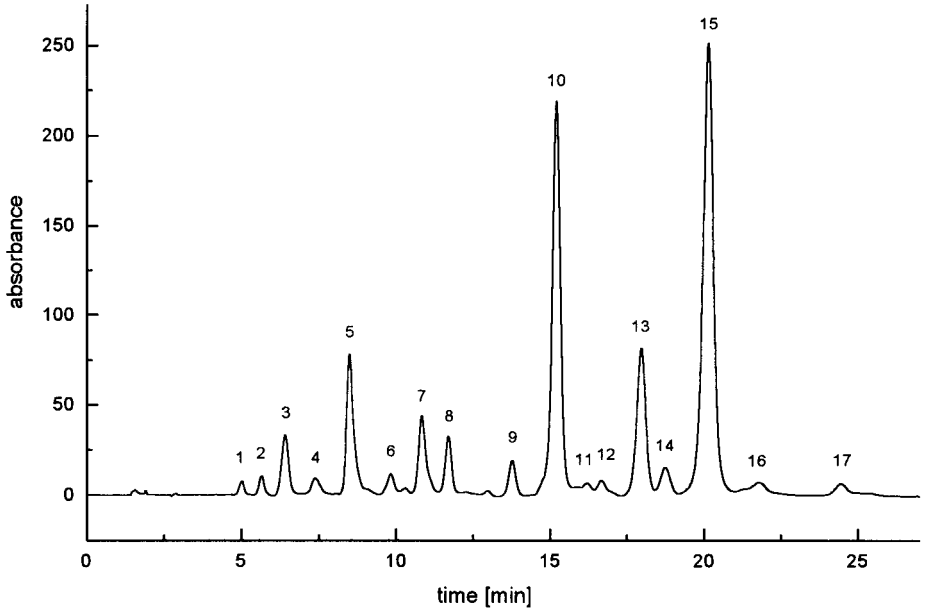



Figure. *Typical HPLC profile of carotenoids of an extract of* C. f. pvar poinsettiae *on a* $C_{18}$ *reversed-phase column* (MeOH/MeCN/$H_2O$/$^i$Pr$_2$EtN 50:47:2.75:0.25). Detection at 460 nm. Peak identification: *1:* ? ($\lambda_{max}$ (eluent) 478 nm)[3]); *2:* ? (477)[3]); *3:* ? (477)[3]); *4*: ?; *5:* **5** ((all-*E*)); *6:* ? (465): *7:* **4** ((all-*E*)); *8:* bisanhydrobacterioruberin (**3**)[4]); *9:* ? (486); *10: C.p. 473* (**2**); *11:* (*Z*)-**2** (473)[4]); *12:* (*Z*)-**2** (470)[4]); *13: C.p. 460* (**6**); *14:* (*Z*)-**2** (469)[4]); *15: C.p. 450* (**1**); *16:* (*Z*)-**1** (451)[4]); *17:* (*Z*)-**1** (447)[4]).

except for the protons at the side chain connected to C(2). Further $^1$H-NMR and CD data confirm the proposed structure of **4** and **5**. For the determination of the absolute configuration of the glucose moieties, **4** and **5** were cleaved by mild methanolysis and the formed carbohydrates perbenzylated. The perbenzylated carbohydrates were isolated by reversed-phase HPLC and their CD spectra (from 200 to 250 nm) compared with authentic samples and literature data [13], thus establishing the D-configuration of the glucose moiety in **4** and **5**.

The $^1$H-NMR signals for $CH_2$ (4″) of **4** and **5** consisting of two *d* demonstrate that the carbohydrate moiety is linked to the primary-alcohol function of **1** and **2**. The identification of the carbohydrate moiety of **4** and **5** is based on the $^1$H-NMR data of the corresponding acetates **7** and **8**. From the cross peaks in the $^1$H,$^1$H-COSY spectra of **7** and **8** and starting with the *d* at 4.51 ppm for the proton at the anomeric C-atom, a series of five consecutive proton signals with coupling constants of 7.8–9.7 Hz, indicating that all the protons are diaxially oriented, are observed. Therefore, the carbohydrate moiety of **7** and **8**, as well as that of **4** and **5**, is identified as $\beta$-glucopyranose; moreover, our NMR data are in full agreement with data previously reported for similar compounds [14]. The determination of the configuration at C(2) and C(2′) of the aglycon is based on the CD spectra (– 180°, $Et_2O$/isopentane/EtOH 5:5:2) of **4** and **5** which are identical with that of synthetic *C.p. 450* ((all-*E*,2*R*,2′*R*); **1**) [9] and *C.p. 473* ((all-*E*,2*R*,2′*S*); **2**) [10], respectively, demonstrating the (2*R*,2′*R*)-configuration for **4** and the (2*R*,2′*S*)-configuration for **5**.

[3]) Probably carotenoid diglycosides.

[4]) Tentative identification based on chromatographic behavior, and UV/VIS and MS data.

Table 1. *$^1$H-NMR* (400 MHz, $CDCl_3$) *Data of* **4** *and* **5**. Chemical shifts $\delta$ in ppm and coupling constants $J$ in Hz.

| | Aglycon of **4** | | Aglycon of **5** | |
|---|---|---|---|---|
| H–C(2) | *ca.* 1.30 | (*m*) | *ca.* 1.31 | (*m*) |
| $H_a$–C(3) | *ca.* 1.30 | (*m*) | *ca.* 1.31 | (*m*) |
| $H_b$–C(3) | *ca.* 1.65 | (*m*) | *ca.* 1.66 | (*m*) |
| $CH_2$(4) | *ca.* 2.00 | (*m*) | *ca.* 1.99 | (*m*) |
| H–C(7) | 6.16 | (*d*, *J* = 15.7) | 6.15 | (*d*, *J* = 15.7) |
| H–C(8) | 6.09 | (*d*, *J* = 15.7) | 6.09 | (*d*, *J* = 15.7) |
| H–C(10) | 6.16 | (*d*, *J* = 11.2) | 6.16 | (*d*, *J* = 11.2) |
| H–C(11) | 6.66 | (*dd*, *J* = 11.2, 15.0) | 6.66 | (*dd*, *J* = 11.2, 14.8) |
| H–C(12) | 6.36 | (*d*, *J* = 15.0) | 6.36 | (*d*, *J* = 14.8) |
| H–C(14) | *ca.* 6.26 | (*m*) | *ca.* 6.26 | (*m*) |
| H–C(15) | *ca.* 6.64 | (*m*) | *ca.* 6.64 | (*m*) |
| Me(16) | 0.92 | (*s*) | 0.92 | (*s*) |
| Me(17) | 1.08 | (*s*) | 1.08 | (*s*) |
| Me(18) | 1.70 | (*s*) | 1.70 | (*s*) |
| Me(19) | 1.98 | (*s*) | 1.99 | (*s*) |
| Me(20) | 1.98 | (*s*) | 1.99 | (*s*) |
| H–C(2′) | *ca.* 1.30 | (*m*) | 2.10 | (*m*) |
| $H_a$–C(3′) | *ca.* 1.30 | (*m*) | 5.50 | (*dd*, *J* = 9.3, 15.6) |
| $H_b$–C(3′) | *ca.* 1.65 | (*m*) | | |
| $CH_2$(4′) | *ca.* 2.00 | (*m*) | | |
| H–C(4′) | | | 6.18 | (*d*, *J* = 15.6) |
| H–C(6′) | | | 6.12 | (*d*, *J* = 11.7) |
| H–C(7′) | 6.16 | (*d*, *J* = 15.7) | 6.58 | (*dd*, *J* = 11.7, 15.5) |
| H–C(8′) | 6.09 | (*d*, *J* = 15.7) | 6.35 | (*d*, *J* = 15.5) |
| H–C(10′) | 6.16 | (*d*, *J* = 11.2) | 6.21 | (*d*, *J* = 11.4) |
| H–C(11′) | 6.66 | (*dd*, *J* = 11.2, 15.0) | 6.62 | (*dd*, *J* = 11.4, 15.3) |
| H–C(12′) | 6.36 | (*d*, *J* = 15.0) | 6.37 | (*d*, *J* = 15.3) |
| H–C(14′) | *ca.* 6.26 | (*m*) | *ca.* 6.24 | (*m*) |
| H–C(15′) | *ca.* 6.64 | (*m*) | *ca.* 6.64 | (*m*) |
| Me(16′) | 0.92 | (*s*) | 1.19 | (*s*) |
| Me(17′) | 1.08 | (*s*) | 1.23 | (*s*) |
| Me(18′) | 1.70 | (*s*) | 1.93 | (*s*) |
| Me(19′) | 1.98 | (*s*) | 1.99 | (*s*) |
| Me(20′) | 1.98 | (*s*) | 1.99 | (*s*) |
| $H_a$–C(1″) | *ca.* 1.81 | (*m*) | *ca.* 1.81 | (*m*) |
| $H_b$–C(1″) | *ca.* 2.22 | (*m*) | *ca.* 2.23 | (*m*) |
| H–C(2″) | 5.50 | (br. *t*, *J* = 6.8) | 5.49 | (br. *t*, *J* = 8.8) |
| Me–C(3″) | 1.71 | (*s*) | 1.70 | (*s*) |
| $H_a$–C(4″) | 4.06 | (*d*, *J* = 11.4) | 4.06 | (*d*, *J* = 11.8) |
| $H_b$–C(4″) | 4.26 | (*d*, *J* = 11.4) | 4.26 | (*d*, *J* = 11.8) |
| $H_a$–C(1‴) | *ca.* 1.80 | (*m*) | *ca.* 2.00 | (*m*) |
| $H_b$–C(1‴) | *ca.* 2.21 | (*m*) | *ca.* 2.33 | (*m*) |
| H–C(2‴) | 5.45 | (br. *t*, *J* = 6.8) | 5.07 | (br. *t*, *J* = 7.2) |
| Me–C(3‴) | 1.69 | (*s*) | 1.61 | (*s*) |
| $CH_2$(4‴)/Me(4‴) | 4.04 | (*s*) | 1.67 | (*s*) |
| | Glycon of **7** (after acetylation) | | Glycon of **8** (after acetylation) | |
| H–C(1⁗) | 4.51 | (*d*, *J* = 7.8) | 4.51 | (*d*, *J* = 8.1) |
| H–C(2⁗) | 5.04 | (*dd*, *J* = 7.8, 9.4) | 5.04 | (*dd*, *J* = 8.1, 9.7) |
| H–C(3⁗) | 5.11 | (*t*, *J* = 9.4) | 5.11 | (*t*, *J* = 9.7) |
| H–C(4⁗) | 5.22 | (*t*, *J* = 9.4) | 5.22 | (*t*, *J* = 9.7) |
| H–C(5⁗) | 3.67 | (*ddd*, *J* = 2.5, 4.7, 9.4) | 3.67 | (*ddd*, *J* = 2.6, 4.6, 9.7) |
| $H_a$–C(6⁗) | 4.16 | (*dd*, *J* = 2.5, 12.3) | 4.15 | (*dd*, *J* = 2.6, 12.4) |
| $H_b$–C(6⁗) | 4.28 | (*dd*, *J* = 4.7, 12.3) | 4.28 | (*dd*, *J* = 4.6, 12.4) |
| AcO | 2.02, 2.04, 2.06, 2.10, 2.11 | | 2.02, 2.04, 2.05, 2.11 | |

The UV/VIS spectrum of **6** with a maximum at 460 nm exhibits a prominent fine structure and is in accordance with the chromophore of $\beta,\psi$-carotene with 11 C=C bonds. In the MALDI-TOF-MS of **6**, the signal for the molecular ion is observed at *m/z* 707.1, which corresponds to $C_{50}H_{74}O_2$ and to *C.p. 473* (**2**) with two additional H-atoms. By means of $^1$H- and $^{13}$C-NMR (*Table 2*), DEPT, $^1$H,$^1$H-COSY, $^1$H,$^{13}$C-COSY, T-ROESY, and HMBC experiments, all protons and C-atoms can unambiguously be assigned, and the presence of the 3,4-dihydroanhydrobacterioruberin end group is established. Based on the comparison of the CD spectra of synthetic **2** ((all-*E*,2*R*,2′*S*)) exhibiting a positive maximum at 235 nm and a negative maximum at 304 nm [10], with isolated **6** exhibiting a positive maximum at 255 nm and a negative maximum at 298 nm, the configuration of **6** is tentatively assigned as (2*R*,2′*R*). The UV/VIS, MS, NMR, and CD data of **6** are consistent with the structure of (all-*E*,2*R*,2′*R*)-2-(4-hydroxy-3-methylbut-2-enyl)-2′-(3-methylbut-2-enyl)-1′,2′-dihydro-$\beta,\psi$-caroten-1′-ol.

Table 2. *$^1$H-NMR* (400 MHz, $CDCl_3$) *Data of* **6**. Chemical shifts $\delta$ in ppm and coupling constants *J* in Hz.

| | | | | | |
|---|---|---|---|---|---|
| H−C(2) | *ca.* 1.30 | (*m*) | H−C(2′) | *ca.* 1.39 | (*m*) |
| $H_a$−C(3) | *ca.* 1.30 | (*m*) | $H_a$−C(3′) | *ca.* 1.29 | (*m*) |
| $H_b$−C(3) | *ca.* 1.67 | (*m*) | $H_b$−C(3′) | *ca.* 1.68 | (*m*) |
| $H_a$−C(4) | *ca.* 1.98 | (*m*) | $H_a$−C(4) | *ca.* 2.09 | (*m*) |
| $H_b$−C(4) | *ca.* 1.98 | (*m*) | $H_b$−C(4) | *ca.* 2.23 | (*m*) |
| | | | H−C(6′) | 5.95 | (*d*, *J* = 11.0) |
| H−C(7) | 6.17 | (*d*, *J* = 15.9) | H−C(7′) | 6.49 | (*dd*, *J* = 11.0, 15.1) |
| H−C(8) | 6.10 | (*d*, *J* = 15.9) | H−C(8′) | 6.25 | (*d*, *J* = 15.1) |
| H−C(10) | 6.16 | (*d*, *J* = 11.2) | H−C(10′) | 6.19 | (*d*, *J* = 11.3) |
| H−C(11) | 6.66 | (*dd*, *J* = 11.2, 14.9) | H−C(11′) | 6.64 | (*dd*, *J* = 11.3, 14.9) |
| H−C(12) | 6.36 | (*d*, *J* = 14.9) | H−C(12′) | 6.36 | (*d*, *J* = 14.9) |
| H−C(14) | *ca.* 6.27 | (*m*) | H−C(14′) | *ca.* 6.27 | (*m*) |
| H−C(15) | *ca.* 6.63 | (*m*) | H−C(15′) | *ca.* 6.63 | (*m*) |
| Me(16) | 0.92 | (*s*) | Me(16′) | 1.19 | (*s*) |
| Me(17) | 1.08 | (*s*) | Me(17′) | 1.23 | (*s*) |
| Me(18) | 1.71 | (*s*) | Me(18′) | 1.81 | (*s*) |
| Me(19) | 1.98 | (*s*) | Me(19′) | 1.98 | (*s*) |
| Me(20) | 1.98 | (*s*) | Me(20′) | 1.98 | (*s*) |
| $H_a$−C(1″) | *ca.* 1.80 | (*m*) | $H_a$−C(1‴) | *ca.* 2.03 | (*m*) |
| $H_b$−C(1″) | *ca.* 2.21 | (*m*) | $H_b$−C(1‴) | *ca.* 2.20 | (*m*) |
| H−C(2″) | 5.45 | (*t*, *J* = 6.6) | H−C(2‴) | 5.23 | (*t*, *J* = 7.2) |
| Me−C(3″) | 1.69 | (*s*) | Me−C(3‴) | 1.66 | (*s*) |
| $CH_2$(4″) | 4.04 | (*s*) | Me(4‴) | 1.72 | (*s*) |

The identification of the major compounds *C.p. 450* (**1**; peak *15*) and *C.p. 473* (**2**; peak *10*) is based on their UV/VIS, MS, and $^1$H- and $^{13}$C-NMR data which are in full agreement with the data reported by *Britton et al.* [8]. In addition, bisanhydrobacterioruberin (**3**) and different (*Z*)-isomers of *C.p. 450* (**1**) and *C.p. 473* (**2**) are identified by means of their chromatographic behavior and UV/VIS and MS data.

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## Experimental Part

1. *General.* Column chromatography (CC): silica gel *60* (*Fluka*, 40–63μm). HPLC: *Hewlett Packard 1100*, consisting of a degasser *G1322A*, a quaternary pump *G1311A*, and a photodiode array detector *G1315*. HPLC: reversed phase, *Nucleosil 120-3* $C_{18}$, *Macherey-Nagel AG*; normal phase; *Nucleosil 300-5*, *Macherey Nagel AG.* UV/VIS: *Perkin-Elmer Lambda-6*; $\lambda_{max}$ in nm. CD: *Jobin-Yvon Dichrograph-6*; in EPA ($Et_2O$/isopentane/EtOH 5:5:2) at −180°. $^1$H- and $^{13}$C-NMR: *Bruker DRX 400* (400 and 100.61 MHz, resp.); in $CDCl_3$ at 20°; chemical shifts $\delta$ in ppm rel. to $CDCl_3$ (= 7.27 and 77.0 ppm, resp.), *J* in Hz. EI-MS: *Varian MAT CH-7A*; ionization energy 70 eV; in *m/z* (rel. intensity in %). MALDI-TOF-MS: home-built linear (1.5 m) instrument; acceleration energy 24 kV; 337-nm $N_2$-laser; matrix 2,5-dihydroxybenzoic acid; in *m/z*.

2. *Cultivation.* The cell material was obtained from the *Deutsche Sammlung für Mikroorganismen und Zellkulturen* (*DSMZ*) in Braunschweig. The cultivation was started on agar plates with culture medium A: glucose (0.5%), $KH_2PO_4$ (0.5%), $K_2HPO_4$ (0.5%), $NH_4Cl$ (0.1%), casein (0.1%), $MgSO_4 \cdot 7\,H_2O$ (0.02%), sodium citrate (0.01%), calcium pantothenate (0.0001%), thiamine (0.0001%), and biotin ($10^{-7}$%) in distilled water. The cells harvested from the plates were filled into ampoules and kept in liquid $N_2$. The frozen material (0.5 ml) was added to 200 ml of culture medium A and shaken for 2 days. Three of these cultures were brought into an industrial 10-l glass fermenter containing 5 l of culture medium A. After 20 h, 5 l of twenty-fold concentrated medium A was slowly added over 50 h. The temp. was kept at 24°, the pH at 6.8, and the air saturation at 25% by adjusting the stirring speed. After centrifugation in a *Heräus* centrifuge, 380 g of dark-orange cell material was obtained and frozen (−20°).

3. *Extraction.* The frozen cell material (138 g) was mixed with 0.1M phosphate buffer pH 7.5 (600 ml). After the formation of a homogeneous suspension, lysozyme (0.7 g; 109000 units/mg; *Fluka*, from hen egg white) was added. The mixture was stirred for 15 h at 25° in the dark. The slimy orange material was frozen in liquid $N_2$ and lyophilized for 4 d. Grinding of the dry material in a mortar gave a fine, orange powder which was extracted 4 times with MeOH (200 ml). The MeOH layers were filtered over *Celite*, combined, and evaporated. The orange solid was dissolved in $CH_2Cl_2$ (400 ml) and the red org. layer washed 4 times with $H_2O$ (a small amount of sat. aq. NaCl soln. was added for a better phase separation). The $CH_2Cl_2$ layer was stored at −20° overnight and the white precipitation then filtered over *Celite.* For the HPLC of the complete extract (*Fig.*), a sample of the filtrate (0.5 ml) was evaporated, redissolved in MeOH/AcOEt, and submitted to reversed-phase HPLC (250 × 4.6 mm, isocratic MeOH/MeCN/$H_2O$/$^i$Pr$_2$EtN 50:47:2.75:0.25, flow 1 ml/min). The remaining filtrate was dried ($MgSO_4$) and evaporated.

4. *Isolation.* The red solid was submitted to CC (12 × 2 cm, AcOEt/MeOH/$Et_3N$ 93:6.5:0.5): *Fr. 1* (**1**/**2**/**3**/**6**) and *Fr. 2* (**4**/**5**). *Fr. 1* was submitted to a second CC (17 × 2 cm, hexane/AcOEt/$^i$Pr$_2$EtN 70:29:1): *Fr. 1.1* (**2**/**6**) and *Fr. 1.2* (**1**). *Fr. 2* was separated by semi-prep. reversed-phase HPLC (250 × 10 mm, isocratic MeOH/MeCN/$H_2O$/$^i$Pr$_2$EtN 57:40:2.7:0.3, flow 3.0 ml/min): **4** and **5**. *Fr. 1.1* was separated and *Fr. 1.2* purified by reversed-phase HPLC (250 × 10 mm, isocratic MeOH/MeCN/$H_2O$/$^i$Pr$_2$EtN 50:47:2.7:0.3, flow 3.5 ml/min): *C.p. 450* (**1**), *C.p. 473* (**2**), and *C.p. 460* (**6**). *C.p. 460* (**6**) was further purified by semi-prep. normal-phase HPLC (250 × 10 mm, isocratic hexane/AcOEt/$^i$Pr$_2$EtN 77:22.5:0.5, flow 3.0 ml/min). The fractions were immediately evaporated and stored at −20° under Ar.

5. *Acetylation of* **4** *and* **5**. To a soln. of **4** (1 mg) in pyridine/$Ac_2O$ 2:1 (3 ml), a catalytic amount of 4-(dimethylamino)pyridine was added. The mixture was stirred at r.t. for 18 h and then diluted with $^t$BuOMe (25 ml). The org. phase was washed with sat. aq. $NH_4Cl$ soln., sat. aq. $NaHCO_3$ soln. and 3 times with $H_2O$, dried ($MgSO_4$), and evaporated and the residue submitted to reversed-phase HPLC (250 × 10 mm, isocratic MeCN/MeOH/$H_2O$/$^i$Pr$_2$EtN 50:47:2.7:0.3, flow 3.2 ml/min): pentaacetate **7**.

The same procedure was applied for the acetylation of **5**: tetraacetate **8**.

6. *Absolute Configuration of the Carbohydrate Moiety of* **4** *and* **5**. Compound **4** or **5** in 0.1M HCl/MeOH (2 ml) was heated under reflux for 8 h. The solvent was evaporated, and pyridine (1 ml), benzoic anhydride (50 mg) and 4-(dimethylamino)pyridine (5 mg) were added. After 50 h at r.t., the mixture was diluted with $^t$BuOMe (10 ml), washed twice with 0.5M HCl and $H_2O$, and evaporated. The benzylated carboydrate was isolated by reversed-phase HPLC (250 × 10 mm, isocratic MeCN/$H_2O$ 75:25, flow 3.2 ml/min): perbenzylated D-glucose.

7. *(all-*E,*2*R,*2′*R*)-2-[4-(β-*D*-Glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(4-hydroxy-3-methylbut-2-enyl)-β,β-carotene* (**4**). Isolated yield 1.1 mg. UV/VIS (EtOH): 424, 450, 477. CD (EPA, −180°): 351, 287, 250, 225, 209. $^1$H-NMR ($CDCl_3$): *Table 1.* EI-MS: 687 (3, $[M-179]^+$), 537 (10), 522 (10), 327 (45), 299 (45), 225 (43), 185 (44), 117 (69), 57 (100), 43 (75). MALDI-TOF-MS: 867.7.

8. *(all-*E*,2*R*,2′*R*)-2-[4-(β-*D*-Glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(4-hydroxy-3-methylbut-2-enyl)-β,β-carotene Pentaacetate* (**7**). UV/VIS (EtOH): 423, 450, 477. $^1$H-NMR ($CDCl_3$): *Table 1*. MALDI-TOF-MS: 1077.5.

9. *(all-*E*,2*R*,2′*S*)-2-[4-(β-*D*-Glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(3-methylbut-2-enyl)-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-1′-ol* (**5**). Isolated yield 1.8 mg. UV/VIS (EtOH): 445, 473, 504. CD (EPA, −180°): 359 (+8), 350 (+10), 305 (−23), 266 (−11), 240 (+12), 221 (−14), 214 (+4), 208 (−10). $^1$H-NMR ($CDCl_3$): *Table 1*. EI-MS: 687 (6, $[M-179]^+$), 610 (7), 522 (10), 386 (22), 327 (43), 299 (51), 225 (48), 185 (60), 103 (75), 57 (100), 43 (69). MALDI-TOF-MS: 867.1.

10. *(all-*E*,2*R*,2′*S*)-2-[4-(β-*D*-Glucopyranosyloxy)-3-methylbut-2-enyl]-2′-(3-methylbut-2-enyl)-3′,4′-didehydro-1′,2′-dihydro-β,ψ-caroten-1′-ol Tetraacetate* (**8**). UV/VIS (EtOH): 444, 473, 504. $^1$H-NMR ($CDCl_3$): *Table 1*. MALDI-TOF-MS: 1019.4.

11. *(all-*E*,2*R*,2′*R*)-2-(4-Hydroxy-3-methylbut-2-enyl)-2′-(3-methylbut-2-enyl)-1′,2′-dihydro-β,ψ-caroten-1′-ol* (**6**). Isolated yield 2.2 mg. UV/VIS (EtOH): 435, 460, 490. CD (EPA, r.t.): 254 (+0.8), 288 (−1.2), 353 (+0.7), 466 (+0.8). CD (EPA, −180°): 213 (+0.8), 226 (−0.5), 255 (+6.4), 298 (−2.0), 363 (+2.5), 380 (+1.8), 422 (+5.0), 450 (+8.7), 475 (+ 11.8), 512 (+10.1). $^1$H-NMR ($CDCl_3$): *Table 2*. $^{13}$C-NMR ($CDCl_3$): *ca.* 12.81 (Me(19), Me(20), Me(19′), Me(20′)); 13.88 (*Me*−C(3″)); 16.87 (Me(18′)); 17.94 (*Me*−C(3‴)); 21.92 (Me(18)); 22.44 (Me(16)); 23.29 (C(3)); 25.91 (C(4‴)); 27.07 (Me(16′)); 27.36 (Me(17)); 27.78 (Me(17′)); 28.30 (C(1″)); 28.98 (C(3′)); 29.03 (C(1‴)); 32.12 (C(4)); 37.64 (C(1)); 39.53 (C(4′)); 45.30 (C(2)); 49.20 (C(2′)); 69.15 (C(4″)); 74.14 (C(1′)); 124.36 (C(2‴)); 124.71 (C(7′)); 125.01 (C(11′)); 125.12 (C(11)); 125.92 (C(6′)); 126.38 (C(2″)); 127.12 (C(7)); 128.91 (C(5)); 130.04 (C(14), C(14′)); 130.87 (C(10)); 131.60 (C(10′)); 131.93 (C(3‴)); 132.48 (C(15)); 132.64 (C(15′)); 135.31 (C(3″)); 135.46 (C(8′)); 135.92 (C(9′)); 136.12 (C(9)); 136.48 (C(13′)); 136.51 (C(13)); 137.32 (C(12)); 137.40 (C(12′)); 138.25 (C(6)); 138.45 (C(8)); 139.73 (C(5′)). EI-MS: 707 (6, $M^+$), 689 (4), 671 (4), 601 (5), 106 (36), 105 (48), 91 (100), 69 (97), 55 (43), 43 (43), 41 (83). MALDI-TOF-MS: 707.1.

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