

63. Synthesis of Carotenoids in the Gliding Bacteria *Taxeobacter*: (all-*E*,2'*R*)-3-Deoxy-2'-hydroxyflexixanthin

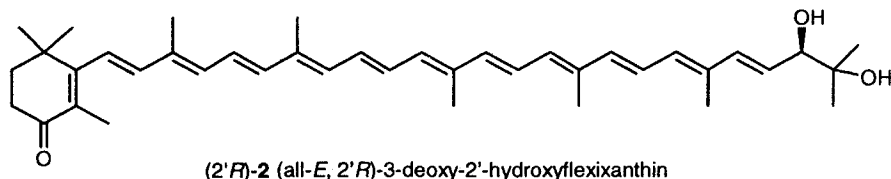
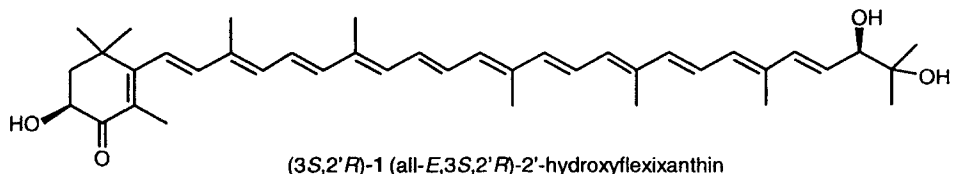
by Christof Bircher¹⁾ and Hanspeter Pfander*

Departement für Chemie und Biochemie, Universität Bern, Freiestrasse 3, CH-3012 Bern

(30.I.97)

The C₄₀-carotenoid (all-*E*,2'*R*)-deoxy-2'-hydroxyflexixanthin (= 1',2'-dihydroxy-3',4'-didehydro-1',2'-dihydro- β , ψ -caroten-4-one; (2'*R*)-**2**) was synthesized according to a C₁₅ + C₁₀ + C₅ + C₁₀ = C₄₀ strategy. The chiral centre was introduced into the C₁₀-end group by the enantioselective *Sharpless* dihydroxylation. The four building blocks were coupled by applying four consecutive *Wittig* reactions. By comparison of the CD spectra of the synthetic (2'*R*)-**2** with those of **2** isolated from the gliding bacteria *Taxeobacter*, the configuration of natural **2** was determined as (2'*R*).

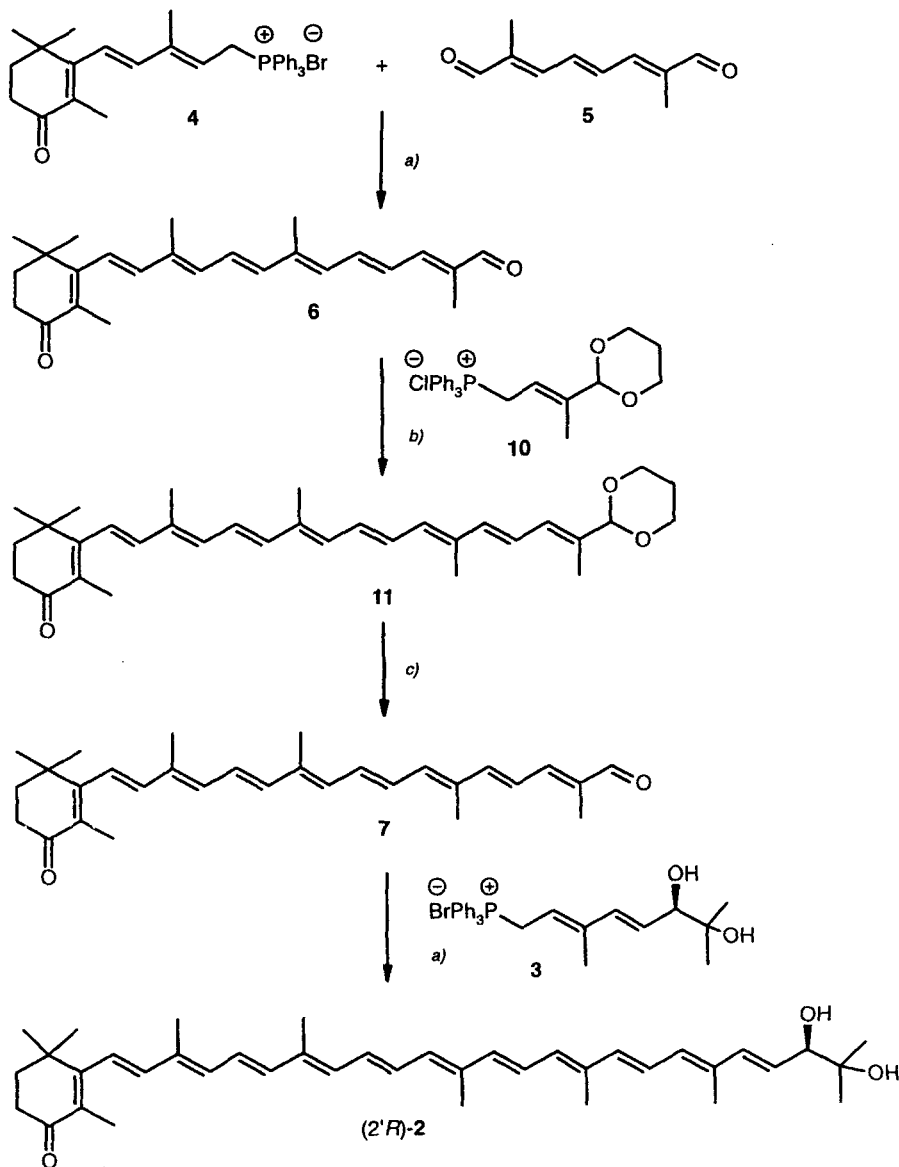
1. Introduction. – Carotenoids are not only encountered in many higher plants and in animals, they are also common in bacteria and archaea. The gliding bacteria *Taxeobacter* was found for the first time by *Reichenbach* [1] [2] in cultures of *E. coli*. Later, several different species of *Taxeobacter* have been isolated from soils [1] [2]. Recently, *Böhlendorf* and *Reichenbach* [3] identified 2'-hydroxyflexixanthin (= 3,1',2'-trihydroxy-3',4'-didehydro-1',2'-dihydro- β , ψ -caroten-4-one; **1**) as major carotenoid of the dark-red-coloured bacteria and, in addition, also the previously unknown 3-deoxy-2'-hydroxyflexixanthin (= 1',2'-dihydroxy-3',4'-didehydro-1',2'-dihydro- β , ψ -caroten-4-one; **2**) was isolated as minor pigment. However, the configuration at the asymmetric C-atoms of **1** and **2** was not established. The 2'-hydroxyflexixanthin (**1**) had been previously isolated by *Andrewes*



¹⁾ Diploma work and part of the Ph.D. thesis of C.B.

et al. from *Flexibacter* (strain NIVA BRG-64), and, based on comparison of the CD spectra with the spectra of plectanixanthin (= 3',4'-didehydro-1',2'-dihydro- β,ψ -carotene-1',2'-diol), the (3*S*,2'*S*)-configuration was postulated [4]. In view of the structure elucidation, especially by comparison of the CD spectra of the natural and the synthetic compounds, we report in the following the synthesis of (all-*E*,2'*R*)-3-deoxy-2'-hydroxyflexixanthin ((2'*R*)-2).

Scheme 1



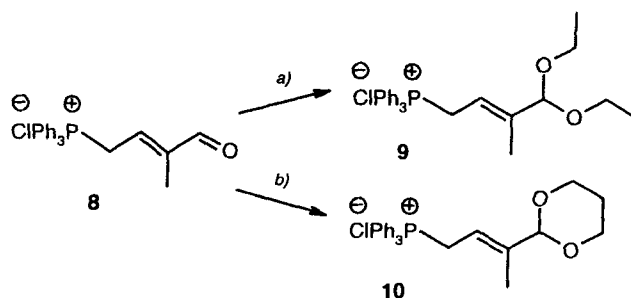
a) 1,2-Ethoxybutane b) NaOMe, MeOH. c) 5% H_2SO_4 , $\text{Et}_2\text{O}/\text{H}_2\text{O}$.

2. Results and Discussion. – For the synthesis of optically active **2**, the strategy $C_{15} + C_{10} + C_5 + C_{10} = C_{40}$ was selected (*Scheme 1*). The C_{10} - ψ -end group **3** containing the chiral centre at the C(2) position was synthesized in eight steps using the pathway of *Traber and Pfander* [5].

The C_{15} -phosphonium salt **4**, the end group of the commercially available carotenoid canthaxanthin ($= \beta, \beta$ -carotene-4,4'-dione), was kindly provided by *F. Hoffmann-La Roche Ltd.*, Basel. The synthesis of **4** is described by *Kienzle and Meyer* [6] and *Widner et al.* [7].

First, **4** was elongated by a *Wittig* reaction in epoxybutane with the C_{10} -dial **5** (*Scheme 1*) to give the C_{25} -apocarotenal **6** accompanied by a small amount (5%) of canthaxanthin, resulting from the double *Wittig* reaction of **5**. By column chromatography (silica gel), **6** was obtained in 51% yield. For the elongation of **6** to the C_{30} -building block **7**, the C_5 -phosphonium salt **8** was protected using a method described by *Freyschlag et al.* [8] [9] to give the diethyl acetal **9** (*Scheme 2*). However, the coupling of **6** with **9** gave a complex mixture of products, partially due to the cleavage of the protection group. Therefore, the cyclic acetal **10** was prepared which was used without purification for the reaction with the C_{25} -synthon **6**. The resulting C_{30} -acetal **11** was transformed to the building block **7** in an overall yield of 42% (referred to **6**) by careful treatment with 5% H_2SO_4 for 30 min in a vigorously stirred H_2O/Et_2O mixture. For the synthesis of (2'*R*)-**2**, the C_{30} -synthon **7** and the phosphonium salt **3** were reacted in 1,2-epoxybutane to give the C_{40} -carotenoid in a yield of ca. 13% after crystallization from benzene/hexane.

Scheme 2



a) $CH(OEt)_3$, MeOH, $TsOH$. b) Propane-1,3-diol, MeOH, $TsOH$.

3. Spectroscopic Studies. – The UV/VIS spectra of (2'*R*)-**2** exhibits a λ_{max} at 474 nm (in hexane) with almost no fine structure due to the carbonyl function at C(4). This value corresponds to the value given for deoxyflexixanthin ($\approx$ 1'-hydroxy-3',4'-didehydro-1',2'-dihydro- β, ψ -caroten-4-one) by *Coman and Weedon* [10]. The mass spectrum shows the molecular ion at m/z 582, which is in agreement with the constitution of **2**. NMR investigations of (2'*R*)-**2**, comprising 1H , ^{13}C , DEPT, 1H , 1H -COSY and H , C correlations experiments, allow the assignment of all signals unambiguously (see *Exper. Part*).

The CD spectrum of (2'*R*)-**2** in Et_2O /isopentane/ $EtOH$ 5:5:2 (EPA 5:5:2) exhibited a strong temperature dependence. At -100° , the spectrum shows a main extremum at 285 ($\Delta\epsilon = -33$; *Fig.*). The spectrum is almost identical to the spectrum obtained from

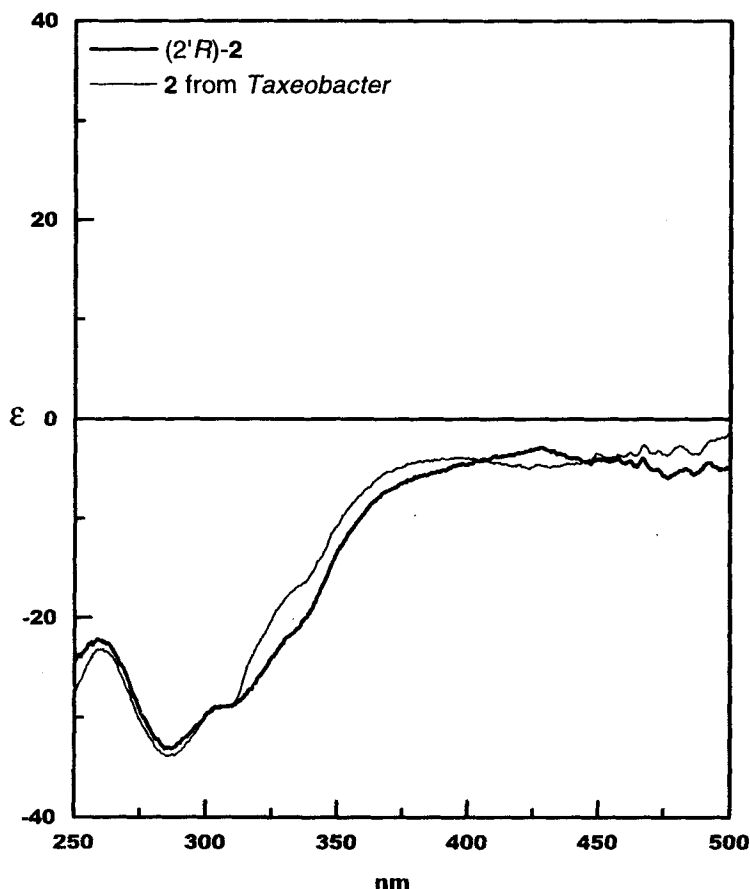


Figure. Comparison of CD spectra (EPA 5:5:2, -100°) of synthetic $(2'R)$ -2 and natural 2

natural 2. Therefore, the structure of the carotenoid isolated from *Taxeobacter* was established as (all-*E*,2'*R*)-3-deoxy-2'-hydroxyflexixanthin ((2'*R*)-2).

The synthesis of the main carotenoid of *Taxeobacter*, (all-*E*,3*S*,2'*R*)-2'-hydroxyflexixanthin ((3*S*,2'*R*)-1), by a $C_{15} + C_{10} + C_{15} = C_{40}$ strategy is in progress.

The authors are grateful to the Swiss National Science Foundation and the Stiftung zur Förderung der wissenschaftlichen Forschung an der Universität Bern for financial support as well as to F. Hoffmann-La Roche Ltd., Basel, for financial support, the gift of chemicals, and the measurement of CD spectra. We thank Dr. A. Steck for the measurement and interpretation of high-resolution NMR spectra and Dr. Bettina Böhlendorf, Novartis, Basel, for the sample of the isolated carotenoids from *Taxeobacter*.

Experimental Part

1. *General.* All experiments were carried out under N_2 or Ar. Solvents were distilled prior to use or purchased in HPLC quality. TLC: silica gel 25 F_{254} (Macherey-Nagel). Column chromatography: silica gel 60 (Merck, 0.040–0.063 mm). M.p.: Büchi 510; not corrected. $[\alpha]_D$: Perkin-Elmer-241 polarimeter. UV/VIS Spectra: Perkin-Elmer-554 spectrometer; λ_{max} in nm. CD Spectra: Jasco 500, wavelength in nm ($\Delta\epsilon$). IR Spectra: Perkin-Elmer-782 spectrometer; ν in cm^{-1} . 1H - and ^{13}C -NMR Spectra: Bruker AC-300 (300 and 75.5 MHz resp.), Bruker AM-400

(400 and 100.6 MHz, resp.), in CDCl_3 ; chemical shifts in ppm as δ values relative to CDCl_3 ($= 7.26$ ppm), J in Hz. Mass spectra: Varian MAT CH-44s; m/z (rel. intensity in %); ionization energy 70 eV.

2. (6*R*,4*E*)-(6,7-Dihydroxy-3,7-dimethyloct-2,4-dien-1-yl)triphenylphosphonium Bromide (**3**) was synthesized according to [5].

3. 4-*Oxo*-12'-*apo*- β -caroten-12'-*al* (**6**). To a soln. of **5** (340 mg, 2.1 mmol) in 1,2-epoxybutane ($=$ 2-ethyloxirane, 20 ml) was added **4** (1.39 g, 2.5 mmol) and the mixture heated to 60° . After 16 h, the deep-red soln. was evaporated at 40° . The crude product was dissolved in EtOH/ H_2O 8:2 (10 ml) and stirred for 3 h at 60° . After cooling slowly to r.t., the product was crystallized in the deep-freezer at -20° overnight. The crystals were separated by filtration and washed carefully with ice-cold MeOH. An additional crystallization was performed with the mother liquor. The combined crystals were dried under h.v. to give 460 mg (51%) of **6**. Deep-red crystals. M.p. 166° . UV/VIS (hexane): 273, 318, 422. IR (KBr): 1655s (C=O), 1610m, 1590m, 1570m, 1350w, 1330w, 1285w, 1185m, 970m. $^1\text{H-NMR}$ (300 MHz, CDCl_3): 1.18 (s, Me(16), Me(17)); 1.86 (s, Me(18)); 1.89 (s, Me(20)); 2.03 (s, Me(19)); 2.07 (s, Me(20)); 2.52 (t, $J = 7$, $\text{CH}_2(3)$); 6.2–6.5 (m, H–C(7), H–C(8), H–C(10), H–C(12), H–C(14)); 6.60–6.83 (m, H–C(11), H–C(15)); 6.98 (d, $J = 10$, H–C(14)); 7.05 (dd, $J = 15, 10$, H–C(15)); 9.42 (s, H–C(12)). MS: 364 (21, M^+), 349 (7), 332 (11), 295 (10), 277 (38), 253 (9), 215 (14), 209 (10), 203 (22), 197 (18), 183 (23), 175 (13), 171 (26), 164 (33), 157 (72), 147 (28), 135 (41), 119 (28), 105 (28), 95 (42), 91 (44), 77 (26), 69 (21), 59 (44), 55 (20), 43 (33), 28 (35), 18 (100).

4. 4-*Oxo*-8'-*apo*- β -caroten-8'-*al* (**7**). To a soln. of **8** (534 mg, 1.44 mmol) in MeOH (30 ml), propan-1,3-diol (560 mg, 6 mmol) was added through a syringe, together with a few crystals of TsOH dissolved in MeOH. The mixture was stirred for 15 h at r.t. After evaporation, the remaining thick oil was dissolved in CH_2Cl_2 , and **10** was precipitated by adding THF. The white residue was separated by filtration and dissolved in MeOH (20 ml) without further purification. Then, **6** (362 mg) was added, and then NaOMe (60 mg) in MeOH (2 ml) was injected. After stirring at 40° for 18 h, the mixture was allowed to cool to r.t. H_2O (50 ml) was added and the mixture extracted with Et_2O ($3 \times$). The combined org. phases were washed twice with sat. NaCl soln., then dried (Na_2SO_4), and evaporated. The crude product was dissolved in Et_2O (10 ml), mixed with 0.5*N* H_2SO_4 (10 ml), and vigorously stirred at r.t. for 30 min. The H_2O phase was separated and extracted with Et_2O ($3 \times$). The combined org. phases were washed with sat. NaHCO_3 soln. ($3 \times$) and sat. NaCl soln. ($2 \times$), dried (Na_2SO_4), and evaporated. The product was crystallized from EtOH/ H_2O 4:1 and dried under h.v. 260 mg (42%), referred to **6** of **7**. Deep-red crystals. M.p. 178° . UV/VIS (hexane): 289, 348, 451, 476. $^1\text{H-NMR}$ (300 MHz, CDCl_3): 1.20 (s, Me(16), Me(17)); 1.89, 1.90 (2s, Me(18), Me(19)); 1.92 (s, Me(19)); 1.99–2.01 (s, Me(20), Me(20)); 2.52 (t, $J = 7$, $\text{CH}_2(3)$); 6.1–6.5 (m, H–C(7), H–C(8), H–C(10), H–C(12), H–C(14), H–C(14)); 6.55–6.80 (m, H–C(11), H–C(11'), H–C(15), H–C(15')); 6.95 (d, $J = 11$, H–C(10)); 9.45 (s, H–C(8')). MS: 430 (10, M^+), 361 (4), 296 (5), 277 (6), 215 (13), 203 (15), 197 (13), 183 (11), 171 (11), 157 (14), 147 (15), 133 (19), 119 (16), 105 (12), 95 (11), 91 (11), 69 (10).

5. (all-*E*,2'*R*)-3-Deoxy-2'-hydroxyflexixanthin ((2'*R*)-**2**). To a soln. of **3** (408 mg, 0.8 mmol) in 1,2-epoxybutane (20 ml), **7** (200 mg, 0.4 mmol) was added. After stirring for 15 h at 60° , more **3** (200 mg) was added to the mixture, and after 8 and 14 h, even more (150 and 100 mg, resp.). The mixture was stirred for another 18 h, then cooled down to r.t., evaporated at 40° . The dark-red product was separated by column chromatography (silica gel 100 g), hexane/AcOEt 1:1 to 1:2 + 0.5% Et_3N . Crystallization from benzene/hexane yielded 36 mg (13%) of (2'*R*)-**2**. Deep-red needles. M.p. 204° (dec.). UV/VIS (hexane): 314, 474, 500. CD (EPA 5:5:2, -100°): 285 (–33), 307 (–29), 427 (–3). $^1\text{H-NMR}$ (400 MHz, CDCl_3): 1.18 (s, Me(16)); 1.19 (s, Me(16), Me(17)); 1.24 (s, Me(17)); 1.83 (t, $J = 6.56$, $\text{CH}_2(2)$); 1.87 (s, Me(18)); 1.93 (s, Me(18)); 1.98 (s, Me(19), Me(20)); 2.00 (s, Me(19)); 2.12 (d, $J = 2.43$, OH); 2.50 (t, $J = 6.56$, $\text{CH}_2(3)$); 4.00 (dd, $J = 7.51, 2.43$, H–C(2')); 5.71 (dd, $J = 15.52, 7.61$, H–C(3')); 6.20 (d, $J = 11.3$, H–C(6')); 6.23 (d, $J = 15.41$, H–C(7)); 6.26 (d, $J = 11.5$, H–C(10')); 6.28 (d, $J = 11.5$, H–C(10)); 6.28 (m, H–C(14')); 6.20 (m, H–C(14)); 6.37 (d, $J = 15.41$, H–C(8)); 6.38 (d, $J = 15.52$, H–C(4')); 6.39 (d, $J = 14.61$, H–C(8')); 6.40 (d, $J = 15.0$, H–C(12')); 6.43 (d, $J = 15.00$, H–C(12)); 6.59 (dd, $J = 13.3, 14.61$, H–C(7)); 6.65 (dd, $J = 11.5, 15.00$, H–C(11)); 6.65 (m, H–C(15)); 6.65 (dd, $J = 11.5, 15.0$, H–C(11)); 6.66 (m, H–C(15')). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): 12.6 (Me(19)); 12.8 (Me(20), Me(20)); 12.9 (Me(19)); 13.0 (Me(18)); 13.8 (Me(18)); 24.0 (Me(16)); 26.5 (Me(17)); 27.7 (Me(16), Me(17)); 34.3 ($\text{CH}_2(3)$); 36.7 (C(1)); 37.4 ($\text{CH}_2(2)$); 73.0 (C(1')); 80.1 ($\text{CH}_2(2')$); 124.0 ($\text{CH}(7)$); 124.3 ($\text{CH}(7')$); 124.5 ($\text{CH}(11)$); 125.2 ($\text{CH}(11')$); 126.3 ($\text{CH}(3')$); 129.9 (C(5)); 130.2 ($\text{CH}(15)$); 130.7 ($\text{CH}(15')$); 132.9 ($\text{CH}(14')$); 133.1 ($\text{CH}(6')$); 133.3 ($\text{CH}(10')$); 133.7 ($\text{CH}(14)$, C(9')); 134.1 (C(5')); 134.5 ($\text{CH}(10)$); 134.7 (C(9)); 136.1 (C(13)); 136.4 (C(13')); 138.1 ($\text{CH}(4')$); 138.3 ($\text{CH}(12')$); 138.7 ($\text{CH}(8')$); 139.3 ($\text{CH}(12)$); 141.3 ($\text{CH}(8)$); 161.2 (C(6)); 199.3 (C(4)). MS: 582 (19, M^+), 564 (3), 548 (2), 524 (9), 476 (32), 419 (16), 326 (14), 295 (6), 283 (7), 269 (8), 263 (6), 255 (8), 247 (7), 237 (9), 223 (16), 215 (20), 209 (26), 203 (70), 197 (27), 185 (24), 169 (23), 161 (19), 145 (51), 139 (43), 133 (82), 119 (57), 105 (69), 91 (100), 81 (30), 69 (38), 59 (54).

REFERENCES

- [1] H. Reichenbach, *Bioforum* **1991**, *14*, 25.
- [2] H. Reichenbach, 'Proceedings of the 2nd Int. Symp. on Flavobacterium, Cytophaga and Related Bacteria', Bloemfontein, South Africa, 1992.
- [3] B. Böhlendorf, H. Reichenbach, Poster Presentation at the 10th Int. Symp. on Carotenoids, Trondheim, Norway, 1993.
- [4] A. G. Andrewes, P. Foss, G. Borch, S. Liaaen-Jensen, *Acta Chem. Scand., Sect. B* **1984**, *38*, 337.
- [5] B. Traber, H. Pfander, *Helv. Chim. Acta* **1996**, *79*, 499.
- [6] F. Kienzle, H. Meyer, *Helv. Chim. Acta* **1978**, *61*, 2609.
- [7] E. Widmer, R. Zell, Th. Lukáč, M. Casadei, P. Schönholzer, E. Broger, *Helv. Chim. Acta* **1981**, *64*, 2405.
- [8] H. Freyschlag, W. Reif, A. Nürrenbach, H. Pommer, Ger. Pat. No. 1211616, 1963.
- [9] H. Freyschlag, W. Reif, A. Nürrenbach, Ger. Pat. No. 1216862, 1964.
- [10] R. E. Coman, B. C. L. Weedon, *J. Chem. Soc., Perkin Trans. 1* **1975**, 2529.