New Synthesis of Natural Carotene Isorenieratene (ϕ,ϕ-Carotene) and its 3,3′-Dimethoxy Analogue

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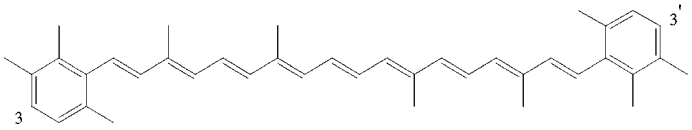
The main pigments of *Brevibacterium linens* are the aromatic carotenoids 3,3′-dihydroxyisorenieratene (φ,φ-carotene-3,3′-diol), the corresponding monohydroxy compound, and the hydrocarbon isorenieratene. We report herein new syntheses of isorenieratene (ϕ,ϕ-carotene) and its xanthophyll analogue, 3,3′-dimethoxy-ϕ,ϕ-carotene, which may be considered a protected form of the natural xanthophyll, 3,3′-dihydroxy-ϕ,ϕ-carotene.

Introduction. – The biosynthesis of the aromatic ϕ,ϕ-carotene (isorenieratene; **1a**) is restricted to genera of anoxygenic photosynthetic bacteria (*Chlorobium*, *Pelochromatium*, and *Phaeobium*) [1] and a few actinomycetes. This compound and xanthophylls with 3-hydroxy or 3,3′-dihydroxy end group(s) have been previously extracted from *Brevibacterium linens* [2], a member of the commercially important group of coryneform bacteria used in cheese ripening, *Mycobacterium aurum* A+ [3], a nonpathogenic, rapidly growing mycobacterium, and *Streptomyces mediolani* [4], a bacterium isolated from soil samples [4]. Isorenieratene and derivatives are responsible, in part, for the color of the rind of red-smear ripened soft cheeses (*e.g.* Livarot, Epoisses, Munster…). The food and cosmetic industries also take advantage of the antioxidant properties of natural carotenoids, often including carotenoids in their products. To test some of their biological properties, we began a project dedicated to the synthetic and biological production of these molecules. We report herein new syntheses of **1a** and its 3,3′-dimethoxy analogue **1b** (3,3′-dimethoxy-ϕ,ϕ-carotene) *via* a $C_{20}+C_{20}$ route. This latter compound could be considered a protected form of the natural xanthophyll 3,3-dihydroxy-ϕ,ϕ-carotene. Carotenoids with tertiary MeO groups are common in phototropic bacteria. A sponge carotenoid possesses a MeO group at C(3) [5].



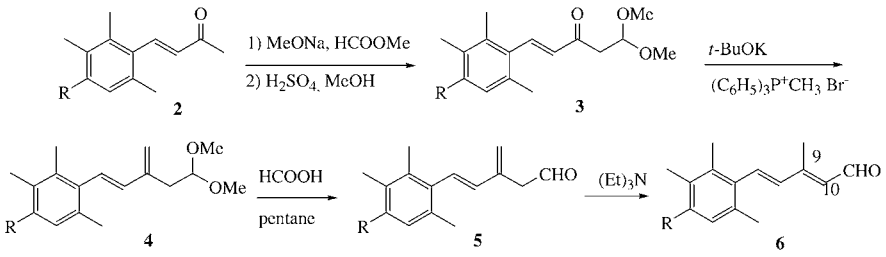
Isorenieratene (**1a**)

Results and Discussion. – The usual process for preparation of carotenoids is a $C_{15}+C_{10}+C_{15}$ strategy (for books related to syntheses, see [6]) but, when C_{20} intermediates are readily available, the synthesis of symmetric carotenoids *via* the coupling of two C_{20} units by *Wittig* olefination [7] (C_{20} phosphonium salt + C_{20} aldehyde) or *Julia* coupling [8] (C_{20} sulfone + C_{20} aldehyde) is also viable. An important contribution to the synthesis of symmetric carotenoids was the discovery by *McMurry* [9] that low-valent titanium succeeds in the reductive coupling of aldehydes or ketones to give high yields of the corresponding alkenes.

The synthesis of isorenieratene and 3,3′-dimethoxyisorenieratene has been previously reported. Apart from *Akiyama et al.* [10], who used a *McMurry* coupling of two C_{20} aldehydes, other reported methods have not made use of the above-outlined strategies. *Yamaguchi* [11] used a $C_{16}+C_8+C_{16}$ method, *Weedon* [12] a $C_{10}+C_{20}+C_{10}$ approach, and *Okukado et al.* [13], for the synthesis of dimethoxyisorenieratene, used a $C_{11}+C_{20}+C_{11}$ strategy.

We use here $C_{15}+C_5$ synthons to generate C_{20} units from methyl isopropylidene cyanoacetate, an easily available C_5 unit, and the C_{15} and C_{16} 'β-methylene aldehyde' synthons **5** [14], thereby avoiding the problems linked to the configuration of the double bond that occur with the usual procedures [15][16]. The C_{15} units were obtained by formylation of ketones **2a** and **2b** ((3*E*)-4-(2,3,6-trimethylphenyl)but-3-en-2-one and (3*E*)-4-(4-methoxy-2,3,6-trimethylphenyl)but-3-en-2-one, resp. [17][18]) with MeONa and HCOOMe, and acetalization of the Na salts of the hydroxymethylene intermediates by H_2SO_4 in MeOH furnished the β-ketoacetals **3** (*Scheme 1*). A *Wittig* reaction with (triphenyl)methylenephosphorane led to the corresponding methylene derivatives, which, after heterogeneous acidic hydrolysis in pentane and HCOOH of the obtained β-methylidene acetals **4**, gave the β-methylidene aldehydes **5** (60% yield based on **2**) [19]. Catalytic conjugation by triethylamine provided regioselectively the desired (*E*,*E*)-α,β-unsaturated aldehydes **6** ((*E*,*E*)/(*E*,*Z*) 97:3), as previously described [14].

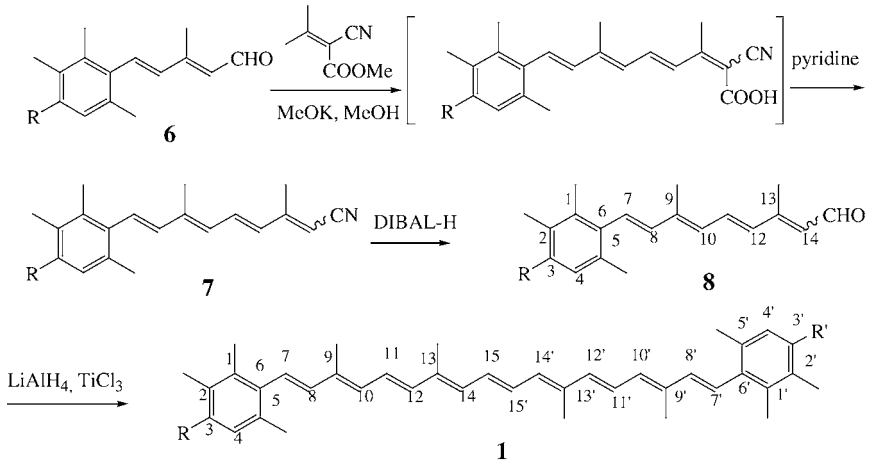
Scheme 1



a R = H; **b** R = OMe.

A *Stobbe*-like condensation with methyl 2-cyano-3-methylbut-2-enoate (*Scheme 2*) followed by decarboxylation of the crude cyano acids in pyridine led to the nitriles **7a** and **7b** in 90% yield (**7a**: (13*E*)/(13*Z*) 80:20; **7b**: (13*E*)/(13*Z*) 72:28). The latter compounds were further reduced with diisobutylaluminium hydride (DIBAL-H) to the aldehydes **8**, and the isomers were separated by column chromatography (SiO_2, CH_2Cl_2). A McMurry (low-valent titanium) coupling of the (all-*E*) isomers gave isorenieratene **1a** and its 3,3′-dimethoxy analogue **1b** in 80% yield. These products were purified first by rapid column chromatography (neutral Al_2O_3, pentane/CH_2Cl_2 50:50) and then by HPLC (*Lichro* 5 μm *CART RP 18* Merck, MeOH/hexane 80:20).

Scheme 2



a: R, R′ = H; **b**: R, R′ = OMe.

Liquid-chromatography/atmospheric-pressure-chemical-ionization mass spectrometry (LC/(APcI)MS) was performed on the products [20]. The MS spectra of both carotenoids clearly showed the respective quasi-molecular ions ($[M+H]^+$) at *m*/*z* 529 (isorenieratene) and *m*/*z* 589 (3,3′-dimethoxyisorenieratene). The mass of isorenieratene was in accordance with that obtained by electron-impact mass spectrometry [10][21]. The fragmentation pattern (*Fig. 1* and *2*) observed was dominated in both cases by loss of one complete ϕ-endring (120 Da and 150 Da) from $[M+H]^+$, resulting in the formation of daughter ions at *m*/*z* 409 and *m*/*z* 439, respectively. The formation of M − 92, M − 106, and M − 158 ions, which is typical for carotenoids under ionization by electron bombardment (*e.g.*, at 70 eV) and direct inlet systems, was not observed [22][23]. As far as we know, aromatic carotenoids have not been studied before by LC/MS analyses, and cleavage of the α-bond has not yet been described. However, the fragmentation unequivocally substantiates the structures of isorenieratene and its 3,3′-dimethoxy analog.

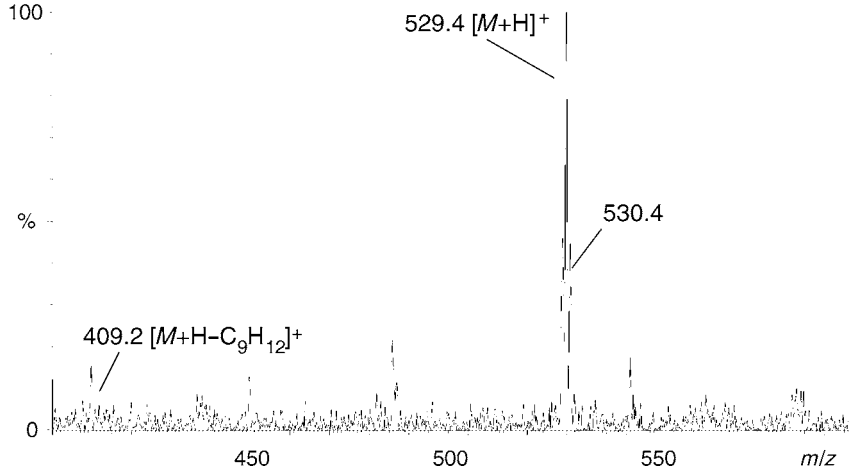


Fig. 1. *Mass spectrum* (LC-(APcI)/MS, positive-ion mode) *of* **1a** (*m/z* 529.4 (100%); *m/z* 409.2 (17%))

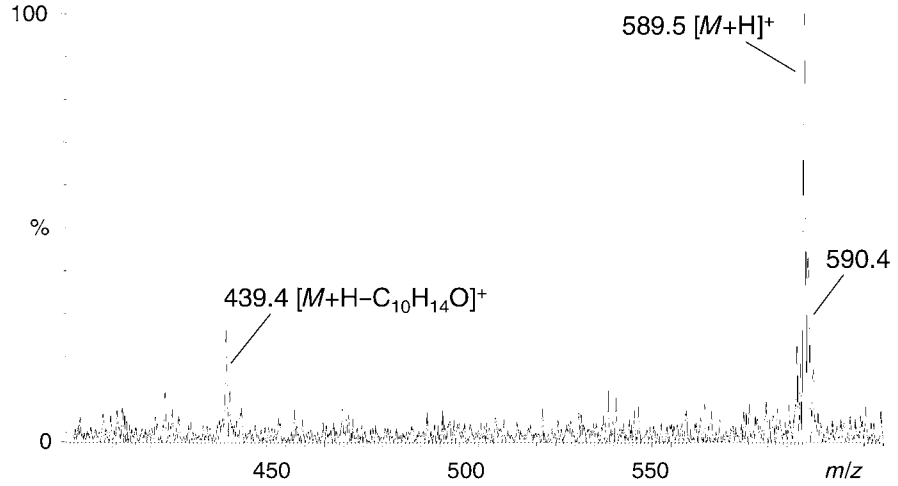


Fig. 2. *Mass spectrum* (LC-(APcI)/MS, positive-ion mode) *of* **1b** (*m/z* 589.5 (100%); *m/z* 439.4 (26%))

Experimental Part

General. All reactions were carried out under Ar. IR Spectra (film): *Bruker IF-55* spectrometer; ν in cm^{-1}. ^{1}H-NMR Spectra ($CDCl_3$, 400 MHz): *Bruker Avance DPX-400*; δ in ppm rel. to $SiMe_4$. LC/MS: *HP-1100* modular HPLC system coupled to a *Micromass VG platform II* quadrupole MS (scan range: *m/z* 400–1000; UV/VIS (DAD): 450 nm; injection vol. 20 µl), carried out in the APcI+ mode; data processed with *MassLynx 3.2* sofware (for further details, see [20]); separation on a *YMC* column (5 µm-RP-C30 (250 × 4.6 mm i.d.) with precolumn (10 × 4 mm i.d.), 35°; mobile phase (flow rate: 1 ml/min.): mixtures of MeOH/*t*-butyl methyl ether/H_2O (A = 81 : 15 : 4 and B = 6 : 90 : 4), gradient program (0/99, 39/44, 45/0, 50/99, 55/99 (min/% A).

*(all-*E*)-3,7-Dimethyl-9-(2,3,6-trimethylphenyl)nona-2,4,6,8-tetraenenitrile* (**7a**) *and (all-*E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenenitrile* (**7b**). General procedure: at 0°, MeOK (2.1 g, 3 mmol) in 10 ml of MeOH was added to a mixture of 1 mmol of **6** [14] and 1 mmol of methyl 2-cyano-3-methylbut-2-enoate. After 2 d at r.t., the crude mixture was poured into 100 ml H_2O and extracted with ether.

The aq. layer was acidified with a 10% HCl soln. and extracted with ether. The solvent was removed under reduced pressure and the crude cyanoacid was dissolved in benzene (20 ml) and decarboxylated by reflux for 20 h with 5 equiv. of pyridine. Benzene and pyridine were removed under reduced pressure, and the crude mixture was extracted with ether and washed successively with 5% aq. HCl and H_2O. The solvent was evaporated and the crude product was purified by column chromatography (CC; SiO_2, CH_2Cl_2).

Data of **7a**: IR: 2940, 2206, 1625, 1465, 1306, 1154, 1119, 974. ^{1}H-NMR[1]): 6.96–7.02 (*m*, H–C(3), H–C(4), H–C(7)); 6.77 (*d*, *J* = 16.4, H–C(8)); 6.17 (*d*, *J* = 11.3, H–C(10)); 6.32 (*d*, *J* = 15.5, H–C(12)); 5.21 (*s*, H–C(14)); 2.27–2.29 (*m*, Me–C(2), Me–C(5), Me–C(13)); 2.24 (*s*, Me–C(1)); 2.14 (*s*, Me–C(9)). Anal. calc. for $C_{20}H_{23}N$: C 86.59, H 8.36, N 5.05; found: C 86.40, H 8.47, N 6.13.

Data of **7b**: IR: 2995, 2928, 2853, 2206, 1620, 1586, 1464, 1384, 1309, 1289, 1121, 972, 837, 797. ^{1}H-NMR[1]): 6.99 (*dd*, *J* = 15.2, 11.3, H–C(11)); 6.74 (*d*, *J* = 16.16, H–C(8)); 6.32 (*d*, *J* = 15.2, H–C(12)); 6.25 (*d*, *J* = 16.16, H–C(7)); 6.17 (*d*, *J* = 11.3, H–C(10)); 3.82 (*s*, MeO); 2.30 (*s*, Me–C(13)); 2.23, 2.17 (2*s*, Me–C(1), Me–C(5)); 2.15 (*s*, Me–C(2)); 2.09 (*s*, Me–C(9)). Anal. calc. for $C_{21}H_{25}NO$: C 82.04, H 8.20, N 4.56, O 5.20; found: C 81.89, H 8.32, N 4.49, O 5.30.

*(all-*E*)-3,7-Dimethyl-9-(2,3,6-trimethylphenyl)nona-2,4,6,8-tetraenal* (**8a**) *and (all-*E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenal* (**8b**). General procedure: DIBAL-H (2 mM, 2.84 g) in toluene (as a 1M soln.) was slowly added at 0° to a stirred soln. of **7** (1 equiv.) in toluene (10 ml). After 2 h at 5°, the mixture was slowly hydrolyzed with 10% aq. HCl (100 ml). The salts were filtered off and washed with ether (2 × 25 ml). The org. layer was washed with brine and dried (Na_2SO_4). The solvent was evaporated and the crude product was purified by CC (SiO_2, CH_2Cl_2/MeOH 97:3).

Data of **8a**: IR: 2954, 2864, 1660, 1583, 1445, 1389, 1154, 1112, 967, 801. ^{1}H-NMR[1]): 10.12 (*d*, *J* = 8.1, CHO); 6.98–7.36 (*m*, H–C(3), H–C(4), H–C(7), H–C(11)); 6.77 (*d*, *J* = 15.2, H–C(8)); 6.42 (*d*, *J* = 15.02, H–C(12)); 6.27–6.29 (*m*, *J* = 15.8, H–C(10)); 5.99 (*d*, *J* = 8.1, H–C(14)); 2.33 (*s*, Me–C(13)); 2.28 (*s*, Me–C(2)); 2.23 (*s*, Me–C(5)); 2.14 (*s*, Me–C(1)); 1.55 (*s*, Me–C(9)). Anal. calc. for $C_{20}H_{24}O$: C 85.67; H 8.63; O 5.70; found: C 85.48, H 8.77, O 5.75.

Data of **8b**: IR: 2995, 2943, 2829, 1659, 1577, 1460, 1399, 1310, 1289, 1262, 1214, 1193, 1152, 1111, 967, 796. ^{1}H-NMR[1]): 10.12 (*d*, *J* = 8.03, CHO); 7.16 (*dd*, *J* = 15.7, 11.1, H–C(11)); 6.76 (*d*, *J* = 15.75, H–C(7)); 6.60 (*s*, H–C(4); 6.40 (*d*, *J* = 15.75, H–C(8)); 6.26 (*m*, 2 H, H–C(10), H–C(12)); 5.98 (*d*, *J* = 8.03, H–C(14)); 3.82 (*s*, MeO); 2.35 (*s*, Me–C(13)); 2.30 (*s*, Me–C(2)); 2.24 (*s*, Me–C(1)); 2.20 (*s*, Me–C(5)); 2.17 (*s*, Me–C(9)). Anal. calc. for $C_{21}H_{26}O_2$: C 81.25, H 8.44, O 10.31; found: C 80.98, H 8.57, O 10.45.

Isorenieratene (= *ϕ,ϕ-Carotene*; **1a**) *and 3,3′-Dimethoxy-ϕ,ϕ-carotene* (**1b**). The reductive coupling of **8** was carried out as described by *Paust* and *Manchand* [24]. General procedure: Under Ar, 190 mg (5 mmol) of powdered $LiAlH_4$ was added to 1.53 g (10 mmol) of $TiCl_3$ in 30 ml of anh. THF. After 2 h at r.t., 5 mmol of **8a** or **8b** in 10 ml THF was added. The mixture was stirred overnight, and 50 ml of 2M HCl was slowly added at 0°. The crude mixture was extracted with ether and washed with brine. The solvents were distilled off under reduced pressure, and the residues obtained were purified first by rapid CC (neutral Al_2O_3, pentane/CH_2Cl_2 50:50) to give 85% of **1a** and 75% of **1b** and, to obtain a pure sample, further by HPLC (*Lichro* 5 μm *CART RP 18 Merck*, MeOH/hexane 80:20). ^{1}H-NMR spectra were identical to those previously described [25].

Data of **1a**: ^{1}H-NMR: 6.95 (*s*, 4 arom. H); 6.1–6.08 (*m*, 14 C=CH); 2.26 (*s*, 4 Me); 2.23 (*s*, 2 Me); 2.08 (*s*, 2 Me); 2.00 (*s*, 2 Me).

Data of **1b**: ^{1}H-NMR: 7.00–6.10 (*m*, 16 H, 2 arom. H, 14 C=CH); 3.82 (*s*, 2 MeO); 2.47 (*s*, Me–C(13,13′)); 2.27 (*s*, Me–C(5,5′)); 2.25 (*s*, Me–C(1,1′)); 2.15 (*s*, Me–C(2,2′)); 2.07 (*s*, Me–C(9,9′)).

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[1]) Retinoid numbering (see *Scheme 2*).

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