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ISOLATION AND STRUCTURAL ELUCIDATION OF CUCURBITAXANTHIN A AND B FROM PUMPKIN *CUCURBITA MAXIMA*

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Key Word Index—*Cucurbita maxima*; Cucurbitaceae; pumpkin; carotenoid; cucurbitaxanthin A; cucurbitaxanthin B.

Abstract—Two new carotenoids, cucurbitaxanthin A [(3*S*,5*R*,6*R*,3′*R*)-3,6-epoxy-5,6-dihydro-β,β-carotene-5,3′-diol] and cucurbitaxanthin B [(3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-3,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol] have been isolated from the pumpkin *Cucurbita maxima*.

INTRODUCTION

β,ε-Carotene, β,β-carotene, lycopene, α-cryptoxanthin, β-cryptoxanthin, lutein, zeaxanthin, lutein-5,6-epoxide, antheraxanthin, violaxanthin and neoxanthin have been reported as the principal carotenoids in the pumpkin [1–4].

In the course of our comparative biochemical studies of carotenoids in plants, we have isolated two new carotenoids, cucurbitaxanthin A (**1**) and cucurbitaxanthin B (**2**) from the flesh of the pumpkin *Cucurbita maxima*. We report in this paper the isolation and structural elucidation of these two new carotenoids.

RESULTS AND DISCUSSION

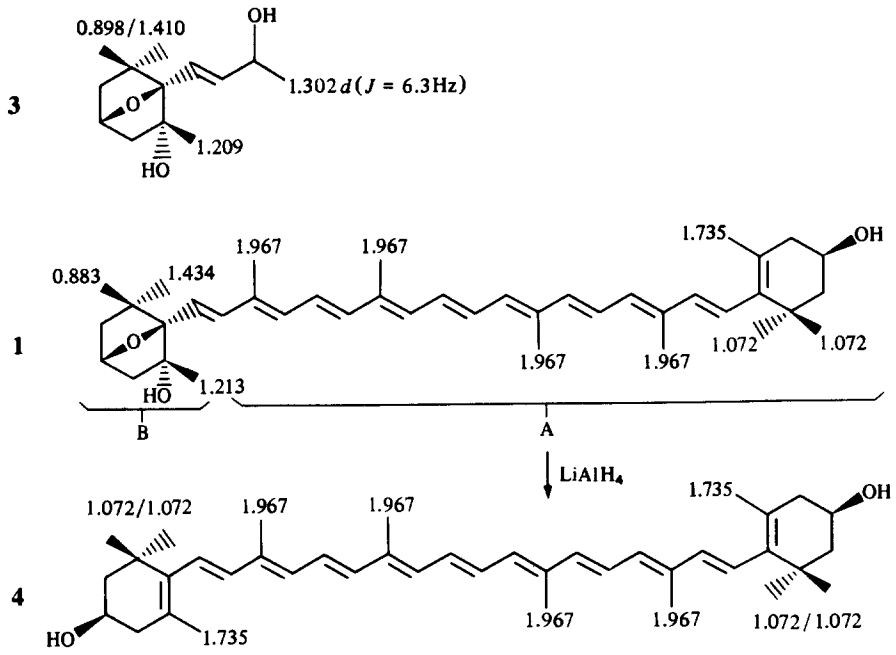
The following carotenoids were identified: β,β-carotene (16.8% of the total carotenoid), (3*R*)-β-cryptoxanthin (1.5%), lutein A [(3*R*,3′*R*,6′*R*)-lutein] [5] (26.1%), (3*R*,3′*R*)-zeaxanthin (4.1%), (3*S*,5*R*,6*S*,3′*R*,6′*R*)-lutein-5,6-epoxide (1.2%), (3*S*,5*R*,6*S*,3′*R*)-antheraxanthin (2.1%), (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-violaxanthin (11.1%) and (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-neoxanthin (3.4%).

Cucurbitaxanthin A (**1**) was isolated as orange needles (yield 2.7 mg from 100 g flesh, 25% of the total carotenoid) and showed mp 175–176°. The molecular ion of **1** (*m/z* 584.4196) was compatible with $C_{40}H_{56}O_3$. Of the three oxygen functions, one was ascribed to a secondary hydroxyl group and one of the remaining two was attributed to a tertiary hydroxyl group by acetylation, trimethyl silylation and ^{1}H NMR data (δ 3.9 *m*, 1H). From the IR spectrum there were no carbonyl, carboxyl, allenic and acetylenic groups. Therefore the third oxygen was ascribed to an epoxide. ^{1}H NMR spectral assignments for **1** are consistent with the presence of the structural moiety A in the molecule of **1** (Scheme 1). Furthermore, the presence of a 5,6-dihydro-5-hydroxy-3,6-epoxy-β end group (B) in **1** was confirmed by comparison with the ^{1}H NMR and the ^{13}C NMR data of (3*S*,5*R*,6*R*)-5,6-dihydro-5-hydroxy-3,6-epoxy-β-ionol (**3**) [6, 7] (Schemes 1 and 2). On the basis of the evidence described above, we have assigned the structure 5,6-dihydro-3,6-epoxy-β,β-carotene-5,3′-diol to cucurbitaxanthin A (**1**). Reduction of **1** with $LiAlH_4$ under forcing conditions provided (3*R*,3′*R*)-zeaxanthin (**4**) (Scheme 1). This result indicated not only the validity of the proposed constitution of **1** but also revealed that **1** possesses 3*S*,6*R* and 3′*R* chiralities in its molecule.

Taking the ^{1}H NMR and ^{13}C NMR data and biosynthetic aspects [6, 7] into the consideration of the structure of cucurbitaxanthin A, the *R*-configuration is favoured for the hydroxyl at C-5. Thus, the structure of cucurbitaxanthin A has been tentatively postulated to be (3*S*,5*R*,6*R*,3′*R*)-3,6-epoxy-5,6-dihydro-β,β-carotene-5,3′-diol (**1**).

Cucurbitaxanthin B (**2**) was isolated as orange needles (0.8 mg from 100 g flesh, 7.7% of the total carotenoid) and showed mp 181–182°. The mass spectrum revealed a molecular weight of 600.4156 compatible with the formula $C_{40}H_{56}O_4$. The presence of one secondary hydroxyl group and one tertiary hydroxyl group is consistent with the formation of a monoacetate and a di-trimethyl silylether and with the ^{1}H NMR data (δ3.9 *m*, 1H). A hypsochromic shift of 20 nm by treatment with HCl indicated the presence of a 5,6-epoxy-β end group in the molecule. From the IR spectrum, there were no carbonyl, carboxyl, allenic and acetylenic groups in the molecule. The ^{1}H NMR signals at δ0.883, 1.434 and 1.213 showed the presence of a 3,6-epoxy-5,6-dihydro-5-hydroxy-β end group and the signals at δ0.978, 1.152 and 1.188 confirmed the presence of a 3′,6′-*cis*-3′-hydroxy-5′,6′-epoxy-β end group in **2** [8]. Consequently the constitution of cucurbitaxanthin B has been assigned as 3′,6′-*cis*-3,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol. In the same manner as for compound **1**, reduction of **2** with $LiAlH_4$ under forcing conditions provided (3*R*,3′*R*)-zeaxanthin (**4**). Therefore the chirality of cucurbitaxanthin B has been assigned as 3*S*,6*R*,3′*S*,5′*R*,6′*S*. From biosynthetic considerations and the ^{1}H NMR data the *R*-configuration is favoured for the hydroxyl group at C-5. On the basis of the evidence described above the structure of cucurbitaxanthin B has been tentatively proposed to be (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-3,6,5′,6′,-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,3′-diol (**2**) (Scheme 3).

In conclusion, the two new carotenoids, cucurbitaxanthin A (**1**) and cucurbitaxanthin B (**2**), possessing a



Scheme 1.

novel 5-hydroxy-3,6-oxabicycloheptane ring, have been isolated from the flesh of pumpkin *C. maxima*.

Naturally occurring carotenoids with a 3,6-oxabicycloheptane ring system, eutreptiellanone, α-cryptoeutreptiellanone and β-cryptoeutreptiellanone, were first isolated from the marine alga *Eutreptiella gymnastica* [9–11].

EXPERIMENTAL

Extraction and isolation of carotenoids. Carotenoids were extracted with Me_2CO from the flesh of *C. maxima* (100 g). After transfer to *n*-hexane–Et_2O (1:1) by adding H_2O, the extracts were evaporated to dryness and saponified with 10% KOH in MeOH at 30° for 12 hr. Individual carotenoids were separated by prep. TLC on silica gel G (0.5 mm). The development solvent used was benzene–EtOAc (3:1).

Spectroscopy. UV-VIS spectra were recorded in Et_2O. Concs were calculated using $E^{1\%}_{1cm} = 2500$ at λ_{max}. IR spectra were recorded in KBr discs. Mass spectra were obtained with a Hitachi M-80 instrument with a direct inlet system at 70 eV, 190–210°. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Varian XL-300 instrument. NMR spectra were recorded in $CDCl_3$ with TMS as standard. CD spectra were recorded on a Jasco J 500-C spectropolarimeter in EPA (Et_2O–*iso*-pentane–EtOH, 5:5:2) at 20°.

HPLC. HPLC was carried out on a Waters Model 510 instrument with a Waters Lambda Max Model 481 LC spectrophotometer set at 450 nm. The column used was a 300 × 8 mm i.d. stainless steel column packed with Sumipax OA-2000 (particle size 5 μm) [12]. The solvent used was *n*-hexane–CH_2Cl_2–EtOH (48:16:0.6) at a flow rate of 2 ml/min.

Chemical derivatizations. Saponification, acetylation, trimethylsilylation, allylic OH test and epoxyfuranoxide rearrangement were carried out by general procedures [13]. Reduction with $LiAlH_4$ was carried out in dry Et_2O for 12 hr at 30°.

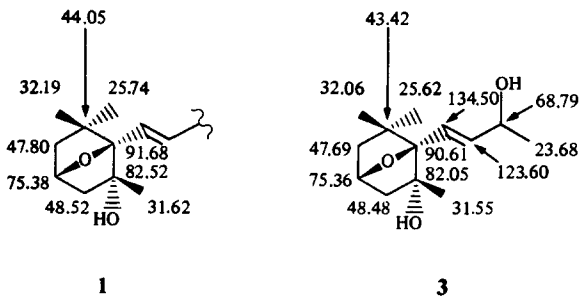
β,β-Carotene. R_f 0.98, inseparable from an authentic sample obtained from *Taraxacum officinale* [14] on co-TLC and co-HPLC; VIS λ_{max} nm: (425), 449, 475; MS *m/z* (rel. int.): 536 $[M]^+$ (100), 444 $[M-92]^+$ (15), 430 $[M-106]^+$ (5).

(3R)-*β-Cryptoxanthin.* R_f 0.78, inseparable from an authentic sample obtained from *T. officinale* [14] on co-TLC and co-HPLC; VIS λ_{max} nm: (425), 449, 475; MS *m/z* (rel. int.): 552 $[M]^+$ (100), 534 $[M-18]^+$ (25), 460 $[M-92]^+$ (5), 446 $[M-106]^+$ (2); CD (EPA) nm (Δε): 224 (−6.0), 236 (0), 245 (+6.0), 260 (0), 280 (−10.0), 350 (+3.0).

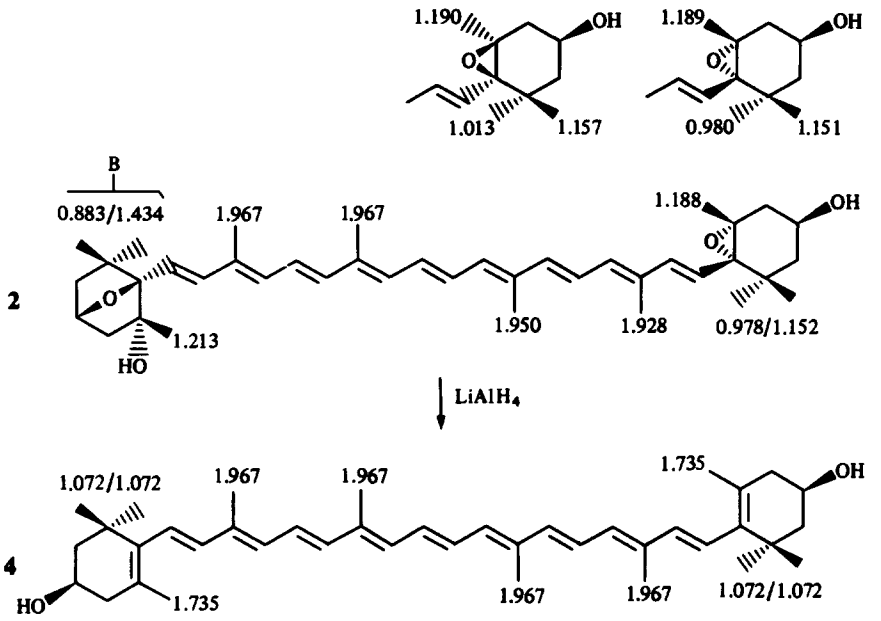
Lutein A [(3R,3'R,6'R)-*lutein*] [5]. R_f 0.40, inseparable from an authentic sample obtained from *T. officinale* [14] VIS λ_{max} nm: (420), 444 and 472; MS *m/z* (rel. int.): 568 $[M]^+$ (60), 550 $[M-18]^+$ (100), 532 $[M-36]^+$ (50), 476 $[M-92]^+$ (10), 462 $[M-106]^+$ (5); CD (EPA) nm (Δε): 220 (+2.0), 245 (+8.0), 275 (0), 285 (−4.5).

(3R,3'R)-*Zeaxanthin.* R_f 0.38, inseparable from an authentic sample obtained from *T. officinale* [14] on co-TLC and co-HPLC; VIS λ_{max} nm: (425), 449, 475; MS *m/z* (rel. int.): 568 $[M]^+$ (100), 550 $[M-18]^+$ (80), 532 $[M-36]^+$ (60), 476 $[M-92]^+$ (10), 462 $[M-106]^+$ (15); CD (EPA) nm (Δε): 224 (−18.0), 236 (0), 245 (+18.0), 260 (0), 284 (−24.8), 325 (0), 350 (+4.0).

(3S,5R,6S,3'R,6'R)-*Lutein-5,6-epoxide.* R_f 0.28, inseparable



Scheme 2.



Scheme 3.

from an authentic sample obtained from *T. officinale* [14] on co-TLC and co-HPLC; VIS λ_{max} nm: 416, 439, 469; MS *m/z* (rel. int.): 584 $[M]^+$ (5), 568 $[M-16]^+$ (2), 566 $[M-18]^+$ (10), 504 $[M-80]^+$ (5), 492 $[M-92]^+$ (5), 478 $[M-106]^+$ (5), 221 (36), 181 (27), 91 (100); CD (EPA) nm ($\Delta\varepsilon$): 234 (+4.1), 273 (−0.2), 330 (+1.4), 352 (+0.4).

(3S,5R,6S,3'R)-*Antheraxanthin*. R_f 0.28, inseparable from an authentic sample obtained from *T. officinale* [14] on co-TLC and co-HPLC; VIS λ_{max} nm: 423, 445, 473; MS *m/z* (rel. int.): 584 $[M]^+$ (100), 568 $[M-106]^+$ (50), 566 $[M-18]^+$ (70), 504 $[M-80]^+$ (40), 492 $[M-92]^+$ (19), 478 $[M-106]^+$ (2), CD (EPA) nm ($\Delta\varepsilon$): 208 (0), 238 (+10.0), 250 (0), 274 (−20.4), 310 (0), 333 (+3.2).

(3S,5R,6S,3'S,5'R,6'S)-*Violaxanthin*. R_f 0.19, inseparable from an authentic sample obtained from *T. officinale* [14] on co-TLC and co-HPLC; VIS λ_{max} nm: 416, 439, 468; MS *m/z* (rel. int.): 600 $[M]^+$ (20), 584 $[M-16]^+$ (5), 582 $[M-18]^+$ (3), 566 $[M-34]^+$ (3), 564 $[M-36]^+$ (2), 500 $[M-100]^+$ (2), 211 (100); CD (EPA) nm ($\Delta\varepsilon$): 225 (0), 230 (+6.1), 240 (0), 267 (−27.6), 310 (0).

(3S,5R,6R,3'S,5'R,6'S)-*Neoxanthin*. R_f 0.10, inseparable from an authentic sample obtained from *T. officinale* [14] on co-TLC and co-HPLC; VIS λ_{max} nm: 414, 436, 468; MS *m/z* (rel. int.): 600 $[M]^+$ (50), 582 $[M-18]^+$ (10), 520 $[M-80]^+$ (5), 508 $[M-92]^+$ (3), 221 (5), 91 (100); CD (EPA) nm ($\Delta\varepsilon$): 219 (0), 225 (−1.8), 243 (−0.7), 265 (−2.8), 293 (−0.6), 311 (−0.8).

Cucurbitaxanthin A (**1**). R_f 0.50, mp 175–176°; VIS λ_{max} nm: 423, 445, 473; IR ν_{max} cm^{-1}: 3360 (*m*) (broad), 2900 (*s*), 2850 (*m*), 1440 (*w*), 1379 (*w*), 1352 (*w*), 1292 (*w*), 1238 (*w*), 1085 (*m*), 1034 (*m*), 956 (*s*), 870 (*w*), 820 (*w*), ^{1}H NMR (300 MHz): δ0.883 *s* and 1.434 *s* (3H + 3H, Me-16, Me-17), 1.072 *s* (6H, Me-16', Me-17'), 1.213 *s* (3H, Me-18), 1.735 *s* (3H, Me-18'), 1.967 *s* (12H, Me-19, Me-20, Me-19', Me-20'), 3.9 *m* (1H, H-3'), 4.4 *m* (1H, H-3), 5.74 *d* (*J* = 15 Hz, 1H, H-7), 6.1–6.7 *m* (13H, olefinic). Assignment of the ^{13}C NMR signals of **1** was consistent with data for (3*R*,3'*R*)-zeaxanthin (**4**) [15] and (3*S*,5*R*,6*R*)-5,6-dihydro-5-hydroxy-3,6-epoxy-β-ionol (**3**): δ12.84 *q* (C-19',20'), 21.62 *q* (C-18'), 25.74 and 32.19 *q* (C-16, C-17), 28.77 *q* (C-16'), 30.29 *q* (C-17'), 31.62 *q* (C-18), 37.14 *s* (C-1'), 42.61 *t* (C-4'), 44.05 *s* (C-1), 47.80 *t* (C-2), 48.52 *t* (C-4, C-2'), 65.13 *d* (C-3'), 75.38 *d* (C-4), 82.52 *s* (C-5), 91.68 *s* (C-6), 124.94 *d* (C-11'), 125.60 *d* (C-7'), 126.17 *s* (C-5'), 130.09 *d* (C-15'), 131.33 *d* (C-10'), 132.68 *d* (C-14'), 135.68 *s* (C-9'), 136.51 *s* (C-13'), 137.61 *s* (C-6'), 137.81 *d* (C-12'), 138.53 *d* (C-8'); remaining sp^2 C signals not assigned 122.89, 124.85, 131.62, 132.60, 134.78, 134.93, 136.43; MS *m/z* (rel. int.): 584.4196 cal. 584.4199 for $C_{40}H_{56}O_3$ $[M]^+$ (100), 566 $[M-18]^+$ (5), 532 $[M-52]^+$ (5), 492 $[M-92]^+$ (3), 463 $[M-121]^+$ (5), 438 $[M-146]^+$ (2), 347 $[M-237]^+$ (15), 228 (20), 106 (1); CD (EPA) nm ($\Delta\varepsilon$): 223 (0), 239 (+2.3), 254 (0), 275 (−7.6), 305 (−1.5), 335 (−2.1).

Acetylation of **1** gave a monoacetate (*m/z* 626) with R_f 0.70. Trimethyl silylation of **1** provided a di-trimethyl silylether (*m/z* 728) with R_f 0.89.

$LiAlH_4$ *reduction of cucurbitaxanthin A* (**1**). Reduction of **1** (0.5 mg) with $LiAlH_4$ in dry Et_2O (8 ml) for 12 hr at 30° provided (3*R*,3'*R*)-zeaxanthin (**4**) (0.3 mg).

Compound **4** *derived from* **1**. R_f 0.38, inseparable from authentic sample of **4** obtained from *T. officinale* [14] on co-TLC and co-HPLC; mp 194–195°; VIS λ_{max} nm: (425), 449, 475; MS *m/z* (rel. int.): 568 $[M]^+$ (100), 550 $[M-18]^+$ (80), 532 $[M-36]^+$ (60), 476 $[M-92]^+$ (10), 462 $[M-106]^+$ (10); ^{1}H NMR (300 MHz): δ1.072 *s* (12H, Me-16, Me-17, Me-16', Me-17'), 1.735 *s* (6H, Me-18, Me-18'), 1.967 *s* (12H, Me-19, Me-20, Me-19', Me-20'), 2.04 *d*, *d* [2H, H-4 (ax), H-4' (ax)], 2.39 *d*, *d* [2H, H-4 (eq), H-4' (eq)], 3.9 *m* (2H, H-3, H-3'), 6.1–6.7 *m* (14H, olefinic); CD (EPA) nm ($\Delta\varepsilon$): 224 (−18.0), 236 (0), 245 (+18.0), 260 (0), 284 (−24.8), 325 (0), 350 (+4.0).

Cucurbitaxanthin B (**2**). R_f 0.40, mp 181 –182°; VIS λ_{max} nm: 415, 438, 468; IR ν_{max} cm^{-1}: 3360 (*m*, broad), 2900 (*s*), 2850 (*m*), 1440 (*w*), 1379 (*w*), 1352 (*w*), 1292 (*w*), 1238 (*w*), 1085 (*m*), 1034 (*m*), 956 (*s*), 870 (*w*), 820 (*w*); ^{1}H NMR (300 MHz): δ0.883 *s* and 1.434 *s* (3H + 3H, Me-16, Me-17), 0.978 and 1.152 *s* (3H + 3H, Me-16', Me-17'), 1.188 *s* (3H, Me-18'), 1.213 *s* (3H, Me-18), 1.928 *s* (3H, Me-19'), 1.950 *s* (3H, Me-20'), 1.967 *s* (6H, Me-19, Me-20), 3.90 *m* (1H, H-3'), 4.38 *m* (1H, H-3), 5.74 *d* (*J* = 15 Hz, 1H, H-7), 5.88 *d* (*J* = 15 Hz, 1H, H-7'), 6.15–6.70 *m* (12H, olefinic), CD (EPA) nm ($\Delta\varepsilon$): 210 (−6.8), 225 (0), 230 (+3.0), 236 (0), 267 (−19.0), 309 (0), 327 (+2.0), 340 (0); MS *m/z* (rel. int.): 600.4156 calc. 600.4154 for $C_{40}H_{56}O_4$ $[M]^+$ (44), 582 $[M-18]^+$ (5), 520

$[M-80]^+$ (20), 508 $[M-92]^+$ (17), 287 (42), 221 (100), 91 (60). The furanoid rearrangement product of **2** showed VIS λ_{max} nm; 395, 419, 448. Acetylation of **2** gave a monoacetate (*m/z* 642) with R_f 0.65. Trimethyl silylation of **2** provided di-trimethyl silylether (*m/z* 744) with R_f 0.85.

Reduction of cucurbitaxanthin B (**2**). Compound **2** (0.5 mg) in dry Et_2O (8 ml) at 30° treated with $LiAlH_4$ for 12 hr provided (3*R*,3'*R*)-zeaxanthin (**4**) (0.3 mg).

Compound **4** *derived from* **2**. R_f 0.38, inseparable from authentic sample of **4** obtained from *T. officinale* [14]; mp 194–195°; VIS λ_{max} nm: (425), 449, 475; MS *m/z* (rel. int.): 568 $[M]^+$ (100), 550 $[M-18]^+$ (80), 532 $[M-36]^+$ (60), 476 $[M-92]^+$ (10), 462 $[M-106]^+$ (10); ^{1}H NMR (300 MHz): δ1.072 *s* (12H, Me-16, Me-17, Me-16', Me-17'), 1.735 *s* (6H, Me-18, Me-18'), 1.967 *s* (12H, Me-19, Me-20, Me-19', Me-20'), 2.04 *d*, *d* [2H, H-4 (ax), H-4' (ax)], 2.39 *d*, *d* [2H, H-4 (eq), H-4' (eq)], 3.9 *m* (2H, H-3, H-3'), 6.1–6.7 *m* (14H, olefinic); CD (EPA) nm ($\Delta\varepsilon$): 224 (−18.0), 236 (0), 245 (+18.0), 260 (0), 284 (−24.8), 325 (0), 350 (+4.0).

(3S,5R,6R)-5,6-*Dihydro-5-hydroxy*-3,6-*epoxy-β-ionol* (**3**). ^{1}H NMR (300 MHz): δ0.898 *s* and 1.410 *s* (3H + 3H, Me-11, Me-12), 1.209 *s* (3H, Me-13), 1.302 *d* (3H, Me-10), 4.4 *m* (2H, H-3, H-9), 5.72 *d* (1H, H-7), 5.78 *d*, *d* (1H, H-8); ^{13}C NMR (75 MHz): δ23.68 *q* (C-10), 25.62 *q* and 32.06 *q* (C-11, C-12), 31.55 *q* (C-13), 43.42 *s* (C-1), 47.69 *t* (C-2), 48.48 *t* (C-4), 68.79 *d* (C-9), 75.36 *d* (C-3), 82.05 *s* (C-5), 90.61 *s* (C-6), 123.60 *d* (C-8), 134.50 *d* (C-7).

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