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ISOLATION OF METHYL 9′*Z*-APO-6′-LYCOPENOATE FROM *BIXA ORELLANA*

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Key Word Index—*Bixa orellana*; Bixaceae; annatto; apocarotenoid; methyl 9′*Z*-apo-6′-lycopenoate

Abstract—The structure of an apocarotenoid (1% of total carotenoid) isolated from the seed coat of *Bixa Orellana* fruit was established as methyl 9′*Z*-apo-6′-lycopenoate (methyl 9′*Z*-6′-apo-ψ-caroten-6′-oate) by means of UV-visible and 500 MHz ^{1}H NMR spectroscopy and mass spectrometry. The well-known C_{40}-carotenes phytoene, phytofluene, ξ-carotene and neurosporene were tentatively identified.

INTRODUCTION

As the demand for annatto and other natural colorants increases [1], more stringent specifications for these products are imposed and better understanding of their chemistry and biochemistry is warranted. Annatto extracts are obtained from the seed coat of *Bixa orellana* L. fruits. *B. orellana* is a large, rapidly growing tree native to tropical America and is now grown in many tropical countries in South and Central America, Africa and Asia [2]. It bears clusters of brown or crimson capsular fruits, containing 10 to 50 seeds covered with thin, highly coloured resinous coatings. The major producers of annatto seeds are Peru, Brazil and Kenya.

There is general agreement [2, 3] that more than 80% of the carotenoids in the *B. orellana* seed coat consists of the apocarotenoid bixin, methyl hydrogen 9′*Z*-6,6′-diapocarotene-6,6′-dioate (**1**), which has been encountered to date only in *B. orellana*. A small amount of norbixin, 9′*Z*-6,6′-diapocarotene-6,6′-dioic acid (**2**), is also found [3]. No detailed investigation of the biosynthesis of bixin has been reported.

Other carotenoids have been detected in trace amounts in the annatto seeds. Tirimanna [4] detected β,β-carotene, cryptoxanthin, lutein, zeaxanthin and methyl bixin but identification was based only on TLC behaviour compared with standards. From UV-visible, mass and ^{1}H NMR spectra, Jondiko and Pattenden [5] established the structure of a new apocarotenoid, methyl 9*Z*-8′-oxo-6,8′-diapocaroten-6-oate. These authors were unable to demonstrate the presence of C_{40}-carotenoids, although extensive chromatography was carried out.

In our preliminary studies of annatto, we detected many carotenoids in small amounts. In this paper we report the isolation and structural elucidation of a geometrical isomer of an apocarotenoid.

RESULTS AND DISCUSSION

Carotenoids were extracted from *B. orellana* seeds with dichloromethane, and the extract was subjected to successive column and TLC. From the carotene fraction, four main components were obtained and tentatively identified by their chromatographic properties and UV-visible and mass spectra as phytofluene, ξ-carotene and neurosporene. Although phytoene could not be isolated in a pure form, its presence was indicated by the UV and mass spectra. Because of the small amounts isolated the geometrical configuration of the C_{40}-carotenes were not established.

On rechromatography on MgO–kieselguhr the third zone was shown to be methyl 9′*Z*-apo-6′-lycopenoate (methyl 9′*Z*-6′-apo-ψ-caroten-6′-oate) (**3**) by ^{1}H NMR spectroscopy. All proton signals could be assigned by means of a ^{1}H–^{1}H COSY experiment. The chemical shifts of the lycopene (ψ) end group were identical to the corresponding data from the literature [6–8]. The separated spin-systems within the polyene chain could be identified by their cross-peaks and typical coupling constant values. COSY cross-peaks to the in-chain methyl groups unequivocally proved these assignments. The singlet for the methyl protons of the ester group appeared at δ 3.76 as did that of all-*E* methyl apo-6′-lycopenoate [9]. The poor resolution of the proton spectrum previously

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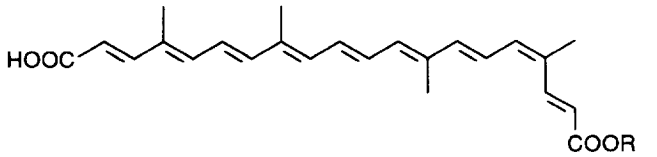
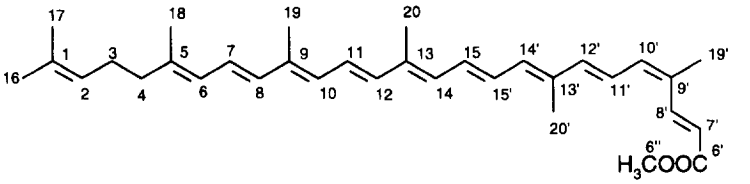


R

(1) CH_3

(2) H



(3)

obtained for all-*E* methyl apo-6′-lycopenoate [9] prevented a complete data comparison with regard to chemical shifts and coupling constant values of the olefinic proton signals that are diagnostically relevant for fixing the in-chain stereochemistry. Therefore, the spectral data of some all-*E* carotenoids with analogous ester end groups [7] were also used for comparison. The chemical shift values δ(H-8′), δ(H-10′), δ(H-11′) and δ(H-12′) of the present apocarotenoid deviated clearly from those of the corresponding protons in all-*E* isomers; no differences, however, were found for the coupling constant values ($^3J_{7'/8'}$, $^3J_{10'/11'}$ and $^3J_{11'/12'}$). Two available pieces of information from the spectrum of all-*E* methyl apo-6′-lycopenoate, δ(H-8′) = 7.39 ppm and $^3J_{7'/8'}$ = 15.4 Hz [9], were in full agreement with these results. According to Englert [7], the isomerization proton shift values $\Delta\delta = \delta(9'Z) - \delta(\text{all-}E)$ should be in the region of + 0.54 ppm for H-8′, − 0.09 ppm for H-10′, + 0.10 ppm for H-11′, and − 0.06 ppm for H-12′. The $\Delta\delta$-values found by comparing the chemical shifts for compound **3** with the average values for the all-*E* isomers mentioned above were + 0.58, − 0.14, + 0.23 and − 0.10 ppm, in agreement with the 9′*Z*-configuration. The NMR analysis unequivocally proves the structure of the compound from *B. orellana* as methyl 9′*Z*-6′-apo-ψ-caroten-6′-oate. The data previously presented for the pigment from *Shepherdia canadensis* [9] are consistent with the reported identification as the all-*E* isomer; whereby the structure elucidation was based on the comparison of the natural compound with a sample prepared by total synthesis. The UV–visible spectrum of methyl 9′*Z*-apo-6′-lycopenoate showed little fine structure and hypsochromic shift of 11 nm compared with the reported λ_{max} value for all-*E* isomer [9]. As expected, the mass spectrum was almost the same as that of the all-*E* isomer [9] with the molecular ion at *m/z* 472 (consistent with $C_{33}H_{44}O_2$). Characteristic loss of 69 mass units from the molecular ion and a peak at *m/z* 69 indicated the presence of a ψ-end-group. The apocarotenoid was reduced with $LiAlH_4$ to verify the presence of a carbonyl function. After purification on silica TLC, the reduced carotenoid was characterized by UV–visible and mass spectra.

All-*E* methyl apo-6′-lycopenoate was previously found, along with lycopene, as a major carotenoid in ripe berries of *S. canadensis* [9]. The 9′*Z*-isomer has not been reported as a natural constituent.

The detection of methyl 9′*Z*-apo-6′-lycopenoate and the C_{40}-carotenoids phytoene, phytofluene, ξ-carotene and neurosporene in *B. orellana* supports oxidative degradation of C_{40}-carotenoids at both ends of the molecule as the possible route to the formation of bixin.

EXPERIMENTAL

Spectroscopy. Mass spectra were obtained with a VG model Quattro instrument with a direct inlet system at 70 eV, 230–240°. The ^{1}H-NMR (500.13 MHz) spectrum of the apocarotenoid was recorded on a Bruker DRX instrument at 23° in $CDCl_3$ (99.5%).

Isolation. The extraction of carotenoids from annatto seeds (100 g) was carried out with CH_2Cl_2 in an ultrasonic bath for 15 min. The extract was evapd to dryness under N_2. The carotenoids were redissolved in petrol and applied to a column of neutral alumina (activity between grades III and IV). The fr. eluted with Et_2O was collected, the polar carotenoids (mostly bixin and norbixin) remaining on the column. The ethereal fraction was concd

in a rotary evaporator at a temp. below 35°, dried under N_2 and applied to a silica gel thin-layer plate (0.5 mm), developed with CH_2Cl_2-petrol (1:1). The first (carotenes) and third (major) bands were scraped off, extracted with Et_2O and submitted to MgO–kieselguhr TLC (0.5 mm) with petrol and Me_2CO–petrol (2:3), respectively, as mobile phases. The four major bands that were obtained from the carotene fraction, one of which was fluorescent, and the only principal band of the third zone were scraped off and extracted with Et_2O. Immediately before ^1H NMR and mass spectrometry, all carotenoids were purified through a neutral alumina minicolumn.

Methyl 9′Z-apo-6′-lycopenoate (**3**). (*ca* 0.17 mg, a minor component < 1% total): UV–visible $\lambda_{max}^{Et_2O}$ nm: 342, 360 (*cis*-peaks), 439 (sh), 460, 493 (sh); EI-MS (probe) 70 eV, *m/z* (rel. int.): 472 $[M]^+$ (10), 403 $[M - 69]^+$ (1), 380 $[M - 92]^+$ (0.3), 366 $[M - 106]^+$ (7), 297 $[M - 69 - 106]^+$ (4), 69 (100); ^1H NMR (500.13 MHz, $CDCl_3$): δ 1.61 (3H, *s*, H-17), 1.69 (3H, *s*, H-16), 1.82 (3H, *s*, H-18), 1.96 (6H, *s*, H-19 and H-19′), 1.98 (3H, *s*, H-20), 1.99 (3H, *s*, H-20′), 2.12 (2H, *m*, H-3), 2.22 (2H, *m*, H-4), 3.76 (3H, *s*, 6′-$COOCH_3$), 5.15 (1H, *m*, H-2), 5.90 (1H, *d*, J = 15.3 Hz, H-7′), 5.96 (1H, *d*, J = 10.9 Hz, H-6), 6.18 (1H, *d*, J = 11.0 Hz, H-10), 6.25 (1H, *d*, J = 14.9 Hz, H-8), 6.27 (1H, X part of ABMX, H-14), 6.31 (1H, M part of ABMX; H-14′), 6.36 (1H, *d*, J = 14.0 Hz, H-12), 6.36 (1H, *d*, J = 11.3 Hz, H-10′), 6.41 (1H, *d*, J = 14.9 Hz, H-12′), 6.50 (1H, *dd*, J = 10.9 and 14.9 Hz, H-7), 6.62 (1H, B part of ABMX, H-15′), 6.66 (1H, *dd*, J = 11.0 and 14.0 Hz, H-11), 6.69 (1H, A part of ABMX, H-15), 6.83 (1H, *dd*, J = 11.3 and 14.9 Hz, H-11′), 7.97 (1H, *d*, J = 15.3 Hz, H-8′). For CH_3 (19′) the assignment is not proven.

9′Z-6′-apo-ψ-Caroten-6′-ol. UV-visible $\lambda_{max}^{Et_2O}$ nm: 329, 343 (*cis*-peaks), 425, 450, 480; EI-MS (probe) 70 eV, *m/z* (rel. int.): 444 $[M]^+$ (24), 426 $[M - 18]^+$ (5), 375 $[M - 69]^+$ (2), 338 $[M - 106]^+$ (23), 269 $[M - 69 - 106]^+$ (9), 69 (93).

Phytoene-like. UV-visible $\lambda_{max}^{Et_2O}$ nm: 275 (sh), 285, 296 (sh); EI-MS (probe) 70 eV, *m/z* (rel. int.): 544 $[M]^+$ (2), 339 $[M - 205]^+$ (3), 205 (30), 137 (69), 69 (100).

Phytofluene-like. UV-visible $\lambda_{max}^{Et_2O}$ nm: 331, 348, 367, %III/II: 90; EI-MS (probe) 70 eV, *m/z* (rel. int.): 542 $[M]^+$ (15), 405 $[M - 137]^+$ (4), 337 $[M - 205]^+$ (16), 69 (100).

ζ-Carotene-like. UV-visible $\lambda_{max}^{Et_2O}$ nm: 296 (*cis*-peak), 378, 400, 424, %III/II: 89; EI-MS (probe) 70 eV, *m/z* (rel. int.): 540 $[M]^+$ (13), 446 $[M - 94]^+$ (1), 403 $[M - 137]^+$ (3), 137 (32), 69 (100).

Neurosporene-like. UV-visible $\lambda_{max}^{Et_2O}$ nm: 416, 438, 467, %III/II: 89; EI-MS (probe) 70 eV, *m/z* (rel. int.): 538 $[M]^+$ (7), 469 $[M - 69]^+$ (0), 446 $[M - 92]^+$ (1), 369 $[M - 137]^+$ (1), 69 (100).

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REFERENCES

1. Ghiraldini, J. E. (1994) II *Simposio Brasileiro de Urucu*, p. 42, Belém, Brasil.
2. Preston, H. D. and Rickard, M. D. (1980) *Food Chem.* **5**, 47.
3. Lauro, G. J. (1991) *Cereal Foods World* **36**, 949.
4. Tirimanna, A. S. L. (1981) *Mikrochim. Acta* **2**, 11.
5. Jondiko, I. J. O. and Pattenden, G. (1989) *Phytochem.* **28**, 3159.
6. Englert, G. (1981) in *Carotenoid Chemistry and Biochemistry* (Britton, G. and Goodwin, T. W., eds). p. 107. Pergamon Press, London.
7. Englert, G. (1995) in *Carotenoids Vol 1B: Spectroscopy* (Britton, G., Liaaen-Jensen, S. and Pfander, H., eds), p. 147. Birkhäuser, Basel.
8. Hengartner, U., Bernhard, K., Meyer, K., Englert, G. and Glinz, E. (1992) *Helv. Chim. Acta* **75**, 1848.
9. Kjøsen, H. and Liaaen-Jensen, S. (1969) *Phytochemistry* **8**, 483.