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SHORT COMMUNICATION

CITRUS CAROTENOIDS-VIII.

THE ISOLATION OF SEMI- β -CAROTENONE AND β -CAROTENONE FROM CITRUS RELATIVES

HENRY YOKOYAMA and MICHAEL J. WHITE

Fruit and Vegetable Chemistry Laboratory,¹ Pasadena, California, U.S.A.

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Abstract—Two new naturally occurring carotenoid ketones have been isolated from the *Citrus* relatives, *Murraya exotica* Linn. and *Triphasia trifolia* (Burm. f.) P. Wils. These pigments were structurally identified as semi- β -carotenone and β -carotenone.

THE formation of semi- β -carotenone (I) and β -carotenone (II) have been reported² as partial oxidation products of β -carotene (III). These pigments, however, have not heretofore been reported as being present in nature.



¹ A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

² R. KUHN and H. BROCKMANN, Ber. 66, 1319 (1933); R. KUHN and H. BROCKMANN, Liebigs Ann. 516, 95 (1935).

We now report the natural occurrence of the monocyclic diketone (I) and the acyclic tetraketone (II) in the fruits of the *Citrus* relatives,³ *Murraya exotica* Linn. and *Triphasia trifolia* (Burm. f.) P. Wils. These compounds appear to be responsible for the bright red color of these fruits.

The monocyclic pigment was isolated as a major constituent from the extractives of the fruit of M. exotica Linn. The isolated compound possessed a decaenone chromophore similar to that observed in the citrus carotenoid citranaxanthin⁴ and reticulataxanthin.⁵ The structure of the isolated carotenoid ketone (I) is firmly supported by the visible, i.r., NMR, and mass spectra data. The visible spectrum conformed to that of a decaenone

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Fig. 1. Nuclear magnetic resonance spectrum of semi- β -carotenone in CDCl₃.

chromophore. Reduction of the isolated pigment with sodium borohydride afforded a product which exhibits a hypsochromic shift (*ca.* 25 nm) in its absorption maxima. The i.r. spectrum indicated saturated carbonyl (1715 cm⁻¹) and conjugated carbonyl (1660 cm⁻¹).⁶ The NMR spectrum (Fig. 1) included signals at 8.95, 8.82 (geminal methyls at C-1 and C-1'), 8.27 (methyl at C-5), 8.00 (methyls at C-9, C-13, C-9', and C-13'), and 7.89 (methyl at C-5'), with the correct relative intensities. No band was observed in the 0.3–0.6 region

- ⁵ A. L. CURL, J. Food Sci. 27, 527 (1962); H. YOKOYAMA, M. J. WHITE and C. E. VANDERCOOK, J. Org. Chem. 30, 2482 (1965).
- 6 L. J. BELLAMY, Infrared Spectra of Complex Molecules, 2nd edition. Methuen, London (1958).

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³ For a discussion of the botanical classification of the genera *Murraya* and *Triphasia*, see W. T. SWINGLE in *The Citrus Industry* (edited by H. H. WEBBER and L. D. BATCHELOR), Vol. I, Chapter IV. University of California Press, Berkeley (1943).

⁴ H. YOKOYAMA and M. J. WHITE, J. Org. Chem. 30, 2481 (1965).

where aldehydic protons with α,β unsaturation exhibit absorption. The mass spectrum indicated the proper molecular weight of 568. Elementary analysis was in agreement with the molecular formula C₄₀H₅₆O₂. The structure of the compound was proved by synthesis from β -carotene (III).

Accompanying the monocyclic ketone (I) in the fruit of the other citrus relative *T. trifolia* (Burm. f.) P. Wils. was a small amount of a keto-carotenoid which exhibited a chromophore quite similar to that of capsorubin (IV). However, the i.r. spectrum of the present pigment exhibited sharp bands at 1715 and 1660 cm⁻¹, indicating the presence of saturated and conjugated carbonyl groups.⁶ Furthermore, the isolated pigment did not display the same



FIG. 2. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF β -carotenone in CDCl₃.

TLC behavior with an authentic sample of capsorubin. This nonidentity with capsorubin was further confirmed by the NMR spectrum. The latter spectrum (Fig. 2) included signals at 8.81 (geminal methyls at C-1 and C-1'), 8.00 (methyls at C-9, C-13, C-9', and C-13'), and 7.88 (methyls at C-5 and C-5'). This, together with other evidences, indicated structure II. Its mass spectrum showed the molecular ion at m/e 600 as required. Elementary analysis indicated the molecular formula $C_{40}H_{56}O_4$. Structure II for the isolated pigment was subsequently confirmed by synthesis from β -carotene.

EXPERIMENTAL SECTION

Melting points were determined in evacuated sealed capillary tubes and are uncorrected. Visible spectra were measured with a Cary Model 14 spectrophotometer. I.r. spectra were recorded in KBr disks on Perkin-Elmer Model 521 spectrophotometer. NMR were determined on a Varian A-60 spectrometer. The NMR

data refer to deuterochloroform solutions, and chemical shifts are in τ values relative to tetramethylsilane as an internal standard. Mass spectral analysis was performed by West Coast Technical Services, San Gabriel, California. Elementary analyses were by L. M. White. The fruit collections were made in April, 1967, at the University of Florida, Subtropical Experiment Station, Homestead, Florida. The fruits were deeply red colored. (Reference to a company or product does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.)

Extraction of Murraya exotica Linn

The seeds were removed from the fruit and the fleshy portion (peel and endocarp, 800 g) extracted with several portions of acetone. The 31. of extract was concentrated at reduced pressure to 500 ml. The carotenoid mixture was diluted with *n*-hexane and 90 per cent methanol. The epiphase was submitted to column chromatography on magnesium oxide-Hyflo Supercel (1:2, w/w). The isolated pigment crystallized from *n*-hexane, yielding 52 mg: m.p. 115–117°; sintered at 105°; λ_{max} in *n*-hexane 440 ($\epsilon \times 10^{-3}$ 82), 467 ($\epsilon \times 10^{-3}$ 105), and 495 nm ($\epsilon \times 10^{-3}$ 84); i.r. bands at 1715 (saturated carbonyl) and 1660 (conjugated carbonyl) cm⁻¹; NMR signals⁷ at 7.89, 8.00, 8.27, 8.82, and 8.95 (relative intensities (1:4:1:2:2); parent ion in the mass spectrum at m/e 568. (Found: C, 84·3; H, 9.84 per cent. C₄₀H₅₆O₂ required: C, 84·45; H, 9.92 per cent; M.W. 568.)

Reduction of the isolated pigment carried out in the usual manner⁴ with NaBH₄ afforded the reduced product: λ_{max} in *n*-hexane, 420, 442, 471 nm.

Semi- β -carotenone (I)²

To a solution of 0.2 g of β -carotene (III) in 200 ml of benzene was added slowly and with swirling 100 ml of the chromic acid solution prepared by diluting 6 ml of a 0.075 N chromic acid to 100 ml with glacial acetic acid. The reaction mixture was allowed to stand at room temperature for 1.5 hr. Isolation of the partial oxidation product by column chromatography on deactivated alumina and crystallization from *n*-hexane gave the diketone (I), yielding 30 mg; m.p. 116–117°, sintered at 106°; (lit.² m.p. 116–117°); both samples exhibited the same TLC behavior (on silica gel G-Ca (OH₂); λ_{max} in *n*-hexane 440 ($\epsilon \times 10^{-3}$ 82), 467 ($\epsilon \times 10^{-3}$ 105), and 495 nm ($\epsilon \times 10^{-3}$ 84). The NMR spectrum 7 (signals at 7.89, 8.00, 8.27, 8.82 and 8.95; relative intensities 1:4:1:2:2) is in full accord with structure I. Further proof of identity with natural semi- β -carotenone was established by i.r. spectroscopy. (Found: C, 84·2; H, 9·78. Calc. for C₄₀H₃₆O₂: C, 84·45; H, 9·92 per cent.)

Extraction of Triphasia trifolia

The fruits (300 g) were extracted with several portions of acetone and methanol. The 4 l. of extract was concentrated at reduced pressure to 500 ml. The carotenoid mixture was diluted with ether and phase-separation effected by addition of saturated NaCl solution. The epiphase, dried (MgSO₄), was stripped of ether, and the residue was phase-partitioned between light petroleum and 95 per cent methanol. The hypophase was chromatographed on Woelm neutral alumina, deactivated. Elution with peroxide-free ether containing 0.25 per cent methanol isolated the ketone. The pigment was crystallyzed from peroxide-free ether-light petroleum, yielding 15 mg: m.p. 175–176°; λ_{max} in CHCl₃ 460 sh ($\epsilon \times 10^{-3}$ 62·4), 490 ($\epsilon \times 10^{-3}$ 97·5) and 522 nm ($\epsilon \times 10^{-3}$ 82·4); i.r. bands at 1715 (saturated carbonyl) and 1660 (conjugated carbonyl) cm⁻¹; NMR signals 7 at 7-88, 8-00 and 8-81 (relative intensities 1:2:2; integrated to 30 methyl protons in agreement with structure II). (Found: C, 79·8; H, 9·18 per cent; M.W. (m/e) 600. C₄₀H₅₆O₄ required: C, 79·96; H, 9·39 per cent; M.W. 600.)

β-Carotenone²

 β -carotenone (II, 36 mg, m.p. 175–176°) was synthesized by the partial oxidation of β -carotene (400 mg) with chromic acid. Reported: m.p. 176–177°.² (Found: C, 79.8; H, 9.21. Calc. for C₄₀H₅₆O₄: C, 79.96; H, 9.39 per cent.)

Comparison with natural sample showed same TLC behavior (on silica gel G-Ca $(OH)_2$); and they had identical visible, i.r. and NMR spectra.

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⁷ Methyl signals only.

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