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IDENTIFICATION OF MONOESTERIFIED CAPSANTHIN IN PAPRIKA (*CAPSICUM ANNUUM*): THE NATURE OF ESTERIFICATION OF CAPSANTHIN

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Capsanthin esters were separated from paprika (*Capsicum annuum*) oleoresin, and their structures were determined without saponification. The major monoesterified capsanthin was identified as 3'-*O*-myristoylcapsanthin. 3-*O*-Esterified capsanthins were not detected, although 3,3'-*O*-diesterified capsanthins with saturated fatty acids were isolated. The results suggest that the rate in esterification of fatty acid to the hydroxyl group on the cyclopentane ring of capsanthin is different from that on the cyclohexene ring.

KEY WORDS capsanthin; *Capsicum annuum*; paprika; esterification; oleoresin

The ripe fruits of paprika (*C. annuum*) have been used worldwide as peppers, vegetables, folk medicines and food colorants. It is well known that paprika is a good source of carotenoid pigments and that substantial amounts of hydroxycarotenoids (xanthophylls), mainly capsanthin and capsorbin in the ripe fruits exist as fatty acid esters. Esterification enhances the liposolubility; therefore, it is believed that esterified xanthophylls accumulate more easily in the lipophilic globules of fruit chromoplastes. According to several earier papers,1) esterified xanthophylls are more stable than nonesterified ones. Thus, it seems that esterification also plays an important part in accumulation of xanthophylls in the fruits.

In 1978, Camara and Moneger reported that capsanthin exists as nonesterified, monoesterified and diesterified forms in the fruits of *C. annuum*, whereas capsorbin and zeaxanthin exist mainly in diesterified form and monoesterified form, respectively.2) Then, several group authors also observed that the degree of esterification is different among various xanthophylls and that the degree of esterification in capsorbin in particular is higher than that of capsanthin.1a, 3) Recently, Minguez-Mosquera and Hornero-Mendez reported that the portion of diesterified forms in the fruit of paprika is constant during ripening, while the total content of xanthophylls and the portion of monoesterified forms increase.3d) However, no explanation has been offered for these somewhat curious results, although esterification is an important step in xanthophyll accumulation in nature. In the course of our studies on natural colorants,4) we first identified a major monoesterified capsanthin without saponification. Since the identification led to a hypothesis which clearly accounted for the curious phenomena, we decided to describe it in this paper.

Paprika oleoresin (450 mg) imported from Spain was subjected to a preparative TLC as described in the literature with minor modifications.5) Each fraction was analyzed by HPLC,6) and a part of the monoester fraction (R_f=0.1) was further subjected to a preparative HPLC7) to afford b2 (1.5 mg), which was observed as a major peak8) in the analytical HPLC. A part of the diester fraction (R_f=0.4) was also purified by the preparative HPLC to afford c19) (lauroylmyristoylcapsanthin, 6 mg) and c39) (dimyristoylcapsanthin, 3 mg), which were also regarded as major pigments.8)

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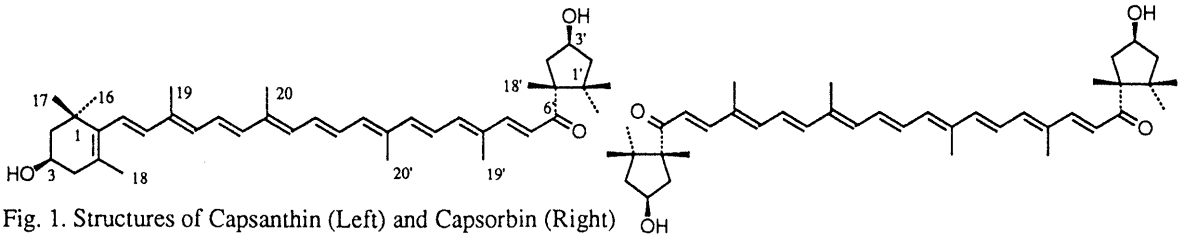

Fig. 1. Structures of Capsanthin (Left) and Capsorbin (Right)

The APCI-MS analysis of b2 showed a quasi-molecular ion peak at m/z 795, which corresponds to myristoylcapsanthin. ^{1}H-NMR spectrum[10)] showed very similar signals to those of authentic capsanthin,[11)] except for signals attributable to an esterified fatty acid and a trimethylcyclopentane ring. Among the signals, H-3' signal at δ 5.24 showed a remarkable down-field sift relative to that at δ 4.51 in capsanthin. These indicated that esterification occurred at 3'-hydroxyl group in the cyclopentane ring rather than 3-hydroxyl group in the cyclohexene ring. Furthermore, ^{1}H-NMR spectra of diesterified capsanthins[12)] provide more information on the esterified position of b2. In the spectra of b2 and capsanthin, the methyl signals of C-16 and C-17 on the cyclohexene ring were observed as equivalent singlet at δ 1.08. On the other hand, in the spectra of c1 and c3, the signals became two singlets at δ 1.08 and δ 1.11. Therefore, the hydroxyl group on the cyclohexene ring of b2 was not substituted, and the structure of b2 was confirmed as 3'-*O*-myristoylcapsanthin.

Analytical HPLC data suggested that the monoester fraction obtained by TLC contained more than fifteen pigments. In order to ascertain whether most of the monoesterified capsanthins are esterified at 3'-position at the cyclopentane ring, the monoester fraction was directly analyzed by ^{1}H-NMR. In the spectrum, the signals at δ 1.11 corresponding to methyl protons of C-16 (or C-17) of 3-*O*-esterified capsanthin were not observed, although the sharp signals at δ 1.08 corresponding to the equivalent methyl protons of nonesterified one were observed. Minor fractions derived from the preparative HPLC of the monoester fraction were also analyzed by ^{1}H-NMR, and the methyl signals at δ 1.11 of 3-*O*-esterified capsanthin were not detected in any spectra. Consequently, it can be deduced that most of the monoesterified capsanthins exist as 3'-*O*-esterified form in paprika oleoresin, although 3,3'-*O*-diesterfied capsanthins are also present.

So far, no one has been able to report which hydroxyl group is esterified in the monoesterified capsanthins since their identifications were performed after saponification. Our finding, that most of the monoesterified capsanthins are esterified at the cyclopentane ring, leads to a hypothesis that esterification at the cyclohexene ring is the rate-determining step in the esterification of xanthophyll, in other words, that 3'-hydroxyl group is esterified much faster than 3-hydroxyl group. If so, the following explanation is conceivable for the above-mentioned phenomena. Capsorbin, which has two hydroxycyclopentane rings, is esterified faster than capsanthin, and zeaxanthin, which has two hydroxycyclohexene rings, is esterified slower. During fruit-ripening, net amounts of xanthophylls increase continuously with increasing esterified forms. Therefore, the esterification of the cyclohexene ring of capsanthin can not catch up with the biosynthetic supply of capsanthin, and, thus, 3'-*O*-monoesterified capsanthins continuously accumulate in the fruits, since capsanthin is the main xanthophyll.

Biacs reported that the monoesterified capsanthin contained mostly unsaturated acid on the basis of saponification-GC analyses of the monoester fraction obtained by HPLC, although the diesters contain saturated acid.[1)] However, we have clarified that the major monoesterified xanthophyll contains saturated acid. Since Philip reported that paprika extract includes a very high amount of triglycerides containing unsaturated acid, 5) the contamination of triglycerides might be conceivable for Biacs' results with saponification, although further studies are needed to clarify the reason.

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5) Philip T., Nawar W.W., Francis F.J., *J. Food Sci.*, **36**, 98 (1971). Our TLC conditions are as follows: plate, Merck Art 5717; developing solvent, 10% acetone in petroleum ether.
6) "HPLC condition" column: Wakosil-II 5C18 AR(250 x 4.6 mm); flow rate: 0.8 ml/min; temperature: 40°C; detection: 460 nm; solvents: 0-5 min, acetone-H_2O (7/3); 5-10 min, a linear gradient from 70% acetone to 90% acetone; 10-13 min, 90% acetone; 13-33 min, a linear gradient from 90% acetone to 100% acetone; 33-38 min 100% acetone. The detailed results of analytical HPLC of paprika oleoresins originating from several countries will be reported elsewhere.
7) "HPLC condition" column: Wakosil-II 5C18 AR (250 x 20 mm); flow rate 10 ml/min; temperature: 40°C; detection: 460 nm; solvents: 80% (b2) and 87% (c1 and c3) acetone in H_2O.
8) A peak which indicates height of 40% or more relative to the largest peak in the chromatogram is regarded as a major peak. Most oleoresins imported from Spain showed five major peaks (b2, b5: β-carotene, c1, c2: a diesterified xanthophyll, c3).
9) Philip and Francis5) first noted that a major diesterified carotenoid of paprika pigments is dilauroyl-capsanthin. Subsequently some studies [*e.g.*, reference 3 and cited therein] with saponification and GC analyses reported that diesterified capsanthins contain mostly middle chain (C_{12}, C_{14} and C_{16}) fatty acid. c1: Frit -FAB-MS m/z, 976(M^+), 777(M^+ - $CH_3(CH_2)_{10}COO$), 749(M^+ - $CH_3(CH_2)_{12}COO$); c3: Frit -FAB-MS m/z, 1004(M^+), 777(M^+ - $CH_3(CH_2)_{12}COO$).
10) b2, 600 MHz, $CDCl_3$, δ : 0.86(3H, s, 16'-Me), 0.88(3H, t, J=7.2 Hz, $-CH_2\underline{CH_3}$), 1.08(6H, s, 16-Me and 17-Me), 1.18(3H, s, 17'-Me), 1.22-1.33(about 15H, brs, $-CH_2-$), 1.32(3H, s, 18'-Me), 1.49(1H, m, H-2a), 1.56(1H, m, H-4'b), 1.60(2H, m, $-COCH_2\underline{CH_2}-$), 1.72(1H, m, H-2'b), 1.72(3H, s, 18-Me), 1.74(1H, m, H-2b), 1.95(3H, s, 19'-Me), 1.97(6H, brs, 19-Me and 20'-Me), 1.99(3H, s, 20-Me), 2.05(1H, m, H-4b), 2.08(1H,dd, J=7.8, 13.8 Hz, H-2'a), 2.26(2H, t, J=7.2 Hz, $-CO\underline{CH_2}CH_2-$), 2.38(1H, dd, J=17.4, 5.4 Hz, H-4a), 2.98(1H, dd, J=8.4, 15.0 Hz, H-4'a), 3.99(1H, m, H-3), 5.24(1H, m, H-3'), 6.11(1H, brd, J=16.2 Hz, H7 or H-8), 6.13(1H, d, J=16.2 Hz, H-7 or H-8), 6.16(1H, d, 12.6 Hz, H-10), 6.26(1H, d, J=11.4 Hz, H-14), 6.35(1H, d, J=10.8 Hz, H-14'), 6.36(1H, d, J=15.0 Hz, H-12), 6.43(1H, d, J=15.2 Hz, H-7'), 6.52(1H, d, J=14.4 Hz, H-12'), 6.55(1H, d, J=10.8 Hz, H-10'), 6.61(1H,dd, J=10.8, 14.4 Hz, H-11'), 6.63(1H, dd, J=10.8, 14.4 Hz, H-15'), 6.68(1H, dd, J=12.6, 15.0 Hz, H-11), 6.70(1H, dd, J=14.4, 11.4 Hz, H-15), 7.34(1H, d, J=15.2 Hz, H-8'). Assignments were confirmed by DQF-COSY analysis.
11) Insufficient data were reported in the paper [Ruttimann A., Englert G., Mayer H., *Helv. Chim. Acta*, **66**, 1939 (1983)]; therefore, we purchased authentic capsanthin from Extrasynthese (Genay, France) and measured it in the same condition as those of isolated samples.
δ : 0.84(3H, s, 16'-Me), 1.07(6H, s, 16-Me and 17-Me), 1.21(3H, s, 17'-Me), 1.37(3H, s, 18'-Me), 1.47(1H, m, H-2a), 1.49(1H, m, H-4'b), 1.71(1H, m, H-2'b), 1.73(3H, s, 18-Me), 1.77(1H, m, H-2b), 1.96(3H, s, 19'-Me), 1.97(6H, brs, 19-Me and 20'-Me), 1.99(3H, s, 20-Me), 2.00(1H, m, H-2'a), 2.05(1H, m, H-4b), 2.39(1H, dd, J=17.4, 5.4 Hz, H-4a), 2.96(1H, dd, J=8.4, 15.0 Hz, H-4'a), 4.00(1H, m, H-3), 4.51(1H, m, H-3'), 6.13(2H, s, H7 and H-8). The signals other than δ 6.13 in the olefinic region are the same as those of b2. Assignments were confirmed by DQF-COSY analysis.
12) 600 MHz, $CDCl_3$, δ : 0.86(3H, s, 16'-Me), 0.88(6H, t x2, J=7.2 Hz, $-CH_2\underline{CH_3}$) x2, 1.08(3H, s, 16-Me or 17-Me), 1.11(3H, s, 16-Me or 17-Me), 1.18(3H, s, 17'-Me), 1.22-1.33(about 35H (c1), about 40H (c3), brs, $-CH_2-$), 1.32(3H, s, 18'-Me), 1.57, 1.58 (each 1H, m, H-2a and H-4'b), 1.62(4H, m, $-COCH_2\underline{CH_2}-$ x2), 1.72(3H, s, 18-Me), 1.74(1H, dd, J=9.6 Hz, 13.8 Hz, H-2'b), 1.78(1H, m, H-2b), 1.95(3H, s, 19'-Me), 1.97(6H, brs, 19-Me and 20'-Me), 1.99(3H, s, 20-Me), 2.08(1H,dd, J=7.8, 13.8 Hz, H-2'a), 2.11 (1H, dd, J=9.6, 17.4 Hz, H-4b), 2.26, 2.28(each 2H, t, J=7.2 Hz, $-CO\underline{CH_2}CH_2-$ x2), 2.43(1H, dd, J=17.4, 5.4 Hz, H-4a), 2.98(1H, dd, J=8.4, 15.0 Hz, H-4'a), 5.07(1H, m, H-3), 5.24(1H, m, H-3'). Signals in the olefinic region are the same as those of b2. Assignments were confirmed by DQF-COSY analysis.

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