Carotenoid composition of the mushroom Scarlet elf cup
(Sarcoscypha coccinea)

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From the extract of the mushroom Scarlet elf cup (Sarcoscypha coccinea) (all-E, 2'R)-plectaniaxanthin, (all-E)-2'-dehydroplectaniaxanthin and a number of sterically unhindered (Z)-isomers of these carotenoids were isolated and partially characterized. The carotenoid composition of the Scarlet elf cup extract was determined by HPLC analysis. The structure elucidation of the isolated compounds was carried out by UV/Vis spectroscopy, 1H and 13C-NMR spectroscopy, IR spectroscopy and mass spectrometry. The NaBH₄-reduction of (all-E)-2'-dehydroplectaniaxanthin resulted in the racemic mixture of (R)- and (S)-plectaniaxanthin. The isolated (Z)-isomers were identified by their UV/Vis spectroscopic properties.

1. Introduction

The mushroom Scarlet elf cup (Sarcoscypha coccinea) is an edible fungus, even though it remains rather tough after cooking. It can be collected in Hungary in the Pécze area in the western Meesek mountains and in the Zselic during February and March. To our knowledge the carotenoid composition of this mushroom has not been investigated and described to date in the literature.

The novelty and the main goal of our work is the determination of the carotenoid composition of the mushroom Scarlet elf cup (Sarcoscypha coccinea) by HPLC and CLC, the isolation of its main carotenoids [(all-E, 2'R)-plectaniaxanthin (1) and (all-E)-2'-dehydroplectaniaxanthin (2); Fig. 1] in highly pure crystalline state, the structure elucidation of these carotenoids by spectroscopic methods (UV/Vis, 1H- and 13C-NMR, MS, IR), and the isolation of numerous sterically unhindered (Z)-isomers of 1 and 2, and their identification on the basis of their UV/Vis spectroscopic properties. The secondary objective of this study is the confirmation of the (2'R) configuration of the naturally occurring (all-E)-pectaniaxanthin (1).

Plectaniaxanthin [(all-E, 2'R)-3',4'-didehydro-1',2'-dihydro-β,β-carotene-1',2'-diol; 1] and its mono- and diester have been isolated for the first time from the mushroom Plectania coccinea (Arpin and Liaaen-Jensen, 1967a,b) and its occurrence was subsequently demonstrated in several other mushrooms, as well (Bae et al., 1971).

Plectania coccinea and Sarcoscypha coccinea are the same species; they are taxonomic synonyms (Korf, 1953; Ronneberg et al., 1982; http://www.mycobank.org/BiologyMICS.aspx?TableKey=14682616000000067&Rec=21926&Fields=All).

The constitution of the compound has been determined using spectroscopic methods (UV/Vis, IR, NMR, MS) (Arpin and Liaaen-Jensen, 1976; Arpin and Liaaen-Jensen, 1967a,b; Hertzberg & Liaaen-Jensen, 1967, 1969; Enzell et al., 1969; Vacheron et al., 1969; Arpin et al., 1970; Bae et al., 1971; Buchecoker et al., 1976), however the configuration at C(2') of the isolated compound remained unknown for a long time. On the basis of CD spectroscopic studies carried out in 1982, it was possible to deduce to the (R) configuration (Ronneberg et al., 1982).

The (2'R) configuration has been verified in 1984: The total synthesis of (2'S)-plectaniaxanthin was reported, using L-serine as starting material, and based on the comparison of the CD spectra of the natural and the synthetic compound, the (2'R)-configuration of the natural compound was established (Dumont and Pfander, 1984). The (2'R)-configuration has been confirmed by the result of later investigation (Ronneberg et al., 1985). The natural occurrence of the free (2'S)-plectaniaxanthin has not been reported to date, but its glucoside [phleixanthophyll; (2'S)-1'-(β-D-glucopyranosyloxy)-3',4'-didehydro-1',2'-dihydro-β,
ψ-caroten-2-ol occurs in nature (Hertzberg and Liaaen-Jensen, 1967; Ronneberg et al., 1985; Britton et al., 2004).

The above mentioned studies reported also the isolation of 2’-dehydroplectaniaxanthin [(all-E)-10-hydroxy-3’0,4’-didehydro-1’2-dihydro-β,ψ-caroten-2’-one; 2] and its esters.

2. Materials and methods

2.1. General experimental procedures

All chemicals used in the extraction, in column liquid chromatography (CLC) and during HPLC analysis were analytical grade quality (Sigma-Aldrich Ltd., Budapest, Hungary).

The UV/Vis spectra were recorded with a Beckman DU-65 spectrophotometer in toluene.

The NMR experiments were carried out on a Varian Unity Inova 400-WB spectrometer, at 400 MHz (1H) or 100 MHz (13C), in CDCl3 (99.8 atom% D; purchased from VWR International) at a probe temperature of 298K. Chemical shift values (δ) are given in ppm referenced to (CH3)4Si (1H) or the residual solvent signals (13C) (Molnár et al., 2006a,b).

FT-IR spectrum was recorded on an IMPACT 400 spectrometer (Nicolet Analytical Instruments, 5225-1 Verona Road, P.O. Box 4508, Madison, WI 53711-0508, USA; DTGS detector; Δν 400–4000 cm⁻¹; resolution 4 cm⁻¹) in KBr pellets (Lőränd et al., 2002a,b; Molnár et al., 2006a,b).

Mass spectra were recorded using a Varian MA–CH–7A mass spectrometer (Molnár et al., 2006a,b).

2.2. Preparative column chromatography

Table 1 Preparative column chromatography of the saponified total extract of mushroom Scarlet elf cup (Sarcoscypha coccinea); UV/Vis data of the isolated carotenoids.

| Zone | λmax (nm, toluene) | Q* | Carotenoid                  |
|------|--------------------|----|-----------------------------|
| 111  | 523, 489, 462, 376 | 12.3 | (all-E)-Plectaniaxanthin   |
| 132  | 515, 483, 457, 374 | 2.3  | (13Z)- or (13’Z)-Plectaniaxanthin |
| 143  | 515, 482, 457, 373 | 2.2  | (13’Z)- or (13Z)-Plectaniaxanthin |
| 151  | 512, 407           | 5.4  | (9Z)- or (9’Z)-Dehydroplectaniaxanthin |
| 152  | 505, 400           | 1.8  | (13Z)- or (13’Z)-Dehydroplectaniaxanthin |
| 222  | 511, 401           | 2.0  | (13’Z)- or (13Z)-Dehydroplectaniaxanthin |
| 23   | 496, 407           | 2.5  | (poly-Z)-Dehydroplectaniaxanthin |
| 241  | 510, 400           | 1.6  | (15Z)-Dehydroplectaniaxanthin |
| 243  | 515, 483, 458, 374 | 1.6  | (15’Z)-Plectaniaxanthin |
| 23   | 496, 407           | 2.5  | (poly-Z)-Dehydroplectaniaxanthin |
| 31   | 511, 406           | 4.9  | (9Z)- or (9’Z)-Dehydroplectaniaxanthin |
| 5    | 507, 403           | 2.7  | (all-E)-Dehydroplectaniaxanthin |
| 6    | 493, 464           | >10  | β-Carotene                  |

Carotenoid of zone 31 [(all-E)-2’-Dehydroplectaniaxanthin (2)] adsorbed with a blue colour on the CaCO3 column. * Q = Amax/Avis-peak.

Fig. 1. Structure of (all-E, 2’R)-plectaniaxanthin (1) and of (all-E)-2’-dehydroplectaniaxanthin (2).

Fig. 2. HPLC analysis of the saponified total extract of mushroom Scarlet elf cup (Sarcoscypha coccinea).
2.2. HPLC measurements

The HPLC separation of the extract of mushroom was performed on a Chromsyl C18 (6 μm; end-capped) column (250 × 4.6 mm i. d.; 298K), using Dionex 580 pump, HP 1050 detector with ChemStation software; detection wavelength was 450 nm. Gradient elution (in linear steps, and flow rate of 1.25 ml/min) was used with solvents A (H2O/MeOH 12 : 88), B (MeOH), and C (acetone/MeOH 1 : 1): 0–2 min, 100% A; 2–10 min, to A/B 80 : 20; 10–18 min, to A/B 50 : 50; 18–25 min, to 100% B; 25–27 min, 100% B; 27–34 min, to 100% C, 34–41 min, 100% C. The peak area was used to determine the percentage of individual components in the extract (Pfander and Riesen, 1995).

The chiral HPLC separation of the racemic mixture of plectaniaxanthin enantiomers and a co-chromatography of this mixture with the natural (all-E, 2′R)-plectaniaxanthin (1) was carried out on a Chiralcel OD (3μm) column [Daicel, Chemical Industries Ltd.; 250 × 4.6 mm i. d.; 303K (thermostated)]. The sample was dissolved in MeOH. Isocratic elution (flow rate of 0.7 ml/min) was used with solvents A (n-hexane) and B (abs. EtOH): 0–40 min, 5.5% B. The analysis was performed with a Dionex HPLC system (Thermo Fischer Scientific) (Turcsi et al., 2015).

2.3. Extraction

The freshly collected mushroom Scarlet elf cup (2500g) was homogenized with MeOH and extracted three times with MeOH and once with diethyl ether (Et2O). The three MeOH extracts and the ethereal extract were combined, transferred to a separatory funnel and diluted with Et2O. The ethereal phase was washed free from MeOH with water and dried over anhydrous Na2SO4. This solution was saponified with 30% KOH–MeOH at room temperature overnight (for 18 h). After this process the ethereal solution was washed free from alkali, evaporated to dryness under vacuum and dissolved in benzene. This solution was stored in darkness under nitrogen at -20 °C until further chromatographic separations (Molnár and Szabolcs, 1979; Schiedt and Liaaen-Jensen, 1995; Molnár et al., 2004; Molnár et al., 2005; Molnár et al., 2006a,b; Horváth et al., 2010; Agocs et al., 2018). The peak area was used to determine the percentage of individual components in the extract (Pfander and Riesen, 1995).

The chiral HPLC separation of the racemic mixture of plectaniaxanthin enantiomers and a co-chromatography of this mixture with the natural (all-E, 2′R)-plectaniaxanthin (1) was carried out on a Chiralcel OD (3μm) column [Daicel, Chemical Industries Ltd.; 250 × 4.6 mm i. d.; 303K (thermostated)]. The sample was dissolved in MeOH. Isocratic elution (flow rate of 0.7 ml/min) was used with solvents A (n-hexane) and B (abs. EtOH): 0–40 min, 5.5% B. The analysis was performed with a Dionex HPLC system (Thermo Fischer Scientific) (Turcsi et al., 2015).
2.4. Column liquid chromatography (CLC)

CaCO₃ (Ph. Hg. VI., Biogal, Hungary) was used as adsorbent (size of the columns 6 x 30 cm), and the solvents n-hexane, 5–10% toluene in n-hexane and 0.1–0.5% acetone in n-hexane were used as eluents (Molnár and Szabolics, 1979).

3. Results and discussion

The HPLC analysis of the saponified total extract of the mushroom gave the following carotenoids in order of their decreasing adsorption affinity: (all-E)-pectaniaxanthin (1), (Z)-isomers of pectaniaxanthin (all-E)-2-dehydropectaniaxanthin (2), (Z)-isomers of 2'-dehydro-pectaniaxanthin, an unidentified carotenoid, β-carotene, and (Z)-isomers of β-carotene (Fig. 2).

During the preparative column liquid chromatography (CLC) (Molnár and Szabolics, 1979) of the saponified total extract the following carotenoids were isolated in order of their decreasing adsorption affinity: (all-E)-pectaniaxanthin (1), (13Z)-pectaniaxanthin (13Z-1), (13Z)-2-dehydropectaniaxanthin ([9Z]-2), (13Z)-2'-dehydro-pectaniaxanthin ([13Z]-2), (13Z)-2'-dehydropectaniaxanthin ([13Z]-2), (15Z,5Z)-2'-dehydro-pectaniaxanthin ([15Z,5Z]-2'), (15Z)-pectaniaxanthin (15Z-1), (15Z)-2-dehydro-pectaniaxanthin (15Z-2), (2',9Z,9Z)-2'-dehydro-pectaniaxanthin ([9Z,9Z]-2'), (9Z,9Z)-2'-dehydro-pectaniaxanthin ([9Z]-2), (9Z,5Z)- or (9Z,5Z)-2-dehydro-pectaniaxanthin ([9Z,5Z]-2 or [9Z,5Z]-2'), β-carotene (Table 1) (all-E)-Pectaniaxanthin (1; 25 mg) and (all-E)-2-dehydro-pectaniaxanthin (2, 21 mg) were isolated in a pure crystalline state (m.p. of 1: 175–176 °C; m. p. of 2: 146–148 °C) and were identified by UV/Vis spectroscopy, 1H- and 13C-NMR spectroscopy and mass spectrometry. The UV/Vis data are shown in Table 1.

The UV/Vis spectra of (all-E)-pectaniaxanthin (1) together with the spectra of its three main (mono-Z)-isomers ([13Z]-1, (13Z)-1 and (13Z)-1), and the UV/Vis spectra of (all-E)-2-dehydro-pectaniaxanthin (2) together with its four main (mono-Z)-isomers ([9Z]-2, (9Z)-2 (13Z)-2 (13Z)-2) are indicated, as examples on Figs. 3, 4, 5, 6.

The UV/Vis spectroscopic properties (λmax-values, fine structure) of (all-E)-1 and (all-E)-2 were in agreement with the corresponding data in the literature (Bae et al., 1971; Rooneberg et al., 1982; Britton, 1995; Britton et al., 2004) confirming the characteristic chromophors (3', 4'-didehydro-1', 2'-dihydro-β,γ-carotene, 3', 4', 5'-didehydro-1', 2'-dihydro-β,γ-caroten-2-one respectively) of these carotenoids.

The (Z)-isomers of pectaniaxanthin (1) and 2'-dehydro-pectaniaxanthin (2) have not been isolated from natural sources to date. The crystallization of the majority of these isomers was unsuccessful because of their small quantity and special solubility, therefore their structure and geometrical configuration was deduced from their UV/Vis spectroscopic properties (λmax-shifts: Q = Amax/Amax_peak; Table 1, Figs. 3, 4, 5, 6) (Zechmeister, 1962; Britton, 1995; Molnár, 2009).

The 13C-NMR measurements of the naturally occurring (all-E)-pectaniaxanthin (1) and (all-E)-2-dehydro-pectaniaxanthin (2) were carried out for the first time in our laboratory. The 1H- and 13C-NMR chemical shifts (δ in ppm) of (all-E)-pectaniaxanthin (1) and (all-E)-2-dehydro-pectaniaxanthin (2) are listed in Table 2. The 1H-NMR chemical shifts of the (all-E)-pectaniaxanthin (1), isolated in our laboratory showed a good agreement with that of the natural (2')- and of the synthetic (2')-pectaniaxanthin, published earlier (Hertzberg and Lliaen-Jensen, 1969; Bae et al., 1971; Dumont and Pfander, 1984; Madhour et al., 2005); The 1H- and 13C-NMR chemical shifts of naturally occurring (all-E)-pectaniaxanthin (1) and of (all-E)-2'-dehydro-pectaniaxanthin (2) showed also

| C-atom | 1H | 13C |
|--------|-----|-----|
| 1      |      |     |
| 2      |      |     |
| 3      |      |     |
| 4      |      |     |
| 5      |      |     |
| 6      |      |     |
| 7      |      |     |
| 8      |      |     |
| 9      |      |     |
| 10     |      |     |
| 11     |      |     |
| 12     |      |     |
| 13     |      |     |
| 14     |      |     |
| 15     |      |     |
| 16     |      |     |
| 17     |      |     |
| 18     |      |     |
| 19     |      |     |
| 20     |      |     |

Table 2
a good agreement with the chemical shifts, which were published for the corresponding end groups (Arpin et al., 1970; Englert, 1995).

The MS data of (all-E)-plectaniaxanthin (1) were in accordance with literature data (Enzell et al., 1969; Vacheron et al., 1969; Arpin et al., 1970; Enzell and Back, 1995; Britton et al., 2004). The peak of molecular ion was observed at m/z 568 (M+), C40H56O2), together with fragments at m/z 552 ([M-O]+), 550 ([M-H2O-O]+), 534 ([M-H2O]+), 532 ([M-2H2O]+), 510 ([M-CH3COCH3]+), 508 ([M-CH3CHOHCH3]+), 476 ([M-C6H6CH3]+), 462 ([M-C6H6(CH2)2]+), 444 ([M-C6H6(CH2)2-H2O]+), 428 ([M-C6H4(CH2)2-H2O-O]+), 404 ([M-C6H4(CH2)2-CH2COCH3]+), 403 ([M-C6H4(CH2)2-CH2COCH3-H]+), 402 ([M-C6H4(CH2)2-CH3CHOHCH3]+), 209, 197, 171, 157, 145, 119, 105, 95, 81, 59, 57, 43.

The MS spectrum of (all-E)-2′-dehydroplectaniaxanthin (2) resulted in the following fragment ions, which were in accordance with the data in the literature (Enzell et al., 1969; Vacheron et al., 1969; Arpin et al., 1970; Enzell and Back, 1995; Britton et al., 2004); m/z: 566 ([M+; C40H54O2]), 550 ([M-O]+), 548 ([M-H2O]+), 507 ([M-CH3COCH3-H]+), 474 ([M-C6H6CH3]+), 460 ([M-C6H6(CH2)2]+), 368 ([M-C6H4(CH2)2-C2H5CH3]+), 209, 197, 183, 145, 119, 105, 95, 81, 69, 59, 43.

In the FT-IR spectrum of (all-E)-2′-dehydroplectaniaxanthin (2) the band ν = 1668 cm⁻¹ (very strong C = O stretching frequency absorption) is characteristic to the 2′-keto group conjugated with the polyene chain (Arpin and Liaaen-Jensen, 1967ab; Hertzberg and Liaaen-Jensen, 1967; Loránd et al., 2002a,b; Molnár et al., 2006a,b; Bernhard and Grosjean, 1995).

The NaBH₄ reduction of (all-E)-2′-dehydroplectaniaxanthin (2) resulted in the formation of the racemic mixture of (R)- and (S)-plectaniaxanthin verifying the presence of the conjugated 2′-keto group (Arpin and Liaaen-Jensen, 1967a,b; Britton et al., 2004).

The racemic mixture of plectaniaxanthin enantiomers was separated by HPLC using ‘Chiralcel OD’ column (Fig. 7) (Turcsi et al., 2015). The co-chromatography of the racemic mixture with the natural (all-E, 2′R)-plectaniaxanthin proved the identity of peak 1 with this carotenoid and of peak 2 with the corresponding (2′S)-enantiomer.

HPLC-ECD measurements were also carried out by stopping the eluent in the HPLC flow cell (Turcsi et al., 2015), but distinct ECD spectra could not be recorded for the separated enantiomers at room temperature, which may be due to the conformational flexibility of plectaniaxanthin.

4. Conclusion

In this study, we described the determination of the carotenoid composition of the mushroom Scarlet elf cup (Sarcoscypha coccinea) by HPLC and CLC, the determination of the ¹³C-NMR chemical shifts of the naturally occurring (all-E)-plectaniaxanthin (1) and (all-E)-2′-dehydroplectaniaxanthin (2), and the isolation and partial characterization of three stericly unhindered mono-cis isomers of plectaniaxanthin (1) [(13Z)-1, (13′Z)-1 and (15Z)-1], five mono-cis isomers of 2′-dehydroplectaniaxanthin (2) [(9Z,2′-Z)-2, (9′Z,2′-Z)-2, (13Z)-2, (13′Z)-2 and (15Z)-2], three di-cis isomers of 2′-dehydroplectaniaxanthin (2) [(9Z,5′Z)-2 or (9′Z,5′Z)-2], and probably (15Z,5′Z)-2), and one poly-cis (tri-cis isomer of 2′-dehydroplectaniaxanthin (2) [probably (9Z,9′Z,5′Z)-2], for the first time. The main carotenoids [(all-E)-plectaniaxanthin (1) and (all-E)-2′-dehydroplectaniaxanthin (2)] were isolated in highly pure crystalline state. The structure elucidation of these carotenoids was carried out by spectroscopic methods (UV/Vis, ¹H- and ¹³C-NMR, MS, IR). The majority of the isolated (Z) isomers were identified and characterized only on the basis of their UV/Vis spectroscopic properties (λmax-shifts; Q-values (Q = Amax/Acis-peak)) (Zechmeister, 1962; Britton, 1995; Molnár, 2009; Molnár et al., 2017).

Besides of the main achievements of our work mentioned above, the (2′R)-configuration of the naturally occurring (all-E)-plectaniaxanthin (1) was also confirmed.

Declarations

Author contribution statement

Péter Molnár: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
Erzsébet Ösz, Erika Turcsi: Performed the experiments; Analyzed and interpreted the data.
József Deli: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.
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