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GLYCEROL TRIDEHYDROCREPENYNATE FROM THE BASIDIOMYCETE *CRATERELLUS CORNUCOPIOIDES*

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Key Word Index—*Craterellus cornucopioides*; Cantharellaceae; Basidiomycetes; triacylglycerols; dehydrocrepenynic (octadeca-9*Z*,14*Z*-dien-12-ynoic) acid.

Abstract—Glycerol tri-dehydrocrepenynate (octadeca-9*Z*,14*Z*-dien-12-ynoate) is the most abundant (66%) triacylglycerol in fruiting bodies of the Basidiomycete *Craterellus cornucopioides*. Glycerol trioleate and a linoleate-dioleate were also isolated from the fungus, in addition to minor unidentified triacylglycerols some of which contained further quantities of dehydrocrepenynic acid.

INTRODUCTION

Dehydrocrepenynic acid is one of the likely precursors of a group of natural acetylenes from higher plants and fungal cultures which are generally referred to as polyacetylenes [1–6]. The acid itself has so far only been identified in hydrolysates of crude seed oils and fungal lipids [6–8]. We now demonstrate that the majority, if not all, of the bound dehydrocrepenynic acid in fruiting bodies of the Basidiomycete *Craterellus cornucopioides* (L.) ex Pers. occurs in the form of triacylglycerols. Moreover, glycerol tri-dehydrocrepenynate is the most abundant neutral lipid in this fungus.

RESULTS AND DISCUSSION

The lipids of *Craterellus* were extracted by a procedure reported to preclude their partial digestion, during isolation and storage, by co-extracted fungal lipases [9, 10]. Test samples prepared by the simpler method of Folch *et al.* [11] were, however, not significantly different in composition.

The bulk of the constituents of the crude fungal lipid had similar chromatographic mobilities (TLC, silica gel, CH_2Cl_2) to those of the triacylglycerols from sunflower seed oil. ^{13}C NMR spectra (25 MHz) in a series of solvents of increasing polarity (C_6D_6; $CDCl_3$; $CDCl_3$–CD_3OD, 2:1) showed, except for minor peaks, only signals which could be preliminarily assigned to esterified glycerol and to dehydrocrepenynoyl, oleoyl, and linoleoyl residues [12, 13], thus indicating the absence of substantial amounts of polar lipids and non-lipid contaminants.

Separation of the *Craterellus* lipids by column chromatography yielded five fractions (A–E, in increasing polarity). Fraction E represented 66% of the bulk and was identified as glycerol tri-dehydrocrepenynate (**1**). The mass spectrum obtained by desorption chemical ionization (DCI) (NH_3) showed the $[M+NH_4]^+$ ion at *m/z* 884, while the fragmentation pattern corresponded closely to that produced by structurally related triacylglycerols [14, 15]. The position and stereochemistry of the multiple bonds were established by high-field NMR. ^{13}C chemical shifts (125 MHz) for the acyl carbons (Table 1)

R	Number of R per glycerol		
	1	**2**	**3**
$CH_3(CH_2)_7-CH=CH(CH_2)_7CO-$	0	3	2
$CH_3(CH_2)_4-CH=CH-CH_2-CH=CH(CH_2)_7CO-$	0	0	1
$CH_3(CH_2)_2-CH=CH-C\equiv C-CH_2-CH=CH(CH_2)_7CO-$	3	0	0

Table 1. ^{13}C NMR chemical shifts for compounds **1** and **2** (CD_2Cl_2, 125 MHz, δ-values, TMS as int. standard)

	C	1	2
Glycerol moiety:	1(3)	62.45 (2C)	62.50 (2C)
	2	69.28 (1C)	69.35 (1C)
Acyl moiety:	1	173.45 (2C)	173.48 (2C)
		173.07 (1C)	173.10 (1C)
	2	34.52 (1C)	34.60 (1C)
		34.35 (2C)	34.43 (2C)
	3	25.27 (1C)	25.35 (1C)
		25.24 (2C)	25.32 (2C)
	4	29.44 (2C)*	29.64 (1C)*
		29.41 (1C)*	29.62 (2C)*
	5	29.49 (1C)*	29.60 (1C)*
		29.47 (2C)*	29.58 (2C)*
	6	29.55 (1C)*	29.54 (2C)*
		29.53 (2C)*	29.50 (1C)*
	7	29.74 (1C)*	30.20 (1C)
		29.73 (2C)*	30.19 (2C)
	8	27.50 (3C)	27.63 (3C)†
	9	132.05 (2C)	130.18 (2C)
		132.02 (1C)	130.16 (1C)
	10	124.73 (1C)	130.40 (1C)
		124.71 (2C)	130.39 (2C)
	11	18.23 (3C)	27.61 (3C)†
	12	92.83 (2C)	30.24 (3C)
		92.81 (1C)	
	13	77.36 (1C)	29.77 (3C)‡
		77.35 (2C)	
	14	143.02 (3C)	29.97 (3C)
	15	109.69 (3C)	29.76 (3C)‡
	16	32.44 (3C)	32.37 (3C)
	17	22.57 (3C)	23.12 (3C)
	18	13.91 (3C)	14.28 (3C)

*,†,‡ Assignments interchangeable within the same column.

closely corresponded to published data for other esters of dehydrocrepenynic acid [12, 13] and to the dominant signals in the 25 MHz spectra of the crude fungal lipids. The widely spaced resonances of the olefinic and acetylenic carbons appear to be particularly characteristic for its 9*Z*,14*Z*-dien-12-yne system. Also, most signals were split into narrow doublets with approximate peak intensity ratios of 2:1, indicating magnetic inequivalence of the corresponding carbons in the acyl residues which are bound to the glycerol 1(3)- and 2-positions. ^1H NMR data (500 MHz; Table 2) confirmed the structure of **1** with respect to both chemical shift values [13] and the ratios of integrated peak areas for the glycerol and acyl residue protons. The *Z*-configuration, while directly evident for the 14,15-double bond ($J = 10.7$ Hz), can also be safely assumed for the 9,10-position: the fingerprint of the respective multiplet, as far as it does not overlap with the solvent (CD_2Cl_2) signal, was near-identical to the corresponding region in the spectrum (in C_6D_6) of indol-3-ylethyl 9*Z*,14*Z*-dehydrocrepenynate, which could be analysed in detail [13].

Fraction A (18%) contained glycerol trioleate (**2**). The mass spectrum showed the expected $[M+NH_4]^+$ ion (*m/z* 902) and fragmentation pattern [14, 15]. The presence of three oleoyl residues was confirmed by comparing ^1H and ^{13}C NMR spectra (Tables 1 and 2) with published data for the free acid and its esters [12, 13, 16]. The ^1H NMR spectrum (Table 2) of the lipid (**3**) eluted in fraction B (7%) indicated a ratio of one linoleoyl to two oleoyl residues per glycerol moiety [13, 16]. As the mass spectrum showed only one $[M+NH_4]^+$ ion (*m/z* 900), **3** appears to be a mixed-type triacylglycerol, rather than a mixture of triacylglycerols. Lipid fractions C (1%) and D (7%) were neither homogeneous nor available in quantities sufficient for complete characterization. Integration of the ^1H NMR signals of the material eluted in fraction D indicated an average of 1 dehydrocrepenynoyl, 1.5 oleoyl, and 0.5 linoleoyl residues per glycerol moiety. The above triacylglycerols probably supply the acyl residues used by *Craterellus* in the esterification of indole-3-ethanol, a plant-hormone related compound, when this is administered to the fungus [13].

Craterellus cornucopioides is a cosmopolitan, ubiquitous species [17], and glycerol tri-dehydrocrepenynate is the main component of the fungal triacylglycerols. The Basidiomycete may therefore be a readily available source of dehydrocrepenynic acid, which should be easily obtained in high purity from the isolated glyceride **1**.

EXPERIMENTAL

General. Solvents for chromatography were redistilled. TLC was on silica gel GF 254, using development with CH_2Cl_2 mixed with cyclohexane as required for optimal separation, and detection by UV absorbance, I_2 staining, and spraying with 10% ethanolic H_2SO_4 followed by heating. Desorption chemical ionisation MS (DCIMS) (NH_3) was at 50 eV and a source pressure of 6×10^{-4} Torr.

Isolation of triacylglycerols. Fruiting bodies (10 g) of *Craterellus cornucopioides* (L.) ex Pers. freshly collected in the surroundings of Zagreb, Yugoslavia, were immersed in boiling *i*-PrOH (200 ml), boiled shortly to inactivate lipases, left to cool (50°), and homogenized. The homogenate was filtered and the residue was extracted with $CHCl_3$–MeOH (2:1, 200 ml). The extracts were combined. Five batches of such combined extracts were pooled and concd *in vacuo*. The residue (5 ml) was dissolved in $CHCl_3$–MeOH (2:1, 1 l). The soln was partitioned against 0.88% aq. KCl (250 ml); the aq. phase was re-extracted with $CHCl_3$–MeOH (2:1, 50 ml), and the combined organic phases were partitioned against MeOH–H_2O (1:1, 250 ml). The lower phase was stored in the dark, under N_2, at −10°. An aliquot (150 ml containing 140 mg of dry matter) was passed through a column of silica gel H (75 g) mixed with celite (25 g), eluted with CH_2Cl_2–cyclohexane (1:1, 300 ml; 2:1, 150 ml; 3:1, 100 ml, 4:1, 350 ml). The effluent was monitored by TLC, and the following fractions were collected: A (210–270 ml, 12 mg), B (270–310 ml, 5 mg), C (310–360 ml, 1 mg), D (380–410 ml, 5 mg), and E (440–490 ml, 44 mg).

Glycerol tri-dehydrocrepenynate (**1**, *fraction E*). DCIMS (NH_3), *m/z*, (rel. int.): 884 $[M+NH_4]^+$(8), 867 $[M+H]^+$ (2), 591 $[M-RCOO]^+$ (3), 565 $[M-RCOO-26]^+$ (0.5), 333 $[RCO+74]^+$ (5), 317 $[RCO+58]^+$ (5), 294 $[RCOOH+NH_4]^+$ (22), 277 $[RCOOH+H]^+$ (14), 259 $[RCO]^+$ (14), 91(100).

Table 2. ^{1}H NMR spectral data of compounds **1**–**3** (CD_2Cl_2, 500 MHz, δ-values)

H	1	2		3	
	Glyceryl residue				
1(3)	4.26 *dd*	4.2 *dd*		4.24 *dd*	
1′(3′)	4.13 *dd*	4.1 *dd*		4.11 *dd*	
2	5.22 *tt*	5.3 *tt*		5.21 *tt*	
	Acyl residues				
			linoleoyl		oleoyl
2	2.29 *t*	2.29 *t*		2.29 *t*	
3	1.6 *m*	1.6 *m*		1.6 *m*	
4→7	1.3 *m*	1.3 *m*		1.3 *m*	
8	2.05 *q*	2.01 *m*	2.04 *q*		2.00 *q*
9,10	5.5 *m*	5.3 *m*		5.3 *m*	
11	3.08 *d*	2.01 *m*	2.77 *t*		2.00 *q*
12,13	—		5.3 *m*		
14	5.5 *m*		2.04 *q*		
15	5.82 *dt*	1.3 *m*		1.3 *m*	
16	2.24 *qd*				
17	1.41 *sex*				
18	0.92 *t*	0.88 *t*	0.88 *t*		0.87 *t*

J[Hz]: Glyceryl residue: 1,2 = 2,3 = 4.3; 1′2 = 2,3′ = 6.0; 1,1′ = 3,3′ = 11.9; Acyl residues: compound **1**: 2,3 = 7.2; 7,8 = 8,9 = 6.9; 10,11 = 6.6; 14,15 = 10.7; 14,16 = 1.4; 15,16 = 16,17 = 17,18 = 7.4; compound **2**: 2,3 = 7.3; 9,10 = 10; 17,18 = 7; compound **3**: linoleoyl: 7,8 = 8,9 = 10,11 = 11,12 = 13,14 = 14,15 = 7.0; oleoyl: 7,8 = 8,9 = 10,11 = 11,12 = 6.5; lineoyl + oleoyl: 2,3 = 7.6; 17,18 = 6.9.

Integrated peak areas: Glyceryl residue: 1(3) = 1′(3′) = 2H, 2 = 1H; Acyl residues: compound **1**: 15 = 3H, 2 = 3 = 8 = 11 = 16 = 17 − 6 H; 9 + 10 + 14 − 18 − 9H; 4→7 = 24H; compound **2**: 2 = 3 = 9 + 10 = 6H; 18 = 9H; 8 + 11 = 12H; (4→7) + (12→17) = 60H; compound **3**: linoleoyl: 11 = 2H; 18 = 3H; 8 + 14 = 4H; 9,10,12,13 under CH_2Cl_2; oleoyl: 18 = 6H; 8 + 11 = 8H; 9,10 under CH_2Cl_2; linoleoyl + oleoyl: 2 = 3 = 6H; 4→7 + (15→17, linoleoyl) + (12→17, oleoyl) = 54 H.

Glycerol trioleate (**2**, *fraction A*). DCIMS (NH_3), *m/z* (rel. int.): 902 $[M+NH_4]^+$ (3), 603 $[M-RCOO]^+$ (20), 577 $[M-RCOO-26]^+$ (10), 393 $[RCO+128]^+$ (5), 339 $[RCO+74]^+$ (42), 323 $[RCO+58]^+$ (55), 300 $[RCOOH+NH_4]^+$ (35), 265 $[RCO]^+$ (66), 264 $[RCO-H]^+$ (50), 81 (100).

Glycerol linoleate dioleate (**3**, *fraction B*). DCIMS (NH_3), *m/z* (rel. int.): 900 $[M+NH_4]^+$ (0.5), 603 $[M-C_{17}H_{31}COO]^+$ (5), 601 $[M-C_{17}H_{33}COO]^+$ (4), 577 $[M-C_{17}H_{31}COO-26]^+$ (3), 339 $[C_{17}H_{33}CO+74]^+$ (25), 337 $[C_{17}H_{31}CO+74]^+$ (8), 323 $[C_{17}H_{33}CO+58]^+$ (24), 321 $[C_{17}H_{31}CO+58]^+$ (8), 300 $[C_{17}H_{33}COOH+NH_4]^+$ (20), 298 $[C_{17}H_{31}COOH+NH_4]^+$ (17), 265 $[C_{17}H_{33}CO]^+$ (37), 264 $[C_{17}H_{33}CO-H]^+$ (26), 263 $[C_{17}H_{31}CO]^+$ (25), 262 $[C_{17}H_{31}CO-H]^+$ (34), 81 (100).

Mixture of triacylglycerols from fraction D. ^{1}H NMR (500 MHz, CD_2Cl_2); glycerol moiety: see Table 2; dehydrocrepenynoyl moiety: δ2.03 (2H, *q*, $J_{7,8} = J_{8,9} = 7.0$ Hz, H-8), 3.06 (2H, *d*, $J_{10,11} = 5.7$ Hz, H-11), 5.81 (1H, *dt*, $J_{14,15} = 10.7$ Hz, $J_{15,16} = 7.4$ Hz, H-15), 2.23 (2H, *qd*, $J_{14,16} = 1.3$ Hz, $J_{15,16} = J_{16,17} = 7.4$ Hz, H-16), 1.39 (*ca* 2H, *sextet*, $J_{16,17} = J_{17,18} = 7.4$ Hz, H-17), 0.90 (3H, *t*, H-18), oleoyl moiety: 1.99 (*ca* 6H, *q*, $J_{7,8} = J_{8,9} = J_{10,11} = J_{11,12} = 6.5$ Hz, H-8, H-11), 0.86 (*ca* 4.5 H, *t*, $J_{17,18} = 6.9$ Hz, H-18) linoleoyl moiety: 2.0 (*ca* 2H, *q*, H-8, H-14 + dehydrocrepenynoyl H-8), 2.75 (*ca* 1H, *t*, $J_{10,11} = J_{11,12} = 6.6$Hz, H-11) 0.87 (*ca* 1.5H, *t*, $J_{17,18} = 7.0$ Hz, H-18), common acyl signals: 2.27 (6H, *t*, $J_{2,3} = 7.6$ Hz, H-2), 1.6 (6H, *m*, H-3), 5.3–5.5 (*m*, CH + solvent), 1.20–1.35 (*ca* 45H, *m*, all CH_2 not specified above).

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