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CONVERSION OF INDOLE-3-ETHANOL TO FATTY ACID ESTERS IN *CRATERELLUS CORNUCOPIOIDES**

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Key Word Index—*Craterellus cornucopioides*; Cantharellaceae; biological conjugation; indol-3-ylacetic acid precursors; plant acetylenes; 2-(indol-3-yl)ethanol (tryptophol); 2-(indol-3-yl)-ethyl dehydrocrepenynate (octadeca-9*Z*,14*Z*-dien-12-ynoate); 2-(indol-3-yl)ethyl oleate; 2-(indol-3-yl)ethyl linoleate.

Abstract—Three new esters of 2-(indol-3-yl)ethanol (tryptophol) containing the acyl residues of dehydrocrepenynic (octadeca-9*Z*,14*Z*-dien-12-ynoic), oleic and linoleic acids have been identified in fruiting bodies of the Basidiomycete *Craterellus cornucopioides* supplied with exogenous tryptophol. The esters were formed reversibly in high yields and are possible storage forms of auxin precursors which may be deposited in lipophilic cell compartments.

INTRODUCTION

2-(indol-3-yl)Ethanol (tryptophol, **1**) and the growth hormone, indol-3-ylacetic acid (IAA) occur in plants in similar concentrations [1–9]. They are also interconvertible, to a variable degree, via the common precursor, indol-3-yl-acetaldehyde. While only the pathogen *Corynebacterium fascians* has so far been reported to reduce IAA to tryptophol [10], an enzyme system capable of maintaining IAA homeostasis by feedback-regulated tryptophol oxidation appears to exist in many plant species. The enzyme has been isolated and characterized from cucumber seedlings [11, 12] and from the fungus *Phycomyces blakesleeanus* [13]. Moreover, when tryptophol levels are artificially enhanced by feeding, many plants will not oxidize that extra tryptophol to a comparable excess of IAA, but will conjugate it instead [14]. The β-D-glucopyranoside of tryptophol is formed preferentially in seed plants, the β-D-galactopyranoside by certain unicellular algae, and the *O*-acetate by a restricted number of cormophytes and thallophytes [14, 15]. A further set of conjugates formed in significant amounts on feeding tryptophol has so far only been characterized as a group of highly lipophilic esters [14, 15]. Lipid-soluble conjugates of plant hormones and their metabolic precursors have so far received little attention. We have therefore isolated and characterized the tryptophol esters formed in relatively large quantities by the Basidiomycete *Craterellus cornucopioides*.

RESULTS AND DISCUSSION

As shown previously [14], fruiting bodies of *Craterellus* contained less than *ca* 1 μg/g fr. wt of endogenous indole derivatives while IAA, indole-3-carboxylic acid, tryptophol acetate and, most abundantly, a series of more lipophilic esters accumulated when tryptophol (**1**) was supplied. Only those esters will be considered in this paper. While their isolation from one sample of tryptophol-incubated *Craterellus* is described in detail in the Experimental section, several smaller batches were also isolated by essentially the same method. Three tryptophol esters were always obtained, although their relative proportion was variable depending on the origin and maturity of the fungal material. Since preliminary spectroscopic evidence indicated that the esters might be tryptophol oleate (**2**), linoleate (**3**) and dehydrocrepenynate (octadeca-9*Z*,14*Z*-dien-12-ynoate, **4**), the authentic compounds were synthesized and compared with the plant metabolites.

Chemical synthesis of tryptophol esters

Esters **2** and **3** were prepared by condensing the alcohol and acid components in the presence of *N*,*N*-dicyclohexylcarbodi-imide (DCC) and 4-dimethylaminopyridine (DMAP) [16]. Compound **4** was synthesized by coupling the tryptophol ester of 9-oxo-nonanoic acid (**5**) with a C-9 Wittig salt (**6**) containing the conjugated enyne system [17, 18]. The crude 9-oxo-nonanoic acid used as the starting material contained *ca* 7% nonanoic and 35% 1,9-nonadioic acids, as estimated from the ratio of CHO and COOH signals in the ^{13}C NMR spectrum and by esterification (see below). Condensation of the aldehydic acid with dithioethane [19] for protection and purification was attempted. However, the tryptophol ester of the derivatized acid could not be deprotected in significant yields using silver and mercury salts [20, 21] or

*Preliminary reports: Laćan, G., Lewer, P., Magnus, V. and Iskrić, S. (1987) in *Proc. Int. Symp. Conjugated Plant Hormones. Structure, Metabolism and Function* (Schreiber, K., Schütte, H. R. and Sembdner, G., eds), p. 69. VEB Deutscher Verlag der Wissenschaften, Berlin; Magnus, V., Lewer, P., Laćan, G., Iskrić, S. and Thaller, V. (1988) in *Proc. Symp. Physiology and Biochemistry of Auxins in Plants* (Kutaček, M., Bandurski, R. S. and Krekule, J., eds), p. 93. Academia, Praha.

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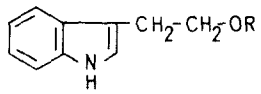


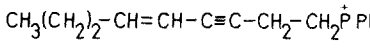
1 R = H

2 R = $CH_3(CH_2)_7-CH=CH(CH_2)_7CO-$

3 R = $CH_3(CH_2)_4-CH=CH-CH_2-CH=CH(CH_2)_7CO-$

4 R = $CH_3(CH_2)_2-CH=CH-C\equiv C-CH_2-CH=CH(CH_2)_7CO-$

5 R = $OHC(CH_2)_7CO-$



6

chloramine T [22] possibly due to side reactions at the indole ring [23]. Surprisingly, the crude 9-oxo-nonanoic acid could be esterified with tryptophol without major problems using DCC/DMAP, and the mixture of esters could be easily separated chromatographically. Condensation of the aldehydic ester **5** with **6** gave **4**. The *Z*-configuration of the newly formed double bond was deduced from H,H coupling constants at the respective hydrogens ($J_{9,10} = 10.6$ Hz).

Comparison of plant metabolites with authentic tryptophol esters

The fungal metabolites co-chromatographed with authentic **2–4**, respectively (TLC, CH_2Cl_2–cyclohexane, 1:1, multiple development), although separation was poor. Mass spectra confirmed the identity of the fungal esters showing the expected molecular ions at *m/z* 425 (**2**), 423 (**3**) and 419 (identified as $C_{28}H_{37}NO_2$ by high resolution MS, **4**). Intense fragment ions at *m/z* 143 ([indole—$CH=CH_2$]$^+$·) and 130 ([quinolinium]$^+$) indicated the presence of a 2-(indol-3-yl)ethyl moiety in all three esters. The positions of the double bonds in the acyl residues, which cannot be reliably deduced from mass spectra [24] were established from ^{13}C and ^1H NMR spectra. These were identical for the fungal esters and the respective authentic standards. The position of the acetylenic carbon resonances and the widely spaced signals for the olefinic carbons are highly characteristic for the subterminal dienyne system of dehydrocrepenynic acid [25]. In the ^1H NMR spectrum [18, 26] the signals of H-9 to H-18 were unequivocally assigned by homonuclear decoupling, in sequence, each of these protons starting from the ω-end of the acyl residue. Both double bonds were in the *Z*-configuration as deduced from the vicinal coupling constants. This was also clearly visible (on decoupling the adjacent CH_2) for the double bonds of both the natural and the synthetic oleoyl, and highly probable for the respective linoleoyl esters, although the exact positions of these double bonds could not be deduced directly from the NMR spectra. A large representative sample of unsaturated fatty acids and their esters including 15 out of 16 possible C-18 *Z*-monoenoic and 8 C-18 *Z,Z*-dienoic acids has, however, been examined by ^{13}C and ^1H NMR [25, 27]. In not a single case were the spectra of different positional isomers identical. In summary, the data obtained show convincingly that the conjugates formed by *Craterellus* from exogenous tryptophol are **2–4**.

Possible artifact formation

Fruiting bodies of *Craterellus* contain oleoyl, linoleoyl and dehydrocrepenynoyl glycerides [28] which are the likely sources of the acyl residues incorporated into tryptophol conjugates. Unfortunately, erroneous transesterifications have frequently been found to occur during isolation of compounds from tissue, as a result of catalysis by either constituents of the plant extracts or by incompletely deactivated lipolytic enzymes [29]. In order to exclude such mechanisms of formation of the tryptophol esters in the present work, tryptophol was added, in separate experiments, to both boiled fungal tissue and to a methanol extract of *Craterellus*, and stored for several weeks. In neither case were esters of the parent compound detected. For fruiting bodies incubated with tryptophol in the standard way, extraction with boiling 2-propanol and then with methanol gave slightly higher recoveries than did methanol alone. Boiling 2-propanol deactivates lipolytic enzymes instantaneously [29] and is a better solvent for lipophilic compounds than methanol. These control experiments show that **2–4** are true fungal metabolites rather than artifacts.

To investigate whether endogenously synthesized tryptophol is conjugated by *Craterellus* in the same way as the exogenous compound, indol-3-yl-acetaldehyde was supplied to the fungus. Tryptophol was the most prominent metabolite, accompanied by smaller amounts of IAA and a product which co-chromatographed with a mixture of authentic **2–4** and was hydrolysed to tryptophol (identified by TLC) by methanolic ammonia, as would be expected for an ester. As none of these indoles was formed by boiled fungal material, the aldehyde must have been converted to tryptophol by (most likely, within) the living cells and then conjugated. Of other reported [1] tryptophol precursors tested, tryptophan furnished no detectable amounts of indolic metabolites. Tryptamine gave only traces of tryptophol and IAA, but no esters; *N*-acetyl tryptamine was formed instead. This observation lends additional support to the conclusion that tryptophol is esterified enzymatically when supplied to *Craterellus*. If there were chemical transfer of acyl residues from triglycerides, both *O–N* and *O–O* transacylations should occur. *N*-acyl tryptamines containing fatty acid residues were not, however, formed by the fungus.

Reversibility of tryptophol conjugation by Craterellus

If fatty acid esters of tryptophol were formed *in vivo*, they could be storage compounds from which IAA could be synthesized during periods of high auxin requirement. While that subject requires further investigation, a necessary pre-requisite for such a function is that tryptophol esterification is reversible. The oleate was, indeed, hydrolysed by *Craterellus* in yields high enough to permit

isolation of the tryptophol formed and its indentification by ^{1}H NMR.

CONCLUSION

This is the first report on fatty acid esters of a plant hormone-related compound. In contrast to the host of hydrophilic conjugates identified to date [1, 30], which are most likely localized in the vacuole, the tryptophol esters described here would be suitable for transport through lipid membranes and storage in lipophilic cell compartments. We have so far only identified the tryptophol esters formed on feeding the free alcohol in amounts which may not occur naturally. This was necessary to obtain sufficient material for NMR analysis, as the acyl residues could not be identified by mass spectrometry. The physiological significance of these fatty acid esters remains to be elucidated, but should not *a priori* be assumed to be negligible. Indeed, the first conjugate of a plant hormone chemically identified, *N*-(indol-3-ylacetyl) aspartic acid, now established as an important plant constituent [30], was originally detected in pea seedlings overfed with IAA [31]. Some other tryptophol esters also occur naturally: the *O*-acetate has been isolated from wine as a yeast metabolite [32]. An alkali-labile tryptophol conjugate, most likely an ester, has been detected in pine seeds [6]. Exogenous tryptophol was converted to esters with about the same chromatographic properties as those formed by *Craterellus*, in 107 species including bacteria and all major taxa of thallophytes and cormophytes [14]. A conjugation mechanism so conservatively maintained during evolution should have some physiological significance.

EXPERIMENTAL

Materials. Solvents were redistilled; Et_2O and THF were free of peroxides. Linoleic acid was isolated from sunflower seed oil [33]. The $NaHSO_3$ adduct of indol-3-yl-acetaldehyde was prepared after ref. [34]. The aldehyde was liberated with 5% $NaHCO_3$ and extracted into Et_2O. 1-(non-5*Z*-en-3-ynyl)-Triphenylphosphonium iodide (**6**) was synthesized according to ref. [18]. Fruiting bodies of *Craterellus cornucopioides* (L.) ex Pers. were collected close to Zagreb, Yugoslavia. Fungal material was coarsely cut immediately before use.

2-(indol-3-yl)Ethyl octadeca-9Z-enoate (**2**). To a soln of redistilled (10^{-2} mm Hg) oleic acid (565 mg, 2 mmol), tryptophol (322 mg, 2 mmol) and 4-dimethylaminopyridine (24 mg, 0.2 mmol) in dry CH_2Cl_2 (5 ml), stirred at 0° under Ar, *N,N'*-dicyclohexylcarbodi-imide (413 mg, 2 mmol) was added. The mixture was stirred at 0° (1 hr), then at room temp (24 hr). *N,N'*-Dicyclohexylurea (420 mg, 94%) was filtered off and the filtrate was extracted sequentially with 4% (v/v) H_3PO_4 (3 × 2 ml), 5% $NaHCO_3$ (2 × 2 ml) and satd NaCl soln (3 × 2 ml). The organic phase was dried (Na_2SO_4), concd and passed through a column of silica gel H–Celite (75 + 25 g) eluted with CH_2Cl_2– cyclohexane (1:1, 50 ml; 2:1, 500 ml) to yield **2** (588 mg, 69%). Repeated crystallization from petrol at −15° (refrigerated centrifuge) gave white crystals, mp 28–30°. (Found: C, 79.29; H 10.38; N, 3.47. $C_{28}H_{43}NO_2$ requires: C, 79.01; H, 10.18; N, 3.29). λ_{max}^{EtOH} nm (log ε): 289 (3.69), 280 (3.76), 273sh (3.74), 220 (4.52), 200 (4.29); IBEIMS 70 eV, *m/z* (rel. int.): 425 $[M]^{+\cdot}$ (3), 265 $[C_{17}H_{33}CO]^+$ (1), 144 $[M-C_{17}H_{33}COO]^+$ (27), 143 $[M-C_{17}H_{33}COOH]^{+\cdot}$ (100), 130 [quinolinium]$^+$ (10), 117 [indole]$^{+\cdot}$ (3), 115 [indole −2H]$^{+\cdot}$ (4), 103 [130 − HCN]$^+$ (2).

2-(indol-3-yl)Ethyl octadeca-9Z,12Z-dienoate (**3**). Linoleic acid (561 mg, 2 mmol) was reacted with tryptophol (322 mg, 2 mmol) and the ester (700 mg, 83%) was isolated as described for the oleate. Repeated crystallization from petrol at −20° gave white crystals melting around 0°. (Found: C, 79.29; H, 10.00; N, 3.32. $C_{28}H_{41}NO_2$ requires: C, 79.38; H, 9.76; N, 3.31). λ_{max}^{EtOH} nm (log ε): 289 (3.70), 280 (3.77), 273sh (3.76), 220 (4.53), 201 (4.44); DCIMS (NH_3) 50 eV, source pressure: 6×10^{-4} Torr *m/z* (rel. int.): 441 $[M+NH_4]^+$ (13), 424 $[M+H]^+$ (18), 423 $[M]^{+\cdot}$ (7), 298 $[C_{17}H_{31}COOH+NH_4]^+$ (7), 280 $[C_{17}H_{31}COOH]^{+\cdot}$ (2), 145 $[M+H-C_{17}H_{31}COO]^{+\cdot}$ (21), 144 $[M-C_{17}H_{31}COO, M+H-C_{17}H_{31}COOH]^+$ (100), 143 $[M-C_{17}H_{31}COOH]^{+\cdot}$ (79), 130 [quinolinium]$^+$ (21), 115 [indole−2H]$^{+\cdot}$ (3).

2-(indol-3-yl)Ethyl 9-oxo-nonoanoate, (**5**) *nonanoate and nona-1,9-dioate.* Redistilled oleic acid was ozonized and the ozonide reduced with Ph_3P [17]. The crude acidic fraction contained about 2.7 COOH per CHO (from ^{13}C NMR). An aliquot (344 mg) was esterified with an empirically optimized amount of tryptophol (483 mg, 3 mmol) in dry CH_2Cl_2 (10 ml) under Ar, using DCC (618 mg, 3 mmol) and DMAP (37 mg, 0.3 mmol) as described for **2**, except for a shorter reaction time (6 hr). The products were separated on a column of silica gel H–Celite (65 + 40 g) eluted with CH_2Cl_2 (100 ml) followed by CH_2Cl_2–Et_2O (100: 1, 100 ml; 100:2, 100 ml; 100:3, 100 ml; 100:4, 100 ml; 100:5, 200 ml). Tryptophol nonanoate (14 mg), **5** (145 mg) and tryptophol nona-1,9-dioate (162 mg) eluted in this order, and were characterized as follows.

2-(indol-3-yl)Ethyl nonanoate. ^{13}C NMR (25 MHz, $CDCl_3$); acyl residue: δ173.76 (1C, C-1), 34.33 (1C, C-2), 24.93 (1C, C-3), 29.14 (1C, C-4), 29.08 (2C, C-5, C-6), 31.76 (1C, C-7), 22.60 (1C, C-8), 14.07 (1C, C-9); 2-(indol-3-yl)ethyl moiety: δ64.34 (1C, C-1), 24.76 (1C, C-2), 121.80 (2C, C-2', C-5'), 111.69 (1C, C-3'), 127.17 (1C, C-3a'), 118.53 (1C, C-4'), 119.11 (1C, C-6'), 110.94 (1C, C-7'), 135.87 (1C, C-7a'); ^{1}H NMR (100 MHz, $CDCl_3$); acyl residue: δ2.30 (2H, *t*, $J_{2,3}$ = 7.1 Hz, H-2), 1.61 (2H, *m*, H-3), 1.26 (10H, *br s*, other CH_2), 0.87 (3H, *t*, $J_{8,9}$ = 5.9 Hz, H-9); 2-(indol-3-yl)ethyl moiety: δ4.35 (2H, *t*, $J_{1,2}$ = 7.2 Hz, H-1), 3.09 (2H, *t*, H-2), 8.03 (1H, *br s*, H-1'), 7.03 (1H, *m*, H-2'), 7.1–7.7 (*ca* 4H, *m*, H-4'→H-7').

2-(indol-3-yl)Ethyl 9-oxo-nonanoate (**5**). IBEIMS 70 eV, *m/z* (rel. int.): 315 $[M]^{+\cdot}$ (12), 144 $[M-C_9H_{15}O_3]^+$ (25), 143 $[M-C_9H_{16}O_3]^{+\cdot}$ (100), 130 [quinolinium]$^+$ (23), 115 [indole −2H]$^{+\cdot}$ (4), 103 [130 − HCN]$^+$ (3); ^{13}C NMR (25 MHz, C_6D_6): acyl residue: δ173.21 (1C, C-1), 34.30 (1C, C-2), 25.18 (1C, C-3), 28.97 (2C, C-4, C-5), 29.11 (1C, C-6), 21.99 (1C, C-7), 43.64 (1C, C-8), 201.03 (1C, C-9); 2-(indol-3-yl)ethyl moiety; 64.57 (1C, C-1) 25.06 (1C, C-2), 121.94 (2C, C-2', C-5'), 111.72 (1C, C-3'), 118.91 (1C, C-4'), 119.40 (1C, C-6'), 111.26 (1C, C-7'), 136.48 (1C, C-7a'); ^{1}H NMR (100 MHz, C_6D_6); acyl moiety: δ2.13 (2H, *t*, $J_{2,3}$ = 7.3 Hz, H-2), 1.47 (2H, *quin*, $J_{3,4}$ = 7.3 Hz, H-3), 1.78 (2H, *td*, $J_{8,9}$ = 1.5 Hz, $J_{7,8}$ = 7.5 Hz, H-8), 1.00 (8H, *m*, other CH_2), 9.29 (1H, *t*, H-9); 2-(indol-3-yl)ethyl moiety: δ4.38 (2H, *t*, $J_{1,2}$ = 7.0 Hz, H-1), 3.01 (2H, *t*, H-2), 6.55 (1H, *m*, H-2'), 7.65 (2H, *m*, H-4' or H-7'), 7.27 (*m*, other indole H).

Di-(2-indole-3-yl)Ethyl nona-1,9-dioate. Rechromatography and repeated crystallization from C_6H_6–petrol (2:1) at 50° gave white crystals, mp 79°. (Found: C, 73.14; H, 7.03; N, 5.74. $C_{29}H_{34}N_2O_4$ requires: C, 73.39; H, 7.22; N, 5.90). ^{13}C NMR (25 MHz, $CDCl_3$); acyl residue: δ173.76 (2C, C-1, C-9), 34.27 (2C, C-2, C-8), 24.81 (2C, C-3, C-7), 28.78 (3C, C-4→C-6); 2-(indol-3-yl)ethyl moiety: δ64.40 (2C, C-1), 24.76 (2C, C-2), 121.85 (2C, C-2'), 111.64 (2C, C-3'), 127.17 (2C, C-3a'), 118.47 (2C, C-4'), 121.74 (2C, C-5'), 119.11 (2C, C-6'), 110.99 (2C, C-7'), 135.93 (2C, C-7a'); ^{1}H NMR (100 MHz, $CDCl_3$); acyl residue: δ2.28 (4H, *t*, $J_{2,3}$ = $J_{7,8}$ = 7.1 Hz, H-2, H-8), 1.55 (4H, *m*, H-3, H-7), 1.21 (6H, *br s*, H-4→H-6); 2-(indol-3-yl)ethyl moiety: δ4.35 (4H, *t*, $J_{1,2}$ = 7.1 Hz,

H-1), 3.07 (4H, *t*, H-2), 8.22 (2H, *br s*, H-1'), 6.93 (2H, *m*, H-2'), 7.0–7.7 (8H, *m*, H-4'→H-7').

2-(indol-3-yl)Ethyloctadeca-9Z,14Z-dien-12-ynoate (**4**). To a suspension of **6** (179 mg, 0.35 mmol) in dry THF (10 ml) stirred under dry argon at 0°, *n*-BuLi (1.6 M in hexane, 0.2 ml) was added dropwise, through a rubber septum, followed immediately by a soln of **5** (125 mg, 0.4 mmol) in dry THF (0.2 ml). After 1 hr at 0°, dry Et_2O (10 ml) and 0.1 M HCl (10 ml) were added. The organic phase was separated and the aq. phase extracted with Et_2O. The combined organic phases were washed with H_2O and satd NaCl soln and dried (Na_2SO_4 + small amount $CaCO_3$). *Purification on a column of silica gel* H–celite (65 + 40 g) eluted with CH_2Cl_2–cyclohexane (2:1, 100 ml; 4:1, 125 ml) followed by CH_2Cl_2 (200 ml) gave **4** (14 mg, 10% based on BuLi) as a colourless oil. λ_{max}^{EtOH} nm (log ε): 289 (3.63), 278 (3.70), 274sh (3.68), 236sh (4.09), 222 (4.60), 203sh (4.28); IBEIMS 70 eV, *m/z* (rel. int.): 419 $[M]^+$ (6), 276 $[C_{17}H_{27}COOH]^+$ (1), 144 $[M-C_{17}H_{27}COO]^+$ (44), 143 $[M-C_{17}H_{27}COOH]^+$ (100), 130 [quinolinium]$^+$ (37), 117 [indole]$^+$ (12), 115 [indole − 2H]$^+$ (13), 103 [130 − HCN]$^+$ (7).

Metabolic studies—general methods. Fungal material was vacuum-infiltrated (15 mm Hg) with solns (*ca* 1.25 ml/g) of the following compounds in 0.067 M KH_2PO_4, pH 4.5; tryptophol (0.8 mg/ml), indol-3-ylacetaldehyde (0.4 mg/ml), tryptamine hydrochloride (0.8 mg/ml), tryptophan (0.1 mg/ml) or **2** (see below). The soln remaining was discarded and the fungal material was incubated at 22° for 5 hr. It was then homogenized with a 5-fold amount (v/w) of MeOH. The homogenate was filtered and the residue extracted twice more in the same way. The pooled extracts were stored at −10° in the dark. They were then repeatedly concd to successively smaller vols followed by dilution with MeOH and, finally, C_6H_6–MeOH (5:1). Precipitates formed on addition of organic solvents were discarded.

Screening for metabolites. The prepurified extract was fractionated on a column of silica gel (0.06–0.2 mm, 3.8 g), eluted with C_6H_6–MeOH (20:1, 30 ml, fraction 1; 15:1, 30 ml, fraction 2) and Et_2O–MeOH–H_2O (30:10:1, 35 ml, fraction 3). Tryptophol and its esters eluted in fraction 1, *N*-acetyl tryptamine in fraction 2, and tryptamine and IAA in fraction 3 [14, 35]. Preliminary identification of metabolites was by TLC, using *p*-dimethylaminobenzaldehyde–HCl for visualization. Suitable solvent systems were $CHCl_3$–C_6H_6 (1:1, for tryptophol esters, R_f *ca* 0.5), $CHCl_3$–EtOH (19:1, for tryptophol, R_f 0.28), $CHCl_3$–EtOH (4:1, for *N*-acetyl tryptamine, R_f 0.57), EtOAc-*i*-PrOH–NH_4OH (9:7:4, for tryptamine, R_f 0.75, and IAA R_f 0.35).

Isolation of tryptophol esters. Aliquots of the prepurified extract corresponding to *ca* 200 g of fungal material were passed through a column of silica gel (0.06–0.2 mm, 35 g) eluted with C_6H_6–MeOH (20:1). Fractions containing the esters (elution vol 100–200 ml) from five such aliquots were pooled and chromatographed on a column of silica gel H–Celite (75 + 25 g) eluted with CH_2Cl_2–cyclohexane (1:1, 300 ml; 2:1, 300 ml) and CH_2Cl_2 (200 ml). Two main fractions of tryptophol esters were detected in the eluent by TLC (CH_2Cl_2–cyclohexane, 2:1, 2 developments): A (apparent R_f 0.38, elution vol. 470–530 ml) and B (apparent R_f 0.33, elution vol. 520–580 ml). Mixed fractions of A and B were rechromatographed. To remove I_2-staining (on TLC) contaminants, aliquots of A were passed through a column of silica gel H–Celite (75 + 25 g) eluted with CH_2Cl_2. Remaining impure material, eluting before the pure fractions, was added to the next aliquot of A and rechromatographed. This also resulted in a partial separation of **2** (at slightly higher R_f) and **3**; the concentration of the latter declined from 70% in the first, to 20% in the last, batch of A. Each batch was separately purified further by prep. TLC (silica gel PF, CH_2Cl_2) followed by a column (27 × 1.5 cm) of Sephadex LH-20 eluted with 95% EtOH (elution vol. 25–35 ml). Fractions with the UV spectrum of **2** were pooled to yield a total of 38 mg (determined spectrophotometrically) containing *ca* 8 mg (by ^{1}H NMR) of **3**. Ester fraction B was purified in essentially the same way omitting the prep. TLC which was found in preliminary tests to involve substantial losses of this compound. The final yield was 59 mg (determined spectrophotometrically) of pure **4**.

Identification of tryptophol esters. Synthetic **4** and the plant metabolite had identical ^{13}C NMR (25 MHz, C_6D_6, Table 1), ^{1}H NMR (500 MHz, C_6D_6, Table 2) and mass spectra. High resolution IBEIMS gave a molecular mass of 419.2826 ($C_{28}H_{37}NO_2$ requires 419.2824) for the natural ester. ^{1}H NMR spectra (300 MHz, C_6D_6) of the presumptive fungal **2** and **3** showed the same signals as the pure synthetic compounds (Table 2). Combined GC/EIMS (BP1, 12 m capillary column, hold 1 min at 100°, then 10°/min to 250°) analysis of the oleate (R_t 37 min), gave a fragmentation pattern identical to that of an authentic sample taken under the same conditions. The MS of the presumptive linoleate (R_t 36 min) showed *m/z* (rel. int.): 423 $[M]^+$ (0.2), 144 $[M-C_{17}H_{31}COO]^+$ (25), 143 $[M-C_{17}H_{31}COOH]^+$ (100), 130 [quinolinium]$^+$ (15), 115 [indole − 2H]$^+$ (9), 103 [103 − HCN]$^+$ (2), which are consistent with the proposed structure and DCIMS data obtained for authentic **3**.

Table 1. ^{13}C NMR chemical shifts (25 MHz, δ-values, TMS as internal standard) for compounds **2**–**4**

C	**2** ($CDCl_3$)	**3** ($CDCl_3$)	**4** (C_6D_6)
Acyl moiety			
1	173.57	173.73	172.77
2	34.34	34.11	34.36
3	24.75	24.68	25.24
4	29.08[a]		29.25[a]
5	29.28[a]	28.84	29.29[a]
6	29.47[a]		29.39[a]
7	29.62[a]	29.33	29.51[a]
8	27.14	26.93[a]	27.29
9	129.46	129.76	131.43
10	129.65	127.84	124.48
11	27.14	25.41	18.29
12	29.08[a]	127.70	92.66
13	29.28[a]	129.91	77.71
14	29.47[a]	26.93[a]	142.11
15	29.62[a]	29.08	110.00
16	31.85	31.25	32.42
17	22.61	22.31	22.46
18	14.10	13.80	13.85
2-(indole-3-yl)Ethyl moiety			
1	64.29	64.24	64.40
2	24.90	24.59	25.21
2'	121.78	121.79	121.97
3'	111.66	111.49	111.81
3a'	127.13	127.17	†
4'	118.47	118.42	118.93
5'	121.63	121.69	121.68
6'	119.05	119.03	119.46
7'	110.88	110.91	111.11
7a'	135.88	135.96	136.33

[a]Assignments interchangeable with the same column.
†Under solvent signal.

Table 2. ^{1}H NMR data (δ-values) for compounds **2**–**4**

H	**2** (C_6D_6, 300 MHz)	**3** (CD_2Cl_2, 500 MHz)	**4** (C_6D_6, 500 MHz)
Acyl moiety			
2	2.15 *t*	2.33 *t*	2.16 *t*
3	1.57 *quin*	1.63 *quin*	1.56 *quin*
4→7	1.2 *m*	1.3 *m*	1.1 *m*
8	2.09 *m*	2.10 *q*	1.89 *m*
9	5.49 *m*	5.4 *m*	5.39 *dtt*
10			5.55 *dtt*
11	2.09 *m*	2.83 *t*	3.03 *dm*
12→13	1.2 *m*	5.4 *m*	—
14		2.10 *q*	5.57 *dtt*
15		1.3 *m*	5.66 *dt*
16			2.37 *qd*
17			1.34 *sex*
18	0.90 *m*	0.93 *t*	0.87 *t*
2-(indole-3-yl)Ethyl moiety			
1	4.37 *t*	4.39 *t*	4.39 *t*
2	3.01 *t*	3.11 *td*	3.02 *td*
1′	6.64 *br s*	8.29 *br s*	6.66 *br s*
2′	6.43 *m*	7.06 *m*	6.45 *m*
4′	7.65 *dd*	7.67 *dm*	7.66 *ddt*
5′	7.19 *m*	7.15 *ddd*	7.2 *m*
6′		7.22 *ddd*	
7′	7.03 *dd*	7.38 *dt*	7.04 *dt*

J [Hz]: oleoyl moiety: 2,3 = 7.4, 3,4 = 7.2; linoleoyl moiety: 2,3 = 7.5; 3,3 = 7,8 = 8,9 = 13,14 = 14,15 = 7.1; 17,18 = 7.0; 10,11 = 11,12 = 6.6; dehydrocrepenynoyl moiety: 14,15 = 10.7; 9,10 = 10.6; 2,3 = 7.5; 3,4 = 17,18 = 7.4; 7,8 = 8,9 = 15,16 = 16,17 = 7.3; 10,11 = 6.9; 11,14 = 2.2; 9,11 = 1.7; 8,10 = 1.6; 14,16 = 1.3; 2-(indole-3-yl)ethyl moiety: 4′,5′ = 7.9; 6′,7′ = 8.0; 1,2 = 7.2; 5′,6′ = 7.0; 4′,6′ = 1.1. 5′,7′ = 0.9; 2,2′ = 4′,7′ = 0.8.

Integrated peak areas: oleoyl moiety: 2 = 3 = (9 + 10) = 2H; 18 = 3H; 8 + 11 = 4H; (4→7) + (12→17) = 10H; linoleoyl moiety: 2 = 3 = 11 = 2H; 18 = 3H; 8 + 14 = (9 + 10 + 12 + 13) = 4H; (4→7) + (15→17) = 14H; dehydrocrepenynoyl moiety: 9 = 10 = 14 = 15 = 1H; 2 = 3 = 8 = 11 = 16 = 17 = 2H; 18 = 3H; 4→7 = 8H; 2-(indol-3-yl)ethyl moiety: 1′→7′ = 1H each; 1 = 2 = 2H.

Controls to exclude artifactual tryptophol esterification during isolation. In parallel experiments, four aliquots (50 g) of fungal material (A–D) were treated as follows: samples A–C were incubated with tryptophol. A was then extracted with MeOH as usual. B was immersed in boiling *i*-PrOH (500 ml) to deactivate lipases, homogenized, filtered, and the residue re-extracted with MeOH; the two extracts were pooled. C was immersed in a boiling water bath (30 min), then incubated as usual, after which time it was extracted as for B. D was directly extracted with MeOH and tryptophol (26 mg; approximate amount infiltrated into the fungal material in A–C) was added to the extract. Extracts A–D were stored under the usual conditions and worked-up simultaneously. Sample B yielded slightly higher amounts of tryptophol esters (spot density on TLC) than A; C and D did not contain such esters.

Metabolism of **2**. The synthetic ester (100 mg) dissolved in EtOH (2 ml) was added to the usual Pi buffer (200 ml) and the resulting emulsion infiltrated into the fungal material (240 g). After incubation, the extract was fractionated on four parallel columns of silica gel (3.8 g each). Fractions 1 and 2, containing **2** and the only major metabolite, were pooled and passed through a column of silica gel (0.06–0.2 mm, 35 g) eluted with CH_2Cl_2 (150 ml) followed by CH_2Cl_2–MeOH (100:1, 100 ml; 50:1, 100 ml; 20:1, 100 ml). The metabolite was further purified by two prep. TLC steps (1:CH_2Cl_2–Et_2O, 100:1, 2:CH_2Cl_2, multiple development). The material (0.9 mg) obtained was identical to authentic 2-(indol-3-yl)ethanol by R_f value (TLC), UV spectrum and showed the following ^{1}H NMR spectrum (90 MHz, $CDCl_3$); δ3.92 (2H, *t*, $J_{1,2}$ = 6.3 Hz, H-1), 3.05 (2H, *t*, H-2), 8.03 (1H, *br s*, H-1′), 7.7–7.1 (*m*, H-2′–H-7′).

Metabolism of tryptophan, tryptamine and indol-3-ylacetaldehyde. Fungal material (50 g) incubated with the above compounds was worked up as described above. While no indolic tryptophan metabolites were detected by TLC, tryptamine gave small amounts of material which co-chromatographed with tryptophol and IAA, a polar metabolite (fraction 3) and, most abundantly, a compound with the same chromatographic mobility as authentic [36] *N*-acetyl tryptamine (fractions 1 and 2). The metabolite was purified on a column of silica gel H–Celite (65 + 40 g) eluted with CH_2Cl_2, CH_2Cl_2–Et_2O mixtures and CH_2Cl_2–EtOH (4:1) followed by prep. TLC (3 × CH_2Cl_2–EtOH, 29:1), to yield material (0.5 mg) with the UV spectrum of *N*-acetyl tryptamine, while, in its ^{1}H NMR spectrum (90 MHz, $CDCl_3$), the following signals of the authentic compound were recognizable: δ7.6–7.0 (*m*, indole ring); 3.59 (*q*, $J_{1,2} = J_{1,NH}$ = 6.5 Hz, H-1); 2.96 (*t*, H-2); 1.91 (*s*, Ac). Indol-3-ylacetaldehyde gave tryptophol, tryptophol esters (presumably **2**, **3**, and **4**), and IAA. Tryptophol and its ester(s) were separated by prep. TLC (CH_2Cl_2). The tryptophol (0.8 mg) was further purified and identified as above (metabolism of **2**). The esters were hydrolysed

using NH_3 (satd soln in MeOH) to yield tryptophol (identified by TLC). IAA was purified by prep. TLC (1:*i*-PrOH–EtOAc–NH_4OH, 7:9:4, 2:*i*-PrOH–petrol–H_2O, 55:30:11) and treated with CH_2N_2 to yield the methyl ester (identified by TLC).

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