Atlanticones, New Protoilludane Sesquiterpenes from the Mushroom *Lactarius atlanticus* (Basidiomycetes)[‡]

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A chemical investigation of the fruiting bodies of *Lactarius atlanticus* (Russulaceae, Basidiomycetes) resulted in the isolation of new protoilludane sesquiterpenes, namely esters of atlanticones A (**1**) and B (**2**), and atlanticones C (**3**) and D (**4**). The former two compounds were isolated from intact carpophores, whereas the remaining two were isolated from injured fruiting bodies. Their structures, including their absolute stereochemistries, were determined by spectroscopic methods including extensive 2D NMR, CD and molecular modelling.

Introduction

In our continuing work on the secondary metabolites of Basidiomycetes as chemotaxonomic markers and potential pharmacological leads, we investigated the chemical content of fruiting bodies of *L. atlanticus* Bon, a mushroom growing in autumn in Mediterranean *Quercus ilex* and *Pinus* mixed forests.[1] Notably, and unlike those of most mushrooms, the fruiting bodies of *L. atlanticus* are rarely attacked by worms, insects, or other parasites. Some other living organisms display similar deterrence, often associated with the presence of secondary metabolites constituting a chemical defence system. In the *Russula* and *Lactarius* species (family Russulaceae), such defensive metabolites are generally not present in intact fruiting bodies, but are formed enzymatically from inactive precursors once the mushrooms are injured.[2,3] With a few remarkable exceptions,[3] the "inactive forms" are long-chain fatty acid esters of different sesquiterpene alcohols, which, upon injury to the mushroom, are very probably exposed to lipases or similar esterase enzymes. As a result, the free alcohols are released and/or further converted enzymatically into rearranged derivatives,[4] usually endowed with potent biological activities, included antibiotic and cytotoxic ones.[2,5] The possibility that a similar biochemical mechanism has also evolved in *L. atlanticus* motivated our chemical investigation of the content of intact fruiting bodies of this species, as well as of the compounds formed in damaged specimens. Indeed, when fruiting bodies of *L. atlanticus* are broken, the initially tasteless latex and flesh slowly turn slightly bitter,[1] indicating chemical transformations occurring inside the mushrooms. Nothing is yet known about the compounds involved in these changes.

Results and Discussion

In order to compare our results with those reported for similar studies on other *Lactarius* species, we followed a well established extraction procedure described in detail in previous papers.[3] Only young specimens of *L. atlanticus* that appeared undamaged were collected, and these were soaked in CH_2Cl_2 at room temperature a few hours after collection. In addition to spots attributable to common glycerides and fatty acids, this crude extract showed three main spots on TLC plates, corresponding to three different sesquiterpenoids; **1**, **2**, and an unidentified one. The last compound was rapidly degraded both on silica gel (even if deactivated with NEt_3) and on neutral alumina; however, spectroscopic analysis of small amounts isolated as a mixture with triglycerides pointed to a structure markedly related to **1** and **2** (Figure 1).

To simulate injury, a few fruiting bodies were minced without the addition of solvent and extracted with CH_2Cl_2 at room temperature at different intervals after injury. The extracts were then analysed by TLC and ^1H NMR spectroscopy. As expected, the content of the extracts changed with time, even if at a much smaller rate than those of related

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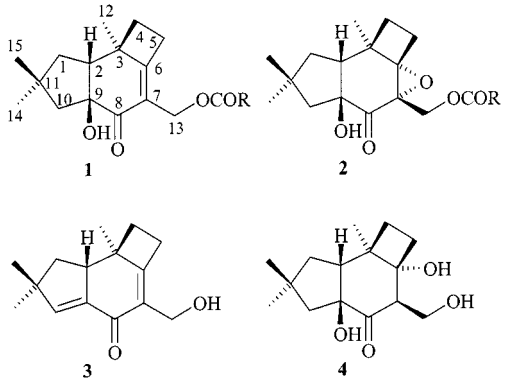


Figure 1. Structures of atlanticones **1**–**4**; R = $C_{17}H_{33}$ (oleic, ca. 90%) and $C_{17}H_{31}$ (linoleic, ca.10%)

species, such as *L. vellereus*[4] and *L. chrysorrheus*,[6] the original metabolites of which are transformed within a few minutes after injury. Indeed, enzymatic hydrolysis of the sesquiterpenoid esters proved largely incomplete even after 3 h and these compounds were still predominant in the extracts. A few, more polar, derivatives were detected, however, and two of these, compounds **3** and **4**, were isolated. Notably, no traces of the sesquiterpenes previously isolated from other *Lactarius* species, in particular the acylated velutinals and their enzymatic transformation products widely occurring in pungent or bitter species,[2,3] were detected in this investigation.

Esters of general formula **1** were present in fairly large amounts in the extract and were readily isolated by chromatographic separations, thanks to their stability both on silica gel and alumina and their strong absorption on TLC plates at 254 nm. No attempt was made to separate the individual esters occurring in the mixture further, because of their similar chromatographic properties. The MS, ^{1}H NMR, and ^{13}C NMR spectra of **1** clearly indicated that the alcoholic moiety was a sesquiterpene alcohol, named atlanticone A. In the terpenoid structure, the presence of a tertiary hydroxy group was indicated by the resonance of a quaternary carbon atom at δ_C = 86.4 in the ^{13}C NMR spectrum, and confirmed by IR and MS spectra. Two additional functional groups could be inferred from the spectroscopic data: an oxo group conjugated with a tetrasubstituted double bond from the resonances at δ_C = 198.2, 176.2, and 123.8, an IR band at 1663 cm^{-1} and a UV maximum at 250 nm, and an ester function from the CO resonance at δ_C = 173.8 (IR band at 1739 cm^{-1}) and the resonance at δ_C = 57.5 attributable to an isolated allylic *C*H_2OCOR group (^{1}H NMR: singlet at δ = 4.72). The remaining signals in the ^{13}C NMR spectrum of the sesquiterpenoid moiety were attributed, with the aid of the DEPT spectrum, to three quaternary methyl groups, four methylene, one methine, and two nonprotonated sp^3 carbon atoms. An HSQC spectrum established the positions of all of the ^{1}H NMR signals for each of the carbon atoms, while all the proton spin systems were firmly determined through homonuclear COSY and selective 1D decoupling experiments. When the partial structures thus established were compared with the two- and three-bond C–H connectivity data from the HMBC spectrum (Figure 2, A and B), structure **1** could be assembled unambiguously. In particular, the HMBC correlations between 2-H and C-12, 12-H_3 and C-2, 2-H and C-4, and 1-H_2 and C-3 allowed the tertiary OH group to be placed unequivocally on C-9 instead of C-2, while the position of the ester group was firmly established by the correlations of 13-H_2 with C-6, C-7 and C-8. The relative stereochemistry of **1** was inferred from NOE and NOESY spectra. Pertinent correlations (Figure 2, C) placed 2-H and 9-OH on the same side as 15-H_3, that is, opposite to 14-H_3 and 12-H_3.

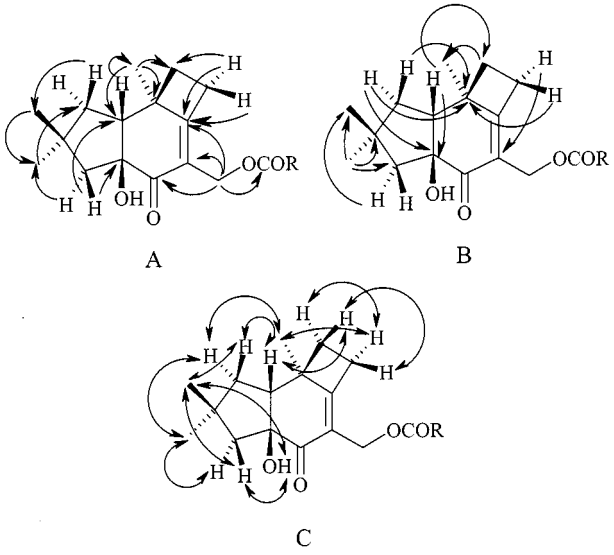


Figure 2. Pertinent HMBC (A and B) and NOESY (C) correlations observed for atlanticone A esters (**1**)

The chiroptical properties of skewed *s-trans* enones can be used for determination of the absolute configuration of organic compounds on the basis of simple empirical helicity rules connecting the signs of the CD bands (both n→π* and π→π*) to the sign of the C=C–C=O dihedral angle ω.[7,8] The enone system of compound **1** was inferred to be nonplanar, both from the molecular modelling and from the relatively low value of ε = 5000 at 250 nm, which has been calculated[9] to decrease with increasing skew angle. Accordingly, the CD spectrum of **1** (Figure 3) showed two Cotton effects above 220 nm, a weak, negative band at longer wavelengths, with Δε = −2 at 335 nm (n→π* transition), and a positive band Δε = +18.5 at 246 nm (I π→π* transition). According to the helicity rules of skewed 2-cyclohexenones,[7,8] these signs correspond to the enantiomer with a positive dihedral angle ω. For esters **1**, an ω angle of about +150° was calculated by PM3, corresponding to the absolute configuration (2*R*,3*R*,9*S*). This assignment was further corroborated by the almost complete superimposition of the CD curve of **1** (Figure 3) upon that of protoilludane sesquiterpene plorantinone B, isolated from *R. pseudodelica* and assigned the same stereochemistry.[10] Thus, all naturally occurring protoilludanes with known absolute configurations belong to the same enantiomeric series.[11]

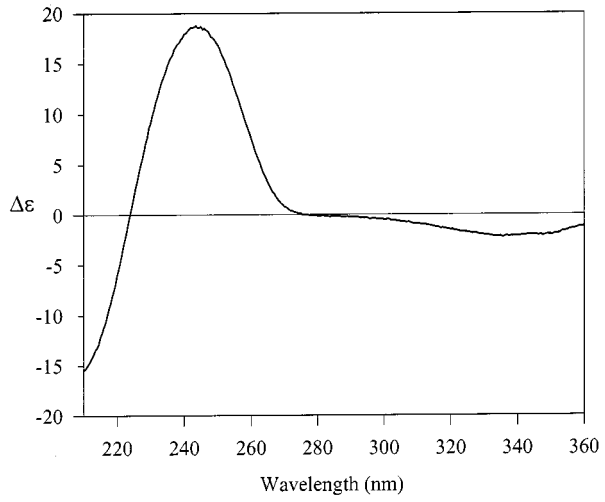
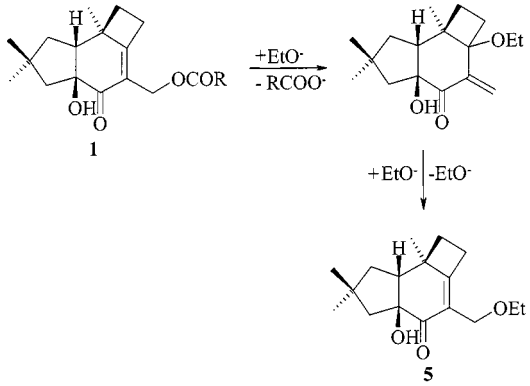


Figure 3. CD spectrum of esters **1** (solvent: cyclohexane)

In order to confirm the structure of **1** and to identify the different fatty acids esterifying the primary allylic alcohol, esters **1** were exposed to NaOH in EtOH/H_2O. However, instead of the expected free alcohol, the main sesquiterpene product of hydrolysis was the ether **5**, readily identified from its NMR spectra. Two consecutive Michael additions of an ethoxide anion, each readily followed by a β-elimination reaction (Scheme 1) could account for the formation of **5**. The high reactivity of the C-6−C-7 double bond towards nucleophiles was probably due to a considerable reduction of strain energy upon saturation of C-6, as previously observed for the related protoilludane sesquiterpene stearoyldelicone,[12] which also possesses a conjugated carbonyl group at C-8. After methylation (CH_2N_2) of the free fatty acid mixture, GC-MS analysis showed oleic acid as the main component (about 90% of the total), the remaining amount being linoleic acid, together with traces of stearic, palmitic, $C_{15:0}$ and $C_{14:0}$ acids. In accordance with this finding, the MS spectrum of **1** showed a weak peak at *m*/*z* = 514, corresponding to the molecular ion of the oleic ester.



Scheme 1

Esters **2**, unlike **1**, were present in only small amounts in the extract and rapidly degraded on silica gel; however, they could be purified on neutral alumina. Inspection of the NMR spectra revealed a structure similar to **1**, a protoilludane sesquiterpene alcohol, named atlanticone B, esterified mainly by oleic and linoleic acids. ^{1}H NMR showed that the two diastereotopic ester protons at C-13, which had resonated as a singlet in the spectrum of **1**, became strongly non-equivalent in **2**, giving rise to two doublets at δ = 4.0 and 4.9, respectively. Moreover, in the ^{13}C NMR spectrum, the two quaternary olefinic carbon atoms C-6 and C-7 of **1** were replaced by two quaternary carbon resonances at δ_C = 69.5 and 58.7, respectively, clearly indicative of an oxirane ring, while HMBC correlations (Figure 4) confirmed the position of the hydroxy group at C-9. Thus, esters **2** corresponded to 6,7-epoxy derivatives of metabolites **1**, as confirmed by the molecular ion (*m*/*z* = 530) of the oleic ester in the MS spectrum. The relative stereochemistry of the epoxy group was assigned as α from the observation of an NOE effect between the 13-H_2 ester protons and the 5-H^β one, combined with an accurate modelling investigation.

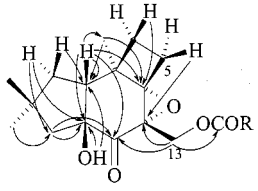
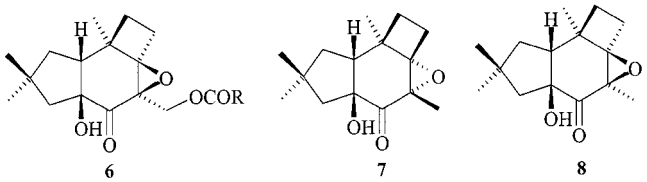


Figure 4. Pertinent HMBC correlations observed for atlanticone B esters **2**

A search of the conformational space of compound **2** and its β isomer **6** was undertaken at semiempirical level through PM3 calculations starting with an initial study of the properties of the tetracyclic skeletons in the simplified structures **7** and **8**, with methyl groups in place of the long acyloxymethyl substituents; the orientation of the hydroxy hydrogen atom was always kept *anti* to the C-2 carbon atom. A certain degree of conformational mobility was observed at the PM3 level as three different conformations were located both for **7** and for **8**. They had some differences in the puckering of the pentacyclic and hexacyclic rings, as can be seen in Table 1.



The multiple minima were in part an artefact of PM3; in fact, when we reoptimized the six **7A**−**C** and **8A**−**C** conformations at a higher level in the DFT framework by B3LYP/6-31G* calculations, only two conformations survived for **7** and one for **8**, as **7A** and **7B** converged to the same **7A′** conformation, and **8A**−**C** all converged to **8A′** (Table 1 and Figure 5). Actually, the global minima of compounds **7** and **8** obtained with the two methods are in close agreement, the main exception being that the cyclobutane ring in the case of **7** is calculated to be too flat at the semiempirical level. However, the strict similarity of the main features of the global minimum conformations, as well as the comparable distances of 5-H^α and 5-H^β from the C-13

Table 1. Relative energies [kcal/mol] and selected geometrical features of PM3- (**7A−C** and **8A−C**) and B3LYP/6-31G*- (**7A′**, **7C′**, and **8A′**) calculated minimum-energy conformations of compounds **7** and **8**

Conform.	E_{rel}	Ring-puckering coordinates 5-membered ring[a]		6-membered ring[b]			4-membered ring[c]	Interatomic distances	
		q_2	φ_2	Q	θ	φ_2	q	5-H$^{\alpha}$/C-13	5-H$^{\beta}$/C-13
7A	0.00	0.30	81	0.50	80	35	0.03	3.90	3.17
7B	0.14	0.28	67	0.45	79	32	0.05	3.88	3.11
7C	3.11	0.32	167	0.54	103	67	0.05	3.91	3.13
8A	0.00[d]	0.29	98	0.59	75	52	0.21	3.74	3.96
8B	0.22	0.26	105	0.60	77	55	0.21	3.75	3.98
8C	1.25	0.18	62	0.60	77	55	0.22	3.74	3.96
7A′	0.00	0.43	71	0.32	54	324	0.24	3.94	2.98
7C′	2.62	0.40	159	0.38	110	64	0.20	3.99	3.10
8A′	0.00[e]	0.35	118	0.61	82	51	0.27	3.80	3.81

[a] C-1/C-2/C-9/C-10/C-11. [b] C-2/C-3/C-6/C-7/C-8/C-9. [c] C-3/C-4/C-5/C-6. [d] E_{rel} = 12.28 with respect to **7A**. [e] E_{rel} = 5.89 with respect to **7A′**.

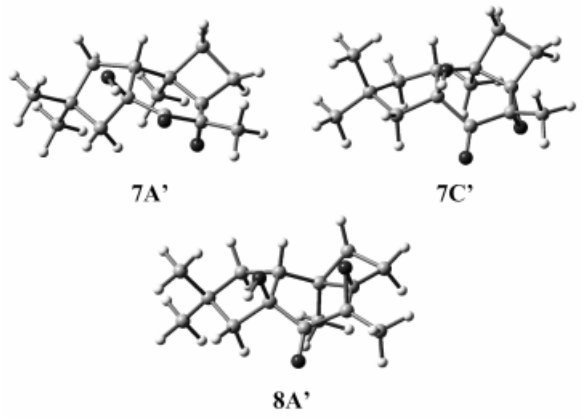


Figure 5. 3D plots of the B3LYP/6-31G*-calculated minimum energy conformations of compounds **7** and **8**

methyl group (Table 1), allowed us to consider the PM3 level of calculations as satisfactory and to come back to esters **2** and **6**. These were studied by PM3 (the B3LYP/6-31G* approach in these cases being excessively time-consuming) after reintroduction of a simplified acyloxy group (acetyloxy) on C-13. With the ester function in the (*Z*) geometry and the C-13−O single bond in the *anti* orientation, the energy profiles for rotation around the C-7−C-13 single bond were determined in 5° steps; three minima were present in both cases. For each of the 72 points of the profiles the distances of 5-H$^{\alpha}$ and 5-H$^{\beta}$ from 13-H^{R} and 13-H^{S} (where the notations *R* and *S* refer to the *pro-R* and *pro-S* diastereotopic hydrogen atoms at C-13) were measured and averaged as the inverse third power weighted on the basis of the Boltzmann coefficients determined from the energy of the 72 conformations. Table 2 reports the calculated averaged distances, which can be correlated with NOE data, and show that only the distances between 5-H$^{\beta}$ and the 13-H_2 hydrogen atoms of compound **2** are compatible with the observed NOE effect, thus allowing **6** to be ruled out as the structure of oleoyl atlanticone B.

Table 2. Averaged interatomic distances in compounds **2** (R = methyl) and **6** (R = methyl)

	5-H$^{\alpha}$/13-H^{R}	5-H$^{\alpha}$/13-H^{S}	5-H$^{\beta}$/13-H^{R}	5-H$^{\beta}$/13-H^{S}
2	3.79	4.09	*3.08*	*3.27*
6	3.85	3.92	4.17	4.04

The α,β-epoxy ketone moiety typical of atlanticone B esters **2** is, to the best of our knowledge, unprecedented among protoilludane sesquiterpenes.[11] Moreover, compounds of this type usually display considerable toxicity and antifeedant activity,[13] owing to their ability to react with biological nucleophiles. We therefore presume that **2** is responsible, at least in part, for insect repellence by fruiting bodies of *L. atlanticus*, even though the amount of **2** in our hands was insufficient to perform extensive bioactivity tests.

Among the metabolites formed in injured fruiting bodies, we isolated small amounts of two novel sesquiterpenes, dienone **3** and triol **4**, existing in the extract as free alcohols (IR). These were identified by comparison of their NMR spectra with those of **1** and **2**. With respect to **1**, atlanticone C (**3**) had an additional double bond conjugated with the carbonyl group at C-8 (IR band at 1651 cm^{-1}), which was located unequivocally between C-9 and C-10 ($\delta_{10\text{-H}}$ = 6.52; $\delta_{C\text{-}10}$ = 147.9; $\delta_{C\text{-}9}$ = 138.8). Atlanticone C (**3**) is isomeric with radulone B, which was isolated from the extracts of the culture fluids of *Radulomyces confluens*[14] and has the OH group at C-2 instead of C-13. Biosynthetically, protoilludane **3** may derive from **1** through ester hydrolysis and H_2O elimination, whereas atlanticone D (**4**) proved to be the product of the formal addition of H_2O across the C-6−C-7 double bond of atlanticone A. The structure of compound **4**, including the α configuration and the *cis* relationship of the 7-H and 6-OH groups, was firmly established from HMBC correlations (Figure 6, A, B, and C) and NOE contacts (Figure 6, D) between 1-H$^{\alpha}$, 10-H$^{\alpha}$ and 7-H, and between 13-H_2 and 5-H$^{\beta}$.

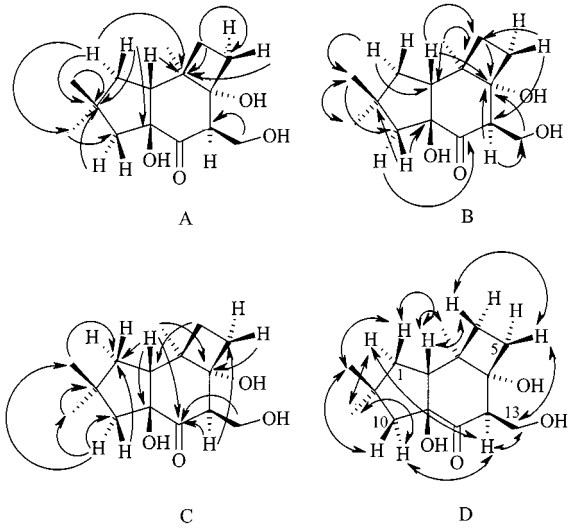


Figure 6. Pertinent HMBC (A, B, and C) and NOESY (D) correlations observed for atlanticone D (**4**)

Atlanticone esters **1** and **2** were inactive towards *Staphylococcus aureus* and *Escherichia coli*.

Conclusions

In conclusion, it appears that the fruiting bodies of *L. atlanticus* originally contain fatty acid esters of at least two new protoilludane sesquiterpenes (**1** and **2**) and that these esters are enzymatically slowly converted into free hydroxy derivatives (**3** and **4**) when fruiting bodies are injured. This behaviour thus seems very general among the *Lactarius* species, but with the original substrates often being different from species to species.[2,3] The class of protoilludane sesquiterpenes is of fairly wide occurrence in higher fungi (Basidiomycetes) and several oxidized variants have been isolated, mostly from mushrooms of the genera *Armillaria*, *Clitocybe*, and *Coprinus*.[11,15−18] In the biosynthesis of Russulaceae sesquiterpenes, protoilludanes have an important position as presumed precursors of marasmane, lactarane, and secolactarane sesquiterpenes, all common and widespread metabolites of *Lactarius* and *Russula* species.[2,3] It is therefore rather surprising that protoilludanes had previously been found only in two species of this family, *L. violascens*[19] and *R. pseudodelica*,[10,12] to which *L. atlanticus* should now be added. This latter finding is of further interest since it unequivocally confirms that the absolute configuration of *Lactarius* protoilludanes corresponds to that of the related sesquiterpene families, thus giving support to the proposed biosynthetic pathway.[2,3]

The sesquiterpenes of *L. atlanticus* are biosynthetically unrelated to the caryophyllane sesquiterpenes isolated from *L. camphoratus*[20] and *L. subumbonatus*,[21] which are grouped by some authorities in the same section *Olentes*.[1] Indeed, our findings would not corroborate this taxonomic assignment. Even more puzzlingly, very similar protoilludane structures have been found in three Russulaceae species *L. violascens*, *L. atlanticus*, and *R. pseudodelica*, which are completely unrelated from a taxonomic point of view. Further study (mainly DNA sequencing, which is now becoming almost routine in Basidiomycetes[22]) will prove whether the ability to biosynthesize similar secondary metabolites is indeed a proof of phylogenetic relationships.

Experimental Section

General: NMR spectra were recorded as $CDCl_3$ solutions with a Bruker CXP 300 or a Bruker Avance 600 MHz spectrometer. Chemical shifts are reported in δ units relative to $CHCl_3$ [δ_H = 7.26, δ_C (central line of t) = 77.0]; the abbreviations s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and br = broad are used throughout. Coupling constants (*J*) are in Hz. The multiplicity (in parentheses) of each carbon signal was determined by DEPT experiments. Mass spectra (direct inlet system) were recorded at 70 eV (0.5 mA) with a Finnigan MAT 8222 instrument. IR spectra were obtained as thin films with a Perkin−Elmer FT-IR Paragon 100 PC spectrometer. Optical rotations were determined with a Perkin−Elmer 241 polarimeter at 20 °C. $[\alpha]_D$ values are given in 10^{-1} deg·cm^2·g^{-1}. UV spectra were recorded with a Perkin−Elmer Lambda 5 instrument and CD spectra with a Jasco J-710 spectropolarimeter, by employing 0.1-cm optical cuvettes. Δε and ε are reported in L·mol^{-1}·cm^{-1}. Analytical TLC was carried out on glass-backed plates, precoated with a 0.25 mm layer of silica gel, and viewing was effected with short-wavelength UV light (254 nm) or with 0.5% vanillin solution in H_2SO_4/EtOH (4:1) followed by heating. Liquid chromatography was accomplished with 60 Kieselgel (40−63 μm) or neutral alumina of activity III−IV.

Collection, Extraction, and Isolation: Fruiting bodies of *L. atlanticus* were collected in two different places in the vicinity of Rome (Italy). They were ground and immediately extracted with CH_2Cl_2 (extract of intact fruiting bodies) or, alternatively, ground and the resulting pulp allowed to rest for about 3 h and then extracted with CH_2Cl_2 (extract of injured fruiting bodies). From approximately 700 g of fresh material, it was possible to obtain 1.1 g of crude extract of intact fruiting bodies and 550 mg of crude extract of injured fruiting bodies. Liquid chromatography on silica gel or on alumina resulted in the isolation of four novel metabolites: **1** (30 mg), **2** (2.5 mg), **3** (6 mg), **4** (4 mg). EtOAc/hexane gradient mixtures were employed as eluents in the chromatographic separations.

Atlanticone A Esters (1): Colourless oil. $[\alpha]_D^{20}$ = −1.5 (*c* = 0.3, $CHCl_3$). EIMS: *m/z* (%) = 514 (4) [M$^+$], 486 (6), 484 (7), 383 (4), 264 (18), 251 (12), 233 (68), 219 (35), 205 (45), 189 (34), 161 (21), 137 (28), 123 (50), 109 (39), 95 (69), 69 (69), 55 (100), 43 (65), 41 (40). UV (CH_2Cl_2): λ_{max} = 250 nm; ε = 5000. CD (cyclohexane): 246 nm (Δε = +18.5), 335 nm (Δε = −2). IR: $\tilde{\nu}$ = 3468 (OH), 2926, 2855, 1739 (ester C=O), 1663 (unsaturated ketone C=O), 1464, 1243, 1172 cm^{-1}. ^{1}H NMR: δ = 5.34 (m, olefinic protons of unsaturated fatty acids); 4.72 (s, 2 H, 13-H$_2$); 3.17 (dt, $J_{5\alpha\text{-}4}$ = 9.4, $J_{5\alpha\text{-}5\beta}$ = 16.7, 1 H, 5-H$^\alpha$); 2.98 (dt, $J_{5\beta\text{-}4}$ = 5.8, 1 H, 5-H$^\beta$); 2.77 [t, *J* = 6.5, bis(allylic) protons of linoleic acid]; 2.64 (brd, $J_{2-1\alpha}$ = 3.6, $J_{2-1\beta}$ = 9.2, 1 H, 2-H); 2.48 (s, exchangeable with D_2O, 1 H, OH); 2.28 (t, *J* = 7.0, 2 H, 2′-H); 2.01 (m, allylic protons of unsaturated fatty acids); 1.98 (m, 2 H, 4-H$_2$); 1.92 (dd, $J_{1\alpha\text{-}1\beta}$ = 14.3, 1 H, 1-H$^\beta$); 1.77 (d, $J_{10\alpha\text{-}10\beta}$ = 14.7, 1 H, 10-H$^\alpha$); 1.69 (brd, 1 H, 10-H$^\beta$), 1.68 (dd, 1 H, 1-H$^\alpha$); 1.60 (br, 2 × CH_2 of fatty acids), 1.35 (s, 3 H, 12-H$_3$); 1.29 (s, 3 H, 15-H$_3$); 1.28 (br, *n* × CH_2 of fatty acids), 1.17 (s, 3 H, 14-H$_3$), 0.88 (brt, 3 H, 18′-H$_3$). ^{13}C NMR: δ = 198.2 (s, C-8); 176.2 (s, C-6); 173.8 (s, C-1′); 130.2, 130.1, 129.9 and 128.2

(d, CH olefinic carbon atoms of unsaturated fatty acids); 123.8 (s, C-7); 86.4 (s, C-9); 57.5 (t, C-13); 56.2 (d, C-2); 50.7 (t, C-1); 46.3 (s, C-3); 40.3 (t, C-10); 38.9 (s, C-11); 35.8 (t, C-4); 34.4 (t, C-2′); 33.5 (q, C-15); 30.8 (q, C-14); 30.0 (t, C-5); 32.1, 29.9−29.3, 27.4, 27.3, 25.1, 22.9 (t, $n \times CH_2$ of fatty acids); 23.5 (q, C-12); 14.3 (q, C-18′).

Hydrolysis of Esters 1: Esters **1** (10 mg) were dissolved in EtOH (3 mL). Aqueous NaOH (2 N, 0.5 mL) was added and the solution was stirred at 45 °C for 30 min until completion of the reaction. EtOH was removed under vacuum and the aqueous phase was extracted with Et_2O. The organic phase was dried with Na_2SO_4 and the solvents were evaporated under vacuum. The neutral products of hydrolysis were separated by chromatography on silica gel; compound **5** (1.5 mg) was the only one obtained in amounts sufficient for partial characterisation. The aqueous phase was acidified with 10% aqueous HCl and extracted with Et_2O; the fatty acids thus obtained were methylated with CH_2N_2 and analysed by GC-MS, which found the methyl esters of the following acids: oleic (MW: 296), stearic (MW: 298), linoleic (MW: 294), palmitic (MW: 270), $C_{15:0}$ (MW: 256), and $C_{14.0}$ (MW: 242).

Compound 5: Colourless oil. CIMS (NH_3): *m/z* = 296 [M + NH_4^+], 279 [M + H^+]. ^{1}H NMR: δ = 4.14 (AB_q, 2 H, 13-H_2); 3.48 (m, 2 H, 1′-H_2); 3.17 (dt, $J_{5\alpha\text{-}5\beta}$ = 16.0, $J_{5\alpha\text{-}4}$ = 9.0, 1 H, 5-H^α); 2.97 (ddd, $J_{5\beta\text{-}4}$ = 7.1, $J_{5\beta\text{-}4'}$ = 5.0, 1 H, 5-H^β); 2.66 (br. d, $J_{2-1\alpha}$ = 3.7, $J_{2-1\beta}$ = 9.0, 1 H, 2-H); 2.51 (s, OH, 1 H); 2.0−1.93 (m, 2 H, 4-H_2); 1.90 (dd, $J_{1\alpha\text{-}1\beta}$ = 14.5, 1 H, 1-H^β); 1.77 (d, $J_{10\alpha\text{-}10\beta}$ = 14.6, 1 H, 10-H^α); 1.71−1.66 (m, 2 H, 10-H^β and 1-H^α); 1.35 (s, 3 H, 12-H_3); 1.30 (s, 3 H, 15-H_3); 1.13 (s, 3 H, 14-H_3); 1.18 (t, *J* = 7.1, 3 H, 2′-H_3). ^{13}C NMR: δ = 198.9 (s, C-8); 86.3 (s, C-9); 66.0 (t, C-1′); 64.1 (t, C-13); 56.2 (d, C-2); 50.5 (t, C-1); 46.3 (s, C-3); 40.2 (t, C-10); 38.7 (s, C-11); 35.8 (t, C-4); 33.4 (q, C-15); 30.7 (q, C-14); 29.6 (t, C-5); 23.3 (q, C-12); 15.1 (t, C-2′). Because of the small amounts isolated the signals of carbon atoms C-6 and C-7 were indistinguishable from the noise.

Atlanticone B Esters (2): Colourless oil. $[\alpha]_D^{20}$ = −36 (*c* = 0.2, CH_2Cl_2). EIMS: *m/z* (%) = 530 (1) [M^+], 512 (1), 500 (10), 498 (10), 482 (9), 470 (4), 426 (21), 249 (19), 216 (64), 205 (62), 187 (53), 95 (45), 83 (55), 69 (68), 55 (100), 43 (80). IR: ṽ = 3450, 2926, 2842, 1732, 1460, 1365, 1358, 1261, 1172, 1110, 1033, 800 cm^{-1}. ^{1}H NMR: δ = 5.35 (m, olefinic protons of unsaturated fatty acids); 4.87 (d, $J_{13a\text{-}13b}$ = 12.6, 1 H, 13-H^a); 4.00 (d, 1 H, 13-H^b); 3.25 (s, 1 H, OH); 2.78 [t, *J* = 6.5, bis(allylic) protons of linoleic acid]; 2.61 (m, 1 H, 5-H^α); 2.56 (dd, $J_{2-1\beta}$ = 6.7, $J_{2-1\alpha}$ = 14.2, 1 H, 2-H); 2.37 (m, 2 H, 2′-H_2); 2.34 (d, $J_{10\alpha\text{-}10\beta}$ = 13.5, 1 H, 10-H^α); 2.18−2.12 (m, 2 H, 5-H^β and 4-H^a); 2.07−1.98 (m, allylic protons of unsaturated fatty acids); 1.69 (m, 1 H, 4-H^b); 1.66−1.57 (br, $n \times CH_2$ of fatty acids); 1.38 (dd, $J_{1\alpha\text{-}1\beta}$ = 13.2, 1 H, 1-H^β); 1.35 (d, 1 H, 10-H^β); 1.33−1.22 (br, $m \times CH_2$ of fatty acids); 1.18 (s, 3 H, 12-H_3); 1.07 (s, 3 H, 15-H_3); 0.99 (s, 3 H, 14-H_3); 0.95 (dd, 1 H, 1-H^α); 0.88 (t, *J* = 6.8, 3 H, 18′-H_3). ^{13}C NMR: δ = 203.5 (s, C-8); 175.5 (s, C-1′); 129.9 and 129.7 (2 d, olefinic carbon atoms of oleic acid); 86.2 (s, C-9); 69.5 (s, C-6); 64.9 (t, C-13); 58.7 (s, C-7); 53.3 (d, C-2); 45.5 (t, C-10); 41.7 (t, C-1); 41.6 (s, C-3); 34.4 (s, C-11); 34.0 (t, C-2′); 32.0 (q, C-14); 30.2 (q, C-15); 31.8, 29.7−29.0, 27.2, 27.1, 24.6, 22.6 (t, $n \times CH_2$ of fatty acids); 28.4 (t, C-4); 23.9 (t, C-5); 19.4 (q, C-12); 14.1 (q, C-18′).

Atlanticone C (3): Colourless oil. $[\alpha]_D^{20}$ = −224 (*c* = 0.1, CH_2Cl_2). MS (ESI): *m/z* = 487 [2 M + Na^+], 255 [M + Na^+], 233 [M + H^+], 215 [233 − H_2O]. IR: ṽ = 3427, 2928, 2860, 1651, 1618, 1454, 1370, 1340, 1247, 1140, 1065, 1015, 986 cm^{-1}. ^{1}H NMR: δ = 6.52 (d, *J* = 2.5, 1H, 10-H), 4.25 (ABq, 2 H, 13-H_2), 3.43 (dt, J_{10-2} = 2.5, $J_{2-1a} \approx J_{2-1b}$ = 8.0, 1 H, 2-H); 3.15 (dt, $J_{5\alpha\text{-}5\beta}$ = 16.0, $J_{5\alpha\text{-}4a} = J_{5\alpha\text{-}4b}$ = 9.3, 1 H, 5-H^α); 2.91 (ddd, $J_{5\beta\text{-}4a}$ = 7.3, $J_{5\beta\text{-}4b}$ = 3.7, 1 H, 5-H^β); 2.06−1.96 (m, 2 H, 4-H_2); 1.79 (dd, $J_{1a\text{-}1b}$ = 12.8, 1 H, 1-H^a); 1.66 (dd, 1 H, 1-H^b); 1.24, 1.21, and 1.10 (3s, 3 H each, 12-H_3, 14-H_3, and 15-H_3). ^{13}C NMR: δ = 188.0 (s, C-8); 172.6 (s, C-6); 147.9 (d, C-10); 138.8 (s, C-9); 129.3 (s, C-7); 57.3 (t, C-13); 53.6 (d, C-2); 47.4 (s, C-3); 45.8 (s, C-11); 39.3 (t, C-1); 32.5 (t, C-4); 28.6 (t, C-5); 28.5 (q, C-15); 27.4 (q, C-14); 19.1 (q, C-12).

Atlanticone D (4): Colourless oil. $[\alpha]_D^{20}$ = −235 (*c* = 0.1, CH_2Cl_2). MS (ESI): *m/z* = 559 [2 M + Na^+], 251 [M + H − H_2O]$^+$, 233 [251 − H_2O]$^+$. IR: ṽ = 3408, 2953, 2860, 1710, 1458, 1369, 1307, 1268, 1188, 1139, 1049, 946, 848 cm^{-1}. ^{1}H NMR: δ = 4.17 (dd, $J_{13a\text{-}13b}$ = 11.4, $J_{13a\text{-}7}$ = 5.8, 1 H, 13-H^a); 4.08 (dd, $J_{13b\text{-}7}$ = 7.2, 1 H, 13-H^b); 3.33 (ddd, $J_{5\alpha\text{-}7}$ = 1.4, 1 H, 7-H); 2.47 (dd, $J_{2-1\beta}$ = 7.5, $J_{2-1\alpha}$ = 13.0, 1 H, 2-H); 2.10 (ddd, $J_{5\alpha\text{-}5\beta}$ = 12.8, $J_{5\beta\text{-}4}$ = 8.6, 3.6, 1 H, 5-H^β), 1.95 (brd, $J_{10\alpha\text{-}10\beta}$ = 14.4, 1 H, 10-H^α); 2.01−1.9 (m, 1 H, 5-H^α); 1.86 (ddd, $J_{1\alpha\text{-}1\beta}$ = 12.8, $J_{10\beta\text{-}1\beta}$ = 2.8, 1 H, 1-H^β); 1.61 (t, 1 H, 1-H^α); 1.57 (dd, 1 H, 10-H^β); 1.40−1.27 (m, 2 H, 4-H_2); 1.23 (s, 3 H, 12-H_3); 1.20 (s, 3 H, 15-H_3); 1.13 (s, 3 H, 14-H_3). ^{13}C NMR: δ = 214.3 (s, C-8); 85.6 (s, C-9); 81.9 (s, C-6); 58.5 (t, C-13); 58.1 (d, C-2); 54.1 (t, C-10); 51.6 (d, C-7); 46.1 (t, C-1); 45.9 (s, C-3); 40.0 (s, C-11); 29.5 (q, C-14); 28.7 (t, C-5); 26.9 (q, C-15); 23.9 (t, C-4); 20.2 (q, C-12).

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