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METABOLITES OF *PESTALOTIOPSIS* SPP., ENDOPHYTIC FUNGI OF *TAXUS BREVIFOLIA*

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Key Word Index—*Pestalotiopsis*; *Taxus brevifolia*; endophytic fungi; caryophyllenes; acetogenins.

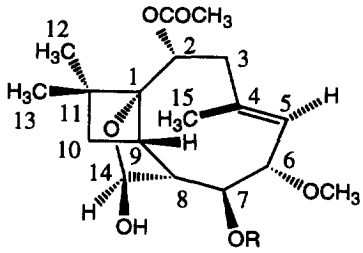
Abstract—Two strains of *Pestalotiopsis* spp. (JCM 9685 and JCM 9686), endophytic fungi of *Taxus brevifolia*, produced several new compounds when grown in liquid culture. Detailed investigation on the first of these strains revealed that five of these metabolites were sesquiterpenes: three of the caryophyllene type, pestalotiopsin A, B and C, one possessing the humulane skeleton, and the last one was a drimane derivative. The remaining compounds were two C-methylated acetogenins: (4*S**,5*R**)-(6*Z*,8*E*)-4,5-dihydroxy-6-hydroxymethyl-6,8-decadiene and (4*S**,5*R**)-(2*Z*,6*Z*,8*E*)-4,5-dihydroxy-6-hydroxymethyl-2,6,8-decatriene. Furthermore, an aldehyde closely related to these two triols was obtained from JCM 9686. The isolation and characterization of pestalotiopsin C and of the three new C-methylated acetogenins is described. The chemotaxonomic significance of the latter is discussed. © 1997 Elsevier Science Ltd

INTRODUCTION

Hardly any natural compound has captured as much attention among the scientific community as the diterpenoid taxol, whose importance as an anticancer agent is nowadays widely recognized and demonstrated by its efficacy in several Phase II clinical trials [1]. Taxol, though isolated more than 25 years ago [2], has been the subject of a great number of investigations in the last few years. These have been aimed at overcoming the toxicity and low solubility of taxol, and finding an alternative source of this compound other than the bark of the Pacific yew tree (*Taxus brevifolia*), a source that, mainly for ecological reasons, is not sustainable [3–6].

Stierle *et al.* [7] claimed production of taxol by means of an endophytic fungus, *Taxomyces andreanae*, associated with the bark of *Taxus brevifolia*, and this observation led us to start our investigation of the metabolites produced by endophytic fungi of the yew tree and related species (*Taxus* sp.). The aim of which is to obtain better understanding of the ecological relationships between the microorganisms and their host. We have already reported on the investigation of two strains of *Pestalotiopsis* spp., JCM 9685 and JCM 9686, obtained respectively from samples of leaves and bark of a *T. brevifolia* tree collected in Bozeman, Montana. The morphological features of the spores in the strains agree well with the concept of the genus *Pestalotiopsis* Stey. However, it is difficult to identify the strains at the species level because of some problems concerning the taxonomy of *Pestalotiopsis* and allied genera [8]: we prefer to follow Steyaert [9], but the strains could be as well included into the genus *Pestalotia sensu lato* [10]. The colonies and spore morphology are different in the strains, so they should be treated as *Pestalotiopsis* spp., and, although some metabolites, like **1**, **2**, **6**, and **7**, are certainly found in both strains, some differences are nevertheless evident, especially with respect to the proportions of the compounds. The results we present here refer to JCM 9685 with the addition of the aldehyde **8**, isolated only from JCM 9686. We have already described some of these unique metabolites: the two sesquiterpenes of the caryophyllene type, pestalotiopsin A and pestalotiopsin B (respectively **1** and **2**) [11], an unusually 2-hydroxy substituted isodrimenol **5** [12], and a highly functionalized humulane **4** [13], the first of fungal origin. In this paper we report on the isolation and characterization of pestalotiopsin C (**3**), a congener of **1** and **2**, the two new C-methylated acetogenins **6** and **7**, and the related aldehyde **8**.

* Authors to whom correspondence should be addressed.



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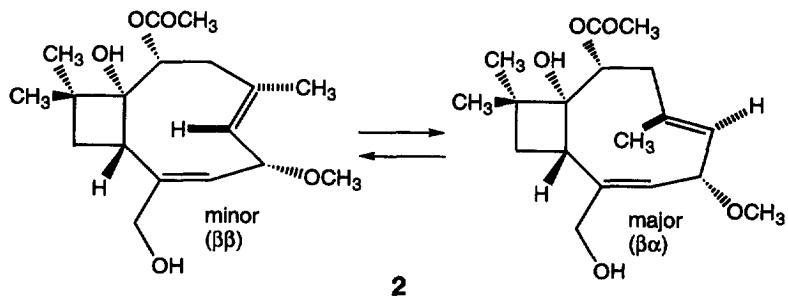

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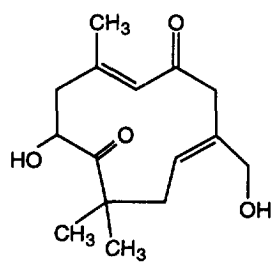

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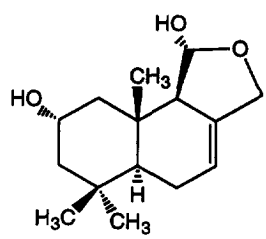

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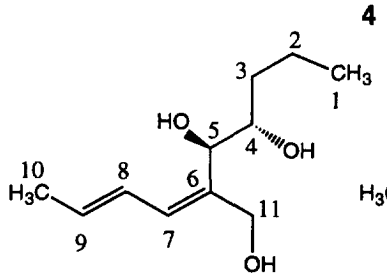

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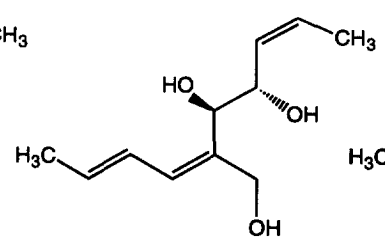

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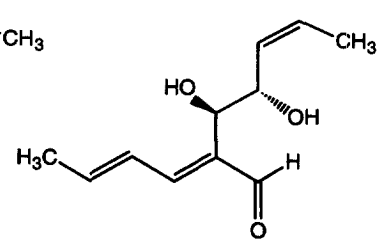

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RESULTS AND DISCUSSION

The fungus was grown on a modified S7 medium for periods varying from 3 to 6 weeks, furnishing, after solvent extraction, a waxy yellow material. The yield, as well as the proportions of the compounds, was found to vary from batch culture to culture. However, the humulane derivative **4** was found only in old cultures (typically 46 days), while pestalotiopsin C (**3**), although observed in traces in all of the extracts, could be obtained in significant amount only occasionally and this probably related to the presence of spores in the medium [14].

We have already described pestalotiopsin A (**1**) and pestalotiopsin B (**2**) [11]. Their structures were determined by the concerted application of several two-dimensional NMR techniques, and confirmed by X-ray diffraction analysis. The structure of **1**, the most polar among the pestalotiopsins, comprises an oxatricyclic sesquiterpene system unprecedented among natural products. Compound **2**, the most abundant among the pestalotiopsins, appears to be a single entity on HPLC and HPTLC analysis, but exists as a mixture of two slowly equilibrating atropisomers, $\beta\alpha$ and $\beta\beta$, whose ratio, determined on the basis of NOE experiments, is 6 to 5, respectively (erroneously reported as 5 to 6 in ref. [11]).

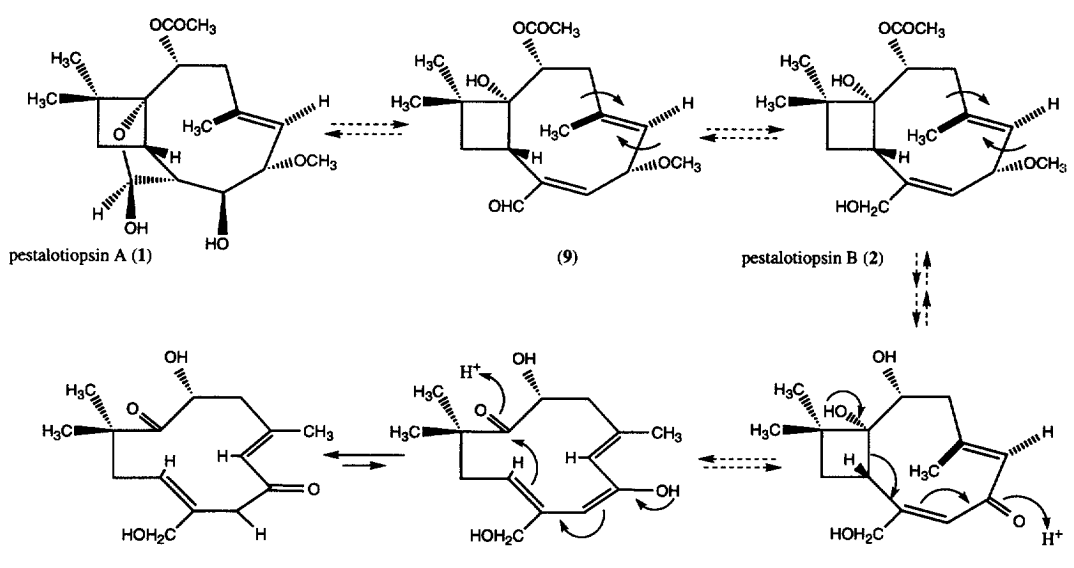
Pestalotiopsin C (**3**) is the least polar among the three natural caryophyllenes produced by this fungus. Its yield was found to greatly change from culture to

culture; in the best case it accounted for 11% of the whole crude extract. The compound can be obtained pure after chromatography of the crude extract over silica gel followed by recrystallization from ethyl acetate. Positive HRFAB-mass spectroscopy furnishes a molecular peak at *m/z* 355.2148 leading to the molecular formula $C_{19}H_{31}O_6$. The ^1H and ^{13}C NMR spectra closely resemble those of pestalotiopsin A, and the only consistent difference is the presence of two signals around δ 3.3 and 56, respectively, accounting for a further methoxyl group. The position of the latter is established by careful comparison of the spectra of the two compounds, and confirmed by the HMBC data. The NOE relations are also very similar to those of **1**, suggesting that **3** exists in solution as a $\beta\alpha$ conformer.

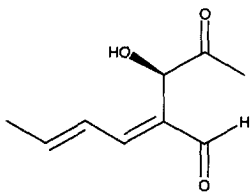
In plants, caryophyllenes are thought to be biosynthesized from farnesyl pyrophosphate via a humulene cation, and a similar pathway has been put forward for the much more uncommon fungal caryophyllenes [15]. However, apart from traces of humulene itself [16] no other sesquiterpene of this kind had been reported from fungi before the isolation of compound **4** [13]. The latter shows an evident structural relationship with pestalotiopsin B, i.e. the humulane is an oxidized derivative of the caryophyllene. This is in agreement with a common biosynthetic route. However, it suggests also that **4** is a catabolite rather than a precursor of **2**, and the fact that the humulane is peculiar to old cultures can be taken as support for this speculation. Nevertheless, the possibility that it is the caryophyllene that originates from the humulane cannot be ruled out.

The degree of functionalization of the pestalotiopsins is in agreement with the idea that compound **2** is also the precursor of compound **1**. However, during an attempted esterification of pestalotiopsin A with (*S*)-*O*-methylmandelic acid at 42°, we obtained, as the only product of the reaction, the aldehyde **9** (unpublished results). This could be regarded as a biosynthetic emulating process, and would suggest that the biosynthetic pathway operates in the opposite direction. The possible biosynthetic relationships of all of these compounds is depicted in Scheme 1. Elimination of water from **1** and subsequent reduction of the aldehyde **9** would yield **2**. Deacetylation at C-2 and oxidation at C-6 of the latter leads to a hypothetical intermediate, the direct precursor of the enolic form of the humulane **4**. Alternatively, the whole process might work in the opposite direction from the humulane **4** through to pestalotiopsin A (**1**).

Pestalotiopsis sp. proved to be a good source of sesquiterpenes. It should be noted that compound **5** was isolated from the liquid culture of this fungus [12]. However, though sesquiterpenes constitute the major part of the fungal metabolites, they are not the only ones. The same strain furnished two other new compounds which are totally unrelated to those described above, and have some structural similarities with the antibiotic avellaneol (**10**) [17], and have the same carbon skeleton as multicolosic acid (**11**) and related compounds [18]. These two compounds **6** and **7**, trivially named pestalotiopsol A and B, were typically obtained with yields of 2.1 and 1.4%, respectively. Comparison of the ^1H NMR spectrum of **6** and **7** clearly shows they are related and very similar. Most of the signals have exactly the same chemical shift and multiplicity, and the only consistent difference is the presence of one more double bond in **7**. This suggests **6** to be a partially saturated derivative of **7**. Negative HRFAB-mass spectroscopy furnishes a molecular peak at *m/z* 199.1329 for pestalotiopsol A, and 197.1176 for pestalotiopsol B, which fit well with the molecular formulae of $C_{11}H_{20}O_3$ and $C_{11}H_{18}O_3$, respectively. Also the mass spectra confirm the resemblance between the two compounds: somewhat better results



Scheme 1. Possible metabolic relationship between the pestalotiopsins (**1**, **2**) and the humulene **4**.



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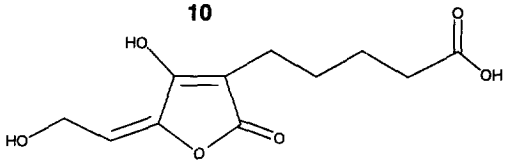

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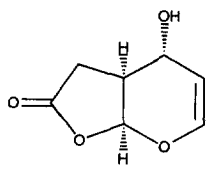

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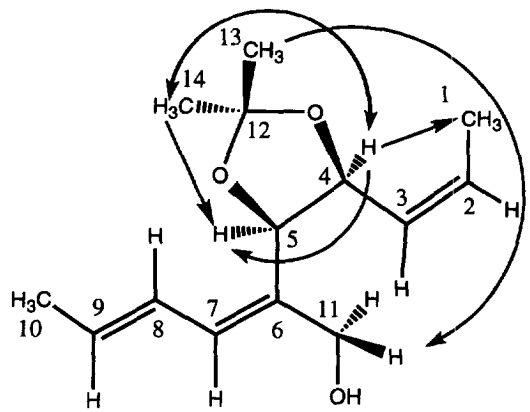

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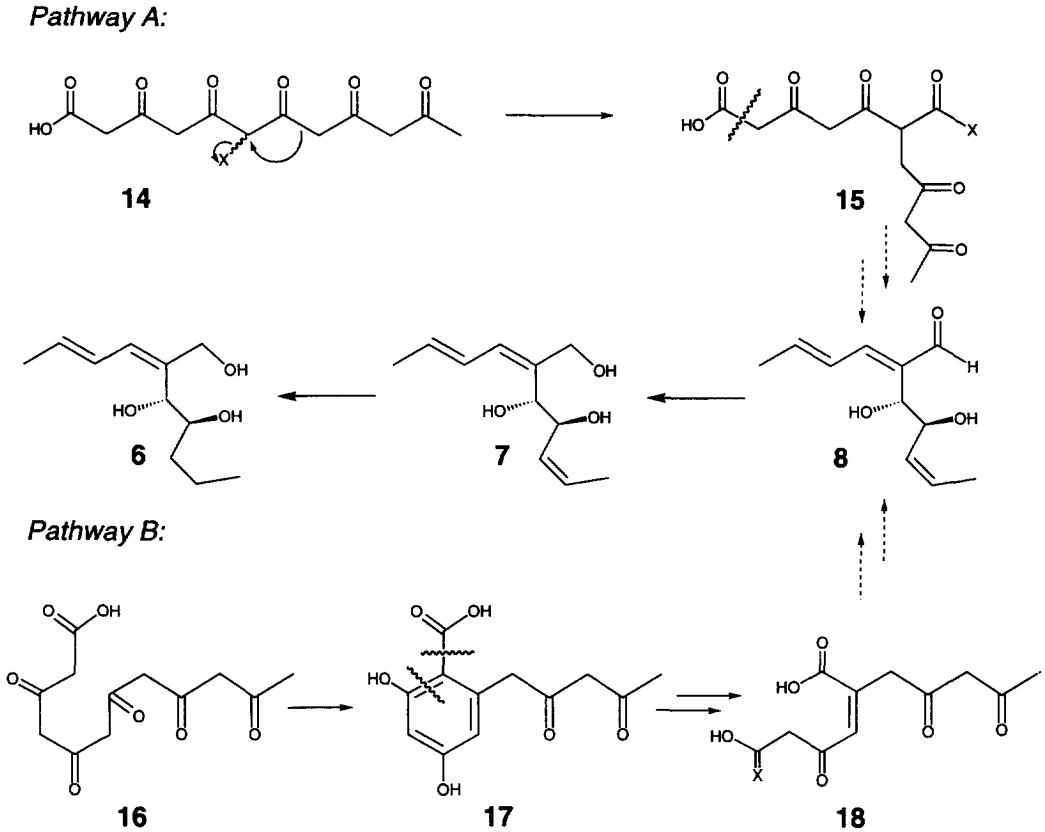
Fig. 1. Structure and observed NOEs of **13**.

are obtained on negative FAB-mass spectroscopy, in this case the spectra are almost identical except for the shift of 2 amu. In the positive FAB-mass spectrum, two subsequent losses of water are evident, and this suggests the presence of at least two hydroxyl groups, which is in good agreement with some of the chemical shifts of the ^{1}H NMR spectra. The primary structure of **7** can be easily obtained by examining the PFG-DQFCOSY, PFG-HMQC and PFG-HMBC spectra, while the stereochemistry of the double bonds is given by the values of the coupling constants as well as the NOEs: irradiation of H-8 enhances the signals of H-10 and H-5, while enhancements of H-9 and H-11 follow the irradiation of H-7; finally, by irradiation of H-4 only the signal of H-1 is enhanced. This clearly suggests a *Z*, *Z* and *E* stereochemistry for Δ2, Δ6, and Δ8, respectively. Assignment of all the signals is less straightforward for **6**, as a consequence of the lack of crosspeaks in the olefinic region of the HMQC spectrum. In particular, ambiguity arises when assigning C-7 and C-6 (signals at δ 134.9 and 133.5). Only the presence in the HMBC spectrum of a weak crosspeak between H-9 and the signal at δ 133.5, suggesting the latter to be therefore C-7, and the comparison between the 2D NMR spectra obtained for **6** and those measured in exactly the same experimental conditions for **7**, allows the full assignment.

The relative configuration of the two stereocentres at C-5 and C-4 can be determined by preparing an acetonide derivative. The reaction has been performed only on compound **7**, on the assumption that the sole difference between pestalotiopsol A and **B** lies in the existence of one more double bond in the latter. The acetonide **13** is easily isolated, and NOE difference spectroscopy clearly indicates a 4*S**,5*R** configuration (Fig. 1).

The same carbon skeleton as that of compounds **6** and **7** is found in the aldehyde **8** which we have isolated in small amounts from the cultures of JCM 9686, and identified on the basis of its ^{1}H NMR and FAB-mass spectrum. This new derivative might be an intermediate in the biosynthesis of pestalotiopsols, as we suggest in Scheme 2 where two possible routes to the biogenesis of these compounds are illustrated. One route parallels the one that has been described for avellaneol (**10**) [17] (pathway A): this requires direct chain fission of a hypothetical polyketide **14** to yield **15**, and its subsequent funtionalization. The close similarity between avellaneol and pestalotiopsols makes this route attractive. However, the biosynthesis of multicolosic acid (**11**) has been shown to follow a different route [18] which, not only could account for the biosynthesis of pestalotiopsols, but closely resembles the one that has been proposed for oxysporone (**12**) and related metabolites produced by *Pestalotiopsis theae* and *P. longiseta* [19]. This (pathway B) requires an aromatic polyketide precursor (**17**) which is oxidatively cleaved, either by means of a monooxygenase, as suggested for oxysporone [19], or by an alternative mechanism, as shown for multicolanic acid [18]. Also in this case, the subsequent functionalization of the hypothetical intermediate **18** eventually leads to **8**. This second pathway has the merit of highlighting the similarity between **6** and **7** on one hand, and **12** and related metabolites on the other; hence it would provide a chemotaxonomic, in addition to a morphological, proof that the strains under investigation here belong to the genus *Pestalotiopsis*. This is very important, in fact, since a survey of the literature reveals that the members of this genus that have been previously chemically investigated, i.e. *P. guepinii*. [21], *P. oenotherae*, a pathogen of the evening primrose [20], and *P. theae* and *P. longiseta*, responsible for the tea gray blight disease [19], all produce exclusively acetogenins and C-methylated acetogenins, though it should be mentioned that among the metabolites produced by the latter, PT-



Scheme 2. Possible pathways for the formation of compounds **6**, **7**, and **8**.

toxin was envisaged as arising from gentisaldehyde by loss of a hydroxymethyl moiety and subsequent addition of an isoprene unit. Furthermore, this holds even if we consider the metabolites produced by species belonging to the genus *Pestalotia*, whose taxonomy, as we have already mentioned, is not always clear [8]. This means that the strains under investigation here are the only ones among *Pestalotiopsis* and allied genera that can produce terpenes. This peculiarity is further stressed by the fact that sesquiterpenes are undoubtedly the major, and the C-methylated acetogenins the very minor components of their metabolic mixtures.

Furthermore, the formerly studied species belonging to this genus are all known to be saprophytes or parasites on plants, and almost all of their metabolites have been recognized as phytotoxins. The two strains under investigation here, are endophytic, that is, in agreement with the definition given by Wilson [22], they invade the stem and leaves of the plant but cause no symptoms of disease. Little is known about the role of endophytic fungi [23]. In this specific case, we suggest that the two main attributes described above (these strains produce terpenes and are endophytes) are probably not only related, but that one might be the consequence of the other. Parasitic fungi are in fact considered to have evolved from saprophytes [24], and, in turn, parasites are thought to have given rise to endophytes [25]. Hence, the strains under investigation here probably represent the last stage in the evolution of this genus, and their metabolites might simply be the adaptations to a new environment.

EXPERIMENTAL

General. All the reagents and fermentation media components were used without further purification. TLC: silica gel (kieselgel 60, F 254), compounds visualized by spraying with a mixt. of anisaldehyde–H_2SO_4 (5%) in MeOH, and subsequent heating at 110° for 2–3 min. CC: Silica gel (70–230 mesh); Mps: uncorr; ^1H and ^{13}C NMR: pulsed field gradient, $CDCl_3$ soln at 600 or 300 and 150 or 75 MHz, and the chemical shifts are given relative to the TMS and $CDCl_3$ solvent peaks at 0.00 and 77.0 ppm, respectively. NOE difference spectra and all 2D spectra were recorded using procedures described earlier [11]. FAB-MS and HRFAB-MS: glycerol or NBA matrix; IR: KBr discs; specific: MeOH soln.

Fungal material and fermentation conditions. Leaves and bark of *Taxus brevifolia* were collected in Bozeman, MT. The strains were selected by culturing several small leaf or bark pieces onto agar, and subsequently transferring the mycelial tips several times. The strains, JCM 9685 and JCM 9686, were identified as *Pestalotiopsis* spp., and the mycelial cultures are deposited in the collection of the Japan Collection of Microorganisms (Riken). Small agar plugs were then

transferred into a 2 l Erlenmayer flask containing 1 l of a culture broth containing (per l of H_2O): 1 mg biotine, 1 mg thiamine, 1 mg Ca^{2+} pantothenate, 1 mg pyrodoxine, 3.6 mg $MgSO_4$, 6.5 mg $Ca(NO_3)_2$, 1 mg $Cu(NO_3)_2$, 2.5 mg $ZnSO_4$, 5 mg $MnCl_2$, 2 mg $FeCl_3$, 5 mg phenylalanine, 100 mg Na^+ benzoate, 1 g glucose, 3 g fructose, 6 g sucrose, 1 g CH_3COONa, 1 g bacto-soytone, 1 ml of 1 M of KH_2PO_4. The fungus was grown in the dark in still culture at 25° for between 21 and 46 days.

Extraction and isolation. The culture was filtered through cheese cloth to remove the mycelia, and the fluid extracted with 0.5 vol. CH_2Cl_2. This was concd *in vacuo* to yield the crude extract which was directly applied to silica gel. Details on the isolation of compounds **1**, **2**, **4** and **5** have already been given [11–13]. The organic extract obtained from a 42-day-old culture (60 mg, from 1 l) and particularly rich in compound **3**, was applied to silica gel (2 × 13 cm), and the column developed with $CHCl_3$–MeOH (97:3). The eluent was collected in 1.0 ml frs. Tubes 32–41 on evapn yielded 6.2 mg of a solid material which was crystallized from EtOAc, giving 6.0 mg of pure compound (pestalotiopsin C, **3**).

Pestalotiopsin C (**3**). R_f ($CHCl_3$–MeOH 49:1) 0.37, light blue spot. Crystals (from EtOAc), Mp 170°, $[\alpha]_D$ +63.5° (*c* = 0.29, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3380, 2982, 2945, 1736 and 1238; ^1H NMR (600 MHz, $CDCl_3$): δ 1.04 (3H, *s*, H-12), 1.19 (3H, *s*, H-13), 1.63 (1H, *dd*, *J* = 12.2, 5.9 Hz, H-10a), 1.89 (3H, *s*, H-15), 2.01 (1H, *dd*, *J* = 12.2, 9.8 Hz, H-10b), 2.07 (3H, *s*, $MeCO_2$), 2.43 (1H, *dd*, *J* = 10.7, 10.7 Hz, H-3a), 2.55 (1H, *ddd*, *J* = 2.9, 2.4, 1.5 Hz, H-8). 2.57 (1H, *dd*, *J* = 10.7, 5.4 Hz, H-3b), 2.60 (1H, *ddd*, *J* = 9.8, 5.9, 1.5 Hz, H-9), 3.18 (1H, *d*, *J* = 2.4 Hz, 14-OH), 3.28 (3H, *s*, 6-OMe), 3.41 (3H, *s*, 7-OMe), 3.55 (1H, *dd*, *J* = 5.4, 2.9 Hz, H-7), 3.97 (1H, *dd*, *J* = 11.7, 5.4 Hz, H-6), 5.15 (1H, *d*, *J* = 11.7 Hz, H-5), 5.26 (1H, *dd*, *J* = 10.7, 5.4 Hz, H-2), 5.89 (1H, *dd*, *J* = 2.4, 2.4 Hz, H-14); ^{13}C NMR (600 MHz, $CDCl_3$): δ 17.40 (*q*, C-15), 21.41 (*q*, $\underline{Me}CO_2$, 24.13 (*q*, C-13), 27.25 (*q*, C-12), 38.67 (*d*, C-9), 39.57 (*s*, C-11), 41.18 (*t*, C-3), 42.52 (*t*, C-10), 55.99 (*q*, 6-OMe), 57.64 (*q*, 7-OMe), 61.64 (*d*, C-8), 73.48 (*d*, C-2), 81.80 (*d*, C-6), 87.07 (*d*, C-7), 98.27 (*s*, C-1), 107.86 (*d*, C-14), 125.24 (*d*, C-5), 136.14 (*s*, C-4), 170.21 (*s*, $\underline{Me}CO_2$); Positive FAB-MS, *m/z*: 355 $[M+H]^+$, 338, 337, 277, 245, 213, 187; HRFAB-MS, *m/z*: $[M+H]^+$ 355.2148 (calcd for $C_{19}H_{31}O_6$: 355.2121).

Another portion (116 mg) of a 42-day-old crude extract was used for the isolation of pestalotiopsol A and B (**6** and **7**). This was applied to silica gel (2 × 14 cm), and the column developed with *n*-hexane–Me_2CO (4:1). The eluent was collected in 1.0 ml frs. On evapn of tubes 105–144 and 156–199, amorphous solids enriched, respectively, in **6** (8.9 mg) and **7** (9.6 mg) were obtained. The material richer in **6** (8.9 mg) was applied to silica gel (1.2 × 9 cm) and the column developed with $CHCl_3$–MeOH (49:1); evapn of the solvent from tubes 76–120 (1.0 ml frs) gave 2.5 mg of pestalotiopsol A (**6**). The same procedure was used for the solid richer in **7** (9.6 mg): on evapn of tubes 75–96, 1.6 mg of pestalotiopsol B (**7**) was obtained.

(4S*,5R*)-(6Z,8E)-4,5-*Dihydroxy*-6-*hydroxymethyl*-6,8-*decadiene* (*pestalotiopsol A*, **6**). R_f (*n*-hexane–Me_2CO 3:1) 0.16, purple–brown spot. Oil. ^1H NMR (600 MHz, $CDCl_3$): δ 0.94 (3H, *t*, *J* = 6.8 Hz, H-1), 1.40 (1H, *m*, H-3a), 1.41 (1H, *m*, H-2a), 1.43, (1H, *m*, H-3b), 1.58 (1H, *m*, H-2b), 1.80 (3H, *d*, *J* = 6.8 Hz, H-10), 3.82 (1H, *m*, H-4), 4.15 (1H, *d*, *J* = 11.7 Hz, H-11a), 4.30 (1H, *d*, *J* = 11.7 Hz, H-11b), 4.69 (1H, *d*, *J* = 4.9 Hz, H-5), 5.83 (1H, *dq*, *J* = 14.5, 6.8 Hz, H-9), 6.22 (1H, *d*, *J* = 11.2 Hz, H-7), 6.29 (1H, *dd*, *J* = 14.5 Hz, 11.2, H-8); ^{13}C NMR (600 MHz, $CDCl_3$): δ 14.1 (*q*, C-1), 18.5 (*q*, C-10), 19.1 (*t*, C-2), 34.9 (*t*, C-3), 65.1 (*t*, C-11), 72.3 (*d*, C-5), 73.8 (*d*, C-4), 126.0 (*d*, C-8), 133.5 (*d*, C-7), 133.8 (*d*, C-9), 134.9 (*s*, C-6); Positive FAB-MS, *m/z*: 201 $[M+H]^+$, 185, 183, 165, 111; Negative FAB-MS, *m/z*: 199 $[M-1]^-$, 183, 151, 127, 109; HRFAB-MS, *m/z*: $[M-H]^-$ 199.1329 (calcd for $C_{11}H_{19}O_3$: 199.1334).

(4S*,5R*)-(2Z,6Z,8E)-4,5-*Dihydroxy*-6-*hydroxymethyl*-2,6,8-*decatriene* (*pestalotiopsol B* **7**). R_f (*n*-hexane–Me_2CO 3:1) 0.12, grey–black spot. Oil. ^1H NMR (600 MHz, $CDCl_3$): δ 1.73 (3H, *dd*, *J* = 6.6, 1.5 Hz, H-1), 1.79 (3H, *d*, *J* = 6.6 Hz, H-10), 4.22 (1H, *d*, *J* = 12.1 Hz, H-11a), 4.24 (1H, *d*, *J* = 12.1 Hz, H-11b), 4.64 (1H, *dd*, *J* = 8.8, 5.9 Hz, H-4), 4.73 (1H, *d*, *J* = 5.9 Hz, H-5), 5.45 (1H, *br. dd*, *J* = 11.0, 8.8 Hz, H-3), 5.75 (1H, *dq*, *J* = 11.0, 6.6 Hz, H-2), 5.82 (1H, *dq*, *J* = 14.7, 6.6 Hz, H-9), 6.20 (1H, *d*, *J* = 11.0 Hz, H-7), 6.27 (1H, *dd*, *J* = 14.7, 11.0 Hz, H-8); ^{13}C NMR (600 MHz, $CDCl_3$): δ 13.7 (*q*, C-1), 18.5 (*q*, C-10), 65.3 (*t*, C-11), 69.6 (*d*, C-4), 72.4 (*d*, C-5), 126.2 (*d*, C-8), 129.0 (*d*, C-3), 129.5 (*d*, C-2), 133.0 (*d*, C-7), 133.6 (*d*, C-9), 134.0 (*s*, C-6); Positive FAB-MS, *m/z*: 197, 181, 179, 163, 151, 145, 135, 111; Negative FAB-MS *m/z*: 197 $[M-H]^-$, 181, 167, 151, 125, 109, 89; HRFAB-MS, *m/z*: $[M-H]^-$ 197.1176 (calcd for $C_{11}H_{17}O_3$: 197.1178).

The extract of a 46-day-old culture (628 mg from 4 ls) from JCM 9686 was applied to silica gel (2 × 13 cm) and roughly fractionated. The second fr. (95 mg) was further purified by flash chromatography, eluted with EtOAc–hexane (2:3) in 150 frs of 2 ml. Evapn of tubes 70–99 yielded 15 mg of the compound, which, however, quickly decomposed. Part of this (3 mg) was further purified by HPLC (column: CAPCELL PAK C18, solvent system: MeOH–H_2O 3:2) to yield a sample (0.9 mg) of the titled compound (**8**).

(4S*,5R*)-(2Z,6Z,8E)-4,5-*Dihydroxy*-6-*oxomethyl*-2,6,8-*decatriene* (**8**). R_f (*n*-hexane–Me_2CO 3:1) 0.22, red–violet spot. Oil. ^1H NMR (600 MHz, $CDCl_3$): δ 1.67 (3H, *dd*, *J* = 6.8, 1.5 Hz, H-1), 1.97 (3H, *dd*, *J* = 6.8, 1.5 Hz, H-10), 4.60 (1H, *m*, H-4), 4.67 (1H, *m*, H-5), 5.42 (1H, *ddq*, *J* = 11.2, 9.3, 1.5 Hz, H-3), 5.69 (1H, *dq*, *J* = 11.2, 6.8 Hz, H-2), 6.39 (1H, *dq*, *J* = 14.6, 6.8 Hz, H-9), 6.64 (1H, *dd*, *J* = 14.6, 11.7 Hz, H-8), 6.93 (1H, *d*, *J* = 11.7 Hz, H-7), 9.37 (3H, *d*, *J* = 1.5 Hz, H-11); Positive FAB-MS, *m/z*: 197

$[M+H]^+$, 179, 161, 133; HRFAB-MS, *m/z*: $[M-H]^-$ 195.1021 (calcd. for $C_{11}H_{15}O_3$: 195.1021).

Synthesis of the 6,7-isopropyliden derivative of pestalotiopsol B (**13**). 1.8 mg of pestalotiopsol B (**7**) was dissolved in 1 ml of Me_2CO–MeOH (9:1) and PTSA (0.2 mg) was added. The soln was stirred for 3 h and then poured in an aq. $NaHCO_3$ and extracted with EtOAc (3 × 3 ml). The organic layers were collected, dried over Na_2SO_4 and the solvent evapd, yielding 1.5 mg of compound **13**.

Pestalotiopsol B acetonide (**13**). Oil. ^1H NMR (600 MHz, $CDCl_3$): δ 1.44 (3H, *s*, H-14), 1.57 (3H, *s*, H-13), 1.63 (3H, *dd*, J = 7.3, 1.5 Hz, H-1), 1.78 (3H, *d*, J = 6.4 Hz, H-10), 3.95 (1H, *d*, J = 12.2 Hz, H-11a), 4.19 (1H, *d*, J = 12.2 Hz, H-11b), 5.18 (1H, *ddd*, J = 8.0, 7.0, 1.0 Hz, H-4), 5.30 (1H, *br. d*, J = 7.0 Hz, H-5), 5.30 (1H, *ddq*, J = 10.8, 8.0, 1.5 Hz, H-3), 5.64 (1H, *ddq*, J = 10.8, 7.3, 1.0 Hz, H-2), 5.72 (1H, *dq*, J = 14.2, 6.4 Hz, H-9), 6.13 (1H, *m*, H-7), 6.13 (1H, *m*, H-8); ^{13}C NMR (600 MHz, $CDCl_3$) δ 13.3 (*q*, C-1), 18.3 (*q*, C-10), 24.4 (*q*, C-14), 27.0 (*q*, C-13), 65.9 (*t*, C-11), 74.4 (*d*, C-4), 77.4 (*d*, C-5), 108.5 (*s*, C-12), 126.2 (*d*, C-8), 126.4 (*d*, C-3), 129.1 (*d*, C-2), 130.0 (*d*, C-7), 132.4 (*d*, C-9), 133.2 (*s*, C-6).

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