Journal of Natural Products
Vol. 52, No. 5, pp. 941-947, Sep-Oct 1989

ANTIMICROBIAL TETRAPRENYLPHENOLS FROM *SUILLUS GRANULATUS*

CORRADO TRINGALI,* MARIO PIATTELLI,

Dipartimento di Scienze Chimiche, Universitá di Catania,

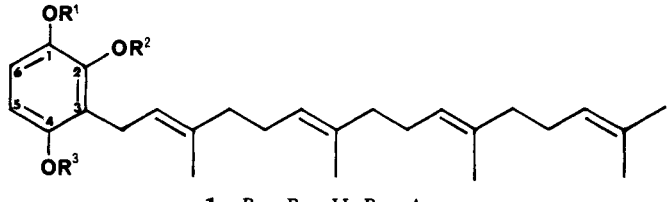
CORRADA GERACI, and GIOVANNI NICOLOSI

Istituto del CNR per lo Studio delle Sostanze Naturali, viale A. Doria 6, 95125 Catania, Italy

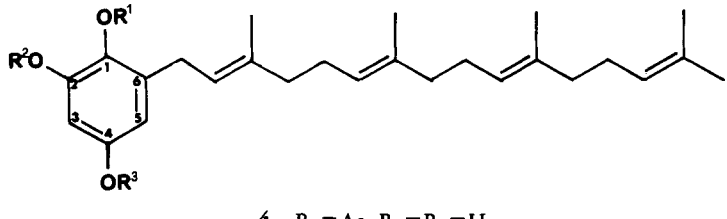
ABSTRACT.—From fruit bodies of the basidiomycete *Suillus granulatus* the tetraprenylphenols **2–6** were isolated. Their structures were elucidated by means of chemical and spectroscopic methods. Compounds **4** and **5** possess antimicrobial properties.

In the course of our research on the isolation and structure determination of bioactive natural compounds, we have found that the CH_2Cl_2 extract of the fruit bodies of *Suillus granulatus* (L. ex Fr.) O. Kuntze (Boletaceae) is active against both *Micrococcus luteus* and KB cells. Biological tests have shown that cytotoxic and antimicrobial activity are associated with different fractions obtained from the crude extract by chromatography on Sephadex LH-20. As reported previously (1), the main cytotoxic principle is suillin [**1**] (2), which also has a significant antitumor activity against leukemia P388 cells. The present paper describes the characterization of five phenols **2–6** closely related to suillin; compounds **4** and **5** possess antimicrobial activity against Gram-positive and/or Gram-negative microorganisms (Table 1).

Chromatography on a Sephadex LH-20 column of the CH_2Cl_2 extract from the fruit bodies of *S. granulatus* gave biologically inactive fractions, containing essentially glycerides and sterols, and fractions with antimicrobial activity. These were further separated or purified by careful chromatography on acetylated polyamide or Diol Si gel. In addition to the known suillin [**1**], four polyprenyl phenols **2–5** and a chromene derivative **6** have been obtained.



1 $R_1=R_2=H$, $R_3=Ac$
2 $R_1=R_3=H$, $R_2=Ac$
3 $R_1=R_2=H$, $R_3=Me$
7 $R_1=R_2=R_3=Ac$
8 $R_1=R_2=R_3=Me$



4 $R_1=Ac$, $R_2=R_3=H$
5 $R_1=Me$, $R_2=R_3=H$
10 $R_1=R_2=R_3=Ac$

TABLE 1. Antibacterial Spectra of Compounds **2–6**.[a]

Bacterial strains[b]	MIC (ug/ml)				
	2	**3**	**4**	**5**	**6**
Aeromonas hydrophyla	>100	>100	12.50	0.78	>100
Escherichia coli	50	>100	6.25	1.56	>100
Micrococcus luteus	50	100	50	6.25	>100
Streptococcus faecalis	100	>100	>100	>100	>100

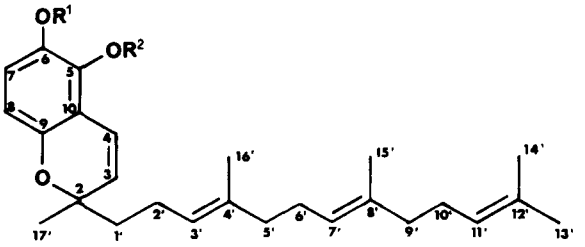
[a]MIC of compounds **2–6** against *Hafnia alvei*, *Proteus mirabilis*, *Bacillus subtilis*, and *Staphylococcus aureus* is >100 ug/ml.

[b]Strain designation and location of culture deposition are given in the Experimental section.

Compound **2**, $C_{28}H_{40}O_4$ (hrms), possesses spectroscopic features closely resembling those of **1**. Evidence for an all-trans geranylgeranyl side chain was obtained from comparison of ^{1}H- and ^{13}C-nmr data (see Experimental) with those of reported compounds (2–4). The ^{1}H-nmr spectrum of **2** also contains an AB system assignable to two *ortho*-coupled aromatic protons (δ 6.50 and 6.68, $J = 9$ Hz) and resonances at δ 5.39 and 5.62 (D_2O-exchangeable) for two phenolic hydroxyls. An aromatic acetate group was evident from nmr spectra [^{1}H nmr δ 2.28 (3H, s); ^{13}C nmr ppm 170.1 s and 20.8 q] and mass fragmentation (loss of 42 amu from the molecular ion). Acetylation of **2** furnished peracetate **7**, identical (ms, ir, uv, nmr) to suillin peracetate (1), thus confirming the substitution pattern of the aromatic ring. Finally, the acetoxy group in **2** was located at C-2 based on chemical shift analysis of the aromatic resonances in the ^{13}C-nmr spectrum (ppm 112.8 d, 113.9 d, 120.0 s, 142.2 s, and 142.8 s) and the observation that **2** is easily oxidized to a *p*-quinone by treatment with Ag_2O (λ max = 242 nm) (5).

Compound **3**, $C_{27}H_{40}O_3$ (hrms), also possesses spectral properties (see Experimental) very similar to those of **1**, apart from those expected for the replacement of the acetoxyl with a methoxyl (^{1}H nmr δ 3.75 s; ^{13}C nmr ppm 56.2). Upon treatment with Me_2SO_4/NaOH, **3** gives a trimethoxyderivative **8**, whose spectral properties (uv, ir, ms, nmr) are identical to those of the product obtained from suillin in the same conditions. ^{13}C chemical shift analysis of the aromatic resonances of compound **3** (ppm 102.9 d, 112.0 d, 116.2 s, 138.5 s, 143.0 s, and 151.3 s) strongly indicated that the methoxyl is located at C-4, and therefore the fungal metabolite was formulated as 3-geranylgeranyl-1,2-dihydroxy-4-methoxybenzene [**3**]. Definite confirmation was obtained from the reaction of **3** with phenylboronic acid, which afforded a phenylboronate **9** (*m/z* 498) only compatible with the presence in the molecule of **3** of two ortho phenolic hydroxyls.

The third compound isolated from *S. granulatus*, **4**, an isomer of **2**, was also iden-

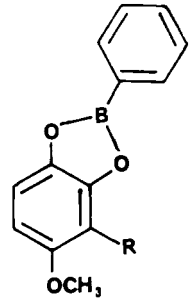


6 $R_1=R_2=H$
11 $R_1=R_2=Ac$

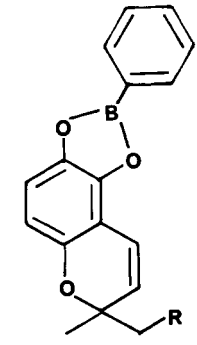
tified from spectral evidence as an all-trans geranylgeranylphenol monoacetate (see Experimental). Upon acetylation it gave a triacetate, in the ^{1}H nmr of which the aromatic protons are seen as meta-coupled ($J = 3$ Hz) (6) sharp doublets (in the original compound the coupling constant was unmeasurable), while in the ^{13}C nmr the aromatic carbons give six distinct resonances, implying lack of symmetry for the substitution pattern. Therefore, the peracetate was formulated as **10**. At this point the acetoxyl group in **4** was positioned at C-1 on the basis of ^{13}C chemical shift analysis of the aromatic resonances, whose values (ppm 102.5 d, 108.4 d, 131.0 s, 137.2 s, 148.0 s, 154.0 s) are in good agreement with those calculated (4). This inference was confirmed by the fact that the metabolite does not reduce Tollens reagent.

Compound **5**, $C_{27}H_{40}O_3$ (hrms), is a dihydroxybenzene (resonances at δ 4.60 and 5.60, D_2O-exchangeable) bearing a methoxyl (^{1}H nmr δ 3.73 s; ^{13}C nmr ppm 61.4 q) and the usual all-trans geranylgeranyl side chain. The number of the possible structures was reduced from seven to two (6-geranylgeranyl-3,5-dihydroxy-1-methoxybenzene and 6-geranylgeranyl-2,4-dihydroxy-1-methoxybenzene) on the basis of the following considerations: (a) two meta-coupled ($J = 3$ Hz) aromatic protons are seen in the ^{1}H-nmr spectrum of **5**; (b) compound **5** is insensitive to oxidants (Ag_2O, Tollens reagent) and therefore the two hydroxyls must bear a meta relationship to each other; (c) six resonances for aromatic carbons are present in the ^{13}C nmr spectrum of **5**, a fact that allows us to eliminate symmetrical structures. The final choice between the two structures compatible with the above data was made on the basis of chemical shift analysis for the aromatic carbons (ppm 100.7 d, 107.5 d, 131.2 s, 136.7 s, 149.4 s, 152.3 s) and biogenetic considerations; accordingly, the new metabolite was formulated as **5**.

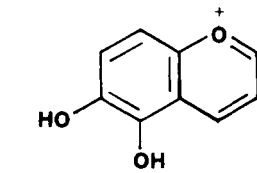
Compound **6** was obtained as an optically active oil, $[\alpha]^{25}D$ +24.8°, molecular formula $C_{26}H_{36}O_3$ (hrms). Upon treatment with Ac_2O/pyridine, **6** furnished a diacetate **11** (loss of two ketene molecules from the molecular ion); therefore, two oxygen atoms in the molecular formula were accounted for by two phenolic hydroxyls. On the basis of a base peak at *m/z* 177, attributable to the dihydroxymethylchromene ion **A**, and uv absorption (λ max 276, 284 and 330 nm) (7), a chromene ring system was assigned to the structure. This was confirmed by the appropriate resonances in the ^{1}H-nmr (δ 5.60 and 6.24, each 1H, d, $J = 10$ Hz, H-3 and H-4; 1.37, 3H, s, 2-Me) and ^{13}C-nmr spectra (ppm 77.8, s, C-2; 114.9, d, C-4; 129.3, d, C-3). The ^{1}H-nmr spectrum of **6** also displays signals for an all-trans alkyl side chain and an AB system arising from ortho-coupled aromatic protons (δ 6.58 and 6.66, d, $J = 9$ Hz). All the above data indicated for the new metabolite a structure related to suillin, in which the geranylgeranyl side chain is cyclized to a chromene nucleus. Moreover, from the observation that **6** gives by reaction with phenylboronic acid a phenylboronate **12**, *m/z* 482, an ortho relationship between the hydroxyls was deduced. All the above data could be accommodated by



9 R=geranylgeranyl



12 R=farnesyl



m/z 177

A

structure **6**, which was definitely confirmed by 2D nmr analysis of diacetate **11**, stable to aerial oxidation and therefore preferred to **6** in overnight experiments. In particular, one-bond and long-range heteronuclear correlations (H,C-CORR, and H,C-COLOC) allowed unambiguous assignment of the ^{13}C resonances of the chromene ring system and in addition indicated a long-range heteronuclear coupling between C-5 and H-4, thus excluding the alternative structure in which the dihydropyran ring is reversed with respect to the aromatic ring. Finally, it must be stated that the optical activity of **6** excludes an artifactual origin for this metabolite.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were obtained at 70 eV on a Kratos MS-50 spectrometer. Ir and uv spectra were recorded on Perkin-Elmer Model 684 and Model 330 spectrophotometers, respectively. The nmr spectra were recorded at 250 (^1H) and 62.9 (^{13}C) MHz, respectively, using a Bruker AC-250 instrument equipped with a carbon-13-proton dual probehead, an Aspect 3000 data system, and a 24 MByte Disk cartridge. For both ^1H- and ^{13}C-nmr spectra, $CDCl_3$ was used as solvent and TMS as internal standard; chemical shifts are measured in δ (ppm). Two-dimensional carbon-proton shift correlations were performed using the commercially available microprograms XHCORR by polarization transfer via J_{CH}. Relaxation delays were 1.2 sec; polarization transfer delays were adapted to the expected averages of one-bond couplings (J_{CH} = 150 Hz) for CH correlation and two- or three-bond couplings ($^{2,3}J_{CH}$ = 7 Hz) for long-range CH correlations. Data matrices (1024 × 256) were applied for the ^{13}C and ^1H chemical shift domains. Optical rotation was determined with a Perkin-Elmer 141 polarimeter. Tlc was carried out using glass-backed, pre-coated Si gel F254 plates (Merck). Spot detection was obtained by spraying with 10% solution of $Ce(SO_4)_2$ in 2 N H_2SO_4, fast red salt B in H_2O, or by uv light (254 nm). Tollens reagent and Ag_2O were prepared and used according to the literature (8,9).

^{13}C CHEMICAL SHIFT ANALYSIS.—Observed ^{13}C chemical shifts of aromatic carbons were compared with the values calculated for different ring substitutions, using empirical additive substituent increments reported for substituted benzenes (4). A base value of 128.5 ppm was assumed for benzene carbons.

ANTIBACTERIAL ACTIVITY.—In the course of fractionation, antibacterial activity was followed by the paper disc-agar diffusion assay using *Micrococcus luteus* as test organism. The MICs for pure metabolites **2–6** were determined by the conventional serial broth dilution assay (10) against eight bacterial strains (*Aeromonas hydrophyla, Bacillus subtilis, Escherichia coli, Hafnia alvei, Micrococcus luteus, Proteus mirabilis, Staphylococcus aureus,* and *Streptococcus faecalis*). For testing compounds **4** and **5**, strains grown in Mueller-Hinton medium (Difco) were used, while for compounds **2**, **3**, and **6** the strains were grown in thioglycolate medium (Difco) in order to minimize oxidation during incubation. Incubation was at 37°. Antibacterial activities are reported in Table 1. All bacteria used, except for *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *M. luteus* (ATCC 4698), were from the collection of the Institute of Microbiology, University of Catania, Italy. They were obtained as clinical isolates from patients diagnosed as having bacterial infections and typed.

EXTRACTION AND SEPARATION.—Fruit bodies of *S. granulatus* were collected on the Etna slopes, freeze-dried, and ground. A voucher specimen is lodged in the Herbarium of the Institute of Botany, University of Catania, Italy. Powdered material (200 g) was extracted 3 times with CH_2Cl_2 under continuous stirring. The extract was taken to dryness to give 18 g of an oily residue. Chromatography on Sephadex LH 20-100 (lipophylic 25–100 μm, Sigma) using *n*-hexane–CH_2Cl_2 (1:4) followed by Me_2CO-CH_2Cl_2 (2:3 and 4:1) as eluents gave fractions (20 ml) which were collected, and those exhibiting similar tlc profiles were pooled. Compounds **1** and **3** were eluted in fractions 75–100; a mixture of compounds **2**, **5**, and **6** was obtained in fractions 111–124; compound **4** was recovered in fractions 125–150. Fractions 75–110 were subjected to preparative liquid chromatography on Polyamide CC 6-Ac (0.05–0.16 mm, Macherey-Nagel) using a gradient of $CHCl_3$ in hexane to give **3** (0.1 g) and **1** (1.5 g). Fractions 111–124 (0.8 g) were rechromatographed on Polyamide CC 6-Ac (gradient of $CHCl_3$ in hexane as the eluent) to give compound **6** (0.12 g) and mixture of **2** and **5**, which was subjected to careful chromatography on Polyamide CC 6-Ac ($CHCl_3$ as the eluent) to afford pure **2** (10 mg) and **5** (20 mg). Fractions 125–150 were subjected to chromatography on LiChroprep DIOL [25–40 μm (Merck), MeOH-$CHCl_3$ (0.5:99.5)] to give compound **4** (0.11 g).

2-ACETOXY-3-GERANYLGERANYL-1,4-DIHYDROXYBENZENE [**2**].—Uv (EtOH) λ max (ε) 280 nm (2360); ir ($CHCl_3$) ν max 3600, 3550, 3420, 3020, 2930, 2860, 1760, 1710, 1220; ^1H nmr δ 1.59 (9H, s), 1.68 (3H, s), 1.79 (3H, s), 2.02 (12H, m), 2.28 (3H, s), 3.26 (2H, d, J = 7 Hz), 5.09 (3H, m), 5.23 (1H, t, J = 7 Hz), 5.39 (-OH, bs), 5.62 (-OH, bs), 6.50, 6.68 (AB system, J = 9 Hz); ^{13}C nmr ppm

20.8 (q, COMe), 112.8 (d, C-5), 113.9 (d, C-6), 120.0 (s, C-3), 142.0 (s, C-2), 142.2 (s, C-1), 142.8 (s, C-4), 170.1 (s, COMe), 16.0, 16.1, 16.2, 17.7, 25.7 (q, 5× =$\dot{C}CH_3$), 24.0 (s, ArCH_2), 26.3, 26.6, 26.7 (t, 3× =$CHCH_2$), 39.7 (t, 3× =$\dot{C}CH_2$), 120.7, 123.6, 124.1, 124.4 (d, 4× =$\dot{C}H$), 131.2, 135.0, 135.8, 139.3 (s, 4× =$\dot{C}Me$); hrms $[M]^+$ 440.2918 (4%), $C_{28}H_{40}O_4$ requires 440.2926; ms *m/z* 440, $[M-CH_2CO]^+$ 398 (11%), 262 ($C_{16}H_{22}O_3$) (10%), 259 ($C_{19}H_{31}$) (8%), 194 ($C_{11}H_{14}O_3$) (21%), 181 ($C_9H_9O_4$) (18%), 139 ($C_7H_7O_3$) (60%), 69 (C_5H_9) (100%).

3-GERANYLGERANYL-1,2-DIHYDROXY-4-METHOXYBENZENE [**3**].—Uv (EtOH) λ max (ε) 285 nm (2200); ir ($CHCl_3$) ν max 3600, 3400, 3020, 2930, 2860, 1760, 1470 cm^{-1}; ^1H nmr δ 1.59 (9H, s), 1.67 (3H, s), 1.81 (3H, s), 2.03 (12H, m), 3.43 (2H, d, *J* = 7 Hz), 3.75 (3H, s), 5.07 (3H, m), 5.09 (1H, t, *J* = 7 Hz), 6.35, 6.69 (AB system, *J* = 9 Hz); ^{13}C nmr ppm 56.2 (q, -OMe), 102.9 (d, C-5), 112.0 (d, C-6), 116.2 (s, C-3), 138.5 (s, C-1), 143.0 (s, C-2), 151.3 (s, C-4), 16.0, 16.1, 16.2, 17.7, 25.7 (q, 5× =$\dot{C}CH_3$), 24.6 (t, ArCH_2), 26.4, 26.6, 26.8 (t, 3× =$CHCH_2$), 39.7 (t, 3× =$\dot{C}CH_2$), 121.8, 123.7, 124.2, 124.4 (d, 4× =$\dot{C}H$), 131.0, 135.0, 135.6, 138.5 (s, 4× =$\dot{C}Me$); hrms $[M]^+$ 412.2968 (15%), $C_{27}H_{40}O_3$ requires 412.2977; ms *m/z* 412, $[M-C_{10}H_{16}]^+$ 276 (2%), 259 ($C_{19}H_{31}$) (3%), 191 (30%), 153 ($C_8H_9O_3$) (95%), 69 (C_5H_9) (100%).

1-ACETOXY-6-GERANYLGERANYL-2,4-DIHYDROXYBENZENE [**4**].—Uv (EtOH) λ max (ε) 279 nm (2190); ir ($CHCl_3$) ν max 3600, 3550, 3350, 3020, 2930, 2860, 1760, 1720, 1610, 1460, 1230 cm^{-1}; ^1H nmr δ 1.60 (9H, s), 1.67 (6H, s), 2.03 (12H, m), 2.33 (3H, s), 3.15 (2H, d, *J* = 9 Hz), 5.11 (3H, bt), 5.19 (1H, t, *J* = 7 Hz), 6.25 (1H, bs), 6.28 (1H, bs); ^{13}C nmr ppm 20.6 (q, COMe), 102.5 (d, C-3), 108.4 (d, C-5), 131.0 (s, C-6), 137.2 (s, C-1), 148.0 (s, C-2), 154.0 (s, C-4), 169.8 (COMe), 16.0 (q, 2× =$\dot{C}CH_3$), 16.1, 17.7, 25.6 (q, 3× =$\dot{C}CH_3$), 22.7 (t, ArCH_2), 26.6, 26.7, 28.7 (t, 3× =$CHCH_2$), 39.7 (t, 3× =$\dot{C}CH_2$), 121.1, 124.0, 124.3, 124.4 (d, 4× =$\dot{C}H$), 131.3, 135.0, 135.2, 135.7 (s, 4× =$\dot{C}Me$); hrms $[M]^+$ 440.2917 (1.5%), $C_{28}H_{40}O_4$ requires 440.2926; ms *m/z* 440, $[M-CH_2CO]^+$ 398 (9%), 265 (12%), 177 (27%), 139 ($C_7H_7O_3$) (29%), 95 (24%), 81 (C_6H_9) (49%), 69 (C_5H_9) (100%).

6-GERANYLGERANYL-2,4-DIHYDROXY-1-METHOXYBENZENE [**5**].—Uv (EtOH) λ max (ε) 283 nm (2120); ir ($CHCl_3$) ν max 3600, 3550, 3350, 3020, 2930, 2860, 1760, 1620, 1500, 1470 cm^{-1}; ^1H nmr δ 1.59 (9H, s), 1.68 (3H, s), 1.71 (3H, s), 2.03 (12H, m), 3.31 (2H, d, *J* = 7 Hz), 3.73 (3H, s), 4.60 (-OH, bs), 5.09 (3H, m), 5.26 (1H, t, *J* = 7 Hz), 5.60 (-OH, bs), 6.17 (1H, d, *J* = 3 Hz), 6.31 (1H, d, *J* = 3 Hz); ^{13}C nmr ppm 61.4 (q, -OCH_3), 100.7 (d, C-5), 107.5 (d, C-3), 131.1 (s, C-6), 136.7 (s, C-1), 149.4 (s, C-2), 152.3 (s, C-4), 16.0, 16.1, 16.2, 17.7, 25.7 (q, 5× =$\dot{C}CH_3$), 26.5, 26.6, 26.7, 27.8 (t, 4× =$CHCH_2$), 39.7 (t, 3× =$\dot{C}CH_2$), 122.0, 124.0, 124.2, 124.4 (d, 4× =$\dot{C}H$), 131.3, 135.0, 135.2, 135.4 (s, 4× =$\dot{C}CH_3$); hrms $[M]^+$ 412.2965 (15%), $C_{27}H_{40}O_3$ requires 412.2977; ms *m/z* 412, $[M-C_{10}H_{16}]^+$ 276 (8%), 259 ($C_{19}H_{31}$) (8%), 208 ($C_{12}H_{16}O_3$) (20%), 153 ($C_8H_9O_3$) (34%), 135 ($C_{10}H_{15}$) (18%), 121 (C_9H_{13}) (16%), 69 (C_5H_9) (100%).

5,6-DIHYDROXY-2-METHYL-2[3′,7′*E*)-4′,8′,12′-TRIMETHYLTRIDECA-3′,7′,11′-TRIEN]-2(H)-CHROMENE [**6**].—Uv (EtOH) λ max (ε) 276 (8490), 284 sh (6790), 330 (1620) nm; ir ($CHCl_3$) ν max 3600, 3550, 3300, 3020, 2930, 2860, 1650, 1590, 1480, 1290, 940 cm^{-1}; ^1H nmr δ 1.37 (3H, s), 1.58 (6H, s), 1.60 (3H, s), 1.68 (3H, s), 1.70 (2H, m), 2.00, 2.05 (10H, m), 5.10 (3H, t, *J* = 3 Hz), 5.60 (1H, d, *J* = 10 Hz), 6.24 (1H, d, *J* = 10 Hz), 6.58, 6.66 (2H, AB system, *J* = 9 Hz); ^{13}C nmr ppm 77.8 (s, C-2), 107.1 (d, C-8), 110.0 (s, C-10), 114.9 (d, C-4), 117.0 (d, C-7), 129.3 (d, C-3), 136.3 (s, C-6), 140.3 (s, C-5), 147.5 (s, C-9), 16.0 (q, 2× =$\dot{C}CH_3$), 17.7, 25.7 (q, 2× =$\dot{C}CH_3$), 22.6, 26.5, 26.7 (t, 3× =$CHCH_2$), 39.6, 40.6 (t, 3× =$\dot{C}CH_2$), 123.9, 124.2, 124.4 (d, 3× =$\dot{C}H$), 131.2, 134.9, 135.3 (s, 3× =$\dot{C}Me$); hrms $[M]^+$ 396.2656 (30%), $C_{26}H_{36}O_3$ requires 396.2664; ms *m/z* 396, 243 (12%), 217 (10%), 178 (38%), 177 ($C_{10}H_9O_3$) (100%), 176 (13%), 139 ($C_7H_7O_3$) (12%), 81 (C_6H_9) (16%), 69 (C_5H_9) (48%).

ACETYLATION OF COMPOUNDS **2**, **4**, AND **6**.—Compounds **2**, **4**, and **6** (20 mg each) were dissolved separately in pyridine (0.5 ml) and treated with Ac_2O (1.0 ml), and the mixtures were kept at room temperature for 5 h. Conventional workup gave the pure acetates.

Compound **7**.—After purification by flash chromatography [Si gel, Et_2O-hexane (3:7) as the eluent] compound **7** had physical properties identical to those of the product obtained from **1** under the same experimental conditions (1).

Compound **10**.—After flash chromatography (Si gel, CH_2Cl_2 as the eluent) compound **10** had the following properties: ^{1}H nmr δ 1.60 (9H, s), 1.66 (3H, s), 1.68 (3H, s), 2.03 (12H, m), 2.27 (6H, s), 2.29 (3H, s), 3.24 (2H, d, J = 7 Hz), 5.11 (3H, m), 5.21 (1H, t, J = 7 Hz), 6.85 (1H, d, J = 3 Hz), 6.91 (1H, d, J = 3 Hz); ^{13}C nmr ppm 20.3, 20.7, 21.1 (q, 3 × $COCH_3$), 114.7 (d, C-5), 119.7 (d, C-3), 138.1 (2C, s, C-1 and C-6), 142.4 (s, C-4), 147.8 (s, C-2), 16.0 (q, 2 × =$\dot{C}CH_3$), 16.2, 17.7, 25.7, (q, 3 × =$\dot{C}CH_3$), 24.7 (t, $ArCH_2$), 26.5, 26.6, 26.7 (t, 3 × =$CHCH_2$), 39.7 (t, 3 × =$\dot{C}CH_2$), 120.1, 123.9, 124.2, 124.3 (d, 4 × =*C*H), 131.3, 134.9, 135.3, 136.1 (s, 4 × =$\dot{C}$Me); cims (isobutane) *m/z* $[MH]^+$ 525 (20%), $[MH - CH_2CO]^+$ 483 (2%), $[MH - 2\,CH_2CO]^+$ 441 (3%), $[MH - 3\,CH_2CO]^+$ 399 (2%), 303 (30%), 261 (12%), 205 (14%), 177 (13%), 137 (44%), 81 (66%), 69 (100%).

Compound **11**.—After purification by flash chromatography [Si gel, Et_2O-hexane (1:4) as the eluent] compound **11** had the following properties: ^{1}H nmr δ 1.38 (3H, s), 1.58 (3H, s), 1.59 (6H, s), 1.68 (3H, s), 1.70 (2H, m), 2.00–2.05 (10H, m), 2.25 (3H, s), 2.31 (3H, s), 5.10 (3H, t, J = 7 Hz), 5.63 (1H, d, J = 10 Hz), 6.31 (1H, d, J = 10 Hz), 6.66, 6.89 (2H, AB system, J = 9 Hz); ^{13}C nmr ppm 20.3, 20.6 (q, 2 × $COMe$), 78.7 (s, C-2), 113.9 (d, C-8), 115.3 (s, C-10), 116.4 (d, C-4), 122.3 (d, C-7), 131.0 (d, C-3), 135.5 (s, C-6), 137.6 (s, C-5), 151.2 (s, C-9), 167.9, 168.7 (s, 2 × *C*OMe), 16.0 (q, 2 × =$\dot{C}CH_3$), 17.7, 25.7 (q, 2 × =CCH_3), 26.7, 26.5, 22.6 (t, 3 × =$\dot{C}HCH_2$), 39.6, 39.7, 41.1 (t, 3 × =$\dot{C}CH_2$), 123.7, 124.1, 124.4 (d, 3 × =*C*H), 131.2, 134.9, 135.5 (s, 3 × =$\dot{C}$Me); hrms $[M]^+$ 480.2869 (6%), $C_{30}H_{40}O_5$ requires 480.2876; ms *m/z* 480, $[M - CH_2CO]^+$ 438 (19%), $[M - 2\,CH_2CO]^+$ 396 (8%), 261 ($C_{14}H_{13}O_5$) (99%), 219 ($C_{12}H_{11}O_4$) (100%), 177 ($C_{10}H_9O_3$) (95%), 139 ($C_7H_7O_3$) (11%), 69 (C_5H_9) (83%).

METHYLATION OF COMPOUNDS **1** AND **3**.—Me_2SO_4 (0.1 ml) and 2 N NaOH (0.5 ml) were added to a solution of **1** (20 mg) in MeOH (0.5 ml), and the mixture was kept overnight at room temperature. The reaction mixture was extracted with Et_2O, and the organic layer was dried (Na_2SO_4) and taken to dryness. The residue was subjected to flash chromatography [Si gel, Et_2O-hexane (1:4) as the eluent] to give **8** (11 mg): ^{1}H nmr δ 1.57 (9H, s), 1.68 (3H, s), 1.77 (3H, s), 2.00 (12H, m), 3.37 (2H, d, J = 7 Hz), 3.77 (3H, s), 3.80 (3H, s), 3.81 (3H, s), 5.09 (3H, m), 5.19 (1H, t, J = 7 Hz), 6.57, 6.71 (2H, AB system, J = 9 Hz); hrms $[M]^+$ 440.3281 (10%), $C_{29}H_{44}O_3$ requires 440.3290; ms *m/z* 440, 259 ($C_{19}H_{31}$) (8%), 235 (55%), 221 (5%), 204 (36%), 181 ($C_{10}H_{13}O_3$) (100%), 179 (8%), 166 ($C_9H_{10}O_3$) (24%), 151 ($C_8H_7O_3$) (6%), 121 (17%), 109 (9%), 95 (8%), 81 (C_6H_9) (18%), 69 (C_5H_9) (40%). In the same experimental conditions, **3** gave a compound indistiguishable (tlc, ^{1}H nmr, ms) from **8**.

REACTION OF COMPOUNDS **3** AND **6** WITH PHENYLBORONIC ACID.—A solution of **3** (5 mg) and phenylboronic acid (3 mg) in Me_2CO (0.5 ml) was refluxed for 4 h. After removal of the solvent, the residue was examined by ms without any further purification. A parent ion at *m/z* 498 (5%) indicated the formation of a phenylboronate **9**; major fragments were observed at *m/z* 440 (15%), 312 (40%), 293 (45%), 262 (84%), 239 (100%), 161 (32%), 149 (22%), 135 (25%), 121 (40%), 109 (45%), 95 (47%), 81 (78%), 89 (90%). When **6** (5 mg) was reacted with phenylboronic acid as above, mass spectral examination of the crude product gave evidence of a parent ion at *m/z* 482 (22%) indicating the formation of a phenylboronate also in this case; major ions were observed at *m/z* 345 (8%), 303 (7%), 263 (100%), 225 (9%), 81 (9%), 69 (20%).

ACKNOWLEDGMENTS

Thanks are due to Ministero della Pubblica Istruzione (Roma) for financial support, and to the Associazione Micologica Bresadola (Catania) for classification of the mushroom. Thanks are also due to the Ispettorato Provinciale Ripartimentale delle Foreste (Catania), for enabling us to collect plant material in State demesne. The authors extend their thanks to Mrs. C. Rocco for skillful technical assistance.

LITERATURE CITED

1. C. Tringali, C. Geraci, G. Nicolosi, J.F. Verbist, and C. Roussakis, *J. Nat. Prod.*, in press.
2. E. Jagers, V. Pasupathy, A. Hovenbitzer, and W. Steglich, *Z. Naturforsch.*, **41b**, 645 (1986).
3. E. Breitmaier, G. Haas, and W. Voelter, "Atlas of Carbon-13 NMR Data," Heyden & Son, London, 1975.
4. E. Breitmaier and W. Volter, "Carbon-13 NMR Spectroscopy," 3rd ed., Verlag Chemie, Weinheim, 1978, p. 319.
5. A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, Oxford, 1964, p. 123.
6. L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed., Pergamon Press, Oxford, 1969, p. 306.

7. A. Langemann and O. Isler, in: "Biochemistry of Quinones." Ed. by R.A. Morton, Academic Press, London, 1965, p. 128.
8. E.C. Bate-Smith and R.G. Westall, *Biochim. Biophys. Acta*, **4**, 427 (1950).
9. L.F. Fieser and M. Fieser, "Reagents for Organic Synthesis," John Wiley, New York, 1976, Vol. 1, p. 1011.
10. E.H. Lennette, A. Balows, W.J. Hausler Jr., and H.J. Shadomy, Eds., "Manual of Clinical Microbiology," 4th ed., American Society for Microbiology, Washington, DC, 1985.

Received 22 December 1988