The Chemical Constituents of the Fungus *Stereum* sp.

by **Guo-Hong Li**, **Lei Li**, **Meng Duan**, and **Ke-Qin Zhang***

Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, Kunming 650091, P. R. China

(phone: +868715034878; fax: +868715034838; e-mail: Kqzhang111@yahoo.com.cn)

Four new compounds, including a sesquiterpene and three aromatic compounds, and a known compound were isolated from a culture broth of the fungus *Stereum* sp. The novel sesquiterpene was determined to be stereumone A ((+)-2,3,4a,5,6,7,8a,9-octahydro-5-hydroxy-6,6,9-trimethyl-4,8a-epoxy-naphtho[2,3-*b*]furan-8(8*H*)-one; **1**), and the three new aromatic compounds were elucidated as 3,5-dihydroxy-4-(3-methylbut-2-enyl)benzene-1,2-dicarbaldehyde (**2**), 5,7-dihydroxy-6-(3-methylbut-2-enyl)-isobenzofuran-1(3*H*)-one (**3**), butyl 2,4-dihydroxy-6-methylbenzoate (**4**), together with the known compound methyl 2,4-dihydroxy-6-methylbenzoate (**5**). The structures were established by spectroscopic methods including 2D-NMR techniques. Compounds **2** and **4** showed evident nematicidal activity against nematode *Panagrellus redivivus*.

Introduction. – Mushrooms produce diversiform compounds, many of which exhibit significant bioactivities. Recent phytochemical studies on some species of the mushroom *Stereum* have brought the discovery of some interesting novel compounds including acetylenic aromatics, phenolic compounds, and sesquiterpenes, some of which showed antimicrobial and phytotoxic activities [1–3]. In the experiments for screening and searching for nematicidal metabolites from fungi, we investigated the secondary metabolites produced by *Stereum* sp. in culture. The basidiomycete *Stereum* sp. was collected in Xishuangbanna, Yunnan Province, and the mycelium of *Stereum* sp. was separated from the fruiting body. In a search for nematicidal agents from microbial sources, we found that the culture of the strain *Stereum* sp. exhibited strong activity against the tested nematode *Panagrellus redivivus*, and the nematicidal active fraction was the BuOH extract of culture broth of the fungus. Herein, we describe our study concerning the isolation and structure elucidation of four new compounds and a known compound from the BuOH extract of the culture broth of *Stereum* sp. 8954. Among them, two compounds showed nematicidal activity against *P. redivivus*.

Results and Discussion. – 1. *Chemistry*. The chromatographic purification of the BuOH extracts of the fermentation broth of a *Stereum* sp. yielded four new compounds, including a sesquiterpene (**1**) and three aromatic compounds (**2**–**4**), and a known aromatic compound (**5**) (*Fig. 1*).

Compound **1** was obtained as yellowish amorphous solid. The HR-ESI-MS data determined the molecular formula to be $C_{15}H_{19}O_4$ (*m/z* 263.1281 ($[M-H]^-$; calc. 263.1283)). The IR spectra revealed the presence of a OH (3438 cm^{-1}), C=O (1718*s* cm^{-1}), C=C (1654 cm^{-1}), and an ether (1138 cm^{-1}) group. The DEPT experiments

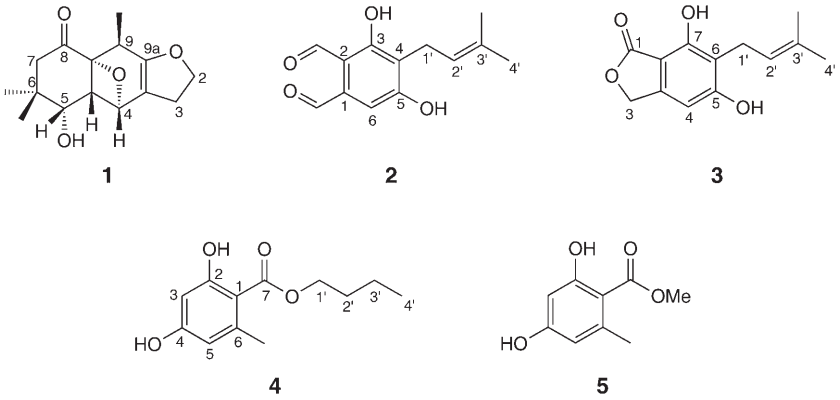

Fig. 1. *Structures of compounds* **1**–**5**

(*Table 1*) showed 15 C signals for three Me, three CH_2, four CH, and five quaternary C-atoms including one C=O (δ 195.9) and a C=C bond (δ 129.0 and 151.0). The HMBC data (*Table 1*) showed the ^{1}H,^{13}C-NMR long-range correlations between the H-atoms at δ(H) 0.93 ppm (6β-Me) and the C-atoms at δ(C) 21.1 (6α-Me), 40.6 (C(6)), 41.3 (C(7)), and 81.6 ppm (C(5)), between the H-atoms at δ(H) 0.99 (6α-Me) and the C-atoms at δ(C) 27.9 (6β-Me), 40.6 (C(6)), 41.3 (C(7)), and 81.6 (C(5)), between the H-atoms at δ(H) 1.26 (CH_2(7)) and the C-atoms at δ(C) 21.1 (6α-Me), 27.9 (6β-Me), 40.6 (C(6)), 195.9 (C(8)(w)), and between the H-atom at δ(H) 2.79 (H–C(4a)) and the C-atoms at δ(C) 40.6 (C(6)), 68.1 (C(8a)), 81.6 (C(5)), and 195.9 (C(8)). Moreover, the ^{1}H,^{1}H-COSY spectra (*Table 1*) showed that the H-atom at δ(H) 2.79 (H–C(4a)) correlated with the H-atom at 3.85 (H–C(5)) to afford the fragment **1a** (*Fig. 2*). Additionally, analysis of ^{1}H,^{1}H-COSY plots (*Table 1*) revealed that the H-atoms at δ(H) 4.34 and 4.27 (CH_2(2)) correlated with the H-atoms at 2.23 and 2.66 (CH_2(3)), and that the H-atoms at δ(H) 1.27 (9-Me) correlated with the H-atom at δ(H) 2.83 (H–C(9)), which together with the HMBC data (*Table 1*) led to the fragment **1b** (*Fig. 2*). Fragments **1a** and **1b** can be connected based on the correlation between the H-atom at δ(H) 2.83 (H–C(9)) and the C-atoms at δ(C) 68.1 (C(8a)) and 195.9 (C(8)), and between the H-atom at δ(H) 2.79 (H–C(4a)) and the C-atom at δ(C) 90.6 (C(4)). The demand of the number of unsaturated degrees and a relative downfield methine signal (H–C(4)) displayed in ^{13}C-NMR spectra implied that, except for the naphthalene and furan ring, there should be an additional ring in the molecular skeleton, linking C(4) and C(8a) through an oxygen bridge. The relative configuration was deduced from ROESY experiments with correlations between H–C(4) and H–C(4a), between H–C(4a) and H–C(5), 9-Me, and between H–C(5) and 6β-Me (*Fig. 2*). These spectroscopic data established the structure of **1** to be 2,3,4a,5,6,7,8a,9-octahydro-5-hydroxy-6,6,9-trimethyl-4,8a-epoxynaphtho[2,3-*b*]furan-8(8*H*)-one [4] (*Fig. 1*), namely stereumone A.

Compound **2** was obtained as yellowish amorphous solid. The HR-ESI-MS data determined the molecular formula to be $C_{13}H_{14}O_4$ (*m*/*z* 257.0799 ([*M* + Na]$^+$; calc. 257.0789)). The IR spectra revealed the presence of a phenolic OH (3422 cm^{-1}) and an

Table 1. *NMR Data of Compound* **1**. In (D_6)acetone; δ in ppm, J in Hz.

Position	δ(^{13}C)	δ(^{1}H)	HMBC (H–C)	^{1}H,^{1}H-COSY
CH_2(2)	59.0 (*t*)	4.34 (*dt*, *J* = 2.4, 16.5),	C(4), C(3a), C(9a)	2.23
		4.27 (*dt*, *J* = 2.1, 16.5)	C(4), C(3a), C(9a)	2.66
CH_2(3)	30.5 (*t*)	2.18–2.23 (*m*),	C(4)(w), C(9a)	4.34
		2.62–2.66 (*m*)	C(3a), C(9a)	4.27
C(3a)	129.0 (*s*)	–	–	–
H–C(4)	90.6 (*d*)	5.04 (br.)	–	–
H–C(4a)	53.9 (*d*)	2.76–2.79 (*m*)	C(4), C(5), C(6), C(8a), C(8)	3.85
H–C(5)	81.6 (*d*)	3.85 (*t*, 4.3)	C(8)(w)	2.79
C(6)	40.6 (*s*)	–	–	–
CH_2(7)	41.3 (*t*)	1.19–1.26 (*m*)	6α-Me, 6β-Me, C(6), C(8)(w)	–
C(8)	195.9 (*s*)	–	–	–
C(8a)	68.1 (*s*)	–	–	–
H–C(9)	46.2 (*d*)	2.80–2.83 (*m*)	C(8a), C(8), C(9a)	1.27
C(9a)	151.0 (*s*)	–	–	–
6α-Me	21.1 (*q*)	0.99 (*s*)	C(5), C(6), C(7), 6β-Me	–
6β-Me	27.9 (*q*)	0.93 (*s*)	C(5), C(6), C(7), 6α-Me	–
9-Me	23.0 (*q*)	1.27 (*d*, *J* = 7.9)	C(8a), C(9), C(9a)	2.83

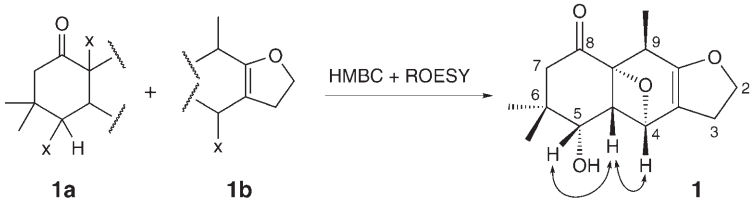

Fig. 2. *Significant ROESY correlations of* **1**

aldehyde group (1712*s* cm^{-1}), a C=C bond (1654 cm^{-1}), and a phenyl ring (1580, 1462 cm^{-1}). The UV absorbance at λ_{max} (log ε) 203.0 (4.39), 221 (4.34), 269 (3.93), 331 nm (3.44) showed the same result. The ^{1}H-NMR spectra (*Table 2*) exhibited a coupling system with signals at δ 3.41 (H–C(1′)) and 5.22 (H–C(2′)), a *singlet* of an aromatic H-atom at δ 7.14 (H–C(6)), two aldehyde *singlets* at δ 10.12 (1-CHO) and 10.71 (2-CHO), and two Me *singlets* at δ 1.64 (3′-Me) and 1.77 (Me(4′)). The ^{1}H-NMR and DEPT spectra showed that **2** contains a C=C bond and a pentasubstituted phenyl ring. In the HMBC experiment (*Table 3*), the ^{1}H,^{13}C-NMR long-range correlations between the H-atom of the CHO group at δ(H) 10.12 (1-CHO) and the phenyl-ring C-atoms at δ(C) 111.7 (C(4)(w)), 115.5 (C(6)), 136.7 (C(1)), and 164.0 (C(3)), and between the H-atom of the CHO group at δ(H) 10.71 (2-CHO) and the phenyl-ring C-atoms at δ(C) 111.7 (C(4)), 120.7 (C(2)), and 164.0 (C(3)) showed that two CHO groups were in consecutive positions at the phenyl ring. The HMBC spectra showed correlations between the H-atoms at δ(H) 3.41 (CH_2(1′)) and the C-atoms at δ(C) 25.0 (3′-Me), 120.7 (C(2′)), 161.6 (C(5)), and 164.0 (C(3)), and between the H-atom of the double-bond methine group at δ(H) 5.22 (H–C(2′)) and the C-atoms at δ(C) 17.1

Table 2. *^{1}H- and ^{13}C-NMR Data for Compounds* **2**–**4**. In (D_6)acetone; δ in ppm, J in Hz.

Position	**2**		**3**		**4**	
	$\delta(^1H)$	$\delta(^{13}C)$	$\delta(^1H)$	$\delta(^{13}C)$	$\delta(^1H)$	$\delta(^{13}C)$
C(1)	–	136.7 (*s*)	–	172.1 (*s*)	–	103.5 (*s*)
C(2)	–	120.7 (*s*)	–	–	–	164.6 (*s*)
C(3), CH_2(3), or H–C(3)	–	164.0 (*s*)	5.26 (*s*)	69.9 (*t*)	6.23 (*d*, $J=1.6$)	99.8 (*d*)
C(3a)	–	–	–	146.6 (*s*)	–	–
C(4) or H–C(4)	–	111.7 (*s*)	6.62 (*s*)	100.8 (*d*)	–	161.4 (*s*)
C(5) or H–C(5)	–	161.6 (*s*)	–	162.8 (*d*)	6.29 (*d*, $J=2.5$)	110.5 (*d*)
H–C(6) or C(6)	7.14 (*s*)	115.5 (*d*)	–	114.7 (*d*)	–	142.5 (*s*)
C(7)	–		–	155.0 (*d*)	–	170.9 (*s*)
C(7a)	–	–	–	103.1 (*s*)	–	–
CH_2(1′)	3.41 (*d*, $J=7.2$)	21.7 (*t*)	3.36 (*d*, $J=7.2$)	21.4 (*t*)	4.36 (*t*, $J=6.6$)	63.9 (*t*)
H–C(2′) or CH_2(2′)	5.22 (*t*, $J=8.3$)	120.7 (*d*)	5.23 (*t*, $J=7.1$)	122.1 (*d*)	1.74–1.80 (*m*)	29.5 (*t*)
C(3′) or CH_2(3′)	–	132.0 (*s*)	–	131.0 (*s*)	1.45–1.52 (*m*)	18.2 (*t*)
Me(4′)	1.77 (*s*)	17.1 (*q*)	1.76 (*s*)	17.0 (*q*)	0.98 (*t*, $J=6.5$)	12.1 (*q*)
1-CHO	10.12 (*s*)	192.3 (*d*)	–	–	–	–
2-CHO	10.71 (*s*)	195.2 (*d*)	–	–	–	–
6-Me	–	–	–	–	2.48 (*s*)	2.6 (*q*)
3′-Me	1.64 (*s*)	25.0 (*q*)	1.64 (*s*)	25.0 (*q*)	–	–

(C(4′)), 21.7 (C(1′)), and 25.0 (3′-Me). The ROESY experiments showed correlations between the aldehyde H-atoms and H–C(6), between the two aldehyde H-atoms, and between the 3′-Me and Me(4′) H-atoms and H–C(2′). On the basis of these data, together with other HMBC data (*Table 3*), the structure of **2** was deduced to be 3,5-dihydroxy-4-(3-methylbut-2-enyl)benzene-1,2-dicarbaldehyde (*Fig. 1*).

Compound **3** was isolated as white amorphous solid. The HR-ESI-MS data determined the molecular formula to be $C_{13}H_{14}O_4$ (*m*/*z* 257.0783 ($[M+Na]^+$; calc. 257.0789)). Analysis of NMR (*Table 2*), UV, and IR data suggested that the structure of

Table 3. *HMBC* (H–C) *Data of Compounds* **2**–**4**

Position	**2**	**3**	**4**
3	–	C(1), C(3a), C(4), C(5), C(7a)	C(2), C(4), C(5)
4	–	C(3), C(7a), C(6)	–
5	–	–	C(1), 6-Me, C(3), C(4)
6	C(1′)(w), C(1), C(2), C(4), C(5), 2-CHO(w), 1-CHO	–	–
1′	3′-Me, C(2′), C(3), C(5)	C(2′), C(3′), C(5), C(6), C(7)	C(2′), C(3′), C(7)
2′	C(1′), C(4′), 3′-Me	C(4′), 3′-Me, C(6)	C(1′), C(4′), C(3′)
3′	–	–	C(1′), C(2′), C(4′)
4′	C(2′), C(3′), 3′-Me	C(2′), C(3′), 3′-Me	C(2′), C(3′)
1-CHO	C(1), C(3), C(4)(w), C(6)	–	–
2-CHO	C(2), C(3), C(4)	–	–
3′-Me	C(2′), C(3′), C(4′)	C(2′), C(3′), C(4′)	–
6-Me	–	–	C(1), C(6), C(5)

3 is similar to that of compound **2**. The IR spectra revealed the presence of a phenolic OH (3469 cm^{-1}) and a C=O group (1699*s* cm^{-1}), a C=C bond (1619 cm^{-1}), and a phenyl ring (1615, 1461, 1447 cm^{-1}). The NMR data (*Tables 2* and *3*) showed that **3** has a COO instead of a CHO group at C(2) of **2** and a OCH_2 instead of a CHO group at C(1) of **2**, and that these two new groups form a lactone. The HMBC (*Table 3*) showed correlations between the H-atoms at δ(H) 5.26 (CH_2(3)) and the phenyl-ring C-atoms at δ(C) 100.8 (C(4)), 103.1 (C(7a)), 146.6 (C(3a)), and 162.8 (C(5)) and the C=O C-atom at δ(C) 172.1 (C(1)), and between the H-atom at δ(H) 6.62 (H−C(4)) and the C-atoms at δ(C) 69.9 (C(3)), 103.1 (C(7a)), 114.7 (C(6)), and 162.8 (C(5)). ROESY Experiments showed correlations between H−C(4) and CH_2(3), and the 3′-Me and Me(4′) H-atoms and H−C(2′). Together with these data, the structure of **2**, and other NMR data, **3** was assigned as 5,7-dihydroxy-6-(3-methylbut-2-enyl)isobenzofuran-1(3*H*)-one [5] (*Fig. 1*).

Compound **4** was also obtained as white amorphous solid. The molecular formula was established to be $C_{12}H_{16}O_4$ by the analysis of its ESI-MS (*m*/*z* 225 ($[M+H]^+$)) and DEPT spectra (*Table 2*). The data of IR, UV, and NMR (*Table 2*) spectra of **4** are highly similar to those of compound **5** except for the methyl ester in compound **5** and the butyl ester moiety in compound **4**. The ^{1}H-NMR data (*Table 2*) showed two coupling phenyl *doublets* at δ 6.29 (*d*, $J=2.5$ Hz, H−C(5)) and 6.23 (*d*, $J=1.6$ Hz, H−C(3)). The experiments of HMBC (*Table 3*) showed correlations between the methine H-atoms at δ(H) 6.29 (H−C(5)) and the C-atoms at δ(C) 22.6 (6-Me), 99.8 (C(3)), 103.5 (C(1)), and 161.4 (C(4)), and between the H-atom at δ(H) 6.23 (H−C(3)) and the C-atoms at δ(C) 110.5 (C(5)), 161.4 (C(4)), and 164.6 (C(2)), and between the H-atoms at δ(H) 2.48 (6-Me) and the C-atoms at δ(C) 103.5 (C(1)), 110.5 (C(5)), and 142.5 (C(6)). So, compound **4** was established as butyl 2,4-dihydroxy-6-methylbenzoate [6] (*Fig. 1*).

Compound **5** was determined to be methyl 2,4-dihydroxy-6-methylbenzoate on the basis of its physical and spectral data [6] (*Fig. 1*).

2. *Nematicidal Activity.* The culture broth of *Stereum* sp. was able to kill 89.5% and the BuOH extract (5 mg/ml) could kill 92.4% of *P. redivivus* in 24 h. The pure compounds **1**–**5** were tested in the nematicidal activity assay. Compound **4** killed 90% of *P. redivivus* at 100 ppm in 12 h, and **2** was also able to kill 50% of *P. redivivus* at 200 ppm within 24 h, but other compounds did not exhibit nematicidal activity against *P. redivivus* at 200 ppm in 48 h.

Interestingly, compounds **2** and **3** have a similar structure, but the latter did not show nematicidal activity at 200 ppm, which implies that the CHO group is crucial for activity. In addition, compounds **4** and **5** consist of the same carboxylic acid, but only the former showed nematicidal activity. This clearly indicates that the ester group is responsible for the compound to be active. From our research, compounds **2** and **4** may have a different functional mechanism according to comparison of their structures. In the screening, the broth of *Stereum* sp. showed strong nematicidal activity toward the nematode, and the active compounds showed only moderate activity, which implies that a synergistic action could exist in the broth.

This work was partially supported by the *Key Applied Foundation Program of Yunnan Province* (1999C0001Z), the *Natural Science Foundation of Yunnan Province* (2004C0003Q) and the *Ministry of*

Science and Technology of P. R. China (2003CB415102). We are also grateful to Mr. *Rong Huang* of Yunnan University, for performing the NMR spectra.

Experimental Part

General. TLC: Precoated plates (*Si gel G*) from *Qingdao Marine Chemical Factory*, Qingdao, P. R. China. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*); silica gel (200–300 mesh) from *Qingdao Marine Chemical Factory.* Optical rotation: *Jasco P-1020* polarimeter. UV Spectra: *Shimadzu UV-2401PC*, λ_{max} (log ε) in nm. IR Spectra: *Paragon 1000pc* spectrometer; KBr pellets; in cm^{-1}. ^{1}H-, ^{13}C-, DEPT, ^{1}H,^{1}H-COSY, ROESY, HMQC, and HMBC NMR experiments were carried out on a *Bruker DRX-500* spectrometer, at 500 MHz (^{1}H) and 125 MHz (^{13}C), resp.; chemical shifts δ in ppm rel. to $SiMe_4$, *J* in Hz. HR-ESI-MS and ESI-MS: *VG Auto-Spec-3000* mass spectrometer and *Finnigan Trace DSQ*; values in *m*/*z*.

Fungal Material. The fungus *Stereum* sp. (No. NN048954) was collected in Xishuangbanna, Yunnan Province, and the mycelium of *Stereum* sp. 8954 was separated from the fruiting body and deposited in the *Laboratory for Conservation and Utilization of Bio-resources of Yunnan Province*, P. R. China. The fungus was grown in shake culture (200 ml per 500 ml triangular flask) on a medium consisting of glucose (20 g), potato (200 g, boiled and filtered) (per liter of water), and incubated for 10 d at 26°. The culture was harvested for further study.

Extraction and Isolation. The culture (10 l) of *Stereum* sp. 8954 was filtered to separate cell and broth. The culture broth was extracted with BuOH and the extract was concentrated under reduced pressure. The crude BuOH extract was subjected to CC (silica gel 200–300 mesh; petroleum ether/acetone 20:1 to 2:1) to yield *Fr.* A_1–A_7. *Fr.* A_6 was chromatographed on CC (silica gel; petroleum ether/acetone 9:1 to 2:1) to give *Fr.* A_{6-3}, which was repeatedly purified on a *Sephadex LH-20* column (acetone) to afford compound **1** (5 mg). *Fr.* A_2 was purified repeatedly on a *Sephadex LH-20* column (acetone) to yield compound **4** (15 mg). *Fr.* A_3 was repeatedly subjected to CC (silica gel; petroleum ether/acetone 9:1) to furnish **5** (8 mg). *Fr.* A_4 was purified on a *Sephadex LH-20* column (acetone) to yield *Fr.* A_{4-2} and *Fr.* A_{4-3}. *Fr.* A_{4-2} was subjected to CC (silica gel; petroleum ether/acetone 9:1) to afford compound **3** (5 mg), and repeated separation of *Fr.* A_{4-2} on a *Sephadex LH-20* column (acetone) yielded compound **2** (8 mg).

Stereumone A (*=(+)-(4*R**,4a*S**,5*S**,8a*S**,9*S**)-2,3,4a,5,6,7,8a,9-Octahydro-5-hydroxy-6,6,9-trimethyl-4,8a-epoxynaphtho[2,3-*b*]furan-8(8*H*)-one*; **1**): Yellowish amorphous solid. $[\alpha]_D^{22} = +112$ ($c = 0.60$, acetone). IR (KBr): 3438, 2958, 1718*s*, 1654, 1138, 1089. UV (MeOH): 203.0 (3.90). ^{1}H- and ^{13}C-NMR: see *Table 1.* ESI-MS: 263 ($[M-H]^-$).

3,5-Dihydroxy-4-(3-methylbut-2-enyl)benzene-1,2-dicarbaldehyde (**2**): Yellowish amorphous solid. IR (KBr): 3422, 2928, 1712, 1654, 1580, 1462, 1352, 1072. UV (MeOH): 203.0 (4.39), 221 (4.34), 269 (3.93), 331 (3.44). ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*. ESI-MS: 257 ($[M+Na]^+$).

*5,7-Dihydroxy-6-(3-methylbut-2-enyl)isobenzofuran-1(3*H*)-one* (**3**): White amorphous solid. IR (KBr): 3469, 3185, 2924, 1699*s*, 1619, 1615, 1461, 1447, 1340, 1253, 1064, 994. UV (MeOH): 203.0 (4.47), 225 (4.47), 262 (3.94), 319 (3.43). ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*. ESI-MS: 257 ($[M+Na]^+$).

Butyl 2,4-Dihydroxy-6-methylbenzoate (**4**): White amorphous solid. IR (KBr): 3462, 2970, 1639*s*, 1584, 1403, 1377, 1319, 1261, 1275, 1060. UV (MeOH): 215.0 (4.38), 263 (4.12), 300 (3.20). ^{1}H- and ^{13}C-NMR: see *Tables 2* and *3*. ESI-MS: 225 ($[M+H]^+$), 247 ($[M+Na]^+$).

Methyl 2,4-Dihydroxy-6-methylbenzoate (**5**): Yellowish amorphous solid. IR (KBr): 3372, 2925, 1701*s*, 1585, 1403, 1325, 1266, 1170, 1160. UV (MeOH): 203.0 (4.24), 217 (4.21), 263 (3.91), 300 (3.46). ^{1}H-NMR ((D_6)acetone): 6.23 (*d*, $J = 1.8$, H–C(3)); 6.29 (*d*, $J = 2.35$, H–C(5)); 3.91 (*s*, MeO); 2.45 (*s*, 6-Me). ^{13}C-NMR ((D_6)acetone): 172.1 (COO); 165.4 (C(2)); 162.5 (C(4)); 143.5 (C(1)); 111.4 (C(5)); 104.5 (C(6)); 100.7 (C(3)); 51.2 (C(1′)); 22.3 (Me). ESI-MS: 183 ($[M+H]^+$).

Nematicidal-Activity Assay. The saprophytic nematode *P. redivivus* was cultured on oatmeal medium (20 g of oatmeal in 80 ml of H_2O) at 25° for 7 d. Then, the cultured nematodes were separated from the culture medium using the *Baerman* funnel technique [7], and an aqueous suspension of the nematode was prepared as a working stock. The nematicidal activity against *P. redivivus* was assayed according to

the method described in [8] with 2 ml of broth (in the 6-cm plate) and with the BuOH extract, diluted to 5 mg/ml with sterile H_2O. Each compound was dissolved in acetone and then diluted to different concentrations (200, 100, and 50 ppm) with sterile H_2O.

REFERENCES

[1] G. M. Bin, A. Fkyerat, R. Tabacchi, *Phytochemistry* **2000**, *53*, 571.
[2] J. O. Omolo, H. Anke, O. Sterner, *Phytochemistry* **2002**, *60*, 431.
[3] B. S. Yun, I. K. Lee, Y. Cho, S. M. Cho, I. D. Yoo, *J. Nat. Prod.* **2002**, *65*, 786.
[4] S. R. Peraza-Sánchez, D. Chávez, H. B. Chai, Y. G. Shin, R. García, M. Mejía, C. R. Fairchild, K. E. Lane, A. T. Menendez, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn, *J. Nat. Prod.* **2000**, *63*, 492.
[5] G. H. Bao, L. Q. Wang, K. F. Cheng, G. W. Qin, *Chin. Chem. Lett.* **2002**, *13*, 237.
[6] G. Nicollier, M. Rebetez, R. Tabacchi, H. Gerlach, A. Thalmann, *Helv. Chim. Acta* **1978**, *61*, 2899.
[7] N. F. Gray, *Soil Biol. Biochem.* **1984**, *16*, 81.
[8] G. H. Li, Y. M. Shen, K. Q. Zhang, *J. Microbiol.* **2005**, *43*, 17.

Received November 28, 2005