Synthesis and Antifungal Activity of 2-Hydroxy-4,5-methylenedioxyaryl Ketones as Analogues of Kakuol

by **Loana Musso***[a]), **Sabrina Dallavalle**[a]), **Lucio Merlini**[a]), and **Gandolfina Farina**[b])

[a]) Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, Via Celoria 2, IT-20133 Milano (phone: +390250316816; fax: +390250316801; e-mail: loana.musso@unimi.it)
[b]) Dipartimento di Protezione dei Sistemi Agroalimentare e Urbano e Valorizzazione delle Biodiversità, Università di Milano, Via Celoria 2, IT-20133 Milano (phone: +390250316779; fax: +390250316748)

In a study aiming to determine the structural elements essential to the antifungal activity of kakuol, we synthesized a series of 2-hydroxy-4,5-methylenedioxyaryl ketones, and we assayed their *in vitro* antifungal activity. The most sensitive target organisms to the action of these class of compounds were *Phytophthora infestans*, *Phytium ultimum*, *Cercospora beticola*, *Cladosporium cucumerinum*, and *Rhizoctonia solani*. Most of the analogs showed a remarkable *in vitro* activity, and some of them appeared significantly more effective than the natural product. The biological activity was mainly affected by introducing structural modification on the carbonyl moiety of the natural-product molecule. In particular, compound **5a**, bearing a C=C bond conjugated to the C=O group, was found active with a *MIC* value of 10 μg ml^{-1} against *Cladosporium cucumerinum*. The results suggest that 2-hydroxy-4,5-methylenedioxyaryl ketones can be considered promising candidates in the development of new antifungal compounds.

Introduction. – Innovation in crop protection is essential for sustainability of agriculture and global food production [1]. In contrast to the growing world population and the increasing demand for higher quantity and quality of food, the area of farmland per head of population has decreased dramatically during the last 50 years. In addition, crop losses due to pests, diseases, and weed damages are still as high as 50% overall. Although these losses may be attenuated by the use of disease-tolerant cultivars, crop rotation, or sanification practices, the use of fungicides will remain the predominant method of crop protection for the foreseeable future.

Despite the availability of effective fungicides [2], new antifungal chemicals are still needed to obtain improved yields and quality benefits, and to combat pathogens which show resistance or reduced sensitivity to existing antifungal compounds. In our study toward the development of new antifungal agents, we focused our interest on naturally occurring substances with antifungal activity as starting points for the rational design of new fungicides.

In 2005, *Hwang* and co-workers [3] isolated kakuol (=2-hydroxy-4,5-(methylenedioxy)propiophenone=1-[2-hydroxy-4,5-(methylenedioxy)phenyl]propan-1-one; **1**; *Fig. 1*) from the rhizomes of *Asarum sieboldii* (MIQ.) MAEK, a perennial herb belonging to the family of Aristolochiaceae, and demonstrated for the first time its antifungal activity against some important plant pathogens such as *Colletotrichum orbiculare*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora capsici*, and *Cladosporium cucumerinum*.

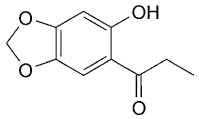


Fig. 1. *Structure of kakuol* (**1**)

Although *Asarum sieboldii* had been reported to have antifungal and insecticidal activity [4][5], the study by *Hwang* and co-workers was the first to show the fungicidal activity of the plant component kakuol, which is considered a metabolic oxidation product of saricin and safrole.

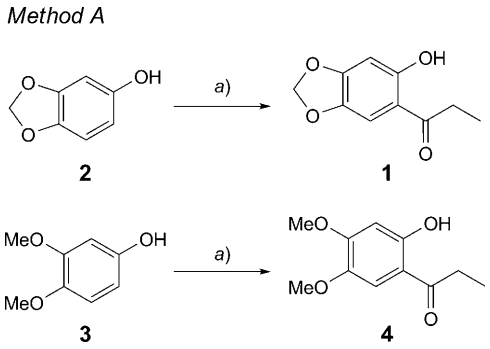
Several analogous propiophenones, such as 2-methoxy-4,5-(methylenedioxy)propiophenone (methylkakuol) [6–8], 2-methoxy-3,4-(methylenedioxy)propiophenone [9], and 2,4,5-trimethoxypropiophenone [10], were recently isolated from *Asarum*, *Anethum*, and *Piper* species, respectively, but their antifungal activity was not investigated. Although some data related to antifungal activity of kakuol and methylkakuol have been reported in a recent patent [11], nothing has been published so far about the synthesis and the determination of a structure–activity relationship (SAR) for a series of kakuol analogues. This lack of information prompted us to investigate this promising class of small molecules.

The results reported in the present study give an insight into some structural features affecting antifungal activity of kakuol.

Results and Discussion. – 1. *Synthesis.* In an effort to define the structural elements essential to the antifungal activity, we have altered kakuol at single sites assumed to be critical for function: the phenol group, and the methylenedioxy and the acyl moieties.

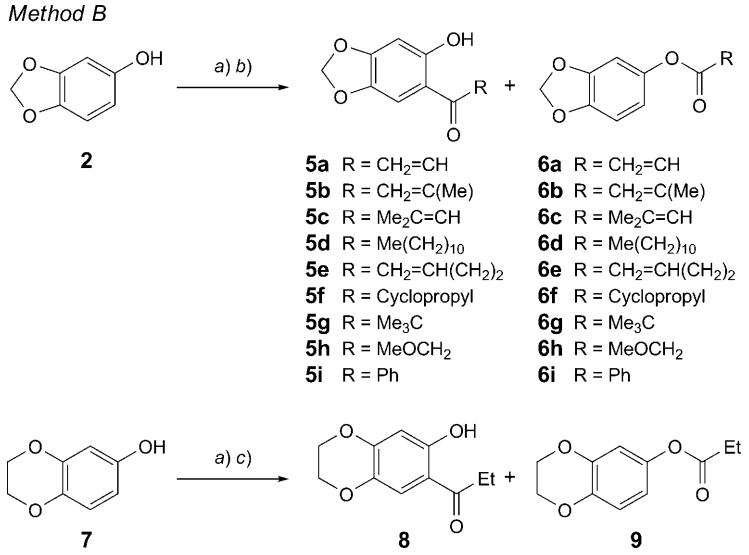
Two different strategies were applied to obtain kakuol and the series of 2-hydroxyaryl ketones; the first one involved *Fries*-type rearrangement [12] of appropriate phenol esters (*Method A*, *Scheme 1*); the second one exploited the reaction of highly coordinating metal phenolates with appropriate acyl chlorides to afford in a single step *ortho*-acyl phenols [13] (*Method B*, *Scheme 2*). For each compound, the strategy was chosen depending on the availability of starting materials and with the aim to minimize the number of synthetic steps.

Scheme 1. *Synthesis of Kakuol* (**1**) *and 4,5-Dimethoxypropiophenone* (**4**)



a) $BF_3 \cdot Et_2O$, propanoic anhydride, 45 min, 80°; yield 73% for **1**, 42% for **4**.

Scheme 2. *Synthesis of 2-Hydroxy-4,5-methylenedioxyaryl Ketones* **5a**–**5i**



a) MeMgBr, Et_2O, 45 min, r.t. *b*) RCOCl, toluene, r.t., 16 h; see *Exper. Part* for yields. *c*) Propanoyl chloride, toluene, r.t., yield 22% for **8**, 46% for **9**.

Endo et al. [11] reported a two-step synthesis of kakuol in a 26% overall yield. In our synthetic approach, kakuol (**1**) was obtained in a single step by reacting sesamol (= 3,4-(methylenedioxy)phenol; **2**) with $BF_3 \cdot Et_2O$ in propanoic anhydride with a 73% yield (*Scheme 1*). Following the same procedure, compound **4** was obtained from 3,4-dimethoxyphenol (**3**) in 42% yield.

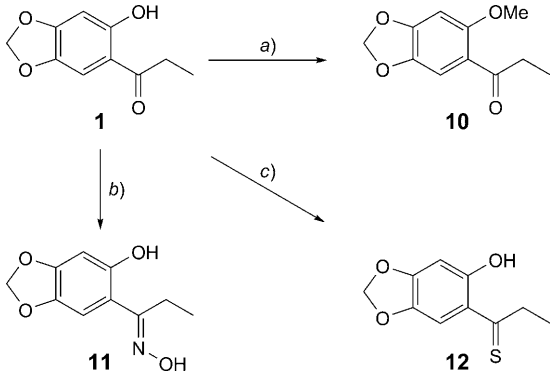
The compounds bearing different chains linked to the CO moiety were obtained by reacting magnesium 3,4-(methylenedioxy)phenolates with appropriate acyl chlorides affording in a single step the *ortho*-acyl phenols **5a**–**5i** (*Scheme 2*). Commercially unavailable acyl chlorides were prepared according to reported methods [14–16].

In some cases, besides the desired *ortho*-acyl phenols, we observed the formation of the corresponding esters **6**. The presence of these compounds in the reaction mixture made the purification of desired *ortho*-acyl phenols very difficult, and, in some cases, a further purification by crystallization after flash chromatography was needed. The best results were obtained for **5a**, obtained in good yield (66%) and without the formation of the corresponding ester, and for **5i**, obtained in 45% yield, together with a small amount of the ester (16%).

For the synthesis of compound **8**, phenol **7** was prepared in 86% yield from 2,3-dihydro-1,4-benzodioxine-6-carbaldehyde by *Bayer–Villiger* oxidation [17] and immediate hydrolysis of the formate. The acylation of the magnesium phenolate of **7** afforded compound **8** in only 22% yield together with 46% of ester **9**.

Compound **10** was obtained from **1** by methylation with MeI and K_2CO_3 in acetone [18] (*Scheme 3*). The synthesis of compound **11** was performed by reacting kakuol (**1**) with $NH_2OH \cdot HCl$ [15] and AcONa in MeOH, and thione **12** was prepared in 13% yield by refluxing a solution of **1** in toluene with *Lawesson*'s reagent [19] for 7 h.

Scheme 3. *Synthesis of Compounds* **10**, **11**, *and* **12**



a) MeI, K_2CO_3, acetone, 48%. *b*) $NH_2OH \cdot HCl$, AcONa, MeOH, reflux, 14 h; 80%. *c*) *Lawesson*'s reagent, toluene, reflux, 12 h; 13%.

2. *Biological Activity.* To integrate the scarce literature data reporting kakuol biological activity, synthetic **1** was tested against the following pathogens: *Phytophthora infestans*, *Pythium ultimum*, *Botrytis cinerea*, *Cercospora beticola*, *Rhizoctonia solani*, and *Cladosporium cucumerinum*. As reported in the *Table*, compound **1** exhibited a broad spectrum of activity and led to a good reduction of mycelial growth on *P. ultimum*, *R. solani*, *B. cinerea* (*ca.* 70% at 62.5 μg ml^{-1}). It was less efficient on *C. beticola* (49% at 62.5 μg ml^{-1}) and *C. cucumerinum* (30% at 62.5 μg ml^{-1}).

To define the most relevant structural features affecting *in vitro* antifungal activity, we first examined the role of the free phenol group. The methoxy derivative **10** presented values of mycelial growth inhibition comparable to those of kakuol (**1**) with the exception for *P. ultimum* (29% at 62.5 μg ml^{-1}; *Table 1*). This result suggests that the presence of the OH group is not crucial for the activity.

Regarding the modification of the methylenedioxy moiety, we prepared the ethylenedioxy derivative **8** and the 4,5-dimethoxy derivative **4**. Both of them were less active than kakuol on all the tested pathogens at the same concentration range.

Transforming the CO group of kakuol (**1**) into an oxime, **11**, led to a decrease of antifungal activity against *B. cinerea*, *C. cucumerinum*, and *R. solani*; on the contrary, in the case of *P. infestans, P. ultimum*, and *C. beticola*, the inhibition of mycelial growth was quite similar to that of **1**. Moreover, the substitution of the CO with the CS group (*cf.* **12**) led to a decrease of activity against the tested organisms.

More useful information was obtained from compounds with a modified chain (compounds **5a**–**5i**). In particular, the introduction of a C=C bond in the α-position to the CO gave compound **5a** with increased activity. In fact, it almost completely inhibited the mycelial growth of the tested pathogens at 62.5 μg ml^{-1}. Only *B. cinerea* was less sensitive to this compound (64% growth inhibition at 125 μg ml^{-1}).

The results listed in the *Table* showed that the introduction of a Me group at the α-position of the CO group (*i.e.*, **5b**) generally did not affect the activity compared to compound **5a**; on the other hand, the introduction of two Me groups (*i.e.*, **5c**) led to a substantial decrease of activity. As regards the relevance of the position of the C=C

Table. *Growth Inhibition* [%] *Induced by Compounds* **1**–**12**

	Conc. [μg ml^{-1}]	*P. infestans*	*P. ultimum*	*B. cinerea*	*C. beticola*	*C. cucumerinum*	*R. solani*
1	125	41	82	83	49	73	81
	62.5	31	70	70	49	28	71
10	125	54	57	80	59	–	90
	62.5	25	29	51	33	–	75
8	125	27	44	22	–	–	23
	62.5	16	45	15	40	–	11
4	125	30	26	18	49	–	44
	62.5	24	20	13	44	–	30
5a	125	100	100	64	97	100	100
	62.5	92	100	32	91	100	100
5b	125	100	100	64	79	100	100
	62.5	100	100	5	66	100	93
5c	125	19	19	20	32	8	29
	62.5	14	15	14	30	5	22
5d	62.5	0	0	0	0	0	0
	31.25	0	0	0	0	0	0
5e	125	31	48	18	41	16	66
	62.5	31	27	26	41	8	60
5f	125	65	85	62	58	48	79
	62.5	45	60	50	51	42	72
5g	125	47	80	25	37	35	38
	62.5	40	77	24	33	33	32
5h	125	15	37	0	33	10	14
	62.5	9	17	0	13	0	3
5i	125	4	24	16	20	0	28
	62.5	3	14	15	20	0	22
11	125	59	87	53	51	42	69
	62.5	41	79	42	41	29	63
12	100	42	56	35	33	47	68
Euparen[a])	100	100	100	–	–	–	–
Sideral[a])	25	–	–	100	–	–	–
Tetraconazole[a])	10	–	–	–	100	100	100

[a]) Euparen (toluilfluanide; 50%), sideral (procimidone; 50%), and tetraconazole; the reported standard concentrations referred to the pure compounds.

bond in the side chain, compound **5e** was much less active than **5a**, and more active than **5c**. Thus, we can hypothesize that the presence of a C=C bond conjugated to the CO group; *i.e.*, **5a** *vs.* **1** and **5e**, plays an important role, most likely due to the increased reactivity of the molecule to nucleophilic addition.

Moreover, the drop of antifungal activity in compound **5c**, which bears two Me groups at the C=C bond in β-position, could be due either to an unfavorable steric hindrance or to an increased lipophilicity.

To have a further insight into the relevance of lipophilicity, we synthesized compounds **5d**, **5g**, **5f**, and **5i**. The lipophilicity values of the compounds **1** and **5a**–**5i**, expressed as logarithm of partition coefficient in octan-1-ol/H_2O (log P) were calculated according to *Ghose* and *Crippen*'s fragmentation method [20] using

ChemDraw Ultra Vers. 5.0 [21]. As previously reported (*Table*), compounds **1** (log *P* 1.39), **5a** (log *P* 1.42), and **5b** (log *P* 1.77) showed higher antifungal activities than the more liphophilic **5c** (log *P* 1.96) and **5e** (log *P* 1.95). The cyclopropyl derivative **5f** (log *P* 1.46) exhibited activity quite similar to kakuol (**1**) on a wide range of organisms. The introduction of a lipophilic Ph residue (*i.e.*, **5i**; log *P* 2.63) led to a decrease of antifungal activity. Conversely, the introduction of a MeO in the chain (*i.e.*, **5h**; log *P* 0.40), led to a much less active compound. This substituent lowered the lipophilicity of the compound causing a detrimental effect on antifungal activity.

Due to its very poor solubility, the lauroyl derivative **5d** (log *P* 4.73) was tested at lower concentrations (62.5 and 31.25 μg ml^{-1}) than the other compounds, and was found completely inactive against all the tested pathogens.

The plots of percent growth inhibition induced by compounds **1**, **5a**–**5c**, and **5e**–**5i** (at 125 μg ml^{-1}) *vs.* log *P* for *C. cucumerinum* and *C. beticola* (*Fig. 2*) showed that the most active compounds have a log *P* value in the range of 1.4–1.8 (compounds **5a**, **5b**, **1**, and **5f**), the only exception being compound **5g** that maintained a moderate antifungal activity, despite its quite high log *P* value (2.66).

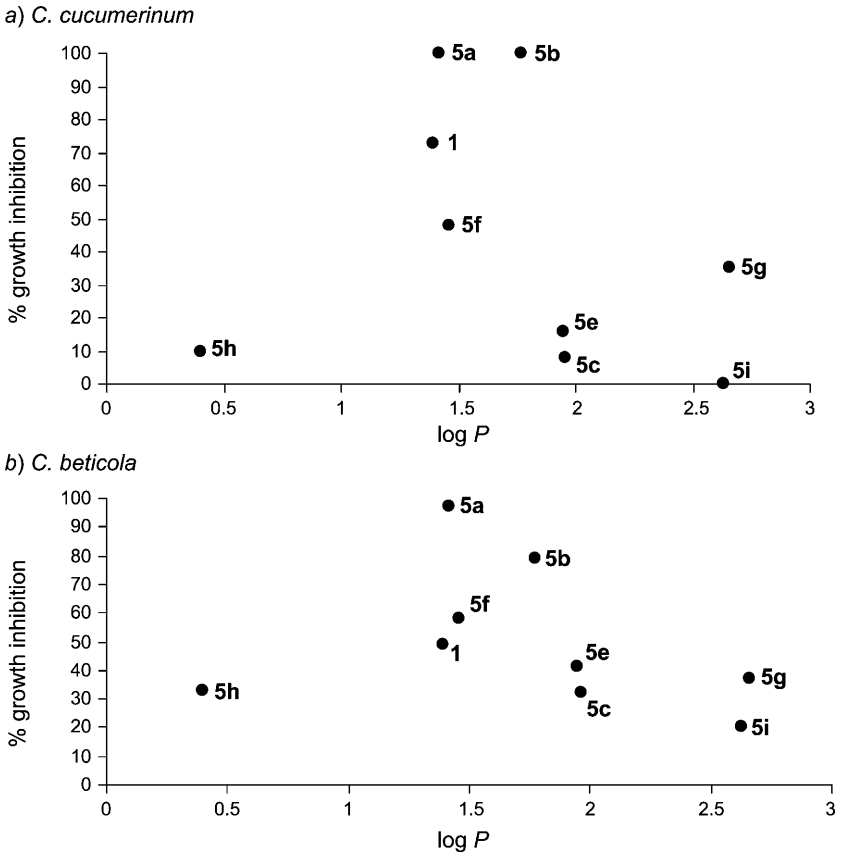


Fig. 2. *Percent growth inhibition of* a) C. cucumerinum *and* b) C. beticola *induced by compounds* **1**, **5a**–**5c**, *and* **5e**–**5i** (at 125 μg ml^{-1}) vs. *log* P

Among the tested compounds, **5a** appeared to be the most promising one. For this reason, it was tested at lower concentrations in order to evaluate the minimum inhibitory concentration (*MIC*) for the most sensitive organisms (*P. infestans*, *P. ultimum*, *C. beticola*, *C. cucumerinum*, and *R. solani*). The lowest *MIC* value for compound **5a** (10 μg ml^{-1}) was observed against *C. cucumerinum*. A slightly higher *MIC* value (*ca.* 25 μg ml^{-1}) was found against the oomycetes *P. infestans* and *P. ultimum*, whereas *R. solani* and *C. beticola* were found less sensitive (*MIC* values of 50 μg ml^{-1} and >100 μg ml^{-1}, resp.).

Observations at the optical microscope of *C. cucumerinum* conidia, inoculated on PDA (potato dextrose agar) amended with **5a** at 25 and 12.5 μg ml^{-1}, showed absence of germination. Moreover, when the same conidia were transferred on new, not amended PDA, they were again unable to germinate. This means that **5a** is featured with a high and persistent fungicidal activity interfering with spore germination, differently from the standard compound tetraconazole. This latter does not interfere with the spore germination but stops the mycelial growth, as expected for a compound belonging to the sterol biosynthesis inhibitors (SBI).

A long-lasting effect of **5a** was also observed in *P. infestans* and *P. ultimum* mycelial growth, while, in cultures treated with the standard Euparen (tolylfluanide) at the same concentration (100 μg ml^{-1}), mycelial growth was visible after 9-d treatment.

Preliminary *in vivo* studies showed that **5a** inhibited, at 1000 μg ml^{-1}, 60% of wheat rust and 80% of tomato downy mildew.

Conclusions. – The results obtained suggest that 2-hydroxy-4,5-methylenedioxyaryl ketones can be considered promising candidates in the development of new antifungal compounds. In particular, compound **5a**, bearing a C=C bond conjugated to the CO group, showed a remarkable *in vitro* antifungal activity. Further biological tests and structure–activity relationship studies on this new series of compounds are currently underway.

The authors gratefully acknowledge Dr. *Luigi Mirenna*, Department of Biology and Agronomy of *ISAGRO Research*, for the *in vivo* tests, Mr. *Elio Burrone* for the *in vitro* test, and the University of Milano (FIRST 2004-5 funds) for financial support.

Experimental Part

General. All reagents and solvents were reagent-grade or were purified by standard methods before use. Solvents were routinely distilled prior to use; anh. THF and Et_2O were obtained by distillation from Na-benzophenone ketyl; dry CH_2Cl_2 and toluene were obtained by distillation from $CaCl_2$. All reactions requiring anh. conditions were performed under a positive N_2 flow, and glassware was oven-dried. Isolation and purification of the compounds were performed by flash column chromatography (FCC) on silica gel *60* (SiO_2; 230–400 mesh). Anal. TLC: *Fluka* TLC plates (silica gel *60* F_{254}, aluminium foil). M.p.: *Stuart Scientific SMP3* instrument; uncorrected. ^{1}H-NMR Spectra: in $CDCl_3$ (if not otherwise stated) at r.t. on a *Bruker AMX-300* spectrometer operating at 300 MHz; chemical shifts (δ) and coupling constants (J) were reported in ppm and in Hz, resp. HR-MS: *Finnigan MAT-TSQ70* spectrometer; in m/z (rel. %).

General Synthetic Procedures. Method A. Propanoic anhydride (10.1 mmol) and $BF_3 \cdot Et_2O$ (7.1 mmol) were added to a soln. of the appropriate phenol (3.6 mmol), and the mixture was heated at 80°. The mixture was then cooled to r.t., and H_2O (3 ml) was added. After extraction with CH_2Cl_2 and evaporation of the org. solvent, the crude material was purified by FCC.

Method B. To a suspension of Mg beads (5.5 mmol) in dry Et_2O (5 ml), MeI (5.5 mmol) was added under stirring at 30–35°. When the Mg was completely dissolved, a soln. of phenol (3.62 mmol) in dry Et_2O (5 ml) was added, and the resulting mixture was stirred for 1 h. The solvent was removed *in vacuo*, and dry toluene (10 ml) was added. The appropriate acyl chloride (3.62 mmol) was added dropwise to the resulting slurry at 0°, and the yellow suspension was stirred at r.t. The mixture was poured into sat. aq. NH_4Cl and then extracted three times with AcOEt. The soln. was dried (Na_2SO_4), the solvent was evaporated under reduced pressure, and the crude material was purified by FCC on SiO_2.

1-(6-Hydroxy-1,3-benzodioxol-5-yl)propan-1-one (**1**). Compound **1** was obtained from 3,4-(methylenedioxy)phenol according to *Method A*. FCC (hexane/AcOEt 95:5): 516 mg (73%) of **1**. M.p. 109–110° (yellow crystals). ^{1}H-NMR: 13.09 (*s*, OH); 7.08 (*s*, 1 arom. H); 6.44 (*s*, 1 arom. H); 5.97 (*s*, OCH_2O); 2.88 (*q*, *J* = 7.4, MeCH_2); 1.21 (*t*, *J* = 7.4, *Me*CH_2). HR-EI-MS: 194.0581 (M^+, $C_{10}H_{10}O_4^+$; calc. 194.0584).

1-(2-Hydroxy-4,5-dimethoxyphenyl)propan-1-one (**4**). Compound **4** was obtained by *Method A*. The crude material was purified by FCC (hexane/Et_2O 1:1): **4** (245 mg, 42%). M.p. 118–119°. ^{1}H-NMR ((D$_6$)DMSO): 12.53 (*s*, OH); 7.29 (*s*, 1 arom. H); 6.54 (*s*, 1 arom. H); 3.82 (*s*, MeO); 3.76 (*s*, MeO); 3.05 (*q*, *J* = 7.1, MeCH_2); 1.09 (*t*, *J* = 7.1, *Me*CH_2). HR-EI-MS: 210.0893 (M^+, $C_{11}H_{14}O_4^+$; calc. 210.0897).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)prop-2-en-1-one (**5a**). Compound **5a** was obtained by *Method B*, starting from *sesamol* (**2**; 1.50 g, 10.86 mmol) and acryloyl chloride. The crude material was purified by FCC (hexane/AcOEt 95:5): **5a** (1.37 g, 66%). M.p. 117–118°. ^{1}H-NMR: 13.49 (*s*, OH); 7.13 (*s*, 1 arom. H); 7.13 (*dd*, *J* = 10.42, 16.7, 1 H of CH=CH_2); 6.52 (*dd*, *J* = 1.49, 16.7, 1 H of CH=CH_2); 6.48 (*s*, 1 arom. H); 5.99 (*s*, OCH_2O); 5.90 (*dd*, *J* = 1.5, 10.4, 1 H of CH=CH_2). HR-EI-MS: 192.0424 (M^+, $C_{10}H_8O_4^+$; calc. 192.0428).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)-2-methylprop-2-en-1-one (**5b**). Compound **5b** was obtained by *Method B* starting from **2** (500 mg, 3.62 mmol) and 2-methylacryloyl chloride. The crude material was purified by FCC (hexane/AcOEt 95:5): 415 mg of a mixture of **5b** (23% yield determined by ^{1}H-NMR) and **6b** (*1,3-benzodioxol-5-yl 2-methylprop-2-enoate*; 32% yield determined by ^{1}H-NMR of the mixture); crystallization of this mixture with Et_2O/hexane gave **5b** (100 mg, 13%).

Data of **5b**. M.p. 78–79°. ^{1}H-NMR: 12.87 (*s*, OH); 7.12 (*s*, 1 arom. H); 6.97 (*s*, 1 arom. H); 6.30 (*s*, 1 H of C=CH_2); 5.99 (*s*, OCH_2O); 5.75 (*s*, 1 H of C=CH_2); 2.02 (*s*, Me). HR-EI-MS: 206.0580 (M^+, $C_{11}H_{10}O_4^+$; calc. 206.0584).

Data of **6b**. ^{1}H-NMR: 6.77 (*d*, *J* = 8.6, 1 arom. H); 6.10 (*d*, *J* = 2.6, 1 arom. H); 6.05 (*dd*, *J* = 8.6, 2.6, 1 arom. H); 5.99 (*s*, OCH_2O); 5.60 (*s*, 1 H of C=CH_2); 5.30 (*s*, 1 H of C=CH_2); 2.02 (*s*, Me).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)-3-methylbut-2-en-1-one (**5c**). Compound **5c** was obtained by *Method B* starting from **2** (500 mg, 3.62 mmol) and 3,3-dimethylacryloyl chloride. The crude material was purified by FCC (hexane/AcOEt 95:5) to afford 386 mg of a mixture of **5c** (29% yield determined by ^{1}H-NMR of the mixture) and **6c** (*1,3-benzodioxol-5-yl 3-methylbut-2-enoate*; 19% yield determined by ^{1}H-NMR); crystallization of this mixture with Et_2O/hexane gave **5c** (192 mg, 24%). M.p. 104–106°. ^{1}H-NMR: 13.64 (*s*, OH); 7.11 (*s*, 1 arom. H); 6.51–6.53 (*m*, C*H*=CMe_2); 6.44 (*s*, 1 arom. H); 5.96 (*s*, OCH_2O); 2.16 (*d*, *J* = 1.1, Me); 2.00 (*d*, *J* = 1.1, Me). HR-EI-MS: 220.0738 (M^+, $C_{12}H_{12}O_4^+$; calc. 220.0741).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)dodecan-1-one (**5d**). Compound **5d** was obtained by *Method B* starting from **2** (400 mg, 2.90 mmol) and dodecanoyl chloride. The crude material was purified by FCC (hexane/AcOEt 95:5) to give 550 mg of a mixture of **5d** and **6d** (*1,3-benzodioxol-5-yl dodecanoate*); crystallization of this mixture with Et_2O/hexane gave of **5d** (136 mg, 15%), together with **6d** (393 mg, 36%).

Data of **5d**. M.p. 83–84.0°. ^{1}H-NMR: 13.18 (*s*, OH); 7.08 (*s*, 1 arom. H); 6.44 (*s*, 1 arom. H); 5.97 (*s*, OCH_2O); 2.82 (*t*, *J* = 7.4, $COCH_2$); 1.79–1.60 (*m*, CH_2); 1.45–1.15 (*m*, 8 CH_2); 0.87 (*t*, *J* = 6.7, Me).

Data of **6d**. ^{1}H-NMR: 6.75 (*d*, *J* = 7.8, 1 arom. H); 6.57 (*d*, *J* = 1.8, 1 arom. H); 6.50 (*dd*, *J* = 7.8, 1.8, 1 arom. H); 5.97 (*s*, OCH_2O); 2.50 (*t*, *J* = 7.4, $COCH_2$); 1.77–1.55 (*m*, CH_2); 1.45–1.15 (*m*, 8 CH_2); 0.75–0.95 (*m*, Me).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)pent-4-en-1-one (**5e**). Compound **5e** was obtained by *Method B* starting from **2** (500 mg, 3.62 mmol) and pent-4-enoyl chloride. The crude material was purified by FCC (hexane/AcOEt 95:5) to provide 287 mg of a mixture of **5e** (26% yield determined by ^{1}H-NMR of the

mixture) and **6e** (*1,3-benzodioxol-5-yl pent-4-enoate*, 16% yield determined by ^{1}H-NMR); crystallization of this mixture with Et_2O/hexane gave **5e** (150 mg, 19%).

Data of **5e**. M.p. 74.6–76.7°. ^{1}H-NMR: 13.09 (*s*, OH); 7.07 (*s*, 1 arom. H); 6.44 (*s*, 1 arom. H); 5.97 (*s*, OCH_2O); 5.95–5.77 (*m*, C*H*=CH_2); 5.09 (*ddd*, *J* = 1.5, 3.3, 17.1, 1 H, CH=CH_2); 5.02 (*ddd*, *J* = 1.5, 3.3, 11.9, 1 H, CH=CH_2); 2.95 (*t*, *J* = 7.1, CH_2CH=CH_2); 2.57–2.42 (*m*, CH_2CH_2CH=CH_2). HR-EI-MS: 220.0738 (M^+, $C_{12}H_{12}O_4^+$; calc. 220.0740).

Data of **6e**. ^{1}H-NMR: 6.74 (*d*, *J* = 8.5, 1 arom. H); 6.53 (*d*, *J* = 2.2, 1 arom. H); 6.50 (*dd*, *J* = 8.5, 2.2, 1 arom. H); 5.97 (*s*, OCH_2O); 5.87–5.72 (*m*, C*H*=CH_2); 5.17–4.92 (*m*, CH=CH_2); 2.70–2.55 (*m*, CH_2CH=CH_2); 2.52–2.35 (*m*, CH_2CH_2CH=CH_2).

Cyclopropyl(6-hydroxy-1,3-benzodioxol-5-yl)methanone (**5f**). Compound **5f** was obtained by *Method B* starting from **2** (500 mg, 3.62 mmol) and cyclopropylcarbonyl chloride. The crude material was purified by FCC (hexane/AcOEt 95 : 5) to obtain **5f** (111 mg, 15%). In this case, the crude material was a very complex mixture, and it was not possible to verify, by ^{1}H-NMR spectra, the presence of ester derived from *O*-acylation reaction. M.p. 83° (dec.). ^{1}H-NMR: 13.24 (*s*, OH); 7.25 (*s*, 1 arom. H); 6.45 (*s*, 1 arom. H); 5.99 (*s*, OCH_2O); 2.55–2.40 (*m*, CH); 1.28–1.40 (*m*, CH_2); 0.95–1.25 (*m*, CH_2). HR-EI-MS (pos.): 206.0581 (M^+, $C_{11}H_{10}O_4^+$; calc. 206.0584).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)-2,2-dimethylpropan-1-one (**5g**). Compound **5g** was obtained by *Method B* starting from **2** (500 mg, 3.62 mmol) and pivaloyl chloride. The crude material was purified by FCC (hexane/AcOEt 95 : 5) to yield **5g** (74 mg, 9%). M.p. 93°. ^{1}H-NMR: 13.06 (*s*, OH); 7.37 (*s*, 1 arom. H); 6.47 (*s*, 1 arom. H); 5.96 (*s*, OCH_2O); 1.41 (*s*, Me_3C). HR-EI-MS: 222.0894 (M^+, $C_{12}H_{14}O_4^+$; calc. 222.0897).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)-2-methoxyethanone (**5h**). Compound **5h** was obtained by *Method B* starting from **2** (250 mg, 1.81 mmol) and 2-methoxyacetyl chloride. The crude material was purified by FCC (hexane/Et_2O 3 : 1) to afford 116 mg of a mixture of **5h** (22% yield determined by ^{1}H-NMR of the mixture) and **6h** (*1,3-benzodioxol-5-yl methoxyacetate*, 9% yield determined by ^{1}H-NMR); crystallization of this mixture from Et_2O/hexane gave **5h** (69 mg,18%).

Data of **5h**. M.p. 125°. ^{1}H-NMR: 12.69 (*s*, OH); 7.25 (*s*, 1 arom. H); 7.04 (*s*, 1 arom. H); 5.98 (*s*, OCH_2O); 4.54 (*s*, MeOCH_2); 3.49 (*s*, *Me*OCH_2). HR-EI-MS: 210.0530 (M^+, $C_{10}H_{10}O_5^+$; calc. 210.0533).

Data of **6h**. ^{1}H-NMR: 6.67 (*d*, *J* = 8.5, 1 arom. H); 6.63 (*d*, *J* = 2.2, 1 arom. H); 6.63 (*dd*, *J* = 8.5, 2.2, 1 arom. H); 5.98 (*s*, OCH_2O); 4.24 (*s*, MeOCH_2); 3.51 (*s*, *Me*OCH_2).

(6-Hydroxy-1,3-benzodioxol-5-yl)(phenyl)methanone (**5i**). Compound **5i** was obtained by *Method B* starting from **2** (500 mg, 3.62 mmol) and benzoyl chloride. The crude material was purified by FCC (hexane/Et_2O 3 : 1) to give **5i** (438 mg, 45%), and 200 mg of a mixture of phenol and ester **6i** (*1,3-benzodioxol-5-yl benzoate*; 72 : 28 determined by ^{1}H-NMR).

Data of **5i**. M.p. 105–106°. ^{1}H-NMR: 13.00 (*s*, OH); 7.70–7.61 (*m*, 2 arom. H); 7.60–7.42 (*m*, 3 arom. H); 6.92 (*s*, 1 arom. H); 6.05 (*s*, 1 arom. H); 6.00 (*s*, OCH_2O). HR-EI-MS: 242.0581 (M^+, $C_{14}H_{10}O_4^+$; calc. 242.0584).

Data of **6i**. ^{1}H-NMR: 8.45–8.05 (*m*, 2 arom. H (Ph)); 7.67–7.35 (*m*, 3 arom. H (Ph)); 6.80 (*d*, *J* = 7.8, 1 arom. H); 6.72 (*d*, *J* = 2.6, 1 arom. H); 6.22 (*dd*, *J* = 7.8, 2.6, 1 arom. H); 6.00 (*s*, OCH_2O).

1-(2,3-Dihydro-7-hydroxy-1,4-benzodioxin-6-yl)propan-1-one (**8**). Compound **8** was obtained by *Method B* starting from 3,4-(ethylenedioxy)phenol (196 mg, 1.29 mmol) and propanoyl chloride. The crude material was purified by FCC (hexane/Et_2O 8 : 2) to give **8** (59 mg, 22% yield) together with compound **9** (*2,3-dihydro-1,4-benzodioxin-6-yl propanoate*; 46%).

Data of **8**. M.p. 111–112°. ^{1}H-NMR ((D_6)DMSO): 12.02 (*s*, OH); 7.40 (*s*, 1 arom. H); 6.40 (*s*, 1 arom. H); 4.35–4.28 (*m*, 2 H of OCH_2CH_2O); 4.25–4.07 (*m*, 2 H of OCH_2CH_2O); 3.00 (*q*, *J* = 7.1, MeCH_2); 1.06 (*t*, *J* = 7.1, *Me*CH_2). HR-EI-MS: 208.0738 (M^+, $C_{11}H_{12}O_4^+$; calc. 208.0741).

Data of **9**. ^{1}H-NMR: 6.80 (*d*, *J* = 8.9, 1 arom. H); 6.61 (*d*, *J* = 2.6, 1 arom. H); 6.54 (*dd*, *J* = 8.9, 2.6, 1 arom. H); 4.37–4.17 (*m*, OCH_2CH_2O); 2.53 (*q*, *J* = 7.4, MeCH_2); 1.23 (*t*, *J* = 7.4, *Me*CH_2).

1-(6-Methoxy-1,3-benzodioxol-5-yl)propan-1-one (**10**). To a soln. of **1** (90 mg, 0.46 mmol) in acetone (2 ml), K_2CO_3 (65 mg, 0.47 mmol) and MeI (162 µl, 2.6 mmol) were added, and the mixture was refluxed for 16 h. The mixture was washed with H_2O and brine, the soln. was dried (Na_2SO_4), and solvent was removed *in vacuo*. The crude material was purified by FCC (hexane/Et_2O 95 : 5) to give **10** (46 mg, 48%).

M.p. 88–89°. ^{1}H-NMR: 7.31 (*s*, 1 arom. H); 6.52 (*s*, 1 arom. H); 5.97 (*s*, OCH_2O); 3.85 (*s*, MeO); 2.94 (*q*, *J* = 7.4, Me*CH*$_2$); 1.13 (*t*, *J* = 7.4, *Me*CH_2). HR-EI-MS: 208.0738 (M^+, $C_{11}H_{12}O_4^+$; calc. 208.0741).

*6-[(1*E*)-*N*-Hydroxypropanimidoyl]-1,3-benzodioxol-5-ol* (**11**). To a suspension of $NH_2OH \cdot HCl$ (31 mg, 0.45 mmol) and AcONa (41 mg, 0.49 mmol) in MeOH (3 ml), **1** (80 mg, 0.41 mmol) was added, and the mixture was refluxed for 6 h. After the addition of a second portion of $NH_2OH \cdot HCl$ (31 mg, 0.45 mmol) and AcONa (41 mg, 0.49 mmol), the mixture was refluxed for 8 h. The solvent was evaporated, and the crude material was dissolved in AcOEt. The org. phase was washed with H_2O, dried (Na_2SO_4), and the solvent was removed *in vacuo*. The crude material was purified by FCC (hexane/AcOEt 9 :1) to give **11** (69 mg, 80%). M.p. 174–176°. ^{1}H-NMR ((D$_6$)DMSO): 11.87 (*s*, OH); 11.25 (*s*, C=NOH); 7.01 (*s*, 1 arom. H); 6.48 (*s*, 1 arom. H); 5.95 (*s*, OCH_2O); 2.71 (*q*, *J* = 7.4, Me*CH*$_2$); 1.02 (*t*, *J* = 7.4, *Me*CH_2). HR-EI-MS: 209.0690 (M^+, $C_{10}H_{11}NO_4^+$; calc. 209.0693).

1-(6-Hydroxy-1,3-benzodioxol-5-yl)propane-1-thione (**12**). To a soln. of **1** (100 mg, 0.5 mmol) in dry toluene (3 ml) *Lawesson*'s reagent (121 mg, 0.3 mmol) was added. The mixture was refluxed for 7 h; in spite of further addition of *Lawesson*'s reagent, the reaction was not complete. The solvent was evaporated and the crude material was purified by FCC (hexane/AcOEt 9 :1) to give **12** (13 mg, 13%). ^{1}H-NMR: 14.41 (*s*, OH); 7.24 (*s*, 1 arom. H); 6.53 (*s*, 1 arom. H); 5.99 (*s*, OCH_2O); 3.25 (*q*, *J* = 7.4, Me*CH*$_2$); 1.36 (*t*, *J* = 7.4, *Me*CH_2). GC/MS: 210 (100, M^+), 181 (56), 147 (89), 119 (22), 91 (33), 69 (89), 55 (22). HR-EI-MS: 210.0353 (M^+, $C_{10}H_{10}O_3S^+$; calc. 210.0356).

Biological Assays. The antifungal activity was tested *in vitro* on *Phytophthora infestans* (Mont.) de Bary, *Pythium ultimum* Trow, *Botrytis cinerea* Pers., *Cercospora beticola* Sacc., *Cladosporium cucumerinum* Ellis & Arthur, *Rhizoctonia solani* J. G. Kühn.

Radial Fungal-Growth Assay. The activity of the compounds was assayed as growth inhibition by the commonly used 'poisoned food' technique. Different solns. of each compound were prepared by dissolving the appropriate amount of compound in DMSO plus *Tween 20* (2%). Equal volumes of DMSO containing diluted compounds were added to sterile cool agar media (*Potato Dextrose Agar Difco*) to give suitable concentrations for each substance.

A zero-concentration treatment, containing the same percentage of DMSO and *Tween 20* to ensure equivalent concentrations of these components in all the treatments, was prepared for each strain. The final DMSO concentration did not exceed 0.3% of the final volume in both control and treated cultures. Compound-amended agar medium was dispersed aseptically onto 9-cm-diameter plastic *Petri* dishes (10 ml/dish). Each dish was inoculated with two mycelial discs cut from the periphery of actively growing colonies. Two replicates were used for each concentration, together with controls containing toxicant-free medium. The growth inhibition was calculated from mean differences between treated and control cultures as a percentage of the latter. The results were compared with those obtained with three standard fungicides, tolylfluanide, procymidon, and tetraconazole. The growth was determined after 24 h of incubation at 24° for *P. infestans* and *P. ultimum*, 3 d for *B. cinerea*, 6 d for *R. solani*, and 9 d for *C. beticola* and *C. cucumerinum*.

In vivo *Assays.* The tests of direct protecting activity of the compounds were performed on the following pathogen-host combinations: *Puccinia graminis*/wheat cv Irnerio (wheat rust) and *Phytophthora infestans*/tomato cv Marmande (downy mildew). Both surfaces of plant leaves were treated with a 20% (*v*/*v*) aq. soln. of acetone of the selected compounds (1000 μg ml^{-1}). Inoculation with a suitable conidia suspension was performed 7 d after the treatment on tomato and 24 h after the treatment on wheat. The area of inoculated leaves covered by disease symptoms was assessed on a 100-point scale from 0 to 100, in which 100 corresponded to completely infected leaves and 0 to not visible symptoms. The results were compared with those obtained with two standard fungicides in commercially available formulations, benalaxil and tetraconazole, and the activity was expressed as percent inhibition of infection in comparison with untreated control.

REFERENCES

[1] 'Pesticide Chemistry. Crop protection, Public Health, Environmental Safety', Eds. H. Ohkawa, H. Miyagawa, P. W. Lee, Wiley-VCH, Weinheim, 2007.

[2] 'Modern Crop Protection Compounds', Eds. W. Krämer, U. Schirme, Wiley-VCH, Weinheim, 2007.
[3] J. Y. Lee, S. S. Moon, B. K. Hwang, *Pest. Manage. Sci.* **2005**, *61*, 821.
[4] M. Miyazawa, Y. Ishikawa, M. Toshikura, H. Kameoka, *Chem. Express* **1991**, *6*, 703.
[5] M. Takasaki, T. Konoshima, L. Yasuda, T. Hamano, H. Tokuda, *Biol. Pharm. Bull.* **1997**, *20*, 776.
[6] A. M. P. de Diaz, O. R. Gottlieb, *Planta Med.* **1979**, *35*, 190.
[7] L. S. Ramos, M. L. da Silva, A. I. R. Luz, M. G. B. Zoghbi, I. G. S. Maia, *J. Nat. Prod.* **1986**, *49*, 712.
[8] G. S. Kim, N. I. Baek, J. D. Seong, Y. H. Kwack, *J. Korean Soc. Agric. Chem. Biotechnol.* **1999**, *42*, 369.
[9] S. S. Tomar, P. Dureja, *Fitoterapia*, **2001**, *72*, 76.
[10] B. V. de Oliveira Santos, M. C. de Oliveira Chaves, *Biochem. Syst. Ecol.* **1999**, *27*, 539.
[11] H. Endo, T. Fujimura, T. Kitaura, JP 2006199619, 2006.
[12] D. T. Witiak, S. K. Kim, A. K. Tehim, K. D. Sternitzke, R. L. McCreery, S. U. Kim, D. R. Feller, K. J. Romstedt, V. S. Kamanna, H. A. I. Newmann, *J. Med. Chem.* **1988**, *31*, 1437.
[13] F. Bigi, G. Casiraghi, G. Casnati, S. Marchesi, G. Sartori, C. Vignali, *Tetrahedron* **1984**, *40*, 4081.
[14] K. Liu, L. Xu, J. P. Berger, K. L. MacNaul, G. Zhou, T. W. Doebber, M. J. Forrest, D. E. Moller, A. B. Jones, *J. Med. Chem.* **2005**, *48*, 2262.
[15] K. A. Aherendt, R. M. Williams, *Org. Lett.* **2004**, *6*, 4539.
[16] M. E. Jung, S.-J. Min, *J. Am. Chem. Soc.* **2005**, *127*, 10834.
[17] J. Hellberg, E. Dahlstedt, M. E. Pelcam, *Tetrahedron* **2004**, *60*, 8899.
[18] P. F. Schuda, W. A. Price, *J. Org. Chem.* **1987**, *52*, 1972.
[19] H. Gao, J. Kawabata, *Bioorg. Med. Chem.* **2005**, *13*, 1661.
[20] A. K. Ghose, G. M. Crippen, *J. Chem. Inf. Comput. Sci.* **1987**, *27*, 21.
[21] ChemDraw Ultra Version 5.0, *Cambridge Soft Corp.*, USA.

Received August 27, 2009