Antimicrobial Depsides Produced by *Cladosporium uredinicola*, an Endophytic Fungus Isolated from *Psidium guajava* Fruits

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Four depsides, all of them new as natural products, were isolated from *Cladosporium uredinicola* solid-media culture and identified as 3-hydroxy-2,5-dimethylphenyl 2,4-dihydroxy-3,6-dimethylbenzoate (**1**), 3-hydroxy-2,4,5-trimethylphenyl 2,4-dihydroxy-3,6-dimethylbenzoate (**2**), 3-hydroxy-2,5-dimethylphenyl 3-[(2,4-dihydroxy-3,6-dimethylbenzoyl)oxy]-6-hydroxy-2,4-dimethylbenzoate (**3**), and 3-hydroxy-2,4,5-trimethylphenyl 3-[(2,4-dihydroxy-3,6-dimethylbenzoyl)oxy]-6-hydroxy-2,4-dimethylbenzoate (**4**). The endophytic fungus was isolated from *Psidium guajava* fruits and cultivated over sterilized rice. The compounds **1**–**4** were purified by classical chromatographic procedures, and the chemical structures were identified by spectroscopic studies, mainly 1D- and 2D-NMR and LC/ESI-MS/MS. Three of the isolated depsides exhibited moderate bacteriostatic and/or bactericide effects on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtillis*.

Introduction. – Plant-associated microorganisms are well known as great producers of natural products, being a rich source for biologically active metabolites with a wide-ranging application [1]. Their ability to utilize various solid substrates for their own metabolism, including vegetal sources, makes them very interesting biochemical-chemical agents in nature, specially affecting their host-plant development [2]. Most fruits contain high concentration of sugar and, therefore, are good targets for microorganism attacks. Nevertheless, according to literature searches, fungi and bacteria isolated from fruits seem to be explored more from the agronomic than from the chemical point of view. Microorganisms living in association with health-plant organs may accumulate chemicals in their tissue. This possibility has to be investigated since these secondary metabolites can be incorporated into human diet due to the vegetal consumption. *Psidium guayava* is a fructiferous tree belonging to the *Myrtaceae* family. It is an important food crop and medicinal plant in tropical and subtropical countries, widely used as food (due to its sweet and vitamin-containing fruits) and in folk medicine around the world [3]. Guava fruits are a source of nutrients for many animals and so for microorganisms, becoming a natural host for them.

This article reports the production of new depsides in sterile-rice media by *Cladosporium uredinicola*, a fungus species isolated as an endophytic microorganism from guava fruits. The new depsides were isolated and identified by spectroscopic

techniques such as NMR and LC/MS. The antimicrobial properties of the isolated depsides were tested against a set of pathogenic bacteria.

Results and Discussion. – 1. *Isolation and Structure Elucidation.* The EtOH extract of rice-media culture of *C. uredinicola* was fractionated by the combination of low-pressure column chromatography (silica gel) and prep. reversed-phase HPLC. Four depsides were isolated and identified as 3-hydroxy-2,5-dimethylphenyl 2,4-dihydroxy-3,6-dimethylbenzoate (**1**), 3-hydroxy-2,4,5-trimethylphenyl 2,4-dihydroxy-3,6-dimethylbenzoate (**2**), 3-hydroxy-2,5-dimethylphenyl 3-[(2,4-dihydroxy-3,6-dimethylbenzoyl)oxy]-6-hydroxy-2,4-dimethylbenzoate (**3**), and 3-hydroxy-2,4,5-trimethylphenyl 3-[(2,4-dihydroxy-3,6-dimethylbenzoyl)oxy]-6-hydroxy-2,4-dimethylbenzoate (**4**) (*Fig. 1*).

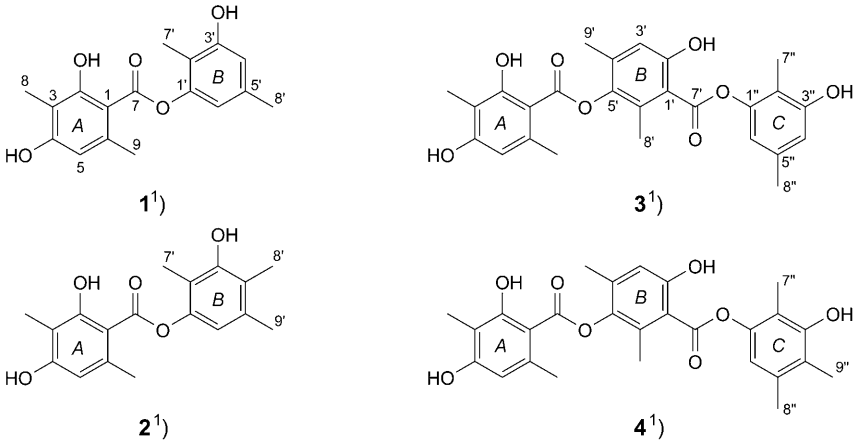

Fig. 1. *Depsides* **1**–**4** *isolated from* C. uredinicola *rice-media culture*

The ^{1}H-NMR data (*Tables 1* and *2*) of the four isolated metabolites **1**–**4** from *C. uredinicola* showed signals in the same regions, with similar chemical shifts and multiplicities. The main spectral characteristic observed for all isolated compounds was the presence of a great number of *s* signals in the ^{1}H-NMR spectra. Aromatic Me groups, aromatic H-atoms with no couplings, and OH groups of the H-bonding type were the structure parts responsible for this profile. These characteristics are compatible with compounds of the depside class, which are compounds derived from orsellinic acid (=2,4-dihydroxy-6-methylbenzoic acid). Due to the ^{1}H-NMR spectra profile of the isolated depsides, the use of ^{13}C-NMR and mainly the 2D-NMR data (HMBC and HSQC) were essential for the signal assignments in the structural determinations of **1**–**4**.

The ^{1}H-NMR spectrum of **1** and **2** showed a great similarity, differing only by one Me group. Thus, their ^{1}H-NMR spectra showed the presence of *s* signals at δ(H) 2.05 (Me(7′)), 2.13 (Me(8)), 2.30 (Me(8′)), and 2.62 (Me(9)) for **1** and 2.03 (Me(7′)), 2.11 (Me(8)), 2.00 (Me(8′)), 2.63 (Me(9)), and 2.23 (Me(9′)) for **2** corresponding to four

[1]) Arbitrary atom numbering

Table 1. *NMR Data* (400 (^{1}H) and 100 MHz (^{13}C)) *of Compounds* **1** *and* **2**. δ in ppm.

	1[1])			**2**[1])		
	δ(C)[a])	δ(H)[b])	HMBC	δ(C)[a])	δ(H)[a])	HMBC
C(1)	103.6	–	Me(9), H–C(5), OH–C(2)	103.4	–	OH–C(2), H–C(5), Me(9)
C(2)	163.9	–	Me(8), OH–C(2)	164.0	–	OH–C(2), Me(8)
C(3)	109.6	–	Me(8), H–C(5)	109.3	–	OH–C(2), H–C(5), Me(8)
C(4)	161.0	–	Me(8), H–C(5)	160.2	–	Me(8)
H–C(5)	111.4	6.30 (*s*)	Me(9)	111.1	6.31 (*s*)	Me(9)
C(6)	140.6	–	Me(9)	140.4	–	Me(9)
C(7)=O	171.4	–	–	170.6	–	–
Me(8)	7.8	2.13 (*s*)	–	7.8	2.11 (*s*)	–
Me(9)	24.6	2.62 (*s*)	H–C(5)	24.8	2.63 (*s*)	–
C(1′)	156.3	–	Me(7′), H–C(6′)	152.8	–	Me(7′)
C(2′)	113.9	–	Me(7′), H–C(6′)	114.5	–	Me(7′), H–C(6′)
C(3′)	149.7	–	Me(7′), H–C(4′)	148.3	–	Me(7′), Me(8′)
H–C(4′) or Me–C(4′)	114.7	6.54 (*s*)	–	135.4	–	Me(8′), Me(9′)
C(5′)	137.0	–	Me(8′)	119.8	–	Me(8′), Me(9′), H–C(6′)
H–C(6′)	114.0	6.56 (*s*)	Me(8′), H–C(4′)	114.6	6.59 (*s*)	Me(9′)
Me(7′)	9.2	2.05 (*s*)	–	9.5	2.03 (*s*)	–
Me(8′)	21.0	2.30 (*s*)	H–C(6′), H–C(4′)	12.5	2.00 (*s*)	–
Me(9′)	–	–	–	19.9	2.23 (*s*)	H–C(6′)
OH–C(2)	–	11.84 (*s*)	–	–	11.99 (*s*)	–
OH–C(4)	–	5.13 (*s*)	–	–	5.34 (br. *s*)	–
OH–C(3′)	–	4.77 (br. *s*)	–	–	4.74 (br. *s*)	Me(8′)

[a]) In $CDCl_3$/CD_3OD 9.75 : 0.25. [b]) In $CDCl_3$.

and five Me groups, respectively, bonded to sp^2 C-atoms. This difference was also supported by ESI-MS data ($[M-H]^-$ at *m*/*z* 301 for **1** and 315 for **2**, *Fig. 2*). Deshielded *s* signals corresponding to aromatic rings and a chelated OH group were present in the spectra of both compounds. As expected, the δ(H) values of **1** and **2** were very similar, due to the similarity between the magnetic environments found in the structures for each substituent. Thus, the NMR spectra contained signals at δ(H) 6.30 (H–C(5)) for **1**, 6.31 (H–C(5)) for **2**, 6.56 (H–C(6′)) for **1**, and 6.59 (H–C(6′)) for **2**. In the NMR spectrum of **1**, there was an extra aromatic H-atom at δ(H) 6.54 (H–C(4′)) when compared with the spectrum of **2**. Chelated and free OH groups, with characteristic shifts were detected for both compounds, at δ(H) 11.84 (OH–C(2)), 5.13 (OH–C(4)), and 4.77 (OH–C(3′)) for **1**, and at δ(H) 11.99 (OH–C(2)), 5.34 (OH–C(4)), and 4.77 (OH–C(3′)) for **2**. The structural similarity of **1** and **2** led basically to the same HMBC data of the aromatic ring *A* (see *Table 1*). The HMBC experiment allowed detection of long-range correlations of O*H*–C(2) with δ(C) 103.6 (C(1)) and 163.9 (C(2)) for **1** as well as with δ(C) 103.4 (C(1)) and 164.0 (C(2)) for **2**. The HMBC data of ring *B* of **1** revealed the correlations of H–C(4′) with δ(C) 21.0 (Me(8′)), 114.0 (C(6′)), and 149.7 (C(3′)), whereas those of **2** showed correlations of Me(8′) with δ(C) 119.8 (C(5′)), 135.4 (C(4′)), and 148.3 (C(3′)). The ^{13}C-NMR data of **1** and **2** were in agreement with the number of CH and Me groups, as well as with that of

Table 2. *NMR Data* (400 (^{1}H) and 100 MHz (^{13}C)) *of Compounds* **3** *and* **4**. δ in ppm.

	3[1])			**4**[1])		
	δ(C)[a])	δ(H)[b])	HMBC	δ(C)[c])	δ(H)	HMBC
C(1)	110.9	–	H–C(5)	108.9	–	OH–C(2), Me(9), H–C(5)
C(2)	164.0	–	OH–C(2), Me(8)	164.0	–	OH–C(2)
C(3)	117.3	–	Me(8)	117.4	–	OH–C(2), H–C(5), Me(8)
C(4)	153.2	–	Me(8), H–C(5)	153.2	–	Me(8), H–C(5)
H–C(5)	116.9	6.31 (*s*)	Me(9)	116.8	6.63 (*s*)	Me(9)
C(6)	140.1	–	Me(9)	140.2	–	Me(9)
C(7)=O	170.2	–	–	170.2	–	–
Me(8)	9.3	2.13 (*s*)	–	9.3	2.12 (*s*)	–
Me(9)	24.4	2.70 (*s*)	H–C(5)	24.8	2.73 (*s*)	H–C(5)
C(1′)	109.2	–	Me(8′), H–C(3′)	108.9	–	OH–C(2′), Me(8), H–C(3′)
C(2′)	164.1	–	Me(8′)	164.2	–	OH–C(2′)
H–C(3′)	111.2	6.30 (*s*)	Me(9′)	111.2	6.32 (*s*)	Me(9′)
C(4′)	140.5	–	Me(9′)	140.7	–	Me(9′)
C(5′)	159.9	–	Me(8′)	159.0	–	Me(8′), H–C(3′)
C(6′)	103.6	–	Me(9′), H–C(3′)	104.1	–	Me(9′), H–C(3′)
C(7′)=O	170.5	–	–	170.3	–	–
Me(8′)	7.7	2.11 (*s*)	–	7.6	2.14 (*s*)	–
Me(9′)	24.5	2.62 (*s*)	H–C(3′)	24.6	2.63 (*s*)	H–C(3′)
C(1″)	155.2	–	Me(7″)	151.9	–	Me(7″), H–C(6″)
C(2″)	114.3	–	Me(7″), H–C(6″), H–C(4″)	114.0	–	Me(7″), H–C(6″)
C(3″)	149.3	–	Me(7″), H–C(4″)	148.0	–	Me(8″), Me(7″)
H–C(4″) or Me–C(4″)	114.2	6.51 (*s*)	Me(8″)	135.8	–	Me(9″), Me(8″)
C(5″)	137.1	–	Me(8″)	120.5	–	Me(9″), M(8″), H–C(6″)
H–C(6″)	114.0	6.58 (*s*)	–	114.9	6.61 (*s*)	Me(9″)
Me(7″)	9.1	2.05 (*s*)	–	9.4	2.05 (*s*)	–
Me(8″)	21.0	2.30 (*s*)	H–C(6″), H–C(4″)	12.4	2.02 (*s*)	–
Me(9″)	–	–	–	19.9	2.25 (*s*)	H–C(6″)
OH–C(2)	–	–	–	–	11.92 (*s*)	–
OH–C(2′)	–	–	–	–	11.70 (*s*)	–
OH–C(4)	–	–	–	–	5.21 (br. *s*)	–
OH–C(3″)	–	–	–	–	4.67 (br. *s*)	–

[a]) In $CDCl_3/CD_3OD$ 9.75 : 0.25. [b]) In CD_3OD. [c]) In $CDCl_3$.

the quaternary sp^2 C-atoms, including the carboxylic C-atoms (δ(C) 171.4 (C(7)) for **1** and 170.6 (C(7)) for **2**).

The depsides **3** and **4** contained each three aromatic rings and two OH groups of the H-bonding type. Six and seven Me-group signals were present in the ^{1}H-NMR spectra of **3** and **4**, respectively. As in the two-ring depsides **1** and **2**, **3** and **4** differed from each other only by one Me group at ring *C*. Thus, a δ(H) 6.51 (H–C(4″)) was observed for **3**, while a *s* of a Me group at δ(H) 2.25 (Me(9″)) was present for **4**. The central ring *B* of **1** and **2** was identical, showing very similar chemical shifts of the corresponding H- and C-atoms. The positions of the substituents at ring *B* of **3** and **4** were determined according to the correlations of H–C(3′) with C(1′) (δ(C) 109.2 (**3**) and 108.9 (**4**)), C(6′) (δ(C)

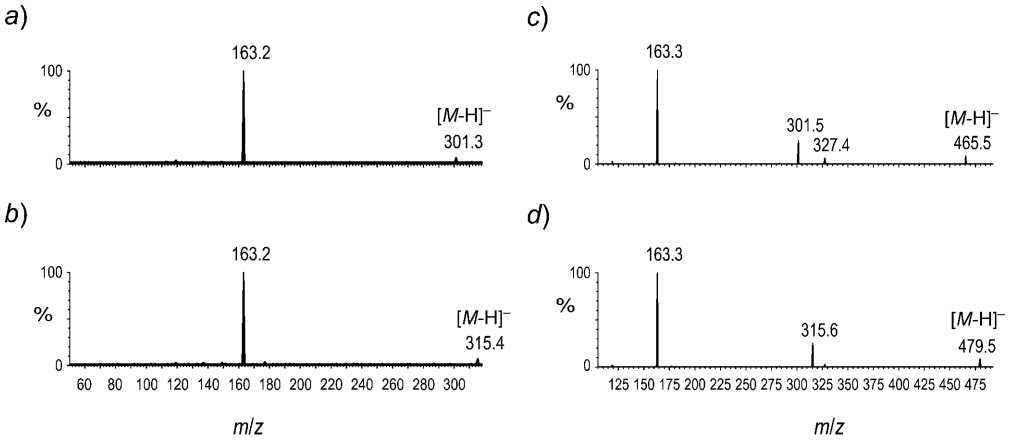

Fig. 2. *ESI-MS/MS* ([$M-H$]$^-$) *of* a) **1** (*m*/*z* 301), b) **2** (*m*/*z* 315), c) **3** (*m*/*z* 465), *and* d) **4** (*m*/*z* 479)

103.6 (**3**) and 104.1 (**4**)), and C(9′) (δ(C) 24.5 (**3**) and 24.6 (**4**)) of Me (8′) with C(1′) (δ(C) 109.2 (**3**) and 108.9 (**4**)) and C(5′) (δ(C) 159.9 (**3**) and 159.0 (**4**)), and of Me(9′) with C(3′) (δ(C) 111.2 (**3**) and 111.2 (**4**)), C(4′) (δ(C) 140.5 (**3**) and 140.7 (**4**)), and C(6′) (δ(C) 103.6 (**3**) and 104.1 (**4**)). The signals of the carboxylate units linking the aromatic ring appeared at δ(C) 170.2 (C(7) of both **3** and **4**) and at δ(C) 170.5 and 170.3 (C(7′) of **3** and **4** resp.). All others correlations obtained from the HMBC experiments are given in *Table 2*. The ^{13}C-NMR data were also in agreement with the number of quaternary C-atoms and CH and Me groups. Besides all data assignments, the ^{1}H- and ^{13}C-NMR data of all new isolated depsides **1**–**4** were compared with closest literature models, mainly with the data of atranorin (= 3-hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenyl 3-formyl-2,4-dihydroxy-6-methylbenzoate) [4] and colletotric acid (=4-carboxy-5-hydroxy-2,3-dimethylphenyl 3-[(2,4-dihydroxy-6-methylbenzoyl)oxy]-5-methoxy-2,4,6-trimethylbenzoate) [5], being in agreement with them.

The ESI mass spectra of **1**–**4** exhibited the quasi-molecular ion [$M-H$]$^-$ at *m*/*z* 301, 315, 465, and 479, respectively (*Fig. 2*), whose elemental compositions were confirmed by HR-MS. According to CID (collisionally induced dissociation) spectra of the product ions of each depside **1**–**4**, it was possible to establish proposals for the fragmentation mechanism, as shown in *Fig. 3* for **3** and **4**. Due to the high structural similarity of **1**–**4**, the fragmentation profile was very similar for all of them, resulting in a fragment ion at *m*/*z* 163. This fragment ion was probably produced by a *McLafferty*-type rearrangement followed by an acyl cleavage. The product-ion MS obtained for compounds **3** and **4** contained peaks at *m*/*z* 301 and 315, respectively (the same precursor ions were detected for **1** and **2**), which arose by a similar fragmentation but eliminating a neutral 164 mass-unit fragment (*Fig. 3*).

Depsides are polyketides commonly produced by lichens and other organisms associated with them, including higher plants and microorganisms [6–8]. These compounds contain two or more aromatic rings linked by a carboxy unit. It appears that orsellinic acid is the key tetra ketide unit that is esterified during the route to depside

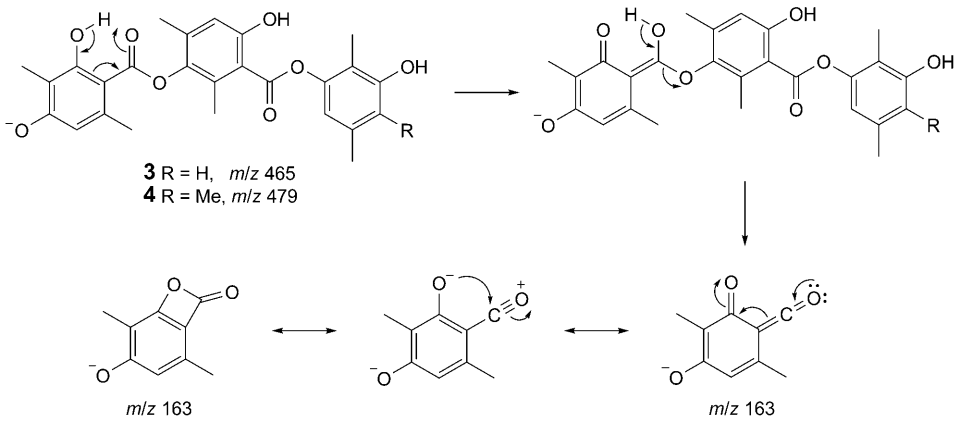

Fig. 3. *Plausible fragmentation mechanism for the isolated depsides* **3** *and* **4** *in the MS*

formation [9]. Due to the biosynthetic pathway of these polyketides, most of the known depsides show at least one carboxy or MeO group as ring substituent. However, this fact is not observed in the *C. uredinicola* depsides **1**–**4**, which is not so common for this kind of compounds in nature. For instance, among more than 15 depsides produced by the lichen species *Ervernia prunastri*, only one of them, lecaronin (= 3-hydroxy-5-methylphenyl 2,4-dihydroxy-6-methylbenzoate), has the same characteristic [10]. Among the compounds produced by *C. uredinicola*, depside 3-hydroxy-2,5-dimethylphenyl 2,4-dihydroxy-3,6-dimethylbenzoate (**1**) is reported for the first time as a natural product. This compound has already been obtained as a product of thermal decomposition of atranorin (= 3-hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenyl 3-formyl-2,4-dihydroxy-6-methylbenzoate) [10].

2. *Antimicrobial Activity.* Several properties of this class of compounds, such as anti-HIV-1 integrase and antiviral activity, analgesic, anticancer, antimicrobial, and herbicide activities have already been reported [6][7][11], which suggest that the isolated depsides **1**–**4** can be promising biologically active compounds.

The activity of compounds **1**, **3**, and **4** was tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis.* A bactericide effect of **1** was observed at a dosage of 25 μg/ml when examined in the presence of *Bacillus subtilis* and *Pseudomonas aeruginosa*. For *Escherichia coli* and *Staphylococcus aureus*, a bactericide effect was observed at 250 μg/ml. Compound **3** showed a bacteriostactic effect for all bacteria, at a dosage of 250 μg/ml, while compound **4** showed a bacteriostatic effect in the presence of *Escherichia coli* and *Staphylococcus aureus* at a dosage of 250 μg/ml, and in the presence of *Bacillus subtilis* and *Pseudomonas aeruginosa* at 25 μg/ml. Compound **2** was not tested yet.

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Experimental Part

General. CC = Column chromatography. Anal. HPLC: *Shimadzu-SPD-M10A-VP* diode-array detector, *LC-10AD* pumps, *SIL-10ADVp* auto sampler, and UV/VIS *SPD-6A* detector. Prep. HPLC: *Shimadzu* UV detector, *LC-6AD* pump, *SPD-10AV* UV detector and manual injector. HPLC: anal. column, *Luna Phenyl-Hexyl* (5 μm, 250 × 21.20 mm; *Phenomenex*); prep. column, *Luna Phenyl-Hexyl* (5 μm, 250 × 4.60 mm; *Phenomenex*). UV Spectra: *Hewlett Packard 8452-A*; in MeOH; λ_{max} (log ε) in nm. IR Spectra: *Bomen MB-102*, KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX400*; at 400 (^1H) and 100 MHz (^{13}C); in $CDCl_3$ or CD_3OD; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Micromass Quattro-LC* equipped with an ESI 'Z-spray' ion source operating in the neg.-ion mode; in m/z (rel. %). HR-MS: ESI/TOF-MS *Waters Synapt HDMS*; desolvation and ion-source block temp., 120 and 90°, resp.; gaseous N_2 was used to nebulize (80 l h^{-1}) and desolvate (788 l h^{-1}); optimal voltages for the probe and ion source: 3.18 kV for the stainless-steel capillary, 32 V for the sample cone, and 3 V for the extractor cone. ESI-MS/MS: Ar was added in the collision cell, to produce a pressure of $1.5 \cdot 10^{-3}$ mbar for collisionally induced dissociation (CID); collisional energy 10 eV.

Fungal Material and Identification. The fungus *Cladosporium uredinicola* was isolated from *Psidium guajava* fruits which were bought in São Carlos local trade, São Paulo state, Brazil. Firstly, the fruits were washed with detergent in current water. After that, the general procedures adopted followed an adapted methodology described by *Petrini* and co-workers [12]. The peeled fruit was sterilized by alternate immersions in 70% EtOH soln., H_2O, 11% aq. NaClO soln., and H_2O, for 1 min each. After peeling, little pulp pieces were deposited on *Petri* dishes containing PDA media (potato/dextrose/agar) and incubated in the dark at 25° for one week. The fungus *Cladosporium uredinicola* grew as brown-grey colonies from guava pulp following these procedures after two collections during October 2007 and November 2008. The microorganism was identified by colleagues of the Laboratório de Produtos de Origem Microbiana, Universidade Federal do Amazonas – ICB-DFCA/UFAM.

Extraction and Isolation. The fungus was grown in 33 *Erlenmeyer* flasks (500 ml), each one containing 90 g of *Uncle Ben*'s® rice and 80 ml of dist. H_2O. The rice media were sterilized twice in an autoclave system (121°). Small pieces of PDA medium with *C. uredinicola* mycelium were transferred to 30 flasks; 3 flasks were maintained as control medium. After 30 d of fungal growth at 25°, EtOH (300 ml) was added into each *Erlenmeyer* flask, and the content was triturated. The EtOH extraction was kept during 12 h under static conditions. The solid and liquid phases were separated by vacuum filtration, and the liquid was evaporated. The EtOH extract was submitted to vacuum CC (silica gel (70–230 mesh), gradient 100% CH_2Cl_2 100% to 100% AcOEt): four fractions (TLC monitoring). The CH_2Cl_2/AcOEt 7:3 fraction was subjected to low-pressure CC (silica gel (230–400 mesh, 5 × 25 cm), CH_2Cl_2, AcOEt, and MeOH gradient). The medium-polarity fractions, obtained with CH_2Cl_2/AcOEt 1:1 contained the mixture of depsides and was analyzed by anal. HPLC (isocratic elution system adjusted to the best resolution of depsides bands, *i.e.*, 80% MeOH, flow rate 0.7 ml/min). The fractions with depsides were submitted to reversed-phase prep. HPLC (80% MeOH, at 15 ml/min, according to the scaling up from the anal. HPLC conditions, UV detection at 275 nm). Seven depsides were isolated but only four of them were obtained in sufficient amounts to acquire good spectroscopic data: **1** (8 mg), **2** (5 mg), **3** (18 mg), and **4** (16 mg)).

3-Hydroxy-2,5-dimethylphenyl 2,4-Dihydroxy-3,6-dimethylbenzoate (**1**): White amorphous powder. UV (MeOH): 222 (4.43), 276 (4.17), 306 (3.87). IR (KBr): 3421, 2925, 1652, 1643, 1635, 1271, 1155. ^1H-NMR (400 MHz, $CDCl_3$ and CD_3OD) and ^{13}C-NMR (100 MHz, $CDCl_3$), HSQC, and HMBC: *Table 1*. ESI-MS/MS daughter ions, 10 eV): 301 (5, $[M-H]^-$), 163 (100). HR-MS: 301.1067 ($[M-H]^-$, $C_{17}H_{17}O_5^-$; calc. 301.1076).

3-Hydroxy-2,4,5-trimethylphenyl 2,4-Dihydroxy-3,6-dimethylbenzoate (**2**): White amorphous powder. UV (MeOH): 222 (4.43), 274 (4.19), 308 (3.85). IR (KBr): 3421, 2979, 1652, 1647, 1635, 1627, 1271, 1155. ^1H- (400 MHz, $CDCl_3$ and CD_3OD) and ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD), HSQC, and HMBC: *Table 1*. ESI-MS/MS (daughter ions, 10 eV): 315 (2, $[M-H]^-$), 163 (100). HR-MS: 315.1227 ($[M-H]^-$, $C_{18}H_{19}O_5^-$; calc. 315.1232).

3-Hydroxy-2,5-dimethylphenyl 3-[(2,4-Dihydroxy-3,6-dimethylbenzoyl)oxy]-6-hydroxy-2,4-dimethylbenzoate (**3**): White amorphous powder. UV (MeOH): 226 (4.39), 278 (4.13), 310 (3.81). IR (KBr):

3419, 2925, 1664, 1623, 1583, 1147, 1257. ^{1}H- (400 MHz, $CDCl_3$ and CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD), HSQC, and HMBC: *Table 2*. ESI-MS/MS (daughter ions, 10 eV): 465 (8, $[M-H]^-$), 301 (25), 163 (100). HR-MS: 465.1522 ($[M-H]^-$, $C_{26}H_{25}O_8^-$; calc. 465.1548).

3-Hydroxy-2,4,5-trimethylphenyl 3-[(2,4-Dihydroxy-3,6-dimethylbenzoyl)oxy]-6-hydroxy-2,4-dimethylbenzoate (**4**): White amorphous powder. UV: 226 (4.37), 278 (4.12), 310 (3.82). IR (KBr): 3421, 2931, 1706, 1654, 1623, 1585, 1143, 1257. ^{1}H- (400 MHz, $CDCl_3$ and CD_3OD) and ^{13}C-NMR (100 MHz), HSQC, and HMBC: *Table 2*. ESI-MS/MS (daughter ions, 10 eV): 479 (10, $[M-H]^-$), 315 (20), 163 (100). HR-MS: 479.1674 ($[M-H]^-$, $C_{27}H_{27}O_8^-$; calc. 479.1705).

Antibacterial Bioassay. The minimal inhibitory concentrations (*MIC*s) were determined by the microbroth dilution assay as recommended by the *Subcommittee on Antifungal Susceptibility Testing* of the *US National Committee for Clinical Laboratory Standards* (*NCCLS*). The assays were performed on 96-well plates with 100 µl of *Mueller–Hinton* broth (MHB), 100 µl of test compound, and 5 µl of test bacteria at 1.0×10^7 UFC ml^{-1}, followed by incubation at 37° (24 h). The test substances obtained from the fungus culture were dissolved in DMSO at the initial concentration 250 µg/ml. The tested microorganisms were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Bioactivity was recorded as blue coloration in the wells (use of resazurin dye), and their bacteriostatic or bactericidal effects were observed on *Mueller–Hinton* agar plates. Pos. (vancomycin and tetracycline) and neg. (DMSO) controls were used during the tests.

REFERENCES

[1] L. A. A. Gunatilaka, *J. Nat. Prod*. **2006**, *69*, 509.
[2] G. A. Strobel, *Microbes Infection* **2003**, *5*, 535.
[3] R. M. P. Gutiérrez, S. Mitchell, R. V. Solis, *J. Ethnopharmacol.* **2008**, *117*, 1.
[4] M. G. Carvalho, G. A. Carvalho, R. Braz Filho, *J. Braz. Chem. Soc.* **2000**, *11*, 143.
[5] W. X. Zou, J. C. Meng, H. Lu, G. X. Chen, T. Y Zhang, R. X. Tan, *J. Nat. Prod.* **2000**, *63*, 1529.
[6] P. C. Lv, Z. P. Xiao, R. Q. Fang, H. Q. Li, H. L. Zhu, C. H. Liu, *Eur. J. Med. Chem*. **2009**, *44*, 1779.
[7] D. Joulain, R. Tabacchi, *Flavour Fragrance J.* **2009**, *24*, 49.
[8] T. Narui, K. Sawada, S. Takatsuki, T. Okuyama, C. F. Culberson, W. L. Culberson, S. Shibata, *Phytochemistry* **1998**, *48*, 815.
[9] N. K. Honda, W. Vilegas, *Quim. Nova* **1998**, *21*, 110.
[10] S. Huneck, J. Schimit, R. Tabacchi, *Z. Naturforsch. B* **1989**, *44*, 1283.
[11] I. S. Rojas, B. Lotina-Hansen, R. Mata, *J. Nat. Prod.* **2000**, *63*, 1396.
[12] O. Petrini, T. N. Sieber, L. Toti, O. Viret, *Nat. Toxins* **1993**, *1*, 185.

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