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Production of phenylacetic acid derivatives and 4-epiradicinol in culture by *Curvularia lunata*

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Abstract

Phenylacetic acid derivatives, methyl 2-acetyl-3,5-dihydroxyphenylacetate (**1**) and methyl 2-acetyl-5-hydroxy-3-methoxyphenylacetate (**3**); curvulin or ethyl 2-acetyl-3,5-dihydroxyphenylacetate (**4**), a known metabolite of *Curvularia siddiqui*, and 4-epiradicinol (**5**) have been isolated from the culture mycelia of *Curvularia lunata* grown on YMG, a medium consisting of yeast, malt extract and glucose. Compounds **1**, **3** and **4** lack antimicrobial and antioxidant activity, but 4-epiradicinol (**5**) inhibited the growth of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella choleraesuis* and *Bacillus subtilis*. The structures of compounds **1**, **3**–**5** were determined by analyses of IR, MS, 1D and 2D NMR data, assisted by chemical shift comparison to related and model compounds. The relative stereochemistry of the vicinal diol in **5** was determined from the ^{1}H NMR signals for the methyl protons of the resulting cyclic acetonide prepared from **5**.

1. Introduction

Fungi are a prolific source of new compounds with fascinating biological activities (Gloer, 1997). The monocultures of the fungus, *Curvularia lunata* for instance have produced several bioactive metabolites including radicinin (2,3-dihydro-3-hydroxy-2-methyl-7-[(1*E*)-1-propenyl]-4H, 5H-pyrano[4,3-b]pyran-4,5-dione), an antimicrobial agent and inhibitor of human rhinovirus 3C-protease (Kadam et al., 1994). (+) Abscisic acid, lunatin, cynodontin, and cytoskyrin A have also been isolated from *C. lunata* (Jadulco et al., 2002; Hobson et al., 1997). Here, we report the isolation of methyl 2-acetyl-3,5-dihydroxyphenylacetate (**1**) and methyl 2-acetyl-5-hydroxy-3-methoxyphenylacetate (**3**), as new phenylacetic acid derivatives and also known ethyl 2-acetyl-3,5-dihydroxyphenylacetate (**4**) and 4-epiradicinol (**5**) from the culture mycelia of *C. lunata*. Phenylacetic acid derivatives such as curvulin or ethyl 3,5-dihydroxyphenylacetate (**4**), curvulinic acid or 2-acetyl-3,5-dihydroxyphenylacetic acid (**2**) and curvulol or 4-acetyl-5-β-hydroxyethyl resorcinol were previously isolated from *Curvularia siddiqui* in 1963 by Kamal and co-workers but their NMR data were absent. Radicinol or 3,4-dihydroxy-2-methyl-7-(1*E*)-1-propenyl-2H,5H-pyrano[4,3-b]-pyran-5-one (**6**) is a phytotoxin produced by the genera *Cochiliobolus* (Nukina and Marumo, 1977), *Alternaria* (Robeson et al., 1982; Sheridan and Canning, 1999; Solfrizzo et al., 2004) and *Bipolaris* (Nakajima et al., 1997) but the occurrence of 4-epiradicinol (**5**) in the genus *Curvularia* is reported here for the first time. *C. lunata* has been reported to cause allergic fungal sinusitis (MacMillan et al., 1987).

The genus *Curvularia* belongs to the Deuteromycetes (Mitosporic fungi) group of fungi and more than eight species have been isolated from the roots and seeds of plants (Watanabe, 2002) (see Fig. 1).

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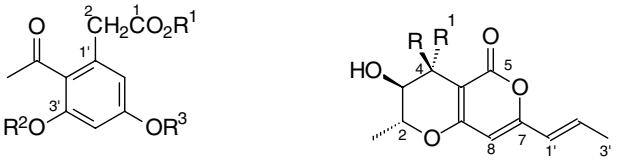


1 $R^1 = CH_3$, $R^2 = R^3 = H$
2 $R^1 = R^2 = R^3 = H$
3 $R^1 = R^2 = CH_3$, $R^3 = H$
4 $R^1 = CH_2CH_3$, $R^2 = R^3 = H$
5 $R^1 = H$, $R = OH$
6 $R^1 = OH$, $R = H$

Fig. 1. The structure of compounds **1** and **3**–**5** isolated from *C. lunata*.

2. Results and discussion

The residues of EtOAc extracts of the culture filtrate and mycelia of *C. lunata*, grown by inoculating YMG, a medium consisting of yeast, malt extract and glucose, with potato dextrose agar plugs from a stock culture of *C. lunata*, exhibited similar antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella choleraesuis*. The EtOAc extract of the mycelia obtained, after the fungi was cultured for 9 days, was subjected to column chromatography over silica gel to give compounds **1** (19 mg), **3** (9 mg), **4** (36 mg) and **5** (10 mg) in addition to the glycerides of linoleic and oleic acids.

Judging from the ^{1}H and ^{13}C NMR data (see Table 1), compounds **1**, **3** and **4** are derivatives of 2-acetyl-3,5-dihydroxyphenyl acetic acid (**2**) or curvulinic acid (Kamal et al., 1963). HREIMS analysis of **1** gave a molecular ion at *m/z* 224.0691 that is compatible with the formula $C_{11}H_{12}O_5$. The molecular formula suggested a total of six unsaturation equivalents in compound **1**, one of which must be due to a ring form. Compound **1** showed absorptions for acetyl and ester groups at 1682 and1723 cm^{-1} in the IR spectrum. The acetyl and methyl ester groups were also observed as singlet signals at δ 2.54 and 3.80 in the ^{1}H NMR and showed cross-peaks with the signals at δ_C 32.1 and 55.7 in the HSQC spectrum. A prominent singlet at δ 3.75, which showed cross-peaks with δ_C 40.7 (C-2) in the HSQC spectrum, was assigned to the benzyl protons (H-2) of **1**. The ^{1}H NMR data of **1** also revealed two meta-coupled aromatic protons, H-4′ and H-6′, each a doublet ($J = 2.3$ Hz) at δ 6.35 and 6.34, respectively; the ^{13}C NMR data displayed six quaternary carbons two of which are acetyl and ester carbonyl carbons and resonated at δ_C 204.2 and 172.5. Alkaline hydrolysis of **1** gave curvulinic acid (**2**). Taken together, compound **1** was identified as methyl 2-acetyl-3,5-dihydroxyphenylacetate.

The IR spectrum of compound **3** showed absorptions at 3400, 1723, and 1704 cm^{-1} indicating the presence of phenol, ester and ketone functional groups. Compound **3** analyzed for $C_{12}H_{14}O_5$ in the HREIMS data, a formula requiring six unsaturation equivalents one of which must be due to a ring form. The ^{1}H and ^{13}C NMR spectra of **3** revealed 12 carbon atoms: three methyl groups, one methylene unit, six aromatic carbons, one ketone and one carboxyl carbon. Of the aromatic carbons, two were not substituted, and of the substituted carbons two were oxygen-bound. The protons of the two unsubstituted aromatic carbons were established to be *meta*-coupled on the basis of coupling constant ($J = 2.3$ Hz). The NMR data (Table 1) showed the presence of an acetyl unit at δ 2.51 and its position was confirmed by HMBC correlations between the protons of the acetyl methyl at δ 2.51 and phenyl carbon C-2′ at δ 120.5. The HMBC spectrum of **3** also showed cross-peaks between δ 6.43 (H-6′), 6.42 (H-4′) and the signals at δ 120.5 (C-2′) and 163.0 (C-5′); between δ 6.43 (H-6′) and the benzyl carbon resonance at δ 40.5 (C-2) and between δ 3.75 (H-2) and the carboxyl carbon and phenyl carbon signals at δ172.4 (C-1) and 137.3 (C-1′), respectively. Similar correlations were observed in the HMBC spectra of **1** and **4** and enabled compounds **1**, **3** and **4** to

Table 1
^{1}H and ^{13}C NMR data for compounds **1**, **3** and **4** in $CDCl_3$

Entry	**1**			**3**			**4**		
	δ_H	*J* (Hz)	δ_C	δ_H	*J* (Hz)	δ_C	δ_H	*J* (Hz)	δ_C
1			172.5			172.4			171.3
2	3.75	(2H, *s*)	40.7	3.75	(2H, *s*)	40.5	3.76	(2H, *s*)	40.1
1′			138.2			137.3			137.4
2′			119.5			120.5			119.0
3′			162.0			163.4			161.3
4′	6.34	(1H, *d*, 2.3)	102.7	6.42	(1H, *d*, 2.3)	100.9	6.30	(1H, *d*, 2.3)	102.3
5′			161.8			163.0			161.2
6′	6.35	(1H, *d*, 2.3)	112.3	6.43	(1H, *d*, 2.3)	111.1	6.35	(1H, *d*, 2.3)	111.9
(C-2′)-COCH_3	2.54	(3H, *s*)	32.1	2.51	(3H, *s*)	31.7	2.51	(3H, *s*)	31.7
(C-2′)-$COCH_3$			204.2			204.2			203.3
(C-3′/C-5′)-OH	3.50	Broad					4.0	Broad	
(C-3′)-OCH_3				3.80	(3H, *s*)	55.7			
(C-5′)-OH				4.0	Broad				
(C-1)-OCH_3	3.77	(3H, *s*)	55.7	3.76	(3H, *s*)	55.7			
(C-1)OCH_2CH_3							4.10	(2H, *q*, 7.1)	60.6
(C-1)OCH_2CH_3							1.19	(3H, *d*, 7.1)	14.0

be identified as phenylacetic acid derivatives. The DEPT and HSQC data of **3** were consistent with the presence of one exchangeable proton and also allowed the three methyl groups at δ 31.7, 55.7 and 55.7 to be attributed to the acetyl, ester and ether groups. In the UV, **3** absorbed at 220 ($\log\varepsilon = 3.6$), 270 ($\log\varepsilon = 3.4$) nm and more intensely at 220 ($\log\varepsilon = 4.6$) and 350 ($\log\varepsilon = 4.6$) nm on addition of NaOH. The shift in the UV absorption is consistent with the presence of a phenolic acetophenone chromophore.

On the basis of the NMR data so far discussed two partial structures **3a** and **3b** shown in Fig. 2 are possible for compound **3**. The correct partial structure was suggested by NOE experiment that was performed with **3** in CD_3COCD_3 to which a drop of water was added. The water signal at δ 3.75 was irradiated; there was saturation transfer to the phenolic hydroxyl group (Feeney and Heinrich, 1966) and a proportional enhancement of the two doublets at 6.42 and 6.43 was observed. This observation supported partial structure **3a**. In addition, a solution of compound **3** in EtOH did not give a color change when mixed with aqueous ferric chloride solution. This further supported the assignment of the methoxy group to position C-3′ in compound **3.**

Compounds **3** and **4** have identical molecular mass and formula but **4** gave compound **2** on alkaline hydrolysis. The NMR data of **4** is listed in Table 1. The presence of the characteristic A_2B_3 signals for $CH_3CH_2O^-$ moiety in its ^{1}H NMR spectrum and of the McLafferty re-arrangement-derived $(M-28)^+$ peak at m/z 210 in the mass spectrum of **4**, which are absent in the ^{1}H NMR and EIMS spectra of **3**, further confirmed that compounds **3** and **4** are structural isomers.

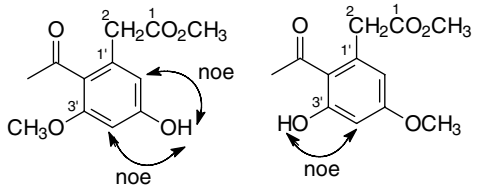


Fig. 2. Two likely substitution patterns for compound **3**; NOE experiment supported **3a**.

Biosynthetically, 2-acetyl-3,5-dihydroxyphenyl acetic acid (**2**), the basic chemotype of compounds **1**, **3** and **4** must be derived through the polyketide pathway.

The spectral data of compound **5** is quite different from those of **3** and **4** with which it has identical molecular formula. Structurally, compound **5** resembles radicinin (Tanabe et al., 1970) and radicinol (Nukina and Marumo, 1977; Robeson et al., 1982) but differs in certain respects. In the IR, compound **5** absorbed at 3233 (broad), 1720 (conjugated ester) and 1584 cm^{-1} (double bonds). The UV showed long wavelengths absorption at 240 ($\log\varepsilon = 3.3$), 280 ($\log\varepsilon = 3.2$) and 320 ($\log\varepsilon = 3.3$) nm, that are compatible with the presence of an α,β unsaturated carbonyl chromophore that is extended by at least two double bonds and some auxochromes (Hesse et al., 1997). Positive EIMS gave the molecular ion of 238 and M−18 peak at m/z 220. HREIMS analysis suggested a molecular formula of $C_{12}H_{14}O_5$, which is consistent with the number of carbon and hydrogen atoms estimated in the NMR spectra. The molecular formula of **5** also suggested a total of six unsaturated equivalents, two of which must be due to ring forms.

The ^{1}H and ^{13}C NMR spectra of compound **5** (Table 2) revealed two methyl groups, six olefinic carbons three of which are protonated, one carboxyl carbon and three sp^3 oxymethine units two of which are secondary alcohols. The UV absorptions and tracking of correlations in the ^{1}H–^{1}H COSY, HSQC and HMBC data revealed the presence of two partial structures **5a** and **5b** in compound **5**.

In substructure **5a** (see Fig. 3), the protons of the methyl group at C-2 resonated as a doublet ($J = 6.6$ Hz) at δ 1.45 because of their coupling to the oxymethine proton at δ 4.28 (H-2). H-2 was a quartet ($J = 6.6$ Hz). H-3 was observed at δ 3.68 as a partially resolved doublet of a doublet ($J = 5.6$, 0.3 Hz), which must have resulted from the coupling of H-3 to H-4 (δ 4.47) and to the hydroxy proton

Table 2
^{1}H and ^{13}C NMR data for compound **5** ^{1}H NMR data for **7** in $CDCl_3$

Entry	δ_H	**5**			**7**	
		J (Hz)	δ_C	HMBC	δ_H	J (Hz)
2	4.28	(1H, *q*, 6.6)	77.9	17.3, 72.8	4.20	(1H, *q*, 7.2)
3	3.68	(1H, *dd*, 5.6, 0.3)	72.8	17.3	4.76	(1H, *d*, 4.0)
4	4.47	(1H, *d*, 5.6)	67.3		4.97	(1H, *d*, 4.5)
5			164.0			
7			159.6			
8	5.97	(1H, *s*)	99.9		5.80	(1H, *s*)
9			159.1			
10			101.9			
1′	6.15	(1H, *dd*, 15.5, 1.7)	124.0	18.3	5.98	(1H, *d*, 15.4)
2′	6.59	(1H, *dd*, 15.5, 6.9)	134.7	18.3	6.72	(1H, *dd*, 15.5, 7.0)
3′	1.89	(3H, *dd*, 6.9, 1.7)	18.3	134.7, 124.0	1.91	(3H, *d*, 7.0)
(C-2)-CH_3	1.45	(3H, *d*, 6.6)	17.3	72.8, 77.9	1.49	(3H, *d*, 7.2)
Acetonyl-CH_3					1.33	(3H, *s*)
Acetonyl-CH_3					1.47	(3H, *s*)

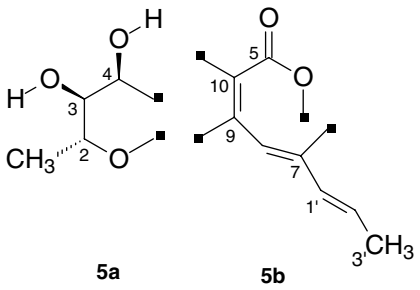


Fig. 3. NMR-derived partial substructures of compound **5**.

of the secondary alcohol at C-3, respectively. The oxymethine proton at H-4, which was observed as a doublet ($J = 5.6$ Hz) at δ 4.47, must be vicinally *cis* to H-3 and flanked by a quaternary carbon. Although the coupling between H-2 and H-3 was not observed in the ^{1}H NMR spectrum of **5**, the signal at δ 4.28 (H-2) showed cross-peaks with the signals at δc 72.8 (C-3) and 17.31 (the methyl group at C-2) in the HMBC spectrum. H-3 and H-2 are therefore vicinally *trans* and about 90° to each other. Taken together, the stereogenic centers 2 and 3 in **5** and radicinol (**6**) must have identical configurations, which are 2*S*,3*R* (Robeson et al., 1982) and compound **5** must be a 2*S*,3*R*,4*R* diastereomer of radicinol (**6**).

Substructure **5b** (see Fig. 3) is discernible in radicinin (Tanabe et al., 1970). The signals of the methyl group protons at δ_H 1.89 (H_3-3′) were observed as a doublet of a doublet ($J = 6.9$, 1.7 Hz). H-1′ and H-2′ are mutually coupled vinyl protons and their coupling constant, $J = 15.5$ Hz, suggested an *E*-configuration for the double bond. Apart from a singlet proton at δ 5.97 (H-8), whose corresponding carbon atom resonated at δ 99.9 in the HSQC spectrum, there were no other signals in the ^{1}H NMR that can be attributed to substructure **5b**. With the help of a molecular model and coupling constant values, the relative configuration of compound **5** was suggested by NOESY correlations shown in Fig. 4. Correlation contours between H-4 and H-3 and the protons of the methyl group at C-2 were observed.

To further establish the stereochemistry of the hydroxy groups at positions C-3 and C-4 in compound **5**, we have **5** converted into a cyclic acetonide (Scheme 1).

In acetonide or cyclic acetal derivative of diols, the oxymethine oxygen atoms are connected but the stereochemistries of the parent diols are not expected to change (Colman-Saizarbitoria et al., 1995; Gu et al., 1994). Significant differences in the acetonyl methyl protons (Colman-Saizarbitoria et al., 1995) or cyclic acetal protons (Gu et al., 1994) in the *cis* or *trans* configurations of the cyclic acetonide or acetal derivatives enable the relative stereochemistries of the parent diols to be assigned.

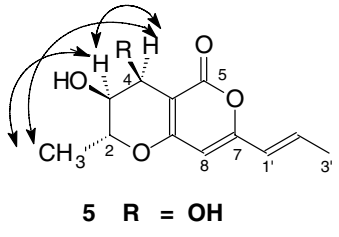
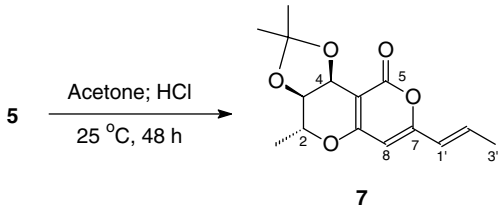


Fig. 4. Significant NOESY correlations in compound **5**.



Scheme 1.

In the ^{1}H NMR spectrum, H-3 and H-4 of acetonide **7** resonated at δ 4.76 and 4.97 and the acetonyl methyl protons were observed as two well-separated singlets at δ 1.33 and 1.46 (Table 2) suggesting a *cis* configuration for the parent diols.

Compound **5**, which is isolated for the first time from genus *Curvularia* is identified as C-4 epi of radicinol (**6**). Radicinol derivatives have previously been isolated from *A. chrysanthemi* (Robeson et al., 1982; Sheridan and Canning, 1999) and *Bipolaris coicis* (Nakajima et al., 1997).

Following standard protocols, compounds **1**, **3**–**5** were evaluated for antioxidant (Chen et al., 1999) and antimicrobial (Anonymous, 2001) activities. They were all inactive in the antioxidant assay, inhibiting DPPH radicals at less than 10% control. The phenylacetic acid derivatives also lacked antibacterial and antifungal properties. However, 4-epi-radicinol (**5**) inhibited the growth of *E. coli* (mic 125 μg/ml), *Salmonella choleraesuis* (mic 125 μg/ml), *Staphylococcus aureus* (mic 62.5 μg/ml), *and Bacillus subtilis* (mic 31.8 μg/ml), but was inactive against *Klebsiella pneumoneae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231), respectively.

3. Experimental

3.1. General

^{1}H–^{1}H COSY, HSQC, DEPT, and HMBC were recorded on Bruker Advance 400 MHz NMR spectrometer in $CDCl_3$ with TMS as internal standard. UV spectra were recorded using UV–Visible HP-8453 spectrophotometer. IR spectra were recorded on a Nicolet FT-IR spectrometer in solution or using KBr disks. HR-EIMS spectra were recorded with a JEOL JMS-SX102A (EIMS, 70 eV, gun high 3.0 kV). For chromatography, EM Science Silica gel 60 (70–230 mesh ASTM) was used and all solvents were distilled prior to use. Whatman precoated silica gel (60A° K6F) plates were used for TLC, with compounds visualized by their UV absorbance at 254 nm and by spraying with 10% (v/v) H_2SO_4 or vanilline–H_2SO_4 followed by heating.

3.2. Fungal material

C. lunata used in this study was obtained from grass (Mseblo) leaf spot collected by Abddullah Al Sa'di at Barka in October 2003 in the Sultanate of Oman. A piece of the leaf spot was removed and thoroughly surface-rinsed with 1% NaOCl, sterile distilled H_2O and dried under a laminar flow hood. Then, tissues of the sample were placed on agar plates. After several days of incubation, hyphal tips of the fungi were removed and sub-cultured on potato dextrose agar (PDA) plates at 25 °C for 5 days. After repeated inoculation on fresh PDA plates, pure fungal cultures were isolated. The isolated fungi was identified by Centraalbureau voor Schimmelcultures (CBS) at the Fungal Biodiversity Centre, Institute of the Royal Academy of Arts and Sciences, Utrecht, The Netherlands and assigned the accession number Det 134-2003-DUF-19. Ten inoculum discs of 9-day-old subcultures were used to inoculate 500 mL of autoclaved YMG (consisting of yeast extract, 4 g; malt extract, 10 g; glucose, 4 g and distilled water, 1 L) in 1 L Erlenmeyer flasks at fermentation pH of 5.8–6.0 and incubated in an orbital shaker (Sanyo Gallenkamp Orbital Incubator) operating at 130 rpm at 25 °C for 9 days. The fungal biomass was separated by filtration, and the culture filtrate and mycelia were extracted separately with EtOAc.

3.3. Extraction and isolation

The wet mycelia were milled using a domestic blender and then soaked in EtOAc (50 g of mycelia in 100 ml of EtOAc). After two days, the mixture was filtered and the mycelia re-extracted by maceration with 100 mL of EtOAc for 24 h. The combined filtrate was dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum to give the mycelia extract. After several fermentation and extraction processes, 550 g of wet mycelia gave 120 mg of the mycelia extract.

The EtOAc extract of the mycelia (530 mg) was applied to an open column (column diameter 2 cm) of silica gel (35 g) and eluted with a gradient of petroleum ether–EtOAc in this order: (solvent mixture, volume eluted) and collecting fractions in portions of 50 mL. Petroleum ether: EtOAc (9.5:0.5, 2.5 L), (9:1, 1 L), (8.5:1.5, 3.5 L), (8:2, 2 L), (7.5:2.5, 1.1 L), (6.5:3.5, 0.9 L), (6:4, 0.6 L), (1:1, 3.9 L), (4.5:5.5, 2.3 L), (4:6, 0.8 L), (3.5:6.5, 0.9 L), (6:4, 0.6 L), (4.5:5.5, 2.3 L), (4:6, 0.8 L) and (3.5:6.5, 0.9 L). Similar fractions were pooled based on TLC analysis. Petroleum ether:EtOAc (9.5:0.5) eluted compound **4**, curvulin (36 mg), petroleum ether–EtOAc (8.5:1.5) gave compound **5** (10 mg) and petroleum ether:EtOAc (1:1) afforded compounds **1** (19 mg) and **3** (9 mg).

3.4. Methyl 2-acetyl-3,5-dihydroxyphenylacetate (*1*)

Semi-solid; UV (CH_2Cl_2) λ_{max} (log ε) 229 (3.7), 300 (3.3) nm; IR (KBr) ν_{max} 3300, 1723, 1682, 1606, 1439, 1264, 1158, 1010, 839 cm^{-1}. ^{13}C NMR and ^1H NMR see Table 1; EIMS *m/z* 224 ([M^+], 25), 209 (10), 181 (100), 164 (53), 135 (11), 28 (35), 18 (70). HREIMS *m/z* 224.0691 (calcd. for $C_{11}H_{12}O_5$, 224.0685).

3.5. Methyl 2-acetyl-5-hydroxy-3-methoxyphenylacetate (*3*)

Semi-solid; UV (CH_2Cl_2) λ_{max} (log ε) 220 (3.6), 270 (3.4) nm; IR (KBr) ν_{max} 3400, 1723, 1704, 1600, 1589, 1473, 1264, 1156, 1025, 870, 800 cm^{-1}. ^{13}C NMR and ^1H NMR see Table EIMS *m/z* 238 ([M^+], 15), 224 (14), 196 (35), 181 (100) 165 (50), 150 (20), 125 (20), 86 (30), 70 (80), 41 (30), 18 (20), HREIMS *m/z* 238.0828 (calcd. for $C_{12}H_{14}O_5$, 238.0841).

3.6. Ethyl 2-acetyl-3,5-dihydroxyphenylacetate (*4*)

Semi-solid. UV (CH_2Cl_2) λ_{max} (log ε) 220 (3.6), 271 (3.4), 302 (3.2) nm; IR 3400, 1720, 1701, 1600, 1505, 1150, 850, 780 cm^{-1}. ^{13}C NMR and ^1H NMR see Table 1; EIMS *m/z* 238 ([M^+], 14), 210 (10), 196 (33), 167 (51), 165 (35), 164 (52), 164, 150 (24), 121 (14), 107 (20), 69 (14), 43 (27), 28 (33), 18 (100). HREIMS *m/z* 238.0858 (calcd. for $C_{12}H_{14}O_5$, 238.0841).

3.7. 4-Epiradicinol (*5*)

Semi solid. $[\alpha]_D^{25} = -10.7°$ (c 0.0014, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3233, 1720 and 1584 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ε) 240 (3.3), 280 (3.2) and 320 (3.3) nm. ^{13}C NMR and ^1H NMR see Table 2; EIMS *m/z* 238 ([M^+], 2), 220 ([$M-18$]$^+$, 5), 181 (100), 111 (25), 69 (35), 32 (25), 18 (80), HREIMS *m/z* 238.0836 (calcd. for $C_{12}H_{14}O_5$, 238.0841).

3.8. Alkaline hydrolysis

A solution of compound **1** (2 mg) in CH_3OH (2 ml) was treated with 1 ml of 20% aqueous NaOH. The mixture was left at room temperature for 48 h, evaporated, neutralized with HCl–H_2O (1:1) in the cold, extracted with $CHCl_3$ and evaporated to give **2**.

3.9. Acetonide derivative 7

Compound **5** (2 mg) was dissolved in 0.5 ml of a solution containing 1.5 mg of HCl in 2 mL of acetone. The mixture was left for 48 h at room temperature, evaporated and dried under vacuum at room temperature to give acetonide derivative **7**. ^1H NMR see Table 2.

3.10. Biological assays

The antimicrobial activities of the EtOAc extracts of the culture filtrate and mycelia extracts of *C. lunata* and isolates were evaluated against *E. coli* ATCC 9637, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, *K. pneumoneae*

ATCC 10031 and *Salmonella choleraesuis* ATCC 14028 using well diffusion assays (Juliani et al., 2002) and contact autobiographical (Hamburger and Cordell, 1987; Saxena et al., 1995). All the isolates were inactive when tested in the DPPH antioxidant assay (Chen et al., 1999). The minimum inhibitory concentration (mic) was performed for pure compounds by the broth micro-dilution method according to the NCCLS protocols (Anonymous, 2001). The tested compounds were dissolved in DMSO and graded concentrations were made in nutrient broth. Radicinol (**5**) inhibited the growth of *E. coli* (mic 125 μg/ml), *Salmonella choleraesuis* (mic 125 μg/ml), *Staphylococcus aureus* (mic 62.5 μg/ml), and *B. subtilis* (mic 31.8 μg/ml), respectively.

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