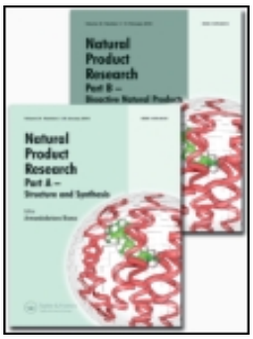

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Biologically Active Metabolites from Fungi, 19: New Isocoumarins and Highly Substituted Benzoic Acids from the Endophytic Fungus, *Scytalidium* SP.

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BIOLOGICALLY ACTIVE METABOLITES FROM FUNGI, 19: NEW ISOCOUMARINS AND HIGHLY SUBSTITUTED BENZOIC ACIDS FROM THE ENDOPHYTIC FUNGUS, *SCYTALIDIUM* SP.*,†

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Six known metabolites, two new isocoumarins **4** and **8**, and one new highly substituted benzoic acid derivative **9** were isolated from the ethyl acetate culture extract of a fungal endophyte, *Scytalidium* sp. In addition, another new benzoic acid **10** with an unusual 1,2-dicarbonyl side chain was indirectly identified from its methylated derivatives **10a**–**10d**.

Keywords: Metabolites; Isocoumarins; *Scytalidium* sp.

INTRODUCTION

As part of our continuous investigation of new metabolites from fungi, we recently analyzed an ethyl acetate culture extract of an endophytic fungus, *Scytalidium* sp., which had been isolated from a leaf of a *Salix* species growing in the Harz Mountains in Lower Saxony, Germany. Crude ethyl acetate extracts, both from malt-soya and biomalt semi-solid agar culture media, showed activities in the agar diffusion tests against *Escherichia coli*, *Ustilago violacea*, *Eurotium repens*, *Mycothypha microspora* and inhibited the growth of *Chlorella fusca* and *Lemna*. From these extracts, nine metabolites **1**–**9** were isolated in pure form by a combination of repeated column and preparative TLC chromatography on silica gel. Three compounds (**4**, **8** and **9**, Fig. 1) were new natural products. A polar component was indirectly identified as a new benzoic acid **10** from its four methylation derivatives (**10a**–**10d**) (Scheme 3).

*This article is dedicated to Professor Günter Adam on the occasion of his 70th birthday.
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RESULTS AND DISCUSSION

The isolated compounds are shown in Fig. 1, numbered according to their polarity on TLC, using dichloromethane/0–8% methanol and toluene/20–40% ethyl acetate as the eluants. The known metabolites **1**–**3**, and **5**–**7** were identified by comparison of their spectral data with those published in the literature. The least polar constituent was identified as (+)-dihydronaphtho(1,2-b)furan-5,6-dicarboxylic anhydride (**1**), previously isolated from *Penicillium herquei* [1], and later also from *Roesleria pallida* [2].

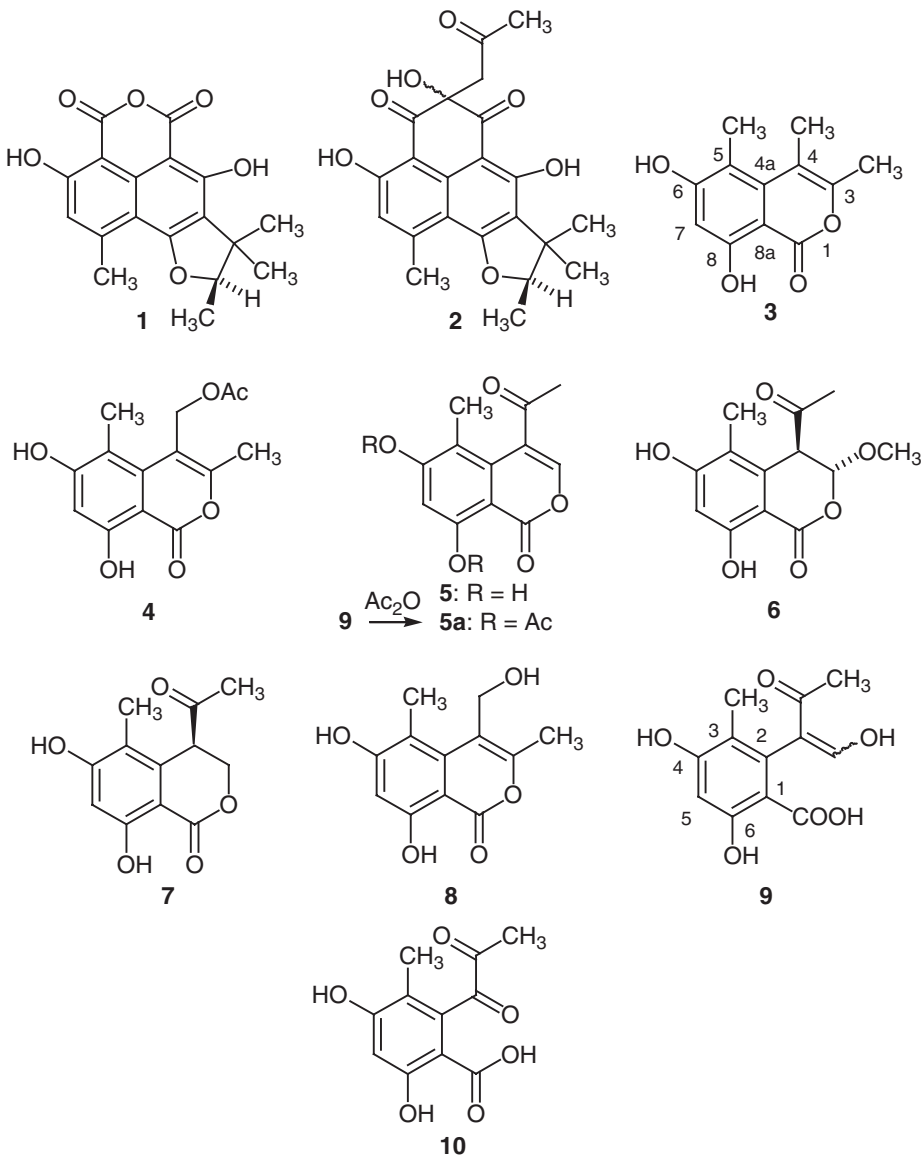


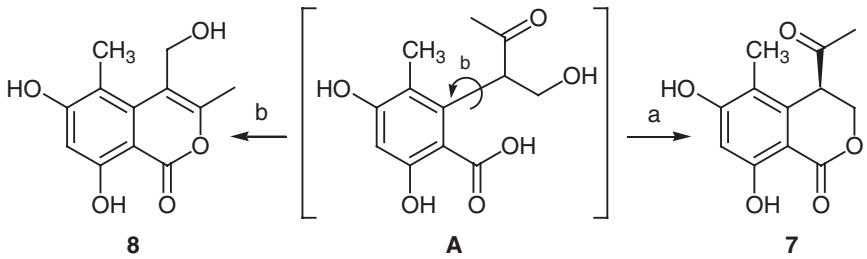
FIGURE 1 Structures of secondary metabolites identified as constituents of the endophytic fungus, strain 5681.

The levorotatory form of the anhydride, *ent*-**1**, was found as a constituent of the fungus *Gremmeniella abietina* [3]. The relative and also the absolute configuration of (+)-**1** were determined by X-ray single crystal analysis [4]. The next compound following in polarity, was a related derivative, the tertiary alcohol **2**, also found in the levorotatory form in *G. abietina* [3]. The tertiary alcohol **2** existed as a mixture of epimers with the same splitting of some signals in the ^{1}H-NMR spectrum as reported in the literature [3]. The natural products following in polarity on TLC, were isocoumarin derivatives. Interestingly, they all had methyl groups at C-5, similar to some natural products previously isolated by Whyte and Gloer [5], Aldridge *et al.* [6] and our group [7]. They were identified as decarboxycitrinone (**3**) [5], 4-acetyl-6, 8-dihydroxy-5-methyl-2-benzopyran-1-one (**5**) [6] (also characterized as the diacetate **5a**), 4-acetyl-3,4-dihydro-6,8,-dihydroxy-3-methoxy-5-methylisocoumarin (**6**) [7], and 4-acetyl-3,4-dihydro-6,8,-dihydroxy-5-methylisocoumarin (**7**) [7].

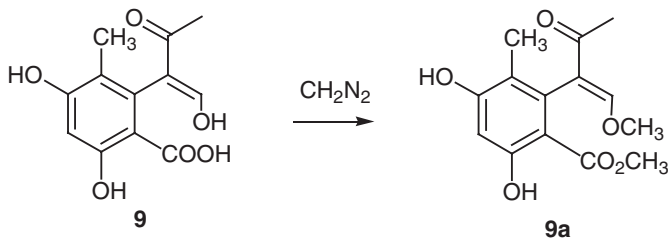
The structures of the new isocoumarins **4** and **8** were elucidated by direct comparison of their spectral data with those of Compound **3** [5]. Although both the ^{1}H-NMR and ^{13}C-NMR spectra of **4** were in close correspondence to those of isocoumarin **3**, some new signals could be observed, such as the resonances at $\delta = 5.10$ ppm in the ^{1}H-NMR and at $\delta = 61.3$ ppm in the ^{13}C-NMR spectra. These signals, in conjunction with the DEPT 135 spectrum, proved the presence of a heteroatom bonded methylene group in **4**, replacing one of the methyl groups in **3**. The resonance at $\delta = 171.4$ ppm in the ^{13}C-NMR spectrum, the carbonyl band at 1740 cm^{-1} in the IR spectrum, and the resonance at $\delta = 2.07$ ppm for a methyl group in the ^{1}H-NMR spectrum were characteristic for the presence of an acetate moiety. This was confirmed by the mass spectrum with $[M+H]^+$ at $m/z = 279$, suggesting the molecular formula $C_{14}H_{14}O_6$. The relative positions of the ester and methyl groups were determined by a 1D NOE experiment. Irradiation of the methylene protons at $\delta = 5.10$ ppm showed considerable interactions with both the methyl groups at C-3 and C-5. The CH_2OCOCH_3 chain therefore had to be attached at C-4 as shown in structure **4** in Fig. 1.

The spectral data of Compound **8** closely corresponded with those of both **3** and **4**. The mass spectrum with $m/z = 237$ $[M+H]^+$ suggested the molecular formula $C_{12}H_{12}O_5$. This showed the presence of an additional oxygen for **8** with respect to **3** and the absence of the acetic ester moiety compared to **4**. The absence of the acetic ester moiety was further confirmed by the missing signals for the ester carbonyl carbon atom and the methyl group in the NMR and relevant bands in the IR spectra. The structure of the corresponding alcohol **8**, related to the ester **4**, was further confirmed by the expected upfield shift ($\delta = 4.52$ ppm) of the methylene protons in the ^{1}H-NMR spectrum with respect to the acetate **4** ($\delta = 5.10$ ppm). The location of the hydroxymethyl group was independently determined by NOE experiments to be located at C-4, as shown by strong interactions of the methylene protons with both the methyl protons at C-3 and C-5.

The alcohol **8** is closely related to the isocoumarin sescandelin B, isolated by Kimura *et al.* [8] and recently synthesized by Kim *et al.* [9], missing only the methyl group at C-5. On the other hand, there is also a close biosynthetic relationship of the hydroxymethyl isocoumarin **8** with the acetyl compound **7**, isolated by our group from another endophytic fungus, *Mycelia sterila* [7]. A presumed open chain precursor **A** can either close directly to the six-membered lactone (path a) or cyclize after rotation of the side chain via the acetyl enol tautomer to form the hydroxymethyl compound **8** (path b), as shown in Scheme 1.

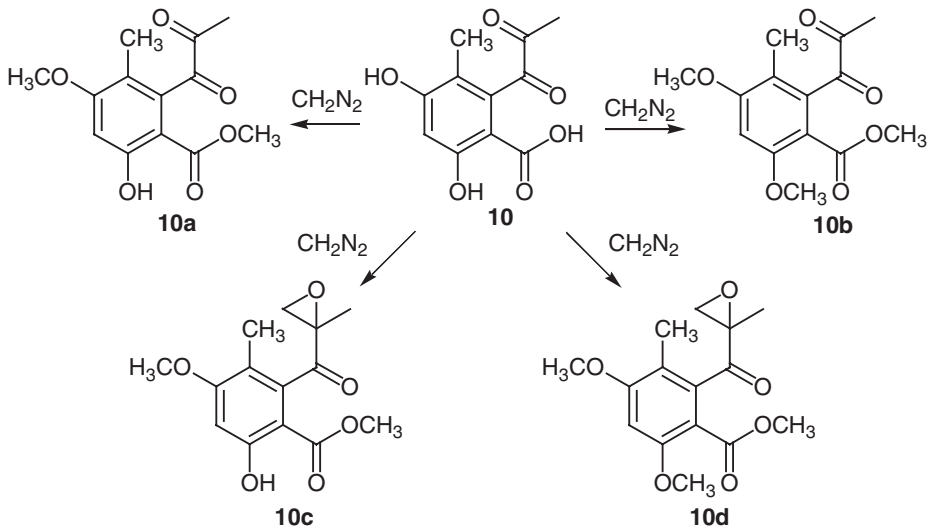


SCHEME 1 Presumed biosynthetic relationship of isocoumarins **7** and **8**.



SCHEME 2 Diazomethane methylation of the hydroxymethylene ketone **9** to the enol ether **9a**.

The most polar compound, isolated in pure state, was the hydroxymethylene ketone **9**. The keto–enol tautomerism and thus the occurrence of signals with different integration in the NMR spectra of these tautomers, complicated a straightforward structure elucidation of this polar component. Therefore, the compound was treated with diazomethane for a few minutes to trap the enol tautomer (Scheme 2), as described similarly by Aldridge *et al.* for a related semi-synthetic derivative [6]. Both the ^{1}H-NMR and ^{13}C-NMR spectra of the major methylation product closely corresponded to those of isocoumarin **5**. However, in the ^{1}H-NMR spectrum of **9a**, two three-proton singlets at $\delta = 3.83$ and $\delta = 3.86$ ppm indicated the presence of additional *O*-methyl groups, whereas the position of the signals for the aromatic methyl group at C-3 and the acetyl group remained essentially unchanged. The mass spectrum displayed the $[M+H]^+$ ion at $m/z = 281$ which suggested the molecular formula $C_{14}H_{16}O_6$, thus the addition of one methyl and one methoxy group with respect to **5**. The location of the methoxy groups was identified by COLOC experiments. The correlation of one of the methoxy groups with the vinylic proton and the absence of any correlation with the aromatic proton proved the methylation of the conjugated enol system in **9a**. On the other hand, a resonance at $\delta = 171.6$ ppm in the ^{13}C-NMR spectrum indicated the presence of an ester group, ca. 6 ppm downfield from the resonance of the corresponding lactone carbonyl in **5** ($\delta = 165.6$ ppm). The resonance of the vinylic carbon (=*C*HOMe) in the open chain arrangement ($\delta = 157.3$ ppm) also showed a significant downfield shift with respect to that of the cyclized form **5** ($\delta = 145.3$ ppm). All of these data were in agreement with the open chain methyl ester enol ether structure **9a**, originating from β-ketoaldehyde **9**. In addition, further evidence for the open chain structure **9** arose from the conversion into the isocoumarin diacetate **5a** upon treatment with acetic anhydride/pyridine.



SCHEME 3 Diazomethane methylation of diketo acid **10** to produce the methylated compounds **10a**–**10d**.

The isolation of metabolites from very polar fractions of the silica gel chromatography was hampered by the presence of dark brown polymeric material. We therefore decided to subject the entire polar fractions to a short methylation with diazomethane, hoping that polar phenolic hydroxyl groups and carboxylic acids would be converted to the less polar methyl ethers and esters, which can be purified more easily. From this experiment, four different compounds **10a**–**10d** with very interesting open chain structures were isolated in pure form after extensive preparative TLC of the reaction mixture (Scheme 3).

The presence of the diketo acid **10** was deduced only indirectly, but the evidence from the structures of the four methylation products **10a**–**10d** unambiguously proved the structure of the precursor **10**. The presence of a methyl ester and a free chelated phenolic hydroxy group could be confirmed by comparison of the ^{1}H-NMR and ^{13}C-NMR spectra of **10a**, the most abundant methylation product, with those of **9a**. The second methyl group was tentatively located at the non-chelated phenolic hydroxy group. No signals for olefinic protons could be detected in **10a**, but instead two resonances at $\delta = 193.8$ and $\delta = 196.5$ ppm indicated the presence of two additional carbonyl groups. Comparison with literature reference [10] of the somewhat unusual chemical shift for these carbonyl groups suggested (in conjunction with the mass spectrum with $[M+H]^+$ at $m/z = 267$ and the molecular formula $C_{13}H_{14}O_6$) the presence of an α-diketo side chain, as shown in the tentatively assumed structure **10a**.

Both the ^{1}H-NMR and ^{13}C-NMR spectra of **10b** were almost identical to those of **10a** with the exception of signals for an additional methoxy group and a missing chelated phenolic hydroxyl. The methylation of a chelated phenolic hydroxy group was confirmed by the mass difference of 14 units and thus structure **10b** was tentatively assumed for the second methylation product.

The chemical nature of the side chain could be determined after analysis of the spectral data of the next two methylation products **10c** and **10d**. They were related to each other as were **10a** and **10b**, also showing the additional methylation of the chelated

hydroxyl group in **10d** with respect to **10c**. An additional methylene group and also a quaternary carbon could be detected both in their ^{1}H-NMR and ^{13}C-NMR spectra, whereas one of the signals for the carbonyl groups present in **10a** and **10b** was missing. The presence of the additional methylene group was also confirmed by the respective mass difference of 14 units. It was thus reasonable to assume, the insertion of a methylene carbene to form an oxirane because the chemical shift of the carbon and hydrogen atoms and also the geminal coupling constant of ca. 5.0–5.5 Hz for the methylene protons were in perfect agreement with the presence of oxiranes both in **10c** and **10d**. The upfield shift of the signal for the acetyl methyl groups from $\delta = 1.94$ and 1.97 ppm in **10a** and **10b** to $\delta = 1.70$ and 1.69 ppm in **10c** and **10d** proved the transformation of the external carbonyl group to an oxirane. Evidently, in addition to possible electronic effects, the pentasubstituted benzene ring effectively shields the benzylic carbonyl group. This spectral evidence and also the fact that the methylenation of 1,2-dicarbonyl compounds has ample precedence in the literature [11], confirms the structures of the methylation products **10a**–**10d** and thus that of the polar natural constituent **10**.

In summary, two isocoumarins with an acetoxy methyl (**4**) and a hydroxymethyl substituent (**8**) and two benzoic acids **9** and **10** with unusual hydroxymethylene ketone and 1,2-diketo side chains related in their substitution pattern to isocoumarines, were identified as constituents of the endophytic fungus, *Scytalidium* sp. Further investigation of their biological properties is underway.

EXPERIMENTAL

For general methods and instrumentation see Ref. [12], and for microbiological methods and conditions of culture see Ref. [7]. UV spectra were measured with a Perkin-Elmer Lambda 15 UV-VIS spectrometer. Plates (20 × 20 cm) from Macherey-Nagel, Germany (1 and 0.5 mm of silica gel) were used for preparative TLC. Compounds were detected on TLC plates (Merck AG, silica gel 60 F_{254}) by spraying with cerium–molybdenum spray reagent (10 g of cerium(IV) sulphate, 25 g of molybdato phosphate, 60 mL of conc. H_2SO_4, 940 mL of H_2O) followed by heating.

Isolation

Strain 5681 was cultivated at room temperature for 111 days on two different culture media, namely, malt-soya and biomalt semi-solid agar media. The culture media were then separately extracted, once with petroleum ether and three times with ethyl acetate to obtain the crude extracts. Ethyl acetate soluble fractions from both media showed similar resolution of components and were therefore combined (4.63 g) and subjected to column chromatography for fractionation on silica gel, using gradients of petroleum ether–dichloromethane, then by dichloromethane and then by gradients of dichloromethane with up to of 10% methanol. A total of 251 fractions were collected. These fractions were screened by TLC under UV light and by spraying with cerium–molybdenum or vannilin–sulfuric acid spray reagents. Similar fractions were combined and were investigated to isolate pure Compounds **1** (54.3 mg), **2** (9.0 mg), **3** (84.8 mg), **4** (11.1 mg), **5** (12.5 mg), **6** (20.2 mg), **7** (9.1 mg), **8** (3.3 mg) and **9** (73.7 mg).

Derivatization by acetylation and methylation

To aid structure elucidation, 12.0 and 50.0 mg of **9** were acetylated using acetic anhydride–pyridine in dichloromethane, and methylated using an ethereal diazomethane solution, following the standard procedures [13]. Derivatives **5a** (6.0 mg) and **9a** (16.8 mg) were purified by PTLC as acetylated and methylated derivatives of **9**, respectively. A combined column fraction of different polarity [CH_2Cl_2–MeOH (95 : 5) to CH_2Cl_2–MeOH (92:8)] showed very poor resolution of its components on TLC. From the polarity of this fraction it was assumed that it may contain compounds of polyhydroxyl and/or carboxyl acid derivatives. Methylation of aliquots of this fraction was done twice following the standard procedure [13]. In the first methylation, a suspension of 260.0 mg of the crude polar fraction, containing largely brown polymeric material, was treated with 5 mL of ethereal diazomethane solution for a period of 3 h at room temperature (20 °C). In the second reaction, 158.0 mg of this fraction was similarly methylated for a period of 5 min at about 0 °C. Four pure compounds **10a** (5.0 mg), **10b** (5.9 mg), **10c** (5.7 mg) and **10d** (6.9 mg) were isolated from this methylated fraction. In the first reaction, **10a** was the minor compound compared to other three and in the second reaction the yield was reversed.

*Acetic acid 6,8-Dihydroxy-3,5-dimethyl-1-oxo-1H-isochromen-4-ylmethyl ester (**4**)* m.p. 230°C; R_f 0.56 (CH_2Cl_2–2%MeOH); IR (KBr): $\nu = 1740\,cm^{-1}$, 1657, 1398, 1269; UV (MeOH): λ_{max} ($\log\varepsilon$) = 242 nm (3.96), 247 (3.96); ^{1}H-NMR (300 MHz, $CDCl_3$–2% CD_3OD): $\delta = 2.07$ (s, 3H, $COCH_3$), 2.26 (s, 3H, 3-CH_3), 2.29 (s, 3H, 5-CH_3), 5.10 (s, 2H, CH_2), 6.38 (s, 1H, 7-H), 8.36 (br s, 1H, 6-OH), 11.65 (s, 1H, 8-OH); ^{13}C-NMR (50 MHz, $CDCl_3$–2%CD_3OD): $\delta = 12.1$ (3-CH_3), 18.2 (5-CH_3), 21.4 ($COCH_3$), 61.3 (CH_2), 100.4 (C-8a), 102.1 (C-7), 109.9 (C-4), 111.3 (C-5), 137.8 (C-4a), 156.3 (C-3), 162.0 (C-8), 164.2 (C-6), 167.0 (C-1), 171.4 (acetate–CO); EIMS (80 eV, 150 °C): *m/z* (%) = 278 [M^+] (78), 236 (6), 219 (58), 218 (100), 217 (37), 193 (33), 175 (36), 148 (15), 43 (16), 28 (4).

*Acetic acid 8-acetoxy-4-acetyl-5-methyl-1-oxo-1H-isochromen-6-yl ester (**5a**)* m.p. 91°C; R_f 0.82; (CH_2Cl_2–MeOH 8%); IR (KBr): $\nu = 3359\,cm^{-1}$, 2929, 2852, 1771, 1745, 1693, 1626, 1455, 1372, 1316, 1264; UV (CH_2Cl_2): λ_{max} ($\log\varepsilon$) = 239 nm (4.0); ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.98$ (s, 3H, 5-CH_3), 2.31 (s, 3H, $OCOCH_3$), 2.34 (s, 3H, $OCOCH_3$), 2.45 (s, 3H, $COCH_3$), 7.01 (s, 1H, 7-H), 7.54 (s, 1H, 3-H); ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 16.5$ (5-CH_3), 21.4 ($OCOCH_3$), 21.6 ($OCOCH_3$), 30.4 ($COCH_3$), 113.3 (C-8a), 119.2 (C-7), 122.7 (C-4), 125.3 (C-5), 136.5 (C-4a), 147.8 (C-3), 151.8 (C-8), 155.6 (C-6), 157.6 (C-1), 168.3 ($OCOCH_3$), 169.9 ($OCOCH_3$), 197.4 ($COCH_3$); EIMS (70 eV, < 50 °C): *m/z* (%) = 318 [M^+] (6), 276 (59), 234 (100), 219 (16), 217 (28), 206 (14), 191 (15), 163 (7), 135 (6), 43 (42).

*6,8-Dihydroxy-4-hydroxymethyl-3,5-dimethyl-isochromen-1-one (**8**)* m.p. 245 °C; R_f 0.39 (CH_2Cl_2–8%MeOH); IR (KBr): $\nu = 2924\,cm^{-1}$, 2851, 1665, 1612; UV (MeOH): λ_{max} ($\log\varepsilon$) = 242 nm (3.56), 247 (3.57); ^{1}H-NMR (300 MHz, CD_3OD): $\delta = 2.25$ (s, 3H, 3-CH_3), 2.35 (s, 3H, 5-CH_3), 4.52 (s, 2H, CH_2OH), 6.24 (s, 1H, 7-H); ^{13}C-NMR (75 MHz, CD_3OD): $\delta = 10.9$ (3-CH_3), 16.3 (5-CH_3), 57.3 (CH_2OH), 99.4 (C-8a), 100.9 (C-7), 111.7 (C-4), 113.7 (C-5), 137.9 (C-4a), 154.6 (C-3), 162.3 (C-8), 165.2 (C-6), 166.8 (C-1); EIMS (70 eV, 240 °C): *m/z* (%) = 236 [M^+] (57), 218 (58), 193 (39), 175 (58), 148 (37), 77 (20), 69 (34), 44 (83), 43 (100), 28 (14).

*2-(1-Acetyl-2-hydroxyvinyl)-4,6-dihydroxy-3-methyl-benzoic acid (**9**)* m.p. 175°C; R_f 0.35 (CH_2Cl_2–8% MeOH); IR (KBr): $\nu = 2924\,cm^{-1}$, 1719, 1652, 1616, 1460, 1398, 1274, 1160, 1031; UV (MeOH): λ_{max} ($\log\varepsilon$) = 224 nm (3.79), 227 (3.80), 231 (3.78); ^{1}H-NMR (200 MHz, CD_3OD): $\delta = 2.07$ (s, 3H, 3-CH_3), 2.24 (s, 3H, COCH_3), 6.03 (s, 1H, 5-H), 6.38 (s, 1H, vinyl-H); EIMS (80 eV, < 50°C): *m/z* (%) = 252 [M^+] (54), 234 (24), 210 (25), 206 (18), 192 (100), 174 (18), 164 (86), 163 (20), 136 (22), 77 (14), 69 (16), 43 (61), 28 (3).

*2-(1-Acetyl-2-methoxyvinyl)-4,6-dihydroxy-3-methylbenzoic acid methyl ester (**9a**)* m.p. 185°C; R_f 0.56 (CH_2Cl_2–MeOH 8%); IR (KBr): $\nu = 2950\ cm^{-1}$, 2845, 2567, 1728, 1654, 1628, 1612, 1439, 1334, 1250, 1198, 1166, 1150; UV (MeOH): λ_{max} ($\log\varepsilon$) = 224 nm (3.81), 259 (3.86); ^{1}H-NMR (300 MHz, $CDCl_3$–2%CD_3OD): $\delta = 2.01$ (s, 3H, 3-CH_3), 2.15 (s, 3H, COCH_3), 3.83 (s, 3H, OCH_3), 3.86 (s, 3H, CO_2CH_3), 6.45 (s, 1H, 5-H), 7.41 (s, 1H, vinyl-H), 11.51 (br s, 1H, 6-OH); ^{13}C-NMR (75 MHz, $CDCl_3$–2% CD_3OD): $\delta = 12.4$ (3-*C*H_3), 27.4 (CO*C*H_3), 52.2 (CO_2*C*H_3), 62.3 (O*C*H_3), 102.9 (C-5), 104.8 (C-1), 118.2 (C-3), 121.7 (*C*=CHOCH_3), 136.5 (C-2), 157.3 (=*C*OCH_3), 161.4 (C-6), 162.6 (C-4), 171.6 (CO_2CH_3), 198.7 (*C*OCH_3); EIMS (70 eV, 240°C): *m/z* (%) = 280 [M^+] (22), 248 (43), 217 (32), 216 (79), 175 (32), 174 (100), 146 (15), 57 (14), 43 (19), 28 (16).

*6-Hydroxy-4-methoxy-3-methyl-2-(2-oxopropionyl)-benzoic acid methyl ester (**10a**)* m.p. 98°C; R_f 0.55 (toluene–ethyl acetate 20%); IR (KBr): $\nu = 2924\,cm^{-1}$, 2852, 1719, 1703, 1657, 1605, 1584, 1440, 1372, 1347, 1253, 1191, 1160; UV (CH_2Cl_2): λ_{max} ($\log\varepsilon$) = 231 nm (3.69), 259 (3.66); ^{1}H-NMR (200 MHz, $CDCl_3$): $\delta = 1.94$ (s, 3H, 3-CH_3), 2.55 (s, 3H, COCH_3), 3.85 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 6.53 (s, 1H, 5-H), 10.60 (br s, 1H, 6-OH); ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 12.3$ (3-*C*H_3), 24.0 (CO*C*H_3), 52.9 (O*C*H_3), 56.4 (O*C*H_3), 100.6 (C-5), 102.5 (C-1), 117.9 (C-3), 139.4 (C-2), 162.4 (C-6), 164.0 (C-4), 168.8 (*C*OOCH_3), 193.8 (CO), 196.5 (CO); EIMS (70 eV, < 50°C): *m/z* (%) = 266 [M^+] (8), 224 (45), 223 (100), 191 (38), 163 (29), 135 (13), 120 (9), 109 (8), 77 (14), 69 (20), 43 (39).

*4,6-Dimethoxy-3-methyl-2-(2-oxopropionyl)-benzoic acid methyl ester (**10b**)* m.p. 134°C; R_f 0.41 (toluene–ethyl acetate 30%); IR (KBr): $\nu = 2924\,cm^{-1}$, 2847, 1719, 1709, 1647, 1590, 1481, 1440, 1341, 1269, 1207; UV (CH_2Cl_2): λ_{max} ($\log\varepsilon$) = 229 nm (3.61); ^{1}H-NMR (200 MHz, $CDCl_3$): $\delta = 1.97$ (s, 3H, 3-CH_3), 2.58 (s, 3H, COCH_3), 3.83 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 6.53 (s, 1H, 5-H); ^{13}C-NMR (50 MHz, $CDCl_3$): $\delta = 12.1$ (3-*C*H_3), 24.2 (CO*C*H_3), 53.0 (O*C*H_3), 56.2 (O*C*H_3), 56.9 (O*C*H_3), 96.6 (C-5), 109.9 (C-1), 118.0 (C-3), 143.2 (C-2), 160.1 (C-6), 162.9 (C-4), 168.5 (*C*OOCH_3), 193.9 (CO), 197.2 (CO); EIMS (70 eV, < 50°C): *m/z* (%) = 280 [M^+] (1), 249 (11), 238 (55), 237 (100), 207 (9), 179 (16), 164 (14), 136 (15), 120 (12), 91 (15), 77 (16), 69 (9), 43 (24), 28 (3).

*6-Hydroxy-4-methoxy-3-methyl-2-(2-methyloxiranecarbonyl)-benzoic acid methyl ester (**10c**)* m.p. 134°C; R_f 0.48 (toluene–20% ethyl acetate); IR (KBr): $\nu = 2919\,cm^{-1}$, 2857, 1703, 1667, 1605, 1445, 1357, 1253, 1166; UV (CH_2Cl_2): λ_{max} ($\log\varepsilon$) = 231 nm (3.65); ^{1}H-NMR (200 MHz, $CDCl_3$): $\delta = 1.70$ (s, 3H, OCCH_3), 1.98 (s, 3H, 3-CH_3), 2.66 (d, $J_{gem} = 5.0$ Hz, 1H, CH_2), 2.84 (d, $J_{gem} = 5.0$ Hz, 1H, CH_2), 3.88 (s, 3H, OCH_3), 3.96 (s, 3H, CO_2CH_3), 6.51 (s, 1H, 5-H), 11.13 (br s, 1H, 6-OH); ^{13}C-NMR (75 MHz, $CDCl_3$): $\delta = 11.9$ (3-*C*H_3), 16.4 (OC*C*H_3), 52.1 (*C*H_2), 52.5 (CO_2*C*H_3), 55.6 (O*C*H_3), 60.0 (R′R″R‴*C*CO), 99.4 (C-5), 116.0 (C-1), 122.0 (C-3), 138.0 (C-2),

161.6 (C-6), 162.8 (C-4), 169.0 (CO_2CH_3), 204.3 (CO); EIMS (70 eV, 240°C): m/z (%) = 280 [M^+] (24), 223 (100), 191 (17), 165 (14), 163 (15), 136 (13), 77 (10), 57 (10), 43 (10).

*4,6-Dimethoxy-3-methyl-2-(2-methyloxiranecarbonyl)-benzoic acid methyl ester (**10d**)* m.p. 87°C; R_f 0.28 (toluene–ethyl acetate 30%); IR (KBr): $\nu = 2940\,cm^{-1}$, 2847, 1734, 1703, 1590, 1476, 1440, 1336, 1269, 1207, 1155, 1021; UV (CH_2Cl_2): λ_{max} (log ε) = 231 nm (3.80); ^{1}H-NMR (200 MHz, $CDCl_3$): $\delta = 1.69$ (s, 3H, OCCH_3), 2.03 (s, 3H, 3-CH_3), 2.78 (d, $J_{gem} = 5.4$ Hz, 1H, CH_2), 2.85 (d, $J_{gem} = 5.4$ Hz, 1H, CH_2), 3.89 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.50 (s, 1H, 5-H); ^{13}C-NMR (50 MHz, $CDCl_3$): $\delta = 12.5$ (3-*C*H_3), 17.2 (OC*C*H_3), 52.7 (O*C*H_3), 53.5 (*C*H_2), 56.1 (O*C*H_3), 56.9 (O*C*H_3), 60.4 (R′R″R‴*C*CO), 96.4 (C-5), 112.0 (C-1), 117.0 (C-3), 140.6 (C-2), 158.9 (C-6), 161.7 (C-4), 166.9 (CO_2CH_3), 205.0 (CO); EIMS (70 eV, 240°C): m/z (%) = 294 [M^+] (26), 264 (11), 249 (14), 238 (65), 237 (100), 221 (29), 207 (17), 179 (24), 177 (15), 164 (17), 149 (15), 136 (17), 120 (17), 91 (19), 77 (18), 57 (11), 43 (14).

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