Chloroisosulochrin, Chloroisosulochrin Dehydrate, and Pestheic Acid, Plant Growth Regulators, Produced by *Pestalotiopsis theae*

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Chloroisosulochrin, Pestheic Acid, *Pestalotiopsis theae*

New plant growth regulators, named chloroisosulochrin, chloroisosulochrin dehydrate and pestheic acid, have been isolated from the culture filtrate of *Pestalotiopsis theae* grown on a Raulin-Thom medium. Their structures have been established by spectroscopic and chemical methods. The biological activities of these compounds have been examined using bioassay methods with lettuce and rice seedlings.

**Introduction**

*Pestalotiopsis theae* has been known as causal fungus for tea gray blight disease [1–3]. PT-toxin [4] and pesthetoxin [5] from the fungus can induce leaf necrosis and have been isolated from the culture filtrate grown on a potato extract medium. Recently, we changed to Raulin-Thom medium in order to study the production of new plant growth regulators from the fungus. These studies led to the isolation of three chlorinated metabolites, chloroisosulochrin, chloroisosulochrin dehydrate, and pestheic acid. In this report, we describe the isolation, structural determination and biological activities of these compounds.

**Results and Discussion**

The fungus was cultured stationarily in a Raulin-Thom medium (120 L) at 24 °C for 21 days. The culture filtrate was adjusted to pH 2.0, before being treated with active charcoal, and successively extracted with acetone. The acetone-soluble acidic fraction was purified by silica gel column chromatography, and final purification by recrystallization afforded isosulochrin (1), isosulochrin dehydrate (2), chloroisosulochrin (3), chloroisosulochrin dehydrate (4), and pestheic acid (5).

Compound 1 and 2 were identified as isosulochrin and isosulochrin dehydrate (Fig. 1) by comparing the physicochemical properties with those reported (see experimental section) [6, 7].

![Fig. 1. Structures of isosulochrin (1), isosulochrin dehydrate (2), chloroisosulochrin (3), chloroisosulochrin dehydrate (4), and pestheic acid (5).](image)

Compound 3 was obtained as pale yellow prisms. The molecular formula of 3 was determined by MS and elemental analysis to be...
C_{17}H_{15}ClO_7. The UV spectrum showed absorption maxima at 275 and 338 nm, which was similar to those of 1 [6, 7]. The IR spectrum showed characteristic absorption bands at 1640 and 1610 cm\(^{-1}\), indicating that 3 possesses a benzophenone skeleton [7]. Comparison of the molecular formula and \(^1\)H NMR data of 3 with those of 1 revealed that 3 differs from 1 only in the presence of a chlorine atom instead of an aromatic proton. A NOE between the methyl protons at \(\delta = 2.25\) and two aromatic protons at \(\delta = 6.23\) indicated that the two aromatic protons were adjacent to the methyl group. On treatment with acetic anhydride-pyridine, 3 afforded the triacetyl derivative. A signal at \(\delta = 6.95\) suggested that a signal at \(\delta = 6.23\) in 3 shifted to a lower magnetic field in the triacetyl derivative. This spectroscopic and chemical evidence indicated that two hydroxy and a methyl group were present in the relationship such as 2,6-dihydroxy-4-methylphenyl in one aromatic ring of a benzophenone skeleton. On treatment with diazomethane, 3 gave the dimethyl derivative. In the differential NOE spectra of the dimethyl derivative of 3, a NOE was observed between an aromatic proton at \(\delta = 7.38\) and methoxy protons at \(\delta = 4.00\), but no NOE was observed between this aromatic proton and methoxy protons at \(\delta = 3.75\). These NOE experiments indicated that the aromatic proton was adjacent to the methoxy group at \(\delta = 4.00\), but not to another methoxy group at \(\delta = 3.75\) which is placed at C-3 instead of a hydroxy group in 3. These spectroscopic and chemical evidences indicated that a chlorine atom instead of an aromatic proton was placed at C-4 of 1. From these results, 3 was identified as methyl-2-(2,6-dihydroxy-4-methylbenzoyl)-4-chloro-3-hydroxy-5-methoxybenzoate and the compound was named chloroisosulochrin (Fig. 1).

Compound 4 was obtained as yellow needles. The molecular formula of 4 was determined by MS and elemental analysis to be C_{17}H_{13}ClO_6. The UV spectrum showed absorption maxima at 241, 253, 308 and 358 nm, which was similar to those of 2 [6, 7]. The IR spectrum showed characteristic absorption bands at 1661, 1640 and 1600 cm\(^{-1}\), indicating that 4 possesses a xanthone skeleton [7]. Comparison of the molecular formula and \(^1\)H NMR data of 4 with those of 2 revealed that 4 differs from 2 only in the presence of a chlorine atom instead of an aromatic proton. A NOE between the methyl protons and two aromatic protons at \(\delta = 6.64\) and 6.84 indicated that the two aromatic protons were adjacent to methyl group. Another NOE was observed between the methoxy protons at \(\delta = 4.06\) and one remaining aromatic proton at \(\delta = 6.94\). The spectroscopic data of 4 were identical with those of the xanthone derivative obtained by treatment of 3 with KOH solution. From these results, 4 was identified as methyl-(5-chloro-1-hydroxy-6-methoxy-3-methylxanthone)-8-carboxylate and the compound was named chloroisosulochrin dehydrate (Fig. 1). Compound 5 was obtained as colorless prisms. The molecular formula of 5 was determined by MS and elemental analysis to be C_{17}H_{15}ClO_6. The presence of an aromatic ring in the molecule was suggested by IR band at 1601 and 1583 cm\(^{-1}\). A band at 3420 cm\(^{-1}\) indicated the presence of a phenolic hydroxy group which was positive to alcoholic ferric chloride, while a band at 1620 cm\(^{-1}\) suggested a chelated carboxy group [8, 9]. The IR band at 1722 cm\(^{-1}\) and two signals at \(\delta = 52.2\) and 164.6 in the \(^{13}\)C NMR spectrum indicated the presence of a methoxycarbonyl group. The \(^1\)H NMR spectrum of 5 indicated the presence of a methyl, two methoxy, three hydroxy groups and three aromatic protons. A NOE between the methyl protons and two aromatic protons at \(\delta = 5.72\) and 6.40 indicated that the two aromatic protons were adjacent to the methyl group. Furthermore, a NOE between methoxy protons at \(\delta = 3.89\) and an aromatic proton at \(\delta = 7.00\) indicated that the aromatic proton was adjacent to the methoxy group. A diacetyl-monomethyl derivative of 5 was obtained by treatment of 5 with acetic anhydride-pyridine and then with diazomethane in ether. This chemical evidence indicated that two hydroxy groups and a carboxy group were present in 5. On treatment with diazomethane, 5 afforded the dimethyl and trimethyl derivatives. In the \(^1\)H NMR spectra of the dimethyl derivative, a signal at \(\delta = 11.47\) indicated that a hydrogen bond was formed between a hydroxy and an adjacent carbonyl group instead of the chelated carboxy group of 5. In the differential NOE spectra of the trimethyl derivative, a NOE was observed between an aromatic proton at \(\delta = 6.39\) and methoxy protons at \(\delta = 3.86\) instead of the hydrogen-bonded hydroxy group in the dimethyl derivative. Furthermore, a NOE was observed between methyl protons at \(\delta =\)
2.19 and two aromatic protons at $\delta = 5.79$ and 6.39. These spectroscopic and chemical evidences indicated that a carboxy, a hydroxy and a methyl group were present in the relationship such as 2-hydroxy-4-methylbenzoic acid in one aromatic ring of 5. On the other hand, a NOE was observed between methoxy protons at $\delta = 3.97$ and an aromatic proton at $\delta = 7.30$, but no NOE was observed between the aromatic proton and methoxy protons at $\delta = 3.92$. These NOE experiments indicated that the aromatic proton was adjacent to the methoxy group at $\delta = 3.97$, but not to another methoxy group which is placed at C-2' instead of a hydroxy group in 5. These spectroscopic and chemical evidences indicated that a chlorine atom instead of an aromatic proton was placed at C-3'.

The UV and IR spectra of the trimethyl derivative were very similar to those of pentamethylosalicic acid [10], which possesses a diphenyl ether linkage. These chemical and spectroscopic evidences indicated that a chlorine atom, a hydroxy, a methoxy and a methoxycarbonyl groups were present in the relationship such as 3-chloro-2-hydroxy-4-methoxy-6-methoxycarbonylphenyl in another aromatic ring of 5. From these results, 5 was identified as 6-(3-chloro-2-hydroxy-4-methoxy-6-methoxycarbonyloxy)-2-hydroxy-4-methyl-benzoic acid and the compound was named pestheic acid (Fig. 1).

5 is structurally similar to asterric acid which is derived from sulochrin produced by the fungus Oospora sulphurea-ochracea [10–12], and the orientation of substituents in 5 is strikingly similar to that in 3. These evidences suggest that 3 may be a precursor of 5 [12]. Interestingly, none of these five compounds was obtained from cultures using potato extract medium [4, 5].

1 and 2, which are produced by the fungus Aspergillus wentii, show no mutagenicity and no toxicity towards animals [6, 7]; no plant growth activities appear to have been studied. Biological activities of five compounds were examined using bioassay with lettuce and rice seedlings (Fig. 2, 3).

With lettuce seedlings, all compounds showed no inhibitory effect on the hypocotyl elongation of the seedlings from 1 mg/l to 100 mg/l. Both 1 and 2 showed no inhibitory effect on the root growth of the seedlings from 1 mg/l to 100 mg/l. On the other hand, 3 and 5 accelerated the root growth to 162% and 161% of control at a concentration of 100 mg/l, respectively. In addition, 4 accelerated the root growth to 160% of control at a concentration of 1 mg/l. With rice seedlings, all compounds showed no inhibitory effect on the stem elongation of the seedlings from 1 mg/l to 100 mg/l. Both 2 and 4 showed no inhibitory effect on the root growth of the seedlings from 1 mg/l to 100 mg/l. On the other hand, 3 accelerated the root growth of the seedlings in proportion to its concentration.
from 1 mg/l to 100 mg/l. In addition, 1 accelerated the root growth to 129% of control at a concentration of 100 mg/l, and 5 accelerated the root growth to 134% of control at a concentration of 10 mg/l. These results suggest that the substitution of an aromatic proton with a chlorine atom is more effective in promoting the root growth of lettuce seedlings than that of rice seedlings.

**Experimental**

UV and IR spectra were recorded on a Hitachi 100–50 and a JASCO FT/IR-7000 spectrometer, respectively. 1H NMR and 13C NMR spectra were obtained on a JEOL JNM GX-270 spectrometer. The MS spectrum was taken on a Hitachi RMU-6U spectrometer.

**Isolation and purification of chloroisosulochrin, chloroisosulochrin dehydrate and pestheic acid**

The isolation procedure of compounds 1–5 from the culture filtrate is summarized in Fig. 4. *P. theae* was cultured stationarily in a Raulin-Thom medium (120 l) at 24 °C for 21 days. The culture filtrate was adjusted to pH 2.0 with 2 N HCl, before being treated with active charcoal, and successively extracted acetone. The combined solvents were concentrated *in vacuo*, and the resulting residue (31.3 g) was first fractionated by column chromatography on silica gel with benzene and acetone (500-ml fractions).

a) Fractions 13–17 (228 mg), obtained by elution with 1% acetone, were recrystallized from MeOH to afford 67 mg of chloroisosulochrin dehydrate (4).

b) Fractions 18–20 (383 mg), obtained by elution with 2% acetone, were recrystallized from MeOH to afford 115 mg of pestheic acid (5).

c) Fractions 22–25 (3667 mg), obtained by elution with 2% acetone, were recrystallized from benzene to afford 339 mg of chloroisosulochrin (3).

d) Fractions 30–33 (1094 mg), obtained by elution with 5% acetone, was further fractionated by column chromatography on silica gel with benzene and EtOAc (50-ml fractions). Fractions 10–15 (17 mg: 5% EtOAc) were recrystallized from EtOAc-hexane to afford 11 mg of isosulochrin dehydrate (2). Fractions 29–31 (420 mg; 20% EtOAc) were recrystallized from EtOAc-hexane to afford 232 mg of isosulochrin (1).

![Fig. 4. Isolation procedure of metabolites from Pestalotiopsis theae.](image-url)
Analytical and spectroscopic data of 1-5:

Isosulochrin (1)

M.p. 182–189 °C. – UV/vis (EtOH): $\lambda_{\text{max}}$ (lg $\varepsilon$) = 215 (4.36), 230sh (4.08), 281 (3.99), 320 nm (3.65). – IR (KBr): $\nu$ = 3350 (OH), 2950 (C–C), 1698 (O–C=O), 1638 (C=O), 1619 (C=C), 1593, 1510, 1488, 1450, 1348, 1368 cm$^{-1}$. – $^1$H NMR (270.05 MHz, acetone-d$_6$): $\delta$ = 2.19 (s, 3H, Ar-Me), 5.69 (q, Ar-COOMe), 6.19 (2H, 3'-H, 5'-H), 6.66 (d, $J = 2.7$ Hz, 1H, 4-H), 6.98 (d, $J = 2.7$ Hz, 1H, 6-H). – $^{13}$C$^1$H NMR (67.80 MHz, acetone-d$_6$): $\delta$ = 22.4 (q, Ar-Me), 35.7 (q, Ar-O), 40.02 (s, 1H, Ar-OH). – MS (EI): $m/z$ (%) = 638 (37) [M$^+$], 366 (100) [M$^+$], 334 (59), 317 (43), 306 (75), 244 (44), 151 (53), 122 (44). – $\mathrm{C}_{13}\mathrm{H}_{15}\mathrm{O}_7$ (366.1): calcld. C 55.67, H 4.12, Cl 9.67; found C 55.80, H 3.98, Cl 9.70.

Chloroisosulochrin dehydrate (4)

M.p. 203–205 °C. – UV/vis (EtOH): $\lambda_{\text{max}}$ (lg $\varepsilon$) = 241 (4.19), 253sh (3.89), 308 (3.88), 358 nm (3.32). – IR (KBr): $\nu$ = 3420 (OH), 2960 (C=O), 1725 (O=C=O), 1661 (C=O), 1621 (C=C), 1600, 1569, 1500, 1460, 1439, 1405, 1341 cm$^{-1}$. – $^1$H NMR (270.05 MHz, CDC$_3$): $\delta$ = 2.43 (s, 3H, Ar-Me), 4.02 (s, 3H, Ar-COOMe), 4.06 (s, 3H, Ar-O), 6.64 (br. s, 1H, 2-H or 4-H), 6.84 (br. s, 1H, 2-H or 4-H), 6.94 (s, 1H, 7-H). 12.07 (s, 1H, OH). – $^{13}$C$^1$H NMR (67.80 MHz, CDC$_3$): $\delta$ = 22.5 (q, Ar-Me), 53.2 (q, Ar-COOMe), 57.0 (q, Ar-O), 106.1 (s), 106.9 (d), 107.6 (d), 111.1 (s), 112.1 (d), 112.2 (s), 132.7 (s), 149.3 (s), 152.9 (s), 155.4 (s), 159.8 (s), 161.3 (s), 169.0 (s), 179.4 (s). – MS (EI): $m/z$ (%) = 350 (26) [M$^+$], 348 (68) [M$^+$], 318 (45), 316 (100), 302 (7), 288 (13). – $\mathrm{C}_{17}\mathrm{H}_{13}\mathrm{ClO}_6$ (348.0): calcld. C 58.55, H 3.76, Cl 10.17; found C 58.58, H 3.61, Cl 9.98.

Pesticide acid (5)

M.p. 192–200 °C. – UV/vis (EtOH): $\lambda_{\text{max}}$ (lg $\varepsilon$) = 212 (4.66), 251 (4.04), 305 nm (3.74). – IR (KBr): $\nu$ = 3420 (OH), 2951 (C=O), 1722 (O=C=O), 1620 (COOH), 1601 (C=O), 1583, 1490, 1470, 1440, 1350, 1310 cm$^{-1}$. – $^1$H NMR (270.05 MHz, DMSO-d$_6$): $\delta$ = 2.08 (s, 3H, Ar-Me), 3.07–4.53 (br. s, 3H, OH), 3.64 (s, 3H, Ar-COOMe), 3.89 (s, 3H, Ar-O), 5.72 (br. s, 1H, 3-H or 5-H), 6.40 (br. s, 1H, 3-H or 5-H), 7.00 (s, 1H, 3'-H). – $^{13}$C$^1$H NMR (67.80 MHz, DMSO-d$_6$): $\delta$ = 21.3 (q, Ar-Me), 52.2 (q, Ar-COOMe), 56.4 (q, Ar-O), 103.9 (d), 104.9 (s), 105.2 (d), 110.7 (d), 114.1 (s), 123.0 (s), 135.9 (s), 143.4 (s), 148.2 (s), 152.4 (s), 157.8 (s), 159.7 (s), 164.6 (s), 170.3 (s). – MS (EI): $m/z$ (%) = 384 (40) [M$^+$], 382 (91) [M$^+$], 366 (55), 364 (100), 329 (82), 301 (59), 277 (41), 171 (41), 151 (45). – $\mathrm{C}_{13}\mathrm{H}_{15}\mathrm{ClO}_8$ (382.0): calcld. C 53.34, H 3.95, Cl 9.26; found C 53.57, H 3.96, Cl 9.31.

Acetylation of 3

3 (100 mg) was acetylated with acetic anhydride (1.0 ml) and pyridine (2.0 ml) for 24 h at room temperature. Purification by recrystallization from hexane-EtOAc gave the triacetyl derivative of 3 (84 mg) as colorless prisms.
Triacetyl derivative of 3: M.p. 149-151 °C. - UV/vis (EtOH): \( \lambda_{\text{max}} \) (lg e) = 212 (4.61), 247 (4.25), 288 nm (3.86). - IR (KBr): \( \nu = 2950 \) (C=O), 1790 (O-C=O), 1731 (O-C=O), 1692 (C=O), 1593 (C=C), 1433, 1375, 1340, 1260 cm\(^{-1}\). - \(^1\)H NMR (270.05 MHz, CDCl\(_3\)): \( \delta = 2.11 \) (s, 6H, Ar-OCOMe), 2.18 (s, 3H, Ar-OCOMe), 2.50 (s, 3H, Ar-Me), 2.80 (s, 3H, Ar-OCOMe), 4.10 (s, 3H, Ar-OMe), 6.95 (s, 2H, 3'-H, 5'-H), 7.49 (s, 1H, 6-H). - \(^13\)C\(^{\text{[H]}}\) NMR (67.80 MHz, CDCl\(_3\)): \( \delta = 19.9 \) (q, Ar-Me), 52.4 (q, Ar-COO Me), 52.5 (q, Ar-COOMe), 56.6 (q, Ar-O Me), 61.2 (q, Ar-O Me), 110.1 (d), 121.7 (s), 122.0 (2C, d), 128.9 (s), 130.3 (s), 134.9 (s), 146.1 (s), 149.9 (2C, s), 156.4 (s), 165.6 (s), 167.0 (s), 168.6 (2C, s), 187.1 (s). - MS (EI): \( m/z \) = 492 (100) \([M^+]\), 367 (19), 364 (44), 344 (9), 335 (99), 333 (100), 164 (67).  

**Methylation of 3**

3 (17 mg) was methylated with ethereal diazomethane (1 ml) and methanol (0.5 ml) for 24 h at room temperature. Purification by preparative TLC in benzene- EtOAc (98 : 2, v/v) and recrystallization from EtOAc gave methyl 4-chloro-2-(2-hydroxy-6-methoxy-4-methylbenzoyl)-3,5-dimethoxybenzoate (17 mg) as pale yellow prisms.

M.p. 166-167 °C. - UV/vis (EtOH): \( \lambda_{\text{max}} \) (lg e) = 212 (4.58), 258 (4.08), 285 (4.17), 333 nm (3.48). - IR (KBr): \( \nu = 2970 \) (C=C), 1723 (O-C=0), 1601 (C=O), 1481, 1439, 1425, 1376 cm\(^{-1}\). - \(^1\)H NMR (270.05 MHz, CDCl\(_3\)): \( \delta = 2.29 \) (s, 3H, Ar-Me), 3.37 (s, 3H, Ar-OMe), 3.72 (s, 3H, Ar-OCOMe), 3.75 (s, 3H, Ar-OMe), 4.00 (s, 3H, Ar-OMe), 6.06 (s, 2H, 3'-H or 5'-H), 6.47 (s, 2H, 3'-H or 5'-H), 7.38 (s, 1H, 6-H), 12.83 (s, 1H, OH). - MS (EI): \( m/z \) (%) = 396 (17) \([M^+]\), 394 (40) \([M^+]\), 367 (19), 364 (44), 344 (9), 335 (99), 333 (100), 164 (67).

**Transformation of 3 into 4**

3 (50 mg) in 10% KOH-methanol solution (1.8 ml) was refluxed for 1 h at 70 °C [7]. The reaction mixture was adjusted to pH 2 with 2 N HCl and extracted with EtOAc. The organic layer was then dissolved in NaHCO\(_3\) solution. The aqueous layer was extracted with EtOAc after acidification to pH 2 and the organic layer was concentrated to 1 ml. 5-chloro-1-hydroxy-6-methoxy-3-methylanthrone-8-carboxylic acid. The derivative was methylated with ethereal diazomethane (2 ml) and methanol (1 ml) for 24 h at room temperature. Purification by preparative TLC in benzene-EtOAc (95 : 5, v/v) and recrystallization from EtOAc-hexane gave 4 (40 mg) as yellow needles. The spectral data were identical to authentic chloroisosulochrin dehydrate (4).

**Acetylation and methylation of 5**

5 (12 mg) was acetylated with acetic anhydride (0.2 ml) and pyridine (0.3 ml) for 24 h at room temperature. Purification by recrystallization from EtOAc-hexane gave diacetyl derivative of 5 (12.6 mg). Then, the diacetyl derivative was methylated with ethereal diazomethane (1 ml) and methanol (0.5 ml) for 24 h at room temperature. Purification by preparative TLC in benzene-EtOAc (98 : 2, v/v) and recrystallization from EtOAc gave methyl 6-(2-acetoxy-3-chloro-4-methoxy-6-methoxybenzoyl)-2-acetoxy-4-methylbenzoate (12.8 mg) as pale red prisms.

M.p. 170-171 °C. - UV/vis (EtOH): \( \lambda_{\text{max}} \) (lg e) = 215 (4.69), 240 (4.29), 300 nm (3.69). - IR (KBr): \( \nu = 2950 \) (C=C), 1736 (O-C=O), 1631 (chelated C=O), 1582 (C=C), 1462, 1440, 1360, 1339 cm\(^{-1}\). - \(^1\)H NMR (270.05 MHz, CDCl\(_3\)): \( \delta = 2.13 \) (s, 3H, Ar-Me), 3.72 (s, 3H, Ar-OCOMe), 3.83 (s, 3H, Ar-OCOMe), 3.95 (s, 3H, Ar-OMe), 3.98 (s, 3H, Ar-OMe), 6.18 (s, 1H, 3-H or 5-H), 6.61 (s, 1H, 3-H or 5-H), 7.44 (s, 1H, 5'-H).

**Methylation of 5**

5 (12 mg) was methylated with ethereal diazomethane (1 ml) and methanol (0.5 ml) for 24 h at room temperature. Purification by preparative TLC in benzene-EtOAc (98 : 2, v/v) and recrystallization from EtOAc gave methyl 6-(3-chloro-2,4-dimethoxy-6-methoxybenzoyl)-2-hydroxy-4-methylbenzoate (4 mg) as colorless prisms and methyl 6-(3-chloro-2,4-dimethoxy-6-methoxybenzoyl)-2-hydroxy-4-methylbenzoate (8 mg) as pale red prisms.

Methyl 6-(3-chloro-2,4-dimethoxy-6-methoxybenzoyl)-2-hydroxy-4-methylbenzoate: M.p. 183-184 °C. - UV/vis (EtOH): \( \lambda_{\text{max}} \) (lg e) = 214 (4.75), 247 (4.28), 253 (4.28), 308 nm (3.79). - IR (KBr): \( \nu = 2950 \) (C=O), 1736 (O-C=O), 1631 (chelated C=O), 1582 (C=C), 1462, 1440, 1360, 1339 cm\(^{-1}\). - \(^1\)H NMR (270.05 MHz, CDCl\(_3\)): \( \delta = 2.13 \) (s, 3H, Ar-Me), 3.72 (s, 3H, Ar-OCOMe), 3.83 (s, 3H, Ar-OCOMe), 3.95 (s, 3H, Ar-OMe), 3.98 (s, 3H, Ar-OMe), 5.69 (br.s, 1H, 5-H), 6.45 (br.s, 1H, 3-H), 7.30 (s, 1H, 3'-H), 11.47 (s, 1H, OH). - \(^13\)C\(^{\text{[H]}}\) NMR (67.80 MHz, CDCl\(_3\)): \( \delta = 22.0 \) (q, Ar-Me), 52.4 (q, Ar-OCOMe), 52.5 (q, Ar-OCOMe), 56.6 (q, Ar-OMe), 61.2 (q, Ar-OMe),
100.6 (d), 105.8 (d), 108.4 (d), 111.6 (s), 123.4 (s), 141.8 (s), 146.4 (s), 151.0 (s), 152.9 (s), 159.3 (s), 163.2 (s), 164.9 (s), 171.4 (s). - MS (EI): m/z (%): 412 (22) [M+], 410 (54) [M+], 381 (42), 379 (98), 320 (100), 305 (58), 292 (48), 178 (75), 149 (52).

Methyl 6-(3-chloro-2,4-dimethoxy-6-methoxy-carbonylphenoxy)-2-methoxy-4-methylbenzoate: M.p. 177-178 °C. - UV/vis (EtOH): λ_{max} (lg ε) = 214 (4.89), 246 (4.31), 281 (3.79), 301 nm (3.79). - IR (KBr): v = 2950 (C=C), 1744 (O-C=O), 1709 (O-C=O), 1593 (C=C), 1469, 1434, 1411, 1349 cm^{-1}. - ^1H NMR (270.05 MHz, CDCl_3): δ = 2.19 (s, 3H, Ar-Me), 3.75 (s, 3H, Ar-COO-Me), 3.84 (s, 3H, Ar-COO-Me), 3.86 (s, 3H, Ar-COO-Me), 3.92 (s, 3H, Ar-OMe), 3.97 (s, 3H, Ar-OMe), 5.79 (br.s, 1H, 5-H), 6.39 (br.s, 1H, 3-H), 7.30 (s, 1H, 3'-H). - ^13C{^1H} NMR (67.80 MHz, CDCl_3): δ = 22.1 (q, Ar-Me), 52.2 (q, Ar-COO-Me), 52.6 (q, Ar-COO-Me), 56.0 (q, Ar-OMe), 56.6 (q, Ar-OMe), 61.3 (q, Ar-OMe), 105.6 (d), 106.1 (d), 108.3 (d), 108.5 (s), 110.1 (s), 123.8 (s), 141.2 (s), 141.6 (s), 151.3 (s), 153.0 (s), 155.9 (s), 157.4 (s), 165.4 (s), 166.5 (s). - MS (EI): m/z (%): 426 (32) [M+], 424 (100) [M+], 393 (19), 391 (47), 360 (23), 348 (37), 259 (80), 192 (78), 163 (41).

Bioassay for the growth of lettuce and rice seedlings

Bioassay methods with lettuce and rice seedlings were described previously [13].