Pavietin, a Coumarin from *Aesculus pavia* with Antifungal Activity[⊥]

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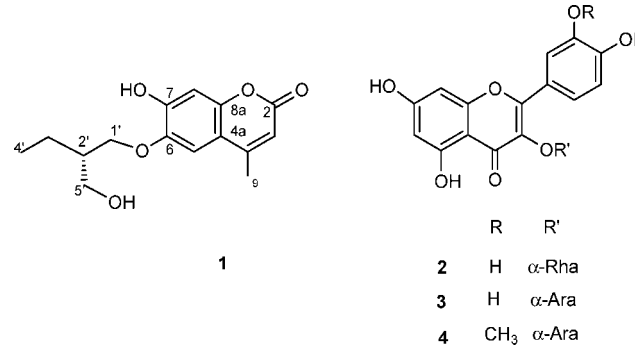
A new prenylated coumarin, *S*-6-[2-(hydroxymethyl)butoxy]-7-hydroxy-4-methyl-2*H*-chromen-2-one (**1**), named pavietin, has been isolated from the leaves of an *Aesculus pavia* genotype along with three known flavonol glycosides, quercetin 3-*O*-α-rhamnoside (quercitrin, **2**), quercetin 3-*O*-α-arabinoside (**3**), and isorhamnetin 3-*O*-α-arabinoside (distichin, **4**). The chemical structure of compound **1** was determined by chemical and spectroscopic methods, inclusive of UV, MS, and 1D and 2D NMR experiments. It showed appreciable antimicrobial properties against several pathogens, displaying a significant antifungal activity toward one of the main fungal parasites of *Aesculus* species, *Guignardia aesculi.* The same biological tests performed with a mixture of flavonoids **2**–**4** resulted in weak or no activity. Compound **1** was undetectable in *Aesculus hippocastanum*, a closely related species lacking resistance to fungal pathogens. The possible role of **1** in plant resistance is discussed.

Coumarin (1,2-benzopyrone) derivatives represent an important class of C_6–C_3 plant metabolites, originating from the shikimate pathway, and studied extensively because of their biological properties related to their medicinal use. Representative coumarins display activity as anticoagulant,[1] antituberculous,[2] anti-inflammatory, and cytotoxic agents.[3] Other coumarin derivatives are used in the preparation of perfumes, soaps, flavorings, and sunscreen cosmetics.[4,5] Moreover, the antimicrobial activity of coumarin derivatives is documented widely against bacteria[6,7] and fungi.[7,8] In this respect, coumarins are considered phytoalexins because they may be biosynthesized by plant tissues as a defensive response to pathogenic attack.[9]

In a search for natural antifungal compounds from plants[10–12] we have performed a phytochemical screening procedure on the leaves of *Aesculus pavia.* This plant is native to North America, but when introduced to Europe, it has produced hybrids,[13] therefore making available several different new genotypes and chemical races. Thus, from the active organic extract of an *A. pavia* genotype, which showed an unusual resistance to fungal attack, we have isolated a new prenylated coumarin, *S*-6-[2-(hydroxymethyl)butoxy]-7-hydroxy-4-methyl-2*H*-chromen-2-one (**1**), named pavietin, along with three known flavonol glycosides, quercetin 3-*O*-α-rhamnoside (quercitrin, **2**), quercetin 3-*O*-α-arabinoside (**3**), and isorhamnetin 3-*O*-α-arabinoside (distichin, **4**). The structure of compound **1** was determined by chemical and spectroscopic methods, including UV, MS, and 1D and 2D NMR experiments. The antimicrobial activity of the four isolated compounds was evaluated to explore their possible involvement in plant resistance to pathogens.

From the alcoholic extract of *A. pavia* leaves, a new coumarin (**1**, 0.8 mg g^{-1} dry wt), named pavietin, has been isolated, along with three known flavonoid glycosides: **2** (3.2 mg g^{-1} dry wt), **3** (4.3 mg g^{-1} dry wt), and **4** (0.6 mg g^{-1} dry wt). The flavonoids were identified as quercetin 3-*O*-α-rhamnoside (quercitrin, **2**),[14] quercetin 3-*O*-α-arabinoside (**3**),[15] and isorhamnetin 3-*O*-α-arabinoside (distichin, **4**),[16] respectively, by comparison of their MS and NMR data with those reported in the literature.



Preliminary hydrolysis of **1**, performed according to the method of Arcelli et al.,[17] indicated the absence of any sugar unit. Its UV spectrum showed two main absorption bands at 220–230 and 310–350 nm and a secondary shoulder at 293 nm that suggested a typical coumarin structure.[18] However, analytical HPLC of **1** (t_R 9.25 min) in comparison with esculin, esculetin, fraxetin, and scopoletin (t_R 6.50, 7.90, 8.12, and 8.45 min, respectively) suggested that **1** differs structurally from the coumarins already described in the common horse chestnut (*Aesculus hippocastanum* L.) leaf extracts.[19] The HRFABMS of **1** gave a molecular ion peak at *m/z* 279.1256 $[M + H]^+$, which, together with the data obtained from the ^{13}C NMR spectrum, indicated the molecular formula to be $C_{15}H_{18}O_5$. Its ^1H NMR spectrum (Table 1) showed three singlet signals at δ 6.08, 6.78, and 7.04, in agreement with a trisubstituted coumarin skeleton. The ^{13}C NMR spectrum of **1** supported this hypothesis by showing resonances of an ester carbonyl (δ 164.5) and of eight sp^2 carbons (Table 1), three of which were protonated. Further signals in the NMR spectra were those attributed to a methyl group (3H, s, δ 2.39) and to a 2-(hydroxymethyl)butoxy moiety (Table 1), for which this partial structure was supported by the COSY and HSQC spectra. This demonstrated the connection of the three protonated sp^2 carbons as well as the other sp^3 protonated carbons of the ^{13}C NMR spectrum with the attached protons. The structure of the coumarin skeleton, including the location of its substituents, was secured from the HMBC spectrum, which also allowed a complete assignment of the ^1H and ^{13}C NMR spectra (Table 1). Thus, HMBC cross-peaks from H-3 to C-2, C-4a, and C-9 and from H-9 to C-3, C-4, and C-4a (Figure 1 and Table 1)

[⊥] In memory of Ferdinando D'Aquila, Istituto Sperimentale per la Floricoltura di Sanremo.

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Table 1. ^{1}H, ^{13}C, and HMBC NMR Data of Pavietin (**1**) [500 MHz, CD_3OD, δ (ppm), and mult. in Hz]

position	δ_H (mult., J in Hz)	δ_C (mult.)	HMBC
2		164.5 (C)	
3	6.08 (s)	111.3 (CH)	2, 4a, 9
4		156.0 (C)	
4a		113.5 (C)	
5	7.04 (s)	110.0 (CH)	4, 4a, 6, 7, 8a
6		149.7 (C)	
7		151.9 (C)	
8	6.78 (s)	103.7 (CH)	2, 4a, 6, 7, 8a
8a		144.6 (C)	
9	2.39 (s)	18.8 (CH_3)	3, 4, 4a
1′a/1′b	4.11 (dd, 10, 3) 3.64 (bd, 10, 3)	69.1 (CH_2)	6, 1′, 5′
2′	2.07 (m)	40.2 (CH)	4′
3′	1.35 (dq, 7, 3)	24.9 (CH_2)	1′, 5′
4′	0.98 (t, 7)	11.4 (CH_3)	2′, 3′
5′a/5′b	3.61 (dd,10, 3) 3.29 (dd,10, 3)	63.9 (CH_2)	1′, 2′, 3′

helped to define the lactone ring, while HMBC correlations from H-5 to C-4, C-4a, C-6, C-7, and C-8a and from H-8 to C-2, C-4a, C-6, C-7, and C-8a were relevant to the aromatic ring. Further correlations of H-1′ with C-6 were used to locate on this oxygenated carbon the 2-(hydroxymethyl)butoxy moiety, thus leaving the free hydroxyl group on the adjacent C-7.

To determine the stereochemistry at C-2′ in **1**, Mosher's method[20] was used. Thus, pavietin (**1**) was treated with (+)- and (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride, affording the 7,5′-di-(+)-MTPA and the 7,5′-di-(−)-MTPA ester, respectively. The ^{1}H NMR spectra (Experimental Section) of these two compounds showed the 5′-methylene proton signals of the (−)-MTPA ester resonating as well-separated signals at δ 4.14 and 4.28 (each dd, J = 6.5, 10.5 Hz), while in the spectrum of the (+)-MTPA ester they appeared as doublets at δ 4.19 and 4.23 (each dd, J = 6.5, 10.5 Hz), in agreement with a 2′ *S*-configuration. In fact, in the spectra of MTPA esters of an *S* isomer, the oxymethylene protons, adjacent to the stereocenter and acylated with MTPA, appear as much closer signals in the spectrum of the (+)-MTPA ester than in that of (−)-MTPA derivative, while the reverse occurs for MTPA esters of an *R* isomer.[20] All these data indicated the structure of **1** as *S*-6-[2-(hydroxymethyl)butoxy]-7-hydroxy-4-methyl-2*H*-chromen-2-one.

Pavietin (**1**) was found exclusively within leaf tissues of the *A. pavia* HBT genotype grown at the Botanical Garden of Turin. However, it was undetectable in *A. hippocastanum* (white flowering) leaves.

The isolated coumarin (**1**) appears to be a constitutive leaf component of the *A. pavia* genotype investigated, as suggested by its concentration of around 0.8 mg g^{-1} dry material, with fluctuations, depending on the observation periods, of about ±12% of the average value. The finding of this metabolite within *A. pavia* HBT leaves is not surprising, since leaves represent the primary site of coumarin biosynthesis.[21] Coumarins have been reported to accumulate on and/or under the surface of leaves, fruits, and seeds, where they may inhibit growth and sporulation of fungal pathogens.[22]

Thus, in a preliminary antifungal screening by filter disk method (top of Table 2) we showed that pavietin (**1**) possesses significant activity against an *Aesculus*-specific fungal parasite, *Guignardia aesculi,* while exerts weaker activity against the generalist polyphagous *Pythium ultimum* and the *Aesculus* nonhost *Fusarium basilici*.

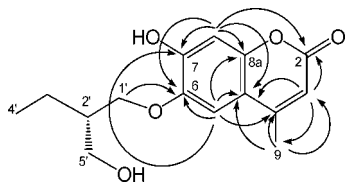

Figure 1. Selected HMBC (H→C) correlations exhibited by pavietin (**1**).

Table 2. Mycelial Growth of Fungal Species in the Presence of Pavietin (**1**) Using a Filter Disk Method (top) and Added to the Growth Medium (bottom)

	concentration of **1** (mg /disk)		
fungal species	0.15	1.5	15.0
Fusarium basilici	87.8[a]	76.4[b]	59.0[b]
Guignardia aesculi	85.3[b]	64.0[a]	33.8[a]
Pythium ultimum	88.4[a]	81.6[b]	78.0[c]

	concentration of **1** (μm)		
fungal species	50	100	200
Alternaria alternata	86.8[d,e]	80.2[d,e]	58.5[c,d]
Alternaria dianthi	89.5[d,e]	77.0[d]	64.9[d]
Aspergillus niger	73.2[b]	68.6[c,d]	50.0[c]
Botrytis cinerea	78.8[c]	64.6[c]	51.4[c]
Cladosporium fulvum	88.6[d,e]	78.3[d,e]	60.8[c,d]
Fusarium oxysporum dianthi	91.8[e]	83.3[e]	66.9[d]
Guignardia aesculi	84.6[d]	46.5[a]	20.7[a]
Penicillium expansum	68.8[a]	48.4[a]	29.9[b]
Polyporus marginatus	93.6[f]	86.2[f]	76.1[e]
Pseudomonas fluorescens	69.9[a]	58.8[b]	50.6[c]
Rhizopus stolonifer	82.0[d]	71.3[c,d]	68.1[d]
Trichoderma viride	78.8[c]	70.4[c,d]	61.5[c,d]

[a–e]Growth percentages were determined by comparison to a control (100% growth). Values in each column followed by the same letter are not statistically different for $p = 0.05$, according to the Student–Neumann–Keuls test. Percentages were transformed in arcsin before the statistical analysis.

More detailed antifungal screening by adding **1** to mycelial growth medium of different fungal pathogens (bottom of Table 2) showed that **1** exhibits an appreciable inhibitory activity toward the assayed phytopathogens, depending on the fungal genotype, while a mixture of flavonoids **2**–**4** showed little or no ability to inhibit mycelial growth (Table S1, Supporting Information). Compound **1** was more active for *G. aesculi* than on the other fungal pathogens and the bacterium *Pseudomonas fluorescens* (Table 2). Among the other fungal pathogens, **1** at the highest dosage was shown to be effective against *Penicillium expansum*, while displaying the lowest activity toward *Alternaria dianthi*, *Fusarium oxysporum dianthi*, and *Rhizopus stolonifer*. An intermediate effect of **1** has been detected toward the other tested fungi. In general, a concentration-related inhibition of mycelial growth has been observed for **1** on the assayed microorganisms. However, the highest concentration used (200 μmol/L) can be still considered a low concentration for a natural antifungal drug.[27] Thus, the selective antifungal activity displayed by **1** against *G. aesculi* suggests a possible defensive role of this compound against fungal attack. Although further investigations are needed to demonstrate an involvement in the protection of *A. pavia* against pathogens, compound **1** may be a useful lead compound,[8] which adds to the growing number of new coumarins with antifungal activity described recently from plants.[8,23–25]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10 cm microcell. FTIR spectra were run both on a Bruker IFS-48 spectrometer in KBr (University of Napoli Federico II) and on a Perkin-Elmer 1600 spectrometer in KBr (University of Molise). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova spectrometer at 500.13 and 125.77 MHz, respectively. Chemical shifts were referred to the residual solvent signal (CD_3OD: δ_H 3.31, δ_C 49.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ^{1}H connectivities were determined by using COSY and HOHAHA experiments; the 2D HOHAHA experiments were performed in the phase-sensitive mode (TPPI) using the MLEV-17 (mixing time 125 ms) sequence for mixing. One-bond heteronuclear ^{1}H–^{13}C connectivities were determined with 2D HSQC pulse sequence with an

interpulse delay set for $^1J_{CH}$ of 130 Hz. Two- and three-bond heteronuclear ^1H–^{13}C connectivities were determined with 2D HMBC experiments, optimized for a $^1J_{CH}$ of 8 Hz. Nuclear Overhauser effect (NOE) measurements were performed by 2D ROESY experiments. HRFABMS (glycerol matrix) were performed on a VG Prospec Fisons mass spectrometer. GC-MS analysis was performed on a Carlo Erba instrument. HPLC analyses were performed on a Perkin-Elmer system (200 lc pump, 795A UV/vis detector) equipped a PE Nelson NCI 900 interface controlled by Turbochrom software.

Plant Material. The *Aesculus* species used throughout the present research is a tree about 70 years old, growing in the University Botanical Garden (Viale Mattioli, Torino, Italy), classified in the garden botanical register as *A. pavia* HBT genotype and identified by Prof. Bruno Peyronel (Università di Torino). A voucher specimen of leaves (No. 2003AP) has been deposited at the Istituto Sperimentale per la Floricoltura, Sanremo. *A. pavia* species frequently originate hybrids that are sterile, due to low pollen viability.[26] The genotype here considered bears red flowers but was not fruiting. This tree represents a living specimen available on request as an explant source.

Five-hundred-gram lots of fresh leaves were harvested, at different times, along a time period from spring (May) to summer (September). The collected leaves were allowed to dry completely on shelves at room temperature, and then they were milled, thus obtaining a dry powder.

Leaves of *Aesculus hippocastanum* trees, which bear white flowers and produce the typical horse chestnut fruit, were used as a reference material.

Extraction, Isolation, and in Situ Quantitation. Powdered leaves were extracted according to a previously published protocol,[11] and the coumarin- and flavonoid-containing fraction were obtained from the crude extracts through reversed-phase column chromatography, following an already described procedure.[10] The coumarin fraction was rechromatographed on a Sephadex LH-20 (Sigma, St. Louis, MO) column (30 × 1 cm) packed and eluted with EtOH–H_2O (50:50), acidified to pH 4.5 with H_3PO_4. Coumarin **1** was finally purified through preparative TLC on 20 × 20 cm glass silica plates, 500 μm thickness (Merck, Darmstadt, Germany), using EtOAc–petroleum ether (bp 40–60 °C), 65:35, as solvent. The flavonoid fraction was rechromatographed on a Sephadex G-25-80 (Sigma, St. Louis, MO) column (350 × 28 mm) eluted with water, acidified to pH 3.5 with HCOOH. Further purification of flavonoids **2**–**4** was carried out by a column (200 × 28 mm) filled with silica gel 100 C_{18} reversed-phase, under controlled eluent flux (by means of a Gilson model peristaltic pump), using a linear solvent gradient from water (5% HCOOH)–MeOH (85:15) to water (5% HCOOH)–MeOH (20:80).

Quantitation of **1** within leaf tissues of *A. pavia* was carried out by means of HPLC analysis on a C_{18} reversed-phase column (25 cm × 4.6 mm × 5 μm) using a mobile phase of water (5% H_3PO_4)–MeOH (70:30) and a flow rate of 1 mL/min.

Four different coumarins, esculin, esculetin, fraxetin, and scopoletin, previously isolated from leaves of the horse chestnut (*A. hippocastanum*),[19] were purchased from Sigma (St. Louis, MO) and run under the same analytical conditions.

Quantitative determinations of coumarin **1** were performed by the calibration curve method. Known amounts of **1**, previously dissolved in the mobile phase at different concentrations (2.0–250 μg/mL), were run to define the corresponding peak integrated areas to be used as reference. Measurements were performed on leaves collected during 2006, in three different months: May, July, and September.

Pavietin [*S*-6-[2-(hydroxymethyl)butoxy]-7-hydroxy-4-methyl-2*H*-chromen-2-one, 1]: yield 0.8 mg g^{-1}; yellow, amorphous solid; $[\alpha]_D^{25}$ –27.0 (*c* 0.1 $CHCl_3$); UV λ_{max} (log ε) 226 (3.95), 293 (sh), 346 (4.26) nm; IR (KBr) ν_{max} 3400, 1680, 1450, 1295, 1162 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRFABMS (positive ion) found *m/z* 279.1256 $[M + H]^+$ (calcd for $C_{15}H_{18}O_5$ *m/z* 279.1233).

MTPA Esters of 1. Compound **1** (1.5 mg) was esterified with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (5 μL) in dry pyridine (0.15 mL) for 1 h at room temperature to give, after removal of the solvent, the 7,5′-di-(+)-MTPA: ^1H NMR (CD_3OD) δ_H 6.08 (1H, s, H-3), 7.04 (1H, s, H-5), 6.95 (1H, s, H-8), 2.39 (3H, s, H_3-9), 4.19 and 4.23 ppm (each 1H, dd, J = 6.5, 10.5 Hz, H_2-5′).

The 7,5′-di-(–)-MTPA ester of **1** (1.5 mg) was prepared using (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride. ^1H NMR (CD_3OD) identical with values reported for (+)-MTPA except for signals of H_2-5′ split at δ 4.14 and 4.28 ppm (each 1H, dd, J = 6.5, 10.5 Hz).

Evaluation of Biological Activity. An initial evaluation of the antifungal activity of **1** was carried out by the filter disk method.[27] Compound **1** was dissolved in a few drops of absolute EtOH to give the needed amounts of 0.15, 1.5, and 15 mg per 5 mm diameter paper disk, respectively. Thirty 12 cm diameter Petri dishes, aseptically filled with potato dextrose agar (PDA) growth medium, were set for each treatment and for each fungal species. The inhibition zones surrounding each filter paper disk were measured in millimeters at the end of an incubation period of 3 days at 27 °C. The absolute EtOH alone showed no inhibition zone (control). The flavonoid fraction, containing 39.5% **2**, 53% **3**, and 7.5% **4** (w/w/w), was simultaneously tested by the same assay at concentrations of 0.25, 2.5, and 25 mg per paper disk.

An additional assay of the antifungal activity of **1** was then carried out by incorporating the compound directly into the growth medium. The molecule to be tested, dissolved in a few drops of DMSO, was added after ultrafiltration to the autoclaved, still molten PDA medium. Concentrations of 50, 100, and 200 μmol L^{-1} were respectively assayed, and 40 Petri dishes/treatment/fungal species were set. Mycelial disks, 1 cm diameter, were inoculated and allowed to grow for 3 days at 22 °C, in darkness. The mycelial development reached for each treatment was then compared to that of the corresponding untreated samples (controls), containing only DMSO, assumed as 100%, and the results were expressed as percentages.

Fungal species used in the experiments were isolated, determined, and kept in culture under in vitro conditions at University of Turin, Grugliasco (TO), Italy.

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Supporting Information Available: Table S1 showing antifungal activity of a mixture of flavonoids. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Anderson, D. M.; Shelley, S.; Crick, N.; Buraglio, M. J. *Clin. Pharmacol.* **2002**, *42*, 1358–1365.
(2) Gürsoy, A.; Karali, N. *Turk. J. Chem.* **2003**, *27*, 545–551.
(3) Lacy, A.; O'Kennedy, R. *Curr. Pharm. Des.* **2004**, *10*, 3797–3811.
(4) Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E.; Thornes, D. *Drug Metabol. Rev.* **1990**, *22*, 503–529.
(5) Wilkinson, J. A.; Brown, A. M. G. *Int. J. Cosmet. Sci.* **1999**, *21*, 437–447.
(6) Dini, A.; Ramundo, E.; Saturnino, P.; Scimone, A.; Stagno d'Alcontres, I. *Boll. Soc. Ital. Biol. Sper.* **1992**, *68*, 453–461.
(7) Rehman, S. U.; Chohan, Z. H.; Gulnaz, F.; Supuran, C. T. *J. Enzyme Inhibit. Med. Chem.* **2005**, *20*, 333–340.
(8) Oliva, A.; Meepagala, K. M.; Wedge, D. E.; Harries, M. D.; Hale, A. L.; Aliotta, G.; Duke, S. O. *J. Agric. Food Chem.* **2003**, *51*, 890–896.
(9) Weinmann, I. In *Coumarins–Biology, Applications and Mode of Action*; O'Kennedy, R., Thornes, R. D., Eds.; Wiley and Sons Ltd: Chichester, UK, 1997; pp 1–22.
(10) Curir, P.; Dolci, M.; Lanzotti, V.; Taglialatela-Scafati, O. *Phytochemistry* **2001**, *56*, 717–721.
(11) Curir, P.; Dolci, M.; Dolci, P.; Lanzotti, V.; De Cooman, L. *Phytochem. Anal.* **2003**, *14*, 8–12.
(12) Curir, P.; Dolci, M.; Corea, G.; Galeotti, F.; Lanzotti, V. *Plant Biosyst.* **2006**, *140*, 156–162.
(13) Hsiao, J. Y.; Li, H. L. *Brittonia* **1973**, *25*, 57–63.
(14) Byron, L. W.; Simon, H. W. *J. Am. Chem. Soc.* **1953**, *75*, 4363–4.
(15) Clark, H. I.; Simon, H. W. *J. Am. Chem. Soc.* **1953**, *75*, 50–52.
(16) Kariyone, T.; Takahashi, M.; Ito, T.; Masutani, K.; Yakugaku, Z. *Yakugaku Zasshi* **1960**, *80*, 102–5.
(17) Arcelli, A.; Paradisi, F.; Porzi, G.; Rinaldi, S.; Sandri, S. J. *Chem. Res., Synop.* **2002**, *199*, 200.
(18) Riberéau-Gayon, P. In *Les Composés Phénoliques des Végétaux*; Riberéau-Gayon, P., Ed.; Dunod: Paris, 1968; pp 111–112.
(19) Bombardelli, E.; Morazzoni, P.; Griffini, A. *Fitoterapia* **1996**, *67*, 483–511.
(20) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
(21) Murray, R. D. H.; Mendez, J.; Brown, S. A. *The Natural Coumarins–Occurrence, Chemistry and Biochemistry*; John Wiley and Sons, Ltd: Chichester, UK, 1982.
(22) Matern, U.; Lüer, P.; Kreusch, D. In *Comprehensive Natural Products Chemistry, Polyketides and Other Secondary Metabolites Including*

Fatty Acids and Their Derivatives; Barton, D., Nakanishi, K., Meth-Cohn, O., Sankawa, U., Eds.; Elsevier Science Ltd.: Oxford, UK, 1999; pp 623–637.
(23) Ojala, T.; Remes, S.; Haansun, P.; Vuorela, H.; Hiltunen, R.; Haachtela, K.; Vuorela, P. *J. Ethnopharmacol.* **2000**, *73*, 299–305.
(24) Moury, T.; Yano, T.; Kochi, S.; Ando, T.; Hori, M. *J. Pestic. Sci.* **2005**, *30*, 209–213.
(25) Prats, E.; Bazzolo, M.; León, A.; Jorrìn, J. *Euphytica* **2006**, *147*, 451–460.
(26) Sherman-Hoar, C. *Bot. Gaz.* **1927**, *84*, 156–170.
(27) Nene, Y. L. Thapliyal, P. N. *Fungicides in Plant Disease Control*; Oxford & IBH Publisher: New Delhi, 1982.

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