Antifungal 3-Butylisocoumarins from Asteraceae-Anthemideae

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Seven new naturally occurring 3-butylisocoumarins were isolated and identified from lipophilic extracts of aerial as well as underground organs: corfin (**17**) and 3′-hydroxycorfin (**18**) from the roots of *Chamaemelum mixtum* and (−)-(*R*)-2′-methoxydihydroartemidin (**5**), (+)-(*S*,*R*)-epoxyartemidin (**6a**), dracumerin (**12**), (+)-(*R*)-(*E*)-3′-hydroxyartemidin (**13**), and capillarin isovalerate (**20**) from various organs of *Artemisia dracunculus* (tarragon). Furthermore, six known derivatives, artemidiol (**7**), (*E*/*Z*)-artemidin (**11**), capillarin (**19**), artemidinol (**21**), 8-hydroxyartemidin (**22**), and 8-hydroxycapillarin (**23**), were obtained. The antifungal activities of all naturally occurring derivatives were determined in a germ-tube inhibition test against a susceptible strain of rice blast fungus *Pyricularia grisea*. The 3-butyl side-chain is a prerequisite for high activity. Eleven structurally related synthetic derivatives were additionally tested to explore the influence of structural characteristics on activity. Benlate, blasticidin S, kresoxim-methyl, griseofulvin, and the carrot phytoalexin 6-methoxymellein all served as positive controls.

The most recent review[1] of isocoumarins listed more than 160 natural products that occur in a wide range of organisms, including bacteria, fungi, and plants. Moreover, these compounds were also detected in secretions of termites and ants. 6-Methoxymellein was one of the first derivatives to be isolated from a plant source. This compound gained some attention because it was identified to cause the bitter taste that spoils the quality of stored carrots.[2] In vitro experiments suggested that the formation of 6-methoxymellein was actually induced by fungal infection, and subsequently, a number of studies explored its function as a phytoalexin.[3]

Polyketide synthases are involved in the biosynthesis of most naturally occurring isocoumarins, e.g., in 6-methoxymellein,[4] or in the mycotoxin ochratoxin A.[5] However, isocoumarins isolated from *Artemisia capillaris* Thunb., *A. dracunculus* L. (tarragon), and several other genera of the Asteraceae tribe Anthemideae were found to descend from fatty acid precursors on the basis of feeding experiments.[6] Thus, we suggest that these isocoumarins deserve a separate status within isocoumarins as 3-butylisocoumarins.

A preliminary TLC bioautography, using *Cladosporium herbarum* (Pers.: Fries) Link, indicated that extracts of various sources of *A. dracunculus* as well the only source of *Chamaemelum mixtum* (L.) All. contained different antifungal 3-butylisocoumarins. These findings and the fact that 6-methoxymellein was already known to form a potent antifungal natural product prompted us to explore the antifungal activities of naturally occurring 3-butylisocoumarins. Previous phytochemical analyses of various sources of the polymorphic *A. dracunculus* and other closely related species had already led to the identification of different 3-butylisocoumarins.[7,8] Thus, we refocused on various sources of *A. dracunculus* grown from seed that were obtained from different botanical gardens. HPLC−UV fingerprinting of lipophilic crude extracts intended to detect as much structural variety as possible. As a result, five derivatives (**5**, **6a**, **12**, **13**, and **20**) turned out to represent hitherto unpublished structures. In addition, two further new isocoumarins, **17** and **18**, were isolated and identified from the roots of *C. mixtum* collected in Corfu Island, Greece.

Aside from additional hydroxyl groups on carbon atom 5 or 8 on the aromatic ring system (**21**−**23**), structural diversity mainly resulted from various transformations of the butyl side-chain. To obtain more information concerning the structure−activity relationships of this type of isocoumarins, 11 additional derivatives were synthesized.[9] In bioautography on TLC plates, diffusion often impairs the determination of inhibitory concentrations.[10] Consequently, germ-tube inhibition tests were carried out in broth microdilutions to obtain a comparability of results. A susceptible strain of the rice blast fungus *Pyricularia grisea* (Cooke) Sacc. [teleomorph *Magnaporthe grisea* (Herbert) Barr],[11] which has already served as a test organism in our laboratory to detect other antifungal natural products,[12] was used to evaluate the antifungal activity of the available 3-butylisocoumarins.

Results and Discussion

3-Butylisocoumarins are characterized by typical UV spectra. Modifications of the chromophore system by substituents affect the intensity and position of maxima, in particular between 270 and 320 nm. Additional hydroxylation of the aromatic ring causes a bathochromic shift of the maximum in the wavelength range from 340 to 360 nm.[13] These spectroscopic characteristics permit the direct detection of isocoumarins in HPLC−UV analyses.

A hexaploid[7] strain of *A. dracunculus* from the Botanical Garden in Novosibirsk, Russia (AR-789), accumulated a larger amount of compound **12**. The UV spectrum could be clearly differentiated from that of the co-occurring isomeric mixture of *E*/*Z*-artemidin (**11**) by a bathochromic shift from 280−305 nm to 300−315 nm. This suggested an extension of the chromophore.[13] Both **11** and **12** occurred as a pair of *E*/*Z*-isomers that were largely inseparable by HPLC: the isomers of artemidin (**11**) were at least partially resolved, and those of **12** eluted as a single peak. Only GC supplied a baseline resolution of the corresponding 2′-(*E*)- and 2′-(*Z*)-isomers of **11** and **12**. In comparison with

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Table 1. ^{1}H and ^{13}C NMR of 3-Butylisocoumarins **11** (*E* and *Z*), **12** (*E* and *Z*), **13**, and **5** ($CDCl_3$, δ/ppm, 250 MHz)[a]

	^{1}H						^{13}C					
	11(*E*)	**11(*Z*)**	**12(*E*)**	**12(*Z*)**	**13**	**5**[b]	**11(*E*)**	**11(*Z*)**	**12(*E*)**	**12(*Z*)**	**13**	**5**[c]
1							162.1 s	162.2 s	161.9 s	161.9 s	162.0 s	
3							152.5 s	153.7 s	152.4 s	153.8 s	152.0 s	
4	6.27 s	6.36 s	6.37 s	6.41 s	6.35 s	6.34 s	103.4 d	105.8 d	105.7 d	107.1 d	105.2 d	104.9 d
4a							137.6 s	137.6 s	137.5 s	137.4 s	137.4 s	
5	7.37 br.d	7.39 br.d	7.39 br.d	7.40 br.d	7.39 br.d	7.37 br.d	125.4 d	125.6 d	125.7 d	128.8 d	125.7 d	125.2 d
6	7.66 ddd	7.67 ddd	7.67 m	7.67 m	7.68 ddd	7.68 ddd	134.5 d	134.6 d	134.8 d	134.7 d	134.8 d	134.7 d
7	7.44 br.dd	7.46 ddd	7.47 ddd	7.45 ddd	7.46 ddd	7.46 ddd	127.5 d	127.9 d	128.1 d	128.2 d	128.1 d	127.7 d
8	8.26 br.d	8.26 br.d	8.27 br.d	8.27 br.d	8.26 br.d	8.26 br.d	129.4 d	129.6 d	129.8 d	129.7 d	129.7 d	129.5 d
8a							120.4 s	120.2 s	120.9 s	120.4 s	120.8 s	
1′	6.04 dt	5.94 dt	6.17 d	5.87 d	6.30 dd	2.71/2.63 dd	138.1 d	140.6 d	123.4 d	119.7 d	138.7 d	38.2 t
2′	6.70 dt	5.82 dt	7.10 dd	6.28 dd	6.68 dd	3.63 ddt	120.8 d	120.1 d	133.8 d	134.5 d	120.4 d	79.3 d
3′	2.27 ddq	2.68 ddq	6.48 ddd	7.64 m	4.55 br.dq	1.62 m	25.6 t	22.9 t	135.8 d	134.0 d	67.7 d	26.3 t
4′	1.10 t	1.12 t	5.50/5.34 d	5.44/5.43 dd	1.39 d	0.96 t	12.8 q	14.2 q	121.7 t	123.2 t	23.4 q	9.2 q

[a] Coupling constants. Benzene ring: $J(5,6) = J(6,7) = J(7,8) = 7.9 \pm 0.1$ Hz, $J(5,7) = J(6,8) = 1.1 \pm 0.1$ Hz. **11(*E*)**: $J(1',2') = 15.6$ Hz, $J(1',3') = 1.2$ Hz, $J(2',3') = 6.7$ Hz, $J(3',4') = 7.4$ Hz. **11(*Z*)**: $J(1',2') = 12.0$ Hz, $J(1',3') = 1.0$ Hz, $J(2',3') = J(3',4') = 7.5$ Hz. **12(*E*)**: $J(1',2') = 15.4$ Hz, $J(2',3') = 11.0$ Hz, $J(3',4'a) = 17.0$ Hz, $J(3',4'b) = 9.9$ Hz. **12(*Z*)**: $J(1',2') = 11.9$ Hz, $J(2',3') = 11.5$ Hz, $J(3',4'a) = 17.7$ Hz, $J(3',4'b) = 8.8$ Hz, $J(2',4') = 0.8$ Hz. **13**: $J(1',2') = 15.5$ Hz, $J(1',3') = 1.4$ Hz, $J(2',3') = 5.5$ Hz, $J(3',4') = 6.5$ Hz. **5**: $J(1'a,1'b) = 14.8$ Hz, $J(1'a,2') = 7.3$ Hz, $J(1'b,2') = 5.7$ Hz, $J(2',3') = \sim 7$ Hz, $J(3',4') = 7.4$ Hz. [b] **5**: OCH_3 3.35 s. [c] **5**: OCH_3 57.2 q.

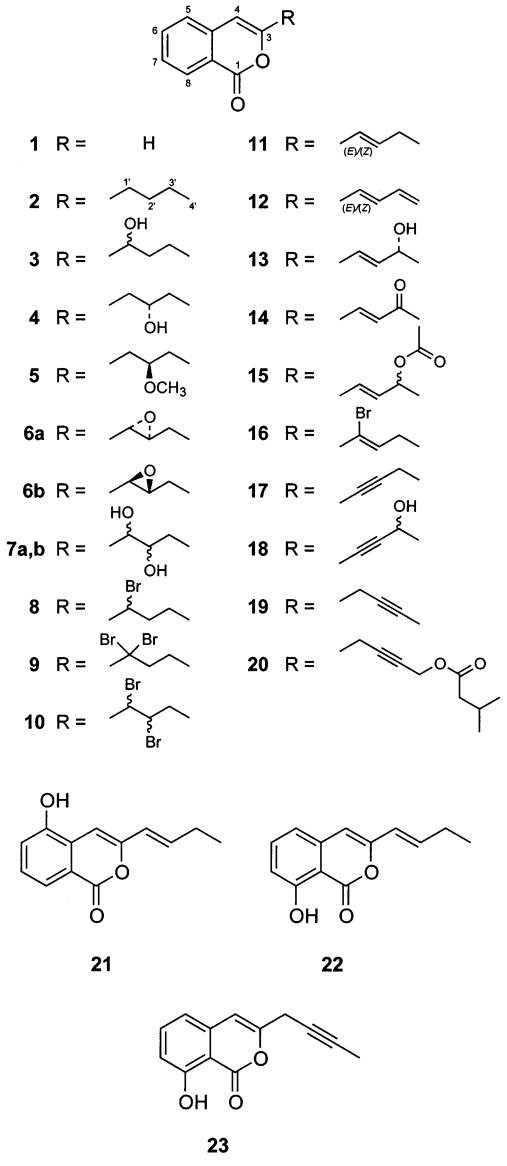

Figure 1.

synthetic (*E*)-artemidin (**11**) the peak with the lower retention time was identified as the *Z*-isomer.[13] The mass spectrum of **12** showed the molecular ion ($m/z = 198$) that differed by 2 mass units from **11** ($m/z = 200$).[14] This could be explained by inferring an additional double bond in the butyl side-chain. A strong band at 1000 cm^{-1} in the IR spectrum also supported this hypothesis. Finally, the structure of **12** was confirmed by ^{1}H and ^{13}C NMR spectra showing the characteristic ABX system for the terminal 4′-methylene groups CH(3′)═CH_2(4′) in conjugation with the CH(1′)═CH(2′) double bond in either *E*- ($J_{1',2'} = 15.4$ Hz) or *Z*-configuration ($J_{1',2'} = 11.9$ Hz) (Table 1). Since only mixtures of (*E*)/(*Z*)-**12** (7:3) were available, the assignments of the ^{1}H and ^{13}C resonances to the isomers were confirmed by C/H correlation (HMQC). With regard to its restricted occurrence in the *A. dracunculus* group, we propose to call **12** dracumerin. *Z*- and *E*-isomers of artemidin (**11**) occurred in distinct ratios in aerial (10:7) as well as underground organs (3:1). The different accumulation rates of different isomers appeared to be the result of specific enzymatic activities rather than a mere consequence of isolation procedures. For instance, *Chamaemelum fuscatum* (Brot.) Wagenitz was shown to accumulate only the *E*-isomer of **11** in its roots.[14]

Three further isocoumarin derivatives were isolated from a decaploid strain[7] of *A. dracunculus* from the Botanical Garden in Krefeld (AR-913). One was identified as the known capillarin (**19**).[15] The second derivative (**20**) was shown to be a hitherto undescribed compound. The molecular formula of $C_{18}H_{18}O_4$ and the UV spectrum similar to that of capillarin (**19**) suggested a related compound with an additional residue in the side-chain. This was supported by the comparison of ^{1}H and ^{13}C NMR data with those of **19**. In contrast to **19**, the terminal methyl group had changed to a methylene group with a chemical shift of 4.76 ppm in the ^{1}H and 52.1 ppm in the ^{13}C NMR spectrum (Table 3). This was indicative for oxygen substitution and, together with the carbonyl resonance at 172.4 ppm in the ^{13}C spectrum, suggested an ester function at C-4′. The mass difference between compounds **19** and **20** corresponded to a $C_5H_8O_2$ unit, and a combined analysis of ^{1}H and ^{13}C data proved that the expected ester was derived from isovaleric acid (Table 3, footnote *b*). Consequently, the new compound was named capillarin isovalerate (**20**). The corresponding ester with senecionic acid was recently described from *Artemisia arctica* Less. as capillarisen.[16] The third derivative proved to be 8-hydroxycapillarin (**23**) on the basis of a comparison of spectral data with published data.[17]

An octoploid[7] strain of *A. dracunculus*, originating from the Botanical Garden in Tashkent, Uzbekistan (AR-312), proved to be an especially rich source of 3-butylisocou-

Table 2. ^{1}H and ^{13}C NMR of (+)-(*S*,*R*)-Epoxyartemidin (**6a**) and Its Decomposition Products **7a** and **7b** ($CDCl_3$, δ/ppm, 250 MHz)[a]

	^{1}H			^{13}C
	6a	**7a**[b]	**7b**[c]	**6a**
1				161.8 s
3				152.8 s
4	6.54 s	6.63 s	6.66 s	103.8 d
4a				136.5 s
5	7.41 br d	7.44 dm	7.44 dm	125.5 d
6	7.71 ddd	7.72 ddm	7.72 ddm	134.9 d
7	7.51 ddd	7.51 ddm	7.51 ddm	128.4 d
8	8.26 br d	8.27 dm	8.27 dm	129.6 d
8a				120.8 s
1′	3.48 d	4.51d	4.34 d	54.4 d
2′	3.29 dt	3.97 ddd	3.99 dt	61.5 d
3′	1.73 m	1.60 m (3′a) 1.53 m (3′b)	1.67 dq	24.7 t
4′	1.07 t	1.02 t	1.04 t	9.5 q

[a] Coupling constants. Benzene ring: $J(5,6) = J(6,7) = J(7,8) = 7.9 \pm 0.1$ Hz, $J(5,7) = J(6,8) = 1.1 \pm 0.1$ Hz. **6a**: $J(1',2') = 2.0$ Hz, $J(1',3') = 1.2$ Hz, $J(2',3') = 5.4$ Hz, $J(3',4') = 7.5$ Hz. **7a**: $J(1',2') = 4.4$ Hz, $J(2',3'a) = 9.0$ Hz, $J(2',3b') = 4.4$ Hz, $J(3',4') = 7.4$ Hz. **7b**: $J(1',2') = 3.4$ Hz, $J(2',3') = 6.7$ Hz, $J(3',4') = 7.4$ Hz. [b] 1′-OH 2.30 br s, 2′-OH 2.77 br s. [c] 1′-OH 2.01 br s, 2′-OH 2.02 br s.

marins.[9] Underground and aerial organs contained (*E*/*Z*)-artemidin (**11**) and several other 3-butylisocoumarins. Among those, 5-hydroxyartemidin, artemidinol (**21**), and 8-hydroxyartemidin (**22**) were already described from roots and stolons in previous reports.[18] Three further unknown isocoumarins **5**, **6a**, and **13** were isolated from the aerial organs of the same source. Although the UV spectrum of **5** was identical with capillarin (**19**), the molecular formula $C_{14}H_{16}O_3$ (HREIMS) and the ^{1}H NMR data suggested a saturated side-chain containing a methoxy group. The coupling pattern of the side-chain determined the position of the methoxy group at carbon atom 2′. The quasi benzylic 1′-H_2 protons were diastereotopic (2.71 and 2.63 ppm), and the resonances appeared as two dd with one geminal and one vicinal coupling each (Table 1). The same vicinal coupling constants (J = 7.3 and 5.7 Hz) were also evident in the signal of 2′-H at the oxygen-substituted position 2′ at δ 3.63 ppm (ddt, J = 7.3, 5.7, 7.0). The absolute configuration of compound **5**, named (−)-(*R*)-2′-methoxydihydroartemidin, was determined by circular dichroism (CD) correlation with synthetic 2′-hydroxydihydroisocoumarin, (+)-(*S*)-**4**.[9] The absolute configuration of the latter was known from the kinetic resolution of racemic 2-phenylbutyric acid anhydride with the optically active alcohol (+)-**4** according to the method of Horeau.[9]

The more polar fractions supplied the two derivatives **6a** and **13**. **6a** showed a capillarin-type and **13** an artemidin-type UV spectrum. Molecular formulas of $C_{13}H_{12}O_3$ for both compounds (HREIMS) were compatible with tentative structures of an epoxide (**6a**) and a hydroxy derivative of artemidin (**13**). In the latter, the conjugated double bond in the side-chain was suggested not only by the UV spectrum but also by a medium band at 1626 cm^{-1} in the IR spectrum. A further medium band at 3625 cm^{-1} in the IR spectrum of **13** was indicative for a hydroxy group. The 1′,2′-oxygen substitution of **6a** followed from the chemical shifts in the ^{13}C NMR at 54.4 and 61.5 ppm and from those of the side-chain protons in the ^{1}H NMR with a characteristic coupling pattern: a doublet (d) for 1′-H at 3.48 ppm and a dt at 3.29 ppm for 2′-H (Table 2). The remaining characteristic ethyl group resonances for CH_2 (3′)−CH_3 (4′) completed the C_4 side-chain. In the case of **13**, the *E*-orientated olefinic protons for C1′-H and C2′-H and the oxygen substitution at C-3′ followed in a straightforward manner from the combined ^{1}H and ^{13}C NMR data (Table 1). Especially the coupling pattern of the side-chain protons was decisive: 1′-H (dd, with a *trans* olefinic coupling of J = 15.5 Hz to 2′-H and a long-range coupling of J = 1.4 Hz to 3′-H), 2′-H (dd, J = 15.5 and 5.5 Hz), 3′-H (br dq, 5.5 and 6.5 Hz with a broadening due to the 1′,3′-long-range coupling), and 4′-H (d, J = 6.5 Hz). The absolute configurations of (−)-(*R*,*S*)-**6a** and (+)-(*R*)-**13** were determined following the kinetic resolution method of Horeau, in the case of **6a** after a reduction to (+)-(*S*)-**4**.[9] Because of the close structural relationship to artemidin (**11**), **6a** was named epoxyartemidin and **13** (+)-(*R*)-3′-hydroxyartemidin.

HPLC−UV analyses of the lipophilic root extract of *C. mixtum* indicated the existence of two further new isocoumarin derivatives (**17**, **18**). The strong absorption maxima in the UV spectra between 270 and 300 nm are characteristic for unsaturated bonds in conjugation with the isocoumarin skeleton,[13] and the IR signals at 2200 cm^{-1} for acetylenic linkages. The molecular formula $C_{13}H_{10}O_2$ of compound **17** derived from HREIMS suggested an isomer of capillarin (**19**) that possessed a triple bond at C1′ instead of C2′. The side-chain C≡C−CH_2−CH_3 was confirmed by ^{1}H NMR (Table 3). The terminal group showed only the coupling between CH_3(4′) and CH_2(3′); the chemical shift of the latter at δ 2.46 ppm was typical for the triple bond directly attached to it (Table 3). The molecular formula $C_{13}H_{10}O_3$ (HREIMS) and the presence of an IR band at 3625 cm^{-1} suggested that **18** was an alcohol of **17**. The optical rotation of $[\alpha]^{20}_D$ −5° and significant CD bands of

Table 3. ^{1}H and ^{13}C NMR of Isocoumarins **17**−**20** with Acetylenic Side Chain Substitution ($CDCl_3$, δ/ppm, 250 MHz)[a]

	^{1}H				^{13}C		
	17	**18**	**19**	**20**[b]	**18**	**19**	**20**[b]
1					161.5 s	162.5 s	162.3 s
3					153.7 s	153.8 s	152.3 s
4	6.63 s	6.72 s	6.62 s	6.62 s	111.6 d	103.1 d	103.5 d
4a					136.4 s	137.2 s	137.1 s
5	7.38 br d	7.41 br d	7.42 br d	7.42 br d	129.2 d	127.9 d	128.2 d
6	7.69 ddd	7.72 ddd	7.70 ddd	7.72 ddd	134.9 d	134.8 d	134.9 d
7	7.50 ddd	7.53 ddd	7.47 ddd	7.49 ddd	129.8 d	129.6 d	129.7 d
8	8.27 br d	8.28 dd	8.26 dd	8.26 br.d	125.8 d	125.4 d	125.5 d
8a			3.46 m		121.6 s	120.1 s	120.2 s
1′				3.55 br s	76.9 s	23.9 t	23.8 t
2′					95.3 s	79.9 s	79.7 s
3′	2.46 q	4.76 q			58.6 d	71.8 s	78.4 s
4′	1.25 t	1.56 d	1.90 t	4.76 t	23.8 q	3.6 q	52.1 t

[a] Coupling constants. Benzene ring (**17**−**20**): $J(5,6) = J(6,7) = J(7,8) = 7.9 \pm 0.1$ Hz, $J(5,7) = J(6,8) = 1.2 \pm 0.2$ Hz. **17**: $J(3',4') = 7.5$ Hz. **18**: $J(3',4') = 6.5$. **19**: $J(1',4') = 2.7$ Hz. **20**: $J(1',4') = 2.0$ Hz. [b] Isovaleric ester side chain of **20**; ^{1}H: CH_2 2.25 d, CH 2.14 m, $(CH_3)_2$ 0.98 d, $J(2'',3'') = 7.0$ Hz, $J(3'',4'') = J(3'',5'') = 6.0$ Hz; ^{13}C: 1″ 172.4 s, 2″ 43.0 t, 3″ 25.7 d, 4″ 22.3 q.

Table 4. Isocoumarin and 3-Butylisocoumarins: Inhibition on Germ-Tube Development of the Plant-Pathogenic Microfungus *Pyricularia grisea* (EC_{50}, EC_{90}, and MIC) and MICs from TLC Bioautography (Indicator Organism Saprophytic Microfungus Using *Cladosporium herbarum* as Test Organism)[i]

	μg mL^{-1}	EC_{50} (95% FL)[a]	EC_{90} (95% FL)[b]	MIC[c]	MIC[d]
1[e]	isocoumarin	83 (31−705)	*f*	>200	n.t.
saturated 3-butylisocoumarins					
2[e]	dihydroartemidin	10 (4−24)	40 (18−401)	50	100
3[e]	1′-hydroxydihydroartemidin	37 (27−53)	83 (57−186)	100	100
4[e]	(+)-(*S*)-2′-hydroxydihydroartemidin	25 (4−68)	*f*	>200	100
5	(−)-(*R*)-2′-methoxydihydroartemidin			inactive	>100
6a	(+)-(*S*,*R*)-epoxyartemidin	93 (51−303)	*f*	>200	25
6b[e]	(−)-(*R*,*S*)-epoxyartemidin			inactive	50
7a	*threo*-artemidiol			inactive	n.t.
7b	*erythro*-artemidiol			inactive	n.t.
8[e]	1′-bromodihydroartemidin	35 (15−87)	96 (49−1718)	100	25
9[e]	1′,1′-dibromodihydroartemidin	22 (5−436)	*f*	>200	50
10[e]	1′,2′-dibromodihydroartemidin	25 (4−68)	*f*	>200	100
olefinic 3-butylisocoumarins					
11	(*E*,*Z*)-artemidin	11 (10−12)	19 (17−23)	25	100
12	(*E*,*Z*)-dracumerin	14 (10−19)	45 (29−92)	50	25
13	(+)-(*R*)-*E*-3′-hydroxyartemidin	140 (112−189)	292 (209−644)	>200	100
14[e]	(*E*)-3′-oxoartemidin	5 (3−8)	21 (13−48)	25	<25
15[e]	(*E*)-3′-acetoxyartemidin	8 (4−19)	30 (22−44)	50	50
16[e]	(*E*)-1′-bromoartemidin	2 (1.6−2.9)	9 (6−15)	12	<25
acetylenic 3-butylisocoumarins					
17	corfin	3 (0.7−7)	99 (33−1717)	100	>100
18	3′-hydroxycorfin	0.9 (0.002−3)	*g*	50	100
19	capillarin	37 (28−49)	92 (65−172)	100	inactive
20	capillarin isovalerate	5 (3−7)	13 (9−31)	25	inactive
ring-oxygenated 3-butylisocoumarins					
21	artemidinol	12 (9−15)	32 (23−62)	50	>100
22	8-hydroxyartemidin	4 (3−7)	19 (11−47)	25	>100
23	8-hydroxycapillarin	2 (0.4−6)	102 (33−2192)	100	inactive
positive controls					
	6-methoxymellein	47 (37−66)	106 (82−153)	200	25
	Benlate[h]	0.06 (0.02−0.2)	*f*	200	<25
	blasticidin S	13 (5−56)	*f*	>200	n.t.
	griseofulvin	4 (0.3−10)	*f*	>200	n.t.
	kresoxim-methyl[h]	0.003 (0.0003−0.03)	*f*	>200	n.t.

[a] EC_{50}, estimated effective dose that caused 50% inhibition. [b] EC_{90}, estimated effective dose that caused 90% inhibition. [c] MIC, first concentration in dilution series that caused total inhibition of germ-tube growth development. [d] MIC in bioautography, first concentration in the dilution series from 100 to 25 μg/spot that caused a homogeneous inhibition zone in the mycelial layer on the TLC plate. [e] Compound of synthetic origin. [f] Probit estimate failed due to missing endpoint. [g] Available data did not allow unambiguous estimation of EC_{90}. [h] Diluted to 0.00002 μg/mL; FL, fiducial limits; n.t., not tested. [i] Stock solution (2 mg test compound/250 μL acetone/4.75 mL 4%-malt extract, emulsified with 0.2% Tween 80) was diluted 2-fold from 400 to 1.56 μg/mL; medium, 4%-malt extract broth; negative control, dilution of pure solvent. Inoculation: 50 μL spore suspension (10^4 CFU, 4% malt extract broth). The final test concentration range was 200−0.78 μg/mL. After incubation for 16 h at room temperature growth was stopped by adding 10 μL of lactophenol blue to each well. Germ-tube size was determined by pixel counts after capturing images of 10 germinated conidia per well on the hard disk. Probit-log estimates were calculated from a concentration range of 200−0.78 μg/mL.

18 hinted toward the presence of a chiral carbon atom in the molecule. Consequently, this fact and data from UV, IR, and MS strongly supported the assumption that the hydroxyl group was localized on carbon atom 3′ in the 3-butyl side-chain. ^{1}H and ^{13}C NMR confirmed structure **18**. The ^{1}H resonance signal of the terminal CH_3(4′) group at 1.56 ppm was a d with $J = 6.5$ Hz, and the signal of the neighboring CH(3′) group was found at 4.76 ppm as a q with $J = 6.5$ Hz. This relatively low-field chemical shift of 4.76 ppm is the result of oxygen substitution together with the anisotropic effect of the triple bond between C(1′) and C(2′). The corresponding acetylenic ^{13}C resonances were found at 76.9 and 95.3 ppm (Table 3). With reference to the source of *C. mixtum*, the Ionian Island of Corfu, we propose to call **17** and **18** corfin and 3′-hydroxycorfin.

Table 4 summarizes EC_{50}, EC_{90}, and MIC values of the tested isocoumarin derivatives that were obtained in the germ-tube inhibition test. Additionally, MIC values from the TLC bioautography against *C. herbarum* are also listed. However, only the former are accounted for in the comparative discussion of the activities. In the EC_{50} range, only kresoxim-methyl and Benlate outperformed the most active naturally occurring isocoumarins. However, in the endpoint range, some of the 3-butylisocoumarins inhibited germ-tube development at concentrations as low as 12 μg/mL. Benlate and kresoxim-methyl failed to produce comparable effects in the range of tested concentrations. Blasticidin S is a metabolite of *Streptomyces griseochromogenes* Fukunaga. Its antifungal activity against *P. oryzae* was also discovered through germ-tube inhibition testing.[19] In our test, it somehow compared to artemidin (**11**) though it lacked the definite endpoint of **11**. The 3-butyl side-chain contributed much to antifungal activity of this type of isocoumarins; the isocoumarin ring system (**1**) alone was only weakly active. In the EC_{50} range, a saturated butyl side-chain (**2**) caused inhibitory effects comparable to those of an unsaturated butyl side-chain, such as **11**. However, the endpoint was less well defined for **2** than for **11**. Subsequent introduction of oxygen functions (**3**−**7**) decreased activities compared to **2**. Brominated dihydro-3-butylisocoumarins (**8**−**10**) showed activities comparable to or slightly better than the hydroxylated derivatives (**3**, **4**, and **7**), but did not outperform **2**. Further unsaturation of the butyl side-chain at carbon atom 3 (**12**) caused effects that compared more to **2** than to **11**. The introduction of a hydroxyl function at position 3′ (**13**) eliminated activity. Oxidation of that hydroxyl group into a ketone (3′-oxoartemidin, **14**) again restored the activity to a level comparable to **2**, **11**, and

12. Esterification of the same oxygen function with acetic acid (**15**) did not affect activity at all. 1′-Bromoartemidin (**16**) turned out to be the most active of all 3-butylisocoumarins tested, though the increase in activity by bromination was only moderate. Oxygenation at carbon atom 5 and 8 of the aromatic ring system (**21**, **22**) did not significantly change activity compared to non-hydroxylated **11**. The replacement of the double bond at carbon atom 1′ by a triple bond (**17**) increased antifungal activity. Additional hydroxylation at carbon atom 3′ of corfin (**18**) further increased activity. 3′-Hydroxycorfin (**18**) showed the lowest EC_{50} of all naturally occurring derivatives. Here, hydroxylation caused an opposite effect than was shown in **11**. A triple bond at carbon 2′ (**19**) notably decreased activity. However, esterification at position 4′ (**20**) or hydroxylation at carbon atom 8 (**23**) of **19** again increased antifungal activity to the levels of **17** and **18**. The obtained pattern of activities suggests that various modes of actions might be involved.

Routine bioautography on TLC plates with *Cladosporium herbarum* as a test fungus preceded the germ-tube inhibition tests. Overall, variable diffusion effects impaired the comparability of results. The MIC values presented in Table 4 were determined on the basis of the criterion that the test concentration caused a homogeneous inhibition zone. In general, the tested 3-butylisocoumarins caused more pronounced inhibition effects in the germ-tube inhibition test, agreeing with observations that were made testing other classes of natural products. However, one result from the bioautography strongly contradicted those obtained in the germ-tube inhibition: (−)-epoxyartemidin (**6a**) caused homogeneous inhibition zones starting at 25 μg. Disagreement of the results of the two tests and the availability of sufficient amounts of **6a** from synthesis work prompted us to investigate the stability of **6a** during the test period that comprised 3 days on TLC plates and 18 h in a liquid medium. HPLC analysis of **6a** that was directly extracted from the silica gel of the incubated TLC plate supplied **6a** as a single peak. Analysis of the malt extract medium containing the test compound for microdilution supplied three peaks. UV spectra and the comparison of retention times identified the last eluting peak as **6a**. The two larger peaks that eluted before **6a** showed similar UV spectra. These findings suggested that the decomposition of **6a** had occurred. **6a** and its decomposition products were silylated and further analyzed by GC−MS. The chromatogram showed five peaks, of which one could be identified as **6a** by co-analysis of a reference compound. The two largest peaks in the GC showed spectra of typical silylated compounds that were characterized by prominent peaks at $m/z = 73$. Those results suggested that the two major peaks might be the corresponding diols of the epoxide. Natural hydrolysis of compounds with epoxide functions in aqueous solutions is known to occur.[20] Synthesis had provided sufficient amounts of **6a** that were available as a precursor to obtain large enough amounts of the two diols for NMR analysis. They were identified by the comparison of obtained spectroscopic data with published data of artemidiol (**7**, described without stereochemistry) as two diastereoisomers, *threo*- and *erythro*-artemidiol.[21] However, low available amounts of the pure diastereoisomers precluded the determination of absolute configurations. Both diastereoisomers did not affect germ-tube growth of *P. grisea* at all.

During the preparation of the manuscript a study about antifungal constituents of the essential oil fraction of *A. dracunculus* was published.[22] Active compounds identified also included capillarin (**19**). In that study, *Botrytis cinerea* Pers.:Fr. and *Colletotrichum fragariae* A.N. Brooks were used as the test fungi. The report suggested that those two isolates may be more susceptible to **19** than our strain of *P. grisea*. However, result comparability of this and our study is somehow hampered by the following issues: (1) standard statistics for quantitative biological assays as recommended by Roberts and Boyce were not employed;[23] and (2) scored range of concentrations comprised only inhibitions from 10 to ∼50% of control growth.

Known biological activities of 3-butylisocoumarins are not solely restricted to antifungal effects. For capillarin (**19**), insect-antifeeding activity[24] and efficacy in treatments of hepatocellular jaundice associated with acute viral hepatitis[25] were demonstrated. In a comparison of cytotoxic activities of lactone-bearing natural products, **19** emerged as one of the compounds that was less active, and it did not compare to values that were recorded for sesquiterpene lactones.[26] Those and our findings suggest that 3-butylisocoumarins represent promising candidates for activity screenings beyond those reported herein. Given that, in other test systems, a similar extent of variation in structure still maintains the level of efficacy, as we have observed in the germ-tube inhibition of *P. grisea*, then 3-butylisocoumarins might have the potential to meet with issues that are required by specificity of action and toxicological risk.

Experimental Section

General Experimental Procedures. Optical rotation, Perkin-Elmer polarimeter 241; CD, Jobin Ivon Dichrograph CD 6; UV, Hewlett-Packard, 8452A diode array spectrophotometer; IR, Perkin-Elmer 16PC FT-IR; NMR, Bruker, AM 400 WB and AC 250; MS, Finnigan MAT 900 S; HPLC−UV, Hewlett-Packard 1090 Series II, 230 nm signal, Hypersil BDS C18, 250 × 4 mm column, 5 μm, mobile phase MeOH (gradient 60−100%) in aqueous buffer (0.015 M *o*-phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH 3), flow rate 1 mL/min; GC-MS, Perkin-Elmer GC Autosystem XL coupled to a Turbomass quadrupole MS, capillary columns PE 5 ms (20 m, 0.18 mm diameter, 0.18 μm film) and PE-1704 (15 m, 0.25 mm diameter, 0.25 μm film), carrier gas helium, constant flow rate 1 mL/min; ion source temp 180 °C, transfer line 250 °C, EI-spectra from 30 to 600 amu, electron energy 70 eV, filament emission 200 μA; temp gradients started at 75 °C and rose to the column's temp limit at 5 °C/min; silylation, BSTFA-TMCS (99:1) in pyridine at ambient temperature; image analysis of digitized germ-tubes, Olympus DF Plan 140× and Sony DXC-C1MDP video camera, software NIH Image 1.62.[27]

Plant Material. Different sources of *A. dracunculus* were grown from achenes that were obtained from various botanical gardens and cultivated under field conditions in the Botanical Garden of the University of Vienna (AR-312, Uzbekistan, Tashkent; AR-789, Russia, Novosibirsk; AR-913, Germany, Krefeld; AR-917, Germany, Marburg). Aerial (leaves and stems) and underground organs (roots and stolons) were collected during the month of May. Roots of *C. mixtum* were collected in Greece, Ionian Islands, northern part of Corfu, on meadows near the sandy beach in the bay of Agios Georgios (Ormos Ag. Georgiu), 2 km NE of the village Acharavi. Voucher specimens were deposited at the Herbarium of the Institute of Botany, University of Vienna (WU). Carrots were obtained from a local supermarket.

Extraction and Isolation. Air-dried leaves and underground organs were ground and extracted with hexane−Et_2O−MeOH (1:1:1) for 3 days in total darkness at ambient temperature. The filtered extract was concentrated to an aqueous residue and repeatedly extracted with $CHCl_3$. Combined $CHCl_3$ fractions were evaporated to dryness, dissolved in MeOH, and stored at −20 °C until proceeding further. Rough fractionation

(50 mL) was performed on CC (silica 60, 0.2−0.5 mm) by starting with 100 mL of hexane and adding increasing portions of Et_2O; eluent of the final fraction was Et_2O−MeOH (97:3). United fractions containing identical compounds, monitored by TLC (silica 60, 0.25 mm), were further purified by MPLC (400 × 40 mm column, Merck LiChroprep silica 60, 25−40 μm, UV detection, 254 nm) using step-gradient elution with pure hexane and hexane with 5, 10, and 15% EtOAc as additives. Inseparable mixtures were additionally subjected to cyclic MPLC using the same eluent composition that was used to elute the fraction in the straight MPLC run.[28] Chopped carrots were inoculated with conidia of *Botrytis cinerea* to obtain 6-methoxymellein. Induction and isolation were carried out as described.[29]

Isolation of Artemidin (11) and Dracumerin (12). Fifty-two grams of leaves of *A. dracunculus* AR-789 yielded 3.6 g of extract that was submitted to CC. The fractions eluting with Et_2O−hexane (5:95) up to Et_2O−hexane (25:75) were combined (180 mg) and further purified by MPLC. The main fraction contained a mixture of **11** and **12** (142 mg). Pure **11** (36 mg) and **12** (59 mg) were obtained by using cyclic MPLC with the same eluent. GC−MS suggested that both compounds were present as a mixture of *Z*- and *E*-isomers, which was confirmed by the ^{1}H NMR spectrum. Determined proportions of isomers amounted to 3:1 (*Z*:*E*) for **11** in underground organs and 10:7 (*Z*:*E*) in aerial organs. The isomer ratio of **12** was 3:7 (*Z*:*E*).

Isolation of Capillarin (19), Capillarin Isovalerate (20), and 8-Hydroxycapillarin (23). The $CHCl_3$ fraction (1.7 g) of 135 g of underground organs of *A. dracunculus* AR-913 was submitted to CC. Fractions (362 mg) eluting with Et_2O−hexane (1:9) were subjected to MPLC (hexane) and, among others, afforded 7 mg of **19**, 3.5 mg of **20**, and 4 mg of **23**.

Isolation of 2′-Methoxydihydroartemidin (5), (−)-(*R*,*S*)-Epoxyartemidin (6a), and (+)-(*R*)-(*E*)-3′-Hydroxyartemidin (13). A portion (1.3 g) of the $CHCl_3$ fraction (3.4 g) obtained from 163 g of dried aerial organs of *A. dracunculus* AR-312 was submitted to CC. Fractions (81 mg) eluted by Et_2O−hexane (25:75) contained **11** and **5**. MPLC with EtOAc−hexane (9:1) yielded 17 mg of **11** and 10.5 mg of **5**; fractions (67 mg) eluted by Et_2O−hexane (1:1) contained **6a** and **13**. Purification by MPLC (EtOAc−hexane, 15:85) afforded 2.5 mg of **6a** and 2 mg of **13**.

Isolation of Artemidinol (21) and 8-Hydroxyartemidin (22). The $CHCl_3$ fraction (520 mg) of 78 g of roots and stolons of *A. dracunculus* AR-917 was submitted to CC. The fraction (184 mg) eluting with Et_2O−hexane (1:9) was subjected to MPLC (EtOAc−hexane, 5:95) and gave 4 mg of **22** and 96 mg of **11**; fractions (64 mg) eluting with Et_2O−hexane (1:1) were purified by MPLC (EtOAc−hexane, 1:9) to give 32 mg of pure **21**.

Isolation of Corfin (17) and 3′-Hydroxycorfin (18). The $CHCl_3$ fraction (283 mg) obtained from 35 g of dried roots of *C. mixtum* was submitted to CC. Fractions (56 mg) eluting with Et_2O and Et_2O−MeOH (97:3) contained **17** and **18** and were purified by repeated MPLC [EtOAc−hexane (15:85)], yielding 4 mg of **17** and 3 mg of **18**.

Isolation of 6-Methoxymellein. Sliced carrots (100 g) inoculated with *B. cinerea* were extracted with MeOH for 3 days at ambient temperature. The concentrated aqueous residue was twice extracted with $CHCl_3$. The combined fractions (633 mg) were subjected to CC. Fractions eluting with Et_2OAc−hexane (1:3) showed the characteristic UV spectrum of 6-methoxymellein. Purification with MPLC (EtOAc−hexane, 1:9) supplied 42 mg of the desired compound. Spectroscopic data were consistent with those reported.[29]

Preparation of Artemidiol (7). A 20 mg portion of (−)-(*R*,*S*)-epoxartemidin (**6a**) was dissolved in 50 mL of 4% malt extract broth and incubated in total darkness at ambient temperature, in concordance with bioassay procedures. After 5 days, the solution was concentrated and lipophilic compounds were extracted twice with $CHCl_3$. The combined fractions (13.5 mg) were separated by MPLC (EtOAc−hexane, 1:9) into *threo*- and *erythro*-**7**.

(−)-(*R*)-2′-Methoxydihydroartemidin [(−)-(*R*)-3-(2′-methoxybutyl)-1*H*-2-benzopyran-1-one] (5): colorless oil; $[\alpha]^{20}_D$ −15° (*c* 0.1 in EtOH); CD (EtOH) $\Delta\epsilon_{232}$ +2.1, $\Delta\epsilon_{273}$ −1.0, $\Delta\epsilon_{315}$ −1.3; UV (MeOH) λ_{max} 228, 240sh, 258sh, 268, 326 nm; IR (CCl_4) γ_{max} 2954, 2926, 2854, 1742, 1658, 1608, 1570, 1486, 1378, 1160, 1120, 1088, 1022 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m*/*z* 232 (M^+, 6), 203 (4), 200 (3), 160 (82), 145 (9), 131 (10), 117 (3), 103 (7), 89 (25), 77 (6), 73 (100), 63 (7); HREIMS *m*/*z* 232.1103 (calcd for $C_{14}H_{16}O_3$, 232.1099).

(−)-(*R*,*S*)-Epoxyartemidin [(−)-(1*R*,2*S*)-3-(1,2-epoxybutyl)-1*H*-2-benzopyran-1-one] (6a): colorless oil; $[\alpha]^{20}_D$ −10° (*c* 0.2 in EtOH); CD (EtOH) $\Delta\epsilon_{266}$ +0.8, $\Delta\epsilon_{275}$ +0.5, $\Delta\epsilon_{327}$ +0.4; UV (MeOH) λ_{max} 230, 241sh, 268, 278sh, 326 nm; IR (CCl_4) γ_{max} 2977, 2941, 1755, 1662, 1607, 1486, 1161, 1043, 1028, 1009 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS (70 eV) *m*/*z* 216 (M^+, 22), 200 (3), 187 (3), 175 (4), 158 (100), 145 (18), 130 (71), 117 (8), 103 (46), 89 (30), 77 (9), 63 (12); HREIMS *m*/*z* 216.0791 (calcd for $C_{13}H_{12}O_3$, 216.0786).

***threo*-Artemidiol (7):** yellowish oil; UV (MeOH) λ_{max} 229, 238sh, 242sh, 256, 264, 273, 325 nm; IR (CCl_4) γ_{max} 3625, 3077, 2967, 2927, 2857, 1741, 1671, 1611, 1586, 1492, 1472, 1387, 1292, 1277, 1212, 1168, 1128, 1083, 1053, 1023, 988 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS (70 eV) *m*/*z* 234 (M^+, 4), 216 (5), 175 (100), 147 (23), 145 (31), 130 (18), 89 (51), $C_{13}H_{15}O_4$.

***erythro*-Artemidiol (7):** yellowish oil; UV (MeOH) λ_{max} 230, 238sh, 243sh, 256, 264, 274, 326 nm; IR (CCl_4) γ_{max} 3625, 2967, 2927, 2857, 1751, 1671, 1611, 1581, 1492, 1467, 1387, 1297, 1277, 1168, 1138, 1083, 1053, 1028, 988 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS (70 eV) *m*/*z* 234 (M^+, 3), 216 (4), 175 (100), 147 (48), 145 (25), 130 (38), 89 (63), $C_{13}H_{15}O_4$.

(*E*,*Z*)-Dracumerin (12): colorless needles (Et_2O); mp 95−97 °C; UV (MeOH) λ_{max} 240, 255sh, 305sh, 310, 352, 364sh nm; IR (CCl_4) γ_{max} 3070, 1626, 1606, 1584, 1574, 1558, 1482, 1364, 1326, 1216, 1050, 1022, 1000, 914 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m*/*z* 198 (M^+, 100), 181 (16), 170 (32), 169 (32), 153 (12), 141 (55), 128 (7), 115 (22), 89 (23), 68 (35), 63 (14), 53 (17); HREIMS *m*/*z* 198.0678 (calcd for $C_{13}H_{10}O_2$, 198.0681).

(+)-(*R*)-(*E*)-3′-Hydroxyartemidin (13): colorless oil; $[\alpha]^{20}_D$ +18 (*c* 2.2 in ethanol); CD (EtOH) $\Delta\epsilon_{227}$ −0.8, $\Delta\epsilon_{242}$ −0.6, $\Delta\epsilon_{344}$ +0.5; UV (MeOH) λ_{max} 228sh, 231, 244sh, 252, 274sh, 278, 283sh, 288, 294sh, 302, 343 nm; IR (CCl_4) γ_{max} 3625, 2966, 2857, 1751, 1661, 1626, 1571, 1551, 1492, 1462, 1367, 1332, 1312, 1247, 1227, 1178, 1143, 1068, 1033, 988, 973, 953 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m*/*z* 216 (M^+, 2), 199 (2), 173 (18), 145 (3), 115 (6), 89 (11), 85 (15), 73 (32), 71 (22), 61 (94), 57 (34), 55 (14), 45 (100); HREIMS *m*/*z* 216.0790 (calcd for $C_{13}H_{12}O_3$, 216.0786).

Corfin (17): colorless oil; UV (MeOH) λ_{max} 247, 256, 281sh, 304, 318, 347 nm; IR (CCl_4) γ_{max} 3077, 3007, 2947, 2887, 2847, 2239, 2189, 1761, 1636, 1616, 1566, 1546, 1492, 1352, 1332, 1312, 1227, 1188, 1078, 1058, 1038, 1013, 973, 893 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS (70 eV) *m*/*z* 198 (M^+, 100), 170 (68), 155 (67), 141 (89), 127 (45), 115 (37); HREIMS *m*/*z* 198.0679 (calcd for $C_{13}H_{10}O_2$, 198.0681).

3′-Hydroxycorfin (18): colorless oil; $[\alpha]^{20}_D$ −5° (*c* 0.15 in EtOH); CD (EtOH) $\Delta\epsilon_{280}$ +0.9, $\Delta\epsilon_{295}$ +0.7, $\Delta\epsilon_{328}$ +0.25; UV (MeOH) λ_{max} 230, 250sh, 270sh, 282, 295, 330sh nm; IR (CCl_4) γ_{max} 3625, 3077, 2997, 2937, 2887, 2239, 1980, 1761, 1686, 1641, 1611, 1576, 1546, 1497, 1347, 1312, 1287, 1262, 1227, 1192, 1107, 1058, 1043, 1028, 988, 933, 888 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS (70 eV) *m*/*z* 214 (M^+, 100), 199 (75), 116 (59), 89 (36), 84 (39); HREIMS *m*/*z* 214.0627 (calcd for $C_{13}H_{10}O_3$, 214.0630).

Capillarin Isovalerate (20): colorless needles (Et_2O); mp 53−54 °C; UV (MeOH) λ_{max} 228, 258sh, 262sh, 290, 302, 326 nm; IR (CCl_4) γ_{max} 2968, 2878, 1752, 1666, 1608, 1570, 1488, 1372, 1327, 1280, 1243, 1201, 1182, 1160, 1141, 1118, 1094, 1044, 1018, 991, 973 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS (70 eV) *m*/*z* 298 (M^+, 37), 269 (5), 214 (67), 201 (13), 196 (47), 186 (21), 168 (24), 157 (32), 145 (31), 141 (27), 129 (25), 115 (31); HREIMS *m*/*z* 298.1201 (calcd for $C_{18}H_{18}O_4$, 298.1205).

Artemidin (11), Capillarin (19), Artemidinol (21), 8-Hydroxyartemidin (22), and 8-Hydroxycapillarin (23). Spectroscopic data of isolated natural products were consistent with those reported previously for **11**,[30] **19**,[31] **21**,[18] **22**,[18] and **23**.[17]

Synthesis of Isocoumarin (1), 1′-Hydroxydihydroartemidin (2), (+)-(*S*)-2′-Hydroxydihydroartemidin (3), (+)-(*S*)-2′-Hydroxydihydroartemidin (4), (+)-(*S*,*R*)-Epoxyartemidin (6a), (−)-(*R*,*S*)-Epoxyartemidin (6b), 1′-Bromodihydroartemidin (8), 1′,1′-Dibromodihydroartemidin (9), 1′,2′-Dibromodihydroartemidin (10), (*E*)-Artemidin (11), (+)-(*R*)-(*E*)-3′-Hydroxyartemidin (13), (*E*)-3′-Oxoartemidin (14), (*E*)-3′-Acetoxyartemidin (15), and (*E*)-1′-Bromoartemidin (16). 1 was synthesized as described[32] with some modifications: reaction products were purified by flash chromatography (silica 60, 0.040−0.063 mm); methyl-(2-ethenyl)-benzoate (step 1) was eluted with hexane−Et_2O (95:5), and the corresponding cyclic acetal (step 2) with hexane−EtOAc (2:1). Separation of **1** from partially hydrolyzed byproducts was achieved with CH_2Cl_2−hexane (6:1). Spectroscopic data were consistent with those reported. Synthesis, structure elucidation of **2**, **3**, **4**, **6a**, **6b**, **8**, **9**, **10**, (*E*)-**11**, **13**, **14**, **15**, and **16**, and determination of absolute configuration for **4** and **6a** are published elsewhere.[9]

Antifungal Bioassays. Bioautography on TLC plates was carried out as described.[10] *Cladosporium herbarum* (Vienna Institute of Applied Microbiology VIAM-MA1511) was used as a test fungus. Microdilution in 4% malt extract broth was performed as described.[10] *Pyricularia grisea* was provided and identified by Ms. Arunee Surin, Rice Disease Research Group, Plant Pathology and Microbiology Division, Ministry of Agriculture, Bangkok, Thailand. The isolate was recovered from infected rice leaves in Saraburi, Central Thailand. The strain is deposited in the culture collection of the Vienna Institute of Applied Microbiology (VIAM-MA1628). Positive controls included Benlate, (DuPont), blasticidine S (ICN Biomedicals, Germany), griseofulvin (Sigma Aldrich, Austria), and kresoxim-methyl (BASF, Germany).

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Supporting Information Available: UV spectra of **11**, **12**, **17**, **19**, **21**, **22**, and **23** to illustrate typical UV chromophores of 3-butylisocoumarins. GC−MS permits analysis of isomers of **11** and **12** that can even be determined in chromatograms of crude extracts by SIM monitoring at the respective masses of the molecule peaks. This material is available free of charge via the Internet at http://pubs.acs.org.

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