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Faramak Almassi, Emilio L. Ghisalberti, Melissa J. Narbey, and Krishnapillai Sivasithamparam

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NEW ANTIBIOTICS FROM STRAINS OF *TRICHODERMA HARZIANUM*

FARAMAK ALMASSI, EMILIO L. GHISALBERTI,* MELISSA J. NARBEY,

Department of Organic Chemistry

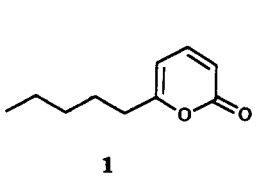
and KRISHNAPILLAI SIVASITHAMPARAM

Soil Science and Plant Nutrition Group, School of Agriculture, The University of Western Australia, Nedlands 6009, Australia

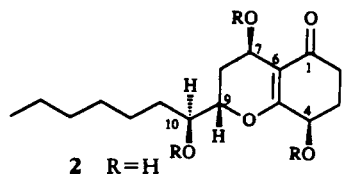
ABSTRACT.—The metabolites produced in liquid cultures by two strains of *Trichoderma harzianum*, isolated from wheat roots, have been examined. Strain 71 produces three compounds with antibiotic activity towards the take-all fungus, *Gaeumannomyces graminis* var. *tritici*. Compounds **1** and **2** have been isolated previously from strains of *Trichoderma koningii* and *T. harzianum*. The structure of the third and major metabolite **3**, an octaketide-derived acetal diol, has been deduced from detailed spectroscopic analysis of **3** and its derivatives. Strain 73 elaborates two new butenolide metabolites containing the 3,4-dialkylfuran-2(5*H*)-one nucleus. Both metabolites antagonize the growth of the take-all fungus.

Trichoderma species (Deuteromycotina) have received considerable attention as biocontrol agents of soil-borne plant pathogens involved in the "damping-off" disease of young seedlings. Cultures of *Trichoderma koningii* Oudem, which were isolated from soil and were suppressive to the saprophytic growth of the take-all fungus *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Waler (Ggt), have been shown to produce 6-*n*-pentylpyrone [**1**] and the hexahydrobenzopyran-5-one **2** (1,2). Both compounds inhibited the growth of Ggt and several other soil-borne plant pathogens in vitro. Similar results have been observed for *Trichoderma hamatum* Bain studied by us (3) and two isolates of *Trichoderma harzianum* Rifai investigated by Claydon *et al.* (4). We have also examined three isolates of *T. harzianum* and found that they varied in their ability to antagonize the take-all fungus on agar plates and glasshouse screening tests (3).

The least effective strain, isolate 70, in liquid medium produced only 1-hydroxy- and 1,8-dihydroxy-3-methylanthraquinone and only marginally inhibited the growth of Ggt (3). Isolate 71 (IMI 311090), the most effective in disease suppression, was shown to produce small quantities of **1** and **2** accompanied by a new metabolite which from preliminary analysis of its spectroscopic properties seemed to be related to **2** (3).



1



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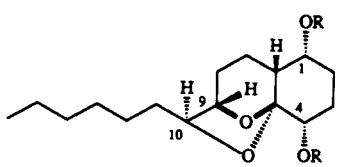
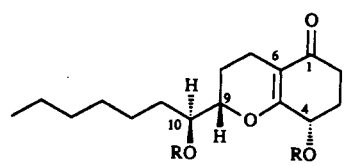
7 R=Ac



3 R=H
4 R=Ac



5 R=H
6 R=Ac

We now report on the structure of this compound and the evidence which allows the structure and stereochemistry to be assigned as shown in **3**. The third strain, isolate 73 (IMI 311092), also had little effect on Ggt growth but increased the shoot length of wheat in the presence or absence of Ggt (3). In a preliminary investigation (3) of the metabolites produced in a pure liquid culture of isolate 73, only a mixture of fatty acids and glycerides was isolated. On aging the cultures (3 months) the proportion of fatty acids and glycerides was significantly reduced and the extract consisted largely of a new metabolite **8** with a smaller amount of a congener **9**. A re-examination of chromatography fractions from the original analysis revealed that **8** had indeed been present, albeit in trace quantities. This report presents evidence for the structures assigned to the new compounds.

RESULTS AND DISCUSSION

Liquid cultures of *T. harzianum* isolate 71 (IMI 311090) were grown over a period of 28 days. The liquid medium was extracted with EtOAc as described previously (2). Tlc analysis of the crude extract showed that it contained amounts of **1**, **2**, and fatty acids as well as a new compound as the major metabolite. The components were isolated by radial plate chromatography to give a mixture of fatty acids (30% of crude extract), **1** (11%), **2** (1%), and **3** (30%). The new compound **3** was obtained after crystallization from Me_2CO as cream-colored needles, mp 77–79°, $[M]^+$ 284 ($C_{16}H_{28}O_4$). The ^{13}C-nmr spectrum of **3** included signals for an acetal carbon (δ_C 109.2, s), four oxymethine carbons (δ_C 79.5, 79.1, 72.8, 69.9, all d), a methine carbon (δ_C 41.3, d), nine methylene carbons, and a methyl group (δ_C 14.1, q), indicating that the compound was tricyclic. The ir spectrum showed hydroxyl absorption (ν max 3500 cm^{-1}),

TABLE 1. ^1H- and ^{13}C-nmr Data of the Acetal **3**.

Position	$\delta\ ^1H$[a]	$\delta\ ^{13}C$[b]
1	3.89 ddd (2.5)	72.8
2a	1.57 m	
2		30.7
2b	1.85 m	
3a	1.85 m	
3		25.2
3b	1.96 m	
4	3.61 dd (5.4, 11.6)	69.9
5		109.2
6	1.57 m	41.3
7	1.72, 2.11 m	20.5
8a	1.55 m	
8		27.2
8b	2.25 dddd (2.1, 3.5, 13, 13)	
9	4.32 ddd (2.1)	79.1
10	4.03 dt (2.1, 7.0)	79.5
11	1.50, 1.60 m	35.2
12	1.30 m	25.6
13	1.30 m	29.1
14	1.30 m	31.7
15	1.30 m	22.6
16	0.89 t (7, 0)	14.1

[a]Assignments are based on ^1H decoupling experiments.

[b]Obtained at 75 MHz, $CDCl_3$. Multiplicities were determined from DEPT/90° and DEPT/135° experiments.

whereas the uv spectrum showed only end absorption. The formation of a diacetate **4** ($[M]^+$ 368) indicated the presence of two hydroxyl groups in **3**. Although analysis of the nmr spectral data (Table 1) strongly supported the gross structure of **3**, the structural analysis was facilitated by the observation that **3**, left in $CDCl_3$ for 2 days (nmr tube), was quantitatively converted into a new compound which has been assigned the structure **5** on the basis of the following evidence. The formula, $C_{16}H_{26}O_4$, was derived from the ms ($[M]^+$ 282) and ^{13}C-nmr spectrum. Compound **5** on acetylation formed the diacetate **6** ($[M]^+$ 366, $C_{20}H_{30}O_6$). Comparison of the ^{13}C-nmr spectra of **5** and **6** with those previously (2) obtained for **2** and its triacetate **7** (Table 2) revealed the following information: (a) the chemical shifts for the side chain carbons (C-10 to C-16) are in good agreement and point to the same configuration at C-10 in **2** and **5**; (b) the ether carbon at C-9 in **5** is deshielded by 3.1 ppm compared to the same carbon in **2**, as is expected for the removal of a β-hydroxyl group at C-7; (c) the vinylic carbon at C-6 in **5** is shielded compared to the same carbon in **2**, supporting the absence of oxygen substitution at C-7 in **5**; (d) the differences in δ_C for C-1 to C-4 are those expected if it is assumed that the configuration of the hydroxyl group at C-4 in **2** (β) has been changed to α in **5**. Supporting evidence for the relative placement of the functional groups comes from extensive decoupling experiments carried out on **5** (Table 3) and the ms which included significant ions at *m/z* 142 and 139 arising from a retro-Diels-Alder-type rearrangement involving the pyran ring. The formation of **5** from **3** requires acid-catalyzed (^2HCl in $CDCl_3$) ring opening of the 1,3-dioxolane ring, followed by air oxidation of the allylic hydroxyl group thus generated at C-1. The regioselectivity of oxidation can be rationalized because the quasi-axial allylic alcohol (at C-1), with a more accessible oxymethine proton, would be expected to be more readily oxidized. These observations allow the relative stereochemistry of **3** to be deduced. Given that in **2** and **5** the configuration at C-9 and C-10 is the same, then only two possibilities (**A** and **B**) are allowed for the tricyclic ring system of **3**. The hydroxyl group at C-4 in **3** is equatorially disposed as the geminal hydrogen shows diaxial ($J = 11.6$ Hz) and axial-equatorial ($J = 5.4$ Hz) coupling to the vicinal methylene group at C-3. The C-1 hydroxyl group is axial be-

TABLE 2. ^{13}C-nmr Data of Selected Compounds.[a]

Carbon	2	5	7[b]	6[c]
C-1	195.8	198.1	194.8	192.0
C-2	33.3	27.1	31.6	27.1
C-3	28.8	29.0	26.4	26.3
C-4	65.7	71.0	66.5	72.3
C-5	171.5	171.3	170.4	170.4
C-6	113.9	109.1	112.1	110.6
C-7	57.1	17.6	59.6	17.6
C-8	31.7	22.7	29.1	22.4
C-9	77.7	80.8	74.2	77.8
C-10	73.2	73.2	72.9	73.5
C-11	32.4	32.8	29.9	29.9
C-12	25.1	25.4	25.1	25.2
C-13	29.2	29.2	29.2	29.0
C-14	32.2	31.7	32.8	31.5
C-15	22.6	22.6	22.5	22.5
C-16	14.0	14.1	14.0	14.0

[a]75 MHz, $CDCl_3$.

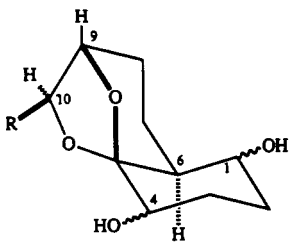
[b]Other signals: acetate carbons, δ 169.8, 168.4 (2), 21.1, 20.9, 20.8.

[c]Other signals: acetate carbons, δ 170.6, 169.8, 20.91, 20.88.

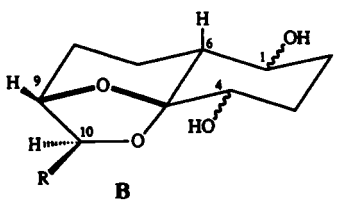
TABLE 3. ^{1}H-nmr Data of the Diol **5**.

Proton	δ ^{1}H	$J_{H,H}$ (Hz)
H-2a	2.5	$J_{2a,2b}=17.0$
H-2b	2.6	$J_{2a,3a}=12.5, J_{2a,3b}=5.0$
H-3a	1.8	$J_{2b,3a}=5.5, J_{2b,3b}=2.0$
H-3b	2.4	$J_{3a,3b}=13.5$
H-4	4.1	$J_{3a,4}=12.5, J_{3b,4}=5.5$
H-7	2.0, 2.1	
H-8a	1.7	$J_{8a,8b}=13.5, J_{8a,9}=10.5$
H-8b	2.0	$J_{8b,9}=2.0$
H-9	3.8	$J_{9,10}=5.5$
H-10	3.7	$J_{10,11}=6.5$
H-11	1.6, 1.7	
H-12	1.3	
H-13	1.3	
H-14	1.3	
H-15	1.3	
H-16	0.9	$J_{15,16}=6.5$

cause the C-1 proton appears as a ddd ($J=2.5, 2.5, 2.5$ Hz), indicative of vicinal axial-equatorial and equatorial-equatorial coupling to three protons. Furthermore nOe studies on **3** showed that irradiation of H-4 enhanced the signal for H-6 (6%) which was also enhanced (12%) on irradiation of H-1, requiring these three protons to be on the same side of the cyclohexane ring. This leads to structures **C** and **D**. As previously mentioned, the C-4 hydroxyl has the α configuration, thus eliminating structure **C**. Therefore, the structure and relative stereochemistry of the major metabolite from *T. harzianum* is that shown in **D** and **3**.



A



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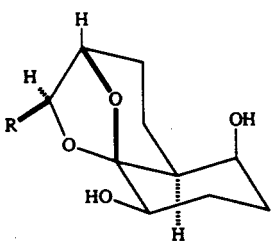
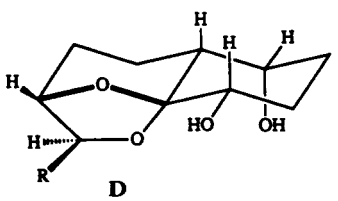


C

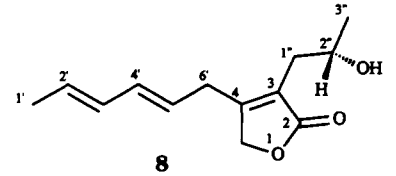


D

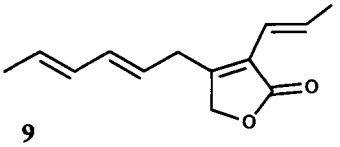
R=$CH_2CH_2CH_2CH_2CH_2CH_3$

While this work was in progress, a paper (5) describing the structure of koninginin A, a metabolite of *T. koningii*, appeared. The gross structure, with no stereochemical descriptors, assigned to this metabolite corresponds to **3**. Comparison of the physicochemical parameters of the two metabolites indicates their identity. Using the bioassay described previously (3), compound **3** was found to inhibit the growth of the take-all fungus at 250 μg. Cutler *et al.* (5) found that **3** did not inhibit the growth of *Curvularia lunata* Boed., *Aspergillus flavus* Link ex Fr., and *Chaetomium* species at concentrations of up to 500 μg/disk. A more detailed study of the antifungal and plant-growth-regulating properties of **3** is being undertaken.

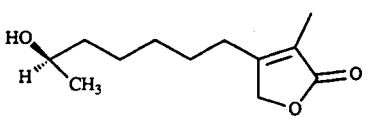
Extraction of the liquid medium of a 3-month-old culture of *T. harzianum*, isolate 73 (IMI 311092), with EtOAc afforded an extract which from tlc and ^{1}H-nmr analysis appeared to contain fatty material and two other major metabolites. The crude extract was partitioned between hexane and MeCN. Cc of the crude extract obtained from the MeCN fraction yielded the major component as an unstable oil, homogeneous by tlc. The structure **8** was assigned on the following evidence. The ^{13}C-nmr spectrum of **8** contained signals for 13 carbons including a carbinol carbon (δ_C 66.1, d) and a carboxyl carbon (δ_C 175.8, s) as part of an oxygen ester (δ_C 71.8, t). Ir absorptions at 3500–3400 and 1740 cm^{-1} supported this assignment, which together with the ms data (*m/z* 222) indicated a molecular formula of $C_{13}H_{18}O_3$. ^{1}H-nmr measurements and decoupling experiments identified a hexa-2,4-dienyl system and a 2-oxypropyl system as part of the molecule. The fact that both groups are linked to a double bond was evident from the chemical shift of their terminal methylene protons at δ_H 3.16 (doubly allylic) and 2.38, respectively. The only remaining signal, a two-proton singlet at δ_H 4.70, was assigned to an allylic methylene linked to an oxygen ester. ^{1}H-^{13}C-HETCOR results showed these protons to be associated with the carbon at δ_C 71.8 (t). The evidence so far indicates that **8** contains a 3,4-dialkylfuran-2(5*H*)-one system, and this was supported by the near coincidence of the δ_C for carbons 2 to 5 with those of model systems, e.g., **10** (6). The orientation of the substituents was deduced from nOe difference measurements. Irradiation of the protons resonating at δ_H 3.16 (H_2-6′) only increased the intensity of signals at δ_H 6.10 (H-4′, 12%), 5.40 (H-5′, 10%), and 4.70 (H_2-5, 3%). Irradiation of the C-5 methylene protons resulted in enhancement of the signals due to H-5′ (4%), H-4′ (2%), and H_2-6′(2%). The stereocenters at C-2′ and C-4′ are assigned the *E,E* configurations because the vicinal couplings between the respective vinylic protons were found to be 14.0 and 14.5 Hz. The configuration at C-2″ was assigned from comparison of the optical rotation of **4** ([α]D +6.6°) with that of related systems. Thus **6** and derivatives with an *R* configuration at the carbinol stereocenter show (6) an [α]D of the same magnitude but opposite sign (−5° to −7°), suggesting that the configuration at C-2″ in **4** is *S*.



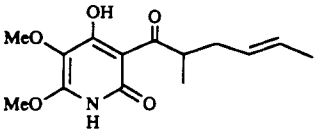
8



9



10



11

The minor component could not be obtained free of plasticizers. However the 300 MHz ^{1}H-nmr spectrum of this fraction indicated the presence of butenolide **9**. Comparison with the ^{1}H-nmr spectrum of **8** showed that the 2-hydroxypropyl chain had been replaced by an (*E*)-propenyl moiety (δ_H 1.87, dd, J = 14.5 Hz; 6.85, dq, J = 6.5, 14.5 Hz). The possibility that **9** is an artifact of the extraction or isolation process cannot be discounted.

Both **8** and **9** show distinct antibiotic activity towards Ggt using the bioassay described previously (3). Details of their antifungal and plant-growth-regulating activity will be published later. The production of **8** and **9** by *T. harzianum*, isolate 73, is of interest particularly when compared to the metabolites produced by other strains of the fungus. From our work and that of others, at least four separate chemical strains can be identified. Two of these produce 6-*n*-pentylpyrone [**1**] but are clearly distinguished by the nature of their other metabolites. Thus whereas *T. harzianum*, isolate 71, in addition produces **2** and **3** (3), the strain studied by Claydon *et al.* (4) and Dickinson *et al.* (7) also elaborates the pyridone **11**. The other two produce, respectively, anthraquinones (3) and the butenolides **8** and **9**. It is worth noting that aging the cultures leads to increased production of **8** and **9**. This presumably is in response to nutrient stress and challenge to the organism.

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—300 MHz ^{1}H-nmr and 75 MHz ^{13}C-nmr spectra were recorded on a Brucker AM-300 Spectrometer. Uv spectra were obtained using a Hewlett-Packard 8450 A UV/VIS Spectrophotometer. Mass spectra were measured with a Hewlett-Packard 5986 GC/MS System (35 eV). Hrms were recorded on a Varian MAT-31 Spectrometer. Gc-eims measurements were recorded using an HP5986 gc-ms fitted with a 25 m column (HP-1 crosslinked methyl silicone gum phase, 25 m × 0.31 mm). A Perkin-Elmer 141 Polarimeter with a 1 dm cell was used to measure [α]D. For tlc Kieselgel 60F_{254} aluminum sheets (Merck) were used. Preparation of liquid cultures and bioassay methods have been described before (2).

ISOLATION OF THE MAJOR METABOLITE FROM THE LIQUID CULTURE OF *T. HARZIANUM*.—The liquid medium (800 ml) of a 28-day-old culture of *T. harzianum* WU 71 (culture deposited with the Commonwealth Mycological Institute IMI 311090) was extracted repeatedly with EtOAc, and the combined organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The extract (94 mg) on tlc analysis (Si gel, 20% petroleum ether/EtOAc) appeared to contain three compounds with one predominating at R_f 0.3. Radial plate chromatography using gradient elution (petroleum ether to EtOAc) gave fractions of a mixture of fatty acids (30 mg), 6-*n*-pentylpyrone [**1**] (10 mg), a new compound **3** (30 mg) and a trace of **2**. The new compound **3** had mp 77–79°; [α]D −22° (c = 0.7, $CHCl_3$); ir ν max 3500 cm^{-1}; uv end absorption only; ^{1}H and ^{13}C nmr see Table 1; eims *m/z* $[M]^+$ 284 (5), 266 (3), 248 (6), 199 (11), 181 (100), 163 (18), 153 (13), 152 (41), 135 (21), 126 (52), 110 (31), 97 (34).

DERIVATIVES OF **3**.—*Diacetate* **4**.—A solution of **3** in pyridine was treated with Ac_2O and left at room temperature overnight. The product, recovered in the usual way, was an oil: ^{1}H nmr (300 MHz, $CDCl_3$) δ 5.00 (1H, dd, $J_{3a,4}$ = 12.3 Hz, $J_{3b,4}$ = 5.0 Hz, H-4), 5.10 (1H, q, J = 2.5 Hz, H-1), 4.30 (1H, t, J = 2.1 Hz, H-9), 3.96 (1H, t, J = 6.5 Hz, H-10), 2.11 and 2.10 (each 3H, acetoxymethyl protons), 0.88 (3H, t, J = 6.4 Hz, H_3-16); ^{13}C nmr (75 MHz, $CDCl_3$) δ 14.1 (q, C-16), 20.9 (t, 2q, C-7 and acetoxy methyl carbons), 22.6 (t, C-15), 25.2 (t, C-3), 25.5 (t, C-12), 27.1 (t, C-8), 29.1 (t, C-13), 30.0 (t, C-2), 31.6 (t, C-14), 35.3 (t, C-11), 41.2 (d, C-6), 70.4 (d, C-4), 72.8 (d, C-1), 78.5 and 78.9 (d, C-9 and C-10), 106.8 (s, C-5), 170.3 and 170.9 (s, ester carbons); eims *m/z* $[M-42]^+$ 324 (10), 309 (87), 248 (47), 194 (21), 181 (10), 163 (100).

Dihydropyran **5**.—A solution of **3** in $CDCl_3$ left standing for 2 days gave a quantitative yield of **5** which was recovered as an oil: $[M]^+$ 282 ($C_{16}H_{26}O_4$); ir ν max ($CHCl_3$) 3600–3520, 1730, 1650, 1620 cm^{-1}; λ max (EtOH) 260 nm, log ε 4.9; ^{13}C nmr see Table 2; ^{1}H nmr see Table 3; eims *m/z* $[M]^+$ 282 (7), 264 (4), 168 (17), 155 (18), 153 (13), 152 (11), 142 (20), 139 (11), 129 (17), 126 (100). Acetylation of the compound with Ac_2O/pyridine yielded the diacetate **6** as an oil: ^{1}H nmr (300 MHz, $CDCl_3$) δ 5.30 (1H, dd, $J_{3a,4}$ = 13 Hz, $J_{3b,4}$ = 5 Hz, H-4), 5.05 (1H, dt, $J_{9,10}$ = 5 Hz, $J_{10,11}$ = 7 Hz, H-10), 3.93 (1H, ddd, $J_{8a,9}$ = 2 Hz, $J_{8b,9}$ = 11 Hz, $J_{9,10}$ = 5 Hz, H-9), 2.18 and 2.10 (each 3H, acetoxymethyl protons), 0.90 (3H, t, J = 7 Hz, H_3-16); ^{13}C nmr see Table 2; eims *m/z* 366 (3), 324 (14), 306 (11), 264 (18), 246

(32), 221 (17), 175 (33), 123 (100); cims (CH_4) $[M+1]^+$ 367 (11%), 307 (8), 247 (11), 85 (17), 71 (18), 57 (100).

ISOLATION OF THE MAJOR METABOLITES FROM LIQUID CULTURES OF *T. HARZIANUM* ISOLATE 73.—The culture broth (4 liters) from a 3-month-old culture of *T. harzianum*, isolate 73 (deposited with the International Mycological Institute, Kew, England, number IMI 311092) was extracted repeatedly with EtOAc, and the combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The extract (0.726 g) was partitioned between petroleum ether and MeCN. The portion (125 mg) of the extract soluble in petroleum ether appeared from ^{1}H-nmr spectroscopy to contain fatty acids and was not investigated further. The MeCN-soluble fraction (601 mg) was chromatographed on Si gel (petroleum ether/Et_2O gradient) to yield fractions of **9** (16 mg) contaminated with plasticizers (gc-eims: T_1 80°, t_1 1.0 min; T_2 240° at 20°/min; **9** (Rt 7.7 min), plasticizers (Rt 8.1 and 8.6 min). Elution with Et_2O gave fractions of **8** (270 mg).

3-(propenyl)-4-(hexa-2E,4E-dien-6-yl)furan-2(5H)-one [**9**].—Oil; ^{1}H-nmr (300 MHz, $CDCl_3$) δ 1.70 (3H, dd, J = 6, 1.5 Hz, H_3-1′), 1.87 (3H, dd, J = 6.5, 1.5 Hz, H_3-3″), 3.21 (2H, br d, J = 7 Hz, H_2-6′), 4.65 (2H, s, H_2-5), 5.48 (1H, m, H-5′), 5.70 (1H, m, H-2′), 5.96–6.13 (3H, m, H-3′, -4′, -1″), 6.85 (1H, dq, J = 6.5, 14.5 Hz, H-2″); eims *m/z* (rel. int.) $[M]^+$ 204 (38), 189 (15), 176 (15), 175 (52), 171 (12), 162 (20), 159 (37), 157 (12), 147 (38), 145 (29), 143 (31), 131 (76), 129 (50), 128 (40), 115 (59), 105 (44), 91 (100).

3-(2-hydroxypropyl)-4-(hexa-2E,4E-dien-6-yl)furan-2(5H)-one [**8**].—Oil, $[\alpha]_D$ +6.6° (c = 3.5, $CHCl_3$), $[\alpha]_{578}$ +8.0°, $[\alpha]_{546}$ +8.3°, $[\alpha]_{436}$ +13.6°; ir ν max (film) 3500–3400, 3010, 1740 cm^{-1}; ^{1}H-nmr (300 MHz, $CDCl_3$) δ 1.16 (3H, d, J = 6 Hz, H_3-3″), 1.70 (3H, dd, $J_{1',2'}$ = 6.5 Hz, $J_{1',3'}$ = 1.5 Hz, H_3-1′), 2.38 (2H, AB system, J_{AB} = 13 Hz, H_2-1″), 3.16 (2H, br d, $J_{5',6'}$ = 7 Hz, H_2-6′), 4.03 (1H, ddq, $J_{1a'',2''}$ = 5 Hz, $J_{1b'',2''}$ = 7.5 Hz, $J_{2'',3''}$ = 6 Hz, H-2″), 4.70 (2H, s, H_2-5), 5.40 (1H, dt, $J_{5',4'}$ = 14.5, $J_{5',6'}$ = 7 Hz, H-5′), 5.62 (1H, dq, $J_{2',3'}$ = 14 Hz, $J_{2',1'}$ = 6.5 Hz, H-2′), 5.90 (1H, ddq, $J_{2',3'}$ = 14 Hz, $J_{3',4'}$ = 10 Hz, $J_{1',3'}$ = 1.5 Hz, H-3′), 6.10 (1H, ddt, $J_{3',4'}$ = 10 Hz, $J_{4',5'}$ = 14.5 Hz, $J_{4',6'}$ = 1.5 Hz, H-4′); ^{13}C-nmr (75.5 MHz, $CDCl_3$) δ 17.9 (q, C-1′), 23.1 (q, C-3″), 30.2 (t, C-6′), 33.1 (t, C-1″), 66.1 (d, C-2″), 71.8 (t, C-5), 123.6 (d, C-5′), 124.3 (s, C-4), 129.9 (d, C-2′), 130.2 (d, C-3′), 133.9 (d, C-4′), 161.5 (s, C-3), 175.8 (s, C-2); eims *m/z* (rel. int.) $[M]^+$ 222 (39), 204 (28), 189 (14), 178 (11), 171 (12), 159 (30), 154 (20), 149 (17), 147 (12), 145 (24), 143 (33), 133 (40), 131 (68), 117 (64), 111 (51), 105 (79), 91 (100).

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