The Microbiological Transformation of Two 15β-Hydroxy-ent-kaurene Diterpenes by Gibberella fujikuroi

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The incubation of 15β-hydroxy-3-oxo-ent-kaur-16-ene (1) with the fungus Gibberella fujikuroi afforded 11β-hydroxy-3,15-dioxo-ent-kauran-16 (6), 11β,15β-dihydroxy-3-oxo-ent-kaur-16-ene (8), 7β,11β,15β-trihydroxy-3-oxo-ent-kaur-16-ene (9), 7α,11β,15β-trihydroxy-3-oxo-ent-kaur-16-ene (7), and 7α,11β,15β-trihydroxy-3-oxo-ent-kaur-16-ene (10). The incubation of 15β-hydroxy-ent-kaur-2,16-diene (3) with the same fungus yielded 7α,11β-dihydroxy-15-oxo-ent-kaur-2-ene (12), 7α,11β,15β-trihydroxy-ent-kaur-2,16-diene (13), 7β,15β-dihydroxy-ent-kaur-2,16-dien-19,6-olide (14), 7β,7α,15β-trihydroxy-ent-kaur-2,16-dien-19,6-olide (15), 7α,11β,16α-trihydroxy-15-oxo-ent-kaur-2-ene (17), and 7α,15β,17-trihydroxy-11β,16β-epoxy-ent-kaur-2-ene (19). These results indicated that a 3-oxo group in ent-kaur-16-ene derivatives inhibits the oxidation at C-19, typical of the biosynthetic pathway of gibberellins and kaurenolides, while a 2,3-double bond or a 15β-OH does not. In both substrates a 15β-alcohol directs hydroxylations at C-11(β) and C-7(α), while in those with a 2,3-double bond the functionalization of C-1(β) is favored.

For several years we have studied the biotransformation of ent-kaurene diterpenes by the fungus Gibberella fujikuroi. The main aims of these works have been to prepare new gibberellin analogues, to study the biosynthetic pathway of these plant hormones, and to obtain information about the substrate specificity of the enzymes involved in the biosynthesis of gibberellins and kaurenolides. We have shown that in ent-kaurene derivatives a 15α-hydroxyl group,1,2 a 16α-alcohol,3 or a 15α,16α-epoxide4 directs hydroxylation at C-11(β) and C-7(α) and inhibits oxidation at C-19. This oxidation is typical of the biosynthetic route of gibberellins.5 We have also studied the microbial transformation of 3α,15β-dihydroxy derivatives by this fungus.6 In the latter neither gibberellins nor kaurenolides were obtained due to the presence of the 3α-hydroxyl group in the molecule, which also inhibits the oxidation at C-19. We have also shown that ent-kaurene diterpenes with a 15-oxo or a 15β,18-dihydroxy8 group can be oxidized at C-19 at the acid level and biotransformed all along the gibberellin and kaurenolide pathway. Here, we report the results obtained in the incubation with G. fujikuroi of two 15β-hydroxy-kaurene diterpenes, 1 and 3, which are not hydroxylated at C-19 and possess a 3-oxo group and a 2,3-double bond, respectively.

Results and Discussion

The substrates 1 and 3 were prepared as follows: (a) 3α-Hydroxy-15β-angeloxy-ent-kaur-16-ene (5) was oxidized with Jones reagent to give 2, which was hydrolyzed with methanolic potassium hydroxide, affording 3-oxo-15β-hydroxy-ent-kaur-16-ene (1). (b) Dehydration of 5 with phosphorus oxychloride led to 4, which was hydrolyzed, giving 15β-hydroxy-ent-kaur-2,16-diene (3).9 The starting compound in both preparations (5) had been isolated from Elaseolium tenuifolium.9,10

The biotransformations with the fungus were carried out in the presence of AMO 1618, a compound that inhibits the production of ent-kaur-16-ene without affecting the post-kaurene metabolism.11,12 The substrates were incubated with G. fujikuroi in different experiments, and the cultures were extracted following the usual procedure.

Chromatography of the extract obtained in the incubation of 1 afforded 6–10. The least polar metabolite 6 showed a molecular ion at m/z 318.2185 in accordance with
the molecular formula C_{20}H_{30}O_{3}. In its ¹H and ¹³C NMR spectra it was observed that the exocyclic double bond and the 15β-alcohol of the substrate had disappeared, being replaced by a carbonyl (δ_c 222.3) and a methyl group (δ_c 1.27, d, J = 6.8 Hz, δ_c 11.1). Thus, the formation of a 15-oxo,17-methyl derivative had occurred during the fermentation. The ¹H NMR spectrum also showed a new hydrogen signal at δ 3.94 (d, J = 5.5 Hz). Its chemical shift, the form of the signal, and the coupling constant were characteristic of a hydroxyl group in an alcohol group at C-11(β) in ent-kaur-16-ene diterpenes.¹² The results of a 2D NMR study (COSY, HSQC, and HMBC) were in accordance with these assertions and permitted us to assign the structure of 11β-hydroxy-3,15-dioxo-ent-(165)-kaurenolide (6) to this substance. The formation of this 15-oxo,17-methyl derivative implies a rearrangement, produced by protonation of the 16,17-double bond with formation of a 16-carboxylation and a 15,16-hydride shift, because this type of rearrangement occurs very readily in acid medium.¹³ Another possibility is that compound 6 could be formed by oxidation of the 15β-OH and subsequent hydrogenation of the 15,16-double bond, because we have shown that G. fujikuroi transforms 15-oxo-ent-kaur-16-ene diterpenes into the corresponding dihydroderivatives.⁷ The 1β-stereochemistry assigned to the 17-methyl was given considering that both reactions, 15,16-hydride shift and hydrogenation of the 16,17-double bond, take place by the α-face.⁷,¹³,¹⁴

The second metabolite isolated (8) had a molecular formula C_{20}H_{30}O_{3} as determined by HRMS, which indicated that a new oxygen had been introduced into the molecule during the fermentation. Its ¹H NMR spectrum showed a proton geminal to a new alcohol group as a doublet at δ 4.00 (J = 4.8 Hz), which was assigned to C-11(β). This was confirmed by its ¹³C NMR spectrum (Table 1), which was unambiguously assigned by two-dimensional NMR analysis. Therefore, the structure of this compound was determined to be 11β,15β-dihydroxy-3-oxo-ent-kaur-16-ene (8). This compound had been isolated from Rabdosia infllexa and named inflexarabdonin J.¹⁵

The third substance obtained from this incubation was (9). Its HRMS was in accordance with the formula C_{20}H_{30}O_{3}. The ¹H NMR spectrum was very similar to that of 8, but now the signal of a proton geminal to a new alcohol appeared at δ 3.81 (t, J = 3 Hz). Its coupling constant indicated that the hydroxyl group must be axial and situated at C-1(β) or C-7(β). The latter position was chosen considering its HMBC spectrum, where correlations of H-7 with C-5 and C-15 were observed. Thus, the structure 7(β, 11β,15β-trihydroxy-3-oxo-ent-kaur-16-ene (9) was assigned to this compound.

Another metabolite was identified as 7α,11β,15β-trihydroxy-3-oxo-ent-kaur-16-ene (10) as follows. The molecular ion at 334.2139 indicated a C_{20}H_{24}O_{4} molecular formula. Its ¹H NMR spectrum, in comparison with that of the substrate, showed two new hydrogens at δ 3.72 (dd, J = 11.6 and 4.3 Hz) and 4.00 (d, J = 4.9 Hz). The coupling constants for the first of these signals were typical of a proton geminal to an equatorial alcohol group at C-1(α) or C-7(α). The 7α-position was chosen considering the ¹³C NMR data (Table 1). The second of these signals was assigned to the proton geminal to an 11β-hydroxy by comparison with the ¹H and ¹³C NMR spectra of compounds such as 8 and 9. A study of the HMOC and HMBC spectra confirmed the structure of 10.

An isomer of 10, with structure 7, was also isolated from this incubation. Its ¹H NMR spectrum showed, as in the case of 6, that during the incubation a rearrangement of ring D had occurred. Thus, the proton geminal to the 15β-OH and the hydrogens of the 16,17-double bond of the substrate were not observed, being substituted by a C-17 methyl (δ_c 1.26, d, J = 6.9 Hz; δ_c 11.1) and a 15-oxo group (δ_c 221.7). The hydrogen geminal to the 7α- and 11β-alcohol appeared at δ 3.93 (dd, J = 11.6 and 4.3 Hz) and 4.00 (d, J = 4.9 Hz), respectively. The chemical shifts and splitting patterns of these protons were similar to those of its isomer 10 and to those of other compounds with these oxygen functions.⁷ Correlations between the H-7 and the 15-oxo group and of H-11 with C-9 were observed in the HMBC spectrum. Therefore, the structure of this compound was determined to be 7α,11β-dihydroxy-3,15-dixo-ent-(165)-kaurenolide (7).

The second incubation with G. fujikuroi was carried out with substrate 3. In comparison with 1, the 3-oxo group of 1, inhibitor of the oxidation at C-19, has been substituted by a 2,3-double bond in 3. This biotransformation yielded the metabolites 12–15, 17, and 19. A compound hydroxylated at C-15(β) and C-19 had been previously incubated with this fungus, giving 1β- and 6β-hydroxy derivatives with C-19 in the form of an acid group.¹⁶

The arguments used above in the assignment of the alcohol groups at C-7(α) and C-11(β) in metabolites 7 and 10 were the same ones utilized to locate them in substances 12 and 13, respectively. Compound 18 was obtained when 13 was left in CDCl₃ in a NMR tube for four weeks. Substance 18 appears to be formed from 13 by protonation of the 16,17-double bond in the acid medium and neutralization of the ion created at C-16 by 11β-OH. Their ¹H and ¹³C NMR spectra showed H-11 at δ 4.24 (br s) and 84.2, respectively. These chemical shifts and forms of resonance are characteristic of this type of compound.⁶

Another substance obtained from this experiment was 7β,15β-dihydroxykaurenolide (14) (C_{20}H_{20}O_{3}). The ¹H NMR spectrum showed that ring B contained the vicinal 6α,7β-oxygen functions characteristic of similar lactones (δ 4.42, d, J = 6.5 Hz, H-7; 4.77, t, J = 6.5 Hz, H-6; 2.28, d, J = 6.5 Hz, H-5). The H-15 signal appeared as a broad singlet at δ 4.68. ¹³C NMR showed the corresponding carbons at 71.4, 83.3 (C-6), 50.6 (C-5), and 73.2 (C-15), and the oxo group of C-19 appeared at δ 178.0.

Compounds 15 was obtained as its triacetate methyl ester 16a by methylation of the extract and subsequent acetylation of the chromatographic fractions containing it. Its ¹H NMR spectrum showed, in comparison with those of the
substrate, the signals of a methyl ester at δ 3.61 and of two new geminal protons to acetoxy groups at δ 4.78 (br s) and 5.07 (d, J = 5.4 Hz). The last one was assigned to C-1(β) by a double radiation experiment. Irradiation of H-1 at δ 5.07 converted the double doublet of H-2 at 5.76 into a clean doublet. Alternatively, irradiation of this last signal transformed the doublet of H-1 into a singlet. Unambiguous assignment of the 13C NMR data (Table 2), using HMQC and HMBC spectra, confirmed the position of this acetoxy group and permitted us to place the other acetate at C-7(α) considering that it is present in the substrate and the other at C-7(β) considering its 13C resonance at δ 69.3, which was similar to that observed in 18 (δ 69.7). The hydroxyacetomethylenic group was located at C-17 observing the chemical shift of C-16, δ 8.24 and 86.7, in 18 and 19, respectively. Thus, 19 was determined to be 7α,15β,17-trihydroxy-11β,16β-epoxy-ent-kaur-2-ene. This was confirmed by the correlations observed in the HMBC spectrum of H-17 with C-16 and C-13, of H-13 with C-14 and C-11, and of H-12 with C-14 and C-16. Substance 19 must be an artifact, probably formed in the medium from 20 by acid opening of the 16α,17α-epoxide with attack of C-16 of the 11β-OH. The oxirane ring in 20 has an α-stereochemistry because the microbiological epoxidation of ent-kaurene derivatives occurs via the α-face.2,10 The β-stereochemistry to the ether bridge in 19 may be given consideration. 3 The other two geminal hydrogens were overlapped in the 1H NMR spectrum. One was assigned to C-15(β) considering that it is present in the substrate and the other at C-7(α) considering its 13C resonance at δ 77.7 and with the molecular formula C20H30O4 (m/z 334.2137). The stereochemistry assigned to the 16α-alcohol was given considering that in this kind of molecule the hydroxylation normally occurs by the α-face.17

The 1H NMR spectrum of compound 19 (C20H30O4) showed three methyls, a hydroxyacetomethylenic group, and three protons geminal to oxygenated functions. The appearance of the exocyclic double-bond hydrogens in this spectrum and the absence of the oxo carbon resonances indicated the existence of an ether bridge between C-11(β) and C-16(β). This was confirmed by the resonance of H-11 at δ 4.32, whose chemical shift and splitting pattern are characteristic of this grouping. The other two geminal hydrogens were overlapped in the 1H NMR spectrum. One was assigned to C-15(β) considering that it is present in the substrate and the other at C-7(α) considering its 13C resonance at δ 69.3, which was similar to that observed in 18 (δ 69.7). The hydroxyacetomethylenic group was located at C-17 observing the chemical shift of C-16, δ 82.4 and 86.7, in 18 and 19, respectively. Thus, 19 was determined to be 7α,15β,17-trihydroxy-11β,16β-epoxy-ent-kaur-2-ene. This was confirmed by the correlations observed in the HMBC spectrum of H-17 with C-16 and C-13, of H-13 with C-14 and C-11, and of H-12 with C-14 and C-16. Substance 19 must be an artifact, probably formed in the medium from 20 by acid opening of the 16α,17α-epoxide with attack of C-16 of the 11β-OH. The oxirane ring in 20 has an α-stereochemistry because the microbiological epoxidation of ent-kaurene derivatives occurs via the α-face.2,10 The β-stereochemistry to the ether bridge in 19 was assigned considering the 11β-alcohol attack at C-16 by the β-face, also favored by the α-opening of the epoxy ring.

The results of these two biotransformations may be summarized as follows:

1. The existence of a 15β-hydroxyl group in the molecule does not inhibit the oxidation at C-19, typical of the biosynthetic pathway of gibberellins and kaurenolides, in contrast with our previous studies that showed that its epimer, a 15α-alcohol, inhibits oxidation thereof.

2. The presence of a 15β-alcohol directs the hydroxylation at C-11β and C-7(α). Similar results were obtained by us with 15α-alcohols1,2 and 15-oxo ent-kaur-16-ene derivatives.

Table 2. 13C NMR Data for Compounds 3, 12, 14, 16a, and 17–19

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*a,b, These values can be interchanged.
3. A 3-oxo group in the molecule inhibits oxidation at C-19. This had also occurred when there was a 3α-alcohol but not when a 3β-OH was present.  
4. The presence of a 2,3-double bond does not affect the oxidation at C-19.  
5. In the biotransformation of substrates with a 15β-hydroxy and a 2,3-double bond such as 3, there are two competitive processes, the oxidation at C-19 and the hydroxylation at C-11(β) and C-7(α). Thus, the amount of substrate that is oxidized in C-19 to an acid group, a prerequisite to be transformed into kaurenolides and gibberellins, is low. Moreover, we have also observed that there is a preference for the formation of kaurenolides with respect to gibberellins.  
6. Another competitive biotransformation is the hydroxylation at C-1(β) in the substrate 3 to give 15, which is directed by the presence of the 2,3-double bond.  
7. Isomerization of the 15β-hydroxy to the 15-oxo derivatives seems not to be produced in the medium by acid rearrangement because compound 11, which should be obtained from rearrangement of substrate 1, was not isolated from the fermentation. Thus, this is a biotransformation produced by the fungus by a garrifoline-cuauchichine type rearrangement or by oxidation of the 15β-alcohol and subsequent hydrogenation of the 16,17-double bond.  
8. The occurrence of 11β-hydroxylated ent-kaur-16-ene diterpenes with oxygen functions of the 15α-OH, 15β-OH, or 15-oxo type in liverworts and higher plants seems to indicate that the 11β-hydroxylation should be directed by the presence in the molecule of a 15-oxidgenated substrate, as occurs in the microbiological transformations with the fungus G. fujikuroi.

**Experimental Section**

**General Experimental Procedures.** Melting points were determined with a Reichert Thermovar apparatus and are uncorrected. IR spectra were recorded in a Perkin-Elmer 1600 FT-IR. 1H NMR spectra were recorded in CDC13 solution at 200.13 and 500.13 MHz with a Bruker AC-200 or a Bruker AMX-500 spectrometer, respectively, and the 13C NMR at 125.03 MHz in a Bruker AMX-500, except those of 3 and 6–10, which were recorded at 50 MHz in a Bruker AC-200. Mass spectra were taken at 70 eV (probe) in a Shimadzu QZ2000, and high-resolution mass spectra in a Micromass Autospec spectrometer. Optical rotations have been measured at 25 °C in a Perkin-Elmer 343 Plus. HPLC was performed using a Beckman System Gold 125P. Purification by HPLC was achieved using a Beckman System Gold 125P.

**Microorganism.** The fungal strain was Gibberella fujikuroi IMI 56289 and was a gift from Dr. J. R. Hanson, School of Chemistry, Physics and Environmental Science (University of Sussex, UK).

**Preparation of 15β-Hydroxy-3-oxo-ent-kaur-16-ene (1).** The fungus G. fujikuroi, inhibited with 5 x 10−5 M AMO 1618, was grown on shake culture at 25 °C for 2 days in 55 conical flasks (250 mL), each containing 50 mL of sterile medium comprising (per dm3) glucose (80 g), NH4NO3 (0.48 g), KH2PO4 (5 g), MgSO4 (1 g), and trace elements solution (2 mL). The trace elements solution contained (per 100 mL) Co(NO3)2 0.016, CuCl2 0.0015, ZnSO4 0.16 g, MnSO4 0.01 g, and (NH4)2MoO4 0.01 g. The incubation was performed for 2 days in 55 conical flasks (250 mL), each containing 50 mL of sterile medium comprising (per dm3) glucose (80 g), NH4NO3 (0.48 g), KH2PO4 (5 g), MgSO4 (1 g), and trace elements solution (2 mL). The trace elements solution contained (per 100 mL) Co(NO3)2 0.016, CuCl2 0.0015, ZnSO4 0.16 g, MnSO4 0.01 g, and (NH4)2MoO4 0.01 g. The metabolites 6, 8, and 10, which were obtained as a mixture of trinorses, were chromatographed on silica gel impregnated with AgNO3 (15%) eluting with petroleum ether–EtOAc (1:1) and EtOAc, respectively.

**11β-Hydroxy-3,15-dioxo-ent-kaur-16-ene (6):** colorless crystals; mp 185–187 °C (petroleum ether–EtOAc; [α]D −82° (c 0.16, CHCl3); 1H NMR (500 MHz) δ 1.04 (3H, s, H-19), 1.05 (3H, s, H-20), 1.12 (3H, s, H-18), 1.25 (1H, br s, H-9), 1.27 (3H, d, J = 8.8 Hz, H-17), 1.65 (1H, dt, J = 13.5 and 8.5 Hz, H-1j), 2.03 (1H, dd, J = 13, 7.5 and 5.5 Hz), 3.31 (1H, quint, J = 6.8 Hz, H-16), 3.24 (1H, dd, J = 12 and 11.1 Hz, H-14), 2.46 (1H, d, J = 16, 8.5 and 7.7 Hz, H-2), 2.48 (1H, br s, H-13), 2.55 (1H, dhd, J = 16, 8.5 and 5.3 Hz, H-2), 3.94 (1H, d, J = 5.5 Hz, H-11); EIMS m/z (rel int) 318[M+]+12, 303 (7), 300 (55), 285 (35), 267 (22), 257 (21), 243 (23), 227 (15), 209 (19), 201 (19), 185 (19); HREIMS m/z[M+]+131:291.3.2185 (calcld for C30H38O4, 318.2195).

**7α,11β-Dihydroxy-3,15-dioxo-ent-kaur-16-ene (7):** colorless crystals; mp 229–232 °C (petroleum ether–EtOAc; [α]D −111° (c 0.53, CHCl3); 1H NMR (500 MHz) δ 1.05 (6H, s, H-19 and H-20), 1.13 (3H, s, H-18), 1.22 (1H, s, H-9), 1.26 (3H, d, J = 6.9 Hz, H-17), 1.50 (1H, dt, J = 12 Hz, H-6x), 1.62 (1H, dt, J = 13.3 and 8.5 Hz, H-1), 1.67 (1H, dd, J = 12 and 2.4 Hz, H-5), 1.77 (1H, ddd, J = 12, 4.2, and 2.4 Hz, H-6j), 2.27 (1H, quint, J = 6.8 Hz, H-16), 2.47 (1H, dt, J = 16 and 8.5 Hz, H-2), 2.52 (1H, br s, H-13), 2.55 (1H, ddd, J = 16, 8.5, and 5.2 Hz, H-2), 3.93 (1H, d, J = 5.1 Hz, H-11), 4.00 (1H, dd, J = 12 and 4.2 Hz, H-7); EIMS m/z (rel int) 334 [M]+12, 316 (21), 298 (8), 288 (24), 260 (65), 243 (30), 231 (12), 225 (14), 213 (14), 196 (48), 178 (68); HREIMS m/z[M+]+134:291.4 (calcld for C30H38O4, 324.2144).
ether—EtOAc; [α]D = −80° (c 0.22, CHCl3); [1]H NMR (500 MHz) δ 0.99 (each 3H, s, H-20), 1.04 (3H, s, H-19), 1.11 (3H, s, H-18), 1.14 (1H, m, H-14), 1.68 (1H, br s, H-9), 1.94 (1H, dj, J = 12.1 Hz, H-14), 2.04 (1H, dd, dj = 14.6, 5.0, and 2.8 Hz, H-12), 2.09 (1H, dd, dd = 13.4, 7.9, and 5.5 Hz, H-11), 2.45 (1H, dt, dj = 16 and 7.9 Hz, H-2), 2.58 (1H, dd, dj = 16, 8.4, and 5.5 Hz, H-2), 2.68 (1H, br s, H-13), 3.82 (1H, br s, H-15), 4.00 (1H, dj, J = 12 and 7.9 Hz, H-13), 5.37 (1H, dd, J = 15.4 and 5.4 Hz, H-17), 5.41 (1H, s, H-17); EIMS m/z (rel int) 318 M+ [78], 300 (48), 285 (37), 267 (22), 243 (22), 229 (8), 215 (4), 201 (19), 197 (17); 1H NMR (500 MHz) δ 3.45 (2H, H-7 and H-15), 4.24 (1H, br s, H-11), 5.38 (1H, dd, J = 10 and 2.5 Hz, H-3), 5.45 (1H, dd, J = 10, 6, and 1.8 Hz, H-2); EIMS m/z (rel int) 318 M+ [29], 302 (28), 253 (6), 208 (5).

7j,15j-Dihydroxy-ent-kaur-2,16-diene-19,6-olide (14): colorless crystals; 253—255 °C (CHCl3); [1]H NMR (500 MHz) δ 0.85 (3H, s, H-20), 1.32 (3H, s, H-18), 1.43 and 2.15 (each 1H, m, H-1), 1.46 (1H, dj, J = 11.9 Hz, H-14), 1.66 (1H, dd, J = 12 and 5.3 Hz, H-13), 2.45 (1H, dd, J = 16.5 and 6.4 Hz, H-2), 2.28 (1H, dd, J = 6.5 Hz, H-5), 2.64 (1H, dj, J = 7 Hz, H-13), 4.42 (1H, dj, J = 6.5 Hz, H-6), 5.00 and 5.10 (each 1H, br s, H-17), 5.73 (1H, dd, J = 9.8, 6.4, and 1.6 Hz, H-2), 5.78 (1H, dd, J = 9.8 and 2.5 Hz, H-3); EIMS m/z (rel int) 312 M+ [58], 284 (41), 269 (15), 240 (19), 235 (17), 207 (8), 195 (16), 169 (16); HREIMS m/z M+ [72] 312.1724 (calc for C20H24O3, 312.2175).

7j,15j-Dihydroxy-ent-kaur-2,16-diene-19-acid (15): This compound was obtained as the triacetate methyl ester 16a by methylation of the extract and subsequent acetylation (Ac2O—py; 80 °C, 12 h) and chromatography of the fractions containing it, eluting with petroleum ether—EtOAc (1:1).

Triacetate methyl ester (16a): a gum; [1]H NMR (500 MHz) δ 1.00 (3H, s, H-20), 1.22 (3H, s, H-18), 1.33 (1H, m, H-14), 1.38 (1H, m, H-11), 1.70 (2H, m, H-11 and H-1), 1.89 (1H, d, J = 12 Hz, H-14), 1.96, 2.05 and 2.09 (each 3H, s, OAc), 2.07 (1H, dd, J = 9 and 3 Hz, H-5), 2.46 (1H, dj, J = 6.9 Hz, H-9), 2.69 (1H, br s, H-13), 3.61 (3H, s, OMe), 4.78 (1H, br s, H-7), 4.87 (1H, br d, J = 9.1 Hz, each 1H, br s, H-17), 5.07 (1H, dd, J = 5.4 Hz, H-11), 5.45 (1H, br s, H-15), 5.76 (1H, dd, J = 10.1 and 5.4 Hz, H-2), 5.88 (1H, dj, J = 10.1 Hz, H-3); EIMS m/z (rel int) 488 M+ [20], 428 (1), 386 (1), 386 (2), 306 (8), 265 (11), 249 (42), 221 (27), 207 (27), 169 (32); HREIMS m/z M+ [72] 488.2425 (calc for C20H24O3, 488.2410).

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References and Notes

