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GUMMIFEROLIC ACID, A NEW *ent*-ATIS-16-ENE DITERPENOID FROM *MARGOTIA GUMMIFERA*

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Key Word Index—*Margotia gummifera* (= *Elaeoselinum gummiferum*); Umbelliferae; diterpenoids; *ent*-kaur-16-en-19-oic and *ent*-7α-angeloxy-atis-16-en-19-oic acids; ^{13}C NMR data.

Abstract—From the roots of *Margotia gummifera* a new diterpenoid with the *ent*-atis-16-ene skeleton, gummiferolic acid, has been obtained in very high yield (2% of the dry plant), together with the known *ent*-kaur-16-en-19-oic acid.

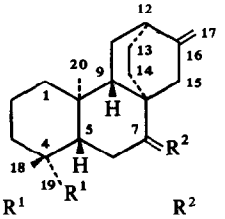
INTRODUCTION

In our search for new natural substances in the Umbelliferous plants endemic at the Iberian Peninsula [1, 2], we have examined the roots of *Margotia gummifera* (Desf.) Lange [= *Elaeoselinum gummiferum* (Desf.) Tutin], from which two diterpenic compounds have been isolated. One of these diterpenoids is the previously known *ent*-kaur-16-en-19-oic acid [3] and the other one is a new substance, gummiferolic acid (**1**), which possesses the *ent*-atis-16-ene skeleton, unusual for oxygenated nitrogen-free compounds obtained from natural sources [4].

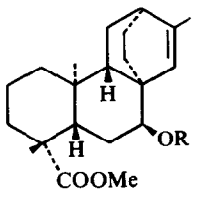
RESULTS AND DISCUSSION

From concentrated petrol extracts of dried roots of the plant, a crystalline white solid of pure gummiferolic acid (**1**) was separated. Compound **1** had a molecular formula of $C_{25}H_{36}O_4$ and its IR spectrum showed typical absorptions for an exocyclic methylene group (3070, 1644, 880 cm^{-1}) and two carbonyl functions, probably an ester (1725 cm^{-1}) and a free carboxylic acid (3320, 1676 cm^{-1}). The PMR spectrum of **1** showed characteristic signals for an angelic ester [δ 6.01 (1H, *qq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz), 1.99 (3H, *dq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz) and 1.94 (3H, *s* (*br*))] [5] axially attached to a carbocyclic secondary carbon atom which is between a methylene group and a sp^3 tetrasubstituted carbon atom as the signal of its geminal equatorial proton showed an apparent triplet at δ 4.84 (J = 3 Hz). The signals assigned to the exocyclic methylene group appeared as two quartets (J = 2 Hz) at δ 4.72 and 4.56. In addition, gummiferolic acid possessed two methyl groups attached to fully substituted carbon atoms (singlets at δ 1.15 and 0.93). The UV absorption of **1** (λ_{max} 218 nm, log ε 3.91) [5] was also indicative of the presence of the angelate moiety in the molecule of the diterpenoid.

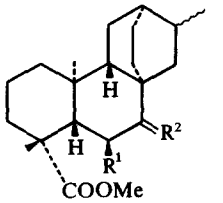
Ethereal diazomethane treatment of **1** gave the methyl ester **2** (COOMe at δ 3.64, 3H, *s*). The MS spectrum of **2** showed an ion fragment at *m/e* 101 (10%) which appeared



	R^1	R^2
1	COOH	OCOMe(Z)=CHMe, H
2	COOMe	OCOCMe(Z)=CHMe, H
3	CH_2OH	OH, H
4	CH_2OAc	OAc, H
5	COOH	OH, H
6	COOMe	OH, H
7	COOMe	O
8	COOMe	H_2
9	COOMe	H, OH
10	COOMe	OAc, H



11 R = Ac
12 R = H



	R^1	R^2
13	H	OH, H
14	H	O
15	Br	O

in compound **1** at *m/e* 87 (7%), this being indicative of the presence of a C-18 or C-19 carboxylic function in a tetracyclic diterpenoid [6]. The IR absorptions at 1160 and 1190 cm^{-1} in compound **2** suggested an axial configuration for this methyl ester [7]. On the other hand, $LiAlH_4$ reduction of **1** gave a $C_{20}H_{32}O_2$ diol (**3**) which was acetylated to give compound **4**. The PMR chemical shifts observed for the hydroxymethylene protons in **3** (AB quartet centred at δ 3.60) and for the CH_2OAc grouping of **4** (δ 4.04) are in complete agreement with a C-19 axial function [8].

Alkaline hydrolysis of **1** under strong conditions (2.5 N KOH, in EtOH solution, 24 hr reflux) yielded tiglic acid (arising from an isomerization of angelic acid) and the hydroxy acid **5**, whereas identical treatment of methyl ester **2** gave the hydroxy ester **6**, thus confirming the C-19 axial position for the COOMe function.

These facts are accommodated most readily, albeit not exclusively, in tetracarbocyclic, monoolefinic, diterpenic structures of *ent*-kaur-16-ene or *ent*-atis-16-ene types with a carboxylic group on C-19 and an angeloxy group attached to C-1, C-3 or C-7 in both skeletons or at C-14 in the latter type. However, the PMR signal pattern showed by the exocyclic methylene group (see above) is only expected for C-15 hydroxylated (or esterified) *ent*-kaur-16-ene compounds [9] but it is normal in all *ent*-atis-16-ene derivatives [10], thus the first skeleton must be eliminated from consideration.

Final proof of the *ent*-atis-16-ene skeleton and the carboxylic function (C-19) of gummiferolic acid was established as follows. Jones' oxidation of compound **6** gave the keto ester **7**, which was subjected to Nagata's modification of the Wolff–Kishner reduction [11] to yield, after ethereal diazomethane treatment, a compound (**8**) identical in all respects with methyl *ent*-atis-16-en-19-oate previously obtained by Coates and Bertram [12] from isosterviol.

Finally, the presence in the molecule of gummiferolic acid of a C-7 angeloxy group axially oriented was confirmed by several facts. The ^{13}C NMR spectra of compounds **2**, **7** and **8** (Table 1) were in agreement with this conclusion because the presence of only a carbon triplet near 20 ppm in compounds **2** and **7** suggested C-1, C-2, C-3, C-6 or C-7 as the site of attachment of the oxygenated function [13], but the diamagnetic shifts experienced by the doublets for C-5 and C-9 (see Table 1) in compound **2** with respects to **7** and **8** clearly pointed to axial C-7 as the locus of the angeloxy group [14, 15].

$NaBH_4$ reduction of **7** gave predominantly compound **9** in which the OH group is equatorial (axial geminal proton as a quadruplet at δ 3.23, $J_{aa'}$ = 9.5 Hz, J_{ae} = 5.5 Hz). The identical chemical shifts for C-Me groups in the PMR spectra of compounds **6** (δ 1.17 and 0.79) and its epimer **9** (δ 1.18 and 0.80) is only compatible with C-7 epimeric hydroxyl functions.

Acetylation of the hydroxy ester **6** yielded the derivative **10** which was subjected to olefinic double bond isomerization by treatment with I_2 in benzene solution [16] to yield the derivative **11** which was saponified to compound **12**. The paramagnetic shift ($\Delta\delta$ = +0.19) showed by the proton geminal to the OAc group in compound **11** with respect to **10** indicated that this proton in the first product (**11**) is near to the deshielding zone of the double bond. On the other hand, the diamagnetic shift ($\Delta\delta$ = −0.33) showed by the olefinic proton in compound **11** with respect to the deacetylated derivative (**12**) was also indicative of the presence of a C-7 axial hydroxyl group, because the same effect has been observed in similar *ent*-7α-hydroxy-kaur-15-ene derivatives [17].

Hydrogenation of compound **6** gave an expected mixture [18] of C-16 epimers (PMR: C-17 Me doublets at δ 0.97 and 0.94, 2:1 ratio) from which a pure substance (**13**) was obtained after several crystallizations. Jones' oxidation of **13** yielded compound **14** which was treated with Br_2 in HOAc solution under controlled conditions to give the monobromoderivative **15**, the PMR spectrum of which showed the bromomethyne proton as a doublet (J = 6 Hz) at δ 5.24. This was only compatible with a keto group at C-7 or, alternatively, at C-14 of the *ent*-atisane skeleton; however the latter position must be discarded because the C-20 methyl group was deshielded ($\Delta\delta$ = +0.19) in compound **7** with respect to derivative **6**. (Bromination of **14** causes a conformational change, chair → boat, in ring B of compound **15**, similar to that observed in *ent*-7-oxo-kaurane derivatives [19]). Finally, application of Horeau's method of partial resolution [20] to epimeric alcohols **6** and **9** established as 7*S* and 7*R*, respectively, the absolute stereochemistry of this center, according to all the above deductions.

Gummiferolic acid is thus *ent*-7α-angeloxy-atis-16-en-19-oic acid (**1**) and belongs to the very small group of nitrogen-free natural substances with this hydrocarbon skeleton [4, 21, 22].

EXPERIMENTAL

Mps were determined in a Kofler apparatus and are uncorr. PMR and ^{13}C NMR spectra were measured at 100 and 25.2 MHz, respectively, in $CDCl_3$ soln with TMS as internal standard. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance and noise-decoupled ^{13}C NMR spectra. Elemental analyses were carried out in this laboratory with the

Table 1. ^{13}C chemical shifts* of compounds **2**, **7** and **8**

Carbon No.	2†	7	8
1	39.7	39.2	39.6
2	18.7	18.6	18.8
3	38.2	38.2	38.2
4	43.3	43.8	43.8
5	49.4	54.0	57.1
6	27.9‡	37.8§	20.2
7	75.7	214.6	39.6
8	36.8	47.4	33.3
9	47.6	51.8	52.0
10	38.0	37.8	38.2
11	27.7‡	27.6‡	27.2
12	36.3	35.8	36.5
13	25.5‡	26.1‡	28.2‡
14	26.9‡	28.1‡	28.7‡
15	41.7	39.9§	48.1
16	151.1	149.8	152.5
17	105.1	106.2	104.3
18	28.5	28.3	28.7
19	177.4	176.7	177.7
20	11.6	11.8	11.9
OMe	51.1	51.3	51.1

* All ^{13}C chemical shifts are given in ppm relative to TMS.

† Angelic ester: C-1, 166.9 (*s*); C-2, 128.3 (*s*); C-3, 136.8 (*d*); C-4, 20.9 (*q*); C-2', 15.8 (*q*).

‡§ These assignments may be reversed, but those given here are considered to be most likely.

help of an automatic analyzer. Plant materials were collected in September 1977 near Batres (Madrid), on acidic sandy soil, identified by Dr J. Borja, Department of Botany, Faculty of Pharmacy (Madrid) and voucher specimens (No. 86747, 71335) were deposited in the Herbarium of this Faculty.

Extraction and isolation of the diterpenoids. Dried and finely powdered *M. gummifera* roots (750 g) were extracted for 60 hr with petrol (6 litre) in a Soxhlet. The extract was concd *in vacuo* to leave a residue (55 g) which was dissolved in Et_2O. From this soln 5 g of pure gummiferolic acid (**1**) were crystallized. The remaining 50 g of the extract were chromatographed on a Si gel column (600 g) (eluent: petrol and petrol–EtOAc, 19:1) yielding the following diterpenoids in order of elution: *ent*-kaur-16-en-19-oic acid [450 mg, identified by physical (mp, $[\alpha]_D$) and spectroscopic (IR, PMR, ^{13}C NMR, MS) data and by comparison with an authentic sample] and an additional amount (10 g) of **1**.

Gummiferolic acid (**1**). Mp 186–188° (MeOH), $[\alpha]_D^{20°}$ −27° (*c* 0.39. $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3320, 1676 (COOH), 3070, 1644, 880 (exocyclic methylene), 1725 (ester). UV λ_{max}^{EtOH} nm (log ε): 218 (3.91). PMR: δ 6.01 (1H, *qq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz, H-3 angelate), 4.84 (1H, *t*, *J* = 3 Hz, H-7), 4.72 and 4.56 (1H each, *q*, *J* = 2 Hz, 2H-17), 1.99 (3H, *dq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz, 3H-4 angelate), 1.94 (3H, *q*, *J* = 1 Hz, 3H-2′ angelate), C-Me singlets at 1.15 (3H-18) and 0.93 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 400 (M^+ 1.1), 300 (100), 285 (21), 281 (6), 272 (6), 271 (4), 255 (29), 254 (23), 239 (25), 211 (17), 199 (17), 185 (32), 146 (25), 131 (27), 105 (34), 91 (40), 87 (7), 83 (66), 55 (96). (Found: C, 74.87; H, 9.05. $C_{25}H_{36}O_4$ requires: C, 74.96; H, 9.06%).

Methyl ester **2**. Treatment of compound **1** (300 mg) with CH_2N_2–Et_2O soln gave **2** (308 mg), mp 113–114° (MeOH), $[\alpha]_D^{20°}$ −25° (*c* 0.37, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3070, 1645, 878 (exocyclic methylene), 1720 (angelate and COOMe), 1160, 1190 (axial COOMe). UV λ_{max}^{EtOH} nm (log ε): 214 (3.96). PMR: δ 6.03 (1H, *qq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz, H-3 angelate), 4.83 (1H, *t*, *J* = 3 Hz, H-7), 4.73 and 4.57 (1H each, *q*, *J* = 2 Hz, 2H-17), 3.64 (3H, *s*, COOMe), 2.00 (3H, *dq*, J_{vic} = 7 Hz, $J_{allylic}$ = 1 Hz, 3H-4 angelate), 1.93 (3H, *q*, *J* = 1 Hz, 3H-2′ angelate), C-Me singlets at 1.08 (3H-18) and 0.82 (3H-20), MS (70 eV, direct inlet) *m/e* (rel. int.): 314 (M^+-angelic acid, 100), 299 (10), 255 (42), 254 (45), 239 (30), 211 (20), 185 (25), 146 (17), 121 (30), 105 (25), 101 (10), 91 (25), 83 (50), 55 (75). (Found: C, 75.54; H, 9.26. $C_{26}H_{38}O_4$ requires: C, 75.32; H, 9.24%).

$LiAlH_4$ *reduction of* **1** *to yield* ent-7α,19-*dihydroxy-atis*-16-*ene* (**3**). $LiAlH_4$ reduction of **1** (100 mg) in Et_2O soln gave **3** (70 mg), mp 130–133° (aq. EtOH), $[\alpha]_D^{20°}$ +3° (*c* 0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 3065, 1640, 875 (exocyclic methylene). UV λ_{max}^{EtOH} nm (log ε): 207.5 (3.59). PMR: δ 4.73 and 4.60 (1H each, *q*, *J* = 2 Hz, 2H-17), 3.60 (2H, AB system, *J* = 11 Hz, 2H-19), 3.44 (1H, *m*, $W_{\frac{1}{2}}$ = 4 Hz, H-7), two C-Me singlets at 0.98 (6H, 3H-18 and 3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 304 (M^+ 3), 273 (8), 271 (6), 268 (8), 255 (100), 199 (17), 173 (18), 123 (38), 107 (32), 93 (47), 81 (42). (Found: C, 78.66; H, 10.51. $C_{20}H_{32}O_2$ requires: C, 78.89; H, 10.59%).

ent-7α,19-*Diacetoxy-atis*-16-*ene* (**4**). Treatment of compound **3** (50 mg) with Ac_2O–Py (0.5–1 ml) as usual gave **4** (52 mg), a syrup, $[\alpha]_D^{20°}$ −10° (*c* 0.57, $CHCl_3$). IR ν_{max}^{film} cm^{-1}: 3065, 1645, 875 (exocyclic methylene), 1725, 1240 (acetates). PMR: δ 4.74 and 4.58 (1H each, *q*, *J* = 2 Hz, 2H-17), 4.66 (1H, *t*, *J* = 3 Hz, H-7), 4.04 (2H, AB system, *J* = 11 Hz, 2H-19), 2.07 and 2.03 (3H each, *s*, two OAc), C-Me singlets at 0.99 and 0.88. MS (70 eV, direct inlet) *m/e* (rel. int.) : 388 (M^+ 0.5), 328 (57), 313 (10), 268 (100), 255 (63), 253 (43), 199 (23), 185 (32), 146 (27), 131 (30), 121 (27), 105 (38), 93 (40), 85 (52), 71 (56), 57 (92). $C_{24}H_{36}O_4$ MW 388.

Alkaline hydrolysis of **1**. A soln of compound **1** (2 g) in 2.5 N ethanolic KOH was refluxed for 24 hr. The soln was then acidified and extracted with $CHCl_3$. The $CHCl_3$ extract was dried, filtered and concd *in vacuo* to leave a residue (2 g) which was separated on PLC Si gel plates (continuously eluted with $CHCl_3$ for 24 hr) into two components. The less polar compound (390 mg) was identical with authentic tiglic acid (mp, mmp; IR, PMR and MS spectra). The most polar compound, *ent*-7α-hydroxy-atis-16-en-19-oic acid (**5**, 1.5 g) had mp 215–221° (MeOH), $[\alpha]_D^{20°}$ −33° (*c* 0.58, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 3300–2800, 1685 (COOH), 3060, 1648, 875 (exocyclic methylene). UV λ_{max}^{EtOH} nm (log ε): 210.5 (3.43). PMR: (DMSO-d_6): δ 4.67 and 4.50 (1H each, *m*, $W_{\frac{1}{2}}$ = 6 Hz, 2H-17). 3.23 (1H, *m*, $W_{\frac{1}{2}}$ = 5 Hz, H-7), C-Me singlets at 1.07 and 0.82. MS (70 eV, direct inlet) *m/e* (rel. int.): 318 (M^+ 6), 303 (8), 300 (100), 285 (27), 255 (42), 254 (27), 199 (24), 164 (35), 146 (61), 121 (60), 105 (64), 91 (85), 87 (11), 79 (72). (Found: C, 75.31; H, 9.64. $C_{20}H_{30}O_3$ requires: C, 75.43; H, 9.50%). Treatment of compound **5** with CH_2N_2–Et_2O soln gave **6** (see below).

Alkaline hydrolysis of **2** *to yield methyl* ent-7α-*hydroxy-atis*-16-*en*-19-*oate* (**6**). Compound **2** (200 mg) was treated under the same conditions described for **1**, to give **6** (140 mg), mp 205–207° (Me_2CO), $[\alpha]_D^{20°}$ −35° (*c* 0.19, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3530 (OH), 1705, 1195, 1160 (axial COOMe), 3065, 3030, 1640, 880, 873 (exocyclic methylene). PMR: δ 4.74 and 4.61 (1H each, *q*, *J* = 2 Hz, 2H-17), 3.64 (3H, *s*, COOMe), 3.49 (1H, *t*, *J* = 3 Hz, H-7), C-Me singlets at 1.17 (3H-18) and 0.79 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 332 (M^+ 7), 317 (8), 314 (100), 299 (14), 273 (14), 272 (20), 255 (64), 254 (55), 121 (68), 107 (68), 101 (14), 93 (68), 91 (68). (Found: C, 75.56; H, 9.80. $C_{21}H_{32}O_3$ requires: C, 75.86; H, 9.70%).

Application of Horeau's method [20] *to* **6**. A mixture of (±)-α-phenylbutyric anhydride (0.38 mmol) and **6** (0.172 mmol) in Py soln (2 ml) was kept at room temp. for 24 hr. α_1 = −1.413; α_2 = −1.202; α_1 − 1.1 α_2 = −0.091. Configuration 7*S*.

Methyl ent-7-*oxo-atis*-16-*en*-19-*oate* (**7**). Excess Jones' reagent was added to an Me_2CO soln (30 ml) of the hydroxy ester **6** (200 mg) and the mixture left for 2 hr at room temp. Workup yielded **7** (185 mg) which was crystallized from Me_2CO, mp 119–121°, $[\alpha]_D^{20°}$ −40.5°, $[\alpha]_{365}^{20°}$ +52.5° (*c* 0.22, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3075, 1645, 885, 875 (exocyclic methylene), 1720 (ester), 1690 (ketone). PMR: δ 4.76 and 4.66 (1H each, *q*, *J* = 2 Hz, 2H-17), 3.67 (3H, *s*, COOMe), C-Me singlets at 1.16 (3H-18) and 0.98 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 330 (M^+ 100), 315 (5), 312 (4), 298 (22), 270 (89), 255 (38), 204 (24), 162 (73), 147 (49), 119 (43), 105 (40), 101 (19), 91 (68), 79 (49). $C_{21}H_{30}O_3$ MW 330.

Wolff-Kishner reduction of **7** *to yield methyl* ent-*atis* 16-*en*-19-*oate* (**8**). Compound **7** (320 mg) was treated under N_2 in the conditions described by Nagata [11] for the Wolff–Kishner reduction, yielding a product which was methylated with ethereal CH_2N_2 to give **8** (220 mg, after PLC purification on 12% $AgNO_3$–Si gel, C_6H_6–EtOAc, 3:1), mp 126.5–128° (MeOH), $[\alpha]_D^{17°}$ −61.4° (*c* 0.95, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3070, 3030, 1640, 880, 875 (exocyclic methylene), 1718, 1210, 1190, 1155 (axial COOMe). PMR: δ 4.68 and 4.52 (1H each, *q*, *J* = 2 Hz, 2H-17), 3.62 (3H, *s*, COOMe), C-Me singlets at 1.16 (3H-18) and 0.78 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 316 (M^+ 52), 301 (65), 273 (26), 257 (100), 256 (35), 241 (69), 213 (26), 121 (61), 107 (61), 101 (20), 91 (70), 81 (56), 79 (58). (Found: C, 79.43; H, 10.01. Calc. for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19%). Identical in all respects with the previously reported compound [12]: mp 126–127°, $[\alpha]_D^{23°}$ −62.5°, IR and PMR spectra superimposable.

$NaBH_4$ *reduction of* **7** *to* **9** *and* **6**. To an EtOH–dioxane (1:1) soln (15 ml) of **7** (105 mg), $NaBH_4$ (200 mg) was slowly added and the mixture kept at room temp. for 2 hr. The reaction products were separated on PLC Si gel (C_6H_6–EtOAc, 3:1) giving **9** (most polar component, 93 mg) and **6** (6 mg). Compound **9** crystallized from Me_2CO–*n*-hexane had mp 170–172°, $[\alpha]_D^{20°}$ −66° (*c* 0.51, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3530 (OH), 3080, 3030, 1640, 888 (exocyclic methylene), 1700, 1195, 1160 (axial COOMe). PMR: δ 4.74 and 4.58 (1H each, *q*, *J* = 2 Hz, 2H-17), 3.63 (3H, *s*, COOMe), 3.23 (1H, *q*, $J_{aa'}$ = 9.5 Hz, $J_{ae'}$ = 5.5 Hz, axial H-7), C-Me singlets at 1.18 (3H-18) and 0.80 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int): 332 (M^+ 32), 317 (5), 314 (14), 303 (3), 299 (6), 289 (100), 276 (9), 273 (9), 255 (41), 254 (24), 203 (32), 171 (48), 164 (25), 123 (46), 121 (51), 107 (41), 101 (14), 93 (48), 91 (50), 79 (51). $C_{21}H_{32}O_3$ MW 332.

Application of Horeau's method to **9**. Performed in the usual manner [20]. **9** (0.095 mmol), (±)-α-phenylbutyric anhydride (0.38 mmol), Py (2 ml). α_1 = −1.306; α_2 = −1.289; α_1 − 1.1: α_2 = +0.112. Configuration 7*R*.

Methyl ent-7α-*acetoxy-atis*-16-*en*-19-*oate* (**10**). Treatment of compound **6** (290 mg) with Ac_2O–Py in the usual manner gave **10** (285 mg), mp 139–142° (aq. EtOH), $[\alpha]_D^{18°}$ −33° (*c* 1.02, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3070, 1645, 875, 870 (exocyclic methylene), 1730, 1245 (acetate), 1718, 1185, 1155 (axial COOMe). PMR: δ 4.75 (2H, *m*, H-7 and one C-17 protons), 4.60 (1H, *q*, *J* = 2 Hz, one C-17 proton), 3.65 (3H, *s*, COOMe), 2.09 (3H, *s*, OAc), C-Me singlets at 1.10 (3H-18) and 0.81 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 374 (M^+ 1), 314 (100), 299 (16), 255 (64), 254 (69), 239 (48), 211 (37), 146 (32), 121 (64), 105 (48), 101 (16), 91 (58), 79 (52). $C_{23}H_{34}O_4$ MW 374.

Isomerization of **10** *to methyl* ent-7α-*acetoxy-atis*-15-*en*-19-*oate* (**11**). A C_6H_6 soln (50 ml) of **10** (300 mg) was treated with I_2 (30 mg) under reflux for 12 hr. After cooling, the soln was diluted with C_6H_6 (100 ml) and washed with aq. $Na_2S_2O_3$ and H_2O. Evapn of the C_6H_6 and final PLC purification on 12% $AgNO_3$–Si gel (C_6H_6–EtOAc, 3:1) gave 110 mg of the starting material (**10**) and 185 mg of its Δ^{15} isomer (**11**), mp 190–193° (aq. EtOH), $[\alpha]_D^{20°}$ −42.5° (*c* 0.48, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3040, 1630, 815 (olefinic double bond), 1730, 1245 (acetate), 1715 (COOMe). PMR: δ 5.61 (1H, *s* (*br*), $W_{\frac{1}{2}}$ = 4 Hz, H-15), 4.94 (1H, *t*, *J* = 3 Hz, H-7), 3.61 (3H, *s*, COOMe), 2.10 (3H, *s*, OAc), 1.72 (3H, *d*, *J* = 1.5 Hz, 3H-17), C-Me singlets at 1.08 (3H-18) and 0.79 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 374 (M^+ 10), 346 (RDA on Δ^{15}, 2), 314 (46), 299 (26), 286 (23), 273 (23), 255 (43), 254 (36), 239 (25), 226 (15), 211 (17), 185 (21), 146 (20), 118 (100), 105 (39), 101 (10), 93 (41), 81 (49). $C_{23}H_{34}O_4$ MW 374.

Alkaline hydrolysis of **11** *to methyl* ent-7α-*hydroxy-atis*-15-*en*-19-*oate* (**12**). Compound **11** (80 mg) was treated under the same conditions described for **1** and **2**, to give **12** (65 mg), mp 190–192° (MeOH), $[\alpha]_D^{18°}$ −37° (*c* 0.47, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3560 (OH), 3030, 825 (olefinic double bond), 1710, 1210, 1195, 1165 (axial COOMe). PMR: δ 5.94 (1H, *s* (*br*), $W_{\frac{1}{2}}$ = 4 Hz, H-15), 3.82 (1H, *t*, *J* = 3 Hz, H-7), 3.62 (3H, *s*, COOMe), 1.75 (3H, *d*, *J* = 1.5 Hz, 3H-17), C-Me singlets at 1.16 (3H-18) and 0.78 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 332 (M^+ 60), 317 (28), 314 (18), 304 (12), 289 (9), 286 (7), 273 (28), 255 (37), 250 (37), 164 (80), 123 (83), 117 (71). 108 (76), 105 (71), 101 (16), 93 (69), 81 (100). $C_{21}H_{32}O_3$ MW 332.

Hydrogenation of **6** *to yield methyl* ent-7α-*hydroxy*-17ξ-*atisan*-19-*oate* (**13**). An EtOH soln (100 ml) of **6** (510 mg) was hydrogenated with 10% Pd/C (60 mg) at room temp. and atm. pres. for 6 hr. to give a 2:1 mixture (508 mg) of C-16 epimers (PMR). After several crystallizations from MeOH, a pure compound (**13**) was isolated. **13**: mp 159–163°. PMR: δ 3.63 (3H, *s*, COOMe), 3.38 (1H, *t*, *J* = 3 Hz, H-7), 0.97 (3H, *d*, *J* = 7 Hz, 3H-17), C-Me singlets at 1.16 (3H-18) and 0.78 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 334 (M^+ 5), 316 (23), 257 (32), 182 (37), 123 (100), 101 (17). $C_{21}H_{34}O_3$ MW 334.

Methyl ent-7-*oxo*-17ξ-*atisan*-19-*oate* (**14**). Compound **13** (240 mg) was treated with excess Jones reagent to yield **14** (230 mg), mp 104–108° (aq. EtOH), $[\alpha]_D^{18°}$ −30°, $[\alpha]_{365}^{18°}$ +71° (*c* 0.43, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1720 (ester), 1693 (ketone). PMR: δ 3.68 (3H, *s*, COOMe), 0.96 (3H, *d*, J = 7 Hz, 3H-17), C-Me singlets at 1.16 (3H-18) and 0.96 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 332 (M^+ 37), 317 (3), 314 (3), 272 (100), 257 (19), 244 (22), 203 (26), 121 (40), 107 (54), 101 (21), 95 (51), 81 (45). $C_{21}H_{32}O_3$ MW 332.

Methyl ent-6α-*bromo*-7-*oxo*-17ξ-*atisan*-19-*oate* (**15**). An HOAc soln of Br_2 (0.036 ml in 10 ml) was added dropwise to compound **14** (216 mg) dissolved in glacial HOAc (10 ml), followed by addition of 5 drops of HBr-saturated HOAc. The reaction was left at room temp. in the dark for 1 week. It was diluted with H_2O (100 ml) and extracted with Et_2O; the Et_2O extract was washed with H_2O, $Na_2S_2O_3$ soln and H_2O. Evapn of the Et_2O gave a residue of **15** (250 mg), mp 100–104° (spontaneously on cooling), $[\alpha]_D^{20°}$ −78° (*c* 0.36, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1720 (ester), 1695 (ketone). PMR: δ 5.24 (1H, *d*, *J* = 6 Hz, H-6), 3.66 (3H, *s*, COOMe), 0.97 (3H, *d*, *J* = 7 Hz, 3H-17), C-Me singlets at 1.40 (3H-18) and 0.59 (3H-20). MS (70 eV, direct inlet) *m/e* (rel. int.): 412 and 410 (M^+ 1.5), 353 and 351 (1), 331 (100), 271 (12), 243 (11), 149 (9), 121 (12), 107 (13), 101 (5), 95 (15), 81 (14), 79 (13). $C_{21}H_{31}O_3Br$ MW 411

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