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A new example of 1α-hydroxylation of drimanic terpenes through combined microbial and chemical processes

Gérard Aranda,[a] Luis Moreno,[b] Manuel Cortés,[b] Thierry Prangé,[c] Michèle Maurs[d] and Robert Azerad[d,*]

[a]*Laboratoire de Synthèse Organique, UMR 7652, Ecole Polytechnique, 91128 Palaiseau Cedex, France*
[b]*Facultad de Química, Pontificia Universidad Católica de Chile, Casilla 306, Correo 22-Santiago, Chile*
[c]*Chimie Structurale Biomoléculaire, UPRES A 7031, UFR Biomédicale, 93017 Bobigny Cedex, France*
[d]*Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR 8601, Université René Descartes-Paris V, 45 rue des Saints-Peres, 75270 Paris 06, France*

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Abstract—Among various filamentous fungi, *Aspergillus niger* ATCC 9142 was the most efficient strain to convert confertifolin in high yield into its 3β-hydroxy derivative, which was then chemically converted to 1α-hydroxy-, 1α,11α-dihydroxy- and 1α-acetoxy-11α-hydroxyconfertifolin, new compounds structurally related to bioactive natural products isolated from plants or marine sponges.

1. Introduction

Microbial hydroxylations of terpenoid compounds[1] have been repeatedly reported and employed for the regioselective functionalization of a number of commonly found sesqui- and diterpenes, representing a unique and inexpensive source of structural diversity. Much attention has been paid to the synthesis of hydroxylated drimanic compounds[2–8] due to their wide range of biological activities and possible industrial applications. The microbial hydroxylation of this type of 4,4-dimethyl sesquiterpenes invariably leads to 3β-hydroxylated derivatives, sometimes naturally found as minor compounds. Hydroxylation at the 1-position, a distinctive feature of several bioactive terpenes, cannot be achieved by this method. However, starting from 3β-hydroxy derivatives, we have previously shown that a simple sequence of reactions can result in a functionalization transfer, allowing the preparation in good yields of the corresponding 1α-hydroxylated compounds.[7,9,10]

In this report, we describe the extension of these reactions to the preparation of 1α-hydroxylated derivatives of

Table 1. 3β-Hydroxylation of confertifolin **1** (0.5 g L^{-1}) upon incubation with some 65 h-grown fungal cultures (27°C, 250 rpm)

Microorganisms	Incubation time (days)	3β-hydroxyconfertifolin **2**[a,b]
Absidia blakesleeana ATCC 6811	8	++
A. niger ATCC 9142	5	++++
Chaetomium indicum LCP 98-4200	3	+++
Cunninghamella echinulata NRRL 3655	3	+++
Cunninghamella elegans ATCC 36112	3	+
Curvularia lunata NRRL 2380	2	+
Mortierella isabellina NRRL 1757	8	++
Mucor plumbeus ATCC 4740	5	+++
Rhizopus arrhizus ATCC 11145	5	+++

[a] GC/MS measurements.
[b] +: 5–15%; ++: 16–40%; +++: 41–65%; ++++: 66–90%

Keywords: confertifolin; biotransformation; microbial hydroxylation; functionalization transfer.
* Corresponding author. Tel.: +33-1-42-86-21-71; fax: +33-1-42-86-83-87; e-mail: robert.azerad@biomedicale.univ-paris5.fr

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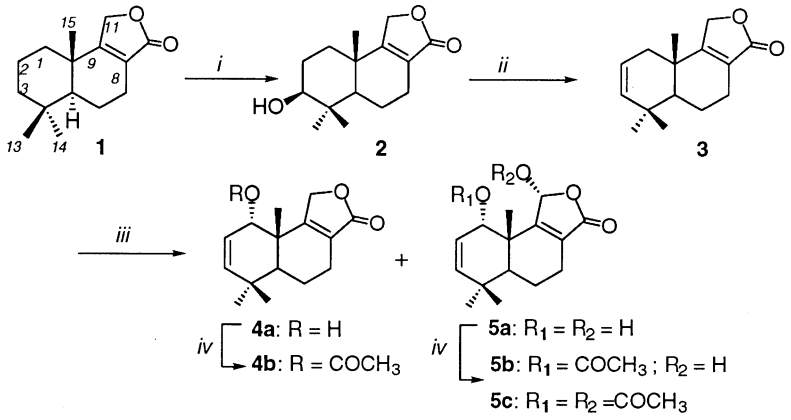


Scheme 1. (i) *A. niger* ATCC 9142. (ii) Ph_3P, DEAD in refluxing THF. (iii) SeO_2, NPO in dioxane, 100°C. (iv) AC_2O, DMAP, 4°C.

confertifolin **1**, a $\Delta^{8\text{-}9}$-drimenic lactone from a South American tree commonly found in Chile and Argentina, *Drimys winteri* Forst.[11]

2. Results and discussion

As a complement to previous studies,[8] a number of fungal or bacterial microorganisms (20 strains) generally employed for terpene metabolism[1] were investigated for the biotransformation of confertifolin. The most significant results are summarized in Table 1. *Aspergillus niger* ATCC 9142 remained the most efficient strain and afforded the corresponding 3β-hydroxylated derivative in 80–90% yield after simple crystallisation from the crude incubation extract.

Application to 3β-hydroxyconfertifolin **2** of the previously described dehydration/oxidation reaction sequence [10] leading to a functionalization transfer to position 1α- afforded (Scheme 1) two hydroxylated derivatives which were more easily separated as their acetate esters. After mild alkaline hydrolysis (K_2CO_3, MeOH), the new alcohols were respectively identified as the 1α-hydroxy-2,3-dehydroconfertifolin **4a** and the 1α,11α-dihydroxy-2,3-dehydroconfertifolin **5a** by high field NMR and mass spectrometry.

The expected 1α-hydroxy derivative **4a** (20–35%, see Table 2) exhibited a broad singlet at 3.87 ppm by ^{1}H NMR, with a characteristic pattern similar to that previously described for 1α-derivatives (an ABX system between 2-, 3- and 1-hydrogens, with a H-1/H-2 coupling constant of 5.7 Hz).[10]

Table 2. Selenium dioxide oxidation of 2,3-dehydroconfertifolin (**3**) (pyridine-*N*-oxide: 7–8 equiv.; dioxane; 100°C)

SeO_2 equivalents	Hours	Conversion %	Total yield (**4a**+**5a**) %	**4a**/**5a** ratio
1.5	48	65	80	43:57
2	67	93	80	26:74

Table 3. ^{13}C Chemical shifts (ppm) of confertifolin derivatives. Unless otherwise indicated, NMR spectra were run in $CDCl_3$ at 50 MHz. Bracketted data indicating the number of hydrogens attached to each carbon atom were determined by distorsionless enhancement by polarization transfer (DEPT) using a flip angle of 135°

Carbon no.	**2**[a]	**3**	**4a**	**4b**	**5a**[b]	**5b**	**5c**	**6**	**7a**	**7b**	**7c**
1	34.2(2)	36.2(2)	69.4(1)	71.5(1)	70.0(1)	71.8(1)	70.9(1)	71.4(1)	71.3(1)	74.7(1)	73.6(1)
2	27.1(2)	119.8(1)	122.6(1)	118.9(1)	123.6(1)	119.2(1)	119.5(1)	25.8(2)	26.2(2)	22.3(2)	22.0(2)
3	78.3(1)	138.7(1)	143.0(1)	144.6(1)	143.0(1)	144.0(1)	144.0(1)	34.5(2)	34.5(2)	35.1(2)	35.0(2)
4	39.0(0)	34.6(0)	35.1(0)	35.0(0)	35.9(0)	35.2(0)	35.2(0)	33.0(0)	33.2(0)	33.4(0)	32.9(0)
5	50.6(1)	48.2(1)	41.3(1)	42.2(1)	42.5(1)	42.7(1)	42.7(1)	43.9(1)	44.2(1)	45.2(1)	45.4(1)
6	18.1(2)	18.9(2)	18.8(2)	18.8(2)	20.0(2)	18.6(2)	18.7(2)	17.8(2)	17.7(2)	17.7(2)	17.9(2)
7	21.6(2)	21.5(2)	21.2(2)	21.3(2)	22.0(2)	21.3(2)	21.7(2)	21.7(2)	21.4(2)	21.6(2)	22.0(2)
8	123.8(0)	124.1(0)	125.9(0)	126.7(0)	131.0(0)	130.6(0)	132.6(0)	124.9(0)	130.6(0)	129.7(0)	132.0(0)
9	170.0(0)	168.5(0)	167.6(0)	165.4(0)	166.9(0)	163.3(0)	161.2(0)	169.8(0)	166.3(0)	164.9(0)	162.9(0)
10	36.4(0)	35.5(0)	41.3(0)	39.7(0)	43.0(0)	40.0(0)	40.5(0)	41.9(0)	43.4(0)	40.8(0)	42.0(0)
11	68.2(2)	68.3(2)	69.2(2)	68.5(2)	99.2(1)	97.3(1)	91.0(1)	68.9(2)	99.9(1)	97.2(1)	90.8(1)
12	174.7(0)	174.1(0)	174.7(0)	174.0(0)	173.6(0)	171.5(0)	170.6(0)	175.0(0)	171.7(0)	171.4(0)	170.5(0)
13	15.6(3)	22.2(3)	21.8(3)	22.0(3)	22.4(3)	22.2(3)	21.9(3)	21.7(3)	21.4(3)	21.5(3)	21.7(3)
14	28.2(3)	31.7(3)	31.4(3)	31.2(3)	31.5(3)	31.3(3)	31.4(3)	33.1(3)	33.1(3)	32.9(3)	33.5(3)
15	20.9(3)	20.6(3)	20.9(3)	21.1(3)	19.6(3)	19.5(3)	20.6(3)	21.3(3)	21.4(3)	21.0(3)	20.6(3)
$COCH_3$	–	–	–	170.5(0)		171.6(0)	169.5(0)			171.3(0)	169.6(0)
	–			21.6(3)		21.3(3)	169.8(0)			21.7(3)	169.9(0)
							21.3(3)				21.5(3)

[a] See Ref. 8.
[b] In CD_3OD (125 MHz).

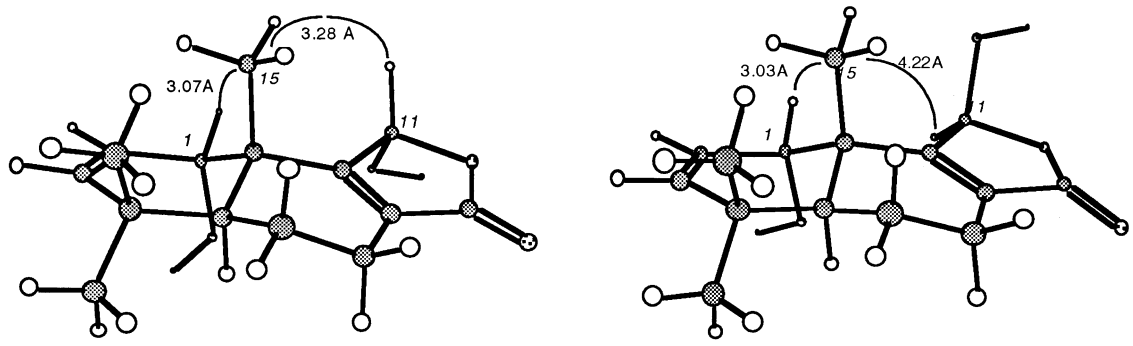


Figure 1. Energy-minimized models for 1α,11α-dihydroxy (left) and 1α,11β-dihydroxy-2,3-dehydroconfertifolin (right).

^{13}C NMR data (Table 3) and heteronuclear 2D studies entirely confirmed this assignement. Acetate **4b** was easily prepared by acetylation. Catalytic hydrogenation (PtO_2 in EtOAc) of **4a** selectively afforded the corresponding 2,3-reduced derivative (1α-hydroxyconfertifolin) **6**. Other catalysts, such as palladium on charcoal or Crabtree's catalyst were ineffective for reducing the $\Delta^{2,3}$ double bond.

Oxidation of 2,3-dehydroconfertifolin **3** with excess selenium dioxide (see Table 2) afforded mainly the unexpected 1α,11α-hydroxy derivative **5a**. The 2,3-dehydro-11-hydroxy derivative was not detected. The assignment of an additional 11α-hydroxylation was based on ^{1}H and ^{13}C NMR data, and particularly on NOESY experiments which showed very similar nuclear Overhauser effects between CH_3-15 and H-11 or CH_3-15 and H-1β, indicating very similar distances. Molecular modelling of 11α- and 11β-hydroxylated derivatives (Fig. 1) showed that this pattern was only compatible with an 11α-hydroxyl group: in minimized models a distance of 3.15±0.1 Å was measured between CH_3-15/H-11β or H-1β for an 11α-OH group, vs CH_3-15/H-11α=4.22 Å and CH_3-15/H-1β=3.03 Å measured for an 11β-OH group.

The additional allylic oxidation by SeO_2 in the lactone ring is strictly dependent on the presence of the 2,3-unsaturation and/or the 1α-hydroxy structure because, in identical reaction conditions, confertifolin remained unchanged. Such 11α-hydroxylated derivatives have been already found as minor natural products such as valdiviolide **8** in various *Drimys* species or fuegine **9** in *Polygonum hydropiper* L., but their stereochemistry at C-11 remains to be clarified.[12–14]

Mild acetylation of the dihydroxy derivative **5a** provided regioselectively the monoacetate **5b** and the diacetate **5c** while catalytic hydrogenation of **5a** (PtO_2 in EtOAc, 15 psi) afforded the corresponding 2,3-reduced derivative (1α,11α-dihydroxy-confertifolin) **7a**. Catalytic hydrogenation of the monoacetate derivative **5b** was not successful, because the 1α-acetyl group was transferred in part to the 11α-position and no pure compound **7b** could be isolated. Hydrogenation of the diacetate **5c** afforded a diacetate **7c**, but this one did not present convincing NMR data for an 11α-(acet)oxy derivative, as previously observed with **5a**. However, an X-ray determination[15] unambiguously demonstrated a 1α,11α-diacetoxy structure (Fig. 2).

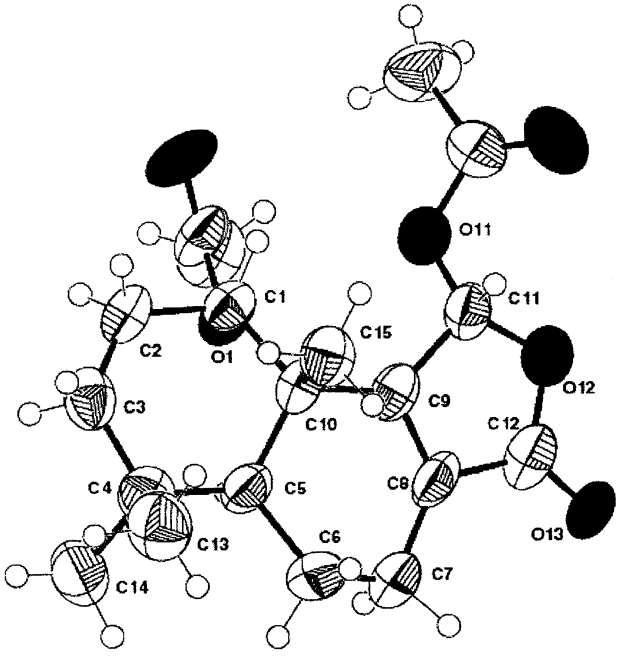
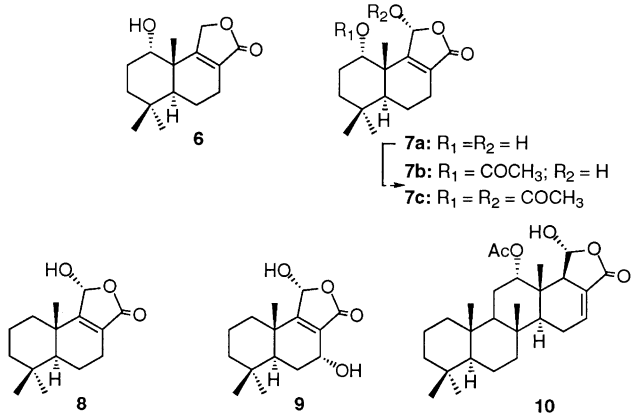


Figure 2. ORTEP representation of **7c** with ellipsoids at 50% probability level. Oxygen atoms are dashed.

3. Conclusions

For the first time, an 11α-hydroxy hemiketal structure in the drimenic lactone series is unambiguously demonstrated and should be compared to previously known natural products, such as valdiviolide, fuegin[12,13] and cinnamolide derivatives.[14]



Moreover, it is of some interest that some of the prepared derivatives and particularly the 2,3-reduced monoacetate **7b** present marked similarities with part of the unusual structures of several cytotoxic sesterpenes of marine sponges,[16–18] such as scalarin **10** for example.

4. Experimental

4.1. General

General experimental methods have been already described.[19] High resolution mass spectrometry (HRMS) was performed on a JEOL MS700 spectrometer. EI- and CIMS were performed on a Hewlett–Packard 5989B instrument. Incubation course was monitored by GC-MS, using a 25 m×0.2 mm Ultra 2 (Hewlett–Packard) capillary column (temp. programmed 110–270° at 8° min^{-1}). Column chromatography was performed on silicagel Merck 60H (70–230 mesh). ^1H NMR and ^{13}C NMR spectra were acquired in $CDCl_3$ or CD_3OD solutions at 200 or 500 and 50 or 125 MHz, respectively.

Acetylations were performed in CH_2Cl_2 at the ice bath temperature with acetic anhydride (3–4 equiv.) in the presence of DMAP (4–5 equiv.). Total conversion was checked by thin layer chromatography. Hydrogenation of 2,3-dehydro derivatives was performed with PtO_2 catalyst in EtOAc (kept on K_2CO_3) at room temperature and atmospheric pressure during 6–8 h.

4.2. Starting material

Pure confertifolin **1** was prepared by vacuum distillation of the natural extract mixture of *D. winteri*, purified by column chromatography (Silicagel, cyclohexane–ethyl ether) and crystallized from CH_2Cl_2–pentane: mp 150–151°C, $[\alpha]_D^{22}=+71.7°$ (*c* 1.83, $CHCl_3$). IR and NMR data were in full agreement with earlier literature.[11,20,21]

Cultivation and incubation of fungal strains with confertifolin **1**, and isolation of 3β-hydroxyconfertifolin **2** have been previously described.[8]

4.2.1. 2,3-Dehydroconfertifolin 3. This compound was prepared, following the earlier described protocol,[9] from 3β-hydroxyconfertifolin **2** (539 mg), triphenylphosphine (2.26 g) and diethyldiazodicarboxylate (1.35 mL) in refluxing anhydrous THF (21.6 mL). After usual work-up, chromatographic purification and two crystallizations from pentane–ethyl ether, dehydroconfertifolin **3** (438 mg, 86%) was obtained: mp 131°C. $[\alpha]_D^{20}=+155°$ (*c* 0.865, $CHCl_3$). HRMS (CI–NH_3) calc. for $C_{15}H_{22}O_2$ (M+1) 233.1542, found 233.1546. IR (CCl_4), ν_{max} (cm^{-1}): 3017, 2963, 2868, 1765, 1676, 1449, 1374, 1344, 1028. ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 0.96, 1.03, 1.17 (9H, 3s, CH_3-15, -14 and -13), 4.70 (2H, AB part of an ABXY system between H_2-11 and H_2-7, J_{AB}=16.9 Hz, J_{AX}=3.6 Hz, J_{AY}=1.5 Hz and $J_{BX}=J_{BY}$=2.7 Hz, H-11α and H-11β), 5.50–5.52 (br.m, 2H, H-2 and H-3). ^{13}C NMR, see Table 3.

4.2.2. 1α-Hydroxy-2,3-dehydroconfertifolin 4a. This compound was prepared from **3** by oxidation with SeO_2 in dry dioxane at 100°C, as previously described.[10] After two crystallizations from pentane–CH_2Cl_2, mp 188–190°C. $[\alpha]_D^{18}=+295°$ (*c* 0.56, $CHCl_3$). HRMS (CI–NH_3) calc. for $C_{15}H_{22}O_3$ (M+1) 249.1491, found 249.1495. IR (CCl_4), ν_{max} (cm^{-1}): 3615, 3446, 3020, 2963, 2868, 1763, 1736 (sh.), 1668, 1388, 1153, 1034. ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 0.96, 1.07, 1.09 (9H, 3s, CH_3-15, -14 and -13), 3.87 (1H, br.s, d after D_2O addition, J_{1-2}=5.2 Hz, H-1β), 4.72, 4.80, 4.94, 5.02 (2H, AB part of an ABXY system, between H_2-11 and H_2-7, J_{AB}=16.5 Hz, $J_{AX}=J_{AY}$=2.7 Hz, J_{BX}=1.4 Hz and J_{BY}=3.5 Hz, H-11α and H-11β), 5.64, 5.69, 5.74, 5.77, 5.78, 5.81 (2H, AB part of an ABX system between H-2, H-3 and H-1β, J_{AB}=10 Hz, J_{AX}=0, J_{BX}=5.7 Hz, H-2 and H-3). ^{13}C NMR, see Table 3.

4.2.3. 1α-Hydroxyconfertifolin 6. 62 mg of **4a** were hydrogenated in the presence of PtO_2 (39 mg) in EtOAc (3.4 mL) during 8 h. After filtration through Celite and two crystallizations from pentane–CH_2Cl_2, mp 185–186.5°C. $[\alpha]_D^{21}=+96°$ (*c* 0.38, $CHCl_3$). HRMS (CI–NH_3) calc. for $C_{15}H_{23}O_3$ (M+1) 251.1647, found 251.1648. IR (CCl_4), ν_{max} (cm^{-1}): 3688, 3608, 3464, 3008, 2943, 2872, 1747, 1674, 1459, 1086, 1028; ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 0.95, 1.02, 1.19 (9H, 3s, CH_3-15, -14 and -13), 3.83 (1H, br.s, H-1β), 4.69, 4.77, 4.94, 5.03 (2H, AB part of an ABXY system, between H_2-11 and H_2-7, J_{AB}=16.6 Hz, $J_{AX}=J_{AY}$=2.7 Hz, J_{BX}=3.4 Hz and J_{BY}=1.5 Hz, H-11α and H-11β). ^{13}C NMR, see Table 3.

4.2.4. 1α-Acetoxy-2,3-dehydroconfertifolin 4b. This compound was obtained by acetylation of **4a**, usual work-up and crystallization from pentane–EtOAc (yield 80–85%): mp 190–200°C dec. $[\alpha]_D^{20}=+358°$ (*c* 0.45, $CHCl_3$). IR (CCl_4), ν_{max} (cm^{-1}): 3026, 2964, 2943, 1767, 1740, 1680, 1449, 1370, 1334, 1028. HRMS (CI–NH_3) calc. for $C_{17}H_{23}O_4$ (M+1) 291.1596, found 291.1594. ^1H NMR (200 MHz, $CDCl_3$) δ ppm: 0.99, 1.10, 1.18 (9H, 3s, CH_3-15, -14 and -13), 1.99 (3H, s, CH_3CO), 4.44, 4.53, 4.72, 4.80 (2H, AB part of an ABXY system, between H_2-11 and H_2-7, J_{AB}=16.7 Hz, J_{AX}=1.3 Hz, J_{AY}=3.7 Hz, and $J_{BY}=J_{BX}$=2.8 Hz, H-11α and H-11β, 4.96 (1H, d, J_{1-2}=4.6 Hz, H-1β), 5.74, 5.79, 5.80, 5.82, 5.87 (2H, complex m, H-2 and H-3). ^{13}C NMR, see Table 3.

From a mixture of **4a**+**5a** (470 mg, 26:74), obtained after oxidation with excess SeO_2 (see Table 2) and acetylated overnight, a chromatographic separation afforded the *monoacetates* **4b** (203 mg) and **5b** (128 mg) and the *diacetate* **5c** (245 mg).

4.2.5. 1α,11α-Dihydroxy-2,3-dehydroconfertifolin 5a. This compound was obtained by mild hydrolysis of **5b** (or **5c**) with K_2CO_3 in aqueous MeOH. After two crystallizations from EtOAc–pentane, mp 179–180°C; $[\alpha]_D^{21}=+321°$ (*c* 0.35, CH_3OH). HRMS (CI–NH_3) calc. for $C_{15}H_{24}O_4N$ (M+18) 282.1704, found 282.1705. ^1H NMR (500 MHz, CD_3OD) δ ppm: 0.99, 1.09, 1.19 (9H, 3s, CH_3-15, -14 and -13), 1.93 (2H, s+dd, *J*=6 and 12 Hz, H-5 and H-6α), 1.65 (1H, m, H-6β), 2.13 (1H, m, H-7α), 4.08 (1H, br.s, H-1β), 2.39 (1H, dd, $J_{7\beta-7\alpha}$=18 Hz, $J_{7\beta-6\alpha}=J_{7\beta-6\beta}$=5 Hz, H-7β), 5.66, 5.68, 5.72, 5.73, 5.74, 5.75 (2H, AB part of an ABX system between H-2, H-3 and H-1β, J_{AB}=10 Hz, J_{AX}=0,

J_{BX}=5 Hz, H-2 and H-3), 6.30 (1H, s, H-11β). ^{13}C NMR, see Table 3.

4.2.6. 1α-Acetoxy-11α-hydroxy-2,3-dehydroconfertifolin 5b. After crystallization from pentane–EtOAc, mp 183–186°C. $[\alpha]_D^{22}$=+307° (*c* 0.305, $CHCl_3$). HRMS (CI–NH_3) calc. for $C_{17}H_{26}O_5$ (M+1) 324.1811, found 324.1804. IR (CCl_4), ν_{max} (cm^{-1}): 3027, 3013, 2986, 2931, 1760, 1728, 1602, 1465, 1422, 1370, 1264, 1083, 1019. ^{1}H NMR (200 MHz, CD_3OD) δ ppm: 0.96, 1.04, 1.26 (9H, 3s, CH_3-15, -14 and -13), 1.98 (3H, s, CH_3CO), 5.11 (1H, d, $J_{1\text{-}2}$=5.3 Hz, H-1β), 5.68, 5.73, 5.76, 5.79, 5.81, 5.84, 5.91 (2H, AB part of an ABX system, between H-2, H-3 and H-1β, J_{AB}=10 Hz, J_{AX}=0, and J_{BX}=5.3 Hz, H-2 and H-3), 6.0 (1H, br.s, H-11β). ^{13}C NMR, see Table 3.

4.2.7. 1α,11α-Diacetoxy-2,3-dehydroconfertifolin 5c. After two crystallizations from pentane–CH_2Cl_2, mp 142–145°C. $[\alpha]_D^{20}$=+238° (*c* 0.5, $CHCl_3$). IR (CCl_4), ν_{max} (cm^{-1}): 2979, 2927, 2854, 1786, 1736, 1371, 1242, 1204, 1075, 1018, 985. HRMS (CI–NH_3) calc. for $C_{19}H_{24}O_6$ (M^+) 348.1573, found 348.1577. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.96, 1.08, 1.18 (9H, 3s, CH_3-15, -14 and -13), 1.98 and 2.09 (6H, 2s, $COCH_3$), 4.85 (1H, d, $J_{1\text{-}2}$=5.4 Hz, H-1β), 5.70, 5.75, 5.79, 5.81, 5.83, 5.86 (2H, AB part of an ABX system, between H-2, H-3 and H-1β, J_{AB}=9.8 Hz, J_{AX}=0, and J_{BX}=5.4 Hz, H-2 and H-3), 6.97 (1H, t, *J*=0.75 Hz, H-11β). ^{13}C NMR, see Table 3.

4.2.8. 1α,11α-Dihydroxyconfertifolin 7a. 68 mg of **5a** were hydrogenated in the presence of PtO_2 (49 mg) in EtOAc (4.5 mL) during 6.5 h. After filtration through Celite and two crystallizations from EtOAc–pentane, mp 152–154°C. $[\alpha]_D^{20}$=+100° (*c* 0.95, CH_3OH). HRMS (CI–NH_3) calc. for $C_{15}H_{23}O_4$ (M+1) 267.1596, found 267.1593. ^{1}H NMR (500 MHz, CD_3OD) δ ppm: 0.95, 0.98, 1.25 (9H, 3s, CH_3-15, -14 and -13), 1.21 and 1.74 (3H, 2m, H_2-3 and H-5), 1.61 and 2.04 (2H, 2m, H_2-3), 1.66 (1H, m, H-6), 1.92 (1H, m, H-6), 2.12 (1H, m, H-7α), 2.33 (1H, dd, $J_{7\beta\text{-}7\alpha}$=18 Hz, $J_{7\beta\text{-}6\alpha}$=$J_{7\beta\text{-}6\beta}$=5.8 Hz, H-7β), 4.05 (1H, br.s, H-1β), 6.30 (1H, br.s, H-11β). ^{13}C NMR, see Table 3.

4.2.9. 1α-Acetoxy,11α-hydroxyconfertifolin 7b. Hydrogenation of **5b** (50 mg) in EtOAc (3.5 mL) in the presence of PtO_2 (45 mg) during 5 h provided quantitatively a 3:1 mixture of 1α-acetoxy,11α-hydroxy and 1α-hydroxy,11α-acetoxy derivatives which were not separated by TLC. Despite repeated crystallization (CH_2Cl_2–pentane), a pure sample of the major constituent (**7b**) could not be obtained. HRMS (CI–NH_3) calc. for $C_{17}H_{25}O_5$ (M+1) 309.1702, found 309.1696. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.94, 0.98, 1.33 (9H, 3s, CH_3-15, -14 and -13), 2.07 (3H, s, $COCH_3$), 5.19 (1H, t, *J*=2.8 Hz, H-1β), 5.84 (1H, d, *J*=1.85 Hz, H-11β). ^{13}C NMR, see Table 3.

4.2.10. 1α,11α-Diacetoxyconfertifolin 7c. Hydrogenation of **5c** (66 mg) in EtOAc (4.5 mL) in the presence of PtO_2 (45 mg) during 4 h provided quantitatively **7c**. After crystallization from CH_2Cl_2–pentane, mp 156–157°C. $[\alpha]_D^{20}$=+15.8° (*c* 0.228, $CHCl_3$). IR (CCl_4), ν_{max} (cm^{-1}): 2957, 2933, 1784, 1735, 1374, 1248, 1203, 1046, 1012, 984. HRMS (CI–NH_3) calc. for $C_{19}H_{27}O_6$ (M+1) 351.1806, found 351.1802. ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 0.93, 1.00, 1.24 (9H, 3s, CH_3-15, -14 and -13), 2.05 and 2.09 (6H, 2s, $COCH_3$), 4.77 (1H, t, *J*=2.7 Hz, H-1β), 6.94 (1H, t, *J*=1.0 Hz, H-11β). ^{13}C NMR, see Table 3.

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15. **7c** was crystallized by slow evaporation of a methanol solution. A crystal 0.1×0.3×0.4 mm^3 in size was recorded on a PHILIPS PW1100 diffractometer operating the CuKα radiation. Space group *P*21. Parameters are *a*=8.501(3); *b*=13.979(4); *c*=7.847(2) Å; β=90.26(7)° and *Z*=2. The structure was solved by direct methods and refined with anisotropic thermal factors (except for hydrogens) to a final *R*=6.9% for 1325 observed structure factors. The coordinates have been deposited with the Cambridge Crystallographic Data Centre (CCDC 157588).
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