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LATHYRANE TYPE DITERPENOID ESTERS FROM *EUPHORBIA CHARACIAS*

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Key Word Index—*Euphorbia characias*; Euphorbiaceae; lathyrane type diterpenes; jolkinol esters; skin irritant; tumor promotion.

Abstract—From the irritant acetone extract of latex of *Euphorbia characias* six macrocyclic diterpene esters of the following lathyrane type were obtained. The compounds isolated were shown by physico-chemical methods to be five new diesters of the parent alcohol jolkinol-5β,6β-oxide, and one triester of the new parent alcohol 2α-hydroxyjolkinol-5β,6β-oxide. All compounds were practically inactive as irritants.

INTRODUCTION

Plants of the families Euphorbiaceae and Thymelaeaceae are known to contain toxic, skin irritant and tumor promoting principles [1, 2]. All of them represent tri- and tetra-cyclic diterpene skeletons of either the tigliane type (e.g. phorbol esters), the ingenane type (e.g. ingenol esters), or the daphnane type (e.g. resiniferonol esters). From some species, macrocyclic diterpenoids belonging to the casbane, jatrophane and lathyrane types have also been isolated. Casbane and jatrophane are the bicyclic carbon skeletons of crotonitenone [3] and jatrophone [4], respectively, and lathyrane is the tricyclic carbon skeleton of 7-hydroxylathyrol [5], ingol [6], and jolkinols A–D [7]. The macrocyclic diterpene parents may be considered the biogenetic precursors of tigliane, ingenane and daphnane derivatives [8, 9]. In contrast to the esters of the tigliane, ingenane and daphnane type, the derivatives of casbane, jatrophane and lathyrane skeletons as tested so far—be they esters or not—are inactive as irritants or as tumor promoters. But some of them, e.g. jatrophone, exhibit weak antileukemic activity [4].

E. characias L., widespread in Mediterranean countries, is known to cause irritation and blistering of human skin [10]. During our investigation of latex and of roots of *E. characias* 14 macrocyclic diterpenoids have been isolated in addition to irritant and tumor promoting principles [11]. Here we report on six of these compounds derived from the lathyrane skeleton.

RESULTS AND DISCUSSION

The irritant and tumor promoting acetone extract of latex of *E. characias* [11] was subjected to two Craig distributions to yield a hydrophilic portion. This material was further separated by means of a third Craig distribution to yield several irritant fractions, from which six new macrocyclic compounds, **1b–1f** and **2b** (Table 1), were isolated by prep. TLC.

Jolkinol-5β,6β-oxide (**1a**) *and the* 3,15-*diesters* **1b–1f**

The diterpene moiety of compounds **1b–1f** is, with reference to spectroscopic data, closely related to that of jolkinol B (**1g**) from *E. jolkini* [7]. Characteristic features are the chemical shifts and/or multiplicity of H_a-1, H-3, H-5, H-12, and H_3-20 in the ^{1}H NMR spectra (Table 2), established by decoupling experiments for **1c** (Table 4), and especially the typical UV absorption band at *ca* 270 nm (Table 3) corresponding to the β-cyclopropyl enone in **1a** as reported also for derivatives of lathyrol (**3**) [5, 12] or for crotonitenone [3]. The parent alcohol, jolkinol-5β,6β-oxide* (**1a**), is obtained by base-catalysed transesterification of **1c**.

The mass spectra of **1b–1f** have similar fragmentation patterns and indicate diacylates of the diterpene moiety $C_{20}H_{30}O_4$ (high resolution), with acetic acid common to all, and a variable second acid moiety. These acid moieties in **1b–1f** are identified as propionic, isobutyric, tiglic, benzoic and nicotinic acid, respectively. Different chemical shifts of H-3 in the ^{1}H NMR spectra (Table 2) point to a variable acid moiety at C-3 and the acetic acid moiety at C-15. This is proved for **1c** by partial transesterification yielding the 3-*iso*-butyrate **1h** (missing signal of the acetyl group, same chemical shift of H-3 as in **1c**). Hence, the structure of **1c** is 15-*O*-acetyl-3-*O*-*iso*-butyryljolkinol-5β,6β-oxide. The structures of **1b**, and **1d–1f** are, therefore, deduced to be the 3-*O*-propionyl, 3-*O*-tigloyl, 3-*O*-benzoyl and 3-*O*-nicotinoyl derivatives, respectively, of 15-*O*-acetyljolkinol-5β,6β-oxide (**1i**).

Isomerization of jolkinol-5β,6β-oxide (**1a**), *isolathyrol* (**4a**) *and its esters*

Acidic treatment of **1c** furnishes two products, **4b** and **4c**, with identical molecular formula (high resolution mass spectrometry) as the starting material but bearing a

*To simplify nomenclature of these macrocyclic diterpenes we propose, in analogy to lathyrol nomenclature, the name jolkinol for the diterpene moiety of jolkinol D, because it contains the least double bond equivalents and the smallest number of oxygen atoms in the series of closely related jolkinols A–D [7]; hence, jolkinol-5β,6β-oxide (**1a**) is the parent of jolkinol B (**1g**).

Table 1. Separation of the hydrophilic portion of latex of *E. characias* into fractions by Craig distribution and some characteristic data of the isolated compounds **1b–1f** and **2b**

Fraction	r (elements)	Compound*	Yield† (%)	$[M]^+$ ion (m/z)	Formula	TLC†† R_f‡‡	TLC†† R_f§§
6	225–248	**2b**	0.011	567.2834‡	$C_{32}H_{41}NO_8$§	0.15	0.20
8	281–304	**1f**	0.014	481.2460‡	$C_{28}H_{35}NO_6$‖	0.11	0.23
12	425–464	**1b**	0.030	432.2507‡	$C_{25}H_{36}O_6$¶	0.44	0.63
14	521–548	**1c**	0.053	446.2663‡	$C_{26}H_{38}O_6$**	0.49	0.67
		1d	0.023	458	—	0.48	0.66
		1e	0.006	480	—	0.50	0.71

*All compounds were practically inactive as irritants (IU > 25 μg/ear) compared to the tigliane derivative 12-*O*-tetradecanoylphorbol-13-acetate (TPA), IU: 0.05 μg/ear, ID_{50}^{24}: 0.01 μg/ear [16].

†Acetone extract: 100%.

‡By high resolution mass spectrometry.

§Calculated for 567.2832.

‖Calculated for 481.2464.

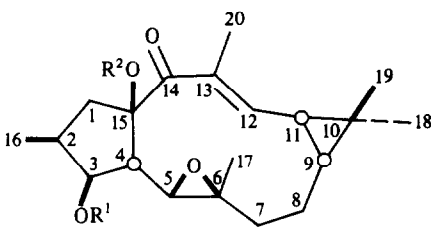
¶Calculated for 432.2512.

**Calculated for 446.2667.

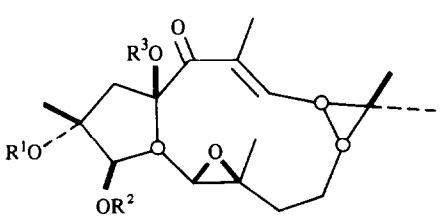
††Staining with vanillin–sulfuric acid reagent: **1b–1f**, red-brown; **2b**, brown.

‡‡Et_2O–petrol (6:1).

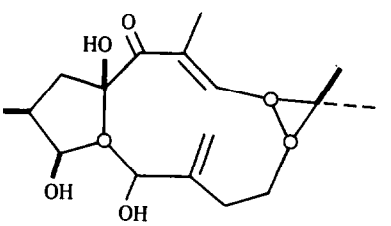
§§CH_2Cl_2–Me_2CO (12:1).



1a $R^1 = R^2 = H$
1b $R^1 = COCH_2Me$, $R^2 = COMe$
1c $R^1 = COCHMe_2$, $R^2 = COMe$
1d $R^1 = COC(Me) \overset{E}{=} CHMe$, $R^2 = COMe$
1e $R^1 = COC_6H_5$, $R^2 = COMe$
1f $R^1 = COC_5H_4N$, $R^2 = COMe$
1g $R^1 = H$, $R^2 = COCH \overset{E}{=} CHC_6H_5$
1h $R^1 = COCHMe_2$, $R^2 = H$
1i $R^1 = H$, $R^2 = COMe$



2a $R^1 = R^2 = R^3 = H$
2b R^1, R^2, $R^3 = COMe$, $COCHMe_2$, COC_5H_4N



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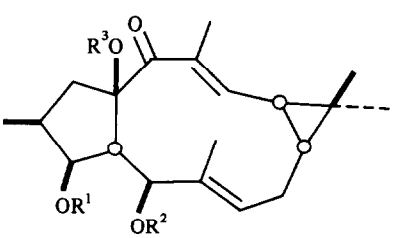

4a $R^1 = R^2 = R^3 = H$
4b $R^1 = COCHMe_2$, $R^2 = H$, $R^3 = COMe$
4c $R^1 = H$, $R^2 = COCHMe_2$, $R^3 = COMe$
4d $R^1 = R^3 = H$, $R^2 = COMe$
4e $R^1 = R^2 = COMe$, $R^3 = H$
4f $R^1 = COCHMe_2$, $R^2 = R^3 = COMe$
4g $R^1 = R^3 = COMe$, $R^2 = COCHMe_2$

hydroxyl. In the ^1H NMR spectrum of **4b**, a doublet at δ 4.95 coupling with H-4 is apparent which is shifted to 6.22 after acetylation, yielding **4f**. Additionally, an olefinic proton is present at *ca* δ 5.2 coupling with a vinylic methyl group at 1.55. These findings are in agreement with a double bond between C-6 and C-7 (configuration remains to be established) and a free hydroxyl group at C-5, which could be formed under acidic conditions via a carbonium ion at C-6, followed by hydrogen abstraction.

In **4c** the doublet of H-5 appears at δ 6.35 indicating esterification of the new hydroxyl group. This can only be explained by acyl migration of the acid moiety from OH-3

Table 2. ^{1}H NMR spectral data of compounds **1b–1f** and **2b** (90 MHz spectra in $CDCl_3$, TMS as internal standard, δ)

Diterpene moiety	Chemical shift, multiplicity [coupling constant (Hz)]					
	1b	**1c**	**1d**	**1e**	**1f**	**2b**
H-12	6.97 *br d* (11)	6.93 *br d* (11)	6.99 *br d* (10)	6.96 *dd* (1.5, 11)	6.94 *dd* (1.5, 11)	6.84 *br d* (11)
H-3	5.43 *dd* (4, 4)	5.37 *dd* (3.5, 3.5)	5.52 *dd* (4, 4)	5.68 *dd* (4, 4)	5.67 *dd* (4, 4)	5.58 *dd* (1.5, 4)
H_a-1	3.52 *dd* (7, 13)	3.52 *dd* (7, 14)	3.59 *dd* (7, 14)	3.64 *dd* (7, 14)	3.63 *dd* (7, 13)	4.48 *dd* (1.5, 16)
H-5	3.26 *d* (9)	3.26 *d* (9)	3.26 *d* (9)	3.27 *d* (9)	3.28 *d* (9)	3.27 *d* (9)
H_3-20	1.90 *s*	1.89 *m*	1.91 *br s*	1.92 *d* (1.5)	1.91 *d* (1.5)	1.69 *s*
H_3-16	0.95 *d* (7)	0.94 *d* (6)	0.95 *d* (7)	1.02 *d* (7)	1.01 *d* (7)	1.69 *s*
Acid moieties*	2.46 *q*, 1.23 *t* (p); 2.10 *s* (a)	2.68 *sept.*, 1.24 *d* (i); 2.09 *s* (a)	6.95 *m*, 1.9 *m* (t); 2.10 *s* (a)	8.2-8.0 *m*, 7.7-7.3 *m* (b); 2.19 *s* (a)	9.25, 8.78, 8.34, 7.41 (n); 2.19 *s* (a)	8.96, 8.69, 8.17, 7.31 (n); 2.71 *sept.*, 1.24 *d* (i); 2.10 *s* (a)

*Abbreviations: (a) acetic acid; (p) propionic acid; (i) *iso*-butyric acid; (t) tiglic acid; (b) benzoic acid; (n) nicotinic acid.

Table 3. UV and IR spectral data of compounds **1b–1f** and **2b**

	1b	**1c**	**1d**	**1e**	**1f**	**2b**
UV(MeOH):	268 (14 020)	267 (15 540)	268 (15 430)	270 (9100)	265 (14 200)	267 (12 620)
λ_{max} (nm) (ε)	—	—	215 (12 420)	228 (12 100)	219 (10 740)	218 (10 750)
λ (nm) (ε)	193 (6530)	193 (5930)	193 (11 270)	195 (32 040)	193 (18 590)	194 (17 080)
IR: wave numbers (cm^{-1})	1735, 1660, 1625	1730, 1650, 1620	1735, 1705, 1650, 1620	1735, 1655, 1625, 1600	1725, 1715, 1625, 1585	1735, 1725, 1655, 1620, 1585

Table 4. ^{1}H NMR decoupling experiments with compounds **1c** and **2b**, isolated, and with reaction product **4b**

Compound	Irradiation at ppm	proton	Observed at ppm	proton	Change of signal(s)
1c	6.96	H-12	1.91	H_3-20	sharpening
	5.41	H-3	1.69	H-4	*dd* ⟶ *d*
	3.27	H-5	1.69	H-4	*dd* ⟶ *d*
	1.91	H_3-20	6.96	H-12	sharpening
	1.69	H-4	5.41	H-3	*dd* ⟶ *d*
			3.27	H-5	*d* ⟶ *s*
2b	5.58	H-3	4.48	H_a-1	*dd* ⟶ *d*
			2.15	H-4	*dd* ⟶ *d*
	4.48	H_a-1	5.58	H-3	*dd* ⟶ *d*
	2.16	H-4	5.58	H-3	*dd* ⟶ *d*
			3.27	H-5	*d* ⟶ *s*
4b	5.62	H-3	2.38	H-4	*dd* ⟶ *d*
	5.18	H-7	1.56	H_3-17	sharpening
	4.97	H-5	2.38	H-4	*dd* ⟶ *d*
	2.38	H-4	5.62	H-3	*dd* ⟶ *s**
			4.97	H-5	*d* ⟶ *s*
	1.56	H_3-17	5.18	H-7	sharpening

*Irradiation nearby the frequency of H-2.

in **4b** to OH-5 in **4c** under acidic conditions. All decoupling experiments with **4b** and **4c** are in agreement with these results (see also Table 4).

The diterpene **4a** ($C_{20}H_{30}O_4$ by high resolution mass spectrometry) can be obtained by transesterification of **4b** or **4c**, from which the 5-monoacetate **4d** is derived in good yield and the 3,5-diacetate **4e** in low yield by treatment with acetic anhydride–pyridine. This different reactivity of OH-3 and OH-5 can also be observed during the acetylation of **4b** and **4c**. The acetate **4f** can be obtained from **4b** with acetic anhydride–pyridine; the acetylation of **4c** is only successful with acetic anhydride–4-(*N*,*N*-dimethylamino)pyridine and longer reaction times, yielding a mixture of **4f** and **4g** (indicated by ^{1}H NMR, signals of H_a-1,

H-3 and H-5 appear twice). Obviously, acyl migration takes place under these conditions as well, affording the unexpected acetate **4f**.

The diterpene moiety **4a** was also obtained from jolkinol B (**1g**) by treatment with neutral alumina at 60° [7]; a second minor product of this reaction proved to be identical with lathyrol (**3**). In the structure elucidation of jolkinols A–D [7], for the OH-5 in lathyrol (**3**) the α-position was erroneously assumed. Therefore, the α-position of the epoxide was derived for jolkinol B (**1g**). By X-ray structure analysis of acylates of 7-hydroxylathyrol or of 6,20-epoxylathyrol, the β-position of the hydroxyl at C-5 was unequivocally established [5, 12]. For this reason the epoxide oxygen of **1b–1f** and of jolkinol B (**1g**) should be in the β-position of the molecule, if the configuration at C-5 is not changed during the isomerization. The diterpene **4a** may, therefore, be named isolathyrol.

2α-Hydroxyjolkinol-5β,6β-oxide (**2a**) *and the* 2,3,15-*triester* **2b**

Compound **2b** exhibits the same UV extinction at *ca* 270 nm as the other compounds isolated (Table 3). The molecular formula, $C_{32}H_{41}NO_8$ (high resolution mass spectrometry), and the fragmentation pattern indicate the presence of acetic, *iso*-butyric and nicotinic acids (confirmed by ^{1}H NMR data) and of a diterpene moiety with the formula $C_{20}H_{30}O_5$, one oxygen more than in **1a**. ^{1}H NMR data indicate an additional acyloxy group at C-2, since the doublet of H_3-16 at *ca* δ 1 is missing (Table 2). The broad singlet of six protons at δ 1.69 is caused by the allylic H_3-20 and the tertiary H_3-16. This new acyloxy group causes additionally a paramagnetic shift for H_a-1 of *ca* δ 0.9, but it has only a small influence on the chemical shift of H-3. H_a-1 and H-3 show a common long-range coupling, which is confirmed by decoupling experiments (Table 4). All other data correspond to those of **1a**. Compound **2b** is, therefore, recognized as a 2,3,15-*O*-acetyl, *iso*-butyryl, nicotinoyl derivative of 2α-hydroxyjolkinol-5β,6β-oxide (**2a**); the esterification type of **2b** could, however, not be determined due to lack of material.

Compounds **1b–1f** and **2b** are inactive as irritants on the mouse ear (Table 1) as has been found for other macrocyclic diterpenoids isolated from Euphorbiaceae. Their occurrence, as well as the existence of derivatives with the tigliane and daphnane skeletons in *E. characias* [11], support their suspected biogenetic derivation from casbane type compounds [9].

EXPERIMENTAL

Material. Latex of *Euphorbia characias* was collected in June 1977 in Ardèche valley, southern France, and preserved under an equal vol. of MeOH until used. Identification of the plant was confirmed by Dr. H.-F. Schölch, Institut für systematische Botanik und Pflanzengeographie, Universität Heidelberg.

Biological assay. Irritant units (IU) and irritant doses 50 (ID_{50}^{24}) were determined according to the standard procedure [13].

Spectra. Mass spectra were measured at 100 eV. UV spectra were obtained in MeOH soln. 90 MHz ^{1}H NMR spectra were recorded in $CDCl_3$ solns with TMS as int. standard.

Separation. The MeOH–H_2O mixture of *ca* 400 ml of latex was decanted and the remaining solid mass was extracted × 4 with 1 l. Me_2CO. The combined liquids yielded an Me_2CO extract (98 g, ID_{50}^{24}: 3.2 μg/ear) after evaporation. The extract (75 g) was subjected to two Craig distributions [14, 15] in systems petrol–MeOH–H_2O (30:20:1) and CCl_4–MeOH–H_2O (40:20:3) carrying out $n = 65$ and $n = 36$ transfers, respectively ($z = 30$ elements, $V = 100$ ml/100 ml). In the first procedure hydrophobic parts and in the second, strong hydrophilic parts were separated, thus, yielding a hydrophilic portion (15.2 g, 20.2%). This material (13.5 g) was subjected to a third Craig distribution in petrol–MeOH–H_2O (30:20:1) ($z = 1020$ elements, $V = 10$ ml/10 ml, single withdrawal procedure, $n = 4000$ transfers). According to their composition by TLC the contents of the tubes were combined to give fractions from which by prep. TLC in several solvent systems the compounds **1b–1f** and **2b** were isolated (Table 1).

Spectroscopic data. The spectroscopic data (^{1}H NMR, IR, UV) of compounds **1b–1f** and **2b** are summarized in Tables 2 and 3. NMR decoupling expts were carried out with **1c** and **2b** (Table 4). ^{13}C NMR of **1c**: δ 194.91 (*s*, C-14), 175.73 (*s*, –COOR), 169.43 (*s*, –COOR), 144.21 (*d*, C-12), 134.46 (*s*, C-13), 91.64 (*s*, C-15), 79.55 (*d*, C-3), 63.24 (*s*, C-6), 57.19 (*d*, C-5), 51.08 (*d*, C-4), 45.95 (*t*, C-1), 38.68 (*t*), 37.95 (*t*), 34.51 (*d*), 33.86 (*t*), 29.83 (*t*), 29.12 (*t*), 26.26 (*s*, C-10), 23.33 (*q*), 21.32 (*q*), 19.89 (*q*), 19.24 (*q*), 19.11 (*q*), 16.38 (*q*), 13.52 (*q*) and 12.41 (*q*).

Reaction of **1c** *with* NaOMe–MeOH. (a) Compound **1c** (12 mg) was treated with 0.1 M NaOMe–MeOH (2 ml) for 5 hr at room temp. Usual work-up (Pi buffer, pH *ca* 7, extraction with EtOAc, drying with $MgSO_4$ and concn) and prep. TLC in Et_2O–petrol (3:1) yielded 5 mg **1h** (R_f 0.50) and 2 mg **1c** (R_f 0.42). 3-*O*-*iso*-butyryljolkinol-5β,6β-oxide (**1h**), ^{1}H NMR: δ 7.66 (*dd*, $J = 1.5$, 11 Hz, H-12), 5.41 (*dd*, $J = 4$, 4 Hz, H-3), 3.39 (*dd*, $J = 8$, 13 Hz, H_a-1), 3.20 (*d*, $J = 9$ Hz, H-5), 1.88 (*d*, $J = 1.5$ Hz, H_3-20), 0.97 (*d*, $J = 6$ Hz, H_3-16), 2.18 (*s*, OH), 2.68 (*sept.*, $J = 7$ Hz, $COCHMe_2$); MS *m/z*: 404 $[M]^+$, 361, 343, 316, 298, 273. 255; UV λ_{max} nm (ε): 269 (16070), 192 (6100); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3680, 3590, 1735, 1640, 1625. (b) Compound **1c** (12 mg) was treated with 1 M NaOMe–MeOH (2 ml) for 23 hr at room temp. (work-up as above) to yield jolkinol-5β,6β-oxide (**1a**, 8 mg, 89%), R_f 0.40 in Et_2O–petrol (2:1); ^{1}H NMR: δ 7.72 (*dd*, $J = 1.5$, 11 Hz, H-12), 4.12 (*dd*, $J = 3.5$, 3.5 Hz, H-3), 3.45 (*d*, $J = 10$ Hz, H-5), 3.35 (*dd*, $J = 8$, 13 Hz, H_a-1), 1.87 (*d*, $J = 1.5$ Hz, H_3-20), 1.10 (*d*, $J = 7$ Hz, H_3-16), 2.96 (*s*, OH), 2.5–2.2 (OH); MS *m/z*: 334.2142 $[M]^+$ ($C_{20}H_{30}O_4$ calcd for 334.2144), 319, 316, 306, 301, 298, 292, 291, 273; UV λ_{max} nm (ε): 270 (15250), 192 (5460); IR $\nu_{max}^{CD_2Cl_2}$ cm^{-1}: 3610, 3600–3300, 1650, 1625.

Reaction of **1c** with acidic solvents. Compound **1c** dissolved in EtOAc or $CHCl_3$ and treated with *p*-toluenesulfonic acid monohydrate reacted to yield two products with lower R_f values than the starting material. By prep. TLC in Et_2O–petrol (2:1) unchanged **1c** was obtained from the zone R_f 0.32, the reaction products (staining grey-brown) were isolated together from the zone R_f 0.23–0.29. The latter were separated by prep. TLC in cyclohexane–Me_2CO (2:1). (a) 15-*O*-Acetyl-3-*O*-*iso*-butyryliso-lathyrol (**4b**) (R_f 0.28); MS *m/z*: 446.2670 $[M]^+$ ($C_{26}H_{38}O_6$ calcd for 446.2668), 428, 404, 386, 358, 316, 315, 303, 299, 298, 283, 255; ^{1}H NMR: δ 6.46 (*br d*, $J = 11$ Hz, H-12), 5.62 (*dd*, $J = 4$, 4 Hz, H-3), 5.18 (*br t*, $J = 9$ Hz, H-7), 4.97 (*br d*, $J = 9$ Hz, H-5), 3.38 (*dd*, $J = 9$, 15 Hz, H_a-1), 2.39 (*dd*, $J = 4$, 9 Hz, H-4), 2.35 (*m*, H-8), 1.76 (*s*, H_3-20), 1.56 (*s*, H_3-17), 1.38 (*d*, $J = 11$ z, H-11), 0.92 (*d*, $J = 7$ Hz, H_3-16), 2.65 (*sept.*, $J = 7$ Hz, $COCHMe_2$), 2.09 (*s*, acetate), for decoupling expts see Table 4; IR ν_{max}^{KBr} cm^{-1}: 3470, 1735, 1715, 1645, 1625; UV λ_{max} nm (ε): 278 (11260), 196 (13110). (b) 15-*O*-Acetyl-5-*O*-*iso*-butyrylisolathyrol (**4c**) (R_f 0.38); MS *m/z*: 446.2671 $[M]^+$ ($C_{26}H_{38}O_6$ calcd for 446.2668), 403, 386, 358, 316, 315, 299, 298, 297, 283, 255; ^{1}H NMR: δ 6.55 (*br d*, $J = 12$ Hz, H-12), 6.36 (*d*, $J = 8$ Hz, H-5), 5.22 (*dd*, $J = 5$, 12 Hz, H-7), 4.28 (*dd*, $J = 4$, 4 Hz, H-3), 3.32 (*dd*, $J = 8$, 14 Hz, H_a-1), 2.71 (*m*, H-8), 2.38 (*dd*, $J = 4$, 8 Hz, H-4), 2.11 (*m*, H-2), 1.76 (*s*, H_3-20).

1.56 (*s*, H_3-17), 1.46 (*m*, H-11), 1.06 (*d*, J = 7 Hz, H_3-16), 2.05–1.90 (OH), 2.53 (*sept.*, J = 7 Hz, $COCHMe_2$), 2.11 (*s*, acetate); IR ν_{max}^{KBr} cm^{-1}: 3560, 1730, 1635, 1610; UV λ_{max} nm (ε): 277 (10 980), 193 (11 780).

Reaction of **4b** *and* **4c** *with* NaOMe–MeOH. Both **4b** and **4c** were treated with 0.1 M NaOMe–MeOH for 6 hr to yield, after prep. TLC in CH_2Cl_2–Me_2CO (4:1), *ca* 70% isolathyrol (6,20-dihydro-6,7-dehydrolathyrol, **4a**, R_f 0.40); MS *m/z*: 334.2138 $[M]^+$ ($C_{20}H_{30}O_4$ calcd for 334.2144), 316, 298, 283, 273, 255, 245, 243; ^1H NMR: δ 7.53 (*dd*, J = 1.5, 11 Hz, H-12), 5.46 (*m*, H-7), 5.21 (*d*, J = 9 Hz, H-5), 4.50 (*dd*, J = 4, 4 Hz, H-3), 3.26 (*dd*, J = 10, 14 Hz, H_a-1), 1.76 (*d*, J = 1.5 Hz, H_3-20), 1.61 (*s*, H_3-17), 1.27 (*s*) and 1.19 (*s*, H_3-18 and H_3-19), 1.14 (*d*, J = 6 Hz, H_3-16), 3.78 (*s*, OH), 2.9 (*br*, OH), 1.8 (*br*, OH); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3580, 3600–3300, 1710, 1615; UV λ_{max} nm^{-1} (ε): 281 nm (11 130), 195 (12 150).

Acetylation of isolathyrol (**4a**). Compound **4a** (12 mg) was treated with Ac_2O–pyridine (1:2) (1 ml) at room temp. for 1 day to yield, after work-up and prep. TLC in CH_2Cl_2–Me_2CO (6:1), two products with higher R_f values. (a) 5-*O*-Acetylisolathyrol (**4d**, 89%, R_f 0.58); MS *m/z*: 376.2243 $[M]^+$, ($C_{22}H_{32}O_5$ calcd for 376.2250), 358, 316, 299, 298, 288, 283, 273, 255; ^1H NMR: δ 7.84 (*dd*, J = 1.5, 11 Hz, H-12), 6.27 (*d*, J = 10 Hz, H-5), 5.18 (*br dd*, J = 4, 11 Hz, H-7), 4.26 (*m*, H-3),3.23 (*dd*, J = 10, 14 Hz, H_a-1), 2.39 (*dd*, J = 4, 10 Hz, H-4), 1.76 (*d*, J = 1.5 Hz, H_3-20), 1.53 (*d*, J = 1 Hz, H_3-17), 1.30 (*s*) and 1.16 (*s*, H_3-18 and H_3-19), 1.10 (*d*, J = 7 Hz, H_3-16), 4.42 (*s*, OH), 2.72 (*d*, J = 3 Hz, OH), 2.06 (*s*, acetate); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3610, 3600–3300, 1735, 1710, 1615; UV λ_{max} nm (ε): 283 (11 240), 193 (12 400); (b) 3,5-Di-*O*-acetylisolathyrol (**4e**, 10%, R_f 0.70); MS *m/z*: 418.2349 $[M]^+$ ($C_{24}H_{34}O_6$ calcd for 418.2355), 390, 358, 340, 330, 316, 315, 299, 298, 283, 280, 265, 255; ^1H NMR: δ 7.76 (*br d*, J = 11 Hz, H-12), 6.20 (*d*, J = 10 Hz, H-5), 5.70 (*dd*, J = 4, 4 Hz, H-3), 5.18 (*br dd*, J = 5, 12 Hz, H-7), 3.33 (*dd*, J = 10, 14 Hz, H_a-1), 2.58 (*dd*, J = 4, 10 Hz, H-4), 1.74 (*s*, H_3-20), 1.53 (*s*, H_3-17), 1.34 (*s*) and 1.20 (*s*, H_3-18 and H_3-19), 0.98 (*d*, J = 7 Hz, H_3-16), 2.10 (*s*) and 1.93 (*s*, two acetates); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3680, 1735, 1620; UV λ_{max} nm (ε): 283 (9300), 194 (14 530).

Acetylation of **4b**. Compound **4b** (11 mg) was treated with Ac_2O–pyridine (1:2) (1.5 ml) at room temp for 20 hr. After usual work-up and prep. TLC in cyclohexane–Me_2CO (2:1) the acetate **4f** was obtained (83%, R_f 0.51); MS *m/z*: 488.2773 $[M]^+$ ($C_{28}H_{40}O_7$ calcd for 488.2774), 428, 385, 368, 358, 316, 315, 299, 298, 297, 293, 280, 265, 255; ^1H NMR: δ 6.65 (*br d*, J = 11 Hz, H-12), 6.22 (*d*, J = 9 Hz, H-5), 5.73 (*dd*, J = 4, 4 Hz, H-3), 5.24 (*br dd*, J = 5, 12 Hz, H-7), 3.43 (*dd*, J = 9, 15 Hz, H_a-1), 1.79 (*d*, J = 1.5 Hz, H_3-20), 1.56 (*d*, J = 1.5 Hz, H_3-17), 0.90 (*d*, J = 7 Hz, H_3-16), 2.14 (*s*) and 1.95 (*s*, two acetates); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1730, 1645, 1625; UV λ_{max} nm (ε): 278 (11 190), 197 (12 170).

Acetylation of **4c**. To a soln of 10 mg (0.022 mmol) of **4c** and 13.5 mg (0.11 mmol) of 4-(*N*,*N*-dimethylamino)pyridine in C_6H_6 (0.4 ml) a soln of 11 mg (0.104 mmol) of Ac_2O in C_6H_6 (0.2 ml) was added at room temp. After 3 days, usual work-up, and prep. TLC in Et_2O–petrol (3:1) a mixture of the acetates **4f** and **4g** was obtained (7 mg, 64%, R_f 0.52); MS *m/z*: 488.2778 $[M]^+$ ($C_{28}H_{40}O_7$ calcd for 488.2774), 446, 445, 429, 428, 401, 386, 368, 358, 357, 316, 315, 299, 298, 297, 283, 280, 265, 255; ^1H NMR: δ 6.67 (*br d*, J = 11 Hz, H-12), 6.28 and 6.23 (*d*, each, J = 8 Hz, two H-5), 5.73 and 5.68 (*dd*, each, J = 4, 4 Hz, two H-3), 5.26 (*dd*, J = 5, 12 Hz, H-7), 3.43 and 3.41 (*dd*, each, J = 9, 15 Hz, two H_a-1), 1.79 (*d*, J = 1.5 Hz, H_3-20), 1.56 (*s*, H_3-17), 0.92 (*d*, J = 7 Hz, H_3-16), 2.14 (*s*), 2.06 (*s*) and 1.95 (*s*, two acetates); the appearance of two signals for H_a-1, H-3, H-5 and one acetyl group indicates two different acyl groups in the 3- and 5-positions; IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1735, 1650, 1625; UV λ_{max} nm (ε): 279 (10 370), 193 (13 400). The acetylation of **4c** was not successful with Ac_2O–pyridine.

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