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DERIVATIVES OF CHARACIOL, MACROCYCLIC DITERPENE ESTERS OF THE JATROPHANE TYPE FROM *EUPHORBIA CHARACIAS*

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Key Word Index—*Euphorbia characias*; Euphorbiaceae; macrocyclic diterpenoids; jatrophane; characiol derivatives.

Abstract—Eight non-irritant macrocyclic diterpene esters of the jatrophane type were obtained from an irritant acetone extract of latex and from an irritant methanol extract of roots of *Euphorbia characias*. They were shown to be diesters of the new parent alcohols characiol, characiol-5β,6β-oxide and 5β-hydroxyisocharaciol and pentaesters of 2,5β,8-trihydroxyisocharaciol.

INTRODUCTION

Euphorbia species have afforded many polyfunctional diterpenoids with the tetracyclic tigliane and ingenane skeletons and the tricyclic daphnane skeleton [1, 2]; most of them are skin irritants and many of them are tumour promoters of mouse skin [1, 2]. Moreover, non-irritant polyfunctional macrocyclic diterpenoids with lathyrane and jatrophane skeletons have been obtained. Their diterpene parents are considered biogenetic precursors of the irritants [3]. Polyfunctional derivatives of the tricyclic lathyrane are lathyrol [4], ingol [5] and jolkinol [6, 7], and of the bicyclic jatrophane are jatrophone and derivatives thereof [8, 9], kansuinines A and B [10, 11], euphornin [12] and euphoscopins A to D [13]. Here we report on the isolation and characterization of eight new jatrophane type diterpenoids (compounds A to H) obtained from latex and roots of *Euphorbia characias* L. [14]. The irritant and tumour promoting factors of the plant [14] will be reported elsewhere.

RESULTS AND DISCUSSION

Compounds A–F were isolated from latex of *E. characias* (Table 1 and figures) by the procedure described in ref. [7]. Compounds G and H were obtained from roots of *E. characias* by a comparable procedure (Table 1).

Compounds A and B had similar R_f-values and colour reactions on TLC plates after spraying with vanillin–sulphuric acid. The presence of molecular ions at *m/z* 474 (A) and 496 (B) in their MS together with the ^{1}H NMR data were suggestive of a diterpene moiety $C_{20}H_{30}O_5$ esterified with acetic acid, together with tiglic acid in A and with benzoic acid in B (Table 1).

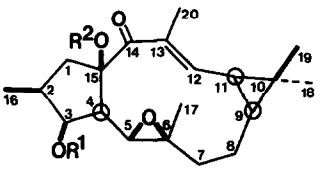
In the ^{1}H NMR spectra of compounds A and B, proton signals of the diterpene moiety (H_a-1, H-3, H-4, H-5, H_3-17 and H_3-20) were present as for diesters of jolkinol-5β,6β-oxide (**1a**) with the lathyrane skeleton [7] (Table 3). They differed, however, from the latter by the lack of a doublet for H-12 at *ca* δ7 and the appearance of a broad triplet at δ6.05. In decoupling experiments with com-

Table 1. Some characteristic data of compounds A–H isolated from fractions obtained by Craig-distributions of the hydrophilic portion of latex [7] and of roots of *E. characias*

Compound*	Fraction		Yield†		TLC		[M]$^+$	Molecular
	no(s).	r(element)	(mg)	(%)	R_f‡	staining§	(*m/z*)	formula‖
A (**3b**)	9,10	305–380	86	0.12	0.30	greyish-brown	474	$C_{27}H_{38}O_7$
B (**3c**)	8–10	281–380	23	0.031	0.33	greyish-brown	496	$C_{29}H_{36}O_7$
C (**4b**)	14	521–548	11	0.015	0.53	brown	432	$C_{25}H_{36}O_6$
D (**5b**)	12	425–464	22	0.029	0.53	brown	462	$C_{26}H_{38}O_7$
E (**5c**)	9,10	305–380	77	0.10	0.51	brown	474	$C_{27}H_{38}O_7$
F (**5d**)	8	218–304	13	0.017	0.48	brown	496	$C_{29}H_{36}O_7$
G (**6b**)	1	1–100	22	0.021	0.17	reddish-grey	717	$C_{39}H_{43}NO_{12}$
H (**6c**)	1	1–100	6	0.006	0.18	reddish-grey	695	$C_{37}H_{45}NO_{12}$

*Compounds A–F isolated from latex, compounds G and H from roots.
†Refers to the acetone extract of latex or to the methanol extract of roots.
‡Precoated silica gel plates in Et_2O–petrol (6:1).
§Vanillin–H_2SO_4 reagent.
‖Determined by peak matching.

pound A (see Table 2) this proton was assigned H-12 coupling with two protons at δ2.49 (H_2-11) and with three protons at δ1.77 (H_3-20, long range coupling). Hence, the cyclopropane ring present in the lathyrane skeleton was absent and it was concluded that compounds A and B were based on the bicyclic jatrophane skeleton. This was supported by the UV spectra. Thus the maxima of derivatives of jolkinol-5β,6β-oxide (**1a**) [7] and lathyrol (**2a**) [4] appear at *ca* 270 nm (β-cyclopropyl enone system) whereas the maxima in compounds A and B were



1a $R^1 = R^2 = H$ (jolkinol-5β,6β-oxide)
1b R^1 = *iso*-butyryl, R^2 = acetyl

Table 2. ^{1}H NMR decoupling experiments with compounds A (**3b**), D (**5b**) and G (**6b**)

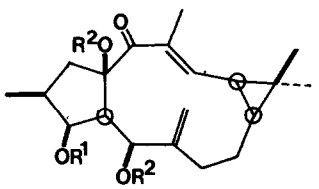
Compound	Irradiated		Observed		Change of signal(s)
	ppm	proton	ppm	proton	
A (**3b**)	6.05	H-12	2.49	H_2-11	$d \rightarrow s$
			1.77	H_3-20	sharpening
	5.50	H-3	2.3–1.7	H-2, H-4	changed multiplicities
	2.48	H_2-11	6.05	H-12	$t \rightarrow s$
	2.13	H-2	5.49	H-3	$dd \rightarrow d$ (J = 4 Hz)
			3.27	H_a-1	$dd \rightarrow d$ (J = 13 Hz)
			0.92	H_3-16	$d \rightarrow s$
	1.79	H-4	5.50	H-3	$dd \rightarrow d$ (J = 4 Hz)
			3.33	H-5	$d \rightarrow s$
		H_3-20	6.05	H-12	sharpening
	1.72	H_b-1	3.27	H_a-1	$dd \rightarrow d$ (J = 7 Hz)
D (**5b**)	5.59	H-3	3.33	H-4	$dd \rightarrow d$ (J = 11 Hz)
			2.1	H-2	changed multiplicity
	5.25	H-5	3.33	H-4	$dd \rightarrow d$ (J = 4 Hz)
		H-12	3.98	H-13	$dq \rightarrow q$ (J = 7 Hz)
	3.98	H-13	5.67	H-11	sharpening
			5.12	H-12	$dd \rightarrow d$ (J = 15 Hz)
			1.18	H_3-20	$d \rightarrow s$
	3.32	H-4	5.59	H-3	$dd \rightarrow d$ (J = 4 Hz)
			5.28	H-5	$d \rightarrow s$
	2.03	H-2	5.59	H-3	$dd \rightarrow d$ (J = 4 Hz)
			0.91	H_3-16	$d \rightarrow s$
		H_2-7	5.06	H_a-17	sharpening
			4.99	H_b-17	sharpening
	0.89	H_3-16	2.1	H-2	changed multiplicity
G (**6b**)	6.15	H-3	3.16	H-4	$dd \rightarrow d$ (J = 10 Hz)
			3.82	H_a-1	sharpening
			2.88	H_b-1	sharpening
	5.87	H-5	3.16	H-4	$dd \rightarrow d$ (J = 4 Hz)
	5.75	H-12	4.98	H-11	$d \rightarrow s$
			3.4	H-13	$m \rightarrow q$ (J = 7 Hz)
	5.27	H_a-17	2.1	H_2-7	sharpening
	4.98	H-11	5.75	H-12	$dd \rightarrow d$ (J = 9 Hz)
		H_b-17	2.1	H_2-7	sharpening
	3.82	H_a-1	6.15	H-3	sharpening
			2.88	H_b-1	$d \rightarrow s$
	3.34	H-13	5.75	H-12	$dd \rightarrow d$ (J = 16 Hz)
			1.26	H_3-20	$d \rightarrow s$
	3.16	H-4	6.15	H-3	$d \rightarrow s$
			5.87	H-5	$d \rightarrow s$
	2.88	H_b-1	6.15	H-3	sharpening
			3.82	H_a-1	$d \rightarrow s$
	2.07	H_2-7	5.27	H_a-17	sharpening
			4.97	H_b-17	sharpening
			5.22	H-8	$t \rightarrow s$
	1.26	H_3-20	3.42	H-13	$m \rightarrow d$ (J = 9 Hz)

Table 3. Comparison of selected ^{1}H NMR spectral data (δ-values) of compounds A (**3b**), C (**4b**), D (**5b**) and G (**6b**) with the *iso*-butyrate, acetate (**1b**) of jolkinol-5β,6β-oxide (**1a**) [7] and the diacetate, benzoate (**2b**) of lathyrol (**2a**) [15] (90 MHz, $CDCl_3$, TMS as int. standard)

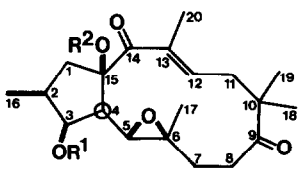
	Chemical shift, multiplicity [coupling constant (Hz)]										
Compound	H_a-1	H-3	H-4	H-5	H-11	H_2-11	H-12	H-13	H_2-17	H_3-17	H_3-20
1b	3.52 *dd* (7; 13)	5.37 *t* (3.5)	1.69†	3.26 *d* (9)	—*	—	6.93 *d* (*br*) (11)	—	—	1.22 *s*	1.89 *m*
A (**3b**)	3.27 *dd* (7; 13)	5.50 *t* (4)	1.79†	3.33 *d* (9)	—	2.49 *d* (7)	6.05 *t* (*br*) (7)	—	—	1.31 *s*	1.77 *m*
C (**4b**)	3.28 *dd* (7; 13)	5.23 *t* (4)	—*	5.41 *d* (10)	—	2.41 *d* (6)	6.36 *t* (*br*) (6)	—	—	1.40 *d* (1.5)	1.70 *d* (1.5)
2b	3.6 *dd* (8.5; 11)	5.81 *t* (3.5)	2.92 *dd* (3.5; 10)	6.2 *d* (10)	—*	—	6.53 *dd* (1.5; 11)	—	5.0 *s* 4.78 *s*	—	1.76 *d* (1.5)
D (**5b**)	—*	5.59 *t* (4)	3.33 *dd* (4; 11)	5.28 *d* (11)	5.67 *d* (15)	—	5.12 *dd* (9; 15)	3.98 *dq* (7; 9)	5.06 *s* 4.99 *s*	—	1.18 *d* (7)
G (**6b**)	3.82 *d* (18)	6.15 *d* (4)	3.16 *dd* (4; 10)	5.87 *d* (10)	4.98 *d* (16)	—	5.75 *dd* (9; 16)	3.4 *m*	5.27 *s* 4.97 *s*	—	1.26 *d* (6)

*Not identified due to complexity of signals.
†Determined by decoupling experiments.

observed at *ca* 230 nm indicating the presence of the enone system C-12 to C-14. In the ^{13}C NMR spectrum of B, two signals for carbonyl functions were present. The NMR data were compatible with the presence of the second carbonyl function at C-9 and two methylene groups at C-7 and C-8 (see also structure elucidation of compound G). These data were in accordance with the structure **3a** for the polyfunctional diterpene moiety.



2a $R^1 = R^2 = H$ (lathyrol)
2b R^1 = benzoyl, R^2 = acetyl



3a $R^1 = R^2 = H$ (characiol-5β,6β-oxide)
3b R^1 = tigloyl, R^2 = acetyl (Compound A)
3c R^1 = benzoyl, R^2 = acetyl (Compound B)
3d R^1 = propionyl, R^2 = acetyl
3e R^1 = tigloyl, $R^2 = H$

The coupling constants of H_a-1, H-3 and H-5 in compound A were similar or identical to those reported for derivatives of lathyrol (**2a**) [4] and of jolkinol-5β,6β-oxide (**1a**) ([6, 7]; Table 3). Therefore, the configuration at C-2 to C-5 and C-15 must be identical. It differed, however, from that of other jatrophane derivatives, e.g. *cis*-ring junction in euphornin [12], α-methyl group at C-2 in euphoscopins A to D [13] or α-hydroxy group at C-5 in kansuinine B [11].

The diterpene moiety (**3a**) of compounds A and B represents a new parent alcohol with the jatrophane skeleton. By analogy to the nomenclature proposed and used for lathyrol (**2a**) [4] and jolkinol-5β,6β-oxide (**1a**) [7] the name characiol-5β,6β-oxide (**3a**) is proposed (for characiol itself, see below).

Partial transesterification of compound A led to the 3-monoester **3e**, indicated by the molecular ion at *m/z* 432 in its MS and by the unchanged shift of H-3 and the missing signal of the acetyl group in the ^{1}H NMR spectrum. Again for H-12 a paramagnetic shift of *ca* 1 ppm was observed as in corresponding jolkinol derivatives [7] which must be attributed to the now free α-ketol group. Thus the structure of compound A was shown to be 15-*O*-acetyl-3-*O*-tigloylcharaciol-5β,6β-oxide (**3b**).

As the signal of H-3 of compound B appeared at lower field than in compound A esterification of the 3-hydroxyl group with benzoic acid was assumed; hence the structure of compound B is 15-*O*-acetyl-3-*O*-benzoylcharaciol-5β,6β-oxide (**3c**). Attempts to bring about the base catalysed complete transesterifications of **3b** or **3c** and reactions under acidic conditions led to no clear-cut reaction products.

Compound C differed from compounds A and B in both its R_f-value and colour reactions. As in A and B, in its UV spectrum the maximum was observed at *ca* 230 nm. In its mass spectrum the molecular ion was at *m/z* 432 and the fragmentation pattern suggested the presence of a

diterpene moiety $C_{20}H_{30}O_4$ esterified with acetic acid and propionic acid (Table 1).

The ^1H NMR spectrum confirmed the presence of the acid moieties. The signals for the diterpene moiety, H_a-1, H-3, H_2-11, H-12, H_3-16, H_3-18 to H_3-20, were identical with those in **3a** (see also Table 3). A broad doublet of one proton at δ5.41 (H-5) coupled (allylic) with a doublet of three protons at δ1.40 (H_3-17) suggested a double bond between C-5 and C-6. This was established by treatment of compound C with 3-chloroperbenzoic acid yielding the 5,6-epoxide **3d** with identical signals for the diterpene moiety as in compounds A and B (**3b** and **3c**), respectively. For the new diterpene moiety of compound C the trival name characiol (**4a**) is proposed. Assignment of the positions of the acid moieties in compound C was impossible due to a lack of material. By analogy to compounds A and B and also to the jolkinol derivatives from *E. characias* [7], it was assumed that the C-15 hydroxyl group was esterified with acetic acid. Thus, compound C was tentatively assigned the structure 15-*O*-acetyl-3-*O*-propionylcharaciol (**4b**).

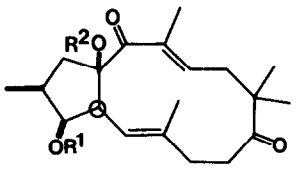
Compounds D–F had similar R_f-values and stained the same as compound C (Table 1). By means of peak matching mass spectrometry and ^1H NMR spectroscopy they were recognized as the acetate, *iso*-butyrate (D), acetate, tigliate (E) and acetate, benzoate (F) of a diterpene moiety $C_{20}H_{30}O_5$; compounds E and F were isomeric with compounds A (**3b**) and B (**3c**), respectively. The signals in the IR spectra of compounds D–F indicated the presence of hydroxyl functions. In the UV spectra, in contrast to compounds A–C, no absorption bands were present at *ca* 230 nm indicating the lack of an enone system in the diterpene moiety.

The proton signals for the diterpene moiety in the ^1H NMR spectra were identical in all three compounds and differed strikingly from those in **3a** (Table 3). From decoupling experiments with compound D (see Table 2), the sequence of protons H-2 (H_3-16), H-3, H-4, H-5, H_2-7 and H_2-17 was derived with similar multiplicity and chemical shifts as in derivatives of lathyrol (**2a**) ([4, 15]; Table 3). Thus, an identical partial structure as in lathyrol (**2a**) involving C-2 to C-7 and C-15 to C-17 was derived. The coupling constant J = 15 Hz between a proton at δ5.67 (H-11) and a proton (*dd*) at δ5.12 (H-12) was characteristic for a *trans*-double bond in compound D. By irradiation at δ3.98 (H-13, doublet of a quartet, see Table 2) H-11 was sharpened, H-12 appeared as a doublet and the doublet of three protons at δ1.18 (H_3-20) as a singlet. Hence the double bond was located between C-11 and C-12. The chemical shift of H-13 at relatively low field may be explained by its vicinity to the double bond and to a carbonyl group at C-14; as in the isomeric compounds **3b** and **3c** a second carbonyl group may be located at C-9 (see below). From these data a new diterpene parent with a jatrophane skeleton carrying an *exo*-methylene group was derived and it is proposed that it is named 5β-hydroxyisocharaciol* (**5a**). The opening of the oxirane ring present in **3a** leading to a partial structure as present in **5a** was synthetically carried out in the lathyrane skeleton with a derivative of jolkinol-5β,6β-oxide (**1a**, jolkinol B) leading to lathyrol (**2a**) [6]. In all derivatives of **1a** and **2a**, H_a-1 appeared at low field (*ca* δ3.6) [4, 6, 7, 15] or in **3b**, **3c** and **4b** at *ca* δ3.3. In derivatives of isocharaciol (**5a**), H_a-1 was strikingly shifted upfield though still adjacent to the ketol structure. This might indicate that due to the lack of an enone system the macrocyclic ring is not as rigid as in **1a**, **2a**, **3a** or **4a**.

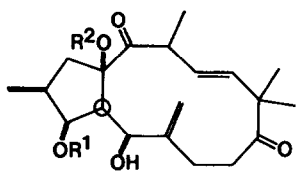
Attempts to determine the positions of the acid moieties of compounds D–F by partial transesterification reactions were not successful. It may be assumed that as in all other jatrophane and lathyrane derivatives isolated from *E. characias* [7] in compounds D–F the C-3 and C-15 hydroxyl groups were esterified, resulting here in a free C-5 hydroxyl group. The chemical shift of H-5 (*ca* δ5.3) in D–F was not unusual in this class of compounds. In 3,15-diesters of isolathyrol the signal of H-5 appears at *ca* δ5, and in 3,5,15-triesters of lathyrol [16] and isolathyrol [7] at *ca* δ6.2. The different chemical shifts for H-3 in compounds D–F were in accordance with the proposition that the variable acid moieties were located at the C-3 hydroxyl group and the (constant) acetic acid moiety at the C-15 hydroxyl group. Hence, for compounds D–F the structures 15-*O*-acetyl-5β-hydroxyisocharaciol-3-*iso*-butyrate (**5b**), -3-tigliate (**5c**) and -3-benzoate (**5d**), respectively, were tentatively assigned.

Of all the compounds isolated compounds G and H exhibited the lowest R_f-values and stained reddish-grey. Their molecular formula, $C_{39}H_{43}NO_{12}$ and $C_{37}H_{45}NO_{12}$, respectively, were determined by means of peak matching mass spectrometry. The loss of five acid moieties indicated a diterpene moiety $C_{20}H_{30}O_7$ (Table 1).

In the ^1H NMR spectrum of compound G signals for three acetyl groups, a benzoic acid and a nicotinic acid moiety were present. By decoupling experiments (Table 2) the same partial structure (C-10 to C-14, C-20) was derived as in **5a**. H-4, a doublet of a doublet at δ3.16 had common coupling constants with a broad doublet (J = 4 Hz) at δ6.15 (H-3) and a doublet (J = 10 Hz) at δ5.87



4a $R^1 = R^2 = H$ (characiol)
4b R^1 = propionyl, R^2 = acetyl (Compound C)



5a $R^1 = R^2 = H$ (5β-hydroxyisocharaciol)
5b R^1 = *iso*-butyryl, R^2 = acetyl (Compound D)
5c R^1 = tigloyl, R^2 = acetyl (Compound E)
5d R^1 = benzoyl, R^2 = acetyl (Compound F)

*5β-Hydroxyisocharaciol (**5a**) is an isomer to characiol (**4a**) with an additional hydroxyl function, i.e. 6,11,12,17-tetradehydro-5,6,12,13-tetrahydro-5β-hydroxycharaciol.

(H-5), indicating the C-5 hydroxyl group was esterified. The multiplicity of H-3 differed from that in **3a**, **4a** and **5a**, but it was identical with that of a derivative of 2α-hydroxyjolkinol-5β,6β-oxide [7]. Hence, the presence of an acyloxy group at C-2 was a possibility; this was supported by the singlet of H_3-16 at δ1.75. Irradiation at the frequency of H-3 caused sharpening of two doublets at δ3.82 and 2.88, which might be related to H_a-1 and H_b-1, respectively. Compared to **5a** a paramagnetic shift of about 1 ppm was observed for H_a-1. A similar shift was reported for corresponding derivatives of jolkinol, and was attributed to the additional acyloxy group at C-2 [7].

Compared to **5a**, the position of the second additional acyloxy group at C-8 was deduced as follows: Irradiation at δ2.09 (H_2-7) caused sharpening of two singlets at δ5.27 and δ4.97 which were assigned H_2-17. Additionally, a triplet at δ5.22 was converted to a singlet and was possibly associated to H-8. Based on the partial structures so far elucidated and the molecular formula, the presence of a second carbonyl group was indicated which could only be located at C-9 as postulated for the structure elucidation of **3a** and **5a** (see above). Hence a new diterpene parent was recognized and named 2,5β,8-trihydroxyisocharaciol (**6a**).

In the ^{1}H NMR of compound H the same signals of the diterpene moiety were present as in that of compound G; tiglic acid was the fifth acid moiety besides nicotinic acid and three acetic acid moieties. As the chemical shifts of H-3 in compounds G and H differ (0.25 ppm), it was assumed that benzoic acid and tiglic acid, respectively, were esterified with the C-3 hydroxyl group. The positions of the acid moieties could, however, not be determined from transesterification reactions. Hence, compounds G and H were recognized as the pentaesters 2,5,8,15-*O*-triacetyl, nicotinoyl-2,5β,8-trihydroxyisocharaciol-3-benzoate (**6b**) and -3-tigliate (**6c**), respectively.

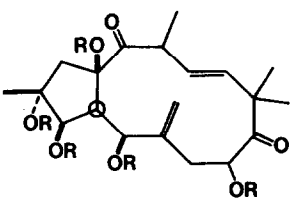
All eight compounds were tested for irritant activity [16] and proved to be practically inactive (IU > 50 μg/ear [14]).

EXPERIMENTAL

For material, methods and separation procedure of latex see ref. [7].

Isolation of compounds A to F. Compounds A to F were obtained from several fractions of the hydrophilic portion of latex of *E. characias* by prep. TLC on silica gel in several solvent systems (Table 1).

Isolation of compounds G and H. Roots (1.8 kg) of *E. characias* were homogenized and exhaustively extracted with MeOH (Ultra turrax) to give a MeOH extract (103 g, ID_{50}: 22.7 μg/ear). On partitioning this material between H_2O (1 l.) and EtOAc (5 × 1 l.) an EtOAc fraction (35 g, 33%) was obtained. This fraction was subjected to two successive Craig distributions (z = 30 elements, V = 100 ml/100 ml) in the systems petrol–MeOH–H_2O (30:20:1) (n = 50 transfers) and CCl_4–MeOH–H_2O (40:20:3) (n = 35 transfers). In the first partition, a hydrophobic fraction, and in the second one, a very polar material were separated, thus yielding a hydrophilic portion (8.1 g, 7.9%). This material (6.6 g) was subjected to a third Craig distribution in petrol–MeOH–H_2O (30:20:1) (z = 1020, V = 5 ml/3 ml, n = 2000 transfers, single withdrawal procedure). According to TLC the contents of the tubes were combined to give eight fractions [14]. Prep TLC of fraction 1 in several solvent systems gave compounds G and H (Table 1).



6a R = H (2,5β,8-trihydroxyisocharaciol)
6b R = acetyl (× 3), benzoyl, nicotinoyl (Compound G)
6c R = acetyl (× 3), tigloyl, nicotinoyl (Compound H)

Compound A (15-O-*acetyl*-3-O-*tigloylcharaciol*-5β,6β-*oxide*) (**3b**). ^{1}H NMR ($CDCl_3$): δ6.05 (*t* (*br*), *J* = 7 Hz, H-12), 5.50 (*t*, *J* = 4 Hz, H-3), 3.33 (*d*, *J* = 9 Hz, H-5), 3.27 (*dd*, *J* = 7, 13 Hz, H_a-1), 3.1–2.7 (*m*, 1H), 2.49 (*d* (*br*), *J* = 7 Hz, H_2-11), 1.77 (*m*, H_3-20), 1.31 (*s*, H_3-17), 1.06 (*s*), 1.04 (*s*, H_3-18 and H_3-19), 0.92 (*d*, *J* = 7 Hz, H_3-16), 2.21 (*s*, acetate), 6.97 (*q* (*br*), *J* = 7 Hz), 1.92 (*m*) and 1.83 (*m*, tigliate); for decoupling experiments see Table 2; MS *m/z*: 474.2621 $[M]^+$ ($C_{27}H_{38}O_7$ calculated for 474.2617), 431, 414, 392, 374, 359, 358, 332, 331, 314, 313, 286, 285; UV λ_{max}^{MeOH} nm (ε): 307 (160), 229 (sh, 15840), 223 (17280), 217 (sh, 16020), 193 (9300); IR ν_{max}^{KBr} cm^{-1}: 1735, 1705, 1665, 1650.

Transesterification of **3b** *affording* **3e**. Compound **3b** (13 mg) was treated for 5 hr with 2% KOH/MeOH (8 ml). Addition of Pi buffer (pH *ca* 7, 25 ml), extraction with EtOAc (2 × 25 ml), drying ($MgSO_4$) and rotary evaporation yielded a mixture of compounds (14 mg). Prep. TLC on silica gel in CH_2Cl_2–Me_2CO (40:1) afforded unchanged **3b** (7 mg, R_f 0.21) and as the main reaction product 3-*O*-tigloylcharaciol-5β,6β-oxide (**3e**) (3 mg, R_f 0.15). ^{1}H NMR ($CDCl_3$): δ7.12 (*t* (*br*), *J* = 6 Hz, H-12), 5.53 (*t*, *J* = 3.5 Hz, H-3), 3.14 (*dd*, *J* = 8, 13 Hz, H_a-1), 3.08 (*d*, *J* = 9 Hz, H-5), 1.90 (*m*, H_3-20), 1.27 (*s*, H_3-17), 1.16 (*s*), 1.00 (*s*, H_3-18 and H_3-19), 0.96 (*d*, *J* = 7 Hz, H_3-16), 6.87 (*m*), 1.90 (*m*) and 1.79 (*m*, tigliate); MS *m/z*: 432 $[M]^+$, 404, 350, 332, 314, 291, 286, 83 (base peak).

Compound B (15-O-*acetyl*-3-O-*benzoylcharaciol*-5β,6β-*oxide*) (**3c**). ^{1}H NMR ($CDCl_3$): δ6.05 (*t* (*br*), *J* = 7 Hz, H-12), 5.65 (*t*, *J* = 4 Hz, H-3), 3.39 (*d*, *J* = 9 Hz, H-5), 3.33 (*dd*, *J* = 7, 13 Hz, H_a-1), 3.1–2.7 (*m*, 1H), 2.49 (*d* (*br*), *J* = 7 Hz, H_2-11), 1.78 (*m*, H_3-20), 1.29 (*s*, H_3-17), 1.10 (*s*), 1.05 (*s*, H_3-18 and H_3-19), 0.98 (*d*, *J* = 6 Hz, H_3-16), 8.2–8.0 (*m*) and 7.65–7.35 (*m*, benzoate), 2.28 (*s*, acetate); MS *m/z*: 496.2456 $[M]^+$ ($C_{29}H_{36}O_7$ calculated for 496.2461), 453, 436, 414, 380, 331, 313, 285, 269; UV λ_{max}^{MeOH} nm (ε): 280 (1020), 273 (1220), 230 (28080), 195 (48920); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1735–1700, 1665, 1645, 1595; ^{13}C NMR ($CDCl_3$): δ213.7 (*s*, C-9), 200.0 (*s*, C-14), 137.0 (*s*, C-13), 135.6 (*d*, C-12), 90.2 (*s*, C-15), 80.8 (*d*, C-3), 61.4 (*s*, C-6), 59.4 (*d*, C-5), 51.5 (*d*, C-4), 48.0 (*s/t*, C-10/1), 39.5 (*t*, C-8), 38.6 (*d*, C-2), 33.0 (*t*, C-11), 32.0 (*t*, C-7), 25.1 (*q*, C-18), 23.3 (*q*, C-20), 16.8 (*q*, C-17), 13.5 (*q*, C-16), 12.1 (*q*, C-19); acetate: 170.3 (*s*, COO), 21.5 (*q*, Me); benzoate: 165.6 (*s*, COO), 133.2 (*d*, C_p), 130.4 (*s*, C_i), 129.9 (*d*, C_o), 128.6 (*d*, C_m).

Compound C (15-O-*acetyl*-3-O-*propionylcharaciol*) (**4b**). ^{1}H NMR ($CDCl_3$): δ6.36 (*t* (*br*), *J* = 6 Hz, H-12), 5.41 (*d br*), *J* = 10 Hz, H-5), 5.23 (*t*, *J* = 4 Hz, H-3), 3.28 (*dd*, *J* = 7, 13 Hz, H_a-1), 2.41 (*d* (*br*), *J* = 6 Hz, H_2-11), 1.70 (*d*, *J* = 1.5 Hz, H_3-20), 1.40 (*d*, *J* = 1.5 Hz, H_3-17), 1.21 (*s*), 1.04 (*s*, H_3-18 and H_3-19), 0.92 (*d*, *J* = 6 Hz, H_3-16); 2.12 (*s*, acetate); 2.43 (*q*, *J* = 7 Hz) and 1.24 (*t*, *J* = 7 Hz, propionate); MS *m/z*: 432.2517 $[M]^+$ ($C_{25}H_{36}O_6$ calculated for 432.2512), 389, 372, 361, 358, 350, 315, 297, 287, 276; UV λ_{max}^{MeOH} nm (ε): 234 (7720), 193 (12860).

Oxygenation of **4b** *affording* **3d**. To **4b** (2 mg) in CH_2Cl_2 (0.5 ml), 3-chloroperbenzoic acid (3 mg) was added and the soln

stirred for 5 hr. After addition of 5% aq. Na_2SO_3 (20 ml), the aq. phase was extracted (× 2) with CH_2Cl_2 (20 ml); the combined organic phases were shaken (× 2) with saturated $KHCO_3$-soln (20 ml) and H_2O (20 ml) and dried ($MgSO_4$). Prep. TLC on silica gel in CH_2Cl_2–Me_2CO (19:1) yielded 15-*O*-acetyl-3-*O*-propionylcharaciol-5β,6β-oxide (**3d**) (1.5 mg, R_f 0.27). ^{1}H NMR ($CDCl_3$): δ6.0 (*t* (*br*), *J* = 7 Hz, H-12), 5.41 (*t*, *J* = 4 Hz, H-3), 3.34 (*d*, *J* = 9 Hz, H-5), 3.23 (*dd*, *J* = 7, 14 Hz, H_a-1), 3.0–2.6 (*m*, 1H), 2.48 (*d* (*br*), *J* = 7 Hz, H_2-11), 1.76 (*m*, H_3-20), 1.30 (*s*, H_3-17), 1.07 (*s*), 1.04 (*s*, H_3-18 and H_3-19), 0.93 (*d*, *J* = 7 Hz, H_3-16), 2.21 (*s*, acetate), 2.48 (*q*, *J* = 7 Hz) and 1.21 (*t*, *J* = 7 Hz, propionate); MS *m/z*: 448 $[M]^+$, 405, 388, 366, 349, 332, 331, 314, 313, 307, 306, 289, 285.

Compound D (15-O-*acetyl-5β-hydroxyisocharaciol*-3-iso-*butyrate*) (**5b**). ^{1}H NMR ($CDCl_3$): δ5.67 (*d*, *J* = 15 Hz, H-11), 5.59 (*t*, *J* = 4 Hz, H-3), 5.28 (*d*, *J* = 11 Hz, H-5), 5.12 (*dd*, *J* = 9, 15 Hz, H-12), 5.06 (*s* (*br*), H_a-17), 4.99 (*s* (*br*), H_b-17), 3.98 (*dq*, *J* = 7, 9 Hz, H-13), 3.33 (*dd*, *J* = 4, 11 Hz, H-4), 1.18 (*d*, *J* = 7 Hz, H_3-20), 0.91 (*d*, *J* = 6 Hz, H_3-16), 2.41 (*s*, OH), 1.97 (*s*, acetate), 2.6 (*m*), and 1.15 (*m*, *iso*-butyrate); for decoupling experiments see Table 2; MS *m/z*: 462.2624 $[M]^+$ ($C_{26}H_{38}O_7$ calculated for 462.2617), 434, 420, 402, 375, 374, 366, 359, 346, 337, 333, 332, 331, 316, 315, 314, 306, 286, 279, 278, 277; UV λ_{max}^{MeOH} nm (ε): 274 (860), 215 (sh, 6400), 193 (9740); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3590 (OH), 1735, 1715 (CO), 1650 (C=C).

Compound E (15-O-*acetyl-5β-hydroxyisocharaciol*-3-*tigliate*) (**5c**). ^{1}H NMR: δ5.71 (*d*, *J* = 16 Hz, H-11), 5.70 (*t*, *J* = 4 Hz, H-3), 5.32 (*d*, *J* = 11 Hz, H-5), 5.17 (*dd*, *J* = 9, 16 Hz, H-12), 5.09 (*s*, H_a-17), 5.03 (*s*, H_b-17), 4.03 (*dq*, *J* = 7, 9 Hz, H-13), 3.37 (*dd*, *J* = 4, 11 Hz, H-4), 1.16 (*d*, *J* = 7 Hz, H_3-20), 0.92 (*d*, *J* = 6 Hz, H_3-16), 2.40 (*s*, OH), 6.83 (*q* (*br*), *J* = 7 Hz), 1.90 (*m*) and 1.82 (*m*, tigliate), 1.98 (*s*, acetate); MS *m/z*: 474.2612 $[M]^+$ ($C_{27}H_{38}O_7$ calculated for 474.2617), 446, 432, 415, 414, 388, 387, 386, 378, 374, 371, 358, 350, 349, 346, 333, 332, 331, 319, 318, 316, 315, 314, 291, 290, 289, 286; UV λ_{max}^{MeOH} nm (ε): 302 (350), 215 (13540), 194 (12370). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3500 (broad, OH), 1730, 1700, 1680 (CO), 1645 (C=C).

Compound F (15-O-*acetyl-5β-hydroxyisocharaciol*-3-*benzoate*) (**5d**). ^{1}H NMR: δ5.87 (*t*, *J* = 4 Hz, H-3), 5.71 (*d*, *J* = 16 Hz, H-11), 5.37 (*d*, *J* = 11 Hz, H-5), 5.17 (*dd*, *J* = 9, 16 Hz, H-12), 5.10 (*s*, H_a-17), 5.02 (*s*, H_b-17), 4.15–3.90 (*m*, H-13), 3.41 (*dd*, *J* = 4, 11 Hz, H-4), 1.24 (*s*) and 1.16 (*s*, H_3-18, H_3-19), 1.16 (*d*, *J* = 7 Hz, H_3-20), 0.96 (*d*, *J* = 6 Hz, H_3-16), 2.47 (*s*, OH), 8.05–7.9 (*m*) and 7.7–7.45 (*m*, benzoate), 1.93 (*s*, acetate); MS *m/z*: 496.2454 $[M]^+$ ($C_{29}H_{36}O_7$ calculated for 496.2461), 468, 454, 437, 436, 418, 414, 410, 409, 408, 371, 355, 354, 341, 340, 332, 331, 315, 314, 313, 312, 311, 296, 286; UV λ_{max}^{MeOH} nm (ε): 297 (460), 280 (990), 273 (1100), 266 (sh, 1000), 227 (16880), 194 (48760); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3590 (OH), 1735–1695 (CO), 1645, 1595 (C=C).

Compound G (2,5,8,15-O-*triacetyl, nicotinoyl*-2,5β,8-*trihydroxyisocharaciol*-3-*benzoate*) (**6b**). ^{1}H NMR: δ6.15 (*d* (*br*), *J* = 4 Hz, H-3), 5.87 (*d*, *J* = 10 Hz, H-5), 5.75 (*dd*, *J* = 9, 16 Hz, H-12), 5.27 (*s*, H_a-17), 4.97 (*s*, H_b-17), 5.22 (*t*, *J* = 5 Hz, H-8), 4.98 (*d*, *J* = 16 Hz, H-11), 3.82 (*d*, *J* = 18 Hz, H_a-1), 3.4 (*m*, H-13), 3.16 (*dd*, *J* = 4, 10 Hz, H-4), 2.88 (*d*, *J* = 18 Hz, H_b-1), 1.75 (*s*, H_3-16), 1.26 (*d*, *J* = 6 Hz, H_3-20), 1.30 (*s*) and 0.99 (*s*, H_3-18, H_3-19), 9.35 (*d*, *J* = 3 Hz), 8.87 (*dd*, *J* = 2, 5 Hz), 8.38 (*dt*, *J* = 2, 8 Hz) and 7.4 (*m*, nicotinoate), 8.2–7.95 (*m*) and 7.8–7.3 (*m*, benzoate), 2.31 (*s*), 2.05 (*s*) and 1.94 (*s*, 3 acetates); for decoupling experiments see Table 2; MS *m/z*: 717.2780 $[M]^+$ ($C_{39}H_{43}NO_{12}$ calculated for 717.2785), 676, 675, 659, 658, 657, 648, 647, 623, 622, 590, 589, 588, 587, 559, 558, 545, 520, 492, 474, 465, 464, 435, 375, 370, 369, 353, 352, 342, 311, 310, 309, 300, 299, 292, 283, 282, 281, 280, 279, 274; UV λ_{max}^{MeOH} nm (ε): 300 (640), 282 (sh, 1530), 270 (sh, 3440), 264 (3950), 257 (sh, 3620), 226 (21270), 194 (57430); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1745, 1725 (CO), 1655, 1605, 1590 (C=C).

Compound H (2,5,8,15-O-*triacetyl, nicotinoyl*-2,5β,8-*trihydroxyisocharaciol*-3-*tigliate*) (**6c**). ^{1}H NMR: δ5.90 (*d* (*br*), *J* = 4 Hz, H-3), 5.79 (*d*, *J* = 11 Hz, H-5), 5.67 (*dd*, *J* = 9, 16 Hz, H-12), 5.23 (*s* (*br*), H_a-17), 4.92 (*s* (*br*), H_b-17), 5.15 (*t*, *J* = 5 Hz, H-8), 4.92 (*d*, J = 16 Hz, H-11), 3.74 (*d*, *J* = 18 Hz, H_a-1), 3.30 (*m*, H-13), 3.14 (*dd*, *J* = 4, 11 Hz, H-4), 2.77 (*d*, *J* = 18 Hz, H_b-1), 1.70 (*s*, H_3-16), 1.21 (*d*, *J* = 7 Hz, H_3-20), 1.26 (*s*) and 0.97 (*s*, H_3-18, H_3-19), 9.37 (*d*, *J* = 2 Hz), 8.83 (*dd*, *J* = 2, 5 Hz), 8.32 (*dt*, *J* = 2, 8 Hz) and 7.45 (*dd*, *J* = 5, 8 Hz, nicotinoate), 6.91 (*m*), 1.89 (*m*) and 1.81 (*m*, tigliate), 2.23 (*s*), 2.06 (*s*) and 1.99 (*s*, 3 acetates); MS *m/z*: 695.2950 $[M]^+$ ($C_{37}H_{45}NO_{12}$ calculated for 695.2941), 655, 654, 653, 637, 636, 626, 625, 608, 601, 600, 594, 593, 572, 568, 567, 566, 565, 540, 537, 536, 523, 512, 498, 497, 477, 471, 470, 458, 452, 444, 443, 442, 414, 413, 412, 402, 399, 370, 353, 329, 328, 319, 310, 300, 299, 292, 287, 286, 283, 275, 274; UV λ_{max}^{MeOH} nm (ε): 302 (375), 270 (sh, 2140), 263 (2680), 258 (2650), 218 (18310), 196 (23500); IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 1745, 1725 (CO), 1655, 1610, 1590 (C=C).

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