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PHYLLOCLADANES (13β-KAURANES) FROM *PLECTRANTHUS AMBIGUUS*

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Key Word Index—*Plectranthus ambiguus*; Labiatae; phyllocladane (13β-kaurane) skeleton; structure elucidation; chemical correlation.

Abstract—Six new diterpenoids of the rare phyllocladane (13β-kaurane) type have been isolated from *Plectranthus ambiguus*. Their structures were determined on the basis of their spectral properties and by correlation with 16*R*,17-dihydroxyphyllocladane-3-one (calliterpenone).

INTRODUCTION

In continuation of our current research concerning biologically active constituents of African and Asian medicinal plants [1], we investigated the labiate *Plectranthus ambiguus* [2] with respect to antioxidative activity and inhibition of the enzymes of arachidonic acid metabolism. Antioxidant activity guided fractionation of the air-dried plant material (see Experimental) and afforded the main antioxidant principle of this species, the quite rare 5,6-dihydroxy-7,4′-dimethoxyflavone (**1**) (ladanein or 4′,7-dimethylscutellarein) [3–7]. The mother liquor of this fraction contained the tetracyclic diterpenoids **2a**, **2b**, **3a**, **3b**, **4** and **5a** as the main components. The present paper reports on the isolation and structural determination of these six novel phyllocladanes (13β-kauranes).

RESULTS AND DISCUSSION

Extraction of the aerial parts of *P. ambiguus* followed by partition (hexane–90% methanol) and column chromatography (Sephadex LH-20, silica gel) yielded from the main fraction exhibiting significant antioxidative activity the crystalline flavone **1** (0.026%). Subsequent column chromatography (silica gel) of the mother liquor and preparative HPLC (C-18) afforded the tetracyclic diterpenoids **2a** (0.55%), **2b**/**3b** (as a mixture, *ca* (6:1, 0.08%), **3a** (0.29%), **4** (0.10%) and **5a** (0.13%).

The molecular formula of compound **2a** was established as $C_{27}H_{42}O_6$ on the basis of its EI-mass spectrum and elemental analysis. Its substituents were assigned as $Me_2C=CHCO_2-$, AcO– and $-CH_2OH$ based on the MS fragmentation pattern and the following spectral data: UV: 218 (4.15) nm (log ε); IR: 3200–3600 (*br*), 1746, 1718 and 1650 cm^{-1}; ^1H NMR: δ1.68 and 2.13 (each 3H, each *d*, ^4J = 1.2 Hz), δ5.55 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz); δ2.11 (*s*, 3H); δ3.62 and 3.76 (each 1H, ABX, J = 10.8, 5.4 Hz, after adding D_2O: AB, ^2J = 10.9 Hz). The presence of three tertiary Me-signals at δ0.86, 0.99 and 1.01 (each 3H, each *s*) and the ^{13}C NMR data (Table 1) suggested that **2a** was a tetracyclic diterpenoid.

The mono-*O*-acetyl derivative **2b** (acetic anhydride–pyridine) still had IR absorption (3590 cm^{-1}) in the hydroxyl region and this was assigned to a tertiary hydroxyl group. The ^1H NMR spectrum showed an additional, primary acetoxy group (δ2.10, 3H, *s*; 4.16 and 4.24, each 1H, AB, ^2J = 11.3 Hz).

Compound **2a** readily gave the acetonide **2c** (acetone–$CuSO_4$ [8]). Its ^1H NMR spectrum showed an AB system for a methylene group at δ3.90 and 4.06 the ^2J = 8 Hz indicating a five-membered ring ketal [9]; hence, **2a** had to be a 1,2-dihydroxy compound [8–10].

Compound **2a** was easily oxidized (H_5IO_6–methanol) to the ketone **2d**. Its strong IR absorption (1745 cm^{-1}) is typical for five-membered ring ketones. Therefore, **2a** is a tetracyclic diterpenoid with a five-membered ring D which is substituted with a primary and a tertiary hydroxyl group. These substituents can only be located at C-17 and C-16, respectively. Based on the low-field chemical shifts of H_2-17 (AB at δ3.62 and 3.76) and C-16 (δ84.2) in **2a**, the 16*R* configuration was tentatively assigned [11, 12].

The positions and the relative stereochemistry of the $Me_2C=CH-CO_2-$ and AcO– substituents were deduced by the ^1H NMR splitting pattern and double resonance experiments: the signals at δ5.01 (1H, *d*, ^3J = 2.7 Hz, H_{eq}) and 5.25 (1H, *ddd*, ^3J = 12.4, 4.5 and 2.7 Hz, H_{ax}) clearly showed a vicinal *cis*-relationship of the corresponding protons. When the doublet at δ5.01 was irradiated, only the signals at δ5.25 and 0.86 (Me-19) and 0.99 (Me-18) showed a NOE. Thus, these two substituents are located at the 2α and 3α position in ring A. Their relative arrangement was established by a heteronuclear ^1H, ^{13}C-decoupling experiment: when the proton at δ5.01 (H-3) was irradiated, the signal of the acetoxy

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Table 1. ^{13}C NMR spectral data (50 MHz, $CDCl_3$)

C	2a	2b/3b	3a	4	5a
1	37.7	38.0	37.9	32.7	44.3
2	66.8	66.9	67.8	22.4	70.2
3	77.0	77.2	77.1	78.2	209.2
4	37.8*	38.1*	38.1*	36.4*	48.3
5	49.5	49.7	49.8	50.3	55.7
6	19.1	19.2†	19.4	19.1[b]	19.3†
7	40.6	40.7	40.9	41.1	40.4
8	43.1	43.6	43.5	43.6	43.0
9	56.0	56.2	56.2	56.3	56.9
10	38.7*	38.9*	39.0*	37.4*	38.1
11	19.1	19.3†	19.4	19.7†	20.6†
12	26.2	26.5	26.5	26.2	26.2
13	43.3	44.3	43.8	43.8	43.4
14	48.1	48.1	48.3	48.4	48.0
15	44.4	44.8	44.9	44.8	44.4*
16	84.2	82.5	84.5	84.6	84.2
17	65.0	67.6	65.5	65.5	65.0
18	27.6†	27.9‡	27.9	28.0	24.8
19	21.3‡	21.6§	21.7†	21.3‡	21.2‡
20	15.3	15.5	15.6	14.5	15.1
21	165.6	165.8/172.3	172.4		165.3
22	115.6	115.9/43.3	43.3		115.1
23	156.5	156.8/25.3	25.4		157.8
24	19.9‡	20.1§/22.3	22.4		20.1‡
25	27.1†	27.3‡/22.3	22.4		27.2
AcO	20.6‡	20.8§	21.0†	22.0‡	
	170.2	170.4	170.5	170.8	
		20.9§			
		171.1			

*†‡§Assignments are interchangable.

carbonyl group (δ170.2, *dq*, $^2J_{CH} = 7$ Hz and $^3J_{CH} = 4$ Hz) was simplified to a *q* ($^2J_{CH} = 7$ Hz), whereas after irradiation of the proton at δ5.25 (H-2) the carbonyl signal of the senecioyl moiety (δ165.6, *m*, $W_{1/2}$ *ca* 7 Hz) was significantly narrowed ($W_{1/2}$ *ca* 4 Hz), i.e. $Me_2C=CH-CO_2-$ is located at C-2 and AcO– at C-3.

The signal at δ15.6 in the ^{13}C NMR spectrum of **2a** corresponded to a methyl group and was assigned to Me-20. Due to this pronounced high-field chemical shift, the compound is considered to be a phyllocladane-type diterpenoid rather than a kaurane-type [13]. Hence, the structure of **2a** is formulated as (16*R*)-2α-(3-methyl-2-butenoyloxy)-3α-acetoxyphyllocladane-16,17-diol.

The CI-mass spectrum of compound **3a** indicated the molecular formula $C_{27}H_{44}O_6$, which suggested it to be a dihydro derivative of **2a**. The ^1H and ^{13}C NMR spectra are very similar to those of **2a** (see Experimental and Table 1); they mainly lack signals corresponding to the senecioyloxy substituent. Extra signals appear at δ0.92 and 0.93 (each 3H, each *d*, $^3J = 6.4$ Hz) for two diastereotopic Me groups of a 2-propyl group [14], and the olefinic carbons are substituted by CH and CH_2 signals.

Acetylation (acetic anhydride–pyridine) of **3a** yielded a mono-*O*-acetyl derivative (**3b**) and catalytic hydrogenation (Pd/C–methanol) of **2a** afforded **3a**, which proved to be identical in every respect with the natural product (R_f, mp, $[\alpha]_D$, mass, IR, ^1H and ^{13}C NMR spectra). Therefore, the structure of **3a** is (16*R*)-2α-(3-methylbutanoyloxy)-3α-acetoxyphyllocladane-16,17-diol.

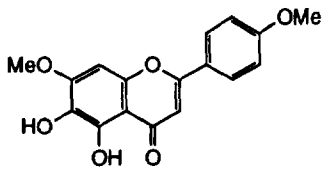
The diacetates **2b** and **3b** were also isolated as natural products (**2b**: **3b** *ca* 6:1, according to ^1H NMR). However, all attempts to separate the mixture were unsuccessful. Comparing the physical data with those of the pure mono-*O*-acetyl derivatives **2b** and **3b** prepared from either **2a** or **3a** fully confirmed the structures. Thus, the natural compounds are established as (16*R*)-2α-(3-methyl-2-butenoyloxy)-3α,17-diacetoxy-16-hydroxyphyllocladane (**2b**) and (16*R*)-2α-(3-methylbutanoyloxy)-3α,17-diacetoxy-16-hydroxyphyllocladane (**3b**), respectively.

Based on its CI-mass spectrum and ^{13}C NMR data, the molecular formula $C_{22}H_{36}O_4$ can be assigned to compound **4**. Its IR spectrum showed absorption bands for hydroxyl groups (3440 and 3325 cm^{-1}) and an ester (1740 cm^{-1}). Its ^1H NMR spectrum revealed three tertiary Me-groups (δ0.85, 0.89 and 0.91, each 3H, each *s*), an ABX-system at δ3.64 and 3.78 (each 1H, $J = 10.9$ and 5.4 Hz, after adding D_2O: AB, $^2J = 10.9$ Hz, $-CH_2OH$), and an axially oriented acetoxy group (δ2.08, 3H, *s*; 4.64, 1H, *t*, $^3J = 2.7$ Hz). These facts, together with the ^{13}C NMR data (Table 1), indicate that **4** is an acetoxy substituted phyllocladane-16,17-diol. Its position at C-3 was determined by a NOE experiment: when the signal at δ4.64 was irradiated, the signals of Me-18 (δ0.89) and Me-19 (δ0.85) showed a NOE, which establishes the structure of **4** as (16*R*)-3α-acetoxyphyllocladane-16,17-diol.

Compound **5a** has the molecular formula $C_{25}H_{38}O_5$ (CI-mass spectrum and elemental analysis). It exhibited the typical spectral features of a senecioyloxy derivative such as **2a** (δ1.92, 2.18, each 3H, each *d*, $^4J = 1.2$ Hz), 5.79 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz) and an additional carbonyl group (IR 1705 cm^{-1}, ^{13}C NMR δ209.2). In the ^1H NMR spectrum there are singlets for three tertiary Me-groups (δ1.12, 1.16 and 1.27, each 3H, each *s*) and the ABX-system for a CH_2OH substituent (δ3.65 and 3.80, each 1H, $J = 10.8$ and 4.7 Hz, after adding D_2O: AB, $^2J = 10.9$ Hz). The compound still has a tertiary hydroxyl group because acetylation (acetic anhydride–pyridine) afforded a monoacetate (**5b**), whose IR spectrum showed an OH-absorption band at 3580 cm^{-1}. Therefore, it is a senecioyloxy substituted 16,17-dihydroxyphyllocladanone derivative. The ^{13}C NMR data supported this assumption (Table 1).

The chemical shift and the multiplicity of the oxymethine proton (δ5.65, 1H, *dd*, $^3J = 13.4$ and 6.1 Hz, H_{ax}) clearly showed the ester substituent to be located in an equatorial position adjacent to a carbonyl group [8]. Because the signals of Me-18 and Me-19 are shifted paramagnetically (δ1.16 and 1.27) compared with those of **2a** (δ0.86, 0.99), this carbonyl group must be located at C-3 and the senecioyloxy substituent at C-2. Thus, the structure of **5a** is (16*R*)-2α-(3-methyl-2-butenoyloxy)-16,17-dihydroxyphyllocladane-3-one.

Phyllocladane-type (or 13β-kaurane) diterpenoids are rather rare in nature; most of the tetracyclic diterpenoids



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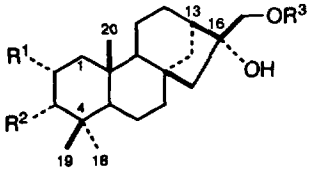
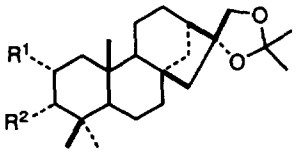
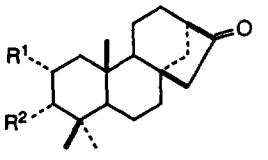


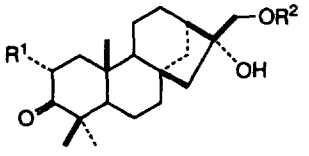
	R^1	R^2	R^3
2a	$OCOCH{=}CMe_2$ (21, 23)	OAc	H
2b	$OCOCH{=}CMe_2$	OAc	Ac
3a	$OCOCH_2CHMe_2$	OAc	H
3b	$OCOCH_2CHMe_2$	OAc	Ac
4	H	OAc	H



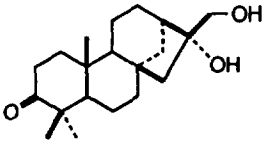
	R^1	R^2
2c	$OCOCH{=}CMe_2$	OAc



	R^1	R^2
2d	$OCOCH{=}CMe_2$	OAc



	R^1	R^2
5a	$OCOCH{=}CMe_2$	H
5b	$OCOCH{=}CMe_2$	Ac



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belong to the *ent*-kaurane series. This fact prompted us to confirm the structures of the new natural products, which were based only on spectroscopic data, and, especially, to determine the absolute configuration unambiguously. Therefore, the main constituent (**2a**) was chemically transformed to the dihydroxyketone **6**. The physical data, the chiroptical and spectral properties of the final product (**6**) corresponded to those of the known 16*R*,17-dihydroxyphyllocladane-3-one (calliterpenone) (**6**) [15–18]. Moreover, since several of the isolated compounds are correlated by interconversions, the new structures are established as **2a**, **2b**, **3a**, **3b**, **4** and **5a**. A full account of the synthetic part will be published elsewhere.

EXPERIMENTAL

General. ^{1}H NMR: $CDCl_3$ at 300 or 400 MHz; ^{13}C NMR: 50 MHz.

Plant material. Cuttings of *P. ambiguus* [2] (L.E. Codd, Acc.-Nr 411-67) from The Royal Botanical Gardens, Kew, U.K., were propagated and cultivated in the open in the surroundings of Zurich (1979). Plants from the original cuttings are kept in the greenhouse of our institute.

Extraction and isolation. Air-dried leaves and stems of *P. ambiguus* (350 g) were extracted with hexane (3 l, overnight) at room temp. and then re-extracted (×2) with Et_2O (3 l, 7 hr; 3 l, overnight) at room temp. The hexane extract was concd *in vacuo* to give a green semi-solid (1.55 g, 0.44%). The Et_2O extract was concd *in vacuo* to afford a green residue (9.01 g, 2.57%), which was partitioned between hexane (400 ml) and 90% MeOH (400 ml). The hexane layer was washed with 90% MeOH (200 ml) and the MeOH layers were combined and washed with hexane (3 × 200 ml) and then evapd *in vacuo* to afford a yellow solid (fr. A, 6.91 g, 1.97%). The hexane

extracts were combined and evapd *in vacuo* to give a green solid (fr. B, 2.02 g, 0.58%).

All frs were tested for their antioxidative activity using a modification of the method of ref. [19]. Among them, the main fr. A showed significant antioxidative activity.

Hexane–CH_2Cl_2 (1:6, 50 ml) was added to fr. A and the mixt. filtered. The filtrate was sepd on Sephadex LH-20 [increasing elution strength from hexane–CH_2Cl_2 (1:6), CH_2Cl_2, CH_2Cl_2–Me_2CO (1:1), Me_2CO] to give frs I–V. Only fr. III had significant antioxidative activity. Hexane–Et_2O (2:1, 100 ml) was added to III, which was filtered and the ppt. recrystallized from CH_2Cl_2–Me_2CO–MeOH to afford the flavone **1**. The filtrate and the mother liquor displayed no antioxidative activity. They were combined and repetitive CC [silica gel, pentane–Et_2O (1:2), CH_2Cl_2–MeOH (50:1), CH_2Cl_2–MeOH (25:1)] yielded a mixt. of **2b** and **3b** and a solid fr. The latter was further sepd by prep. HPLC [column: Nucleosil C-18, 10 μm, 25 × 2 cm, H_2O–MeOH (2.5:1), 17 ml min^{-1}; 70 bar] to give **2a**, **3a** and a mixt. of **4** and **5a**, which was crystallized from Et_2O to yield **5a**. The mother liquor was sepd by CC [silica gel, pentane–Et_2O (1:1), pentane–Et_2O (2:1), Et_2O] to yield **4** and more **5a**. CC of fr. II [silica gel, pentane, Et_2O–pentane (1:1)] afforded another portion of the mixt. of **2a** and **3b**. The following amounts of the pure compounds were isolated: **1** (0.092 g, 0.026%), **2a** (1.940 g, 0.55%), **2b**/**3b** (0.281 g, 0.08%), **3a** (1.015 g, 0.29%), **4** (0.364 g, 0.10%), and **5a** (0.450 g, 0.13%).

5,6-Dihydroxy-4',7-dimethoxyflavone (**1**) (*Ladanein or 4',7-dimethylscutellarein*). Yellow needles (CH_2Cl_2–Me_2CO–MeOH), mp 213–215°. The spectral data (UV/VIS, IR, ^{1}H NMR, EI and CIMS) were in full agreement with the reported values [3–7].

(16R)-2α-(3-*Methyl*-2-*butenoyloxy*)-3α-*Acetoxyphyllocladane*-16,17-*diol* (**2a**). Needles (Et_2O–hexane), mp 88–92°; $[\alpha]_D^{25}$ = + 5.7° (*c* 0.575, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 218 (4.15); IR ν_{max}^{KBr} cm^{-1}: 3200–3600 (*br*), 2940, 2870, 1746, 1718, 1650, 1452, 1375, 1240, 1142, 1075, 1048, 1032, 1010, 990; ^{1}H NMR: δ0.86 (3H, *s*, Me-19), 0.99 (3H, *s*, Me-18), 1.01 (3H, *s*, Me-20), 1.86, 2.13 (each 3H, each *d*, ^4J = 1.2 Hz, Me-24, Me-25), 2.11 (3H, *s*, AcO), 3.62, 3.67 (each 1H, ABX, ^2J = 10.8, $^3J_{17,OH}$ = 5.4 Hz, after adding D_2O: AB, ^2J = 10.9 Hz, H_2-17), 5.01 (1H, *d*, $^3J_{3,2}$ = 2.7 Hz, H-3), 5.25 (1H, *ddd*, $^3J_{2,lax}$ = 12.4, $^3J_{2,leq}$ = 4.5, $^3J_{2,3}$ = 2.7 Hz, H-2), 5.55 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz, H-22); ^{13}C NMR: Table 1: EIMS 70 eV, *m/z* (rel. int.): 431 (25) [M − CH_2OH]$^+$, 389 (6) [M − CH_2OH − ketene]$^+$, 362 (11): [M − Me_2C = $CHCO_2H$]$^+$, 331 (21) [M − CH_2OH − Me_2C = $CHCO_2H$]$^+$, 320 (76) [M − Me_2C = $CHCO_2H$ − ketene]$^+$, 302 (30) [M − Me_2C = $CHCO_2H$ − AcOH]$^+$, 289 (20) [331 − ketene]$^+$, 285 (21), 284 (22), [302 − H_2O]$^+$, 271 (100) [289 − H_2O]$^+$, 253 (53) [271 − H_2O]$^+$, 245 (11), 229 (14), 178 (10), 163 (11). Found: C, 69.91; H, 9.35. $C_{27}H_{42}O_6$ requires: C, 70.10; H, 9.15%.

(16R)-2α-(3-*Methyl*-2-*butenoyloxy*)-3α,17-*diacetoxy*-16-*hydroxyphyllocladane* (**2b**). A soln of **2a** (30 mg) in dry pyridine and excess Ac_2O was kept overnight at room temp. Work-up and CC [silica gel, pentane–Et_2O (1:2)] afforded **2b** (32 mg, 98%) as an oil. $[\alpha]_D^{25}$ + 10.0° (*c* 1.085, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 218 (4.14); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 2980, 2940, 2870, 1735 (*br*), 1648, 1452, 1375, 1248 (*br*), 1145, 1075, 1034, 990; ^{1}H NMR: δ0.87 (3H, *s*, Me-19), 1.00 (3H, *s*, Me-18), 1.01 (3H, *s*, Me-20), 1.87, 2.14 (each 3H, each *d*, ^4J = 1.1 Hz, Me-24, Me-25), 2.10, 2.11 (each 3H, each *s*, 2AcO), 4.16, 4.24 (each 1H, AB, ^2J = 11.3 Hz, H_2-17), 5.01 (1H, *d*, $^3J_{3,2}$ = 2.7 Hz, H-3), 5.25 (1H, *ddd*, $^3J_{2,lax}$ = 12.4, $^3J_{2,leq}$ = 4.4, $^3J_{2,3}$ = 2.7 Hz, H-2), 5.55 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz, H-22); ^{13}C NMR: Table; CIMS (2-methylpropane), *m/z* (rel. int.): 505 (36) [M + 1]$^+$, 488 (21), 487 (100), [M + 1 − H_2O]$^+$, 463 (12) [M + 1 − ketene]$^+$, 445 (26) [M + 1 − AcOH]$^+$, 427 (10) [M + 1 − H_2O − AcOH]$^+$, 405 (34) [M + 1 − Me_2C = $CHCO_2H$]$^+$, 345 (47) [405 − AcOH]$^+$, 327 (15) [345 − H_2O]$^+$. Comparison of the R_f, UV, IR, ^{1}H, ^{13}C NMR and MS proved this monoacetate to be identical in every respect with **2b**, present in a mixt. with **3b**.

Acetonide **2c**. A mixt. of **2a** (102 mg) in dry Me_2CO (10 ml) and $CuSO_4$ (150 mg) was heated under reflux for 5 hr under N_2. Work-up and CC [silica gel, hexane–CH_2Cl_2 (1:2)] afforded **2c** (101 mg, 91%) as crystals: mp 70°. IR ν_{max}^{KBr} cm^{-1}: 2980, 2938, 2868, 1745, 1715, 1650, 1454, 1375, 1240, 1142, 1055, 1034, 988, 895, 850; ^{1}H NMR: δ 0.87 (3H, *s*, Me-19), 1.00 (3H, *s*, Me-18), 1.01 (3H, *s*, Me-20), 1.34, 1.38 (each 3H, each *s*, 2Me of the acetonide), 1.86, 2.13, (each 3H, each *d*, ^4J = 1.1 Hz, Me-24, Me-25), 2.11 (3H, *s*, AcO), 2.26 (1H, *dd*, $^2J_{15\beta,15\alpha}$ = 14.6, $^4J_{15\beta,14\alpha}$ = 1.7 Hz, H-15β), 3.90, 4.06 (each 1H, AB, ^2J = 8.0 Hz, H_2-17), 5.01 (1H, *d*, $^3J_{3,2}$ = 2.7 Hz, H-3), 5.25 (1H, *ddd*, $^3J_{2,lax}$ = 12.4, $^3J_{2,leq}$ = 4.4, $^3J_{2,3}$ = 2.7 Hz, H-2), 5.55 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz, H-22); CIMS (2-methylpropane), *m/z* (rel. int.): 503 (60) [M + 1]$^+$, 487 (9), 461 (10) [M + 1 − ketene]$^+$, 445 (37), 443 (15) [M + 1 − AcOH]$^+$, 403 (44) [M + 1 − Me_2C = $CHCO_2H$)]$^+$, 386 (51), 385 (100) [403 − H_2O]$^+$, 344 (13), 343 (57) [M + 1 − Me_2C = $CHCO_2H$)]$^+$, 285 (36).

Oxidation of **2a** *to ketone* **2d**. A mixt. of **2a** (31 mg) in MeOH (2 ml) and H_5IO_6 (17 mg) was stirred for 40 min at room temp., then poured into H_2O and extracted with CH_2Cl_2. The organic phase was washed with H_2O, dried over $MgSO_4$, filtered, evapd and purified by CC (silica gel, CH_2Cl_2) to yield **2d** (25 mg, 87%) as crystals, mp 47–50°; $[\alpha]_D$ + 50.4° (*c* 0.28, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 218 (4.22); IR ν_{max}^{KBr} cm^{-1}: 2950, 2870, 1745, 1715, 1648, 1454, 1374, 1238, 1142, 1075, 1045, 1025, 1012; ^{1}H NMR: δ0.91 (3H, *s*, Me-19), 1.02 (3H, *s*, Me-18), 1.03 (3H, *s*, Me-20), 1.88, 2.15 (each 3H, each *d*, ^4J = 1.1 Hz, Me-24, Me-25), 2.13 (3H, *s*, AcO), 2.38 (1H, *m*, H-13), 2.66 (1H, *dd*, $^2J_{15\beta,15\alpha}$ = 18.6, $^4J_{15\beta,14\alpha}$ = 3.6 Hz, H-15β), 5.04 (1H, *d*, $^3J_{3,2}$ = 2.7 Hz, H-3), 5.28 (1H, *ddd*, $^3J_{2,1ax}$ = 12.4, $^3J_{2,1eq}$ = 4.4, $^3J_{2,3}$ = 2.7 Hz, H-2), 5.56 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz, H-22); CIMS (NH_3), *m/z* (rel. int.): 448 (100) [M + 1 + NH_3]$^+$, 406 (4) [M + 1 + NH_3 − ketene]$^+$.

(16R)-2α-(3-*Methylbutanoyloxy*)-3α-*acetoxyphyllocladane*-16,17-*diol* (**3a**). Flakes (Et_2O–hexane), mp 35°; $[\alpha]_D^{25}$ + 2.5° (*c* 0.16, $CHCl_3$); IR ν_{max} KBr cm^{-1}:

3600–3200 (*br*), 2940, 2870, 1745 (*br*), 1465, 1455, 1374, 1295, 1236 (*br*), 1190, 1154, 1120, 1050, 1032, 1015, 988; ^{1}H NMR: δ 0.86 (3H, *s*, Me-19), 0.98 (3H, *s*, Me-18), 1.00 (3H, *s*, Me-20), 0.92, 0.93 (each 3H, each *d*, ^3J = 6.4 Hz, Me-24, Me-25), 2.11 (3H, *s*, AcO), 3.62, 3.76 (each 1H, AB, ^2J = 10.9 Hz, H_2-17), 4.97 (1H, *d*, $^3J_{3,2}$ = 2.7 Hz, H-3), 5.23 (1H, *ddd*, $^3J_{2,1ax}$ = 12.4, $^3J_{2,1eq}$ = 4.4, $^3J_{2,3}$ = 2.7 Hz, H-2); ^{13}C NMR: Table 1; CIMS (NH_3), *m/z* (rel. int.): 482 (100) $[M + 1 + NH_3]^+$, 464 (6) $[M]^+$, 451 (11), 450 (45) $[M - CH_3OH]^+$, 440 (1) $[M -$ ketene$]^+$, 382 (14), 322 (9) $[382 - AcOH]^+$.

Catalytic reduction of **2a**. A mixt. of **2a** (14 mg) and Pd/C (9 mg) in dry MeOH (3 ml) was shaken for 2 hr under H_2 at room temp. Work-up afforded a solid (13 mg, 93%) which proved to be identical in every respect with the natural product **3a** (mp, mixed mp, R_f, $[\alpha]_D$, IR, ^{1}H, ^{13}C NMR, MS).

(16R)-2α-(3-*Methylbutanoyloxy*)-3α,17-*diacetoxy*-16-*hydroxyphyllocladane* (**3b**). A soln of **3a** (10 mg) in dry pyridine and excess Ac_2O was kept overnight at room temp. Work-up and CC [silica gel, Et_2O–pentane (1:1)] afforded **3b** (10 mg, 92%) as a viscous oil, $[\alpha]_D^{25}$ + 3.6° (*c* 0.45, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 2950, 2870, 1732, 1465, 1455, 1375, 1295, 1250, 1190, 1155, 1120, 1035, 988, 922; ^{1}H NMR: δ 0.87 (3H, *s*, Me-19), 0.94, 0.95 (each 3H, each *d*, ^3J = 6.3 Hz, Me-24, Me-25), 0.99 (3H, *s*, Me-18), 1.01 (3H, *s*, Me-20), 2.12 (6H, *s*, 2AcO), 4.18, 4.25 (each 1H, AB, ^2J = 11.3 Hz, H_2-17), 4.98 (1H, *d*, $^3J_{3,2}$ = 2.7 Hz, H-3), 5.25 (1H, *ddd*, $^3J_{2,1ax}$ = 12.4, $^3J_{2,1eq}$ = 4.4, $^3J_{2,3}$ = 2.7 Hz, H-2); ^{13}C NMR: Table 1; CIMS (NH_3), *m/z* (rel. int.): 524 (100) $[M + 1 + NH_3]^+$, 489 (31) $[M + 1 - H_2O]^+$, 464 (34), $[M -$ ketene$]^+$, 429 (1) $[M + 1 - AcOH]^+$. Comparison of the R_f, ^{1}H, ^{13}C NMR and MS proved this monoacetate to be identical in every respect with natural **3b**.

(16R)-3α-*Acetoxyphyllocladane*-16,17-*diol* (**4**). Needles (Et_2O), mp 122–123°; $[\alpha]_D^{25}$ − 32.3° (*c* 0.505, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3440, 3325, 2940, 2855, 1740, 1450, 1435, 1384, 1374, 1365, 1252, 1056, 1040, 1032, 1012, 990; ^{1}H NMR: δ0.85 (3H, *s*, Me-19), 0.89 (3H, *s*, Me-18), 0.91 (3H, *s*, Me-20), 2.08 (3H, *s*, AcO), 3.64, 3.78 (each 1H, ABX, ^2J = 10.9, $^3J_{17,OH}$ = 5.4 Hz, after adding D_2O: AB, ^2J = 10.9 Hz, H_2-17), 4.64 (1H, *t*, $^3J_{3,2}$ = 2.7 Hz, H-3); ^{13}C NMR: Table 1; CIMS (NH_3) *m/z*: 382 (29) $[M + 1 + NH_3]^+$, 364 (19) $[M]^+$, 346 (5) $[M - H_2O]^+$, 304 (10) $[M - AcOH]^+$, 288 (21), 287 (100) $[M + 1 - H_2O - AcOH]^+$, 273 (27), 269 (52) $[287 - H_2O]^+$.

(16R)-2α-(3-*Methyl*-2-*butenoyloxy*)-16,17-*dihydroxyphyllocladane*-3-*one* (**5a**). Flakes (Et_2O), mp 175–177°; $[\alpha]_D^{25}$ + 8.3° (*c* 1.20, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 219 (4.24); IR ν_{max}^{KBr} KBr cm^{-1}: 3525, 3580–3400 (*br*), 2930, 2860, 1720, 1705, 1648, 1480, 1458, 1390, 1380, 1235, 1158, 1125, 1075, 1068, 1050, 1023, 1008, 982, 968, 924, 870, 852; ^{1}H NMR: δ1.12 (3H, *s*, Me-20), 1.16 (3H, *s*, Me-19), 1.27 (3H, *s*, Me-18), 1.92, 2.18 (each 3H, each *d*, ^4J = 1.2 Hz, Me-24, Me-25), 3.65, 3.80 (each 1H, ABX, ^2J = 10.8, $^3J_{17,OH}$ = 4.7 Hz, after adding D_2O: AB, ^2J = 10.9 Hz, H_2-17), 5.65 (1H, *dd*, $^3J_{2,1ax}$ = 13.4, $^3J_{2,1eq}$ = 6.1 Hz, H-2), 5.79 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz, H-22); ^{13}C NMR: Table 1; CIMS (2-methylpropane), *m/z* (rel. int.): 419 (69) $[M + 1]^+$, 401 (100) $[M + 1 - H_2O]^+$, 387 (4) $[M + 1 -$ MeOH$]^+$, 336 (9) $[M + 1 - Me_2C{=}CHCO]^+$, 319 (12) $[M + 1 - Me_2C{=}CHCO_2H]^+$, 301 (40) $[M - H_2O - Me_2C{=}CHCO_2H]^+$, 283 (17) $[301 - H_2O]^+$, 101 (6), 83 (49). Found: C, 72.02; H, 9.38. $C_{25}H_{38}O_5$ requires: C, 71.74; H, 9.15%.

Acetate **5b**. A soln of **5a** (30 mg) in dry pyridine and excess Ac_2O was kept overnight at room temp. After work-up and prep. TLC [silica gel, CH_2Cl_2–MeOH (25:1)], crystallization from hexane–Et_2O afforded **5b** (30 mg, 91%) as plates, mp 136–138°; $[\alpha]_D^{25}$ + 5.9° (*c* 0.17, $CHCl_3$); IR ν_{max} KBr cm^{-1}: 3580, 2940, 2860, 1748, 1730, 1720, 1650, 1456, 1386, 1372, 1350, 1300, 1240 (*br*), 1146, 1124, 1068, 1030, 978, 918, 872, 850; ^{1}H NMR: δ1.12 (3H, *s*, Me-20), 1.16 (3H, *s*, Me-19), 1.27 (3H, *s*, Me-18), 1.92, 2.18 (each 3H, each *d*, ^4J = 1.1 Hz, Me-24, Me-25), 2.13 (3H, *s*, AcO), 4.19, 4.26 (each 1H, each *d*, AB, ^2J = 11.3 Hz, H_2-17), 5.65 (1H, *dd*, $^3J_{2,1ax}$ = 13.4, $^3J_{2,1eq}$ = 6.0 Hz, H-2), 5.79 (1H, *m*, *t*-like, $W_{1/2}$ *ca* 3 Hz, H-22); CIMS (NH_3), *m/z* (rel. int.): 478 (23) $[M + 1 + NH_3]^+$, 462 (25), 461 (100) $[M + 1]^+$, 443 (7) $[M + 1 - H_2O]^+$, 401 (7) $[M + 1 - AcOH]^+$, 378 (10) $[M + 1 + NH_3 - Me_2C{=}CHCO_2H]^+$, 362 (10), 361 (61) $[M + 1 - Me_2C{=}CH - CO_2H]^+$, 345 (11), 289 (7).

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