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5-METHYLCOUMARIN DERIVATIVES FROM *APHYLLOCLADUS DENTICULATUS*

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Key Word Index—*Aphyllocladus denticulatus* var. *denticulatus*; Compositae; 5-methylcoumarin derivatives; sesquiterpenes.

Abstract—The aerial parts of *Aphyllocladus denticulatus* afforded, in addition to some widespread compounds, six 5-methylcoumarin derivatives isolated previously from a *Lycoseris* species, and eight new ones. The structures were elucidated by high field NMR techniques. The chemotaxonomic relevance of the results is briefly discussed.

INTRODUCTION

The small genus *Aphyllocladus* (Compositae, tribe Mutisieae), distributed in the Andes from S Bolivia to N Chile, has been placed in the subtribe Gochnatiinae [1]. So far, no reports have been produced on its chemistry. As a continuation of our investigations of the tribe Mutisieae, we have studied *A. denticulatus* (Remy) Cabr. var. *denticulatus*.

RESULTS AND DISCUSSION

The extract of the aerial parts afforded, in addition to widespread compounds (see Experimental), the known 5-methylcoumarin derivatives **1** [2], **6** [2], **8** [2], **10** [2], **12** [2] and **13** [2], as well as eight further ones, the hydroxylycoserin **2**, which was isolated as its acetate **2a**, an isomer named isolycoserone (**3**) and its 10′-hydroxy derivative **4**, the 1′,2′,6′,7′,8′-epimer of **8** (**5**), a 10′,11′-dehydro derivative of **8** (**7**), a 10-hydroxy derivative of **8** (**9**), the nor compound **11** named aphyllocladone, and the partly rearranged nor derivative **14** named aphyllodenticulide.

The structure of **2** followed from the ^{1}H NMR data of the corresponding acetate **2a** (Table 1) which were similar to those of the corresponding 10′-desoxy derivative [2]. Spin decoupling indicated that the doublet at δ5.61 was the H-10′ signal. Accordingly, the additional oxygen function was at C-10′.

The ^{1}H NMR spectral data of **3**, **4** and the corresponding acetate **4a** (Table 1) were similar. Spin decoupling showed that in coumarin **4** an oxygen function at C-10′ was present. Furthermore, a broadened singlet around δ1.5 (3H) indicated an olefinic methyl group. The down field shift of H-5′ showed, in agreement with the mass spectrum, the presence of a 8′-keto group. A pair of doublets with a large geminal coupling in the spectra of all three compounds around δ2.9 was assigned to H-1′. This was supported by the MS which showed the fragments 189 ($C_{14}H_9O_3$), and 222 ($C_{14}H_{22}O_2$) indicating a preferred splitting of the 1′,2′-bond. In the MS of **4** elimination of Me_2CHCHO (*m/z* 354) was visible. Inspection of a model led to the proposal that a hydrogen bond between the 10′-hydroxyl and the 8′-keto group may be present. This as well as the whole stereochemistry was established by the observed NOEs. Thus clear effects were present between H-10′, H-3′, H-11′ and H-12′, between H-9 and H-6, between H-15′ and H-1′ as well as between H-13′ and H-9. The resulting configurations of C-2′, C-3′ and C-9′ are the same in compound **3**, as indicated by the similarity of the ^{1}H NMR signals. The ^{13}C NMR data of **4** also supported the structure. INEPT experiments were necessary for the complete assignment. Compound **3** has been named isolycoserone.

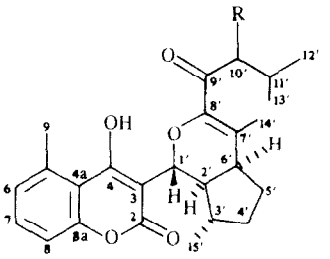
The ^{1}H NMR spectrum of **4a** at room temperature was highly broadened indicating a mixture of conformers most likely due to restricted rotation. At elevated temperature clear signals were observed which allowed spin decoupling.

The ^{1}H NMR spectrum of **5** (Table 1) was in part similar to that of cyclolycoserone (**8**) [2]. However, some chemical shifts differed and the optical rotation had the opposite sign. The observed NOEs indicated that the configurations of C-1′, C-2′, C-6′, C-7′ and C-8′ were reversed but not that of C-3′. Thus, clear effects were observed between H-14′, H-6′, H-9 and H-7′, between H-15′, H-7′, H-1′ and H-3′, between H-6′, H-14′ and H-2′, as well as between H-7′, H-14′ and H-15′. The stereochemistry agrees with the results of a chiral synthesis of lycoserone, cyclolycoserone and also of the epimer **5** [3]. Therefore, the absolute configuration of these compounds could also be established.

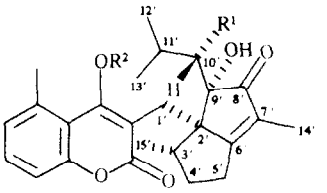
The ^{1}H NMR data of **7** (Table 1) were similar to those of cyclolycoserone [2]. The presence of a 10′,11′-double bond followed from its typical signals [δ6.45 *qq*, 2.24 *d* (3H), 1.99 *d* (3H)]. Similarly, the spectrum of **9** pointed to a 10′-hydroxy derivative of **8**. Thus, an additional low field signal at δ4.81 was observed which showed a 7 Hz coupling with a signal at δ2.13 *d* (hydroxy proton). Most likely, a hydrogen bond between the hydroxy group and an ether oxygen was present. However, the relative configuration at C-10′ could not be assigned.

Compounds **12** and **13** were isolated previously as a mixture [2]. After acetylation, the corresponding acetates **12a** and **13a** could be separated by repeated TLC.

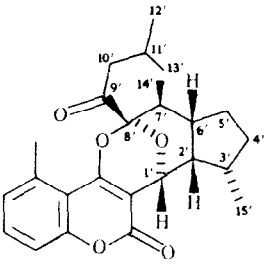
The ^{1}H NMR data (Table 1) and the molecular formula of **11** indicated that most likely a benzofuran de-



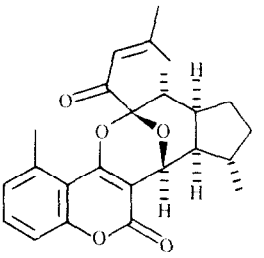
1 R = H(1″-*epi*)
2 R = OH
2a R = OAc



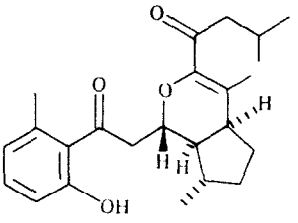
3 $R^1 = R^2 = H$
4 R^1 = OH, R^2 = H
4a R^1 = OAc, R^2 = Ac



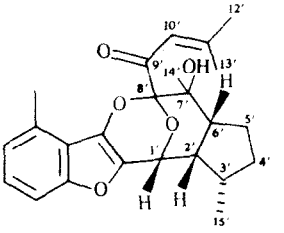
5
6 6.7 epoxide



7
8 10.11 H
9 10-OH; 10.11 H



10

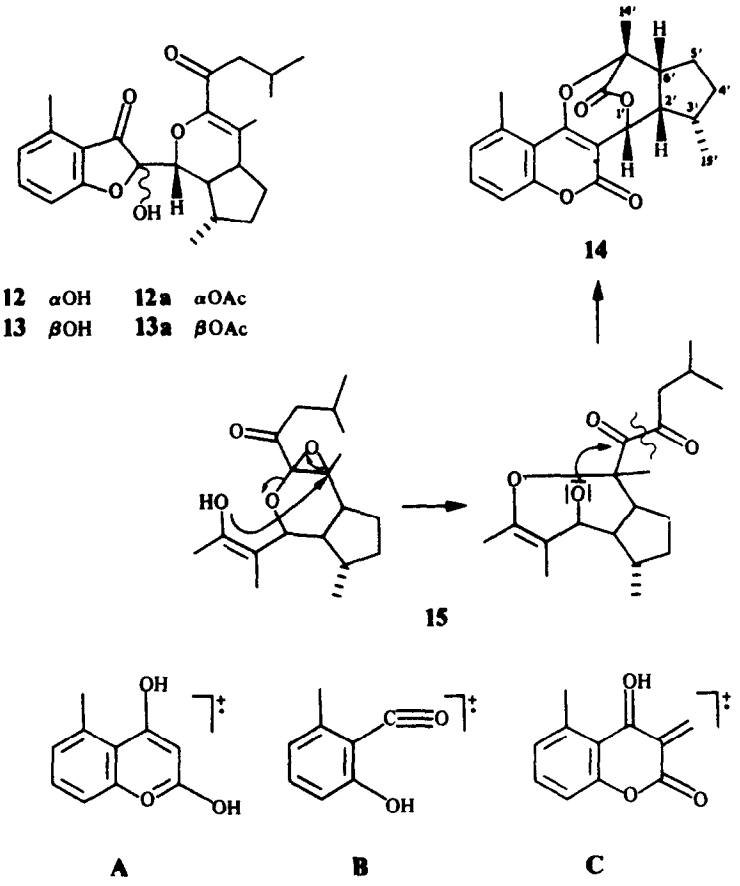


11

rivative was present. In agreement with this proposal the signals of the aromatic protons were shifted up field. Spin decoupling further showed that the sequence H-1′ to H-6′ was identical with that of **12**. The nature of the side chain at C-8′ followed from the typical senecioyl signals. The configurations of C-7′ could not be assigned. Most likely ketone **11** is closely related to **12**. If the 7′,8′-epoxide of the 2-desoxy derivative of 10′,11′-dehydro **12** were assumed as a precursor, the corresponding enol of the 3-keto group could open the epoxide by formation of the proposed ketal. Ketone **11** has been named aphyllocladone.

The structure of **14** was deduced from the molecular formula ($C_{20}H_{20}O_5$) and the ^{1}H NMR spectrum (Table 1). Spin decoupling gave the sequence H-1′ to H-6′ and the position of the secondary methyl (H-15). The chemical shift of the methyl singlet (δ1.79) required several deshielding effects which are present only at the proposed carbon. The presence of a lactone followed from the IR band at 1760 cm^{-1} and by INEPT of H-14′ with C-7′ and the carbonyl carbon as well as of H-1′ with the latter. The observed NOEs supported the structure and allowed the assignment of the stereochemistry. Thus, clear effects were present between H-15′ and H-1′, between H-14′, H-6′ and H-9, between H-3′ and H-2′, between H-9, H-14′ and H-6′, between H-2′, H-3′, H-6′ and H-1′, as well as between H-6′ and H-2′. The ketone **14**, which has been named aphyllodenticulide, is most likely formed via the proposed precursor epoxide **15**, by isomerization and oxidative cleavage of the diketone **16** (see Scheme). The structure was established by synthesis [3].

The isolation of the 5-methylcoumarins is of chemotaxonomic interest. These unusual compounds have so far only been reported from representatives of the subtribe Mutisiinae with the exception of *Lycoseris*. This latter genus, as well as *Aphyllocladus*, is placed in subtribe Gochnatiinae [1, 4], where sesquiterpene lactones are widespread. However, these compounds are missing in the Mutisiinae. Therefore a reinvestigation of the placement of *Lycoseris* and *Aphyllocladus*, both of which contain very similar constituents and are shrubs with secretory canals as in *Mutisia* [1], may be worthwhile, especially since the systematics of this tribe seems still to be a problem [1].



12 αOH **12a** αOAc
13 βOH **13a** βOAc

14

15

A **B** **C**

EXPERIMENTAL

The air-dried aerial parts (560 g, collected E of Puquios, Region de Atacama, Chile, voucher Conc. 72992) were extracted and worked-up as reported previously [5]. CC fractions were combined into four fractions [1: petrol; 2: Et_2O–petrol, (1:9); 3: Et_2O–petrol, (1:1); 4:Et_2O]. TLC of fraction 1 (petrol) gave 100 mg γ- and 20 mg δ-cadinene. Fraction 2 gave 500 mg lupeyl-acetate and fraction 3 gave by HPLC (MeOH–H_2O, 9:1; RP 8, flow rate 3 ml/min in all separations) six fractions (3/1–3/6). Fraction 3/1 gave after separation by HPLC 2 mg **11** (R_t 1.6 min). Fraction 3/2 was separated by TLC (Et_2O–petrol 1:1) affording 15 mg spathulenol and 20 mg of a mixture of **12** and **13** (*ca* 1:1), which was acetylated (Ac_2O, $CHCl_3$, DMAP, 1 hr 60°) affording **12a/13a**. Separation was achieved by TLC [Et_2O–petrol (1:9), six developments]. Fraction 3/3 gave by TLC [Et_2O–petrol (1:3)] 10 mg caryophyllenepoxide, 15 mg cadinol T, 3 mg **10** and 2 mg **9** [purified by HPLC [MeOH–H_2O (17:3), R_t 11.0 min]. Fraction 3/4 contained 30 mg **1**, and fraction 3/5 gave by TLC (Et_2O–petrol, 1:3) 5 mg **6** and 3 mg **7** (R_f 0.38). TLC of fraction 3/6 (Et_2O–petrol, 1:3) afforded 15 mg **8** and 17 mg **5** (R_f 0.48). CC of fraction 4 was separated by flash chromatography (Si gel, 30–60 μ, Et_2O–petrol mixtures) into three fractions (4/1–4/3). Fraction 4/1 turned out to be inseparable mixture (^{1}H NMR showed no acetate methyl) which was acetylated (s.a.). TLC of the acetates (Et_2O–petrol, 1:1, two developments) gave 20 mg **2a** (R_f 0.70), 2 mg **14** (R_f 0.50) and 10 mg **3** (R_f 0.35). Fraction 4/2 contained a mixture of unidentified triterpenes and fraction 4/3 gave by TLC (Et_2O) 15 mg **4** (R_f 0.35). Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material.

10′-Acetoxy-1′-epilycoserone (**2a**). Colourless gum; IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3300 (OH), 1730 (C=O), 1630, 1610, 1570 (aromatic); MS *m/z* (rel. int.): 468 $[M]^+$ (1), 408.194 $[M-HOAc]^+$ (29) (calc. for $C_{25}H_{28}O_5$: 408.194), 365 $[408-C_3H_7]^+$ (36), 325 $[408-C_4H_7CO]^+$ (26), 229 (44), 189 $[C]^+$ (95), 177 $[A]^+$ (48), 135 $[B]^+$ (100); $[\alpha]_D^{24°}$ +104 ($CHCl_3$; *c* 1.3).

Isolycoserone (**3**). Colourless gum; IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3500–2600, 1735, 1640, 1630, 1580 (4-hydroxycoumarin), 1735 (C=O), 1670 (C=CC=O); MS *m/z* (rel. int.): 410.210 $[M]^+$ (8) (calc. for $C_{25}H_{30}O_5$: 410..209), 396 $[M-H_2O]^+$ (4.5), 323 (8), 222 (44), 221 (40), 204 (100), 189 $[C]^+$ (51), 135 $[B]^+$ (57); $[\alpha]_D^{24}$ −21 ($CHCl_3$; *c* 0.42).

10-Hydroxyisolycoserone (**4**) Colourless gum, IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3500–2600, 1730, 1640, 1620, 1570 (hydroxycoumarin); 1670 (C=CC=O); MS *m/z* (rel. int.). 426.204 $[M]^+$ (1.3) (calc. for $C_{25}H_{30}O_6$: 426.204), 354 $[M-OCHCHMe_2]^+$ (20), 248 (20), 189 (40), 177 $[A]^+$ (100), 135 $[B]^+$ (66); ^{13}C NMR ($CDCl_3$) C-2–C-9: δ166.7 *s*, 100.1 *s*, 164.4 *s*, 115.1 *s*, 138.1 *s*, 127.4 *d*, 130.8 *d*, 114.5 *d*, 154.0 *s*, 21.9 *q*; C-1′–C-15′: 32.9 *t*, 61.8 *s*, 29.9 *t*, 27.2 *t*, 26.4 *t*, 184.7 *s*, 130.3 *s*, 206.0 *s*, 86.8 *s*, 78.3 *d*, 37.4 *d*, 14.9 *q*, 14.1 *q*, 8.5 *q*, 23.5 *q*. Acetylation (Ac_2O, 1 hr, 70°) gave **4a**; colourless gum; IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3520 (OH), 1785 (C=C–OAc), 1730 (C=O, OAc); MS *m/z* (rel. int.): 510.225 $[M]^+$ (1) (calc. for $C_{29}H_{34}O_8$: 510.225), 450 $[M-ketene]^+$ (5.5), 390 $[450-HOAc]^+$ (2.5), 262 (28), 220 (100), 219 (88), 177 $[A]^+$ (36), 135 $[B]^+$ (26).

1′,2′,6′,7′,8′-Epicyclolycoserone (**5**). Colourless gum; IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1730 (C=O), 1720, 1630, 1605 (coumarin); MS

Table 1. ^{1}H NMR spectral data of **2a**, **3**, **4**, **4a**, **5**, **7**, **9**, **11** **12a**, **13a** and **14** (400 MHz, $CDCl_3$, δ-values)

H	**2a***	**3*‡**	**4†**	**4a***(75§)	**5***	**7†**	**9§**	**11†**	**12a†**	**13a†**	**14†**
6	6.61 *br d*	6.67 *br d*	6.97 *br d*	6.60 *br d*	6.68 *br d*	7.08 *br d*	6.64 *br d*	6.74 *br d*	6.84 *br d*	6.84 *br d*	7.05 *br d*
7	6.85 *t*	6.85 *t*	7.28 *t*	6.83 *t*	6.91 *t*	7.39 *t*	6.88 *t*	7.09 *t*	7.42 *t*	7.41 *t*	7.40 *t*
8	6.93 *br d*	6.96 *br d*	7.04 *br d*	6.87 *br d*	6.99 *br d*	7.20 *br d*	6.96 *br d*	6.63 *br d*	6.84 *br d*	6.80 *d*	7.17 *br d*
9	2.68 *br s*	2.84 *br s*	2.70 *br s*	2.34 *br s*	2.71 *br s*	2.70 *br s*	2.58 *br s*	2.60 *br s*	2.55 *br s*	2.61 *br s*	2.66 *br s*
1′	5.13 *d*	2.93 *d* 2.67 *d*	2.87 *d* 2.77 *d*	3.02 *d* 2.81 *d*	5.34 *br s*	5.08 *br s*	5.19 *br s*	4.47 *s*	3.98 *d*	4.00 *d*	5.85 *d*
2′	1.85 *m*	—	—	—	2.18 *m*	1.72 *br dd*	1.62 *br dd*	2.24 *t*	2.18 *m*	2.18 *m*	2.77 *br t*
3′	2.31 *m*	2.04 *m*	2.24 *m*	2.25 *m*	2.12 *m*	2.32 *m*	2.22 *m*	2.17 *m*		2.24 *m*	2.40 *br dq*
6′	2.01 *m*	—	—	—	1.37 *m*	1.82 *m*	1.39 *m*	2.47 *m*	2.46 *m*	2.58 *m*	3.27 *m*
7′	—	—	—	—	1.94 *dq*	2.11 *dq*	2.19 *dq*	—	—	—	—
$10'_1$	5.61 *d*	1.42 *dd*	3.51 *br d*	4.80 *d*	2.54 *dd*	6.45 *qq*	4.81 *dd*	6.32 *qq*	2.03 *dd*	1.94 *dd*	—
$10'_2$		1.27 *dd*			2.46 *dd*				1.74 *dd*	1.50 *dd*	—
11′	2.20 *dqq*	1.61 *tqq*	1.50 *m*	2.14 *dqq*	2.32 *tqq*	—	2.33 *dqq*	—	1.88 *tqq*	1.83 *tqq*	—
12′	0.94 *d*	0.78 *d*	0.75 *d*	1.03 *d*	0.92 *d*	2.24 *d*	0.83 *d*	2.02 *d*	0.73 *d*	0.72 *d*	—
13′	0.96 *d*	0.70 *d*	0.90 *d*	0.91 *d*	0.90 *d*	1.99 *d*	1.12 *d*	1.82 *d*	0.65 *d*	0.58 *d*	—
14′	2.13 *br s*	1.52 *br s*	1.54 *br s*	1.51 *br s*	0.83 *d*	0.94 *d*	0.72 *d*	1.48 *s*	1.96 *s*	1.95 *s*	1.79 *s*
15′	0.71 *d*	0.98 *d*	1.20 *d*	1.12 *d*	1.16 *d*	1.18 *d*	1.05 *d*	1.16 *d*	1.19 *d*	1.21 *d*	1.12 *d*
OAc	1.76 *s*			1.98 *s* 1.86 *s*	—	—	—	—	2.13 *s*	2.12 *s*	—

*In C_6D_6; in $CDCl_3$; ‡H-5′ 3.38 *br dd* (J = 18, 9 Hz) and 1.99 *m*; §OH 2.13 *d*; J [Hz]: 6′,7′ = 7,8 = 8; 3′,15′ = 11′,12′ = 11′,13′ = 7; compound **2a**: 1′,2′ = 11.5; 10′,11′ = 5.5; compound **3**, **4** and **4a**: $1'_1$,$1'_2$ = 14; compound **3**: $10'_1$,$10'_2$ = 14; 10′,11′ = 5; compounds **4** and **4a**: 10′,11′ = 4; compound **5**: 6′,7′ = 7′,14′ = 7; $10'_1$$10'_2$ = 17.5; 10′,11′ = 7; compounds 7 and **9**: 2′,3′ = 10; 2′,6′ = 6; 6′,7′ = 10; (compound **7**: 10′,12′ = 10′,13′ = 1; compound **9**: 10′,11′ = 2.3; 10′, OH = 7); compound **11**:'2′,3′ = 2′,6′ = 9; 10′,12′ = 10′,13′ = 1; compounds **12a** and **13a**: 7.5; 10_1',$10'_2$ = 17; 10′,11′ = 7; compound **14**: 1′,2′ = 1; 2′,3′ = 2′,6′ = 9.5.

m/z (rel. int.): 410.209 $[M]^+$ (10) (calc. for $C_{25}H_{30}O_5$: 410.209), 392 $[M-H_2O]^+$ (2), 326 $[M-C_5H_8O]^+$ (36), 325 $[M-COCH_2CHMe_2]^+$ (56), 308 $[326-H_2O]^+$ (58), 189 $[C]^+$ (100), 135 $[B]^+$ (84); $[\alpha]_D^{24°}$ −78 ($CHCl_3$; *c* 1.62).

10′,11′-*Dehydrocyclolycoserone* (**7**). Colourless gum; IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1710 (coumarin, C=CC=O); MS *m/z* (rel. int.): 408.194 $[M]^+$ (6.4) (calc. for $C_{25}H_{28}O_5$: 408.194), 326 $[M-C_5H_6O]^+$ (2), 325 $[M-C_5H_7O]^+$ (6), 308 $[326-H_2O]^+$ (24), 189 $[C]^+$ (12), 135 $[B]^+$ (14), 83 $[C_4H_7CO]^+$ (100).

10′-*Hydroxycyclolycoserone* (**9**). Colourless gum; IR $\nu_{max}^{CCl_4}$, cm^{-1}: 3500 (OH), 1720, 1625, 1600 (coumarin), 1720 (C=O); MS *m/z* (rel. int.): 426.204 $[M]^+$ (12) (calc. for $C_{25}H_{30}O_6$: 426.204), 326 $[M-C_5H_8O_2]^+$ (42), 325 $[M-C_5H_9O_2]^+$ (52), 308 $[326-H_2O]^+$ (7), 246 (73), 189 $[C]^+$ (100), 177 $[A]^+$ (11), 135 $[B]^+$ (76).

Aphyllocladone (**11**). Colourless gum; IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1700 (C=CC=O); MS *m/z* (rel. int.): 396.194 $[M]^+$ (10) (calc. for $C_{24}H_{28}O_5$: 396.194), 233 (28), 177 $[A]^+$ (8), 135 $[B]^+$ (34), 83 $[C_4H_7CO]^+$ (100).

3-*Acetoxycoumarolycoserone* (**13a**). Colourless gum; MS *m/z* (rel. int.): 440.220 $[M]^+$ (35) (calc. for $C_{26}H_{32}O_6$: 440.220), 380 $[M-HOAc]^+$ (5), 296 (76), 295 $[380-C_5H_9O]^+$ (100), 235 (60), 175 (26), 161 (38), 137 (43), 136 (62), 135 (50), 91 (58); ^{13}C NMR ($CDCl_3$) C-3–C-9: δ101.8 *s*, 196.9 *s*, 119.7 *s*, 138.9 *s*, 124.0 *d*, 137.0 *d*, 109.3 *d*, 142.8 *s*, 22.2 *q*; C-1′–C-15′: δ76.2 *d*, 43.1 *d*, 36.8 *d*, 33.9 *t*, 31.5 *t*, 43.0 *d*, 167.5 *s*, 124.1 *s*, 198.2 *s*, 47.5 *t*, 22.3 *d*, 17.8 *q*, 17.0 *q*, 22.2 *q*, 23.2 *q*; OAc: δ20.5 *q*, 170.1 *s*.

Aphyllodenticulide (**14**). Colourless crystals, mp 221–223° (Et_2O); IR $\nu_{max}^{CCl_4}$, cm^{-1}: 1760 (lactone), 1715, 1620, 1610 (coumarin); MS *m/z* (rel. int.). 340.131 $[M]^+$ (100) (calc. for $C_{20}H_{20}O_5$: 340.131), 214 $[M-C_7H_{10}O_2]^+$ (93), 190 (82), 189 [214–Me]$^+$ (67), 135 $[B]^+$ (70), 107 $[135-CO]^+$ (54).

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