Phytochemistry, Vol. 28, No. 1, pp. 173-175, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

β-AGAROFURANE SESQUITERPENE ESTERS FROM *MAYTENUS CANARIENSIS*

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(*Received in revised form* 7 *June* 1988)

Key Word Index—*Maytenus canariensis*; Celastraceae; β-dihydroagarofurane sesquiterpenes.

Abstract—Two sesquiterpene polyesters with new polyhydroxy skeletons were isolated from the aerial part of *Maytenus canariensis*. These compounds were identified by chemical degradation and spectroscopy as 1α,9α-dibenzoyloxy-6β,8α,15-triacetoxy-4β-hydroxy-dihydro-β-agarofurane and 1α,9α-dibenzoyloxy-2α,6β,8α,15-tetracetoxy-4β-hydroxydihydro-β-agrofurane.

INTRODUCTION

Maytenus canariensis (Loes.) Kunk. et Sund. [1] was once considered a species of the genus *Catha* Forsk [2] among others [3, 4], but at the present is placed in *Maytenus* Feuill. It is one of the Celastraceae used in folk medicine and, hence, of interest in our programme of intensive research into such species. Various nortriterpene quinonoid compounds [5] with antitumour properties [6] have already been reported together with triterpenes [7] and chemotaxonomic evidence connecting this plant to the monospecies *Catha edulis* [8]. A further study of the minor constituents from the aerial part of *M. canariensis* has yielded epifriedelinol [9] and cerin [10], known triterpenes not previously isolated from this plant, together with two new sesquiterpene esters (**1** and **5**).

RESULTS AND DISCUSSION

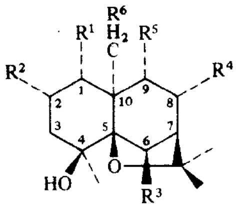
Compound **1** was assigned the molecular formula $C_{35}H_{40}O_{12}$ and the structure 1α,9α-dibenzoyloxy-6β, 8α,15-triacetoxy-4β-hydroxydihydro-β-agarofurane on the basis of the following evidence: IR absorption bands at 3670 (OH), and 1725 and 1720 (COO) cm^{-1}, a benzoate chromophore in the UV spectrum [11] which was confirmed by the loss of m/z 122 (C_6H_5COOH) units in the mass spectrum, and signals for 10 protons between δ6.86 and 7.60 in the ^1H NMR spectrum. The three singlets of three protons each which appear at δ2.04, 2.14 and 2.35 confirm the presence of three acetate groups as do fragments corresponding to the repeated loss of 60 (AcOH) and 42 ($CO=CH_2$) units in the MS.

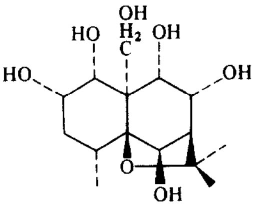
Compound **1** is unaltered when treated with acetic anhydride in pyridine. This, taken together with the IR data and the presence of a singlet proton exchangeable with D_2O at δ2.70 in ^1H NMR, indicates that the molecule contains a tertiary hydroxy group.

Thus **1** has two benzoates, three acetates and a tertiary hydroxyl group. The ^1H and ^{13}C data (see Experimental) being those characteristic of a dihydro-β-agarofurane skeleton [12]. NOE experiments sited the tertiary hydroxy group at C-4 in the β configuration. An ABX system was also visible in the ^1H NMR spectrum with signals at δ5.76 (*d*, $J=5.8$), 5.54 (*dd*, $J=4.1$, 5.8) and 2.37 (*d*, $J=4.1$) for H-9, H-8 and H-7, respectively, according to double resonance experiments. No NOE effect between H-6 (with α disposition, $J_{6,7} < 1$ Hz) and H-8 being observed. Therefore H-8 must have the β configuration, like H-9 in alatol (**9**) [13], where the J_{7-8} and J_{8-9} are identical to those of **1**. The NOE effect seen between H-6 and the methyl at C-4 means that the configuration of this latter is α and consequently that of the tertiary hydroxy group is β. A proton which appears as a doublet at δ5.56 ($J=4$, 12.5) was assigned to H-1 with axial disposition, as in all the dihydroagarofurane-skeleton sesquiterpenes isolated from Celastraceae [14]. An AB system at δ4.72 and 5.22 ($J=13.3$) was assigned to the protons of a methylene attached to an ester group. Its chemical shift and coupling constant [15] placed it at C-15.

As the signals for acetate methyls (δ2.04–2.35) and aromatic protons (δ6.86–7.60) appear in the usual regions, the substituents at C-1 and C-9 cannot be 1α–9β since this would have produced important diamagnetic shifts of the signals [16, 17], and so the 1α, 9α stereochemistry was confirmed. The distribution of the ester groups remained to be solved. Partial hydrolysis of **1** with 0.1 M sodium hydrogen carbonate in methanol gave three products, **2**–**4**, from the structures of which it was possible to arrive at an unequivocal assignment for **1**. Compound **2** proved to be the 6-deacetylderivative of **1** as shown by its ^1H NMR spectrum where the signal for the H-6 proton was shifted from δ6.86 in **1** to 5.34 in **2** while the acetate methyl signal which in **1** was at δ2.14 disappeared completely. Thus one acetate was located at C-6. Product **3** is the 1α-debenzoyloxy-6β-deacetylderivative of **1** as is shown by the disappearance of the signals for aromatic protons and the shift of H-1 from δ5.56 in **1** to 5.35 in **3** and that of H-6 from δ6.86 in **1** also to 5.35 in **3**; one of the benzoates can therefore be sited at C-1. Compound **4** proved to be the 6β,8α,15-trideacetylderivative of **1**: the three acetate methyls disappear while the C-6, C-8 and C-15 acetate protons shift from δ6.86, 5.54 and 4.72–5.22 in **1** to 5.62, 4.43 and 4.31–4.57, respectively, in **4**, which

	R^1	R^2	R^3	R^4	R^5	R^6
1	OBz	H	OAc	OAc	OBz	OAc
2	OBz	H	OH	OAc	OBz	OAc
3	OH	H	OH	OAc	OBz	OAc
4	OBz	H	OH	OH	OBz	OH
5	OBz	OAc	OAc	OAc	OBz	OAc
6	OBz	OAc	OH	OAc	OBz	OAc
7	OBz	OAc	OH	OH	OBz	OAc
8	OBz	OH	OH	OAc	OBz	OH





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establishes the structure of **1** quite definitely. The basic polyhydroxy skeleton of compound **1** is an isomer of alatol (**9**) [18].

The natural product **5** has the formula $C_{37}H_{42}O_{14}$ and its spectroscopic properties are similar to those of **1**, the most notable difference being that **5** has an extra acetate on C-2 with the same stereochemistry as in alatol, i.e. 2α on the basis of the following data. The protons on C-1 and C-2 and C-3 are observed as an ABX_2 system at δ 5.77, 5.47 and 2.24 and have coupling constants similar to those found in alatol derivatives [13]. The rest of the data indicate OH functionalization at 4, i.e. δ 2.75 exchangeable with D_2O. The data for the 8α, 9α and 15 groups (See 1H NMR data, Table 1) agree with those of **1**. The four acetates and two benzoates were assigned by a study of the partial hydrolysis products of **5**, namely. **6–8** (Table 1), which indicate that the polyhydroxy skeleton of this compound is that of 4β-hydroxyalatol [18].

EXPERIMENTAL

The plant was collected in Icod, Tenerife and a voucher specimen is lodged with the Departamento de Biologia Vegetal of the Facultad de Ciencias Biologicas, Universidad de La Laguna.

The aerial part of the plant (7 kg) was extracted with cold EtOH. 100 g of the extract was chromatographed on Sephadex LH20 using a *n*-hexane–HCl_3–MeOH (2 : 1 : 1) as eluent, followed by repeated chromatography on silica gel with mixtures of *n*-hexane–$CHCl_3$–MeOH and *n*-hexane–EtOAc in dioxane. Friedelin, *epi*-friedelinol, cerin and 30-hydroxyfriedelan-3-one were obtained as were **1** (52 mg) and **5** (38 mg). The ^1H NMR spectra were taken in $CDCl_3$.

1α,9α-Dibenzoyloxy-6β,8α,15-triacetoxy-4β-hydroxydihydro-β-agarofurane (**1**). Isolated as a crystalline solid, mp 212–213°, $[M - MeCOOH]^+$ at *m/z* 592.2394 (calc. for $C_{33}H_{36}O_{10}$, 592.2480); UV λ_{max}^{EtoH} nm: 230, 270; IR ν_{max} cm^{-1}: 3670, 3540, 3010, 2990, 2840, 1725, 1720, 1450, 1365, 1275, 1230, 1105, 1090, 710; ^1H NMR (200 MHz) δ: 1.41 (3H, *s*), 1.57 (3H, *s*), 1.68 (3H, *s*), 2.04 (3H, *s*), 2.14 (3H, *s*), 2.35 (3H, *s*), 2.37 (1H, *d*, $J = 4.1$ Hz), 2.70 (1H, *s*), 4.72, 5.22 (2H, d_{AB}, $J = 13.3$ Hz), 5.54 (1H, *dd*, $J = 5.8, 4.1$ Hz), 5.56 (1H, *dd*, $J = 12.5, 4.0$ Hz), 5.76 (1H, *d*, $J = 5.8$ Hz), 6.86 (1H, *br s*), 6.86 (1H, *t*), 7.18 (3H, *m*), 7.39 (2H, *m*), 7.60 (4H, *m*); ^{13}C NMR δ: 72.67 (C-1), 25.17 (C-2), 37.94 (C-3), 70.59 (C-4), 92.20 (C-5), 78.59 (C-6), 53.24 (C-7), 75.45 (C-8), 70.17 (C-9), 52.57 (C-10), 82.68 (C-11), 24.27 (C-12), 29.45 (C-13), 22.75 (C-14), 60.58 (C-15); EIMS *m/z* (rel. int.) 592 $[M - 60]^+$ (3), 550 (3), 530 (1), 488 (1), 470 (1), 428 (2), 306 (2), 288 (2), 268 (4), 122 (2), 105 (100), 83 (100).

Table 1. ^1H NMR data for compounds **1–8** (200 MHz, $CDCl_3$)

	H-1	H-2	H-6	H-7	H-8	H-9	H-15	AcO–
1	5.56 *dd* $J = 4.0, 12.5$		6.86 *br s*	2.37 *d* $J = 4.1$	5.54 *dd* $J = 4.1, 5.8$	5.76 *d* $J = 5.8$	4.72–5.22 *d* $J_{AB} = 13.3$	2.04, 2.14, 2.35
2	5.62 *dd***		5.34 *br s*	2.45 *d* $J = 4.2$	5.59 *d*** $J = 4.2, 5.3$	5.76 *d* $J = 5.3$	4.85–5.07 $J_{AB} = 13.4$	2.24, 2.45
3	5.35 *m***		5.35 *br s*	2.67 *d* $J = 4.0$	5.76 *dd* $J = 4.0, 5.5$	5.83 *d* $J = 5.5$	4.83–5.02 $J_{AB} = 13.1$	2.00
4	5.62 *dd* $J = 4.5, 12.5$		5.62 *br s*	2.45 *d* $J = 4.1$	4.43 *dd* $J = 4.1, 5.3$	5.71 *d* $J = 5.3$	4.31–4.57 *d* $J_{AB} = 12.6$	
5	5.77 *d* $J = 3.8$	5.47 *m*	6.97 *br s*	2.42 *d* $J = 4.0$	5.57 *dd* $J = 4.0, 6.2$	5.72 *d* $J = 6.2$	5.05–5.29 $J_{AB} = 13.5$	2.05, 2.07, 2.15, 2.37
6	5.76 *d* $J = 3.0$	5.44 *m*	5.43 *br s*	2.40 *d* $J = 4.0,$	5.60 *dd* $J = 4.0, 5.0$	5.73 *d* $J = 5.0$	5.00–5.23 *d* $J_{AB} = 12.5$	2.00, 2.06, 2.28
7	5.80 *d* $J = 5.0$	5.47 *m***	5.77 *br s*	2.70 *d* $J = 4.0$	5.47 *m***	5.70 *d* $J = 6.0$	4.90–5.35 *d* $J_{AB} = 12.5$	2.03, 2.28
8	5.76 *d* $J = 3.0$	4.42 *m*	5.56 *br s*	2.49 *d* $J = 4.0$	5.37 *dd* $J = 6.0, 4.0$	5.58 *d* $J = 6.0$	4.46–4.75 *d* $J_{AB} = 12.5$	2.09

*The signal could not be observed owing to overlapping. *J* values in Hz.

A soln of **1** (37 mg) in MeOH (4 ml) was treated with 0.1 M $NaHCO_3$ (2 ml) and then refluxed at 50° for 8 hr. After extraction and chromatography, **2–4** were obtained.

Compound **2** was an amorphous solid, mp 52–54°; UV λ_{max}^{EtOH} nm: 275, 281; IR ν_{max} cm^{-1}: 3680, 3540, 3450, 3010, 3000, 2930, 2850, 1750, 1450, 1365, 1275, 1235, 1100, 710; ^1H NMR (200 MHz) δ: 1.62 (3H, *s*), 1.68 (3H, *s*), 1.70 (3H, *s*), 2.01 (3H, *s*), 2.24 (3H, *s*), 2.45 (1H, *d*, $J = 4.2$ Hz), 4.85, 5.07 (2H, d_{AB}, $J = 13.4$ Hz), 5.34 (1H, *s*), 5.59 (1H, *dd*, $J = 5.3, 4.2$ Hz), 5.62 (1H, *dd*, $J = 4.5$, could not be measured; overlapping signals), 5.76 (1H, *d*, $J = 5.3$ Hz), 6.87 (2H, *t*), 7.19 (3H, *t*), 7.40 (2H, *m*), 7.59 (3H, *m*); EIMS m/z (rel. int.): 592 $[M-18]^+$ (1), 550 (1), 488 (1), 470 (1), 428 (1), 105 (100).

Compound **3** was isolated as an amorphous solid, mp 52–53°; UV λ_{max}^{EtOH} nm 283, 290; IR λ_{max} cm^{-1}: 3680, 3400, 3010, 3000, 2920, 2850, 1750, 1600, 1370, 1280, 1230, 1100, 710; ^1H NMR (200 MHz) δ: 1.47 (3H, *s*), 1.64 (3H, *s*), 1.68 (3H, *s*), 1.73 (3H, *s*), 2.00 (3H, *s*), 2.67 (1H, *d*, $J = 4.0$ Hz), 4.83, 5.02 (2H, d_{AB}, $J = 13.1$ Hz), 5.35 (1H, *br s*), 5.35 (1H, *m*, overlapping with proton at position 6), 5.76 (1H, *dd*, $J = 4.0, 5.5$ Hz), 5.83 (1H, *d*, $J = 5.5$ Hz), 7.47 (3H, *m*), 7.96 (2H, *t*); EIMS m/z (rel. int.): 488 $[M-18]^+$ (6), 470 (0) 428 (2), 366 (1), 351 (1), 350 (2), 306 (4), 264 (3), 105 (100).

Compound **4** was obtained as an amorphous solid, mp 51–53°; UV λ_{max}^{EtOH} nm: 227, 273, 282; IR ν_{max} cm^{-1}: 3680, 3650, 3010, 2910, 1720, 1270, 1190, 1100, 710; ^1H NMR (200 MHz) δ: 1.62 (6H, *s*), 1.77 (3H, *s*), 2.45 (1H, *d* $J = 4.1$ Hz), 4.31, 4.57 (2H, d_{AB}, $J = 12.6$ Hz), 4.42, 4.45 (1H, *dd*, $J = 4.1, 5.3$ Hz), 5.62 (1H, *br s*), 5.62 (1H, *dd*, $J = 12.5, 4.5$ Hz), 5.71 (1H, *d*, $J = 5.3$ Hz), 6.90 (2H, *t*), 7.21 (3H, *m*), 7.35 (2H, *m*), 7.63 (3H, *m*); EIMS m/z (rel. int.): 508 $[M-18]^+$ (3), 490 (5), 475 (4), 404 (7), 386 (41), 371 (11), 105 (100).

1α,9α-Dibenzoyloxy-2α,6β,8α,15-tetracetoxy-4β-hydroxydihydro-β-agarofurane (**5**) was isolated as an amorphous solid, mp 50–52°; $[M-MeCOOH]^+$ at m/z 650.2297 (Calcd for $C_{35}H_{38}O_{12}$, 650.2232); IR ν_{max} cm^{-1}: 3560, 3010, 2990, 2920, 1725, 1365, 1275, 1240, 1110, 1090, 1040, 710: ^1H NMR (200 MHz) δ: 1.53 (3H, *s*), 1.58 (3H, *s*), 1.68 (3H, *s*), 2.05 (3H, *s*), 2.07 (3H, *s*), 2.15 (3H, *s*), 2.37 (3H, *s*), 2.42 (1H, *d*, $J = 4.0$ Hz), 2.75 (1H, *s*), 5.05, 5.29 (2H, d_{AB}, $J = 13.5$ Hz), 5.47 (1H, *m*), 5.57 (1H, *dd*, $J = 4.0, 6.2$ Hz), 5.72 (1H, *d*, $J = 6.2$ Hz), 5.77 (1H, *d*, $J = 3.8$ Hz), 6.89 (2H, *m*), 6.97 (1H, *br s*), 7.18 (3H, *m*), 7.40 (2H, *m*), 7.55 (3H, *t*); EIMS m/z (rel. int.): 650 $[M-60]^+$ (6), 608 (2), 590 (1), 546 (1), 528 (1), 468 (1), 426 (1), 105 (100).

When a soln of **5** (37 mg) in MeOH (4 ml) was treated with 0.1 M $NaHCO_3$ (2 ml) at 50° for 5 hr, then extracted and chromatographed, compounds **6–8** were obtained.

Compound **6**, gum: ^1H NMR (200 MHz) δ: 1.60 (3H, *s*), 1.68 (3H, *s*), 1.80 (3H, *s*), 2.00 (3H, *s*), 2.06 (3H, *s*), 2.28 (3H, *s*), 2.40 (1H, *d*, $J = 4.0$ Hz), 5.00, 5.23, (2H, d_{AB}, $J = 12.5$ Hz), 5.43 (1H, *br s*), 5.44 (1H, *m*), 5.60 (1H, *dd*, $J = 4.0, 5.0$ Hz), 5.73 (1H, *d*, $J = 5.0$ Hz), 5.76 (1H, *d*, $J = 3.0$ Hz), 6.85 (2H, *m*), 7.16 (3H, *m*), 7.38 (2H, *m*), 7.52 (3H, *m*); EIMS m/z (rel. int.): 626 $[M-42]^+$ (1), 608 (1), 546 (1), 481 (1), 428 (1), 386 (2), 355 (4), 279 (6), 105 (42).

Compound **7**, gum: ^1H NMR (200 MHz) δ: 1.64 (3H, *s*), 1.68 (3H, *s*), 1.75 (3H, *s*),1 1.91 (3H, *s*), 2.03 (3H, *s*), 2.70 (1H, *d*, $J = 4.0$ Hz), 4.90, 5.35 (2H, d_{AB}, $J = 12.5$ Hz), 5.47 (2H, *m*), 5.70 (1H, *d*, $J = 6.0$ Hz), 5.77 (1H, *br s*), 5.80 (1H, *d*, $J = 6.0$ Hz), 6.80 (2H, *m*), 7.04 (2H, *m*), 7.33 (3H, *m*), 7.52 (3H, *m*); EIMS m/z (rel. int.): 608 $[M-18]^+$ (2), 590 (1), 546 (1), 486 (1), 426 (1), 408 (1), 368 (3), 304 (2), 244 (4), 202 (7), 105 (100).

Compound **8**, gum; ^1H NMR (200 MHz) δ: 1.60–1.70 (angular methyls), 2.09 (3H, *s*), 2.49 (1H, *d*, $J = 4.0$ Hz), 4.42 (1H, *m*), 4.46, 4.75 (2H, d_{AB}, $J = 12.5$ Hz), 5.37 (1H, *dd*, $J = 6.0, 4.0$ Hz), 5.56 (1H, *br s*), 5.58 (1H, *d*, $J = 6$ Hz), 5.76 (1H, *d*, $J = 3.0$ Hz), 6.90 (2H, *m*), 7.19 (3H, *m*), 7.50 (2H, *m*), 7.90 (3H, *m*); EIMS m/z (rel. int.): 415 $[M-109]^+$ (60), 386 (2), 372 (2), 344 (2), 330 (2), 305 (5), 284 (6), 279 (9), 256 (8), 105 (15).

Acknowledgements—We are indebted to CAICYT (PR694-84) and the AIETI Foundation for subsidizing this project.

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