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FURTHER SESQUITERPENE PYRIDINE ALKALOIDS FROM *MAYTENUS AQUIFOLIUM*

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Key Word Index—*Maytenus aquifolium*; Celastraceae; root bark; sesquiterpene evoninate alkaloids.

Abstract—Two new sesquiterpene evoninate alkaloids, aquifoliunine E-III and aquifoliunine E-IV, were isolated from the root bark of *Maytenus aquifolium*. The structures of the new compounds were elucidated on the basis of spectroscopic methods, including ES-MS.

INTRODUCTION

Various sesquiterpene pyridine alkaloids derived from dihydro-β-agarofuran skeleton have been isolated from members of the Celastraceae [1]. They are characterized by a macrocyclic structure formed by two ester linkages between a sesquiterpene and various dicarboxylic acids (e.g. evoninic acid, wilfordinic acid, edulinic acid, etc.) at positions 3 and 15 [2]. Previous work on the root bark of *Maytenus aquifolium* has resulted in the isolation of the bioactive sesquiterpene evoninate alkaloids, aquifoliunine E-I and aquifoliunine E-II [3]. In this paper, we describe the isolation and structure elucidation of two new sesquiterpene pyridine alkaloids, aquifoliunine E-III (**1**) and aquifoliunine E-IV (**2**) from the same source. The structures and relative stereochemistry of isolated compounds are based on 2D NMR spectroscopic techniques, including DQCOSY, HETCOR, HMBC and HMQC.

RESULTS AND DISCUSSION

Aquifoliunine E-III (**1**) and E-IV (**2**) were isolated from the CH_2Cl_2-soluble fraction of the methanolic extract of the root bark of *M. aquifolium*. The resulting extract was subjected to silica gel column chromatography (CC). The fractions obtained were further separated by silica gel (CC) and prep. HPLC to give the two new sesquiterpene pyridine alkaloids.

Aquifoliunine E-III (**1**) has the molecular formula $C_{36}H_{45}NO_{17}$ by analysis of electrospray mass spectral analysis (ES-MS). The ^1H and ^{13}C NMR spectra of **1** revealed the presence of five acetyl groups (δ_H 1.84, 2.07, 2.13, 2.19, 2.23) and a further oxygenated functional group (δ_C 74.1) around the basic skeleton was assumed to be a hydroxyl group. Proton assignments around the bicyclic skeleton were determined based on an interpretation of the DQCOSY spectrum. The spin systems derived from H-1α, H-2α, H-3β, H-8β, H-7β and H-9α were readily recognized by starting with the 1H doublet at δ 5.57 assigned to H-1α ($J_{1\alpha,2\alpha}=3.5$ Hz) which showed a cross-peak with the 1H doublet of doublets at δ 5.23 assigned to H-2α ($J_{2\alpha,1\alpha}=3.5$, $J_{2\alpha,3\beta}=2.6$ Hz). This latter signal also showed a cross-peak with the 1H doublet at δ 4.71 assigned to H-3β ($J_{3\beta,2\alpha}=2.6$ Hz). The 1H doublet of doublets at δ 4.32 assigned to H-8β ($J_{8\beta,7\beta}=3.4$, $J_{8\beta,9\alpha}=9.4$ Hz) showed cross-peaks with the 1H doublets at δ 2.40 (H-7β) and δ 5.56 (H-9α). The position of this hydroxyl group was confirmed at C-8, due to H-8β resonating at higher field than the corresponding protons bearing acetyl groups [3]. In addition, H-8β did not show any cross-peak with a carbonyl carbon resonance in the HMBC spectrum. The relative stereochemistry of the H-8β and H-9α was deduced by their *axial*, *axial* coupling constant ($J_{8,9}$ and $J_{9,8}=9.4$ Hz) [3]. The two sets of methylenic protons, consisting of two pairs of doublet of doublets, were assigned to H-11 and H-15, respect-

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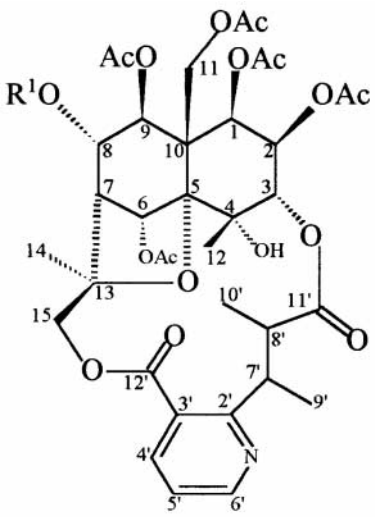

1. $R^1 = H$

2. $R^1 = Nic$

ively. The presence of an esterified evoninic acid was indicated by two doublets at δ 1.20 and 1.40 assigned to two secondary methyl groups (H-10′ and H-9′) which showed cross-peaks with a pair of resonances at δ 2.57 and 4.63 assigned to H-8′ and H-7′, respectively (q, $J_{8',\ 10'}$ and $J_{7',\ 9'} = 7.0$ Hz). The configuration of the methyl groups of the evoninate-type compounds were determined as 7′*S*,8′*S* (evoninic acid: 2*S*,3*S*) by hydrolysis [4] and X-ray analysis [5]. The 2,3-disubstituted pyridine unit was determined by the signals at δ 7.27 (*dd*, J = 7.8, 4.5 Hz), 8.08 (*dd* J = 1.8, 7.8 Hz) and 8.70 (*dd*, J = 1.8, 4.5 Hz) assigned to H-5′, H-4′ and H-6′, respectively. The location of the ester groups around the basic skeleton was solved by examination of the HMBC spectrum. Five acetoxy carbonyl resonances were correlated with the corresponding proton.The attachment of these acetoxy groups at C-2, C-1, C-9, C-6 and C-11 was established by defining cross-peaks between corresponding proton signals and the acetoxy carbonyl resonances. The carbon signal at δ 164.5 was attributed to C-2′ based on cross-peaks with the proton signals at δ 2.57, 4.63 (H-8′, H-7′) and δ 1.40 (H-9′). Further correlations between the carbon signal at δ 173.5 (C-11′) with the proton signal at δ 4.71 (H-3) and the carbon signal at δ 167.8 (C-12′) with proton signals at δ 3.69–5.92 (H-15) proved the attachment of the evoninate moiety at C-3 and C-15. HETCOR analysis was used to assign the signals for all proton-bearing carbons.

Aquifoliunine E-IV (**2**), was shown to have a molecular formula $C_{42}H_{48}N_2O_{18}$ by analysis of the ES-MS spectrum. It also was an evoninate type sesquiterpene pyridine alkaloid derivative of **1**. The ^{1}H and ^{13}C NMR spectra of **2** revealed the presence of five acetyl groups and one nicotinoyl group (Tables 1 and 2). The position of this latter was assigned at C-8, where H-8β resonating at lower field than the corresponding protons of **1**. The signal at δ 164.5 was assigned to the carbonyl carbon of the nicotinate moiety based upon its cross-peak with the pyridine *o*-protons at δ 9.15 and 8.85 in the HMBC spectrum. Again, HMBC analysis was used to confirm each ester group around the basic skeleton.

EXPERIMENTAL

Instrumentation and chromatographic materials

Silica gel (Merck, 230–400 and 70–230 mesh) was used for all column chromatography unless otherwise stated and solvents were redistilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity spectrometer at 400 and 100.57 MHz, respectively, with TMS as a internal standard. IR spectra were obtained on a Nicolet spectrometer. ES-MS were recorded on a VG Platform II spectrometer. Optical activities were measured on a Polamat A, Carl Zeiss Jena. HPLC separations were performed on a Shimadzu LC-10 AS using a reverse phase column (Supelcosil, C_{18}; 3.9 × 150 mm) eluted with MeOH–H_2O (45:55), at a

Table 1. ^{1}H NMR spectral data of alkaloids **1** and **2** (400 MHz, $CDCl_3$, ppm relative to internal TMS)

H	1	2
1	5.57 *d* (3.5)	5.63 *d* (3.5)
2	5.23 *dd* (3.5, 2.6)	5.26 *dd* (3.5, 2.6)
3	4.71 *d* (2.6)	4.74 *d* (2.6)
4-OH	4.45 *d* (1.2)	4.53 *d* (1.2)
6	6.45 *s*	6.73 *s*
7	2.40 *d* (3.4)	2.66 *d* (3.5)
8	4.32 *dd* (3.4, 9.4)	5.75 *dd* (3.5, 9.7)
9	5.56 *d* (9.4)	5.87 *d* (9.7)
11	4.51–4.88 *d* (ABq, 13.3)	4.61–4.82 *d* (ABq, 13.3)
12	1.53 *d* (1.2)	1.54 *d* (1.2)
14	1.75 *s*	1.81 *s*
15	3.69–5.92 *d* (ABq 11.4)	3.63–5.97 *d* (ABq 11.5)
4′	8.08 *dd* (7.8, 1.8)	8.05 *dd* (7.8, 1.8)
5′	7.27 *dd* (7.8, 4.5)	7.23 *dd* (7.8, 4.8)
6′	8.70 *dd* (4.5, 1.8)	8.69 *dd* (4.8, 1.8)
7′	4.63 *q* (7.0)	4.65 *q* (7.0)
8′	2.57 *q* (7.0)	2.60 *q* (7.0)
9′	1.40 *d* (7.0)	1.39 *d* (7.0)
10′	1.20 *d* (7.0)	1.24 *d* (7.0)
1-CH_3CO	1.84 *s*	1.87 *s*
2-CH_3CO	2.13 *s*	2.16 *s*
6-CH_3CO	2.07 *s*	1.97 *s*
9-CH_3CO	2.19 *s*	2.23 *s*
11-CH_3CO	2.23 *s*	2.35 *s*
8-ONic		
2″		8.85 *d* (1.9, 4.8)
3″		7.43 *dd* (4.8, 7.9)
4″		8.19 *dt*-like (1.9, 7.9)
6″		9.15 *d* (1.9)

Coupling constants in Hz are given in parentheses.

Table 2. ^{13}C NMR (100 MHz, $CDCl_3$) chemical shifts for **1** and **2**

C	1	2
1	72.2 (*d*)	72.4 (*d*)
2	68.3 (*d*)	68.5 (*d*)
3	75.1 (*d*)	75.2 (*d*)
4	70.6 (*s*)	70.6 (*s*)
5	94.2 (*s*)	94.5 (*s*)
6	74.8 (*d*)	74.9 (*d*)
7	51.1 (*d*)	49.4 (*d*)
8	74.1 (*d*)	74.6 (*d*)
9	76.9 (*d*)	73.7 (*d*)
10	51.3 (*s*)	52.6 (*s*)
11	60.7 (*t*)	60.7 (*t*)
12	23.5 (*q*)	23.8 (*q*)
13	85.5 (*s*)	85.6 (*s*)
14	19.6 (*q*)	19.7 (*q*)
15	70.2 (*t*)	69.8 (*t*)
2′	164.5 (*s*)	165.4 (*s*)
3′	125.0 (*s*)	125.0 (*s*)
4′	139.3 (*d*)	137.7 (*d*)
5′	121.5 (*d*)	121.1 (*d*)
6′	150.1 (*d*)	151.5 (*d*)
7′	36.1 (*d*)	36.4 (*d*)
8′	44.6 (*d*)	44.9 (*d*)
9′	11.7 (*q*)	12.0 (*q*)
10′	9.8 (*q*)	9.8 (*q*)
11′	173.5 (*s*)	173.9 (*s*)
12′	167.8 (*s*)	168.6 (*s*)
1-OCO**CH$_3$**	20.3 (*q*)	20.5 (*q*)
2-OCO**CH$_3$**	20.7 (*q*)	21.0 (*q*)
6-OCO**CH$_3$**	20.8 (*q*)	21.0 (*q*)
9-OCO**CH$_3$**	21.3 (*q*)	21.5 (*q*)
11-OCO**CH$_3$**	21.0 (*q*)	21.2 (*q*)
1-**OCO**CH$_3$	168.8 (*s*)	169.1 (*s*)
2-**OCO**CH$_3$	168.3 (*s*)	168.6 (*s*)
6-**OCO**CH$_3$	170.8 (*s*)	169.8 (*s*)
9-**OCO**CH$_3$	169.7 (*s*)	169.7 (*s*)
11-O*C*OCH$_3$	169.6 (*s*)	169.9 (*s*)
8-O*C*ONic		164.5 (*s*)
2″		151.1 (*s*)
3″		125.0 (*d*)
4″		137.0 (*d*)
5″		123.6 (*d*)
6″		154.2 (*d*)

Multiplicity in the DEPT spectrum is given after the respective chemical shifts: s (singlet), d (doublet), t (triplet) and q (quartet).

flow rate of 1.0 ml/min and detection at 420 nm to isolate compound **1**.

Plant material

M. aquifolium root bark was collected at Ribeirão Preto, São Paulo and identified by Dr Rita Maria de Carvalho. The voucher specimen is deposited in the Herbarium of the University of Campinas, São Paulo, Brazil.

Extraction and isolation of constituents

The dried and powdered root bark of *M. aquifolium* (32.0 g) was extracted with MeOH. The resulting MeOH extract was filtered and concentrated *in vacuo* to afford a brown gum (2.35 g). The CH_2Cl_2-soluble part of MeOH extract (0.523 g) was applied to a Si gel column (300 g) and eluted with hexane containing increasing amounts of EtOAc to give 51 fractions. The fraction 47 (120 mg) was applied to prep. TLC, eluted with $CHCl_3$/MeOH (95:05) to give the fraction 47-1 (16 mg) and compound **2** (7.1 mg). The fraction 47-2 was applied to HPLC to give **1** (3.3 mg).

Aquifoliunine E-III (**1**): Amorphous solid. $[\alpha]_D - 20.8^\circ$ ($CHCl_3$; *c* 3.3). IR_{max}^{film} KBr cm^{-1}: 3480, 1748, 1560, 1440, 1380, 1100. ES-MS, *m/z* (rel. int.): 764 $[M + 1]^+$ (100). ^1H NMR: Table 1, ^{13}C NMR: Table 2.

Aquifoliunine E-IV (**2**): $[\alpha]_D - 15.2^\circ C$ ($CHCl_3$; *c* 2.9). IR_{max}^{film} KBr cm^{-1}: 3490, 1750, 1570, 1440, 1382, 1100. ES-MS, *m/z* (rel. int.): 869 $[M + 1]^+$ (100). ^1H NMR: Table 1, ^{13}C NMR: Table 2.

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