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BIOACTIVE SESQUITERPENE PYRIDINE ALKALOIDS FROM *MAYTENUS AQUIFOLIUM*

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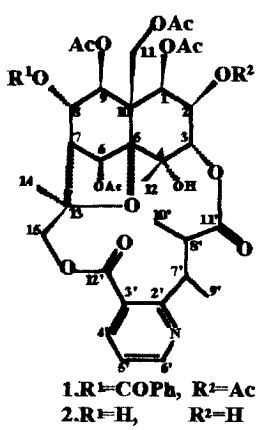
Key Word Index—*Maytenus aquifolium*; Celastraceae; root bark; sesquiterpene evoninate alkaloids.

Abstract—Two new sesquiterpene evoninate alkaloids, were isolated together with the known compounds syringaresinol and 4′-*O*-methyl-(−)-epigallocatechin from the root bark of *Maytenus aquifolium*. The structures of the two alkaloids were elucidated by interpretation of their spectral data, and both exhibited very weak activity in a mechanism-based DNA-modifying yeast assay. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The Celastraceae family is a rich source of sesquiterpene pyridine alkaloids derived from polyester sesquiterpenes based on the dihydro-β-agarofuran skeleton [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]. In our search for anticancer agents employing a mechanism-based yeast bioassay for DNA-modifying agents [3, 4] we have identified two sesquiterpene pyridine alkaloids from *Maytenus aquifolium* Martius. In this paper we report the isolation and structural elucidation of two sesquiterpene pyridine alkaloids aquifoliunine E-I (**1**) and aquifoliunine E-II (**2**) from *M. aquifolium* by application of 2D NMR spectroscopic techniques, including DQCOSY, HETCOR, HMBC, HMQC and NOESY, and their biological evaluation using the yeast bioassay system.



RESULTS AND DISCUSSION

A CH_2Cl_2 soluble fraction from the methanolic extract of root bark of *M. aquifolium* was subjected to silica gel column chromatography (CC). The fractions obtained were further separated by silica gel (CC) to give the sesquiterpene pyridine alkaloids **1** and **2**.

Aquifoliunine E-I (**1**), was shown to have a molecular formula $C_{43}H_{49}NO_{18}$ by analysis of its HRFABMS spectrum. Its IR spectrum revealed absorption bands at 3488, 1750 and 1443 cm^{-1} characteristic of hydroxy, ester and phenyl groups, respectively [5, 6]. The ^1H and ^{13}C NMR spectra of **1** revealed the presence of five acetyl groups (δ_H 1.82, 1.88, 2.14, 2.20, 2.33) and one benzoyl group [δ_H 7.43 (*m*), 7.57 (*p*), 7.92 (*o*)] (Tables 1 and 2). Proton assignments around the bicyclic skeleton were determined based on interpretation of the DQCOSY spectrum. The spin system derived from H-1α, H-2α, H-3β, H-8β and H-9α was readily recognized by starting with the 1H doublet at δ 5.63 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 5.25 assigned to H-2α ($J_{2\alpha,1\alpha}$ = 3.5, $J_{2\alpha,3\beta}$ = 2.5 Hz). This latter signal also showed a cross-peak with the 1H doublet at δ 4.73 assigned to H-3β

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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	5.63 *d* (3.5)	5.46 *d* (3.7)
2	5.25 *dd* (3.5, 2.5)	4.06 *dd* (3.5, 2.6)
3	4.73 *d* (2.5)	4.73 *d* (2.6)
4-OH	4.53 *d* (1.2)	4.45 *d* (1.2)
6	6.73 *s*	6.50 *s*
7	2.66 *d* (3.5)	2.35 *d* (3.4)
8	5.67 *dd* (3.6, 9.9)	4.30 *dd* (3.5, 9.4)
9	5.89 *d* (9.9)	5.56 *d* (9.4)
11	4.62–4.82 *d* (ABq, 13.6)	4.65–4.97 *d* (ABq, 13.7)
12	1.54 *d* (1.2)	1.56 *d* (1.2)
14	1.81 *s*	1.73 *s*
15	3.63–5.96 *d* (ABq 11.6)	3.65–5.95 *d* (ABq 11.5)
4′	8.03 *dd* (8.0, 1.6)	8.03 *dd* (7.8, 1.6)
5′	7.23 *dd* (8.0, 4.8)	7.15 *dd* (7.8, 4.8)
6′	8.68 *dd* (4.6, 1.6)	8.66 *dd* (4.8, 1.8)
7′	4.65 *q* (7.2)	4.63 *q* (7.2)
8′	2.58 *q* (7.2)	2.54 *q* (7.3)
9′	1.39 *d* (7.0)	1.35 *d* (7.0)
10′	1.20 *d* (7.0)	1.15 *d* (7.0)
OCMe	(C-1) 1.82 *s*	(C-1) 1.95 *s*
OCMe	(C-2) 2.14 *s*	—
OCMe	(C-6) 1.88 *s*	(C-6) 2.19 *s*
OCMe	(C-9) 2.20 *s*	(C-9) 2.09 *s*
OCMe	(C-11) 2.33 *s*	(C-11) 2.21 *s*
OBz		
o-COPh	(C-8) 7.92 *dd* (1.2, 7.8)	
m-COPh	(C-8) 7.43 *t* like (7.4)	
p-COPh	(C-8) 7.57 *dt* like (1.0, 7.3)	

Coupling in Hz are given in parentheses.

($J_{3\beta,2\alpha}$ = 2.5 Hz). The 1H doublet of doublets at δ 5.67 assigned to H-8β ($J_{8\beta,7\beta}$ = 3.6, $J_{8\beta,9\alpha}$ = 9.9 Hz) showed cross-peaks with the 1H doublets at δ 2.66 (H-7β) and δ 5.89 (H-9α). The latter proton signal was also correlated with the H-8β.

The relative stereochemistry of the H-8β and H-9α was deduced from NOE difference experiment, in which irradiation of the methine resonance at δ 6.73 (H-6) caused an enhancement of the signal at δ 5.67 (H-8β). The irradiation of the methyl resonance at δ 1.81 (H-14) caused an enhancement of the signal at δ 5.89 corroborating the axial orientation of H-9 (Fig. 1). The two sets of methylenic protons consisting of two pairs of doublet of doublets at δ 4.62, 4.82 ($J_{11a,12b}$ = 13.6 Hz) and δ 3.63, 5.96 (J_{15a15b} = 11.6 Hz) were assigned to H-11 and H-15, respectively. The presence of an esterified evoninic acid was indicated by two doublets at δ 1.20 and 1.39 assigned to two secondary methyl groups (H-10′ and H-9′) which showed cross-peaks with a pair of resonances at δ 2.58 and 4.65 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: 2S, 3S) by hydrolysis [7] and X-ray analysis [8]. The 2,3-disubstituted pyridine unit was determined by the signals at δ 7.23 (*dd*, J = 8.0, 4.8 Hz), 8.03 (*dd*, J = 1.6, 8.0 Hz) and 8.68 (*dd*, J = 1.6, 4.6 Hz) assigned to H-5′, H-4′ and H-6′, respectivity. The location of the ester groups around the basic skeleton was solved by examination of the HMBC spectrum. The four acetoxy carbonyl resonances at δ 168.6, 169.1, 169.7, 169.8 and 170.0 were correlated with 3H singlets at δ 2.14, 1.82, 2.20, 1.88 and 2.33, respectively, and 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 the acetoxy carbonyl resonances and proton signals at δ 5.25 (H-2α), 5.63 (H-1α), 5.89 (H-9α), 6.73 (H-6β) and 4.62–4.82 (H-11). The signal at δ 164.5 was assigned to the carbonyl carbon of the benzoate moiety based upon its cross-peak with the phenyl *o*-protons at δ 7.92. The signals of a carbonyl carbon at δ 165.6 was assigned to C-2′ based on it cross-peaks with the proton signals at δ 2.58, 4.65 (H-8′, H-7′) and δ 1.20, 1.39 (H-10′, H-9′). Further correlations between the carbon signal at δ 174.0 (C-11′) and δ 168.4 (C-12′) with proton signals at δ 3.63–5.96 (H-15) and δ 4.73 (H-3) proved the attachment of the evoninate moiety to be at C-3 and C-15.

Comparison of the ^{13}C NMR data for **1** with those of horridine [9] showed a discrepancy in the literature assignments for some carbons in the structure and

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

C	1	2
1	72.4 *d*	74.5 *d*
2	68.5 *d*	69.3 *d*
3	75.2 *d*	77.1 *d*
4	70.6 *s*	70.7 *s*
5	94.5 *s*	94.4 *s*
6	74.6 *d*	74.6 *d*
7	49.3 *d*	51.8 *d*
8	74.5 *d*	74.5 *d*
9	73.7 *d*	77.2 *d*
10	51.3 *s*	52.0 *s*
11	60.7 *t*	61.3 *t*
12	23.8 *q*	23.8 *q*
13	85.7 *s*	85.5 *s*
14	19.6 *q*	19.5 *q*
15	69.8 *t*	70.1 *t*
2′	165.6 *s*	165.1 *s*
3′	125.0 *s*	125.4 *s*
4′	137.7 *d*	137.6 *d*
5′	121.0 *d*	121.1 *d*
6′	151.5 *d*	151.3 *d*
7′	36.4 *d*	36.4 *d*
8′	44.9 *d*	44.8 *d*
9′	12.0 *q*	12.0 *q*
10′	9.8 *q*	9.1 *q*
11′	174.0 *s*	174.5 *s*
12′	168.4 *s*	168.4 *s*
OCOMe (C-1)	20.5 *q*	20.9 *q*
OCOMe (C-2)	21.0 *q*	
OCOMe (C-6)	20.7 *q*	21.6 *q*
OCOMe (C-9)	21.5 *q*	21.2 *q*
OCOMe (C-11)	21.2 *q*	21.3 *q*
OCOMe (C-1)	169.1 *s*	169.3 *s*
OCOMe (C-2)	168.6 *s*	—
OCOMe (C-6)	169.8 *s*	169.7 *s*
OCOMe (C-9)	169.7 *s*	171.2 *s*
OCOMe (C-11)	170.0 *s*	170.0 *s*
OCOPh (C-8)	164.5 *s*	
i-OCOPh (C-8)	129.7 *s*	
o-OCOPh (C-8)	129.7 *d*	
m-OCOPh (C-8)	128.8 *d*	
p-OCOPh (C-8)	133.7 *d*	

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

these have been revised based on HETCOR and HMBC data (see Table 2).

Aquifoliunine E-II (**2**) has the molecular $C_{34}H_{43}NO_{16}$ by analysis of its HRFABMS spectrum. It also was an evoninate type sesquiterpene pyridine alkaloid derivative of **1** and its sesquiterpene core contained four acetyl groups. Further oxygenated functional groups were assumed to be hydroxyl groups. The positions of these hydroxyl groups were confirmed at C-2 and C-8, where the H-2 and H-8 are methine protons (δ 4.06, *dd*, $J_{2\alpha\text{-}1\alpha} = 3.5$, $J_{2\alpha\text{-}3\beta} = 2.6$ Hz and 4.30, *dd*, $J_{8\beta\text{-}7\beta} = 3.5$, $J_{8\beta\text{-}9\alpha} = 9.4$ Hz) resonating at higher field than the corresponding protons of **1**. In addition, H-2α and H-8β did not show any cross-peaks with a carbonyl carbon resonance in the HMBC spectrum. Again, HETCOR analysis was used to assign the signals for all proton-bearing carbons.

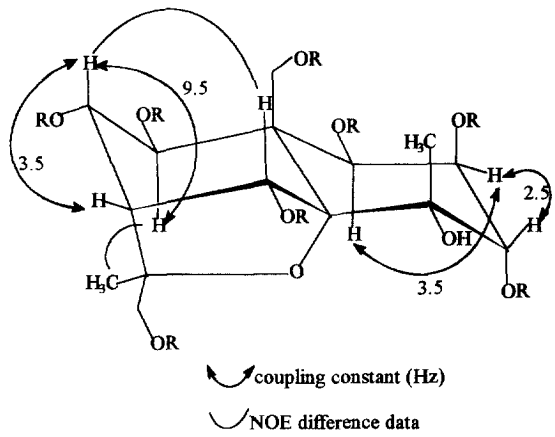

Fig. 1.

The known compound 4′-*O*-methyl-(−)-epigallocatechin (**3**) was earlier isolated from *M. evonymoides* [10], *M. rigida* [11] and *Ourotea* sp. [12], while syringaresinol (**4**) [13], a 3,7-dioxabicyclo[3.3.0]octane lignan derivative, was isolated for the first time from *Maytenus* species.

In the bioassay utilizing genetically engineered mutants of the yeast *Saccharomyces cerevisiae* the sesquiterpene alkaloids aquifoliunine E-I (**1**) and aquifoliunine E-II (**2**) exhibited very weak bioactivity. Compounds **1** and **2** showed weak but selective activities with IC_{12} values of 164 and 266; 160 and 167; 160 and 180 μg/mL in the mutant yeast strains RS 321N, RS 322YK (*rad* 52Y), and RS 167N (*rad* 6), respectively; they were inactive ($IC_{12} > 1000$ μg/mL) in the "wild type" strain RS 188N (*rad*+). These results indicate these compounds might serve as the main components responsible for the slight DNA-damaging activity of *M. aquifolium* extract.

EXPERIMENTAL

Instrumentation and chromatography materials

Silica gel (Merck, 230–400 and 70–230 mesh) were used for all column chromatography unless otherwise stated and solvents were redistilled prior to use. ^1H 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. HRFABMS: at 70 EV VG 7070E-HF. Optical activities were measured on a Polamat A, Carl Zeiss Jena.

Plant material

M. aquifolium root bark was collected at Ribeirão Preto, São Paulo and classified by Dr Rita Maria de

Carvalho. The voucher specimen is deposited in the Herbarium of the University of Campinas, São Paulo, Brazil.

Bioassays

The experimental method utilized in the screening procedure has been described elsewhere [3, 4]. The IC_{12} values refer to the concentration in μg/mL required to produce a zone of inhibition of 12 mm diameter around a 100 μl well during a 48 h incubation period at 37° C.

Extraction and isolation of constituents

The dried and powdered root bark of *M. aquifolium* (153.5 g) was extracted with MeOH. The resulting MeOH extract was filtered and concd *in vacuo* to afford a brown gum (17.35 g). The CH_2Cl_2 soluble part of MeOH extract (1.43 g) was applied to a Si gel column (300 g), and eluted with hexane containing increasing amounts of EtOAc to give 68 frs. Fr. 10 (720 mg) was applied to a Si gel column (2.0 g) 230–400 mesh, and eluted with $CHCl_3$ with increasing amounts of MeOH to give **1** (9.8 mg). Fr. 14 (22.2 mg) was subjected to Si gel CC, eluted with $CHCl_3$ containing an increasing amount of MeOH to give **4** (5.1 mg). Fr. 20 (23.6 mg) was chromatographed on silica gel (CC) eluted with $CHCl_3$ containing increasing amounts of MeOH to give **2** (2.6 mg). Fr. 30 (149.8 mg) was applied to Si gel (CC) eluted with $CHCl_3$ containing increasing amount of MeOH to give **3** (3.7 mg).

Aquifoliunine E-I (**1**). Amorphous solid. $[\alpha]_D -3.1$ ($CHCl_3$; c 3.2). IR_{max}^{film} KBr cm $^{-1}$: 3488, 1750, 1570, 1443, 1380, 1100. HRFABMS, *m/z* (rel. int.): 868.3010 $[M+1]^+$ (100). ^{1}H NMR: Table 1, ^{13}C NMR: Table 2.

Aquifoliunine E-II (**2**). $[\alpha]_D -17.2°$ ($CHCl_3$; c 2.9). IR_{max}^{film} KBr cm^{-1}: 3490, 1755, 1572, 1443, 1383, 1100. HRFABMS, *m/z* (rel. int.): 722.2668 $[M+1]^+$ (100). ^{1}H NMR: Table 1, ^{13}C NMR: Table 2.

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