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ACANTHOTHAMINE, A SESQUITERPENOID ALKALOID FROM *ACANTHOTHAMNUS APHYLLUS**

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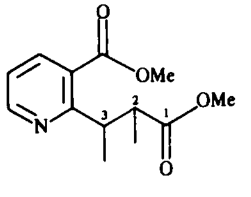
Key Word Index—*Acanthothamnus aphyllus*; Celastraceae; polyhydroxydihydroagarofurane *iso*-evoninate.

Abstract—Acanthothamine, a new sesquiterpene alkaloid, with an *iso*-evoninic acid residue, was isolated from *Acanthothamnus aphyllus* and characterized by chemical and spectroscopic data as 8-epieuonyminol tetraacetate *iso*-evoninate.

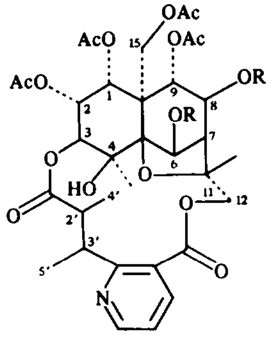
INTRODUCTION

The presence of alkaloids in plants belonging to the Celastraceae has been known for a long time, and their structures and biological activities have been examined by several groups of investigators [1–3]. One type of these compounds comprises the evoninic alkaloids [1, 4], which are characterized by the presence of evoninic acid (**1a**) esterified to a highly oxygenated sesquiterpene nucleus. Pailer and Libiseller [5] studied the structure of **1a** and established it as (2*S*,3*S*)-2-methyl-3-methyl-3-(*β*-carboxy-*α*-pyridyl)-propionic acid.

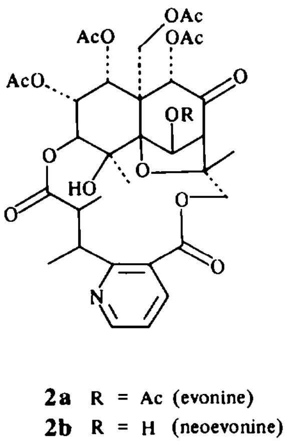
As part of our studies on Mexican plants of the Celastraceae [6], we have isolated from *Acanthothamnus aphyllus* T. S. Brandegee a new sesquiterpene alkaloid,



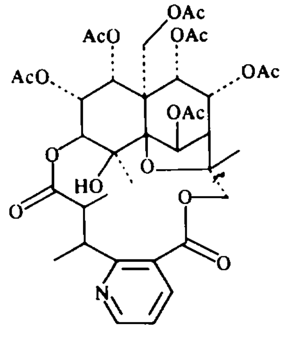
1a 2*S*, 3*S*
1b 2*R*, 3*S*



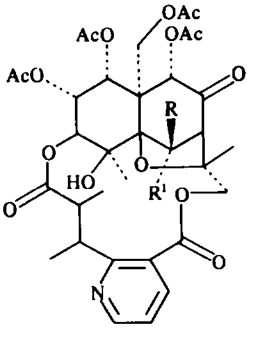
3a R = H (acanthothamine)
3b R = Ac



2a R = Ac (evonine)
2b R = H (neoevonine)



4 (euonymine)



5a R = OH, R¹ = H
5b R, R¹ = O

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Table 1. ^{1}H NMR spectral data for compounds **2**–**5** (80 MHz, $CDCl_3$, TMS as internal standard)

Compound	H-1	H-2	H-3	H-6	H-7	H-8	H-9	H-12	H-13 (Me)	H-14 (Me)	H-15 (–CH_2–)
3a	5.58	5.28	4.70	5.05	2.57	4.18	5.55	4.1, 5.55	1.72	1.85	4.67 *d*
	d (4)*	*t* (4)	*d* (3)	*s* (*br*)	*d* (*br*)	*d*, *d*	*d* (10)	ABq (12)	*s*	*s*	
					(3)	(10, 3)					
3b	5.57	5.30	4.70	6.4	2.5	5.62	5.67	4.3, 5.1	1.57	1.67	4.7
	d (4)	*t* (3)	*d* (3)	*s* (*br*)	*d* (*br*) (3)	*dd* (8, 3)	*d* (8)	ABq (11)	*s*	*s*	*s*
5a	5.70 *d*	5.30	4.77	5.37	3.15	—	5.55	4, 5.68	1.55	1.87	4.46, 4.9
	(3)	*t* (3)	*d* (3)	*d* (1)	*d* (1)		*s*	ABq (12)	*s*	*s*	ABq (14)
5b	5.8 *d*	5.37	4.82	—	3.52	—	5.8	4.08, 4.6	1.57	1.57	4.55, 4.76
	(4)	*t* (4)	*d* (4)		*s*		*s*	ABq (12)	*s*	*s*	ABq (14)
2b [4]	5.72	5.34	4.82	5.41	3.02	—	5.59	3.8, 6.1	1.64	1.90	4.47, 4.92
	d (3.2)	*t* (3.2)	*d* (4)	*d* (1.5)	*d* (1.5)		*s*	ABq (12)	*s*	*s*	ABq (13)
4 [7]	5.55	5.23 *dd*	4.72	7.02	2.3 *dd*	5.5 *dd*	5.34	3.7, 5.94	—	—	4.5, 5.13
	d (4)	(4, 2.5)	*d* (2.5)	*d* (1.0)	(3.8, 1)	(3.8, 6.2)	*d* (6.2)	ABq (12)			ABq (13.5)
2a [4]	5.71	5.29	4.78	6.72	3.04	—	5.57	3.76, 6.04	1.61	1.61	4.58, 4.82
	d (3.2)	*t* (3.2)	*d* (3.2)	*d* (1.0)	*d* (1.0)	—	*s*	ABq (11.7)	*s* (*br*)	*s* (*br*)	ABq (13.0)
Evoninic acid residue values											
	H-2′	H-3′	H-4′ (Me)	H-5′ (Me)							
3a	2.35	4.37	0.95	1.37							
	m	*m*	*d* (7)	*d* (7)							
5a	2.80	4.40	0.87	1.42							
	m	*m*	*d* (7)	*d* (7)							
2b [4]	2.58	4.85	1.20	1.44							
	m	*m*	*d*	*d*							

*Coupling constants (in Hz) are given in parentheses.

named acanthothamine (**3a**), which possesses the epimeric evoninic acid (**1b**) residue. The details of the characterization of **3a** are described in this paper. This work constitutes the first report on the chemistry of an *Acanthothamnus* species.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of acanthothamine (**3a**), $C_{34}H_{43}NO_{16}$ ($[M]^+$, *m/z* 721), contained signals due to an evoninic acid residue (Table 2) and indicated the presence of four acetate groups [δ 1.85, 2.05 (*s*, 3H each) and 2.15 (*s*, 6H)]. It also contained two additional singlets (3H each) at δ 1.72 and 1.85 which were attributed to methyl groups on fully substituted carbon atoms. Addition of deuterium oxide revealed the presence of three exchangeable protons and thus the presence of three hydroxyl groups. One of these groups was the typical tertiary alcohol at C-4 and the other two secondary hydroxyl functions in the sesquiterpene moiety.

The ^{1}H NMR spectra of acanthothamine (**3a**), evonine (**2a**) [4], neoevonine (**2b**) [4] and euonymine (**4**) [7] (Table 1) were very similar and suggested that acanthothamine (**3a**) was the bidesacetyl derivative of euonymine (**4**). The ^{1}H NMR spectrum of the peracetylated derivative of **3a**, acanthothamine diacetate (**3b**), and that of euonymine (**4**) [7] (Table 1), however, differed in the chemical shifts of H-6 and the C-12 and C-15 methylene groups. A broad singlet observed at δ 6.4 in the ^{1}H NMR spectrum of **3b** was assigned to H-6 by comparison with similar structures (see Table 1) since it usually appears as a broad singlet or doublet ($J = 1$ Hz) when the substituent at C-6 is equatorial [1, 4, 7]. In euonymine, H-6 is observed at δ 7.02 (*d*, $J = 1$ Hz). The C-12 and C-15 methylene groups in euonymine are responsible for two AB systems at δ 3.7 and 5.94 ($J = 12$ Hz) and 4.5 and 5.13 ($J = 13.5$ Hz). In acanthothamine diacetate (**3b**), they are observed as an AB quartet at δ 4.3 and 5.1 ($J = 11$ Hz) and a singlet (2H) at δ 4.7. This information suggested a difference in stereochemistry at one or more chiral centres in both alkaloids.

In order to establish the site of attachment of the secondary hydroxyl groups in acanthothamine (**3a**), it was treated with pyridinium dichromate, yielding **5a** and **5b**. A broad doublet observed at δ 2.57 ($J = 3$ Hz) in the ^{1}H NMR spectrum of **3a** was shifted to δ 3.15 and observed as a doublet ($J = 1$ Hz) in the ^{1}H NMR spectrum of **5a** and as a sharp singlet at δ 3.52 in **5b**. The chemical shifts and multiplicities shown by this proton allowed the unambiguous assignment of these signals to H-7. The formation of compounds **5a** and **5b** proved that the secondary hydroxyl groups in acanthothamine (**3a**) are attached to C-6 and C-8. The stereochemistry of the secondary hydroxyl group at C-8 in **3a** was established by double resonance experiments. Irradiation at δ 2.57 transformed the double doublet at δ 4.18 ($J = 10$ and 3 Hz) into a doublet ($J = 10$ Hz) indicating a *trans*-diaxial relationship between H-8 and H-9*. As expected, H-9 was observed in the ^{1}H NMR spectra of **5a** and **5b** as a singlet (Table 1). The partially oxidized derivative **5a** is structurally identical to neoevonine (**2b**) but their ^{1}H NMR spectra differ in the chemical shifts shown by the hydrogens at C-7 and of the methylene at C-12 (Table 1).

The data presented indicate that the difference between **5a** and neoevonine (**2b**) must be due to different configurations at the chiral centre C-11 or in the evoninic acid residue.

Methanolysis of **3a** led to the isolation of **1b**. Comparison of its ^{1}H NMR spectrum with the spectrum described for **1a** (Table 2) indicated that acanthothamine (**3a**) contained an *iso*-evoninic acid residue. This was confirmed by X-ray diffraction analysis of a single crystal of **3a** [8].

Acanthothamine (**3a**) constitutes the first example of an alkaloid in which the evoninic acid residue is a (2*R*,3*S*)-2-methyl-3-methyl-3-(β-carboxy-α-pyridyl)-propionic acid, if we consider that C-7 has an *R*-absolute configuration, as has been found in all the products of this type described so far [1, 2]. It is also the first example of a natural 8-*iso*-euonyminol [1, 7] derivative.

The ^{13}C NMR chemical shifts assignments (Table 3) were based on data described for neoevonine [9].

EXPERIMENTAL

Mps (Fisher Jones apparatus): uncorr.; CC: Merck silica gel 60 (0.063–0.2 mm); ^{1}H NMR and ^{13}C NMR: 80 and 20 MHz, $CDCl_3$ and CCl_4 solns with TMS as internal standard. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance, noise-decoupled and APT ^{13}C NMR spectra. MS: 70 eV. Plant material was collected in Puebla, 3 km north from Tehuacán. A voucher specimen (MEXU-348134) has been deposited at the herbarium of the Instituto de Biología, UNAM.

Isolation of acanthothamine (**3a**). The stems of *Acanthothamnus aphyllus* (5 kg) were air-dried, powdered and extracted (× 2) with MeOH (18 l.) for 8 days. The combined filtrates were concentrated (2 l.), diluted with H_2O, filtered over celite and extracted with EtOAc (4 × 4 l.). The EtOAc extracts were washed with saturated NaCl soln, dried (Na_2SO_4) and concentrated. The extract (60 g) was chromatographed on silica gel (1500 g) with

Table 2. ^{1}H NMR spectral data for the dimethyl derivatives of compounds **1a** and **1b** (60 MHz, CCl_4, TMS as internal standard)

H	Dimethyl evoninate [4]	Dimethyl *iso*-evoninate
2	2.97 *m*	3.01*m*
3	3.90 *m*	3.95 *m*
Me-2	1.23 *d*	0.9*d* $J = 7$*
Me-3	1.25 *d*	1.25 *d* $J = 7$
α	8.47 *dd*	8.65 *dd* $J = 3, 6$
β	7.03 *dd*	7.15 *dd* $J = 6, 9$
γ	7.95 *dd*	8.09 *dd* $J = 3, 9$
OMe	3.38, 3.89 2*s*	3.67, 3.91 2*s*

*Coupling constants are given in Hz.

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*8-*iso*-Euonymine was obtained on sodium borohydride reduction of evonine followed by acetylation. The ^{1}H NMR spectrum described for this product [4] does not coincide with that of acanthothamine diacetate.

Table 3. ^{13}C NMR spectral data for neoevonine and acanthothamine (20 MHz, $CDCl_3$, DMSO-d_6)

C	Neoevonine [9]	Acanthothamine
1	62–75	68–77
2	62–75	68–77
3	62–75	68–77
4	78.4	72.01
5	95.4	92.60
6	62–75	68–77
7	61.9	53.25
8	196.1	68–77
9	62–75	68–77
10	52.5	50.75
11	85.8	84.96
12	70.7	71.17
13	—	20–25
14	—	20–25
15	60.3	60.83
2′	44.9	46.64
3′	36.4	40.19
4′	12.0	19.77
5′	9.9	14.83

hexane–EtOAc as eluant. Elution with hexane–EtOAc (1:3) gave an oily mixture which on repeated chromatography and recrystallizations (CH_2Cl_2) afforded pure **3a** (150 mg), mp 287–290°; IR ν_{max}^{Nujol} cm^{-1}: 3580, 3500, 3280, 1755, 1745, 1725, 1720, 1715, 1585, 1565; CIMS *m/z*: 721 $[M]^+$; ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 3.

Acetylation of **3a**. A soln of **3a** (28 mg) in C_5H_5N (1.5 ml) was treated with Ac_2O (1.5 ml) and allowed to stand at room temp. for 18 hr. The mixture was washed with dilute HCl and sat $NaHCO_3$ soln, dried and concentrated under vacuum, affording a mixture (25 mg), which was chromatographed on silica gel. Elution with hexane–EtOAc (3:2) yielded **3b** (18 mg), mp 145–148°, $[\alpha]_D^{20}$ + 43.25° (*c* 0.004; $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 3567, 1752, 1585, 1565; ^{1}H NMR: see Table 1; CIMS *m/z*: 763 $[M-42]^+$.

Oxidation of **3a** *with pyridinium dichromate* (*PDC*). A soln of **1** (25 mg) in CH_2Cl_2 was treated with PDC (25 mg) for 12 hr. The mixture was percolated through silica gel and concentrated under vacuum to give 20 mg of a mixture of **3** and **4**, which were separated by chromatography using hexane–EtOAc as eluant. Early fractions gave 8 mg **5b**, mp 295–297°; IR ν_{max}^{Nujol} cm^{-1}: 3530, 1785, 1755, 1740, 1585, 1565; ^{1}H NMR: see Table 1; CIMS *m/z*: 717 $[M]^+$; and the last fractions afforded 10 mg **5a**, mp 256–258°; IR ν_{max}^{Nujol} cm^{-1}: 3520, 3400, 1753, 1733, 1720, 1580, 1560; ^{1}H NMR: see Table 1; CIMS *m/z*: 719 $[M]^+$.

Dimethyl evoninate (**1b**). Acanthothamine (**3a**) (98 mg) was treated with 5 ml of a soln of NaOMe in MeOH (prepared by dissolving 102 mg Na in 8 ml MeOH), with stirring overnight, at room temp. After the usual work-up, the reaction mixture was separated by TLC (silica gel), yielding 13.5 mg **1b**, $[\alpha]_D^{20}$ − 99.22° (*c* 0.0129; $CHCl_3$); ^{1}H NMR: see Table 2.

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