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AMARISOLIDE, A NEO-CLERODANE DITERPENE GLYCOSIDE FROM *SALVIA AMARISSIMA**

EMMA MALDONADO, JORGE CÁRDENAS, HUMBERTO BOJÓRQUEZ, ELEAZAR M. ESCAMILLA† and ALFREDO ORTEGA‡

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, DF; †Departamento de Química, Instituto Tecnológico de Celaya, Av. Tecnológico e Irrigación, 38010, Celaya, Gto., México

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Key Word Index—*Salvia amarissima*; Labiatae; neo-clerodane diterpene glycoside; diterpenoids; amarisolide.

Abstract—A new neo-clerodane glycoside, amarisolide, has been isolated from the aerial parts of *Salvia amarissima*. Its structure was established as 2β-*O*-β-D-glucopyranosyl neo-cleroda-3,13(16),14-trien-15,16-epoxy-18,19-olide by chemical and spectroscopic means.

INTRODUCTION

In the course of our phytochemical investigation of the *Salvia* genus [1, 2], we have investigated *S. amarissima* Ort. (Family Labiatae, section *Uricae* subgenus *Calosphace*). From an acetone extract of the aerial parts of this plant, we have isolated, in addition to ursolic and oleanolic acids and pedalitin (5,6,3′,4′-tetrahydroxy-7-methoxyflavone) [3–5], the new diterpenoid glucoside amarisolide (**1**). This is the first report about the occurrence of this type of glucosides in *Salvia* species.

RESULTS AND DISCUSSION

The new compound **1** was assigned the molecular formula $C_{26}H_{36}O_9$ (mass spectrum). The ^{1}H NMR spectrum revealed it to be a glucosyl derivative of a clerodane diterpene. Thus, it showed the signals for a secondary and a tertiary methyl group at δ 0.87 and 0.59, respectively, as well as the signals for a β-substituted furan ring at δ 6.28 (H-14), 7.35 (H-15) and 7.24 (H-16) (IR: 1503 and 874 cm^{-1}) and for an α,β-unsaturated-γ-lactone at δ 6.96 *d* (H-3), 3.93 *dd* (H-19$_{pro\text{-}S}$) and δ 4.34 *d* (H-19$_{pro\text{-}R}$) (IR: 1782 and 1766 cm^{-1}). The COSY spectrum revealed that the H-3 signal was coupled with the signal at δ 4.56 which was ascribed to H-2 The presence of a hexose unit in **1** was inferred from the strong IR absorption at 3386 cm^{-1} and from the fragment at *m/z* 313 $[M - C_6H_{11}O_6]^+$ in the mass spectrum. The signals between δ 3.0 and 4.5 in the ^{1}H NMR spectrum, especially the value of $J_{1'\text{-}2'} = 7.5$ Hz (Table 1) and the ^{13}C NMR signals for C-1′-C-6′ (Table 2) [6] suggested it was a β-D-glucopyranosyl moiety. Acetylation of **1** with acetic anhydride in pyridine afforded the tetraacetyl derivative **2**. Its IR spectrum did not show hydroxyl absorptions and in its ^{1}H NMR spectrum only the signals for H-1′ to H-6′ were shifted downfield. This indicated no additional hydroxyl groups other than those of the glucose unit in **1**. The chemical shift of the H-2 signal was in agreement with the presence of an oxygenated function at C-2, which must be the glucopyranosyl moiety. The β-orientation of this moiety was supported by the H-2 coupling constants ($J_{1\alpha\text{-}2} = J_{1\beta\text{-}2} = 2.4$ Hz and $J_{2\text{-}3} = 6.3$ Hz), which established the equatorial disposition for this proton [7, 8].

The relative configuration of **1** was ascertained by the ^{13}C NMR signals for C-20 (δ 17.4) and C-17 (δ 15.3), which were in agreement with an α-orientation of these methyl groups on an A/B *trans*-neo-clerodane skeleton [7, 8]. In addition, the H-1α–H-10 coupling constant (13.8 Hz) established a *trans* diaxial relationship between them and therefore a β-orientation for H-10. The long range coupling shown by H-19 *pro-S* and H-6β indicated an α-axial orientation of C-19.

Compound **1** was resistant to hydrolysis under acid or basic conditions. Nevertheless, the aglycone **3** was obtained by the fungal action of *Fusarium moniliforme*, which is known to use the sugar moieties of glucosides as a carbon source [9]. Compound **3** showed IR absorptions for a hydroxyl group, an α,β-unsaturated-γ-lactone and a double bond. In its ^{1}H NMR spectrum the signal for H-2 appeared as a *dt* at δ 4.55 ($J = 6$ and 3 Hz) and the signals for the β-D-glucopyranosyl were not present.

*Contribution No. 1429 from Instituto de Química, UNAM.
‡Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR spectral data for compounds **1–3** (300 MHz, $CDCl_3$, TMS as int. standard)

H	**1***	**2**†	**3**‡
H-1α	1.29 *td*	1.23 *td*	
	(13.8, 2.4)	(13.8, 3.3)	
H-1β	2.00 *br d*	1.92 *m*	
	(13.8)		
H-2	4.56 *dt*	4.55 *dt*	4.50 *dt*
	(6.3, 2.4)	(6, 3)	(6, 3)
H-3	6.96 *d*	6.68 *d*	6.68 *d*
	(6.3)	(6)	(6)
H-6α	1.92 *br d*		
	(12)		
H-6β	1.33 *br t*	1.19 *m*	
	(12)		
H-7α	1.51 *m*	1.47 *m*	
H-7β	1.66 *m*	1.67 *m*	
H-8	1.80 *m*	1.70 *m*	
H-10	2.39 *d*	2.25 *d*	
	(13.8)	(13.8)	
H-11	1.54 *m*	1.47 *m*	
H-11′	1.66 *m*	1.67 *m*	
H-12	2.55 *td*	2.41 *td*	
	(14.1, 4.2)	(13.1, 4.5)	
H-12′	2.33 *td*	2.23 *td*	
	(14.1, 5.1)	(13.1, 6.1)	
H-14	6.28 *dd*	6.31 *dd*	6.31 *m*
	(1.5, 0.6)	(1.5, 0.6)	
H-15	7.35 *t*	7.32 *t*	7.32 *t*
	(1.5)	(1.5)	(2)
H-16	7.24 *br s*	7.24 *br s*	7.22 *m*
Me-17	0.87 *d*	0.85 *d*	0.87 *d*
	(6.6)	(6)	(6)
H-19	3.93 *dd*	3.97 *dd*	3.87 *dd*
pro-S	(8.1, 1.8)	(8.2, 1.8)	(8, 2)
H-19	4.34 *d*	4.29 *d*	4.30 *d*
pro-R	(8.1)	(8.2)	(8)
Me-20	0.59 *s*	0.54 *s*	0.60 *s*
H-1′	4.46 *d*	4.63 *d*	
	(7.5)	(7.9)	
H-2′	3.24 *dd*	4.94 *dd*	
	(9, 7.5)	(9.5, 7.9)	
H-3′	3.43 *t*	5.15 *t*	
	(9)	(9.5)	
H-4′	3.40 *t*	5.01 *t*	
	(9)	(9.5)	
H-5′	3.31 *m*	3.68 *ddd*	
		(9.5, 4.9, 2.4)	
H-6′	3.65 *dd*	4.20 *dd*	
	(11.7, 4.5)	(12.3, 4.9)	
H-6″	3.80 *dd*	4.09 *dd*	
	(11.7, 3)	(12.3, 2.4)	

*Run in $CDCl_3/CD_3OD$ solution.
†AcO signals at δ 2.06, 1.99, 1.97 and 1.95.
‡Run at 80 MHz.

EXPERIMENTAL

Mps: uncorr; MS: 70 eV, direct inlet; ^{1}H NMR: 80 or 300 MHz, TMS as int. standard; ^{13}C NMR: 75 MHz, $CDCl_3$ was taken as reference at 77 ppm. Plant material was collected at 53 km SE from Oaxaca City (Oaxaca, México) and a voucher specimen (MEXU 598853) is deposited at the Herbarium of the Instituto de Biología, UNAM.

Isolation of the constituents of S. amarissima. Dried and finely powdered aerial parts of *S. amarissima* (458 g) were extracted with Me_2CO and MeOH at room temp. to obtain, after solvent evapn, 52 and 29 g extract, respectively. Partition of the Me_2CO extract between MeOH–hexane gave 39.4 and 12.1 g residue, respectively. The same treatment applied to the MeOH extract afforded 21.2 and 7.1 g residue. Both hexane frs were combined (19.2 g), decolourized with activated

Table 2. ^{13}C NMR spectral data for compounds **1** and **2** (75 MHz, $CDCl_3$)

C	1*	2
1	26.4 *t*	26.0 *t*
2	69.9 *d*	69.2 *d*
3	129.6 *d*	128.6 *d*
4	144.0 *s*	145.3 *s*
5	45.7 *s*	46.0 *s*
6	33.9 *t*	34.3 *t*
7	27.7 *t*	27.7 *t*
8	36.4 *d*	36.7 *d*
9	38.0 *s*	38.0 *s*
10	39.8 *d*	39.8 *d*
11	37.9 *t*	37.8 *t*
12	17.2 *t*	17.2 *t*
13	125.4 *s*	125.3 *s*
14	110.8 *d*	111.0 *d*
15	142.6 *d*	142.6 *d*
16	138.4 *d*	138.6 *d*
17	15.3 *q*	15.5 *q*
18	169.5 *s*	168.8 *s*
19	71.1 *t*	71.0 *t*
20	17.4 *q*	17.7 *q*
1′	101.6 *d*	98.7 *d*
2′	73.9 *d*	71.6 *d*
3′	76.6 *d*	72.8 *d*
4′	70.6 *d*	68.5 *d*
5′	75.8 *d*	72.1 *d*
6′	62.2 *t*	61.9 *t*

*Run in $CDCl_3/CD_3OD$ solution.
Multiplicities were obtained by DEPT experiments.

charcoal and chromatographed over silica gel. Mixts of hexane–EtOAc of increasing polarity were used as eluents to obtain 1.87 g a mixt. of ursolic and oleanolic acids. The methanolic fr. of the Me_2CO extract was chromatographed over celite (hexane–EtOAc, gradient elution). Frs eluted with EtOAc contained **1** and pedalitin. These frs were chromatographed over silica gel (Me_2CO–hexane, 4:1) to obtain pedalitin (154.5 mg), which was identified by comparison of its physical and spectral data as well as those reported in the lit. [3–5]. Frs eluted with EtOAc–Me_2CO (9:1 and 4:1) contained **1**. These frs were decolourized with activated charcoal and crystallized from MeOH–H_2O to obtain hydrated **1** (13.88 g) mp 120–132°. Anhydrous **1**, mp 206–208°, $[\alpha]_D$ −18.48° (MeOH; *c* 0.178); UV (MeOH), λ_{max} nm (ε): 208 (17,500); IR (nujol) ν_{max} cm^{-1}: 3386, 1782, 1766, 1666, 1503, 1457, 1377, 1201, 1076, 1032, 969, 944, 874; ^1H NMR: Table 1; ^{13}C NMR: Table 2; EIMS *m/z* (rel. int.): 492 $[M]^+$ (10), 474 $[M-H_2O]^+$ (1), 443 $[474-CH_2OH]^+$ (2), 425 $[443-H_2O]^+$ (1), 398 $[M-C_6H_6O]^+$ (29), 380 $[398-H_2O]^+$ (7), 368 $[398-CH_2O]^+$ (5), 313 $[M-C_6H_{11}O_6]^+$ (14), 283 $[313-CH_2O]^+$ (7), 218 $[C_{14}H_{18}O_2]^-$ (25), 203 $[218-Me]^+$ (15), 95 $[C_6H_7O]^-$ (100), 81 $[C_5H_5O]^+$ (55), 43 (10), 41 (11).

Acetylation of **1**. A soln of **1** (225.7 mg) in pyridine (1.5 ml) and Ac_2O (1.5 ml) was left to stand overnight at room temp. and worked-up as usual to give 299.5 mg of **2** as a syrup: IR ($CHCl_3$) ν_{max} cm^{-1}: 1759, 1502, 1452, 1375, 1049, 970, 908, 874; ^1H NMR: Table 1; ^{13}C NMR: Table 2; FABMS *m/z* (rel. Int.): 661 $[C_{34}H_{44}O_{13}+H]^+$ (27), 601 $[M+H-HOAc]^+$ (2), 331 $[M+H-C_{14}H_{18}O_9]^+$ (48), 313 $[331-H_2O]^-$ (67), 283 $[313-CH_2O]^-$ (5), 218 $[C_{14}H_{18}O_2]^+$ (12), 203 $[218-Me]^-$ (33), 169 $[C_8H_9O_4]^+$ (96), 109 $[C_6H_5O_2]^+$ (47), 95 $[C_6H_7O]^+$ (23), 81 $[C_5H_5O]^+$ (69), 43 $[Ac]^+$ (100).

Preparation of **3**. *Fusarium moniliforme* was inoculated into Czapek medium (20 ml) and incubated at 27±1° for 24 hr with continuous shaking. The culture was then transferred to Czapek medium (250 ml) without saccharose. Compound **1** (200 mg), 5 mg l^{-1} biotin and 100 mg l^{-1} thiamin were added. The culture was incubated at 27±1° for 14 days in a shaker bath (110 oscillations min^{-1}). The resulting suspension was extracted with $CHCl_3$, the solvent was removed under red. pres. and chromatographed over silica gel (hexane–EtOAc, 3:7) to yield **3** (76 mg). Mp 162–163° (Me_2CO–hexane); $[\alpha]_D$ = −13.2° ($CHCl_3$; *c* 0.34); IR ($CHCl_3$) ν_{max} cm^{-1}: 3612, 1771, 1601, 1502, 1471, 1186, 1141, 1023, 972, 935, 873; ^1H NMR: Table 1; MS *m/z* (rel. int.): 330 $[C_{20}H_{26}O_4]^+$ (9), 312 $[M-H_2O]^-$ (8.5), 217 $[C_{14}H_{17}O_2]^-$ (5), 187 $[217-CH_2O]^+$ (6), 173 $[217-CO_2]^+$ (15), 95 $[C_6H_7O]^+$ (100), 81 $[C_5H_5O]^+$ (46).

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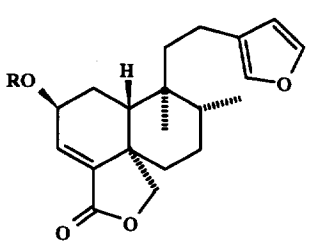

1 R= β-D-Glucopyranosyl
2 R= Tetraacetyl β-D-Glucopyranosyl
3 R= H

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