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GUAIANOLIDES FROM *LIATRIS SQUARROSA**

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Key Word Index—*Liatris squarrosa*; Compositae; Eupatorieae; sesquiterpene lactones; guaianolides, spicatin analogs.

Abstract—The isolation of three new guaianolides related to spicatin from *Liatris squarrosa* is reported.

INTRODUCTION

In continuation of our studies of *Liatris* species (Compositae, Eupatorieae) which have resulted in the isolation of a number of cytotoxic and antileukemic sesquiterpene lactones [1–12] we have investigated *Liatris squarrosa* (L.) Michx. and have isolated three new guaianolides, desacetylspicatin (**1a**), desacetylspicatin hydrochloride (**2a**) and **2b**. Sitosterol, β-amyrin and lupeol were also found.

RESULTS AND DISCUSSION

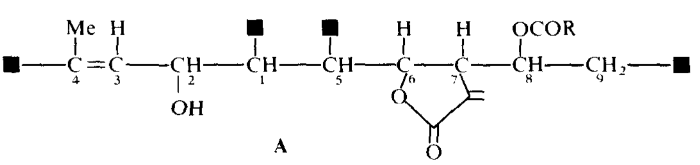
^{1}H and ^{13}C NMR spectra of the three new sesquiterpene lactones, all of which were non-crystalline, are given in Tables 1 and 2. In each case, spin-decoupling experiments established the sequence shown in partial structure **A** (numbering as in final structure), with H-3 being allylically coupled to the methyl group on C-4. In the case of **1a**, the empirical formula from the MS and the spectroscopic evidence also showed the presence of partial structure **B** which on biogenetic grounds was inserted between C-1 and C-9. The spectrometric evidence (MS, Tables 1 and 2) showed clearly that the acyl function attached to C-8 of all three compounds was the *cis*-sarracenoyl-sarracenate ester moiety first encountered in spicatin (**1b**) and its congeners. [5, 6].

On this basis and because of the coupling constants, **1a** was deduced to be desacetylspicatin as can be seen by comparing its ^{1}H and ^{13}C NMR spectra with those of spicatin (**1b**) whose structure has been settled by X-ray crystallography [5,6]. The only significant differences in the ^{13}C NMR spectra are the expected upfield shift of C-2 and the downfield shift of C-3 in going from **1b** to **1a**. Confirmatory evidence was obtained by acetylation of **1a** and **1b** which gave the same diacetate **1c**.

MS and NMR evidence (Tables 1 and 2) indicated that the second lactone was the desacetyl analog **2a** of spicatin hydrochloride (**2c**). This was confirmed by converting **1a** to **2a**. Although **2a** could conceivably be an artefact, a number of similar compounds have been reported as natural products.

The third lactone was a *cis*-sarracenoyl analog of **2a** (MS), in which the new sarracenoyl group esterified a primary hydroxyl as in **2b** or **2d**. A decision in favor of **2b** was reached as follows. Close inspection of the ^{13}C NMR spectra of **1a**, **1b** and **2a** indicated that the signals of the terminal and non-terminal *cis*-sarracenoyl ester moieties differ significantly not so much in the chemical shifts of C-5′ and C-5″, which are surprisingly similar, but in the chemical shifts of C-2′ and C-2″, the C-2 carbon of the sarracenoyl group experiencing an upfield shift of *ca* 4 ppm, from ~131.5 to 127.5, on acylation. This is also evident on comparing the ^{13}C NMR spectra of **3a** and **3b** [13]. As the ^{13}C NMR spectrum of the third lactone exhibited only one singlet near 127.5 and two singlets near 131.50 ppm, it must contain two terminal *cis*-sarracenoyl ester functions as in **2b**. A formula in which the attachment of the C_5 and C_{10} ester functions to C-8 and C-14 is reversed is not excluded by the NMR spectra, but seems less likely for reasons of analogy; an attempt at selective hydrolysis to verify this failed.

Our results differ from those of a previous study of *L. squarrosa* which recorded the absence of sesquiterpene lactones [14]. *L. squarrosa* has been segregated into several varieties [15]. Our material was difficult to classify but may well be var. *squarrosa*. The sesquiterpene lactone chemistry



A

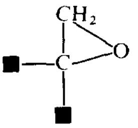


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Table 1. ^{1}H NMR spectra of compounds from *Liatris squarrosa**

	1a	**1a**†	**1b**	**1c**	**2a**	**2a**†	**2b**
H-1	2.0‡	1.42‡	1.63 *dd*	1.61	2.42‡	2.27	2.36 *dd*
H-2	4.62 *ddq*	4.20‡	5.44	5.44	5.46	4.42	4.52
H-3	5.70 *dq*	5.45	5.48	5.50	5.75	5.57	5.75
H-5	2.76 *dd*	2.21	2.18	2.22	2.75	2.45	2.76
H-6	4.64 *dd*	4.63	4.61	4.61	4.50	4.65	4.57
H-7	3.56 *dddd*	3.40	3.38	3.40	3.90	3.87	3.95
H-8	5.62 *ddd*	5.77	5.72	5.76	5.70	5.88	5.71
H-9a	2.65 *dd*	2.75	2.33	2.37	2.42	2.60	2.40
H-9b	2.22 *dd*	2.13	2.05	2.06	2.42	2.40	2.40
H-13a	6.29 *d*	6.24	6.25	6.24	6.25	6.28	6.24
H-13b	5.56 *d*	5.18	5.20	5.24	5.47	5.33	5.46
H-14	2.57 §	2.24§	2.22§	2.25§	3.65 *d* 3.90 *d*	3.46 3.79	4.38§
H-15‖	1.99 *dd*	1.89	1.84	1.87	2.02 *dq*	2.01	2.02
H-3′	7.10 *q*	7.04	6.94	6.94	7.14	7.11	7.12
H-4′‖	1.95	1.35	1.39	1.40	1.95	1.47	1.96
H-5′	4.89 *d* 4.80 *d*	4.80 4.59	4.87 4.65	4.90 4.70	4.93 4.76	4.84 4.72	4.90 4.80
H-3″	6.91 *q*	6.80	6.83	6.99	6.91	6.95	6.91
H-4″‖	1.90 *d*	1.48	1.5	1.58	1.90	1.59	1.91
H-5″	4.30§	4.24§	4.31§	4.89§	4.30§	4.29§	4.31§
Misc			¶	¶			¶

$J_{1,2}$ = 7 Hz for **1**–**3**, 6 Hz for **4** and **5**; $J_{1,5}$ = 9 Hz for **1**–**3**, 6 Hz for **4** and **5**; $J_{2,3} = 2$; $J_{2,15} = 0.5$; $J_{3,15} = 1$; $J_{5,6} = 11$; $J_{6,7} = 8.5$; $J_{7,8} = 3.9$, $J_{3,13a} = 3.5$; $J_{7,13b} = 3$; $J_{8,9a} = 8$; $J_{8,9b} = 7$; $J_{9a,9b} = 15$; $J_{3',4'} = J_{3'',4''} = 7$; $J_{4a',4b'} = 13$.

* Run at 270 MHz in $CDCl_3$ unless indicated otherwise. Frequencies in ppm downfield from TMS.

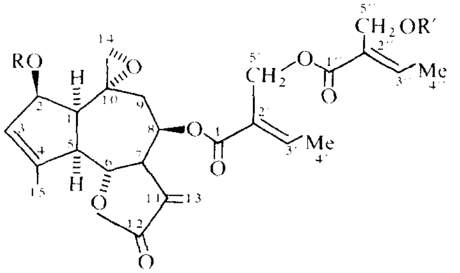
† In C_6D_6.

‡ Obscured signal.

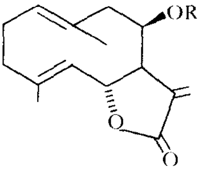
§ Intensity two protons; centre of AB system.

‖ Intensity three protons.

¶ In **1b**, 1.72 (Ac). In **1c**, 1.73 and 1.63 (Ac). In **2b**, 7.02 *q* (7 Hz, H-3‴), 1.94 *d* (7, H-4‴), 4.31 *br* (H-5‴).



1a R, R′ = H
1b R = Ac, R′ = H
1c R, R′ = Ac



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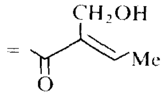

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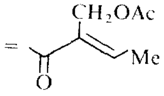
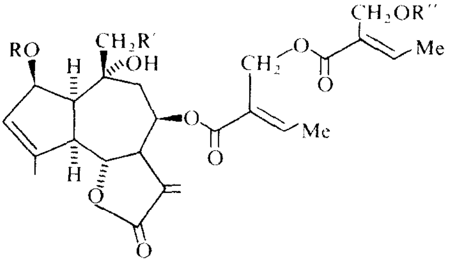
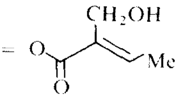




2a R, R″ = H, R′ = Cl

2b R, R″ = H, R′ =


2c R = Ac, R′ = Cl, R″ = H

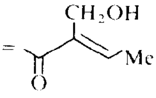
2d R = H, R′ = OH, R″ =


Table 2. ^{13}C NMR spectra of compounds from *Liatris squarrosa**

	1a	**1b**	**2a**	**2b**
C-1	55.37 *d*	55.56 *d*†	54.66 *d*	54.77 *d*
C-2	76.37 *d*	78.68 *d*†	74.70 *d*	74.75 *d*
C-3	129.02 *d*	125.34 *d*†	128.91 *d*	128.99 *d*
C-4	149.51	150.59	149.06	149.18
C-5	52.44 *d*	51.20 *d*†	51.90	52.29
C-6	81.15	80.21 *d*†	82.06 *d*	82.12 *d*
C-7	47.64 *d*	47.53 *d*†	47.22 *d*	47.30 *d*
C-8	67.46 *d*	67.19 *d*†	67.27 *d*	67.38 *d*
C-9	36.15 *t*	35.68 *t*	36.21 *t*	35.99 *t*
C-10	56.05	55.21	73.56	77.30
C-11	134.21	133.65	134.68	134.80
C-12	169.55	169.88	169.87	169.85
C-13	122.53 *t*	123.02 *t*	121.83 *t*	121.69 *t*
C-14	56.33 *t*‡	56.59 *t*‡	54.91 *t*	71.43 *t*
C-15	17.43 *q*	17.46 *q*	17.97 *q*	17.97 *q*
C-1′	165.38	165.33	165.70	165.76
C-2′	127.57	127.60	127.50	127.60
C-3′	145.49 *d*	145.61 *d*	145.80 *d*	145.69 *d*
C-4′	14.53 *q*	14.53 *q*	14.57 *q*	14.57 *q*
C-5′	57.38 *t*	57.52 *t*	57.46 *t*	57.46 *t*
C-1″	166.63	166.68	166.82	166.80
C-2″	131.69	131.90	131.44	131.77‡
C-3″	141.87 *d*	141.11 *d*	142.30 *d*	142.31 *d*§
C-4″	14.23 *q*	14.17 *q*	14.31 *q*	14.30 *q*
C-5″	56.49 *t*‡	56.18 *t*‡	56.16 *t*	56.05 *t*‖
C-1‴		169.12		167.46
C-2‴		21.30 *q*		131.50‡
C-3‴				142.15 *d*§
C-5‴				56.27 *t*‖

* Run in $CDCl_3$ at 67.9 MHz. Unmarked signals are singlets.

† Assignment verified by single frequency off-resonance decoupling.

‡ § ‖ Interchangeable assignments.

of our collection resembles that of *L. spicata*, *pycnostachya*, *graminiflora* and *tenuifolia* all of which yielded similar guaianolides [5, 6, 10], whereas examination of other *Liatris* species gave heliangolides, [1–4, 7–9, 11, 12]. Further studies are required to establish whether such differences provide useful taxonomic information at the subgeneric level.

EXPERIMENTAL

Aerial parts of *Liatris squarrosa* (L.) Michx., collected by Dr. Norlan Henderson on 18 July 1978 along Missouri Highway No. 13 *ca* 3 miles north of Lowry City, St. Clair County, Missouri (Henderson 78-11 on deposit in herbarium of University of Missouri–Kansas City), wt 5.6 kg, were extracted with $CHCl_3$ and worked up as usual [16]. The crude gum (200 g) was adsorbed on 220 g of Si gel (Mallinckrodt 100 mesh) and chromatographed on a 2 kg Si gel column packed with toluene–$CHCl_3$ (1:). The column was eluted with solvents of increasing polarity, 500 ml fractions being collected and monitored by TLC in the following order: 1–6, $CHCl_3$–toluene (1:0); 7–15, $CHCl_3$; 16–20, $CHCl_3$–MeOH (99:1); 21–25, $CHCl_3$–MeOH (97:3) and 26–36 $CHCl_3$–MeOH (19:1).

Fractions 1–6 contained waxy material which was not studied further. Fractions 7–15 which showed one major spot on TLC were combined and crystallized from $CHCl_3$–MeOH, yield 20 g, mp 202° which was a 1:1 mixture of lupeol and β-amyrin by direct comparison. Fractions 16–20 were recrystallized from MeOH, yield 5 g, mp 135°, which was identified as sitosterol by direct comparison.

TLC of fractions 21–25 showed the presence of a major constituent which was purified by prep. TLC (EtOAc–hexane, 3:1). The non-crystalline lactone **2a** had IR ν_{max} cm^{-1}: 3500 (br), 1760 (γ-lactone), 1720 (sh), 1710 (esters), 1650 (sh), 1640 and 1630 (sh) (double bonds); $[\alpha]_D - 40°$ (*c* 0.02, $CHCl_3$); MS (low resolution) *m/e*: 510 (M^+), 492 (both extremely weak), 474, 456, 438, 376, 360, 358, 340, 322, 309, 278, 260, 242, 224; 99 (base peak, $C_5H_7O_2$). The composition of the peak at *m/e* 474 was shown to consist of $M - 2H_2O$ and $M - HCl$ (weak) by peak matching (Calc. for $C_{25}H_{27}O_7Cl$, $C_{25}H_{30}O_9$: 474.1449; 474.1890. Found (MS): 474.1431; 474.1876).

Fractions 26–31, which contained one major constituent, were combined and purified by prep. TLC ($CHCl_3$–MeOH, 9:1). The product **1a** (100 mg) could not be induced to crystallize and had $[\alpha]_D - 25.5°$ (c 0.03, $CHCl_3$); IR ν_{max} cm^{-1}: 3420 (br, –OH), 1770 (γ-lactone), 1730 (sh) and 1720 (strong esters), 1655 (sh), 1645, 1630 (sh) and 1620 (sh); MS m/e: 474 (M^+, very weak), 456, 438, 420, 359, 358, 340, 260, 24, 99 (base peak, $C_5H_7O_2$). The molecular ion was not observed.

Acetylation of 50 mg **1a** with Ac_2O–Py at 0° overnight followed by the usual work-up and purification by prep. TLC gave 35 mg **1c** which was identical with acetylspicatin in all respects (TLC, IR, NMR). A solution of 20 mg **1a** in 5 ml dry EtOH and 0.2 ml 12 % HCl was stirred at room temp. for 4 hr and evapd *in vacuo*. The residue was purified by prep. TLC. The product which was less polar than **1a** was identical in all respects with **2a** from the plant.

Fractions 32–36 showed the presence of a major constituent which was purified by prep. TLC (EtOAc–hexane, 9:1, two developments). The product **2b** (50 mg) could not be induced to crystallize and had $[\alpha]_D - 30.5°$ (c 0.015, $CHCl_3$); IR ν_{max} cm^{-1}: 3414 (br, –OH), 1765, 1725 (sh) and 1710 (v. strong, esters), 1660 (sh), 1655, 1635 (sh) and 1620 (sh); MS m/e: 572 (M − 18, very weak), 554, 474, 456, 438, 358, 340, 260, 242, 99 (base peak, $C_5H_7O_2$). The molecular ion was not observed.

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