New Melampolides from *Schkuhria schkuhrioides*

Guillermo Delgado,*,† Verónica Tejeda,† América Salas,† María Isabel Chávez,† Salvador Guzmán,† Alberto Bolaños,† María Isabel Aguilar,‡ Víctor Navarro,§ and María Luisa Villarreal§

Instituto de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Circuito Exterior, Coyoacán 04510, Mexico, D. F., Facultad de Química, Departamento de Farmacia, Universidad Nacional Autónoma de México, Ciudad Universitaria, Circuito Exterior, Coyoacán 04510, Mexico, D. F., and Unidad de Investigación Biomédica del Sur, Instituto Mexicano del Seguro Social, Argentina 1, Xochitepec, Morelos, Mexico

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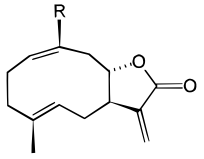
The novel melampolides (11*R*)-11,13-dihydro-schkuhriolide (**7**), (11*S*)-11,13-dihydro-schkuhriolide (**8**), and schkuhrioidiol (**11**), along with the known constituents, frutescin (**1**), schkuhriolide (**2**), frutescinic acid (**4**), *allo*-schkuhriolide (**5**), and epoxyschkuhriolide (**6**) were isolated from the aerial parts of *Schkuhria schkuhrioides*. The structures of the new compounds were determined by spectroscopic methods. Compounds **1**, **2**, **4**, **5**, and **6** displayed no significant cytotoxic or antimicrobial activities.

Species belonging to the genus *Schkuhria*[1] are known sources of sesquiterpene lactones,[2−6] diterpenes,[7] polyacetylenes, and other constituents.[8,9] Some species and their varieties are used in traditional medicine,[1,2] and different biological activities have been reported for some constituents.[10,11] Previous papers have reported a series of melampolides,[12,13] elemanolides,[14,15] and flavonoids from the aerial parts of *S. schkuhrioides* (Link & Otto) Thellung (Compositae). We have now characterized additional sesquiterpene lactones from this source, and the antimicrobial and cytotoxic activities of some melampolides were evaluated.

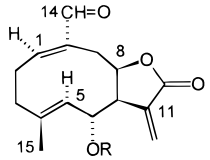
Aerial parts of *S. schkuhrioides* were extracted with *n*-hexane and then with acetone. This extract was chromatographed using vacuum liquid chromatography (VLC)[16,17] to yield frutescin (**1**),[18,19] schkuhriolide (**2**),[12,13] frutescinic acid (**4**),[20] *allo*-schkuhriolide (**5**),[12,21,22] epoxyschkuhriolide (**6**),[13,23] and the novel natural sesquiterpenes **7**, **8**, and **11.** Spectroscopic data of **1**, **2**, **4**, **5**, and **6** were identical to those reported previously.

Some fractions containing a complex mixture of minor constituents were acetylated and separated by repeated column chromatography and preparative TLC, to afford epimers **9** and **10**. The structures were deduced from their ^{1}H NMR data (Table 1), which were very closely related to those of acetyl schkuhriolide (**3**), previously characterized.[13] The structures **9** and **10** were established as the 11,13-dihydroderivatives of acetyl schkuhriolide, in agreement with the molecular formula and expected changes in the NMR data. The configurations at C-11 in **9** and **10** were determined by observing the changes in the chemical shifts of H-11 and H-13 (in $CHCl_3$ and C_6D_6).[24,25] The major difference in the chemical shifts of H-13 ($\Delta\delta = \delta_{CDCl_3} - \delta_{C_6H_6}$), due to the shielding effect of the solvent, observed for **9** ($\Delta\delta_{H(13)} = 0.3$) with respect to that of **10** ($\Delta\delta_{H(13)} = 0.17$) indicated that the secondary methyl group in **9** is oriented to the α-(convex) face of the macrocycle. The same trend is observed for H-11 in **10** ($\Delta\delta_{H(11)} = 0.84$) when compared to **9** ($\Delta\delta_{H(11)} = 0.17$), corroborating the α-orientation of H-11 in **10**. Therefore, **7** [(11*R*)-11,13-dihydro-schkuhriolide] and **8** [(11*S*)-11,13-dihydro-schkuhriolide] are natural constituents of *S. schkuhrioides*.

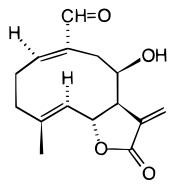
The most polar compound, schkuhrioidiol (**11**), was also a sesquiterpene lactone as suggested by the EIMS and ^{13}C NMR data. The ^{1}H NMR data, which also closely resembled those of **2**, indicated the presence of an hydroxymethylene at C(14). ^{1}H COSY, HMBC, and HMQC experiments[26] of **11** and **12** (obtained by acetylation of **11**) allowed the assignment of all ^{1}H and ^{13}C signals (See Table 2), confirming the structures. The



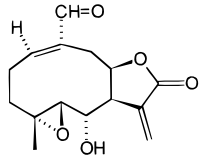
1 R = CHO Frutescin
4 R = COOH



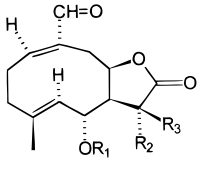
2 R = H Schkuhriolide
3 R = Ac



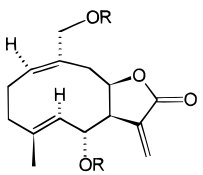
5 *Allo*-schkuhriolide



6 Epoxy-schkuhriolide



	R_1	R_2	R_3
7	H	CH_3	H
8	H	H	CH_3
9	Ac	CH_3	H
10	Ac	H	CH_3



11 R = H Schkuhrioidiol
12 R = Ac

* To whom correspondence should be addressed. Tel.: (525) 622-4446. Fax: (525) 616-2217. E-mail: delgado@servidor.unam.mx.
† Instituto de Química, UNAM.
‡ Facultad de Química, UNAM.
§ Unidad de Investigación Biomédica del Sur, IMSS.

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Table 1. ^{1}H NMR (300 MHz, $CDCl_3$, δ, Coupling Constants in Parentheses) Spectral Data for Melampolides **9** and **10**

hydrogen	**9**	**9**[a]	**10**	**10**[a]
H-1	6.62 ddd (9.4,7,2)	5.57 ddd (8.1,7,2)	6.63 ddd (9.6,7,2)	5.57 ddd (9.4,7,3)
H-2	*b*	*b*	*b*	*b*
H-3				
H-5	4.69 br d (10.5)	4.50 br d (10.2)	4.65 br d (9.6)	4.30 br d (9.1)
H-6a				
H-6b	5.14 t (10.6)	5.29 t (10.6)	5.29 t (10.6)	5.38 dd (10,10.6)
H-7	*b*	*b*	*b*	*b*
H-8	5.77 dt (12,5.1)	5.86 dt (11.4,5)	5.53 dt (12,5.8)	5.43 dt (12,5.8)
H-9a	*b*	*b*	*b*	
H-9b				
H-11	2.62 q (7.8)	2.63 q (7.7)	2.96 m	2.12 m
H-13a				
H-13b	1.36 d (7.8)	1.06 d (8.1)	1.20 d (6.8)	1.07 d (7.3)
H-14	9.44 d (1.8)	9.02 d (1.8)	9.46 d (1.8)	9.06 d (1.9)
H-15	1.94 br s	1.59 br s	1.92 br s	1.53 s
H-16				
H-17a				
H-17b				
OAc	2.01 s	1.52 s	1.96 s	1.50 s

[a] Taken in C_6D_6. [b] Superimposed signals.

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for Melampolides **6**, **11**, and **12** (δ Values in ppm from TMS)[a]

	6			**11**			**12**		
position	δ_H, mult	*J* (Hz)	δ_C	δ_H, mult	*J* (Hz)	δ_C	δ_H, mult	*J* (Hz)	δ_C
1	6.82 ddd	8.5, 8.0, 2.5	155.86	5.53 br t	8.1	128.30	5.64 br t	7.5	131.58
2a	2.50 br t	4.1	24.43	2.13 m		24.22	2.20 m		24.37
2b	2.52−2.55 m			1.87 dd	2.9, 12.3		1.92 m		
3a	2.35 br t	4.5	35.82	2.20 m		38.09	2.22 m		38.04
3b	2.32−2.34 m			1.75 m			1.75 dd	12, 10.5	
4			62.86			135.89			131.44
5	2.80 d	9	63.87	4.95 d	10.2	125.37	4.83 d	10.5	122.12
6	3.31 dd	10, 9	65.02	4.03 t	10.2	65.74	5.25 t	10.5	68.97
7	2.89 m		48.67	2.88 dd	10.2, 5.1	49.90	3.02 br dd	10.5, 5.0	47.08
8	5.59 ddd	10, 9, 2.5	75.35	4.91 ddd	12.3, 5.1, 4.5	79.36	4.74 ddd	13, 5, 5	78.22
9a	3.05 br dd	14.5, 2.5		2.59 dd	12.3, 4.5	29.77	2.60 m		29.79
9b	2.59 br dd	14.5, 10		2.29 m			2.32 br t	13	
10			140.17			136.48			131.77
11			136.89			138.48			137.70
12			169.24			169.91			169.37
13a	6.33 d	2.0	126.05	5.82 t	1	124.76	6.27 t	0.5	124.70
13b	5.84 d	2.0		6.30 t	1		5.73 t	0.5	
14a	9.46	2.0	196.60	4.13 d	12	67.58	4.61 d	12.5	68.31
14b				4.07 d	12.0		4.47 d	12.5	
15	153 s		17.55	1.77 s		16.90	1.91 d	1.5	17.04
C(6)OAc							2.00		170.42, 20.86
C(14)OAc							2.08		168.82, 20.75

[a] Assignments were made on the basis of HMBC, HMQC, and NOESY correlation methods.

relative stereochemical assignments of **12** were accomplished by NOESY experiments. The observation of a strong NOE between H-14 methylene protons and H-1, and between H-7 and H-8 confirmed the *cis*-configuration of the C(1)−C(10) double bond and the γ-lactone, respectively. The NOE observed between H-14 and H-7 and H-8, as well as the NOE between H-1 and H-5, allowed us to establish the $[_1D_{14}\ ;\ ^{15}D_5]$[27] conformation for **12**, which is similar to that found for **2**. $NaBH_4$ reduction of **2** afforded **11**, confirming the structure of the new melampolide.

The acetone extract of *S. schkuhrioides* investigated was found not to be active against several microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella tiphi*, *Candida albicans*, *Tricophyton mentagrophytes*, *Microsporum gypseum*; MIC > 400 μg/mL),[28,29] tumor cancer cell lines (KB, nasopharingeal carcinoma, UISO, cervix carcinoma, COLON, colon carcinoma; DE_{50} > 20 μg/mL),[30] or *Artemia salina* (LC_{50} > 800 ppm).[31] Compounds **1**, **2**, **4**, **5**, and **6** displayed no significant activities in the above-mentioned bioassays. Compound **5** showed the best cytotoxicities with ED_{50} values of 5.7×10^{-4}, 1.82, and 0.9 μg/mL against KB, UISO, and COLON, respectively.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on Varian VXR-300 and Varian Unity Plus-500 instruments, and the chemical shifts are expressed in parts per million (δ) relative to TMS. Samples for NOE experiments were degassed (freeze, pump, thaw, 3×) and sealed under argon. IR spectra were recorded with a Nicolet Magna IR TM 750 and Perkin−Elmer 283B instruments. MS data were recorded with a JEOL JMS-AX 505 HA mass spectrometer. EIMS were obtained at 70 eV ionization energy. Vacuum chromatography was performed on Merck Kieselgel 60 (0.040−0.863 mm).[16,17] All separations were carried out using distilled solvents. TLC analyses were performed on Alugram Sil G/UV_{254} Si gel plates.

Plant Material. Aerial parts of *S. schkuhrioides* were collected near Teoloyucan (State of Mexico), in September 1993. A voucher specimen (MEXU 636061) has been deposited at the National Herbarium, Instituto de Biología de la Universidad Nacional Autónoma de México.

Extraction and Isolation. The air-dried plant material (5 kg) was powdered and extracted with *n*-hexane (twice, 48 h) and then with Me_2CO (twice, 48 h) at room temperature, to give 177 g of residue. This extract (170 g) was chromatographed using VLC over Si gel (670 g) with a *n*-hexane−EtOAc gradient to obtain 14 fractions. The residue obtained from the fraction 7 eluted with *n*-hexane−EtOAc (9:1) (15 g) was subjected to column rechromatography over Si gel to afford 85 mg of **1**.[18,19] Repeated rechromatography over Si gel of fraction 8 (12.5 g, eluted with *n*-hexane−EtOAc 7:3) with *n*-hexane−EtOAc gradient gave a residue that was further purified by column chromatography over Si gel using CH_2Cl_2−MeOH (4:1), to afford 240 mg of frutescinic acid (**4**).[20] Fraction 9 (8.1 g, eluted with *n*-hexane−EtOAc 3:2) was rechromatographed using VLC over Si gel (*n*-hexane−EtOAc gradient), and some fractions were further purified by column chromatography using CH_2Cl_2−MeOH (81:19) as eluent to obtain 35 mg of *allo*-schkuhriolide (**5**).[12,21,22] Subsequent fractions of this rechromatography, which contained the mixture **7** + **8** (960 mg), were acetylated following the standard procedure to afford a residue that was chromatographed over Si gel using *n*-hexane−EtOAc mixtures of increasing polarity to obtain **9** (12 mg) and **10** (16 mg). Compound **2**[12,13] (2g) crystallized from the eluates of the chromatography of fraction 10 (eluted with *n*-hexane−EtOAc, 1:1). Some polar fractions of the rechromatography of fraction 10 (7 g) were rechromatographed over Si gel using VLC with *n*-hexane−EtOAc gradient, and some fractions were further subjected to repeated column chromatography on Si gel (*n*-hexane−EtOAc and CH_2Cl_2−MeOH gradients) to obtain epoxyschkuhriolide (**6**)[13,23] (52 mg). Schkuhrioidiol (**11**) (53 mg) was isolated from fraction 11 (eluted with *n*-hexane−EtOAc 2:3) after repeated column chromatography over Si gel followed by preparative TLC (CH_2Cl_2−MeOH (4:1). Standard acetylation of **11** afforded **12**.

(11*R*)-11,13-Dihydro-acetyl-schkuhriolide (9): colorless oil, $[\alpha]^{25}_D$ + 9.1 (*c* 0. 11, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.89), 226 (3.80) nm; IR ($CHCl_3$) ν_{max} 2953, 2928, 2855, 1773, 1732, 1685, 1630, 1460, 1373, 1179, 1009, 945 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ and in C_6D_6), see Table 1; ^{13}C NMR (75 MHz, C_6D_6) δ 195.6 s (C-14), 178.1 (s, C-12), 154.8 (d, C-1), 141.3 (s, C-10), 137.3 (s, C-4), 123.7 (s, C-5), 70.4 (d, C-8), 68.9 (d, C-6), 41.2 (d, C-7), 38.9 (d, C-11), 37.3 (t, C-3), 29.7 (t, C-9), 27.6 (t, C-2), 17.1 (q, C-15), 14.8 (q, C-13); EIMS *m/z* 306 $[M]^+$ (2), 277 (4), 262 (4), 246 (25), 240 (17), 217 (40), 203 (10), 173 (25), 143 (45), 131 (15), 105 (22), 83 (32), 69 (30), 43 (100).

(11*S*)-11,13-Dihydro-acetyl-schkuhriolide (10): colorless oil, $[\alpha]^{25}_D$ + 2.63 (*c* 0. 19, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.77), 227 (3.77) nm; IR ($CHCl_3$) ν_{max} 3686, 2937, 2857, 1769, 1730, 1687, 1522, 1429, 1011, 932 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ and in C_6D_6), see Table 1; EIMS *m/z* 306 $[M]^+$ (1), 217 (33), 143 (47), 43 (100), 41 (35).

(4*R*,5*R*)-4(5)-Epoxyschkuhriolide (6): colorless needles (Me_2CO) mp 132−134 °C [lit:[13] 134−136 °C]; IR ($CHCl_3$) ν_{max} 3597, 2932, 2865, 1766, 1713, 1688, 1635, 1522, 1425, 1367, 1337, 997 cm^{-1}; ^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 278 $[M]^+$ (2), 263 (6), 250 (6), 240 (15), 217 (32), 198 (24), 171 (25), 138 (31), 135 (22), 105 (33), 95 (38), 83 (58), 55 (46), 43 (100), 41 (54).

Schkuhrioidiol (11): pale yellow oil, $[\alpha]^{25}_D$ + 124.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.13) nm; IR ($CHCl_3$) ν_{max} 3600, 3027, 2938, 2875, 1765, 1672, 1456, 1384, 1296, 1046 cm^{-1}; ^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 264 $[M]^+$(1), 246 (3), 231 (2), 180 (20), 145 (29), 143 (28), 135 (34), 117 (48), 105 (67), 84 (89), 83 (100), 79 (65), 67 (52), 55 (50), 41 (76), 39 (58); *anal.* C 68.29%, H 7.88%, calcd for $C_{15}H_{20}O_4$, C 68.16%, H 7.63%..

Compound 11 obtained via reduction of 2. To a stirred solution of $NaBH_4$ (6 mg, 0.15 mmol) in MeOH (5 mL) was added dropwise a solution of **2** (80 mg, 0.30 mmol) and $CeCl_3 \cdot 8H_2O$ (149 mg, 0.38 mmol) in MeOH (5 mL). The resultant mixture was stirred for 5 min at room temperature, decomposed with diluted 10% HCl (to pH 6), and extracted with EtOAc (× 3, 25 mL). The combined organic layer was washed with H_2O and dried over Na_2SO_4. Solvent was removed under reduced pressure, and the resultant material was purified by column chromatography (*n*-hexane−EtOAc gradient) to give **11** (74 mg).

Diacetylschkuhrioidiol (12): obtained by standard acetylation of **11**; pale yellow oil, IR ($CHCl_3$) ν_{max} 2960, 1770, 1737, 1672, 1460, 1372, 1164, 1116, 1010, 957, 947 cm^{-1}; ^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$), see Table 2; EIMS *m/z* 318$[M]^+$ (1), 290 (4), 303 (2), 258 (6), 162 (12), 143 (15), 105 (33), 84 (70), 83 (100), 55 (45).

Bioassays. The antimicrobial studies,[28,29] the cytotoxicity assays,[30] and the brine shrimp (*Artemia salina* Leach) lethality tests[31] for the Me_2CO extract, fractions, and isolated compounds (**1**, **2**, **4**, **5**, and **6**) from the title plant were performed using standard protocols.

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