Phytochemistry, Vol. 26, No. 3, pp. 755–759, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00

SCHKUHRIDIN A AND SCHKUHRIDIN B, *C*-14β,*H*-5α-ELEMANOLIDES FROM *SCHKUHRIA SCHKUHRIOIDES**

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(*Received* 7 *April* 1986)

Key Word Index - *Schkuhria schkuhrioides*; Compositae; Heliantheae; sesquiterpene lactones; $C_{14}\beta,H_5\alpha$-elemanolides; schkuhridin A; schkuhridin B.

Abstract From *Schkuhria schkuhrioides*, two new sesquiterpene lactones, schkuhridin A and schkuhridin B, were isolated. Their structures and relative stereochemistry were assigned by chemical and spectral evidence. A separate proof for the structures of the novel compounds was provided by the transformation of budlein B to schkuhridin B via basic hydrolysis, Cope rearrangement and relactonization.

INTRODUCTION

The genus *Schkuhria*, which comprises *ca* 15 taxa [1], belongs to the tribe Heliantheae of the Compositae. It is endemic to the American continent and has been introduced into Africa. Organic extracts of some species exhibit antifeedant, antimicrobial and antileukaemic activity [2–4], and phytochemical reports indicate that acetylenic compounds [5], labdanes [6], heliangolides [3, 4, 7], germacrolides [7, 8], melampolides [8, 9] and *C*-14α,*H*-5β-elemanolides [10] are constituents of this genus. We recently reported the conversion of schkuhriolide (**1**) to elemanschkuhriolide (**2**) via Cope rearrangement and intramolecular hemiacetalization. This and the occurrence of both products in *S. schkuhrioides*, allowed us to propose the melampolides as possible biogenetic precursors of *C*-14α,*H*-5β-elemanolides [10].

During the phytochemical analysis of this plant it was observed that several lactonic constituents displayed a great tendency to decompose when they were purified from a mixture containing a flavonoid compound, and these constituents could not be characterized. Therefore, it was decided to reinvestigate this species and in the present paper we report the structure of these minor and unstable sesquiterpene lactones, namely, schkuhridin A (**3**) and B (**4**). The proposed structures of these compounds were confirmed by chemical correlation with the germacrolide budlein B (**5**), a natural product isolated from several *Viguiera* species [11–13].

RESULTS AND DISCUSSION

Extraction of the aerial parts of *S. schkuhrioides* with acetone afforded, after careful chromatographic separation, schkuhriolide (**1**) [9], elemanschkuhriolide (**2**) [10], and schkuhrioidin (**6**) [14], whose structures were established by direct comparison with authentic samples. Two novel non-crystalline and unstable sesquiterpene lactones, schkuhridin A (**3**) and B (**4**), were also isolated and their structures established as follows.

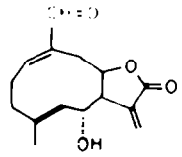
Mass spectrometry of schkuhridin A (**3**) indicated the molecular formula $C_{20}H_{28}O_6$ and its IR spectrum was consistent with the presence of an hydroxyl group (3580 cm^{-1}), an ester carbonyl (1730 cm^{-1}) and an α,β-unsaturated-γ-lactone (1765, 1635 cm^{-1}). The ^{13}C NMR spectrum of schkuhridin A (**3**, Table 1) showed signals arising from a quaternary carbon atom, three methyl groups, one methylene carbon, three methines, a methylene carbon bonded to an oxygen atom, three methines bearing oxygen, six olefinic carbons and two carbonyls. In agreement with these assignments, the ^1H NMR spectrum of **3** (Table 2) showed signals for an olefinic methyl and two secondary methyl groups. Olefinic protons between δ6.30 and 4.75 include an ABX system of a monosubstituted double bond, the characteristic hydrogens β to the lactonic carbonyl and the hydrogens of a *gem*-disubstituted double bond. All the above data allowed us to propose an elemanolide skeleton for schkuhridin A (**3**), esterified at C-14 with a five-carbon chain (α-hydroxyisovalerate), in agreement with the molecular formula requirements.

Mild catalytic hydrogenation of **3** afforded the tetrahydro derivative **8** as an oil, $C_{20}H_{32}O_6$, in whose ^{13}C and ^1H NMR spectra (Tables 1 and 2) the signals of the C(1)–C(2) and C(11)–C(13) double bonds were replaced by new signals at higher field. The presence of two secondary hydroxyl groups in schkuhridin A (**3**) was confirmed by acetylation to obtain the non-crystalline diacetyl derivative **9**, $C_{24}H_{32}O_8$, as indicated by the downfield shift in the ^1H NMR spectrum (Table 2) of the protons geminal to these oxygenated functions (Δδ 1.45 for H-6 and Δδ 0.83 for H-2′), and the appearance of two new methyl signals in the ^{13}C and ^1H NMR spectra (Tables 1 and 2).

Catalytic hydrogenation of the diacetyl derivative **9** afforded **10** in whose ^{13}C and ^1H NMR spectra were observed the above described changes.

Coupling constants and the proton connectivity

*Contribution No. 788 of Instituto de Química de la UNAM.



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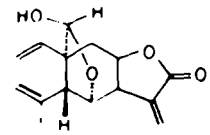

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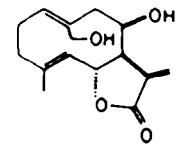

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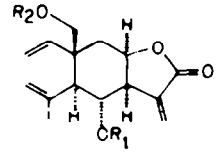
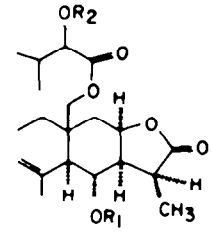




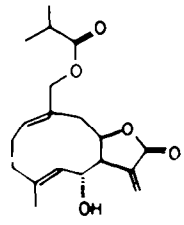
3 R_1 = H, R_2 = COCH(OH)CH(CH_3)$_2$

4 R_1 = H, R_2 = H

9 R_1 = Ac, R_2 = COCH(OAc)CH(CH_3)$_2$

8 R_1 = R_2 = H

10 R_1 = R_2 = Ac



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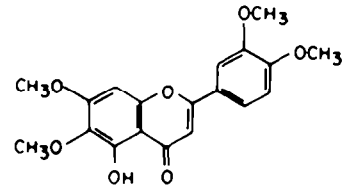

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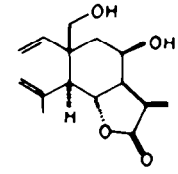

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sequence of the cyclohexane ring of 3 were determined by proton decoupling experiments. In particular, the antiperiplanar relationship between H-5, H-6 and H-7 is indicated by their *trans*-diaxial coupling constants ($J_{5,6}$ = 11 Hz, $J_{6,7}$ = 9 Hz), and assuming that H-7 is α [15], H-6 is β and H-5 is α oriented. Vicinal coupling of H-7 and H-8 ($J_{7,8}$ = 6 Hz) as well as the allylic coupling between H-7 and H-13, H-13' showed that the lactone ring is *cis* and belongs to the A type ($^4J_{7,13}$ < 3 Hz) [16]. The low chemical shift of H-14 of 3 (δ4.17) suggested an axial orientation of the oxymethylene group [17], in agreement with biogenetic considerations [18]. Therefore this substance possesses the *C*-14β,*H*-5α stereochemistry common in natural elemanolides.

The second new sesquiterpene lactone isolated from *S. schkuhrioides*, schkuhridin B (4), was a colourless oil with a molecular formula of $C_{15}H_{20}O_4$. Its IR spectrum showed hydroxyl (3570 cm^{-1}) and α,β-unsaturated-γ-lactone absorptions (1760, 1635 cm^{-1}), and its NMR spectral data correlated directly with those of schkuhridin A (3) expected for the absence of the α-hydroxyisovaleroyl moiety at C-14, since the oxymethylene AB system showed an up-field shift ($\Delta\delta$0.59, Table 2), and the characteristic signals of this residue were absent. Alkaline hydrolysis of schkuhridin A (3) afforded a substance identical in all respects with schkuhridin B (4), confirming the proposed structure for the natural product.

Budlein B (5) [11, 12], a germacrolide isolated from several *Viguiera* species [13], could be converted to schkuhridin B (4) via relactonization to C-8 and Cope rearrangement. However, initial attempts at thermal rearrangement or relactonization of budlein B gave unsatisfactory results. Examination of the Dreiding models showed that the appropriate crown ($_1D^{14}$, $^{15}D_5$) conformation [16] for the [3,3]sigmatropic reaction is more easily achieved when the lactonic ring is open. Furthermore, a preferential C-8 and *cis* (rather than C-6 and *trans*) relactonization may be anticipated for C-14β,H-5α-elemanolides and, therefore, we decided to attempt the Cope rearrangement under the hydrolysis

Table 1. ^{13}C NMR spectral data of 3, 8 and 9 (20 MHz, $CDCl_3$)

	3	8	9
1	142.16 (*d*)	31.30 (*t*)	142.03 (*d*)
2	115.58 (*t*)	7.86 (*c*)	116.59 (*t*)
3	114.31 (*t*)	117.20 (*t*)	114.76 (*t*)
4	142.33 (*s*)	142.43 (*s*)	140.21 (*s*)
5	56.25 (*d*)	56.95 (*d*)	54.26 (*d*)
6	69.72 (*d*)	67.99 (*d*)	71.04 (*d*)
7	46.51 (*d*)	46.95 (*d*)	45.27 (*d*)
8	75.04 (*d*)	75.13 (*d*)	74.99 (*d*)
9	33.02 (*t*)	32.84 (*t*)	32.61 (*t*)
10	42.20 (*s*)	39.66 (*s*)	42.44 (*s*)
11	138.17 (*s*)	41.64 (*d*)	137.17 (*s*)
12	169.82 (*s*)	178.72 (*s*)	168.89 (*s*)
13	123.96 (*t*)	11.04 (*c*)	123.57 (*t*)
14	67.27 (*t*)	66.23 (*t*)	66.85 (*t*)
15	25.88 (*c*)	23.74 (*c*)	24.77 (*c*)
16	174.65 (*s*)	174.73 (*s*)	169.72 (*s*)
17	75.36 (*d*)	77.11 (*d*)	76.41 (*d*)
18	39.11 (*d*)	39.20 (*d*)	36.58 (*d*)
19	15.41 (*c*)	15.40 (*c*)	15.34 (*c*)
20	11.67 (*c*)	11.67 (*c*)	11.43 (*c*)
Me			20.70
Me			20.51
CO			170.51
CO			169.57

conditions. In this manner, treatment of budlein B (5) with methanolic KOH, heating to reflux for 1 hr, taking the solution to dryness, and acidification of the residue, afforded material identical with schkuhridin B (4, Scheme 1), thus confirming the proposed structures for the new natural products.

EXPERIMENTAL

Aerial parts of *S. schkuhrioides* (Link. & Otto.) Thellung were collected along Hwy 45, 10 km N Lagos de Moreno, State of Jalisco, México. Voucher (GD 1156) deposited in the National Herbarium, Instituto de Biologia de la Universidad Nacional Autónoma de México.

Isolation of compounds 1-4, 6 *and* 7. Dried and comminuted material (4.5 kg) was extracted with Me_2CO (× 2) at room temp. for 5 days. The concentrate of the extract (47 g) was chromatographed on silica gel, eluted with hexane and hexane–EtOAc mixtures. Initial fractions gave fats and waxes, which were discarded. Subsequent chromatographic development yielded the following compounds in order of increasing polarity: elemanschkuhriolide (2, 245 mg) [10], schkuhrioidin (6, 340 mg) [8], a mixture of schkuhridin A (3), schkuhridin B (4), 3'-methylcirsilineol (7) [14] (600 mg) and schkuhriolide (1, 1.3 g).

The hexane–EtOAc (1:1) eluates of the initial CC afforded on standing at 0–5° yellow crystals which were sepd by filtration. Successive crystallization of this material from EtOH, gave a solid mp 190–192° (lit.: 190–191° [14]) which was identified as 3'-methyl-cirsilineol (7) by direct comparison. The two remaining substances from this residue, displayed great reactivity toward light, temp. or air, and this instability increases on separation from the flavonoid compound, which could be considered a stabilizer of the mixture [19]. Therefore, these two substances were handled at low temp. and protected from light, under an Ar atm. Both substances showed very similar R_f values in several solvent systems, but careful CC on silica gel–$AgNO_3$ (10%), using hexane EtOAc, allowed partial resolution of the mixture. Evapn of the eluates of the more mobile compound gave 117 mg of schkuhridin A (3) as a pale yellow oil $[\alpha]_D^{25} = +104.58$ ($CHCl_3$, *c* 0.10); UV λ_{max}^{MeOH} nm (log ε): 204 (3.9); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3579, 2969, 1765, 1730, 1640, 1602, 1459, 1392, 1348, 1263, 1238, 1218, 1136, 1048, 982, 913; MS *m/z* (rel. int.): 364 [M]$^+$ (1), 346 (1), 264 (8), 246 (3), 239 (3), 238 (3), 201 (4), 200 (3), 183 (5), 135 (11), 119 (12), 117 (11), 107 (18), 105 (21), 91 (30), 79 (18), 77 (16), 57 (34), 45 (53), 43 (100), 41 (81); ^{13}C and ^1H NMR: see Tables 1 and 2.

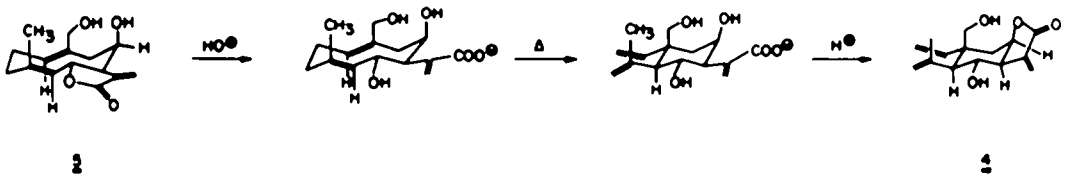
Subsequent fractions of this rechromatography eluted a mixture of 3 and 4, but some final fractions afforded pure schkuhridin B (4) (^1H NMR maintaining) as a pale yellow oil (21 mg) $[\alpha]_D^{25} = +60.54$ (MeOH, *c* 0.18); UV λ_{max}^{MeOH} nm (log ε): 204 (4.1); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 2960, 1761, 1662, 1635, 1372, 1342, 1260, 1144, 1085, 1045, 975, 950, 908; MS *m/z* (rel. int.): 264 [M]$^+$ (1), 246 (22), 228 (5), 216 (6), 215 (7), 201 (4), 169 (13), 149 (18), 143 (18), 107 (42), 105 (60), 93 (41), 91 (94), 79 (45), 69 (53), 55 (61), 43 (58), 41 (100), 39 (67); ^1H NMR: see Table 2.

Catalytic hydrogenation of schkuhridin A (3). A soln of 46.9 mg of 3 in 10 ml of EtOAc was hydrogenated using 10% Pd/C (6 mg) as catalyst during 3 hr at room temp. The Pd/C was filtered off and the solvent removed under vacuum. The non-crystalline product 8 (44.1 mg) showed: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 2958, 2920, 1760, 1725, 1632, 1598, 1453, 1375, 1160, 1131, 1040, 1010, 905; MS *m/z* (rel. int.): 366 [M]$^+$ (1), 348 (3), 330 (5), 248 (18), 118 (40), 117 (45), 43 (100); ^{13}C and ^1H NMR: see Tables 1 and 2.

Acetylation of schkuhridin A (3). The acetate was prepared in the usual manner with pyridine–Ac_2O affording, after CC using hexane EtOAc, a 69% yield of 9 as a pale yellow oil $[\alpha]_D^{25} = +11.35$ ($CHCl_3$, *c* 0.185); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3005, 2929, 2936, 2879, 1755, 1744, 1460, 1374, 1239, 1194, 1112, 1042, 1029, 954, 909; EM *m/z* (rel. int.): 448 [M]$^+$ (3), 388 (5), 328 (5), 345 (5), 160 (16), 120 (15), 43 (100); ^{13}C and ^1H NMR: see Tables 1 and 2.

Catalytic hydrogenation of 9. 9 (102.5 mg) was reduced as described above for 3 affording 90.3 mg of 10 as a pale yellow oil, after CC purification using hexane–EtOAc (4:1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3030, 2929, 2938, 2882, 1774, 1740, 1637, 1460, 1432, 1374, 1243, 1194, 1166, 1131, 1042, 907, 860; MS *m/z* (rel. int.): 452 [M]$^+$ (3), 424 (7), 393 (2), 372 (2), 232 (3), 176 (2), 157 (21), 144 (8), 129 (22), 115 (11), 109 (10), 93 (10), 91 (9), 69 (32), 43 (100); ^1H NMR: see Table 2.

Hydrolysis of schkuhridin A (3). A 144 mg sample of 3 was



Scheme 1. Conversion of budlein B (5) to schkuhridin B (4).

Table 2. ^{1}H NMR data of 3, 4 and 8–10*

	3	4	8	9	10
H-1	5.78 *dd* (11.5, 15)	5.78 *dd* (11.5, 15)	†	5.75 *dd* (11, 16)	
H-2a	5.16 *dd* (11.5, 1.5)	5.16 *dd* (11.5, 1.5)	†	5.24 *d* (11)	
H-2b	5.07 *dd* (15, 1.5)	5.07 *dd* (15, 1.5)	†	5.10 *d* (16)	
H-3a	5.24 *br s* $W_{1/2}$ = 2	5.24 *br s* $W_{1/2}$ = 2	4.89 *br s* $W_{1/2}$ = 3	5.01 *br s* $W_{1/2}$ = 3	
H-3b	4.85 *br s* $W_{1/2}$ = 3	4.85 *br s* $W_{1/2}$ = 3	5.21 *br s* $W_{1/2}$ = 3	4.75 *br s* $W_{1/2}$ = 2	
H-5	2.18 *d* (11)	2.18 *d* (11)	2.25 *d* (11)	2.40 *d* (11)	2.31 *d* (11)
H-6	3.81 *dd* (11, 9)	3.88 *dd* (11, 9)	3.83 *dd* (11, 9)	5.26 *dd* (11, 9)	5.10 *dd* (11, 9)
H-7	2.9 *dddd* (9, 6, 25, 2)	2.87 *dddd* (9, 6, 2.5, 2)	†	3.06 *dddd* (9, 6, 2, 2)	
H-8	4.7 *ddd* (6, 6, 6)	4.76 *ddd* (6, 6, 6)	4.55 *ddd* (5, 4, 3)	4.67 *ddd* (6, 6, 6)	4.56 *ddd* (5, 4, 3)
H-9, H-9′	2.30–1.85	2.35–1.80	†	†	†
H-13a	6.29 *dd* (2.5, 1)	6.27 *dd* (2.5, 1)	1.38 *d* CH_3 (8)	6.20 *d* (2)	1.19 *d* CH_3 (8)
H-13b	5.94 *dd* (2.0, 1)	5.96 *dd* (2, 1)		5.58 *d* (2)	
H-14a	4.33 *d* (12)	3.61 *br s* $w/2$ = 4	4.05 *d* (10)	4.35 *d* (12)	4.32 *d* (12)
H-14b	4.01 *d* (12)	3.61 *br s* $w/2$ = 4	4.22 *d* (10)	3.98 *d* (12)	4.00 *d* (12)
H-15	1.83 *br s* $W_{1/2}$ = 3	1.60 *br s* $W_{1/2}$ = 3	1.85 *br s* $W_{1/2}$ = 3	1.75 *br s* $W_{1/2}$ = 3	1.75 *br s* $W_{1/2}$ = 3
H-2′	4.00 *d* (4.0)		4.04 *d* (4)	4.83 *d* (4)	4.88 *d* (4)
CH_3 CH	0.99 *d* (7)		0.95 *d* (7)	0.96 *d* (7)	0.93 *d* (7)
CH_3	0.94 *d* (7)		1.05 *d* (7)	0.96 *d* (7)	1.01 *d* (7)
CH_3C					1.94 *s*
O				2.00 *s*	
				2.11 *s*	2.12 *s*

*Recorded at 80 MHz using $CDCl_3$ as solvent and TMS as internal standard; coupling constants in Hz are in parentheses.

†Obscured complex signals.

refluxed in 10 ml of 2 N NaOH–MeOH for 45 min. The soln was acidified with HCl (10%), the MeOH removed at red. press. and the residue diluted with H_2O. The mixture was extracted with EtOAc. The washed and dried extract was filtered and concd. The residue was sepd by prep. TLC eluting with hexane–EtOAc (3:2). The less polar band yielded 50 mg of an oily compound, which was found to be identical to an authentic sample of 4 by TLC, IR, ^{1}H NMR and $[\alpha]_D^{25}$.

Cope rearrangement of budlein B (5). 5 (35 mg) was heated at 200° during 5 min under an Ar atm. The Cope rearrangement product 11 [13, 20] was obtained after CC using hexane–EtOAc (7:3). Mp 159–160° (lit. [20]: 158–160°).

Transformation of budlein B (5) *to schkuhridin B* (4). A soln of 250 mg of 5 in 7 ml MeOH was refluxed for 1 hr with MeOH–KOH (260 mg in 8 ml). The reaction mixture was taken to dryness and the residue dissolved in H_2O (30 ml). This soln was acidified with HCl (10%) and extracted with EtOAc (× 3, 10 ml). The organic layer was washed, dried and concd, affording 155 mg of a mixture which afforded, after prep. TLC (eluted × 3 with hexane–EtOAc), 32 mg of 4 as a pale yellow oil, identified by direct comparison with an authentic sample.

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