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GERMACRANOLIDES FROM *SCHKUHRIA ANTHEMOIDEA**

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Key Word Index—*Schkuhria anthemoidea*; Bahiinae; Heliantheae; Compositae; heliangolides; germacranolides; X-ray crystallography.

Abstract—The isolation of eucannabinolide and three new sesquiterpene lactones from *Schkuhria anthemoidea* is reported. The structures and stereochemistries of the new compounds were established by chemical and spectroscopic means. The structure of santhemoidin B was confirmed by X-ray crystallography.

INTRODUCTION

Previous studies have shown [1–5] that species belonging to the genus *Schkuhria* (subtribe Bahiinae, tribe Heliantheae) are rich in sesquiterpene lactones having the germacrane skeleton.

In a continuation of our interest in this field, we undertook a chemical examination of the annual *S. anthemoidea* (DC.) Coult which resulted in the isolation of three new compounds. Two of them are the heliangolides santhemoidin A (**1a**) and santhemoidin B (**1b**). The third new compound is the germacrolide santhemoidin C (**2**). *S. anthemoidea* also contains the known lactone eucannabinolide (**1c**), previously found in other species of this genus [1, 3, 4].

RESULTS AND DISCUSSION

The chloroform extract of *S. anthemoidea* was chromatographed on silica gel to give two fractions (A and B); the less polar fraction A yielded only santhemoidin A.

Santhemoidin A (**1a**), $C_{22}H_{24}O_7$, mp 167–169°, showed IR bands due to a γ-lactone and an acetate (1750 and 1730 cm^{-1}). A band at 1722 cm^{-1} was attributed to a furoyloxy group. The ^1H NMR spectrum of **1a** (Table 1) was almost superimposable on that of **1c**, differing only in the signals of the ester group at C-8, which was identified as a 3-furoate [6].

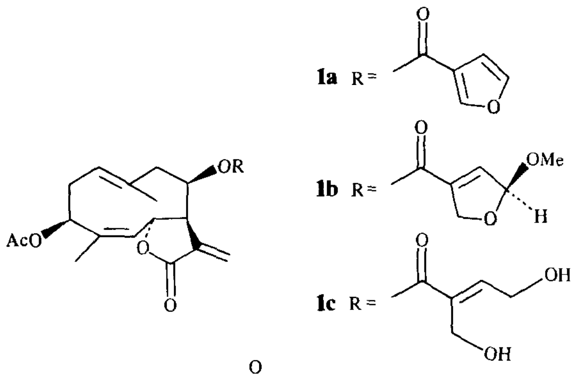
Treatment of eucannabinolide (**1c**) with benthonitic earth (Tonsil Optimum Extra) or charcoal afforded santhemoidin A, thus establishing its structure as **1a**. The nature of the ester attached to C-8 was confirmed by its mass spectrum, which in addition to the base peak (m/z 95, $C_5H_3O_2$) showed a fragment at m/z 67 (C_4H_3O) in agreement with the presence of this group.

The more polar fraction B afforded, after silica gel chromatography, santhemoidin A (**1a**), santhemoidin B (**1b**), santhemoidin C (**2**) and eucannabinolide (**1c**).

Santhemoidin B (**1b**), $C_{23}H_{28}O_8$, mp 147–149°, showed the characteristic band of a γ-lactone and an acetate (1760 and 1740 cm^{-1}) in its IR spectrum.

Analysis of the ^1H NMR spectrum and spin decoupling experiments in C_6D_6 solution (Table 1) suggested structure **1b** for santhemoidin B. The ^1H NMR spectrum of santhemoidin B (**1b**) showed the presence of a methoxy group; the rest of the spectrum was almost superimposable on that of santhemoidin A (**1a**). The methoxy group should be attached at C-4′ since this C-atom showed a high-field shift in the ^{13}C NMR spectrum (Table 2) as compared with the same atom in **1a**. Another significant change was observed for C-5′ which was a high-field triplet, appropriate for the proposed 4-methoxydihydrofuroyloxy group. The relative configuration at C-4′ of santhemoidin B was found to be *S* by means of an X-ray analysis; thus its structure was confirmed as **1b**.

Santhemoidin B could be the precursor of santhemoidin A (**1a**) as indicated by the following experiment. On treatment of santhemoidin B with benthonitic earth, methanol was eliminated to yield santhemoidin A (**1a**).



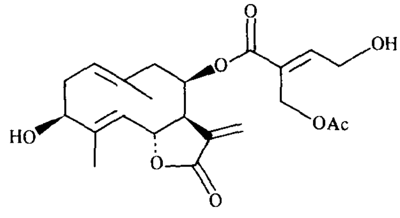


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Table 1. ^1H NMR spectral data of compounds **1a**, **1b** and **2** (80 MHz, $CDCl_3$, TMS as internal standard)*

	1a	**1b**	**1b**†	**2**‡	**2** + TAI
H-1	5.25§	5.25§	4.74 *m*	4.85 *m*	5.15§
H-2	2.5‖	2.5‖	2.03 *br dd* (3, 14)	2.5§	2.5‖
H-3	5.25§	5.25§	5.15 *dd* (2, 4)	4.32 *dd* (7, 10)	5.15§
H-5	5.2 *dd* (10.5)	5.15 *m*	4.78 *m*	4.8 *m*	5.05 *m*
H-6	5.92 *dd* (2.5, 10.5)	5.8 *m*	5.75 *dd* (2.5, 10.5)	5.23 *dd* (8, 10)	5.35 *dd* (7, 10)
H-7	2.96 *m*	2.9 *m*	2.36 *m*	2.9 *m*	2.9 *m*
H-8	5.25§	5.25§	5.06 *m*	5.8 *m*	5.85 *m*
H-9	2.5‖	2.5‖	2.56 *br dd* (4, 14)	2.5§	2.5‖
H-13a	6.32 *d* (2.5)	6.33 *d* (2.5)	6.28 *d* (2.5)	6.27 *d* (3.5)	6.32 *d* (3.5)
H-13b	5.75 *d* (2.0)	5.72 *d* (2.0)	5.32 *d* (2.0)	5.6 *d* (3.0)	5.64 *d* (3.5)
H-14	1.75 *br*	1.75 *br*	1.55 *br*	1.5 *br*	1.55 *br*
H-15	1.82 *br*	1.8 *d* (1.5)	1.51 *d* (1.5)	1.82 *br d* (1.5)	1.86 *br*
H-3′	6.6 *br d* (2.0)	6.55 *m*	6.55 *dd* (2, 1.5)	7.05 *t* (6.0)	7.0 *t* (6.0)
H-4′	7.37 *br t* (1.5)	5.75 *m*	5.58 *dd* (2, 4)	4.46 *d* (6.0)	5.12 *d* (6.0)
H-5′	7.9 *br*	4.7 *m*	4.78 *m*	4.83	4.86
MeCO–	2.1	2.07	1.74	2.0	2.04
MeO		3.36	3.18		

*Unmarked signals are singlets. Coupling constants (Hz) in parentheses. TAI = trichloroacetyl isocyanate.

†100 MHz, C_6D_6.

‡$CDCl_3$ + DMSO.

§, ‖Overlapping signals.

This experiment indicated that santhemoidin A may have been formed during the isolation process.

The third substance, santhemoidin C (**2**), $C_{22}H_{28}O_8$, mp 169–172°, showed characteristic IR bands for free hydroxyl groups, an α-methylene-γ-lactone, an acetate and an α,β-unsaturated ester (3430, 1750, 1740 and 1720 cm^{-1}, respectively). Santhemoidin C displayed a ^1H NMR spectrum characteristic of a germacrolide (Table 1) whose chemical shifts and coupling constants for H-6 and H-8 were similar to those of authentic *trans*-1(10)-*trans*-4-germacradien-6α,12β-olides with a β-oriented ester function at C-8 [7–9]. This compound contained, in addition to the ester group attached at C-8, a free hydroxyl group at the C-3 position whose chemical shift and coupling constants (δ4.32 *dd*, $J = 7$, 10 Hz) indicated the β-configuration [10, 11]. The nature of the ester group was deduced from its ^1H NMR spectrum and double-resonance experiments. Irradiation at the frequency of H-3′ (δ7.05) simplified the doublet at δ4.46 (H-4′) to a singlet. This result is congruent with the presence of a hydroxyl group at C-4′ and an acetate at C-5′. The mass spectrum was also in accord with the above deduction since it showed the base peak at m/z 97 ($C_5H_5O_2$) and abundant fragments at m/z 157 ($C_7H_9O_4$), 115 ($C_5H_7O_3$), 69 (C_4H_5O) and 43 (C_2H_3O). As santhemoidin C (**2**) was unstable, we prepared its diurethane *in situ*, by treatment with trichloroacetyl isocyanate (TAI) [12]. The presence of two low-field singlets (δ8.5 and 8.6) confirmed the formation of a diurethane, whose ^1H NMR spectrum showed the expected paramagnetic shifts of the H-3 and H-4′ signals (δ5.05 and 5.3, respectively) in agreement with structure **2**.

EXPERIMENTAL

Mps are uncorr. The benthonitic earth employed consisted of SiO_2 (72.5%), Al_2O_3 (13.0%), Fe_2O_3 (5.0%), MgO (1.5%), CaO (0.8%) and humidity (8.5%) with pH 3. A plant specimen voucher has been deposited at the herbarium of the Instituto de Biologia, U.N.A.M.

Extraction and separation. The dried and ground aerial part of *S. anthemoidea* (DC.) Coult. (3.06 kg), collected in Cuernavaca, Morelos in October 1981 (voucher MEXU 340472), was extracted with $CHCl_3$ and the resulting residue (149 g) percolated through Kieselgel G using solvents of increasing polarity (hexane, $CHCl_3$, EtOH, Me_2CO). The less polar fractions (hexane and $CHCl_3$) were combined, yielding 37.4 g of a residue

Table 2. ^{13}C NMR spectral data of compounds **1a** and **1c** (20 MHz, $CDCl_3$, TMS as internal standard)*

Carbon	**1c**†	**1a**	**1c**
1	125.07 *d*‡	125.34 *d*‡	125.60 *d*‡
2	39.38 *t*	29.61 *t*	29.60 *t*
3	76.83 *d*§	76.99 *d*§	76.92 *d*§
4	135.27	137.76	137.26 ¶
5	126.03 *d*‡	126.78 *d*‡	126.60 *d*‡
6	79.07 *d*§	78.68 *d*§	79.00 *d*§
7	48.32 *d*	48.68 *d*	48.48 *d*
8	76.05 *d*§	75.69 *d*§	75.46 *d*§
9	43.25 *t*	43.52 *t*	43.39 *t*
10	136.56 ‖	136.52 ‖	136.69 ‖
11	135.44 ‖	135.50 ‖	135.06 ‖
12	170.25	169.56	169.31
13	125.07 *t*	124.32 *t*	124.51 *t*
14	19.39 *q*	19.45 *q*	19.54 *q*
15	22.99 *q*	23.09 *q*	23.06 *q*
1′	165.49	161.87	161.09
2′	131.09	119.17	137.48 ¶
3′	145.45 *d*	109.56 *d*	136.04 *d*
4′	58.78 *t*	144.23 *d*	109.35 *d*
5′	56.52 *t*	147.78 *d*	72.79 *t*
1″	169.90	169.25	169.19
2″	21.13 *q*	21.16 *q*	21.14 *q*
3‴			54.37 *q*

*Unmarked signals are singlets.

†Data taken from ref. [3].

‡, §, ‖, ¶These assignments may be interchanged.

designated as fraction A. The EtOAc and Me_2CO fractions were combined to afford fraction B (85 g).

Fraction A was percolated on benthonitic earth with solvents of increasing polarity: hexane, $CHCl_3$, EtOAc, Me_2CO. The $CHCl_3$ and EtOAc fractions (20 g) were chromatographed through silica gel, eluting with hexane and increasing proportions of $CHCl_3$. Santhemoidin A (**1a**) was obtained from the fractions eluted with hexane–$CHCl_3$ (1.236 g), mp 167–169°, $[\alpha]_D$ $-184°$ (MeOH; *c* 0.221). UV λ_{max}^{MeOH} nm (ε): 204 (24240), 230 (6570); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1730, 1722, 1662, 1658. EIMS 70 eV, *m/z* (rel. int.): 400 $[M]^+$ (1.1), 358 $[M-C_2H_2O]^+$ (1.0), 340 $[M-HOAc]^+$ (3.0), 228 $[M-HOAc-RCO_2H]^+$ (17.9), 95 $[C_5H_3O_2]^+$ (100), 67 $[C_4H_3O]^+$ (5.0), 43 $[C_2H_3O]^+$ (22.9).

Fraction B was treated with charcoal in MeOH. Evapn of the solvent left a residue which was chromatographed on a silica gel column. Elution with $CHCl_3$ gave 15 mg **1a**. The $CHCl_3$–EtOAc (9:1) fractions gave **1b** (1.310 g) as white crystals from hexane–$CHCl_3$, mp 147–149°. $[\alpha]_D$ $-146°$ (MeOH; *c* 0.181); UV λ_{max}^{MeOH} nm (ε): 205 (30980); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1740, 1735, 1650. CIMS (CH_4) 200 eV, *m/z* (rel. int.): 433 $[M+1]^+$ (0.8), 401 $[M+1-MeOH]^+$ (0.4), 358 $[M+1-MeOH-C_2H_3O]^+$ (0.5), 341 $[M+1-MeOH-HOAc]^+$ (2.5), 289 $[M+1-RCO_2H]^+$ (4.8), 229 $[M+1-RCO_2H-HOAc]^+$ (100), 127 $[C_6H_7O_3]^+$ (6.0).

The structure of santhemoidin B (**1b**) was confirmed by means of an X-ray crystallographic analysis. The compound crystallized from hexane–EtOAc in space group $P2_12_12_1$, $a = 11.2940$ (2) Å, $b = 13.2970$ (4) Å, $c = 14.8744$ (4) Å, $z = 4$. The intensities of 1651 independent reflexions were collected on a Nicolet-R 3m four-circle diffractometer ($CuK_\alpha\omega$ scan, $2\theta \leqslant 115°$). The structure was solved by direct methods using the SHELXTL system [13] and refined (all hydrogen atoms were fixed to an isotropic temp. factor, $U = 0.060$ Å^2) to a final *R* value of 3.7% for 1681 reflexions with $I > 2.5\,\sigma(I)$. The atomic co-ordinates have been deposited at the Cambridge Crystallography Data Centre.

The $CHCl_3$–EtOAc (6:4) eluates gave **2** (78 mg). Recrystallization from hexane–Me_2CO, mp 169–172°, $[\alpha]_D$ $+75°$ (MeOH; *c* 0.193); UV λ_{max}^{MeOH} nm (ε): 205 (29500); IR ν_{max}^{nujol} cm^{-1}: 3430, 1750, 1740, 1720, 1660. EIMS 70 eV, *m/z* (rel. int.): 420 $[M]^+$ (0.2), 360 $[M-HOAc]^+$ (0.4), 246 $[M-RCO_2H]^+$ (4.0), 157 $[C_7H_9O_4]^+$ (39.5), 115 $[C_5H_7O_3]^+$ (85.0), 97 $[C_5H_5O_2]^+$ (100), 69 $[C_4H_5O]^+$ (43.4), 43 $[C_2H_3O]^+$ (74.6). CIMS (CH_4) 200 eV, *m/z* (rel. int.): 421 $[M+1]^+$ (12.7), 403 $[M+1-H_2O]^+$ (8.5), 247 $[M+1-RCO_2H]^+$ (79.7), 229 $[M+1-RCO_2H-H_2O]^+$ (100).

The $CHCl_3$–EtOAc (1:1) fractions gave a gum which was rechromatographed on silica gel, eluting with C_6H_6 and increasing proportions of EtOAc. The C_6H_6–EtOAc (1:1) fractions gave 8.9 g **1c** as a pale yellow oil. The IR and ^{1}H NMR data were identical to those published [3].

Transformation of **1b** with benthonitic earth. A soln of 115 mg **1b** in 15 ml $CHCl_3$ was stirred with 1 g benthonitic earth. The reaction was followed by TLC. After 2 hr, the mixture was filtered and the solvent evapd. The residue was percolated over Kieselgel-G, eluting with hexane–Me_2CO (4:1) to give 70 mg **1a** and 22 mg **1b**.

Transformation of **1c** *with benthonitic earth.* A soln of 140 mg **1c** in 15 ml $CHCl_3$ was stirred with 2 g benthonitic earth for 7 hr at room temp. The mixture was filtered, the solvent evapd and the residue purified in the same manner as described for **1b**, yielding 18.6 mg **1a** and 82.9 mg **1c**.

Transformation of **1c** *with charcoal.* A soln of 150 mg **1c** in 25 ml MeOH was stirred with 1.66 g charcoal. The reaction was followed by TLC. After 10 hr, the mixture was filtered and the solvent evapd. The residue was purified in the same manner as described for **1b**, yielding 8.7 mg **1a** and 110.6 mg **1c**.

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