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## ISOLATION AND IDENTIFICATION OF SPICIGERA LACTONE: COMPLETE $^1\text{H}$ AND $^{13}\text{C}$ ASSIGNMENTS USING TWO- DIMENSIONAL NMR EXPERIMENTS

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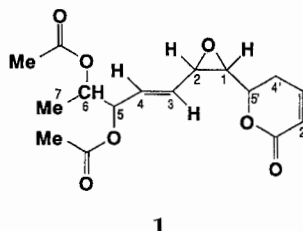
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**ABSTRACT.**—Spicigera  $\delta$ -lactone [**1**] [5,6-diacetoxy-1,2-epoxy-1,5'-(2'-pentene-5'-olide)-3E-heptene] was isolated from the inflorescence of *Hyptis spicigera* (Lamiaceae). The identification and structure elucidation were achieved using spectrometric techniques. Complete  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr assignments of this new natural lactone have been made using 2D homonuclear and heteronuclear-correlated spectroscopy.

*Hyptis spicigera* Lam. (Lamiaceae) is an aromatic annual plant growing wild in most parts of the tropical vegetation of Africa (1). This species is used by Burkinabe farmers, as in many African countries (2), to control insect infestation of grains. The whole plant is strongly scented and it is also burned in rooms to repel mosquitoes (3). As part of our continuing phytochemical investigation of *H. spicigera* (4,5), we report herein the isolation, identification, and spectral data of spicigera  $\delta$ -lactone **1**, a new natural product, isolated from the petroleum extract of the inflorescences. The structural formula of **1** is 5,6-diacetoxy-1,2-epoxy-1,5'-(2'-pentene-5'-olide)-3E-heptene.

### RESULTS AND DISCUSSION

The molecular formula for spicigera lactone **1** [levorotatory white powder,  $[\alpha]_D^{25} -114.3$  ( $\text{C}_6\text{H}_5\text{CH}_3$ ,  $c=0.0054$  g/ml)] was established as  $\text{C}_{16}\text{H}_{20}\text{O}_7$  by elemental analysis. Ir absorption bands at 1741 and 1727  $\text{cm}^{-1}$  coupled with the  $^{13}\text{C}$ -nmr resonances at 170.3, 169.9, and 162.5 ppm, were indicative of two carboxy and an  $\alpha,\beta$ -unsaturated  $\delta$ -lactone func-



tionality. In the eims, a major fragmentation was observed at  $m/z$  97 (rel. int. 20%). In the  $^1\text{H}$ -nmr spectrum, three signals attributable to Me groups were observed as a doublet at 1.20 ppm ( $J=6.6$  Hz) and two singlets at 2.09 and 2.04 ppm; these latter resonances can be assigned to acetoxy Me groups. The presence of a *cis*-1,2-disubstituted epoxide function was evident from ir (6) and  $^1\text{H}$ -nmr (7) spectral data [1251  $\text{cm}^{-1}$ , 3.63 ppm (dd,  $J=4.2$ , 5.2 Hz) and 3.32 ppm (dd,  $J=4.2$ , 8.4 Hz)]. The  $^1\text{H}$  spectrum also revealed four ethylenic signals [6.91 ppm (ddd,  $J=3.6$ , 5.0, 9.9 Hz), 6.07 ppm (dt,  $J=2.0$ , 2.0, 9.9 Hz), 5.86 ppm (ddd,  $J=0.7$ , 6.3, 15.7 Hz), and 5.76 ppm (ddd,  $J=1.0$ , 5.2, 15.7)] and three protons on oxygen-bearing carbons [5.37 ppm (dd,  $J=3.9$ , 6.3 Hz), 5.07 ppm (dq,

$J=3.9, 6.6, 6.6, 6.6$  Hz), and 4.18 ppm (dt,  $J=6.2, 8.5, 8.5$  Hz)].

The  $^{13}\text{C}$ -nmr spectrum confirmed the presence of two secondary ( $-\text{CH}=\text{CH}-$ ) double bonds with cis and trans geometry, one of which (cis geometry) was conjugated with the lactone moieties. The multiplicities of the  $^{13}\text{C}$ -nmr signals, determined from the DEPT pulse sequence (8), indicated nine methines, one methylene, three methyls, and three quaternary carbons.

The molecular framework and the complete  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr chemical shift assignments (Table 1) for **1** were deduced from the concerted application of homonuclear and heteronuclear chemical shift correlations and are in agreement with literature values (9). These 2D experiments established proton connectivities from the proton-proton couplings and correlated these  $^1\text{H}$  assignments with the  $^{13}\text{C}$  spectrum. Consideration of these connectivity networks, in conjunction with the inferences drawn from the conventional spectra, permitted structural segments to be assembled to give spicigera

lactone **1**. Fragmentation observed in the ms showed a peak at  $m/z$  227 (13%), which was characteristic of the diacetoxyepoxyheptene moiety.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—All nmr spectra were recorded on a Bruker AMX-400 spectrometer in  $\text{CDCl}_3$  solutions. Chemical shifts were measured in  $\delta$  relative to TMS. Resonance multiplicities for  $^{13}\text{C}$  were established via the acquisition of DEPT spectra obtained for proton pulse  $P=3\pi/4$  (CH and Me differentiated from  $\text{CH}_2$ ). For the DEPT pulse sequence, the width of a  $^{13}\text{C}$   $90^\circ$  pulse was 4.5  $\mu\text{sec}$ , the width of a  $^1\text{H}$   $90^\circ$  pulse was 10  $\mu\text{sec}$ , and the  $(2J)^{-1}$  delay was set equal to 3.7 msec. The homonuclear  $^1\text{H}$ - $^1\text{H}$  shift-correlated 2D diagram was obtained using the COSY-45 pulse sequence. The spectral width was 4807 Hz, allowing a digital resolution of 4.7 Hz. The spectrum was processed by sinusoidal multiplication in each dimension followed by symmetrization of the final data matrix. Other parameters were as follows: number of increments in  $t_1$ , 256; scans, 16; phase cycling, 16 and relaxation delay, 1 sec. The heteronuclear 2D  $^1\text{H}$ - $^{13}\text{C}$  chemical shift correlation experiment was obtained with proton decoupling in the F1 dimension (10,11). The spectrum was acquired with  $4\text{K} \times 256$  data points and a data acquisition of  $256 \times 128$  increments in  $t_1$  and a zero filling in the F1 dimension. Spectral

TABLE 1.  $^1\text{H}$  and  $^{13}\text{C}$  nmr Assignments of Spicigera Lactone **1**.

Position <sup>a</sup>	$^{13}\text{C}$	$^1\text{H}$		
	$\delta^b$	$\delta^b$	multiplicity	$J^c$
7 .....	15.18	1.20	d	$J_{7,6}=6.6$
6 .....	70.23	5.07	dq	$J_{7,6}=6.6, J_{6,5}=3.9$
5 .....	73.69	5.37	dd	$J_{6,5}=3.9, J_{5,4}=6.3$
4 .....	130.41	5.86	ddd	$J_{5,4}=6.3, J_{4,3}=15.7, J_{4,2}=0.7$
3 .....	127.34	5.76	dd	$J_{4,3}=15.7, J_{3,2}=5.2, J_{3,5}=1.0$
2 .....	55.99	3.63	dd	$J_{2,3}=5.2, J_{2,1}=4.2$
1 .....	57.95	3.32	ddd	$J_{2,1}=4.2, J_{1,3'}=8.4$
5' .....	74.15	4.18	dt	$J_{1,5'}=8.4, J_{5',4'}=8.5, 6.2$
4' .....	144.38	6.91	dt	$J_{4',3'}=5.0, 3.6, J_{4',2'}=9.9$
3' .....	27.19	2.60	m	$J_{5',3'}=8.5, 6.2, J_{3',4'}=5.0, 3.6, J_{3',2'}=2.0$
2' .....	121.58	6.07	dt	$J_{2',4'}=9.9, J_{2',3'}=2.0$
1' .....	162.53			
MeCO .....	21.01	2.04 or	s	
	21.12	2.09	s	
MeCO .....	170.32			
	169.91			

<sup>a</sup>Hydrogen and carbon atoms have the same labelling.

<sup>b</sup>In ppm from TMS.

<sup>c</sup>Values in Hz.

widths of 20000 and 4807 Hz were employed in the F2 ( $^{13}\text{C}$ ) and F1 ( $^1\text{H}$ ) domains, respectively. Data were processed using unshifted sine-bell functions in both dimensions. The refocusing delay was 2.5 msec, the mixing delay was 3.7 msec, and the relaxation delay was 1 sec. Sixteen phase cycling steps were employed.

Tlc was performed on Si gel 60 (F254) 0.2 mm plates (E. Merck, Darmstadt) using EtOAc as eluent ( $R_f=0.43$ ). Analyses by glc were made on an FID type DELSI 30 gas chromatograph. Detector and injector temperatures were set at 220 and 230°, respectively. The gc column used was a DB1 WEOT Si gel capillary column (25 m, 0.32 mm i.d., 0.15  $\mu\text{m}$  phase thickness, programmed temperature 70–210° at 2° min $^{-1}$ , inlet pressure of helium used as carried gas 1.5 bar. The mp's were determined on a Buchi 510 apparatus and were uncorrected. Optical rotation was recorded on an electronic polarimeter Bendix NPL Type 143. The ir spectrum was recorded on a Mattson 1000 FT-IR Spectrometer using a KBr pellet. The eims was recorded on a Varian MAT 311 (15 and 70 eV, direct inlet, ei mode).

ISOLATION PROCEDURES AND PHYSICAL DATA.—*H. spicigera* was collected in the Ouagadougou area of Burkina-Faso. The species is cultivated continuously at Ouagadougou, so living plants are available as vouchers [a voucher specimen identified by Professor S. Guinko is deposited (n° 1019) in the Laboratory of Biologie Végétale et Ecologie, Université de Ouagadougou]. Air-dried inflorescences (200 g) were extracted with petroleum ether (35–70°) using a Soxhlet extractor. The solvent was evaporated, and **1** (52 mg) precipitated as a white powder from a neutral aqueous solution of a crude extract (mp 78°). The material appeared chromatographically pure using tlc and glc (DB1 phase).

Elemental analysis of **1** showed  $\text{C}_{16}\text{H}_{20}\text{O}_7$ : found C% 59.71, H% 46.0, O% 33.83 (calcd C% 59.26, H% 46.17, O% 34.57);  $[\alpha]_{\text{D}}^{25} -114.3^\circ$  in

toluene ( $\lambda=5461 \text{ \AA}$ ); ir 3043, 3010, 2917, 2936, 1741, 1727, 1425, 1384, 1251, 1095, 1050, 968, 875, 815, 787  $\text{cm}^{-1}$ ; eims 227 (13), 204 (3), 178 (4), 177 (4), 141 (5), 125 (15), 111 (4), 99 (13), 97 (20), 81 (15), 69 (6), 68 (13), 43 (100), 41 (15), 39 (12);  $^1\text{H}$  and  $^{13}\text{C}$  nmr see Table 1.

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