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A TRIS-NORSESQUITERPENE LACTONE AND OTHER SESQUITERPENES FROM *CALEA CROCINERVOSA**

Alfredo Ortega, José Del C. López and Emma Maldonado

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F

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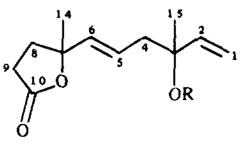
Key Word Index—*Calea crocinervosa*, Heliantheae; compositae, tris-norsesquiterpene lactone; eudesmanolides, furanoheliangolide

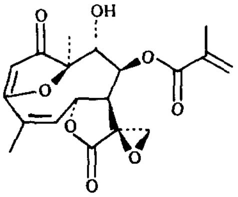
Abstract—The aerial parts of *Calea crocinervosa* afforded, in addition to known compounds, the novel tris-norsesquiterpene lactone, crocinervolide, whose structure was elucidated by spectroscopic methods.

INTRODUCTION

From the many *Calea* species that have been so far chemically analysed, only *C. rotundifolia* [1] and *C. prunifolia* [2] contained norterpene derivatives. We have studied *C. crocinervosa* Wussow, Urbastch and G. A. Sullivan sp *nov* [3] and we have found, in addition to several known compounds, the new tris nornerolidol derivative (**1**) which we have named crocinervolide. *Calea crocinervosa* was collected at two different growing stages and the differences in their composition will be presented here.



1 R = H
3 R = CONHCOCCl$_3$



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RESULTS AND DISCUSSION

The first collection of *C. crocinervosa* was carried out before the plant had flowered. The aerial parts gave the known flavones 7-*O*-methylacacetin and genkwanin, as well as the furanoheliangolide **2** [4, 5] and septuplinolide [6]. Dehydration of the latter afforded a mixture of isoalantolactone [7] and alloalantolactone [7], which allowed us to confirm the structure of septuplinolide.

The second collection of *C crocinervosa* was performed when the plant was in bloom. On this occasion, in addition to β-sitosterol, stigmasterol, 7-*O*-methylacacetin genkwanin, septuplinolide and telekin [8], we isolated crocinervolide (**1**). The furanoheliangolide was not present in this collection.

Crocinervolide (**1**) exhibited IR absorption bands for hydroxyl, γ-lactone and double bonds. Its ^{13}C NMR spectrum (see Experimental) showed only 12 signals, attributable to one carbonyl, two double bonds, two tertiary carbons bonded to oxygen, three methylenes and two methyl groups.

The ^{1}H NMR spectrum (CDCl$_3$) shows the presence of two tertiary Me groups on carbons that bear an oxygen function. The vinyl group is evident by an ABC system, where proton A (H-2) is a *dd* signal with $J = 17.5$ and 10.5 Hz, while the B (H-1) and C (H-1′) protons appear as two *dd* signals with $J = 17.5$ and 2 and 10.5 and 2 Hz, respectively. The multiplicity of H-2 indicate that the vinyl group is bonded to a tetrasubstituted carbon. The integral in the region of δ5.5 to 5.75 reveals the existence of two additional protons In order to improve the resolution in this region, the spectrum was run in benzene-d_6. This allowed us to observe an ABX$_2$ pattern attributed to the protons H-5 and H-6 bonded to a *trans*

Table 1. ^{1}H NMR spectral data of compounds **1** and **3** (80 MHz, TMS as internal standard

H	**1** (CDCl$_3$)	**1** (C$_6$D$_6$)	**3** (CDCl$_3$)
1 *t*	5.17 *dd*	5.14 *dd*	5.28 *br d*
	(17.5, 2)	(17.5, 2)	(16.5)
1 *c*	5.05 *dd*	4.91 *dd*	5.22 *br d*
	(10.5, 2)	(10.5, 2)	(12)
2	5.91 *dd*	5.76 *dd*	6.02 *dd*
	(17.5, 10.5)	(17.5, 10.5)	(16.5, 12)
4	2.29 *d*	2.09 *d*	2.68 *d*
	(6)	(6.5)	(6)
5	*	5.21 *br d*	5.58 *d*
		(15.5)	(15.5)
6	*	5.69 *dt**	5.81 *dt**
		(15.5, 6.5)	(15.5, 6)
8	2.35–1.95 *m*	1.65–1.25 *m*	2.2–1.95 *m*
9	2.7–2.45 *m*	2.2–1.95 *m*	2.7–2.4 *m*
14	1.50 *s*	1.12 *s*	1.48 *s*
15	1.27 *s*	1.11 *s*	1.63 *s*

*Partially overlapped signals.

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double bond vicinal to a methylene group (H-4). Irradiation of H-4 transformed the H-5 signal into a doublet. As **1** also possesses a γ-lactone function, as shown by the IR and ^{13}C NMR spectra, it is proposed that this function is close to the tertiary carbon (C-7). In this way structure **1** or its $\triangle^4$ isomer were proposed for crocinervolide The formation *in situ* of the carbamate **3** by addition of TAI allowed us to discard the second structure since in the ^1H NMR spectrum of **3** the signals for H-4 and H-15 were shifted to lowfield 0.4 and 0.36 ppm, respectively, establishing the vicinity of these protons to the hydroxyl group At this point, the structure of crocinervolide was determined as the tris nornerolidol derivative **1**. The stereochemistry at C-3 and C-7 was not determined.

The presence of **1** in *C. crocinervosa* has antecedence in the isolation of nor and tris norsesquiterpenes from another *Calea* species [1, 2]. Futhermore, a tris nornerolidol aldehyde which is a suitable precursor of **1** has been obtained from a nerolidol hydroperoxide [9].

EXPERIMENTAL

Calea crocinervosa was collected in September and December 1985 in Chiapas, Mexico A voucher specimen is deposited in the National Herbarium (AOH 201)

First collection Ground, dried aerial parts of the plant (400 g) were extracted with hexane and $CHCl_3$ to yield 9 and 30 g of extract, respectively The hexane extract was chromatographed over silica gel eluted with a hexane–EtOAc gradient Fractions eluted with hexane–EtOAc (9 1) gave 34 mg of 7-*O*-methylacacetin Fractions eluted with hexane–EtOAc (7 3) were purified by successive silica gel CC to obtain 32 8 mg of septuplinolide The $CHCl_3$ extract was chromatographed over silica gel using a hexane–Me_2CO gradient 7-*O*-Methylacacetin (161 mg) was obtained from fractions eluted with hexane–Me_2CO (19 1) Fractions eluted with hexane–Me_2CO (4 1) yielded genkwanin (79 6 mg) and subsequently **2** (30 9 mg)

Second collection Ground dried material (1 31 kg) was extracted with hexane, Me_2CO and MeOH Each extract was percolated through celite with hexane, $CHCl_3$, EtOAc and the solvents combined to give hexane fraction (78 g), $CHCl_3$ fraction (128 g), EtOAc fraction (22 g), Me_2CO fraction (52 g) and MeOH fraction (16 g)

Repeated CC (silica gel Merck G, hexane–EtOAc gradient elution) of the hexane fraction gave, 120 mg of β-sitosterol and stigmasterol, 870 mg of 7-*O*-methylacacetin, 287 mg of genkwanin, 109 mg of septuplinolide and 4 7 mg of telekin After repeated CC (silica gel Merck G, hexane–EtOAc gradient elution) of the $CHCl_3$ fraction 7-*O*-methylacacetin (187 mg), genkwanin (724 mg), septuplinolide (43 mg), telekin (21 mg) and crocinervolide (**1**) (295 mg) were isolated A similar work-up of the EtOAc fraction gave 23 mg of 7-*O*-methylacacetin, 38 mg of genkwanin and 23 mg of crocinervolide (**1**), while the Me_2CO fraction afforded 7-*O*-methylacacetin (58 mg) and genkwanin (124 mg)

Crocinervolide (**1**) Colourless oil, $[\alpha]_D + 41\ 9$ ($CHCl_3$, *c* 0 264), UV λ^{EtOH} 201 nm ($\varepsilon = 1733$), IR ν_{max}^{film} cm^{-1} 3435, 3086, 3039, 1767, 1669, 1643, 1141, 997, 976, 929, CIMS *m/z* (rel int) 211 $[M + 1]^+$ ($C_{12}H_{18}O_3$, 22), 193 $[M + 1 - H_2O]^+$ (86), 181 $[M + 1 - CH_2O]^+$ (4), 141 $[M + 1 - C_4H_7O]^+$ (100), 123 $[141 - H_2O]^+$ (23), ^{13}C NMR (20 MHz, $CDCl_3$, TMS as int standard) δ111 92 (*t*, C-1), 144 86 (*d*, C-2), 72 47 (*s*, C-3), 45 08 (*t*, C-4), 135 68 (*d*, C-5), 125 41 (*d*, C-6), 85 35 (*s*, C-7), 34 13 (*t*, C-8), 28 95 (*t*, C-9), 176 70 (*s*, C-10), 26 58 (*q*, C-14) and 27 30 (*q*, C-15)

Dehydration of septuplinolide To a soln of septuplinolide (61 mg) in pyridine (1 ml) was added to a 0 $SOCl_2$ (0 5 ml) The reaction mixture was allowed to reach room temp (10 min) After addition of H_2O and extraction with EtOAc, the organic soln was washed with aq HCl (5%), satd $NaHCO_3$, H_2O and dried over Na_2SO_4 The reaction mixture (two spots on TLC) was purified by silica gel CC to give 32 1 mg of isoalantolactone [7] and 18 1 mg of alloalantolactone [7]

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