Anti-inflammatory Constituents of Topically Applied Crude Drugs. IV.[1] Constituents and Anti-inflammatory Effect of Paraguayan Crude Drug "Alhucema" (*Lavandula latifolia* VILL.)[2]

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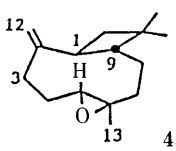
The anti-inflammatory active fraction of the Paraguayan crude drug, "Alhucema," *Lavandula latifolia* VILL. afforded four compounds: coumarin (1), 7-methoxycoumarin (2), *trans*-phytol (3) and caryophyllene oxide (4). 1 showed a weakly inhibitory effect on carrageenin-induced paw edema in rats on topical application and 4 showed an inhibitory effect on histamine-induced contraction in guinea pig ileum.

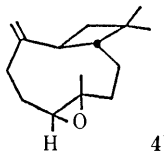
Keywords *Lavandula latifolia*; coumarin; 7-methoxycoumarin; *trans*-phytol; caryophyllene oxide; anti-inflammatory effect; carrageenin edema; histamine-induced contraction

In the course of a search for biologically active substances from Paraguayan medicinal plants, "Alhucema," the aerial portion of *Lavandula latifolia* VILL. (Labiatae) was found to have inhibitory activity when topically applied to carrageenin-induced paw edema (CPE) in rats. Alhucema is a folk medicine used for the treatment of bronchitis, asthma and rheumatism[3] and has also been topically applied to a swellings in Paraguay. It is of interest that a 70% ethanolic extract of Alhucema showed an inhibitory effect on CPE by our experimental method.[4] Studies on constituents of the essential oil of this plant have been made,[5] but no report has been found on the biological activities including its anti-inflammatory benefit.

In this paper, we report the isolation of chemical constituents and their anti-inflammatory effect. The 70% ethanolic extract was partitioned between chloroform and water to give an active chloroform soluble fraction which showed potent inhibition to CPE, and was chromatographed on a silica gel column by elution with a chloroform–methanol mixture to give an effective fraction (fr. 1) (1% methanol eluate) (Table I). Fraction 1 was further separated and purified by centrifuge liquid chromatography (CLC) and preparative thin layer chromatography (TLC) (each silica gel) to yield four compounds, **1**—**4**. Compounds **1** and **2** were identified as coumarin and 7-methoxycoumarin, respectively, by direct comparison with authentic samples. The spectral data of compounds **3** and **4** matched those published for *trans*-phytol[6] and caryophyllene oxide.[7] **4** has four possible conformers due to the conformation of epoxide (**4a**, **b** and two *cis*-oxides). It was reported that the chemical shifts of 13-methyl group and exomethylene group were different by each isomer in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum.[8] We concluded the structure of **4** to be **4a** by comparison with published data. We tested for the anti-inflammatory activity of the isolated compounds (**1**—**4**) in the CPE test using topical application; coumarin (**1**) showed a weakly positive effect (Table I), but the other compounds provided no reliable data owing to their limited sample availability. We previously reported that inhibition of histamine-induced contraction in guinea pig ileum (HCl) was responsible for the effect in the CPE test which could be achieved with a smaller amount of samples than in the CPE test.[1,9] The inhibitory effect of compounds **2**—**4** on HCl was therefore examined along with compound **1** and fr. 1 to deduce their anti-inflammatory activity; **4** showed the strongest effect ($IC_{50} = 1.0 \times 10^{-4}$ M) among them (Table II); this compound might be expected to show an inhibitory effect on CPE and further separation is in progress. In conclusion, the anti-inflammatory effect of Alhucema when topically applied is seen when combined with coumarins (**1** and **2**) and caryophyllene oxide (**4**).



4a



4b

Experimental

Melting points were determined on a Yanagimoto micromelting point

TABLE I. Inhibitory Effect on Carrageenin-Induced Paw Edema in Rats[a] by Topical Application

	Dose (mg/site × 4)	Inhibition of swelling (%)			
		1 h	2 h	3 h	4 h[b]
70% EtOH ext.	5	—	18.1	30.1[c]	18.5
$CHCl_3$ soluble	5	31.8	10.0	32.5[d]	24.3[c]
Fr. 1	5	44.8[d]	40.0[d]	45.5[d]	50.1[d]
Coumarin (**1**)	5	20.5	11.5	22.6[c]	19.0

a) $n = 4$ or 5. *b*) Time after carrageenin injection. —: no effect (less than 10%), *c*) $p < 0.05$, *d*) $p < 0.01$.

TABLE II. Inhibitory Effect on Histamine[a]-Induced Contraction in Guinea Pig Ileum

	IC_{50}	
	(g/ml)	(M)
Fr. 1	7.0×10^{-5}	
Coumarin (**1**)	$> 10^{-4}$	$> 10^{-4}$
7-Methoxycoumarin (**2**)	7.9×10^{-5}	4.5×10^{-4}
trans-Phytol (**3**)	$> 10^{-4}$	$> 10^{-4}$
Caryophyllene oxide (**4**)	2.4×10^{-5}	10^{-4}
Diphenhydramine·HCl	3.2×10^{-8}	1.1×10^{-7}

a) At 10^{-7} g/ml.

apparatus and were uncorrected. Spectral data were obtained as follows: infrared (IR) spectra with a Hitachi 260-0611 spectrophotometer; ultraviolet (UV) spectra with a Hitachi 270S spectrophotometer; mass spectra (MS) with a JEOL JMS-D 200 spectrometer; ^{1}H-NMR spectra with a JEOL FX 90Q (90 MHz) spectrometer; ^{13}C-NMR spectra with a JEOL FX 90Q (22.5 MHz) spectrometer. Chemical shifts were given in δ (ppm) values referred to internal tetramethylsilane (TMS). The following abbreviations were used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet.

Plant Materials Aerial parts of *Lavandula latifolia* were collected at Caacupe, Paraguay in November, 1985 and 1986 (voucher specimen deposited).

Bioassay Anti-inflammatory activity by topical application toward CPE and inhibitory effect on HCl were assessed as described in a previous report.[4]

Extraction and Separation The aerial parts of *Lavandula latifolia* (0.65 kg) were extracted with hot 70% EtOH and concentrated *in vacuo* to give 70% EtOH extract (164 g). The 70% EtOH extract was partitioned between H_2O and $CHCl_3$ to give $CHCl_3$ soluble fraction (22.6 g), H_2O soluble fraction (120.2 g) and precipitate (6.0 g). $CHCl_3$ soluble fraction was applied to column chromatography on silica gel with the $CHCl_3$–MeOH gradient system as eluent to afford fr. 1 (0.84 g, from 99 : 1), fr. 2 (5.39 g, from 97 : 3), fr. 3 (6.44 g, from 9 : 1) and fr. 4 (8.07 g, from 1 : 1) on monitoring with TLC. Fraction 1 was further separated and purified by CLC, column chromatography and preparative TLC to yield compounds **1** (0.22 g), **2** (0.027 g), **3** (0.026 g) and **4** (0.006 g). **1** (mp 70 °C) and **2** (mp 120—125 °C) were identifield as coumarin and 7-methoxycoumarin by direct comparison with authentic samples.

***trans*-Phytol** Colorless oil. EI-MS *m/z*: 296 (M^+), 278 ($M^+ - H_2O$), 263, 236, 196. IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 3600—3200, 2930, 2840, 1460. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (12H, d, J=6.0), 1.64 (3H, s, 3a-Me), 1.95 (2H, m), 4.05 (2H, d, J=6.4, H-1), 5.33 (1H, t, J=6.4, H-2). ^{13}C-NMR ($CDCl_3$) δ: 140.26 (s, C-3), 123.08 (d, C-2), 59.39 (t, C-1), 39.87 (t, C-4), 39.38 (t, C-14), 37.43 (t, C-10), 37.36 (t, C-8), 37.28 (t, C-12), 36.66 (t, C-6), 32.79 (t, C-11), 32.70 (t, C-7), 27.99 (d, C-15), 25.14 (t, C-5), 24.82 (t, C-13), 24.48 (t, C-9), 22.72, 22.64 (each q, C-15a, 16), 19.73 (q, C-7a, 11a), 16.16 (q, C-3a).

Caryophyllene Oxide (4) Colorless oil, $[\alpha]_D^{23}$ −64.2° (c=0.6, $CHCl_3$). HR-MS Calcd for $C_{15}H_{24}O$: 220.1826. Found: 220.1779. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3075, 1630, 1450. ^{1}H-NMR ($CDCl_3$) δ: 0.99, 1.01 (each 3H, s, 14,15-Me), 1.20 (3H, s, 13-Me). 4.86 (1H, d, J=1.5, $=CH_2$), 4.98 (1H, d, J=1.5, $=CH_2$). ^{13}C-NMR ($CDCl_3$) δ: 151.8 (s, C-2), 112.7 (t, C-12), 63.7 (d, C-5), 59.8 (s, C-6), 50.9 (d, C-9), 48.7 (d, C-1), 39.8[a] (t, C-3), 39.2[a] (t, C-7), 34.0 (s, C-11), 30.2 (t, C-4), 29.9[b] (t, C-10), 29.9[b] (q, C-13), 27.3 (t, C-8), 21.6 (q, C-14), 17.0 (q, C-15). *a*) The assignments may be interchanged. *b*) Confirmed by INEPT experiment.

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