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ANTI-INFLAMMATORY ACTIVITY OF TWO FLAVONOIDS FROM *TANACETUM MICROPHYLLUM*

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ABSTRACT.—The CH_2Cl_2 extract of *Tanacetum microphyllum* aerial parts exhibited anti-inflammatory activity and yielded two anti-inflamatory flavonoids: 5,7,3′-trihydroxy-3,6,4′-trimethoxyflavone (centaureidin) [**1**], and 5,3′-dihydroxy-4′-methoxy-7-carbomethoxyflavonol [**2**]. This is the first report of the chemical composition of *Ta. microphyllum* and of the isolation of centaureidin from the genus *Tanacetum*.

In Spanish traditional medicine, the aerial parts of *Tanacetum microphyllum* DC. (Compositae) are widely used for the treatment of inflammatory and rheumatic diseases and for their beneficial effects on the digestive tract.

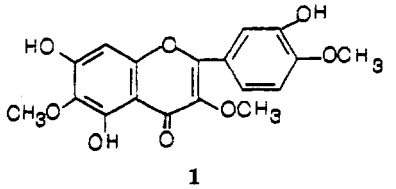
In a previous work (1), this laboratory reported anti-inflammatory and antiulcerogenic activity for organic extracts of *Ta. microphyllum* in rats. Continuing our investigation, the CH_2Cl_2 extract, the most active extract, has yielded two flavonoids. Their characterization and biological activity are reported in this paper.

RESULTS AND DISCUSSION

The CH_2Cl_2 extract of *Ta. microphyllum* exhibited significant dose-related antiedema activity at 200–600 mg/kg po, using carrageenan-induced paw edema test in albino mice. This extract was fractionated by chromatographic methods, yielding compounds **1** and **2**.

The spectral characteristics of compound **1** identified this isolate to be 5,7,3′-trihydroxy-3,6,4′-trimethoxyflavone (centaureidin) (2). The positions of the substituents in rings A and B were confirmed by the uv spectra and diagnostic reagents. Within the Compositae, centaureidin has already been isolated in diverse genera, specifically the genera *Tetragonotheca* (3), *Brickellia* (4), and *Achillea* (5), but this is the first report of the isolation of centaureidin from the genus *Tanacetum*.

The spectral characteristics of compound **2**, when compared with the existing bibliographic literature, identified this compound as 5,3′- dihydroxy-4′-methoxy-7-carbomethoxyflavonol. Regarding the presence of the carbomethoxy group, references have been found only in some models of isoflavones (6). All spectral data agree with a flavonol tetrasubstituted nature, very likely with two methyl groups. The ^{1}H-nmr spectrum gave a multiplet at δ 7.65, corresponding to H-2′ and H-6′, and two doublets at 6.254 and 6.517 (J=2 Hz) for H-6 and H-8. The spectrum also gave a doublet at 7.099 (J=8 Hz), assigned to H-5′. The ^{13}C-nmr spectrum gave 17 signals, corresponding to a 17-carbon-atom skeleton. The δ at 56.3 and 60.2 were assigned to two methyl groups. The ms of compound **2** gave a prominent peak at 360, corresponding to a



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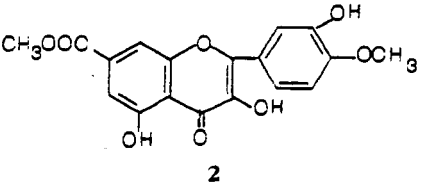

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molecular formula of $C_{18}H_{16}O_8$. Only by postulating a carbomethoxyl group as one of the substituents can all the carbon atoms required by this formula be accounted for. In the ^{13}C-nmr spectrum, the signal from the carbomethoxyl -CO- group appears at 166.8, overlapping with the C-7 signal at 164.9, while it is absent in the ^{13}C-nmr spectrum, in accordance with the 18-atom skeleton showed by the ms. The positions of the substituents of compound **2** were confirmed by the uv spectra and diagnostic reagents, and they allow assignment of position 7 to the carbomethoxyl group.

The CH_2Cl_2 extract and flavonoids **1** and **2** obtained from *Ta. microphyllum* exhibited anti-inflammatory activity as shown in Tables 1 and 2. The extract showed a dose-related

TABLE 1. Anti-inflammatory Activity at 3 h Exhibited by *Tanacetum microphyllum* CH_2Cl_2 Extract and Flavonoids **1** and **2**.

Group[a]	Oral Dose (mg/Kg)	Mean increase in paw volume (ml±SD)	Inhibition of edema (%I)
Control	—	0.37±0.02	—
Phenylbutazone	80	0.18±0.01[b]	51.4
CH_2Cl_2 extract	200	0.27±0.01[b]	27.0
CH_2Cl_2 extract	400	0.24±0.01[b]	35.1
CH_2Cl_2 extract	600	0.15±0.01[b]	59.5
Compound **1**	25	0.12±0.01[b]	67.6
Compound **2**	10	0.13±0.02[b]	64.9

[a]Ten mice per treatment group.
[b]Dunnett's *t*- test: $p \leq 0.01$.

response at 600 mg/kg, similar to that of the reference drug at the same time (+3 h). The anti-inflammatory activity of centaureidin and flavonoid **2** was found to be also highly significant, although the effect of these compounds and extract continues at +7 h, exhibiting higher durations of action than that of phenylbutazone (Figures 1 and 2).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectra were recorded on a vis/uv Beckmann DU-40 spectrophotometer in MeOH with subsequent addition of the usual reagents (6,7): NaOMe, $AlCl_3$, HCl, NaOAc, and H_3BO_3. The ^{1}H-nmr and ^{13}C-nmr spectra were determined in a Varian XL 300 (operating at 300 MHz) and a Bruker AM 200 (50 MHz) spectrometer, respectively, with $CDCl_3$ as eluent and TMS as

TABLE 2. Anti-inflammatory Activity at 5 h Exhibited by *Tanacetum microphyllum* CH_2Cl_2 Extract and Flavonoids **1** and **2**.

Group[a]	Oral Dose (mg/Kg)	Mean increase in paw volume (ml±SD)	Inhibition of edema (%I)
Control	—	0.36±0.03	—
Phenylbutazone	80	0.19±0.01[b]	47.2
CH_2Cl_2 extract	200	0.30±0.03[c]	16.7
CH_2Cl_2 extract	400	0.28±0.03[c]	22.2
CH_2Cl_2 extract	600	0.16±0.02[b]	55.5
Compound **1**	25	0.16±0.01[b]	55.5
Compound **2**	10	0.17±0.01[b]	52.7

[a]Ten mice per treatment group.
[b]Dunnett's *t*- test: $p \leq 0.01$.
[c]Dunnett's *t*- test: not significant.

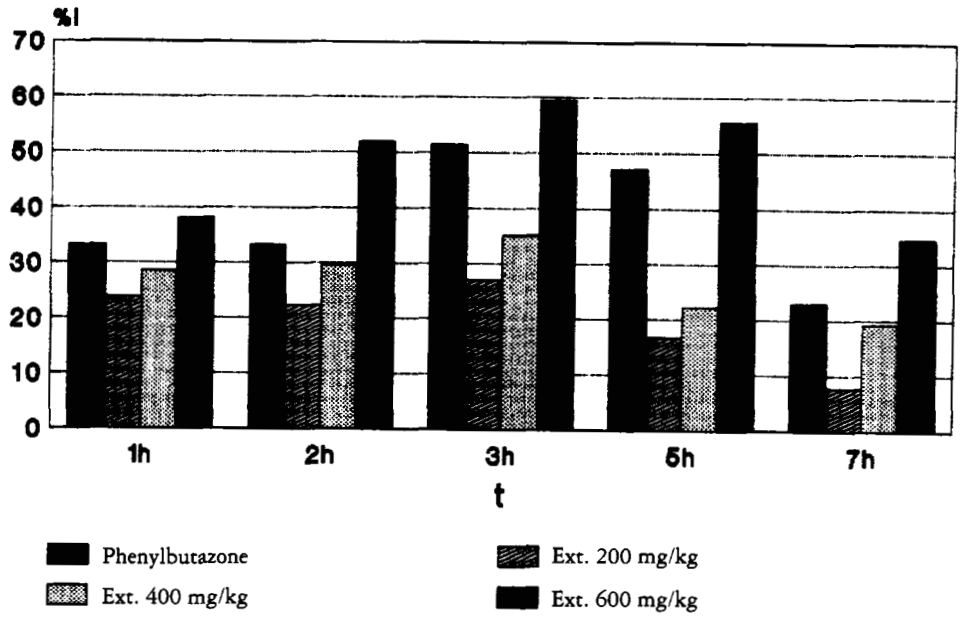

FIGURE 1. Inhibitory effects of *Tanacetum microphyllum* CH_2Cl_2 extract against swelling of mouse paw edema induced by carrageenan.

internal standard. Mass spectra were obtained with a VG 12-250 mass spectrometer. Merck Si gel (70–230 mesh) was used for cc separation, and analytical tlc was done on Merck Si gel 60 F_{254} plates.

PLANT MATERIAL.—The aerial parts of *Ta. microphyllum* were collected in September–October 1990, near Algete (Madrid). A voucher specimen was deposited in the Botany Department Herbarium at the Faculty of Pharmacy, Complutense University, Madrid, Spain.

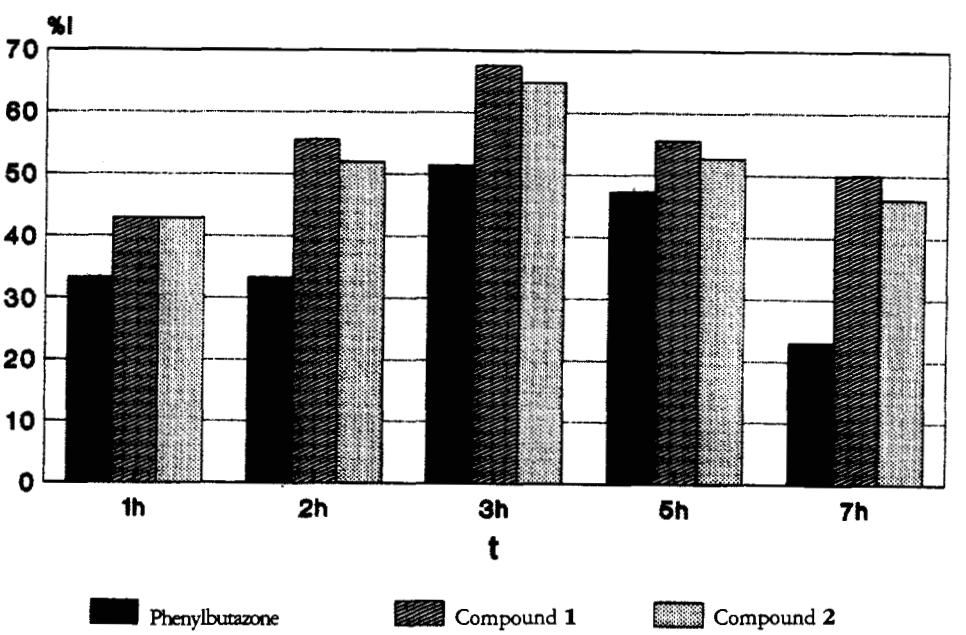

FIGURE 2. Inhibitory effects of flavonoids **1** and **2** against swelling of mouse paw edema induced by carrageenan.

EXTRACTION AND FRACTIONATION OF COMPOUNDS **1** AND **2**.—The air-dried plant material (2 kg) was extracted sequentially in a Soxhlet extractor with hexane, CH_2Cl_2, EtOAc, and MeOH. The CH_2Cl_2 extract was obtained by concentration in a vacuum and separated by medium-pressure liquid chromatography (mplc). Elution with CH_2Cl_2/EtOAc/MeOH gradient solvent system, starting with 100% CH_2Cl_2 with increasing amounts of EtOAc and MeOH to 100%, afforded seven fractions (A–G). Fraction C (4.5 g) was chromatographed by mplc, using CH_2Cl_2-MeOH (98:2) as eluent, and afforded five fractions (C_1–C_5). Fraction C_3 (845 mg), was found to be a mixture of two compounds and was purified by "flash" chromatographic column using CH_2Cl_2-MeOH (98:2), which allowed the spontaneous crystallization of compounds **1** (288 mg) and **2** (125 mg).

Compound **1**.—This compound was identified as centaureidin. The uv, ^{1}H-nmr, and ^{13}C-nmr data of this compound agreed with the literature (2–5).

Compound **2**.—This compound was identified as 5,3′-dihydroxy-4′-methoxy-7-carbomethoxyflavonol: uv λ max (MeOH) nm 252, 266, 353; λ max (NaOMe) nm 272, 396; λ max ($AlCl_3$) nm 268, 298, 362, 404; λ max ($AlCl_3$/HCl) nm 264, 276, 294 sh, 355, 404; λ max (NaOAc) nm 254, 270, 362; λ max (NaOAc/H_3BO_3) nm 252, 266, 355; ^{1}H nmr ($CDCl_3$) δ 6.254 (H-6), 6.517 (H-8), 7.099 (H-5′), 7.65 (H-2′ and H-6′); ^{13}C-nmr ($CDCl_3$) δ 157.90 (C-2), 136.60 (C-3), 179.50 (C-4), 162.90 (C-5), 99.30 (C-6), 164.90 (C-7), 94.50 (C-8), 156.50 (C-9), 105.80 (C-10), 121.75 (C-1′), 112.20 (C-2′), 147.30 (C-3′), 150.85 (C-4′), 115.75 (C-5′), 124.20 (C-6′).

ANTI-INFLAMMATORY ACTIVITY.—Anti-inflammatory activity was determined by the method of Winter *et al.* (8) as modified by Sugishita *et al.* (9), using female Swiss mice weighing 20±5 g. The animals were divided into three groups (control, standard, and test) consisting of 10 mice for each set of experiments. Edema was induced by subcutaneous injection of 0.05 ml of carrageenan type IV (3% w/v in saline solution), into the subplantar region of each animal's left hindpaw. The paw edema was measured by water plethysmography (Letica, Spain) before the injection of carrageenan, and 1, 2, 3, 5, and 7 h later. The drug, vehicle alone [Tween 80-carboxymethylcellulose-H_2O (5.7:1:94.3; v/w/v)], *Ta. microphyllum* crude extract (at 200, 400, and 600 mg/kg doses), and the isolated compounds (at a dose equivalent to 12.5 g dry plant/kg body wt) were administered po 1 h before the carrageenan injection. Phenylbutazone (80 mg/kg, po), suspended in the same dosage vehicle, was given to the animals of the standard group. The effect observed at +3 h was found to be maximum, and the results are expressed as arithmetic means ± SD.

The inhibition percentage of edema was calculated for each animal group in comparison with its vehicle-treated group. Differences between the control and the treatments in these experiments were tested for significance by Dunnett's *t*- test. The results are given in Tables 1 and 2 and Figures 1 and 2.

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