Journal of Natural Products
Vol. 58, No. 11, pp. 1749-1752, November 1995

SANTOLINDIACETYLENE, A POLYACETYLENE DERIVATIVE ISOLATED FROM THE ESSENTIAL OIL OF *SANTOLINA CANESCENS*

M.P. UTRILLA,* M.C. NAVARRO, J. JIMENEZ, M.P. MONTILLA, and A. MARTIN

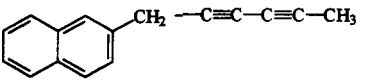
Department of Pharmacology, Faculty of Pharmacy, University of Granada, 18071 Granada, Spain

ABSTRACT.—The yield, composition, and some pharmacological activities (hepatoprotective and antioxidant) of the essential oil of *Santolina canescens* aerial parts have been investigated. The essential oil qualitative data were determined by gc and gc-ms. The main component, santolindiacetylene [**1**], was isolated and characterized by spectral methods, and the structure assigned as 1-(2′-naphthyl)hexa-2,4-diyne. The protective activities of the essential oil and its main component [**1**] were evaluated against carbon tetrachloride-induced hepatotoxicity in a rat model. In both cases a significant hepatoprotective effect was observed, as evident from the strong decrease of elevated GPT serum levels caused by carbon tetrachloride-induced hepatic damage.

The genus *Santolina* (Asteraceae), widely represented in the Mediterranean and Iberian flora, is a taxonomically complex group of species used in traditional medicine. Flowers and leaves of different *Santolina* species have been used in the treatment of liver and digestive disorders. Although studies of various members of this genus have been reported (1,2), *Santolina canescens* Lag. has not been hitherto investigated in detail, since a survey of the literature showed that little is currently known about the chemical and pharmacological properties of this species. We report herein the composition of *S. canescens* essential oil, from which we have isolated a new polyacetylene derivative as its main component. The hepatoprotective and antioxidant activities of *S. canescens* essential oil and its main component, santolindiacetylene [**1**], were studied.

The essential oil content obtained by steam distillation of the aerial parts of *S. canescens* was less than 0.75% v/w. The essential oils obtained from *Santolina* spp. contain a large proportion of sesquiterpene derivatives (1), of which sesquiterpene hydrocarbons are the most prevalent group identified (approximately 50%). The predominant component of the *S. canescens* essential oil proved to be santolindiacetylene [**1**] (28.5%).

The molecular formula of **1**, determined by ms, was $C_{16}H_{12}$ ([M]$^+$ 204).



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The base peak occurred at *m/z* 153, suggesting the loss of a highly unsaturated chain of four carbons by the fragmentation of the naphthalene ring and the loss of a C_4H_3 unit whose mass (*m/z* 51) appeared as a moderate intensity signal (34%) in the spectrum. Moreover, the *m/z* 128 and *m/z* 76 peaks were characteristic of a naphthalene unit. The ^{13}C-nmr spectrum of **1** indicated the presence of a naphthalene ring with signals from 10 aromatic carbons between δ 126.6 and δ 135.7, and DEPT nmr revealed the presence of three quaternary carbons. One methyl carbon at δ_c 4.2 and one methylene carbon at δ_c 25.5 were also observed. Furthermore, four signals from quaternary carbons at δ 64.4, δ 67.5, δ 73.8, and δ 74.2 were detected, suggesting the existence of a diacetylene chain. The ^{1}H-nmr spectrum of **1** showed a methyl triplet at δ 1.92 (J=1.2 Hz) linked to an acetylenic carbon, and a methylene doublet at δ 3.65 (d, J=0.7 Hz) also linked to an acetylenic carbon. Their assignments were made by COSY, ^{1}H-^{13}C correlation, and DEPT nmr spectra. The multiplicity of the methyl is only possible if long-range coupled with the methylene of the acetylenic chain, and

this was confirmed by the COSY nmr spectrum. In the ir spectrum, typical acetylenic bands were observed. The location of the side-chain in the naphthalene ring was determined by comparing the spectra of **1** with those obtained for derivatives of alpha and beta naphthalenes. In the ^{1}H-nmr spectrum of **1**, the singlet at 7.25 ppm corresponded to the H-1′ of the naphthalene ring. On the basis of these spectral data, the structure of **1** was assigned as 1-(2′-naphthyl)hexa-2,4-diyne.

In the course of hepatic detoxification of xenobiotic and toxic substances, large amounts of reactive oxygen intermediates are produced. These intermediates can affect the antioxidant defense system, causing hepatic damage. Compounds with scavenger activity could contribute to the partial or total alleviation of this damage.

Table 1 shows the scavenger activity of the *S. canescens* essential oil and its different constituents, as well as correlation coefficients. The scavenger activity shown by the *S. canescens* essential oil, and mainly by santolindiacetylene [**1**], demonstrated that this major constituent is an antioxidant, which could exert a beneficial action against pathological alterations caused by the presence of free radicals that occur in certain hepatic disorders. Also, these results show that **1** can be held largely responsible for the scavenger activity of *S. canescens* essential oil.

TABLE 1. Scavenger Activity of the Components from *S. canescens* Essential Oil Against the Free Radical DPPH.

Sample	EC_{50} (mg/ml)	r^a
Essential oil	5.0	0.9780
α-Pinene	330.6	0.9910
Sabinene	232.7	0.9890
β-Pinene	220.1	0.9853
β-Phellandrene	127.7	0.9875
Myrcene	248.0	0.9912
γ-Terpinene	22.0	0.9897
p-Cymene	408.0	0.9879
Carvone	11.7	0.9932
Camphor	2.4	0.9961
Borneol	2.4	0.9908
Terpinen-4-ol	53.7	0.9998
γ-Caryophyllene	3.7	0.9987
α-Terpineol	146.3	0.9859
Santolindiacetylene [1]	1.7	0.9880
L-Ascorbic acid[b]	2.7[c]	0.9993
Silymarin[b]	24.6[c]	0.9989

[a]*r*=Correlation coefficient.
[b]Standard compound.
[c]EC_{50} data expresseed as μg/ml.

Carbon tetrachloride-induced liver damage is assumed to be mediated by hepatic microsome production of free radicals during toxin metabolism. These radicals peroxidize the membrane lipids when oxygen is present, causing a structural change in the membrane (3,4). When the cell membrane is damaged several cytoplasmatic enzymes are released into the bloodstream such as GPT (5). Determination of GPT serum levels is thus a useful indicator of hepatocellular damage. On the other hand, lipoperoxidation, the result of free radical reactions, is detectable by several methods. In this case, determination of antioxidant activity was made by quantification of malondialdehyde. This quantification is based on the acid-catalyzed decomposition of lipid peroxides, in the course of which malondialdehyde is produced (6,7).

A single intraperitoneal dose of CCl_4 caused a marked rise in GPT serum levels and lipid peroxides in the liver. The antihepatotoxic activities of *S. canescens* essential oil and **1** were demonstrated by their capacity to prevent these increases (Table 2). Levels of GPT were clearly lower in both cases than in the group treated only with CCl_4, without significant differences between the results of the groups treated with the essential oil or **1** and those treated with silymarin, a known antihepatotoxic agent. In the group of rats treated with CCl_4 only, the hepatic concentrations of malondialdehyde, which indicate the degree of lipoperoxidation, were abnormally high. The results obtained with the groups pretreated with *S. canescens* essential oil or **1** showed a stronger antilipoperoxidative activity in both cases than the effect caused by silymarin. The decrease in the

TABLE 2. Effects of *S. canescens* Essential Oil and Santolindiacetylene [**1**] on CCl_4-Induced Hepatotoxicity in Rats.[a]

Sample	Dose	GPT		Malondialdehyde	
		(IU/liter)	%	nmol/ml	%
Blank	0.5 ml	10.9± 2.6	—	0.160±0.07	—
CCl_4	1 ml/kg	117.3±47.0[b]	100	0.546±0.13[b]	100
Essential oil	66 mg/kg	21.7± 9.6[d]	18.5	0.165±0.04[d]	30.2
Santolindiacetylene [**1**]	7 mg/kg	23.3± 8.8[d]	19.8	0.182±0.03[d]	33.3
Silymarin[e]	35 mg/kg	27.9±11.7[d]	23.7	0.200±0.05[c]	36.6

[a]Mean values ± SE.
[b]$p<0.001$ vs. a blank group.
[c]$p<0.01$ vs. a CCl_4-treated group.
[d]$p<0.001$ vs. a CCl_4-treated group.
[e]Standard compound.

malondialdehyde concentration was a maximum for the group pretreated with the essential oil, without significant differences from the blank group.

From the results obtained, a clear antihepatotoxic activity was exhibited by *S. canescens* essential oil and its main component, **1**. This was emphasized by a significant drop in the serum GPT levels and the decline in hepatic malondialdehyde values. The latter could be due, at least in part, to the scavenger activity found in both samples during the in vitro experiments.

Hepatoprotective and antioxidant effects have been described for many species of the Asteraceae but generally they have not been related to acetylenic derivatives. However, Kim *et al.* (8) have shown hepatoprotective activity against CCl_4-induced hepatotoxicity by some polyacetylenes (panaxydol, panaxynol, and panaxytriol) isolated from Korean ginseng, which operate by inhibiting lipoperoxidation.

Santolindiacetylene [**1**], a new polyacetylene, can be considered as the principle responsible for the antioxidant and hepatoprotective properties shown by *S. canescens* essential oil.

EXPERIMENTAL

PLANT MATERIAL.—The aerial parts of *Santolina canescens* Lag. were collected during the flowering period (June 1993), in Cogollos Vega, Granada, Spain, as populations observed in this area were homogenous and did not co-occur with other species of *Santolina*. The identity of the plant was confirmed by Dr. Oswaldo Socorro, Department of Vegetal Biology, Faculty of Pharmacy, University of Granada. The specimen is currently deposited in the GDA, Faculty of Pharmacy, Herbarium, University of Granada, under catalogue number 18099. The plants were dried at 20°–25° for 48 h. The leaves and flower capitula were then separated from the rest of the plant, weighed, and stored in flasks hermetically sealed until use.

EXTRACTION AND ISOLATION.—*Santolina canescens* plant material was distilled in a Clevenger device for 5 h. The purified oil (0.75%, v/w) was dried over anhydrous $NaSO_4$ and then stored at 4°–6°.

The most abundant compound in *S. canescens* essential oil was isolated using cc with Si gel as stationary phase and C_6H_6 as mobile phase. The isolated compound was identified by ms, ^{1}H-nmr, ^{13}C-nmr, DEPT, and COSY techniques using a Bruker AM 300 apparatus with $CDCl_3$ as solvent.

1-(2′-Naphthyl)hexa-2,4-diyne (santolindiacetylene) [**1**].—Compound **1** was isolated as a yellow oil; ir ($CHCl_3$) ν max 2261, 2241, 2147, 1453, 1416, 1375, 773, 751 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 1.92 (3H, t, J=1.2 Hz, H-6), 3.65 (2H, d, J=0.7 Hz, H-1), 7.25 (1H, s, H-1′), 7.20–7.53 (6H, m, H-3′–H-8′); ^{13}C nmr (300 MHz, $CDCl_3$) δ 4.2 (C-6), 25.5 (C-1), 64.4 (C-4), 67.5 (C-3), 73.8 (C-5), 74.2 (C-2), 126.6 (C-7′), 126.8 (C-8′), 127.9 (C-1′, C-4′), 128.6 (C-2′), 128.6 (C-3′), 128.7 (C-6′), 129.5 (C-9′), 134.7 (C-5′), 135.7 (C-10′); gc-ms (70 eV) *m/z* [M]$^+$ 204 (6), 153 (100), 128 (14), 76 (32), 51 (34), 43 (40).

QUALITATIVE AND QUANTITATIVE ANALYSIS.—Analysis of the essential oil of *S. canescens* was performed by gc and gc-ms. Gc analysis was performed using a Perkin-Elmer gas chromato-

graph (model 8310 B) equipped with a flame-ionization detector and a Perkin-Elmer computing integrator (model GP-100). A semi-capillary column (25 m×0.53 mm) coated with Carbowax 20M was employed, using a temperature program of 75° (5 min), 140° (5 min), and 170° (7 min), with an increase of 8°/min. The carrier gas was He, at a flow rate of 10 ml/min.

Gc-ms analysis was performed using a Hewlett-Packard mass spectrometer (model 5988A), combined with a Hewlett-Packard gas chromatograph (model 5980A), with a dimethylsilicone capillary column (25 m×0.20 mm). Other conditions were as follows: initial temperature 50°, final temperature 200°, initial time 5 min, rate 10°/min, and final time 5 min. The carrier gas was H_2 and the flow rate was 2 ml/min. Components were identified by co-injection of the oil with pure components. A ms library and comparison with mass spectra of pure components and/or with literature data were also used for peak identity confirmation. The quantitative analysis was performed by the relative proportion method under the conditions specified in the qualitative analysis section.

HEPATOPROTECTIVE ACTIVITY.—Female Wistar rats (180–225 g body wt) were used. Food was withdrawn approximately 18 h before each experiment, and H_2O was provided *ad libitum*. Intoxication with CCl_4 (1 ml/kg body wt in a 20% v/v olive oil solution) was intraperitoneal, and the test substances were administered i.p. 24 h before intoxication. Both the plant essential oil (66 mg/kg body wt) and compound **1** (7 mg/kg body wt) were administered in an emulsion with Tween 80 (9:1). The blank group received the same volume of vehicle used in the treated groups. Silymarin, an antihepatotoxic agent (9,10), was used as control substance. GPT levels were measured according to the method of Reitman and Frankel (11) (Boehringer kit). The amount of malondialdehyde present in the rat livers was determined by the Uchiyama and Mihara procedure (12).

SCAVENGER ACTIVITY.—The method described by Lamaison *et al.* (13) using the colored free radical DPPH (1,1-diphenyl-2-picryl-hydrazine) was employed. This activity was expressed as an effective concentration at 50% (EC_{50}), i.e., the concentration of the solution required to give a 50% decrease in absorbance from that of DPPH solution. Ascorbic acid was used as a control substance (EC_{50} value of 1.5×10^{-5}M). Various components of the essential oil used in this study were purchased from Sigma, with exception of **1**.

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Received 14 March 1995