Journal of Ethnopharmacology, 31 (1991) 43–48
Elsevier Scientific Publishers Ireland Ltd.

TRADITIONAL MEDICINE OF BAJA CALIFORNIA SUR (MEXICO) III. CARNOSOL: A DITERPENE ANTIBIOTIC FROM *LEPECHINIA HASTATA*

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(Accepted March 25, 1989)

Summary

The medicinal plant *Lepechinia hastata*, used as a remedy against uterine infections in Baja California Sur (Mexico), was shown to contain carnosol as the main diterpenoid secondary metabolite. Carnosol has potent in vitro antimicrobial activity. Detailed spectroscopical properties of carnosol are presented.

Introduction

During an investigation of traditional medicine as practised in Baja California Sur our attention was attracted to *Lepechinia hastata* (A. Gray) Epling (Labiatae) (basionym: *Sphacele hastata* A. Gray (Morley and Dutkiewicz, 1978)) (Encarnacion et al., 1987). This plant, locally known as Chicura de la Sierra, is reported to cure uterine infections if ingested as decoctions of the root. Subsequent investigations of extracts of dried plant material revealed antimicrobial activity against test cultures of selected microorganisms. Bioassay guided isolation and purification gave a pure compound identified as carnosol (Fig. 1).

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Published and Printed in Ireland

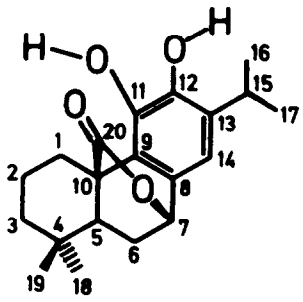

Fig. 1. Structure of carnosol.

Experimental

Instrumentation

Mass spectra were obtained from a VG 20-250 Quadropole mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL FX90Q NMR spectrometer operating on 90 and 22.5 Mhz, respectively, the ^{1}H-NMR spectra used to determine coupling constants originate from a Bruker instrument operating on 250 Mhz. UV data were collected using a Perkin-Elmer lambda 17 spectrophotometer while IR data were obtained from a Perkin-Elmer 580 spectrometer.

Plant material

Lepechinia hastata was collected in Sierra de la Laguna, B.C.S., Mexico, in December 13 of 1980. The plant was identified at the Pharmacognosy Laboratory of the Marine Biological Department of Universidad Autonoma de Baja California Sur, Mexico. A voucher specimen has been deposited in the Biological Institute of Universidad Nacional Autonoma de Mexico. The air dried aerial parts of *L. hastata* (422.3 g) were exhaustively extracted (6–7 days) successively with petrol ether (b.p. 30–60 °C, 2 l), chloroform (2 l) and ethanol (2 l) in a soxhlet extractor. Evaporation of the solvents under reduced pressure left three residues, 58.1 g (petrol ether), 22.0 g ($CHCl_3$) and 37.7 g (EtOH) all of which were active (approximately 2.8 mg per disc) against *Staphylococcus aureus*, *Streptococcus faecalis* and *Bacillus subtilis*. The most potent activity against *Strep. faecalis* was exhibited by the $CHCl_3$ extract which deposited a solid. After recrystallisation from $CHCl_3$ the crude crystalline mixture (200 mg) was further purified by column chromatography (20 g silica gel) using $CHCl_3$/MeOH (9:1) as eluent. Fractions of 2 ml were collected and the combined fractions 6–24 afforded 191 mg of a solid subsequently recrystallised from $CHCl_3$ to yield 70 mg colorless crystals, m.p. 235–236 °C. A further crop of 20 mg slightly impure product was isolated from the mother liquid giving a combined yield of 0.02%.

Elemental analysis

Calculated for $C_{20}H_{28}O_4$, C: 72.73%, H: 7.88%. Found C: 72.30%, H: 8.12%.

Mass spectrometry

m/z 330 (20%, M^+), 286 (100%, $M^+ - 44$), 271 (15%, $286 - CH_3$), 215 (80%).

IR (cm^{-1})

0.95 mg in 300 mg KBr: 3495 (m sharp, free OH), 3290 (m broad, hydrogen bonded OH); 2960 (m, aliphatic C—H stretch), 1715 cm^{-1} (s, C=O stretching vibration).

UV

1.10 mg in 25 ml abs. ethanol: 208 nm ($\log \varepsilon = 4.39$), 284 nm ($\log \varepsilon = 3.35$).

Results and Discussion

The structure of the active agent was inferred from the data given in Experimental as carnosol. The results from low resolution mass spectrometry and elemental analysis gave the molecular formula $C_{20}H_{28}O_4$. UV-spectroscopy revealed the presence of an aromatic system supported by the six ^{13}C-NMR resonances between 112.6 and 144.7 ppm (Table 1). The aromatic ring must be penta-substituted and one substituent is an isopropyl group according to the ^1H-NMR data (Table 1). From a 30-nm bathochromic shift on addition of base the presence of an *o*-dihydric phenol (Scott, 1964) was inferred. The IR-spectrum supported this assignment and in addition showed one of the phenolic hydroxyl groups to be strongly hydrogen bonded (3290 cm^{-1}, broad) in contrast to the other phenolic hydroxyl group (3495 cm^{-1}, sharp). The intense carbonyl stretching vibration appearing at 1715 cm^{-1} must, according to ^{13}C-NMR, originate from an ester or lactone group.

The presence of a lactone group was substantiated by the fact that the UV-spectrum of the base did not revert to the original spectrum on acidification. The unusual position of the carbonyl band was noted by Brieskorn and Fuchs (1952), who suggested the displacement to be effected by strong intermolecular hydrogen bonding. The remaining number of unsaturation sites coupled with the presence of two methyl groups and a benzylic terminus of the lactone ring defined the structure as an abietane type. Assignment of the proton and carbon resonances then unambiguously identified the compound as carnosol.

The ^{13}C assignments were made by comparison with similar structures (Wehrli and Nishida, 1979; Canigueral et al., 1988; Galicia et al., 1988) combined with off-resonance decoupled spectral data. The latter not only revealed the multiplicity of the signals, but in addition proved the signal at 20.1 ppm to arise from superimposed signals from CH_3 and CH_2 groups. With a few exceptions (marked in Table 1 with an [a]) all signals could be unambiguously assigned. The ^1H assignments were investigated partially by spin

TABLE 1

NMR DATA OF CARNOSOL IN CD_3OD

Position	^{13}C/ppm	^{1}H/ppm
1	30.9t[a]	2.85 ddd and 2.61 ddd
2	20.1t	1.98 m + not resolved
3	30.9t[a]	1.57 m and 1.35 add
4	35.5s	
5	42.2d	1.73 dd
6	30.1t[a]	2.25 ddd and 1.89 ddd
7	79.8d	5.46 dd
8	133.4s	
9	123.1s	
10	48.6s	
11	144.3s[a]	
12	144.7s[a]	
13	136.1s[a]	
14	112.6d	6.73 s
15	32.2d	3.29 sept
16	23.2q	1.22 d
17	23.2q	1.25 d
18	28.0q	0.91 s
19	2.01q	0.91 s
20	179.4s	0.91 s

Coupling constants (Hz): $J_{15,16} = J_{15,17} = 6.9$; $J_{6,7} = 1.5$ and 4.0; $J_{6,6} = 13.6$; $J_{5,6} = 10.6$ and 5.6; $J_{3,3} = 12.5$; $J_{2,2} = 13.7$; $J_{1,1} = 14.3$.
[a]The assignments of the pairs 30.1/30.9, 133.4/136.1 and 144.3/144.7 ppm may be reversed.

decoupling with irradiation of the resonances at 1.99 and 2.69 ppm. This served to verify the presence of the $O-CH-CH_2-CH$ moiety in the molecule. The very complicated $CH_2-CH_2-CH_2$ pattern in the 1.2–2.3 ppm range was not further explored, but the integrated intensity added up to the expected six protons.

Carnosol was initially isolated from *Salvia carnosa* (Dougl.) (White and Jenkins, 1942a,b) and is presumably identical with a compound obtained from *Salvia officinalis* L. (Janot et al., 1952). The correct structure was eventually deduced from, mainly, chemical studies of material isolated from *Rosmarinus officinalis* L. (Brieskorn et al., 1964). The latter authors also showed carnosol to be identical to pikrosalvin (picrosalvin) from *S. officinalis* L. (Brieskorn and Fuchs, 1952; Linde, 1964). The structural assignment of carnosol has been confirmed by total synthesis (Shew and Meyer, 1968). Carnosol has later been detected in *Rosmarinus officinalis* (Nakatani and Inatani, 1981; Hayashi et al., 1987; Wu et al., 1982), *Salvia canariensis* flowers (Gonzales et al., 1987), *Salvia calycina* and *Salvia triloba* (Doganis, 1971).

Since the structure elucidation rested mainly on chemical evidence, spectroscopic data of carnosol is scattered in the literature and not easily accessi-

ble. We have therefore included these data in the Experimental part. Table 1 gives the assignment of ^{1}H and ^{13}C resonances, since only data of acetone-d_6 solutions exist (Inatani et al., 1982). It should also be noted that the melting point determined was more than 10 °C higher than the reported value (Brieskorn and Fuchs, 1952).

Carnosol was active against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* (approximately 0.4 mg per disc). Brieskorn et al. (1958) noted activity against *Staphylococcus aureus*, *E. coli* and *Epidermophyton*.

Acknowledgments

Financial support (C-86-01-0208) from the Direccion General de Investigación Cientifica y Superación Academica of the Secretaria de Educación Publica is gratefully acknowledged.

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