High Antioxidant Activity of Extracts Obtained from Sage by Supercritical CO_2 Extraction

Z. Djarmati*[a], R.M. Jankov[b], E. Schwirtlich[c], B. Djulinac[c] and A. Djordjevic[c]

[a]Technical High School and Institute of Technology "Servo Mihalj," 23000 Zrenjanin, Yugoslavia, [b]Department of Biochemistry, Faculty of Science, 11000 Belgrade, Yugoslavia, and [c]Institute of Technology "Servo Mihalj," 23000 Zrenjanin, Yugoslavia

The ethanolic extract of sage (*Salvia officinalis* L.) was separated into five fractions through reextraction with supercritical CO_2. Further fractionation of the most active antioxidant fractions by means of liquid chromatography, with silicic acid as absorbent, yielded 2H-10,4α-(epoxy methano)-phenantren-12-one-1,3,4,9,10,10α-hexahydro-5,6-dihydroxy-9α-ethoxy-1,1-dimethyl-7-(1-methylethyl), (rosmanol-9-ethyl ether). The same compound was isolated from the alcoholic extract of the hyssop (*Hyssopus officinalis* L.). Rosmanol-9-ethyl ether was shown to be one of the active antioxidant components in sage and hyssop, with activity much greater than butylated hydroxytoluene (BHT).

KEY WORDS: Hyssop extract, natural antioxidants, rosmanol-9-ethyl ether, sage extract, supercritical CO_2 extraction.

Natural products, isolated from spices, can act as antioxidants either alone or synergistically with chemical additives. In 1977, Chang *et al.* (1) reported a patented method for extraction of active antioxidants from rosemary and sage. Since methanol and ethanol were found to be the most suitable solvents for extraction of antioxidants from plant material, a number of publications have dealt with further purification of the alcoholic extracts.

To obtain a useful natural food additive from alcoholic extracts, it is necessary to improve the antioxidant properties and to create an odorless, tasteless and colorless product, if possible.

Some methods of purification and fractionation, such as vacuum steam distillation (1) or molecular distillation (2) can be used on production scale. However, it appears drastic for heat-sensitive natural products. On the other hand, fractionation with column liquid chromatography on silicic acid (3) or Sephadex (4), or high pressure liquid chromatography (5) are useful methods for purification, although not suitable for large-scale production for economic reasons.

This paper reports fractionation of the alcoholic extract of sage (*Salvia officinalis* L.) with supercritical CO_2, and the isolation, structure determination and activity test of rosmanol-9-ethyl ether, which was recently found in rosemary (6) and sage (7). For the first time we wish to report the presence of the same compound in hyssop (*Hyssopus officinalis* L.). For this compound we have established an antioxidant activity higher than butylated hydroxytoluene (BHT).

MATERIALS AND METHODS

The plant material originated from The Institute for Hop, Sorghum and Medicinal Plants, Bački Petrovac, Yugoslavia. For the extraction we were using the aerial parts of the plant during its flowering period.

Melting points (m.p.; uncorrected) were taken on a Büchi melting point apparatus. Infrared spectra (IR) were recorded on a Perkin-Elmer (Norwalk, CT) spectrophotometer FT-IR Model 1725X in KBr pellets. Nuclear magnetic resonance (NMR) spectra were determined in a Varian spectrometer FT-80A (Palo Alto, CA) with $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard.

Supercritical extractions with CO_2 were performed in the UHDE GmbH pilot plant, Frankfurt, Germany (1986).

EXPERIMENTAL PROCEDURES

Preparation of sage extracts. Steam-distilled and ground sage herb (9.35 kg) was divided into three parts, and each part was extracted with 20 L of ethanol (96%) at room temperature with occasional stirring, and was left overnight. The mixture was filtered and the residue was reextracted with 15 L of fresh ethanol at 60°C, in the same manner as the initial extraction at room temperature. After the third extraction, which was carried out in the same way as the second, the solid residue was discarded. The combined filtrates were concentrated by rotary evaporator to 10 L (to contain approximately 10 g of residue per 100 mL of extract). The concentrated extract was passed through a column filled with 2 kg of active carbon. The column was washed with ethanol, and the resulting light brown filtrate was concentrated again to approximately 10 L.

Fractionation of sage antioxidants. For the preparation of suitable material for batch extraction with CO_2, the alcoholic extract is mixed with thermally treated alumino silicate (approximately 1200 g), and the resulting suspension was evaporated under reduced pressure. The solid residue was dried and passed through a 2-mm sieve.

The solid material was extracted with CO_2 at 60°C at 100, 200, 300 and 400 bars, and at 100°C at 500 bars, for 6 hr, at CO_2 flow of 20 kg/hr.

Isolation of rosmanol-9-ethyl ether. The amorphous mixture (25 g), which resulted from reextraction of the alcoholic extract from sage with supercritical CO_2 (temperature 60°C, pressure 350 bar, time 6 hr, flow rate 20 kg CO_2/hr), was dissolved in benzene (120 mL) and chromatographed over silicyc acid (900 g, Merck, Darmstadt, Germany, silica gel 0.063–0.200 mm and silica gel under 0.08 mm in ratio of 1:1).

Elution of the column with benzene and benzene-ethyl acetate mixture (up to 10%) as eluent yielded I (1.35 g). The recrystallization of crude I from ethyl acetate gave orthorhombic colorless crystals.

From the corresponding extract of hyssop, by chromatographic separation as above, a crude rosmanol-9-ethyl ether (I) was obtained. Several recrystallizations from ethyl acetate gave colorless crystals, which was in all re-

*To whom correspondence should be addressed at Technical High School and Institute of Technology "Servo Mihalj," 23000 Zrenjanin, P. Drapšina 15, Yugoslavia.

TABLE 1

Fractionation of the Alcoholic Extract of Sage Supercritical with CO_2

Code number	t (°C)	Pressure bars	Time (hr)	Flow rate kg/hr	Yield[a] (g)	Yield[a] (%)
100/60	60	100	6	20	28.46	0.30
200/60	60	200	6	20	59.97	0.64
300/60	60	300	6	20	22.05	0.23
400/60	60	400	6	20	19.75	0.21
500/100	100	500	6	20	119.25	1.27
Total:					249.48	2.67

[a]Yields were calculated on the basis of 9.35 kg of the air-dried herb.

TABLE 2

Antioxidant Properties of Five Fractions Made by Reextraction of the Ethanolic Extract of the Sage Using CO_2 Under Supercritical State as the Solvent

Additive 0.01%	Peroxide value (mmol O_2/kg) at 98°C after 0	12	24	48	72 hr
None	0.85	10.76	45.31	<100	
BHT		1.26	2.07	17.83	<100
Ethanolic extract		1.35	5.23	68.84	<100
Fraction:					
100/60		1.57	2.50	54.54	<100
200/60		1.25	1.53	3.58	56.08
300/60		1.19	1.73	3.48	58.26
400/60		1.20	1.74	4.07	78.77
500/60		1.35	1.55	19.78	90.19

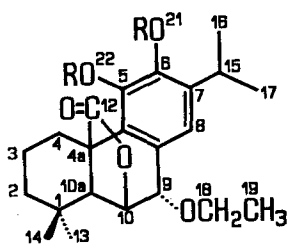

FIG. 1. Formula for rosmanol-9-ethyl ether (I) and its diacetate (II): I R = H; II R = $COCH_3$.

spects identical with the crystalline product isolated from the sage extract.

Peroxide values. Peroxide values were determined by the official method ISO 3960 (1970).

RESULTS AND DISCUSSION

To improve the antioxidant properties of the bleached alcoholic extract of sage, it was fractionated by reextraction with supercritical CO_2 as solvent. As shown in Table 1, five fractions were obtained. The fractions were light brown-colored and (except for fraction 100/60) slightly spice-flavored. A total yield (calculated on the basis of air-dry plant material) of 2.67% was obtained.

The obtained fractions showed excellent antioxidant activities when added to lard at the concentration of 0.01% (Table 2).

The peroxide value of the lard was determined after the samples were aged at 98°C in the dark for 6, 12, 24, 48, and 72 hr. The decrease in the rate of peroxide formation was used as the measure of antioxidant activity.

At the concentration of 0.01%, the antioxidant activities of fractions 200/60, 300/60 and 400/60 were comparable to those of butylated hydroxytoluene (BHT).

Identification of rosmanol-9-ethyl ether. After the column-chromatographic isolation, colorless crystals were obtained, which were identified as rosmanol-9-ethyl ether, whose formula is given in Figure 1.

The characteristics of rosmanol-9-ethyl ether (I) $C_{22}H_{30}O_5$ are as follows: m.p. 222-224°C. ν_{max}^{KBr} cm^{-1} 3480 and 3370 (–O–H), 1775 (=C=O), 1120 and 1050 (C–O–C). ^1H **NMR** (80 MHz, $CDCl_3$): δ 0.90 and 1.00 (3H, *s*, H-13 and H-14), 1.20 and 1.20 (3H, *d*, J = 7 Hz, H-16 and H-17), 1.32 (3H, *t*, J = 7 Hz, H-19), 2.25 (1H, *s*, H-10a), 3.10 (1H, *m*, $W_{1/2}$ = 20 Hz, H-15), 3.85 (2H, *q*, H-18), 4.32 (1H, *d*, J = 3 Hz, H-9β), 4.62 (1H, *d*, J = 3 Hz, H-10α), 5.60 (2H, *m*, $W_{1/2}$ = 12 Hz, O_{22}-H and O_{21}-H), and 6.75 (1H, *s*, H-8).

IR and NMR spectra have been given in Figures 2 and 3, respectively.

Rosmanol-9-ethyl ether diacetate (II) was obtained by usual acetylation with acetic anhydride in pyridine. The corresponding acetate, $C_{22}H_{34}O_7$, was obtained with the following data: m.p. 205-207°C. ν_{max}^{KBr} cm^{-1} 1775 (=C=O) and 1755 (=C=O), 1200, 1100 and 1030 (C–O–C). ^1H **NMR** (80 MHz, $CDCl_3$): δ 0.90 and 1.00 (3H, *s*, H-13 and H-14), 1.20 and 1.20 (3H, *d*, J = 7 Hz, H-16 and H-17), 1.33 (3H, *t*, J = 7 Hz, H-19), 2.27 and 2.30 (3H, *s*, O_{22}-$COCH_3$ and O_{21}-$COCH_3$), 2.85 (1H, *m*, $W_{1/2}$ = 20 Hz, H-15), 3.87 (2H, *q*, H-18), 4.40 (1H, *d*, J = 3 Hz, H-9β), 4.63

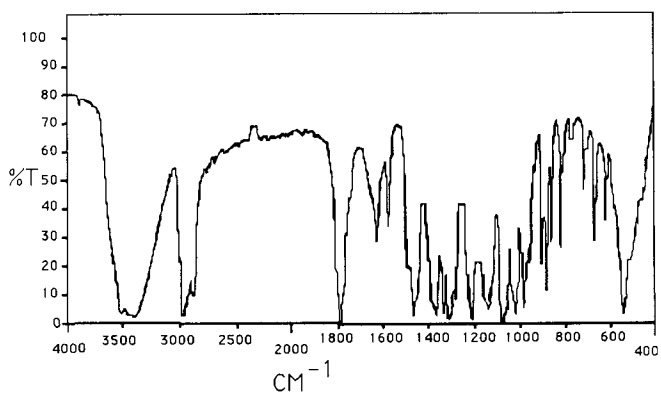

FIG. 2. IR spectrum of rosmanol-9-ethyl ether (I).

(1H, *d*, J = 3 Hz, H-10α), and 7.20 (1H, *s*, H-8).

The molecular structure of rosmanol-9-ethyl ether (I), inferred from the spectroscopic data, was substantiated by X-ray diffraction (7).

Because the structure of rosmanol-9-ethyl ether, unambiguously determinated, contained two phenolic functions with the isopropyl group in the *o*-position, the same as carnosol and carnosic acid [the natural antioxidants isolated from rosemary and sage (8)], it was to be expected for this compound to have antioxidant activity. Therefore, we tested the antioxidant property of rosmanol-9-ethyl ether and the corresponding diacetate, and the data are shown in Table 3.

At the concentration of 0.01%, the antioxidant activity

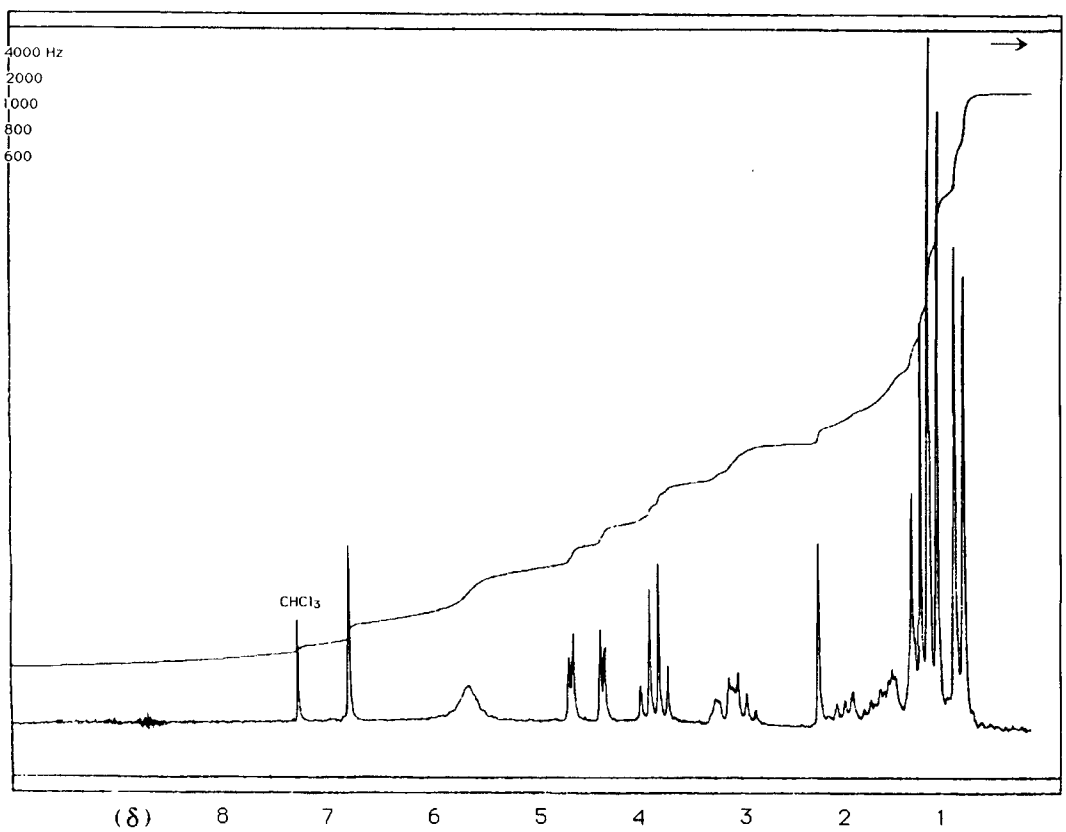

FIG. 3. NMR spectrum of rosmanol-9-ethyl ether (I).

TABLE 3

Antioxidant Properties of Rosmanol-9-ethyl ether (I) and the Corresponding Diacetate (II)

	Peroxide value (mmol O_2/kg) at 98°C			
Additive 0.01%	after 0	6	18	24 hr
None	0.92	1.33	28.40	53.60
Propyl gallate		0.85	1.18	1.44
Rosmanol-9-ethyl ether (R-9EE), (I)		1.04	1.76	1.80
Rosmanol-9-ethyl ether, diacetate (II)		1.26	32.42	55.36
Butylated hydroxy toluene (BHT)		0.95	1.73	2.27

TABLE 4

Antioxidant Property of Rosmanol-9-ethyl ether (R-9EE) and Butylated Hydroxytoluene (BHT)

Additive	Peroxide value (mmol O_2/kg) at 98°C					
	after 0	6	18	24	48	72 hr
None	0.92	1.33	28.40	53.60	>100	
BHT (0.00125%)		1.33	3.75	10.90	>100	
BHT (0.0025%)		1.23	2.50	3.95	93.3	>100
BHT (0.0050%)		1.14	1.92	2.74	70.40	>100
BHT (0.0100%)		0.98	1.55	2.20	22.40	>100
R-9EE (0.00125%)		1.09	1.71	2.07	76.00	100
R-9EE (0.0025%)		1.09	1.86	2.16	4.59	29.76
R-9EE (0.0050%)		1.16	2.02	2.27	3.19	5.53
R-9EE (0.0100%)		1.25	1.51	2.04	3.30	4.36

of rosmanol-9-ethyl ether was at least equal with that exhibited by BHT and propyl galate. The derivatization of phenolic groups resulted in the expected loss of antioxidant activity (8).

Further investigation of the antioxidant properties of rosmanol-9-ethyl ether at 0.01%, 0.005%, 0.0025% and 0.00125% concentration (Table 4) has shown that this substance, one of the antioxidant components in sage and hyssop, has activity much greater than BHT.

ACKNOWLEDGMENT

This work is financially supported by Research Fund of Serbia, Belgrade, Yugoslavia.

REFERENCES

1. Chang, S.S., B. Ostrić-Matijasević, O.A.-L. Hsieh and C.-L. Huang, *J. Food Sci. 42*:1102 (1977).
2. Bracco, U., J. Löliger and J.L. Viret, *J. Am. Oil Chem. Soc. 58*:686 (1981).
3. Wu, J.W., M.-H. Lee, C.-T. Ho and S.S. Chang, *Ibid. 59*:339 (1982).
4. Hayashi, T., M. Arisawa, T. Bandome, Y. Namose, M. Shimizu, S. Suzuki, M. Yoshizaki, M. Kawasaki, A. Fujita, H. Ueno, T. Horie, S. Wada, H. Shogawa, N. Morita, L.H. Berganza, E. Ferro and I. Basualdo, *Planta Med. 53*:394 (1987).
5. Kramer, R.E., *J. Am. Oil Chem. Soc. 62*:111 (1985).
6. Masterova, I., D. Uhrin, V. Kettmann and V. Suchy, *Chem. Rap. 43*:797 (1989).
7. Argay, Gy., A. Kalman, B. Ribar, Z. Djarmati, N. Tbt, E. Schwirtlich and R.M. Jankov, *J. Cryst. Spectr. Res. 21*:625 (1991).
8. Brieskorn, C.H., and H.-J. Dömling, *Arch. Pharm. 302*:10 (1969).

[Received January 24, 1991; accepted June 27, 1991]