THE ISOLATION OF CARNOSIC ACID 12-METHYL ETHER FROM *Salvia officinalis* L. AND NMR STUDY OF ITS METHYL ESTER

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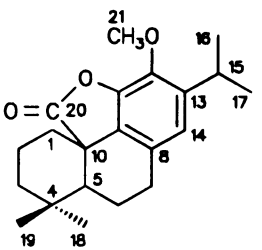
In continuation of our studies on the chemical compositions of the aerial parts of cultivated *Salvia officinalis* L., we have isolated the title substance for the first time in this type of Sage. The structure of the isolated compound was established by spectroscopic data and some chemical reactions. Investigation of ^{1}H and ^{13}C NMR, homonuclear (COSY) (^{1}H–^{1}H), heteronuclear (HETCOR) (^{1}H–^{13}C), and selective INEPT spectra, permitted the unambiguous assignment of the ^{1}H and ^{13}C NMR spectra, what gave the possibility to resolve the signals of similar natural products.

In the previous work[1] we reported the isolation and structure determination of carnosic acid 12-methyl ether-γ-lactone (*I*), which was isolated from the neutral fraction of the extract, derived from the aerial parts of the flowering *Salvia officinalis* L., cultivated in north-east of Yugoslavia (Vojvodina).

Further investigation of the acidic fractions, which was obtained by the washing of the total extract with 10% aqueous potassium carbonate, and acidifying the obtained solution with 5% hydrochloric acid (up to pH 1), led to the separation of the substance carnosic acid 12-methyl ether (*II*), which was previously described[2] as it was isolated from *Salvia lanigera*, but not from *S. officinalis* L.



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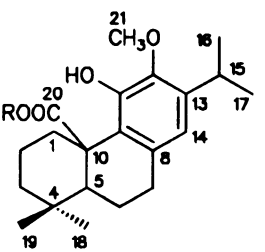

II, R = H
III, R = CH_3

EXPERIMENTAL

Infrared spectra were measured in potassium bromide pellets; wavenumbers are in cm^{-1}. NMR spectra were measured on Bruker AC 250E apparatus (250 MHz for ^1H and 62.9 MHz for ^{13}C) in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Assignments of ^{13}C NMR chemical shifts were made with the aid of APT, selective INEPT and 2D homo- and heteronuclear chemical shifts correlated techniques. For column chromatography Merck silica gel (0.063 – 0.2 mm and under 0.08 mm in 1 : 1 ratio) was used, while analytical TLC was carried out on Merck silica gel HF. UV active materials were detected visually (254 or 350 nm).

Carnosic Acid 12-Methyl Ether (*II*)

The plant material (aerial parts of the flowering *Salvia officinalis* L.) and method of extraction, as well as the separation technique are given in all details in refs[1,3]. Total CO_2 extract (20 g), obtained at 60 °C, and pressure 40.53 MPa, time 6 h, flow rate 15 kg CO_2 h^{-1} was dissolved in 250 ml of benzene, underlaid with aqueous 10% K_2CO_3 solution, two layers were stirred on the magnetic stirrer overnight, in order to avoid the emulsification. The procedure was repeated three times. The combined water layers were cooled with crushed ice, and acidified to pH 1 with 5% HCl. Turbid water layer was extracted with ether, ethereal extract was washed and dried in vacuo. The solid residue was dissolved in benzene and chromatographed.

Compound *II* was obtained as pale yellow heavy liquid in 2.17 g yield from 20 g of total CO_2 extract. IR spectrum: 3 511, 3 383, 2 949, 2 873, 2 845, 2 633, 1 694, 1 610, 1 570, 1 490, 1 461, 1 245, 1 202, 1 041, 912. ^1H NMR spectrum: 0.85 s, 3 H (3 × H-19); 0.95 s, 3 H (3 × H-18); 1.18 and 1.20 2 × d, 2 × 3 H (3 × H-16 and 3 × H-17, J = 6.9); 3.15 h, 1 H (H-15, J = 6.9); 3.60 s, 3 H (OCH_3); 6.15 s, 1 H (phenolic OH); 6.50 s, 1 H (H-14); 8.45 b, 1 H (COOH).

Methyl Carnosate 12-Methyl Ether (*III*)

A) Diazomethane in ether solution was added dropvise to the solution of *II* (1.5 g) in ether (100 ml). The progress of the reaction was monitored on TLC until disappearing of the starting compound, visualizing the mixture by spraying the plate with 2,6-dibromoquinone-4-chloroimide, and obtaining an intensive blue colour for the upper spot of compound *III*. Crude compound *III* was rechromatographed and obtained as pale yellow liquid in the yield 1.1 g (72 %). IR spectrum: 3 400, 2 963, 1 731, 1 614, 1 574, 1 413, 1 261, 1 024, 912. For ^1H NMR and ^{13}C NMR see Table I.

B) Carnosic acid 12-methyl ether γ-lactone (*I*) (120 mg, 0.37 mmol) was dissolved in methanol (20 ml), a drop of concentrated HCl was added, and heated under reflux for 5 min, monitoring the course of the reaction on TLC, giving just one product (120 mg, 99%), which was more polar than the starting substance. The product was identified as compound *III*, on the basis of identical spectra.

RESULTS AND DISCUSSION

The IR spectrum of carnosic acid 12-methyl ether (*II*) showed bands at 3 511 and 3 383 cm^{-1}, the broad band characteristic for the strongly hydrogen bound hydroxyl group in the region 3 100 – 2 500 cm^{-1}, carbonyl group band at 1 694 cm^{-1}, as well as the aromatic bands (1 610, 1 570 cm^{-1}).

The ^1H NMR of the *II* showed one proton signal only in the aromatic region at δ 6.65, indicating the presence of a highly substituted aromatic ring. Two tertiary methyls

TABLE I
^{1}H and ^{13}C NMR spectra (in $CDCl_3$) of methyl ester of carnosic acid 12-methyl ether (*III*), for other conditions see Experimental

Position	^{1}H NMR, ppm		^{13}C NMR, ppm
1	H-1α(a)	1.17 m[a]	33.9
	H-1β(e)	3.54 dt[b]	
2	H-2α(e)	2.16 m[c]	19.8
	H-2β(a)	1.53 m	
3	H-3α(a)	1.28 ddd[d]	41.2
	H-3β(e)	1.51 m	
4		–	33.6
5	H-5α(a)	1.54 m	53.9
6	H-6α(e)	2.27 m[e]	18.3
	H-6β(a)	1.82 m	
7	2 × H-7	2.85 m	31.8
8		–	134.0
9		–	125.5
10		–	47.6
11		6.01 s[f]	147.5
12		–	142.3
13		–	139.0
14		6.52 s	117.6
15		3.17 h[g]	26.2
16		1.21 d[h,i]	23.5[i]
17		1.19 d[i,j]	23.2[i]
18		0.97 s	32.4
19		0.78 s	19.9
20		–	176.1
OCH_3		3.73 s	61.3
$COOCH_3$		3.62 s	51.1

[a] $J(1\alpha,2\alpha) = 4$; [b] $J(1\beta,1\alpha) = 13.1$, $J(1\beta,2\beta) = 3$; [c] $J(2\alpha,2\beta) = 11.9$; [d] $J(3\alpha,2\beta) = 13.1$, $J(3\alpha,3\beta) = 12.5$, $J(3\alpha,2\alpha) = 4.5$; [e] $J(6\alpha,6\beta) = 12.5$; [f] signal of OH group; [g] $J(15,16) = J(15,17) = 6.9$; [h] $J(15,16) = 6.9$; [i] signals can be interchanged; [j] $J(15,17) = 6.9$.

were at δ 0.85 and 0.95, in addition to two doublets (J = 6.90 Hz), centered at δ 1.18 and 1.20 which represent six protons of the two methyls of an isopropyl group. A single proton heptet at δ 3.15 (J = 6.90 Hz) was assigned to H-15. Further evidence for the proposed structure came from the methylation of *II* to give methyl ester of carnosic acid 12-methyl ether (*III*). Its IR spectrum showed bands at 3 400 cm^{-1} (for the OH group), at 1 731 cm^{-1}, (for carbomethoxy group), as well as two aromatic bands (1 610, 1 570 cm^{-1}), while no broad peaks were observed in the region 3 100 – 2 500 cm^{-1}.

The same methyl ester *III* was also obtained synthetically, and, thus, chemically correlated with the compound of the known structure, by acid-catalyzed methanolysis of lactone *I*, whose structure was unambiguously assigned by X-ray crystallography[1].

Methyl ester of carnosic acid 12-methyl ether (*III*) has not been described in the literature so far, although some similar derivatives of carnosic acid such as carnosic acid 12-methyl ether, methyl carnosoate, and methyl ester of carnosic acid 11,12-dimethyl ether have been studied recently[2,4]. However, the NMR spectral assignment for these compounds are partial (^{1}H NMR) and the the assignments for ^{13}C NMR were

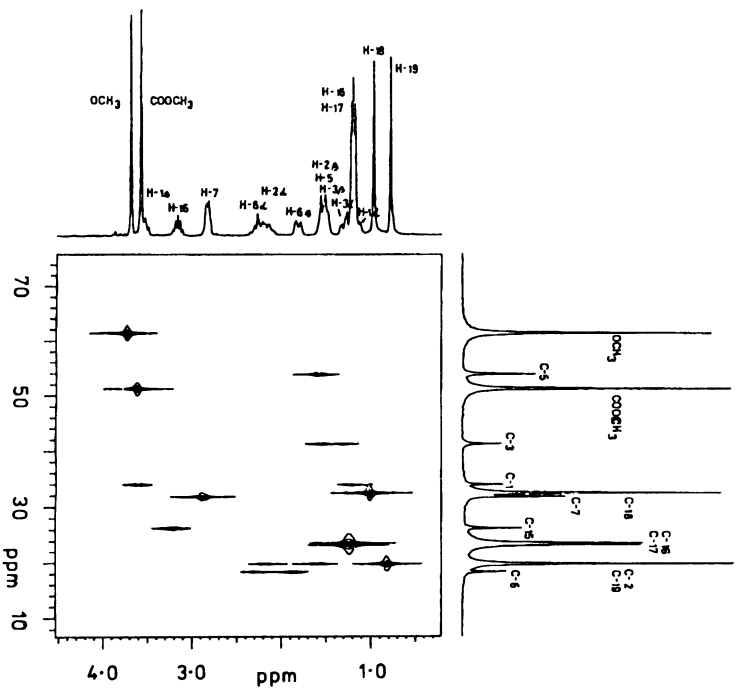

FIG. 1
Heterocorrelated ^{1}H–^{13}C 2D NMR spectrum of methyl ester of carnosic acid 12-methyl ether (*III*); aromatic region has not been shown

made by empirical correlation with similar structures, which are correct in general, but leaves the space for the reasonable doubts.

Using the abundance of the isolated pure compound *II*, and the simple reaction for obtaining *III*, as well as the advanced NMR instrumentation, in order to have a complete picture of the assignments of all C and H atoms in this type of molecules we have studied these molecules in details.

In our study the special attention has been paid to these non-aromatic C-atoms which are the "lower part" of the tricyclic system (rings A and B), namely carbons: C-1, C-2, C-3, C-5, C-6, C-7. Unambiguous assignments for carbons (see Table I) were made by the use of different ^{13}C NMR technique, such as APT experiment, two-dimensional proton–carbon chemical shift correlation and selective INEPT[5] spectroscopy. The selective INEPT experiment has been utilized for the selective enhancement of signals for carbons which are (three bonds) away from the irradiated proton. Magnetization transfer via irradiation of H-7 (at δ 2.85) resulted in enhancements at δ 18.3, 53.9, 117.6, 125.5, and 134.0, what could be assigned to C-6, C-5, C-14, C-9, and C-8, respectively. Irradiation of H-1β at δ 3.54 (what also affected the $COOCH_3$ at 3.62) enhanced the carbons at δ 20.0, 41.2, 47.6, 53.9, and 176.1, which could be assigned to C-2, C-3, C-10, C-5, and C-20, respectively.

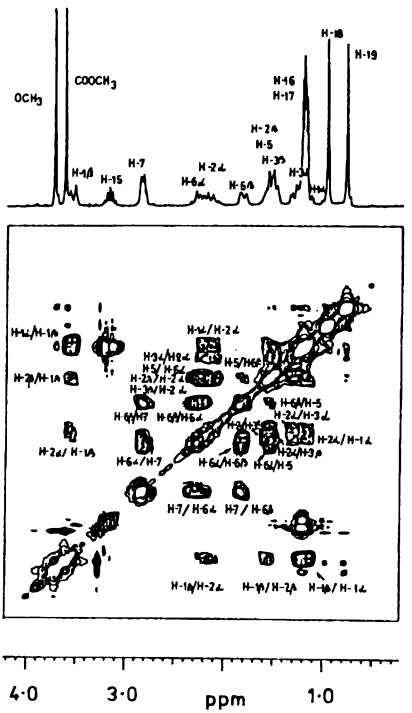

FIG. 2
250 MHz COSY spectrum of methyl ester of carnosic acid 12-methyl ether (*III*)

The heteronuclear chemical shift correlation 2D spectrum (^1H–^{13}C correlation, Fig. 1) clearly showed the signals for the protons H-1, H-2, H-3, H-5, H-6, and H-7, and it is obvious that the protons at δ 3.54 and 1.17 are satellite of C-1 (δ 33.9), whereas the protons at 2.85 are bound to C-7 (δ 31.8).

The chemical shifts difference of δ approx. 2.4 ppm for H-1 protons is expected. Rather deshielded position of H-1β (equatorial) at δ 3.54 can be due to its forced coplanarity with the aromatic C-ring and its close proximity to the oxygen lone pairs of the C-11 hydroxyl group, the phenomenon known as "rabbit ear effect" (ref.[6]). Similar "dramatic" deshielding has been observed for C-11 oxygenated diterpenes with methyl group at C-10 (ref.[7]).

The other values are within the limits of the normal, expected values, according to their axial or equatorial orientation. The narrow signal (within 10 Hz) for two C-7 protons can be explained by the deshielding of the aromatic ring C, which affects equally protons H-7α and H-7β, if the ring B is in the supposed half-chair conformation.

Homonuclear COSY spectrum (^1H–^1H) correlation (Fig. 2) confirms the conclusions drawn from the ^1H–^{13}C correlation, and clearly shows the interaction within two groups of protons, one group consisting of ring A protons, and the other consisting of ring B protons.

The data presented above and series of experiments of the selective homodecoupling of the examined protons enables us the complete assignments of all protons in NMR spectrum (Table I).

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