COMPONENTS OF THE WOOD-ROTTING FUNGUS *Sarcodontia setosa**

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From the wood-rotting fungus *Sarcodontia setosa* (PERS.) DONK a mixture of benzoquinone acids was obtained which was converted to methyl esters *Ia*—*IIIa* and separated by preparative HPLC. The presence of the known sarcodontic acid (*II*), *i.e.* 15-(6-methoxy-1,4-benzoquinon-2-yl)-2-pentadecenoic acid, and further of 15-(6-methoxy-1,4-benzoquinon-2-yl)pentadecanoic acid (*I*) and 15-(6-methoxy-1,4-benzoquinon-2-yl)-2,5-pentadecadienoic acid (*III*) was proved in the mixture. The structures were determined by spectral methods.

During the isolation of sarcodontic acid (*II*), *i.e.* 15-(6-methoxy-1,4-benzoquinon-2-yl)-2-pentadecenoic acid from the wood-rotting fungus *Sarcodontia setosa* (PERS.) DONK[1,2] was detected in it together with two so far unknown acids, *I* and *III*. The aim of this study was the isolation and the structure determination of these compounds.

Extraction of dry, ground fruit bodies of the fungus with benzene, further extraction with diethyl ether and crystallization from acetone gave a mixture of acids *I*—*III* which were converted to corresponding methyl esters *Ia*—*IIIa* with diazomethane (method *A*). This mixture was separated by HPLC on silica gel. The proportion of individual components is 10% of *Ia*, 45% of *IIa* and 45% of *IIIa*. On hydrogenation of the mixture of the acids *I*—*III* (method *B*), subsequent oxidation with ferric chloride and methylation with diazomethane, methyl ester *Ia* was obtained.

The structure of compounds *Ia*—*IIIa* was confirmed by elemental analysis and spectroscopic methods (^{1}H NMR, MS, IR, UV). On the basis of mass spectrometry the values of molecular ions were obtained: *Ia* *m/z* 392/394, *IIa* 390/392, *IIIa* 388/390. The two close values may be explained by partial reduction of benzoquinones to quinols in the mass spectrometer. The structure of the reduced form of the benzoquinone nucleus was confirmed by fragments *m/z* 154, 153.

In the infrared spectrum the quinone moiety is characterized by the wave number 1 602, 1 651 and 1 681 cm^{-1}. In the case of the stretching vibrations of the carbonyl group of the esters a shift to lower frequencies takes place with the increase of the number of the conjugated double bonds in the side chain (*Ia* 1 740, *IIa* 1 726, *IIIa*

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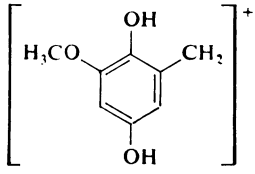
1 721 cm^{-1}). In the UV spectrum compounds *Ia*–*IIIa* are characterized by two maxima. The absorption maximum of the fragment of compound *IIa*, —CH=CH—CH=CH—$COOCH_3$, lies under the limit of 208 nm and the increase in the extinction coefficient, in comparison with *Ia* and *IIIa*, corresponds to tabulated data (CH_3—CH=CH—CH=CH—COOH λ_{max} = 205 nm, ε = 14·000; *IIa* 18 810; *Ia* 7200; *IIIa* 8 630 l . mol^{-1} cm^{-1}) (ref.[3]). The tabulated values of the fragment of compound *IIIa*, —CH=CH—CH=CH—CH=CH—$COOCH_3$, correspond to the measured values (CH_3—CH=CH—CH=CH—CH=CH—COOH λ_{max} = 259 nm, ε = 20 000 l mol^{-1} cm^{-1}; *IIIa* 265, 45 920; *IIa* 269, 15 000; *Ia* 269, 12 750) (ref.[3]). ^{1}H NMR spectroscopy (Table I) confirmed the number of double bonds in the side chain of the benzoquinone nucleus and indicated their position and configuration. In compound *IIIa* there were two double bonds conjugated with the carboxyl group, in configuration *trans-trans*



m/e 153

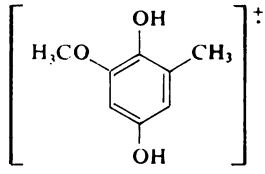


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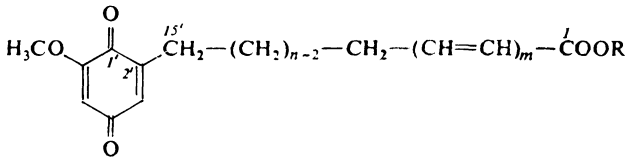
TABLE 1

^{1}H NMR parameters of compounds *Ia*—*IIIa*

Proton	Chemical shifts[a]			Coupling constants[a]	*Ia*	*IIa*	*IIIa*[b]
	Ia	*IIa*	*IIIa*[b]				
$H_{(2)}$	2·30	5·81	5·78	$J_{2,3}$	7·6	15·2	15·2
$H_{(3)}$	[c]	6·97	7·59	$J_{2,4}$	[c]	1·8	[c]
$H_{(4)}$	[c]	2·19	6·03	$J_{3,4}$	[c]	7·0	11·0
$H_{(5)}$	[c]	[c]	5·83	$J_{4,5}$	[c]	7·0	15·4
$H_{(6)}$	[c]	[c]	1·96	$J_{5,6}$	[c]	[c]	7·0
$H_{(15)}$	2·43	2·42	2·28	$J_{6,7}$	[c]	[c]	7·0
$H_{(3')}$	6·48	6·48	6·38	$J_{14,15}$	7·6	7·2	6·5
$H_{(5')}$	5·87	5·87	5·56	$J_{3',5'}$	2·4	2·4	2·3
$COOH_3$	3·66	3·72	2·93				
OCH_3	3·81	3·81	3·57				

[a] Chemical shifts in ppm, tetramethylsilane used as internal standard, coupling constants in Hz; [b] spectrum measured in deuteriobenzene; [c] position of signals not determined.

($J_{2,3}$ = 15·2; $J_{4,5}$ = 15·4 Hz), in compound *IIa* one conjugated double bond in configuration *trans* ($J_{2,3}$ = 15·2 Hz). The side chain in the quinone *Ia* is saturated.



I, $n = 14$, $m = 0$, R = H *Ia*; $n = 14$, $m = 0$, R = CH_3
II, $n = 12$, $m = 1$, R = H *IIa*; $n = 12$, $m = 1$, R = CH_3
III; $n = 10$, $m = 2$, R = H *IIIa*; $n = 10$, $m = 2$, R = CH_3

EXPERIMENTAL

The temperature data are not corrected. The melting points were measured on a Boetius block. Samples for analysis were dried in a 13—1·3 Pa vacuum for 5—10 h, over phosphorus pentoxide. The mass spectra were measured on an AE I MS 902 instrument, the infrared spectra (in chloroform) on a UR-20 and the UV spectra (in methanol) on a Specord UV-VIS spectrophotometer, and the ^{1}H NMR spectra (in deuteriochloroform) on a Varian XL-200 instrument, using tetramethylsilane as internal reference. HPLC was carried out on an apparatus composed of a linear injector 4003 (Laboratory Apparatus, Prague), a dosing valve (Knauer), a UV detector UVM-4 (Developmental Workshops of the Czechoslovak Academy of Sciences) and a recorder TZ 4 100 (Laboratory Apparatus, Prague). For column chromatography silica gel Silpearl (Kavalier, Votice) deactivated with 13% of water was used, for HPLC silica gel Si VKS 8 (Laboratory Apparatus, Prague). The fungus *Sarcodontia setosa* was identified by Dr F. Kotlaba, Botanical Institute, Czechoslovak Academy of Sciences, Prague[4].

Mixture of Acids *I*—*III*

The fruit bodies of the fungus were collected in September of 1980 in the surroundings of Kosmonosy (Mladá Boleslav region). They were dried at room temperature, ground (1 410 g) and extracted with benzene (20 l). The solvent was evaporated in a vacuum and the residue (32·1 g) extracted with diethyl ether until the dark components had disappeared. After filtration the yellow residue (11·0 g) was crystallized from acetone (4·9 g, m.p. 112—116°C).

Methyl Ester *Ia*

A mixture of acids *I*—*III* (0·750 g; method *A*) was dissolved in toluene (15 ml) and an ethereal diazomethane solution (4·7 ml; 17·8 mg/ml) was added dropwise under cooling with ice. After five min standing the solution was evaporated under reduced pressure (0·759 g) and the residue purified by column chromatography on silica gel (100 g, 5—15% of ethyl acetate in toluene), affording 0·582 g of a mixture of *Ia*—*IIIa*. 0·160 g of it was separated by HPLC on silica gel (12·7 × 500 mm; 0·5% 2-propanol in hexane; detection at 283 nm; 8 ml/min; 10 MPa). Yield, 0·0147 g of crude *Ia* (K = 12·8), after purification by HPLC 0·0047 g; yellow crystals, m.p. 104—106°C. For $C_{23}H_{36}O_5$ (392·5) calculated: 70·38% C, 9·24% H; found: 69·73% C, 9·12% H. UV spectrum: λ_{max} = 269, 366 nm; ε = 2 750, 730 l mol^{-1} cm^{-1}. IR spectrum: $COCH_3$ 1 740, quinone skeleton 1 602, 1 651, 1 681 cm^{-1}. Mass spectrum m/z (% r.i.): 394/392 (32·3/100),

362/360 (42·0/46·2), 334/332 (1·6/3·2), 193 (12·9), 179 (12·9), 166 (6·5), 154 (45·2), 153 (51·6), 95 (6·5), 81 (6·5), 74 (6·5), 69 (11·3), 67 (8·1), 59 (6·5), 55 (22·6), 43 (12·9), 41 (19·4), 29 (6·5), 28 (17·8).

The mixture of acids *I*—*III* (0·472 g; method *B*) was dissolved in a toluene–acetic acid mixture (32 + 4 ml) and hydrogenated under catalysis with 10% palladium on charcoal (0·049 g) at room temperature for 1·5 h. The mixture was filtered under suction through a layer of kieselguhr (diatomaceous earth) on a fritted filter, and washed with three 5 ml portions of hot toluene. A 10% aqueous chloride solution (21 ml) was added to the filtrate and the mixture was stirred at 30 to 40°C for 3 h. The layers were then separated, the aqueous layer extracted twice with 3 ml portions of toluene and the combined organic layers were dried over magnesium sulfate. After filtration the solvent was evaporated in a vacuum. The residue (0·443 g) was dissolved in 35 ml of toluene and an ethereal diazomethane solution (3 ml; 16·5 mg/ml) was added to it under cooling with ice. After 5 min standing the solution was evaporated (0·458 g). 0·075 g of the crude product were purified by column chromatography on silica gel (25 g; 5% ethyl acetate in tetrachloromethane; 5 ml/min). Yield, 0·074 g of *Ia*; yellow crystals; m.p. 108—109°C. For $C_{23}H_{36}O_5$ (392·5) calculated: 70·38% C, 9·24% H; found: 70·62% C, 9·26% H. Mass spectrum *m/z* (% r.i.): 394/392 (19·6/$C_{23}H_{36}O_5$ 25), 362/360 (42·9/$C_{22}H_{32}O_5$ 42·9), 334/332 (5·4/$C_{21}H_{32}O_3$ 10·7), 193 ($C_{11}H_{13}O_5$ (10·7), 179 ($C_{10}H_{11}O_3$ 10·7), 166 ($C_9H_{10}O_3$ 12·5), 154 (71·4), 153 ($C_8H_9O_3$ 48·2), 28 (100).

Methyl Ester *IIa*

A mixture of *Ia*—*IIIa* (0·160 g) was separated by HPLC on silica gel (see methyl ester *Ia*). Yield, 0·0588 g of a crude product (K = 14·5). After purification by HPLC, yield 0·0243 g, yellow crystals m.p. 91—92°C. For $C_{23}H_{34}O_5$ (390·5) calculated: 70·74% C, 8·78% H; found: 70·92% C, 8·79% H. UV spectrum: λ_{max} = 269, 367 nm; ε = 15 000, 840 l mol^{-1} cm^{-1}. IR spectrum: $COOCH_3$ 1 726, quinone skeleton 1 602, 1 651, 1 681 cm^{-1}. Mass spectrum *m/z* (% r.i.): 392/390 (26·5/100), 360/358 (17·6/94·1), 332/330 (8·8/8·8), 193 (11·8), 179 (11·8), 166 (8·8), 154 (52·9), 153 (64·7), 123 (5·9), 121 (5·9), 109 (5·9). 107 (7·4), 95 (10·3), 93 (5·9), 81 (20·6), 69 (11·8), 67 (14·7), 59 (5·9), 55 (23·5), 53 (7·4), 43 (8·8), 39 (5·9), 29 (7·4), 28 (10·3).

Methyl Ester *IIIa*

A mixture of *Ia*—*IIIa* (0·160 g) was separated by preparative HPLC on silica gel (see methyl ester *Ia*). Yield 0·0747 g of a crude product (K = 16·5). After purification by HPLC, yield 0·0226 g, yellow crystals, m.p. 94—96°C. For $C_{23}H_{32}O_5$ (388·5) calculated: 71·11% C, 8·30% H; found: 71·07% C, 8·36% H. UV spectrum: λ_{max} = 265, 376 nm, ε = 45 920, 870 l mol^{-1} cm^{-1}. IR spectrum: $COOCH_3$ 1 721, quinone skeleton 1 602, 1 651, 1 681 cm^{-1}. Mass spectrum *m/z* (% r.i.): 390/388 (16·1/45·2), 358/356 (35·5/100), 330/328 (3·2/1·6), 193 (6·5), 179 (6·5), 166 (9·7), 154 (58·1), 153 (67·7), 152 (6·5), 147 (9·7), 133 (16·1), 119 (12·9), 111 (12·9), 107 (29), 95 (9·7), 94 (9·7), 93 (9·7), 91 (9·7), 81 (19·4), 79 (16·1), 77 (8·1), 69 (12·9), 67 (19·4), 66 (8·1), 65 (6·5), 57 (12·9), 53 (12·9), 43 (8·1), 41 (25·8), 39 (9·7), 29 (8·1), 28 (14·5), 27 (6·6).

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