TWO ANTIBIOTIC BENZOQUINONE–HYDROQUINONE PAIRS FROM THE PYRENOMYCETE *Camarops microspora* (KARST.) SHEAR

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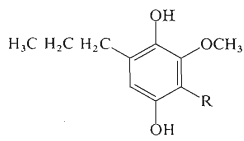
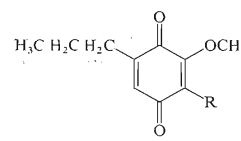
Structures of two antibacterial quinones from the culture medium of the fungus *Camarops microspora* (KARST.) SHEAR were determined. One substance is identical with 2-methoxy-6-(1-propyl)--1,4-benzoquinone (*III*) and the other substance is the 3-methoxy derivative (*IV*) of compound *III*. In addition to substances *III* and *IV*, the corresponding hydroquinones *I* and *II* were isolated and identified. The identity of substances *I*—*IV*, hitherto unobserved in nature, was established by spectral methods.

In assays on submersion cultures of pyrenomycetes (family *Ascomycetes*) with respect to the presence of antibiotical metabolites, Bandre and Šašek[1] observed a marked antibacterial activity in the culture medium of the fungus *Camarops microspora* (KARST.) SHEAR. The corresponding antibiotics have been now isolated and characterised on the basis of chromatography and spectral analysis. By means of thin--layer chromatography (solvent system S_1) of the ethereal extract of the cultivation liquid, four antibiotic compounds were separated possessing the hR_F values 10 (compound *I*), 24 (*II*), 51 (*III*), and 67 (*IV*). After purification, compounds *I*—*IV* inhibited in our tests the growth of *Bacillus subtilis* in concentrations of 40 μg/ml.



I, R = H
II, R = OCH_3



III, R = H
IV, R = OCH_3

As indicated by NMR (Table I), related compounds are present in the extract. Thus, compounds *I*—*IV* exhibit the presence of a *n*-propyl group and six-membered

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ring formed by sp^2-hybridised carbon atoms. One methoxyl group is present in compounds *I* and *III* and two methoxyl groups are present in compounds *II* and *IV*.

Compounds *III* and *IV* ($C_{10}H_{12}O_3$ and $C_{11}H_{14}O_4$) are quinones since their IR spectra contain ν(C=O) bands, the mass spectrum exhibits an intensive M + 2 peak[2,3], and the ^{13}C-NMR spectrum shows two signals in the 180–195 ppm region. As inferred from the chemical shift of these signals, compounds *III* and *IV* are *para*-

TABLE I

The ^{1}H- and ^{13}C-NMR Spectra of Compounds *I*–*IV*

Deuteriochloroform as solvent, hexamethyldisiloxane as internal standard (δ = 0·06 ppm), chemical shifts in the δ scale, coupling constants *J* in Hz (in parentheses), multiplicity: s singlet, d doublet, t triplet, q quartet, mt multiplet.

Nucleus	Assignment	*I*	*II*	*III*	*IV*
^{1}H	CH_3	0·94 t	0·87 t	0·90 t	0·90 t
		(6·5)	(7·3)	(7·3)	(7·3)
	β-CH_2	1·63 mt	1·47 mt	1·42 mt	1·40 mt
	α-CH_2	2·56 dd	2·48 t	2·35 t	2·34 t
		(6·8; 6·5)	(7·3)	(7·3)	(7·3)
	OCH_3	3·84 s	3·81 s	3·75 s	3·93 s
		—	3·84 s	—	3·95 s
	OH	—	5·11 s, 2 H	—	—
	H-3	6·31 d[d]	—	5·82 d	—
		(2·7)	—	(2·4)	—
	H-5	6·23 d	6·42 s	5·41 dt	6·31 t
		(2·7)	—	(2·4; 1·5)	(1·5)
^{13}C[c]	CH_3	13·6 q	13·8 q	13·5 q	13·6 q
	β-CH_2	22·7 t	22·6 t	20·8 t	20·9 t
	α-CH_2	31·7 t	31·4 t	30·4 t	30·4 t
	OCH_3	55·9 q	60·5 q[a]	56·0 q	61·1 q[a]
	C-1	128·8 s[b]	123·9 s[b]	187·4 s	184·0 s
	C-2	137·4 s[b]	139·9 s[b]	158·6 s	144·9 s
	C-3	97·2 d[e]	136·9 s[b]	106·9 d	144·5 s
	C-4	146·7 s[b]	138·9 s[b]	193·2 s	184·2 s
	C-5	108·0 d[e]	110·3 d	132·8 d	130·3 d
	C-6	148·3 s[b]	141·4 s[b]	147·0 s	147·5 s

[a] Two carbon atoms (determined by gated decoupling which suppresses NOE (ref.[14]). [b] Assignments in the column are interchangeable. [c] The multiplicity determined by SFORD (Single Frequency Off-Resonance Decoupling). [d] Assigned on the basis of NOE with a methoxy group. [e] Assigned by correlation with ^{1}H-NMR by means of J_r obtained by SFORD.

-benzoquinone derivatives[4]. Coupling constant of aromatic protons in *III* unequivocally indicates the *meta* position of substituents and thus the structure of a 2-methoxy-6-(1-propyl)-1,4-benzoquinone (*III*). The structure *IV* was determined on the basis of an *ortho*-benzylic coupling of the aromatic proton with protons of the CH_2 group in the side chain[5] (established by double resonance) and on the basis of an undecoupled ^{13}C-NMR spectrum in which C-5 affords a doublet of a triplets with ^3J from CH_2 protons in the propyl group[6]. This spectrum also permits an unambiguous assignment of all signals since $^2J_{C,H}$ is equal to zero with quinones[7]. Consequently, compound *IV* is a 3-methoxy derivative of compound *IV*.

Compounds *I* and *II* ($C_{10}H_{14}O_3$ and $C_{11}H_{16}O_4$) are readily air-oxidised with the formation of quinones *III* and *IV*. The IR spectra of *I* and *II* lack the ν(C=O) bands but exhibit the ν(OH) bands. The presence of two exchangeable hydrogen atoms is indicated by labelling with perdeuteriomethanol in the ion source of a mass spectrometer. The ^{1}H- and ^{13}C-NMR spectral data are in accordance with structures of 2-methoxy-6-(1-propyl)hydroquinone (*I*) and the 3-methoxy derivative (*II*) of compound *I*.

The proposed structures *I* and *III* correspond to known compounds[8] which, however, have not been spectrometrically characterised except for the UV absorption. From natural sources, about ninety benzoquinones have been isolated, approximately one third from fungi[9,10]. The natural occurrence of compounds *I* to *IV* has not been so far reported. Of a special interest is the demonstrated plastoquinone activity[11] of the synthetic compound *III*. The pairs *I* and *III*, or, *II* and *IV* could thus with *Camarops microspora* represent an efficient redox system participating on the transfer of electrons. The hydroquinones appear to be produced primarily into the cultivation medium[12].

From the related fungus pyrenomycete *Nectaria coryli*, another benzoquinone–hydroquinone pair has been isolated[10], antibiotically active against *Staphylococcus aureus*. A closely related to quinones from *Camarops microspora* is primine, 2-(1-pentyl)-6-methoxy-1,4-benzoquinone, detected in *Primula obconica* HANCE (ref.[13]).

EXPERIMENTAL

Melting points (uncorrected) were taken on a heated microscope stage (Kofler block). The UV-VIS spectra were recorded in ethanol on a Cary 118 C apparatus. The IR spectra were taken in chloroform on a UR-20 (Carl Zeiss, Jena) spectrometer. The ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Jeol FX-60 apparatus under conditions shown in Table I. Molecular weights were determined by mass spectrometry on a Varian MAT 311 apparatus; relative peak intensities (%) and formulae are given in parentheses after the *m/e* values. The thin-layer chromatography was performed on ready-for-use Silufol UV_{254} or the thick-layer Silufol 20 UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent systems S_1, tetrachloromethane–methanol (100 : 5), and S_2, benzene. The spots of antibiotics were detected by a biological

technique (the chromatogram was pressed to an agar-agar plate inoculated with *Bacillus subtilis*) and viewing under UV light.

The laboratory *Camarops microspora* (KARST) SHEHR culture was obtained from the fungal collection of the Institute of Botany, Charles University, Prague. The submersion cultivation was performed in an 18-litre laboratory fermentor in a worth medium, pH 5·5 (10 l), with a 4% inoculant in the same medium. The extracellular antibiotic activity was checked in the course of the cultivation by a diffuse plate test with *Bacillus subtilis*.

Isolation

The cultivation liquid from a 15-day culture (5·6 l) was concentrated under diminished pressure on a rotatory evaporator at 35°C to the consistence of a sirup which was triturated with 350 g of a cellulose powder and extracted in two portions with ethanol (three 1 000 ml portions) at room temperature. The extracts were combined and evaporated. The resulting thick sirup was dissolved in water (400 ml) and the aqueous solution extracted with three 400 ml portions of ether, the last extraction being performed at pH 2 (aq. HCl). The combined ethereal extract was taken down and the residual antibiotic components (1·1 g) separated and purified by repeated chromatography on thin-layer silica gel sheets in the solvent system S_1. The developed sheets were cut into strips while moist and the antibiotics extracted immediately with ether from the appropriate strips. The hR_F 51 fraction contained in addition to the benzoquinone derivative *III* a substantial amount of a non-antibiotic aromatic compound (not identified) which was separated by rechromatography in S_2. The ethereal extracts were separately evaporated and the antibiotics subjected to a further purification by crystallisation.

2-Methoxy-6-(1-propyl)-hydroquinone (*I*)

The hR_F 10 substance crystallised from 10 : 1 light petroleum–chloroform; yield, 48 mg. Recrystallisation from light petroleum (b.p. 70—75°C) afforded compound *I*, m.p. 104—106°C (reported[8], m.p. 106°C). UV spectrum: λ_{max} 290 nm (log ε 3·56). IR spectrum: 3 605 (free OH), 3 555 and 3 450 (intramolecular and intermolecular hydrogen bond), 1 609 and 1 500 cm^{-1} (C=C). Mass spectrum: 182 (98, $C_{10}H_{14}O_3$, M^+), 167 (10, $C_9H_{11}O_3$), 154 (47, $C_8H_{10}O_3$), 153 (100, $C_8H_9O_3$), 139 (17, $C_7H_7O_3$), 125 (28, $C_6H_5O_3$, $C_7H_9O_2$), 111 (6, $C_6H_7O_2$), 110 (9, $C_6H_6O_2$), 107 (15, C_7H_7O), 69 (19, C_4H_5O, C_5H_9). For $C_{10}H_{13}O_3$ (182·22) calculated: 65·92% C, 7·74% H; found: 66·20% C; 7·64% H.

2,3-Dimethoxy-6-(1-propyl)-hydroquinone (*II*)

The ethereal extract of the appropriate substance (hR_F 24) was evaporated to the consistence of a sirup which solididified. The solid was recrystallised from light petroleum–ether to afford 53 mg of compound *II*, needles, m.p. 88—89°C. UV spectrum: λ_{max} 290 nm (log ε 3·58). IR spectrum: 3 545 and 3 450 (OH), 1 600 and 1 503 cm^{-1} (C=C). Mass spectrum: 212 (59, $C_{11}H_{16}O_4$ M^+), 197 (7, $C_{10}H_{13}O_4$), 183 (100, $C_9H_{11}O_4$), 169 (6, $C_8H_9O_4$), 168 (7, $C_8H_8O_4$), 155 (5, $C_7H_7O_4$, $C_8H_{11}O_3$), 150 (3, $C_8H_6O_3$), 147 (2, $C_9H_7O_2$), 140 (5, $C_7H_8O_3$), 137 (6, $C_7H_5O_3$, $C_8H_9O_2$). For $C_{11}H_{16}O_4$ (212·25) calculated: 62·25% C, 7·60% H; found: 62·19% C, 7·20% H.

2-Methoxy-6-(1-propyl)-1,4-benzoquinone (*III*)

The crystalline yellow antibiotic substance (hR_F 51) was obtained in 44 mg yield from light petroleum–chloroform. Recrystallisation from light petroleum afforded compound *III*, m.p. 77—78°C

(reported[8], m.p. 78—79°C). UV-VIS spectrum: λ_{max-1} 267 nm (log ε 4·14), λ_{max-2} 364 nm (log ε 2·95); reported[8], λ_{max} 266 nm (log ε 4·25). IR spectrum: 1 659 (C=O) 1 603 cm^{-1} (C=C). Mass spectrum: 182 (34, $C_{10}H_{14}O_4$, M^+ +2), 180 (100, $C_{10}H_{12}O_3$,M^+), 165 (21, $C_9H_9O_3$), 163 (6, $C_{10}H_{11}O_2$), 153 (24, $C_8H_9O_3$), 152 (22, $C_9H_{12}O_2$), 148 (11, $C_9H_8O_2$), 137 (41, $C_8H_9O_2$), 121 (23, $C_7H_5O_2$, C_8H_9O), 120 (17, C_8H_8O), 109 (22, $C_6H_5O_2$, C_7H_9O), 95 (17, C_6H_7O), 91 (19, C_7H_7), 69 (82, C_4H_5O, C_5H_9).

2,3-Dimethoxy-6-(1-propyl)-1,4-benzoquinone (*IV*)

The hR_F 67 antibiotic was purified by distillation under diminished pressure in a Hickman flask and isolated in 135 mg yield as a brownish red viscous liquid *IV*, b.p. 80—81°C/6·7 Pa. UV-VIS spectrum: λ_{max-1} 267 nm (log ε 4·13), λ_{max-2} 402 nm (log ε 2·90). IR spectrum 1 659 (C=O), 1 603 cm^{-1} (C=C). Undecoupled ^{13}C-NMR spectrum: 184·2 (s, C-4), 184·0 (dt, ^2J = = 11·7 Hz, ^3J = 3·9 Ht, C-1), 147·5 (dt, ^2J = 8·8 and 2·9 Hz, C-6), 144·4 (q, ^3J = 2·9 Hz, C-2), 144·5 (mt, ^3J = 2·8 and 2·9 Hz, C-3), 130·3 (dt, ^1J = 167·0 Hz, ^3J = 5·9 Hz, C-5). Mass spectrum: 212 (21, $C_{11}H_{16}O_4$, M^+ +2), 210 (100, $C_{11}H_{14}O_4$, M^+), 195 (26, $C_{10}H_{11}O_4$), 192 (7, $C_{11}H_{12}O_3$), 183 (32, $C_9H_{11}O_4$), 181 (28, $C_9H_9O_4$, $C_{10}H_{13}O_3$), 177 (16, $C_{10}H_9O_3$), 167 (23, $C_8H_7O_4$, $C_9H_{11}O_3$), 165 (45, $C_9H_9O_3$), 163 (35, $C_9H_7O_3$), 153 17, $C_8H_9O_3$), 139 (16, $C_7H_7O_3$, $C_8H_{11}O_2$), 137 (23, $C_7H_5O_3$, $C_8H_9O_2$), 135 (11, $C_8H_7O_2$), 125 (9, $C_6H_5O_3$, $C_7H_9O_2$), 123 (17, $C_6H_3O_3$, $C_7H_7O_2$), 121 (10, $C_7H_5O_2$, C_8H_9O), 111 (28, $C_6H_7O_2$, $C_6H_{11}O$,C_8H_{15}). For $C_{11}H_{14}O_4$ (210·23) calculated: 62·85% C, 6·71% H; found: 62·79% C, 6·44% H.

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REFERENCES

1. Bandre T. R., Šašek V.: Folia Microbiol. (Prague) *22*, 1977 (in press).
2. Budzikiewicz H., Djerassi C., Williams D. H.: *Mass Spectrometry of Organic Compounds*, p. 527. Holden-Day, San Francisco 1967.
3. Thomson R. H.: *Naturally Occurring Quinones*, II. Ed., p. 39. Academic Press, London and New York 1971.
4. Berger S., Rieker A.: Tetrahedron *28*, 3123 (1972).
5. Barfield M., Chakrabarti B.: Chem. Rev. *69*, 757 (1969).
6. Karliner J., Rodebaugh R.: Tetrahedron Lett. *1975*, 3783.
7. Höfle G.: Tetrahedron *32*, 143 (1976).
8. Dean F. M., Osman A. M., Robertson A.: J. Chem. Soc. *1955*, 11.
9. Ref. 3, p. 93.
10. Nair M. S. R., Anchel M.: Tetrahedron Lett. *1972*, 795.
11. Redfearn E. R., Friend J.: Biochem. J. *84*, 34P (1962).
12. Pettersson G.: Acta Chem. Scand. *20*, 45 (1966).
13. Schildknecht H., Bayer I., Schmidt H.: Z. Naturforsch. *22b*, 36 (1967).
14. Freeman R., Hill H. D. W.: J. Magn. Resonance *5*, 278 (1971).

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