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PHENOLIC MEROTERPENOIDS FROM THE BASIDIOMYCETE *ALBATRELLUS OVINUS*

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Key Word Index—*Albatrellus ovinus*; *Polyporus ovinus*; Basidiomycetes; meroterpenoids; farnesylresorcinols; structural determination.

Abstract—From the lipophilic fraction of fresh *Albatrellus ovinus*, the following substances were isolated and identified: *E,E*-5-methyl-2-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1,3-benzenediol (grifolin), *E,E*-5-methyl-2-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1,3-dimethoxybenzene, *E,E*-5-methyl-2-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1-hydroxy-3-methoxybenzene, *E,E*-5-methyl-4-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1,3-benzenediol (neogrifolin), and *E,E*-5-methyl-4-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1,3-dimethoxybenzene.

INTRODUCTION

The metabolites of *Albatrellus ovinus* (Schaeff. ex Fr.) Kotl et Pouz. [*Polyporus ovinus* (Schaeff. ex Fr.)] have not been previously examined to our knowledge.

RESULTS AND DISCUSSION

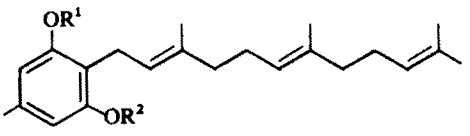
The lipophilic fraction of the acetone extract of fresh *Albatrellus ovinus* collected in the neighbourhood of Zbiroh in Bohemia (Czechoslovakia), contained as main components two closely related antibiotically active phenolic compounds (64 and 22%).

The less polar compound **1**, mp 41–43°, $C_{22}H_{32}O_2$ (MS), exhibited in the IR spectrum bands of hydroxylic functions (3430 and 1043 cm^{-1}) and aromatic bands (1815, 1631, 1590 cm^{-1}). The presence of two hydroxyls was established by acetylation with the formation of the diacetate **2** (oil), $C_{26}H_{36}O_4$ (MS), IR: 1770, 1370, 1197 (AcO—arom), 1482 and 1580 cm^{-1} (arom.), and by methylation to afford the dimethoxy derivative **3** (oil), $C_{24}H_{36}O_2$ (MS), which shared IR absorption bands 1118 cm^{-1} (C—O—C) and 1492, 1566 and 1608 cm^{-1} (arom.).

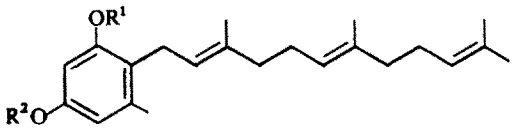
The ^{1}H-NMR spectrum of **1** indicated the presence of two equivalent aromatic protons (δ 6.22, *bs*, 2H), a methyl group on the aromatic ring (δ 2.20, *bs*, 3H), and a C_{15}-isoprenoid chain (multiplet of three olefinic protons at δ 5.07, 2H and 5.26, 1H, a doublet of Ar—CH_2—C = protons at 3,39, J = 7.0 Hz, an eight-proton multiplet of —CH_2—CH_2— protons at 2.04 and broadened singlets of four sp^2-methyl groups at 1. 58, 6H, 1.67, 3H and 1.80, 3H). In accordance with the presence of two phenolic hydroxyls in the parent compound **1**, the spectra of the acetylated derivative **2** and the methylated derivative **3** exhibited the corresponding signal of two equivalent acetoxy groups (δ 2.26, *s*, 6H) and methoxy groups (δ 3.78, *s*, 6H), while the signals of other protons (except for the 2 aromatic ones) remained without any considerable change. The chemical shift equivalence of aromatic protons indicates symmetrical substitution of the aromatic nucleus, the methyl group and the isoprenoid chain being in the *para* position and the two hydroxyls being in the *ortho* positions with respect to one or the other alkyl substituent. The structure with hydroxylic functions situated *ortho* with respect to the isoprenoid chain is favoured by observation of long-range interactions of aromatic hydrogens that exhibit J = 0.4 Hz with methyl protons and $J \approx 0$ Hz with benzylic —CH_2— protons. An unambiguous assignment could be made on the basis of the nuclear Overhauser effect experiment with the dimethoxy derivative **3** since irradiation of protons of the aromatic methyl group resulted in a 10% increase of the intensity of signals belonging to aromatic protons. Compound **1**, *E,E*-5-methyl-2-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1,3-benzenediol is identical with the antibiotically active metabolite grifolin, isolated by Hirata and Nakanishi [1] from the closely related Japanese fungus *Grifola confluens*. Our NMR data are in a fair accord with those reported for grifolin by other authors [2, 3] (except for the obviously erroneous assignment of the signal of the aromatic methyl in the paper of Goto *et al.* [2]). These authors [2] also established the *trans–trans* configuration of the isoprenoid chain in grifolin.

The more polar compound **6**, mp 43–45°, $C_{22}H_{32}O_2$ (MS), exhibited IR absorption bands of hydroxylic groups (3400 and 1110 cm^{-1}) and aromatic bands (1496, 1516 and 1600 cm^{-1}). The presence of two hydroxylic functions was established by acetylation affording the diacetate **7** (oil), $C_{26}H_{36}O_4$ (MS), IR: 1770, 1370 and 1206 (AcO—arom) and 1481 and 1588 cm^{-1} (arom.), NMR: 2 × OAc (δ 2.25 and 2.26), and by methylation yielding the dimethoxy derivative **8** (oil), $C_{24}H_{36}O_2$ (MS), IR: 1118 (C—O—C) and 1492, 1586 and 1608 cm^{-1} (arom.), NMR: 2 × OMe (δ 3.77).

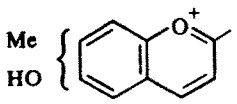
The ^{1}H-NMR spectrum of compound **6** strikingly resembled that of compound **1** and indicated the presence of the same structural groups (for NMR data see Table 1). A single characteristic difference consisted in the behaviour of two aromatic protons which were not equivalent in the case of compound **6** (broadened doublets at δ 6.19 and 6.24, resp.). The substituents on the ring are consequently not placed in symmetrical positions. The magnitude of the coupling constant (J = 2.5 Hz) of aromatic



(1) $R^1 = R^2 = H$
(2) $R^1 = R^2 = COMe$
(3) $R^1 = R^2 = Me$
(4) $R^1 = H, R^2 = Me$
(5) $R^1 = COMe, R^2 = Me$



(6) $R^1 = R^2 = H$
(7) $R^1 = R^2 = COMe$
(8) $R^1 = R^2 = Me$



9

hydrogens indicated the *meta* position. This finding corresponds to four isomeric structures. The double resonance experiments showed $J \approx 0$ of aromatic hydrogens with hydrogens of the benzyl residue (similar to compound **1**) and $J \neq 0$ with the aromatic methyl group. For this reason, we prefer the structure **6**, *E,E*-5-methyl-4-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1,3-benzenediol, in which the *ortho* positions with respect to the isoprenoidal substituent are occupied by the methyl and hydroxylic group while the second hydroxylic function is in the *meta* position with respect to these groups. This structure is also most probable from the biogenetic point of view. Compound **6** is thus identical with the 4-farnesyl isomer of compound **1** which was prepared by several authors [2–5] in the form of an oil as a by-product of the synthesis of grifolin, and which was named neogrifolin by Manners *et al.* [5].

The isomers **1** and **6** exhibited almost identical MS. The main fragmentation processes of these compounds considered in the benzylic cleavage to the $M - C_{14}H_{23}$ ion and the allylic cleavage between the double bonds of the side chain affording the $M - C_{10}H_{17}$ fragments. The highly abundant $M - C_{10}H_{17}$ ions appeared to be stabilised by the electron pair of the oxygen atom in the aromatic substituent. The isomers exhibited significant ions $C_{14}H_{23}$, $C_{13}H_{21}$ and $M - C_{11}H_{19}$. These fragments were probably formed after migration of the double bond in the side chain. In the case of the $M - C_{11}H_{19}$ ion, we may assume migration of the double bond into conjugation with the aromatic ring and the subsequent benzylic cleavage. The spectra of both isomers exhibited a relatively intensive peak at *m/e* 175 ($C_{11}H_{11}O_2$) which is formulated by the oxonium structure **9**.

The branched isoprenoid character of the side chain was supported by the absence of the $M - C_2H_5$ peak and the negligible intensity of $M - C_7$ alk(en)ylions. The higher region of the spectrum mainly contained fragments formed from the ionised molecule by removal of portions of the side chain. The degree of unsaturation of the removed hydrocarbon residues was variable and

Table 1. Chemical shifts in the ^{1}H-NMR spectra of grifolin and neogrifolin derivatives in deuterochloroform

Compound	R^1	R^2	Me—Ar	H—Ar	—CH_2—Ar	Me—C=	—CH=	—CH_2—CH_2—
1	—	—	2.20	6.22 (2H)	3.38	1.58 (6H) 1.67 (3H) 1.80 (3H)	5.07 (2H) 5.26 (1H)	2.04
2	2.26	2.26	2.31	6.76 (2H)	3.14	1.58 (6H) 1.68 (3H) 1.71 (3H)	5.05 (3H)	2.00
3	3.78	3.78	2.32	6.36 (2H)	3.32	1.58 (6H) 1.68 (3H) 1.76 (3H)	4.95–5.30 (3H)	1.99
4	—	3.77	2.26	6.30 (2H)	3.37	1.58 (6H) 1.67 (3H) 1.79 (3H)	4.95–5.35 (3H)	2.03
5	2.26	3.77	2.25	6.47 (1H) 6.51 (1H)	3.32	1.58 (6H) 1.68 (3H) 1.75 (3H)	5.07 (3H)	2.00
6	—	—	2.22	6.19 (1H) 6.24 (1H)	3.29	1.58 (6H) 1.67 (3H) 1.78 (3H)	5.10 (3H)	2.03
7	2.25	2.26	2.29	6.71 (1H) 6.80 (1H)	3.22	1.58 (6H) 1.67 (3H) 1.73 (3H)	5.06 (3H)	2.00
8	3.77	3.77	2.25	6.32 (2H)	3.28	1.58 (6H) 1.67 (3H) 1.75 (3H)	5.07 (3H)	1.99

suggested migration of double bonds in the side chain. The migration of double bonds must be for example anticipated in the formation of M − C_4H_8, M − C_6H_{10}, and M − C_8H_{15} ions.

Grifolin monomethyl and dimethyl derivatives and the neogrifolin dimethyl derivative were also isolated as minor components of the acetone extract. This is the first report of these naturally occurring compounds. Grifolin monomethyl derivative **4**, a liquid, $C_{23}H_{34}O_2$ (MS), exhibited the IR absorption band of the hydroxyl group (3450 cm^{-1}) and aromatic bands (1510, 1585, and 1615 cm^{-1}). The presence of one hydroxyl function was established by acetylation yielding the monoacetyl derivative **5**, a liquid, $C_{25}H_{36}O_3$ (MS), IR: 1768 and 1212 (AcO—arom.) and 1485 and 1595 cm^{-1} (arom.), NMR: 1 × OAc (δ 2.26). Methylation of **4** with diazomethane afforded the dimethyl derivative **3**, the IR, MS, and NMR of which were identical with those of grifolin dimethyl derivative.

Grifolin dimethyl derivative **3**, a liquid, $C_{24}H_{36}O_2$ (MS) exhibited IR, MS, and NMR data identical with those of the specimen obtained by methylation of grifolin with diazomethane.

Neogrifolin dimethyl derivative **8**, a liquid, $C_{24}H_{36}O_2$ (MS), was identified on the basis of IR, MS, and NMR data which were identical with those of a specimen prepared by methylation of neogrifolin with diazomethane.

EXPERIMENTAL

Mps were taken on a Kofler block. TLC was performed on Si gel G. IR spectra were measured in KBr micropellets, high resolution MS at 70 eV, NMR on a 100 MHz apparatus. GC was carried out on a Gas Chrom Q column (3 mm × 1.5 m) coated with 3% QF-1 at 220° and on a Gas Chrom column (3 mm × 1.5 m) coated with 3% OV-17 at 240°.

Extraction. Fresh *Albatrellus ovinus* (0.9 kg was disintegrated in Me_2CO. The Me_2CO extract was concnd and the concentrate distributed between 60% aq. EtOH and C_6H_6. The C_6H_6 layer was dried and evapd. The residue (4.6 g) was repeatedly chromatographed on Si gel (hundred fold 15% H_2O) using petrol–C_6H_6 (3:1), C_6H_6, and C_6H_6–Et_2O (9:1). The purity of chromatographic fractions was checked by TLC (C_6H_6–Et_2O, 9:1) and GC.

E,E-5-*Methyl*-2-(3,7,11-*trimethyl*-2,6,10-*dodecatrienyl*)-1,3-*benzenediol* **1** (*grifolin*). TLC R_f = 0.60; mp 41–43°; IR ν_{max}^{KBr} cm^{-1}: 3430, 1631, 1590, 1515, and 1043; MS *m/e* (rel. int.): M^+ 328 ($C_{22}H_{32}O_2$, 11), 313 ($C_{21}H_{29}O_2$, 1.9), 285 ($C_{19}H_{25}O_2$, 0.9), 272 ($C_{18}H_{24}O_2$, 0.5), 259 ($C_{17}H_{23}O_2$, 1.2), 246 ($C_{16}H_{22}O_2$, 2.4), 245 (1.7), 217 ($C_{14}H_{17}O_2$, 1.3), 215 ($C_{14}H_{15}O_2$, 1.5), 205 (*d* 7:1, $C_{13}H_{17}O_2$, $C_{15}H_{25}$, 1.5), 204 (2.4), 203 ($C_{13}H_{15}O_2$, 2.7), 192 (6), 191 (*d* 4:5, $C_{12}H_{15}O_2$, $C_{14}H_{23}$, 14), 190 (5), 189 ($C_{12}H_{13}O_2$, 5), 177 (*d* 5:1, $C_{11}H_{13}O_2$, $C_{13}H_{21}$, 11), 175 ($C_{11}H_{11}O_2$, 18), 163 (7), 149 (7), 138 (10), 137 ($C_8H_9O_2$, 100), 121 (12), 109 (9), 95 (7), 93 (7), 81 (12), 69 (23), 55 (7), 41 (20).

Grifolin diacetate **2** (C_5H_5N—Ac_2O). A liquid; IR ν_{max}^{KBr} cm^{-1}; 1197, 1370, 1482, 1580, 1770; MS *m/e* (rel. int.): M^+ 412 ($C_{26}H_{36}O_4$, 16), 369 (*d* 2:1, $C_{23}H_{29}O_4$, $C_{24}H_{33}O_3$, 6), 327 (2), 288 ($C_{17}H_{20}O_4$, 7), 259 (*d* 1:3, $C_{16}H_{19}O_3$, $C_{17}H_{23}O_2$, 10) 246 (6), 245 ($C_{15}H_{17}O_3$, 11), 233 ($C_{14}H_{17}O_3$, 20), 221 ($C_{12}H_{13}O_4$, 15), 217 (10), 203 (8), 191 (*d* 3:1, $C_{12}H_{15}O_2$, $C_{14}H_{23}$, 79), 179 ($C_{10}H_{11}O_3$, 46), 175 ($C_{11}H_{11}O_2$, 39), 137 (84), 136 (*d* 10:1, $C_{10}H_{16}$, $C_8H_8O_2$, 77), 81 (C_6H_9, 52), 69 (C_5H_9, 100), 43 (63), 41 (61).

E,E-5-*Methyl*-2-(3,7,11-*trimethyl*-2,6,10-*dodecatrienyl*)-1,3-*dimethoxybenzene* **3**. A liquid; TLC R_f = 0.85; IR ν_{max}^{KBr} cm^{-1}: 1608, 1566, 1492, 1118; MS *m/e* (rel. int.): M^+ 356 ($C_{24}H_{36}O_2$, 13), 219 ($C_{14}H_{19}O_2$, 46) 165, ($C_{10}H_{13}O_2$, 100), 151 (8), 150 (8), 69 (17). IR, MS and NMR spectra were identical with those of dimethylderivative of grifolin prepared by methylation of grifolin with diazomethane.

E,E-5-*Methyl*-2-(3,7,11-*trimethyl*-2,6,10-*dodecatrienyl*)-1-*hydroxy*-3-*methoxybenzene* **4**. A liquid, TLC R_f = 0.68, IR ν_{max}^{KBr} cm^{-1}: 3450, 1615, 1585, 1510; MS *m/e* (rel. int.): M^+ 342 ($C_{23}H_{34}O_2$, 7), 327 (0, 3), 299 (0, 3), 286 (0, 5), 273 (1), 260 (1, 1), 231 (1.3), 229 (1, 5), 217 (2), 205 ($C_{13}H_{17}O_2$, 21), 204 (3), 203 (1, 6), 191 (12), 190 (7), 189 (11), 177 (5), 163 (5), 151 ($C_9H_{11}O_2$, 100), 121 (15), 69 (28), 43 (4), 41 (26).

E,E-5-*Methyl*-2-(3,7,11-*trimethyl*-2,6,10-*dodecatrienyl*)-1-*acetoxy*-3-*methoxybenzene* **5**. A liquid; IR ν_{max}^{KBr} cm^{-1}: 1768, 1595, 1485, 1212; MS *m/e* (rel. int.): M^+ 384 ($C_{25}H_{36}O_3$, 8), 342 (0.8), 273 (1.3), 260 (1.8), 248 ($C_{15}H_{20}O_3$, 11), 247 (3.5), 246 (2.5), 231 (1.5), 218 (4.6), 217 (3.3), 205 ($C_{13}H_{17}O_2$, 100), 204 (6), 193 ($C_{11}H_{13}O_3$, 33), 191 (*d* 2:1, $C_{14}H_{23}$, $C_{12}H_{15}O_2$, 10), 175 (10), 163 (7), 151 ($C_9H_{11}O_2$, 95), 137 (13), 121 (*d* 1:1, C_9H_{13}, C_8H_9O, 23), 109 (7), 107 (8), 95 (8), 93 (6), 91 (7), 81 (C_6H_9, 19), 69 (61), 67 (8), 57 (2), 55 (9), 53 (5), 43 (14), 41 (47).

E,E-5-*Methyl*-4-(3,7,11-*trimethyl*-2,6,10-*dodecatrienyl*)-1,3-*benzenediol* **6** (*neogrifolon*). Mp 43–45°, TLC. R_f = 0.26; IR ν_{max}^{KBr} cm^{-1}: 3400, 1600, 1516, 1496, 1110. MS identical with that of compound **1** except for the intensity of some peaks.

Neogrifolin diacetate **7** (C_5H_5N—Ac_2O). A liquid; IR ν_{max}^{KBr} cm^{-1}: 1770, 1581, 1481, 1370, 1206. MS identical with that of compound **2** except for the intensity of some peaks.

E,E-5-*Methyl*-4-(3,7,11-*trimethyl*-2,6,10-*dodecatrienyl*)-1,3-*dimethoxybenzene* **8**. A liquid; TLC R_f = 0.75; IR ν_{max}^{KBr} cm^{-1}: 1608, 1586, 1492, 1118. MS identical with that of compound **3** except for the intensity of some peaks.

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