Phytochemistry, Vol. 34, No. 4, pp. 1057–1059, 1993
Printed in Great Britain.

0031-9422/93 $6.00+0.00

UNUSUAL HYDROXY FATTY ACIDS FROM SOME HIGHER FUNGI

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(*Received in revised form* 15 *March* 1993)

Key Word Index—Basidiomycetes; Ascomycetes; fungi; hydroxy fatty acids; novel unusual fatty acids; 7-hydroxy-8,14-dimethyl-9-hexadecenoic acid; 7-hydroxy-8,16-dimethyl-9-octadecenoic acid; 7-hydroxy-8,18-dimethyl-9-icosenoic acid; 7-hydroxy-8,20-dimethyl-9-docosenoic acid.

Abstract—Nine fungal species belonging to the Ascomycetes and Basidiomycetes were examined for their fatty acid compositions. Using capillary GC-MS, MS, HPLC, ^{1}H and ^{13}C NMR spectrometry some novel unusual hydroxy fatty acids were identified. They include four homologues, three minor and one major acid, from C_{18} to C_{24}. They were identified as 7-hydroxy-8,14-dimethyl-9-hexadecenoic acid, 7-hydroxy-8,16-dimethyl-9-octadecenoic acid, 7-hydroxy-8,18-dimethyl-9-icosenoic acid and 7-hydroxy-8,20-dimethyl-9-docosenoic acid.

INTRODUCTION

Hydroxy fatty acids make up an interesting group of natural compounds widespread among fungi [1–3]. As a rule, the major hydroxy acids are 2-hydroxy acids [1–7] and, in particular, 2-hydroxystearic acid which constitutes over 30% of total fatty acids (FA) in a number of fungal species [8]. Dihydroxy acids also occur in ceramides and sphingolipids of various fungal species [2, 9].

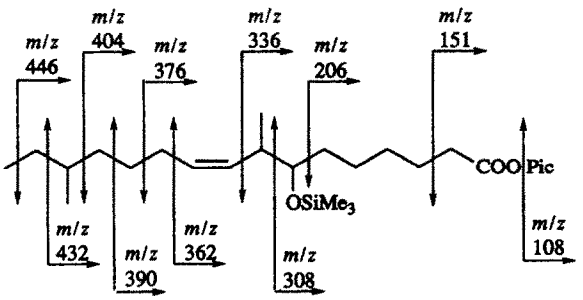
Our earlier screening analysis of FAs from various Basidiomycetes [4, 6] and Ascomycetes [5] revealed a diversity of hydroxy FAs which ranged from C_{12} to C_{30}. A more detailed analysis of nine fungal species is reported in the present paper where small amounts of hydroxy FAs with unusual structures were identified.

RESULTS AND DISCUSSION

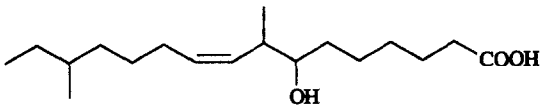
Our previous examination of more than 25 higher fungal species for their fatty acid content had shown that this varied from 10 to 15% of the total lipid extracts [4–6]. A number of acids required additional physicochemical investigation.

Using HPLC on silica gel, we separated hydroxy fatty acid methyl esters from normal chain esters. Furthermore, using preparative gradient elution reverse phase (RP)-HPLC, we isolated the main (C_{18}) hydroxy acid. This acid from *Pleurotus* sp. was obtained at 96% purity. Fractions of hydroxy acids (as picolinyl–TMSi derivatives) were subsequently identified by GC-MS.

On the basis of ^{1}H, ^{13}C NMR, high-resolution mass spectrometry, GC-MS and chemical degradation, we identified the structure of the C_{18} acid as 7-hydroxy-8,14-dimethyl-9-hexadecenoic acid (**1**). Comparison of mass spectra of this acid with those from the other three homologues suggested analogous structures (Table 1). From ^{1}H NMR spectra it followed that two methyl groups are present, one terminal and the second in the middle of the chain. One double bond and a hydroxyl group were also detected. This observation was confirmed by means of ^{13}C NMR and further structural details were elucidated, i.e. one double bond and an anteiso-methyl group. The hydroxyl groups in the mid-chain position were also identified. From high resolution mass spectrometry the structure **1** was proposed. By means of chemical degradation–hydrogenation and oxidative cleavage of the carbon chain, 7-methylnonanoic acid and heptanedioic acid were identified. By oxidation of the native molecule, 9-methylundecanoic acid, 2-oxo-10-methyldodecanoic and heptanedioic acid were obtained. On the basis of all the above evidence we thus proved the structure as **1**. The structures of the three minor components were identified by means of GC-MS (Table 1). These FAs are ubiquitous, but occurred in only very small quantities. From single ion monitoring of ions *m/z* 73 and



Scheme 1. Mass spectral fragmentation of the picolinyl–TMSi derivative of **1**.



1

371 (C_{18}), 73 and 399 (C_{20}), 73 and 427 (C_{22}) and 73 and 455 (C_{24}) [quantified only by these ions, not by means of total ion current] the values shown in Table 1 were determined. This analysis showed that only nine out of the 25 fungal species examined contained unusual hydroxy acids. The C_{18} acid was always the predominant one.

Hydroxy FAs, including 12-OH-18:1, 16-OH-16:0, 17-OH-18:0 and 2-OH-16:0 are present in fungal lipids, and the most important of these is D-12-hydroxy-9-octadecenoic acid (ricinoleic acid). Biosynthesis of the latter is described by Morris [10]. Tetrahydroxyalkanoic acids are known to occur in some lichen species [11, 12]. A number of unusual hydroxy acids: 2,15,16-trihydroxyhexadecanoic, 15,16-dihydroxyhexadecanoic and 2,3,15,16-tetrahydroxyhexadecanoic were isolated as the corresponding glycosides from *Ustilago zeae* [13]. Hydroxy acids from 14:0 to 18:2 having a hydroxyl group in positions 13–18 were obtained from sophorolipids produced by *Torulopsis bombicola* [14]. Iso- and anteiso-2-hydroxy acids belong to acids previously isolated by us from many higher fungi [4–6]. Very long-chain FAs, 2-OH-25:0 and 2-OH-26:1 were isolated from *Agaricus fungi*; iso-2-OH-24:1 (*n*-15) and iso-2-OH-26:1 (*n*-17) were detected in lipids of Ascomycetes [4–6]. Thus, our analysis has shown that some higher fungi contain homologous acids, viz., C_{18}, C_{20}, C_{22} and C_{24}. As follows from the above survey of the literature, the described acids have not been identified before.

Table 1. Content of unusual hydroxy fatty acids from some fungi (% of total fatty acids)

Species	C_{18}	C_{20}	C_{22}	C_{24}
Clitocybe sp.	0.03	0.02	—	—
Coriolus zonatus	0.01	—	—	—
Daedaleopsis confragosa var. *tricolor*	0.02	0.01	—	—
Daedalia quercina	0.05	0.02	0.01	—
Mycena polygramma	0.05	0.01	—	—
Phellinus sp. 1	0.08	0.06	0.02	0.01
Phellinus sp. 2	0.12	0.04	0.02	0.01
Pleurotus sp.	0.06	0.01	0.01	—
Stropharia aeroginosa	0.04	0.03	0.01	—

C_{18}: 7-hydroxy-8,14-dimethyl-9-hexadecenoic acid; C_{20}: 7-hydroxy-8,16-dimethyl-9-octadecenoic acid; C_{22}: 7-hydroxy-8,18-dimethyl-9-icosenoic acid; C_{24}: 7-hydroxy-8,20-dimethyl-9-docosenoic acid.

EXPERIMENTAL

Extraction and isolation. Fungal species were sampled in the nature reserve 'Samarskaya Luka' near Togliatti in August 1990. Freshly collected specimens were thoroughly cleansed of extraneous matter and homogenized in a high-speed unit. Lipids were extracted in 3 stages and the extracts combined as described previously [6, 15]. Lipid extracts were submitted to triple washing in cooled 0.9% KCl.

Preparation of fatty acids. Me esters of the corresponding FAs were prepd by alkaline hydrolysis and reaction of free acids with BF_3–MeOH [15]. Me esters were sepd by HPLC on a silica gel column (250 × 10 mm) with hexane–THF (4:1). After elution of non-hydroxy Me esters, the hydroxy Me esters were collected and analysed by GC-MS as the corresponding picolinyl ester–TMSi ethers [16].

GC-MS. Picolinyl ester–TMSi ethers were sepd on a fused silica capillary column (60 m × 0.32 mm i.d.) coated with a 0.25 μm layer of SPB-1 (Supelco) using splitless injection and He as carrier. The oven temp. was programmed from 100 to 320° at 4° min^{-1}.

Isolation of 7-hydroxy-8,14-dimethyl-9-hexadecenoic acid (**1**). Total hydroxy FA Me esters were sepd using a semiprep. column (RP-C18, 250 × 10 mm) and eluting with a gradient of MeOH–THF from 3:2 to 2:3 during 25 min. Chemical degradation, hydrogenation and oxidation of isolated FA was carried out according to ref. [17].

NMR and MS. ^1H and ^{13}CNMR were measured ($CDCl_3$, ppm, TMS) at 300 and 75 MHz, respectively. ^1H NMR: 0.84 *d* (Me on C8), 0.87 *d* (anteiso-Me), 0.88 *t* (terminal Me), 1.27–1.29 *br s* (bulk CH_2), 3.6 *br s* (hydroxyl), 3.65 *s* (COOMe), 5.27 *m* (olefinic 2H). ^{13}C NMR: C−1, 181.2, C−2, 37.3, C−3, 28.4, C−4+5, 29.1, C−6, 28.3, C−7, 72.1, C−8, 28.5, C−9, 129.8, C−10, 129.1, C−11, 27.4, C−12+13, 29.3, C−14, 29.7, C−15, 31.6, C−16, 14.2, C−17, 19.8, C−18, 22.7, COOMe 51.5. HR-MS was determined (as picolinyl ester–TMSi ether) in EI mode at 70 eV. $[M]^+$ *m/z* 461.7548 (found); 461.7665 $C_{17}H_{47}NO_3Si$ (calcd). GC-MS of **1**: *m/z* (rel. int.) 461 ($[M]^+$, 14), 446 ($[M-Me]^+$, 5), 432 ($[M-C_2H_5]^+$, 8), 404 ($[M-C_4H_9]^+$, 4), 390 (37), 376 (35), 371 ($[M-TMSiOH]^+$, 53), 362 (10), 336 (7), 308 (17), 206 (8), 192 (5), 178 (14), 164 (28), 151 (45), 108 (100).

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