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Constituents of various wood-rotting basidiomycetes

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Abstract

Phytochemical investigation of *n*-hexane and methanol extracts of fruiting bodies of the wood-rotting fungi *Fomitopsis pinicola*, *Ganoderma lipsiense*, *Fomes fomentarius* and *Gloeophyllum odoratum* led to the isolation and identification of several triterpene derivatives and some aromatic compounds derived from lignin. These are the new natural products, namely, pinicolic acid E (16α-hydroxy-3-oxolanosta-8,24-dien-21-oic acid) and pinicolol C (3-oxolanosta-7,9(11),24-trien-15α,21-diol) from the crust of *F. pinicola*, ganoderenic acid D {(*E*)-7β-hydroxy-3,11,15,23-tetraoxolanosta-8,20(22)-dien-26-oic acid} and ganoderic acid N (7β,20-dihydroxy-3,11,15,23-tetraoxolanost-8-en-26-oic acid) from *G. lipsiense* and ergosterol peroxide (5α,8α-epidioxyergost-6-en-3β-ol) as well as ergost-7-en-3-one from *F. fomentarius*. From *G. odoratum*, dehydroeburicoic acid {24-methylene-3-oxolanosta-7,9(11)-dien-21-oic acid}, the dimethylacetal of 4,4,14α-trimethyl-24-oxo-5α-chol-8-en-21-oic acid and some aromatic compounds, of which 1-(4′-methoxyphenyl)-1,2-ethandiol is a new natural product, were isolated. Furthermore, a complete set of ^{13}C NMR data of the steryl esters 3β-linoleyloxyergosta-7,24(28)-diene, 3β-linoleyloxyergosta-7,22-diene and 3β-linoleyloxyergost-7-ene, which could be identified as a mixture in all investigated fungi, could be recorded. It was proved by HPLC and TLC investigations, that the crust on top of the fruiting bodies of *F. pinicola* consists of lanostane derivatives.

Keywords: *Fomitopsis pinicola*; *Ganoderma lipsiense*; *Fomes fomentarius*; *Gloeophyllum odoratum*; Polyporaceae; Basidiomycetes; Crust; Triterpenes; Steroids; Aromatic compounds

1. Introduction

In the course of the analysis of wood-rotting basidiomycetes for triterpenes and steroidal constituents we have investigated extracts of the brown-rot saprophyte *Fomitopsis pinicola* (Swartz ex Fr.) Karst (syn. *Polyporus pinicola* [Fr.], *Fomes marginatus* [Fr.] Gillet, *Fomes pinicola*), that grows on weak or dead spruces or pine trees, the white-rot saprophyte *Ganoderma lipsiense* (Batsch) G.F. Atk. (syn. *Ganoderma applanatum* [Pers.] Pat., *Fomes applanatum* [Pers.] Gillet), that grows on dead deciduous trees, the white-rot parasite *Fomes fomentarius* (L.) Fr., which grows mainly on beech and birch trees and the brown-rot saprophyte *Gloeophyllum odoratum* (Wulf. ex Fr.) Imazeki (syn. *Osmoporus odoratus* [Wulf. ex Fr.] Singer, *Trametes odorata* [Wulf.] Fr.) which grows on stumps of spruces and pine trees. Recently, we have described the isolation of 12 triterpenes from *F. pinicola* (Rösecke and König, 1999). Numerous triterpene derivatives were previously isolated from *G. lipsiense*, including common fungal steroids derived from ergosterol (Strigina et al., 1970; Protiva et al., 1979) and highly oxygenated lanostane derivatives with biological activities of which some can be found in *Ganoderma lucidum*, too (Nishitoba et al., 1989; Chairul et al., 1991, 1994). *G. lucidum* is used since ancient times in traditional Chinese medicine and is known as "Reishi" or "Lin-Zhi". To our knowledge, the only triterpenes which have been isolated from *F. fomentarius* are ergosterol, ergosta-7,22-dien-3-one and isoergosterone (ergosta-4,6-dien-3-one) (Arthur et al., 1958; Singh and Rangaswami, 1965).

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Because of its widespread occurrence in northern Germany, we have included this species in our investigations. From *G. odoratum*, the volatile constituents of which have been investigated intensely because of its anise- and fennel-like scent, only ergosterol and some lanostane derivatives have been isolated in the past (Halsall et al., 1959). In the present paper we describe the isolation and identification of two new natural products from *F. pinicola* and of triterpene derivatives from *G. lipsiense*, *F. fomentarius* and *G. odoratum*. In addition, some aromatic compounds have been identified from the extracts of *G. odoratum*. Furthermore, ^{13}C-NMR data of three linoleic acid steryl esters, which are constituents of all investigated species, are reported here. In addition, differences between the concentration of steroids in the crust of *F. pinicola*, *G. lipsiense* and *F. fomentarius* and the rest of their fruiting bodies are described.

2. Results and discussion

The crusts of fresh fruiting bodies of *F. pinicola*, *G. lipsiense* and *F. fomentarius* were separated from the rest and these materials as well as the fruiting bodies of *G. odoratum* were in all cases extracted first with *n*-hexane and then with methanol. The solid methanol extracts were extracted again with dichloromethane.

2.1. Fomitopsis pinicola

The methanol extract of the crust, the dichloromethane extract of the rest of the fruiting bodies and the methanol extract of a very young specimen, which did not possess a crust, were investigated by reversed-phase HPLC. Because previously isolated triterpenoid compounds were available as reference (Rösecke and König, 1999), many peaks corresponding to triterpenes could be assigned. In comparing the HPLC chromatograms, it becomes obvious that the concentration of triterpenes in the crust is remarkably higher than in the rest of the fruiting bodies and that in young specimens of this fungus the overall triterpene concentration is lower than in mature ones (Fig. 1). In addition, a small amount of the crust was extracted with dichloromethane and was almost completely dissolved in it. This indicated that the crust actually consists of triterpenes. All this led to the conclusion that production of the triterpenes begins in young specimens and that these compounds are accumulated on top of the fruiting bodies to create a crust, while the concentration of triterpenes in the fruiting bodies themselves is held at a constant level.

Silica gel chromatography of the dichloromethane extract of the crust yielded 12 already described compounds (Rösecke and König, 1999) and, additionally, two new compounds **1** and **2**.

The ^{13}C-NMR of **1** showed four olefinic carbons (δ 123.41, 132.47, 133.24, 135.08) one methine carbon at

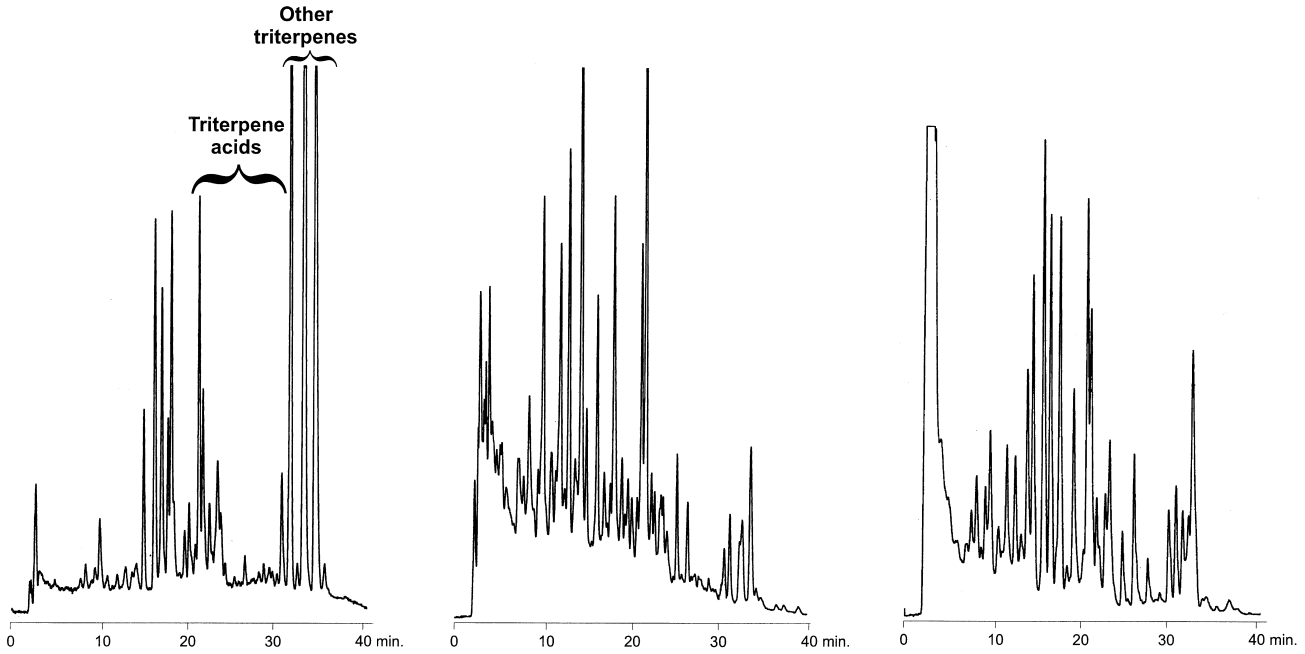


Fig. 1. HPLC chromatograms of different extracts of *F. pinicola* [CH_2Cl_2 extract of the crust (left), CH_2Cl_2 extract of the fruiting bodies separated from the crust (middle), methanol extract of a young specimen (right)].

δ 77.04, one carboxylic (δ 181.01) and one keto moiety (δ 217.52). This indicated the presence of a lanosta-8,24-diene structure. A hydroxylic methine proton (δ 4.14) which is connected to the carbon at δ 77.04 showed long-range coupling with carbon atoms at δ 42.64, 48.34, 46.06 and 56.96. Comparison of these data with those of pachymic acid (Keller et al., 1996) left carbon 16 as the only possible position for this hydroxyl group which must have α-configuration. Comparison of the complete NMR data with the data of 21-hydroxylanosta-8,24-dien-3-one (Rösecke and König, 1999) and pachymic acid (Keller et al., 1996) indicated a structure of 16α-hydroxy-3-oxolanosta-8,24-dien-21-oic acid for which we propose the name pinicolic acid E.

The ^{13}C-NMR spectrum of **2** showed six olefinic carbons (δ 116.82, 121.27, 124.57, 131.77, 140.97 and 144.90), indicating a lanosta-7,9(11),24-triene structure, one methine carbon at δ 74.57, one methylene carbon at δ 62.46 and one keto function at δ 216.60. Comparison of the NMR data with the data of 21-hydroxylanosta-8,24-dien-3-one (pinicolol B) (Rösecke and König, 1999) and lanosta-8,24-dien-3β,15α-diol (polycarpol) (Jung et al., 1990) indicated the structure of 3-oxolanosta-7,9(11),24-trien-15α,21-diol for which we propose the name pinicolol C.

One fraction of the *n*-hexane extract of the crust (separated on silica gel) contained a mixture of three linoleic acid steryl esters which could be identified by comparison of their ^{13}C-NMR data with the known data of ergosta-7,22-dien-3β-ol (Keller et al., 1996), ergost-7-en-3β-ol, ergosta-7,24(28)-dien-3β-ol (Shirane et al., 1996) and linoleic acid (Lie Ken Jie and Mustafa, 1997) as 3β-linoleyloxyergosta-7,22-diene (**3**), 3β-linoleyloxyergost-7-ene (**4**) and 3β-linoleyloxyergosta-7,24(28)-diene (**5**) which occurred in a ratio of approximately 3 (**4**):2 (**5**):1 (**3**). The ^{13}C-NMR data of **3**–**5** are listed in Table 1. This mixture could also be isolated from the other basidiomycetes species investigated in almost the same ratio thus indicating the importance of the three free sterols for fungal growth, as the linoleic acid esters are most likely a modification suitable for transport via lipoproteins.

2.2. Fomes fomentarius

The *n*-hexane extract of the crust was separated into two fractions by GPC on Sephadex LH-20. NMR studies of the oils obtained, indicated that they consist of aliphatic compounds and contain no triterpenes. Separation of the *n*-hexane extract of the rest of the fruiting bodies by silica gel chromatography led to the isolation of ergosta-7,22-dien-3-one (**6**) (Singh and Rangaswami, 1965). In the NMR spectra of **6** a minor compound could be detected, the existence of which was also proved by capillary gas chromatography. About 0.1 mg of this new compound could be obtained by reverse-phase HPLC separation on an RP30 column. This compound could now be identified by mass spectrometry and comparison of its NMR

Table 1
^{13}C shifts for identified linoleic acid steryl esters

C	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	C	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)
1	36.89 *t*	36.89 *t*	36.89 *t*	24	42.85 *d*	39.10 *d*	156.87 *s*
2	34.79 *t*	34.79 *t*	34.79 *t*	25	33.13 *d*	31.49 *d*	33.84 *d*
3	73.21 *d*	73.21 *d*	73.21 *d*	26	17.62 *q*	17.62 *q*	22.03 *q*
4	31.56 *t*	31.56 *t*	31.56 *t*	27	19.67 *q*	20.55 *q*	21.90 *q*
5	40.11 *d*	40.11 *d*	40.11 *d*	28	19.98 *q*	15.48 *q*	106.00 *t*
6	29.63 *t*	29.63 *t*	29.63 *t*	1′	173.47 *s*	173.47 *s*	173.47 *s*
7	117.36 *d*	117.32 *d*	117.36 *d*	2′	33.89 *t*	33.89 *t*	33.89 *t*
8	139.60 *s*	139.60 *s*	139.53 *s*	3′	25.11 *t*	25.11 *t*	25.11 *t*
9	49.31 *d*	49.31 *d*	49.31 *d*	4′	29.13 *t*	29.13 *t*	29.13 *t*
10	34.25 *s*	34.25 *s*	34.25 *s*	5′	29.13 *t*	29.13 *t*	29.13 *t*
11	21.51 *t*	21.51 *t*	21.51 *t*	6′	29.13 *t*	29.13 *t*	29.13 *t*
12	39.43 *t*	39.53 *t*	39.53 *t*	7′	29.13 *t*	29.13 *t*	29.13 *t*
13	43.31 *s*	43.39 *s*	43.43 *s*	8′	27.22 *t*	27.22 *t*	27.22 *t*
14	55.09 *s*	55.02 *s*	55.02 *s*	9′	130.10 *d*	130.10 *d*	130.10 *d*
15	22.98 *t*	22.98 *t*	22.95 *t*	10′	128.07 *d*	128.07 *d*	128.07 *d*
16	28.12 *t*	27.94 *t*	27.94 *t*	11′	25.67 *t*	25.67 *t*	25.67 *t*
17	755.97 *d*	56.04 *d*	56.04 *d*	12′	127.95 *d*	127.95 *d*	127.95 *d*
18	12.13 *q*	11.89 *q*	11.89 *q*	13′	130.25 *d*	130.25 *d*	130.25 *d*
19	12.98 *q*	12.98 *q*	12.98 *q*	14′	27.22 *t*	27.22 *t*	27.22 *t*
20	40.52 *d*	36.67 *d*	36.22 *d*	15′	29.20 *t*	29.20 *t*	29.20 *t*
21	21.15 *q*	19.05 *q*	18.87 *q*	16′	31.56 *t*	31.56 *t*	31.56 *t*
22	135.70 *d*	33.70 *t*	34.67 *t*	17′	22.61 *t*	22.61 *t*	22.61 *t*
23	131.92 *d*	30.74 *t*	31.12 *t*	18′	14.11 *q*	14.11 *q*	14.11 *q*

data with the data of ergost-7-en-3β-ol (fungisterol) (Jung et al., 1990) and **6** as ergost-7-en-3-one (**7**). Furthermore, the biologically active compound 5α,8α-*epi*-dioxyergost-6-en-3β-ol (ergosterol peroxide) (**8**), which was previously found in many other basidiomycetes (Turner and Aldridge, 1983) and which was shown to have antiviral activity (Lindequist et al., 1989), was isolated. This compound was described earlier as an artifact which arises from oxidation of ergosterol by air. Other constituents of the extracts are supposed to act as sensitizers for this reaction (Adam et al., 1967). As noted above, the three linoleic acid steryl esters **3**–**5** could also be identified in the *n*-hexane extract.

2.3. Ganoderma lipsiense

TLC and HPLC investigations of the extracts led to the conclusion that, unlike in *F. pinicola*, the numerous lanostane derivatives are distributed in the whole fruiting body of this species and that the crust contains the ergostane-type steroids and only small amounts of other triterpenes.

Silica gel chromatography of the dichloromethane extract of the fruiting bodies yielded the major triterpene ganoderenic acid A (**9**) (Nishitoba et al., 1989), ganoderenic acid D (**10**) which was previously isolated from *G. lucidum* (Komoda et al., 1985) together with a minor constituent, which appeared to be ganoderic acid N (**11**). **11** was previously isolated as its methyl ester from *G. lucidum* (Nishitoba et al., 1987). The NMR assignments could be completed for this compound.

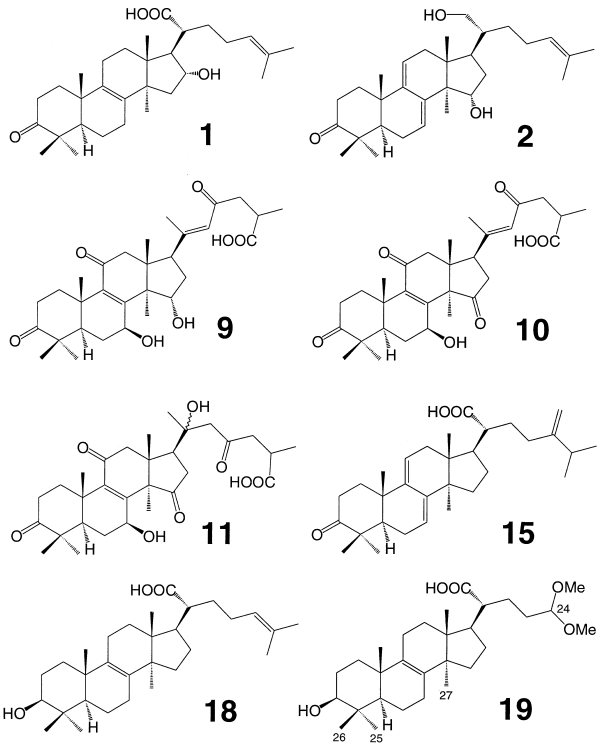
From the *n*-hexane extract of the crust, the known common fungal constituents ergosterol (**12**), ergosta-7,22-dien-3β-ol (**13**) (Shirane et al., 1996; Keller et al., 1996) and ergosta-7,22-dien-3-one (**6**) could be identified. In the NMR spectra of **6** again the signals of **7** could be detected. Again, the three linoleic acid steryl esters **3**–**5** could be identified.

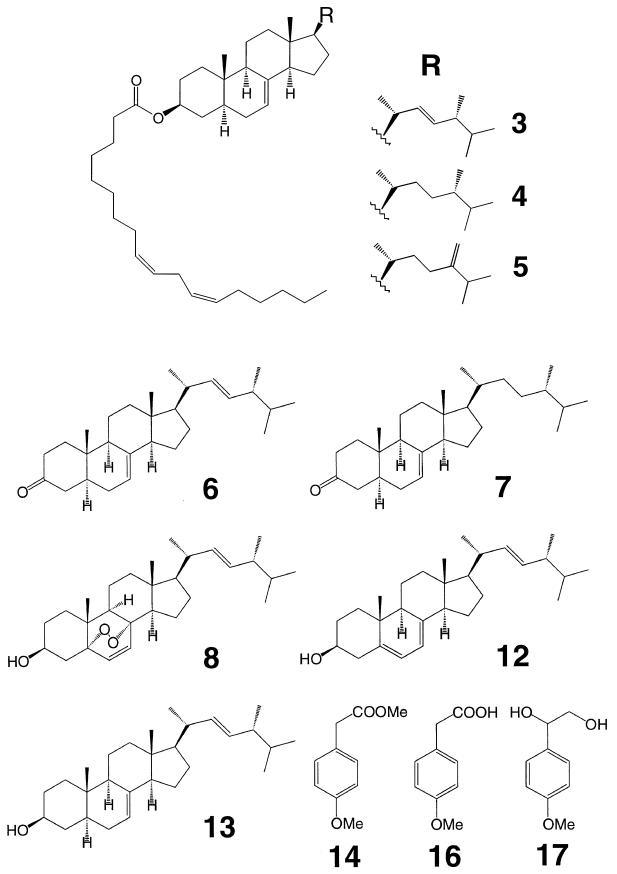
2.4. Gloeophyllum odoratum

Silica gel chromatography of the *n*-hexane extract yielded a mixture of **3**–**5** and methyl 4-methoxyphenylacetate (**14**), the major constituent of the hydrodistillate of this species (Kahlos et al., 1994). Dehydroeburicoic acid (**15**), previously obtained from the fungus *Poria cocos* (Tai et al., 1995; Schulte et al., 1967), was also isolated.

Chromatography of the dichloromethane extract afforded 4-methoxyphenylacetic acid (**16**) (Rösecke and König, 2000). In one of the fractions containing **16** another compound was present, which, by examination of the NMR data of the mixture, was identified as 1-(4′-methoxyphenyl)-1,2-ethandiol (**17**) (Kawasaki and Katsuki, 1997; Ferraboschi et al., 1990). This was confirmed by comparison of the spectral properties with those of an authentic sample of **17**, prepared by methylation (CH_2N_2) of *p*-hydroxymandelic acid and reduction ($LiAlH_4$) of the ester (Elsenbaumer and Mosher, 1979). Enantioselective GC showed that **17** from *G. odoratum* was a racemic mixture.

Trametenolic acid B (**18**), previously isolated from this (Halsall et al., 1959) and other species, e.g. *F. pinicola* (Rösecke and König, 1999) was also identified. The last compound isolated from this extract was assigned structure **19** from the following evidence. The NMR data showed good correspondence with the data of **18**, but it obviously contained a different side-chain. The ^{13}C-NMR spectrum indicated, that the main skeleton contained 27 carbons with two additional methyl groups connected to two oxygen atoms. These methyl groups showed long-range coupling with a tertiary carbon atom at δ 104.34. The proton at this carbon (δ 4.59) showed long-range coupling with the carbons 22 and 23. The latter ones also showed long-range coupling with the hydrogen at carbon 20. This led to the conclusion, that this compound was the dimethylacetal of 4,4,14α-trimethyl-24-oxo-5α-chol-8-en-21-oic acid for which we propose the name gloeophyllic acid A. It is very likely, that the identified dimethylacetal is formed from the corresponding aldehyde during the extraction in the reasonably acidic medium in the presence of the huge excess of methanol. Unfortunately, this could not be proven, but neither **19**, nor gloeophyllic acid A were found in nature before.





3. Experimental

3.1. Plant material

Fruiting bodies were collected in Hamfelde near Hamburg (*Gloeophyllum odoratum*, June 1999; *Fomitopsis pinicola*, September 1998; *Ganoderma lipsiense*, October 1998), near Salzgitter, Germany, (*Fomitopsis pinicola*, September 1998), in the Harz Mountains near Ilmenstein (*Fomitopsis pinicola*, August 1999), in Aumühle near Hamburg (*Ganoderma lipsiense*, October 1998, *Fomes fomentarius*, July 1998) and in the Harburger Berge near Hamburg (*Fomes fomentarius*, May 1998) (Gerhardt, 1996).

3.2. Extraction

The crust was separated from the fruiting bodies of *F. pinicola*, *G. lipsiense* and *F. fomentarius* and all fractions were crushed in liquid nitrogen. The resulting powders were in all cases extracted with *n*-hexane (crust: 2 × 1 l, rest: 2 × 2 l, 6 days each) and then with methanol (crust: 1 l, rest: 2 l, 6 days each). The methanol extracts were extracted with 250 ml of dichloromethane (each 1 day under stirring). The amounts of the fresh material and the extracts and their consistencies are listed in Table 2.

3.3. Isolation

3.3.1. Fomitopsis pinicola

The dichloromethane extract (crust, 4.8 g of a dark red solid) was submitted to flash column chromatography on silica gel with a step gradient of chloroform–methanol (from 49:1 to 1:1, v/v) and was rechromatographed several times using step-gradients of petroleum ether–ethyl acetate (from 39:1 to 1:1, v/v). This yielded compounds **1** (4 mg) and **2** (4 mg).

The *n*-hexane extract of the crust was submitted to flash column chromatography on silica gel with a step gradient of chloroform–methanol (from 49:1 to 1:1, v/v). This yielded previously described compounds (Rösecke and König, 1999) and a mixture of **3**, **4** and **5** (50 mg).

3.3.2. Fomes fomentarius

The *n*-hexane extract of the crust was submitted to gel permeation chromatography on Sephadex LH-20 with chloroform–methanol (1:1, v/v) as eluent. By NMR spectroscopy only aliphatic compounds were detected in both fractions obtained.

The *n*-hexane extract of the rest of the fruiting bodies was submitted to flash column chromatography on silica gel with a step gradient of chloroform–methanol (from 49:1 to 1:1, v/v). This yielded a mixture of **3**, **4** and **5** (30 mg), a 2:1 mixture of compounds **6** and **7** (60 mg) and compound **8** (20 mg). A small amount (~0.1 mg) of **7** could be obtained in a pure state by preparative HPLC on an RP-30 column. From this only a mass spectrum could be obtained.

3.3.3. Ganoderma lipsiense

The dichloromethane extract of the fruiting bodies was flash chromatographed several times on silica gel with step gradients of chloroform–methanol (from 49:1 to 1:1, v/v). This yielded compounds **9** (30 mg), **10** (10 mg) and **11** (15 mg).

The *n*-hexane extract of the crust was submitted to flash column chromatography on silica gel with a step gradient of chloroform–methanol (from 49:1 to 1:1, v/v). This yielded a mixture of **3**, **4** and **5** (20 mg) and a mixture of the compounds **12** and **13** (40 mg).

3.3.4. Gloeophyllum odoratum

The *n*-hexane extract was submitted to flash column chromatography on silica gel with a step gradient of petroleum ether–ethyl acetate (from 49:1 to 0:1, v/v). This yielded a mixture of **3**, **4** and **5** (100 mg), compounds **14** (4 mg) and **15** (5 mg).

The dichloromethane extract was flash chromatographed several times on silica gel with step gradients

of chloroform–methanol (from 1:0 to 1:1, v/v) and petroleum ether–ethyl acetate (from 9:1 to 1:1, v/v). This yielded **16** (10 mg), 1 mg of a mixture of **16** and **17** and compound **18**. One fraction was separated by GPC on Sephadex LH-20 using chloroform–methanol (1:1, v/v) as eluent. This yielded **19** (15 mg).

3.4. TLC

Silica gel $60F_{254}$ (Merck); eluent chloroform-2-propanol 9:1, v/v, and petroleum ether–ethyl acetate, 1:1, v/v; detection by spraying with sulfuric acid (10% in ethanol).

3.5. Column chromatography and GPC

Various column sizes with silica gel 0.063–0.040 mm (Macherey–Nagel) and Sephadex LH-20 (0.025–0.100 mm, Pharmacia Fine Chemicals).

3.6. HPLC

Bischoff "Prontosil 200-3-C30", particle size 3 μm, pore width 20 nm, column size 250 × 4.6 mm, eluent: methanol-2-propanol 1:1 (v/v) at 1 ml/min, detection: refractive index.

Phenomenex "Prodigy ODS(3)", particle size 5 μm, pore width 10 nm, column size 250 × 4.6 mm, gradient: methanol-2% acetic acid 75:25 (5 min), 75:25 to 95:5 during 30 min at 1 ml/min, detection: UV at 254 nm.

3.7. Capillary GC

Carlo Erba HRGC 5300 Mega Series equipped with a fused silica column coated with CPSil-5CB (25 m), 110–230°C, 3°C/min or with a 25 m fused silica column coated with heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (50% in polysiloxane OV1701, w/w). Temperature: 170°C, detection: FID.

3.8. NMR spectroscopy

Bruker WM 400 at 400.16 (^{1}H) and 100.61 MHz (^{13}C) and Bruker DRX 500 at 500.13 (^{1}H) and 125.76 MHz (^{13}C). All NMR shifts are relative to TMS.

3.9. Mp

Uncorr., apotech "Schmelzpunkt–Bestimmer".

3.10. Polarimetry

Perkin–Elmer 241, $l = 1$ dm, $\lambda = 589$ nm.

3.11. EI-MS

70 eV, VG Analytical 70-250S, exact mass measurement at resolution 10.000, direct probe sample introduction.

3.12. Compound **1**, 16α-hydroxy-3-oxolanosta-8,24-dien-21-oic acid (pinicolic acid E)

Waxy yellow solid. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.78 (3H, *s*, H-18), 0.96 (3H, *s*, H-30), 1.07 (3H, *s*, H-29), 1.09 (3H, *s*, H-28), 1.10 (3H, *s*, H-19), 1.61 (3H, *s*, H-26), 1.69 (3H, *s*, H-27), 4.14 (1H, *t*, $J = 6.1$ Hz, H-16), 5.13 (1H, *t*, $J = 7.1$ Hz, H-24). ^{13}C−NMR (100.61 MHz, $CDCl_3$): see Table 3.

3.13. Compound **2**, 3-oxolanosta-7,9(11),24-trien-15α,21-diol (pinicolol C)

Waxy yellow solid. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.66 (3H, *s*, H-18), 0.96 (3H, *s*, H-30), 1.09 (3H, *s*, H-28), 1.13 (3H, *s*, H-19), 1.20 (3H, *s*, H-29), 1.62 (3H, *s*, H-26), 1.70 (3H, *s*, H-27), 2.77 (1H, *dt*, $J = 14.2$, 6.1 Hz), 3.62 (1H, *dd*, $J = 11.2$, 4.1 Hz, H-21_a), 3.72 (1H, *dd*, $J = 11.2$, 2.5 Hz, H-21_b), 4.31 (1H, *dd*, $J = 9.1$, 6.1 Hz, H-15), 5.11 (1H, *tt*, $J = 7.1$, 1.5 Hz, H-24), 5.39 (1H, *d*, $J = 6.1$ Hz, H-11), 5.92 (1H, *d*,

Table 2
Weights and consistencies of the plant material and the obtained extracts

Species name	Weight of the crust (g)	*n*-Hexane extract (g) (consistency)	Methanol extract (g) (consistency)	CH_2Cl_2 extract (g) (consistency)
F. pinicola	150	4.5 (dark orange oil)	5.3 (orange solid)	4.8 (orange solid)
G. lipsiense	100	2.5 (yellow oil)	4.5 (dark red solid)	1.5 (orange solid)
F. fomentarius	10	2.0 (orange oil)	1.5 (crimson solid)	–
	Weight of the rest (g)	*n*-Hexane extract (g) (consistency)	Methanol extract (g) (consistency)	CH_2Cl_2 extract (g) (consistency)
F. pinicola	2000	7.5 (orange oil)	8.6 (orange solid)	0.9 (orange solid)
G. lipsiense	2000	8.1 (orange oil)	6.8 (dark red solid)	1.2 (orange solid)
F. fomentarius	3000	8.5 (yellow oil)	5.0 (orange solid)	0.3 (orange solid)
G. odoratum	4000	8.0 (dark red oil)	10.1 (dark red solid)	2.0 (red solid)

$J = 6.1$ Hz, H-7). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 3.

3.14. Compound 7, ergost-7-en-3-one

^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 3. EI-MS *m/z* (rel. int): 398 $[M]^+$ (88), 383 $[M-Me]^+$ (24), 271 (84), 229 (51), 95 (76), 40 (100).

3.15. Compound **14**, methyl 4-methoxyphenylacetate

Colorless liquid. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 3.58 (2H, *s*), 3.69 (3H, *s*), 3.80 (3H, *s*), 6.87 (2H, *dt*, $J = 8.6$ Hz, 2.5 Hz), 7.21 (2H, *dt*, $J = 8.6$, 2.5 Hz). EI-MS *m/z* (rel. int): 180 $[M]^+$ (18), 121 $[C_7H_6(OMe)]^+$ (100), 91 (5), 77 (10).

3.16. Compound **16**, 4-methoxyphenylacetic acid

Amorphous colorless solid, Mp 80–83°. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 7.21 (2H, *dt*, $J = 8.6$, 1.5 Hz), 6.88 (2H, *dt*, $J = 8.6$, 1.5 Hz), 3.80 (3H, *s*), 3.60 (2H, *s*). ^{13}C-NMR (100.61 MHz, $CDCl_3$): δ 178.05 (*s*, Ar–CH_2–COOH), 158.89 (*s*, C-4), 130.43 (*d*, C-2 + C-6), 125.37 (*s*, C-1), 114.11 (*d*, C-3 + C-5), 55.28 (*q*, Ar–OCH_3), 40.15 (*t*, Ar-CH_2–COOH). EI-MS *m/z* (rel. int): 166 $[M]^+$ (25), 121 $[C_7H_6(OMe)]^+$ (100), 78 (16), 77 (16).

3.17. Compound **17**, 1-(4′-methoxyphenyl)-1,2-ethanediol

Obtained only as a mixture with **16**. ^{1}H-NMR (500.13 MHz, $CDCl_3$): δ 7.31 (2H, *dt*, $J = 8.6$, 1.5 Hz), 6.91 (2H, *dt*, $J = 8.6$, 1.5 Hz), 4.79 (1H, *dd*, $J = 8.2$, 3.8 Hz), 3.82 (3H, *s*), 3.74 (1H, *dd*, $J = 11.4$, 3.8 Hz), 3.68 (1H, *dd*, $J = 11.4$, 8.2 Hz). EI-MS *m/z* (rel. int): 168 $[M]^+$ (4), 150 $[M-H_2O]^+$ (5), 137 $[M-CH_2OH]^+$ (100), 121 (27), 109 (21), 94 (22), 77 (30).

3.18. Synthesis of 1-(4′-methoxyphenyl)-1,2-ethanediol

50 mg (0.30 mmol) of racemic *p*-hydroxymandelic acid were dissolved in CH_2Cl_2:MeOH (1:1, v/v) and treated with a diazomethane solution. After removal of the solvent the resulting methyl *p*-methoxymande-

Table 3
^{13}C shifts for identified compounds

C	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**7** ($CDCl_3$)	**11** ($CDCl_3$)	**19** (pyridine-d_5)
1	36.04 *t*	36.62 *t*	38.80 *t*	35.67 *t*	35.72 *t*
2	34.55 *t*	34.81 *t*	38.14 *t*	34.29 *t*	28.32 *t*
3	217.52 *s*	216.60 *s*	211.98 *s*	216.88 *s*	77.61 *d*
4	47.41 *s*	47.44 *s*	44.27 *t*	46.79 *s*	39.14 *s*
5	51.21 *d*	50.51 *d*	42.90 *d*	48.88 *d*	50.50 *d*
6	19.35 *t*	23.64 *t*	30.08 *t*	27.59 *t*	18.32 *t*
7	26.25 *t*	121.27 *d*	116.98 *d*	66.32 *d*	26.43 *t*
8	133.24 *s*	140.97 *s*	139.60 *s*	157.65 *s*	133.88 *s*
9	135.08 *s*	144.90 *s*	48.88 *d*	141.00 *s*	134.77 *s*
10	36.97 *s*	37.29 *s*	34.43 *s*	38.23 *s*	37.00 *s*
11	20.56 *t*	116.82 *d*	21.72 *t*	197.79 *s*	20.87 *t*
12	28.99 *t*	37.86 *t*	39.45 *t*	50.45 *t*	29.02 *t*
13	46.33 *s*	44.12 *s*	43.38 *s*	45.29 *s*	44.49 *s*
14	48.34 *s*	52.03 *s*	54.96 *d*	59.71 *s*	49.45 *s*
15	42.64 *t*	74.57 *d*	22.98 *t*	217.86 *s*	30.48 *t*
16	77.04 *d*	39.62 *t*	27.92 *t*	36.20 *t*	26.98 *t*
17	56.96 *d*	42.99 *d*	55.96 *d*	49.29 *d*	47.34 *d*
18	17.52 *q*	16.37 *q*	11.92 *q*	19.24 *q*	16.02 *q*
19	18.64 *q*	22.17 *q*	12.47 *q*	18.12 *q*	19.03 *q*
20	46.06 *d*	42.38 *d*	36.65 *d*	72.95 *s*	48.56 *d*
21	181.01 *s*	62.46 *t*	19.03 *q*	26.58 *q*	178.18 *s*
22	32.16 *t*	29.78 *t*	33.66 *t*	52.56 *t*	30.76 *t*
23	26.42 *t*	25.01 *t*	30.71 *t*	210.36 *s*	27.56 *t*
24	123.41 *d*	124.57 *d*	39.08 *d*	47.45 *t*	104.35 *d*
25	132.47 *s*	131.77 *s*	31.49 *d*	34.33 *d*	15.96 *q*
26	17.71 *q*	17.76 *q*	20.54 *q*	180.11 *s*	28.24 *q*
27	25.74 *q*	25.72 *q*	17.61 *q*	16.87 *q*	24.10 *q*
28	21.30 *q*	22.47 *q*	15.47 *q*	20.76 *q*	
29	26.10 *q*	25.01 *q*		27.00 *q*	
30	25.29 *q*	25.45 *q*		25.08 *q*	
OMe[a]					51.89 *q*
OMe[b]					52.57 *q*

late (56 mg, 0.29 mmol, 97%) was dissolved in 1 ml of dry 1,2-dimethoxyethane and added to a stirred suspension of 14 mg (0.37 mmol) of $LiAlH_4$ in 10 ml of dry 1,2-dimethoxyethane. After stirring for 15 h at room temperature, first 0.5 ml of a saturated NH_4Cl solution and finally 1 ml of diluted HCl solution (1.2 mol/l) were added. The solvent was removed under reduced pressure and the salts were extracted once with dichloromethane. After that, the salts were dissolved in diluted HCl solution and extracted three times with dichloromethane. The combined organic layers were dried over Na_2SO_4 and the solvent removed. This yielded 34 mg (0.20 mmol, 70%) of 1-(4′-methoxyphenyl)-1,2-ethanediol. Its spectroscopic and spectrometric data are identical to those given above.

*3.19. Compound **19**, 4,4,14α-trimethyl-24-oxo-5α-chol-8-en-21-oic acid dimethylacetal (gloeophyllic acid A dimethylacetal)*

Amorphous yellow powder, Mp 205–210°. ^{1}H-NMR (500.13 MHz, $CDCl_3$): δ 1.01 (3H, *s*, H-27), 1.02 (3H, *s*, H-19), 1.06 (3H, *s*, H-18), 1.07 (3H, *s*, H-25), 1.24 (3H, *s*, H-26), 2.44 (1H, *m*, H-17), 2.61 (1H, *m*, H-20), 3.32 (3H, *s*, OMe), 3.33 (3H, *s*, OMe), 3.43 (1H, *dd*, $J = 8.5$, 7.2 Hz, H-3), 4.59 (1H, *t*, $J = 5.2$ Hz, H-24). ^{13}C-NMR (125.76 MHz, $CDCl_3$): see Table 3. EI-MS *m*/*z* (rel. int): 445 $[M-OMe]^+$ (21), 444 $[M-OMe-H]^+$ (61), 430 $[M-OMe-Me]^+$ (34), 429 $[M-OMe-Me-H]^+$ (100), 411 (61), 397 (45), 379 (66), 71 (98).

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