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Constituents of the fungi *Daedalea quercina* and *Daedaleopsis confragosa* var. *tricolor*

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Abstract

Phytochemical examination of solvent extracts of the wood-rotting fungi *Daedalea quercina* and *Daedaleopsis confragosa* var. *tricolor* led to the isolation of five new triterpene derivatives and some known fungal constituents. All structures were identified by one- and two-dimensional NMR spectroscopy and mass spectrometry. From *Daedalea quercina*, the new natural products 16-*O*-acetylpolyporenic acid C, 16α-acetoxy-24-methylene-3-oxolanost-8-en-21-oic acid, (+)-24-methylene-3,23-dioxolanost-8-en-26-oic acid, (+)-3β,12β-dihydroxy-24-methyl-23-oxolanost-8-en-26-oic acid and 12β,23-epoxy-3α,23-dihydroxy-24-methyllanost-8-en-26-oic acid could be isolated. From *Daedaleopsis confragosa* var. *tricolor*, the compounds 3α-carboxyacetoxyquercinic acid, 3α-carboxyacetoxy-24-methylene-23-oxolanost-8-en-26-oic acid and 5α,8α-epidioxyergosta-6,22-dien-3β-ol were identified. These are the first described triterpene derivatives isolated from this fungus.

Keywords: *Daedalea quercina*; *Daedaleopsis confragosa* var. *tricolor*; Polyporaceae; Basidiomycete; Triterpene derivatives

1. Introduction

Little efforts have been made in the past to identify compounds from the brown-rot fungus *Daedalea quercina* (L.) Pers. [syn. *Trametes quercina* (L.) Pilát], which grows on weak or dead stumps of deciduous trees, mainly oak trees, and from the white-rot species *Daedaleopsis confragosa* var. *tricolor* (Bulliard) Bondarzew [syn. *Lenzites tricolor* (Bulliard)], which grows on various deciduous trees. Only ergosterol, ergosta-7,22-dien-3β-ol, ergosterol peroxide (5α,8α-epidioxyergosta-6,22-dien-3β-ol) and 3α-carboxyacetoxyquercinic acid (3α-carboxyacetoxy-24-methyl-23-oxolanost-8-en-26-oic acid) have been isolated from fruiting bodies of *D. quercina* (Adam et al., 1967a; Turner and Aldridge, 1983). Ergosterol peroxide is believed to be an artefact (Adam et al., 1967b). From *D. confragosa* var. *tricolor* only some unusual fatty acids have been described earlier (Dembitsky et al., 1993). In this article, we describe the isolation and characterization of 12 compounds, five of which are new natural products.

2. Results and discussion

Fresh fruiting bodies of both species were cut in small pieces and crushed in liquid nitrogen. The resulting powders were extracted with *n*-hexane. After removing the solvent, *D. quercina* was first extracted with methanol and the obtained solid extract finally extracted with dichloromethane. *D. confragosa* var. *tricolor* was directly extracted with dichloromethane. Both dichloromethane extracts and the *n*-hexane extract of *D. quercina* were repeatedly chromatographed on silica gel with step-gradients of petroleum ether–ethyl acetate and chloroform–methanol.

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2.1. Daedalea quercina

Six compounds were identified by one- and two-dimensional NMR spectroscopy, mass spectrometry and comparison with literature data as polyporenic acid C (**1**), 16α-hydroxy-24-methylene-3-oxolanost-8-en-21-oic acid (**2**), 16-*O*-acetylpolyporenic acid C (**3**), 16α-acetoxy-24-methylene-3-oxolanost-8-en-21-oic acid (**4**), 3α-carboxyacetoxyquercinic acid (**5**) and ergosterol peroxide (**6**). To our knowledge, compounds **3** and **4** are new natural products. In addition, compounds **1** and **2** are reported here for the first time as constituents of *D. quercina*, and the NMR data of compounds **2** and **4** were not reported before. Compound **1** was previously isolated from various fungi, e.g. *Fomitopsis pinicola*, and converted to its 16-*O*-acetyl derivative (Keller et al., 1996). Compound **2** was isolated previously from the closely related fungus *Daedalea dickinsii* (Inouye et al., 1970). The NMR data of compound **5** was described earlier (Chairul et al., 1990). Some of the earlier assignments appeared to be incorrect.

Compound **7** showed four olefinic carbons (δ 133.31, 135.16, 125.46, 148.05), one acid (δ 178.31) and two ketone carbon signals (δ 201.83, 217.90). The chemical shifts of the keto function at δ 201.83, the exocyclic methylene group at δ 125.46 and observed long-range correlations indicated a lanost-8-ene structure with a 23-oxo-24-methylene functionalized side-chain and an acid function in position 26 which is common to previously identified triterpenic acids from this species. Analysis of the NMR spectra (DEPT, HMQC, HMBC) and their comparison with the data of other isolated metabolites indicated the structure 24-methylene-3,23-dioxolanost-8-en-26-oic acid for which we propose the name quercinic acid B.

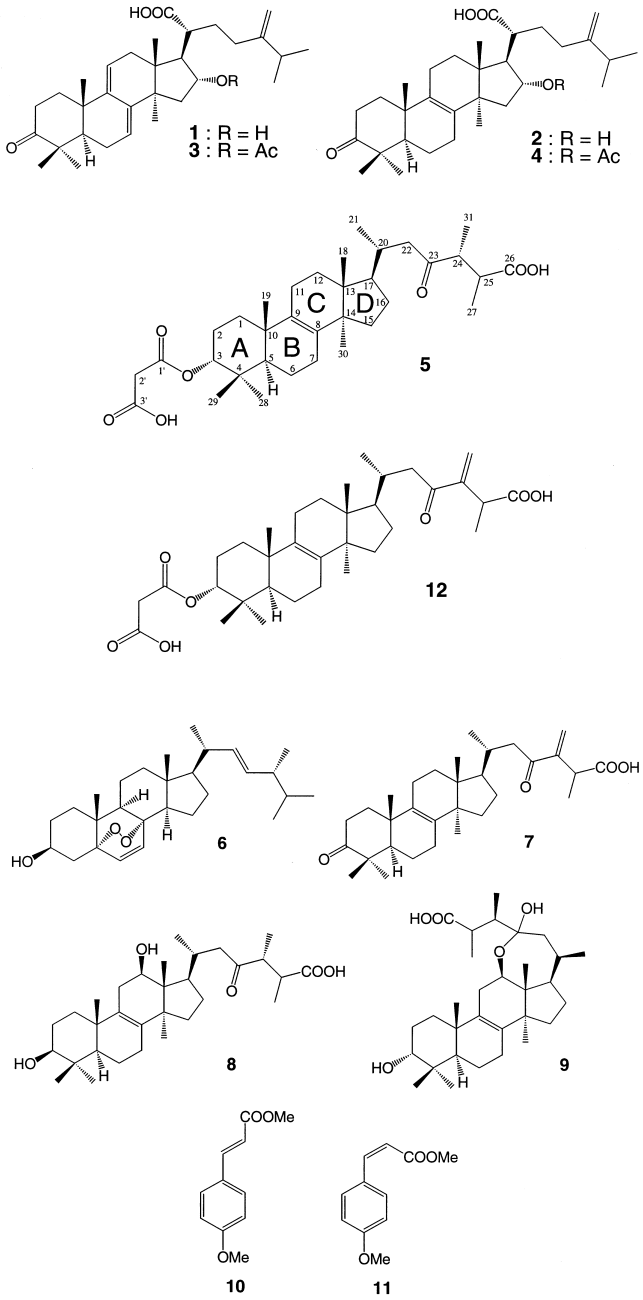
Compound **8** showed two olefinic (δ 133.33, 136.74) and two hydroxylic methine carbons (δ 71.57, 74.64) as well as one ketone (δ 213.31) and one carboxylic function (δ 178.04). The hydroxylic methine proton at δ 71.57 showed long-range coupling with the quaternary carbons 13 and 14 (δ 52.04, 49.36) as well as with a methylene group at δ 34.62. Further analysis left carbon 12 as the only possible position for this hydroxy function. The other hydroxyl group could be assigned to carbon 3 as the proton showed long-range couplings with carbons 2, 4, 5 and with the methyl carbons 28 and 29. Futher analysis of the NMR data indicated presence of a 24-methyl-23-oxo side chain with an acid function in position 26. In the NOESY spectrum, the hydroxylic methine proton (δ 4.39) only showed a correlation with the methyl protons at carbon 30 (δ 0.94) indicating a β-configuration. The hydroxylic methine proton at carbon 3 (δ 3.63) only showed a correlation with the protons connected to carbon 28 (δ 0.92) also indicating a β-configuration. Therefore, this compound was 3β,12β-dihydroxy-24-methyl-23-oxolanost-8-en-26-oic acid for which we propose the name quercinic acid C.

Compound **9** showed two olefinic (δ 133.84, 134.85) and two methine carbon signals (δ 75.79, 79.73) as well as one carboxylic carbon at δ 177.36. In addition, a quaternary carbon signal at δ 109.96 was found which corresponds to a semi-acetalic carbon atom. The latter showed long-range couplings with the hydrogen atoms at carbons 25 (δ 2.22), 24 (δ 2.12), 20 (δ 2.24) and with the hydroxylic methine proton at carbon 12 (δ 4.34) (HMBC). This led to the conclusion that formation of a cyclic acetal had taken place involving the oxygen at position 12 and the carbonyl group in position 23 of quercinic acid C (**8**). Comparison of the chemical shifts of the carbons in ring A of the triterpene skeleton with the data of many previously identified compounds indicated a 3α-configuration, so that this compound was not a simple artefact of compound **8**. Analysis of all spectral data led to the conclusion that compound **9** was 12β,23-epoxy-3α,23-dihydroxy-24-methyllanost-8-en-26-oic acid for which we propose the name 3α-oxepanoquercinic acid C.

From the *n*-hexane extract, a 15:1 mixture of (*E*)- (**10**) and (*Z*)-methyl 4-methoxycinnamate (**11**) was isolated. The identification was carried out by comparison of the GC-MS and NMR spectra with literature data (**10**: van Heerden et al., 1996, **11**: Jacobsen et al., 1994). Compound **10** was previously isolated from the fungus *Lentinus lepideus* (Wat and Towers, 1977). A reference sample of methyl (*E*)-4-methoxycinnamate was prepared by treatment of authentic (*E*)-4-methoxycinnamic acid with diazomethane. The spectroscopic data corresponded to those of compound **10**.

2.2. Daedaleopsis confragosa var. tricolor

From the dichloromethane extract, compounds **5** and **6** were isolated. These compounds are the first triterpene derivatives isolated from this species and were identified by comparison of their spectral data with literature data. Furthermore, a mixture of compound **5** and a very similar compound was obtained. The latter one showed different carbon shifts for the side chain compared with compound **5**. The additional presence of an olefinic methylene carbon at δ 125.34 and a quaternary carbon at δ 148.05 as well as the upfield shift of the carbonyl carbon to δ 201.54 indicated that this compound was 3α-carboxyacetoxy-24-methylene-23-oxolanost-8-en-26-oic acid (**12**). This was confirmed by analysis of the two-dimensional NMR spectra. This compound has been isolated previously from an unidentified Indonesian *Ganoderma* species (Chairul et al., 1990) but some NMR assignments appeared to be incorrect.



3. Experimental

3.1. Plant material

Fruiting bodies of *Daedalea quercina* were collected in October 1998 near Salzgitter in the Harz Mountains and fruiting bodies of *Daedaleopsis confragosa* var. *tricolor* in September 1999 in the Sachsenwald near Hamburg (both in northern Germany) (Gerhardt, 1996).

3.2. Extraction

3.2.1. Daedalea quercina

One kilogram of fresh fruiting bodies were crushed in liquid nitrogen. The resulting powder was extracted twice with *n*-hexane (2 × 2 l, 6 days each) and then with methanol (2 l, 6 days). The methanol extract (5.3 g of a green solid) was extracted with 250 ml of dichloromethane (1 day under stirring) yielding 4.8 g of a pale green solid.

3.2.2. Daedaleopsis confragosa var. tricolor

750 g of fresh fruiting bodies were crushed in liquid nitrogen. The resulting powder was extracted twice with *n*-hexane (2 × 2 l, 6 days each), with dichloromethane (2 l, 6 days), yielding 6.7 g of a pale green solid, and finally with methanol (2 l, 6 days).

3.3. Isolation

3.3.1. Daedalea quercina

The dichloromethane extract was submitted to flash column chromatography on silica gel with a stepwise gradient of petroleum ether–ethyl acetate (from 39:1 to 0:1, v/v) and was rechromatographed several times on silica gel using stepwise gradients of petroleum ether–ethyl acetate (from 39:1 to 0:1, v/v) and chloroform-methanol (from 49:1 to 9:1, v/v). The following yields were obtained: **1** (see below), **2** (15 mg), **3**, **4** (see below), **5** (30 mg), **6** (55 mg), **7** (14 mg), **8** (10 mg), **9** (50 mg). compounds **3** and **4** were obtained only as a mixture and **1** was only obtained admixed with **2**, but because of the existence of similar reference compounds, further separation was not necessary. The *n*-hexane extract was chromatographed in the same manner and yielded 5 mg of a 15:1 mixture of **10** and **11**.

3.3.2. Daedaleopsis confragosa var. tricolor

The dichloromethane extract was chromatographed in the same manner as described for the extracts of *D. quercina*. Yields: **5** (5 mg), a 3:1 mixture of **5** and **12** (100 mg) and **6** (10 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 sulphuric acid (10% in ethanol).

3.5. Column chromatography

Various column sizes with silica gel 0.063–0.040 mm (Macherey–Nagel).

3.6. Gas chromatography

Carlo Erba HRGC 5300 Mega Series instrument equipped with a fused silica capillary (25 m) coated with CPSil-5CB (Chrompack), split injector and FID. Temperature program: 110–230°C, 3°C/min. Carrier gas: hydrogen.

3.7. GC-MS

VG Analytical VG 70-250S mass spectrometer (EI, 70 eV) coupled to a Hewlett-Packard HP 5890 gas chromatograph. Carrier gas: helium.

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. Polarimetry

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

3.10. EI-MS

70 eV, VG Analytical 70-250S, exact mass measurement at resolution 10,000, direct probe sample introduction.

3.11. Compound *1*, polyporenic acid C, 16α-hydroxy-24-methylene-3-oxolanosta-7,9(11)-dien-21-oic acid

Obtained only as a mixture with **2**. ^{13}C-NMR (100.61 MHz, pyridine-d$_5$): see Table 1.

3.12. Compound *2*, 16α-hydroxy-24-methylene-3-oxolanost-8-en-21-oic acid

^{1}H-NMR (400.13 MHz, pyridine-d$_5$): δ 0.98 (3H, *d*, $J = 3.1$ Hz), 0.99 (3H, *d*, $J = 3.1$ Hz), 1.00 (3H, *s*, H-

Table 1
^{13}C shifts of identified compounds (pyr = pyridine-d$_5$)

C	**1** (pyr)	**2** (pyr)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** ($CDCl_3$)	**6** ($CDCl_3$)	**7** ($CDCl_3$)	**8** (pyr)	**9** ($CDCl_3$)	**12** ($CDCl_3$)
1	36.38 *t*	35.72 *t*	36.70 *t*	36.07 *t*	30.70 *t*	34.69 *t*	36.10 *t*	30.55 *t*	31.79 *t*	30.69 *t*
2	34.54 *t*	34.29 *t*	34.75 *t*	34.50 *t*	23.23 *t*	30.08 *t*	34.63 *t*	26.49 *t*	23.18 *t*	23.22 *t*
3	214.79 *s*	215.83 *s*	216.47 *s*	217.48 *s*	80.50 *d*	66.46 *d*	217.90 *s*	74.64 *d*	79.73 *d*	80.47 *d*
4	47.08 *s*	46.95 *s*	47.48 *s*	47.42 *s*	36.86 *s*	36.90 *t*	47.43 *s*	37.76 *s*	36.78 *s*	36.85 *s*
5	50.64 *d*	50.90 *d*	50.67 *d*	51.18 *d*	45.36 *d*	82.16 *s*	51.28 *d*	44.18 *d*	45.21 *d*	45.35 *d*
6	23.47 *t*	19.22 *t*	23.66 *t*	19.30 *t*	18.01 *t*	135.42 *d*	19.44 *t*	18.17 *t*	17.81 *t*	18.00 *t*
7	120.31 *d*	26.27 *t*	121.16 *d*	26.30 *t*	25.97 *t*	130.73 *d*	26.35 *t*	25.83 *t*	25.58 *t*	25.97 *t*
8	142.43 *s*	133.00 *s*	141.29 *s*	133.35 *s*	134.17 *s*	79.43 *s*	135.16 *s*	133.33 *s*	134.85 *s*	134.17 *s*
9	144.35 *s*	135.26 *s*	144.48 *s*	134.63 *s*	134.49 *s*	51.08 *d*	133.31 *s*	136.74 *s*	133.84 *s*	134.47 *s*
10	37.13 *s*	36.69 *s*	37.28 *s*	36.95 *s*	36.86 *s*	36.96 *s*	36.94 *s*	36.89 *s*	36.78 *s*	36.83 *s*
11	117.24 *d*	20.51 *t*	116.51 *d*	20.58 *t*	20.93 *t*	23.40 *t*	21.08 *t*	34.62 *t*	29.45 *t*	20.94 *t*
12	35.85 *t*	29.25 *t*	35.43 *t*	28.95 *t*	30.89 *t*	39.74 *t*	30.90 *t*	71.57 *d*	75.79 *d*	30.88 *t*
13	44.63 *s*	45.82 *s*	43.82 *s*	45.12 *s*	44.59 *s*	44.56 *s*	44.69 *s*	52.04 *s*	52.65 *s*	44.58 *s*
14	48.96 *s*	48.48 *s*	48.55 *s*	48.16 *s*	50.03 *s*	51.68 *d*	50.06 *s*	49.36 *s*	49.18 *s*	50.02 *s*
15	43.95 *t*	43.31 *t*	40.92 *t*	40.28 *t*	30.82 *t*	20.63 *t*	28.40 *t*	31.27 *t*	30.94 *t*	30.81 *t*
16	75.98 *d*	76.17 *d*	78.71 *d*	78.98 *d*	28.37 *t*	28.64 *t*	21.08 *t*	25.61 *t*	23.62 *t*	28.45 *t*
17	57.20 *d*	56.93 *d*	53.06 *d*	52.91 *d*	50.26 *d*	56.20 *d*	50.72 *d*	50.38 *d*	42.99 *d*	50.72 *d*
18	17.20 *q*	17.44 *q*	17.11 *q*	17.38 *q*	15.80 *q*	12.87 *q*	15.94 *q*	10.44 *q*	11.79 *q*	15.81 *q*
19	21.96 *q*	18.22 *q*	22.00 *q*	18.63 *q*	18.97 *q*	18.17 *q*	19.73 *q*	19.01 *q*	18.86 *q*	18.96 *q*
20	48.12 *d*	48.27 *d*	46.07 *d*	46.20 *d*	32.68 *d*	39.74 *d*	33.96 *d*	29.91 *d*	28.07 *d*	34.05 *d*
21	178.24 *s*	178.37 *s*	180.53 *s*	180.77 *s*	13.45 *q*	20.88 *q*	18.71 *q*	22.55 *q*	19.65 *q*	19.67 *q*
22	31.04 *t*	31.18 *t*	29.94 *t*	30.05 *t*	48.76 *t*	135.20 *d*	44.69 *t*	48.21 *t*	40.74 *t*	44.66 *t*
23	32.83 *t*	32.83 *t*	31.66 *t*	31.66 *t*	213.53 *s*	132.30 *d*	201.83 *s*	213.31 *s*	109.96 *q*	201.54 *q*
24	155.64 *s*	155.69 *s*	154.82 *s*	154.82 *s*	48.13 *d*	42.77 *d*	148.05 *s*	48.46 *d*	50.61 *d*	148.05 *s*
25	33.73 *d*	33.73 *d*	34.02 *d*	34.02 *d*	40.79 *d*	33.06 *d*	40.19 *d*	41.58 *d*	42.25 *d*	40.31 *d*
26	21.63 *q*	21.63 *q*	21.85 *q*	21.85 *q*	181.41 *s*	19.64 *q*	178.31 *s*	178.04 *s*	177.36 *q*	179.76 *s*
27	21.48 *q*	21.48 *q*	21.90 *q*	21.90 *q*	14.26 *q*	19.95 *q*	15.89 *q*	14.41 *q*	13.68 *q*	15.84 *q*
28	21.63 *q*	20.93 *q*	22.42 *q*	21.27 *q*	21.78 *q*	17.56 *q*	21.32 *q*	22.21 *q*	21.82 *q*	21.78 *q*
29	25.95 *q*	26.00 *q*	25.33 *q*	26.19 *q*	27.64 *q*		26.20 *q*	28.68 *q*	27.58 *q*	27.65 *q*
30	25.24 *q*	25.00 *q*	25.65 *q*	24.85 *q*	24.29 *q*		24.41 *q*	23.82 *q*	24.71 *q*	24.28 *q*
31	106.64 *t*	106.64 *t*	106.85 *t*	106.85 *t*	19.82 *q*		125.46 *t*	13.26 *q*	13.01 *q*	125.34 *t*
1′			170.79 *s*	170.79 *s*	166.74 *s*					166.74 *s*
2′			21.33 *q*	21.33 *q*	40.78 *t*					40.78 *t*
3′					171.49 *s*					171.49 *s*

29), 1.05 (3H, *s*, H-18), 1.13 (3H, *s*, H-19), 1.14 (3H, *s*, H-28), 1.46 (3H, *s*, H-30), 4.53 (1H, *dd*, $J = 7.6, 6.6$ Hz, H-16), 4.85 (1H, *s*, H-31$_a$), 4.98 (1H, *s*, H-31$_b$). ^{13}C-NMR (100.61 MHz, pyridine-d$_5$): see Table 1.

3.13. Compound **3**, 16-O-acetylpolyporenic acid C, 16α-acetoxy-24-methylene-3-oxolanosta-7,9(11)-dien-21-oic acid

Obtained only as a mixture with **4**. ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1.

3.14. Compound **4**, 16α-acetoxy-24-methylene-3-oxolanost-8-en-21-oic acid

Obtained only as a mixture with **3**. ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1.

3.15. Compound **5**, 3α-carboxyacetoxyquercinic acid, 3α-carboxyacetoxy-24-methyl-23-oxolanost-8-en-26-oic acid

^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.74 (3H, *s*, H-18), 0.87 (3H, *d*, $J = 7.6$ Hz, H-21), 0.89 (3H, *s*, H-29), 0.93 (3H, *s*, H-30), 0.94 (3H, *s*, H-28), 1.00 (3H, *s*, H-19), 1.09 (3H, *d*, $J = 7.1$ Hz, H-31), 1.21 (3H, *d*, $J = 6.9$ Hz, H-27), 3.45 (2H, *s*, H-2′), 4.76 (1H, *br*. *s*, H-3). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EI-MS *m*/*z* (rel. int.): 528 $[M - CO_2]^+$ (9), 513 $[M - CO_2 - Me]^+$ (13), 453 (80), 435 (21), 309 (52), 153 (71), 43 (100).

3.16. Compound **6**, ergosterol peroxide, 5α,8α-epidioxyergosta-6,22-dien-3β-ol

Waxy brown solid, $[\alpha^{20} - 20.0]$ ($CHCl_3$, *c* 0.2). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.82 (3H, *d*, $J = 12.6$ Hz, H-26), 0.82 (3H, *s*, H-18), 0.83 (3H, *d*, $J = 17.1$ Hz, H-27), 0.88 (3H, *s*, H-19), 0.91 (3H, *d*, $J = 6.6$ Hz, H-21), 1.00 (3H, *d*, $J = 6.6$ Hz, H-28), 3.97 (1H, *m*, H-3), 5.14 (1H, *dd*, $J = 15.3, 8.2$ Hz, H-23), 5.22 (1H, *dd*, $J = 15.2, 7.6$ Hz, H-22), 6.24 (1H, *d*, $J = 8.7$ Hz, H-7), 6.51 (1H, *d*, $J = 8.7$ Hz, H-6). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EI-MS *m*/*z* (rel. int.): 396 $[M - O_2]^+$ (30), 363 (15), 81 (41), 69 (100).

3.17. Compound **7**, quercinic acid B, 24-methylene-3,23-dioxolanost-8-en-26-oic acid

Waxy yellow solid, $[\alpha^{20} + 37.5]$ ($CHCl_3$, *c* 1.0). ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.77 (3H, *s*, H-18), 0.90 (3H, *d*, $J = 8.1$ Hz, H-21), 0.91 (3H, *s*, H-30), 1.07 (3H, *s*, H-28), 1.10 (3H, *s*, H-29), 1.13 (3H, *s*, H-19), 1.36 (3H, *d*, $J = 7.2$ Hz, H-27), 5.95 (1H, *s*, H-31$_a$), 6.20 (1H, *s*, H-31$_b$). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1. EI-MS *m*/*z* (rel. int.): 482 $[M]^+$ (8), 467 $[M - Me]^+$ (23), 449 $[M - Me - H_2O]^+$ (13), 325 (87), 151 (100), 69 (94).

3.18. Compound **8**, quercinic acid C, 3β,12β-dihydroxy-24-methyl-23-oxolanost-8-en-26-oic acid

Amorphous light brown solid, $[\alpha^{20} + 86.0]$ (CH_2Cl_2, *c*0.05). ^{1}H-NMR (400.13 MHz, pyridine-d$_5$): δ 0.92 (3H, *s*, H-28), 0.94 (3H, *s*, H-30), 1.05 (3H, *s*, H-18), 1.08 (3H, *s*, H-19), 1.15 (3H, *d*, $J = 6.9$ Hz, H-31), 1.23 (3H, *s*, H-29), 1.34 (3H, *d*, $J = 7.2$ Hz, H-27), 1.35 (3H, *d*, $J = 7.3$ Hz, H-21), 2.57 (1H, *dd*, $J = 17.3, 10.1$ Hz, H-20), 2.86 (1H, *dd*, $J = 17.6, 6.9$ Hz, H-22$_a$), 3.17 (2H, *m*, H-24, H-25), 3.63 (1H, *br*. *s*, H-3), 4.39 (1H, *t*, $J = 7.9$ Hz, H-12). ^{13}C-NMR (100.61 MHz, pyridine-d$_5$): see Table 1. EI-MS *m*/*z* (rel. int.): 502 $[M]^+$ (7), 484 $[M - H_2O]^+$ (11), 469 $[M - H_2O - Me]^+$ (100), 451 $[M - 2\ H_2O - Me]^+$ (47), 325 (38), 309 (31), 69 (74).

3.19. Compound **9**, 3α-oxepanoquercinic acid C, 12β,23-epoxy-3α,23-dihydroxy-24-methyllanost-8-en-26-oic acid

Amorphous white solid. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.76 (3H, *s*, H-18), 0.84 (3H, *s*, H-29), 0.89 (3H, *s*, H-28), 0.98 (3H, *d*, $J = 8.2$ Hz, H-21), 0.99 (3H, *s*, H-19), 0.99 (3H, *s*, H-30), 1.10 (3H, *d*, $J = 6.7$ Hz, H-31), 1.24 (3H, *d*, $J = 7.1$ Hz, H-27), 2.65 (1H, *m*, H-17), 4.34 (1H, *t*, $J = 8.1$ Hz, H-12), 4.74 (1H, *br*. *s*, H-3). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1.

3.20. Compound **10**, methyl (*E*)-4-methoxycinnamate

Obtained only as a mixture with **11**. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 3.80 (3H, *s*, OMe), 3.85 (3H, *s*, COOMe), 6.32 (1H, *d*, $J = 16.2$ Hz, H-8), 6.92 (2H, *dt*, $J = 9.1, 1.6$ Hz, H-3, H-5), 7.49 (2H, *dt*, $J = 9.1, 1.6$ Hz, H-2, H-6), 7.66 (1H, *d*, $J = 16.2$ Hz, H-7). ^{13}C-NMR (100.61 MHz, $CDCl_3$): δ 51.61 (*q*, COOMe), 55.41 (*q*, OMe), 114.36 (*d*, C-3, C-5), 115.31 (*d*, C-8), 127.16 (*s*, C-1), 129.75 (*d*, C-2, C-6), 144.56 (*d*, C-7), 153.74 (*s*, C-4), 161.43 (*s*, C-9). EI-MS *m*/*z* (rel. int.): 192 $[M]^+$ (72), 161 $[M - OMe]^+$ (100), 133 $[M - COOMe]^+$ (38), 118 (13), 89 (18).

3.21. Preparation of methyl (*E*)-4-methoxycinnamate

Commercially available (*E*)-4-methoxycinnamic acid was recrystallized from chloroform and 2 mg were treated with diazomethane solution until a yellow colour remained, indicating an excess of diazomethane. Finally, the solvent and the diazomethane were removed in a stream of nitrogen yielding pure methyl (*E*)-4-methoxycinnamate.

3.22. Compound **11**, methyl (*Z*)-4-methoxycinnamate

Obtained only as a mixture with **10**. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 3.80 (3H, *s*, OMe), 3.85 (3H, *s*, COOMe), 5.85 (1H, *d*, $J = 12.7$ Hz, H-8), 6.86 (1H, *d*, $J = 12.7$ Hz, H-7), 6.90 (2H, *d*, $J = 8.7$ Hz, H-3, H-5), 7.70 (2H, *d*, $J = 8.7$ Hz, H-2, H-6). EI-MS *m*/*z* (rel. int.): 192 $[M]^+$ (69), 161 $[M - OMe]^+$ (100), 133 $[M - COOMe]^+$ (44), 118 (21), 89 (32).

3.23. Compound **12**, 3α-carboxyacetoxy-24-methylene-23-oxolanost-8-en-26-oic acid

Obtained only as a mixture with **5**. ^{1}H-NMR (400.13 MHz, $CDCl_3$): δ 0.74 (3H, *s*, H-18), 0.89 (3H, *s*, H-29), 0.90 (3H, *d*, $J = 7.6$ Hz, H-21), 0.93 (3H, *s*, H-30), 0.94 (3H, *s*, H-28), 1.00 (3H, *s*, H-19), 1.36 (3H, *d*, $J = 7.2$ Hz, H-27), 3.46 (2H, *s*, H-2′), 4.76 (1H, *br*. *s*, H-3), 5.94 (1H, *s*, H-31$_a$), 6.20 (1H, *s*, H-31$_b$). ^{13}C-NMR (100.61 MHz, $CDCl_3$): see Table 1.

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