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Concentriols B, C and D, three squalene-type triterpenoids from the ascomycete *Daldinia concentrica*

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Abstract

Three squalene-type triterpenoids named concentricols B, C and D (**1**–**3**) were isolated from the ethyl acetate extract of fruiting bodies of the xylariaceous ascomycete *Daldinia concentrica*. Their absolute structures were elucidated by analysis of 2D NMR, MS, IR and UV spectra, and the modified Mosher's method.

Keywords: *Daldinia concentrica*; Xylariaceae; Fungus; Concentricols; Squalene-type triterpenoid

1. Introduction

Fungi of the ascomycete genus *Daldinia* Ces. & De Not. have been shown to be a good source of unique secondary metabolites. Already several decades ago, 4:5:4′:5′-tetrahydroxy-1:1′-binaphthyl and dihydroxyperylene quinone were reported by Allport and Bu'Lock (1958) from stromata of the fungus, while the same authors later reported 2,6-dihydroxy- butyrophenone, 8-methoxy-1-naphthol and 2-hydroxy-5-methylchromone from its mycelia (Allport and Bu'Lock, 1960). Some of these compounds were later found to exhibit antimicrobial and nematicidal activities (Anke et al., 1995).

In the course of our investigation of the biologically active substances from xylariaceous fungi, we previously reported the isolation of novel binaphthyl, benzophenone derivatives (Hashimoto et al., 1994a), three azaphilone derivatives named daldinins A–C (Hashimoto et al., 1994b, Hashimoto and Asakawa, 1998), and 16 10-phenyl- [11]-cytochalasans (Buchanan et al., 1995, 1996a,b) from *Daldinia* sp. Along with other *Daldinia* sp. from throughout the world, the producer organisms of the aforementioned compounds were recently reclassified using a combination of morphological methods, HPLC profiling and PCR-based genetic fingerprinting, and their current taxonomy is outlined in Stadler et al. (2001a). These data not only emphasized the importance of secondary metabolites as chemotaxonomical markers for species discrimination in Xylariaceae as previously outlined by Whalley and Edwards (1995) but also gave rise to the hope that further unprecedented metabolites may be encountered in these fungi as well.

This paper thus deals with the isolation and structural elucidation of three new compounds from *Daldina concentrica* (Bolt: Fr) Ces. and De Not (a European species) for which we propose the trivial names concentricols B (**1**), C (**2**) and D (**3**), as well as that of the synthetic methyl ester **4** derived from **1**. Previously, a new triterpenoid concentricol (**5**) was also found, whose occurrence in *Daldinia* sp. proved to be taxonomically significant (Stadler et al., 2001b).

2. Results and discussion

The ethyl acetate extract prepared from the freeze-dried fruiting bodies of *D. concentrica* was subjected repeatedly to silica gel and Sephadex LH-20 column

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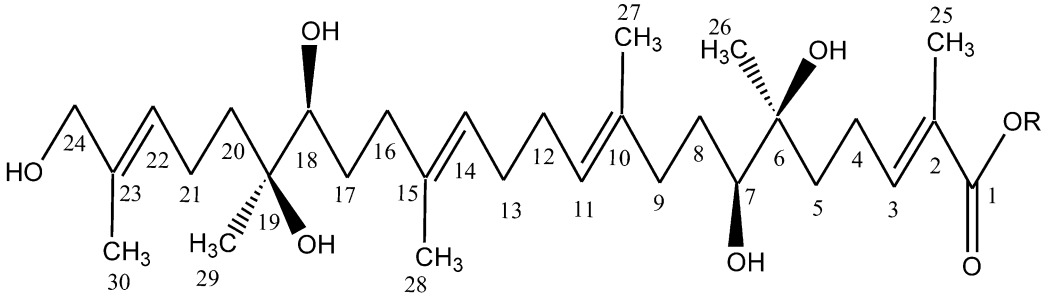
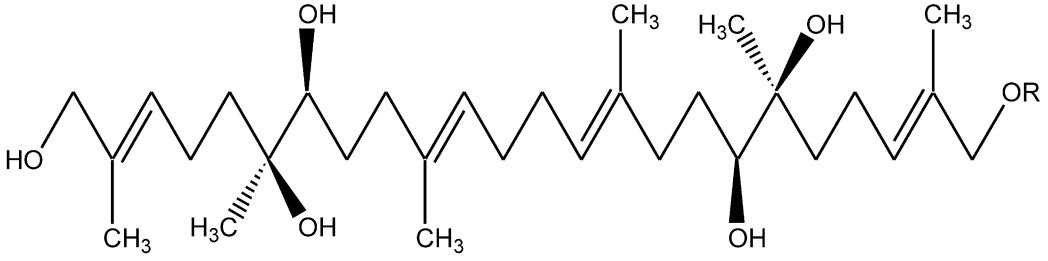
chromatography, followed by prep. HPLC as described in the Experimental to give three new concentricol derivatives (**1**–**3**) (Chart 1).

Concentricol B (**1**) was obtained as an oil and its molecular formula was determined to be $C_{30}H_{52}O_7$ by HR-FABMS. The IR spectrum indicated the presence of a hydroxyl group (3359 cm^{-1}). The ^1H and ^{13}C NMR spectral data (Table 1 and 2) of **1** showed the presence of two tertiary methyls (δ_H 1.01 and 1.01), four vinyl methyls (δ_H 1.63, 1.63, 1.66 and 1.83), one primary alcohol (δ_H 3.91; δ_C 69.0), two secondary alcohols (δ_H 3.25; δ_C 78.1), two tertiary alcohols (δ_C 75.2), four trisubstituted olefinic protons (δ_H 5.22, 5.22, 5.41 and 5.68), 10 methylenes (δ_C 23.8, 39.1, 30.6, 38.0, 29.3, 29.3, 38.0, 30.6, 39.1 and 22.6), and one unsaturated carboxyl group (δ_C 171.8; 1687 cm^{-1}; 207 nm). On the basis of the above spectral data, **1** was deduced to have the same carbon skeleton as that of concentricol (**5**) (Stadler et al., 2001b), but it differed from **5** in the following points: Compound **1** possessed a signal at δ_C 171.8 ppm in place of a secondary hydroxy carbon in **5**. The position of the carboxyl group in **1** was shown to be at C-1 based on



1 R= H
4 R= CH_3



2 R = Ac
5 R = H

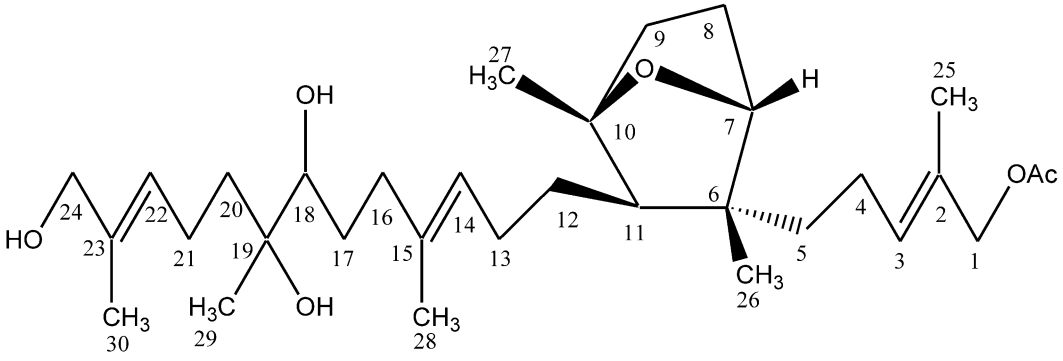


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Chart 1.

Table 1
^{1}H NMR spectral data for **1**, **2**, **3** (600 MHz, CD_3OD)

H	1	2	3
1		4.44 (*s*)	4.45 (*s*)
2			
3	6.80 (*t*, 7.1)	5.49 (*t*, 7.1)	5.47 (*t*, 7.1)
4	2.07 (*m*)	2.12 (*dd*, 5.2, 12.6)	1.96 (*m*)
	2.33 (*dd*, 5.5, 13.7)	2.17 (*dd*, 5.5, 12.6)	2.11 (*m*)
5	1.49 (*ddd*, 1.9, 5.2, 13.7)	1.48 (*ddd*, 1.3, 5.2, 12.8)	1.44 (*m*)
	1.57 (*ddd*, 1.9, 5.0, 13.7)	1.60 (*ddd*, 1.1, 5.2, 12.8)	1.52 (*dd*, 4.9, 11.5)
6			
7	3.25 (*dd*, 4.4, 6.5)	3.28 (*dd*, 1.9, 8.0)	3.85 (*d*, 5.5)
8	1.38 (*dd*, 4.4, 10.2)	1.38 (*ddd*, 4.9, 9.1, 13.7)	1.51 (*m*)
	1.75 (*m*)	1.75 (*dd*, 7.7, 13.7)	1.67 (*m*)
9	2.00 (*m*)	2.00 (*m*)	1.71 (*dd*, 5.2, 12.4)
	2.26 (*m*)	2.25 (*ddd*, 4.4, 9.6, 13.5)	1.97 (*m*)
10			
11	5.22 (*brd*, 1.1)	5.21 (*br s*)	1.28 (*t*, 6.9)
12	2.04 (*m*)	2.04 (*m*)	1.35 (*m*)
13	2.04 (*m*)	2.04 (*m*)	1.99 (*m*)
14	5.22 (*brd*, 1.1)	5.21 (*br s*)	5.19 (*t*, 7.2)
15			
16	2.00 (*m*)	2.00 (*m*)	2.01 (*m*)
	2.26 (*m*)	2.25 (*ddd*, 4.4, 9.6, 13.5)	2.25 (*ddd*, 4.7, 9.6, 14.3)
17	1.38 (*dd*, 4.4, 10.2)	1.38 (*ddd*, 4.9, 9.1, 13.7)	1.38 (*m*)
	1.75 (*m*)	1.75 (*dd*, 7.7, 13.7)	1.75 (*m*)
18	3.25 (*dd*, 4.4, 6.5)	3.28 (*dd*, 1.9, 8.0)	3.28 (*dd*, 1.4, 10.2)
19			
20	1.49 (*ddd*, 1.9, 5.2, 13.7)	1.48 (*ddd*, 1.3, 5.2, 12.8)	1.48 (*m*)
	1.57 (*ddd*, 1.9, 5.0, 13.7)	1.60 (*ddd*, 1.1, 5.2, 12.8)	1.57 (*dd*, 4.7, 11.8)
21	2.11 (*dd*, 6.3, 12.2)	2.12 (*dd*, 5.2, 12.6)	2.10 (*m*)
	2.17 (*dd*, 6.3, 12.2)	2.17 (*dd*, 5.5, 12.6)	2.15 (*m*)
22	5.41 (*dd*, 6.5, 7.8)	5.41 (*t*, 7.1)	5.41 (*t*, 7.2)
23			
24	3.91 (*s*)	3.91 (*s*)	3.91 (*s*)
25	1.83 (*s*)	1.67 (*s*)	1.66 (*s*)
26	1.01 (*s*)	1.10 (*s*)	1.03 (*s*)
27	1.63 (*s*)	1.62 (*s*)	1.34 (*s*)
28	1.63 (*s*)	1.62 (*s*)	1.63 (*s*)
29	1.01 (*s*)	1.10 (*s*)	1.11 (*s*)
30	1.66 (*s*)	1.66 (*s*)	1.66 (*s*)
1-OAc		2.03 (*s*)	2.04 (*s*)

HMBC correlations between H-25/C-1, and H-3/C-1. In addition, it was confirmed by the low field position of proton H-3 (δ_H 6.80) due to conjugation between the methylene and carbonyl groups (Edwards et al., 1991; Adeboya et al., 1995). Since the absolute configuration of concentricol (**5**) was not established (Stadler et al., 2001b), the modified Mosher's method (Ohtani et al., 1991) was applied to determine the absolute stereochemistry of the methyl ester (**4**) of **1**. This was followed by a NOE experiment with the acetonide of **4**. Methylation of **1** with $(CH_3)_3SiCHN_2$ in methanol afforded **4** as an oil. HR-FABMS of **4** at *m*/*z* 561 indicated the molecular formula $C_{31}H_{54}O_7Na$. The ^{1}H and ^{13}C NMR spectral data of **4** were similar to those of **1** except for the signals at (δ_H 3.79 and δ_C 51.7) due to a methoxyl group. The cross peak between 1-OCH_3 and C-1 was clearly detected in the HMBC spectrum and indicated the presence of a carbomethoxyl group. Compound **4** was esterified with (−)- and (+)-α-methoxy-α-trifluoromethyl-phenyl acetic acid (MTPA), DCC and DMAP in CH_2Cl_2 afforded (−)-MTPA- ester **4a** and (+)-ester **4b** derivatives at C- 7, C-18 and C-24. Both MTPA-esters were the mixture of two diastereoisomers and could not be isolated in pure state. Based on the result of integration of the ^{1}H NMR spectrum, the (−)-MTPA-ester **4a** and (+)-MTPA-ester **4b** were calculated to be about 66% as major components in each mixture. The modified Mosher's method was carried out based on the chemical shift of the major compound only. Examination of the $\Delta\delta$ values [$\delta_{(-)}-\delta_{(+)}$] showed a negative and positive chemical shift distribution to various protons as shown in Fig. 1. Consequently, the configuration of both C-7 and C-18 were determined to be *S*. Furthermore, in order to determine the configuration of C-6 and C-19, **4** was converted into the acetonide derivative **6** by using 2,2-dimethoxypropane and *p*-TsOH. The

Table 2
^{13}C NMR spectral data for **1**, **2**, **3** (150 MHz, CD_3OD)

C	1	2	3
1	171.8 (*s*)	71.3 (*t*)	71.2 (*t*)
2	128.7 (*s*)	135.7 (*s*)	131.3 (*s*)
3	144.4 (*d*)	131.2 (*d*)	130.7 (*d*)
4	23.8 (*t*)	22.6 (*t*)	24.7 (*t*)
5	39.1 (*t*)	39.0 (*t*)	40.9 (*t*)
6	75.2 (*s*)	75.3 (*s*)	50.0 (*s*)
7	78.1 (*d*)	78.1 (*d*)	86.4 (*d*)
8	30.6 (*t*)	30.6 (*t*)	39.9 (*t*)
9	38.0 (*t*)	38.0 (*t*)	26.6 (*t*)
10	136.3 (*s*)	136.3 (*s*)	88.2 (*s*)
11	125.6 (*d*)	125.6 (*d*)	56.9 (*d*)
12	29.3 (*t*)	29.3 (*t*)	29.0 (*t*)
13	29.3 (*t*)	29.3 (*t*)	29.2 (*t*)
14	125.6 (*d*)	125.6 (*d*)	125.8 (*d*)
15	136.3 (*s*)	136.3 (*s*)	136.4 (*s*)
16	38.0 (*t*)	38.0 (*t*)	37.9 (*t*)
17	30.6 (*t*)	30.6 (*t*)	30.6 (*t*)
18	78.1 (*d*)	78.1 (*d*)	78.0 (*d*)
19	75.2 (*s*)	75.3 (*s*)	75.3 (*s*)
20	39.1 (*t*)	39.0 (*t*)	39.2 (*t*)
21	22.6 (*t*)	22.6 (*t*)	22.6 (*t*)
22	127.9 (*d*)	127.3 (*d*)	127.3 (*d*)
23	135.7 (*s*)	135.7 (*s*)	135.7 (*s*)
24	69.0 (*t*)	69.0 (*t*)	69.0 (*t*)
25	12.4 (*q*)	14.0 (*q*)	13.7 (*d*)
26	21.8 (*q*)	21.9 (*q*)	20.1 (*q*)
27	16.3 (*q*)	16.3 (*q*)	19.1 (*q*)
28	16.3 (*q*)	16.3 (*q*)	16.4 (*q*)
29	21.8 (*q*)	21.9 (*q*)	22.0 (*q*)
30	13.7 (*q*)	13.7 (*q*)	14.0 (*q*)
1-$\underline{CH_3}CO$		20.8 (*q*)	20.8 (*q*)
1-$\underline{CH_3CO}$		172.8 (*s*)	172.9 (*s*)

molecular formula of **6** was determined to be $C_{37}H_{62}O_7Na$ [*m*/*z* 641.4399] by HR–FABMS. The ^{1}H and ^{13}C NMR spectra of **6** resembled those of **4** except for the presence of two acetal carbons (δ_C 106.7), and the lower field chemical shifts (Amico et al., 1982) of C-6, C-7, C-18 and C-19, as well as protons H-7 and H-19 due to two 1,3-dioxolane rings as shown in Fig. 2. NOEs between (i) H-7 and H-26, (ii) H-18 and H-29 were observed in the NOESY spectrum of **6**, indicating that they were *cis* or α-face. Accordingly, the absolute configurations of both C-6 and C-19 were established to be *S*. The geometries of all four olefines were determined as being in the *E*-form by a NOESY spectrum of **6** as shown in Fig. 2.

Concentricol C (**2**) was obtained as an oil; the molecular formula was found to be $C_{32}H_{56}O_7$ by HR-FABMS. The IR spectrum indicated absorption bands of a hydroxyl (3414 cm^{-1}) and a carbonyl (1721 cm^{-1}) group. The NMR spectral data of **2** (Tables 1 and 2) were similar to those of concentricol (**5**) and **1**, except for the presence of an acetyl group (δ_C 20.8, 172.8) in place of a carboxylic acid group indicating that **2** was a monoacetate of concentricol. The position of the acetyl group was determined to be at C-1 due to the observed HMBC correlation between H-1 and the acetyl group. Therefore, the structure of **2** was determined to be a monoacetate of concentricol.

The absolute configuration of compound **2** was determined by the modified Mosher's method. Compound **2** was esterified with (−)-MTPA and (+)-MTPA in DCC and DMAP to give (−)-MTPA ester (**2a**) and (+)-MTPA-ester (**2b**), respectively. The $\Delta\delta$ values [$\delta_{(-)}-\delta_{(+)}$] indicated that the absolute configuration of both C-7 and C-18 were *S* (Fig. 3). To establish the configurations of C-6 and C-19, **2** was treated in the same manner as **4** to give **7**, the molecular formula of which was found to be $C_{38}H_{64}O_7Na$ by HR-FABMS. The ^{1}H NMR spectrum of **7** was closely related to that of **6**. The configuration of both C-6 and C-19 of **6** were determined to be *S* by the NOEs correlations between (i) H-7 and H-26, (ii) H-18 and H-29 (Fig. 4).

Concentricol D (**3**) had a *quasi*-molecular ion peak at *m*/*z* 557 in HR-FABMS suggesting that the molecular formula of **3** was $C_{32}H_{54}O_6Na$. Its IR spectrum showed absorption bands of a hydroxyl (3365 cm^{-1}) and a carbonyl (1735 cm^{-1}) group. The ^{1}H and ^{13}C NMR spectral data of **3** (Tables 1 and 2) were very similar to those of **2** except for chemical shifts of C_5–C_{11}, C_{26} and C_{27}. Compound **3** showed long-range correlations between (i) H-26/C-6, C-7 and C-11; (ii) H-27/C-8, C-9, C-10 and C-11; (iii) H-7/C-8, C-10 and C-11; (iv) H-11/C-6 and C-10 in the HMBC spectrum (Fig. 5). Thus, compound **3** was found to contain a six-membered ring. The connection between C-7 and C-10 via an ether bond was confirmed by correlation between H-7 and C-10 in the

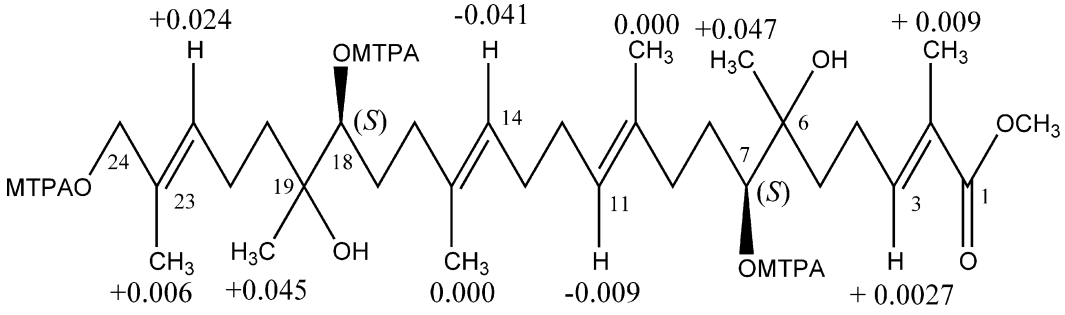

Fig. 1. $\Delta\delta$ values [$\delta(-)-\delta(+)$] for (+)- and (−)-MTPA esters of compound **4**.

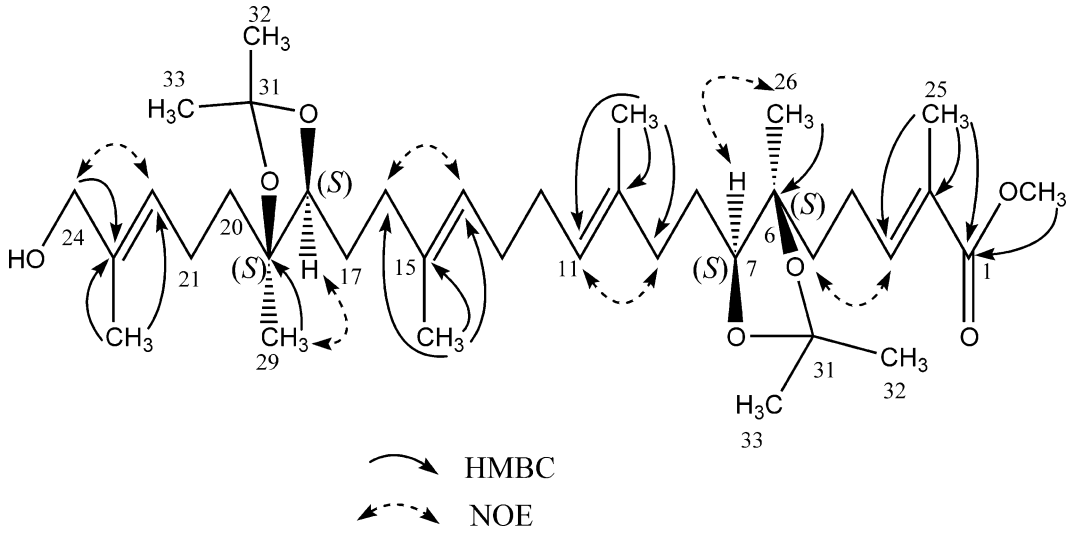

Fig. 2. The important HMBC and NOESY correlations of compound **6**.

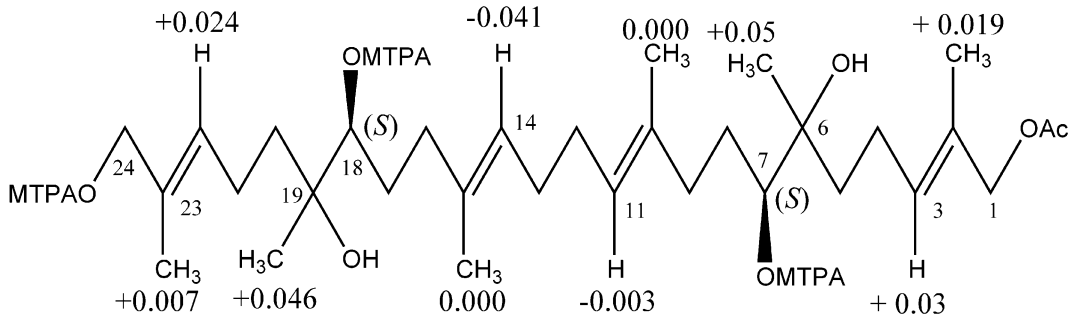

Fig. 3. $\Delta\delta$ values [$\delta(-)-\delta(+)$] for (+)- and (−)-MTPA esters of compound **2**.

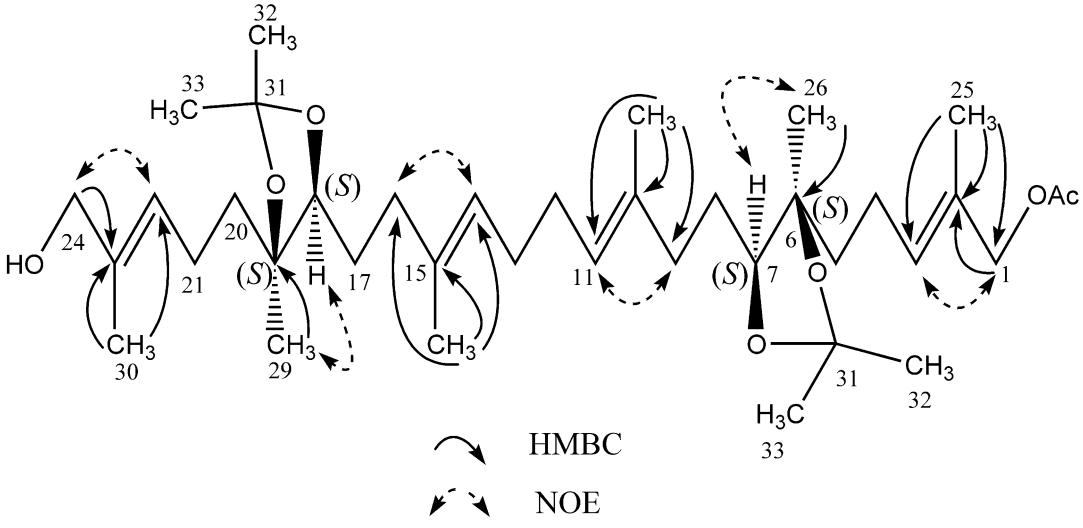

Fig. 4. The important HMBC and NOESY correlations of compound **7**.

HMBC spectrum (Fig. 5). The NMR spectral data of this ring system were very similar to those of farnesyl hydroxybenzoic acid derivatives (Chen et al., 2001). The configuration of C-6, C-7, C-10 and C-11 were determined to be 6*R**, 7*R**, 10*S** and 11*R** due to the correlations between H-7 and H-26, H-26 and H-27 in the NOESY spectrum (Fig. 5). Therefore, the structure of **3** was elucidated as shown in Fig. 1. The absolute structure of **3** remains unclear.

Formally, concentricols might be derived from squalene (Stadler et al., 2001b). Possible biosynthetic pathways **1**–**5** are illustrated in Fig. 6. Starting from

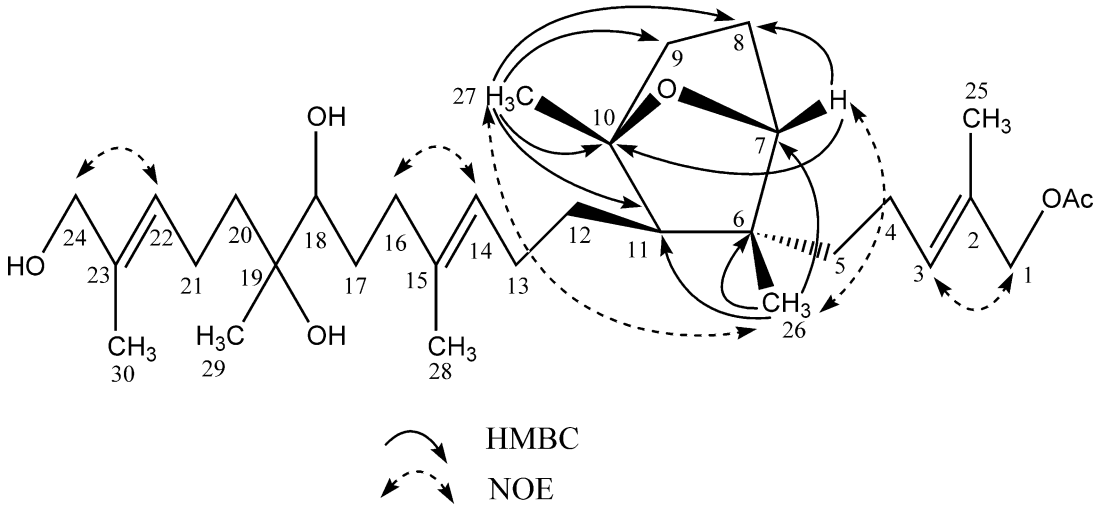

Fig. 5. The important HMBC and NOESY correlations of compound **3**.

squalene as a precursor, concentricol may be formed by oxidases and later converted into compound **1** by oxidative introduction of a terminal carboxyl group. Whereas **2** is formed from concentricol by acetylation, compound **3** may be derived from an intramolecular attack, starting from an epoxy intermediate which occurs during the biosynthesis of concentricol as a common precursor. From this intermediate compound, an acid-catalysed radical-mediated cyclisation would finally result in the formation of compound **3**. However plausible, the hypothesis outlined in Fig. 6 stands in contradiction to previous results reported by O'Hagan et al. (1992), who proposed that cubensic acid from *Xylaria cubensis* (i.e., a structurally related metabolite from a fungal species taxonomically closely related to *Daldinia*) was produced via the acetate–malonate pathway rather than from a terpenoid precursor such as squalene. The biogenesis of concentricols in *Daldinia* should therefore be studied, e.g., by incorporation experiments using labeled mevalonate to verify that

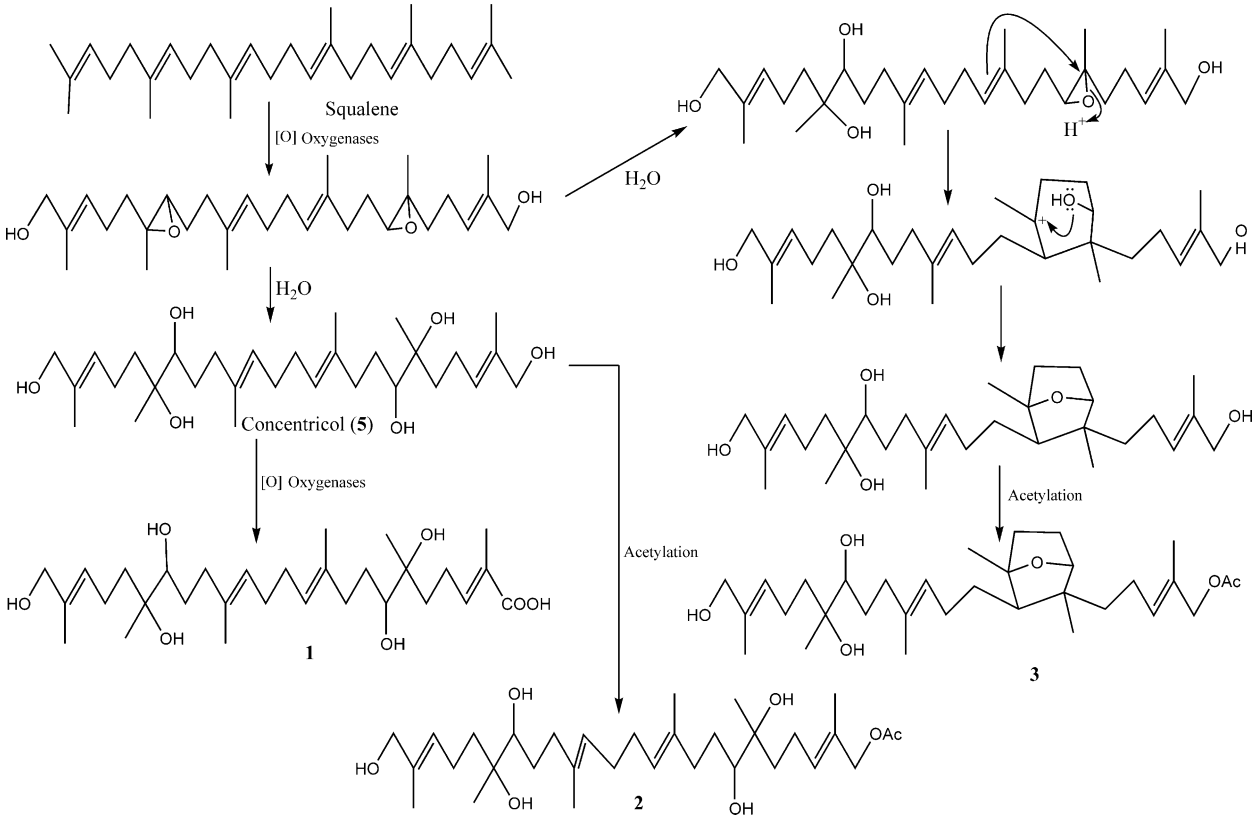

Fig. 6. Possible biosynthetic pathway for compounds **1**–**3**.

squalene from the terpene metabolism is actually involved. Furthermore, it remains to be seen whether HPLC-based studies on the distribution of these new metabolites in *Daldinia* and other Xylariaceae will result in additional evidence on the chemotaxonomy of these fungi.

3. Experimental

3.1. General

NMR spectra were recorded on Varian Unity 600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR), using either $CDCl_3$ or CD_3OD as solvent. Chemical shifts are given with TMS used as internal standard (^{1}H NMR), and δ 77.03 (ppm) from $CDCl_3$ and δ 49.00 (ppm) from CD_3OD as a standard (^{13}C NMR). Mass spectra including high-resolution FAB mass spectra were recorded on a JEOL JMS AX-500 spectrometer. IR spectra were measured on JASCO FT/IR-5300 spectrophotometer. The UV spectra were obtained on a Shimadzu UV-1650PC in MeOH solution. The specific optical rotations were measured on a JASCO DIP-1000 polarimeter with MeOH as solvent. HPLC was performed on Shimadzu liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II or 5 SL-II column. TLC was performed on silica gel plates (Kieselgel 60 F254, Merck) and reversed phase C_{18} silica gel plates (Merck), using solvent system A: $CHCl_3$–MeOH–H_2O (65:15:10, lower phase) and solvent system B: CH_3CN–H_2O (70:30). The spots on TLC were detected under UV 254 nm and by spraying with 10% H_2SO_4 or Godin reagent (Godin, 1954), followed by heating at 120 °C.

3.2. Materials

Fruiting bodies of *Daldinia concentrica* were collected and identified by M.S., J.D. Rogers and H. Wollweber from trunks of *Fraxinus excelsior* in the Neandertal near Haan-Gruiten, North Rhine Westphalia, Germany, in April 2000 and June 2001 and freeze-dried immediately after harvest. Voucher specimens, which show the characteristics of the genus and species sensu Rogers et al. (1999) and Wollweber and Stadler (2001) are deposited at WSU (Washington State University, Pullman, WA), at the mycological herbarium of the Fuhlrott Museum, Wuppertal, Germany (accession numbers Ww 3912) and at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Japan (numbered VN02002).

3.3. Extraction and isolation

Dried fruit bodies (133.0 g) of *D. concentrica* were extracted with EtOAc at room temp. The EtOAc extract was concentrated in vacuo to give a residue (8.49 g), which was applied to a silica gel (CC) column using hexane: EtOAc (5:1) as eluent to give 10 fractions (Fractions 1–10). Fraction 9 (872.1 mg) was purified by Sephadex LH-20 with solvent MeOH–$CHCl_3$ (1:1) and then silica gel CC using $CHCl_3$:MeOH:H_2O (13:3:2, lower phase) to give concentricol B (**1**) (95.9 mg). Fraction 8 (1034.6 mg) was also subjected to Sephadex LH-20 chromatography using $CHCl_3$:MeOH (1:1) and then silica gel CC using $CHCl_3$:MeOH:H_2O (25:2:0.1) as an eluent to give two sub-fractions, which were purified by prep. HPLC with reversed phase column, flow rate 1 ml/min, solvent system CH_3CN–H_2O (7:3) to give concentricol C (**2**) (28.6 mg) and concentricol D (**3**) (2.3 mg).

3.3.1. Concentricol B (*1*)

Oil, $[\alpha]_D^{20}$ −6.8° (*c* 0.97; CH_3OH). HR-FABMS: *m/z* 547.3644 $[M+Na]^+$, $C_{30}H_{52}O_7Na$, requires 547.3611. UV λ_{max} (CH_3OH) nm (log ε): 207 (4.25). IR (KBr) cm^{-1}: 3359, 2929–2525, 1687, 1644, 1448, 1384, 1255, 1153, 1071, 981. ^{1}H and ^{13}C NMR (CD_3OD) (Tables 1 and 2).

3.3.2. Concentricol C (*2*)

Oil, $[\alpha]_D^{20}$ −4.5° (*c* 1.2; CH_3OH). HR-FABMS: *m/z* 575.3916 $[M+Na]^+$, $C_{32}H_{56}O_7Na$, requires 575.3924. IR (KBr) cm^{-1}: 3414, 2936, 1722, 1670, 1451, 1380, 1262, 1073, 1024, 855. ^{1}H and ^{13}C NMR (CD_3OD) (Tables 1 and 2).

3.3.3. Concentricol D (*3*)

Oil, $[\alpha]_D^{20}$ −5.6° (*c* 0.5, CH_3OH). HR-FABMS: *m/z* 557.3799 $[M+Na]^+$, $C_{32}H_{54}O_6Na$, requires 557.3818. IR (KBr) cm^{-1}: 3365, 2935, 1735, 1600, 1458, 1382, 1232, 1067, 1024, 980. ^{1}H and ^{13}C NMR (CD_3OD) (Tables 1 and 2).

3.3.4. Methylation of concentricol B (*1*)

Compound **1** (29.5 mg) was methylated with MeOH (3 ml) and $(CH_3)_2SiCHN_2$ (1 ml) at 5 °C. After stirring for 120 min, one drop of acetic acid was added and then evaporated. The residue was purified by prep. HPLC using EtOAc as solvent, flow rate 1 ml/min to give **4** (16 mg). HR-FABMS: *m/z* 561.3766 $[M+Na]^+$, $C_{31}H_{54}O_7Na$, requires 561.3767. IR (KBr): 3386, 2950, 1700, 1647, 1437, 1386, 1282, 1193, 1072, 938 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 6.78 (1H, *ddd*, *J* = 1.4, 6.0, 8.8 Hz, H-3), 5.43 (1H, *ddd*, *J* = 1.1, 6.0, 8.2 Hz, H-22), 5.19 (2H, *br d*, *J* = 1.1 Hz, H-11 and H-14), 4.00 (2H, *s*, H-24), 3.79 (3H, *s*, 1-OMe), 3.38 (2H, *br d*, *J* = 10.4 Hz, H-7 and H-18), 2.34 (1H, *m*, H-4), 2.22 (1H, *m*, H-4), 2.20 (2H, *m*, H-9 and H-16), 2.18 (1H, *m*, H-21), 2.08 (3H, *m*, H-9, H-16 and H-21), 2.07 (2H, *m*, H-12 and H-13), 1.85 (3H, *s*, H-25), 1.70 (1H, *m*, H-5), 1.68 (3H, *s*, H-30), 1.64 (1H, *m*, H-20), 1.62 (6H, *s*, H-27 and H-28),

1.59 (2H, *m*, H-8 and H-17), 1.42 (1H, *m*, H-5), 1.41 (2H, *m*, H-8 and H-17), 1.38 (1H, *m*, H-20), 1.16 (6H, *s*, H-26 and H-29); ^{13}C NMR ($CDCl_3$): δ 168.7 (C-1), 142.8 (C-3), 135.3 (C-10 and C-15), 134.9 (C-23), 127.4 (C-2), 126.2 (C-22), 125.2 (C-11 and C-14), 78.2 (C-7 and C-18), 74.6 (C-6), 74.3 (C-19), 68.7 (C-24), 51.7 (1-OMe), 36.7 (C-9 and C-16), 34.5 (C-20), 34.4 (C-5), 29.1 (C-8 and C-17), 27.8 (C-12 and C-13), 23.1 (C-26 and C-29), 22.8 (C-4), 21.6 (C-21), 16.0 (C-27 and C-28), 13.7 (C-30), 12.3 (C-25).

3.3.5. Preparation of (+)-MTPA ester of compound 4

To compound **4** (5 mg) in anhydrous CH_2Cl_2 (4.5 ml) was added (+)-MTPA (65 mg), DCC (70 mg), DMAP (50 mg) and stirred at room temp for 2 h 15 min. The reaction mixture was concentrated in vacuo and then the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with brine, 1 N HCl and 5% $NaHCO_3$ and brine successively and dried over $MgSO_4$. The filtrate was evaporated in vacuo to give crude oil (92.2 mg), which was purified by silica gel CC (hexane/EtOAc, 5.1) to afford (+)-MTPA ester (**4b**) (6.9 mg). HR-FABMS: *m/z* 1209.5000 $[M+Na]^+$, $C_{61}H_{75}O_{13}F_9Na$, requires 1209.4962. IR (KBr) cm^{-1}: 3504, 2951, 1745, 1718, 1650, 1496, 1451, 1379, 1271, 1169, 1123, 1081, 1018, 922. ^{1}H NMR ($CDCl_3$): δ 6.65 (1H, *t*, $J=7.4$ Hz, H-3), 5.42 (1H, *t*, $J=7.1$ Hz, H-22), 5.11 (1H, *brd s*, H-14), 5.02 (1H, *dd*, $J=1.9$, 4.7 Hz, H-11), 1.80 (3H, *s*, H-25), 1.58 (3H, *s*, H-30), 1.56 (3H, *s*, H-27), 1.52 (3H, *s*, H-28), 1.14 (3H, *s*, H-26), 1.12 (3H, *s*, H-29).

3.3.6. Preparation of (−)-MTPA ester of compound 4

To compound **4** (5 mg) in anhydrous CH_2Cl_2 (3 ml) was added to (−)-MTPA (26.5 mg), DCC (35 mg), DMAP (17.5 mg) and stirred at room temperature for 1 day. To the reaction mixture was added (−)-MTPA (27.0 mg), DCC (34.6 mg), DMAP (18.1 mg) and further stirred for 1 day. The reaction mixture was washed in the same manner as described above to give the crude oil (121 mg) which was purified by silica gel CC with hexane/EtOAc (4/1) to give (−)-MTPA ester (**4a**) (6.7 mg). HR-FABMS: *m/z* 1209.4940 $[M+Na]^+$, $C_{61}H_{75}O_{13}F_9Na$, requires 1209.4962. IR (KBr) cm^{-1}: 3420, 2931, 1746, 1715, 1637, 1517, 1451, 1377, 1270, 1169, 1123, 1082, 1018, 922. ^{1}H NMR ($CDCl_3$): δ 6.68 (1H, *t*, $J=7.4$, H-3), 5.44 (1H, *t*, $J=7.1$, H-22), 5.07 (1H, *brd s*, H-14), 5.01 (1H, *dd*, $J=1.9$, 4.7 Hz, H-11), 1.81 (3H, *s*, H-25), 1.59 (3H, *s*, H-30), 1.56 (3H, *s*, H-27), 1.52 (3H, *s*, H-28), 1.18 (3H, *s*, H-26), 1.16 (3H, *s*, H-29).

3.3.7. Acetonide of compound 4

To a solution of compound **4** (11.5 mg) in anhydrous CH_2Cl_2 (2 ml) was added *p*-TsOH (3.5 mg), 2,2-dimethoxypropane (0.5 ml) and stirred at room temperature for 5 h. The reaction mixture was evaporated and then partitioned between $CHCl_3$ and H_2O. The organic layer was washed with 5% $NaHCO_3$ and brine and then dried by $MgSO_4$. Filtration and evaporation gave crude oil (12 mg) followed by methylation as described above and then purified by prep. HPLC using EtOAc as solvent to give **6** (4 mg). HR-FABMS: *m/z* 641.4399 $[M+Na]^+$, $C_{37}H_{62}O_7Na$, requires 641.4393. IR (KBr) cm^{-1}: 3491, 2982, 2933, 2858, 1715, 1650, 1437, 1377, 1248, 1215, 1191, 1097, 1014, 932. ^{1}H NMR ($CDCl_3$): δ 6.78 (1H, *t*, $J=7.6$ Hz, H-3), 5.43 (1H, *t*, $J=7.4$ Hz, H-22), 5.19 (2H, *br s*, H-11 and H-14), 4.00 (2H, *d*, $J=4.9$ Hz, H-24), 3.73 (3H, *s*, 1-OMe), 3.70 (2H, *t*, $J=9.9$ Hz, H-7 and H-18), 2.38 (1H, *m*, H-4), 2.24 (1H, *m*, H-21), 2.21 (1H, *m*, H-4), 2.20 (2H, *m*, H-9 and H-16), 2.10 (1H, *m*, H-21), 2.05 (2H, *m*, H-9 and H-16), 2.03 (4H, *m*, H-12 and H-13), 1.85 (3H, *s*, H-25), 1.68 (3H, *s*, H-30), 1.64 (1H, *m*, H-5), 1.62 (2H, *m*, H-8 and H-17), 1.61 (6H, *s*, H-27 and H-28), 1.59 (1H, *m*, H-20), 1.47 (2H, *m*, H-8 and H-17), 1.42 (6H, *s*, H-33), 1.35 (6H, s, H-32), 1.30 (1H, *m*, H-5), 1.23 (6H, *s*, H-26 and H-29), 1.20 (1H, *m*, H-20); ^{13}C NMR ($CDCl_3$): δ 168.7 (C-1), 142.6 (C-3), 134.7 (C-23), 134.5 (C-10 and C-15), 127.5 (C-2), 126.2 (C-22), 124.8 (C-11 and C-14), 106.7 (C-31), 83.9 (C-7 and C-18), 81.4 (C-6 and C-19), 68.9 (C-24), 51.7 (1-OMe), 36.8 (C-9 and C-16), 34.7 (C-20), 33.8 (C-5), 28.3 (C-12 and C-13), 28.2 (C-32), 27.1 (C-8 and C-17), 26.8 (C-33), 22.8 (C-26 and C-29), 22.7 (C-4), 21.6 (C-21), 16.0 (C-27 and C-28), 13.6 (C-30), 12.3 (C-25).

3.3.8. Preparation of (+)-MTPA ester of concentricol C (2)

To a solution of compound **2** (4.7 mg) in CH_2Cl_2 (4.5 ml) was added (+)-MTPA (59.2 mg), DCC (68.5 mg), DMAP (47.6 mg) and stirred at room temp. for 2.5 h. Work-up in the same manner as mentioned above was conducted to yield (+)-MTPA ester (**2b**) (6.1 mg). HR-FABMS: *m/z* 1223.5160 $[M+Na]^+$, $C_{62}H_{77}O_{13}F_9Na$, requires 1223.5118. IR (KBr) cm^{-1}: 3527, 2951, 1744, 1496, 1452, 1378, 1269, 1169, 1123, 1082, 1019, 921. ^{1}H NMR ($CDCl_3$): δ 5.42 (1H, *t*, $J=7.4$ Hz, H-22), 5.36 (1H, *t*, $J=7.1$ Hz, H-3), 5.11 (1H, *br s*, H-14), 5.01 (1H, *t*, $J=6.9$ Hz, H-11), 1.63 (3H, *s*, H-25), 1.58 (3H, *s*, H-30), 1.55 (3H, *s*, H-27), 1.52 (3H, *s*, H-28), 1.12 (3H, *s*, H-26), 1.11 (3H, *s*, H-29).

3.3.9. Preparation of (−)-MTPA ester of concentricol C (2)

To a solution compound **2** (6.1 mg) in CH_2Cl_2 (5 ml) was added (−)-MTPA (61.4 mg), DCC (64.7 mg) and DMAP (50.0 mg) and stirred at room temp. for 14 h. To the reaction mixture was added (−)-MTPA (60.0 mg), DCC (64.5 mg), DMAP (50.3 mg), and futher stirred at room temp. for 6 h. Work-up in the same manner as mentioned above was conducted to yield (−)-MTPA ester (**2a**) (6.5 mg). HR-FABMS: *m/z* 1223.5070 $[M+Na]^+$, $C_{62}H_{77}O_{13}F_9Na$, requires 1223.5118. IR

(KBr) cm^{-1}: 3528, 2951, 1745, 1497, 1452, 1378, 12969, 1170, 1122, 1081, 1019. ^1H NMR ($CDCl_3$): δ 5.44 (1H, *t*, $J=7.4$ Hz, H-22), 5.39 (1H, *t*, $J=7.1$ Hz, H-3), 5.07 (1H, *br s*, H-14), 5.01 (1H, *t*, $J=7.2$ Hz, H-11), 1.65 (3H, *s*, H-25), 1.59 (3H, *s*, H-30), 1.55 (3H, *s*, H-27), 1.52 (3H, *s*, H-28), 1.17 (3H, *s*, H-26), 1.16 (3H, *s*, H-29).

3.3.10. Acetonide of concentricol C (2)

To a solution of compound **2** (5.7 mg) in CH_2Cl_2 (1 ml) was added *p*-TsOH (1.8 mg) and 2,2-dimethoxypropane (0.5 ml). The reaction mixture was treated in the same manner as described above to give **7** (5.6 mg). HR–FABMS: *m/z* 655.4509 $[M+Na]^+$, $C_{38}H_{64}O_7Na$, requires 655.4550. IR (KBr) cm^{-1}: 3481, 2982, 1740, 1457, 1376, 1220, 1107, 1019, 919. ^1H- NMR ($CDCl_3$): δ 5.98 (1H, *t*, $J=7.4$ Hz, H-3), 5.43 (1H, *t*, $J=7.4$ Hz, H-22), 5.19 (2H, *brd s*, H-11 and H-14), 4.45 (2H, *s*, H-1), 4.40 (2H, *s*, H-24), 3.69 (2H, *dd*, $J=3.6$, 9.6 Hz, H-7 and H-18), 2.25 (2H, *dd*, $J=7.1$, 12.1 Hz, H-4 and H-21), 2.19 (2H, *dd*, $J=3.6$, 9.6 Hz, H-9 and H-16), 2.09 (2H, *m*, H-4 and H-21), 2.08 (3H, s, 1-$\underline{CH_3}CO$), 2.05 (2H, *m*, H-9 and H-16), 2.03 (4H, *m*, H-12 and H-13), 1.68 (3H, *s*, H-30), 1.67 (3H, *s*, H-25), 1.63 (2H, *dd*, $J=4.7$, 9.1 Hz, H-8 and H-17), 1.62 (6H, *s*, H-27 and H-28), 1.58 (2H, *dd*, $J=4.7$, 12.6 Hz, H-5 and H-20), 1.49 (2H, *m*, H-8 and H-17), 1.42 (6H, s, H-33), 1.35 (6H, *s*, H-32), 1.25 (2H, *dd*, $J=4.9$, 12.6 Hz, H-5 and H-20), 1.22 (6H, *s*, H-26 and H-29). ^{13}H NMR ($CDCl_3$): δ 171.0 (1-$CH_3\underline{CO}$), 134.7 (C-2), 134.4 (C-10 and C-15), 131.7 (C-23), 129.8 (C-3), 126.2 (C-22), 124.8 (C-11), 124.7 (C-14), 106.6 (C-31), 83.9 (C-7 and C-18), 81.5 (C-6 and C-19), 70.3 (C-1), 68.9 (C-24), 36.8 (C-9 and C-16), 34.7 (C-20), 34.5 (C-5), 28.4 (C-33), 28.3 (C-12 and C-13), 27.0 (C-8 and C-17), 26.9 (C-32), 22.8 (C-26 and C-29), 21.7 (C-4), 21.6 (C-21), 21.0 (1-$\underline{CH_3}CO$), 16.0 (C-27 and C-28), 13.9 (C-25), 13.6 (C-30).

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