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Terpenoids from *Tripterygium doianum* (Celastraceae)

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Abstract

The extract of *Tripterygium doianum* (Celastraceae) afforded three triterpenoids [3β-acetoxy-11-ursen-13α,30-olide, 25-chloro-24-hydroxytirucall-7-en-3-one and tirucall-7-en-3,24-dione], two sesquiterpenoids [5α-acetoxy-1β,8α-bis-cinnamoyl-4α-hydroxydihydroagarofuran and 5α-acetoxy-1β-benzoyl-8α-cinnamoyl-4α-hydroxydihydroagarofuran] and nine known triterpenoids. Their structures were established based on spectroscopic studies.

Keywords: *Tripterygium doianum*; Celastraceae; Triterpenoid; Sesquiterpenoid

1. Introduction

Members of the genus *Tripterygium* have been used as traditional Chinese drugs for the treatment of cancer and as insecticides for hundreds of years. Recently, some Chinese clinics have used *T. wilfordii* Hook to treat rheumatoid arthritis and ankylosing spondylitis (Matlin et al., 1993; Qian, 1987; Qian et al., 1995). We previously studied some of the constituents of *T. wilfordii* var. *regelii*, *T. wilfordii* and *T. hypoglaucum,* and reported their isolation, structure determination and activities (anti-HIV, immunosuppressive, and inhibitory effects on Epstein—Barr virus activation (Shishido et al., 1994; Fujita et al., 2000; Duan et al., 2000, 2001a,b).

In continuation of our previous studies in this area, we examined the constituents of *T. doianum*, which is a scrubby deciduous vine found in southern Japan. We report here isolation and structure elucidation of three triterpenes, two sesquiterpenes and nine known compounds.

2. Results and discussion

Compound **1** was assigned a molecular formula of $C_{32}H_{48}O_4$ based on HR–FAB–MS. Its IR spectrum showed a lactone carbonyl (1757 cm^{-1}) and a carbonyl band (1728 cm^{-1}). The ^1H NMR spectrum of **1** indicated the presence of two methine protons [δ_H 5.95 (1H, *d*, $J=10.2$ Hz) and 5.54 (1H, *dd*, $J=10.2$, 3.0 Hz)] attached to a double bond, one oxygenated methine proton [δ_H 4.50 (1H, *dd*, $J=10.4$, 6.0 Hz)], one acetyl group [δ_H 2.06 (3H, *s*)], six methyls [δ_H 1.17, 1.06, 0.94, 0.94, 0.87, 0.87 (each 3H, *s*)] and one secondary methyl [δ_H 1.01 (3H, *d*, $J=6.1$ Hz)]. The ^{13}C NMR spectrum of **1** showed two carbonyl carbons [δ_C 179.9, 171.1], one double bond [δ_C 133.4, 129.0], and six methines, eight methylenes, eight methyls and six quaternary carbon signals. Based on these data, compound **1** was assumed to be an ursen-type triterpene. In comparison with the data for triterpenes from Celastraceae, its ^{13}C NMR spectral data for C-1–C-10, C-23 and C-24 were to similar those of grahamidiol acetate (Sukumar et al., 1995). In its HMBC spectrum, the correlation of H-11 (δ_H 5.95) with C-8 (δ_C 41.8), C-9 (δ_C 53.0), C-10 (δ_C 36.4) and C-13 (δ_C 89.7), and H-12 (δ_H 5.54) with C-9 and C-14 (δ_C 42.0) indicated that the double bond can be placed between C-11 and C-12. The correlations of H_3-27 (δ_H 1.17) with C-8, C-14, C-15 (δ_C 25.6) and C-13, and H-21 (δ_H 2.13) with C-30 (δ_C 179.9) indicated that the lactone ring was located between C-13 and C-20. The configuration of the acetoxyl on C-3 was determined from the coupling constants of H-3 ($J=10.4$, 6.0 Hz) and the correlation of H-3 with H-5 in the NOESY spectrum. The relative configurations of C-13, C-19 and C-20 were determined based on the following NOESY results: H-12 with H_3-29; and H_3-29 with H-20. Based

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on these results, the structure of **1** was assigned to be 3β-acetoxy-11-ursen-13α, 30-olide (Fig. 1). Furthermore, the generated conformations were optimized by the molecular mechanics calculations with AMBER force field. One conformation having the boat form in D-ring has 71.74 kcal/mol, and the another having the chair form has 60.87 kcal/mol (Fig. 2). This fact clearly indicate that the conformation of D-ring is the chair form. This is the first 13α,30-olide ursen-type triterpenoid isolated from a natural source.

Compound **2** was assigned a molecular formula of $C_{30}H_{49}O_2Cl$ based on HR–EIMS. The IR spectrum showed a hydroxyl group (3460 cm^{-1}) and a ketone group (1703 cm^{-1}). The ^1H NMR spectrum revealed the presence of one methine proton that was associated with the double bond [δ_H 5.31 (1H, *d*, $J=3.1$ Hz)], one oxygenated methine [δ_H 3.43 (1H, *brd*, $J=9.9$ Hz)], one doublet methyl [δ_H 0.87 (3H, *d*, $J=6.2$ Hz)], and seven methyl groups [δ_H 1.61, 1.56, 1.12, 1.06, 1.03, 1.01, 0.84 (each 3H, *s*)]. The ^{13}C NMR spectral data of **2** were very similar to those of 24*S*,25-dihydroxytirucall-7-en-3-one (**6**), except for the chemical shifts of C-25, C-26, and C-27. In the HMBC spectrum, the signals of H-2 (δ_H 2.73), H_3-30 (δ_H 1.12) and H_3-29 (δ_H 1.06) were correlated with the signal of C-3 (δ_C 216.9), the signal of H_3-28 (δ_H 1.03) was correlated with the signal of C-8 (δ_C 146.0), and the signals of H_3-26 (δ_H 1.61) and H_3-27 (δ_H 1.56) were correlated with the signals of C-24 (δ_C 80.0) and C-25 (δ_C 77.1). These findings indicated that the ketone group was located at C-3, the double bond was at C-7, and the hydroxyl or chloride was at C-24 or C-25, which suggested that **2** had the same framework as **6**. Acetylation of **2** gave monoacetate **2a**, for which the H-24 signal at δ_H 3.43 in ^1H NMR was shifted downfield to δ_H 4.97. This clearly indicated that the hydroxyl group and chlorine function were located at C-24 and C-25, respectively.

Compound **3**, $C_{30}H_{48}O_2$, exhibited one methine proton attached to the double bond [δ_H 5.31 (1H, *d*, $J=3.0$ Hz)], three secondary methyls [δ_H 1.10 (6H, *d*, $J=7.0$ Hz), 0.84 (3H, *d*, $J=6.2$ Hz)], and five methyls [δ_H 1.12, 1.06, 1.02, 1.01, 0.84 (each 3H, *s*)] in its ^1H NMR spectrum. The ^{13}C NMR spectrum of **3** was very similar to that of **2** (Table 1), except for the chemical shifts of C-22–27. In the HMBC spectrum of **3**, the correlation of H_3-30 (δ_H 1.12) and H_3-29 (δ_H 1.06) with C-3 (δ_C 216.9) indicated that a ketone was located on C-3, and the correlation of H_3-26 (δ_H 1.10) and H_3-27 (δ_H 1.10) with C-24 (δ_C 215.2) indicated that a ketone was located on C-24. Therefore, the structure of **3** was determined to be 3,24-dioxotirucall-7-en.

Compound **4** showed a hydroxyl absorption band at 3549 cm^{-1} and a carbonyl absorption band at 1715 cm^{-1} in its IR spectrum, and its UV spectrum showed the presence of an aromatic moiety (222 nm). The ^1H NMR spectrum indicated the presence of two benzene rings, four olefinic protons [δ_H 7.52, 6.27 (each 1H, *d*, $J=16.0$ Hz), and 7.36, 6.34 (each 1H, *d*, $J=16.0$ Hz)], three oxygenated methine protons [δ_H 5.50, 5.48, 4.88], one acetylmethyl proton [δ_H 2.14 (3H, *s*)], and four methyls [δ_H 1.54, 1.51, 1.44, 1.36 (each 3H, *s*)]. The ^{13}C NMR spectrum indicated the presence of two cinnamoyl groups, one acetyl group, two quaternary carbons attached to an oxygen function, three methine carbon attached to an oxygen function, two quaternary carbons, one methine carbon, three methylene carbons, and four methyl carbons. These data agree with a molecular formula of $C_{35}H_{40}O_8$, which was supported by HR–EIMS. It was concluded that **4** was a sesquiterpene derived from dihydroagarofuran polyol esters found in Celastraceae, and had one acetyl and two cinnamoyl groups. In the HMBC spectrum, the signal of H-8 (δ_H 4.88) was correlated with the signal of the carbonyl carbon of the cinnamoyl group (δ_C 166.1), as well

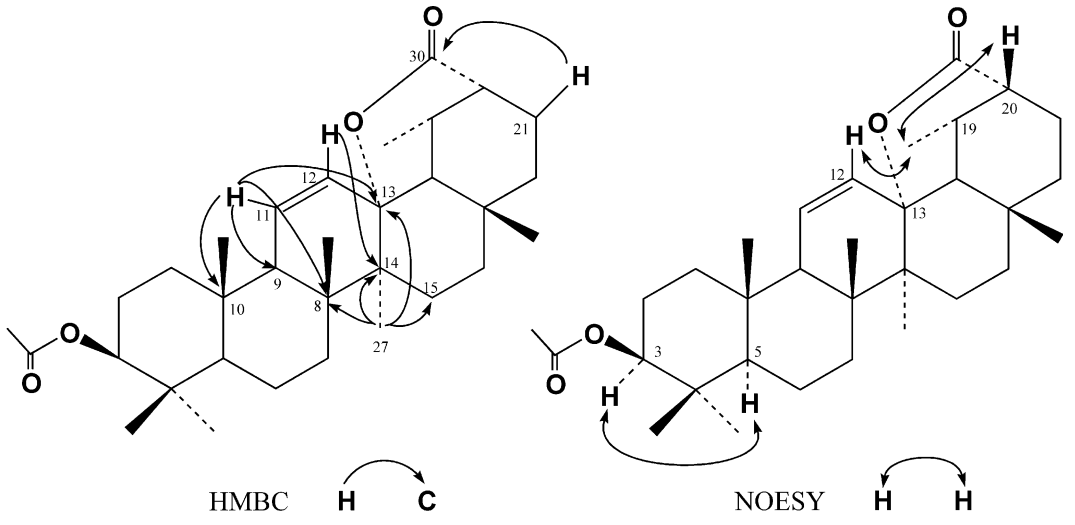


Fig. 1. The HMBC and NOESY spectral data of **1**.

as with C-9 (δ_C 51.6), C-10 (δ_C 91.7), C-7 (δ_C 32.0), C-6 (δ_C 49.2), and C-11 (δ_C 19.9), the signal of H-5 (δ_H 5.50) was correlated with the signal of the carbonyl carbon of the acetyl group (δ_C 170.6), as well as C-9, C-10, C-6, and C-13 (δ_C 84.4); the signal of H-1 (δ_H 5.48) was correlated with the signal of C-11, and the signal of H_3-12 (δ_H 1.36) was correlated with the signals of C-3 (δ_C 38.7), C-4 (δ_C 70.6), and C-10 (δ_C 91.7). These findings indicated that two cinnamoyl groups, one acetyl group, and one hydroxyl group were attached to C-1, C-8, C-5, and C-4, respectively. In the NOESY spectrum, the signal of H_3-11 (δ_H 1.44) was correlated with those of H-8, H-5 and H_3-12, and the signal of H-5 was correlated with that of H_3-12. These findings showed that the structure of **4** was 5α-acetoxy-1β,8α-bis-cinnamoyl-4α-hydroxydihydroagarofuran.

The ^{13}C NMR spectrum of compound **5** ($C_{33}H_{38}O_8$) was very similar to that of **4**, except for one benzoyl group in **5** and one cinnamoyl group in **4**. In the HMBC spectrum, the correlation of H-1 (δ_H 5.46) with carbonyl carbon of the cinnamoyl (δ_C 165.9), H-5 (δ_H 5.54) with the carbonyl carbon of the acetyl (δ_C 170.6), and H-8 (δ_H 5.09) with the carbonyl carbon of the benzoyl (δ_C 165.5) indicated that the acetyl group, benzoyl group, and cinnamoyl group were attached to C-5, C-8, and C-1, respectively.

The following known compounds were identified by comparison with literature data: 24*S*,25-dihydroxytirucall-7-en-3-one (**6**) (Mulholland et al., 1998), 2-hydroxy-3-oxo-12-ursen-28-oic acid (**7**) (Tsichritzis and Jakupovic, 1990), cangoronine (**8**) (Itokawa et al., 1991), 3β-hydroxy-2-oxofriedelan-20α-carboxylic acid (**9**) (Sousa et al., 1990), wilforic acid C (**10**) (Li et al., 1997), polpunoic acid (**11**) (Itokawa et al., 1991), triptohypol B (**12**) (Duan et al., 1997), triptocalline A (**13**) (Nakano et al., 1997), 3-epikatonic acid (**14**) (Coxon and Wells, 1980).

3. Experimental

3.1. General

NMR experiments were run on a Bruker ARX-400 instrument ^1H NMR: 400 MHz, ^{13}C NMR 100 MHz, using TMS as int. stand. MS were obtained on a Jeol JMSD-300 instrument. Chromatography column: silica gel 60 (Merck), Sephadex LH-20 (Pharmacia), and Toyopearl HW-40 (TOSOH); HPLC: (Shodex H-2001, 2002, $CHCl_3$; Asahipak, GS-310 2G, MeOH), silica gel HPLC (YMC-Park SIL-06 SH-043–5-06, 250×20 mm). IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin-Elmer), and UV spectra were measured on a UV 2100 UV–vis recording spectrometer (Shimadzu). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

3.2. Plant material

The branches of *Tripterygium doianum* were collected in October 1998 from Miyazaki Prefecture, the southern part of Kyushu island, Japan and deposited (specimen number UTP98008) in the botanical garden of the University of Tokushima.

3.3. Extraction and isolation of compounds

The branches of *Tripterygium doianum* (15.6 kg) was crushed and extracted (3×50 l) with MeOH at 60 °C for 6 h. The MeOH extracts were concentrated in vacuo to give a residue (1.08 kg), which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (238 g), which was subjected to silica gel chromatography (1.2 kg, 90×850 mm). The column was eluted with solvents of increasing polarity to give 14 frs. (fr. 1–14). Fr. 3 (11 g) was subjected to chromatography on medium pressure liquid chromatography (MPLC), eluting with $CHCl_3$, $CHCl_3$–MeOH (97:3, 9:1), and MeOH to give 11 frs. (fr. 3.1– 3.11). Fr. 3.5 (390 mg) on GPC ($CHCl_3$) and Si gel HPLC (hexane:EtOAc, 5:2) yielded **8** (13.5 mg). Fr. 3.3 was subjected to a Toyopearl column with $CHCl_3$–MeOH (2:1), and HPLC ($CHCl_3$: hexane, 8:2) to give frs. (fr. 3.3.1–3.3.5). Fr. 3.3.1 was subjected to GPC ($CHCl_3$) yielded 4 frs. (fr. 3.3.1.1–3.3.1.4). Frs. 3.3.1.2 was separated by prep. TLC (hexane: EtOAc, 2:1 and $CHCl_3$:hexane, 8:2) to give **3** (7.8 mg). Fr. 3.3.4 on GPC ($CHCl_3$) and prep. TLC ($CHCl_3$:hexane, 8:2) yielded **1** (7.7 mg) and **2** (3.6 mg). Fr. 8 (10.0 g) was sub-

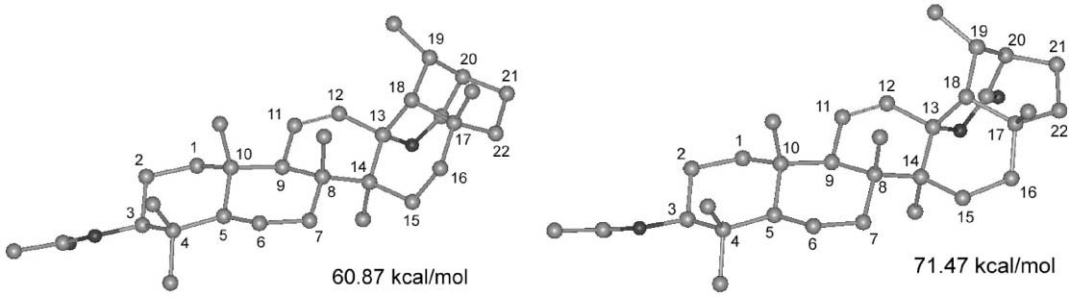


Fig. 2. Possible conformations of **1**.

jected to a Toyopearl column ($CHCl_3$:MeOH, 1:1) to give frs. (fr. 8.1–8.7). Fr. 8.2 was subjected to chromatography on a MPLC, eluting with $CHCl_3$–MeOH ($CHCl_3$:MeOH, 99:1, 95:5, 9:1) and MeOH to give 12 frs. (fr. 8.2.1–8.2.12). Fr. 8. 2. 1 on GPC ($CHCl_3$) and HPLC (hexane:EtOAc, 2:1) yielded **4** (5.8 mg) and **5** (10.3 mg). Fr. 8.2.5 and fr. 8.2.8 were separated by GPC ($CHCl_3$) and HPLC (hexane:EtOAc, 2:1 and 3:2, respectively) to give **13** (4.6 mg), **14** (4.5 mg), and **6** (67.6 mg). On the other hand, fr. 8 (9.7 g) was subjected to a Si gel chromatography column with $CHCl_3$–MeOH ($CHCl_3$:MeOH, 9:1) to give frs. (fr. 8.8–8.14). Fr. 8.14 was separated by a Sephadex LH-20 chromatography column with MeOH to yield 5 frs. (fr. 8.14.1–8.14.5). Fr. 8.14.3 on GPC (MeOH) yielded 6 frs. (fr. 8.14.3.1–8.14.3.6) and **7** (51.1 mg). Fr. 8.14.3.6 was applied to on Si gel HPLC (hexane:EtOAc, 3:2) to give **11** (12.3 mg) and **9** (3.8 mg).

Table 1
^{13}C NMR spectral data compounds **1–5** and grahamidiol acetate

	1	Grahamidiol acetate	**2**	**3**	**4**	**5**
C-1	38.1	38.5	28.6	38.6	72.6	73.0
C-2	23.4	25.4	35.0	35.0	23.8	23.6
C-3	80.7	80.9	216.9	216.9	38.7	38.8
C-4	37.9	37.7	48.0	48.0	70.6	70.6
C-5	55.0	55.3	52.4	52.4	79.9	79.9
C-6	17.7	18.3	24.5	24.5	49.2	49.2
C-7	31.4	32.8	117.9	118.0	32.0	32.0
C-8	41.8	40.0	146.0	146.0	72.9	73.2
C-9	53.0	47.6	48.5	48.5	51.6	51.7
C-10	36.4	36.0	35.1	35.1	91.7	91.7
C-11	133.4	23.5	18.4	18.4	19.9	20.0
C-12	129.0	125.1	33.8	33.7	24.1	24.2
C-13	89.7	138.5	43.7	43.6	84.4	84.5
C-14	42.0	42.0	51.4	51.4	25.9	25.9
C-15	25.6	26.9	34.1	34.1	29.8	29.7
C-16	30.9	29.3	28.5	29.0	–	–
C-17	45.2	33.6	53.3	53.0	–	–
C-18	40.4	58.8	22.3	22.1	–	–
C-19	38.2	36.8	12.9	12.9	–	–
C-20	60.7	43.9	36.5	35.7	–	–
C-21	22.9	28.1	18.9	18.6	–	–
C-22	31.3	40.8	32.3	28.5	–	–
C-23	27.8	28.1	29.0	37.9	–	–
C-24	16.1	15.7	80.0	215.2	–	–
C-25	19.2	16.1	77.1	40.9	–	–
C-26	19.0	16.8	29.4	18.4	–	–
C-27	16.2	23.5	27.1	18.5	–	–
C-28	18.1	28.5	27.5	27.6	–	–
C-29	17.9	17.1	24.7	24.7	–	–
C-30	179.9	68.2	21.7	21.7	–	–
3β-OAc	171.1	171.3	–	–	–	–
	21.4	21.3	–	–	–	–
30-OAc	–	170.9	–	–	–	–
	–	21.0	–	–	–	–

Measured in $CDCl_3$; **4**: (1β,8α-O-Cin; 166.3, 166.1, 144.7, 145.4, 118.2, 118.0, 134.5, 134.4, 130.3, 130.2, 128.9, 128.8, 128.4, 128.2; 5α-OAc; 170.6, 21.7); **5**: (1β-O-Cin; 165.9, 144.3, 134.5, 128.8, 128.1, 130.1; 1α-OCO-Bz; 165.5, 129.7, 130.1, 128.2, 133.1; 5α-OAc; 170.6, 21.7).

Fr. 8.14.4 was separated by GPC ($CHCl_3$) to give 5 frs. (fr. 8.14.4.1–8.14.4.5). Fr. 8.14.4.2 was recrystalized (MeOH) to give **8** (30.0 mg). Fr. 9 (6.7 g) was subjected to silica gel chromatography with $CHCl_3$–MeOH (9:1) to give five frs. (fr. 9.1–9.5). Fr. 9.2 was subjected to a Sephadex LH-20 chromatography with MeOH to give five frs. (fr. 9.2.1–9.2.5). Fr. 9.2.4 on GPC ($CHCl_3$) and Si gel HPLC (hexane:EtOAc, 1:1) yielded **12** (9.1 mg).

3.4. 3β-Acetoxy-11-ursen-13α,30-olide (*1*)

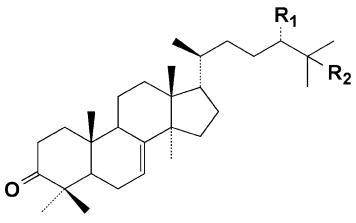
Amorphous powder. $[\alpha]_D^{25}+46.9^\circ$ (MeOH, *c* 0.3); IR (KBr) ν_{max} cm^{-1}: 3732, 2964, 2925, 2863, 2360, 1757, 1728, 1469, 1365, 1242, 1144, 1093, 1024, 1242, 1144, 1093, 1024, 991, 903, 866, 648; HR–FABMS: *m/z* 496.3544 $[M]^+$, calc. for $C_{32}H_{48}O_4$, 496.3553; ^1H NMR ($CDCl_3$): 5.95 (1H, *d*, $J=10.2$ Hz, H-11), 5.54 (1H, *dd*, $J=10.2$, 3.0, H-12), 4.50 (1H, *dd*, $J=10.4$, 6.0 Hz, H-3), 2.13 (1H, *m*, H-21), 2.06 (3H, *s*, -OAc), 1.98 (1H, *brs*, H-9), 1.17 (3H, *s*, H_3-27), 1.06 (3H, *s*, H_3-26), 1.01 (3H, *d*, $J=6.1$, H_3-29), 0.94 (6H, *s*, H_3-28, H_3-25), 0.87 (6H, *s*, H_3-23, H_3-24); ^{13}C NMR ($CDCl_3$): Table 1.

3.5. Theoretical computations of *1*

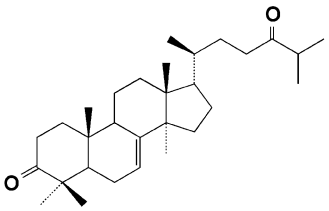
In this complicated molecule, the conformational search computation cannot be performed by the conventional program CONFLEX based on the corner-flapping/edge-flipping algorithm (Goto et al., 1989, 1998; Goto and Osawa, 1993). Thus, an exhaustive generation of conformations, our FORTRAN program was developed with the modifications from the corner-flapping/edge-flipping algorithm. In our program, the generated conformations were optimized by the molecular mechanics calculations with AMBER force field (Weiner and Kollman, 1981; Weiner et al., 1986; Damm et al., 1997). Atomic charges of the computed molecule were estimated by the semi-empirical molecular orbital calculation with PM3 Hamiltonians in MOPAC program.

3.6. 25-Chloro-24-hydoroxytirucall-7-en-3-one (*2*)

Amorphous powder. $[\alpha]_D^{25}$ -15.8° (MeOH, *c* 0.6); IR (KBr)ν_{max} cm^{-1}: 3460, 2969, 1703, 1458, 1388; HR–EIMS: *m/z* 476.3395 (calc. for $C_{30}H_{49}O_2Cl$, 476.3421); ^1H NMR ($CDCl_3$): 5.31 (1H, *d*, $J=3.1$ Hz, H-7), 3.43 (1H, *brd*, $J=9.9$ Hz, H-24), 2.73 (1H, *ddd*, $J=14.5$, 14.5, 5.4 Hz, H-2), 2.29 (1H, *m*, 9H), 1.61 (3H, *s*, H_3-26), 1.56 (3H, *s*, H_3-27), 1.12 (3H, *s*, H_3-30), 1.06 (3H, *s*, H_3-29), 1.03 (3H, *s*, H_3-28), 1.01 (3H, *s*, H_3-19), 0.87 (3H, *d*, $J=6.2$, H_3-21), 0.84 (3H, *s*, H_3-18); ^{13}C NMR ($CDCl_3$): Table 1; Acetylation of **2**: Compound **2** was subjected to acetylation with Ac_2O-pyridine overnight at room temperature to give **2a**. **2a**: ^1H NMR ($CDCl_3$): 5.32 (1H, *d*, $J=3.1$ Hz, H-7), 2.76 (1H, *ddd*, $J=14.7$, 14.7, 5.2 Hz,



	R_1	R_2
2	**OH**	**Cl**
2a	**OAc**	**Cl**
6	**OH**	**OH**



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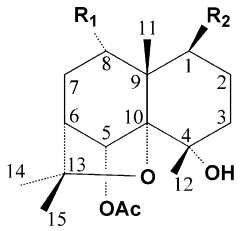
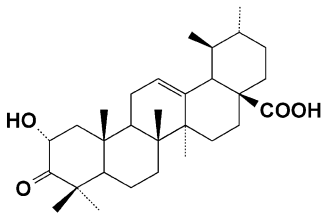


	R1	R2
4	OCin	OCin
5	OBz	OCin



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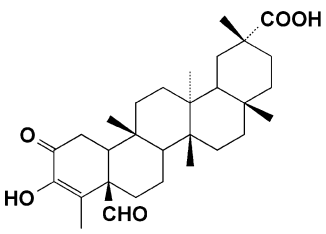
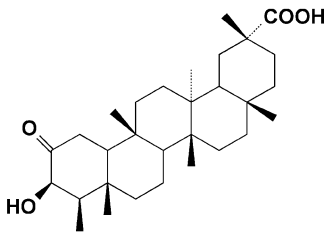
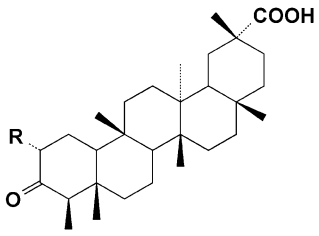


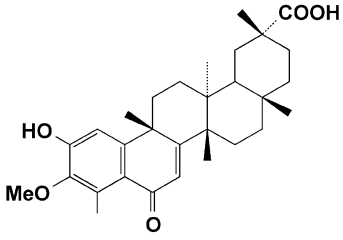
8



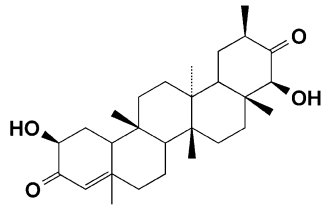
9



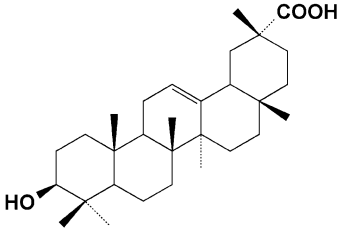
	R
10	OH
11	H



12



13



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H-2), 2.12 (3H, *s*, -OAc), 1.56 (3H, *s*, H_3-26), 1.55 (3H, *s*, H_3-27), 1.12 (3H, *s*, H_3-30), 1.06 (3H, *s*, H_3-29), 1.02 (3H, *s*, H_3-28), 1.01 (3H, *s*, H_3-19), 0.87 (3H, *d*, $J=6.4$ Hz, H_3-21), 0.80 (3H, *s*, H_3-18).

3.7. Tirucall-7-en-3,24-dione (*3*)

Amorphous powder. $[\alpha]_D^{25} = +5.93^\circ$ (MeOH, *c* 0.3); IR (KBr) ν_{max} cm^{-1}: 2965, 1709, 1466, 1384, 1273; HR–EIMS: *m/z* 440.3691 (calc. for $C_{30}H_{48}O_2$, 440.3654); ^1H NMR ($CDCl_3$) : 5.31 (1H, *d*, $J=3.0$ Hz, H-7), 2.75 (1H, *ddd*, $J=14.4$, 14.4, 5.5 Hz, H-2), 1.12 (3H, *s*, H_3-30), 1.10 (6H, *d*, $J=7.0$, H_3-26, H_3-27), 1.06 (3H, *s*, H_3-29), 1.02 (3H, *s,* H_3-28), 1.01, (3H, *s*, H_3-19), 0.84 (3H, *d*, $J=6.2$ Hz, H_3-21), 0.84 (3H, *s*, H_3-18); ^{13}C NMR ($CDCl_3$): Table 1.

3.8. 5α-Acetyl-1β,8α-bis-cinnamoyl-4α-hydroxydihydroagarofuran (*4*)

Amorphous powder. $[\alpha]_D^{25} + 198.0^\circ$ (MeOH, *c* 0.3); IR (KBr) ν_{max} cm^{-1}: 3549 2930, 1715, 1637, 1336, 1313,

1243, 1169, 1096, 975, 768; UV (MeOH) λ_{max} nm (log ε): 222 (4.3); HR–EIMS: m/z 588.2732 (calc. for $C_{35}H_{40}O_8$, 588.2723); ^{1}H NMR ($CDCl_3$) : 7.52 (1H, *d*, $J=16.0$ Hz, Cin-H), 7.47–7.31 (10H, *m*, Cin-H), 7.36 (1H, *d*, $J=16.0$ Hz, Cin-H), 6.34 (1H, *d*, $J=16.0$ Hz, Cin-H), 6.27 (1H, *d*, $J=16.0$ Hz, Cin-H), 5.50 (1H, *s*, H-5), 5.48 (1H, *m*, H-1), 4.88 (1H, *d*, $J=7.0$ Hz, H-8), 2.45 (1H, *m*, H-7), 2.14 (3H, *s*, 5-Ac), 1.54 (3H, *s*, H_3-15), 1.51 (3H, *s*, H_3-14), 1.44 (3H, *s*, H_3-11), 1.36 (3H, *s*, H_3-12); ^{13}C NMR ($CDCl_3$): Table 1.

*3.9. 5α-Acetoxy-1β-benzoyl-8α-cinnamoyl-4α-hydroxydihydroagarofuran (**5**)*

Amorphous powder. $[\alpha]_D^{25}$ 109.4 (MeOH, *c* 1.3); IR (KBr) ν_{max} cm^{-1}: 2954, 1716, 1637, 1283, 1241, 1171, 1095, 973; UV (MeOH) λ_{max} nm (log ε): 222 (4.6); HR–EIMS: m/z 562.2607 (calc. for $C_{33}H_{38}O_8$, 562.2567); ^{1}H NMR ($CDCl_3$): 7.95 (2H, *d*, $J=7.4$ Hz, Bz-H), 7.53 (1H, *t*, $J=7.6$ Hz, Bz-H), 7.39 (2H, *t*, $J=7.7$ Hz, Bz-H), 7.33 (5H, *m*, Cin-H), 7.05 (1H, *d*, $J=16.0$ Hz, Cin-H), 6.05 (1H, *d*, $J=16.0$ Hz, Cin-H), 5.54 (1H, *s*, H-5), 5.46 (1H, *dd*, J = 11.9, 3.6 Hz, H-1), 5.09 (1H, *d*, $J=7.0$ Hz, H-8), 2.50 (1H, *m*, H-7), 2.15 (3H, *s*, 5-Ac), 1.55 (3H, *s*, H_3-15), 1.51 (3H, *s*, H_3-14), 1.47 (3H, *s*, H_3-11), 1.37 (3H, *s*, $H_3$12); ^{13}C NMR ($CDCl_3$): Table 1.

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