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Trichiol and 3-epitrichiol acetate, novel cytotoxic sterols with an unprecedented 2,6-dioxabicyclo[2.2.2]octan-3-one ring system from the myxomycete *Trichia favoginea* var. *persimilis*

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Abstract—Trichiol (**1**) and 3-epitrichiol acetate (**2**), two new sterols, have been isolated from field-collected fruit bodies of the myxomycete, *Trichia favoginea* var. *persimilis*, and their structures elucidated by spectral data. Trichiol (**1**) and 3-epitrichiol acetate (**2**) possess an unprecedented 2,6-dioxabicyclo[2.2.2]octan-3-one ring system. Trichiol (**1**) was cytotoxic against HeLa cells, while compound **2** proved to exhibit reversal effect against TNF-related apoptosis inducing ligand (TRAIL)-resistant Jurkat cell lines.

1. Introduction

In our search for bioactive natural products from myxomycetes,[1,2] we recently investigated a field-collected material of the fruit bodies of *Trichia favoginea* var. *persimilis* (Triciaceae) and reported the isolation of three new dibenzofurans, Kehokorins A–C.[3] Further investigation of the extract of this myxomycete has now resulted in isolation of two new sterols, trichiol (**1**), and 3-epitrichiol acetate (**2**), with a novel 2,6-dioxabicyclo[2.2.2]octan-3-one ring system. Here, we describe the isolation and structure elucidation of these sterols. Trichiol (**1**) was cytotoxic against HeLa cells, while 3-epitrichiol acetate (**2**) proved to exhibit reversal effect against TNF-related apoptosis inducing ligand (TRAIL)-resistant Jurkat cell lines.

2. Results and discussion

The fruit bodies of myxomycetes, *T. favoginea* var. *persimilis*,[4] collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extracts were subjected to flash chromatography on silica gel and ODS, followed by further purification with reversed-phase HPLC (Develosil C30-UG-5) to give trichiol (**1**) and 3-epitrichiol acetate (**2**) in 0.01% and 0.02% yield, respectively.[5]

Trichiol (**1**)[6] showed a quasi-molecular ion peak at *m*/*z* 459 $(M+H)^+$ in its positive FAB mass spectrum, and its molecular formula was revealed as $C_{29}H_{46}O_4$ by the HRFABMS data [*m*/*z* 459.3429, $(M+H)^+$, Δ −4.6 mmu]. The IR absorption bands at 3419 and 1773 cm^{-1} indicated the presence of hydroxy and carbonyl groups, and no particular UV absorption was observed for **1**. The ^1H NMR spectrum of **1** in $CDCl_3$ (Table 1) showed signals for a dioxymethine proton at δ_H 5.73 (1H, s), two oxymethine protons at δ_H 3.59 (1H, tt, $J = 9.5$ and 4.8 Hz) and 4.09 (1H, dt, $J = 6.8$ and 1.5 Hz), and four methyl groups [one primary methyl at δ_H 0.87 (3H, t, $J = 7.0$ Hz), two secondary methyls at δ_H 0.82 (3H, d, $J = 7.0$ Hz) and at δ_H 0.86 (3H, d, $J = 7.0$ Hz), and one tertiary methyl at δ_H 0.83 (3H, s)]. The ^{13}C NMR spectrum of **1** (Table 1) gave 29 signals assignable to one carbonyl (δ_C 173.2), 2 sp^3 quaternary carbons (δ_C 35.8 and 48.0), 11 sp^3 methines (δ_C 100.4, 72.5, 71.2, 56.9, 55.2, 46.3, 45.0, 41.8, 36.6, 34.7, and 28.2), 11 sp^3 methylenes (δ_C 38.0, 36.7, 35.7,

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Table 1. ^{1}H and ^{13}C NMR data of trichiol (**1**) and 3-epitrichiol acetate (**2**)

Positions	**1** ($CDCl_3$)		**2** ($CDCl_3$)	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	(α) 0.99 m (β) 1.68 m	36.7	1.23 m (2H)	32.7
2	(α) 1.80 m (β) 1.41 m	31.4	(α) 1.72 m (β) 1.64 m	26.0
3	3.59 tt (9.5, 4.8)	71.2	5.01 br s	70.0
4	(α) 1.56 m (β) 1.29 m	38.0	1.46 m (2H)	32.8
5	1.13 m	45.0	1.47 m	40.2
6	1.22 m and 1.30 m	28.5	(α) 1.25 m (β) 1.18 m	28.1
7	0.95 m and 1.73 m	32.4	1.00 m and 1.69 m	32.3
8	1.65 m	34.7	1.65 m	34.7
9	0.85 m	55.2	0.97 m	55.1
10		35.8		36.1
11	(α) 1.68 m (β) 1.38 m	21.9	(α) 1.68 m (β) 1.36 m	21.5
12	(α) 1.44 m (β) 2.36 m	35.7	(α) 1.44 m (β) 2.37 m	35.7
13		48.0		48.1
14	1.43 m	56.9	1.43 m	57.0
15	1.52 m (2H)	26.3	1.52 m (2H)	26.3
16	(α) 1.87 m (β) 1.60 m	30.2	(α) 1.87 m (β) 1.60 m	30.2
17	2.39 dd (10.8, 3.9)	36.6	2.40 dd (9.8, 3.9)	36.6
18	5.73 s	100.4	5.74 s	100.5
19	0.83 s (3H)	12.2	0.82 s (3H)	11.3
20	2.65 dd (3.9, 1.5)	46.3	2.66 dd (3.9, 1.5)	46.4
21		173.2		173.2
22	4.09 dt (6.8, 1.5)	72.5	4.09 dt (6.8, 1.5)	72.6
23	1.48 m and 1.68 m	31.7	1.49 m and 1.68 m	31.8
24	1.19 m	41.8	1.19 m	41.8
25	1.78 m	28.2	1.78 m	28.3
26	0.82 d (7.0) (3H)	18.7	0.82 d (7.0) (3H)	18.7
27	0.86 d (7.0) (3H)	18.9	0.86 d (7.0) (3H)	18.9
28	1.19 m and 1.36 m	22.7	1.19 m and 1.36 m	22.7
29	0.87 t (7.0) (3H)	11.9	0.87 t (7.0) (3H)	11.9
				170.6
			2.05 s (3H)	21.5

32.4, 31.7, 31.4, 30.2, 28.5, 26.3, 22.7, and 21.9), 4 sp^3 methyls (δ_C 18.9, 18.7, 12.2, and 11.9). Since only one out of the seven unsaturation degrees was accounted for by the presence of a carbonyl group, **1** was inferred to contain six rings. The ^{1}H–^{1}H COSY spectrum of **1** revealed the following five partial structures: (i) from H-1 to H-7, (ii) from H-8 to H-12 and H-14, (iii) from H-15 to H-20, (iv) from H-22 to H_3-29, and (v) from H-25 to H_3-27 (Fig. 1). Evidence for the connections of these partial structures was provided by the HMBC ^{1}H–^{13}C long-range connectivity data (Fig. 1). The presence of a 2,6-dioxabicyclo[2.2.2]octan-3-one ring was suggested by the following HMBC correlations: H-17/C-21, H-18/C-13, H-18/C-17, H-18/C-21, H-18/C-22, H-20/C-13, H-20/C-17, H-20/C-21, H-22/C-17, and H-22/C-21. HMBC correlations for H-23/C-20, H-23/C-22, H-23/C-24, H-23/C-25, H-23/C-28, H_3-26/C-24, H_3-26/C-25, H_3-26/C-27, H_3-27/C-25, H_3-27/C-26, H_2-28/C-23, H_2-28/C-24, H_2-28/C-25, H_2-28/C-29, H_3-29/C-24, and H_3-29/C-28 indicated the existence of a 2-ethyl-3-methylbutyl side chain (C-23 to C-29) attached on C-22. Thus, the planar structure of trichiol was revealed as **1**. The relative stereochemistry of trichiol (**1**) was elucidated by a combination of observed coupling constants and NOESY data (Fig. 2). The NOESY correlations observed for H_3-19/H-2β, H_3-19/H-4β, H-3/H-2α, H-3/H-4α, and H-3/H-5, as well as the coupling constants observed for H-3 ($J_{3,2\beta} = J_{3,4\beta} = 9.8$ Hz and $J_{3,2\alpha} = J_{3,4\alpha} = 4.8$ Hz) suggested that the A-ring exists in a chair conformation, and the secondary hydroxy

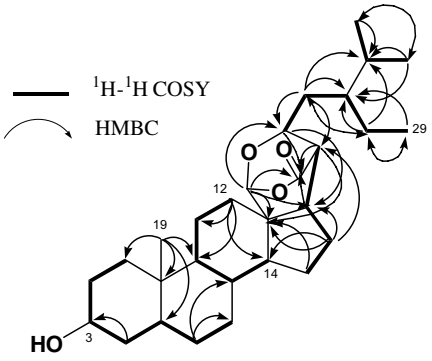


Figure 1. Key ^{1}H–^{1}H COSY and HMBC Data of **1**.

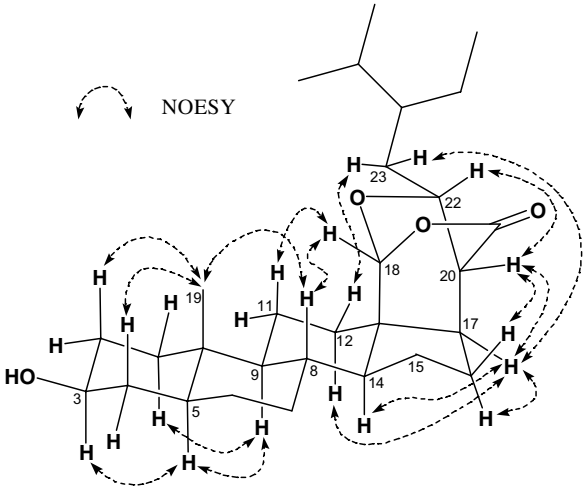
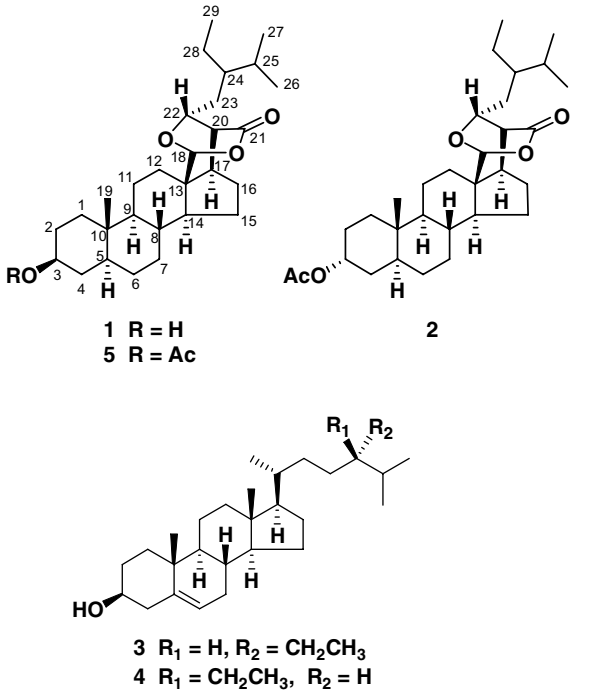


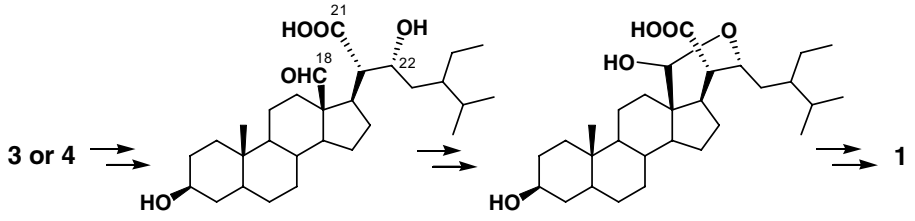
Figure 2. Key NOESY Data of **1**.



group on C-3 was oriented in a β-equatorial arrangement, while the tertiary methyl group on C-10 (C-19) was in a β-axial position. The NOESY cross-peaks observed for H-8/H_3-19, H-8/H-18, H-9/H-1α, H-9/H-5, H-12α/H-17, H-17/H-14, H-18/H-11β implied that the B- and C-rings exist in a chair conformation, and the C/D rings were fused with a *trans*-orientation and the H-18 (C-18 methine carbon) was therefore located in the β-position. Since NOESY correlations were observed for H-20/H-16β, H-17/H-12α, H-17/H-14, and H-17/H-16α, the C-20 methine carbon was revealed to have β-position. The presence of cross-peaks between H-12β/H-23 (δ_H 1.68) and H-17/H-23 (δ_H 1.48) provided evidence that the C-23 side chain was in the α-position and the H-22 in the β-position. The configuration of the C-24 chiral center bearing the ethyl group remained undetermined due to conformational lability. From several species of myxomycetes such as *Physarum*[7] and *Didymium*,[8] clionasterol (**3**) was isolated as a major sterol, and **3** was a C-24 epimer of β-sitosterol (**4**) which is known as a major plant sterol.[9]

The molecular formula of 3-epitrichiol acetate (**2**)[10] was revealed as $C_{31}H_{48}O_5$ by the HRFABMS data [*m*/*z* 501.3557, $(M+H)^+$, Δ −2.3 mmu], having a C_2H_2O unit more than that of trichiol (**1**). The ^1H and ^{13}C NMR spectral data of **2** (Table 1) were parallel to those of compound **1**, except for the fact that the NMR signal due to a methyl group was observed at δ_H 2.05 (3H, s) and an oxymethine proton on C-3 resonated at relatively lower field for **2** (H-3, δ_H 5.01). Although analysis of the ^1H–^1H COSY, HMQC, HMBC, and NOESY data of **2** showed that the planar structure of **2** corresponded to an acetate of compound **1**, the acetoxy-bearing methine proton (H-3) appeared as a broad singlet; this observation suggested that this proton (H-3) has small coupling constants with vicinal methylene protons on C-2 and C-4, implying β-equatorial orientation of H-3. Thus, compound **2** was inferred to be 3-epitrichiol acetate [= an acetate of C-3 epimer of trichiol (**1**)], which was further supported by the following findings. Trichiol (**1**) was treated with acetic anhydride and pyridine to give its acetate (**5**), FABMS *m*/*z* 501 $(M+H)^+$.[11] The ^1H NMR data of **5** were almost parallel but not identical with those of compound **2**. The H-3 of **5**, having α-axial configuration, resonated at relatively higher field (δ_H 4.69) as a triplet of triplet ($J_{3,2\beta} = J_{3,4\beta} = 10.6$ Hz and $J_{3,2\alpha} = J_{3,4\alpha} = 5.4$ Hz), while H-3 of **2** was observed as a broad singlet at δ_H 5.01 (vide supra).

Trichiol (**1**) and 3-epitrichiol acetate (**2**) belong to a new class of sterols possessing a 2,6-dioxabicyclo[2.2.2]-octan-3-one ring system. This unique ring system may be generated, from a sterol such as **3** or **4**, through oxidation of the C-18 methyl into an aldehyde, the C-21 methyl into a carboxyl group, and the C-22 methylene into a hydroxyl-bearing methine carbon, followed by formation of a lactone and acetal functionalities (Scheme 1). Although several types of sterols with



Scheme 1. A conceivable biogenetic pathway of **1**.

chemically-unique structures had been isolated from a variety of natural sources,[12] the 2,6-dioxabicyclo[2.2.2]-octan-3-one ring system is so far unknown as a partial structure, to the best of our knowledge. Trichiol (**1**) exhibited cell growth inhibitory activity against the HeLa human epithelial carcinoma cell line (>90% inhibition at 25 μM, IC_{50} 12.5–25 μM), while compound **2** and clionasterol (**3**)[13] were almost inactive (IC_{50} > 25μM). On the other hand, compound **2** proved to exhibit reversal effect against TNF-related apoptosis inducing ligand (TRAIL)-resistant Jurkat cell lines.[14] Although 50 μM of compound **2** and 0.125 μg/mL of TRAIL alone did not show growth inhibition on Jurkat cells, the combination of these compounds produced a remarkable inhibitory activity on the growth of Jurkat cells (T/C% = 15.8).

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4. The fruit bodies of *Trichia favoginea* var. *persimilis* were collected at Tosayamada-shi in Kochi Prefecture, Japan, in January 2004. A voucher specimen (#25634) is maintained by Y.Y. (Ohtsu-ko, Kochi).
5. The wild fruit bodies (17.6 g) were extracted with 90% MeOH (200 mL × 3) and 90% acetone (200 mL × 1). The combined MeOH and acetone extracts (1.0 g) were subjected to silica gel column chromatography (30 × 180 mm) eluted with 0–100% methanol in chloroform. The fraction eluted with 20% MeOH in $CHCl_3$ afforded arcyriaflavin C (40 mg),[15,16] and the fraction (86 mg) eluting with 100% $CHCl_3$ was separated by the second silica gel chromatography (15 × 280 mm) eluted with 0–10% acetone in chloroform, and the fraction (10 mg) eluting with 5–10% acetone in $CHCl_3$ followed by ODS column ($MeOH/H_2O$, 3:1) to afford trichiol (**1**, 2.4 mg). The fraction of the first silica gel column (52 mg) eluting with 100% $CHCl_3$ was separated by the second ODS column ($MeOH/H_2O$, 95:5), followed by further purification with reversed-phase HPLC (Develosil C30-UG-5, 90–100% MeOH; ODS-UG-5, 60% CH_3CN) to afford 3-epitrichiol acetate (**2**, 3.1 mg).
6. Trichiol (**1**): amorphous solid; $[\alpha]_D^{22}$ +98 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 3419 and 1773 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 459 $(M+H)^+$; HRFABMS calcd for $C_{29}H_{46}O_4$ $(M+H)^+$ 459.3474, found *m/z* 459.3429.
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10. 3-Epitrichiol acetate (**2**): amorphous solid; $[\alpha]_D^{22}$ +90 (*c* 0.5, $CHCl_3$); IR (film) ν_{max} 1775 and 1733 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m/z* 501 $(M+H)^+$; HRFABMS calcd for $C_{31}H_{49}O_5$ $(M+H)^+$ 501.3580, found *m/z* 501.3557.
11. Trichiol (**1**, 0.4 mg) was treated with Ac_2O/pyridine (1:1, 0.2 mL) overnight at room temperature. The solvent was then evaporated and the residue purified by silica gel chromatography (hexane/EtOAc, 8:2), followed by Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1) to afford compound **5** (0.5 mg): ^1H NMR ($CDCl_3$) δ_H 4.69 (1H, tt, J = 10.6 and 5.4 Hz; H-3), 5.72 (1H, s; H-18), 4.09 (1H, dt, J = 7.2 and 1.5 Hz; H-22), 2.65 (1H, dd, J = 4.2 and 1.5 Hz; H-20), 2.40 (1H, dd, J = 9.6 and 4.2 Hz; H-17), and 2.02 (3H, s; CH_3CO); FABMS *m/z* 501 $(M+H)^+$.
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13. Clionasterol (**3**) was isolated from cultured *Didymium minus* previously in our laboratory.[8]
14. For Jurkat cells, 3.5×10^5 cells/mL of the cells were seeded in 95 μL of culture medium per well in 96-well microtiter plates, and were treated with 5 μL of graded concentrations of samples in the absence or presence of 0.125 μg/mL of TRAIL, and were then incubated for 42 h at 37 °C in a 5% CO_2–95% air atmosphere. Cell viability was determined by the colorimetric assay using alamer blue: Fields, R. D.; Lancaster, M. V. *Am. Biotechnol. Lab.* **1993**, *11*, 48–50.
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