

Antitermitic Activities of Abietane-type Diterpenes from *Taxodium distichum* Cones

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Abstract Eight known abietane-type diterpenes were isolated from the weak acidic fraction of the *n*-hexane extract from cones of *Taxodium distichum*, one of the extant, living fossil conifers. They were identified as 6,7-dehydroroleanone (**1**), taxodal (**2**), taxodione (**3**), salvinolone (**4**), 14-deoxycoleon U (**5**), 5,6-dehydrosugiol (**6**), sandaracopimaric acid (**7**), and xanthoperol (**8**). The structures of these compounds were determined by comparison of NMR spectral data with published data. The antitermitic (termicidal and antifeedant) activities of the compounds **1–8** against the subterranean termite, *Reticulitermes speratus* Kolbe, were evaluated. Compounds **1** and **3** showed potent termicidal activity, and **5** and **8** showed potent antifeedant activity. Compound **1** was found to be one of the representative bioactive compounds in the *n*-hexane extract of *T. distichum* cones. Compounds **1–8**, with the exception of **7**, were oxides of ferruginol (**9**). Therefore, the presence of various oxidation forms of the abietane-type structure reflects their various bioactivities.

Keywords *Taxodium distichum* · Cone · Taxodiaceae · Abietane · Diterpene · Oxidation · Antitermite · *Reticulitermes speratus* · Bioactivity

Introduction

Taxodium distichum Rich (Taxodiaceae), commonly known as “bald” or “swamp” cypress, is well known as an extant deciduous, living fossil conifer indigenous to the southern part of North America. *T. distichum* heartwood is used for building materials, and has been reported to resist the attacks of the subterranean termite, *Coptotermes formosanus* Shiraki (Scheffrahn et al. 1988). The cones and seeds of *T. distichum* tend to be discovered from ancient stratum, and there are several reports concerning the fossil conifer and sediments of ancient flora (Otto et al. 2003, 2005). The cones produced by the conifers are essential parts for self-propagation. Consequently, the potential for having anti-feedant, antifungal, as well as other phytochemical activities against external influences is suggested (Yano and Furuno 1994).

Abietane-type diterpenes are widely distributed in the plant kingdom as natural compounds. They reveal characteristic bioactivities including cytotoxic, anti-tumor, anti-microbial, and anti-bacterial effects (Gao and Han 1997; Ulubelen et al. 1999; Gigante et al. 2003; Son et al. 2005; Marques et al. 2006). From previous studies, it is known that the abietane-type diterpenes are the major compounds in the cones of *Taxodium* species (Otto et al. 2003). The isolation of several kinds of abietane-type diterpenes from the cones of *T. distichum* has been reported (Yamamoto et al. 2003). Some of the compounds isolated, such as taxodione, taxodone, and taxodistine A and B, have been reported to have anti-tumor and cytotoxic activity (Kupchan et al. 1969; Hirasawa et al. 2007). Taxodione has been reported to exhibit antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) as well (Yang et al. 2001). In addition, nezukol,

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ferruginol, and manool, which have been isolated from the *T. distichum* heartwood (Scheffrahn et al. 1988), were shown to have antitermitic activities (Kang et al. 1993; Kano et al. 2004).

Natural compounds isolated from Taxodiaceae extant fossil conifers, such as from the genus *Metasequoia* and *Taxodium*, mostly have been taken from the leaf (Si et al. 2005) or the heartwood (Sato et al. 1966; Enoki et al. 1977). Only a few reports have considered bioactive compounds in living fossil conifer cones. Their bioactive compounds might have been important for the persistence, and thus, evolutionary success of *T. distichum*.

The aim of this research was to investigate the bioactive compounds of *T. distichum* cones and to evaluate their antitermitic activities by using a bioassay with the subterranean termite *Reticulitermes speratus* Kolbe. Subterranean termites are important decomposers in the terrestrial environment, and there has been interest in the activities of natural compounds against these termites (Bultman et al. 1979; Cornelius et al. 1997; Bläske and Hertel 2001). The termites *R. speratus*, *C. formosanus*, and *Cryptotermes domesticus* are harmful insects that damage wooden structures of buildings in Japan (Kang et al. 1993; Fukumoto et al. 2007).

Methods and Materials

Termite Collections Colonies of *Reticulitermes speratus* Kolbe were collected in Tsuruoka city, Yamagata prefecture, and maintained in well-humidified chambers at 27°C±1°C for 1 month before the bioassay.

Terminicidal and Antifeedant Tests Terminicidal and antifeedant activities were tested simultaneously. Samples were applied to paper discs (Advantec, 8 mm diam, 1.5 mm thickness, ca. 30 mg disk weight) according to a previously described method (Tellez et al. 2002; Ganapaty et al. 2004). Paper discs were treated with 60 µl of each sample solution (5.0 mg/ml in MeOH). After being dried in a vacuum desiccator for 24 h, the weights of the paper discs were measured. The concentration of the sample in the dried paper disc was prepared to 1.0% (sample weight/paper disc weight×100). This dried paper disc was put on top of sea sand (3 g) that was spread uniformly at the bottom of a glass petri dish (45 mm diam, 20 mm high). Ten *R. speratus* workers were placed in the petri dish. The sea sand was kept moistened with water by using a sprayer. These petri dishes were maintained in a well-humidified chamber at 27°C±1°C for 10 d together with blank paper discs (no samples) and controls (no paper discs). Each of the tests included three replicates. The mortality of termites was recorded every 24 h, and terminicidal activities were

evaluated from the mortality average. The mass loss of each paper disc was measured at the end of the experimental period. Antifeedant activities were evaluated from the average of the mass that one termite fed per 24 h (mass loss of paper disc in 10 d/total number of termites in 10 d), and the relative rates were calculated from the blank readings.

Plant Material The fallen cones of *T. distichum* (Taxodiaceae) were gathered in the Yamagata Field Science Center (Faculty of Agriculture, Yamagata University, Japan). Identification was confirmed by the above institution.

General Experimental Procedures GLC analysis was performed with a HITACHI G-3000 Gas Chromatograph under the following conditions: DB-1 capillary column (30 m×0.32 mm i.d.; 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA); column temperature from 180°C (0 min) to 280°C (15 min) at 4°C/min; injection temperature 250°C; detection temperature 250°C. GC/MS data were measured with a SHIMADZU QP-5000 GC-MS: DB-1 capillary column (30 m×0.32 mm i.d.; 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA); column temperature from 100°C (1 min) to 320°C (10 min) at 5°C/min; injection temperature of 230°C; detection temperature of 250°C; acquisition mass range of 450–50 amu using helium as the carrier gas (3.6 ml/min). 1D and 2D NMR spectra were measured with a JEOL JNM-EX400 (¹H 400 MHz/¹³C 100 MHz) spectrometer. The IR spectra were taken by a HORIBA FT-710 IR spectrometer with KBr pellets. UV spectra were taken by a SHIMADZU UV-1600PC spectrometer. Optical rotation values were measured by a HORIBA SEPA-300 polarimeter.

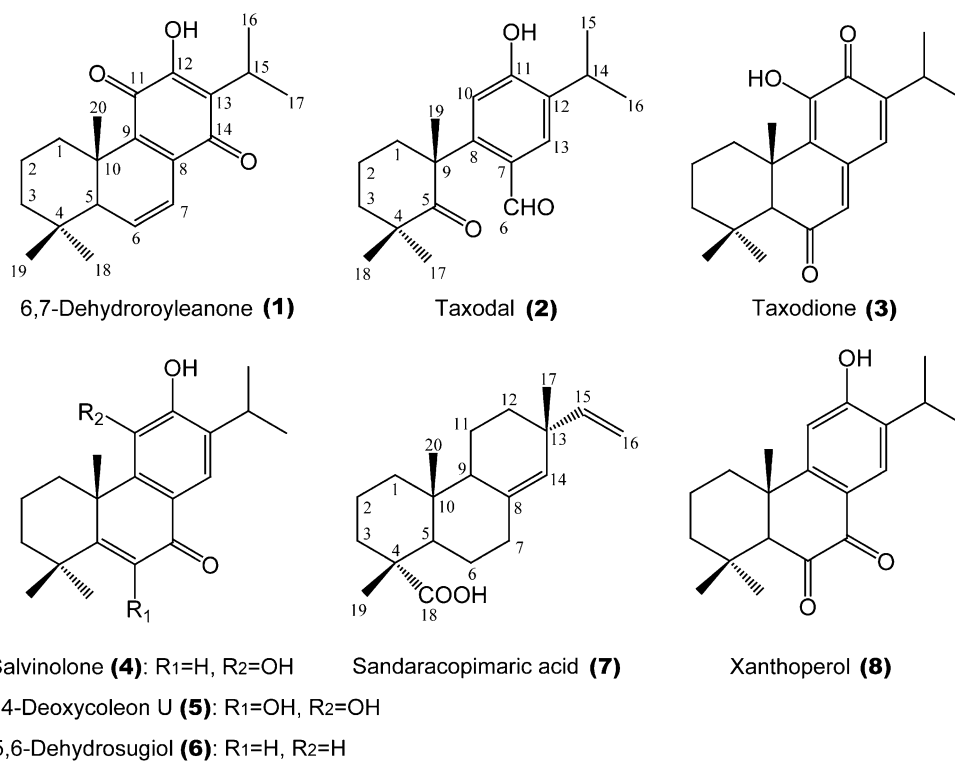
Extraction and Isolation The *n*-hexane extract of air-dried *T. distichum* cones (800.0 g) was extracted by the same method as previously reported (Kusumoto et al. 2008). The *n*-hexane soluble fraction (88.6 g) was extracted by partition extraction with saturated NaHCO₃, 10% NaCO₃, and 1% NaOH aqueous solution in a separatory funnel to yield 1.4 g, 1.2 g, and 11.2 g fractions, respectively, along with 74.8 g residue fraction. Part of the weak acidic fraction (11.0 g) was applied to silica gel 60 N (spherical 63–210 µm, neutral, Kanto Chemical Co., Japan) column chromatography (CC) with solvent systems of *n*-hexane / EtOAc (100:1 to EtOAc only), and eight fractions (fr. A–H) were collected. Fractions C, D, and E (566.3 mg, *n*-hexane / EtOAc, 100:1) were recrystallized by using benzene to give a red needle of 6,7-dehydroroyleanone (**1**, 306.3 mg). Further, fr. G (541.9 mg, *n*-hexane / EtOAc, 100:3) was recrystallized by using *n*-hexane to give a colorless needle of taxodal (**2**, 44.6 mg). Fraction F (304.7 mg, *n*-hexane /

EtOAc, 100:1 to 50:1) was purified by silica gel 60 N CC with a solvent system of *n*-hexane / chloroform (3:1 to chloroform only), and 40 fractions (fr. 1–40) were collected. Fractions 9, 10, 11, and 12 (110.0 mg, *n*-hexane / chloroform, 3:1) were purified by silica gel 60 N CC using a solvent system of *n*-hexane / chloroform (3:1) to give a dark yellow amorphous solid of taxodione (**3**, 29.0 mg). Fraction H (10.2 g) contained a polymerized mixture, which was separated to yield the EtOAc soluble (9.8 g) and insoluble fractions (333.7 mg). The EtOAc soluble fraction (6 g) was applied to silica gel 60 N CC using chloroform, and 48 fractions (fr. H1–H48) were collected. Fractions H9, H10, and H11 (233.1 mg) were recrystallized by using benzene, which gave a pale yellow powder of salvinolone (**4**, 123.2 mg). Successively, fractions H16, H17, H18, and H19 (461.1 mg) were recrystallized by using benzene, which yielded a pale yellow crystal of 14-deoxycoleon U (**5**, 182.8 mg). Fractions H23 to H27 (921.8 mg) were applied to silica gel 60 N CC using a solvent system of benzene / acetone (9:1) to give pale yellow needle of 5,6-dehydrosugiol (**6**, 21.3 mg), a dark brown needle of sandaracopimaric acid (**7**, 11.6 mg), and a yellow needle of xanthoperol (**8**, 37.6 mg), respectively. Ferruginol (**9**) and sugiol (**11**) were isolated from *Cryptomeria japonica* in previous studies (Nagahama et al. 2000; Ashitani et al. 2001). 6,7-Dehydroferruginol (**10**) was synthesized from **11** by a chemical conversion according to a previous report (Matsui et al. 2004).

6,7-Dehydroroyleanone (1) 306.3 mg. Red needle. IR γ_{\max} (KBr) cm^{-1} : 3360.4, 2961.2, 2925.5, 2910.1, 2869.6, 1663.3, 1642.1, 1625.7, 1551.5, 1457.9, 1377.9, 1329.7, 1298.8, 1272.8, 1253.5, 1164.8, 1106.0, 913.1, 769.5, 756.0, 714.5, 651.8; UV ($C=0.05$ mg/ml, *n*-hexane) nm: 451.0, 327.0, 255.5; mp: 171°C; $[\alpha]_{\text{D}}^{24.3} = -364.8^\circ$ ($C=0.3$ mg/ml); EI-MS: m/z 314 (M^+ , $C_{20}H_{26}O_3$, 79%), 299 (27), 281 (6), 271 (21), 258 (16), 253 (16), 246 (15), 245 (68), 244 (74), 243 (23), 232 (100), 231 (45), 229 (22), 217 (23), 213 (25), 201 (15), 187 (25), 185 (19), 129 (18), 128 (21), 115 (32), 91 (24), 83 (41), 69 (30), 55 (75); $^1\text{H-NMR}$ (CDCl_3): δ 0.98 (3H, *s*, 18- CH_3), 1.01 (3H, *s*, 19- CH_3), 1.03 (3H, *s*, 20- CH_3), 1.21 (3H, *d*, $J=7.1$ Hz, 16- CH_3), 1.22 (3H, *d*, $J=7.2$ Hz, 17- CH_3), 1.25 (2H, *m*, 3- CH_2), 1.43 (2H, *td*, $J=13.3$ Hz, 3.9 Hz, 1- CH_2), 1.51 (2H, *m*, 3- CH_2), 1.62 (2H, *m*, 2- CH_2), 1.71 (2H, *m*, 2- CH_2), 2.14 (1H, *t*, $J=3.1$ Hz, 5-CH), 2.89 (2H, *d*, $J=3.4$ Hz, 1- CH_2), 3.17 (1H, *hept*, $J=7.1$ Hz, 15-CH), 6.46 (1H, *dd*, $J=3.0$ Hz, 9.7 Hz, 6-CH), 6.81 (1H, *dd*, $J=3.1$ Hz, 9.8 Hz, 7-CH), 7.34 (1H, *s*, 12-OH); $^{13}\text{C-NMR}$ (CDCl_3): δ 15.2 (20- CH_3), 18.7 (2- CH_2), 19.8 (16- CH_3), 20.0 (17- CH_3), 22.8 (19- CH_3), 24.1 (15-CH), 32.6 (18- CH_3), 33.3 (4-C), 35.2 (1- CH_2), 39.3 (10-C), 40.5 (- CH_2), 52.1 (5-CH), 121.1 (7-CH), 122.6 (13-C), 138.5 (8-C), 139.6 (6-CH), 140.5 (9-C), 151.2 (12-C), 183.5 (11-C), 186.1 (14-C). (Figs. 1 and 2).

Taxodal (2, Fig. 1) 44.6 mg. Colorless needle. IR γ_{\max} (KBr) cm^{-1} : 3012.3, 2969.8, 2948.6, 2861.8, 2775.1, 2751.9,

Fig. 1 Structures of compounds isolated from *Taxodium distichum* cones



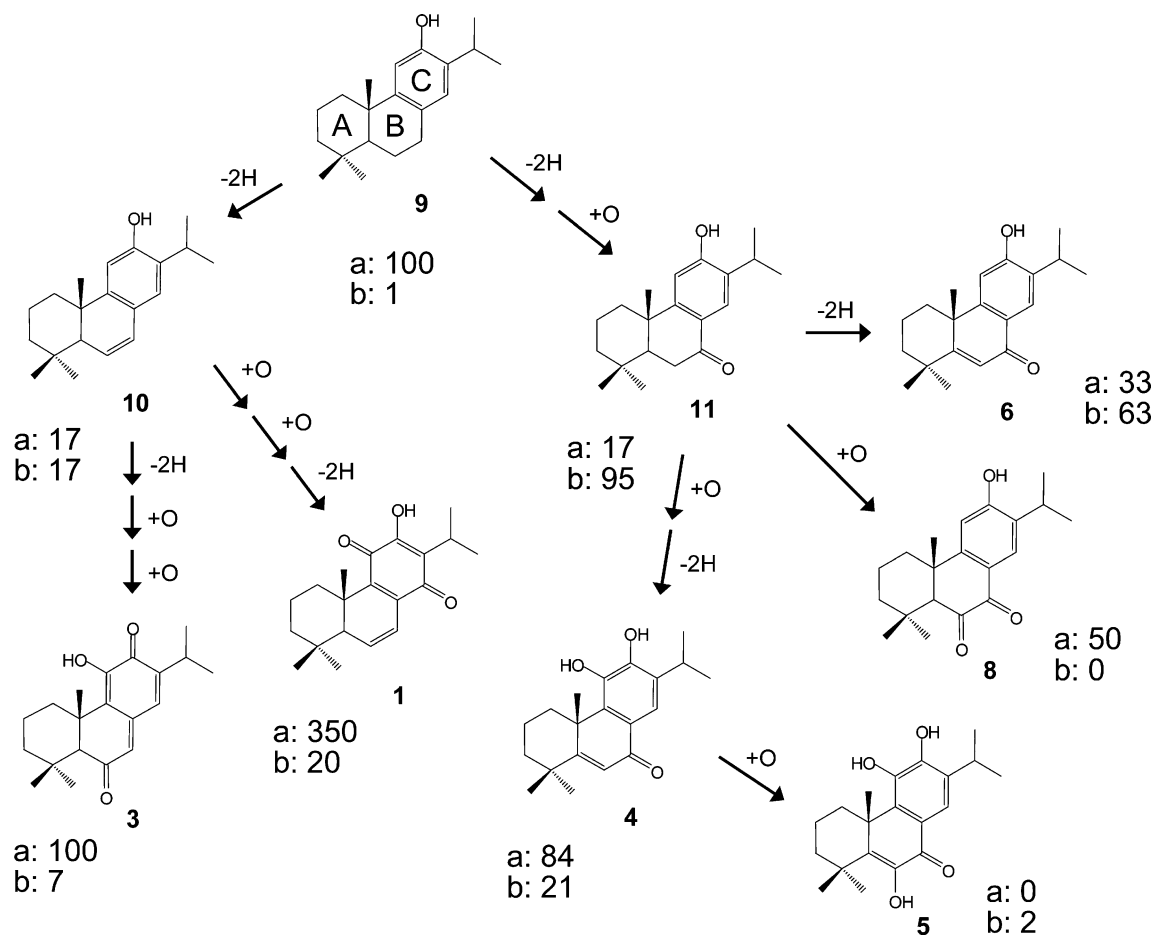


Fig. 2 Route of ferruginol (9) oxidation via 6,7-dehydroferruginol (10) and sugiol (11), and bioactivities of each compound. **a** Relative mortality (%) of *Reticulitermes speratus* caused by the compounds; mortality caused by compound 9 in 10 d=100 (A higher rate means a

higher termiticidal activity). **b** Relative mass (μg) of paper disc that one termite fed in 24 h, mass of disc treated with 9=1 (A lower rate means a higher antifeedant activity)

2503.2, 1689.3, 1666.2, 1614.1, 1581.3, 1469.5, 1461.8, 1425.1, 1400.1, 1346.1, 1276.7, 1253.5, 1211.1, 1172.5, 979.7, 898.7, 763.7; UV ($C=0.005$ mg/ml, EtOAc) nm: 285.5; mp: 234–240°C; $[\alpha]_D^{20}=-131.4^\circ$ ($C=0.5$ mg/ml); EI-MS: m/z 302 (M^+ , $C_{19}H_{26}O_3$, 26%), 287 (3), 274 (8), 269 (2), 259 (9), 256 (3), 241 (5), 232 (9), 231 (51), 220 (55), 219 (36), 205 (14), 204 (18), 203 (100), 191 (29), 190 (22), 189 (15), 187 (16), 177 (17), 175 (16), 161 (22), 159 (10), 147 (12), 128 (14), 115 (15), 91 (16), 77 (11), 69 (11), 55 (27); $^1\text{H-NMR}$ (acetone- d_6): δ 1.17 (3H, s, 17- CH_3), 1.22 (3H, s, 18- CH_3), 1.26 (3H, d, $J=7.0$ Hz, 16- CH_3), 1.27 (3H, d, $J=7.0$ Hz, 15- CH_3), 1.55 (3H, s, 19- CH_3), 1.62 (2H, m, 1- CH_2), 1.67 (2H, m, 2- CH_2), 2.43 (2H, ddd, $J=13.3$ Hz, 13.3 Hz, 4.0 Hz, 3- CH_2), 3.31 (1H, hept, $J=7.0$ Hz, 14-CH), 7.16 (1H, s, 10-CH), 7.73 (1H, s, 13-CH), 9.20 (1H, s, 11-OH), 9.68 (1H, s, 6-CH); $^{13}\text{C-NMR}$ (CDCl_3): δ 17.6 (2- CH_2), 20.8 (15- CH_3), 20.8 (16- CH_3), 24.0 (19- CH_3), 25.5 (14-CH), 25.6 (17- CH_3), 28.4 (18- CH_3), 36.9 (3- CH_2), 38.9 (1- CH_2), 43.1 (4-C), 51.4 (9-C), 114.3 (10-CH), 124.8 (7-C),

131.4 (12-C), 136.5 (13-CH), 146.9 (8-C), 158.7 (11-C), 190.2 (6-CH), 213.2 (5-C).

Taxodione (3, Figs. 1 and 2) 29.0 mg. Dark yellow amorphous solid. IR γ_{max} (KBr) cm^{-1} : 3322.8, 2935.1, 2361.4, 1669.1, 1612.2, 1594.84, 1354.8, 639.3; mp: 104–109°C; EI-MS m/z : 314 (M^+ , $C_{20}H_{26}O_3$, 100%), 299 (17), 286 (58), 272 (19), 271 (83), 253 (13), 245 (69), 245 (69), 244 (25), 243 (25), 232 (38), 231 (44), 229 (25), 217 (33), 215 (26), 206 (22), 203 (25), 189 (22), 187 (22), 175 (14), 173 (15), 165 (12), 157 (11), 141 (17), 129 (20), 128 (24), 115 (30), 109 (41), 91 (26), 77 (24), 69 (31), 55 (51); $^1\text{H-NMR}$ (CDCl_3): δ 1.12 (3H, s, 18- CH_3), 1.16 (3H, d, $J=7.0$ Hz, 17- CH_3), 1.18 (3H, d, $J=7.0$ Hz, 16- CH_3), 1.22 (2H, m, 3- CH_2), 1.27 (3H, s, 19- CH_3), 1.27 (3H, s, 20- CH_3), 1.40 (2H, m, 3- CH_2), 1.61 (2H, m, 2- CH_2), 1.73 (2H, m, 1- CH_2), 1.75 (2H, m, 2- CH_2), 2.60 (1H, s, 5-CH), 2.93 (2H, m, 1- CH_2), 3.07 (1H, hept, $J=7.0$ Hz, 15-CH), 6.21 (1H, s, 7-CH), 6.88 (1H, s, 14-CH), 7.58 (1H, s, 11-OH);

^{13}C -NMR (CDCl_3): δ 18.5 (2- CH_2), 21.2 (16- CH), 21.6 (17- CH_3), 21.8 (20- CH_3), 22.1 (19- CH_3), 27.1 (15- CH), 32.8 (4- C), 33.3 (18- CH_3), 37.0 (1- CH_2), 42.9 (10- C), 63.0 (5- CH), 125.6 (9- C), 134.0 (7- CH), 136.1 (14- CH), 139.9 (8- C), 145.0 (11- C), 145.3 (13- C), 181.7 (12- C), 201.0 (6- C).

Salvinolone (**4**, Figs. 1 and 2) 123.2 mg. Pale yellow powder. IR γ_{max} (KBr) cm^{-1} : 3373.9, 3250.4, 2960.2, 2869.6, 2361.4, 2341.2, 1625.7, 1583.3, 1506.1, 1456.0, 1371.1, 1314.3, 1260.3, 1178.3, 1048.1; mp: 204–206°C; EI-MS m/z : 314 (M^+ , $\text{C}_{20}\text{H}_{26}\text{O}_3$, 99%), 299 (11), 286 (5), 272 (11), 271 (44), 258 (10), 255 (5), 245 (89), 244 (100), 229 (34), 215 (29), 203 (20), 175 (14), 128 (10), 115 (12), 83 (18), 55 (24); ^1H -NMR (CDCl_3): δ 1.27 (3H, d , $J=7.0$ Hz, 16- CH_3), 1.30 (3H, d , $J=7.0$ Hz, 17- CH_3), 1.43 (3H, s , 18- CH_3), 1.43 (3H, s , 19- CH_3), 1.44 (2H, m , 3- CH_2), 1.50 (3H, s , 20- CH_3), 1.75 (2H, m , 2- CH_2), 1.78 (2H, m , 1- CH_2), 1.90 (2H, m , 2- CH_2), 1.93 (2H, m , 3- CH_2), 2.28 (2H, m , 1- CH_2), 3.19 (1H, *hept*, $J=7.0$ Hz, 15- CH), 5.78 (1H, s , 11- OH), 6.86 (1H, s , 6- CH), 7.15 (1H, s , 12- OH), 8.01 (1H, s , 14- CH); ^{13}C -NMR (CDCl_3): δ 17.6 (2- CH_2), 22.3 (16- CH_3), 22.5 (17- CH_3), 26.9 (15- CH), 27.6 (18- CH_3), 28.2 (19- CH_3), 33.6 (1- CH_2), 35.9 (4- C), 37.9 (3- CH_2), 40.3 (10- C), 111.4 (14- CH), 120.9 (8- C), 125.6 (6- CH), 133.8 (13- C), 141.0 (11- C), 143.8 (9- C), 154.9 (12- C), 157.7 (5- C), 179.7 (7- C).

14-Deoxycoleon U (**5**, Figs. 1 and 2) 182.8 mg. Pale yellow crystal. IR γ_{max} (KBr) cm^{-1} : 3523.3, 3380.6, 3232.1, 2962.1, 2935.1, 2875.3, 1768.4, 1631.5, 1583.3, 1554.3, 1488.8, 1467.6, 1411.6, 1342.2, 1270.9, 1187.9, 1135.9, 1060.7, 997.0, 908.3, 885.2, 784.9, 572.8, 464.8; mp: 210–212°C; EI-MS m/z : 330 (M^+ , $\text{C}_{20}\text{H}_{26}\text{O}_4$, 35%), 315 (6), 287 (12), 274 (9), 262 (16), 261 (94), 260 (100), 248 (19), 247 (12), 245 (28), 233 (15), 232 (12), 231 (15), 219 (14), 217 (14), 191 (8), 128 (8), 115 (9), 82 (15), 77 (8), 69 (8), 55 (17); ^1H -NMR (pyridine- d_5): δ 1.30 (3H, d , $J=7.0$ Hz, 16- CH_3), 1.30 (3H, d , $J=7.0$ Hz, 17- CH_3), 1.64 (3H, s , 18- CH_3), 1.68 (3H, s , 19- CH_3), 1.95 (3H, s , 20- CH_3), 3.64 (1H, *hept*, $J=7.0$ Hz, 15- CH), 7.10 (1H, s , -OH), 8.24 (1H, s , 14- CH), 8.39 (1H, s , -OH); ^{13}C -NMR (pyridine- d_5): δ 18.3 (2- CH_2), 22.8 (16- CH_3), 23.1 (17- CH_3), 27.5 (19- CH_3), 27.6 (15- CH), 28.3 (18- CH_3), 28.4 (20- CH_3), 30.6 (1- CH_2), 36.7 (4- C), 36.9 (3- CH_2), 41.4 (10- C), 116.4 (14- CH), 121.6 (8- C), 135.6 (13- C), 140.3 (11- C), 142.4 (9- C), 144.2 (5- C), 144.3 (6- C), 150.2 (12- C), 180.7 (7- C).

5,6-Dehydrosugiol (**6**, Figs. 1 and 2) 21.3 mg. Pale yellow needle. IR γ_{max} (KBr) cm^{-1} : 2964.1, 2935.1, 2867.6, 1637.3, 1612.2, 1560.1, 1504.2, 1459.9, 1388.5, 1324.9, 1263.2, 1184.1, 891.0, 879.4, 869.7, 651.8; mp: 256°C; EI-MS m/z : 298 (M^+ , $\text{C}_{20}\text{H}_{26}\text{O}_2$, 61%), 283 (25), 255 (32), 242 (14), 241 (16), 230 (35), 229 (84), 228 (42), 214 (18),

213 (100), 199 (34), 187 (30), 185 (10), 171 (11), 170 (12), 165 (15), 157 (13), 152 (11), 128 (11), 115 (12), 83 (10), 55 (23); ^1H -NMR (acetone- d_6): δ 1.18 (3H, d , $J=7.0$ Hz, 16- CH_3), 1.20 (3H, d , $J=7.0$ Hz, 17- CH_3), 1.21 (3H, s , 18- CH_3), 1.32 (3H, s , 19- CH_3), 1.47 (3H, s , 20- CH_3), 2.40 (2H, m , 1- CH_2), 3.23 (1H, t , $J=1.7$ Hz, 15- CH), 6.30 (1H, s , 6- CH), 6.95 (1H, s , 11- CH), 7.84 (1H, s , 14- CH); ^{13}C -NMR (acetone- d_6): δ 19.2 (2- CH_2), 22.6 (16- CH_3), 22.7 (17- CH_3), 27.5 (15- CH), 29.7 (20- CH_3), 32.8 (18- CH_3), 32.8 (19- CH_3), 38.0 (4- C), 38.5 (1- CH_2), 41.0 (3- CH_2), 41.9 (10- C), 111.4 (11- CH), 123.2 (8- C), 124.6 (6- CH), 124.9 (14- CH), 135.0 (13- C), 155.3 (9- C), 160.5 (12- C), 174.5 (5- C), 185.7 (7- C).

Sandaracopimaric acid (**7**, Fig. 1) 11.6 mg. Dark brown needle. IR γ_{max} (KBr) cm^{-1} : 2929.3, 2869.6, 1695.1, 1637.3, 1560.1, 1540.9, 1508.1, 1457.9, 1382.7, 1363.4, 1276.7, 997.0, 908.3; mp: 157–163°C; EI-MS m/z : 302 (M^+ , $\text{C}_{20}\text{H}_{30}\text{O}_2$, 17%), 287 (30), 257 (8), 241 (8), 167 (15), 159 (9), 148 (12), 139 (22), 135 (21), 134 (18), 133 (28), 123 (25), 121 (100), 119 (28), 107 (34), 105 (33), 93 (42), 91 (51), 81 (30), 79 (43), 77 (24), 67 (27), 55 (45); ^1H -NMR (CDCl_3): δ 0.84 (3H, s , 20- CH_3), 1.04 (3H, s , 17- CH_3), 1.14 (2H, m , 1- CH_2), 1.21 (3H, s , 19- CH_3), 1.26 (2H, m , 6- CH_2), 1.36 (2H, m , 12- CH_2), 1.54 (2H, m , 11- CH_2), 1.60 (2H, m , 2- CH_2), 1.62 (2H, m , 3- CH_2), 1.66 (2H, m , 1- CH_2), 1.77 (1H, m , 9- CH), 1.93 (1H, dd , $J=2.5$ Hz, 12.4 Hz, 5- CH), 2.21 (2H, d , $J=7.0$ Hz, 7- CH_2), 4.89 (2H, dd , $J=1.5$ Hz, 10.4 Hz, 16- CH_2), 4.91 (2H, dd , $J=1.5$ Hz, 17.6 Hz, 16- CH_2), 5.22 (1H, s , 14- CH), 5.77 (1H, dd , $J=10.5$ Hz, 17.4 Hz, 15- CH); ^{13}C -NMR (CDCl_3): δ 15.5 (20- CH_3), 17.0 (19- CH_3), 18.4 (2- CH_2), 18.8 (11- CH_2), 24.9 (6- CH_2), 26.3 (17- CH_3), 34.7 (12- CH_2), 35.7 (7- CH_2), 37.3 (3- CH_2), 37.7 (10- C), 38.0 (13- C), 38.6 (1- CH_2), 47.6 (4- C), 49.1 (5- CH), 50.8 (9- CH), 110.4 (16- CH_2), 129.4 (14- CH), 136.9 (8- C), 149.2 (15- CH), 185.2 (18- COOH).

Xanthoperol (**8**, Figs. 1 and 2) 37.6 mg. Yellow needle. IR γ_{max} (KBr) cm^{-1} : 3371.0, 2961.2, 2938.0, 2903.3, 2871.5, 2361.4, 1715.4, 1655.6, 1592.0, 1566.9, 1466.6, 1328.7, 1290.1, 1263.2; mp: 246–249°C; EI-MS m/z : 314 (M^+ , $\text{C}_{20}\text{H}_{26}\text{O}_3$, 28%), 286 (7), 272 (9), 271 (43), 229 (11), 217 (25), 205 (22), 204 (100), 203 (33), 187 (12), 173 (7), 161 (33), 128 (8), 115 (14), 91 (10), 77 (8), 69 (11), 55 (22); ^1H -NMR (CDCl_3): δ 0.46 (3H, s , 19- CH_3), 0.97 (3H, s , 18- CH_3), 1.22 (3H, s , 20- CH_3), 1.28 (2H, s , 1- CH_2), 1.28 (3H, d , $J=7.0$ Hz, 17- CH_3), 1.30 (3H, d , $J=7.0$ Hz, 16- CH_3), 1.31 (2H, m , 3- CH_2), 1.45 (2H, m , 3- CH_2), 1.57 (2H, m , 2- CH_2), 2.47 (2H, d , $J=14.8$ Hz, 1- CH_2), 2.64 (1H, s , 5- CH), 3.22 (1H, *hept*, $J=7.0$ Hz, 15- CH), 6.86 (1H, s , 11- CH), 8.04 (1H, s , 14- CH); ^{13}C -NMR (CDCl_3): δ 18.9 (2- CH_2), 22.3 (16- CH_3), 22.3 (17- CH_3), 24.1 (19- CH_3), 27.0 (15- CH), 31.4

Table 1 Termicidal activities of extracts from *Taxodium distichum* cones against *Reticulitermes speratus*

Fraction	Yields (%)	Mortality of termites ^a	
		5days	10days
Strong acidic	1.59	6.67±6.67	13.3±8.82
Medium acidic	1.39	10.0±5.77	33.3±20.3
Weak acidic	12.61	10.0±5.77	33.3±12.0
Neutral	84.41	n.m. ^b	6.67±3.33
Blank	-	3.33±3.33	3.33±3.33

^a Concentration of compounds on paper disc (100×compound weight/paper disc weight) were 1.0%. *N*=3 replicates. 100%=30 termites. Means ± SE are given. Mortality rates were recorded every 24 hr during the test periods

^b No mortality observed

(18-CH₃), 35.4 (4-C), 36.3 (1-CH₂), 38.5 (20-CH₃), 39.4 (10-C), 42.0 (3-CH₂), 68.9 (5-CH), 111.2 (11-CH), 127.2 (8-C), 129.8 (14-CH), 134.7 (13-C), 150.6 (9-C), 160.5 (12-C), 179.9 (7-C), 200.1 (6-C).

Results and Discussion

Bioactivities of the Compounds from T. distichum Cones The *n*-hexane soluble fraction (88.6 g) of *T. distichum* cones

was separated by partition extraction and yielded strong (1.59%), medium (1.39%), and weak (12.61%) acidic fractions and a neutral fraction (84.41%). Termicidal activities of these four fractions were tested, and the results are shown in Table 1. The medium (33.3±20.3%) and the weak (33.3±12.0%) acidic fractions exhibited potent termicidal activity during a 10-d bioassay period. Based on the amount of extraction and the results of the bioassays, the weak acidic fraction seemed to contain the bioactive compounds. Therefore, it was further examined by silica gel column chromatography.

On a preparative scale, compounds **1–8** (Fig. 1) were isolated from the weak acidic fraction. The structures of these compounds, 6,7-dehydroroyleanone (**1**) (Hensch et al. 1975; Tezuka et al. 1998), taxodal (**2**) (Kusumoto et al. 2008), taxodione (**3**) (Kupchan et al. 1969; Tezuka et al. 1998), salvinolone (**4**) (Lin et al. 1989; Yamamoto et al. 2003), 14-deoxycoleon U (**5**) (Hueso-Rodriguez et al. 1983; Yamamoto et al. 2003), 5,6-dehydrosugiol (**6**) (Kupchan et al. 1969; Lin et al. 1989), sandaracopimaric acid (**7**) (Wenkert and Buckwalter 1972), and xanthoperol (**8**) (Kondo et al. 1963; Li et al. 2003), were determined by EI mass spectra, IR data, melting points, and NMR spectral data, and these were compared with published data. This is the first report of **2**, **7**, and **8** from *T. distichum* cones. Compounds **1–8**, except **2** and **7**, were diterpenes with an abietane-type structure.

Table 2 Antitermitic activities of compounds against *Reticulitermes speratus*

Compound	Mortality of termites ^a		Mass of paper fed by termites ^d	
	5days (%)	10days (%)	Mass (μg) ^d	Relative rate (%) ^e
6,7-Dehydroroyleanone (1)	46.7±3.33	70.0±10.0	8.97±1.91	20.3
Taxodal (2)	n.m. ^b	n.m. ^b	16.7±2.21	37.7
Taxodione (3)	n.m. ^b	20.0±11.6	2.97±0.72	6.7
Salvinolone (4)	3.33±3.33	16.7±6.67	9.33±0.86	21.1
14-Deoxycoleon U (5)	n.m. ^b	n.m.	0.83±0.55	1.9
5,6-Dehydrosugiol (6)	6.67±6.67	6.67±6.67	27.9±2.64	63.1
Sandaracopimaric acid (7)	n.t. ^c	n.t. ^c	n.t. ^c	n.t. ^c
Xanthoperol (8)	6.67±3.33	10.0±5.77	0.0	0.0
Ferruginol (9)	10.0±5.77	20.0±11.6	0.44±0.19	1.0
6,7-Dehydroferruginol (10)	n.m. ^b	3.33±3.33	7.35±2.56	16.6
Sugiol (11)	3.33±3.33	3.33±3.33	41.9±2.33	94.7
No feed	3.33±3.33	13.3±3.33	-	-
Blank	1.67±1.67	1.67±1.67	44.3±1.54	100

^a The concentration of the sample in the dried paper discs was prepared to 1.0% (sample weight/paper disc weight×100). *N*=3 replicates. 100%=30 termites. Means ± SE are given. Mortality rates were recorded every 24 h during the test periods

^b No mortality observed

^c None tested

^d The mass of paper discs fed by one termite per 24 hr (calculated by mass loss of paper disc in 10 d divided by total number of feeding termites in 10 d)

^e Relative rates were calculated from blank reading (100×mass of each sample/mass of blank)

The termiticidal and antifeedant activities of compounds **1–8** against *R. speratus* were tested, (except for **7**, which was isolated in too low amounts), together with ferruginol (**9**), 6,7-dehydroferruginol (**10**), and sugiol (**11**). The results are shown in Table 2. Compounds **9–11** are well-known abietane-type diterpenes that exist in Taxodiaceae (Otto et al. 2002). Compound **9** has been shown previously to have antitermitic and antifungal activities (Kofujita et al. 2001; Kano et al. 2004).

When testing termiticidal activity, **1** caused strong mortality (46.7±3.33% in 5 d, 70.0±10.0% in 10 d). Compound **3** also caused notable mortality after 10 d (20.0±11.6%), with the same value as **9**. The mortality rates caused by **8** after 10 d (10.0±5.77%) and the rates observed in the “no feed” assay (13.3±3.33%) were similar.

When testing antifeedant activity, **8** showed effective activity against *R. speratus*. The termites fed on paper treated with **8**. Compounds **3** and **5** also showed potent relative activities in the antifeedant test.

From these results, we conclude that *T. distichum* cones contain strong bioactive compounds (**1**, **3**, **5**, and **8**). In particular, **1** is a specific bioactive compound in the *n*-hexane extract of *T. distichum* cones, and has a quinone structure at the C ring (Fig. 2). The abietane-type diterpenes, which have quinone structures at the C rings, such as cryptoquinone and 7-hydroxy-11,14-dioxo-8,12-abietadiene, previously have been reported to show antifungal and cytotoxic activities (Kofujita et al. 2002, 2006). Herein, we report that **1**, the abietane-type quinone, exhibited strong activities against subterranean termite.

Bioactivities of the Oxidized Abietane-type Compounds It was assumed that the bioactive compounds **1–8** were oxides of **9** (Yamamoto et al. 2003). In order to compare the bioactive value of **9** with its oxides, the oxidation routes of **9** and the relative values on the basis of **9**, as a standard compound, are indicated in Fig. 2.

First, we discuss the termiticidal activity (Fig. 2a). The activity decreased with dehydrogenation of C-6 and C-7 at **10**, but the activity increased with the synthesis of a quinone structure in the oxidation progression to **1** or **3**. Activity also decreased at **11**, but increased again through oxidation to **4**, **6**, and **8**. Moreover, oxidation of **4** to **5** resulted in loss of all activity.

Second, a similar change was observed with antifeedant activity (Fig. 2b). The activity of **3** was stronger than that of **1**. Furthermore, the activity completely disappeared at **11**, but high activities were obtained through oxidation to **5** or **8**.

These findings suggest that the various forms of the abietane-type structure due to the degree of oxidation reflect their various bioactivities. As several compounds that are active against *R. speratus* were discovered, they

may be related to the defense of *T. distichum*. Hence, we consider that this may be one of the important factors that explains why *T. distichum* have prospered on the earth.

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