Phytochemistry

ent*-Eudesmane sesquiterpenoids, galloyl esters of the oak lactone precursor, and a 3-*O*-methylellagic acid glycoside from the wood of *Platycarya strobilacea

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Abstract

Three *ent*-eudesmane sesquiterpenoids, 8,11-dihydroxy-2,4-cycloeudesmane, 11-hydroxy-2,4-cycloeudesman-8-one and 2,4-cyclo-7(11)-eudesmen-8-one, were isolated from the wood of *Platycarya strobilacea*, which has been used as an aromatic tree since at least the 18th century. On charring the wood, 2,4-cyclo-7(11)-eudesmen-8-one was detected in the smoke. In the charred wood, the concentrations of ellagitannins, such as galloyl pedunculagin, dramatically decreased, whereas concentrations of pentagalloyl glucose, and other gallotannins were relatively stable. In addition, two other compounds, the 6'-*O*-*m*- and *p*-digalloyl oak lactone precursor and the 3-*O*-methylellagic acid 4'-*O*-(4''-*O*-galloyl)-xylopyranoside, were isolated from the charred wood along with *m*- and *p*-digallic acid.

Keywords: *Platycarya strobilacea*, Juglandaceae, eudesmane, tannin, pyrolysis

1. Introduction

Platycarya strobilacea Sieb. et Zucc. (Juglandaceae) is a woody plant present across East Asia (Atkinson et al., 2006), whose fruits, leaves, bark and wood contain a large amount of ellagitannins used for dyeing (Tanaka et al., 1993; Tanaka et al., 1998). The use of the old wood as an incense appeared originally in an old Japanese scripture, "Yamato-Honzo," written in 1709. The wood appears to be used as a substitute for expensive Agarwood (*Aquilaria agallocha*) (Naf et al., 1995; Ito et al., 2005). For a previous study, the occurrence of (3*S*,4*S*)-3-methyl-4-hydroxyoctanoic acid 4-*O*-β -D-glucopyranoside and its 6'-O-gallate (**5**) in *P. strobilacea* wood was establishd (Tanaka et al., 1996). These compounds are precursors of oak lactone (3-methyl-4-octanolide), and subsequently, the presence of the precursors in oak wood was confirmed (Masson et al., 2000). Oak lactone is an important compound contributing to the flavor of whisky, wine and brandy aged in barrels made of oak wood (Onishi et al., 1977; Otsuka et al., 1974). In the course of research to identify new plant sources containing functional compounds in the Nagasaki region of Japan (Maeda et al., 2009), the non-polar constituents of the wood of *P. strobilacea* were examined, because it may represent a promising fragrance material. Furthermore, the chemical composition of the wood was compared before and after charring because charred wood is used as

incense.

2. Results and Discussion

2.1. Structures of terpenoids

The MeOH and aqueous acetone extracts of the wood of *P. strobilacea* were successively partitioned with *n*-hexane and EtOAc. Three new terpenoids, 8,11-dihydroxy-2,4-cycloeudesmane (**1**), 11-hydroxy-2,4-cycloeudesman-8-one (**2**) and 2,4-cyclo-7(11)-eudesmen-8-one (**3**), were isolated along with 3-hydroxy-α-calacorene (**4**) from the *n*-hexane extract. The EtOAc fraction primarily contained phenolic compounds, and the 6'-*O*-galloyl oak lactone precursor (**5**) (Tanaka et al., 1996), gallic acid, ellagic acid, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**6**) (Nishizawa et al., 1982), eugeniin (**7**) (Nonaka et al., 1980), pedunculagin (**8**) (Schmidt et al., 1965), 1(β)-galloylpedunculagin (**9**) (Gupta et al., 1980), casuariin (**10**) and casuarinin (**11**) (Okuda et al., 1983) were isolated by a combination of column chromatography steps using Sephadex LH-20, MCI-gel CHP20P, Diaion HP20P and Chromatorex ODS materials, respectively (Fig. 1).

Compound **1** was obtained as colorless needles from *n*-hexane-acetone and its molecular formula, $C_{15}H_{26}O_2$, was confirmed by HR EIMS. The ^{13}C-NMR spectrum

exhibited 15 aliphatic carbon signals assignable to three quaternary, four methine, four methylene and four methyl groups (Table 1). The chemical shifts of the quaternary (δ 73.2) and methine (δ 69.2) carbons indicated that these carbons are attached to oxygen atoms. The presence of hydroxy groups was supported by IR absorption bands at 3,300 cm^{-1}. The ^{1}H-NMR spectrum exhibited signals attributable to four methyl groups (Me-12, Me-13, Me-14 and Me-15) and one oxygen-bearing methine (δ 4.62, dt, J = 3.0 Hz, H-8) protons. In addition, a pair of characteristic methylene protons observed at δ 0.54 (t, J = 3.9 Hz) and 0.99 (dd, J = 3.9, 8.2 Hz) suggested the presence of a cyclopropyl ring. This was supported by the appearance of an IR absorption band at 3,042 cm^{-1} and the degree of unsaturation (3) calculated from the molecular formula. With the aid of ^{1}H-^{1}H COSY analysis, two strings of carbon connections C-1 – C-2 – C-3 and C-5 – C-6 – C-7 – C-8 – C-9 were demonstrated (Fig. 2). The aforementioned cyclopropyl methylene protons at δ 0.54 and 0.99 were assigned to the C-3methylene protons. Furthermore, the HMBC correlations illustrated in Fig. 2 established the structure of **1**: HMBC correlations of H-15 with C-3, C-4, and C-5 indicated that the C-15 methyl group was located at the C-4 quaternary carbon. Similarly the C-14 methyl carbon was shown to be attached to the C-10 quaternary carbon by HMBC correlations of H-14 with C-1, C-10, C-9 and C-5. This observation and the correlation of H-5 with

C-10 indicated a connection between C-5 and C-10. The location of the hydroxyisopropyl group at C-7 was established by correlations of C-7 with H-12 and H-13. Therefore, **1** was identified as a 2,4-cycloeudesmane-8,11-diol. As for its configuration, the small coupling constant (J = 3.0 Hz) of H-8 with H-7 and H-9 indicated that the C-8 hydroxy group was in an axial orientation. Furthermore, NOESY correlations between H-14 and H-15, between H-3 and H-5, and between H-5 and H-7, unambiguously confirmed the relative configuration of **1** (Fig. 2).

Compound **2** was isolated as a colorless syrup and the molecular formula was determined to be $C_{15}H_{24}O_2$ by HR-EIMS. The ^{13}C-NMR spectra (Table 1) were related to those of **1** except for the appearance of a carbonyl carbon signal at δ 215.4 in the spectra of **2** instead of the C-8 oxymethine of **1**. The HMBC correlations of the carbonyl carbon with H-6 and H-7 indicate that the carbonyl group is located at C-8 (Fig. 3). The ^{1}H-^{1}H couplings between H-1, H-2 and H-3, and between H-5, H-6 and H-7 were similar to those of **1**, suggesting that the remaining portion of the structure was the same as that of **1**. Thus, compound **2** was concluded to be 11-hydroxy-2,4-cycloeudesman-8-one.

Compound **3** was obtained as a colorless syrup and the the molecular formula was determined to be $C_{15}H_{22}O$ by HR-EIMS. UV absorption at 251 nm suggested the

presence of a conjugated carbonyl group, and this was confirmed by observation of a carbonyl carbon signal at δ 203.6 (C-8) and olefinic carbon resonances at δ 130.5 (C-7) and 146.3 (C-11). In the ^{1}H-NMR spectrum, the signals attributable to H-1 – H-3 were related to those of **1** and **2**. Two methyl groups resonating at δ 1.83 (H-12) and 2.01 (H-13) were found to be attached to a double bond based on their chemical shifts. In the ^{1}H-^{1}H COSY spectrum, one of the methyl groups (H-12) showed a long-range allyl-coupling with the C-6 methylene protons. Furthermore, HMBC correlations of **3** (Fig. 3), including a ^{4}J correlation between H-13 and C-8, confirmed the planar structure of **3**.

Efforts to determine the absolute configuration by application of a modified Mosher's method to **1** failed (Ohtani et al., 1991). The MTPA esters of **1** were unstable because of the axial orientation of the C-8 hydroxy group and the presence of the bulky substituent at the adjacent C-7. Therefore, we compared the CD data of **3** with those of steroidal and related *cisoid*-α,β-unsaturated ketones. Based on computer aided molecular modeling, the torsion angle of the *cisoid*-enone moiety was estimated to be about 29º, and the CD spectrum of **3** showed a negative Cotton effect at 330 nm (Δε – 5.0). It has been reported that *cisoid*-α,β-unsaturated ketones with negative torsion angles show negative Cotton effects in the range of 320–340 nm (Snatzke, 1965;

Jadwiga et al., 1996). Therefore, the molecular structure of compound **3** is presented in Fig. 4. Oxidation of **1** with $K_2Cr_2O_7$ afforded **2**, and subsequent treatment of **2** with trifluoroacetic acid yielded **3**. Thus, the absolute configuration of **1** and **2** are determined to be the same as that of **3**.

2.2. Charring of wood

The wood of *P. strobilacea* is charred when it is used as incense; therefore, we next examined whether the terpenoids are detectable in the smoke generated from charring the wood. The smoke was collected using a large stainless funnel connected to an aspirator and compounds in the smoke were trapped by $CHCl_3$ placed between the funnel and the aspirator. TLC and HPLC of the $CHCl_3$ soluble compounds revealed the presence of **3** in the smoke; however, **1**, **2** and **4** were not detected. In addition, despite the presence of a relatively high concentration of the oak lactone precursor (**5**) in the wood, oak lactone (β-methyl-γ-octalactone) was also detected within the *n*-hexane layer of the extract. Compound **3** may be responsible for the characteristic odor of this wood.

TLC analysis showed that the *n*-hexane soluble fraction of the charred wood contained compounds **1—4.** The amount of compound **3** was higher than the levels found in fresh wood, probably due to the dehydration of compound **2**. Important

differences of constituents were observed in the EtOAc fractions: the HPLC of the EtOAc soluble fraction obtained from the charred wood showed some peaks that were not observed in the analysis of the soluble fractions obtained from fresh wood (Fig. 5). New strong peaks at 18.0 and 20.3 min were identified to be an equilibrium mixture of *m*- and *p*-digallic acid (**15**) (Fig. 6) (Nishizawa et al., 1982). Production of **15** was deduced to be via acyl migration of galloyl groups or dehydrative dimerization of gallic acid. Heating of either 1,2,3,4,6-penta-*O*-galloylglucose (**6**) or gallic acid at 200 ºC for 30 min afforded **15**. However, it is also possible that **15** was generated by partial hydrolysis of unknown galloyl esters with *m*- and *p*-digalloyl esters originally contained in the fresh wood. Another remarkable difference observed in the chromatograms is the significant decrease of ellagitannins pedunculagin (**8**), 1(β)-*O*-galloyl pedunculagin (**9**), casuariin (**10**) and casuarinin (**11**). This phenomenon is probably because ellagitannins are more sensitive to radical oxidation (Barbehenn et al., 2006). The products produced from ellagitannins remain unknown. The peak area of ellagic acid did not increase, suggesting that simple hydrolysis is not important.

From the EtOAc layer of charred wood, coniferylaldehyde, sinapylaldehyde, vanillin, syringaldehyde and (-)-episyringareginol (**14**), were identified. These compounds were presumably generated by the pyrolysis of lignins, and the sweet aroma

of vanillin contributed to the characteristic scent of the charred wood (Sarni et al., 1990). In addition, quercetin 3-*O*-rhamnoside (**12**), myricetin 3-*O*-rhamnoside (**13**) and two new compounds **16** and **17** were isolated. The ^{1}H- and ^{13}C-NMR spectra of compound **16** was similar to the spectra of the oak lactone precursor (**5**) and *m*- and *p*-digallic acid (**15**). The ESI-TOF MS (*m/z*: 663, M+Na) indicated that the additional galloyl ester was attached to **5**. From ^{13}C NMR spectroscopic comparison with **15**, the location of the additional galloyl group was deduced to be the phenolic hydroxygroup of the galloyl group at the glucose C-6. The structure was also confirmed by enzymatic hydrolysis of **16**, which yielded gallic acid and (3*S*,4*S*)-3-methyl-4-hydroxyoctanoic acid 4-*O*-β -D-glucopyranoside (Tanaka et al., 1996). Accordingly, compound **16** was characterized to be 6'-*O*-*p*- and *m*-digalloyl-(3*S*,4*S*)-3-methyl-4-hydroxyoctanoic acid 4-*O*-β -D-glucopyranoside.

Characteristic UV absorptions at 361 and 250 nm of compound **17** suggested that this compound is a derivative of ellagic acid. The ^{1}H NMR spectrum showed signals attributable to an *O*-methyl (δ 4.12, 3H, s) and a galloyl group (δ 7.07, 2H, s), along with two aromatic singlets of the ellagic acid moiety (δ 7.39, 7.22) and sugar proton signals. The sugar was shown to be β-D-xylose based on the large coupling constants of the proton signals ($J_{1,2}$ = 7.3, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz). The acylation of the xylose C-4

hydroxy group was apparent from the large low field shift (δ 5.03). Location of the xylose and the methyl group on the ellagic acid moiety was determined by HMBC spectroscopic analysis (Fig. 6), and confirmed by enzymatic hydrolysis of the galloyl ester with tannase yielding 3-*O*-methylellagic acid 4'-*O*-xylopyranoside (Tanaka et al., 1998). Thus, the structure of **17** was determined to be 3-*O*-methylellagic acid 4'-*O*-(4"-*O*-galloyl)-xylopyranoside. The new galloyl esters **16** and **17** are also detected in fresh wood; however, HPLC comparison (Fig. 5) indicates that the concentration of **17** is higher in fresh wood.

3. Conclusions

In this study, presence was demonstrated new *ent*-eudesmane type sesquiterpenes in the wood of *P. strobilacea*, which has been recorded as an incense wood in since the 18th century. On charring the wood, compound **3** with an enone structure was detected in the smoke. The presence of **3** is probably responsible for the characteristic odor of this wood on charring, and this observation is important in the use of the wood as a fragrance material. The observation of a rapid decrease of ellagitannins compared to gallotannins on wood charring is also an interesting finding. The preparation of oak lactone by hydrolysis of the precursor in wood and the application of the wood or its

extract as a fragrance material is currently under investigation.

4. Experimental

4.1. General experimental procedures

UV spectra were obtained with a Jasco V-560 UV/Vis spectrophotometer and optical rotations were measured with a Jasco DIP-370 digital polarimeter (Jasco Co., Tokyo, Japan). ^{1}H- and ^{13}C NMR spectra were measured in CD_3OD at 27 ºC using a Varian Unity plus 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) (Varian, Palo Alto, CA, USA). Coupling constants are expressed in Hz and chemical shifts are given on a δ (ppm) scale. MS were recorded on a Voyager DE-PRO (Applied Biosystems, USA) and a JEOL JMS-700N spectrometer (JEOL Ltd.**,** Tokyo, Japan). 2,5-Dihydroxybenzoic acid and glycerol were used as the matrix for MALDI-TOF-MS and FAB-MS measurements, respectively. HR ESI TOF MS was measured with a JMS-T100TD system (JEOL Ltd.**,** Japan). Column chromatography (CC) was performed using Diaion HP20SS (Mitsubishi Chemical, Japan), Sephadex LH-20 (25–100 μm; GE Healthcare Bio-Science AB, Uppsala), and Chromatorex ODS (100–200 mesh; Fuji Silysia Chemical, Tokyo, Japan) columns. TLC was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick; Merck, Darmstadt, Germany) with

n-hexane-EtOAc (9:1, v/v), *n*-hexane-acetone (5:1, v/v) and toluene- HCO_2Et-HCO_2H (1:7:1, v/v) as a solvent, and spots were detected by UV illumination (254 nm) and by spraying with a 2% ethanolic $FeCl_3$ and 10% H_2SO_4 reagent, followed by heating. Analytical HPLC was performed on a Cosmosil 5C_{18}-AR II (Nacalai Tesque Inc., Kyoto, Japan) column (4.6 mm i.d. × 250 mm) with gradient elutions from 4–30% (39 min) and 30–75% (15 min) of CH_3CN in 50 mM H_3PO_4 (for polyphenols) or from 70–100% (15 min) and 100% (10 min) CH_3CN in 50 mM H_3PO_4 (for terpenoids). The flow rate was 0.8 mL/min and detection was achieved using a JASCO photodiode array detector MD-910.

4.2. Plant material

The wood of *Platycarya strobilacea* was collected at Tsushima Island, Nagasaki, Japan, in May 2008, and was identified by Associate Professor K. Yamada of laboratory in Medical Plants Garden of Nagasaki University. A voucher specimen (N-0805T1) was deposited at the Nagasaki Agricultural and Forestry Technical Development Center, Japan.

4.3. Extraction and separation

The wood of *P. strobilacea* (2.0 kg) was mechanically chipped into small pieces (about 0.5–1 cm long, 0.2–0.5 cm thick) and extracted (each 2 days, 25 °C) with acetone-H_2O (5 L, 3:2, v/v) (2 times) and MeOH (5 L, 3 times). The extracts were combined, concentrated and successively partitioned with *n*-hexane and EtOAc to give a *n*-hexane extract (10.3 g) and an EtOAc extract (50.6 g). The *n*-hexane extract was separated by silica gel CC eluted with *n*-hexane containing increasing proportions of EtOAc (0–100 %, 10% stepwise) to give six fractions. Fr. H1 (5.2 g), mainly containing wax and triglycerides, was further separated by silica gel CC (10–100 % $CHCl_3$ in *n*-hexane, and then 2–10 % MeOH in $CHCl_3$) into 7 fractions. Fractions H1-5 and H1-6 were separately subjected to silica gel CC with *n*-hexane-$CHCl_3$-acetone (90:9:1 and then 80:18:2) to give 3-hydroxy-α-calacorene (**4**) (43 mg) from Fr. H1-5 and 2,4-cyclo-7(11)- eudesmen-8-one (**3**) (163 mg) from Fr. H1-6. Fr. H3 was applied to silica gel CC with *n*-hexane-EtOAc (9:1–8:2) to yield 11-hydroxy-2,4-cycloeudesman-8-one (**2**) (193 mg). Fr. H5 was crystallized from *n*-hexane-acetone to give 8,11-dihydroxy-2,4-cycloeudesmane (**1**) (426 mg) as colorless fine needles.

The EtOAc extract was separated by Sephadex LH-20 CC with EtOH containing

increasing proportions of H_2O (0–40 %, 10% stepwise) and finally 60% acetone to give nine fractions: Fr. A1–A9. Fr. A2 (6.9 g) was successively subjected to Sephadex LH-20 (60% MeOH) and MCI-gel CHP20P (0–70% MeOH, 10% stepwise) CC to yield gallic acid (78 mg) and oak lactone presursor (**5**) (1.3 g). Recrystallization of Fr. A4 (1.6 g) from H_2O afforded colorless needles of ellagic acid (691 mg). Fr. A8 (8.8 g) was successively separated by Diaion HP20SS and Chromatorex ODS CC with H_2O containing increasing amount of MeOH (0–60% MeOH, 10% stepwise) to give casuariin (**10**) (22 mg), pedunculagin (**8**) (222 mg), casuarinin (**11**) (225 mg), 1,2,3,4,6-penta-O-galloyl-β-D-glucose (**6**) (286 mg), and eugeniin (**7**) (374 mg). Fr. A9 (1.5 g) was identified to be 1(β)-galloylpedunculagin (**9**).

*4.3.1. 8,11-Dihydroxy-2,4-cycloeudesmane (**1**)*

Colorless needles (*n*-hexane-acetone), mp 147–149 ºC, $[\alpha]_D$ +0.1 (*c* 0.3, EtOH), HR EI-MS *m/z* 238.1914 ($C_{15}H_{26}O_2$ requires 238.1934)**,** IR ν_{max} cm^{-1}: 3302, 3042, 2947, 1454, 1378. For ^1H (in C_5D_5N) and ^{13}C (in $CDCl_3$) for NMR spectroscopic data, see Table 1. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.34 (1H, t, 3.9, H-3), 0.87 (2H, m, H-1, H-3), 1.14, 1.19, 1.27, 1.37 (each 3H, s, H-12, H-13, H-14, H-15), 1.69–1.84 (4H, m, H-1, H-9, H-6) and 4.37 (1H, br s, H-8).

*4.3.2. 11-Hydroxy-2,4-cycloeudesman-8-one (**2**)*

Colorless syrup, $[\alpha]_D$ −34.2 (*c* 0.3, EtOH), HR EI-MS *m/z* 236.1754 ($C_{15}H_{24}O_2$ requires 238.1772), IR ν_{max} cm^{-1}: 3509, 2951, 1688, 1455. For ^1H and ^{13}C NMR spectroscopic data, see Table 1.

*4.3.3. 2,4-Cyclo-7(11)-eudesmen-8-one (**3**)*

Colorless syrup, $[\alpha]_D$ −86.5 (*c* 0.3, EtOH), HR EI-MS *m/z* 218.1665 ($C_{15}H_{22}O$ requires 218.1672), IRν_{max} cm^{-1}: 2941, 1673, 1590, 1454, UV λ_{max} nm (log ε): 251 (3.73). CD (EtOH, 9.72 × 10^{-5} mol/L) nm (Δε): 330 (-5.0), 243 (13). For ^1H and ^{13}C NMR spectroscopic data, see Table 1.

*4.3.4. Oxidation of **1***

A solution of **1** (20 mg) and $K_2Cr_2O_7$ (50 mg) in AcOH (0.3 mL) was heated at 80°C for 40 min with stirring. The mixture was treated with *i*-PrOH (1.5mL) at room temperature for 3 h to decompose excess oxidizing agent. After concentration, products were extracted with *n*-hexane (2 mL × 3). TLC analysis of the *n*-hexane soluble showed presence of **2** and **3**. The *n*-hexane extract was treated with 10 % CF_3CO_2H in MeOH (3 mL) for 12 h at room temperature. The mixture was concentrated and separated by silica

gel CC (*n*-hexane-EtOAc) to give **3** (10.3 mg), $[\alpha]_D$ −74.7 (*c* 0.4, EtOH).

*4.3.5. Conversion of **2** to **3***

A solution of **2** (10 mg) in 10 % CF_3CO_2H in MeOH (1 mL) was stirred at room temperature for 12 h. The mixture was concentrated and separated by silica gel CC (*n*-hexane-EtOAc) to give **3** (6.3 mg), $[\alpha]_D$ −86.6 (*c* 0.2, EtOH).

4.4. Separation of the compounds from the charred wood.

Wood blocks (10 cm wide × 10 cm long × 3 cm high) were burned with a gas burner until the color of the surface was black and then mechanically chipped into small pieces as described for the experiment using fresh wood. The charred chips (2.1 kg) were extracted and fractionated as described above to give a *n*-hexane soluble fraction (13.7 g) and an EtOAc fraction (48.2 g). TLC and HPLC analysis of the *n*-hexane fraction showed that the composition was similar to that of the fraction obtained from fresh wood. The EtOAc fraction was separated by Sephadex LH-20 CC [EtOH, EtOH-H_2O (4:1, v/v), and then acetone-H_2O (3:2, v/v)] into four fractions: CA-1 (5.0 g), CA-2 (16.6 g), CA-3 (6.5 g), and CA-4 (15.7 g). The CA-1 fraction was successively subjected to the Sephadex LH-20 (MeOH-H_2O, 4:1, v/v) and silica gel

(*n*-hexane-acetone, 1:0–1:1) to give **5** (329 mg), coniferylaldehyde (47.3 mg), sinapylaldehyde (71.2 mg) and (-)-episyringareginol (**14**) (27.7 mg). The presence of vanillin and syringaldehyde in Fr. CA-1 was confirmed by HPLC comparison with authentic samples. Fr. CA-2 was separated by Sephadex LH-20 (EtOH) to give three fractions: CA-2-1 (4.9 g), CA-2-2 (10.2 g) and CA-2-3 (0.6 g). CA-2-2 was crystallized from H_2O to give gallic acid (4.3 g). Successive chromatography of Fr. CA-2-1 over MCI-gel CHP20P CC with H_2O containing increasing proportions of MeOH (0-80%, 10% stepwise) and Sephadex LH-20 CC with H_2O containing MeOH (40%) yielded gallic acid (2.4 g) and compound **16** (52.6 mg). Fr. CA-3 was dissolved in H_2O to give crystalline ellagic acid (0.8 g), and the mother liquor was separated by MCI-gel CHP20P CC with H_2O containing increasing proportions of MeOH (0–100%, 10% stepwise) to give gallic acid (108 mg), quercetin 3-*O*-rhamnoside (**12**) (355 mg), myricetin 3-*O*-rhamnoside (**13**) (122 mg), and *meta*- and *para*-digallic acid (**15**) (295.3 mg). Fr. CA-4 separated by Diaion HP20SS CC with H_2O containing increasing proportions of MeOH (0–100%, 10% stepwise) to yield pentagalloylglucose (**6**) (2.19 g) and compound **17** (116.2 mg) along with a mixture of tetragalloyl glucoses (3.83 g).

*4.4.1. 6'-O-p- and m-Digalloyl-(3S,4S)-3-methyl-4-hydroxyoctanoic acid 4-O-β -D-glucopyranoside (**16**)*

Tan amorphous powder, $[\alpha]_D$ −19.1 (*c* 0.13, MeOH), IR ν_{max} cm^{-1}: 3391, 1699, 1327, 1211; UV λ_{max} nm (log ε): 276 (4.35); HR ESI TOF MS *m/z* 663.1907 $[M+Na]^+$ ($C_{29}H_{36}O_{16}Na$ requires 663.1901); ^1H NMR (400MHz, acetone-d_6) *m*-digalloyl isomer δ: 7.48, 7.37 (1H, d, *J* = 2 Hz, *m*-diG H-2, 6), 7.26 (2H, s, *m*-diG H-2', 6'), 4.60 (1H, dd, *J* = 10 Hz, 2Hz, glc H-6), 4.39 (1H, d, *J* = 7 Hz, glc H-1), 4.35 (1H, dd, *J* = 12 Hz, 7 Hz, glc H-6), 3.60 (2H, m, H-4, glc H-5), 3.46-3.40 (2H, m, H-3', H-4'), 3.22–3.18 (1H, m, H-2'), 2.59 (1H, dd, *J* = 12 Hz, 5 Hz, H-2), 2.21–2.12 (2H, m, H-2, H-3), 1.5–1.1 (6H, m, H-5, H-6, H-7), 0.89 (3H, d, *J* = 7 Hz, H-9), 0.74 (3H, t, *J* = 7 Hz, H-8), *p*-digalloyl isomer 7.25, 7.21 (each 2H, s, p-diG H-2,6 and H-2', 6'). ^{13}C NMR (100 MHz, acetone-d_6) δ: 14.3 (C-8), 15.1 (C-9), 23.1 (C-7), 28.6 (C-6), 31.5 (C-5), 34.1 (C-3), 37.3 (C-2), 65.0 (glc C-6), 71.6 (glc C-4), 74.7 (glc C-2), 75.2 (glc C-5), 78.0 (glc C-3), 83.0 (C-4), 103.9 (glc C-1), 109.9 (p-diG C-2, 6), 109.9, 110.6 (*p*- and *m*-diG C-2', 6'), 114.6 (*m*-diG C-6), 117.4 (*m*-diG C-2), 120.7, 121.8 (*m*-diG C-1, 1', *p*-diG C-1'), 129.0 (*p*-diG C-1), 132.4 (*p*-diG C-4), 139.5, 139.8 (*m*-diG C-4, 4', *p*-diG C-4'), 143.5 (*m*-diG C-3), 146.1, 146.2 (*m*- and *p*-diG C-3', 5'), 147.0 (*m*-diG C-5), 151.3 (*p*-diG C-3, 5), 164.9, 166.1 (*m*- and *p*-diG C-7, 7') and 175.2 (C-1).

*4.4.2. 3-O-Methylellagic acid 4'-O-(4''-O-galloyl)-xylopyranoside (**17**)*

Tan amorphous powder, $[\alpha]_D$ +0.87 (*c* 0.13, MeOH); IR ν_{max} cm^{-1}: 3409, 1716, 1608, 1348; UV λ_{max} nm (log ε): 361 (4.02), 250 (4.69); FABMS *m/z* 623 $[M+Na]^+$; HRFABMS *m/z* 601.0823 $[M+H]^+$ ($C_{27}H_{21}O_{16}$ requires 601.0828); ^1H NMR (CD_3OD, 400 MHz) δ: 7.39 (1H, s, H-5'), 7.22 (1H, s, H-5), 7.07 [2H, s, galloyl (G)-2, 6], 5.03 (1H, m, xyl-4), 5.00 (1H, d, *J* = 7.3 Hz, xyl-1), 4.24 (1H, dd, *J* = 11.0, 5.3 Hz, xyl-5), 4.12 (3H, s, 3-OCH_3), 3.92 (1H, t, *J* = 9.0 Hz, xyl-3), 3.69 (1H, dd, *J* = 9.0 Hz, 7.3 Hz, xyl-2) and 3.62 (1H, dd, *J* = 11.0, 10.0 Hz, xyl-5). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 167.7 (G C-7), 160.1 (C-7), 160.1 (C-7'), 153.6 (C-4), 148.0 (C-4'), 146.4 (G C-3,5), 142.4 (C-3'), 142.0 (C-2), 141.3 (C-3), 140.0 (G C-4), 136.8 (C-2'), 120.9 (G C-1), 115.7 (C-1'), 113.7 (C-6), 113.2 (C-5'), 1128 (C-5), 112.1 (C-1), 110.3 (G C-2,6), 108.1 (C-6'), 104.0 (xyl-1), 74.6 (xyl-2), 74.4 (xyl-3), 72.8 (xyl-4), 64.0 (xyl-5) and 62.0 (OCH_3).

*4.5. Pyrolysis of **6***

Compound **6** (400 mg) in a flat bottom glass vial (20 mL) was heated at 200 ºC for 30 min. The mixture was separated by MCI-gel CHP20P CC (2 cm i.d. × 24 cm) with H_2O containing increasing proportions of MeOH (0–100%, 10% stepwise) to give

gallic acid (53.8 mg), pyrogallol (6.9 mg), *meta*- and *para*-digallic acid (**15**) (10.5 mg) and **6** (66.0 mg), and a mixture of a polymeric substance (46.8 mg). Gallic acid (2 mg) was heated under similar conditions and HPLC analysis of the reaction mixture showed the generation of a small amount of **15**.

4.6. Detection of terpenoids in smoke

The dried wood chips were placed in a roasting pan and heated with a gas burner until the surface of the wood became dark brown. The smoke was collected using a large stainless funnel connected to an aspirator and compounds in the smoke were trapped by $CHCl_3$ placed between the funnel and the aspirator. The $CHCl_3$ solution was concentrated and examined by TLC and HPLC, and the presence of **3** in the smoke was confirmed.

*4.7. Hydrolysis of **16***

An aqueous solution of **16** (10 mg) was treated with tannase (5 mg, from *Aspergillus nigar*) at room temperature for 10 h. The mixture was separated by Sephadex LH-20 CC with H_2O containing increasing proportions of MeOH (0–40%, 10% stepwise) and silica gel ($CHCl_3$-MeOH-H_2O, 40:10:1 and 14:6:1) to give the oak

lactone precursor (2.4 mg) (Tanaka et al., 1996).

*4.8. Hydrolysis of **17***

A solution of **17** (10 mg) in MeOH-H_2O (1:4, v/v) was treated with tannase at room temperature for 12 h. The solution was concentrated and the resulting precipitate (1.5 mg) was collected by filtration. The product was identified as 3-*O*-methylellagic acid 4'-*O*-xylopyranoside by the comparison of spectroscopic data (Tanaka et al., 1998). Determination of absolute configuration of the xylose moiety was as follows: a solution of **17** (4 mg) in 0.05 M H_2SO_4 (0.2 mL) was heated at 100 °C for 5 h. After neutralization with Amberlite IRA400 (OH form), the resin was removed by filtration and the filtrate was concentrated to dryness. The residue was dissolved in pyridine (0.5 mL) containing L-cysteine methyl ester (5 mg) and heated at 60 °C for 1 h. The mixture was mixed with a solution (0.5 mL) of pyridine *o*-tolylisothiocyanate (5 mg) in pyridine and heated at 60°C for 1 h. The final mixture was directly analyzed by HPLC [Cosmosil 5C_{18} AR II (250 × 4.6 mm i.d., Nacalai Tesque Inc.) with isocratic elution at 25% CH_3CN in 50 mM H_3PO_4]. The t_R of the peak at 20.1 min coincided with that of the thiocarbamoyl thiazolidine derivative of D-xylose (the tR of the L-diastereomer was 18.6 min) (Tanaka et al., 2007).

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Figure Captions

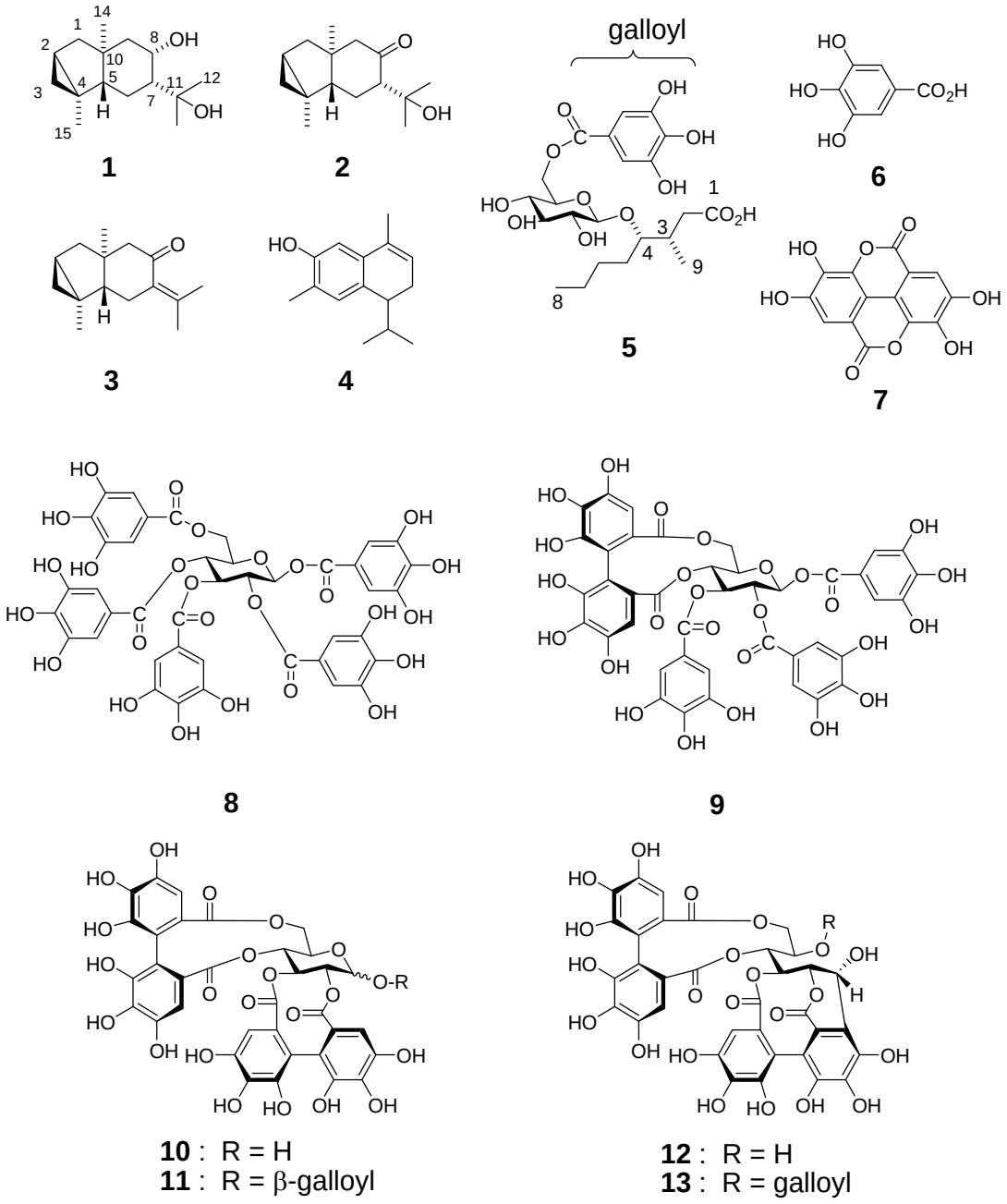

Fig. 1. Structures of compounds isolated from fresh wood.

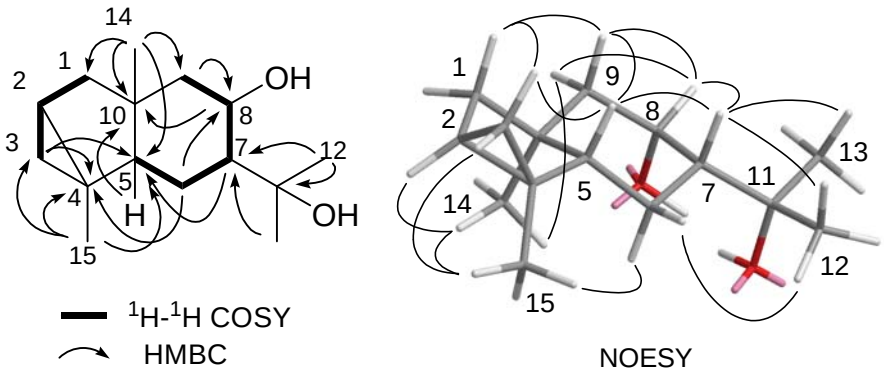

Fig. 2. Selected ^{1}H-^{1}H COSY, HMBC and NOESY correlations observed for **1**.

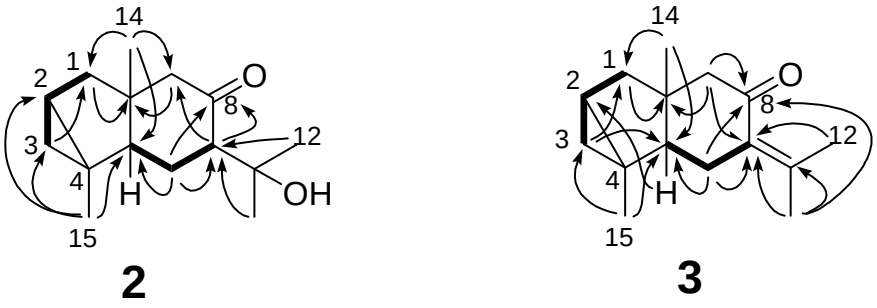

Fig. 3. Selected ^{1}H-^{1}H COSY and HMBC correlations observed for **2** and **3**.

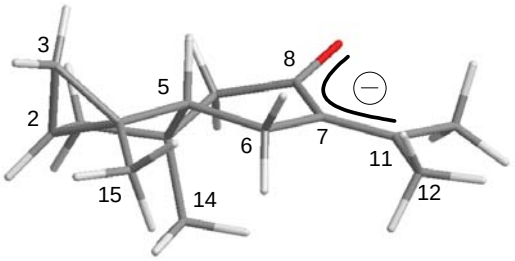

Fig. 4. Stereostructure of **3**.

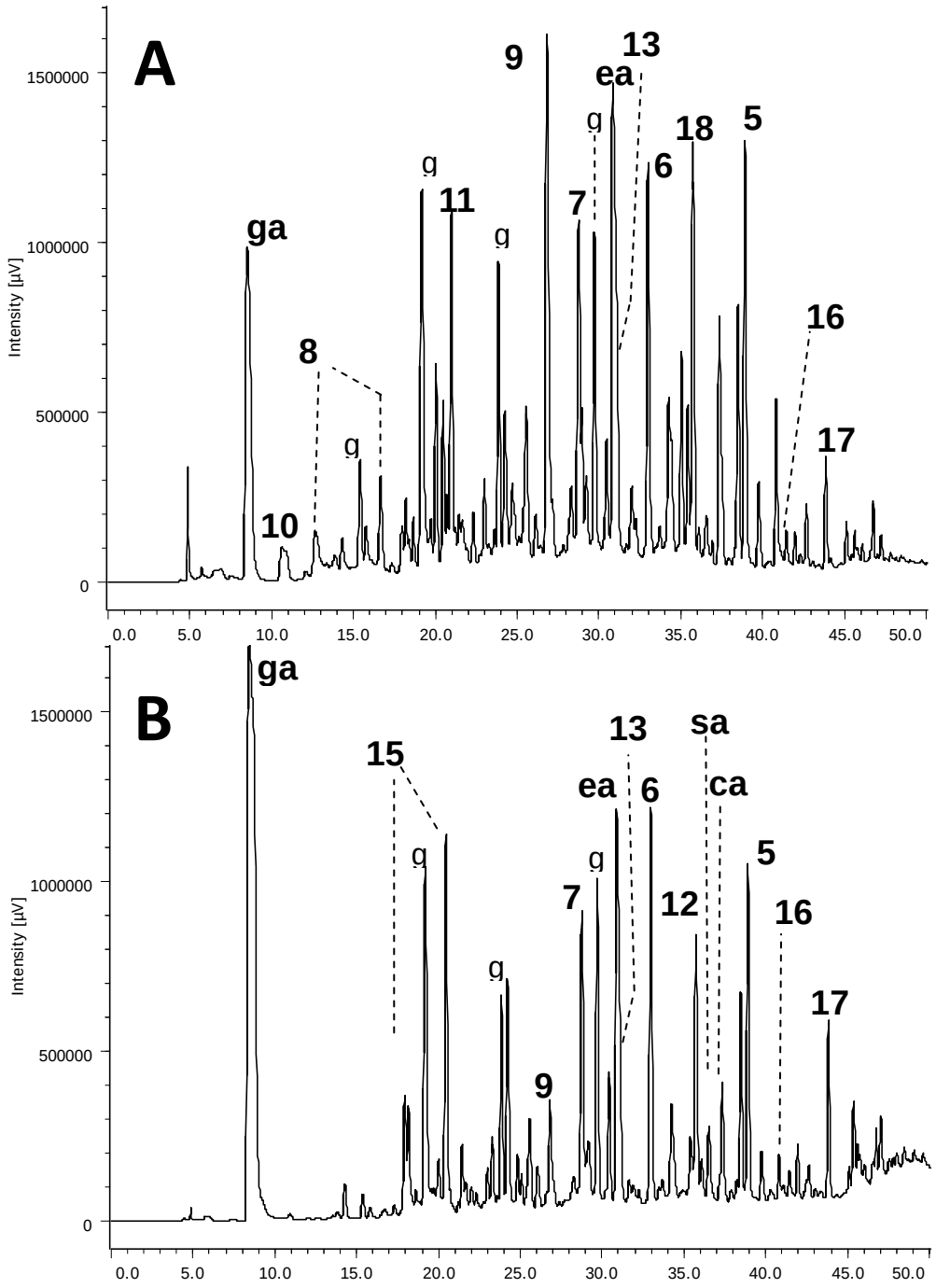

Fig. 5. Reversed-phase HPLC of EtOAc soluble fraction of *P. strobilacea* wood.

A: EtOAc fraction of fresh wood, **B:** EtOAc fraction of charred wood. g: Unidentified galloyl glucoses , ga: Gallic acid, ea: Ellagic acid, ca: Coniferylaldehyde, sa: Sinapylaldehyde.

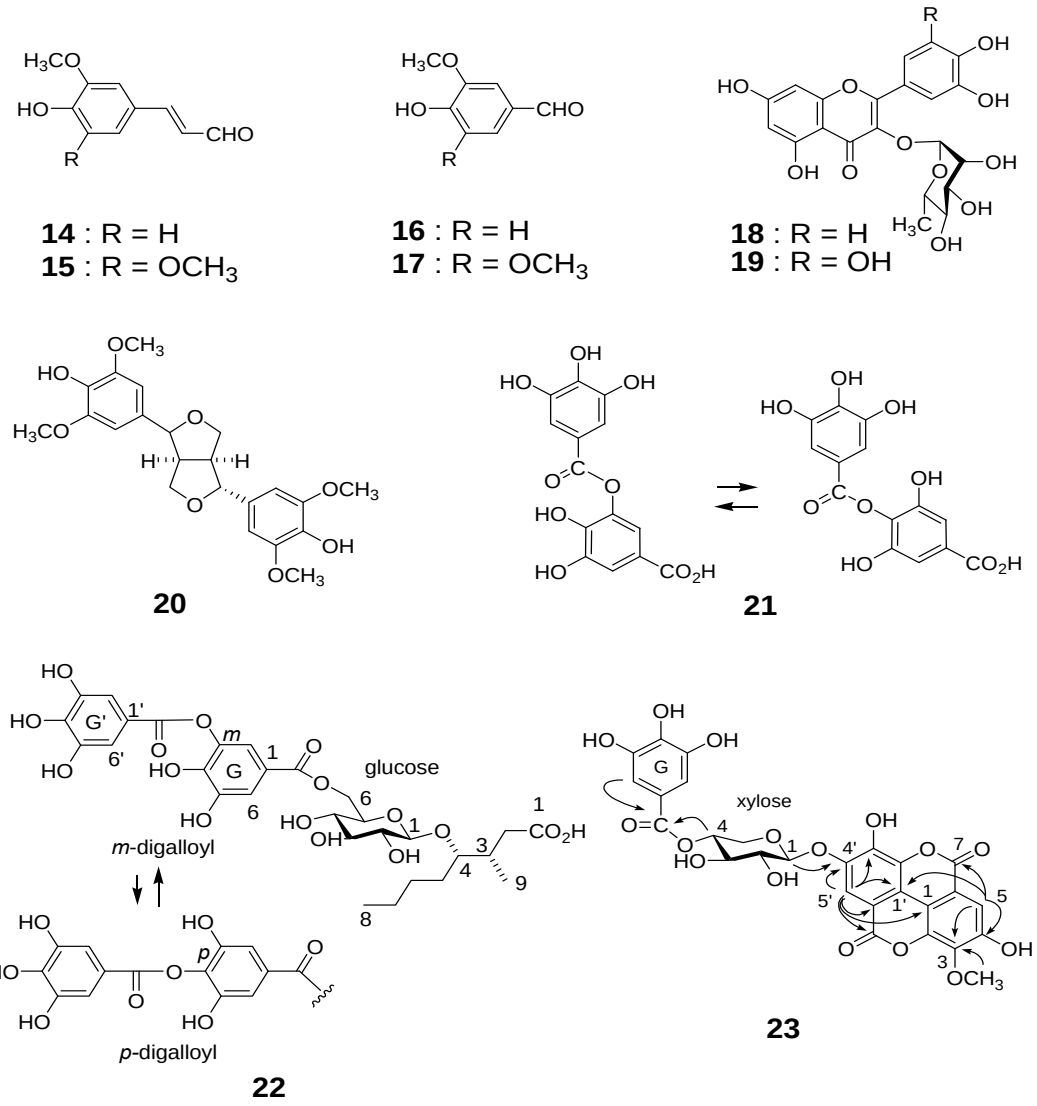

Fig. 6. Structures of compounds isolated from charred wood.

Table 1 ^{1}H-(500 MHz), ^{13}C-(125 MHz) NMR spectroscopic data for compounds **1**, **2**, and **3** (δ in ppm, J in Hz)

	1		**2**		**3**	
Position	^{1}H[a]	^{13}C[b]	^{1}H[b]	^{13}C[b]	^{1}H[b]	^{13}C[b]
1	0.86 (br dd, 3.4, 12.0)	45.7	0.98 (br d, 4.0, 12.7)	43.9	0.95 (br dd, 3.2, 12.7)	44.6
	1.84 (dd, 6.6, 12.0)		1.76 (dd, 6.8, 12.7)		1.82 (dd, 6.8, 12.7)	
2	1.22 (m)	18.7	1.20 (m)	26.8	1.21 (dddd, 3.2, 4.0, 6.8, 8.3)	26.8
3	0.54 (t, 3.9)	33.6	0.44 (t, 4.4)	33.5	0.39 (t, 4.0)	33.4
	0.99 (dd, 3.9, 8.2)		0.92 (dd, 4.4, 8.3)		0.91 (dd, 4.0, 8.3)	
4		27.1		26.0		26.4
5	1.05 (dd, 3.0, 13.0)	56.8	1.52 (m)	54.0	1.42 (dd, 5.2, 14.2)	52.2
6	2.06 (dt, 13.0, 3.0)	19.3	2.13 (m)	25.2 [c]	2.28 (dddd, 0.9, 2.1, 14.2, 15.1	27.0
	2.16 (q, 13.0)		2.23 (m)		2.71 (ddd, 0.7, 5.2, 15.1)	
7	1.33 (dt, 13.0, 3.0)	51.1	2.21 (m)	58.8		130.5
8	4.62 (dt, 3.0, 3.0)	69.2		215.4		203.6
9	1.36 (dd, 3.0, 14.2)	45.2	2.18 (d, 13.2)	54.8	2.18 (br d, 15.9)	54.2
	2.06 (dd, 3.0, 14.2)		2.25 (d, 13.2)		2.31 (d, 15.9)	
10		50.9		54.3		52.1
11		73.2		71.9		146.3
12	1.50 (s)	29.1	1.25 (s)	28.6	1.83 (d, 0.9)	23.1
13	1.62 (s)	28.8	1.20 (s)	25.2 [c]	2.01 (dd, 0.7, 2.1)	23.6
14	1.51 (s)	21.7	0.88 (s)	20.2	1.00 (d, 0.9)	20.6
15	1.23 (s)	26.3	1.11 (s)	18.9	1.17 (s)	19.1

[a] Measured in C_5D_5N, [b] measured in $CDCl_3$, [c] assignments may be interchanged.