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A sesquiterpene hydrocarbon from the bogwoods of *Cryptomeria japonica* D. Don, presumably formed by diagenetic hydrogenation

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Abstract

Structure of an hitherto unknown component of the essential oils from two bogwoods of *Cryptomeria japonica* D. Don was determined by mass spectrometry and NMR analyses. It was identified as cadina-1(10)-ene, a new cadinane-type sesquiterpene hydrocarbon with a single double bond.

Keywords: *Cryptomeria japonica*; Taxodiaceae; Sugi; Sesquiterpene; Cadina-1(10)-ene

1. Introduction

Sugi, *Cryptomeria japonica* (Taxodiaceae), a conifer tree, is one of the most popular and commercially important lumber sources in Japan. It is also found as unearthed materials as natural bogwoods or artifacts, such as dugout canoes, several thousand years old.

Recently, natural bogwood samples of *C. japonica* were discovered in the southwestern part of the main island of Japan. One was 2950 ± 70 years old in the sediment of a muddy field of Abu-cho, Yamaguchi, Japan (Y-bogwood) (Narita et al., 2006), and the other was 3500–3800 years old in the volcanic ash of Sanbe-cho, Shimane, Japan (S-bogwood) (Narita and Yatagai, 2006). The GC/MS analyses of the essential oils from these bogwoods identified thirty-one sesquiterpenes for the Y-bogwood, and eight for the S-bogwood. Of these, several compounds have remained unidentified. These unidentified compounds were supposed to be derived from original terpenes in fresh wood via geochemical processes, because they were not present in the essential oil from fresh wood collected in the forest 40 km east of the Y-bogwood site. In the present study, we isolated one component common to the essential oils of Y- and the S-bogwoods, and determined its structure as a new cadinane-type sesquiterpene hydrocarbon (**1**, *m*/*z* 206).

2. Results and discussion

Analyzing the essential oils from *Y*- and *S*-bogwoods by GC/MS, compound **1** showed its molecular ion signal at *m*/*z* 206. It was likely to have the molecular formula of $C_{15}H_{26}$, possibly with three degrees of unsaturation. However, there were no mass spectral data corresponding to it in the databases of Adams (2001) and the NIST (National Institute of Standardization, USA) 62 Mass spectral library. It is well known that deposited organic materials such as fossil woods, coals, petroleum, and sediments often contain hydrogenated hydrocarbons *m*/*z* 208 originating from sesquiterpenes with *m*/*z* 204 (Staccioli et al., 1993; Elias et al., 1996). Since the major sesquiterpene hydrocarbons in *sugi* fresh wood are δ-cadinene (**2**) 204 ($C_{15}H_{24}$) and α-muurolene (**3**) 204 ($C_{15}H_{24}$), compound **1** was considered to be a partially hydrogenated product formed from one of those components. It was previously reported

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Table 1
NMR spectroscopic data in $CDCl_3$ for cadina-1(10)-ene (**1**)

C	^{13}C (ppm)		^1H (ppm) (J = Hz)	DQF-COSY	HMBC	NOESY
1	132.5	C	–	–	2a, 3a, 3b, 5a, 7, 9, 14	–
2	29.7	CH_2	a, 2.69 (3.5, 3.5, 13.5), ddd	2b, 3b	3a,3b	2b, 3a, 14
			b, 1.60,m	2a, 3a, 3b	–	2a
3	36.1	CH_2	a, 1.71, m	2b, 3b	2a 4, 5a, 5b, 15	2a, 3b, 4
			b, 0.85, m	2a, 2b, 3a, 4	–	3a
4	33.0	CH	1.51, m	3b, 5a, 15	2a, 3a, 3b, 5a, 5b, 15	3a, 15
5	43.1	CH_2	a, 0.58 (12.0, 12.0, 12.0), ddd	4, 5b, 6	3a, 4, 7, 15	7, 9, 15
			b, 1.91, m	5a	–	5a, 6, 11
6	40.8	CH	1.78, m	5a, 7	2a, 5a, 5b, 7, 8a, 8b, 11	11, 12, 13
7	46.8	CH	0.99, m	6, 8b	5a, 5b, 8a, 8b, 9, 11, 12, 13	5a, 6, 8a, 8b, 9
8	21.3	CH_2	a, 1.55 (3.5), d	8b, 9	7, 11	7, 8b, 9, 13
			b, 1.16, m	7, 8a, 9	–	3a, 7, 9, 12
9	31.8	CH_2	1.90, br s	8a, 8b	7, 8a, 8b, 14	3a, 5a, 6, 7, 8b, 14, 15
10	124.2	C	–	–	2a, 8b, 9, 14	–
11	27.5	CH	1.84, m	12, 13	7, 8a, 12, 13	6, 12, 13
12	17.0	CH_3	0.78 (6.5), d	11	11, 13	6, 8b, 11, 13
13	22.0	CH_3	0.94 (6.5), d	11	7, 11, 12	6, 8a, 11, 12, 15
14	19.1	CH_3	1.63, br s	–	–	2a, 3b, 6, 9
15	22.5	CH_3	0.87 (6.0), d	4	3a, 5a, 5b	3a, 4, 5a, 9

that five unknown compounds with m/z 206 were detected by GC/MS in fossil wood and they were considered to be diagenetic hydrogenation intermediates of cadinane-type (MW 204) sesquiterpene hydrocarbons (Staccioli et al., 1993). However, compound **1** showed a MS pattern different from them.

The wood powders of Y-bogwood were extracted with hexane and separated by column chromatography (CC). Compound **1** was isolated by following preparative thin layer chromatography (TLC). The 1D and 2D NMR spectroscopic analysis of compound **1** (Table 1) revealed its structure as a new cadinane-type hydrocarbon with one double bond, cadina-1(10)-ene (Fig. 1). The ^1H NMR spectrum in $CDCl_3$ showed three doublets and one broad singlet for methyl groups at 0.78 (3H, *d*, $J = 6.5$ Hz, H-12), 0.87 (3H, *d*, $J = 6.0$ Hz, H-15), 0.94 (3 H, *d*, $J = 6.5$ Hz, H-13) and 1.63 (3H, *brs*, H-14), respectively. The downfield broad singlet of methyl group (δ 1.63) indicated that it was attached to a double bond. The ^{13}C NMR spectrum of **1**, together with the information from the Edit-HSQC spectrum, showed the presence of 15 carbon signals assigned to four CH_3 groups (δ 17.0, 19.1, 22.0 and 22.5), five CH_2 groups (δ 21.3, 29.7, 31.8, 36.1 and 43.1), four CH groups (δ 27.5, 33.0, 40.8 and 46.8) and two olefinic quarternary carbons (δ 124.2 and 132.5). The DQF-COSY and HMBC spectra established the carbon skeleton of **1** (Fig. 1a). The proton–proton correlations observed in DQF-COSY revealed that the C-15 methyl group was attached to C-4, that C-12 and 13 methyl groups were attached to C-11 to form a isopropyl group and that the highly shielded proton at δ 0.58 (1H, *ddd*, $J = 12.0$, 12.0, 12.0 Hz, H-5a) was one of the C-5 methylene protons

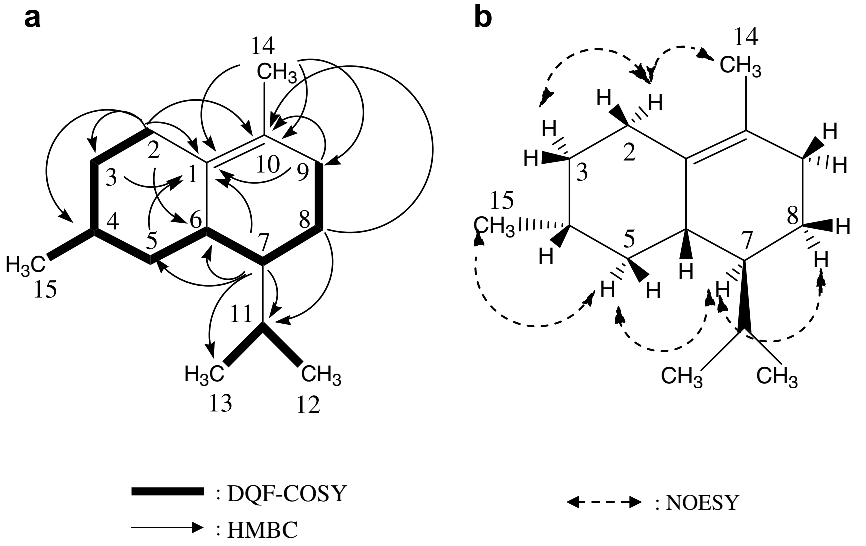


Fig. 1. Correlations seen in NMR spectrum of cadina-1(10)-ene (**1**). (a) DQF-COSY and HMBC, (b) NOESY.

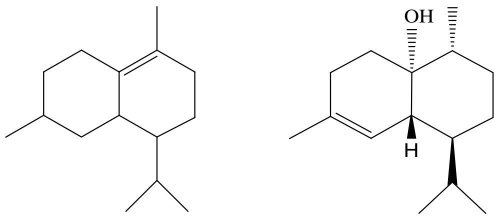

hydrocarbon "**III**" Cubenol "**I**"

Fig. 2. A synthesized hydrocarbon "**III**" and the starting compound, Cubenol "**I**".

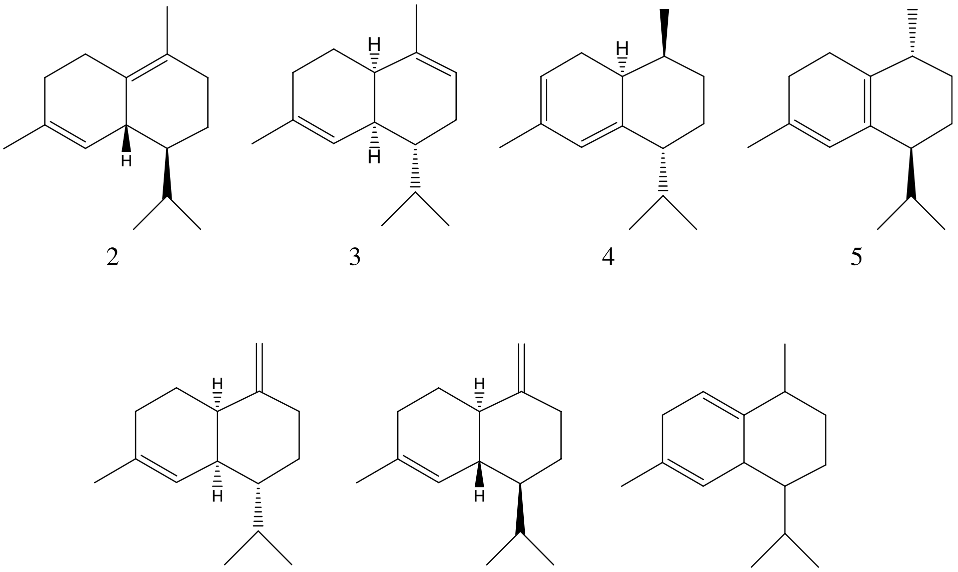
shielded by the double bond. The large vicinal coupling constants ($J_{4,5a} = J_{5a,6} = 12.0$ Hz) indicated the *trans*-dispositions of H-5a/H-4 and H-5a/H-6. It was found that H-5a had couplings with H-4, H-5b and H-6, respectively in the fine structures of cross peaks in DQF-COSY. Thus H-5a, H-4 and H-6 are supposed to be all axial. It was confirmed by the strong NOE interactions of H-5a/H-7 and H-5a/H-15 and no interactions of H-5a/H-4 and H-5a/H-6 in NOESY spectrum. The downfield shifted signal at δ 2.69 (1H, *ddd*, J = 3.5, 3.5, 13.5 Hz, H-2a) was assigned one of allylic C-2 methylene protons which was in-plane with the double bond. The C-14 methyl group at δ 1.63 showed HMBC correlations with two double bond carbons at δ 132.5 (C-1) and δ 124.2 (C-10), as well as a methylene carbon at δ 31.8 (C-9). One of the methylene protons, H-2a at δ 2.69, showed HMBC correlations with the double bond carbons (C-1 and C-10), a methylene carbon at δ 36.1 (C-3) and two methine carbons at δ 33.0 (C-4) and δ40.8 (C-6). The methylene protons H-9 at δ 1.9 showed HMBC correlations with the double bond carbons (C-1 and C-10) and a methine carbon at δ 46.8 (C-7). The methine proton H-7 at δ 0.99 showed HMBC correlations with the double bond carbon (C-1), a methyl carbon at δ 22.0 (C-13), a methylene carbon at δ 43.1 (C-5) and two methine carbons at δ 40.8 (C-6) and δ 27.5 (C-11). The relative configuration of **1** was derived from analysis of data from a NOESY experiment (Fig. 1b).

As an artificial product, hydrocarbon "**III**" was reported derived from cubenol "**I**" by catalytic hydrogenation and subsequent dehydration with formic acid (Fig. 2) (Ohta and Hirose, 1967). While the derivative "**III**" was characterized only by ^{1}H NMR (CCl_4) signals of four methyl groups at δ 0.79 and 0.94 {6H, two doublets, J = 6.6 cps, $(C\underline{H}_3)_2CH$–}, 0.89 (3H, *d*, J = 5.7 cps, $C\underline{H}_3CH$<) and 1.60 {3H, *s*, –$(C\underline{H}_3)C{=}C$<}, it seems to be identical with **1**. Thus cadina-1(10)-ene (**1**) has been known as a synthetic compound only from partial characterisation of ^{1}H NMR data, but was identified here as a natural geochemical product for the first time. Seven non-aromatic cadinane-type sesquiterpenes {δ-cadinene (**2**), α-muurolene (**3**), *trans*-3,5-muuroladiene (**4**), cadina-1(6),4-diene (**5**), γ-muurolene (**6**), γ-cadinene (**7**), 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methy ethyl)-naphthalene (**8**)} were previously identified in the fresh wood (Narita et al., 2006) (Fig. 3). In these compounds, only δ-cadinene (**2**) has a double bond at the same position as that of **1**. Compound **2** was present also in the Y-bogwood in a moderate amount, and in the S-bogwood in a small amount. This situation strongly suggests that **1** was induced from δ-cadinene (**2**) by diagenetic hydrogenation.

3. Experimental

3.1. General experimental procedures

GC-FID was carried out by a Shimadzu GC-14C with 30 m × 0.25 mm TC-FFAP capillary column, film thickness


2 3 4 5

6 7 8

Fig. 3. Cadinane-type non-aromatic sesquiterpene hydrocarbons reported from fresh wood of *Cryptomeria japonica*.

0.25 μm. The temperature of the oven was held at 60 °C for 15 min, and it was programmed to rise at 5 °C/min to 220 °C, at which it was kept for 23 min. Helium was used as carrier gas with flow rate of 1.22 ml/min. The temperatures of detector and injector were 220 °C.

The GC/MS analysis was carried out by a Shimadzu QP5050A Ver. 1 with 30 m × 0.25 mm TC-FFAP capillary column, with film thickness of 0.25 μm (GL Sciences, Tokyo, Japan). The oven was maintained at 60 °C for 15 min, and then was heated at 5 °C/min to 220 °C, at which it was kept for 23 min. Helium at flow rate of 0.7 ml/min was used as carrier gas. The head pressure was 30.0 kPa at 220 °C. The temperature of both transfer line and injector was 220 °C. Mass spectra were obtained in the electron impact (EI) mode at 70 eV.

NMR measurements were carried out with a JEOL JNM-A500spectrometer (500 MHz) with TMS as an internal standard in $CDCl_3$.

Silicagel 60 (Merck) was used as the liquid chromatography column.

For preparative thin layer chromatography (TLC) plates of RP-18 F254s (Merck) were used.

3.2. Plant materials

A stump of *C. japonica* (Y-bogwood) was collected from a muddy sediment in Abu-cho, Yamaguchi, Japan. It was estimated to have been more than 180 years old at the time of burial and was shown to be 2950 ± 70 years old by ^{14}C dating, carried out at the Laboratory for Radiocarbon Dating of the University Museum of the University of Tokyo. The stump was dried for approximately three years. The area where the bogwoods were collected used to be a sedimentary basin (Hatanaka and Miyoshi, 1980) and is currently used as a paddy field for rice cultivation. A trunk of *C. japonica* (S-bogwood) was collected from volcanic ash in a buried forest at the base of Sanbe volcano in Sanbe-cho, Shimane, Japan. It is estimated to have been more than 93 years old at the time of burial. Bogwoods collected in the same place were shown to be c. 3500–3800 years old by ^{14}C dating (Watanabe, 2000).

3.3. Extraction and separation

The heartwoods of the samples were ground in a Wiley mill to pass a 2-mm screen. Essential oils were obtained by the distillation procedure described previously (Narita et al., 2006; Narita and Yatagai, 2006).

The *n*-hexane extract was obtained by Soxhlet extraction of milled dry sample 120 g of Y-bogwood with 2,000 ml of *n*-hexane. The solvent was removed from the extracted solution by evaporation, providing a brown oil (10.3 g). An aliquot of the oil (5.0 g) was next subjected to silica gel(120 g)CC with *n*-hexane, ethyl acetate and methanol successively to give 30 fractions. Then the first fraction (0.20 g) eluted with *n*-hexane (200 ml) was separated into 10 fractions by silica gel (10 g) CC with *n*-hexane. The first fraction (0.10 g) of the second chromatography eluted with *n*-hexane (10 ml) was separated into 16 fractions by silica gel (12 g) CC with *n*-hexane. The 9th and 10th fractions eluted with *n*-hexane (1 ml), respectively, were combined, and purified by reversed-phase TLC with methanol. The fraction of R_f 0.32 provided compound **1** (8 mg, colorless oil). The constituents of the fractions were monitored with GC and GC/MS.

*3.4. Cadina-1(10)-ene(**1**)*

Colorless oil; R.I.$_{TC-FFAP}$:1592; MS (EI, 70 eV), *m/z*: 206$[M]^+$ (15), 163 (100), 136 (9), 121 (18), 107 (38), 95 (13), 93 (22), 91 (19), 81 (24), 79 (20), 77 (10), 69 (9), 67 (10), 55 (14), 41 (21); for ^1H and ^{13}C NMR spectroscopic data, see Table 1.

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