Phytochemistry 69 (2008) 3080–3086

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Phloroglucinol diglycosides accompanying hydrolyzable tannins from *Kunzea ambigua*

Naoki Kasajima [b], Hideyuki Ito [a,*], Tsutomu Hatano [a,*], Takashi Yoshida [c]

[a] *Department of Pharmacognosy, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Tsushima, Okayama 700-8530, Japan*
[b] *School of Pharmacy, Shujitsu University, Nishigawara, Okayama 703-8516, Japan*
[c] *Matsuyama University, Bunkyo-cho, Matsuyama 790-8578, Japan*

ARTICLE INFO

*Article history:*
Received 17 January 2008
Received in revised form 20 February 2008
Available online 23 April 2008

*Keywords:*
*Kunzea ambigua*
Myrtaceae
Phloroglucinol
Tannin
Kunzeaphlogins
Kunzeatannin

ABSTRACT

Six phloroglucinol diglucosides—kunzeaphlogins A–F (**1**–**6**) and a hydrolyzable tannin, kunzeatannin A (**7**)—were isolated along with 10 known polyphenols from the leaf extract of *Kunzea ambigua*. Structural elucidation of these compounds was based on spectroscopic analyses and chemical properties.



## 1. Introduction

*Kunzea ambigua* (SM.) Druce belonging to the Myrtaceae is native to Australia (Tasmania, New South Wales, and Victoria). Its essential oil containing monoterpenes and sesquiterpenes has been used in New Zealand as a remedy for the treatment of diarrhoea, cold, inflammation, and wounds (David, 1997; Khambay et al., 2002). Besides the reported phloroglucinol derivatives, we previously isolated six galloylated chromone *C*-glucosides (kunzeachromones A–F) and a dimeric flavonol glycoside (kunzeagin A) (Ito et al., 2004b), as well as acylated phloroglucinol derivatives (kunzeanones A–C) (Ito et al., 2004a), from the leaf extract of this plant. Further investigation on phenolic constituents has led to isolation of 17 polyphenols including seven new compounds (six phloroglucinol glucosides and a hydrolyzable tannin). We describe herein the structural elucidation of these compounds.

## 2. Results and discussion

### 2.1. Structure elucidation of new compounds

The ethyl acetate and *n*-butanol extracts of an aqueous acetone homogenate of the dried leaves of *K. ambigua* were purified by a combination of various column chromatographic techniques (Toyopearl HW-40, Diaion HP-20, MCI GEL CHP-20P, YMC-GEL ODS-AQ 120-S50 and/or Sephadex LH-20 columns) to afford three new compounds [kunzeaphlogins C–E (**3**–**5**)] and three known compounds [3-*O*-*p*-coumaroylquinic acid (Haslam et al., 1961), 4′,6′-dihydroxyisobutrophenone 2′-*O*-β-glucoside (Vancraenenbroeck et al., 1965) and pedunculagin (**8**) (Hatano et al., 1988)] from the ethyl acetate extract, and five new compounds [kunzeaphlogins A (**1**), B (**2**), D (**4**), F (**6**) and kunzeatannin A (**7**)] along with eight known compounds [4-*O*-β-glucopyranosyl-*cis*-coumaric acid (Cui et al., 1990), 3-*O*-*p*-coumaroylquinic acid, 4-*O*-*p*-coumaroylquinic acid, ellagic acid, 3,3′-di-*O*-methylellagic acid (Duc Do et al., 1990), 3,3′-di-*O*-methylellagic acid 4-*O*-β-glucoside (Pakulski and Budzianowski, 1996), casuarinin (Okuda et al., 1983) and praecoxin A (Hatano et al., 1991)] from the *n*-butanol extract.

Kunzeaphlogin A (**1**) was obtained as a pale yellow amorphous powder. Its molecular formula of $C_{23}H_{34}O_{14}$ was determined by high-resolution electrospray ionization mass spectroscopy (HRESIMS) [*m/z* 535.1991].

The $^1$H NMR spectrum of **1** showed signals due to an isovaleroyl group {two secondary methyl groups [$\delta_H$ 0.91, 0.87 (3H each, *d*, *J* = 7.0 Hz)], a methylene signal [$\delta_H$ 3.15 (1H, *dd*, *J* = 6.5, 16.5 Hz), 2.88 (1H, *dd*, *J* = 7.5, 16.5 Hz)] and a methine signal [$\delta_H$ 2.16 (1H, *m*)]}, and an aromatic proton [$\delta_H$ 6.28 (1H, *s*)]. The presence of two glucose residues was also suggested by two anomeric proton resonances [$\delta_H$ 5.04 (*d*, *J* = 7.5 Hz, H-1′) and 4.80 (*d*, *J* = 9.5 Hz, H-1″)] and other aliphatic proton signals characteristic of $^4C_1$

* Corresponding authors. Tel./fax: +81 86 251 7937.
*E-mail address:* hito@cc.okayama-u.ac.jp (H. Ito).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.03.003

glucopyranose residue, which were assigned by $^1$H–$^1$H COSY. The $^{13}$C NMR spectrum of **1** supported the presence of a phloroglucinol moiety ($\delta_C$ 165.5, 164.1, 161.0, 106.4, 105.9, 95.4), an isovaleroyl group ($\delta_C$ 206.8, 53.5, 25.4, 23.1, 22.6) and two glucose moieties (Table 1). In the HMQC spectrum of **1**, the anomeric proton signals at $\delta_H$ 5.04 and 4.80 correlated with carbon resonances at $\delta_C$ 101.3 and 74.7, respectively, suggesting that kunzeaphlogin A shares *C*- and *O*-glucosidic characters (see Fig. 1).

The HMBC spectrum of **1** showed long-range correlations between H-1′ ($\delta_H$ 5.04) and C-2 ($\delta_C$ 161.0) and between the H-1″ signal ($\delta_H$ 4.80) and carbon resonances at $\delta_C$ 164.1 (C-4), 106.4 (C-5) and 165.5 (C-6) (Fig. 2). The aromatic proton at $\delta_H$ 6.28 correlated with the carbons at $\delta_C$ 105.9 (C-1), 161.0 (C-2), 164.1 (C-4) and 106.4 (C-5). Structure **1** thus assigned to kunzeaphlogin A was verified by the NOESY measurement, which showed NOEs between the H-1′ and the aromatic proton at $\delta_H$ 6.28. The *O*-glycosylated sugar obtained from acid hydrolysis of **1** was identified as D-glucose by co-chromatography with an authentic sample on HPLC equipped with an optical rotation detector and also by positive reaction to D-glucose oxidase (Miwa et al., 1972). Although the *C*-glycosidic sugar in **1** lacks direct evidence, its assignment is likely based on the analogy of the $^1$H and $^{13}$C NMR spectroscopic data to those of the values reported in the literature (Agrawal, 1989). Based on these findings, the structure of kunzeaphlogin A was determined as formula **1**.

**Table 1**
$^{13}$C NMR spectroscopic data for kunzeaphlogins A (**1**)–C (**3**) (126 MHz, acetone-$d_6$ + $D_2O$)

| Carbon | 1 | 2 | 3 |
|---|---|---|---|
| C-1 | 105.9 | 106.1 | 106.18 |
| C-2 | 161.0 | 160.8 | 160.9 |
| C-3 | 95.4 | 95.6 | 95.7 |
| C-4 | 164.1 | 164.4 | 164.5 |
| C-5 | 106.4 | 106.4 | 106.19 |
| C-6 | 165.5 | 165.4 | 165.7 |
| C-7 | 206.8 | 206.7 | 206.9 |
| C-8 | 53.5 | 53.5 | 53.4 |
| C-9 | 25.4 | 25.5 | 25.5 |
| C-10 | 23.1 | 23.0 | 22.9 |
| C-11 | 22.6 | 22.6 | 22.5 |
| *O-Glucose* | | | |
| C-1’ | 101.3 | 101.0 | 101.0 |
| C-2’ | 74.1 | 74.0 | 74.0 |
| C-3’ | 77.7[a] | 79.1[d] | 77.3[g] |
| C-4’ | 70.8[b] | 70.9[e] | 70.3 |
| C-5’ | 77.6[a] | 78.9[d] | 71.7[g] |
| C-6’ | 62.1[c] | 63.7[f] | 63.8[h] |
| *C-Glucose* | | | |
| C-1” | 74.7 | 74.7 | 74.7 |
| C-2” | 72.2 | 72.3 | 72.2 |
| C-3” | 79.3 | 77.3[d] | 77.0[g] |
| C-4” | 70.7[b] | 70.2[e] | 72.2 |
| C-5” | 81.6 | 75.1 | 75.1[g] |
| C-6” | 61.9[c] | 64.8[f] | 64.2[h] |
| *Galloyl* | | | |
| C-1”’ | | 120.92 | 120.93 |
| | | 120.85 | 120.86 |
| | | | 120.8 |
| C-2”’, 6”’ | | 109.8 (2C) | 110.1 (4C) |
| | | 109.7 (2C) | 109.9 (2C) |
| C-3”’, 5”’ | | 145.78 (2C) | 145.8 (2C) |
| | | 145.77 (2C) | 145.7 (2C) |
| | | | 145.69 (2C) |
| C-4”’ | | 138.91 | 139.12 |
| | | 138.87 | 138.91 (2C) |
| C-7”’ | | 167.4 | 167.3 |
| | | 167.2 | 167.2 |
| | | | 166.8 |

[a–h] Interchangeable.

Kunzeaphlogins B (**2**) and C (**3**) were shown to have molecular formulas of $C_{37}H_{42}O_{22}$ and $C_{44}H_{46}O_{26}$, respectively, by HRESIMS. Their UV spectral patterns (**2**; $\lambda_{max}$ 219, 280 and 322sh, **3**; $\lambda_{max}$ 219, 280 and 321sh) were similar to that of **1**. The $^1$H and $^{13}$C NMR spectroscopic data of **2** and **3** were also similar to those of compound **1**, except for the appearance of galloyl signals [$\delta_H$ 7.07, 7.06 (2H each, *s*) in **2**; $\delta_H$ 7.17, 7.115, 7.109 in **3**]. The location of the two galloyl groups at O-6′ and O-6″ of the glucose residues in **2** was determined by comparison of the $^1$H NMR spectrum of **2** with that of **1**, which showed a remarkable downfield shift ($\Delta$ 0.67–0.87 ppm) of H-6′and H-6″ in **2**. Three galloyl groups in compound **3** were similarly allocated at O-6′, O-6″ and O-4″ by a large downfield shift of H-4″ ($\Delta$ 1.82 ppm) of **3** on comparing the $^1$H NMR spectroscopic data with those of **2**. The structural relationship between **2** and **3** was confirmed by enzymatic hydrolysis of 3 with tannase, which afforded **2** and **1**, along with gallic acid. Consequently, the structures of kunzeaphlogins B and C are represented by formulas **2** and **3**, respectively.

Kunzeaphlogin D (**4**), a pale yellow amorphous powder, had the molecular formula of $C_{25}H_{38}O_{14}$ as indicated by HRESIMS. A phloroglucinol skeleton of **4** was deduced from analysis of the UV ($\lambda_{max}$ 217, 271 and 321sh) and NMR spectra. The $^1$H and $^{13}$C NMR spectra of **4** exhibited the presence of an isovaleroyl group [$\delta_C$ 210.4, 53.8, 26.0, 22.9, 22.7; $\delta_H$ 3.16 (1H, *dd*, *J* = 7.0, 15.5 Hz), 2.93 (1H, *dd*, *J* = 7.0, 15.5 Hz), 2.08 (1H, *m*), 0.84, 0.83 (3H each, *d*, *J* = 7.0 Hz)]. The spectra also showed signals due to two aromatic methyl groups [$\delta_C$ 10.9, 9.8; $\delta_H$ 2.27, 2.14 (3H each, *s*)] and a phloroglucinol moiety [$\delta_C$ 159.4, 157.3, 153.4, 118.5, 117.3, 116.8] as well as two glucose entities (Table 2). The HMBC spectrum of **4** showed correlations of H-1′ to C-2; H-1″ to C-4; 3-Me to C-2, C-3 and C-4; 5-Me to C-4, C-5 and C-6; H-8 to C-7, C-9, C-10 and C-11; H-9 to C-8, C-10, C-11; H-10, 11 to C-9 (Fig. 2). The NOESY spectrum of **4** showed correlations of 3-Me with both anomeric proton signals H-1′ and H-1″, and a correlation between 5-Me and H-1″. Acid hydrolysis of **4** yielded glucose and aglycone (**4a**). A liberated sugar from **4** was identified as D-glucose by reversed-phase HPLC with an optical rotation detector and positive reaction to D-glucose oxidase. The structure of **4a** was consistent with its $^1$H NMR spectroscopic data. Based on these data, the structure of kunzeaphlogin D was established as **4**.

The molecular formula of kunzeaphlogin E (**5**) was determined to be $C_{32}H_{42}O_{18}$ by HRESIMS. The $^1$H and $^{13}$C NMR spectra of **5** assigned from COSY, TOCSY, HMQC and HMBC were very similar to those of **4** except for the presence of an extra signal [$\delta_H$ 7.12 (2H, *s*)] due to a galloyl group and downfield shifts of H-6″ [$\delta_H$ 4.57 (1H, d, *J* = 2.0, 12.0 Hz), 4.28 (*d*, *J* = 4.0, 12.0 Hz)]. The downfield-shifted signals (H-6″) were assigned by correlation with the anomeric proton at $\delta_H$ 4.78 (H-1″) in the TOCSY spectrum of **5**. Enzymatic hydrolysis of **5** with tannase afforded **4** and gallic acid. Consequently, kunzeaphlogin E (**5**) was characterized as a monogallate of kunzeaphlogin D (**4**).

Kunzeaphlogin F (**6**) showed a pseudomolecular ion peak $(M + H)^+$ at *m/z* 549, which is 14 mass units ($CH_2$) less than that of **4**, and its molecular formula ($C_{24}H_{36}O_{14}$) was confirmed by HRESIMS. The UV, $^1$H and $^{13}$C NMR spectra of **6** were similar to those of **4**. A distinguishing feature from **4** in the NMR spectra was a lack of methylene signals, suggesting that the isovaleroyl group in **4** is replaced by an isobutyryl group {two methyl signals [$\delta_C$ 19.8, 17.5; $\delta_H$ 1.12, 0.92 (3H each, *d*, *J* = 6.0 Hz)], methine signal [$\delta_C$ 48.8; $\delta_H$ 3.90 (1H, *m*)], carbonyl carbon signal ($\delta_C$ 211.8)} in **6**. The HMQC, HMBC and NOESY spectroscopic data were fully consistent with structure **6** for kunzeaphlogin F.

Kunzeatannin A (**7**) showed the $(M + NH_4)^+$ ion peak at *m/z* 1586 in ESI-MS, corresponding to the molecular formula of $C_{68}H_{48}O_{44}$. Methylation of **7** with diazomethane followed by methanolysis gave methyl tri-*O*-methylgallate (**10**), dimethyl hexamethoxydi-

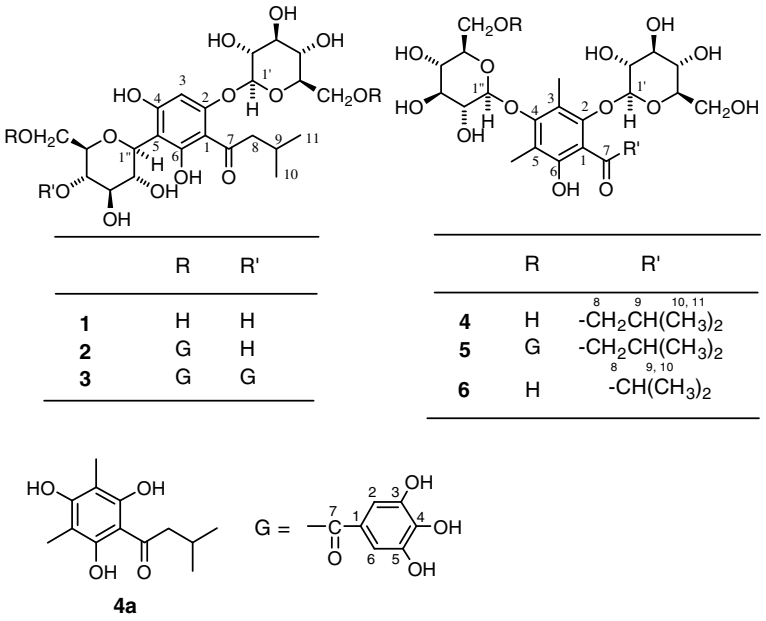


**Fig. 1.** Chemical structures of compounds **1**–**6**.

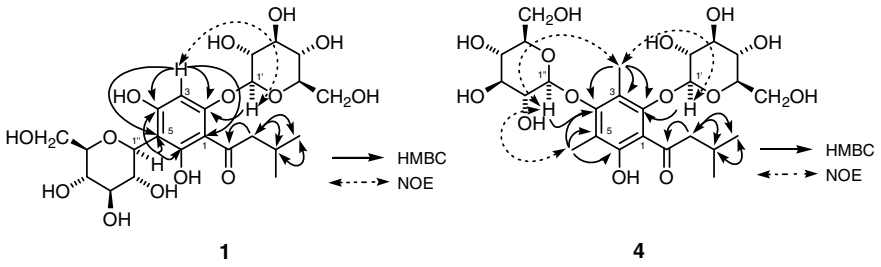


**Fig. 2.** HMBC and NOESY correlations of kunzeaphlogin A (**1**) and kuzneaphlogin D (**4**).

**Table 2**
$^{13}$C NMR spectroscopic data for kunzeaphlogins D (**4**)–F (**6**) (126 MHz, acetone-$d_6$ + $D_2O$)

| Carbon | 4 | 5 | 6 |
|---|---|---|---|
| C-1 | 116.8 | 116.8 | 116.4 |
| C-2 | 157.3 | 158.1 | 151.2 |
| C-3 | 118.5 | 118.4 | 117.5 |
| C-4 | 159.4 | 159.4 | 157.5 |
| C-5 | 117.3 | 117.4 | 117.1 |
| C-6 | 153.4 | 153.6 | 154.6 |
| C-7 | 210.4 | 210.3 | 211.8 |
| C-8 | 53.8 | 53.8 | 48.8 |
| C-9 | 26.0 | 26.0 | 19.8 |
| C-10 | 22.9 | 23.1 | 17.5 |
| C-11 | 22.7 | 22.9 | |
| 3-$CH_3$ | 10.9 | 10.8 | 10.5 |
| 5-$CH_3$ | 9.8 | 9.9 | 10.0 |
| *O-Glucose* | | | |
| C-1', 1'' | 104.9, 104.8 | 105.3, 104.8 | 104.3 (2C) |
| C-2', 2'' | 75.04, 74.97 | 75.5, 75.1 | 74.34, 74.25 |
| C-3', 3'' | 77.3, 77.1[a] | 77.7, 77.5[b] | 77.0 (2C)[c] |
| C-4', 4'' | 71.0, 70.8 | 71.8, 70.7 | 70.4, 70.1 |
| C-5', 5'' | 77.1, 77.0[a] | 77.3, 75.6[b] | 76.6, 76.3[c] |
| C-6', 6'' | 62.3, 62.1 | 63.2, 62.9 | 61.6, 61.3 |
| *Galloyl* | | | |
| C-1''' | | 121.8 | |
| C-2''', 6''' | | 110.1 (2C) | |
| C-3''', 5''' | | 146.0 (2C) | |
| C-4''' | | 138.8 | |
| C-7''' | | 166.7 | |

[a–c] Interchangeable.

phenate (**11**), and trimethyl octa-*O*-methyltergallate (**12**) (Fig. 2), indicating that **7** is a dimeric hydrolyzable tannin with a tergalloyl group as the connecting unit of monomers. The $^1$H NMR spectrum of **7** was complicated owing to a formation of multiple signals for each proton, suggesting that this compound exists as an equilibrium mixture of α- and β-anomers at sugar portion(s). Nevertheless, the presence of a galloyl, two HHDP and a tergalloyl group was demonstrated by aromatic proton signals composed of a 2H singlet and seven 1H singlets, most of which were paired by doubly duplicated (four lines) signals.

The atropisomerism of chiral HHDP and tergalloyl groups in **7** was shown to be in the (*S*)-series by a strong positive Cotton effect at 240 nm in the CD spectrum (Okuda et al., 1982). The sugar carbon resonances in the $^{13}$C NMR spectrum of **7** showed close correspondence with those of pedunculagin (**8**) and tellimagrandin I (**9**) (Wilkins and Bohm, 1976) (Table 3), which indicated that **7** is an ellagitannin dimer biogenetically produced through C–O oxidative coupling forming a tergalloyl group between a hexahydroxydiphenoyl group of **8** and a galloyl group of **9**. Structure **8** was presumed to be the most plausible one for kunzeatannin A, taking into consideration the most frequently encountered coupling mode in biogenesis of ellagitannin oligomers. The tergalloyl group is known to easily isomerize into an isomeric valoneoyl group by Smiles rearrangement (Yoshida et al., 1993). To verify the proposed structure, **7** was treated with phosphate buffer (pH 7.2) to yield an isomerized product, which was identified with the known hydrolyzable tannin, camelliin A (**13**) (Yoshida et al.,

**Table 3**
$^{13}$C-NMR spectroscopic data for the sugar moieties of kunzeatannin (**7**), pedunculagin (**8**) and tellimagrandin I (**9**) (126 MHz, acetone-$d_6$ + $D_2O$)

| Carbon | 7 | | 8 | | 9 | |
|---|---|---|---|---|---|---|
| | α | β | α | β | α | β |
| C-1 | 91.2 | 94.9 | 91.8 | 95.4 | | |
| C-2 | 75.3 | 77.9 | 75.6 | 78.3 | | |
| C-3 | 75.5 | 77.3 | 75.8 | 77.6 | | |
| C-4 | 69.8 | 69.4 | 69.9 | 69.6 | | |
| C-5 | 66.7 | 71.6 | 67.4 | 72.5 | | |
| C-6 | 63.83[b] | 63.79[b] | 63.6 | | | |
| C-1' | 90.6 | 96.0 | | | 91.2 | 96.7 |
| C-2' | 73.2 | 74.5 | | | 72.9 | 74.1 |
| C-3' | 71.3[a] | 73.6 | | | 71.1 | 73.5 |
| C-4' | 70.9[a] | | | | 71.1 | |
| C-5' | 66.9 | 71.9 | | | 67.2 | 72.0 |
| C-6' | 63.4[b] | | | | 63.5 | |

[a,b] Interchangeable.

1990) (Fig. 3). Based on these data, kunzeatannin A is represented by the structure **7**.

### 2.2. Concluding remarks

In this study, *K. ambigua* was demonstrated to contain novel diglucosidic phloroglucinols, kunzeaphlogins. Although only a phloroglucinol diglucoside having both *O*- and *C*-glucosidic linkages in the molecule has been isolated from *Dryopteris crassirhizome* (Dryopteridaceae) (Chang et al., 2006), kunzeaphlogins A (**1**), B (**2**) and C (**3**) are the second examples of this type of phloroglucinol. In addition, kunzeaphlogins B (**2**), C (**3**) and E (**5**) were also the first isolated products of the galloylated phloroglucinol diglucosides from natural sources. These galloylated glucosides in addition to previously reported kunzeachromones A–F may be characteristic constituents of this plant.

## 3. Experimental

### 3.1. General

$^1$H and $^{13}$C NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for $^1$H and 126 MHz for $^{13}$C), and the chemical shifts are given in $\delta$ (ppm) values relative to that of the solvent [acetone-$d_6$ ($\delta_H$ 2.04; $\delta_C$ 29.8)] and tetramethylsilane. The standard pulse sequences that were programmed into the instrument (VXR-500) were used for each two-dimensional measurement. The $J_{CH}$ value was set at 6 Hz in the HMBC spectra. Optical rotations were measured with a Jasco DIP-1000 polarimeter. UV spectra were measured with a Hitachi U-2000 spectrophotometer. ESIMS was recorded on a Micromass Auto Spec OA-TOF mass spectrometer (solvent: MeOH:$H_2O$ (1:1, v/v) containing 0.1% $NH_4OAc$; flow rate: 0.02 mL/min). Normal-phase HPLC was conducted on a YMC-Pack SIL A-003 column (4.6 mm i.d. × 250 mm; YMC Co., Japan), and developed at room temperature with a solution of *n*-hexane/MeOH/tetrahydrofuran/$HCO_2H$ (55:33:11:1 containing 450 mg/L oxalic acid) (solvent A) (detection: UV 280 nm). Reversed-phase HPLC was performed with a YMC-Pack ODS-A A-302 column (4.6 mm i.d. × 150 mm), and developed at 40 °C with 10 mM $H_3PO_4$/10 mM $KH_2PO_4$/MeCN (42.5:42.5:15, solvent B), 10 mM $H_3PO_4$/10 mM $KH_2PO_4$/AcOEt/EtOH (45:45:8:2, solvent C) and MeOH/$H_2O$ (60:40, solvent D). Column chromatography was performed on Diaion HP-20, MCI GEL CHP-20P (Mitsubishi Kasei Co., Japan), Toyopearl HW-40 (coarse grade; Tosoh Co., Japan), YMC-GEL ODS-AQ 120-50S (YMC Co. Ltd.) or Mega Bond Elut $C_{18}$ (Varian Inc.).

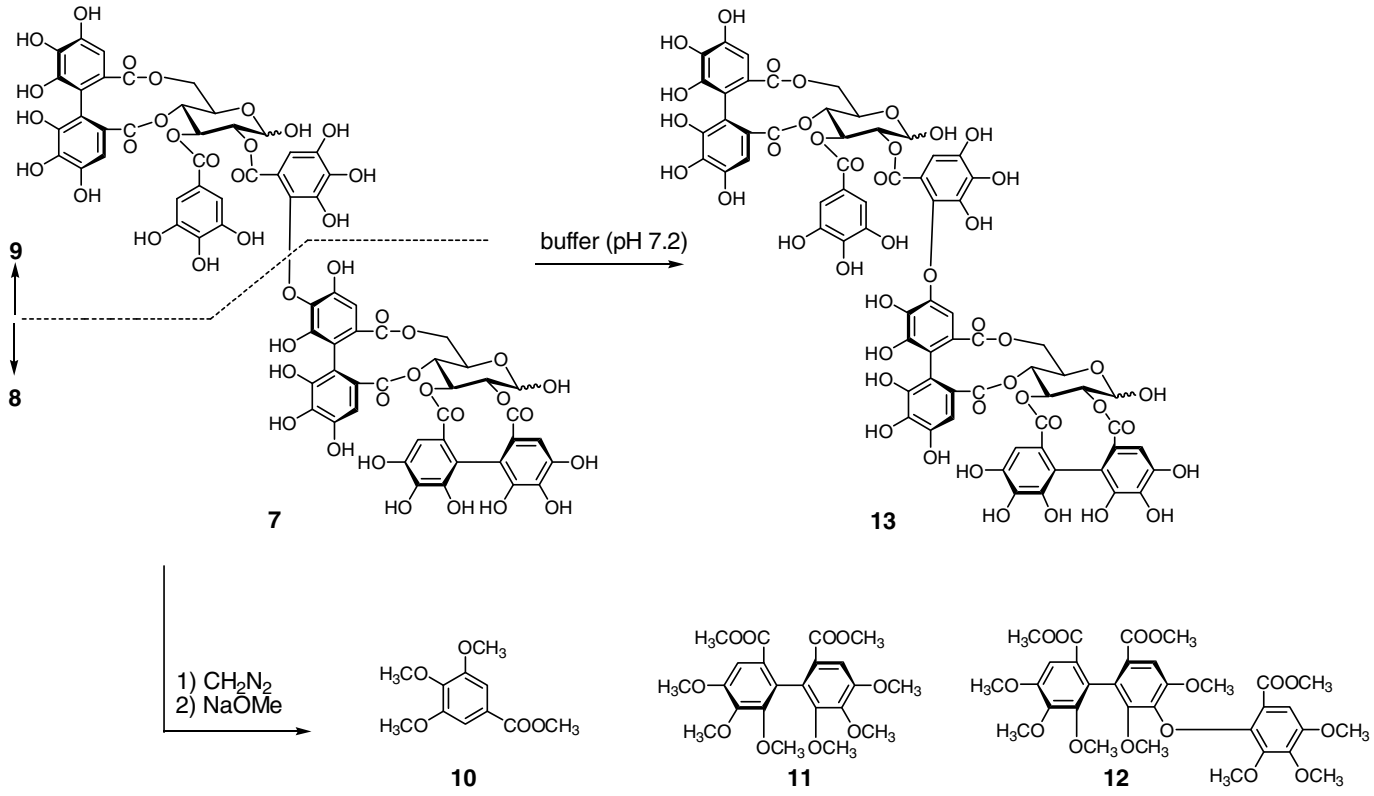


**Fig. 3.** Isomerization of kunzeatannin A (**7**) to camelliin A (**13**) by smiles rearrangement.

### 3.2. Plant materials

*K. ambigua* (SM.) Druce was collected in April 1998 from the herbal garden of Pola Co. Ltd. (Japan). A voucher specimen has been deposited in the Medicinal Herbal Garden of Okayama University (specimen No. OKP-MY98003).

### 3.3. Extraction and isolation

Dried leaves (700 g) of *K. ambigua* were homogenized in acetone–$H_2O$ (7:3, v/v) (10 L), and the homogenate was filtered and concentrated. The concentrated solution was extracted successively with ether, EtOAc and water-saturated *n*-BuOH. A portion (2.0 g) of the EtOAc extract (11.0 g) was applied to a Toyopearl HW-40 column (2.2 cm i.d. × 40 cm) and eluted with $H_2O$ containing increasing amounts of MeOH in a stepwise gradient. The MeOH–$H_2O$ (1:4, v/v) eluate was subjected to column chromatography over an MCI GEL CHP-20P (1.1 cm i.d. × 25 cm) with aqueous MeOH, to yield kunzeaphlogin D (**4**) (23.7 mg). The MeOH–$H_2O$ (3:7, v/v) eluate was further purified by column chromatography over an MCI GEL CHP-20P column (1.1 cm i.d. × 25 cm), to yield 3-*O*-*p*-coumaroylquinic acid (8.9 mg), 4′,6′-dihydroxyisobutrophenone 2′-*O*-β-glucoside (11.0 mg) and kunzeaphlogin E (**5**) (37.4 mg). The MeOH–$H_2O$ (6:4, v/v) eluate was similarly subjected to chromatographic purification over an YMC-gel ODS-AQ 120-S50 (1.1 cm i.d. × 44 cm) with aqueous MeOH as eluant to yield kunzeaphlogin C (**3**) (11.0 mg). The other portion (3.3 g) of the EtOAc extract was subjected to column chromatography over Toyopearl HW-40 (2.2 cm i.d. × 42 cm) with aqueous MeOH in a stepwise gradient. The MeOH–$H_2O$ (6:4, v/v) eluate was similarly applied to a YMC-GEL ODS-AQ 120-S50 (1.1 cm i.d. × 44 cm) column eluted with aqueous MeOH, yielding pedunculagin (**8**) (156.8 mg) and **3** (21.2 mg). A 10.1-g portion of the *n*-BuOH extract (24.6 g) was fractionated by column chromatography over Diaion HP-20 (5.0 cm i.d. × 50 cm), and developed with $H_2O$ and increasing amounts of MeOH in a stepwise gradient. The MeOH–$H_2O$ (3:7, v/v) eluate was purified by column chromatography over Toyopearl HW-40 (2.2 cm i.d. × 40 cm) and/or a Mega Bond Elut $C_{18}$ cartridge with aqueous MeOH to afford 3-*O*-*p*-coumaroylquinic acid (4.9 mg), 4-*O*-*p*-coumaroylquinic acid (3.1 mg), casuarinin (4.9 mg), 4-*O*-β-glucopyranosyl-*cis*-coumaric acid (20.2 mg), ellagic acid (4.0 mg), kunzeaphlogin A (**1**) (3.1 mg) and praecoxin A (28.4 mg). The MeOH–$H_2O$ (1:1, v/v) eluate was further purified by chromatography on Toyopearl HW-40 (2.2 cm i.d. × 40 cm) and YMC-GEL ODS-AQ 120-S50 column (1.1 cm i.d. × 22 cm) with aqueous MeOH, or a Sephadex LH-20 (1.1 cm i.d. × 28 cm) with EtOH–MeOH to yield **1** (10.2 mg), **2** (5.7 mg) and **6** (4.2 mg).

### 3.4. Kunzeaphlogin A (*1*)

Pale yellow amorphous powder; $[\alpha]_D^{23}$ − 19 (*c* 1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 226 (4.15), 287 (4.02), 321sh (3.55) nm; $^1$H NMR (acetone-$d_6$ + $D_2O$, 500 MHz) $\delta_H$; 6.28 (1H, s, H-3), 5.04 (1H, *d*, *J* = 7.5 Hz, glc-1′), 4.80 (1H, *d*, *J* = 9.5 Hz, glc-1″), 4.00 (1H, *t*, *J* = 9.5 Hz, glc-2″), 3.91 (1H, *dd*, *J* = 2.5, 11.5 Hz, glc-6′), 3.52 (1H, *t*, *J* = 7.5 Hz, glc-4″), 3.48 (1H, *t*, *J* = 9.5 Hz, glc-3″), 3.3–3.8 (overlapping DHO, H-2, 6, glc-2′–6′ glc-5″–6″), 3.15 (1H, *dd*, *J* = 6.5, 16.5 Hz, H-8), 2.88 (1H, *dd*, *J* = 7.5, 16.5 Hz, H-8), 2.16 (1H, *m*, H-9), 0.91, 0.87 (3H each, *d*, *J* = 7.0 Hz, H-10, 11); for $^{13}$C NMR spectroscopic data, see Table 1; ESIMS *m/z* 535 $[M + H]^+$, 552 $[M + NH_4]^+$; HRESIMS *m/z* 535.1991 $[M + H]^+$ ($C_{23}H_{34}O_{14}$ + H, 535.2027).

### 3.5. Acid hydrolysis of *1*

A solution of **1** (0.5 mg) in 1 M HCl (1.0 mL) was heated at 100 °C for 1 h in a boiling water bath. After cooling, the reaction mixture was passed through a Mega Bond Elut $C_{18}$ cartridge column and washed with $H_2O$. The aqueous solution was concentrated and analyzed by reversed-phase HPLC with an optical rotation detector (Shodex OR-2; Showa Denko Co.) [column, TSK-gel Amide-80 (4.6 mm i.d. × 250 mm) (Tosoh Co.); solvent, MeCN/$H_2O$ (75:25); column temp., 35 °C; flow rate, 1.0 mL/min] to detect D-glucose (R*t*; 9.2 min, R*t*; 9.8 min) as positive peaks identical with those of authentic glucose. Positive reaction to D-glucose oxidase was also confirmed.

### 3.6. Kunzeaphlogin B (*2*)

Pale yellow amorphous powder; $[\alpha]_D^{23}$ − 45 (*c* 1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 219 (4.67), 280 (4.31), 322sh (3.98) nm; $^1$H NMR (acetone-$d_6$ + $D_2O$, 500 MHz) $\delta_H$; 7.07, 7.06 (2H each, *s*, galloyl-H, H′), 6.26 (1H, *s*, H-3), 5.17 (1H, *d*, *J* = 6.5 Hz, glc-1′), 4.88 (1H, *d*, *J* = 9.5 Hz, glc-1″), 4.58 (1H, br *d*, *J* = 10.5 Hz, glc-6′), 4.52 (1H, br *d*, *J* = 10.5 Hz, glc-6″), 4.41 (1H, *dd*, *J* = 5.0, 12.0 Hz, glc-6″), 4.32 (1H, *dd*, *J* = 4.0, 12.0 Hz, glc-6′), 4.00 (1H, *t*, *J* = 9.5 Hz, glc-2″), 3.5–3.9 (overlapping DHO, glc-2′–5′, 3″–5″), 3.09 (1H, *dd*, *J* = 6.01, 15.5 Hz, H-8), 2.84 (1H, *dd*, *J* = 7.5, 15.5 Hz, H-8), 2.13 (1H, *m*, H-9), 0.85, 0.81 (3H each, *d*, *J* = 6.5 Hz, H-10, 11); for $^{13}$C NMR spectroscopic data, see Table 1; ESIMS *m/z*: 677 [M-galloyl group]$^+$, 839 $[M + H]^+$, 861 $[M + Na]^+$; HRESIMS *m/z* 839.2347 $[M + H]^+$ ($C_{37}H_{42}O_{22}$ + H, 839.2246).

### 3.7. Kunzeaphlogin C (*3*)

Pale yellow amorphous powder; $[\alpha]_D^{23}$ − 41 (*c* 1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 219 (4.88), 280 (4.88), 321sh (3.98) nm; $^1$H NMR (acetone-$d_6$ + $D_2O$, 500 MHz) $\delta_H$; 7.17, 7.115, 7.109 (2H each, *s*, galloyl-H, H′, H″), 6.31 (1H, *s*, H-3), 5.34 (1 H, *t*, *J* = 9.5 Hz, glc-4″), 5.20 (1H, *d*, *J* = 7.0 Hz, glc-1′), 5.07 (1H, *d*, *J* = 9.5 Hz, glc-1″), 4.66 (1H, *dd*, *J* = 2.5, 12.0 Hz, glc-6′), 4.46 (1H, br *d*, *J* = 11.0 Hz, glc-6″), 4.36 (1H, *dd*, *J* = 5.0, 12.0 Hz, glc-6′), 4.29 (1H, *dd*, *J* = 5.0, 11.0 Hz, glc-6″), 4.11 (1H, *m*, glc-5″), 4.05 (1H, *t*, *J* = 9.5 Hz, glc-2″), 3.93 (1H, *t*, *J* = 9.5, glc-3″), 3.87 (1H, *m*, glc-5′), 3.6–3.7 (3H, *m*, glc-2′–4′), 3.16 (1H, *dd*, *J* = 6.0, 15.5 Hz, H-8), 2.96 (1H, *dd*, *J* = 6.0, 15.5 Hz, H-8), 2.23 (1H, hept, *J* = 6.0 Hz, H-9), 0.92, 0.89 (3H each, *d*, *J* = 6.0 Hz, H-10, 11); for $^{13}$C NMR spectroscopic data, see Table 1; ESIMS *m/z* 991 $[M + H]^+$, *m/z* 1013 $[M + Na]^+$; HRESIMS *m/z* 991.2350 $[M + H]^+$ ($C_{44}H_{46}O_{26}$ + H, 991.2356).

### 3.8. Kunzeaphlogin D (*4*)

Pale yellow amorphous powder; $[\alpha]_D^{23}$ + 38 (*c* 1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 217 (4.34), 271 (4.11), 321sh (3.83) nm; $^1$H NMR (acetone-$d_6$ + $D_2O$, 500 MHz) $\delta_H$; 11.6 (1H, *s*, 6-OH), 4.70 (1H, *d*, *J* = 7.5, glc-1″), 4.50 (1H, *d*, *J* = 7.5, glc-1′), 3.85 (2H, *m*, glc-6′, 6″), 3.64 (1H, *dd*, *J* = 2.5, 10.0 Hz, glc-6″), 3.62 (1H, *dd*, *J* = 5.5, 10.0 Hz, glc-6′), 3.46–3.55 (overlapping DHO, glc-2′, 3′, 2″, 3″), 3.40 (1H, br *t*, *J* = 10.0 Hz, glc-4″), 3.35 (1H, br *t*, *J* = 10.0 Hz, glc-4′), 3.20 (1H, *ddd*, *J* = 2.5, 5.5, 10.0 Hz, glc-5″), 3.11 (1H, *ddd*, *J* = 2.5, 5.5, 10.0 Hz, glc-5′), 3.16 (1H, *dd*, *J* = 7.0, 15.5 Hz, H-8), 2.93 (1H, *dd*, *J* = 7.0, 15.5 Hz, H-8), 2.27 (3H, *s*, 3-Me), 2.14 (3H, *s*, 5-Me), 2.08 (1H, *m*, H-9), 0.84, 0.83 (3H each, *d*, *J* = 7.0 Hz); for $^{13}$C NMR spectroscopic data, see Table 2; ESIMS *m/z* 563 $[M + H]^+$, *m/z* 580 $[M + NH_4]^+$; HRESIMS *m/z* 580.2567 $[M + NH_4]^+$ ($C_{25}H_{38}O_{14}$ + H, 580.2605).

### 3.9. Acid hydrolysis of *4*

A solution of **4** (5.1 mg) in 1 M HCl (2.0 mL) was heated at 100 °C for 1 h in a boiling water bath. After cooling, reaction mixtures were partitioned between AcOEt and water to yield **4a** from the AcOEt soluble portion and glucose from the water-soluble

portion. D-glucose was identified by HPLC with an optical rotation detector and a positive reaction to D-glucose oxidase.

### 3.10. Kunzeaphlogin D aglycone (**4a**)

Brown oil: $^1$H NMR (acetone-$d_6$, 500 MHz) $\delta_H$; 2.98 (2H, *d*, *J* = 7.5, H-8), 2.23 (1H, *m*, H-9), 2.08 (6H, *s*, 3-Me, 5-Me), 0.93 (6H, *d*, *J* = 7.0, H-10, 11).

### 3.11. Kunzeaphlogin E (**5**)

Pale yellow amorphous powder; $[\alpha]_D^{23} + 31$ (*c* 1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 216 (4.71), 274 (4.40), 337sh (3.90) nm; $^1$H NMR (acetone-$d_6$ + $D_2O$, 500 MHz) $\delta_H$; 7.12 (2H, *s*, galloyl-H), 4.78 (1H, *d*, *J* = 7.0 Hz, glc-1″), 4.57 (1H, *dd*, *J* = 2.0, 12.0 Hz, glc-6″), 4.53 (1H, *d*, *J* = 7.5 Hz, glc-1′), 4.28 (1H, *d*, *J* = 4.0, 12.0 Hz, glc-6″), 3.35–3.60 (overlapping DHO, glc-2′–4′, 6′, 2″–5″), 3.15 (1H, *m*, H-8), 3.14 (1H, *m*, glc-5′), 2.95 (1H, *dd*, *J* = 6.5, 16.5 Hz, H-8), 2.22 (3H, *s*, 3-Me), 2.14 (3H, *s*, 5-Me), 2.12 (1H, *m*, H-9), 0.88, 0.86 (3H each, *d*, *J* = 7.0 Hz, H-10, 11); for the $^{13}$C NMR data, see Table 2; ESIMS *m/z* 715 $[M + H]^+$, *m/z* 732 $[M + NH_4]^+$, *m/z* 737 $[M + Na]^+$; HRESIMS *m/z* 732.2590 $[M + NH_4]^+$ ($C_{32}H_{42}O_{18}$ + H, 732.2715).

### 3.12. Partial hydrolysis of **2**, **3** and **5** with tannase

A solution of each compound (0.2–1.0 mg) in $H_2O$ (0.2–0.5 mL) was treated at 37 °C for an appropriate time with two drops of tannase, which was obtained from *Aspergillus niger*. After addition of EtOH, the reaction mixture was evaporated to dryness. The residue was analyzed by normal and reversed-phase HPLC. The products besides gallic acid from each compound were as follows and identified by co-chromatography with authentic specimens, respectively:

Production of **1** and **2** from **3**: reaction time 20 h; normal and reversed-phase HPLC (solvents A, B).
Production of **4** from **5**: reaction time 48 h; normal and reversed-phase HPLC (solvents A, B).

### 3.13. Kunzeaphlogin F (**6**)

Pale yellow amorphous powder; $[\alpha]_D^{23} + 30$ (*c* 1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 218 (4.11), 267 (3.73), 320sh (3.31) nm; $^1$H NMR (acetone-$d_6$ + $D_2O$, 500 MHz) $\delta_H$; 4.70 (1H, *d*, *J* = 7.0 Hz, glc-1″), 4.39 (1H, *d*, *J* = 8.0 Hz, glc-1′), 3.90 (1H, m, H-8), 3.72 (1 H, *dd*, *J* = 2.5, 12.5 Hz, glc-6″), 3.65 (1H, *dd*, *J* = 2.5, 12.5 Hz, glc-6′), 3.60 (1 H, *dd*, *J* = 5.5, 12.5 Hz, glc-6″), 3.46–3.52 (overlapping DHO, glc-3′, 6′, 2″, 3″), 3.45 (1H, *t*, *J* = 8.0 Hz, glc-2′), 3.40 (1H, *t*, *J* = 9.0 Hz, glc-4″), 3.29 (1H, br *t*, *J* = 9.0 Hz, glc-4′), 3.20 (1H, *ddd*, *J* = 2.5, 5.5, 9.0 Hz, glc-5″), 3.10 (1H, *ddd*, *J* = 2.5, 6.0, 9.0 Hz, glc-5′), 3.90 (1H, *m*, H-8), 2.27 (3H, *s*, 3-Me), 2.14 (3H, *s*, 5-Me), 1.12, 0.92 (3H each, *s*, *J* = 6.0 Hz, H-9, 10); for $^{13}$C NMR spectroscopic data, see Table 2; ESIMS *m/z* 549 $[M + H]^+$, *m/z* 566 $[M + NH_4]^+$, *m/z* 571 $[M + Na]^+$; HRESIMS *m/z* 566.2439 $[M + NH_4]^+$ ($C_{24}H_{36}O_{14}$ + H, 566.2449).

### 3.14. Kunzeatannin A (**7**)

Pale brown amorphous powder; $[\alpha]_D^{23} + 54$ (*c* 1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 214 (5.13), 261 (4.72) nm; CD (MeOH) [$\theta$]nm; + $2.2 \times 10^5$ (240), $7.5 \times 10^4$ (263), +$3.6 \times 10^4$ (286), −$1.9 \times 10^4$ (314), $^1$H NMR (acetone-$d_6$ + $D_2O$, 500 MHz) $\delta_H$; 7.02, 6.99 (2H in total, each *s*, galloyl-H), 6.88, 6.86, 6.632, 6.629, 6.61, 6.603, 6.600, 6.58, 6.572, 6.570, 6.56, 6.53, 6.51, 6.48, 6.47, 6.351, 6.345 (7H in total, each s, HHDP-H and tergalloyl-H), 5.86 (*t*, *J* = 10.5 Hz, H-3′α), 5.64 (*t*, *J* = 9.5 Hz, H-3′β), 5.61 (d, *J* = 4.0 Hz, H-1′α), 5.45 (*dd*, *J* = 3.5, 10.0 Hz, H-3α), 5.42 (*d*, *J* = 3.0 Hz, H-1α), 5.29 (*dd*, *J* = 8.0, 9.5 Hz, H-2′β), 5.22 (*m*, H-3β, 6′α), 5.21 (*m*, H-6′β), 5.19 (*m*, H-6β, 2′α), 5.18 (*m*, H-6α), 5.16 (*m*, H-4′β), 5.10 (*m*, H-4′α), 5.09 (*m*, H-4α), 5.08 (*m*, H-4β), 5.03 (*m*, H-2α), 5.024 (*d*, *J* = 8.5 Hz, H-1β), 5.018 (*d*, *J* = 8.0 Hz, H-1′β), 4.83 (*t*, *J* = 8.5 Hz, H-2β), 4.65 (br *dd*, *J* = 8.0, 10.0 Hz, H-5′α), 4.59 (*m*, H-5α), 4.24 (br *dd*, *J* = 3.5, 10.0 Hz, H-5′β), 4.18 (*m*, H-5β); for $^{13}$C NMR spectroscopic data, see Table 3; ESIMS *m/z* 1586 $[M + NH_4]^+$.

### 3.15. Methylation of **7** followed by methanolysis

Compound **7** (1.0 mg) in EtOH was methylated with $CH_2N_2$–$Et_2O$ at room temperature for 8 h. The residue obtained after removal of the solvent was directly methanolyzed with 1% NaOMe in MeOH (1.0 mL) at room temperature for 4 h to give methyl tri-*O*-methylgallate (**10**), dimethyl hexamethoxydiphenate (**11**) and trimethyl octa-*O*-methyltergallate (**12**), which were identified by co-chromatography on the reversed-phase HPLC (solvent D).

### 3.16. Isomerization of **7** to camelliin A by smiles rearrangement

A solution of **7** (0.2 mg) in a phosphate buffer (pH 7.2) (0.5 mL) was treated at room temperature for 1 h. The reactant was identified as camelliin A (**13**) by co-chromatography on normal phase HPLC (R*t* 15.2 min, solvent A) and reversed-phase HPLC (R*t* 5.4, 6.0 min, solvent C).

## Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 10557207) from the Ministry of Education, Science, Sports and Culture, Japan. Thanks are due to Mr. Y. Kitada of the Central Research Laboratory of the Pola Corporation for the kind supply of the plant materials. The NMR experiments were performed at the SC-NMR Laboratory of Okayama University.

## References

Agrawal, P.K., 1989. Carbon 13 NMR of Flavonoids. Elsevier, New York.
Chang, X., Li, W., Koike, K., Wu, L., Nikaido, T., 2006. Phenolic constituents from the rhizomes of *Dryopteris crassirhizoma*. Chem. Pharm. Bull. 54, 748–750.
Cui, C.B., Tezuka, Y., Kikuchi, T., Nakano, H., Tamaoki, T., Park, J.H., 1990. Constituents of a fern, *Davallia mariesii* Moore I. Isolation and structures of davallialactone and a new flavanone glucuronide. Chem. Pharm. Bull. 38, 3218–3225.
David, H.J.J., 1997. PCT Int. Appl. 14pp.
Duc Do, K., Sung Tran, V., Campos, A.M., Lallemand, J.Y., Fetizon, M., 1990. Ellagic compounds from *Diplopanax stachyanthus*. Phytochemistry 29, 251–256.
Haslam, E., Haworth, R.D., Makinson, G.K., 1961. Synthesis of 3-*O*-*p*-coumaroylquinic acid. J. Chem. Soc., 5153–5156.
Hatano, T., Yoshida, T., Shingu, T., Okuda, T., 1988. Carbon-13 nuclear magnetic resonance spectra of hydrolyzable tannins. II. Tannins forming anomer mixtures. Chem. Pharm. Bull. 36, 2925–2933.
Hatano, T., Yazaki, K., Okonogi, A., Okuda, T., 1991. Tannins of Stachyurus species. II. Praecoxins A, B, C and D, four new hydrolyzable tannins from *Stachyurus praecox leaves*. Chem. Pharm. Bull. 39, 1689–1693.
Ito, H., Iwamori, H., Kasajima, N., Kaneda, M., Yoshida, T., 2004a. Kunzeanones A, B, and C: novel alkylated phloroglucinol metabolites from *Kunzea ambigua*. Tetrahedron 60, 9971–9976.
Ito, H., Kasajima, N., Tokuda, H., Nishino, H., Yoshida, T., 2004b. Dimeric flavonol glycoside and galloylated *C*-glucosylchromones from *Kunzea ambigua*. J. Nat. Prod. 67, 411–415.
Khambay, B.P., Beddie, D.G., Simmonds, M.S., 2002. An insecticidal mixture of tetramethylcyclohexenedione isomers from *Kunzea ambigua* and *Kunzea baxterii*. Phytochemistry 59, 69–71.
Miwa, I., Okudo, J., Maeda, K., Okuda, G., 1972. Mutarotase effect on colorimetric determination of blood glucose with D-glucose oxidase. Clin. Chim. Acta 37, 538–540.
Okuda, T., Yoshida, T., Hatano, T., Koga, T., Toh, N., Kuriyama, K., 1982. Circular dichroism of hydrolyzable tannins. I. Ellagitannins and gallotannins. Tetrahedron Lett. 23, 3937–3940.

Okuda, T., Yoshida, T., Ashida, M., Yazaki, K., 1983. Tannins of *Casuarina* and *Stachyurus* species. Part 1. Structures of pendunculagin, casuarictin, strictinin, casuarinin, casuariin, and stachyurin. Journal of the Chemical Society, Perkin Transactions 1, 1765–1772.

Pakulski, G., Budzianowski, J., 1996. Ellagic acid derivatives and naphthoquinones of *Dionaea muscipula* from *in vitro* cultures. Phytochemistry 41, 775–778.

Vancraenenbroeck, R., Vanclef, A., Lontie, R., 1965. Characterization of flavonols and glucoside of phloroisobutyrophenone in hops. Proceedings of the Congress – European Brewery Convention, 360–371.

Wilkins, C.K., Bohm, B.A., 1976. Chemotaxonomic studies of Saxifragaceae. Part 3. Ellagitannins from *Tellima grandiflora*. Phytochemistry 15, 211–214.

Yoshida, T., Chou, T., Maruyama, Y., Okuda, T., 1990. Tannins of theaceous plants. II. Camelliins A and B, two new dimeric hydrolyzable tannins from flower buds of *Camellia japonica* L. and *Camellia sasanqua* Thunb. Chem. Pharm. Bull. 38, 2681–2686.

Yoshida, T., Ahmed, A.H.F., Memon, M.U., Okuda, T., 1993. Tannins and related polyphenols of Tamaricaceous plants. Part 4. Dimeric hydrolysable tannins from *Tamarix pakistanica*. Phytochemistry 33, 197–202.