Triterpenoid Ketones from *Lingnania chungii* McClure: Arborinone, Friedelin and Glutinone

Toshihiro Akihisa,*,[a] Kazuhiro Yamamoto,[a] Toshitake Tamura,[a] Yumiko Kimura,[b] Takashi Iida,[c] Toshio Nambara,[d] and Frederic C. Chang[e]

College of Science and Technology, Nihon University,[a] 1–8 Kanda Surugadai, Chiyoda-ku, Tokyo 101, Japan, College of Pharmacy, Nihon University,[b] 7–7–1, Narashinodai, Funabashi, Chiba 274, Japan, College of Engineering, Nihon University,[c] Tamura-machi, Koriyama, Fukushima 963, Japan, Pharmaceutical Institute, Tohoku University,[d] Aobayama, Sendai, Miyagi 980, Japan, and Harvey Mudd College,[e] Claremont, California 91711, U.S.A.

Received August 26, 1991

The powder coating of a bamboo, *Lingnania chungii* McClure (=*Bambusa chungii*) was found to be a rich source of the 3-oxo pentacyclic triterpenes (25% on the recovery basis by chromatography on silica gel) which contained friedelin, arborinone and glutinone as the major components accompanied by minor amounts of α- and β-amyrenones. A simple procedure for isolation of friedelin is described. All proton and carbon-13 nuclear magnetic resonance signals for arborinone, friedelin and glutinone were assigned.

Keywords *Lingnania chungii*; *Bambusa chungii*; Gramineae; arborinone; friedelin; glutinone; ^{1}H-NMR; ^{13}C-NMR

A species of bamboo indigenous to South China, described by McClure as *Bambusa chungii* and later reclassified by him as *Lingnania chungii*[1)] (family Gramineae), has on its developing culm an exudate, a white waxy powder, the copiousness of which is a distinguishing characteristic of the species. Over 50 years ago, one of us (FCC)[2)] had isolated from the powder three triterpenoid ketones: one was identified as friedelin, and the others, only partially characterized, were named bambuselins I and II. (perhaps named whimsically by Professor L. F. Fieser [*cf.*, bamboozle].)

No further chemical work has been reported on the species in the five decade interval. We have obtained from the same source a supply of the powder to resume the interrupted study. In this paper we confirm the isolation of the same three ketones and an additional two known triterpenoid ketones, α- and β-amyrenones.

A mixture of triterpenoid ketones was separated from the bamboo powder of *Lingnania* (*Bambusa*) *chungii* by preparative thin-layer chromatography (TLC) on silica gel. Preparative high-performance liquid chromatography (HPLC) of the mixture allowed the isolation of three compounds, **1**, **4** and **7**, and two minor compounds, **8** and **10**, which were identified as arborinone [D:C-friedo-B′:A′-5α-neogammacer-9(11)-en-3-one] (26% of the 3-oxo triterpene fraction as determined based on the gas-liquid chromatography [GLC] and HPLC data), glutinone (D:B-friedo-olean-5-en-3-one) (26%), friedelin (D:A-friedo-olean-3-one) (37%), α-amyrenone (5α-urs-12-en-3-one) (3%) and β-amyrenone (5α-olean-12-en-3-one) (5%), respectively, by direct comparison of the chromatographic (HPLC, GLC) and spectroscopic (mass spectrometry [MS] and proton nuclear magnetic resonance [^{1}H-NMR] spectroscopy) data with those of the reference compounds. The two triterpenoid ketones, **1** and **4**, were then subjected to $NaBH_4$ reduction in which **1** yielded isoarborinol (3β-OH) (**2**) and arborinol (3α-OH) (**3**) whereas **4** gave glutinol (3β-OH) (**5**) and 3-epiglutinol (3α-OH) (**6**). Comparison of physical properties revealed that the two pentacyclic triterpenoid ketones, arborinone (**1**) and glutinone (**4**), are identical with bambuselins I and II, respectively.[3)]

Arborinone (**1**), first isolated from a Rubiaceae plant,[4)] has since been found in several plants. **1** has been found in only one species (*Imperata cylindrica*) in an extensive survey of the large Gramineae (Grass) family, while the corresponding 3-hydroxy epimers (**2** and **3**) have been reported to be present in many plants.[5−8)] Glutinone (**4**), first isolated from a Betulaceae species,[9)] and further studied under the name alnusenone,[10,11)] is found in several species of Gramineae plants,[6)] accompanied by 3-hydroxy derivatives. Whereas α-(**8**)[12)] and β-amyrenones (**10**)[13,14)] were found in some plants as natural products, neither of these has been reported to be present in Gramineae plants.[6)] The corresponding 3-hydroxy compounds, α- (**9**) and β-amyrins (**11**), are known to be among the most widely dispersed triterpenoids in the plant kingdom. Friedelin (**7**), first isolated from the bark of

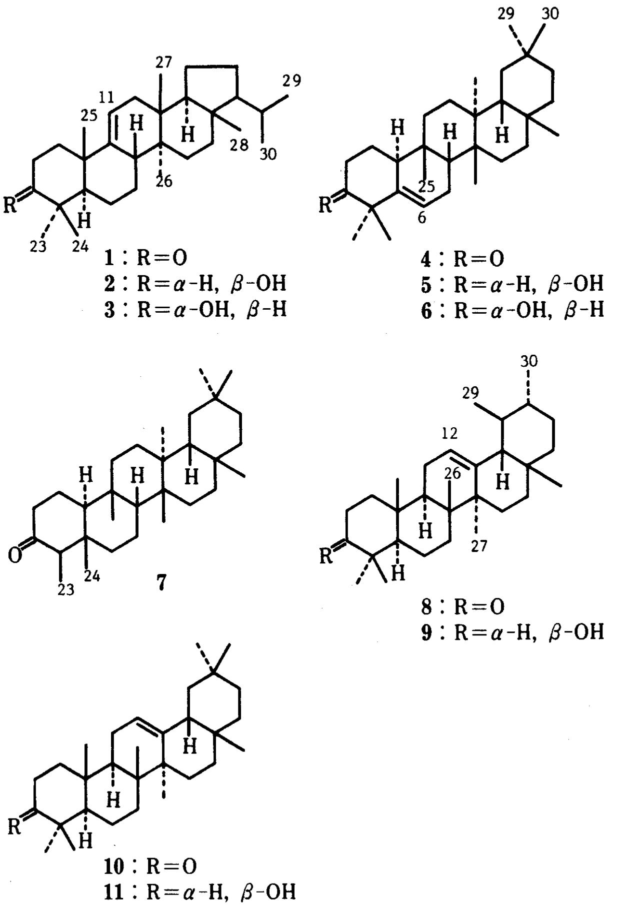

Chart 1

TABLE I. ^{1}H- and ^{13}C-NMR Spectral Signal Assignments of **1**, **4** and **7** Isolated from *Lingnania chungii* Powder[a]

C No.	Arborinone (**1**)		Glutinone (**4**)		Friedelin (**7**)	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	36.6	1.80 (m), 2.07 (m)	21.6	1.63 (m), 1.88 (m)	22.3	1.69 (m), 1.97 (m)
2	34.8	2.39 (m), 2.72 (m)	38.1	2.38 (m), 2.46 (m)	41.5	2.31 (m), 2.39 (m)
3	217.1	—	215.4	—	213.2	—
4	47.6	—	50.0	—	58.2	2.25 (m)
5	53.2	1.38 (m), 1.86 (m)	142.4	—	42.1	—
6	26.3	1.36 (m)	121.3	5.69 (br d, 5)	41.3	1.29 (m), 1.75 (m)
7	22.6	1.56 (m), 1.66 (m)	23.6	1.97 (m), 2.01 (m)	18.2	1.38 (m), 1.49 (m)
8	41.0	2.10 (dd, 3, 6)	47.0	1.68 (m)	53.1	1.41 (m)
9	147.4	—	35.1	—	37.4	—
10	39.3	—	50.7	2.24 (m)	59.4	1.55 (m)
11	115.6	5.29 (br d, 6)	34.1	1.49 (m), 1.56 (m)	35.6	1.26 (m), 1.46 (m)
12	36.1	1.52 (m), 1.72 (m)	30.3	1.38 (2H, m)	30.5	1.34 (2H, m)
13	36.7	—	39.3	—	39.7	—
14	38.2	—	37.9	—	38.3	—
15	29.6	1.27 (m), 1.50 (m)	31.9	1.35 (m), 1.47 (m)	32.4	1.32 (m), 1.51 (m)
16	35.8	1.41 (m), 1.68 (m)	35.9	1.40 (m), 1.55 (m)	36.0	1.39 (m), 1.59 (m)
17	42.8	—	30.1	—	30.0	—
18	52.0	1.62 (m)	43.1	1.60 (m)	42.8	1.56 (m)
19	20.1	1.37 (2H, m)	35.1	1.26 (m), 1.38 (m)	35.3	1.21 (m), 1.38 (m)
20	28.2	1.22 (m), 1.85 (m)	28.2	—	28.1	—
21	59.6	0.98 (m)	33.1	1.27 (m), 1.45 (m)	32.7	1.28 (m), 1.43 (m)
22	30.7	1.47 (m)	38.9	0.93 (m), 1.57 (m)	39.2	0.95 (m), 1.53 (m)
23	22.0[b]	1.07 (s)	24.4[b]	1.23 (s)[b]	6.8	0.88 (d, 7)
24	25.5[b]	1.07 (s)	28.5[b]	1.24 (s)[b]	14.6	0.73 (s)
25	21.6	1.22 (s)	15.6	0.82 (s)	17.9	0.87 (s)
26	16.9	0.81 (s)	19.3	1.10 (s)	20.2	1.01 (s)
27	15.3	0.80 (s)	18.4	1.03 (s)	18.6	1.05 (s)
28	13.9	0.77 (s)	32.0	1.17 (s)	32.1	1.18 (s)
29	22.1[c]	0.89 (d, 6)[c]	34.5	0.96 (s)	35.0	0.95 (s)
30	23.0[c]	0.83 (d, 6)[c]	32.4	0.99 (s)	31.8	1.00 (s)

a) Determined at 400 MHz (^{1}H-NMR) and 100.62 MHz (^{13}C-NMR) in $CDCl_3$ with tetramethylsilane as internal standard. Figures in parentheses on ^{1}H-NMR denote *J* values (Hz). *b*, *c*) Assignment in each column may be interchanged.

the cork tree,[15] and the structure of which was finally determined by X-ray studies,[16] has been reported to be one of most ubiquitous triterpenes found in nature.[17] Compound **7** in appreciable quantity is still produced by the method[15,18] of extraction of a commercially abundant by-product obtained from the manufacture of cork board. However, for preparation of smaller quantities of **7**, limited only by the availability of the *L. chungii* powder, a product of excellent purity can be obtained by a much simpler procedure, as described in the Experimental section.

In comparison, the predominance of the five triterpenoid ketones in the exudate of *L. chungii*, is noteworthy. According to our TLC results, the corresponding 3-hydroxy compounds, if present, are in minimal concentrations. We are pursuing this point further.

Since the ^{1}H- and carbon-13 (^{13}C)-NMR spectral assignments of arborinone (**1**) and glutinone (**4**) have not been reported, we have made full assignments of the spectra for the three 3-oxo triterpenes, **1**, **4** and friedelin (**7**), with the aid of ^{13}C distortionless enhancement by polarization transfer (DEPT), ^{1}H-^{1}H correlated spectroscopy (COSY), long-range ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and ^{1}H–^{13}C correlation spectroscopy for long-range couplings (COLOC) experiments[19]; results are shown in Table I.

Experimental

Crystallizations were performed in acetone–MeOH. Melting points were measured with a Yanagimoto melting point apparatus, and are uncorrected. Preparative TLC on silica gel (Kieselgel 60G, Merck; 0.5 mm thick) was developed two times using hexane–EtOAc (6 : 1, v/v). Preparative HPLC was carried out on an Altex Ultrasphere ODS 5 μ column (25 cm × 10 mm i.d.) (Beckman Instruments, Inc., San Ramon, California) with MeOH (4 ml/min) using a SSC Flow System 3100K (Senshu Scientific Co.) and an ERC-7520 reflective index detector (Erma Optical Works, Ltd.). GLC was run on a Shimadzu GC-14A apparatus using a DB-17 fused silica capillary column (30 m × 0.3 mm i.d., column temp. 275 °C). Relative retention time (Rt_R) on HPLC and GLC was expressed relative to cholesterol (cholest-5-en-3β-ol) acetate. MS and high-resolution (HR) MS were taken on a Hitachi M-80B double focusing gas chromatograph-mass spectrometer (70 eV) using a direct inlet system. NMR spectra were recorded with a JEOL JNM GX-400 spectrometer at 400 MHz (^{1}H-NMR) and 100.62 MHz (^{13}C-NMR) in $CDCl_3$ with tetramethylsilane as an internal standard, and chemical shifts are recorded in δ values. Ultraviolet (UV) spectra were determined on a Shimadzu UV-300 spectrometer in EtOH. Reference samples of arborinone (**1**), glutinone (**4**), α-amyrenone (**8**) and β-amyrenone (**10**) were prepared from the corresponding 3-alcohols described in our previous paper,[20] *viz.*, isoarborinol (**2**), glutinol (**5**), α-amyrin (**9**) and β-amyrin (**11**), respectively, by oxidation with CrO_3 in pyridine as described recently.[21] The fresh sample of *L. chungii* used for this study was obtained from the bamboo grove at Lingnan Universiy in Canton (Guangzhou) from which the original powder[2] had been collected.

Isolation of 3-Oxo Triterpenes from *L. chungii* Powder by Preparative TLC and Preparative HPLC *L. chungii* powder (2.5 g) was directly subjected to preparative TLC fractionation which gave five bands. The fraction (494 mg) recovered from the second band (*Rf* 0.62) from the solvent front, which cochromatographed with a reference 3-oxo triterpene, **7**, constituted the most abundant one among the other fractions. This fraction was subjected to preparative HPLC which yielded **1** (110 mg), **4** (85 mg), **7** (130 mg), **8** (9 mg), **10** (23 mg) and several other unidentified compounds.

Arborinone (**1**): Colorless needles, mp 217—219 °C (lit.,[5] mp 216 °C). Rt_R: 0.97 (HPLC), 2.15 (GLC). UV λ_{max} nm: 205. HR-MS *m/z*: 424.3691 (M^+) (Calcd for $C_{30}H_{48}O$, *m/z* 424.3702).

Glutinone (**4**): Colorless needles, mp 246—249 °C (lit.,[9] mp 245—246 °C). Rt_R: 0.67 (HPLC), 1.81 (GLC). UV λ_{max} nm: 207. HR-MS *m/z*: 424.3689 (M^+) (Calcd for $C_{30}H_{48}O$, *m/z*: 424.3702).

Friedelin (**7**): Colorless needles, mp 268—269 °C (lit.,[22] mp 265 °C). Rt_R: 0.74 (HPLC), 2.41 (GLC). UV λ_{max} nm: 202. HR-MS *m/z*: 426.3852 (M^+) (Calcd for $C_{30}H_{50}O$, *m/z* 426.3858).

α-Amyrenone (**8**): Colorless needles, mp 128—129 °C (lit.,[22] mp 126 °C). Rt_R: 0.65 (HPLC), 1.65 (GLC). HR-MS *m/z*: 424.3674 (M^+) (Calcd for $C_{30}H_{48}O$, *m/z* 424.3702). ^{1}H-NMR δ: 0.80 (3H, d, $J=6$ Hz, H-29), 0.81 (3H, s, H-28), 0.92 (3H, br s, H-30), 1.06 (6H, s, H-24, H-26), 1.08 (6H, s, H-25, H-27), 1.10 (3H, s, H-23), 5.15 (1H, dd, $J=3$, 4 Hz, H-12).

β-Amyrenone (**10**): Colorless needles, mp 187—188 °C (lit.,[22] mp 180 °C). Rt_R: 0.58 (HPLC), 1.47 (GLC). HR-MS *m/z*: 424.3719 (M^+) (Calcd for $C_{30}H_{48}O$, *m/z* 424.3702). ^{1}H-NMR δ: 0.84 (3H, s, H-28), 0.88 (6H, s, H-29, H-30), 1.02 (3H, s, H-24), 1.05 (3H, s, 25-H), 1.07 (3H, s, H-26), 1.10 (3H, s, H-23), 1.15 (3H, s, H-27), 5.21 (1H, dd, $J=4$, 4 Hz, H-12).

$NaBH_4$ Reduction of Arborinone (1) and Glutinone (4) A solution of **1** (50 mg) in MeOH (25 ml), was treated with $NaBH_4$ (50 mg), and the reaction mixture was stirred at room temp. for 16 h. After neutralization by the addition of 5% HCl, the mixture was diluted with water and extracted with Et_2O. The extract was washed with water, dried over anhydrous Na_2SO_4, and concentrated to dryness. Preparative HPLC of the residue yielded isoarborinol (**2**) (87% of the mixture as determined by HPLC) and arborinol (**3**) (13%). The same reduction of **4** followed by HPLC fractionation gave glutinol (**5**) (14% of the reduction mixture) and 3-epiglutionol (**6**) (86%).

Isoarborinol (**2**): Colorless needles, mp 298—301 °C (lit.,[22] mp 299 °C). Rt_R: 0.85 (HPLC). HR-MS *m/z*: 426.3855 (M^+) (Calcd for $C_{30}H_{50}O$, *m/z* 426.3858). ^{1}H-NMR δ: 0.76 (3H, s, H-28), 0.77 (3H, s, H-26), 0.81 (3H, s, H-27), 0.82 (3H, s, H-24), 0.83 (3H, d, $J=7$ Hz, H-29 or H-30), 0.89 (3H, d, $J=7$ Hz, H-30 or H-29), 0.99 (3H, s, H-23), 1.03 (3H, s, H-25), 3.22 (1H, dd, $J=4$, 12 Hz, H-3α), 5.23 (1H, br d, $J=6$ Hz, H-11).

Arborinol (**3**): Colorless needles, mp 270—272 °C (lit.,[22] mp 275 °C). Rt_R: 0.73 (HPLC). MS *m/z*: 426 (M^+). ^{1}H-NMR δ: 0.76 (3H, s, H-28), 0.77 (3H, s, H-26), 0.82 (3H, s, H-27), 0.83 (3H, d, $J=7$ Hz, H-29 or H-30), 0.88 (3H, s, H-23 or H-24), 0.89 (3H, d, $J=7$ Hz, H-30 or H-29), 0.96 (3H, s, H-24 or H-23), 1.05 (3H, s, H-25), 3.43 (1H, br s, H-3β), 5.26 (1H, br d, $J=6$ Hz, H-11).

Glutinol (**5**): Colorless needles, mp 210—212 °C (lit.,[22] mp 211 °C). Rt_R: 0.51 (HPLC). MS *m/z*: 426 (M^+). ^{1}H-NMR δ: 0.85 (3H, s, H-25), 0.95 (3H, s, H-29), 0.99 (3H, s, H-30), 1.00 (3H, s, H-27), 1.04 (3H, s, H-23), 1.09 (3H, s, H-26), 1.14 (3H, s, H-24), 1.16 (3H, s, H-28), 3.49 (1H, m, H-3α), 5.63 (1H, br d, $J=6$ Hz, H-6).

3-Epiglutinol (**6**): Colorless needles, mp 197—200 °C. Rt_R: 0.58 (HPLC). HR-MS *m/z*: 426.3833 (M^+) (Calcd for $C_{30}H_{50}O$, *m/z* 426.3858). ^{1}H-NMR δ: 0.78 (3H, s, H-25), 0.95 (3H, s, H-29), 0.96 (3H, s, H-23 or H-24), 0.99 (3H, s, H-30), 1.00 (3H, s, H-27), 1.09 (3H, s, H-26), 1.14 (3H, s, H-24 or H-23), 1.16 (3H, s, H-28), 3.23 (1H, dd, $J=4$, 12 Hz, H-3β), 5.65 (1H, br d, $J=6$ Hz, H-6).

Isolation of Friedelin (7) *L. chungii* powder (8.8 g) was stirred overnight in 400 ml of 95% EtOH, then filtered. The air-dried residue was stirred for 1 h in 200 ml of EtOAc, and filtered. The residue was then refluxed overnight in 400 ml of EtOAc, and filtered. The colorless filtrate, on standing after concentration to 300 ml, deposited 1.08 g of cottony needles (mp 260—263 °C). From the mother liquor on further concentration two additional crops of needles were obtained, 0.52 and 0.30 g (total yield, 1.90 g, 21.5%).

Acknowledgements We thank Dr. M. Aimi (Coll. Sci. Technol., Nihon Univ.) for mass spectra and Mr. Y. Kawasaki for technical assistance. We also wish to thank Drs. W. C. Campbell[15] and R. Stevenson[18] for reference samples of friedelin, and Prof. W. H. Huang of Zhongshan (formerly, Lingnan) University for procuring our present supply of *L. chungii* powder.

References and Notes

1) F. A. McClure, *Lingnan Sci. J.*, **15**, 689 (1936); *idem*, *Lingnan Sci. Bull.*, **9**, 34 (1940).
2) F. C. Chang, *Lingnan Sci. J.*, **17**, 617 (1938); *idem*, Harvard University Thesis, 1941, p. 17.
3) The names arborinone and glutinone have been established in the literature for many years, we acknowledge that they are more appropriate than the prior names, bambuselins I and II, and will follow the current usage.
4) H. H. Hui and C. N. Lam, *Phytochemistry*, **4**, 333 (1965).
5) T. Ohmoto, *Yakugaku Zasshi*, **89**, 1682 (1965).
6) T. Ohmoto, M. Ikuse, and S. Natori, *Phytochemistry*, **9**, 2137 (1970).
7) T. Ohmoto, S. Uzawa, and N. Imazeki, *Shoyakugaku Zasshi*, **26**, 47 (1972).
8) T. Ohmoto, S. Uzawa, and R. Tanabe, *Shoyakugaku Zasshi*, **28**, 1 (1974).
9) S. Chapon and S. David, *Bull. Soc. Chim. Fr.*, **1953**, 333.
10) J. M. Beaton, F. S. Spring, and R. Stevenson, *J. Chem. Soc.*, **1955**, 2616.
11) J. M. Beaton, F. S. Spring, and J. L. Stewart, *Tetrahedron*, **2**, 246 (1958).
12) R. K. Gupta and V. Mahadevan, *Indian J. Pharm.*, **29**, 289 (1967).
13) A. K. Pal and K. C. Basuchaudhary, *Curr. Sci.*, **45**, 739 (1976).
14) L. Guang-Yi, A. I. Gray, and P. G. Waterman, *Phytochemistry*, **27**, 2283 (1988).
15) N. L. Drake and J. K. Wolfe, *J. Am. Chem. Soc.*, **62**, 3018 (1940); and references cited therein.
16) M. Laing, M. E. Burk-Laing, R. Bartho, and M. Weeks, *Tetrahedron Lett.*, **1977**, 3839.
17) R. F. Chandler and S. N. Hooper, *Phytochemistry*, **18**, 711 (1979).
18) R. Stevenson, *J. Org. Chem.*, **26**, 2142 (1961).
19) W. R. Croasmun and R. M. K. Carlson, "Two-Dimensional NMR Spectroscopy. Applications For Chemists and Biochemists," VCH Publishers, Weinheim, 1987.
20) T. Itoh, H. Tani, K. Fukushima, T. Tamura, and T. Matsumoto, *J. Chromatogr.*, **234**, 65 (1982).
21) T. Akihisa, T. Tamura, T. Matsumoto, D. S. Eggleston, W. C. M. C. Kokke, and N. Shimizu, *J. Chem. Soc.*, *Perkin Trans. 1*, **1988**, 439.
22) R. McCrindle and K. H. Overton, "Rodd's Chemistry of Carbon Compounds," Vol. II, Part C, ed. by S. Coffey, Elsevier Publ. Co., Amsterdam, 1969, Chapter 14.