Studies on Cytotoxic Constituents in Pericarps of *Mallotus japonicus*. IV

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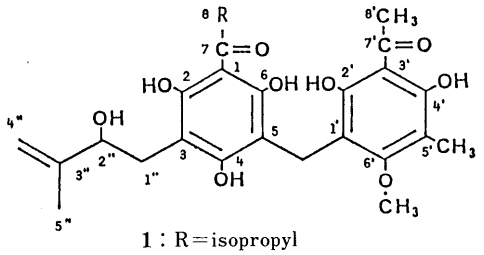
Two new phloroglucinol derivatives, isomallotolerin (1) and isomallotochromanol (2), were isolated from the cytotoxic fraction of the pericarps of *Mallotus japonicus*. The new derivatives were identified as 3-(3-methyl-2-hydroxybut-3-enyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phlorisobutyrophenone (1) and 6-acetyl-5,7-dihydroxy-8-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-2,2-dimethyl-3-hydroxychroman (2) from chemical and spectral data. Isomallotolerin and its acetate were found to be cytotoxic to KB cell line.

Keywords *Mallotus japonicus*; Euphorbiaceae; phloroglucinol derivative; cytotoxicity; ^{13}C-NMR

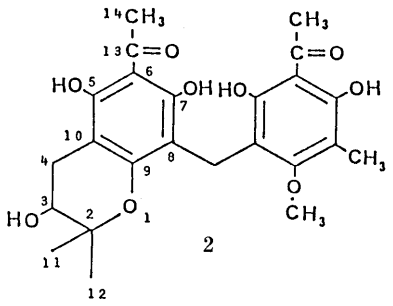
Previously, we reported several cytotoxic phloroglucinol derivatives in pericarps of *Mallotus japonicus* MUELL. ARG. (Euphorbiaceae).[1-3] In a continuing search for cytotoxic constituents in the $CHCl_3$-soluble fraction of the pericarps of *M. japonicus*, two new compounds named isomallotolerin (**1**) and isomallotochromanol (**2**) were isolated. We wish to report the structural elucidation and the cytotoxic activities of these new compounds. Separation of the extract of *M. japonicus* by column chromatography on Si gel[3] yielded **2** and a mixture of **1** and mallotolerin (**3**). The isolation of **1** was performed by high-performance liquid chromatography (HPLC).

Compound **1**, $C_{26}H_{32}O_9$, gave a positive $FeCl_3$ reaction. The ultraviolet (UV) spectrum was similar to that of **3**. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum closely resembled that of **3**, except for the appearance of the signals of an isopropyl ketone group at δ 1.19 (6H, d, J=6.7 Hz) and 4.05 ppm (1H, sept, J=6.7 Hz) instead of the signals of a propyl ketone. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** was also similar to that of **3**, except for the appearance of the carbon signals of an isopropyl group at δ 18.93(q), 19.12(q) and 38.97 ppm(d) instead of the signals of a propyl group. The mass spectrum (MS) of **1** showed a molecular ion peak at m/z 488 and prominent peaks at m/z 274, 259, 221, 209, 196 and 181, indicating a 3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl moiety.[1-3] Reductive alkaline cleavage of **1** afforded 2,6-dihydroxy-3-methyl-4-methoxyacetophenone.[1] From these chemical and spectral data and from biosynthetic considerations, the structure of **1** is proposed to be 3-(3-methyl-2-hydroxybut-3-enyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phlorisobutyrophenone, and it was named isomallotolerin (**1**). A Cotton effect was not detected in a circular dichroism (CD) study on its benzoate[4,5] and **1** was converted into the L-menthoxyacetyl derivatives, which showed two peaks on HPLC, the areas of which were equal.[6] From these results, the allylic alcohol on its side chain is considered to be racemic.

Compound **2**, $C_{24}H_{28}O_9$, also gave a positive $FeCl_3$ reaction. The UV, IR and MS were similar to those of mallotochromanol (**4**). The ^{1}H-NMR spectrum closely resembled that of **4** with a slight difference in the methylene signals at δ 3.07 (1H, dd, J=15.1, 8.3 Hz) and 3.18 ppm (1H, dd, J=15.1, 9.3 Hz) and a methine proton at δ 4.87 ppm (1H, dd, J=9.3, 8.3 Hz). The signal of the methylene protons between the rings of **4** shifted upfield on acetylation, whereas the signal of the methylene protons between the rings of **2** showed a down-



1 : R=isopropyl
3 : R=propyl



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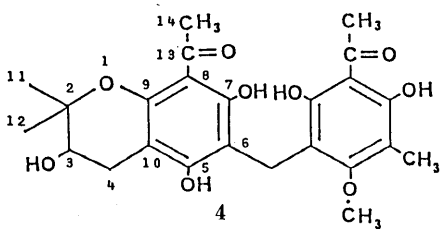

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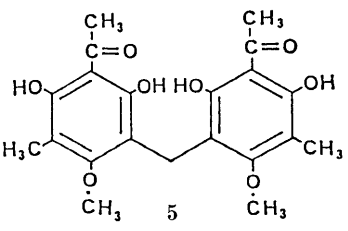

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Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of **1**—**4**

Carbon No.	**1**[a]	**3**[b]	**2**[c]	**4**[b]
1	104.32[d]	104.94[d]		
2	159.68[e]	159.71[e]	69.96	78.49
3	106.31[d]	106.07[d]	89.96	68.49
4	159.79[e]	159.81[e]	26.99	26.09
5	104.61[d]	105.32[d]	154.54	160.72
6	160.12[e]	160.12[e]	104.77	105.20
7	211.87	207.55	158.69	160.91
8	38.97	46.04	101.52	105.01
9	18.93	18.13	165.05	155.08
10	19.12	14.02	103.66	99.47
11			22.94	22.02
12			26.34	24.84
13			202.46	203.79
14			32.44	32.89
1′	108.53	108.73	112.10	108.79
2′	157.16	157.39	163.54	157.22
3′	108.67	109.15	108.22	108.79
4′	162.02	162.47	158.97	162.56
5′	109.17	109.48	108.57	109.95
6′	159.99	159.90	163.65	159.95
7′	205.57	205.62	204.68	205.56
8′	33.50	33.77	33.09	33.75
1″	29.20	29.31		
2″	78.11	78.16		
3″	146.81	146.75		
4″	110.02	110.67		
5″	18.10	18.39		
Ar–CH_2–Ar	16.83	17.04	16.85	16.55
Ar–Me	8.52	8.82	8.84	8.27
OMe	61.58	61.83	60.01	61.81

a) Measured in $CDCl_3$+CD_3OD. *b*) Measured in $CDCl_3$. *c*) Measured in DMSO-d_6. *d, e*) Assignments may be interchanged.

TABLE II. Cytotoxicities of Phloroglucinol Derivatives Against KB Cells

Compound	ED_{50} (μg/ml)	Compound	ED_{50} (μg/ml)
1	0.84	**1**-Hexaacetate	1.5
2	>20	**2**-Tetraacetate	8.5
3	0.95	**3**-Hexaacetate	3.5
4	>20	**4**-Pentaacetate	8.6

field shift like that found in mallotophenone **5** after conversion to acetates.[1] The ^{13}C-NMR spectrum was also similar to that of **4**, except for the signal due to C-3 which was shifted significantly downfield in comparison with that of **4**. The assignment of the ^{1}H- and ^{13}C-NMR signals of **2** was done by means of two-dimensional NMR with long range ^{1}H–^{13}C shift correlation and by making use of the deuterium induced upfield shifts of alcoholic carbons in the ^{13}C-NMR spectrum.[7] From these spectral data, the sturcture of **2** is proposed to be 6-acetyl-5,7-dihydroxy-8-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-2,2-dimethyl-3-hydroxychroman, and it was named isomallotochromanol (**2**). The hydroxy group on the chroman ring is considered to be racemic based on the CD study[8] and separation of diastereomeric derivatives by HPLC, as in the case of compound **1**.

The isolated compounds **1**, **2** and their acetates were tested for cytotoxic activity in the KB system.[1] While both **2** and its tetraacetate were inactive, as was **4**, **1** and its hexaacetate showed significant cytotoxicity with ED_{50} of 0.84 and 1.5 μg/ml, respectively. Further cytotoxic constituents of this plant are now under investigation.

Experimental

General Procedures All melting points were determined on a Yanagimoto micro melting point apparatus and are recorded uncorrected. UV and IR spectra were recorded on a Hitachi 220S double beam spectrophotometer and 260-10 infrared spectrometer with polystyrene calibration at 1601 cm^{-1}, respectively. Specific rotations were determined on a JASCO DIP-140 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were taken on a Varian XL-200 spectrometer at 200 and 50.3 MHz, respectively, and 2D-NMR spectra were taken on a JEOL JNM-GX 400 spectrometer with tetramethylsilane as an internal standard. The chemical shifts are recorded in δ (ppm) values. Mass spectra were obtained on a JEOL JMS-D-200 mass spectrometer operating at 70 eV. HPLC was performed on a Shimadzu LC-6A liquid chromatograph with an SPD-MIA spectrophotometric detector.

Extraction and Separation The extraction and separation of the dried pericarps of *M. japonicus* have been described previously.[1-3] The 1% MeOH/$CHCl_3$ eluent (26 g) was rechromatographed on a silica gel column (benzene : AcOEt = 4 : 1) to give **2** (25 mg). The crude **1** and **3** from the 15% AcOEt/hexane eluate of the rechromatography on a silica gel column[3] were purified by preparative HPLC (column, Cosmosil $5C_{18}$ 20 mm i.d. × 25 cm; solvent, $CHCl_3$: MeOH : 0.005 M phosphate buffer = 1 : 35 : 5; flow rate 5 ml/min; detection, UV 282 nm) to give **1** (13 mg, t_R 110 min) and **3** (30 mg, t_R 115 min), respectively.

Isomallotolerin (1) Yellow needles, mp 216—217 °C ($CHCl_3$). $[\alpha]_D^{23}$ ±0° (*c* = 0.63, $CHCl_3$). *Anal.* Calcd for $C_{26}H_{32}O_9$: C, 63.91; H, 6.61. Found: C, 63.72; H, 6.55. UV λ_{max}^{EtOH} nm (log ε): 285 (4.04), 323 (3.86). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 3280, 1620 (C=O), 1605, 1420, 1300, 1280, 1145. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (6H, d, *J* = 6.7 Hz, Me × 2), 1.86 (3H, s, 5″-Me), 2.12 (3H, s, 5′-Me), 2.69 (1H, dd, *J* = 15.0, 8.9 Hz, 1″-Ha), 2.73 (3H, s, Ac), 3.14 (1H, dd, *J* = 15.0, 1.8 Hz, 1″-Hb), 3.74 (2H, s, Ar–CH_2–Ar), 3.98 (3H, s, OMe), 4.05 (1H, sept, *J* = 6.7 Hz, 8-H), 4.32 (1H, br d, *J* = 8.9 Hz, 2″-H), 4.90 (1H, br s, 4″-Ha), 5.03 (1H, br s, 4″-Hb). MS *m/z* 488 (M^+), 470, 417, 274, 259, 221, 209, 196, 181. High-resolution MS measurement *m/z* 488.2079 ($C_{26}H_{32}O_9$ requires 488.2044). ^{13}C-NMR see Table I.

Acetylation of 1 Compound **1** was treated overnight with Ac_2O and pyridine at room temperature, and the reaction mixture was worked up as usual to give a hexaactate as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (6H, d, *J* = 6.8 Hz, Me × 2), 1.71 (3H, s, Me), 1.94 (3H, s, OAc), 2.06 (3H, s, Ar–Me), 2.13 (3H, s, OAc), 2.14 (3H, s, OAc), 2.23 (3H, s, OAc), 2.26 (3H, s, OAc), 2.29 (3H, s, OAc), 2.36 (3H, s, Ac), 2.56 (1H, m, 1″-Ha), 2.82 (1H, m, 1″-Hb), 2.88 (1H, m, 8-H), 3.56 (3H, s, OMe), 3.67 (2H, s, Ar–CH_2–Ar), 4.80 (2H, br s, 4″-H_2), 5.28 (1H, dd, *J* = 7.3, 6.6 Hz, 2″-H). MS *m/z*: 740 (M^+).

Isomallotochromanol (2) Yellow needles, mp 150—152 °C (MeOH). $[\alpha]_D^{23}$ ±0° (*c* = 0.27, EtOH). *Anal.* Calcd for $C_{24}H_{28}O_9$: C, 62.59; H, 6.09. Found: C, 62.48; H, 6.09. UV λ_{max}^{EtOH} nm (log ε): 288 (4.40), 345 (3.74). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 3430, 2930, 1630 (C=O), 1595, 1440, 1370, 1130, 1095. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, s, Me), 1.41 (3H, s, Me), 2.14 (3H, s, Ar–Me), 2.68 (3H, s, Ac), 2.71 (3H, s, Ac), 3.07 (1H, dd, *J* = 15.1, 8.3 Hz, 4-Ha), 3.18 (1H, dd, *J* = 15.1, 9.3 Hz, 4-Hb), 3.98 (3H, s, OMe), 3.70 (2H, d, *J* = 1.71 Hz, Ar–CH_2–Ar), 4.87 (1H, dd, *J* = 9.3, 8.3 Hz, 3-H). MS *m/z*: 460 (M^+), 265, 209, 196, 181. High-resolution MS measurement *m/z* 460.1712 ($C_{24}H_{28}O_9$ requires 460.1732). ^{13}C-NMR see Table I.

Acetylation of 2 After acetylation as described for **1**, a tetraacetate was obtained as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, s, Me), 1.02 (3H, s, Me), 2.03 (3H, s, Ar–Me), 2.09 (3H, s, OAc), 2.27 (6H, s, OAc × 2), 2.30 (3H, s, OAc), 2.36 (3H, s, Ac), 2.39 (3H, s, Ac), 2.92 (1H, dd, *J* = 15.7, 9.5 Hz, 4-Ha), 3.00 (1H, dd, *J* = 15.7, 8.4 Hz, 4-Hb), 3.67 (1H, d, *J* = 15.8 Hz, Ar–HCH–Ar), 3.72 (3H, s, OMe), 3.90 (1H, d, *J* = 15.8 Hz, Ar–HCH–Ar), 4.55 (1H, m, 3-H). MS *m/z*: 628 (M^+), 570, 528, 486, 249, 238, 209, 196.

Preparation of Benzoates of 1 and 2 Two drops of benzoyl chloride were added to a dry pyridine solution of **1** (5 mg), with ice cooling. The reaction mixture was evaporated *in vacuo* to obtain the crude benzoate, which was purified by preparative TLC with benzene–AcOEt (19 : 1) as the developing solvent. Recrystallization from MeOH gave the benzoate as colorless prisms, mp 110—112 °C. UV λ_{max}^{EtOH} nm: 233. Similar treatment of **2** (5 mg) gave its benzoate as colorless prisms, mp 94—96 °C. UV λ_{max}^{EtOH} nm: 234. These benzoates were examined by CD (EtOH) but showed no Cotton effect.

Analysis of L-Methoxyacetyl Derivatives of 1 and 2 Two drops of L-menthoxyacetyl chloride were added to a dry pyridine solution of **1** (3 mg), at room temperature. After 12 h, water was added to the solution and the solvent was removed *in vacuo*. The residue was dissolved in chloroform (5 ml) and washed with water (3 ml × 2). After being dried (Na_2SO_4), the solution was evaporated *in vacuo* and the residue was purified by preparative TLC with hexane–ethyl acetate (10 : 3) as the developing solvent. The diastereomeric mixture was obtained as a colorless viscous oil, which showed two peaks (t_R 18 and 19 min, respectively), the areas of which were equal on HPLC, under the following conditions: column, TSKgel Silica-60 (4.6 mm i.d. × 250 nm); mobile phase, cyclohexane–ether (9 : 1); flow rate, 0.5 ml/min; temperature, 32 °C; detection, UV 254 nm. Similar treatment of **2** gave its L-menthoxyacetyl derivative as a colorless viscous oil, which showed two peaks (t_R 12 and 13 min, respectively) the areas of which were equal on HPLC, under the following conditions: column, Cosmosil $5C_{18}$ (4.6 mm i.d. × 150 mm); mobile phase, acetonitrile–H_2O (99 : 1); flow rate, 1.0 ml/min; temperature, 32 °C; detection, UV 254 nm.

Reductive Alkaline Cleavage Reductive alkaline cleavage was carried out as described previously.[1)]

Cytotoxicity Test The test employing KB cell line was carried out as described previously.[1)]

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References and Notes

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