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STUDIES ON CYTOTOXIC CONSTITUENTS IN PERICARPS OF *MALLOTUS JAPONICUS*, PART I[1]

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ABSTRACT.—Two new phloroglucinol derivatives, named mallotophenone (**3**) and mallotochromene (**4**), were isolated from the pericarps of *Mallotus japonicus* together with two known compounds, 3-(3,3-dimethylallyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone (**2**) and 2,6-dihydroxy-3-methyl-4-methoxyacetophenone (**1**). The new derivatives were confirmed to be 5-methylene-*bis*-2,6-dihydroxy-3-methyl-4-methoxyacetophenone (**3**) and 8-acetyl-5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)2,2-dimethylchromene (**4**) by their respective chemical and spectral data. Several of the phloroglucinol derivatives isolated from this tree were cytotoxic against the KB system and L-5178Y in cell culture.

Mallotus japonicus Muell. Arg. (Euphorbiaceae) is a deciduous tree that sprouts red-colored buds and is widely distributed in Japan. The tree has been used in folk medicine; the bark is a medication for ulcers and for cancer; the leaves are used as a treatment for boils.

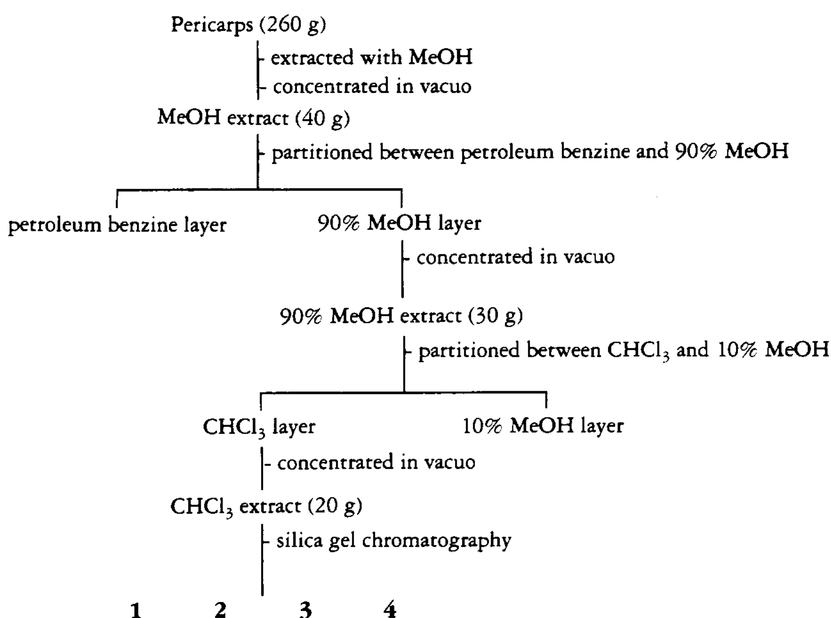
Earlier investigations of the constituents of the tree resulted in the isolation of bergenin from the bark (1), rutin from the leaves (2), and cardiac glycosides from the seed (3, 4). Recently, several new rottlerin-like phloroglucinol derivatives were isolated from the fruits, and their structures were confirmed by the Kawano group (5, 6).

We have now examined cytotoxic constituents in the pericarps and obtained some cytotoxic compounds by bioactivity-directed fractionation. We wish to report the isolation of the cytotoxic compounds, the cytotoxicities of the isolates, and the structural elucidation of two new rottlerin-like compounds named mallotophenone (**3**) and mallotochromene (**4**). The MeOH extract of the pericarps was separated as shown in Scheme 1. The $CHCl_3$ extract was chromatographed on silica gel to afford four compounds (**1-4**), of which **2** was the major component.

Compound **1** was identified as the known compound, 2,6-dihydroxy-3-methyl-4-methoxyacetophenone, from its uv, nmr, and ms spectral data, and this was confirmed by comparison with an authentic sample (6). Compound **2** was identified as 3-(3,3-dimethylallyl)-5-(3-acetyl-2, 4-dihydroxy-5-methyl-6-methoxybenzyl) - phloracetophenone from its uv, nmr, and ms spectral data, and this was confirmed by direct comparison with an authentic sample (5).

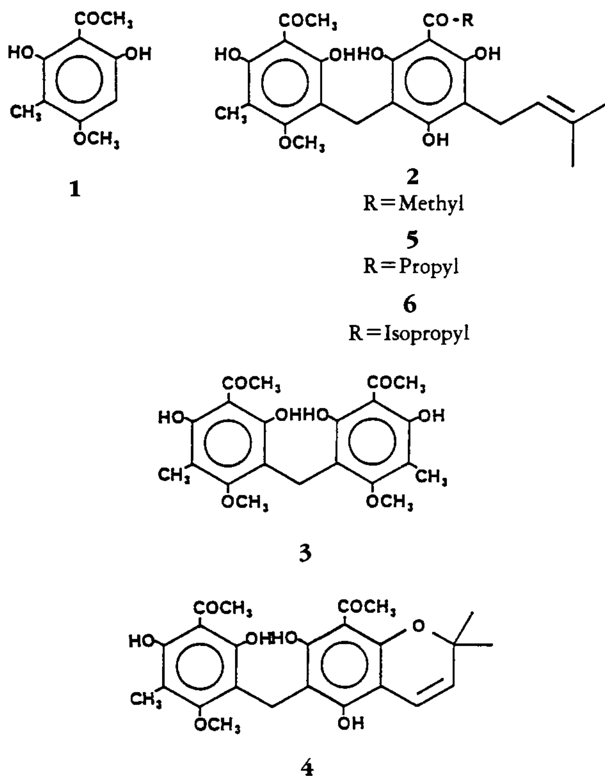
Compound **3**, $C_{21}H_{24}O_8$, gave a positive $FeCl_3$ reaction. The uv spectrum was similar to that of **1**. The ^{1}H-nmr spectrum showed four singlets for the two methyl, acetyl, methoxyl, and hydroxyl groups at δ 2.14 (6H), 2.72 (6H), 4.00 (6H), and 9.00 (2H) ppm, respectively. A methylene proton signal was observed at δ 3.69 ppm, but

[1]A part of this work was presented at the 62nd Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Toyama, June 1984.



SCHEME 1. Fractionation Scheme.

aromatic protons were not observed. The ms spectrum of **3** exhibited a molecular ion peak at *m/z* 404 and prominent peaks at *m/z* 208, 196, and 181. Acetylation of **3** with Ac_2O and pyridine afforded a tetraacetate as a colorless oil which had a molecular ion at *m/z* 572 in the ms spectrum. These spectral data were consistent with a symmetrical structure, **3**. This was further supported by the ^{13}C-nmr spectrum which showed 11



signals (see Experimental section). Reductive alkaline cleavage of **3** afforded 2,6-dihydroxy-3-methyl-4-methoxyacetophenone (**1**). From these chemical and spectral data, the structure of **3** was determined to be 5-methylene-*bis*-2,6-dihydroxy-3-methyl-4-methoxyacetophenone, and it was named mallotophenone (**3**).

Compound **4**, $C_{24}H_{26}O_8$, also gave a positive $FeCl_3$ reaction. The uv spectrum was similar to those of **1-3**. The ^{1}H-nmr spectrum closely resembled that of **2**, except for the appearance of the signals of the 2,2-dimethylchromene at δ 1.47 ppm (6H, s, Me×2), 5.45, and 6.61 ppm (each, 1H, d, J = 10 Hz) instead of the signals of two vinyl methyl groups, a vinyl proton and a methylene group. The ms spectrum of **4** showed a molecular ion peak at *m/z* 442 and prominent peaks at 409, 247, 246, 231, 219, 196, and 181, indicated a 3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl moiety. The reductive cleavage of **4** afforded **1**. From these chemical and spectral data and biosynthetic considerations, the structure of **4** was considered to be 8-acetyl-5,7-dihydroxy-6- or 6-acetyl-5,7-dihydroxy-8-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)2,2-dimethylchromene. The signal of the methylene protons between the rings of **4** closely resembled the upfield shift found in **2** after conversion of these to acetates (see Table 1). Consequently, the structure of **4** is proposed to be 8-acetyl-5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)2,2-dimethylchromene. It was named mallotochromene (**4**).

TABLE 1. ^{1}H-nmr Data of Methylene Proton between Rings on **2, 3, 4** and Their Acetates

Compounds	Chemical shifts		Δ=Original-Acetate
	Original	Acetate	
2	3.83	3.71	+0.12[a]
	3.78	3.71	+0.02[b]
3	3.69	3.89	−0.20
4	3.72	3.65	+0.07

[a]in $CDCl_3$/pyridine-d_5 [b] see Shigematsu *et al.* (5).

The isolates, **1-4**, their acetate derivatives, and a mixture of **5** and **6** (6) were tested for cytotoxic activity in the KB system (7, 8). Three isolates, **2-4**, their acetate derivatives, and a mixture of **5** and **6** displayed activity, but **1** was inactive. They were also tested against mouse leukemia L-5178Y cells *in vitro* (9), and the results are shown in Table 2. Further cytotoxic constituents of the pericarps are under investigation.

TABLE 2. Cytotoxic Activity of the Rottlerin-Like Substances from *Mallotus japonicus* (in vitro.)

Compounds	ED_{50} μg/ml	
	KB	L-5178Y
1	>100	>100
1-Acetate	>100	>100
2	0.58	0.74
2-Acetate	1.50	3.20
3	2.40	6.10
3-Acetate	4.80	5.20
4	2.10	1.25
4-Acetate	0.29	1.04
5+6	0.26	1.07

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Yanagimoto micro melting point apparatus and are recorded uncorrected. Uv spectra were recorded on a Hitachi 220 S double beam spectrophotometer, and ir spectra were obtained on a Hitachi 260-10 infrared spectrometer with polystyrene caribration at 1601 cm^{-1}. ^{1}H-nmr and ^{13}C-nmr spectra were taken on a Varian Xl-200 spectrometer at 200 MHz and 50.3 MHz, respectively, and chemical shifts are given in δ (ppm) with TMS as an internal standard. Ms spectra were obtained on a JEOL JMS-D-200 mass spectrometer operating at 70 eV.

EXTRACTION AND SEPARATION.—The dried pericarps of *M. japonicus*, a voucher specimen of which is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama, Japan,were collected at Sugitani, Toyama, Japan, October 1982, were extracted with MeOH at room temperature for 3 days. The MeOH extract was separated as shown in Scheme 1.[2] The $CHCl_3$ extract was chromatographed on silica gel by stepwise elution with $CHCl_3$, 1% MeOH/$CHCl_3$, 2% MeOH/$CHCl_3$, 5% MeOH/$CHCl_3$, 10% MeOH/$CHCl_3$, and MeOH. The $CHCl_3$ elution gave **2** (900 mg) as yellow needles. The filtrate of **2** and 1% MeOH/$CHCl_3$ elution were combined and rechromatographed on a silica gel column by stepwise elution with the following solvents to give **1, 3** and **4** as follows: **4** (15 mg) from 5% EtOAc/hexane, **3** (43 mg) from 10% EtOAc/hexane, **1** (15 mg) from 20% EtOAc/hexane.

IDENTIFICATION OF **1**.—Yellow needles, mp 196-198° (MeOH) [Lit. (5) mp 197-200°]. Identification was established by comparison (uv, ir, ^{1}H nmr, ms) with an authentic sample of 2,6-dihydroxy-3-methyl-4-methoxyacetophenone (5). The diacetate, colorless needles, mp 84-85°, was also identified by direct comparison (ir, ^{1}H nmr, ms) with an authentic sample.

IDENTIFICATION OF **2**.—Yellow needles, mp 190-191° (MeOH) [Lit. (5) mp 188-189°]. Identification was established by comparison (uv, ir, ^{1}H nmr, ms) with an authentic sample of 3-(3,3-dimethylally)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone (5). The pentaacetate, a colorless oil, was also identified by direct comparison (ir, ^{1}H nmr, ms) with an authentic sample.

CHARACTERIZATION OF MALLOTOPHENONE (**3**).—Yellow needles, mp 223-225° (MeOH); uv λ max (EtOH) (log ε) 281 (4.23) and 352 nm (3.58);ir ν max (KBr) 3250, 1615, 1600, 1420, 1370, 1305, 1280, 1140, 1100, 1025, and 920 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 2.14 (6H, s, aromatic Me×2), 2.72 (6H, s, Ac×2), 3.69 (2H, s, Ar-CH_2-Ar), 4.00 (6H, s, OMe×2), and 9.00 ppm (2H, s, OH×2); ms *m/z* 404 (M^+), 371, 210, 208, 196, 193 and 181; mass measurement *m/z* 404.1478 ($C_{21}H_{24}O_8$ requires 404.1470); ^{13}C nmr ($CDCl_3$) δ 8.84 (q, Me×2), 17.89 (t, Ar-CH_2-Ar), 33.81 (q, acetyl Me×2), 62.02 (q, OMe×2), 108.24, 108.82, 109.89 (each, s, aromatic C×2), 156.94, 159.67, 162.76 (each, s, aromatic C-O×2), and 205.41 ppm (s, CO×2).

ACETYLATION OF **3**.—Compound **3** was treated overnight with Ac_2O and pyridine at room temperature, and the reaction mixture was worked up as usual to give a tetraacetate as colorless oil: ^{1}H nmr ($CDCl_3$) δ 2.09 (12H, s, aromatic Me×2 and OAc×2), 2.29 (6H, s, OAc×2), 2.35 (6H, s, Ac×2), 3.66 (6H, s, OMe×2), and 3.89 ppm (2H, s, Ar-CH_2-Ar); ms *m/z* 572 (M^+).

CHARACTERIZATION OF MALLOTOCHROMENE (**4**).—Yellow needles, mp 190-192° (MeOH); uv λ max (EtOH) (log ε) 283 (4.46) and 327 nm (4.06); ir ν max (KBr) 3260, 2940, 1625, 1610, 1430, 1370, 1300, 1140, and 1105 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.47 (6H, s, Me×2), 2.12 (3H, s, aromatic Me), 2.69 (3H, s, Ac), 2.72 (3H, s, Ac), 3.72 (2H, s, Ar-CH_2-Ar), 3.98 (3H, s, OMe), 5.45 (1H, d, J=10 Hz, 3-H), and 6.61 ppm (1H, d, J=10 Hz, 4-H); ms *m/z* 442 (M^+), 409, 247, 246, 231, 219, 213, 196, 193, and 181; mass measurement *m/z* 442.1634 ($C_{24}H_{26}O_8$ requires 442.1625); ^{13}C nmr ($CDCl_3$) δ 8.74 (q, Me), 16.60 (t, Ar-CH_2-Ar), 27.95 (q, 2-Me×2), 32.78 (q, acetyl Me), 33.79 (q, acetyl Me), 61.81 (q, OMe), 78.22 (s, 2-C), 94.15, 103.38, 105.03, 105.29, 108.81, 109.69 (each s, aromatic C), 116.00 (d, 3-C), 125.16 (d, 4-C), 156.07, 157.12, 157.89, 159.96, 160.91, 162.55 (each, s, aromatic C-O), 204.04, and 205.47 ppm (each, s, CO).

ACETYLATION OF **4**.—Using acetylation as described for **3**, a colorless oil was obtained. ^{1}H nmr ($CDCl_3$) δ 1.46 (6H, s, 2-Me×2), 2.07 (3H, s, aromatic Me), 2.16 (3H, s, OAc), 2.19 (6H, s, OAc×2), 2.29 (3H, s, OAc), 2.36 (3H, s, Ac), 2.50 (3H, s, Ac), 3.63 (3H, s, OMe), 3.65 (2H, s, Ar-CH_2-Ar), 5.65 (1H, d, J=10 Hz, 3-H), 6.12 (1H, d, J=10 Hz, 4-H); ms *m/z* 610 (M^+).

REDUCTIVE ALKALINE CLEAVAGE.—The isolate (**3**, 5 mg) dissolved in 5% NaOH (2 ml) was mixed with Zn powder (20 mg) and warmed for 5 min at 100°. The filtrate of the reaction mixture was acidified

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[2]In the separation, the $CHCl_3$ extract was found to exhibit the highest growth inhibition against KB.

with dilute HCl and extracted with Et_2O. After evaporation of the Et_2O, the residue was purified through a silica gel column to afford yellow needles, mp 198-200°. This compound was identified as **1** by comparison with an authentic sample of **1**. The isolate **4** was also cleaved similarly, and the reaction mixture afforded **1**.

KB AND L-5178Y CELL CULTURE ASSAYS.—Substances were evaluated for cytotoxic activity as previously noted (7-9). All assays were performed in duplicate, and the results were expressed as the effective dose needed to inhibit 50% of the growth observed in control tubes which were treated with solvent only. The cytotoxic activity of the rottlerin-like substances from *M. japonicus* are shown in Table 2.

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