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STUDIES ON CYTOTOXIC CONSTITUENTS IN PERICARPS OF *MALLOTUS JAPONICUS*, V.[1] THREE NEW PHLOROGLUCINOL DERIVATIVES, BUTYRYLMALLOTOCHROMANOL, ISOBUTYRYLMALLOTOCHROMANOL, AND MALLOTOJAPONOL

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ABSTRACT.—Three new phloroglucinol derivatives, butyrylmallotochromanol [**1**], isobutyrylmallotochromanol [**2**], and mallotojaponol [**3**], were isolated from the pericarps of *Mallotus japonicus*. The new derivatives were identified as 5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-8-butyryl-2,2-dimethyl-3-hydroxychroman [**1**], 5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-8-isobutyryl-2,2-dimethyl-3-hydroxychroman [**2**], and 3-(2,3-dihydroxyisopentyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone [**3**] by their respective chemical and spectral data. The pentaacetyl derivatives of **1** and **2** were found to be weakly cytotoxic against a KB cell line.

We previously reported several cytotoxic phloroglucinol derivatives from the pericarps of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae) (1–4). In a continuing search for cytotoxic constituents in the $CHCl_3$-soluble fraction of the pericarps of *M. japonicus*, three new compounds, named butyrylmallotochromanol [**1**], isobutyrylmallotochromanol [**2**], and mallotojaponol [**3**], were isolated. We report the structural elucidation and the cytotoxic activities of these new compounds and propose the naming and renaming of some additional phloroglucinol derivatives from the pericarps.

Separation of the extract of *M. japonicus* by cc on Si gel (3,4) yielded **3** and a mixture of **1** and **2**. The isolation of **1** and **2** was performed by hplc.

Compound **1**, $C_{26}H_{32}O_9$, gave a positive $FeCl_3$ reaction. The uv and ir spectra were similar to those of mallotochromanol [**4**]. The ^{1}H-nmr spectrum closely resembled that of **4**, except for the appearance of the signals of a propyl ketone group at δ 1.01 (3H, t, $J=7.4$ Hz), 1.71 (2H, m, $J=7.4$ Hz), and 3.02 ppm (2H, t, $J=7.4$ Hz) instead of the signals of a methyl ketone. The ^{13}C-nmr spectrum of **1** was also similar to that of **4**, except for the appearance of the carbon signals for a propyl group at δ 14.02 (q), 18.58 (t), and 46.44 ppm (t), instead of a signal for a methyl group. The ms spectrum of **1** exhibited a molecular ion at *m/z* 488 and prominent fragment ions at *m/z* 293, 280, 237, 221, 209, 196, and 181, indicating a 3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl moiety (1–4). Reductive alkaline cleavage of **1** afforded 2,6-dihydroxy-3-methyl-4-methoxyacetophenone (1). In the ^{1}H-nmr, the signal for the methylene protons between the rings of isomallotochromanol [**5**] exhibited a downfield shift on acetylation, whereas the signal for the methylene protons between the rings of **1** resembled the upfield shift found in **4** after conversion of these to acetates (4). From these chemical and spectral data and from biosynthetic considerations, the structure of **1** is proposed to be 5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-8-butyryl-2,2-dimethyl-3-hydroxychroman, and it was named butyrylmallotochromanol [**1**]. Compound **1** was converted into the L-menthoxyacetyl derivatives, which produced two peaks; the areas of the peaks were equal on hplc (5). From these results, the hydroxyl group on the chroman ring was considered to be racemic.

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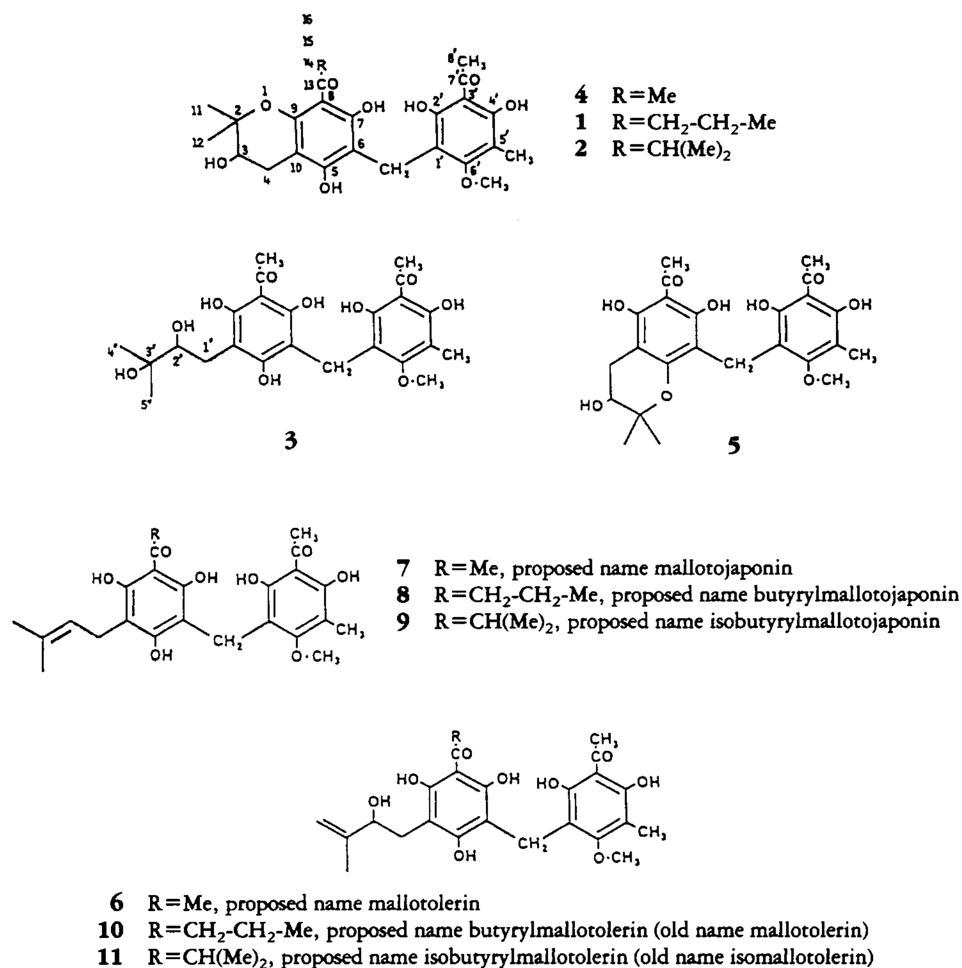

FIGURE 1. Isolated compounds from pericarps of *Mallotus japonicus*.

Compound **2**, $C_{26}H_{32}O_9$, also gave a positive $FeCl_3$ reaction. The uv, ir, and ms spectra were similar to those of compound **1**. The ^{1}H-nmr spectrum closely resembled that of **1**, except for the appearance of signals due to the isopropyl ketone group at δ 1.20 (6H, d, $J=6.9$ Hz) and 3.86 ppm (1H, sept, $J=6.9$ Hz) instead of the signals of a propyl ketone. Reductive alkaline cleavage of **2** afforded 2,6-dihydroxy-3-methyl-4-methoxyacetophenone, and the ms of **2** also indicated the presence of a 3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl moiety. In the ^{1}H nmr, the signal of the methylene protons between the rings of **2** resembled the upfield shift found in compounds **1** and **4** after conversion of these to acetates (4). The ^{13}C-nmr spectrum was also similar to that of **1**, except for the appearance of the carbon signal for an isopropyl group at δ 19.31 (q), 19.50 (q), and 39.45 ppm (t). From these spectral data, the structure of compound **2** was proposed to be 5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-8-isobutyryl-2,2-dimethyl-3-hydroxychroman, and **2** was named isobutyrylmallotochromanol. The hydroxyl group on the chroman ring is considered to be racemic based on the separation of diastereomeric L-menthoxyacetyl derivatives by hplc similar to the procedure used with compound **1**.

Compound **3**, $C_{24}H_{30}O_{10}$, also gave a positive $FeCl_3$ reaction. The molecular formula of **3** was 18 mass units larger than **4**, **5**, and mallotolerin [**6**]. The uv and ir

TABLE 1. Nmr Data of Compounds **1**–**4** (in $CDCl_3$, δ ppm, J = Hz).

Carbon	**1**		**2**		**4**	Proton	**3**
	^{13}C-nmr	^{1}H-nmr	^{13}C-nmr	^{1}H-nmr	^{13}C-nmr		^{1}H-nmr
2	78.49 (s)		78.51 (s)		78.49 (s)		
3	68.49 (d)	3.78 (dd, J = 5.2, 5.5)	68.41 (d)	3.79 (dd, J = 5.2, 5.5)	68.49 (d)	2″	3.60 (brd, J = 9.3)
4	26.15 (t)	2.62, 2.86 (dd, J = 5.5, 17.3)	26.16 (t)	2.63, 2.87 (dd, J = 5.5, 17.3)	26.09 (t)	1″	2.38 (dd, J = 9.3, 14.7) 3.12 (brd, J = 14.7)
5	160.33 (s)		160.34 (s)		160.72 (s)		
6	105.19 (s)		105.35 (s)		105.20 (s)		
7	160.33 (s)		160.69 (s)		160.91 (s)		
8	104.72 (s)		104.00 (s)		105.01 (s)		
9	154.90 (s)		154.56 (s)		155.08 (s)		
10	99.60 (s)		99.59 (s)		99.47 (s)		
11	21.96 (q)	1.36 (s)	21.80 (q)	1.36 (s)	22.02 (q)	4″	1.28 (s)
12	24.83 (q)	1.40 (s)	24.70 (q)	1.40 (s)	24.84 (q)	5″	1.36 (s)
13	205.45 (s)		205.46 (s)		203.79 (s)		
14	46.44 (t)	3.02 (t, J = 7.4)	39.45 (d)	3.86 (sept, J = 6.9)	32.89 (q)		2.71 (s)
15	18.58 (t)	1.71 (m)	19.31 (q)	1.20 (d, J = 6.9)			
16	14.02 (q)	1.01 (t, J = 7.4)	19.50 (q)	1.20 (d, J = 6.9)			
1′	108.89 (s)		108.90 (s)		108.79 (s)		
2′	157.09 (s)		157.14 (s)		157.22 (s)		
3′	108.89 (s)		108.90 (s)		108.79 (s)		
4′	162.23 (s)		162.23 (s)		162.56 (s)		
5′	109.57 (s)		109.57 (s)		109.56 (s)		
6′	159.92 (s)		159.95 (s)		159.95 (s)		
7′	205.45 (s)		205.46 (s)		205.56 (s)		
8′	33.82 (q)	2.72 (s)	33.85 (q)	2.72 (s)	33.75 (q)		2.71 (s)
Ar-CH_2-Ar	16.63 (t)	3.72 (s)	16.69 (t)	3.78 (s)	16.55 (t)		3.72 (s)
Ar-Me	8.78 (q)	2.12 (s)	8.78 (q)	2.12 (s)	8.72 (q)		2.11 (s)
OMe	61.83 (q)	3.98 (s)	61.83 (q)	3.98 (s)	61.81 (q)		3.98 (s)
OH		9.21, 9.73, 13.68, 16.14		9.22, 9.40, 13.69, 16.22			9.38, 13.63

spectra were similar to those of **1**, **2**, and **4**. The [1]H-nmr spectrum closely resembled that of **4** or **5** with a slight difference in the methyl protons at δ 1.28 (s) and 1.36 ppm (s), methylene protons at δ 2.38 (1H, dd, J = 9.3, 14.7 Hz) and 3.12 ppm (1H, brd, J = 14.7 Hz), and a methine proton at δ 3.60 ppm (1H, brd, J = 9.3 Hz). The ms of **3** also indicated a 3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl moiety. The acetylation of **3** with Ac_2O and pyridine afforded a hexaacetate that exhibited a molecular ion peak at *m/z* 730 $[M]^+$ in the mass spectrum. From these spectral data and biosynthetic considerations, the structure of **3** is proposed to be 3-(2,3-dihydroxy-isopentyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone, and it was named mallotojaponol [**3**]. The secondary alcohol on its side chain is considered to be racemic based on the specific rotation and biosynthetic considerations. The isolated compounds **1**–**3** and their acetates were tested for cytotoxic activity in the KB system (1). The isolated compounds **1**, **2** and **3**, which have a saturated side chain or chromanol ring, were inactive, similar to isomallotochromanol [**5**] (4); the hexaacetyl derivative of **3** was also inactive. However, the pentaacetyl derivatives of **1** and **2** were weakly cytotoxic with ED_{50} values of 2.8 and 4.5 μg/ml, respectively (Table 2).

TABLE 2. Cytotoxic Activities of Isolated Compounds and Their Derivatives Against KB Cell in vitro.

Compound	ED_{50}, μg/ml	Derivative	ED_{50}, μg/ml
1	13.5	**1**-Acetate	2.8
2	14.6	**2**-Acetate	4.5
3	22.0	**3**-Acetate	22.5
4	21.0	**4**-Acetate	8.6

We propose the naming and renaming of some reported compounds isolated from the pericarps of this plant. Our proposed names are mallotojaponin [**7**], butyrylmallotojaponin [**8**], isobutyrylmallotojaponin [**9**], and mallotolerin [**6**], and revised names are butyrylmallotolerin [**10**] and isobutyrylmallotolerin [**11**] to replace, respectively, the older names mallotolerin and isomallotolerin. These proposed and revised names are shown with their structures in Figure 1. Further cytotoxic constituents of this plant are now under investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were determined on a Yanagimoto micro mp apparatus and are recorded uncorrected. Uv and ir spectra were recorded on a Hitachi 220S double beam spectrophotometer and 260-10 ir spectrometer with polystyrene calibration at 1601 cm^{-1}, respectively. Specific rotations were determined on a JASCO DIP-140 digital polarimeter. [1]H-nmr and ^{13}C-nmr spectra were taken on a JEOL JNM-GX 270 spectrometer at 270 MHz and Varian XL-200 spectrometer at 50.3 MHz, respectively, with TMS as an internal standard. The chemical shifts are recorded in δ (ppm) values. Eims 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 instrument with an SPD-MIA spectrophotometric detector.

EXTRACTION AND SEPARATION.—The source, extraction, and separation of the dried pericarps of *M. japonicus* have been described previously (1–4). The 1% $MeOH/CHCl_3$ eluent (26 g) was rechromatographed on a Si gel column [solvent C_6H_6-EtOAc (4:1)] to give isomallotochromanol [**5**], the crude compound **3**, and a mixture of compounds of **1** and **2**. The crude compound **3** was purified by Si gel preparative layer chromatography (plc) [solvent C_6H_6-EtOAc-HOAc (15:5:2)] to give pure **3** (2 mg). The mixture of **1** and **2** was separated and purified by preparative hplc [column Cosmosil $5C_{18}$, 20 mm i.d. × 25 cm; solvent MeOH-0.005 M phosphate buffer-$CHCl_3$ (80:20:1); flow rate 5 ml/min; detection uv 282 nm] to give **1** (11 mg, Rt 70 min) and **2** (12 mg, Rt 66 min).

BUTYRYLMALLOTOCHROMANOL [**1**].—Yellow needles, mp 197–198° (hexane/EtOAc); $[\alpha]^{23}D$ ±0° (c = 0.5, $CHCl_3$); uv λ max (EtOH) (log ε) 282 (4.28), 335 nm (3.89); ir ν max (KBr) 3500, 3300, 2940, 1610, 1420, 1290, 1160, 1130 cm^{-1}; ^1H nmr see Table 1; eims m/z $[M]^+$ 488, 293, 280, 237, 221, 209, 196, 181; eims m/z 488.2066 ($C_{26}H_{32}O_9$ requires 488.2044); ^{13}C nmr see Table 1.

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 the pentaacetate as a colorless oil: ^1H nmr ($CDCl_3$) δ 0.94 (3H, t, J = 7.3 Hz, -CH_2-*Me*), 1.30 (3H, s, 2-Me), 1.31 (3H, s, 2-Me), 1.66 (2H, m, -CH_2-CH_2-Me), 2.06 (6H, s, 3-OAc and Ar-Me), 2.12 (3H, s, OAc), 2.16 (3H, s, OAc), 2.18 (3H, s, OAc), 2.28 (3H, s, OAc), 2.35 (3H, s, Ac), 2.48 (1H, m, Ha-4), 2.78 (3H, brt, J = 7.2 Hz, CO-CH_2-CH_2- and Hb-4), 3.62 (3H, s, OMe), 3.64 (2H, s, Ar-CH_2-Ar), 4.96 ppm (1H, t, J = 5.0 Hz, H-3); eims m/z $[M]^+$ 698.

ISOBUTYRYLMALLOTOCHROMANOL [**2**].—Yellow needles; mp 211–212° (MeOH); $[\alpha]^{23}D$ ±0° (c = 0.5, $CHCl_3$); uv λ max (EtOH) (log ε) 282 (4.24), 335 nm (3.81); ir ν max (KBr) 3500, 3250, 2940, 1620, 1605, 1425, 1290, 1150 cm^{-1}; ^1H nmr see Table 1; eims m/z $[M]^+$ 488, 445, 249, 237, 221, 209, 196, 181; eims m/z 488.2055 ($C_{26}H_{32}O_9$ requires 488.2044); ^{13}C nmr see Table 1.

ACETYLATION OF **2**.—Using acetylation as described for **1**, a pentaacetate was obtained as a colorless oil: ^1H nmr ($CDCl_3$) δ 1.12 (3H, d, J = 6.8 Hz, -CH-*Me*), 1.14 (3H, d, J = 6.8 Hz, -CH-*Me*), 1.29 (3H, s, Me), 1.30 (3H, s, Me), 2.05 (3H, s, Ar-Me)*, 2.06 (3H, s, OAc)*, 2.11 (3H, s OAc), 2.15 (3H, s, OAc), 2.17 (3H, s, OAc), 2.28 (3H, s, OAc), 2.35 (3H, s, Ac), 2.47 (1H, dd, J = 15.2, 4.9 Hz, Ha-4), 2.75 (1H, dd, J = 15.2, 4.9 Hz, Hb-4), 3.11 (1H, sept, J = 6.8 Hz, Me-*CH*-Me), 3.62 (5H, s, OMe and Ar-CH_2-Ar), 4.96 ppm (1H, t, J = 4.9 Hz, H-3) (assignments with an asterisk may be exchangeable); eims m/z $[M]^+$ 698.

MALLOTOJAPONOL [**3**].—Yellow needles; mp 150–151° (MeOH/$CHCl_3$); $[\alpha]^{23}D$ ±0° (c = 0.1, $CHCl_3$); uv λ max (EtOH) (log ε) 282 (4.00), 325 nm (3.59); ir ν max (KBr) 3470, 3290, 2940, 1610, 1430, 1365, 1315, 1130 cm^{-1}; ^1H nmr see Table 1; eims m/z $[M]^+$ 478, 404, 209, 196, 181; eims m/z 478.1787 ($C_{24}H_{30}O_{10}$ requires 478.1837).

ACETYLATION OF **3**.—Using acetylation as described for **1**, a hexaacetate was obtained as a colorless oil: ^1H nmr ($CDCl_3$) δ 1.18 (3H, s, Me), 1.20 (3H, s, Me), 1.84 (3H, s, OAc), 2.06 (3H, s, Ar-Me), 2.16 (3H, s, OAc), 2.17 (3H, s, OAc), 2.21 (3H, s, OAc), 2.27 (3H, s, OAc), 2.28 (3H, s, OAc), 2.37 (3H, s, Ac), 2.38 (3H, s, Ac), 2.68 (2H, m, H-1″), 3.53 (3H, s, OMe), 3.68 (2H, s, Ar-CH_2-Ar), 4.96 (1H, m, H-2″); eims m/z $[M]^+$ 730.

REDUCTIVE ALKALINE CLEAVAGE OF **1**, **2**, and **3**.—Reductive alkaline cleavage was carried out as described previously (1) to give 2,6-dihydroxy-3-methyl-4-methoxyacetophenone.

ANALYSIS OF L-MENTHOXYACETYL DERIVATIVES OF **1** AND **2**.—To a dry pyridine solution of 3 mg of compound **1**, **2** or **3**, 2 drops of L-menthoxyacetyl chloride were added at room temperature. After 12 h, H_2O was added, and the reaction mixture was evaporated in vacuo. The reaction product was dissolved in $CHCl_3$ and washed with H_2O. After being dried with Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by plc with hexane-EtOAc (10:3) as the developing solvent. The diastereomeric mixture was obtained as a colorless viscous oil. The L-menthoxyacetyl derivative of **1** showed two peaks, Rt 39 min and 40 min, which showed equivalent areas on hplc under the following conditions: column, Cosmosil $5C_{18}$ (4.6 mm i.d. × 150 mm); mobile phase, MeCN-H_2O (99:1); flow rate 1.0 ml/min; temperature 40°; detection uv 254 nm. The L-menthoxyacetyl derivative of **2** also showed two peaks, Rt 38 min and 39 min, which showed equal areas on hplc under the same conditions as described for **1**.

CYTOTOXICITY TEST.—The test employing KB cell line was carried out as described perviously (1).

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