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

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ABSTRACT.—Two new cytotoxic chromene derivatives, butyrylmallotochromene [**10**] and isobutyrylmallotochromene [**11**], were isolated from the pericarps of *Mallotus japonicus*. The new derivatives were identified as 5,7-dihydroxy-6-(2′,4′-dihydroxy-3′-acetyl-5′-methyl-6′-methoxybenzyl)-8-butyryl-2,2-dimethylchromene [**10**] and 5,7-dihydroxy-6-(2′,4′-dihydroxy-3′-acetyl-5′-methyl-6′-methoxybenzyl)-8-isobutyryl-2,2-dimethylchromene [**11**] by means of spectral data. These compounds and their respective acetylation and methylation products were found to be cytotoxic against KB cell lines.

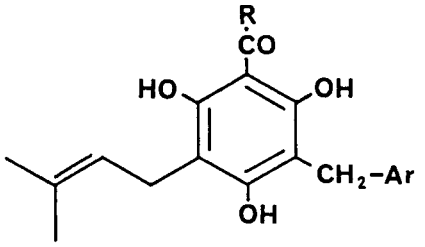
Previously, we reported on the cytotoxicity of several phloroglucinol derivatives **1**–**9** from the pericarps of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae) (1,2). In a continuing investigation of the $CHCl_3$-soluble fraction, which was found to show significant cytotoxic activity in the KB cell culture system, two new chromene derivatives named butyrylmallotochromene [**10**] and isobutyrylmallotochromene [**11**] were isolated. We wish to report the structure elucidation, including unambiguous assignments of the ^{13}C-signals of the chromene derivatives, and the cytotoxic activities of the isolated compounds and their derivatives.

The isolation of two new compounds, **10** and **11**, was performed by hplc after cc and preparative tlc separation.

Compound **10**, $C_{26}H_{30}O_8$, gave a positive $FeCl_3$ reaction. The uv spectrum was similar to that of mallotochromene [**7**] (1). The ^1H-nmr spectrum closely resembled that of **7**, except for the appearance of the signals of a propyl ketone group at δ 1.02 (3H, t, $J=7.4$ Hz), 1.73 (2H, apparent sext, $J=7.4$ Hz), and 3.05 ppm (2H, t, $J=7.4$ Hz) instead of the signal of a methyl ketone. The ^{13}C-nmr spectrum of **10** was also similar to that of **7**, except for the appearance of the carbon signals for a propyl group at δ 14.02 (q), 18.44 (t), and 46.05 ppm (t) instead of the signal of a methyl group at δ 32.78 ppm (q). The mass spectrum of **10** showed a molecular ion peak at *m/z* 470 and prominent peaks at *m/z* 275, 259, 247, 221, 219, 196, and 181, indicating a 2′,4′-dihydroxy-3′-acetyl-5′-methyl-6′-methoxybenzyl moiety (1–4). The signal of the methylene protons between the aromatic rings of **10** shifted upfield on acetylation, as did those of compounds **1**, **2**, **7**, and **8** (1,2). From these spectral data and biosynthetic considerations, the structure of **10** is proposed to be 5,7-dihydroxy-6-(2′,4′-dihydroxy-3′-acetyl-5′-methyl-6′-methoxybenzyl)-8-butyryl-2,2-dimethylchromene, and it was named butyrylmallotochromene [**10**].

Compound **11**, $C_{26}H_{30}O_8$, also gave a positive $FeCl_3$ reaction. The uv spectrum of **11** was similar to those of **7** and **10**. The ^1H- and ^{13}C-nmr spectra of **11** closely resembled those of **10**, except for the appearance of the signals of an isopropyl ketone group instead of the signals of a propyl ketone. The ms of **11** was similar to that of **10**, and the acetylation shift of the signal of the methylene proton between the aromatic rings of **11** was similar to those of **1**, **2**, **7**, **8** (1,2), and **10**. From these spectral data and biosyn-

[1]A part of this work was presented at The Japanese-United States Congress of Pharmaceutical Sciences, Honolulu, December 1987. For Part II in this series, see Arisawa *et al.* (2).



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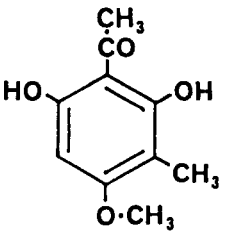
4 R=Propyl
5 R=Isopropyl



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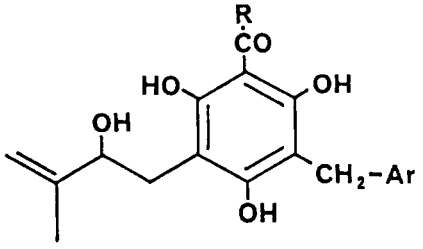

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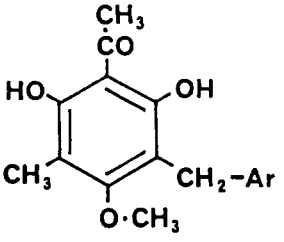

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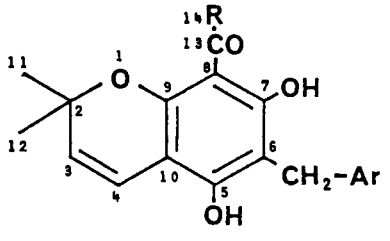
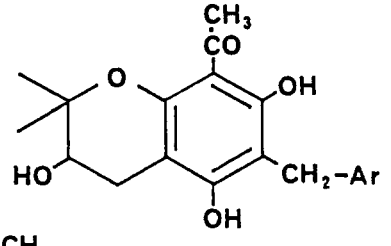


7 R=Me
10 R=Propyl
11 R=Isopropyl



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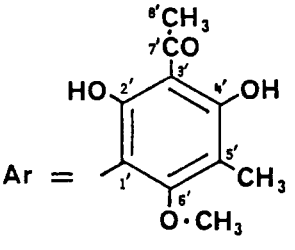

thetic considerations, the structure of **11** is proposed to be 5,7-dihydroxy-6-(2′,4′-dihydroxy-3′-acetyl-5′-methyl-6′-methoxybenzyl)-8-isobutyryl-2,2-dimethylchromene, and it was named isobutyrylmallotochromene [**11**]. In the previous reports (1–4) on the isolated compounds **1–9** the singlet carbon signals were unassigned in their ^{13}C-nmr spectra. In the present work, the unambiguous assignments of ^{13}C-nmr spectra of compounds **6**, **7**, **10**, and **11** (Table 1) were performed by means of 2D nmr of long range C-H shift correlation. Compounds **10** and **11** and their acetylated and methylated derivatives were tested for cytotoxic activity in the KB system previously reported (1,2), and the results are shown in Table 2. Compound **11** showed the best activity among the isolated compounds **1–11** from the pericarps of this plant. Both acetylated derivatives

TABLE 1. ^{13}C-nmr Assignments for Isolated Compounds **6**, **7**, **10**, and **11** (in $CDCl_3$, δ ppm).

Carbon No.	Compounds			
	6	**7**	**10**	**11**
2	—	78.22, s	78.23, s	78.25, s
3	—	125.16, d	125.16, d	125.05, d
4	8.84, q	116.00, d	116.85, d	116.84, d
5	159.67, s	157.89, s	157.83, s	157.85, s
6	108.24, s	105.29, s	105.63, s	105.65, s
7	156.94, s	160.91, s	161.34, s	161.80, s
8	108.24, s	105.03, s	104.77, s	104.02, s
9	162.72, s	156.07, s	155.83, s	155.46, s
10	109.86, s	103.38, s	103.31, s	103.26, s
11	—	27.95,q	27.95, q	27.87, q
12	—	27.95, q	27.95, q	27.87, q
13	205.41, s	204.04, s	206.83, s	211.19, s
14	33.81, q	32.78, q	46.05, t	39.13, d
15	—	—	18.44, t	19.35, q
16	—	—	14.02, q	19.35, q
1′	108.24, s	108.81, s	108.85, s	108.89, s
2′	156.94, s	157.12, s	157.24, s	157.27, s
3′	108.24, s	108.81, s	108.85, s	108.84, s
4′	162.72, s	162.55, s	162.66, s	162.64, s
5′	109.98, s	109.69, s	109.62, s	109.56, s
6′	159.67, s	159.96, s	159.96, s	159.96, s
7′	205.41, s	205.47, s	205.32, s	205.55, s
8′	33.81, q	33.79, q	33.79, q	33.79, q
Ar-CH_2-Ar	17.87, t	16.60, t	16.66, t	16.73, t
Ar-Me	8.84, q	8.74, q	8.78, q	8.79, q
OMe	62.02, q	61.81, q	61.83, q	61.83, q

showed less but similar levels of activity, and the methylated derivatives were considered inactive ($ED_{50} > 4$ μg/ml). Further cytotoxic constituents of this plant are now under investigation.

EXPERIMENTAL

GENERAL EXPERIMENTAL 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 220 S double-beam spectrophotometer and 260-10 infrared spectrometer with polysyrene calibration at 1601 cm^{-1}, respectively. ^{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 TMS as 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 Shimazu LC-6A liquid chromatograph instrument equipped with a SPD-M1A spectrophotometric detector.

TABLE 2. Cytotoxic Activities of Chromene Derivatives Against KB Cells in Culture.

Compound	ED_{50}, μg/ml
10	3.3
10-tetraacetate	2.55
di-*O*-methyl **10**	5.9
11	0.4
11-tetraacetate	2.65
di-*O*-methyl **11**	5.5

EXTRACTION AND SEPARATION.—The extraction and separation of the dried pericarps of *M. japonicus* have been described previously (1,2). The $CHCl_3$ eluent (69 g) of the Si gel column of the $CHCl_3$ extract (204 g) was chromatographed on a Si gel column by stepwise elution with C_6H_6, C_6H_6-$CHCl_3$ (1:1), and MeOH-$CHCl_3$ (1:1). The C_6H_6 eluent (47 g) was rechromatographed on a Si gel column by stepwise elution with *n*-hexane, EtOAc/*n*-hexane, and EtOAc. The 2.5% EtOAc/*n*-hexane eluent (10 g) was further separated on plc [plate: Kieselgel 60 F_{254}, 0.5 mm; solvent: *n*-hexane-EtOAc (10:1)] to afford crude crystals (60 mg) from the band of R_f 0.5 on tlc. The isolation of butyrylmallotochromene [**10**] and isobutyrylmallotochromene [**11**] from the crude crystals was performed on an hplc. Compounds **10** and **11** (20 mg each) were obtained by repeated hplc (Rt 14 and 13 min, respectively) under the following conditions: column, Cosmosil 5C 18 (4.6 mm i.d. × 150 mm); mobile phase, MeOH-0.02 M phosphate buffer (49:1); flow rate, 1.0 ml/min; temperature, 30°; detection, uv 282 nm.

CHARACTERIZATION OF BUTYRYLMALLOTOCHROMENE [**10**].—Yellow needles, mp 175–176° (MeOH); uv λ max (EtOH) (log ε) 225 (sh) (4.64), 280 (4.62), 335 nm (4.04); ir ν max (KBr) 3300, 2950, 1650, 1615, 1425, 1365, 1290, 1200, 1165, 1130, 1090 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.02 (3H, t, J = 7.4 Hz, Me), 1.47 (6H, s, Me × 2), 1.73 (2H, apparent sext, J = 7.4 Hz, -CH_2-CH_2-Me), 2.12 (3H, s, Ar-Me), 2.73 (3H, s, Ar-Ac), 3.05 (2H, t, J = 7.4 Hz, -CO-CH_2-CH_2), 3.72 (2H, s, Ar-CH_2-Ar), 3.98 (3H, s, OMe), 5.45 (1H, d, J = 10.0 Hz, 3-H), 6.61 (1H, d, J = 10.0 Hz, 4-H), 9.21 (1H, s, 5-OH, D_2O exchangeable), 9.30 (1H, s, 2′-OH, D_2O exchangeable), 13.62 (1H, s, 7-OH, D_2O exchangeable), 16.04 ppm (1H, s, 4′-OH, D_2O exchangeable); ms *m/z* $[M]^+$ 470, 275, 259, 247, 241, 233, 231, 221, 219, 209, 196, 181; mass measurement *m/z* 470.1910 ($C_{26}H_{30}O_8$ requires 470.1939); ^{13}C nmr see Table 1.

CHARACTERIZATION OF ISOBUTYRYLMALLOTOCHROMENE [**11**].—Yellow needles, mp 180–181° (MeOH); uv λ max (EtOH) (log ε) 225 (sh) (4.41), 281 (4.44), 335 nm (3.89); ir ν max (KBr) 3280, 2940, 1640, 1610, 1480, 1425, 1385, 1370, 1350, 1290, 1150, 1130, 1085, 1015, 995, 920 cm^{-1}; ^1H nmr ($CDCl_3$) δ 1.21 [6H, d, J = 6.7 Hz, -CH-(Me)$_2$], 1.47 (6H, s, Me × 2), 2.12 (3H, s, Ar-Me), 2.73 (3H, s, Ar-Ac), 3.72 (2H, s, Ar-CH_2-Ar), 3.87 [1H, q, J = 6.7 Hz, -CO-CH-(Me)$_2$], 3.98 (3H, s, OMe), 5.45 (1H, d, J = 10.0 Hz, 3-H), 6.62 (1H, d, J = 10.0 Hz, 4-H), 9.23 (1H, s, 5-OH, D_2O exchangeable), 9.33 (1H, s, 2′-OH, D_2O exchangeable), 13.65 (1H, s, 7-OH, D_2O exchangeable), 16.06 ppm (1H, s, 4′-OH, D_2O exchangeable); ms *m/z* $[M]^+$ 470, 275, 259, 247, 241, 231, 219, 209, 196, 181; mass measurement *m/z* 470.1977 ($C_{26}H_{30}O_8$ requires 470.1939); ^{13}C nmr see Table 1.

ACETYLATION OF **10**.—Compound **10** 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 a colorless oil. ^1H nmr ($CDCl_3$) δ 0.95 (3H, t, J = 7.3 Hz, -CH_2-Me), 1.44 (6H, s, Me × 2), 1.69 (2H, m, J = 7.3 Hz, -CH_2-CH_2-Me), 2.07 (3H, s, Ar-Me), 2.14 (3H, s, OAc), 2.18 (3H, s, OAc), 2.20 (3H, s, OAc), 2.28 (3H, s, OAc), 2.36 (3H, s, Ar-Ac), 2.81 (2H, t, J = 7.3 Hz, -CO-CH_2-CH_2-), 3.64 (5H, s, OMe and Ar-CH_2-Ar), 5.64 (1H, d, J = 10.0 Hz, 3-H), 6.10 ppm (1H, d, J = 10.0 Hz, 4-H); ms *m/z* $[M]^+$ 638, 596, 554, 539, 512, 497, 469, 451, 317, 304, 289, 275, 259, 247, 231, 209, 197, 181.

ACETYLATION OF **11**.—Using acetylation as described for **10**, a colorless oil was obtained. ^1H nmr ($CDCl_3$) δ 1.14 [6H, d, J = 6.8 Hz, -CH-(Me)$_2$], 1.43 (6H, s, Me × 2), 2.07 (3H, s, Ar-Me), 2.15 (3H, s, OAc), 2.16 (3H, s, OAc), 2.22 (3H, s, OAc), 2.29 (3H, s, OAc), 2.36 (3H, s, Ar-Ac), 3.18 [1H, m, J = 6.8 Hz, -CH-(Me)$_2$], 3.62 (2H, s, Ar-CH_2-Ar), 3.64 (3H, s, OMe), 5.63 (1H, d, J = 10.0 Hz, 3-H), 6.09 ppm (1H, d, J = 10.0 Hz, 4-H); ms *m/z* $[M]^+$ 638, 596, 554, 539, 512, 511, 497, 469, 451, 317, 303, 289, 275, 261, 259, 231, 209, 197, 181.

METHYLATION OF **10**.—To an MeOH solution of **10**, an Et_2O solution of CH_2N_2 was added. The reaction mixture was evaporated to afford a crude methylated product. The methylated product was purified on plc to afford the 5,2′-di-*O*-methyl derivative. ^1H nmr ($CDCl_3$) δ 1.00 (3H, t, J = 7.5 Hz, Me), 1.49 (6H, s, Me × 2), 1.72 (1H, apparent sext, J = 7.5 Hz, -CH_2-CH_2-Me), 2.10 (3H, s, Ar-Me), 2.72 (3H, s, Ar-Ac), 3.05 (2H, t, J = 7.5 Hz, Ar-CH_2-CH_2-), 3.52 (3H, s, OMe), 3.57 (3H, s, OMe), 3.69 (3H, s, OMe), 3.95 (2H, s, Ar-CH_2-Ar), 5.47 (1H, d, J = 9.8 Hz, 3-H), 6.43 ppm (1H, d, J = 9.8 Hz, 4-H); ms *m/z* $[M]^+$ 498, 483, 451, 289, 273, 245, 210, 195, 167, 149.

METHYLATION OF **11**.—Using methylation as described for **10**, the 5,2′-di-*O*-methyl derivative was obtained. ^1H nmr ($CDCl_3$) δ 1.19 [6H, d, J = 6.6 Hz, -CH-(Me)$_2$], 1.48 (6H, s, Me × 2), 2.10 (3H, s, Ar-Me), 2.72 (3H, s, Ar-Ac), 3.53 (3H, s, OMe), 3.56 (3H, s, OMe), 3.69 (3H, s, OMe), 3.87 [1H, q, J = 6.6 Hz, -CH-(Me)$_2$], 3.96 (2H, s, Ar-CH_2-Ar), 5.47 (1H, d, J = 9.8 Hz, 3-H), 6.44 ppm (1H, d, J = 9.8 Hz, 4-H); ms *m/z* $[M]^+$ 498, 483, 451, 289, 273, 245, 210, 195, 149.

CYTOTOXICITY TEST.—The bioassay employing KB cell lines was performed as described previously (1,2). Results are shown in Table 2.

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