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

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ABSTRACT.—Two new phloroglucinol derivatives, mallotolerin (**8**) and mallotochromanol (**9**), 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)-phlorbutyrophenone (**8**) and 8-acetyl-5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)2,2-dimethyl-3-hydroxychroman (**9**) by their respective chemical and spectral data. These compounds were found to be cytotoxic against KB and L-5178Y cell lines in culture and to be moderately effective in inhibiting the growth of Ehrlich carcinoma in mice. Cytotoxic activities of the isolated phloroglucinol derivatives (**1-9**) from the pericarps are also discussed.

The pericarps of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae) have previously afforded several phloroglucinol derivatives, 3-(3,3-dimethylallyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone (**1**) (1,3); 3-(3-methyl-2-hydroxybut-3-enyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone (**2**) (1); 2, 6-dihydroxy-3-methyl-4-methoxyacetophenone (**3**) (2,3); 3-(3,3-dimethylallyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phlorbutyrophenone (**4**) and -phlorisobutyrophenone (**5**) (2); mallotophenone (**6**) (3); and mallotochromene (**7**) (3). Besides, the cytotoxic activities of these isolated compounds against KB and L-5178Y cell lines in culture were reported (3).

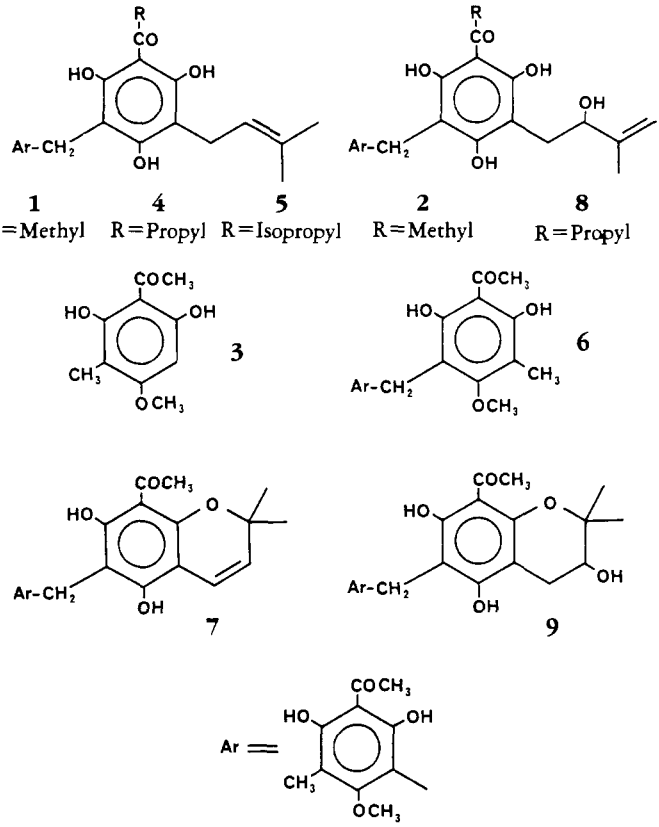
In a continuing search for cytotoxic constituents in the $CHCl_3$ soluble fraction of the pericarps of *M. japonicus*, two compounds named mallotolerin (**8**) and mallotochromanol (**9**) were newly isolated. We wish to report the structural elucidation and the cytotoxic activities of these new rottlerin-like compounds, together with the effect of compound **1** on solid and ascites tumors of Ehrlich carcinoma in mice.

RESULTS

Separation of the extract of *M. japonicus* by column chromatography on silica gel (3) yielded three compounds, a known compound (**2**) and two new compounds, **8** and **9**.

Compound **2** was identified as the known compound, 3-(3-methyl-2-hydroxybut-3-enyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone, from its uv, ir, nmr, and ms spectral data, and this was confirmed by comparison with an authenic sample (1). The allylic alcohol on its side chain is considered to be racemic based on the absence of a Cotton effect in the cd of its benzoate (4,5).

[1]This work was presented at the 105th Annual Meeting of Pharmaceutical Society of Japan, Kanazawa, April, 1985. For Part I, see Arisawa *et al.* (3).



Compound **8**, $C_{26}H_{32}O_9$, gave a positive $FeCl_3$ reaction. The uv spectrum was similiar to that of **2**. The ^{1}H-nmr spectrum closely resembled that of **2**, except for the appearance of the signals of a propyl ketone group instead of the signal of a methyl ketone. The ms of **8** showed a molecular ion peak at *m/z* 488 and prominent peaks at *m/z* 470, 445, 417, 221, 209, 196, and 181, indicating a 3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl moiety (1-3). Reductive alkaline cleavage of **8** afforded 2,6-dihydroxy-3-methyl-4-methoxyacetophenone (3). From these chemical and spectral data and from biosynthetic considerations, the structure of **8** is proposed to be 3-(3-methyl-2-hydroxybut-3-enyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phlorbutyrophenone, and it was named mallotolerin (**8**). The allylic alcohol on its side chain was considered to be racemic for the same reason as compound **2**.

Compound **9**, $C_{24}H_{28}O_9$, also gave a positive $FeCl_3$ reaction. The uv spectrum was similar to those of **2** and **8**. The ^{1}H-nmr spectrum closely resembled that of **7** (3), except for the appearance of the signals of the 2,2-dimethyl-3-hydroxychroman ring instead of the signals of the 2,2-dimethylchromene ring. The ms of **9**, which showed a molecular ion peak at *m/z* 460 and prominent peaks at *m/z* 265, 252, 196, and 181, also indicated a 3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl moiety. The reductive cleavage of **9** also afforded **3**. The signal of the methylene protons between the rings of **9** was shifted upfield on acetylation, as was those of compounds **1**, **2**, and **7** (3). From these chemical and spectral data and from biosynthetic considerations, the structure of **9** is proposed to be 8-acetyl-5,7-dihydroxy-6-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)2,2-dimethyl-3-hydroxychroman, and it was named mallotochromanol (**9**). The hydroxy group on the chromen ring is considered to be pseudoaxial from the coupling constant (J=5.19 Hz) of the methine proton, but a Cotton effect was not detected in a cd study on its benzoate; therefore, compound **9** is racemic (6).

TABLE 1. Cytotoxic Activity of the Phloroglucinol Derivatives from *Mallotus japonicus* (in vitro)

Substances	ED_{50} (μg/ml)	
	KB	L-5178Y
1[a]	0.58	0.74
1-Acetate[a]	1.50	3.20
2	1.22	2.45
2-Acetate	1.11	0.80
3[a]	>100	>100
3-Acetate[a]	>100	>100
4+**5**	0.26	1.07
6[a]	2.40	6.10
6-Acetate[a]	4.80	5.20
7[a]	2.10	1.25
7-Acetate[a]	2.90	1.04
8	0.95	0.82
8-Acetate	3.50	10.05
9	>100	>100
9-Acetate	8.60	4.50

[a]See Arisawa *et al.* (3).

The isolated compounds, **2**, **8**, and **9**, and their acetate derivatives were tested for cytotoxic activities in KB and L-5178Y cell lines in culture, and the results are shown in Table 1, which also includes the potencies of **1** and **3-7** as reported previously. Most of these phloroglucinol derivatives showed cytotoxic activity, while the monomeric derivative **3** and compound **9** were almost inactive. Compounds **1** and **2** having a side chain at the 3-position were found to be more active than **6**, and **8** having a propyl ketone at position-1 showed more activity than **2**. Compound **1** showed good cytotoxicity against two tumor cell lines, with ED_{50} of 0.58 μg/ml for KB cells and 0.74 μg/ml for L-5178Y cells, respectively. Meanwhile, the effects of compound **1** on solid and ascites tumors of Ehrlich carcinoma in mice were examined. As shown in Tables 2 and 3, the results indicated that the response to compound **1** was moderately potent in inhibiting solid tumor growth, but was observed to a lesser degree in ascites tumor. Further antitumor experiments are now in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Uv spectra were recorded on a Hitachi 220 S double beam spectrophotometer, and ir spectra were obtained on a Hitachi 260-10 ir spectrometer with poly-

TABLE 2. Effect of Compound **1** on Solid Tumor of Ehrlich Carcinoma in Mice

Dose (mg/kg/day)	Mortality	Tumor weight (g) Mean±S.E.	T/C (%)
20	0/6	0.59±0.11	49.5
7	0/6	0.93±0.19	78.1
Control (0.25% CMC)	0/8	1.19±0.18	—

Mouse: Male 5-week-old ddY mice; 6 mice/group (8 mice for control group).
Inoculum: Ehrlich carcinoma cells 3×10^6/head, sc (inguinal region).
Treatment: Qd, day 3-8; ip. The compound to be tested was suspended in 0.25% carboxymethylcellulose (CMC) solution.
Determination: Day 10.

TABLE 3. Effect of Compound **1** on Ehrlich Ascites Carcinoma in Mice

Dose (mg/kg/day)	MST[a] (day)	Survival day
20	14	11, 12, 14, 14, 34, 36
7	20	12, 15, 19, 21, 24, 29
Control (0.25% CMC)	19	11, 13, 17, 18, 18, 20, 24, 26, 26, 26

[a]Median survival time.
Mouse: Male 6-week-old ddY mice; 6 mice/group (10 mice for control group).
Inoculum: Ehrlich carcinoma cells 1×10^6/head, ip.
Treatment: Qd, day 2-8; ip.

styrene caribration at 1601 cm^{-1}. Specific rotations were determined on a JASCO DIP-140 digital polarimeter, and cd curves were obtained on a JASCO J-500 C spectropolarimeter. ^{1}H- 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. Mass spectra were obtained on a JEOL JMS-D-200 mass spectrometer operating at 70 eV.

EXTRACTION AND SEPARATION.—The extraction and separation of the dried pericarps of *M. japonicus* have been described previously (3). The crude compounds of **2, 8,** and **9** from 15% EtOAc/hexane elutions of the rechromatography on a silica gel column (3) were purified on plc to afford 18 mg, 5 mg, and 40 mg as yellow needles, respectively.

IDENTIFICATION OF **2**.—Yellow needles, mp 208-210° (MeOH) [lit. (1) mp 197-199°], $[\alpha]^{23}D$ −0.53 (c=1.0, $CHCl_3$) (may be experimental error). Identification was established by comparison (uv, ir, nmr, ms, and mixed mp) with an authentic sample of 3-(3-methyl-2-hydroxybut-3-enyl)-5-(3-acetyl-2,4-dihydroxy-5-methyl-6-methoxybenzyl)-phloracetophenone (1). The hexaacetate, colorless oil, was also identified by direct comparison (^{1}H nmr and ms) with an authentic sample.

CHARACTERIZATION OF MALLOTOLERIN (**8**).—Yellow needles, mp 197-198° (MeOH); $[\alpha]^{23}D$ −0.30 (c=1.0, $CHCl_3$) (may be experimental error); uv λ max (EtOH) (log ε) 230 (4.20), 287 (4.12), and 320 nm (4.00); ir ν max (KBr) 3430, 3260, 1620, 1610, 1420, 1370, 1300, 1285, 1180, 1160, 1130 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.99 (3H, t, J=7.4 Hz, Me), 1.72 (2H, m, -CH_2-), 1.86 (3H, s, 3′-Me), 2.12 (3H, s, 5″-Me), 2.70 (1H, m, 1′-Ha), 2.73 (3H, s, Ac), 3.12 (2H, t, J=7.4 Hz, -CO-CH_2-), 3.14 (1H, m, 1′-Hb), 3.74 (2H, s, Ar.-CH_2-Ar.), 3.98 (3H, s, OMe), 4.32 (1H, d, J=8.4 Hz, 2′-H), 4.90 (1H, brs, 4′-Ha), and 5.03 ppm (1H, brs, 4′-Hb); ms *m/z* 488 (M^+), 470, 417, 262, 219, 209, 196, and 181; mass measurement *m/z* 488.2066 ($C_{26}H_{32}O_9$ requires 488.2044); ^{13}C nmr ($CDCl_3$) δ 8.80 (q, Me), 14.03 (q, acetyl Me), 17.05 (t, Ar.-CH_2-Ar. and acyl-CH_2-), 18.36 (q, vinylic Me), 29.21 (t, Ar.-CH_2-), 33.77 (q, acetyl Me), 46.01 (t, carbonyl -CH_2-), 61.85 (q, OMe), 78.27 (d, -O-CRH-) 105.00, 105.52, 106.10, 108,84, 109.10, 109.24 (each s, aromatic C), 110.68 (d, =CH-), 157.41, 160.10, and 162.55 ppm (each s, aromatic C).

ACETYLATION OF **8**.—Compound **8** was treated overnight with Ac_2O and pyridine at room temperature, and the reaction mixture was worked up as usual to give a hexaacetate as a colorless oil: ^{1}H nmr ($CDCl_3$) δ 0.93 (3H, t, J=7.4 Hz, Me), 1.62 (2H, m, -CH_2- overlapping with H_2O), 1.71 (3H, s, Me), 1.94 (3H, s, OAc), 2.07 (3H, s, aromatic Me), 2.15 (6H, s, OAc), 2.23 (3H, s, OAc), 2.28 (6H, s, OAc), 2.37 (3H, s, Ac), 2.57 (1H, m, 1′-Ha), 2.66 (2H, t, J=7.4 Hz, CO-CH_2-), 2.82 (1H, m, 1′-Hb), 3.57 (3H, s, OMe), 3.68 (2H, s, Ar.-CH_2-Ar.), 4.82 (2H, brs. =CH_2), and 5.25 ppm (1H, dd, J=8.6, 6.8 Hz, -CRH-O-).

CHARACTERIZATION OF MALLOTOCHROMANOL (**9**).—Yellow needles, mp 216-218° (MeOH); $[\alpha]^{23}D$ −0.74 (c=1.0, $CHCl_3$) (may be experimental error); uv λ max (log ε) 288 (4.08) and 323 nm (3.97); ir ν max (KBr) 3555, 3450, 3250, 1600, 1425, 1370, 1290, 1135, 1105, 1080, and 1025 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.35 (3H, s, 2-Me), 1.40 (3H, s, 2-Me), 2.12 (3H, s, Ar.-Me), 2.62 (1H, dd, J=17.2, 5.2 Hz, 4-Ha), 2.67 (3H, s, Ac), 2.72 (3H, s, Ac), 2.86 (1H, dd, J=17.2, 5.2 Hz, 4-Hb), 3.72 (2H, s, Ar.-CH_2-Ar.), 3.79 (1H, t, J=5.2 Hz, 3-H), and 3.98 ppm (3H, s, OMe); ms *m/z* 460 (M^+), 265, 247, 209, 196, and 181; mass measurement *m/z* 460.1751 ($C_{24}H_{28}O_9$ requires 460.1732); ^{13}C nmr ($CDCl_3$) δ 8.27 (q, Me), 16.55 (t, Ar.-CH_2-Ar.), 22.02 (q, Me), 24.84 (q, Me), 26.09 (t, 4-C), 32.89 (q, acetyl Me), 61.81 (q, OMe), 68.41 (d, 3-C), 78.49 (s, 2-C), 94.13, 99.47, 105.20, 108.79, 109.56, 155.08, 159.95, 160.72, 160.91, 162.56 (each s, aromatic C-O), 203.79, and 205.56 ppm (each s, CO).

ACETYLATION OF **9**.—Using acetylation as described for **8**, a colorless oil was obtained. ^{1}H nmr ($CDCl_3$) δ 1.33 (3H, s, 2-Me), 1.34 (3H, s, 2-Me), 2.07 (6H, s, 3-OAc and aromatic Me), 2.15 (3H, s, OAc), 2.18 (3H, s, OAc), 2.19 (3H, s, OAc), 2.29 (3H, s, OAc), 2.36 (3H, s, Ac), 2.49 (3H, s, Ac), 2.79 (1H, dd, J=17.0, 5.0 Hz, 4-H), 3.62 (3H, s, Me), 3.67 (1H, s, Ar.-CH_2-Ar.), and 4.98 ppm (1H, t, J=5.0 Hz, 3-H); ms *m/z* 670 (M^+).

PREPARATION OF BENZOATES OF COMPOUNDS **2, 8,** AND **9**.—To a pyridine solution of a sample, 2 drops of benzoyl chloride were added with ice cooling. The reaction mixture was evaporated in vacuo to obtain crude benzoate, which was purified on a silica gel column and examined by cd.

REDUCTIVE ALKALINE CLEAVAGE.—Reductive alkaline cleavage was carried out as described previously (3).

CYTOTOXICITY TEST.—The test methods employing KB and L-5178Y cell lines were carried out as described previously (3).

IN VITRO ANTITUMOR TEST.—Male ddY mice, which were implanted intraperitoneally or subcutaneously with Ehrlich carcinoma cells, were treated with a test compound in the stated schedule as presented in the footnotes of Tables 2 and 3.

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