Anti-allergic Agents from Natural Sources (4[1]): Anti-allergic Activity of New Phloroglucinol Derivatives from *Mallotus philippensis* (Euphorbiaceae)

Akihiro DAIKONYA,[a] Shigeki KATSUKI,[b] Jin-Bin WU,[c] and Susumu KITANAKA[a]

[a] *College of Pharmacy, Nihon University; 7–7–1 Narashinodai, Funabashi, Chiba 274-8555, Japan:* [b] *Tanegashima Experimental Station for Medicinal Plants, National Institute of Health Sciences; 17007–1 Noma-Matsubarayama, Nakatate-cho, Kumage-gun, Kagoshima 891–3604, Japan: and* [c] *Institute of Pharmaceutical Chemistry, China Medicinal University; Taichung 404, Taiwan.* Received July 10, 2002; accepted August 21, 2002

Two new phloroglucinol derivatives, mallotophilippen A (1) and B (2) were isolated from the fruits of *Mallotus philippensis*. These compounds were identified, using chemical and spectral data, as 1-[5,7-dihydroxy-2,2-dimethyl-6-(2,4,6-trihydroxy-3-isobutyryl-5-methyl-benzyl)-2*H*-chromen-8-yl]-2-methyl-butan-1-one and 1-[6-(3-Acetyl-2,4,6-trihydroxy-5-methyl-benzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]-2-methyl-butan-1-one, respectively. They inhibited nitric oxide (NO) production and inducible NO synthase (iNOS) gene expression by a murine macrophage-like cell line (RAW 264.7), which was activated by lipopolysaccharide (LPS) and recombinant mouse interferon-γ (IFN-γ). Furthermore, they inhibited histamine release from rat peritoneal mast cells induced by Compound 48/80. These results suggest that the novel phloroglucinol derivatives have anti-inflammatory effects.

Key words *Mallotus philippensis*; Euphorbiaceae; macrophage; nitric oxide

Mallotus philippensis (LAM.) MUELL. ARG. is a deciduous tree widely distributed throughout tropical Asia, Australia and the Philippines. Kamara, a red powder consisting of glandular hairs from the capsules of the plant, has long been used as an anthelminticum[2,3] and an orange dye for silk.[4] Previous studies have isolated cardenolides[5—7] and kamalins[8—10] from this genus, and cytotoxic,[11—13] anti-tumor,[14] and human immunodeficiency virus (HIV) reverse transcriptase inhibitory activities[15] have also been described.

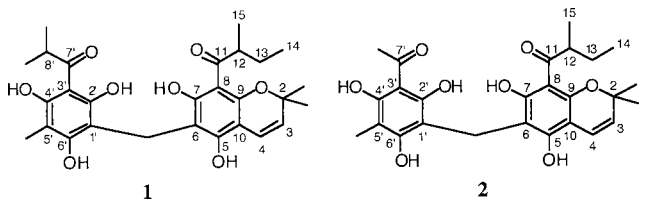
Macrophages play major roles in host defense, immunity and inflammatory responses, where once activated they result in cytokine, oxygen and nitrogen species, and eicosanoid production. In macrophages, the best characterized stimuli to induce the transcription of genes encoding pro-inflammatory proteins is bacterial lipopolysaccharide (LPS) alone or in combination with recombinant mouse interferon-γ (IFN-γ). Such stimulation results in cytokine release and the synthesis of enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). The nitric oxide (NO) radical is known to play a central role in inflammatory and immune reactions. However, the excessive production of NO may lead to tissue damage. In inflammatory disease such as rheumatoid arthritis, excessive NO production by activated macrophages has been observed.

Mast cells and basophils play a central role in immediate allergic reactions mediated by immunoglobulin E (IgE). Binding of multivalent allergens to specific IgE, located bound to the high-affinity IgE receptor on mast cells or basophils, leads to the release of inflammatory mediators such as histamine, serotonin and arachidonic metabolites. These mediators ultimately cause various allergy symptoms including dermatitis and asthma.

We recently discovered that the acetone extract of the *M. philippensis* fruit inhibited activated macrophage NO production. The acetone fraction was partitioned with hexane, ethyl acetate, *n*-butanol, and water-soluble fraction, successively. Both the hexane and the ethyl acetate-soluble fractions had stronger inhibition than quercetin and the degree of inhibition are similar. It was demonstrated that the hexane-soluble fraction involved major constituents of this plant with the thin-layer chromatographic technique. Therefore, we have focused our research efforts on elucidating the chemical constituents of the hexane-soluble fraction, attempting to identify the anti-allergic agents. Here we describe the isolation and structural identification of two new phloroglucinol derivatives, as well as their anti-allergic activities (Fig. 1).

Results and Discussion

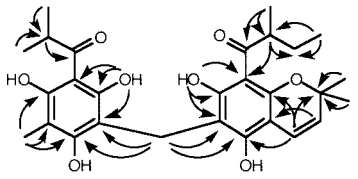
Compound **1** was obtained as a yellow powder and its molecular formula was determined to be $C_{28}H_{34}O_8$ (*m*/*z* 498.2251 [M^+]) by high resolution electron impact (HR-EI)-MS. The UV spectrum showed maxima at 293, 205 nm. The ^{1}H- and ^{13}C-NMR spectra were similar to mallotochromene.[16] The presence of two *cis*-coupled olefinic protons, together with two methyl group resonances and a quaternary carbon at δ 78.0 in the ^{13}C-NMR spectrum, indicated a 2,2-dimethylpyran system. The arrangement of the substituents and the placement of the pyran at 9/10 positions was established from the results of the heteronuclear multiple-bond connectivity (HMBC) experiments. HMBC correlation presented in Fig. 2. The presence of geminal methyl protons at δ 1.47 (3H, s, Me-2), coupling with *cis*-coupled olefinic protons at δ 5.44 (1H, d, J=9.9 Hz, H-3) and δ 6.65 (1H, d, J=9.9 Hz, H-4), chelated hydroxyl proton at δ 16.11 (1H, s, OH-7) and δ 16.20 (1H, s, OH-2′), with one aromatic methyl proton at δ



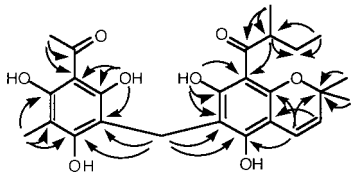
1 **2**

Fig. 1. Phloroglucinol Derivatives from *M. philippensis*

* To whom correspondence should be addressed. e-mail: daikonya@pha.nihon-u.ac.jp



1



2

Fig. 2. HMBC Correlation of Compounds **1** and **2**

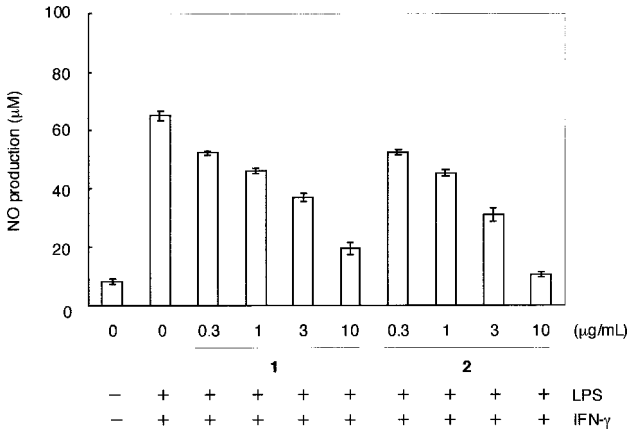


Fig. 3. Inhibitory Effects of Compounds **1** and **2** on NO Production Stimulated by LPS and IFN-γ

2.09 (3H, s, Me-5′) were identified. In addition, compound **1** exhibited extra signals indicating a 2-methyl-1-one-butyryl side chain. In the ^{1}H-NMR spectra these signals appeared at two methyl protons at δ 0.92 (3H, t, J=6.7 Hz, Me-14) and δ 1.17 (3H, d, J=6.7 Hz, Me-15), one methylene proton at δ 1.17 (2H, m, H-13), and one methine proton at δ 3.78 (1H, overlapped, H-12). A 2-methyl-1-one-propyl side chain appeared at two methyl protons, at δ 1.19 (3H, d, J=6.7 Hz, Me-8′) and one methine proton at δ 3.94 (1H, m, H-8′). The combination of the ^{13}C-NMR and lack of distortions, enhanced by polarization transfer (DEPT) spectra of compound **1**, revealed the presence of seven methyls, two methylenes, two methines, two olefine methines, one quaternary carbon, twelve quaternary aromatic carbons, and two carbonyl carbons. From these spectral data, the structure of compound **1** was determined to be 1-[5,7-dihydroxy-2,2-dimethyl-6-(2,4,6-trihydroxy-3-isobutyryl-5-methyl-benzyl)-2*H*-chromen-8-yl]-2-methyl-butan-1-one and named mallotophilippen A (**1**).

Compound **2** was also obtained as a yellow powder and its molecular formula of was determined as $C_{26}H_{30}O_8$ by (*m/z* 470.1939 [M$^+$]) by HR-EI-MS spectral analysis. The UV spectrum showed maxima at 290, 206 nm. The structure of compound **2** was very similar to the main skeleton of compound **1**. The ^{1}H-NMR indicated the presence of geminal methyl protons at δ 1.47 (3H, s, Me-2), coupling with *cis*-configured olefinic protons at δ 5.44 (1H, d, J=9.9 Hz, H-3) and δ 6.65 (1H, d, J=9.9 Hz, H-4), having a chelated hydroxyl proton at δ 16.11 (1H, s, OH-7) and δ 16.20 (1H, s, OH-2′), and one aromatic methyl proton at δ 2.09 (3H, s, Me-5′). Compound **2** showed signals for a 2-methyl-1-one-butyryl side chain. In the ^{1}H-NMR spectrum these signals appeared at two methyl protons δ 0.92 (3H, t, J=6.7 Hz, H-14) and δ 1.17 (3H, d, J=6.7 Hz, H-15), one methylene proton at δ 1.17 (2H, m, H-13), and one methine proton at δ 3.78 (1H, overlapped, H-12). Acethyl methyl signals appeared at δ 2.70 (3H, s, Me-7′). The ^{13}C-NMR and DEPT spectra of compound **2** revealed the presence of six methyls, two methylenes, two methines, two olefine methines, one quaternary carbon, twelve quaternary aromatic carbons, and two carbonyl carbons. Long-range correlations were observed in the HMBC spectrum. HMBC correlation presented in Fig. 2. Therefore, the structure of compound **2** was determined to be 1-[6-(3-acetyl-2,4,6-trihydroxy-5-methyl-benzyl)-5,7-dihydroxy-2,2-dimethyl-2*H*-chromen-8-yl]-2-methyl-butan-1-one and named mallotophilippen B (**2**).

We found that the acetone extract from the fruits of *M. philippensis* inhibited activated macrophage NO production and the major inhibitory activity was due to the phloroglucinol derivatives. These findings are supported by similar results obtained in RAW 264.7, mouse macrophage-like cells.[17)]

Compounds **1** and **2** had stronger inhibition than quercetin on NO production (**1**: IC_{50}=4.2 μM; **2**: IC_{50}=3.2 μM) (Fig. 3). Quercetin exhibited a similar effect (IC_{50}=26.8 μM). Quercetin is reported to have an inhibitory effect on the production of NO by LPS stimulated macrophage cell RAW 264.7.[18,19)] Furthermore, two new phloroglucinol derivatives inhibited iNOS mRNA expression in a dose-dependent fashion (Fig. 4). The cytotoxic effects of these compounds were measured using the MTT assay. Their compounds (0.3—10 μg/ml) did not demonstrated any significant cytotoxic effects with LPS/IFN-γ treatment for 24 h. NO is synthesized by a family of enzymes termed NOS, which utilize arginine as a substrate in the generation of NO. Of the three NOS isoforms, the isoform expressed in the macrophage is termed iNOS. Its activity is regulated at the transcription level by cytokines as well as through cell exposure to immune and inflammatory stimuli. The RT-PCR analysis in the present study indicated that LPS/IFN-γ treatment increased the level of iNOS mRNA expression, and that the phloroglucinol derivatives inhibited this increase. Therefore, inhibition of iNOS induction by the phloroglucinol derivatives may be mediated through the suppression of these transcription activating factors, thereby inhibiting iNOS transcription.

Apart from maintaining normal physiological function, NO is required to combat infectious agents and tumors. However, production of excessive amounts of NO in response to endotoxin can have adverse effects on host survival ranging from direct cellular cytotoxity to cellular component damage, which then leads to mutagenesis. Therefore, the expression of pathological NO titer, if inhibited by any pharmacological agent, will be of great medical interest. Once the mechanism of action is elucidated, phloroglucinol derivatives may be principle molecules used in the discovery of a new generation of drugs for controlling various acute and chronic inflammatory diseases.

We have also shown that the two phloroglucinol derivatives

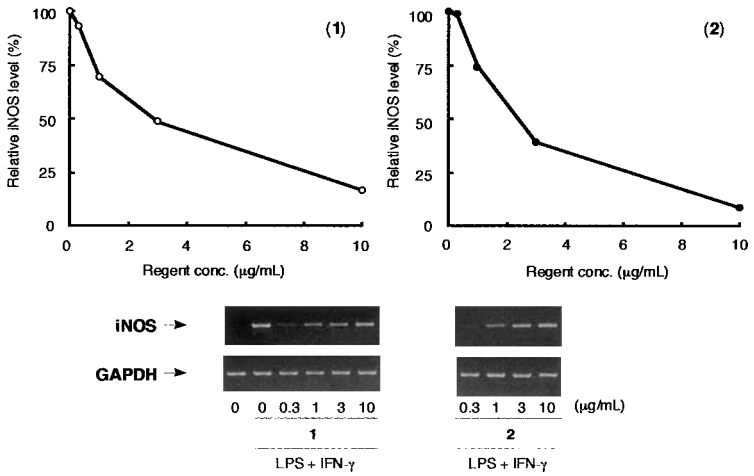


Fig. 4. Inhibitory Effects of Compounds **1** and **2** on iNOS Gene Expression Stimulated by LPS and IFN-γ

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for Compounds **1** and **2** (δ values in $CDCl_3$)

Position	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
2		78.1 s		78.1 s
3	5.44 (1H, d, J=9.9 Hz)	125.0 d	5.44 (1H, d, J=9.9 Hz)	125.0 d
4	6.65 (1H, d, J=9.9 Hz)	117.2 d	6.65 (1H, d, J=9.9 Hz)	117.2 d
5		158.2 s		158.2 s
6		106.3 s		106.1 s
7		162.2 s		161.8 s
8		104.5 s		104.5 s
9		155.3 s		155.3 s
10		103.4 s		104.5 s
11		210.9 s		210.9 s
12	3.78 (1H, m)	45.8 d	3.78 (1H, m)	45.8 d
13	1.17 (2H, m)	26.7 t	1.17 (2H, m)	26.7 t
14	0.92 (3H, t, J=6.7 Hz)	12.0 q	0.92 (3H, t, J=6.7 Hz)	12.0 q
15	1.17 (3H, d, J=6.7 Hz)	16.7 q	1.17 (3H, d, J=6.7 Hz)	16.7 q
1′		106.3 s		106.2 s
2′		162.2 s		162.1 s
3′		104.5 s		104.3 s
4′		155.3 s		155.6 s
5′		101.9 s		101.9 s
6′		160.2 s		159.6 s
7′		211.2 s		204.1 s
8′	3.94 (1H, m)	39.2 d		
2-Me	1.47 (3H, s)	27.8 q	1.47 (3H, s)	27.8 q
5′-Me	2.09 (3H, s)	7.5 q	2.09 (3H, s)	7.5 q
7′-Me			2.70 (3H, s)	32.6 q
8′-Me	1.19 (3H, d, J=6.7 Hz)	19.3 q		
Ar-CH_2-Ar	3.78 (2H, s)	15.9 t	3.78 (2H, s)	15.8 t

The multiplicities of carbon signals were determined using the DEPT method, and are indicated as s, d, t and q.

inhibit Compound 48/80-induced histamine release from peritoneal mast cells in rats (**1**: IC_{50}=8.6 μM; **2**: IC_{50}=13.8 μM). Indomethacin also inhibited histamine release (IC_{50}=250 μM). Compound 48/80 has been used on numerous occasions as a selective histamine release agent from mast cells in rats and mice. Mast cells have been shown to generate intercellular reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, hydroxy radical and nitric oxide, following incubation with several pharmacological agents, such as Compound 48/80, the calcium-ionophore A23187. ROS have also been shown to enhance histamine release from mast cells. Phloroglucinol derivatives may inhibit histamine release through their inhibitory effect on NO production.

In the present study, we demonstrated the anti-inflammatory effects of novel phloroglucinol derivatives. These compounds could serve as prominent molecules in the discovery of a new generation of drugs for controlling various acute and chronic inflammatory diseases.

Experimental

General Procedures UV spectra were obtained by a Shimadzu UV-160 spectrophotometer. The NMR spectra were taken on a JOEL JNM GX-400 instrument (400 MHz for ^{1}H-NMR), and chemical shifts were given in ppm relative to internal tetramethylsilane (TMS). Mass spectra were obtained on a Hitachi M-80B spectrometer. Column chromatography was carried out

using silica gel (Wako gel C-300, Wako Pure Chemical Ind., Ltd.). TLC was performed on Merck TLC plates (0.25 mm thickness) and compounds visualized by 5% (v/v) H_2SO_4 in ethanol solution.

Extraction and Isolation The air-dried capsules of plants (500 g) were extracted with acetone. The solvent was evaporated under reduced pressure from the combined extract producing the acetone extract (53.4 g: NO production inhibition IC_{50}=6.9 μg/ml). The extract was suspended in water. And the extract was partitioned with hexane, ethyl acetate and *n*-butanol, successively. Evaporation of the solvent yielded a hexane fraction (48.2 g: IC_{50}=6.7 μg/ml), ethyl acetate fraction (4.0 g: IC_{50}=6.8 μg/ml), *n*-butanol fraction (764 mg: IC_{50}=10.7 μg/ml), and the aqueous fraction (438 mg: IC_{50}>30 μg/ml). The hexane fraction was subjected to Sephadex LH-20 column chromatography with $CHCl_3$: MeOH=1 : 1 to give fractions A1 (749 mg), A2 (10.4 g), A3 (29.1 g), and A4 (216 mg). Fraction A3 was chromatographed on silica gel using hexane–$CHCl_3$ (0→100) to give fractions B1 (2.7 g), B2 (8.4 g), B3 (10.6 g), B4 (829 mg), B5 (1.3 g), and B6 (6.6 g). Fractions B1 and B2 were purified by reverse phase HPLC (Fluofix ODS) using aqueous MeOH to give compounds **1** (31.4 mg) and **2** (68.7 mg), respectively.

Mallotophilippen A (**1**): A yellow powder, $[\alpha]_D^{23}$ ±0° (*c*=0.1, MeOH), IR_{max}^{KBr} cm^{-1}: 3276, 2969, 2932, 2873, 1609, 1465, 1426, 1382, 1364, 1278, 1132. UV λ_{max}^{MeOH} nm (log ε): 293 (4.42), 205 (4.38). HR-EI-MS *m*/*z*: 498.2251 (Calcd for $C_{28}H_{34}O_8$: 498.2254). EI-MS *m*/*z*: 498 [M^+], 484, 289, 276, 261, 231, 179, 167. ^{1}H- and ^{13}C-NMR presented in Table 1.

Mallotophilippen B (**2**): A yellow powder, $[\alpha]_D^{23}$ ±0° (*c*=0.1, MeOH), IR_{max}^{KBr} cm^{-1}: 3282, 2969, 2932, 2873, 1610, 1465, 1428, 1366, 1321, 1279, 1234. UV λ_{max}^{MeOH} nm (log ε): 290 (4.47), 209 (4.45). HR-EI-MS *m*/*z*: 470.1939 (Calcd for $C_{26}H_{30}O_8$: 470.1940). EI-MS *m*/*z*: 470 [M^+], 289, 276, 261, 231, 195, 182, 179, 167. ^{1}H- and ^{13}C-NMR presented in Table 1.

Nitrite Assay[20] The cells were seeded at 1.2×10^6 cells/ml onto 96-well flat bottom plate (Sumitomo Bakelite, #8096R, Tokyo) and then incubated at 37 °C for 2 h. Next, the test compound was added to the culture simultaneously with both *Escherichia coli* LPS (100 ng/ml) and recombinant mouse IFN-γ (0.33 ng/ml). Then cells were incubated at 37 °C for approximately 16 h and subsequently chilled on ice. One hundred microliters of the culture supernatant was placed in duplicate in the wells of 96-well flat-bottomed plates. A standard solution of $NaNO_2$ was placed in alternate wells on the same plate. To quantify nitrite, 50 μl of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-1-naphthyletylenediamide dihydrochloride) was added to each well. After 10 min the reaction products were colorimetrically quantified at 550 nm using a Model 3550 Microplate Reader (BIO-RAD) and the background absorbance (630 nm) was subtracted. Cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method.

Assay of Inhibitory Activity on Histamine Release[21] All the isolated compounds were assayed using a modified HPLC-fluorometry method. Male Wister rats (Japan SLC, Shizuoka) weighing 180—200 g were exsanguinated and injected intraperitoneally with 10 ml of Tyroad solution. The abdominal region was gently massaged for 3 min and then the peritoneal exudates were collected. The peritoneal cavity fluid containing mast cells was suspended in phosphate-buffered saline (PBS), then layered on bovine serum albumin (*d*=1.068) in a test tube at room temperature for 20 min. After centrifugation at 300×***g*** at 4 °C for 10 min, the layer containing mast cells was pipetted out. The cells were washed three times with 3 ml PBS (pH 7.0) and suspended in the same medium. Cell viability was determined using trypan blue (10 μl) at 37 °C for 10 min, followed by the addition of histamine releasers (Compound 48/80, 5 μg/ml). The mixture was incubated again for 10 min; the quantity of histamine released was expressed in peak height and percent inhibition was then calculated.

Reverse Transcriptase-Polymerase Chain Reaction Analysis of iNOS mRNA The cells were cultured at 1.2×10^6 cells/ml onto 96-well flat bottom plate at 37 °C for 2 h. Then the test compound was added to the culture simultaneously with both LPS (100 ng/ml) and IFN-γ (0.33 ng/ml). The cells were incubated at 37 °C for approximately 8 h. Total RNA was isolated from the cell pellet using a RNA isolation kit (QIAGEN, Hilden, Germany). Total RNA (250 ng) was reverse-transcribed into cDNA by oligo $(dT)_{12—18}$ primer. The PCR samples contained 30 μl of the reaction mixture, comprised of 50 mM KCl, 5 mM $MgCl_2$, 0.2 mM dNTP, 0.6 units of Ampli Taq GOLD (Applied Biosystems, CA, U.S.A.), and 0.4 μmol of sense and antisense primers. The sense primer for iNOS was 5′-ACCTACTTCCTGGACATTACGACCC-3′ and the antisense primer was 5′-AAGGGAGCAATGCCCGTACCAGGCC-3′. The sense primer for glyceraldehydes-3-phosphatedehydrogenase (GAPDH) was 5′-ACCACAGTCCATGCCATCAC-3′, and the antisense primer was 5′-TCCACCACCCTGTTGCTGTA-3′. The PCR reaction was performed under the following conditions: 25 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1.5 min, using a thermal cycler (GeneAmp PCR Systems 9700; PE Applied Biosystems, U.S.A.). The PCR products were run on a 2% agarose gel and visualized by ethidium bromide staining. The bands in the gel were then photographed.

Acknowledgements This work was supported by grants to Nihon University from the Promotion and Mutual Aid Corporation for Private Schools of Japan; a Grant from the Ministry of Education, Culture, Sports, Science, and Technology to promote multi-disciplinary research projects; and a Health Sciences Research Grant for Research on Eye and Ear Science, Immunology, Allergy and Organ Transplantation from the Ministry of Health, Labour and Welfare, Japan.

References

1) Part 3: Wang N., Yao X., Ishii R., Kitanaka S., *Chem. Pharm. Bull.*, **49**, 938—942 (2001).
2) Srivastava M. C., Singh S. W., Tewari J. P., *Ind. J. Med. Res.*, **55**, 746—748 (1967).
3) Gupta S. S., Verma P., Hishikar K., *Indian J. Physiol. Pharmacol.*, **19**, 103—110 (1984).
4) Lounasmaa M., Widen C. J., Tuuf C. M., Huhtikangas A., *Planta Med.*, **28**, 16—31 (1975).
5) Roberts K. D., Weiss Ek., Reichstein T., *Helv. Chim. Acta*, **46**, 2886—2892 (1963).
6) Roberts K. D., Weiss Ek., Reichstein T., *Helv. Chim. Acta*, **49**, 316—329 (1966).
7) Roberts K. D., Weiss Ek., Reichstein T., *Helv. Chim. Acta*, **50**, 1645—1664 (1967).
8) McGookin A., Robertson A., Tittensor E., *J. Chem. Soc.*, **1939**, 1579—1587 (1939).
9) Narang K. S., Ray J. N., Ray B. S., *J. Chem. Soc.*, **1937**, 1862—1865 (1937).
10) Cardillo G., Merlini L., Mondell R., Moreshini L., *Gazz. Chim. Ital.*, **95**, 725—734 (1965).
11) Arisawa M., Fujita A., Suzuki R., Hayashi T., Morita N., Kawano N., Koshimura S., *J. Nat. Prod.*, **49**, 298—302 (1986).
12) Fujita A., Hayashi T., Arisawa M., Shimizu M., Morita N., Kikuchi T., Tezuka Y., *J. Nat. Prod.*, **51**, 708—712 (1988).
13) Arisawa M., Fujita A., Hayashi T., Morita N., Kikuchi T., Tezuka Y., *Chem. Pharm. Bull.*, **38**, 698—700 (1990).
14) Arisawa M., Fujita A., Morita N., Koshimura S., *Planta Med.*, **56**, 377—379 (1990).
15) Nakane H., Arisawa M., Fujita A., Koshimura S., Ono K., *FEBS Lett.*, **286**, 83—85 (1991).
16) Arisawa M., Fujita A., Suzuki R., Hayashi T., Morita N., Kawano N., Koshimura S., *J. Nat. Prod.*, **48**, 455—459 (1985).
17) Ishii R., Horie M., Saito K., Arisawa M., Kitanaka S., *Biochim. Biophys. Acta*, **1568**, 74—82 (2001).
18) Rao Manjeet K., Ghosh B., *Int. J. Immunopharmacol.*, **21**, 435—443 (1999).
19) Kim H. K., Cheon B. S., Kim Y. H., Kim S. Y., Kim H. P., *Biochem. Pharmacol.*, **58**, 759—765 (1999).
20) Ishii R., Saito K., Horie M., Shibano T., Kitanaka S., Amano F., *Biol. Pharm. Bull.*, **22**, 647—653 (1999).
21) Hirai Y., Takase H., Kobayashi H., Yasuhara T., Nakajima T., *Shoyakugaku Zasshi*, **37**, 374—380 (1983).