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THE ANTIALLERGIC ACTIVITIES OF SYNTHETIC ACROPHYLLINE AND ACROPHYLLIDINE

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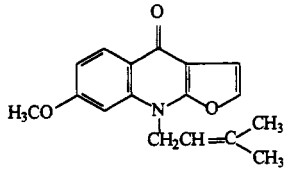
ABSTRACT.—The antiallergic activities of synthetic acrophylline [**1**] and acrophyllidine [**2**] have been demonstrated. Both compounds **1** and **2** at 30 μmol/kg reduced the plasma leakage in mouse ear in a passive cutaneous anaphylactic (PCA) reaction. In addition, compound **1** suppressed mast cell degranulation in a dose-dependent manner, while compound **2** at 100 μM produced no significant inhibition of the release of preformed inflammatory mediators. These results suggest that the antiallergic effect of compound **1** probably occurs through the suppression of mast cell degranulation, and that of compound **2** by protection of the vasculature against challenge by mediators of inflammation.

In the course of a project directed toward the synthesis and determination of the biological activities of furo[2,3-b]quinolin-4-one alkaloids, work on the total synthesis of glycarpine (1), taifine [**3**] (2), isomaculosidine (3), isotaifine (4), and acrophylline [**1**] (5) has been published, along with reports of the biological activity of taifine (6) and isodictamine (6). The present report describes the synthesis of acrophyllidine [**2**] and compares the antiallergic activity of compounds **1** and **2** on the permeability change of cutaneous vasculature elicited by the passive cutaneous anaphylactic reaction (PCA). The effect of both compounds on the release reaction of mast cells was also examined.

The synthetic pathway to compound **2** is shown in Scheme 1. When compound **1** was oxymercurated with mercuric acetate in THF/H_2O, followed by treatment with $NaBH_4$, oxymercuration followed by demercuration gave the target compound **2**.

The ms (M^+ 300) and elemental analysis of compound **2** suggested a molecular formula of $C_{17}H_{19}NO_4$. The uv spectrum showed a λ max (MeOH) at 259 nm. Its ir spectrum had a carbonyl absorption band at 1640 cm^{-1}. The ^1H-nmr spectral data of the *N*-(3-hydroxy-3-methylbutyl) group of compound **2** were almost superimposable on those reported for natural acrophyllidine (7) (Table 1). The ^{13}C-nmr spectrum of compound **2** further substantiated the structure assignment. Interpretation of the ^{13}C-nmr spectrum was conducted by comparison with data reported for compounds **1** (5) and **3** (2), and by 2D nmr techniques, including ^1H-^1H-COSY,



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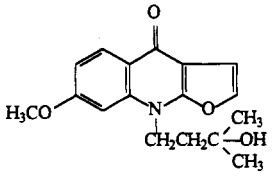

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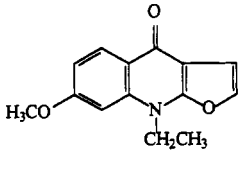

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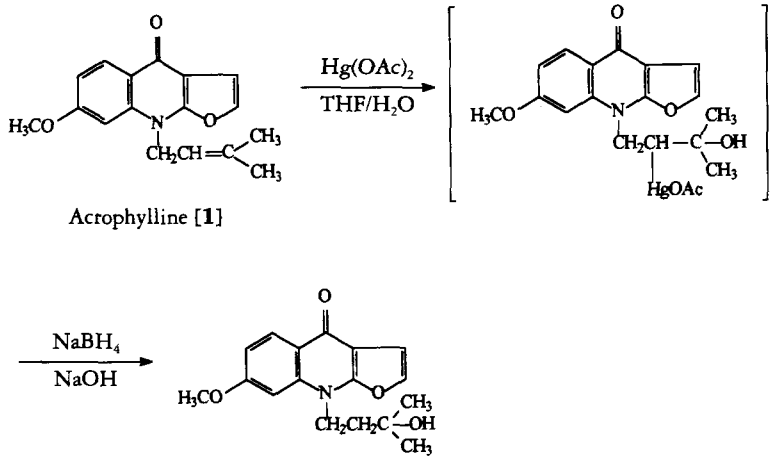

Acrophylline [**1**]

Acrophyllidine [**2**]

SCHEME 1

HMQC, HMBC, and NOESY experiments.

In the PCA reaction studies, intravenous injection of DNP-albumin in mice pretreated with anti-DNP caused ear edema. All three test compounds (diphenhydramine, compounds **1** and **2**) were intraperitoneally injected at 30 μmol/kg 30 min prior to the DNP-albumin challenge, and significantly reduced the volume of plasma exudation in the mouse ear (Figure 1). The reduction of edema formation in mice pretreated with compound **1** was greater than that in mice pretreated with compound **2**. Because mast cells, which release various inflammatory mediators during cell activation and thereby increase vascular permeability (8) and participate as prominent inflammatory cells in this animal test (9), the effect of the test compounds on the release reaction of mast cell was examined thereafter.

TABLE 1. Selected ^{1}H-Nmr (300 MHz) Chemical Shifts of Compound **2** and Natural Acrophyllidine [**2**] in $CDCl_3$.[a]

	Compound	
	Natural **2**	**2**
H_2-1′	4.58 (t, J=8)	4.50 (d, J=8.4)
H-2′	2.03 (t, J=8)	1.97 (t, J=8.4)
CH_3-4′,-4″	1.40 s	1.37 s

[a]Chemical shifts (relative to TMS) are in ppm and coupling constants in Hz.

Mepacrine, a phospholipase inhibitor, has been reported to inhibit mast cell degranulation (10). Like mepacrine, compound **1** at 10 and 30 μM significantly reduced the amount of β-glucuronidase released from compound 48/80-challenged mast cells (Figure 2). However, increase of concentrations of compound **2** up to 100 μM had no effect on mast cell degranulation. The changes of histamine content in the supernatant of the reaction mixture is not presented here, because both compounds **1** and **2** interfered with the fluorometric assay of histamine.

Drugs which inhibit the generation and release of mediators from the inflammatory cells and/or protect the vasculature against mediator challenge are presumed to suppress the edematous response in the PCA reaction (11–13). Diphenhydramine, a histamine antagonist, prevented the interaction of histamine with its receptor on the vasculature and thereby reduced the vascular permeability caused by histamine.

In this study, the antiallergic and mast cell stabilizing effects were greatly

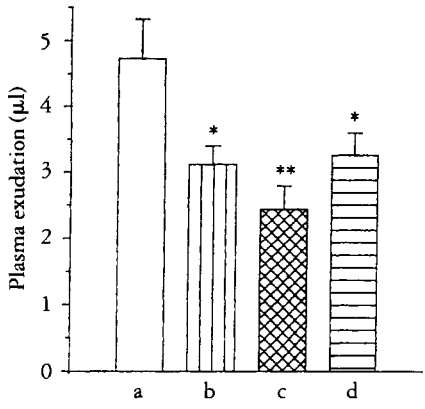

FIGURE 1. Inhibitory effect of diphenhydramine and compounds **1** and **2** on mouse ear edema in the PCA reaction. Mice were injected intraperitoneally with (a) DMSO as control, or (b) diphenhydramine, (c) compound **1**, or (d) compound **2** (30 μmol/kg), 30 min before intravenous injection of DNP-albumin. Forty-five min later, the volume of plasma exuded in the ear was determined. In the control group, the average plasma exudation was 4.7±0.5 μl. Values are presented as means±S.E.M. of 5–6 animals. * and ** denote significant differences from the control at $p<0.05$ and $p<0.01$ (ANOVA followed by the Newman-Keuls test), respectively.

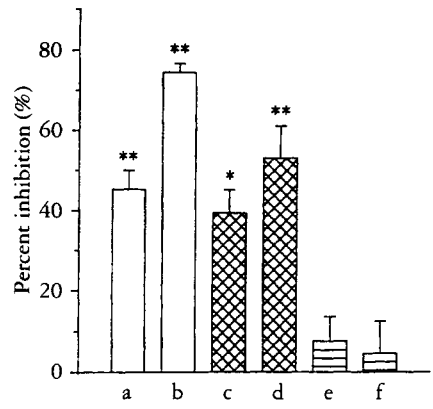

FIGURE 2. Inhibitory effect of mepacrine and compounds **1** and **2** on rat peritoneal mast cell degranulation. Cell suspension was preincubated with DMSO as control; mepacrine (a) 30 μM or (b) 100μM; compound **1** (c) 10 μM or (d) 30 μM; compound **2** (e) 30 μM or (f) 100 μM, 3 min prior to the addition of compound 48/80 (10 μg/ml) to start the release reaction. Fifteen min later, β-glucuronidase in the supernatant was detected. Mean β-glucuronidase release in the control group was 34.1±0.6% of the total content. Spontaneous release was less than 10%. Values are presented as means±S.E.M. of 4–5 tests. * and ** denote significant differences from the control at $p<0.05$ and $p<0.01$ (ANOVA followed by the Newman-Keuls test), respectively.

increased as the *N*-(3-hydroxy-3-methylbutyl) group of **2** was changed to a *N*-isoprenyl group. The action mechanism by which compound **1** reduced plasma extravasation in the PCA reaction more likely includes suppression of the release of chemical mediators from mast cells. However, the antiallergic effect of compound **2** probably occurs largely through the protection of the vasculature against chemical mediator-induced plasma extravasation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps are uncorrected. The ir spectra were recorded on a Shimadzu ir 440 spectrometer in KBr. The nmr spectra were taken at 90 MHz on a JEOL FX-90Q spectrometer and Varian VXR-300 FT-nmr spectrometer, with TMS as internal standard in $CDCl_3$ or DMSO-d_6. The mass spectra were measured with a Hewlett-Packard 5995 GC-MS instrument and a JMS-D-300 spectrometer. The uv spectra were recorded on a Hewlett-Packard Diode Array uv-vis spectrometer (HP-8452A). Elemental analyses were performed by Chung Shan Institute of Science and Technology, Taiwan, Republic of China, and National Cheng-Kung University, Tainan, Taiwan, Republic of China.

ASSAY MATERIALS.—Compound **1** was synthesized as previously described (5), whereas compound **2** was synthesized as described in the present study. Dinitrophenyl (DNP)-albumin, monoclonal anti-DNP (clone SPE-7; mouse IgE), Evans blue, diphenhydramine, compound 48/80, sodium pentobarbital, dimethylsulfoxide (DMSO), Triton X-100 and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO).

SYNTHESIS OF ACROPHYLLIDINE [**2**].—Mercuric acetate (3.19 g, 10.0 mmol) was dissolved in H_2O (10 ml) and THF (20 ml) was added. Then,

acrophylline (**1**, 2.38 g, 10.0 mmol) was added and the reaction mixture was stirred for 30 min at 25±2° to complete the oxymercuration stage. Next, 10 ml of 3.0 M NaOH was added, followed by 10 ml of a solution of 0.5 M $NaBH_4$ in 3.0 M NaOH. Stirring was continued for a further 10 min, and the mercury formed was allowed to settle. NaCl was added to saturate the aqueous layer. The upper layer of THF was separated and dried over $MgSO_4$. The solvent was evaporated off and the residue was purified by cc on Si gel. Elution with $CHCl_3$-EtOH (97:3) yielded compound **2** (2.2 g, 73%): mp 179–181°; ir (KBr) ν max 1628 (C=O), 3300–3600 (OH) cm^{-1}; uv (MeOH) λ max (ϵ) 259 nm (5.67×10^4); eims (70 eV) *m/z* $[M]^+$ 301; *anal.*, calcd for $C_{17}H_{19}NO_4$, C, 67.76, H, 6.36, N, 4.64; found C, 67.90, H, 6.42, N, 4.37; ^1H nmr ($CDCl_3$, 300 MHz) δ 1.37 (6H, s, CH_3-4′,-4″), 1.97 (1H, t, J=8.4 Hz, H-2′), 3.87 (3H, s, OCH_3-7), 4.50 (2H, d, J=8.4 Hz, H-1′), 6.91 (1H, dd, J=2 and 2.9 Hz, H-6), 7.02 (1H, d, J=2.1 Hz, H-3), 7.02 (1H, d, J=2 Hz, H-8), 8.41 (1H, d, J=9 Hz, H-5); ^{13}C nmr ($CDCl_3$, 75.4 MHz) δ 29.62 (C-4′, C-4″), 40.48 (C-1′), 40.69 (C-2′), 55.55 (-OCH_3), 97.64 (C-8), 105.90 (C-3a), 107.75 (C-3), 110.65 (C-6), 119.61 (C-4a), 129.24 (C-5), 137.54 (C-2), 139.05 (C-8a), 155.87 (C-9a), 173.00 (C-4).

PASSIVE CUTANEOUS ANAPHYLACTIC (PCA) REACTION.—The PCA reaction was measured as previously described (11). Briefly, monoclonal anti-DNP (0.05 μg) or sterile saline was injected into the right and left ears, respectively, of sodium pentobarbital-anesthetized mice (ICR, 20–25 g). After 48 h, 0.5% Evans blue with 1.5% sodium pentobarbital in saline (4 ml/kg) was administered intravenously, followed 5 min later by the DNP-albumin (10 mg/kg iv) challenge. Animals were killed 45 min after the induction of edema. Exuded blue dye of the 9-mm-diameter tissue sample punched out from both ears of each mouse was extracted (14). The volume of plasma exudation was calculated by interpolation on a O.D.-plasma volume standard curve (12).

MAST CELL DEGRANULATION.—Rats of the Sprague Dawley strain (250–300 g) were used. Peritoneal mast cells were isolated (15,16). Cells were washed and suspended in Tyrode's solution with BSA 0.1% at $1–1.5\times10^6$ cells/ml. This cell suspension was preincubated at 37° with DMSO or test compound for 3 min, then compound 48/80 (10 μg/ml) was added to trigger the release reaction. The released β-glucuronidase in the supernatant was determined (17). The total content of β-glucuronidase in the mast cells was measured after treatment of the cell suspension with Triton X-100. The percent release was determined.

ACKNOWLEDGMENTS

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