Antipruritic Effects of 1,4-Naphthoquinones and Related Compounds

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The antipruritic effects of orally administered 1,4-naphthoquinone derivatives and related compounds on compound 48/80-induced scratching behavior in mice were studied. 2-Hydroxy-3-(2-hydroxyethyl)-1,4-naphthoquinone, ferulic acid, 2,2’-methylenebis(3-hydroxy-1,4-naphthoquinone), and 2,2’-ethylidenebis(3-hydroxy-1,4-naphthoquinone) (impatienol) all exhibited significant antipruritic activity. However, 2-methoxy-3-(2-hydroxyethyl)-1,4-naphthoquinone (balsaquinone), which was isolated from a natural source for the first time, did not show any activity. The present results indicate that these compounds are promising for treating allergic diseases with chronic and severe pruritus.

Key words antipruritic activity; 1,4-naphthoquinone derivative; Impatiens balsamina; balsaquinone; scratching; phenolic compound

The number of patients with allergic disease has recently been increasing. To treat such diseases, we have isolated natural compounds with antianaphylactic,1,2) antihistaminic,3) and anti-platelet-activating factor (PAF)4) activity using in vivo assay systems that we have developed. However, in cases of allergic disease with chronic and severe pruritus, such as atopic dermatitis, not only treatment of the allergy but also inhibition of scratching at the lesion is very important. We therefore searched for natural compounds with antipruritic activity using our in vivo assay systems.5) We discovered that a 2-hydroxy-1,4-naphthoquinone (lawsone) (1) isolated from Impatiens balsamina L., could significantly inhibit scratching behavior5) induced in mice by histamine-releasing agents, such as Dextran T40 and compound 48/80 (COM). We were also able to show that 1 can be effective as an antipruritic agent against atopic dermatitis, because it inhibited6) chronic and serious scratching behavior in atopic dermatitis model NC mice with dermatitis.

Here we report the antipruritic effects of natural and synthesized 1,4-naphthoquinones and related compounds for new antipruritic medicines that can be effective for the treatment of allergic disease.

MATERIALS AND METHODS

General Experimental Procedures Melting points were determined with a Yanagimoto micro melting point apparatus. IR spectra were recorded on a Shimadzu 435 spectrometer and UV absorption spectra with a Hitachi 323 spectrometer. 1H-NMR and 13C-NMR spectra were recorded with JEOL JNM-GSX 400 spectrometers (using tetramethylsilane (TMS) as an internal reference). Electron-impact (EI)-MS spectra (70 eV) were obtained with a JEOL JMS-DX 303 spectrometer.

Materials COM was obtained from Sigma. 2,3-Dihydroxy-1,4-naphthoquinone (2), 2-hydroxy-3-methoxy-1,4-naphthoquinone (3), and 2-hydroxy-3-(2-hydroxyethyl)-1,4-naphthoquinone (4) were synthesized by known methods.5,6) Compound 1, p-hydroxybenzoic acid (6), p-coumaric acid (7), ferulic acid (8), scopoletin (9), 2,2’-methylenebis(3-hydroxy-1,4-naphthoquinone) (10), and 2,2’-ethylidenebis(3-hydroxy-1,4-naphthoquinone) (impatienol) (11) were isolated from I. balsamina using methods described previously.1,9,10) Balsaquinone (5) was isolated from the pericarp of I. balsamina as described in the following section.

Isolation and Identification of Compound 5 Fresh pericarp (75 g) of I. balsamina was extracted with 50% ethanol 400 ml over 3 d at room temperature and filtered. The extract (2.9 g) was subjected to silica gel chromatography using a CHCl3-MeOH gradient system followed by TLC to give compound 5 (6 mg). The UV spectrum showed characteristic absorption maxima of 1,4-naphthoquinone. The IR spectrum suggested the presence of a hydroxyl group and an α,β-unsaturated function. The EI-MS spectrum exhibited a [M]+ at m/z 232. The 1H- and 13C-NMR spectral data were similar to those of 2-methoxy-1,4-naphthoquinone except for the presence of the hydroxyethyl group and absence of 3-H, suggesting that 5 is 2-methoxy-1,4-naphthoquinone with a hydroxyethyl group substituting for H-3. The spectral data (UV, IR, MS, 1H-, and 13C-NMR) of 5 were identical with those of the synthetic5) 5. Therefore 5 is 2-methoxy-3-(2-hydroxyethyl)-1,4-naphthoquinone [CA index name: 3-methoxy-2-(2-hydroxyethyl)-1,4-naphthalendione]. This is the first report of its isolation from a natural source, although it has been synthesized previously.5) We called this compound balsaquinone (5). All signal assignments of 1H- and 13C-NMR spectra of 5 were confirmed by H-H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectra.

Compound 5: mp: 107—108°C. El-MS m/z: 232 [M]+ (100), 217 (18.1), 202 (48.7), 187 (17.8), 171 (26.0), 115 (28.9), 105 (15.4). IR (KBr): 3360—3150, 1670, 1650, 1610, 1590, 1215, 1040, 720 cm−1. UV (MeOH) λmax (log ε): 204 (4.23), 252 (4.23), 278 (4.17), 334 (3.55) nm. 1H-NMR (CDCl3) δ: 1.85 (t, J=6.2 Hz, OCH2), 2.92 (2H, t, J=6.2 Hz, CH2CH2OH), 3.81 (2H, q, J=6.2 Hz, CH3CH2OH), 4.17 (3H, s, OCH3), 7.68—7.73 (2H, m, 6, 7-H), 8.03—8.09 (2H, m, 5, 8-H). 13C-NMR (CDCl3) δ: 7.47 (t, CH3CH2OH), 61.4 (q, CH2OH), 61.8 (t, CH3CH2OH), 126.2 (d, 5C), 126.3 (d, 4aC), 133.4 (d, 3-H), 133.9 (d, 6C), 158.6 (s, 2C), 181.3 (s, 1C), 186.1 (s, 4C).

Animals Male ddY mice (SPF grade), 7 weeks old, were obtained from Japan SLCl (Shizuoka, Japan) and housed at

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Assay for Antipruritic Activity against COM-Induced Scratching Behavior The antipruritic activity was measured by a method examining the incidence of scratching.\(^5\) The histamine-releasing agent COM (3 mg/kg) was injected subcutaneously into the base of the neck on the back of mice to provoke scratching behavior. Compound 1 was administered at 10 mg/kg p.o. 24, 18, 12, 6, 3, or 1 h before injection with COM in a previous study,\(^7,6\) and it was found that 24 h premeditation was the most effective. Thus compounds 1—11 were orally administered at a dose of 10 mg/kg 24 h before challenge with COM. As a control, untreated mice were injected with COM alone. The incidence of scratching behavior on the whole body was recorded for 20 min.

Statistical Analysis Each value represents the mean±S.E. The data were evaluated by Student’s t-test (\(n=7\) per group).

RESULTS AND DISCUSSION

Antipruritic activity has been reported\(^5,7\) for 1 and 2-methoxy-1,4-naphthoquinone isolated from the petals of \(I.\) \(balsamina.\) We thought it worth investigating the same biological effect for 1,4-naphthoquinones and related compounds from \(I.\) \(balsamina\) and synthetic 1,4-naphthoquinone derivatives. Figure 1 shows the antipruritic activity of compounds 1—11. Compound 1 was used as a positive control because previous studies had shown it to have a significant antipruritic effect.\(^7,6\)

Compounds 4, 10, and 11 significantly inhibited COM-induced scratching behavior. Compound 4 showed antipruritic activity, but 5, which had a methylated 2-OH group of 4, did not show any activity. Compound 3, in which the 3-(2-hydroxyethyl) group of 4 was replaced with a methoxy group, showed weaker inhibitory effects than 4. The pharmacologic activities of 4 have not previously been reported. To our knowledge, this is also the first report of the antipruritic activity of 10 and 11 among bisnaphthoquinone derivatives.

Previous investigations have shown the antiprotease\(^15\) and antitumor promoting\(^2\) activity of 10, and the anti-HIV (weak)\(^13\) and 5α-reductase inhibitory\(^9\) activity of 11. The active mechanisms of 4, 10, and 11 are currently under investigation.

Ferulic acid (8) significantly inhibited COM-induced scratching behavior in mice, but p-coumaric acid (7), did not have a methoxy group, did not show any activity. \(p\)-Hydroxybenzoic acid (6) and scopoletin (9) did not showed significant inhibitory effects. However, as the inhibitory effects of 7 and 9 on histamine release from mast cells have been previously reported in an in vitro assay, in the future experiments should be conducted using other routes, time schedules, and doses. In natural product pharmacy therapeutic studies, in vivo assays usually yield more information for actual application. The antipruritic effect of 8 is reported here for the first time, although the inhibitory effects of 8 have previously been reported in several inflammatory\(^1\) and I—IV-type allergic reactions.\(^1\) One mechanism of the antipruritic activity of 8 would inhibit histamine release from mast cells.\(^1\) In addition, 8 would inhibit the synthesis of both metabolic substances from arachidonic acid\(^18,19\) and related substances in the cytokine network.\(^20,21\) These substances are related to the induction and transmission of the itching sensation.\(^22\)

The results of our experiments suggest the possibility that 4, 8, 10, and 11 would be useful in the treatment of allergic disease with chronic and severe pruritus. At present, the depressive effects of these compounds on type I and IV allergic reactions and the pruritus and dermatitis in atopic dermatitis model NC mice\(^6,23\) are under investigation.

The results of the present study suggest possibilities for the development of new agents for treating allergic diseases with chronic and severe pruritus.

REFERENCES