Screening for Molluscicidal Activity in Crude Drugs

Osamu TAKEDA,[a] Seiji TANAKA,[a] Kazuo YAMASAKI,[a] Hiroshi KOHDA,*[,a] Yuzuru IWANAGA[b] and Moriyasu TSUJI[b]

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,[a] 1–2–3 Kasumi, Minami-ku, Hiroshima 734, Japan and School of Medicine, Hiroshima University School of Medicine,[b] 1–2–3 Kasumi, Minami-ku, Hiroshima 734, Japan. *Received August 4, 1988*

Thirty-four extracts of crude drugs and medicinal plants have been screened for activity against *Oncomelania nosophora*, the intermediate host of the Japanese strain of *Schistosoma japonicum*. Strong molluscicidal activity was found in the MeOH extract of Anemarrhenae Rhizoma. Although timosaponin A-III, one of the main saponins of Anemarrhenae Rhizoma, showed very strong killing activity, markogenin-3-*O*-β-D-glucopyranosyl (1—2)-*O*-β-D-galactopyranoside having the same glycosidic linkage was found to be inactive.

Keywords molluscicide; *Oncomelania nosophora*; Anemarrhenae Rhizoma; timosaponin A-III; steroidal saponin

Schistosomiasis afflicts millions of people living in African, Asian, and South-American countries. This disease is related to aquatic snails because they serve the parasite as intermediate hosts. There are 4 main geographical strains of *Schistosoma japonicum*, the Chinese, Formosan, Philippine and Japanese strains. Various attempts are presently being made to control schistosomiasis by killing the transmitter of this endemic disease. Although chemotherapy is one of the most common methods for the control of schistosomiasis, synthetic compounds are more expensive than natural products such as plant extracts in developing countries. Naturally occurring molluscicides isolated from various plant sources have consequently received considerable attention since the discovery of the active *Phytolacca* saponins.[1] Adewunmi and Sofowora[2] reported a preliminary screening of Nigerian plant extracts for molluscicidal activity. In many cases, the molluscicidal activity is probably due to the presence of various saponins. However, not only saponins but also some terpenes,[3,4] a chalcone,[5] and a flavonoid glycoside[6] show molluscicidal properties.

In connection with our systematic isolation and structure studies on biologically active constituents from crude drugs and medicinal plants, we noticed that the crude methanol extract of Anemarrhenae Rhizoma possessed strong molluscicidal activity. In present paper, we report the results of preliminary molluscicidal screening of crude drugs and medicinal plants using *Oncomelania nosophora* snails, and describe the isolation of the active principle of Anemarrhenae Rhizoma.

Materials and Methods

Bioassay was done with snails of the species *Oncomelania nosophora* reared in a soil-filter circulating tank in the laboratory of the Department of Parasitology, Hiroshima University School of Medicine. Snails used in this experiment were mature (6 to 8 mm in length).

The tests were carried out by exposing three snails to a solution of a known concentration of test sample in distilled water at the temperature of 24±2 °C for 24 h. Following the exposure period, the snails were placed in distilled water. At intervals the snails were placed on a Petri dish, light was shone from below and the decision was made, whether the snails were living or dead, based on observation through a microscope.

Crude drugs used in this study were purchased at Kunmin in China and medicinal plants were obtained from the Experimental Station of Medicinal Plants, Hiroshima University School of Medicine. The Anemarrhenae Rhizoma, imported from China, was purchased from Kojima Pharmacy (Osaka, Japan).

A sample of each material was extracted with boiling MeOH and the extracts were concentrated, frozen and dried. The powder-like sample obtained by lyophilization was tested for its activity at a concentration of 1000 ppm.

Results and Discussion

Thirty-four extracts of crude drugs and medicinal plants have been screened for molluscicidal activity against *Oncomelania nosophora*, one of the snail vectors of schistosomiasis. The results are summarized in Table I. Of these samples tested, 19 samples showed molluscicidal activity. However, the snails in 9 cases revived when they were

TABLE I. Results of Preliminary Molluscicidal Screening of Crude Drug Extracts[a]

	After 24 h in test solv.[b]	After 48 h in distilled water[b]
Lysimachia shikokiana	3/3	2/3
Ilex pubescens (root)	3/3	0/3
Anemarrhenae Rhizoma	3/3	3/3
Cinnamomi Sieboldii Cortex	3/3	0/3
Zanthoxyli Bungeani Fructus	3/3	0/3
Bupleurii Radix	3/3	0/3
Phellodendri Cortex	3/3	3/3
Araliae Radix	3/3	0/3
Lysimachiae Herba	0/3	0/3
Ilex chinensis (leaf)	0/3	0/3
Coicis Semen	0/3	0/3
Gardeniae Fructus	0/3	0/3
Platycodi Radix	3/3	3/3
Polygalae Radix	3/3	3/3
Picrorhizae Rhizoma	0/3	0/3
Nothopteryngi Radix	3/3	0/3
Coptidis Rhizoma	3/3	3/3
Catechu	3/3	3/3
Adenophorae Radix	0/3	0/3
Codonopsis Pilosulae Radix	0/3	0/3
Meliae Tossendae Fructus	3/3	3/3
Chrysanthemi Flos	2/3	0/3
Aristolochiae Fructus	1/3	0/3
Schizandrae Fructus	1/3	0/3
Premna japonica (leaf)	2/3	2/3
Hippophae rhamnoides (leaf)	3/3	3/3
Lycii Fructus	0/3	0/3
Ephedrae Herba	3/3	1/3
Magnoliae Cortex	3/3	3/3
Uncaliae Ramulus et Hook	2/3	2/3
Salviae Miltiorrhizae Radix	0/3	0/3
Cynomorium coccineum	3/3	3/3
Shiraia bambusicola (fungi)	3/3	0/3
Cordyceps sinensis (fungi)	0/3	0/3

a) Concentration 1000 ppm. *b*) Number of dead snails/number of tested snails.

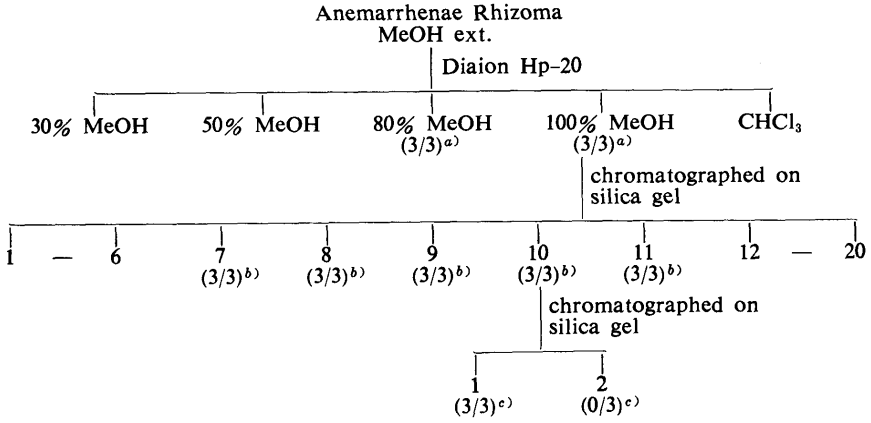

Fig. 1. Fractionation of the Constituents of Anemarrhenae Rhizoma

Values in parenthesis are number of dead snails/number of tested snails at the concentration of the assay sample; a) 800 ppm, b) 200 ppm, c) 100 ppm.

moved into distilled water within 48 h. The remaining 10 of these extracts showed strong activity against the snails at a concentration of 1000 ppm. Many extracts that have been reported to show strong activity contained various saponin components, for example, Anemarrhenae Rhizoma,[7] Platycodon Radix,[8] and Polygalae Radix.[9] However, saponins of *Ilex pubescens*,[10] Bupleurii Radix,[11] and *Ilex chinensis*,[12] have been found to be inactive. It was noteworthy that Phellodendri Cortex, Coptidis Rhizoma, and Magnoliae Cortex, which have alkaloid components, also showed molluscicidal activity.

Among the active extracts, Anemarrhenae Rhizoma was selected for further study. Anemarrhenae Rhizoma is the rhizoma of *Anemarrhena asphoderoides* (Liliaceae). Anemarrhenae Rhizoma is used as an anti-inflammatory, diuretic, and antifebrile component of Chinese prescriptions. The crude drug (1 kg) was extracted with MeOH. The MeOH extract, at a concentration of 800 ppm, killed the snails within 24 h. The MeOH extract was fractionated using highly porous polymer, DIAION HP-20 (Mitsubishi Chem. Ind. Tokyo, Japan) and successively eluted with 30%, 50%, 80%, 100% MeOH, and $CHCl_3$ to give 5 fractions. The 80% and 100% MeOH fractions, crude steroid saponin fraction, showed molluscicidal activity. The 100% MeOH fraction was separated into 20 fractions by silica gel column chromatography. The molluscicidal activity of each fraction was checked at each stage of the isolation process (Fig. 1). The biologically active 7th to 11th fractions showed 2 main spots on thin layer chromatography (TLC). The molluscicidal active fraction was again subjected to silica gel column chromatography to obtain two saponins (**1** and **2**) in yields of 1.1 and 0.29 g, respectively.

Acid hydrolysis of **1** and **2** afforded sarsasapogenin and markogenin, respectively. The sugars obtained from the saponin hydrolysates were galactose and glucose from both **1** and **2**. Compound **1** was suggested to be tiomosaponin A-III by carbon-13 and proton nuclear magnetic resonance (^{13}C- and ^{1}H-NMR) spectroscopy. Compound **1** was identified as timosaponin A-III[7] by comparing its TLC behavior and ^{1}H- and ^{13}C-NMR spectra with those of an authentic sample. The ^{13}C-NMR spectrum of **2** was very similar to that of **1**. However, the spectrum of **2** indicated the presence of one more OH group than in **1**. Eventually, **2** was identified as markogenin-3-*O*-β-D-glucopyranosyl(1—2)-*O*-β-D-galactopyranoside[7] mainly by ^{13}C- and ^{1}H-NMR spectroscopy. Compound **1** showed strong molluscicidal activity, whereas **2** was found to be inactive (100 ppm). Compound **1** killed snails at a concentration of 10 ppm within 24 h. Molluscicidal activity of **1** against *Biomphalaria glabrata* has already been described by Hostettmann *et al.*[13] In our recent study, although **1** showed a strong homolytic effect, **2** had no effect.[14] Further, both **1** and **2** showed strong anti-platelet aggregation activity.[14] The structure and activity relationship should be examined in more detail. Further investigations on the active principles of other potent extracts are in progress.

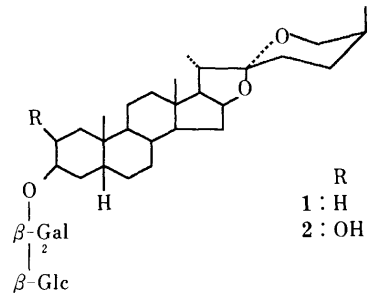

Chart 1

References

1) C. B. Lugt, *Planta Medica*, **38**, 68 (1980).
2) C. O. Adewunmi and E. A. Sofowora, *Planta Medica*, **39**, 57 (1980).
3) K. Nakanishi and I. Kubo, *Israel J. Chem.*, **16**, 28 (1977).
4) T. C. B. Tomassini and M. E. O. Matos, *Phytochemistry*, **18**, 663 (1979).
5) A. Maradufu and J. H. Ouma, *Phytochemistry*, **17**, 823 (1978).
6) S. Dossaji and I. Kubo, *Phytochemistry*, **19**, 482 (1980).
7) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.*, **11**, 1221 (1963).
8) A. Tada, Y. Kaneiwa, J. Shoji and S. Shibata, *Chem. Pharm. Bull.*, **23**, 2965 (1975).
9) S. Sakuma and J. Shoji, *Chem. Pharm. Bull.*, **29**, 2431 (1981).
10) K. Hidaka, M. Ito, Y. Matsuda, H. Kohda, K. Yamasaki, J. Yamahara, T. Chisaka, Y. Kawakami, T. Sato and K. Kagei, *Chem. Pharm. Bull.*, **35**, 3349 (1987).
11) K. Shimizu, S. Amagaya and Y. Ogihara, *Chem. Pharm. Bull.*, **33**, 3349 (1985).
12) A. Inada, M. Kobayashi, H. Murata and T. Nakanishi, Abstracts of Papers, 106th Annual Meeting of Pharmaceutical Society of Japan, Chiba, April 1986, p. 226.
13) K. Hostettmann, M. Hostettmann and K. Nakanishi, *Helv. Chim. Acta*, **61**, 1990 (1978).
14) A. Niwa, O. Takeda, M. Ishimaru, Y. Nakamoto, K. Yamasaki, H. Kohda, H. Nishio, T. Segawa, K. Fujimura and A. Kuramoto, *Yakugaku Zasshi*, **108**, 555 (1988).