A Screening Procedure for Repellents against a Sea Snail

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A convenient procedure to assay the repellent activities of organic and inorganic substances against a sea snail *Monodonta neritoides* has been developed. By the use of this procedure, 8 authentic compounds and the benzene extracts of 11 species of plants were found to show such repellent activities.

Inorganic copper¹⁾ and organic tin compounds²⁾ have been commonly used throughout the world to protect ships, fishnets, buoys and other structures in the sea from attaching marine animals (fouling animals). In order to minimize the amount of pollutants in the sea, heavy metallic antifouling agents should be replaced by safer chemicals which are less toxic to mammals and not accumulated in organisms. This necessity prompted us to survey natural products for the presence of repellents against fouling animals.

As part of our investigation, we have recently developed a procedure of detecting repellents against a sea snail *Monodonta neritoides* (Japanese name, Kurotsukegai), and the activities of a variety of authentic compounds of our laboratory collection and plant extracts have been assayed.

This paper deals with the procedure of the bioassay and results of the screening study.

MATERIALS AND METHODS

Test animals. Sea snails *M. neritoides*, 8~10 mm in shell diameter, were collected from the intertidal zone of the Okuzure coast of Shizuoka City, and left unfed overnight in a glass box which contained a 1-cm depth of seawater.

Assay of repellent activity. The bioassay was carried out at about 25°C. A test sample was dissolved in ethylacetate, and a glass capillary was used to have 0.2 ml of the solution evenly absorbed into the sample zone on a filter paper (Toyo Filter Paper, No. 2, Toyo Roshi Co., Ltd.), 9 cm in diameter (Fig. 1). After

evaporating the solvent, the filter paper was set at the bottom of a petri dish, 9.5 cm in diameter, which contained 3 ml of seawater. After 1 min, 5 snails were placed at the center of the filter paper. Touching the surface of the filter paper frequently with their antennae, the snails immediately began to move away from the center. On an inactive sample, all the snails could arrive at the corner of the petri dish within 1 min across the sample zone (Fig. 2). If no snail could get away from the central area within 15 min, the sample was judged to be active, and the weight (μg) of the sample absorbed into 1 cm² of the sample zone was recorded as the criterion of the repellent activity.

FIG. 1. Filter Paper Used in the Bioassay.

A: Snails were left here at the start. B: Solution of the test sample was absorbed here (sample zone).

Screening procedure for plant extracts. Fresh plant was extracted with benzene for a week at room temperature. After filtration, benzene was evaporated. The residue was dissolved in ethylacetate, and the repellent activity was assayed by the same procedure as described above.

Chemicals. All the chemicals tested were commercially supplied.

FIG. 2. Response of Snails *M. neritoides*.

The area between the coaxial circles, marked with vertical lines, is the sample zone. A: start of the bioassay. B: an inactive sample; snails could move across the sample zone and arrive at the periphery of the petri dish.

RESULTS AND DISCUSSION

Repellent activities of authentic compounds

The activities of 20 fatty acids, 9 aromatic acids and phenols and 8 basic compounds were assayed. As shown in Table I, among a series of fatty acids, enanthic (C_7), *n*-caprylic (C_8), pelargonic (C_9) and capric (C_{10}) acids were active at the concentration of $50 \mu\text{g}/\text{cm}^2$, and the last two even at $30 \mu\text{g}/\text{cm}^2$. Benzoic, *p*-coumaric and cinnamic acids showed repellent activities at $50 \mu\text{g}/\text{cm}^2$. Basic compounds including basic amino acids were inactive except indole and quinoline which showed activities at $30 \mu\text{g}/\text{cm}^2$. Copper (II) sulfate was expected to be a potent repellent because the copper ion had been used as an antifouling

agent. A clear repellent activity, in fact, was observed at $5 \mu\text{g}/\text{cm}^2$.

TABLE I. REPELLENT ACTIVITIES OF AUTHENTIC COMPOUNDS AGAINST A SEA SNAIL *M. neritoides*

Compounds	Repellent activity at ($\mu\text{g}/\text{cm}^2$)		
	100	50	30
<u>Fatty acids</u>			
Propionic (C_3)	—		
<i>n</i> -Butyric (C_4)	—		
Isobutyric (C_4)	—		
<i>n</i> -Valeric (C_5)	—		
<i>n</i> -Caproic (C_6)	—		
Isocaproic (C_6)	—		
Enanthic (C_7)	+	+	—
<i>n</i> -Caprylic (C_8)	+	+	—
Pelargonic (C_9)	+	+	+
Capric (C_{10})	+	+	+
Undecanoic (C_{11})	—		
Lauric (C_{12})	—		
Myristic (C_{14})	—		
Palmitic (C_{16})	—		
Stearic (C_{18})	—		
Oleic (C_{18}, Δ_1)	—		
Linoleic (C_{18}, Δ_2)	+	—	
Linolenic (C_{18}, Δ_3)	—		
Arachidic (C_{20})	—		
Behenic (C_{22})	—		
<u>Other acids and phenols</u>			
Benzoic acid	+	+	—
<i>p</i> -Coumaric acid	+	+	—
Cinnamic acid	+	+	—
Phenol	+	—	
Catechol	+	—	
Phloroglucinol	—		
Salicylic acid	+	—	
Ferulic acid	—		
Caffeic acid	—		
<u>Basic compounds</u>			
Aniline	—		
Pyridine	—		
Purine	—		
Indole	+	+	+
Quinoline	+	+	+
Arginine	—		
Histidine	—		
Ornithine	—		
<u>Inorganic compounds</u>			
Copper (II) sulfate	+	+	+

Repellent activities of plant extracts

Twenty species of terrestrial plants (leaves) and 7 species of seaweeds were collected, and the repellent activities of their benzene extracts

TABLE II. REPELLENT ACTIVITIES OF BENZENE EXTRACTS OF PLANTS (LEAVES) AND SEAWEEDS AGAINST A SEA SNAIL *M. neritoides*

Family	Species	Repellent activity at 100 $\mu\text{g}/\text{cm}^2$
<u>Terrestrial plants</u>		
Amaryllidaceae	<i>Narcissus Taxetta</i> L. var. <i>chinensis</i> Roem.	—
Apocynaceae	<i>Nerium indicum</i> Mill.	—
Araliaceae	<i>Gilibertia trifida</i> Makino	—
	<i>Fatsia japonica</i> Decne. et Planch.	—
Caprifoliaceae	<i>Lonicera japonica</i> Thunb.	—
	<i>Viburnum Awabuki</i> K. Koch	—
Compositae	<i>Artemisia vulgaris</i> L. var. <i>indica</i> Maxim.	—
	<i>Chrysanthemum morifolium</i> Ramat. var. <i>sinense</i> Makino	—
	<i>Erigeron sumatrensis</i> Retz.	+
	<i>Petasites japonicum</i> Miq.	—
	<i>Taraxacum platycarpum</i> Dahlst.	—
Cupressaceae	<i>Chamaecyparis obtusa</i> Endl.	+
Lauraceae	<i>Cinnamomum japonicum</i> Sieb.	+
	<i>Machilus japonica</i> Sieb. et Zucc.	—
Leguminosae	<i>Astragalus sinicus</i> L.	—
	<i>Trifolium repens</i> L.	—
Podocarpaceae	<i>Podocarpus macrophylla</i> D. Don	—
	<i>P. Nagi</i> Zoll. et Moritzi	—
Taxodiaceae	<i>Cryptomeria japonica</i> D. Don	+
	<i>Sequoia semperivirens</i> Endl.	+
<u>Seaweeds</u>		
Codiaceae	<i>Codium latum</i> Suringar	+
Ulvaceae	<i>Ulva</i> sp.	—
Laminariaceae	<i>Undaria pinnatifida</i> (Harvey) Suringar	+

Corallinoideae	<i>Marginisporum aberrans</i> (Yendo) Johansen et Chihara	+
Grateloupiaceae	<i>Grateloupia turuturu</i> Yamada	+
	<i>G. sp.</i>	+
Rhodomelaceae	<i>Laurencia intermedia</i> Yamada	+

were assayed at the concentration of 100 $\mu\text{g}/\text{cm}^2$. The results are shown in Table II. Among the terrestrial plants, 5 species were active. Notably, all the species of seaweeds except *Ulva* sp. were active. Active compounds in seaweeds may play the role of attaching or feeding inhibitors against marine animals.

The procedure of the bioassay makes it possible to examine the repellent activities of a number of samples in a short time. The efficacy of the bioassay procedure was evidenced by the discovery of some active authentic compounds and 11 species of plants. The isolation and identification of active principles in plants are in progress.

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- 1) See, for example, cuprous oxide, "The Merck Index," 9th ed., ed. by M. Windholz, Merck & Co., N. J., 1976, p. 347.
- 2) K. Nakagawa, *Tosō no Gijutsu*, 11, 32 (1976).