Insecticidal and Fungicidal Compounds from *Isatis tinctoria*

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Tryptanthrin (**1**), indole-3-acetonitrile (**2**) and *p*-coumaric acid methylester (**3**) were isolated from the aerial parts of *Isatis tinctoria* L. The compounds show insecticidal and antifeedant activity against termites *(Reticulitermis santonensis)*, insect preventive and control activity against larvae of the house longhorn beetle (*Hylotrupes bajulus*) and fungicidal activity against the brown-rot fungus *(Coniophora puteana)*.

Introduction

In the search for naturally occurring insecticides and fungicides we have found woad (*Isatis tinctoria* L., Brassicaceae) having a history of use in folk medicine [1] and in the field of wood preservatives [2, 3]. Woad (leaves, juice, balsam) was used for example as a styptic surgical agent or laxative [1]. An extract of *Isatis tinctoria* L. has also shown an antimicrobial activity [4]. In the Middle Ages indigo, the blue dye, was produced from woad by a special procedure.

Materials and Methods

Bioassay

Insect preventive activity against Hylotrupes bajulus

Pine sap-wood samples (50 × 25 × 15 mm) were impregnated with solutions of tryptanthrin (**1**), indole-3-acetonitrile (**2**) or p-coumaric acid methylester (**3**) in dioxane. Thirty egg larvae of *Hylotrupes bajulus* L. were allowed to attack the wood for 28 days. At the end of the assay the mortality of the egg larvae and the depth of nibbling at the impregnated wood were determined.

Insect control activity against Hylotrupes bajulus

Six medium-sized larvae of *Hylotrupes bajulus* L. (weight 50–150 mg) were placed on each pine sap-wood sample (100 × 60 × 30 mm). After 12 weeks one of the surfaces (100 × 60 mm) of each wood sample was treated with a solution of one of the compounds in dioxane. The other surfaces were sealed with a solution of gelatine. The wood samples were seasoned for 24 weeks at 22 °C and 75% relative humidity and then the mortality was determined in 3 parallel assays.

Insecticidal and antifeedant activity against Reticulitermis santonensis

Screening test

Filter paper sheets (FN 4 – paper for chromatography, 10 mm in diameter) were impregnated with 0.1 ml of solutions of **1–3** in dioxane. After drying, the filter paper sheets were placed in petri dishes (20 mm in diameter) and 20 working termites (*Reticulitermis santonensis* De Feytaud) were allowed to attack the paper for 14 days in 6 parallel assays. The intestines of the dead termites were placed in physiological NaCl solution and the mortality of the intestine flagellates was determined by means of a microscope.

Laboratory assay

The pine sap-wood samples (30 × 30 × 10 mm) were put into glass vessels (120 mm in diameter × 70 mm) containing quartz sand at the bottom. For the food force assay, one impregnated pine sap-wood sample was in the glass vessel; for the food alternative assay, an impregnated and an untreated wood sample were in the glass vessel. Each vessel contained 200 working termites, 1 soldier and 1 nymphae (3 parallel 4 week assays).

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Fungicidal activity against Coniophora puteana

Eight pine sap-wood samples (40 × 15 × 4 mm) were impregnated with the solutions of **1–3** in acetone, dried at 22 °C for 8 days (4 wood samples were leached with water for 135 h at 22 °C), placed in petri dishes (90 mm in diameter) containing *Coniophora puteana* (Schum.: Fr.) Karst. on malt agar and incubated for 6 weeks. At the end of the assay the mass loss of the wood samples was determined.

Antifungal bioassay

The antifungal compound-containing fractions were subjected to TLC in $CHCl_3$–MeOH (99:1), the plates then sprayed with a spore suspension of *Cladosporium cucumerinum* (obtained from the firm Fahlberg-List Magdeburg), and incubated at 25 °C in the dark for 2 days. The fungitoxic compounds on the TLC plates were visible as white spots bare of mycelium in a dark grey layer of the mycelium covering the plates [5].

Product analysis

General

M.p.: corr. ^1H NMR spectra were recorded at 300 MHz and ^{13}C spectra at 75.5 MHz. The mass spectra were measured at 70 eV. Column chromatography was carried out on silica gel 60 (0.063–0.2 mm), TLC on silica gel sheets (0.4 mm, HF_{254}) and prep. TLC on silica gel sheets (1 mm, PF_{254}).

Isolation of compounds

Plants of *Isatis tinctoria* L. were cultivated from seeds (Botanical Garden Oldenburg, Germany) in the garden of the Institute for Plant Biochemistry, Halle, Germany. Fresh minced aerial parts (35.2 kg) were extracted with acetone. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$–MeOH–H_2O (19:1:20). The organic phase was separated and the aqueous phase extracted (×3) with $CHCl_3$–MeOH (19:1).

The organic layers were collected, the solvent was evaporated under reduced pressure and the residue was chromatographed (×3) on silica gel. The column was eluted with $CHCl_3$ and mixtures of $CHCl_3$–MeOH (49:1–1:1). The fractions containing the appropriate compounds were further purified by prep. TLC $CHCl_3$–MeOH (99:1) to give tryptanthrin (**1**, 98 mg R_f 0.66), indole-3-acetonitrile (**2**, 400 mg, R_f 0.44) and *p*-coumaric acid methylester (**3**, 140 mg, R_f 0.31).

Tryptanthrin (**1**)

M.p. 268–269 °C; ref. [6] m.p. 267 °C; MS *m/z* (rel. int.): 248 (M^+, 100), 220 (71), 192 (23), 165 (7), 144 (3); ^1H NMR ($CDCl_3$) δ (ppm): 8.58 (ddd, J = 8.1, 0.9, 0.6 Hz, H-10), 8.39 (ddd, J = 7.9, 1.5, 0.4 Hz, H-1), 7.99 (ddd, J = 8.1, 1.2, 0.4 Hz, H-4), 7.88 (ddd, J = 7.6, 1.4, 0.6 Hz, H-7), 7.82 (ddd, J = 8.1, 7.3, 1.5 Hz, H-3), 7.75 (ddd, J = 8.1, 7.6, 1.4 Hz, H-9), 7.63 (ddd, J = 7.9, 7.3, 1.2 Hz, H-2), 7.39 (ddd, J = 7.6, 7.6, 0.9 Hz, H-8); ^{13}C NMR ($CDCl_3$) δ (ppm): 182,5 (C-6), 158.0 (C-12), 146.6 (C-5a), 146.3 (C-4a), 144.3 (C-10a), 138.2 (C-9), 135.1 (C-3), 130.7 (C-4), 130.2 (C-2), 127.5 (C-1), 127.2 (C-8), 125.4 (C-7), 123.7 (C-12a), 121.9 (C-6a), 117.9 (C-10).

Indole-3-acetonitrile (**2**)

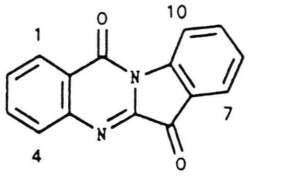
M.p. 36 °C; ref. [7] m.p. 36–36.5 °C; MS *m/z* (rel. int.): 156 (M^+, 79), 155 (100), 130(48), 101 (7); ^1H NMR ($CDCl_3$) δ (ppm): 8.31 (br, H-1), 7.65 (ddd, J = 7.7, 1.3, 0.7 Hz, H-4), 7.44 (ddd, J = 8.0, 1.2, 0.7 Hz, H-7), 7.33 (ddd, J = 7.7, 7.1, 1.2 Hz, H-5), 7.28 (ddd, J = 7.1, 8.0, 1.3 Hz, H-6), 7.21 (m, H-2), 3.86 (2H, d, J = 1.1 Hz, CH_2); ^{13}C NMR ($CDCl_3$) δ (ppm): 136.2 (C-8), 125.9 (C-9), 122.8 (C-2), 122.7 (C-5), 120.1 (C-6), 118.3 (CN), 117.9 (C-4), 111.5 (C-7), 104.4 (C-3), 14.2 (CH_2).

p-Coumaric acid methylester (**3**)

M.p. 138 °C; ref. [8] m.p. 137 °C; MS *m/z* (rel. int.): 178 (M^+, 45), 147 (100), 119 (20), 91 (15); ^1H NMR ($CDCl_3$) δ (ppm): 7.62 (d, J = 16.0 Hz, H-7), 7.46 (d, J = 8.7 Hz, H-2, H-6), 6.83 (d, J = 8.7 Hz, H-3, H-5), 6.32 (d, J = 16.0 Hz, H-8), 3.77 (3H, s, CH_3O); ^{13}C NMR ($CDCl_3$) δ (ppm): 169.8 (C-9), 161.1 (C-4), 146.5 (C-7), 131.1 (C-2, C-6), 127.1 (C-1), 116.8 (C-3, C-5), 114.9 (C-8), 52.0 (CH_3O).

Results and Discussion

The crude acetone extract of the fresh aerial parts of *Isatis tinctoria* L. was checked for fungicidal compounds using TLC. The developed TLC plates of the extract were treated with a conidial suspension



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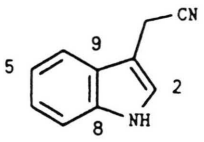

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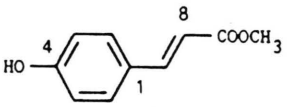

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Formel **1–3.**

of *Cladosporium cucumerinum* and incubated for 2 days. Inhibition zones proved the compounds to be fungitoxic. Bioassay-directed fractionation of the woad extract by means of silica gel column chromatography and prep. TLC, yielded tryptanthrin (**1**), indole-3-acetonitrile (**2**) and *p*-coumaric acid methylester (**3**) as compounds with fungicidal activity.

Tryptanthrin (**1**), indole-3-acetonitrile (**2**) and *p*-coumaric acid methylester (**3**) were identified by physical and spectral data (MS, NMR) and by comparison with authentic synthesized samples of these compounds. Tryptanthrin has already been isolated from *Isatis tinctoria* and characterized as an antidermatophytic [6, 9]. Since detailed ^{13}C NMR data are not yet published, the known assignments of the proton signals of **1** [10] were transferred to carbons with attached protons using a one-bond heteronuclear shift correlation 2D NMR experiment. [^{14}C]-Indole-3-acetonitrile could be detected in tissue of *Isatis tinctoria* after incubation with [^{14}C]-indole-3-acetaldoxim [11]. Tryptanthrin (**1**), indole-3-acetonitrile (**2**) and *p*-coumaric acid methylester (**3**) were synthesized and tested for biological activity.

Tryptanthrin (**1**) and indole-3-acetonitrile (**2**) showed an insecticidal activity against larvae of the house longhorn beetle (*Hylotrupes bajulus* L.).

In the assay for insect preventive activity the total mortality of egg larvae was 88% (42% outside and 46% inside the wood) after treatment of the pine sap-wood samples with indole-3-acetonitrile (concentration of the impregnation solution 0.1%) (Table I). Tryptanthrin (**1**) and *p*-coumaric acid methylester (**3**) showed no activity against egg larvae at a concentration of 0.1% in this assay.

At a concentration of 4% of the impregnation solution (absorption capacity 350 g/m^3) tryptanthrin (**1**) had a good insecticidal activity in the assay for insect control activity. The mortality of medium-sized larvae in the wood is 65% compared with 3% in the control.

The termite species *Reticulitermis santonensis* De Feytaud was influenced in the screening test differently by **1, 2** and **3.** The intestine flagellates react more sensitively than the termites (Fig. 1). This finding is in accordance with results obtained by

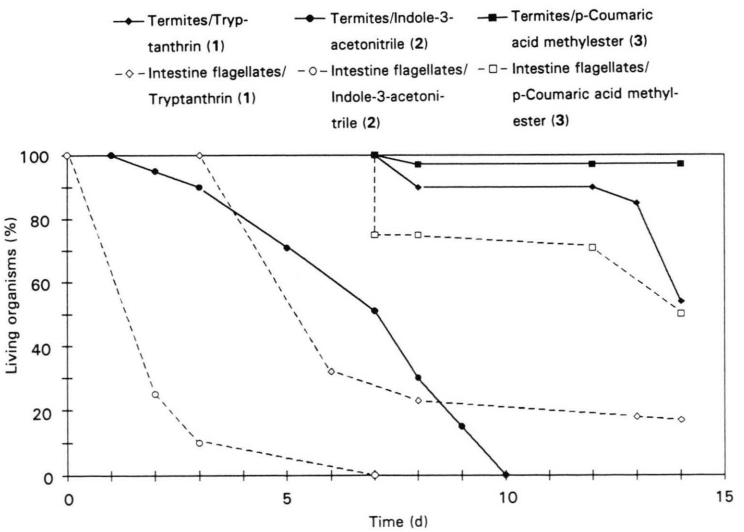

Fig. 1. Mortality of termites and their intestine flagellates (concentration of the impregnation solution: 1%).

Table I. Insect preventive activity of indole-3-acetonitrile (**2**) on the egg larvae of *Hylotrupes bajulus* L. (Test period: 4 weeks).

Concentration of the impregnation solution	Absorption capacity of the wood	Egg larvae not penetrated in the wood		Egg larvae penetrated in the wood		Total mortality	Depth of nibbling at the wood
		dead	living	dead	living		
%	[g/m³]	%	%	%	%	%	[mm]
0.04	130	45	0	22	33	67	1.7
0.1	360	42	0	46	12	88	1.4
Control		0	0	0	100	0	3.2

treatment of termites with extracts of mold fungi and tensides [12]. Indole-3-acetonitrile (**2**) had the highest activity. The mortality of the intestine flagellates was 100% after 7 days of the treatment. Three days later all termites were dead. In the case of treatment with tryptanthrin (**1**) the mortality of termites was 46% and of the intestine flagellates 83% after 14 days. The insecticidal activity of *p*-coumaric acid methylester (**3**) is low in comparison with **2** (Fig. 1).

When pine sap-wood was treated with 0.1% solution of tryptanthrin (**1**) and used in a food force assay of termites, the mass loss of the wood was only 44% of that of the control wood (Fig. 2). In a food alternative assay the tryptanthrin treated wood was avoided by the termites (Fig. 2). The mass loss of the wood was only 0.3% in comparison with 12% of the untreated wood. So compound **1** showed an antifeedant activity against termites.

The fungicidal activity against *Coniophora puteana* (Schum.: Fr.) Karst. of compounds **1–3** is given in Table II. The mass loss of the pine sap-wood of the control (20.6%) was reduced by impregnation of the wood with **1–3.** Tryptanthrin (**1**) showed a good activity (mass loss of the wood in the concentration range 0.05–0.5%, 3.5–2.4%) even after treatment of the wood samples with water.

Table II. Fungicidal activity of tryptanthrin (**1**), indole-3-acetonitrile (**2**) and *p*-coumaric acid methylester (**3**) against *Coniophora puteana* (Schum.: Fr.) Karst. (Test period: 6 weeks).

Compound	Concentration of the impregnation solution	Absorption capacity of the wood	Leaching with water	Mass loss of the wood samples
	%	[kg/m³]		%
Tryptanthrin (**1**)	0.05	0.25		3.1
	0.1	0.50	without	2.4
	0.5	2.87		2.7
	0.05	0.26		3.5
	0.1	0.50	with	2.9
	0.5	2.79		2.4
Indole-3-aceto-nitrile (**2**)	0.05	0.28		5.3
	0.1	0.55	without	3.8
	0.5	2.81		1.1
	0.05	0.27		10.9
	0.1	0.55	with	10.9
	0.5	2.85		1.5
p-Coumaric acid methylester (**3**)	0.1	0.45	without	7.2
	0.5	2.32		3.6
	0.1	0.44	with	10.0
	0.5	2.35		3.7
Control				20.6

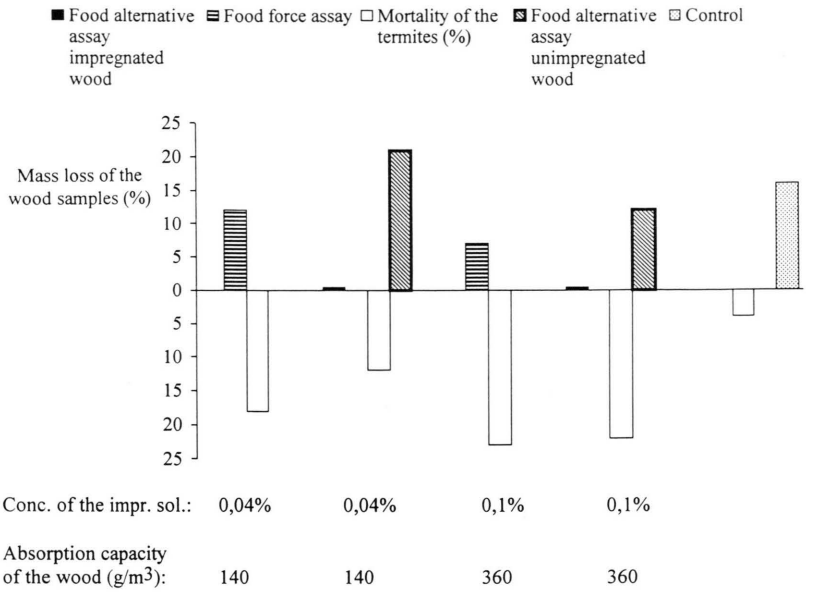

Fig. 2. Insecticidal and antifeedant activity of tryptanthrin (**1**) against termites.

Indole-3-acetonitrile (**2**) was not resistant to leaching with water at an impregnation solution concentration of 0.05–0.1%. A 0.5% solution of **2** showed the highest activity of the tested substances without as well as with leaching.

The natural compounds **1–3** from *Isatis tinctoria* L. improve the biostability of the wood due to their insecticidal, fungicidal and antifeedant activity.

Acknowledgements

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[1] J. B. Hurry, in: The Wood Plant and its Dye (H. Milford, ed.), Oxford University Press, London (1930).
[2] W. Feige, Ger. (East) Patent 232 060, Chem. Abstr. **105,** 210540 p (1986).
[3] W. Feige, Ger. (East) Patent 243 823, Chem. Abstr. **108,** 57934 k (1988).
[4] K. Dornberger and H. Lich, Pharmazie **37,** 215 (1982).
[5] A. L. Homans and A. Fuchs, J. Chromatogr. **51,** 327 (1970).
[6] G. Honda, V. Tosirisuk, and M. Tabata, Planta Medica **38,** 275 (1980).
[7] H. B. Henbest, E. R. H. Jones, and G. F. Smith, J. Chem. Soc. 3796 (1953).
[8] Beilstein's Handbook of Organic Chemistry, p. 299, Springer Verlag, Berlin H 10 (1927).
[9] M. Tabata and G. Honda, Japan. Patent 55-47684, Chem. Abstr. **93,** 186400 d (1980).
[10] M. Y. Jarrah and V. Thaller, J. Chem. Res. (S) 186 (1980).
[11] J. Helmlinger, T. Rausch, and W. Hilgenberg, Phytochem. **24,** 2497 (1985).
[12] W. Unger, Dissertation, University Greifswald (1975).