

Sesquiterpene lactones. XXXV. Constituents of *Reichardia tingitana* L. Roth. and their antifeedant activity

WŁODZIMIERZ M. DANIEWSKI¹, PIOTR SKIBICKI¹, MARIA GUMUŁKA², BOHDAN DROŹDŹ^{2*},
HALINA GRABARCZYK^{2*}, ELŻBIETA BŁOSZYK^{2*}

¹Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

^{2*}Chair and Department of Medicinal Plants, Medical Academy, Mazowiecka 33, 60-623 Poznań, Poland

(Received: January 28, 1988. Accepted: June 7, 1988)

Abstract

Reinvestigation of the components of *Reichardia tingitana* L. Roth. afforded besides already known sesquiterpene lactones, 14-deoxylactucin (II) and desacetylmaticarin (III) cholesterol (V), stigmasterol (VI), β -sitosterol (VII) and ceryl alcohol (VIII). The insect deterrent activity of compounds II and III has been determined.

Key words: *Reichardia tingitana*, sesquiterpene lactones, cholesterol, stigmasterol, β -sitosterol, ceryl alcohol, chemotaxonomy, antifeedants

INTRODUCTION

The constituents of *Reichardia tingitana* L. Roth. var. *orientalis* (L.) Asch. et Schweinf. have already been investigated (Sawsan El-Masry et al. 1980). Two sesquiterpene lactones, lactucin (I) and 14-deoxylactucin (II) have been reported. In seeking antifeedants of plant origin we investigated an extract of *Reichardia tingitana* L. Roth. in order to isolate the compounds and to check their deterrent activity. Several sesquiterpene lactones showed marked insect feeding deterrence (Nawrot et al. 1983); having many examples of such compounds may enable us to draw conclusions on the structure activity relationship.

RESULTS AND DISCUSSION

Investigation of the methylene chloride extract of aerial parts of *Reichardia tingitana* L. Roth. gave two sesquiterpene lactones, desacetylmaticarin (III) and 14-deoxylactucin (II); no lactucin (I) was found (Fig. 1). 14-Deoxylactucin

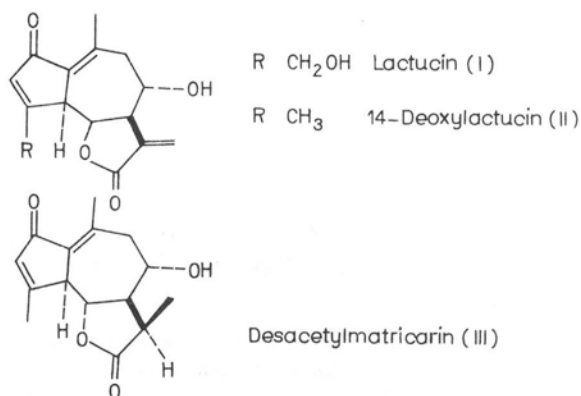


Fig. 1. The chemical structure of sesquiterpene lactones isolated from *Reichardia tingitana* L. Roth.

upon acetylation under standard conditions gave the already described 11,12-dehydromatricarin (IV) (Bohlmann and Zdero 1978). The ¹H NMR spectrum of (IV) was identical with that published by Bohlmann and Zdero (1978).

The ¹H NMR spectrum of (II) published by Sawsan El-Masry et al. (1980) was obtained using a 60 MHz spectrometer, therefore we report our spectrum in detail which was obtained using a 400 MHz high-field spectrometer (Table 1).

Table 1

¹H NMR (400 MHz) spectrum of compound II

Proton	Chemical shift	J Hz
H-3	6.13dq	3-14 = 1.3, 3-5 = 0.8
H-5 α	3.42dd	5 α -6 β = 10.0
H-6 β	3.60dd	6 β -7 α = 10.0
H-7 α	2.96dddd	7 α -13 = 3.2, 7 α -8 β = 10.0
H-8 β	3.87dddd	8 β -13 = 5.2, 8 β -9 α = 10.8, 8-9 = 2.4
H-9 α	2.80dd	9 α -9 β = 13.7
H-9 β	2.35dd	9 β -8 α = 2.4
H-13	6.20ddq	7 α -13 = 3.2, 8 β -13 = 5.2, 13-13 = 1.0
H-14	2.28dd	3-14 = 1.3, 3-5 = 0.8
H-15	2.38s	

The sterols in the methylene chloride extract of *Reichardia tingitana* existed in two forms, as esters (TLC-spots, R_f = 0.2-0.3 benzene/acetone, 9:1). The sterol ester fraction was hydrolysed in 10% MeOH KOH solution under argon and gave a mixture of sterols which was acetylated under standard conditions.

TLC analysis of the acetates using silver nitrate-impregnated Si-gel plates, (benzene/hexane, 15:85, five times developed) showed the presence of at least three compounds. A similar mixture was obtained from acetylation of free sterols. GLC was used in order to identify the compounds. The retention times of eluting peaks were compared with those of known standards. The following sterols were identified: cholesterol V (20%), stigmasterol VI (50%) and β -sitosterol VII (30%). The identification was confirmed by GLC coupled with a mass spectrometer. Mass spectra of V, VI and VII exhibited $M^+ - 60$ peaks (order of elution) at m/z 368 (cholesterol), 394 (stigmasterol), 396 (β -sitosterol), respectively.

Column chromatography on Si-gel of the extract gave a pink spot (TLC — $R_f = 0.6$, hexane/ethylacetate, 8:2, spraying H_2SO_4 anisaldehyde) which was identified as ceryl alcohol (VIII) — $C_{26}H_{54}O$, its mass spectrum exhibited $M^+ - 18$ ion at m/z 364. The 1H NMR spectrum of (VIII) showed a broad singlet at 1.26 ppm of aliphatic methylenes 48H, a 2H triplet at 3.6 ppm and a 3H triplet at 0.85 ppm substantiating the structure.

Compound (VIII) when acetylated gave a monoacetyl derivative (IX) as expected. 1H NMR spectrum confirmed its structure.

The tests of the deterrent (antifeedant) activity of sesquiterpene lactones II and III were carried out by the method of Nawrot et al. (1982). These substances were tested on adult specimens of *Sitophilus granarius* L. and *Tribolium confusum* Duv. and on the larvae of the latter species and of *Trogoderma granarium* Ev. The results are summarized in Table 2.

Desacetylmatricarin (III) is the effective deterrent. The activity (III class — average deterrent activity in Table 2) is almost twice as strong as 14-deoxylactucin (II). A very high deterrent activity (the value of "total coefficients" in Table 2 between 151-200) against adults of *Tribolium confusum* Duv. was found in compound (III) and against larvae of *Trogoderma granarium* Ev. in compounds II and III.

Previous papers refer to a series of data on the high deterrent activity of sesquiterpenoids containing an exomethylene double bound conjugated with the free or the bound carboxyl function in the molecule (Nawrot et al. 1983, Daniewski et al. 1986, in press). The experiments showed that 14-deoxylactucin (II) with the methylene double bound in the γ -lactone ring has, in general a lower antifeedant activity than desacetylmatricarin (III). 14-Deoxylactucin (II) showed feedant attractivity for adults of *Tribolium confusum* Duv. (Table 2). The highest deterrence index was observed in the larvae of *Trogoderma granarium* Ev. which are strongly resistant to periodic starvation. On the other hand, the beetles of *Sitophilus granarius* L. were relatively least affected by the presence of antifeedants in comparison with the other species of insects. This is perhaps connected with the number of taste receptors which this species possesses. *Sitophilus granarius* L. has only one pair of basiconic sensiles at the tip of the snout (Donat 1970).

Table 2

A test of the deterrent activity of the sesquiterpene lactones from *Reichardia tingitana* L. Roth.

Compound	<i>Tribolium confusum</i> Duv.						<i>Trogoderma granarium</i> Ev. larvae			<i>Sitophilus granarius</i> L. adults			Average deterrent activity (for four tested insectes)
	adults			larvae									
	coefficients			coefficients			coefficients			coefficients			
	rel.	abs.	total	rel.	abs.	total	rel.	abs.	total	rel.	abs.	total	
14-Deoxy-lactucin (II)	-18.1	-15.4	-33.5	50.2	6.6	56.8	94	65.3	159.3	7.9	57.6	65.5	62 (II class)
Desacetyl-matricarin (III)	92.4	65.3	157.7	46	39.6	85.6	100	100	200	26.7	61.6	88.3	132.9 (III class)

rel. — relative, abs. — absolute

Below "O" — attractant, 0-50 — poor deterrent, 51-100 — medium deterrent, 101-150 — good deterrent, 151-200 — very good deterrent.

The following numbers of insects were used in the tests: *Sitophilus granarius* L. adults — 3; *Tribolium confusum* Duv. adults — 20, larvae — 10; *Trogoderma granarium* Ev. larvae — 10.

EXPERIMENTAL

The plant used in this study came from the garden of the Department of Medicinal Plants of the Poznań (Poland) Medical Academy, Voucher No.13/85. All m.ps. were measured with a Kofler block and they were not corrected. The mass spectra were measured on an LKB spectrometer. The IR spectra were measured on a Beckman Aculab spectrometer. Optical rotations were determined with a Perkin-Elmer polarimeter in CHCl_3 solutions. The ^1H NMR spectra were measured using various instruments. TLC analysis was done on Merck TLC plates.

ISOLATION OF STEROLS AND CERYL ALCOHOL

Dried aerial parts of *Reichardia tingitana* (2.0 kg) were ground and extracted exhaustively with methylene and gave 18 g of an extract. The extract was purified by passing through a short column with aluminium oxide (1:15 ratio) in an ethanol/benzene, 3:7, system. The purified extract was subjected to column chromatography on Si-gel in a hexane/ethylacetate gradient (5-20%) solvent system. Fractions having R_f values of 0.8 (sterol esters), 0.6 (ceryl alcohol) and 0.45 (free sterols) in hexane/ethylacetate, 8:2, were collected, evaporated and gave the desired products, sterol esters mixture oil: ceryl alcohol, $\text{C}_{26}\text{H}_{54}\text{O}$, m.p. 79-80°C; MS: m/z $\text{M}^+ - \text{H}_2\text{O}$ 364; ^1H NMR (CDCl_3 , 60 MHz): 3H,t, 0.95, 52H, br.s. 1.25, 2H,t, 3.6 ppm; IR (nujol): 3600, 2850 cm^{-1} .

Ceryl alcohol acetate was obtained by acetylation of (VIII): $\text{C}_{28}\text{H}_{56}\text{O}$; m.p. 60°C; MS: m/z M^+ 424; IR (nujol): 2850, 1725 cm^{-1} C=O; ^1H NMR (CDCl_3 , 60 MHz): 3H,t, 0.9, 53H, br.s. 1.32, 3H,s. 1.75 CH C=O, 2H,t, 4.0 ppm.

Free sterols (mixture) m.p. 124-132°C.

Hydrolysis of sterol esters with 10% KOH MeOH, H_2O solution under standard conditions under argon.

Acetylation of free sterols in pyridine solution standard procedure.

Gas liquid chromatography Willy-Giede 18.3 gas chromatograph fitted with a dual column dual flame ionization detector. Column: 2m SS, int.dia. 4 mm, filled with Gas-chrom Q coated with 3% F-60 silicone oil, maintained at 260°C, with carrier gas N flow rate 60 $\text{cm}^3 \text{min}^{-1}$. Inj.port. temp. 270°C. Det.temp. 300°C. Retention times: cholesterol – 21.3 min., stigmasterol – 29.4 min., β -sitosterol – 33.9 min.

Isolation of sesquiterpenes. Purified chloroform extract of R.t. (700 mg) was chromatographed on a Si-gel column using a benzene/acetone 1-20% gradient eluting system. Ten fractions were obtained and two of them, $R_f=0.4-0.3$, contained the lactones II (176mg) and III (123 mg). The larger fraction upon dissolving in ethylacetate gave a precipitate which after recrystallization from chloroform/hexane gave 14-deoxylactucin II (40 mg), m.p. 214-216°C;

(α)_D = +101.9°; (c=1.07); IR (nujol): 3450 (OH), 1745 (C=O), 1680 cm⁻¹ (C=C); MS: m/z (%) M⁺260(100), M⁺-H₂O 242(8.1), 227(16.5), 213(12.8), 199(17.5), 196(12.), 185(11.1), 171(13.5), 169(7.2), 143(10.8), 147(18.4), 145(10.4), 136(23.5), 91(50.9), 77(27.0), 69(46.0), 41(53.8). The mother liquors and the smaller fraction were combined and rechromatographed using an HPLC system (pack of 5.8 mm × 30 cm Si-gel column) joined in series, Waters SDS-6000, RI detector, in hexane/ethylacetate 60:40 eluting solvent. Additional amounts of II and pure III (20 mg, after chromatography) were obtained. M.p. 146-148°C; (α)_D = +62.2° (c=1.0); IR (nujol); MS: m/z M⁺262; ¹H NMR the same as that reported by Dominguez and Cardenas (1975).

Acetylation of II. Compound II was acetylated by standard procedure and gave 11,13-dehydromatricarin (IV) with its ¹H NMR spectrum the same as that reported by Bohlmann and Zdero (1978).

DETERRENT ACTIVITY TEST

The test is described in detail in one of our previous papers (Nawrot et al. 1982). Insects (adults and larvae) used for the tests were reared under laboratory conditions at a temp. of 26°C and 75% relative humidity. All compounds investigated were diluted in 96° ethanol to a concentration of 10 mg cm⁻³. Wheat wafer discs were used as the test food. The discs (1cm in diameter) were saturated with ethanol solutions of pure compounds II and III. Feeding of insects was recorded under three conditions: 1 = on pure food (control), 2 = on food with the possibility of choice (choice test), 3 = on food with the tested compounds (no-choice test). The wafer discs were weighed after drying in air for 30 min. before the experiments, and again after 7 days of feeding by beetles or larvae. On the basis of the eaten food, the index of the activity of the tested compounds was obtained in the following way: three coefficients were calculated from the weight of food eaten in the control KK, in the no-choice test EE, and in the choice test KE.

a. The absolute coefficient of deterrence, $A = \frac{KK - EE}{KK + EE} \times 100$,

b. The relative coefficient of deterrence, $R = \frac{K - E}{K + E} \times 100$,

c. The total coefficient of deterrence, $T = A + R$.

The total coefficient served as the index of activity.

The examined compounds are shown in Table 2.

Acknowledgement

This study was financed through the interdepartmental program CPBP 01.13.2.21.

REFERENCES

- Bohlmann F., Zdero C., 1978. New sesquiterpenes and acetylenes from *Athanasia* and *Pentia* species. *Phytochemistry* 17: 1595-1597.
- Daniewski W. M., Kroszczyński W., Błoszyk E., Drożdż B., Nawrot J., Rychlewska U., Buděšinsky M., Holub M., 1986. Sesquiterpenoids from *Dittrichia viscosa* (L.) Greuter. Their structure and deterrent activity. *Coll. Czechoslov. Chem. Commun.* 51: 1710-1721.
- Daniewski W. M., Kroszczyński W., Błoszyk E., Dudek A., Kosturkiewicz Z., Rychlewska U., Nawrot J., Buděšinsky M., Vasičkova S., Holub M., (in press). Sesquiterpene lactones of *Cephalophora aromatica* (Hook) Schrader and their deterrent activity. The stereostructure of geigerynin and hymenoratin. Revision of the stereostructure of Linifolin A. *Coll. Czechoslov. Chem. Commun.*
- Dominquez X. A., Cardenas G., 1975. Achillin and desacetylmaticarin from two species *Artemisia*. *Phytochemistry* 14: 2511-2512.
- Donat H. J., 1970. Zur Kenntnis des chemoreceptorischen Verhaltens der Kornkäfers, *Sitophilus granarius* L., *Coleoptera* beim Auffinden seiner Nahrung. *Z. angew. Ent.* 65: 1-11.
- Nawrot J., Błoszyk E., Grabarczyk H., Drożdż B., 1982. Deterrent properties of sesquiterpene lactones for the selected storage pests. *Pr. Nauk. IOR.* XXIV (1): 27-36.
- Nawrot J., Błoszyk E., Grabarczyk H., Drożdż B., Daniewski W. M., Holub M., 1983. Further evaluation of feeding deterency of sesquiterpene lactones to storage pests. *Pr. Nauk. IOR.* XXV (1): 91-98.
- Sawsan El-Masry, Saleh M. R. I., Ghazy N. M., Vuihorgne M., 1980. Isolation and structure elucidation of sesquiterpene lactones from *Reichardia tingitana* L. Roth. var. *orientalis* (L.) Asch. et Schweint. *Acta Pharm. Suecica* 17(3): 137-142.

Laktony seskwiterpenowe. XXXV. Składniki chemiczne wyciągu z Reichardia tingitana L. Roth, i ich własności deterrentne

Streszczenie

Ponowne przebadanie zawartości wyciągu z *Reichardia tingitana* L. Roth. wykazało obok znanych laktonów seskwiterpenowych, 14-deoksylaktucyny (II) i desacetylomatrykaryny (III), również obecność cholesterolu (V), stigmasterolu (VI), β -sitosterolu (VII) i alkoholu cerylowego (VIII). Stwierdzono aktywność deterrentną związków II i III w stosunku do wybranych gatunków owadów — szkodników magazynów zbożowych.