Eur. J. Entomol. **90**: 65–70, 1993
ISSN 1210–5759

Antifeedant activity of some sesquiterpenoids of the genus *Lactarius* (Agaricales: Russulaceae)

WŁODZIMIERZ M. DANIEWSKI[1], MARIA GUMULKA[1], KATARZYNA PTASZYŃSKA[1], PIOTR SKIBICKI[1], ELŻBIETA BŁOSZYK[2], BOHDAN DROŻDŻ[2], STURE STROMBERG[3], TORBJÖRN NORIN[3] and MIROSLAV HOLUB[4]

[1]Institute of Organic Chemistry, Polish Academy of Sciences, ul. Kasprzaka 44, 01-224 Warsawa, Poland

[2]Department of Medicinal Plants, Academy of Medicine, ul. Mazowiecka 33, 60-623 Poznań, Poland

[3]Royal Institute of Technology, Department of Organic Chemistry, S-100 44 Stockholm, Sweden

[4]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nám. 2, 166 10 Praha 6, Czech Republic

Antifeedants, *Sitophilus granarius*, *Tribolium confusum*, *Trogoderma granarium*, substances of Fungi, *Lactarius* sp.

Abstract. A test with wafer discs was used to examine the ability of 20 compounds to inhibit feeding in adults of *Sitophilus granarius* and *Tribolium confusum* as well as in the larvae of *Tribolium confusum* and *Trogoderma granarium*. The compounds tested were sesquiterpenoids of lactarane, isolactarane, marasmane and 13-normarasmane skeletons, either isolated from species of *Lactarius* or their synthetically prepared derivatives. The obtained results of the antifeedant activity are discussed.

INTRODUCTION

Some of us (W.M.D., M.G. and P.S.) have been studying the isolation and structural estimation of sesquiterpenoids from *Lactarius* Pers. ex S.F. Gray (Agaricales: Russulaceae) (Daniewski et al. 1989; 1990; 1991; references cited in Ayer & Browne, 1981). The majority of sesquiterpenoids of this genus is formed from velutinal (1)* (Favre-Bonvin et al., 1982; Sterner et al., 1983) – a common precursor – by enzymatic process (Sterner et al., 1985). Sterner (1985) studied the process in *Lactarius vellereus* (Fr.) Fr. and found that during the first stages – among others – the unstable compounds, velleral (2) (Magnusson et al., 1973) and isovelleral (3) were formed (Magnusson et al., 1972; List et al., 1969). Compounds 2 and 3 were found to possess bacteriostatic activity (Sterner, 1985) and isovelleral (3) showed very strong mutagenic activity (Sterner et al., 1982). It is thought that the formation of sesquiterpenoids from velutinal esters (1b) could represent one of the chemical defence mechanisms of individuals of *Lactarius* species against pests (e.g., insects, moulds, snails etc.) (Sterner, 1985).

* Chemical structures of all discussed substances 1–23 are shown in Fig. 1

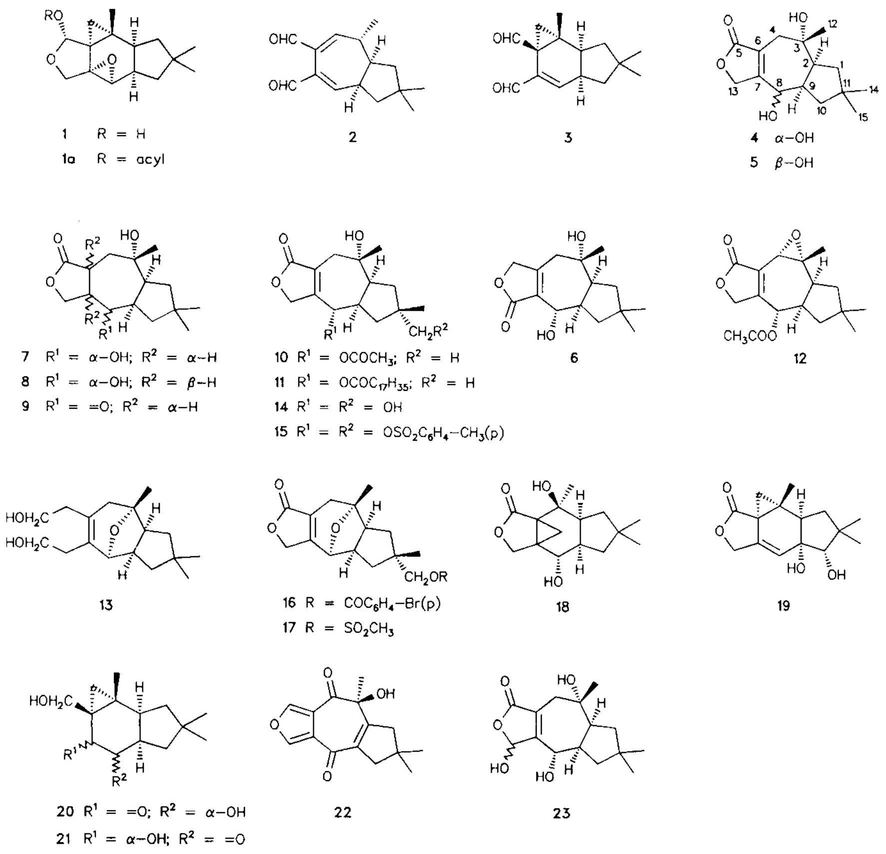

Fig. 1: Chemical structures of substances 1–23.

MATERIAL AND METHODS

Twenty sesquiterpenoids (4–23) were tested, (Table 1), some of them were natural products (4, 14, 18–23), the others were the synthetically prepared derivatives of natural substances (5–13, 15–17). Two of these derivatives (11 and 12) have not been described in the literature.

TABLE 1. List of tested compounds (name of compound, number of its formula, reference).

Lactarorufin A (4) Daniewski & Kocór, 1970
8-Epilactarorufin A (5) Daniewski & Kocór, 1971
5-Desoxylactarolide B (6) Daniewski et al., 1983
6αH,7αH-Dihydrolactarorufin A (7) Daniewski et al., 1983
6βH,7βH-Dihydrolactarorufin A (8) Daniewski et al., 1983
6βH,7βH-Dihydro-8-oxolactarorufin A (9) Daniewski & Kocór, 1971
Lactarorufin A-8-O-acetate (10) Daniewski & Kocór, 1970
Lactarorufin A-8-O-stearate (11) this communication

3α,4α-epoxy-13-hydroxy-8-acetoxylactar-6-en-5-oic acid γ-lactone (12) this communication
3α,8α-epoxy-5,13-dihydrolactar-6-en (13) Daniewski & Kocór, 1971
Lactarorufin B (14) Daniewski & Kocór, 1970
Lactarorufin B-8,14-ditosylate (15) Daniewski et al., 1973
Lactarorufin B-3,8-epoxy-14-p-brombenzoate (16) Daniewski et al., 1973
Lactarorufin B-3,8-epoxy-14-mesylate (17) Daniewski et al., 1973
Isolactarorufin (18) Daniewski & Kocór, 1970
9α,10α,13-trihydroxymarasm-7-en-5-oic acid γ-lactone (19) Daniewski et al., 1988
5β,8α-dihydroxy-13-normarasm-7-one (20) Daniewski et al., 1988
5β,7α-dihydroxy-13-normarasm-8-one (21) Daniewski et al., 1988
5,13-epoxy-3β-hydroxylactara-2(9),5,7(13)-trien-4,8-dione (22) Daniewski et al., 1991
Lactarolide A (23) Daniewski et al., 1983

Antifeedant activity tests

The tests using the adults of *Sitophilus granarius* L. (Coleoptera) and *Tribolium confusum* Duv. (Coleoptera) and larvae of *T. confusum* and *Trogoderma granarium* Ev. (Coleoptera) were described, in detail, in previous papers (Nawrot et al., 1982; Nawrot et al., 1983; Nawrot et al., 1986; Daniewski et al., 1988). The results of these tests are summarised in Table 2 and 3.

Synthesis of lactarorufin A-8-O-stearate (11)

Lactarorufin A (4) (20 mg, 0.075 mmol) was dissolved in pyridine (2 ml), and stearoyl chloride (55 mg, 0.18 mmol) was added, and the mixture was heated at 60° for 10 hours. The reaction mixture was diluted with chloroform (50 ml) and washed with 5%-aqueous hydrochloric acid, water and dried (magnesium sulphate). After removal of the solvent the residue was passed through a column filled with neutral alumina (2 g) in benzene-ethanol (9 : 1) solvent system. The proper fractions were collected [TLC : benzene-acetone (4 : 1), R_f 0.8] gave lactarorufin A-8-O-stearate (11) (26 mg, 66% yield) as a thick oil $[\alpha]_D^{20}$ -17.7 (c 0.78, methanol). IR spectrum ($CHCl_3$; cm^{-1}): 3 600 (hydroxyl), 1 760 (γ-lactone and ester), 1 700 (double bond). ^{1}H-NMR spectrum (200 MHz; $CDCl_3$): 1.2–1.8 m (4H): 1αH, 1βH, 10αH, 10βH; 2.45–2.8 (4H): H(2), H(4), H(9); 5.77 d (J = 8.5 Hz): H(8); 4.65 q: H(13); 0.88 t: H(33); 1.25 m: H(18)-H(32); 2.37 t: H(17). For $C_{33}H_{56}O_5$ (563.8) calculated: 74.4% C, 10.6% H; found: 74.2% C, 10.7% H.

Synthesis of 3α,4α-epoxy-13-hydroxy-8-acetoxylactar-6-en-5-oic acid γ-lactone (12)

Anhydrolactarorufin A-8-O-acetate (Daniewski & Kocór, 1971) (600 mg, 2.5 mmol) was dissolved in dry chloroform (30 ml) and m-chloroperbenzoic acid (200 mg) was added and the mixture was stirred, at room temperature, for 18 hours. Then the mixture was diluted with chloroform (100 ml) and washed with aqueous 5%-sodium hydrocarbonate and water. After drying (magnesium sulphate) and filtration, the solvent was removed and the residue was chromatographed on silica gel (50 g) in a hexane-acetone gradient solvent system. Proper fractions were collected and gave the mixture of α- and β-epoxides (425 mg; 67% yield). The analytical HPLC of this mixture of epoxides in triple solvent system hexane-chloroform-ethylacetate (23 : 23 : 4) gave small amount of substance 12, m.p. 141–142°C. IR spectrum (Nujol; cm^{-1}): 1 760 (γ-lactone), 1 740 (acetate), 1 600 (double bond). ^{1}H-NMR spectrum (500 MHz; $CDCl_3$): 1.71 dd: H(1); 1.60 t: H(1'); 2.81 ddd: H(2); 3.56 s: H(4); 6.66 d: H(8); 1.54 dd: H(10); 1.38 dd: H(10'); 1.41 s: H(12); 4.72 d: H(13); 4.52 d: H(13'); 1.07 s: H(14); 1.07 s: H(15); 2,14 s: H(17). J (Hz): 2, 1 = 5.8; 1,1' = 12.7; 1',2 = 12.7; 2,9 = 7.2; 8,9 = 10.4; 10,10' = 14; 9, 10' = 2.4; 13,13' = 17.9. Mass spectrum (70 Ev; m/z; relative intensity in %): 264 ($M-CH_2CO$; 55), 246 (M-60; 34), 228 (M-60-18; 25), 217 (13), 203 (45), 190 (21), 175 (18), 159 (20), 139 (40), 123 (13), 105 (15), 95 (24), 81 (23), 69 (26), 43 (100).

The melting point was determined on a Kofler block and it was not corrected. Thin layer chromatography was performed on silica gel G according to Stahl (Merck). The mass spectrum was measured with Finnigan Mat 8222 mass spectrometer. The IR spectra were measured on a Beckman 42–40 spectrometer. HPLC was performed using Waters liquid chromatograph. The ^{1}H-NMR spectra were measured on a Brucker AM 500 (500 MHz) and Varian Gemini 200 (200 MHz) spectrometers.

TABLE 2. Total coefficients of deterrency[a].

Compound number	*Tribolium confusum*		*Trogoderma granarium*	*Sitophilus granarius*
	adults	larvae	adults	larvac
4	163.8	144.9	148.6	88.6
5	78.9	59.3	−17.4	−11.9
6	108.7	150.0	93.3	73.4
7	103.4	88.4	9.7	18.0
8	92.5	−3.2	16.6	35.3
9	83.8	114.0	136.0	−1.2
10	78.2	117.7	b	b
11	74.4	52.6	b	b
12	119.2	22.4	8.4	40.0
13	98.2	102.2	135.7	41.4
14	133.3	67.9	92.5	37.2
15	93.7	76.2	−24.2	17.5
16	10.1	−34.0	−33.6	2.8
17	21.9	15.8	27.9	26.0
18	57.8	7.7	8.7	90.3
19	83.7	47.9	162.7	70.7
20	166.7	125.4	b	b
21	98.4	81.2	b	b
22	−31.6	124.0	122.0	190.0
23	70.9	94.9	91.7	86.0

[a]The total coefficient of deterrency between 200–150 is thought as very strong antifeedant, between 150–100 as strong one, between 100–50 as medium one, between 50–0 as week one and between 0 to −100 as atractant.

[b]Not tested in respect of the insufficient amount of the substance of question.

TABLE 3. The average intensity of deterrency[a]

Compound number	Average intensity	Compound number	Average intensity
4	136.5	14	82.7
5	27.2	15	40.8
6	106.3	16	−13.7
7	54.8	17	22.9
8	35.3	18	41.1
9	83.2	19	91.2
10	97.9[b]	20	146.0[b]
11	63.5[b]	21	89.8[b]
12	47.5	22	101.1
13	94.4	23	85.9

[a] The sum of total coefficients of deterrency of one compound divided by the number of total coefficients summarised.

[b] Based on the experiments with two instars of *Tribolium confusum*.

RESULTS AND DISCUSSION

The results of the antifeedant activity tests of sesquiterpenes 4–23 are summarised in Tables 2 and 3. The natural substances tested possessing lactarane skeleton included compounds 4, 14, 22 and 23. Considering the antifeedant activity of these compounds, it was obvious that within the lactarane skeleton the activity depended upon the functional groups present in each molecule and, therefore, synthetic derivatives were prepared and their antifeedant properties were measured, to find possible structure activity relationship.

By comparison with an average intensity of deterrence of all the compounds tested (Table 3) we found that, in general, the deterrence of natural products (4, 14, and 18–23, isolated from some species of *Lactarius* genus) was substantially higher, than that of synthetic derivatives. This is especially expressive in the case of both adults of *Sitophilus granarius* and larvae of *Trogoderma granarium.* The natural products could be classified as strong antifeedants according to the simplified scale of deterrency (footnote in Table 2). In both mentioned instars of *Tribolium confusum* the difference in the activity of natural products and their derivatives is not so large, and all the compounds tested may be regarded as strong or medium, antifeedants. The antifeedant activity in *Tribolium confusum* larvae ranges from almost very strong (substance 4) to opposite reaction (attraction of insects by compounds 8 and 16). If the deterrency coefficients of all of the compounds tested against adults only are considered, it can be seen that the compounds are more effective antifeedants against *Tribolium confusum* (2 – very strong, 5 – strong, 11 – medium, 2 – week) than they are against *Sitophilus granarius* (1 – very strong, 5 – medium, 6 – week). The comparison of the antifeedant activity of all the compounds tested against adults and larvae within one species, i.e., *Tribolium confusum*, showed that in 70% cases the compounds have stronger activity against adults and in the remaining 30% cases the activity is reversed.

Compounds 4 and 22 showed very strong antifeedant activity. Compound 4 is formed in all of the bitter and acrid tasting species of *Lactarius* and it is an universal antifeedant. Only in *Sitophilus granarius* is the deterrency coefficient smaller (88.6). Compound 22, the diketo-hydroxy-furan, is a very strong – almost perfect – antifeedant against *Sitophilus granarius*, (190.0), *Tribolium confusum* and *Trogoderma granarium* larvae, but, to our surprise, it is an attractant for *Tribolium confusum* adults.

With reference to the antifeedant activity of sesquiterpenes of lactarane skeleton (4–17, 22 and 23) it may be said, that their activity depends mainly on the functional groups. The change of stereochemistry of hydroxyl group at C(8) in compound 4 deprived its activity (see Table 2). Similarly the saturation of C(6)–C(7) double bond of compound 4 in order to produce compounds 8 and 9 decreased the activity, especially in the case of the dihydroderivative 8. Oxidation of the secondary hydroxyl group of the substance 7 to produce the keto derivative (compound 9) increased its activity. Esterification of the C(8) hydroxyl group of the substance 4 to give the compound 10 (acetate) or 11 (stearate) decreased the deterrent activity. The same decrease of activity was observed when both hydroxyl groups [on C(8) and C(14)] of compound 14 were esterified (formation of substance 15). Among sesquiterpenes containing marasmane and C(13)-nor marasmane skeletons (compounds 19–21) it was found, that the substance 19 showed very strong antifeedant activity against *Trogoderma granarium* larvae (162.7). Also compound 20 showed very strong activity against adults of *Tribolium confusum.* Unfortunately small amounts of substances 20 and 21 did not allow us to perform the tests on all tested insect species.

Considering all the results we can say that among the compounds tested there was no universally very strong antifeedant for all the storage pests (compound 4 being the best). Mutilation of the flesh of bitter tasting *Lactarius* species triggers an enzymatic process in which velutinal (1) is transformed into a series of sesquiterpenes which are complementary in their protection of the species against such predators as bacteria, insects, snails, etc. In order to draw more conclusions on structure antifeedant activity relationship more examples of compounds must be tested.

This study was financed through the interdepartmental program C.P.B.P. 01.13.2.21.

REFERENCES

AYER W.A. & BROWNE L. 1981: Terpenoid metabolites of mushrooms and related Basidiomycetes. *Tetrahedron* **37**: 2199–2248.

DANIEWSKI W.M., GUMULKA M., PTASZYŃSKA K., SKIBICKI P., FRONZA G. & VIDARI G. 1989: New sesquiterpenoid triol of marasmane skeleton from Lactarius vellereus. *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **37**: 283–287.

DANIEWSKI W.M., GUMULKA M. & SKIBICKI P. 1990: Furantriol, a lactarane sesquiterpene from Lactarius mitissimus. *Phytochemistry* **29**: 527–529.

DANIEWSKI W.M., GUMULKA M., SKIBICKI P., KRAJEWSKI J. & GLUZIŃSKI P. 1991: 2(3)-8(9)-Bisanhydrolactarorufin A and a highly oxygenated furanol from Lactarius vellereus. *Phytochemistry* **30**: 1326–1328.

DANIEWSKI W.M. & KOCÓR M. 1970: Isolation and structure of some new sesquiterpenes from Lactarius rufus. *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **18**: 585–593.

DANIEWSKI W.M. & KOCÓR M. 1971: Structure of lactarorufin A. *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **19**: 553–561.

DANIEWSKI W.M., KOCÓR M. & ŻÓŁTOWSKA B. 1973: Structure of lactarorufin B. *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **21**: 785–792.

DANIEWSKI W.M., KROSZCZYŃSKI W. & KRÓL J. 1983: Regioselectivity of furan oxidation induced by neighbouring hydroxyl groups. Transformation of furandiol into lactarorufin A. *Polish J. Chem.* **57**: 483–487.

DANIEWSKI W.M., KROSZCZYŃSKI W., SKIBICKI P., DE BERNARDI M., FRONZA G., VIDARI G. & VITA-FINZI P. 1988: Nor-marasmane sesquiterpenes from Lactarius vellereus. *Phytochemistry* **27**: 187–191.

DANIEWSKI W.M., SKIBICKI P., GUMULKA M., DROŻDŻ B., GRABARCZYK H. & BŁOSZYK E. 1988: Constituents of Reichardia tingitana (L.) Roth. and their antifeedant activity. *Acta Soc. Botan. Polon.* **57**: 539–545.

FAVRE-BONVIN J., GLUCKHOFF-FIASSON K. & BERNILON J. 1982: Structure du stéaryl-velutinal, sesquiterpénoide naturel de Lactarius velutinus Bert. *Tetrahedron Letters* **23**: 1907–1908.

LIST P.H. & HACKENBERG H. 1969: Velleral und iso-Velleral, scharf schmeckende Stoffe aus Lactarius vellereus Fries. *Arch. Pharm.* **302**: 125–143.

MAGNUSSON G., THOREN S. & DRAKENBERG T. 1973: Structure of a novel sesquiterpene dialdehyde from Lactarius by spectroscopic methods. *Tetrahedron* **29**: 1621–1624.

MAGNUSSON G., THOREN S. & WICKBERG B. 1972: Structure of a sesquiterpene dialdehyde from Lactarius by computer simulation of the NMR spectrum. *Tetrahedron Letters* **13**: 1105–1108.

NAWROT J., BŁOSZYK E., GRABARCZYK H. & DROŻDŻ B. 1982: Deterrent properties of sesquiterpene lactones for selected storage pests. *Pr. Nauk. Inst. Ochr. Rośl.* **24**: 36–37.

NAWROT J., BŁOSZYK E., GRABARCZYK H., DROŻDŻ B., DANIEWSKI W.M. & HOLUB M. 1983: Further evaluation of feeding deterrency of sesquiterpene lactones to storage pests. *Pr. Nauk. Inst. Ochr. Rośl.* **25**: 91–98.

NAWROT J., BŁOSZYK E., HARMATHA J., NOVOTNÝ L. & DROŻDŻ B. 1986: Action of antifeedants of plant origin on beetles infesting stored products. *Acta Entomol. Bohemoslov.* **83**: 327–335.

STERNER O. 1985: *The Russulaceae Sesquiterpenes.* Doctoral thesis. University of Lund, 123 pp.

STERNER O., BERGMAN R., KESLER E., MAGNUSSON G., NILSSON L., WICKBERG B., ZETTERBERG G. & ZIMERSON E. 1982: Forty-eight species screened for mutagenic activity in the salmonella/microsome assay. *Mutation Res.* **101**: 269–276.

STERNER O., BERGMAN R., KESLER E., NILSSON L., OLUWADIYA J. & WICKBERG B. 1983: Velutinal esters of Lactarius vellereus and L. necator. The preparation of free velutinal. *Tetrahedron Letters* **24**: 1415–1418.

STERNER O., BERGMAN R., KIHLBERG J. & WICKBERG B. 1985: The sesquiterpenes of Lactarius vellereus and their role in a proposed chemical defence system. *J. Nat. Prod.* **48**: 279–288.

Received December 2, 1991, accepted May 25, 1992