



## SESQUITERPENES OF *LACTARIUS* ORIGIN, ANTIFEEDANT STRUCTURE-ACTIVITY RELATIONSHIPS

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**Key Word Index**—*Lactarius*; Agaricaceae; sesquiterpenes antifeedant activity; storage pests; *Tribolium confusum*; *Trogoderma granarium*; *Sitophilus granarius*; structure-activity relationships.

**Abstract**—Antifeedant activity of 53 sesquiterpenes of *Lactarius* origin was measured against the storage pests *Tribolium confusum*, *Trogoderma granarium* and *Sitophilus granarius*. The activities of the compounds are discussed in relation to their structures and oxidation state. Conclusions concerning structure-activity relationships have been drawn. It was found that compounds with lactarane and marasmane skeletons were more active than isolactaranes. Also an increasing number of hydroxyl groups present in a molecule decreased its activity. Generally compounds of natural origin, with the exception of keto derivatives, possessed stronger activity than their chemically modified analogues.

### INTRODUCTION

Sesquiterpenes of mushrooms of *Lactarius* are formed in the flesh of the mushrooms by enzymatic transformation of the common precursor called velutinal (1) (Fig. 1) [1, 2]. The precursor is stored in the lipid layers of the cell membranes, and in this way is protected against the action of lipases and other enzymes [3, 4]. It was found that when the flesh of a mushroom is broken, the chemical transformation cascade is triggered and a series of compounds is formed [5]. At the beginning of the cascade two very pungent taste dialdehydes, velleral (2) [6] and isovelleral (3) (Fig. 1), [7] are formed. The enzymes responsible for these transformations are different in every species. This can be demonstrated by the fact that different sets of monohydroxylactones are produced in different species [8]. Similarly, it was shown recently [9] that the yellowing milky cellular juice is formed in *Lactarius chrysorrhoeus* and *L. scrobiculatus*, whereas in other mushrooms *L. rufus*, *L. vellereus* and *L. mitissimus*, the phenomenon is not observed, although sesquiterpenes with the lactarane skeleton are formed in all the species. The compound responsible for yellowing of the mushroom juice has a lactarane skeleton and is very unstable [9]. It was found that the sesquiterpenes formed in the mushrooms constitute their chemical defence against various predators such as bacteria, fungi, animals [10] and insects. The compounds also showed antifeedant properties against the

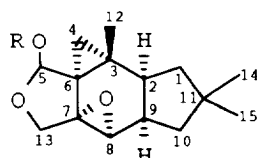
storage pests *Tribolium confusum* Duv., *Trogoderma granarium* Ev. and *Sitophilus granarius* L. as was demonstrated in our previous papers [11-13], where a group of 24 compounds was tested. The tests showed some interesting results; however, because of the limited number of examples, conclusions on structure-activity relationships could not be drawn. Subsequently we have investigated another series (30 compounds) of sesquiterpenes of *Lactarius* origin using the same pests, and after having tested more than 50 compounds, reliable conclusions on structure-activity relationships can now be proposed. For simplicity in the presentation of the antifeedant activity test results, the compounds investigated have been divided into four groups: marasmanes (Fig. 1), lactarane furans (Fig. 2), lactarane lactones (Fig. 3), and isolactaranes (Fig. 4). Each figure is followed by the appropriate table, which includes numbers of compounds, references and activity coefficients.

### RESULTS AND DISCUSSION

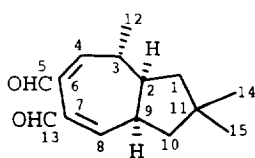
#### *Marasmanes*

Marasmanes (Fig. 1, Table 1) are the sesquiterpenes derived directly from velutinal (1) without change in the carbon skeleton. We have investigated isovelleral (3), the very important bacteriostatic and mutagenic dialdehyde [7], which is believed to be at the beginning of the transformation cascade, and the compound showed very strong selective activity against *Tribolium confusum*, being

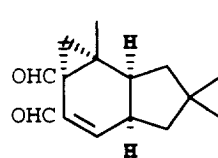
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**1.** R=stearate

Velutinal

**2.**

Velleral

**3.**

Isovelleral

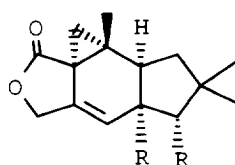
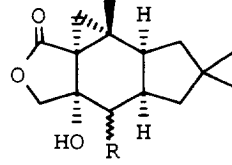
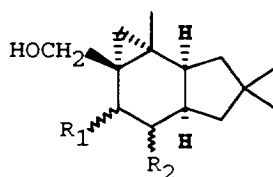
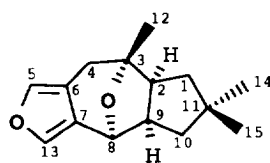
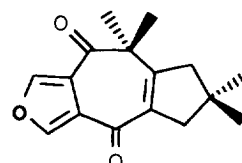
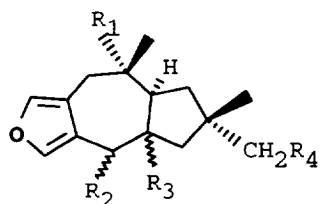
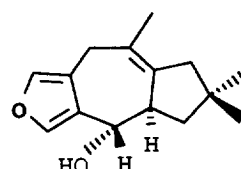
**4.** R=H(13-hydroxy-marasm-7(8)-en-5-oic-acid  $\gamma$ -lactone)**6.** R= $\alpha$ OH,(7 $\alpha$ ,8 $\alpha$ -13-trihydroxy-marasm-5-oic-acid  $\gamma$ -lactone)**5.** R=OH(9 $\alpha$ ,10 $\alpha$ ,13-trihydroxy-marasm-7-en-5-oic acid  $\gamma$ -lactone)**7.** R= $\beta$ OH.(7 $\alpha$ ,8 $\beta$ -13-trihydroxy-marasm-5-oic-acid  $\gamma$ -lactone)**8.** R<sub>1</sub>=O, R<sub>2</sub>= $\alpha$ OH(5,8 $\alpha$ -dihydroxy-13-nor-marasm-7-one)**9.** R<sub>1</sub>= $\alpha$ OH, R<sub>2</sub>=O(5,7 $\alpha$ -dihydroxy-13-nor-marasm-8-one)

Fig. 1.

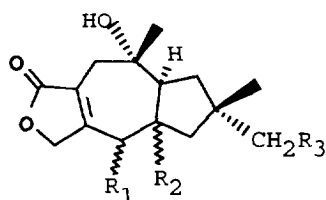
**10.**

(Furanether)

**12.**(5,13-epoxy-3 $\beta$ -hydroxy-lactara-2(9),5,7(13)-trien-4,8-dione)**11.** R<sub>1</sub>=OEt, R<sub>2</sub>= $\alpha$ OH, R<sub>3</sub>= $\alpha$ H, R<sub>4</sub>=H (3-O-ethylfurandiol)**14.** R<sub>1</sub>=OH, R<sub>2</sub>= $\alpha$ OH, R<sub>3</sub>= $\alpha$ H, R<sub>4</sub>=H (Furandiol)**15.** R<sub>1</sub>=OH, R<sub>2</sub>= $\beta$ OH, R<sub>3</sub>= $\alpha$ H, R<sub>4</sub>=H (8-Epi-furandiol)**16.** R<sub>1</sub>=OH, R<sub>2</sub>= $\beta$ OH, R<sub>3</sub>= $\beta$ H, R<sub>4</sub>=H (8-Epi-9-epi-furandiol)**17.** R<sub>1</sub>=OH, R<sub>2</sub>= $\alpha$ OH, R<sub>3</sub>= $\alpha$ H, R<sub>4</sub>=OH (Furantriol).**13.**

(Furanol)

Fig. 2.



18.  $R_1=\alpha\text{OH}$ ,  $R_2=\alpha\text{H}$ ,  $R_3=\text{H}$  (Lactarorufin A)

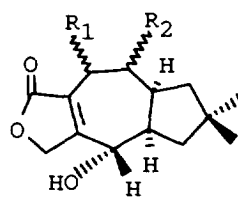
19.  $R_1=\beta\text{OH}$ ,  $R_2=\alpha\text{H}$ ,  $R_3=\text{H}$  (8-Epi-lactarorufin A)

28.  $R_1=\alpha\text{OCOCH}_3$ ,  $R_2=\alpha\text{H}$ ,  $R_3=\text{H}$  (Lactarorufin A-8-O-acetate)

29.  $R_1=\alpha\text{OCOC}_{17}\text{H}_{35}$ ,  $R_2=\alpha\text{H}$ ,  $R_3=\text{H}$  (Lactarorufin A-8-O-stearate)

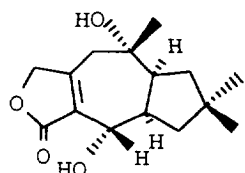
30.  $R_1=\alpha\text{OH}$ ,  $R_2=\alpha\text{H}$ ,  $R_3=\text{OH}$  (Lactarorufin B)

31.  $R_1=\alpha\text{OSO}_2\text{C}_6\text{H}_4\text{-CH}_3$  (p),  $R_2=\alpha\text{H}$ ,  
 $R_3=\text{OSO}_2\text{C}_6\text{H}_4\text{-CH}_3$  (p) (Lactarorufin B-8,14-O-ditosylate)



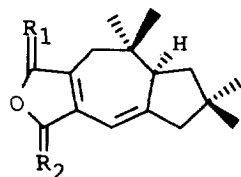
20.  $R_1=\alpha\text{OH}$ ,  $R_2=\beta\text{CH}_3$   
 (Lactarorufin E)

21.  $R_1=\beta\text{OH}$ ,  $R_2=\alpha\text{CH}_3$   
 (3-Epi-lactarorufin D)



22.

(5-Deoxy-lactarolide B)

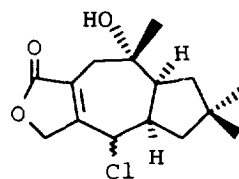


25.  $R_1=\text{O}$ ,  $R_2=2\text{H}$

(8,9-Anhydro-lactarorufin A)

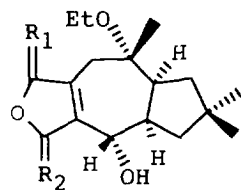
26.  $R_1=2\text{H}$ ,  $R_2=\text{O}$

(Lactaroscrobiculide B)



27.

(8-Chloro-5-desoxy-lactarolide B)

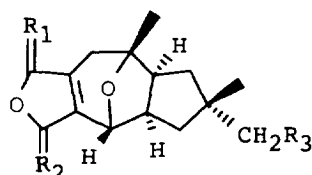


32.  $R_1=\text{O}$ ,  $R_2=2\text{H}$

(3-O-Ethyl-lactarorufin A)

33.  $R_1=2\text{H}$ ,  $R_2=\text{O}$

(3-O-Ethyl-5-deoxylactarolide B)



34.  $R_1=\text{O}$ ,  $R_2=2\text{H}$ ,  $R_3=\text{H}$

(3,8-Oxa-13-hydroxy-lactar-6-en-5-oic acid  $\gamma$ -lactone)

35.  $R_1=2\text{H}$ ,  $R_2=\text{O}$ ,  $R_3=\text{H}$

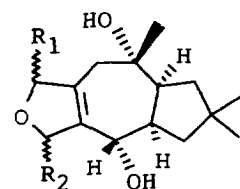
(3,8-Oxa-5-hydroxy-lactar-6-en-5-oic acid  $\gamma$ -lactone)

36.  $R_1=\text{O}$ ,  $R_2=2\text{H}$ ,  $R_3=\text{OCOC}_6\text{H}_4\text{Br}$  (p)

(Lactarorufin B-3,8-epoxy-14-p-benzoate)

37.  $R_1=\text{O}$ ,  $R_2=2\text{H}$ ,  $R_3=\text{OSO}_2\text{CH}_3$

(Lactarorufin B-3,8-epoxy-14-mesylate)

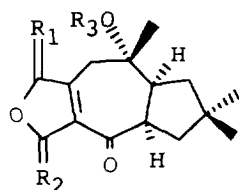


43.  $R_1=\text{O}$ ,  $R_2=\text{OH}$

(Lactarolide A)

44.  $R_1=\text{OH}$ ,  $R_2=\text{O}$

(Lactarolide B)



39.  $R_1=\text{O}$ ,  $R_2=2\text{H}$ ,  $R_3=\text{Et}$

(3-O-Ethyl-8-dehydro-lactarorufin A)

40.  $R_1=\text{O}$ ,  $R_2=2\text{H}$ ,  $R_3=\text{H}$

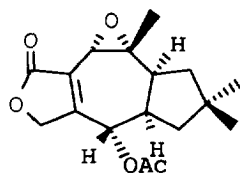
(Lactarorufin A-8-dehydro)

41.  $R_1=2\text{H}$ ,  $R_2=\text{O}$ ,  $R_3=\text{H}$

(5-Deoxy-8-dehydro-lactarolide B)

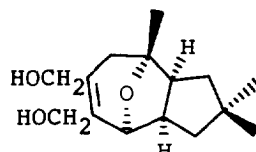
42.  $R_1=2\text{H}$ ,  $R_2=\text{O}$ ,  $R_3=\text{Et}$

(3-O-Ethyl-5-deoxy-8-dehydro-lactarolide B)



45.

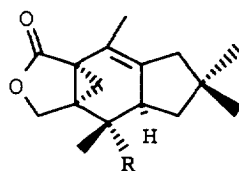
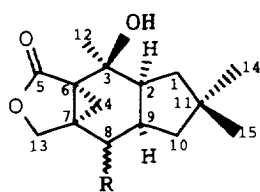
(3 $\alpha$ ,4 $\alpha$ -epoxy-13-hydroxy-8-acetoxylactar-6-en-5-oic acid  $\gamma$ -lactone)



46.

3 $\alpha$ ,8 $\alpha$ -epoxy-5,13-dihydroxylactar-6-en

Fig. 3.



47. R=αOH (Isolactarorufin)

48. R=αOAc (Isolactarorufin-8-O-acetate)

49. R=βOH (8-Epi-isolactarorufin)

50. R=O (8-dehydro-isolactarorufin)

51. R=OH, (3,4-anhydro-isolactarorufin)

52. R=OAc (3,4-anhydro-8-O-acetyl-isolactarorufin)

Fig. 4.

Table 1. Antifeedant activity test (coefficients) of marasmanes of *Lactarius* origin

Compound number [ref.]	<i>Tribolium confusum</i>		<i>Trogoderma granarium</i>	<i>Sitophilus granarius</i>	Average activity Class*
	Adults	Larvae	Larvae	Adults	
3 [7]	163.6	84.5	— 20.3	40.3	64.1 II
4 [19]	162.3	124.3	116.2	82.4	121.3 III
5 [20]	83.7	47.9	162.7	70.7	91.2 II
6 [19]	63.5	109.3	—	—	86.4 II
7 [19]	95.8	75.4	4.1	38.2	53.4 II
8 [20]	—	166.7	125.4	—	146.0 III
9 [20]	—	98.4	82.2	—	90.3 II

\*Below '0', attractant, class I (0–50), poor antifeedant; class II (51–100), medium antifeedant; class III (101–150), good antifeedant; class IV (151–200), very good antifeedant.

inactive against the other species of insects. Within the group a very strong antifeedant activity was exhibited by the 13-C norketone **8**, which is a very good deterrent against *Tribolium confusum* and *Trogoderma granarium*. Also all round good activity was observed for the unhydroxylated lactone **4**. Other compounds of this group showed only moderate activity, with the exception of lactone **5** which was a very good antifeedant against *Trogoderma granarium*.

#### Lactaranes

Compounds which belong to this group are the most numerous and can be subdivided into (a) furans (Fig. 2, Table 2), (b) lactones, lactarolides and miscellaneous (Fig. 3, Table 3).

**Furans.** The best antifeedant properties among the lactaro-furans were recorded for the furanether (**10**), which is an all round very good antifeedant, and the compound has no hydroxyl group. Subsequently strong antifeedant activity was found for compound **11** ('artefact'). Similarly the 4,8-diketo-furanol **12** was a very good antifeedant. The furanol **13** showed good antifeedant activity against *Tribolium confusum* and *S. granarius*, and surprisingly it was an attractant for *Trogoderma granar-*

*ium*. The furans **14–16** possess hydroxyl groups in positions 3 and 8 and showed only moderate activity. The change in stereochemistry at C-8 and C-9 did not have any influence on activity. Introduction of a third hydroxyl group into the molecule (furantriol **17**) at C-14 slightly decreased the antifeedant activity.

**Lactones.** Enzymatic oxidation of furans is responsible for the formation of lactones. The lactones play an important role in chemical defence of bitter tasting *Lactarius* mushrooms. Most of the lactones possess the lactonic carbonyl at position C-5; however, compounds with carbonyl at C-13 were also isolated. The lactones can be obtained by oxidation of furans, as good methods of preparation of desired regioisomers were elaborated [14]. A very potent antifeedant lactarorufin A (**18**) is formed in very hot-tasting *Lactarius* mushrooms. To study the influence of functional groups on activity of lactarane-lactones, **18** was subjected to a series of chemical transformations and compounds thus obtained were tested. The results of biological tests of compounds found in nature, and those of compounds obtained by chemical transformations allowed us to draw several conclusions on structure–activity relationships. Introduction of another hydroxyl group at C-14 (compound **30**) decreased activity similarly, as happened in the case of furandiol.

Table 2. Antifeedant activity test (coefficients) of lactarane furans of *Lactarius* origin

Compound number [ref.]	<i>Tribolium confusum</i>		<i>Trogoderma granarium</i>	<i>Sitophilus granarius</i>	Average activity Class*
	Adults	Larvae	Larvae	Adults	
10 [21]	37.6	178.3	149.5	137.1	125.6 III
11 [11, 22]	178.6	163.8	41.6	173.7	139.4 III
12 [23]	31.6	124.0	122.0	190.0	116.9 III
13 [24]	175.0	152.7	21.0	137.0	110.7 III
14 [14, 24]	115.5	95.7	9.2	60.0	70.1 II
15 [25]	113.7	97.5	22.6	97.9	82.9 II
16 [25]	71.9	23.6	102.7	0.0	49.3 I
17 [26]	110.4	3.2	38.8	64.6	54.2 II

\*Below '0', attractant, class I (0–50), poor antifeedant; class II (51–100), medium antifeedant; class III (101–150), good antifeedant; class IV (151–200), very good antifeedant.

Table 3. Antifeedant activity test (coefficients) of lactarane lactones of *Lactarius* origin

Compound number [ref.]	<i>Tribolium confusum</i>		<i>Trogoderma granarium</i>	<i>Sitophilus granarius</i>	Average activity Class*
	Adults	Larvae	Larvae	Adults	
18 [27]	163.8	144.9	148.6	88.6	136.4 III
19 [28]	78.9	59.3	–17.4	–11.9	27.2 I
20 [29]	—	124.9	—	—	124.9 III
21 [30]	—	103.7	—	—	103.7 III
22 [31]	108.7	150.0	93.3	73.4	106.3 III
23 [27]	103.4	88.4	9.7	18.0	54.8 II
24 [27]	92.5	–3.2	16.6	35.3	35.3 I
25 [32]	145.3	44.3	145.5	–26.1	77.2 II
26 [33]	58.1	58.9	110.5	74.1	75.4 II
27 [this commun.]	134.0	108.8	23.7	32.2	74.6 II
28 [28]	164.7	95.8	94.5	92.0	111.7 III
29 [13]	—	74.4	52.6	—	63.5 II
30 [27]	133.3	67.9	92.5	37.2	83.7 II
31 [34]	93.7	76.2	–24.2	17.5	40.8 I
32 [11]	98.4	150.2	52.3	69.0	92.4 II
33 [11]	123.3	46.7	65.2	94.9	82.5 II
34 [32]	160.9	126.7	73.7	112.1	118.3 III
35 [32]	40.7	—	—	—	40.7 I
36 [34]	10.1	–34.0	33.6†	2.8‡	3.0 I
37 [34]	21.9	15.8	27.9†	26.0‡	22.9 I
38 [27]	83.8	114.0	136.0	–1.2	83.1 I
39 [11]	157.9	174.9	132.0	103.2	142.0 III
40 [27]	134.4	143.6	117.0	119.0	128.5 III
41 [this commun.]	149.0	187.0	100.0	110.8	136.9 III
42 [11]	131.5	118.1	160.0	–2.7	101.7 III
43 [14]	70.9	94.9	91.7	86.0	85.8 II
44 [14]	126.2	88.9	85.6	117.4	105.5 III
45 [13]	119.2	22.4	8.4†	40.0‡	47.5 I
46 [28]	98.2	102.2	135.7†	41.4‡	94.3 II
53 [8, 15]	127.3	118.2	127.5	102.5	118.8 III

\*Below '0', attractant, class I (0–50), poor antifeedant; class II (51–100), medium antifeedant; class III (101–150), good antifeedant; class IV (151–200), very good antifeedant.

†Adults.

‡Larvae.

The change of configuration at C-8 in **18** to produce 8-*epi*-lactarorufin A (**19**) almost deprived **18** of its activity. The shift of the C-3 $\alpha$  hydroxyl group into position 4 $\alpha$  (**20**, lactarorufin E) or 4 $\beta$  (**21**) produced compounds with decreased activity. These, however, were tested on one species only, as only small amounts of these compounds were available. Regioisomeric lactone **22** (carbonyl group at C-13) possessed decreased activity, as compared to that of **18**. Saturation of the 6,7-double bond in **18** gave two dihydro derivatives (**23**, **24**) with much lower activity. Dehydration of **18** and **22** gave two regioisomeric 8,9-anhydro-derivatives **25** and **26**. The activity of **25** was good against *Tribolium confusum* and *Trogoderma granarium* but against *S. granarius* compound **25** is an attractant. The other dehydrated regioisomer **26** was much less active than the parent **22**. Replacement of C-8 hydroxyl group in **22** by chlorine gave a chloroderivative (**27**) which was less active than the parent **22**. C-8 Ester derivatives (**28**, **29**) of **18** were also less active than their parent compound. The prolongation of the acid chain decreased activity even further. Also the 8,14-ditosyl derivative of lactarorufin B (**31**) was less active than the parent **30**. Oxidation of the 'artefact' (**11**) gave two regioisomeric lactones **32** and **33**. The artefact itself is a very strong antifeedant and lactonization of the furan ring was expected to produce more active compounds. Unfortunately the effect was opposite, and **32** and **33** were much less active than their parent compound. The activity of **32** and **33** was similar to that of the mixture of monohydroxy lactones (**53**) from *Lactarius necator* [8, 15], where the oxygen function at C-3 was removed. The regioisomeric lactonic counterpart (**34**, **35**) of the furan ether (**10**) possessed lower activity than the parent compound, this was manifested especially in the C-13 carbonyl derivative (**35**). Ester derivatives of the 14-hydroxylated internal ether, i.e. 14-*p*-bromo-benzoate **36** and 14-mesylate **37** were almost inactive. Therefore the conclusion can be drawn that introduction of an oxygen function in position 14 decreases activity (see Table 3).

The C-8 secondary hydroxyl group in various derivatives of sesquiterpenes with a lactarane skeleton can be oxidized to a keto group. The ketones were found to be

good or very good antifeedants, being the only examples where synthetic derivatives are better or at least not worse antifeedants than the compounds isolated from natural sources. Already the oxidation of the poor antifeedant **21** gave the keto derivative **38** with much improved activity against *Tribolium confusum* and *Trogoderma granarium*. Transformation of **32** into the ketone **39** doubled its antifeedant activity against all the insect species tested. Oxidation of lactarorufin A (**18**), already a potent antifeedant, produced the keto derivative **40** with improved activity against *S. granarius*. The regioisomer of lactarorufin A (**22**) gave the keto derivative **41** with all round improved activity. The moderate activity of **33** upon transformation to its 8-keto derivative (**42**) was much improved against *Tribolium confusum* and *Trogoderma granarium* but decreased against *S. granarius*. Lactarolides (hydroxy-lactones) can be prepared by oxidation of furans and were isolated from extracts of *Lactarius scrobiculatus* [16] and *L. mitissimus* [17]. Introduction of a hydroxyl group into the lactone ring of **18** decreased its activity two-fold (**43**). However this effect was much smaller in the case of **22**, as the 5-hydroxylactone **44** had roughly the same activity as the parent compound.

*Miscellaneous.* Lithium aluminium hydride reduction of the lactone ring in **34** gave the diol **45** which had lower activity than the parent compound. 3 $\alpha$ ,4 $\alpha$ -Epoxy-13-hydroxy-8-acetoxylactar-6-en-5-oic acid  $\gamma$ -lactone **46** was also a weak antifeedant. Perhaps the activity of the last compound could be improved by reinstatement of the C-8 hydroxyl group.

#### Isolactaranes

Isolactarorufin (Fig. 4, Table 4) (**47**) appeared to be, on average, a very weak antifeedant when tested against the storage pests. Strong activity was shown only against the adults of *Tribolium confusum*. Also chemical transformations such as acetylation (**48**), dehydration (**51**, **52**) and epimerization at C-8 (**49**) did not improve antifeedant activity. A slight increase in activity was obtained by introduction of the keto group at C-8 (**50**), as took place in the case of lactarane derivatives.

Table 4. Antifeedant activity test (coefficients) of isolactaranes of *Lactarius* origin

Compound number [ref.]	<i>Tribolium confusum</i>		<i>Trogoderma granarium</i>	<i>Sitophilus granarius</i>	Average activity Class*
	Adults	Larvae	Larvae	Adults	
<b>47</b> [35]	161.0	15.3	-50.4	-7.0	29.7 I
<b>48</b> [35]	97.0	11.0	29.3	19.4	39.2 I
<b>49</b> [35]	128.0	-22.8	42.6	35.3	45.8 I
<b>50</b> [35]	142.0	23.0	9.0	52.0	56.5 II
<b>51</b> [35]	-36.0	57.0	5.0	12.0	12.0 I
<b>52</b> [35]	-49.7	31.1	5.6	-53.4	-16.6atr.

\*Below '0', attractant, class I (0-50), poor antifeedant; class II (51-100), medium antifeedant; class III (101-150), good antifeedant; class IV (151-200), very good antifeedant.

## GENERAL CONCLUSIONS

Considering the results, it was found that sesquiterpenes with lactarane and marasmane skeletons were much more active than those with an isolactarane skeleton. The activity of furans is generally higher than their lactonic counterpart. The activity of furans depends upon the presence of hydroxyl groups in their molecules; the greater the number of hydroxyl groups the lower the activity. No simple correlation can be found between the antifeedant activity of lactones and the number of hydroxyl groups in their molecules. A change in the position of a carbonyl group from C-5 to C-13 in the lactone ring does not improve antifeedant activity. Alteration in the natural characteristic configuration at C-8 causes a decrease in antifeedant activity in both lactones and furans of lactarane skeleton. The principle, stated earlier [15], that the biological activity of natural compounds is higher than those of chemically modified compounds is not always true, as the 8-dehydro-derivatives were more active than their parent compounds.

## EXPERIMENTAL

All the compounds tested were isolated from mushrooms (*Lactarius necator*, *L. rufus*, *L. vellereus*, voucher numbers 33551, 33550 and 32260, respectively, deposited at Department of Systematics and Geography of Plants of University of Warsaw) or synthesized by transformations of natural products; the procedures are given in references in Tables following the Figures.

**Preparation of 8-chloro-5-desoxy-lactarolide B (27).** 5-Desoxylactarolide B (**22**, 120 mg, 0.45 mmol) dissolved in pyridine (8 ml) was treated with mesyl chloride (0.15 ml) at 0°. The reaction was followed by TLC ( $C_6H_6/Me_2CO$  7:3). After 5 hr water was added to the reaction mixture and the resulting products were extracted with  $CHCl_3$ . The extract was washed with 5% aq. HCl soln,  $H_2O$  and dried over  $MgSO_4$ . After removal of the solvent, the resulting products were separated by CC in  $C_6H_6/Me_2CO$  (19:1) and the following compounds were obtained: 1. lactaroscrobiculide B (**26**, 13 mg, 12%); 2. unreacted **22** (60 mg, 50%) and **27** (33 mg, 26%) mp 118–120°; UV  $\lambda_{max}^{EtOH}$  nm 217 ( $\epsilon$  6365);  $[\alpha]_D^{20} + 20.8^\circ$  ( $CHCl_3$ ;  $c$  1.0); IR  $\nu_{max}^{CHCl_3}$   $cm^{-1}$ : 3550, 1770, 1690;  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  1.82 (1H, *dd*,  $J_1 = 13.0$  Hz,  $J_2 = 8.3$  Hz H1 $\alpha$ ), 1.20 (1H, *m*, H1 $\beta$ ), 2.81 (1H, *m*, H2 $\alpha$ ), 2.87, 2.52 (2H, *AB*<sub>q</sub>,  $J = 19.1$  Hz, H4a, 4b), 4.71, 4.66 (2H, *AB*<sub>q</sub>,  $J = 17.4$  Hz, H5a, 5b), 5.12 (1H, *d*,  $J = 2.7$  Hz, H8 $\beta$  or H8 $\alpha$ ), 3.10 (1H, *m*, H9 $\alpha$ ), 1.60 (1H, *dd*,  $J_1 = 12.0$  Hz,  $J_2 = 6.6$  Hz, H10 $\alpha$ ), 1.15 (1H, *m*, H10 $\beta$ ), 1.23 (3H, *s*, H12), 1.05 (3H, *s*, H14), 1.02 (3H, *s*, H15); MS 70 eV  $m/z$  (rel. int.): 284 ( $[M]^+$  2), 249 (20), 231 (15), 215 (10), 206 (12), 191 (18), 174 (11), 161 (13), 153 (23), 137 (11), 123 (11), 105 (20), 95 (40), 83 (23), 69 (33), 55 (49), 43 (100); analysis: found: C, 63.18; H, 7.60%.  $C_{15}H_{21}ClO_3$  required: C, 63.27; H, 7.38; Cl, 12.31%.

**Preparation of 8-dehydro-5-desoxy-lactaroscrobiculide B (41).** 5-Desoxylactarolide B (**22**, 100 mg, 0.38 mmol) was oxidized by the standard method with Jones reagent and

gave **41** (68 mg, 69%) oil;  $[\alpha]_D^{20} = +23^\circ$  ( $CHCl_3$ ;  $c$  1.0); UV  $\lambda_{max}^{EtOH}$  nm 221 ( $\epsilon$  3851), 258 ( $\epsilon$  1293); IR  $\nu_{max}^{nujol}$   $cm^{-1}$ : 1760, 1740, 1680;  $^1H$  NMR (500 MHz,  $CDCl_3$ ): 1.73 (1H, *dd*,  $J_1 = 12.8$  Hz,  $J_2 = 7.9$  Hz, H1 $\alpha$ ), 1.28 (1H, *t*,  $J_1 = 11.1$  Hz, H1 $\beta$ ), 3.35 (1H, *td*, H2 $\alpha$ ), 2.87, 2.74 (2H, *AB*<sub>q</sub>,  $J = 19.8$  Hz, H4a, 4b), 4.76, 4.70 (2H, *AB*<sub>q</sub>,  $J = 18.0$  Hz, H5a, 5b), 2.79 (1H, *td*, H9 $\alpha$ ), 1.66 (1H, *dd*,  $J_1 = 11.1$  Hz,  $J_2 = 12.3$  Hz, H10 $\alpha$ ), 1.53 (1H, *t*, H10 $\beta$ ), 1.35 (3H, *s*, H12), 1.10 (3H, *s*, H14), 0.99 (3H, *s*, H15); high resolution MS  $[M]^+$   $m/z$  264.136315 calc. for  $C_{15}H_{20}O_4$   $[M]^+$  264.13616; MS 70 eV  $m/z$  (rel. int.) 264 ( $[M]^+$ , 11), 246 (51), 231 (60), 217 (43), 190- (72), 125 (43), 111 (58), 97 (80), 71 (81), 57 (100).

**Antifeedant activity test.** The test is described in detail in ref. [18]. Insects (adults and larvae) used for the test were reared under laboratory conditions at a temp. of 26° and 75% humidity. All compounds investigated were dissolved in EtOH at 10 mg ml<sup>-1</sup>. Air-dried wheat wafer discs were used as the test food. The discs (1 cm in diameter) were saturated with EtOH solns of pure compounds to produce 0.5% (by weight) contamination of the wafer in every test. 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 compounds tested (no-choice test). The wafer discs were weighed after satn and drying in air for 30 min before the experiments and again after 7 days of feeding by beetles or larvae. On the basis of eaten food, the index of activity of the compounds tested was calculated in the following way: three values of the food eaten were obtained in the control KK, in the no-choice test EE, and in the choice test K,E.

Thus:

The absolute coefficient of antifeedancy

$$A = \frac{KK - EE}{KK + EE} \times 100,$$

the relative coefficient of antifeedancy

$$R = \frac{K - E}{K + E} \times 100.$$

The total coefficient of antifeedancy is equal to  $T = A + R$ , and its values for compounds **1–53** are presented in Tables 1–4. The maximum value of the coefficient can reach 200 for a perfect antifeedant.

## REFERENCES

1. Favre-Bonvin, J., Gluchoff-Fiasson, K. and Bernillon, J. (1982) *Tetrahedron Letters* **23**, 1907.
2. Sterner, O., Bergman, R., Kesler, E., Nilsson, L., Oluvadiya, J. and Wickberg, B. (1983) *Tetrahedron Letters* **24**, 1415.
3. De Bernardi, M., Vidari, G., Vita-Finzi, P. and Fronza, G. (1992) *Tetrahedron* **48**, 7331.
4. Hansson, T. and Sterner, O. (1991) *Tetrahedron Letters* **32**, 2541.
5. Sterner, O. (1985) *The Russulaceae Sesquiterpenes*, p. 123. Doctoral thesis, University of Lund.

6. Magnusson, G., Thoren, S. and Drakenberg, T. (1973) *Tetrahedron* **29**, 1621.
7. Magnusson, G., Thoren, S. and Wickberg, B. (1972) *Tetrahedron Letters* **13**, 1105.
8. Daniewski, W. M., Kroszczyński, W., Wawrzun, A. and Rymkiewicz, A. (1984) *J. Liquid Chromatography* **7**, 2915.
9. De Bernardi, M., Garlaschelli, L., Toma, L., Vidari, G. and Vita-Finzi, P. (1993) *Tetrahedron* **49**, 1489.
10. Camazine, S. M., Resch, J. F., Eisner, T. and Meinwald, J. (1983) *J. Chem. Ecol.* **9**, 1439.
11. Daniewski, W. M., Gumulka, M., Gluziński, P., Krajewski, J., Pankowska, E., Ptasińska, K., Sitkowski, J. and Błoszyk, E. (1992) *Polish J. Chem.* **66**, 1249.
12. Daniewski, W. M., Gumulka, M., Pankowska, E., Ptasińska, K., Błoszyk, E., Jacobsson, U. and Norin, T. (1993) *Phytochemistry* **32**, 1499.
13. Daniewski, W. M., Gumulka, M., Ptasińska, K., Skibicki, P., Błoszyk, E., Drożdż, B., Stromberg, S., Norin, T. and Holub, M. (1993) *Eur. J. Entomol.* **90**, 65.
14. Daniewski, W. M., Gumulka, M., Ptasińska, K., Skibicki, P., Jacobsson, U. and Norin, T. (1992) *Polish J. Chem.* **66**, 791.
15. Nawrot, J., Błoszyk, E., Grabarczyk, H., Drożdż, B., Daniewski, W. M. and Holub, M. (1983) *Pr. Nauk. IOR.* **25**, 91.
16. De Bernardi, M., Fronza, G., Mellerio, G., Vidari, G. and Vita-Finzi, P. (1979) *Phytochemistry* **18**, 293.
17. Daniewski, W. M., Gumulka, M., Ptasińska, K., Sitkowski, J., Skibicki, P., Jacobsson, U. and Norin, T. (1991) *Bull. Acad. Sci., Ser. Sci. Chim.* **39**, 251.
18. Nawrot, J., Błoszyk, E., Grabarczyk, H. and Drożdż, B. (1982) *Pr. Nauk. IOR.* **24**, 27.
19. Daniewski, W. M., Gumulka, M., Ptasińska, K., Skibicki, P., Krajewski, J. and Gluziński, P. (1992) *Phytochemistry* **31**, 913.
20. Daniewski, W. M., Kroszczyński, W., Skibicki, P., De Bernardi, M., Fronza, G., Vidari, G. and Vita-Finzi, P. (1988) *Phytochemistry* **27**, 187.
21. Andina, P., De Bernardi, M., Del Vecchio, A., Fronza, G., Mellerio, G., Vidari, G. and Vita-Finzi, P. (1980) *Phytochemistry* **19**, 93.
22. Magnusson, G., Thoren, S., Dahmen, J. and Leander, K. (1974) *Acta Chem. Scand. B* **28**, 841.
23. Daniewski, W. M., Gumulka, M., Skibicki, P., Krajewski, J. and Gluziński, P. (1991) *Phytochemistry* **30**, 1326.
24. Nozoe, S., Matsumoto, H. and Urano, S. (1971) *Tetrahedron Letters* **12**, 3125.
25. Daniewski, W. M., Gluziński, P., Gumulka, M., Krajewski, J. and Ptasińska, K. (1994) *Polish J. Chem.* **68**, 287.
26. Daniewski, W. M., Gumulka, M. and Skibicki, P. (1990) *Phytochemistry* **29**, 527.
27. Daniewski, W. M. and Kocór, M. (1970) *Bull. Acad. Sci., Ser. Sci. Chim.* **18**, 585.
28. Daniewski, W. M. and Kocór, M. (1971) *Bull. Acad. Sci., Ser. Sci. Chim.* **19**, 553.
29. Daniewski, W. M., Wawrzun, A., De Bernardi, M., Vidari, G., Vita-Finzi, P., Fronza, G. and Gatti, G. (1984) *Tetrahedron* **40**, 2757.
30. Daniewski, W. M., Gumulka, M., Kamieński, B., Ptasińska, K., Skibicki, P., Jacobsson, U. and Norin, T. (1989) *Bull. Acad. Sci., Ser. Sci. Chim.* **37**, 289.
31. Daniewski, W. M., Kroszczyński, W. and Król, J. (1983) *Polish J. Chem.* **57**, 483.
32. Daniewski, W. M., Gumulka, M., Pankowska, E., Ptasińska, K., Błoszyk, E., Jacobsson, U. and Norin, T. (1993) *Phytochemistry* **32**, 1499.
33. Battaglia, R., De Bernardi, M., Fronza, G., Mellerio, G., Vidari, G. and Vita-Finzi, P. (1980) *J. Nat. Prod.* **43**, 319.
34. Daniewski, W. M., Kocór, M. and Zóltowska, B. (1973) *Bull. Acad. Sci., Ser. Sci. Chim.* **21**, 785.
35. Daniewski, W. M., Kocór, M. and Thoren, S. (1978) *Roczn. Chemii* **52**, 561.