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3,8-ETHERS OF LACTARANE SESQUITERPENES

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Key Word Index—*Lactarius necator*; Basidiomycetes; fruiting body, lactarane 3,8-ethers; antifeedant activity, storage pests: *Tribolium confusum, Trogoderma granarium, Sitophylus granarius.*

Abstract—An ethanol extract of *Lactarius necator* gave 3,8-oxa-13-hydroxy-lactar-6-en-5-oic acid γ-lactone and furanether A, besides other known sesquiterpenes. Syntheses of these internal ethers, as well as the 13-oxa-lactonic analogue, were performed. The antifeedant properties of the internal ethers measured against the storage pests *Tribolium confusum, Trogoderma granarium* and *Sitophylus granarius* are reported.

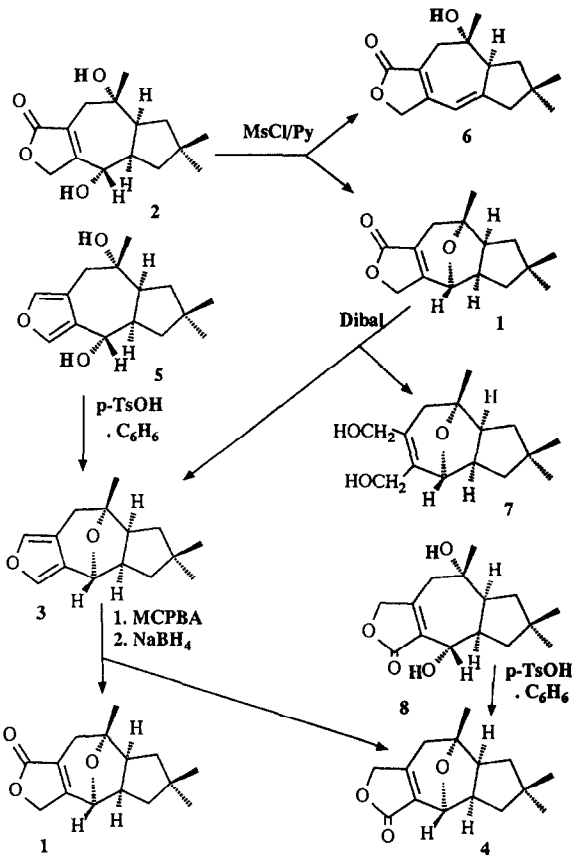
INTRODUCTION

The 3,8-internal ethers of lactarane sesquiterpenes have already been reported [1–3]. The first compound (3,8-oxa-13-hydroxy-lactar-6-en-5-oic-acid γ-lactone, **1**) whose structure has been published was a synthetic derivative obtained by dehydration of lactarorufin A (**2**) [1]. Later, Italian workers isolated furanether A (**3**) from an ethanolic extract of *Russula sardonia* [2] and furanether B from *Lactarius scrobiculatus* [3]. We report now the isolation of compounds **1** and **3** from an ethanolic extract of *Lactarius necator*, transformation of **3** into **1**, synthesis of **3** from furandiol (**5**), and synthesis of a new 3,8-internal ether, 13-oxa-lactone (**4**). Very high antifeedant activity of the 3-*O*-ethyl ether of furandiol [4] against storage pests *Tribolium confusum, Trogoderma granarium* and *Sitophylus granarius* prompted us to check the activity of compounds **1** and **3**, as well as of the synthetic products **4** and **6** shown in Scheme 1.

RESULTS AND DISCUSSION

An ethanolic extract of *Lactarius necator* was obtained by the procedure described earlier [5]. Compound **1** was isolated by HPLC from the fraction containing monohydroxylactones. The structure of **1** was well substantiated by spectroscopy and intercorrelation with lactarorufin A (**2**) [1]. The ^{1}H NMR spectrum (500 MHz) of **1** is presented in Table 1 and is consistent with structure **1**. Assignments of all signals were confirmed by decoupling experiments. Also, the ^{13}C NMR spectrum confirmed structure **1**. The formation of **3** from velutinal has already been demonstrated [6] and one can postulate the formation of **1** from **3** by enzyme assisted oxidation.

The antifeedant activity of **1** as an average appeared to be moderate. However, in the case of *Tribolium confusum* adults the coefficient was very high (Table 2) and almost



Scheme 1.

the same as that of the well known antifeedant azadirachtin [7]. Since lactarorufin A (**2**) and furandiol (**5**) were available we decided to synthesize **1**, **3** and **4** and to check their antifeedant activity against the storage pests

Table 1. ^{1}H NMR spectral data of compounds **1** (400 MHz), **3**, **4** and **6** (500 MHz, $CDCl_3$, TMS as int. standard)

H	1	3	4	6
1α	1.40 *ddd*	1.27 *m*	1.42 *ddd*	1.85 *ddd*
1β	0.90 *m*	1.02 *t*	0.79 *m*	1.68 *t*
2α	2.82 *td*	2.74 *td*	2.80 *td*	3.16 *t*
4α	2.44 *d* ABq	2.67 *d* ABq	2.58 ABq	2.80 *d* ABq
4β	2.34 *d* ABq	2.59 *dd* ABq	2.35 ABq	2.71 *d* ABq
5	—	7.13 *s*	4.77 ABq 4.73 ABq	—
8β	4.61	4.98 *d*	4.72 *d*	5.86 *d*
9α	3.33 *m*	3.20 *m*	3.29 *m*	
10α	1.44 *ddd*	1.27 *m*	1.51 *m*	2.39 ABq
10β	0.90 *m*	0.88 *dd*	0.96 *m*	2.32
12	1.48 *s*	1.45 *s*	1.49 *s*	1.17 *s*
13	4.83 ABq 4.74 BBq	7.14 *s*	—	4.68 *m*
14	1.00 *s*	0.95 *s*	0.98 *s*	1.11 *s*
15	0.98 *s*	0.84 *s*	0.97 *s*	1.05 *s*

J (Hz) **1**: 1α,1β = 12.0; 1α,2α = 8.0; 1β,2α = 11.6; 2α,9α = 11.6; 4α,4β = 18.0; 8β,9α = 7.1; 10α,9α = 4.2; 13a,13b = 17.6; **3**: 1α,1β = 11.7; 1α,2α = 8.0; 1β,2α = 11.7; 2α,9α = 11.7; 4α,4β = 16.2; 8β,9α = 7.0; 10α,10β = 12.6; 10β,9α = 8.4. **4**: 1α,1β = 11.9; 1α,2α = 8.0; 1β,2α = 11.8; 2α,9α = 11.8; 4α,4β = 18.9; 5a,5b = 17.6; 8β,9α = 6.5; **6**: 1α,1β = 12.0; 1α,2α = 8.0; 1β,2α = 12.0; 4α,4β = 18.0; 10α,10β = 17.3.

Table 2. ^{1}H NMR spectral data of compounds **7**, **7a**, **8** and **9** (500 MHz, $CDCl_3$, TMS as int. standard)

H	7	7a	8	9
1α	1.66 *ddd*	1.59 *dd*	1.64 *ddd*	1.61 *m*
1β	1.13 *t*	1.38 *t*	1.32 *t*	1.28 *t*
2α	2.78 *m*	2.66 *m*	2.85 *m*	2.91 *m*
4α	2.70 ABq	2.71 ABq	2.81 ABq	2.78 ABq
4β	2.63 ABq	2.56 ABq	2.61 ABq	2.74 ABq
5	4.65 ABq 4.57 ABq	4.63 ABq 4.57 ABq	—	4.78 ABq 4.65 ABq
8β	4.40 *d*	5.71 *d*	—	—
9α	2.93 *m*	2.66 *m*	3.37 *m*	3.32 *m*
10α	1.55 *ddd*	1.65 *dd*	1.72 *ddd*	1.76 *ddd*
10β	0.96 *t*	1.30 *dd*	1.34 *t*	1.41 *dd*
12	1.23 *s*	1.18 *s*	1.27 *s*	1.28 *s*
13	—	—	5.09 ABq 4.71 ABq	—
14	1.03 *s*	1.07 *s*	1.09 *s*	1.09 *s*
15	1.00 *s*	1.01 *s*	1.03 *s*	1.01 *s*
$MeCH_2$	1.19 *t*	1.14 *t*	0.99 *t*	1.04 *t*
$MeCH_2$	3.60 *qd*	3.44 *qd*	3.39 *qd*	3.43 *qd*
	3.33 *qd*	3.35 *qd*	3.08 *qd*	3.27 *qd*
MeCO	—	2.12 *s*	—	

J (Hz) **7**: 1α,1β = 12.3; 1α,2α = 8.1; 1β,2α = 11.8; 4α,4β = 20.0; 5a,5b, = 17.0; 8β,9α = 2.9; 10α,10β = 12.7; 10α,9α = 6.9; 10β,9α = 12.7; **7a**: 1α,1β = 12.7; 1α,2α = 6.0; 1β,2α = 12.1; 4α,4β = 17.6; 5a,5b = 17.3; 8β,9α = 6.4; 10α,10β = 13.5; 10α,9α = 5.8; 10β,9α = 7.0; **8**: 1α,1β = 12.5; 1β,2α = 12.2; 1α,2α = 7.5; 4α,4β = 19.8; 10α,10β = 12.4; 10α,9α = 7.9; 10β,9α = 12.2; 13a,13b = 17.6. **9**: 1α,1β = 12.2; 1α,2α = 7.4; 1β,2α = 11.9; 4α,4β = 20.5; 5a,5b = 17.9; 10α,10β = 12.8; 10α,9α = 8.0; 10β,9α = 11.5.

to gain information on the structure–activity relationships.

The dehydration of **2** with mesyl chloride in pyridine was investigated. As a side product 8,9-anhydrolactarorufin A (**6**) was isolated in 20% yield. In view of our earlier results [8], where the reduction of lactones to furans with DIBAL gave good yields, it seemed logical to prepare furanether A by the reduction of **1** with DIBAL. Unfortunately, even at low temperature, the reduction of **1** with DIBAL gave only a low yield of **3**, the diol **7** [1] was predominantly obtained. The dehydration of **5** with mesyl chloride in pyridine has been performed [2] but the reported yield of furanether A was only 8%. Therefore, a new method using azeotropic removal of water in the presence of an acid catalyst was developed. The yield of furanether A was improved (18%) but two unidentified products were still formed. Nevertheless, a sufficient amount of **3** was obtained. The oxidation of **3** with MCPBA gave a mixture of hydroxylactones which, after removal of the excess of MCPBA and MCBA, was reduced with sodium borohydride to give the mixture of regioisomeric lactones **1** and **4**. The mixture of lactones was separated by HPLC and gave **1** and **4** in the ratio 2.7 : 1, with the 5-oxo-lactone predominating. The ratio of regioisomers obtained here is opposite to that obtained when **5** or its acetate were oxidized by the same method [8]. A similar ratio of regioisomers was obtained when the 3-deoxy-8-*O*-acetyl-furandiol is oxidized using the same method [Daniewski, W. M., unpublished result]. Therefore, in order to prepare the 13-oxo analogue, a method of dehydration of 5-deoxylactarolide B (**8**) was elaborated. Similarly, as in the case of dehydration of **5** the azeotropic method of removal of water from the acid catalysed reaction was used and **4** was formed in 52% yield. The structure of **4** was substantiated by spectroscopy. The high field ^{1}H NMR spectrum of **4** is presented in Table 1 and is consistent with structure **4**. The only difference between the spectra of **1** and **4** is the chemical shift of protons H-13a, H-13b in the spectrum of **1** (lower field, δ4.83, 4.74), whereas protons H-5a and H-5b in the spectrum of **4** exhibit a signal at slightly higher field (δ4.77, 4.73) because of the larger distance from the oxygen atom. Also the difference in chemical shifts between the signals of protons 'a' and 'b' is larger in the case of **1** for the same reason (i.e. a shorter distance from the oxygen). The structure of **6** is well substantiated by spectroscopy. The UV spectrum of **6** showed typical linear conjugated absorption at λ_{max} 285 nm. In the ^{1}H NMR spectrum of **6** instead of the usual signal for an H-8 doublet, a signal of the H-8 vinylic proton (δ5.86) was observed. Protons H-10α and H-10β showed the typical ABq relationship.

The antifeedant activity tests showed some interesting results, which are presented in Table 3. The strongest antifeedant properties were exhibited by furanether (**3**) which is a very good deterrent. Good deterrent activity was also obtained for the internal-ether-lactone (**1**). Compound **4**, the synthetically prepared regioisomer of **1**, showed poor antifeedant activity against adults and larvae of *Tribolium confusum* (the most sensitive insect)

Table 3. The antifeedant activity test (coefficients) of compounds **1**, **3**, **4** and **6**

Compound	*Tribolium confusum* Duv.		*Trogoderma granarium* Ev.	*Sitophilus granarius* L.	Class*
	Adults	Larvae	Larvae	Adults	
1	160.9	126.7	73.7	112.1	139.4 III
3	37.6	178.0	149.5	137.1	141.1 III
4	40.7	0.0	—	—	20.3 I
6	145.3	44.3	146.5	−26.1	77.5 II

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

and, therefore, its deterrent activity for the remaining pests was not tested. The 3-hydroxylactone (**6**), obtained by dehydration of lactarorufin A (**2**), showed good deterrent activity only against *Tribolium confusum* adults and *Trogoderma granarium* larvae, for *Sitophylus granarius* it is an attractant.

EXPERIMENTAL

Lactarius necator was collected in October 1990 in Zalesie mixed forest near Warsaw and was authenticated by the mycologist Prof. A. Skirgiełło (Warsaw University). A specimen (Voucher no. 33551) is deposited at the Department of Systematics and Geography of Plants, University of Warsaw.

Isolation of compounds **1** *and* **3**. The preparation of an ethanolic extract of *Lactarius necator* and coarse chromatography were carried out in the same way as described earlier [5]. The fraction containing monohydroxylactones (TLC, C_6H_6–Me_2CO, 4:1; R_f 0.7–0.8) was rechromatographed by MPLC using a hexane–EtOAc gradient system monitored by TLC. Fractions possessing R_f 0.3–0.4 (hexane–EtOAc, 4:1) were collected and the solvent evapd. The residue was chromatographed using a series of five 8 mm i.d. × 30 cm columns filled with Lichrosorb 10 μ silica gel with hexane–EtOAc (41:9) solvent system. Peak ($k' = 6.7$) was collected evapd and gave **1** with physicochemical properties the same as reported earlier [1]. ^{13}C NMR (100.614 MHz, $CDCl_3$); δ 31.2 (*t*, C-1), 54.1 (*d*, C-2), 80.7 (*s*, C-3), 42.0 (*t*, C-4), 172.6 (*s*, C-5), 165.6 (*s*, C-6), 124.8 (*s*, C-7), 74.6 (*d*, C-8), 55.9 (*d*, C-9), 40.0 (*t*, C-10), 47.0 (*s*, C-11), 29.1 (*q*, C-12), 71.5 (*t*, C-13), 27.0 (*q*, C-14), 27.2 (*q*, C-15); MS 70 eV *m/z* (rel. int.): 248 (100) $[M]^+$, 233 (30), 230 (17), 218 (35), 206 (40), 191 (32), 187 (25), 175 (22), 161 (25), 149 (40), 145 (25), 133 (17), 119 (35), 105 (51), 95 (60), 91 (43), 77 (30), 69 (33), 55 (22), 43 (93).

Preparation of 3,8-*oxa*-13-*hydroxy-lactar*-6-*en*-5-*oic acid* γ-*lactone* (**1**) *and* 8,9-*anhydrolactarorufin A* (**6**). Lactarorufin A (**2**) (100 mg, 0.38 mmol) dissolved in pyridine (8 ml) was treated with mesyl chloride (355 mg, 3.10 mmol). The reaction mixture was left at room temp. for 2 hr and TLC (C_6H_6–Me_2CO) showed that all **2** was transformed into the mesyl ester. Subsequently, H_2O (70 ml) was added and the mesylate was extracted with $CHCl_3$ (3 × 50 ml). The extract was washed with H_2O (40 ml) dried ($MgSO_4$), filtered and the solvent removed. The residue was dissolved in pyridine (10 ml) and heated under reflux for 2 hr. The reaction course was followed by TLC. From the mesylate two products were formed: a less polar compound **1** and **6**. The reaction mixture was cooled, H_2O (100 ml) was added, and extracted with $CHCl_3$ (3 × 50 ml). The extract was dried ($MgSO_4$), filtered and the solvent removed leaving a residue which was chromatographed on silica gel with C_6H_6–Me_2CO (19:1) and gave **1** (50.3 mg, 54%) and **6** (18.0 mg, 19%) mp 75–78°; $[\alpha]_D^{20}$ + 334.5° ($CHCl_3$; *c* 0.6); UV λ_{max}^{EtOH} nm 285.2 (ε 10 220); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1690; High resolution MS: $[M]^+$ *m/z* 248.1411, calculated for $C_{15}H_{20}O_3$ $[M]^+$ *m/z* 248.1412; MS 70 eV *m/z* (rel. int.): 248 $[M]^+$ (74), 233 (37), 215 (17), 205 (20), 187 (17), 159 (32), 145 (27), 131 (23), 117 (25), 105 (50), 91 (42), 77 (36), 43 (100).

Preparation of 3,8-*oxa*-5-*hydroxy-lactar*-6-*en*-13-*oic acid* γ-*lactone* (**4**). 5-Deoxy-lactarolid B (**8**) (48 mg, 0.18 mmol) was dehydrated by boiling (8 hr) in C_6H_6 soln in a flask equipped with a Dean–Stark adapter in the presence of a trace of *p*-TsOH. Subsequently the solvent was evapd and the residue chromatographed on silica gel in C_6H_6–Me_2CO (49:1) to give the unreacted **8** (15 mg, 31.1%) and **4** (16 mg, 36%); oil; $[\alpha]_D^{20}$ + 24.61° ($CHCl_3$; *c* 0.4); UV λ_{max}^{EtOH} nm: 217.2 (ε 3123); IR ν_{max}^{film} cm^{-1}: 1760, 1680; High resolution MS: $[M]^+$ 248.1408, calculated for $C_{15}H_{20}O_3$ $[M]^+$ 248.1412; MS 70 eV *m/z* (rel. int.): 248 $[M]^+$ (40), 233 (33), 215 (35), 206 (55), 191 (72), 174 (46), 159 (28), 145 (36), 119 (25), 105 (50), 95 (65), 81 (33), 69 (27), 55 (29), 43 (100).

Preparation of furanether A (**3**). Furandiol (**5**) was dehydrated by the same method as described for the preparation of **4**. Thus, from **5** (285 mg, 1.14 mmol) a mixture was obtained from which by CC on silica gel in C_6H_6–Me_2CO (49:1) furanether A (**3**) (51.3 mg, 18%) plus two other unidentified products were isolated.

Oxidation of furanether A with MCPBA. Compound **3** (10 mg, 0.04 mmol) was oxidized with a large excess of MCPBA by the method elaborated by us [8]. The crude reaction mixture was passed through alumina (1 g) in C_6H_6–EtOH (9:1) to remove the acids. The resulting mixture of hydroxylactones was reduced in EtOH with $NaBH_4$ and gave a mixture of **1** and **4**. Pure lactonic 3,8-ethers were separated by HPLC in hexane–EtOAc–*i*-PrOH (655:327:18) using 8 mm i.d. × 30 cm column

filled with silica gel. Thus, **1** (5.1 mg, 47.3%) and **4** (1.9 mg, 17.5%) were obtained.

Antifeedant activity test. The test is described in detail in ref. [9]. Insects (adults and larvae) used for the test were reared under laboratory conditions at a temp. of 26° and 75% relative humidity. All compounds investigated were dissolved in 96% EtOH at a concn of 10 mg ml^{-1}. Wheat wafer discs were used as the test food. The discs (1 cm in diameter) were satd with EtOH solns of pure compounds. 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 the 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 deterrence,

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

the relative coefficient of deterrence,

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

The total coefficient of deterrence is equal to $T = A + R$, and its values for compounds **1**, **2**, **4**–**9** are presented in Table 3. The maximum value of the coefficient can reach 200 for a perfect antifeedant.

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