Phytochemistry, Vol. 27, No. 1, pp. 187–191, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

NORMARASMANE SESQUITERPENES FROM *LACTARIUS VELLEREUS*

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(*Revised received* 13 *May* 1987)

Key Word Index—*Lactarius vellereus*; Basidiomycetes; marasmane and normarasmane sesquiterpenes; configuration and conformation determination.

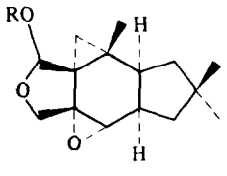
Abstract—Ethanolic extracts of *Lactarius vellereus* gave, in addition to known sesquiterpenes, one new highly oxygenated marasmane lactone and two new 13-normarasmane sesquiterpenes, which are the first representatives of such a class of compounds. Molecular configurations and conformations have been established by spectroscopic methods.

INTRODUCTION

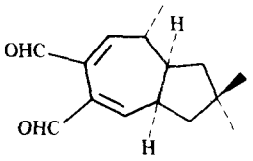
Lactarius vellereus Fr. is one of the most thoroughly investigated species of mushroom because of its peppery taste and apparent resistance to attack by predators such as insects, snails and mammals [1, 2]. Originally only a single marasmane sesquiterpene, the chemically very labile velutinal (**1a**), was isolated from the mushrooms, where it is present as the stearic acid ester **1b** [3]. Whenever fresh mushrooms are injured and then extracted with organic solvents, different sesquiterpenes are isolated which are believed to be formed from stearoyl velutinal (**1b**) by chemical and/or enzymatically triggered reactions [4]. For instance velleral (**2**) and isovelleral (**3**), two biologically active pungent-tasting dialdehydes, are considered to take part in a chemical defence mechanism which is activated when the mushrooms are damaged [4, 5]. The present paper describes the results obtained in a study of the polar sesquiterpenes of *L. vellereus* extracted with ethanol. They could well have escaped detection in earlier investigations because of their tiny amounts (*ca* 30–60 mg each from 52 kg of mushrooms!).

RESULTS AND DISCUSSION

Following the MPLC separation of the extract on a silica gel column, fractions containing dihydroxy sesquiterpenes were collected and further separated by prep. HPLC using reversed phase RP-18 columns (see Experimental). Lactarorufin A (**4**), isolactarorufin (**5**) and furandiol (**6**) were identified by comparison with our own authentic samples isolated from other mushrooms [6, 7]. Besides a mixture of monoglycerides, three new sesquiter-



1a R = H
1b R = Stearoyl



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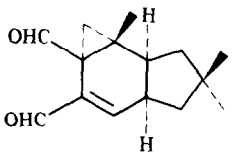

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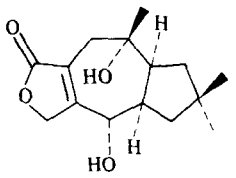

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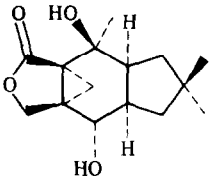

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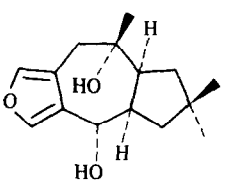

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penes **7a**, **8a** and **12a** were isolated and their structures were established in the following way.

The first isolated compound (**7a**) is a sticky oil with molecular formula $C_{15}H_{20}O_4$ (high resolution MS, NMR counting of H and C atoms) and two easily exchangeable protons (M^+ increased by two units after deuteration). IR bands were attributable to alcoholic hydroxyl and γ-lactone carbonyl stretchings and to olefinic bonds. These features were confirmed by the appropriate signals in the ^{13}C NMR spectrum at δ176.8 (C=O), 141.5 and 115.4 (C=CH-), 86.4 (-CHO), 78.1 (-C-O) and 68.8 (CH_2O). On acetylation with pyridine–acetic anhydride at room temperature a mixture of two compounds was obtained, the less polar compound **7b** contained no free hydroxyl groups (IR), but instead two new acetyl groups (NMR δ1.96*s* and 2.05*s*) were present. Moreover, on acetylation, a significant downfield shift was observed for only one methine proton indicating the presence of one secondary and one tertiary hydroxyl group.

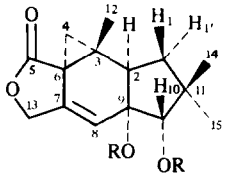
The chemical shift of the olefinic proton in the NMR and the end absorption in the UV spectra of compound **7a** exclude any conjugation of the γ-lactone carbonyl with the double bond; furthermore, useful structural information was inferred from the presence of an allylic coupling between the olefinic CH and the lactone methylene protons. The remaining signals of the high resolution ^1H NMR spectrum (300 MHz) were assigned to three quaternary methyls, to an isolated $-CH_2-CH$ system (AMX pattern) and to one isolated methylene group. The last of these gave rise to an ABq system whose small coupling constant (3.4 Hz) is characteristic for two geminal cyclopropane protons. Their unusually rather low chemical shifts (δ1.64 and 1.92 in $CDCl_3$, respectively) could indeed be due to the withdrawing effect of the carbonyl group [compare isovelleral (**3**): doublets at δ0.95 and 1.88 [8], and methyl marasmate: doublets at δ1.18 and 2.34 [9]].

Attempts to draw the entire structure of the compound also had to accomodate the presence of four quaternary sp^3 carbon atoms (^{13}C NMR spectrum) and the absence of any coupling of the secondary CH-OH.

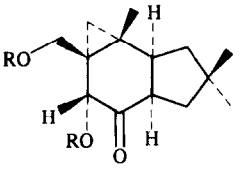
These restrictions and obvious biogenetic considerations made **7a** the structure of choice, not excluding the alternative possibility of placing the two hydroxyl groups at C-1 and C-2 instead of C-9 and C-10. In fact the absence of any vicinal coupling of H-8 does not exclude *a priori* the presence of a proton at C-9, as the $J_{8,9}$ can became negligibly small for some conformations of compounds of this type [1]. In either case the two hydroxyl groups must be vicinal and *syn* to each other, in order to explain the easy acetylation of the tertiary hydroxyl group by C-10 → C-9 migration of the acetyl group [10].

The structure of sesquiterpene **7a**, including the relative configuration at the stereogenic centres, was confirmed by NOEDS experiments in C_6D_6 solution. The selective irradiation of the olefinic proton (H-8) induced in fact a positive NOE on the C-13 protons (1.5%) and on the CH-OH signal (7.3%), indicating that all these protons were on the same side of the polycyclic system, i.e. the 1,2-diol system was at C-9 and C-10. Moreover, irradiation of the methyl signal at δ0.94 resulted in an enhancement of H-2 (6.1%) and only one of the protons at C-1 (5.2%), whereas the saturation of the methyl group at δ0.79 affected the signals of H-8 (2.4%), H-10 (6.1%) and the other proton at C-1 (4.4%), but left H-2 unaffected. These effects clearly indicated that H-2 and H-10 are *trans* to each other on the cyclopentane ring and allowed the assignment of the chemical shifts for H-1, H-1′ and the geminal methyl groups. Finally, the small but clean enhancement of H-2 (2%) by irradiation of H-4 *endo* at δ1.83 established the *cis* relationship between H-2 and the cyclopropane methylene group. The values of $J_{1,2}$ = 12.4 Hz and $J_{1',2}$ = 7.5 Hz, in addition to the observed NOE's showed that H-1 and H-2 are pseudoaxially oriented and that the cyclopentane ring possibly exists in the $^2T_0$ or ^2E conformation [for five- and six-membered rings conformation nomenclature, we followed the rules given originally for aldofuranoses and aldopyranoses, *J. Chem. Soc., Chem. Commun.* (1973), 505]. For such a conformations H-1 lies in the shielding cone of the C-7,8 double bond, which explains the unusual high field shift of this proton (δ0.94 instead of *ca* 1.3 in $CDCl_3$ solution).

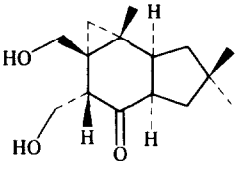
Compound **8a**, contained hydroxyl and saturated ketone groups, but no lactone function (IR). From the NMR data the formula $C_{14}H_{22}O_3$ was calculated, which corresponds to a norsesquiterpene structure. The ^1H NMR spectrum showed the presence of one CH_2OH and one CHOH group, both attached to fully substituted carbon atoms as deduced from the absence of any vicinal coupling. In the diacetate **8b** the methine proton of the secondary acetate (δ 5.82) showed a long range coupling (J = 2.0 Hz) to a very high field shifted signal (δ0.52) which, with the proton at δ0.68, formed an AB system attributable to a cyclopropane methylene group (18.96 ppm in the ^{13}C NMR spectrum). Interestingly these two protons showed an accidental chemical equivalence in the original compound **8a**. The remaining features of the NMR spectra best fitted a tricarbocyclic structure with a 13-normarasmane skeleton. Indeed, direct comparison of the ^1H NMR signal pattern with that of the marasmane lactaropallidine (**9**) [11] clearly indicated that C-7 of sesquiterpene **8a** carries a hydroxyl group instead of the CH_2OH group found for structure **9**. Selective irradiation of the H-7 signal (marasmane numbering) produced an enhancement of the resonances of H-5 (1.3%), H-1 and H-10 (2.5%), whereas H-2, H-9 and C(12)H_3 were not affected. The observed NOEDS effects (performed on diacetate **8b**) firmly established that H-7 was *trans* to the bridgehead protons H-2 and H-9 and that the CH_2OH group had to be placed at C-6, and the methyl at C-3 (not *vice versa*). Moreover, to account for the long-range coupling constant of H-7 (see above), a *trans* relationship must exist between H-7 and C(4)H_2, so that H-7 and H-4$_{exo}$ could interact through a four-bond *W* pathway [12]. Reduction of diacetate **8b** with sodium borohydride in methanol gave a complex mixture of four compounds, **10a**,**b** and **11a**,**b**, as a result of a non-stereoselective reduction of the carbonyl group and acyl migration from C(7)OH to C(8)OH. Acetylation of this mixture gave an inseparable mixture of two stereoisomers **10c** and **11c**, in the ratio 1:6. The major isomer **11c** showed $J_{8,9}$ = 11.5 Hz and $J_{7,8}$ = 4.3 Hz, in accordance with an axial–axial and axial–equatorial interaction of βH-8 with H-9 and H-7 respectively, whereas, for the minor stereoisomer **10c**, $J_{8,9}$ = 7.0 Hz and $J_{7,8}$ = 9.5 Hz suggested an α axial orientation for H-8. These data confirmed the relative configuration of H-7 and H-9 and the location of the carbonyl group at C-8 in the starting compound **8a**. Finally the calculated values of dihedral angles and the observed NOEDS effects suggest, by analogy with lactaropallidine **9**, that the cyclohexanone ring approaches the skew conformation $^3S_1$ and that



7a R = H
7b R = Ac



8a R = H
8b R = Ac



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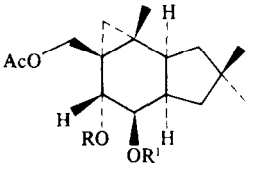
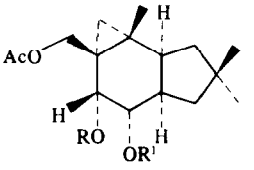
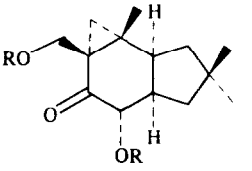


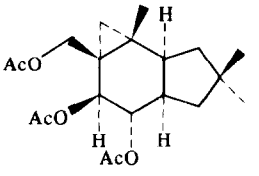
10a R = Ac, R¹ = H
10b R = H, R¹ = Ac
10c R = R¹ = Ac



11a R = Ac, R¹ = H
11b R = H, R¹ = Ac
11c R = R¹ = Ac



12a R = H
12b R = Ac



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the cyclopentane ring probably adopts the envelope conformation ^{11}E or E_1.

The last product isolated from *L. vellereus* was an isomer of the normarasmane **8a**. Detailed analysis of the NMR data showed no proton at C-7 and the presence of a secondary hydroxyl group at C-8, the two protons H-8 and H-9 mutually interacting with $J = 11.5$ Hz. Reduction of the diacetate **12b** with sodium borohydride in methanol followed by acetylation, gave an inseparable mixture of two triacetyl derivatives, in the ratio 2.5:1. The minor compound showed NMR signals identical with those of **11c**, previously obtained from ketone **8b** by analogous reactions, while the major isomer is the C-7 epimer **13**. These data are consistent with structure **12a** for this new 13-normarasmane. The reversed hybridation of C-8 and C-9, with respect of ketol **8a**, has a marked effect on the molecular shape of compound **12a**. The coupling constants $J_{1-2} = 13.0$ and $J_{9-10} = 1.1$ Hz indicate that H-2 and H-1 are *trans* diaxially oriented while H-9 and H-10 are *trans* diequatorially oriented, therefore the cyclopentane ring probably exists in the E_9 or $^2T_9$ conformation. Dreiding models showed that in either conformation Me-14 and H-8 are close in space, while Me-15 and H-9 are much more distant. This conclusion has been confirmed by the appropriate NOE effects: enhancements of H-1 (3%), H-10 (5.3%) and H-8 (6%) by irradiation of Me-14; enhancement of H-1′, (5.7%), H-2 (7.3%) and H-10′, (8%) by irradiation of Me-15. Moreover the preferred conformation of the cyclohexanone ring now approaches the chair-boat with C-8 below the mean plane.

Sesquiterpene **7a** is the first marasmane with an oxygenated function on the cyclopentane ring, while compounds **8a** and **12a** represent the first examples of the 13-normarasmane class; the latter ketones can derive form lactaropallidine **9** by β-elimination and oxidation at C-7. As they are not formed during the solvolysis of stearoyl velutinal in alcohols [13], we believe they are formed from stearoyl velutinal by some enzyme catalysed reactions. For biogenetic reasons the formulae **7a**, **8a** and **12a** must then represent the absolute configuration of the molecules. The new compounds showed no activity against *Bacillus subtilis*, *Staphylococcus oxford*, *Escherichia coli* and *Candida albicans*.

EXPERIMENTAL

Mps (Fisher Johns hot plate): uncorr.; ^{1}H NMR: 300 MHz, in $CDCl_3$ soln unless otherwise indicated with TMS as int standard. ^{13}C NMR: 75.47 MHz, $CDCl_3$ (which also provided the lock signal), TMS as int. ref. Assignments of ^{13}C chemical shifts were made with the aid of off-resonance and noise decoupled ^{13}C NMR spectra. Compounds were visualized on GF_{254} silica gel plates as coloured spots by spraying with a vanillin–H_2SO_4 soln and then heating at 120° for 10 min. The identity of the

mushroom was checked by Prof. A. Skirgiello (University of Warsaw).

Extraction and isolation of sesquiterpenes from L. vellereus. Fresh mushrooms (52 kg) collected in Zalesie mixed forest near Warsaw (Poland) in September 1985 were ground and then soaked in EtOH for 45 days and the extract was processed essentially in the same way as described in a previous paper [14]. The residue (25 g), by MPLC on a silica gel column (1 kg) with a C_6H_6–Me_2CO gradient system (TLC monitoring), gave a fraction containing dihydroxy sesquiterpenes (R_f 0.2–0.4, C_6H_6–Me_2CO 4:1). The latter (4.8 g) was separated into 5 groups of compounds (I–V) on a reversed phase RP-18 column (15–25 μm, MeOH–H_2O 3:2). I (1.1 g): mainly isolactarorufin (**5**); II (550 mg): a mixture of lactarorufin A (**4**) and compound **7a**; III (400 mg): mixture of **8a** and **12a**; IV (950 mg): furandiol (**6**); V (2.0 g): monoglycerides, washed out with MeOH. Fraction II was separated using a series of five HPLC columns (30 cm length × 8 mm i.d.; Waters instrument, RI detector), filled with Lichrosorb Si 60 (10 μm) and a mixture of $CHCl_3$–hexane–*iso*-PrOH (12:12:1) as eluant, to afford 43 mg of pure diol **7a**. Analogous prep. HPLC of fraction III, with the same solvent system, gave 5,7α-dihydroxy-13-normarasman-8-one (**8a**, 62 mg) and the corresponding isomer **12a** (32 mg).

9α,10α,13-*Trihydroxy-marasm*-7(8)*en*-5-*oic acid* γ-*lactone* (**7a**). Sticky oil which could not be induced to crystallize, $[\alpha]_D^{20}$ + 11.2° (CH_2Cl_2, c = 0.5); IR ν_{max}^{film} cm^{-1}: 3420 (OH), 1760 (γ-lactone (C=O), 1685 (C=C), 1455, 1400, 1380, 1365, 1330, 1290, 1215, 1150, 1065, 1005, 930, 910, 880, 845, 800, 768, 735; ^{1}H NMR (C_6D_6): δ0.60 (1H, *t*, $J_{1-1'}$ = J_{1-2} = 12.4 Hz, H-1), 0.79 (3H, *s*, H_3-14), 0.94 (3H, *s*, H_3-15), 1.34 (1H, *dd*, $J_{1'-1}$ = 12.4, $J_{1'-2}$ = 7.5 Hz, H-1'), 1.41 (3H, *s*, H_3-12), 1.49 (1H, *d*, $J_{4-4'}$ = 3.4 Hz, H-4$_{exo}$), 1.83 (1H, *d*, $J_{4-4'}$ = 3.4 Hz, H-4$_{endo}$), 2.26 (1H, *dd*, J_{1-2} = 12.4 Hz, $J_{1'-2}$ = 7.5 Hz, H-2), 2.58 and 2.73 (2 × 1H, 2 *brs* exchangeable with D_2O, 2 OH), 3.00 (1H, *s*, H-10) 4.26 (1H, *dd*, $J_{13-13'}$ = 13.5, J_{13-8} = 1.8 Hz, H-13), 4.37 (1H, *dd*, $J_{13'-13}$ = 13.5, $J_{13'-8}$ = 2.5 Hz, H-13'), 4.65 (1H, *br t*, $J_{13'-8}$ = 2.5, J_{13-8} = 1.8 Hz, H-8); ^{13}C NMR: 17.05 (*q*, C-12), 23.97 (*q*, C-15), 29.73 (*s*, C-3 or C-6), 30.15 (*q*, C-14), 35.63 (*s*, C-11), 36.81 (*t*, C-4), 38.46 (*s*, C-6 or C-3), 42.37 (*t*, C-1), 46.05 (*d*, C-2), 68.84 (*t*, C-13), 78.06 (*s*, C-9), 86.40 (*d*, C-10), 115.35 (*d*, C-8), 141.54 (*s*, C-7), 176.77 (*s*, C-5) ppm; HR EIMS (probe) 70 ev, *m/z* (rel. int.): 264.1362 [M$^+$, calculated for $C_{15}H_{20}O_4$: 264.1361] (20), 249 [M − Me]$^+$ (19), 246 [M − H_2O]$^+$ (41), 231 [M − H_2O − Me]$^+$ (69), 213 [M − 2H_2O − Me]$^+$ (10), 208 [M − isobutene]$^+$ (11), 203 [231 − CO]$^+$ (21), 193 (31), 177 (33), 164 (100), 149 (22), 119 (30), 117 (30), 105 (37), 101 (53), 77 (47), 55 (42), 43 (53), 41 (67). On deuteration, peaks at *m/z* 264, 249, 246, 231, 164 and 101 were shifted to *m/z* 266, 251, 247, 232, 165 and 103 respectively.

Acetylation of compound **7a** with Ac_2O–Py at room temp. afforded a mixture of the mono- and diacetyl derivatives, which could be separated on a short column of silica gel 60 (0.040–0.063 mm) eluted with C_6H_6–EtOAc (3:1).

Diacetyl derivative of compound **7a**, **7b**. Sticky oil. IR ν_{max}^{film} cm^{-1}: 1775 (γ-lactone CO), 1740 (acetate CO), 1245 (C-O); ^{1}H NMR: δ0.94 (1H, *t*, $J_{1-1'}$ = J_{1-2} = 12.7 Hz, H-1), 0.96 and 1.07 (3H each, *s* and *s*, H_3-14 and H_3-15), 1.52 (3H, *s*, H_3-12), 1.65 (1H, *d*, $J_{4-4'}$ = 3.7 Hz, H-4), 1.74 (1H, *dd*, $J_{1'-1}$ = 12.7 Hz, $J_{1'-2}$ = 8.0 Hz, H-1'), 1.79 (1H, *d*, $J_{4-4'}$ = 3.7 Hz, H-4'), 1.96 (3H, *s*, MeCO), 2.05 (3H, *s*, MeCO), 2.77 (1H, *dd*, J_{1-2} = 12.7 Hz, $J_{1'-2}$ = 8.0 Hz, H-2), 4.93 (1H, *dd*, $J_{13-13'}$ = 13.8 Hz, J_{13-8} = 1.8 Hz, H-13), 5.01 (1H, *dd*, $J_{13-13'}$ = 13.8 Hz, $J_{13'-8}$ = 2.5 Hz, H-13'), 5.25 (1H, *s*, H-10), 5.75 (1H, *br t*, *J* = 2.2 Hz, H-8); EIMS (probe) 15eV, *m/z* (rel. int.): 306 [M − CH_2CO]$^+$ (20), 264 [M − 2 × CH_2CO]$^+$ (8), 246 [M − AcOH − CH_2CO]$^+$ (15), 228 [M − 2 × AcOH]$^+$ (100), 213 [M − 2 × AcOH − Me]$^+$ (93), 164 (18), 119 (20).

5,7α-*Dihydroxy*-13-*normarasman*-8-*one* (**8a**). Mp 88–91°, $[\alpha]_D^{20}$ − 125.9° ($CHCl_3$; c 1.6); IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1712 (ketone CO), 1380 and 1365 [$\rangle$C(Me$_2$)], 1140, 1100, 1082, 1060, 1030, 1018, 910, 882; ^{1}H NMR: δ0.43 (2H, *s*, H-4 and H-4'), 0.97 and 1.11 (3H each, *s* and *s*; H_3-15 and H_3-14), 1.21 (3H, *s*, H_3-12), 1.37 (1H, *ddd*, $J_{10-10'}$ = 13.0 Hz, J_{10-4} = 8.7 Hz, J_{10-15} = 0.5 Hz, H-10), 1.47 (1H, *t*, $J_{1'-1}$ = $J_{1'-2}$ = 12.0 Hz, H-1'), 1.59 (1H, *br s*, OH), 1.82 (1H, *ddd*, $J_{10'-10}$ = 13.0 Hz, $J_{10'-9}$ = 9.0 Hz, $J_{10'-1'}$ = 2.2 Hz, H-10'), 1.85 (1H, *ddd*, $H_{1'-1}$ = 12.0 Hz, $J_{1'-2}$ = 6.7 Hz, $J_{1'-10'}$ = 2.2 Hz, H-1'), 2.44 (1H, *br s*, OH), 2.84 (1H, *ddd*, J_{1-2} = 12.0 Hz, J_{1-2} = 6.7 Hz, J_{2-9} = 10.0 Hz, H-2), 2.94 (1H, *dt*, J_{9-8} = 10.0 Hz, J_{9-10} ≥ J_{9-10} ≥ 9.0 Hz, H-9), 3.75 (1H, *d*, $J_{5-5'}$ = 11.5 Hz, H-5), 3.93 (1H, *br d*, $J_{5'-5}$ = 11.5 Hz, H-5'), 4.91 (1H, *br s*, H-7); ^{13}C NMR: 18.66 (*t*, C-4), 19.83 (*q*, C-12), 24.94 (*s*, C-3 or C-6), 26.49 (*q*, C-15 or C-14), 28.88 (*q*, C-14 or C-15), 34.61 (*s*, C-11), 40.53 (*s*, C-6 or C-3), 45.29 (*t*, C-10 or C-1), 46.19 (*d*, C-9 or C-2), 47.58 (*d*, C-2 or C-9), 48.36 (*t*, C_1 or C_{10}), 64.15 (*t*, C-5), 70.71 (*d*, C-7), 213.5 (*s*, C-8) ppm; HR EIMS (probe) 70 eV, *m/z* (rel. int.): 220.1456 [M − H_2O, calculated for $C_{14}H_{20}O_2$: 220.1463] (14), 205 [M − Me]$^+$ (12), 202 [M − H_2O]$^+$ (4), 192 [M − CO]$^+$ (23), 187 [M − Me − H_2O]$^+$ (8), 177 [M − CO − Me]$^+$ (24), 167 (46), 123 [calculated for C_9H_{15}: 123.1174, found: 123.1174] (100), 107 (24), 96 (53), 95 (39), 81 (56), 79 (28), 77 (27), 69 (29), 67 (35), 55 (37), 43 (37), 41 (74).

5,7α-*Diacetoxy*-13-*normarasman*-8-*one* (**8b**). Prepared from **8a** in the usual way, sticky oil, $[\alpha]_D^{20}$ −76.4° ($CHCl_3$; c = 1.0); IR ν_{max}^{film} cm^{-1}: 1750 and 1720 (acetate and ketone CO), 1370, 1242, 1230, 1190, 1155, 1045, 1025, 975, 890; ^{1}H NMR: δ0.52 (1H, *dd*, $J_{4-4'}$ = 6.5 Hz, J_{4-4} = 2.0 Hz, H-4$_{exo}$), 0.68 (1H, *d*, $J_{4'-4}$ = 6.5 Hz, H-4 *endo*), 0.97 and 1.14 (3H each, *s* and *s*, H_3-15 and H_3-14) 1.20 (3H, *s*, H_3-12), 1.50 (1H, *t*, $J_{1'-1}$ = $J_{1'-2}$ = 11.8 Hz, H-1'), 1.57 (1H, *dd*, $J_{10-10'}$ = 13.0 Hz, J_{10-9} = 8.5 Hz, H-10), 1.83 (1H, *ddd*, $J_{10'-10}$ = 13.0 Hz, $J_{10'-9}$ = 9.0 Hz, $J_{10'-1'}$ = 2.5 Hz, H-10'), 1.91 (1H, *ddd*, $J_{1-1'}$ = 11.8 Hz, J_{1-2} = 6.5 Hz, $J_{1-10'}$ = 2.5 Hz, H-1'), 2.10 (3H, *s*, MeCOO–), 2.18 (3H, *s*, MeCOO–), 2.82 (1H, *m*, H-2), 2.88 (1H, *m*, H-9), 3.70 (1H, *d*, $J_{5-5'}$ = 12.0 Hz, H-5), 4.57 (1H, *br d*, $J_{5'-5}$ = 12.0 Hz, H-5'), 5.82 (1H, *d*, $J_{7-4'exo}$ = 2.0 Hz, H-7); EIMS (probe) 15 eV, *m/z* (rel. int.): 280 [M − CH_2CO]$^+$ (8), 262 [M − AcOH]$^+$ (13), 247 [M − AcOH − Me]$^+$ (7), 220 [M − CH_2CO − AcOH]$^+$ (100), 174 (43), 167 (51), 159 (30), 123 (91), 91 (4), 81 (5), 67 (2), 55 (2), 43 (23).

5,7α,8α-*Triacetoxy*-13-*normarasmane* (**11c**). Compound **8b**, by reduction with $NaBH_4$ in MeOH, followed by acetylation with Ac_2O in Py, yielded a sticky oil, consisting of an inseparable mixture of **11c** and **10c** (6:1). ^{1}H NMR signals of major stereoisomer **11c**: δ0.39 (1H, *d*, $J_{4-4'}$ = 5.5 Hz, H-4), 1.00, 1.07 and 1.08 (3H each, 3*s*, H_3-15, -14 and 12), 1.03 (1H, *d*, $J_{4'-4}$ = 5.5 Hz, H-4'), 1.32 (1H, *dd*, $J_{10-10'}$ = 13.5 Hz, J_{10-9} = 2.7 Hz, H-10), 1.53 (1H, *dd*, $J_{10'-10}$ = 13.5 Hz, $J_{10'-9}$ = 7.5 Hz, H-10'), 1.55 (1H, *t*, $J_{1'-1}$ = $J_{1'-2}$ = 12.6 Hz, H-1'), 1.67 (1H, *dd*, $J_{1-1'}$ = 12.6 Hz, J_{1-2} = 7.0 Hz, H-1), 2.02, 2.06 and 2.08 (3H each, 3*s*, 3 × MeCOO–), 2.12 (1H, *m*, H-9), 2.63 (1H, *dt*, $J_{1'-2}$ = 12.6 Hz, $J_{1'-2}$ = J_{2-9} = 7.0 Hz, H-2), 3.81 (1H, *d*, $J_{5-5'}$ = 12.0 Hz, H-5), 4.36 (1H, *d*, $J_{5'-5}$ = 12.0 Hz, H-5'), 4.79 (1H, *dd*, J_{8-9} = 11.5 Hz, J_{7-8} = 4.3 Hz, H-8), 5.46 (1H, *d*, J_{7-8} = 4.3 Hz, H-7); IR ν_{max}^{film} cm^{-1}: 1735 (acetate CO), 1365, 1240, 1105, 1020, 970.

5,8α-*Dihydroxy*-13-*normarasman*-7-*one* (**12a**). Sesquiterpene **12a**, obtained from prep. HPLC of fraction III, was further purified by 'flash' chromatography (Silica Woelm TSC), using hexane–Et_2O (1:1) as eluant. Thick oil, $[\alpha]_D^{20}$ −83.33° ($CHCl_3$, c 0.3). IR ν_{max}^{film} cm^{-1}: 3430 (OH), 1695 (cyclopropyl ketone), 1450, 1385, 1365, 1320, 1295, 1225, 1192, 1150, 1130, 1092, 1070, 1050, 1030, 1000, 970, 910, 820; ^{1}H NMR: δ0.86 (1H, *d*, $J_{4-4'}$ = 5.4 Hz, H-4), 1.09 (1H, *d*, $J_{4'-4}$ = 5.4 Hz, H-4'), 1.09 (3H, *s*, H_3-15), 1.17 (3H, *s*, H_3-14), 1.28 (3H, *s*, H_3-12), 1.59 (1H, *t*, $J_{1'-2}$ = 13.0 Hz, $J_{1'-1}$ = 13.0 Hz, H-1'), 1.66 (1H, *dd*, $J_{10-10'}$ = 13.8 Hz, J_{10-9}

= 6.8 Hz, H-10'), 1.83 (1H, *dd*, J_{1-1} = 13.0 Hz, $J_{1'-2}$ = 6.5 Hz, H-1'), 1.87 (1H, *dd*, $J_{10-10'}$ = 13.8 Hz, J_{10-9} = 1.1 Hz, H-10), 1.96 (1H, *dt*, J_{8-9} = 11.5 Hz, J_{2-9} = 6.5 Hz, $J_{9-10'}$ = 6.8 Hz, H-9), 2.63 (1H, *dt*, J_{2-9} = 6.5 Hz, J_{2-1} = 13.0 Hz, $J_{2-1'}$ = 6.5 Hz, H-2), 3.59 (1H, *d*, $J_{5-5'}$ = 11.8 Hz, H-5), 3.73 (1H, *d*, J_{8-9} = 11.5 Hz, H-8), 4.20 (1H, *d*, $J_{5-5'}$ = 11.8 Hz, H-5'); EIMS (probe) 70 eV, *m/z* (rel. int.): 238 $[M]^+$ (1), 220 $[M-H_2O]^+$ (23), 205 $[M-H_2O-Me]^+$ (20), 202 $[M-2H_2O]^+$ (7), 192 (26), 191 (24), 187 (15), 177 (22), 167 (28), 163 (14), 161 (23), 159 (19), 149 (16), 137 (14), 135 (18), 124 (25), 123 (100), 121 (28), 119 (17), 109 (30), 107 (41), 105 (26), 97 (25), 96 (29), 95 (53), 93 (27), 91 (30), 85 (19), 83 (25), 81 (69), 79 (32), 77 (21), 71 (16), 69 (48), 67 (37), 57 (43), 55 (61), 43 (98), 41 (79).

5,8α-*Diacetoxy*-13-*normarasman*-7-*one* (**12b**). Obtained from compound **12a** in the standard way. Oil $[\alpha]_D^{20}$ −22.3° ($CHCl_3$, *c* 1); IR ν_{max}^{film} cm^{-1} 1740 (acetate CO), 1710 (ketone CO), 1385, 1365, 1330, 1230, 1130, 1075, 1050, 1030, 980, 930, 895, 815; ^1H NMR: δ0.87 (1H, *d*, $J_{4-4'}$ = 5.4 Hz, H-4), 1.9 (3H, *s*, H_3-15), 1.18 (3H, *s*, H_3-14), 1.23 (3H, *s*, H_3-12), 1.31 (1H, *d*, $J_{4-4'}$ = 5.4 Hz, H-4'), 1.48 (1H, *dd*, $J_{10-10'}$ = 13.9 Hz, J_{10-9} = 1.2 Hz, H-10), 1.62 (1H, *dd*, $J_{10-10'}$ = 13.9 Hz, $J_{10'-9}$ = 6.2 Hz, H-10'), 1.64 (1H, *t*, $J_{1-1'} = J_{1-2}$ = 12.5 Hz, H-1), 1.85 (1H, *dd*, $J_{1-1'}$ = 12.5 Hz, $J_{1'-2}$ = 6.5 Hz, H-1'), 2.04 (3H, *s*, MeCOO–), 2.15 (3H, *s*, MeCOO–), 2.22 (1H, *dt*, J_{8-9} = 11.9 Hz, J_{2-9} = 6.5 Hz, $J_{9-10'}$ = 6.2 Hz, J_{9-10} = 1.2 Hz, H-9), 2.70 (1H, *dt*, $J_{2-9} = J_{2-1'}$ = 6.5 Hz, J_{2-1} = 12.5 Hz, H-2), 3.81 (1H, *d*, $J_{5-5'}$ = 11.6 Hz, H-5), 4.95 (1H, *br d*, $J_{5-5'}$ = 11.6 Hz, H-5'), 4.95 (1H, *d*, J_{8-9} = 11.9 Hz, H-8); ^{13}C NMR: 19.54 (*q*, C-12), 20.62 and 20.77 (*q* and *q*, 2 COMe), 23.56 (*t*, C-4), 27.57 (*s*, C-3 or C-6), 32.62 and 32.70 (*q* and *q*, C-14 and C-15), 36.36 (*s*, C-11), 37.15 (*s*, C-6 or C-3), 39.22 (*d*, C-2 or C-9), 43.91 (*t*, C-1 or C-10) 44.51 overlapped *d* and *t*, C-9 or C-2 and C-10 or C-1), 73.79 (*t*, C-5), 74.79 (*d*, C-8), 170.38 and 170.80 (*s* and *s*, 2-OCOMe), 201.34 (*s*, C-7) ppm.

5,7β,8α-*Triacetoxy*-13-*normarasmane* (**13**). The diacetyl derivative **12b** was reduced with $NaBH_4$ in 95% EtOH, according to the standard procedure, followed by acetylation with Ac_2O in Py. An inseparable mixture of two diastereomeric triacetates was obtained, in the ratio 2.5:1, showing a molecular ion at *m/z* 366 (EIMS) and IR bands at 1730 and 1245 cm^{-1}. The ^1H NMR signals of the minor isomer corresponds to 5,7α,8α-triacetoxy-13-normarasmane (**11c**), whereas the major stereoisomer is 5,7β,8α-triacetoxy-13-normarasmane (**13**). ^1H NMR: δ0.60 (1H, *d*, $J_{4-4'}$ = 5.4 Hz, H-4), 0.90 (1H, *d*, $J_{4-4'}$ = 5.4 Hz, H-4'), 0.98, 1.10 and 1.15 (3H each, 3*s*, H_3-15, -14 and -12), 1.33 (1H, *dd*, $J_{10-10'}$ = 13.6 Hz, J_{9-10} = 4.5 Hz, H-10), 1.50–1.70 (3H, *m*, H-10', H-1 and H-1'), 1.92 (1H, *qd*, J_{9-10} = 4.5 Hz, $J_{2-9} \simeq J_{9-10'} \simeq J_{8-9} \simeq$ 8.0 Hz, H-9) 2.02, 2.03 and 2.04 (3H each, 3*s*, 3 MeCOO–), 2.57 (1H, *dt*, J_{1-2} = 12.9 Hz, $J_{2-9} \simeq J_{2-1'} \simeq$ 7.5 Hz, H-2), 4.08 (1H, *d*, $J_{5-5'}$ = 12.0 Hz, H-5), 4.12 (1H, *d*, $J_{5-5'}$ = 12.0 Hz, H-5'), 4.81 (1H, *dd*, J_{8-9} = 8.0 Hz, J_{7-8} = 6.5 Hz, H-8), 5.14 (1H, *d*, J_{7-8} = 6.5 Hz, H-7).

Acknowledgements—We warmly thank Dr G. Mellerio and Dr D. Vercesi, The University of Pavia, for the mass spectra and specific optical rotation measurements, respectively. This work has been done according to a scientific cooperation program between Italy (CNR) and Poland (The Polish Academy of Sciences). Partial financial support was given by the MPI.

REFERENCES

1. Sterner, O. (1985) *The Russulaceae Sesquiterpenes*, Doctoral Thesis dissertation, University of Lund.
2. Camazine, S. M., Resch, J. F., Eisner, T. and Meinwald, J. (1983) *J. Chem. Ecol.* **9**, 1439.
3. Sterner, O., Bergman, R., Kesler, E., Nilsson, L., Oluwadiya, J. and Wickberg, B. (1983) *Tetrahedron Letters* **24**, 1415.
4. Sterner, O., Bergman, R., Kihlberg, J. and Wickberg, B. (1985) *J. Nat. Prod.* **48**, 279.
5. Sterner, O., Bergman, R., Franzén, C. and Wickberg, B. (1985) *Tetrahedron Letters* **26**, 3163.
6. Ayer, W. A. and Browne, L. M. (1981) *Tetrahedron* **37**, 2199.
7. Turner, W. B. and Aldridge, D. C. (1983) *Fungal Metabolites II*. Academic Press, London.
8. Magnusson, G., Thorén, S. and Wickberg, B. (1972) *Tetrahedron Letters* 1105.
9. Dugan, J. J., de Mayo, P., Nisbert, M., Robinson, J. R. and Anchel, M. (1966) *J. Am. Chem. Soc.* **88**, 2838.
10. de Mayo, P. (1963–1964) *Molecular Rearrangements*. Interscience Publishers, New York.
11. De Bernardi, M., Fronza, G., Mellerio, G., Valla, V., Vidari, G. and Vita-Finzi, P. (1984) *Gazz. Chim. It.* **114**, 163.
12. Jackman, L. M. and Sternhell, S. (1969) *Applications of NMR Spectroscopy in Organic Chemistry*. Pergamon Press, Oxford.
13. Sterner, O., Bergman, R., Kihlberg, J., Oluwadiya, J., Wickberg, B., Vidari, G., De Bernardi, M., De Marchi, F., Fronza, G. and Vita-Finzi, P. (1985) *J. Org. Chem.* **50**, 950.
14. Daniewski, W. M. and Kocór, M. (1970) *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **18**, 585.