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STRUCTURES OF TWO GLUTINOPALLAL ESTERS, NEW NATURAL SESQUITERPENOIDS FROM *LACTARIUS GLUTINOPALLENS*

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Key Word Index—*Lactarius glutinopallens* Lange, Russulaceae, sesquiterpenoid esters, palmityl- and stearyl-glutinopallal.

Abstract—Stearyl- and palmityl glutinopallal, new natural sesquiterpenoids isolated from *Lactarius glutinopallens* Lange, have structures **1** and **2**; these compounds are responsible for the green reaction of the fungus to the 'sulfovanillic' mixtures.

INTRODUCTION

In the course of our researches about the nature of fungal substances responsible for the positive reaction to the 'sulfovanillin' [1], our attention was drawn towards *Lactarius glutinopallens* Lange which offers the originality to develop a green coloration with this reagent [2]. Thus, we were able to isolate two esters of the same sesquiterpenoid hemicetal to which we assigned formulae **1** and **2** and gave the names of palmityl- and stearyl-glutinopallal.

RESULTS AND DISCUSSION

Fatty acids were identified, after methanolysis, by GC to palmitic and stearic acids, ^{1}H NMR and mass spectra corroborating this attribution.

The structure of the sesquiterpenoid moiety was determined, as previously [1], from the spectral study of natural products and methanolysis product **3**.

The electron impact mass spectra gave, as ions of highest mass with very weak intensities, 558 and 530 respectively, for each natural products. This indicated that the terpenoid had M_r 292. This result was corroborated by the presence of an intense ion (base peak) at m/z 275 (in each spectrum).

The mass and ^{1}H NMR spectra of **1** and **2** are identical with the exception of molecular ions and CH_2 integration. The ^{1}H NMR decoupling experiments, carried out on **1** and **3**, showed the configuration of the hydrocarbonated links **A**, **B** and **C** (continuous lines) that led to the determination of the glutinopallal skeleton.

The 3-position of –COOMe (function detected by IR and ^{13}C NMR) was given by the absence of coupling of cyclopropanic protons with other protons as observed with stearyl-velutinal [1]. The ^{13}C NMR spectrum of **3** compared to that of **5** (see Table 1) agreed perfectly with the proposed structure.

EXPERIMENTAL

Fungal material. Fungi were collected near Monlet (F-43), in October 1983 and identified by Pr R. Kuhner, mycologist at University Lyon 1.

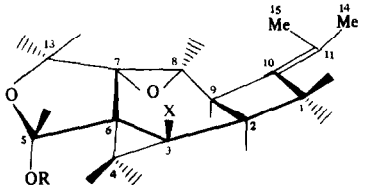
Isolation procedure. The frozen fruitbodies were extracted twice with CH_2Cl_2. The extract, from which H_2O was removed after freezing, was coned to dryness and taken up in acetone. Prep. TLC (silica gel, hexane–EtOAc 10:1) gave a single green band (**1** + **2**) after spraying with vanillin (2.5 g) in H_2SO_4–H_2O 1:1 (80 ml). The two esters were separated by prep. HPLC on a RP-18 column with MeOH–H_2O 95:5 (1 ml/min); elution volumes (ml): 14.5 (**1**) and 20.5 (**2**) (RI detection).

Methanolysis. The **1** + **2** mixture from prep. TLC was submitted to methanolysis (17 hr at 60°). **3** was purified by prep. TLC on alumina (with hexane–$CHCl_3$–EtOAc 4:1:1) then on silica gel (with hexane–$CHCl_3$–EtOAc 12:1:1).

Stearyl-glutinopallal **1**. R_f 0.64 (System 'a'; silica gel TLC F_{254}; hexane–$CHCl_3$–EtOAc 6:1:1). UV λ_{max}^{Hexane} = 209 nm (ε 6500). ^{1}H NMR (CAMECA 350 MHz, $CDCl_3$, δ: ppm TMS): 6.67 (1H, s, H-5), 4.22 (1H, *d*, *J* = 10.3 Hz, H-13a), 4.16 (1H, *d*, *J* = 10.3 Hz, H-13b), 3.70 (3H, s, OMe-17), 2.76 (1H, ddd, J_{2-3a} = 10.3 Hz, J_{2-1b} = 7.3 Hz, J_{2-9} = 7.3 Hz, H-2), 2.71 (1H, *br s*, H-8), 2.65 (1H, *br dd*, J_{gem} = 16 Hz, J_{1b-2} = 7.3 Hz, H-1b), 2.37 (2H, *t*, *J* = 7.5 Hz, CO–CH_2–$(CH_2)_n$–CH_3), 2.31 (1H, *br d*, J_{9-2} = 7.3 Hz, H-9), 2.10 (1H, *m*, H-1a), 1.73 (3H, *br s*, Me-14), 1.66 (2H, *m*, CO–CH_2–CH_2–), 1.64 (3H, *br s*, Me-15), 1.36 (1H, *d*, *J* = 5.2 Hz, H-4b), 1.26 (24H, *br s*, CO–CH_2–CH_2–$(CH_2)_{12}$–Me), 1.17 (1H, *d*, *J* = 5.2 Hz, H-4a), 0.88 (3H, *t*, *J* = 6.7 Hz, Me–$(CH_2)_n$–), MS (EI, 70 eV) *m/z*: 558 (M^+, 2, 558.393 calc. for $C_{34}H_{54}O_6$: 558.392), 527 (M − 31, 0.4), 499 (0.4), 284 (10, 284.270 calc. for $C_{18}H_{36}O_2$: 284.272 (= stearic acid)), 275 (100), 274 (61), 252 (6), 245 (33), 242 (81), 227 (6), 215 (38), 214 (34), 213 (14), 197 (17), 187 (15), 185 (15), 165 (10).

Palmityl-glutinopallal **2**. R_f 0.64 (system 'a'). MS (EI, 70 eV) *m/z*: 530 (M^+, 0.8, 530.358 calc. for $C_{32}H_{50}O_6$: 530.361), 499 (M − 31, 0.4), 471 (0.3), 275 (100, 275.1274 calc. for $C_{16}H_{19}O_4$: 275.1283), 274 (31, 274.1195, calc. for $C_{16}H_{18}O_4$: 274.1205), 256 [4, 256.2407, calc. for $C_{16}H_{32}O_2$: 256.2402 (= palmitic acid)], 245 (19), [245.1178, calc. for $C_{15}H_{17}O_3$: 245.1178], 242 (45, 242.0944, calc. for $C_{15}H_{14}O_3$: 242.0943) 227 (3), 215 (44, 215.1069, calc. for $C_{14}H_{15}O_2$: 215.1072), 214 (20), 197 (10), 187 (9), 185 (11), 165 (5). IR (FT, $CHCl_3$, ν cm^{-1}) 2950, 2925, 2855, 1723, 1440, 1395, 1280, 1245, 1195, 1147, 1095, 1037, 990, 965, 935, 895, 835.

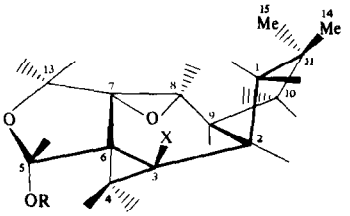
Methyl-glutinopallal **3**. R_f 0.37 (system 'a'). ^{1}H NMR (CAMECA-350 MHz, $CDCl_3$, LR = long range coupling); δ:



Glutinopallal skeleton

X = $^{12}_{}$COO$^{16}_{}$Me

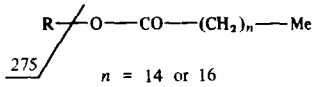
1 R = stearyl
2 R = palmityl
3 R = Me (17)



Velutinal skeleton

X = $^{12}_{}$Me

4 R = stearyl
5 R = Me (17)



n = 14 or 16

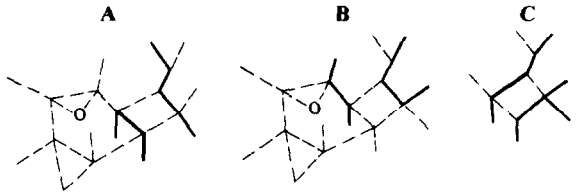


Table 1. ^{13}C NMR spectra of methylated velutinal **5** and glutinopallal **3** (CAMECA 350 MHz, $CDCl_3$, δ ppm/TMS)

C	**5**	δ	**3**	δ		J
1	CH_2	46.4*	CH_2	42.4	*tm*	127
2	CH	38.6	CH	37.3	*dm*	133
3	C	23.4	C	37.2	*s*	
4	CH_2	17.2	CH_2	18.8	*t*	167
5	CH	105.4	CH	105.2	*dm*	184
6	C	31.3	C	31.0	*s*	
7	C	65.8	C	64.8	*sl*	
8	CH	58.3	CH	54	*dl*	184
9	CH	43.2	CH	47.4	*dl*	136
10	CH_2	45.8*	C=	133.8	*s*	
11	C	36.7	C=	131	*s*	
12	Me	20.6	C=0	173.2	*s*	
13	CH_2	68.3	CH_2	67.7	*td*	150[a]
14	Me	31.8	Me	13.8	*q*	124
15	Me	31.8	Me	12.4	*q*	124
16	OMe	54.3	OMe	51.7	*qd*	142[b]
17			OMe	55	*q*	147

*These values can be exchanged, [a] and 6.8 Hz, [b] and 3.6 Hz, J: Hz, undecoupled spectrum.

ppm/TMS) 1.12 (1H, *d*, J = 5.2 Hz, H-4a), 1.42 (1H, d, J = 5.2 Hz, H-4b), 1.63 (3H, *br s*, Me-14), 1.71 (3H, *br s*, Me-15), 2.09 (1H, *br t*, J_{gem} = 16 Hz, J_{1a-2} = 11 Hz, J_{LR} with Me-14, H-1a), 2.30 (1H, *br d*, J_{9-2} = 7.3 Hz, J_{LR} with H-8, Me-14 and Me-15, H-9), 2.60 (1H, *br dd*, J_{gem} = 16 Hz, J_{1b-2} = 7.3 Hz, J_{LR} with Me-14, H-1b), 2.67 (1H, *br s*, J_{LR} with H-9, H-8), 2.75 (1H, *ddd*, J_{2-1a} = 11 Hz, J_{2-1b} = 7.3 Hz, J_{2-9} = 7.3 Hz, H-2), 3.42 (3H, *s*, OMe-5), 3.70 (3H, *s*, OMe-12), 4.16 (1H, *d*, J = 10.3 Hz, H-13a), 4.22 (1H, *d*, J = 10.3 Hz, H-13b) 5, 32 (1H, *s*, H-5); ^{13}C NMR: see Table 1.

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