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# MARASMANE SESQUITERPENES FROM THE BASIDIOMYCETE *CLITOCYBE HYDROGRAMMA**

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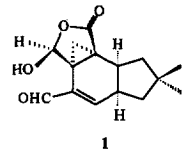
**Key Word Index**—*Clitocybe hydrogramma*; Basidiomycetes; marasmane sesquiterpene dialdehydes; 10-hydroxy-isovelleral; hydrogrammic acid; structural elucidation.

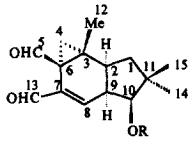
**Abstract**—Investigations of solid cultures of *Clitocybe hydrogramma* gave rise to the isolation of two new α,β-unsaturated dialdehydes having the marasmane skeleton. Their structures have been determined on the basis of $^1H$ and $^{13}C$ NMR evidence. © 1997 Elsevier Science Ltd

## INTRODUCTION

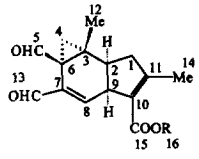
Basidiomycetes including the genus *Clitocybe* are known to produce a series of biologically active compounds when grown in pure culture. The majority of these compounds belong to the protoilludane class and have antibacterial and antitumour activity [2]. In the present paper, we describe the isolation of two new marasmanes and marasmic acid (**1**), a toxic metabolite isolated first from *Marasmius congenius* [3] from *C. hydrogramma*. Marasmanes containing an unsaturated dialdehyde group as isovelleral were isolated from *Lactarius vellereus* and *L. pergamenus* [4]. The interest in this class of sesquiterpenoid derives from the presence of the unsaturated dialdehyde function. These compounds possess a pungent taste and are antimicrobial, cytotoxic and antifeedant [5] and are not present in intact fungal bodies, but are formed rapidly from a common precursor (stearoylvelutinal) [6] when the fungus is injured. Subsequently, they are rapidly degraded and rendered harmless to the fungus. The degradation products are formed by autoxidation and include 9-hydroxy-isovelleral [7] and 9-hydroxy-marasmic acid [8]. Other marasmanes containing one or more oxygenated functions, in particular on the cyclopentane ring, have been isolated from *Lactarius vellereus* [9].



**1**



**2a** R = H
**2b** R = Ac



**3a** R = H
**3b** R = Me

## RESULTS AND DISCUSSION

When *C. hydrogramma* was grown on MPGA (malt extract–peptone–glucose–agar) for three weeks, three main metabolites, marasmic acid (**1**) and two new compounds (**2a** and **3a**), were isolated by silica gel chromatography.

10-Hydroxy-isovelleral (**2a**), solid mp 75°, analysed for $C_{15}H_{20}O_3$. Its $^{13}C$ NMR spectrum (Table 1) contained signals for one aldehyde carbon and a —CH=C(7)C(13) HO grouping in which C-7 presented a characteristic $^2J_{C\text{-}7,\ H\text{-}13} = 26$ Hz [10], in addition to those for three methyl groups, two methylene groups (one of which was assigned to a cyclopropane carbon since it showed a $^1J = 163.5$ Hz), three methine groups and three quaternary (one of which was attached to the aldehyde group since it exhibited a $^2J_{C\text{-}6,\ H\text{-}5} = 25$ Hz) $sp^3$ carbon atoms. The $^1H$ NMR spectrum (Table 2) showed one hydroxyl resonance vicinally coupled to H-10 and revealed the presence of the sequence

—C(1)$H_2$—C(2)H—C(9)H|—C(10)HOH.
C(8)H=

* Part 54 in the series "Secondary Mould Metabolites". For Part 53 see ref. [1].

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Table 1. $^{13}C$ NMR data for compounds **2a** and **3b** in $CDCl_3$

| | **2a** | | **3b** | |
|---|---|---|---|---|
| | $\delta_C$ | $^1J$(CH)* | $\delta_C$ | $^1J$(CH)* |
| **1** | 44.45 *t* | 130 | 43.73 *t* | 131.5 |
| **2** | 36.11 *d* | 133 | 40.17 *d* | 131 |
| **3** | 35.00 *s* | | 32.58 *s* | |
| **4** | 26.41 *t* | 163.5 | 27.91 *t* | 163.5 |
| **5** | 197.82 *d* | 179.5 | 197.49 *d* | 180.5 |
| **6** | 35.26 *s* | | 34.73 *s* | |
| **7** | 141.06 *s* | | 140.15 *s* | |
| **8** | 147.09 *d* | 162 | 146.97 *d* | 163 |
| **9** | 44.65 *d* | 132 | 45.08 *d* | 140 |
| **10** | 81.02 *d* | 140 | 126.32 *s* | |
| **11** | 39.81 *s* | | 157.44 *s* | |
| **12** | 17.73 *q* | 127.5 | 18.35 *q* | 128 |
| **13** | 192.53 *d* | 177.5 | 192.49 *d* | 178 |
| **14** | 30.20 *q* | 125 | 16.89 *q* | 128.5 |
| **15** | 25.47 *q* | 125 | 165.24 *s* | |
| **16** | | | 51.44 *q* | 147 |

*C-6 and C-7 exhibited $^2J_{C,H}$ = 25 and 26 Hz with H-5 and H-13, respectively.

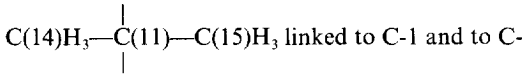
Table 2. $^1H$ NMR chemical shifts ($\delta$, ppm) and couplings constants ($J$/Hz) of compounds **2a**, **2b** and **3b** in $CDCl_3$

| | **2a** | **2b** | **3b** | | **2a** | **3b** |
|---|---|---|---|---|---|---|
| 1α | 1.83 | 1.84 | 2.60 | 1α, 1β | 13.1 | 17.1 |
| 1β | 1.35 | 1.37 | 2.41 | 1α, 2 | 7.6 | 7.6 |
| 2 | 2.52 | 2.61 | 2.91 | 1α, 14 | | 1.0 |
| 4α | 0.98 | 0.98 | 0.96 | 1β, 2 | 11.7 | 11.1 |
| 4β | 1.92 | 1.94 | 1.95 | 1β, 9 | | 1.8 |
| 5 | 9.75 | 9.75 | 9.77 | 1β, 14 | | 1.3 |
| 8 | 7.02 | 6.70 | 6.58 | 2, 9 | 7.9 | 9.0 |
| 9 | 2.81 | 2.99 | 3.61 | 4α, 4β | 4.5 | 4.5 |
| 10 | 4.10 | 5.03 | | 8, 9 | 2.4 | 2.2 |
| 12 | 1.10 | 1.12 | 1.14 | 9, 10 | 7.1 | |
| 13 | 9.54 | 9.56 | 9.49 | 9, 14 | | 1.2 |
| 14 | 1.11 | 1.14 | 2.16 | 10, OH-10 | 7.2* | |
| 15 | 0.93 | 0.93 | | | | |
| 16 | | | 3.81 | | | |
| OR-10 | 4.12* | 2.18 | | | | |

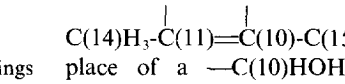
*Value observed in acetone-$d_6$.

The formation of the monoacetate **2b** upon acetylation confirmed the presence of the OH function.

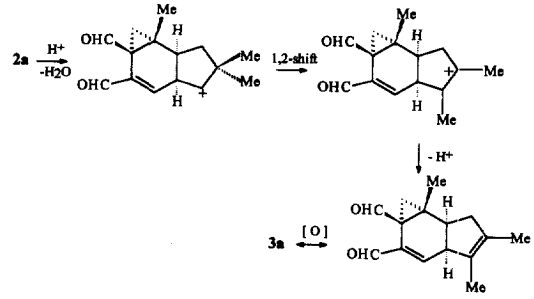
The cross-peaks observed between both $H_3$—14 and $H_3$—15 and C-1, C-10 and C-11 in the COLOC spectrum indicated the presence of a fragment



to form a cyclopentane ring while the cross-peaks observed between $H_3$-12 and C-2, C-3 and C-6 and between $H_2$-4 and C-12 allowed us to join C-3 with C-2 and C-12. NOE difference experiments led to 3*R*, 6*S*, 9*R*, 10*S* as the relative conformation of **2a**. The second metabolite, hydrogrammic acid (**3a**), isolated from the more polar fractions of the chromatography, was a solid, mp 189°. HR mass spectrometry indicated a molecular formula of $C_{15}H_{16}O_4$. Methylation of hydrogrammic acid gave rise to the corresponding methyl ester **3b** which showed a peak at *m/z* 274 in agreement with a molecular formula of $C_{16}H_{18}O_4$. A comparison of the $^{13}C$ and $^1H$ NMR spectra of **2a** and **3b** (Tables 1 and 2) revealed that they shared the same ring system, the most significant differences being the presence in **3b** of signals attributable to a



moiety in place of a —C(10)HOH—C(11)$Me_2$ unit. NOE difference experiments carried out on **3b** confirmed that **2a** and**3b** have the same relative configuration.



Scheme. Possible biosynthetic route for the formation of **3a** from **2a**.

From a biogenetic point of view, hydrogrammic acid (**3a**) may arise directly from 10-hydroxy-isovelleral (**2a**) *via* the formation of the carbocation shown in the scheme and the subsequent 1,2 shift of a methyl group according to the Wagner-Meerwein rearrangement [11]. 10-Hydroxy-isovelleral and hydrogrammic acid showed antibacterial activity against *Bacillus cereus* and *B. subtilis* (50 μg/disc) but not against *Escherichia coli*; no activity was found against *Saccharomyces cerevisiae* and *Cladosporium cladosporioides*.

## EXPERIMENTAL

*General.* Mps: uncorr.; Flash CC: Merck silica gel (0.04–0.06 mm); TLC: Merck $HF_{254}$ and RP-18 $F_{254}$; MS: Finnigan-MAT-TSQ70 spectrometer; NMR: 250.1 MHZ $^1H$ and 62.9 MHz $^{13}C$, chemical shifts are in ppm ($\delta$) from TMS as int. standard.

*Cultivation of the fungus and isolation of compounds* **1**, **2a** *and* **3a**. A mycelium suspension of *Clitocybe hydrogramma* (CBS 316.85) was inoculated into 15 Roux flasks containing MPGA (100 ml) (malt extract–peptone–glucose–agar 20:5:20:15 g $l^{-1}$). After four weeks the cultures were extracted with EtOAc containing 1% of MeOH. The combined extracts were dried ($Na_2SO_4$) and evapd to give a crude extract (0.96 g) which was chromatographed on a silica gel column using $CH_2Cl_2$–MeOH (gradient) as eluant, and purified by RP-TLC (PLC) with MeCN–$H_2O$ (1:1) to yield: marasmic acid (**1**) (50 mg), $^1H$ NMR and mass spectroscopy data in agreement with literature [3], 10-hydroxy-isovelleral (**2a**) (40 mg) and hydrogrammic acid (**3a**) (90 mg).

10-*Hydroxy-isovelleral* (**2a**). $[\alpha]_D+119°$ ($CHCl_3$; *c* 0.1); UV $\lambda_{max}^{EtOH}$ nm: 250 ($\varepsilon$:4600); IR $\nu_{max}$ ($CHCl_3$) $cm^{-1}$:3400, 1705, 1680; HRMS: $[M]^+$ 248.14126, calc. for $C_{15}H_{20}O_3$ 248.14124. MS *m/z* (rel. int.): 248 $[M]^+$(3), 215(4), 177(17), 159(22), 91(49), 77(47), 55(45), 41(100); $^{13}C$ and $^1H$ NMR: Tables 1 and 2; NOE experiments ($CDCl_3$): {H-2} enhanced H-1$\alpha$ (3%), H-9 (2%), H-10 (2%), $H_3$-12 (1%); {H-4$\alpha$} enhanced H-2 (1%), H-4$\beta$ (17%), H-9 (5%); {H-4$\beta$} enhanced H-4$\alpha$ (16%), H-5 (1%), $H_3$-12; {H-5} enhanced H-4$\beta$ (0.5%), $H_3$-12 (1%); {H-8} enhanced H-9 (3%), H-13 (11%); {H-9} enhanced H-2 (3.5%), H-4$\alpha$ (3.5%), H-8 (2%), H-10 (4%); {H-10} enhanced H-2 (3%), H-9 (4%), $H_3$-14 (1%); {H-13} enhanced H-8 (10%); and {$H_3$-15} enhanced H-1$\beta$ (4.5%), H-8 (2.5%).

10-*Hydroxy-isovelleral acetate* (**2b**). Compound **2a** (30 mg) was dissolved in $CH_2Cl_2$ (5 ml) and treated with $Ac_2O$ (0.2 ml), pyridine (0.05 ml) and DMAP (10 mg). After 6 hr at 0°, standard work-up followed by PLC using hexane–EtOAc (2:1) as eluant gave **2b** (15 mg) as an oil. $^1H$ NMR: Table 1.

*Hydrogrammic acid* (**3a**). $[\alpha]_D+429°$ ($CHCl_3$; *c* 0.07); UV $\lambda_{max}^{EtOH}$ nm: 255 ($\varepsilon$ 8100); IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3020, 1705, 1680; HRMS: $[M]^+$ 260.1052, calc. for $C_{15}H_{16}O_4$ 260.1049. MS *m/z* (rel. int.): 260 $[M]^+$(17), 242(18), 213(14), 171(18), 149(75), 136(55), 128(36), 105(29), 91(49), 79(39), 57(52), 43(100); $^1H$ NMR ($CDCl_3$): 0.95 (H-4$\alpha$), 1.12($H_3$-12), 1.95(H-4$\beta$), 2.18($H_3$-14), 2.40(H-1$\beta$), 2.60(H-1$\alpha$), 2.90(H-2), 3.6(H-9), 6.58(H-8), 9.38(H-13), 9.67(H-5).

*Hydrogrammic acid methyl ester* (**3b**). Compound **3a** (20 mg) was methylated with $CH_2N_2$. Evapn of the solvent and PLC using hexane–EtOAc (2:1) as eluant gave ester **3b** (15 mg) as an oil; MS, *m/z* (rel. int.): 274 $[M]^+$(29), 242(64), 215(56), 171(59), 157(72), 143(81), 136(100), 128(87), 115(68), 91(61), 77(68). $^{13}C$ and $^1H$ NMR: Tables 1 and 2; NOE experiments ($CDCl_3$): {H-2} enhanced H-1$\alpha$ (1.5%), H-9 (4%); {H-4$\alpha$} enhanced H-2 (1%), H-4$\beta$ (19%), H-9 (5%); {H-4$\beta$} enhanced H-4$\alpha$ (18.5%), H-5 (1%), $H_3$-12 (1%); {H-5} enhanced H-4$\beta$ (0.5%), $H_3$-12 (0.5%), H-13 (0.5%); {H-8} enhanced H-9 (3%), H-13 (16%); {H-9} enhanced H-2 (3.5%), H-4$\alpha$ (3%), H-8 (3%); {$H_3$-12} enhanced H-1$\alpha$ (3.5%), H-1$\beta$ (2.5%), H-2 (5%), H-4$\beta$ (5%), H-5 (2.5%); {H-13} enhanced H-8 (15%); {$H_3$-14} enhanced H-1$\alpha$ (2.5%), H-1$\beta$ (1.5%), $H_3$-16 (0.5%); and {$H_3$-16} enhanced H-8 (1%), $H_3$-14 (0.5%).

*Biological tests.* Antibacterial and antifungal activity were tested with paper discs (6 mm diam.), soaked with metabolites **2a** and **3a** (100 and 50 $\mu$g dissolved in EtOH) and placed on suitable culture medium, which had been cooled at 45° and poured into Petri dishes with *Bacillus cereus* (ATCC 10702), *Bacillus subtilis* (ATCC, 6633), *Escherichia coli* (IPV 287) and *Saccharomyces cerevisiae* (NCYC 729) as test micro-organisms.

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