116. Gilmicolin and Mycorrhizinol, Two New Metabolites of *Gilmaniella humicola* BARRON

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(19.III.79)

Summary

The structure of gilmicolin and mycorrhizinol, two new metabolites isolated from culture filtrates of *Gilmaniella humicola* BARRON, have been shown to be **3** and **15** respectively by spectral and chemical studies. The X-ray analysis of gilmicolin (**3**) is also reported.

1. Introduction. – Many times the knowledge of the minor metabolites of a microorganism is of great help in understanding the mode of biogenesis of major secondary metabolites. These minor components can be biogenetic intermediates or shunt metabolites on the biogenetic pathway or even catabolic products. As a part of our biosynthetic studies of mikrolin (**1**) and dechloromikrolin (**2**) [1], the two novel metabolites of *Gilmaniella humicola* BARRON [2], we have examined the effect of changing the culture medium on the nature of secondary metabolism. Experiments with six different synthetic and semisynthetic media were performed. The fungus grew quite well on four media (A to D) and not at all on two (E and F) (see Experimental Part). Chemical investigations of ethyl acetate extracts of these fermentations revealed, in addition to mikrolin (**1**) and dechloromikrolin (**2**), the presence of mycorrhizin A (**11**) [3], (–)-6-hydroxy-mellein (**14**) [4], and of two new compounds named glimicolin (**3**) and mycorrhizinol (**15**). Structural investigations of these compounds by chemical and physical methods as well as an X-ray crystal analysis of **3** are presented in this report. The significance of these metabolites to the biosynthetic pathway is also discussed.

2. Gilmicolin (3). – Gilmicolin crystallized from acetone/hexane as stout needles, m.p. 120°; $[\alpha]_D^{20} = -48 \pm 2°$ (methanol). The presence of signals for 18 protons in

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0018-019X/79/4/1129-14$01.00/0

Table 1. *Assignment of H-atoms in the ^{1}H-NMR. spectra*

Compound	C(1)	C(2)	C(3)	C(5)	C(8)	C(9)	C(13) (14)	Others
3a	1.50 *d* *J*=6.2	4.45 *m* *J*=6; 6.2; 7.2	2.98 *d*×*d*×*d* *J*=15.3; 6.2; 1.2 1H 2.81 *d*×*d*×*d* *J*=15.3; 7.2; 2.7 1H	6.06 *d*×*d* *J*=2.7; 1.2	1.76 *d*×*d* *J*=6.2; 4.1 1H 1.67 *d*×*d* *J*=9; 4.1 1H	2.34 *d*×*d* *J*=9; 6.2	1.38 *s* 1.47 *s*	3.8; 4.08 2×OH
3b	1.50 *d* *J*=6	4.42 *m*	2.80–3.0 *m*	6.79 *t* *J*=0.9	~1.28 ~1.8	*ca.* 2.1	1.38 *s* 1.46 *s*	3.89; 4.17 2×OH
5	1.13 *d* *J*=6.2	5.10 *m*	2.87 *d*×*d*×*d* *J*=4.6; 0.9; 14 1H 2.56 *d*×*d*×*d* *J*=14; 0.9; 7.4 1H	6.77 *t* *J*=0.9	1.95 *d*×*d* *J*=4.4; 8.6 1H 1.42 *d*×*d* *J*=4.4; 3.5 1H	2.20 *d*×*d* *J*=5.5; 8.6	1.31 *s* 1.37 *s*	1.98; 2.05 2×CH_3–CO
6	1.27 *d* *J*=6	~4.12 *m*	2.56 *m* *J*=7.6; 4.4; 1.2	6.46 *t* *J*=1.2	1.22 *d* *J*=7	3.12 *qa* *J*=7	1.42 *s* 1.46 *s*	OH
7	1.27 *d* *J*=6.1	~4.16 *m*	2.73 *d* *J*=4.6	6.12 *s*	4.21 *d*×*d* *J*=7.4; 13.5 1H 4.38 *d*×*d* *J*=13.5; 7.4 1H	3.37 *t* *J*=7.4	1.49 *s* 2×CH_3	2.13 *s* CH_3–CO 3.65 br. OH
8	1.18 *d* *J*=6.5	*ca.* 5.04 *m*	2.76 *d* *J*=5	6.46 *s*	4.46 *d*×*d* *J*=6; 13 1H 4.14 *d*×*d* *J*=13; 10 1H	3.38 *d*×*d* *J*=10; 6	1.44 *s*	2.01; 2.04; 2.31; 2.34 4×CH_3–CO
9	1.86 *d* *J*=6.7	5.84 *qa* *J*=6.7	–	6.64 *s*	4.5 *d*×*d* *J*=11.4 1H 4.06 *d*×*d* *J*=11.9 1H	3.6 *d*×*d* *J*=4.9	4.1 *s* 1.49 *s*	2.0; 2.05; 2.24; 2.30 4×CH_3–CO

10	2.05 *d* *J*=7	7.05 *qa* *J*=7	-	7.18 *s*	1.68 *t* *J*=5 1H 1.92 *d*×*d* *J*=5; 8 1H	2.36 *d*×*d* *J*=5; 8	4.15 (13-CH_2) 1.40 *s*	1.92; 2.1 2× CH_3–CO
11	2.03 *d* *J*=6.8	7.01 *qa* *J*=6.8	-	7.12 *s*	1.60 *d*×*d* *J*=4.5; 5.6 1H 1.90 *d*×*d* *J*=8; 4.5 1H	2.24 *d*×*d* *J*=8.1; 5.6	1.25 *s* 1.35 *s*	
12	2.04 *d* *J*=7	7.04 *qa* *J*=7	-	7.16 *s*	1.68 *t* *J*=5 1H 1.91 *t* *J*=5; 8 1H	2.30 *d*×*d* *J*=5; 8	1.30 *s* 1.37 *s*	1.96 CH_3–CO
15	2.46	-	-	6.43	2.73 *d*×*d* *J*=18; 4.7 1H 3.07 *d*×*d* *J*=18; 5 1H	3.9 *d*×*d* *J*=5; 4.7	1.41 *s* 1.46 *s*	OH at 3.9
16	2.46	-	-	6.7	2.73 *d*×*d* *J*=18; 4.6 1H 3.07 *d*×*d* *J*=18; 5 1H	5.07 *d*×*d* *J*=5; 4.6	1.38 *s* 1.44 *s*	2.05; 2.38 2× CH_3–CO
17	2.45	-	-	6.47	2.66 *d*×*d* *J*=18; 5 1H 3.02 *d*×*d* *J*=18; 5 1H	3.9 (under OCH_3 signal)	1.4 *s* 1.46 *s*	3.87 OCH_3

All spectra were measured in $CDCl_3$ with TMS as internal standard. *s*=singlet, *d*=doublet, *t*=triplet, *qa*=quartet, *m*=multiplet, br.=broad. Spin-spin coupling constant (*J*) is in Hz. Numbering is shown on formula **3**.

the ^{1}H-NMR. spectrum, signals for 14 carbon atoms in the ^{13}C-NMR. spectrum and M^+ and $M^+ + 2$ peaks at 266 and 268 respectively in the mass spectrum lead to the assignment of $C_{14}H_{18}O_5$ (found 266.1154; calc. for $C_{14}H_{18}O_5$: 266.1131) as the molecular formula for **3**. The $M^+ + 2$ peak most probably arises due to the presence of moisture in the mass spectrometer. Similar phenomena have been observed previously with certain quinones and are due to reduction of the quinones by moisture in the mass spectrometer [5]. Like mikrolin (**1**) and dechloromikrolin (**2**) [1], glimicolin (**3**) also exists in solution as an equilibrium mixture of structures **3a** and **3b**, the former being the major component. Since **3b** was found to be more soluble in ether than **3a** we were able to obtain **3a** in a pure state and to crystallize it from acetone/hexane mixtures. On acetylation with acetic anhydride and pyridine both **3a** and **3b** gave the same di-*O*-acetyl derivative **5**, thereby confirming that **3b** is an open form of the hemiacetal **3a**. This equilibrium is a slow process and in the ^{1}H-NMR. spectrum of **3a** no signals attributable to **3b** were observed. On the other hand in the ^{13}C-NMR. spectrum one always observes two sets of signals (data collection time *ca.* 16 hours) in a ratio of 3:1. We were never able to isolate **3b** in a pure state and most of the studies reported herein were carried out either on pure **3a** or the equilibrium mixture.

IR. absorption at 1680 cm^{-1} and UV. absorption at 290 nm ($\log\varepsilon = 3.9$) suggested the presence of an α,β-unsaturated carbonyl unit in the molecule. Absorptions at 3530 and 1380 cm^{-1} in the IR. spectrum of **3a** indicated the presence of hydroxyl and geminal dimethyl groups respectively. The ^{1}H-NMR. spectrum of **3a** *(Table 1)* displayed signals for geminal dimethyl groups at 1.38 and 1.47 ppm, an *AMX* (CH_2–CH) system at 1.6–2.34 ppm, two hydroxyl protons (exchangeable with D_2O) at 3.8 and 4.08 ppm and a $CH{=}C{-}CH_2{-}\underset{\displaystyle O}{\underset{|}{CH}}{-}CH_3$ unit. These assignments were confirmed by decoupling experiments. The ^{13}C-NMR. spectrum *(Table 2)* corroborated the above mentioned conclusions and further indicated the presence of one carbonyl carbon atom (at 192.66 ppm) and four quaternary carbon atoms (at 44.7, 85.51, 99.41, 104.28 ppm) in the molecule. Comparison of these chemical shifts to those exhibited by the anomeric carbon atom of five membered sugars (*ca.* 101 ppm) suggested the presence of two five membered rings incorporating hemiacetal units. The signals for the minor component in the ^{13}C-NMR. spectrum are in agreement with the structure **3b**. The change in chemical shifts of C(2), C(4), C(6) and C(12) is consistent with the transformation from **3a** to **3b**.

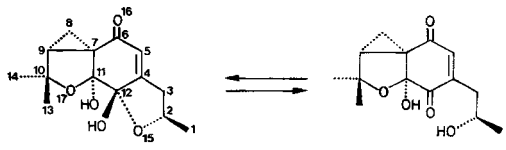
Comparison of spectral properties of glimicolin with those of mikrolin (**1**) and dechloromikrolin (**2**) strongly suggested that glimicolin should have structure **3**. The following experiments provide strong support for this conclusion. The crude product from catalytic hydrogenation of **3** upon purification (silica gel) yielded a yellow gum, the UV. spectrum (272 nm; $\log\varepsilon = 4.21$) of which indicated the presence of a 1,4-quinonoid moiety in the molecule. The set of *AMX*-signals (cyclopropane unit) in the ^{1}H-NMR. spectrum of **3a** was replaced by signals due to a CH_3–CH unit *(Table 1)* in the ^{1}H-NMR. spectrum of **6**. Reduction of **3** with zinc in acetic acid gave the 1,4-dihydroquinone derivative **7** as the major product. The ^{1}H-NMR. spectrum of **7** exhibited signals for a $CH_3{-}COOCH_2{-}CH$ unit instead of those for the cyclo-

Scheme 1

1a X = Cl
2a X = H

Mikrolin
Dechloromikrolin

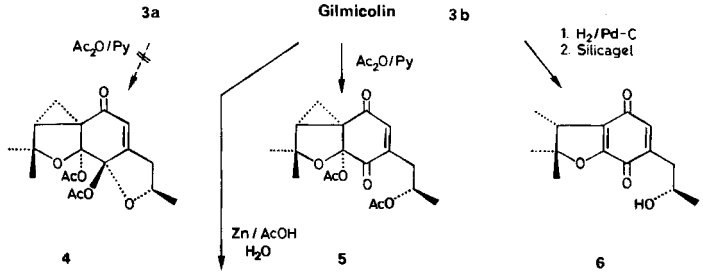
1b X = Cl
2b X = H



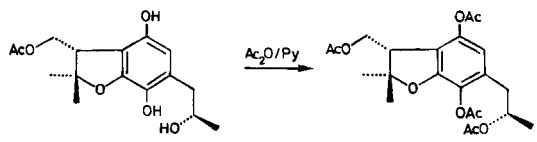
3a

Gilmicolin

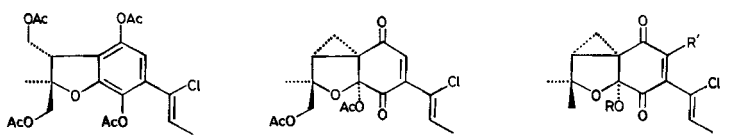
3b



4 5 6

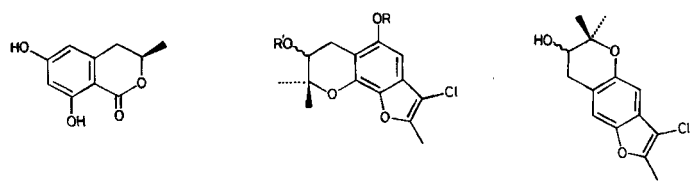


7 8



9 10

11 R=R'=H: **Mycorrhizin A**
12 R=Ac; R'=H
13 R=H ; R'=Cl



14

15 R=R'=H: **Mycorrhizinol**
R=R'=Ac
R=CH_3; R'=H

18

Table 2. *Assignments of the C-atoms in the ^{13}C-NMR. spectra*

Compound	3a	3b	5	10
C(1)	23.13 *qa*	23.65 *qa*	19.95 *qa*	16.44 *qa*
C(2)	76.77 *d*	66.93 *d*	68.74 *d*	135.47 *d*
C(3)	37.30 *t*	39.44 *t*	35.74 *t*	126.77 *d*
C(4)	159.71 *s*	149.31 *s*	146.26 *s*	144.51 *s*
C(5)	122.48 *d*	139.58 *d*	139.83 *d*	137.68 *d*
C(6)	192.46 *s*	194.34* *s*	190.57* *s*	190.70 *s*
C(7)	44.77 *s*	43.01 *s*	44.96 *s*	44.90 *s*
C(8)	19.36 *t*	14.10 *t*	14.16 *t*	14.55 *t*
C(9)	42.43 *d*	43.47 *d*	46.13 *d*	41.71 *d*
C(10)	85.51 *s*	82.13 *s*	84.09 *s*	85.51 *s*
C(11)	104.28 *s*	99.93 *s*	103.05 *s*	104.55 *s*
C(12)	99.41 *s*	194.36* *s*	190.12* *s*	187.50 *s*
C(13)	29.56* *qa*	29.31 *qa*	30.02 *qa*	69.72 *t*
C(14)	27.03* *qa*	24.89 *qa*	24.89 *qa*	20.92 *qa*
Other C-Atoms			20.21; 20.53; 170.04	170.17; 169.78; 20.53; 20.92

All spectra were measured in $CDCl_3$ with TMS as internal standard. *s* = singlet, *d* = doublet, *t* = triplet, *qa* = quartet. Assignments marked with * may be reversed in a particular column.

propane ring in the ^1H-NMR. spectrum of **3a**, thereby indicating that the addition of **1** equivalent of acetic acid has occurred across the C(7), C(8) bond. Acetylation of **7** with acetic anhydride/pyridine gave the tetra-*O*-acetyl derivative **8**. The formation of these products can be explained by the same mechanisms as recently discussed for the formation of **9** from mikrolin (**1**) [1].

Treatment of **3** with pyridine/acetic anhydride gave the di-*O*-acetyl derivative **5** as an oil. The appearance of H–C(5) at 6.77 ppm, a downfield shift of *ca.* 1 ppm for H–C(2) in the ^1H-NMR. spectrum, and most importantly the ^{13}C chemical shift of 192.4 ppm for C(12) (shifted from 99.4 ppm in the ^{13}C-NMR. spectrum of **3a**) clearly suggest the presence of the tricyclic structure **5** and not of the tetracyclic structure **4**. Comparison of the spectral properties of **5** with those of di-*O*-acetyl mikrolin (**10**) (*Tables 1* and *2*) also tends to support this conclusion.

With the overall skeleton of gilmicolin firmly established, the objective was to determine the configuration of the five chiral centers. The configuration at chiral carbon atoms No. 7, 9 and 11 was assigned by analogy to that of mikrolin (**1**) [2][2]). The answer to the question of the relative configuration of the 11,12-diol was provided by the relative rate of oxidation with CrO_3/H_2SO_4 in acetone. Since *cis*- and *trans*-ditertiary glycols are known to undergo oxidative cleavage with CrO_3/H^+ at very different rates [6], the relative inertness of **3** to CrO_3/H^+ conditions (*ca.* 80% recovery of the starting material after a reaction time of 5 minutes) as compared to mikrolin (**1**) (*ca.* 10% recovery of starting material after 5 minutes) indicated a *trans*-relationship of 11,12-glycol system in gilmicolin (**3**), in contrast to the *cis*-relation-

[2]) Numbering is shown on formula **3**. This numbering system was coined by *Bollinger et al.* [2] for the mikrolin (**1**) skeleton and is retained for all the related compounds.

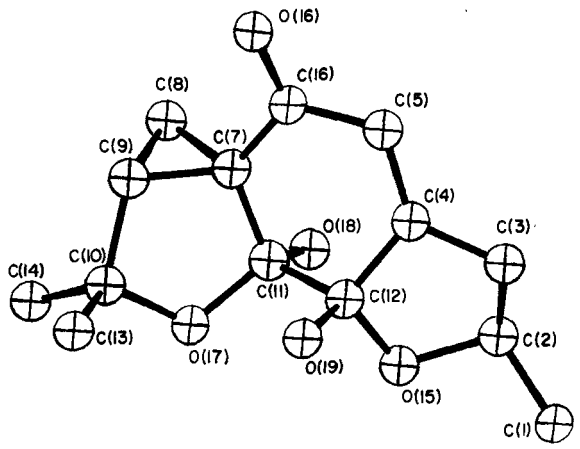


Fig. *Computer Generated Perspective Drawing of the Final X-Ray Model of Gilmicolin* (**3**).

ship observed in mikrolin (**1**) as established by X-ray analysis [2]. In order to confirm these results and to find out the configuration of the methyl group at C(2) an X-ray analysis was carried out.

The crystals of **3a** were monoclinic with systematic extinctions conforming to the common chiral space group $P2_1$. Accurate cell constants, determined from a least-squares fit of fifteen high angle reflections, were: $a = 6.809(8)$, $b = 9.869(9)$, $c = 10.927(11)$ Å and $\beta = 113.42(8)°$. A density of ~1.31 g/cc indicated one molecule of $C_{14}H_{18}O_5$ formed the asymmetric unit. All unique diffraction maxima with $2\theta \leqslant 114.1°$ were recorded on a four-circle diffractometer using graphite monochromated Cu*Ka* radiation(1.54178 Å) and a one-degree, variable speed ω-scan. Of the 973 unique reflections surveyed, 938 (96%) were judged observed ($I \geqslant 3\sigma(I)$) after correction for *Lorentz,* polarization and background effects.

The structure was determined uneventfully by a multisolution weighted tangent formula approach. All hydrogen atoms were located on difference electron density syntheses and included in subsequent calculations. Full matrix least-squares refinement with anisotropic temperature factors for the nonhydrogen atoms and isotropic temperature factors for the hydrogen atoms converged to a conventional crystallographic discrepancy index of 0.054. The *Figure* is a computer generated perspective drawing of the final X-ray experiment [7]. It does not define the absolute configuration.

All bond distances and angles agree well with generally accepted values. Further details can be found in *Table 3* (fractional coordinates and temperature factors), *Table 4* (bond distances) and *Table 5* (bond angles)[3]). Ring A has a slightly puckered conformation. All five atoms are nearly in one plane since C(7), C(10), C(11) and C(17) are coplanar within 0.01 Å and C(9) is only 0.11 Å out of plane *(cf. Fig.).* Ring C also has an envelope conformation: C(2), C(4), C(12) and O(15) are coplanar within 0.01 Å and C(3) is 0.52 Å out of the plane. There are two intermolecular

[3]) The observed and calculated structure factors are available from the authors[1]).

Table 3. *Fractional coordinates and temperature factors for gilmicolin* (3). Standard deviations of the least significant figures are given in parentheses. Hydrogens are assigned the same numbers as the heavy atoms to which they are bonded

A	X	Y	Z	B11 OR B	B 22	B 33	B 12	B 13	B 23
C (1)	−0.0503 (14)	0.2550 (16)	−0.2731 (6)	0.0425 (25)	0.0139 (9)	0.0069 (7)	0.0022 (19)	0.0111 (11)	0.0002 (7)
C (2)	−0.1623 (10)	0.2684 (13)	−0.1784 (5)	0.0316 (16)	0.0085 (6)	0.0043 (5)	0.0031 (10)	0.0057 (7)	0.0008 (5)
C (3)	−0.3130 (10)	0.1550 (13)	−0.1797 (5)	0.0238 (17)	0.0110 (7)	0.0051 (5)	−0.0002 (10)	0.0028 (8)	−0.0005 (5)
C (4)	−0.2972 (8)	0.1627 (12)	−0.0398 (5)	0.0201 (13)	0.0077 (6)	0.0068 (5)	−0.0009 (8)	0.0046 (7)	−0.0000 (5)
C (5)	−0.4470 (9)	0.1378 (13)	0.0095 (6)	0.0154 (15)	0.0129 (8)	0.0090 (6)	−0.0026 (9)	0.0036 (8)	0.0000 (6)
C (6)	−0.3964 (8)	0.1655 (12)	0.1491 (5)	0.0162 (12)	0.0088 (6)	0.0098 (6)	−0.0012 (8)	0.0065 (7)	0.0021 (5)
C (7)	−0.2149 (8)	0.2544 (12)	0.2215 (5)	0.0196 (12)	0.0057 (5)	0.0079 (5)	0.0014 (7)	0.0085 (7)	0.0015 (5)
C (8)	−0.2365 (11)	0.3554 (12)	0.3208 (6)	0.0298 (19)	0.0111 (9)	0.0107 (7)	0.0021 (10)	0.0139 (10)	−0.0013 (6)
C (9)	−0.0815 (8)	0.2420 (12)	0.3671 (5)	0.0259 (15)	0.0081 (6)	0.0067 (5)	−0.0016 (9)	0.0094 (8)	−0.0010 (5)
C (10)	0.1509 (8)	0.2689 (12)	0.3886 (4)	0.0237 (14)	0.0081 (6)	0.0041 (4)	−0.0009 (8)	0.0056 (6)	−0.0008 (5)
C (11)	−0.0675 (7)	0.3053 (11)	0.1566 (4)	0.0177 (12)	0.0034 (5)	0.0058 (5)	0.0011 (6)	0.0053 (6)	0.0011 (4)
C (12)	−0.0758 (8)	0.2057 (11)	0.0450 (5)	0.0160 (12)	0.0053 (5)	0.0055 (5)	0.0006 (6)	0.0058 (6)	0.0004 (4)
C (13)	0.2776 (11)	0.1368 (13)	0.4262 (6)	0.0243 (18)	0.0111 (8)	0.0075 (6)	0.0015 (10)	0.0052 (8)	0.0018 (6)
C (14)	0.2613 (12)	0.3801 (13)	0.4874 (6)	0.0347 (22)	0.0126 (9)	0.0073 (7)	−0.0045 (13)	0.0072 (10)	−0.0025 (6)
C (15)	0.0000 (5)	0.2725 (11)	−0.0434 (3)	0.0247 (9)	0.0071 (4)	0.0059 (3)	−0.0018 (5)	0.0078 (5)	0.0004 (3)
O (16)	−0.5052 (6)	0.1170 (12)	0.2069 (4)	0.0231 (11)	0.0163 (7)	0.0136 (5)	−0.0044 (7)	0.0111 (7)	0.0024 (5)
O (17)	0.1388 (5)	0.3140 (11)	0.2582 (3)	0.0184 (8)	0.0068 (4)	0.0050 (3)	−0.0012 (4)	0.0048 (4)	0.0001 (3)
O (18)	−0.1352 (6)	0.4337 (11)	0.1001 (4)	0.0280 (10)	0.0063 (4)	0.0077 (4)	0.0029 (5)	0.0093 (6)	0.0016 (3)
O (19)	0.0472 (7)	0.0904 (11)	0.0986 (4)	0.0168 (12)	0.0065 (4)	0.0068 (4)	0.0002 (5)	0.0050 (5)	−0.0004 (3)
H (1A)	−0.169 (11)	0.268 (8)	−0.375 (7)	4.4 (13)					
H (1B)	0.067 (13)	0.314 (9)	−0.249 (7)	3.9 (15)					
H (1C)	0.044 (13)	0.192 (9)	−0.248 (8)	4.1 (17)					
H (2)	−0.249 (11)	0.344 (9)	−0.201 (7)	3.8 (14)					
H (3A)	−0.235 (8)	0.065 (6)	−0.202 (5)	2.0 (10)					
H (3B)	−0.450 (12)	0.161 (7)	−0.237 (6)	3.3 (12)					
H (5)	−0.589 (11)	0.101 (7)	−0.039 (6)	3.3 (11)					
H (8A)	−0.365 (12)	0.352 (8)	0.324 (6)	4.2 (14)					
H (8B)	−0.181 (10)	0.444 (8)	0.307 (6)	3.5 (13)					
H (9)	−0.117 (7)	0.168 (6)	0.412 (5)	1.4 (8)					
H (13A)	0.401 (14)	0.149 (9)	0.427 (7)	4.6 (16)					
H (13B)	0.289 (11)	0.116 (8)	0.519 (8)	4.7 (15)					
H (13C)	0.189 (10)	0.073 (7)	0.360 (7)	3.0 (12)					
H (14A)	0.168 (11)	0.467 (9)	0.462 (6)	4.0 (13)					
H (14B)	0.272 (11)	0.361 (8)	0.581 (8)	5.2 (16)					
H (14C)	0.417 (13)	0.405 (8)	0.506 (8)	5.1 (16)					
H (18)	−0.084 (12)	0.473 (8)	0.047 (8)	4.5 (16)					
H (19)	0.161 (11)	0.112 (6)	0.132 (5)	1.6 (12)					

Table 4. *Bond distances of gilmicolin* (3). The standard deviation of the least significant figure of each distance is given in parentheses

C(1)-C(2)	1.516 (12)	C(7) -C(11)	1.526 (9)
C(2)-C(3)	1.515 (15)	C(8) -C(9)	1.482 (14)
C(2)-C(15)	1.451 (5)	C(9) -C(10)	1.528 (8)
C(3)-C(4)	1.491 (8)	C(10)-C(13)	1.527 (15)
C(4)-C(5)	1.352 (10)	C(10)-C(14)	1.513 (14)
C(4)-C(12)	1.484 (8)	C(10)-O(17)	1.463 (8)
C(5)-C(6)	1.450 (9)	C(11)-C(12)	1.550 (11)
C(6)-C(7)	1.465 (11)	C(11)-O(17)	1.405 (5)
C(6)-O(16)	1.244 (10)	C(11)-O(18)	1.405 (14)
C(7)-C(8)	1.523 (13)	C(12)-C(15)	1.424 (10)
C(7)-C(9)	1.490 (7)	C(12)-O(19)	1.396 (13)

Table 5. *Bond angles of gilmicolin* (3). The standard deviation of the least significant figure of each distance is given in parentheses

C(1)-C(2)-C(3)	116.9 (9)	C(9) -C(10)-C(13)	109.5 (9)
C(1)-C(2)-C(15)	108.1 (5)	C(9) -C(10)-C(14)	115.2 (7)
C(3)-C(2)-C(15)	105.6 (7)	C(9) -C(10)-O(17)	104.4 (4)
C(2)-C(3)-C(4)	100.4 (6)	C(13)-C(10)-C(14)	111.1 (5)
C(3)-C(4)-C(5)	129.9 (5)	C(13)-C(10)-O(17)	109.2 (7)
C(3)-C(4)-C(12)	107.2 (5)	C(14)-C(10)-O(17)	107.1 (8)
C(5)-C(4)-C(12)	122.9 (5)	C(7) -C(11)-C(12)	109.4 (7)
C(4)-C(5)-C(6)	118.9 (6)	C(7) -C(11)-O(17)	106.8 (4)
C(5)-C(6)-C(7)	118.5 (7)	C(7) -C(11)-O(18)	109.9 (6)
C(5)-C(6)-O(16)	121.4 (7)	C(12)-C(11)-O(17)	111.9 (6)
C(7)-C(6)-O(16)	120.1 (6)	C(12)-C(11)-O(18)	108.9 (5)
C(6)-C(7)-C(8)	118.5 (6)	O(17)-C(11)-O(18)	110.1 (8)
C(6)-C(7)-C(9)	123.6 (8)	C(4) -C(12)-C(11)	112.5 (5)
C(6)-C(7)-C(11)	121.1 (5)	C(4) -C(12)-C(15)	105.1 (4)
C(8)-C(7)-C(9)	58.9 (6)	C(4) -C(12)-O(19)	108.6 (9)
C(8)-C(7)-C(11)	112.5 (8)	C(11)-C(12)-C(15)	109.3 (8)
C(9)-C(7)-C(11)	106.8 (4)	C(11)-C(12)-O(19)	111.0 (5)
C(7)-C(8)-C(9)	59.4 (5)	C(15)-C(12)-O(19)	110.1 (6)
C(7)-C(9)-C(8)	61.7 (5)	C(2) -C(15)-C(12)	110.0 (5)
C(7)-C(9)-C(10)	107.7 (5)	C(10)-O(17)-C(11)	113.8 (5)
C(8)-C(9)-C(10)	118.4 (9)		

hydrogen bonds, O(19)–H– – –O=C of 2.809 Å and O(18)–H– – –O(19) of 2.915 Å. All the other intermolecular distances correspond to *van der Waals'* contacts.

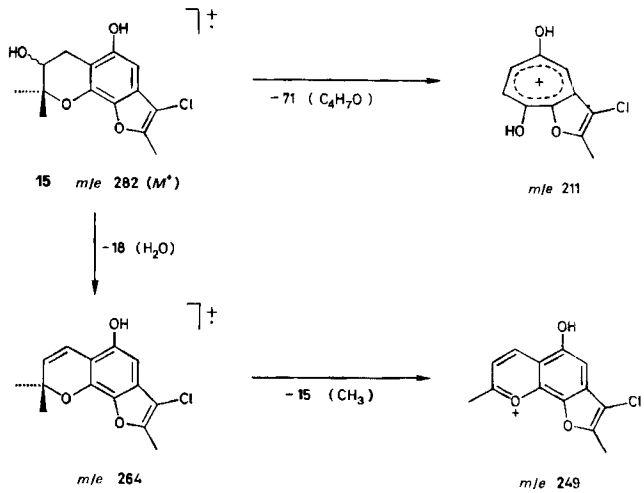
3. Mycorrhizin A (11). – The metabolite crystallized from methylene chloride/heptane mixtures as yellow crystals; m.p. 161–162°, $[\alpha]_D^{25} = +32 \pm 2°$ (ethanol). The molecular formula $C_{14}H_{15}ClO_4$ (M^+ 282.0646, calc. for $C_{14}H_{15}ClO_4$: 282.0659) was determined by high resolution mass spectrometry. The major fragmentations can be summarized as *m/e* 282; *m/e* 264 ($M^+ - H_2O$); *m/e* 229 ($M^+ - H_2O - Cl$); *m/e* 201 ($M^+ - H_2O - Cl - CO$); and *m/e* 173 ($M^+ - H_2O - Cl - 2\,CO$). The IR. spectrum exhibited absorptions for hydroxyl, carbonyl and geminal methyl groups at 3500, 1720 and 1390 cm^{-1} respectively. The ^{1}H-NMR. spectrum of **11** *(Table 1)* displayed signals for 3 methyl groups at 1.25, 1.35 and 2.03 ppm. The latter was coupled to an olefinic proton at 7.01 ppm ($J = 6.8$ Hz). The second proton in the olefinic region appeared

as a singlet at 7.12 ppm. The remaining signals were consistent with a tertiary hydroxyl group and an *AMX* multiplet (confirmed by decoupling experiments). Treatment of **11** with pyridine/acetic anhydride yielded an *O*-acetyl derivative **12** as an oil. On comparing the ^{1}H-NMR. spectra of mycorrhizin A and its *O*-acetyl derivative with those of **5** and **10** *(Table 1)*, structures **11** and **12** respectively were assigned. At this time *Trofast & Wickberg* [3] reported on the isolation and structure elucidation of mycorrhizin A and chloromycorrhizin A. These compounds were isolated from an unidentified fungus D and structures **11** and **13** respectively were assigned on the basis of chemical studies [3] and X-ray analysis of **13** [8]. Comparison of the spectral properties reported for mycorrhizin A (**11**) with those of **11** in our hands clearly suggested that these two compounds are identical. Direct comparison of **11** with the mycorrhizin A by thin-layer chromatography and mixed melting point left no doubt about the identity of the two compounds[4]).

4. Mycorrhizinol (15). – The metabolite crystallized as plates from methylene chloride/heptane mixtures, m.p. 115–116°. It was sensitive to air oxidation. Accurate mass measurement of the molecular ion at *m/e* 282 gave $C_{14}H_{15}ClO_4$ as the molecular formula (found 282.0656, calc. for $C_{14}H_{15}ClO_4$: 282.0659) and indicated its isomeric nature to mycorrhizin A (**11**). The fragmentation pattern in the mass spectrometer of **15** was quite different from that of **11**. The molecular ion (*m/e* 282) showed a major loss of a C_4H_7O unit giving rise to the base peak at *m/e* 211 *(Scheme 2)*.

Positive iron(III)chloride test in conjunction with the formation of a mono-*O*-methyl ether upon treatment with methyl iodide and potassium carbonate in acetone suggested the presence of one phenolic unit in the molecule. Presence of a substituted benzofuran unit was indicated by absorptions at 250 (4.00), 264 (4.18) and 269 (3.48) nm ($\log\varepsilon$) in the UV. spectrum [9]. The ^{1}H-NMR. spectrum of **15** *(Table 1)* revealed

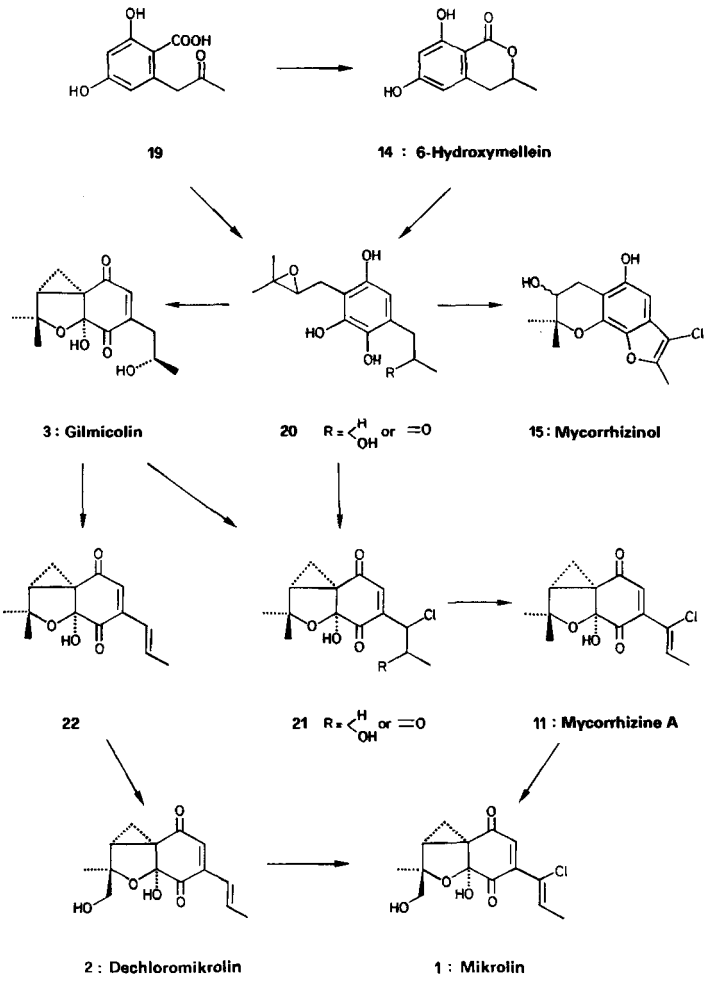
Scheme 2



[4]) We thank Prof. *B. Wickberg*, Lund Institute of Technology, Lund, Sweden, for sending us a sample of his natural material.

the presence of two aliphatic tertiary methyl groups, one aromatic methyl group, one uncoupled aromatic proton, an *ABX*-system in which the methylene protons were of benzylic nature, and a secondary hydroxyl group in its structure. On acetylation with pyridine/acetic anhydride mycorrhizinol (**15**) yielded the di-*O*-acetyl derivative **16**. After a detailed comparison of the chemical shifts in the ^{1}H-NMR. spectrum with the chemical shift values for many possible alternative structures and on biosynthetic grounds (especially its co-occurrence with mycorrhizin A (**11**), mikrolin (**1**) and dechloromikrolin (**2**)), it was concluded that mycorrhizinol must have either structure **15** or **18**. The structure **15** is favoured over **18** due to the following evidence. The ^{1}H-NMR. spectrum of *O*-methyl-mycorrhizinol (**17**) in C_6D_6 exhibited an upfield shift of 0.48 ppm for the methoxy protons, compared with the spectrum in $CDCl_3$. This indicates that at least one of the positions ortho to the

Scheme 3



methoxy group is unsubstituted [10]. Due to paucity of the material the absolute configuration at C(9) could not be determined.

5. Discussion. – In the preceding publication [1] we have suggested that mikrolin (**1**) and dechloromikrolin (**2**) are derived from 6-hydroxy-mellein (**14**) or 2-carboxy-3,5-dihydroxybenzyl methyl ketone (**19**) (not isolated) by decarboxylation and hydroxylation at the same carbon atom and by introduction of prenyl unit from mevalonate, followed by epoxidation to give the hypothetical intermediate **20**. From the latter, the formation of the mikrolins may be postulated as occurring by further transformations as outlined in *Scheme 3*. The co-occurrence of compounds **1**, **2** and **14** along with gilmicolin (**3**) and mycorrhizin A (**11**) in the culture filtrates tends to support this hypothesis. Although we have not been able to detect the presence of the hypothetical intermediate **20** in our extracts it is possible that biological transformations of such precursors to mikrolin (**1**), dechloromikrolin (**2**) and gilmicolin (**3**) are so efficient as to prevent its accumulation. The biosynthesis of mikrolin (**1**) from the hypothetical intermediate **20**, requires its conversion to gilmicolin (**3**) followed by dehydration to compound **22**, which has not been isolated yet, and hydroxylation to dechloromikrolin (**2**) and chlorination of the latter. It is not certain which process occurs first. Another possibility is the chlorination taking place at the polyketide stage or at the intermediate **20**. Thus pathways for mikrolin (**1**) and dechloromikrolin (**2**) diverge at one of these points. It is also unknown whether compound **21**, which has not been isolated, acts as a precursor of mycorrhizin A (**11**). Studies for the elucidation of the later states of the biosynthesis of the mikrolins are in progress and will be published in due course.

The support of these investigations by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* (Projects No. 2.435.0.75 und 2.629.0.76) and by *Sandoz AG.*, Basel, is gratefully acknowledged.

Experimental Part

1. *General Methods.* The melting points were determined on a *Kofler* block and are uncorrected. IR. (cm^{-1}), UV. (λ_{max} nm ($\log\varepsilon$)) and optical rotations were measured on a *Perkin Elmer* Model 125 grating spectrometer, *Beckman* D.K.2 spectrophotometer and *Perkin Elmer* Model 141 polarimeter respectively. The 90-MHz-^{1}H-NMR. and 22.63-MHz-^{13}C-NMR. spectra were recorded with a *Bruker* WH-90 spectrometer with *Fourier* Transform in the spectral laboratory of our institute. The low resolution mass spectra were recorded in the Physikalisch-chemisches Institut der Universität Basel, on an *A.E.I.* MS-30 instrument *(A. Raas)*. We thank Dr. *H. Lichti, Sandoz AG.*, Basel, for measurements of high resolution mass spectra on a *CEC* 21-110B instrument. Preparative thin-layer chromatography (TLC.) was carried out on silica gel PF 254 *(Merck)* and for column chromatography, silica gel 0.05–0.2 mm from *E. Merck AG.*, Darmstadt, was used.

2. *Culture Media and Isolation of Products.* In general 500 ml *Erlenmeyer* flasks containing 150 ml of sterilized medium were inoculated with 1 ml of spore suspension under aseptic conditions. These flasks were shaken on a rotary shaker at 200 revolutions/minute at 25° for 6 days. Both the mycelium and the medium were then extracted with ethyl acetate. The organic extracts were washed twice with water, dried with sodium sulfate (anhydrous) and evaporated *in vacuo* (i.V.) at 40°. The crude extract was chromatographed on silica gel using increasing amounts of methanol in methylene chloride. In a preparative experiment the fermentations were performed in a 10 l fermenter. The sterilized culture medium (10 l) was inoculated with 50 ml of spore suspension under sterile conditions and stirred at 300 rpm with air circulation of 0.4 l per liter of culture medium per minute at 27°.

Culture media A to F per liter:

A: 20 g glucose H_2O, 2 g malt extract, 2 g peptone, 2 g yeast extract, 2 g KH_2PO_4, 2 g $MgSO_4 \cdot 7\ H_2O$;

B: 55 g glucose H_2O, 10 g ammonium nitrate, 5 g KH_2PO_4, 2.5 g $MgSO_4 \cdot 7\ H_2O$ and 30 mg $FeCl_3 \cdot 6\ H_2O$;

C: 85 g sucrose, 10 g L-asparagine, 3.5 g ammonium sulfate, 10 g KH_2PO_4, 2 g $MgSO_4 \cdot 7\ H_2O$, 75 mg $CaCl_2 \cdot 2\ H_2O$, 5 mg $ZnSO_4 \cdot 7\ H_2O$, 5 mg $Na_2B_2O_7$, 2 mg $FeSO_4 \cdot 7\ H_2O$ and pH was adjusted to 4.5 by addition of HCl;

D: 85 g glucose H_2O, 10 g L-aspartic acid, 10 g KH_2PO_4, 3.5 g $(NH_4)_2SO_4$, 2 g $MgSO_4 \cdot 7\ H_2O$, 75 mg $CaCl_2 \cdot 2\ H_2O$, 5 mg $ZnSO_4$, 5 mg $Na_2B_2O_7$ and pH was adjusted to 4.5 by adding NH_4OH;

E: 40 g glucose H_2O, 1.2 g urea, 0.6 g K_2HPO_4, 0.4 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.5 g KCl, 10 mg $FeSO_4 \cdot 7\ H_2O$;

F: 10 g sucrose, 5 g peptone, 3 g beef extract, 2 g NaCl, 0.5 g KH_2PO_4, 125 mg $MgSO_4 \cdot 7\ H_2O$, 0.5 g $Ca(NO_3)_2$ and 1 ml of trace element solution.

The yield of the isolated metabolites are listed in *Table 6.*

3. *Gilmicolin* (**3**). From acetone/hexane crystals of m.p. 120°, $[\alpha]_D^{20} = -48 \pm 2°$ ($c = 0.143$, methanol). – IR. ($CHCl_3$): bands at 3530, 1680, 1380. – UV. (Ethanol) 290 (3.9). – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – ^{13}C-NMR. ($CDCl_3$): *Table 2.* – MS.: 266.1154 (calc. for $C_{14}H_{18}O_5$: 266.1131).

Table 6. *Yield of Metabolites* (mg/l culture medium)

Medium	(1)	(2)	(3)	(11)	(15)	(14)
A	75–80	70–80	0	0	0	0
B	20–30	20–30	75–80	0	15–20	0
C	25–30	25–30	0	4–5	10–15	3–4
D	15–20	15–20	0	3–4	10–15	3–4
E	0	0	0	0	0	0
F	0	0	0	0	0	0

4. *Acetylation of* **3** *to di-*O*-acetyl derivative* **5**. A solution of 50 mg of **3** in 3 ml of pyridine and 1.5 ml of acetic anhydride was stirred at RT. for 18 h. The solvent was removed i.V. at ambient temperature and reaction product purified by preparative TLC. (methylene chloride/methanol 97:3) to give 42 mg of di-*O*-acetyl derivative **5** as an oil, $[\alpha]_D^{20} = +65.7 \pm 2°$ ($c = 1.86$, methanol). – IR. ($CHCl_3$): bands at 1730, 1702, 1678, 1375. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – ^{13}C-NMR. ($CDCl_3$): *Table 2.* – MS.: 350 (M^+, calc. for $C_{18}H_{22}O_7$: 350).

5. *Hydrogenation of gilmicolin* (**3**) *to* **6**. A solution of 50 mg of **3** in 5 ml of ethanol was hydrogenated with 25 mg of Pd/C (5%) at room temperature. After 2 h the solution was filtered and evaporated i.V. The residue was purified by preparative TLC. (methylene chloride/methanol 95:5) to yield 10 mg of **6** as an orange gum. – UV. (Ethanol): 272 (4.21). – IR. ($CHCl_3$): bands at 3500, 1680, 1375. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – MS.: 250 (M^+, calc. for $C_{14}H_{18}O_4$: 250).

6. *Reduction of gilmicolin* (**3**) *with zinc in acetic acid.* A solution of 100 mg of **3** in 3 ml of 90% aqueous acetic acid was stirred with 17 mg of zinc dust at RT. After 2 h the reaction mixture was diluted with 50 ml of ethyl acetate and neutralized with 1N $NaHCO_3$. The organic layer was washed with water, dried with sodium sulfate (anhydrous) and evaporated i.V. Preparative TLC. (methylene chloride/methanol 95:5) of the crude reaction product yielded 41 mg of unreacted gilmicolin (**3**), 38 mg of mono-*O*-acetyl-trihydroxy derivative **7** and traces of tetrahydroxy derivative (not persued further).

Compound **7**, an oil, $[\alpha]_D^{20} = +17 \pm 2°$ ($c = 0.063$ in chloroform). – UV. (Ethanol): 256, 302 (4.2; 3.9). – IR. ($CHCl_3$): bands at 3400–3600, 1748, 1605. – ^{1}H-NMR.: *Table 1.* – MS.: 310 (M^+, calc. for $C_{16}H_{22}O_6$: 310).

7. *Acetylation of mono-*O*-acetyl-trihydroxy derivative* **7** to *tetra-*O*-acetyl derivative* **8**. A solution of 12 mg of **7** in 2 ml of pyridine and 1 ml of acetic anhydride was stirred at RT. for 18 h. After removing the solvent i.V. the residue was purified by preparative TLC. (methylene chloride/methanol 97:3) to yield 9 mg of tetra-*O*-acetyl derivative as oil. – IR. ($CHCl_3$): bands at 1765, 1748, 1378. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – MS.: 436 (M^+, calc. for $C_{22}H_{28}O_9$: 436).

8. *Mycorrhizin A* (**11**). From methylene chloride/hexane crystals of m.p. 161–162°, $[\alpha]_D^{25} = +32 \pm 2°$ ($c = 0.16$ in ethanol). – UV. (Ethanol): 295 (3.55). – IR. ($CHCl_3$): bands at 3500, 1720, 1390. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – MS.: 282.0646 (M^+, calc. for $C_{14}H_{15}ClO_4$: 282.0659).

9. *Acetylation of mycorrhizin A* (**11**) *to* O-*acetyl-mycorrhizin A* (**12**). A solution of 5 mg of mycorrhizin A (**11**) in 1 ml of pyridine and 0.5 ml of acetic anhydride was stirred at RT. After 14 h the solvent was removed i.V. and the residue purified by preparative TLC. to give 3.2 mg of *O*-acetyl-mycorrhizin A (**12**) as an oil. – UV. (Ethanol): 290 (3.8). – IR. ($CHCl_3$): bands at 1735, 1702, 1680, 1390. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – MS.: 324 (M^+, calc. for $C_{16}H_{17}ClO_5$: 324).

10. *Mycorrhizinol* (**15**). It crystallized as plates from methylene chloride/heptane mixtures, m.p. 115–116°, $[\alpha]_D^{20} = -30 \pm 2°$ ($c = 0.27$, methanol). – UV. (Ethanol): 250, 264, 269 (4.00, 4.18, 3.48). – IR. ($CHCl_3$): bands at 3600, 3350, 1600, 1370. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – MS.: 282.0656 (M^+, calc. for $C_{14}H_{15}ClO_4$: 282.0659).

11. *Acetylation of mycorrhizinol* (**15**) *to di*-O-*acetylmycorrhizinol* (**16**). A solution of 5 mg of **15** in 3 ml of pyridine and 1.5 ml of acetic anhydride was stirred at RT. After 18 h the solvent was evaporated and the residue purified. Preparative TLC. (methylene chloride/methanol 99:1) yielded 3 mg of di-*O*-acetylmycorrhizinol (**16**) as a gum, $[\alpha]_D^{20} = -27 \pm 2°$ ($c = 0.09$, chloroform). – IR. ($CHCl_3$): bands at 1760, 1745, 1385. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – MS.: 366 (M^+, calc. for $C_{18}H_{19}ClO_6$: 366).

12. *Methylation of mycorrhizinol* (**15**) *to mono*-O-*methyl derivative* **17**. The solution of 6 mg of **15** in 10 ml of dry acetone was treated with 0.5 ml of methyl iodide and 30 mg of potassium carbonate at RT. After 8 h the solution was filtered and dried i.V. The product was purified by preparative TLC. (methylene chloride/methanol 99:1) to yield 3.6 mg of *O*-methyl mycorrhizinol as crystals; m.p. 68–70°, $[\alpha]_D^{20} = -29 \pm 2°$ ($c = 0.07$, chloroform). – IR. ($CHCl_3$): bands at 3500, 1604, 1390. – ^{1}H-NMR. ($CDCl_3$): *Table 1.* – ^{1}H-NMR. (C_6D_6): *Table 1.* – MS.: 296 (M^+, calc. for $C_{15}H_{17}ClO_4$: 296).

13. (–)-*6-Hydroxymellein* (**14**). It crystallized as prisms from acetone/hexane, m.p. 212–213° (lit. 214–215°); sublimed at 139°/0.06 Torr (lit. 140°/0.06 Torr); $[\alpha]_D^{20} = -64 \pm 2°$ ($c = 0.134$, methanol) (lit. $[\alpha]_D = -63°$, $c = 0.6$, ethanol). – UV. (Ethanol): 270, 305 (4.11, 3.76). – IR.: 1648. – ^{1}H-NMR. ($CDCl_3$): 1.38 (*d*, $J = 6$ Hz, 3 H); *ca.* 2.85 (*m*, 2 H); *ca.* 4.67 (*m*, 1H); 6.19 (*d*, $J = 2.6$ Hz, 1H); 6.22 (*d*, $J = 2.6$ Hz, 1H); 10.59 (1H, exchangeable with D_2O); 11.12 (1H, exchangeable with D_2O). – MS.: 194 (M^+, calc. for $C_{10}H_{10}O_4$: 194).

REFERENCES

[1] *K.K. Chexal & Ch. Tamm*, Helv. *61*, 2002 (1978).

[2] *P. Bollinger & T. Zardin-Targalia*, Helv. *59*, 1809 (1976); *H.P. Weber & T.J. Petcher*, Helv. *59*, 1821 (1976).

[3] *J. Trofast & B. Wickberg*, Tetrahedron *33*, 875 (1977).

[4] *R.F. Curtis, P.C. Harries, C.H. Hassall, J.D. Levi & D.M. Phillips*, J. chem. Soc. *C168* (1966); *H. Arakawa, N. Torimoto & Y. Masui*, Liebigs Ann. Chem. *728*, 152 (1969).

[5] *R.T. Alpin & W.T. Pike*, Chemistry & Ind. *1966*, 2009; *S. Ukai, K. Hirose, D. Tatematsu & T. Goto*, Tetrahedron Letters *1967*, 4999.

[6] *M. Uskokovic, M. Gut, E.N. Trachtenberg, W. Klyne & R.I. Dorfman*, J. Amer. chem. Soc. *82*, 4965 (1960); *J. Rocek & F.H. Westheimer*, J. Amer. chem. Soc. *84*, 2241 (1962).

[7] *G. Germain, P. Merin & M.M. Woolfson*, Acta crystallogr. Sect. A, *27*, 368 (1971); *W.R. Busing, K.O. Martin & H.A. Levi*, 'ORFLS, A Fortran Crystallographic Least Square Programme, U.S. Atomic Energy Commission Report ORNL-TM-305', Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A. (1970); *C.A. Johnson*, 'ORTEP-II, A Fortran Thermal Ellipsoid Plot Programme for Crystal Structure Illustrations', U.S. Atomic Energy Commission Report ORNL-3794 (2nd revision with supplemental instructions) Oak Ridge National Laboratory, Oak Ridge, Tenn. U.S.A. 1971.

[8] *C. Stalhandske, C. Svensson & C. Särnstrand*, Acta Crystallogr. *B33*, 870 (1977).

[9] *cf. A.I. Scott*, 'Interpretation of the Ultraviolet Spectra of Natural Products', Pergamon Press, London 1964, p. 138.

[10] *K.K. Chexal, B.K. Handa, W. Rahman, R. Warren & A. Pelter*, Tetrahedron *27*, 1625 (1971) and references cited therein.