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# NITROGEN CONTAINING METABOLITES OF *FUSARIUM SAMBUCINUM*

**Daniel Niederer, Christoph Tamm* and Werner Zürcher**

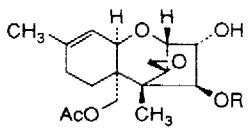
Institut für Organische Chemie der Universität
St. Johanns-Ring 19, CH-4056 Basel, Switzerland

*Key Words: Fusarium sambucinum*; 2-(1-Hydroxyethyl)-quinazolin-4(3H)-one; 2-Pyruvylaminobenzamide; 2-Acetylquinazolin-4(3H)-one; Absolute configuration

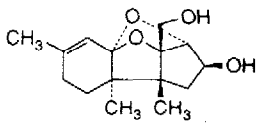
**Abstract:** 2-(1-Hydroxyethyl)-quinazolin-4(3H)-one (chrysogine, **7**), 2-pyruvylaminobenzamide (**8**), 2-acetylquinazolin-4 (3H)-one (**9**) and N-carbobenzoxy-L-phenylalanilol (**10**) have been isolated from cultures of *Fusarium sambucinum*. The absolute configuration of **7** was determined by the use of the *Helmchen* method. 2-Acetylquinazolin-4(3H)-one (**9**) is probably an artefact originating from **8**. It is doubleful whether compound **10** is a genuine metabolite.

A few years ago we reported the structure elucidation of sambucinol (**3**), sambucoin (**4**) and sambucinic acid (**5**) which were isolated from cultures of *Fusarium sambucinum* (ATCC No 11 852) (*Fungi imperfecti*) as minor metabolites[1,2]. The major compound was diacetoxyscirpenol (anguidine, **1**)[3-6]. Whereas **1** possesses the normal trichothecane skeleton, the minor metabolites **3**, **4** and **5** as well as apotrichothec-9-ene-3α,13-diol (**6**), a fourth minor metabolite isolated by *ApSimon* and coworkers[7-9], are characterized by modified ring systems. Working up a large-scale fermentation we have found five additional minor metabolites four of which surprisingly, contain nitrogen, namely 2-(1-hydroxyethyl)-quinazolin-4(3*H*)-one (chrysogine, **7** which was first isolated from *Penicillium chrysogenum*) m.p. 176-179°, $[\alpha]_D^{21}$ = -25 (c=0.39, MeOH), spectral data[10]; $C_{10}H_{10}N_2O_2$, 1.3 mg/l; 2-pyruvylaminobenzamide (**8**), m.p. 187-194°, $C_{10}H_{10}N_2O_3$, 3.5 mg/l, spectral data[11], and 2-quinazolin-4(3*H*)-one (**9**), m.p. 200-206°, $C_{10}H_8N_2O_2$, 1.1 mg/l. The latter compound is probably an artefact originating from **8**. An additional substance isolated, was identified as N-carbobenzoxy-L-phenylalanilol, m.p. 78°/91-92°; $C_{17}H_{19}NO_3$, 49 mg/l may also not be a genuine metabolite. The fifth compound found proved to be identical with monoacetoxyscirpenol (**2**).

The compounds **7**, **8**, **9**[12,13] and **10** are known, but the configuation of the chiral centre of metabolite **7** has never been determined. Because it was neither possible to grow crystals of



**1** R = Ac
**2** R = H



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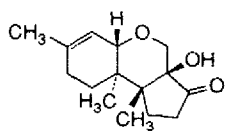

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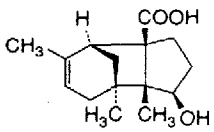

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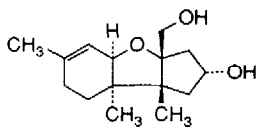

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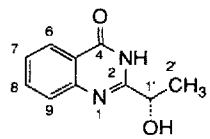
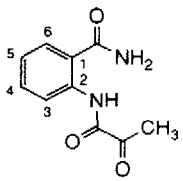
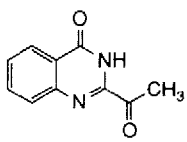


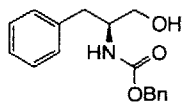
**7**



**8**

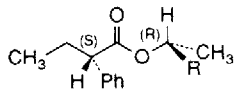


**9**



**10**

compound **7** nor to prepare derivatives thereof suitable for X-ray diffraction, we made use of the *Helmchen* method[14]. The chiral alcohol **7** was esterfied with both enantiomers of α-phenylbutyric acid in two separate experiments. Because of diamagnetic deshielding effected by the phenyl ring present in the acyl group, characteristic differences of the chemical shifts of structurally equivalent groups of the alkoxy moiety are observed in the $^1$H-NMR spectra. They allow the assignment of the configuration of a secondary alcohol. If C(1') of compound **7** possesses the (*R*)-configuration, the signal of the methyl group at C(1') of the corresponding (*S*)-α-phenylbutyric acid ester **11** is shifted to a higher field as it is the case in the corresponding ester **12** of (*R*)-α-phenylbutyric acid. The reversed effect is to be expected if C(1') of **7** has the (*R*)-configuration. The methyl group at C(1') in the (*R*)-α-phenylbutyric acid ester **13** will appear at a higher field as compared to the corresponding ester **14** of (*S*)-α-phenylbutyric acid. The esterification of metabolite **7** with α-phenylbutyric acid was readily accomplished by treatment with dicyclohexylcarbodiimide/4-dimethylaminopyridine.[15] In the $^1$H-NMR spectrum of the ester derived from (*R*)-α-phenylbutyric acid the signal of the methyl group at C(1') appeared at 1.57 ppm, whereas **7** was observed at 1.67 ppm in the ester formed with the enantiomeric α-phenylbutyric acid[16]. On the basis of the difference of the chemical shifts of 0.1 ppm the (*S*)-configuration can be assigned to C(1') of metabolite **7**. A comparison with esters of structurally related alcohols[14] as well as with those of (*R*)-α-phenylethanol clearly shows that observed difference of 0.1 ppm is significant. It allows an unequivocal assignment of the configuration.



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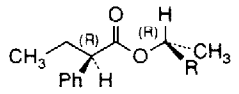

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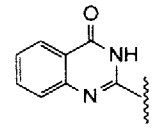
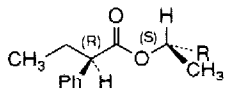
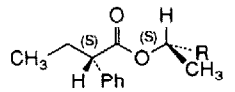




13



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The simultaneous presence of trichothecenes and N-containing minor metabolites in *Fusarium sambucinum* has also been observed in *Fusarium culmorum*[17]. These findings might indicate a close biological relationship of both species.

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9. Greenhalgh, B.; Pare, B.A.R.J.; Miller, J.D.; Levandrer, D.; Meier R.M.; Taylor, A.; ApSimon, J.W., *Bioact. Mol.* **1986**, 137.
10. IR (KBr): 3400, 3180, 3040, 2980, 2920, 1670, 1600cm$^{-1}$; $^{1}$H-NMR (400 MHz), acetone($d_6$), TMS): 8.17 (*m*, H-C(6) or H-C(9)), 7.80 (*m*, H-C(7) or H-C(8)), 7.64 (*m*, H-C(9) or H-C(6)), 7.49 (*m*, H-C(8) or H-C(7)), 4.79 (*q*, $J$ = 6.7 Hz, H-C(1')); 1.57 (*d*, $J$ = 6.6 Hz, $H_3$C(2')) ppm. $^{13}$C-NMR (101 MHz, acetone($d_6$)): 161.9 and 160.2 (2*s*, C(4) and C(2)), 149.9 (*s*, C(10)), 135.1, 128.0, 127.0, and 126.9 (4*d*, C(6), C(7), C(8), and C(9)), 122.5 (*s*, C(5)), 68.0 (*d*, C(1$^{1}$)), 22.4 (*q*, C(2'))ppm.

11. IR (KBr): 3410, 3340, 3300, 3220, 3100, 3080, 1730, 1690, 1660 $cm^{-1}$; $^1$H-NMR (90 MHz, $CDCl_3$, TMS): 12.4 (*br*, H-N-C(2)), 8.8-7.1 (*m*, H-C(3), H-C(4), H-C(5), and H-C(6)), 6.0 (*br*, $H_2N$-CO), 2.56 (*s*, $H_3C$-CO).
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16. (*R*)-α-Phenylbutyric acid ester **11**:
$^1$H-NMR (300 MHz, $CDCl_3$): 8.27 (d, J = 7.7, H-C(6) oder H-C(9)); 7.8 - 7.6 (*m*, 2H,H-C(9) oder H-C(6) und H-C(7) oder H-C(8); 7.49 (*m*, H-C(8) oder H-C(7)); 7.35 - 7.27 (*m*, 5H,, 2 *o*-H, 2 *m*-H, 1 *p*-H); 5.76 (*q*, J = 6.7, H-C(1')); 3.65 (*t*, *J* = 7.7, H-C(2")); 2.15 und 1.87 (2 m, $H_2C$(3")); 1.57 (*d*, *J* = 6.7, $H_3C$(2')); 0.091 (*t*, *J* = 7.4, $H^3C$(4")).
EI-MS (70 eV): 336 (25, *M*+), 217 (5), 189 (83), 173 (42), 146 (21), 119 (41), 91 (100).
(*S*)-α-Phenylbutyric acid ester (**12**):
$^1$H-NMR (300 MHz, $CDCl_3$): 9.31 (*s* br., HN); 8.21 (*dd*, J = 1.5, 8 H-C(6) oder H-C(9)); 7.74 (*m*, H-C(7) oder H-C(8)); 7.65 (*m*, H-C(9) oder H-C(6)); 7.45 (*m*, H-C(8) oder H-C(7)); 7.36 - 7.26 (*m*, 5H, 2 *o*-H, 2 *m*-H, 1 *p*-H)); 5.77 (*q*, *J* = 6.7, H-C(1')); 3.60 (*t*, *J* = 7.7, H-C(2")); 2.15 und 1.88 (2 *m*, $H_2C$(3"); 1.67 (*d*, *J* = 6.7, $H_3C$(2)); 0.93 (*t*, *J* = 7.4, $H_3C$(4").
EI-MS (70 eV): identical with EI-MS of compound 13.
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