48. Sambucinol and Sambucoin, Two New Metabolites of *Fusarium Sambucinum* Possessing Modified Trichothecene Structures

41st Communication on Verrucarins and Roridins[1])

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Summary

Sambucinol (**2**) and sambucoin (**4**), two C_{15}-trichothecenes, have been isolated from cultures of *Fusarium sambucinum*. The structures were deduced from spectroscopic data and by X-ray diffraction. A hypothetical biogenetic pathway is proposed.

The trichothecenes are a growing class of closely related sesquiterpenoid secondary metabolites produced by moulds, especially various species of *Fungi imperfecti* [2]. Many members of the family display a wide range of biological effects, such as cytostatic (antileukemic) activity, but they are also highly toxic.

In 1965 *Flury et al.* [3] have isolated diacetoxyscirpenol (anguidine) (**1**) from cultures of *Fusarium diversisporum* and *Fusarium sambucinum*. Working up a large-scale fermentation we have found that the latter microorganism (ATCC No. 11852) produces besides anguidine (**1**), which is the major metabolite, a series of hitherto unknown minor metabolites. In this communication we report the isolation and structural elucidation of two of these new compounds, which we name sambucinol (**2**) and sambucoin (**4**).

The culture filtrate of a 70-liter fermentation[2]) was extracted with AcOEt. The extracts yielded after workup according to known procedures [3b] 46.7 g of crude product. After separation of *ca.* 20 g of **1** by crystallization, the mother liquor was further purified by repeated chromatography on silica gel (AcOEt/petroleum ether and CH_2Cl_2/acetone). By this method pure samples of sambucinol (**2**) and of the less polar sambucoin (**4**) were obtained.

Sambucinol (**2**) (yield *ca.* 3 mg/l) crystallized from acetone, m.p. 227°; $[\alpha]_D^{r.t.} = -22.6°$ (c = 1.3, pyridine); IR (KBr): 3350 (br.), 1675. A composition of $C_{15}H_{22}O_4$ was secured by elemental analysis (found: C 67.28%, H 8.31%, calc.: C 67.65%, H 8.33%) and by

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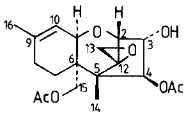
[1]) 40th Commun.: [1].

[2]) We thank Dr. *E. Härri, Sandoz AG*, Basel, for carrying out this fermentation.



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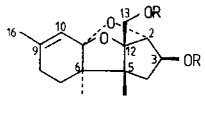
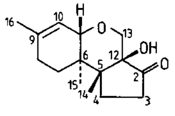


2 R = H
3 R = Ac



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MS (EI, m/z 266, 251, 161, 124). The ^{1}H-NMR spectrum of **2** showed the three characteristic singlets for the CH_3-groups of the trichothecene skeleton, a broad *s* at 5.4 ppm for H–C(10), a *dd* at 4.23 ppm for H–C(3) (no coupling with H–C(2)!) and a *s* at 3.92 ppm for H–C(2). Noteworthy is also the absence of the C(13)-methylene *AB*-pattern. However, acetylation (Ac_2O/pyridine) led to the di-*O*-acetyl derivative **3** (MS: m/z 350, 335, 291), in which the CH_2OAc-signal appeared as a clear *AB*-system centered at 4.45

Table 1. *^{13}C- and ^{1}H-NMR Data for Sambucinol* (**2**) *and Sambucoin* (**4**)[a])

Position	**2**		**4**	
	^{13}C-shifts[b])	^{1}H-shifts[c])	^{13}C-shifts[b])	^{1}H-shifts[d])
2	89.0 (*d*)	3.92 (*s*)	216.1 (*s*)	–
3	72.7 (*d*)	4.23 (*dd*, *J* = 4.8)	34.7 (*t*)	2.58 (*ddd*, *J* = 3, 12, 19.5) 2.29 (*ddd*, *J* = 8, 10.5, 19.5)
4	46.3 (*t*)	2.59 (*dd*, *J* = 8, 15); ~ 1.5 (*m*)	27.2 (*t*)[i])	2.02 (*ddd*, *J* = 3, 10.5, 14) 1.77 (*ddd*, *J* = 8, 12, 14)
5	50.5 (*s*)[e])	–	46.9 (*s*)	–
6	48.3 (*s*)[e])	–	38.0 (*s*)	–
7	29.4 (*t*)[f])	1.3–2.2 (*m*)	27.8 (*t*)[i])	1.47 (*ddd*, *J* = 2, 6, 13.5);
8	30.4 (*t*)[f])		27.7 (*t*)[i])	~ 1.6 (*m*, 2H); 1.9 (*m*, 1H)
9	144.1 (*s*)	–	135.2[k])	–
10	118.3 (*d*)	5.4 (br. *s*)	122.9[k])	5.21 (br. *s*)
11	108.0 (*s*)	–	75.9 (*d*)	3.96 (br. *s*)
12	95.1 (*s*)	–	74.3 (*s*)	–
13	59.6 (*t*)	4.07 (*s*)	67.4 (*t*)	3.45; 4.21 (*AB*, *J* = 11)
14	15.2 (*q*)[g])	0.83 (*s*)[h])	14.3 (*q*)[j])	0.66 (*s*)
15	16.7 (*q*)[g])	1.07 (*s*)[h])	16.5 (*q*)[j])	1.15 (*s*)
16	22.8 (*q*)	1.76 (br. *s*)	22.5 (*q*)	1.64 (br. *s*)

[a]) The data for diacetoxyscirpenol (**1**) are reported in [3b] (^{1}H-NMR) and in [4] (^{13}C-NMR).
[b]) Spectra recorded at 22.63 MHz in (D_5)-Pyridine. Chemical shifts are given in ppm with TMS at 0.0.
[c]) Spectrum taken at 90 MHz in $CDCl_3$.
[d]) Spectrum recorded at 360 MHz in $CDCl_3$.
[e])–[j]) Assignments may be reversed.
[k]) Measured in $CDCl_3$.

ppm ($J = 12$) indicating that a conformational change is associated with the esterification, whereas the H–C(3)-resonance was shifted to 5.05 ppm upon acetylation. The ^{13}C-NMR spectrum revealed an sp^3 C-atom at unusual low field (108.0 ppm) suggesting an acetal function. All these data (*cf. Table 1*) are in agreement with the structure assignment given, which was finally deduced from an X-ray study.

Sambucoin (**4**) (yield *ca.* 2 mg) crystallized from acetone/petroleum ether, m.p. 205–210°; IR (KBr): 3380, 1735, 1670. Elemental analysis (found: C 71.46%, H 9.30%; calc.: C 71.97%, H 8.86%) and MS (EI, *m/z* 250, 235, 188) suggested a composition of $C_{15}H_{22}O_3$. The ^{1}H-NMR spectrum revealed an *AB*-system at 3.45 and 4.21 ppm ($J = 11$), respectively, assigned to $H_2C(13)$, the olefinic proton at 5.21 and a br. *s* at 3.96 ppm

Table 2. *Positional and Thermal Parameters of Structure* **2** *($C_{15}H_{22}O_4$) and their Standard Deviations*

Atom	X/A	Y/B	Z/C	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.2971	0.2544	0.2879	0.0262	0.0288	0.0392	0.0020	0.0045	−0.0042
	0.0010	0.0005	0.0004	0.0035	0.0032	0.0035	0.0032	0.0032	0.0031
C2	0.2360	0.2636	0.1979	0.0199	0.0345	0.0383	−0.0017	−0.0064	−0.0049
	0.0014	0.0007	0.0006	0.0045	0.0051	0.0052	0.0048	0.0044	0.0045
C3	0.0118	0.2945	0.1939	0.0193	0.0397	0.0455	−0.0091	−0.0028	0.0024
	0.0014	0.0008	0.0007	0.0047	0.0055	0.0058	0.0051	0.0050	0.0045
C2	−0.0705	0.2525	0.1157	0.0320	0.0612	0.0466	−0.0055	−0.0072	0.0045
	0.0011	0.0006	0.0005	0.0039	0.0047	0.0040	0.0043	0.0037	0.0042
C4	0.0146	0.4146	0.1894	0.0218	0.0284	0.0486	0.0022	−0.0025	0.0042
	0.0015	0.0007	0.0007	0.0047	0.0046	0.0062	0.0045	0.0050	0.0042
C5	0.2386	0.4484	0.2067	0.0266	0.0284	0.0296	0.0008	0.0051	0.0012
	0.0015	0.0007	0.0006	0.0048	0.0045	0.0048	0.0042	0.0047	0.0045
C6	0.3048	0.4396	0.3050	0.0304	0.0335	0.0257	0.0032	0.0010	−0.0092
	0.0015	0.0007	0.0006	0.0051	0.0050	0.0044	0.0046	0.0046	0.0045
C7	0.4605	0.5243	0.3318	0.0441	0.0210	0.0455	−0.0035	−0.0028	0.0021
	0.0017	0.0007	0.0007	0.0060	0.0043	0.0056	0.0045	0.0056	0.0048
C8	0.5481	0.5040	0.4224	0.0390	0.0417	0.0393	−0.0051	−0.0081	0.0080
	0.0018	0.0008	0.0007	0.0059	0.0055	0.0059	0.0052	0.0056	0.0055
C9	0.6092	0.3927	0.4340	0.0264	0.0572	0.0297	−0.0026	0.0000	−0.0089
	0.0016	0.0009	0.0007	0.0054	0.0066	0.0051	0.0051	0.0046	0.0051
C10	0.5586	0.3172	0.3795	0.0209	0.0468	0.0362	0.0012	−0.0019	−0.0013
	0.0016	0.0008	0.0007	0.0044	0.0056	0.0050	0.0050	0.0047	0.0049
C11	0.4290	0.3389	0.3029	0.0223	0.0261	0.0346	−0.0049	0.0032	0.0019
	0.0014	0.0007	0.0006	0.0043	0.0044	0.0048	0.0042	0.0045	0.0041
O3	0.5397	0.3475	0.2239	0.0184	0.0416	0.0349	−0.0116	0.0036	0.0034
	0.0010	0.0005	0.0004	0.0031	0.0036	0.0034	0.0033	0.0027	0.0033
C12	0.3639	0.3563	0.1690	0.0211	0.0275	0.0304	−0.0046	0.0000	0.0075
	0.0013	0.0007	0.0006	0.0042	0.0044	0.0046	0.0045	0.0040	0.0043
C13	0.4221	0.3626	0.0748	0.0296	0.0520	0.0359	−0.0042	0.0059	−0.0011
	0.0016	0.0009	0.0006	0.0056	0.0061	0.0052	0.0056	0.0048	0.0057
O4	0.5325	0.2707	0.0527	0.0388	0.0687	0.0371	−0.0094	0.0015	0.0067
	0.0012	0.0006	0.0004	0.0043	0.0050	0.0036	0.0042	0.0037	0.0045
C14	0.2827	0.5540	0.1660	0.0466	0.0372	0.0443	0.0115	−0.0062	0.0011
	0.0018	0.0008	0.0007	0.0065	0.0053	0.0056	0.0052	0.0058	0.0056
C15	0.1238	0.4353	0.3704	0.0298	0.0624	0.0360	−0.0078	0.0118	0.0192
	0.0016	0.0009	0.0007	0.0052	0.0068	0.0052	0.0059	0.0050	0.0057
C16	0.7291	0.3684	0.5163	0.0524	0.0596	0.0340	0.0068	−0.0123	0.0016
	0.0019	0.0010	0.0007	0.0071	0.0071	0.0051	0.0058	0.0056	0.0070

(H–C(11)). Sambucoin (**4**) remained unchanged when treated with CrO_3/H_2SO_4/acetone, thus the OH-group being tertiary. While **4** was not changed by treatment with Ac_2O/pyridine, more forcing conditions (Ac_2O/Et_3N/DMAP) yielded an unstable acetyl derivative. The chemical shift of the carbonyl group (216.1) pointed to a cyclopentanone system, which was compatible with the high geminal coupling constant (19.5 Hz) observed for the H-atoms at C(3). Irradiation experiments at 360 MHz allowed to separate the four spin-system in the five-membered ring from that in ring A, the latter showing a slightly different pattern from other trichothecenes owing to the unusual *trans*-relationship. Again an X-ray study ascertained the structural formula.

Crystallographic Data. – Crystallographic data of structures **2** ($C_{15}H_{22}O_4$) and **4** ($C_{15}H_{22}O_3$): orthorhombic, space group $P2_12_12_1$, $a = 6.609(5)$, $b = 13.057(4)$, $c = 15.531(7)$ Å and $a = 7.248(2)$, $b = 11.918(3)$, $c = 15.289(4)$ Å, respectively, $Z = 4$, $d_{exp} = 1.32$, $d_{calc} = 1.320$ g/cm^3 (**2**), $d_{exp} = 1.26$, $d_{calc} = 1.259$ g/cm^3 (**4**).

Table 3. *Positional and Thermal Parameters of Structure* **4** *($C_{15}H_{22}O_3$) and their Standard Deviations*

Atom	*X/A*	*Y/B*	*Z/C*	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
O1	0.2678	0.2320	0.7567	0.0452	0.0265	0.0523	−0.0013	0.0034	−0.0032
	0.0010	0.0005	0.0004	0.0040	0.0029	0.0042	0.0032	0.0042	0.0033
C2	0.2435	0.1630	0.9602	0.0356	0.0456	0.0348	−0.0121	−0.0150	0.0031
	0.0014	0.0008	0.0006	0.0050	0.0049	0.0052	0.0047	0.0053	0.0050
C3	0.1404	0.0678	1.0031	0.0568	0.0370	0.0518	−0.0035	0.0093	−0.0038
	0.0017	0.0009	0.0007	0.0070	0.0056	0.0066	0.0052	0.0063	0.0057
C4	0.1638	−0.0327	0.9374	0.0516	0.0467	0.0496	0.0095	0.0144	−0.0077
	0.0016	0.0009	0.0008	0.0064	0.0056	0.0063	0.0056	0.0059	0.0057
C5	0.2500	0.0161	0.8497	0.0314	0.0248	0.0440	−0.0060	−0.0078	0.0032
	0.0015	0.0007	0.0006	0.0045	0.0043	0.0057	0.0041	0.0053	0.0044
C6	0.1055	0.0584	0.7803	0.0343	0.0269	0.0451	−0.0097	0.0022	−0.0028
	0.0014	0.0007	0.0007	0.0046	0.0057	0.0044	0.0051	0.0043	
C7	0.0191	−0.0428	0.7305	1.0000	0.0362	0.0596	−0.0087	−0.0089	−0.0102
	0.0016	0.0008	0.0008	0.0000	0.0051	0.0074	0.0054	0.0063	0.0051
C8	−0.1276	−0.0021	0.6659	0.0656	0.0554	0.0489	0.0018	−0.0205	−0.0152
	0.0019	0.0010	0.0008	0.0079	0.0067	0.0067	0.0060	0.0067	0.0069
C9	−0.0703	0.1042	0.6152	0.0551	0.0516	0.0475	−0.0169	−0.0125	0.0107
	0.0017	0.0009	0.0008	0.0069	0.0062	0.0067	0.0058	0.0062	0.0057
C10	0.0738	0.1638	0.6400	0.0508	0.0471	0.0292	−0.0086	0.0087	0.0056
	0.0015	0.0009	0.0006	0.0068	0.0057	0.0051	0.0049	0.0051	0.0055
C11	0.1960	0.1312	0.7140	0.0314	0.0312	0.0348	0.0026	0.0102	−0.0021
	0.0013	0.0007	0.0006	0.0048	0.0044	0.0051	0.0042	0.0045	0.0043
C12	0.3604	0.1213	0.8829	0.0303	0.0304	0.0491	−0.0046	0.0058	−0.0014
	0.0013	0.0008	0.0007	0.0048	0.0046	0.0061	0.0048	0.0052	0.0044
C13	0.4137	0.2054	0.8135	0.0355	0.0421	0.0462	0.0026	−0.0052	−0.0089
	0.0014	0.0008	0.0008	0.0055	0.0055	0.0061	0.0052	0.0054	0.0050
C14	0.3852	−0.0728	0.8110	0.0472	0.0293	0.0673	−0.0198	0.0077	0.0013
	0.0016	0.0008	0.0008	0.0062	0.0046	0.0075	0.0055	0.0065	0.0050
C15	−0.0556	0.1262	0.8269	0.0314	0.0528	0.0542	−0.0190	0.0136	0.0019
	0.0014	0.0009	0.0007	0.0051	0.0062	0.0069	0.0058	0.0054	0.0055
C16	−0.1921	0.1403	0.5415	0.0743	0.0669	0.0637	−0.0098	−0.0369	0.0089
	0.0018	0.0010	0.0008	0.0092	0.0075	0.0081	0.0075	0.0081	0.0075
O17	0.2426	0.2618	0.9842	0.0458	0.0440	0.0515	−0.0174	−0.0023	0.0039
	0.0010	0.0006	0.0005	0.0041	0.0037	0.0043	0.0036	0.0043	0.0038
O18	0.5242	0.0837	0.9278	0.0326	0.0363	0.0624	0.0001	−0.0106	0.0050
	0.0009	0.0005	0.0005	0.0037	0.0035	0.0047	0.0038	0.0039	0.0034

Data Collection. – Intensity data for both structures were collected with MoK_a radiation out to $\theta = 27°$ using θ-2θ-scan mode. Unit cell parameters were obtained from accurate centering of 25 strong independent reflexions. Intensities of 1772 independent reflexions for structure **2** and 1745 for structure **4** were measured with a computer-controlled *Enraf-Nonius CAD4* diffractometer equipped with a graphite monochromator. No correction for absorption was applied (μMo = 2.9 cm^{-1}).

Solution and Refinement of the Structures. – The structures were solved with the help of direct methods [5]. For structure **2** an E-map revealed the positions of 2 O-atoms and 10 C-atoms. Difference maps and subsequent least squares refinements then led to the location of the remaining atoms of the molecule. For structure **4** an E-map produced the positions of all non-H-atoms. Programs used were developed partly by *Sheldrick* [6] and partly by ourselves. Unit weights were used for anisotropic refinements with all reflexions; the final R-indices were 0.076 and 0.069, respectively ($R = \Sigma\|F_o| - |F_c\|/|F_o|$). Scattering factors for neutral atoms including anomalous dispersion were taken from *Cromer et al.* [7]. Atomic positional parameters are listed in *Tables 2* and *3*. Stereopairs and numbering schemes are given in *Fig. 1* and *2*.

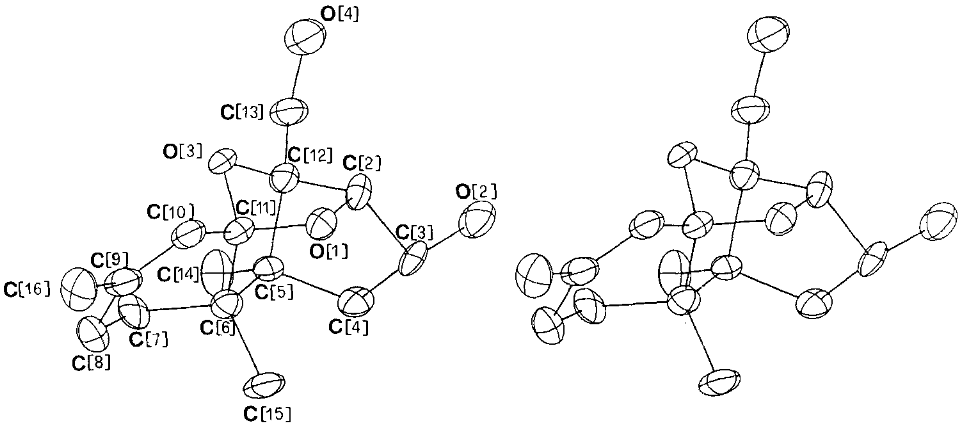

Fig. 1. *Stereopairs and numbering scheme of* **2**

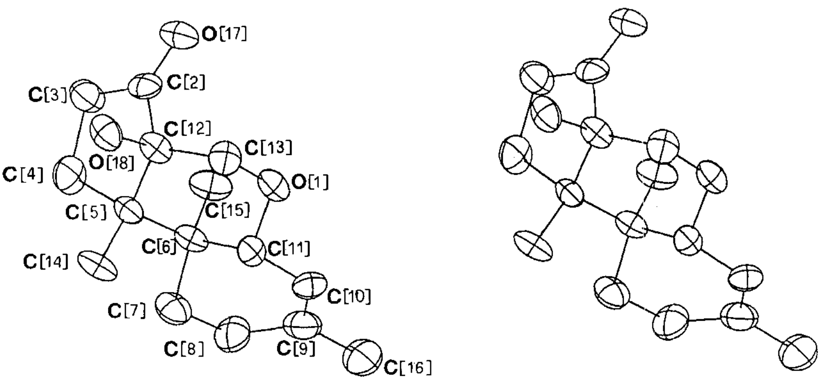

Fig. 2. *Stereopairs and numbering scheme of* **4**

Sambucinol (**2**) and sambucoin (**4**) are the first C_{15}-trichothecenes, which lack the 12,13-epoxy group. Verrucarin K [8], a macrocyclic trichothecene, and 12,13-deoxytrichodermodiene [9], a trichoverroid, are the other known examples from natural sources being devoid of an oxirane ring. Although no incorporation studies have been carried out yet, a biogenetic relationship seems to be obvious. However, it remains unclear, at which stage the ramification of the pathways occurs leading to the different sesquiterpenoid metabolites. Two possibilities are proposed, both starting with trichodiene (**5**), the well-established precursor of the trichothecenes [10] [11]. In the first one, **5** is transformed to trichodiol (**6**), whose intermediacy in the biogenetic scheme has been postulated since it was isolated from cultures of *Trichothecium roseum* [12]. Pyran ring formation by attack of the 2-OH-group leads to 12,13-epoxytrichothec-9-ene (**7**), which is also a naturally occurring metabolite. Subsequent oxidations at C(11) and C(3), epoxide opening, and acetalization would finally complete the biosynthesis of **2**. On the other hand, hydrolysis of **6** would yield the tetrol **8**, which, by attack of the primary OH-group, can directly cyclize to form the sambucoin skeleton. It is noteworthy, that rings A and B are *trans*-fused, a fact which must be associated with the type of ring closure, and that the (*S*)-configuration at C(12), which is typical for all naturally occurring 12,13-epoxytrichothecenes, is retained. The alternative pathway would involve two allylic hydroxylations leading after epoxidation to the key intermediate **9**. Nucleophilic attack at C(13) produces **10**, the immediate precursor of sambucoin (**4**), whereas oxidation to the *α,β*-unsaturated ketone, epoxide opening, acetalization and oxygenation at C(3) complete, *via* **11**, the formation of sambucinol (**2**). However, it should be emphasized that in both pathways leading to sambucinol (**2**) inversion at C(12) must take place.

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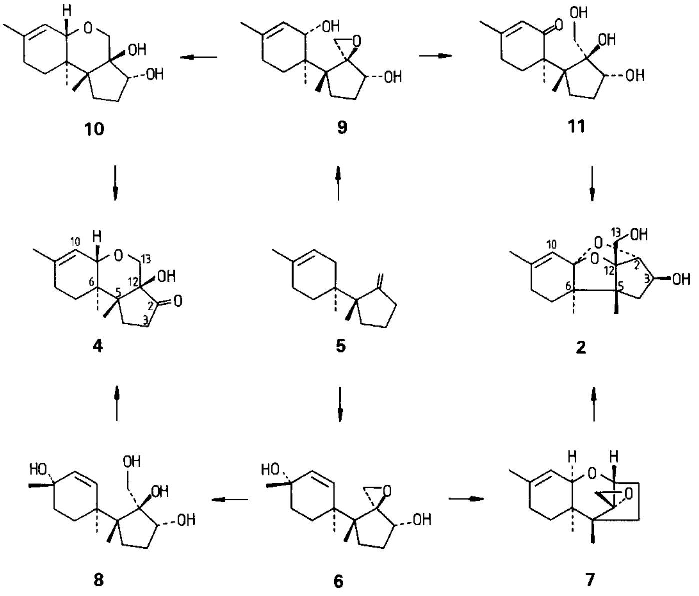

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