154. Verrucarin K, the First Natural Trichothecene Derivative Lacking the 12,13-Epoxy Group

Verrucarins and Roridins. 34th Communication [1]

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Dedicated to Professor Dr. *T. Reichstein* on the occasion of his 80th birthday

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Summary

A new metabolite, verrucarin K ($C_{27}H_{34}O_8$) has been isolated from a strain of *Myrothecium verrucaria* (ALBERTINI et SCHWEINITZ) DITMAR ex FRIES. On the basis of spectral and chemical evidence structure **1** was assigned to the new compound. Base-catalysed hydrolysis yielded verrucarinolactone (**5**), *E,Z*-muconic acid (**6**) and trichotheca-9,12-diene-4,15-diol (**3**). The implications of **1** in the trichothecane biosynthesis are discussed.

1. Introduction. – During the past fifteen years a number of metabolites have been isolated from various strains of *Myrothecium verrucaria* and *Myrothecium roridum* [2]. This class of natural products, characterized by antibiotic, antifungal and even cytostatic activity, includes verrucarin A (**8**) [3], verrucarin B [4], verrucarin J [5] and 2'-dehydroverrucarin A [6] as well as roridin A [7], roridin D [8], roridin E [9] and roridin H [10]. These eight mould metabolites, whose structures have been elucidated, are closely related to each other [11] and characterized either as macro-cyclic diesters (roridin series) or triesters (verrucarin series) of the sesquiterpene alcohol verrucarol (**7**) [12] which belongs to the trichothecane group. Vertisporin [13], a metabolite of *Verticimonosporium diffractum*, satratoxin H [14], the highly toxic principle of the mould *Stachybotrys atra* and the antileukemic baccharins [15], isolated from the Brazilian shrub *Baccharis megapotamica*, also belong to the same class of natural products.

We have now isolated verrucarin K (**1**) a further member of the family of the macrocyclic trichothecene diesters from the mycelium of strain S 118 of *Myrothecium verrucaria*. The new metabolite, formed only in minor amounts, possesses a rather unusual structure in which the normal 12,13-epoxy group is replaced by an exocyclic double bond. In this communication we describe the isolation and the structural elucidation of the new compound.

2. Isolation. – Silica gel chromatography of the ethyl acetate extract of the mycelium of a 500 l fermentation[1]) of *Myrothecium verrucaria* (ALBERTINI et SCHWEINITZ)

Scheme 1

1 R = H   Verrucarin K

2 R = Ac

3 R = H

4 R = Ac

1. LiAlH₄

2. Ac₂O

K₂CO₃

5 Verrucarinolactone

6 E,Z – Muconic acid

7 Verrucarol
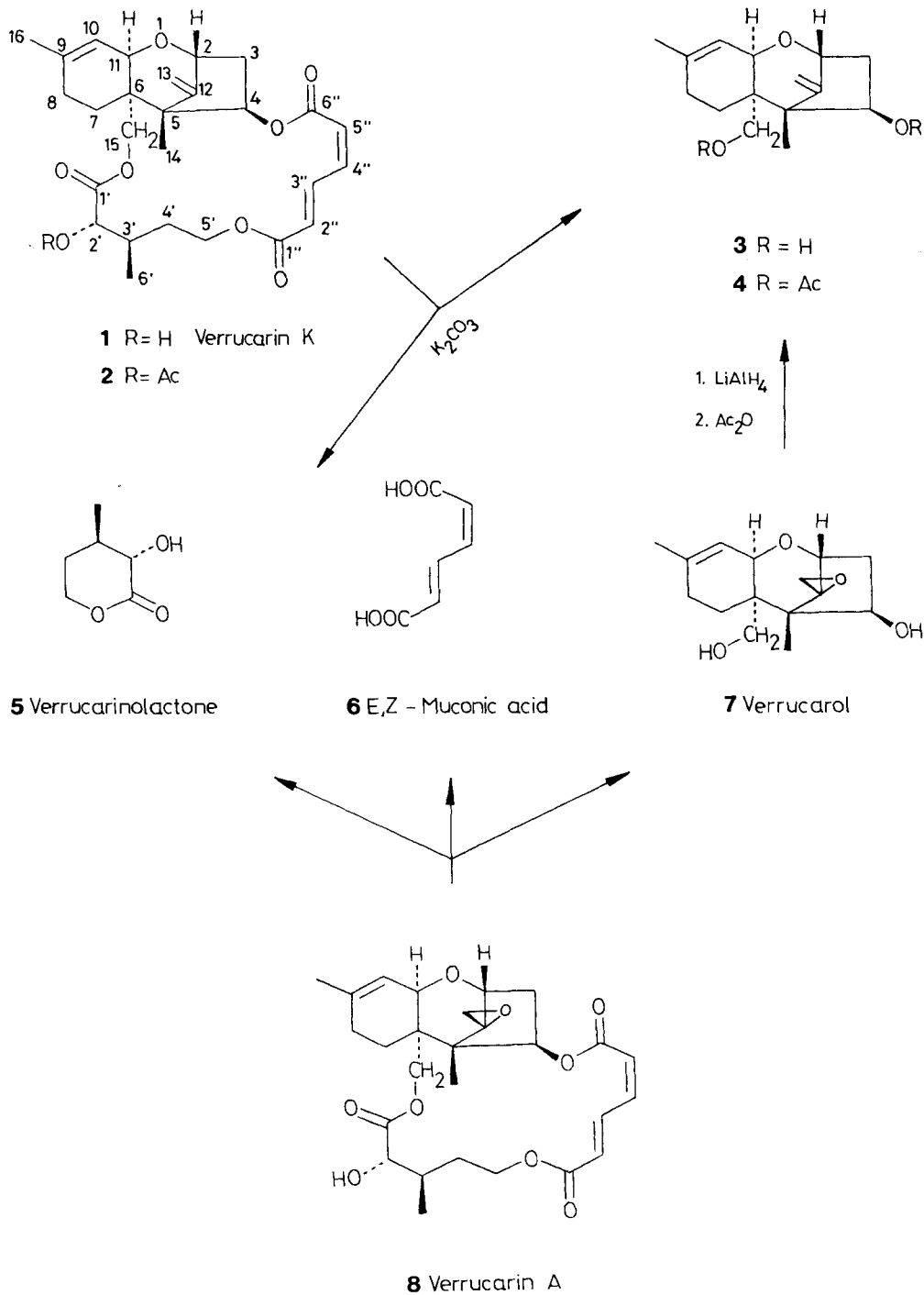
8 Verrucarin A

Table 1. *Assignments of the H-at*

Compound	C(4)	C(10)[a]	C(13)	C(14)	C(1
Verrucarin A (8)	5.83 $d \times d$ (5.5; 7.5)	5.46 d (5)	2.97 AB (4)	0.87 s	
Verrucarin K (1)	5.81 $d \times d$ (4; 8)	5.39 d (5.5)	4.71 s 5.18 s	1.09 s	
Mono-O-acetylverrucarin K (2)	5.80 $d \times d$ (3.5; 8)	5.36 d (5.5)	4.71 s 5.17 s	1.11 s	
Diol 3	4.72 $d \times d$ (3.5; 8)	5.41 d (5.5)	4.71 s 5.14 s	1.16 s	3.57 AB
Di-O-acetyl derivative 4	5.80 $d \times d$ (3.5; 7.5)	5.41 d (5.5)	4.72 s 5.14 s	1.03 s	4.08 AB

[a]   Measured in $CDCl_3$ solution. Chemical shift values are given in δ (ppm) relative to TMS. The spin coupling constants J (Hz) are noted in brackets. Abbreviations: s = singlet, d = doublet, $d \times d$ = d doublet, t = triplet, qa = quartet.
[b]   These signals often show fine structure.

DITMAR ex FRIES (strain S 118) yielded verrucarin A (8) and B as major products, the roridins A, D and E as minor products and a fraction of verrucarin K (1) with verrucarin J as an impurity. Pure verrucarin K (1) was obtained after separation by preparative thin layer chromatography on silica gel plates.

3. Structure. – As observed for the closely related verrucarin A (8) [3], verrucarin K (1) does not show a definite melting point below 320°. The molecular formula, $C_{27}H_{34}O_8$, was deduced from the elemental analyses and the high resolution mass spectrum (calc. m/e 486.2254, found m/e 486.2236). The UV. spectrum of metabolite 1 exhibits a maximum at 259 nm (log ε = 4.19) characteristic for the $\alpha,\beta,\gamma,\delta$-unsaturated ester group. In the IR. spectrum a strong carbonyl absorption at 1710 cm^{-1} and a hydroxyl band at 3550 cm^{-1} are observed. In the ^{1}H-NMR. spectrum the hydroxyl group appears as a doublet at 2.71 ppm exchanged in D_2O. Treatment of verrucarin K (1) with acetic anhydride and pyridine yielded exclusively the mono-O-acetyl derivative 2 without any hydroxyl group (IR.). The ^{1}H-NMR. spectrum of verrucarin K (1) was similar to that of verrucarin A (8), except that the AB-system at *ca*. 3 ppm, typical of the C(13)-protons of the oxirane group, has been replaced by additional singlets at 4.71 ppm and 5.18 ppm, assigned to the two protons of the exocyclic double bond. The presence of this olefinic double bond is supported by the resonances at 106.3 ppm and 151.6 ppm in the ^{13}C-NMR. spectrum.

More light was shed on the structure of verrucarin K (1) by the base-catalysed hydrolysis. Treatment either of the metabolite 1 or its O-acetyl derivative 2 with K_2CO_3 in aqueous methanol yielded verrucarinolactone (5) and the diol 3. The isolation of the third hydrolysis product, E,Z-muconic acid (6), was not attempted because its existence in the genuine natural product was supported sufficiently by the spectral data. The IR. spectrum of the diol 3 was characterized by an intense hydroxyl absorption band at 3620 cm^{-1}. In the ^{1}H-NMR. spectrum the AB-system centered at 3.66 ppm due to the two protons at C(15), is easily recognized. The two singlets at 1.16 ppm and 1.70 ppm are assigned to the methyl groups. Acetylation

the ¹*H-NMR. spectra* (selected data)[a]

| C(16)[a]	C(6')	C(2″)[b]	C(3″)[b]	C(4″)[b]	C(5″)[b]	Ac
1.79 s	0.89 d	6.06 d	8.08 d × d	6.70 t	6.17 d	
	(7)	(16)	(11; 16)	(11)	(11)	
1.72 s	0.89 d	6.05 d	8.05 d × d	6.67 t	6.08 d	
	(6.5)	(16)	(11; 16)	(11)	(11)	
1.71 s	1.05 d	6.06 d	8.03 d × d	6.66 t	6.09 d	2.16 s
	(6.5)	(16)	(11; 16)	(11)	(11)	
1.70 s						
1.68 s						2.03 s
						2.09 s

of the diol **3** with acetic anhydride and pyridine gave the di-*O*-acetyl derivative **4** without hydroxyl group (IR.). According to the chemical shifts and the splitting patterns in the ¹H-NMR. spectrum the compound was identical to the corresponding derivative **4** which was obtained earlier from verrucarol (**7**) [12]. Our attempts to remove the 12,13-epoxy group in verrucarol (**7**) with potassium selenocyanate [16] were unsuccessful.

4. Discussion. – The structure of verrucarin K (**1**), especially the replacement of the 12,13-epoxy group by a double bond in the trichothecane moiety, is most interesting for biogenetic reasons. The biosynthesis of the verrucarins and related systems has been investigated thoroughly [17]. In this connection several interesting com-

Table 2. *Assignments of the C-atoms in the* ¹³*C-NMR. spectra*[a]

	1	2	3		1	2
C(2)	78.5 d	78.6 d	78.7 d	C(1')	174.5 s	170.6 s[e]
C(3)	35.9 t	36.1 t	40.2 t	C(2')	74.0 d	76.0 d[d]
C(4)	75.5 d	75.8 d[d]	73.2 d	C(3')	33.1 d	30.9 d
C(5)	52.0 s	52.1 s	52.4 s	C(4')	32.1 t	32.8 t
C(6)	44.2 s	44.4 s	43.7 s	C(5')	61.1 t	60.8 t
C(7)	18.7 t	18.9 t	20.3 t	C(6')	10.0 qa	11.4 qa
C(8)	27.4 t	27.2 t	28.1 t	C(1″)	165.9 s[b]	165.9 s[b]
C(9)	140.5 s	140.9 s	140.3 s	C(2″)	127.4 d[f]	127.3 d[f]
C(10)	118.2 d	118.1 d	119.1 d	C(3″)	138.7 d	138.6 d[e]
C(11)	66.5 d	66.7 d	64.4 d	C(4″)	138.7 d	138.8 d[e]
C(12)	151.6 s	151.8 s	152.7 s	C(5″)	125.6 d[f]	125.9 d[f]
C(13)	106.3 t	106.0 t	105.5 t	C(6″)	165.3 s[b]	165.2 s[b]
C(14)	12.1 qa	12.2 qa	11.4 qa	CH₃(Ac)		20.4 qa
C(15)	63.5 t	63.1 t	62.8 t	C=O(Ac)		168.9 s[e]
C(16)	23.2 qa	23.3 qa	23.3 qa			

[a] See footnote [a] in *Table 1*.   [b]–[f] These assignments could be reversed.

Scheme 2

9 Trichodiene   **10** Trichodiol   **11**
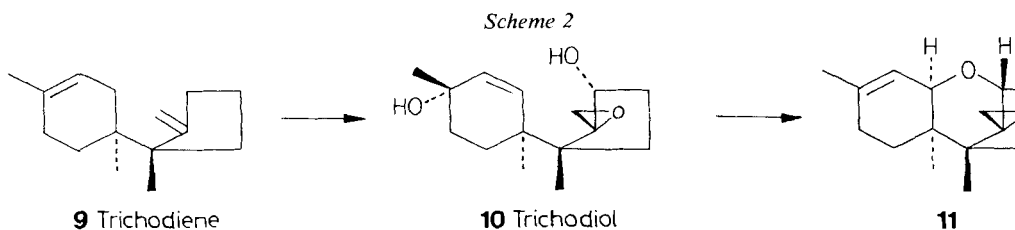
pounds, such as trichodiene (**9**), trichodiol (**10**) and 12, 13-epoxytrichothec-9-ene (**11**), which are supposed to be interrelated biogenetically (*cf. Scheme 2*), have been isolated from microorganisms [18].

If the biogenesis of verrucarin K (**1**) involves trichodiol (**10**) as an intermediate, as anticipated at present for all known natural 12, 13-epoxytrichothec-9-ene derivatives, the reductive removal of the preformed epoxy function would be required at a later stage. This possibility cannot be precluded on the basis of the experimental evidence available. However, an alternative to the reaction sequence is offered by the direct cyclization of an intermediate of type **12** to the trichotheca-9, 12-diene system **13**. It is interesting to note that such a compound was a key intermediate for the cyclization reaction in a recent biomimetic total synthesis [19].
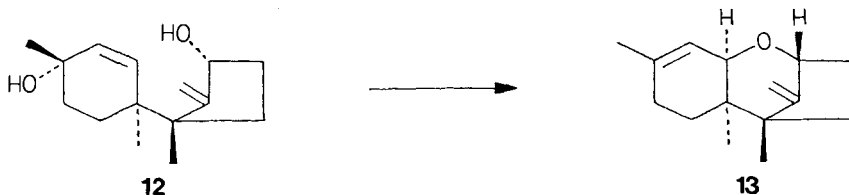
12   **13**

In conclusion, our findings demonstrate that it is not yet clear whether the formation of the tricyclic skeleton or the epoxidation of the 12, 13-double bond are the final steps in trichothecane biosynthesis. Nature can also make alternative use of both biogenetic pathways.

Experimental Part

1. *General.* Melting points were determined on a *Kofler*-block and are corrected. Microanalyses were performed in the microanalytical laboratory of the Institute (*E. Thommen*). IR. (cm^{-1}) and UV. (λ_{max} nm (log ε)) spectra were measured on a *Perkin Elmer* Model 125 grating spectrometer and a *Beckman* D.K. 2 spectrophotometer, respectively. The 90 MHz-^{1}H-NMR. spectra (*Table 1*) and the 22.63 MHz-^{13}C-NMR. spectra (*Table 2*) were determined on a *Bruker* WH 90 spectrometer with *Fourier* transform in the spectral laboratories of the Institute (*K. Aegerter*). The mass spectra (*m/e*) were recorded on an *AEI*-MS 30 instrument in the Physikalisch-Chemisches Institut, Basel (*A. Raas*). We thank Dr *H. Lichti, Sandoz AG.*, Basel, for measurement of the high resolution mass spectrum of verrucarin K (**1**) which was carried out on a *CEC* 21-110 B instrument. Rotations and additional IR. spectra were determined on a *Perkin Elmer* Model 141 polarimeter and a *Perkin Elmer* Model 177 grating spectrometer. For column chromatography, silica gel 0.063–0.200 mm (70–230 mesh ASTM) from *E. Merck AG.*, Darmstadt, was used. Preparative thin-layer chromatography (TLC.) was carried out on silica gel 60 PF 254 (*E. Merck AG.*).

2. *Isolation.* The mycelium (7.8 kg) from a 500 l fermentation of the strain S 118 of *Myrothecium verrucaria* was extracted with ethyl acetate. Evaporation of the solvent yielded a crude extract which on column chromatography gave a mixture of verrucarins A, B and roridins A, D and E along with verrucarin J and verrucarin K (**1**). Further purification by preparative TLC., with petroleum ether/ ethyl acetate, followed by crystallization from CH_2Cl_2/ether afforded 350 mg of pure verrucarin K (**1**), m.p. > 320° (dec.). $[a]_D^{23} = +218 \pm 2°$ ($c = 0.58$, $CHCl_3$). – UV. (ethanol): 259 (4.19). – IR. (KBr): 3550 (OH), 1710 (C=O), 1630, 1585. – MS.: 486.2236 (M^+, calc. for $C_{27}H_{34}O_8$ 486.2254).

$C_{27}H_{34}O_8$ (486.22)   Calc. C 66.65 H 7.04%   Found C 66.64 H 7.21%

3. *Mono-O-acetyl-verrucarin K* (**2**). A solution of 90 mg of verrucarin K (**1**) in 1.5 ml of abs. pyridine and 0.8 ml of acetic anhydride was kept at 35° for 15 h. Evaporation of the solvent i.V. with benzene followed by crystallization from acetone/ether/petroleum ether yielded 57 mg of the acetate **2** as colourless needles, m.p. 199–202°. $[a]_D^{23} = +143 \pm 2°$ ($c = 0.83$, $CHCl_3$). – IR. (CH_2Cl_2): 1750 (sh.), 1740, 1715, 1635, 1590, 1190, 1030. – MS.: 528 (M^+).

$C_{29}H_{36}O_9$ (528.58)   Calc. C 65.89 H 6.87%   Found C 65.88 H 7.07%

4. *Hydrolysis.* A stirred solution of 95 mg of verrucarin K (**1**) in 15 ml of methanol was treated with 1.3 g of K_2CO_3 in 5 ml of water. After 4 h the mixture was concentrated i.V., diluted with 10 ml of water and extracted with CH_2Cl_2. Evaporation of the solvent and purification by preparative TLC., with CH_2Cl_2/methanol, yielded **3** as a colourless amorphous solid. $[a]_D^{23} = -98 \pm 2°$ ($c = 0.71$, $CHCl_3$). – IR. (CH_2Cl_2): 3620 (OH), 1675 (C=C).

The aqueous phase was acidified with H_2SO_4, extracted in a continuous liquid-liquid extractor with ether and the organic phase evaporated i.V. Purification by preparative TLC. with CH_2Cl_2/ methanol followed by crystallization from ether yielded 8 mg of verrucarinolactone (**5**), m.p. 103.5– 104°. $[a]_D^{23} = -11 \pm 2°$ ($c = 0.33$, $CHCl_3$).

5. *Di-O-acetyl derivative* **4**. A solution of 62 mg of **3** in 1.2 ml of abs. pyridine and 1.5 ml of acetic anhydride was kept at 30° for 16 h. Evaporation of the solvent i.V. with benzene gave a crude product. Purification by preparative TLC., using CH_2Cl_2/methanol, yielded 73 mg of the diacetate **4** as a colourless oil. $[a]_D^{23} = -68 \pm 2°$ ($c = 0.90$, $CHCl_3$). – IR. (CH_2Cl_2): 1730 (C=O), 1675 (C=C), 1225, 1070. – MS.: 334 (M^+).

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