175. Isolation of Four Hexaketides from *Verticillium intertextum*

Preliminary Communication

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Zusammenfassung

Aus dem Kulturmedium von *Verticillium intertextum* wurden vier Metabolite isoliert: Vertinolid (**1**) und Bisvertinochinol (**2**), beide bisher unbekannt, sowie das auch von *Penicillium chrysogenum (= P. notatum)* gebildete Sorbicillin (**3**) und das früher synthetisch hergestellte, aber noch nicht als Naturprodukt bekannte Dihydrosorbicillin (**4**). Hypothesen über gemeinsame Biosynthesewege werden erörtert.

1. Source and Isolation. - *Verticillium intertextum* [1] has - with one exception [2] - not been studied hitherto with respect to the chemical nature of its metabolites. When cultivating the fungus, excretion of a yellow material into the nutrient broth was observed. The coloured compounds, together with colourless ones, could be extracted from the acidified spent culture medium with chloroform after removal of the mycelium. Chromatography of the extract on *Sephadex* LH-20 afforded three crude fractions: *a*) a yellow, *b*) a pale yellow and *c*) an orange-yellow fraction, containing at least two, four and three components, respectively. By fractional crystallization of fraction *b*, two new compounds were isolated, namely vertinolide (**1**, 5% of the extract) and bisvertinoquinol (**2**, 1.5%). Preparative thin layer chromatography on silica gel of the material from the mother liquid of the crystallization yielded two known compounds, sorbicillin (**3**, 0.11%) and dihydrosorbicillin (**4**, 0.02%). Of the remaining components, those of fractions *a* and *c*, two have been isolated but their structures are still unknown and others have not yet been purified.

2. Vertinolide. - The substance was obtained as colourless prisms, m.p. 149–152° (dec.); $[\alpha]_D^{20} = -25.0°$ ($c = 0.05$, $CHCl_3$); UV. (nm (ε), CH_3OH): 230 (9900), 270 (28460). The structure (without absolute configuration) of a *4-hydroxy-3, 5-dimethyl-*

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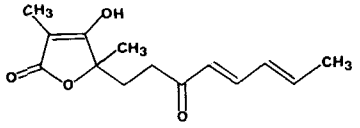
5-(3-oxo-4, 6-octadienyl)-2(5 H)-furanone (**1**) resulted from an X-ray analysis[1]). The ^{1}H- and ^{13}C-NMR. spectra exhibit highly characteristic features for this structure. Vertinolide (**1**) was converted to an *O*-acetyl, an *O*-methyl and a side-chain-tetrahydro derivative.



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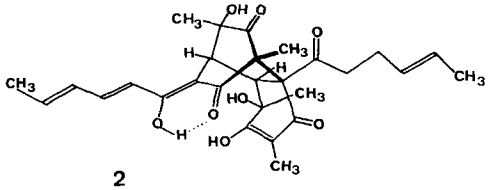
3. Bisvertinoquinol. – This pigment was obtained as yellow triangular prisms, m.p. 160–163° (dec.); $[\alpha]_D^{20} = +329°$ ($c = 0.20$, $CHCl_3$); UV. (nm (ε), CH_3OH): 250 *S* (~9000), 301 (18040), 370 (13430), 380 *S* (~13000). The structure (without absolute configuration) of a *5, 6, 9-trihydroxy-12-(1-hydroxy-2, 4-hexadienylidene)-1, 4, 6, 9-tetramethyl-2-(1-oxo-4-hexenyl)tricyclo [6.2.2.0^{2,7}]dodec-4-ene-3, 10, 11-trione* (**2**) was established by X-ray analysis[1]) and by ^{1}H- and ^{13}C-NMR. spectroscopy. The mass spectrum was dominated by two peaks, which correspond to a *retro-Diels-Alder*-type fragmentation. Bisvertinoquinol (**2**) was converted to a monomethyl enol ether. This product, as well as **2**, showed strong colouring with ferric chloride.



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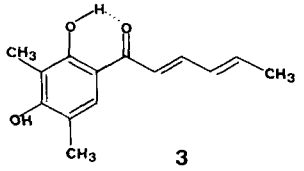
4. Sorbicillin. – This pigment was obtained as orange prisms, m.p. 122–125°. It was found to be identical with the yellow pigment sorbicillin (**3**) isolated previously from *Penicillium notatum* [3] [4] = *P. chrysogenum* [5]. A synthetic sample of sorbicillin (**3**), as orange (and occasionally as yellow, m.p. 124–143°) prisms, was also prepared according to the method described in [6]. The conventional spectra of our isolated and synthetic samples were superimposable.



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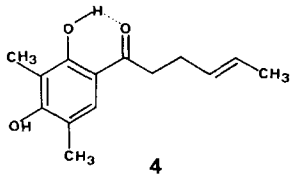
5. Dihydrosorbicillin. – This substance was obtained as colourless needles, m.p. 67–70°. Structure **4** was derived by its ^{1}H-NMR., ^{13}C-NMR. and mass spectra. Its UV. spectra in neutral and basic ethanol were identical with those reported for **4** which had been obtained [4] from a partial hydrogenation of sorbicillin (**3**).

[1]) Performed by *J. H. Bieri & R. Prewo* of the Institute of Organic Chemistry, University of Zürich.



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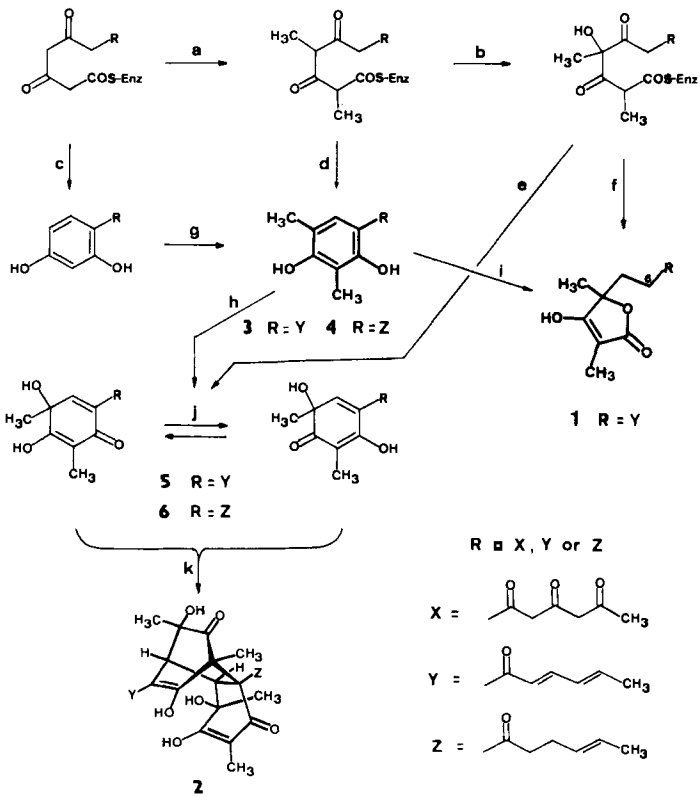
6. Hypothetical Biosynthetic Scheme. – As a deuteromycete *V. intertextum* can be expected to follow the polyketide route for the synthesis of its secondary metabolites [7a]. This pathway has already been postulated [7b] for sorbicillin (**3**), which was the only case known so far within the (relatively rare [7c]) class of hexaketide metabolites where monocyclization presumably involves the carboxyl end [7b]. The present work adds three more examples (compounds **1**, **2** and **4**) to this class. For their biosynthesis we speculatively consider several alternative pathways, which are illustrated in the *Scheme*. Some steps are based on reasonable analogies in fungal and bacterial secondary metabolism, others illustrate unsolved problems in this field.

We first consider a pathway not involving step **b** (and thus also not steps **e** and **f**): We find analogies for step **a** in [7d] [8] [9a], for steps **c** and **d** in [7e], for step

Scheme



g in [7f] and for step **h** in [7g] [9b]. Step **i** with oxidative ring opening by way of the usual oxygenase-type mechanism would lead one to expect an O-atom at C(6)[2]) of **1**. However, for the removal of an equivalently situated O-atom there is a certain analogy in the biosynthesis of penicillic acid [10].

Since the arguments for step **i** are somewhat circumstantial we also consider an alternative pathway, which includes step **b**. Even though 'extra' O-atoms are most often introduced only after stabilized products have been formed [7h], step **b** opens up the attractive possibility for the formation of **1** by way of step **f**. Step **e** is included even though no analogy was found.

Both pathways mentioned above are sufficient to explain the formation of **1**, **3** and **4**, as well as of **5** and **6** as precursor of **2**. Step **j** is a simple tautomerism and step **k** is a *Diels-Alder* reaction, which might be spontaneous or enzymatic (*cf.* [11]). Metabolites which are believed to be the result of intermolecular *Diels-Alder* reactions of cyclohexadienones have been observed previously [12]. The present case is of interest because the diene (an *o*-quinol) and the dienophile (a *p*-quinol) are tautomers of very similar compounds, one with R = Y, the other with R = Z.

The propionate pathway, as an explanation for the two 'extra' methyl groups, is not included in the *Scheme* since the intervention of an acyl group other than acetyl is rare in fungi [7i]. Furthermore, the methyl groups of clavatol, which has the same substitution pattern on the aromatic ring as sorbicillin (**3**), have been shown to be derived from methionine [7f]. The omission of a specification of R under a formula in the *Scheme* is intended to indicate that the conversion of X to Y or Z might or might not yet have taken place.

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[2]) Biogenetic-type numbering.