*Phytochemistry*, 1977, Vol. 16, pp. 125–126. Pergamon Press. Printed in England.

# DOTHISTROMIN AND 2-EPIDOTHISTROMIN FROM *CERCOSPORA SMILACIS**

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(*Received* 14 *June* 1976)

**Key Word Index**—*Cercospora*; Deuteromycetes; dothistromin; 2-epidothistromin; averufin; averythrin; furano-furanoanthroquinones.

**Abstract.**—Examination of a strain of *Cercospora smilacis* Thüm grown on potato–agar medium showed the presence of the known phytotoxin dothistromin together with the new 2-epidothistromin. Mutual conversion by epimerization at C-2 occurred during derivatization.

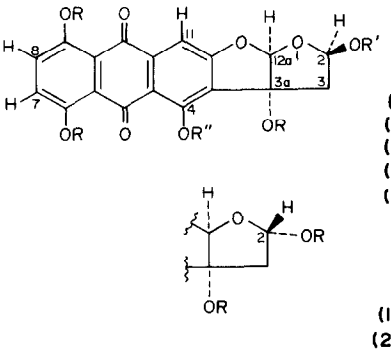
## INTRODUCTION

During a screening of the genus *Cercospora* for secondary metabolites [1] we found that some species, namely *C. smilacis*, *C. ferruginea*, *C. fusca*, *C. microsora*, *C. rosicola* and *C. rubi*, when grown on potato-agar medium, produced a red pigmentation. Extraction, purification and derivatization showed the pigment to be dothistromin, the furanofuranoanthraquinone (**1**), recently isolated from *Dothistroma pini* [2, 3], the fungus responsible for necrotic disease of *Pinus radiata*. The structure (**1**) for dothistromin was elucidated [2, 3] by chemical degradation, spectra and finally by X-ray analysis [4] of the tetraacetylbromoethylacetal (**5**). This analysis established also the absolute configuration of dothistromin, except for C-2, as a ready epimerization could have occurred during the derivatization.

## RESULTS AND DISCUSSION

Extraction of the mycelium of *C. smilacis* afforded a crude product, that appeared from the PMR spectrum as a mixture of two compounds in the ratio of *ca* 7:3. Attempts to further separate the two components were unsuccessful. The substances were also partly soluble which made the measurement of spectra difficult.

Derivatization of this material afforded substances that are derivatives of dothistromin (**1**) or of the epimer 2-epidothistromin (**1a**) depending on the reaction conditions. This is not surprising, because of the well-known susceptibility to ring opening and closure of such cyclic acetal structures in the presence of acid or base. Thus acetylation with $Ac_2O$ in $H_2SO_4$, according to Gallagher *et al.* [3] gave dothistromin pentaacetate (**2**) [3] and dothistromin tetraacetate (**3**). Treatment of the starting material with MeOH and $SOCl_2$ [3] afforded a mixture of dothistromin Me acetal (**4**) and 2-epidothistromin Me acetal, with a predominence of the former. By contrast, when acetylation was carried out in pyridine at 0°, the new 2-epidothistromin pentaacetate (**2a**) was



(**1**) R, R′, R″ = H
(**2**) R, R′, R″ = Ac
(**3**) R, R′ = Ac, R″ = H
(**4**) R, R″ = H; R′ = Me
(**5**) R, R″ = Ac; R′ = $CH_2CH_2$–Br



(**1a**) R = H
(**2a**) R = Ac

obtained almost exclusively. At higher temperature and with sodium acetate, a 1:1 mixture of dothistromin tetraacetate and of (**2a**) was obtained. Again a 1:1 mixture of mono Me ethers (with the OMe in an unknown position in the aromatic ring) was isolated by methylation with MeI and $K_2CO_3$, and acetylation of this mixture gave the same ratio of monomethyltetraacetates.

The assignment of an *exo* (2-epidothistromin) or *endo* (dothistromin) structure to these compounds was based on the PMR spectra, where the 2-acetoxy group appears strongly shielded (1.67 $\delta$ in (**2**) vs. 2.04 in (**2a**)) in the *endo* forms by the polycyclic aromatic ring [3]. Moreover, in all the derivatives the H-2 appears constantly as a three-line signal (X part of ABX) with a separation of 4 Hz in the *exo* form, whereas the same proton shows a multiplet, often superimposed on the 12a signal, and with lesser separations, for the *endo* isomer.

With these means of identification at hand, reexamination of the PMR spectrum of the starting material showed unambiguously that it is a mixture *ca* 7:3 of 2-epidothistromin (**1a**) and dothistromin (**1**), as the signals from H-2 appear clearly in the spectrum. The suspicion

* Part 5 of a series on metabolites of *Cercospora*. For Part 4 see ref. [1].

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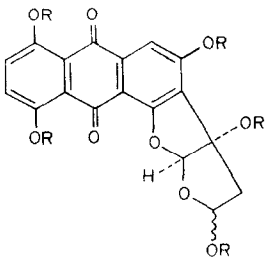
that equilibration of the two epimers could have occurred during chromatography through Si gel was ruled out by running the spectrum on the EtOAc extract of the mycelium, without further purification, which confirmed the presence of the mixture. Therefore it seems that both dothistromin and 2-epidothistromin are produced by the fungus.

According to Gallagher *et al.* [3] their extract of the mycelium of *Dothistroma pini* is a mixture of dothistromin, and of 10–20% of deoxydothistromin, which could not be separated. The only evidence given by these workers for this latter compound is the presence of the corresponding peaks in the MS of dothistromin. We have found no evidence of the presence of deoxydothistromin in our extract of *C. smilacis*, nor was any derivative with a MS corresponding to this compound found in any of the derivatives.

The product of acetylation of (**1**) and (**1a**) in pyridine at 0° contained another compound (**7**) besides (**2a**), that was isolated by TLC. The MS of (**7**) is consistent with a dothistromin pentaacetate structure. The PMR spectrum is very similar to that of this derivative, and shows that



(**6**) R = H
(**7**) R = Ac

(**7**) is again a mixture *ca* 1:1 of *endo* and *exo* 2-acetates. (The spectrum in $C_6H_6$ particularly shows all the signals well separated), so that the assignment of the ABX system of the 2 and 3 protons, and of all the other protons is straightforward. The only difference between the spectrum of (**2**) and (**2a**) is the slight downfield shift of the signal of H-12a (6.67 $\delta$ vs 6.50 $\delta$). The only possible explanation for such data that can be given is ring opening and ring closure to the angular isomer (**6**), although we cannot offer any proof for this structure. Attempts to deacetylate (**7**) to the possible angular dothistromin isomer (**6**) gave only intractable mixtures, whereas 2-epidothistromin pentaacetate afforded the parent compound.

From some cultures of the fungus, we have isolated also the anthraquinones averufin [5] and averythrin [6], both also found by Danks and Hodges [7] in *Dothistroma pini*.

## EXPERIMENTAL

Mps are uncorrected. UV spectra were measured in 95% EtOH. PMR spectra were recorded at 100 MHz, chemical shifts are in ppm ($\delta$), from TMS as internal standard. Column chromatography and TLC were performed with Si gel. Where not otherwise indicated the purity of the products was checked by TLC, PMR and MS and deemed sufficient for the purposes of structural elucidation.

*Isolation of the metabolite.* A culture of *C. smilacis*. 556.7l obtained from Centraalbureau voor Schimmelcultures, Baarn, grown on potato–agar in 26 Roux flasks, was extracted twice with EtOAc after 2 weeks growth; the extracts were evaporated to yield 340 mg (13 mg/flask) of dark solid material, which was further purified by crystallization from $Me_2CO$. A sample of this product had MW 372 and the PMR spectrum showed a mixture of two isomers (**1a**, **1**) in the ratio *ca* 7:3. $\delta$ (DMSO), 2.0–2.4 (*m*, $H_2$–3), 5.30 (H-2,1a) and 5.70 (H-2, **1**), 6.02 and 6.06 (H-12a), 7.20 (H-11), 7.30–7.44 (H-7, H-8), 15.68 (2 chelated OH) and 16.34 (chelated OH).

*2-Epidothistromin pentaacetate* (**2a**) *and* (**7**). Acetylation (Py, $Ac_2O$, 0° for 1 week) of (**1**, **1a**) gave after usual work up (PLC in $C_6H_6$-$Et_2O$-HOAc; 9:1:0.2) two pentaacetates: the former being 2-epidothistromin pentaacetate (**2a**), mp 125° (from $CHCl_3$–hexane), $[\alpha]_D^{20} = -58.5°$ (in $CHCl_3$, $c = 0.43$), $M^+$ 582, uv $\lambda_{max}$ (nm) 241, 270.5, 350 ($\varepsilon$ 2900, 32.700, 5370), PMR $\delta$ ($CDCl_3$) 2.04 and 2.11 (aliphatic Ac), 2.40 (Ac) and 2.44 (2Ac), 2.82 (*m*, $H_2$–3), 6.33 (H-2), 6.50 (*s*, H-12a), 7.37 (H-7, H-8) and 7.58 (*s*, H-11). The second product, (**7**), had mp 160°, $M^+$ 582, uv $\lambda_{max}$ (nm) 224, 253, 270*sh*, 370 ($\varepsilon$ 24 000, 20 900, 12 500, 6100); the PMR spectrum ($CDCl_3$) showed signals for a mixture of pentaacetates, and particularly $\delta$1.64 (2-acetoxy of *endo*-form), 6.37 (H-2 of the *endo*-form), 6.52 (H-2 of the *exo*-form), 6.67 (H-12a of both forms).

*Hydrolysis of the pentaacetate* (**2a**). A few mg of NaOMe were added to 10 mg of (**2a**) dissolved in MeOH (5ml), under $N_2$, at room temp; after 10 min the spot corresponding to dothistromin appeared on TLC.

*Acetylation with $Ac_2O$ and AcONa.* 100 mg of the mixture (**1**, **1a**) with $Ac_2O$ (5 ml) and AcONa (200 mg) were left for 4 hr at 60°, filtration and concentration gave after PLC (**2a**) and a *ca* 50% mixture of the tetraacetates of (**1**, **1a**). PMR $\delta$ ($CDCl_3$) 1.67 (Ac) and 6.70 (H-2) for (**1**) derivative; 2.15 (Ac) and 6.32 (H-2) for (**1a**) derivative. All the others signals are identical.

*Dothistromin pentaacetate* (**2**) *and tetraacetate* (**3**). Acetylation of (**1**, **1a**) under acidic conditions according to [3], gave principally the compound (**2**); another product was a tetraacetate of (**1**), with a free OH at position 4 (the PMR spectrum showing one chelated OH and the signal of H-11 at 7.23 $\delta$, (vs 7.56 in the pentaacetate). (**3**) is a glassy solid mp 105–110°, $M^+$ 540 PMR $\delta$ ($CDCl_3$) 1.70 (Ac-2) and 2.09 (Ac-3a), 2.44 and 2.46 (Ac), 2.80–2.98 (*m*, $H_2$-3), 6.50 (H-2 and H-12a), 7.23 (*s*, H-11), 7.4 (H-7, H-8), 12.95 (chelatee OH).

*The methyl acetai* (**4**). Prepared with $SOCl_2$ in MeOH, [3]; the reaction mixture, after solvent evaporation, was crystallized from EtOH–$H_2O$ to give red crystals of (**4**) mp 230°; PMR $\delta$ (DMSO) 2.4–2.9 (*m*, $H_2$-3), 3.19 (OMe), 5.39 (H-2), 6.10 (*s*, H-12a), 7.08 (H-7, H-8), 14.88, 15.18 and 15.46 (chelated OH); other signals in the PMR spectrum indicated that the sample contained a small amount of the Me acetal of (**1a**).

*Methylation of the mixture* (**1**, **1a**). Methylation with MeI, $K_2CO_3$ and dry $Me_2CO$ under reflux for 6 hr, gave a mono Me ether as a red solid, mp 180°, identified from the tetraacetate derivative (Py, $Ac_2O$), as a glassy solid mp 135°, PMR $\delta$ ($CDCl_3$) 1.63 (Ac-2, endo-form), 2.06 (Ac-3a), 2.10 (Ac-2, exo-form), 2.28 (2Ac), 2.76–2.92 (*m*, $H_2$-3), 4.4 (OMe), 6.32 (H-2 exo), 6.53 (H-2 endo), 6.63 (*s*, H-12a), 7.36 (H-7, H-8).

*Averufin and averythrin.* Averufin and averythrin were identified by comparison of mp, UV, NMR and MS with published data [5, 6].

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