of the known GAGs[3,5]. In enzymatic studies it was found that chondroitinase ABC hydrolyzed this mucopolysaccharide almost completely[8]. Further, this compound accounted for about 90% of the lMW-GAGs and for approximately 1% of the total GAGs[3] of the aorta.

Discussion. Little attention has been paid to the possible existence of lMW-GAGs in various tissues and fluids. To date because of the existing separation techniques, these compounds are overlooked and discarded during fractionation. However, with the availability of the new 2-dimensional electrophoretic procedure utilized in the present study, μg-quantities of GAGs can now successfully be investigated. Thus, we decided to modify the existing fractionation procedure and utilize this electrophoretic method to determine whether hitherto unknown lMW-GAGs exist.

As to the characterization of the isolated lMW-GAG, the digestion with a specific hydrolase (chondroitinase ABC) indicated that this GAG is a chondroitin sulfate. Moreover, the 2 electrophoretic mobilities of this compound suggest the presence of an oversulfated mucopolysaccharide.

The significance of an oversulfated lMW-chondroitin sulfate is difficult to assess on the basis of the available information. Although it could easily be speculated that the aortic lMW-GAGs may be due to incomplete degradation of the oversulfated portion of the high MW-GAGs or due to incomplete synthesis, it is nevertheless important to consider that this hitherto unknown GAG may in fact be a new entity requiring detailed chemical and biological analysis for its complete identification.

8 In control experiments carried out to assess the effect of papain and of incubation at pH 1–2 on high mol.wt GAGs, it could be established that to dialyzable mucopolysaccharides were formed.

Cynodontin, the tetrahydroxyanthraquinone of *Curvularia* and *Drechslera* species

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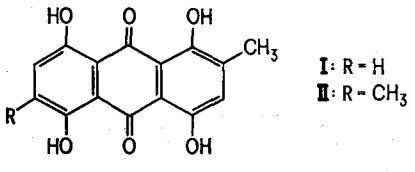
Summary. The red anthraquinone derivative isolated from several Drechslera and Curvularia species has been identified as cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone), and not as 1,4,5,8-tetrahydroxy-2,6-dimethylanthraquinone as reported earlier. Both pigments showed similar physico-chemical properties. A good distinction could be obtained by gas-liquid chromatography.

In the course of a study on the production of metabolites, especially anthraquinone pigments, produced by species of Drechslera, we always identified the isolated red tetrahydroxyanthraquinone as cynodontin (1,4,5,8-tetrahydroxy-2-methylanthraquinone) (**I**), but found never 1,4,5,8-tetrahydroxy-2,6-dimethylanthraquinone (**II**), the pigment reported by Bohlmann et al.[1] for Curvularia lunata (Wakker) Boedijn (st. asc. Cochliobolus lunatus Nelson et Haasis), and subsequently for Drechslera spicifera (Bain.) v. Arx (st. asc. C. spicifer Nelson)[2], D. sorokiniana (Sacc.) Subram. et Jain [st. asc. C. sativus (S. Ito et Kuribayashi) Drechsler ex Dastur][3] and also several other Curvularia species[2]. Since the dimethylanthraquinone is somewhat unusual[4] and provides the only example of an anthraquinone in which C-methylation would occur in its biosynthesis[5], we decided to make more intensive examinations of the existence of this pigment as a natural substance.

The original natural **II** was not available[6] and, therefore, we re-isolated the pigment from cultures of the C. lunata strain used by Bohlmann and his co-workers. The isolated product was compared with natural cynodontin (**I**) and synthetic **II**, which we synthesized according to Flumiani[7]. The identity of the synthetic product was confirmed by high resolution mass spectrometry (MS).



Curvularia lunata NRRL 2380[8] was cultivated in a medium containing peptone (microbiotone Oxoid) 5 g, glucose 50 g, $MgSO_4 \cdot 7\ H_2O$ 0.25 g and K_2HPO_4 0.25 g per liter deionized water. Erlenmeyer flasks of 300 ml, each containing 100 ml of medium, were incubated as still cultures at 24 °C for 18 days. Mycelium collected from 9 flasks was extracted with ethyl acetate using a Waring blendor. After evaporation of the solvent, the residue (447 mg) was treated with light petroleum, bp 40–60 °C, for removal of the fatty material. The remaining material (21 mg) was recrystallized from ethyl acetate. Brown crystals (4 mg) with a bronze lustre, designated as compound **III**, were obtained. Dry weight of the mycelium after extraction was 4.4 g.

Drechslera halodes (Drechsler) Subram. et Jain CBS 273.52 was grown as surface cultures on Czapek Dox medium supplemented with 0.05% yeast extract (Oxoid) and 1 ml/l medium of a trace elements solution, prepared according to Kitto et al.[9], for 14 days at 24 °C. Mycelium from 6 Erlenmeyer flasks (100 ml medium/flask) was collected and also extracted with ethyl acetate. Removal of the solvent followed by washing with light petroleum

1 F. Bohlmann, W. Lüders and W. Plettner, Arch. Pharm. Ber. Dt. Chem. Ges. *294*, 521 (1961).
2 R. G. Coombe, J. J. Jacobs and T. R. Watson, Aust. J. Chem. *21*, 783 (1968).
3 R. G. Coombe, personal communication (1968), in: R. H. Thomson, Naturally Occurring Quinones, 2nd ed., p. 504. Academic Press, London 1971.
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5 W. G. Turner, Fungal Metabolites, p. 163. Academic Press, London 1971.
6 F. Bohlmann, personal communication.
7 G. Flumiani, Monatsh. Chemie *45*, 43 (1924).
8 Thanks are due to Dr K. Kieslich, Schering AG, Berlin-West, for providing the NRRL 2380 strain of Curvularia lunata.
9 G. B. Kitto, M. E. Kottke, L. H. Bertland, W. H. Murphey and N. O. Kaplan, Archs Biochem. Biophys. *121*, 224 (1967).

left a residue of 76 mg which was recrystallized from ethyl acetate and again gave brown bronze crystals (35 mg). This compound was identified as cynodontin by means of MS, IR and UV-visible spectra in full agreement with the data presented by other authors[10,11].

On comparing the compounds **II** and **III** with cynodontin (**I**), we remarked a striking similarity between the physico-chemical properties generally used for their identification. We measured the same UV-visible spectra, detected slight differences in the IR-spectra only when using pure samples (figure 1) and were unable to obtain any separation on silica gel thin-layer plates using 8 solvent systems. However, a very good separation between **I** and **II** was achieved by applying gas-liquid chromatography (GLC) using a recently described method[12] (figure 2). It was surprising to measure the same relative retention time for the trimethylsilyl ether of **I** and **III**, indicating that the pigment of C. lunata is in fact cynodontin. This was proved by high resolution MS. A molecular formula was found in agreement with that of cynodontin.

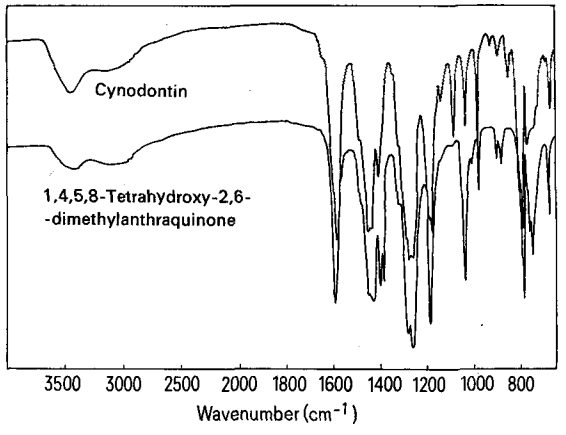

Fig. 1. IR-spectra of **I** and **II** (in KBr).

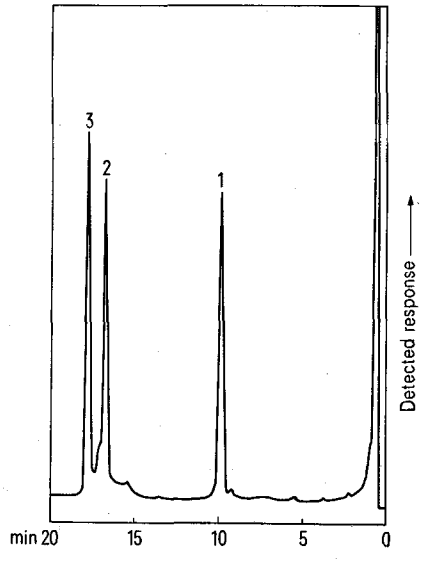

Fig. 2. GLC separation of **I** and **II** as trimethylsilyl ethers. Peaks: *1* chrysophanol (1,8-dihydroxy-3-methylanthraquinone, RRT: 1.00); *2* **I** (RRT: 1.68); *3* **II** (RRT: 1.78).

This result prompted us to re-investigate the nature of the red pigment produced by the several species of fungi as described by Coombe et al. Unfortunately it was not possible to investigate the original strains since they had been discarded[13]. Therefore we studied the pigment production in some CBS strains of the same species. The red pigment was isolated from cultures of Curvularia geniculata (Tracy et Earle) Boedijn (st. asc. Cochliobulus geniculatus Nelson) CBS 220.52, C. ramosa (Bain.) Boedijn CBS 179.36, C. trifolii (Kauffman) Boedijn CBS 187.49, Drechslera spicifera CBS 274.52 and D. sorokiniana CBS 139.29 and CBS 140.31. In addition the red compound was isolated from C. lunata (Wakker) Boedijn var. aeria (Batista et al.) M. B. Ellis CBS 294.61 and from D. dematioidea (Bubák et Wroblewski) Subram. et Jain CBS 303.50 and D. setariae (Sawada) Subram. et Jain [st. asc. C. setariae (S. Ito et Kuribayashi) Drechsler ex Dastur] CBS 143.29. The red pigment of all these strains was identified unambiguously as cynodontin (**I**).

From the results, it may be concluded that **II** does not exist as a natural substance. In their article, Bohlmann et al.[1] described the elemental analysis of the isolated pigment and of 3 of its derivatives, viz. the tetraacetate, the dibenzoate and the tetrabenzoate. The percentages C and H reported for the pigment agrees most closely with the calculated percentages C and H of the dimethylcompound **II**. The values C and H reported for the tetraacetate, however, correlate well with the calculated values of the tetraacetate of cynodontin. Exact conclusions cannot be drawn from the percentages C and H given for the di- and tetrabenzoate of their isolate. Coombe et al.[2] claimed that **II** was present in fungi because the isolated compound showed the same UV-visible spectrum in pentane as synthetic **II**, and the natural and synthetic pigments could not be separated by TLC using 4 solvent systems. The present work shows that this is insufficient for complete characterization.

Physico-chemical data. Cynodontin (**I**): MS m/e 286 (M^+, $C_{15}H_{10}O_6$); M^+ 232.7: transition $286^+ \rightarrow 258^+ + 28$ (CO)[14]; λ_{max} (EtOH): 243, 297, 474 sh, 484, 506 sh, 517, 542, 554, 603 nm. 1,4,5,8-Tetrahydroxy-2,6-dimethylanthraquinone (**II**): mol.wt 300.06598, calc. for $C_{16}H_{12}O_6$ 300.06338; λ_{max} (EtOH): 247, 300, 475 sh, 484, 508 sh, 517, 543, 555, 602 nm. Compound **III** (identified as cynodontin): mol.wt 286.04945, calc. for $C_{15}H_{10}O_6$ 286.04773.

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13 P. G. Valder, University of Sydney, School of Biological Sciences, Sydney, personal communication.
14 The authors are indebted to Mr C. Versluys, Analytical Laboratory, State University of Utrecht, for measuring the mass spectra.