

2-CARBOXYMETHYL-3-N-HEXYL-MALEIC ACID ANHYDRIDE,

A NOVEL METABOLITE FROM AN ASPERGILLUS

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In the course of our antibiotic screening program we isolated the Aspergillus FH-X-213 from an apple which had been kept in earth for three weeks. The strain belongs to the group Aspergillus niger and is similar to Aspergillus awamori (1). The metabolite described below shows a weak in vitro activity against grampositive bacteria. It is produced by the above-mentioned strain in a shaking culture as well as in a fermentor. We cultivated the strain by batch fermentation in a 10 l fermentor under the following broth and culture conditions:

10 g glucose, 2.5 g NaCl, 30 g sucrose, 1 g malt extract, 10 g caseinpeptone, tap water ad 1000 ml, pH 6.8, 28°C, 280 r.p.m. aeration: 50 l/l/h; after 24 hours 300 l/l/h up to the harvest.

Fermentation was stopped after 46 hours and 10 l of this culture broth, at pH 3.9, were centrifuged, the solution was adjusted to pH 2 with concentrated H<sub>2</sub>SO<sub>4</sub> and extracted with 4 l of butyl acetate. The aqueous phase was discarded and the organic phase was evaporated to a brown oily residue at 50°C/20 mm Hg (yield 7 g). The oil was purified by preparative tlc on Silicagel (Merck) using CHCl<sub>3</sub>/CH<sub>3</sub>COOH 95:5 v/v. The active UV absorbing zone was eluted and evaporated in vacuo to a yellow oil, which was desiccated and freed from solvent over NaOH, CaCl<sub>2</sub> and paraffin (yield 40 % of the initial material). This product, which is chromatographically uniform, had the following physical properties:

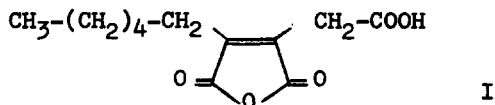
$n_D^{20} = 1.4787$ ,  $\alpha_D^{21} = 0^\circ$  (dioxane,  $c = 1$ ),  $E_{1\text{cm}}^{1\%} = 210$  at 253 nm (THF)  
 $M = 259$  ( $\text{C}_6\text{H}_6$ ), 226 ( $\text{CH}_3\text{COCH}_3$ ) osmometrically, equivalent weight 84 and 85.  
 Found: C 59.2 %; H 6.8 %; O 33.3 %. Calculated for  $\text{C}_{12}\text{H}_{16}\text{O}_5$ ,  $M = 240$ :  
 C 60.0 %; H 6.7 %; O 33.3 %.

On treatment with cyclohexylamine the oil gave a crystalline derivative,  
 m.p.  $146-148^\circ\text{C}$  ( $\text{CH}_3\text{OH}$ /diethyl ether).

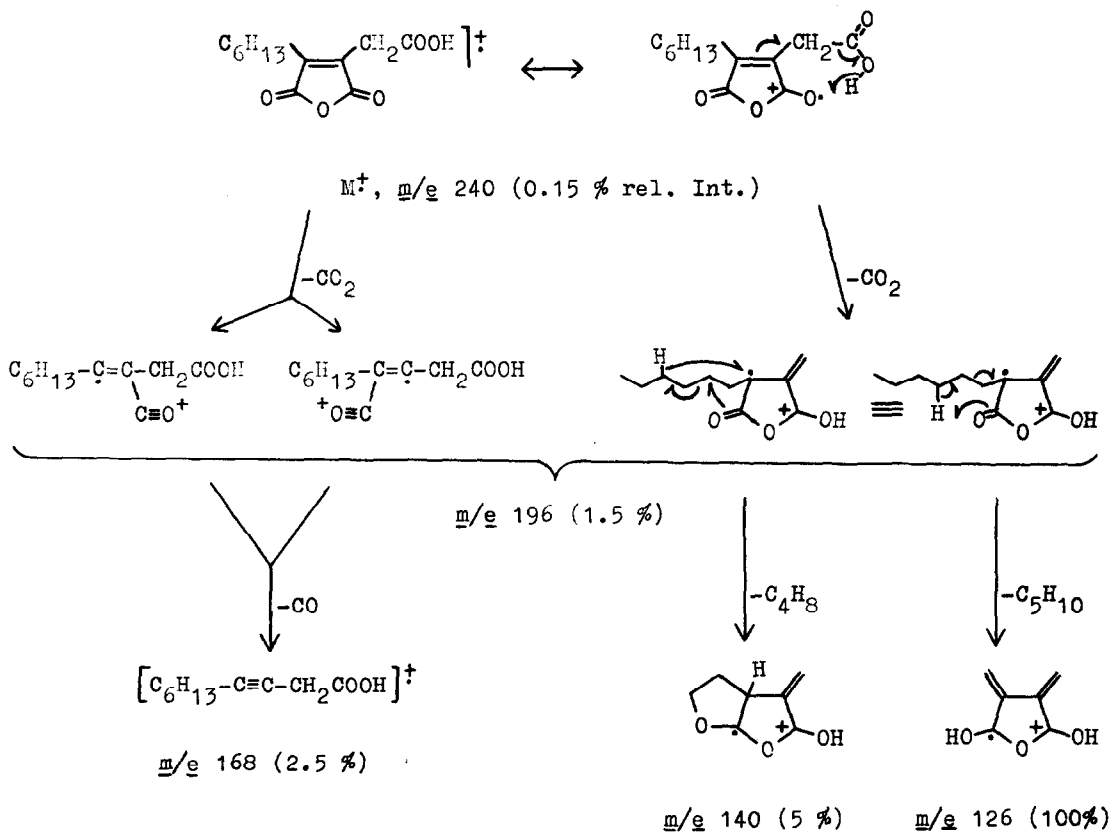
Found: C 66.1 %; H 10.5 %; O 15.7 %; N 7.5 %. Calculated for  $\text{C}_{30}\text{H}_{55}\text{O}_5\text{N}_3$ ,  
 $M = 537$ : C 67.0 %; H 10.3 %; O 14.9 %; N 7.8 %.

The addition product of three moles of cyclohexylamine suggested the presence of a tribasic acid, the absence of optical activity an achiral molecule. In the IR spectrum a broad structured OH absorption band from 3600 to  $2300\text{ cm}^{-1}$  as well as a CO band at  $1720\text{ cm}^{-1}$  refer to the presence of a COOH group. Another intensive CO band at  $1770\text{ cm}^{-1}$  and two weak bands at 1823 and  $1850\text{ cm}^{-1}$  can be interpreted as resulting from an acid anhydride. The UV maximum of 253 nm (250 nm in diethyl ether) is typical of the 2,3-dialkylmaleic acid chromophore (2).

In the NMR spectrum, a triplet at  $\delta = 0.9$  ppm refers to a terminal methyl group, a multiplet at 1.1-1.8 ppm to 4 methylene groups in an alkyl chain and another triplet at 2.5 ppm to a  $\text{CH}_2$  group which is deshielded by connection to a double bond. This combination is interpreted as an n-hexyl group. Furthermore, a singlet at 3.6 ppm relates to an isolated  $\text{CH}_2$  group which is flanked by two unsaturated groups. Summarizing these data we propose structure I, which is confirmed by the mass spectrum.



The main fragment peaks viz.  $m/e$  240, 196, 168, 140, and 126, are interpreted by the following fragmentation scheme:




The proton noise-decoupled CMR spectrum is in accordance with structure I. The twelve C's give rise to 6 resolved signals between 13.4 and 31.3 ppm from the internal TMS reference for  $sp^3$  carbons, and 4 signals between 135 and 174 ppm for  $sp^2$  carbons. Inspection of the intensities and consideration of Overhauser effects and spin-lattice relaxation times suggest that two methylene carbons (29.1 ppm) and the anhydridic carbons (165.07 ppm) overlap.

Our assignments are as follows:

173.37 ppm  $\longrightarrow$  COOH

165.07 ppm  $\longrightarrow$  O=C-O-C=O

148.0 ppm  $\longrightarrow$  

135.4 ppm

22.3-31.27 ppm  $\longrightarrow$  CH<sub>2</sub>

13.4 ppm  $\longrightarrow$  CH<sub>3</sub>

A related compound (Rubratoxin B) with the uncommon maleic anhydride group has recently been isolated from Penicillium rubrum (3).

#### REFERENCES

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- (3) M.O. Moss, F.V. Robinson, A.B. Wood, J. Chem. Soc. (C) 1971, 619

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