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ПРАГА 1975 ПРАГА
PYROLYSIS-GAS-CROMATOGRAFÍA OF THE PIGMENT OF EURO-
TNUM ECHINULATUM DELACR

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_Eurotium echinulatum_ Delacr., a fungus isolated from a southern Spain vertisol, synthesizes phenols, anthraquinones, two complex structure compounds: flavoglaucin and echinulin, as well as a humic acid-like pigment.

The phenols are integrated in some transformation pathways, having as key compounds p-hydroxycinnamic acid, orsellinic acid, acetylphloroglucinol, 3-methylorsellinic acid and 6-methylsalicylic acid. The two key anthraquinones are endocrocín and emodín. These precursors are altered by introduction of additional hydroxyl groups, by decarboxylation and by oxidation of methyl groups to form a series of derivative compounds.

All the metabolites of the pathways have been identified, except some from the 3-methylorsellinic and 6-methylsalicylic acids, which require a more profound study to demonstrate the suggested transformations.

In the culture media from _E. echinulatum_ forty metabolites have been identified, which represent sixty per cent of the compounds separated by TLC (SÁIZ-JIMÉNEZ et al. 1975, a).

The formation of the humic acid-like pigment starts to 18-20 days reaching to 3 g/1 after 3 months. During the period of rapid polymer formation the phenols and anthraquinones disappeared from the medium. At the termination of incubation only small amounts of anthraquinones were still present in free forms and the simple phenols had largely disappeared. It is highly probably that these phenols and anthraquinones were linked into the developing polymer. Both enzymatic and autooxidative reactions and coupling mechanisms appeared to be active in the polymer formation and no anthraquinones or phenols could be extracted with ether or alcohol from the polymer even after exhaustive methylation with diazomethane (SÁIZ-JIMÉNEZ et al. 1975, b).

Some analytical data from the pigment are shown in Table 1.

Reductive degradation of the polymer with Na-amalgam yielded about 45 to 60% ether soluble products of which 19 to 32% correspond to phenolic compounds. In addition to anthraquinones, anthrones, anthranols and anthracene derivatives were present. The anthrones, anthranols and anth-
racenes, however, appear to be formed by the reductive treatment and are
not present as such in the polymer as supported by the release of anth-
raquinones only upon Na-dithionite treatment (SÁIZ-JIMÉNEZ et al., 1975,
c).

Pyrolysis—gas—chromatography have not been often utilized in the se-
paration and eventual identification of volatile compounds from humic sub-
stances.

PGC with a microreactor unit permits the pyrolysis of materials in
few seconds and with a short time of residence in the pyrolyser, which
greatly reduce the secondary reactions.

Fig. 1 shows the pyrogram from several standard aromatic compoun-
ds, where the production of artefacts was not found.

Pyrolysis of the pigment was carried out at 700°C during 10 sec.
and the chromatograph equipped with columns of 10 ft x 1/8 in stainless
steel packed with 10% UCC W 982 on Chromosorb W AW DMCS 80–100
or 5% OV-17 on Chromosorb W AW DMCS 80–100. Identification of produc-
tes was made by pyrolysis of standard compounds, and the agreement of
retention times in both columns were taken as identity criteria.

Table 2 shows the tentative identification of some peaks from the py-
rograms. Dimethylphenols, dihydroxytoluenes and dimethylresorcinols have
been also identified in the culture media as reductive degradation produc-
tes from the pigment (SÁIZ-JIMÉNEZ 1974). Anthracene and methylanthra-
cene were, probably, produced by the thermal fission of anthraquinones as
well as benzene, toluene, phenol, xylenes and cresols from phenolic deri-
vatives (Fig. 2).

SÁIZ-JIMÉNEZ (1974) stated that anthraquinones must be linked to
the periphery of the polymer, according to their active positions and the
data of Na-dithionite reduction. It seems also possible that a part of the
phenols occupies similar positions. KIMBER and SEARLE (1970) pointed
out that benzene and toluene were produced from peripheral and non
condensed aromatic structures in humic acids, therefore, it is possible that
the portion of the molecule pyrolysed in the pigment was the more peripher-
ical, having in account that after pyrolysis 50% of total weight can be re-
covered.

PGC has been applied to taxonomic studies of bacteria and fungi
(REINER et al., 1969, KULIK and VINCENT 1973), KIMBER and SEARLE
(1970) and MURSAKOV (1972) utilized this technique to compare differen-
ce due to the extractant and to determine the dynamics of organic mat-
ter extraction. WERSHAW and BOHNER (1969) were able to identify low boiling point products from pyrolysis of humic matter.

In this study, PGC has been utilized as a technique for tentative identification of volatile compounds from fungal polymers; whose structural units were, previously, identified by other degradative methods.

**Summary**

Pyrolysis-gas-chromatography of the humic acid-like pigment of *Eurotium echinulatum* Delacr. was studied. Volatile compounds were similar to the structural units obtained by reductive methods. Pyrolysis seem to affect the periphery of the molecule, having in account that 50% in weight can be recovered after thermal fission.

**References**


**ПИРОЛИЗ И ГАЗОВАЯ ХРОМАТОГРАФИЯ ПИГМЕНТА ИЗ EUROTIIUM ECHINULATUM**

При помощи газовой хроматографии изучались продукты пиролиза пигмента *Eurotium echinulatum* Delacr., похожего по своим свойствам на гуминовые кислоты. Лёгкие компоненты пиролиза отвечают структурным единицам приобретённым методом восстановления. В связи с тем, что свыше 50% исходного веса может быть определено и после термической обработки, предполагается только периферийное воздействие пиролиза на молекулы пигмента.
Table 1   Analytical data from the *Eurotium echinulatum* pigment  

<table>
<thead>
<tr>
<th></th>
<th>b&lt;sup&gt;2&lt;/sup&gt;</th>
<th>a&lt;sup&gt;3&lt;/sup&gt;</th>
<th>weight loss on 6N HCl(%)</th>
<th>nitrogen loss on 6N HCl (%)</th>
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<tr>
<td>C (%)</td>
<td>61.5</td>
<td>64.0</td>
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<tr>
<td>H (%)</td>
<td>4.2</td>
<td>3.7</td>
<td>12.8</td>
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<td>O (%)</td>
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<td>32.0</td>
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<tr>
<td>N (%)</td>
<td>2.5</td>
<td>0.3</td>
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<tr>
<td>methyl</td>
<td>b</td>
<td>a</td>
<td>O&lt;sub&gt;as&lt;/sub&gt; carboxyl</td>
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<tr>
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<tr>
<td>total hydroxyl</td>
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<td>8.0</td>
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<tr>
<td>total carbonyl</td>
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<tr>
<td>methoxyl</td>
<td>0.4</td>
<td>n.d.</td>
<td></td>
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1) Average value from four different culture media  
2) Before 6N HCl hydrolysis  
3) After 6N HCl hydrolysis

Table 2   Tentative identification of compounds from *E. echinulatum* pigment's pyrolysis

<table>
<thead>
<tr>
<th></th>
<th>UCC</th>
<th>OV-17</th>
<th>UCC</th>
<th>OV-17</th>
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<tr>
<td>benzene</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>toluene</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>xylenes</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>phenol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>o-cresol</td>
<td>+</td>
<td>+</td>
<td>1,2-dimethylresorcinol</td>
<td>+</td>
</tr>
<tr>
<td>m- and p-cresol</td>
<td>+</td>
<td>+</td>
<td>2,5-dimethylresorcinol</td>
<td>-</td>
</tr>
<tr>
<td>2,5-dimethylphenol</td>
<td>+</td>
<td>+</td>
<td>fluorene</td>
<td>+</td>
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<tr>
<td>2,3- and 3,5-dimethylphenol</td>
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<td>+</td>
<td>anthracene</td>
<td>+</td>
</tr>
<tr>
<td>3,4-dimethylphenol</td>
<td>+</td>
<td>+</td>
<td>methylanthracene</td>
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<tr>
<td>p-hydroxybenzaldehyde</td>
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Figure 1 Pyrogram from standard compounds. Column: 5% OV-17 on Chromosorb W AW DMCS 80-100.
Figure 2  Pyrogram from Eurotium echinulatum pigment. Column: 5% OV-17 on Chromosorb W AW DMCS 80-100