and at *m/e* 70 (39%), 69 (45%), 56 (25%), 55 (35%), 43 (27%), 42 (32%) and 41 (30%). Metastable ion peaks were detected for the following transitions: *m/e* 154 → 126 + CO (m* = 103.1); *m/e* 154 → 111 + HNCO (m* = 80.0); *m/e* 111 → 83 + CO (m* = 62.1); and *m/e* 83 → 55 (*m** = 36.4). Expulsion of the elements of HNCO from the molecular ion strongly suggested a cyclic lactam structure containing other unsaturated groups in the ring.

Excellent crystals of the amide were obtained by slow cooling of a methanol-acetone-hexane (trace) solution. The observed Laué symmetry and extinctions correspond to the space group $P2_12_12_1$ with a = 9.666 ± 0.006, b = 5.870 ± 0.004, c = 13.067 ± 0.010, Å; Z = 4; $\varrho$ calc. = 1.381 g/cm³; and $\varrho$ obsd. = 1.37 g/cm³. Diffraction intensities were measured in the variable speed $\theta$–$2\theta$ scan mode with monochomated $MoK_\alpha$ radiation on a Syntex Pi diffractometer; of the 1280 independent reflections investigated ($2\theta \leq 60.0°$), a total of 1234 were retained as objectively observed. No corrections were applied for either absorption or extinction.

The equal atom structure was solved by direct methods[3,4] using a computerized[5] multiple-formula single-solution procedure based on the generalized tangent formula[6]. Full-matrix least-squares refinement of the structure (130 independent variables) with anisotropic thermal parameters and hydrogen positional parameters yielded a standard residual $R = 0.046$ (for all observed data and a weighted residual $R_w = 0.054$). Refinement of the structure without the hydrogen atoms yielded the residuals $R = 0.109$ and $R_w = 0.143$.

The perspective view in the Figure represents fully the molecular configuration and conformation of Gly-L-Pro lactam[7,8]. This is, to the best of our knowledge, the first time this dipeptide lactam has been isolated from a marine organism or indeed from any natural source[8,9]. Since the natural specimen gives the same ORD spectrum as the synthetic material prepared from L-proline, the absolute configuration is assigned as (S)–$C_2$.

As can be seen from the Figure neither of the rings in the molecule is planar; both in fact show pronounced folding. In the pyrrolidine ring the atoms $C_2$, $N_1$, $C_8$, and $C_7$ are quite coplanar (average deviation 0.015 Å) with $C_9$ considerably out of the plane (0.55 Å). This particular ring conformation has been observed only in 2,3-*cis*-3,4-*trans*-3,4-dihydroxyproline[10]; typically $C_8$ is out of the plane[11–13]. The dioxopiperazine ring has a pronounced fold about the line $C_2$–$C_5$; the dihedral angle between the planes $C_2, C_3, O_3, N_4, H_4, C_5$ and $C_2, N_1, C_7, C_6, O_6, C_5$ is 38.3 deg. The peptide bond in the first of these planes is flat with a dihedral angle about $N_4$–$C_3$ of only 0.8° ($\omega = 179.2°$); the atoms in this plane show average deviations of only 0.005 Å. The second plane is considerably less planar (average deviation 0.030 Å) due in part to a slight twist of 7.2° about the peptide bond $N_1$–$C_6$ ($\omega = 172.8°$). The only other example of a nonplanar dioxopiperazine ring is that from L-Ala-L-Ala[14], where the dihedral angle between the nearly planar peptide groups is 25.7°.

*Zusammenfassung.* Es wird über die Isolierung und Charakterisierung der Titelverbindung (Gly-L-Pro Lactam, I) aus dem Seestern *Luidia clathrata* berichtet. Die Kristallstruktur dieses Dioxopiperazinderivats wurde ermittelt. Der Piperazinring liegt in der Wannen-Konformation vor.

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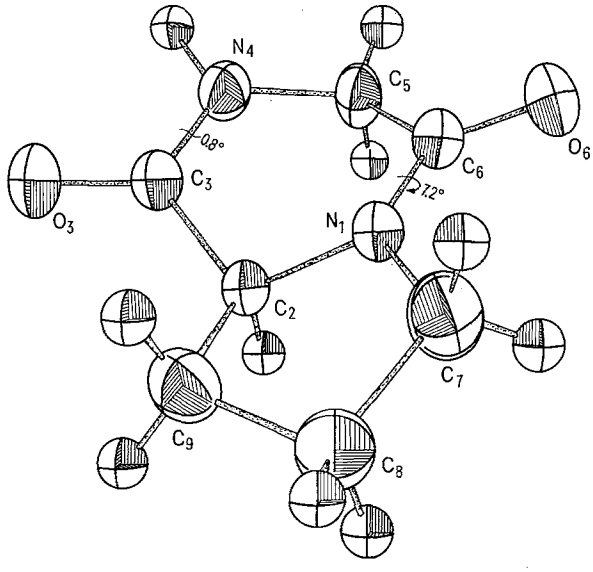


A perspective representation of the structure of Gly-L-Pro lactam.

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## Anthraquinones in the Fungus *Talaromyces stipitatus*

In the course of morphological studies on the genus *Talaromyces*[1], it was observed that the strain CBS 349.72 of *Talaromyces stipitatus* C. R. Benjamin ex STOLK and SAMSON (st. con. *Penicillium stipitatum* Thom) showed different pigmentation from the other strains examined. This strain developed a red-brown reverse while the others became yellowish. It was decided to investigate the nature of this red colour.

The fungus was grown on Czapek-Dox agar in petri dishes at 24 °C. The cultures (35 plates) were extracted with ethyl acetate after 14 days. The residue obtained on evaporating the solvent was extracted twice with light petroleum b.p. 40–60 °C and then recrystallized from chloroform-methanol (2:1). Bright red needles (18 mg) were obtained. Colour reactions, UV- and visible spectra and the decomposition pattern of the mass spectrum were characteristic for an anthraquinone derivative. The pigment proved to be erythroglaucin (1,4,8-trihydroxy, 3-methyl, 6-methoxyanthraquinone), since it was completely identical with preparations of erythroglaucin isolated from *Eurotium rubrum* CBS 110.31 (st. con. *Aspergillus sejunctus* = *A. ruber*[2]) and synthesized from catenarin[3].

From the mother liquor 2 other anthraquinone derivatives were isolated after chromatographing several times on preparative silica gel G layers using different solvent systems. One compound (1 mg) turned out to be catenarin (1,4,6,8-tetrahydroxy, 3-methylanthraquinone) and showed complete agreement in all respects with catenarin obtained from *Drechslera catenaria* CBS 191.29 = *Helminthosporium catenarium*[4]. The other substance (0.5 mg) could be identified as emodin (1,6,8-trihydroxy, 3-methylanthraquinone) by direct comparison with a commercial sample (Fluka).

Erythroglaucin occurs in cultures of several *Aspergillus* spp.[2] and in the lichen *Xanthoria elegans*[5]. So far it has not been detected in a species of *Talaromyces* or *Penicillium*. Catenarin was isolated from several *Drechslera* = *Helminthosporium* spp.[4], *Eurotium amstelodami* (st. con. *Aspergillus amstelodami*)[6], and only once detected in a *Penicillium* species viz. *P. islandicum*[7]. The simultaneous presence of catenarin and its 6-methylether erythroglaucin in a fungus has not been reported previously. Emodin is an ubiquitous natural anthraquinone. It occurs in both higher and lower fungi. The isolation from *Hamigera avellanea* = *Talaromyces avellaneus* (con. st. *Penicillium avellaneam*) has been described[8].

Physico-chemical data of the *T. stipitatus* metabolites[9]. Erythroglaucin: m.p. 204–206°; mol. wt. 300.06227, calc. for $C_{16}H_{12}O_6$ 300.06338[10]; $\lambda_{max}$ (MeOH): 232, 256, 277, 305, 465 sh, 480 sh, 490, 510 sh, 523 nm; $\lambda_{max}$ (MeOH/KOH): 245, 312, 550, 590 sh nm; $\nu_{max}$ (KBr): 1598 $cm^{-1}$. Catenarin: mol. wt. 286.046126, calc. for $C_{15}H_{10}O_6$ 286.047731; $\lambda_{max}$ (EtOH); 232, 257, 272 sh, 282, 302 sh, 468 sh, 482, 491, 512, 525 nm; $\lambda_{max}$ (EtOH/KOH): 221, 257, 300, 324, 530 nm; $\nu_{max}$ (KBr): 1600 $cm^{-1}$. Emodin: mol. wt. 270.053787, calc. for $C_{15}H_{10}O_5$ 270.052817; $\lambda_{max}$ (MeOH): 223, 253, 267, 288, 304 sh, 438 nm; $\lambda_{max}$ (MeOH/KOH): 221, 255, 310, 498 nm.

*Zusammenfassung.* Drei Anthrachinonpigmente wurden aus dem Schimmelpilz *Talaromyces stipitatus* CBS 349.72 isoliert. Die Untersuchung zeigte, dass sie Verbindungen identisch sind mit Erythroglaucin, Catenarin und Emodin. Durch Vergleich mit authentischen Proben konnte diese Ansicht bestätigt werden.

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## Inhibition of Arylpyruvate Oxidase by Chelating Agents[1]

*p*-Hydroxyphenylpyruvate oxidase and phenylpyruvate oxidase have been shown to be inhibited by several chelating agents[2,3], especially diethyldithiocarbamate, which suggests that it may be a copper-containing enzyme. With some copper-containing enzymes the metal is so firmly bound that chelating agents do not remove it from the enzyme molecule[4]. Most metalloenzymes, however, are inhibited by chelating agents through the removal of the cation from the holoenzyme. The present study was undertaken to decide which mechanism applies to *p*-hydroxyphenylpyruvate oxidase.

*Methods.* *p*-Hydroxyphenylpyruvate oxidase[5] and phenylpyruvate oxidase[6] were assayed by the change in optical density of the enol-borate complex of the substrate. Enzyme was purified as outlined previously[7]; tests on inhibition were carried out on material purified to step (c). All reagents were the best commercially available, and distilled water was deionised to a concentration of less than 1 part per million. Copper, nickel and cobalt were assayed as complexes with diethyldithiocarbamate[8] and iron as its complex with 1,10-phenanthroline[9]. Cadmium was assayed as its complex with dithizone[10]. Unless otherwise stated all buffers were 1 mM in ascorbate.

*Results and discussion.* The degree of inhibition of both activities by chelating agents is shown in Table I. Diethyldithiocarbamate was the most effective chelating agent tested. In each case phenylpyruvate oxidase was inhibited more completely than *p*-hydroxyphenylpyruvate.

Reactivation was observed on addition of several cations to *p*-hydroxyphenylpyruvate oxidase that had

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