natal life[3]. Nevertheless, the enzyme system responsible for ATP cleavage and energy disposal does exhibit the same properties found in the adult retina, no changes in the ratio Mg^{++}–Na^+–K^+/Mg^{++} stimulated ATPase activity being measured during the first 4 months of life. CÔTÉ[8] has shown that the enzyme activity is measured in the brain before the occurrence of electrical activity. Though the alteration of the ERG in the affected animals is easily related to the damage of photoreceptor cells, it is worth noting that the early decline of Na^+-K^+ stimulation of ATPase activity offers a suggestive dynamic explanation of the transitory appearance and subsequent disappearance of the electroretinogram in this same rat strain[3]. One could suppose that the ERG appears because at the 12th day ATPase is still largely stimulated by Na^+ and K^+ ions and declines soon after, since the ionic stimulation of enzyme activity becomes insufficient. If ATPase is an allosteric enzyme, as suggested by SQUIRES[9], a conformational alteration of the catalytic protein at the allosteric site for Na^+ and K^+ ions would be responsible for the observed changes in ATPase activities. Whatever the possible speculations, it can only be stated that ATPase stimulation by Na^+ and K^+ ions declines in the dystrophic retina with age in comparison with non-Na^+–K^+ stimulated ATPase[10].

Riassunto. Viene descritto un aumento significativo dell'attività specifica dell'ATPasi (attività/mg proteine) durante lo sviluppo post-natale della retina in ratti normali ed in ratti con retinite pigmentosa ereditaria. Il rapporto tra attività ATPasica in presenza di Mg^{++}–Na^+–K^+ ed attività ATPasica in presenza di Mg^{++} non varia nella retina normale, mentre esso va incontro ad un precoce declino durante lo sviluppo della retina distrofica. Il significato di questi dati è discusso molto brevemente.

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The Production of Leucylphenylalanine Anhydride by a Variant of *Streptomyces noursei*

Streptomyces noursei, variant No. 5286, which produces the antibiotic phalamycin[1] also produces several substituted dioxopiperazines[2]. Four of them have been reported, namely: 3,6-dibenzylidene-2,5-dioxopiperazine (I), its dihydro derivative 3-benzyl-6-benzylidene-2,5-dioxopiperazine (II), and its tetrahydro derivative 3,6-dibenzyl-2,5-dioxopiperazine (III), and 3-benzylidene-6-isobutylidene-2,5-dioxopiperazine (IV). Because of the relationship between I, II, and III a subsequent search was made for further products which might be similarly related to IV by saturation of either or both of the olefinic double bonds. Only the fully saturated product, namely 3-benzyl-6-isobutyl-2,5-dioxopiperazine or leucylphenylalanine anhydride (V) was found. This note reports its isolation and proof of identity by methods previously described[2].

For the isolation, ethyl acetate extracts of 4-day shake cultures in yeast extract broth were concentrated to dryness in vacuo and the residue extracted repeatedly with each of the following solvents in succession: water, and 10, 20, 30, 40, 50, 60, 70, 80, and 90% acetone. Based on IR-spectra, pools were made and solids obtained by concentration in vacuo. In the 70–90% acetone extracts there was evidence for IV as well as V. In the 10–60% acetone extracts there was strong evidence (in particular, 3 characteristic absorption bands at 1347, 1336, and 1325 cm^{-1}) for the presence of V. These extracts contained also much highly colored material, most of which could be removed by several washings with ether. The residue was further purified by repeated sublimations at a temperature of approximately 180 °C and a pressure of about 1 mm Hg. Waxy resinous solids collected on the cold finger in the early cuts of this process, and clean white amorphous solids sublimed thereafter. Efforts to crystallize this latter material revealed a striking difference from the other dioxopiperazines. Crystallization could not be initiated or was practically negligible from most of the ordinary organic solvents or solvent pairs; either a stiff gel formed when the hot solutions were cooled, or no crystals appeared if the solutions were dilute enough to avoid gel formation. From aqueous acetic acid, approximately 60%, long fibrous, glistening white crystals were sometimes obtained after several days at – 5 °C, but only in very low yield. However, when crystals failed to appear from the acetic acid-water solutions or when a gel formed, a moderately good yield of crystals could be obtained by first freezing the solution or gel and then thawing it at room temperature. The tiny white rod-shaped crystals thus formed melted a few degrees lower than the larger crystals mentioned above, but nevertheless this step brought about considerable improvement in purity.

This natural V was identified by comparison of its properties with those of the synthetic product prepared by cyclization of L-leucyl-L-phenylalanine. Both natural and synthetic products show the same principal absorption maxima: 3210, 3070, 2960, 2905, 1665, 1605, 1499, 1465, 1458, 1390, 1370, 1347, 1336, 1325, 1093, 1012, 912, 868, 836, 766, 752, and 700 cm^{-1}. The natural product melts with decomposition at 272–273 °C, the synthetic at 273–274 °C, and the mixture melting point shows no depression. Analysis of the natural substance gave: C, 69.46; H, 7.52; N, 10.65; calculated for $C_{15}H_{20}N_2O_2$: C, 69.18;

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H, 7.75; N, 10.77. For the natural product $[\alpha]_D^{29}$ −50 °C (c 0.316 pyridine); for the synthetic product, $[\alpha]_D^{27}$ −28 °C (c 0.261 pyridine). The difference here is attributed to partial racemization during synthesis and perhaps also during purification. This compound can also be formed by catalytic hydrogenation (10% palladium-carbon in glacial acetic acid) of natural IV. Both the natural and the synthetic compound in 95% ethanol show only end absorption in the UV. On acid hydrolysis they yield equimolar amounts of leucine and phenylalanine.

Résumé. A partir de cultures de *Streptomyces noursei*, variante No. 5286, on a isolé la 3-benzyl-6-isobutyl-2,5-dioxopipérazine, en plus des quatre dioxopipérazines disubstituées dont la présence a été signalée précédemment; on n'a pas obtenu de preuve de l'existence d'un dérivé 3-benzylidène-6-isobutyl- ou 3-benzyl-6-isobutylidène-correspondant à la 3-benzyl-6-benzylidène-2,5-dioxopipérazine déjà connue.

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Isolation of O-Acetyltyrosylvaline from Pig Neurohypophysis

The isolation of peptides from neurohypophysis without using biological tests has been reported by RAMACHANDRAN[1], GROS[2] and WITTER[3]. In the present paper we report the isolation of *O*-acetyltyrosylvaline and some other peptides.

A 0.2 *M* pyridine-0.05 *M* acetic acid extract of 250 g of acetone powder of pig neurohypophysis (donated by N.V. Organon, The Netherlands) was separated on an 80 · 8 cm Sephadex G25 column in the same buffer system. A high molecular fraction (43.6 g) and a low molecular fraction (29.4 g) were obtained. In the high molecular fraction the hormones oxytocine and lysine-vasopressine were present, coupled to the Van Dyke protein. The low molecular fraction contained among others a large quantity of amino acids and a small amount of peptides. Further separation of the low molecular fraction occurred with Amberlite IRC50.

At pH 4.0 the amino acids leave the Amberlite IRC50 column (in the H^+ form) unretarded, while the peptides are absorbed[4]. We obtained a peptide fraction I (1.651 g) and an 'amino acid fraction'. Indications in the literature[5] concerning the presence of small peptides in this amino acid fraction urged us to attempt their concentration. It was possible to isolate a peptide fraction II (2.25 g) by chromatography over a DEAE-cellulose column after conversion into the copper II complexes. The effectiveness of the separation technique developed in our laboratory by TOMMEL[6] is shown in Figure 1.

The peptide fractions I and II were each further separated into fractions on a 144 · 2 cm column of Dowex 1 · 2 (200–400 mesh, acetate form) by stepwise elution with 1% pyridine-acetic acid buffers of pH 9.3, 6.5, 5.5, 4.5, and 3.5. Finally pure peptides were obtained by preparative paper chromatography in the solvent systems *n*-butanol-acetic acid-water 4:1:5 v/v (BAW) and *n*-butanol-pyridine-acetic acid-water 30:20:6:24 v/v (BPAW).

One of the purified peptides of peptide fraction II (yield 3.6 mg, Rf in BAW = 0.60, Rf in BPAW = 0.72) consisted of the amino acid residues tyrosine and valine. To determine the structure of this peptide, a mass spectrometric analysis was performed after the conversion of this peptide into the 2,4-dinitrophenyl (DNP)-peptide methylester[7,8].

The mass spectrum and the proposed structure is given in Figure 2, and the exact mass of some peaks and the corresponding empirical formulas are outlined in the Table.

The structure of the original peptide is *O*-acetyltyrosylvaline, the DNP-peptide methylester derivative of which has an empirical formula of $C_{23}H_{26}N_4O_9$. The peak 626 is due to replacement of the acetyl group by a DNP group during the reaction with 1-fluoro-2,4-dinitrobenzene. The presence of an acetyl group in the original peptide was verified with the gas-chromatographic method of WARD[9]; in contrast to the non-hydrolysed product, the hydrolysed product gave an acetic acid peak.

That the acetyl group is bound to the hydroxyl group of tyrosine was clear from a positive reaction of the pep-

m/e	Measured exact mass	Calculated exact mass	Empirical formula
626	626.1629	626.1608	$C_{27}H_{26}N_6O_{12}$
502	502.1691	502.1699	$C_{23}H_{26}N_4O_9$
485	485.1677	485.1672	$C_{23}H_{25}N_4O_8$
468	468.0830	468.0790	$C_{20}H_{14}N_5O_9$
293	293.0878	293.0886	$C_{12}H_{13}N_4O_5$
261	261.0992	261.0988	$C_{12}H_{13}N_4O_3$

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