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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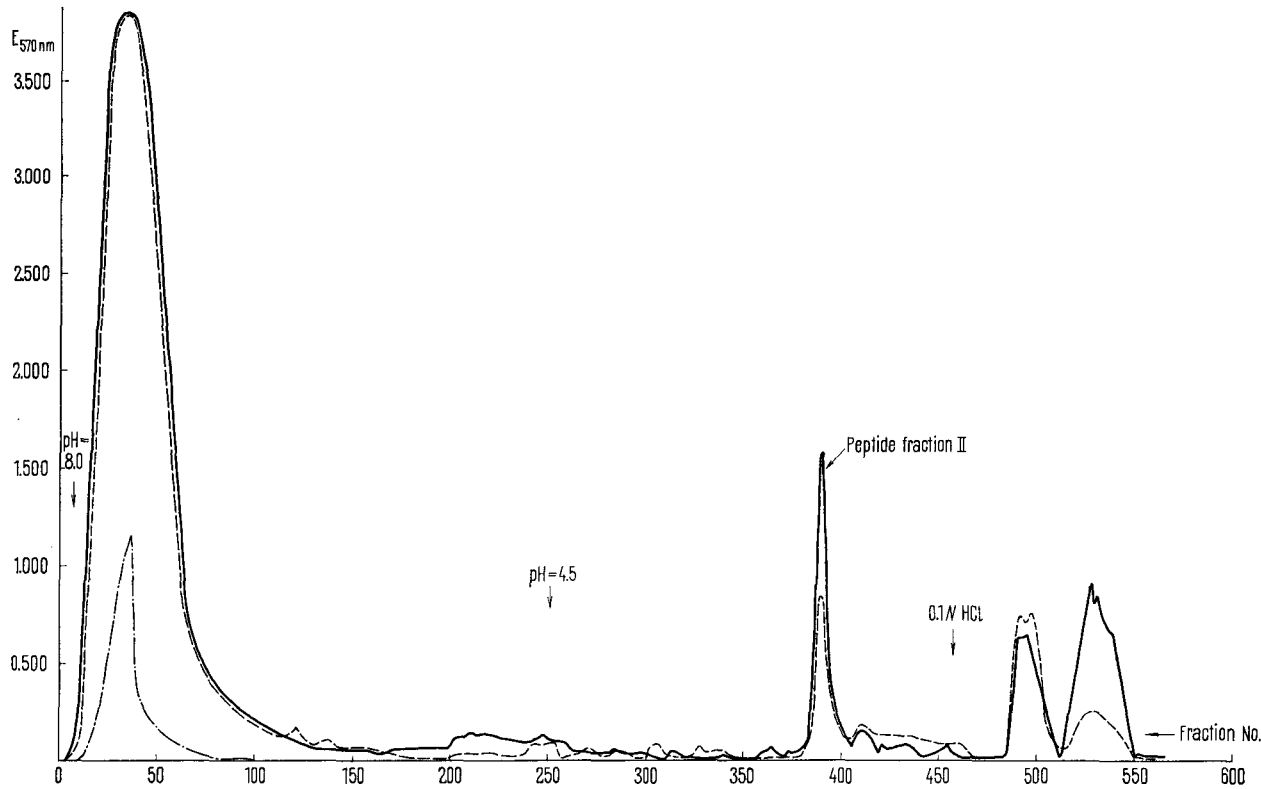

Fig. 1. Isolation of peptide fraction II from a mixture containing a large amount of amino acids. The separation occurred on a DEAE-cellulose column after conversion into Cu(II)-complexes[7]. —— Ninhydrin colour after alkaline hydrolysis (570 nm); ------- ninhydrin colour without preceding hydrolysis (570 nm); ·—·—· extinction at 620 nm.

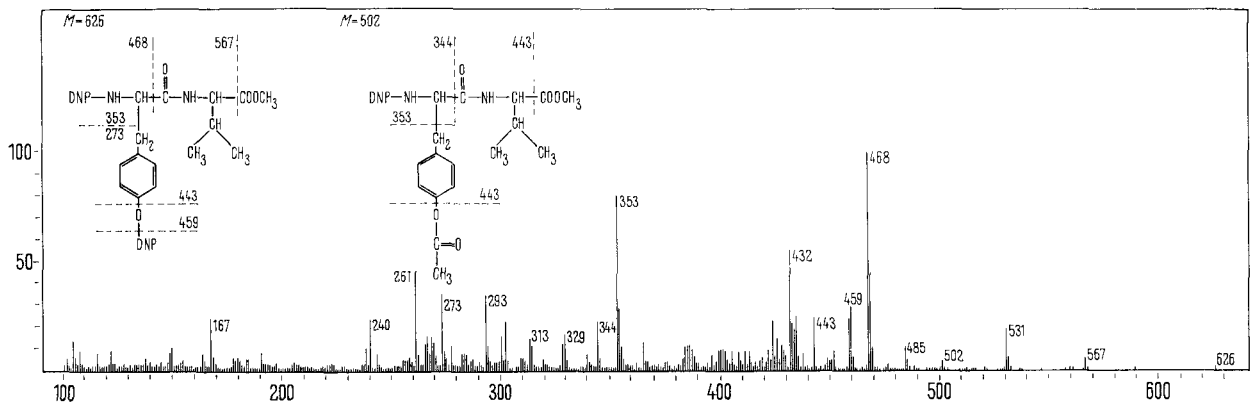

Fig. 2. A part of the mass spectrum of the DNP-peptide methylester. The peaks of the spectrum could be explained by the proposed structures.

tide with ninhydrin and the neutral electrophoretic behaviour. As far as the authors know, this is the first *O*-acetyl peptide isolated from natural sources. Using mass spectrometry we also determined the structure of some other simple peptides isolated from pig neurohypophysis; again the DNP-peptide methyl esters[7,8] were employed. The structures of ala.val.leu, val.leu and tyr.gly[7,8], as well as of arg.tyr[10] and of ala.ala.ala[10] were established.

Zusammenfassung. Die Isolierung des Peptids *O*-Acetyltyrosylvalin aus Schweins-Hypophyse wurde beschrieben und die Strukturaufklärung mittels Massenspektrometrie des DNP-Peptid-methylesters ermittelt.

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