stirred with tetrameric PNC (0.25 mmole) in chloroform at room temperature for 30 min, then freshly prepared L-alanine methyl ester (1 mmole) was added, and the mixture was allowed to stand for 24 h. Removal of the solvent, followed by standard washing procedures, gave N-benzyloxycarbonyl-L-phenylalanyl-L-alanine methyl ester: mp 130–131° (65%). Application of the nuclear magnetic resonance spectroscopy method for the determination of racemization revealed no DL dipeptide had formed in the reaction[4].

The reagent was tested on a broader scale by another synthesis of N-*t*-butoxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine, made previously in a study on the C-terminal sequence of gastrin[5]. The di-, tri-, and tetrapeptide units were formed with the aid of PNC without undue difficulty. The physical properties of the intermediates were in agreement with the literature values. Recently, the trimeric PNC has been shown to produce other tetrapeptides in the glucagon series in moderate amounts[6].

The mechanism here may involve the initial formation of an active ester intermediate, followed by a displacement. Shortly after this investigation was begun, the use of commercial PNC in the formation of various amides and hydrazides, but not peptides, was described in a short note[7,8].

Zusammenfassung. Phosphonitrilchlorid, dessen Verbindung razemisierungsfrei erfolgt, kann als Reagens zur Synthese von Peptiden verwendet werden.

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Synthesis of a Biologically Active Analog of Deamino-8-Arginine-Vasopressin which Does not Contain a Disulphide Bond[1]

The preparation of an analog of deamino-oxytocin, in which the disulphide bond is replaced by an ethylene linkage, was first published in 1967 by RUDINGER and JOŠT[2] who demonstrated that this compound possessed weak but definite oxytocin-like activity. This compound is called deamino-dicarba-oxytocin. Subsequently, we reported the synthesis of the same compound obtained by an independent route[3], and it was found that the rat uterotonic activity of this compound was almost ten-fold higher than that recorded previously[2]. By the same technique we have prepared the corresponding Lys[8]-vasopressin[4]. The preliminary biological activities of this peptide along with those of deamino-dicarba-oxytocin are given in the Table. We now report the synthesis and some preliminary biological activities of deamino-dicarba-Arg[8]-vasopressin.

[1] The tentatively proposed rules by the IUPAC-IBC were followed in the use of abbreviations: J. biol. Chem. *241*, 2491 (1966). Asu, α-aminosuberic acid; –OSu, N-hydroxysuccinimide ester; Aoc–, t-amyloxycarbonyl. The amino acids used were in the L-form.
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Biological activities of deamino-dicarba-Arg[8]-vasopressin and its related compounds

Compounds	Biological activities (IU/mg) Oxytocic (rat)	Depressor (fowl)	Pressor (rat)	Antidiuretic (rat)
Deamino-oxytocin[a]	803 ± 36	975 ± 24	1.44 ± 0.06	19
Deamino-dicarba-oxytocin[b]	96	52	< 0.25	1.78
Deamino-Lys[8]-vasopressin[c]	12 ± 0.5	61 ± 2	126 ± 2	301 ± 11
Deamino-dicarba-Lys[8]-vasopressin[d]	5.1	4.2	10.4	7.8
Deamino-Arg[8]-vasopressin[e]	27 ± 4	150 ± 4	370 ± 20	1300 ± 200
Deamino-dicarba-Arg[8]-vasopressin	11.9[f]	8[g]	23.0[f]	84.5[h]

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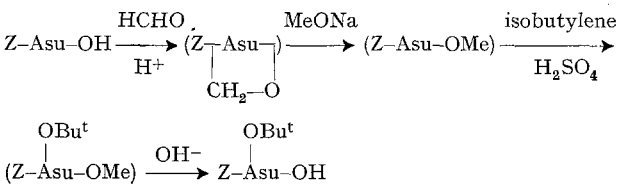
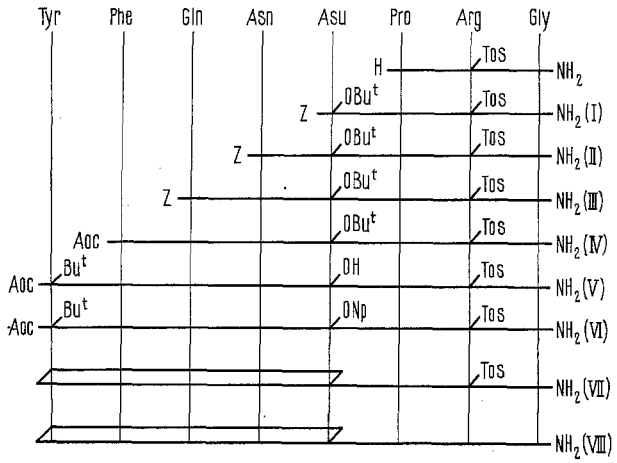
Benzyloxycarbonyl-L-α-aminosuberic acid ω-t-butyl ester (Z-Asu(OBu^t)-OH)[3,4], a key intermediate for the preparation of dicarba-analogs, was synthetized according to a procedure modified by MURAKAMI et al.[5] for the preparation of α-esters. The sodium methoxide treatment,



the second step in the reaction scheme, was carried out overnight at 10 °C in order to avoid possible racemization. Since all intermediates were obtained as oily compounds, completion of each reaction was ascertained by thin-layer chromatography, and the over-all yield of the key intermediate was satisfactory (66%). The partially protected octapeptide was synthetized by step-wise elongation of H-Pro-Arg(Tos)-Gly-NH_2[6] with the appropriate amino acid active esters[3,4] (Figure). Compound V was smoothly converted to the active ester VI by the trifluoroacetate method[7]. After removal of the remaining protecting groups from the tyrosyl residue, VI was treated with pyridine at 50 °C under high-dilution conditions (1 mmol/l), and a cyclic compound VII was obtained in about 60% yield. Removal of the tosyl group from VII was achieved by the HF-procedure[8] at 0 °C for 1 h in the presence of anisole. Remaining hydrogen fluoride in the crude product was removed by passing the aqueous solution through a short column of Amberlite IR-45 (OH^-). The HF-free product was purified on a column of Sephadex G-25 with 0.1 *N* AcOH as the solvent, and the homogeneous compound was obtained as lyophilizate in a yield of 51%. Paper chromatography showed this compound to give a single spot in 2 different solvent systems; Rf 0.36 (*n*-BuOH:AcOH:water = 4:1:1) and Rf 0.19 (*n*-BuOH:pyridine:water = 4:1:1); $[\alpha]_D^{16}$ – 69° (c 0.38, 0.1 *N* AcOH). Anal. calcd. for $C_{48}H_{67}N_{14}O_{12}\cdot CH_3COOH\cdot 2.5\,H_2O$: C 52.81; H 6.74; N 17.24. Found for a sample dried at 100 °C for 10 h in vacuo: C 52.87; H 6.71; N 17.18. Ratio of amino acids after acid hydrolysis: $Tyr_{0.99}$, $Phe_{1.08}$, $Glu_{1.08}$, $Asp_{1.00}$, $Asu_{1.06}$, $Pro_{0.96}$, $Arg_{1.00}$, $Gly_{1.01}$. Preliminary bioassay revealed that deamino-dicarba-Arg^8-vasopressin possesses approximately $^1/_{15}$ of the principal activities of natural-type deamino-Arg^8-vasopressin (see Table).



Synthesis of deamino-dicarba-Arg^8-vasopressin.

Zusammenfassung. Die Synthese und Resultate einer vorläufigen biologischen Prüfung von Desamino-dicarba-Arg^8-vasopressin, einem Neurohypophysenhormon-Analogon, in dem die Schwefelbrücke durch eine Äthylengruppe ersetzt ist, werden beschrieben.

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Inhibition of DNA Synthesis in Regenerating Rat Liver by Hydrocortisone

The inhibition of growth of lymphoid tissues by adrenal corticosteroids is well documented[1–3]. Natural corticosteroids or their analogs have also been shown to inhibit erythropoiesis[4] and the proliferation of fibroblasts[5], to induce growth failure in neonatal rats[6], to cause the abnormal development or death of embryonic tissues[7,8], and to cause the regression of tumors of lymphoid origin[9–11].

In a previous study[12] it was shown that pharmacological doses of hydrocortisone markedly inhibit RNA synthesis in the regenerating liver of hypophysectomized, but not of intact rats. The results of other studies suggest that adrenal corticosteroids inhibit mitosis in the regenerating liver of the mouse[13,14] and of the rat[15]. Furthermore, adrenocorticotrophic hormone was shown[16] to decrease the incorporation of tritiated thymidine into nuclei of the regenerating liver of the rat. In view of their importance as anticancer agents, the growth inhibiting action of the corticosteroids was studied further, using the regenerating liver system.

Male Sprague-Dawley rats (240–300 g) were maintained in plastic cages with purina chow and water ad libitum. Lighting was controlled from 06.00–18.00 h. Partial hepatectomy involved the surgical removal of approximately 70% of the liver[17]; the operations were routinely performed between 10.00–13.00 h. The rats were fasted in wire bottom cages following the operation. The labelled precursors, (^{3}H-methyl) thymidine or 6-^{14}C orotic acid, were injected i.p. 26 h after partial hepatectomy, when the rate of DNA synthesis is approximately