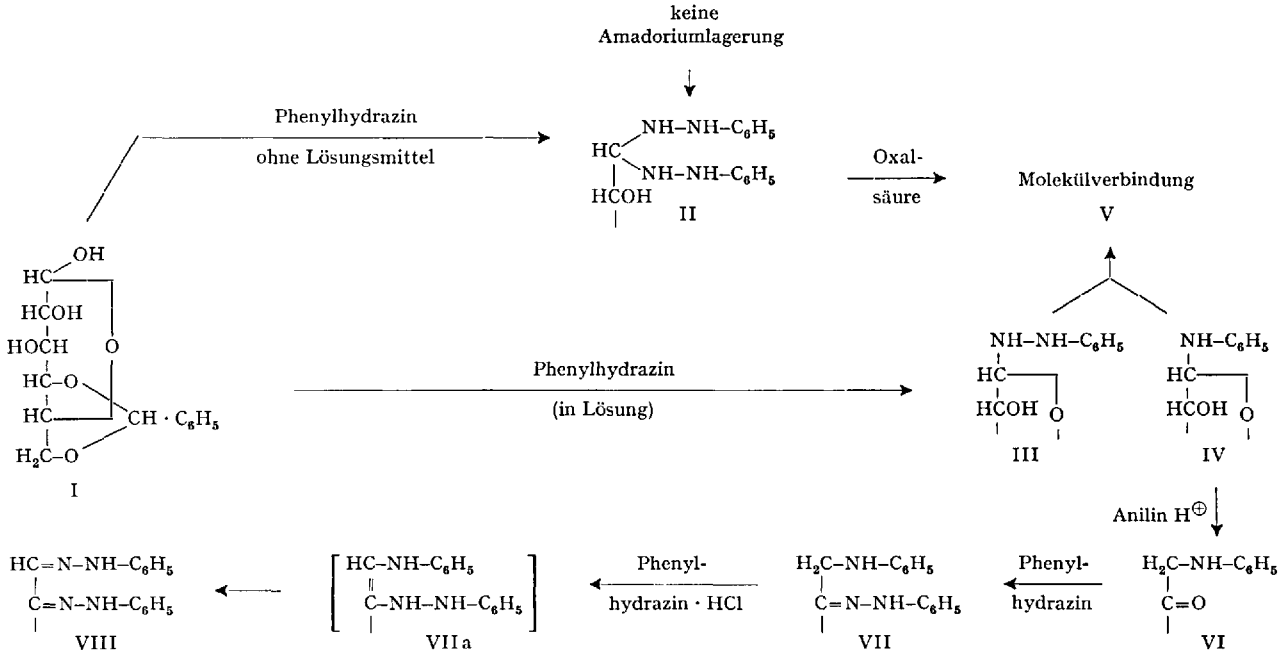

Übergang der Bis-hydrazino-verbindung (II) zum Amadoriprodukt nicht erfolgt, dürfte auf die Bildung einer stabilen Wasserstoffbrücke zwischen N- und H-Atomen der beiden Phenylhydrazinreste zurückzuführen sein. Statt dessen bildet sich in schneller Reaktion neben 4,6-Benzal-D-glucose-phenylhydrazon (III) durch teilweisen Zerfall von Phenylhydrazin[5–7] (in Anilin, Ammoniak, Benzol und Stickstoff) 1-Phenylamino-4,6-benzal-D-glucosid (IV), das mit III eine Molekülverbindung (V) bildet. Die Struktur von V wurde durch die Darstellung aus den Komponenten (III und IV) bewiesen. Nach Acetylierung von V kann das N-D-Glucosid (IV) als 1-[Acetyl-phenylamino]-2,3-diacetyl-4,6-benzal-D-glucosid abgetrennt werden. 4,6-Benzal-D-glucose (I) und Anilin oder das N-D-Glucosid (IV) gehen unter geeigneten Bedingungen (H^+-Ionen-Katalyse) ausserordentlich schnell in 1-Desoxy-1-phenylamino-4,6-benzal-D-fructose (VI)[8] über. Die Umlagerung, die offenbar über das 1,1-Bis-phenylamino-derivat verläuft[4], wird bei VI durch Zugabe geringer Mengen von Anilin sehr stark beschleunigt. Die 1,1-Bis-arylamino-derivate der 4,6-Benzal-D-glucose konnten bisher nicht in reiner Form gewonnen werden. Sie sind stets mit N-D-Glucosid und schnell sich bildendem Amadoriprodukt verunreinigt. Die Amadoriverbindung (VI) reagiert mit Phenylhydrazin schnell und fast quantitativ zu 1-Desoxy-1-phenylamino-4,6-benzal-D-fructose-phenylhydrazon (VII), das unter sehr milden Bedingungen mit 2 Moll. Phenylhydrazin-hydrochlorid in hoher Ausbeute das Phenylosazon (VIII) liefert.

Es ist nicht erforderlich, dass zur Bildung des N-D-Glucosids (IV) noch freie Glucose in der Reaktionslösung vorliegt. Das Phenylhydrazon (III) tauscht den Phenylhydrazinrest sehr leicht gegen Amin aus.

Die Osazonreaktion verläuft demnach über die Amadoriumlagerung einer 1-Desoxy-1-phenylamino-verbindung (VI), die sich primär aus dem Phenylhydrazon mit Anilin durch Umaminierung bildet. Letzteres verdankt seine Entstehung dem disproportionierenden Zerfall von überschüssigem Phenylhydrazin[5–7]. Die Dehydrierung des Phenylhydrazons (VII) verläuft wahrscheinlich über die stark reduzierende En-diamin-form[9] (VIIa). Hierauf wurde bereits in einer früheren Mitteilung hingewiesen. Wasserstoffakzeptor ist vermutlich weiteres Phenylhydrazin.

Summary. For the osazone reaction of the reducing sugars, the following mechanism is suggested: the initially formed phenylhydrazone is converted with aniline into the N-glycoside. Aniline arises from disproportional decomposition of phenylhydrazine. The N-glycoside undergoes an Amadori-rearrangement to 1-deoxy-1-amino-2-ketose derivative. From this is formed the phenylhydrazone. The latter is converted by dehydrogenation and transamination into the phenylosazone.

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Synthetic Peptides Related to Eledoisin (Part I)

After the structure of Eledoisin, a powerful vasodilating and hypertensive peptide isolated from the salivary glands of a mollusc[1], had been elucidated[1] and confirmed by synthesis[2], we have prepared a large number of analogues of this substance in order to investigate the influence of structural modifications on its biological properties. Those few analogues or partial sequences which have been found to possess high biological activity will be the subject of a later report. In the present communication, we are listing in a Table those analogues and partial

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Nr.	Chemical formula	Activity on the guinea-pig ileum	$[\alpha]_D^{22}$ in 95% acetic acid (c = 1)	Mp.	Electrophoretic mobility in 80% formic acid		Elemental formula		Elemental analysis C	H	O	N	S
1	H–Glu(OH)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-NH_2 Eledoisin	100	−44°	230° dec.	0.48	Try	$C_{51}H_{85}O_{15}N_{13}S$ + H_2O	C F	53.8 53.2	7.2 7.9	21.2 21.4	15.0 14.6	2.7 2.8
2	H–Glu(OH)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——NH_2	<1	−59°	210° dec.	0.53	Try	$C_{49}H_{76}O_{14}N_{12}$	C F	55.7 55.2	7.3 7.7	21.2 21.7	15.9 15.4	– –
3	H–Glu(OH)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——*OH*	<1	−65°	~250° dec.	0,60	Try	$C_{49}H_{75}O_{15}N_{11}$	C F	55.6 55.2	7.2 7.9	22.7 22.4	14.6 14.6	– –
4	H–Glu(*NH_2*)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——NH_2	<1	−59°	~210° dec.	0.54	Try	$C_{49}H_{77}O_{13}N_{13}$ + CF_3COOH	C F	52.3 51.1	6.7 7.1	– –	15.6 15.7	– –
5	H–Glu(*NH_2*)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——*OH*	<1	−59°	~240° dec.	0.48	Try	$C_{49}H_{76}O_{14}N_{12}$ + $2H_2O$	C F	53.8 53.9	7.3 7.3	23.4 23.4	15.4 15.3	– –
6	H–Glu(*NH_2*)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——NH_2	<1	−50°	~200° dec.	0.86	Try	$C_{49}H_{79}O_{14}N_{13}$ + $6H_2O$	C F	49.8 50.2	7.7 7.5	27.1 26.9	15.4 15.1	– –
7	H–Glu(*NH_2*)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——*OH*	<1	−55°	~190° dec.	0.85	Try	$C_{49}H_{78}O_{15}N_{12}$	C F	54.8 54.4	7.3 7.5	22.4 22.4	15.6 15.5	– –
8	*Bz*-Glu(OH)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——NH_2	<1	−50°	~185° dec.	0.55	Try	$C_{56}H_{82}O_{14}N_{12}$ + H_2O	C F	57.7 57.5	7.1 7.5	20.6 20.7	14.5 14.3	– –
9	*Bz*-Glu(OH)-Pro-Ser-Lys-Asp(OH)-Ala-Phe-Ile-Gly-Leu——*OH*	<1	−58°	~240° dec.	0.55	Try	$C_{56}H_{81}O_{15}N_{11}$ + H_2O	C F	57.6 57.2	7.2 7.6	22.0 22.1	13.2 13.1	– –
10	H——Asp(OH)-Ala-Phe-Ile-Gly-Leu——NH_2	<1	−30°	~250° dec.	0.65	Try	$C_{30}H_{47}O_8N_7$	C F	56.9 56.9	7.5 8.2	20.2 19.6	15.4 15.2	– –
11	H——Asp(OH)-Ala-Phe-Ile-Gly-Leu——*OH*	<1	−29°	~250° dec.	0.65	Try	$C_{30}H_{46}O_9N_6$	C F	56.8 56.8	7.3 7.5	22.7 22.3	13.3 13.3	– –
12	H——Asp(*NH_2*)-Ala-Phe-Ile-Gly-Leu——NH_2	<1	−30°	260° dec.	0.68	Try	$C_{30}H_{48}O_7N_8$	C F	56.9 56.8	7.7 7.8	17.7 17.3	17.7 18.0	– –
13	H——Asp(*NH_2*)-Ala-Phe-Ile-Gly-Leu——*OH*	<1	−28°	240° dec.	0.68	Try	$C_{30}H_{47}O_8N_7$	C F	56.8 56.5	7.5 7.9	20.2 20.3	15.5 15.4	– –
14	H——Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-*Gly*-NH_2	<1	−27°	~250° dec.	0.63	Try	$C_{37}H_{59}O_{10}N_9S$ + CF_3COOH	C F	50.0 49.9	6.5 6.5	– –	13.5 14.3	3.4 3.4
15	H——Asp(OH)-Ala-Phe-Ile-Gly-Leu-Met-*OH*	<1	−30°	~250° dec.	0.61	Try	$C_{35}H_{55}O_{10}N_7S$ + CF_3COOH	C F	50.6 50.6	6.4 6.8	– –	11.2 11.3	3.6 3.6
16	H——Asp(OH)-Ala-Phe-Ile-Gly-Leu-*Met*(O)-NH_2	~1	−22°	~240° dec.	0.62	Try	$C_{35}H_{56}O_{10}N_9S$ + CF_3COOH	C F	49.7 48.1	6.4 6.4	– –	12.6 13.0	3.6 3.7
17	H——Asp(OH)-Ala-Phe-Ile-Gly-Leu-*But*-NH_2	<1	−33°	~260° dec.	0,61	Try	$C_{34}H_{54}O_9N_8$ + CF_3COOH	C F	51.9 51.7	6.7 6.9	– –	13.5 14.3	– –

Nr.	Chemical formula	Activity on the guinea-pig ileum	$[\alpha]_D^{22}$ in 95% acetic acid (c = 1)	Mp.	Electrophoretic mobility in 80% formic acid		Elemental formula		Elemental analysis C	H	O	N	S
18	H—(Asp(OH))-Ala-Phe-Ile-Gly-Leu-*Nva*-H_2N	<1	−32°	~260° dec.	0.60	Try	$C_{35}H_{56}O_9N_8$	C	52.5	6.8	–	13.2	–
							$+CF_3COOH$	F	52.0	7.1	–	13.5	–
19	H—(Asp(OH))-Ala-Phe-Ile-Gly-Leu-*Nle*-NH_2	~3	−34°	~260° dec.	0.57	Try	$C_{36}H_{58}O_9N_8$	C	52.9	6.9	–	13.0	–
							$+CF_3COOH$	F	53.2	7.3	–	13.6	–
20	H—Ala-Phe-Ile-Gly-Leu-Met-NH_2	~5	−20°	225° dec.	0.58	Try	$C_{31}H_{51}O_6N_7S$	C	57.3	7.9	–	15.1	–
								F	57.0	8.1	–	14.8	–
21	H—Ala-Phe-Ile-Gly-Leu-Met-*Met*-NH_2	<1	−39°	~300° dec.	0.58	Try	$C_{36}H_{60}O_7N_8S_2$	C	51.7	7.6	–	13.4	7.7
							$+HCl+H_2O$	F	51.7	7.8	–	13.2	7.6
22	H—Ala-Phe-Ile-Gly-Leu-*Leu*-NH_2	<1	−37°	~230° dec.	0.54	Try	$C_{32}H_{53}N_7O_6$	C	52.6	7.6	–	13.4	–
							$+HBr+H_2O$	F	52.2	7.9	–	13.1	–
23	H—Ala-Phe-Ile-Gly-Leu-*Val*-NH_2	<1	−25°	~310° dec.	0.62	Try	$C_{31}H_{51}O_6N_7$	C	55.4	8.1	–	14.6	–
							$+HCl+H_2O$	F	55.4	8.2	–	14.9	–
24	H—Ala-Phe-Ile-Gly-Leu-D-*Val*-NH_2	<1	−18°	~290° dec.	0.63	Try	$C_{31}H_{51}O_6N_7$	C	53.9	8.2	–	14.2	–
							$+HCl+2H_2O$	F	53.8	8.3	–	13.8	–
25	H—Ala-Phe-Ile-Gly-*Met*-Met-NH_2	<1	−20°	~300° dec.	0.61	Try	$C_{30}H_{49}O_6N_7S_2$	C	49.9	7.3	–	13.6	–
							$+HCl+H_2O$	F	50.0	7.4	–	13.8	–
26	H—Ala-Phe-*Pro*-Gly-*Ile*-Met-NH_2	<1	−45°	166° dec.	0.58	Try	$C_{30}H_{47}O_6N_7S$	C	53.8	7.2	–	14.6	–
							$+HCl$	F	53.5	7.6	–	14.4	–
27	H—Ala-Phe-*Pro*-Gly-Leu-Met-NH_2	<1	−44°	158° dec.	0.58	Try	$C_{30}H_{47}O_6N_7S$	C	53.8	7.2	–	14.6	–
							$+HCl$	F	54.0	7.5	–	14.3	–
28	H—Ala-*Gly*-Ile-Gly-Leu-Met-NH_2	<1	−14°	199° dec.	0,63	Try	$C_{34}H_{45}O_6N_7S$	C	46.3	6.9	–	14.6	–
							$+CF_3COOH$	F	46.1	7.2	–	14.1	–
29	H—Phe-Ile-Gly-Leu-Met-NH_2	~2	−14°	170° dec.	0.64	Try	$C_{28}H_{46}O_5N_6S$	C	56.5	7.9	–	13.0	–
							$+CH_3COOH$	F	55.6	7.5	–	12.9	–
30	H—Pro-Ser-Lys—Ile-Gly-Leu-Met-NH_2	<1	−44°	~150° dec.	1.00	Try	$C_{33}H_{61}O_8N_9S$	C	45.2	6.5	–	–	3.3
							$+2CF_3COOH$	F	43.9	6.5	–	–	3.3
31	H—Ser-Lys—Ile-Gly-Leu-Met-NH_2	<1	−35°	~160° dec.	1.03	Try	$C_{23}H_{54}O_7N_8S$	C	43.9	6.5	–	–	3.7
							$+2CF_3COOH$	F	42.7	6.2	–	–	4.0
32	H-Glu-Pro-Ser-Lys-(Asp(OH))-Ala-Phe-Ile-Gly—OH	<1	−66°	~190° dec.	0.45	Try	$C_{43}H_{64}O_{14}N_{10}$	C	50.2	6.3	–	13.0	–
							$+CF_3COOH$	F	50.0	6.8	–	13.2	–
33	H-Glu-Pro-Ser-Lys-(Asp(OH))-Ala-Phe-Ile—*OH*	<1	−61°	~140° dec.	0.47	Try	$C_{41}H_{61}O_{13}N_9$	C	51.5	6.2	–	12.5	–
							$+CF_3COOH$	F	51.2	6.0	–	12.6	–

Abbreviations: -Glu- = -pyroglutamyl-, -But- = -α-amino-butyryl-, -Nva- = -norvalyl-, -Nle = -norleucyl-.

sequences, which have been found to be devoid or almost devoid of biological activity. In addition to the physical properties and elemental analyses of these peptides, the Table also indicates the level of activity on the guinea-pig ileum compared with that of Eledoisin itself taken as 100. The methods used for the synthesis of these peptides will be described elsewhere in detail[3].

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Zusammenfassung. Die Eigenschaften einer ersten Serie von synthetischen Peptiden, die mit Eledoisin strukturell verwandt sind, wurden beschrieben.

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Synthesis of N-Acetylneuraminic Acid

N-acetylneuraminic acid (NANA) occurs in a wide variety of biological materials, e.g. mucins, colostrum, erythrocytes, gangliosides, gonadotropins, enzymes, bacterial cell walls, etc.[1]. Its biochemical role, as yet largely unknown, is likely to be associated with the electrical charge imparted to the structure to which it is bound. The ever increasing interest in this ubiquitous substance prompted us to evaluate the available synthetic methods for large scale preparation. The synthesis of NANA from N-acetyl-D-glucosamine and oxaloacetic acid at pH 11 in 1–2% yield has been recorded by CORNFORTH et al.[2,3]. After the finding[4–6] that NANA is structurally related to N-acetyl-D-mannosamine and not to N-acetyl-D-glucosamine, BRUG et al.[7] and later CARROLL and CORNFORTH[8] sought to improve this synthesis by employing N-acetyl-D-mannosamine in the condensation reaction. The latter authors thus claimed an 8–10% yield of NANA. Since N-acetyl-D-mannosamine epimerizes rapidly in alkaline medium to an equilibrium with N-acetyl-D-glucosamine the potential yield of NANA in the above condensation obviously depends on the relative rates of the two reactions: (a) epimerization of N-acetyl-D-mannosamine and (b) condensation with oxaloacetic acid. We have followed the rate of the latter reaction spectrophotometrically at 565 mμ[9] by the development of Ehrlich-positive material[1]. Our reaction conditions were somewhat different from those reported by CARROLL and CORNFORTH[8].

To 10 ml of water were added alternately in small portions 5.4 g of oxaloacetic acid and 10 *N* sodium hydroxide in such a manner as to keep the pH between 5 and 10. The temperature was maintained at 10–12°C by external cooling. When all oxaloacetic acid had been added 10 g of N-acetyl-D-mannosamine was added to the mixture, the pH adjusted to 11 and the temperature raised to 25°C, the reaction mixture was stirred until all N-acetyl-D-mannosamine had dissolved and the pH was adjusted to 11 frequently by the addition of small amounts of sodium hydroxide. Spectrophotometric determination of Ehrlich-positive material in samples withdrawn at 15 min intervals indicated that the condensation was essentially complete in approximately 1 h. After dilution with water the reaction mixture was applied directly to 45 ml of Dowex-2 (acetate) in a 1.8 cm dia. column. The column was washed thoroughly with water until the effluent gave a negative direct and indirect Ehrlich test[1]. The products were then eluted with a gradient of pH 6.0 ammonium acetate buffer (0.1–1.5 *M*). The eluate fractions were tested with Ehrlich's reagent. Two distinct peaks were obtained as indicated in the elution diagram (A, Figure). The pooled fractions under each peak were passed through separats Dowex 50 (H-form) columns. The individual effluente were evaporated to a small volume under reduced pressure and then lyophilized to give hygroscopic powders. Paper chromatograms (Schleicher & Schuell ≠589) of the first fraction developed in an *n*-butanol-pyridine-water system (6:4:3) by the descending technique and sprayed with orcinol reagent indicated the presence of NANA-isomer with glucosamine configuration (R_{NANA}: 1.5) along with a slightly smaller amount of NANA (R_{NANA}: 1.0). The second peak consisted of a main component with an *R* value identical with that of NANA, and a trace of another with the *R* value of NANA isomer. However, all attempts to crystallize this material failed. The ion-exchange separation was further refined by stepwise elution with 0.2 and 0.65 *M* pH 6.0 ammonium acetate buffer (A, Figure). Nonetheless almost equal amounts of the two NANA isomers were still found to be present in the first peak, and the material from the second peak still contained the two components noted above. These contradictory observations can be reconciled by the assumption that the material eluted in the second peak was not NANA, but a mixture of the intermediate dicarboxylic acids (I) formed in the initial step of the condensation of oxaloacetic acid and N-acetyl-D-mannosamine.

This presumed mixture of isomers would then have to possess *R* values on paper chromatograms identical with that of NANA in order to conform to the observed results. The presence of a trace of NANA-isomer shown on paper chromatograms could readily be explained by the occurrence of some decarboxylation in the work-up of this mixture. The two monocarboxylic acids, NANA and its isomer with glucosamine configuration (II) would accordingly be expected to emerge in the first peak, a feature which is

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