The combined fractions (0.87 g) were dissolved in 10% aqueous hydrochloric acid, and the solution was diluted with water to a total volume of 100 ml and was extracted with chloroform (4 × 75 ml).

The acidic chloroform extract was washed with 2% aqueous sodium carbonate and then with water, and, after it had been dried over anhydrous sodium sulfate, the solvent was distilled off. The residue (0.43 g) was chromatographed on alumina (1:25) with elution by chloroform. This gave 0.21 g of taurenine, mp 100-102°C [petroleum ether–chloroform (1:2)], M^+ 495(0.9%), 480(1.6), 478(4.6), 435(43), 420(100), 418(21.4), 404(18), 390(53.5), 60(8.0).

Amino Alcohol of Taurenine (II). Taurenine (0.05 g) was heated in 10 ml of a 2% aqueous methanolic solution of KOH on the water bath for one hour. After the solvent had been distilled off, the residue was dissolved in water and the solution was extracted with ether (3 × 50 ml). An amino alcohol with mp 205-207°C (0.04 g) was obtained. IR: 3545, 3470, 3320 cm^{-1} (OH). M^+ 453(18.5), 438(32.5), 436(100), 420(9), 397(6.0), 366(12.0%).

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SYNTHESIS OF A NUMBER OF COMBINED ANALOGUES OF SUBSTANCE P AND LITORIN

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With the aim of obtaining new biologically active compounds, we have synthesized nine combined peptides (I)-(IX) consisting of combinations of the C-terminal tripeptide litorin and the hydrophobic central fragments of substance P, and also modified analogues of them. The synthesis of these compounds was achieved by the methods of classical peptide chemistry with the condensation of their N-terminal moieties with the C-terminal tripeptide H-His-Phe-Met-NH_2.

At the present time, when the localization of neuropeptides in the organism has been basically established, the problem remains of elucidating their role in the functioning of the nervous system. An important place among such peptides is occupied by peptides of the tachykinin series – in particular, substance P (SP) and bombesin-like peptides. They possess a broad spectrum of biological action both on the central nervous system and on other vitally important functions of the organism, frequently exerting an effect on identical processes. Attention is attracted by the remarkable coincidence of the distribution in the brain of the bombesin-like and SP-like immunoreactivities [1]. It is known that some syn-

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thetic modified analogues of substance P are antagonists of bombesin-like peptides in their influence on the outflow of amylase from isolated acini of the pancreas [2] and some other digestive processes [3]. It has been shown by the method of theoretical conformational analysis in the pairwise additive approximation that a complex conformation of the peptide backbone in stable structures is characteristic for the central hydrophobic fragments of SP and of the bombesin-like nonapeptide litorin [4, 5].

Starting from what has been said above, with the aim of obtaining new biologically active compounds we have undertaken the synthesis of a number of combined peptides consisting of combinations of the C-terminal tripeptide of litorin and the hydrophobic central fragments of SP, and also modified analogues of them:

H-Phe-Phe-Gly-His-Phe-Met-NH_2 (I),
H-Gln-Phe-Phe-Gly-His-Phe-Met-NH_2 (II)
H-Gln-Gln-Phe-Phe-Gly-His-Phe-Met-NH_2 (III),
H-Trp-Phe-Phe-Gly-His-Phe-Met-NH_2 (IV),
H-Gln-Trp-Phe-Phe-Gly-His-Phe-Met-NH_2 (V),
H-Gln-Phg-Phe-Gly-His-Phe-Met-NH_2 (VI),
H-Gln-Phe-Phg-Gly-His-Phe-Met-NH_2 (VII),
H-Gln-Phg-Phg-Gly-His-Phe-Met-NH_2 (VIII),
H-Phg-Phe-Phe-Gly-His-Phe-Met-NH_2 (IX).

These peptides were synthesized by condensing their N-terminal moieties with H-His-Phe-Met-NH_2 (X). We used the tert-butoxycarbonyl (Boc) group to protect the α-amino groups of the amino acids. It was eliminated with solutions of HCl in AcOEt or with glacial AcOH or, for peptides containing glutamine residues, with a 20% solution of CF_3COOH in CH_2Cl_2 at a lowered temperature. The carboxy group of the glycine residue was protected by the formation of the p-nitrobenzyl (Nb) ester. This permitted the ready identification of the reaction products by the TLC method with the aid of UV irradiation. The Nb group was easily eliminated by catalytic hydrogenolysis over palladium black with a high yield.

The synthesis of the C-terminal tripeptide of litorin (X) has been described in detail in [6]. The peptide Boc-Phe-Phe-Gly-ONb was obtained by the successive growth of the chain from the C-end with the aid of N,N'-dicyclohexylcarbodiimide (DCC) in DMFA. The addition of phenylglycine residues was effected by the same method. Tryptophan and glutamine residues were added, respectively, by the pentafluorophenyl and p-nitrophenyl ester methods. The intermediate peptides formed that contained up to four amino acid residues were purified by reprecipitation from various solvents. The glutamine-containing pentapeptides required purification by column chromatography on silica gel L 40/100.

The condensation of the N-terminal fragments with peptide (X) was carried out with the aid of DCC in the presence of N-hydroxybenzotriazole (HOBT) in DMFA. The peptides formed were purified by chromatography on silica gel L 40/100. After elimination of the Boc group, the peptides (I)-(IX) formed were purified by gel filtration on Sephadex LH-20.

An investigation has been made of the action of the peptides synthesized (I)-(IX) on the contraction of rat and guinea-pig smooth musculature and on the inhibition of the binding of radioactive [^{125}I]-labeled substance P with a tachykinin receptor of the NK-1 type, and also on thermoregulation in the rat.

EXPERIMENTAL

In synthesis we used amino acids of the L- series and their derivatives from Reanal (Hungary), Fluka (Switzerland), the Biokhimreaktiv Scientific Production Combine, and the Voikov Chemical Factory. Melting points were determined in open capillaries and are given without correction. Solutions were evaporated in a rotary vacuum evaporator at a residual pressure of 10-15 mm Hg and a temperature not exceeding 40°C. DMFA was evaporated at 0.5-1 mm Hg. The homogeneity of the compounds obtained was checked by the TLC method on Silufol and Silufol UV_{254} plates (Kavalier, Czechoslovakia) and silica gel 60 F_{254} plates (Merck, FRG) using the following solvent systems: $CHCl_3$–MeOH (9:1) (A); $CHCl_3$–MeOH (7:1) (B); $CHCl_3$–MeOH (4:1) (C); n-BuOH–AcOH–H_2O (4:1:5; upper phase) (D); $CHCl_3$–MeOH–25% NH_4OH (60:45:20) (E); $CHCl_3$–EtOH–AcOEt–AcOH–H_2O (85:5:8:2:0.25) (F); and $CHCl_3$–EtOH–n-BuOH–AcOEt–H_2O (10:6:4:3:1) (G). The substances were detected on the chromatograms with the aid of chlorine/benzidine and ninhydrin, tryptophan-containing peptides being identified with the aid of the Pauly reagent. The specific rotaations of the compounds were measured on a J-20

TABLE 1. Physicochemical Characteristics of Compounds (XXIV)-(XXXI)

Compound	Yield %	mp, °C	$[\alpha]_D^{20}$, deg	R_f	
XXIV	85	201–204	−23,2 (*c* 1; DMFA)	0,40 (A)	0,13 (F)
XXV	85	241–243	−2',0 (*c* 0,5; DMFA)	),58 (C)	0.49 (D)
XXVI	95	190–192	−32,0 (*c* 1; DMFA)	0 55 (A)	0,46 (D)
XXVII	85	129–132	−23,4 (*c* 0,67; DMFA)	0,54 (A)	0,44 (F)
XXVIII	91	183–184	+9:0 (*c* 1; DMFA)	0,36 (A)	0,52 (D)
XXIX	88	192–193	+21;0 (*c* 1; DMFA)	0,43 (C)	0,48 (C)
XXX	92	209–210	+37,2 (*c* 1; DMFA)	0,53 ( )	0,55 (D)
XXXI	94	174–176	+4,6 (*c* 1; DMFA)	0,62 (C)	0,44 (F)

TABLE 2. Physicochemical Characteristics of Compounds (XXXIII)-(XL)

Compound	Yield %	mp, °C	$[\alpha]_D^{20}$, deg	R_f	
				C	F
XXXIII	61	182–186	−35,5 (*c* 0,5; DMFA)	0,46	0,25
XXXIV	55	173–175	−16,0 (*c* 0,5; DMFA)	0,48	0,34
XXXV	59	174–177	−26,3 (*c* 1; DMFA)	0,51	0,21
XXXVI	48	163–166	−7,81 (*c* 1; DMFA)	0,49	0,2)
XXXVII	54	Oil	−9,8 (*c* 1; DMFA)	0,57	0,42
XXXVIII	58	Oil	−7,8 (*c* 1; DMFA)	0,54	0,41
XXXIX	53	154–155	+14,8 (*c* 1; DMFA)	0,49	0,34
XL	66	179–183	−17,8 (*c* 0,5; DMFA)	0,60	0,44

spectrometer (Jasco, Japan). Acid hydrolysis was conducted under standard conditions (6 N HCl, 110°C, 24 h). The amino acids in the hydrolysates were determined quantitatively with the aid of an LKB (Sweden) automatic amino acid analyzer.

Boc-Phe-Phe-Gly-ONb (XI). With stirring, 2.02 g (9.78 mmoles) of DCC and, after 10 min, a solution of 3.50 g (8.89 mmoles) of H-Phe-Gly-ONb·HCl and 1.24 ml (8.89 mmoles) of Et_3N in 15 ml of DMFA were added to a solution of 2.36 g (8.89 mmoles) of Boc-Phe-OH in 15 ml of DMFA cooled to 5°C. The reaction mixture was stirred at 2-5°C for 1.5 h and 20°C for 3 h and the precipitate of Et_3N·HCl and the partial precipitate of N,N'-dicyclohexylurea were filtered off. The filtrate was evaporated, the residue was dissolved with 10% $NaHCO_3$ solution (3 × 30 ml), H_2O (2 × 30 ml), 10% $KHSO_4$ solution (2 × 30 ml), and again H_2O (2 × 30 ml). The residue formed after the over-anhydrous $MgSO_4$ and evaporation of the AcOEt was triturated under hexane and was purified by reprecipitation from AcOEt with hexane. The yield of peptide (XI) was 3.71 g (69%), mp 93-95°C, $[\alpha]_D^{20}$ −10.7° (c 1; DMFA), R_f 0.67 (A); 0.63 (D); 0.65 (E).

The following peptides were obtained in a similar way to (XI): Boc-Phg-Phe-Gly-ONb (XII), mp 144-145°, $[\alpha]_D^{20}$ +10.3° (c 1; DMFA), R_f 0.79 (A); 0.47 (E); Boc-Phe-Phg-Gly-ONb (XIII), mp 176-177°, $[\alpha]_D^{20}$ +16.1° (c 1; DMFA), R_f 0.75 (C); 0.43 (F); Boc-Phg-Phg-Gly-ONb (XIV), mp 174-175°, $[\alpha]_D^{20}$ +56.1° (c 1; DMFA), R_f 0.75 (A); 0.47 (F); Boc-Phg-Phe-Phe-Gly-ONb (XV), mp 178-179°, $[\alpha]_D^{20}$ −18.1° (c 0.5; DMFA), R_f 0.75 (C); 0.60 (F).

Boc-Trp-Phe-Phe-Gly-ONb (XVI). A solution of 0.52 g (0.96 mmole) of H-Phe-Phe-Gly-ONb·HCl in 20 ml of DMFA was treated with 0.15 ml (1.11 mmoles) of Et_3N, and the reaction mixture was stirred at 20°C for 30 min. Then it was cooled to 0°C, and 0.56 g (1.21 mmoles) of Boc-Trp-OPfp was added, and stirring was continued at 0°C for 1 h and at 20°C for 15 h. The precipitate of Et_3N·HCl was filtered off, the filtrate was evaporated to 5 ml, and 40 ml of ether was added. The resulting precipitate was filtered off, washed with ether, and reprecipitated with ether from MeOH. This gave 0.62 g (82%) of peptide (XVI), mp 156-158°C, $[\alpha]_D^{20}$ −33.0° (c 1; $CHCl_3$), R_f 0.67 (B); 0.75 (C).

Boc-Gln-Phe-Phe-Gly-ONb (XVII). A solution of 2.03 g (5.53 mmoles) of Boc-Gln-Onp and 2.99 g (5.53 mmoles) of H-Phe-Phe-Gly-ONb·HCl in 25 ml of DMFA was treated with 0.77 ml (5.53 mmoles) of Et_3N and two drops of AcOH, and the reaction mixture was stirred at 20°C for 20 h. The solvent was evaporated off, and the residue was stirred with 80 ml of a mixture of AcOEt and H_2O (1:4). The resulting precipitate was filtered off and was washed on

TABLE 3. Physicochemical Characteristics of Compounds (I)-(IX)

Compound	Yield in the deblocking stage, %	mp, °C	$[\alpha]_D^{20}$, deg	R_f	
				C	D
I	95	131—133	−4,8 (c 1; DMFA)	0,21	0,32
II	92	143—146	−7,2 (c 0,5; MeOH)	0,16	0,28
III	85	Oil	−3,6 (c 1; DMFA)	0,08	0,26
IV	93	157—159	−6,4 (c 0,5; DMFA)	0,23	0,35
V	92	209—212	−1,4 (c 0,5; DMFA)	0,18	0,27
VI	86	99—102	+6,9 (c 0,40; MeOH)	0,09	0,28
VII	87	116—118	+7,4 (c 2,05; DMFA)	0,12	0,39
VIII	87	110—112	+28,0 (c 1; MeOH)	0,10	0,32
IX	96	171—173	+1,6 (c 0,05; MeOH)	0,13	0,30

the filter with 10% $KHSO_4$ solution, H_2O, 10% $NaHCO_3$ solution (10 × 10 ml), and H_2O again to neutrality and was dried. The residue was washed on the filter with AcOEt, with AcOEt–ether (1:1), and with ether, and, after drying, 3.85 g (95%) of peptide (XVII) was obtained, with mp 190-192°C, $[\alpha]_D^{20}$ −16.0° (c 1; DMFA), R_f 0.91 (D); 0.52 (E); 0.22 (F).

After the deblocking of the peptides (XVII), (XVI), (XII), (XIII), and (XIV), the following peptides, respectively, were obtained by analogy with the synthesis of (XVII): Boc-Gln-Gln-Phe-Phe-Gly-ONb (XVIII), yield 93%, mp 217-220°, $[\alpha]_D^{20}$ −26.8° (c 1; DMFA), R_f 0.79 (C); 0.83 (D); 0.21 (F); Boc-Gln-Trp-Phe-Phe-Gly-ONb (XIX), yield 81%, mp 149-152°, $[\alpha]_D^{20}$ −23.0° (c 1; DMFA), R_f 0.62 (C); 0.89 (D); 0.52 (E); Boc-Gln-Phg-Phe-Gly-ONb (XX), yield 92%, mp 231-232°, $[\alpha]_D^{20}$ +1.2° (c 0.5; DMFA), R_f 0.58 (A); 0.71 (D); 0.29 (F); Boc-Gln-Phe-Phg-Gly-ONb (XXI), yield 90%, mp 184-185°, $[\alpha]_D^{20}$ +16.1° (c 1; DMFA), R_f 0.57 (C), 0.62 (D); Boc-Gln-Phg-Phg-Gly-ONb (XXII), yield 94%, mp 203-204°, $[\alpha]_D^{20}$ +42.2° (c 1; DMFA), R_f 0.56 (B); 0.61 (G).

Boc-Phe-Phe-Gly-OH (XXIII). The hydrogenation of 1.20 g (1.98 mmoles) of peptide (XI) in solution in 20 ml of DMFA–AcOH (1:1) was carried out with hydrogen in the presence of 0.1 g of Pd black at 20°C for 4 h. After the completion of the reaction (monitoring by TLC) the catalyst was filtered off and was washed with small portions of DMFA; the filtrate was evaporated, and the residue was reevaporated several times with ether and was crystallized under ether. The precipitate was washed with water and was dried in a vacuum desiccator over P_2O_5. This gave 0.85 g (91%) of compound (XVIII), mp 150-152°C, $[\alpha]_D^{20}$ −24.0° (c 1; EtOH), R_f 0.63 (C); 0.36 (F); 0.34 (G).

The hydrogenation of compounds (XVII), (XVIII), (XVI), (XIX)-(XXII), and (XV), by analogy with the preparation of peptide (XXIII) gave, respectively, Boc-Gln-Phe-Phe-Gly-OH (XXIV), Boc-Gln- Gln-Phe-Gly-OH (XXV), Boc-Trp-Phe-Phe-Gly-OH (XXVI), Boc-Gln-Trp-Phe-Phe-Gly-OH (XXVII), Boc-Gln-Phg-Phe-Gly-OH (XXVIII), Boc-Gln-Phe-Phg-Gly-OH (XXIX), Boc-Gln-Phg-Phg-Gly-OH (XXX), and Boc-Phg-Phe-Phe-Gly-OH (XXXI). The physicochemical characteristics of peptides (XXIV)-(XXXI) are given in Table 1.

Boc-Phe-Phe-Gly-His-Phe-Met-NH_2 (XXXII). A solution of 0.33 g (0.70 mmole) of the hydrochloride of tripeptide (X) in 3 ml of DMFA, 0.14 g (1.04 mmoles) of HOBT, 0.21 g (1.02 mmoles) of DCC, and 0.10 ml (0.70 mmole) of Et_3N was added to a solution of 0.36 g (0.77 mmole) of peptide (XXIII) in 5 ml of DMFA at 0°C. The reaction mixture was stirred at 0-5°C for 2 h and at 20°C for 16 h and was filtered, and the filtrate was evaporated to dryness. The residue was triturated under ether, and the precipitate formed was separated off and was purified on a column of silica gel L 100/160, with elution by chromatographic system F. This gave 0.35 g (56%) of peptide (XXXII), mp 157-160°C, $[\alpha]_D^{20}$ −15.0° (c 1; EtOH), R_f 0.57 (C); 0.72 (E); 0.29 (F).

By the condensation of compounds (XXIV)-(XXXI) with (X) in a similar manner to the preparation of peptide (XXXII) we obtained, respectively, Boc-Gln-Phe-Phe-Gly-His-Phe-Met-NH_2 (XXXIII), Boc-Gln-Gln-Phe-Phe-Gly-His-Phe-Met-NH_2 (XXXIV), Boc-Trp-Phe-Phe-Gly-His-Phe-Met-NH_2 (XXXV), Boc-Gln-Trp-Phe-Phe-Gly-His-Phe-Met-NH_2 (XXXVI), Boc-Gln-Phg-Phe-Gly-His-Phe-Met-NH_2 (XXXVII), Boc-Gln-Phe-Phg-Gly-His-Phe-Met-NH_2 (XXXVIII), Boc-Gln-Phg-Phg-Gly-His-Phe-Met-NH_2 (XXXIX), and Boc-Phg-Phe-Phe-Gly-His-Phe-Met-NH_2 (XL). The physicochemical characteristics of peptides (XXXIII)-(XL) are given in Table 2.

Peptides (XXXII)-(XL) were deblocked by treatment with a 2.16 N solution of HCl in AcOH at 0-5°C for 1-2 h, giving, respectively, compounds (I)-(IX). The final purification of the deblocked peptides (I)-(IX) was achieved by gel chromatography on Sephadex LH-20 with elution by the solvent system MeOH–H_2O–0.1 N AcOH (1:0.5:0.2). Results of the amino acid analysis of peptides (I)-(IX): (I) – Phe 2.93 (3), Gly 1.02 (1), His 0.89 (1), Met 0.96 (1); (II) – Glx 1.01 (1), Phe 2.94 (3), Gly 1.01 (1), His 0.91 (1), Met 0.94 (1); (III) – Glx 1.99 (2), Phe 2.97 (3); Gly 0.99 (1), His 0.92 (1), Met 0.92 (1); (IV) – Phe 2.97 (3), Gly 0.99 (1), His 0.92 (1), Met 0.92 (1); (V) – Glx 0.97 (1), Phe 2.98 (3), Gly 1.03 (1), His 0.95 (1), Met 1.01 (1); (VI) – Glx 0.96 (1), Phg 0.99 (1), Phe 1.97 (2), Gly 1.02 (1), His 0.89 (1), Met 0.95 (1); (VII) – Glx 0.97 (1), Phe 2.01 (2), Phg 1.02 (1), Gly 0.97 (1), His 0.92 (1), Met 0.98 (1); (VIII) – Glx 0.94 (1), Phg 1.96 (2), Gly 1.03 (1), His 0.84 (1), Phe 0.92 (1), Met 0.95 (1); (IX) – Phg 1.02 (1), Phe 3.03 (3). Gly 0.97 (1), His 0.86 (1), Met 0.89 (1). The physicochemical characteristics of compounds (I)-(IX) are given in Table 3.

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INSECT PHEROMONES AND THEIR ANALOGUES.

XXXIV. CHIRAL PHEROMONES FROM (S)-(+)-3,7-DIMETHYLOCTA-1,6-DIENE.

2. SYNTHESIS OF OPTICALLY ACTIVE (S)-(–)-DIPRIONYL ACETATE CONFIGURATIONALLY HOMOGENEOUS WITH RESPECT TO THE C^3 ATOM

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A four-stage synthesis has been performed of the optically active (S)-(–)-diprionyl acetate in the form of an equimolar mixture of erythro-(2S,3S,7SR)- and threo-(2R,3S,7SR)-2-acetoxy-3,7-dimethylpentadecanes from a readily available chiral compound – (S)-(+)-3,7-dimethylocta-1,6-diene – with an overall yield of 13%.

The sex pheromone of dangerous pests of coniferous trees – pine sawflies of the genera Diprion and Neodiprion – includes acetates of optically active forms of 3,7-dimethylpentadecan-2-91 [1-3]. Several syntheses of racemic (for the latest, see [4]) and optically active forms configurationally homogeneous with respect to one [5], two [1, 6], or all three [7-9] chiral centers of the pheromone have been described in the literature.

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