compatible to the conjugate addition condition with vinyl iodides that have alkyl substituents at C_4.[4]
(12) A. F. Kluge, K. G. Untch, and J. H. Fried, *J. Am. Chem. Soc.*, **94**, 7827 (1972).
(13) C. E. Castro, E. J. Gaughan, and D. C. Owsley, *J. Org. Chem.*, **31**, 4071 (1966).
(14) E. J. Corey and D. J. Beames, *J. Am. Chem. Soc.*, **94**, 7210 (1972).
(15) The synthesis of cyclopentenones **9** and **11–15** is the subject of a paper in preparation; see also K. F. Bernady, J. F. Poletto, and M. J. Weiss, U.S. Patent 3 836 581 (Sept 17, 1974).
(16) Cyclopentenone **9b** was prepared by a modification of the procedure of Grieco and Reap[17] (see Experimental Section).
(17) P. A. Grieco and J. J. Reap, *J. Org. Chem.*, **38**, 3413 (1973).
(18) K. F. Bernady, J. F. Poletto, and M. J. Weiss, *Tetrahedron Lett.*, 765 (1975).
(19) M. B. Floyd and M. J. Weiss, *Prostaglandins*, **3**, 921 (1973).
(20) M. B. Floyd, *Synth. Commun.*, **4**, 317 (1974).
(21) F. Dessy, M. R. Maleux, and A. Cognioul, *Arch. Int. Pharmacodyn. Ther.*, **206**, 368 (1973).
(22) L. I. Kleinman and E. P. Radford, *J. Appl. Physiol.*, **19**, 360 (1964).
(23) A long-acting bronchial spasm is induced by an injection of 400 μg/kg followed by perfusion of 4 μg/kg/min of pilocarpine. The increase of the airway's resistance must be at least 200–300% of the resting value. After a 15-min control period the compound is administered by means of an ultrasonic aerosol device (Monaghan 650). All the parameters are then followed for at least 1 h. The activity of the compound is expressed as percentage of inhibition of the induced spasm. Further details of this method are described by Lulling and co-workers.[24]
(24) (a) J. Lulling, F. El Sayed, and P. Lievens, *Med. Pharmacol. Exp.*, **16**, 481 (1967); (b) J. Lulling, P. Lievens, F. El Sayed, and J. Prignot, *Arzneim.-Forsch.*, 18, 995 (1968).

Cholecystokinin (Pancreozymin). 3.[1] Synthesis and Properties of an Analogue of the C-Terminal Heptapeptide with Serine Sulfate Replacing Tyrosine Sulfate

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The influence of tyrosine *O*-sulfate, the 27th residue in the sequence of cholecystokinin (pancreozymin) (CCK-PZ) on calcium outflux in isolated pancreatic cells of guinea pigs, was studied by replacing this residue in the biologically active C-terminal heptapeptide, CCK-PZ-(27–33) (I), with L-serine *O*-sulfate. The synthetic analogue Ser-(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (IV), produced the half-maximal outflux observed with I, if applied at about 250 times higher concentration. The unsulfated form of IV was about ten times less potent than unsulfated I. Thus, in the effect on the calcium outflux, serine cannot replace tyrosine without a major loss in potency; a sulfate ester group in position 27 is important but in itself not sufficient for full potency. Interestingly, if the terminal amino group of the heptapeptide is left protected by a *tert*-butyloxycarbonyl group, the potencies of the derivatives of both I and IV were slightly, but significantly, higher than those of the free peptides.

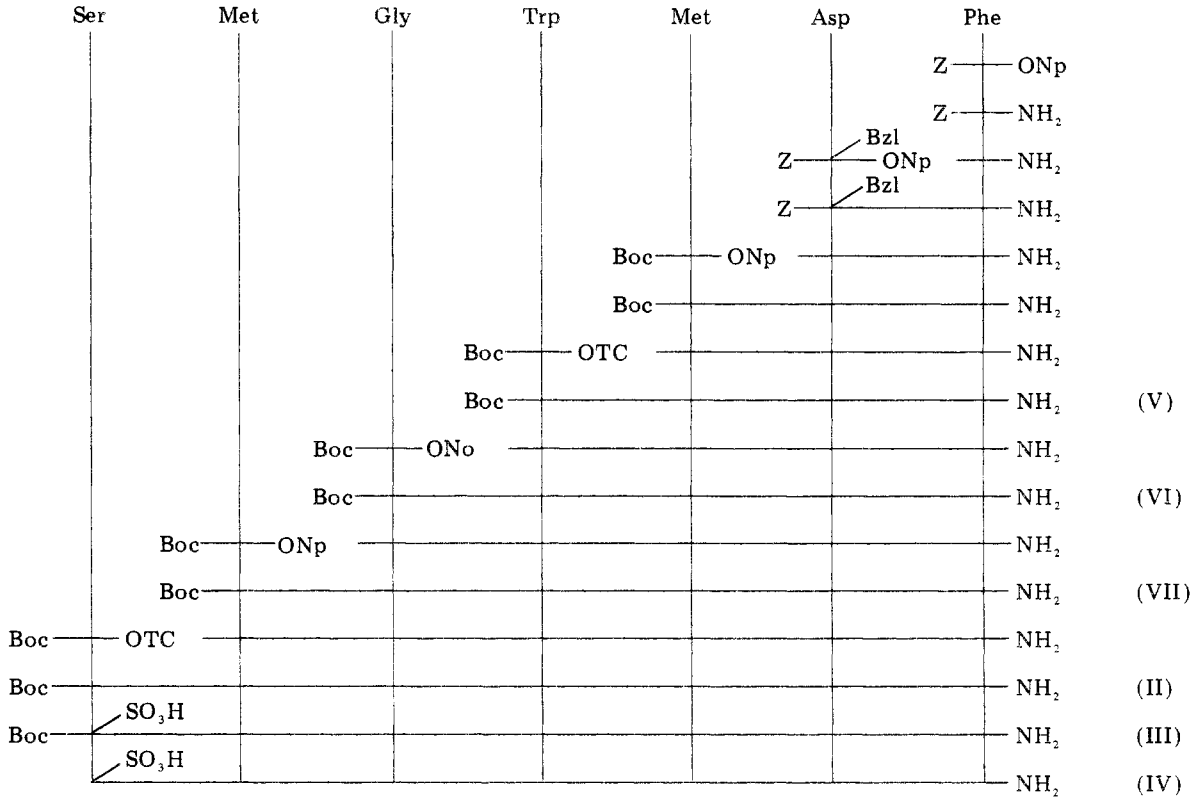
A gastrointestinal hormone stimulating the contraction of the gall bladder was discovered by Ivy and Oldberg[2] and was named cholecystokinin (CCK), while a factor causing the release of digestive enzymes from the pancreas was found by Harper and Raper[3] in the intestinal mucosa of the hog and was designated as pancreozymin (PZ). Subsequently, Jorpes and Mutt[4] demonstrated that a single compound is responsible for both kinds of activities. The sequence of the 33 amino acid residues in the peptide chain of CCK-PZ was elucidated by Mutt and Jorpes.[5] The C-terminal dodecapeptide of CCK-PZ, synthesized by Ondetti and his co-workers,[6] and even the C-terminal heptapeptide, Tyr(*O*-SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (I), showed the hormonal properties of the entire molecule. Similar biological activities are exhibited by caerulein, a peptide isolated from the skin of *Hyla caerulea* by Anastasi and her associates.[7] The C-terminal sequences of CCK-PZ and caerulein are quite similar, except that methionine in position 28 of CCK-PZ is replaced by threonine in caerulein. Among the synthetic peptides related to caerulein, the heptapeptide with the C-terminal sequence of CCK-PZ was also investigated.[8] A closely related sequence is found also in the antral hormone gastrin.[9]

Unlike in gastrin, where the presence or absence of a sulfate ester group on the phenolic hydroxyl of tyrosine has no major influence on biological activity, in CCK-PZ the fact that a tyrosine *O*-sulfate residue and not a simple tyrosine is present is quite significant. The hormonally active C-terminal peptides tested before sulfation had only a small fraction of the potency of the corresponding sulfated materials.[6,8,10] Also, the recently reported[11] synthesis of Tyr^{27}-CCK-PZ produced a peptide which was 250 times less active than the parent hormone. Yet, while these observations provided ample evidence on the importance of the sulfate ester grouping, it was still questionable whether or not a sulfate ester in the side chain of the 27th residue is *sufficient* for full hormonal activity. The role of the structural features of *tyrosine* remained to be investigated. We decided, therefore, to synthesize the shortest part of the CCK-PZ molecule with significant biologic activity, the C-terminal heptapeptide CCK-PZ-(27–33), with serine *O*-sulfate instead of tyrosine *O*-sulfate in position 27. The potency, if any, of this analogue was expected to shed light on the role of the tyrosine moiety.

In previous studies CCK-PZ as well as synthetic C-terminal fragments have been shown to increase amylase secretion from dispersed acinar cells from the guinea pig pancreas.[10] The initial steps in the action of CCK-PZ on enzyme secretion are release of exchangeable cellular calcium followed by an increase in cellular 3′,5′-guanosine monophosphate.[12,13] Previous results[10,12,13] indicate that stimulation of calcium outflux from pancreatic acinar cells is a sensitive, reproducible bioassay system for CCK-PZ and structurally related peptides.

Synthesis. The protected heptapeptide amide *tert*-butyloxycarbonyl-L-seryl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (II) was synthesized stepwise,[14] starting with the C-terminal

Chart I. Synthesis of IV [27-Ser(SO_3H)-CCK-PZ-(27-33)]



residue, with active esters as acylating agents. A characteristic feature of the synthesis was the presence, from the dipeptide stage on, of an unprotected side chain carboxyl, that of the aspartyl residue. This resulted in amphoteric amino components, which were acylated as such, *without the addition of tertiary base*. In the presence of a tertiary base, the side-chain carboxyl as a nucleophile attacks active esters and gives rise to undesired by-products.[15,16] The N-terminal serine residue was introduced in the form of *tert*-butyloxycarbonyl-L-serine 2,4,5-trichlorophenyl ester,[17] which in turn was prepared by hydrogenolysis from its *O*-benzyl derivative. In this way the need for the removal of the *O*-benzyl protecting group after incorporation of the serine residue into a methionine-containing peptide[18] becomes unnecessary.

The free hydroxyl group in the side chain of serine in the protected heptapeptide II was esterified with the aid of a pyridine–SO_3 complex[19] to afford the sulfate ester III. The *tert*-butyloxycarbonyl group was then removed by treatment with trifluoroacetic acid, and the sulfated heptapeptide IV was secured by chromatography on a column of silica gel, in a system of ethyl acetate–pyridine–acetic acid–water in homogeneous form. The synthesis is summarized in Chart I. For comparisons, the unaltered CCK-PZ-(27–33) (I), was also prepared.

Calcium Outflux.[10] Outflux of ^{45}Ca from control cells was 5.3 ± 0.8%/min (mean of five separate experiments ± 1 SD). Adding 10^{-8} M Tyr^{27}(SO_3H)-CCK-PZ-(27–33) (I) increased ^{45}Ca outflux to 26.2 ± 1.9%/min. Significant stimulation of ^{45}Ca outflux by I could be detected at 3×10^{-11} M and maximal stimulation occurred at 10^{-8} M (Figure 1). The synthetic analogue IV in which the amino terminal Tyr(SO_3H) residue was replaced by Ser(SO_3H) was approximately 250 times less potent than I. The unsulfated form of I was approximately 100 times less potent than the sulfated form. The unsulfated form of IV was approximately ten times less potent than the sulfated form. For I or IV if the terminal amino group was left protected by a *tert*-butyloxycarbonyl group the potency with which the peptide stimulated ^{45}Ca outflux was slightly but significantly increased.

Conclusions

The importance of the sulfate ester group on the hydroxyl of the tyrosine residue in position 27 of CCK-PZ was known before. However, the effect of the side chain of tyrosine on biological potency has not been determined so far. The experiments here reported show that the aromatic ring in this side chain cannot be omitted without a major loss in potency. Whether the aromatic character of this partial structure or the distance between the sulfate ester group and the peptide backbone created by the presence of the ring is important, for full activity, remains to be explored.

Experimental Section

Capillary melting points are reported uncorrected. The homogeneity of the intermediates and products was examined on silica gel thin-layer plates (Merck) in the solvent system EtOAc–pyridine–acetic acid–H_2O (60:20:6:11). The spots were revealed by charring or with Ehrlich's reagent. Peroxide-free ether was used; reagent grade DMF was dried over a molecular sieve (Linde Type 4A). For amino acid analysis, samples were hydrolyzed with constant boiling HCl in evacuated, sealed ampules at 110 °C for 16 h and analyzed by the Spackman–Stein–Moore method on a Beckman-Spinco instrument. The following abbreviations are used: DMF = dimethylformamide, DIEA = diisopropylethylamine, TFA = trifluoroacetic acid.

***N*-*tert*-Butyloxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (V).** The trifluoroacetate salt of L-methionyl-L-aspartyl-L-phenylalanine amide[15] (3.5 g) was dissolved in water (100 mL). A suspension of Dowex-1 acetate (20 mL) was added to this solution and the mixture was stirred gently for 10 min. The solution was filtered and the resin washed with water (2 × 25 mL). The combined filtrates and washings were evaporated; during evaporation, crystals separated. They were filtered and dried in vacuo (2.2 g, mp 230–232 °C). From the filtrate, a further amount of the amphoteric peptide was

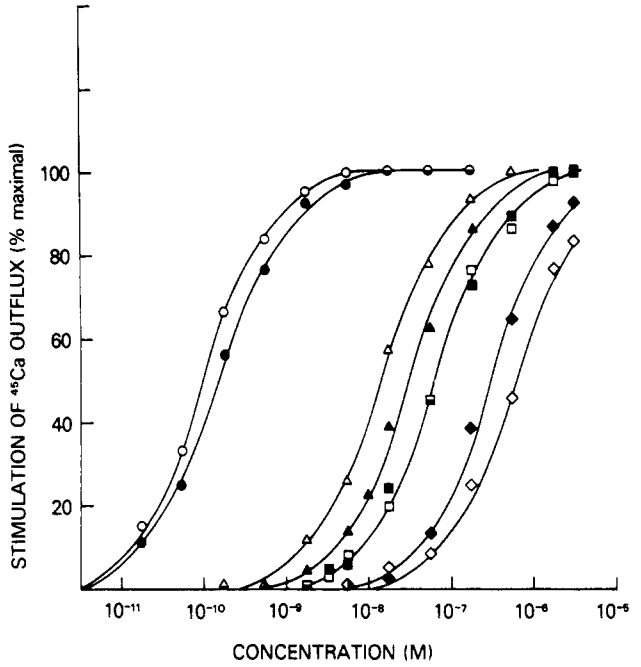


Figure 1. Stimulation of ^{45}Ca outflux from guinea-pig pancreatic acinar cells by analogues of the C-terminal heptapeptide of CCK-PZ. Dispersed acinar cells were preincubated in Krebs–Ringer bicarbonate (pH 7.4) containing 1% (wt/vol) albumin and 0.5 mM ^{45}Ca for 60 min at 37 °C. Calcium outflux was determined from the loss of cellular ^{45}Ca during a 5-min incubation after adding 5 mM EDTA. Stimulation of calcium outflux is given as the percent of maximal stimulation obtained with I at 10^{-7} M. Each point was determined in duplicate and results shown are means of five separate experiments. The symbols in Figure 1 represent: ○, Boc-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (IX); ●, H-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2·CF_3COOH (I); △, Boc-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (VIII); ▲, H-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2·CF_3COOH (VIIIa); □, Boc-Ser(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (III); ■, H-Ser(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2·CF_3COOH (IV); ◇, Boc-Ser-Met-Gly-Trp-Met-Asp-Phe-NH_2 (II); ◆, H-Ser-Met-Gly-Trp-Met-Asp-Phe-NH_2·CF_3COOH (IIa).

obtained: 0.3 g; mp 210–214 °C.

To a stirred suspension of L-methionyl-L-aspartyl-L-phenylalanine amide (2.2 g) in DMF (30 mL), *tert*-butyloxycarbonyl-L-tryptophan 2,4,5-trichlorophenyl ester[20] (2.9 g) was added. Gradually, a clear solution formed. After 72 h, the ninhydrin reaction was still positive. A second portion of the active ester (200 mg) was added and stirring continued for an additional 48 h; the ninhydrin reaction became negative. The solvent was evaporated in vacuo and the residue triturated with ether (100 mL). It was filtered, and the solid was resuspended in EtOAc (50 mL), filtered, washed with EtOAc, and dried: 3.53 g (87%); mp 206–209 °C dec; TLC R_f 0.49; $[\alpha]^{25}_D$ −34.7° (*c* 2, DMF). According to TLC, this was a single, homogeneous compound; no succinimide derivative[13] could be detected.

***N*-*tert*-Butyloxycarbonylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (VI).** A sample of the protected tetrapeptide V (900 mg) was dissolved in TFA containing 5% anisole (2.5 mL). After 30 min in an ice–water bath and under nitrogen, the TFA was evaporated in vacuo and the residue triturated with ether, filtered, washed with ether, and dried to yield the tetrapeptide amide trifluoroacetate (900 mg). This was dissolved in a mixture of water (30 mL) and ethanol (20 mL). A suspension of Dowex-1 acetate (5 mL) was added, and the mixture was stirred for 20 min. A white precipitate started to separate. The suspension of the precipitate was decanted from the resin, the solid collected by centrifugation, and the supernatant used to recover more precipitate from the resin. Finally, the resin was washed with the same solvent mixture (100 mL), the combined extracts were evaporated, and the residue was combined with the material collected by centrifugation: VIa, 710 mg; mp 223–225 °C dec. The amphoter was suspended in DMF (10 mL) and *tert*-butyloxycarbonylglycine *o*-nitrophenyl ester[21] (425 mg) was added. After overnight stirring, the ninhydrin reaction became negative. The solvent was removed in vacuo and the residue triturated with ether (50 mL). The product was filtered, resuspended in ethyl acetate (15 mL), stirred for 10 min, and refiltered: 750 mg (76%); mp 196–198 °C dec; TLC R_f 0.42; $[\alpha]^{25}_D$ −24.5° (*c* 2, DMF). Only a trace of the corresponding succinimide derivative[13] and slight impurity with R_f 0.48 could be detected.

***N*-*tert*-Butyloxycarbonyl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (VII).** A. The *tert*-butyloxycarbonyl group was removed from compound VI (1.55 g) with TFA containing 5% anisole (5.5 mL) as described before. The trifluoroacetate salt VIa was dissolved in DMF (15 mL); DIEA (0.64 mL) was added, followed by *tert*-butyloxycarbonyl-L-methionine *p*-nitrophenyl ester[22] (0.95 g). The solution was stirred at room temperature overnight; the ninhydrin and fluorescamine reactions became negative. The solvents were evaporated in vacuo, and the residue was triturated with ether, filtered, and dried: 1.57 g (86%); mp 180–182 °C dec. This contained some of the corresponding succinimide derivative,[15] R_f 0.78. Crystallization from ethanol raised the melting point to 200–201 °C dec; TLC R_f 0.42; $[\alpha]^{25}_D$ −30.3° (*c* 2, DMF). Amino acid analysis: Asp 1.05, Gly 0.96, Met 2.01, Phe 1.00. Anal. ($C_{41}H_{56}N_8O_{10}S_2$) C, H, N.

B. A sample of VIa (3.0 g) was dissolved in about 40% aqueous methanol (200 mL), a suspension of Dowex-1 acetate (30 mL) was added, and the amphoteric pentapeptide amide (2.56 g, mp 214–215 °C dec) was secured as described in the preparation of VI. It was suspended in DMF (30 mL), and *tert*-butyloxycarbonyl-L-methionine *p*-nitrophenyl ester[22] (1.7 g) was added. After stirring for 16 h, the ninhydrin reaction became negative. The solvent was evaporated in vacuo, and the residue was triturated and stirred with ether (60 mL), filtered, and suspended in EtOAc (25 mL), then stirred, filtered, and washed with EtOAc (10 mL): 2.8 g (81%); mp 187–189 °C dec; TLC R_f 0.42; $[\alpha]^{25}_D$ −26.3° (*c* 2, DMF). This product contained a very small amount of the succinimide derivative.[15]

***N*-*tert*-Butyloxycarbonyl-L-serine 2,4,5-Trichlorophenyl Ester.** An ethyl acetate solution (40 mL) containing *tert*-butyloxycarbonyl(*O*-benzyl)-L-serine (Beckman, 2.95 g, 10 mmol) and 2,4,5-trichlorophenol (2.4 g, 12 mmol) was cooled in ice, and DCC (2.06 g, 10 mmol) was added in portions. After 20 min, the ice bath was exchanged for the water bath of room temperature and stirring continued for 3 h more. Glacial AcOH (1 mL) was added and after 5 min the solution was filtered to removed the *N*,*N*′-dicyclohexylurea. The solvent was removed and the residue applied to column of silica gel (220 g, Baker) in chloroform. The elution was carried out with the same solvent. After the void volume (140 mL), 8-mL fractions were collected. The active ester was recovered from fractions 1–35 as an oil, contaminated with some trichlorophenol. One-third of this material was dissolved in EtOAc containing 1% HOAc (30 mL), 10% Pd-on-charcoal catalyst (400 mg) was added, and the mixture was hydrogentated for 18 h. On removal of the catalyst and the solvents, the product crystallized in a desiccator. On trituration with ether–hexane (1:1, 24 mL), the active ester was obtained in pure form: 195 mg; mp 104–105 °C; TLC R_f 0.49 ($CHCl_3$–MeOH 19:1); $[\alpha]^{25}_{578}$ −41° (*c* 2, DMF). Anal. ($C_{14}H_{16}O_5Cl_3N$) H, N; C: calcd, 43.7; found, 44.2.

Two further crops (250 and 245 mg) with the same melting point were obtained from the ether–hexane mother liquor: total yield, 54%.

***N*-*tert*-Butyloxycarbonyl-L-seryl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (II).** The *tert*-butyloxycarbonyl group was removed from compound VII (600 mg) with TFA containing 5% anisole (3 mL), as described before. The trifluoroacetate salt VIIa was converted to the amphoter as described in the previous paragraphs: 480 mg; mp 202–203 °C. The amphoteric hexapeptide (480 mg, 0.61 mmol) was suspended in DMF (5 mL) and *tert*-butyloxycarbonyl-L-serine 2,4,5-trichlorophenyl ester (260 mg, 0.67 mmol) was added, and the mixture was stirred overnight. The ninhydrin reaction was negative. The solvent was removed, the residue triturated with ether, and the separated solid filtered and dried: 575 mg (97%). A sample (50 mg) was triturated with EtOAc–pyridine–AcOH–H_2O (60:20:6:11, 1 mL) to yield 28 mg; mp 195–196 °C dec; TLC R_f 0.34; $[\alpha]^{25}_D$ −27.5° (*c* 2, DMF). Amino

acid analysis: Asp, 1.05; Ser 0.91, Gly 0.96, Met 2.09, Phe 1.00. Anal. ($C_{44}H_{61}N_9O_{12}S_2$) H, N; C: calcd, 54.4; found, 53.9.

***N*-*tert*-Butyloxycarbonyl(*O*-sulfate)-L-seryl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (III).** A sample of II (200 mg) was dissolved in dry pyridine (2 mL) and DMF (5 mL) and pyridine–sulfur trioxide complex (1.2 g) was added. This mixture was stirred for 48 h maintaining the anhydrous conditions. The product (170 mg) was isolated as described by Ondetti et al.[6] An aliquot of the crude material (50 mg) was applied to a column of silica gel (Baker, 18 × 0.6 cm) poured from a slurry in EtOAc–pyridine–AcOH–H_2O (60:20:6:11) and the same solvent mixture was used for elution. Fractions of 2 mL were collected. Fractions 4–9 contained the desired sulfated material: 23 mg; mp 172–176 °C dec; TLC R_f 0.29 (double development); $[\alpha]^{25}_D$ −24.3° (*c* 2, 0.1 M NH_4OH). The UV spectrum showed the presence of 1 mol of pyridine. Anal. ($C_{44}H_{61}N_9O_{15}S_3 \cdot C_5H_5N \cdot 3H_2O$) C, H, N.

A sample of III (10 mg) was deblocked with TFA containing 5% anisole (0.1 mL) to yield the trifluoroacetate IV: 9 mg. Hydrolysis of this sample with aminopeptidase M (Rohm)[23] gave Asp 0.92, Gly 0.86, Met 1.99, Phe 1.00. Ser-*O*-sulfate was eluted before Asp; their peaks had about the same area. TLC gave R_f 0.13 (double development).

***N*-*tert*-Butyloxycarbonyl-L-tyrosyl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (VIII). A.** The trifluoroacetate salt VIIa (3.4 g) was dissolved in DMF (33 mL), and DIEA (1.28 mL) was added, followed by *tert*-butyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester[24] (1.76 g). The solution was stirred at room temperature overnight. The solvents were removed in vacuo; the residue was triturated with a mixture of EtOAc (15 mL) and ether (75 mL) for 10 min and filtered. It was resuspended in EtOAc (20 mL), stirred, filtered, and dried: 3.8 g (95%); mp 178–179 °C; TLC R_f 0.42; $[\alpha]^{25}_D$ −26° (*c* 2, DMF). A small amount of a faster moving material (R_f 0.78), presumably the succinimide derivative,[15] could be detected. Amino acid analysis: Asp 1.06, Gly 0.97, Met 2.11, Tyr 0.99, Phe 1.00. Anal. ($C_{50}H_{65}N_9O_{12}S_2$) C, H, N.

B. A sample of compound VIIa (4.1 g) was converted to its amphoteric form as described before. This material (3.5 g) was suspended in DMF (21 mL); *tert*-butyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester (1.98 g) was added and stirred for 48 h, when the ninhydrin reaction became negative. The solvent was removed in vacuo and the residue triturated and stirred with ether (150 mL) for 30 min. It was then filtered, resuspended in EtOAc (20 mL), stirred for 15 min, filtered, and dried: 4.3 g (90%); mp 181–182.5 °C; TLC R_f 0.42; $[\alpha]^{25}_D$ −26.6° (*c* 2, DMF). This product contained only a trace of the succinimide derivative.[15]

L-Tyrosyl(*O*-sulfate)-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (I). The protected heptapeptide amide VIII (400 mg) was sulfated, as described for III, to yield 360 mg. A sample (100 mg) of the sulfated crude product was dissolved in the solvent system EtOAc–pyridine–HOAc–H_2O (60:20:6:11, 1 mL) and applied to a column of silica gel (Baker, 36 × 0.6 cm). Fractions of 3 mL were collected. They were examined by TLC and also by plotting the weight of fractions. Fractions 3–8 contained the unsulfated starting material and other minor faster moving impurities (9 mg), fractions 9–16 contained the desired Boc-heptapeptide amide sulfate IX (53 mg), and fractions 17–30 contained materials with lower R_f values (30 mg). The solid obtained from fractions 9–16 showed a strong absorption in IR at 1040 cm^{-1}, characteristic for the phenol–sulfate esters (cf. ref 6): TLC R_f 0.23.

A second sample (100 mg) of the crude sulfated Boc-heptapeptide was deblocked with TFA containing 5% anisole (1.5 mL) and the product chromatographed on a silica gel column (10 g), in the system used for the protected peptide. Fractions of 3 mL were collected, and the elution was monitored by TLC. Unsulfated heptapeptide amide (VIIIa, 30 mg) was collected from fractions 12–17, and the sulfated heptapeptide amide X (50 mg) was secured from fractions 22–28: mp (183) 189–206 °C dec; TLC R_f 0.13. The UV spectrum showed the presence of 1 mol of pyridine. Anal. ($C_{45}H_{57}N_9O_{13}S_3 \cdot C_5H_5N \cdot CF_3COOH \cdot 3H_2O$) C, H, N; F: calcd, 4.47; found, 3.94.

Method for ^{45}Ca Outflux. Outflux of ^{45}Ca from pancreatic acinar cells was determined as described previously.[10] Dispersed acinar cells from guinea pig pancreas were prepared as described previously[10] and were suspended in Krebs–Ringer bicarbonate (pH 7.4; equilbrated with 95% O_2 and 5% CO_2) containing 1% (wt/vol) bovine serum albumin and 0.5 mM ^{45}Ca. Cells were preincubated for 60 min at 37 °C and gassed with 95% O_2–5% CO_2. Cellular ^{45}Ca was determined at the end of the preincubation and again after a 5-min incubation with 5 mM ethylenediaminetetraacetate (EDTA). EDTA chelates extracellular ^{45}Ca and abolishes calcium influx; therefore, the loss of cellular radioactivity during the 5-min incubation is a measure of calcium outflux.

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References and Notes

(1) For the preceeding paper in this series, cf. Y. Klausner and M. Bodanszky, *J. Org. Chem.*, **42**, 147 (1977).
(2) A. C. Ivy and E. Oldberg, *Am. Physiol.*, **86**, 599 (1928).
(3) A. A. Harper and M. S. Raper, *J. Physiol. (London)*, **102**, 115 (1942).
(4) J. E. Jorpes, V. Mutt, and K. Toczko, *Acta Chem. Scand.*, **18**, 2408 (1966); J. E. Jorpes and V. Mutt, *Acta Physiol. Scand.*, **66**, 196 (1966).
(5) V. Mutt and J. E. Jorpes, *Biochem. Biophys. Res. Commun.*, **26**, 392 (1967); *Eur. J. Biochem.*, **6**, 156 (1968); *Proc. Int. Union Physiol. Sci.*, **6**, 193 (1968); J. E. Jorpes, *Gastroenterology*, **55**, 157 (1968); V. Mutt and J. E. Jorpes, *Biochem. J.*, **125**, 57 (1971).
(6) (a) M. A. Ondetti, J. Pluŝĉec, E. F. Sabo, J. T. Sheehan, and N. Williams, *J. Am. Chem. Soc.*, **92**, 195 (1970); (b) M. A. Ondetti, B. Rubin, S. L. Engel, J. Pluŝĉec, and J. T. Sheehan, *Digestive Dis.* **15**, 149 (1970).
(7) A. Anastasi, V. Erspamer, and R. Endean, *Experientia*, **23**, 699 (1967).
(8) A. Anastasi, L. Bernardi, G. Bertaccini, G. Bosisio, R. de Castiglione, V. Erspamer, O. Goffredo, and M. Impicciatore, *Experientia*, 24, 771 (1968).
(9) For a review on gastrin, cf. J. H. Walsh and M. I. Grossman, *N. Engl. J. Med.*, **292**, 1324–1377 (1975).
(10) J. D. Gardner, T. P. Conlon, H. L. Klaeveman, T. D. Adams, and M. A. Ondetti, *J. Clin. Invest.*, **56**, 366 (1975).
(11) H. Yajima, Y. Mori, Y. Kiso, K. Koyama, T. Tobe, M. Setoyama, H. Adachi, T. Kanno, and A. Saito, *Chem. Pharm. Bull.*, **24**, 1110 (1976).
(12) J. P. Christophe, E. K. Frandsen, T. P. Conlon, G. Krishna, and J. D. Gardner, *J. Biol. Chem.*, **251**, 4640 (1976).
(13) H. T. Shelby, L. P. Gross, P. Lichty, and J. D. Gardner, *J. Clin. Invest.*, **58**, 1482 (1976).
(14) M. Bodanszky, *Ann. N.Y. Acad. Sci.*, **88**, 655 (1960).
(15) M. Bodanszky and S. Natarajan, *J. Org. Chem.*, **40**, 2495 (1975).
(16) S. Natarajan and M. Bodanszky, *J. Org. Chem.*, **41**, 1269 (1976).
(17) J. Pless and R. A. Boissonnas, *Helv. Chim. Acta*, **46**, 1609 (1963).
(18) Cf. footnote 8 in ref 13 and also H. Medzihradszky-Schweiger, *Ann. Univ. Sci. Budap. Rolando Eötvös Nominatae, Sect. Chim.*, **13**, 35 (1972).
(19) H. C. Reitz, R. E. Ferrel, H. Fraenkel-Conrat, and H. S. Olscotti, *J. Am. Chem. Soc.*, **68**, 1024 (1940); cf. also ref 6a.
(20) W. Broadbent, J. S. Morley, and B. E. Stone, *J. Chem. Soc. C*, 2632 (1967).
(21) M. Bodanszky and K. W. Funk, *J. Org. Chem.*, **38**, 1296 (1973).
(22) E. Scoffone, F. Marchiori, A. M. Tamburro, and R. Roochi, *Gazz. Chim. Ital.*, **94**, 695 (1974).
(23) K. Hofmann, F. M. Finn, M. Limetti, J. Montibeller, and Z. Zanetti, *J. Am. Chem. Soc.*, **88**, 3633 (1966).
(24) J. Meienhofer, Y. Sano, and R. P. Patel in "Peptides, Chemistry and Biochemistry", B. Weinstein and S. Lande, Ed., Marcel Dekker, New York, N.Y., 1970, p 419.