Studies on Peptides. CXLVI.1,2) Synthesis of Gln\textsuperscript{15}-Motilin and Examination of Its Immunological Properties

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Gln\textsuperscript{15}-motilin was synthesized in a conventional manner by assembling seven peptide fragments followed by deprotection with 1 m trifluoromethanesulfonic acid–thioanisole in trifluoroacetic acid. The usefulness of phenylthiotrimethylsilane for the reduction of Met(O) was confirmed. This motilin analog was as biologically active as synthetic motilin in terms of contraction of rabbit duodenal muscles, but in radioimmunoassay it was only about 25% as reactive with antiserum raised against synthetic porcine motilin.

Keywords—Gln\textsuperscript{15}-motilin synthesis; Met(O) reduction; phenylthiotrimethylsilane; trimethylsilyl trifluoromethanesulfonate; trifluoromethanesulfonic acid deprotection; smooth muscle contraction; motilin immunogenicity

Recently it has become evident that there are multiple forms of motilin immunoreactive materials in the duodenum, as well as in the central nervous system, which can be selectively detected by particular antibodies. In order to examine whether our motilin antiserum raised against synthetic motilin\textsuperscript{4}) and used in the above experiments possesses adequate specificity, we synthesized Gln\textsuperscript{15}-motilin and examined its immunological properties, since deamination of Gln to Glu is often observed during the isolation of natural hormones, as reported in the sequence analysis of adrenocorticotropic\textsuperscript{5}) and more recently in that of neutrotensin.\textsuperscript{6}) A similar problem was also reported with a new gastrointestinal polypeptide, PHI.\textsuperscript{7}) We wish to report that synthetic Gln\textsuperscript{15}-motilin is indistinguishable from the parent molecule in terms of biological activity, but is distinguishable immunologically.

From the synthetic viewpoint, compared with our previous synthesis of porcine motilin,\textsuperscript{4}) Arg(Tos) was replaced by Arg(Mts),\textsuperscript{8}) a more suitable derivative for thioanisole-mediated deprotection with TFMSA\textsuperscript{9}) (Fig. 1). Met(O) was reduced with phenylthiotrimethylsilane\textsuperscript{10}) before deprotection as described in the synthesis of human gastrin-releasing polypeptide\textsuperscript{11}) and a time-consuming reduction step with 2-mercaptoethanol\textsuperscript{12}) was eliminated. Thus, the usefulness of this reducing reagent in practical peptide synthesis was further demonstrated. The entire peptide backbone of this analog was constructed by successive azide condensations of 7 fragments. Of these, two fragments, [1] and [6], were identical with those employed for the previous motilin synthesis.\textsuperscript{4}) In the N-terminal fragment [7], the Z(OME) group was employed as an N\textsuperscript{ε}-protecting group, instead of the Z group previously employed, since we are considering the synthesis of possible larger forms of motilin in the future.

For the preparation of fragment [2], Z(OME)–Glu–Arg(Mts)–NH\textsubscript{2}, Z(OME)–Glu(Obzl)–Arg(Mts)–OME was prepared by the Su procedure\textsuperscript{13}) and this, after removal of the Bzl group by hydrogenolysis, was treated with hydrazine hydrate as usual to give [2].
Fragment [3], Z(OMe)-Gln-Gln-Lys(Z)-NHNH₂, was prepared by the Np condensation \(^{14}\) of Z(OMe)-Gln-ÖH with a TFA-treated sample of Z(OMe)-Gln-Lys(Z)-OMe,\(^{15}\) followed by the usual hydrazine treatment. Fragment [4], Z(OMe)-Gln-Arg(Mts)-Met(O)-NHNH₂, was prepared in a stepwise manner, Z(OMe)-Arg(Mts)-OH\(^{8}\) was introduced by the mixed anhydride method (MA)\(^{16}\) and Z(OMe)-Gln-ÖH by the Np method. The resulting protected tripeptide ester was converted to [4] by the usual hydrazine treatment. For the preparation of fragment [5], Z(OMe)-Glu-Leu-NHNH₂, Z(OMe)-Glu(Obzl)-Leu-ÖMe was prepared by the Su method, then the Bzl group was removed by hydrogenolysis as described for the preparation of fragment [2]. The resulting dipeptide ester was converted to [5] as usual The N-terminal pentapeptide hydrazide [7], Z(OMe)-Phè-Val-Pro-Ile-Phe-NHNH₂, was prepared in essentially the same manner as described previously, except for the Nª-protecting group. Z-Phè-Val-Pro-ÔH\(^{41}\) was converted to the corresponding Z(OMe)-derivative by hydrogenolysis followed by treatment with \(p\)-methoxybenzylazidoformate.\(^{17}\) This Z(OMe)-tripeptide was condensed with H-Ile-Phe-ÖMe\(^{42}\) by using DCC in the presence of HOBT\(^{48}\) to give the protected pentapeptide ester, which was converted to [7] as usual.

### Table 1. Amino Acid Ratios in 6 N HCl Hydrolysates of Synthetic Gln\(^{15}\)-Motilin and Its Protected Intermediates

<table>
<thead>
<tr>
<th></th>
<th>17—22</th>
<th>14—22</th>
<th>11—22</th>
<th>9—22</th>
<th>6—22</th>
<th>1—22</th>
<th>Syn. Gln(^{15})-motilin</th>
<th>Theory</th>
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<tr>
<td>Asp</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>Thr</td>
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</tr>
<tr>
<td>Glu</td>
<td>1.95</td>
<td>3.92</td>
<td>4.98</td>
<td>6.00</td>
<td>6.11</td>
<td>5.44</td>
<td>5.73</td>
<td>(6)</td>
</tr>
<tr>
<td>Pro</td>
<td></td>
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<td></td>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>Gly</td>
<td>0.98</td>
<td>0.98</td>
<td>1.00</td>
<td>1.01</td>
<td>2.03</td>
<td>2.05</td>
<td>2.15</td>
<td>(2)</td>
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<tr>
<td>Val</td>
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<td>(1)</td>
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<tr>
<td>Met(^a)</td>
<td>0.78</td>
<td>0.83</td>
<td>0.91</td>
<td></td>
<td></td>
<td></td>
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<td>(1)</td>
</tr>
<tr>
<td>Ile</td>
<td></td>
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<td>(1)</td>
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<tr>
<td>Leu</td>
<td>1.02</td>
<td>1.06</td>
<td>0.98</td>
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<td>(1)</td>
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<tr>
<td>Tyr</td>
<td>1.01</td>
<td>0.98</td>
<td>0.93</td>
<td></td>
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<td>(1)</td>
</tr>
<tr>
<td>Phe</td>
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<td></td>
<td></td>
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<td>(1)</td>
</tr>
<tr>
<td>Lys</td>
<td>0.99</td>
<td>1.91</td>
<td>1.97</td>
<td>2.05</td>
<td>2.25</td>
<td>2.01</td>
<td>1.83</td>
<td>(2)</td>
</tr>
<tr>
<td>Arg</td>
<td>0.96</td>
<td>0.90</td>
<td>1.86</td>
<td>1.90</td>
<td>2.07</td>
<td>1.84</td>
<td>1.90</td>
<td>(2)</td>
</tr>
<tr>
<td>Recov. (%)</td>
<td>79</td>
<td>78</td>
<td>83</td>
<td>80</td>
<td>92</td>
<td>92</td>
<td>77</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Met + Met(O).
The seven fragments thus obtained were assembled successively by the azide\textsuperscript{19} procedure as shown in Fig. 1. The amount of the acyl component was increased from 1.2 to 2 eq as the chain elongation progressed. Each product was purified either by precipitation from DMF with AcOEt or MeOH or by gel-filtration on Sephadex LH-20 or 60 using DMF as an eluant. Throughout this synthesis, Asp was selected as a diagnostic amino acid in acid hydrolysis (Table I). By comparison of the recovery of Asp with those of newly added amino acids after each condensation reaction, satisfactory incorporation of each fragment was ascertained.

The Met(O) residue in protected Gln\textsuperscript{15}-motilin thus obtained was reduced by treatment with phenylthiotrimethylsilane\textsuperscript{10} in the presence of a small amount of trimethylsilyl trifluoromethanesulfonate at 25°C for 30 min. Next all protecting groups were removed from the reduced peptide by treatment with 1 M TFMSA–thioanisole in TFA\textsuperscript{8} in the presence of m-cresol in an ice-bath for 120 min. The deprotected peptide was then treated with dil. ammonia to reverse a possible N→O shift\textsuperscript{20} and submitted to gel-filtration on Sephadex G-25. The product was purified by ion-exchange chromatography on carboxymethyl (CM)-cellulose followed by high performance liquid chromatography (HPLC) on a Cosmosil 5C\textsubscript{18} column. The purity of synthetic Gln\textsuperscript{15}-motilin was ascertained by thin-layer chromatography (TLC), analytical HPLC, amino acid analyses after acid hydrolysis and enzymatic digestion.

Synthetic Gln\textsuperscript{15}-motilin and synthetic porcine motilin were found to be equipotent in their ability to contract the rabbit duodenal muscle, but as shown in Fig. 2, in radioimmunoassay the Gln\textsuperscript{15}-motilin was only about 25% as reactive with antiserum raised against synthetic porcine motilin.\textsuperscript{31} Thus porcine motilin and Gln\textsuperscript{15}-motilin was judged to be biologically equivalent, but immunologically different from each other, and our motilin antiserum was found to have regional specificity involving the Glu residue at position 15 of porcine motilin.

**Experimental**

General experimental methods employed in this investigation are essentially the same as described in part LXXXVIII\textsuperscript{21} of this series. Mixed anhydrides were prepared according to Vaughan and Osato\textsuperscript{16} with isobutyl chloroformate; the reaction was performed in an ice-bath. Azides were prepared according to Honzl and Rudinger,\textsuperscript{19} with isoamyl nitrite at 4°C. The active ester reaction was carried out at room temperature.

TLC of products was performed on silica gel (Kieselgel G, Merck). The Rf values refer to the following v/v solvent systems: Rf\textsubscript{1} CHCl\textsubscript{3}–MeOH–H\textsubscript{2}O (8:3:1), Rf\textsubscript{2} CHCl\textsubscript{3}–MeOH–AcOH (9:1:0.5), Rf\textsubscript{3} n-BuOH–AcOH–pyridine–H\textsubscript{2}O (4:1:1:2), Rf\textsubscript{4} n-BuOH–AcOH–AcOEt–H\textsubscript{2}O (1:1:1:1). For HPLC, a Waters compact model 204 was employed. Motilin radioimmunoassay was conducted according to Tai and Chey.\textsuperscript{22}

**Syntheses of Fragments, [2],[3],[4], and [5]**——The dipeptide and tripeptide hydrazides were prepared as described in the text. After vaporization of the solvent, products were purified by one of the following procedures. Procedure A: For purification of products soluble in AcOEt, the extract was washed with 5% citric acid, 5% NaHCO\textsubscript{3}, and H\textsubscript{2}O, dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated. The residue was recrystallized or precipitated from appropriate solvents. Procedure B: For purification of products less soluble in AcOEt, the crude product was
### Table II. Characterization of Dipeptide Derivatives

<table>
<thead>
<tr>
<th>Puri.</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
<th>$[\alpha]_D^{20}$</th>
<th>$R_f$</th>
<th>Formula</th>
<th>Analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Caled (Found)</td>
<td></td>
</tr>
<tr>
<td>OBzl</td>
<td>Mts</td>
<td>A-1</td>
<td>80</td>
<td>73—76</td>
<td>-15.6°</td>
<td>C$<em>{32}$H$</em>{46}$N$_2$O$_9$S</td>
</tr>
<tr>
<td></td>
<td>Boc—Glu—Arg—OMe</td>
<td>B-1</td>
<td>93</td>
<td>89—92</td>
<td>-23.0°</td>
<td>C$<em>{26}$H$</em>{46}$N$_2$O$_9$S</td>
</tr>
<tr>
<td>Mts</td>
<td>Boc—Glu—Arg—OMe</td>
<td>B-2</td>
<td>94</td>
<td>119—122</td>
<td>-18.7°</td>
<td>C$<em>{22}$H$</em>{46}$N$_2$O$_9$S</td>
</tr>
<tr>
<td></td>
<td>Boc—Glu—Arg—NHNH$_2$</td>
<td>Mts</td>
<td>A-2</td>
<td>99</td>
<td>68—73</td>
<td>C$<em>{26}$H$</em>{46}$N$_2$O$_9$S</td>
</tr>
<tr>
<td></td>
<td>Z(OMe)−Arg—Met—OMe</td>
<td>Boc—Glu—Leu—OMe</td>
<td>B-1</td>
<td>99</td>
<td>142—145</td>
<td>C$<em>{17}$H$</em>{36}$N$_2$O$_7$</td>
</tr>
<tr>
<td>OBzl</td>
<td>Boc—Glu—Leu—OMe</td>
<td>B-3</td>
<td>73</td>
<td>148—151</td>
<td>-20.3°</td>
<td>C$<em>{18}$H$</em>{36}$N$_2$O$_6$</td>
</tr>
</tbody>
</table>

$a)$ A, extraction procedure; B, without extraction; 1, precipitation from AcOEt with n-hexane; 2, recrystallization from MeOH and ether; 3, recrystallization from MeOH and EtOH. $b)$ $R_f$.  

### Table III. Characterization of Tripeptide Derivatives

<table>
<thead>
<tr>
<th>Puri.</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
<th>$[\alpha]_D^{20}$</th>
<th>$R_f$</th>
<th>Formula</th>
<th>Analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Caled (Found)</td>
<td></td>
</tr>
<tr>
<td>Z(OMe)—Gln—Gln—Lys—OMe</td>
<td>A-1</td>
<td>84</td>
<td>213—216</td>
<td>-6.9°</td>
<td>0.50</td>
<td>C$<em>{34}$H$</em>{46}$N$<em>2$O$</em>{11}$</td>
</tr>
<tr>
<td>Z(OMe)—Gln—Gln—Lys—NHNH$_2$</td>
<td>B-3</td>
<td>69</td>
<td>193—195</td>
<td>-10.2°</td>
<td>0.41</td>
<td>C$<em>{33}$H$</em>{46}$N$<em>2$O$</em>{10}$</td>
</tr>
<tr>
<td>Z(OMe)—Gln—Arg—Met—OMe</td>
<td>Mts</td>
<td>A-1</td>
<td>89</td>
<td>102—105</td>
<td>-10.5°</td>
<td>C$<em>{35}$H$</em>{51}$N$<em>2$O$</em>{14}$S</td>
</tr>
<tr>
<td></td>
<td>Z(OMe)—Gln—Arg—Met—NHNH$_2$</td>
<td>Mts</td>
<td>B-2</td>
<td>69</td>
<td>172—174</td>
<td>C$<em>{35}$H$</em>{51}$N$<em>2$O$</em>{16}$S</td>
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</tbody>
</table>

$a)$ A, extraction procedure; B, without extraction; 1, precipitation from DMF with AcOEt; 2, from DMF with MeOH; 3, from DMSO with MeOH.

Recrystallized or precipitated from appropriate solvents. Yields, physical constants, and analytical data of the hydrazides and their intermediates are listed in Tables II and III.

**Synthesis of Protected Gln$^{15}$-Motilin**—The protected form of Gln$^{15}$-motilin was prepared by successive azide condensation of the seven fragments as shown in Fig. 1. The amount of the acyl component was increased from 1.2 to 2 eq as the chain elongation progressed, as described in the text. Every reaction was performed in DMF at 4 °C until the solution became ninhydrin-negative. After evaporation of the solvent, products were purified by one of the following procedures. Procedure C: The crude product was treated with 5% citric acid and ether. The resulting
TABLE IV. Characteristics of Protected Gln\textsuperscript{15}-Motilin and Its Intermediates

<table>
<thead>
<tr>
<th>Puri \textsuperscript{a}</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
<th>[\eta] \textsuperscript{D} (DMF)</th>
<th>Rf</th>
<th>Formula</th>
<th>Analysis (%)</th>
<th>Calcd</th>
<th>Found</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C H N</td>
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<tr>
<td>Boc-(17-22)-OH</td>
<td>C\textsubscript{1}</td>
<td>63</td>
<td>134-137</td>
<td>-6.0\textdegree</td>
<td>0.60</td>
<td>(3) C\textsubscript{50}H\textsubscript{34}N\textsubscript{12}O\textsubscript{17}S</td>
<td>52.34</td>
<td>6.50</td>
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<td>(52.16</td>
<td>6.49</td>
<td>14.40</td>
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<tr>
<td>Z(OMe)-(14-22)-OH</td>
<td>C\textsubscript{1}</td>
<td>66</td>
<td>212-215</td>
<td>-11.0\textdegree</td>
<td>0.48</td>
<td>(1) C\textsubscript{18}H\textsubscript{108}N\textsubscript{18}O\textsubscript{25}S</td>
<td>53.36</td>
<td>6.34</td>
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<td>(53.36</td>
<td>6.35</td>
<td>14.44</td>
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<td>Z(OMe)-(11-22)-OH</td>
<td>D\textsubscript{1}</td>
<td>68</td>
<td>245-248</td>
<td>-12.3\textdegree</td>
<td>0.53</td>
<td>(3) C\textsubscript{103}H\textsubscript{147}N\textsubscript{25}O\textsubscript{35}S</td>
<td>51.02</td>
<td>6.49</td>
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<td>Boc-(9-22)-OH</td>
<td>C\textsubscript{2}</td>
<td>79</td>
<td>260</td>
<td>-4.6\textdegree</td>
<td>0.58</td>
<td>(3) C\textsubscript{110}H\textsubscript{165}N\textsubscript{27}O\textsubscript{35}S</td>
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<td>Z(OMe)-(6-22)-OH</td>
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<td>142-146</td>
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<td>(3) C\textsubscript{129}H\textsubscript{184}N\textsubscript{30}O\textsubscript{41}S</td>
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<td>(52.40</td>
<td>6.42</td>
<td>13.91</td>
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<tr>
<td>Z(OMe)-(1-22)-OH</td>
<td>D\textsubscript{2}</td>
<td>49</td>
<td>156-160</td>
<td>-14.3\textdegree</td>
<td>0.28</td>
<td>(3) C\textsubscript{10}H\textsubscript{122}N\textsubscript{35}O\textsubscript{46}S</td>
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<td>6.75</td>
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<td>(53.72</td>
<td>6.49</td>
<td>13.29</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Precipitation from DMF with AcOEt (C\textsubscript{1}) or from DMF with MeOH (C\textsubscript{2}). Gel-filtration on Sephadex LH-20 (D\textsubscript{1}) or Sephadex LH-60 (D\textsubscript{2}) using DMF. b) CHCl\textsubscript{3}-MeOH-H\textsubscript{2}O (8:4:1).

powder was washed with 5\% citric acid, 5\% NaHCO\textsubscript{3} and H\textsubscript{2}O and precipitated from DMF with AcOEt or MeOH. Procedure D: The crude product was purified by gel-filtration on Sephadex LH-20 (procedure D\textsubscript{1}) or Sephadex LH-60 (procedure D\textsubscript{2}) using DMF as an eluant. In these cases, individual fractions (8 ml each) were examined by measuring the ultraviolet (UV) absorption at 275 nm. Fractions corresponding to the front main peak were combined and the solvent was removed by evaporation. The residue was treated with ether to afford the product as a powder. Amino acid ratios in 6 n HCl hydrolysates are listed in Table I. Purification procedures, physical constants, and analytical data are listed in Table IV.

H-Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Gln-Lys-Glu-Arg-Asn-Lys-Gly-Glu-OH — The above protected form of Gln\textsuperscript{15}-motilin (50 mg, 14.2 \mu mol) in DMF (1 ml) was treated with phenylthiotrimethylsilane (130 \mu l, 50 eq) and trimethylsilyl trifluoroacetate (6.3 \mu l, 2 eq) under an Ar atmosphere at room temperature for 30 min; the starting material disappeared and a new spot, Rf\textsubscript{i} 0.30, was detected on TLC. The solution, after being neutralized with Et\textsubscript{3}N, was concentrated and the residue was treated with ether to form a powder; yield 50 mg (100\%).

The reduced form of protected Gln\textsuperscript{15}-motilin (49 mg) was treated with 1 M TFA in TFA (0.7 ml) in the presence of m-cresol (38 \mu l, 25 eq) and EDT (13 \mu l, 10 eq) in an ice-bath for 120 min, then dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets in vacuo for 2 h and dissolved in H\textsubscript{2}O (3 ml). The pH of the solution was adjusted to 8.0 with 5\% NH\textsubscript{4}OH and after 30 min to 5.0 with 1 N AcOH. The solution was

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{hplc.png}
\caption{HPLC of Synthetic Gln\textsuperscript{15}-Motilin
\(a\): CM-purified sample, OD\textsubscript{275}. \(b\): Purified sample, OD\textsubscript{275}.}
\end{figure}
applied to a column of Sephadex G-25 (2.3 × 131 cm), which was eluted with 0.5 N AcOH. The fractions (5 ml each, monitored by UV absorption measurement at 275 nm) corresponding to the front main peak (tube Nos. 46–56) were combined and the solvent was removed by lyophilization to give a powder; yield 33 mg (88%).

The crude product thus obtained was purified by ion-exchange chromatography on CM-cellulose (1.6 × 8 cm) using gradient elution with AcONH₄ buffer (0→0.2 M). The fractions (5 ml each) corresponding to the main peak (tube Nos. 17→20, monitored by UV absorption measurement at 275 nm) were combined and the solvent and the salt were removed by repeated lyophilization to give a white powder; yield 12 mg (32%). The CM-purified product (7 mg) was further purified by HPLC on a Cosmosil 5C₁₈ (10 × 250 mm) using gradient elution with acetonitrile (25→28%, 60 min) in 0.1% TFA at a flow rate of 1.5 ml per min (Fig. 3-a). The eluate corresponding to the main peak (retention time 45.6 min) was collected and the solvent was removed by lyophilization to give a white fluffy powder; yield 3.5 mg. The rest of the sample was similarly purified; yield 5.9 mg. The overall yield from the protected peptide was 16% (ε = 68.7 ° (c = 0.1, 1 N AcOH), Rf₅ = 0.21, Rf₆ = 0.19; retention time, 19.8 min in HPLC on an analytical Nucleosil 5C₁₈ column (4 × 150 mm) by gradient elution with acetonitrile (22→32%, 30 min) in 0.1% TFA at a flow rate of 0.8 ml per min (Fig. 3-b). Since the sample was sensitive to air-oxidation, rechromatography of the HPLC-purified sample gave a side peak corresponding to the Met(O)-derivative. Amino acid ratios in a 6 N HCl hydrolysate are shown in Table I. Amino acid ratios in an aminopeptidase (Sigma Lot. L-6007) digest (numbers in parentheses are theoretical): Thr 0.91 (1), Glu 2.00 (2), Pro 0.90 (1), Gly 2.10 (2), Val 0.94 (1), Met 0.93 (1), Ile 0.99 (1), Leu 1.09 (1), Tyr 0.97 (1), Phe 1.94 (2), Lys 1.84 (2), Arg 1.86 (2), Gln and Asn were not determined (recovery of Glu, 77%).

References and Notes


2) Amino acids used in this investigation are of the L-configuration. The following abbreviations are used: Z = benzylxoycarbonyl, Z(OMe) = p-methoxybenzoylcarbonyl, Bzl = benzyl, Tos = p-toluene sulfonyl, Mts = mesitylenesulfonyl, TFA = trifluoroacetic acid, TFMSA = trifluoromethanesulfonyl acid, Np = p-nitrophenyl, Su = N-hydroxysuccinimidyl, DCC = dicyclohexylcarbodiimide, HOBT = N-hydroxybenzotriazole, DMF = N,N-dimethylformamide, DMSO = dimethyl sulfoxide, EDT = ethanedithiol.


