condition relative to a control suspension lysed with 0.05% Triton-X-100 (30 min on ice, centrifuged at 400g, 4 °C). The "% inhibition" of lysozyme release was determined as follows:

$$\% \text{ inhibition} = 100 - [(\% \text{ lysozyme released + drug})/(\% \text{ lysozyme released - drug})]$$

Cyclooxygenase Inhibition Assay. Human platelets (1×10^7 platelets/0.5 mL) in Ca^{2+}-free pH 7 buffer were incubated with test compound 15 min at 37 °C with shaking. [^{14}C]Arachidonic acid (0.25 μCi, 0.155 μg/mL) was added and the mixture incubated another 30 min. The reaction was terminated by addition of 0.025 mL of 0.8 N HCl. The mixture was then extracted with ethyl acetate–methylene chloride (2:3) supplemented with 30 μg/mL cold arachidonic acid to reduce degradation of the metabolites. After concentrating the organic phase, the number of microliters containing 1×10^5 cpm was determined and that volume applied to a silica gel plate. The plate was developed in methylene chloride–methanol–acetic acid–water (90:8:1:0.8), air-dried, and counted on a Berthold linear TLC analyzer. The integrated area of the prostaglandin–thromboxane envelope was determined and compared with the control (no drug).

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Synthesis and Binding Affinities of Analogues of Cholecystokinin-(30–33) as Probes for Central Nervous System Cholecystokinin Receptors

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CCK-30–33 has been identified as the minimum fragment of CCK with nanomolar affinity for the *central* CCK receptors, as assayed by displacement of [^{3}H]-Boc-β-alanyl-CCK-30–33 (pentagastrin) in homogenized mouse cerebral cortex. Examination of binding using this assay in the two series Boc-Trp-X-Phe-NH_2 when X = Met-Asp (Boc-CCK-30–33), Gly-Asp, Met-Gly, and Gly-Gly and when X = $(CH_2)_n$ (n = 0–4) reveals that modification of the tetrapeptide reduces affinity to a maximum of micromolar affinity (Boc-Trp-Gly-Asp-Phe-NH_2; $K_i = 2 \times 10^{-6}$ M), whereas in the series when n = 0 and 2 pentamolar affinity is still retained (Boc-Trp-Phe-NH_2, $K_i = 7 \times 10^{-5}$ M; Boc-Trp NH CH_2-CH_2-CO-Phe-NH_2, $K_i = 3 \times 10^{-5}$ M). Modification of the tetrapeptide CCK-30–33 reduces affinity 1000-fold, whereas di- and tripeptide fragments are identified that reduce affinity only a further 10-fold. This structure–activity relationship establishes a basis to design "peptoid" analogues of CCK that have therapeutic potential.

Several fragments of the polypeptide cholecystokinin (CCK) have been identified in both brain and gut of several species.[1–5] It appears that the C-terminal octapeptide CCK-26–33 retains all peripheral CCK-like activity, including gall bladder contraction, gastric acid secretion, and pancreatic secretion.[6] However, CCK-30–33, the C-terminal fragment common with the gastrin family including CCK, gastrin, and caeurulin, appears to retain these activities but has more selective effects in releasing hormones from endocrine pancreas.[3–7]

Recently, CCK fragments have been found in the brain,[4] and CCK has been implicated as a putative satiety factor.[4,5,8] Specific high-affinity sites have been located in many brain areas in rats and mice, including cerebral cortex.[3,4,9] CCK-26–33, -27–33, and -30–33 have all been shown to bind to these high-affinity sites.[9] It appears that the C-terminal tetrapeptide CCK-30–33 is the minimum fragment required for nanomolar affinity in the brain, but the full octapeptide (sulfated CCK-26–33) is required for nanomolar affinity in pancreatic tissue.[3]

This paper describes some structure–activity work on analogues of CCK-30–33 in order to characterize the chemical features necessary for binding to central nervous system (CNS) receptors, using the mouse brain cerebral cortex binding assay previously described.[9] The results are summarized in Table I.

Chemistry. The dipeptide **11** and the methylene-bridged Boc-Trp-HN$(CH_2)_n$CO-Phe-NH_2 (n = 1–4) analogues **12–15** (see Table I) were synthesized by coupling the *N*-Boc-Trp with the corresponding $H_2N(CH_2)_n$CO-Phe-NH_2 by the mixed-anhydride method with ethyl chloroformate and triethylamine. β-Alanyl-Phe-NH_2 and (δ-aminovaleryl)-Phe-NH_2 were obtained by deprotection of CBZ-β-alanyl-Phe-NH_2 and CBZ-δ-valeryl-Phe-NH_2, respectively, by catalytic hydrogenation over 10% Pd/C in acetic acid. (γ-Aminobutyryl)-Phe-NH_2 was obtained

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Table I. Binding Affinities of Fragments of CCK-26–33 (Compounds 1–10) and Modified CCK-31–33 Analogues (Compounds 11–19)

no.	ligand	binding affinities: K_i, M	n
1	Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	$(3 \pm 0.5) \times 10^{-9}$	5
2	Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2	$(180 \pm 58) \times 10^{-9}$	5
3	Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2	$(2 \pm 0.5) \times 10^{-9}$	4
4	Boc-β-Ala-Trp-Met-Asp-Phe-NH_2	$(0.6 \pm 0.04) \times 10^{-9}$	5
5	Trp-Met-Asp-Phe-NH_2	$(3 \pm 0.1) \times 10^{-9}$	6
6	Met-Asp-Phe-NH_2	$(1.0 \pm 0.2) \times 10^{-4}$	3
7	Asp-Phe-NH_2	inactive at 10^{-3}	3
8	Phe-NH_2	inactive at 10^{-3}	1
9	Trp-Met-Asp-NH_2	inactive at 10^{-3}	3
10	Trp-Met-NH_2	inactive at 10^{-3}	1
11	Boc-Trp-Phe-NH_2	$(7.3 \pm 0.5) \times 10^{-5}$	2
12	Boc-Trp-$NHCH_2CO$-Phe-NH_2	$(1.1 \pm 0.1) \times 10^{-5}$	3
13	Boc-Trp-$NHCH_2CH_2CO$-Phe-NH_2	$(4.5 \bullet 0.8) \times 10^{-5}$	3
14	Boc-Trp-$NHCH_2CH_2CH_2CO$-Phe-NH_2	$(5.3 \pm 0.2) \times 10^{-3}$	1
15	Boc-Trp-$NHCH_2CH_2CH_2CH_2CO$-Phe-NH_2	inactive at 10^{-3}	1
16	Boc-Trp-Met-Asp-Phe-NH_2	$(1.8 \pm 0.2) \times 10^{-9}$	3
17	Boc-Trp-Gly-Gly-Phe-NH_2	$(5.2 \pm 0.2) \times 10^{-3}$	3
18	Boc-Trp-Met-Gly-Phe-NH_2	$(2.4 \pm 0.4) \times 10^{-5}$	3
19	Boc-Trp-Gly-Asp-Phe-NH_2	$(1.6 \pm 0.2) \times 10^{-6}$	3

by condensation of Boc-γ-aminobutyric acid with Phe-NH_2 by the mixed anhydride method, followed by removal of the Boc group with trifluoroacetic acid (TFA).

The Boc-tetrapeptides **16–19** were prepared by stepwise solution coupling and deprotection of commercially available amino acids and dipeptides, by the mixed-anhydride method and TFA, respectively. Compound **17** was prepared by mixed-anhydride condensation of Boc-Trp with Gly-Gly-Phe-NH_2; compound **18** was prepared in six steps by sequential mixed-anhydride condensation of the *N*-Boc-amino acids, starting with Phe-NH_2, and removal of the Boc group with TFA. For the synthesis of compound **19**, Asp-Phe-NH_2 was commercially available, and protection of the aspartic acid side chain was not necessary under the mixed-anhydride conditions.

Biological Results and Discussion

Extensive studies on the role of analogues of the tetrapeptide CCK-30–33 on gastric acid secretion in vivo have implicated the importance of all three Trp, Asp, and Phe side chains in eliciting agonist secretory activity in this peripheral assay.[11-14] It appears that a variety of substituents on the N-terminus may be tolerated, but substitution on the C-terminus is more limited. However, complete removal of the C-terminal amide moiety has been reported to both eliminate[14] and maintain[13] gastric acid secretory activity.

Our results of specific binding of ligands to CCK *central* receptors from mouse cerebral cortex are given in Table I. Affinities are expressed as K_i values with displacement of [^{3}H]-Boc-β-alanyl-CCK-30–33 (pentagastrin) as previously described.[9] Compounds **1** and **3**, CCK-26–33 and CCK-27–33 (both sulfated), show nanomolar affinity, whereas compound **2** (desulfated CCK-26–33) shows a 70-fold decrease in affinity. Interestingly, pentagastrin, Boc-CCK-30–33, and CCK-30–33 (compounds **4**, **16**, and **5**) all retain nanomolar affinity, illustrating the vital role of the C-terminal tetrapeptide in the receptor recognition process and that the N-terminal nitrogen atom of this tetrapeptide fragment is tolerated as the free primary amine or derivatized in either an amide or cabamate bond. The tri- and dipeptide and amino acid amide fragments of CCK-30–33 (compounds **6–10**) have at least 10^5-fold less affinity than the tetrapeptide. These data suggest the Trp and Phe residues *together* are important for high affinity if the primary structural features alone are considered.

In order to test the hypothesis that both Trp and Phe residues were necessary for high-affinity binding, the dipeptide **11** and the methylene-bridged Trp-NH-$(CH_2)_n$CO-Phe-NH_2 derivatives (compounds **12–15**), when n = 1–4, were prepared. Modest affinity was found with compounds **11–13**. Interestingly, the distance through the bonds of compound **15** corresponds most closely with that in CCK-30–33 ($-CH_2CH_2CH_2CH_2-$ = 4.62 Å; $-CHCONHCH-$ = 4.31 Å), yet this compound is at least 100-fold less active than the dipeptide **11** or the ethane-bridged analogue **13** and at least 10^6 less active than CCK-30–33. This may be rationalized in several ways, for example, (i) the importance of the amide peptide bonds in determining a folded secondary structure (e.g., a stabilized β-bend), (ii) omission of the Met-Asp side chains does not favor optimum orientation of the Trp and Phe residues at the receptor, (iii) a cooperativity between all four amino acid side chains, (iv) the large difference in lipophilicity of the bridging groups in the peptide and the methylene isosteres (log *P* CH_2CH_2 = +1.54, log *P* CONH (aliphatic) = −2.18), which would decrease favorable receptor affinity.

We therefore synthesize the three tetrapeptides **17–19** in order to gain more insight into the relative contribution of each amino acid residue to the overall binding affinity. Compound **17** has the Met-Asp residues of Boc-CCK-30–33 **16** replaced by a Gly-Gly linkage and showed minimal affinity ($K_i = 5 \times 10^{-3}$ M). Modest affinity is restored by replacing the Gly with the Met residue (**18** $K_i = 2.7 \times 10^{-5}$ M), but micromolar affinity is seen when the Asp group is replaced (**19**, $K_i = 2 \times 10^{-6}$ M).

These binding data for compounds **16–19** indicate an important role for *both* Met and Asp side chains, the Asp

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perhaps being more important, each increasing affinity by 10-fold and 100-fold over compound **17**, respectively. However, the full tetrapeptide structure **16** imparts a further 1000-fold increased affinity over compound **19**. Our results indicate an important *cooperative* role for the four amino acid side chains for optimum binding affinity and that secondary structural features must contribute to this cooperativity. NMR (**19**), X-ray (**15**), ORD (**16**), and theoretical studies (**17**) are not in agreement on the overall secondary structure of CCK-30–33, but do suggest some rigidity around the Trp residue, flexibility of the Asp chain, and the molecule exhibiting some folding to bring the Trp and the aromatic rings to within 5–8 Å of each other. Examination of Dreiding models shows that the Boc dipeptides **11** and **13**, each with pentamolar affinity, can overlay the Trp indole and Phe benzene rings on this folded conformation of CCK-30–33.

We are continuing to explore the design of "peptoid"[18] analogues of CCK-30–33, where the Trp, Asp, and Phe side chains are considered important components. Molecular graphics and the three ligand analysis[18] are in progress to determine optimal orientation of the amino acid chains, in order to produce potentially therapeutically useful CCK-like ligands.

Experimental Section

Melting points were determined on a Reichart Köfler-block and are uncorrected. Reactions were followed by thin-layer chromatography (Merck silica gel GF-254 plates) and developed by a methylene chloride–methanol–concentrated ammonia mixture (19:2:1) and developed by spraying with aqueous potassium permanganate solution. All intermediates and final products were routinely monitored by TLC, NMR and IR spectroscopy, and final products were purified on the Harrison chromatotron, in the solvent system described above, before biological testing. All final products were homogeneous by TLC and had IR and NMR consistent with their structures. IR spectra were recorded on a Perkin-Elmer 1420 ratio recording instrument in a Nujol mull or chloroform solution and NMR spectra were recorded on a JEOL PMX-60 and Bruker WP 200 NMR spectrometer in $CDCl_3$, Me_2SO-d_6, D_2O, and MeOD-d_3, and the spectra for compounds **16–18** were compared with published proton NMR data on CCK-30–33 fragments.[10,11] Compounds **1–11** were obtained from commercial sources and used without further purification.

(*tert*-Butyloxycarbonyl)-L-tryptophylglycyl-L-phenylalanine Amide (12). A solution of *N*-carbobenzoxyglycyl-L-phenylalanine amide (1.78 g) in acetic acid (20 mL) was hydrogenated at 60 °C for 1.5 h over 10% Pd/C. The solution was filtered and evaporated to give glycyl-L-phenylalanine amide acetate as an oily solid (0.84 g, 60%), which was used directly in the next step.

The mixed anhydride of *N*-(*tert*-butyloxycarbonyl)-L-tryptophan was prepared from ethyl chloroformate (180 μL), triethylamine (297 μL), and *N*-(butyloxycarbonyl)-L-tryptophan (0.644 g). A solution of the crude glycyl-L-phenylalanine acetate (described above), THF (5 mL), water (2 mL), and triethylamine (297 μL) was added, and the solution was stirred for 1 h. Water (20 mL) and ethyl acetate (40 mL) were added, the mixture was shaken, and the organic layer was dried and evaporated to dryness. Tituration with methylene chloride, methanol, and ammonia gave a white solid, which was filtered to give the product as a white solid (0.53 g, 50%): ^1H NMR ($(CD_3)_2SO$–D_2O) δ 1.16 (s, *tert*-butyl), 2.4–4.6 (m, 8-H), 7.0–7.7 (m, 10-H, Trp, Phe); mp 125–129 °C; $[\alpha]^{20}_D$ (MeOH) +6.67°. Anal. ($C_{27}H_{33}N_5O_5 \cdot 0.2H_2O$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl-β-alanyl-L-phenylalanine Amide (13). Carbobenzoxy-β-alanyl-L-phenylalanine amide (1.1 g) was hydrogenated in acetic acid (25 mL) at 60 °C over 10% Pd/C. Filtration and evaporation gave **β-alanyl-L-phenylalanine amide acetate** as a white solid (1.57 g, 66%, mp 105 °C). This crude product was condensed with *N*-(*tert*-butyloxycarbonyl)-L-tryptophan (0.304 g) by the mixed-anhydride method, as described for **12** above, to give **13** as a white solid (0.25 g, 48%): mp 196.5 °C; $[\alpha]^{20}_D$ (MeOH) +6.24°; ^1H NMR ($(CD_3)_2SO$–D_2O) δ 1.2 (s, *tert*-butyl), 2.1–4.5 (m, 10-H), 6.9–7.6 (m, 10-H, Trp, Phe). Anal. ($C_{28}H_{35}N_5O_5 \cdot 2.5H_2O$) C, N; H: calcd, 7.12; found, 6.28.

(*tert*-Butyloxycarbonyl)-L-tryptophyl(γ-aminobutyryl)-phenylalanine Amide (14). *N*-(*tert*-butyloxycarbonyl)-γ-aminobutyric acid (1.18 g) was condensed with phenylalanine amide by the mixed-anhydride method described above, to give ***N*-(*tert*-butyloxycarbonyl)(γ-aminobutyryl)phenylalanine amide** (1.76 g) as a white solid. The crude product was stirred in methylene chloride (10 mL) at 0 °C, and TFA (1 mL) was added. The solution was stirred for 1 h and then evaporated to give **(γ-aminobutyryl)-L-phenylalanine amide** as an oily solid (1.17 g, 64% over two steps).

The mixed anhydride of Boc-Trp (0.608 g) and ethyl chloroformate (170 μL) was prepared as described above with Et_3N (280 μL). The crude (γ-aminobutyryl)-L-phenylalanine amide TFA salt was added in a solution of water (4 mL) and triethylamine (280 μL). Workup as described above gave an oil, which solidified on tituration with ethyl acetate to give the product **14** as a white solid (0.57 g, 49%): mp 162–164 °C; $[\alpha]^{20}_D$ (MeOH) +9.92°; ^1H NMR ($(CD_3)_2SO$ + $CDCl_3$) δ 1.4 (s, *tert*-butyl), 1.4–4.4 (m, 12-H), 6.8–7.9 (m, 10-H, Trp, Phe). Anal. ($C_{29}H_{37}N_5O_5 \cdot 0.5H_2O$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-tryptophyl(δ-aminovaleryl)-L-phenylalanine Amide (15). *N*-Carbobenzoxy(δ-aminovaleryl)-L-phenylalanine amide (0.26 g) was hydrogenated in acetic acid (10 mL) over 10% Pd/C at 60 °C for 1 h. The solution was filtered and evaporated to yield (δ-aminovaleryl)-L-phenylalanine amide as an oil (0.23 g).

The mixed anhydride of Boc-Trp (0.304 g) and ethyl chloroformate (85 μL) was prepared as described for **14**. The crude (δ-aminovaleryl)-L-phenylalanine amide was added, and the solution was stirred for 1 h. Workup as described above gave **15** (0.27 g, 48% over two steps) as a white solid on tituration with ethyl acetate followed by filtration and drying in vacuo: mp 136–141 °C; $[\alpha]^{20}_D$ (MeOH) +6.55°; ^1H NMR ($(CD_3)_2SO$ + $CDCl_3$) δ 1.38 (s, *tert*-butyl), 2–4.9 (m, 14-H), 6.9–8.1 (m, 10-H). Anal. ($C_{30}H_{39}N_5O_5 \cdot 0.5H_2O$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-L-tryptophylglycylglycyl-L-phenylalanine Amide (17).** Carbobenzoxyglycylglycine (0.266 g) was condensed with phenylalanine amide by the mixed-anhydride method described above to give **carbobenzoxyglycylglycyl-L-phenylalanine amide** as a white solid (0.197 g, 48%). This reaction was repeated on a 5× scale to give 1.23 g of product, which was used without further purification. A solution of the protected tripeptide (1.2 g) in acetic acid (20 mL) was hydrogenated over 10% Pd/C at 60 °C for 1 h. The solution was filtered and evaporated to give **glycylglycyl-L-phenylalanine amide diacetate** as a white solid (0.98 g, 86%) on tituration with ether, filtration, and drying in vacuo. The crude tripeptide diacetate was condensed with Boc-Trp (0.304 g) under the mixed-anhydride conditions described above, to give an oily solid, which was chromatographed through a short column to remove base-line material. Evaporation of this eluate gave **17** as a white solid (0.14 g): mp 126–139 °C; $[\alpha]^{20}_D$ (MeOH) +8.90°, ^1H NMR ($(CD_3)_2SO$ + D_2O) δ 1.28 (s, *tert*-butyl), 3.15 (m, C-β-H for Trp; Phe), 4.2–4.5 (m, C-α-H for Gly, Gly, Trp, Phe), 7.0–7.3 (m, 9 H, Trp, Phe), 7.4 (d, indole C-2-H). Anal. ($C_{29}H_{36}N_6O_6 \cdot 2H_2O$) C, H; N: calcd, 14.01; found, 14.80.

***N*-(*tert*-Butyloxycarbonyl)-L-tryptophyl-L-methionylglycyl-L-phenylalanine Amide (18).** Phenylalanine amide (1.64 g) was condensed with Boc-glycine (1.75 g) by the mixed-anhydride method described above, to give **(*tert*-butyoxycarbonyl)-glycyl-L-phenylalanine amide** as a white solid (2.43 g, 76%). The crude product was suspended in methylene chloride (20 mL) and cooled to 0 °C, and TFA (2 mL) was added. The solid dissolved on being stirred. The resulting solution was warmed to ambient temperature and another 2 mL of TFA was added. After 1 h, the solution was evaporated and excess TFA azeotroped with ether to give **glycyl-L-phenylalanine amide** as a white solid (2.15 g).

The crude glycylphenylalanine amide TFA salt (2.15 g) was condensed with Boc-methionine (1.59 g) by the mixed-anhydride method described above to give **(*tert*-butyloxycarbonyl)-L-methionylglycyl-L-phenylalanine amide** as a hygroscopic white solid (1.94 g).

A suspension of the crude Boc-methionylglycylphenylalanine amide (1.94 g) in dichloromethane (20 mL) was stirred at ambient temperature and TFA (3 mL) was added dropwise. The solution was stirred for 1 h, evaporated to dryness, and triturated with ether to give **L-methionylglycyl-L-phenylalanine amide** as a very hygroscopic white solid (0.65 g). The crude product was condensed with Boc-tryptophan (0.425 g) by the mixed-anhydride method described above, to give **18**, which was purified on a Harrison chromatotron using methylene chloride, ethanol, and ammonia (19:2:1) (0.174 g): mp 178–181 °C; $[\alpha]^{20}_D$ (MeOH) −10.16; ^{1}H NMR ($(CD_3)_2SO$) δ 1.3 (s, *tert*-butyl), 2.03 (s, SCH_3, Met), 3.7 (m, C-α-H, Gly), 4.45 (m, C-α-H, Phe), 4.30 (m, C-α-Met), 1.90 (m, C-β-H, Met), 2.45 (m, C-γ-H, Met), 2.8–3.2 (m, C-β-H for Trp, Phe), 7.55 (d, indole C-2-H). Anal. ($C_{32}H_{42}N_6O_6S\cdot0.5H_2O$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-L-tryptophylglycyl-L-aspartyl-L-phenylalanine Amide (19).** Glycine (1.5 g) in aqueous sodium hydroxide solution (0.8 g in 2 mL of water) was condensed with Boc-Trp (3.04 g) by the mixed-anhydride method described above, to give Boc-trptophylglycine (1.06 g, 29%), which was used without further purification.

L-Aspartyl-L-phenylalanine amide hydrate (0.088 g) was condensed with crude Boc-trptophylglycine by the mixed-anhydride method described above, to give Boc-tryptophylglycylaspartylphenylalanine amide, which was recrystallized from ethyl acetate–methylene chloride as a white solid (0.035 g, 19%): mp 143–154 °C; $[\alpha]^{20}_D$ (MeOH) −24.74°; ^{1}H NMR ($(CD_3)_2SO$) δ 1.38 (s, *tert*-butyl), 3.78 (q, C-α-H-Gly), 4.05 (m, C-α-H-Asp), 4.39 (m, C-α-H-Phe), 2.4–3.4 (m, C-β-H for Trp, Asp, Phe), 6.9–7.4 (m, 9-H, Trp, Phe), 7.6 (d, indole C-2-H). Anal. ($C_{31}H_{38}N_6O_8\cdot1.5H_2O$) C, H, N.

Registry No. **1**, 25126-32-3; **2**, 25679-24-7; **3**, 20988-64-1; **4**, 5534-95-2; **5**, 1947-37-1; **6**, 5934-92-9; **7**, 5241-71-4; **8**, 5241-58-7; **9**, 90736-02-0; **10**, 97533-28-3; **11**, 33900-27-5; **12**, 63069-11-4; **13**, 106651-39-2; **14**, 106651-40-5; **15**, 106651-41-6; **16**, 5235-21-2; **17**, 88463-50-7; **18**, 17338-87-3; **19**, 106651-42-7; Boc-Trp, 13139-14-5; *N*-carbobenzoxyglycyl-L-phenylalanine amide, 5513-69-9; glycyl-L-phenylalanine amide acetate, 13467-26-0; carbobenzoxy-β-alanyl-L-phenylalanine amide, 17471-89-5; β-alanyl-L-phenylalanine amide, 106757-00-0; *N*-(*tert*-butyloxycarbonyl)-γ-aminobutyric acid, 57294-38-9; *N*-(*tert*-butyloxycarbonyl)-(γ-aminobutyryl)phenylalanine amide, 106651-43-8; (γ-aminobutyryl)-L-phenylalanine amide, 106651-45-0; *N*-carbobenzoxy-(δ-aminovaleryl)-L-phenylalanine amide, 106651-46-1; (δ-aminovaleryl)-L-phenylalanine amide, 106651-48-3; carbobenzoxyglycylglycine, 2566-19-0; carbobenzoxyglycylglycyl-L-phenylalanine amide, 75501-76-7; glycylglycyl-L-phenylalanine amide diacetate, 106651-49-4; Boc-glycine, 4530-20-5; (*tert*-butyloxycarbonyl)-glycyl-L-phenylalanine amide, 33900-05-9; glycyl-L-phenylalanine amide (TFA salt), 97587-48-9; Boc-methionine, 2488-15-5; (*tert*-butyloxycarbonyl)-L-methionylglycyl-L-phenylalanine amide, 60058-46-0; L-methionylglycyl-L-phenylalanine amide, 106651-51-8; glycine, 56-40-6; Boc-tryptophylglycine, 25691-58-1; L-aspartyl-L-phenylalanine amide, 5241-71-4; cholecystokinin, 9011-97-6.

A Novel Synthesis of Colchicide and Analogues from Thiocolchicine and Congeners: Reevaluation of Colchicide as a Potential Antitumor Agent

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Desulfurization of thiocolchicine with Raney nickel in a hydrogen atmosphere yielded tetrahydromethoxycolchicine (**2**), which was readily separated from unreacted thiocolchicine by chromatography and was smoothly oxidized to 10-demethoxycolchicine (colchicide) by Pd/C in refluxing toluene. Several analogues of colchicide were prepared from the corresponding thiocolchicines by this procedure. Treatment of colchicide with concentrated sulfuric acid yielded 2-demethylcolchicide. Colchicide and its analogues were found to be inactive in a tubulin-binding assay. Evidence is presented that colchicide prepared earlier from thiocolchicine with Raney nickel in aerial atmosphere was contamination with 1–2% thiocolchicine.

Methylthiocolchicide, commonly named thiocolchicine (**1**),[1-3] loses the methylthio group at C-10 when treated with Raney nickel catalyst in acetone under a hydrogen atmosphere, to afford tropone **3** named colchicide.[2-4] Reports that colchicide (**3**) binds well to tubulin in vitro[2-4] and showed, in addition, considerable activity in the P388 lymphocytic leukemia screen[4] prompted an extension of our earlier work to include a similar study of analogues of colchicide. In the course of these investigations, we found that the previously reported activity of **3** was attributable to a small amount of contamination by thiocolchicine (**1**). We now report results of such an investigation.

Chemistry

The procedure[2,4] that we previously used to remove the methylthio group in **1** to produce colchicide (**3**) with Raney nickel catalyst in an aerial atmosphere was not satisfactory in our hands, produced erratic results, and usually afforded besides **3** hydrogenated congeners of 10-demethoxycolchicine, which had to be removed by chromatography. Reductive desulfurization of thiocolchicine (**1**) over Raney nickel catalyst under a hydrogen atmosphere, on the other hand, afforded the well-known 10-demethoxy-8,10,11,12-tetrahydrocolchicine (**2**)[2,5,6] accompanied by another tetrahydro compound, probably the C-12,C-12a dehydro

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(1) To avoid the complicated *Chemical Abstracts* nomenclature for 10-demethoxycolchicine = colchicide = *N*-(5,6,7,9-tetrahydro-1,2,3-trimethoxy-9-oxobenzo[*a*]heptalen-7-yl)acetamide, we have named the novel analogues described here as colchicides, derived from the name colchicide given to tropone **3** and with the numbering indicated in its formula. In this way colchicides nicely correspond to their relatives of the colchicine family of compounds = colchicinoids. We prefer the name thiocolchicine over that of 10-thiomethylcolchicide, used by others in earlier communications.
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