better predictor of pK_m than eq 7. The use of σ^+ did not improve the correlation. Thus it was not possible to demonstrate any dependence of the reaction on electronic effects.

The demonstration of a decrease in K_m with an increase in log P is especially interesting in view of the recent studies by Lu, *et al.*,[15] on the subfractionation of the microsomal NADPH oxidase system. These workers have separated the microsomes into 3 components which are necessary for oxidation: (1) a NADPH-dependent reductase, (2) cytochrome P450 (which binds the substrates), and (3) a heat-stable, lipid fraction. The lipid fraction is not necessary for substrate *binding* but is necessary for *oxidation* of the substrate.

It seems likely that the increase in pK_m with increasing log P can be translated to *in vivo* rate and/or extent of metabolism. For example, it is known that pentobarbital is extensively and rather quickly metabolized whereas barbital is largely excerted unchanged.[16]

These structure–activity relationships observed with the nonspecific microsomal oxidases are different from those previously reported for three other common pathways of drug metabolism; oxidative deamination by monoamine oxidase and the formation of glucuronide and glycine conjugates.[17] For these reactions there was evidence of an optimum log P of approximately 2. Table I includes compounds with log P values ranging from −0.07 to 3.64; no evidence of an optimum log P is seen. In the cases of oxidative deamination and glucuronide formation there was also evidence of a steric requirement for maximal oxidation. As noted above no such effect was seen with the microsomal oxidases.

The relationships discussed above may help explain why it is not always possible to predict *in vivo* pharmacological activity of a series of drugs from *in vitro* studies. For example, *in vitro* there may be no dependence of activity on log P. Thus two drugs might appear equally active *in vitro* but differ dramatically *in vivo* when the difference in metabolic rates must also be considered. The medicinal chemist should remember that it may be possible to alter the rate of metabolism of a "lead" compound by synthesizing a derivative with a different partition coefficient.

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Analogs of Bradykinin Containing β-2-Thienyl-L-alanine[1]

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Three analogs of bradykinin containing β-2-thienyl-L-alanine in place of Phe were synthesized by the solid phase method: [5-thienylalanine]-, [8-thienylalanine]-, and [5,8-bis(thienylalanine)]bradykinin. These analogs were more active than bradykinin in the rat uterus and rat blood pressure assays.

The problem of finding a generally applicable rule for the synthesis of antimetabolites of peptide hormones is as yet unsolved. Many structural analogs of these hormones have been synthesized, but the relatively few examples of antihormonal activity found do not seem to exemplify any general rule for structural modification of the parent compounds for the purpose of producing inhibitors. Among the analogs of bradykinin described,[2] there are only a few peptides with any antibradykinin activity. The biological properties of these peptides leave much to be desired for a good antibradykinin.

One logical approach to the synthesis of peptide antimetabolites would be the incorporation into peptides of amino acids which in other systems have been found to be antimetabolites of the amino acids they replace. This approach was followed by Nicolaides, *et al.*,[3] who synthesized [8-*p*-fluoro-L-phenylalanine]-bradykinin, but they found it to have only bradykinin-like activity; indeed, its activity was higher than that of bradykinin itself. A likely candidate for replacement of phenylalanine would be β-(2-thienyl)alanine, which has long been recognized as having antiphenylalanine activity in both microbial and mammalian systems.[4,5] β-(2-Thienyl)alanine has been used in the synthesis of several di- and oligopeptides.[6–9] Dunn and coworkers have shown that under their testing conditions most thienylalanine-containing peptides are much more potent inhibitors of bacterial growth than is

(1) This work was aided by U. S. Public Health Service Grant HE12325 and Population Council Grant M70.64C (JMS) and a grant from The Robert A. Welch Foundation of Houston, Texas (FWD). All optically active amino acid residues are of the L configuration. Abbreviations used are: Boc = *tert*-butyloxycarbonyl; Thi = β-(2-thienyl)alanine; TFA = trifluoroacetic acid.

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TABLE I

CHARACTERISTICS OF THIENYLALANINE-CONTAINING BRADYKININ ANALOGS

Peptide	Electrophoretic mobility[a]	Distribution coefficient (CCD)	Amino acid composition, moles per mole of peptide					
			Arg	Pro	Gly	Ser	Phe	Thi
[Thi[3]]bradykinin	0.69	0.66	1.84	2.83	1.00	0.92	0.89	1.08
[Thi[5]]bradykinin	0.68	0.77	1.96	3.00	1.00	0.92	0.95	0.87
[Bis(Thi[5,8])]bradykinin	0.69	0.63	1.97	2.95	1.00	0.92		1.70

[a] Distance peptide moved divided by distance arginine moved.

TABLE II

BIOLOGICAL ACTIVITIES OF [THIENYLALANINE]BRADYKININS COMPARED WITH BRADYKININ

Compound	Rat uterus[a]	Guinea pig ileum[a]	Rat blood pressure[b]		Pulmonary inactivation,[c] %
			ia	iv	
Bradykinin	1	1	0.1	3.0	97
[Thi[5]]bradykinin	2	0.4	0.1	2.0	95
[Thi[3]]bradykinin	3–5	0.4	0.1	2.0	95
[Bis(Thi[5,8])]bradykinin	5–10	2	0.15	3.0	95

[a] Activity of analogs compared with bradykinin, based on the dose required to cause a half-maximal isotonic contraction. [b] Dose (in μg) required to effect a depressor response of 20–25 mm of Hg by the intraaortic (ia) and iv routes. [c] Percentage of an iv dose destroyed by a single passage through the pulmonary circulation in the rat.

thienylalanine itself. Although thienylalanine has not been used to replace Phe in a peptide hormone, it was used to replace histidine in angiotensin II,[10,11] and to replace tyrosine in a hexapeptide fragment of angiotensin II.[12] Details of the synthesis and properties of these [thienylalanine]angiotensins were not given; they had little or no biological activity in the studies reported. Neither of these syntheses represented a test of the effect on a peptide hormone of a classical isosteric ring replacement. In the first case thiophene has long been recognized as being isosteric with benzene,[13] not with imidazole. In the second case, not only is thienylalanine not an antimetabolite of tyrosine,[14] but the Tyr-containing hexapeptide itself is practically devoid of angiotensin II activity. It should be pointed out that thienylalanine does not contain the thiazole ring as was stated in one report.[11]

In order to establish the effect of replacing Phe with β-(2-thienyl)alanine in a peptide of known biological activity, 3 new bradykinin analogs were synthesized in which the two Phe residues were systematically replaced by β-(2-thienyl)-L-alanine: [5-thienylalanine]bradykinin, [8-thienylalanine]bradykinin, and [5,8-bis(thienylalanine)]bradykinin. The nonapeptides were synthesized by the Merrifield solid phase method,[15] using both manual and automatic procedures.[16]

Certain difficulties were encountered when standard chemical procedures were applied to peptides containing the thiophene group. When peptides were cleaved from the resin by treatment with HBr in CF_3CO_2H, subsequent hydrogenation for deprotection of nitroarginine was extremely slow. On the other hand, the use of anhyd HF for simultaneous cleavage of the peptide from the resin and deblocking of nitroarginine gave satisfactory products. Thienylalanine in the bradykinin analogs was partially destroyed by the usual acid hydrolysis, but better amino acid analyses were obtained from peptides hydrolyzed in HCl containing PhOH and mercaptoethanol.[17] Chemical properties of the peptides synthesized are reported in Table I.

Biological Activity.—The results of the biological comparisons of the 3 bradykinin analogs are shown in Table II. In some of the assays the analogs showed greater bradykinin-like activity than did bradykinin itself. No antibradykinin activity was seen in any system. In the rat uterus assay [5,8-bis(thienylalanine)]-bradykinin was as much as 10 times as active as bradykinin, making it the most potent bradykinin analog known in this system. The other 2 analogs were less active than this one, but they were still more active than bradykinin.

In the rat blood pressure study, 2 of the analogs were almost identical with bradykinin in potency by the intraaortic route, while the third, [5,8-bis(thienylalanine)]bradykinin, was slightly less active. The analogs were destroyed to the extent of about 95% (somewhat less than the 98% typical for bradykinin[18]) by one passage through the pulmonary circulation, causing them to be more potent than bradykinin by the iv route of administration.

The enhanced bradykinin activity observed in the thienylalanine-containing analogs is reminiscent of that reported for [8-fluorophenylalanine]bradykinin. Both kinds of analogs represent isosteric ring replacements and substantiate the classical concept that the thienyl and FC_6H_4 groups are very similar to Ph.

Numerous drugs have been modified in structure through replacement of the benzene ring with the thiophene ring, often with improvement of biological activity.[19,20] The presence of the thiophene group may

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allow more effective penetration of the molecule to the site of action, or the molecular conformation of the analog may be favorably altered to allow more effective interaction with receptor sites. Another possibility is that the presence of the thiophene ring may make the peptides resistant to kininases. This was indeed the case in the pulmonary circulation, which is the primary site of kininase action *in vivo*. However, since the rat uterus is thought to possess little or no kininase, this can hardly be the explanation of the very high potency observed in that tissue.

In an attempt to shed further light on the question of the high activity of the thienylalanine analogs, the effect of "bradykinin potentiating factor" was examined. PyroGlu-Lys-Trp-Ala-Pro (BPP_{5a}) is a peptide which occurs in the venom of the snake *Bothrops jararaca*, and which potentiates the action of bradykinin on a number of assay systems, including guinea pig ileum.[17] The mechanism of the potentiation of the hypotensive effect of bradykinin has been shown to be inhibition of at least one of the pulmonary kininases. It potentiates the action of bradykinin on guinea pig ileum, a tissue rich in kininases, but not on rat uterus, which has no demonstrable kininase activity. The ability of BPP_{5a} to potentiate the action of the [thienylalanine]bradykinins on ileum was essentially identical to its ability to potentiate the action of bradykinin on that tissue. This would seem to indicate that the thienylalanine analogs are not particularly resistant to kininases in that tissue. It should not be assumed, however, that this experiment provides an unequivocal answer to the question. Certain of the features of the action of BPP_{5a} are difficult to explain on the basis of kininase inhibition. It has been suggested that BPP_{5a} may modify kinin receptors or displace bradykinin from nonspecific binding sites. Further investigations of this phenomenon are under way at the present time.

Experimental Section[21]

Boc amino acids were obtained from Schwarz BioResearch, with the exception of Boc-thienylalanine, which is described below.

Boc-β-(2-thienyl)-L-alanine.—*tert*-Butyl carbazate (16.5 g) was diazotized and coupled with 8.5 g of β-(2-thienyl)-L-alanine[5] by the method of Schwyzer, *et al.*[22] The product was obtained as an oil which slowly crystd after several weeks; yield, 8.8 g (79%). A sample was recrystd from Et_2O–hexane; mp 79°; $[\alpha]^{24}D$ + 25° (*c* 2, 95% EtOH). *Anal.* ($C_{12}H_{17}NO_4S$) C, H, N.

Solid Phase Synthesis of Peptides.[15]—Boc-nitroarginine was esterified to 2% cross-linked chloromethyl resin by the standard procedure. For automatic synthesis, an amount of Boc-nitroarginine resin was used which contd 0.4 mmole of arginine. The Boc protecting group was removed at each stage of the synthesis by a 30-min treatment with 4 *M* anhyd HCl in peroxide-free dioxane. The α-amine hydrochloride form of the peptide resin was neutralized by treatment with 10% Et_3N in $CHCl_3$ for 10 min. The Boc amino acids (1.0 mmole) were coupled to the peptide resin with DCI (1.0 mmole) in $CHCl_3$, using a 2-hr reaction time. Because of its low solubility, Boc-nitroarginine was coupled in 1:1 DMF–$CHCl_3$. After assembly of the peptide on the resin was complete, the peptide–resin was treated with 4 *M* HCl-dioxane for 30 min, washed successively with dioxane, EtOH, and CH_2Cl_2, and finally was dried *in vacuo*. Peptides were deblocked and cleaved from the resin by treatment with anhyd HF (10 ml/g of resin) in the presence of anisole (50 moles/mole of peptide) at 0° for 30 min. After thorough evaporation of the HF *in vacuo*, the resin and peptide mixt was extd with EtOAc to remove the last traces of anisole and its reaction products. Peptides were extd from the resin with H_2O and were dried by lyophilization.

The peptides were purified by countercurrent distribution for 100 transfers in *n*-BuOH–1% TFA (1:1). Homogeneity of the peptides was demonstrated by paper electrophoresis in pyridine–AcOH buffer (pH 5, 0.1 *M* in pyridine); the spots were visualized with ninhydrin and Sakaguchi reagents. Peptides for amino acid analyses were hydrolyzed in sealed N_2-flushed tubes for 22 hr in 6 *N* HCl contg 1 mg/ml each of PhOH and 2-mercaptoethanol.

Bioassays.—Rat uterus assays were done on tissue from estrus virgin females suspended in oxygenated de Jalon's soln which contd only 20 mg/l. of $CaCl_2$.[23] Isotonic contractions were measured under 1-g load. Guinea pig ileum from animals starved overnight was suspended in oxygenated atropinized Tyrode's soln; measurements were made as above.

Rat blood pressure measurements were made on male rats (Sprague–Dawley, 180–250 g) fasted for 18–20 hr and prepd by anesthetization with allobarbital, 1.3 ml/kg, ip (each 100 ml contd 10 g of diallylbarbituric acid, 40 g of urethane, and 40 g of monomethyl urea). The animals were tracheotomized and heparinized (10 mg/kg, iv). The left jugular vein was cannulated with 12 cm of polyethylene tubing (Intramedic PE-10) for iv injection (afferent to lungs); another 12-cm cannula (PE-50 or PE-10) was placed in the ascending aorta through the right carotid artery until its tip lay just above the aortic valve for intraarterial injection (efferent from lungs). Systemic arterial blood pressure was recorded on a Grass Model 7 polygraph by a Statham force transducer P23-DC connected to a femoral artery cannula.

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