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Synthesis of [1-Isoleucine-, 3-Proline-, and 5-Alanine]-angiotensins. II*

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ABSTRACT: [1-Isoleucine,5-isoleucine]-angiotensin II and [3-proline,5-isoleucine]-angiotensin II were synthesized by the solid-phase method using dicyclohexylcarbodiimide as the condensing agent. The formation of the arginyl-proline bond was extremely difficult under conditions used. The former had about 25% pressor and 50% oxytocic activities giving further evidence the acidity of the β -carboxyl is unnecessary. The latter possessed 40% pressor and 80% oxytocic activities of the parent angiotensin. This relatively high biological

activity was surprising because of the limitation on possible peptide conformations imposed by this cyclic amino acid.

[5-Alanine]-angiotensin II was prepared by solid phase using *N*-ethyl-5-phenylisoxazolium-3-sulfonate as the condensing agent. This peptide possessed approximately 5% of pressor activity of angiotensin II indicating the importance of branched side chain of valine or isoleucine occurring naturally in this position.

The β -carboxyl group of aspartic acid in angiotensin II is not essential for biological activity since its replacement by an amide group produced little change in biological activity (Rittel *et al.*, 1957; Schwyzer *et al.*, 1957). However, with β -aspartyl-angiotensin II, the side chain is lengthened by one methylene group and the duration of the pressor response is increased about 50% from that of the natural isomer (Brunner and Regoli, 1962; Regoli *et al.*, 1963). Since this apparent increase in pressor activity may be due to resistance to angiotensinase, it was of interest to determine the effect of a hydrophobic side chain at position 1 in angiotensin II. Position 3 of angiotensin II seems to have little side-chain specificity (Schwyzer *et al.*, 1957; Schwyzer, 1961; Khosla *et al.*, 1967a,b); therefore, proline was substituted here to modify the conformation of the peptide and determine the effect of this modification on biological activity.

Position 5 of angiotensin plays some role in biological activity, possibly because of binding of the aliphatic side chain on to the receptor protein as has been suggested for oxytocin (Rudinger and Krejci, 1962; Nesvadba *et al.*, 1963). Position of branching of side chain

rather than its length appears to affect the activity. Thus, while 5-valine and 5-isoleucine, both with the branching at C₃, have similar activity, shifting the branching from C₃ to C₄ in leucine reduces the activity to 25% (Schwyzer and Turrian, 1960). Complete removal of all side-chain branching by replacing isoleucine with alanine in position 5 should further prove its importance.

Several workers have reported difficulties when using dicyclohexylcarbodiimide as a coupling reagent with amino acids containing an unprotected hydroxyl group (Bodanszky and Ondetti, 1966) or with amino acids such as asparagine and glutamine (Gish *et al.*, 1956; Ressler, 1956; Liberek, 1962; Paul and Kende, 1964; Kashelkar and Ressler, 1964). For these reasons we wanted to test the usefulness of Woodward's reagent K (*N*-ethyl-5-phenylisoxazolium-3-sulfonate) in solid-phase peptide synthesis using [5-alanine]-angiotensin II as the model peptide even though it contained neither of these amino acids.

Results and Discussion

The octapeptides [1,5-diisoleucine]-angiotensin II and [3-proline,5-isoleucine]-angiotensin II were synthesized by stepwise addition of succeeding amino acids to *t*-Boc-phenylalanine resin as described by Marshall and Merrifield (1965). The total amount of *t*-Boc-phenylalanine esterified to the polymer was determined by weight increase and by spectrophotometric estimation

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of unreacted phenylalanine in the filtrate (Khosla *et al.*, 1967a,b). The cycle for each amino acid consisted of the removal of the *t*-butyloxycarbonyl group by 1 N HCl in acetic acid, neutralization of the resulting hydrochloride with triethylamine in dimethylformamide, and then coupling the free base with the next amino acid using dicyclohexylcarbodiimide. Some difficulty was experienced when *t*-Boc-nitroarginine was coupled to the proline polymer hexapeptide using either dicyclohexylcarbodiimide or Woodward's reagent K as condensing agent. Even with a fourfold excess of *t*-Boc-nitroarginine, the arginine-proline bond could not be formed, and the compound isolated at the end of the synthesis was found to be a heptapeptide lacking the arginine residue. When this coupling step was carried out with a tenfold excess of amino acid, the required octapeptide was still contaminated with about 20% of this heptapeptide. However, this contaminant was removed by chromatography on a column of Sephadex G-25.

[5-Alanine]-angiotensin II was synthesized through the use of Woodward's reagent K as a condensing agent. When using this reagent, the triethylammonium salt of the *t*-Boc-amino acid was treated at 0° with the reagent in dimethylformamide in a separate flask, and the activated ester so obtained was added to the free-base peptide polymer. In the case of *t*-Boc-valine, the active ester did not give a clear solution even on stirring for 4-5 hr at room temperature; therefore, the milky suspension was used as such for condensation. Excess reagent and by-products were removed from the polymer by washing successively with methylene dichloride, dimethylformamide, ethanol, and acetic acid.

At the end of all syntheses the protected peptides were cleaved from the polymer by HBr dissolved in trifluoroacetic acid and the partially protected peptides were catalytically hydrogenated to obtain free octapeptides. It was impossible to predict the amount of protected peptide which would be cleaved from the polymer by subtracting the weight of the polymer from the weight of the polymer containing peptide. To check this point, a small amount of *t*-Boc-phenylalanine polymer was hydrolyzed by refluxing in a mixture of 6 N HCl and acetic acid for 24 hr and the amount of phenylalanine in the filtrate was determined with ninhydrin (Merrifield, 1963). This gave a value of 0.37 mmole/g of polymer compared with 0.45 mmole/g determined spectroscopically by the difference method (Khosla *et al.*, 1967a,b). Similarly, a prolonged HBr-trifluoroacetic acid treatment of phenylalanine polymer releases no more than 0.36 mmole of phenylalanine/g of polymer. It, therefore, appears that a part of the total phenylalanine on the polymer is resistant to hydrolytic cleavage and also unable to enter into peptide synthesis. This may also explain the appearance of shorter peptide chains at the end of the syntheses.

All peptides were purified by chromatography and shown to be homogeneous by paper electrophoresis and by paper and thin-layer chromatography in various solvent systems. Acid hydrolysis and subsequent amino acid analyses showed the amino acid composition of all peptides to be within the expected range. In common with many other angiotensin II analogs, these peptides

apparently contain acetic acid and water (Schwyzer *et al.*, 1958; Riniker and Schwyzer, 1961; Schroeder and Hempel, 1965; Schroeder, 1966). All octapeptides were completely degraded to component amino acids by partially purified leucine aminopeptidase.

Biological assay of [1-isoleucine]-angiotensin II showed this peptide possessed 25% of the pressor activity and 50% of the oxytocic activity of the natural peptide. [Proline³]-angiotensin possessed 40% of the pressor activity and 80% of the oxytocic activity of the natural peptide. [5-Alanine]-angiotensin possessed 5% of the pressor activity of natural angiotensin. The heptapeptide H-Asp-Pro-Tyr-Ile-His-Pro-Phe-OH was also tested and found to possess 1% of the pressor activity and 4% of the oxytocic activity of angiotensin II.

Proline in position 3 has more effect than other amino acids having an aliphatic side chain. However, the high biological activity of this analog is somewhat surprising because of the marked effect this amino acid may have on limiting possible conformations of a peptide. The aliphatic residue in position 5 seems to have more specificity than the aliphatic residue in position 3. The side-chain branching at the β -carbon atom of the amino acid in position 5 seems to be essential for pressor activity. This would suggest that this side chain may possibly be involved in hydrophobic bonding with the receptor protein.

Experimental Section¹

t-Boc-amino acids were synthesized according to the procedure of Schwyzer *et al.* (1959). Purity was determined by melting point, thin-layer chromatography, and optical rotation. Solvents used for ascending paper chromatography on Whatman No. 1 paper and thin-layer chromatography were (a) 1-butanol-acetic acid-water (BAW) (4:1:5) and (b) 1-butanol-acetic acid-water-pyridine (BAWP) (30:6:24:20). Thin-layer chromatography was conducted on Eastman Kodak silica gel chromatogram sheets type K₃₀₁R₂. Electrophoresis was carried out on S & S 2043A filter paper strips at 450 V, using formic acid-acetic acid buffer (pH 1.9) for 3 hr at room temperature. Migration is expressed as the ratio the compound moved compared with glutamic acid. For example, $E_{Glu} = 1.15$ indicates the substance migrated 1.15 times the distance of glutamic acid. Protected or semiprotected compounds were detected on paper strips or thin-layer plates by spraying with ninhydrin and/or diazotized sulfanilic acid. For amino acid analyses the samples were hydrolyzed in 6 N HCl in a sealed tube at 110° for 36 hr and the analysis was performed on a Technicon amino acid autoanalyzer. For enzymatic hydrolysis 2 mg of the corresponding peptide was dissolved in 3 ml of 0.001 M magnesium chloride in 0.01 M sodium bicarbonate (pH 8.1) mixed with 60 mg of crude hog kidney leucine aminopeptidase (Schwarz and Bumpus, 1959). The mixture was incubated at 37° for 48 hr. Aliquots were taken intermittently and spotted on paper and silica gel thin-layer

¹ All amino acids used were the L isomer.

plates for chromatography on BAW and BAWP ascending solvent systems. While considerable splitting occurred within 1-hr incubation, complete degradation to component amino acids required 20 hr.

t-Boc-phenylalanine Polymer. *t*-Boc-phenylalanine (20 mmoles) and triethylamine (20 mmoles) in ethanol were refluxed for 24 hr with 20 g of chloromethylated copolystyrene-2% divinylbenzene. The total amount of *t*-Boc-phenylalanine esterified to the polymer was determined by spectrophotometric estimation of the unreacted phenylalanine in the filtrate. The polymer was found to contain 0.45 mmole of *t*-Boc-phenylalanine/g by this method. However, when 30 mg of this esterified resin was hydrolyzed by refluxing for 24 hr in the mixture of 5 ml of acetic acid and 5 ml of 6 N HCl, only 0.37 mmole of phenylalanine/g of resin was released. The amount of phenylalanine released was determined by ninhydrin after filtration of the resin and removal of acid *in vacuo*. The yield of peptide at the end of each synthesis was calculated using the value of 0.37 mmole of *t*-Boc-phenylalanine/g of polymer.

t-Boc-isoleucylnitroarginylvalyl-*O*-benzyltyrosylisoleucyl-*N*tm-benzylhistidylprolylphenylalanine Polymer. *t*-Boc-phenylalanine polymer (5 g containing 1.85 mmoles of *t*-Boc-phenylalanine) was introduced into a reaction vessel (Khosla *et al.*, 1967a,b) and the following steps were used to introduce each new amino acid residue: (1) wash with glacial acetic acid (three 60-ml portions); (2) remove *t*-Boc group by treatment with 60 ml of 1 N HCl in acetic acid for 30 min; (3) wash with glacial acetic acid (three 60-ml portions); (4) wash with absolute ethanol (three 60-ml portions); (5) wash with dimethylformamide (three 60-ml portions); (6) neutralize the hydrochloride salt with 6 ml of triethylamine in 60 ml of dimethylformamide for 10 min; (7) wash with dimethylformamide (three 60-ml portions); (8) wash with methylene chloride (three 60-ml portions); (9) with cooling in ice bath, add 7.5 mmoles of the appropriate *t*-Boc-amino acid dissolved in 45 ml of methylene chloride and mix for 10 min; (10) introduce 7.5 mmoles of DCCI dissolved in 15 ml of methylene chloride and shake the mixture 2 hr with cooling in ice, and then overnight at room temperature; (11) wash with methylene chloride (three 60-ml portions); and (12) wash with ethanol (three 60-ml portions).

When coupling the amino acids *t*-Boc-*N*tm-benzylhistidine and *t*-Boc-nitroarginine, step 8 was deleted and dimethylformamide was used as the solvent in place of methylene chloride in steps 9-11.

Isoleucylarginylvalyltyrosylisoleucylhistidylprolylphenylalanine-[1-isoleucine,5-isoleucine]-angiotensin II. The protected octapeptide polymer was washed three times with 60 ml of glacial acetic acid, suspended in 100 ml of trifluoroacetic acid, and a slow stream of hydrogen bromide was bubbled through the suspension for 60 min. The polymer was removed by filtration and washed three times with 10-ml portions of trifluoroacetic acid. The combined filtrates were evaporated on a rotary evaporator *in vacuo* at room temperature and the syrupy product obtained was triturated with dry ether. Ether was removed by decantation and the residue was washed three times with ether. The amorphous

powder was collected on a sintered-glass funnel, dissolved in a mixture of methanol-acetic acid-water (10:1:1), and reduced by bubbling hydrogen through the solution at atmospheric pressure for 36 hr. Palladium black (1 g) was used as the catalyst. After hydrogenation, the catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The oily residue was dissolved in deionized water and extracted with ethyl acetate. The aqueous layer was evaporated to dryness to give 1.3 g of free octapeptide. This represents an over-all yield of 60% based on the amount of phenylalanine initially esterified to the resin. On paper electrophoresis a major spot at E_{Glu} equal to 1.25 was evident when sprayed with ninhydrin or Sakaguchi reagent, and traces of peptide at $E_{Glu} = 1.3$. Paper chromatography in BAW also showed a minor spot of slightly higher R_F than the desired octapeptide.

A 500-mg portion was chromatographed on a column (3.0 × 60 cm) of Sephadex G-25 using butanol-acetic acid-water (4:1:5) as developing solvent (Smeby *et al.*, 1966). Fractions of 12 ml were collected and fractions 80-120 contained a mixture of contaminant peptide and desired octapeptide. Fractions 120-145 contained only the major compound and were pooled and solvent was removed by evaporation. The residue was dissolved in 5 ml of dimethylformamide and an amorphous solid precipitated by the addition of dry ether: yield 300 mg of [1-isoleucine-5-isoleucine]-angiotensin II, mp 252-254° dec; loss of water at 100° 2.88%; $[\alpha]_D^{23} -65.8^\circ$ (*c* 0.5, 1 N HOAC); paper chromatography R_F (BAW) 0.59, R_F (BAWP) 0.74; thin-layer chromatography R_F (BAW) 0.36, R_F (BAWP) 0.73; E_{Glu} 1.20. Amino acid ratios found were Arg, 1.07; Tyr, 0.98; Ile, 2.15; Val, 1.18; His, 1.00; Pro, 0.94; and Phe, 1.09. *Anal.* Calcd for $C_{22}H_{37}N_{13}O_{10} \cdot 2 CH_3COOH$ (1164.37): C, 57.42; H, 7.27; N, 15.83. Found: C, 57.10; H, 7.09; N, 15.46.

Benzylloxycarbonyl-β-benzylaspartylnitroarginylprolyl-O-benzyltyrosylisoleucyl-Ntm-benzylhistidylprolylphenylalanine Polymer. The desired sequence was made on the polymer in a manner similar to [1-isoleucine]-angiotensin II by step wise addition of succeeding amino acids to 5 g of *t*-Boc-phenylalanine polymer containing 1.85 mmoles of *t*-Boc-phenylalanine. A fourfold excess of all *t*-Boc-amino acids was used for all steps except for *t*-Boc-nitroarginine where a tenfold excess was used.

Aspartylarginylprolyltyrosylisoleucylhistidylprolylphenylalanine ([3-Proline,5-isoleucine]-angiotensin II). Protected peptide polymer was suspended in 100 ml of trifluoroacetic acid and a slow stream of hydrogen bromide was bubbled through the suspension for 45 min under anhydrous conditions. The polymer was removed by filtration, washed three times with 10-ml portions of trifluoroacetic acid, and the combined filtrate was evaporated at room temperature *in vacuo*. The residual syrup was triturated with ether and the amorphous white powder was removed by filtration. It was dissolved in 75 ml of methanol-acetic acid-water (10:1:1) and reduced by bubbling hydrogen through the solution for 48 hr at atmospheric pressure. Palladium black (1 g) was used as the catalyst. The catalyst was removed

by filtration and the filtrate was evaporated to dryness to give 1.5 g (70%) of compound.

A 600-mg portion of this compound was chromatographed on a column of Sephadex G-25 using butanol-acetic acid-water (4:1:5). Fractions of 12 ml each were collected. Fractions 70-100 contained a mixture of hepta- and octapeptides. Fractions 140-170, which contained the pure [3-proline]-angiotensin II, were pooled, concentrated to 5 ml, filtered through Hyflo-supercel, and evaporated to dryness at room temperature *in vacuo*. Addition of dry ether to the residual syrup yielded an amorphous white solid which was removed by filtration and washed with dry ether to give 350 mg of [3-proline,5-isoleucine]-angiotensin II: mp 225-230° dec, loss of H₂O at 100°, 2.83%; $[\alpha]_D^{23} -94.2^\circ$ (c 5, 1 N AcOH); paper chromatography R_F (BAW) 0.26, R_F (BAWP) 0.46; thin-layer chromatography R_F (BAW) 0.3, R_F (BAWP) 0.5; $E_{Glu} = 1.17$. Amino acid ratio found were Asp, 1.00; Arg, 1.09; Pro, 2.1; Tyr, 0.72; Ile, 1.02; His, 1.06; and Phe, 0.92. *Anal.* Calcd for C₅₀H₆₉N₁₃O₁₂·CH₃COOH·2H₂O (1140.26): C, 54.78, H, 6.81, N, 15.97. Found: C, 55.02, H, 6.67, N, 15.62. Leucine aminopeptidase digestion, like that for [1-isoleucine]-angiotensin II, showed a considerable amount of aspartic acid split during the first hour and a complete hydrolysis to component amino acids within 24 hr.

Aspartylprolyltyrosylisoleucylhistidylprolylphenylalanine. Fractions 70-100 in the above-mentioned purification of [3-proline,5-isoleucine]-angiotensin II were pooled, concentrated *in vacuo*, and rechromatographed on a column of Sephadex G-25 using butanol-acetic acid-water (4:1:5). Fractions of 10 ml each were collected. Fractions 75-90 were pooled, filtered through Hyflo-supercel, concentrated *in vacuo*, and triturated with dry ether to give 100 mg of peptide: mp 195-198°; $[\alpha]_D^{23} -64.6$ (c 0.5, 1 N AcOH); paper chromatography R_F (BAW) 0.64, R_F (BAWP) 0.71; thin-layer chromatography R_F (BAW) 0.5, R_F (BAWP) 0.4; $E_{Glu} = 1.13$. Amino acid ratios found were Asp, 0.9; Pro, 2.2; Tyr, 1.0; Ile, 1.1; His, 1.0; and Phe, 1.1. Leucine aminopeptidase digestion showed that the heptapeptide is split completely in 24 hr.

t-Boc-β-benzylaspartylnitroarginylvalyl-O-benzyltyrosylalanyl-Ntm-benzylhistidylprolylphenylalanine Polymer. *t*-Boc-phenylalanyl polymer (10 g with 3.7 mmoles of phenylalanine) was introduced into the reaction vessel, and the following cycle of reactions was used to add each new residue. The amino acid or peptide polymer was prepared for coupling as described above as steps 1-8 in the preparation of [1-isoleucine]-angiotensin II. Then in a separate flask 10 mmoles of *t*-Boc-amino acid, dissolved in dimethylformamide containing exactly 10 mmoles of triethylamine, was added to a suspension of exactly 10 mmoles of Woodward's reagent K in 50 ml of the same solvent. The mixture was stirred at 0° or room temperature until the zwitterion went into solution and then for an additional 2 hr. The clear solution was then introduced into the reaction vessel and the mixture was shaken at room temperature for 4 hr. The reagents were filtered and the polymer was washed with dimethylformamide (three

75-ml portions) and ethanol (three 75-ml portions).

Aspartylarginylvalyltyrosylalanylhistidylprolylphenylalanine-[5-Alanine]-angiotensin II. At the end of the synthesis, the protected peptide polymer was washed with glacial acetic acid (three 75-ml portions), suspended in approximately 100 ml of trifluoroacetic acid, and a slow stream of HBr was passed through with occasional shaking for about 30 min under anhydrous conditions. The suspension was filtered and the polymer was washed three times with 10-ml portions of trifluoroacetic acid. The combined filtrates were evaporated at room temperature *in vacuo*. The residual syrup was treated slowly with excess ether (approximately 300 ml) with stirring; the supernatant was decanted and the residue was washed with ether three to four times. The residual solid was separated by filtration on a sintered-glass funnel, dissolved in methanol-acetic acid-water (10:1:1), treated with charcoal (Darco G-60) at room temperature, and filtered. The filtrate was hydrogenated over palladium black (1 g) at atmospheric pressure by continuously passing hydrogen for 36 hr; the catalyst was removed by filtration and solvent was removed *in vacuo*. The residue was extracted with deionized water, the insoluble material was removed by filtration, and the filtrate was extracted with ethyl acetate (three 50-ml portions). The aqueous layer was lyophilized, then chromatographed on a column (78 × 4.6 cm) of Sephadex G-25 using 1-butanol-acetic acid-water-pyridine (30:6:24:20) as the developing solvent. Fractions (200) of 12 ml each were collected. Fractions 29-52, which contained the major component, were pooled and evaporated to dryness. The yellowish residue was dissolved in deionized water and passed through a column of Amberlite IR-45 (40 × 2.5 cm). The peptide was eluted with 150 ml of deionized water. The colorless eluate was concentrated to a small volume, passed through Hyflo-supercel, and the clear aqueous filtrate was evaporated to dryness on a rotary evaporator *in vacuo*. The residual solid was precipitated twice from a small volume of dimethylformamide with ether as an amorphous solid to yield 2.1 g of (50%) [5-alanine]-angiotensin II. The combined mother liquors were treated with ethanol to turbidity to yield a further 10% of the above analog: mp 205° dec loss of water at 100°, 3.05% $[\alpha]_D^{23} -65.5^\circ$ (c 0.54, 1 N AcOH); paper chromatography R_F (BAW) 0.33, R_F (BAWP) 0.55; thin-layer chromatography R_F (BAW) 0.26, R_F (BAWP) 0.56; $E_{Glu} = 1.11$. Amino acid ratios found were Asp, 1.1; Arg, 0.95; Val, 1.00; Tyr, 0.85; Ala, 1.09; His, 1.00; and Pro, 1.15. In a separate experiment, the molar ratio of tyrosine was found to be fairly low when valyltyrosine was hydrolyzed under similar conditions. *Anal.* Calcd for C₄₇H₆₆N₁₃O₁₂·CH₃COOH·2H₂O (1100.20): C, 53.50; H, 6.68; N, 16.55. Found: C, 53.74; H, 6.50; N, 16.35. Leucine aminopeptidase digestion showed that the peptide is split completely in 20 hr.

Bioassay. The pressor activity of these peptides was determined by intravenous injection into a vagotomized, ganglion-blocked rat (Pickens *et al.*, 1965) and oxytocic activity was determined using an isolated rat uterus. These are expressed in percentage activity of [5-isoleucine]-angiotensin II.

	Pressor (%)	Oxytocic (%)
[5-Alanine]-angiotensin II	7.5	
[1-Isoleucine]-angiotensin II	25	50
[3-Proline]-angiotensin II	40	80
Asp-Pro-Tyr-Ile-His-Pro-Phe	1	4

References

- Bodanszky, M., and Ondetti, M. A. (1966), *Chem. Ind. (London)* 1, 26.
- Brunner, H., and Regoli, D. (1962), *Experientia* 18, 504.
- Gish, D. T., Katsoyannis, P. G., Hess, G. P., and Stedman, R. J. (1956), *J. Am. Chem. Soc.* 78, 5954.
- Kashelkar, D. V., and Ressler, C. (1964), *J. Am. Chem. Soc.* 86, 2467.
- Khosla, M. C., Smeby, R. R., and Bumpus, F. M. (1967a), *Science* 156, 253.
- Khosla, M. C., Smeby, R. R., and Bumpus, F. M. (1967b), *Biochemistry* 6, 754.
- Liberek, B. (1962), *Bull. Acad. Polon. Sci.* 10, 227.
- Marshall, G. R., and Merrifield, R. B. (1965), *Biochemistry* 4, 2394.
- Merrifield, R. B. (1963), *J. Am. Chem. Soc.* 85, 2149.
- Nesvadba, H., Honzl, J., and Rudinger, J. (1963), *Collection Czech. Chem. Commun.* 28, 1961.
- Paul, R., and Kende, A. S. (1964), *J. Am. Chem. Soc.* 86, 741.
- Pickens, P. T., Bumpus, F. M., Lloyd, A. M., Smeby, R. R., and Page, I. H. (1965), *Circulation Res.* 17, 438.
- Regoli, D., Riniker, B., and Brunner, H. (1963), *Biochem. Pharmacol.* 12, 637.
- Ressler, C. (1956), *J. Am. Chem. Soc.* 78, 5956.
- Riniker, B., and Schwyzer, R. (1961), *Helv. Chim. Acta* 44, 658.
- Rittel, W., Iselin, B., Kappeler, H., Riniker, B., and Schwyzer, R. (1957), *Helv. Chim. Acta* 40, 614.
- Rudinger, J., and Krejci, I. (1962), *Experientia* 18, 585.
- Schroeder, E. (1966), *Ann. Chem.* 691, 232.
- Schroeder, E., and Hempel, R. (1965), *Ann. Chem.* 684, 243.
- Schwarz, H., and Bumpus, F. M. (1959), *J. Am. Chem. Soc.* 81, 890.
- Schwyzler, R. (1961), *Helv. Chim. Acta* 44, 667.
- Schwyzler, R., Iselin, B., Kappeler, H., Riniker, B., Rittel, W., and Zuber, H. (1957), *Chimia* 11, 335.
- Schwyzler, R., Iselin, B., Kappeler, H., Riniker, B., Rittel, W., and Zuber, H. (1958), *Helv. Chim. Acta* 41, 1287.
- Schwyzler, R., Sieber, P., and Kappeler, H. (1959), *Helv. Chim. Acta* 42, 2622.
- Schwyzler, R., and Turrian, H. (1960), *Vitamins Hormones* 18, 237.
- Smeby, R. R., Khairallah, P. A., and Bumpus, F. M. (1966), *Nature* 211, 1193.