(7) M. M. Abdel-Monem, N. E. Newton, and C. E. Weeks, *J. Med. Chem.*, 18, 945 (1975).
(8) N. Relyea and R. R. Rando, *Biochem. Biophys. Res. Commun.*, **67**, 392 (1975).
(9) J. S. Heller, E. S. Canellakis, D. L. Bussolotti, and J. K. Coward, *Biochim. Biophys. Acta*, **403**, 197 (1975).
(10) H. G. Williams-Ashman and A. Schenone, *Biochem. Biophys. Res. Commun.*, **46**, 288 (1972).
(11) A. E. Pegg and C. Conover, *Biochem. Biophys. Res. Commun.*, **69**, 766 (1976).
(12) R. H. Fillingame and D. R. Morris, *Biochemistry*, **12**, 4479 (1973).
(13) R. H. Fillingame, C. M. Jorstad, and D. R. Morris, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4042 (1975).
(14) S. Otani, Y. Mizoguchi, I. Matsui, and S. Morisawa, *Mol. Biol. Rep.*, 1, 431 (1974).
(15) H. Inoue, Y. Kato, M. Takigawa, K. Adachi, and Y. Takeda, *J. Biochem.*, **77**, 879 (1975).
(16) S. I. Harik, M. D. Hollenberg, and S. H. Snyder, *Nature (London)*, **249**, 250 (1974).
(17) G. J. Dixon, E. A. Dulmadge, and F. M. Schabel, Jr., *Cancer Chemother. Rep.*, **50**, 247 (1966).
(18) N. E. Newton, K. Ohno, and M. M. Abdel-Monem, *J. Chromatogr.*, **124**, 277 (1976).
(19) P. S. Mamont, P. Böhlen, P. P. McCann, P. Bey, F. Schuber, and C. Tardif, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1626 (1976).
(20) D. H. Russell, *Cancer Res.*, **32**, 2459 (1972).
(21) O. Heby, L. J. Marton, C. B. Wilson, and H. M. Martinez, *FEBS Lett.*, **50**, 1 (1975).
(22) M. Field, J. B. Block, V. T. Oliverio, and D. P. Rall, *Cancer Res.*, **24**, 1939 (1964).
(23) C. Dave and L. Caballes, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 736 (1973).
(24) J. L. Clark and J. L. Fuller, *Biochemistry*, **14**, 4403 (1975).
(25) T. C. Theoharides and Z. N. Canellakis, *Nature (London)*, **255**, 733 (1975).
(26) J. E. Kay and K. J. Lindsay, *Biochem. J.*, **132**, 791 (1973).
(27) C. B. Favour in "Immunological Methods", J. F. Ackroyd, Ed., F. A. Davis Co., Phiiadelphia, Pa., 1964, p 195.
(28) W. C. Schneider, *Methods Enzymol.*, **3**, 680 (1957).

Synthesis of [1-Sarcosine,8-*O*-methylserine]angiotensin II and 1-Substituted Analogues of [8-Threonine]angiotensin II as Antagonists of Angiotensin II[1]

M. C. Khosla,* M. M. Hall, H. Munoz-Ramirez, P. A. Khairallah, F. M. Bumpus,

Research Division, The Cleveland Clinic Foundation, Cleveland, Ohio 44106

J. H. Seely, and A. M. Thomas

Division of Antibiotics and Natural Products, Abbott Laboratories, North Chicago, Illinois 60064. *Received March 22, 1976*

[1-*N*-methylisoleucine,8-threonine]- (I), [1-dimethylglycine,8-threonine]- (II), [1-guanidineacetic acid,8-threonine]- (III), des-1-aspartic acid-[8-threonine]- (IV), and [1-sarcosine,8-*O*-methylserine]angiotensin II (V) were synthesized by Merrifield's solid-phase procedure to study the effect of (a) substituents in position 1 on the antagonistic activity of [1-sarcosine,8-threonine]angiotensin II, and (b) a change in size and branching in position 8 of [1-sarcosine,-8-*O*-methylthreonine]angiotensin II. The analogues I–V caused an initial rise in blood pressure (30 min of infusion, 250 ng/kg/min in vagotomized ganglion-blocked rats) of 8.05, 11.7, 3.50, 4.5, and 11.16 mmHg. The pA_2 values (rabbit aortic strips) obtained were 7.68, 7.53, 7.23, 7.53, and 9.66, and the dose ratios (in vagotomized ganglion-blocked rats infused at 250 ng/kg/min) obtained were 2.37, 4.49, 1.02, 1.47, and 24.04, respectively. The results obtained indicate that (a) the nature of the substituent in position 1 has an important influence on the biological activity of these peptides, and (b) the potency of antagonists I–IV (all less potent antagonists than [1-sarcosine,8-threonine]angiotensin II) is very much influenced by the length and branching of the side chain in position 8. The in vivo antagonistic activity of [1-sarcosine,8-*O*-methylthreonine]angiotensin II is reduced considerably by shortening the chain length by one carbon atom as is in V.

We have previously reported that all known antagonists of the pressor action of angiotensin II, e.g., [Sar[1],Ala[8]]- and [Sar[1],Ile[8]]angiotensin II, cause an initial agonist activity which is equal to 1–2% of that of the parent hormone.[2,3] In continuation with our earlier work,[3] the present investigation is an attempt to find potent antagonists of the pressor and myotropic activity of angiotensin II having no or low agonist activity. Comparative infusion studies in rats with several analogues indicate that [Sar[1],Thr[8]] angiotensin II[4] shows the lowest agonist to antagonist ratio,[5] while [Sar[1],Thr(Me)[8]]angiotensin II is the most potent antagonist thus far synthesized.[3] In order to study the effect of substituents in the one position on the antagonistic activity of [Sar[1],Thr[8]]angiotensin II, we report the synthesis of some analogues in which position 1 has been replaced with a hydrogen atom, *N*-methylisoleucine, dimethylglycine, or guanidineacetic acid. Similarly, [Sar[1],Ser(Me)[8]]angiotensin II has been synthesized to study the effect of a change in size and branching in an ether-linked side chain in position 8.

All the analogues reported in this paper have been synthesized by the solid-phase procedure.[6] Determinations of the pressor activity[7] of the analogues as compared to angiotensin II (expressed as percent) and the comparative antagonistic activity [2] (expressed as dose ratio) have been carried out on vagotomized ganglion-blocked rats, as are the infusion studies[2] to determine the initial pressor activity (expressed as mmHg). Inhibition of contractile activity of angiotensin II has been studied on isolated spirally cut rabbit aortic strips (expressed as pA_2 values).[4,8,9]

Results and Discussion

Comparative initial agonist properties (bolus injection in rats) (Table I) indicate that the replacement of position 1 in [Sar[1],Thr[8]]angiotensin II with a hydrogen atom, guanidineacetic acid, dimethylglycine, or *N*-methylisoleucine reduces the initial pressor activity. Similar results

Table I. Comparative Agonist and Antagonist Effects of Analogues of Angiotensin II

Compd no.	Angiotensin II analogue	Pressor act.[a]	Antagonist act. (rabbit aortic strips)[b]		
			Log K_2	n	pA_2
I	[MeIle^1,Thr^8]-	0.12	2.48 ± 0.09	0.32 ± 0.03	7.68 ± 0.38 (7)
II	[Me$_2$Gly^1,Thr^8]-	0.38	2.83 ± 0.32	0.37 ± 0.03	7.53 ± 0.18 (7)
III	[GdnAc^1,Thr^8]-	0.15	2.12 ± 0.28	0.28 ± 0.02	7.23 ± 0.51 (6)
IV	Des-Asp^1-[Thr^8]-	0.24	2.83 ± 0.32	0.37 ± 0.03	7.53 ± 0.18 (7)
V	[Sar^1,Ser(Me)^8]-	0.49	5.58 ± 0.63	0.58 ± 0.07	9.66 ± 0.08 (6)
	[Sar^1,Thr^8]-[c]	0.60	8.10 ± 0.05	0.93 ± 0.13	8.79 ± 0.14
	[Sar^1,Thr(Me)^8]-[d]	0.48	8.39 ± 0.62	0.95 ± 0.06	8.76 ± 0.08

[a] Bolus injection in ganglion-blocked vagotomized rats; pressor activity relative to [Asp^1,Ile^8]angiotension II = 100. [b] The pA_2 value has been defined[8,9] as the negative logarithm of the molar concentration of a competitive antagonist that reduces the effect of a double concentration of agonist to that of a single one or, in other words, $pA_2 = (\log K_2)/n$ wherein K_2 and n are constants. n = slope of the line obtained from a plot of (log dose ratio − 1) vs. (−log antagonist concentration) and K_2 = intersect of the line on the y axis and represents the affinity constant. [c] See ref 4 and 5. [d] See ref 3.

are obtained when the hydroxyl function in the position 8 side chain[3] (viz. in Ser or Thr) is replaced with an *O*-methyl function. On the contrary, infusion studies in rats (Table II) at 30 min indicate that [Me$_2$Gly^1,Thr^8]-, [Sar^1,Ser(Me)^8]-, and [Sar^1,Thr(Me)^8]angiotensin II caused greater rise in blood pressure (by 2–6 mmHg) than [Sar^1,Thr^8]angiotensin II under the same conditions.

Studies with the inhibition of the contractile activity of angiotensin II on rabbit aortic strips (Table I) or comparative dose ratios in the rat (Table II) indicate that the analogues I–IV are less potent as antagonists than [Sar^1,Thr^8]angiotensin II.

Comparative antagonistic activities of [Sar^1,Ser(Me)^8]- (V) and [Sar^1,Thr(Me)^8]angiotensin II[3] indicate that V is more potent on the rabbit aortic strips but is less potent in the in vivo (rat) studies than the *O*-methylthreonine analogue.

The results obtained indicate that the nature of the substituent in position 1 has an important influence on the biological activity of these peptides. Although the initial pressor activity in these peptides is reduced by either reducing the chain length at the N terminus by one amino acid residue (IV) or by replacing sarcosine with a basic moiety (guanidineacetic acid) (III), these changes also reduce the in vitro and in vivo antagonistic activities. Similarly, dimethylglycine in position 1 (II) reduces the antagonistic activity but, contrary to other substituents, it induces an increase in the initial pressor activity of [Thr^8]angiotensin II.

The important influence of branching of the side chain in position 8 is evidenced by the fact that the in vivo antagonistic activity of [Sar^1,Thr(Me)^8]angiotensin II is reduced considerably by a nonbranched side chain as in *O*-methylserine. In spite of this, [Sar^1,Ser(Me)^8]angiotensin II is equipotent to [Sar^1,Ile^8]- (dose ratio 27.79 ± 6.25) or [Sar^1,Thr^8]angiotensin II in its in vivo antagonistic activity. However, in vitro (rabbit aortic strips) the antagonistic activity (pA_2 9.66) of this analogue (V) is higher than any other competitive antagonist of angiotensin II.

Recently, it has been observed that des-Asp^1-[Ile^8]angiotensin II is a better antagonist of angiotensin II or des-Asp^1-angiotensin II induced steroidogenesis than [Sar^1,Ile^8]angiotensin II.[10-12] In view of these results des-Asp^1-[Thr^8]angiotensin II (IV) has also been tested as an antagonist of aldosterone secretion. A preliminary investigation (Bravo et al., unpublished results) indicates that IV is almost as potent as des-Asp^1-[Ile^8]angiotensin II.

Experimental Section

Ascending TLC on cellulose, supported on glass plates (Brinkmann Cel 300-25), were performed using the following solvent systems: (a) *n*-BuOH–HOAc–H_2O (BAW, 4:1:5); (b) *n*-BuOH–HOAc–H_2O–Prd (BAWP, 30:6:24:20); (c) *n*-BuOH–AcOEt–HOAc–H_2O (BEAW, 1:1:1:1); (d) *n*-BuOH–Prd–H_2O (BPW, 10:2:5). Compounds were detected on the chromatogram with ninhydrin and/or with diazotized sulfanilic acid. For amino acid analysis, samples were hydrolyzed with 6 N HCl at 105 °C for 24 h, in sealed evacuated tubes, and analyzed on a Beckman-Spinco 120-B. The NMR spectra were recorded with a Varian HA-100 spectrometer using HOAc-d_4 as solvent. Chemical shifts were reported in parts per million, δ, from internal Me_4Si ($\delta = 0$); δ values for multiplets refer to the center of the observed peaks. Optical rotations were measured with a Hilger and Watts polarimeter on solutions in 0.1 M HOAc. Derivatives of optically active amino acids, used as starting materials, were of the L configuration.

Synthesis and Purification of Analogues. The protected octapeptide polymer esters were synthesized by the solid-phase procedure of Merrifield.[6] The procedure used consisted of esterification of the chloromethyl resin with the C-terminal amino acid followed by coupling of each amino acid residue utilizing the cycle of reactions and washes given below. Unless specified all washings were 3 min each. The esterified polymer was swollen for 12 h in CH_2Cl_2 prior to use. The swollen resin was (1) washed four times with dioxane; (2) the Boc group removed by treatment with 4 N HCl in dioxane, 5 min and then 25 min; (3) washed with dioxane five times; (4) washed three times with EtOH; (5) washed three times with $CHCl_3$; (6) the free amino group was liberated with 10% Et_3N–$CHCl_3$, three 5-min treatments; (7) washed four times with $CHCl_3$; (8) washed six times with CH_2Cl_2; (9) coupled with a threefold excess of *tert*-butyloxycarbonylamino acid and DCCI in CH_2Cl_2, except for Boc-Arg(Tos) and Boc-His(Tos) which were dissolved in DMF–CH_2Cl_2 (1:1), for 4 h; (10) washed six times with MeOH–$CHCl_3$ (1:2); (11) washed four times with EtOH. Subsequent cycles were repeated as above; completeness of coupling at each stage was checked by the ninhydrin color test.[13] In cases where a second coupling was required to ensure complete reaction, steps 8–11 were repeated. At the end of the synthesis, the peptide was cleaved from the polymer and partially deblocked with HBr–TFA at room temperature, as previously described.[14] Complete deblocking of the peptide was accomplished by treatment with anhydrous HF[15] in the presence of an equal weight of anisole. The various [Thr^8]angiotensin II analogues were purified, after HF, by chromatography on Bio-Rad AG-1-X2, acetate form, eluted with 0.05 M NH_4OAc and 0.25 M pyridine buffer, pH 7.1.[16] When required this was followed by additional purifications on Sephadex as described under the individual compounds. Fractions in the column chromatography were cut without regard for yield to obtain the desired compound in the pure form and no attempt was made to rechromatograph the minor fractions for identification purposes. The homogeneity of the compound was determined by thin-layer chromatography in solvent systems of different pH and amino acid analysis.

Side-chain functional protecting groups employed were Thr(Bzl), His(Tos), Tyr(2,6-dichlorobenzyl), Arg(Tos); α-amino groups were blocked by the *tert*-butyloxycarbonyl function.

Nitroguanidineacetic acid was prepared according to the literature procedure:[17] mp 185–187 °C. *tert*-Butyloxycarbonyl-*N*-methyl-L-isoleucine was prepared as described previously.[17] *tert*-Butyloxycarbonyl-*O*-methyl-L-serine was prepared according

Table II. Comparative Agonist and Antagonist Effects of Analogues of Angiotensin II. Infusion into Ganglion-Blocked Vagotomized Rats

Compd no.	Angiotensin II analogue	Dose, ng/kg/min	Rise in blood pressure during analogue infusion (mmHg ± SEM)			Angiotensin II ED_{20} (g × 10^9) ± SEM		Dose ratio[a]	p^b
			3 min	10 min	30 min	Before infusion of analogue	During infusion of analogue		
I	[MeIle^1,Thr^8]-	500	4.33 ± 0.71	6.66 ± 0.95	8.05 ± 2.30	1.75 ± 0.20	3.59 ± 0.64 (6)	2.37 ± 0.64 (6)[c]	<0.02
II	[Me$_2$Gly^1,Thr^8]-	250	7.50 ± 0.94	12.57 ± 1.60	11.71 ± 3.83	1.81 ± 0.11	7.70 ± 1.16	4.49 ± 0.79 (9)	<0.001
III	[GdnAc^1,Thr^8]-	500	0.50 ± 0.50	0.75 ± 1.25	3.50 ± 1.65	1.21 ± 0.15	1.14 ± 0.17	1.02 ± 0.26 (4)	>0.05
IV	Des-Asp^1-[Thr^8]-	500	0.50 ± 1.32	1.50 ± 1.93	4.5 ± 4.5	1.97 ± 0.23	3.01 ± 0.79	1.47 ± 0.21 (4)	<0.05
V	[Sar^1,Ser(Me)^8]-	250	15.66 ± 1.20	16.16 ± 1.51	11.16 ± 3.41	1.55 ± 0.15	37.0 ± 9.12	24.04 ± 5.30 (6)	>0.005
	[Sar^1,Thr^8]-[d]	250	9.46 ± 0.79	9.66 ± 2.00	9.20 ± 4.26	1.71 ± 0.26	44.55 ± 8.20	26.9 ± 3.30 (10)	<0.001
	[Sar^1,Thr(Me)^8]-[e]	250	13.82 ± 0.98	15.88 ± 0.85	15.06 ± 1.63	1.55 ± 0.10	95.26 ± 23.83	62.52 ± 14.93 (17)	<0.001

[a] ED_{20} of angiotensin II was determined before infusion of the analogue and during infusion of the analogue. The dose ratio was calculated by dividing ED_{20} of angiotensin II during infusion of the analogue by ED_{20} of angiotensin II determined before infusion of the analogue. [b] Level of significance between ED_{20} values. [c] The numbers in parentheses are the number of rats used. [d] See ref 4 and 5. [e] See ref 3.

to the procedure of Hodges and Merrifield.[18] Quantitation of Me_2Gly and GdnAc is not possible by normal amino acid analysis techniques (no amino group). In the case of MeIle quantitation is theoretically possible but is not practical. MeIle has a color constant only $^1/_{150}$th that of other amino acids and in order to have a reasonable peak size excessive amounts of compound would have to be hydrolyzed. It is precisely for these reasons that ^{1}H NMR spectra were obtained. For example, in the case of MeIle the CH_3– shows up as a singlet which can be intergrated relative to the other amino acids. Further, the unusual amino acids in question are all in position 1; any failure to achieve complete coupling of these amino acids would manifest itself in the presence of a failure sequence, namely the heptapeptide, des-Asp^1-[Thr^8]-angiotensin II. This material was synthesized and cochromatographed with the octapeptides on the TLC. All octapeptides were homogeneous, gave correct amino analysis (for des-Asp^1-[Thr^8]angiotensin II), but did not show identical behavior on the TLC with the heptapeptide.

[MeIle^1,Thr^8]angiotensin II. The protected heptapeptide resin ester [Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-Ile-His(Tos)-Pro-Thr(Bzl)-P] (4 mmol) was deblocked and neutralized in the usual way. Boc-MeIle (12 mmol) and DCCI (12 mmol) in CH_2Cl_2 were shaken with the resin overnight. The resin beads were still ninhydrin positive, so the coupling was repeated using 12 mmol of Boc-Ile and DCCI, but also including 12 mmol of HOBt. After shaking for several hours a negative ninhydrin test was obtained. The washed resin was dried under reduced pressure (P_2O_5) and treated with HF (10 ml/g of resin) in the presence of anisole (1 ml/g of resin). The product was purified by chromatography on AG-1-X2 (pH 7.1 buffer) followed by Sephadex G-25 chromatography on two successive columns using BAWP and BAW solvent systems, respectively: TLC (cellulose) R_f 0.61 (BAW), 0.71 (BAWP), 0.31 (BPW), 0.84 (BEAW); amino acid analysis Arg 1.04, Val 1.05, Tyr 0.87, Ile 0.98, His 1.04, Pro 0.95, Thr 0.93 (the chromatographic peak for MeIle merged into that for valine; the color value for this amino acid was also 150-fold less than that of valine; hence, only a qualitative estimation could be obtained); $[\alpha]^{22}D$ –85.2° (*c* 0.5); ^{1}H NMR δ 0.8–1.0 (m, Val, Ile, and MeIle-CH_3), 1.25 (d, Thr-CH_3), 2.78 (s, MeIle-NCH_3), 6.72 and 7.02 (d, Tyr-arom), 7.40 and 8.78 (s, His-arom).

[Me_2Gly^1-Thr^8]angiotensin II. *N,N*-Dimethylglycine was coupled to the heptapeptide polymer using Woodward's Reagent K, as described previously.[17] The product obtained after HBr–TFA cleavage and HF treatment was purified by ion-exchange chromatography on AG-1-X2 (pH 7.1 buffer) followed by partition chromatography on Sephadex G-25 on two successive columns using BAWP and BPW solvent systems: TLC (cellulose) R_f 0.40 (BAW), 0.50 (BAWP), 0.60 (BEAW), 0.16 (BPW); amino acid analysis Arg 1.07, Val 0.92, Tyr 0.93, Ile 0.76, His 0.81, Pro 1.06, Thr 1.02; $[\alpha]^{22}D$ –69.8° (*c* 0.5); ^{1}H NMR δ 0.8–1.0 (m, Val, Ile-CH_3), 1.26 (d, Thr-CH_3), 3.02 (s, Me_2Gly-NCH_3), 6.74 and 7.04 (d, Tyr-arom), 7.38 and 8.77 (s, His-arom).

[Gdn-Ac^1,Thr^8]angiotensin II. Nitroguanidineacetic acid was coupled to the heptapeptide polymer using a threefold molar excess of *N,N'*-carbonyldiimidazole in DMF until a negative ninhydrin test was obtained. After cleavage with HBr–TFA and HF treatment, the product was purified on AG-1-X2 followed by partition chromatography on Sephadex G-25 (BPW system): TLC (cellulose) R_f 0.03 (BPW), 0.60 (BAWP), 0.43 (BAW), 0.66 (BEAW); amino acid analysis Arg 1.05, Val 1.03, Tyr 1.00, Ile 0.89, His 1.07, Pro 0.96, Thr 0.99; $[\alpha]^{22}D$ –79.6° (*c* 0.5); ^{1}H NMR δ 0.8–1.0 (m, Val and Ile-CH_3), 1.26 (d, Thr-CH_3), 4.15 (s, GdnAc), 6.73 and 7.04 (d, Tyr-arom), 7.40 and 8.77 (s, His-arom).

[Sar^1,Thr^8]angiotensin II. The product prepared by the present method was shown to be identical with that prepared previously:[4] TLC (cellulose) R_f 0.16 (BPW), 0.58 (BAW), 0.67 (BAWP); amino acid analysis Sar 1.12, Arg 1.08, Val 0.95, Tyr 0.95, Ile 0.81, His 0.94, Pro 1.07, Thr 1.07; $[\alpha]^{22}D$ –86.0° (*c* 0.5); ^{1}H NMR δ 0.8–1.0 (m, Val and Ile-CH_3), 1.26 (d, Thr-CH_3), 2.83 (s, Sar-NCH_3), 4.05 (s, Sar-CH_2), 6.74 and 7.02 (d, Tyr-arom), 7.40 and 8.77 (s, His-arom).

Des-Asp^1-[Thr^8]angiotensin II. The protected heptapeptide resin ester [Boc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-Ile-His(Tos)-Pro-Thr(Bzl)-P] (2 mmol) was treated with HBr–TFA followed by HF. The product was purified by chromatography on AG-1-X2 (pH 7.1 buffer) followed by partition chromatography on Sephadex

G-25 (BPW system). Several trace impurities revealed by TLC (cellulose) were successfully removed by countercurrent distribution (BAW, 10 ml each phase, 900 transfers): TLC (cellulose) R_f 0.52 (BAW), 0.53 (BAWP), 0.72 (BEAW), 0.24 (BPW); amino acid analysis Arg 1.03, Val 0.93, Tyr 0.90, Ile 0.96, His 0.95, Pro 1.05, Thr 1.08; $[\alpha]^{24}D$ −67.0° (c 0.5); ^1H NMR δ 0.87 (m, Val and Ile-CH_3), 1.25 (d, Thr-CH_3), 6.73 and 7.03 (d, Tyr-arom), 7.38 and 8.76 (s, His-arom).

[Sar^1,Ser(Me)^8]angiotensin II. After cleavage of the peptide from the resin support with HBr–TFA, the monotosyl octapeptide was obtained in a homogeneous state by chromatography on Sephadex G-25 (0.1 M HOAc). The tosyl group was removed with HF, and the free peptide was chromatographed on AG-1-X2 in the usual manner. The product obtained was homogeneous in three TLC systems without further purification: TLC (cellulose) R_f 0.28 (BPW), 0.51 (BAWP), 0.4 (BAW); amino acid analysis Sar 0.96, Arg 1.00, Val 1.06, Tyr 0.99, Ile 0.85, His 1.07, Pro 1.03 (*O*-methylserine was partially degraded during hydrolysis and was not calculated); $[\alpha]^{21}D$ −71.4° (c 0.5); ^1H NMR δ 0.8–1.0 (m, Val and Ile-CH_3), 2.83 (s, Sar-NCH_3), 3.37 [s, Ser(Me)-OCH_3], 4.06 (s, Sar-CH_2), 6.73 and 7.03 (d, Tyr-arom), 7.43 and 8.78 (s, His-arom).

Acknowledgment. The authors are indebted to Dr. R. S. Egan and Mrs. Ruth Stanszek for ^1H NMR spectra, to Mr. David McKinney and Mr. John Fraser for amino acid analyses, and to Mr. Eugene Bacznsky for the excellent technical assistance.

References and Notes

(1) Abbreviated designation of amino acid derivatives and peptides is according to the recommendation of the IUPAC–IUB commission (IUPAC information bulletin no. 26). In addition, the following abbreviations have been used: Prd = pyridine, GdnAc = guanidineacetic acid, Ser(Me) = *O*-methylserine, Thr(Me) = *O*-methylthreonine, HOBt = 1-hydroxybenzotriazole.
(2) H. Munoz-Ramirez, M. C. Khosla, F. M. Bumpus, and P. A. Khairallah, *Eur. J. Pharmacol.*, **31**, 122 (1975).
(3) M. C. Khosla, H. Munoz-Ramirez, M. M. Hall, R. R. Smeby, P. A. Khairallah, F. M. Bumpus, and M. J. Peach, *J. Med. Chem.*, **19**, 244 (1976).
(4) M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **17**, 1156 (1974).
(5) H. Munoz-Ramirez, M. C. Khosla, and P. A. Khairallah, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, Abstract No. 3113 (1975).
(6) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(7) P. T. Pickens, F. M. Bumpus, A. M. Lloyd, R. R. Smeby, and I. H. Page, *Circ. Res.*, **17**, 438 (1965).
(8) R. F. Furchgott and S. Bhadrakom, *J. Pharmacol. Exp. Ther.*, **103**, 129 (1953).
(9) O. Arunlakshna and H. O. Schild, *Br. J. Pharmacol.*, **14**, 48 (1959).
(10) E. L. Bravo, M. C. Khosla, and F. M. Bumpus, *Am. J. Physiol.*, **228**, 110 (1975).
(11) E. L. Bravo, M. C. Khosla, and F. M. Bumpus, *J. Clin. Endocrinol. Metab.*, **40**, 530 (1975).
(12) C. A. Sarstedt, E. D. Vaughan, Jr., and M. J. Peach, *Circ. Res.*, **37**, 350 (1974).
(13) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(14) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Biochemistry*, **6**, 754 (1967).
(15) S. Sakakibara, Y. Shimonishi, Y. Kisheda, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164 (1967).
(16) M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **16**, 829 (1973).
(17) M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **17**, 431 (1974).
(18) R. S. Hodges and R. B. Merrifield, *J. Org. Chem.*, **39**, 1870 (1974).

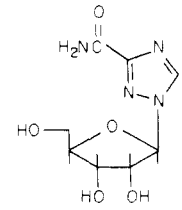
Synthesis and Antiviral Activity of Certain Thiazole *C*-Nucleosides

Prem C. Srivastava,* Michael V. Pickering, Lois B. Allen, David G. Streeter, Marie T. Campbell, Joseph T. Witkowski, Robert W. Sidwell, and Roland K. Robins

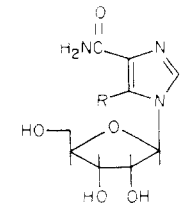
ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715. *Received May 10, 1976*

A general reaction of glycosyl cyanides with liquid hydrogen sulfide in the presence of 4-dimethylaminopyridine to provide the corresponding glycosylthiocarboxamides is described. These glycosylthiocarboxamides were utilized as the precursors for the synthesis of 2-D-ribofuranosylthiazole-4-carboxamide and 2-β-D-ribofuranosylthiazole-5-carboxamide (**23**). The structural modification of 2-β-D-ribofuranosylthiazole-4-carboxamide (**12**) into 2-(2,3,-5-tri-*O*-acetyl-β-D-ribofuranosyl)thiazole-4-carboxamide (**15**), 2-β-D-ribofuranosylthiazole-4-thiocarboxamide (**17**), and 2-(5-deoxy-β-D-ribofuranosyl)thiazole-4-carboxamide (**19**) is also described. These thiazole nucleosides were tested for in vitro activity against type 1 herpes virus, type 3 parainfluenza virus, and type 13 rhinovirus and an in vivo experiment was run against parainfluenza virus. They were also evaluated as potential inhibitors of purine nucleotide biosynthesis. It was shown that the compounds (**12** and **15**) which possessed the most significant antiviral activity were also active inhibitors (40–70%) of guanine nucleotide biosynthesis.

Certain naturally occurring *C*-glycosyl nucleosides possess a variety of biological properties that are of potential medicinal importance.[1-5] In recent years, considerable work has been directed toward the synthesis of such compounds. In search of a potent antiviral drug several *N*-glycosyl nucleosides[6,7] including ribavirin[8] (**1**) have been prepared and studied in this Laboratory. Studies with ribavirin[9] and certain imidazole nucleosides[6] (**2**) have shown that those compounds which exhibited significant antiviral activity were also active inhibitors of guanine nucleotide biosynthesis. This suggested that the selective regulation of this important pathway to nucleic acid biosynthesis may have specific chemotherapeutic application. The present report describes the synthesis and antiviral evaluation of certain *C*-glycosylthiazoles structurally related to ribavirin.



1



2, R = H or halogen

Various approaches for the synthesis of *C*-nucleosides have been summarized in the literature.[10] Of these, the conversion of β-D-aldofuranosyl cyanides into suitably functionalized anhydroalditols, in which further elabo-