Synthesis and Biological Activities of Analogues of Angiotensins II and III Containing *O*-Methyltyrosine and D-Tryptophan

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Analogues of angiotensin II and III (ANG II and ANG III) in which the tyrosine and/or phenylalanine residues were substituted have been synthesized by the solid-phase method and purified by (carboxymethyl)cellulose chromatography and reversed-phase HPLC. The antagonist and agonist potencies of these peptides were determined in the rat isolated uterus assay. [Sar^1,Tyr(Me)^4]ANG II, [Tyr(Me)^3]ANG III, [Sar^1,D-Trp^4]ANG II, [D-Trp^3]ANG III, [Sar^1,D-Trp^8]ANG II, [D-Trp^7]ANG III, [Sar^1,Tyr(Me)^4,Ile^8]ANG II, [Tyr(Me)^3,Ile^7]ANG III, [Sar^1,D-Trp^4,Ile^8]ANG II, [D-Trp^3,Ile^7]ANG III, [Sar^1,Tyr(Me)^4,D-Trp^8]ANG II, and [Tyr(Me)^3,D-Trp^7]ANG III had antagonist activities (pA_2) respectively of 8.1, <6, <6, <6, (7.7), (6.7), 7.2, <6, <6, <6, 7.1, and <6. The agonist activity of each peptide was less than 0.1% of that of ANG II. Analogues in which only the Phe residue was substituted were not readily reversible in the bioassay, whereas analogues in which only the Tyr residue or both the Tyr and Phe residues were substituted were reversible antagonists. Peptides that were twice substituted had lower antagonist activities than peptides having a single aromatic residue substitution. Substitution of the Tyr residue in ANG II, but not ANG III, provides a new route for the synthesis of potent and competitive angiotensin antagonists. Differences in the biological properties of ANG II and ANG III analogues substituted at the Tyr residue suggest different binding/conformation requirements for the two endogenous ligands at angiotensin receptors in smooth muscle.

The octapeptide angiotensin II (ANG II, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and the desaspartyl heptapeptide angiotensin III (ANG III) act at receptors in different target tissues to elicit contractile and secretory responses. In general, ANG III is less potent than or equipotent with ANG II in a variety of bioassays. Structure–activity studies of ANG II and ANG III have demonstrated that the C-terminal Phe residue in both peptides is important in eliciting the biological activity and that replacement of this residue with a nonaromatic hydrophobic amino acid produces antagonists.[1] For the octapeptide, substitution of Sar at position 1 results in an increase in potency[1] which may be attributable to an increased biological half-life of the peptide. The peripheral and central actions of ANG II combine to produce increased vascular resistance and extracellular fluid volume, thus implicating this peptide in blood pressure regulation in both normotensive and hypertensive states. The substitution of D-Trp for aromatic residues in luteotropin,[2] neurotensin,[3] and substance P[4] has resulted in the production of analogues with antagonist properties. Similarly, the substitution of *O*-Me-Tyr for Tyr in vasopressin[5] and oxytocin[5] results in the production of antagonists. We have investigated these same substitutions in ANG II and ANG III and measured the biological activities of the resulting molecules, with the possibility in mind that certain ground rules for substitution of aromatic amino acids in small peptides may be becoming evident as the available literature on structure–activity relationships expands. Initially we found that [Sar^1,Tyr(Me)^4]ANG II is a potent competitive antagonist both in vitro and in vivo,[6] and further exploration of the effects of methylation of the Tyr hydroxyl in angiotensin analogues seemed warranted. In addition, equivalent chemical modifications to the ANG II and ANG III molecules may provide some clues as to the differential potencies of these two endogenous ligands at angiotensin receptors. Finally, we undertook these investigations in the hope of preparing functionally new angiotensin antagonists that might find application in the diagnosis and treatment of hypertension. With the finding that converting enzyme inhibitors are effective in both renovascular and essential hypertensive states, a resurgence in the importance of angiotensin in mediating various forms of hypertension has become apparent, together with the need for improved angiotensin antagonists.

Table I. Agonist and Antagonist Activities of Angiotensin Analogues in the Rat Isolated Uterus Assay[a]

peptide	agonist act.,[b] %	antagonist act. (pA_2)[c]
[Sar^1,Tyr(Me)^4]ANG II	<0.1	8.1 ± 0.1 (7)
[Tyr(Me)$_3$]ANG III	<0.1	<6
[Sar^1,D-Trp^4]ANG II	<0.1	<6
[D-Trp^3]ANG III	<0.1	<6
[Sar^1,D-Trp^8]ANG II	<0.1	(7.7)[d]
[D-Trp^7]ANG III	<0.1	(6.7)[d]
[Sar^1,Tyr(Me)^4,Ile^8]ANG II	<0.1	7.2 ± 0.1 (4)
[Tyr(Me)^3,Ile^7]ANG III	<0.1	<6
[Sar^1,D-Trp^4,Ile^8]ANG II	<0.1	<6
[D-Trp^3,Ile^7]ANG III	<0.1	<6
[Sar^1,Tyr(Me)^4,D-Trp^8]ANG II	<0.1	7.1 ± 0.1 (4)
[Tyr(Me)^3,D-Trp^7]ANG III	<0.1	<6
[Sar^1,Ile^8]ANG II	<0.1	(8.7)[d]

[a] Values are given as mean ± SEM (number of animals). [b] Relative to ANG II = 100%. [c] The pA_2 is the negative logarithm of the concentration of antagonist that reduces the response to an ED50 dose of ANG II to the response to half the ED50 dose. [d] The antagonist appeared not to be purely competitive and may not have a true pA_2; the value given was obtained by challenging each tissue only once with a "pA_2 dose" of antagonist and measuring the response to ANG II after 2 min (see text); mean of four determinations.

Results

The biological activities of the peptides synthesized during this investigation are illustrated in Table I. None of these peptides demonstrated a significant agonist activity in the rat isolated uterus assay, and the antagonist activities of the different analogue were varied with respect to potency and mechanism of action. The most potent of these new analogues of angiotensin was [Sar^1,Tyr(Me)^4]-ANG II, which demonstrated all the properties of a reversible competitive antagonist.[6] In contrast, the equivalent ANG III analogue [Tyr(Me)^3]ANG III had no

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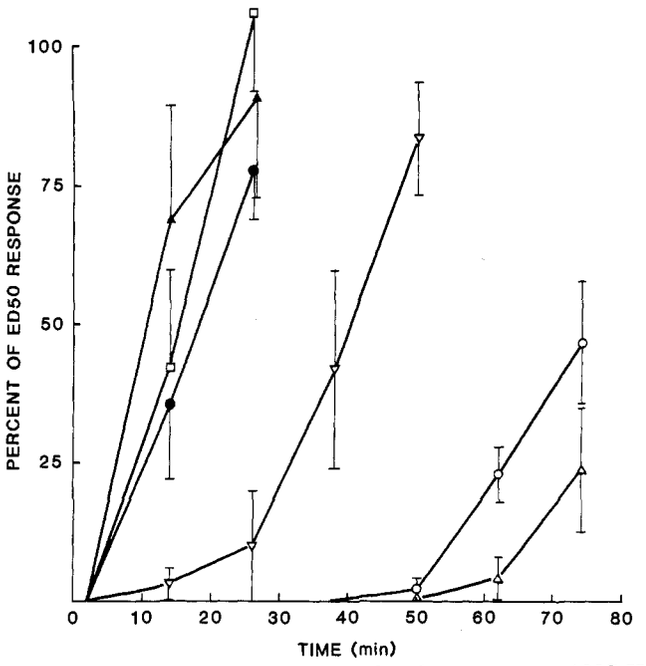

Figure 1. Desensitization of the isolated rat uterus to ANG II after treatment with angiotensin antagonists. The antagonist (10^{-5} M) was given at time zero and washed out after 2 min. Subsequently, ED50 doses of ANG II followed by washing out were given at 12-min intervals. Points represent mean ± SEM. □, [Sar^1,Tyr(Me)^4]ANG II, N = 5; ▲, [Sar^1,Tyr(Me)^4,Ile^8]ANG II, N = 4; ●, [Sar^1,Tyr(Me)^4,D-Trp^8]ANG II, N = 4; ▽, [D-Trp^7]ANG III, N = 3; ○, [Sar^1,D-Trp^8]ANG II, N = 3; △, [Sar^1,Ile^8]ANG II, N = 3.

measurable affinity ($pA_2 < 6$) for angiotensin receptors in the rat isolated uterus. Unlike the substitution of Tyr(Me) for Tyr in ANG II, the substitution of D-Trp for Tyr in ANG II did not produce an antagonist. Thus [Sar^1,D-Trp^4]ANG II turned out to have an immeasurably low binding affinity ($pA_2 < 6$; Table I) for angiotensin receptors, as did its heptapeptide counterpart, [D-Trp^3]ANG III.

However, substitution of D-Trp for Phe in the octapeptide resulted in an analogue with relatively high antagonist potency, and its heptapeptide counterpart, [D-Trp^7]ANG III, was also an antagonist. Both of these peptides modified at the C-terminal residue were found to have a protracted antagonist activity in the rat isolated uterus assay. In both cases, and unlike [Sar^1,Tyr(Me)^4]-ANG II, the tissue did not regain full responsiveness to ANG II 12 min after washing out of a pA_2 dose of the antagonist. Furthermore, high doses (10^{-5} M) of [Sar^1,D-Trp^8]ANG II and [D-Trp^7]ANG III incapacitated the tissues for prolonged periods of time, whereas the same dose of [Sar^1,Tyr(Me)^4]ANG II was completely reversed within 24 min after washing out (Figure 1). Thus the analogues containing D-Trp at the C-terminus are slowly reversing and may not be purely competitive antagonists. For this reason, the pA_2 value given in Table I (in parentheses) was obtained by challenging each tissue only once with each antagonist and measuring the ANG II response 2 min later. The behavior of these two peptides was characteristic of that observed with [Sar^1,Ile^8]ANG II,[7] although their effects on the tissue were not as long lasting (Figure 1).

We have also examined the effects of substituting both the Tyr and the Phe residues in angiotensin simultaneously. [Sar^1,D-Trp^4,Ile^8]ANG II and [D-Trp^3,Ile^7]ANG III were not antagonists. Similarly, the heptapeptides [Tyr(Me)^3,Ile^7]ANG III and [Tyr(Me)^3,D-Trp^7]ANG III were inactive. However, [Sar^1,Tyr(Me)^4,Ile^8]ANG II and [Sar^1,Tyr(Me)^4,D-Trp^8]ANG II were antagonists and, moreover, appeared to be competitive reversible antagonists (Figure 1). Thus the slow reversal properties associated with C-terminal amino acid substitution were essentially eliminated with concomitant methylation of the Tyr hydroxyl group (Figure 1). Substitution at both Tyr and Phe in [Sar^1]ANG II resulted in peptides that were weaker antagonists than any of the parent antagonists substituted either in position 4 or in position 8, this being the case when the C-terminal amino acid was either Ile or D-Trp (Table I).

Discussion

The finding that [Sar^1,Tyr(Me)^4]ANG II is a potent and competitive antagonist of ANG II in smooth muscle is reminiscent of similar findings with vasopressin and oxytocin, where methylation of the Tyr residue produces antagonists.[5] Methylation of a tyrosine residue in small peptides may turn out to be a generalized tactical approach for producing antagonists. It has been shown previously that substitution of fluorophenylalanine for Tyr in ANG II produces an antagonist[8] and it is somewhat surprising that this strategic finding has not been investigated more extensively in the angiotensin molecule. [Tyr(Me)^4]ANG II has been synthesized previously,[9] but its antagonist activity was not determined. Interestingly, it has been suggested previously that the Tyr hydroxyl proton in ANG II may form a hydrogen bond with the angiotensin receptor.[10]

Methylation of the Tyr hydroxyl in ANG III produces a peptide that has an immeasurably low binding affinity ($pA_2 < 6$; Table I) for angiotensin receptors in uterine smooth muscle. This finding was unexpected and may be indicative of the existence of different conformation/receptor binding requirements for the two endogenous ligands.

The finding that substitution of D-Trp for Tyr in neurotensin analogues produces antagonists[3] is not a general rule that can be applied to ANG II, since both [Sar^1,D-Trp^4]ANG II and [D-Trp^3]ANG III have very low binding affinities ($pA_2 < 6$; Table I) at angiotensin receptors in smooth muscle. In contrast, substitution of D-Trp for Phe in ANG II or ANG III produces antagonists. This finding was not unexpected because it has been known for some time that a variety of substitutions at the C-terminus of ANG II or ANG III produces antagonists. The antagonism by angiotensin analogues substituted with D-Trp at the C-terminus was characterized by slow reversibility (Figure 1), properties that, in our hands, are characteristic of many angiotensin analogues in which the C-terminal residue is replaced by a nonaromatic hydrophobic amino acid, e.g., Ile. [Sar^1,Ile^8]ANG II has been studied in some detail in this laboratory, and these investigations have led us to conclude that this analogue is not a reversible competitive antagonist of ANG II. We have proposed a model to explain the mixed competitive/quasi-noncompetitive antagonist activity of [Sar^1,Ile^8]ANG II, in which a C-terminal aromatic residue in the correct orientation is required for directing the necessary conformational alignment of the

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Tyr residue for activation of the receptors.[7] When the Tyr hydroxyl is methylated and the Phe residue of ANG II is substituted simultaneously, the resulting peptides have decreased antagonist activity compared to peptides having either of the two single substitutions, illustrating that the "additivity rule" does not hold for these antagonists. [The addivity rule has generally been applied to agonists rather than antagonists. In one sense, the additivity rule does hold for these angiotensin antagonists, since twice substitution reduces the binding affinity (pA_2) further than either of the two single substitutions.] Thus [Sar[1],Tyr(Me)[4],Ile[8]]ANG II is a weaker antagonist than either [Sar[1],Tyr(Me)[4]]ANG II or [Sar[1],Ile[8]]ANG II. Similarly, [Sar[1],Tyr(Me)[4],D-Trp[8]]ANG II is a weaker antagonist than either [Sar[1],Tyr(Me)[4]]ANG II or [Sar[1],D-Trp[8]]ANG II. Furthermore, the twice-substituted peptides are readily reversible and appear to have the properties of competitive antagonists, apparently losing the slow reversal effects associated with substitution of only the C-terminal amino acid (Figure 1). Thus, a free tyrosine hydroxyl group is required, in association with substitution of the C-terminal residue, to produce protracted antagonism. In a broader context, it appears likely that the desensitization effect induced by ANG II and ANG III, and many analogues, in a variety of smooth muscle tissues, is intimately associated with the Tyr hydroxyl group in these peptides.

In conclusion, the observations reported here suggest that modification of the Tyr residue in ANG II, but not in ANG III, offers an alternative route for the synthesis of potent angiotensin antagonists. Such antagonists would be expected to be purely competitive antagonists, and therefore useful probes for investigating angiotensin receptors and the physiological roles of angiotensin. It appears that the tyrosine hydroxyl group in ANG II is of critical importance in the mechanism of activation of angiotensin receptors mediating the myotropic response and is also important in eliciting the desensitization phenomena (Figure 1) associated with certain angiotensin analogues. The pronounced difference in biological activities between ANG II and ANG III analogues modified at the tyrosine residue suggests the existence of different conformation/receptor-binding requirements for the two endogenous ligands. It appears, on the basis of data reported herein and previous investigations on the substitution of the proline residue in ANG II and ANG III analogues,[11] that the ANG III molecule is much more intolerant to chemical modification than the ANG II molecule. This difference in susceptibility to chemical modification with retention of biological activity between ANG II and ANG III may provide insight into the mechanism(s) of action of the two endogenous ligands at angiotensin receptors and may provide information necessary for understanding the physiological role of ANG III in blood flow regulation.

Recent conformational analyses have delineated differences in the conformational properties of angiotensin analogues that may have direct relevance to the observations reported herein. In particular, the antagonist activity of analogues of angiotensin in which the Phe residue is substituted appears to result from elimination of a His/Phe ring stacking interaction in ANG II.[15] The significance of the Tyr hydroxyl group in angiotensin may be related to its proposed participation in a tyrosine charge relay system (TyrOH–His–carboxylate interaction) and its possible involvement in (transient) covalent bonding to the receptor.[14] On the basis of these findings and more recent pharmacological studies in this laboratory (Scanlon and Moore, unpublished work), [Sar[1],Ile[8]]ANG II and related analogues may eventually have to be classified as essentially irreversible antagonists.

Experimental Section

tert-Butyloxycarbonyl-blocked amino acids were purchased from Peninsula Laboratories and were checked for purity by TLC before use. ANG II, ANG III, and [Sar[1],Ile[8]]ANG II were products of Peninsula Laboratories and were found to be highly purified by reversed-phase HPLC. TLC of synthetic peptides was carried out on silica gel on glass plates (Brinkmann Instruments, 60F-254) in the following solvent systems: 1-butanol–pyridine–acetic acid–water (BPAW; 15:10:3:6, v/v) and chloroform–methanol–acetic acid–water (CMAW; 15:10:2:3, v/v). Detection of peptides was sequentially by UV fluorescence quenching, ninhydrin spray reagent, and chlorination followed by starch–KI spray reagent. Peptides were hydrolyzed in sealed tubes in 6 M HCl, or 4 M methanesulfonic acid (Trp-containing peptides), containing 1% cresol at 110 °C for 18 h in vacuo. Amino acid analyses were obtained with a Beckman 121 M amino acid analyzer. Tyr(Me) was partially hydrolyzed to Tyr during acid hydrolysis and the amount of Tyr(Me) present in each peptide was taken as the sum of Tyr(Me) and Tyr. Mass spectra were obtained by high-field fast atom bombardment (FAB) with a Finnigan-Mat 4600 and a Kratos MS-50 high magnet FAB. Solid-phase peptide synthesis was conducted with a Beckman 990 peptide synthesizer with use of methods essentially as described previously.[12] Peptides were synthesized on a 0.5-mmol scale up to the heptapeptide stage, and thereafter the protected resin was divided in half and Boc-Sar was added to one half. The deprotection reagent (CF_3CO_2H:$CHCl_3$ = 1:3) contained indole (0.5 g/L) and ethyl methyl sulfide (0.5 mL/L). The tosyl group was used to protect His and Arg and the 2-bromobenzyloxycarbonyl group was used to protect Tyr. Two coupling reactions were used for each amino acid, the first utilizing dicyclohexylcarbodiimide in the presence of 1-hydroxybenzotriazole for 3 h and the second mediated by *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline for 8 h. This strategy enabled reliable syntheses without the necessity of checking the completeness of reactions. The completed peptide was removed from the resin and simultaneously deprotected by reaction with anhydrous HF (20 mL) at 0 °C for 1 h in the presence of *p*-cresol (2 mL), indole (0.2 g), and ethyl methyl sulfide (0.2 mL). HF was removed in vacuo, the peptide was dissolved in trifluoroacetic acid (60 mL), and the resin was removed by filtration. The solvent was removed on a rotary evaporator and the peptide was obtained as a white amorphous solid by trituration with ether. Yields of the crude peptides were in excess of 90%. Initial purification of each peptide was carried out by cation-exchange chromatography with a column (80 × 1.6 cm) of (carboxymethyl)cellulose (Whatman CM23) with a linear gradient of ammonium acetate, 0.01 M and pH 5.0 to 0.5 M and pH 8.0, at a flow rate of 40–50 mL/h. The absorbance of the effluent was measured at 280 nm and fractions (10 mL) of the major product were pooled, lyophilized, and relyophilized twice from 1% acetic acid. Final purification of the product was achieved by reversed-phase HPLC with a semipreparative automated repetitive injection technique that has been described in detail previously.[12] The major peak detected from the absorbances of the effluent at 254 and 230 nm was collected, acetonitrile removed by rotary evaporation, and the product lyophilized and relyophilized from 1% acetic acid. Yields of the final products ranged from 7% to 24% (based on starting Boc-amino acid–resin). Peptides were stored at −20 °C in the lyophilized state. Stock solutions (1 mg/mL) were prepared at weekly intervals, and dilutions for assays were prepared daily. The rat isolated uterus assay was carried out on uteri from diethylstilbestrol-primed female Sprague–Dawley rats (150–250 g) as described previously.[13]

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The tissue was challenged every 12 min in all experiments. For the measurement of the antagonist potencies of analogues, the antagonist was given 2 min before ANG II. Antagonist potencies (pA_2) were determined as the negative logarithm of the concentration of antagonist required to reduce the response to an ED50 dose of ANG II to the response to half the ED50 dose. The antagonist effects of some peptides were found not to be reversible within the 12-min time frame between ANG II challenges, and for these peptides each tissue was challenged only once with "pA_2 dose" of the irreversible antagonist. The "pA_2 values" so obtained are considered tentative (see Table I) since these antagonists do not appear to have purely competitive mechanisms of action.[7]

[Sar^1,D-Trp^4,Ile^8]angiotensin II: yield 18%; TLC R_f (BPAW) 0.37, R_f (CMAW) 0.41. Amino acid analysis: Arg, 1.10; Val, 1.00; Trp, 0.88; Ile, 2.11; His, 1.08; Pro, 0.92.

[Sar^1,D-Trp^4]angiotensin II: yield 16%; TLC R_f (BPAW) 0.38, R_f (CMAW) 0.43. Amino acid analysis: Arg, 1.12; Val, 1.00; Trp, 0.87; Ile, 0.89; His, 1.05; Pro, 0.95; Phe, 1.12.

[Sar^1,Tyr(Me)^4]angiotensin II: yield 16% TLC R_f (BPAW) 0.32, R_f (CMAW) 0.32. Amino acid analysis: Arg, 1.12; Val, 1.00; Tyr(Me)+Tyr, 0.90; Ile, 0.88; His, 1.05; Pro, 0.99; Phe, 1.08. FAB-MS, MH^+ = 1017.

[Sar^1,Tyr(Me)^4,Ile^8]angiotensin II: yield 15%; TLC R_f (BPAW) 0.35, R_f (CMAW) 0.38. Amino acid analysis: Arg, 1.08; Val, 1.00; Tyr(Me)+Tyr, 0.92; Ile, 2.04; His, 1.06; Pro, 0.96. FAB-MS, MH^+ = 983.

[Sar^1,D-Trp^8]angiotensin II: yield 7%; TLC R_f (BPAW) 0.36, R_f (CMAW) 0.40. Amino acid analysis: Arg, 1.10; Val, 1.00; Tyr, 1.05; Ile, 0.89; His, 1.15; Pro, 1.05; Trp, 0.89.

[Sar^1,Tyr(Me)^4,D-Trp^8]angiotensin II: yield 10%; TLC R_f (BPAW) 0.39, R_f (CMAW) 0.43. Amino acid analysis: Arg, 1.05; Val, 1.00; Tyr(Me)+Tyr, 0.95; Ile, 0.96; His, 1.06; Pro, 1.04; Trp, 0.89.

[D-Trp^3,Ile^7]angiotensin III: yield 22%; TLC R_f (BPAW) 0.55, R_f (CMAW) 0.42. Amino acid analysis: Arg, 1.08; Val, 1.00; Trp, 0.92; Ile, 2.08; His, 1.06; Pro, 1.12.

[D-Trp^3]angiotensin III: yield 24%; TLC R_f (BPAW) 0.56, R_f (CMAW) 0.40. Amino acid analysis: Arg, 1.12; Val, 1.00; Trp, 0.87; Ile, 0.89; His, 1.05; Pro, 0.95; Phe, 1.12.

[Tyr(Me)^3)]angiotensin III: yield 14%; TLC R_f (BAPW) 0.53, R_f (CMAW) 0.41. Amino acid analysis: Arg, 1.10; Val, 1.00; Tyr(Me)+Tyr, 0.92; Ile, 0.94; His, 1.06; Pro, 0.93; Phe, 1.07.

[Tyr(Me)^3,Ile^7]angiotensin III: yield 15% TLC R_f (BAPW) 0.52, R_f (CMAW) 0.40. Amino acid analysis: Arg, 1.05; Val, 1.00; Tyr(Me)+Tyr, 0.93; Ile, 1.95; His, 1.08; Pro, 0.90.

[D-Trp^7]angiotensin III: yield 8%; TLC R_f (BPAW) 0.53, R_f (CMAW) 0.41. Amino acid analysis: Arg, 1.12; Val, 1.00; Tyr, 0.97; Ile, 0.92; His, 1.12; Pro, 0.92; Trp, 0.96.

[Tyr(Me)^3,D-Trp^7]angiotensin III: yield 10%; TLC R_f (BPAW) 0.57, R_f (CMAW) 0.44. Amino acid analysis: Arg, 0.93; Val, 1.00; Tyr(Me)+Tyr, 0.95; Ile, 1.06; His, 1.10; Pro, 0.95; Trp, 0.91.

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Registry No. [Sar^1,D-Trp^4,Ile^8]angiotensin II, 95798-24-6; [Sar^1,D-Trp^4]angiotensin II, 95798-25-7; [Sar^1,Tyr(Me)^4]angiotensin II, 88874-29-7; [Sar^1,Tyr(Me)^4,Ile^8]angiotensin II, 92780-94-4; [Sar^1,D-Trp^8]angiotensin II, 95841-12-6; [Sar^1,Tyr(Me)^4,D-Trp^8]angiotensin II, 95841-13-7; [D-Trp^3,Ile^7]angiotensin III, 95798-26-8; [D-Trp^3]angiotensin III, 95798-27-9; [Tyr(Me)^3]angiotensin III, 95936-83-7; [Tyr(Me)^3,Ile^7]angiotensin III, 95798-28-0; [D-Trp^7]angiotensin III, 95798-29-1; [Tyr(Me)^3,D-Trp^7]angiotensin III, 95798-30-4; [Sar^1,Ile^8]angiotensin II, 37827-06-8.

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Hashish:[1] Synthesis and Central Nervous System Activity of Some Novel Analogues of Cannabidiol and Oxepin Derivatives of Δ^9-Tetrahydrocannabinol[2]

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Several C-10 substituted cannabidiol (CBD) derivatives and novel oxepin derivatives of Δ^9-tetrahydrocannabinol (Δ^9-THC) were synthesized and evaluated for biological activity in mice and dogs. Treatment of 10-bromocannabidiol diacetate (**3**) with various amines in Me_2SO gave the corresponding 10-aminocannabidiol derivatives **4–6**. Similarly, treatment of **3** with NaN_3 gave the azido compound **7**, which with $LiAlH_4$ afforded the 10-aminocannabidiol **9**. However, reduction of **7** with $CrCl_2$ formed the amide **8**, which on further reduction with $LiAlH_4$ gave the *N*-ethyl analogue **10**. Coupling of **9** with **11** in the presence of dicyclohexylcarbodiimide formed **12**, which was then deprotected with HCl to give the analogue **13**. The oxepin analogue **14a** was synthesized from **3** by treatment with Na_2CO_3 in CH_3OH/H_2O at room temperature. The dimethylheptyl analogue **14b** was similarly prepared. Incorporation of *N*-ethyl (**10**), *N*-methyl-*N*-propargyl (**6**), and morpholino (**4**) groups in CBD at position 10 resulted in analogues that were more potent than CBD in producing hypoactivity in mice. These analogues had relatively little effect on rectal temperature. Selected substitutions at C-10 also resulted in analogues that were partially effective in blocking Δ^9-THC antinociceptive activity. This blockade was observed particularly in compound **10**, which also showed unusually toxic properties. Incorporation of a seven-membered oxepin in the Δ^9-THC structure eliminated cannabinoid activity although substitution of the pentyl side chain with a 1,2-dimethylheptyl in the oxepin **14b** resulted in CNS depression in mice.

In continuation of our work[3] to study structure–activity relationships (SAR) and develop cannabinoids that are more specific in their effects, we directed our efforts toward cannabidiol derivatives. Interest in cannabidiol (CBD, **1**) and related compounds arises from the fact that CBD is

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(1) Paper 35 in the Hashish Series. For paper 34, see: Jorapur, V. S.; Duffley, R. P.; Razdan, R. K. *Synth. Commun.* **1984**, *14*, 655.

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