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Relationship of Structures and Microbiological Activities of the 16-Membered Macrolides

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The 16-membered macrolide antibiotics were divided into three types depending on the number and position of carbonyl groups in the aglycone. The role of the carbonyl groups in the microbial activity of the macrolides was presented.

During the past 15 years, the structures of a number of macrolide antibiotics have been elucidated. Of particular interest to us is the group of macrolides containing the 16-membered lactone ring which include such compounds as leucomycins,[1-4] YL-704 antibiotics,[5,6] espinomycin,[7] SF-837 antibiotics,[8,9] spiramycins,[10] niddamycin,[11,12] magnamycins,[10,13,14] cirramycin,[15] B-58941,[16] tylosin,[17] chalcomycin,[18] and neutramycin.[19] While these antibiotics have many similar structural features, there are some marked structural differences which appear, however, to be of little microbiological consequence. All these antibiotics have about the same antibacterial spectra exhibiting pronounced activity against Gram-positive bacteria, Gram-negative cocci, and some species of mycoplasma. It is for this reason that we felt it appropriate and timely to ascertain which functional groups are essential for microbiological activity and which are not.

The 16-membered macrolide antibiotics may be divided into three classes depending on the number and position of carbonyl groups in the molecule. The first class represented by leucomycins, spiramycins, etc. (Figure 1) possesses an aldehyde group attached to the six position of the ring structure through a methylene group. The second class includes the magnamycins, cirramycin, tylosin, etc. (Figure 2) and is similarly characterized by the aldehyde group attached to carbon 6 through a methylene group but also by a carbonyl group in position 9. The antibiotics of the third class, illustrated by chalcomycin and neutramycin, have only one carbonyl group, the keto function at position 9 (Figure 3).

In previous publications it was shown that the aldehyde group in leucomycins[20] and spiramycins[21] is important for antibiotic activity. In the case of chalcomycin, it had been assumed that the carbonyl group at position 9 may be important for microbial activity but no data were presented. The present report offers new data which demonstrate further the importance of the carbonyl groups in the microbial activity of the 16-membered macrolides and also summarizes on a broader basis the relationship of microbial activities of these antibiotics with chemical structures.

Results and Discussion

The importance of the 9-keto group in chalcomycin is evident from a comparison of the microbial activity of chalcomycin with its hexahydro derivative **2** and its octahydro derivative **3** (Scheme I), both derivatives having been prepared from the parent by catalytic hydrogenation.[18] As seen in Table I, in which is listed our microbial data of compounds discussed in this report, compound **2**, which still possesses the keto group, showed activity close to that of its parent whereas the octahydro compound **3**, with no keto group, possesses negligible microbial activity. The high activity of the hexahydro compound **2** also demonstrates the unimportance of the conjugated double bonds between carbons 10 and 13 for microbiological activity. The same conclusion was reached previously for leucomycin A_3.[20]

The 9-keto group does not appear to be important in those antibiotics of the magnamycin type so long as the aldehyde group remains intact. While leucomycin A_3 (**4**) and its 9-dehydro derivative, magnamycin B (**5**), prepared from **4** by oxidation with MnO_2 (Scheme II), showed about the same activity, 18-dihydroleucomycin (**6**), prepared from 4 by reduction with $NaBH_4$, possesses very little microbial activity. The contribution of the 9-keto group was noted further from the microbial activity of 9-dehydro-18-dihydroleucomycin (**7**) prepared from **6** by allylic oxidation. Compound **7** with its 9-keto group but without the aldehyde group shows a low order of microbial activity, although greater than its parent **6**.

A similar structure-activity relationship obtains with isoleucomycin A_3 (**8**), an isomer of **4** prepared by rearrangement[2] of the 9-hydroxyl group to the C-13 position. While **8** has almost the same activity as **4**, its 18-dihydro derivative (**9**) has very little or no activity. Similarly, 13-dehydro-18-dihydroisoleucomycin A_3 (**10**), prepared from **9** by oxidation with MnO_2, shows, in contrast to **9**, small but definite activity.

Tylosin (**11**) which like magnamycins has carbonyl functions at C_9 and C_{20} also loses its antimicrobial activity by reduction of these groups with $NaBH_4$ to the tetrahydro

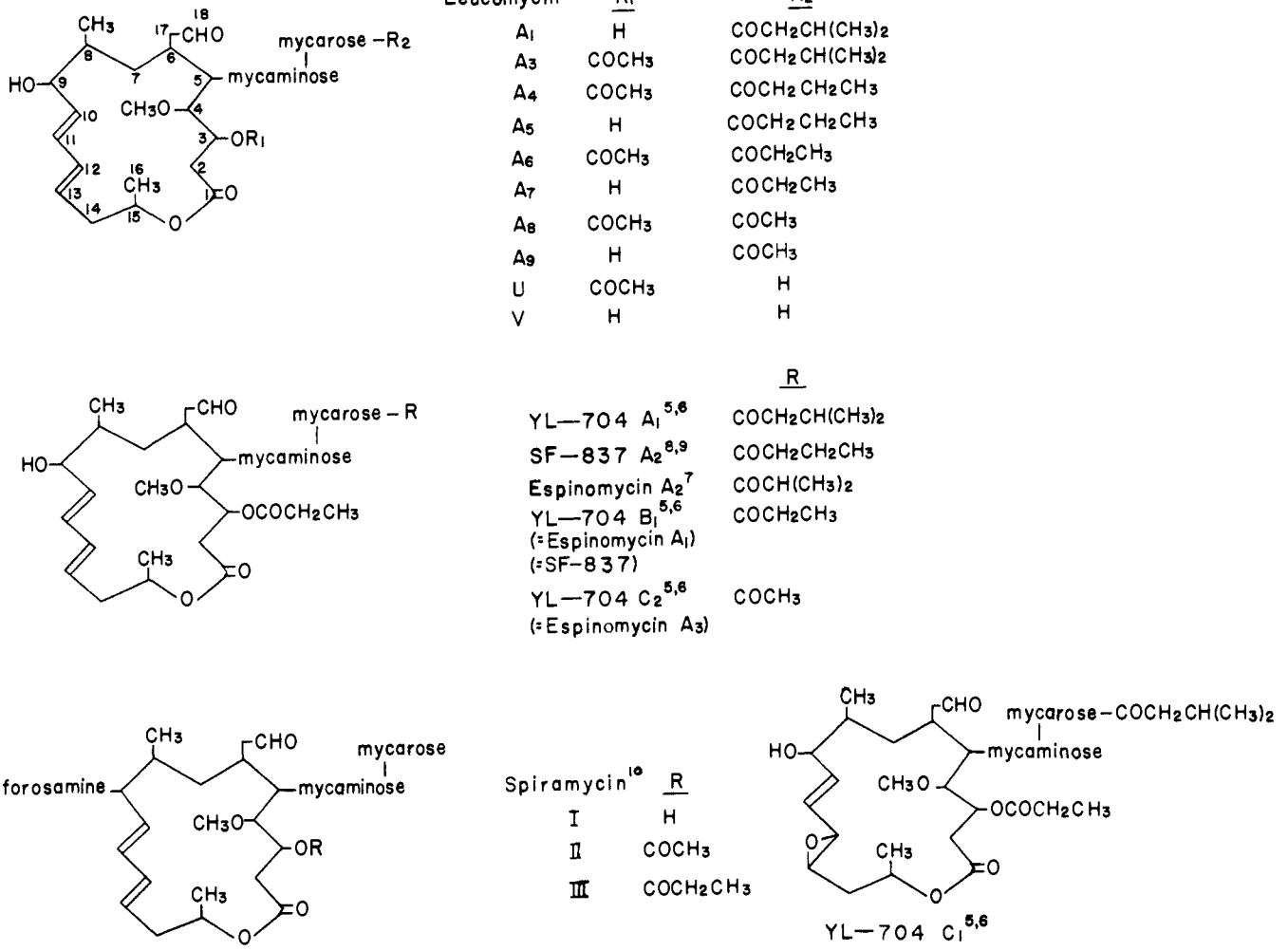


Figure 1.

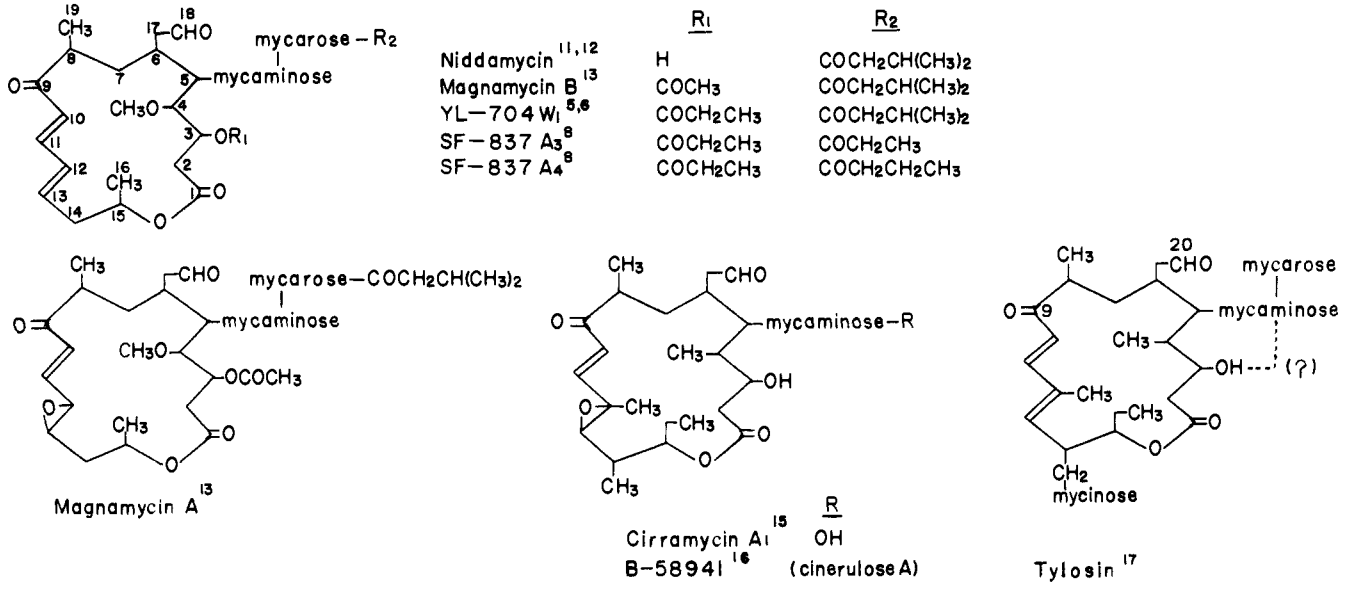


Figure 2.

compound (**12**). While attempts to oxidize **12** to the corresponding 9-dehydro derivative (**13**) were unsuccessful, the latter was obtained directly from **11** by selective reduction.[21] Compound **13** shows pronounced microbial activity compared with **12** (Scheme III).

A survey of all the 16-membered macrolide antibiotics indicates that groups attached to the ring system at C_3, C_4, and C_{12} through C_{13} have little significance in terms of mi-

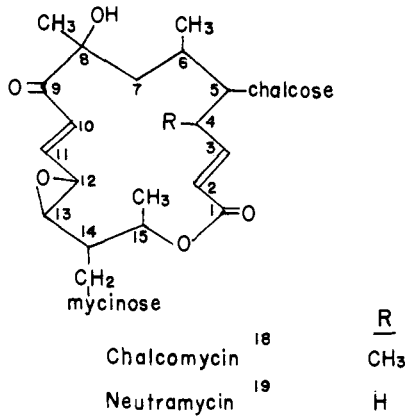
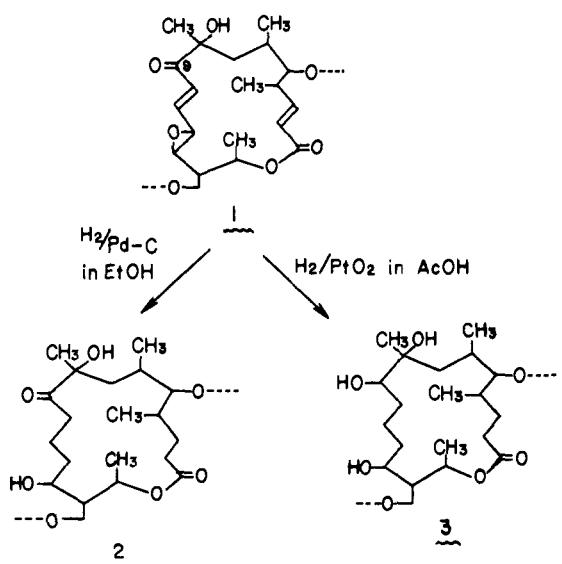


Figure 3.

Scheme I



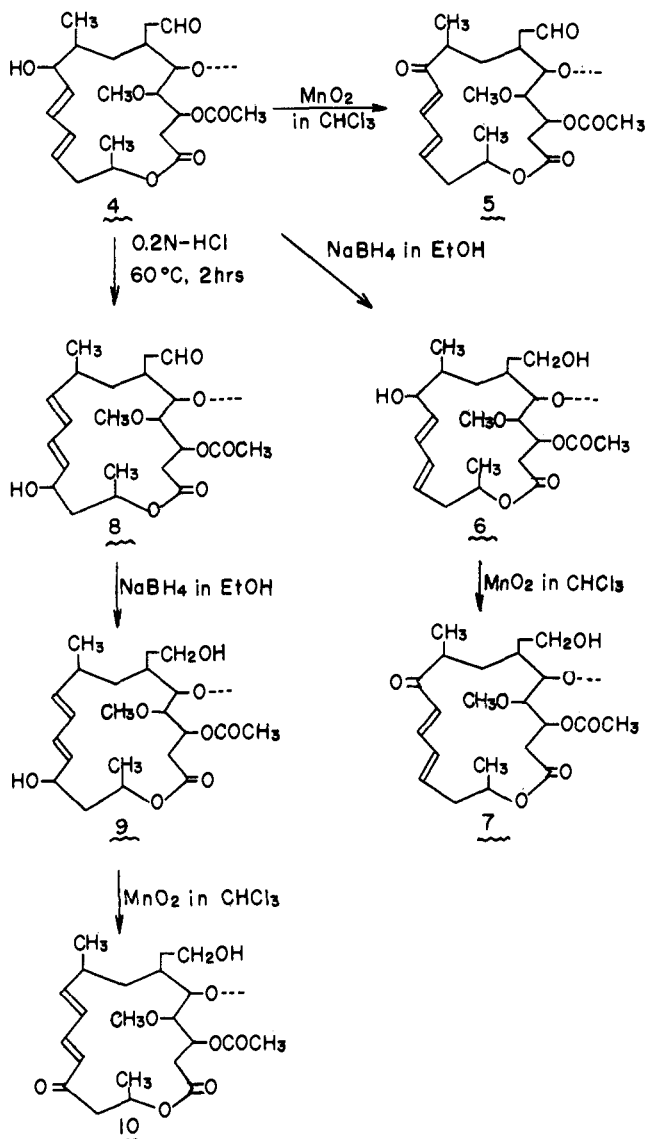
crobial activity. For example, the replacement of the methoxy group of leucomycin with methyl, as in chalcomycin and tylosin, or by hydrogen, as in neutramycin, produces insignificant change.

One other important group for antibiotic activity already reported is the dimethylamino group of the mycaminose moiety of leucomycin† and spiramycin.[22] In the case of the chalcomycin, a methoxyl has replaced the dimethylamino group of mycaminose without impairing microbial activity.

It is known that the microbial activity of the macrolides involves binding of the antibiotics with ribosomes and inhibition of protein synthesis.[23,24] In the 16-membered macrolides, binding must involve the aldehyde or the 9-keto group (or both), the dimethylamino or the *O*-methyl group (as in chalcomycin), the hydroxyl group at 2′ and possibly the lactonic ester group. Each 16-membered macrolide has methyl groups attached to C-8 and methyl or ethyl groups attached to C-15 which possibly play a role by bringing about hydrophobic bonding with ribosomal lipophylic groups. To account for the activity of chalcomycin and leucomycin and indeed for other 16-membered macrolides, one must assume that these different antibiotics can take on similar topochemical[25] and isosteric conformations in order to affect their ribosomal binding. The conformational analysis[26] of leucomycin suggested that

†S. Ōmura, unpublished data, Kitasato Institute, Tokyo, Japan.

Scheme II



the 16-membered lactone ring was mobile and dependent on the solvent from CD data. This mobile conformation of 16-membered lactone may promote their taking on various isosteric conformations.

Scheme III

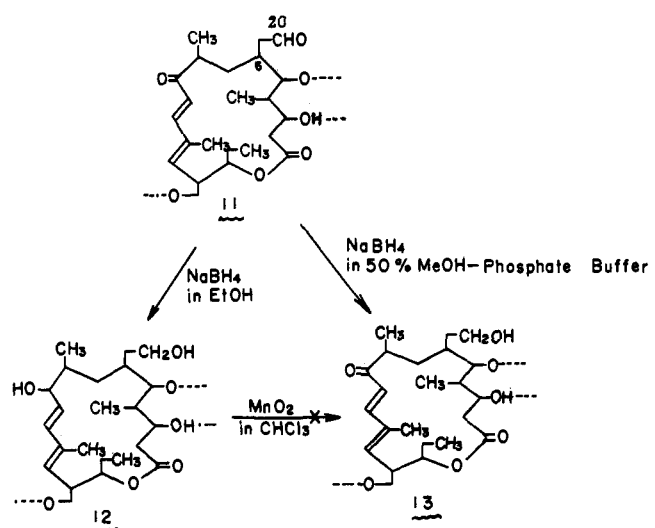


Table I. Antimicrobial Activities of Leucomycin, Chalcomycin, Tylosin, and Their Derivatives

Compounds	MIC, μg/ml[a]						
	1	2	3	4	5	6	7
Chalcomycin (1)	25	0.8-3.12	0.2	>100	>100	3.13	<0.20
Hexahydrochalcomycin (2)	50	1.56	0.2-12.5	>100	>100	50	25
Octahydrochalcomycin (3)	>100	>100	25	>100	>100	>100	>100
Leucomycin A_3 (4)	0.2	0.4	0.05	12.5	50	<0.20	<0.20
9-Dehydroleucomycin A_3 (5) (Magnamycin B)	0.4	0.4	0.05	>100	25		
18-Dihydroleucomycin A_3 (6)	>100	>100	50-100	>100	>100	>100	>100
9-Dehydro-18-dihydroleucomycin A_3 (7)	25	25	1.56	>100	>100	1.56	0.39
Isoleucomycin A_3 (8)	0.05	0.05	0.05	50	>100	<0.20	<0.20
18-Dihydroisoleucomycin A_3 (9)	>100	>100	>100	>100	>100	100	25
13-Dehydro-18-dihydroisoleucomycin A_3 (10)	50	100	6.25	>100	>100	25	3.13
Tylosin (11)	0.2	0.4	0.05	50	25	0.78	<0.20
9,20-Tetrahydrotylosin (12)	>100	>100	3.12	>100	>100	>100	3.12
18-Dihydrotylosin (13)	0.4	6.25	0.05	>200	>200		

[a] Agar dilution streak method. Test microorganisms: 1, *Bacillus subtilis* PCI 219; 2, *Staphlococcus aureus* FDA 209P; 3, *Sarcina lutea* PCI 1001; 4, *Klebsiella pneumoniae* PCI 607; 5, *Escherichia coli* NIHJ; 6, *Mycoplasma hominis* type 1 C; 7, *Mycoplasma salivarium* C Hup.

Experimental Section

Examination of Antibacterial Activity. The minimum inhibitory concentration (MIC) of each compound was examined by an agar dilution streak method with five different bacteria, listed in Table I.

MIC against Mycoplasma. Following the procedure of Hoshino, *et al.*,[27] Hokken mycoplasma medium was used for the cultivation of mycoplasma, and Hokken mycoplasma agar medium was used for the determination of the minimum inhibitory concentration (MIC) of each compound against mycoplasma strains. MIC of each antibiotic was obtained by the agar dilution method using mycoplasma grown in a liquid medium at 37° for 3 days. The organisms were transplanted on agar plate and incubated at 37° for 8 days, and the growth of mycoplasma colonies was examined under a microscope of low magnification (×100).

Hexahydrochalcomycin (2),[18] octahydrochalcomycin (3),[11] 9-dehydroleucomycin A_3 (5),[2] isoleucomycin A_3 (8),[2] and 18-dihydrotylosin (13)[21] were obtained by the methods described in the literature.

18-Dihydroleucomycin A_3 (6). To a solution of 500 mg (0.605 mmoles) of 4 in 10 ml of absolute EtOH, 20 mg (0.701 mmoles) of $NaBH_4$ was added at room temperature. After standing for 1 hr, the reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was chromatographed over silica gel (25 g) and developed with C_6H_6-Me_2CO (5:1). Effluents containing 18-dehydroleucomycin A_3 were collected and on evaporation of solvent, a white powder, 280 mg (0.340 mmoles) was obtained as product: yield, 55.8%; $[\alpha]^{22}D$ −48.0° (*c* 0.5, EtOH); uv λ_{max} 229 nm (ε 26,300, EtOH). *Anal.* ($C_{42}H_{71}O_{15}N$) C, H, N.

9-Dehydro-18-dihydroleucomycin A_3 (7). 18-Dihydroleucomycin A_3 (6) (120 mg, 0.145 mmoles) was dissolved in 10 ml of $CHCl_3$ and 1.2 g of activated MnO_2[28] was added. After stirring for 8 hr at room temperature, the reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was chromatographed over silica gel (20 g) and developed with C_6H_6-Me_2CO (5:1). Effluents containing 9-dehydro-18-dihydroleucomycin A_3 (7) were collected. Concentration of the combined effluents gave 30 mg (0.036 mmoles) of a white powder (7): yield, 25.1%; $[\alpha]^{22}D$ −61.6° (*c* 0.33, EtOH); uv λ_{max} 280 nm (ε 21,000, EtOH); ir ($CHCl_3$) 3460 (ν_{OH}), 1725 (lactone and ester ν_{CO}), 1679 (α,β,γ,δ-unsatd ketone ν_{CO}), and 1595 cm^{-1} (trans, trans double bond). *Anal.* ($C_{42}H_{69}O_{15}N$) C, H, N.

18-Dihydroisoleucomycin A_3 (9). Isoleucomycin A_3 (8) (260 mg, 0.314 mmoles) was treated with 10 mg (0.350 mmoles) of $NaBH_4$ in the same way as was 4. A yield of 180 mg (0.217 mmoles) of 18-dihydroisoleucomycin A_3 (9) was obtained: yield, 69.0%; $[\alpha]^{22}D$ −52.0° (*c* 0.5, EtOH); uv λ_{max} 233 nm (ε 29,200, EtOH). *Anal.* ($C_{42}H_{71}O_{15}N$) C, H, N.

13-Dehydro-18-dihydroisoleucomycin A_3 (10). 18-Dihydroisoleucomycin A_3 (9) (100 mg, 0.121 mmoles) was treated with 1.1 g of activated MnO_2 in 9 ml of $CHCl_3$. In a similar manner as 7, 18 mg (0.022 mmoles) of the 13-dehydro compound (10) was obtained: yield, 18.1%; $[\alpha]^{22}D$ −59.8° (*c* 0.31, EtOH); uv λ_{max} 279 nm (ε 28,600, EtOH). *Anal.* ($C_{42}H_{69}O_{15}N$) C, H, N.

Tetrahydrotylosin (12). Tylosin (300 mg, 0.328 mmoles) was dissolved in 4 ml of absolute EtOH and 17 mg (0.611 mmoles) of $NaBH_4$ was added. After stirring for 2 hr at room temperature, the reaction mixture filtered and the filtrate was concentrated to dryness under the reduced pressure. To the residue was added 10 ml of H_2O, and the solution was extracted three times with 5 ml of $CHCl_3$. After drying with Na_2SO_4, the $CHCl_3$ extract was concentrated to dryness giving 280 mg of crude 12. This residual powder was chromatographed over silica gel (50 g) and developed with C_6H_6-Me_2CO (6-3:1). Evaporation of the effluents to dryness gave 200 mg (0.218 mmoles) of a white powder (12): yield, 66.4%; $[\alpha]^{22}D$ +38.2° (*c* 0.5, $CHCl_3$); uv λ_{max} 237 nm (ε 18,300, EtOH); ir ($CHCl_3$) 3460 (ν_{OH}), 1710 cm^{-1} (ν_{CO}). *Anal.* ($C_{46}H_{81}NO_{17}$) C, H, N.

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Angiotensin II Antagonism. Structure–Activity Relationships of 8-Substituted Angiotensin II Analogs

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Six analogs of angiotensin II were synthesized by the solid-phase method: [Asn^1,Val^5,Gly^8]-, [Asn^1,-Val^5,Ala^8]-, [Asn^1,Val^5,β-Ala^8]-, [Asn^1,Val^5,Abu^8]-, [Asn^1,Val^5,Val^8]-, and [Asn^1,Val^5,Leu^8]angiotensin II. These analogs were potent inhibitors of the vascular action of angiotensin II.

Many analogs of the pressor peptide hormone angiotensin II, Asp-Arg-Val-Tyr-Ile(or Val-)-His-Pro-Phe,[1] have been synthesized to delineate the relationship between chemical structure and biologic activity. Although these studies have provided a great wealth of information on the structural requirements for pressor activity, little is known about the role angiotensin II plays in hypertension. Elucidation of this role has been hampered by the lack of a specific, competitive inhibitor of the pressor activity of angiotensin II. With such an inhibitor the structural requirements for binding to the receptor sites could be differentiated from those necessary for biologic activity.

Recently specific inhibitors of the action of angiotensin II on smooth muscle have been reported. Khairallah, *et al.*,[2] and Türker, *et al.*,[3] found that [Ile^5,Ala^8]angiotensin II antagonized the action of angiotensin II on strips of guinea pig ileum and rabbit aorta, but found no *in vivo* activity. Marshall, *et al.*,[4] reported that [Phe^4,Tyr^8]angiotensin II inhibited angiotensin II activity on isolated rat uterus and also inhibited its pressor activity in rats. Park and Regoli[5] found that [D-Phe^8]angiotensir II and [8-(1-aminocyclopentanecarboxylic acid)]angiotensin inhibited the pressor and myotropic action of angiotensin I and angiotensin II. Pals, *et al.*,[6] have reported that [Asn^1,Val^5,Ala^8]angiotensin II[6a] and [Sar^1,Val^5,Ala^8]angiotensin II[6b] are specific, competitive antagonists of the action of angiotensin II on vascular smooth muscle both *in vitro* (rabbit aorta) and *in vivo* (pithed rats). Further investigation of analogs with aliphatic amino acids in the 8 position resulted in a series of inhibitors (Table I) which is the subject of this report.

Results and Discussion

The octapeptides in Table I were prepared by the basic solid-phase procedure described by Marshall and Merrifield[7] for the synthesis of [Ile^5]angiotensin II and were evaluated as antagonists of the action of angiotensin II on vascular smooth muscle as described in the Experimental Section.

This series of angiotensin II antagonists provides information on the role of the 8-phenylalanine residue of angiotensin II and allows some speculation on the molecular requirements for competitive antagonistic activity of analogs of the parent hormone. The aromatic ring in the 8 position is necessary for the pressor effect of the parent hormone.[5] This effect could be brought about either by π bonding of the aromatic ring to the receptor or by hydrophobic interaction of the ring with a specific part of the receptor. Further work will be necessary to elucidate the mechanism of the pressor effect caused by the phenylalanine residue. The fact that [Phe^4,Tyr^8]angiotensin II was also an antagonist would seem to contradict this theory. Its lack of pressor activity cannot be attributed to the substitution of tyrosine for phenylalanine, since [Tyr^8]angiotensin II is a potent pressor agent. However, Regoli and Park[5] have reported that [Phe^4,Tyr^8]angiotensin II, when given in relatively high doses, evoked pressor effects similar to angiotension II. In addition, the dose-response curve for [Ala^8]-angiotensin II was not parallel to the curves for either angiotensin II or [Phe^4,Tyr^8]angiotensin II. Thus it appears that the interaction of [Phe^4,Tyr^8]angiotensin II with the receptor may be more like that of angiotensin II than the 8-aliphatic substituted analogs.

Secondly, it is apparent from the pA_2 values for compounds **1**–**6** that the side chain of the 8 substituent does participate in binding to the receptor, since a steady increase in potency was observed as the length of the side chain was increased. The most dramatic increase was observed between peptide **2** with 8-alanine and peptide **4** with 8-α-aminobutyric acid. The ethyl side chain in **4** caused 20-fold greater binding than the methyl group in peptide **2**.

The free carboxyl group on the residue in position 8 has been shown *via* esterification or amide formation to be an essential feature for pressor activity.[8] From comparison of peptide **3** (8-β-alanine) to peptide **2** (8-alanine) it is apparent

Table I. Activity of 8-Substituted Angiotensin II Analogs
Asn-Arg-Val-Tyr-Val-His-Pro-R

No.	R	pA_2[a]
1	Gly	6.54 ± 0.01
2	Ala	6.84 ± 0.03
3	β-Ala	5.08 ± 0.08
4	Abu	7.93 ± 0.02
5	Val	8.31 ± 0.03
6	Leu	8.26 ± 0.07

[a] Values = mean ± standard error.