nication) has obtained a similar value for TPE-d_1 in $CDCl_3$.
(24) A. Abragam, "The Principles of Nuclear Magnetism", Clarendon Press, Oxford, 1961, p 504.
(25) See, for example, C. Brevard, J. P. Kintzinger, and J. M. Lehn, *Chem. Commun.*, 1193 (1969); C. Brevard and J. M. Lehn, *J. Am. Chem. Soc.*, **92**, 4987 (1970).
(26) J. P. Kintzinger, J. M. Lehn, and R. L. Williams, *Mol. Phys.*, **17**, 135 (1969).
(27) T_1 for the methine resonance (^1H) of TPE is ~0.7 s. The value of T_2^* employed in the line-shape calculations is based on the width at half height (1.0 Hz) of this resonance.
(28) For another analysis of a spin coupled to a relaxing nucleus, cf. J. A. Pople, *Mol. Phys.*, **1**, 168 (1958), and ref 21b.
(29) The TPE used in the present study contains ~25% TPE-d_0, and the d_0 line effectively "hides" the spectrum of the d_1 species. Analyses based on width at half height therefore measure essentially only the line width of the d_0 species. It is significant, however, that over the temperature range 310–370 K (toluene-d_8) the methine proton resonance of this sample does not perceptibly broaden ($w_{1/2h}$ ~ 1.6 Hz; $w_{baseline}$ ~ 5 Hz), nor does the ^2H coupling become apparent.
(30) Estimated from the Debye–Einstein equation (ref 21c).

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Rapid Access to Analogues of Phalloidin by Replacing Alanine-1 in the Natural Toxin by Other Amino Acids[1]

Sir:

In extensive studies of structure-activity correlations of the phallotoxins from the toxic mushroom *Amanita phalloides*,[2] the amino acid in position 1 (alanine) of phalloidin (**1a**) has

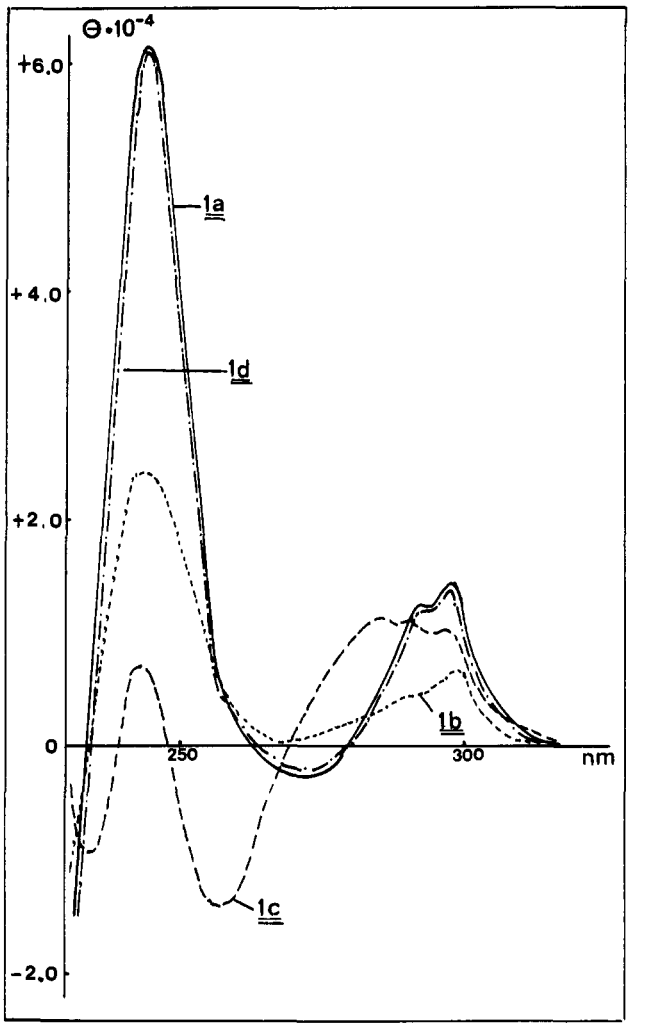


Figure 1. CD spectra of phalloidin **1a** and its analogues **1b**, **1c**, and **1d** measured in water solution.

Scheme I

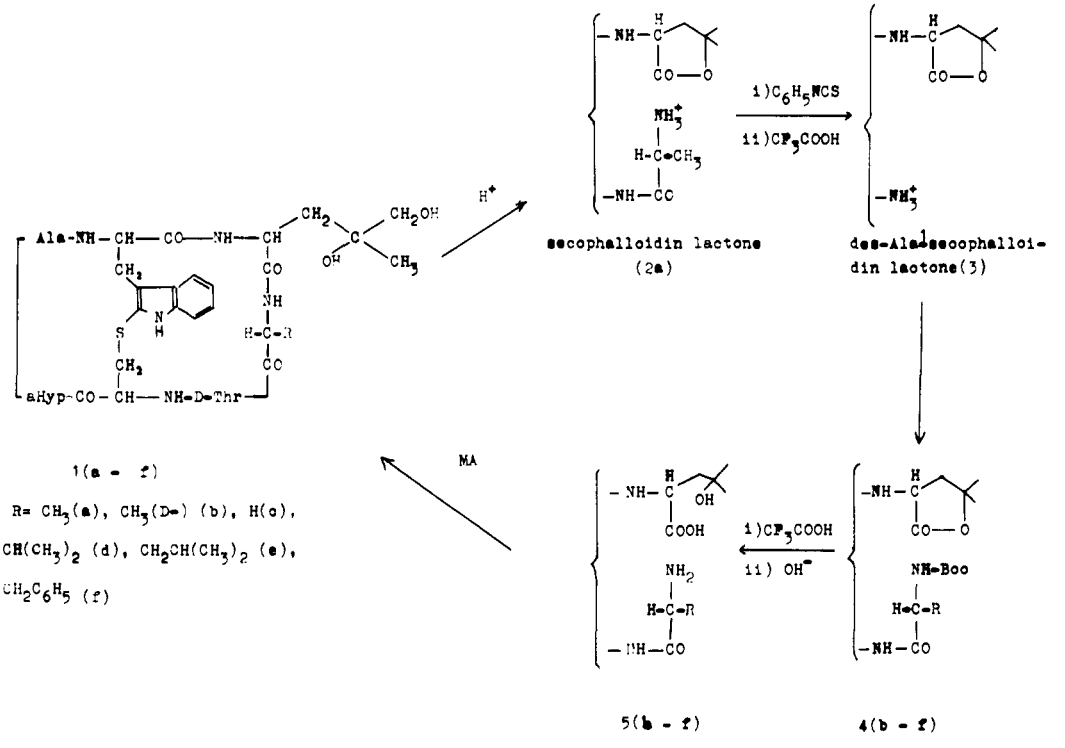


Table I. Yields of Cyclization, R_f Values (on Silica Gel TLC Plates, Kieselgel 60 F_{254} Merck, in 65:25:4 Chloroform-Methanol-Water by Volume), Amino Acid Analyses, and Toxicities (LD_{50}, Milligrams/Kilogram in White Mice)

Amino acid in position 1	No.	Yield of cyclization, %	R_f value	Ratio of amino acids		Toxicity
Ala[a]	**1a**	8.7	0.31	Ala Thr	2.00 0.90	2.0
D-Ala	**1b**	6.6	0.32	Ala Thr	2.00 0.95	*b*
Gly	**1c**	22.0	0.30	Gly Ala Thr	1.00 0.98 1.02	7.5
Val	**1d**	2.4	0.45	Val Ala Thr	1.06 1.00 0.89	2.5
Leu	**1e**	2.7	0.48	Leu Ala Thr	1.03 1.00 0.93	2.5
Phe	**1f**	2.2	0.48	Phe Ala Thr	0.97 1.00 0.92	20.0
des-Ala	**6**	7.7	0.32	Ala Thr	1.00 0.89	*b*
Ala_2	**8**	28.0	0.40	Ala Thr	3.00 1.04	*b*

[a] Substance obtained by recyclization of secophalloidin **5a**.[3] [b] Tested in doses up to 30 mg/kg.

been replaced by several amino acids. Recently, we described the recyclization of the nontoxic secophalloidin (**5a**) to yield phalloidin (**1a**) by the mixed anhydride method.[3] The exchange of 1-alanine of the seco compound **2a** was carried out by one Edman degradation step, followed by coupling of the shortened peptide **3** with the Boc derivative of the desired amino acid to provide the different Boc seco compounds **4b–f**. Removal of the protecting group, hydrolytic opening of the γ-lactone in position 7 (γ,δ-dihydroxyleucine), and cyclization of the seco compounds **5b–f** afforded the phalloidin analogues **1b–f**.

Secophalloidin lactone[4] (**2a**, 600 mg, 0.76 mmol) gave phenylthiocarbamoyl secophalloidin lactone (591 mg, 84.0%) on reaction with an excess of phenyl isothiocyanate (12.5 mL) in 50% aqueous pyridine (400 mL) at 40 °C for 1 h.[5] The phenylthiocarbamoyl derivative (500 mg, 0.54 mmol) was treated with trifluoroacetic acid as described[5] and chromatographed on Sephadex G-15 in 0.1 M acetic acid, to yield [des-Ala^1]-secophalloidin lactone (**3**, 343 mg, 88.3%). Compound **3** (200 mg, 0.28 mmol) was acylated with, for example, Boc-valine-*N*-hydroxysuccinimido ester (450 mg, 5.1 equiv) and *N*-methylmorpholine (0.3 mL) in dimethylformamide (5 mL) at 0 °C for 1 h and 20 °C for 35 h. The resulting [Boc-Val^1]-secophalloidin lactone (**4d**) was purified chromatographically on Sephadex LH-20 in methanol (241 mg, 83.1% with respect to **3**). The Boc group of **4d** (440 mg, 0.48 mmol) was removed with trifluoroacetic acid and the deprotected γ-lactone was hydrolytically opened via chromatography on Sephadex LH-20 in 4 mM aqueous ammonia[3] to afford **5d** (358 mg, 88.8%). Compound **5d** (300 mg, 0.36 mmol) was cyclized via its mixed anhydride with isobutyloxycarbonyl chloride in 10^{-4} M solution to give [Val^1]-phalloidin (**1d**, 7.1 mg, 2.4%) (Scheme I).

Starting from **3**, a bicyclic hexapeptide, [des-Ala^1]-phalloidin (**6**), and a bicyclic octapeptide, [*endo*-Ala^{1a}]-phalloidin (**8**), have also been obtained. Compound **6** was synthesized by cyclization of **3** after opening of the lactone ring. Cyclization of the seco compound **7** afforded compound **8**. The seco compound **7** was prepared by coupling of **2a** with Boc-alanine-*N*-hydroxysuccinimido ester and subsequent removal of the

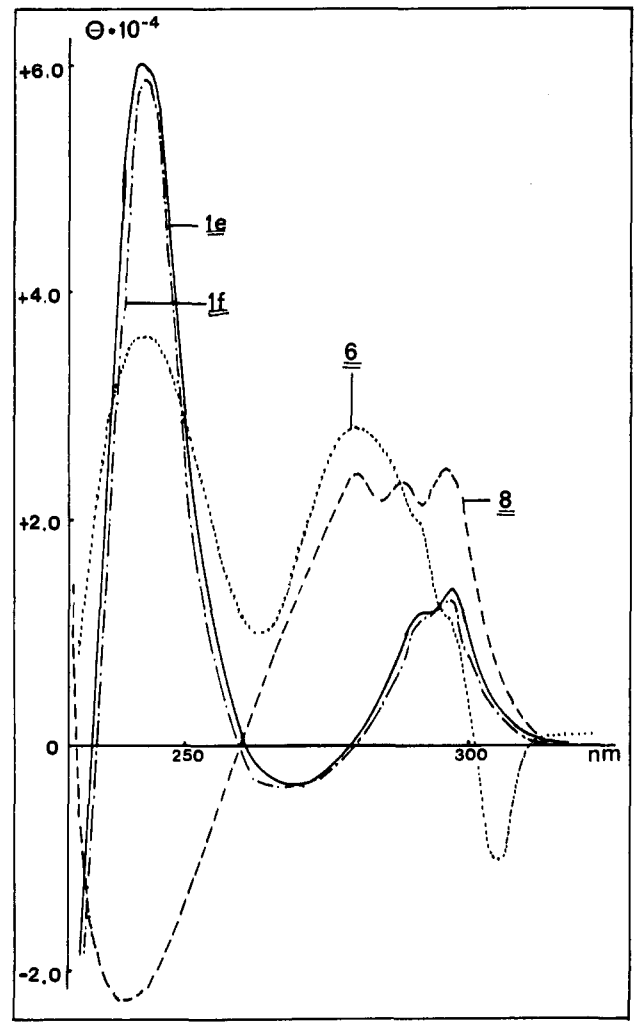
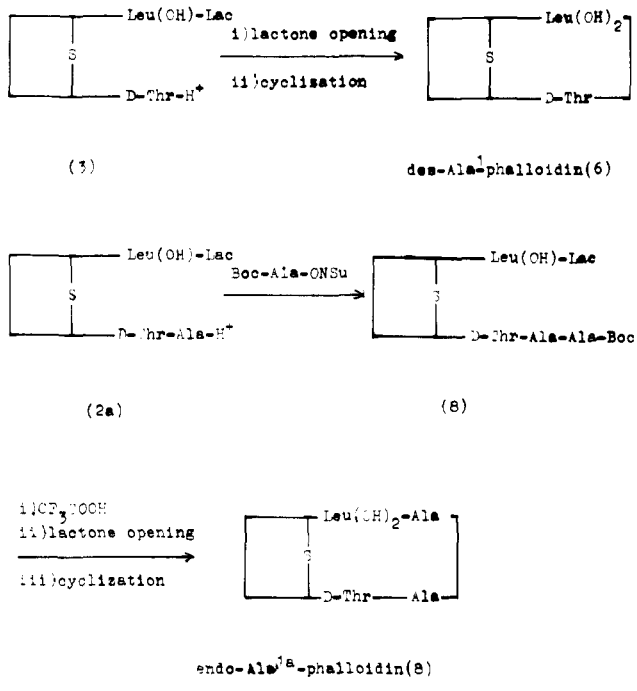


Figure 2. CD spectra of analogues **1e**, **1f**, **6**, and **8** measured in water solution.

Scheme II



Boc group and opening of the lactone ring (Scheme II).

The yields of the cyclization reactions, R_f values of the analogues on TLC, amino acid analyses, and toxicities in white mice are compiled in Table I.

The CD spectra of the analogues **1d, 1e,** and **1f** are almost identical with that of **1a**, whereas the curve of analogue **1c** is significantly different (Figures 1 and 2). The same is true for the UV-difference spectra of the complexes with rabbit muscle actin,[2b,6] where the Gly^1 analogue **1c** shows a curve deviating from the normal one. Interestingly **1c** possesses toxicity, although to a reduced extent. The hexapeptide **6** and the octapeptide **8** also show abnormal CD spectra and no binding to actin as evidenced by the lack of difference spectra.

The present results extend our knowledge on the structure-toxicity relationships of the phallotoxins as follows. (1) In order to be toxic the bicyclic peptide must consist of seven amino acids, since the hexapeptide **6** and octapeptide **8** are nontoxic. (2) The methyl group of L-alanine may be replaced by an isopropyl (**1d**) or an isobutyl group (**1e**) without loss of toxicity. Toxicity is reduced by substitution of the methyl group by either a hydrogen atom (**1c**) or benzyl group (**1f**). (3) Change of configuration at L-alanine from L to D eliminates the toxic properties of the cyclic peptide. Details of the preparation of the analogues and their binding to actin will be reported in a forthcoming publication.

Acknowledgment. Ms. A. Schmitz, Ingelheim, is thanked for performing the toxicological experiments.

References and Notes

(1) Paper 54. Communication on the Components of the Green Deathcap Toadstool *Amanita phalloides*. 53: E. Munekata, H. Faulstich, and T. Wieland, *Justus Liebigs Ann. Chem.*, in press.
(2) For reviews, see (a) T. Wieland and O. Wieland, "Microbial Toxins", Vol. 8, S. Kadis, A. Ciegler, and S. J. Ajl, Ed., Academic Press, New York, N.Y., 1972, pp 249–280; (b) T. Wieland, "26. Colloquium Mosbach, 1975", L. Heilmeyer, J. C. Rüegg, and T. Wieland, Ed., Springer-Verlag, Berlin–Heidelberg, 1976, pp 203–214.
(3) E. Munekata, H. Faulstich, and T. Wieland, *Angew. Chem.*, **89**, 274 (1977); *Angew. Chem., Int. Ed. Engl.*, **16**, 267 (1977).
(4) T. Wieland and W. Schön, *Justus Liebigs Ann. Chem.*, **593**, 157 (1955). For the purpose of preparation, phalloidin was treated overnight with 50% aqueous trifluoroacetic acid and the seco compound purified chromatographically on Sephadex G-15 in 0.1 M acetic acid.
(5) P. Edman, "Protein Sequence Determination", S. B. Needleman, Ed., Chapman and Hall, London, Springer-Verlag, Berlin–Heidelberg–New York, 1972, pp 211–255.
(6) T. Wieland, J. X. de Vries, A. Schäfer, and H. Faulstich, *FEBS Lett.*, **54**, 73 (1975).
(7) Research fellow of Alexander von Humboldt Foundation, 1974–1976.

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Additions and Corrections

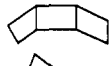
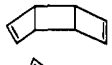
A Study on the Mechanism of the Reaction of *N*-(2,4-Dinitrophenyl)-3-carbamoylpyridinium Chloride with Amines and Amino Acids with Reference to Effect of Polyelectrolyte Addition [*J. Am. Chem. Soc.*, **98**, 2282 (1976)]. By S. KUNUGI, T. OKUBO, and N. ISE,* Department of Polymer Chemistry, Kyoto University, Kyoto, Japan.

On page 2285, in Table II, footnote *a*, "[amine] = 2.5×10^{-3} M" should be deleted.

On page 2286, second column, line 46 should read: "The τ_{s1} process was . . .".

Thermally Promoted Ring Cleavage Reactions of Stereoisomeric Tetracyclo[4.3.0.$0^{2,5}$.$0^{7,9}$]non-3-enes, Pentacyclo[5.3.0.$0^{2,6}0^{3,5}.0^{8,10}$]decanes, and Their Epoxide Counterparts [*J. Am. Chem. Soc.*, **98**, 8175 (1976)]. By LEO A. PAQUETTE* and MICHAEL J. CARMODY, Evans Chemical Laboratories, The Ohio State University, Columbus, Ohio 43210.

The lower section of Table III (p 8177) should read as follows:

	$\Delta H^{\pm}$, kcal/mol	$\Delta S^{\pm}$, eu	E_a, kcal/mol	Log A
	*a* 30.8	+1.05		
	31.2	−1.63		
			30.49 ± 0.16	14.22 ± 0.09
			32.59 ± 0.17	14.01 ± 0.09

1,3-Dicarbonyl-2-ketimines. Hydrolysis of 1,3-Dimethyl-5-(*p*-tolylimino)barbituric Acid [*J. Am. Chem. Soc.*, **99**, 2665 (1977)]. By J. M. SAYER* and MARTHA DEPECOL, Department of Chemistry, University of Vermont, Burlington, Vermont 05401.

On p 2668, headings for the last two columns of Table I