

- nication) has obtained a similar value for TPE- $d_1$  in  $\text{CDCl}_3$ .
- (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 ( $^1\text{H}$ ) of TPE is  $\sim 0.7$  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  $\sim 25\%$  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 ( $w_{1/2h} \sim 1.6$  Hz;  $w_{\text{baseline}} \sim 5$  Hz), nor does the  $^2\text{H}$  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<sup>1</sup>

Sir:

In extensive studies of structure–activity correlations of the phallotoxins from the toxic mushroom *Amanita phalloides*,<sup>2</sup> the amino acid in position 1 (alanine) of phalloidin (**1a**) has

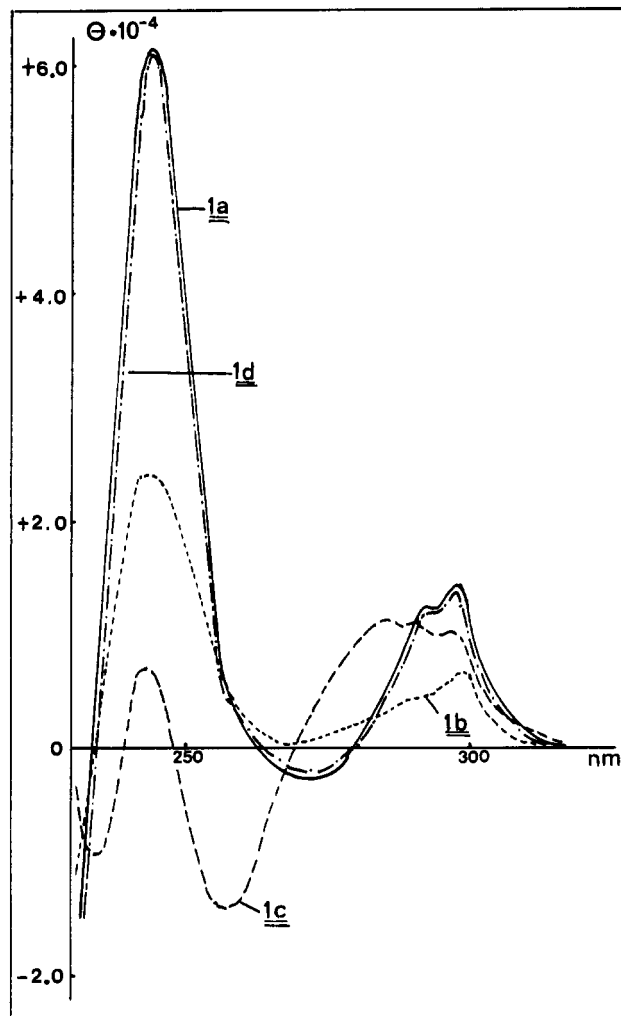
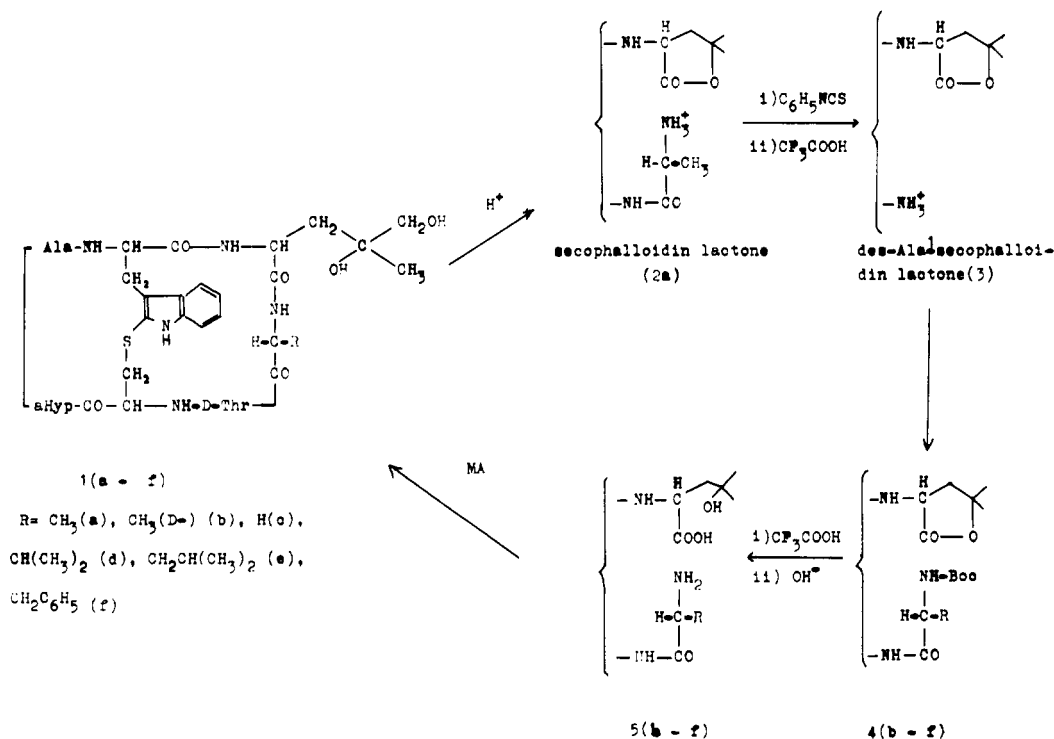


Figure 1. CD spectra of phalloidin **1a** and its analogues **1b**, **1c**, and **1d** measured in water solution.

## Scheme I



**Table I.** Yields of Cyclization,  $R_f$  Values (on Silica Gel TLC Plates, Kieselgel 60 F<sub>254</sub> Merck, in 65:25:4 Chloroform–Methanol–Water by Volume), Amino Acid Analyses, and Toxicities (LD<sub>50</sub>, Milligrams/Kilogram in White Mice)

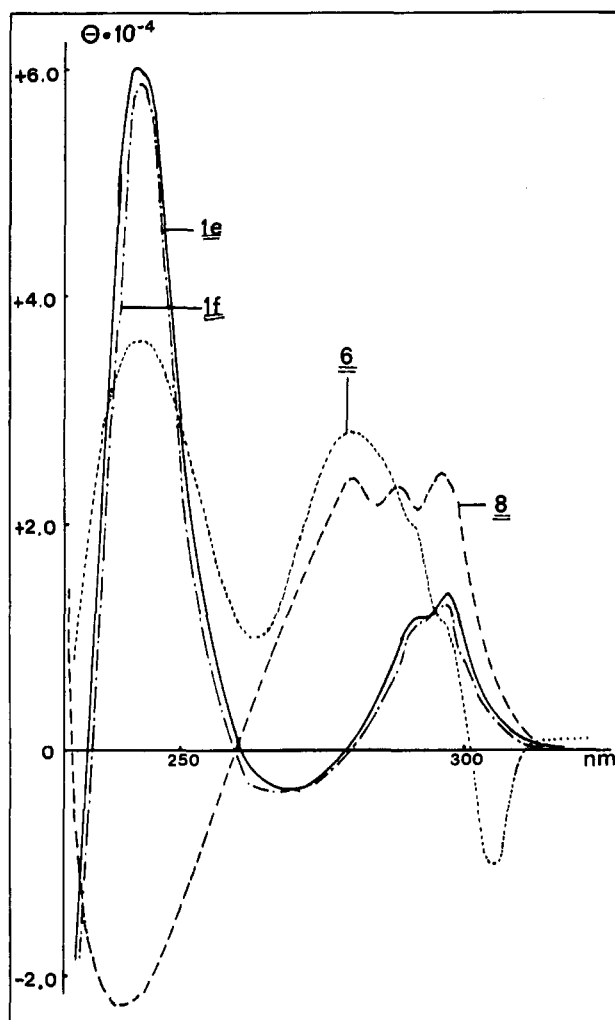
| Amino acid in position 1 | No.       | Yield of cyclization, % | $R_f$ value | Ratio of amino acids             | Toxicity |
|--------------------------|-----------|-------------------------|-------------|----------------------------------|----------|
| Ala <sup>a</sup>         | <b>1a</b> | 8.7                     | 0.31        | Ala 2.00<br>Thr 0.90             | 2.0      |
| D-Ala                    | <b>1b</b> | 6.6                     | 0.32        | Ala 2.00<br>Thr 0.95             | <i>b</i> |
| Gly                      | <b>1c</b> | 22.0                    | 0.30        | Gly 1.00<br>Ala 0.98<br>Thr 1.02 | 7.5      |
| Val                      | <b>1d</b> | 2.4                     | 0.45        | Val 1.06<br>Ala 1.00<br>Thr 0.89 | 2.5      |
| Leu                      | <b>1e</b> | 2.7                     | 0.48        | Leu 1.03<br>Ala 1.00<br>Thr 0.93 | 2.5      |
| Phe                      | <b>1f</b> | 2.2                     | 0.48        | Phe 0.97<br>Ala 1.00<br>Thr 0.92 | 20.0     |
| des-Ala                  | <b>6</b>  | 7.7                     | 0.32        | Ala 1.00<br>Thr 0.89             | <i>b</i> |
| Ala <sub>2</sub>         | <b>8</b>  | 28.0                    | 0.40        | Ala 3.00<br>Thr 1.04             | <i>b</i> |

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

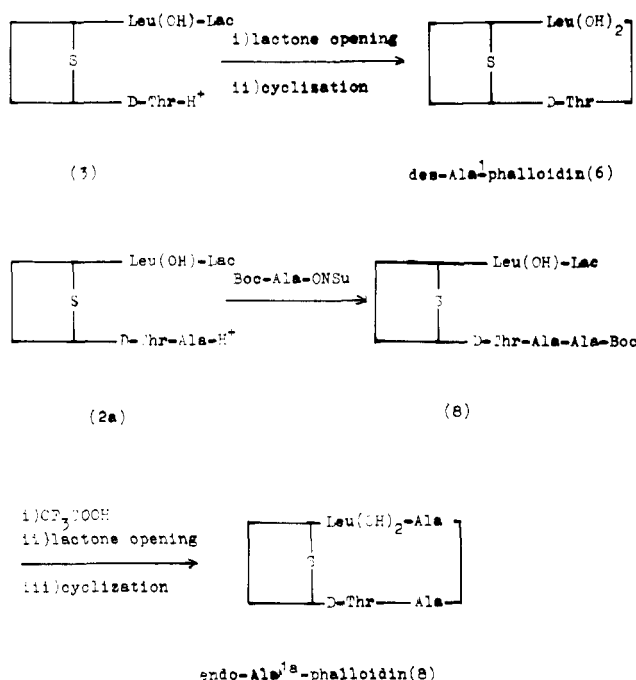
been replaced by several amino acids. Recently, we described the recyclization of the nontoxic secophalloidin (**5a**) to yield phalloidin (**1a**) by the mixed anhydride method.<sup>3</sup> The exchange of 1-alanine of the seco compound **2a** was carried out by one Edman degradation step, followed 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  $\gamma$ -lactone in position 7 ( $\gamma,\delta$ -dihydroxyleucine), and cyclization of the seco compounds **5b–f** afforded the phalloidin analogues **1b–f**.

Secophalloidin lactone<sup>4</sup> (**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.<sup>5</sup> The phenylthiocarbamoyl derivative (500 mg, 0.54 mmol) was treated with trifluoroacetic acid as described<sup>5</sup> and chromatographed on Sephadex G-15 in 0.1 M acetic acid, to yield [des-Ala<sup>1</sup>]-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<sup>1</sup>]-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  $\gamma$ -lactone was hydrolytically opened via chromatography on Sephadex LH-20 in 4 mM aqueous ammonia<sup>3</sup> 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<sup>−4</sup> M solution to give [Val<sup>1</sup>]-phalloidin (**1d**, 7.1 mg, 2.4%) (Scheme I).

Starting from **3**, a bicyclic hexapeptide, [des-Ala<sup>1</sup>]-phalloidin (**6**), and a bicyclic octapeptide, [endo-Ala<sup>1a</sup>]-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,<sup>2b,6</sup> where the Gly<sup>1</sup> 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. Clegler, 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. Ruegg, 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

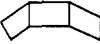
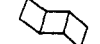


**A Study on the Mechanism of the Reaction of *N*-(2,4-Dinitrophenyl)-3-carbamoylpyridinium Chloride with Amines and Amino Acids with Reference to Effect of Polyelectrolyte Addition** [*J. Am. Chem. Soc.*, **98**, 2282 (1976)]. By S. KUNUGI, T. OKUBO, and N. ISE,\* Department of Polymer Chemistry, Kyoto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

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

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

**Thermally Promoted Ring Cleavage Reactions of Stereoisomeric Tetracyclo[4.3.0.0<sup>2,5</sup>.0<sup>7,9</sup>]non-3-enes, Pentacyclo[5.3.0.0<sup>2,6</sup>.0<sup>3,5</sup>.0<sup>8,10</sup>]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:

|   | $\Delta H^\ddagger$ ,<br>kcal/mol | $\Delta S^\ddagger$ ,<br>eu | $E_a$ , kcal/mol. | Log A        |
|---|-----------------------------------|-----------------------------|-------------------|--------------|
|  | <sup>a</sup><br>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