sensitive to the environment of the chromophore. It is qualitatively similar to the emission properties of the anilinonaphthalenesulfonates studied by others.[3-6] These compounds have been useful in studying protein structure, but according to Weber and Laurence[3] they can be used only with proteins which bind the chromophore rather tightly. We feel that the mansyl moiety, covalently attached, may provide some advantages in the use of protein fluorescent probes. In the preliminary studies reported here it has been shown that denaturing the protein leads to marked changes in the quantum yield of the chromophore. As would be expected in the presence of 5 *M* guanidine hydrochloride, the emission is decreased when the protein acquires a more random structure. This may be due to the decreased interaction of the mansyl residue with nonpolar regions of the protein.

However, the results obtained by comparing the emission of the mansyl BSA at pH 5.5 and pH 2.0 were not expected. Foster[11] reported that BSA is unfolded at pH 2.0. Wishnia and Pinder[12] found that butane and pentane were bound to BSA only 20% as much at pH 2 as at pH 5.5. Their data suggest that there are fewer effective hydrophobic binding sites at pH 2 than at pH 5.5. Our data suggest that upon denaturation by acid the covalently bound mansyl moeity is in a less polar environment. There is, thus, no conflict between these results; each technique probably measures a different property of BSA.

(11) J. F. Foster in "The Plasma Proteins," F. W. Putnam, Ed., Academic Press Inc., New York, N. Y., 1960, pp 179–239.
(12) A. Wishnia and T. Pinder, *Biochemistry*, **3**, 1377 (1964).

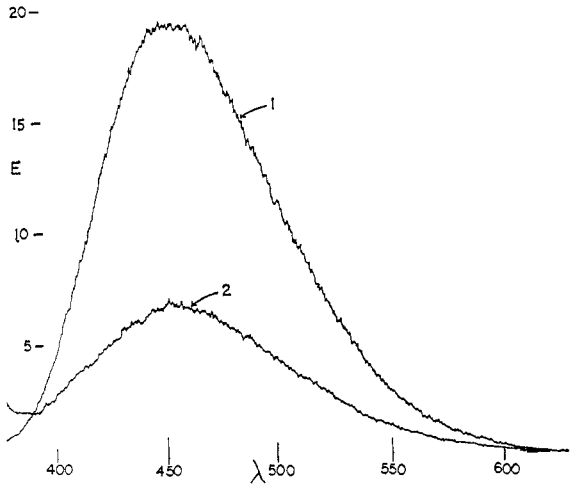


Figure 3. Effect of 5 *M* guanidine hydrochloride on the fluorescence emission of 2,6-mansyl BSA. Emission (*E*) in relative units *vs.* wavelength (λ) in nanometers. Excited at 360 nm; curve 1, mansyl BSA in buffer at pH 5.5; curve 2, mansyl BSA in 5 *M* guanidine hydrochloride at pH 5.5. Both samples are 6×10^{-6} *M* in mansyl.

McClure and Edelman[13] reported a rise in the fluorescence of 2-*p*-toluidinylnapthalene-6-sulfonate as the pH was lowered. However, their data may not be comparable to ours since, in our case, the chromophore is covalently linked to the protein.

Acknowledgment. The authors thank Dr. F. T. Bond for nmr spectra.

(13) W. O. McClure and G. M. Edelman, *ibid.*, **6**, 559 (1967).

Communications to the Editor

The Stereospecific Total Synthesis of *dl*-Lycopodine

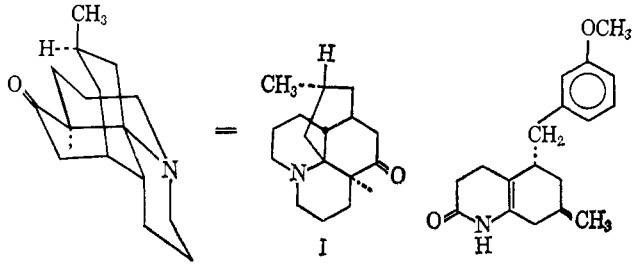
Sir:

Lycopodine (I) is one of many structurally related alkaloids found in numerous *Lycopodium* species.[1] We now report the total synthesis of *dl*-lycopodine.

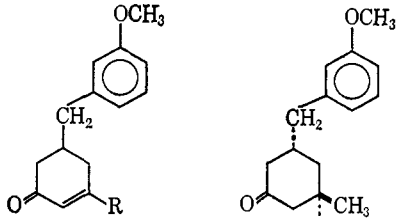
The initial goal of our approach was the substituted quinolone II. Reaction of *m*-methoxybenzaldehyde with ethyl acrylate and triphenylphosphine[2] gave ethyl 4-(*m*-methoxyphenyl)-3-butenoate, bp 125–128° (0.1 mm), which on subsequent heating with ethyl acetoacetate (sodium ethoxide–ethanol overnight) followed by hydrolysis and decarboxylation (15% aqueous potassium carbonate, 30-hr reflux) led in 36% yield to 5-(*m*-methoxybenzyl)-1,3-cyclohexanedione (III), mp 110–110.5°. The dione was converted *via* the usual lithium aluminum hydride reduction of the ethyl enol ether into 5-(*m*-methoxybenzyl)cyclohexenone (IV), bp 139–146° (0.1 mm), λ^{film} 5.85 μ, and the latter then led (methylmagnesium iodide, catalytic amount of cupric chloride in ether) in 90% yield to *trans*-3-(*m*-methoxybenzyl)-5-methylcyclohexanone[3] (V), bp 140–142° (0.1 mm), λ^{film} 5.85 μ, δ 0.96 (d, $J \sim 7$ cps).

Transformation of V into II was accomplished by reaction of the pyrrolidinenamine of V with acrylamide (overnight reflux in dioxane followed by addition of water and heating 2 hr). The two isomeric quinolones anticipated from this reaction[4] could be readily separated by crystallization which gave in about 20–25% yield the desired II, mp 132–133°, λ^{CHCl_3} 6.0 μ, δ ($CDCl_3$) 0.95 (3 H d, $J = 5$ cps), accompanied by the incompletely purified lower melting isomer II′ (= II with methyl and methoxybenzyl interchanged), δ 1.0 (d, $J \sim 7$ cps). The structural assignment follows from the mass spectra which show, in addition to the molecular ion at *m/e* 285, a much larger peak at M − 121 (loss of methoxy benzyl) than at M − 15 (loss of methyl) for II, while the reverse is true of II′.

(1) *Cf.* K. Wiesner, *Fortschr. Chem. Org. Naturstoffe*, **20**, 271 (1962).
(2) *Cf.* R. Oda, T. Kawabata, and S. Tanimoto, *Tetrahedron Letters*, 1653 (1964).
(3) *Cf.* N. L. Allinger and C. K. Riew, *ibid.*, 1269 (1966).
(4) This method of synthesis of 3,4,5,6,7,8-hexahydro-2-quinolones was first investigated by Dr. G. P. Moss of these laboratories, using the pyrrolidine enamine of cyclohexanone.



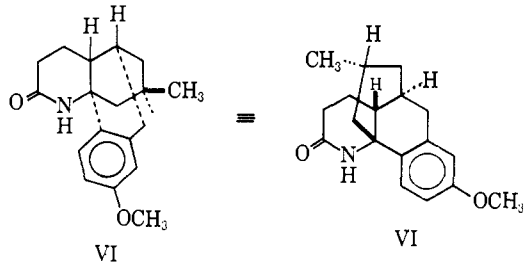
I = I II



III, R = OH
IV, R = H

V

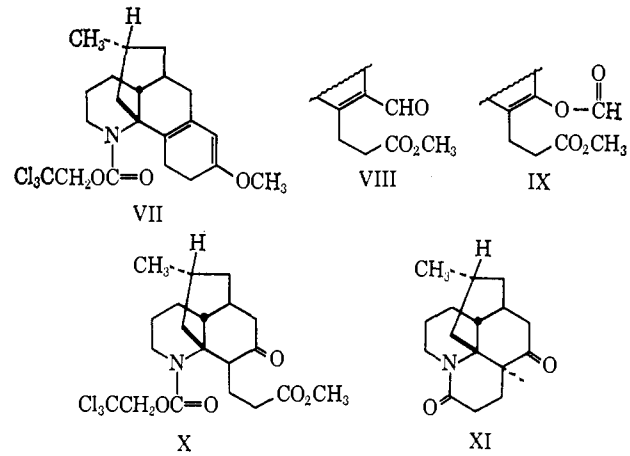
It was anticipated that only one of the two (reversibly) protonated species derivable from II would cyclize readily to the stereochemistry required of VI. This proved to be the case: treatment of II with 1:1 80% phosphoric acid–formic acid at room temperature for 20 hr gave, in addition to some *ortho* cyclization isomer, mp 201–202°, easily recognized by its nmr spectrum, a 55% yield of VI, mp 213.5–215.5° (δ ($CDCl_3$) 0.83 (broad, 3 H)), as the sole product of cyclization *para* to the methoxy group. The following sequence of steps



VI ≡ VI

served to convert VI into the N-protected keto ester X. Removal of the amide carbonyl (lithium aluminum hydride–tetrahydrofuran) was followed by reduction of the anisole ring (lithium–ammonia, *t*-butyl alcohol, ether), conjugation (potassium *t*-butoxide in dimethyl sulfoxide, 4 hr, room temperature) to the homoannular diene (λ^{EtOH} 275 mμ (ε ~3800), λ^{film} 6.02 and 6.22 μ), the amino group of which was then protected as the trichloroethyl carbamate[5] VII, and ozonolysis (methanol, −80°) led to the aldehydo methyl ester VIII (λ^{EtOH} 249 mμ, λ^{film} 5.80, 5.85, and 6.01 μ, δ 3.67 (3 H, s), 10.2 (s)). Cleavage of the unsaturated aldehyde proceeded (selenium dioxide–anhydrous hydrogen peroxide in *t*-amyl alcohol, 3 hr at 70°) to the enol formate IX (δ 8.14 (s, 1 H)) which gave (1% sodium methoxide, 30 min, room temperature) the keto ester X, mp 141–141.5°, thus obtained in ~30% over-all yield starting from VI.

Heating with zinc dust in methanol (150°, 20 hr) removed the protecting group and formed the tetracyclic



VII VIII IX

X XI

keto lactam XI, mp 143–144° (λ^{CHCl_3} 5.88 and 6.18 μ; *m/e* 261 and 204 (base; loss of bridge)) from which lithium aluminum hydride reduction[6] (40-hr reflux in tetrahydrofuran) gave *dl*-dihydrolycopodine (*dl*-complanatine), mp 182–184°. The infrared spectrum in $CHCl_3$ of this substance was identical in every detail with that of a sample of authentic dihydrolycopodine,[7] mp 169°, prepared from natural lycopodine.[8] Oxidation[9] with chromic acid–acetone–sulfuric acid then gave *dl*-lycopodine, mp 130–131°, the mass spectrum of which was essentially identical with that of the natural alkaloid.[10]

Acknowledgment. We thank the National Institutes of Health and the National Science Foundation for their support of this work.

(5) *Cf.* T. B. Windholz and D. B. R. Johnston, *Tetrahedron Letters*, 2555 (1967).

(6) *Cf.* W. A. Ayer, J. A. Berezowsky, and G. G. Iverach, *Tetrahedron*, **18**, 567 (1962); W. A. Harrison, M. Curcumelli-Rodostamo, D. F. Carson, L. R. C. Barclay, and D. B. MacLean, *Can. J. Chem.*, **39**, 2086 (1961).

(7) R. H. F. Manske, D. G. Lewis, and L. Marion, *ibid.*, **B20**, 87 (1942).

(8) We thank Professor L. Marion for a sample of natural lycopodine.

(9) *Cf.* B. Douglas, D. G. Lewis, and L. Marion, *Can. J. Chem.*, **31**, 272 (1953).

(10) W. A. Ayer, W. R. Bowman, T. C. Joseph, and P. Smith have also succeeded in synthesizing lycopodine (*cf. J. Am. Chem. Soc.*, **90**, 1648 (1968)), while a synthesis of an epimer has been described by H. Dugas, M. E. Hazenberg, Z. Valenta, and K. Wiesner (*Tetrahedron Letters*, 4937 (1967)).

Gilbert Stork, R. A. Kretchmer, R. H. Schlessinger

Department of Chemistry
Columbia University, New York, New York 10027

Received December 22, 1967

The Synthesis of *dl*-Lycopodine

Sir:

Lycopodine, the most widely occurring of the *Lycopodium* alkaloids, has been shown[1] to possess structure **1**. We report herein a synthesis of lycopodine *via* the natural relay **2**.

The immonium salt **3**, the synthesis of which has been reported,[2] was treated in tetrahydrofuran with the

(1) W. A. Harrison and D. B. MacLean, *Chem. Ind.* (London), 261 (1960); F. A. L. Anet, *Tetrahedron Letters*, **No. 20**, 13 (1960); W. A. Harrison, M. Curcumelli-Rodostamo, D. F. Carson, L. R. C. Barclay, and D. B. MacLean, *Can. J. Chem.*, **39**, 2086 (1961).

(2) W. A. Ayer, W. R. Bowman, G. A. Cooke, and A. C. Soper, *Tetrahedron Letters*, 2021 (1966).