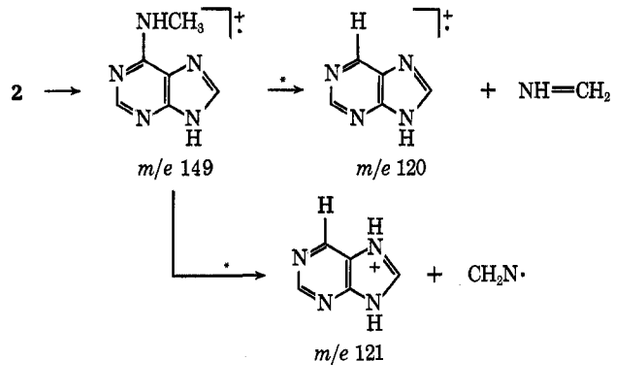
SCHEME I



that of **5** but shifted one mass unit higher, showing the presence of ^{15}N originating at N^6 in **1**, in support of the ring-opening mechanism. This interpretation is confirmed by the singly labeled derivative **6**, which produces a pattern which is experimentally indistinguishable from **1** but offset one mass unit higher as required by the Dimroth mechanism. The absence of contributions from products such as **9** which would contain an exocyclic ^{15}N label shows the absence of a methyl migration mechanism (eq 2) at the detectable limit of approximately 2% of the total reaction yield.

Conversion of 1-methyladenosine was more than 90% complete at pH 11 under the conditions employed, but less than 50% complete at pH 7. However, the identity of patterns from compound **7** at the two pH values shows that transformation to N^6-methyladenosine occurs exclusively by ring opening in both the protonated (pH 7) and neutral (pH 11) forms of 1-methyladenosine, a result which is consistent with earlier kinetic studies of Macon and Wolfenden.[7]

Registry No.—**1**, 34308-25-3; **2**, 1867-73-8.

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Biosynthesis of Ergot Alkaloids. Synthesis of 6-Methyl-8-acetoxymethylene-9-ergolene and Its Incorporation into Ergotoxine by *Claviceps*

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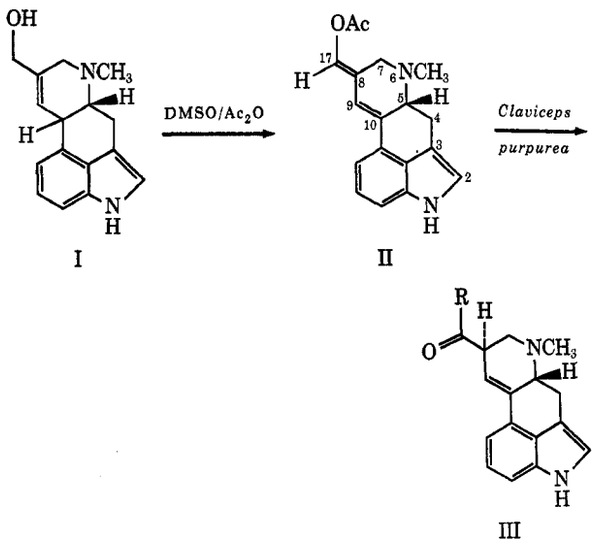
It is well known that simple clavine alkaloids, particularly agroclavine and elymoclavine (I), are precursors of the lysergic acid (III, R = OH) moiety of more complex amide and peptide type ergot alkaloids;[1-5]



however, the sequence of steps from I to the lysergic acid stage is unknown. Lysergene, lysergol, isolysergol, and penniclavine apparently are not precursors of lysergic acid derivatives.[2,3] Therefore, shift of the double bond into the 9,10 position is not the first step. 6-Methyl-8-ergolene-8-carboxylic acid ($\Delta^{8,9}$-lysergic acid), a natural constituent of certain ergot strains,[6] was found to be incorporated into lysergic acid amides, although not so efficiently as lysergic acid.[7] While this could indicate biological double bond isomerization at the lysergic acid stage, the fact that the same reaction also occurs spontaneously at a measurable rate[6] makes the interpretation of this experimental result somewhat ambiguous. In order to examine this question further, we attempted to prepare $\Delta^{8,9}$- and/or $\Delta^{9,10}$-lysergaldehyde (III, R = H) from elymoclavine. Surprisingly, it turns out that the hydroxymethyl group of elymoclavine is extremely resistant to most of the usual oxidizing agents. This fact has apparently been noted before in extensive attempts to produce lysergic acid commercially by chemical oxidation of elymoclavine.[8] The only defined oxidation products obtained were penniclavine and isopenniclavine, the products of hydroxylation in the 8 position.[9,10]

Treatment of elymoclavine with a mixture of dimethyl sulfoxide and acetic anhydride at room temperature for 12 hr[11] produced, in addition to elymoclavine *O*-acetate, a new compound which was identified as 6-methyl-8-acetoxymethylene-9-ergolene (II), the enol acetate of lysergaldehyde. Separation of the two

(1) K. Mothes, K. Winkler, D. Gröger, H. G. Floss, U. Mothes, and F. Weygand, *Tetrahedron Lett.*, 933 (1962).
(2) S. Agurell and M. Johansson, *Acta Chem. Scand.*, **18**, 2285 (1964).
(3) H. G. Floss, H. Günther, D. Gröger, and D. Erge, *Z. Naturforsch. B*, **21**, 128 (1966).
(4) R. Voigt, M. Bornschein, and G. Rabitzsch, *Pharmazie*, **22**, 326 (1967).
(5) T. Ohashi, S. Aoki, and M. Abe, *J. Agr. Chem. Soc., Jap.*, **44**, 527 (1970).
(6) H. Kobel, E. Schreier, and J. Rutschmann, *Helv. Chim. Acta*, **47**, 1052 (1964).
(7) S. Agurell, *Acta Pharm. Suecica*, **3**, 65 (1966).
(8) A. Hofmann, private communication.
(9) M. Abe, S. Yamatodani, Y. Yamano, and M. Kusumoto, *Bull. Agr. Chem. Soc. Jap.*, **19**, 92 (1955).
(10) A. Hofmann, R. Brunner, H. Kobel, and A. Brack, *Helv. Chim. Acta*, **40**, 1358 (1957).
(11) J. D. Albright and L. Goldmann, *J. Amer. Chem. Soc.*, **89**, 2416 (1969).

products, which in most systems have very similar R_f values, was achieved by chromatography on a column of Sephadex LH-20 using ethanol as the developing solvent. Upon repeated rechromatography of those fractions containing mixtures of the two products and crystallization from ethanol, II was obtained in 15.6% yield as yellow needles, mp 178–180° dec. The molecular formula of II was established as $C_{18}H_{18}N_2O_2$ by high-resolution mass spectrometry (mol wt calcd 294.13682, obs 294.13551). The presence of strong peaks at *m/e* 251 and 252 ($C_{16}H_{15}N_2O$ and $C_{16}H_{16}N_2O$) due to loss of acetyl and ketene, respectively, and lack of any peak due to loss of acetic acid indicated the presence of an enol acetate moiety in the molecule. This is supported by the infrared spectrum, which showed the typical enol acetate carbonyl absorption at 1750 cm^{-1}. The nmr spectrum confirmed the presence of the acetyl group, showing a methyl signal at δ 2.18 in addition to the NCH_3 signal at δ 2.60. Assignment of the signal at δ 8.03 to the indole proton was confirmed by D_2O exchange. The ultraviolet spectrum [λ_{max}^{MeOH} 248 nm (log ϵ 4.19) and 339 (4.11)] closely resembled that of lysergene (6-methyl-8-methylene-9-ergolene) [λ_{max}^{MeOH} 246 nm (log ϵ 4.46) and 337 (4.11)], indicating the presence of the same chromophore. The dienol acetate moiety ($AcOCH_{17}{=}CCH_9{=}C$–) was further supported by the nmr spectrum, which showed broad singlets at δ 6.84 and 7.35 which could be assigned to the protons at C-9 and C-17, respectively. Further evidence for the proposed structure is the fact that II gives lysergol upon reduction with $LiAlH_4$.

A variety of attempts were made to convert II into lysergaldehyde or lysergic acid. These included hydrolysis with dilute acetic acid or ammonium hydroxide, attempted generation of the lithium enolate anion from II by treatment with CH_3Li,[12] hydrolysis–oxidation with silver oxide in alkaline or acidic medium, and oxidation with molecular oxygen following alkaline hydrolysis. All these attempts were unsuccessful, producing no reaction or leading to decomposition or formation of very complex mixtures.

To evaluate whether II can be converted into lysergic acid biosynthetically, a tritiated sample was prepared by oxidation of [*indole*-^{3}H]elymoclavine. While II is not expected to be an intermediate in the biosynthetic conversion of elymoclavine to lysergic acid derivatives, it might serve as a source of lysergaldehyde inside the cells, because the ergot fungus is apparently quite capable of cleaving acetate esters.[13] [*indole*-^{3}H]-II was fed to two 100-ml shake cultures of *Claviceps purpurea* strain PEPTY 695 as described earlier.[14,15] For comparison, [*indole*-^{3}H]elymoclavine and [*indole*-^{3}H]elymoclavine *O*-acetate were each fed to two parallel cultures. The experiments were terminated 24 hr later, the alkaloid content of the cultures was determined colorimetrically, and ergotoxine (III) was isolated and purified by tlc (silica gel, chloroform–ethanol 9:1) as described previously.[14,15] The ergotoxine was then hydrolyzed with methanolic KOH to give lysergic acid, which was purified to constant specific radioactivity. The results of these experiments, which are summarized in Table I, clearly indicate that II can be converted into lysergic acid by the ergot fungus. The efficiency of its

TABLE I

INCORPORATION OF PRECURSORS INTO THE LYSERGIC ACID PORTION OF ERGOTOXINE BY *Claviceps purpurea*

	Precursor fed[a]		
	II	Elymoclavine	Elymoclavine *O*-acetate
Amount fed, μmol	4.15	7.88	17.94
Radioactivity fed, dpm	1.23×10^6	2.34×10^6	5.33×10^6
Alkaloid formed, μmol	175	213	184
Specific radioactivity of lysergic acid, dpm/μmol	109	164	1200
Total radioactivity of lysergic acid in alkaloid, dpm	1.9×10^4	3.5×10^4	2.2×10^5
Incorporation, %	1.55	1.49	4.15

[a] All precursors labeled with tritium in the indole portion.

incorporation about equals that of elymoclavine, an established lysergic acid precursor. The better utilization of elymoclavine *O*-acetate confirms an earlier report by Agurell[13] and may be due to permeability differences. While these experiments by no means establish the intermediacy of lysergaldehyde in lysergic acid biosynthesis, they certainly do suggest that the possibility of double-bond isomerization at the aldehyde rather than the acid stage should be kept in mind.

Experimental Section

General.—Infrared spectra were recorded on a Perkin-Elmer 237B grating spectrophotometer, ultraviolet spectra on a Perkin-Elmer Coleman 124 spectrophotometer, and nmr spectra on a Jeol MH 60 or Varian HR 220 instrument in deuteriochloroform with TMS as internal standard. Mass spectra were obtained using a Hitachi RMU-6D low-resolution and a CEC 2110 high-resolution mass spectrometer. Melting points were determined in vacuum-sealed tubes on samples dried for 18 hr at room temperature over P_2O_5 under vacuum. Ehrlich's reagent was used to visualize ergoline derivatives on chromatograms. Radioactivity determinations were carried out in a Packard Tricarb Model 3365 liquid scintillation counter using PPO and POPOP in toluene as scintillator solution. [*indole*-^{3}H]Elymoclavine was material which had been prepared earlier[3] by biosynthesis from [*indole*-^{3}H]tryptophan. The feeding experiments with *Claviceps purpurea* strain PEPTY 695 and the isolation, purification, and degradation of the alkaloid were carried out as described previously.[14,15] Nonlabeled elymoclavine was prepared by fermentation of *Claviceps* strain SD 58.[16]

6-Methyl-8-acetoxymethylene-9-ergolene (II).—To 476 mg (1.87 mmol) and 503 mg (1.98 mmol) of elymoclavine were added 8.3 and 9.3 ml, respectively, of a mixture (3:2 by volume) of dimethyl sulfoxide (Baker AR, redistilled over NaH, dried over molecular sieves) and acetic anhydride (Mallinckrodt AR). The reaction mixtures were stirred at room temperature in the dark under nitrogen for 12 hr and then combined. Distilled water (500 ml) was added with shaking and the aqueous layer was extracted with 6 × 100 ml of chloroform. The aqueous phase was then basified with 14% ammonium hydroxide to pH 5–6, 7–8, and 9–10 and extracted at each stage with 3 × 100 ml of chloroform. The combined chloroform extract was washed with 4 × 500 ml of distilled water, dried by filtering through anhydrous sodium sulfate, concentrated to a syrup on the rotary evaporator at 23–25°, and further dried under vacuum for 20 hr to give 1.2 g of a dark brown residue. This crude reaction product was dissolved in a small volume of absolute ethanol and poured on top of a column (2.5 × 35 cm) packed with 25 g of Sephadex LH-20 which had been swelled in ethanol for at least 3 hr. The column was eluted with absolute ethanol, 60 fractions of

(12) H. O. House and B. M. Trost, *J. Org. Chem.*, **30**, 2502 (1965).
(13) S. Agurell, *Acta Pharm. Suecica*, **3**, 23 (1966).
(14) D. Gröger and D. Erge, *Z. Naturforsch. B*, **25**, 196 (1970).
(15) W. Maier, D. Erge, and D. Gröger, *Biochem. Physiol. Pflanzen (Jena)*, **161**, 559 (1971).
(16) H. G. Floss and D. Gröger, *Z. Naturforsch. B*, **18**, 519 (1963).

3 ml were collected, and the elution was monitored by tlc (silica gel, ethyl acetate–acetone–dimethylformamide 5:5:1, R_f 0.62). Fractions containing only II were combined, concentrated, and crystallized from ethanol to give 77 mg of yellow needles, which showed a single spot upon chromatography in several systems. Fractions containing mixtures of II and elymoclavine *O*-acetate were combined and chromatographed in the same way a second and a third time to give 52 and 48 mg of product, bringing the total yield to 177 mg (0.6 mmol, 15.6%). II has a melting point of 178–180° dec, gives a green color with Ehrlich's spray reagent, and shows a strong blue fluorescence under uv; ir (KBr) 1750, 1368, 1210, and 1049 cm^{-1}; uv (MeOH) λ_{max} 248 nm (log ϵ 4.19) and 339 (4.11); major mass spectral peaks *m/e* (rel intensity) 294.13551 (M^+) (calcd 294.13682) (100), 252 (22), 251 (45.6), 249 (13.4), 235 (24.2), 223 (32.9), 222 (14.4), 221 (32.2), 192 (16.2), 154 (15.4). The 220-MHz nmr spectrum ($CDCl_3$) shows signals at δ 2.18 (singlet, 3 H) for the acetyl methyl group, 2.60 (singlet, 3 H) for the *N*-methyl, 2.73 (dd, J = 10, 14 Hz, 1 H, 4α proton), 3.07 (d, J = 14 Hz, 1 H, 7β proton), 3.32 (dd, J = 6, 10 Hz, 1 H, C-5 proton), 3.52 (dd, J = 6, 14 Hz, 1 H, 4β proton), 3.94 (d, J = 14 Hz, 1 H, 7α proton), 6.84 (broad singlet, 1 H, for the vinyl proton at C-9), 6.89 (broad singlet, 1 H, indole 2 H), 7.22 (multiplet, 3 H, aromatic protons), 7.35 (broad singlet, 1 H, vinyl proton at C-17) and 8.03 (broad singlet, indole NH).

Registry No.—I, 548-43-6; I acetate, 5080-45-5; II, 39717-29-8; III (R = H), 39717-30-1; III (R = OH), 82-58-6; dimethyl sulfoxide, 67-68-5; acetic anhydride, 108-24-7.

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Rearrangement in the Indan-1,3-dione System

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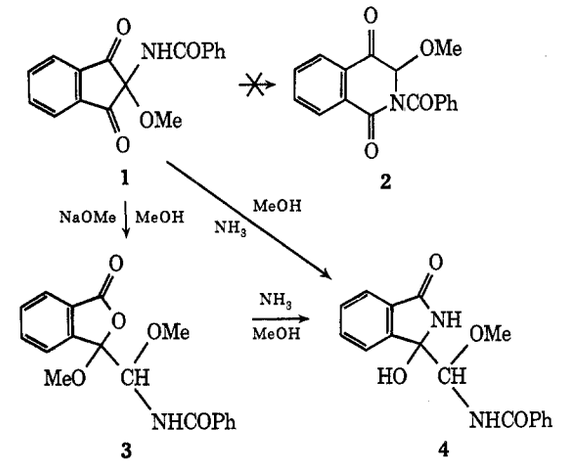
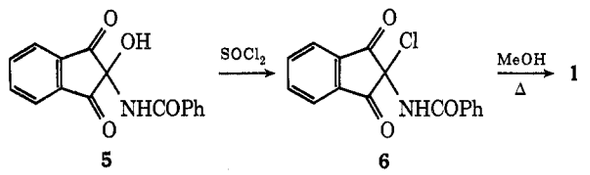
In an attempt to synthesize azanaphthaquinone[1] we tried to prepare the precursor 2-benzoyl-3-methoxytetrahydroisoquinoline-1,4-dione (**2**) by a base-catalyzed internal displacement ring enlargement[2] of 2-benzamido-2-methoxyindan-1,3-dione (**1**).

Instead of the expected ring enlargement product **2**, we obtained the rearranged phthalide derivative **3**, which was further converted to the isoindolone **4** on treatment with methanolic ammonia solution. The same isoindolone derivative **4** was also obtained from the starting material **1** on treatment with methanolic ammonia solution. Attempts to induce an acid-catalyzed ring enlargement on the isoindolone derivative were also unsuccessful.

The starting material 2-benzamido-2-methoxyindan-1,3-dione (**1**) was prepared in high yield from ninhydrine hydrate and benzamide. The addition product **5** was converted to the methoxybenzamidoindandione (**1**) by treatment with thionyl chloride followed by refluxing a methanolic solution of the chloroamide **6**, for 1 hr.



1 **2** **3** **4**



5 **6**

Experimental Section

Melting points are corrected; infrared spectra were measured in chloroform solutions and nmr in deuteriochloroform (unless otherwise indicated).

2-Benzamido-2-hydroxyindan-1,3-dione (5).—A mixture of ninhydrin hydrate (10.0 g, 0.05 mol) and benzamide (6.8 g, 0.05 mol) in benzene (100 ml) was refluxed for 1 hr. The water, formed in the reaction, was removed by azeotropic distillation. The mixture was cooled and the solid formed was filtered to give 15.1 g (86%) of product: mp 143–144°; ir 1760, 1720, and 1650 (CO), 3500 (OH), and 3300 cm^{-1} (NH). The hydroxyamide was used in the following procedure without further purification.

2-Benzamido-2-chloroindan-1,3-dione (6).—A mixture of the hydroxyamide **5** (2.0 g) and thionyl chloride (1.2 ml, 2 equiv) in methylene chloride (40 ml) was refluxed for 2 hr. The solvent was evaporated and the residue was triturated with benzene, filtered, and crystallized from benzene to give 1.97 g (93)% of product which melted at 173–174°: ir 1775, 1735, and 1660 (CO) and 3430 cm^{-1} (NH); *m/e* 399.

Anal. Calcd for $C_{16}H_{10}NO_3Cl$: C, 64.10; H, 3.34; N, 4.68; Cl, 10.80. Found: C, 64.17; H, 3.44; N, 4.32; Cl, 10.49.

2-Benzamido-2-methoxyindan-1,3-dione (1).—A solution of the chloroamide **6** in absolute methanol (25 ml) was refluxed for 1 hr. The solvent was evaporated and the residue was triturated with ether–hexane and filtered. It was crystallized from benzene–hexene: mp 166–167°; yield 66%; ir 1725, 1760, 1660 (CO), and 3420 cm^{-1} (NH); nmr δ 8.2–7.3 (m, 10 H) and 3.57 (s, 3 H).

Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.14; H, 4.44; N, 4.74. Found: C, 68.87; H, 4.52; N, 5.06.

3-Methoxy-3-(α-methoxy-α-benzamidomethyl)phthalide (3).—A solution of 2-benzamido-2-methoxyindan-1,3-dione (**1**, 1.0 g) and sodium methylate (20 mg) in absolute methanol (20 ml) was left at room temperature for 48 hr. The solvent was evaporated and the residue was dissolved in ethyl acetate (50 ml) and washed with water. The organic layer was dried over magnesium sulfate and evaporated. The residue was triturated with ether, filtered, and crystallized from ethyl acetate–hexane: mp 178°; yield 60%; ir 1780, 1670 (CO), and 3430 cm^{-1} (NH); nmr δ 8.2–7.3 (m, 9 H), 6.85 (d, 1 H, J = 10 cps), 5.80 (d, 1 H, J = 10 cps), 3.32 (s, 3 H), and 3.10 (s, 3 H).

Anal. Calcd for $C_{18}H_{17}NO_5$: C, 66.05; H, 5.28; N, 4.28. Found: C, 65.92; H, 5.27; N, 4.04.

3-Hydroxy-3-(α-methoxy-α-benzamidomethyl)isoindolone (4).—A mixture of the lactone **3** (1.0 g) in methanolic ammonia

(1) D. Ben-Ishai, Z. Inbal, and A. Warshawski, *J. Heterocycl. Chem.*, **7**, 615 (1970).

(2) C. R. Hauser and S. W. Kantor, *J. Amer. Chem. Soc.*, **73**, 1440 (1951).