Formal Synthesis of Clavicipitic Acid Based on a Biosynthetic Proposal

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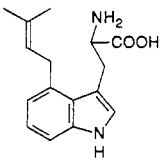
The clavicipitic precursor **10** was synthesized by an efficient route culminating in an intramolecular, acid-catalyzed aminoalkylation between an amine and an alcohol to form the azepino ring system characteristic of this indolic amino acid. A ring closure utilizing these functionalities has been proposed as a plausible biosynthetic step in the biosynthesis of the alkaloid.

The complete elucidation of the biosynthetic pathway of the ergot alkaloids produced by various members of the genus *Claviceps* remains obscure. What is known with certainty is that 4-(γ,γ-dimethylallyl)tryptophan (DMAT, **1**) is the principal substrate from which arises the variety of structures associated with the genus. Also, numerous feeding experiments have pointed toward a reasonable sequence of transformations for other intermediates of the pathway.[1]

Clavicipitic acid **4**, currently regarded as a derailment product of normal ergot metabolism,[2] has received considerable attention by synthetic organic chemists over the past 7 years. much of this attention seems to have been directed toward achieving a biomimetic synthesis of this indolic amino acid. The literature records four syntheses,[3] two of which stem from Kozikowski and his group.[3a,b,d] These syntheses—particularly the most recent—serve as interesting examples of how synthetic approaches may suggest alternative mechanisms for biosynthetic consideration, in addition to enlarging our appreciation of the facility with which nature may deal with a given substrate.



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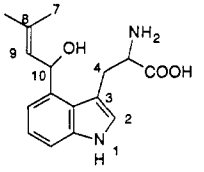

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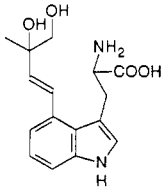

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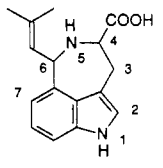
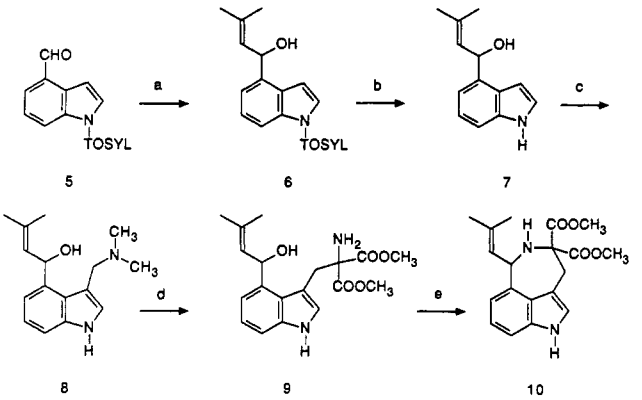


Clavicipitic Acid, 4

We now report the synthesis of clavicipitic acid by an efficient route, which is based upon a functional equivalent of 10-hydroxy-4-(γ,γ-dimethylallyl)tryptophan (10-hydroxylated DMAT, **2**). Although this compound has been specifically proposed as a hypothetical biochemical precursor of the clavicipitic acids,[2] a laboratory synthesis based on this substrate or its functional equivalent has not yet appeared in the literature.

On the other hand, Kozikowski's "biomimetic" synthesis[3d] is based upon Floss' proposed sequence for the biosynthesis of the ergot alkaloid chanoclavine I.[1a] The key intermediate common to both Floss' proposed biosynthetic pathway and to the Kozikowski laboratory synthesis is the bis(hydroxy)-4-(γ,γ-dimethylallyl)tryptophan (bis-hydroxylated DMAT, **3**). We note that 10-hydroxylated DMAT is, curiously, also a postulated biosynthetic precursor of this diol in Floss' scheme as well. More significant, however, is the recent finding that the N-methylated derivative of **3** is not, in fact, an intermediate in ergoline biosynthesis.[4]

Scheme I[a]



5 6 7 8 9 10

[a] (a) $BrCH{=}C(CH_3)_2$, Mg, THF; (b) Na/Hg, MeOH; (c) H_2CO, $HN(CH_3)_2$, HOAc; (d) $H_2NCH(COOCH_3)_2$, $P(n\text{-}Bu)_3$, CH_3CN; (e) TsOH, CH_3CN.

The development of a synthetic route to the clavicipitic acids based on 10-hydroxylated DMAT would therefore appear to be of some interest. Such a synthesis might suggest that the derailment of metabolism leading to clavicipitic acid may occur much earlier than at the bis-hydroxylated DMAT stage, i.e., at the 10-hydroxylated DMAT stage instead.

Accordingly, we prepared the requisite alcohol **6** in good yield by the Grignard reaction of N-tosylated indole-4-carboxaldehyde[5] with 2-methyl-1-propenylmagnesium bromide (Scheme I). Deprotection of the indole nitrogen to afford indole **7** was smoothly accomplished in nearly quantitative yield by an application of Trost's buffered

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amalgam method[6] as similarly utilized by Fuchs for the detosylation of sulfonamides to amines.[7]

Under the classic Mannich conditions as described by Plieninger,[8] the new indole was converted to its gramine derivative 8. Although obtained essentially pure in 77% yield, judging from TLC and spectral data, the perceived sensitivity of this material dictated that it be converted directly to the amino acid derivative rather than further purified. Subjection to the conditions reported by Somei[9] afforded an 80% yield of the amino alcohol 9 as a light yellow oil after chromatography.

By analogy to the ring closure recently reported by Hegedus in his synthesis of aurantioclavine,[10] we subjected the amino alcohol to acid catalysts in refluxing acetonitrile. Thin-layer chromatography indicated the disappearance of starting material after 2 h. Chromatography afforded a 48% yield of a material having an ^{1}H NMR spectrum consistent with that of closely analogous ring systems.[3b,d,e] The particular stability of the nascent, doubly allylic benzylic carbocation must permit its intramolecular interception by any unprotonated amine. Since the corresponding diethyl ester has been shown to undergo hydrolysis with base to a cis–trans mixture of the clavicipitic acids,[3e] this therefore represents a formal synthesis of the alkaloid. These results also suggest that the biosynthesis of clavicipitic acid may proceed from 2 without the intermediacy of diol 3.

Of additional interest is the speculation that clavicipitic acid may be an artifact derived during isolation.[11] Although no details have accompanied such speculations, in each case the workup of crude fungal extracts to obtain the clavicipitic acids has indeed involved the use of acidic resins or conditions.[12] In light of the acid-catalyzed ring closure described here, the possibility arises that any naturally occurring 10-hydroxylated DMAT present in crude fungal extracts may undergo ring closure to the clavicipitc acids under the acidic isolation procedures employed. Should this be the case, the clavicipitic acids may very well be more correctly viewed as artifacts of an isolation procedure rather than as true secondary metabolites of the *Claviceps* fungus.

Since Kozikowski had converted bis(hydroxy)-4-(γ,γ-dimethylallyl)tryptophan (a vinylogue of 10-OH DMAT) to the clavicipitic acids by way of Mitsunobu conditions,[3d] we were curious to see whether 9 would also afford the azepino ring system with a similar approach. Subjection of the amino alcohol 9 to standard Mitsunobu conditions with either triphenylphosphine or tri-*n*-butylphosphine[13] with diethyl azo dicarboxylate led to recovery of the starting amino alcohol upon workup, with no trace of the desired clavicipitic system.

Experimental Section

General Procedures. Melting points were taken on a Fisher-Johns melting point apparatus. All melting points are uncorrected. Infrared spectra were recorded neat (NaCl) or as the KBr pellet on a Beckman IR-33 spectrometer. ^{1}H NMR spectra were determined in chloroform-d_1 solution with tetramethylsilane as internal standard on a Varian FT-80 or a Chemagnetics XL-200 instrument. Splitting patterns: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra were performed on a Finnigan 4000 mass spectrometer; exact mass determinations were performed on a Kratos MS-50 spectrometer. All reactions were performed under a positive pressure of dry nitrogen in oven-dried glassware fitted with rubber septa for introduction of reagents via syringe when appropriate. Reactions were monitored by TLC on precoated thin-layer Baker-flex silica gel 1B2-F plates. Visualization was with short-wave ultraviolet and, in the case of some N-deprotected indoles, also with Van Urks' reagent.[14] Flash chromatography was carried out as described by Still[15] with silica gel 60 (230–400 mesh). Radial chromatography was performed on a chromatotron (Harrison Research).

***N*-Tosyl-4-(1-hydroxy-3-methyl-2-butenyl)indole (6).** To a flask containing 0.729 g (30.0 mmol) of magnesium turnings covered with 20 mL of tetrahydrofuran was added dropwise 4.09 g (3.1 mL, 30 mmol) of 1-bromo-2-methylpropene. After being stirred for 5 h at room temperature, the mixture was gently warmed over a steam bath for 1 h. After the mixture was cooled in an ice bath, a solution of 3.0 g (10 mmol) of *N*-tosylindole-4-carboxaldehyde (5) in 10 mL of dry tetrahydrofuran was added dropwise over 30 min. The reaction mixture was allowed to stir overnight (10 h). The mixture was poured onto 50 g of ice and 20 mL of saturated ammonium chloride and was extracted with 70 mL of ether. The aqueous layer was reextracted with 2 × 30 mL of ether. The organic extracts were dried ($MgSO_4$), filtered, and concentrated by rotary evaporation to afford 3.55 g of a crude, pale yellow oil. Flash chromatography (50% ethyl acetate–hexane) afforded a colorless syrup, which crystallized to a white powder upon trituration: 3.02 g (85%); mp 105–106 °C; TLC (50% EtOAc–hexane) R_f 0.48; IR (KBr) 3490, 3400, 3100, 2940, 2900, 1655, 1585, 1165, 1115, 740, 650 cm^{-1}; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.96–7.54 (m, 4, arom), 7.29–7.12 (m, 4, arom), 6.83 (dd, J = 1 Hz, 4 Hz, 1, indole 3C-H), 5.72 (d, J = 9 Hz, 1, benzylic), 5.49 (dt, J = 1 Hz, 9 Hz, 1, vinylic), 2.32 (s, 3, tosyl CH_3), 1.87 (br s, 1, OH), 1.81 (d, J = 1 Hz, 3, CH_3), 1.72 (d, J = 1 Hz, 3, CH_3); exact mass for $C_{20}H_{21}NSO_3$ calcd 355.1242, found 355.1242.

4-(1-Hydroxy-3-methyl-2-butenyl)indole (7). To a solution of 1.5 g (4.2 mmol) of *N*-tosylindole 6 in 100 mL of anhydrous MeOH were added 2.4 g (16.9 mmol) disodium hydrogen phosphate and 6.3 g finely pulverized 6% Na/Hg amalgam. The suspension was stirred magnetically for 15 min, after which time TLC indicated complete disappearance of starting material. The suspension was vacuum filtered through 2 cm of Celite in a sintered-glass funnel and washed with 2 × 20 mL of MeOH. The filtrate was poured onto 50 mL of saturated ammonium chloride, mixed, and extracted with ether (3 × 100 mL). The combined organic layers were washed once with 80 mL of H_2O and then with brine (1 × 50 mL), dried ($MgSO_4$), filtered, and concentrated by rotary evaporation to a colorless oil, 0.828 g (98%): TLC ($CHCl_3$ saturated with NH_3) R_f 0.22; IR (KBr) 3400, 3200, 1600, 1020, 730 cm^{-1}; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.47 (br s, 1, indole NH), 7.12–6.94 (m, 4, arom), 6.59 (t, J = 3 Hz, 1, indole 3C-H), 5.79 (d, J = 9 Hz, 1, benzylic), 5.60 (dt, J = 1 Hz, 9 Hz, 1, vinylic), 2.32 (s, 1, OH), 1.78 (d, J = 1 Hz, 3, CH_3), 1.67 (d, J = 1 Hz, 3, CH_3); exact mass for $C_{13}H_{15}NO$ calcd 201.1154, found 201.1151.

4-(1-Hydroxy-3-methyl-2-butenyl)-3-[(dimethylamino)-methyl]indole (8). To 1.66 g (8.25 mmol) of alcohol 7 was added 2.89 mL of stock Mannich reagent, prepared according to the procedure of Plieninger et al.,[8] at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h and then at room temperature for 3 h. The mixture was cooled to 0 °C and acidified dropwise with 4.12 mL of cold 2 N HCl. The solution was extracted with ethyl acetate (4 × 10 mL). The combined organic layers were back-extracted with cold, dilute HCl (3 × 10 mL). The combined aqueous layers

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were made basic to pH 10 with ice-cold 40% sodium hydroxide. The product was extracted with ethyl acetate (3 × 30 mL), dried (Na_2SO_4), and concentrated to a pink foam. TLC ($CHCl_3$ saturated with NH_3) indicated a single product: R_f 0.10; van Urks, immediate sky blue drying to forest green; IR (KBr) 3380, 3220, 2940, 1440, 1350, 1325, 1130, 980, 720 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.56 (br s, 1, indole NH), 7.65 (d, J = 16 Hz, 1, methine), 7.29–6.96 (m, 4, arom), 6.29 (d, J = 16 Hz, 1, vinyl), 3.57 (s, 2, CH_2N), 2.24 (s, 6, $N(CH_3)_2$), 1.71 (s, 1, OH), 1.44 (s, 6, $C(CH_3)_2$); mass (m/z, %) 259 (M + 1, 63), 241 (M - H_2O, 100), 214 (M - $N(CH_3)_2$, 61), 196 (M - H_2O - $N(CH_3)_2$, 21); exact mass for $C_{16}H_{22}N_2O$ calcd 258.1732, found 258.1730.

Methyl 2-Amino-2-carbomethoxy-3-[4-(1-hydroxy-3-methyl-2-butenyl)-3-indolyl]propionate (9). This compound was prepared in accordance with the procedure of Somei.[9] To 0.300 g (1.16 mmol) of dimethyl aminomalonate and 0.300 g (1.16 mmol) of gramine derivative 8 in 2 mL of acetonitrile was added, in one portion, a solution of 116 L of tri-*n*-butylphosphine (95%, 0.55 mmol) in 1 mL of acetonitrile. The reaction was heated at gentle reflux over a steam bath for 4 h. After cooling, the solution was poured into 8 mL of H_2O and extracted with hexanes (1 × 2 mL), the hexane layer was discarded, and the aqueous layer was extracted with methylene chloride (4 × 5 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated to yield 0.40 g of a golden residue. Radial chromatography (1% MeOH–$CHCl_3$) afforded 356 mg (85%) of product as a pale yellow oil: TLC ($CHCl_3$ saturated with NH_3) R_f 0.13; IR (neat, NaCl) 3360, 3210, 3010, 2930, 1725, 1290, 1200 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.65 (br s, 1, indole NH), 7.53, (d, J = 16 Hz, 1, methine), 7.25–6.97 (m, 4, arom), 6.18 (d, J = 16 Hz, 1, vinyl), 3.72 (s, 8, $COOCH_3$ obscuring methylene), 2.33 (br s, 2, NH_2), 1.74 (br s, 1, OH), 1.46 (s, 6, $C(CH_3)_2$); mass (m/z, %) 361 (M + 1, 8), 343 (M - H_2O, 100); exact mass for $C_{19}H_{24}N_2O_5$ calcd 360.1685, found 360.1695.

Dimethyl 3,4,5,6-Tetrahydro-6-(2-methyl-1-propenyl)-azepino[5,4,3-*cd*]indole-4,4-dicarboxylate (10). A solution of 97.9 mg (0.27 mmol) of amino alcohol **9**, 0.005 g (0.018 mmol) of *p*-toluenesulfonic acid, and 5 mL of acetonitrile was heated at gentle reflux for 2 h. After cooling, the solution was poured onto 20 mL of saturated sodium bicarbonate and extracted with ether (3 × 10 mL). The combined ethereal layers were dried (Na_2SO_4), filtered, and concentrated by rotary evaporation, and radially chromatographed (1% EtOAc–methylene chloride) to afford a golden oil: 45 mg (48%); TLC ($CHCl_3$ saturated with NH_3) R_f 0.49; van Urks slowly developing pale yellow drying to rose; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.99 (br s, 1, indole NH), 7.16 (d, 1, J = 8.2 Hz, C-14), 7.01 (m, 1, C-13), 6.94 (br s, 1, C-12), 6.77 (d, 1, J = 7.3 Hz, indole 2C-H), 5.45 (d, J = 7.8 Hz, 1, vinyl), 5.30 (d, J = 8.8 Hz, 1, methine), 3.93 (d, J = 15.6 Hz, 1, methylene), 3.77 (s, 3, $COOCH_3$), 3.71 (s, 3, $COOCH_3$), 3.50 (d, J = 15.5 Hz, 1, methylene), 2.92 (d, J = 14.1 Hz, 1, NH), 1.88 (s, 3, CH_3), 1.74 (s, 3, CH_3); mass (m/z, %) 343 (M + 1, 100), 399 (isobutane adduct, 5); exact mass for $C_{19}H_{22}N_2O_4$ calcd 342.1580, found 342.1577.

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Prodrugs Based on Masked Lactones. Cyclization of γ-Hydroxy Amides

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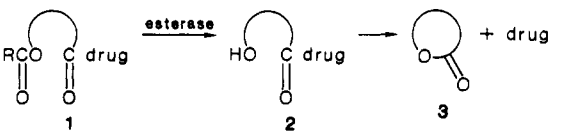
A versatile approach to prodrug design based on the lactonization of γ-hydroxy carbonyl compounds is investigated. A range of γ-hydroxy amides have been synthesized as models for amide-linked prodrugs. The rates of lactonization of these compounds have been measured, and the effects of pH, leaving group pK_a, buffer species, and ionic strength are investigated. The kinetic data are consistent with changes in the rate-determining step with the nature of the buffer and with pH over the range 6–10. Some compounds show only small changes in rate over the pH range 7–9. The best model prodrugs studied have rates of amine expulsion that would probably be adequate for therapeutic use, but precise rates of drug liberation in vivo cannot be predicted from these data due to the problems of estimating the magnitude of biological buffer catalysis and effects due to tissue binding. However, drug liberation half-lives in vivo in the region of 1 h for aromatic amides, less for aliphatic amides, may be achieved by using prodrugs that yield 4,4-dialkyl(or spiroalkyl)-(*Z*)-but-2-enoic acid lactones during drug release.

Many potentially useful drugs are not used therapeutically since an optimum concentration at the site of action cannot be achieved or cannot be maintained for an adequate period of time. These and other deficiencies may arise from poor oral absorption, inadequate permeation of cell membranes, chemical instability, rapid clearance, or toxicity to specific tissues, etc., depending on the nature of the drug.

Attempts to overcome such problems have led to the development of a number of drug delivery systems,[1] which can be mechanical or chemical in nature. The former class centers around the use of microparticulate materials and is not the subject of this paper. The chemical approach often involves the investigation of salt formation, but we

Scheme I



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