A typical laboratory-scale hydrogenation is illustrated as follows. To a vivid reddish yellow solution of $Ru(OCOCH_3)_2[(R)\text{-binap}]$[5] (806 mg, 0.957 mmol) in degassed dichloromethane (20 mL) was added 1.42 N HCl in 90% methanol (1.41 mL, 2.00 mmol). After the resulting dark red solution was stirred at 23 °C for 2.5 h, the solvent was removed under reduced pressure to give $RuCl_2$-[(*R*)-binap] (722 mg) as a reddish brown solid, which was used as the hydrogenation catalyst.[12] A solution of methyl 3-oxobutanoate (**1a**) (100 g, 0.862 mol) in degassed anhydrous methanol (100 mL) was placed in a 300-mL Schlenk vessel and degassed by 3 freeze–thaw cycles. With use of a cannula this was then mixed with the solid Ru–BINAP catalyst (341 mg, 0.429 mmol) in another 300-mL Schlenk tube under argon, and the resulting light yellow solution was transferred to a glass vessel placed in a 500-mL stainless steel autoclave. Hydrogen was pressurized to 100 atm, and the solution was stirred at 30 °C for 36 h. The solvent was removed under reduced pressure, and the residue was distilled to give methyl (*R*)-3-hydroxybutanoate (**2a**) (97.5 g, 96% yield), bp 40 °C/2 mmHg, $[\alpha]_D^{25}$ −24.2° (neat) (lit.[1d] $[\alpha]_D^{22}$ −23.5° (neat)). The enantiomeric excess was determined to be 99.4% by HPLC analysis after converting an aliquot of the product to the (*R*)-MTPA ester[13] (1.4 equiv of (*R*)-MTPACl, 9 equiv of pyridine, CH_2Cl_2, 20 °C, 12 h, 94% yield). HPLC analysis of this ester (column, YMC 003-3 SIL and 002-3 SIL; eluent, 1:3 ether–hexane mixture; flow rate, 1 mL/min; detection, 254-nm light) showed two signals with t_R = 15.2 and 16.0 min in 99.7:0.3 ratio assignable to the *R,R*- and *R,S*-diastereomers, respectively.

(11) Bakers' yeast with the aid of sucrose reduces ethyl 3-oxobutanoate to ethyl (*S*)-3-hydroxybutanoate in 88–97% ee in 70–80% yield. In order to obtain high (95–97%) enantioselectivity, the substrate concentration should be kept below 1 g/L. Wipf, B.; Kupfer, E.; Bertazzi, R.; Leuenberger, H. G. W. *Helv. Chim. Acta* **1983**, *66*, 485. Seebach, D.; Sutter, M. A.; Weber, R. H.; Züger, M. F. *Org. Synth.* **1985**, *63*, 1. Ehrler, J.; Giovannini, F.; Lamatsch, B.; Seebach, D. *Chimia* **1986**, *40*, 172.

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Characterization of an Endogenous Factor Controlling the Cell Cycle of Complex Tissues

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The study and characterization of factors called chalones which are capable of regulating specific cellular arrest in complex tissues has been ongoing since their discovery in 1960.[1] Their obvious potential chemotherapeutic use and more recently their potential involvement in circadian rhythms[2,3] has stimulated continual interest in these factors. Trigonelline, *N*-methylnicotinic acid, became the first structurally defined substance capable of promoting specific G2 cellular arrest.[4,5] It was subsequently found that the activity of trigonelline was regulated by the age of the organism.[6] We now describe the isolation and characterization

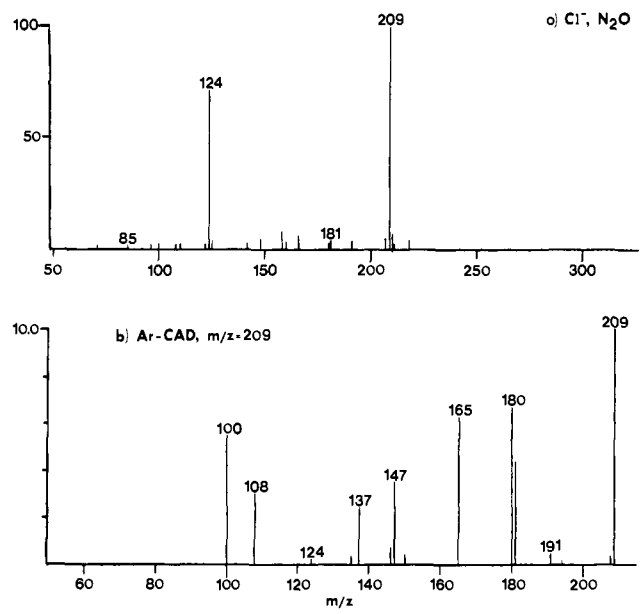

Figure 1. Negative ion chemical ionization (CI, N_2O) spectrum of **1** (a) and Cl^- spectrum (b). Selected m/z 209 ion collisionally decomposed with argon gas.

of an unusually substituted pyrrole **1** produced by older seedlings of *Pisum sativum* (Leguminasae) which functions as a specific endogenous regulator of trigonelline-induced G2 arrest.

The roots of 10-day-old etiolated *P. sativum* seedlings from ~1500 seeds were excised and blended (3×) at low speed for 45 min with sufficient methanol to cover the tissue. The extract was filtered through Celite, concentrated to 70 mL, applied to an open bed reverse phase filter, and step gradient washed with 150-mL portions of 0%, 25%, 50%, 75%, and 100% aqueous MeOH. The biologically active[5] 25% and 50% MeOH fractions were dried in vacuo onto a minimum amount of silica gel and washed with $MeOH/CHCl_3$, 1:1. The eluent was concentrated and chromatographed (SiO_2, 1–10% $CH_3CN/CHCl_3$) and preparatively purified on reverse phase HPLC (Zorbax C-8, 10% CH_3CN/H_2O, 2 mL/min, R_t = 4.2 min, 254-nm detection). Final purification was afforded on C-18 HPLC (Zorbax, 10% CH_3CN/H_2O, 2 mL/min, R_t = 4.2 min) yielding 20 μg of a clear oil. This procedure was repeated several times through the course of the characterization.

The UV spectrum (MeOH) of the purified material showed absorption maxima at 292 (9200) and 258 (3800) nm characteristic of a pyrrole bearing an α-carbonyl substituent.[7] IR bands at 1707 and 2840 cm^{-1} indicated an aldehyde, and stretching frequencies at 3300 and 1770 cm^{-1} suggested hydroxyl and γ-lactone functionality.

Negative ion chemical ionization (CI, N_2O),[8] Figure 1, and electron impact (EI, 70 eV) mass spectrometry gave an intense M^- ion at m/z 209 (EI 209.0691, calcd 209.0688) indicating a composition of $C_{10}H_{11}NO_4$. The CI spectra showed clean fragmentation to a single even mass ion at m/z 124. The mass of this ion, together with its subsequent fragmentation, led to the assignment of a formyl, hydroxymethyl pyrrole. Such intense C–N cleavage has proven to be characteristic of the *N*-alkyl-2-carbonyl pyrroles analyzed under $Cl(N_2O)$ negative ion conditions (unpublished).

Collision-activated decomposition[8] greatly accentuated the fragmentation of the molecular ion showing losses of H_2O (m/z 191), CO (m/z 181), CHO (m/z 180), and CO_2 (m/z 165). This induced fragmentation confirmed the functional groups suggested

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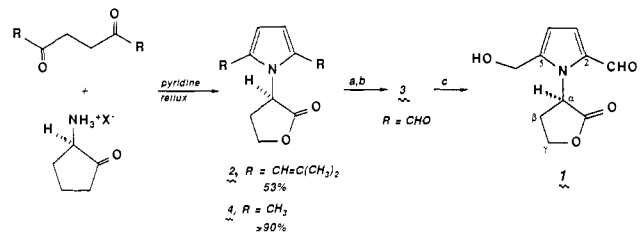
Scheme I Synthesis of **1**[a]



[a] a, O_3; b, Me_2S; c, B_2H_6.

by IR and restricted the neutral fragment of $C_4H_5O_2$ to a γ-lactone bound to the pyrrole nitrogen. Two ^1H NMR resonances,[9a] which appeared on cooling and resolved as a double doublet (δ 5.70, α-) and a pentet (δ 2.67, β-H) at −20 °C, were anisotropically broadened by hindered rotation of the N–C bond.[9b] Conversion to the symmetrical dialdehyde **3** (400 μg, MnO_2, CH_3CN, 1 h, ~100%)[10] gave sharp ^1H NMR resonances at ambient temperature,[11] further supporting **1**.

Paal–Knorr condensation[12] of acetonyl acetone with α-aminobutyrolactone–HBr (pyridine, reflux, 4 h, 89%) afforded the dimethylpyrrole **4**. Oxidation of **4** with benzeneseleninic anhydride[13] (1.3 equiv, PhCl, 18 h, reflux, 34%) gave the monoaldehyde **5** which could not be further oxidized. However, condensation of the aminolactone with 2,9-dimethyldeca-2,8-dien-4,7-dione[14] gave the bis(isobutenyl)pyrrole **2** (53%), which on ozonolysis (O_3, CH_2Cl_2, Me_2S workup) followed by diborane reduction (THF, 0 °C) gave **1** in good yields (Scheme I). Condensation of the chiral α-aminobutyrolactone[15] gave **2** (~20% racemization, resolved by repeated recrystallization from ether/petroleum ether (R, $[\alpha]_D^{23}$ −288 (c 0.0036, $CHCl_3$)) afforded both (+)- and (−)-**1**. Mosher's acid esterification[17] (1.2 equiv, R-(+), pyridine, 0 °C, 12 h) allowed for HPLC analysis (Zorbax ODS, 48% MeCN/H_2O 2 mL/min; $R_t(R,R)$ = 7.8 min, (S,R = 7.3 min) of the resulting diastereomers. Similar derivatization of the natural product confirmed its R absolute configuration.

The ED_{50}[5] of exogenously added **1** (S isomer shows no activity) in the inhibition of trigonelline-induced cellular arrest in G2 is 5×10^{-7} M. By harvesting tissues from roots of various ages, we have found that this concentration is reached within the tissue only in roots greater than 7 days of age. Thus, the presence of **1** could completely account for the inability of trigonelline to induce G2 arrest in older roots.[6] The biological reason for this antagonism is not known, but **1** *becomes the first chemically characterized substance which overrides hormonally induced cellular arrest in complex tissues.*

MS/MS experiments on the hybrid BEQQ spectrometer very efficiently established the functional groups and their structural arrangement as seen in **1**. The substitution pattern of the pyrrole does not suggest obvious biosynthetic pathways; however, one possibility would involve a reductive amination of amino and keto acid precursors similar to the opines.[19] This class of eucaryotic non-protein amino acids are biosynthesized in higher plants only when plasmid genes (T-DNA) of *A. tumefaciens* coding for the appropriate dehydrogenase are transferred and genetically incorporated into the plant genome. The characteristic (R) configuration in the products of these dehydrogenases is seen in **1** and preliminary experiments suggest that opines either serve as precursors or activate the synthesis of **1**. The significance of such a pathway and its role in cell cycle control are under investigation.

Acknowledgment. We are grateful to Jeffrey Shabanowitz for the initial MS/MS studies, S. Neighbors and S. Walker for preliminary isolation work, and The University of Chicago Cancer Center and Ciba-Geigy Corporation for support. D.G.L. is a Sloan Foundation Fellow and a Camille and Henry Dreyfus Teacher-Scholar.

(9) (a) ^1H NMR (500 MHz, C_3D_6O, −20 °C) δ 9.48 (1 H, s, CHO), 7.20 (1 H, d, J = 4 Hz, H-3), 6.37 (1 H, d, J = 4 Hz, H-4), 5.70 (1 H, dd, J = 12, 10 Hz, α-H), 4.80 (2 H, AB, J = 15 Hz, CH_2OH), 4.67 (1 H, dt, J = 10, 1.6 Hz, γ-H), 4.53 (1 H, ddd, J = 11, 10, 9.5 Hz, γ-H), 2.82 (1 H, bq, J = 10, 1.6 Hz, β-H), 2.67 (1 H, p, J = 11 Hz, β-H). (b) The coalescence temperature for **1** is 40 °C with an approximate $\Delta G^{\ddagger}$ of 14.7 cal/m. Oki, M. *Methods in Stereochemical analysis*; Marchand, A. P., Ed.; VCH Publishers: 1985; Chapter 5 and references therein.

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(11) ^1H NMR (500 MHz, $CDCl_3$) δ 9.80 (2 H, s, CHO), 7.10 (2 H, s, H-3, 4), 6.66 (1 H, dd, J = 10.5, 9 Hz, α-H), 4.74 (1 H, dt J = 10, 2.5 Hz, γ-H), 4.50 (1 H, m, γ-H), 2.76 (1 H, m, β-H), 2.69 (1 H, m, β-H).

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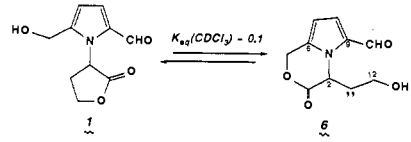
(18) During isolation, **1** was found to be sensitive to racemization and in $CDCl_3$ to intramolecularly transacylate to the fused ring system **6**: ^1H NMR (500 MHz, $CDCl_3$) δ 9.49 (1 H, s, CHO), 7.04 (1 H, d, J = 3 Hz, H-8), 6.25 (1 H, d, J = 3 Hz, H-7), 5.62 (1 H, d, J = 14.7 Hz, H-5), 5.37 (1 H, d, J = 14.7 Hz, H-5'), 5.84 (1 H, t, J = 6.3 Hz, H-2), 3.73 (1 H, m, J = 12.8, 6 Hz, H-12), 3.44 (1 H, m, J = 12, 8, 4 Hz, H-12), 2.40 (2 H, m, H-11).



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Retro Aza Diels–Alder Reactions: Acid-Catalyzed Heterocycloreversion of 2-Azanorbornenes in Water at Ambient Temperature

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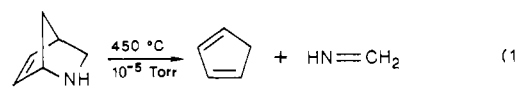
The retro Diels–Alder reaction has received considerable attention during the past 20 years with emphasis on synthetic and mechanistic aspects.[1] In sharp contrast the imino variant of the retro Diels–Alder reaction[2] has received very little attention which is undoubtedly due in part to the fact that such retro Diels–Alder reactions have commonly been conducted at temperatures in the range of 400–600 °C (cf. eq 1).[2a] These observations are not



(1)

surprising since it is well known that norbornene is resistant to thermolysis, requiring temperatures in excess of 250 °C.[3] In striking contrast to the chemistry outlined in eq 1 we now report that *2-azanorbornene as well as its N-alkyl derivatives undergoes acid-catalyzed retro Diels–Alder reaction at room temperature in water.*

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