was applied directly to a preparative layer plate (20 × 20 × 0.2 cm layer of silica gel) and eluted with 20% ether in petroleum ether (R_f 0.8). Isolation yielded 53 mg (0.27 mmol, 52%) of lactone **7**; ^{1}H NMR ($CDCl_3$) δ 1.26 (d, 3 H, J = 7 Hz), 1.50 (m, 8 H), 2.30 (m, 6 H), 5.18 (m, 1 H), 5.35 (m, 2 H).

Vapor phase chromatographic comparisons were performed with a Hewlett-Packard 5750 flame ionization instrument using a 6 ft × ⅛ in. column of 3% OV-17 on 80–100 mesh Chromosorb W at a temperature of 145° and a nitrogen flow rate of 60 ml/min; a retention time of 14 min was observed for synthetic and naturally derived lactone **7**.

Acknowledgment. This research was assisted financially by the National Institutes of Health and the National Science Foundation.

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Total Synthesis of Gentiocrucine, an Unusual Alkaloid Containing a Stable Primary Enamide

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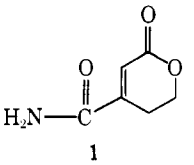
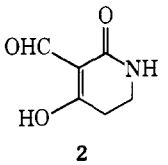
Abstract: A total synthesis of the naturally occurring alkaloid gentiocrucine is reported which unambiguously confirms its structure **3a, 3b** as the first example of a stable primary enamide. Treatment of methyl 2-(methoxymethylene)acetoacetate (**4**) with aniline produced methyl 2-(*N*-phenylaminomethylene)acetoacetate (**5**). Addition of *n*-butyllithium (2 equiv) to **5** at −78° followed by gaseous formaldehyde afforded a 60% yield of *N*-phenylgentiocrucine **8**, mp 116–118.5°. Stirring **8** in liquid ammonia produced gentiocrucine (66%, mp 144–145°), identical with an authentic sample. The reactivity of this substance as well as factors responsible for its stability is discussed.

Gentiocrucine, a terpene alkaloid derivative first isolated from *Gentiana cruciata*, was assigned structure **1** by Popov and Marekov on the basis of its spectral and chemical properties.[1] These workers also demonstrated that gentianaine,[2] previously thought to be **2**, was identical with gentiocrucine.[3] Recently the same substance was discovered in another plant, *Enicostemma hyssopifolium*, and its structure reinvestigated.[4] During this work, comprehensive spectroscopic studies ruled out **1** and **2** in favor of the isomeric enamides **3a** and **3b**. If structurally correct, this pair of isomers would represent the first examples, naturally occurring or synthetic, of a stable primary enamide. This article discloses a short total synthesis of gentiocrucine which unambiguously confirms the revised structure **3a, 3b** and establishes a simple new approach to substituted 3-keto-δ-valerolactones.



1



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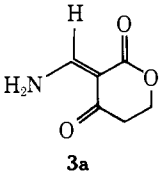
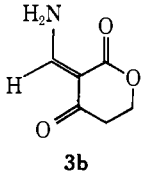


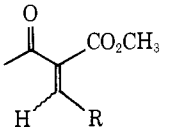
3a



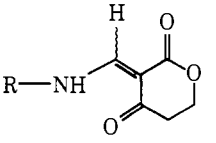
3b

Results

Treatment of methyl 2-(methoxymethylene)acetoacetate (**4**)[5] with two equiv of aniline in $CHCl_3$ produced the corresponding enaminoketoester **5** in 97% yield as a mixture of isomers.[6,7] Addition of *n*-butyllithium (2 mol equiv) to **5** at −78° followed immediately by passage of anhydrous gaseous formaldehyde (1.5 equiv, generated from paraformaldehyde) into the clear orange dianion solution using a stream of nitrogen resulted in a rapid uptake of the gas at −78° and concomitant disappearance of color. After warm-



4, R = OCH_3
5, R = NHPh
6, R = NH_2
7, R = $NHCH_2Ph$

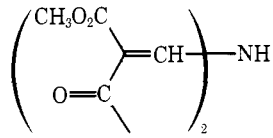


8, R = Ph
9, R = $PhCH_2$

ing to room temperature, a 60% yield of the lactone **8**, mp 116–118.5°, was realized.[6,7] The NMR spectrum verified that **8**, like gentiocrucine,[4] exists as a nearly 2:1 mixture of stereoisomers about the enamide double bond. Conversion to the natural product was effected merely by dissolving **8** in anhydrous liquid ammonia, then evaporating the solvent, and washing the solid white residue with ether to remove aniline. Silica gel chromatography ($CHCl_3$) afforded a 66% yield of crystalline gentiocrucine (mp 144–145°; authentic sample[8] mp 145–147°; mmp 144–146°) whose infrared, NMR, and mass spectra were in complete agreement with reference spectra of the naturally occurring alkaloid.[9] In particular, the Me_2SO-d_6 NMR spectrum revealed overlapping triplets (δ 2.45, 2.50, J = 6 Hz) of unequal intensity (~1.5:2.5) for the carbonyl-adjacent methylene protons and a complex multiplet (δ 8.1) originating from unequal coupling of the stereoisomeric vinyl hydrogens with nonequivalent NH_2 protons.[4] Natural and synthetic material also exhibited identical TLC behavior (single spot) in four different solvent systems.

Discussion

The stability of gentiocrucine's primary enaminoketone reflects the vinylic nitrogen's unusually low nucleophilic character. For example this substance did not react with such alkylating agents as methyl iodide or benzyl bromide in refluxing $CHCl_3$. It was unaffected by aqueous nitrous acid at 0° and only slowly oxidized by cold $KMnO_4$ in aqueous acetone. Furthermore no disproportionation or appreciable decomposition occurred when gentiocrucine was refluxed in toluene for 24 hr. Such behavior may well be due to extensive hydrogen bonding with the ring carbonyls, as evidenced by the infrared NH absorption at 2.81 μ. Mere conjugation with the two carbonyl groups in **3** does not sufficiently explain this stability; attempts to prepare the analogous primary enaminoketoester **6** by the addition of ammonia to **4** furnished instead the dimeric secondary dienamide **10**, mp 107–108°. We conclude that hydrogen bonding in **6**, probably formed as a transient intermediate to **10**,



10

must be of little consequence compared with that in gentiocrucine, where the ring-imposed *s*-cis geometry between each NH_2 and carbonyl pair (cf. **3a** and **3b**) makes possible such highly attractive forces.

Other geometric as well as electronic requirements were carefully considered while designing the key lactone-forming alkylation. Rather than introduce the aminomethylene group after cyclization, we specifically employed the olefinic linkage in **5** to maintain the necessary geometry for spontaneous lactonization during the aldol condensation, while the residual nitrogen-based anion was expected to protect the conjugated alkene from nucleophilic attack. Efforts to cyclize in a similar fashion related ketoesters which fail to meet these two criteria were uniformly unsuccessful. Notably the enolate of **4** rapidly polymerized during generation at −78°, and the dianion of methyl acetoacetate, prepared by a published procedure,[10] underwent polycondensation with formaldehyde at a variety of temperatures but afforded no 3-keto-δ-valerolactone.[11]

To demonstrate the general feasibility of this method, we have also prepared *N*-benzylgentiocrucine (**9**) as a 2:1 stereoisomeric mixture (mp 165–168°) in 80% yield from **7**. The simple three-step synthesis described here makes available appreciable quantities of these α-methylene lactone alkaloids for the first time. Results of preliminary pharmacological screening tests currently in progress on gentiocrucine and its derivatives will be reported elsewhere.

Experimental Section

Melting points were determined in capillaries and are uncorrected. NMR spectra were recorded on a Varian A-60A spectrometer with tetramethylsilane as an internal standard. Infrared spectra of $CHCl_3$ solutions were determined on a Perkin-Elmer 137 spectrophotometer. Mass spectra were carried out using a computerized ADI MS-902 instrument. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Preparation of Methyl 2-(*N*-Phenylaminomethylene)acetoacetate (5). A solution of methyl 2-(methoxymethylene)acetoacetate (**4**)[5] (4.50 g, 28.5 mmol) in $CHCl_3$ (60 ml) was cooled in ice during the addition of aniline (5.58 g, 60 mmol). Upon completion of addition, the contents of the flask were brought to room temperature and washed with ice-cold 5% HCl (100 ml) and then saturated $NaHCO_3$ solution. The organic layer was dried ($MgSO_4$) and concentrated to yield 6.1 g (97%) of white crystals, mp 80–82.5°. One recrystallization from ethanol yielded needles: mp 82–83.5°; NMR δ ($CDCl_3$) 2.50 (s, 3 H), 3.75 (s, 3 H), 7.18 (broad m, 5 H), 8.24 (d, 1 H, J = 14 Hz), 12.21 (broad d, 1 H, J = 14 Hz); ir ($CHCl_3$) 5.86, 6.11, 6.24, dm 6.33 μ.

Anal. Calcd for ($C_{12}H_{13}NO_3$) C, H, N.

Preparation of Methyl 2-(*N*-Benzylaminomethylene)acetoacetate (7). A solution of **4** (7.12 g, 45 mmol) in $CHCl_3$ (50 ml) was treated with benzylamine (5.36 g, 50 mmol) at 0°. Work-up as described above for **5** afforded 10.45 g (99%) of white crystals: mp 77–79°; NMR δ ($CDCl_3$) 2.46 (s, 3 H), 3.70 (s, 3 H), 4.45 (d, 2 H, J = 7 Hz), 7.29 (broad s, 5 H), 8.07 (d, 1 H, J = 14); ir ($CHCl_3$) 5.87, 6.13, 6.26 μ.

Preparation of *N*-Phenylgentiocrucine (8). A flame-dried 100-ml round-bottomed three-necked flask containing **5** (0.633 g, 2.9 mmol) was equipped with magnetic stir bar and a gas inlet tube on the center joint. The inlet was connected by Tygon™ to the sidearm of a dry 50-ml erlenmeyer filter flask containing paraformaldehyde (0.14 g). A nitrogen inlet was attached to the mouth of the erlenmeyer and the three-necked flask also fitted with rubber septum and gas exit tube. After the entire system was flushed with N_2, **5** was dissolved in dry tetrahydrofuran (THF, 20 ml, distilled from $LiAlH_4$) and cooled to −78° (dry ice-2-propanol). To this solution was added slowly *n*-butyllithium (Alfa; 2.0 *M* in hexane, 2.9 ml) down the side of the flask. A pale orange color developed immediately, and the formaldehyde generator was immersed in a 140° oil bath with a fast N_2 flow carrying formaldehyde gas directly into the vortex of the rapidly stirred dianion solution. This process took ca. 10 min. A precipitate appeared at first which dissolved upon removal of the low temperature bath. After 6 hr at room temperature, water was added and the bulk of THF removed under reduced pressure. The residue was extracted three times with $CHCl_3$. These combined extracts were dried ($MgSO_4$) and concentrated to an oily solid. Chromatography over silica gel (W.R. Grace) using $CHCl_3$ eluted five fractions identical by TLC (R_f 0.4 in ether). These were combined to yield 0.39 g (60%) of crystals: mp 116–118.5°; NMR δ ($CDCl_3$) 2.58, 2.70 (two overlapping triplets, 2 H, J = 6.5 Hz), 4.44, 4.47 (two overlapping triplets, 2 H, J = 6.5 Hz), 7.3 (broad m, 5 H), 8.43, 8.50 (two doublets, 1 H, J = 14, ratio 65:35); ir ($CHCl_3$) 5.86, 5.88, 6.11, 6.23, 6.31 μ mass spectrum 217 (molecular ion, also base peak).

Anal. Calcd for ($C_{12}H_{11}NO_3$) C, H, N.

Preparation of *N*-Benzylgentiocrucine (9). Using the apparatus and procedure described above for **8**, a solution of **7** (1.95 g, 8.4 mmol) in THF (40 ml) was cooled to −78° during the addition of *n*-butyllithium (2.0 *M*, 8.4 ml). A deep red dianion solution resulted which decolorized completely during addition of CH_2O gas (from 0.54 g paraformaldehyde). Work-up and chromatography as before furnished 1.56 g (80%) of crystals: mp 165–168°; NMR δ ($CDCl_3$) 2.55 (broad t, 2 H, J = 7), 4.38 (broad t, 2 H, J = 7 Hz), 5.58 (d, 2 H, J = 8), 7.2–7.3 (broad m, 5 H), 8.16, 8.26 (two doublets, 1 H, J = 14, ratio 2:1); ir ($CHCl_3$) 5.87, 6.10, 6.21 μ; mass spectrum 231 (parent ion, also base peak).

Conversion of 8 to Gentiocrucine. *N*-Phenylgentiocrucine (0.140 g, 0.645 mmol) was dissolved in liquid NH_3 (20 ml) and stirred at

reflux for 3 hr. Evaporation left an off-white powder which was triturated with ether (5 ml) to remove aniline. The residual solid was chromatographed on silica gel (W.R. Grace) eluting with $CHCl_3$ to afford 60 mg (66%) of fine white crystals: mp 144–145°; NMR δ (D_2O) 2.58 (t, 2 H, J = 6.5 Hz), 4.40 (t, 2 H, J = 6.5 Hz), 8.10 (broad s, 1 H); ir ($CHCl_3$) 2.81, 5.86, 6.08, 6.11 μ; mass spectrum 141 (M^+, base peak), 114, 113, 70, 69. Synthetic and natural gentiocrucine had identical TLC behavior in the following systems: R_f 0.05 in ether; 0.15 in CH_3OH–ether 1:9; 0.49 in CH_3OH–ether 1:4; 0.17 in ethyl acetate.

Anal. Calcd for ($C_6H_7O_3N$) C, H, N.

Addition of Ammonia to 4. Preparation of 10. Ammonia was bubbled through $CHCl_3$ solution of **4** (0.356 g, 2.25 mmol) at 0° until the characteristic odor of NH_3 became evident. Stirring was continued for an additional hour at room temperature and then the solvent evaporated at reduced pressure to afford 0.300 g (98%) of white crystals: mp 107–108°; NMR δ ($CDCl_3$) 2.42, 2.46 (2 singlets, 6 H), 3.71, 3.81 (2 singlets, 6 H), 7.0–7.3 (broad s, 1 H, NH), 8.07, 8.16 (2 doublets, broad, 2 H, J = 16 Hz); ir ($CHCl_3$) 5.85, 5.89, 6.10 (strong), 6.16 μ; mass spectrum 269 (M^+).

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for their support of this research.

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(8) Kindly provided by Dr. S. Ghosal, Banaras Hindu University, Varanasi-5, India.
(9) Thanks are due to Drs. N. Marekov and S. Popov for providing these spectra as well as a small sample of gentiocrucine.
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