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Structure of the Lycorinine Alkaloid Nobilisitine A

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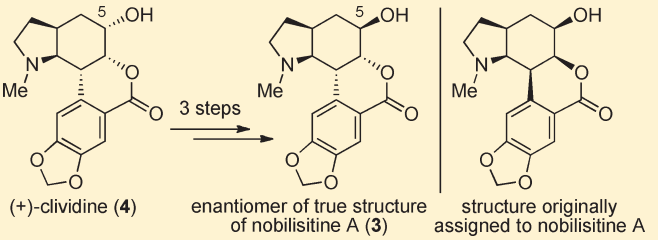
Supporting Information

ABSTRACT: The structure **3** recently proposed, on the basis of computed NMR chemical shifts, for the natural product nobilisitine A has been synthesized from its C5-epimer (+)-clividine (**4**). The spectral data derived from compound **3** match those reported for the natural product.



The natural product nobilisitine A was isolated from the ornamental plant *Clivia nobilis* found growing in Egypt.[1] On the basis of the derived ^{1}H and ^{13}C NMR spectroscopic data, Evidente and co-workers assigned structure **1** (Figure 1) to this compound, thus implying it is a member of the marasmane or lycorinine subclass of Amaryllidaceae alkaloid.[1] While almost no biological evaluation of nobilisitine A appears to have been undertaken, other compounds within the subclass and other natural products possessing related structures display various interesting biological effects, including antifungal, antitumor, and apoptosis-inducing activities.[2]

For the purposes of checking Evidente's structural assignment, which incorporates an all-*cis* arrangement of substituents about the central C-ring, we recently completed[3] a synthesis of compound *ent*-**1** using the enantiomerically pure *cis*-1,2-dihydrocatechol **2** as starting material.[4] As a result, we determined that the derived spectral data did not match those recorded for nobilisitine A and concluded, therefore, that its structure had been assigned incorrectly.[3]

Prompted by our revelation of this erroneous assignment and following certain suggestions we made about the true structure of the natural product, Lodewyk and Tantillo[5] conducted a series of DFT calculations to predict the ^{1}H and ^{13}C NMR chemical shifts of the various diastereoisomeric forms of compound **1**. As a consequence, they suggested that the correct structure for nobilisitine A is likely to be **3** or its enantiomer (Figure 2). Coincidentally, and using chemistry related to that employed in the synthesis of compound *ent*-**1**, we recently completed a synthesis of (+)-clividine (**4**),[6] the C5-epimer of the new structure proposed for nobilisitine A. Accordingly, it seemed appropriate to pursue the conversion **4** → **3** in an effort to determine if the revised structure proposed for nobilisitine A is correct. The outcomes of relevant studies are reported here.

Our initial attempts to convert (+)-clividine (**4**) into epimer **3** involved treating the former compound under Mitsunobu conditions[7] using α-chloroacetic acid as the nucleophile. However, no reaction was observed, an outcome in keeping with the known sensitivity of this reaction to steric effects.[8] In another approach, compound **4** was oxidized to the corresponding

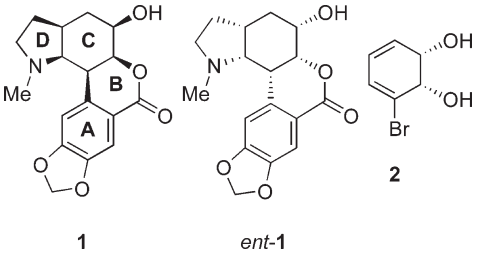


Figure 1. Structures of compounds **1**, *ent*-**1**, and **2**.

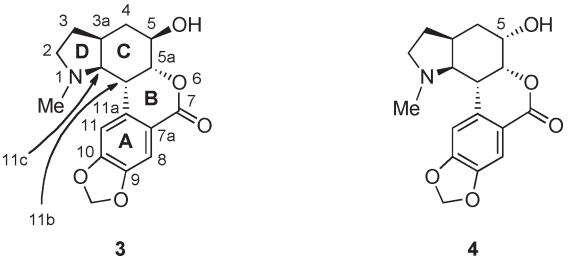


Figure 2. Structures of compounds **3** and **4**.

ketone but when this was, in turn, subjected to reaction with zinc borohydride a complex mixture of products, including small quantities of the original alcohol **4**, was obtained. The ultimately successful means for preparing compound **3** is shown in Scheme 1. The pivotal step involved exploiting a novel but underutilized protocol developed by Zard and co-workers[9] for the inversion of secondary alcohols. Thus, the starting material **4** was converted into the corresponding propargyl xanthate **5** (67%) by successive treatment of the former compound with sodium hydride, carbon disulfide, and propargyl bromide. Ester **5** was, in turn, heated in refluxing chlorobenzene in the presence of benzoic acid, thereby effecting, via sigmatropic rearrangement,

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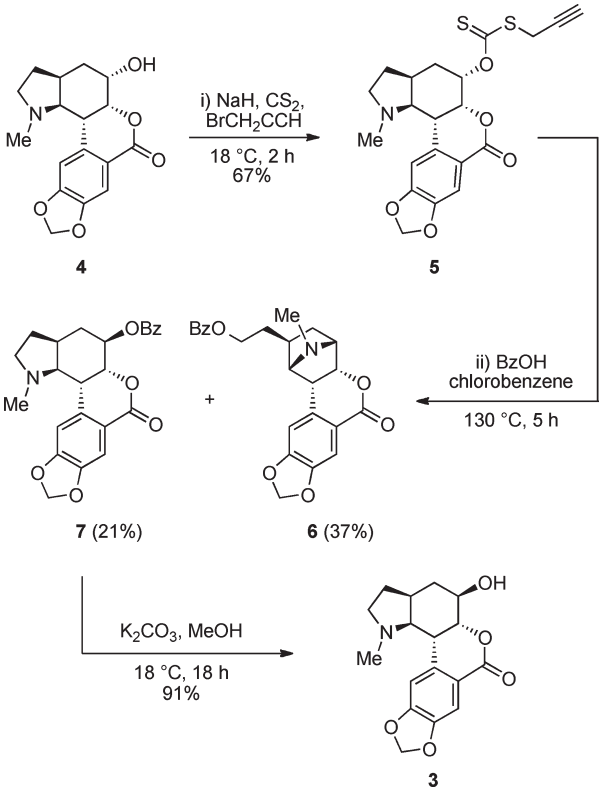
Scheme 1. Synthesis of Compound 3



the formation of a betaine[9] that reacted with the added acid to give a chromatographically separable mixture of the 7-azabicyclo-[2.2.1]heptane **6** (37%) and the desired benzoate **7** (21%). Compound **6** is thought to arise via a transannular nucleophilic displacement of the betaine by the D-ring nitrogen followed by fragmentation of the ensuing quaternary ammonium salt with benzoic acid in a von Braun-type process. Its structure was established by a single-crystal X-ray analysis (see the Supporting Information for details).[10] In the final step of the reaction sequence, benzoate **7** was treated with potassium carbonate in methanol, and in this manner the target alcohol **3** was obtained in 91% yield and as a white, crystalline solid. The derived spectral data were in complete accord with the assigned structure, but final confirmation of this followed from a single-crystal X-ray analysis (see the Supporting Information for details).[10]

The melting range of the synthetically derived material (195−196 °C) matched reasonably well with that reported[1] for nobilisitine A (186−187 °C). Comparisons of the ^{13}C and ^{1}H NMR spectral data obtained on compound **3** with those reported for nobilisitine A and compound *ent*-**1** are presented in the Supporting Information in Tables S1 and S2, respectively. Similar comparisons of the infrared and mass spectral data are presented in Table S3 of the Supporting Information. All of these quite clearly demonstrate that compound **3** does indeed correspond to the structure of nobilisitine A. Unfortunately, the specific rotation of the natural product has not been reported, so it has not been possible to establish the absolute configuration of this alkaloid.[11] However, given that nobilisitine A is the C5-epimer of clividine (which occurs naturally in the form *ent*-**4**[12]), there is some likelihood this is represented by structure *ent*-**3**.

The results reported here clearly show that compound **3** or (more likely) its enantiomer corresponds to the true structure of the lycorinine alkaloid nobilisitine A. The present work reinforces the notion that the de novo prediction of NMR chemical shifts using DFT-based techniques represents a powerful tool for assisting with the assignment of molecular structures.[13] This study also suggests that Zard's protocol[9] for the inversion of configuration of secondary alcohols deserves more attention than it has received thus far.

EXPERIMENTAL SECTION

General Experimental Procedures. Unless otherwise specified, proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded at 18 °C in base-filtered $CDCl_3$ at 400 MHz for proton and 100 MHz for carbon nuclei. For ^{1}H NMR spectra, signals arising from the residual protio forms of the solvent were used as the internal standards. ^{1}H NMR data are recorded as follows: chemical shift (δ) [multiplicity, coupling constant(s) J (Hz), relative integral] where multiplicity is defined as s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet or combinations of the above. The signal due to residual $CHCl_3$ appearing at δ_H 7.26 and the central resonance of the $CDCl_3$ "triplet" appearing at δ_C 77.0 were used to reference ^{1}H and ^{13}C NMR spectra, respectively. Infrared spectra (ν_{max}) were analyzed as thin films on KBr plates. Melting points are uncorrected. Analytical thin-layer chromatography (TLC) was performed on aluminum-backed 0.2 mm thick silica gel 60 F_{254} plates. Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable dip followed by heating. These dips included phosphomolybdic acid/ceric sulfate/sulfuric acid (conc)/water (37.5 g/7.5 g/37.5 g/720 mL) or potassium permanganate/potassium carbonate/5% sodium hydroxide aqueous solution/ water (3 g/20 g/5 mL/300 mL). Flash chromatographic separations were carried out following protocols defined by Still et al.[14] with silica gel 60 (40−63 μm) as the stationary phase and using the AR- or HPLC-grade solvents indicated. Starting materials and reagents were generally available from commercial suppliers and were used as supplied. Drying agents and other inorganic salts were purchased from commercial suppliers. Tetrahydrofuran (THF), methanol, and dichloromethane (DCM) were dried using a solvent purification system that is based upon a technology originally described by Grubbs et al.[15] Where necessary, reactions were performed under an argon atmosphere.

Compound 5. A magnetically stirred solution of *ent*-clividine (**4**)[6] (237 mg, 0.75 mmol) in THF (8 mL) maintained at 18 °C under an atmosphere of nitrogen was treated with NaH (30 mg of a 60% dispersion in mineral oil, 0.90 mmol). After 1 h, the reaction mixture was treated with carbon disulfide (54 μL, 0.90 mmol), stirred at 18 °C for 0.5 h, and then treated with propargyl bromide (100 μL of an 80% w/v solution in toluene, 0.90 mmol). After a further 0.5 h, the reaction mixture was quenched with $NaHCO_3$ (10 mL of a saturated aqueous solution) and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated under reduced pressure at 40 °C. The brown oil so obtained was subjected to flash chromatography (silica, 1:20 v/v ammonia saturated methanol/CH_2Cl_2 elution), and concentration of the appropriate fractions (R_f = 0.8) afforded *xanthate* **5** (217 mg, 67%) as a viscous orange oil: $[\alpha]_D$ −2.5 (*c* 0.4, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.51 (s, 1H), 6.92 (s, 1H), 6.06 (d, J = 1.3 Hz, 1H), 6.05 (d, J = 1.3 Hz, 1H), 5.93 (ddd, J = 11.3, 4.9, and 2.4 Hz, 1H), 4.84 (m, 1H), 3.89 (dd, J = 16.6 and 2.7 Hz, 1H), 3.84 (dd, J = 16.6 and 2.7 Hz, 1H), 3.29−3.23 (complex m, 1H), 2.75 (dd, J = 8.9 and 2.5 Hz, 1H), 2.64 (m, 1H), 2.53−2.45 (complex m, 2H), 2.40 (dd, J = 17.6 and 9.1 Hz, 1H), 2.23 (t, J = 2.7 Hz, 1H), 2.20−2.15 (complex m, 1H), 2.17 (s, 3H), 1.95−1.89 (complex m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 212.0, 164.4, 151.9, 147.4, 139.6, 117.8, 109.7, 107.6, 101.9, 77.6, 76.3, 71.9, 67.0, 55.7, 44.9, 42.5, 36.2, 29.9, 29.6, 25.1, 24.3; IR (KBr) ν_{max} 3293, 2930, 1717, 1616, 1503, 1478, 1448, 1384, 1258, 1218, 1110, 1038, 939 cm^{-1}; MS (ESI) m/z 454

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[(M + Na)$^+$, 10], 432 [(M + H)$^+$, 100], 318 (20), 300 (92); HRMS (M + H)$^+$ calcd for $C_{21}H_{21}NO_5S_2$ 432.0939, found 432.0934.

Compounds 6 and 7. A magnetically stirred solution of xanthate **5** (217 mg, 0.50 mmol) in freshly distilled chlorobenzene (10 mL) maintained under a nitrogen atmosphere was treated with benzoic acid (184 mg, 1.51 mmol) and the ensuing mixture heated at reflux for 5 h. The cooled reaction mixture was quenched with $NaHCO_3$ (10 mL of a saturated aqueous solution) and the separated aqueous layer extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were then dried (Na_2SO_4), filtered, and concentrated under reduced pressure at 40 °C. The malodorous residue obtained in this way was subjected to flash chromatography (silica, 1:19 v/v methanol/CH_2Cl_2 elution), thus affording three fractions, A, B, and C.

Concentration of fraction A (R_f = 0.8 in 1:20 v/v ammonia saturated MeOH/CH_2Cl_2) afforded xanthate **5** (26 mg, 12% recovery) that was identical, in all respects, with an authentic sample.

Concentration of fraction B (R_f = 0.7 in 1:20 v/v ammonia saturated MeOH/CH_2Cl_2) afforded *benzoate* **7** (45 mg, 21%) as an orange foam: $[\alpha]_D$ −47.8 (*c* 0.8, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04−8.01 (complex m, 2H), 7.59−7.52 (complex m, 1H), 7.55 (s, 1H), 7.43 (t, *J* = 7.7 Hz, 2H), 6.97 (s, 1H), 6.06 (d, *J* = 1.2 Hz, 1H), 6.06 (d, *J* = 1.2 Hz, 1H), 5.33 (m, 1H), 4.86 (dd, *J* = 6.4 and 4.3 Hz, 1H), 3.35−3.26 (complex m, 1H), 3.25 (m, 1H), 2.79−2.76 (complex m, 1H), 2.43−2.31 (complex m, 2H), 2.22 (s, 3H), 2.20−2.14 (complex m, 1H), 2.03−1.95 (complex m, 1H), 1.89 (dt, *J* = 13.6 and 9.1 Hz, 1H), 1.75 (broad s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.7, 163.7, 152.3, 147.3, 133.1, 129.9, 129.7, 128.4, 118.8, 110.0, 106.7, 102.0, 78.0, 70.9, 66.0, 55.4, 42.6, 37.8, 35.0, 30.2, 29.7 (one signal obscured or overlapping); IR (KBr) ν_{max} 2939, 1717, 1616, 1503, 1480, 1449, 1383, 1264, 1111, 1071, 1035, 934, 713 cm^{-1}; MS (EI, 70 eV) *m*/*z* 421 (M$^{+\bullet}$, 50), 316 (16), 299 (26), 96 (100); HRMS M$^{+\bullet}$ calcd for $C_{24}H_{23}NO_6$ 421.1525, found 421.1526.

Concentration of fraction C (R_f = 0.4 in 1:20 v/v ammonia saturated methanol/CH_2Cl_2) afforded *7-azabicyclo[2.2.1]heptane* **6** (78 mg, 37%) as a white crystalline solid: mp 145−147 °C; $[\alpha]_D$ −50.3 (*c* 1.2, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.81−7.77 (complex m, 2H), 7.56−7.51 (complex m, 1H), 7.52 (s, 1H), 7.42−7.37 (complex m, 2H), 6.46 (s, 1H), 5.87 (d, *J* = 1.4 Hz, 1H), 5.52 (d, *J* = 1.4 Hz, 1H), 5.18 (ddd, *J* = 11.0, 5.0, and 1.4 Hz, 1H), 4.22 (m, 1H), 4.11 (dt, *J* = 11.0 and 5.7 Hz, 1H), 3.68 (td, *J* = 4.6 and 0.8 Hz, 1H), 3.63 (dd, *J* = 11.0 and 4.9 Hz, 1H), 3.37 (dd, *J* = 4.9 and 0.9 Hz, 1H), 2.55 (s, 3H), 2.01 (dd, *J* = 12.8 and 8.8 Hz, 1H), 1.82−1.74 (complex m, 1H), 1.71−1.62 (complex m, 1H), 1.57−1.50 (complex m, 1H), 1.30 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.3, 162.7, 152.8, 147.0, 135.0, 132.8, 130.1, 129.3, 128.3, 116.8, 109.2, 106.4, 101.7, 77.3, 70.5, 66.6, 62.8, 35.6, 35.0, 34.1, 33.0, 30.1; IR (KBr) ν_{max} 2947, 1710, 1619, 1503, 1481, 1449, 1410, 1274, 1251, 1126, 1035 cm^{-1}; MS (EI, 70 eV) *m*/*z* 421 (M$^{+\bullet}$, 12), 299 (19), 232 (100), 231 (98), 162 (25), 126 (77); HRMS M$^{+\bullet}$ calcd for $C_{24}H_{23}NO_6$ 421.1525, found 421.1522.

Compound 3. A magnetically stirred solution of benzoate **7** (50 mg, 0.12 mmol) in methanol (10 mL) was treated with anhydrous $KHCO_3$ (20 mg, 0.13 mmol) and the resulting mixture stirred at 18 °C for 18 h while being maintained under an atmosphere of nitrogen. The reaction mixture thus obtained was acidified with glacial acetic acid (∼1 mL) and the aqueous layer extracted with diethyl ether (10 mL). The separated aqueous layer was then brought to pH = 8 using sodium bicarbonate (saturated aqueous solution) and extracted with dichloromethane (3 × 10 mL). The combined organic phases were washed with brine (1 × 10 mL) and dried (Na_2SO_4) before being filtered and concentrated under reduced pressure at 40 °C. The residue thus obtained was subjected to flash chromatography (silica, 2:4:5 v/v/v ammonia saturated methanol/ethyl acetate/hexane elution), and concentration of the appropriate fractions (R_f = 0.2 in 1:20 v/v ammonia saturated methanol/CH_2Cl_2 elution) afforded a white foam. Crystallization (ethyl acetate) of this material gave compound **3**[1] (28 mg, 91%) as small, white crystalline masses: mp 195−196 °C (lit.[1] mp 186−187 °C); $[\alpha]_D$ −47.0 (*c* 0.2, $CDCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ see Table S2 in the Supporting Information; ^{13}C NMR ($CDCl_3$, 100 MHz) δ see Table S1 in the Supporting Information; IR (KBr) ν_{max} 3389, 2931, 1710, 1616, 1503, 1480, 1447, 1385, 1263, 1122, 1073, 1034 cm^{-1}; MS (EI, 70 eV) *m*/*z* 317 (M$^{+\bullet}$, 48), 301 (3), 273 (2), 242 (5), 228 (5), 172 (8), 126 (8), 96 (100); HRMS M$^{+\bullet}$ calcd for $C_{17}H_{19}NO_5$ 317.1263, found 317.1265.

■ ASSOCIATED CONTENT

Supporting Information. CIF files, crystallographic data, and anisotropic displacement ellipsoid plots derived from the single-crystal X-ray analyses of compounds **3** and **6**; Tables S1−S3 comparing spectral data derived from compound *ent*-**1**, nobilistine A, and compound **3**; ^{1}H and ^{13}C NMR spectra for compounds **3** and **5**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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