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# Two novel Amaryllidaceae alkaloids from *Hippeastrum equestre* Herb.: 3-*O*-demethyltazettine and egonine

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**Abstract**

From the bulbs of vietnamese *Hippeastrum equestre* Herb. (Amaryllidaceae), two novel Amaryllidaceae alkaloids 3-*O*-demethyltazettine and egonine have been isolated. Their structures were established by spectroscopic analysis (UV, MS, NMR and CD) as well as by comparison with respective spectroscopic data of tazettine and tazettadiol. 

*Keywords:* *Hippeastrum equestre* Herb; Amaryllidaceae; Alkaloids; 3-*O*-demethyltazettine; Egonine

## 1. Introduction

Continuing our investigations into basic components of the bulbs of *Hippeastrum equestre* Herb. (Döpke, Pham, Gründemann, Bartoszek, & Flatau, 1995a; Döpke, Pham, Gründemann, Bartoszek, & Flatau, 1995b; Pham, Gründemann, & Döpke, 1997; Wagner, Pham, & Döpke, 1996) the isolation and identification of two new Amaryllidaceae alkaloids 3-*O*-demethyltazettine (**2**) and egonine (**3**) was achieved. The alkaloid **2** of natural origin was isolated for the first time, although this compound has been synthesized on the way toward establishing the structure of tazettine (**1**) (Tsuda, & Uyeo, 1961). For the first time a complete NMR study of tazettine (**1**) is also presented in this paper. The new alkaloid **3** was identified by means of spectroscopic methods (UV, MS, NMR and CD) as 3α-hydroxy-3aβ-(2-hydroxymethyl-4,5-methylenedioxyphenyl)-6β-methoxy-*N*-methyl-2,3,6,7-tetrahydro-7a-H-indole and designated as egonine[1]. Alkaloid **3** belongs to mesembrine-type alkaloids, which have been originally found in Aizoaceae. Besides amisine from *Hymenocallis arenicola* (Döpke, Sewerin, & Trimino, 1980) and mesembrenol from *Crinum oliganthum* (Döpke, Sewerin, Trimino, & Gutierrez, 1981) egonine represents therefore the third alkaloid of mesembrine-type structure as an exception from the common rule (Mothes, Schütte, & Luckner, 1985; Frohne, & Jensen, 1992) that amaryllidaceae alkaloids do not appear in any other plant family.

## 2. Results and discussion

Compound **2** was isolated from the methanol extract of the dried bulbs of *H. equestre* by chromatography on silica gel. The EIMS of this new alkaloid ($C_{17}H_{19}NO_5$) showed beside the molecular ion peak at *m*/*z* 317 characteristic fragments with a base peak at *m*/*z* 247, which originated from a cleavage reaction of the tazettine structure (Duffield et al., 1965). The UV spectrum of **2** in MeOH exhibited maxima ($\lambda_{max}$: nm ($\varepsilon$: $m^2$/mol)) at 205.1 (2367), 239.2 (486) and 292.0

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[1] In order to appreciate Dr. Egon Gründemann for his contributions to the establishment of the structures of the alkaloids from *Hippeastrum equestre*, especially of this novel compound.

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(360) in agreement to those of tazettine (DeAngelis, & Wildman, 1969).

The presence of 19 H- and 17 C-atoms of **2** was confirmed by $^{1}H$ and $^{13}C$ NMR spectra. The $^{1}H$ spectrum exhibited two singlets at $\delta$ 6.45 and 6.78 for the aromatic protons H-9 and H-12, an AB-system at $\delta$ 5.85 for two protons of the methylenedioxy group and two double double doublets at $\delta$ 5.52 and 5.99 for the olefinic protons H-1 and H-2, respectively. Two AB-systems, one at $\delta$ 4.89 and 4.58, and the other at $\delta$ 3.26 and 2.62, could be assigned to the protons of the methylenes on C-8 and C-6. These assignments were achieved according to the NOESY spectrum of **1** by the NOEs of the protons H-8α and H-8β with H-9; H-8α ($\delta$ 4.89) and H-6α ($\delta$ 3.24) with the signal of the hydroxy group at C-6a ($\delta$ 2.6-2.9), and H-6β ($\delta$ 2.62) with the *N*-methyl protons at $\delta$ 2.43. The multiplet at $\delta$ 2.80, corresponding to the proton of C-4a, and the signal of H-3 at $\delta$ 4.53 showed their couplings with the protons α and β of C-4 at $\delta$ 2.18 and 1.57 in the COSY spectrum. The stereochemical α position of H-3 and β position of H-4a are confirmed by the NOE between H-3 and H-4α, H-4a and H-4β. In the NOESY spectrum, the proton H-12 also exhibited the NOEs due to the protons H-1, H-4a and H-4β.

The assignments of the chemical shifts and the multiplicities of all the protons of **1** and **2** (see Table 1) demonstrate the similarity of these two compounds. The largest difference found for the chemical shift of H-3 is due to the OH substitution of the methoxy group at C-3, and the resonance of the proton H-3 of **2** is shifted to lower field.

Table 1 also presents the carbon shifts of **1** and **2**, assigned by attached proton test (APT) $^{13}C$ NMR spectra. The assignments of five quaternary C-atoms C-10, C-11, C-8a, C-12a and C-12b have been made by lowest differences to values obtained by the chemical shift calculation program SPECINFO from Chemical Concepts Company, carried on 99,000 $^{13}C$ NMR spectra. They have also been confirmed by the cross-peaks of H-12 with C-8a and C-10, of H-9 with C-11 and C-12a, of H-2 with C-12b and C-4 in the long-range HETCOR $^{1}H$–$^{13}C$ NMR spectrum of **1**. In comparison with the carbon shifts of **1** the resonance of C-4a of **2** is shifted by about 5 ppm to higher field because of the substitution of the methoxy group at C-3 by the hydroxy group in *equatorial* position (Eliel, Bailey, & Kopp, 1975).

The structure of compound **3** (amorphous powder) was established on the basis of MS, UV, one- and two-dimensional $^{1}H$ and $^{13}C$ NMR and the CD spectrum.

The molecular ion $[M + H]^{+}$ ($C_{18}H_{24}NO_5$) at *m/z* 334 was only found in the FAB and CIMS. The EIMS showed this molecular ion with very low intensity

Table 1
Carbon and proton shifts ($CDCl_3$) of tazettine (**1**) and 3-*O*-demethyltazettine (**2**); chemical shifts are in $\delta$-values (ppm) from TMS; coupling constants are in Hz

| Atom | $\delta_C$ (ppm) | | $\delta_H$ (ppm) multiplicity (*J* (Hz)) | |
|---|---|---|---|---|
| | **1** | **2** | **1** | **2** |
| 1 | 128.6 | 128.3 | 5.54 ddd | 5.52 ddd (10.4; 2.1; 1.6) |
| 2 | 130.6 | 133.6 | 6.08 ddd | 5.99 ddd (10.4; 1.8; 1.3) |
| 3α | 72.9 | 70.2 | 4.06 dddd | 4.53 dddd (10.1; 6.1; 2.1; 1.8) |
| 4α | 26.7 | 30.3 | 2.23 dddd | 2.18 dddd (−13.7; 6.1; 3.8; 1.3) |
| 4β | | | 1.56 ddd | 1.57 ddd (−13.7; 10.1; 2.4) |
| 4a | 70.0 | 64.3 | 2.79 m | 2.80 m (3.8; 2.4; 1.6) |
| 6α | 65.6 | 65.5 | 3.24 d | 3.26 d (−10.6) |
| 6β | | | 2.62 d | 2.62 d (−10.6) |
| 6a | 102.1 | 102.0 | | |
| 8α | 62.1 | 62.1 | 4.89 d | 4.89 d (−14.7) |
| 8β | | | 4.58 d | 4.58 d (−14.7) |
| 8a | 125.5 | 125.6 | | |
| 9 | 104.0 | 104.1 | 6.44 s | 6.45 s |
| 10 | 146.4 | 146.4 | | |
| 11 | 146.6 | 146.6 | | |
| 12 | 109.3 | 109.2 | 6.79 s | 6.78 s |
| 12a | 128.0 | 127.9 | | |
| 12b | 49.9 | 49.7 | | |
| -$OCH_2O$- | 100.9 | 101.0 | 5.84; 5.85 AB (1.8) | 5.85; 5.86 AB (1.8) |
| -$OCH_3$ | 56.2 | | 3.40 s | |
| -$NCH_3$ | 41.9 | 41.9 | 2.43 s | 2.34 s |
| 3-OH | | | | 1.5–2.8 |
| 6a-OH | | | 2.6–2.9 | 1.5–2.8 |

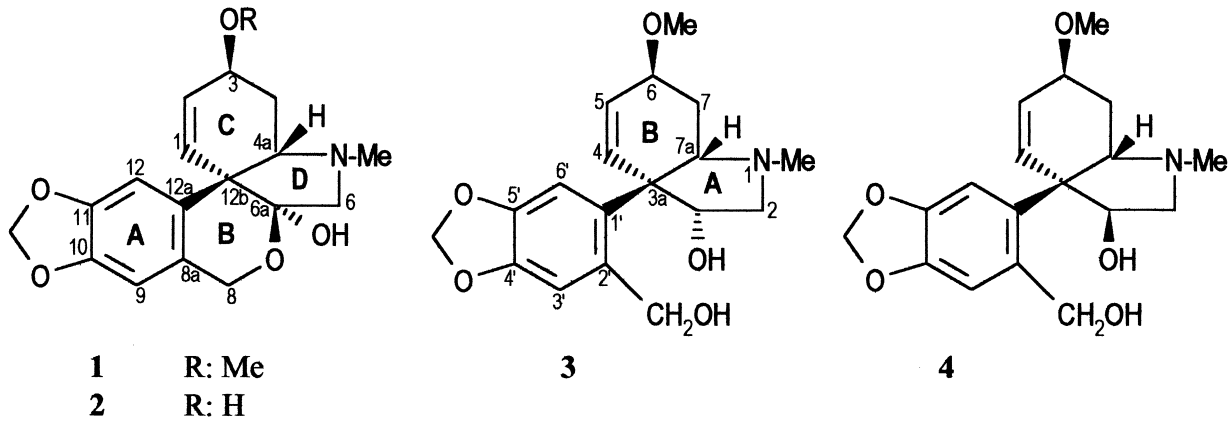


Fig. 1. The structures of tazettine (**1**), 3-*O*-demethyltazettine (**2**), egonine (**3**) and tazettadiol (**4**).

(0.6%) and one other peak at *m/z* 302, which is typical for the loss of the $CH_2OH$ group in the aromatic ring and which has been found in the EIMS of tazettadiol as well as criwellinediol (NIST MS library). The base peak at *m/z* 70 as well as the characteristic peaks at *m/z* 318 and 249 originate from a cleavage reaction of the tazettine structure and indicate the stereochemical β position of the methoxy group at C-6 (Duffield et al., 1965).

The presence of 23 H- and 18 C-atoms of **3** was confirmed by the $^1H$ and $^{13}C$ NMR spectra. The $^1H$ NMR spectrum exhibited two singlets at $\delta$ 6.91 and 6.67 for the protons H-3′ and H-6′ of the 1,2,4,5-tetrasubstituted aromatic ring, an AB-system at $\delta$ 5.87 and 5.86 for two protons of a methylenedioxy group, a multiplet and a double double doublet at $\delta$ 5.92 and 5.73 for the olefinic protons H-5 and H-4, respectively. An AB-system at $\delta$ 4.79 and 4.69 was assigned to the methylene protons at C-7′. A singlet at $\delta$ 3.33 belongs to the protons of the methoxy group on C-6. A multiplet at $\delta$ 2.93, corresponding to the proton of C-7a, showed the couplings with the protons α and β of C-7 at $\delta$ 2.32 and 1.69 in the COSY spectrum. The NOESY spectrum verified the proximity of the olefinic proton H-5 ($\delta$ 5.92) to the other olefinic proton H-4 ($\delta$ 5.73), to the proton H-6α ($\delta$ 3.94), and to the protons of the methoxy group in the α position at C-6 ($\delta$ 3.33), as well as the proximity of the aromatic proton H-6′ at $\delta$ 6.67 to the methylene proton H-7β ($\delta$ 1.69) and to the proton H-7a ($\delta$ 2.93), proving the stereochemical β position of the proton H-7a and the *cis*-3 A:B ring junction of the compound **3** (Figs. 1, Wagner, Pham, & Döpke, 1996). The methylene protons of the primary alcohol at $\delta$ 4.69 and 4.79 exhibited NOEs due to the protons H-4 ($\delta$ 5.73), H-3′ ($\delta$ 6.91) and H-3 ($\delta$ 4.45). The assignments of the protons H-2α, H-2β and H-3β of the ABX system at $\delta$ 3.07, 2.49 and 4.45 were achieved according to the NOESY and the COSY spectra. The COSY spectrum showed a strong coupling of H-3 in the β position at $\delta$ 4.45 with the proton H-2β at $\delta$ 2.49 but no coupling with the proton H-2α. Therefore, the dihedral angle of H-2α and H-3β is proved to be 90°.

The assignments of the $^1H$- as well as $^{13}C$-shifts (Table 2) were confirmed by a HETCOR $^1H$–$^{13}C$ NMR spectrum. The measurement of $^{13}C$ NMR in $CDCl_3/D_2O$ proved the assignments of the OH group at C-3 and OMe group at C-6. Only the signals of C-3 and C-7′ in $CDCl_3/D_2O$ are shifted toward high field (0.018 and 0.09 ppm) by isotopic effect.

The proposed structure of the new alkaloid egonine (**3**), presented in Fig. 2, was also supported by the UV spectrum which exhibits maxima [$\lambda_{max}$: nm (log ε)] at 290.0 (2.835), 242.8 (0.919) and 211.3 (2.835) indicating the presence of a methylenedioxyphenylene chromophore (Wagner et al., 1996).

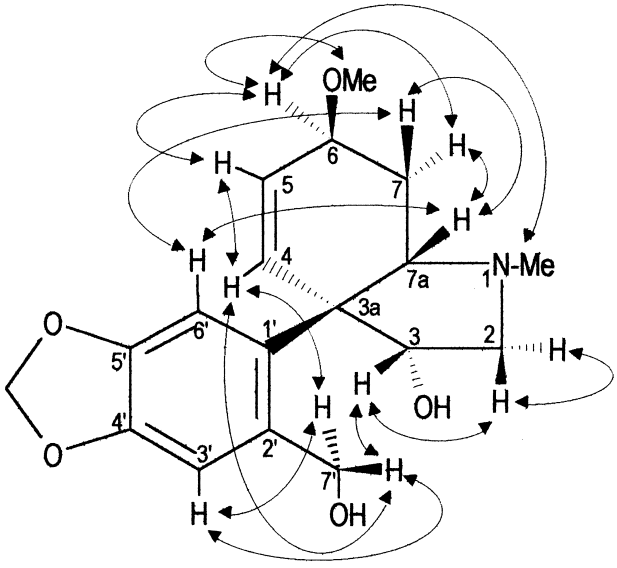


Fig. 2. Structure and NOEs of egonine (**3**).

Table 2
Carbon and proton shifts ($CDCl_3$) of egonine (**3**); chemical shifts are in $\delta$-values (ppm) from TMS; coupling constants are in Hz

| Atom | $\delta_C$ (ppm) | $CH_n$ | $\delta_H$ (ppm) multiplicity ($J$ (Hz)) |
|---|---|---|---|
| 1 | 40.1 | $-NCH_3$ | 2.38s |
| 2α | 64.8 | $CH_2$ | 3.07 d (−10.6) |
| 2β | | | 2.49 m |
| 3β | 78.4 | CH | 4.45 d (5.5) |
| 3a | 55.0 | C | |
| 4 | 130.7 | CH | 5.73 ddd (10.6; 2.1; 1.8) |
| 5 | 129.3 | CH | 5.92 m |
| 6α | 71.3 | CH | 3.94 dddd (10.6; 5.6; 1.8; 1.6) |
| 7α | 27.6 | $CH_2$ | 2.32 m |
| 7β | | | 1.69 ddd (−13.2; 10.6; 2.6) |
| 7a | 69.7 | CH | 2.93 m |
| 1′ | 133.1 | C | |
| 2′ | 136.0 | C | |
| 3′ | 112.0 | CH | 6.91 s |
| 4′ | 147.1 | C | |
| 5′ | 146.3 | C | |
| 6′ | 107.1 | C | 6.67 s |
| 7′ α and β (Ar–$CH_2$OH) | 62.2 | $CH_2$ | 4.69 and 4.79 *AB* (−11.9) |
| -$OCH_2O$- | 101.3 | $CH_2$ | 5.87 and 5.86 *AB* (1.8) |
| -$OCH_3$ | 55.9 | $CH_3$ | 3.33 s |
| 3-OH | | | 3.1–2.3 |
| 7′-OH | | | 3.1–2.3 |

The absolute configurations of **2** and **3** were also established by the similarities of the CD spectra of **1**, **2**, **3** and **4** (Fig. 3). The unique CD pattern of both alkaloids **1** and **2** as well as of **3** and **4** clearly indicates that they belong to the same enantiomeric series possessing a *cis* B:D ring junction (Wagner et al., 1996) for alkaloid **2** and a *cis* A:B ring fusion for alkaloid **3** proving the α position of the hydroxy group at C-6a of **2** and β position of the methylenedioxyphenylene segment at C-3a of **3**.

The CD spectra shown in Fig. 3 also allow some concluding remarks concerning the CD behavior of derivatives of the [2]benzopyrano[3,4-c]-indole alkaloids (**1** and **2**) in comparison with open ring analogues (**3** and **4**). The CD and UV transitions of the open ring derivatives (**3** and **4**) are also determined by the methy-

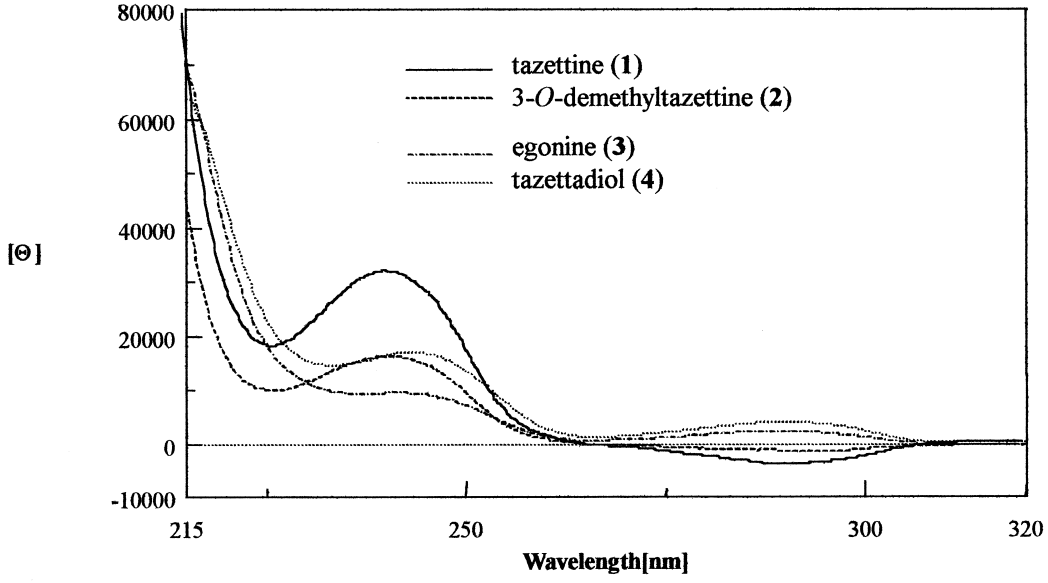


Fig. 3. CD spectra of tezattine (**1**), 3-*O*-demethyltazettine (**2**), egonine (**3**) and tazettadiol (**4**) in methanol.

lendioxyphenylene chromophore like in the case of tazettine (**1**).

The alkaloids **3** and **4** are hindered in their free rotation about the C-3a–C-1 bond because of the voluminous $CH_2OH$-group at the A-ring and OH-group at the B-ring. Therefore, one energetically preferred conformation, obviously similar to the B-ring of tazettine, is assumed. Thus, similarities in the CD spectra of **3**, **4** and tazettine in the region of 225 to 260 nm can also be explained.

The alkaloids **3** and **4** are 3α- and 3β-hydroxy isomers. From the CD results the conclusion might be drawn that the substituents in the B-ring obviously do not influence the shape of the CD spectra significantly.

Egonine possesses the same constitution as the substance named epi-tazettadiol, which was obtained by a reaction described by Kobayashi, Kihara, Shingu, and Shingu (1980). We carried out this reaction in order to prove our result, but instead of the described epitazettadiol we obtained a mixture of two substances ($C_{17}H_{19}NO_3$ and $C_{17}H_{21}NO_4$) of equal $R_f$-value but of quite different structure. Thus, egonine was isolated and identified for the first time from natural origin.

The 3-D structures of the new alkaloids egonine and 3-*O*-demethyl-tazettine (see graphical abstract) were established by energy minimization with the SPARTAN program and on the basis of the NMR and NOE data (Fig. 4). The configuration obtained is also confirmed by the CD investigations.

## 3. Conclusion

Two novel Amaryllidaceae alkaloids 3-*O*-demethyltazettine (**2**) and egonine (**3**) have been isolated from vietnamese *Hippeastrum equestre* Herb. (Amaryllidaceae) and their structures were established by spectroscopic analysis (UV, MS, NMR and CD). With respect to the common rule that Amaryllidaceae alkaloids do not appear in any other plant family, egonine was found to be the third alkaloid of mesembrine-type isolated from amaryllidaceae as an exception.

## 4. Experimental

### 4.1. General

UV: PU 8735 (Philips Analytical, Cambridge, UK); CD: JASCO J-710 (Jasco, Japan), ($d = 0.5$ mm, $c = 0.5$ mg/ml); HRMS (70 eV): Autospec, Fa. Fisons; EIMS (70 eV): HD 5995 A, Fa. Hewlett Packard); NMR spectra: 300/75.5 MHz (Gemini 300, Fa. Varian), 500/125.7 MHz (Unity 500, Fa. Varian), $CDCl_3$; $\delta$: ppm; CC, FCC and TLC: see Döpke et al. (1995b).

### 4.2. Plant material and extraction

For details see Döpke, Pham, Gründemann, Bartoszek, & Flatau (1995b).

### 4.3. Isolation of alkaloids

The crude alkaloid extract (extract B in Döpke, Pham, Gründemann, Bartoszek, & Flatau (1995b)) was subjected to column chromatography on silica gel (40 cm × 3 cm), using *n*-hexane–$CHCl_3$ (2:8, 1:9), $CHCl_3$ and $CHCl_3$–MeOH (99:1, 98:2, 95:5, 9:1, 8:2, 7:3, ...) mixtures of increasing polarity until pure MeOH was used. Frs (50 ml, 200 fractions) were examined by TLC (silica gel). Frs of similar content were combined. Frs 22–108 (*n*-hexane–$CHCl_3$, 2:8, 1:9 and $CHCl_3$) yielded a large quantity of tazettine (816 mg, crystallized from acetone). Frs 112–141 ($CHCl_3$–MeOH, 99:1, 98:2, 223 mg) were separated by CC on silica gel (20 cm × 2.1 cm) eluting with $CHCl_3$, $CHCl_3$–MeOH (99:1, 98:2, 97:3 and 9:1) to give tazettine, hip-

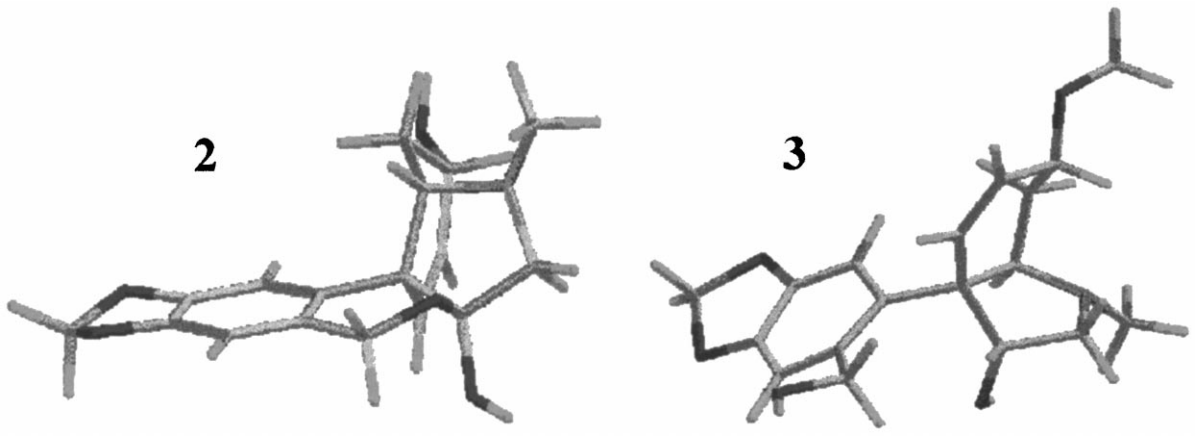


Fig. 4. Energy-minimized 3D structures of 3-*O*-demethylazettine (**2**) and egonine (**3**).

peastrine, lycorine and 14 mg of egonine (frs 30–42, $CHCl_3$–MeOH, 99:1, frs 8–11, *n*-hexane–acetone, 7:3). Frs 157–162 ($CHCl_3$–MeOH, 95:5, 800 mg) were separated by CC on silica gel (20 cm × 2.1 cm) eluting with *n*-hexane–acetone (8:6, 7:3 and 1:1) to give 13 mg of 3-*O*-demethyl-tazettine (frs 38–49, *n*-hexane–acetone, 7:3, crystallized from acetone).

*4.3.1. Egonine*

$[\alpha]_D^{22}$ + 86° (*c* 1.0, MeOH). HRMS: $[M + H]^+$ found: 334.1708; $C_{18}H_{24}NO_5$ requires: 334.1655. EIMS: [70 eV, *m*/*z* (rel. int.)]: 334 [M + H] (0.7), 318 (0.9), 303 (3.4), 302 (15), 249 (4.3), 71 (15), 70 (100). CD (MeOH, $[\theta]_\lambda$): $[\theta]_{213.4}$ +74650; $[\theta]_{236.6}$ +8955; $[\theta]_{240.4}$ +9170; $[\theta]_{265.0}$ +459; $[\theta]_{290.4}$ +2145.

*4.3.2. Tazettine*

Mp, $[\alpha]_D$, MS: see Döpke, Pham, Gründemann, Bartoszek, & Flatau (1995b). CD (MeOH, $[\theta]_\lambda$): $[\theta]_{207.2}$ +194500; $[\theta]_{240.4}$ +32600; $[\theta]_{271}$ 0; $[\theta]_{289.2}$ −3090.

*4.3.3. 3-O-demethyltazettine*

Mp 179–181°. $[\alpha]_D^{22}$ + 85° (MeOH, *c* 1.0). HRMS: $[M]^+$ found: 317.12697; $C_{17}H_{19}NO_5$ requires: 317.12632. EIMS, *m*/*z* (rel. int.): 317 $[M]^+$ (24), 298 (1.9), 247 (100), 230 (12), 71 (42), 70 (52). CD (MeOH, $[\theta]_\lambda$): $[\theta]_{207.2}$ +124180; $[\theta]_{226.4}$ +9530; $[\theta]_{240.0}$ +15860; $[\theta]_{262.6}$ 0; $[\theta]_{295.0}$ −1760.

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