HETEROCYCLES, Vol. 55, No.4, 2001, pp. 641 - 652, Received, 30th, November, 2000

FOUR NEW AMARYLLIDACEAE ALKALOIDS FROM *GALANTHUS GRACILIS* AND *GALANTHUS PLICATUS* SUBSP. *BYZANTINUS*

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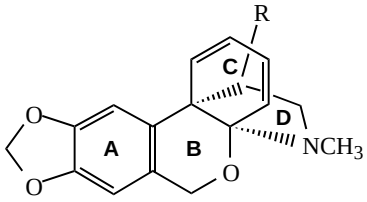
Abstract - (+)-3-Epi-3,4-dihydro-3-hydroxygraciline (**5**), isolated from *Galanthus gracilis* of Turkish origin, is a new member of the gracilines, a recently established subgroup of the Amaryllidaceae alkaloids. *G. plicatus* subsp. *byzantinus* has furnished three new alkaloids, namely (+)-plicane (**12**), which is a new plicamine-type alkaloid, along with (+)-3-*O*-(3-hydroxybutyryl)tazettinol (**13**), and *N*-formylismine (**15**).

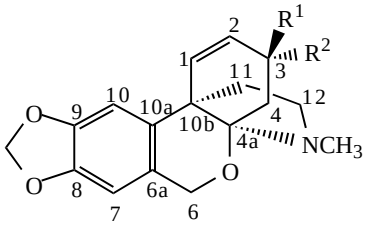
INTRODUCTION

Recently, *Galanthus gracilis* of Turkish origin yielded three novel alkaloids, (+)-graciline (**1**), (+)-3,4-dihydro-3-hydroxygraciline (**2**) and an analogous dimeric compound, (-)-digracine (**3**), while another *Galanthus* species, *G. plicatus* subsp. *byzantinus* afforded (+)-11-acetoxygraciline (**4**), all of which contain an incorporated 10b,4a-ethanoiminodibenzo[*b,d*]pyrane skeleton, thus establishing a new subgroup for the Amaryllidaceae alkaloids.[1] The latter plant had been shown also to elaborate three tazettine-type alkaloids, (+)-tazettine (**6**),[2] (+)-3-epimacronine (**7**),[2] and (+)-3-*O*-demethylepimacronine (**8**)[3] along with a simple phenanthridine derivative, ismine (**9**).[2] A following study has reported the isolation of two novel dinitrogenous compounds, (+)-plicamine (**10**) and (-)-secoplicamine (**11**), which are the first representatives of another new subgroup of the Amaryllidaceae alkaloids.[4] Our ongoing investigations on the chemical constituents of the above-mentioned *Galanthus* species have now

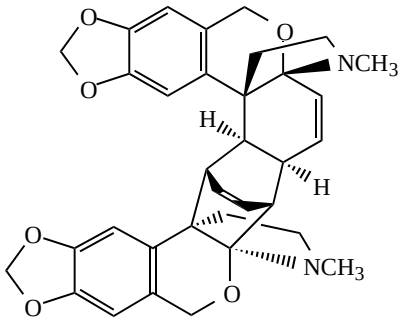
resulted in the isolation and characterization of four new alkaloids, (+)-3-epi-3,4-dihydro-3-hydroxygraciline (**5**), (+)-plicane (**12**), (+)-3-*O*-(3-hydroxybutyryl)tazettinol (**13**), and *N*-formylismine (**15**).



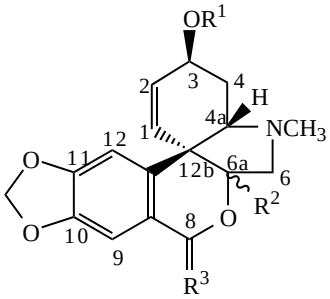
1 R = H
4 R = $OCOCH_3$



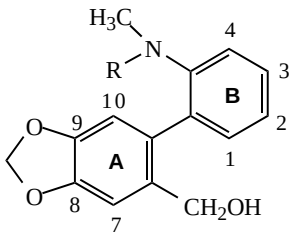
2 R^1= OH, R^2= H
5 R^1= H, R^2= OH



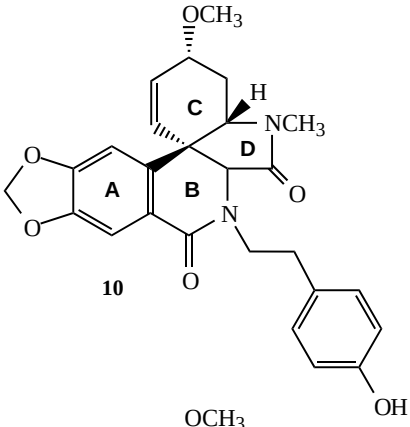
3



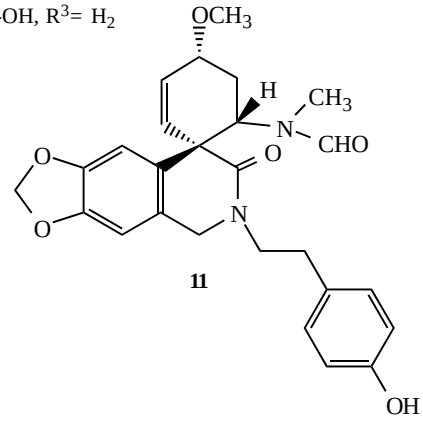
6 R^1= CH_3, R^2= α-OH, R^3= H_2
7 R^1= CH_3, R^2= β-H, R^3= O
8 R^1= H, R^2= β–H, R^3= O
13 R^1= $COCH_2CHOHCH_3$, R^2= α-OH, R^3= H_2
14 R^1= H, R^2= α-OH, R^3= H_2



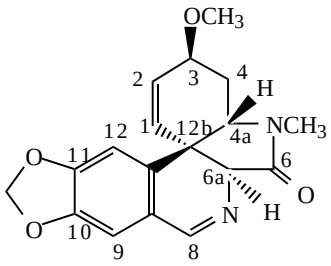
9 R = H
15 R = CHO



10



11



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RESULTS AND DISCUSSION

The 600 MHz ^{1}H-NMR spectrum of the new compound (**5**), $C_{17}H_{19}NO_4$, isolated from *G. gracilis*, accounts for the presence of two aromatic hydrogens (δ 6.88 and 6.43), two olefinic protons (δ 5.90 and 5.61), a methylenedioxy substituent (δ 5.87) and an *N*-methyl group at δ 2.36. The signals in the aliphatic region belong to an isolated deshielded methylene (δ 4.41 and 4.70), one methine hydrogen

(δ 4.26), two methylene protons (δ 2.95 and 2.73) and a shielded hydrogen of a geminal pair (δ 2.13 and 1.68). A multiplet at δ 211-2.21 accounts for further three hydrogens.

The key features of the ^{13}C-NMR spectrum are the signals at δ 47.0 and 94.5 for two quaternary carbons, hinting at the presence of a 10b,4a-ethanoiminodibenzo[*b,d*]pyrane structure, a unique framework first exemplified by four novel alkaloids isolated from two different *Galanthus* species.[1]

The evaluation of the detailed 2D NMR experiments (^{1}H-^{1}H DQF COSY, TOCSY, HSQC, HMBC) not only confirm the expected analogy to the previously encountered framework of the gracilines, but also reveal that the structural details are identical with those of (+)-3,4-dihydro-3-hydroxygraciline (**2**).[1] However, even upon a visual comparison of the ^{1}H-NMR spectra of **5** and **2**, obvious differences are detected, pointing to a possible stereoisomerism. Noteworthy of the ^{1}H-NMR spectrum of **2** were the relatively unresolved, broad signals for almost all of the hydrogens, postulated to be a consequence of conformational flexibility in solution.[1] In contrast, the ^{1}H-NMR spectrum of **5** can easily be described as having well-resolved signals with fine and interpretable couplings. Moreover, the chemical shifts of the corresponding hydrogens of **5** and **2** are noticeably different.

The CD curve of **5** closely resembles that of the other known gracilines,[1] suggesting the same absolute configuration at 4a and 10b both as (*R*). For the determination of the configuration at C-3, the site of differentiation between the two molecules (**5**) and (**2)**, a detailed NOESY experiment was undertaken, in which no interaction is detected between H-4β and H-6β, opposed to that of **2**, where these two hydrogens share a substantial cross signal. Moreover, H-3 of **5** is interrelated spatially neither to H-11, nor to H-12, whereas strong interactions are observed for the corresponding hydrogens of compound (**2)**. Since a H-3α configuration has been established for **2**,[1] compound (**5)** must have the reverse configuration at C-3. Application of this information on Dreiding models suggests a probable intramolecular hydrogen bonding between the nitrogen and hydroxyl group positioned at C-3, providing conformational stability to compound (**5)**, which consequently results in better resolution of the ^{1}H-NMR signals.

Like that of its epimeric counterpart, **5** has a simple EIMS, where the base peak is found at *m/z* 225,

representing the stable cation formed by the facile expulsion of $CH_3CH_2NCH_3$ and water from the molecular ion. The only observable differences between the spectra of these two epimeric compounds rest in the relative intensities of some peaks.[1]

The isolation of (+)-3-epi-3,4-dihydro-3-hydroxygraciline (**5**) from *G. gracilis* not only expands the recently established subgroup of the Amaryllidaceae alkaloids, the gracilines, but also suggests that in the biosynthetic pathway leading to these epimeric compounds, the formation of the secondary alcohol at position 3 probably proceeds in a nonstereospecific manner.

The ESI-MS spectrum of the second new compound, (+)-plicane (**12**), $C_{18}H_{18}N_2O_4$, isolated from *G. plicatus* subsp. *byzantinus* furnishes an $[M+H]^+$ peak at *m/z* 327, suggesting that it may be a dinitrogenous alkaloid of the plicamine series, another newly established subgroup of the Amaryllidaceae alkaloids named after the prototypic compound, (+)-plicamine (**10**), obtained from the same plant.[4]

The ^{1}H-NMR spectrum of **12** , the relevant signals for two aromatic (δ 6.93 and 6.83) and two olefinic hydrogens (δ 6.13 and 5.49) , a methylenedioxy substituent (δ 6.02 and 6.01) and an aliphatic methoxyl group (δ 3.45) are the usual features encountered in the ^{1}H-NMR spectra of many Amaryllidaceae alkaloids. Of the aliphatic hydrogens, two appear upfield as a geminal pair (δ 2.22 and 1.91), whereas the others are methine hydrogens resonating at relatively high frequencies (δ 4.25, 3.78 and 3.65). Of interest is the relatively downfield chemical shift of the *N*-methyl signal at δ 2.89, as was also the case with that of (+)-plicamine (**10**).[4] Even more noteworthy, however, is the one-proton singlet at δ 8.21, which is correlated by an HSQC experiment to a carbon resonating at δ 158.1. In the HMBC experiment, the prominent $^3J_{CH}$ correlations both from H-9 (δ 6.83) and from H-6a (δ 4.25) to this carbon clearly fixes the position of the latter at position 8. Thus, the hydrogen resonating at δ 8.21 is a benzylic methine, flanked further downfield by the neighboring nitrogen.

The carbon chemical shifts in the ^{13}C-NMR spectrum of **12** display marked resemblance to those of the prototypic compound, (+)-plicamine (**10**). Immediately noticeable characteristic signals at δ 40.6 and 171.8 belong to quaternary carbons, the former confirming the presence of a spiro center and the latter

of a carbonyl function, further verified by a strong 1695 cm^{-1} absorption in its IR spectrum. A thorough evaluation of the 2D NMR experiments (^{1}H,^{1}H DQF COSY, TOCSY, HSQC, HMBC) indicate that **12** is indeed an alkaloid of the plicamine subgroup, where position 7 of the basic tazettine skeleton is occupied by a nitrogen instead of an oxygen atom. As deduced from the HMBC experiment, ring D is a five-membered lactam, where the $^3J_{CH}$ correlations of the *N*-methyl hydrogens to C-4a (δ 62.4) and to the carbonyl function (δ 171.8) place the latter at C-6. The relatively downfield shift of the *N*-methyl signal (δ 2.89) is, therefore, easily explainable by the lactam nature of ring D.

The CD spectrum of **12** resembles closely that of (+)-plicamine (**10**), thus suggesting a (12b*S*) configuration. The configuration at C-3 is deduced from the lack of an observable coupling between H-2 (δ 6.13) and H-3 (δ 3.78) in the ^{1}H-NMR spectrum, which indicates a dihedral angle of about 90^o between these two hydrogens.[5] This is possible only when H-3 is α oriented, thus indicating the (3*S*) configuration. Considering the (3*R*) configuration of the previously encountered analogous compounds, (**10** and **11)**, the (3*S*) configuration of **12** suggests that the alkaloids of the plicamine subgroup elaborate both configurations at C-3.

The majority of the signals observed in the ^{1}H- and ^{13}C-NMR spectra of our third new compound (**13)**, $C_{21}H_{25}NO_7$, isolated from *G. plicatus* subsp. *byzantinus*, points at its close structural resemblance to that of (+)-tazettine (**6**), the latter being a well-known alkaloid isolated in good yield from the same plant.[2] The 1D and 2D NMR spectra (^{1}H, ^{1}H DQF COSY, TOCSY, HSQC, HMBC) provide sound evidence for the structure of **13** as having the basic tazettine framework. For example, informative features in the ^{13}C-NMR spectrum are the resonances at δ 101.6 and 49.6 for C-6a and the spiro center at C-12b, respectively. In the ^{1}H-NMR spectrum, many of the signals also compare closely to those of (+)-tazettine (**6**). However, alerting differences are the presence of additional signals in the aliphatic region of the spectrum, the lack of a signal for an aliphatic methoxyl group, and the relatively downfield chemical shift of H-3 at δ 5.73, as compared to that of (+)-tazettine resonating at δ 4.13.[2] The magnitude of difference observed at the latter case suggests an *O*-acylation at C-3.[6]

In the ^{13}C-NMR spectrum, the additional signals at δ 22.4, 43.0 and 64.3, along with a downfield signal at δ 172.6 throw light into the nature of the acyl group, the presence of which is also supported by the

1715 cm^{-1} carbonyl absorption in its IR spectrum. In the ^{1}H-NMR spectrum, the signals observed at δ 1.25 as a doublet (*J* 6.3 Hz, 3H), at δ 2.48 and 2.57 as two doublet of doublets representing geminal hydrogens (J_{gem} 16.5 Hz), and at δ 4.24 as a multiplet accounting for a methine proton corroborate the presence of a butyryl moiety. A HSQC experiment correlates the methyl hydrogens with the carbon resonating at δ 22.4, the following two geminal hydrogens with the one at δ 43.0 and the methine hydrogen with the carbon chemical shift at δ 64.3. The ^{1}H,^{1}H DQF COSY and TOCSY experiments interrelate these protons only among themselves, but with no other hydrogens of the main tazettine structure. The fact that the methyl and the methylene both couple to the downfield methine at δ 4.24, but not to each other provides a further clue to their sequence on this chain. Furthermore, in the HMBC experiment, the methylene and the methine hydrogens are connected to the carbonyl function resonating at δ 172.6 through the $^2J_{CH}$ and $^3J_{CH}$ correlations. The foregoing data firmly establishes that compound **13** is tazettinol (**14**)[7,8] esterified at the secondary alcohol at C-3 with 3-hydroxybutyric acid.

In the EIMS of **13**, a molecular ion observed at *m/z* 403 (rel. int. 90%) is in accordance with the molecular weight. As expected, the base peak is at *m/z* 316 ($[M-87]^+$), corresponding to the ion formed by the loss of the hydroxybutyryl moiety.

The CD spectrum of **13** bears a great similarity to that of (+)-tazettine (**6**), thus suggesting a (12b*S*) configuration. The α orientation of H-3, as is the case with (+)-tazettine, is deduced from the small magnitude coupling constant (*J* 0.8 Hz) between H-2 and H-3.[5] The reciprocating NOESY correlations between H-12 (δ 6.82) and H-4a (δ 2.96) ascribe a β-configuration to the latter. H-4a, in turn, interacts with the H-6 resonating at δ 2.77, which allows the assignment of orientations of the methylene hydrogens at position 6. The mutual spatial correlations between H-6β (δ 2.77) and the *N*-methyl hydrogens establish the orientation of the latter as projecting behind the mean plane of the molecule. Moreover, the NOESY interactions among H-12 (δ 6.82), H-4a (δ 2.96) and one of the methylene hydrogens resonating at δ 1.75, interpreted on Dreiding models, suggest a chair-like conformation for ring C.

The ESI-MS of the last new compound (**15)** $C_{16}H_{15}NO_4$ ($M^{+\bullet}$ at *m/z* 285) thus suggesting that we are dealing with a relatively simple monomeric structure. Interestingly, however, the ^{1}H-NMR spectrum

displays a doubling of most of the signals in the ratio of 3:1, pointing to two configurations at the amide.

The high frequency region of the ^{1}H-NMR spectrum of **15** displays signals for six aromatic hydrogens, two as singlets and four with relevant couplings for a 1,2-disubstituted benzene ring, and a methylenedioxy substituent. The signals of the aliphatic region reveal the presence of an *N*-methyl group, along with an isolated methylene, the downfield chemical shifts and couplings of which are in accordance with the hydrogens of a benzylic primary alcohol. This spectral evidence suggests that compound **15** is an analog of ismine (**9**).[2] A comparison of the ^{1}H-NMR data of **15** and **9** confirms that the majority of the corresponding chemical shifts are indeed very similar, except for the noticeably downfield chemical shifts of the *N*-methyl group (δ 2.92 and 3.18 for the major and minor isomers, respectively) as compared to that of ismine at δ 2.71.[2] Moreover, the presence of another interesting downfield resonance at δ 8.13 for the major and δ 7.94 for the minor isomers focus our attention to the substitution at C-4a as the site of diversion from the ismine structure. An HSQC experiment correlates this deshielded hydrogen to the quaternary carbon (δ 163.2 for the major and δ 162.5 for the minor isomers), indicating the presence of an aldehyde, a fact further verified by the 1720 cm^{-1} absorption in its IR spectrum. Prominent $^{3}J_{CH}$ interactions between the relevant hydrogens and carbons clearly establish the (*N*-formyl-*N*-methyl)amino substitution at C-4a.

As expected, the 2,2'-disubstitution forces the rings out of coplanarity, therefore, resulting in a drastically diminished resonance interaction between the two aryl moieties. This is clearly reflected in the absence of the K-band in the UV spectrum of **15**.

The NOESY experiment allows the differentiation between the spatial arrangements of the two isomers. The *N*-methyl hydrogens of the major isomer display a prominent spatial interaction with H-10, while the formyl hydrogen interacts as strongly with the benzylic methylene hydrogens. On the other hand, the formyl hydrogen of the minor isomer interacts neither with H-10, nor with the benzylic hydrogens. Application of the NOESY information on Dreiding models suggests that the rings are at about a 60^{o} angle to each other. In the major isomer, the substituted amino group at C-4a is in front of the perpendicular plane formed by ring A, and behind it in the minor isomer. Thus **15** is a mixture of

atropisomers.

The present report on the minor alkaloids of two different *Galanthus* species proves that the Amaryllidaceae plants comprise a rich repository for alkaloids with intriguing structural diversions. The structure elucidation of these minor alkaloids may in time throw light upon the biosynthetic pathway of the major alkaloids, some of which are already acknowledged as potential remedies in some threatening diseases.[9-11]

EXPERIMENTAL

General Methods

Optical rotations: Perkin-Elmer 241 Polarimeter; UV: Perkin-Elmer UV/VIS/NIR Lambda 19 Spectrophotometer; IR: Perkin-Elmer 297 Infrared Spectrophotometer; ^{1}H-NMR, ^{13}C-NMR, ^{1}H,^{1}H DQF-COSY, gs-HSQC, gs-HMBC, TOCSY, NOESY: Bruker DRX-500 and Bruker AMX-600 Spectrometers at 300 K; EI-MS: Finnigan MAT SSQ 700; ESI-MS: Finnigan MAT TSQ 700; CD: Jasco J-715 Spectropolarimeter.

Plant material

Galanthus gracilis Celak and *G. plicatus* Bieb. subsp. *byzantinus* (Baker) D. A. Webb were collected in 1995.[1]

Extraction and isolation

Dried and powdered total plant material of *G. gracilis* (5.25 kg) was extracted with EtOH (100 L) for 40 h at rt. The crude extract (681 g) thus obtained was dissolved in 2% aqueous HCl and filtered. The acidic filtrate was made alkaline with 10% aqueous NH_4OH and then extracted with $CHCl_3$. Evaporation of the organic solvent furnished the crude basic extract (9.58 g), which was fractionated by CC (silica gel, 70-230 Mesh, Merck, 380 g) using $CHCl_3$ gradually enriched with MeOH as the eluent. 500 mL fractions were collected. Combined fractions 4-9 (0.339 g) eluted with $CHCl_3$ were

refractionated by preparative CC (silica gel H, type 60, Merck, 20 g) with toluene/MeOH (50:1). Of the 30 mL fractions taken, fraction 7 (13 mg) was further purified by two consecutive preparative TLC procedures on ready-made plates (silica gel, 0.50 mm, Merck), with development first with toluene/ $CHCl_3$/Me_2CO (13:5:2) and then with toluene/Me_2CO (4:1) yielded 3.5 mg of pure **5**.

Dried and powdered plant material (3.7 kg) of *G. plicatus* subsp. *byzantinu*s was extracted with EtOH (80 L) at rt to furnish the crude alcoholic extract (425 g), which was then dissolved in 2% aqueous HCl and filtered. The acidic solution was basified with 10% aqueous NH_4OH and extracted with $CHCl_3$, the evaporation of which afforded the crude basic extract (10.28 g). The preliminary CC fractionation (silica gel, 70-230 Mesh, Merck, 410 g) was performed by gradient elution with increasing amounts of MeOH in $CHCl_3$; 600 mL fractions were collected. Fraction 6-16 (0.436 g), eluted with $CHCl_3$, was subjected to preparative CC (silica gel H, type 60, Merck, 25 g) using hexane/EtOAc (7:3); 30 mL fractions were taken. Fraction 27-29 (26.1 mg), further purifed by preparative TLC on silica gel plates (0.50 mm, Merck) using $CHCl_3$ for development, afforded 19.3 mg of **15** as a colorless, amorphous powder. Combined fractions 17-22 (0.434 g) of the preliminary CC, eluted with 1% MeOH in $CHCl_3$, were refractionated by preparative CC (silica gel H, type 60, Merck, 25 g). During elution with C_6H_6/EtOAc/EtOH (16:3:1), 20 mL factions were collected. Fraction 24-27 (17.5 mg), purified by preparative TLC on ready-made silica gel plates (0.50 mm, Merck) using C_6H_6/$CHCl_3$/Et_2NH (6:3:1) afforded 5.3 mg of pure **12**. Fraction 40-49 (0.353 g) of the preliminary column, eluted with 2.5% MeOH in $CHCl_3$, was subjected to preparative CC (silica gel H, type 60, Merck, 20 g) using C_6H_6/$CHCl_3$/MeOH/NH_4OH (25%) (7:2:1:0.5) (20 mL fractions). Fraction 5 (46.6 mg) was purified by two consecutive preparative TLC [solvent 1= C_6H_6/$CHCl_3$/MeOH/ Et_2NH (11:6:1:2); solvent 2= C_6H_6/$CHCl_3$/MeOH (12:7:1; saturated with NH_3 vapor)] on silica gel plates (0.50 mm, Merck) to furnish 10.7 mg of **13**.

(+)-3-Epi-3,4-dihydro-3-hydroxygraciline (5): Colorless amorphous solid; $[\alpha]_D$ + 110.6° (c 0.15, MeOH). UV λ_{max} (MeOH) nm (log ε) 215 (3.81), 233 sh (3.56), 292 (3.62). CD (MeOH) nm (log ε) 326 (0), 275 (+3.31), 254 (+2.03), 227 (+5.12), negative tail beyond 227 nm. IR ν_{max} (KBr) 3422, 2927, 1502, 1484, 1385, 1356, 1234, 1154, 1095, 1038, 935, 861 cm^{-1}. ^{1}H-NMR (600 MHz, CD_3OD) δ 6.88 (1H, s, H-10) , 6.43 (1H, s, H-7), 5.90 (1H, dd, *J*= 10.1, 1.4 Hz, H-1), 5.87 (2H, s, OCH_2O), 5.61 (1H,

dd, *J*= 10.2, 2.2 Hz, H-2), 4.70 (1H, d, *J*= 14.4 Hz, H-6α), 4.41 (1H, d, *J*= 14.4 Hz, H-6β), 4.26 (1H, ddd, *J*= 10.0, 5.3, 2.4 Hz, H-3), 2.95 (1H, td, *J*= 9.1, 5.8 Hz, H-12), 2.73 (1H, m, H-12), 2.36 (3H, s, NCH_3), 2.21-2.11 (2H, m, H-11), 2.13 (1H, dd, *J*= 12.9, 4.8 Hz, H-4α), 1.68 (1H, dd, *J*= 12.9, 8.2 Hz, H-4β). ^{13}C-NMR (150 MHz, CD_3OD) δ 33.4 (NCH_3), 34.0 (C-4), 38.8 (C-11), 47.0 (C-10b), 50.5 (C-12), 62.5 (C-6), 66.5 (C-3), 94.5 (C-4a), 102.3 (OCH_2O), 104.6 (C-7), 108.4 (C-10), 127.3 (C-6a), 128.8 (C-2), 134.5 (C-10a), 135.9 (C-1), 147.2 (C-8), 148.5 (C-9). EIMS *m/z* (rel. int.) 301 ($M^{+\bullet}$, 25), 284 (11), 244 (11), 243 (10), 226 (28), 225 (100), 178 (10), 139 (10), 133 (10), 128 (10), 115 (10), 58 (11), 57 (20).

(+)-Plicane (12): Colorless amorphous solid; $[\alpha]_D$ + 205.9° (c 0.117, MeOH). UV λ_{max} (MeOH) nm (log ε) 231 (4.30), 285 (3.35), 319 (3.18). CD (MeOH) nm (log ε) 350 (0), 323 (+0.98), 300 (-0.08), 293 (+0.09), 291 (0), 273 (-1.43), 257 (0), 238 (+30.19), 221 (+9.63), 212 (+12.53), negative tail beyond 212 nm. IR ν_{max} ($CHCl_3$) 3450, 2920, 2850, 1695, 1645, 1600, 1485, 1380, 1265, 1215, 1160, 1090, 1040, 935 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.21 (1H, s, H-8), 6.93 (1H, s, H-12), 6.83 (1H, s, H-9), 6.13 (1H, d, *J*= 10.1 Hz, H-2), 6.02 and 6.01 (2H, 2s, OCH_2O), 5.49 (1H, d, *J*= 10.1 Hz, H-1), 4.25 (1H, br s, H-6a), 3.78 (1H, m, H-3), 3.65 (1H, m, H-4a), 3.45 (3H, s, OCH_3), 2.89 (3H, s, NCH_3), 2.22 (1H, dt, *J*= 14.1, 5.2, H-4β), 1.91 (1H, ddd, *J*= 14.1, 9.4, 2.6 Hz, H-4α). ^{13}C-NMR (150 MHz, $CDCl_3$) δ 26.7 (C-4), 28.1 (NCH_3), 40.6 (C-12b), 56.4 (OCH_3), 62.4 (C-4a), 68.0 (C-6a), 70.9 (C-3), 101.9 (OCH_2O), 108.5 (C-12), 108.8 (C-9), 120.1 (C-8a), 129.6 (C-2), 130.5 (C-1), 130.9 (C-12a), 147.6 (C-10), 150.8 (C-11), 158.1 (C-8), 171.8 (C-6). ESI-MS *m/z* 327 $[M+H]^+$.

(+)-3-*O*-(3-Hydroxybutyryl)tazettinol (13): Colorless amorphous solid; $[\alpha]_D$ + 63.5^0 (c 0.22, MeOH). UV λ_{max} (MeOH) nm (log ε) 215 (4.12), 234 (3.87), 292 (3.60). CD (MeOH) nm (log ε) 309 sh (-0.37), 292 (-1.00), 265 (0), 241 (+7.93), 226 (+3.96), 221 (+4.52), negative tail beyond 221 nm. IR λ_{max} ($CHCl_3$) 3580, 2940, 2860, 1715, 1500, 1485, 1375, 1355, 1310, 1245, 1185, 1100, 1055, 1040, 1010, 885 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.82 (1H, s, H-12), 6.52 (1H, s, H-9), 5.98 (1H, dd *J*= 10.8, 0.8 Hz, H-2), 5.93 (2H, s, OCH_2O), 5.739 (1H, br d *J*≈ 10.4, 1.2 Hz, H-1), 5.737 (1H, H-3 obscured by H-1 signal), 4.99 (1H, d, *J*= 14.8 Hz, H-8), 4.65 (1H, d, *J*= 14.8 Hz, H-8), 4.24 (1H, m, H-3'), 3.40 (1H, d, *J*= 10.8 Hz, H-6α), 2.96 (1H, br s, H-4a), 2.77 (1H, d, *J*= 10.8 Hz, H-6β), 2.57 (1H, dd, *J*= 16.5, 3.3

Hz, H-2'), 2.48 (1H, dd, *J*= 16.5, 8.5 Hz, H-2'), 2.47 (3H, s, NCH_3), 2.31 (1H, m, H-4eq), 1.75 (1H, m, H-4ax), 1.25 (3H, d, *J*= 6.3 Hz, H-4'). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 22.4 (C-4'), 25.9 (C-4), 41.9 (NCH_3), 43.0 (C-2'), 49.6 (C-12b), 62.0 (C-8), 64.3 (C-3'), 65.2 (C-6), 68.2 (C-3), 69.8 (C-4a), 101.0 (OCH_2O), 101.6 (C-6a), 104.2 (C-9), 109.1 (C-12), 125.7 (C-8a), 126.9 (C-12a), 129.1 (C-2), 130.1 (C-1), 146.6 (C-10 and C-11), 172.6 (C-1'). EIMS *m/z* (rel. int.) 403 ($M^{+\bullet}$, 90), 317 (19), 316 (100), 300 (20), 299 (12), 298 (53), 247 (22), 238 (14), 230 (11), 227 (10), 211 (17), 197 (11), 181 (29), 153 (12). ESI-MS *m/z* 404 $[M+H]^+$.

***N*-Formylismine (15):** Colorless amorphous solid; UV λ_{max} (MeOH) nm (log ε) 208 (4.64), 292 (3.71). IR ν_{max} (KBr) 3350, 2980, 1720, 1670, 1505, 1485, 1450, 1370, 1215, 1165, 1105, 1080, 1040, 1005, 935 cm^{-1}. ^{1}H-NMR of major isomer (600 MHz, $CDCl_3$) δ 8.13 (1H, s, CHO), 7.43 (1H, td, *J*= 7.6, 1.6 Hz, H-3), 7.37 (1H, td, *J*= 7.5, 1.3 Hz, H-2), 7.31 (1H, dd, *J*= 7.5, 1.5 Hz, H-1), 7.21 (1H, dd, *J*= 7.8, 1.0 Hz, H-4), 7.03 (1H, s, H-7), 6.55 (1H, s, H-10), 5.99 (2H, s, OCH_2O), 4.33 (1H, d, *J*= 12.4 Hz, H-6), 4.30 (1H, d, *J*= 12.4 Hz, H-6), 2.92 (3H, s, NCH_3). ^{1}H-NMR of minor isomer (600 MHz, $CDCl_3$) δ 7.94 (1H, s, CHO), 7.44 (1H, td, *J*= 7.6, 1.5 Hz, H-3), 7.37 (1H, td, *J*= 7.5, 1.3 Hz, H-2), 7.31 (1H, dd, *J*= 7.5, 1.5 Hz, H-1), 7.24 (1H, dd, *J*= 7.5, 1.5 Hz, H-4), 7.03 (1H, s, H-7), 6.55 (1H, s, H-10), 5.97 (2H, s, OCH_2O), 4.45 (1H, d, *J*= 12.2 Hz, H-6), 4.23 (1H, d, *J*= 12.2 Hz, H-6), 3.18 (3H, s, NCH_3). ^{13}C-NMR of major isomer (150 MHz, $CDCl_3$) δ 33.2 (NCH_3), 62.5 (C-6), 101.4 (OCH_2O), 108.8 (C-7), 109.7 (C-10), 127.0 (C-4), 127.7 (C-2), 129.0 (C-3), 130.5 (C-10a), 132.1(C-1), 132.1 (C-6a), 137.4 (C-10b), 140.5 (C-4a), 147.1 (C-9), 147.9 (C-8), 163.2 (CHO). ^{13}C-NMR of minor isomer (150 MHz, $CDCl_3$) δ 38.3 (NCH_3), 62.3 (C-6), 101.2 (OCH_2O), 108.5 (C-7), 110.0 (C-10), 128.0 (C-4, C-2), 129.0 (C-3), 130.8 (C-10a), 132.4 (C-1), 132.4 (C-6a), 138.7 (C-4a), 139.0 (C-10b), 146.4 (C-9), 147.6 (C-8), 162.5 (CHO). ESI-MS *m/z* 286 $[M+H]^+$. CI-MS (NH_3) 303 (18, $[M + NH_4]^+$) 286 (24, $[M+H]^+$), 268 (100), 258 (16), 240 (34).

ACKNOWLEDGEMENTS

The authors are grateful for the financial support of the Swiss National Science Foundation, of the Turkish Scientific and Technical Research Organization (TÜBITAK) (Grant Numbers SBAG-2194 and SBAG-AYD-263) and of the Ege University Research Fund. Special thanks are extended to Dr. G.

Rentsch and N. Walch for the NMR service and to Dr. K. Drandarov for recording the CD spectra.

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