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A new alkaloid from *Narcissus serotinus* L.

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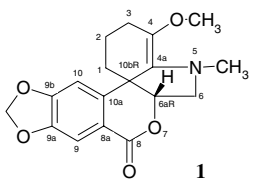
A new alkaloid derivative of [2]benzopyrano-[3,4-c] indole, isomer of 3-epimacronine, 4-methoxy-5-methyl-1,2,3,5,6,6αR-hexahydro-[1,3]dioxolo[4′,5′:6,7]isochromeno [3,4-*c*]indol-8-one, has been isolated from *Narcissus serotinus* L. (Amaryllidaceae) and its structure was elucidated by mass and spectral analysis.

1. Introduction

In Greece the genus *Narcissus* is represented by the species *N. tazetta* L., *N. poeticus* L. and *N. serotinus* L. (Tutin et al. 1980). An alkaloid of the pretazettine-type, an isomer of 3-epimacronine (Viladomat et al. 1990) which differs in the position of the methoxy-group and the double bond was isolated. The structure of the new compound is 4-methoxy-5-methyl-1,2,3,5,6,6αR-hexahydro-[1,3]dioxolo[4′,5′:6,7]-isochromeno[3,4-*c*]indol-8-one (**1**). Most of the Amaryllidaceae alkaloids have biological and pharmacological properties and recent research in a series of alkaloids of this family and this genus demonstrated cytotoxic and antiviral activity (Furusawa et al. 1980; Gabrielsen et al. 1992; Weniger et al. 1995; Lopez et al. 2002). This is the first report of this alkaloid structure in the Amaryllidaceae family and provides scope for further investigation regarding a biological-pharmacological use.



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2. Investigations, results and discussion

The proposed structure of this new alkaloid (**1**), was based on the following spectroscopic data obtained by ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, NOESY, ^{1}H-^{13}C COSY, DEPT, EI-MS, CI-MS and IR.

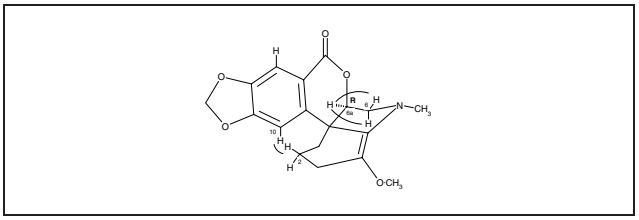


Fig.: Configuration of compound **1**

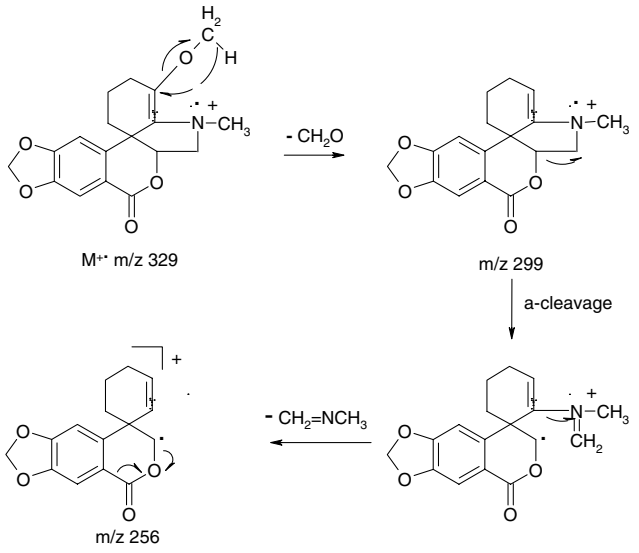
Better assignment was proposed by selective proton decoupling experiments, which showed that irradiation of H-6α (δ 4.20, dd) induced simplification of the m signal at δ 1.80 (H-6, H-6′) to a dd. This reveals that after decoupling, H-6 is no longer coupled with H-6α, giving two doublets and the signal of H-6α (dd) turns to s. The ^{1}H-^{1}H COSY demonstrated a correlation between H-6α (δ 4.20) and each of the H-6 protons (δ 1.80–2.30), as well as between H-3 and H-2 protons (δ 1.80–2.30). The configuration of $C_{6\alpha}$ is R as can be shown based on the following considerations: $H_{6\alpha}$ appears at 4.20 ppm as a doublet of doublets ($J_{6\alpha,6} = J_{6\alpha,6'} = 6$Hz), which suggests a conformation where $H_{6\alpha}$ is located at equal angles from the two neighbouring protons. On this ground, the configuration of $C_{6\alpha}$ becomes R, whereas proximity in space appears between one of the two protons on carbon 2 and H_{10}, as is shown by the reconstruction of the molecule using molecular models (Fig.). This proximity is evidenced by NOE Spectroscopy (NOESY), where correlation appears between H_{10} and the multiplet from 2.30 to 1.80 ppm where H_6, H_2 and H_1 appear.

The ^{1}H-^{13}C COSY spectrum demonstrated the following assignments: H-9 (7.65s) to C-9 (107.04); H-10 (7.25s) to C-10 (103.14); $-OCH_2O$ (6.10–6.08 dd) to OCH_2O (102.20); H-6α (δ 4.20 dd) to C-6α (74.86); OCH_3 (3.55 s) to OCH_3 (58.04) and H-3 (2.55–2.70 m) to C-3 (34.81). Finally, the carbon multiplicities were established by DEPT spectrum.

The EI-MS showed a molecular ion at *m/z* 329 (50%) and CI-MS (CH_4) M + 1, *m/z* 330 (100%). The most important and characteristic fragments in EI-MS with their relative intensities are: 329 ([$M^{+\bullet}$], 50), 314 (8), 299 (40), 272 (60), 271 (35), 256 (68), 241 (86), 240 (90), 228 (23), 213 (30), 212 (18), 135 (30), 57 (100).

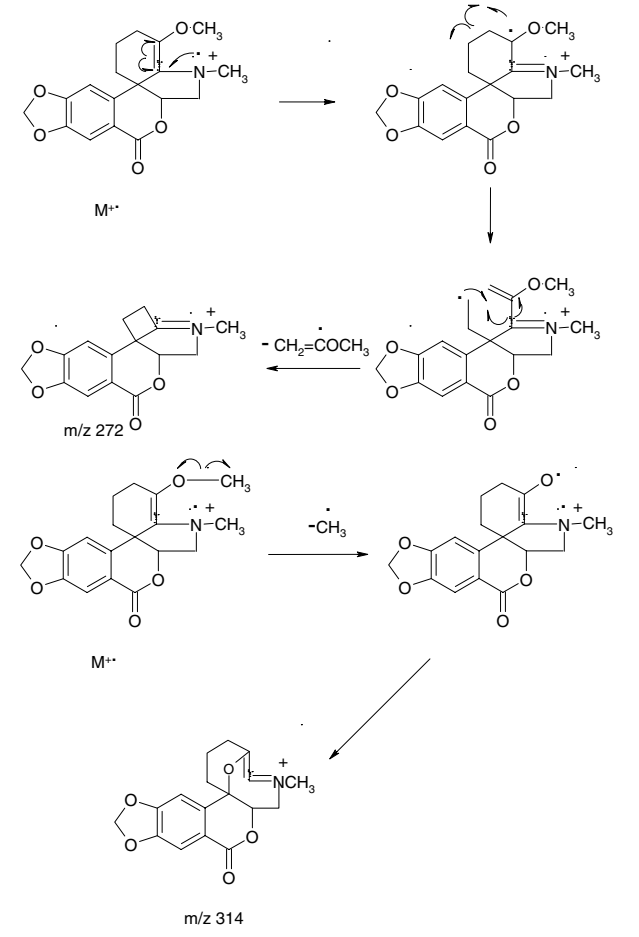
The fragment at *m/z* 299 is due to the single cleavage decomposition of the parent ion. The origin of the *m/z* 256 might have been the α-cleavage next to the nitrogen atom and subsequent loss of the fragment $CH_2 = N-CH_3$. Loss of CO_2 resulted in the formation of the fragment ion *m/z* 212 (Scheme 1). The fragment at *m/z* 314 originated after the loss of $-CH_3$ radical from $M^{+\bullet}$. The low intensity (8%) of this fragment could be explained by the unfavorable stereochem-

Scheme 1



istry of the $-OCH_3$ group; it is known in the case of Amaryllidaceae alkaloids that minor changes in the stereochemistry are frequently sufficient to cause appreciable differences in the MS of many stereoisomers. This happens for tazettine and criwelline which differ only in the configuration of the $-OCH_3$ group, but this is sufficient to cause considerable variations in the MS of these alkaloids. Also, experiments with deuterium labeling of the $>NCH_3$ group demonstrated that only $-OCH_3$ was implicated in the $M^{+\bullet} - CH_3$ process (Duffield et al. 1965). Fragmentation after RDA process, followed by a hydrogen transfer to the nucleus with the double bond, constitutes a general process for these systems (Schoes et al. 1968), but in the case of this compound such type of fragmentation does not seem to appear. On the contrary, a proposed possible fragmentation way of $M^{+\bullet}$, results in the formation of ion *m/z* 272 after the loss of the fragment $[-CH_2COCH_3]$ as radical (Scheme 2). Also the fragment at *m/z* 57 is given as base peak, due to its stability as positive ion and that seems to favor this type of fragmentation.

Scheme 2



3. Experimental

3.1. Apparatus

The IR spectrum was obtained using a Paragon 500 Perkin Elmer spectrophotometer, ^{1}H-, ^{13}C-, ^{1}H-^{1}H COSY, NOESY, ^{1}H-^{13}C COSY, DEPT and irradiation spectra were obtained on the Bruker DRX-400 apparatus. EI-MS, CI-MS were recorded on a VG-Trio 2000 FISONS mass spectrometer.

3.2. Plant material

Plant material was collected from the hillsides at Drapano cape near Chania-Crete, at an altitude of 50 m and was identified by Dr. C. Fournaraki; a voucher specimen is deposited in the herbarium of the Mediterranean Agronomic Institute of Chania, No 3593.

3.3. Extraction and isolation

500 g of the air dried aerial parts and bulbs collected in October 2001 were crushed and extracted with 3×500 ml MeOH. The extract was filtered, concentrated and partitioned between a 2% HCl aqueous phase solution and $CHCl_3$. The aqueous phase was made basically with $NaHCO_3$ and re-extracted with $CHCl_3$ affording 42 mg of a residue, after concentration under vacuum. The residue was subjected twice to silica gel 60 (0.04–0.063 mm) flash column chromatography (1×20 cm), CH_2Cl_2/MeOH 9.5 : 0.5, approxim. 5 ml/min t_R of the substance 120–260 ml; detection of the elutes by TLC (SiO_2, CH_2Cl_2/MeOH/NH_4OH 9.5 : 0.5 : 0.1, Rf 0.50; CH_2Cl_2/i-PrOH/NH_4OH 7 : 3 : 0.1, Rf 0.30; CH_2Cl_2/MeOH/NH_4OH 9 : 1 : 0.1, Rf 0.80; Dragendorff reagent). After a second purification with the same chromatographic conditions the selected fractions yielded 14 mg of pure product.
4-Methoxy-5-methyl-1,2,3,5,6,6αR-hexahydro-[1,3]dioxolo[4′,5′:6,7]isochromeno[3,4-*c*]indol-8-one (**1**): IR (KBr): 1721 (lactone C=0), 1448, 1416, 1257, 1163, 1105 cm^{-1}. ^{1}H NMR ($CDCI_3$, TMS): $\delta = 1.80-2.30$ (6 H, m, H-1, H-2, H-6), 2.41 (3 H, s, $CH_3N<$), 2.70–2.55 (2 H, m, H-3), 3.55 (3 H, s, $-OCH_3$), 4.20 (1 H, dd, $J_{6a,6} = J_{6a,6'} = 6$ Hz, H-6a), 6.08 (1 H, d, $J_{gem} = 1.2$ Hz, $-OCH_2O-$), 6.10 (1 H, d, $J_{gem} = 1.2$ Hz, $-OCH_2O-$), 7.25 (1 H, s, H-10) 7.65 (1 H, s, H-9). ^{13}C NMR ($CDCl_3$, TMS): $\delta = 29.34$ (C-1), 31.26 (C-2), 34.81 (C-3), 42.09 ($CH_3N<$), 53.39 (C-6), 58.04 (CH_3O-), 74.86 (C-6a), 102.20 ($-OCH_2O-$), 103.14 (C-10), 107.04 (C-9), 111.40 (C-10b), 116.33 (C-4a), 135.13 (C-4), 148.08 (C-8a), 152.37 (C-10a), 153.51 (C-9a), 161.43 (C-9b), 185.86 (C-8).

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