Pergamon

Phytochemistry, Vol. 42, No. 4, pp. 1227–1229, 1996

0031-9422/96 $15.00 + 0.00

S0031-9422(96)00070-2

MACEDONINE, A NON-GLYCOSIDIC IRIDOID FROM *GALIUM MACEDONICUM*

MAYA MITOVA, NEDJALKA HANDJIEVA,* STEFAN SPASSOV and SIMEON POPOV

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

(*Received in revised form* 5 *January* 1996)

Key Word Index—*Galium macedonicum*; Rubiaceae; iridoids; macedonine.

Abstract—A new non-glycosidic iridoid, macedonine, was isolated from *Galium macedonicum*. The structure, stereochemistry and conformation were determined by spectral methods and molecular mechanics calculations. Macedonine lacks the 3,4-double bond and the C-1 substituent.

INTRODUCTION

In our recent chemotaxonomic investigation on Balkan *Galium* endemics, we reported on the isolation of a series of known iridoids from the aerial parts of *G. macedonicum* Krendl [1]. Asperuloside was shown to be the main component, followed by deacetylasperulosidic acid. The present paper deals with the isolation and characterization of a new non-glycosidic iridoid, isolated from the same plant.

RESULTS AND DISCUSSION

A methanolic extract of the aerial parts of *G. macedonicum* was partitioned between chloroform and water. The aqueous layer was further treated with charcoal and eluted with H_2O and methanol. The methanolic eluate was subjected to droplet counter current chromatography (DCCC), followed by low-pressure liquid chromatography on Lobar RP-8 to give the new iridoid, macedonine (**1**).

Compound **1** has a molecular formula of $C_{10}H_{14}O_5$ as determined by ^{13}C NMR analysis (Table 1) and mass spectrometry. The ^{13}C NMR showed 10 carbon signals, one arising from a carbonyl group (δ 185.0) and two olefinic carbons ($-CH{=}C{<}$). The remaining seven signals were attributed to four methine groups, one of which belonged to an oxygenated carbon (δ 91.5), and three methylene groups adjacent to oxygen ($-CH_2-O-$). The NMR data and the proton connectivity pattern deduced from the H–H COSY and the selective decoupling experiments supported structure **1**.

In the EI mass spectrum no molecular peak was observed. Eliminations of one and two molecules of water from the molecular peak (*m/z* 196 and 178), followed by elimination of CH_2O (*m/z* 166 and 148), were observed in the region of the higher masses. A series of fragments at *m/z* 122, 121, 120, 104 and 103 were due to eliminations of CO_2, CO_2H and HCO_2H. Only one prominent fragment with a highly conjugated structure at *m/z* 132 $[MH - HCO_2H - 2 \times H_2O]^+$ was observed in the CI mass spectrum.

The relative stereochemistry of the chiral centres C-4, C-5, C-6 and C-9 was determined on the basis of the values of the vicinal H–H coupling constants, the NOE effects observed and the results of the molecular mechanics calculations. The latter were performed using the standard force field MM2 of Burkert and Allinger [2]. The computer-generated minimum energy conformations for all possible combinations of relative configurations at C-4, C-5, C-6 and C-9 were used as a basis for calculation of the vicinal coupling constants from the dihedral angles, using the equation of Haasnoot *et al.* [3]. The calculated conformations provided also the inter-proton distances, which were compared to the NOE results.

The results indicated that a satisfactory agreement between the calculated and experimental vicinal J values within 1.5 Hz was obtained only for the stereostructure with β-orientation of H-4, H-5, H-6 and H-9. All other diastereoisomers showed very significant deviations for some or all of the $J_{4,5}$, $J_{5,6}$, $J_{5,9}$ and $J_{1,9}$ values. For instance, the minimum-energy diastereoisomers with H-4α require a very large $J_{4,5}$ value (>12 Hz, almost anti-periplanar orientation of H-4 and H-5), whereas for H-6α the calculated $J_{4,5}$ value (*ca* 3.5 Hz; dihedral angle 56°) became too small compared to that measured. If both H-4 and H-6 were α-oriented, the calculated $J_{4,5}$ value was even lower (0.5–1 Hz; 70–80°). The *trans*-orientation (α, β or β, α) of H-5 and H-9, observed so far in only one case of all known natural iridoids [4], required either a high $J_{5,9}$ value (>10 Hz; >170°) or too large a value for the other coupling constants.

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*Author to whom correspondence should be addressed.

Table 1. ^1H and ^{13}C NMR parameters for macedonine (**1**) in D_2O

H/C	$\delta\,^1H$, multiplicity and J(Hz)*	Calculated J(Hz), (dihedral angle)†	$\delta\,^{13}C$ and multiplicity‡
1	3.88 *dd* (J = 11.3, 4.2) 3.98 *dd* (J = 11.3, 4.2)	$J_{1,9}$ = 3.2(49°), 1.2(68°)	61.5 *t*§
3	3.77 *m*		61.9 *t*§
4	3.16 *m* ($J_{4,5}$ = 7.8)		45.1 *d*‖
5	3.29 *td* (J = 4.3, 7.4)	$J_{4,5}$ = 6.3(41°)	48.3 *d*‖
6	5.53 *dd* ($J_{5,6}$ = 7.4)	$J_{5,6}$ = 6.4(38°)	91.5 *d*
7	5.94 *d* (J = 1.7)		126.4 *d*
8			155.3 *s*
9	3.00 *q* (J = 4.1); ($J_{5,9}$ = 4.3)	$J_{5,9}$ = 5.6(43°)	50.7 *d*‖
10	4.27 AB-*q* (J = 15.4)		64.5 *t*§
11			185.0 *s*

The ^1H NMR parameters were obtained by first-order approximation.

* The J values with subscripts indicating the coupled protons were obtained from the selective decoupling experiments and are considered more accurate.

† J calculated from the minimum-energy conformation given in Fig. 1.

‡ From DEPT-135 experiments.

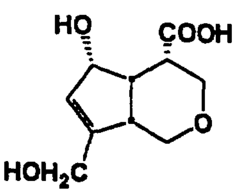
§,‖ Interchangeable.

The DNOE results also supported the proposed stereostructure **1**. Thus, upon irradiation of H-5 a very strong enhancement of the H-9 signal (calculated interproton distance 2.34 Å) and smaller positive NOE effects for H-6 and H-4 (in both cases 2.45 Å) were observed, whereas H-1 and H-3 were unaffected (distances >2.8 Å).

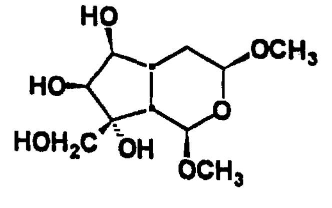
Literature data for iridoids lacking a double bond in the six-membered ring are scarce [5, 6] and in most cases the relevant vicinal couplings were not determined. The $J_{5,9}$ value of 4.3 Hz, determined by us for **1**, is in very good agreement with the corresponding value of 4.5 Hz found for eccremocarpol B (**2**) [7].

An attempt to methylate **1** with diazomethane was unsuccessful probably due to a strong intramolecular O–H · · · O=C bonding (confirmed by IR). Only a low absorbance for the carboxylic monomer (1741 cm^{-1}) was observed and not the expected strong absorbance for the dimer. For this reason, **1** was consecutively acetylated and methylated. The resulting compound showed ^1H NMR signals for two acetoxy groups (δ 2.04 and 2.10) and a CO_2Me group (δ 3.66).

The MM2-calculated minimum energy conformation of **1** (Fig. 1) indicated that the six-membered ring is close to a half-chair conformation with the oxygen atom below the plane C-4,5,9,1. There are some alternative chair- or boat-type conformations (even of lower energy), but they yield vicinal J values which are



1



2

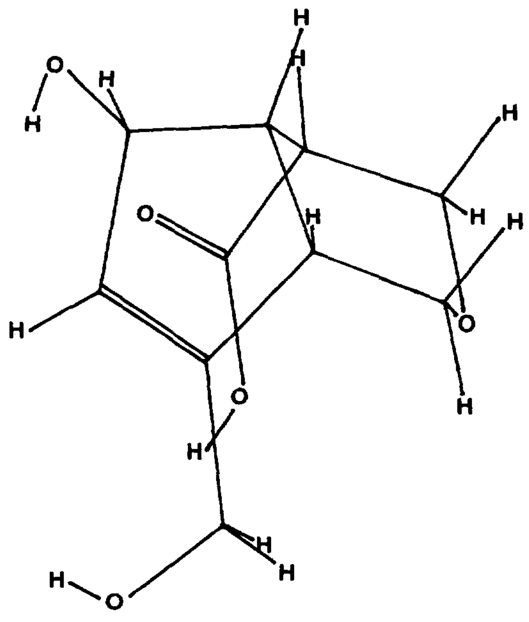


Fig. 1. MM2-calculated minimum energy conformation of compound **1**.

either totally or partially incompatible with the experimental results. In any case one should keep in mind the very approximate approach of the MM-2 calculations. On the other hand, the conformation shown on Fig. 1 could be additionally stabilized by intramolecular O-H···O=C bonding (interatomic H···O distance 2.2 Å), which was not accounted for in our MM calculations.

Macedonine belongs to the rare type iridoids lacking the 3,4-double bond and the C-1 substituent found in *Gelsemium sempervirens* [8], *Rothmannia globosa* [9], etc.

EXPERIMENTAL

NMR: 250.1 MHz (^{1}H) and 62.9 MHz (^{13}C), D_2O, int. standard TSPA. The ^{1}H spectra were measured with solvent presatn. The DEPT sequence was used for ^{13}C multiplet selection. The H,H COSY-45 spectrum was measured with standard Bruker software. The NOE experiments were performed in the difference mode with a pre-irradiation time of 5 sec. MS: JEOL JMS D-300 double focusing mass spectrometer: EI—70 eV, ionizing current 300 μA, accelerating voltage 3 kV; CI—400 eV, 50 μA, 3 kV.

Plant material. Galium macedonicum Krendl. was collected north of Kresna in the Struma valley in July 1994. A voucher specimen (SOM A9275) is deposited in the Institute of Botany, Bulgarian Academy of Sciences.

Extraction and isolation. Dried aerial parts (318 g) were extracted with EtOH. The residue was partitioned between H_2O and $CHCl_3$. The aq. fr. was passed through active C and eluted with H_2O and MeOH. The MeOH fr. (3.9 g) was subjected to ascending DCCC with the solvent system $CHCl_3$–MeOH–H_2O (7:13:8). Frs 7–18 (323 mg) were sepd on Lobar RP-8 and 10% MeOH to yield **1** (4 mg).

Macedonine (**1**). Amorphous powder. EI MS *m/z*: 196 (7), 178 (30), 166 (30), 150 (20), 148 (41), 136 (50), 135 (31), 134 (41), 122 (71), 121 (60), 120 (50), 107 (71), 105 (90), 104 (100), 103 (41); CI MS *m/z*: 132 (100) $[MH - HCO_2H - 2 \times H_2O - H]^+$; ^{1}H and ^{13}C NMR: Table 1.

Acknowledgement—This work was supported by the National Foundation of Scientific Research of Bulgaria under contracts B-423 and X-472.

REFERENCES

1. Mitova, M., Handjieva, N., Anchev, M. and Popov, S. (1996) *Z. Naturforsch.* (in press).
2. Burkert, U. and Allinger, N. L. (1982) *Molecular Mechanics*, ACS Monograph 177. American Chemical Society, Washington, DC.
3. Haasnoot, C. A. G., De Leeuw, F. A. M. and Altona, C. (1980) *Tetrahedron* **36**, 2783.
4. Foderaro, T. A. Stermitz, F. R. and Hope, H. (1992) *Tetrahedron Letters* **33**, 2953.
5. Boros, C. A. and Stermitz, F. R. (1990) *J. Nat. Prod.* **53**, 1055.
6. Boros, C. A. and Stermitz, F. R. (1990) *J. Nat. Prod.* **54**, 1173.
7. Garbarino, J. A. and Nicolleti, M. (1989) *Heterocycles* **28**, 697.
8. Jensen, S. R. Kirk, O., Nielsen, B. J. and Norrestam, R. (1987) *Phytochemistry* **26**, 1725.
9. Jensen, S. R. (1983) *Phytochemistry* **22**, 1761.