(-)-PADOCIN: A NOVEL EPOXYLIGNAN FROM *HAPLOPHYLLUM CAPPADOCICUM*

Belkıs Gözler [1], Bijen Kıvçak [1], Gökay Arar [1], Tekant Gözler [1], and Manfred Hesse [2,*]

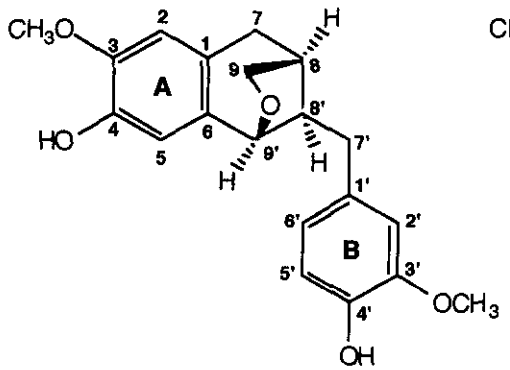
Dedicated to Arnold Brossi on the occasion of his 70th birthday

[1] Ege University, Faculty of Pharmacy, 35100 Bornova, İzmir, Turkey

[2] Organisch-chemisches Institut, Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Abstract- *Haplophyllum cappadocicum* (Rutaceae) of Turkish origin yielded a novel lignan, incorporating an unusual 6,9' fusion in a 9,9'-epoxylignan structure. Extensive 2D nmr experiments and catalytic hydrogenolysis are undertaken for the elucidation of structure with the relative configuration given in **1**.

Our previous studies on *Haplophyllum cappadocicum* Spach. (Rutaceae) have resulted in the isolation of two new compounds, (-)-malatyamine, a 4-quinolone alkaloid (1) and (-)-isodaurinol, an arylnaphthalene lignan (2), along with several known quinoline alkaloids, coumarins and lignans (2, 3). As a part of an ongoing study of the lignans of this annual herb native to eastern Turkey, we had an occasion to investigate the content of a fraction obtained by elution through a silica gel column using 5% MeOH in $CHCl_3$. This fraction was further purified by tlc to yield an optically active



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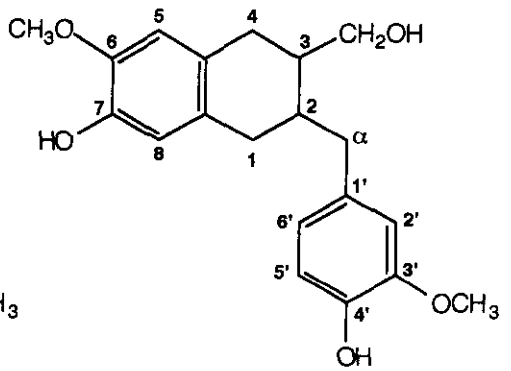

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amorphous compound, (-)-padocin (**1**), $C_{20}H_{22}O_5$, incorporating a unique variation of a 9,9'-epoxylignan structure.

The 1D and 2D nmr (homonuclear, heteronuclear, and ^1H-^{13}C long range COSY, TOCSY, and ROESY) experiments performed on (-)-padocin provide precise information for the correct assignment of carbon and hydrogen chemical shifts as well as for the determination of the connectivities and spatial arrangements of the atoms present in the molecule. In the ^1H-nmr spectrum of **1**, taken in $CDCl_3$, the signals for five aromatic protons are confined to a relatively narrow range, where two appear as singlets at δ 6.60 and 6.64, indicating *para*-oriented hydrogens on a 1,2,4,5-tetrasubstituted benzene ring (Ring A). The other three form an ABX system as two doublets at δ 6.71 (J 1.8 Hz) and at δ 6.84 (J 7.8 Hz) and a doublet of doublets at δ 6.70 (Ring B) In the aliphatic region, well-resolved signals integrate for nine protons, where only the most downfield one is a singlet (δ 4.45). The remaining protons form two groups, one as a three-spin and the other as a five-spin system, the coupling modes of which are clearly established by spin decoupling and ^1H-^1H COSY experiments. The attachment of the five-spin system to Ring A through a terminal methylene carbon is unequivocally deduced from the information provided by nOe, ROESY, TOCSY and ^1H-^{13}C long range COSY spectra. This moiety comprises one of the two phenylpropanoid portions that make up the lignan structure and is attached to the second C_6-C_3 unit through its secondary carbon positioned between the two methylenes of the five-spin system.

The methylene of the three-spin system forms the benzylic carbon of the second phenylpropanoid

unit, whereas the methine carbon is the site of attachment to the first C_6-C_3 portion. Thus, the fusion of the two phenylpropanoid units by a β,β' (or 8,8')-linkage indicates that (-)-padocin fully complies with the definition of a lignan (4).

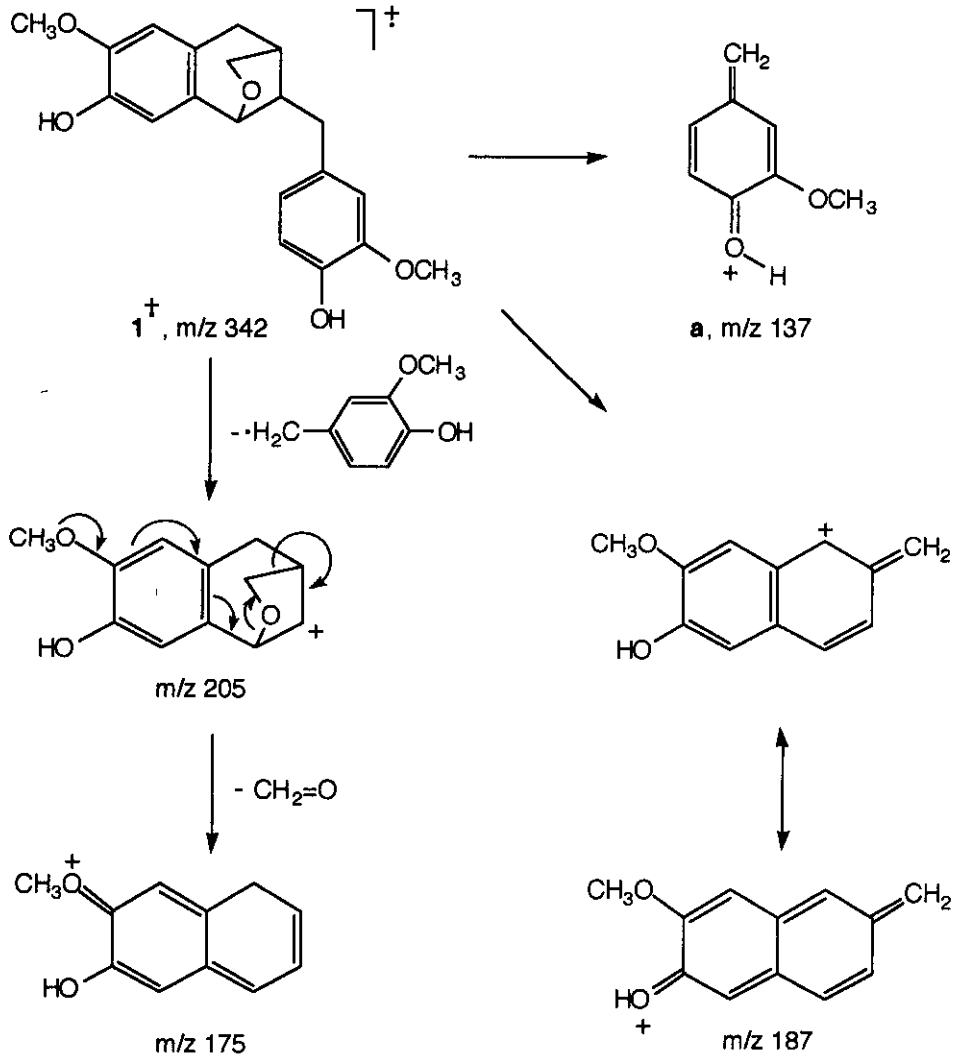
As observed in the ^{13}C-nmr spectrum of **1**, the γ-carbons of both portions, a methylene carbon (δ 71.16) and an aliphatic methine carbon (δ 79.62) , respectively, are clearly downfield as compared to the others, pointing to their proximity to an oxygen atom. This finding is in accord with the presence of three downfield signals (δ 4.45, 4.16, and 3.69) in the ^{1}H-nmr spectrum. This data strongly suggests the presence of an epoxy bridge between the γ-carbons of the two C_6-C_3 units.

The multiplicities of the twenty carbons accounted for in the ^{13}C-nmr spectrum of **1** are determined by a DEPT experiment. Of the seven quaternary aromatic carbons, four with the most downfield chemical shifts are attached to oxygenated substituents. Since two of these substituents are methoxyls, as evidenced by the two three-proton singlets at δ3.83 and 3.86 in the ^{1}H-nmr spectrum, the other two must be phenolic hydroxyl groups. The presence of the latter is confirmed by the reversible base-induced bathochromic shift in the uv spectrum of **1**. The ROESY and the nOe data furnish efficient information for defining the substitution modes on Rings A and B as well as for assigning the chemical shifts of the methoxyl substituents. The irradiation of the δ 3.83 signal produces a strong reversible nOe with H-2 (δ 6.60), thus positioning this methoxyl group on C-3. On the other hand, irradiation of the other methoxyl singlet at δ 3.86 effects only the enhancement of the aromatic H-2' (δ 6.71), identifying the position of the second methoxyl as being on C-3'.

In the low resolution eims of **1**, the molecular ion is at m/z 342, also verified by cims. The facile cleavage of a benzylic bond results in the base peak at m/z 137, represented by the 4'-hydroxy-3'-methoxybenzyl cation (**a** in Scheme 1).

The cd curve of (-)-padocin displays two positive maxima at 229 and 248 nm, and two negative minima at 239 and 278 nm. The information derived from the coupling constants of the aliphatic protons, evaluated on Dreiding models, allows the assignments of relative configurations at the chiral centers as 8R, 8'R, and 9'R.

The spectral data suggests that (-)-padocin (**1**) {1,3,4,5-tetrahydro-10-[(4'-hydroxy-3'-methoxyphenyl)methyl]-7-methoxy-1,4-methano-2-benzoxepin-8-ol} is a novel lignan, encompassing

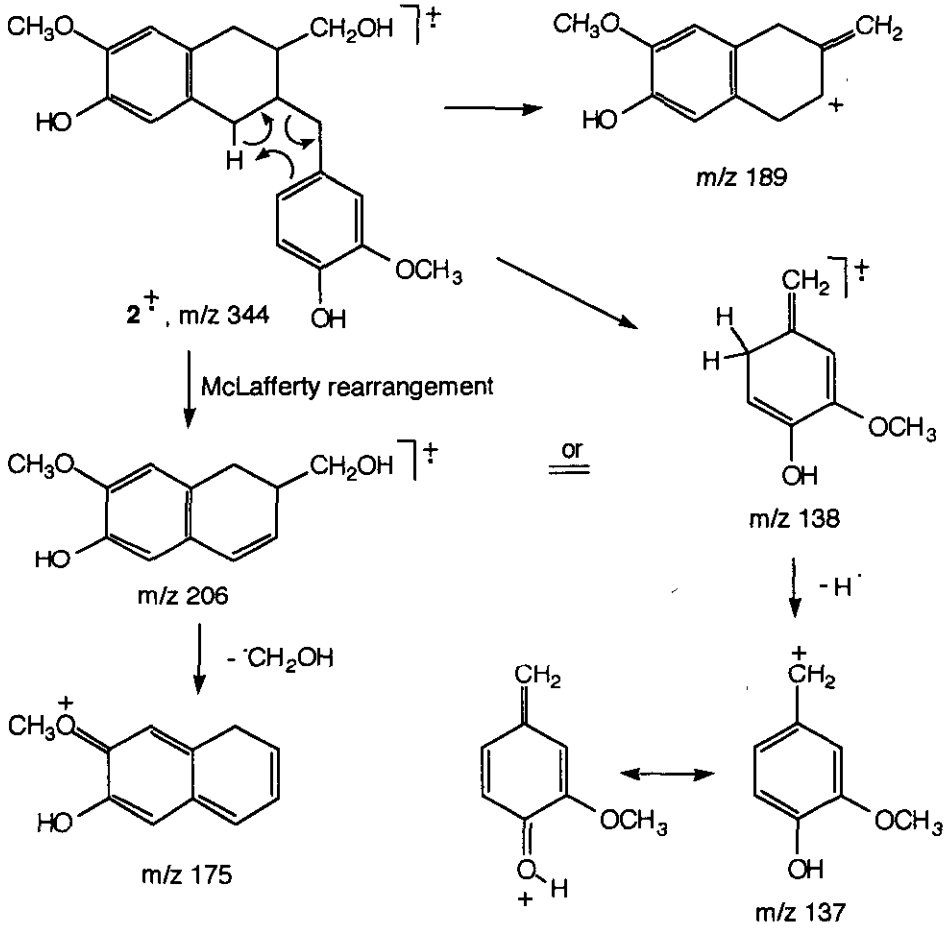


Scheme 1

an interesting C-6-C-9' junction in an otherwise structurally simple 9,9'-epoxylignan species.

Since the proposed structure of (-)-padocin is expected to undergo a benzylic cleavage at the epoxy bond, a catalytic hydrogenolysis of **1** in glacial acetic acid using 10% palladium / carbon as catalyst was undertaken to provide a final proof. In the ^{1}H-nmr spectrum of the reaction product (**2**), the easily noticeable lack of the aliphatic signal at δ 4.45 (H-9') provides an unambiguous proof to the benzylic cleavage of the epoxy bridge at the C-9'-oxygen bond. The most downfield signals now belong to the geminal hydrogens of the newly formed primary alcohol, which resonate at δ 3.81 (Jgem 10.5 Hz,

Jvic 6.3 Hz) and 3.62 (Jvic 7.9 Hz). The signals for the remaining eight protons are observed as multiplets in the range of δ 2.80-2.15. The aromatic region of the spectrum bears close resemblance to that of **1** , while the two methoxyls are observed as a singlet of six hydrogens at δ 3.83. Both in the eims and cims of **2**, the molecular ion is two mass units higher (m/z 344) than that of **1**. As is the case in the parent compound **1**, the base peak results from the facile cleavage of the benzylic bond, affording the m/z 137 ion. The cleavage pattern of **2** is presented in Scheme 2.



Scheme 2

EXPERIMENTAL

Optical rotation: Perkin-Elmer 241 Polarimeter; uv: Perkin-Elmer 555 Spectrophotometer; ir:

Perkin-Elmer 297 Infrared Spectrophotometer; ^{1}H-nmr, ^{13}C-nmr and 2D nmr spectra of **1**: Bruker AMX 600 (600 MHz) Spectrometer; ^{1}H-nmr spectrum of **2**: Bruker AC 300 (300 MHz) instrument; ms: Varian-MAT 112S (eims 70 eV, cims NH_3 as carrier gas).

Plant Material

Haplophyllum cappadocicum Spach. was collected from old Malatya, Turkey, in July 1988 and identified by Dr. M. A. Önür (Ege University). A voucher specimen, No 642, is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Extraction and Isolation

Dried and powdered total plant material (27.8 kg) was extracted with EtOH (250 l) at room temperature for 60 h to furnish the crude alcoholic extract (2650 g), which was dissolved in 2% HCl (20 l) and filtered. The acidic solution was basified with 10% NH_4OH and then extracted with $CHCl_3$. The organic solvent was evaporated to furnish the crude basic extract (24.3 g). During the preliminary separation of the components through a silica gel column, elution with $CHCl_3$:MeOH (95:5) afforded a fraction (1.044 g), which was further purified by successive preparative tlc on silica gel using C_6H_6:Me_2CO:MeOH (7:1:2) saturated with NH_3 vapors to yield 180 mg of pure (-)-padocin (**1**).

(-)-Padocin (**1**): white amorphous solid; $[a]_D^{25}$ -11.3° (c= 0.270, MeOH); uv (MeOH) λ_{max} (log ε) 206 (4.68), 229 (4.04), 284 (3.78) nm; uv (MeOH + OH^-) λ_{max} (log ε) 207 (4.72), 230 sh (4.00), 288 (3.75) nm; ir ($CHCl_3$) ν_{max} 3540, 3005, 2940, 2850, 1515, 1465, 1455, 1435, 1370, 1335, 1310, 1275, 1240, 1155, 1115, 1050, 1035, 940, 905, 870, 825 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 2.31 (1H, t, J= 7.9 Hz, H-8'), 2.44 (1H, m, H-8), 2.59 (1H, dd, J= 13.8 and 7.6 Hz, H-7'), 2.72 (1H, dd, J= 13.9 and 8 2 Hz, H-7'), 2.76 (1H, dd, J= 16.5 and 2.1 Hz, H-7α), 3.05 (1H, m, J_{gem}= 16.4 Hz, H-7β), 3.69 (1H, d, J= 8.5 Hz, H-9, *syn* to ring A), 3.83 (3H, s, 3-OMe), 3.86 (3H, s, 3'-OMe), 4.16 (1H, ddd, J= 8.5, 5.9 and 2.1 Hz, H-9, *anti* to ring A), 4.45 (1H, s, H-9'), 6.60 (1H, s, H-5), 6.64 (1H, s, H-2), 6.70 (1H, dd, J= 7.8 and 1.8 Hz, H-6'), 6.71 (1H, d, J= 1.8 Hz, H-2'), 6.84 (1H, d, J= 7 8 Hz, H-5'); ^{13}C-nmr ($CDCl_3$) δ 36.84 (C-7'), 37.92 (C-7), 38.30 (C-8), 48.65 (C-8'), 55.93 (3'-OMe), 56.02 (3-OMe), 71.16 (C-9), 79.62 (C,9'), 111.63 (C-2 and C-2'), 113.57 (C-5), 114.36 (C-5'), 121.60 (C-6'), 125.54 (C-1), 132.16 (C-1'), 133.16 (C-6), 143.61 (C-4), 143.97 (C-4'), 146.45 (C-3), 146.50 (C-3'); eims m/z (%) 342 ($M^{+\cdot}$, 70),

311 (6), 205 (7), 187 (16), 175 (35), 174 (6), 150 (6), 138 (14), 137 (100), 131 (8), 122 (6), 115 (7); cd (MeOH) nm ($\Delta\varepsilon$) 290 (0), 278 (-0.31), 255 (0), 248 (0 14), 245 (0), 239 (- 0.80), 233 (0), 229 (0.67), 224 (0), negative tail beyond 224 nm.

Catalytic Hydrogenolysis of **1**

14 mg of **1** in 6 ml of AcOH was subjected to catalytic hydrogenolysis for 6 h under pressure (50 kg/cm^2) using 10% Pd/C (25 mg). After filtering the catalyst through Celite, the solvent was evaporated *in vacuo* . The residue was taken into 10 ml of NH_4OH and extracted with 3 X 10 ml of $CHCl_3$. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to give the crude reduction product, which was then purified by preparative tlc on silica gel using C_6H_6:Me_2CO:MeOH (7:1:2) saturated with NH_3 vapors to afford **2** (8 mg). ir ($CHCl_3$) ν_{max} 3540, 3000, 2930, 2850, 1515, 1465, 1450, 1435, 1370,1270, 1240, 1200, 1185, 1150, 1120, 1075, 1035, 910, 870 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 2.15 (1H, m, H-2), 2.27 (1H, m, H-3), 2.30-2.46 (2H, m, CH_2's), 2.56-2.80 (4H, m, CH_2's), 3.62 (1H, dd, J= 7.9 and 10.5 Hz, C$\underline{H}_2$OH), 3.81 (1H, dd, J= 6.3 and 10.5 Hz, C$\underline{H}_2$OH), 3.83 (6H, s, 2 X OCH_3), 6.56 (2H, s, H-5 and H-8), 6.60 (1H, d, J= 1.8 Hz, H-2'), 6.61 (1H, dd, J= 7.8 and 1.8 Hz, H-6'), 6.81 (1H, d, J= 7.8 Hz, H-5'); eims m/z (%) 344 ($M^{+\cdot}$, 40), 206 (12), 189 (20), 175 (32), 138 (54), 137 (100), 122 (16), 115 (17), 107 (11).

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