

Chlorosmaridione; A Novel Chlorinated Diterpene Quinone Methide from *Rosemarinus officinalis* L.

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Abstract – A novel chlorinated diterpene quinone methide; chlorosmaridione (7-chloro-11-hydroxy-abeita-7,9(11),13-triene-6,12-dione) was isolated from petroleum ether extract of the stems of *Rosemarinus officinalis* L. growing in Egypt. In addition, β -sitosterol, stigmasterol, lupeol acetate, α -amyrin, β -amyrin, lupeol, acetyloleanolic acid, acetylursolic acid taxodione, horminone, and cryptotanshinone were also identified. Chemical structures of the isolated compounds have been elucidated on the bases of physical, chemical, and spectral data including IR, UV, MS, 1D- and 2D-NMR spectra.

Keywords – *Rosemarinus officinalis* L., Lamiaceae, abietane diterpene quinone methide, chlorosemaridione, diterpenoids, sterols and triterpenoids.

Introduction

Rosemarinus officinalis L. (Lamiaceae) is a well-known and greatly valued evergreen perennial shrub, native to the Mediterranean region. It has been used since antiquity to improve and strengthen the memory and it also has a long-standing reputation as a tonic herb improving food absorption by stimulating the secretions of digestive enzymes. In addition, Rosemary is a rich source of volatile oils, flavonoids, and phenolic diterpenoids with strong antiseptic and anti-inflammatory properties (Bown, 1995). 70% crude hydro-alcoholic extract of *R. officinalis* leaves (Dias *et al.*, 2000) and a methanol extract (Huang *et al.*, 1994) were found to have anti-ulcerogenic activity and significantly inhibited TPA-induced skin tumors in experimental animals, respectively. These effects were attributed to antioxidant compounds found in both extracts. A number of antioxidant compounds have been isolated and identified from the leaves of this plant. Among these compounds are rosemariquinone (Houlihan *et al.*, 1985), rosmanol that showed remarkably high anti-oxidant activity (Duke and Ayensu, 1985), and carnosol and carnosic acid that were found to be powerful inhibitors of lipid peroxidation in microsomal and liposomal systems (Aruoma *et al.*, 1992). The diterpenoids carnosol, carnosic acid, rosmanol, and epirosmanol isolated from the leaves, inhibited superoxide anion production and the mitochondrial and microsomal lipid peroxidation induced

by NADH or NADPH oxidation. Thus they are effective in protecting biological systems against oxidative stresses (Haraguchi *et al.*, 1995). Furthermore, rosmic acid and rosmanol-related compounds, isolated also from the leaves of *R. officinalis*; showed significant anti-microbial activity against *Streptomyces scabies* (Takenaka *et al.*, 1997). In addition to the diterpenoids and volatile oils, some phytochemical investigations were concerned with the triterpenoids of the leaves (Ganeva *et al.*, 1993; Ganeva *et al.*, 1994) and only one publication considered the diterpene quinones from the roots (Abou-Donia *et al.*, 1989). This foundation prompted us to carry out a phytochemical study on the stems of this valuable plant.

Experimental

Rosemarinus officinalis L. was collected in September 2001 from local gardens in Alexandria, Egypt. The plant was kindly identified by Prof. Dr. Nabil El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University. A voucher specimen is deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University.

General – ^1H - and ^{13}C -NMR spectra were recorded on a Jeol 500 and 125 MHz Instrument, respectively and the samples were dissolved in CDCl_3 . UV spectra were recorded on a Perkin Elmer 550 spectrophotometer. IR spectra were determined on a Perkin Elmer 1600 spectrophotometer. EIMS were recorded on a Finnigan SSQ/7000, 70 ev. Silica gel for column chromatography (70-230 mesh, Merck) was used. TLC and pTLC were carried out on pre-coated silica

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gel plates (60F-245, Merck). Spots were detected by UV lamp before and after exposure to NH_3 vapors and by spraying with anisaldehyde/ H_2SO_4 followed by heating at 105°C for 5 minutes. Reference samples were supplied through Pharmacognosy Department, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

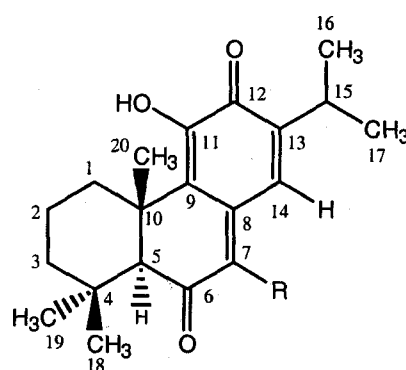
Extraction and isolation – The air-dried, and powdered stems of *R. officinalis* (500 g) were extracted exhaustively with petroleum ether in a Soxhlet apparatus. The residue left after evaporation of the solvent (5 g) was chromatographed over a column of silica gel (200 g). Elution was started with petroleum ether and the polarity was gradually increased by addition of CHCl_3 to give the known compounds, lupeol, β -sitosterol, stigmaterol, lupeol acetate, α -amyrin, β -amyrin, lupeol acetyloleanolic acid, acetylursolic acid, taxodione, hominone, and cryptotanshinone. Fractions obtained using 40% CHCl_3 in petroleum ether were subjected to repeated pTLC using solvent systems with different proportions of petroleum ether and EtOAc to afford 12 mg of compound **1**, R_f 0.55 (Solvent, petroleum ether: EtOAc, 8:2).

7-chloro-11-hydroxy-abeita-7,9(11),13-triene-6,12-dione chlorosemaridione (**1**) (12 mg): dark orange thick oil, $[\alpha]_D^{25} + 89.2$ (MeOH; $c = 0.1$), UV λ_{max} (log ϵ) MeOH: 223 (4.0), 335 (4.3), 409 (2.1) nm. IR ν_{max} (KBr): 3305 (*pseudo* enolic OH), 1685, 1665, 1640, 1625 (quinoidal carbonyls), 1390, 1375, 1185 ($=\text{C}-\text{Cl}$ cm^{-1}). EIMS, m/z (rel. int.): 350 (3.77), 348 (9.38), 336 (14.98), 334 (38.14), 322 (33.24), 320 (100), 307(13.08), 305 (24.66), 251 (16.1), 225 (16.61), 223 (46.39), 211 (22.59), 209 (64.73), 199 (13.56), 197 (31.67), 183 (17.71), 167 (42.90). $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 7.61 (1H, s, OH-12), 6.42 (1H, d, $J = 1.8$ Hz, H-14), 2.98 (1H, br. d, $J = 11.9$ Hz, $\beta\text{H-1}$), 2.91 (1H, d, hept., $J = 1.8, 9.5$ Hz, H-15), 2.89 (1H, s, H-5), 1.32 (3H, s, CH_3 -20), 1.26 (3H, s, CH_3 -19), 1.07 (3H, s, CH_3 -18), 0.94 (3H, d, $J = 9.5$ Hz, CH_3 -16 or 17), 0.84 (3H, d, $J = 9.5$ Hz, CH_3 -17 or -16). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 36.83 (C-1), 18.75 (C-2), 42.42 (C-3), 32.95 (C-4), 62.25 (C-5), 201.01 (C-6), 144.88 (C-7), 141.15 (C-8), 126.83 (C-9), 41.79 (C-10), 145.61 (C-11), 181.28 (C-12), 139.63 (C-13), 133.22 (C-14), 27.07 (C-15), 21.14 (C-16), 21.46 (C-17), 32.58 (C-18), 21.60 (C-19), 21.89 (C-20).

Results and Discussion

Compound **1** was isolated from the petroleum ether extract of the stems of *R. officinalis* in the form of dark orange thick oil. It gives greenish black and purple colors with FeCl_3 and aqueous KOH, respectively, indicating its phenolic nature. The molecular formula of compound **1** was established based on chemical and spectroscopic methods. Qualitative

chemical analysis indicated compound **1** to be a chlorinated derivative. $^{13}\text{C-NMR}$ spectrum revealed the presence of 20 resolved carbon signals, and DEPT experiment indicated these carbons to be: five CH_3 , three CH_2 , three CH , and nine quaternary carbons, of which two were observed at δ 181.28 and 201.01, due to chelated and non-chelated carbonyl groups, respectively. $^1\text{H-NMR}$ spectrum of compound **1**, integrated for 25 protons, was very similar to that of taxodione (Abou-Donia *et al.*, 1989), but only lacking the one-proton singlet of H-7 (δ 5.92) along with its corresponding carbon (δ 134.9) and the appearance of a quaternary carbon at δ 144.88. EIMS confirmed the presence of one chlorine atom, as shown by the appearance of characteristic fragments, with two mass units difference, at m/z 350 (3.77%) and 348



Chlorosmaridione **1** R = Cl
Taxodione R = H

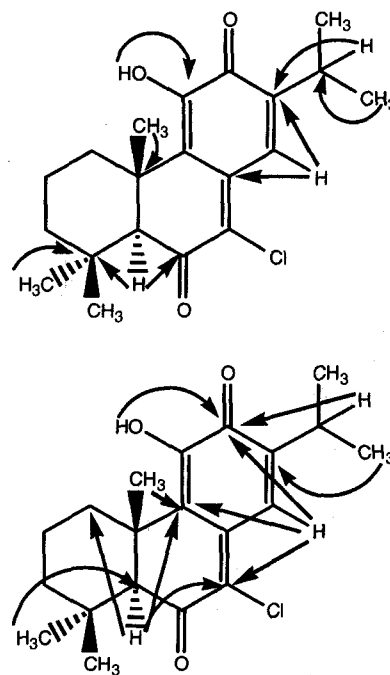


Fig. 1. ^1H , ^{13}C -Correlations as observed by HMBC.

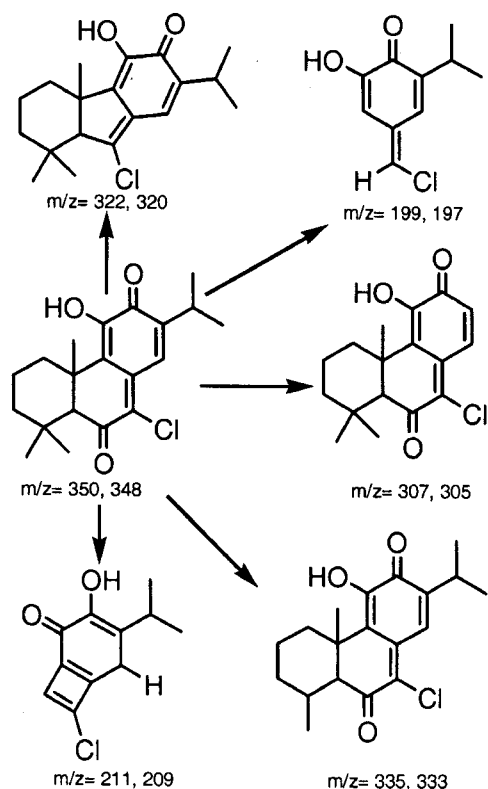


Fig. 2. A suggested fragmentation pathway of Chlorosmaridione.

(9.38%) corresponding to the molecular formula $C_{20}H_{25}ClO_3$. IR spectrum revealed the presence of an absorption band at 1185 cm^{-1} , most probably due to $=C-Cl$ group. The 1H -NMR spectrum showed also three singlet signals; each integrated for three protons; at $\delta 1.07$, 1.26 , and 1.32 due to three CH_3 groups at positions 18, 19, and 20, along with their corresponding carbons resonating at $\delta 32.58$, 21.60 , and 21.89 , respectively. EIMS indicated the presence of two mass fragments at m/z 305 (22.22%) and 307 (7.77%) most probably due to loss of an isopropyl group. COSY spectrum confirmed this suggestion through the appearance of two doublets ($J = 9.5\text{ Hz}$, CH_3-16 , CH_3-17) at $\delta 0.84$ and 0.94 , coupled with the benzylic proton resonating at $\delta 2.91$ (H-15), and appearing as a double quartet ($J = 9.5, 1.8\text{ Hz}$). The H-15 signal showed a vinylic coupling with the aromatic proton doublet ($J = 1.8\text{ Hz}$) observed at $\delta 6.42$ (H-14). The spectrum showed also the presence of a singlet signal at $\delta 2.89$ attributed to H-5, flanked between two quaternary carbons. HMBC experiments (Fig. 1) correlated H-5 to carbons at $\delta 36.83$ (C-1), 42.42 (C-3), 32.95 (C-4), 201.01 (C-6), 144.88 (C-7), 126.83 (C-9), and 41.79 (C-10). In addition, 1H -NMR spectrum revealed the presence of a D_2O -exchangeable signal due to pseudo enolic \underline{OH} at position 12. HMBC correlated this proton to carbons at δ

145.61 (C-11) and 181.28 (C-12). The final chemical structure of compound **1** was unambiguously assigned based on 2D-NMR spectral analyses including COSY, HMQC, and HMBC to be 7-chloro-11-hydroxy-abeita-7,9(11),13-triene-6,12-dione, to which the trivial name chlorosemaridione was given. Finally, a suggested MS fragmentation pathway of chlorosemaridione was postulated (Fig. 2). It is worth mentioning that, this seems to be the first report for the isolation of a chlorinated diterpene quinone methide as a natural product.

Acknowledgement

The author thanks Dr. Mohamed M. Abdul-Ghani, lecturer of Organic Chemistry, Faculty of Science, Beirut Arab University, Beirut, Lebanon, for carrying out IR, UV, and MS analyses.

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(Accepted March 25, 2004)