Diterpene and other Constituents from Stemodia maritima (Scrophulariaceae)

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Um novo diterpeno, ($5^S$,$8^S$,$9^R$,$10^S$)-11β,12β-epoxi-9α-hidróxi-19(4→3)abeo-abieta-3,13-diene-19,18-olide, e as substâncias conhecidas estemodina, D-mannitol, ácido betulínico, uma mistura de 3β-O-β-D-glicopiranósil-β-sitosterol e 3β-O-β-D-glicopiranósilstigmasterol, e 5,7,4′-tridróxi-3,8,3′-trimetoxiflavona, foram isolados das folhas e talos de Stemodia maritima. A elucidação estrutural de todas as substâncias baseou-se na interpretação de dados espectrais, principalmente RMN (1D e 2D) e espectrometria de massa (EM), envolvendo comparação com valores descritos na literatura.

Keywords: Stemodia maritima, Scrophulariaceae, diterpenes, steroids, flavonoids

Introduction

Stemodia Benth. is one of Scrophulariaceae genus and occurs in tropical and subtropical regions of the world.1 Although Stemodia comprises about 40 species, the chemical investigation of this genus is restricted to five species4 from which flavonoids,2,3 labdane diterpenes4,5 and diterpenes derivatives with a rare tetracyclic skeletal, named stemodane, were isolated. This later class of diterpenes seems to be chemomarkers of Stemodia.6

S. maritima Linn. is a very common shrub that widely grows in Northeast Region of Brazil, near the sea coast, where it is known as “melosa”. It has been used to treat stomachache, dropsy and swelling by local population, although toxic symptoms was reported in cattle.7 Stemodane diterpenes, including glycosides derivatives, possessing antiviral and cytotoxic properties were isolated from this species.8-10 The chemical composition and larvicidal activity of its essential oil were recently reported.11

On the course of the phytochemical investigation of S. maritima from the Northeast Region of Brazil, herein we report the non-volatile composition of this species. A new diterpene, ($5^S$,$8^S$,$9^R$,$10^S$)-11β,12β-epoxi-9α-hidróxi-19(4→3)abeo-abieta-3,13-diene-19,18-olide (1), together with the known compounds stemodin (2) (Figure 1), D-mannitol, betulínico acid, a mixture of 3β-O-β-D-glucopiranósil-β-sitosterol and 3β-O-β-D-glucopiranósilstigmasterol and 5,7,4′-trihidróxi-3,8,3′-trimetoxiflavona were isolated from the leaves and stems of Stemodia maritima. Structural elucidation of all compounds was based on interpretation of spectral data, mainly NMR (1D and 2D) and MS, including comparison with values described in the literature.

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based on the interpretation of spectral data, mainly NMR (1D and 2D) and MS, and comparison with literature data.

**Results and Discussion**

The molecular formula of compound 1 was established through HR-ESI-MS, which showed the quasi-molecular ion peak at \( m/z \) 331.1799 ([M+1] \(^{+}\), corresponding to the molecular formula \( \text{C}_{20}\text{H}_{26}\text{O}_{4} \) and indicating eight degrees of unsaturation. EIMS from 1 showed the molecular ion peak at \( m/z \) 330 (\( \text{C}_{20}\text{H}_{26}\text{O}_{4} \), 5%) and additional peaks at \( m/z \) 315 (\( \text{C}_{19}\text{H}_{23}\text{O}_{4} \), 7%) and \( m/z \) 287 [\( \text{C}_{17}\text{H}_{19}\text{O}_{4} \), 100%], attributed to fragments 1a and 1b, respectively (Figure 2).

The presence of a hydroxyl absorption (\( \nu_{\text{max}} \) 3433 cm\(^{-1}\)) and an \( \alpha,\beta-\text{unsaturated-}\gamma\text{-lactone} \) (\( \nu_{\text{max}} \) 1729 cm\(^{-1}\)) was inferred from its IR spectrum.

The \(^1\text{H}\) NMR spectrum (Table 1) revealed the presence of an isopropyl group (\( \delta_{\text{H}} \) 1.03, d, \( J \) 6.8 Hz, 3H-16; \( \delta_{\text{H}} \) 1.05, d, \( J \) 6.8 Hz, 3H-17; \( \delta_{\text{H}} \) 2.62, sep, \( J \) 6.8 Hz, H-15), a methyl group at \( \delta_{\text{H}} \) 1.01 (3H, s, 3H-20) attached to quaternary carbon, two oxygenated methine hydrogens at \( \delta_{\text{H}} \) 3.66 (dd, \( J \) 2.5 and 1.9 Hz, H-11) and \( \delta_{\text{H}} \) 4.40 (brs, H-12), compatible with the presence of an epoxy ring, two deshielded hydrogen from a oxygenated methylene group at \( \delta_{\text{H}} \) 4.72 (brd, \( J \) 17.2 and 1.6 Hz, H-19\( \alpha \)) and \( \delta_{\text{H}} \) 4.68 (brdd, \( J \) 17.2 and 1.6 Hz, H-19\( \beta \)), and an olefinic hydrogen at \( \delta_{\text{H}} \) 5.24 (brd, \( J \) 5.0 Hz, H-14).

The \(^{13}\text{C}\) NMR spectra (Table 1) revealed 20 lines, in accordance with the molecular formula \( \text{C}_{20}\text{H}_{26}\text{O}_{4} \). From these data it is possible to deduce the presence of the six non-protonated carbons: one carbonyl group (\( \delta_{\text{C}} \) 173.9), three sp\(^2\) carbons, one oxygenated sp\(^3\) carbon and one non-oxygenated sp\(^3\) carbon. Additionally, it was observed six methine carbons, including two sp\(^3\) oxygenated at \( \delta_{\text{C}} \) 66.6 and 59.7 and one sp\(^3\) at \( \delta_{\text{C}} \) 121.8; five methylene carbons, one of them oxygenated at \( \delta_{\text{C}} \) 70.4, and three methyl carbons.

The aforementioned data were coherent with a non-aromatic abietane-type diterpene that displays an epoxy ring, a tertiary hydroxyl group, an \( \alpha,\beta\)-unsaturated-\gamma-lactone system and two double bonds, having some similarities with the diterpene triptolide.\(^{12}\)

The location of these functions in the abietane skeleton was deduced through additional HMBC analysis (Table 1), which revealed the following long-range correlations: the epoxy hydrogens at \( \delta_{\text{H}} \) 3.66 (H-11) with C-13 (\( \delta_{\text{C}} \) 140.1, \(^1\text{J}\)) and at \( \delta_{\text{H}} \) 4.4 (H-12) with C-13 (\( \delta_{\text{C}} \) 140.1, \(^2\text{J}\)) and C-14 (\( \delta_{\text{C}} \) 121.8, \(^3\text{J}\)); the isopropyl hydrogen at \( \delta_{\text{H}} \) 2.62 (H-15), with C-13 (\( \delta_{\text{C}} \) 140.1, \(^3\text{J}\)) and C-12 (\( \delta_{\text{C}} \) 66.6, \(^2\text{J}\)); the olefin hydrogen at \( \delta_{\text{H}} \) 5.24 with C-12 (\( \delta_{\text{C}} \) 66.6, \(^3\text{J}\)), C-13 (\( \delta_{\text{C}} \) 140.1, \(^2\text{J}\)) and C-15 (\( \delta_{\text{C}} \) 28.6, \(^1\text{J}\)).

The position of the hydroxyl group at C-9 was established based in the correlations of this oxymethine carbon (\( \delta_{\text{C}} \) 67.9) with the hydrogen of the methyl group (3H-20, \( \delta_{\text{H}} \) 1.01, \(^3\text{J}\)), which

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**Figure 1.** Compounds 1 and 2 isolated from *Stemodia maritima*.

**Figure 2.** Fragments postulated to justify some of principal peaks observed in EIMS of 1.
is generally present in abietane-type diterpenoids.\textsuperscript{13} Finally, the butenolide ring involving the carbons C-3, C-4, C-18 and C-19 was located by the correlations of the methylene hydrogens at $\delta_H$ 4.72 and 4.68 (2H-19) with C-4 ($\delta_C$ 162.0, 2$\tilde{J}_{CH}$), C-3 ($\delta_C$ 125.3, 3$\tilde{J}_{CH}$) and C-18 ($\delta_C$ 173.9, 3$\tilde{J}_{CH}$).

The relative configuration of 1 (Figure 3) was assigned by the analysis of the $^1$H-$^1$H-NOESY spectrum. The $\beta$-orientation of the epoxy function (11,12$\beta$-epoxide) was determined by the dipolar interactions of the hydrogen at $\delta_H$ 3.66 (H-11) with 2H-1 ($\delta_H$ 1.77 and 1.36). In addition, the methyl signal at $\delta_H$ 1.01 (3H-20) exhibited cross-peaks with the hydrogens at $\delta_H$ 2.86 (H-8), $\delta_H$ 2.20 (H-2$\beta$) and $\delta_H$ 1.62 (H-6$\beta$). The hydrogen at $\delta_H$ 2.51 (H-5) showed dipolar interaction with the hydrogens at $\delta_H$ 1.77 (H-1$\alpha$), 1.67 (H-6$\alpha$) and 1.07 (H-7$\alpha$). Based on these correlations, the hydroxyl group at C-9 was established at $\alpha$ position (Figure 3). Therefore, all these data allowed to establish the structure of 1 as (5$S^*$_*,8$S^*_*$,9$R^*_*$,10$S^*$*)-11$\beta$,12$\beta$-epoxy-9$\alpha$-hydroxy-19(4$\rightarrow$3)abeo-abieta-3,13-diene-19,18-olide.

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<td>140.1</td>
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<td>H-12, H-14, H-15</td>
<td>H-8, H-11, 3H-16, 3H-17</td>
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<td>18</td>
<td>173.9</td>
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The relative configuration of 1 (Figure 3) was assigned by the analysis of the $^1$H-$^1$H-NOESY spectrum. The $\beta$-orientation of the epoxy function (11,12$\beta$-epoxide) was determined by the dipolar interactions of the hydrogen at $\delta_H$ 3.66 (H-11) with 2H-1 ($\delta_H$ 1.77 and 1.36). In addition, the methyl signal at $\delta_H$ 1.01 (3H-20) exhibited cross-peaks with the hydrogens at $\delta_H$ 2.86 (H-8), $\delta_H$ 2.20 (H-2$\beta$) and $\delta_H$ 1.62 (H-6$\beta$). The hydrogen at $\delta_H$ 2.51 (H-5) showed dipolar interaction with the hydrogens at $\delta_H$ 1.77 (H-1$\alpha$), 1.67 (H-6$\alpha$) and 1.07 (H-7$\alpha$). Based on these correlations, the hydroxyl group at C-9 was established at $\alpha$ position (Figure 3). Therefore, all these data allowed to establish the structure of 1 as (5$S^*$_*,8S^**,9R*,10S*)-11$\beta$,12$\beta$-epoxy-9$\alpha$-hydroxy-19(4$\rightarrow$3)abeo-abieta-3,13-diene-19,18-olide.

**Compound 2** was obtained as colorless crystal and its molecular formula C$_{20}$H$_{34}$O$_2$ was deduced by EIMS ([M]+, m/z 306) and $^1$H and $^1$C NMR analysis. Its IR spectrum showed hydroxyl absorption at $\nu_{\text{max}}$ 3311 cm$^{-1}$. All spectral data were in accordance with the structure of the stemodin (2), a stemodane-type diterpene previously isolated from *Stemodia* species.\textsuperscript{6,8}

<table>
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Table 1. $^1$H and $^1$C NMR data assignments for the compound 1 (CDCl$_3$, 500/125 MHz)

**Table 1.** $^1$H and $^1$C NMR data assignments for the compound 1 (CDCl$_3$, 500/125 MHz)
Compound 2 was submitted to acetylation with pyridine and acetic anhydride (see Experimental section), yielding 2a (Figure 1). The 1D and 2D NMR spectral data of 2 and of its acetyl derivative (2a) were also used to complete 1H and 13C chemical shifts described in Table 2. Dipolar interactions observed from 1H-1H-NOESY analysis of 2 are summarized in Figure 3.

The other isolated compounds were identified on the basis of their spectral analysis and comparison with the literature data.

Table 2. 1H and 13C NMR data assignments for the compounds 2 and 2a (CDCl3, 500/125 MHz)

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Experimental

General experimental procedures

Melting points were obtained from a Mettler FP82HT apparatus and are uncorrected. IR spectra were recorded using a Perkin Elmer 1000 FT-IR spectrophotometer. Optical rotations were measured on a Perkin Elmer 341 polarimeter. High resolution electrospray ionization mass spectra (ESI-MS/MS), in positive mode, was performed on a QTOF Micromass spectrometer (QqTOF, Micromass-UK). 1H and 13C NMR spectra were recorded on a Bruker Avance DRX-500 (500 MHz for 1H and 125 MHz for 13C); chemical shifts are given in ppm relative to residual CHCl3 (7.27 and 77.23 ppm). Silica Gel 60 (Merck, 230-400 mesh) was used for analytical TLC. Silica gel 60 (Merck, 60 F254, 0.2 mm) was used for column chromatography. All compounds were visualized on TLC by spraying with vanillin/perchloric acid/EtOH followed by heating.

Plant material

S. maritima was collected during the flowering stage in September 2006 along the Flexeiras Beach, Ceará Cost, Northeast of Brazil. The plant was identified by Dr. F. S. Cavalcanti and Prof. E. P. Nunes from the Herbário Prisco Bezerra (EAC), Universidade Federal do Ceará, Fortaleza, Brazil, where a voucher specimen (# 38483) is deposited.

Extraction and isolation

The fresh stems (200.0 g) of S. maritima were exhaustively extracted with ethanol, at room temperature, to obtain a crude material, composed by a precipitate, which was recrystallized from methanol to give D-mannitol14 (80.0 mg, 0.04%).

The aqueous extract obtained after the essential oil extraction (hydrodistillation) of the fresh stems of S. maritima was submitted to liquid-liquid partition with hexane/MeOH (3:7). The hexane fraction (340.0 g) was submitted to column chromatography on silica gel column, using a gradient solvent system of hexane and CHCl3, Chromatography of the subfraction hexane (380.0 mg) using hexane/EtOAc mixtures with increasing polarity yielded betulinic acid15 (380.0 mg, 0.019%). Successive flash chromatography of CH2Cl2 subfraction (2.0 g) using 0-100% CH2Cl2/EtOAc provided a mixture of 3β-O-β-D-glucopyranosyl-β-sitosterol and 3β-O-β-D-glucopyranosylstigmasterol16 (8.2 mg, 0.0024%).

After extraction of the essential oils from the leaves of S. maritima by hydrodistillation, the aqueous extract was subjected to liquid-liquid partition with ethyl acetate. The organic fraction (4.0 g) was chromatographed over silica gel with CHCl3, EtOAc and MeOH to afford three subfractions F1-F3. Successive flash column chromatography of F1 (1.2 g), previously eluted from CHCl3, yielded 2 (45.3 mg, 1.13%) after elution with CHCl3/hexane 7:3. From these same column, fraction CHCl3/hexane 9:1 (180.0 mg) was also obtained and rechromatographed over silica gel using the same eluent system to afforded 5,7,4′-trihydroxy-3,8,3′-trimethoxyflavone17 (6.5 mg, 0.0019%) and 1 (15.6 mg, 0.39%).


Crystalline Solid; mp 264.6-266.5 °C; IR (film, KBr) νmax/cm-1: 3433, 2962, 2866, 1729, 1663, 1453, 1344, 1036; HREIMS, m/z 331.1799, required m/z 331.1909; [α]D25 = -12.9° (c 1.0, CHCl3).

Stemodin (2)

Crystalline Solid; mp 189.9-192.4 °C; IR (film, KBr) νmax/cm-1: 3311, 2954, 1463, 1367, 1217, 1032; EIMS, m/z 306 (M+), 291, 288, 273, 232, 217, 161, 94.

The structures of known compounds were established by 1D 1H and 13C (1H and DEPT) and 2D 1H-1H-COSY, HSQC and HMBC NMR spectral data (Table 2) and by comparison of their spectroscopy data with those reported in the literature.

Acetylation of 2

To a solution of compound 2 (24.0 mg) in pyridine (0.5 mL) were added Ac2O (1.0 mL) and catalytic amount of DMAP. The mixture was stirred for 5 h at room temperature. Subsequent workup afforded a residue that was chromatographed using hexane/CHCl3 (1:1), hexane/CHCl3 (1:3) as eluent to yield compound 2a (12.0 mg, 50.0%) as a colorless solid.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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Magnética Nuclear (CENAUREMN), Universidade Federal do Ceará (UFC), Fortaleza-CE, for the support to perform of the NMR (1D and 2D) spectra and to the Laboratório Thomson de Espectrometria de Massas, IQ-Unicamp, Campinas-SP, Brazil, for the high resolution mass spectra.

References


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*Figure S1a. EI-MS of compound (1) isolated from leaves of *Stemodia maritima*. 

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Figure S1b. $^1$H NMR spectrum (CDCl$_3$, 500 MHz) of compound (1) isolated from leaves of Stemodia maritima.

Figure S2. Expansion $^1$H NMR spectrum (CDCl$_3$, 500 MHz) of compound (1) isolated from leaves of Stemodia maritima.
Figure S3. Expansion 2 $^1$H NMR spectrum (CDCl$_3$, 500 MHz) of compound (1) isolated from leaves of Stemodia maritima.

Figure S4. COSY NMR experiment (CDCl$_3$, 500 MHz) of compound (1) isolated from leaves of Stemodia maritima.
Figure S5. $^{13}$C RMN spectrum (CDCl$_3$, 125MHz) of compound (1) isolated from leaves of *Stemodia maritima*.

Figure S6. DEPT NMR experiment (CDCl$_3$, 125 MHz) of compound (1) isolated from leaves of *Stemodia maritima*. 
Figure S7. NOESY NMR experiment (CDCl₃, 500 MHz) of compound (1) isolated from leaves of Stemodia maritima.

Figure S8. Expansion NOESY NMR experiment (CDCl₃, 500MHz) of compound (1) isolated from leaves of Stemodia maritima.
Figure S9. ESI-MS/MS of compound (1) isolated from leaves of *Stemodia maritima*.

Figure S10. IR spectrum of compound (1) isolated from leaves of *Stemodia maritima*.
Figure S11. HSQC NMR experiment (CDCl₃, 500×125 MHz) of compound (1) isolated from leaves of *Stemodia maritima*.

Figure S12. Expansion 1 HSQC NMR experiment (CDCl₃, 500×125 MHz) of compound (1) isolated from leaves of *Stemodia maritima*. 
Figure S13. Expansion 2 HSQC NMR experiment (CDCl₃, 500×125 MHz) of compound (1) isolated from leaves of Stemodia maritima.

Figure S14. HMBC NMR experiment (CDCl₃, 500×125 MHz) of compound (1) isolated from leaves of Stemodia maritima.
Figure S15. Expansion 1 HMBC NMR experiment (CDCl₃, 500×125 MHz) of compound (1) isolated from leaves of Stemodia maritima.

Figure S16. Expansion 2 HMBC NMR experiment (CDCl₃, 500×125 MHz) of compound (1) isolated from leaves of Stemodia maritima.
Figure S17. Expansion 3 HMBC NMR experiment (CDCl₃, 500×125 MHz) of compound (1) isolated from leaves of *Stemodia maritima*.

Figure S18. ¹H NMR spectrum (CDCl₃, 500 MHz) of compound (2) isolated from leaves of *Stemodia maritima*. 
Figure S19. COSY NMR experiment (CDCl₃, 500 MHz) of compound (2) isolated from leaves of Stemodia maritima.

Figure S20. ¹³C RMN spectrum (CDCl₃, 125MHz) of compound (2) isolated from leaves of Stemodia maritima.
Diterpene and other Constituents from *Stemodia maritima* (Scrophulariaceae)

**Figure S21.** DEPT NMR experiment (CDCl₃, 125 MHz) of compound (2) isolated from leaves of *Stemodia maritima*.

**Figure S22.** IR spectrum of compound (2) isolated from leaves of *Stemodia maritima*.
Figure S23. MS of compound (2) isolated from leaves of *Stemodia maritima*.

Figure S24. HMBC NMR experiment (CDCl₃, 500×125 MHz) of compound (2) isolated from leaves of *Stemodia maritima*. 
Figure S25. HSQC NMR experiment (CDCl₃, 500×125 MHz) of compound (2) isolated from leaves of Stemodia maritima.

Figure S26. NOESY NMR experiment (CDCl₃, 500 MHz) of compound (2) isolated from leaves of Stemodia maritima.
Figure S27. Expansion NOESY NMR experiment (CDCl₃, 500MHz) of compound (2) isolated from leaves of *Stemodia maritima*.

Figure S28. ¹H NMR spectrum (CDCl₃, 500 MHz) of d-mannitol isolated from stems of *Stemodia maritima*. 
Figure S29. $^{13}$C NMR spectrum (CDCl$_3$, 125 MHz) of D-mannitol isolated from stems of *Stemodia maritima*.

Figure S30. DEPT NMR experiment (CDCl$_3$, 125 MHz) of D-mannitol isolated from stems of *Stemodia maritima*. 
Figure S31. $^1$H NMR spectrum (C$_5$D$_5$N, 500 MHz) of betulinic acid isolated from stems of *Stemodia maritima*.

Figure S32. $^{13}$C RMN spectrum (C$_5$D$_5$N, 125MHz) of betulinic acid isolated from stems of *Stemodia maritima*.
Figure S33. DEPT NMR experiment (C$_5$D$_5$N, 125 MHz) of betulinic acid isolated from stems of Stemodia maritima.

Figure S34. HMBC NMR experiment (C$_5$D$_5$N, 500x125 MHz) of betulinic acid isolated from stems of Stemodia maritima.
Figure S35. HSQC NMR experiment (C₅D₅N, 500×125 MHz) of betulinic acid isolated from stems of *Stemodia maritima*.

Figure S36. ¹H NMR spectrum (C₅D₅N, 500 MHz) of steroidal mixture of β-O-β-D-glucopyranosyl-β-sitosterol and 3β-O-β-D-glucopyranosylstigmasterol isolated from stems of *Stemodia maritima*. 
Figure S37. $^{13}$C NMR spectrum (C$_{5}$D$_{5}$N, 125MHz) of steroidal mixture of $\beta$-O-$\beta$-D-glucopyranosyl-$\beta$-sitosterol and 3$\beta$-O-$\beta$-D-glucopyranosylstigmasterol isolated from stems of *Stemodia maritima*.

Figure S38. DEPT NMR experiment (C$_{5}$D$_{5}$N, 125 MHz) of steroidal mixture of $\beta$-O-$\beta$-D-glucopyranosyl-$\beta$-sitosterol and 3$\beta$-O-$\beta$-D-glucopyranosylstigmasterol isolated from stems of *Stemodia maritima*.
Figure S39. 1H NMR spectrum (CD$_3$OD, 500 MHz) of 5,7,4’-trihydroxy-3,8,3’-trimethoxyflavone isolated from leaves of Stemodia maritima.

Figure S40. COSY NMR experiment (CD$_3$OD, 500 MHz) of 5,7,4’-trihydroxy-3,8,3’-trimethoxyflavone isolated from leaves of Stemodia maritima.
Figure S41. $^1$C NMR spectrum (CD$_3$OD, 125MHz) of 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone isolated from leaves of Stemodia maritima.

Figure S42. HMBC NMR experiment (CD$_3$OD, 500×125 MHz) of 5,7,4'-trihydroxy-3,8,3'-trimethoxyflavone isolated from leaves of Stemodia maritima.
Figure S43. HSQC NMR experiment (CD$_2$OD, 500×125 MHz) of 5,7,4’-trihydroxy-3,8,3’-trimethoxyflavone isolated from leaves of *Stemodia maritima*.