P-Coumaric Acid Ester with Potential Antioxidant Activity from the Genus *Salvia*

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ABSTRACT

A phytochemical analysis of the acetone extract of the aerial parts of the plants *Salvia splendens* and *Salvia lanigra* yielded a long chain alkyl *p*-coumaric acid ester; eicosanyl-*cis*-*p*-coumarate **(1)**, which has not previously been isolated from *Salvia* genus, together with two triterpenoids; oleanolic acid **(2)** and echinocystic acid **(3)** and two flavonoids; 7-methoxyapigenin **(4)** and luteolin-7-O-glucoside **(5)**. The structures of these compounds were assigned by spectroscopic analysis and comparison with literature data of known compounds. The antioxidant potential of the *p*-coumaric acid ester **(1)** was evaluated, in *vitro*, by using DPPH for free radical scavenging activity. A moderate free radical scavenging ability was observed.

Keywords: *p*-Coumaric acid ester, NMR, *Salvia*, antioxidant activity.

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INTRODUCTION

Salvia is an important genus widely cultivated and used in flavoring and folk medicines. Phytochemical investigations have shown that *Salvia* genus is mainly rich in diterpenoids, tanshinones,[1-6] triterpenoids,[7-9] as well as flavonoids and other phenolic compounds.[10, 11] These compounds constitute the major secondary metabolites of *Salvia* genus and show interesting spectra of biological activities.[12,13] Previous phytochemical studies with *S. splendens* and *S. lanigra* have led to the isolation of diterpenes, flavonoids, anthocyanin, caffiec acid dimmer, sterol and triterpenes.[14-17] This study was undertaken to perform a phytochemical analysis of the acetone extract of the aerial parts of the plants *S. splendens* and *S. lanigra* which yielded one phenolic compound which proved to be a *p*-coumaric acid ester of a long chain fatty acid, and has been identified as eicosanyl-*cis*-*p*-coumarate **(1)**. This is the first report of isolation of eicosanyl-*p*-coumarate from *Salvia* genus. This compound is also tested for the antioxidant activity. Triterpenoids; oleanolic acid **(2)** and echinocystic acid **(3)** and flavonoids; 7-methoxyapigenin **(4)** and luteolin-7-O-glucoside **(5)** were also isolated.

MATERIAL AND METHODS

UV spectra were determined with a Hitachi 340 spectrophotometer; IR spectra were carried out on a Nicollet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. The ^{1}H and ^{13}C NMR measurements were obtained with a Bruker NM spectrometer operating at 300, and 400 MHz (for ^{1}H) and 75 and 100 MHz (for ^{13}C) in DMSO-d_6 or Acetone-d_6 solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (J) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135°). FAB mass was performed on a VGZAB-HF reversed geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.). EIMS was carried on Scan EIMS-TIC, VG-ZAB-HF, X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.). Si gel (Si gel 60, Merck), were used for open column chromatography. Flash column liquid chromatography was performed using J.T. Baker glassware with 40 μm Si gel (Baker) and Sepralyte C_{18} (40 μm) as the stationary phase. TLC was carried out on precoated silica gel 60 F_{254}

(Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 for 5 min and diazotized sulfanilic acid (Pauly's reagent) for phenols. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), and α-tocopherol (TOC), were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany).

Plant Material

The plant materials used in this work consisted of the leaves and stems of *Salvia splendens* Sello collected in April, 2002 from Zoo garden Giza, Egypt. The leaves and stems of *Salvia lanigra* Poir**,** collected in April, 2002, from Borg Al-Arab Desert, Alexandria, Egypt. The two plants were authenticated by Late Dr. Nabil El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University, and Agricultural Engineer Badia Hassan Aly Dewan, Consultant of Egyptian Flora, Agricultural Museum, Dokki, Giza, Egypt.

Extraction and Isolation.

Salvia splendens **Sello:** The air-dried aerial parts of the plant (2 kg) were extracted with acetone to give 76 g of crude extract. The acetone extract was chromatographed on silica gel column, eluted with solvent systems of n-hexane, n-hexane: EtOAc (95:05-70:30), to give seven fractions (A-G). Fr. D (1.5g) was rechromatographed on a silica gel flash column (Petroleum ether: EtOAc- 90:10-75:25) and Sepralyte C_{18} flash column (H_2O: MeOH-60:40-85:15) to give two fractions of D1 and D2. Fr. D1 (140mg) was further subjected to silica gel column using Petroleum ether: EtOAc (85:15-80:20) and sephadex LH-20 (MeOH) to afford 25 mg of compound **1**. Fr. E (1.25g) was subjected to silica gel column (Petroleum ether: EtOAc- 90:10-75:25), silica gel flash column using n-hexane: EtOAc (90:10-70:30) and Sepralyte C_{18} flash column (H_2O: MeOH- 70:30-90:10) to give two fractions of E1 and E2. Fr. E2 (76mg) was rechromatographed on a silica gel column, eluted with *n*-hexane-ethyl acetate: 85:15 -75:25 to afford compounds **2** (30 mg) and **3** (25 mg).

Salvia lanigra **Poir:** Three kg of air-dried powdered (leaves and stems) of *Salvia lanigra* were subjected to exhaustive extraction with acetone (3 x 12 L). The combined acetone extract was concentrated under *vacuo* at 40°C to dryness (140 g). The marc was then partitioned several times with *n*-butanol (3 x 3 L), the concentrated *n*-butanol extract was concentrated under *vacuo* at 40°C to dryness (14 g). The acetone extract was applied to silica gel column using n-hexane: EtOAc (100:00-70:30), to yield six fractions (A-F). Fr. F (4.6g) was chromatographed over silica gel flash column using n-hexane: EtOAc (90:10-80:20) to give two fractions of F1 and F2. Fr. F1 (1.05g) was further subjected to silica gel column using n-hexane: EtOAc (90:10-80:20), Sepralyte C_{18} flash column (H_2O: MeOH- 65:35-100:0) and Sephadex LH-20 column (MeOH) to afford 73mg of compound **4**. The n-butanol fraction was subjected to silica gel column chromatography using $CHCl_3$: MeOH (100:00-70:30) to give six fractions (A-F). Fraction B (370mg) was further subjected to silica gel column using $CHCl_3$: MeOH (95:5-80:20), silica gel flash column using CH_2Cl_2: MeOH (90:10-75:25), Sepralyte C_{18} flash column (H_2O: MeOH-40:60-20:80) and Sephadex LH-20 column using MeOH and $CHCl_3$: MeOH (15:85) to afford 37 mg of compound **5**.

Free radical scavenging activity

The free radical scavenging activity was determined by the DPPH assay described by Blois (1958).[18] In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of sample solutions in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.[19]

$$\text{DPPH Scavenging Effect (\%)} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}}\right] \times 100$$

RESULTS

Eicosanyl-*cis-p*-coumarate (1): Colorless amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 315, 225, 215; IR υ_{max} (KBr) cm^{-1}: 3420 (br, OH) 1715 (C=O) 1612, 1465, 1169, 905, 735; ^1H and ^{13}C NMR (see **Table 1**); EIMS, (70 ev) *m/z* 444 $[M]^+$, 166, 164, 147, 125, 107, 83, 69, 57, 43.

Oleanolic acid (2): An amorphous solid [MeOH]; IR υ_{max} (KBr) cm^{-1}: 3430, 2955, 1695, 1640; ^1H NMR (400 MHz, Acetone-d_6) δ 5.20 (1H, brs, H-12), 2.99 (1H, dd, *J*= 10.0, 5.0 Hz, H-3), 1.11 (3H, s, H_3-27), 0.90 (3H, s,

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H_3-30), 0.88 (6H, s, H_3-23, 25), 0.84 (3H, s, H_3-29), 0.72 (3H, s, H_3-26), 0.68 (3H, s, H_3-24); ^{13}C NMR (100.0 MHz, Acetone-d_6) δ 179.74 (C-28, *s*), 144.61 (C-13, *s*), 122.56 (C-12, *d*), 78.16 (C-3, *d*), 55.79 (C-5, *d*), 48.10 (C-9, *d*), 47.48 (C-17, *s*), 46.49 (C-19, *t*), 42.13 (C-14, *s*), 41.80 (C-18, *d*), 40.12 (C-8, *s*), 38.96 (C-4, *s*), 38.86 (C-1, *t*), 37.41 (C-10, *s*), 33.22 (C-21, *t*), 32.98 (C-29, *q*), 32.26 (C-22, *t*), 32.10 (C-7, *t*), 31.02 (C-20, *s*), 28.53 (C-23, *q*), 29.01 (C-2, *t*), 28.04 (C-15, *t*), 27.47 (C-27, *q*), 23.78 (C-30, *q*), 23.75 (C-16, *t*), 23.35 (C-11, *t*), 18.78 (C-6, *t*), 17.41 (C-26, *q*), 16.12 (C-24, *q*), 15.51 (C-25, *q*); EIMS (70 eV) *m\z* 456 $[M]^+$, 248, 203.

Echinocystic acid (3): An amorphous solid [MeOH]; IR υ_{max} (KBr) cm^{-1}: 3435, 2950, 1690, 1640; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.20 (1H, brs, H-12), 3.00 (1H, dd, *J*= 10.50, 5.0 Hz, H-3), 1.30 (3H, s, H_3-27), 0.92 (3H, s, H_3-30), 0.88 (3H, s, H_3-23), 0.85 (3H, s, H_3-25), 0.84 (3H, s, H_3-29), 0.68 (3H, s, H_3-26), 0.66 (3H, s, H_3-24); ^{13}C NMR (100.0 MHz, DMSO-d_6) δ 177.50 (C-28, *s*), 144.16 (C-13, *s*), 121.17 (C-12, *d*), 76.80 (C-3, *d*), 54.79 (C-5, *d*), 46.15 (C-9, *d*), 47.40 (C-17, *s*), 46.40 (C-19, *t*), 41.00 (C-14, *s*), 40.01 (C-18, *d*), 39.05 (C-8, *s*), 38.30 (C-4, *s*), 38.11 (C-1, *t*), 36.50 (C-10, *s*), 35.30 (C-21, *t*), 32.82 (C-29, *q*), 31.39 (C-22, *t*), 32.67 (C-7, *t*), 30.30 (C-20, *s*), 28.22 (C-23, *q*), 26.99 (C-2, *t*), 34.64 (C-15, *t*), 26.50 (C-27, *q*), 24.16 (C-30, *q*), 72.99 (C-16, *t*), 22.79 (C-11, *t*), 18.02 (C-6, *t*), 16.83 (C-26, *q*), 16.00 (C-24, *q*), 15.20 (C-25, *q*); EIMS (70 eV) *m\z* 472 $[M]^+$, 264, 246, 231, 207, 201, 105, 57.00.

Table 1. ^{1}H and ^{13}C NMR data of compound (1) (300.0 MHz for ^{1}H and 75.0 MHz for ^{13}C, DMSO- d_6)

Position	^{1}H (*J* in Hz)	^{13}C	DEPT
1	-	128.71	C
2	7.64, d, 8.7	132.38	CH
3	6.74, d, 8.7	114.93	CH
4	-	157.52	C
5	6.74, d, 8.7	114.93	CH
6	7.64, d, 8.7	132.38	CH
7	6.85, d, 13.0	143.89	CH
8	5.80, d, 13.0	118.64	CH
9	-	166.40	C
1’	4.15, t, 6.5	65.48	CH_2
2’	1.69, m	28.75	CH_2
3’-17’	1.35, m	28.72-29.78	CH_2
18’	1.35, m	31.94	CH_2
19’	1.25, m	22.35	CH_2
20’	0.86, t, 7.4	14.78	CH_3

7-methoxyapigenin (4): A pale yellow needles [MeOH]; UV λ_{max} (MeOH) nm: 270, 335; IR ν_{max} (KBr) cm^{-1}: 3420, 1695, 1590; ^{1}H NMR spectral data (400 MHz, acetone -d_6) δ 12.90 (1H, *brs*, 5-OH), 10.50 (1H, *brs*, 4’-OH), 7.96 (2H, *d*, *J*= 8.7 Hz, H-2`,6`), 7.04 (2H, *d*, *J*= 8.7 Hz, H-3`,5`), 6.68 (1H, *d*, *J*= 2.0 Hz, H-8), 6.66 (1H, *s*, H-3), 6.32 (1H, *d*, *J*= 2.0 Hz, H-6), 3.92 (3H, *s*, OCH_3); ^{13}C NMR spectral data (100 MHz, acetone-d_6) δ 183.17 (*s*, C-4), 166.56 (C-2), 165.28 (*s*, C-7), 163.10 (*s*, C-5), 163.08 (*s*, C-4` ), 158.66 (*s*, C-9 ), 129.30 (*d*, C-2`,6`), 119.76 (*s*, C-1`), 116.88 (*d*, C-3`,5`), 104.28 (*s*, C-10), 104.21 (*d*, C-3), 98.67 (*d*, C-6), 93.20 (*d*, C-8), 56.40 (*s*, OCH_3); EIMS *m/z* 284 $[M]^+$.

Luteolin-7-O-glucoside (5): A pale yellow needles [MeOH]; UV λ_{max} (MeOH) nm: 270, 340; IR ν_{max} (KBr) cm^{-1}: 3450, 1690, 1595; ^{1}H NMR spectral data (400 MHz, DMSO-d_6) aglycon δ 13.00 (1H, *brs*, 5-OH), 7.47 (1H, *dd*, *J*= 8.5, 2.2 Hz, H-6`), 7.40 (1H, *d*, *J*= 2.2 Hz, H-2`), 6.85 (1H, *d*, *J*= 8.5 Hz, H-5`), 6.68 (1H, *brs*, H-8), 6.65 (1H, *s*, H-3), 6.45 (1H, *brs*, H-6); sugar moiety δ 5.10 (1H, *d*, *J*= 7.5 Hz, H-1``), 3.82 (1H, *dd*, *J*= 11.5/3.2 Hz, H-$6_b$``), 3.70 (1H, *dd*, *J*= 11.5, 5.5 Hz, H-6_a``), 3.55 (1H, *m*, H-5``), 3.24 (1H, *t*, *J*= 9.3 Hz, H-3``), 3.20 (1H, *dd*, *J*= 7.5, 9.3 Hz, H-2``), 3.19 (1H, *t*, *J*= 9.3 Hz, H-4``); ^{13}C NMR spectral data (100 MHz, DMSO-d_6) aglycon δ 181.29 (*s*, C-4), 166.50 (*s*, C-2), 164.23 (*s*, C-7), 162.90 (*s*, C-5), 157.14 (*s*, C-9), 148.54 (*s*, C-4`), 145.90 (*s*, C-3`), 120.80 (*s*, C-1`), 119.50 (*d*, C-6`), 115.95 (*d*, C-5`), 113.27 (*d*, C-2`), 105.55 (*s*, C-10), 102.83 (*d*, C-3), 99.84 (*d*, C-6), 94.27 (*d*, C-8); sugar moiety δ 99.45 (*d*, C-1``), 77.14 (*d*, C-5``), 76.38 (*d*, C-3``), 73.09 (*d*, C-2``), 69.51 (*d*, C-4``), 61.00 (*t*, C-6``); FABMS *m/z* 451 $[M+H]^+$, 289 $[\text{Aglycon} + H]^+$.

Table 2. DPPH radical scavenging activity (%) of compound (1), BHT, and α-tocopherol*

Sample	10 μg	25 μg	50 μg	100 μg
Compound (1)	29.64 ± 0.23	62.60± 0.64	81.53 ± 0.23	88.46 ± 0.32
BHT	52.30 ± 0.57	79.55 ± 0.58	90.72 ± 0.41	93.64 ± 0.45
α-Tocopherol	65.52 ± 0.89	91.78 ± 0.42	92.85 ± 0.37	95.27 ± 0.74

* Values expressed are means ± SD of three parallel measurements ($p < 0.05$).

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DISCUSSION

The air-dried leaves and stems of *S. splendens* and *S. lanigra* were subjected to exhaustive extraction with acetone. The acetone extract of *S. splendens* was repeatedly chromatographed over normal-phase silica gel, silica gel for flash and reversed-phase silica gel to give one phenolic ester; eicosanyl-*cis*-*p*-coumarate **(1)**, together with the known triterpenoids; oleanolic acid **(2)** and echinocystic acid **(3)**. The acetone extract of *S. lanigra* was concentrated and partitioned with *n*-butanol. Both acetone and *n*-butanol extracts of *S. lanigra* were subjected separately to a series of column chromatography to give two known flavonoids; 7-methoxyapigenin **(4)** and luteolin-7-O-glucoside **(5)**, respectively. Compounds **2-5** were identified by comparing their physical and spectral data with those reported previously.[20-23]

Compound **1**: was obtained as an amorphous solid. The $[M]^+$ at *m/z* 440 was in agreement with the molecular formula $C_{29}H_{48}O_3$, which is also supported by NMR. A strong peak at m/z 147 and a base peak at m/z 164 corresponded to hydroxy cinnamaldehyde and hydroxy cinnamic acid ions,[24] thus clearly indicating *p*-coumaric acid moiety in **1**. The IR spectrum of **1** displayed an ester carbonyl at 1715 cm^{-1} and an aromatic hydroxyl at 3420 cm^{-1} as a weak absorption band. The UV spectrum showed maxima at 315, 225 and 215 nm. The ^1H NMR spectrum (**Table 1**) indicated the presence of a *p*-coumaric acid moiety attached to a large hydrocarbon chain. It showed prominent signals for *cis*-*p*-coumarate moiety at δ 7.64 (2H, *J*=8.7 Hz, C-2, 6), δ 6.74 (2H, *J*=8.7 Hz, C-3, 5), suggested a *para* substituted benzene ring. Together with two olefinic proton signals at δ 6.85 (1H, *d*, *J*=13 Hz, C-7) and δ 5.80 (1H, *d*, *J*=13 Hz, C-8). The coupling constant of 13 Hz between H-7 and H-8 confirm the *cis* geometry.[25] In addition, the peaks at δ 4.15 (*J*=6.5 Hz, H_2-1') for methylene adjacent to oxycarbonyl function,[26] δ 1.69 (2H, m, H-2'), δ 1.35 (30 H, m, H-3'-H-18'), 1.25 (2H, m, H-19'), δ 0.86 (3H, *t*, *J* = 7.4 Hz, H_3-20') indicated a long chain eicosanyl moiety. ^1H NMR spectrum of **1** (**Table 1**) was almost identical with that isolated previously,[27] having a *p*-coumaric acid moiety esterified with long chain alkyl group, thus confirming **1** as eicosanyl-*cis*-p-coumarate. The ^{13}C NMR (DEPT) spectrum of **1** was in close agreement with the given structure (**Table 1**). It showed signals for *cis*-*p*-coumarate moiety; methine carbon peaks observed at δ 132.38 and δ 114.93 corresponding to two pairs of unsubstituted aromatic carbons (C-2, 6 and C-3, 5, respectively), two peaks at δ 157.52 and δ 128.71 for quaternary aromatic carbons (C-1 and C-4, respectively), two olifenic carbon peaks at δ 143.89 (C-7) and δ 118.64 (C-8) and a peak for an ester carbonyl at δ 166.40 (C-9). Compound **1** also showed signals for a methylene attached to oxycarbonyl function at δ 65.48 (C-1'), an end methyl group at δ 14.78 (C-20'), of the eicosanyl moiety. Methylene signals were also observed for eicosanyl moiety carbons at δ 31.94 (C-18'), 29.78-28.72 (C-3'- C-17'), 28.75 (C-2') and 22.35 (C-19'). The length of the non

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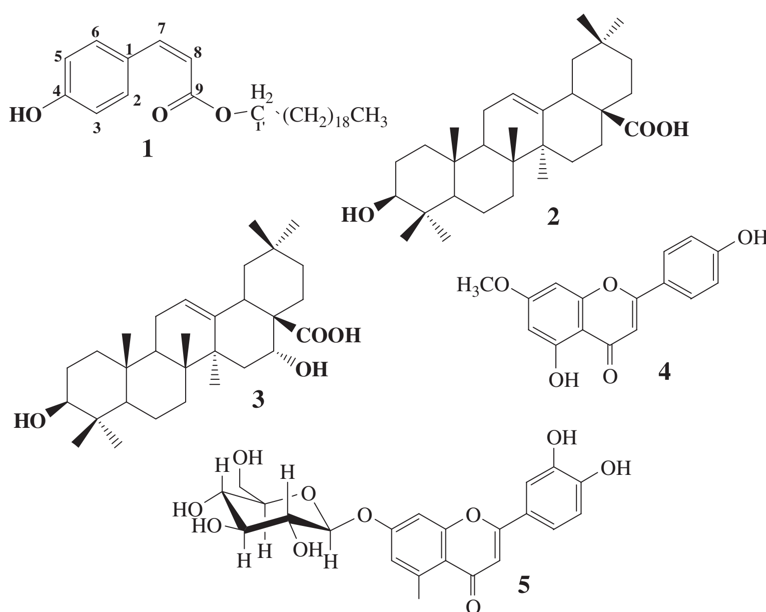

Figure 1. Structure of compounds 1-5.

oxygenated methylene chain in eicosanyl moiety was determined as 18 carbons, based on ^{1}H NMR and mass spectral data. In view of the data, **1** was identified as eicosanyl-*cis*-*p*-coumarate **(1)**. Though this ester was also isolated from the genus *Leptospermum* and genus *Psiadia,*[27,28] this is the first report of isolation of eicosanyl-*p*-coumarate from the genus *Salvia*.

The antioxidant activity of compound **1** was evaluated by free radical scavenging activities. It exhibited a moderate DPPH free radical scavenging activity competing with the standards BHT, and -tocopherol (**Table 2**) except for 10 μg/mL concentration. The radical scavenging activity was dependent on concentration and was increased with increased amount of sample.

Statistical analysis

All data of antioxidant activity tests are mean values of triplicate analyses. The data were recorded as mean ± standard deviation. Significant differences between means were determined by student's t-test, p values <0.05 were regarded as significant.

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