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CAFFEIC ACID DECYL ESTER: AN ANTIOXIDANT PRINCIPLE FROM *Phleum pratense*

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*The isolation, comprehensive structure elucidation, and assessment of the free radical scavenging activity of caffeic acid decyl ester (**1**) from the seeds of* Phleum pratense *have been described. The RC_{50} value of this compound in the DPPH assay was found to be 3.60×10^{-3} mg/mL, whereas that of positive control, trolox, was 3.07×10^{-3} mg/mL.*

Key words: *Phleum pratense*, Poaceae, DPPH, antioxidant, caffeic acid decyl ester.

Phleum pratense L. of the family Poaceae (alt. Gramineae), commonly known as 'Timothy grass' or 'meadow cat's-tail,' is an erect perennial, native to Algeria, Morocco, and Tunisia of northern Africa, Armenia, Azerbaijan, China, Georgia, Iran, Iraq, Kazakhstan, Kyrgyzstan, Mongolia, Russian Federation, and Turkey of temperate Asia, India and Pakistan of tropical Asia, and Austria, Albania, Belarus, Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Moldova, Norway, Poland, Portugal, Romania, Spain, Sweden, Switzerland, United Kingdom, Ukraine, and former Yugoslavia of Europe [1, 2]. This species has also been naturalized in many other countries throughout the temperate region. Stored sterile extracts of *P. pratense* have been used in folkloric medicine to arrest the growth of Sarcoma 45 and other tumor types [3]. The extracts of the seeds of *P. pratense* has been reported to possess antibacterial properties against *Citrobacter freundii* and methicillin-resistant *Staphylococcus aureus*, antioxidant activity in the DPPH assay, and to show significant general toxicity towards brine shrimps [4]. Previous phytochemical investigations revealed the presence of various organic acids in the aerial parts, mono- and disaccharides in the roots, flavonoids and their glycosides in the pollens of *P. pratense*, but none from the seeds [5, 6]. We here report on the isolation, structure elucidation, and assessment of free radical scavenging activity of caffeic acid decyl ester (**1**) from the seeds of *P. pratense*.

A combination of vacuum liquid chromatography (VLC) and preparative thin layer chromatography (PTLC) of the dichloromethane (DCM) extract of *P. pratense* yielded compound **1**. The HRFABMS analysis of **1** showed the $[M+Na]^+$ ion at *m/z* 343.1884 calc. 343.1885 for $C_{19}H_{28}O_4Na$, confirming the molecular formula $C_{19}H_{28}O_4Na$.

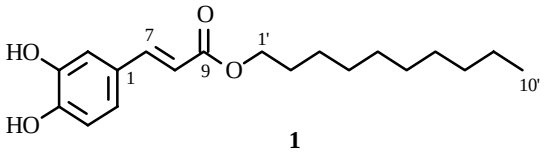

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TABLE 1. ^{1}H NMR (400 MHz, Coupling Constant J/Hz in Parentheses) and ^{13}C NMR (100 MHz) Data of **1** Together with Key ^{1}H-^{13}C Long-Range HMBC Correlation

Atom C	Chemical shift (δ) in ppm		^{1}H-^{13}C HMBC long-range correlation	
	^{1}H	^{13}C	^{2}J	^{3}J
1	-	127.2	-	-
2	7.03 (d, J = 1.8)	115.4	C-1, C-3	C-4, C-6, C-7
3	-	146.3	-	-
4	-	147.1	-	-
5	6.90 (d, J = 8.2)	116.1	C-4, C-6	C-1, C-3
6	7.05 (dd, J = 1.8, 8.2)	122.9	C-1, C-5	C-2, C-4, C-7
7	7.59 (d, J = 16.2)	147.8	C-1, C-8	C-2, C-6, C-9
8	6.29 (d, J = 16.2)	115.6	C-7, C-9	C-1
9	-	169.1	-	-
1′	4.68 (br.t, J = 6.8)	64.8	C-2′	C-9, C-3′
2′	2.75 m	31.7	C-1′, C-3′	C-4′
3′	1.26-2.40 m*	29.9	-	C-1′
4′	1.26-2.40 m*	28.8	-	C-2′
5-7′	1.26-2.40 m*	27.8	-	-
8′	1.26-2.40 m*	26.9	C-9′	-
9′	1.26-2.40 m*	25.6	C-10′	C-7′
10′	1.03 (t, J = 6.7)	14.8	C-9′	C-8′

Spectra were obtained in $CDCl_3$.
*Overlapped peaks.

The UV absorption maxima at 323 and 303 (sh) nm indicated the presence of aromaticity and $\alpha\beta$-unsaturated carbonyl functionality, and were in good agreement with the UV absorption maxima of caffeic acid derivatives. The IR spectrum displayed the absorption bands at 3442, 1741 and 1514–1463 cm^{-1}, corresponding to hydroxyl, carbonyl, and aromatic functionalities. The ^{1}H NMR spectrum showed signals characteristic for a caffeic acid moiety (δ 6.29, d, J = 16.2 Hz; 6.90, d, J = 8.2 Hz; 7.03, d, J = 1.8 Hz; 7.05, dd, J = 8.2, 1.8; 7.59, d, J = 16.2 Hz) and a decyl unit (δ 1.03, t, J = 6.7 Hz; 1.26–2.75 m; 4.68, br.t, J = 6.8 Hz).

The deshielded nature of the oxymethylene signal (δ 4.68) confirmed its link to the carbonyl of the caffeic acid moiety and thus provided evidence for ester formation. The ^{13}C NMR spectrum (Table 1) displayed signals for all 19 carbons including two oxygenated aromatic quaternary (δ 146.3 and 147.1), one aromatic quaternary (δ 127.2), three aromatic methine (δ 115.4, 116.1 and 122.9), two olefinic methine (δ 147.8 and 115.6), an ester carbonyl (δ 169.1), one oxymethylene (δ 64.8), one methyl (δ 14.8), and eight methylene carbons (δ 25.6, 26.9, 27.8 for three carbons, 28.8, 29.9 and 31.7), corresponding to the structure of caffeic acid decyl ester (**1**). The conclusive evidence for the ester formation was obtained from a ^{3}J correlation from the oxymethylene protons (δ 4.68) to the carbonyl carbon (C-9), observed in the ^{1}H–^{13}C HMBC spectrum (Table 1). Thus the compound was unambiguously identified as caffeic acid decyl ester (**1**), which is a new natural product. This is the first report on the occurrence of a caffeic acid ester in the seeds of *P. pratense*. To our knowledge, caffeic acid esters have not previously been reported from the genus *Phleum* either.

The DPPH assay is an easy and straightforward method for determining the free radical scavenging property of a compound. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant which can donate an electron to DPPH, the purple color, which is typical to free DPPH radical, decays, and the change in absorbance at 517 nm can be followed spectrophotometrically. Compound **1** exhibited remarkable antioxidant activity in the DPPH assay, and the RC_{50} value (3.60×10^{-3} mg/mL) was similar to that of the positive control, trolox (3.07×10^{-3} mg/mL). Caffeic acid and its derivatives, isolated from various plant species, have been found to have various degrees of free radical scavenging activities [7–11]. Since caffeic acid and its derivatives have been shown to have antitumor and immunomodulatory properties [12, 13], the free radical scavenging property of **1** may, at least to some extent, contribute to the antitumor properties of the *P. pratense* (folkloric use).

EXPERIMENTAL

General Procedures. IR spectra (KBr) were obtained using an AVATAR 360 FT-IR spectrometer. NMR spectra were recorded in $CDCl_3$ on a Varian Unity INOVA 400 MHz NMR Spectrometer 400 (400 MHz for ^1H and 100 MHz for ^{13}C) using the residual solvent peaks as internal standard. MS analyses were performed on a Finnigan MAT95 XP spectrometer. VLC and PTLC were carried out using, respectively, Merck Silica gel 60H and Merck Silica gel GF_{254}. HMBC spectra were optimized for a long range J_{H-C} of 9Hz.

Plant Material. The seeds of *P. pratense* (Cat No. 15231) were purchased from B & T World Seeds sarl, Paguigan, 334210 Olonzac, France. A voucher specimen (PH100 003) has been deposited in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

Extraction, Isolation and Structure Elucidation. The ground seeds of *P. pratense* (96.2 g) were Soxhlet extracted, successively, with *n*-hexane, DCM, and methanol (MeOH). The DCM extract was subject to VLC using a step gradient of *n*-hexane:ethyl acetate (100:0, 90:10, 80:20, 60:40, 40:60, 20:80, 0:100). Further purification of *n*-hexane:EtOAc (80:20) fraction was carried out by PTLC using chloroform ($CHCl_3$) as the mobile phase, resulting in the isolation of compound **1** (9.2 mg, R_f 0.51). The structure of **1** was determined by a combination of UV, IR, HR-FABMS, and NMR analyses.

Caffeic acid decyl ester (1) (yield: 9.56×10^{-3} % from dried seeds). UV (λ_{max}, MeOH, nm): 323, 303 (sh); IR (KBr, ν_{max}, cm^{-1}): 3442, 3012, 2926, 2853, 1741, 1514, 1463, 1377, 1267, 1169, 1040; HR-FABMS (positive ion mode) *m/z* found 343.1884 calc. 343.1885 for $C_{19}H_{28}O_4Na$. ^1H NMR (Table 1); ^{13}C NMR (Table 1).

Free Radical Scavenging Activity (DPPH assay). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs. The method used by Takao et al. [14] was adopted with appropriate modifications [15]. DPPH (4 mg) was dissolved in $CHCl_3$ (50 mL) to obtain a concentration of 80 μg/mL.

Qualitative assay: Test compound **1** was applied on a TLC plate and sprayed with DPPH solution using an atomizer. It was allowed to develop for 30 min. The color change (purple on white) was noted.

Quantitative assay: Compound **1** was dissolved in $CHCl_3$ to obtain a concentration of 0.5 mg/mL. Dilutions were made to obtain concentrations of 5×10^{-2}, 5×10^{-2}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6}, 5×10^{-7}, 5×10^{-8}, 5×10^{-9}, 5×10^{-10} mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control, trolox, a well-known commercial antioxidant.

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