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CAFFEIC ACID DERIVATIVES IN FRONDS OF THE LADY FERN (*ATHYRIUM FILIX-FEMINA*)

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Key Word Index—*Athyrium filix-femina*; fern; caffeic acid conjugates.

Abstract—A new caffeic acid amide, *N*-caffeoyl-phenylalanine, has been isolated from fronds of *Athyrium filix-femina* along with the known compounds *N*-caffeoyl-tryptophan, 5-*O*-caffeoyl-shikimic acid, 3,4-di-*O*-caffeoyl-quinic acid and chlorogenic acid. Their structures have been identified by spectroscopic analysis.

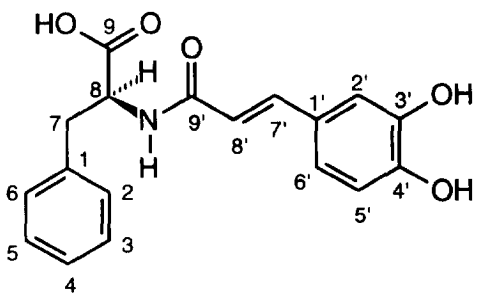
INTRODUCTION

The lady fern, *Athyrium filix-femina*, is one of the most abundant fern species in temperate regions of the northern hemisphere. Despite the fact that there are some reports on the occurrence of caffeic acid derivatives in this fern [1–3], these constituents have not been investigated in detail. This paper describes the isolation and identification of five caffeic acid conjugates from *A. filix-femina* fronds.

RESULTS AND DISCUSSION

Air-dried fronds were sequentially extracted with dichloromethane and methanol. The evaporated methanolic extract was taken up in water and partitioned between ethyl acetate and *n*-butanol. A combination of column chromatography (Sephadex LH-20), vacuum liquid chromatography (VLC) [4] and HPLC of the ethyl acetate-soluble fraction afforded the previously unreported caffeic acid amide *N*-caffeoyl-phenylalanine (**1**) along with *N*-caffeoyl-tryptophan 5-*O*-caffeoyl-shikimic acid, 3,4-di-*O*-caffeoyl-quinic acid and chlorogenic acid. Further purification of the butanol-soluble fraction by VLC and reversed phase HPLC led to the isolation of larger amounts of chlorogenic acid. Compound **1** was obtained as an amorphous powder. The IR spectrum displayed absorption bands characteristic of hydroxyl groups (3400 cm^{-1}), carboxyl and carbamide groups (1725, 1660, 1600 cm^{-1}) and aromatic rings (1515 cm^{-1}). The pseudo molecular ion peak at m/z 326.3 $[M - H]^-$ in the FAB-MS was in agreement with the molecular formula of $C_{18}H_{17}O_5N$.

The ^1H NMR in MeOH-d_4 showed compound **1** to contain 13 unexchangeable protons, as listed in Table 1. These consisted of eight aromatic, two olefinic and three aliphatic protons. The signals of the two olefinic protons at δ_H 6.65 and 7.49 in *trans*-configuration as shown by their coupling constant of 15.8 Hz, and the protons of



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a 1,3,4-substituted benzene ring (δ_H 6.56 (*dd*, J = 1.9, 8.0 Hz, H-6'), 6.67 (*d*, J = 8.1 Hz, H-5'), 6.69 (*d*, J = 2.0 Hz, H-2')) indicated the presence of a caffeic acid moiety. The remaining five aromatic proton signals appearing in the range of δ_H 7.33 to 7.38 were attributed to the monosubstituted benzene ring of a phenylalanine residue. According to their multiplicity, the three aliphatic proton signals at δ_H 2.91 (*dd*, J = 8.3, 14.0 Hz, H-7α), 3.10 (*dd*, J = 5.2, 14.0 Hz, H-7β) and 4.72 (*dd*, J = 5.3, 8.3 Hz, H-8) could be assigned to the 'ABX' spin system of the $-CH_2-CH <$ partial structure of phenylalanine.

The ^1H NMR in DMSO-d_6 showed three additional signals. The two singlets at δ_H 8.71 and 8.76 correspond to the phenolic OH groups of the caffeic acid moiety. The doublet at δ_H 8.31 (J = 8.0) coupling with the methine proton (H-8) of the phenylalanine side chain was attributed to an acidic amide proton involved in the amide linkage between the caffeic acid and phenylalanine moieties.

Acid hydrolysis of compound **1** yielded phenylalanine which was shown to be in the L-configuration based on the comparison of its optical rotation with that of authentic L-phenylalanine. Thus, compound **1** is *N-trans*-caffeoyl-L-phenylalanine.

The structures of *N*-caffeoyl-tryptophan, 5-*O*-caffeoyl-shikimic acid, 3,4-di-*O*-caffeoyl-quinic acid and

Table 1. ^{1}H NMR spectral data of compound **1** in DMSO-d_6 and MeOH-d_4

H	DMSO-d_6	MeOH-d_4
H-2, H-6	7.55, 2H *br d* (8.0)	7.53, 2H *br d* (8.0)
H-3, H-4, H-5	7.37–7.43, 3H *m*	7.33–7.38, 3H *m*
H-7α	2.74 *dd* (8.6, 13.9)	2.91 *dd* (8.3, 14.0)
H-7β	2.92 *dd* (4.9, 13.9)	3.10 *dd* (5.2, 14.0)
H-8	4.45 *ddd* (5.0, 8.1, 8.7)	4.72 *dd* (5.3, 8.3)
H-N	8.31, *d* (8.0)	
H-2′	6.62 *d* (2.0)	6.69 *d* (2.0)
H-5′	6.60 *s* (8.1)	6.67 *d* (8.1)
H-6′	6.48 *dd* (2.0, 8.0)	6.56 *dd* (1.9, 8.0)
H-7′	6.72 *d* (15.8)	6.65 *d* (15.8)
H-8′	7.38 *d* (15.8)	7.49 *d* (15.9)
OH-3′	8.71 s*	
OH-4′	8.76 *s**	

*Signals may be interchanged.

chlorogenic acid have been deduced from ^{1}H NMR, ^{13}C NMR and mass spectral data, in agreement with reported data in the literature [3, 5, 6].

The presence of 5-*O*-caffeoylshikimic acid and chlorogenic acid is not surprising. They represent common constituetnts of ferns [7]. Furthermore, 5-*O*-caffeoylshikimic acid has previously been detected in *A. filix-femina* by HPLC analysis [3]. The occurrence of the two caffeic acid amides, *N*-caffeoyl-tryptophan and *N*-caffeoyl-phenylalanine, in this fern is quite unusual. So far, *N*-caffeoyl-tryptophan is only known from green beans of *Coffea robusta* [5]. To date, the only reported caffeic acid conjugates related to *N*-caffeoyl-phenylalanine (**1**) are *N*-caffeoyl-tyrosine, also present in green *Coffea robusta* beans [8] and the *cis trans* isomeric clovamides with DOPA as amino acid moiety, found in red clover (*Trifolium pratense*) [9, 10] and ebony (*Dalbergia melanoxylon*) [11].

EXPERIMENTAL

Plant material. Athyrium filix-femina fronds were collected in Losheim-Scheiden (Saarland, Germany) in June 1993 and identified by the author. A voucher specimen is retained in the author's laboratory.

Extraction and isolation. Powdered air-dried plant material (320 g) was sequentially extracted with CH_2Cl_2 and MeOH at room temp. The MeOH extract (35 g) was dissolved in H_2O (500 ml), washed with EtOAc (500 ml × 3) and extracted with *n*-BuOH (300 ml × 3). Both EtOAc and *n*-BuOH solns were evapd. under red. pres. to afford 6 g and 12 g residue, respectively. The conc. EtOAc extract was chromatograped on Sephadex LH-20 (1500 mm × 25 mm i.d.) with MeOH–CH_2Cl_2 (4:1) as eluent to give 5 frs (EP 1–5). Fr. EP-1 was further separated by VLC (RP 8, 60 mm × 35 mm i.d., stepwise with MeOH–H_2O (1:4) and MeOH–H_2O (1:1) to afford **1** (160 mg). Fr. EP-2 was subjected to VLC on RP8 silica gel as described for fr. EP-1 and further purified by HPLC (RP 18, 250 mm × 8 mm i.d., MeOH–H_2O–HCOOH (8:12:1) to yield 3 mg of chlorogenic acid. Fr. EP-4 was separated by HPLC (RP18, 8 mm i.d. × 250 mm, MeOH–H_2O–HCOOH (8:12:1) to give *N*-caffeoyl-tryptophan (26 mg), 5-*O*-caffeoyl-shikimic acid (195 mg) and 3,4-di-*O*-caffeoyl-quinic acid (22 mg). The *n*-BuOH extract was chromatographed on RP 8 (VLC, 60 mm × 35 mm i.d.), eluting with a MeOH–H_2O gradient (30–70% MeOH in 10% steps). Fr. BP-1 was purified by HPLC (RP18 250 mm × 8 mm i.d., MeOH–H_2O–HCOOH (10:35:2)) to afford 320 mg of chlorogenic acid.

Acid hydrolysis. 20 mg of **1** were refluxed in 5 ml of M HCl for 1 hr. The mixture was then extracted with EtOAc. Phenylalanine was isolated from the evpd. aq. soln. by repeated prep. TLC on silica gel (0.5 mm, EtOAc–HOAc–HCOOH–H_2O (100:11:11:25)).

NMR spectra. Recorded in MeOH-d_4 and DMSO-d_6 (^{1}H NMR (400 MHz), ^{13}C NMR (100.5 MHz)) relative to MeOH-d_4 at δ_H 3.30, δ_C 49.0, DMSO-d_6 at δ_H 2.50, δ_C 40.8, respectively. ^{13}C multiplicities were determined using the DEPT pulse sequence. UV and optical rotations were measured in MeOH.

N-trans-Caffeoyl-L-phenylalanine. A light yellow amorphous powder, $[\alpha]_D^{20}$ − 20.0° (MeOH; *c* 0.72). FAB-MS *m/z*: 326.3 $[M-H]^-$. UV λ_{max} nm: 216, 222, 277, 305 (*sh*). IR ν^{KBr} cm^{-1}: 3400, 2550, 1725, 1660, 1600, 1515, 1450, 1350, 1280, 1205, 1110, 1170, 1025, 975, 855, 810, 765. ^{1}H NMR: see Table 1. ^{13}C NMR (MeOH-d_4) δ: 175.0 (*s*, C-9), 168.3 (*s*, C-9′), 146.0, 145.0 (*s* × 2, C-4′, C-3′), 142.3 (*d*, C-7′), 135.1 (*s*, C-1), 130.7 (*d*, C-4), 129.7 (*s*, C-1′), 129.7 (*d*, C-3, C-5), 128.7 (*d*, C-2, C-6), 121.7, 121.2 (*d* × 2, C-6′, C-2′), 117.4, 116.4 (*d* × 2, C-5′, C-8′), 55.5 (*d*, C-8), 37.8 (*t*, C-7).

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