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SHORT REPORTS

CAFFEOYLTRYPTOPHAN FROM GREEN ROBUSTA COFFEE BEANS

HIDEKO MORISHITA, YOSHIO TAKAI,* HITOSHI YAMADA,* FUSAKO FUKUDA,* MASAMI SAWADA,* HIDEO IWAHASHI† and RYO KIDO†

Faculty of Education, Wakayama University, Wakayama 640, Japan; *Material Analysis Center, The Institute of Scientific and Industrial Research, Osaka University, Ibaraki-shi 567, Japan; †Department of Biochemistry, Wakayama Medical College, Wakayama 640, Japan

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Key Word Index—*Coffea canephora*; Rubiaceae; robusta coffee bean; caffeoyltryptophan.

Abstract—A new compound, caffeoyltryptophan, was isolated from the coffee beans *Coffea canephora* and its structure was determined by FD mass, IR and ^{1}H NMR spectroscopy.

INTRODUCTION

The main group of phenolic compounds found in the green coffee bean are known as chlorogenic acids, which are esters of hydroxycinnamic acid with quinic acid [1–8]. During an investigation of phenolic compounds in unroasted robusta coffee bean (*Coffea canephora* var. *robusta*), we separated them into 11 components by HPLC [9]. Ten of them have been identified as quinic acid derivatives of caffeic acid, ferulic acid or both [6, 8, 9]. In this paper we describe the isolation and identification of an additional derivative of caffeic acid, caffeoyltryptophan (**1**).

RESULTS AND DISCUSSION

Compound **1** was isolated from the 70% 2-propanol extract of unroasted robusta coffee beans (*C. canephora*) by HPLC, as described in our previous paper [6]. The compound (R_t 25.5 min) eluted between 3,4-*O*-dicaffeoylquinic acid (R_t 24.6 min) and 3-*O*-caffeoyl-4-*O*-feruloylquinic acid (R_t 26.4 min) from the ODS column. The relative retention times to 3-*O*-caffeoylquinic acid were as follows: compound **1** (1.34), 3,4-*O*-dicaffeoylquinic acid (1.29) and 3-*O*-caffeoyl-4-*O*-feruloylquinic acid (1.39). (The free-IUPAC system of numbering for chlorogenic acids is used here.)

The structure of compound **1** was deduced from careful ^{1}H NMR investigations (Table 1). In $CDCl_3$–DMSO-d_6 (40:1), all ^{1}H NMR signals of the compound were assigned by spin decoupling. Two *trans*-vinylic protons at δ7.38 and 6.15 and a trisubstituted benzene ring at δ6.94, 6.79 and 6.75 indicated the presence of a cinnamoyl moiety. The remaining signals were close to those of tryptophan. The triple-triplets at δ4.90 were obviously due to a Cα-proton which was coupled with two Cβ-protons at δ3.36 and 3.32 and a NH proton at 6.43. A low-field signal at δ9.25 was due to N_1H. Two triplets at δ6.99 and 7.05 and two doublets at 7.01 and 7.28 were attributed to indole ring protons. In $CDCl_3$–CD_3OD (15:1), three signals at δ9.25 (NH), 7.9 (OH) and 6.43 (NH) disappeared owing to H–D exchange, which supported the above assignment of these signals.

Furthermore, the ^{1}H NMR spectrum of compound **1** in $CDCl_3$–DMSO-d_6 was compared with those of authentic tryptophan and caffeic acid, as summarized in Table 1. The data of compound **1** were similar to the sum of the two compounds. The peak positions and intensities clearly showed that the compound consisted of tryptophan and caffeic acid moieties in a molar ratio of 1:1.

The IR spectrum of compound **1** supported the structure deduced from the above ^{1}H NMR data. The strong bands of the IR spectrum were assigned as follows: IR (KBr) cm^{-1}: 3380 (OH), 3100 (NH), 1710 (C=O), 1650 (amido I), 1590 (amido II), 1416 (C=N in indole ring), 1510 (CH in benzene), and 1270 and 1210 (CO in phenol).

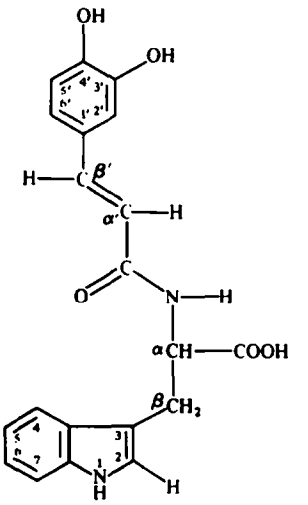

Table 1. ^{1}H NMR spectral data of compound 1, tryptophan and caffeic acid

Position	Compound 1*	Tryptophan†	Caffeic acid‡
N1-H	9.25 (*d*)		
C2-H	7.01 (*d*)	7.38	
C4-H	7.54 (*d*)	7.73	
C5-H	6.99 (*t*)		
C6-H	7.05 (*t*)	7.28	
C7-H	7.28 (*d*)	7.59	
Cα-H	4.90 (*tt*)	4.43	
Cβ-H	3.36 and 3.32 (*dd*, *dd*)	3.45 and 3.50	
Nα-H	6.43 (*d*)		
Cα'-H	6.15 (*d*)		6.17 (*d*)
Cβ'-H	7.38 (*d*)		7.41 (*d*)
C2'-H	6.94 (*d*)		7.02 (*s*)
C5'-H	6.75 (*d*)		6.76 (*d*)
C6'-H	6.79 (*dd*)		6.91 (*d*)
C3'-OH	7.9 (*br*)§		9.12 (*s*)§
C4'-OH			9.52 (*s*)§

J (Hz): compound 1: N1, 2 = 1.9; 4, 5 = 8.1; 5,6 = 7.9; 6,7 = 7.9; α,β = 5.5 and 5.5; NH, α = 7.4; α',β' = 15.7; 5',6' = 8.1; 2',6' = 2.0.

*Spectrum run in $CDCl_3$–DMSO-d_6 (40:1) at 360 MHz.

†The resonance lines of the tryptophan spectrum were in accordance with the data of ref. [10].

‡Spectrum run in DMSO-d_6 at 360 MHz.

§The discrepancy of the chemical shifts between caffeoyltryptophan and caffeic acid may be due to the two different solvents used.

The FD mass spectrum of compound 1 exhibited $[M+K]^+$, the pseudo-molecular ion, at *m/z* 405 as a base peak, and other cluster ions, $[M+H]^+$ and $[M+Na]^+$, at *m/z* 367 and 389, respectively. This indicates that the M_r was 366.

Elemental microanalysis of compound 1 gave C, 62.02; H, 4.99; and N, 6.33%. The existence of two nitrogen atoms in the molecule was assumed on the basis of elementary analysis and the nitrogen rule in mass spectrometry. Thus, the molecule calculated for $C_{20}H_{18}N_2O_5$ (C, 65.57; H, 4.92; N, 7.65%).

On the basis of the data described above the structure of compound 1 was determined as caffeoyltryptophan. The distribution and physiological significance of caffeoyltryptophan in plants remain to be elucidated.

EXPERIMENTAL

Plant material. Coffea canephora var. *robusta* (robusta coffee) beans from Java, harvested in 1983, were obtained commercially. The procedures of extraction and separation of constituents have been described in detail in previous papers [6, 8, 9].

Isolation of compound 1. Fractions on reversed-phase high-performance liquid chromatography (eluant 10 mM H_3PO_4–MeOH) showing an HPLC peak of R_t 25.5 min were collected to provide compound 1. After rechromatography with H_2O–MeOH as eluant, the eluant was lyophilized. A white amorphous powder was obtained (150 μg). UV $\lambda_{max}^{H_2O}$ nm: 290, 320.

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