INEPTRD microprogram using delays $D_2 = D_3 = 36$ msec, corresponding to $J_{C,H} = 7$ Hz.

Droplet counter-current chromatography (DCCC) separations were achieved on a Tokyo Rikakikai model A apparatus, equipped with 300 glass tubes. TLC was carried out on precoated silica gel layers (0.25 mm).

Isolation of **1**. Fr. tubers (5 kg) of *Asphodelus ramosus* L. (collected in the spring in Campania, Italy, and authenticated by the Botanical Garden of the University of Naples) were homogenized in a mechanical stirrer, freeze-dried and extd in a Soxhlet apparatus with petrol (12 hr) and then with Et_2O (12 hr). The Et_2O ext was evapd (3 g) and submitted to DCCC sepn (ascending mode) with $CHCl_3$–MeOH–H_2O (4:4:3, lower phase); flow rate 1 ml/hr. Fractions (30 ml) were collected, then the tubes were discharged (total vol 900 ml) and their contents, on the basis of TLC analysis (silica gel; $CHCl_3$–MeOH, 9:1), collected into six fractions: A (1.8 g), B (120 mg), C (130 mg), D (50 mg), E (80 mg), and F (220 mg) (in increasing order of polarity). Compound **1** was obtained by evapn of fraction F as an amorphous yellow solid, $[\alpha]_D$ −74° (MeOH; *c* 1.1). Elemental analysis: C 64.20%, H 4.89% (calc. for $C_{36}H_{32}O_{13}$: C 64.27%, H 4.80%). Spectral data: see text, Table 1 and Figs.

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CRASSIFOLIOSIDE, A CAFFEIC ACID GLYCOSIDE ESTER FROM *PLANTAGO CRASSIFOLIA*

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Key Word Index *Plantago crassifolia*; Plantaginaceae; caffeic acid glycoside ester; crassifolioside.

Abstract—The structure of crassifolioside, a phenylpropanoid glycoside isolated from *Plantago crassifolia* was shown to be β-(3′,4′-dihydroxyphenyl)-ethyl-(2,3-α-L-dirhamnosyl)-(4-*O*-caffeoyl)-β-D-glucopyranoside.

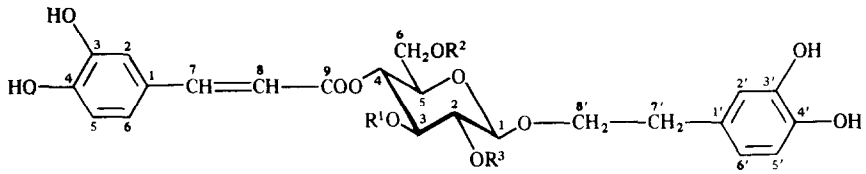
INTRODUCTION

During recent years, a large number of caffeic acid glycoside esters has been isolated from different plants and from callus cultures [1–6]. Most commonly studied is verbascoside (=acteoside), first isolated in 1963 from *Verbascum sinuatum L.* (Scrophulariaceae) [7], the structure of which was completely elucidated in 1982 [1]. While studying the chemotaxonomy by TLC of the genus *Plantago* [8], we observed in the extracts a new caffeic acid derivative. In this paper we describe the isolation and structure elucidation of this new molecule together with the well known verbascoside using extracts from leaves of *Plantago crassifolia* Forskäl.

RESULTS AND DISCUSSION

Crassifolioside (**1**) was found in leaves and in a higher concentration in roots of *Plantago crassifolia*. The compound was extracted from dried and finely powdered roots and purified as described in Experimental.

It was obtained as an amorphous pale yellow powder, with the elementary composition $C_{35}H_{46}O_{19}$. The FABMS gave, upon addition of Na^+ $[M+Na]^+$ at m/z =793, confirming the M_r as 770. Mild and total acid hydrolysis of **1** were carried out as described by Andary *et al.* [9] to give the hydrolytical products which were in good agreement with the ^1H NMR spectra. The ^1H NMR spectra of compound **1** (Table 1) showed the typical



		R^1	R^2	R^3
1	Crassifolioside	Rha(R′)	H	Rha(R″)
2	Poliumoside	Rha	Rha	H
3	Verbascoside	Rha	H	H

signals for the 3,4-dihydroxyphenylethanol moiety and the caffeoyl moiety. The glycosidic moiety indicated the presence of three sugars: two rhamnoses and one glucose, establishing that **1** was an isomer of poliumoside (**2**) [2].

The linkage determination between the three sugars and the aglycone was established with examination of chemical shift values of glucose which were in good agreement with a disubstitution by two rhamnose moieties. The deshielding effect ($\Delta\delta = 0.17$ ppm) of the anomeric proton H-1G (in comparison with that of verbascoside) indicated a substitution at the 2 position of the glucose. As a confirmation in orobanchoside [1] the resonance of the anomeric proton of the glucose, which is 2 substituted, shows a same effect. This deshielding was also observed with H-2G ($\Delta\delta = 0.14$ ppm) and H-3G ($\Delta\delta = 0.15$ ppm) for the same reason. The chemical shift values for H-4G, H-5G and H-6aG, H-6bG were at their usual values. The resonance of the rhamnose R′ was similar to those observed with verbascoside except that H-1R′ was shifted upfield ($\Delta\delta = 0.25$ ppm) due to the steric effect of the second rhamnose R″ at the 2 position of the glucose. This steric effect influenced in the same manner H-1R″ which was shifted upfield ($\Delta\delta = 0.23$ ppm). On the other hand H-3R″ and H-5R″ were deshielding in comparison with those of R′ unit. In order to confirm this linkage between sugar units, **1** was acetylated. A deshielding effect was not shown in the ^{1}H NMR spectrum of undecaacetyl crassifolioside in the glucose moiety, except for the two protons in the 6 position, confirming that the primary alcohol function was free. Moreover, acetylation conducted to typical lower field shifted signals for all the R′ and R″ protons except for H-1, H-5 and methyl group protons. Consequently, the structure of **1** is established as: β-(3′,4′-dihydroxyphenyl)-ethyl-(2,3-α-L-dirhamnosyl)-(4-*O*-caffeoyl)-β-D-glucopyranoside.

Table 1. ^{1}H NMR spectral data of crassifolioside in DMSO-d_6 + CF_3CO_2H (values in parenthesis are coupling constant in Hz)

	H	δ	
Aglycone	2′	6.61 *d*	(2)
	5′	6.63 *d*	(8)
	6′	6.46 *dd*	(2–8)
	7′	2.70 *m*	
	8′	3.56 *m*	(10.5)
		3.87 *m*	(10.5)
Caffeic acid	2	7.03 *d*	(2)
	5	6.76 *d*	(8)
	6	6.97 *dd*	(2–8)
	6	7.46 *d*	(16)
	8	6.20 *d*	(16)
Glucose	1	4.50 *d*	(8)
	2	3.37 *dd*	(8–9.5)
	3	3.85 *t*	(9.5)
	4	4.77 *t*	(9.5)
	5	3.50 *ddd*	(9.5–3–6.5)
	6	3.32 *dd*	(11.5–6.5)
		3.39 *dd*	(11.5–3)
Rhamnose′	1	4.80 *d*	(1.5)
	2	3.63 *dd*	(1.5–3.5)
	3	3.30 *dd*	(3.5–9.5)
	4	3.12 *t*	(9.5)
	5	3.34 *dd*	(9.5–6)
	6	0.98 *d*	(6)
Rhamnose″	1	4.78 *d*	(1.5)
	2	3.69 *dd*	(1.5–3.5)
	3	3.44 *dd*	(3.5–9.5)
	4	3.22 *t*	(9.5)
	5	3.85 *dd*	(9.5–6)
	6	1.14 *d*	(6)

EXPERIMENTAL

General. ^{1}H NMR spectra were recorded at 360 MHz (Bruker WM 360 WB) and chemical shifts are given in δ (ppm) relative to TMS as int. standard. FABMS were taken using argon atoms at 8 keV. Positive ion FAB-spectra were sampled on a Varian SS 200 Data system, calibrated in the electron impact mode.

TLC systems. TLC was conducted on cellulose plates (Merck 5552) with EtOAc–MeOH–H_2O–Me_2CO–$CHCl_3$ (120:22:22:10:3) using 'Neu' reagent (2-aminoethyl diphenylborinate 1% in MeOH) for detection. The TLC systems for partial and total hydrolyses are the same as described in ref. [9].

The chromatographic system, (A) to isolate **1** and **3** was a MPLC column (50 × 450 mm with precolumn: 10 × 100 mm, Büchi, Switzerland) packed with microcrystalline cellulose MN (Machery Nagel Co., ref. 81529) with EtOAc–MeOH–Me-$(CH_2)_4$Me–H_2O (90:15:5:11) as eluent; fractions were detected at $\lambda = 330$ nm.

Compounds **1** and **3** was purified in a chromatographic system (B) using an ordinary glass column (20 × 200 mm) packed with Sephadex LH-20 suspended in MeOH–H_2O (1:4).

Plant material. Plantago crassifolia plants were found in Carnon dunes, France, June 1983. Voucher specimens are deposited at Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Montpellier, France.

Extraction and isolation. 50 g finely powdered roots of *Plantago crassifolia* was extracted twice with 500 ml

MeOH–H_2O (7:3) for 30 min, with rotation, at 40°. The suspension was filtered and the filtrate was evapd *in vacuo* at 30° to 50 ml and shaked with 4 × 50 ml petrol–Et_2O (3:1). The water phase was separated and extracted with 8 × 50 ml EtOAc–MeOH (97:3). The organic phase was dried, filtered and evapd at 30° (442 mg). A part from this crude extract was dissolved in 3 ml MeOH, filtered and added to the chromatographic system A. The faster eluting zones were coned to yield **3** which was purified by system B and identified by partial hydrolysis and direct comparison with an authentic compound. The later zones yielded **1** which was purified by system B (5 mg), $[\alpha]_D^{20} = -110.94$ (MeOH; *c* 0.32), $R_f = 0.41$. FABMS: *m/z* 793 $[M+Na]^+$. For ^1H NMR (in DMSO-d_6) see Table 1.

Acetylation of **1**. Compound **1** was acetylated with Ac_2O (1 ml) and pyridine (1 ml) at room temp. overnight. After evapn of the reagents under vacuum and lyophilization, the undecaacetate was obtained. ^1H NMR ($CDCl_3$) for the glucose moiety, δ: 4.14 (1H, *dd*, $J = 11.5$; 6, H-6_aG), 4.08 (1H, *dd*, $J = 11.5$; 3.5, H-6_bG), 3.57 (1H, *m*, H-5G), 5.10 (1H, *t*, $J = 9.5$, H-4G), 3.98 (1H, *t*, $J = 9.5$, H-3G), 3.80 (1H, *dd*, $J = 7.5$; 9.5, H-2G), 4.43 (1H, *d*, $J = 7.5$, H-1G).

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DEFUSCIN, A NEW PHENOLIC ESTER FROM *DENDROBIUM FUSCESCENS*: CONFORMATION OF SHIKIMIC ACID*

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Key Word Index—*Dendrobium fuscescens*; Orchidaceae; defuscin; *n*-triacontyl *p*-coumarate; (−)-shikimic acid; conformation.

Abstract—Defuscin, a new phenolic ester shown to be *n*-triacontyl *p*-coumarate, and (−)-shikimic acid have been isolated from the whole plant of *Dendrobium fuscescens* Griff. The ^1H NMR spectrum of shikimic acid is indicative of the weighted average of its two half-chair conformers, the one with 4-OH and 5-OH in equatorial orientations being the major contributor.

INTRODUCTION

Dendrobium fuscescens Griff (Orchidaceae) [1, 2] is an epiphytic herb growing in Sikkim Himalayas, Khasia Mountains and Naga Hills at an altitude of 2300 m. It possesses pseudobulb stems with oblong lanceolate leaves and has purplish brown flowers. There is no previous report of work on this species though other *Denbrobium* species have been found to elaborate fluorenone derivatives [3–5], coumarins [6], a phenanthraquinone derivative [7], a polyoxygenated phenanthrene [8], alkaloids [9], spirophthalides [10], sesquiterpenes [11] and steroids [12]. From the whole plant, collected from the vicinity of Darjeeling, West Bengal, we have isolated and characterised a new phenolic ester designated defuscin (**1**), shown to be *n*-triacontyl *p*-coumarate, in addition to (−)-shikimic acid. To our knowledge, this is the first isolation of (−)-shikimic acid from an Orchidaceae plant.

RESULTS AND DISCUSSION

The petrol extract of the whole plant of *Dendrobium fuscescens* on extensive chromatography over silica gel furnished defuscin (**1**) from the petrol–ethyl acetate (17:3)

* Part V in the series 'On the Chemistry of Indian Orchidaceae Plants', For Part IV see ref [5].

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