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Long Chain Alkyl Esters of Ferulic and *p*-Coumaric Acid from *Bauhinia manca*

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Dedicated to Professor Hans Grisebach on the occasion of his 60th birthday

Bauhinia manca, Long Chain Esters of Ferulic and *p*-Coumaric Acid

17 Esters of ferulic acid and *p*-coumaric acid were isolated from the stem extract of *Bauhinia manca*; the alcohol components range from $C_{22}H_{45}$ to $C_{28}H_{57}$.

Introduction

Bauhinia manca (Leguminosae) is a vine growing in the lowland forests of Costa Rica and Panama [2]. In Costa Rica, an infusion of the stems and leaves is taken as an adstringent and diuretic, and as a remedy for diabetes [3, 4]. An antidiabetic effect is also claimed by a pharmacological study [5].

Since nothing is known on the constituents of *Bauhinia manca* we started phytochemical investigations of the woody parts of this plant.

From the petrol extract a number of long chain alkyl esters of ferulic and *p*-coumaric acid was isolated.

Results and Discussion

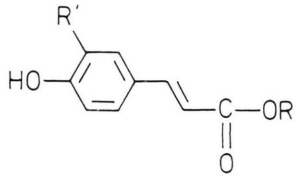
Isolation

Successive flash-chromatography of the petrol extract from the dried woody parts gave besides fatty materials, chlorophyll, β-sitosterol and related sterols two fractions, which consisted each of a mixture of esters of ferulic acid and *p*-coumaric acid, respectively. Separation of these mixtures into the individual components was achieved by hplc using a reversed phase column.

The separation procedure was accompanied and monitored by spectroscopic measurements (mainly MS and ^{1}H-NMR) and this yielded two major and one minor series of esters.

The major series were esters of E-ferulic acid (series **1**) and *E*-*p*-coumaric acid (series **2**).

As alcohol components of these esters the same homologous group of unbranched alcanols was detected ranging from C_{22} to C_{28} (**1a**–**1g** and **2a**–**2g**).



1 R' = OCH_3

2 R' = H

1a **2a**	R = *n* · $C_{22}H_{45}$
1b **2b**	R = *n* · $C_{23}H_{47}$
1c **2c**	R = *n* · $C_{24}H_{49}$
1d **2d**	R = *n* · $C_{25}H_{51}$
1e **2e**	R = *n* · $C_{26}H_{53}$
1f **2f**	R = *n* · $C_{27}H_{55}$
1g **2g**	R = *n* · $C_{28}H_{57}$

The relative concentrations of the individual esters were measured by hplc and by hydrolysis of the mixture of esters followed by gc analysis of the long chain alcohols.

The results are presented in Table I.

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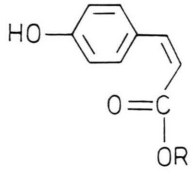
Table I. Relative concentrations of individual esters (Total alkyl esters of *E*-ferulic and *E*-*p*-coumaric acid esters = 100% each).

Alkyl component:	$n \cdot C_{22}H_{45}$	$n \cdot C_{23}H_{47}$	$n \cdot C_{24}H_{49}$	$n \cdot C_{25}H_{51}$	$n \cdot C_{26}H_{53}$	$n \cdot C_{27}H_{55}$	$n \cdot C_{28}H_{57}$
Esters of:							
ferulic acid	2.5	1	10	3	53	9	21
p-coumaric acid	1	0.2	14	2	56	3	23.5

As Table I shows, maximum concentrations of esters are found with hexacosanol and octacosanol in both major series.

The esters with alcohols possessing an uneven number of carbon atoms were present only in trace amounts and this is in accordance with biogenetic considerations.

The minor ester series consists of esters **3a**–**3c** of *Z*-*p*-coumaric acid.



3a: R = $n \cdot C_{24}H_{49}$
3b: R = $n \cdot C_{26}H_{53}$
3c: R = $n \cdot C_{28}H_{57}$

However, no esters of *Z*-ferulic acid were found, and this fact might give evidence that **3a**–**3c** need not necessarily be artefacts produced during work-up but could be genuine natural products.

Model experiments on solutions of long chain alkyl esters of *E*-*p*-coumaric acid as well as *E*-ferulic acid demonstrated that photo-induced isomerization occurs at similar rates and leads in both cases to an *E*/*Z* ratio of about 1:1 (after 2 weeks, day light).

Structure determinations

The structures were determined by spectroscopic studies of the mixtures of esters and of individual compounds.

Alkaline hydrolysis of the mixtures of esters **1**, **2** and **3** yielded the corresponding acids and a series of homologous alcohols, which was subjected to gc analysis and compared with authentic alcanols. Finally, most of the esters of ferulic acid and *E*-*p*-coumaric acid were synthesized: the phenolic groups of ferulic or *p*-coumaric acid were protected by acetylation. Subsequently, the esters were prepared from the protected acids and the alcanols using the dicylohexylcarbodiimide method [6]. In the last step the acetyl group was removed by reductive cleavage with $NaBH_4$ according to [7].

To make sure that the esters are those of ferulic acid and not of isoferulic acid the mixture of esters **1** was treated with MeOH/HCl. After acetylation the resulting methylester was compared by capillary gc with the acetylation products of authentic *E*-ferulic and *E*-isoferulic acid methyl ester.

Long chain alkyl esters of ferulic acid and *p*-coumaric acid have been frequently found in the bark and wood of conifers [8] but were occasionally also detected in other plants [9].

These compounds usually occur as mixtures of homologous and in most cases pure compounds have not been isolated.

In plants of the Leguminosae family these esters are rare and up to now they have not been detected in any *Bauhinia* species. According to our knowledge the isolated esters of *Z*-*p*-coumaric acid (esters **3**) have not been reported before.

From a plant-physiological point of view, the isolated compounds might be important for the plants as antioxidants [10] and as precursors of the suberin polymer [11].

Experimental

General procedures

Mps. uncorr., ^{1}H-NMR (in $CDCl_3$) at 250 MHz (WM 250 Bruker-Physik); chemical shifts in δ (ppm); TMS as internal standard. Mass spectra on a Finnigan 4000 (Finnigan-MAT) at 70 eV. IR spectra in $CHCl_3$ or KBr; UV spectra in CH_3OH (or C_2H_5OH). Gc on 5% OV 17 (packed column: 2 m × 2 mm), isothermal at 230 °C. Capillary gc on SE 54 (25 m × 0.2 mm); isothermal at 220 °C. Column chromatography on silica gel 60 (Macherey-Nagel, 70–230 mesh). Hplc carried out on LiChro-

sorb RP-18, 7 μ (Merck) with CH_3OH; column: 25 cm × 2,0 cm; pressure: 600 psi; flow: 10 ml/min; detection at 312 (or 326) nm. Tlc on silica gel UV_{254} ready-made glass plates (Macherey-Nagel); solvent system: toluene/acetone (98:2); detection by UV.

Plant material

Object of our investigations were the wood and twigs of *Bauhinia manca* Standl., collected in Costa Rica by M. A. Constenla. Herbarium specimen is held under No. 8210 in Erlangen.

Isolation procedure

Air-dried stems (5 kg) were ground and extracted with 15 l petrol at 60 °C in a Soxhlet apparatus for 160 h. The solution was evaporated *in vacuo* yielding 7 g residue. The residue was separated on 300 g silica gel at a pressure of 1.2 kg/cm^2 first with 3 l methylene chloride and then with $CHCl_3/CH_3OH$ mixtures (1 l each: 98:2, 95:5 and 9:1); this yielded the main fractions A1 (1.2 g), A2 (1.7g), A3 (3.6 g).

A2 was chromatographed over 160 g silica gel with petrol/acetone (9:1) giving 6 fractions of A2. On rechromatography over 16 g silica gel with toluene/acetone (998:2) fraction A2.5 (165 mg) yielded the "ester" fractions A2.5.2 (14 mg) and A2.5.4 (40 mg).

Esters of E-ferulic acid

Fraction A2.5.2 consisted of a mixture of *E*-ferulic acid esters. Dc: R_f = 0.35. – Ms: (> *m/z* 100) = 586 (9%, M_1^+), 572 (~ 1%, M_2^+), 558 (28%, M_3^+), 544 (< 1%, M_4^+), 530 (~ 3%, M_5^+), 516 (< 1%, M_6^+), 502 (< 1%, M_7^+), 196 (27), 194 (100), 179 (14), 177 (95), 150 (36), 145 (26), 137 (64), 117 (10). – Uv $\lambda_{max}^{C_2H_5OH}$ nm (lgε): 326 (4.28) 299 sh (4.15), 236 (4.09), 218 (4.15); $\lambda_{max}^{C_2H_5OH + KOH}$ nm (lgε): 376 (4.47), 310, 253. – Ir ν_{max}^{KBr} cm^{-1}: 3400, 2920, 2860, 1700, 1630, 1590, 1270, 1160. – ^{1}H-NMR: δ 7.62 (1H, d, *J* =16.5 Hz, H-3), 7.08 (1H, dd, J_1 = 8.5 Hz, J_2 = 2 Hz, H-6′), 7.03 (1H, d, *J* = 2 Hz, H-2′), 6.93 (1H, d, *J* = 8.5 Hz, H-5′), 6.30 (1H, d, *J* = 16.5 Hz, H-2), 5.83 (1H, s, $-O\underline{H}$), 4.19 (2H, t, *J* = 7 Hz, $-OC\underline{H}_2-$), 3.93 (3H, s, $-OC\underline{H}_3$), 1.70 (2H, m, $-OCH_2-C\underline{H}_2-$), 1.26 (ca. 40H, broad, $-(C\underline{H}_2-)_{\sim 20}$), 0.88 (3H, t, *J* = 6.7 Hz, $-CH_2-C\underline{H}_3$).

The mixture was dissolved in 1 ml $CHCl_3$ and separated by hplc (repeated injections 0.1 ml each). The result is summarized in Table II.

Esters of p-coumaric acid

Fraction A2.5.4 consisted of a mixture of *E*- and *Z*-*p*-coumaric acid esters. Ms: (> *m/z* 100) = 556 (3%, M_1^+), 542 (< 1%, M_2^+), 528 (13%, M_3^+), 514 (< 1%, M_4^+), 500 (1%, M_5^+), 486 (< 1%, M_6^+), 472 (< 1%, M_7^+), 166 (64), 164 (100), 149 (18), 147 (65), 120 (29), 107 (27).

Separation into the *Z*-*p*-coumaric acid esters and the *E*-*p*-coumaric acid esters was achieved on silica gel using toluene/acetone (99:1).

a) Esters of *E*-*p*-coumaric acid

Dc: R_f = 0.15 – Uv $\lambda_{max}^{CH_3OH}$ nm (lgε): 312 (4.38), 299 sh, 228 (4.11); $\lambda_{max}^{CH_3OH + KOH}$ nm (lgε): 358 (4.56), 311 (4.04), 240 (4.02). – Ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 2925, 2830, 1700, 1635, 1610, 1165, 990. – ^{1}H-NMR: δ 7.63 (1H, d, *J* = 16.5 Hz, H-3), 7.43 (2H, d_{br}, *J* = 8.5 Hz, H-2′, 6′), 6.84 (2H, d_{br}, *J* = 8.5 Hz, H-3′, 5′), 6.31 (1H, d, *J* = 16.5 Hz, H-2), 5.00 (1H, s, $-O\underline{H}$), 4.19 (2H, t, *J* = 7 Hz, $-OC\underline{H}_2-$), 1.66 (2H, m, $-OCH_2-C\underline{H}_2-$), 1.28 (ca. 40 H, broad, $-(C\underline{H}_2-)_{\sim 20}$), 0.88 (3H, t, *J* = 6.7 Hz, $-CH_2-C\underline{H}_3$).

b) Esters of *Z*-*p*-coumaric acid

Dc: R_f = 0.19. – Uv $\lambda_{max}^{CH_3OH}$ nm (lgε): 309 (3.99), 296 (sh), 225 (sh); $\lambda_{max}^{CH_3OH + KOH}$ nm (lgε): 357 (4.14), 309 sh. – Ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 2925, 2830, 1705,

Table II. Individual ferulic acid esters by hplc separation.

Compound	M^+	Yield [mg]	Retention time [min]
E-ferulic acid docosylester (**1a**)	502	0.2	54.5
E-ferulic acid tricosylester (**1b**)	516	0.1	64
E-ferulic acid tetracosylester (**1c**)	530	1.0	74
E-ferulic acid pentacosylester (**1d**)	544	0.3	88
E-ferulic acid hexacosylester (**1e**)	558	5.9	101.5
E-ferulic acid heptacosylester (**1f**)	572	1.1	123
E-ferulic acid octacosylester (**1g**)	586	2.5	147

Table III. Individual *p*-coumaric acid esters by hplc separation.

Compound	M^+	Yield [mg]	Retention time [min]
E-*p*-coumaric acid docosylester (**2a**)	472	0.3	53.5
E-*p*-coumaric acid tricosylester (**2b**)	486	0.1	62.5
Z-*p*-coumaric acid tetracosylester (**3a**)	500	0.8	67.5
E-*p*-coumaric acid tetracosylester (**2c**)	500	3.8	73
E-*p*-coumaric acid pentacosylester (**2d**)	514	0.7	86.5
Z-*p*-coumaric acid hexacosylester (**3b**)	528	3.0	93
E-*p*-coumaric acid hexacosylester (**2e**)	528	15.4	102
E-*p*-coumaric acid heptacosylester (**2f**)	542	0.8	119.5
Z-*p*-coumaric acid octacosylester (**3c**)	556	1.3	129
E-*p*-coumaric acid octacosylester (**2g**)	556	6.4	141.5

1630, 1610, 1165. – ^{1}H-NMR: δ 7.64 (2H, d_{br}, J = 8.5 Hz, H-2′, 6′), 6.84 (1H, d, J = 13 Hz, H-3), 6.81 (2H, d_{br}, J = 8.5 Hz, H-3′, 5′), 5.83 (1H, d, J = 13 Hz, H-2), 5.00 (1H, s, $-O\underline{H}$), 4.12 (2H, t, J = 7 Hz, $-OC\underline{H}_2-$), 1.66 (2H, m, $-OCH_2-C\underline{H}_2-$), 1.28 (ca. 40H, broad, $-(CH_2-)_{\sim 20}$), 0.88 (3H, t, J = 6.7 Hz, $-C\underline{H}_2-C\underline{H}_3$).

Mixtures a) and b) were dissolved in $CHCl_3$ and separated by hplc. The result is summarized in Table III.

Hydrolysis

5 mg of the mixture of ferulic acid esters were hydrolized according to [12]. This yielded from the organic layer a mixture of alcanols, which was analyzed by gc (packed column) and compared with authentic alcohols. The retention times and results are compiled in Table IV.

Table IV. Gc of the alcanols from hydrolysis of the ferulic acid esters and *E*-*p*-coumaric acid esters.

Alcohol	Retention time [min]
docosanol	4.2
tricosanol	4.9
tetracosanol	6.9
pentacosanol	9.3
hexacosanol	12.3
heptacosanol	16.4
octacosanol	22.0

The aqueous layer was purified (silica gel; $CHCl_3$/CH_3OH (95:5)) and crystallized from acetone to give needles with m.p. 168/169 °C, identical with *E*-ferulic acid.

About 8 mg of the *E*-*p*-coumaric acid esters were hydrolized by the same method. Gc analysis of the alcohol components yielded exactly the same alcanol composition (Table IV), and from the aqueous layer *E*-*p*-coumaric acid was isolated (m.p. 212 °C; identical with authentic compound).

Likewise the compounds were identified from hydrolysis of the mixture of *Z*-*p*-coumaric acid esters.

Identification of ferulic acid by gc

5 mg of the mixture of ferulic acid esters (**1**) were refluxed in 5 ml CH_3OH/HCl for 6 h to yield *E*-ferulic acid methyl ester. After acetylation (Ac_2O/py) the product was subjected to capillary gc and compared with authentic acetyl derivatives of ferulic acid methyl ester (T_R = 9.5 min) and isoferulic acid methyl ester (T_R = 10 min), respectively. The acidic component from the plant extract was proved to be identical with acetylated ferulic acid methyl ester.

Synthesis

112 mg 4′-acetoxy-3′-methoxy-cinnamic acid (or 97 mg 4′-acetoxycinnamic acid), 0.55 mmol of the alcanol and 5 mg *p*-toluenesulfonic acid were dissolved in 2.5 ml pyridine and kept at 0 °C. Over a period of 30 minutes 110 mg dicyclohexylcarbodiimide dissolved in 2 ml pyridine were added dropwise. Then the reaction mixture was stirred at room temperature for 48 h. After evaporation 5 ml ethyl acetate were added and dicyclohexyl urea was removed by filtration: the filtrate was evaporated and the product purified on silica gel (petrolether/acetone 95:5); (yields: 25% to 40%).

The acetyl-group was split off according to [7] in 2.5 ml dimethoxyethane solution by reaction with 100 mg sodium borohydride (18 h at 45 °C). Work-

up was done by addition of 5 ml aqueous NH_4Cl solution. The ester was extracted with $CHCl_3$ and purified on silica gel (petrolether/acetone 9:1); (yield: 85%).

Acknowledgements

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