Pergamon

0031-9422(95)00395-9

Phytochemistry, Vol. 40, No. 5, pp. 1543-1548, 1995

0031-9422/95 $9.50 + 0.00

STACHYSETIN, A DIAPIGENIN-7-GLUCOSIDE-*p,p'*-DIHYDROXY-TRUXINATE FROM *STACHYS AEGYPTIACA*

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(*Received in revised form* 11 *April* 1995)

Key Word Index—*Stachys aegyptiaca*; Labiatae; flavonoids; acylated flavone glucosides; diapigenin-7-*O*-glucosyl-*p,p'*-dihydroxytruxinate; NMR.

Abstract—Aerial parts of *Stachys aegyptiaca* contain the unique acylated flavonoid, diapigenin-7-*O*-(6″-*trans*,6″-*cis*-*p,p'*-dihydroxy-μ-truxinyl)glucoside, stachysetin, and the hitherto unknown, apigenin 7-*O*-(3″-*p*-coumaryl)glucoside. In addition, the known compounds, apigenin 7-*O*-(6″-*p*-coumaryl)glucoside and naringenin were also identified. Structures were established by conventional methods of analysis and confirmed by ^{1}H, ^{13}C NMR and mass spectral analysis. 2D-chemical shift correlation NMR was also used in the case of the new flavonoids.

INTRODUCTION

In a previous communication, we reported the occurrence of 24 flavonoids in the aerial parts of *Stachys aegyptiaca* [1]. In the present study, we describe the isolation and elucidation of the unique diapigenin 7-*O*-(6″-*trans*, 6″-*cis*-*p,p'*-dihydroxy-μ-truxinyl) glucoside or stachysetin (**1**) and the new apigenin, 7-*O*-(3″-*p*-coumaryl) glucoside (**2**). The known compounds, apigenin, 7-*O*-(6″-*p*-coumaryl)glucoside (**3**) and naringenin (**4**) were also isolated and identified, in addition to the previously identified flavonoids [1]. Diglycoside flavone esters of dicarboxylic acids are rare plant constituents. Thus, Itokawa *et al.* [2] tentatively identified the malonyl diglucosylflavone, agastachin, from *Agastache rugosa* (Labiatae). More recently, Horie *et al.* [3] characterized the unique 3-hydroxy-3-methylglutaryl diglucosylflavone, sudachin, from *Citrus sudachi* (Rutaceae).

The new compound (**1**) is of special interest as it represents the first reported natural occurrence of a flavone glucoside truxinate ester. However, esters of truxinic acid together with those of its positional isomer, truxillic acid, are known in association with alkaloid chemistry, as in the case of α- and β-truxillines, the alkaloid components of *Erythroxylum coca* and *E. movogramatens* [4]. Esters and amides of hydroxylated truxinic and truxillic acids have also been reported to occur in graminaceous cell walls [5] and in *Verbesina caracas* [6].

RESULTS AND DISCUSSION

Two flavonoid fractions were successively eluted by ethanol–water mixtures (7:3 and 9:1) from a polyamide column of the aqueous ethanolic aerial part extract of *S. aegyptiaca*. Compounds (**1–3**) were isolated individually from the 7:3 fraction through a combination of column chromatography (CC) on polyamide and Sephadex LH-20 followed by preparative paper chromatography (PC). Compound **4** was separated and purified from the 9:1 fraction by standard methods (CC and prep. PC). The known compounds (**3** and **4**) gave chromatographic, UV (Table 1), FAB-mass spectral and hydrolytic data typical of apigenin 7-*O*-(6″-*p*-coumaryl)-β-glucoside (**3**) [7] and naringenin (**4**) [8]. The structures of **3** and **4** were confirmed by ^{1}H NMR spectrometry.

Compound **1**, isolated as an off-white amorphous powder, exhibited a $[M + H]^+$ at *m/z* 1157 in the positive FAB-mass spectrum and at $[M - H]^-$ *m/z* 1155 in the negative FAB-mass spectrum, corresponding to a M_r of 1156. Fragments at *m/z* 269, 431 and 577 were also observed in the negative FAB-mass spectrum. On PC, **1** appeared as a dark-purple spot in UV light, which turned lemon-yellow with ammonia vapour. It gave an olive green ferric chloride reaction and a positive Mg–HCl reduction test for flavones. These results, together with the UV spectral data (Table 1) suggested **1** to be a dimeric structure in which the monomer is apigenin 7-*O*-(*p*-coumaryl)hexoside. Complete acid hydrolysis (methanolic 2 M HCl, for 7 hr), as well as controlled acid hydrolysis (aqueous 0.1 M HCl, for 1 hr) of **1** confirmed this view. Thus, complete acid hydrolysis gave apigenin (^{1}H NMR), glucose (CoPC) and a phenolic carboxylic acid (**1a**) (yellowish-brown on PC when sprayed with $FeCl_3$ and dark-purple when sprayed with aniline–xylose [9]). On the other hand, mild acid hydrolysis yielded **1a** together with apigenin 7-*O*-β-glucoside (^{1}H NMR). Pure **1a** separated from a chloroform extract of both hydrolysates of **1**, through prep. PC showed chromato-

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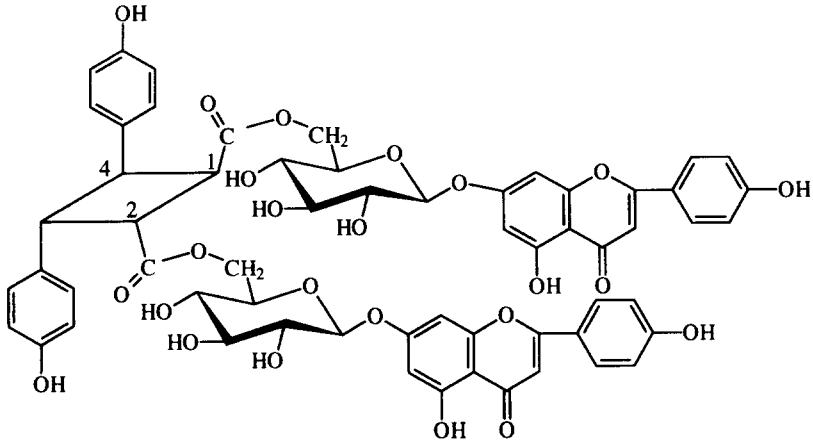

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graphic properties and UV spectral data (Table 1) different from those of known hydroxylated cinnamic acids. The FAB-mass spectrum of **1a** exhibited a $[M-H]^-$ *m/z* 327, corresponding to a M_r of 328, thus suggesting a dimeric *p*-coumaric acid structure of the cyclobutane-type, in which dimerization takes place without loss in mass units resulting in either *p*-hydroxytruxinic acid or its positional isomer, *p*-hydroxytruxillic acid. Consequently, the parent compound (**1**) is based on esterification of the two carboxylic groups of *p*-hydroxy turxinic (or truxillic) acid by two alcoholic sugar groups originating from two different apigenin 7-*O*-*β*-glucoside moieties.

The EI-mass spectrum of the silylated derivative of **1a** revealed a $[M]^+$ at *m/z* 616 (38%, relative intensity), corresponding to a tetra-TMSi derivative. Both symmetrical and asymmetrical fragmentation occurred during mass spectral measurements. This followed from the recognition of an ion at *m/z* 236, assignable to the monosilylated *p*-coumaric acid fragment which is produced by symmetrical splitting and also from the recognition of the two peaks at *m/z* 356 (35% relative intensity) and at *m/z* 260 (6% relative intensity), assignable, respectively, to TMSi–C_6H_4–CH–CH–C_6H_4–TMSi as well as to TMSi–COO–CH–CH–COO–TMSi, fragments which were released during asymmetrical splitting of **1a**. This confirmed that **1a** is the head-to-head dimer of *p*-coumaric acid, namely *p*-hydroxytruxinic acid [10, 11]. Fragmentation of the analogous head-to-tail dimer, *p*-hydroxytruxillic acid, would only proceed symmetrically and the latter two ion peaks would never exist in its spectrum.

In order to determine the configuration of the substituents on the cyclobutane ring in **1a**, it was subjected to ^{1}H NMR analysis. The spectrum (DMSO-d_6, at room temperature) revealed two aromatic resonances (Table 2) at δ6.73 (*d*, J = 8.5 Hz, H-2 and H-6 in both aromatic rings) and at δ6.45 (*d*, J = 8.5 Hz, H-3 and H-5 in both aromatic rings). It also showed two aliphatic proton signals at δ4.12 (*m*, H-3 and H-4 in the cyclobutane ring) and at δ3.77 (*m*, H-1 and H-2 in the cyclobutane ring). These data are best interpreted in terms of a cyclobutane substituent configuration of *p*-hydroxy-*μ*-truxinic acid [*t*-3,*c*-4-di-(4-hydroxyphenyl)cyclobutane-*r*-1,*t*-2-dicarboxylic acid]. This was confirmed by direct comparison with ^{1}H NMR data reported for different configurational isomers of truxinic acids [12, 13].

In conclusion, the structure of the parent compound **1** is identified as diapigenin 7-*O*-(6″ → 1,6″ → 2-*p*,*p*′-dihydroxy-*μ*-truxinyl)glucoside. The site of attachment of the *p*,*p*′-dihydroxy-*μ*-truxinyl moiety to the two apigenin glucosyl moieties is confirmed by ^{1}H NMR. The spectrum (DMSO-d_6, room temperature) revealed two distinct patterns of proton signals (Table 2) belonging to two configurationally non-equivalent apigenin 7-*O*-*β*-glucoside moieties. Each pattern was found to contain well-separated signals of a distinct apigenin moiety together with one *β*-$^4C_1$-glucose anomeric proton signal at δ5.35 (*d*, J = 8.3 Hz) in the first pattern and at δ5.0 (*d*, J = 8.5 Hz) in the second. Both patterns contain, in addition, other sugar proton resonances at δ4.6 (*d*, J = 12.5 Hz) in the first pattern) and at δ4.35 (*d*, J = 12.5 Hz, in the second pattern), assignable to the H-6 methylenic glucose protons in both moieties. Signals of the second methylenic glucose protons at H-6′ (of both glucose moieties) were found in the recorded spectrum of **1** as an overlapped multiplet, located at δ3.85. This characteristic downfield shift of the methylenic glucose proton resonances (in comparison with the corresponding signals in the spectrum of apigenin 7-*O*-*β*-glucoside) is obviously due to esterification of their geminal alcoholic groups by the *p*,*p*′-dihydroxy-*μ*-truxinyl moiety. Other sugar proton signals in this spectrum revealed themselves in the region from δ3.27 to δ4.0. The *p*,*p*′-dihydroxy-*μ*-truxinyl moiety showed its resonances as two aromatic proton doublets appearing in the form of an AB-system centred at δ6.33, attributable to the eight aromatic protons of this moiety. The remaining resonances of the cyclobutane ring protons were detected and assigned, after the measurement of a COSY spectrum, as four distinct signals located at δ3.44 and δ3.4 (H-3 and H-4 cyclobutane protons) and at δ3.3 and δ3.15 (H-1 and H-2 cyclobutane protons). Furthermore, there is an appreciable upfield shift of the proton resonance frequencies of

Table 1. Chromatographic and UV data of flavonoids isolated from *Stachys aegyptiaca*

	Chromatographic properties R_f s values (×100)				UV spectral data, λ_{max} (nm)					
	H_2O	HOAc	BAW	PhOH	MeOH	NaOMe	$AlCl_3$	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3
Stachysetin	5	22	55	83	268, 287*sh*, 328	268, 388	277, 300, 347, 383	277, 298, 343, 380	268, 287*sh*, 332	268, 287*sh*, 355, 390*sh*
Apigenin-7-*O*-glucoside	2	10	68	78	268, 318	262, 308, 370	278, 298, 328, 380	278, 298, 328, 378	268, 318	268, 300*sh*, 320 382
Apigenin-7-*O*-(3″-*p*-coumaryl) glucoside	3	21	80	89	268, 317	262, 310*sh*, 367	278*sh*, 298, 323, 382	278*sh*, 298, 323, 378	268, 317	268, 300*sh*, 317, 380
Apigenin-7-*O*-(6″-*p*-coumaryl) glucoside	5	21	67	88	268, 317	262, 310*sh*, 370	278, 298, 322, 380	278, 298, 323, 377	268, 317	268, 300*sh*, 317, 385
Naringenin	0	6	85	85	287, 327*sh*	325, 395*sh*	310, 370	308, 370	285*sh*, 322	288, 322*sh*
p,p'-Dihydroxy-*μ*-truxinic acid	—	74	53	65	278, 285*sh*, 310*sh*	287, 295	—	—	—	—
p-Coumaric acid	34	30	79	74	290, 308*sh*	260, 308*sh*, 333	—	—	—	—
Apigenin	2	7	82	87	267, 278*sh*, 332	269, 300*sh*, 325, 392	—	—	275, 300*sh*, 340	—

Table 2. ^{1}H chemical shifts (δ) and coupling constants (Hz) of flavonoids 1–3

Compound	Protons of apigenin moiety					Protons of glucose moiety					
	H-6	H-8	H-3	H-3′ and H-5′	H-2′ and H-6′	H-1	H-2	H-3	H-4	H-5	H-6	H-6′
(1) (1 → 6″ *r*)	6.32 (*d*, *J* = 2.5)	6.73 (*d*, *J* = 2.5)	6.52 (*s*)	6.88 (*d*, *J* = 8)	7.9 (*d*, *J* = 8)	5.35 (*d*, *J* = 8.5)	3.35 (*m*)	3.78 (*t*, *J* = 8.5)	4.0 (*m*)	4.0 (*m*)	4.6 (*d*, *J* = 12)	3.85 (*m*)
(1) (1 → 6″ *t*)	6.12 (*d*, *J* = 2.5)	6.38 (*d*, *J* = 2.5)	6.50 (*s*)	6.78 (*d*, *J* = 8)	7.68 (*d*, *J* = 8)	5.0 (*d*, *J* = 8.5)	3.35 (*m*)	3.65 (*t*, *J* = 8.5)	4.0 (*m*)	4.0 (*m*)	4.3 (*d*, *J* = 12)	3.85 (*m*)
(2)	6.48 (*d*, *J* = 2.5)	6.78 (*d*, *J* = 8)	6.75 (*s*)	6.95 (*d*, *J* = 8)	7.97 (*d*, *J* = 8)	5.28 (*d*, *J* = 8)	3.50 (*t*, *J* = 8)	5.10 (*t*, *J* = 8)	3.55 (*m*)	3.6–3.8 (*m*)	3.6–3.8 (*m*)	3.6–3.8 (*m*)
(3)	6.44 (*d*, *J* = 2.5)	6.72 (*d*, *J* = 2.5)	6.72 (*s*)	6.82 (*d*, *J* = 8)	7.94 (*d*, *J* = 8)	5.18 (*d*, *J* = 7.5)	3.2–3.5 (*m*)	3.2–3.5 (*m*)	3.2–3.5 (*m*)	3.8 (*t*, *J* = 12)	4.49 (*d* = 12)	4.2 (*dd*, *J* = 12 and *J* = 5)

	Protons of acyl moiety
(1) *p,p′*-Dihydroxy-truxinyl	6.33 (AB-system, eight aromatic protons), 3.44* (*dd*, *J* = 9 and *J* = 3.5, H-3), 3.4* (*m*, H-4), 3.3** (*t*, *J* = 9, H-1), 3.15** (*t*, *J* = 9, H-2).
(1a) *p,p′*-Dihydroxy-truxinic acid	6.73 (*d*, *J* = 8.5, aromatic H-2 and H-6), 6.45 (*d*, *J* = 8.5, aromatic H-3 and H-5), 4.12 (*m*, cyclobutane H-3 and H-4), 3.77 (*m*, cyclobutane H-1 and H-2).
(2) *p*-Coumaryl	6.43 (*d*, *J* = 16, H-α), 6.80 (*d*, *J* = 8, H-3‴ and H-5‴), 7.55 (*d*, *J* = 8, H-2‴ and H-6‴), 7.60 (*d*, *J* = 16, H-β)
(3) *p*-Coumaryl	6.25 (*d*, *J* = 16, H-α), 6.62 (*d*, *J* = 8, H-3‴ and H-5‴), 7.35 (*d*, *J* = 8, H-2‴ and H-6‴), 7.51 (*d*, *J* = 16, H-β).

*, ** Chemical shifts with the same superscript may be interchanged.

one of the apigenin 7-*O*-β-glucoside, moieties when compared with those of the corresponding protons in the known apigenin, 7-*O*-β-(6″-*p*-coumaryl)-glucoside. In addition, a detectable upfield shift of the resonances frequencies of the cyclobutane ring protons was also recognised in comparison with those of the corresponding protons in free *p,p′*-dihydroxy-μ-truxinic acid. Both shifts are due to not only the configurational non-equivalence of the two apigenin glucoside moieties, but also to the probable puckering of the cyclobutane ring [13] which brought the *trans*-2-apigenin glucoside moiety in front of the plane of the *trans*-3-*p*-hydroxyphenyl ring of the *p,p′*-dihydroxyl-μ-truxinyl moiety, thus shifting the proton resonances of the former moiety (2-apigenin glucoside) upfield.

Final confirmation of the assigned structure of **1** was obtained by ^{13}C NMR analysis. This spectrum exhibited double patterns of carbon signals for the two apigenin 7-*O*-β-glucoside moieties, in addition to the expected carbon resonances of the *p,p′*-dihydroxy-μ-truxinyl moiety. The two β-glucose anomers were recognized from the downfield anomeric carbon resonances at δ99.4 and δ99.7, while the most upfield glucose signal located at δ64.6 and δ65.0, were assigned to the esterified glucose carbons C-6. Assignments of the *p,p′*-dihydroxy-truxinyl carbon resonances (see Experimental) were aided by comparison with reported chemical shifts of analogous compounds [14]. Consequently, compound (**1**) is assigned the structure *t*-3,*c*-4-di-(4-hydroxyphenyl)-cyclobutane-*r*-(1 → 6″*r*),*t*-(2 → 6″*t*)-di-(apigenin-7-*O*-β-glucopyranosyl)-carboxylate, hereby named stachysetin. This is a flavonoid structure which has not been reported in nature before. That this compound is not an artifact is proved by 2D-PC of the fresh crude aqueous ethanolic extract of the aerial parts and the subsequent routine analysis (co-PC against the identified compound **1** and UV absorption) of the material eluted from the corresponding spot detected on several chromatograms.

The new isolated minor compound (**2**), also obtained as an off-white powder was found to possess chromatographic properties and UV absorption spectrum (Table 1) similar to those reported for apigenin 7-*O*-(*p*-coumaryl) glycosides [7]. Complete acid hydrolysis (2 M methanolic HCl, 7 hr) yielded apigenin and *p*-coumaric acid (co-PC, UV and ^{1}H NMR), together with glucose (co-PC), in addition to FAB-mass spectral analysis in both positive and negative modes which proved that (**2**) is apigenin-7-*O*-(*p*-coumaryl)-glucoside with a M_r of 578 $[M + H]^+$ *m/z* 579, in positive mode and $[M - H]^-$ *m/z* 577, in negative mode. However, its R_f values did not agree with those of the known apigenin-7-*O*-(6″-*p*-coumaryl)-glucoside. The structure of **2** was eventually established by ^{1}H NMR (Table 2) and COSY analysis. The ^{1}H NMR spectrum (DMSO-d_6, room temperature) revealed the presence, in the aromatic region, of the characteristic proton signal for a 7-*O*-substituted apigenin moiety, as well as that of the esterifying *p*-courmaric acid moiety (see Experimental). The spectrum also showed, in addition, a pair of sugar resonances localized downfield in the sugar region at δ5.34 (*d*,

J = 8.5 Hz) and at 5.17 (t, J = 8.5 Hz). The chemical shift value, multiplicity and coupling constant of the former signal indicated that it belongs to an anomeric-β-$^4C_1$-glucose proton whose geminal hydroxyl group is substituted. On the other hand, the mode of splitting (triplet) and the low-field position of the latter sugar proton resonance would suggest its correlation to either the H-3 or H-4 glucose proton and its esterification with *p*-coumaric acid, at the geminal hydroxy group. Exclusion of the H-2 glucose proton followed from the chemical shift value of the anomeric proton (δ5.34) which reflects the absence of esterification at the vicinal hydroxyl group, 2-OH. Measurement of a COSY spectrum of **2** solved this ambiguity and led to the assignment of this triplet to the H-3 glucose proton, as well as the assignment of the most upfield glucose proton (δ3.15; t, J = 8.5 Hz) to the H-2 proton and the assignment of the broad multiplet between δ3.2–3.95 to the remaining glucose protons, H-4, H-5, H-6 and H-6′. Consequently, the structure of the isolated minor flavonoid (**2**) is confirmed as apigenin 7-*O*-(3″-*p*-coumaryl)-β-$^4C_1$-glucoside, which represents, to the best of our knowledge, a new natural product.

EXPERIMENTAL

^{1}H NMR chemical shifts were measured relative to TMS, ^{13}C NMR relative to DMSO-d_6 and converted to the TMS scale by adding 39.5. Typical conditions: spectral width = 4000 Hz for ^{1}H and 17500 Hz ^{13}C, 32 K data points and a flip-angle of 45°. PC was carried out on Whatman No. 1 paper, using the solvent systems: (1) H_2O; (2) HOAc (HOAc–H_2O, 3:17); (3) BAW (*n*-BuOH–HOAc–H_2O 4:1:5); (4) benzene–*n*-BuOH–pyridine–H_2O, 1:5:3:3). Solvent systems 2 and 3 were used for prep. PC on Whatman No. 3MM paper, while solvent systems 3 and 4 were used for sugar analysis.

Plant material and fractionation. An aq. EtOH extract (70%) of *S. aegyptiaca* aerial parts was worked up as described in ref. [1].

Isolation and identification. Compounds **1**–**3** were isolated in a pure form from the 70% aq. EtOH fr., eluted from the main polyamide column, by refractionation over polyamide, followed by Sephadex LH-20, using MeOH–H_2O (1:1) in each case. Final purification was achieved by prep. PC using solvent system 3. Compound **4** was separated from the 90% fr. of the polyamide column and purified by prep. PC using solvent system 2. The known compounds, apigenin 7-*O*-(6″-*p*-coumaryl)-glucoside (**3**) and naringenin (**4**) were identified by standard methods. For R_f values and UV data see Table 1. Complete acid hydrolysis of (**3**) yielded apigenin, *p*-coumaric acid and glucose (co-PC). The M_r of (**3**): 578, negative FABMS ([M − H]$^-$ m/z 577). For ^{1}H NMR of **3**: see Table 2. M_r of **4**: 272, negative FABMS ([M − H]$^-$ m/z 271). ^{1}H NMR of **4**: δ2.70 (*dd*, J = 11 and J = 2, H-3 axial), 3.12 (*dd*, J = 11 and J = 9, H-3 equatorial), 5.4 (*dd*, J = 11 and J = 2, H-2), 5.9 (*m*, H-6 and H-8), 6.85 (*d*, J = 8, H-3′ and H-5′), 7.35 (*d*, J = 8, H-2′ and H-6′).

t-3,c-4-*Di*-(4-*hydroxyphenyl*)-*cyclobutane*-r-(1 → 6″),t-(2 → 6″)-*di*-(*apigenin*-7-O-β-*glucopyranoside*)-*carboxylate*, *stachysetin* (**1**). For R_f values and UV data see Table 1. M_r 1156, positive FABMS m/z 1157 [M + H]$^+$, negative FABMS m/z 1155 [M − H]$^-$, 577 [apigenin *p*-coumaryl-glucoside − H]$^-$, 431 [apigenin glucoside − H]$^-$ 269 [apigenin − H]$^-$. **1** was hydrolysed with 2 M HCl in MeOH at 100° for 7 hr to give apigenin, glucose (co-PC) and *p*,*p*′-dihydroxy-μ-truxinic acid (**1a**). Apigenin and *p*,*p*′-dihydroxy-μ-truxinic acid were extracted by $CHCl_3$ and separated by prep. PC using solvent system 3. ^{1}H NMR: apigenin: δ6.18 (*d*, J = 2.5 Hz, H-6), 6.47 (*d*, J = 2.5 Hz, H-8), 6.92 (*d*, J = 8 Hz, H-3′ and H-5′), 6.9 (*s*, H-3), 7.92 (*d*, J = 8 Hz, H-2′ and H-6′), *p*,*p*′-Dihydroxy-μ-truxinic acid (**1a**). For R_f values and UV data see Table 1. M_r 328, negative FABMS: m/z 327 [M − H]$^-$. On silylation [11], the two COOH and OH groups were derivatized. EIMS, (m/z rel. int.): 616 [M]$^+$ (38), 236 (40), 356 (35), 260 (6). Mild acid hydrolysis of **1** with 0.1 m HCl 100° for 1 hr yielded *p*,*p*′-dihydroxy-μ-truxinic acid and apigenin-7-*O*-β-glucoside (**1b**), ^{1}H NMR of released glucoside (**1b**): glucose moiety: δ5.05 (*d*, J = 7.5 Hz, H-1″), 3.15–3.50 (*m*, six glucose proton); apigenin: 6.42 (*d*, *s*, J = 2.5 Hz, H-6), 6.81 (*d*, J = 2.5 Hz, H-8), 6.88 (*s*, H-3), 6.94 (*d*, J = 8 Hz, H-3′ and H-5′), 7.94 (*d*, J = 8 Hz, H-2′ and H-6′). For ^{1}H NMR of **1** and *p*,*p*′-dihydroxy-μ-truxinic acid (**1a**) see Table 2. ^{13}C NMR of **1**: apigenin moiety: δ164.0 (C-2*t* and C-2*r*), 102.9 and 102.7 (C-3*t* and C-3*r*), 181.7 (C-4*t* and C-4*r*), 162.2 (C-5*t* and C-5*r*), 99.6 (C-6*t* and C-6*r*), 162.7 and 162.3 (C-7*t* and C-7*r*), 94.2 and 94.1 (C-8*t* and C-8*r*), 156.8 and 156.7 (C-9*t* and C-9*r*), 105.3 (C-10*t* and C-10*r*), 120.7 and 120.6 (C-1′*t* and C-1′*r*), 128.8 and 128.6 (C-2′*t* and C-2′*r*), 115.6 and 116.0 (C-3′*t* and C-3′*r*), 160.5 and 160.8 (C-4′*t* and C-4′*r*), 115.8 and 116.0 (C-5′*t* and C-5′*r*), 128.8 and 128.6 (C-6′*t* and C-6′*r*); glucose moiety: δ99.4 and 99.1 (C-1″*t* and C-1″*r*), 73.0 (C-2″*t* and C-2″*r*), 76.7 and 76.1 (C-3″*t* and C-3″*r*), 70.6 (C-4″*t* and C-4″*r*), 73.9 (C-5″*t* and C-5″*r*), 64.6 and 65.0 (C-6″*t* and C-6″*r*); *p*,*p*′-dihydroxy-μ-truxinyl moiety: *p*-hydroxyphenyl moieties: δ130.1 and 129.4 (C-1*t* and C-1*r*), 128.7 and 128.6 (C-2*t* and C-2*r*), 116.4 and 116.3 (C-3*t* and C-3*r*), 155.6 (C-4*t* and C-4*r*), 116.4 and 116.3 (C-5*t* and C-5*r*), 128.7 and 128.6 (C-6*t* and C-6*r*); cyclobutane moiety: δ42.5 and 43.2 (C-1 and C-2), 43.7 and 43.9 (C-3 and C-4); carboxylate moieties: δ172.5 and 172.2 (two C=O).

Apigenin-7-O-β-(3″-*p*-*coumaryl*) *glucoside* (**2**). For R_f values and UV data see Table 1. M_r 578, FABMS: negative ion m/z 577 [M − H]$^-$. **2** was hydrolysed with 2 M HCl in MeOH at 100° for 7 hr to give apigenin, glucose and *p*-coumaric acid (co-PC). For ^{1}H NMR of **2** see Table 2.

REFERENCES

1. El-Ansari, M. A., Barron, D., Abd-Alla, M. F., Saleh, N. A. M. and LeQuere, J. L. (1991) *Phytochemistry* **30**, 1169.
2. Itokawa, H., Sato, K. and Takeya, K. (1981) *Chem. Pharmacol. Bull.* **29**, 1777.

3. Horie, T., Tsukayama, M., Yamada, T., Miura, I. and Nakayama, M. (1986) *Phytochemistry* **25**, 2621.
4. Novak, M., Salemink, C. A., Khan, I. J. (1984) *Ethnopharmacology* **10**, 261.
5. Ford, C. W. and Hartley, R. D. C. and (1989) *J. Sci. Food Agric.* **46**, 301.
6. Botta, M., Corelli, F. and De Luca, C. (1991) *Proceedings of Sino-Italian Symposium on Chemistry of Natural Products*, Shanghai, p. 114.
7. Nawwar, M. A. M., El-Mousallamy, A. M., Barakat, H. H., Buddrus, J. and Lindsheid, M. (1989) *Phytochemistry* **28**, 3201.
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, Berlin.
9. Harborne, J. B. (1973) *Phytochemical Methods.* Chapman and Hall, London.
10. Hartley, R. D., Whately, F. R. and Harris, P. J. (1988) *Phytochemistry* **27**, 349.
11. Lewis, F. D., Quillen, S. I. Hale, P. D. and Oxman, J. D. (1988) *J. Am. Chem. Soc.* **110**, 1261.
12. D'Auria, M. and Vantaggi, A. (1992) *Tetrahedron Letters* **48**, 2523.
13. Riddell, F. G. (1980) in *The Conformational Analysis of Heterocyclic Compounds*, p. 49. Academic Press, London.
14. Kalinowski, H. O., Berger, S. and Braun, S. (1984) *13C NMR Spektroskopie*, p. 239. Georg Thieme, Stuttgart.