New Cinnamoyl Esters of Quinic Acid from *Meum athamanticum*

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Two new cinnamoyl quinic acid esters were isolated from the methanolic extract of *Meum athamanticum* rhizome. They were identified as 1-*trans*-O-caffeoyl quinic acid methyl ester and 1-*trans*-O-feruloyl quinic acid methyl ester by the spectral data of the natural products and those of the acetylated derivatives.

Introduction

Meum athamanticum Jacq. (Umbelliferae) is a wild plant common to siliceous soils and growing at an altitude of 400 to 1500 m [1]. Its occurrence is widespread in most of western and central european mountains [2]. Until the present time, *Meum athamanticum* is the only known species of the genus [3] and is valued for its stimulating, stomachic, diuretic [4] and expectorant [5] properties in traditional medicine. Recently, *in vitro* tests have demonstrated inhibition of aggregation of human platelets by aqueous extracts of rhizome tissues [6].

Most of the constituents reported now, have been recovered from nonpolar extracts of the underground parts. Some of these are very ubiquitous in higher plants as 3-carene, β-pinene, terpinolene [7] and β-sitosterol [8]. Others, such as falcarinolone [9] and ligustilide [7], are of common occurrence in the Umbelliferae. In addition to those components, a new hydrocarbon named viridene is presently specific to *Meum* [7].

This paper reports on the isolation and identification of two new cinnamoyl quinic esters from the methanolic extract of *Meum athamanticum* Jacq. rhizome.

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Results and Discussion

The plant material was successively lixivied with *n*-hexane, chloroform and methanol. The concentrated methanolic extract was redissolved in water and then partitioned with benzene, ethyl acetate and finally *n*-butanol. From the last layer, two compounds **1a** and **2a** were isolated and purified by column chromatography on Sephadex LH 20 and polyamide SC 6. The structures of both compounds were established on the basis of spectral data of the natural products and their acetylated derivatives.

1-trans-O-caffeoyl quinic acid methyl ester **1a**

When compared with caffeic acid, this compound exhibited similar UV spectrum as well as the same white bluish fluorescence. However, its R_f values on cellulose and polyamide TLC were different from those of caffeic acid. Therefore, it was strongly suggested the presence of a caffeic acid unit in this compound, the *o*-diphenol group being free, as shown by the $AlCl_3$ UV shift. Furthermore, the ^1H NMR spectrum (CD_3OD) of the natural product exhibited three aromatic protons of which an AB system ($J = 8$ Hz) at δ 6.94, 6.78 ppm, one singlet at δ 7.02 ppm and two *trans*-coupled ethylenic protons ($J = 16$ Hz) at δ 7.52 and 6.20 ppm. These observations were confirmed by the ^1H NMR spectrum ($CDCl_3$) of the acetylated derivative **1b** which indicated two additional isochrone phenolic acetates at δ 2.30 ppm. Moreover, the fragmentation pattern recorded for this part of the molecule conformed with the presence of a caffeic acid moiety as indicated by the fragment ions at *m/z* 180, 163, 162 and 135 in the MS of **1a**.

Examination of the ^1H NMR spectrum of the acetylated derivative **1b**, after irradiation experiments, gave evidence for three methine groups involved in the same chain $-CH(a)-CH(b)-CH(c)-$, with CH(a): δ 5.55 ppm – ddd – J 10, 10 and 4.5 Hz; CH(b): δ 5.10 ppm – dd – J 10 and 3.5 Hz; CH(c): δ 5.59 ppm – dd – J 3.5 and 3.5 Hz, those chemical shift values indicating their O-binding. In addition, the coupling constant $J_{ab} = 10$ Hz corresponding to a *trans*-diaxial orientation permitted their incorporation in a cycle bearing two methylene groups observed as overlapped multiplets centered at δ 2.52 ppm. After irradiation of H(b), only two couplings ($J = 10$ and 4.5 Hz) for

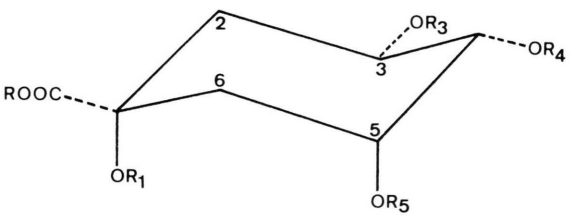
H(a) and one ($J = 3.5$ Hz) for H(c) remained, specifying their positioning on the endocyclic methylene groups. Considering the molecular formula $C_{17}H_{20}O_9$ deduced from measurement by mass spectrometry, in the high resolution mode, of **1a**, those data do not account for three carbon atoms. Two of them were assigned to belong to a methyl ester characterized conjointly by a sharp singlet at δ 3.68 ppm on both ^{1}H NMR spectra of **1a** and **1b** and IR absorptions at 1735 and 1430 cm^{-1} presented by **1a**. The third one, which must be quaternary, was included between the two endocyclic methylene groups leading to a pentasubstituted C_6 ring. The loss of a neutral molecule (205 m.u.), directly from the molecular ion of the natural compound **1a** and the occurrence of the fragment ion at m/z 315 = $C_{14}H_{19}O_8$ in the MS of the acetylated derivative **1b**, clearly identified the last C_6 ring with an O-bound quinic acid methyl ester.

The position of esterification of the methyl quinate with caffeic acid could be deduced from the comparative analysis of ^{1}H NMR chemical shifts of H-3, H-4 and H-5 in the quinic acid derivative. The results listed in Table I, for quinic acid **3** and the four isomeric forms of caffeoyl quinic acid **4**–**7**, indicate that the methine group involved in acylation is largely deshielded in comparison with the two others, even in the case of CH-4 which is usually more shielded [10]. On the basis of the recorded chemical shift values for H-3, H-4 and H-5 of both the natural product **1a** and its acetylated derivative **1b**, one can certainly conclude that none of the methine groups is esterified with the phenolic acid. Consequently, the latter must be bound to position 1 of methyl quinate, resulting in 1-*trans*-O-caffeoyl quinic acid methyl ester **1a**.

Table I. Chemical shift values in ^{1}H NMR (δ ppm/TMS) of H-3, H-4 and H-5 for quinic acid (**3**) and cinnamoyl derivatives (spectra were recorded in MeOH–D_4 for **1a** and **2a**, $CDCl_3$ for **1b** and in C_5D_5N for **3**–**7**).

	H-3	H-4	H-5
1a	ca. 5.20	4.10	5.25
1b	5.55	5.10	5.59
2a	ca. 5.19	4.12	5.24
3[a]	4.69	3.91	4.55
4[a]	4.78	4.04	4.63
5[a]	6.05	4.14	4.61
6[a]	5.01	5.48	4.80
7[a]	4.86	4.29	6.02

[a] From reference 10.



1a R_1 = caffeoyl; $R_3 = R_4 = R_5 = H$
1b R_1 = diacetyl caffeoyl; $R_3 = R_4 = R_5 = Ac$ } R = Me
2a R_1 = feruloyl; $R_3 = R_4 = R_5 = H$

3 $R_1 = R_3 = R_4 = R_5 = H$
4 R_1 = caffeoyl; $R_3 = R_4 = R_5 = H$
5 $R_1 = H$; R_3 = caffeoyl; $R_4 = R_5 = H$ } R = H
6 $R_1 = R_3 = H$; R_4 = caffeoyl; $R_5 = H$
7 $R_1 = R_3 = R_4 = H$; R_5 = caffeoyl

1-trans-O-feruloyl quinic acid methyl ester **2a**

As for 1-*trans*-O-caffeoyl quinic acid methyl ester **1a**, two parts were found again for compound **2a**, a *trans*-cinnamic acid esterifying the same quinic acid methyl ester. On the ^{1}H NMR spectrum, all the protons assigned to the skeleton of the *trans*-O-cinnamoyl quinic acid methyl ester were similar to those of the caffeoyl derivative **1a**, except for a supplementary methyl group. This one was indicated by a sharp singlet at δ 3.88 ppm. Effectively, the molecular ion of **2a** was found at m/z 382 corresponding to molecular formula $C_{18}H_{22}O_9$ (obtained by high resolution mass spectrometry) and the shift of 14 m.u. due to the additional methyl was reflected on the fragmentation pattern of the cinnamoyl part, leading to ions at m/z 194, 177 and 150. Those results, which agreed with the presence of a *trans*-feruloyl part in this molecule, were confirmed both by the characteristic blue fluorescence and the UV spectral data exhibited by **2a**, in comparison with *trans*-ferulic acid.

Even weak, the whole ions resulting from the fragmentation of the acetylated quinic acid methyl ester were present in the MS of the acetylated derivative **2b**. Situated at m/z 315, 273, 255, 213, 195 and 153, they arose, the one from the other by loss of a ketene or a molecule of water probably induced by the vicinality of the three acetyl functions on C-3, C-4 and C-5 of the quinic ring, as observed for the caffeoyl derivative **1b**. The similarity in the chemical shift values of H-3, H-4

and H-5 on the ^{1}H NMR spectra of the natural products **2a** and **1a** (Table I), associated with preceeding results, strongly suggest the same phenolic acid esterification pattern in both compounds. Compound **2a** is consequently considered 1-*trans*-O-feruloyl quinic acid methyl ester, also showing the IR band at 1735 cm^{-1}.

Chlorogenic acid methyl ester was described once in *Sambucus sieboldiana* Blume. (Caprifoliaceae) [11]. On the other hand, the occurrence of 1-caffeoyl and 1-feruloyl quinic acid methyl ester, in the plant kingdom, is reported here for the first time.

Experimental

Vegetal material

Meum athamanticum Jacq. (Umbelliferae) was collected from Col du Lautaret, Hautes Alpes, France, at the beginning of its fruiting stage. The material was dried during a week at about 40 °C before use. A sample of the rhizome has been deposited at Laboratoire de Pharmacognosie, UER de Pharmacie de Grenoble, Domaine de La Merci, F-38700 La Tronche.

Extraction and separation method

The rhizome tissue (1180 g) was powdered and successively extracted with 12 l *n*-hexane, 13 l $CHCl_3$ and 12 l MeOH. The methanolic extract was allowed to stand for 15 days when 60 g sugar crystallized. After removal of the latter, the extract was evaporated under *vacuum* to residue (153 g) which was dissolved in 100 ml MeOH and precipitated by addition of an equal volume of benzene. The supernatant was dried by evaporation *in vacuo*, taken into 500 ml of water and successively partitioned with 200 ml benzene, 300 ml AcOEt and 300 ml *n*-BuOH.

The butanolic layer was chromatographed on a Sephadex LH 20 column which was eluted with MeOH and gave three fractions SI_A, SI_B and SI_C. The last one was rechromatographed on polyamide DC 6 TLC with MeOH and yielded 20 mg of **1a**. Fraction SI_A was passed first through a Sephadex LH 20 column eluted with MeOH-$CHCl_3$ (1:1) then through a Sep-Pack C_{18} cartridge successively eluted with 30% and 50% aq. MeOH. The last layer was rechromatographed on a polyamide CC 6 column, eluted with MeOH and yielded 6 mg of **2a**.

Analytical controls

TLC controls of the natural products were performed on polyamide 6 F_{254} Macherey Nagel with MeOH (system 1) or benzene-MEK-MeOH (75:10:15) (system 2). The R_f of **1a** and **2a** were 0.70 and 0.80 respectively in system 1 and 0.57 for **2a** in system 2.

HPLC controls used a reversed phase column (μBondapack C_{18} 30 cm × 3.9 mm I.D.) and H_2O-MeOH-AcOH (70:30:0.5) (flow rate 1 ml/mn, UV detection at 320 nm). Retention times observed were 14.8 mn for **1a** and 29.6 mn for **2a**.

Acetylated derivatives

Acetylation was effected with acetic anhydrid and pyridine at room temperature. Purification of **1b** was carried out on a silica column with an elution gradient from $CHCl_3$ up to $CHCl_3$–Et_2O (2:8). Isolation of **2b** was achieved on silica gel F_{254} TLC with benzene-Et_2O (7:3).

1-trans-O-caffeoyl quinic acid methyl ester (**1a**): MS (70 eV) *m/z* (%): 368 (M^+; 8; 368.1108; $C_{17}H_{20}O_9$ = 368.1107), 336 (M-MeOH; 21), 180 (66), 163 (100), 162 (45), 136 (25), 135 (18), 134 (19), 129 (13), 110 (86), 94 (100), 89 (18), 83 (14), 81 (17), 77 (13). IR ν_{max}^{KBr} cm^{-1}: 3480, 2980, 2940, 1735, 1730, 1690, 1630, 1600, 1515, 1430, 1380, 1280, 1190, 1135, 1090, 1045, 980, 850, 810, 745, 690. UV λ_{max}^{MeOH} nm: 325, 297 sh, 243 sh, 232 sh, 220 sh; $\lambda_{max}^{MeOH+NaOMe}$ nm: 370, 302 sh, 260; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 355, 307 sh, 260, 255 sh; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 325, 297 sh, 243 sh, 232 sh, 215; $\lambda_{max}^{MeOH+NaOAc}$ nm: 385 sh, 325, 297 sh, 243; $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 350, 297 sh, 253. ^{1}H NMR (CD_3OD; 250 MHz): δ 2.10 (4H, m, H-2, H-6), 3.68 (3H, s, –COOMe), 4.10 (1H, m, H-4), ca. 5.20 (1H, m, H-3), 5.25 (1H, m, H-5), 6.20, 7.52 (AB pattern, J = 16 Hz, H-7′, H-8′), 6.78, 6.94 (AB pattern, J = 8 Hz, H-5′, H-6′), 7.02 (1H, s, H-2′).

Acetylated derivative (**1b**): MS (70 eV) *m/z* (%): 578 (M^+; 4), 494 (100; 494.1409; $C_{23}H_{16}O_{12}$ = 494.1423), 452 (10; 452.1318; $C_{21}H_{24}O_{11}$ = 452.1318), 434 (20; 434.1222; $C_{21}H_{22}O_{10}$ = 434.1670), 392 (8), 315 (41; 315.1084; $C_{14}H_{19}O_8$ = 315.1079), 273 (6; 273.0977; $C_{12}H_{17}O_7$ = 273.0974), 255 (5), 247 (20), 213 (13; 213.0765; $C_{10}H_{13}O_5$ = 213.0762), 205 (19), 195 (5; 195.0659; $C_{10}H_{11}O_4$ = 195.0657), 180 (19; 180.0429; $C_9H_8O_4$ = 180.0422), 171 (6), 163 (47), 162 (90; 162.0322; $C_9H_6O_3$ = 162.0316),

153 (28; 153.0554; $C_8H_9O_3$ = 153.0651), 135 (6), 134 (20; 134.0367; $C_8H_6O_2$ = 134.0367), 111 (7; 111.0445; $C_6H_7O_2$ = 111.0446), 43 (67). IR ν_{max}^{KBr} cm^{-1}: 3510, 3000, 2898, 1780, 1745, 1735, 1715, 1640, 1615, 1590, 1510, 1450, 1430, 1380, 1375, 1330, 1308, 1260, 1240, 1225, 1195, 1190, 1185, 1165, 1130, 1095, 1080, 1050, 1020, 1000, 970, 950, 925, 915, 885, 880, 845, 810, 780, 760, 740, 680, 670, 650. UV λ_{max}^{MeOH} nm: 275, 215 sh. ^{1}H NMR ($CDCl_3$; 250 MHz): δ 2.00 (3H, s, −CH−OAc), 2.07 (3H, s, −CH−OAc), 2.14 (3H, s, −CH−OAc), 2.30 (6H, s, ArOAc × 2), 2.52 (4H, m, H-2, H-6), 3.68 (3H, s, −COOMe), 5.10 (1H, dd, J = 10 and 3.5 Hz, H-4), 5.55 (1H, ddd, J = 10, 10 and 4.5 Hz, H-3), 5.59 (1H, dd, J = 3.5 and 3.5 Hz, H-5), 6.30, 7.58 (AB pattern, J = 16 Hz, H-7′, H-8′), 7.20 (1H, d, J = 8 Hz, H-5′), 7.34 (1H, d, J = 2 Hz, H-2′), 7.38 (1H, dd, J = 8 and 2 Hz, H-6′).

1-trans-O-feruloyl quinic acid methyl ester (**2a**): MS (70 eV) *m/z* (%): 382 (M^+; 30; 382.1250; $C_{18}H_{22}O_9$ = 382.1264), 350 (M-MeOH; 47), 194 (75), 177 (100), 176 (11), 150 (50), 149 (12), 148 (5), 135 (11), 117 (10), 91 (4). IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1735, 1705, 1695, 1630, 1600, 1595, 1590, 1515, 1450, 1430, 1380, 1365, 1275, 1190, 1150, 1130, 1090, 1035, 980, 845, 815, 745. UV λ_{max}^{MeOH} nm: 320, 285 sh, 245 sh; $\lambda_{max}^{MeOH+NaOMe}$ nm: 375, 307 sh, 255 sh; ^{1}H NMR (CD_3OD; 250 MHz): δ 2.10 (4H, m, H-2, H-6), 3.68 (3H, s, −COOMe), 3.88 (3H, s, ArOMe), 4.12 (1H, m, H-4), ca. 5.19 (1H, m, H-3), 5.24 (1H, m, H-5), 6.32, 7.58 (AB pattern, J = 16 Hz, H-7′, H-8′), 6.82 (1H, d, J = 8.5 Hz, H-5′), 7.04 (1H, dd, J = 8.5 and 1.5 Hz, H-6′), 7.18 (1H, d, J = 1.5 Hz, H-2′).

Acetylated derivative (**2b**): MS (70 eV) *m/z* (%): 550 (M^+; 5), 508 (100), 466 (5), 406 (2), 315 (6), 284 (7), 273 (3), 256 (13), 255 (4), 219 (12), 213 (5), 194 (35), 177 (57), 176 (62), 173 (40), 153 (29), 149 (76), 148 (12), 145 (10), 117 (7), 111 (29), 91 (14), 60 (60), 43 (100). UV λ_{max}^{MeOH} nm: 310 sh, 277, 235 sh.

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