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Quinic acid esters from *Pavetta owariensis* var. *owariensis* (Rubiaceae)

Baldé A. M.[1, *], Pieters L. A.[2], Claeys M.[2], Traoré M. S.[1], Baldé M. A.[1], Diallo, M. S. T.[1], Baldé E. S.[1], Diané S.[1], Vlietinck A. J.[2]

[1]Centre de Recherche et de Valorisation des Plantes Médicinales de Dubréka, Dubréka, Guinée
[2]Department of Pharmaceutical sciences, University of Antwerp (UIA), Antwerpen, Belgium

Email address:
bmaliou2002@yahoo.fr (Baldé A. M.)

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Abstract: The stem-bark of *Pavetta owariensis var. owariensis* is used in Guinean traditional medicine as an anthelmintic. Previous biological and chemical investigations reported the *in vivo* and/or *in vitro* anthelmintic, antischistosomal, antiviral and antibacterial properties along with the presence of a wide range of proanthocyanidins possessing a doubly-linked structure and ferulic acid esters substances. From the stem-bark, five previously known quinic acid esters have been isolated for the first time. Their structures have been established by means of FAB-mass, ^{1}H and $^{13}CNMR$ spectroscopy.

Keywords: Rubiaceae, *Pavetta owariensis* var. *owariensis*, Quinic Acid Esters, Spectroscopic Methods

1. Introduction

Quinic acid esters are widespread plant constituents with diverse biological effects such as inhibition of lipoxygenase, antibacterial, antihelmintic activity, anti-inflammatory, antispasmodic, antihepatotoxic/hepatoprotective properties, antioxidant, free radical scavenging properties, vasodilatory actions,antimutagenic, anticarcinogenic, antiinflammatory, antimicrobial [1-5].

Plant species from the Rubiaceae have been found to contain a wide variety of phenolic acid compounds. Previous chemical investigations on te stem-bark of *P. owariensis* var. *owariensis* P. Beauv. (Rubiaceae) have revealed the presence of a wide range of proanthocyanidins possessing a doubly-linked structure and ferulic acid esters substances [6-13]. The stem-bark extracts have shown a wide biological activities including in *vivo* and/or *in vitro* anthelmintic, antischistosomal, antiviral and antibacterial properties [14-17]. Extensive investigations on the stem-bark of the plant have resulted in the isolation of five quinic acid esters.

2. Material and Method

2.1. Plant Material

The stem-bark sample was collected in Sérédou in March 1986. The plant was taxonomically identified by the department of Botany in the Research Center of medicinal Plants, Sérédou. Voucher specimens have been deposited at the herbarium of the Center and the herbarium of the National Botanical Garden of Belgium at Meise.

2.2. Fractionation

Air-dried powdered bark (1,5 kg) of *P. owariensis* var. *owariensis* was percolated with acetone-water (7:3 v/v) at room temperature. The extract was concentrated *in vacuo* to yield 65 g of a brown residue. The residue was partitioned between toluene (3000 ml) and distilled water (1500 ml). The aqueous phase was successively treated with ethylacetate/toluene(1:1 v/v, 3x 1000 ml), and n-butanol (3x 1000ml); each grouped extract was evaporated to dryness yielding 4.0 g of a yellow solid, 16.8 g of a pink solid, and 20.3 g of a red-brown residue, respectively. By portion of 4 to 6 g, each solid was treated with 10 ml of the upper phase of butanol/propanol/water (2:1:3), and insoluble materials were removed by filtration. The filtrate was applied to droplet counter current chromatography

(n-butanol/n-propanol/H_20 2:1:3, ascending mode). Fractions of 15 ml were collected. The chromatographically identical fractions were combined. Eight subfractions were obtained: A (2.23 g), B (6.15g), C (6.05 g), D (7.23 g), E (6.42 g), F (5.40 g), G (1.70 g), and H (0.35 g). They all showed a positive vanillin-chlorhydric reaction, except subfraction A. The subfraction C was subjected to a repetitive chromatography on sephadex LH-20 (2 x 90 cm) with ethanol as eluent to yield compounds 1–5.

The isolation of pure compounds was not made to exhaustion so that the percentages of yield for each compound were only approximate.

2.3. Experimental

Compound 1 (Caffeoylquinic acid)

Amorphous brown compound; Rf= 0.79(Solvent B)

FAB-MS: m/z 377 $(M+Na)^+$, 355 $(M+H)^+$, 163 $(Caffeoyl)^+$

^{1}H-NMR (199.50MHz, CD_3OD): δ 2.02 (4H, m, H-2 and H-6), 3.69-3.75 (1H, m, H-3), 4.13 (1H, brs, H-4), 5.20 (1H, m, H-5), 6.19 (1H, d, J=16Hz, H-α), 6.76 (1H, d, J=8Hz, H-5′), 6.92 (1H, d, J=8Hz, H-6′), 7.03 (1H, s, H-2′), 7.45 (1H, d, J=16Hz, H-β)

^{13}C-NMR (50.10MHz, CD_3OD) δ: 36.9 (C-6), 37.7 (C-2), 69.9 (C-3), 70.9 (C-4), 72.1 (C-3′), 146.2 (C-β), 147.9 (C-4), 168.1 (C-8), 176.8 (C-7).

Compound 2 (Caffeoylquinic acid)

Amorphous compound, Rf= 0.71(Solvent B)

FAB-MS: m⁄z 377$(M+Na)^+$, 355$(M+H)^+$, 163(Caffeoyl) +

^{1}H-NMR (199.50MHz; CD3OD): δ 2.23 (4 H, m, H-2 and H-6), 4.40 (1H, m, H-3), 5.20 (1H, m, H-5), 5.65 (1H, m, H-4), 6.35(1H, d, J=16 Hz, H-β), 6.75 (1H, d, J=8Hz, H-5′), 6.89 (1H, d, J=8 Hz, H-6′), 7.52 (1H, d, J=16Hz, H-α).

^{13}C-NMR (50.10 MHz; CD_3OD): δ 38.6 (C-6), 39.9 (C-2), 69.2(C-3), 69.9(C-5), 76.3 (C-4), 76.5 (C-1), 114.8 (C-2′), 115.1 (C-α), 116.5(C-5′), 123.1(C-6′), 127.7 (C-1′), 146.7 (C-3′), 147.5 (C-β), 149.6 (C-4′), 168.3 (C-8), 178.6 (C-7).

Compound 3 (Feruloylquinic acid)

Amorphous substance; Rf=0.75(Solvent B)

FAB-MS: m /z 391 $(M+Na)^+$, 369$(M+H)^+$, 195(Methylcaffeic ACID +H)$^+$, 177 (Mthylcaffeoyl) $^+$

^{1}H-NMR (199.50 MHz; CD3OD): δ 2.11 (4H, m, H-2 and H-6), 3.71 (1H, m, H-4), 3.80 (MeO-), 4.28 (1H ,m,H-3) , 5.35 (1H,m,H-5), 6.33 (1H,d,J=16Hz ,H-β), 6.91 (1H,m,H-6′), 7.09 (1H,m,H-5′), 7.31 (1H,brs,H-2′), 7.57 (1H,d,J=16Hz,H-α)

^{13}C-NMR (50.10MHz ; CD_3OD) : δ 38.6 (C-6) , 39.7 (C-2), 56.1 (MeO), 71.6 and 72.3 (C-4, C-3), 78.7 (C-5), 115.2 (C-2′), 115.5 (C-α), 116.5 (C-5′), 122.8 (C-6′), 127.8 (C-1′), 146.9 (C-β),149.5 (C-4′), 150.8 (C-3′), 168.9 (C-8), 174.8 (C-7).

Compound 4 (Dicaffeoylquinic acid)

Amorphous compound; Rf= 0.21(solvent B)

^{1}H-NMR (199.50MHz ;CD_3OD) :δ 2.08-2.22 (4H ,m,H-2 and H-6), 4.32 (1H,J < 2 Hz, H-3) , 5.08 (1H,dd,J=9 Hz, J < 2 Hz, H-4),5.67 (1 H,m,H-5), 6.18 (1H,d,J= 16Hz, H-α), 6.26 (1H,d,J=16 Hz, H-a), 6.72 (2H,d,J=8 Hz, H-5′ and H-5″), 6.89 (2H,d,J=8Hz, H-6′ and , H-6″), 6.99 (2H,s,H-2′ and H-2″), 7.46 (1H,d,J=16Hz, H-β),7.57 (1H,d,j=15.4Hz,H-β.

^{13}C-NMR (50.10 MHz; CD_3OD): δ 38.7 (C-6), 40.3 (C-2), 69.4 (C-3), 70.2 (C-‘or C-5), 76.6 (C-5 or C -4), 114.8 (C-2′ and C-2″), 115.1 (2C-α), 116.4 (C-5′ and C- 5″), 122.9 (C-6′ and C-6″),′ 127.7 (C-1′ and C-1″),′ 146.6 (C-3′ and C-3″), 147.3 (C-β), 147.5 (C-β), 149.5 (C-4′ and C-4″), 168.4 (C-8),168.5 (C-8),178.8 (C-7).

Compound 5 (Caffeoyl and feruloylquinic acid)

Amorphous substance; Rf= 0.24 (solvent B)

FAB-MS: m/z 553$(M+Na)^+$, 531$(M+H)^+$

^{1}H-NMR (199.50MHz; CD_3OD): δ 2.08- 2.11 (H-2; H-6), 3.71, 4.28, 4.40, 5.22, 5.35, 5.58 (H-3, H-4, H-5), 3.85 (MeO), 6.23, 6.35, 6.37(H-α), 6.75-6.78 (H-6′ H-6″), 6.92-7.11

^{13}C-NMR (50.10 MHz ; CD_3OD) :δ 38.6, 38.8 (C-6), 39.7, 3.9 (C-2), 72.378.8 (C-3, C-4, C-5), 115.2 (C-2′, C-2″), 115.7, 116.0 (C-5′, C-5″), 122.8 (C-6′, C-6″), 127.8 (C-1′ C-1″), 150.5(C-3′ of the feruloylmoiety), 146.5 (C-3″ of the feruloylmoiety), 146.9 (H-β),149.5 (C-4′ , C-4″), 168.9 (C-8), 174.8 (C-7).

3. Results

Compound 1 was isolated in a yield of 0.007%. Its showed an UV λ max at 325 nm. As shown in table 1, the positive FAB- mass spectrum gave an ion at m/z 355$[M+H]^+$. The ^{1}H and ^{13}C-NMR spectra of 1 were superimposable with those of authentic chlorgenic acid. Thus compound 1 was identified as 5-caffeoylquinic acid or chlorogenic acid.

Compound 2 was isolated, in a yield of 0.005%. On TLC (solvent), 2 showed a Rf value of 0.76. The UV spectrum indicated a λ max at 325 nm. The FAB- mass spectrum of 2 was similar to that of 19, indicating an ion at m/z 355 [M+H] +. The ^{1}H-NMR spectrum of 2 was close to that of 1. However, 2 clearly differed from compound 2 with respect to the chemical shifts of the heterocyclic protons *viz.* δ 5.83 (m), 4.40 (m) and 4.13 for 2 (δ 4.13, 3.69, and 5.19 for 1). The quinic acid protons in 2 were more close to those described by Corse *et al.* (1966)[18] for 4-caffeoylquinic acid [δ5.01 (H-3), 5.48 (H-4), and 4.80 (H-5)], than those of 3-caffeoyquinic acid (δ 6.05, 4.15, and 4.61, respectively). Furthermore, the ^{13}C-NMR spectrum showed a deshielding position of C-1(Δδ 1.5), C-2 (Δδ 2.2), C-4(Δδ 5.4), a shielding position of C-5 (Δδ -2.2) in 2 relative to those of 1. Conspicuous differences were also observed in the aromatic part of these two compounds. These results were consistent with the structure of 4-caffeoyquinic acid.

Compound 3 was isolated in a yield of 0.007%. Its FAB-mass spectrum showed a $[M+H]^+$ ion at m/z 369. The ^{1}H-NMR spectrum of 3 was similar to that of 1 or 2. However, compound 3 differed from these compounds by the presence of an additional singlet of three protons at 3.85 ppm, which was indicative for the presence of a methoxyl group in the molecule. The ^{13}C-NMR spectrum of 3 was also similar to those of 1 and 2, except for the presence of the signal belonging to methoxyl group at δ 56.1. Since the proton signals of the quinic acid moiety of 3 (δ 5.35, H-5; 4.28, H-3; 3.71, H-4) were close to those of 5-Feruloylquinic acid (δ 5.09, 3.93, and 3.55, respectively) described by Morishita *et al.*, (1984) [19] as 3-Feruloylquinic acid (obsolete pre-IUPAC numbering system), compound 3 was identified as

5-feruloyquinic acid.

Compound 4 was isolated in a yield of 0.008%. The FAB-mass spectrum exhibited a $[M+H]^+$ ion at m/z 517, suggesting a dicaffeoylquinic acid structure. The ^{1}H-NMR spectrum showed a presence of four *trans* vinyl protons at δ 7.58 (J=15.4 Hz), 6.26 (J=16 Hz) and 6.18 (J= 16 Hz). This was indicative for a quinic acid acylated with two caffeoyl moieties. Since the spin-spin coupling constant between a C-5 proton (axial) and a C-4 proton (axial) is larger than J 4,3 (axial-equatorial) [17], the proton signals at δ 5.08 (J=9 Hz), 4,32 (J<2Hz), and δ 5.67 (m) were attributed to H-4, H-3, and H-5, respectively. Based on the fact that the paramagnetic chemical shifts have a direct relation to substitution on the hydroxyl groups of quinic acid [18], the deshielding position of H-5 (Δδ = 0.47) and H-4 (Δδ 0.95) relative to 5-CQA (1) were in agreement with two caffeic acid residue attached to C-5 and C-4. Furthermore, the ^{13}CNMR spectrum of 4 was close to those of 5-CQA (1) and 4-CQA (2). However, compound 4 differed from the ^{13}C-NMR spectra of these two chlorogenic moieties by the presence of additional signals. Evidence for two caffeoyl moieties in compound 4 were indicated by the pair of signals at δ 147.3 and 147.5 corresponding to two C-7, and at δ 168.4 and 168.6, corresponding to two carbonyl esters (2C-8). Accordingly, compound 4 was identified as 4, 5-dicaffeoylquinic acid.

Compound 5 was obtained in a yielded of 0.003%. The FAB- mass spectrum of compound 5 gave an ion at m/z 553$[M+Na]^+$ and 531$[M+H]^+$. The ^{1}H-NMR spectrum showed signals that were characteristic for quinic, ferulic and caffeic acid moieties. The proton signals of the quinic acid at δ 5.58, 5.35, 5.22, 4.40, 4.28, and 3.71 suggested that 5 was a mixture of two compounds in the relative ratio of 1: 1. Moreover, the ^{13}CNMR spectrum showed signal pairs for the quinic acid carbon resonances, indicating the presence of two close compounds. Due to the little amount of compound 5, the position of the two acylation sites could not be determined. From the available data, compound 5 was identified as caffeoyl and feruloylquinic acid.

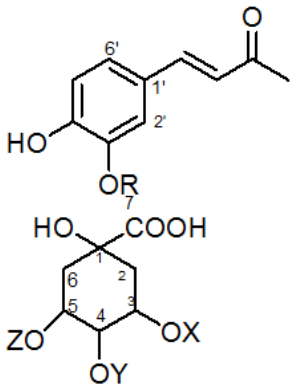

Figure 1. *Structure of compounds 1-5*

R	
H	Caffeoyl- (C)
Me	Feruloyl- (F)

Compound	X	Y	Z
1	H	H	C
2	H	C	H
3	H	H	F
4	H	C	C
5	H*	C*	F*

*Assignements may be interchanged

4. Discussion and Conclusion

Quinic acid esters compounds, in particular chlorogenic acid derivatives are widespread in dicotyledons. Some plant species from the Rubiaceae have been found to contain a wide variety of these phenolic compounds viz Coffea, Uncaria sp, Galium sp, Isertia sp. [1; 20-21].The simultaneous presence of quinic acid esters of caffeic and ferulic acid has been found in Asteraceae, Saxifragaceae, Rosaceae, Ericaceae and Brassicaceae [22], Convolvulaceae [23] etc. *P. owariensis* represents the second exemple of a species from Rubiaceae to contain such wide variety of quinic acid esters, the first exemple being Coffea sp.

Apart from their chemotaxonomic importance, the presence of quinic acid esters of caffeic and ferulic acid (Caffeoylquinic acid, feruloyquinic acid, dicaffeoylquinic acid) in *Pavetta owariensis* could support at least partly some biological properties of *P. owariensis* such as the anti-inflammatory effect, the anthelmintic and the weak antispasmodic of the plant extract observed during our previous therapeutic investigations.

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