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CAFFEOYLMALIC AND TWO PYRROLE ACIDS FROM *PARIETARIA OFFICINALIS*

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Key Word Index—*Parietaria officinalis*, Urticaceae; caffeoylmalic acid, 1*H*-pyrrole-2,3-dicarboxylic acid, 1-[(caffeoyloxy)(carboxy)methoxy]-1*H*-pyrrole-2,3,5-tricarboxylic acid

Abstract—A methanolic extract from leaves and flowers of *Parietaria officinalis* afforded three acids, namely caffeoylmalic, 1*H*-pyrrole-2,3-dicarboxylic and 1-[(caffeoyloxy)(carboxy)methoxy]-1*H*-pyrrole-2,3,5-tricarboxylic.

INTRODUCTION

Parietaria officinalis (Urticaceae) is considered to be a medicinal plant [1] and is included in the pharmacopoeias of several countries [2]. Previous chemical studies showed the presence of 10 flavonol glycosides [3] and *p*-coumaric, ferulic and isoferulic acids [4]. In the present work a search for further phenolics led to isolation of three water soluble carboxylic acids (**1**–**3**) [5].

RESULTS AND DISCUSSION

A methanolic extract of the leaves and flowers, after fractionation with solvents and chromatography, yielded compounds **1**–**3**. Compound **1** was identified as *trans*-caffeoyl-*l*-malic acid from its FD mass spectrum, ^{1}H and ^{13}C NMR (both measured in DMSO-d_6 and pyridine-d_5), UV and $[\alpha]_D$ data [6–11].

Compound **2** revealed in its ^{1}H NMR spectrum in DMSO-d_6 typical pyrrole coupling between NH (δ 11.51) and two C-protons (δ 6.72 and 6.43) [12], observed before addition of D_2O. The ^{13}C NMR spectrum indicated a pyrrole substituted with two carboxylic groups (δ 166.4, 163.7), in agreement with the EI mass spectral fragmentation [13] and supported by conversion of **2** into the expected dimethyl ester (**2a**) and its N-methyl derivative (**2b**). Of the four isomers possible, viz. 2, 3; 2, 4; 2, 5 and 3, 4, the former was chosen because (i) all ring carbons gave separate signals in the ^{13}C NMR spectra of **2**, **2a**, and **2b** [13, 14], (ii) the magnitude of coupling between the pyrrolic C-protons in ^{1}H NMR was 2.9 Hz (**2**) and 2.7 Hz (**2a**, **2b**) (lit. [12, 15] $J_{2,3}$ = 2.3–3.2 Hz). Hence, **2** is 1*H*-pyrrole-2,3-dicarboxylic acid.

The ^{1}H and ^{13}C NMR spectra of **3** showed the presence of the caffeoyl moiety [6, 7]. The ^{13}C NMR spectrum exhibited nine additional signals, of which those at δ 129.7 *s*, 128.3 *s*, 119.8 *s* and 113.2 *d*, suggested a pyrrole unsubstituted only at the one β-position [14]. The remaining signals suggested four carboxylic groups (δ range 169–163) and one isolated methine group (δ 71.7 *d*).

Methanolysis of **3** with NaOMe afforded methyl caffeate and **3a**, whose product of permethylation **3b**, exhibited an EI mass spectrum ($[M]^+$ at *m/z* 255, losses of MeOH [13]) compatible with the structure of trimethyl 1-methyl-1*H*-pyrrole-2,3,5-tricarboxylate. The strong ion at *m/z* 216 (Fig. 1) in the FAB mass spectrum of **3** required the presence of an oxygen atom attached to the nitrogen of the pyrrole. The above observations, together with the FAB mass spectral peak at *m/z* 454, ascribable to the protonated molecular ion ($[M+3H]^+$), led to the conclusion, that the molecule of **3** contains glyoxylic acid hydrate esterified with caffeic acid and attached to the pyrrolic moiety via C–O–N or –CO–O–N linkages. The latter structure can be discarded, because in this case methanolysis should produce a N–OMe derivative (by analogy to closely related N-acyloxyindoles [16]) or at least a N–OH compound. However, no evidences of such functions were noticed in the ^{1}H NMR [17–19] or UV [20] spectra of **3a**. Moreover, no traces of glyoxylic acid could be detected in the alkaline hydrolysate of **3** by application of reaction with tryptophan [21]. The splitting of N–O bond in **3a** (now identified as 1-[(carboxy)(hydroxy)methoxy]-1*H*-pyrrole-2,3,5-tricarboxylic acid) during permethylation (to **3b**) must have resulted from the action of DMSO [22] and/or a base [17]. Thus, the structure of 1-[(caffeoyloxy(carboxy)methoxy]-1*H*- pyrrole-2,3,5-tricarboxylic acid is proposed for **3**.

While caffeic acid esters of various aliphatic hydroxy acids are widespread [23–25], the occurrence of simple pyrrole acids seems to be exceptional. 1*H*-Pyrrole-2-carboxylic acid has been reported from nature either as an acyl moiety in some alkaloids [e.g. 26] or as the free compound [27]. 1H-pyrrole-2,3-dicarboxylic and -2,3,5-tricarboxylic acids are known in the chemistry of melanins [28–32] as products of their oxidation. N-Alkoxy (OMe) pyrrole derivatives are represented by the closely related indoles [33 and refs cited therein].

EXPERIMENTAL

^{1}H and ^{13}C NMR were recorded at 89.6 and 22.5 MHz, respectively. EIMS (probe) at 75 eV.

Parietaria officinalis L. was collected in July 1984 at Plewiska near Poznań, Poland. A voucher specimen is deposited at our department.

Dry leaves and flowers (966 g) were extracted with hot MeOH and the dry extract dissolved in H_2O and successively extracted with $CHCl_3$ and *n*-BuOH. The concd *n*-BuOH extract was fractionated by polyamide CC with H_2O, H_2O–*n*-BuOH (100 9), H_2O–*n*-BuOH–Me_2CO (10·1:1, 20 3 3 and 5:1:1), MeOH and 0 01% NH_4OH–MeOH, respectively. The latter eluate afforded **2** (324 mg) after repeated Sephadex LH-20 CC using H_2O and 90% MeOH. The H_2O-phase from partitioning was sepd by CC on polyamide (H_2O, H_2O–MeOH and 0 01% NH_4OH–MeOH, respectively, and PC (*n*-BuOH–HOAc–H_2O, 6.1·2) of the latter fr yielded **1** (R_f 0 8), **2** (R_f 0 45), and **3** (R_f 0 25). Compound 3 was further purified by PC in 15% HOAc Bands were eluted with 50% MeOH. Pure samples of **1** (350 mg) and **3** (30 mg) were obtained after clean-up on polyamide and Sephadex LH-20 (MeOH or H_2O) columns, respectively Detection **1**, **3** (UV at 365 nm, blue), **2** (UV at 254, absorbance, daylight, diazotized sulphanilic acid, red-brown)

trans-caffeoyl-l-*malic acid* (**1**) Yellowish glass. $[\alpha]_D$ +20 4° (MeOH: *c* 2 2) (lit. [11]: +28.3°, [12] 21 2°). FDMS *m/z* (rel int) 297 $[M+H]^+$ (100), 296 $[M]^+$ (13), 163 [caffeoyl]$^+$ (11)

1*H-Pyrrole-2,3-dicarboxylic acid* (**2**) White needles, mp 242–243° (dec) FDMS *m/z* (rel. int): 155 $[M]^+$ HR EIMS *m/z* (rel int) 155.02178 $[M]^+$ (100) (Calc for $C_6H_5NO_4$ 155 02181), 137 $[M-H_2O]^+$ (38), 120 $[M-H_2O-OH]^+$ (39), 111 (37), 93 (38), 64 (14), 65 (15) IR ν_{max}^{KBr} cm^{-1} 3410 (NH) UV λ_{max}^{MeOH} nm 206, 243, 277, +HCl 207, 243, 286, +NaOMe 245, 273 ^1H NMR (DMSO-d_6) δ 11 51 (1H, *br*, H-1), 7 30 (2H, *br s*, 2 × CO_2H), 6 72 (1H, *t*, $J \sim 2.9$ Hz, H-5), 6.43 (1H, *t*, $J \sim 2$ 9 Hz, H-4). ^1H NMR (DMSO-d_6 + D_2O) δ 6.81 (1H, *d*, $J = 2$ 9 Hz, H-5), 6 51 (1H, *d*, $J = 2.9$ Hz, H-4) ^{13}C NMR (DMSO-d_6) δ 166 4, 163 7 (each *s*, 2 × CO_2H), 128.5 (*s*, C-2), 120.2 (*s*, C-3), 111 7 (*d*, C-4), 118.8 (*d*, C-5)

Dimethyl 1H-*pyrrole-2,3-dicarboxylate* (**2a**). Methylation of **2** with CH_2N_2 in 90% MeOH (1hr) gave **2a** as colourless crystals, mp 69–73° EIMS *m/z* (rel int.)· 183 $[M]^+$ (72), 152 $[M-MeOH]^+$ (98), 120 (100), 93 (37) IR ν_{max}^{KBr} cm^{-1} 3360 (NH) UV λ_{max}^{MeOH} nm 205, 242, 278 ^1H NMR ($CDCl_3$) δ 10 10 (1H, *br*, H-1), 6 89 (1H, *t*, $J \sim 2$ 9 Hz, H-5), 6 66 (1H, *t*, $J \sim 2.9$ Hz, H-4), 3 86 (6H, *s*, 2 × CO_2Me) ^1H NMR ($CDCl_3$ + D_2O) δ· 6 90 (1H, *d*, $J = 2$ 7 Hz, H-5), 6.66 (1H, *d*, $J = 2$ 7 Hz, H-4), 3.86 (6H, *s*, 2 × CO_2Me) ^{13}C NMR ($CDCl_3$) δ 164 7, 160 7 (each *s*, 2 × $\underline{C}O_2Me$), 122 7 (*s*, C-2), 120 3 (*s*, C-3), 121 4 (*d*, C-4), 113 5 (*d*, C-5), 52.0, 51 7 (each *q*, 2 × $CO_2\underline{Me}$)

Dimethyl 1-*methyl*-1H-*pyrrole-2,3-dicarboxylate* (**2b**). Methylation of **2a** (MeI–NaH–DMSO, 1 hr) gave **2b** as a mass, mp 59–63° EIMS *m/z* (rel int.) 197 $[M]^+$ (50), 166 $[M-MeO]^+$ (100), 165 $[M-MeOH]^+$ (23), 136 $[M-MeOH-MeO]^+$ (33), 120 (4), 107 (11) UV λ_{max}^{MeOH} nm 206, 249 sh, 273 ^1H NMR ($CDCl_3$) δ· 6 65 (1H, *d*, $J = 2$ 7 Hz, H-5), 6.47 (1H, *d*, $J = 2.7$ Hz, H-4), 3.88 (3H, *s*, N-Me), 3 82 (6H, *s*, 2 × CO_2Me) ^{13}C NMR ($CDCl_3$) δ 165 0, 161.8 (each *s*, 2 × $\underline{C}O_2Me$), 122 4 (*s*, C-2), 120.8 (*s*, C-3), 110 4 (*d*, C-4), 126 4 (*d*, C-5), 51 9, 51.6 (each *q*, 2 × $CO_2\underline{Me}$), 36 6 (*q*, N-Me)

1-[(*caffeoyloxy*) (*carboxy*)*methoxy*]-1H-*Pyrrole*-2,3,5-*tricarboxylic acid* (**3**) Off-white granules which did not melt up to 360° No EIMS, undefined FDMS FABMS *m/z* (rel. int.)· 454 $[M+3H]^+$ (7), 306 $[M+4H-3\times CO_2-OH]^+$ (44), 289 (26), 279 $[M-4H-4\times CO_2]^+$ (40), 274 (59), 257 (25), 253 (12), 237 (15), 216 (89), 199 (62), 181 [caffeic acid + H]$^+$ (100), 179 (60), 163 (73), 149 (96), 147 (76) For assignments of ions see Fig 1 UV λ_{max}^{MeOH} nm 221, 260 sh, 271, 328, +NaOMe 271, 372 ^1H NMR (DMSO-d_6 + D_2O) δ [caffeoyl moiety] 7 49 (1H, *d*, $J = 16$ Hz, H-7), 7 12 (1H, *s*, H-2), 7 05 (1H, *d*, $J = 8$ Hz, H-6), 6 80 (1H, *d*, $J = 8$ Hz, H-5), 6 36 (1H, *d*, $J = 16$ Hz, H-8), [glyoxylic acid moiety] 5 10 (1H, *s*, CH), [pyrrole moiety] 6 80 (1H, *s*-also before addition of D_2O, H-4) ^{13}C NMR (DMSO-d_6) δ· [caffeoyl moiety]: 125 5 (*s*, C-1), 114.8 (*d*, C-2), 145 7 (*s*, C-3), 148.6 (*s*, C-4), 113.9 (*d*, C-5), 121 2 (*d*, C-6), 145 1 (*d*, C-7), 115.8 (*d*, C-8), 165.8 (*s*, C-9), [glyoxylic acid hydrate moiety]. 71 7 (*d*, CH), [pyrrole moiety]. 129.7 (*s*, C-2), 119.8 (*s*, C-3), 113.2 (*d*, C-4), 128 3 (*s*, C-5), carboxylic groups 168 8, 165 4, 164 0, 163.4 (each *s*)

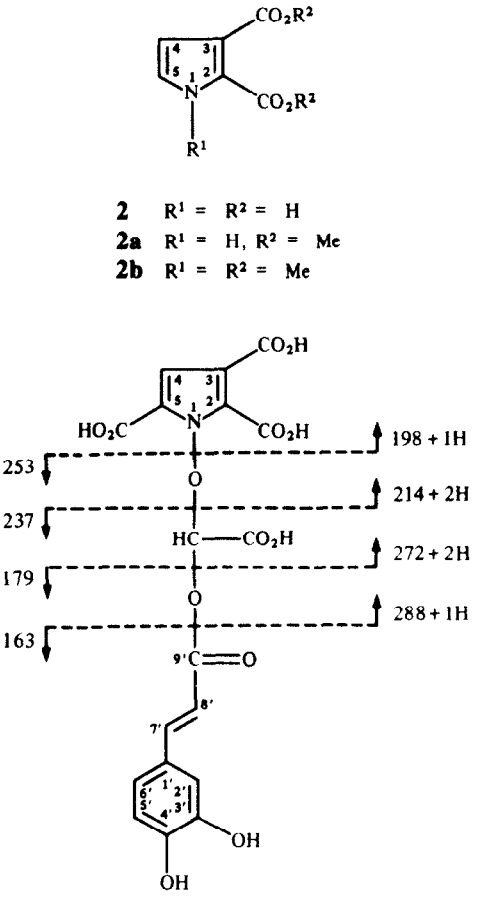

Fig 1 Structures of compounds **2** and **3** and the FAB mass spectral fragmentation pattern of **3**

Methanolysis of **3** *with* NaOMe A soln of **3** (24 mg) in 2% NaOMe–MeOH was kept at room temp. for 1 hr, then neutralized, evapd and sepd between Et_2O (Me caffeate) and H_2O The latter yielded **3a** (12 mg) after purification by HPTLC silica gel in *n*-BuOH–HOAc–H_2O (4 1 5, upper phase), R_f 0 37 Compound **3a** white needles. No EIMS UV λ_{max}^{MeOH} nm 222, 227, 242, 272, +NaOMe 222 sh, 227, 240, 271 ^1H NMR (CD_3OD) δ: 7.31 (1H, *s*, pyrrole H-4), 4 95 (1H, *s*, glyoxylic acid CH) Compound **3a** was permethylated (as described for **2b**) to give **3b** (1.5 mg) by prep TLC on HPTLC silica gel ($CHCl_3$) Compound **3b**. trimethyl 1-methyl-1H-pyrrole-2,3,5-tricarboxylate EIMS *m/z* (rel int)· 255 $[M]^+$ (45), 224 $[M-MeO]^+$ (100), 193 $[M-2\times MeO]^+$ (17), 165 (15), 149 (7), 137 (8), 134 (3), 106 (6), 78 (5), 64 (4) UV λ_{max}^{MeOH} nm 206, 249 sh, 273

Attempted detection of glyoxylic acid in alkaline hydrolysate of **3** Solns of **3** and glyoxylic acid hydrate (each 1 mg) in 1 M aq NaOH (0 5 ml) were kept at room temp. for 1hr, acidified (HCl), treated with tryptophan (1 mg), kept for a further 24 hr, then spotted onto a strip of Whatman 3 paper and dried. Only the latter compound showed a strong yellow fluorescence under UV at 365 nm

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