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MALONATED FLAVONOL GLYCOSIDES AND 3,5-DICAFFEOYLQUINIC ACID FROM PEARS

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Key Word Index—*Pyrus communis*; Rosaceae; pears; malonated flavonol glucosides; 3,5-dicaffeoylquinic acid.

Abstract—3-*O*-(6″-*O*-malonyl)-β-Glucosides of quercetin, kaempferol, isorhamnetin and 3,5-dicaffeoylquinic acid were isolated and identified from leaves of pears. The compounds are also present in the fruits.

INTRODUCTION

During our investigation of flavonoids in pears and apples necessary for the development of analytical methods for detecting the adulteration of juices made from these fruits, we found compounds that could not be initially identified. Chromatographic behaviour and spectral UV data indicated there were three flavonol glycosides and one caffeic acid derivative, each of which contained a free carboxylic acid group. Several authors have recently described compounds in peels of pears which have not been identified [1, 2]. Nortjé reports the presence of isorhamnetin malonylglucoside in pears but does not indicate its exact structure [3]. The presence of 3-*O*-malonylglucosides of quercetin [4], and kaempferol [5, 6], and 3,5-dicaffeoylquinic acid [7–9] has been described as existing in various plants but not in pears.

RESULTS AND DISCUSSION

The varieties of pears examined and the contents of the compounds found are listed in Table 1. 3,5-Dicaffeoylquinic acid (**1**, 205 mg) and 3-*O*-(6″-*O*-malonyl)-β-glucosides of quercetin (**2**, 97 mg), kaempferol (**3**, 48 mg), and isorhamnetin (**4**, 45 mg) were isolated from the leaves of pears by means of repeated prep. HPLC. The purity of the compounds were examined with the help of analytical HPLC with diode-array detection. For identification, standard methods were used. Basic hydrolysis of the flavonoids with mild alkaline (solution of 2% barium hydroxide) cleaves the acid moiety [10]. The resulting flavonol glucosides were identified by HPLC co-chromatography and from their UV spectra recorded with a diode-array detector. After the enzymatic hydrolysis with an unspecific esterase (Röhm EL 1-77, Darmstadt) and after silylation glucose and malonic acid were detected by capillary-GC. The enzymatic hydrolysis of **1** with the unspecific esterase yielded caffeic and quinic acid. Caffeic acid was identified by HPLC and diode-array UV spectrum. After benzoylation [11] both acids could be detected (HPLC). The results were confirmed by capillary-GC.

The ^{1}H and ^{13}C NMR spectra identified the flavonoids as 3-*O*-substituents of quercetin, isorhamnetin, and kaempferol [12]. The number and characteristic shifts of the ^{13}C hexose signals indicated the presence of glucose in each flavonoid. The vicinal proton–proton coupling constants indicated a β-glycosidic linkage. Their identity was confirmed from the vicinal couplings to H-3″. The low field shifts of the methylene protons H-6″A and H-6″B and the shifts of C-6″ and C-5″, compared with values found in literature for glucosides of quercetin, kaempferol, and isorhamnetin [12] indicated acylation of the sugar moieties at C-6″. The nature of the acyl function

Table 1. Contents (ppm fr. wt) of 3,5-dicaffeoylquinic acid and malonated flavonol glycosides in fruits of pears

	Compound			
Variety	**1**	**2**	**3**	**4**
1	4	6	12	54
2	5	60	11	84
3	2	1	3	35
4	3	1	3	20
5	4	1	1	8
6	1	—	—	1
7	3	1	1	3
8	2	9	14	57
9	8	32	9	44
10	1	1	1	5
11	3	28	8	46
12	5	13	14	102

Compounds: **1** 3,5-dicaffeoylquinic acid, **2** quercetin 3-*O*-(6″-*O*-malonyl)-β-glucoside, **3** kaempferol 3-*O*-(6″-*O*-malonyl)-β-glucoside, **4** isorhamnetin 3-*O*-(6″-*O*-malonyl)-β-glucoside.

Varieties: (1) Williams Christ, (2) Clapps Liebling, (3) Gellerts Butterbirne, (4) Köstliche von Charneu, (5) Gute Luise, (6) Conference, (7) Alexander Lucas, (8) Vereinsdechant, (9) Gräfin von Paris, (10) Packhams Triumpf (11) Treveux, (12) Jules Guyot.

—: Not detectable.

was apparent from the remaining ^1H and ^{13}C signals. The methylene protons and ^{13}C shifts identified the presence of malonic acid.

The structure of **1** was unambiguously identified from the characteristic proton–proton coupling constants, ^1H and ^{13}C chemical shifts. The chemical shifts of H-3 and H-5 determined by ^1H 1D and the cross peaks in ^1H 2D COSY spectra indicated a connection of the caffeic acid with the C-3 and C-5 of the quinic acid, which was confirmed by the shifts in the ^{13}C NMR spectrum.

As far as we know this is the first report of **1**–**3** in pears. Although **1**–**4** have been described before, this is the first time that the structures of **3** and **4** have been fully documented by spectral analysis.

EXPERIMENTAL

Plant material. Fruits and leaves were obtained from the Institut für Obstbau und Baumschule, Universität Hannover, F.R.G.

Isolation. Leaves (220 g) of 'Clapps Liebling' were homogenized in MeOH (2 l). After extraction for 15 min at 40° the pulp was filtered and the extraction repeated twice with 70% MeOH. The combined extracts were evapd *in vacuo* at 40° to *ca* 150 ml and filled with H_2O to 500 ml. After two days at 4° chlorophyll was deposited. The soln was carefully siphoned off and purified with the help of polyamide columns [13]. After application of the sample the column was washed with H_2O (800 ml) and MeOH (2 l) and eluted with MeOH–NH_3 (199:1). The eluates were evapd at 40° and filtered (0.2 μm Satorius Minisart, Göttingen, F.R.G.).

Prep. HPLC. HPLC system: LCX PU (Philips), injection valve: Rheodyne 7125 with 2 ml sample loop, column: 250 × 16 mm, LiChrosorb, RP-18, 10 μm (Knauer), detection: UV 360 and 325 nm, solvent: I. 23% MeCN in 1% HOAc aq., II 48% MeOH in 1% HOAc aq., III. as I., flow: 11.2 ml/min. Collected fractions were lyophilized.

Analytical HPLC. HPLC system: LCX PU (Philips), injection valve: Rheodyne 7125 with 20 μl sample loop, column: 250 × 4.6 mm, Shandon ODS-Hypersil, 5 μm (Gynkotek), detection: UV 360 and 325 nm, solvent: (A) 1% HOAc aq. (B) MeOH, 15% B in A to 30% B in A in 30 min, flow: 0.8 ml/min. Benzoate: column: 120 × 4.6 mm SC-Hypersil, 3 μm (Gynkotek), detection: UV 231 nm, solvent: *iso*-octane–Et_2O–MeCN (30:10:1), flow: 1.0 ml/min.

GC conditions. Derivatization with BSA-TMCS (20:1), FID, SE-30 glass capillary, WCOT 30 m × 0.3 mm i.d., 80°, 2 min, to 120° at 5°/min, 120° to 270° at 10°/min.

NMR. ^1H and ^{13}C NMR spectra were recorded at ambient temperature (^1H: 300 and 400 MHz, ^{13}C: 75 and 100 MHz) locked to the deuterium resonance of the solvent. Chemical shifts are relative to TMS and coupling constants are in Hz. Glycerol was used as the matrix for negative ion FABMS.

^1H NMR (CD_3OD), **1** (IUPAC numbering scheme used): δ = 7.659, 7.618 [$d \times 2$, H-7′ × 2, $J_{7'-8'}$ 16.0], 7.108 [*br s*, H-2′ × 2], 7.014, 7.004 [*d*, $d \times 2$, H-6′ × 2, $J_{6'-2'}$ 2.2 $J_{6'-5'}$ 8.2], 6.826 [*br d*, H-5′ × 2], 6.390, 6.307 [$d \times 2$, H-8′ × 2], 5.50–5.40 [*m*, H-3, H-5], 4.022 [*d*, *d*, H-4, J_{4-5} 7.4, J_{4-3} 3.0], 2.364 [*d*, *d*, H-6A, J_{6A-6B} 13.8, $J_{6A-5} \sim 3$], 2.268 [*br m*, H-2AB], 2.213 [*d*, *d*, H-6B, $J_{6B-5} \sim 7$]. ^{13}C NMR (CD_3OD): δ = 177.42 (*s*, C-7), 168.88, 168.43 (*s* × 2, C-9′ × 2), 149.51, 149.42, 147.22 (× 2) (*s* × 4, C-4′ × 2, C-3′ × 2), 147.02, 146.73 (*d* × 2, C-7′ × 2), 128.02, 127.89 (*s* × 2, C-1′ × 2), 123.00, 122.94 (*d* × 2, C-6′ × 2), 116.53 (× 3), 115.69, 115.37, 115.27 (*d* × 6, C-2′ × 2, C-5′ × 2, C-8′ × 2), 74.79 (*s*, C-1), 72.64, 72.13 (*d* × 2, C-3, C-5), 70.83 (*d*, C-4), 37.79, 36.08 (*t* × 2, C-2, C-6).

^1H NMR (DMSO-d_6), **2**: δ 12.581 (5-OH), 7.536 (*d*, H-2′), 7.519 (*d*, *d*, H-6′, $J_{6'-2'}$ = 1.9, $J_{6'-5'}$ = 8.1 Hz), 6.849 (*d*, H-5′), 6.426 (*d*, H-8, J_{8-6} = 1.5 Hz), 6.213 (*d*, H-6), 5.387 (*d*, H-1″, $J_{1''-2''}$ = 7.1 Hz), 4.205 (*d*, H-6″A, $J_{6''A-6''B}$ = (−)11.9 Hz), 4.002 (*d*, H-6″B, $J_{6''B-5''}$ = 5.6 Hz), 3.36–3.16 (*m*, H-2″–H-5″), 3.164 (*d*, H-8″). ^{13}C NMR (DMSO-d_6): δ 177.23 (*s*, C-4), 167.51 (*s*, C-9″), 166.44 (*s*, C-7″), 164.12 (*s*, C-7), 161.08 (*s*, C-5), 156.46, 156.24 (*s* × 2, C-2, C-9), 148.37 (*s*, C-4′), 144.68 (*s*, C-3′), 133.18 (*s*, C-3), 121.37 (*d*, C-6′), 121.01 (*s*, C-1′), 116.16 (*d*, C-5′), 115.11 (*d*, C-2′), 103.79 (*s*, C-10), 101.14 (*d*, C-1″), 98.59 (*d*, C-6), 93.45 (*d*, C-8), 76.23 (*d*, C-3″), 73.88, 73.85 (*d* × 2, C-2″, C-5″), 69.62 (*d*, C-4″), 63.52 (*t*, C-6″), 41.10 (*t*, C-8″). FABMS *m/z*: 550 $[M-H]^-$.

^1H NMR (DMSO − d_6), **3**: δ 12.551 (5-OH), 7.919 (*d* × 2, H-2′, H-6′, $J_{2'-3'} + J_{2'-5'}$ = 8.6 Hz), 6.876 (*d* × 2, H-3′, H-5′), 6.435 (*d*, H-8, J_{8-6} = 2.0 Hz), 6.210 (*d*, H-6), 5.353 (*d*, H-1″, $J_{1''-2''}$ = 7.3 Hz), 4.174 (*d*, H-6″A, $J_{6''A-6''B}$ = (−) 11.8 Hz), 3.996 (*d*, H-6″B, $J_{6''B-5''}$ = 5.5 Hz), 3.65–3.03 (*m*, H-2″–H-5″), 3.079 (*d*, H-8″). ^{13}C NMR (DMSO-d_6): δ 177.25 (*s*, C-4), 167.60 (*s*, C-9″), 166.52 (*s*, C-7″), 164.14 (*s*, C-7), 161.08 (*s*, C-5), 159.91 (*s*, C-4′), 156.58, 156.30 (*s* × 2, C-2, C-9), 133.09 (*s*, C-3), 130.66 (*d* × 2, C-6′, C-2′), 120.65 (*s*, C-1′), 114.96 (*d* × 2, C-5′, C-3′), 103.81 (*s*, C-10), 101. 25 (*d*, C-1″), 98.63 (*d*, C-6), 93.61 (*d*, C-8), 76.07 (*d*, C-3″), 73.94, 73.82 (*d* × 2, C-2″, C-5″), 69.53 (*d*, C-4″), 63.42 (*t*, C-6″), 41.30 (*t*, C-8″). FABMS *m/z*: 534 $[M-H]^-$.

^1H NMR (DMSO-d_6), **4**: δ 12.555 (5-OH), 7.828 (*d*, H-2′), 7.532 (*d*, *d*, H-6′, $J_{6'-2'}$ = 2.1, $J_{6'-5'}$ = 8.4 Hz), 6.910 (*d*, H-5′, $J_{5'-6'}$ = 8.5 Hz), 6.456 (*d*, H-8, J_{8-6} = 2.0 Hz), 6.213 (*d*, H-6), 5.444 (*d*, H-1″, $J_{1''-2''}$ = 7.3 Hz), 4.138 [*d*, H-6″A, $J_{6''A-6''B}$ = (−) 12.2 Hz], 4.072 (*d*, H-6″B, $J_{6''B-5''}$ = 5.3 Hz), 3.840 (*s*, H-7′), 3.50–3.00 (*m*, H-2″–H-5″), 3.071 (*d*, H-8″).

^{13}C NMR (DMSO-d_6): δ 177.19 (*s*, C-4), 167.53 (*s*, C-9″), 166.57 (*s*, C-7″), 164.14 (*s*, C-7), 161.07 (*s*, C-5), 156.26 (*s* × 2, C-2, C-9), 149.50 (*s*, C-3′), 146.83 (*s*, C-4′), 132.99 (*s*, C-3), 122.26 (*d*, C-6′), 120.83 (*s*, C-1′), 115.14 (*d*, C-5′), 113.31 (*d*, C-2′), 103.83 (*s*, C-10), 101.17 (*d*, C-1″), 98.63 (*d*, C-6), 93.65 (*d*, C-8), 76.08 (*d*, C-3″), 74.11, 73.83 (*d* × 2, C-2″, C-5″), 69.63 (*d*, C-4″), 63.46 (*t*, C-6″), 55.65 (*q*, C-7′), 41.25 (*t*, C-8″). FABMS *m/z*: 564 $[M-H]^-$.

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