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# 2′-(*E*)-*O*-*p*-COUMAROYLGALACTARIC ACID AND 2′-(*E*)-*O*-FERULOYLGALACTARIC ACID IN CITRUS

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**Key Word Index**—*Citrus sinensis*; Rutaceae; 2′-*O*-*p*-coumaroyl- and 2′-*O*-feruloylgalactaric acid; galactaric acid esters.

**Abstract**—2′-(*E*)-*O*-*p*-Coumaroyl- and 2′-(*E*)-*O*-feruloylgalactaric acids, hitherto unknown in nature, have been isolated and identified from orange peel.

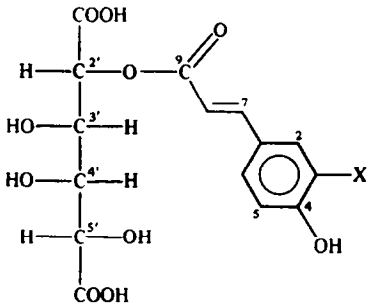
## INTRODUCTION

Knowledge of hydroxycinnamic acid derivatives in citrus species is limited. Wheaton and Stewart [1] reported the isolation of feruloylputrescine from grapefruit leaves. The compound is also present in grapefruit juice [1]. Enzymatic hydrolysis of citrus peel and endocarp extracts yielded significant amounts of *p*-coumaric acid, caffeic acid, ferulic acid, iso-ferulic acid and sinapic acid [2–4]. It is supposed that most of these acids originally occur as glycosides or esters. Maier and Metzler [5] concluded that glycosides were present. They found free hydroxycinnamic acids after treatment with β-glucosidase. The presence of the allergen chlorogenic acid (5′-caffeoylquinic acid) in orange has been discussed [6–8].

## RESULTS AND DISCUSSION

In a survey of hydroxycinnamic acid derivatives in citrus, peel and endocarp extracts of orange, grapefruit and lemon were compared after polyamide chromatography. The late fractions, available after elution with MeOH–2% $HCO_2H$, gave several unidentified *p*-coumaric and ferulic acid derivatives, as shown by analyt. HPLC (gradient system). The chromatograms and UV spectra obtained are comparable in many cases. Therefore, orange peel was taken as representative of citrus and was used for the isolation by prep. HPLC. The carefully concd and filtered extracts were fractionated on polyamide columns by elution with $H_2O$, MeOH and MeOH–2% $HCO_2H$. The elution with $H_2O$ and MeOH was successful in separating free acids, sugars, glycosides, hydroxycinnamoylglucoses and most of the flavonoids from hydroxycinnamic acid esters with organic acids such as quinic, tartaric or malic acid. The purity of the white crystalline hygroscopic substances isolated by means of prep. HPLC was examined by analytical HPLC with diode array detection. The normalized UV spectra of the isolated compounds showed bathochromic shifts of 4 nm compared to the normalized spectra of *p*-coumaric and ferulic acid. Enzymatic hydrolysis with unspecific esterase (Röhm, El 1–77) yielded *p*-coumaric acid and ferulic acid. The 2′-*O*-galactaric acid esters, **1** and **2** (Fig. 1), were identified by means of $^1$H NMR, $^{13}$C NMR, FAB MS and comparison by capillary GC of the silylated compound (after hydrolysis) with reference compounds.

The nature of the aromatic moieties in each compound was unambiguously identified from their characteristic proton–proton coupling constants, $^1$H and $^{13}$C chemical shifts. The detection of vicinal proton–proton couplings, determined from the $^1$H 1D and 2D COSY spectra, allowed unambiguous identification of the fragment H-2′–5′ in **2** and the low field shift of H-2′ indicated attachment of the acyl group at C-2′. The $^{13}$C spectra and 2D $^{13}$C–$^1$H correlation corroborated these findings and indicated the presence of three carbonyl carbons. The highest field signal belonged to the ester group while the two remaining signals were characteristic of free carboxylic acid groups with the high field one being adjacent to the esterification site. The aliphatic acid moiety of **1** showed similar $^1$H and $^{13}$C spectra to those of **2**.




**1** X = H
**2** X = $OCH_3$

Fig. 1. 2′-(*E*)-*O*-*p*-Coumaroylgalactaric acid, **1**, and 2′-(*E*)-*O*-feruloylgalactaric acid, **2** (showing numbering scheme used in the experimental section).

In both cases the negative ion FAB mass spectra confirmed these data. Each compound showed a single deprotonated molecular ion and characteristic fragment ions corresponding to loss of the aromatic ester group from the hexaric acid moiety.

The exact nature of the hexaric acid could not be deduced from the NMR and MS data. However, careful gas chromatographic analysis on three different stationary phases of the TMS-derivatives of the compounds, produced by enzymatic hydrolysis, indicated that galactaric acid was the aliphatic moiety in both **1** and **2**. A clear distinction between glucaric acid, galactaric acid and their lactones was possible. In all cases the TMS-derivatives had the same retention time as the TMS-derivative of galactaric acid.

According to our knowledge this is the first report of 2′-*O*-*p*-coumaroyl- and 2′-*O*-feruloylgalactaric acids in the plant kingdom. The esterification of hexaric acid moieties with cinnamic acid derivatives has only been reported by Ellinger *et al.* [9] and Strack *et al.* [10]. The former found the caffeoyl ester of glucaric acid and lactone forms in tomato leaves, while the latter found feruloylgluconic acid and *O*-feruloyl-4-methoxyaldaric acid (probably glucaric acid) in the primary leaves of rye.

## EXPERIMENTAL

*Isolation.* Orange peel (4.5 kg, Maroc, Shamouti), as fresh or frozen macerated material, was homogenized and extracted by stirring at room temp. with MeOH (2.7 l.). The separated pulp was extracted twice with 80% aq. MeOH (4.7 l.). The combined extracts were concd under red. pres. at 40° and resuspended in $H_2O$ (1.3 l.) and filtered. The filtrate was purified on polyamide columns (250 × 35 mm i.d., MN-SC-6 polyamide without traces of iron, washed with MeOH and finally with $H_2O$). After sample application (50 ml/column) the column was washed with 680 ml $H_2O$, 1 l. MeOH and eluted with 1 l. MeOH–2% $HCO_2H$. The eluates were combined, concd, microfiltered (0.2 μm Sartorius Minisart, Göttingen W. Germany) and used for isolation by means of repeated prep. HPLC.

*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 (Gynkotek), detection: UV 320 nm, isocratic systems, solvent: I, 18% acetonitrile in 1% aq. HOAc, II, 12% MeOH in 1% aq. HOAc, III (*p*-coumaroyl-), 5% MeOH in 1% aq. HOAc, IV (feruloyl-), 3% acetonitrile in 1% aq. MeOH, flow: 10–12 ml/min, collected fractions were carefully freeze dried.

*Analyt. HPLC.* Purity was established by analyt. 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 320 nm, 1040 A HP (diode array detector) with HP 85 and HP 82901 M flexible disc drive (Hewlett Packard), solvent: A, 2% aq. HOAc B, MeOH, 10% B in A to 30% B in A in 45 min., flow: 1.0 ml/min, integrator: CR-3A Shimadzu.

*GC conditions.* Derivatization with BSA–TMCS (20:1), reference: mucic acid, Merck-Schuchardt. Carlo Erba, Fractovap 4160, FID, glass capillary WCOT, 25–35 m × 0.3 mm i.d., SE 30: 150–270° at 8°/min, Dexsil 300: 130–200° at 2°/min, 200–250° at 3°/min, OV-1701: 150–270° at 5°/min, detector, injector: 300°, carrier-$N_2$: 1.5 ml/min, $H_2$: 30 ml/min, air: 400 ml/min (make up-$N_2$: 30 ml/min), split: 1:20, integrator HP 3390A.

*NMR and MS.* $^1H$ (400 MHz) and $^{13}C$ (100 MHz) NMR spectra were recorded at ambient temp. on a Bruker WM 400 NMR spectrometer, locked to the deuterium resonance of the solvent, $CD_3OD$. A 2D $^1H$ COSY spectrum of **2** was recorded with a 90°–$t_1$–90°–FID($t_2$) pulse sequence, while the $^{13}C$ and $^1H$ spectra of the same compound were correlated by a standard heteronuclear shift-correlated $^{13}C$–$^1H$ 2D spectrum with decoupling in the F1 domain. All 1D and 2D spectra were recorded using the standard Bruker software package. Chemical shifts are reported in ppm relative to TMS. The following abbreviations are used to indicate the multiplicities of the signals in the $^1H$ NMR spectra and the multiplicities of the $^{13}C$ signals in the single-frequency $^1H$-decoupled off-resonance $^{13}C$ NMR spectra: *s* = singlet, *d* = doublet and *q* = quartet. Negative ion fast atom bombardment mass spectra (FAB MS) were recorded on a Kratos MS 50 mass spectrometer equipped with a Kratos FAB source. Glycerol was used as matrix.

2′-(E)-O-p-*Coumaroylgalactaric acid*, **1**. $^1H$ NMR ($CD_3OD$): $\delta$ = 7.812 [*d*; H-7: *J* (7–8) 15.9], 7.539 [*d*; H-2, H-6; *J* (2–3) + (2–5) 8.1], 6.860 (*d*; H-3, H-5), 6.511 (*d*; H-8), 5.495 (broad, H-2′), 4.579 (v. broad, H-5′), 4.368 (broad, H-3′), 4.182 (v. broad, H-4′). Couplings of H-2′, H-3′; H-4′ and H-5′ were not resolved. $^{13}C$ NMR ($CD_3OD$): $\delta$ = 168.57 (*s*, C-9), 161.23 (*s*, C-4), 147.34 (*d*, C-7), 131.38 (*d*, C-2, C-6), 127.25 (*s*, C-1), 116.82 (*d*, C-3, C-5), 114.69 (*d*, C-8), 72.72, 72.67 (*d* × 2, C-2′, C-4′), 71.89, 71.84 (*d* × 2, C-3′, C-5′). The signals of C-1′ and C-6′ were not observed, presumably due to paramagnetic broadening. FAB MS *m/z*: 355 $[M-H]^-$, 209 $[M-C_9H_7O_2]^-$, 163 $[C_9H_7O_3]^-$.

2′-(E)-O-*Feruloylgalactaric acid*, **2**. $^1H$ NMR ($CD_3OD$): $\delta$ = 7.810 [*d*; H-7; *J* (7–8) 15.9], 7.263 [*d*; H-2; *J* (2–6) 1.9], 7.153 [*dd*; H-6; *J* (6–5) 8.2], 6.863 (*d*, H-5), 6.536 (*d*, H-8), 5.507 [*d*; H-2′; *J* (2′–3′) 1.5], 4.557 [broad, H-5′; *J* (5′–4′) small], 4.373 [*dd*; H-3′; *J* (3′–4′) 9.8] 4.081 (broad *d*, H-4′), 3.938 (*s*, H-10). $^{13}C$ NMR ($CD_3OD$): $\delta$ = 176.76 (*s*, C-6′), 172.54 (*s*, C-1′), 168.49 (*s*, C-9), 150.68, 149.38 (*s* × 2, C-3, C-4), 147.62 (*d*, C-7), 127.85 (*s*, C-1), 124.16 (*d*, C-6), 116.52 (*d*, C-5), 115.02 (*d*, C-8), 111.96 (*d*, C-2), 73.66 (*d*, C-2′), 72.74 (*d*, C-4′), 71.65 (*d*, C-3′), 71.29 (*d*, C-5′), 56.52 (*q*, C-10). FAB MS *m/z*: 385 $[M-H]^-$, 209 $[M-C_{10}H_9O_3]^-$, 193 $[M-C_{10}H_9O_4]^-$ and $[C_{10}H_9O_4]^-$.

## REFERENCES

1. Wheaton, T. A. and Stewart, J. (1965) *Nature* **205**, 620.
2. Feldman, A. W. (1965) *Nature* **207**, 985.
3. Horowitz, R. M. and Gentili, B. (1960) *J. Org. Chem.* **25**, 2183.
4. Stöhr, H. and Herrmann, K. (1975) *Z. Lebensm. Unters. Forsch.* **159**, 305.
5. Maier, V. P. and Metzler, D. M. (1967) *Phytochemistry* **6**, 1127.
6. Freedman, S. D. (1962) *Am. J. Med. Sci.* **244**, 548.
7. Freedman, S. D. (1964) *Allergy* **35**, 108.
8. Siddiqi, A. and Freedman, S. D. (1963) *Can. J. Biochem. Physiol.* **41**, 947.
9. Ellinger, C. A., Lundin, R. E. and Haddon, W. A. (1981) *Phytochemistry* **20**, 1133.
10. Strack, D., Engel, U., Weissenböck, G., Grotjahn, L. and Wray, V. (1986) *Phytochemistry* **25**, 2605.