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(E)-O-*p*-COUMAROYL-, (E)-O-FERULOYL-DERIVATIVES OF GLUCARIC ACID IN CITRUS

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Key Word Index—*Citrus sinensis*; Rutaceae; *p*-coumaroylglucaric acid, feruloyl- and diferuloylglucaric acid.

Abstract—2-(E)-O-*p*-Coumaroyl-, 2-(E)-O-feruloyl- and 2,4-(E,E)-O-diferuloylaldaric acid esters from orange peel have been isolated and identified spectroscopically. GC-analysis of the TMS-derivatives of the hydrolysis products indicated the aldaric acid was glucaric acid.

INTRODUCTION

In a previous study of the hydroxycinnamic acid derivatives in citrus, we reported the isolation and identification of 2-(E)-O-*p*-coumaroyl- and 2-(E)-O-feruloylgalactaric acid from orange peel [1]. The esters were shown by HPLC to be two compounds of a group of several unidentified *p*-coumaric and ferulic acid derivatives. Here we present evidence of the identity of more of these closely related compounds. Esters of hexaric acid moieties with a hydroxycinnamic acid have been found in the leaves of various solanaceous plants [2, 3] and in rye [4].

RESULTS AND DISCUSSION

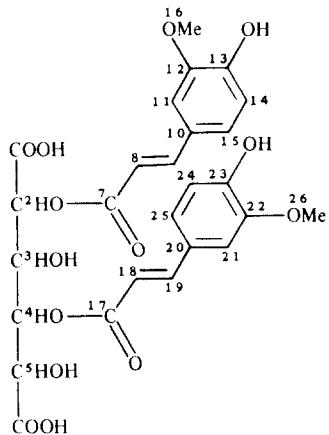
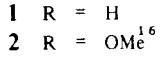
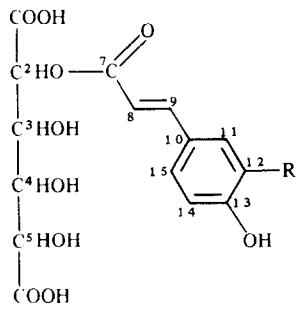
The fractions studied were obtained by preparative HPLC. In the case of the monoesters several narrow eluting fractions were isolated which gave highly hygroscopic white crystalline substances upon freeze drying. The purity and stability of each isolated fraction was established by analytical HPLC. This was also used to check for rearrangements during the isolation and purification procedures.

The ferulic and *p*-coumaric acid fractions each showed a pattern of four peaks by analytical HPLC. These could be unambiguously differentiated from the previously identified *p*-coumaroyl- and feruloylgalactaric acid peaks which had similar retention times. Hydrolysis of each fraction gave the free hydroxycinnamic acid. GC-analysis of the TMS-derivatives of the compounds produced by enzymatic hydrolysis indicated glucaric acid. A clear distinction between glucaric acid, galactaric acid and

their lactones was possible. One peak from each fraction was isolated in amounts sufficient for NMR- and MS-analysis. The nature of the various moieties in the molecules were unambiguously identified from the cross peaks in the 2D ¹H COSY spectra (1) or by homodecoupling (2). The low field shift of H-2 of the aldaric acid moiety was a clear indication of the point of attachment of the hydroxycinnamic acid substituent. As the absolute geometry of this asymmetric centre is not determinable by NMR the unambiguous identity of the glucaric acid derivatives cannot be established. This would only be possible by X-ray analysis of a suitable derivative. Thus the compounds are either 2- or 5-substituted glucaric acids.

The *p*-coumaroylglucaric acid is one of two main components which could not be separated by HPLC. From the FABMS the second compound has a *M*, 14 mass units higher with a methoxyl group replacing one of the hydroxyl groups in the glucaric acid moiety. The feruloylglucaric acid ester showed a small additional component, which is probably the benzyloylglucaric acid from the FABMS. The other peaks in the two fractions could not be obtained in a form suitable for NMR and MS studies. However, it is quite probable that these correspond to the other open chain isomeric forms as positional isomerism is a well-known phenomenon which occurs with esters of phenolic acids with polyhydroxy compounds [2]. Lactone forms were not present, as an orange extract showed the same pattern of peaks before and after treatment with alkali.

The purified diferulic compound was a white crystalline substance that was less hygroscopic than the monoesters.



ters. Enzymatic hydrolysis gave ferulic acid (GC, TMS; HPLC) and glucaric acid (GC, TMS). The similarity of the IR-spectrum with that from the monoester and the detection of the monoester during the enzymatic hydrolysis were all pointers to a multiple-substituted glucaric acid. The NMR and MS data unambiguously showed that the compound was a 2,4-diferuloylaldaric acid. As with the monoesters the absolute identity of the compound could not be established. Hence this new compound is either 2,4- or 3,5-diferuloylglucaric acid.

EXPERIMENTAL

Isolation. The isolation was carried out as described previously [1] with the same work-up procedure. Orange peel (4.5 kg, Maroc, Shamouti), as fresh or frozen macerated material, was homogenized and extracted in 500 g portions 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 H₂O (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 H₂O). After sample application (50 ml/column) the column was washed with 680 ml H₂O, 1 l MeOH and eluted with 1 l MeOH-2% HCO₂H. The eluates were combined, concd, filtered (0.2 μm Sartorius Minisart, Göttingen, F.R.G) 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 (for monoester): I 18% MeCN in 1% aq. HOAc, II 12% MeOH in 1% aq. HOAc, III (p-coumaroyl-) 5% MeOH in 1% aq. HOAc, IV (feruloyl-) 5% MeCN in 1% aq. HOAc; (diferuloyl): I 18% MeCN in 1% aq. HOAc, II 27% MeOH in 1% aq. HOAc, III 23% MeOH in 1% aq. HOAc. Flow: 10–12 ml/min, collected fractions were carefully freeze-dried.

Analytical HPLC. Purity was established by analytical HPLC. HPLC system: as above except sample loop: 20 μl, 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 compounds: glucaric acid, monopotassium salt and glucaric acid 1,4-lactone (Sigma). Carlo Erba, Fractovap 4160, FID, glass capillary WCOT, 25–35 m × 0.3 mm i.d., SE 30: 150–270° at 8°/min, OV-1701: 150–270° at 5°/min, detector, injector: 300°, carrier-N₂: 1.5 ml/min, H₂: 30 ml/in, air: 400 ml/min, make up N₂: 30 ml/min, split: 1:20, integrator: CR-3A Shimadzu.

NMR and MS. ¹H and ¹³C NMR spectra were recorded at ambient temp on Bruker WM 400 and AM 300 NMR spectrometers, locked to the major deuterium resonance of the solvent, CD₃OD. 2D ¹H COSY spectra of 1 and 3 were recorded with 90° − *t*₁ − 90° − FID(*t*₂) pulse sequences. All 1D and 2D spectra were recorded using the standard Bruker software package. 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(or 5)-(E)-O-p-Coumaroylglucaric acid (1). was one of two main components which could not be separated. ¹H NMR (CD₃OD): δ 7.797 (d, *J*_{9,8} = 16.1 Hz, H-9), 7.545 (d, *J*_{11,12} + *J*_{11,14} = 8.7 Hz, H-11, H-15), 6.861 (d, H-12, H-14), 6.478 (d, H-8), 5.343 (d, *J*_{2,3} = 3.2 Hz, H-2), 4.407 (d, d, *J*_{3,4} = 5.8 Hz, H-3), 4.339 (d, *J*_{5,4} = 2.8 Hz, H-5), 3.68 (br, H-4). FAB MS *m/z*: 355 [M − H][−], 209 [M − C₉H₇O₃][−], 163 (C₉H₇O₃)[−]. The second compound was also a *p*-coumaroylglucaric acid derivative which had one OCH₃-group instead of one OH-group. ¹H NMR (CD₃OD): δ 7.765 (d, *J*_{9,8} = 15.9 Hz, H-9), 7.534 (d, *J*_{11,12} + *J*_{11,14} = 8.7 Hz, H-11, H-15), 6.855 (d, H-12, H-14), 6.460 (d, H-8), 5.212 (d, *J*_{2,3} = 3.5 Hz, H-2), 4.22 (br, H-3, H-4), 4.472 (br, H-5, H-5), 3.587 (s, OMe). FAB MS *m/z*: 369 [M − H][−], 223 [M − C₉H₇O₂][−].

2(or 5)-(E)-O-Feruloylglucaric acid (2): ¹H NMR (CD₃OD): δ 7.778 (d, *J*_{9,8} = 15.9 Hz, H-9), 7.251 (d, *J*_{11,15} = 1.6 Hz, H-11), 7.142 (dd, *J*_{14,15} = 8.2 Hz, H-15), 6.860 (d, H-14), 6.499 (d, H-8), 5.380 (d, *J*_{2,3} = 4.0 Hz, H-2), 4.376 (dd, *J*_{3,4} = 5.0 Hz, H-3), 4.232 (d, *J*_{5,4} = 5.6 Hz, H-5), 4.006 (dd, H-4), 3.934 (s, H-16). ¹³C NMR (CD₃OD): δ 175.90 (s, C-6), 171.85 (s, C-1), 168.33 (s, C-7), 150.73, 149.39 (s × 2, C-12, C-13), 147.73 (d, C-9), 127.84 (s, C-10), 124.18 (d, C-15), 116.55 (d, C-14), 114.94 (d, C-8), 112.10 (d, C-11), 74.91 (d, C-2), 73.95 (d, C-4), 71.58 (d, C-3), 73.16 (d, C-5), 56.58 (q, C-16). FAB MS *m/z*: 385 [M − H][−], 209 [M − C₁₀H₉O₃][−], 193 [C₁₀H₉O₄][−]. A second minor component is present in the feruloylglucaric acid fraction which was presumably benzoylglucaric acid from the FABMS with *m/z* 313 [M − H][−] and 209 [M − C₇H₅O][−].

2,4(or 3,5)-(E,E)-O-Diferuloylglucaric acid (3): ¹H NMR (CD₃OD): δ 7.724, 7.623 (d × 2, *J*_{9,8} = *J*_{19,18} = 15.9 Hz, H-9, H-19), 7.01–6.99 (br, H-11, H-21), 6.987, 6.957 (br, d × 2, *J*_{15,14} = *J*_{25,24} = 8.0 Hz, H-15, H-25), 6.772, 6.693 (d × 2, H-14, H-24), 6.329, 6.368 (d × 2, H-8, H-18), 5.451 (br, H-2), 5.347 (br, H-4), 4.749 (br, H-5), 4.431 (br, H-3), 3.799, 3.711 (s × 2, H-16, H-26). ¹³C NMR (CD₃OD): δ 175.19 (s, C-6), 171.32 (s, C-1), 168.17, 168.11 (s × 2, C-7, C-17), 150.61 (s × 2, C-12, C-22), 149.26, 149.18

($s \times 2$, C-13, C-23), 147.84, 147.64 ($d \times 2$, C-9, C-19), 127.74, 127.63 ($s \times 2$, C-10, C-20), 124.17, 124.05 ($d \times 2$, C-15, C-25), 116.45, 116.37 and 115.39, 114.77 ($d \times 4$, C-14, C-24 and C-8, C-18), 111.84, 111.78 ($d \times 2$, C-11, C-21), 75.74, 71.21, 69.85 ($d \times 2$, dd, C-2, C-3, C-4, C-5), 56.34, 56.23 ($q \times 2$, C-16, C-26). FABMS m/z : 561 [$M - H$]⁻, 385 [$M - C_{10}H_9O_3$]⁻, 209 [$M - C_{20}H_{17}O_6$]⁻.

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p-HYDROXYACETOPHENONE DERIVATIVES OF THE MONOTYPIC GENUS *PLATYPODANTHERA*

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Key Word Index—*Platypodanthera melissaefolia*; Compositae; *p*-hydroxyacetophenone derivatives; tremetone derivatives.

Abstract—The aerial parts of *Platypodanthera melissaefolia* afforded several known *p*-hydroxyacetophenone derivatives and two new ones. Furthermore, coumarins and a guaianolide were isolated. The chemotaxonomic relevance of these findings is discussed.

The monotypic genus *Platypodanthera* (Compositae, tribe Eupatorieae) is placed in the subtribe Gyptidinae [1]. The only species *P. melissaefolia* (DC) K. et R. was originally described as *Ageratum melissaefolium* DC and later transferred to *Trichogonia* by Mattfeld. However, the morphology clearly differs from both genera [2]. We now have studied the chemistry of this species. In addition to the coumarins herniarin and ayapin the *p*-hydroxyacetophenone derivative **1** [3] and the derived vinyl compounds **2** [4] and **3** [4] were isolated. Furthermore, the tremetone derivative **5** [5] and the guaianolide **7** [6] were present. Two further compounds (**4** and **6**) were closely related to **3** and **5** respectively.

The structure of **4** followed from its ¹H NMR spectrum which was in part similar to that of **3**. However, the signals of the vinylic protons were replaced by a singlet at δ 9.91, clearly indicating an aldehyde proton. Furthermore, the signals of the aromatic protons were shifted down field while the coupling remained unchanged. Accordingly, we were dealing with the aldehyde **4** most likely formed by oxidative degradation of **3**.

The structure of **6** also could be deduced from the ¹H NMR spectrum which was similar to that of 3α -hydroxy-11-acetoxytoxol [7]. However, some signals were clearly different. Especially, the pair of broadened doublets at δ 4.18 and 4.34 (H-11) required a more rigid

structure. Therefore, in agreement with the molecular formula, the corresponding cyclic anhydro derivative was present. The observed coupling of H-2 required a *cis*-ring closure if the value was compared with those of *cis*- and *trans*-substituted toxol derivatives [3, 4]. We have named compound **6**, platypodantherone.

The chemistry of *Platypodanthera* is in part related to that of some *Trichogonia* species, where vinyl derivatives like **2** and **3** and other *p*-hydroxyacetophenone derived compounds are present [4, 9]. From one species also guaianolides are reported [9]. Derivatives of *p*-hydroxyacetophenone are also isolated from representatives of *Bahianthus* [10], *Stylotrichium* [11] and *Campuloclinium* [unpublished], all placed in the subtribe Gyptidinae. However, these compounds are relatively widespread in many genera of the Compositae. The isolation of **2–4** and **7** indicated closest relationship of *Platypodanthera* to *Trichogonia* and not to *Ageratina*.

EXPERIMENTAL

The air-dried aerial parts (80 g, collected in February 1987 in Peru, voucher RMK 8597, deposited in the US National Herbarium Washington) was extracted and worked-up as reported previously [12]. The extract was first separated by CC into five fractions (petrol with increasing amounts of Et₂O). The first