Phytochemistry, Vol. 25, No. 12, pp. 2904–2905, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

QUERCETIN 3-*O*-[6″-(3-HYDROXY-3-METHYLGLUTAROYL)-β-GALACTOSIDE] FROM BLACKBERRIES

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(*Received* 17 *March* 1986)

Key Word Index—*Rubus*; Rosaceae; blackberries; acylated quercetin glycoside; quercetin 3-*O*-[6″-(3-hydroxy-3-methylglutaroyl)-β-galactoside].

Abstract—Quercetin 3-[6″-(3-hydroxy-3-methylglutaroyl)-galactoside] was isolated and identified from blackberries.

INTRODUCTION

Flavonol glycosides have been found acylated with *p*-coumaric, ferulic, caffeic, *p*-hydroxybenzoic, gallic, acetic and malonic acids [1]. In blackberries we have found a new flavonol glycoside acylated with an acid hitherto unknown in plants, 3-hydroxy-3-methylglutaric acid.

RESULTS AND DISCUSSION

Quercetin 3-[6″-(3-hydroxy-3-methylglutaroyl)-galactoside] (**1**, 80 mg) was isolated by means of repeated prep. HPLC as a new acylated flavonol glycoside. The purity was examined by analytical HPLC with diode-array detection. Standard methods were used for identification. Acidic hydrolysis with 1 N H_2SO_4 caused cleavage of the glycoside linkage, while alkaline hydrolysis with a solution of 2% barium hydroxide cleaved the acid moiety. The products were identified by HPLC co-chromatography and from UV spectra taken during the analytical run. Various chromatographic systems were used for the underivatized and benzoylated products. Quercetin was found after acidic hydrolysis, while quercetin 3-*O*-β-galactoside and 3-hydroxy-3-methylglutaric acid were detected after alkaline hydrolysis. The acid was identified by means of GC using capillary columns with stationary phases of various polarities.

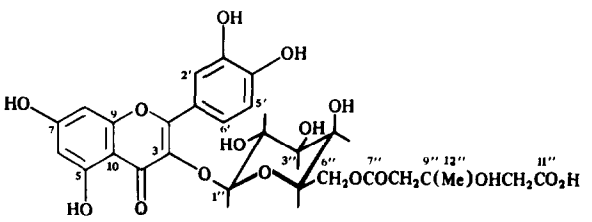
Standard UV spectral analysis [2] confirmed the linkage of the sugar to the aglycone at C-3. The ^{1}H NMR and ^{13}C NMR spectra unambiguously identified the compound as a quercetin derivative with a 3-*O*-substituent [3]. The number and characteristic shifts of the ^{13}C hexose signals indicated the presence of galactose. The vicinal proton–proton coupling constants indicated a β-glycosidic linkage and its identity was confirmed from the vicinal couplings to H-3″. The low field shift of the methylene protons H-6″A and H-6″B and the shifts of C-6″ and C-5″, compared with literature values for quercetin 3-galactoside [3], indicated acylation of the sugar moiety at C-6″. The nature of the acyl function was apparent from the fragmentation in the negative ion FAB mass spectrum and from the remaining ^{1}H and ^{13}C signals. The presence of two sets of non-equivalent methylene protons and characteristic ^{13}C shifts identified the presence of a 3-methyl-3-hydroxyglutaric acid function.

According to our knowledge this is the first report of **1** in the plant kingdom. The varieties Thornless Evergreen, Theodor Reimers, Black Thornfree, Dirkson Thornless, Lucretia, Amtrong, Jackson and James Fellblack were examined and **1** was identified in each variety.

EXPERIMENTAL

Plant material. Fruits of Thornless Evergreen, Theodor Reimers and Black Thornfree were received from Institut für Obstbau und Baumschule, Universität Hannover, West Germany; Dirkson Thornless, Lucretia, Amtrong, Jackson and James Fellblack from Staatl. Lehr- und Versuchsanstalt für Wein- und Obstbau in Weinsberg, Weinsberg, West Germany.

Isolation. Fruits (4 kg) were homogenized in MeOH (6 l.). After extraction for 15 min at 40° the pulp was filtered and the extraction repeated twice with 70% MeOH. The combined



Quercetin 3-*O*-[6″-(3-hydroxy-3-methylglutaroyl)-β-galactoside]

extracts were evaporated in vac. at 40° to about 1 l. The final conc. soln was purified using polyamide columns [4]. After sample application the column was washed with H_2O (800 ml) and MeOH (2 l.) and eluted with MeOH–NH_3 (995 + 5). The eluates were evaporated at 40° and filtered (0.2 μm Satorius Minisart, Göttingen, West Germany).

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 360 nm, solvent: I. 21% MeCN in 1% HOAc aq., II. 42% MeOH in 1% HOAc aq., III. as I., flow: 14 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 nm, integrator: HP 3390 A, 1040 A HP (diode-array detector) with HP 85 and HP 82901 M flexible disc drive (Hewlett–Packard), solvent: (A) 1% HOAc aq. (B) MeCN, 5% B in A to 30% in 45 min, flow: 0.8 ml/min. Benzoate: column: 120 × 4.6 mm SC-Hypersil, 3 μm (Gynkotek, solvent (aglycone): *iso*-octane–Et_2O–MeCN (150:65:15), solvent (glycosides) (150:80:20), flow: 1 ml/min.

GC conditions. Derivatization with BSA–TMCS (20:1). Carlo Erba GC 2150, FID, SE-30, Dexsil 400, OV-1701 glass capillary, WCOT 30 m × 0.3 mm i.d., 150–270° at 5°/min.

NMR. ^1H and ^{13}C NMR spectra were recorded at ambient temperature, at 400 and 100 MHz, respectively, on a Bruker WM-400 NMR spectrometer locked to the deuterium resonance of the solvent, DMSO-d_6. A negative ion fast atom bombardment mass spectrum (FAB MS) was recorded on a Kratos MS 50 mass spectrometer equipped with a Kratos FAB source. Glycerol was used as the matrix. ^1H NMR (DMSO-d_6 + D_2O) δ7.612 (*d*, *d*, H-6′, $J_{6'-2'}$), 2.2 ($J_{6'-5'}$ = 8.5 Hz), 7.517 (*d*, H-2′), 6.818 (*d*, H-5′), 6.414 (*d*, H-8, J_{8-6} = 2.0 Hz), 6.200 (*d*, H-6), 5.326 (*d*, H-1″, $J_{1''-2''}$ = 7.8 Hz), 3.995 (*d*, *d*, H-6″A, $J_{6''A-6''B}$ (−) 11.5, $J_{6''A-5''}$ 7.6 Hz), 3.948 (*d*, *d*, H-6″B, $J_{6''B-5''}$ = 4.7 Hz), 3.64–3.52 (*m*, H-2″, H-4″, H-5″), 3.391 (*d*, *d*, H-3″, $J_{3''-2''}$) 9.5, ($J_{3''-4''}$ = 3.4 Hz), 2.412 (*d*, 8″A or 10″A, J_{A-B} (−) 14.0), 2.288 (*d*, 10″A or 8″A, J_{A-B} (−) 14.9), 2.273 (*d*, 8″ B or 10″B), 2.260 (*d*, 10″B or 8″B), 1.026 (*s*, 12″). ^{13}C NMR (DMSO-d_6): δ177.58 (*s*, C-4), 172.59 (*s*, C-11″), 170.37 (*s*, C-7″), 164.17 (*s*, C-7), 161.10 (*s*, C-5), 156.56 (*s* × 2, C-2, C-9), 148.47 (*s*, C-4′), 144.82 (*s*, C-3′), 133.61 (*s*, C-3), 122.03 (*d*, C-6′), 121.33 (*s*, C-1′), 116.20 (*d*, C-5′), 115.38 (*d*, C-2′), 104.13 (*s*, C-10), 101.85 (*d*, C-1″), 98.83 (*d*, C-6), 93.79 (*d*, C-8), 72.96 (*d* × 2, C-5″, C-3″), 71.10 (*d*, C-2″), 68.90 (*s*, C-9″), 68.32 (*d*, C-4″), 63.29 (*t*, C-6″), 45.43, 45.20 (*t* × 2, C-8″, C-10″), 27.23 (*q*, C-12″). FAB MS *m/z*: 607 $[M-H]^-$; 463 $[M-C_6H_9O_4]^-$; 301 $[M-C_{12}H_{19}O_9]^-$; 285 $[M-C_{12}H_{19}O_{10}]^-$.

Acknowledgement—We wish to thank the Forschungskreis der Ernährungsindustrie and Arbeitsgemeinschaft Industrieller Forschungsvereinigung (AIF) for financial support.

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