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Cyanogenic Glycosides and 4-Hydroxycoumarin Glycosides from *Gerbera jamesonii hybrida*

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A new 4-hydroxycoumarin glycoside (V), named 5-methyl-4-rutinosyloxycoumarin, $C_{22}H_{28}O_{12}$, mp 240—241 °C (dec.), $[\alpha]_D^{22}$ −79.4° (pyridine), was isolated from the underground parts of *Gerbera jamesonii hybrida* (Compositae), in addition to three cyanogenic glycosides (prunasin (I), amygdalin (II) and vicianin (III)) and a coumarin glycoside (4-β-D-glucopyranosyloxy-5-methylcoumarin (IV)). The chemical structure of V was determined to be 5-methyl-4-[α-L-rhamnopyranopyranosyl-(1→6)-β-D-glucopyranosyloxy]coumarin on the basis of chemical and spectral evidence.

Keywords——*Gerbera jamesonii hybrida*; Compositae; prunasin; amygdalin; vicianin; 4-β-D-glucopyranosyloxy-5-methylcoumarin; 5-methyl-4-rutionosyloxycoumarin; coumarin glycoside; cyanogenic glycoside

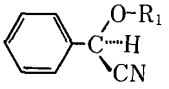
In the previous paper,[1] we reported the isolation of prunasin and 4-β-D-glucopyranosyloxy-5-methylcoumarin from *Leibnitzia anandria* (L.) NAKAI (Fam. Compositae, Tribe Mutisieae). Genus *Gerbera*, including about fifty species distributed in Asia and Africa, has a close taxonomical relationship to *Leibnitzia*. Chemical components of several species of *Gerbera* have been reported: acetylenes, isoprenylated phenolics, methylated or isoprenylated 4-oxygenated coumarins, isoprenylated chromenes and terpenoids.[2-4] Prunasin is the only glycoside so far isolated from *Gerbera* plants.[5] We investigated the distribution of glycosidic components of *Gerbera jamesonii hybrida*, a familiar garden breed developed from *G. jamesonii* BOLUS, and isolated three cyanogenic glycosides (I—III) and two 4-hydroxycoumarin glycosides (IV, V) from the underground parts of the plant.

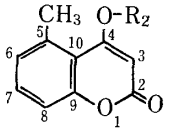
On enzymatic hydrolysis, compounds I—III liberated hydrocyanic acid, which gave positive colorations in the picrate test[6] and the nitrobenzaldehyde–dinitrobenzene test.[7] The spectrometric evidence indicated that I, mp 146—148 °C (dec.) and II, mp 207—209 °C (dec.) are identical with prunasin and amygdalin, respectively, and these identifications were confirmed by direct comparison of I and II with authentic samples. Compound III, mp 170—172 °C,[8,9] $[\alpha]_D^{20}$ −19.6° yielded glucose and arabinose as sugar components on treatment with 5% sulfuric acid, while it afforded prunasin on milder hydrolysis with 0.4 N hydrochloric acid. Accordingly, III is an arabinosylprunasin. The arabinose moiety of III was proved to be attached to C-6′ of the glucose residue (G-6′) on the basis of the ^{13}C-nuclear magnetic resonance (NMR) signal of G-6′ (δ 69.5 ppm)[13] in III as compared with that (δ 62.5 ppm)[13] in prunasin (I). From these results and comparisons with the reported physical data, III was concluded to be vicianin, a known cyanogenic glycoside isolated from *Vicia angustifolia* ROTH. (Leguminosae) and other species.[8]

Compound IV, mp 152—154 °C, $[\alpha]_D^{20}$ −106.0 °C (MeOH) was identified as 4-β-D-glucopyranosyloxy-5-methylcoumarin by direct comparison with an authentic sample, which was isolated from *L. anandria* in a high yield.[1] Compound V, $C_{22}H_{28}O_{12}$, mp 240—241 °C

(dec.), $[\alpha]_D^{22} -79.4°$ (pyridine) showed absorption maxima at 279, 287, 307 (sh) and 321 (sh) nm in its ultraviolet (UV) spectrum, and absorptions due to hydroxyl groups (3500—3300, 1050 cm^{-1}), a lactone (1685 cm^{-1}) and an aromatic ring (1605, 1560 cm^{-1}) in its infrared (IR) spectrum. Since the UV absorptions are very similar to those of compound IV,[1] V seems to be a 4-hydroxycoumarin glycoside. On acid hydrolysis, V yielded glucose and rhamnose as sugar moieties, while enzymatic hydrolysis using hesperidinase V afforded IV as a partial hydrolysis product. This finding indicated that V is a rhamnoside of 4-β-D-glucopyranosyloxy-5-methylcoumarin (IV). The ^{13}C-NMR spectrum of V showed twenty-two signals, out of which sixteen signals exhibited a good correspondence with those of IV except for the signal due to G-6′ (δ G-6′ (IV), 61.9 ppm; δ G-6′ (V), 67.3 ppm).[13a] This result suggested that the rhamnose unit of V is linked to IV at G-6′ through a glycoside bond.[13b,c] The other six signals due to the rhamnose moiety, especially those ascribable to rhamnose C–3′ (R–3′) (δ 72.0 ppm) and R–5′ (δ 69.5 ppm), were in good agreement with those of the α-L-rhamnopyranosyl residue in a rutinoside such as gynosaponin TN-2.[10] Application of Klyne's rule[11] also supported this conclusion: $[M]_D$ of V minus $[M]_D$ of IV[1] is equal to −195°; $[M]_D$ (methyl α-L-rhamnopyranoside) −111°, $[M]_D$ (methyl β-L-rhamnopyranoside) +170°. Accordingly, the chemical structure of V was established as 5-methyl-4-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy] coumarin, and V was named 4-rutinosyloxy-5-methylcoumarin.

Genera *Gerbera*, *Leibnitzia*, *Pertya* and *Ainsliaea* belong to Tribe Mutisieae. Glycosides of sesquiterpene lactones have been isolated characteristically from *Pertya*[12] and *Ainsliaea*[18] plants, but not from *Gerbera* and *Leignitzia* species. On the other hand *Gerbera jamesonii hybrida* and *Leibnitzia anandria* contain common constituents, not only cyanogenic glycosides, but also 4-hydroxycoumarin glycosides, which might help to establish the existence of a close taxonomical relationship between Genera *Gerbera* and *Leibnitzia*.





I (prunasin): R_1 = β-D-Glc(*p*)
II (amygdalin): R_1 = β-D-Glc(*p*)-(1→6)-β-D-Glc(*p*)
III (vicianin): R_1 = α-L-Ara(*p*)-(1→6)-β-D-Glc(*p*)

IV: R_2 = β-D-Glc(*p*)
V: R_2 = α-L-Rha(*p*)-(1→6)-β-D-Glc(*p*)

Chart I

Experimental

All melting points were taken on a Shimadzu micro melting point determination apparatus and are uncorrected. IR spectra were obtained with a Shimadzu IR-400 spectrometer. UV spectra were recorded on a Shimadzu UV-250. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. NMR spectra were recorded with a JEOL FX-100 spectrometer with tetramethylsilane as an internal standard; chemical shifts are given on the δ scale (ppm), coupling constants (*J* values) are expressed in Hz, and the following abbreviations are used: s = singlet, d = doublet and br = broad. Thin layer chromatography (TLC) was performed on Kiesel gel 60 F_{254} precoated plates (Merck) and spots were located by UV detection at 254 nm and by spraying 10% H_2SO_4 followed by heating. Solv. A and B refer to solvent systems for chromatography: $CHCl_3$–MeOH–EtOAc–H_2O (40 : 10 : 70 : 1) and $CHCl_3$–MeOH–H_2O (50 : 15 : 1), respectively.

Extraction and Separation——Commercial *Gerbera jamesonii hybrida* was used as the plant material. The air-dried underground parts of the plant (131.4 g) were extracted with MeOH (1 l × 5) under reflux. The total MeOH solution was concentrated under reduced pressure as far as possible. The residue (11.5 g) was dissolved in water (250 ml), and successively extracted with hexane (200 ml × 3), ether (200 ml × 3), and EtOAc (200 ml × 3) in a separatory funnel. The hexane, the ether and the EtOAc extractives, after removal of the solvent, weighed 1.61, 2.77 and 0.78 g, respectively.

The aqueous layer was concentrated under reduced pressure in order to evaporate off the EtOAc present in it, and the concentrate was applied to a column of polyamide (36 g) (polyamide C–100 from Wako Pure Chemical

Industries, Ltd.). The column was eluted with water (600 ml) and the total eluate was applied to a column of Amberlite XAD-2 (36 g). This column was washed with water (600 ml), and then eluted with MeOH (400 ml). The residue (1.49 g) obtained after concentration of the MeOH eluate was chromatographed over silica gel (150 g) and divided into the following three fractions (Fr.). Fr. 1: EtOAc–MeOH (9 : 1→4 : 1), 0.57 g; Fr. 2: EtOAc–MeOH (2 : 1→1 : 1), 0.50 g; Fr. 3: MeOH, a very small quantity.

Fr. 1 (0.57 g) was re-chromatographed on silica gel. Elution with solv. A afforded I (118 mg) and IV (364 mg). Re-chromatography of Fr. 2 over silica gel with Solv. B as the eluent yielded II (224 mg), III (74 mg) and V (111 mg).

The above EtOAc extractive (0.78 g) was chromatographed on silica gel. Elution with EtOAc–MeOH (9 : 1→4 : 1) afforded I (64 mg) and IV (192 mg). The total yields of I—V were as follows: I, 0.14%; II, 0.17%; III, 0.056%; IV, 0.42%; V, 0.084%.

Prunasin (I) and Its Tetraacetate——I, colorless needles (benzene–99%EtOH), mp 146—148 °C (dec.), $[\alpha]_D^{21}$ −27.8° (c=0.41, H_2O) (lit.[14] mp 150—151 °C, $[\alpha]_D^{28}$ −30.1° (H_2O)). Tetraacetate of I, colorless needles (H_2O–99%EtOH), mp 138—139 °C (lit.[14] mp 139—140 °C). I and its tetraacetate were identical with prunasin (mp, TLC) and prunasin tetraacetate (mixed fusion, IR), respectively.

Amygdalin (II) and Its Heptaacetate——II, colorless needles (99% EtOH), mp 207—209 °C (dec.), $[\alpha]_D^{20}$ −37.8° (c=0.96, H_2O) (lit.[15] mp 215 °C, $[\alpha]_D$ −39.8°). Heptaacetate of II, colorless needles (from H_2O–MeOH), mp 169—171 °C (lit[16] mp 171—172 °C). II and its heptaacetate were identical with amygdalin (mp, TLC) and amygdalin heptaacetate (mixed fusion, IR), respectively.

Vicianin (III) and Its Hexaacetate——III, colorless needles (benzene–MeOH), mp 170—172 °C (lit.[8] mp 175—176 °C, mp 147—148 °C[9]), $[\alpha]_D^{20}$ −19.6° (c=1.04, H_2O) (lit.[8] $[\alpha]_D^{20}$ −20.0° (c=0.5, H_2O). *Anal.* Calcd for $C_{19}H_{25}NO_{10}\cdot H_2O$: C, 51.16; H, 6.11; N, 3.14. Found: C, 51.31; H, 6.18; N, 3.10. ^{13}C-NMR (DMSO-d_6) δ: 134.1 (s), 130.2 (d), 129.5 (d×2), 127.8 (d×2), 119.3 (nitrile), 67.8 (C_6H_5–$\underline{C}H<$), 102.1 (C-1′ of the glucosyl residue (G-1′)), 73.5 (G-2′), 76.6 (G-3′ or G-5′), 70.4 (G-4′), 76.8 (G-5′ or G-3′), 68.6 (G-6′), 104.2 (C-1′ of the arabinosyl residue (A-1′)), 71.3 (A-2′), 73.0 (A-3′), 68.2 (A-4′), 65.8 (A-5′). Hexaacetate of III, colorless needles (H_2O–MeOH), mp 169—170 °C $[\alpha]_D^{20}$ −33.1° (c=0.47, $CHCl_3$) (lit.[8] mp 170—171 °C, $[\alpha]_D^{20}$ −29.6° (c=0.5, $CHCl_3$)).

Hydrolysis of III with 5%H_2SO_4——A solution of III (10 ml) in 5% H_2SO_4 (2 ml) was refluxed for 1.5 h. The solution was neutralized with $Ba(OH)_2$, and then filtered. The filtrate was passed through a column of Amberlite BM-3, and concentrated to a small volume. Glucose and arabinose were found on TLC (Cellulose F_{254} (Merck), BuOH–AcOH–H_2O (6 : 1 : 2), detection with aniline-H_3PO_4).

Partial Hydrolysis of III with 0.4 N HCl——A solution of III (360 mg) in 0.4 N HCl (12 ml) was refluxed for 45 min. The solution was passed through a column of Amberlite XAD-2 (1 g). The column was washed with water (45 ml) , and then eluted with MeOH (78 ml). After evaporation of the solvent, the residue was purified by column chromatography on silica gel. Elution with $CHCl_3$–MeOH–H_2O (80 : 15 : 1) afforded a product as colorless needles (57 mg), mp 147 °C (dec.) (benzene–99%EtOH), $[\alpha]_D^{18}$ −27.0° (c=0.35, H_2O); this product was identical with an authentic sample of prunasin (mp, TLC, $[\alpha]_D$).

4-β-D-Glucopyranosyloxy-5-methylcoumarin (IV)——Colorless needles (H_2O), mp 152—154 °C, $[\alpha]_D^{20}$ −106.0° (c=0.2, MeOH), $[\alpha]_D^{20}$ −57.2° (c=0.25, pyridine) (lit.[17] mp 150 °C, $[\alpha]_D$ −117.5° (MeOH); $[\alpha]_D^{18}$ −56.0° (c=0.5, pyridine)).[1] This compound was identical with an authentic sample isolated from *L. anandria*[1] (mixed fusion, IR).

5-Methyl-4-rutinosyloxycoumarin (V)——Colorless needles (H_2O), mp 240—241 °C (dec.), $[\alpha]_D^{22}$ −79.4° (c=0.2, pyridine). *Anal.* Calcd for $C_{22}H_{28}O_{12}$: C, 54.54; H, 5.83. Found: C, 54.58; H, 5.86. UV λ_{max}^{EtOH} nm (log ε): 279 (3.95), 287 (3.94), 307 (sh, 3.59), 321 (sh, 3.34). ^{1}H-NMR (DMSO-d_6) δ: 7.50 (1H, dd(br), J=7.1, 7.8 Hz, 7–H). 7.22 (1H, d(br), J=7.1 Hz, 8–H), 7.14 (1H, d(br), J=7.8 Hz, 6–H), 5.93 (1H, s, 3–H), 2.69 (3H, s, Ar–CH_3). ^{13}C-NMR (C_5D_5N) δ: 167.3 (C-4), 162.0 (C-2), 155.1 (C-9), 137.6 (C-5). 131.6 (C-7), 127.6 (C-6), 115.0 (C-8), 114.7 (C-10), 94.2 (C-3), 23.5 (Ar-CH_3), 101.3 (G-1′), 74.1 (G-2′), 78.6 (G-3′), 71.0 (G-4′), 77.7 (G-5′), 67.3 (G-6′), 102.3 (C-1′ of the rhamnosyl residue (R-1′)), 72.5 (R-2′), 72.0 (R-3′), 73.7 (R-4′), 69.5 (R-5′), 18.5 (R-6′).

Hydrolysis of V with 5% H_2SO_4——A solution of V (10 mg) in 5% H_2SO_4 was refluxed for 1 h. The reaction mixture was treated in the same manner as in the case of III, and glucose and rhamnose were detected on TLC.

Partial Hydrolysis of V with Hesperidinase——Hesperidinase (40 mg) (hesperidinase "TANABE" from Tanabe Seiyaku Co., Ltd.) was added to a solution of V (80 mg) in Na_2HPO_4–citric acid buffer (pH 4.0) (11 ml), and the mixture was stirred for 2 h at 40 °C, then neutralized with 2 N Na_2CO_3, and concentrated to dryness. The residue was applied to a column of silica gel. Elution with $CHCl_3$–MeOH–H_2O (70 : 10 : 1) afforded a product as colorless needles (47 mg) from H_2O, mp 151—152 °C; this product was identical with 4-β-D-glucopyranosyloxy-5-methylcoumarin isolated from *L. anandria*[1] (mixed fusion, IR).

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