New Glucosides of a 4-Hydroxy-5-methylcoumarin and a Dihydro-α-pyrone from *Gerbera jamesonii hybrida*

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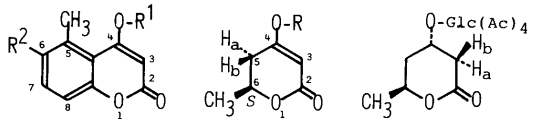
Two new glucosides, 2, $C_{16}H_{18}O_9$, mp 202—203 °C, $[\alpha]_D^{20}$ −96.4° (MeOH) and gerberin (5), $C_{12}H_{18}O_8$, $[\alpha]_D^{19}$ +11.2° (MeOH), were isolated from the aerial part of *Gerbera jamesonii hybrida* (Compositae). On methanolysis, 2 yielded 6-hydroxy-4-methoxy-5-methylcoumarin. The tetraacetate of 5 yielded tetraacetylparasorboside on catalytic hydrogenation. The chemical structures of 2 and 5 were established as 4-β-D-glucopyranosyloxy-6-hydroxy-5-methylcoumarin and (6*S*)-5,6-dihydro-4-β-D-glucopyranosyloxy-6-methyl-2*H*-pyran-2-one, respectively. The yield of gerberin (5) was quite high, amounting to about 3.7% based on the weight of the dried plant material.

Keywords *Gerbera jamesonii hybrida*; Compositae; 4-hydroxy-5-methylcoumarin; 5-methylcoumarin; dihydro-α-pyrone; gerberin

Gerbera (*Gerbera jamesonii hybrida*, Compositae) is widely cultivated as a garden plant. We have already reported the isolation of two 4-hydroxy-5-methylcoumarin glycosides in addition to two cyanogenic glycosides from the underground parts of this plant.[1] In the previous paper we performed a biosynthetic study on 4-β-D-glucopyranosyloxy-5-methylcoumarin (**1**), one of the constituents of this plant, and proposed that its coumarin skeleton is formed *via* a pentaketide derived from the acetate–malonate pathway.[2] This paper deals with the structure determination of two new glycosides **2** and **5**, the latter being named gerberin. These were isolated from the aerial parts of this plant together with **1**, α- and β-amyrin, prunasin, and amygdalin.



1: R^1=Glc, R^2=H
2: R^1=Glc, R^2=OH
3: R^1=H, R^2=OH
4: R^1=CH_3, R^2=OH
5 (gerberin): R=Glc
6: R=Glc(Ac)$_4$
7: R=CH_3
8

Glc=β-D-glucopyranosyl

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts

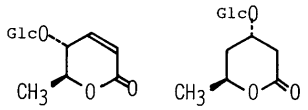
	1	2	3	5	6[a]	7
Solvent	C_5D_5N	DMSO-d_6	DMSO-d_6	C_5D_5N	$CDCl_3$	$CDCl_3$
C-2	162.0	161.5	161.7	166.5	166.0	167.1
C-3	94.1	93.0	91.4	94.6	95.1	90.3
C-4	167.1	166.8	168.7	170.4	170.5	172.6
C-5	137.7	121.1	121.1	34.1	34.0	34.6
C-6	127.8	151.7	151.4	72.0	72.9	72.2
C-7	131.7	119.5	119.1			
C-8	115.1	114.4	114.1			
C-9	155.2	147.5	148.1			
C-10	114.8	114.4	114.9			
CH_3–C	23.7	13.2	13.2	20.5	20.6	20.6
CH_3–O						55.9
Glucose-1	101.3	99.8		100.5	96.5	
-2	74.3	73.0		74.2	70.7	
-3	79.1[b]	77.3[b]		78.9[b]	72.5[b]	
-4	70.7	69.5		70.7	68.1	
-5	78.6[b]	76.5[b]		78.1[b]	72.2[b]	
-6	61.9	60.6		62.0	61.7	

a) The chemical shifts of four acetyl groups were eliminated. *b*) Assignments may be interchanged in each column.

Compound **2**, $C_{16}H_{18}O_9$, mp 202—203 °C, $[\alpha]_D^{20}$ −96.4° (MeOH) showed absorption maxima at 225, 277 (sh), 284, 295 (sh) and 339 nm in its ultraviolet (UV) spectrum, and absorptions due to hydroxy groups (3310 cm^{-1}), an aromatic ring (1610, 1560 cm^{-1}), and an unsaturated lactone (1640 cm^{-1}) in its infrared (IR) spectrum. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of **2** showed sixteen signals comprising a coumarin skeleton (six singlets and three doublets), a methyl group, and a β-glucopyranosyloxy group. These spectra are similar to those of **1**, but **2** has an additional oxygen atom in the coumarin skeleton of **1**. In the ^{1}H-NMR spectrum, **2** showed a one-proton doublet at δ 5.44 ppm (J=5.8 Hz, anomeric proton of the glucose residue), a three-proton singlet at δ 2.50 ppm, a one-proton singlet at δ 5.90 ppm, and a pair of *ortho*-coupled aromatic protons at δ 7.06 and 7.12 ppm (J=8.9 Hz). These findings suggested that **2** is a 6- or 8-hydroxylated 4-hydroxy-5-methylcoumarin glucoside.

On enzymatic hydrolysis using emulsin **2** afforded glucose and an aglycone (**3**), $C_{10}H_8O_4$, mp 258—260 °C. From the following observation, the glucosyl moiety of **2** could be deduced to be attached to C-4 of the aglycone (**3**). The ^{1}H-NMR spectrum of **3** showed a singlet at δ 5.52 ppm ascribable to the proton on C-3 (3-H) and this signal disappeared on addition of deuterium oxide, while the corresponding signal at δ 5.90 ppm in the spectrum of **2** was unaffected by the proton exchange. Moreover, the UV absorption maxima of **3** were observed at 221 and 288 nm, resembling those of **2** except for the appearance of bathochromic shifts with sodium acetate.

In order to clarify the position of the hydroxy group in **2**, the chemical shifts of the aromatic carbons were calculated on the basis of the additivity rule of substituent effects for **1**.[3] The observed chemical shifts of **2** are in better agreement with the calculated ones for 6-hydroxylation of **1** rather than those for 8-hydroxylation. Namely, the hydroxylated aromatic carbon and its protonated *ortho* carbon were observed at δ 151.7 and 119.5 ppm, respectively: in 6-hydroxylation of **1**, those carbon signals are expected to appear at δ 154.7 and 119.0 ppm; in 8-hydroxylation, at δ 142.0 and 129.2 ppm. In fact, treatment of **2** with *p*-TsOH in MeOH gave a methoxy derivative (**4**), $C_{11}H_{10}O_4$, mp



osmundalin parasorboside

Glc = β-D-glucopyranosyl

Chart 2

TABLE II. ^{1}H-NMR Data and Results of Spin Decoupling Experiments (400 MHz)[a]

Compound	3-H	5-H	6-H	6-CH_3	CH_3O–	Coupling constant
6[a]	5.29 d	H_a 2.35 dd H_b 2.51 ddd	4.80 ddq	1.45 d		$J_{3,5b}=1.47$, $J_{5a,5b}=17.33$, $J_{5b,6}=11.23$, $J_{5a,6}=3.91$, $J_{6,CH_3}=6.34$
7	5.14 d	H_a 2.34 dd H_b 2.47 ddd	4.53 ddq	1.45 d	3.75 s	$J_{3,5b}=1.47$, $J_{5a,5b}=17.09$, $J_{5a,6}=3.91$, $J_{5b,6}=11.48$, $J_{6,CH_3}=6.35$

a) Measured in $CDCl_3$. *b*) The data for the tetraacetylglucosyl moiety are omitted.

264—265 °C. The physicochemical data of **4** were identical with the reported values for 6-hydroxy-4-methoxy-5-methylcoumarin, which is one of the constituents of *Gerbera jamesonii*.[4] Consequently **2** was concluded to be 4-β-D-glucopyranosyloxy-6-hydroxy-5-methylcoumarin.

Gerberin (**5**), amorphous powder, $[\alpha]_D^{19}$ −11.2° (MeOH) showed an absorption band at 232 nm in its UV spectrum and absorptions in the IR spectrum assignable to an α, β-unsaturated lactone (1680, 1630 cm^{-1}). Since **5** was acetylated to a tetraacetate (**6**), $C_{20}H_{26}O_{12}$, mp 175—176 °C, $[\alpha]_D^{21}$ +11.6° (MeOH), the molecular formula of **5** was determined as $C_{12}H_{18}O_8$. In the ^{13}C-NMR spectrum of **5**, twelve carbon signals were observed, comprising a methyl, a methylene, a methine joined to oxygen, two olefinic carbons (a singlet and a doublet), a lactone carbonyl and six carbons of the β-glucopyranosyl moiety. These spectral data led us to conclude that **5** is a glucoside of a dihydro-α-pyrone such as osmundalin isolated from *Osmunda japonica*.[5]

Spin-decoupling experiments on the tetraacetate (**6**) were performed and the results are summarized in Table II. A proton at δ 2.51 ppm (5-H_b) coupled with three protons resonating at δ 2.35 (5-H_a, $^2J=17.33$ Hz), 4.80 (6-H, $^3J=11.23$ Hz) and 5.29 ppm (3-H, $^4J=1.47$ Hz). This observation revealed that the chemical structure of the tetraacetate (**6**) of gerberin is as shown by **6** in Chart 1.

In order to derive it into a known compound, **6** was submitted to methanolysis in the presence of an acid catalyst, yielding a methoxy derivative **7**, $C_7H_{10}O_3$, mp 59—60 °C, $[\alpha]_D^{17}$ +256° (MeOH), whose ^{1}H- and ^{13}C-NMR spectra indicated that its chemical structure is 5,6-dihydro-4-methoxy-6-methyl-2*H*-pyran-2-one. The spectral data of **7** were coincident with the reported values of an authentic sample, which is known as a racemate.[6] In the circular dichroism (CD) spectrum of **6** a positive Cotton effect was observed at 256 nm, which revealed that the α,β-unsaturated lactone chromophore has left-handed chirality from Beecham's rule,[7] and at the same time the absolute configuration at C-6 should be *S*. Moreover, **6** was subjected to catalytic hydrogenation, and a dihydro derivative **8**, $C_{20}H_{28}O_{12}$, mp 150—151 °C, $[\alpha]_D^{15}$ −16.1° (acetone), was isolated. The physicochemical properties of **8** were identical with those of tetraacetylparasorboside, an acetate of parasorboside isolated from *Sorbus aucuparia*.[8] Accordingly, the chemical structure of gerberin (**5**) is (6*S*)-5,6-dihydro-4-β-D-glucopyranosyloxy-6-methyl-2*H*-pyran-2-one.

Compounds related to **7** are biologically active in insects and fungi,[9] so synthetic studies have been performed.[6] Since this plant contains a large amount of gerberin (**5**) (in a 3.7% yield by dry weight), it might be of value as a natural resouce for synthetic studies.

Experimental

All melting points were taken on a Yanagimoto melting point determination apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. IR spectra were obtained with a Shimadzu IR-400 spectrometer. UV spectra were recorded on a Shimadzu UV-250. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL JNM-FX-100 or JNM-GX-400 spectrometer with tetramethylsilane as an internal standard; chemical shifts are given on the δ scale (ppm), coupling constants (*J* values) are expressed in herz (Hz), and the following abbreviations are used. s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. Mass spectra (MS) were recorded with a JEOL JMS-D 300 machine. The CD spectrum was measured with a JASCO J-40A in a 1 cm tube. Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} pre-coated plates (Merck) and detection was carried out by UV absorption.

Isolation and Separation Commercial *Gerbera jamesonii hybrida* in bloom was used as the plant material. The air-dried aerial parts of the plant (272 g) were extracted with MeOH (2 l × 7) under reflux. The total MeOH solution was concentrated under reduced pressure. Hot water (1.4 l) was added to the residue (81.7 g), and the mixture was separated into a water-soluble part and an insoluble part. The insoluble part (10 g) afforded a mixture of α- and β-amyrin on silica gel column chromatography and the two compounds were identical with authentic samples on gas chromatography. The hot water-soluble part was applied to a column of polyamide (polyamide C-200, Wako Pure Chemical Industry). The column was eluted with water to give fractions (frs.) 1—20 (each 1 l). Fractions 6—20 were combined and concentrated under reduced pressure to about 1 l, giving 4-β-D-glucopyranosyloxy-5-methylcoumarin (**1**, 1.5 g) as a precipitate. The total aqueous solution of frs. 1—5 was applied to a column of Amberlite XAD-2 and the column was eluted with water (5 l) and then with MeOH (5 l). The water eluate was further applied to a column of Amberlite XAD-4. After being washed with water, the column was eluted with MeOH. The residue obtained on concentration of the MeOH eluate was chromatographed over Sephadex LH-20 with water to give **5** (9.8 g). The MeOH eluate of the Amberlite XAD-2 column was repeatedly chromatographed over silica gel, affording **2** (103.6 mg), amygdalin (66.5 mg) and prunasin (663 mg). The known compounds (**1**, amygdalin, and prunasin) were identified by direct comparison with authentic samples.

Compound 2 Colorless needles (MeOH), mp 202—203 °C, $[\alpha]_D^{20}$ −96.4° (*c* = 1.0, MeOH). *Anal.* Calcd for $C_{16}H_{18}O_9$: C, 54.24; H, 5.12. Found: C, 54.10; H, 5.10. IR ν_{max}^{KBr} cm^{-1}: 3310 (OH), 1640 (C=O), 1610, 1560 (arom.). UV λ_{max}^{MeOH} nm (log ε): 225 (4.29), 277 (sh, 4.05), 284 (4.09), 295 (sh, 3.96), 339 (3.55). (unchanged on addition of NaOAc). ^{1}H-NMR (DMSO-d_6) δ: 2.50 (3H, s, CH_3–) 5.44 (1H, d, $J=5.8$, 1-H of glucose moiety), 5.90 (1H, s, 3-H), 7.06, 7.12 (each 1H, d, $J=8.9$, 7 or 8-H), 9.64 (1H, s, 6-OH, disappeared on addition of D_2O).

Enzymatic Hydrolysis of 2 An EtOH solution (5 ml) of **2** (36 mg) was added to a solution of emulsion in Na_2HPO_4–citric acid buffer (pH 5.0, 15 ml), and the mixture was stirred for 24 h at 37 °C. The EtOH in the mixture was removed *in vacuo*. The resulting aqueous solution was extracted with EtOAc (10 ml × 4). The total EtOAc solution was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated to afford **3** (11 mg). The water-soluble fraction of the hydrolysate was passed through a column of Amberlite MB-3, and concentrated to a small volume. Glucose was detected on TLC (Cellulose F_{254} (Merck), BuOH–AcOH–H_2O (6:1:2), visualized with aniline–H_3PO_4). **3**, pale yellow needles

(MeOH–H_2O), mp 258—260 °C. *Anal.* Calcd for $C_{10}H_8O_4$: C, 62.50; H, 4.20. Found: C, 62.36; H, 4.14. MS *m/z*: 192 (M^+), 150 ($M^+ - CH_2CO$), 122 (150 − CO). IR ν_{max}^{KBr} cm^{-1}: 3150, 1630, 1610, 1460. UV λ_{max}^{MeOH} nm (log ε): 221 (4.50), 288 (4.07); $\lambda_{max}^{MeOH+NaOAc}$ nm: 297 (bathochromic shift). ^{1}H-NMR (DMSO-d_6) δ: 5.52 (1H, s, 3-H, disappeared on addition of D_2O) 6.98, 7.11 (each 1H, d, J=9.0, 7- or 8-H), 9.48 (1H, s, 6-OH, disappeared on addition of D_2O).

Methanolysis of 2 A solution of **2** (20 mg) and *p*-TsOH (120 mg) in anhydrous MeOH (8 ml) was refluxed for 4 h. After concentration to dryness, the residue was chromatographed over silica gel. Fractions eluted with $CHCl_3$ gave **4** (6 mg). **4**, pale yellow needles (MeOH–H_2O), mp 264—265 °C. High-resolution MS *m/z*: Calcd for $C_{11}H_{10}O_4$ (M^+): 206.058. Found: 206.057. UV λ_{max}^{MeOH} nm: 222, 273 (sh), 280, 290, 332. ^{1}H-NMR (DMSO-d_6) δ: 2.46 (3H, s, CH_3-arom.), 3.95 (CH_3-O, 5.75 (1H, s, 3-H), 7.05, 7.11 (each 1H, d, J=8.9, 7- or 8-H), 9.50 (6-OH). The MS, IR, and ^{1}H-NMR data of **4** were identical with those reported for 6-hydroxy-4-methoxy-5-methylcoumarin.[4)]

Gerberin (5) Colorless amorphous powder, $[\alpha]_D^{19}$ −11.2° (*c*=1.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1680, 1630 (α,β-unsaturated lactone). UV λ_{max}^{MeOH} nm (log ε): 232 (4.05).

Acetylation of 5 **5** (500 mg) was acetylated with Ac_2O–pyridine. After work-up in the usual manner, the tetraacetate (**6**, 490 mg) was obtained. **6**, colorless needles (MeOH), mp 175—176 °C, $[\alpha]_D^{21}$ +11.6° (*c*=1.0, MeOH). *Anal.* Calcd for $C_{20}H_{26}O_{12}$: C, 52.40; H, 5.72. Found: C, 52.40; H, 5.72. CI-MS *m/z*: 459 (M^++H). UV λ_{max}^{MeOH} nm (log ε): 229 (4.04). CD ($c = 5.8 \times 10^{-3}$, MeOH) [θ] (nm): $+2.57 \times 10^4$ (256).

Acid Hydrolysis of 5 A solution of **5** (500 mg) in 10% HCl (10 ml) was refluxed for 30 min. The solution was passed through a column of Amberlite MB-3, and processed in the same manner as in the case of **2**. Glucose was identified as a sugar product.

Methanolysis of 6 A solution of **6** (200 mg) and *p*-TsOH (50 mg) in absolute MeOH was refluxed for 7 h and treated in the same way as in the case of **2**. The product (**7**, 85 mg) was purified by sublimation. **7**, mp 59—60 °C, $[\alpha]_D^{17}$ +256° (*c*=0.5, MeOH). UV λ_{max}^{MeOH} nm (log ε): 233 (4.04). High-resolution MS *m/z*: Calcd for $C_7H_{10}O_3$ (M^+): 142.063. Found: 142.063. IR ν_{max}^{KBr} cm^{-1}: 1690, 1625. CD ($c = 3.9 \times 10^{-3}$, MeOH) $[\theta]^{20}$ (nm): $+3.65 \times 10^4$ (253). The UV, IR, and ^{1}H-NMR spectral data of **7** were coincident with those of *dl*-5,6-dihydro-4-methoxy-6-methyl-2*H*-pyran-2-one.[6)]

Catalytic Hydrogenation of 6 A solution of **6** (1 g) in EtOAc (20 ml) was hydrogenated at atmospheric pressure in the presence of 5% Pd–C (100 mg) until absorption of hydrogen ceased. After removal of the catalyst by filtration, the solvent was evaporated off *in vacuo*. The residue was recrystallized from 50% EtOH to give **8** (180 mg). **8**, mp 150—151 °C, $[\alpha]_D^{21}$ −20.7° (*c*=0.9, MeOH), $[\alpha]_D^{20}$ −16.1° (*c*=0.6, acetone). *Anal.* Calcd for $C_{20}H_{28}O_{12}$: C, 52.19; H, 6.13. Found: C, 52.03; H, 6.22. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, d, J=6.10, 6-CH_3), 1.57 (1H, ddd, J=10.25, 11.71, 13.67, 5-Ha or Hb), 2.23 (1H, dddd, J=1.46, 2.70, 5.05, 13.67, 5-Hb or Ha), 2.59 (1H, dd, J=8.30, 17.58, 3-Ha or Hb), 2.98 (1H, ddd, J=1.46, 6.35, 17.58, 3-Hb or Ha), 4.15 (1H, m, 4-H), 4.33 (1H, ddq, J=2.70, 6.10, 11.71, 6-H). The MS, IR, and ^{1}H-NMR data of **8** coincided with those of tetraacetylparasorboside. (lit.[8)] mp 157—158 °C, $[\alpha]_D^{20}$ −17° (acetone)).

References and Notes

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8) *a*) R. Tschesche, H.-J. Hoppe, G. Snatzke, G. Wulff, and H.-W. Fehlhaber, *Chem. Ber.*, **104**, 1420 (1971); *b*) The melting point of **7** is lower by six degrees than that reported for tetraacetylparasorboside,[8a)] though their spectral data and optical rotations coincided with each other. As the original specimen of tetraacetylparasorboside was unavailable to the authors, direct comparison could not be carried out.
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