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New Highly in Vitro Antioxidative 3,8″-Linked Biflav(an)ones and Flavanone-C-glycosides from *Garcinia buchananii* Stem Bark

Timo D. Stark,*[†] Daniel Germann,[†] Onesmo B. Balemba,[‡] Junichiro Wakamatsu,[†] and Thomas Hofmann[†]

[†]Lehrstuhl für Lebensmittelchemie und Molekulare Sensorik, Technische Universität München, Lise-Meitner Strasse 34, D-85354 Freising, Germany

[‡]Department of Biological Sciences, University of Idaho, Moscow, Idaho, United States

Supporting Information

ABSTRACT: Very recently, we described highly antioxidative polyphenols isolated from the stem bark extract of the *Garcinia buchananii* tree. In this study, we describe additional antioxidants from *Garcinia buchananii* bark extract using hydrogen peroxide scavenging, oxygen radical absorbance capacity (ORAC), and trolox equivalent antioxidant capacity (TEAC) assays. UPLC-HR-ESI-TOF-MS^e analysis, 1- and 2D-NMR, and circular dichroism (CD) spectroscopy led to the unequivocal identification of the antioxidative molecules as a series of five 3,8″-linked biflav(an)ones and two flavanone-C-glycosides. (2*S*,3*R*)-Taxifolin-6-*C*-*β*-D-glucopyranoside (**2**), (2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**), (2*R*,3*S*)-buchananiflavonol (**4**), and (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**) are new compounds, and (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**) was described so far only in one other plant. The structure of (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**) and (2*R*,3*S*,2″*S*)-GB2a (**7**) were confirmed. The H_2O_2 scavenging, TEAC, and the ORAC assays demonstrated that these natural products have an extraordinarily high antioxidative power, especially (2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**) with an EC_{50} value of 3.0 μM, 4.00 mmol TE/mmol, and 10.30 μmol TE/ μmol.

KEYWORDS: *3,8″-linked biflav(an)ones, flavanone-6-C-glycosides, Garcinia buchananii, (2R,3S,2″S,3″S)-manniflavanone, (2R,3S)-buchananiflavonol, antioxidants*

INTRODUCTION

Recently, we showed that the stem bark extract of *G. buchananii* has extraordinary strong in vitro antioxidative activity compared to other plant extracts. By means of antioxidative activity-guided separation using hydrogen peroxide scavenging assay dilution analysis as well as ORAC assay on *Garcinia buchananii* fractions in combination with spectroscopic analyses, we found (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside, (2*R*,3*R*)-aromadendrin-6-*C*-*β*-D-glucopyranoside, (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone, (2*R*,3*S*,2″*R*,3″*R*)-GB-2, and (2*R*,3*S*,2″*S*)-buchananiflavanone as highly antioxidative active main constituents.[1] As in this recently performed study the medium pressure liquid chromatography (MPLC) fractions M1 and M3−M5 were investigated, the purpose of the present study was to isolate and elucidate the chemical structures of further constituents and of compounds from antioxidative MPLC fractions M2 and M6 to enable structure−activity investigations. Additionally, all already known constituents from *G. buchananii* stem bark extract and further isolated compounds as well as the crude extract were evaluated with the third antioxidative assay, namely, TEAC with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS).

MATERIALS AND METHODS

Chemicals. The following reagents were obtained commercially: hydrogen peroxide (Merck, Hohenbrunn, Germany); 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), peroxidase from horseradish (HRP), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), fluorescein sodium salt (FL), 2,2′-azobis(2-methylpropinamidine) (AAPH), quercetin, (−)-epicatechin, (±)-naringenin, and ascorbic acid (Sigma-Aldrich, Steinheim, Germany); and rutin, (+)-(2*R*,3*R*)-taxifolin (AppliChem, Darmstadt, Germany). Water for chromatographic separations was purified with a Milli-Q Gradient A10 system (Millipore, Schwalbach, Germany), and solvents used were of HPLC-grade (Merck, Darmstadt, Germany). Deuterated solvents were obtained from Euriso-Top (Gif-sur-Yvette, France).

General Experimental Procedure. 1D and 2D NMR spectroscopy ^{1}H, ^{1}H−^{1}H-*g*COSY, *g*HSQC, *g*HMBC, and ^{13}C were performed on an Avance III 500 MHz spectrometer with a CTCI probe or an Avance III 400 MHz spectrometer with a BBO probe (Bruker, Rheinstetten, Germany). Mass spectra of the compounds were measured on a Waters Synapt G2-S HDMS (details are in the Supporting Information) mass spectrometer (Waters, Manchester, UK) coupled to an Acquity UPLC core system (Waters, Milford, MA, USA). For CD spectroscopy, methanolic solutions of the samples were analyzed by means of a Jasco J810 Spectro polarimeter (Hachioji, Japan). HPLC separations were performed using a preparative HPLC system (PrepStar, Varian, Darmstadt, Germany). Medium pressure liquid chromatography (MPLC) separations were performed on a Büchi Sepacore (Flawil, Switzerland) system using a YMC (YMC Europe, Dinslaken, Germany) DispoPackAT ODS-25 flash cartridge (120g, id. 40 mm, l. 150 mm).

Plant Material. *Garcinia buchananii* stem bark was collected from plants in their natural habitats in Karagwe, Tanzania, and processed as described previously.[2] A sample of bark powder was deposited at the University of Idaho Stillinger herbarium (voucher # 159918).

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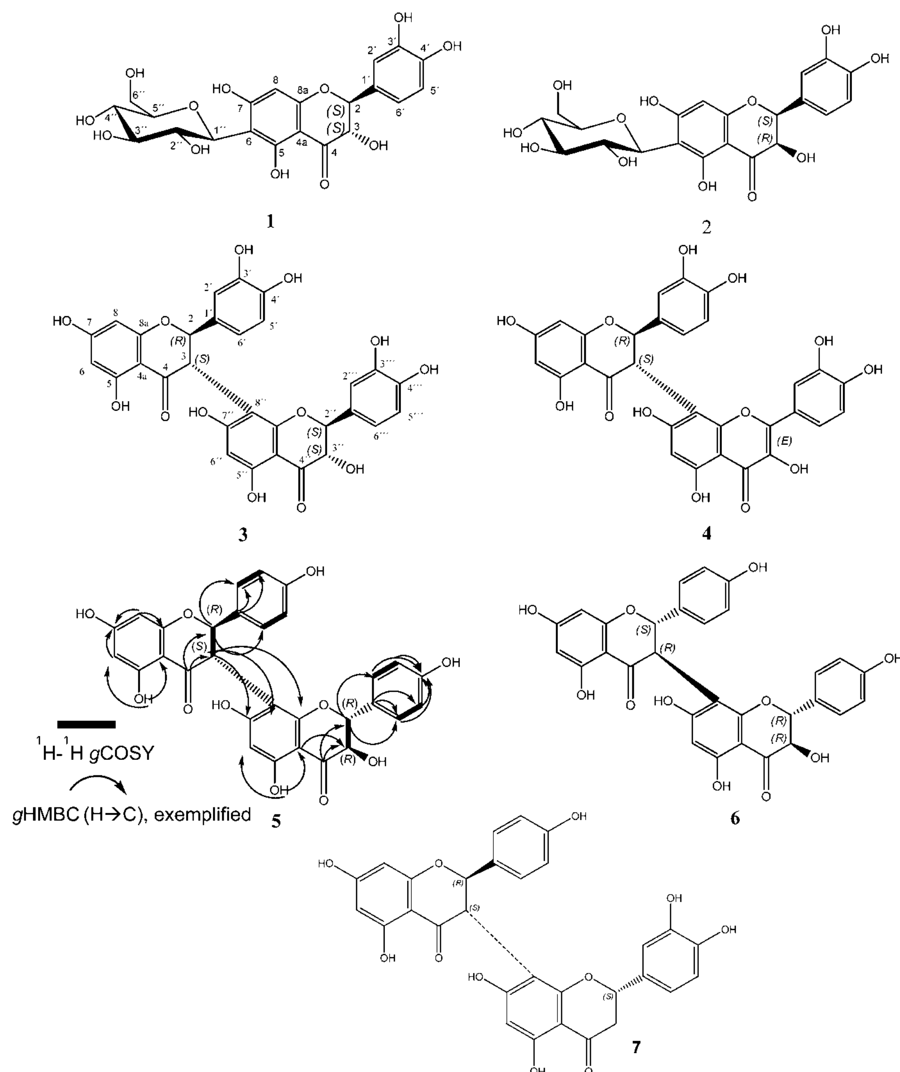

Figure 1. Chemical structures of compounds **1**−**7**.

Extraction and Isolation. Chromatography and extraction of *G. buchananii* bark powder was exactly as done described recently.[1] Eight fractions were collected (Supporting Information, Figure 1), concentrated under reduced pressure, and freeze-dried (M1-M8, 160, 70, 401, 63, 101, 51, 51, and 52 mg).

Antioxidant Assays. *Hydrogen Peroxide Scavenging Assay.* A hydrogen peroxide scavenging assay was performed in accordance with the method of Ichikawa et al.[3,4] Sample solutions at appropriate concentrations were prepared using phosphate buffer (100 mM, pH 6.0). Sample solution (100 μL), phosphate buffer (30 μL, 100 mM, pH 6.0), and hydrogen peroxide solution (10 μL, 500 μM) were mixed in a 96-well clear microplate (VWR, Ismaning, Germany). Then peroxidase (40 μL, 150 U/mL) and ABTS (40 μL, 0.1%) were added. The microplate was incubated at 37 °C for 15 min. The absorbance (A) of each well was measured at 414 nm with FLUOstar OPTIMA (BMG LABTECH, Offenburg, Germany). The scavenging effect (E) was calculated as shown using the formula below (blank stands for solution without hydrogen peroxide, and control did not include a test compound), and EC_{50} was calculated by the probit analysis. After freeze-drying in triplicate, MPLC fractions M1−M8 were analyzed in "natural" ratios.

$$E = [(A - A_{blank})_{control} - (A - A_{blank})_{test}]/(A - A_{blank})_{control} \times 100$$

Oxygen Radical Absorbance Capacity (ORAC) Assay. An ORAC assay was carried out according to the method of Ou et al.[5] with some modifications.[4] Trolox and FL were used as a standard and a fluorescent probe, respectively. Free radicals were produced by AAPH to oxidize FL. Different dilutions of Trolox (200, 100, 50, 25, and 12.5 μM) and appropriate dilutions of the tested sample were prepared with phosphate buffer (10 mM, pH 7.4). Trolox dilution (25 μL) or sample solution were pipetted into a well of a 96-well black microplate (VWR), and then FL (150 μL, 10 nM) was added. The reaction mixture was incubated at 37 °C for 30 min. Afterward, fluorescence was measured every 90 s at the excitation of 485 nm and the emission of 520 nm using FLUOstar OPTIMA. After 3 cycles, AAPH (25 μL, 240 mM) was added quickly, and then the measurement was resumed and continued up to 90 min (60 cycles in total). The background signal was determined using the first 3 cycles. The ORAC values were calculated according to the method of Cao et al.[6] and expressed as a Trolox equivalent (μmol TE/μmol). After freeze-drying in triplicate, MPLC fractions M1−M8 were analyzed in "natural" ratios.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay with ABTS. The ABTS radical scavenging activity was measured employing the ABTS assay as previously described by Floegel et al.[7] with some modifications. Briefly, the ABTS (2.5 mM) solution was prepared with phosphate buffered saline (PBS, 10 mM, pH 7.4), and AAPH (1 mM) was added to the solution. The mixture was heated in a water bath at 68 °C for 40 min. The blue-green ABTS radical solution was cooled to room temperature, filtered through a syringe membrane (0.45 μm), and diluted with PBS buffer until absorbance of 0.325 ± 0.01 at 730 nm was reached. Then Trolox standards (1000, 500, 250, 125, and

62.5 μM, 4 μL each) or samples were mixed with the diluted ABTS radical solution (196 μL) in a 96-well clear microplate. The microplate was incubated at 37 °C for 10 min. The absorbance of each well was measured at 730 nm with FLUOstar OPTIMA. ABTS radical scavenging activity was calculated employing standard curves and expressed as the Trolox equivalent (mmol TE/mmol).

Isolation and Structural Characterization of Compounds. Fractions in "natural" ratios that showed higher levels of antioxidant activities were subjected to the identification and characterization of chemical compounds. The MPLC fractions, which were further purified by means of HPLC, are M2x, M3, and M6a−c.

M2x. Chromatography was performed using an RP column (10 × 250 mm, ThermoHypersil ODS, 5 μm; Kleinostheim, Germany) as the stationary phase. The effluent (4.2 mL/min) was monitored at 290 nm. The isocratic separation was performed with a mixture (90/10, v/v) of aqueous formic acid (0.1% in water, pH 2.5) and acetonitrile (ACN) for 15 min. Collected fractions were concentrated under reduced pressure and freeze-dried twice, affording the recently described (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside and (2*R*,3*R*)-aromadendrin-6-*C*-*β*-D-glucopyranoside,[1] as well as (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside, Ulmoside A (**1**, Figure 1), and (2*S*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**2**, Figure 1).

M3. Using the same column, mobile phases, and the flow rate as described above, isocratic chromatography was performed with a mixture (70/30, v/v) of aqueous formic acid (0.1% in water, pH 2.5) and ACN. The collected fraction was concentrated under reduced pressure and freeze-dried twice, affording (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone[1] and (2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**, Figure 1).

M6a-c. Using the same flow rate and mobile phases as described above, isocratic chromatography was performed using a RP column (10 × 250 mm, Phenylhexyl, 5 μm; Phenomenex, Aschaffenburg, Germany) as the stationary phase and a mixture (70/30, v/v) of aqueous formic acid (0.1% in water, pH 2.5) and ACN. The collected fraction was concentrated under reduced pressure and freeze-dried twice, affording (2*R*,3*S*)-buchananiflavonol (**4**, Figure 1), (2*R*,3*S*,2″*S*)-buchananiflavanone,[1] 2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**), (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**), (2*R*,3*S*,2″*S*)-GB-2a (**7**, Figure 1).

*(2S,3S)-Taxifolin-6-C-β-D-glucopyranoside, Ulmosid A, (**1**, Figure 1).* Colorless powder; UV (MeOH) λ_{max} = 225, 290, 345 nm. (−) HRESIMS: *m*/*z* = 465.1035 [M − H]$^-$ (calcd for $C_{21}H_{21}O_{12}$, 465.1033). CD (MeOH, 0.69 mmol/L): λ_{max} ($\Delta\varepsilon$) = 333 (−1.0), 296 (+6.8), 254 (−1.1), 225 (−6.9), 210 (−0.3), 204 (−2.0). ^{1}H NMR (500 MHz, DMSO-d_6, COSY): δ 3.10 [m, 1H, H−C(4″)], 3.11 [m, 1H, H−C(5″)], 3.15 [dd, 1H, *J* = 7.8, 8.4 Hz H−C(3″)], 3.33 [d, 1H, *J* = 11.1 Hz, H−C(6″α)], 3.66 [d, 1H, *J* = 11.1 Hz, H−C(6″β)], 4.01 [t, 1H, *J* = 9.1, 9.3 Hz, H−C(2″)], 4.43 [d, 1H, *J* = 10.6 Hz, H−C(3)], 4.48 [d, 1H, *J* = 9.8, H−C(1″)], 4.90 [d, 1H, *J* = 10.8, H−C(2)], 5.72 [brs, 1H, HO-C(3)], 5.82 [s, 1H, H−C(8)], 6.73 [s, 2H, H−C(5′,6′)], 6.86 [s, 1H, H−C(2′)], 9.04 [brs, HO-C(3′,4′)], 12.53 [s, HO-C(5)]. ^{13}C NMR (125 MHz, DMSO-d_6, HSQC, HMBC): δ 61.5 [C-6″], 70.2 [C-2″], 70.7 [C-4″], 71.5 [C-3], 73.1 [C-1″], 79.1 [C-3″], 81.4 [C-5″], 82.8 [C-2], 95.2 [C-8], 99.4 [C-4a], 106.1 [C-6], 115.1 [C-5′], 115.2 [C-2′], 119.3 [C-6′], 128.2 [C-1′], 145.0 [C-4′], 145.7 [C-3′], 161.1 [C-8a], 162.6 [C-5], 167.7 [C-7], 196.8 [C-4].

*(2S,3R)-Taxifolin-6-C-β-D-glucopyranoside, (**2**, Figure 1).* Colorless powder; UV (MeOH) λ_{max} = 225, 289, 345 nm. (−) HRESIMS: *m*/*z* = 465.1033 [M − H]$^-$ (calcd for $C_{21}H_{21}O_{12}$, 465.1033). CD (MeOH, 0.75 mmol/L): λ_{max} ($\Delta\varepsilon$) = 341 (−2.8), 295 (+6.6), 252 (−0.2), 237 (+1.0), 220 (−1.8), 216 (−1.2), 211 (−1.8). ^{1}H NMR (500 MHz, DMSO-d_6, COSY): δ 3.09 [m, 1H, H−C(4″)], 3.12 [m, 1H, H−C(5″)], 3.16 [dd, 1H, *J* = 8.2, 8.4 Hz H−C(3″)], 3.37 [d, 1H, *J* = 10.9 Hz, H−C(6α″)], 3.65 [d, 1H, *J* = 10.9 Hz, H−C(6β″)], 4.02 [d, 1H, *J* = 9.3 Hz, H−C(2″)], 4.03 [m, 1H, H−C(3)], 4.47 [d, 1H, *J* = 9.8 Hz, H−C(1″)], 5.28 [d, 1H, *J* = 1.8 Hz, H−C(2)], 5.87 [s, 1H, H−C(8)], 6.12 [s, 1H, HO-C(3)], 6.69 [d, 1H, *J* = 8.1, H−C(5′)], 6.73 [dd, 1H, *J* = 1.9, 8.2, H−C(6′)], 6.94 [d, 1H, *J* = 1.8, H−C(2′)], 8.92 [2xbrs, 2H, HO-C(3′,4′)], 12.53 [s, 1H, HO-C(5)]. ^{13}C NMR (125 MHz, DMSO-d_6, HSQC, HMBC): δ 62.1 [C-6″], 70.8 [C-2″], 71.2 [C-4″], 71.3 [C-3], 73.6 [C-1″], 79.6 [C-3″], 81.2 [C-2], 82.0 [5″], 95.5 [C-8], 102.4 [C-4a], 106.8 [C-6], 115.4 [C-5′], 115.7 [C-2′], 119.4 [C-6′], 127.3 [C-1′], 145.5 [C-4′], 146.5 [C-3′], 161.6 [C-8a], 163.0 [C-5], 165.1 [C-7], 197.6 [C-4].

*(2R,3S,2″S,3″S)-Manniflavanon (**3**, Figure 1).* Colorless powder; UV (MeOH) λ_{max} = 210, 290, 346 nm. (−) HRESIMS *m*/*z* 589.0984 [M − H]$^-$ (calcd for $C_{30}H_{21}O_{13}$, 589.0982). CD (MeOH, 0.34 mmol/L): λ_{max} ($\Delta\varepsilon$) = 337 (−2.7), 309 (+2.3), 283 (+13.4), 250 (+2.9), 235 (+8.8), 215 (−8.2), 204 (+7.3). ^{1}H NMR (500 MHz, DMSO-d_6, COSY): δ 3.85 [d, *J* = 11.5 Hz, H−C(3″)], 4.15 [d, *J* = 11.2, H−C(3″)], 4.30 [d, *J* = 12.0, H−C(3)], 4.66 [d, *J* = 12.2, H−C(3)], 4.76 [d, *J* = 11.5, H−C(2″)], 4.89 [d, *J* = 10.9, H−C(2″)], 5.28 [dd, *J* = 12.1, H−C(2)], 5.56 [s, HO-C(3″)], 5.64 [d, *J* = 12.1, H−C(2)], 5.75 [s, HO-C(3″)], 5.71−5.85 [m, H−C(6″,6,8)], 5.89 [s, H−C(6″)], 6.49 [d, *J* = 7.9, H−C(6′/6‴)], 6.56 [d, *J* = 7.8, H−C(6′/6‴)], 6.59 [m, H−C(5′/5‴)], 6.64 [d, *J* = 8.5, H−C(6′/6‴)], 6.67 [d, *J* = 8.7, H−C(6′/6‴)], 6.70 [s, H−C(2′/2‴)], 6.73 [d, *J* = 8.0, H−C(5′/5‴)], 6.79 [m, H−C(2′/2‴)], 8.98 [brs, HO-C(3′,4′,3‴,4‴)], 12.25 [s, HO-C(5/5″)]. ^{13}C NMR (125 MHz, DMSO-d_6, HSQC, HMBC): δ 47.1, 47.3 [C-3], 72.0, 72.3 [C-3″], 81.5, 82.0 [C-2], 82.5, 82.7 [C-2″], 94.8, 94.9 [C-8], 96.0 [C-6,6″], 96.5 [C-6], 98.3 99.2 [C-4a″], 101.1, 101.3 [C-4a,8″], 114.8, 114.9, 115.1, [C-5′,5‴], 115.3, 115.4, 115.6, [C-2′,2‴], 117.5, 118.0, 118.5, 118.9 [C-6′,6‴], 128.4 [C-1′], 128.5, 128.6 [C-1‴], 128.8 [C-1′], 144.6, 144.7, 145.4, 145.59, 145.63 [C-3′,3‴C-4′,4‴], 159.3 [C-8a″], 159.9 [C-8a″], 162.0 [C-5″], 162.4 [C-5″], 162.6, 162.8, 163.6, 163.7, 164.8 [C-8a,5,7″], 166.4 [C-7], 195.8, 197.1 [C-4,4″].

*(2R,3S)-Buchananiflavonol, (2R,3S)-2-(3,4-Dihydroxyphenyl)-2,3-dihydro-3′-5,5′-7,7′-pentahydroxy-2′-(3,4-dihydroxyphenyl)-[3,8′-Bi-1-benzopyran]-4,4′-dione (**4**, Figure 1).* Yellow powder; UV (MeOH) λ_{max} = 385, 284, 253, 228 nm. (−) HRESIMS *m*/*z* 587.0828 [M − H]$^-$ (calcd for $C_{30}H_{19}O_{13}$, 587.0826). CD (MeOH, 0.41 mmol/L): λ_{max} ($\Delta\varepsilon$) = 370 (+2.6), 324 (−0.2), 290 (+5.1), 266 (−0.6), 256 (+0.6), 218 (−10.7), 206 (−6.7). ^{1}H NMR (500 MHz, DMSO-d_6, COSY): δ 4.72 [d, *J* = 12.0 Hz, H−C(3)], 4.94 [d, *J* = 12.4 Hz, H−C(3)], 5.53 [d, *J* = 12.0 Hz, H−C(2)], 5.73 [d, *J* = 12.1 Hz, H−C(2)], 5.92 [brs, H−C(6,6″,8)], 5.99 [s, H−C(8)], 6.03 [s, H−C(6″)], 6.11 [s, H−C(6)], 6.37 [d, *J* = 7.9, H−C(5′)], 6.41 [d, *J* = 8.6, H−C(6′)], 6.43 [d, *J* = 8.5 Hz, H−C(5‴)], 6.52 [d, *J* = 7.8 Hz, H−C(5′)], 6.63, 6.67 [2xs, H−C(2′,2‴)], 6.65 [d, *J* = 8.2 Hz, H−C(6′)], 6.90 [d, *J* = 8.5 Hz, H−C(5‴)], 6.92 [d, *J* = 8.5 Hz, H−C(6‴)], 7.46 [d, *J* = 8.1, H−C(6‴)], 7.62 [s, H−C(2‴)], 7.75 [s, H−C(2′)], 9.00 [brs, HO-C(3′,4′,3‴,4‴)], 12.30 [brs, HO-C(5″/5)], 12.52 [brs, HO-C(5/5″)]. ^{13}C NMR (125 MHz, DMSO-d_6, HSQC, HMBC): δ 48.0, 48.7 [C-3], 81.5, 82.4 [C-2], 95.9 [C-6,6″,8], 96.7 [C-8], 98.1 [C-6″], 99.0 [C-6], 100.1 [C-8″], 100.8 [C-8″], 101.4, 102.0 [C-4a], 102.7, 103.3 [C-4a″], 115.0 [C-2/2‴], 115.2 [C-5′,2/2‴], 115.5 [C-5‴], 116.1 [C-5‴], 116.6 [C-2′], 118.3 [C-6′], 118.5 [C-6‴], 118.9 [C-6′], 120.4 [C-6‴], 122.4 [C-1‴], 128.6 [C-1′], 129.5 [C-1′], 135.2 [C-3″], 144.8, 145.1, [C-3′], 145.4 [C-3‴], 145.6 [C-4′], 146.6 [C-4‴], 148.0 [C-2″], 148.4 [C-4‴], 153.9 [C-8a″], 154.6 [C-8a″], 160.0 [C-5″], 163.4 [C-7″], 164.0 [C-7″], 164.3 [C-8a], 165.1 [C-8a], 166.9 [C-5], 167.8 [C-7], 175.1 [C-4″], 196.8 [C-4], 197.1 [C-4].

*(2R,3S,2″R,3″R)-GB-1 (**5**, Figure 1).* Colorless powder; UV (MeOH) λ_{max} = 340, 287, 225 nm. (−) HRESIMS *m*/*z* 557.1080 (calcd for $C_{30}H_{21}O_{11}$, 557.1084). CD (MeOH, 0.37 mmol/L): λ_{max} ($\Delta\varepsilon$) = 338 (+1.9), 316 (−1.4), 302 (−7.8), 279 (+8.3), 247 (−1.2), 237 (+2.9), 215 (−11.3), 206 (−8.6). ^{1}H NMR (500 MHz, DMSO-d_6, COSY): δ 3.92 [d, *J* = 11.4, H−C(3″)], 4.17 [d, *J* = 11.0, H−C(3″)], 4.34 [d, *J* = 12.0 Hz, H−C(3)], 4.68 [d, *J* = 12.1 Hz, H−C(3)], 4.89 [d, *J* = 11.3 Hz, H−C(2″)], 5.08 [d, *J* = 11.2 Hz, H−C(2″)], 5.29 [d, *J* = 12.2 Hz, H−C(2)], 5.64 [s, HO-C(3″)], 5.67 [d, *J* = 12.1 Hz, H−C(2)], 5.73−5.89 [6xs, H−C(6,8,6″)], 5.81 [s, HO-C(3″)], 6.63 [d, *J* = 7.5 Hz, H−C(3′,5′/3‴,5‴)], 6.74 [d, *J* = 8.2 Hz, H−C(3′,5′/3‴,5‴)], 6.82 [d, *J* = 8.2 Hz, H−C(3′,5′/3‴,5‴)], 7.07 [2xd, *J* = 8.5, 8.7 Hz, H−C(2′,6′/2‴,6‴)], 7.15 [d, *J* = 8.4 Hz, H−C(2′,6′/2‴,6‴)], 9.52, 9.59 [2xs, HO-C(4′,4‴)], 11.77, 11.93 [s, HO-C(5″)], 12.23, 12.32 [s, HO-C(5)]. ^{13}C NMR (125 MHz, DMSO-d_6, HSQC, HMBC): δ 47.1, 47.4 [C-3], 71.8, 72.3 [C-3″], 81.2, 81.8 [C-2], 82.3 [C-2″], 95.0 95.6, 96.1, 96.3 [C-8,6″,6], 101.3, 101.4, 101.7 [C-4a,8″,4a″], 114.6, 114.7, 114.9 [C-3‴,5‴,3′,5′], 127.9, 128.1, 128.2, 128.4, 128.8, 129.1 [C-1′,1‴,2‴,6‴,2′,6′], 157.3, 157.6, 157.7 [C-

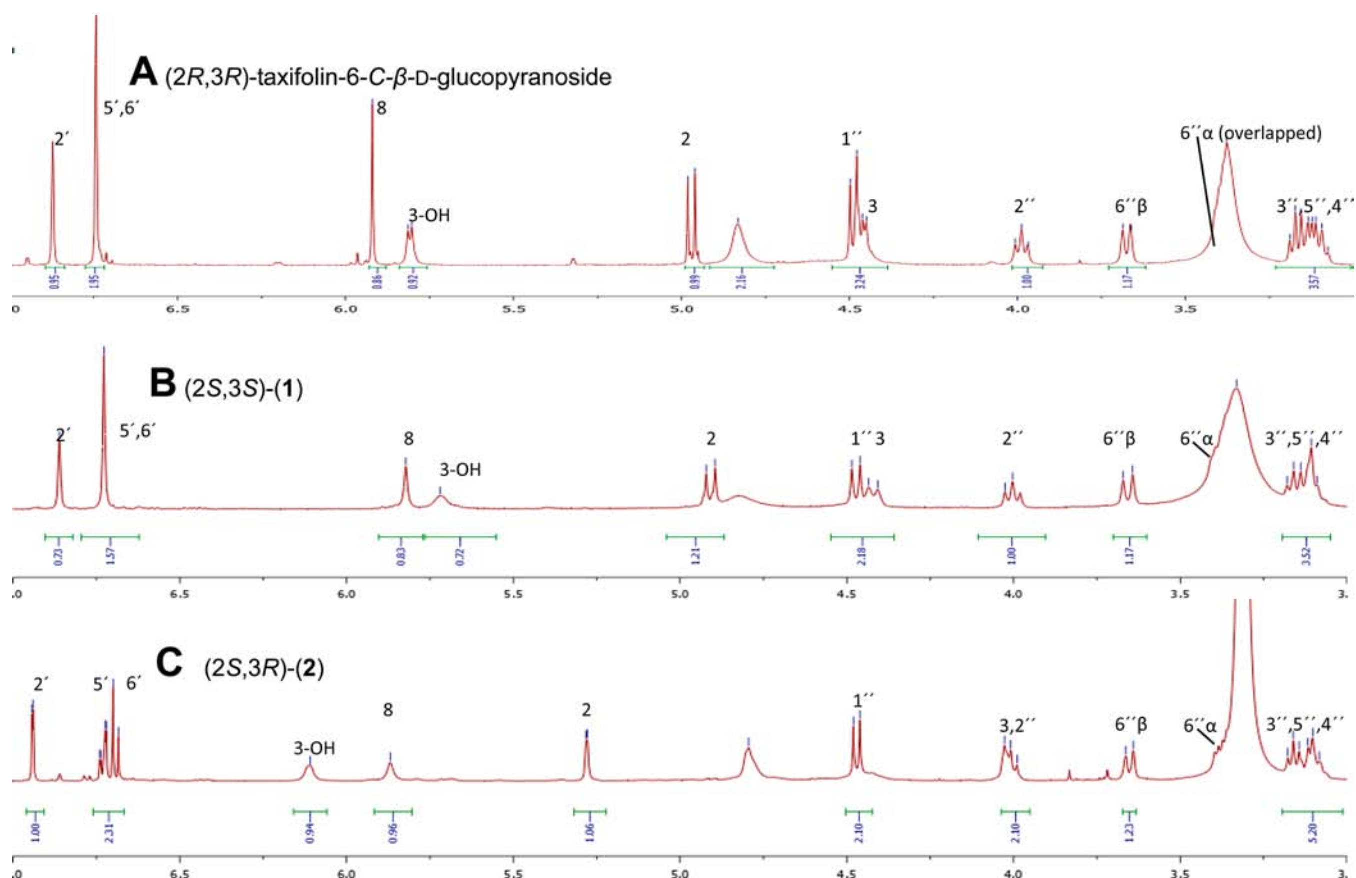

Figure 2. ^{1}H NMR data of (A) (2*R*,3*R*)-, (B) (2*S*,3*S*)-(**1**), and (**C**) (2*S*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**2**).

4′,4‴], 159.3 [C-8a″], 159.9 [C-8a″], 161.9 [C-5″/8a], 162.4 [C-5″/8a], 162.6 [C-8a/C-5″], 162.7 [C-8a/C-5″], 163.6 [C-5,7], 163.8 [C-7], 164.1 [C-5], 166.4 [C-7″], 196.8, 197.1 [C-4,4″].

*(2S,3R,2″R,3″R)-GB-1 (**6**, Figure 1).* Colorless powder; UV (MeOH) λ_{max} = 340, 288, 222 nm. (−) HRESIMS *m/z* 557.1083 (calcd for $C_{30}H_{21}O_{11}$, 557.1084). CD (MeOH, 0.40 mmol/L): λ_{max} ($\Delta\varepsilon$) = 329 (+2.2), 294 (−14.5), 249 (+1.8), 239 (+4.6), 229 (−3.6), 216 (+9.0), 205 (+3.3), 202 (7.9). ^{1}H NMR (500 MHz, DMSO-d_6, COSY): δ 3.98 [d, *J* = 11.4 Hz, H−C(2″)], 4.21 [d, *J* = 11.1 Hz, H−C(3)], 4.27 [d, *J* = 11.6 Hz, H−C(3″)], 5.65 [d, *J* = 12.0 Hz, H−C(2)], 5.73 [s, H−C(6″)], 5.79 [s, H−C(8)], 5.85 [s, H−C(6)], 6.60 [d, *J* = 8.4 Hz, H−C(3′,5′)], 6.80 [d, *J* = 8.4 Hz, H−C(3‴,5‴)], 6.90 [d, *J* = 8.4 Hz, H−C(2′,6′)], 7.23 [d, *J* = 8.4 Hz, H−C(2‴,6‴)], 9.57 [s, HO-C(4′,4‴)], 12.17 [s, HO-C(5″)], 12.40 [s, HO-C(5)]. ^{13}C NMR (125 MHz, DMSO-d_6, HSQC, HMBC): δ 47.3 [C-3], 71.3 [C-3″], 81.1 [C-2], 82.7 [C-2″], 94.8 [C-8,6″], 95.9 [C-6], 101.0 [C-4a″], 101.6 [C-4a], 103.9 [C-8″], 114.5 [C-3′,5′], 114.9 [C-3‴,5‴], 128.1 [C-1′,1‴], 128.5 [C-2′,6′], 129.2 [C-2‴,6‴], 157.4 [C-4′], 157.5 [C-4‴], 159.5 [C-8a″], 162.2 [C-5″], 162.7 [C-8a], 163.6 [C-5], 165.5 [C-7], 166.3 [C-7″], 197.5 [C-4,4″].

*(2R,3S,2″S)-GB-2a (**7**, Figure 1).* Colorless powder; UV (MeOH) λ_{max} = 340, 285, 225. (−) HRESIMS *m/z* 557.1085 (calcd for $C_{30}H_{21}O_{11}$, 557.1084). CD (MeOH, 0.43 mmol/L): λ_{max} ($\Delta\varepsilon$) = 340 (+1.4), 300 (−5.5), 279 (+7.3), 245 (−2.6), 234 (−1.8), 214 (−12.8), 205 (−0.04). ^{1}H NMR (500 MHz, DMSO-d_6, COSY): δ 2.51 [m, H−C(3α″)], 2.61 [m, *J* = 12.8, 15.4 Hz, H−C(3$\alpha\beta$″)], 2.89 [t, *J* = 13.7, 15.6 Hz, H−C(3β″)], 4.48 [d, *J* = 11.8 Hz, H−C(3)], 4.74 [d, *J* = 11.9 Hz, H−C(3)], 5.25 [d, *J* = 12.5 Hz, H−C(2″)], 5.36 [d, *J* = 12.0 Hz, H−C(2″)], 5.45 [d, *J* = 12.1 Hz, H−C(2)], 5.76 [H−C(2)], 5.64−5.87 [m, H−C(8,6″,6)], 6.60−6.80 [m, *J* = 7.7, 8.2, 8.6 Hz, H−C(5‴,3′,5′,6‴,2‴)], 6.86 [s, H−C(2‴)], 7.12 [m, *J* = 7.8 Hz, H−C(2′,6′)], 12.24 [brs, HO-C(5,5″)]. ^{13}C NMR (125 MHz, DMSO-d_6, HSQC, HMBC): δ 42.1, 42.8, 43.1 [C-3″], 47.1, 47.3, 47.4 [C-3], 78.3, 78.8 [C-2″], 81.2, 81.4, 81.8 [C-2], 94.9, 95.0, 95.5, 95.9, 96.0, 96.2 [C-8,6″,6], 100.2, 100.3, 101.0, 101.1, 101.2, 101.3, 101.5, 101.9 [C-4a″,8″,4a], 113.4, 113.6, 113.9, 114.6, 114.7, 115.3, 115.4, 116.3 [C-3′,5′,2‴,5‴], 117.3, 117.5 [C-6‴], 127.9, 128.1 [C-1′], 128.6, 129.0 [C-2′,6′], 129.6, 130.1 [C-1‴], 145.1, 145.2, 145.3, 145.6 [C-3‴,4‴], 157.5 157.6 [C-4′], 159.7, 160.4 [C-8a″], 162.1, 162.5, 162.6, 162.8 163.6 [C-8a,5″,5], 166.6 [C-7], 167.3 [C-7″], 194.7, 195.1 [C-4″], 196.9, 197.1 [C-4].

■ RESULTS AND DISCUSSION

The aqueous ethanolic extract of *Garcinia buchananii* was analyzed in a first antioxidative screening by means of ORAC and H_2O_2 assays and revealed an extraordinary high antioxidant value of 1359 μmol TE/100 mg.[1] Known natural product extracts such as bilberry, elderberry, red wine, and grape seed extract have high antioxidative activity (ORAC) values of 265, 222, 694, and 1189 μmol TE/100 mg,[5] respectively. Additionally, the TEAC assay with ABTS revealed a radical scavenging activity of 4.23 mmol TE/g of the aqueous ethanolic extract of *Garcinia buchananii*, whereas Gibis and Weiss[8] determined for bay berry, rosemary, and grape seed extract values of 0.3−0.4, 1.9, and 4.5 mmol TE/g, respectively. This shows again the enormous in vitro antioxidative power of the *Garcinia buchananii* stem bark extract.

To facilitate the isolation and structure elucidation of the in vitro antioxidative compounds, RP-18 MPLC was performed and afforded eight fractions (M1−M8). As fraction M2 and M6 were collected over a time range of 10 and of 5 min, respectively, these fractions were subdivided into M2a−h and M6a−d to speed up and alleviate the isolation process (Supporting Information).

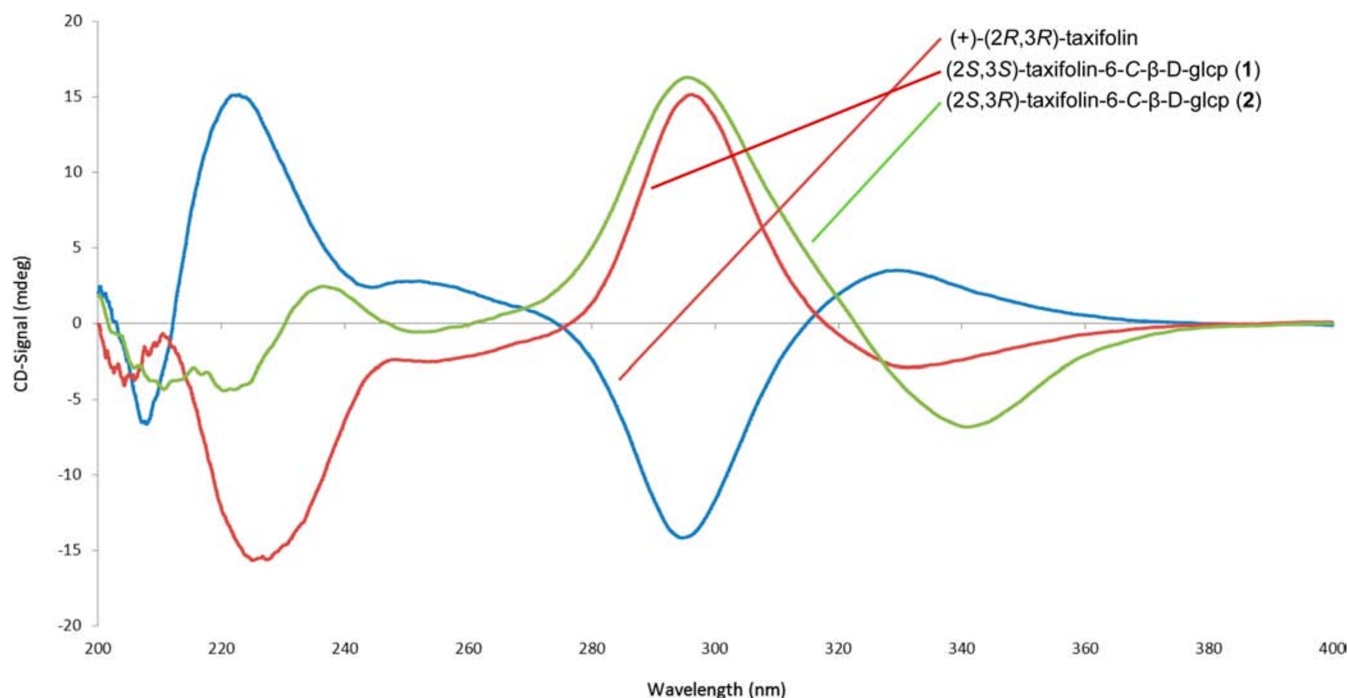

Figure 3. CD-spectra of (+)-(2*R*,3*R*)-taxifolin, (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**), and (2*S*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**2**).

The antioxidative compounds **1** and **2** isolated from fraction M2a were obtained as colorless amorphous powders with typical absorption maxima expected for flavanones. Results from electrospray ionization (ESI) MS indicated the same pseudomolecular $[M - H]^-$ ion with m/z 465, as well as a fragment ion with m/z 345, as expected for a *C*-glycoside. High resolution UPLC-ESI-TOF-MS analysis confirmed the target compound to have the molecular formula $C_{21}H_{22}O_{12}$ and the fingerprint fragment $C_{17}H_{14}O_{18}$, which are identical to the recently published (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside.[1] The ^{1}H and ^{13}C NMR data of **1** was in terms of chemical shifts and signal splitting nearly identical to (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside (Figure 2). Also, the intramolecular *C*-linkage of the *β*-D-glucopyranose could be clearly assigned to the C(6) position of the aglycone taxifolin. The coupling constants of protons H−C(2) and H−C(3) showed in both cases a value of ∼10.8 Hz, indicating the trans-diaxial or trans arrangement of the protons to each other. As the constitution of **1** and (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside is the same and the NMR data are nearly identical, the assumption of enantiomeric aglycones of taxifolin in both molecules arose, whereas both compounds are stereoisomers and are more precisely very similar diastereomers. The ^{1}H and ^{13}C NMR data of **2** showed in comparison to **1** and (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside (Figure 2) characteristic differences for, e.g., the downfield shifted protons H−C(2) and HO-(3) as well as the upfield shifted proton H-(3). Interestingly, the coupling constant of H−C(2) was 1.8 Hz indicating a cis arrangement of the protons H−C(2) and H−C(3). Again the intramolecular *C*-linkage of the *β*-D-glucopyranose in **2** was assigned to the C(6) position of the aglycone taxifolin, and therefore, **2** could be identified as a stereoisomer to **1** and (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside, more precisely a diastereomer as well as an epimer, respectively.

To clarify the absolute configuration of the carbon atoms C(2) and C(3) present in the aglycone taxifolin of compound **1** and **2**, circular dichroism (CD) spectroscopic measurements were performed using the commercially available reference isomer (+)-(2*R*,3*R*)-taxifolin as well as the isolated *C*-glycosides **1** and **2** (Figure 3). The CD spectra of (+)-(2*R*,3*R*)-taxifolin was well in line with literature data.[9−11] The data obtained clearly demonstrated that the spectrum of *C*-glycoside **1** isolated from fraction M2x was a mirror image to the spectrum of (+)-(2*R*,3*R*)-taxifolin and to the recently described (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside as well as (2*R*,3*R*)-aromadendrin-6-*C*-*β*-D-glucopyranoside;[1] therefore, the stereochemistry could be deduced as (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**). In 2009, Rawat et al.[12] first isolated (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**) from *Ulmus wallichiana* and named the compound Ulmosid A, which is not reported so far in any other plant material. NMR and CD data of (**1**) are in line with Ulmosid A.[12] Gaffield[13] showed that the Cotton effect due to the $\pi \rightarrow \pi^*$ transition near 290 nm is more reliable for the determination of the C-2 stereochemistry than the Cotton effect of the $n \rightarrow \pi^*$ transition at a longer wavelength 330−340 nm. *C*-Glycoside **2** (Figure 3) showed a negative Cotton effect at 341 nm and a positive Cotton effect at 295 nm, which is similar to Ulmosid A (**1**) and follows the Gaffield rule, and, therefore, indicates the 2*S* configuration. As the protons of C(2) and C(3) in **2** have a cis arrangement (see coupling constants), the absolute stereochemistry in **2** could be deduced as 2*S*,3*R*. It is interesting to note that the CD curve of **2** between 210 and 250 nm is influenced by the different oppositely signed electron transitions of the two chiral centers, but a slight positive Cotton effect at 237 nm follows the CD curve of (+)-(2*R*,3*R*)-taxifolin and supports the 3*R* configuration. Additional unequivocal evidence comes from the CD spectra of the four stereoisomers of taxifolin. (−)-(2*S*,3*R*)- and (+)-(2*R*,3*S*)-epitaxifolin (2,3-*cis*) showing a stronger Cotton effect at 330−340 nm compared to (+)-(2*R*,3*R*)- and (−)-(2*S*,3*S*) taxifolin (2,3-*trans*).[11] Consequently, the two isolated compounds from fraction M2x could be unequivocally identified by means of HR-MS, 1/2D-NMR, and CD spectroscopy as well as literature study as (2*S*,3*S*)-taxifolin-6-

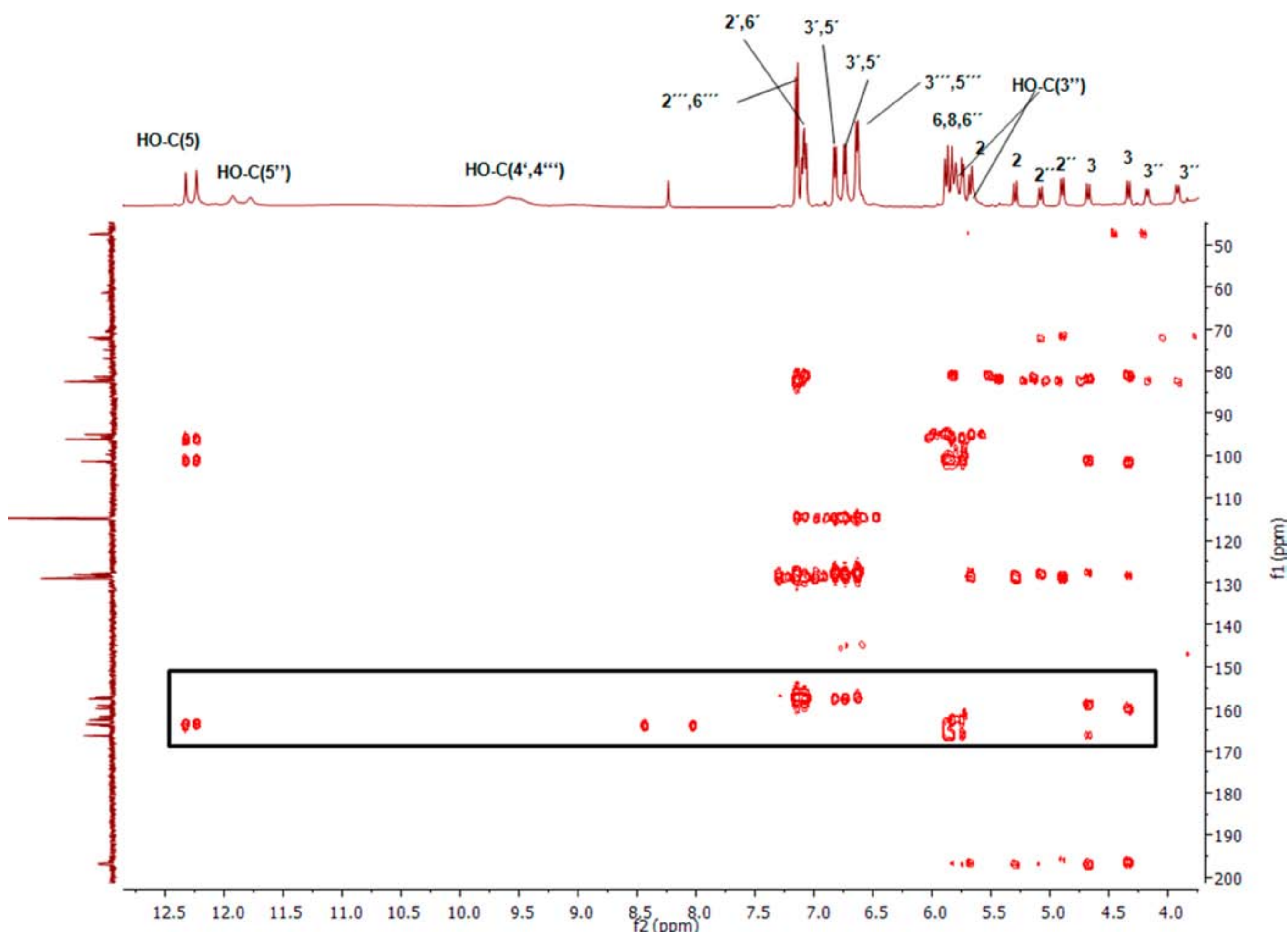

Figure 4. HMBC spectrum (500 MHz, d_6-DMSO) of (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**).

C-*β*-D-glucopyranoside (**1**), known as Ulmoside A, and a new compound (2*S*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**2**).

The antioxidative compounds **3**−**7** isolated from fractions M3 and M6a−c showed the typical absorption maxima expected for biflavanones, and high resolution UPLC-ESI-TOF-MS analysis confirmed the target compound to have the molecular formula $C_{30}H_{22}O_{13}$ for **3**, $C_{30}H_{20}O_{13}$ for **4**, and $C_{30}H_{22}O_{11}$ for **5**−**7**, respectively. Compound **3** was detected in traces in the HPLC separation of fraction M3 with a peak area of about 1/50 next to the recently published (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone.[1] UPLC-ESI-TOF-MS^e experiments of (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone (Supporting Information, Figure 2A) compared to **3** (Supporting Information Figure 2B) revealed characteristic fragment ions of *m*/*z* 463, 435, 419, 285, 151, and 125 each, which are in line with the literature[14−18] for GB dimers and manniflavanone and as indicated for **3**, a so far unknown stereoisomer of (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone.[1] Unequivocal assignment of all carbon atoms and the hydrogen substituted carbon atoms, respectively, was successfully achieved by means of 1- and 2-D-NMR spectroscopy. The close similarity of the NMR data to manniflavanone[1] and the fingerprint correlation between the proton H−C(3) resonating at 4.30 and 4.66 ppm and neighboring carbon atom C(8a″) and C(7″), as well as the lack of correlation to C(5″), demonstrated clearly the intramolecular C-3/C-8″-linkage of the two flavanone monomers. Further, the vicinal coupling constants of 11.2−12.2 Hz of the two sets of aliphatic protons H−C(3,3″) and H−C(2,2″) showed the trans-diaxial relative configuration, and therefore, **3** could be proven as a diastereomer of (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone.

Compounds **4**−**7** were isolated from fractions M6a−c. UPLC-ESI-TOF-MS^e experiments of **4**−**7** compared to manniflavanone isomers (Supporting Information, Figure 2A,B) revealed for compound **4** (Supporting Information, Figure 2C) fingerprint fragment ions of *m*/*z* 461 (−2 Da), 433 (−2 Da), and 311 (−1 Da), which indicated an additional double bond in the heterocycle of I−C or II−C and correlates with the elemental composition $C_{30}H_{20}O_{13}$ (−2 Da). Compounds **5** and **6** showed identical mass spectra (Supporting Information, Figure 2E,F), and the key fragments *m*/*z* 403 and 431 exhibited a difference of −32 Da in comparison to manniflavanone isomers, which correlates with the elemental composition $C_{30}H_{22}O_{11}$ and with less than two atoms of oxygen. Also, the fragments *m*/*z* 269 and 296 are identical to GB-2 (Supporting Information, Figure 2D) and are less than 16 Da in comparison to manniflavanone isomers and fit with the general biflavanone structures consisting of flavanone and 3-hydroxyflavanone units differing only in the substitution pattern of the rings I−B and II−B. At this point, compounds **5** and **6** were proposed as GB-1[14−17] isomers. Compound **7** showed the same elemental composition as GB-1 isomers **5**−**6**, but the fragment ion spectrum differed significantly (Supporting Information, Figure 2G). Similarity

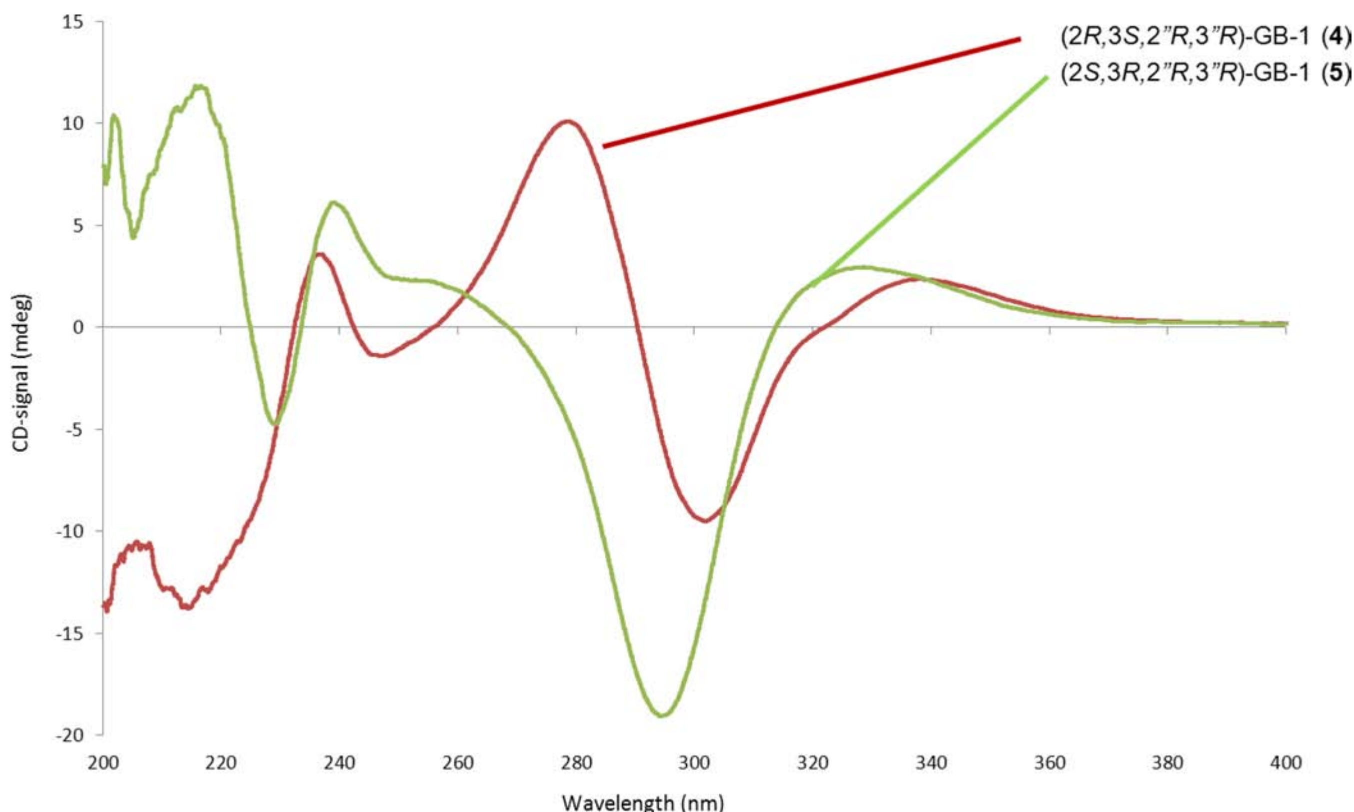

Figure 5. CD-spectra of (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**) and (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**).

could be observed to buchananiflavanone (Supporting Information, Figure 2H), whereas the key fragments of *m*/*z* 431 and especially 295 differ by −16 Da and therefore by one atom oxygen. As a consequence, compound **7** could be proposed as a buchananiflavanone derivative having one oxygen less at ring I−B or II−B. As described above and exemplified in Figures 4 and 1, 1- and 2-D-NMR spectroscopy of compounds **4**−**7** confirmed the intramolecular C-3/C-8″-linkage of the two corresponding monomers, respectively. Diagnostic NMR signals for compound **4** in comparison to manniflavananone isomers were the missing signals for H−C(2″ and 3″), and again, the vicinal coupling constants of ∼12 Hz of the two sets of aliphatic protons H−C(3″) and H−C(2″) showed the trans-diaxial relative configuration. Therefore, the constitution of **4** was identified as a eridictyol-3,8″-quercetin biflavanone or 2″,3″-dehydromanniflavanone, which has not been described in the literature so far. The NMR data of **4** is very similar to pancibiflavonol, a naringenin-3,8″-quercetin biflavanone, which was isolated first in 1999 from Ito et al. from *Calophyllum panciflorum*.[19]

NMR data of the GB-1 isomer **5** (Figure 4) is in line with the first complete NMR assignment of Han et al. in 2005,[20] and again, the trans-diaxial relative configuration was deduced. Extraordinary and eye-catching were the NMR data of GB-1 isomer **6** because no signal doubling was observable at 18 °C, and therefore, the assumption of a stereoisomer of GB-1 arose, which shows no steric hindrance. Further evidence for a diastereomer of GB-1 was found in the chemical shifts of the protons H−C(2″and 3″), which was inverse to all so far described 3−8″ linked biflavanoids[1,9,14−21] and absolutely in line with the observation of Ferrari et al.,[10] which described these observations for the two diastereomers (2*R*,3*S*,2″*R*,3″*R*) and (2*S*,3*R*,2″*R*,3″*R*) of GB-4. Because of their different magnetic-anisotropy effects of their three-dimensional environment, the order of the protons H−C(2″and 3″) was inverse, and also the trans-diaxial relative configuration was deduced from the coupling constants of 11−12 Hz. Compound **7** showed NMR signals very similar to those of GB-2[1,21] as well as to the recently published buchananiflavanone,[1] and therefore, compound **7** was identified as a GB-2a[14−17] isomer.

To clarify the configuration of the carbon atoms C(2) and C(3) in compounds **3**−**7**, CD spectroscopic measurements were performed with compounds **3**−**7**. Since the CD spectrum of GB-1 (**5**) (Figure 5) and GB-2a (**7**) was identical to (2*R*,3*S*,2″*R*,3″*R*)-GB-2,[1,10] (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone,[1] and (2*R*,3*S*,2″*R*,3″*R*)-GB-4,[10] the absolute configurations must be the same, and thus, the absolute configurations of **5** and **7** could be deduced as (2*R*,3*S*,2″*R*,3″*R*)-GB-1 and (2*R*,3*S*,2″*R*,3″*R*)-GB-2a (**7**) (Figure 1). Since both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the 3-hydroxyflavanone units were the same in (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**) and the GB-1 (**6**) isomer (Figure 5), their 3-hydroxyflavanone units had the same configuration of (2″*R*,3″*R*). As both GB-1 isomers showed the trans-diaxial relative configuration, and the absolute stereochemistry of (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**) is already deduced, **6** as chromatographically separated from **5** must be the only remaining possible diastereomer and consequently (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**). This fits perfectly with the inverted $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the flavone unit in **6** compared to **5**, e.g., the enhanced intensity of $\pi \rightarrow \pi^*$ transition at 294 nm situated between that of 3-hydroxyflavanone and flavanone transitions of (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**) and again confirmed its absolute configuration as (2*S*,3*R*). These observations are in line with the investigations of Ferrari et al.,[10] who determined the stereochemistry of (2*R*,3*S*,2″*R*,3″*R*)-GB-4 and (2*S*,3*R*,2″*R*,3″*R*)-GB-4. Note, that it is puzzling and yields to

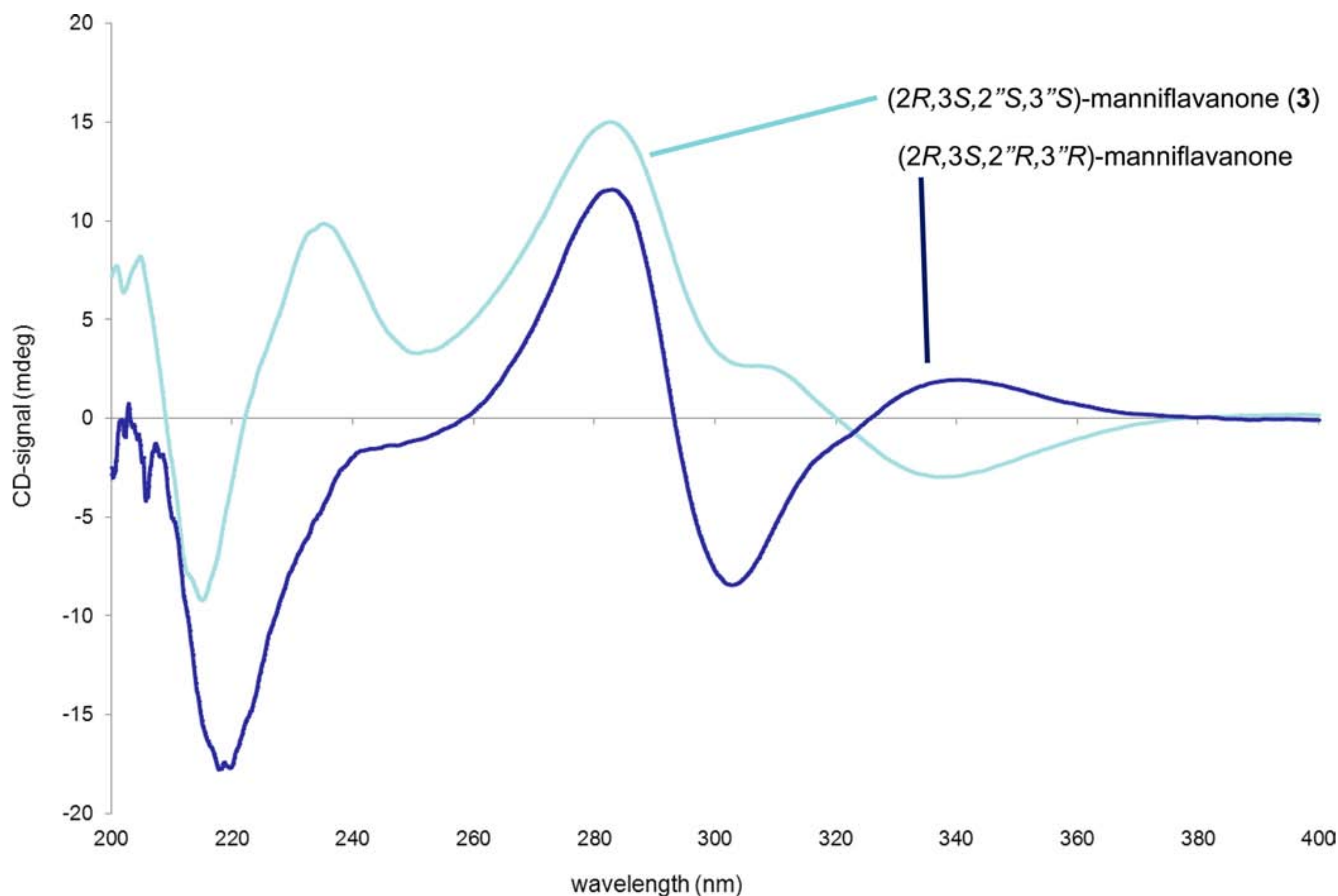

Figure 6. CD-spectra of (2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**) and (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone.

considerable confusion in the literature regarding interpretation of the CD data of biflavanoids or biflavonoids possessing one or both stereogenic units[9,10,20−22] that absolute configuration will change when going from flavanone (e.g., 2*S*-naringenin) to (2*R*, 3*R*)-taxifolin, although both show the same Cotton effects for $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions.[13] To the best of our knowledge, only the structure of (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**) is proven in the literature. As the stereochemistry for some GB dimers was revised in 2003 by Ferrari et al.,[10] in this case from (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**) to (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**), so far there was no NMR and CD evidence for (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**). All molecules in the literature described so far were the same, namely, (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**). Consequently, (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**) is a new molecule, and its structure based on the spectroscopic data described here is unique.

The new compound 2″,3″-dehydromanniflavanone (**4**) showed a positive Cotton effect at 290 nm and a negative Cotton effect at 324 nm, which indicates the 2*R* configuration. As the protons of C(2) and C(3) in **4** have a cis arrangement, the absolute stereochemistry in **4** could be deduced as 2*R*,3*S*. Also, **4** showed a CD spectrum identical to that described for (2*R*, 3*S*)-morelloflavon,[9,22] and therefore the stereochemistry was confirmed as (2*R*, 3*S*)-2″,3″-dehydromanniflavanone (**4**), which we have named (2*R*,3*S*)-buchananiflavonol.

The CD spectrum of **3** (Figure 6) revealed inverted $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the 3-hydroxyflavanone unit compared to (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone (Figure 6), and so, the stereochemistry 2″*S*,3″*S* of the 3-hydroxyflavanone in **3** could be deduced. As both manniflavanone isomers showed the trans-diaxial relative configuration, and the absolute stereochemistry of (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone is already determined, **3** as chromatographically separated from (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone must be the only remaining possible diastereomer and is consequently (2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**). From a theoretical point of view, the 2*S*,3*R*,2″*S*,3″*S* configuration for **3** should be possible, but if this would be the case, then **3** should be the enantiomer of (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone and consequently chromatographically not separated and be the CD mirror image of it. However, this obviously not the case (Figure 6) and fits perfectly with the whole CD spectra of **3** compared to (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone. Both molecules show the same positive Cotton effect for the flavanone unit at 283 nm, and consequently, to the best of our knowledge (2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**) as a new stereoisomer of known (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone[1,18] could be described for the first time.

Antioxidative Activity of Isolated Compounds 1−7. Very recently, we demonstrated strong in vitro antioxidative power (ORAC and H_2O_2 assay) for (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside, (2*R*,3*R*)-aromadendrin-6-*C*-*β*-D-glucopyranoside, (2*R*,3*S*,2″*S*)-buchananiflavanone, and especially for (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone and (2*R*,3*S*,2″*R*,3″*R*)-GB-2 by comparing the activity with known antioxidative reference compounds like (+)-taxifolin, quercetin, rutin, (−)-epicatechin, ascorbic acid, and (±)-naringenin and available and comparable literature data.[1,5,23] We completed these data now with a third antioxidative assay, namely, TEAC with the ABTS assay, for all of these compounds (Table 1). The extraordinary antioxidative

Table 1. Antioxidant Activities of Isolated Compounds 1, 3−7, and Reference Compounds

	H_2O_2 assay[a,b] EC_{50} (μM)	ORAC assay[c,d] (μmol TE/μmol)	ABTS assay[c,d] (mmol TE/mmol)	literature[e,f,h]
(2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside	11.0 (9.0−11.4)[g]	9.57 (±0.50)[g]	1.55 (±0.04)	
(2*R*,3*R*)-aromadendrin-6-*C*-*β*-D-glucopyranoside	10.9 (9.5−12.9)[g]	4.23 (±0.08)[g]	0.83 (±0.10)	
(2*R*,3*S*,2″*R*,3″*R*)-manniflavanone	2.8 (2.4−3.2)[g]	13.73 (±0.43)[g]	5.58 (±0.31)	
(2*R*,3*S*,2″*R*,3″*R*)-GB-2	2.2 (1.9−2.6)[g]	12.10 (±0.26)[g]	2.38 (±0.26)	
(2*R*,3*S*,2″*S*)-buchananiflavanone	14.4 (12.8−16.5)[g]	10.50 (±0.43)[g]	5.16 (±0.26)	
Ulmosid A, (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**)	6.2 (5.4−9.4)	10.14 (±0.26)	1.96 (±0.03)	n.r.[h]
(2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**)	3.0 (2.1−6.1)	10.30 (±0.28)	4.00 (±0.09)	n.r.[h]
(2*R*,3*S*)-buchananiflavonol (**4**)	3.7 (3.6−5.7)	7.20 (±0.07)	3.61 (±0.27)	n.r.[h]
(2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**)	10.9 (3.9−12.9)	8.34 (±0.16)	1.84 (±0.07)	n.r.[h]
(2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**)	7.8 (3.9−10.2)	7.46 (±0.06)	1.84 (±0.08)	n.r.[h]
(2*R*,3*S*,2″*S*)-GB-2a (**7**)	5.1 (4.4−7.0)	5.50 (±0.33)	1.59 (±0.08)	n.r.[h]
(+)-taxifolin	11.3 (9.7−13.2)[g]	7.63 (±0.68)[g]	2.18 (±0.05)	9.74[f]
ascorbic acid	16.5 (15.0−18.3)[g]	0.34 (±0.10)[g]	0.93 (±0.06)	0.95[e]
rutin	6.9 (5.9−8.0)[g]	6.45 (±0.28)[g]	3.16 (±0.01)	6.01[e]; 13.70[f]
quercetin	6.1 (5.3−7.1)[g]	5.61 (±0.07)[g]	4.44 (±0.15)	7.28[e]; 8.04[f]
(−)-epicatechin	4.1 (3.7−4.6)[g]	9.65 (±0.53)[g]	3.17 (±0.10)	9.14[f]
(±)-naringenin	8.6 (6.8−11.9)[g]	3.96 (±0.19)[g]	1.12 (±0.07)	9.23[f]

[a]Each sample was analyzed by means of the H_2O_2 assay by triplicate studies. [b]The range in parentheses represents 95% confidence interval. [c]Each sample was analyzed by means of the ORAC and ABTS assays by quadruplicate studies. [d]The numerical value in parentheses represents SD. [e]Values from Ou et al. [f]Values from Wolfe and Liu in which the stereochemistry of naringenin and taxifolin is not stated. [g]Values from Stark et al. [h]n.r. not reported.

capacity of these natural products was again confirmed, whereas (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone and (2*R*,3*S*,2″*S*)-buchananiflavanone showed the strongest activities of 5.58 and 5.16 mmol TE/mmol, respectively.

Compounds **1** and **3−7** were analyzed by means of ORAC, TEAC, and hydrogen peroxide scavenging assays (Table 1). In comparison to known very antioxidative single compounds, compounds **1** and **3−7** generally revealed again relative high activity. By far the highest activity in all three assays was observed for the new compound (3*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**), which showed outstanding activity in comparison to that of quercetin, rutin, (−)-epicatechin, ascorbic acid, and (±)-naringenin as well as available literature data.[5,23] Also, all EC_{50} values of the hydrogen peroxide scavenging activity of all isolated compounds are lower than those of ascorbic acid.

Structure−activity investigations revealed that the manniflavanone isomers showed in all three assays the highest activity, which is generally explainable with the highest amount of hydroxyl functions and also with two sets of vicinal ones. (2*R*,3*S*,2″*S*)-buchananiflavanone and (2*R*,3*S*,2″*R*,3″*R*)-GB-2 followed by one less hydroxyl function compared to the manniflavanone isomers, but the constitution of these two molecules is different. The position of the corresponding OH function plays an important role, yielding strongly different activities in TEAC and H_2O_2 assays for these two molecules (Table 1). (2*R*,3*S*,2″*S*)-GB-2a compared to (2*R*,3*S*,2″*R*,3″*R*)-GB-2 showed only half the activity in all three assays and has only less one oxygen atom at position 3″. A comparison of manniflavanone, GB-2, and GB-1 (**5**) having all the same stereochemistry of (2*R*,3*S*,2″*R*,3″*R*) reveals a decrease in activity, which comes along with an decreasing amount of vicinal hydroxyl functions at the B-ring. Evidence for the influence of stereochemistry on antioxidative activity is given by comparing GB-1 (**5** and **6**) and manniflavanone isomers as well as (2*R*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside and (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**). Here, it has to be mentioned that the change in stereochemistry revealed differences in activities, but the change in stereochemistry itself for all molecules was not always the same. As a consequence, the stereochemistry itself plays an important role in the activity, but it seems that the number and position of hydroxyl functions play a more important role.

In conclusion, on the basis of UPLC-HR-ESI-TOF-MS, 1/2-D-NMR, as well as CD spectroscopy (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**) (2*S*,3*R*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**2**) (2*R*,3*S*,2″*S*,3″*S*)-manniflavanone (**3**), (2*R*,3*S*)-buchananiflavonol (**4**), (2*R*,3*S*,2″*R*,3″*R*)-GB-1 (**5**), (2*S*,3*R*,2″*R*,3″*R*)-GB-1 (**6**), and (2*R*,3*S*,2″*S*)-GB2a (**7**) were isolated from *Garcinia buchananii* and their chemical structures identified. Compounds **2−4** and **6** are new compounds, and (2*S*,3*S*)-taxifolin-6-*C*-*β*-D-glucopyranoside (**1**) was so far only described in *Ulmus wallichiana*.[12] For compound **7**, the stereochemistry was described and proven. Compounds **1−7** revealed very high activity in comparison to known very antioxidative single compounds in ORAC, TEAC, and hydrogen peroxide scavenging assays. These findings confirmed our previous data that *G. buchananii* bark extract is a rich natural source of antioxidants with potential to be utilized as food supplements in the future.

■ ASSOCIATED CONTENT

Supporting Information

Chromatogram of the MPLC separation of the aqueous ethanolic bark extract; MS^e spectra of compounds **3−7**, (2*R*,3*S*,2″*R*,3″*R*)-manniflavanone, and (2*R*,3*S*,2″*S*)-buchananiflavanone as well as instrument parameters for the HR-UPLC-ESI_{neg}-TOF-MS^e analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +49-8161-71-2911. Fax: +49-8161-71-2949. E-mail: timo.stark@tum.de.

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Notes

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