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Benzophenone glycosides from *Gnidia involucrata*

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Abstract

Six compounds have been isolated from the methanol extract of the aerial parts of *Gnidia involucrata* (Thymelaeaceae). They were identified as 2,3,4′,5,6-pentahydroxybenzophenone-4-*C*-glucoside and 2,4′,6-trihydroxy-4-methoxybenzophenone-2-*O*-glucoside, together with mangiferin, kaempferol-3-*O*-glucoside, yuankanin and manniflavanone by chemical and spectroscopic means. The structures of three additional *C*-glycosyl flavones — vitexin, isovitexin and isoorientin — were determined on-line by LC/UV/APCI-MS^n analysis of the crude extract.

Keywords: *Gnidia involucrata*; Thymelaeaceae; Benzophenone glycosides; Biflavanone; Yuankanin; Mangiferin; Manniflavanone; Flavonoid *C*-glycosides

1. Introduction

The Thymelaeaceae represent a very heterogeneous and widespread taxon of about fifty genera, found mainly in Africa (Evans, 1996). Most of the studies on the representatives of this family concern the lipophilic extracts, rich in irritant diterpenes (Evans and Soper, 1978; Evans and Taylor, 1982). As no phytochemical study has yet been reported on *Gnidia involucrata* Steud. ex A. Rich., the investigation of the methanol extract of its aerial parts was undertaken. The powdered roots of this herbaceous plant and also other Thymelaeaceae species are used in African traditional medicine, especially in Zimbabwe, to reduce the size of the vaginal orifice (Gelfand et al., 1985). In Ethiopia, the same drug is used internally for its laxative and vermifuge properties (Borris et al., 1988). The roots of another species, *Gnidia kraussiana* Meissn., are used as constituents of several hunting and fishing poisons in countries like Nigeria, Congo, Zimbabwe, Malawi and Sudan (Neuwinger, 1994).

2. Results and discussion

The dried aerial parts of *G. involucrata* were extracted at room temperature with solvents of increasing polarity (dichloromethane and methanol). Due to the high tannin content of the plant material, the polar extract was treated with hide powder, in order to avoid any interference during further separation procedures. The abundance of these astringent polyphenols in *G. involucrata* may explain the traditional use of the plant in cases of dilated vagina.

The tannin-free methanol extract was fractionated by a combination of silica gel column chromatography, RP-18 low pressure LC (LPLC), RP-18 medium pressure LC (MPLC) and gel filtration on Sephadex LH-20 to afford compounds **1–6** (see Section 3).

The molecular formula $C_{19}H_{20}O_{11}$ of compound **1** was established on the basis of atmospheric pressure chemical ionization mass spectrometry (APCI-MS), de-

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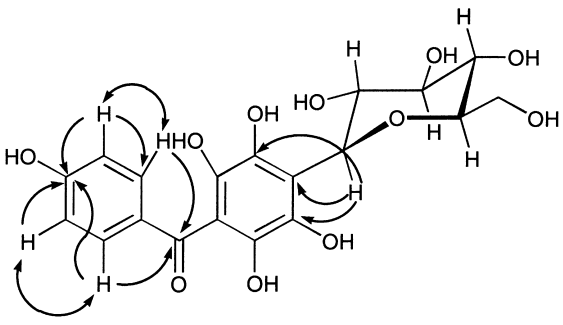
sorption/chemical ionization-MS (D/CI-MS) and electron impact-MS (EI-MS) data, together with ^{1}H and ^{13}C NMR spectra. In addition, the UV spectrum suggested a polyphenolic structure. The D/CI-MS and APCI-MS spectra in the positive ion mode (P.I.) displayed an ion at m/z 408 ($[M + H - 17]^+$) and other fragment ions at m/z 318 ($[M + H - 17 - 90]^+$) and 288 ($[M + H - 17 - 120]^+$), corresponding to the typical fragmentation of *C*-glucosides (Rath et al., 1995). According to the molecular formula deduced from NMR data, the molecular weight (MW) of **1** was expected to be 424 Da. As shown, the D/CI-MS and APCI-MS provided only a dehydrated molecular ion at m/z 408. Furthermore, the EI-MS spectrum presented a peak at m/z 390 corresponding to the loss of two hydroxyl substituents. In order to determine the correct MW for **1**, this compound was peracetylated. The APCI-MS (P.I.) of the nona-acetylated compound **1 (1a)**, showed a protonated molecular ion at m/z 803 ($[M + H]^+$), confirming thus a molecular mass of 424 Da for compound **1**. Two additional doublets at δ 7.60 ($J = 8.8$ Hz) and δ 6.77 ($J = 8.8$ Hz), each integrating for two protons, revealed the presence of a symmetric phenolic derivative with two equivalent pairs of *ortho*-coupled protons, suggesting a second substitution in *para*. The ^{13}C NMR spectrum displayed a signal at δ 198.91, a chemical shift attributable to the carbonyl group of a benzophenone (Tanaka et al., 1984). Signals corresponding to 12 aromatic carbons were also detected, some of them being superimposed (δ 162.78, 2C; δ 160.77, 2 C; δ 132.83, 2 C; δ 115.43, 2 C). These data were in good correlation with the hypothesis of a benzophenone derivative, with a second fully substituted aromatic ring. Multiplicities were revealed by a DEPT experiment and complete attribution was performed by gradient HSQC, gradient HMBC and gradient COSY experiments (Fig. 1). The ^{1}H NMR showed a signal at δ 4.86 ($J = 8.0$ Hz), indicative of the C-1 proton of a β-D-glucosyl moiety (Agrawal, 1992). This proton was attributed using an HSQC experiment to a carbon at δ 76.48, a shift found in *C*-glucosides (Markham, 1982). A clear HMBC correlation between the anomeric proton of this β-D-glucose and C-4 in the benzophenone moiety confirmed the linkage position. Finally, compound **1** was identified as 2,3,4′,5,6-pentahydroxybenzophenone-4-*C*-glucoside. This benzophenone is a new natural product and the first compound of this type to be reported in Thymelaeaceae. It is also a structural isomer of 3-*C*-glucosylmaclurin, the biosynthetic precursor of mangiferin (Franz and Grün, 1983).

The molecular formula $C_{20}H_{22}O_{10}$ of compound **2** was determined on the basis of D/CI-MS and EI-MS data together with ^{1}H and ^{13}C NMR spectra. Its UV spectrum was similar to that of **1**, suggesting again a benzophenone structure. The D/CI-MS (P.I.) showed ions at m/z 440 ($[M + NH_4]^+$) and 423 ($[M + H]^+$) and a fragment ion at m/z 261 ($[M + H - 162]^+$), characteristic of the loss of a *O*-hexosyl unit. Moreover, the EI-MS analysis confirmed the loss of this sugar moiety with a peak at m/z 260 ($[M - 162]^{+\cdot}$). Deshielded signals appeared at δ 7.69 (2H, $J = 8.8$ Hz) and 6.78 (2H, $J = 8.8$ Hz), showing the presence of the same *para*-substituted symmetric phenol ring as in compound **1**. The aromatic protons of the other ring displayed two doublets at δ 6.39 ($J = 2.0$ Hz) and 6.17 ($J = 2.1$ Hz), characteristic for a *meta* coupling. ^{1}H and ^{13}C NMR suggested the presence of a methoxyl group (^{1}H: δ 3.79; ^{13}C: δ 55.24). Its position was assigned in C-4 thanks to an HMBC correlation exper-



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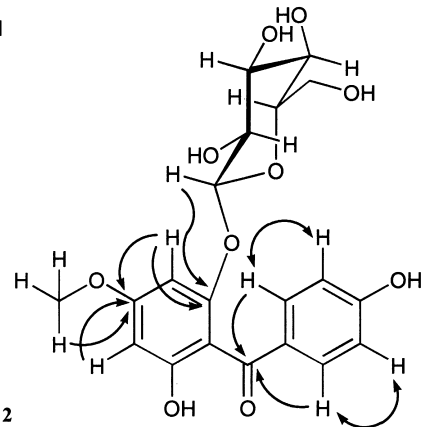


2

Fig. 1. Selected ^{1}H–^{13}C long range correlations (gradient HMBC; →) and ^{1}H–^{1}H COSY (↔) of compounds **1** and **2**.

iment. A signal at δ 196.34, typical for the carbonyl group of a benzophenone, and 12 other signals of aromatic carbons, two pairs being superimposed, confirmed the structure hypothesis. Multiplicities were revealed by a DEPT experiment. Gradient HSQC, HMBC and COSY experiments (Fig. 1), allowed complete assignment and identification of the aglycone of **2** as 2,4′,6-trihydroxy-4-methoxybenzophenone. The presence of an *O*-β-D-glucose was confirmed by the characteristic ^{1}H signal for the anomeric proton at δ 4.86 ($J = 7.8$ Hz). Its linkage position at C-2 was ascertained by HMBC. Thus, compound **2** is 2,4′,6-trihydroxy-4-methoxybenzophenone-2-*O*-glucoside, a new natural product.

Compound **4** exhibited UV, ^{1}H NMR, ^{13}C NMR and APCI-MS data corresponding to a 3′, 3″, 3‴, 4′, 4‴, 5, 5″, 7, 7″-nonahydroxy-biflavanone (Duddeck et al., 1978; Crichton and Waterman, 1979). In order to find out whether the linkage between the monomers occurred through C-3/C-6″ or C-3/C-8″, methylation of **4** was undertaken, as described by Crichton and Waterman (1979), followed by 1D (^{13}C) and 2D (gradient HSQC) NMR experiments. The absence of a low field signal for the OMe (δ 59–61) indicated that these methoxyl groups were not *ortho*-disubstituted and therefore that position C-6″ was not involved in an attachment with a flavanone moiety. Consequently, the linkage of the two flavanone parts is 3/8″ and compound **4** was identified, by comparison with the spectroscopic data, as manniflavanone, a biflavanoid previously found in the stem bark of *Garcinia mannii* Oliv. (Guttiferae) (Crichton and Waterman, 1979). Iwu et al. (1990) also described a diastereoisomer of manniflavanone on positions 2 and 3, garciniflavanone, which is easy to distinguish as it exhibits a supplementary maximum at 250 nm in the UV spectrum. Biflavonoids have a very sporadic distribution among the Angiospermae, while they are very common in the Gymnospermae, and have already been reported in two other Thymelaeaceae species: *Stellera chamaejasme* Linn. (Niwa et al., 1986) and *Wikstroemia sikokiana* Franch. & Sav. (Niwa et al., 1986; Baba et al., 1994).

Compounds **5** and **6** were identified by comparison of their spectral data (UV, ^{1}H and ^{13}C NMR, EI-MS and D/CI-MS) with literature values as kaempferol-3-*O*-glucoside (**5**; Markham, 1982; Markham and Chari, 1982) and yuankanin (genkwanin-5-*O*-xylosylglucoside; **6**; Núnez-Alarcón et al., 1973). The latter is a relatively rare flavone 5-*O*-glycoside which has only been isolated from some species belonging to the Thymelaeaceae, including *G. kraussiana*, and exhibits an activity against cells with fragile membranes, such as isolated lymphoblasts and human leukaemic cells (Ragot et al., 1988).

The spectral data of compound **3** (UV, ^{1}H and ^{13}C NMR, EI-MS and D/CI-MS) clearly indicated the presence of mangiferin (Miura et al., 1978). This common constituent is reported for the first time in the Thymelaeaceae. The presence of this xanthone *C*-glycoside is interesting because it occurs together with **1** and **2** in *G. involucrata* and it has been shown that benzophenones are biosynthetic precursors of xanthones (Atkinson et al., 1968). In order to search for the presence of other xanthones, a LC/UV/APCI-MS analysis of the crude extract was carried out. No xanthone other than mangiferin was found. According to previous papers (Gupta and Lewis, 1971), the biosynthesis of xanthones derives generally from shikimate-acetate to give an intermediate benzophenone, which finally yields the dibenzo-γ-pyrone ring by oxydative coupling.

In the case of mangiferin, the postulated benzophenone intermediate might be formed by condensation of *p*-coumarate with two malonates (Franz and Grün, 1983). This biosynthetic pathway is also common to the flavonoids (Swain, 1975) and indeed, often mangiferin co-occurs with related *C*-glucosyl flavonoids (Hostettmann and Wagner, 1977). In order to check if this was the case for *G. involucrata*, a HPLC/UV/APCI-MS (P.I.) analysis of the methanol extract was undertaken. A broad acetonitile–water gradient revealed peaks at *m/z* 449 and 433, which showed characteristic UV spectra of flavonoids. Losses of 120 Da in the LC/MS spectra were indicative for *C*-glycosides (Rath et al., 1995). Based on this preliminary analysis, these peaks could be related to the ubiquitous compounds orientin, vitexin and/or their isomers. In order to definitely characterize these compounds on-line, an isocratic elution was performed for an additional LC/APCI-MS^n (P.I.) experiment. In multiple stage tandem mass spectrometry (MS^n), the molecular ions of interest are isolated in an ion trap mass analyzer, selectively excited and their fragments analyzed. This MS^2 step can be repeated on fragments, generating multiple stage MS^n spectra. Under these conditions, it has been shown that clear distinction could be made between isomeric pairs of *C*-glycosyl flavones (Rath et al., 1995; Wolfender et al., 1999). On the single ion traces of this isocratic LC/MS analysis (Fig. 2), one peak (**7**) with a protonated molecular ion at *m/z* 449 and two peaks (**8, 9**) with $[M + H]^+$ ions at *m/z* 433 were recorded at 17, 31 and 33 min, respectively. As described previously (Wolfender et al., 1999), the LC/APCI-MS of the $[M + H]^+$ ions of the isomers **8** and **9** were similar, showing characteristic losses of 90 and 120 Da. However, MS^2 analyses on the $[M + H - 120]^+$ ions of these two isomers revealed clear differences. The MS^2 of **9** presented ions at *m/z* 295, 284, 283 and 267 characteristic of losses of 18 ($-H_2O$), 29 ($-CHO$), 30 ($-CHO$, $-H$) and 46 ($-CO$, $-H_2O$) Da,

while the MS^2 of **8** only showed losses of 29 and 30 Da (Fig. 2). In comparison to results already obtained on known standards, the MS^2 spectrum of **8** was characteristic for a *C*-glycoside flavone substituted in position 8, while the MS^2 spectrum of **9** corresponded to its isomer substituted in position 6. Therefore, **8** and **9** were unambiguously identified as vitexin and isovitexin respectively. The flavonoid having an $[M + H]^+$ ion at *m/z* 449 (**7**), displayed an LC/MS^2 of its respective $[M + H - 120]^+$ ion presenting the same losses as for **9** and was identified as isoorientin. No trace of orientin was observed. The peak at 27 min (Fig. 2) displayed also an ion at *m/z* 433, but its $[M + H]^+$ ion was at *m/z* 451. In this case, the ion at *m/z* 433 is most probably issued from a loss of H_2O. A protonated aglycone ion at *m/z* 289 ($[M + H - 162]^+$), together with the UV data, suggested a flavonoid *O*-glycoside structure for this peak. Based on these on-line data only, it was not possible to definitely identify this compound.

A deeper LC/MS investigation of the crude extract of *G. involucrata* is currently underway and studies to determine the chemotaxonomical relationships between several species of *Gnidia* and other Thymelaeaceae, using LC/UV/APCI-MS, will be performed.

3. Experimental

3.1. General

Melting point: Mettler FP 80/82 hot stage apparatus (uncorr.). UV: Perkin Elmer Lambda 20 spectrophotometer, spectra recorded in MeOH. $[\alpha]_D$: Perkin

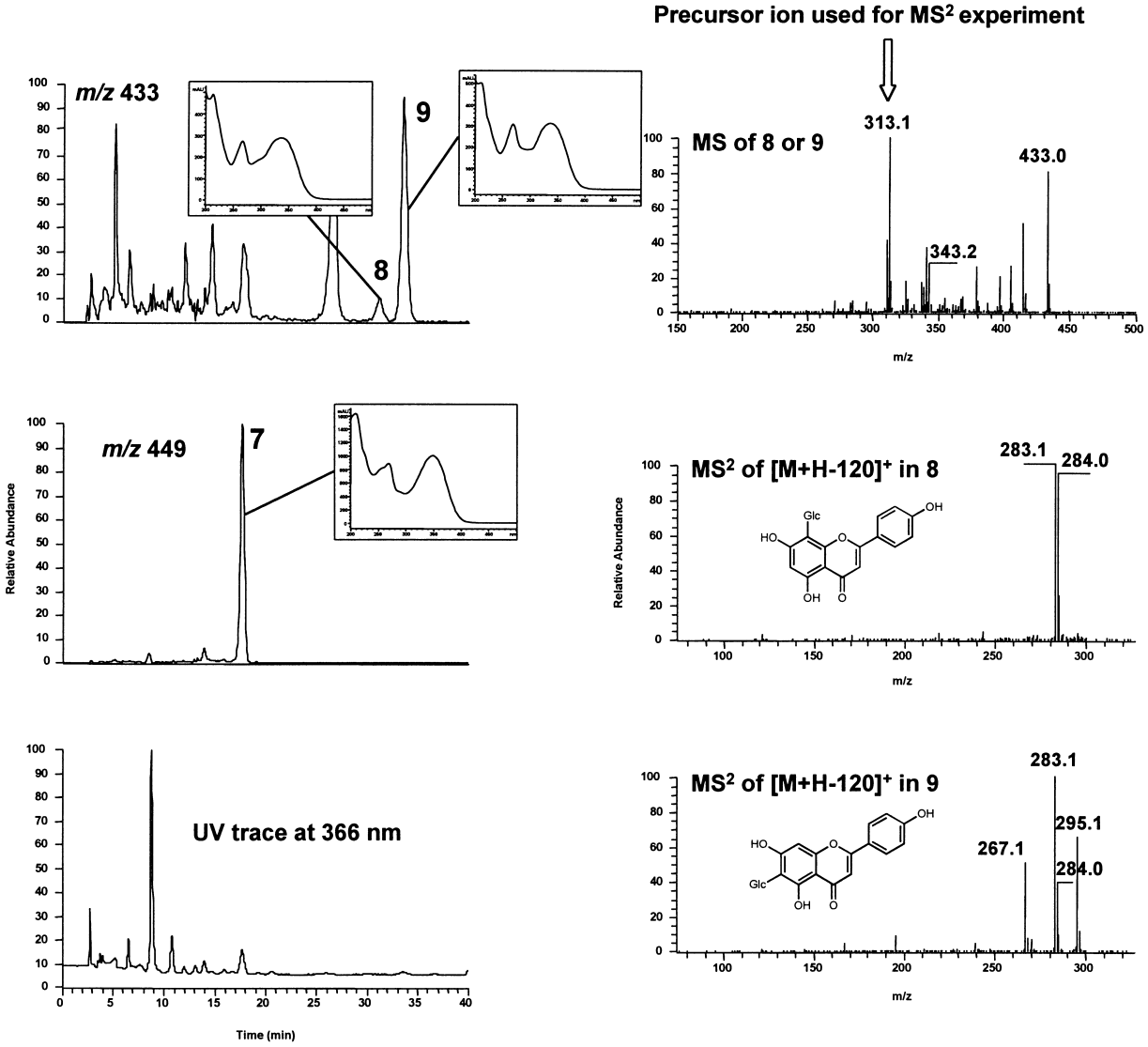
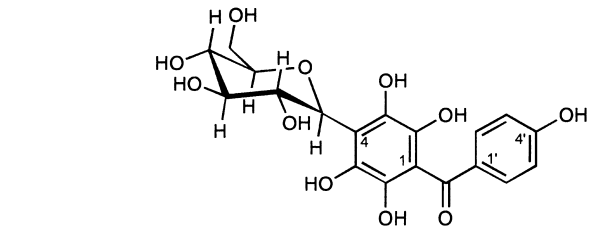


Fig. 2. LC/UV/APCI-MS^n (P.I.) analysis of the aerial parts of *Gnidia involucrata*, methanol extract. (For chromatographic/spectrometric protocols, see Section 3.)



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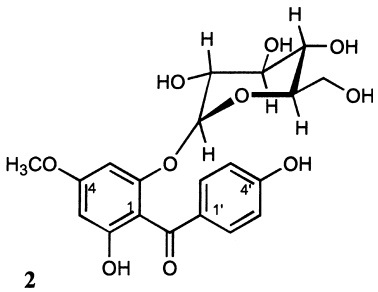

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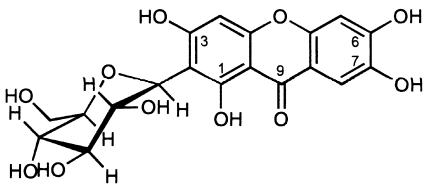

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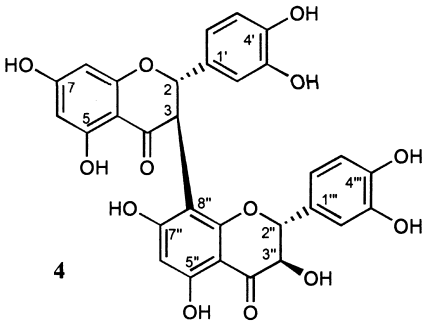

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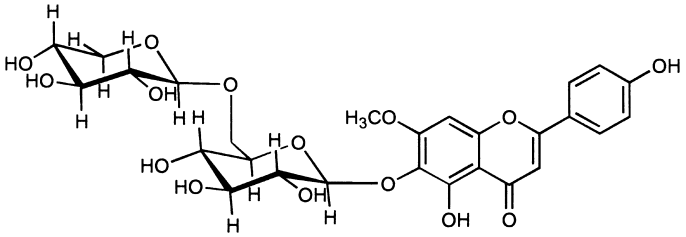

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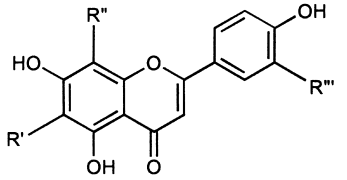

	R'	R"	R"'
7	Glc	H	OH
8	H	Glc	H
9	Glc	H	H

Elmer 241 MC polarimeter at 21° (Na lamp, 589 nm). EI-MS (70 eV) and D/CI-MS (NH_3, positive ion mode): Finnigan-MAT TSQ-700 triple stage quadrupole instrument. ^1H and ^{13}C NMR spectra were measured on an Unity Inova-500 spectrometer (Varian) at 499.87 and 125.70 MHz, respectively: samples were dissolved in either CD_3OD or DMSO-d_6 and TMS was used as internal standard. Complete attribution was performed on the basis of 2D-experiments (gradient COSY, DEPT, gradient HMBC, gradient HSQC). For open CC, silica gel 60 (70–230 and 230–400 mesh; Merck) was used. Gel filtrations were performed on Sephadex LH-20 (Pharmacia). LPLC was completed on a Lobar LiChroprep RP-18 column (40–63 μm; 310 × 25 mm; Merck) and MPLC on a home packed LiChroprep RP-18 stationary phase (15–25 μm; 450 × 40 mm; Merck).

3.2. LC/APCI-MS^n analysis

APCI-MS conditions: Finnigan MAT LCQ ion trap instrument (positive ion mode; corona discharge: 5.0 μA; vaporizer temperature: 450°C; sheath gas (N_2): 80 psi; capillary temperature: 200°C). The MS^n experiments were performed by programming dependent scan events: a full MS scan (MS^1), followed by the second event during which the $[M + H - 120]^+$ ions at m/z 313 and 329 recorded in MS^1 were isolated and selectively fragmented in the ion trap (MS^2). HPLC separations were carried out using a Symmetry C_{18} column (4 μm; 300 × 4.0 mm i.d.; Waters) equipped with a Guard-Pak C_{18} (Waters) pre-column, eluted at 1 ml min^{-1} with an acetonitrile–water 14:86 (+0.05% TFA) mixture during 40 min and finally washed with 100% acetonitrile. The LC/UV traces were recorded

on-line with a Hewlett Packard HP-1100 photodiode-array detector with detection at 366 nm.

3.3. Plant material

Gnidia involucrata was collected near Rukuru Bridge (Nyika Plateau, Malawi) in November 1991 and identified by H. Patel, National Herbarium of Malawi (Zomba, Malawi). A voucher specimen (No. 91078) has been deposited at the Institute of Pharmacognosy and Phytochemistry, University of Lausanne, Switzerland.

3.4. Extraction and isolation

The dried aerial parts of *G. involucrata* (250 g) were extracted at room temperature successively with CH_2Cl_2 3 × 1.5 l and MeOH 3 × 1.5 l. A portion (10 g) of the latter (31 g) was treated to remove tannins with hide powder as described by Hostettmann et al. (1998). This led to a tannin-free extract (6 g) that was further separated on a silica gel open column (60 × 4.5 cm) with a step gradient of $CHCl_3$–MeOH–H_2O 80:20:1 (3 l), 70:30:1 (2 l), 60:40:1 (1 l), 50:50:1 (1 l), 20:80:1 (1 l) and finally MeOH to give 10 fractions (A–J). Fraction D (650 mg) was filtered on a Sephadex LH-20 column (50 × 3 cm; MeOH–H_2O 5:1), affording compounds **2** (225 mg) and **5** (15 mg). Evaporation at room temperature of fraction F to a small volume led to the crystallization of compound **6** (20 mg). Fraction G (500 mg) was separated on a silica gel column (50 × 4 cm) with $CHCl_3$–MeOH–H_2O 65:35:5 (1.5 l) followed by MeOH (0.5 l) to give compound **1** (80 mg). Another portion (1 g) of tannin-free methanol extract was fractionated by LPLC on a Lobar LiChroprep RP-18 column (310 × 25 mm with a step gradient of MeOH–H_2O 30:70 (1.3 l), 40:60 (0.8 l) and finally 70:30 (0.5 l) at a flow rate of 3 ml min^{-1} to yield compound **3** (80 mg) and 7 fractions (K–Q). Further filtration of fraction P (360 mg) on a Sephadex LH-20 column (45 × 3 cm; MeOH) yielded compound **4** (25 mg). For complete identification, **4** was reisolated in a larger amount by fractionation of further 4 g of tannin-free methanol extract by MPLC on a LiChroprep RP-18 column (450 × 40 mm; MeOH–H_2O 40:60, 6 l, 6 ml min^{-1}) (180 mg).

3.5. 2,3,4′,5,6-Pentahydroxybenzophenone-4-C-glucoside (*1*)

Light-yellow powder, $C_{19}H_{20}O_{11}$, M_r 424. mp 168–171°C. $[\alpha]_D^{21} = +21°$; (MeOH; *c* 0.1). TLC: ($CHCl_3$–MeOH–H_2O, 65:35:5) R_f 0.35; UV λ_{max}^{MeOH} nm (log ε): 204 (2.74), 222 (*sh*, 2.27), 290 (2.29); ^{1}H NMR (500 MHz, CD_3OD): δ 7.60 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.77 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 4.86 (1H, *d*, *J* = 8.0 Hz, H-1″), 3.90 (1H, *m*, H-2″), 3.84 (1H, *dd*, *J* = 2.5 Hz, 12.5 Hz, H-6″), 3.73 (1H, *dd*, *J* = 5.0 Hz, 12.0 Hz, H-6″), 3.47 (2H, *dd*, *J* = 2.4 Hz, 7.0 Hz, H-3″ and H-4″), 3.39 (1H, *m*, H-5″); ^{13}C NMR (125 MHz, CD_3OD): δ 198.91 (C=O), 162.78 (C-2 and C-6), 161.24 (C-4′), 160.77 (C-3 and C-5), 133.18 (C-1′), 132.83 (C-2′ and C-6′), 115.43 (C-3′ and C-5′), 107.22 (C-1), 104.56 (C-4), 82.49 (C-5″), 79.81 (C-3″), 76.48 (C-1″), 73.60 (C-2″), 71.40 (C-4″), 62.41 (C-6″); D/CI-MS *m*/*z* (rel. int.): 408 $[M + H - 17]^+$ (10), 391 (100), 247 (98), 196 (52), 179 (98), 137 (97), 109 (98), 108 (99), 92 (38); EI-MS (probe), 70 eV, *m*/*z* (rel. int.): 390 $[M - 34]^{+\cdot}$ (10), 259 (42), 245 (68), 165 (100), 153 (43), 121 (92), 93 (36).

Acetylation of compound **1**: 10 mg of **1** were dissolved in 2 ml of acetic anhydride/pyridine (1:1) and stirred at room temperature for 24 h. Progress of the reaction was checked by TLC ($CHCl_3$–MeOH–H_2O, 65:35:5) and HPLC. The reaction mixture was then poured into ca. 20 ml of icy water. Further partition with ethyl acetate, followed by washing with aq. $CuSO_4$ 2.5% and water yielded a pure organic fraction of nona-acetylated compound **1** (**1a**, 6 mg). A loop APCI-MS experiment in the positive ion mode was performed to confirm the molecular weight of compound **1**.

3.6. 2,4′,6-Trihydroxy-4-methoxybenzophenone-2-O-glucoside (*2*)

Light-yellow powder, $C_{20}H_{22}O_{10}$, M_r 422. Mp 133–135°C. $[\alpha]_D^{21} = -23°$; (MeOH; *c* 0.1). TLC: ($CHCl_3$–MeOH–H_2O, 65:35:5) R_f 0.54; UV λ_{max}^{MeOH} nm (log ε): 208 (2.51), 223 (*sh*, 2.21), 296 (*sh*, 2.02), 312 (2.06); ^{1}H NMR (500 MHz, CD_3OD): δ 7.69 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 6.78 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.39 (1H, *d*, *J* = 2.0 Hz, H-5), 6.17 (1H, *d*, *J* = 2.1 Hz, H-3), 4.86 (1H, *d*, *J* = 7.8 Hz, H-1″), 3.85 (1H, *m*, H-6″), 3.79 (3H, *s*, H-4-methoxy), 3.64 (1H, *dd*, *J* = 5.8 Hz, 12.2 Hz, H-6″), 3.37 (2H, *m*, H-3″ and H-5″), 3.25 (1H, *m*, H-4″), 3.13 (1H, *dd*, *J* = 7.8 Hz, 9.2 Hz, H-2″); ^{13}C NMR (125 MHz, CD_3OD): δ 196.34 (C=O), 163.52 (C-4), 163.45 (C-4′), 158.29 (C-6), 157.71 (C-2), 132.87 (C-2′ and C-6′), 131.02 (C-1′), 115.31 (C-3′ and C-5′), 111.05 (C-1), 101.79 (C-1″), 96.09 (C-5), 94.22 (C-3), 77.60 (C-5″), 77.12 (C-3″), 74.06 (C-2″), 70.55 (C-4″), 61.89 (C-6″), 55.24 (OCH_3); D/CI-MS *m*/*z* (rel. int.): 440 $[M + NH_4]^+$ (44), 423 $[M + H]^+$ (100), 261 $[M + H - Glc]^+$ (100), 260 (61), 167 (10); EI-MS (probe), 70 eV, *m*/*z* (rel. int.): 260 $[M - Glc]^{+\cdot}$ (77), 259 (100), 166 (50).

Hydrolysis of compound **2**: 1 mg of **2** was dissolved in 5 ml of 2N HCL and refluxed for 1 h. The identity of the sugar was confirmed by TLC analysis (EtOAc–MeOH–H_2O–AcOH, 65:15:15:20) of the reaction mix-

ture with a genuine sample of glucose (detection: diphenylamine).

Methylation of compound **4**: 10 mg of **4** were dissolved in 1 ml methanol. 10 ml of freshly prepared diazomethane (CH_2N_2; 35 mM in Et_2O) were then added and the mixture was shaken at room temperature for 4 h. The progress of the reaction was checked by TLC ($CHCl_3$–MeOH–H_2O, 65:35:5). After solvent removal, a ^{13}C NMR analysis was performed on the product to confirm the absence of a sterically hindered methoxy group in position 5.

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