Isolation and On-Line LC/CD Analysis of 3,8″-Linked Biflavonoids from *Gnidia involucrata*

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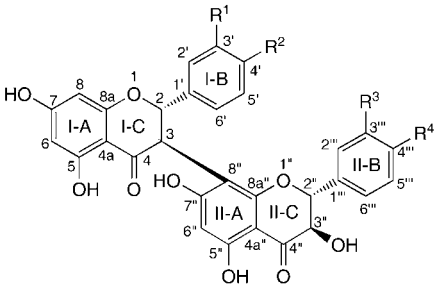
Investigation of the methanol extract of the roots of *Gnidia involucrata* (Thymelaeaceae) led to the isolation and characterization of two new 3,8″-biflavonoid diastereoisomers, named GB-4 (**6a**) and GB-4a (**6b**). Their absolute configurations were determined in mixture by on-line LC/CD measurements, which also allowed the revision of absolute configurations of the biflavanoids GB-1 and GB-2, and the configurational assignment of GB-3.

Introduction. – The investigation of the aerial parts of *Gnidia involucrata* STEUD. EX A. RICH., an African Thymelaeaceae, was reported in a recent publication by *Ferrari et al.* [1]. This study enabled the isolation and characterization of two new benzophenone glycosides, identified as 2,3,4′,5,6-pentahydroxybenzophenone-4-*C*-glucoside and 2,4′,6-trihydroxy-4-methoxybenzophenone-2-*O*-glucoside together with four known compounds: mangiferin, astragalin, yuankanin, and a 3,8″-biflavanoid, manniflavanone. This was the first time that an *O*-glycosylbenzophenone was described, and interesting aspects about the role of this class of molecules in the biosynthesis of xanthones were further discussed by *Kitanov* and *Nedialkov* [2]. The powdered root of *G. involucrata* is traditionally used in Africa for the treatment of dilated vagina [3] or for the benefits of its laxative and vermifuge properties [4]. Thus, in our ongoing search for new natural products from the Thymelaeaceae family, the methanolic extract of the roots of *G. involucrata* was investigated, leading to two new 3,8″-linked biflavanoids.

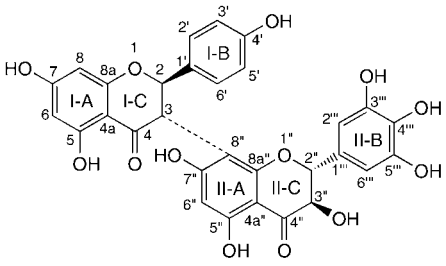
The 3,8″-biflavanoids were mainly isolated from *Garcinia*, *Rheedia*, and *Allanblackia* species (Clusiaceae), and their remarkable pharmacological activities, such as antihepatotoxic [5] and bactericidal properties [6], have been intensely studied and reported. Moreover, manniflavanone (**5**) was patented for the treatment of diseases resulting from disorders of vascular permeability and fragility, and for the prevention of complications of diabetes mellitus [7]. This type of secondary metabolite also exhibits very promising analgesic properties, which have to be further investigated [8]. Most of them consist of flavanone and 3-hydroxyflavanone units and are represented by GB-1 (**1**), GB-2 (**2**), GB-3 (**3**), kolaflavanone (**4**), and manniflavanone (**5**), whose structures differ only in the substitution pattern of the rings I-B and II-B. Their *trans*-diaxial

[1]) Deceased 20 July 2002.

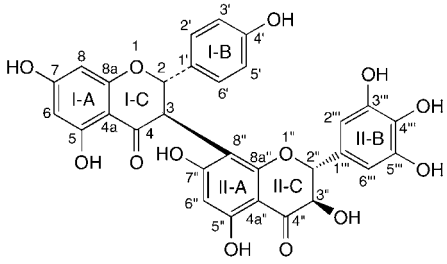
relative configurations were determined by ^{1}H-NMR coupling constants, while the absolute configurations of GB-1 (**1**) and GB-2 (**2**) were deduced from their CD spectra as (2*S*,3*R*,2″*R*,3″*R*) [9]. This was also used as a basis for the configurational assignments of biflavanoids **3**–**5** [9b], and was accepted and applied in the subsequent pharmacological studies.



	R^1	R^2	R^3	R^4
GB-1 (**1**)	H	OH	H	OH
GB-2 (**2**)	H	OH	OH	OH
GB-3 (**3**)	OH	MeO	OH	OH
kolaflavanone (**4**)	H	OH	OH	MeO
manniflavanone (**5**)	OH	OH	OH	OH



6a (2*R*,3*S*,2″*R*,3″*R*)



6b (2*S*,3*R*,2″*R*,3″*R*)

Isolation, NMR, MS, and on-line LC/CD studies of two new 3,8″-biflavanoid isomers, **6a** and **6b**, closely related to the former biflavanoids **1**–**5**, were accomplished in this study, which revealed that the configurational assignments of GB-1 (**1**) and GB-2 (**2**) should be revised, and those of biflavanoids **3**–**5** should be reconsidered.

Results and Discussion. – The dried roots of *G. involucrata* were first extracted at room temperature with CH_2Cl_2 and MeOH, respectively. As described in [1], the high tannin content of the plant material (5–6%) would hinder any further separation, and this class of compounds was, therefore, removed *via* the hide-powder method described by *Hostettmann et al.* [10]. The tannin-free MeOH extract was then fractionated by a combination of *RP-18* medium-pressure LC (MPLC) and silica-gel *60* open-column chromatography to afford a mixture of the diastereoisomers **6a** and **6b**. Since a good separation of these two compounds could be achieved by neither HPLC nor crystallization from solvent, NMR and MS studies were carried out on the mixture.

The molecular formulae and connectivity of isomers **6a** and **6b** were investigated by high-resolution electrospray-ionization mass spectrometry (HR-ESI-MS), desorption/chemical-ionization MS (D/CI-MS), electron-impact MS (EI-MS), and ^{1}H- and

^{13}C-NMR experiments. Moreover, the UV spectrum of the mixture was typical of biflavanones with a maximum at 294 nm, followed by a shoulder at 334 nm [11]. The D/CI (positive ion) mass spectrum of the mixture showed ions at *m/z* 608 ($[M+NH_4]^+$) and 591 ($[M+H]^+$), while the EI mass spectrum confirmed the mass of 590 Da with a molecular peak at *m/z* 590 ($M^{+\bullet}$). In addition, the EI-MS fragments at *m/z* 464, 446, and 126 ($[C_6H_6O_3]^{+\bullet}$) recalled the fragmentation pattern of manniflavanone (**5**) [12]. To confirm the proposed molecular mass of 590 Da and to obtain the molecular formula of the isomers, an HR-ESI-MS measurement was performed, which clearly showed a $[M+Na]^+$ ion at *m/z* 613.09561, corresponding to the molecular formula $C_{30}H_{22}O_{13}Na$ (calc. *m/z* 613.09526) and consistent with a biflavonoid structure.

In the ^{1}H-NMR spectrum of the mixture recorded in (D_6)DMSO at 30°, two series of signals characteristic of GB-type biflavanoids[2]) were distinguished based on the correlations obtained from gradient COSY, HMBC, and HSQC experiments (see *Fig. 1* for some of them). In both series of signals, duplication of certain resonances was observed, indicating the presence of two main rotational isomers for both **6a** and **6b** at ambient temperature. This observation has been previously reported for the biflavanone GB-1 (**1**), which showed very similar NMR features [9a]. For this type of molecules, the existence of rotational isomers at ambient temperature is explained by the strong intra- and intermolecular H-bonding that occurs due to the many OH groups present [13].

The four sharp *singlets* at δ 12.19, 11.94, 12.14, and 11.85 ppm were attributed to the H-bonded OH–C(5) and OH–C(5″), whereas the broad resonance between δ 10.6 and 11.5 ppm resulted from the remaining nonchelated OH groups (see the *Table*). The *doublets* at δ 7.18, 6.81, and δ 6.99, 6.69 ppm ($J = 8.0$ Hz) could be assigned to the *AA′BB′* spin system of the symmetric *para*-substituted I-B rings of **6a** and **6b**, respectively. The large *singlet* at δ 6.45 ppm similarly revealed the presence of an *AA′* spin system in the 3′,4′,5′-trisubstituted II-B rings of both **6a** and **6b** [11]. The ill-defined region between δ 5.91 and 5.85 ppm showed a broad *singlet* corresponding to H–C(6″) and a *multiplet* that grouped together *doublets* of H–C(6) and H–C(8) [12]. The H-atoms of the C-rings appeared in the range of δ 5.72–3.95 ppm, and there was quite a significant difference between the chemical shifts of the H–C(2″) and H–C(3″) in **6a** and **6b** (δ 4.81 and 3.95 ppm in **6a** and δ 4.10 and 4.38 ppm in **6b**, resp., see the *Table*). These chemical-shift differences of the corresponding H-atoms were due to the different magnetic-anisotropy effects of their environment, indicating that **6a** and **6b** may be diastereoisomers. The vicinal coupling constants *J*(H–C(2),H–C(3)) and *J*(H–C(2″),H–C(3″)) (11.5–12.0 Hz) established the *trans*-diaxial arrangements of the H–C(2)/H–C(3) and H–C(2″)/H–C(3″) pairs in both **6a** and **6b**.

The ^{13}C-NMR spectrum displayed lowfield signals at δ 196–198 ppm, typical of C(4)=O groups of *C*(3)-substituted flavanones [14]. The large resonance observed at δ 107.0 ppm could be attributed to the tertiary C-atoms of the 3′,4′,5′-trihydroxy I-B rings of both **6a** and **6b**, while those observed at δ 128.8 and 115.2 ppm for **6a**, and δ 129.4 and 115.0 ppm for **6b** could be assigned to the tertiary C-atoms of the 4′-hydroxy II-B rings [14]. The signals at δ 72.2 and 47.1 ppm for **6a**, and δ 71.9 and 47.3 ppm for **6b**

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[2]) Trivial names and numbering of biflavonoids are used in the *Results and Discussion* part; for systematic names of biflavonoids **6a** and **6b**, see the *Exper. Part*.

represented the aliphatic C-atoms of the 3-hydroxyflavanone and flavanone moieties, respectively [14]. By comparison with ^{13}C-NMR data of other 3,8″-biflavonoids [14][15], the measured resonances of **6a** and **6b** could be attributed to two isomeric 3,8″-biflavonoids consisting of 3-hydroxyflavanone and flavanone moieties, namely isomeric 3″,3‴,4′,4‴,5,5″,5‴,7,7″-nonahydroxy-3,8″-biflavanones. The connectivities in the molecules, particularly the 3,8″-linkage of the two flavonoid moieties, were confirmed by clear HMBC and COSY correlations (*Fig. 1*). An on-line LC/NMR analysis of **6a** and **6b** was also performed, but, due to the multiple residual solvent peaks, it could not provide more information than was already gained from the NMR study of the mixture.

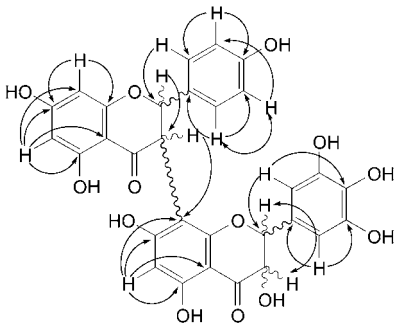

Fig. 1. *Selected ^{1}H,^{13}C long-range correlations* (gradient HMBC; →) *and ^{1}H,^{1}H-COSY* (↔) *of compounds* **6a** *and* **6b**

Although the biflavanoid mixture had a distinctive CD spectrum with maxima at 334, 299, and 271 nm (see *Exper. Part*), it contained the contributions of the two biflavanoid isomers, and, hence, cannot be used with confidence for stereochemical purposes. This was clearly established from the CD-detected HPLC chromatogram monitored at 281 nm, which gave opposite *Cotton* effects (CEs) for the first and second eluted biflavanoid isomers, **6a** and **6b**, respectively (*Fig. 2*). Although baseline separation of **6a** and **6b** could not be achieved by HPLC (*Symmetry C_{18}* column; see *Exper. Part*), the best conditions provided a separation (separation factor (α) 1.04 and resolution (R_s) 1.04), which was found suitable for an on-line LC/CD analysis (*Fig. 2*). This could afford the full on-line CD spectra of the isomers **6a** and **6b** recorded with stopped-flow technique in the maxima of their LC/CD or LC/UV chromatogram, therefore, providing stereochemical information about the individual isomers.

Duddeck et al. [15b] stated that, due to the relative arrangement of the electron-transition moments, there is no exciton coupling between the flavanone and 3-hydroyxflavanone units of the 3,8″-linked biflavanoids if the substituents at the stereogenic centres are *trans*-diequatorial. Thus, from the CD point of view, the flavanone and 3-hydroxyflavanone units of these biflavanoids can be considered independent chromophore systems, which may enable their configurational assignments. The conformations of the heterocyclic rings in flavanones and 3-hydroxyflavanone were correlated with the signs of the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ CD transitions by *Gaffield* (*Fig. 3*) [16]. This correlation can lead to the absolute configuration of flavanones and 3-hydroxyflavanones if the relative configuration is known from NMR.

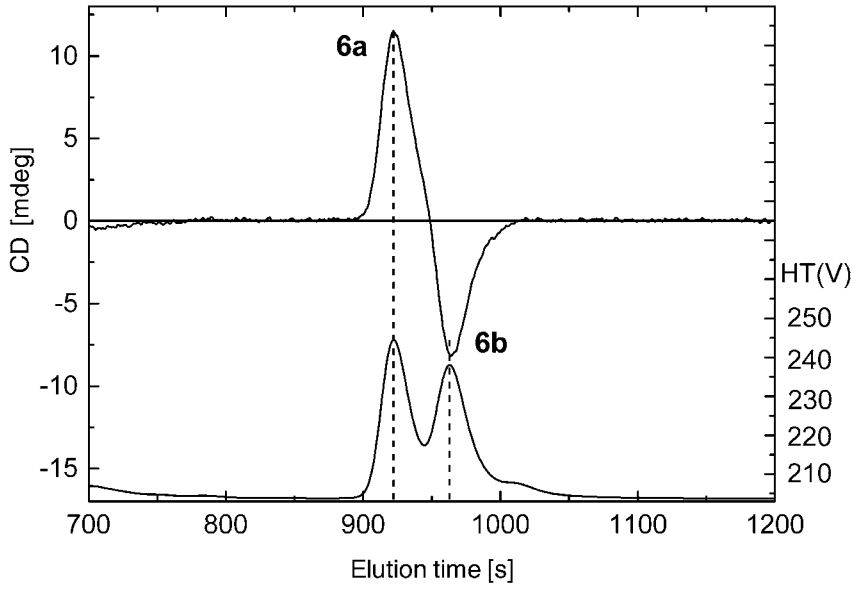

Fig. 2. *LC/UV* (lower curve) *and LC/CD* (upper curve) *chromatograms of the diastereoisomeric mixture of* **6a** *and* **6b** *monitored at 281 nm* (separation factor (α) 1.04 and resolution (R_s) 1.04 for **6a** and **6b**). For chromatographic protocols, see *Exper. Part*.

According to this rule, flavanones of (2*S*)-configuration and 3-hydroxyflavanones of (2*R*,3*R*)-configuration, with the 2-aryl group substituted equatorially to the heterocyclic ring in the former or with the 2,3-groups substituted diequatorially in the latter, exhibit a positive CE due to the n $\rightarrow \pi^*$ transition and a negative CE in the $\pi \rightarrow \pi^*$ region. The flavanone (+)-(2*R*)-naringenin (**7**) and the 3-hydroxyflavanone (+)-(2*R*,3*R*)-taxifolin (**8**), closest analogues of the building blocks of biflavanoids **6a** and **6b**, show characteristic CD bands in the n $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ region (*Fig. 3*), which reflect the helicity of their heterocyclic rings and their absolute configurations, and are in accordance with *Gaffield*'s rule. The corresponding CD transitions of flavanones appear at somewhat lower wavelength than those of 3-hydroxyflavanones, and the $\pi \rightarrow \pi^*$ CD transitions of flavanones become even more blue-shifted when there is an additional aryl moiety at C(3) as in **6a** and **6b**. Thus, it was reported by *Bekker et al.* [17] that the ^1L_b $\pi \rightarrow \pi^*$ CD band of the flavanone derivative **9**, which contains an achiral aryl moiety at C(3), are blue-shifted to 275 nm compared to the 290-nm maximum of naringenin (*Fig. 4*). In this work, the zeyherin derivatives **10** and **11** (*Fig. 4*), which are close analogues to **6a** and **6b**, were also studied, since they contain a flavanone unit linked to a benzofuranone moiety in the same position as was disclosed for **6a** and **6b**. In the CD spectra of **10** or **11**, exciton coupling between the flavanone and benzofuranone units could not be observed. In the derivative **10**, the n $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of the flavanone and benzofuranone units had opposite sign and appeared at different wavelengths, although some of them were partially overlapping (*Fig. 4*). When the configuration was changed in the benzofurane unit resulting in **11**, the CD transitions of

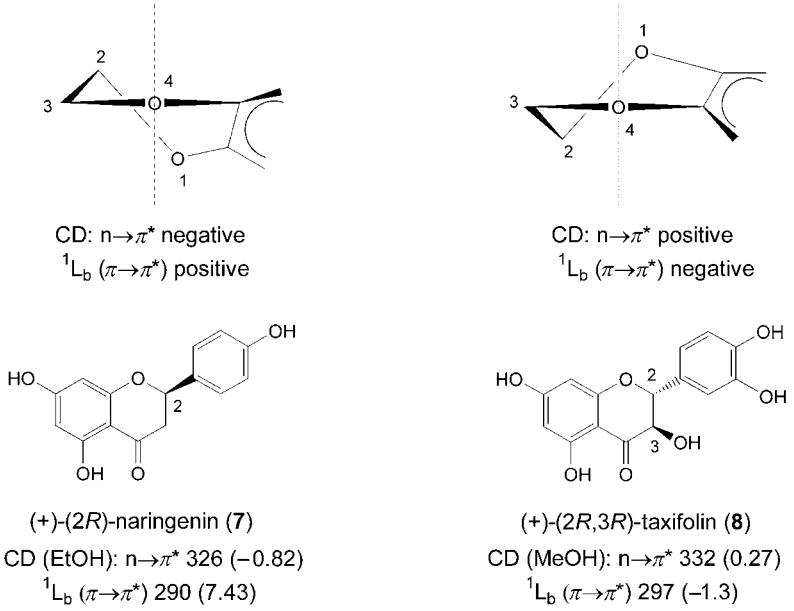

Fig. 3. Gaffield's *rule for correlation between absolute conformation of flavanone and 3-hydroxyflavanone heterorings and their characteristic CD bands.* CD Data of (+)-naringenin and (+)-taxifolin.

the benzofuranone moiety changed sign, which gave an intense and broad CD transition in the ^1L_b region.

As the *trans*-diequatorial arrangement of the substituents of the aliphatic C-ring C-atoms were established in both the first and second eluted isomers (**6a** and **6b**, resp.) by the NMR coupling constants (11.5 – 12.0 Hz), the possible sets of absolute configurations were restricted to four. The comparison of the on-line LC/CD spectra of the first and second peaks (*Fig. 5*) revealed that the $n \rightarrow \pi^*$ and ^1L_b CD bands of the flavanone and 3-hydroxyflavanone moieties appeared at different wavelengths and with opposite signs in the CD spectrum of the first eluted isomer, which allowed assignment of its CD bands. Since the corresponding CD transitions of the 3-hydroxyflavanone were proved to appear at higher wavelengths than those of the flavanone, the highest-wavelength CD transition (344 nm (1.90)) of the first eluted isomer (**6a**) was assigned as $n \rightarrow \pi^*$ transition of the 3-hydroxyflavanone unit, and the negative shoulder at 324 nm ($\Delta\varepsilon = -2.55$) as $n \rightarrow \pi^*$ transition of the flavanone unit (*Fig. 5*). Similarly, the higher-wavelength $\pi \rightarrow \pi^*$ CD band at 302 nm ($\Delta\varepsilon = -11.87$) derives from the 3-hydroxyflavanone and the 278.5-nm band ($\Delta\varepsilon = 12.36$) from the flavanone unit. Thus, according to *Gaffield*'s rule, the positive 3-hydroxyflavanone (344 nm (1.90)) and the negative flavanone (324 nm (sh, –2.55)) $n \rightarrow \pi^*$ transitions define the absolute configuration as (2*R*,3*S*,2″*R*,3″*R*), which is also confirmed by the negative 3-hydroxyflavanone and the positive flavanone $\pi \rightarrow \pi^*$ transitions.

In the on-line CD spectrum of the second eluted peak (**6b**), there is apparently only one $\pi \rightarrow \pi^*$ transition at 292 nm ($\Delta\varepsilon = -20.71$), which clearly originates from the sum of the overlapping previous negative 3-hydroxyflavanone ^1L_b transition and the

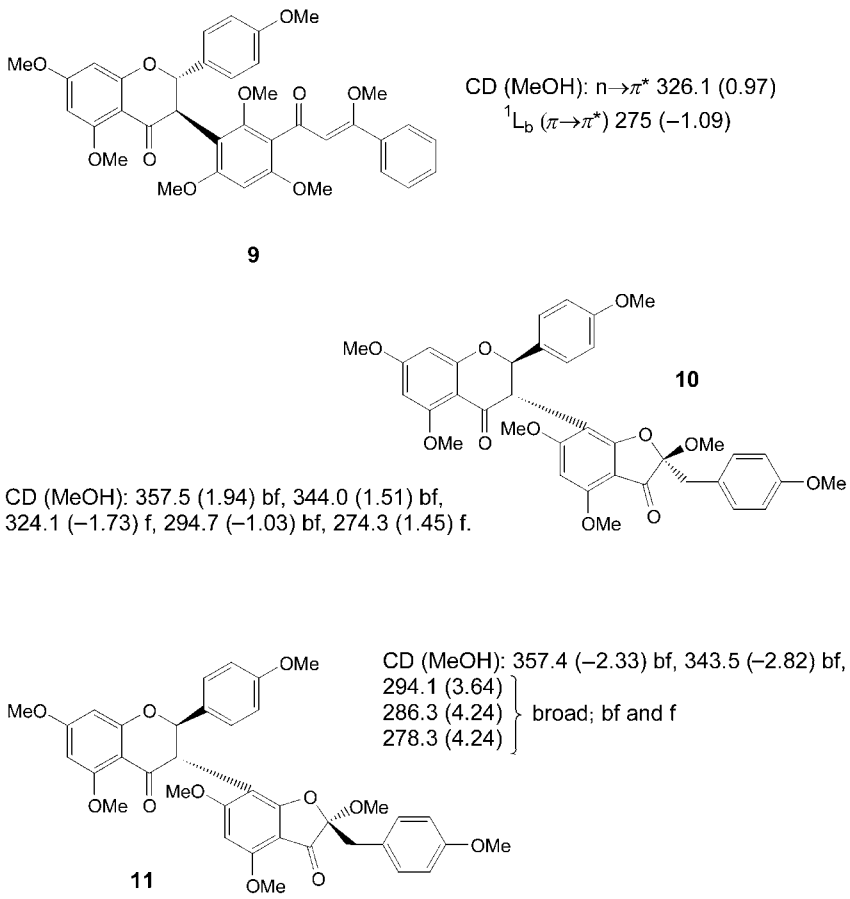

Fig. 4. *CD Data of the flavanone derivative* **9**, *and the zeyherin derivatives* **10** *and* **11**. The abbreviations f and bf stand for flavanone and benzofuranone, respectively.

inverted ^1L_b transition of the flavanone unit (*Fig. 5*). This also corroborated well with the enhanced intensity of this band and its maximum at 291.5 nm, situated between that of the 3-hydroxyflavanone and flavanone ^1L_b transitions of the first eluted peak (**6a**). Similarly, the flavanone $n \rightarrow \pi^*$ transition of the second eluted peak (**6b**) was inverted to positive, which, with the contribution of the positive 3-hydroxyflavanone $n \rightarrow \pi^*$ transition, resulted in an intense, overlapping positive CD band in the $n \rightarrow \pi^*$ region (322.0 nm (3.92)). Since both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the 3-hydroxyflavanone units were the same in **6a** and **6b**, their 3-hydroxyflavanone units had the same configuration, namely (2″*R*,3″*R*). However, the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the flavanone unit were inverted in **6b**, which define its absolute configuration as (2*S*,3*R*). Thus, the first (**6a**) and second (**6b**) eluted peaks are diastereoisomers in which the configurations of the flavanone units are enantiomeric, while the configurations of the 3-hydroxyflavanone units are the same.

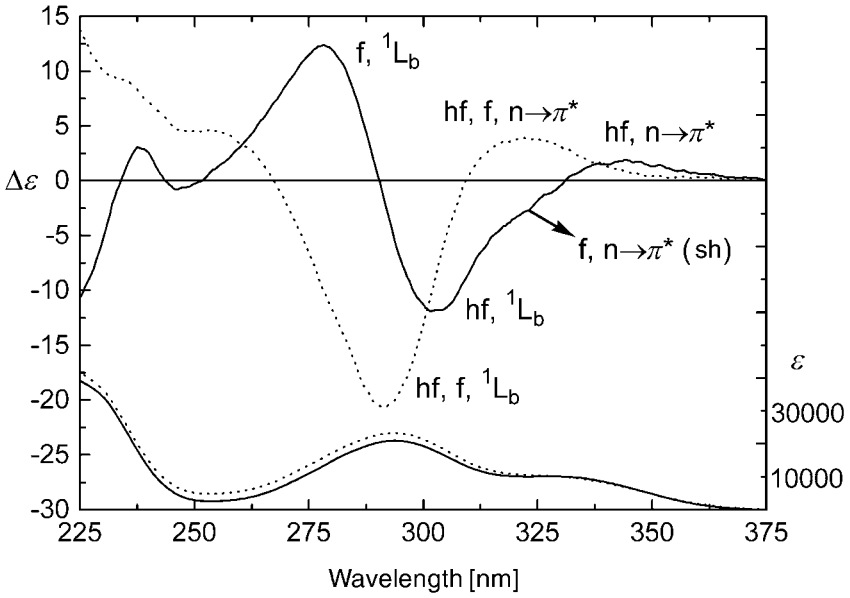

Fig. 5. Upper curves: *LC/CD spectra of* **6a** (solid line) *and* **6b** (dotted line). The abbreviations f and hf stand for flavanone and 3-hydroxyflavanone, respectively. Lower curves: *LC/UV spectra of* **6a** (solid line) *and* **6b** (dotted line).

The 3,8″-biflavanones **6a** and **6b** are new natural products, and, since they show close similarity to the compounds of the GB series, the names GB-4 and GB-4a are proposed for them, respectively. Only few compounds of this type have yet been isolated from species of the Thymelaeaceae family: daphnodorins D_1 and D_2 from *Daphne odora* THUNB. [18], wikstrols A and B from *Wikstroemia sikokiana* FRANCH. ET SAV. [19] and manniflavanone from *G. involucrata* [1].

Sonnenbichler et al. [9a] disclosed the CD spectra of the 3,8″-biflavanones GB-1 (**1**) and GB-2 (**2**), which were practically the same as that of **6b**. However, their intense opposite CEs at 300 and 280 nm were attributed to an exciton coupling due to the presence of atropisomers, and their absolute configurations were deduced as (2*S*,3*R*,2″*R*,3″*R*) based on the positive n → π* transitions at 330 nm and the negative CD band at 290 nm. *Iwu et al.* [9b] determined the same configuration for GB-1 (**1**) and GB-2 (**2**) on the basis of their 325- and 300-nm CD transitions and, without any CD evidence, assumed that manniflavanone (**5**) had also the same configuration. Although the presence of rotational isomers was also observed for **6a** and **6b**, their LC/CD study revealed that the opposite CEs of **6a** at 302 and 279 nm, and hence those of GB-1 (**1**) and GB-2 (**2**) did not derive from exciton coupling but partial overlap of oppositely signed ^1L_b transitions of the flavanone and 3-hydroxyflavanone moieties. This was also confirmed by the CD spectrum of **6b** in which the π → π* CD bands of the flavanone and 3-hydroxyflavanone moieties have the same signs, and, thus, are manifested in an overlapping, broad negative CD band with increased intensity (292 nm (−20.71)). Moreover, it was also asserted that the n → π* transitions of the flavanone and 3-

hydroxyflavanone moieties (344 nm (1.90) and 323.5 nm (sh, −2.55)) appear at different wavelengths and with opposite signs in **6a**, which allowed its (2*R*,3*S*,2″*R*,3″*R*)-configurational assignment in full agreement with the assignment made by its $\pi \rightarrow \pi^*$ CD bands. Since the CD data of GB-1 and GB-2 were the same as those of **6a**, their absolute configurations must be the same, and, thus, the absolute configurations of GB-1 and GB-2 have to be revised to (2*R*,3*S*,2″*R*,3″*R*). *Kabangu et al.* [15c] established the *trans*-diaxial arrangement of the aliphatic H-atoms in GB-3 (**3**) and observed the same CD pattern as for GB-1 and GB-2, which also allows its configurational assignment as (2*R*,3*S*,2″*R*,3″*R*). To the best of our knowledge, there have been no CD data reported for kolaflavanone (**4**) and manniflavanone (**5**), and, thus, further studies are required to determine their absolute configurations.

Experimental Part

General. Open Column chromatography (CC): silica gel *60* (35–70 μm; *Merck*). MPLC: home-packed *LiChroprep RP-18* stationary phase (15–25 μm; 450 × 40 mm; *Merck*). ^{1}H- and ^{13}C-NMR spectra: *Unity Inova-500* spectrometer (*Varian*) at 499.87 and 125.70 MHz, resp.; samples dissolved in (D_6)DMSO, TMS as internal standard; complete attribution performed on the basis of 2D experiments, gradient COSY, gradient HMBC, and gradient HSQC. EI-MS (70 eV) and D/CI-MS (NH_3, positive-ion mode): *Finnigan-MAT TSQ-700* triple-stage quadrupole instrument. HR-ESI-MS: *Bruker FTMS 4.7T Bio APEX II*.

LC/CD Analysis. HPLC Separation was carried out with a *Symmetry* C_{18} column (4 μm; 300 × 4.0 mm i.d.; *Waters*), eluted at 1 ml · min^{-1} with MeCN/H_2O/MeOH 29.3 : 68.3 : 2.4 during 20 min, and finally washed with 100% MeCN (separation factor (α) 1.04 and resolution (R_s) 1.04 for **6a** and **6b**). The LC/CD and LC/UV traces were recorded on-line at 281 nm with a *Jasco J-810 CD* spectropolarimeter (dichrograph) equipped with an HPLC flow cell. The on-line CD and UV spectra (200–400 nm) were recorded simultaneously at the maxima of the UV peaks where the flow was stopped.

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

Extraction and Isolation. The dried roots of *G. involucrata* (250 g) were extracted at r.t. successively with CH_2Cl_2 (3 × 1.5 l) and MeOH (3 × 1.5 l). A portion (15 g) of the latter (38 g) was treated to remove tannins with hide powder as described by *Hostettmann et al.* [10]. This led to a tannin-free extract (7 g) that was further separated by MPLC on a *LiChroprep RP-18* column (450 × 40 mm), eluted at 6 ml · min^{-1} with linear gradients of MeOH/H_2O 5 : 95 → 30 : 70 (4 h), 30 : 70 → 50 : 50 (8 h), 50 : 50 → 70 : 30 (8 h), 70 : 30 → 100 : 0 (2 h), and finally washed during 2 h with 100% MeOH to afford 10 fractions (A–J). *Fr. G* (1680 mg) was then separated on a silica-gel open column (50 × 4.0 cm) with a step gradient of $CHCl_3$/MeOH 75 : 25 (1.5 l), 65 : 35 (0.5 l), 40 : 60 (0.5 l), and finally MeOH (1 l) to give the mixture of the diastereoisomers **6a** and **6b** (280 mg) and eight other fractions (G1–G8).

*(2*R*,2″*R*,3*S*,3″*R*)-3″,3‴,4′,4‴,5,5″,5‴,7,7″-Nonahydroxy-3,8″-biflavanone* (*GB-4*; = *(2*R*,3*R*)-8-[(2*R*,3*S*)-3,4-Dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-2*H*-1-benzopyran-3-yl]-2,3-dihydro-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4*H*-1-benzopyran-4-one*; **6a**). $C_{30}H_{22}O_{13}$, M_r 590. UV and CD data recorded on-line in MeCN/H_2O/MeOH 29.3 : 68.3 : 2.4; UV (λ_{max} [nm] (ε 10^4)): 206 (7.94), 229 (sh, 3.62), 294 (2.09), 334 (sh, 0.97); CD (λ_{max} [nm] ($\Delta\varepsilon$)): 200 (−32.34), 219 (−14.29), 238 (3.06), 246 (−0.77), 279 (12.36), 302 (−11.87), 324 (sh, −2.55), 344 (1.90), 376 (0.05). ^{1}H- and ^{13}C-NMR: see the *Table*.

*(2*S*,2″*R*,3*R*,3″*R*)-3″,3‴,4′,4‴,5,5″,5‴,7,7″-Nonahydroxy-3,8″-biflavanone* (*GB-4a*; = *(2*R*,3*R*)-8-[(2*S*,3*R*)-3,4-Dihydro-5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-2*H*-1-benzopyran-3-yl]-2,3-dihydro-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4*H*-1-benzopyran-4-one*; **6b**). $C_{30}H_{22}O_{13}$, M_r 590. UV and CD data recorded on-line in MeCN/H_2O/MeOH 29.3 : 68.3 : 2.4; UV (λ_{max} [nm] (ε 10^4)): 206 (7.80), 229 (sh, 3.91), 294 (2.37), 334 (sh, 0.97); CD (λ_{max} [nm] ($\Delta\varepsilon$)): 204 (38.68), 217 (sh, 21.68), 236 (sh, 9.19), 256 (sh, 4.45), 292 (−20.71), 322 (3.92). ^{1}H- and ^{13}C-NMR: see the *Table*.

Mixture of **6a** *and* **6b**. Light-yellow powder. CD (MeCN/H_2O/MeOH 29.3 : 68.3 : 2.4; λ_{max} [nm] ($\Delta\varepsilon$)): 334 (1.98), 299 (−14.08), 271 (4.47), 236 (4.80), 221 (−1.50), 209 (11.74). D/CI-MS: 608 (6, $[M + NH_4]^+$), 591 (10,

Table 1. *^{1}H- and ^{13}C-NMR Data of Compounds* **6a** *and* **6b**

	6a		**6b**	
Position	^{1}H	^{13}C	^{1}H	^{13}C
2	5.63 (*d*, *J* = 11.5)	83.4[a])	5.72 (*d*, *J* = 11.5)	84.1[a])
3	4.51 (*d*, *J* = 11.5)	47.1	4.53 (*d*, *J* = 11.5)	47.3
4 (C=O)	–	196.8[b])	–	196.8[b])
5	–	163.0[c])	–	163.0[c])
6	5.85 (*m*)	96.3[d])	5.85 (*m*)	96.3[d])
7	–	166.6[c])	–	166.6[c])
8	5.85 (*m*)	95.2[d])	5.85 (*m*)	95.2[d])
9	–	162.4[c])	–	162.4[c])
10	–	101.5[e])	–	101.2[e])
1′	–	127.5[f])	–	127.3[f])
2′	7.18 (*d*, *J* = 8.0)	128.8	6.99 (*d*, *J* = 8.0)	129.4
3′	6.81 (*d*, *J* = 8.0)	115.2	6.69 (*d*, *J* = 8.0)	115.0
4′	–	158.0	–	158.0
5′	6.81 (*d*, *J* = 8.0)	115.2	6.69 (*d*, *J* = 8.0)	115.0
6′	7.18 (*d*, *J* = 8.0)	128.8	6.99 (*d*, *J* = 8.0)	129.4
2″	4.81 (*d*, *J* = 12.0)	81.7[a])	4.10 (*d*, *J* = 12.0)	81.4[a])
3″	3.95 (*d*, *J* = 12.0)	72.2	4.38 (*d*, *J* = 12.0)	71.9
4″ (C=O)	–	197.8[b])	–	197.8[b])
5″	–	162.4[c])	–	162.4[c])
6″	5.91 (*m*)	96.3[d])	5.91 (*m*)	96.3[d])
7″	–	163.8[c])	–	163.8[c])
8″	–	100.4[e])	–	99.9[e])
9″	–	160.4[c])	–	160.4[c])
10″	–	101.5[e])	–	101.2[e])
1‴	–	127.9[f])	–	127.9[f])
2‴	6.45 (*s*)	107.0	6.45 (*s*)	107.0
3‴	–	145.9	–	145.9
4‴	–	133.8	–	133.8
5‴	–	145.9	–	145.9
6‴	6.45 (*s*)	107.0	6.45 (*s*)	107.0
OH–C(5)/C(5″)	12.19 (*s*), 11.94 (*s*)	–	12.14 (*s*), 11.85 (*s*)	–
other OHs	11.5–10.6	–	11.5–10.6	–

[a])–[f]) These assignments may be interchanged.

$[M+H]^+$), 481 (61), 465 (63), 462 (36), 429 (10), 397 (16), 287 (18), 144 (82), 127 (100). EI-MS (70 eV): 590 ($M^{+\cdot}$), 464, 446, 297, 270, 242, 180, 166, 152, 139, 126 $[C_6H_6O_3]^{+\cdot}$, 97, 51. HR-ESI-MS: 613.09561 ($C_{30}H_{22}NaO_{13}^+$ $[M+Na]^+$; calc. 613.09526).

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