Studies on Bignoniaceae: Newbouldiosides D–F, Minor Phenylethanoid Glycosides from *Newbouldia laevis*, and New Flavonoids from *Markhamia zanzibarica* and *Spathodea campanulata*#

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ABSTRACT

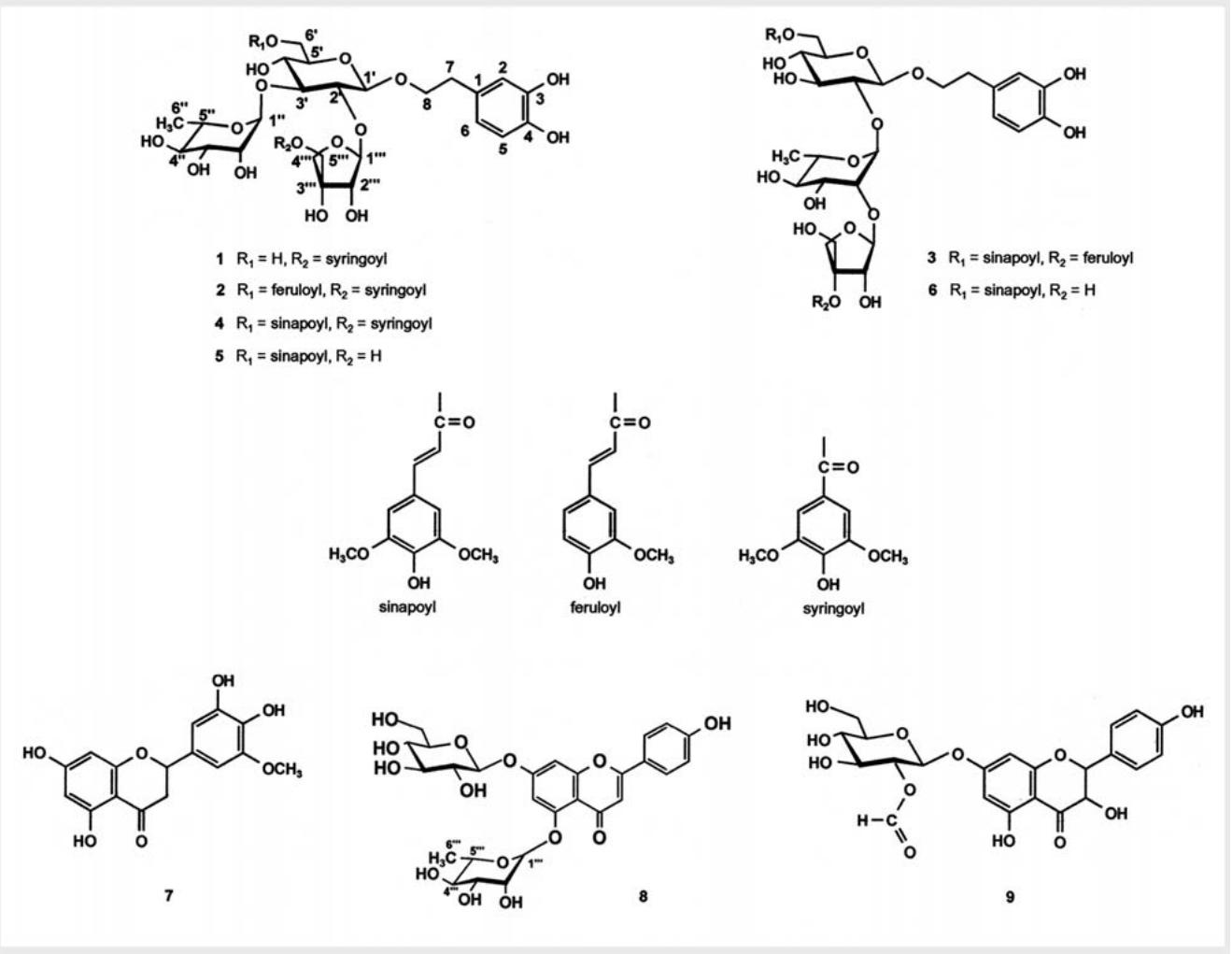
Continued examination of the stem bark of *Newbouldia laevis* afforded three minor phenylethanoid glycosides, designated as newbouldiosides D–F. Their structures were elucidated by spectroscopic methods as *β*-(3,4-dihydroxyphenyl)ethyl 5-*O*-syringoyl-*β*-*D*-apiofuranosyloxy-(1 → 2)-*O*-[*α*-L-rhamnopyranosyl-(1 → 3)]-6-*O*-E-sinapoyl-*β*-D-glucopyranoside, *β*-(3,4-dihydroxyphenyl)ethyl *β*-D-apiofuranosyloxy-(1 → 2)-*O*-[*α*-L-rhamnopyranosyl-(1 → 3)]-6-*O*-E-sinapoyl-*β*-D-glucopyranoside, and *β*-(3,4-dihydroxyphenyl)ethyl *β*-D-apiofuranosyloxy-(1 → 2)-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-6-*O*-E-sinapoyl-*β*-*D*-glucopyranoside, respectively. These metabolites are the first members possessing a sinapoyl structural element. In addition, the series of naturally occurring flavonoids is extended by the identification of 3′,4′,5,7-tetrahydroxy-5′-methoxyflavanone and apigenin-5-*O*-*α*-L-rhamnopyranosyl-7-*O*-*β*-D-glucopyranoside obtained from leaf extracts of *Markhamia zanzibarica* and aromadendrin-7-*O*-(2″-*O*-formyl)-*β*-D-glucopyranoside isolated from *Spathodea campanulata*. The latter compound is the first example of a flavonoid possessing a formylated glucosyl moiety.

Introduction

The family Bignoniaceae, divided into 8 tribes, is represented by 82 genera and more than 820 species [1]. *Newbouldia, Markhamia*, and *Spathodea* are classified in tribe Tecomeae, with geographical distributions in tropical Africa. African Bignoniaceae form a relatively small group of the Neotropical family and information about their constituents are hitherto generally limited. Previous work revealed the occurrence of naphthoquinones [2], phenylethanoid glycosides [3–5], and pyrazole alkaloids [6,7] as major metabolites of *Newbouldia laevis* Seem. *Markhamia zanzibarica* (Bojer ex DC.) K. Schum. ([syn. M. acuminata (Klotzsch) K. Schum.] stands almost unstudied [8], while a broad range of metabolites was documented for *Spathodea campanulata* P. Beauvois including flavonoids [9], iridoids [10], and triterpenoids [11,12]. The species under study are traditionally used by indigenous peoples for the treatment of a variety of ailments including dysentery, rheumatoid arthritis, epilepsy, skin infections, and general malaise [13,14].

We previously reported the isolation of a range of furanonaphthoquinones and phenylethanoid glycosides obtained from the stem bark of *N. laevis* [15,16] and presented a comparative study on cuticular wax compositions [17]. The present report deals with the isolation and structure elucidation of some minor phenylethanoid glycosides obtained from *N. laevis*, and new flavonoids encountered in *M. zanzibarica* and *S. campanulata*.

Dedicated to Professor Arnold Vlietinck on the occasion of his 80th birthday.



▶ **Fig. 1** Structures of compounds **1**–**9**.

Results and Discussion

Dried and powdered stem bark of *N. laevis* was successively extracted with dichloromethane, acetone, and methanol. The acetone extract was chromatographed on Sephadex LH-20 using acetone as the mobile phase to afford yellow eluents that were pooled according to their TLC patterns. Distinct fractions were further purified by repeated column chromatography followed by HPLC to afford three chromatographically homogeneous minor phenylethanoid glycosides (**4, 5**, and **6**) (▶ **Fig. 1**). Their structures were determined by analyses of MS and NMR spectroscopic data. Allocation of signals was facilitated by COSY, HETCOR, and HMBC experiments.

Compounds **4, 5**, and **6** were visualized by the typical light violet fluorescence on TLC plates, while treatment with the Naturstoff A reagent showed conspicuous greenish spots in the daylight. A similar response was noted for newbouldiosides A (**1**), B (**2**), and C (**3**) (▶ **Fig. 1**), previously obtained from the same plant source [16]. This behavior suggested the presence of structural analogues. Consistent with this conjecture, their UV spectra showed similar absorption maxima at ca. 215, 285, and 320 nm and comparable signal patterns in their ^{1}H NMR spectra. An aromatic ABX spin system (δ 6.4–6.7) associated with two mutually coupled methylene groups (δ 2.6–3.9) and an anomeric proton signal arising from a β-D-glucopyranosyl moiety ($J_{1,2}$ = ca. 7.5 Hz) (▶ **Table 1**) were reminiscent of the (3,4-dihydroxyphenyl)ethyl β-*D*-glucopyranoside core of phenylethanoid glycosides. The location of the phenylethoxy residue at C-1 of the glucosyl subunit followed from three-bond correlations between C-8 (δ_C ca. 72) and the corresponding anomeric proton in HMBC experiments of **4, 5**, and **6** (δ 4.30, 4.32, and 4.52, respectively). Additional common diagnostic features included resonances of two anomeric proton signals appearing at ca. δ 5.0 (*d, J* = 1.3 Hz) and 5.2 (*d, J* = 1.4 Hz), which were assignable to an α-rhamnosyl and a β-apiosyl moiety, respectively (▶ **Table 1**), taking into account the corresponding ^{13}C chemical shift data at ca. δ_C 103.5 and 110.7 (▶ **Table 2**). Based on these data, compounds **4, 5**, and **6** were readily found to be phenylethanoid glycosides that are related to previously reported newbouldiosides **1, 2**, and **3** [16] having the same carbohydrate constituent units. In addition, the common negative sign

▶ **Table 1** ^{1}H NMR data of compounds **4**, **5**, and **6** (400 MHz, CD_3OD).

Position	4	5	6
Aglycone			
2	6.64 *d* (2.0)	6.62 *d* (1.8)	7.16 *d* (2.0)
5	6.56 *d* (8.0)	6.58 *d* (8.1)	6.34 *d* (8.0)
6	6.42 *dd* (8.0, 2.0)	6.40 *dd*, (8.1, 1.8)	7.01 *dd* (8.0, 2.0)
7	2.67 *t-like* (7.6)	2.67 *t-like* (7.4)	2.75 *t-like* (7.6)
8a	3.53 (m)	3.55 (m)	3.73 (m)
8b	3.88 (*m*)	3.90 (*m*)	3.94 (*m*)
Glucosyl			
1′	4.30 *d* (7.3)	4.32 *d* (7.6)	4.52 *d* (7.6)
2′	3.50 *dd* (7.3, 9.5)	3.50 *dd* (7.3, 9.5)	3.56–3.61*
3′	3.62*	3.62*	3.56–3.61*
4′	3.35–3.60*	3.35–3.60*	3.56–3.61*
5′	3.35–3.60*	3.60[b]	3.59*
6′a	4.35 *dd*, (5.3, 11.8)	4.33 *dd* (5.3, 11.9)	4.24 (*m*)
6′b	4.51 *dd*, (2.0, 11.8)	4.50 *dd* (2.0, 11.9)	4.24 (*m*)
Rhamnosyl			
1″	5.01 *d* (1.4)	5.02 *d* (1.4)	5.04 *d* (1.0)
2″	3.98*	3.96*	3.98*
3″	3.70 *dd*,(3.2, 9.5)	3.69 *dd*,(3.2, 9.5)	3.56–3.61*
4″	3.44 *t-like* (9.5)	3.42 *t-like* (9.5)	3.56–3.61*
5″	3.96*	3.96*	3.56–3.61*
H_3C-6″	1.26 *d* (6.2)	1.26 *d* (6.1)	1.14 *d* (6.2)
Apiosyl			
1‴	5.23 *d* (1.5)	5.23 *d* (2.2)	5.20 *d* (1.4)
2‴	4.00 *d* (1.5)	3.94 *d* (1.5)	3.96 *d* (1.4)
4‴a	3.82 *d* (9.8)	3.75 *d* (9.8)	3.78 *d* (9.8)
4‴b	4.14 *d* (9.8)	4.01 *d* (9.8)	4.03 *d* (9.8)
5‴A	4.32*	3.57 *br s*	3.58 *br s*
5‴b	4.45 *d* (11.5)	3.57 *br s*	3.58 *br s*
Syringoyl			
2/6	7.35 (*s*)	–	–
3/5-OCH_3	3.84 (*s*)	–	–
Sinapoyl			
2/6	6.84 *s*	6.92 *s*	6.79 *s*
7	7.60 *d* (16.0)	7.64 *d* (15.6)	7.58 *d* (15.6)
8	6.34 *d* (16.0)	6.45 *d* (15.6)	6.32 *d* (15.6)
-OCH_3	3.79 *br s*	3.79 *br s*	3.79 *br s*

Chemical shifts (relative to TMS) are in δ (ppm). Multiplicities and coupling constants in Hz are in parentheses. *Signal pattern unclear due to overlapping.

of the optical rotations suggested a close configurational relationship between the phenylethanoid glycosides **1**–**6** (▶ **Fig. 1**).

The negative FABMS (fast atom bombardment mass spectrometry) of **4**, isolated as a yellow solid, showed a pseudomolecular $[M - H]^-$ ion peak at *m/z* 979, corresponding to the empirical molecular formula $C_{45}H_{56}O_{24}$. Key fragmentation ions suggested that both rhamnose and apiose were terminal units and that a syringoyl residue was attached to the apiosyl moiety in **4** {*m/z* 833 [(M – H)-rha]$^-$; *m/z* 667 [(M – H)-syringoyloxyapiosyl]$^-$}. This conjecture was corroborated by HREIMS as judged from the fragments at *m/z* 489.1258 (calcd. for $C_{20}H_{25}O_{14}$, 489.1244; [syringoyloxyapiosylglucosyl]$^{+\cdot}$) and *m/z* 181.0522 (calcd. for $C_9H_9O_4$, 181.0500; [syringoyl]$^{+\cdot}$), while the ion at *m/z* 153.0624 (calcd. for $C_8H_9O_3$, 153.0577; [2-(3,4-dihydroxyphenyl)ethoxy]$^{+\cdot}$) affirmed the identity of a phenylethanoid entity. The fragmentation pattern of **4** closely resembled that of **2** [16], and also its ^{1}H and ^{13}C

▶ **Table 2** ^{13}C NMR data of compounds **4**, **5**, and **6** (100 MHz, CD_3OD).

C	4	5	6
Aglycone			
1	131.1	131.2	130.2
2	117.0	116.8	116.0
3	144.7	144.7	144.7
4	146.2	146.2	143.8
5	117.2	117.0	116.1
6	121.2	121.2	120.2
7	36.7	36.7	35.7
8	72.3	72.5	71.5
Glucosyl			
1′	102.9	102.9	102.4
2′	78.9	78.7	79.5
3′	86.3	86.2	71.7[a]
4′	70.5[a]	70.3	70.5[b]
5′	77.8	77.8	73.1
6′	64.8	64.6	63.6
Rhamnosyl			
1″	103.5	103.6	102.5
2″	72.2	72.4	79.9
3″	72.1	72.3	71.8[a]
4″	73.9	73.6	70.6[b]
5″	70.7[a]	70.9	69.8
6″	17.9	17.7	17.2
Apiosyl			
1‴	110.7	110.8	109.7
2‴	79.0	80.1	77.5
3‴	79.3	79.3	79.2
4‴	75.5	75.0	73.4
5‴	68.2	64.3	64.8
Syringoyl			
1	121.2		
2	108.7		
3	148.9		
4	142.4		
5	149.0		
6	108.6		
7	167.9		
-OCH_3	57.2		
Sinapoyl			
1	125.1	125.1	126.1
2	106.6	106.6	105.6
3	148.0	148.0	147.8
4	138.7	138.7	138.2
5	147.8	147.8	147.8
6	106.4	106.4	105.4
7	146.0	146.0	146.0

continued

▶ **Table 2** *Continued*

C	4	5	6
8	115.2	115.2	114.0
9	167.2	169.2	167.6
-OCH_3	57.2	57.2	55.6

[a] Assignment of signals with the same superscript may be interchanged; [b] Assignment of signals with the same superscript may be interchanged

NMR spectra showed excellent agreement with the chemical shift data of **2**. Significant differences included replacement of an aromatic ABX spin system by a two-proton singlet at δ 7.35 and a methoxy signal at δ 3.79, integrating for two methoxy functionalities. Taking into account two *trans*-olefinic proton signals (δ 6.34 and 7.60, each *d*, J = 16.0 Hz), these spectral features were in full agreement with the presence of a sinapoyl moiety. The carbonyl carbon at δ_C 167.2 showed a long-range correlation to the H_2-6′ of the central glucose core in the HMBC experiments, establishing the placement of the sinapoyl residue at this position. Independent support for the point of esterification was available from the similar downfield position of H_2-6′ in the ^{1}H NMR spectra of **4** (δ 4.35 and 4.51) (▶ **Table 1**) and newbouldioside B (**2**) (δ 4.33 and 4.49) [16]. The apiosyl and rhamnosyl units were attached at C-2′ and C-3′ of the glucosyl moiety, respectively, as inferred from long-range correlations between these carbons and the anomeric H-1 (δ 5.23, apiosyl) and H″-1 (δ 5.01, rhamnosyl). Compound **4** was thus differentiated from **2** in that the C-6′ feruloyl residue was replaced by a sinapoyl entity. On the basis of these data, the structure of **4** was identified as the new *β*-(3,4-dihydroxyphenyl)ethyl 5-*O*-syringoyl-*β*-*D*-apiofuranosyloxy-(1 → 2)-*O*-[*α*-L-rhamnopyranosyl-(1 → 3)]-6-*O*-E-sinapoyl-*β*-D-glucopyranoside, named newbouldioside D.

Compound **5** was obtained as an amorphous powder. Its molecular weight was deduced from the $[M - H]^-$ ion at *m/z* 799 in the negative FABMS, in complete agreement with the molecular constitution $C_{36}H_{48}O_{20}$. Diagnostic fragments observed at *m/z* 653 [M – H-rhamnosyl]$^-$ and 667 [M – H-apiosyl]$^-$ established that both apiose and rhamnose were again terminal units. Supporting evidence for this conclusion was available from the key fragments at *m/z* 653.6095 (calcd. for $C_{30}H_{37}O_{16}$, 653.6052) and *m/z* 667.6343 (calcd. for $C_{31}H_{39}O_{16}$, 667.6318) observed in the HREIMS. The NMR spectra of **5** and **4** were virtually superimposable, except for the absence of signals assignable to a syringoyl entity in **5**. Unambiguous proton and carbon signal allocations together with the displayed placement of the rhamnosyl and apiosyl units were similarly achieved on the basis of 2D NMR experiments (COSY, HETCOR, HMBC). The data showed that **5** was a desyringoyl derivative of **4**. Accordingly, the structure of **5** was identified as *β*-(3,4-dihydroxyphenyl)ethyl *β*-D-apiofuranosyloxy-(1 → 2)-*O*-[*α*-L-rhamnopyranosyl-(1 → 3)]-6-*O*-E-sinapoyl-*β*-D-glucopyranoside, designated as newbouldioside E.

Compound **6** was obtained as an amorphous powder, possessing the elemental composition of $C_{36}H_{46}O_{20}$ as concluded from the $[M - H]^-$ peak at *m/z* 799 in the negative FABMS. In the HREIMS of **6**, the peaks at *m/z* 207.0664 (calcd. for $C_{11}H_{11}O_4$, 207.0657) and *m/z* 154.0638 (calcd. for $C_8H_{10}O_3$, 154.0629) were in full agreement with the presence of a sinapic acid and 2-(3,4-dihydroxyphenyl)ethanol, respectively. Due to spectroscopic complexity with severe overlap of carbohydrate resonances, the structural assessment of **6** necessitated extensive 2D NMR techniques including HMBC, HMQC, DEPT, HETCOR, and COSY experiments. Close structural similarity of **6** and newbouldioside C (**3**) [16] followed tentatively from the general congruence of ^{1}H and ^{13}C resonances. A significant difference was the presence of just one acyl residue in **6**, identified as a sinapoyl moiety. The carbonyl carbon at δ_C 167.6 showed a long-range correlation to the H_2-6′ signal at δ 4.24 in the HMBC experiments, providing evidence for the esterification at this position. Further support of this placement was similarly available from the downfield position of H_2-6′ (δ 4.24) and C-6′ (δ_C 63.6) as compared to δ 3.76 and δ_C 62.6 of **1**, being devoid of an acyl moiety in this position. Following crucial three-bond associations, the sequence of the sugars was firmly established. The anomeric H-1′ (glucosyl) at δ 4.52 correlated with C-8 (δ_C 71.5) in support of the glycosidic linkage to the phenylethoxy moiety, while the anomeric C-1″ (rhamnosyl) at δ_C 102.5 showed a long-range correlation with the H-2′ at ca. δ 3.58, establishing the corresponding disaccharide unit. Taking into account the conspicuous deshielding of the C-2″ resonance (δ_C 79.9), the collective evidence verified the apiofuranosyl-(1 → 2)-rhamnopyranosyl-(1 → 2)-glucosyl partial structure in **6**. A similar downfield position of the analogous ^{13}C signal was detected for the structurally related newbouldioside C (**3**) [16]. In addition, the apiofuranosyl ^{13}C resonances of **6** were consistent with those of non-acylated analogues [3, 18, 19]. The data showed that **6** was a deferuloyl derivative of **3**. Accordingly, the structure of **6** was unambiguously elucidated as *β*-(3,4-dihydroxyphenyl) ethyl *β*-*D*-apiofuranosyloxy-(1 → 2)-*O*-*α*-*L*-rhamnopyranosyl-(1 → 2)-6-*O*-E-sinapoyl-*β*-D-glucopyrano-side, designated as newbouldioside F.

Worth mentioning is that a wealth of members of this class of metabolites is reported, with structural variants of the central core, which, in turn, is abundantly coupled with various (non)acylated carbohydrate entities [20]. The identification of **4, 5**, and **6** not only extends the range of known phenylethanoid glycosides, but also introduces rare examples with a sinapoyl entity located in a branched (**4** and **5**) and unbranched (**6**) glucose-rhamnose-apiose carbohydrate chain, hitherto limited to newbouldiosides. It is also noteworthy that very few phenylethanoid glycosides have been reported possessing this type of linear arrangement, represented by samioside [18] and picfeosides A and B [21].

The metabolic flavonoid pool of the Bignoniaceae is commonly dominated by flavones and associated flavonols [22–24]. As expected, an initial TLC screening of species of the tribe Tecomeae, including *N. laevis, M. zanzibarica*, and *S. campanulate*, revealed the occurrence of apigenin- and luteolin-based derivatives. The chemical analysis was facilitated by means of hydrolyzed extract samples. A closer examination of the leaf extracts showed co-occurrence of typical members of the above-mentioned subtypes of flavonoids with the flavanones naringenin, naringenin-7-*O*-rutinoside, and eriocitrin in *M. zanzibarica*, while well-known quercetin glycosides were associated with the flavones apigenin, luteolin, and diosmetin in *S. campanulata*. However, limited sampling and information restrict the support of taxonomic boundaries on chemical ground, an issue which is not in the focus of this paper. Noteworthy in the context of our survey was the visualization of some intriguing TLC spots and subsequent isolation of some minor metabolites. Here, we disclose the characterization of three new flavonoids obtained from the latter two plant sources.

Compound **7** (▶ **Fig. 1**) was readily shown to be a flavonoid by a light yellow color on TLC plates and the characteristic coloration upon treatment with Naturstoff spray reagent. Its empirical formula $C_{16}H_{14}O_7$ was deduced from HREIMS, displaying the $[M]^{+\cdot}$ at *m/z* 318.2765 (calcd. for $C_{16}H_{14}O_7$, 318.2790). The EIMS peaks at *m/z* 153 and 139, resulting from RDA fragmentation, provided clues regarding the oxygenation patterns on the aromatic nuclei A and B, respectively. Diagnostic features in the ^{1}H NMR spectrum of **7** were the appearance of signals at *δ* 2.69 (*dd, J* = 3.0 and 12.6 Hz) and 3.06 (*dd, J* = 12.6 and 17.0 Hz) due to a methylene group, which, in turn, was coupled to an aliphatic methin proton (*δ* 5.28, *dd, J* = 3.0 and 12.6 Hz). Besides these signals, typical of the heterocyclic protons of a flavanone skeleton, the ^{1}H NMR spectrum of **7** showed aromatic *meta*-coupled protons (ring A) and two broadened singlets at *δ* 6.79 and 6.92 for the magnetically non-equivalent 2′- and 6′-protons on ring B. This finding suggested the presence of a methoxy group appearing at *δ* 3.33 on this phenyl ring. Consistent evidence was provided by the corresponding ^{13}C resonance at δ_C 56.5, indicating an adjacent unsubstituted carbon. Collectively, the ^{1}H and ^{13}C NMR data (▶ **Table 3**) were compatible with the structure of 3′,4′,5,7-tetrahydroxy-5′-methoxyflavanone for **7**, being reported from a natural source for the first time. This is the second report on the occurrence of flavanones in the Bignoniaceae, notably again from a species of the tribe Tecomeae [25]. The occurrence of naringenin and eriodictyol in *M. zanzibarica*, demonstrated in this study, may lead to the assumption that **7** had the same more common configuration at C-2.

Compound **8** (▶ **Fig. 1**) was readily characterized as a flavone derivative on the basis of its UV absorption (λ_{max} 366 nm) and the presence of the characteristic C-3 one-proton singlet at *δ* 6.67 in the ^{1}H NMR spectrum. Acid hydrolysis produced apigenin, L-rhamnose, and D-glucose, identified by cochromatography with authentic samples. Definition of the D- and L-form verified the configuration of the carbohydrate entities of **8**, thus presumably neglecting the measurement of the vital optical property. The positive FABMS showed the $[M + H]^{+\cdot}$ at *m/z* 579, and the peak at *m/z* 270.2359 (calcd. for $C_{15}H_{10}O_5$, 270.2365) observed in the HREIMS was consistent with the molecular weight of apigenin. Owing to the deficient successive elimination of the sugar entities in the FABMS, the presence of a disaccharide unit could be excluded. Analysis of the ^{1}H NMR spectrum of **8** revealed the presence of an A_2B_2 spin system for the B-ring and aliphatic proton signals arising from a *β*-glucosyl ($J_{1,2}$ = 7.5 Hz) and an *α*-rhamnosyl moiety ($J_{1,2}$ = 1.7 Hz), respectively. Supporting evidence was available from the ^{13}C NMR data (▶ **Table 3**). Confirmation for the bonding points of the rhamnosyl and the glucosyl moieties was obtained from NOE measurements. Irradiation of H-1‴ (*δ* 5.29) and H_3-5‴ (*δ* 6.47) gave an enhancement of H-6 (*δ* 6.47) only, suggesting the location of the rhamnosyl residue at C-5 of apigenin. Using the anomeric proton of the glucosyl moiety (*δ* 5.20) as a reference signal, a prominent NOE association with H-8 (*δ* 6.80) established its attachment to C-7 of the aglycone. These features unambiguously defined **8** as the new apigenin-5-*O*-*α*-L-rhamnosyl-7-*O*-*β*-D-glucoside.

Compound **9** (▶ **Fig. 1**) had the molecular formula $C_{22}H_{22}O_{12}$ according to EIMS data, indicating the $[M]^+$ peak at *m/z* 478 and a diagnostic fragment at *m/z* 272 [$M^+ - C_7H_{11}O_7$]. The low relative abundance of $[M]^{+\cdot}$ (3%) reflected the instability of the molecule under the conditions of ionization. Indication of decomposition precluded the measurement of HREIMS. Analysis of the ^{1}H NMR spectrum of **9** revealed *meta*-coupled signals at *δ* 5.87 (H-6) and 5.89 (H-8), a low-field A_2B_2 spin system, which was reminiscent of a 1,4-disubstitued aromatic ring, while two doublets at *δ* 5.33 (H-3, *J* = 11.5) and 5.35 (H-2, *J* = 11.5) indicated a dihydroflavonol substructure with a relative 2,3-*trans*-configuration [26]. A notable feature was a one-proton signal at *δ* 8.12 in the ^{1}H NMR spectrum, suggesting the presence of a formyl group. This conjecture was supported by the fragment ion at *m/z* 272, which may be plausibly explained by the loss of a formylated glucosyl entity. The solvent signal at *δ* 4.83 unfortunately coincided with the resonances of H-1″ and H-2″. The overlap of the solvent peak with the anomeric proton was similarly seen for taxifolin 7-*O*-glucoside [27], suggesting that glycosylation occurred at the 7-hydroxyl of the aglycone, also taking into account the chemical shift of H-1″. The conspicuously deshielded H-2″ assisted in indicating the site of formylation on the glucosyl residue. The chemical shifts of the anomeric proton and the formyl group correlated well with the analogous signals of formylated *β*-glucose-2,6-diformate [28], again giving credence to the structural proposal. The remaining glucosyl protons appeared in the region *δ* 2.67–3.47 with overlapping patterns. Proof of structure **9** was further attempted by comparison of ^{1}H NMR data of the glucosyl protons to those reported for dihydrokaempferol 7-*O*-glucoside [29] vs. 3-*O*-glucoside [30], signifying that the chemical shifts agreed favorably with the 7-*O*-glucosylation. Insufficient sample quantity and compound instability excluded useful chemical shift correlation experiments and the acquisition of an optical rotation. To date, there is only one report describing the occurrence of a dihydroflavanol (dihydrokaempferol 3-*O*-*α*-L-rhamnosyl-5-*O*-*β*-D-glucoside) in Bignoniaceae, but with no information regarding the configuration [25]. Since different stereoisomers of naturally occurring dihydroflavanols are known, the configuration of **9** remains speculative in the absence of firm proof. The above evidence tentatively defined the structure of **9** as the new aromadendrin 7-*O*-(2″-*O*-formyl)-*β*-D-

▶ **Table 3** ^{1}H NMR (400 MHz, CD_3OD) data of compounds **7**, **8**, and **9** along with ^{13}C NMR (100 MHz) data of compounds **7** and **8**.

Position	7		8		9
	δ_H	δ_C	δ_H	δ_C	δ_H
Aglycone					
2	5.28 *dd* (3.0, 12.6)	79.5		165.8	5.35 *d* (11.5)
3	2.69 *dd* (3.0, 17.0) 3.06 *dd* (12.6, 17.0)	43.1	6.67 *s*	103.1	5.33 *d* (11.5)
4		196.7		183.1	
4a		95.2		94.9	
5		162.0[a]		158.0	
6	5.87 *d* (2.0)	95.6	6.47 *d* (2.0)	99.9	5.89 *d* (2.0)
7		163.6[a]		163.3	
8	5.89 *d* (2.0)	95.2	6.80 *d* (2.0)	94.9	5.87 *d* (2.0)
8a		102.2		106.2	
1′		130.8		122.0	
2′	6.92 *br s*	115.1	7.89 *d* (9.0)	128.7	7.35 *d* (8.5)
3′		118.2	6.93 *d* (9.0)	116.1	6.82 *d* (8.5)
4′		145.5[b]		161.9	
5′		145.9[b]	6.93 *d* (9.0)	116.1	6.82 *d* (8.5)
6′	6.79 br s	113.4	7.89 *d* (9.0)	128.7	7.35 *d* (8.5)
H_3CO	3.33 br s	56.5			
Glucosyl					
1″			5.20 *d* (7.5)	98.8	4.83[c]
2″			3.69 *t-like* (7.5)	77.2	4.83[c]
3″				78.0	
4″			3.40–3.73 *m*[d]	71.2	2.67–3.15 *m*[d]
5″			3.40–3.73 *m*[d]	78.1	
6″			3.40–3.73 *m*[d]	61.4	
Formyl					8.12 *s*
Rhamnosyl					
1‴			5.29 *d* (1.7)	101.5	
2‴				72.9	
3‴			3.48–3.70 *m*[d]	72.8	
4‴			3.48–3.70 *m*[d]	70.3	
5‴			3.48–3.70 *m*[d]	69.0	
H_3C-6‴			1.33 *d* (6.3)	17.2	

Chemical shifts (relative to TMS) are in ppm (δ). Multiplicities and coupling constants in Hz are in parentheses. [a, b] Assignment of signals with the same superscript may be interchanged; [c] Signal overlap with solvent; [d] Signal pattern unclear due to overlapping

glucopyranoside [or dihydrokaempferol 7-*O*-(2″-*O*-formyl)-*β*-D-glucopyranoside].

While naturally occurring C-formylated flavonoids have been found in various natural sources, compound **9** is the first member possessing a formylated constituent carbohydrate unit. Worthy of mention is the demonstrated occurrence of C-formylated iridoids in distinct members of the tribe Tecomeae [31–34]. The search for natural products in Bignoniaceae possessing this uncommon structural feature may provide useful chemotaxonomic information.

Materials and Methods

Plant materials

The stem bark of *N. laevis* was collected in Abuja (Nigeria) and authenticity of the plant material was confirmed by Dr. M. Azuine, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. Leaves of *M. zanzibarica* and *S. campanulata* were obtained from the Botanical Garden of Berlin (Germany). Voucher specimens (BKOL RG 1–3) are deposited at the Institute of Pharmacy, Freie Universität Berlin, Germany.

Extraction and isolation

General details of the experimental equipment have been described previously [16]. Dried and powdered stem bark of *N. laevis* (1 kg) was successively extracted with CH_2Cl_2 (18 L), acetone (20 L), and MeOH (40 L) at room temperature to afford a brownish residue (9.3, 6.6, and 33.1 g, respectively) on evaporation of the solvent. Among the subfractions, the acetone fraction showed several spots on TLC plates with fluorescence and color responses reminiscent of previously identified newbouldiosides obtained from the MeOH extract [16]. Thus, a portion of the acetone-soluble extract (5 g) was repeatedly chromatographed on a Sephadex LH-20 column (3 × 75 cm) with acetone as the eluent, collecting 18 mL fractions. Subsequent HPLC purification of the content of test tubes 141–230 (89 mg) with an H_2O-MeOH gradient system (9 : 1 → 3 : 7 in 40 min, flow rate 3 mL/min, detection at 275 nm) yielded compound **4** (6 mg; R_t 36.1 min). Similar HPLC purification of a portion of the content of test tubes 213–318 (65 mg) afforded compounds **5** (7 mg, R_t 32.0 min) and **6** (4 mg, R_t 28.2 min). Qualitative TLC analysis was carried out on silica gel (*n*-butanol/H_2O/acetic acid 4 : 5 : 0.1 v/v).

Dried leaves of *M. zanzibarica* (100 g) and *S. campanulata* (100 g), respectively, were exhaustively extracted with MeOH (4 L in each instance). The combined extracts were defatted with petroleum ether and the subsequent partition with ethyl acetate yielded a residue (1.5 g) on evaporation of the organic solvent. A portion (150 mg) was subjected to repeated HPLC separations under the above-mentioned conditions to afford compounds **7** (7 mg; R_t 32.5 min) and **8** (8 mg; R_t 31.7 min) from *M. zanzibarica*, and **9** (4 mg; R_t 33.4 min) from *S. campanulata*.

Newbouldioside D (4)

Yellow amorphous material (6 mg). R_f 0.67; R_t 36.1 min.; UV (MeOH) λ_{max} nm: 219, 288, 320. $[\alpha]_D$ −49.7 (MeOH; *c* 2.5). ^{1}H NMR and ^{13}C NMR, see ▶ **Tables 1** and **2**. Negative FABMS *m/z* 979 $[M - H]^-$; HREIMS *m/z* 489.1258 (calcd. for $C_{20}H_{25}O_{14}$, 489.1244), *m/z* 181.0522 (calcd. for $C_9H_9O_4$, 181.0500), *m/z* 153.0624 (calcd. for $C_8H_9O_3$, 153.0577); EIMS *m/z* (rel. int.): 489 (65), 198 (39), 181 (18), 168 (9), 154 (52), 150 (20), 139 (61).

Newbouldioside E (5)

Yellow amorphous material (7 mg). R_f 0.58; R_t 32.0 min. UV (MeOH) λ_{max} nm: 219, 290, 325. $[\alpha]_D$ −48.7 (MeOH; *c* 2.6). ^{1}H NMR and ^{13}C NMR, see ▶ **Tables 1** and **2**. Negative FABMS *m/z* 799 $[M - H]^-$; 653 [M – H-rhamnosyl]$^-$; 667 [M-H-apiosyl]$^-$; HREIMS *m/z* 667.6343 (calcd. for $C_{31}H_{39}O_{16}$, 667.6318), *m/z* 653.6095 (calcd. for $C_{30}H_{37}O_{16}$, 653.6052); EIMS *m/z* (rel. int.): 207 (65), 181 (14), 150 (24), 150 (20), 137 (18).

Newbouldioside F (6)

Yellow amorphous material (6 mg). R_f 0.63; R_t 28.2 min. UV (MeOH) λ_{max} nm: 219, 282. $[\alpha]_D$ −46.3 (MeOH; *c* 2.4). ^{1}H NMR and ^{13}C NMR, see ▶ **Tables 1** and **2**. Negative FABMS *m/z* 779 $[M - H]^-$; HREIMS *m/z* 207.0664 (calcd. for $C_{20}H_{25}O_{14}$, 207.0657), *m/z* 154.0638 (calcd. for $C_8H_{10}O_3$, 154.0629); EIMS *m/z* (rel. int.): 198 (95), 181 (32), 154 (53), 139 (38).

3′,4′,5,7-Tetrahydroxy-5′-methoxyflavanone (7)

Yellow amorphous material (7 mg). R_f 0.90; R_t 32.5 min. UV (MeOH) λ_{max} nm: 219, 290, 325. ^{1}H NMR and ^{13}C NMR, see ▶ **Table 3**. HREIMS *m/z* 318.2895 $[M]^{+\cdot}$ (calcd. for $C_{16}H_{14}O_7$, 318.2790). EIMS *m/z* (rel. int.): 318 (47) $[M]^{+\cdot}$, 317 (27), 153 (52), 139 (15).

Apigenin-5-O-α-L-rhamnosyl-7-O-β-D-glucoside (8)

White yellowish solid material (8 mg). R_f 0.58; R_t 31.7 min. UV (MeOH) λ_{max} nm: 219, 290, 325. ^{1}H NMR and ^{13}C NMR, see ▶ **Table 3**. Positive FABMS *m/z* 579 $[M + H]^{+\cdot}$; HREIMS *m/z* 270.23459 (calcd. for $C_{15}H_{10}O_5$, 270.2365; apigenin). EIMS *m/z* (rel. int.): 270 (65), 181 (14), 150 (24), 137 (18). Compound **8** (2 mg) on hydrolysis with 2 N HCl in 50% aqueous MeOH (80 °C, 1 h) gave glucose, rhamnose, and an aglycone, extracted with EtOAc. The aqueous layer was examined by TLC in comparison with sugar standards using EtOAc-MeOH-CH_3COOH-H_2O (12 : 3 : 3 : 1 v/v) as the mobile phase and thymol-sulfuric acid for visualization.

Aromadendrin-7-O-(2″-O-formyl)-β-D-glucopyranoside (9)

Yellow amorphous material (2 mg). R_f 0.45; R_t 29.0 min. ^{1}H NMR, see ▶ **Table 3**. EIMS *m/z* (rel. int.): 478 $[M]^{+\cdot}$ (3), 272 [M – $C_7H_{11}O_7]^{+\cdot}$.

Contributors' Statement

Conception and design of the work: H. Kolodziej; analysis and interpretation of data: H. Kolodziej; drafting the manuscript: H. Kolodziej; revision of the manuscript: H. Kolodziej.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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