Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 67 (2006) 805–811
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Newbouldiosides A–C, phenylethanoid glycosides from the stem bark of *Newbouldia laevis*

Rainer Gormann [a], Maki Kaloga [a], Daneel Ferreira [b,c], Jannie P.J. Marais [c], Herbert Kolodziej [a,*]

[a] *Freie Universität Berlin, Institute of Pharmacy, Pharmaceutical Biology, Konigin-Lise-Str. 2+4, D-14195 Berlin, Germany*
[b] *Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*
[c] *National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677, USA*

Received 18 October 2005; received in revised form 9 January 2006
Available online 23 February 2006

Abstract

From the stem bark of *Newbouldia laevis* three phenylethanoid glycosides, designated as newbouldioside A–C, were isolated together with a sodium salt of analogue B and the known compounds, verbascoside, 5-hydroxydehydro-*iso*-α-lapachone, 3,8-dihydroxydehydro-*iso*-α-lapachone, apigenin and luteolin. The structures of the phenylethanoid glycosides were elucidated by spectroscopic methods as ß-(3,4-dihydroxyphenyl)ethyl 5-*O*-syringoyl-ß-D-apiofuranosyloxy-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-ß-D-glucopyranoside, ß-(3,4-dihydroxyphenyl)ethyl 5-*O*-syringoyl-ß-D-apiofuranosyloxy-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-6-*O*-*E*-feruloyl-ß-D-glucopyranoside, and ß-(3,4-dihydroxyphenyl)ethyl 3-*O*-*E*-feruloyl-ß-D-apiofuranosyloxy-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 2)-6-*O*-*E*-sinapoyl-ß-D-glucopyranoside, respectively.

Keywords: *Newbouldia laevis*; Bignoniaceae; Phenylethanoid glycosides; Flavonoids; Furanonaphthoquinones

1. Introduction

We previously reported (Gormann et al., 2003), the isolation of five furanonaphthoquinones, atraric acid and a benzofuran derivative obtained from a dichloromethane extract of the stem bark of *Newbouldia laevis* SEEM. (Bignoniaceae). The stem bark is traditionally used among the native populations in areas of Western Africa for the treatment of a variety of ailments including dysentery, rheumatoid arthritis, epilepsy and skin infections (Burkill, 1985; Azuine, 1999). During the earlier isolation the presence of a number of other components in the acetone and methanol extracts were noted and the present report deals with the isolation and structure elucidation of some of these constituents.

2. Results and discussion

Dried and powdered stem bark of *N. laevis* were initially extracted with dichloromethane, the constituents of this fraction have been the subject of a recent paper (Gormann et al., 2003). The plant material was subsequently extracted with acetone and methanol. After removal of a precipitate, the latter extract was further partitioned between methanol and *n*-hexane. Subsequent

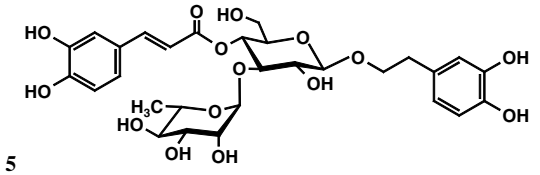
* Corresponding author. Tel.: +49 30 838 53731; fax: +49 30 838 53729.
E-mail address: kolpharm@zedat.fu-berlin.de (H. Kolodziej).

0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.01.016

column chromatography on silica gel of the *n*-hexane soluble portion using a cyclohexane-ethyl acetate gradient system, followed by HPLC purification with water containing increasing amounts of methanol, yielded the known furanonaphthoquinones, 5-hydroxydehydro-*iso*-α-lapachone (**1**) and 3,8-dihydroxydehydro-*iso*-α-lapachone (**2**), identified by comparison of their physical and spectroscopic data with those previously reported (Wagner et al., 1989; Gafner et al., 1996).

The methanol soluble extractives were chromatographed on Sephadex LH-20 in methanol. Subsequent HPLC purification of appropriate fractions afforded apigenin (**3**), luteolin (**4**) and verbascoside (**5**). While the flavonoids **3** and **4**, being reported for the first time from this plant species, were readily recognized by direct comparison with authentic specimens, compound **5** was identified on the basis of its spectroscopic data, identical with those reported previously (Andary et al., 1982).

The acetone extract was chromatographed on Sephadex LH-20 using acetone as the mobile phase to afford bright-yellow eluants that were grouped according to their TLC patterns. Subsequent chromatographic purification of distinct fractions by HPLC afforded four chromatographically homogeneous phenylethanoid glycosides (**6**–**9**). Structural assessment was effected by analyses of MS, and ^{1}H and ^{13}C NMR spectroscopic data. Allocation of signals was facilitated by COSY, HETCOR and HMBC experiments.



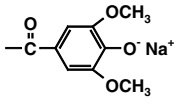
5

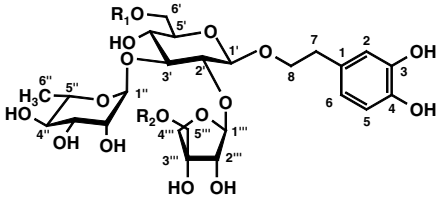
6 R_1 = H, R_2 = syringoyl

7 R_1 = feruloyl, R_2 = syringoyl

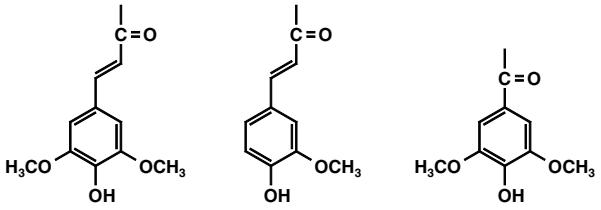
8 R_1 = feruloyl,

R_2 =

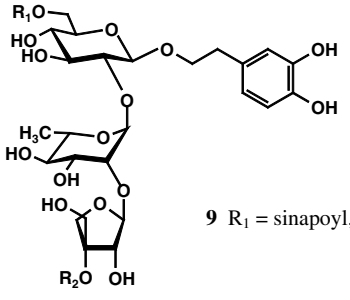




10 R_1 = feruloyl, R_2 = H



sinapoyl feruloyl syringoyl



9 R_1 = sinapoyl, R_2 = feruloyl

These compounds 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 verbascoside (**5**) and a number of structural analogues in a TLC spotting test, thus suggesting the presence of phenylethanoids. Supporting evidence for this assumption was obtained from their UV spectra with absorption maxima at ca 215, 285 and 320 nm (except for **6**), which were reminiscent of those of newbouldioside (**10**), previously obtained from the roots of *N. laevis* (Gafner et al., 1997). In this context it should be noted that **10** was concurrently obtained from *Markhamia lutea* and was designated luteoside C (Kernan et al., 1998). Initial identification of these phenylethanoids was accomplished by analysis of their ^{1}H NMR spectra which displayed the following common key features: signals (δ 6.4–6.7) for an aromatic ABX spin system and the presence of two mutually coupled methylene groups (δ 2.6–3.9), arising from a ß-(3,4-dihydroxyphenyl)ethyl moiety, and three anomeric protons appearing at ca δ 4.30 (*d*, J ca 7.5 Hz), 5.0 (*d*, J ca 1.3 Hz) and 5.2 (*d*, J ca 1.4 Hz), which were assignable to a ß-glucosyl, an α-rhamnosyl and a ß-apiosyl moiety, respectively (Table 1). These features established that compounds **6**–**9** were related to phenylethanoid glycosides such as myricoside (Cooper et al., 1980), forthysioside B (Endo et al., 1982), pedicularioside A (Zimin and Zhongjian, 1991), luteosides A–C (Kernan et al., 1998), and markhamiosides A–E (Kanchanapoom et al., 2002), that possess similar units.

Compound **6** was obtained as a yellow amorphous powder, possessing the elemental composition of $C_{34}H_{46}O_{20}$ as concluded from (−)-FABMS ($[M-H]^-$ at *m/z* 773) and the key fragment $[syringyloxyapiosylglucosyl]^{+\cdot}$ (*m/z* 489.12562, calc. 489.12442 for $C_{20}H_{25}O_{14}$) observed in the HREIMS. Its ^{1}H NMR spectrum displayed, in addition to the key features noted above, signals for a syringoyl residue. An *m/z* 181 ion in the EIMS spectrum of **6** confirmed the ^{1}H NMR notion, while the peaks at *m/z* 154 and 198 corresponded to 2-(3,4-dihydroxyphenyl)ethanol and syringic acid, respectively. The identity of these fragments was proved by HREIMS (see Section 3). Furthermore, key fragmentation ions observed in the negative FABMS established that both rhamnose and apiose were terminal units and that the syringoyl residue was attached to the apiose in **6** {*m/z* 627 [(M − H)-rha]$^-$; *m/z* 461 [(M − H)-syringoyloxyapiosyl]$^-$}.

Although some of the glycosidic protons overlapped in the ^{1}H NMR spectrum, the corresponding signals in **6** were well dispersed and were assigned from a COSY spectrum. Heteronuclear ^{1}H–^{13}C correlations then allowed assignment of the structure and the remaining NMR signals, summarized in Tables 1 and 2. In the ^{13}C NMR spectrum, the apiofuranosyl C-5 (δ_C 68.1) was conspicuously deshielded, compared to the chemical shift of the analogous carbon resonance of newbouldioside **10** (δ_C 64.6) (Gafner et al., 1997) and a non-acylated apiofuranosyl unit (δ_C 65.6) (Sugiyama and Kikuchi, 1993), respectively, supporting the esterification at this position. Confirmatory evidence was similarly available from the downfield position of H_2-5 (δ 4.32 and 4.46, apiosyl) as compared to δ 3.57 in the non-acylated analogue **10**. On the basis of these data, the structure of **6** was elucidated as ß-(3,4-dihydroxyphenyl)ethyl 5-*O*-syringoyl-ß-D-apiofuranosyl-oxy-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-ß-D-glucopyranoside, designated as newbouldioside A.

The negative FABMS of **7**, isolated as a yellow solid, showed an $[M-H]^-$ peak at *m/z* 949, consistent with a molecular formula $C_{44}H_{54}O_{23}$. Again, the series of abundant key ions at *m/z* 489, 198, 181 and 154 (*vide supra*) were detected in the EIMS. Close structural similarity of **6** and **7** followed from the general congruence of ^{1}H and ^{13}C NMR data (Tables 1 and 2). Notable differences were signals from a *trans*-feruloyl moiety in **7**. Comparison of the NMR spectra of **6** and **7** also showed a conspicuous deshielding of H_2-6 ($\Delta\delta$ 0.6) and C-6 ($\Delta\delta$ 2.0) of the central glucose core, thus localizing the acyl moiety at C-6′. Supporting evidence for this placement was also evident from comparing the ^{1}H and ^{13}C NMR data of **7** with those of newbouldioside (**10**) (Gafner et al., 1997; *syn*. luteoside C, Kernan et al., 1998), which differ in the presence of an acylated vs. nonacylated apiosyl moiety, respectively. Thus, compound **7** was identified as the new ß-(3,4-dihydroxyphenyl)ethyl 5-*O*-syringoyl-ß-D-apiofuranosyloxy-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 3)]-6-*O*-*E*-feruloyl-ß-D-glucopyranoside, named newbouldioside B.

Compound **8**, a white amorphous powder, co-eluted with **7** from a Sephadex LH-20 column and was separated by HPLC (R_t 38.2 vs 36.2 min). The peculiar chromatographic behaviour of **8** on the RP-18 column was reproducible and cannot satisfactorily be explained. It showed the peak of highest mass at *m/z* 971 $[M-H]^-$ and an ion at *m/z* 768 [M-phenolate entity]$^-$ in the negative FABMS, corresponding to its molecular formula $C_{44}H_{53}O_{23}Na$. Taking into account that the ^{1}H and ^{13}C NMR spectra of **7** and **8** were superimposable, except for the upfield shifts of H-2/H-6 ($\Delta\delta$ 0.5) and C-2/C-6 ($\Delta\delta$ 1.7) as well as C-4 ($\Delta\delta$ 2.6) of the syringoyl residue (Tables 1 and 2), this finding suggested **8** to be a sodium salt of **7**. Since a glycosidic linkage between the apiosyl and syringoyl units was excluded by the spectroscopic features, the observed chemical shift differences associated with the syringoyl moiety indicated a phenolate structure at C-4 of this acyl unit. Compound **8** represents the first example of a salt form within this group of secondary metabolites. The possibility that **8** represents an artefact was excluded by the work-up procedure, the relatively high yields and the absence of analogous derivatives of the structurally related compounds **6**, **7** and **9**.

The ESI-TOF mass spectrum of **9** showed an $[M+Na]^+$ peak at *m/z* 999.3095, consistent with a molecular formula of $C_{46}H_{56}O_{23}$ for the phenylethanoid glycoside. Analysis of the ^{1}H NMR spectrum indicated again the presence of two acyl moieties in **9**, as inferred from the set of signals

Table 1
^{1}H NMR data of compounds **6–9** (CD_3OD) isolated from *N. laevis*[A]

Position	6	7	8	9
Aglycone				
2	6.62 *d* (2.0)	6.61 *d* (2.0)	6.64 *d* (2.0)	6.69 *d* (1.8)
5	6.60 *d* (8.0)	6.57 *d* (8.0)	6.58 *d* (8.0)	6.64 *d* (8.1)
6	6.40 *dd* (8.0, 2.0)	6.39 *dd* (8.0, 2.0)	6.46 *dd*, (8.0, 2.0)	6.55 *dd*, (8.1, 1.8)
7	2.67 *t-like* (7.6)	2.67 *t-like* (7.6)	2.75 *t-like* (7.6)	2.79 *t-like* (7.4)
8a	3.53 (*m*)	3.53 (*m*)	3.53 (*m*)	3.74 (*m*)
8b	3.94 (*m*)	3.88 (*m*)	3.88 (*m*)	3.97 (*m*)
Glucosyl				
1′	4.28 *d* (7.7)	4.30 *d* (7.3)	4.37 *d* (7.5)	4.52 *d* (7.6)
2′	3.45 *dd* (7.7, 9.0)	3.49 *dd* (7.3, 9.5)	3.49 *dd* (7.5, 9.5)	3.58[B]
3′	3.58 *t-like* (9.0)	3.62[B]	3.62[B]	3.56–3.61[B]
4′	3.36 *t-like* (9.0)	3.35–3.50[B]	3.35–3.50[B]	3.56–3.61[B]
5′	3.23 (*m*)	3.50–3.60[B]	3.50–3.60[B]	3.60[B]
6′a	3.66 *dd* (5.45, 12.5)	4.33 *dd*, (5.3, 11.8)	4.33 *dd*, (5.1, 11.9)	4.24 (*m*)
6′b	3.85 *dd*, (2.0, 12.5)	4.49 *dd*, (2.0, 11.8)	4.50 *dd* (2.0, 11.9)	4.24 (*m*)
Rhamnosyl				
1″	4.98 *d* (1.4)	5.01 *d* (1.3)	5.01 *d* (1.2)	5.06 (*br s*)
2″	3.98[B]	3.98[B]	3.95b	3.96[B]
3″	3.69[B]	3.70[B] *dd*,(3.20, 9.5)	3.69 *dd,* (3.2, 9.6)	3.56–3.61[B]
4″	3.42 *t-like* (9.3)	3.44 *t-like* (9.5)	3.44 *t-like*,(9.6)	3.56–3.61[B]
5″	3.90–3.96[B]	3.95–3.98b	3.95–3.98[B]	3.56–3.61[B]
H_3-6″	1.26 *d* (6.3)	1.26 *d* (6.2)	1.27 *d* (6.2)	1.12 *d* (6.1)
Apiosyl				
1‴	5.23 *d* (1.2)	5.23 *d* (1.5)	5.22 *d* (1.4)	5.21 *d* (2.2)
2‴	4.07 *d* (1.2)	4.02 *d* (1.5)	3.99 *d* (1.4)	3.94[B]
4‴a	3.80 *d* (9.8)	3.81 *d* (9.8)	3.79 *d* (9.8)	3.74 *d* (9.8)
4‴b	4.14 *d* (9.8)	4.14 *d* 89.8)	4.12 *d* (9.8)	4.01 *d* (9.8)
5‴a	4.32 *d* (11.4)	4.30–4.33[B]	4.30–4.33[B]	3.58 (*br s*)
5‴b	4.46 *d* (11.4)	4.46 *d* (11.5)	4.47 *d* (11.5)	3.58 (*br s*)
Syringoyl				
2‴′/6‴′	7.35 (*s*)	7.35 (*s*)	6.86 (*s*)	–
3‴′/5‴′-OCH_3	3.87 (*s*)	3.84 (*s*)	3.84 (*s*)	–
Feruloyl				
2⁗	–	7.15 *d* (1.7)	7.15 *d* (1.8)	7.11 *d* (1.7)
5⁗	–	6.79 *d* (8.1)	6.79 *d* (8.1)	6.77 *d*,(8.2)
6⁗	–	6.99 *dd* (8.1, 1.7)	7.00 *dd* (8.1, 1.8)	7.03 *dd* (8.2, 1.7)
7⁗	–	7.60 *d* (15.9)	7.61 *d* (15.4)	7.62 *d* (15.5)
8⁗	–	6.37 *d* (15.9)	6.39 *d* (15.4)	6.36 *d* (15.5)
5⁗-OCH_3	–	3.84 (*s*)	3.84 (*s*)	3.82 (*s*)
Sinapoyl				
2⁗/6⁗	–	–	–	6.79 (*s*)
7⁗	–	–	–	7.58 *d* (15.6)
8⁗	–	–	–	6.32 *d* (15.6)
5⁗/	–	–	–	3.79 (*br s*)
7⁗-OCH_3				

[A] Chemical shifts (relative to TMS) are in (δ) in ppm, multiplicities and coupling constants in Hz in parentheses.
[B] Signal pattern unclear due to overlapping.

reminiscent of a *trans*-feruloyl residue (*vide supra*), and resonances arising from a sinapoyl moiety (Table 1). In the EI-MS of **9**, the ions at *m*/*z* 177 and 207 were in full agreement with the presence of a feruloyl and sinapoyl moiety, respectively. In contrast to compounds **6–8**, structural assessment of **9** required several HMBC, HMQC, TOCSY, HETCOR, DEPT and COSY experiments due to increased spectroscopic complexity with severe overlap of ^{1}H NMR signals, and a mass fragmentation pattern that did not readily show the sequence of the sugars. Structural assessment of **9** was thus accomplished using a combination of NMR techniques, along with comparisons to the assignments of analogues **6–8**. The carbonyl carbon at δ_C 167.5 showed in the HMBC experiments a three-bond correlation to the H_2-6′ signal (δ 4.24), and the anomeric proton at δ 4.52 correlations to C-8 (δ_C 71.3). This evidence suggested the placement of the sinapoyl residue at C-6 of the glucose unit that, in turn, was again linked to the phenylethoxy moiety. In addition, the anomeric C-1″ (rhamnosyl) at δ_C 102.1 correlated to both the H-2′ at δ 3.58 and H-5″ at ca δ 3.60.

Table 2
^{13}C NMR data of compounds **6**–**9** (CD_3OD) isolated from *N. laevis*

C	6	7	8	9
Aglycone				
1	131.3	131.1	131.2	130.0
2	117.1	116.5	116.4	115.8
3	144.7	144.7	144.7	144.5
4	146.1	146.2	146.2	143.0
5	116.3	117.1	117.2	115.1
6	121.2	121.2	121.3	119.9
7	36.6	36.7	36.8	35.5
8	71.9	72.4	72.2	71.3
Glucosyl				
1′	102.8	103.0	103.0	101.9
2′	78.7	78.9	78.9	79.6
3′	87.1	86.3	86.4	71.0[a]
4′	70.5	70.7	70.7	70.2
5′	77.8	77.8	77.8	72.3
6′	62.6	64.6	64.6	63.2
Rhamnosyl				
1″	103.6	103.5	103.5	102.1
2″	72.5	72.2	72.2	80.6
3″	72.2	72.0	72.1	71.5
4″	73.7	73.8	73.7	70.6[a]
5″	70.7	70.7	70.7	69.6
6″	17.9	17.9	17.9	17.4
Apiosyl				
1‴	110.7	110.7	110.5	109.8
2‴	79.0	78.8	79.1	77.3
3‴	79.2	79.2	79.2	79.2
4‴	75.3	75.3	75.3	73.9
5‴	68.1	68.1	67.7	64.3
Syringoyl				
1⁗′	121.1	121.1	121.1	–
2⁗′	108.7	108.7	107.0	–
3⁗′	149.0	149.0	149.5	–
4⁗′	142.4	142.4	139.8	–
5⁗′	149.0	149.0	149.5	–
6⁗′	108.7	108.7	107.0	–
7⁗′	167.9	167.9	168.9	–
3⁗′/5⁗′-OCH_3	57.0	57.0	56.9	
Feruloyl				
1⁗″	–	127.7	127.7	126.2
2⁗″	–	111.6	111.6	110.4
3⁗″	–	149.4	149.4	149.2
4⁗″	–	150.7	150.7	149.3
5⁗″	–	116.5	116.5	115.1
6⁗″	–	124.4	124.4	122.9
7⁗″	–	147.2	147.2	146.4
8⁗″	–	115.3	115.3	114.0
9⁗″	–	169.1	169.1	166.7
5⁗″-OCH_3	–	56.6	56.5	55.2
Sinapoyl				
1⁗‴	–	–	–	125.1
2⁗‴	–	–	–	105.4
3⁗‴	–	–	–	147.8
4⁗‴	–	–	–	138.0
5⁗‴	–	–	–	147.8
6⁗‴	–	–	–	105.4
7⁗‴	–	–	–	146.0
8⁗‴	–	–	–	113.8
9⁗‴	–	–	–	167.5
5⁗‴/7⁗‴-OCH_3	–	–	–	55.6

[a] Assignment of signals may be interchanged.

Consequently, the rhamnosyl unit was glycosidically linked to C-2′ of the glucose. Owing to the very similar chemical shifts of the C-2 protons of the apiosyl and rhamnosyl moieties (δ 3.94, 3.96), the crucial three-bond correlations required for establishing the apiosyl-rhamnosyl connectivity, could not be established unambiguously. However, the conspicuous deshielding of the C-2″ resonance at δ_C 80.6 supported the ß-D-apiofuranosyl-(1 → 2)-α-L-rhamnopyranosyl partial structure in **9** when compared to the analogous signal in compounds **6**–**8** (Table 2), taking also into account the HMBC associations of C-1″ (*vide supra*). Allocation of signals arising from the apiosyl moiety was clearly established by HMBC experiments, which showed the three-bond correlations of δ 5.21 (H-1‴) with δ_C 79.2 (C-3‴), δ_C 77.3 (C-2‴) with δ 3.58 (H_2-5‴), and δ_C 109.8 (C-1‴) with both δ 3.74 and 4.01 (H_2-4‴). Following these definite associations, the feruloyl unit was placed at C-3‴ because the ^{13}C resonances of C-2‴ and C-5‴ appeared relatively upfield at δ_C 77.3 and 64.3, respectively, when compared with the chemical shifts of the analogous signals of compounds **6**–**8** (ca δ_C 79 and 68 ppm, respectively) being devoid of an acyl moiety in this position. Accordingly, the structure of **9** was identified as ß-(3,4-dihydroxyphenyl)ethyl 3-*O*-*E*-feruloyl-ß-D-apiofuranosyloxy-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 2)-6-*O*-*E*-sinapoyl-ß-D-glucopyranoside, designated as newbouldioside C.

The identification of the newbouldiosides A–C (**6**–**9**) extends the range of analogues of this group of natural products containing glucose, rhamnose and apiose. It is significant that compound **8** represents the first phenolate known hitherto within this group, while newbouldioside C (**9**) not only introduces the first member with a linear glc-rha-api chain, but also possesses a sinapoyl moiety in the molecule.

3. Experimental

3.1. General

UV spectra were recorded on a Shimadzu UV-240 spectrophotometer and optical rotations were measured on a Perkin–Elmer MC 241 polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were obtained using a Bruker DPX-400 and a Varian Unity Inova 600 spectrometer; the chemical shifts are given in δ (ppm) relative to $(Me)_4Si$. HMBC experiments were optimised for $^{2\text{-}3}J_{H/C} = 8$ Hz. EIMS and HR–EIMS were acquired with a Varian MAT CH_7A and a Finnigan MAT 711 spectrometer, respectively. FABMS spectra were recorded on a Finnigan MAT CH_5DF, while the ESI-TOF data were acquired on an Agilent MSD-TOF spectrometer. HPLC separations were done with a Shimadzu instrument, equipped with a gradient former and a photodiode array detector, and Class M10-A software. Experimental conditions: Eurospher 100C-18 (5 μm; 8 × 250 mm); oven temperature, 40 °C; mobile phase A,

H_2O–MeOH gradient 9:1 → 3:7 (40 min, flow rate 3 ml/min); detection at 275 nm; mobile phase B (for naphthoquinones), H_2O–MeOH gradient 1:0 → 0:1 (40 min, flow rate 4 ml/min); detection at 254 nm. Column chromatography was carried out on silica gel (63-200 mesh; Merck) and Sephadex LH-20 (Fluka). After the emergence of yellow material, fractions were collected. Precoated TLC plates were used without activation (silica gel 60 F_{254}, 0.25 mm, Merck). Naphthoquinones were visualized by their typical yellow colour in the daylight and by exposure to UV (366 nm). Phenylethanoid glycosides and flavonoids were spotted by UV (366 nm) and Naturstoff A.

3.2. Plant material

The stem bark of *N. laevis* were collected in Abuja (Nigeria) in 1997 and authenticity of the plant material was confirmed by Dr. M. Azuine, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. A voucher specimen (BKOL 1) is deposited at the Institut für Pharmazie, Pharmazeutische Biologie, Berlin, Germany.

3.3. Extraction and isolation

Dried and powdered stem bark of *N. laevis* (1 kg) were successively extracted with CH_2Cl_2, acetone and MeOH at room temperature to afford a brownish residue (9.3, 6.6 and 33.1 g, respectively) on evaporation of the solvent. A portion of the MeOH extract (30 g) was re-suspended in MeOH and treated with *n*-hexane to yield 378 mg of a solid. The *n*-hexane soluble fraction (300 mg) was subsequently applied to a silica gel G 60 column (2.5 × 36 cm) using a cyclohexane-EtOAc gradient system (10:0 → 4:1). The content (10 ml fractions) of test tubes 41-90 (17.5 mg) and 91-150 (22.3 mg) were subjected to HPLC purification using the H_2O–MeOH gradient system B to afford compounds **1** (3.9 mg, R_t 10.6 min) and **2** (3.7 mg, R_t 14.1 min), respectively.

The MeOH soluble portion (23.7 g) was fractionated on Sephadex LH-20 using MeOH as eluant. A portion (72 mg) of the content of test tubes 17-34 (25 ml fractions; 7.1 g) was further purified by HPLC separation with the H_2O–MeOH gradient system A to afford compound **5** (5.2 mg, R_t 25.7 min).

Re-chromatography of fraction 35-56 (15 ml fractions; 1.3 g) on Sephadex LH-20 using EtOAc as eluant, followed by HPLC purification of the content of test tubes 21-57 (112 mg) yielded compounds **3** (2.9 mg; R_t 42.9 min) and **4** (2.1 mg; R_t 39.8 min), respectively.

A portion of the acetone soluble extract (5 g) was initially chromatographed on a Sephadex LH-20 column (3 × 75 cm), collecting 18 ml fractions. Elution with acetone and subsequent HPLC purification of a portion of fraction 141-230 (89 mg) with a H_2O–MeOH gradient-system (9:1 → 3:7 in 40 min, flow rate 3 ml/min, detection at 275 nm) yielded compounds **7** (11.3 mg; R_t 36.2 min) and **8** (9.1 mg; R_t 38.2 min). Similar HPLC purification of a portion of the content of test tubes 213-318 (65 mg) afforded compounds **6** (8.1 mg, R_t 25.3 min) and **9** (13.2 mg, R_t 38.9 min).

The known furanonaphthoquinones **1** and **2** (Wagner et al., 1989; Gafner et al., 1996) as well as verbascoside (**5**) (Andary et al., 1982) were identified on the basis of their physical data, consistent with those previously reported, while the flavonoids **3** and **4** corresponded to authentic specimens.

3.4. Newbouldioside A (*6*)

Yellow amorphous material (8 mg). R_f 0.53; R_t 25.1–25.3 min. $[\alpha]_D$ – 53.3 (MeOH; c 3.15). UV (MeOH) λ_{max} nm (log ε): 219 (3.99), 281 (3.98). Negative FAB-MS *m/z* 773 $[M-H]^-$; positive FAB-MS *m/z* 797 $[M+Na]^+$; HR–EIMS *m/z* 489.12562 calc. for $C_{20}H_{25}O_{14}$ 489.12442, *m/z* 198.05322 calc. for $C_9H_{10}O_5$ 198.05283, *m/z* 181.05112 calc. for $C_9H_9O_4$ 181.05008, *m/z* 154.06366 (calc. for $C_8H_{10}O_3$ 154.06299; EI-MS *m/z* (rel. int.): 198 (95), 181 (32), 154 (53), 139 (38). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

3.5. Newbouldioside B (*7*)

Yellow amorphous material (11 mg). R_f 0.69; R_t 36.2–36.4 min. $[\alpha]_D$ – 50.5 (MeOH; c 2.75). UV (MeOH) λ_{max} nm (log ε): 219 (3.99), 286 (3.98), 320 (2.4). Negative FAB-MS *m/z* 949 $[M-H]^-$; HR–EIMS *m/z*489.13968, calc. for $C_{20}H_{25}O_{14}$ 489.12442; EI-MS *m/z* (rel. int.): 489 (65), 198 (39), 181 (18), 168 (9), 154 (52), 150 (20), 139 (61). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

3.6. Newbouldioside B sodium salt (*8*)

Yellow amorphous material (9.1 mg). R_f 0.69; R_t 38.0–38.3 min. $[\alpha]_D$ – 52.7 (MeOH; c 4.3). UV (MeOH) λ_{max} nm (log ε): 214 (3.99), 290 (3.98), 327 (2.4). Negative FAB-MS *m/z* 971 [M – H]; 768 [M-phenolate entity]$^-$. EI-MS *m/z* (rel. int.): 489 (66), 207 (65), 181 (14), 150 (24), 150 (20), 137 (18). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

3.7. Newbouldioside C (*9*)

White amorphous material (9.1 mg). R_f 0.69; R_t 38.0–38.3 min. $[\alpha]_D$ – 47.9 (MeOH; c 2.4). UV (MeOH) λ_{max} nm (log ε): 219 (3.99), 294 (3.98), 327 (2.4). MALDI-TOF MS *m/z* 999 $[M+Na]^+$; EI-MS *m/z* (rel. int.): 207 (65), 181 (14), 150 (24), 150 (20), 137 (18). ^1H NMR and ^{13}C NMR see Tables 1 and 2.

Acknowledgements

The authors would like to thank Dr. G. Holzmann, Freie Universität Berlin, Institute of Organic Chemistry,

for the HR–EIMS data, and Mrs. D. Emmrich and Ch. Köhler for skilled HPLC separations. This work was supported in part by the United States Department of Agriculture, ARS Specific Cooperative Research Agreement No. 58-6408-2-0009.

References

Andary, C., Wylde, R., Lafitte, C., Privat, G., Winternitz, F., 1982. Structures of verbascoside and orobanchoside, caffeic acid and sugar esters from *Orobanche rapum-genistae*. Phytochemistry 21, 1123–1127.

Azuine, M.A., 1999. Cancer and Ethnobotany of Nigeria. Shaker Verlag, Aachen, Germany, pp. 115–116.

Burkill, H.M., 1985. The Useful Plants of West Tropical Africa, vol. 1. Royal Botanic Gardens, Kew, London.

Cooper, R., Solomon, P.H., Kubo, I., Nakanishi, K., Shoolery, J.N., Occolowitz, J.L., 1980. Myricoside, an african armyworm antifeedant: separation by droplet countercurrent chromatography. J. Am. Chem. Soc. 102, 7953–7955.

Endo, K., Takahashi, K., Abe, T., Hikino, H., 1982. Structure of forsythoside B, an antibacterial principal of *Forsythia koreana* stems. Heterocycles 19, 261–264.

Gafner, St., Wolfender, J.-L., Nianga, M., Stoeckli-Evans, H., Hostettmann, K., 1996. Antifungal and antibacterial naphthoquinones from *Newbouldia laevis* roots. Phytochemistry 42, 1315–1320.

Gafner, St., Wolfender, J.-L., Nianga, M., Hostettmann, K., 1997. Phenylpropanoid glycosides from *Newbouldia laevis* roots. Phytochemistry 44, 687–690.

Gormann, R., Kaloga, M., Li, X.-C., Ferreira, D., Bergenthal, D., Kolodziej, H., 2003. Furanonaphthoquinones, atraric acid and a benzofuran from the stembarks of *Newbouldia laevis*. Phytochemistry 64, 583–587.

Kanchanapoom, T., Kasai, R., Yamasaki, K., 2002. Phenolic glycosides from *Markhamia stipulata*. Phytochemistry 59, 557–563.

Kernan, M.R., Amarquaye, A., Chen, J.L., Chan, J., Sesin, D.F., Parkinson, N., Ye, Z.-J., Barrett, M., Bales, C., Stoddart, C.A., Sloan, B., Blanc, P., Limbach, C., Mrisho, S., Rozhon, E.J., 1998. Antiviral phenylpropanoid glycosides from the medicinal plant *Markhamia lutea*. J. Nat. Prod. 61, 564–570.

Sugiyama, M., Kikuchi, M., 1993. Phenylethanoid glycosides from *Osmanthus asiaticus*. Phytochemistry 32, 1553–1555.

Wagner, H., Kreher, B., Lotter, H., Hamburger, M.O., Cordell, G.A., 1989. Structure determination of new isomeric naphtha[2,3-*b*]furan-4,9-diones from *Tabebuia avellanedae* by the selective-INEPT technique. Helv. Chim. Acta 72, 659–667.

Zimin, L., Zhongjian, J., 1991. Phenylpropanoid and iridoid glycosides from *Pedicularis striata*. Phytochemistry 30, 1341–1344.