Bioactive Constituents of the Stem Bark of *Beilschmiedia zenkeri*

Bruno Ndjakou Lenta,*[,†,‡] Ferdinand Tantangmo,[§] Krishna Prasad Devkota,[‡] Jean Duplex Wansi,[‡] Jean Rodolphe Chouna,[§] Rene Cosme Fongang Soh,[§] Beate Neumann,[⊥] Hans-Georg Stammler,[⊥] Etienne Tsamo,[§] and Norbert Sewald[‡]

Department of Chemistry, Higher Teachers' Training College, University of Yaoundé 1, P.O. Box 47, Yaoundé, Cameroon, Chemistry Department, Organic and Bioorganic Chemistry, Bielefeld University, P.O. Box 100131, 33501 Bielefeld, Germany, Department of Organic Chemistry, University of Yaoundé 1, P.O. Box 812, Yaoundé, Cameroon, and Chemistry Department, Inorganic and Structural Chemistry, Bielefeld University, P.O. Box 100131, 33501 Bielefeld, Germany

Received June 8, 2009

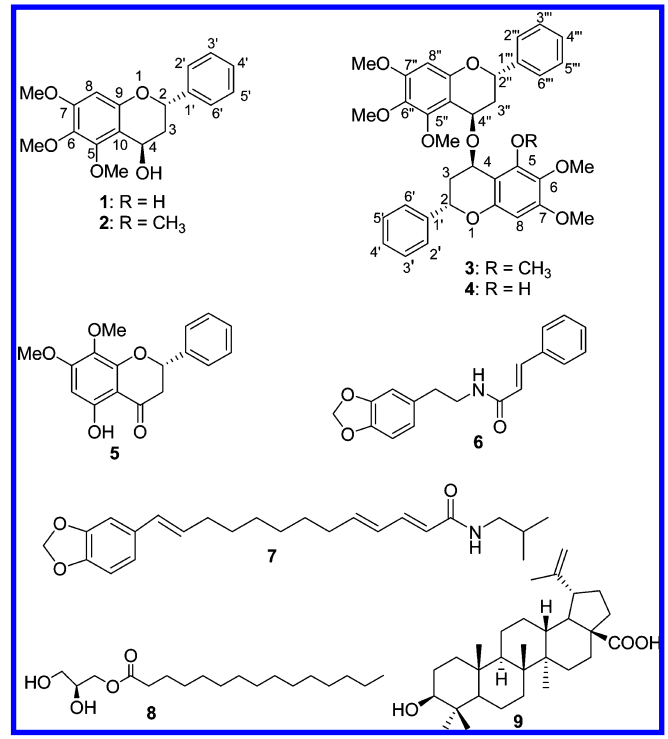
Phytochemical investigation of the stem bark of *Beilschmiedia zenkeri* led to the isolation of four new methoxylated flavonoid derivatives, (2*S*,4*R*)-5,6,7-trimethoxyflavan-4-ol (**1**), (2*S*,4*R*)-4,5,6,7-tetramethoxyflavan (**2**), beilschmieflavonoid A (**3**), and beilschmieflavonoid B (**4**), together with seven known compounds. The structures of **1**−**4** were established by spectroscopic methods, and their relative configurations confirmed by X-ray crystallographic and CD analysis. The isolated compounds were evaluated in vitro for their antibacterial activity against three strains of bacteria, *Pseudomonas agarici*, *Bacillus subtilis*, and *Streptococcus minor*, and for their antiplasmodial activity against *Plasmodium falciparum*, chloroquine-resistant strain W2.

Beilschmiedia (Lauraceae) is a pantropical genus of about 250 species most commonly represented in tropical regions of Africa and Asia. These plants are sources of alkaloids, arylpropanoids, benzopyrans, endiandric acids, and flavonoids.[1−5] Some of these constituents have shown activities in bioassay systems related to bacterial infections, malaria, and tuberculosis.[4,6,7] In Africa, the bark and leaves of some *Beilschmiedia* species are used in traditional medicine for the treatment of uterine tumors, rheumatism, and pulmonary disorders.[8] In Cameroon, the fruits of *B. manii*, *B. gabonensis*, and *B. zenkeri* are used as appetite stimulants and also as spices.[8] *Beilschmiedia zenkeri* Engl. is an evergreen tree, distributed throughout Central Africa. No phytochemical or pharmacological studies have been reported to date on this plant. In a continuing search for bioactive compounds from Cameroonian medicinal plants, we have investigated a dichloromethane extract of the stem bark of *B. zenkeri*, which was found to be active in vitro against *Pseudomonas agarici*, *Bacillus subtilis*, and *Streptococcus minor* with a MIC value of 250 μg/mL in each case. In this contribution, we report the isolation and structure elucidation of four new flavonoid derivatives (**1**−**4**) together with the antibacterial and in vitro antiplasmodial activities of several of the compounds isolated.

Results and Disscussion

The MeOH extract of the dried stem bark of *B. zenkeri* was partitioned with CH_2Cl_2 and water (1:1). Further fractionation and purification of the CH_2Cl_2 extract was carried out by column chromatography over silica gel to yield 11 compounds: (2*S*,4*R*)-5,6,7-trimethoxyflavan-4-ol (**1**), (2*S*,4*R*)-4,5,6,7-tetramethoxyflavan (**2**), beilschmieflavonoid A (**3**), beilschmieflavonoid B (**4**), 5-hydroxy-7,8-dimethoxyflavanone (**5**),[9] zanthomamide (**6**),[10] pipyahyine (**7**),[11] 2,3-dihydroxypropylpentadecanoate (**8**),[12] betulinic acid (**9**),[13] β-sitosterol, and sitosterol 3-*O*-β-D-glucopyranoside.[14]

Compound **1** was obtained as a white powder, $[\alpha]_D^{20}$ +10.4 (*c* 0.3, $CHCl_3$). The molecular formula, $C_{18}H_{20}O_5$, representing nine double-bond equivalents, was deduced from the HRESIMS, which showed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 339.11977 (calcd 339.11895 for $C_{18}H_{20}O_5Na$). The UV spectrum of compound



1 exhibited absorptions at λ_{max} 227 and 280 nm, suggesting a flavanol skeleton.[15−17] The broadband decoupled ^{13}C NMR spectrum of compound **1** (Table 1) displayed 18 carbon signals, which were sorted by DEPT and HSQC spectra into six quaternary carbons, eight methine groups [with two oxymethine groups at δ_C 74.0 (C-2) and 60.1 (C-4)], a methylene at δ_C 38.1 (C-3), and three methoxy groups at δ_C 56.3, 61.4, and 61.6. The ^1H NMR spectrum of compound **1** (Table 1) exhibited signals for a monosubstituted benzene ring between δ_H 7.35 and 7.50 (5H, m) and a shielded aromatic proton at δ_H 6.33 (s). The ^1H NMR spectrum of **1** also showed a signal for a free hydroxy group at δ_H 2.58 (br s, exchangeable with D_2O), two oxymethines at δ_H 5.15 (1H, dd, J = 12.6, 1.1 Hz, H-2) and 4.99 (1H, br d, J = 2.3 Hz, H-4), and two geminal protons at δ_H 2.05 (ddd, J = 14.4, 12.1, 3.8 Hz, H-3ax) and 2.27 (ddd, J = 14.4, 1.8, 1.8 Hz, H-3eq), suggesting the presence of a −CH−CH_2−CH− moiety. This unit was confirmed by the ^1H−^1H COSY experiment, from the correlations observed between H-2 and the pair H-3ax/H-3eq and from the coupling of

* To whom correspondence should be addressed. Tel: +237 75097561. E-mail: lentabruno@yahoo.fr.
† Higher Teachers' Training College, University of Yaoundé 1.
‡ Organic and Bioorganic Chemistry, Bielefeld University.
§ Department of Organic Chemistry, University of Yaoundé 1.
⊥ Inorganic and Structural Chemistry, Bielefeld University.

10.1021/np900341f
Published on Web 11/11/2009

Table 1. ^{13}C ($CDCl_3$, 125 MHz) and ^{1}H ($CDCl_3$, 500 MHz) NMR Data for Compounds **1** and **2**

	1			**2**		
position	δ_C	mult.	δ_H (J in Hz)	δ_C	mult.	δ_H (J in Hz)
2	74.0	CH	5.15, dd (12.1, 1.1)	73.5	CH	5.19, dd (12.6, 1.8)
3	38.1	CH_2	2.05, ddd (14.4, 12.1, 3.8)	34.2	CH_2	1.91, ddd (14.4, 12.6, 3.1)
			2.27, ddd (14.4, 1.8, 1.8)			2.35, ddd (14.4, 1.8, 1.8)
4	60.1	CH	4.99, br d (2.26)	68.1	CH	4.52, br t (2.5)
5	152.2	qC		152.5	qC	
6	135.8	qC		135.9	qC	
7	154.9	qC		154.6	qC	
8	96.5	CH	6.33, s	96.1	CH	6.31, s
9	151.6	qC		151.4	qC	
10	110.2	qC		108.1	qC	
1′	141.3	qC		141.1	qC	
2′,5′	126.7	CH	7.49, m	126.5	CH	7.5, m
3′,6′	129.0	CH	7.42, m	128.6	CH	7.42, m
4′	128.5	CH	7.35, m	128.1	CH	7.37, m
OH-4			2.58, br s			
OMe-4				56.1	CH_3	3.53, s
OMe-5	61.4	CH_3	4.07, s	61.0	CH_3	4.02, s
OMe-6	61.6	CH_3	3.84, s	61.3	CH_3	3.85, s
OMe-7	56.3	CH_3	3.84, s	55.9	CH_3	3.82, s

H-4 with the pair H-3ax/H-3eq. The presence of the methoxy groups in **1** was substantiated by signals observed in the ^{1}H NMR spectrum of compound **1** at δ_H 3.84 (6H, s) and 4.07 (3H, s). All these spectroscopic data clearly indicated that compound **1** is a trimethoxylated flavan-4-ol derivative. The positions of the methoxy groups were determined from the HMBC spectrum (Figure 2), which showed correlations between the aromatic singlet at δ_H 6.33 (H-8) and carbons C-7 (δ_C 154.9), C-9 (δ_C 151.6), and C-10 (δ_C 110.2) and between the methoxy protons at both δ_H 3.84 (6H, s) and 4.07 (3H, s) and C-6 (δ_C 135.8), C-7 (δ_C 154.9), and C-5 (δ_C 152.2), respectively. The location of the free hydroxy group at C-4 was deduced from the correlations observed in the HMBC spectrum between the proton at δ_H 2.58 (br s) and carbons C-3 (δ_C 38.1), C-4 (δ_C 60.1), and C-10 (δ_C 110.2). A NOESY experiment was performed to determine the configurations at C-2 and C-4, in which no cross-peak was observed between H-2 and H-4, suggesting their *trans* stereochemical relationship. Assignment of the axial proton at C-3 as the high-field proton of the H-3eq/H-3ax pair agrees with results obtained in cyclohexanes.[18] However, the value $J_{2,3ax} = 12.1$ Hz in compound **1** is so large that it could only arise from a *trans*-diaxial coupling, indicating that H-2 is axial and the 2-phenyl group is located in an equatorial orientation. The lower value of $J_{4,3ax}$ indicated that H-4 is in a quasi-equatorial position. The CD spectrum of **1** was compared with those of other flavan-4-ol derivatives, isolated from *Abacopteris penangiana,* to establish the absolute configurations at C-2 and C-4.[15,19] The CD spectrum of compound **1** showed a negative Cotton effect at 217 nm ($\Delta\varepsilon$ −0.33) and positive Cotton effects at 238 nm ($\Delta\varepsilon$ +0.43) and 281 nm ($\Delta\varepsilon$ +0.17). From these data, the 2*S*, 4*R* configuration was assigned to compound **1**.[15,19] Thus, the structure of **1** was established as (2*S*,4*R*)-5,6,7-trimethoxyflavan-4-ol.

Compound **2** was obtained as a yellow oil, $[\alpha]_D^{20}$ +16 (*c* 0.9, $CDCl_3$). Its HREIMS showed the molecular ion $[M]^+$ at *m/z* 330.14810, supporting the formula $C_{19}H_{22}O_5$ (calcd 330.14672), consistent with nine double-bond equivalents. This value is 14 mass units higher than that of compound **1**, suggesting the presence of an additional CH_2 unit in compound **2**. The spectroscopic data of compound **2** pointed to a close similarity with the structure of **1**. The major differences between **1** and **2** were the disappearance of the hydroxy group of **1** and the appearance of an additional methoxy group, resonating at δ_C 56.1 and δ_H 3.53. Accordingly, compound **2** is a methylated derivative of compound **1**. This was confirmed in its HMBC spectrum by the correlation observed between the methoxy protons at δ_H 3.53 and C-4 (δ_C 68.1). The *trans* stereochemical relationship between H-2 and H-4 was also deduced from the NOESY spectrum of compound **2**. Its CD spectrum showed positive Cotton effects at 237 nm ($\Delta\varepsilon$ +2.5) and 278 nm ($\Delta\varepsilon$ +1.4), indicating that **2** has the configuration 2*S*, 4*R*.[15,19] Thus, the structure of **2** was elucidated as (2*S*,4*R*)-4,5,6,7-tetramethoxyflavan.

Compound **3** was isolated as colorless needles, mp 236−237 °C, $[\alpha]_D^{20}$ +30.4 (*c* 0.3, $CHCl_3$). Its molecular formula, $C_{36}H_{38}O_9$, with 18 double-bond equivalents, was deduced from the HRESIMS, which showed a quasi-molecular ion peak $[M + Na]^+$ at *m/z* 637.24130 (calcd 637.24315). The UV spectrum showed absorptions at λ_{max} 227 and 280 nm, and the IR spectrum showed the

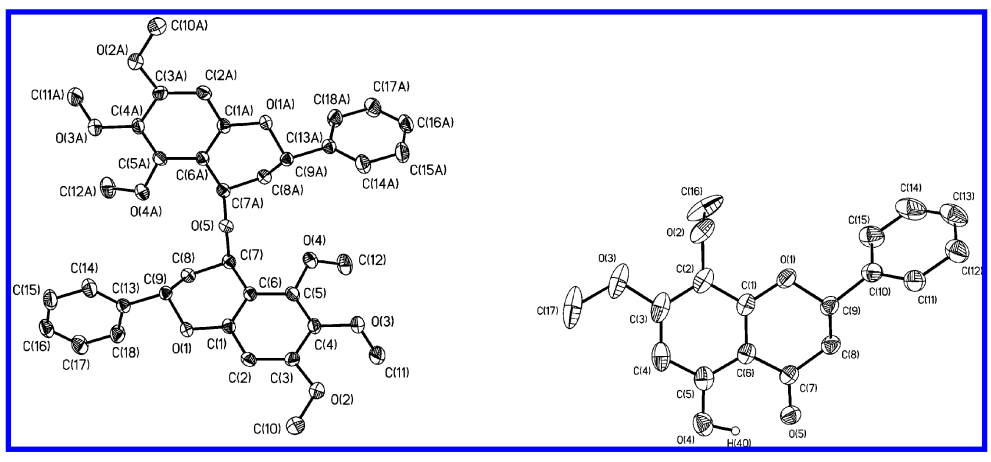


Figure 1. ORTEP diagrams of the crystal structures of compounds **3** and **5**.

Table 2. ^{13}C ($CDCl_3$, 125 MHz) and ^{1}H ($CDCl_3$, 500 MHz) NMR Data for Compounds **3** and **4**

	3			**4**		
position	δ_C	mult.	δ_H (J in Hz)	δ_C	mult.	δ_H (J in Hz)
2,2″	74.4	CH	5.28, br d (12.1)	74.1, 73.7	CH	5.38 br d (12.1); 5.42 (br d, 12.1)
3,3″	34.4	CH_2	1.87, ddd (14.7, 12.1, 3.0) 2.65, br d (14.7)	33.6, 33.9	CH_2	1.94, ddd (14.5, 12.1 2.5); 1.99, ddd (14.4, 12.1, 3.1) 2.78 (br d, 14.4); 2.93 (br d, 14.5)
4,4″	64.2	CH	4.72, br t (2.7)	63.5, 63.7	CH	4.95, br s; 4.86, br s
5,5″	152.6	qC		152.3, 152.5	qC	
6,6″	135.8	qC		129.4, 135.5	qC	
7,7″	155.0	qC		153.1, 154.3	qC	
8,8″	96.3	CH	6.19, s	92.6, 95.9	CH	6.1, s; 6.29, s
9,9″	152.5	qC		148.0, 152.0	qC	
10,10″	108.0	qC		102.4, 107.7	qC	
1′,1 ‴	141.9	qC		141.5, 141.6	qC	
2′,2 ‴	127.2	CH	7.51, m	126.8, 126.5	CH	7.60, m; 7.60, m
3′,3‴	128.9	CH	7.37, m	128.6, 128.5	CH	7.43, m; 7.47, m
4′,4‴	128.5	CH	7. 30, m	128.1, 128.0	CH	7.39, m; 7.39, m
5′,5‴	128.9	CH	7.37 m	128.6, 128.5	CH	7.43, m; 7.47, m
6′,6‴	127.2	CH	7.51 m	126.8, 126.5	CH	7.60, m; 7.60, m
OH-5						5.95, s
OMe-5,5″	60.9	CH_3	3.75, s	−, 60.6	CH_3	−; 3.85, s
OMe-6,6″	61.4	CH_3	3.75, s	61.0, 61.3	CH_3	3.85, s; 3.86, s
OMe-7,7″	56.3	CH_3	3.70, s	55.8, 55.7	CH_3	3.81, s; 3.81, s

presence of aromatic bands (1612 cm^{-1}) but lacked hydroxy and carbonyl absorptions. The ^{1}H NMR spectrum of **3** (Table 2) was similar to that of compound **1**, with characteristic signals of a monosubstituted aromatic ring between δ_H 7.30 and 7.51 (5H, m), an aromatic proton at δ_H 6.19 (s), geminal protons of one methylene group at δ_H 1.87 (ddd, J = 14.7, 12.1, 3.0 Hz, H-3ax) and 2.65 (br d, J = 14.7, H-3eq), two oxymethine groups at δ_H 4.72 (br t, J = 2.7 Hz, H-4) and 5.28 (1H, dd, J = 12.1, 1.2 Hz, H-2), and three methoxy groups at δ_H 3.70 (3H, s) and 3.75 (6H, s), but no hydroxy group. The disappearance of the C-4 free hydroxy group in compound **3** indicated that it may be a substituted derivative of compound **1**. The ^{13}C NMR spectrum of compound **3** (Table 2) exhibited signals for 18 carbons, which were sorted from the *J*mod and HSQC spectra into six quaternary carbons, eight methine groups [with two oxygenated carbons at δ_C 74.4 (C-2) and 64.2 (C-4)], one methylene at δ_C 34.4 (C-3), and three methoxy groups at δ_C 56.3, 60.9, and 61.4. The dimeric and symmetrical nature of this compound was deduced from the comparison of the number of carbons in the broadband decoupled ^{13}C NMR spectrum with the molecular formula. In the HMBC spectrum of this compound (Figure 2), the methoxy protons at δ_H 3.70 (3H, s) and 3.75 (6H, s) gave cross-peaks with C-7 (δ_C 155.0), C-5 (δ_C 152.6), and C-6 (δ_C 135.8), respectively, supporting their location. In the same spectrum, the singlet at δ_H 6.19 (H-8) gave cross-peaks with the carbons C-6 (δ_C 135.8), C-9 (δ_C 152.5), and C-10 (δ_C 108.0). All these data indicated clearly that compound **3** is a dimer of compound **1**. The $C_4-O-C_{4''}$ linkage of the two flavanol moieties was deduced from the correlations observed in the HMBC spectrum between proton H-4 (δ_H 4.72) and carbons C-2 (δ_C 74.4), C-4″ (δ_C 64.2), and C-9 (δ_C 152.5). X-ray crystallographic analysis of compound **3** showed that H-2/H-4 and H2″/H4″ have a *trans* relationship. The CD spectrum of compound **3** showed positive Cotton effects at 239 nm ($\Delta\varepsilon$ +0.83) and 280 nm ($\Delta\varepsilon$ +1.39). From these data, the 2*S*, 4*R* configuration was assigned to compound **3**.[15,19] Thus, compound **3** is a new biflavonoid that was named beilschmieflavonoid A, with the structure as shown.

Compound **4** was obtained as a white powder, $[\alpha]_D^{20}$ +18.2 (*c* 0.2, $CHCl_3$), and gave a positive reaction with ferric chloride, indicating its phenolic nature. A molecular formula of $C_{35}H_{36}O_9$ with 18 unsaturations was deduced from its HRESIMS, which exhibited a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 623.22540 (calcd 623.22700). Its IR spectrum showed the presence of aromatic (1606 cm^{-1}) and hydroxy (3400 cm^{-1}) bands, but lacked carbonyl absorptions. The ^{13}C NMR spectrum of compound **4** (Table 2) displayed 35 carbon signals, which were sorted by DEPT and HSQC into 12 quaternary carbons, 16 methine groups with four oxygenated carbons at δ_C 74.1 (C-2), 73.7 (C-2′), 63.5 (C-4), and 63.7 (C-4′), two methylene groups at δ_C 33.6 (C-3) and 33.9 (C-3′), and five methoxy groups at δ_C 55.7, 55.8, 60.6, 61.0, and 61.3. The ^{1}H NMR spectrum of compound **4** (Table 2) showed signals for two monosubstituted aromatic rings between δ_H 7.38 and 7.60 and three singlets each integrating for one proton at δ_H 5.95, 6.12 (H-8), and 6.29 (H-8″). The ^{1}H NMR spectrum also exhibited resonances for five methoxy groups at δ_H 3.81 (6H, s), 3.85 (6H, s), and 3.86 (3H, s). The ^{1}H NMR spectrum with the aid of the HSQC and COSY spectra also revealed the presence of two $-CH-CH_2-CH-$ moieties and indicated that the signal at δ_H 5.95 (1H, s, exchangeable with D_2O) was due to a free hydroxy group. All these data suggested that the structure of compound **4** is very close to that of compound **3** but with one methoxy group missing. In fact, in the HMBC spectrum of compound **4** (Figure 1), correlations were observed between the methoxy protons at δ_H 3.81 (6H, s) and C-7 (δ_C 153.1) and C-7″ (δ_C 154.3), between the protons at δ_H 3.85 (6H, s) and the carbons C-6″ (δ_C 135.5) and C-5″ (δ_C 152.5), and between the methoxy at δ_H 3.86 (3H, s) and C-6 (δ_C 129.4). These findings indicated clearly that the methoxy groups are located on two different rings, bearing two and three methoxy groups, respectively. The $C_4-O-C_{4''}$ linkage of the two flavanol moieties was also deduced from the correlations observed in the HMBC spectrum between H-4 (δ_H 4.95) and C-2 (δ_C 74.1) and C-4″ (δ_C 63.7) and between H-4″ (δ_H 4.86) and C-2″ (73.7) and C-4 (δ_C 63.5). No NOESY correlation was observed between H-2 and H-4 nor between H-2″ and H-4″, which suggested a *trans* relationship between these protons. This was confirmed by the value of 12.1 Hz for $J_{2,3ax}$ and $J_{2'',3ax''}$. The 2*S*, 4*R* and 2″*S*, 4″*R* configurations were determined from the CD spectrum of compound **4**, which showed a negative Cotton effect at 216 nm ($\Delta\varepsilon$ −4.55) and positive Cotton effects at 238 ($\Delta\varepsilon$ +3.48) and 281 nm ($\Delta\varepsilon$ +1.49).[9,12] Thus, compound **4** was assigned as a new biflavonoid named beilschmieflavonoid B, with the structure as shown.

In addition, the X-ray crystallographic analysis of the known 5-hydroxy-7,8-dimethoxyflavanone (**5**) was also performed and is reported herein. Beilschmieflavonoids A (**3**) and B (**4**) possess an unusual $C_4-O-C_{4''}$ linkage that has been found also in tepicanol A, a biflavonoid isolated from *Tephrosia tepicana* (Leguminosae).[20]

The antibacterial activity of the isolated compounds from the stem bark of *B. zenkeri* was evaluated in vitro against *Pseudomonas agarici*, *Bacillus subtilis*, and *Streptococcus minor.* Their activities were moderate compared to the reference drugs, ampicillin and gentamicin. Pipyahyine (**7**) showed some activity against *B. subtilis*

(MIC 163 μM) and *P. agarici* (MIC 81.5 μM), while compound **4** exhibited weak activity against *S. minor* (MIC 197.5 μM). The other compounds were found to be inactive against the test bacteria.

Compounds **1**−**9** were also tested for their antiplasmodial activity against the W2 strain of *P. falciparum*, which is resistant to chloroquine. 5-Hydroxy-7,8-dimethoxyflavanone (**5**), pipyahyine (**7**), and betulinic acid (**9**) exhibited antiplasmodial activities, with IC_{50} values of 9.3, 3.7, and 5.2 μM, respectively. Chloroquine (IC_{50} 0.13 μM) was used as the positive control.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi-540 melting point apparatus. Optical rotations were measured on a JASCO digital polarimeter (model DIP-3600). UV spectra were determined on a Spectronic Unicam spectrophotometer. CD spectra were measured in solution on a JASCO J-810 spectropolarimeter. IR spectra were determined on a JASCO Fourier transform IR-420 spectrometer. ^{1}H and ^{13}C NMR spectra were run on a Bruker spectrometer equipped with 5 mm ^{1}H and ^{13}C probes operating at 500 and 125 MHz, respectively, with TMS as internal standard. Silica gel 230−400 mesh (Merck) and silica gel 70−230 mesh (Merck) were used for flash and column chromatography, while precoated aluminum-backed silica gel 60 F254 sheets were used for TLC. Spots were visualized under UV light (254 and 365 nm) or using MeOH−H_2SO_4 reagent.

Plant Material. The stem bark of *Beilschmiedia zenkeri* was collected in June 2008 at Mount Kalla at Yaoundé in the central province of Cameroon. The plant was identified by Mr. Nana Victor, botanist at the National Herbarium of Cameroon, where a voucher specimen (no. HNC 18408) has been deposited.

Extraction and Isolation. The ground stem bark of *B. zenkeri* (4.3 kg) was extracted with methanol at room temperature for 72 h. Solvent was evaporated under reduced pressure and yielded 152 g of a methanol extract. The methanol extract was partitioned with dichloromethane and water (1:1) at room temperature to afford 30 g of a dichloromethane-soluble residue. This extract was fractionated by column chromatography over silica gel (0.023−0.20 mesh, Merck), eluting with mixtures of hexane−ethyl acetate of increasing polarity, resulting in the collection of 135 subfractions of 300 mL each, which were combined on the basis of TLC analysis to yield four main fractions, F1−F4. Fraction F1 (8 g) was a complex mixture that was not further studied. Fraction F2 (6.7 g) was subjected to column chromatography over silica gel (70−230 mesh), eluting with gradient mixtures of hexane−ethyl acetate (1:0 to 8:2), and resulted in 97 subfractions of 200 mL being collected. These were combined on the basis of TLC analysis into two subfractions, F21 and F22. Fraction F21 (3.2 g) was separated by column chromatography over silica gel (70−230 mesh), eluting with hexane−ethyl acetate (9:1), to yield sitosterol (1300 mg) and 5-hydroxy-7,8-dimethoxyflavanone (**5**) (153.2 mg). Fraction F22 (1.37 g) was separated by column chromatography over silica gel (70−230 mesh), eluted with hexane−ethyl acetate (9:15), to afford a fluorescent mixture, which was further purified by preparative TLC to afford (2*S*,4*R*)-4,5,6,7-tetramethoxyflavan (**2**) (8.0 mg), as a yellow oil.

Fraction F3 (7 g) was subjected to column chromatography over silica gel (70−230 mesh), for which elution with hexane−ethyl acetate mixtures of increasing polarity (8:2 to 1:1) resulted in 123 subfractions of 200 mL, which were combined on the basis of TLC analysis into three main subfractions, F31, F32, and F33. Fraction F31 (1.83 g) was separated by column chromatography eluting with petroleum ether−acetone (85:15), resulting in 63 subfractions of 100 mL each, which were combined on the basis of TLC analysis into two further subfractions, F31A and F31B. Column chromatography of F31A over silica gel eluted with hexane−ethyl acetate mixtures (8:2 to 7:3) afforded beilschmieflavonoid A (**3**) (9 mg) and beilschmieflavonoid B (**4**) (79.8 mg). Chromatography of F31B over silica gel eluted with a hexane−ethyl acetate mixture (7:3) afforded (2*S*,4*R*)-5,6,7-trimethoxyflavan-4-ol (**1**) (83.7 mg). Subfraction F32 was separated by column chromatography over silica gel eluting with hexane−ethyl acetate mixtures (8:2 to 6:4). Altogether, 75 subfractions of 100 mL each were collected and combined on the basis of TLC analysis into two additional subfractions, F32C and F32D. Successive chromatography of fraction F32C over silica gel eluted with hexane−ethyl acetate mixtures (7.5:2.5 to 6.5:3.5) yielded pipyahyine (**7**) (38.1 mg) and zanthonamide (**6**) (7.5 mg). Subfraction F33 was subjected to column chromatography over silica gel (70−230 mesh), eluting with hexane−ethyl acetate mixtures (6:4 to 1:1), and afforded betulinic acid (**9**) (11.7 mg) and 2,3-dihydroxypropylpentadecanoate (**8**) (4.9 mg). Chromatography of fraction F4 yielded sitosterol 3-*O*-β-D-glucopyranoside (131 mg).

(2*S*,4*R*)-5,6,7-Trimethoxyflavan-4-ol (1): white powder; $[\alpha]_D^{20}$ +10.4 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (4.06), 280 (3.35) nm; CD (*c* 1, MeOH) (nm), λ ($\Delta\varepsilon$) 217 (−0.33), 238 (+0.43), 281 (+0.17); IR (KBr) ν_{max} 3434, 2937, 1612, 1462, 1239, 1104, 701 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m*/*z* 316 (35), 298 (100), 283 (28), 267 (8), 197 (50), 169 (28), 127 (11), 115 (15), 104 (15), 91 (12), 69 (24); HRESIMS *m*/*z* 339.11977 (calcd for $C_{18}H_{20}O_5Na$, 339.11895).

(2*S*,4*R*)-4,5,6,7-Tetramethoxyflavan (2): yellow oil; $[\alpha]_D^{20}$ +16 (*c* 0.9, $CDCl_3$); UV (MeOH) λ_{max} (log ε) 227 (4.03), 280 (3.37) nm; CD (*c* 1, MeOH) (nm), λ ($\Delta\varepsilon$) 237(+2.5), 278 (+1.4); IR ($CHCl_3$) ν_{max} 2950, 1623, 1432, 1190, 1016, 710 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; EIMS *m*/*z* 330 (19), 298 (100), 283 (42), 268 (5), 221 (31), 141(6), 115 (7), 69 (11); HREIMS *m*/*z* 330.14810 (calcd for $C_{19}H_{22}O_5$, 330.14672).

Beilschmieflavonoid A (3): colorless crystal needles; mp 236−237 °C; $[\alpha]_D^{20}$ +30.4 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (5.28), 280 (3.52) nm; CD (*c* 1, CH_3CN) (nm), λ ($\Delta\varepsilon$) 239 (+0.83), 281 (+1.39); IR ($CHCl_3$) ν_{max} 2937, 1612, 1462, 1195, 1016, 701 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz,) and ^{13}C NMR ($CDCl_3$, 125 MHz,), see Table 2; EIMS *m*/*z* 614 (21), 316 (7.81), 299 (100), 283 (17), 269 (10), 197 (19), 169 (8), 104 (5), 91 (13), 69 (8); HRESIMS *m*/*z* 637.24130 (calcd for $C_{36}H_{38}O_9Na$, 637.24315).

X-ray Crystallography Data of Beilschmieflavan A (3). A colorless crystal of approximate dimensions 0.04 × 0.12 × 0.30 mm^3 was obtained from hexane−ethyl acetate (8.5:1.5). The data were collected on a Bruker Nonius Kappa CCD instrument using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystal data: $C_{36}H_{38}O_9$, *M* = 614.66, monoclinic, space group *C*2, *a* = 25.0637(15) Å; *b* = 4.7474(2) Å; *c* = 16.5619(10) Å; β = 127.470(2)°; *V* = 1564.05(15) Å^3, *Z* = 2, D_{calc} = 1.305 mg/m^3, *F*(000) = 652, μ(Mo Kα) = 0.093 mm^{-1}, *T* = 100(2) K. A total of 9744 reflections were collected (2013 unique, R_{int} = 0.042). The structure was solved by direct methods and refined using SHELX-97.[21] The final *R* value was 0.0399 for 1704 reflections with $I > 2\sigma(I)$ and 0.1010 for all unique reflections, 207 parameters. Due to the lack of any heavy atom, the absolute configuration was not reliably determined from the X-ray data. The complete crystallographic data for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number 715722. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

Beilschmieflavonoid B (4): white powder; $[\alpha]_D^{20}$ +18.2 (*c* 0.2, $CHCl_3$,); UV (MeOH) λ_{max} (log ε) 227 (5.18), 280 (3.69) nm; CD (*c* 1, MeOH) (nm), λ ($\Delta\varepsilon$) 217 (−4.55), 238 (+3.48), 281 (+1.49); IR ($CDCl_3$) ν_{max} 3408, 1612, 1487, 1370, 1103, 700; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS *m*/*z* 600 (2), 316 (3), 298 (100), 283 (46), 269 (12), 221 (25), 207 (9), 197 (7), 169 (7), 127 (11), 115 (14), 104 (5), 91 (11), 68 (17).43 (85), 39 (86); HRESIMS *m*/*z* 623.22540 (calcd for $C_{35}H_{36}O_9Na$, 623.22700).

X-ray Crystallography Data of 5-Hydroxy-7,8-dimethoxyflavanone (5): A colorless needle of approximate dimensions 0.07 × 0.09 × 0.30 mm^3 was obtained from hexane−ethyl acetate (9:10). The data were collected on a Bruker Nonius Kappa CCD instrument using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystal data: $C_{17}H_{16}O_5$, *M* = 300.30, monoclinic, space group *C*2, *a* = 23.7529(13) Å; *b* = 4.7381(2) Å; *c* = 14.3636(8) Å; β = 117.8029°; *V* = 1429.93(13) Å^3, *Z* = 4, D_{calc} = 1.395 mg/m^3, *F*(000) = 632, μ(Mo Kα) = 0.103 mm^{-1}, *T* = 100(2) K. A total of 9420 reflections were collected (3092 unique, R_{int} = 0.042). The structure was solved by direct methods and refined using SHELX-97.[21] The final *R* value was 0.0435 for 2687 reflections with $I > 2\sigma(I)$ and 0.1056 for all unique reflections, 206 parameters. Due to the lack of any heavy atom, the absolute configuration was not reliably determined from the X-ray data. The complete crystallographic data for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number 715723. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

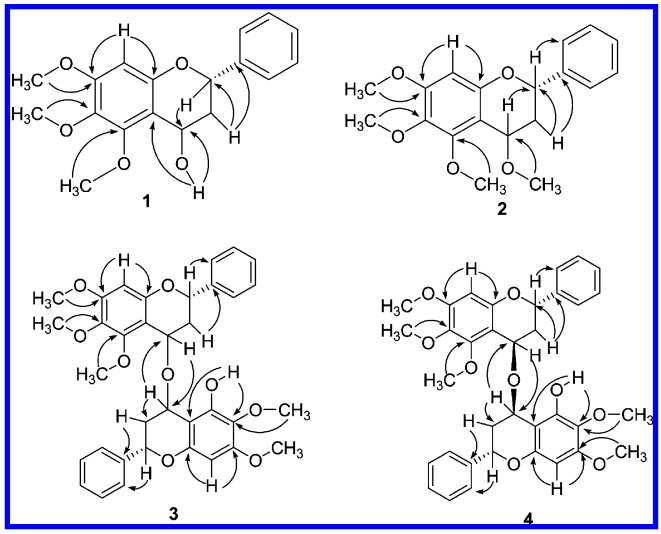


Figure 2. Selected HMBC correlations for compounds **1**−**4**.

In Vitro Antibacterial Assay. In vitro antibacterial activity tests of the extracts and pure compounds were performed by the agar well diffusion method as described in previous reports.[22,23] Ampicillin was used as positive control for *B. subtilis* and *S. minor* and exhibited a MIC value of 1.05 μM for both strains, while gentamicin was used as positive control for *P. agarici* and exhibited a MIC value of 5.6 μM.

In Vitro Antiplasmodial Assay. In vitro antiplasmodial activities of the pure compounds were performed according to the method previously described by Ngouamegne et al.[24] Chloroquine was used as positive control and exhibited an IC_{50} of 0.13 μM.

Acknowledgment. The authors wish to acknowledge the European Commission for awarding a Marie Curie postdoctoral fellowship to B.N.L. (MIF1-CT-2006-021591) and the Alexander von Humboldt Foundation, Germany, for awarding Georg Forster postdoctoral fellowships to K.P.D. and J.D.W., all at Bielefeld University. They also wish to thank Prof. Phillip J. Rosenthal for the antiplasmodial testing.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compound **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

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NP900341F