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Antimicrobial Isopropenyl-dihydrofuranoisoflavones from *Crotalaria lachnophora*

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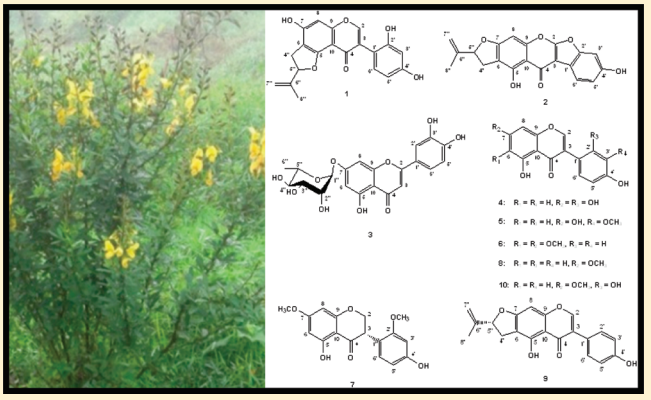
Supporting Information

ABSTRACT: Two new isopropenyl-dihydrofuranoisoflavones exhibiting antimicrobial properties have been isolated along with eight known compounds from the Cameroonian medicinal plant *Crotalaria lachnophora*. The structures of the new compounds were elucidated by 1D and 2D NMR spectroscopy and high-resolution mass spectrometry as 7,2′,4′-trihydroxy-5″-isopropenyl-4″,5″-dihydrofurano[2″,3″:5,6]isoflavone (**1**) and 4,8-dihydroxy-2-isopropenyl-2,3-dihydro-5*H*-[1]benzofuro-[2,3-*b*]furo[3,2-*g*]chromen-5-one (**2**). The CH_2Cl_2/MeOH (1:1) extract and the compounds isolated were subjected to in vitro antimicrobial assays against a panel of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria and fungi. The new compounds, named lachnoisoflavones A (**1**) and B (**2**), showed moderate inhibitory activities against *Escherichia coli* and *Klebsiella pneumoniae*.



The genus *Crotalaria*, which belongs to the family Fabaceae, subfamily Papilionoideae, and tribe Crotalarieae, contains approximately 600 species distributed throughout the temperate, subtropical, and tropical regions of the world.[1] About 500 species of *Crotalaria* occur in Africa,[2] of which ca. 10 species have been found in Cameroon. *Crotalaria lachnophora* (A. Rich.) is a perennial shrub or subshrub, which is found in grassland or woodland at 900−2200 m altitude. The seeds of the plant are considered edible in the Democratic Republic of Congo, while the whole plant is promoted in Rwanda as a green manure crop in rotation systems.[3] In traditional medicine, the leaf sap of *C. lachnophora* is dropped into the ear or drunk to treat otitis and a decoction of the whole plant is used for the treatment of ear diseases, helminthes, and skin diseases.

A literature survey indicated that one pyrrolizidine alkaloid has been identified from the seeds of *C. lachnophora*,[4] and no phytochemical investigation has been done so far on the whole plant. Nevertheless, previous phytochemical studies of members of this genus have revealed the presence of pyrrolizidine alkaloids,[5−8] flavonoids,[9] chalcones,[10] lectins,[11] polysaccharides,[12] and triterpenoids.[13]

As part of our ongoing search for antimicrobial compounds from Cameroonian medicinal plants, two new isopropenyl-dihydrofuranoisoflavones named lachnoisoflavones A (**1**) and B (**2**) together with eight known compounds (**3**−**10**) were isolated from the whole plant of *C. lachnophora*. In addition, the antimicrobial efficacies of the CH_2Cl_2/MeOH (1:1) crude extract and compounds isolated (**1**−**10**) have been investigated against the Gram-positive bacterium *Staphylococcus aureus* subsp. *aureus*, Gram-negative bacteria *Klebsiella pneumoniae* subsp. *ozaenae*, *Pseudomonas aeruginosa*, and *Escherichia coli*, and the fungal organisms *Aspergillus niger* and *Candida albicans*.

The chloroform-soluble fraction of the CH_2Cl_2/MeOH (1:1) crude extract of the whole plant of *C. lachnophora* was fractionated by reversed-phase HPLC-UV to yield lachnoisoflavones A (**1**) and B (**2**), together with eight known compounds: luteolin-7-*O*-α-L-rhamnoside (**3**),[14] 2′-hydroxygenistein (**4**),[15] 3′-*O*-methylorobol (**5**),[16] 7-*O*-methyltectorigenin (**6**),[17] cajanol (**7**),[18] prunetin (**8**),[19] licoagroisoflavone (**9**),[20] and cajanin (**10**)[21] (Figure 1). The known compounds were identified by comparison of their experimental and reported physical data.

Lachnoisoflavone A (**1**) was obtained as an amorphous, pale yellow powder. Its molecular formula $C_{20}H_{16}O_6$ was obtained from its FT-APCI-MS, which showed a quasi-molecular ion peak $[M + H]^+$ at *m*/*z* 353.1021. The ^{1}H NMR spectrum (Table1, Figure S1, Supporting Information (SI)) showed a singlet at δ

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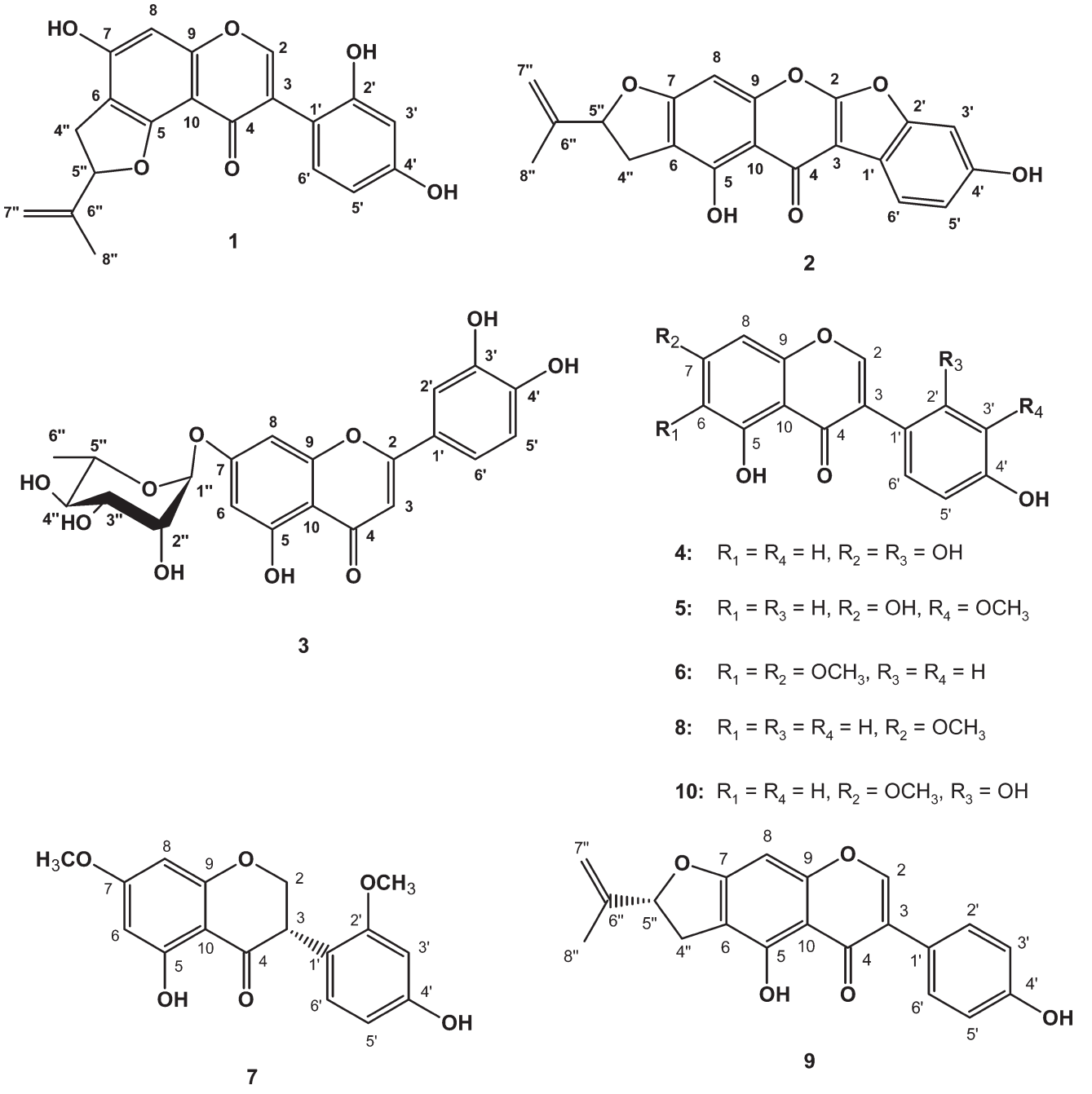

Figure 1. Compounds (**1**−**10**) isolated from *Crotalaria lachnophora*.

8.06, which is characteristic of the olefinic C-2 proton of isoflavones.[22] Moreover, the ^{1}H NMR spectrum exhibited the presence of ABX-type aromatic proton signals at δ 7.07 (1H, d, J = 8.2 Hz), 6.43 (1H, d, J = 2.2 Hz), and 6.39 (1H, dd, J = 8.2, 2.2 Hz) due to B-ring protons and a singlet aromatic proton signal at δ 6.46 (1H, s) due to an A-ring proton. The relative shielded value of the chemical shift of the aromatic carbon at δ 104.3 (C-3′) was indicative of its *ortho* position to the hydroxylated carbons at δ 157.8 (C-2′) and 160.3 (C-4′).[15] Proton signals at δ 1.80 (3H, brs, H_3-8″), 2.99 (1H, dd, J = 15.6, 7.4 Hz, H_a-4″), 3.39 (1H, dd, J = 15.6, 9.6 Hz, H_b-4″), 4.97 (1H, brs, H_a-7″), 5.12 (1H, brs, H_b-7″), and 5.42 (1H, brdd, J = 9.6, 7.4 Hz, H-5″) were indicative of an isopropenyl-dihydrofuran group.[20,23] Important fragments obtained by LC-MS^2 of the quasi-molecular ion peak (m/z 353.1018) confirmed the presence of the isopropenyl-dihydrofuran group that is attached to the A-ring. This is supported by the prominent fragment observed at m/z 219 via a retro-Diels−Alder cleavage. The HMBC spectrum (Figure S5, SI) showed correlations between the methylene protons [δ = 2.99 (H_a-4″); 3.39 (H_b-4″)] of the isopropenyl-dihydrofuran moiety and some carbons [δ = 157.9 (C-5); 109.0 (C-6); 167.8 (C-7)] of the A-ring. The position of the isopropenyl-dihydrofuran side chain was therefore assigned as located at C-6 and C-5 on the basis of LC-MS^2, HMBC, and HMQC experiments [δ = 8.44 (7-OH)]. From the spectroscopic data above and by comparison to the previously reported isopropenyl-dihydrofuranoisoflavone,[19,22] the structure of lachnoisoflavone

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Lachnoisoflavone A (1) in Methanol-d_4 [δ (ppm)]

position	δ_C	δ_H, mult. (J in Hz)	HMBC (H → C)
2	156.8	8.06, s	C-1′, 3, 4, 9
3	122.7		
4	182.9		
5	157.9		
6	109.0		
7	167.8		
8	89.8	6.46, s	C-7, 9, 10
9	159.9		
10	109.9		
1′	110.7		
2′	157.8		
3′	104.3	6.43, d (2.2)	C-1′, 2′, 5′
4′	160.3		
5′	108.2	6.39, dd (8.2, 2.2)	C-1′, 3′
6′	133.2	7.07, d (8.2)	C-2′, 3, 4′
4″	31.5	3.39, dd (15.6, 9.6), H_b-4″	C-5″, 5, 6″, 6, 7
		2.99, dd (15.6, 7.4), H_a-4″	C-5″, 5, 6″, 6, 7
5″	89.5	5.42, brdd (9.6, 7.4)	C-7″, 8″
6″	145.0		
7″	112.8	5.12, brs, H_b-7″	C-5″, 6″, 7″, 8″
		4.97, brs, H_a-7″	C-5″, 7″, 8″
8″	17.1	1.80, s	C-5″, 6″, 7″
7-OH		8.44, brs	C-7

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Lachnoisoflavone B (2) in $CDCl_3$ [δ (ppm)]

position	δ_C	δ_H, mult. (J in Hz)	HMBC (H → C)
2			
3	99.1		
4			
5	158.9		
6	111.4		
7	166.7		
8	91.5	6.53, s	C-6, 7, 9, 10
9	156.7		
10	106.2		
1′			
2′	156.3		
3′	100.8	7.06, d (2.9)	
4′	151.8		
5′	115.2	6.94, dd (8.3, 2.9)	
6′	123.6	7.89, d (8.3)	C-2′, 4′
4″	31.9	3.41, dd (15.5, 9.7), H_b-4″	C-5, 6, 6″, 7
		3.06, dd (15.5, 7.9), H_a-4″	C-5, 6, 6″, 7
5″	89.9	5.38, brdd (9.7, 7.9)	
6″	144.6		
7″	114.2	5.12, brs, H_b-7″	C-5″, 6″, 8″
		4.97, brs, H_a-7″	C-7″, 8″
8″	18.7	1.80, s	C-5″, 6″, 7″
5-OH		13.09, brs	

A (**1**) was assigned as 7,2′,4′-trihydroxy-5″-isopropenyl-4″,5″-dihydrofurano[2″,3″:5,6] isoflavone.

Lachnoisoflavone B (**2**) was obtained as a yellow, amorphous powder. Its FT-APCI-MS spectrum showed a quasi-molecular ion peak $[M + H]^+$ at m/z 351.0862, corresponding to the molecular formula $C_{20}H_{14}O_6$, which is 2 amu lower than that of lachnoisoflavone A (**1**) and consistent with 14 double-bond equivalents. This difference is probably due to a cyclization process involving 2′-OH and C-2. This is substantiated by the lack of the signal corresponding to H-2 in its ^{1}H NMR spectrum (Table 2, Figures S8 and S9, SI). Moreover, the ^{1}H NMR spectrum (Table 2) exhibited signals at δ 6.53 (s) and 13.09 (brs) owing to H-8 and 5-OH, respectively. Furthermore, the ^{1}H NMR spectrum showed a similar isopropenyl-dihydrofuran group compared to that of lachnoisoflavone A (**1**) (Table 1). The HMBC spectrum (Figure S12, SI) showed correlations between the methylene protons [δ = 3.06 (H_a-4″); 3.41 (H_b-4″)] of the isopropenyl-dihydrofuran moiety and some carbons [δ = 158.9 (C-5); 111.4 (C-6); and 166.7 (C-7)] of the A-ring. The isopropenyl-dihydrofuran side chain was therefore assigned as located at C-6 and C-7 on the basis of the HMBC experiment. From the NMR data above and by comparison to the data obtained from lachnoisoflavone A (**1**), the structure of this new compound was assigned as 4,8-dihydroxy-2-isopropenyl-2,3-dihydro-5*H*-[1]benzofuro[2,3-*b*]furo[3,2-*g*]chromen-5-one, to which the trivial name lachnoisoflavone B (**2**) was assigned.

A correlation study between the theoretical electronic structures and optical properties as well as experimental electronic absorption and CD spectra was performed. The optimized most stable geometries of two possible enantiomers of **1** and **2**, considering C-5″, are depicted in Figure S15 (SI). The corresponding electronic absorption and CD spectra in solution are summarized in Table S16 (SI). The good correlation between the different theoretical models shows the suitable applicability of the method for determination of enantiomers with further possible application for elucidation of this type of compounds.[26−29] The n → σ^* transition of the carbonyl group is obtained about 320 nm. The experimental CD spectrum and $[\alpha]_D$ value of **1** and **2** in methanol are in accord with the result of a chiral HPLC-MS/MS separation of **1**, which exhibited two signals with the same peak area (Figure S17, SI). This indicated the presence of a racemic mixture. However, the chiral separation of compound **2** was not successful.

The CH_2Cl_2/MeOH (1:1) crude extract and compounds **1**−**10** were tested for their in vitro antimicrobial activities (Table 3). Our results showed that, of those tested, compounds **3**, **6**, **7**, **8**, **9**, and **10** were the most potent. From the results obtained, it is evident that the activities of compounds **7**−**10** were quite pronounced on the clinically important Gram-negative bacterium *K. pneumoniae*. On the other hand, the crude extract and compounds **3** and **6** were significantly effective against *E. coli*. Interestingly, none of these compounds were active at the concentration tested against the bacteria *S. aureus* and *P. aeruginosa* or against the fungal organisms *A. niger* and *C. albicans*. This may suggest the specificity of such compounds against *K. pneumoniae* and *E. coli*. Comparison of compounds **1**−**10** with the antibiotics streptomycin, tetracycline, ampicillin, and gentamycin as reference positives revealed that the degrees of efficacy were less than those of the established antibiotics. Nevertheless, it was noted that, of all the compounds tested, **7**−**10** were effective against *K. pneumoniae* in a range putatively comparable to the standard antibiotic ampicillin. It is evident that the C-7 sugar moiety of **3** is responsible for its pronounced antimicrobial efficacy against *E. coli*. In general, it was observed that C-7 substitution greatly influenced the overall antimicrobial

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Table 3. Antimicrobial Activity (diameters of growth inhibition zones[b]) of the Crude Extract and Compounds 1−10 as Well as the Standard Compounds

sample	*Staphylococcus aureus* subsp. *aureus* (DSM 799)	*Klebsiella pneumoniae* subsp. *ozaenae* (DSM 681)	*Pseudomonas aeruginosa* (DSM 1128)	*Escherichia coli* (DSM 682)	*Aspergillus niger* (DSM 1988)	*Candida albicans* (DSM 1386)
streptomycin	na[a]	$21.3 \pm 0.6^{a}_{I}$	na	$19.3 \pm 1.5^{d}_{I}$	NA[e]	NA
tetracycline	$20.7 \pm 0.6^{a}_{J}$	$26.3 \pm 1.5^{d}_{J}$	$17.7 \pm 0.6^{a}_{J}$	$22.3 \pm 0.6^{a}_{J}$	NA	NA
ampicillin	na	$11.7 \pm 0.6^{a}_{K}$	na	$24.3 \pm 0.6^{a}_{K}$	NA	NA
gentamycin	$15.3 \pm 0.6^{a}_{L}$	$14.3 \pm 0.6^{a}_{L}$	$20.3 \pm 0.6^{a}_{L}$	$16.3 \pm 0.6^{a}_{L}$	NA	NA
crude extract	na[b,c,d]	$7.3 \pm 0.6^{a}_{A}$	na	$9.7 \pm 0.6^{a}_{A}$	na	na
1	na	$7.0 \pm 0.0^{b}_{D}$	na	$8.3 \pm 0.6^{a}_{D}$	na	na
2	na	$7.3 \pm 0.6^{a}_{H}$	na	$7.0 \pm 0.0^{b}_{H}$	na	na
3	na	$7.7 \pm 0.6^{a}_{B}$	na	$10.7 \pm 0.6^{a}_{B}$	na	na
4	na	$7.0 \pm 0.0^{b}_{C}$	na	$7.0 \pm 0.0^{b}_{C}$	na	na
5	na	$8.3 \pm 0.6^{a}_{D}$	na	$7.0 \pm 0.0^{b}_{D}$	na	na
6	na	$7.0 \pm 0.0^{b}_{E}$	na	$9.3 \pm 0.6^{a}_{E}$	na	na
7 and **8**	na	$9.3 \pm 1.2^{c}_{F}$	na	$7.3 \pm 0.6^{a}_{F}$	na	na
9	na	$9.3 \pm 0.6^{a}_{G}$	na	$7.3 \pm 0.6^{a}_{G}$	na	na
10	na	$9.7 \pm 0.6^{a}_{A}$	na	$7.3 \pm 0.6^{a}_{A}$	na	na

[a] na − Not active at the tested concentration against the specific microorganism. [b] Mean values ± SD (in mm) of three independent experiments, including the diameter of the disk (6.0 mm). [c] The mean values followed by different superscripts within each column indicate their variance at a given probability level ($\alpha \leq 0.05$) according to the one-way ANOVA test. [d] The mean values followed by different subscripts within each column indicate that they were significantly different at that particular probability level according to the one-way ANOVA test ($\alpha \leq 0.05$). [e] NA − Not applicable to the specific microorganism.

effectiveness against specific microorganisms. For example, the C-7 methoxy- or hydroxy-substituted compounds **7**, **8**, and **10** significantly increased the efficacy against the clinically relevant *K. pneumoniae*. However, when a 7-OCH_3 group was supported by a similar group at C-6, the specificity of activity broadened to *E. coli* too, as evidenced with compound **6**. We could evaluate the differential potentials for these compounds by correlating the efficacy of each compound with its structure and by comparing the activities of all the compounds with their structures. Thus, our results on the antimicrobial activities of compounds **1**−**10** have enabled us to establish the basic pattern of action for this class of compounds and might facilitate the development of new derivatives by substituting various groups at the effective positions. These may serve as more potent antimicrobial agents against specific pathogenic microorganisms.

Our investigation on the in vitro antimicrobial activity of *C. lachnophora* indicated moderate activities either for the crude extract or for the compounds isolated. Nevertheless, the most active were luteolin-7-*O*-α-L-rhamnoside (**3**) (10.7 mm) and the crude extract (9.7 mm) against *E. coli* and cajanin (**10**) (9.7 mm), cajanol (**7**)/prunetin (**8**) (9.3 mm), and licoagroisoflavone (**9**) (9.3 mm) against *K. pneumoniae*. Flavonoids have been reported to possess antimicrobial activities. Thus, our results on the antimicrobial activities of the two new isopropenyl-dihydrofuranoisoflavones (**1** and **2**), the flavone (**3**), the isoflavanone (**7**) and isoflavones (**4**−**6**, **8**−**10**) isolated from *C. lachnophora* have, therefore, reinforced the previous findings on the efficiency of these classes of compounds against pathogenic microorganisms.

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-500 at 500 and 125 MHz, respectively. Chemical shifts (δ) are quoted in parts per million (ppm) from internal standard tetramethylsilane (TMS). Preparative reversed-phase HPLC was run for 40 min on a Gilson apparatus with UV detection at 254 nm using a Nucleodur column C_{18}, 5 μm (250 × 16 mm), with H_2O (0.1% HCOOH) (A)−MeOH (0.1% HCOOH) (B) with a gradient program as follows (flow rate 8 mL min^{-1}): 70% A linear to 75% B for 15 min, linear gradient to 20% A over 13 min, linear gradient to 0% A over 2 min, after 100% B isocratic for 7 min, the system was then returned to its initial condition (70% A) over 1 min and was equilibrated for 2 min. CD spectra were recorded on a Jasco J-715 CD spectrometer.

The FT-APCI-MS spectra were obtained with an LTQ-Orbitrap spectrometer (Thermo Fischer, USA). The spectrometer was operated in positive mode (1 spectrum s^{-1}; mass range 150−800; with nominal mass resolving power of 60 000 at m/z 400 at a scan rate of 1 Hz, spray voltage 5 kV, tube lens 80 V) with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using an internal calibration standard; bis(2-ethylhexyl)phthalate: m/z = 391.284586. The spectrometer was equipped with a surveyor HPLC system (Thermo Fisher, USA) consisting of LC-pump, PDA detector, and autosampler (injection volume 10 μL). N_2 was employed as both the sheath (50 arbitrary units) and auxiliary (10 arbitrary units) gas, and helium served as the collision gas. The capillary temperature for the TSQ was set to 190 °C. The vaporizer temperature was set to 400 °C. MS/MS measurements were performed by collision-induced dissociation (CID) in the linear trap of the LTQ-Orbitrap at different energy levels. The separations were performed using a Phenomenex Synergi Fusion RP column (4 μm, 3 × 150 mm) (Torrance, CA, USA) with a H_2O (+0.1% HCOOH/+10 mM NH_4Ac) (C) −MeCN (+0.1% HCOOH) (D) gradient (flow rate 500 μL min^{-1}). Samples were analyzed using a gradient program as follows: 95% C isocratic for 4 min, linear gradient to 0% C over 20 min, after 100% D isocratic for 13 min, the system returned to its initial condition (95% C) within 1 min and was equilibrated for 7 min. Chiral separation of **1** was achieved after dissolving the compound in MeOH and analyzing directly using a chiral LUX 5 μ Cellulose-1 column (150 × 4.60 mm, Phenomenex, Torrance, CA, USA). Isocratic elution was performed after injection of 5 μL using acetonitrile/water/2-propanol (48:45:7, v/v/v) with 0.05% formic acid and 0.05 mM ammonium acetate at a flow rate of 0.9 mL min^{-1}. Selected reaction monitoring of m/z 353.1 → 219.0 ($M + H^+$) was performed at a collision energy of 25 V using a TSQ Quantum Ultra AM mass

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spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an HESI-II ion source operating in positive mode. Nitrogen was employed as both the drying and nebulizer gas. Dwell time was 0.3 s at a peak width of 0.5.

Plant Material. The whole plant of *Crotalaria lachnophora* (Fabacaea) was collected during the flowering period in Dschang (west region of Cameroon) in September 2007 and identified by Mr. Nana, a retired botanist of the Cameroon National Herbarium, where a plant specimen (voucher no. 17313/SRF/Cam) was deposited.

Extraction and Fractionation. The dried and powdered whole plant (1 kg) of *C. lachnophora* was percolated (3 L × 3) in a mixture of CH_2Cl_2/MeOH (1:1). After three days, the solution was filtered, and removal of the solvent in vacuo yielded 25 g of residue, which was dissolved in a mixture of MeOH/$CHCl_3$/H_2O (2:2:1). The $CHCl_3$-soluble portion (5 g) was subjected to reversed-phase HPLC as described above to afford 16 main fractions (I−XVI). Analytical HPLC of each of these fractions showed that fractions II (**3**, 50 mg), XII (**1**, 16 mg), and XVI (**2**, 20 mg) are pure. Mixture fractions with separable quantities were further purified by repeated semipreparative HPLC at the same wavelength and solvent system using an Alltech (Alltech Associates, Inc., Deerfield, IL) Alltima C_{18} column, 5 μm particle size (250 × 10 mm), to give **4** (5 mg), **5** (5 mg), **6** (3 mg), **7** (4 mg), **8** (3 mg), **9** (6 mg), and **10** (5 mg).

Lachnoisoflavone A (1): amorphous, pale yellow powder (CH_3COCH_3); molecular formula $C_{20}H_{16}O_6$; UV (MeOH) λ_{max} 262, 289 nm; $[\alpha]_D^{20}$ 0.002 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3448, 2928, 1618, 1508, 1209, 1030, 780 cm^{-1}; ^1H (methanol-d_4, 500 MHz) and ^{13}C (methanol-d_4, 125 MHz) NMR (Table 1, S1, S2); FT-APCI-MS^2 (CID at 50 eV) (rel int) (S6) *m/z* 335 (95), 325 (30), 311 (35), 299 (85), 283 (30), 265 (10), 219 (100), 201 (15), 165 (10); FT-APCI-MS $[M + H]^+$ (S7) *m/z* 353.1021 (calcd for $C_{20}H_{17}O_6$, 353.1019).

Lachnoisoflavone B (2): amorphous, pale yellow powder (CH_3COCH_3); molecular formula $C_{20}H_{14}O_6$; UV (MeOH) λ_{max} 262, 289 nm; $[\alpha]_D^{20}$ 0.002 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3424, 2929, 1718, 1625, 1508, 1162, 1050, 780 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C ($CDCl_3$, 125 MHz) NMR (Table 2, S8); FT-APCI-MS^2 (CID at 50 eV) (rel int) (S13) *m/z* 336 (30), 333 (40), 323 (35), 309 (30), 305 (23), 297 (100), 281 (20)); FT-APCI-MS $[M + H]^+$ (S14) *m/z* 351.0862 (calcd for $C_{20}H_{15}O_6$, 351.0863).

Luteolin-7-*O*-α-L-rhamnoside (3): yellow powder (CH_3COCH_3); molecular formula $C_{21}H_{20}O_{10}$; UV (MeOH) λ_{max} 255, 350 nm; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.89 (1H, brs, 5-OH), 8.24 (1H, brs, 4′-OH), 7.45 (1H, d, *J* = 2.0 Hz, H-2′), 7.43 (1H, d, *J* = 8.0 Hz, H-5′), 6.88 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.80 (1H, d, *J* = 2.0 Hz, H-8), 6.73 (1H, s, H-3), 6.43 (1H, d, *J* = 2.0 Hz, H-6), 5.56 (1H, d, *J* = 2.0 Hz, H-1″), 3.84−3.40 (4H, rhamnose), 1.12 (3H, d, *J* = 5.6 Hz, H-6″); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 181.9 (C, C-4), 164.5 (C, C-2), 161.7 (C, C-7), 161.2 (C, C-5), 156.9 (C, C-9), 150.1 (C, C-4′), 145.9 (C, C-3′), 121.3 (C, C-1′), 119.2 (CH, C-2′), 116.1 (CH, C-6′), 113.6 (CH, C-5′), 105.4 (C, C-10), 103.1 (CH, C-3), 99.7 (CH, C-6), 98.5 (CH, C-1″), 94.7 (CH, C-8), 71.6 (CH, C-4″), 70.3 (CH, C-2″), 70.1 (CH, C-3″), 69.9 (CH, C-5″), 18.0 (CH_3, C-6″); FT-AP CI-MS^2 (CID at 50 eV) (rel int) *m/z* 287 $[M + H - 146]^+$ (100); FT-APCI-MS $[M + H]^+$ *m/z* 433.1126 (calcd for $C_{21}H_{21}O_{10}$, 433.1129).

2′-Hydroxygenistein (4): yellow powder (CH_3COCH_3); mp 270−271 °C; molecular formula $C_{15}H_{10}O_6$; UV (MeOH) λ_{max} 230, 255, 265, 350 nm; IR (KBr) ν_{max} 3485, 2945, 1620, 1198, 817, 790 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.50 (1H, s, H-2), 7.37 (1H, dd, *J* = 8.2, 2.0 Hz, H-5′), 7.35 (1H, d, *J* = 2.0 Hz, H-3′), 6.88 (1H, d, *J* = 8.2 Hz, H-6′), 6.42 (1H, d, *J* = 1.7 Hz, H-8), 6.18 (1H, d, *J* = 1.7 Hz, H-6); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 179.0 (C, CO), 165.0 (C, C-7), 163.0 (C, C-5), 161.5 (C, C-9), 160.2 (C, C-2′), 158.1 (C, C-4′), 154.5 (CH, C-2), 133.0 (CH, C-6′), 125.3 (C, C-3), 112.5 (C, C-1′), 109.0 (C, C-10), 108.3 (CH, C-3′), 104.7 (CH, C-5′), 97.7 (CH, C-6), 96.1 (CH, C-8); FT-APCI-MS^2 (CID at 50 eV) (rel int) *m/z* 269 (20), 259 (15), 245 (35), 241 (30), 231 (15), 219 (20), 261 (10), 153 (100); FT-APCI-MS $[M + H]^+$ *m/z* 287.0550 (calcd for $C_{15}H_{11}O_6$, 287.0550).

3′-*O*-Methylorobol (5): yellow powder (CH_3COCH_3); mp 218−222 °C; molecular formula $C_{16}H_{12}O_6$; UV (MeOH) λ_{max} 245, 255, 295, 325 nm; IR (KBr) ν_{max} 3480, 2955, 1615, 1580, 1301, 1198, 817, 790 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.94 (1H, brs, 5-OH), 9.63 (1H, brs, 7-OH), 8.33 (1H, brs, 4′-OH), 8.29 (1H, s, H-2), 7.36 (1H, d, *J* = 8.5 Hz, H-5′), 6.80 (1H, dd, *J* = 8.5, 1.5 Hz, H-6′), 6.35 (1H, d, *J* = 1.5 Hz, H-8), 6.19 (1H, d, *J* = 1.5 Hz, H-6), 3.78 (3H, s, 3′-OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 180.1 (C, C-4), 165.2 (C, C-7), 161.9 (C, C-5), 157.6 (C, C-4′), 157.3 (C, C-9), 153.8 (CH, C-2), 147.2 (C, C-3′), 130.0 (CH, C-5′), 122.2 (C, C-3), 121.2 (C, C-1′), 115.0 (CH, C-6′), 113.3 (CH, C-2′), 104.1 (C, C-10), 99.1 (CH, C-6), 93.7 (CH, C-8), 55.7 (CH_3, 3′-OCH_3); FT-APCI-MS^2 (CID at 50 eV) (rel int) *m/z* 253 (35), 243 (85), 225 (20), 215 (93), 153 (100), 149 (30); FT-APCI-MS $[M + H]^+$ *m/z* 301.0706 (calcd for $C_{16}H_{13}O_6$, 301.0707).

7-*O*-Methyltectorigenin (6): yellow powder (CH_3COCH_3); mp 228−229 °C; molecular formula $C_{17}H_{14}O_6$; UV (MeOH) λ_{max} 255, 295, 340 nm; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.77 (1H, brs, 5-OH), 8.43 (1H, s, H-2), 7.37 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 6.82 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.61 (1H, s, H-8), 3.91 (3H, s, 7-OCH_3), 3.76 (3H, s, 6-OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 180.8 (C, C-4), 166.6 (C, C-5), 158.4 (C, C-7), 157.6 (C, C-4′), 154.6 (CH, C-2), 149.3 (C, C-9), 130.0 (2CH, C-2′, C-6′), 129.1 (C, C-6), 122.3 (C, C-3), 121.1 (C, C-1′), 115.2 (2CH, C-3′, C-5′), 104.5 (C, C-10), 96.2 (CH, C-8), 61.2 (CH_3, 6-OCH_3), 56.7 (CH_3, 7-OCH_3); FT-APCI-MS^2 (CID at 50 eV) (rel int) *m/z* 300 (100), 282 (15), 271 (30); FT-APCI-MS $[M + H]^+$ *m/z* 315.0863 (calcd for $C_{17}H_{15}O_6$, 315.0863).

Cajanol (7): yellow powder (CH_3COCH_3); mp 149 °C; molecular formula $C_{17}H_{16}O_6$; UV (MeOH) λ_{max} 275, 285, 325 nm; $[\alpha]_D^{20}$ +3.5 (*c* 0.01, MeOH); ^1H NMR (DMSO-d_6, 500 MHz) δ 12.24 (1H, brs, 5-OH), 6.90 (1H, d, *J* = 8.2 Hz, H-6′), 6.42 (1H, d, *J* = 2.2 Hz, H-3′), 6.31 (1H, dd, *J* = 8.2, 2.2 Hz, H-5′), 6.09 (1H, d, *J* = 2.3 Hz, H-6), 6.07 (1H, d, *J* = 2.3 Hz, H-8), 4.48 (1H, t, *J* = 11.0 Hz, H-2b), 4.41 (1H, dd, *J* = 11.0, 5.6 Hz, H-2a), 4.28 (1H, dd, *J* = 11.0, 5.6 Hz, H-3), 3.86 (3H, s, 4′-OCH_3), 3.79 (3H, s, 7-OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 197.5 (C, C-4), 167.0 (C, C-7), 165.5 (C, C-5), 165.1 (C, C-4′), 162.7 (C, C-9), 157.9 (C, C-2′), 130.7 (CH, C-6′), 113.1 (C, C-1′), 106.8 (C, C-5′), 102.6 (C, C-10), 99.5 (CH, C-3′), 94.3 (CH, C-6), 93.5 (CH, C-8), 69.6 (CH_2, C-2), 55.9 (CH_3, 2′-OCH_3), 55.6 (CH_3, 7-OCH_3), 45.8 (CH, C-3); FT-APCI-MS^2 (CID at 50 eV) (rel int) *m/z* 299 (25), 289 (15), 193 (80), 181 (5), 165 (100), 149 (10), 137 (98); FT-APCI-MS $[M + H]^+$ *m/z* 317.1019 (calcd for $C_{17}H_{17}O_6$, 317.1019).

Prunetin (8): yellow powder (CH_3COCH_3); mp 246−248 °C; molecular formula $C_{16}H_{12}O_5$; UV (MeOH) λ_{max} 240, 260 nm; ^1H NMR (DMSO-d_6, 500 MHz) δ 12.96 (1H, brs, 5-OH), 8.40 (1H, s, H-2), 7.38 (2H, d, *J* = 8.6 Hz, H-2′, H-6′), 6.81 (2H, d, *J* = 8.6 Hz, H-3′, H-5′), 6.66 (1H, d, *J* = 2.2 Hz, H-8), 6.42 (1H, d, *J* = 2.2 Hz, H-6), 3.67 (3H, s, 7-OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 180.1 (C, C-4), 165.5 (C, C-5), 157.9 (C, C-7), 157.5 (2C, C-4′, C-9), 154.3 (CH, C-2), 129.8 (2CH, C-2′, C-6′), 122.5 (C, C-3), 120.7 (C, C-1′), 114.9 (2CH, C-3′, C-5′), 105.3 (C, C-10), 99.5 (CH, C-6), 92.3 (CH, C-8), 55.3 (CH_3, 7-OCH_3); FT-APCI-MS^2 (CID at 50 eV) (rel int) *m/z* 253 (35), 243 (85), 225 (20), 215 (93), 153 (100), 149 (30); FT-APCI-MS $[M + H]^+$ *m/z* 285.0757 (calcd for $C_{16}H_{13}O_5$, 285.0757).

Licoagroisoflavone (9): pale yellow powder (CH_3COCH_3); mp 196−198 °C; molecular formula $C_{20}H_{16}O_5$; UV (MeOH) λ_{max} 214, 264 nm; $[\alpha]_D^{20}$ +67.0 (*c* 0.013, MeOH); ^1H NMR (DMSO-d_6, 500 MHz) δ 13.20 (1H, brs, 5-OH), 8.51 (1H, brs, 4′-OH), 8.37 (1H, s, H-2), 7.37 (2H, d, *J* = 8.5 Hz, H-2′, H-6′), 6.80 (2H, d, *J* = 8.5 Hz, H-3′, H-5′), 6.60 (1H, s, H-8), 5.44 (1H, brdd, *J* = 9.2, 7.7 Hz, H-5″), 5.08

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(1H, brs, H-7″b), 4.94 (1H, brs, H-7″a), 3.35 (1H, dd, J = 15.6, 9.2 Hz, H-4″b), 2.92 (1H, dd, J = 15.5, 7.7 Hz, H-4″a), 1.72 (3H, s, H-8″); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 180.7 (C, C-4), 165.9 (C, C-7), 158.0 (C, C-9), 157.6 (C, C-4′), 156.5 (C, C-5), 154.3 (CH, C-2), 143.0 (C, C-6″), 130.6 (2CH, C-2′, C-6′), 122.5 (C, C-3), 120.9 (C, C-1′), 115.2 (2CH, C-3′, C-5′), 112.8 (CH_2, C-7″), 108.6 (C, C-6), 105.7 (C, C-10), 89.2 (CH, C-8), 87.8 (CH, C-5″), 29.9 (CH_2, C-4″), 17.0 (CH_3, C-8″); FT-APCI-MS^2 (CID at 50 eV) (rel int) m/z 337 (100), 319 (30), 295 (20), 283 (40), 267 (15); FT-APCI-MS $[M + H]^+$ m/z 337.1069 (calcd for $C_{20}H_{17}O_5$, 337.1070).

Cajanin (10): pale yellow powder (CH_3COCH_3); mp 208−210 °C; molecular formula $C_{16}H_{12}O_6$; UV (MeOH) λ_{max} 255, 280, 335 nm; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.94 (1H, brs, 5-OH), 10.25 (1H, brs, 4′−OH), 8.49 (1H, s, H-2), 7.72 (1H, d, J = 8.2 Hz, H-6′), 7.13 (1H, d, J = 1.7 Hz, H-3′), 6.93 (1H, dd, J = 8.2, 1.7 Hz, H-5′), 6.86 (1H, d, J = 2.0 Hz, H-8), 6.50 (1H, d, J = 2.0 Hz, H-6), 3.87 (3H, s, 7-OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 180.1 (C, C-4), 165.1 (CH, C-2), 164.5 (C, C-7), 161.8 (C, C-5), 156.5 (C, C-4′), 154.7 (C, C-9), 149.8 (C, C-2′), 122.2 (C, C-3), 120.7 (C, C-1′), 120.6 (CH, C-6′), 113.5 (CH, C-5′), 104.0 (C, C-10), 98.3 (CH, C-6), 97.3 (CH, C-3′), 93.4 (CH, C-8), 56.1 (CH_3, 7-OCH_3); FT-APCI-MS $[M + H]^+$ m/z 301.0634 (calcd for $C_{16}H_{13}O_6$, 301.0637).

Compounds and Microorganisms Used for Antimicrobial Assay. The in vitro antimicrobial activities of the compounds **1**−**10** and the crude extract were tested against a panel of standard pathogenic control strains belonging to the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, DSMZ), Braunschweig, Germany. The Gram-positive bacterium *Staphylococcus aureus* subsp. *aureus* (DSM 799), Gram-negative bacteria *Klebsiella pneumoniae* subsp. *ozaenae* (DSM 681), *Pseudomonas aeruginosa* (DSM 1128), and *Escherichia coli* (DSM 682), and the fungal microorganisms *Aspergillus niger* (DSM 1988) and *Candida albicans* (DSM 1386) were used. The activation, maintenance, and preparation of working culture suspensions were carried out in accordance with established procedures.[24] The crude extract and compounds tested (**1**−**10**) were dissolved in HPLC grade MeOH at a concentration of 1 μg/μL. Additionally, four antibiotics, namely, streptomycin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), tetracycline (Sigma-Aldrich), ampicillin (Sigma-Aldrich), and gentamycin (Sigma-Aldrich), were used simultaneously as reference standards. The standards were also prepared at the concentration of 1 μg/μL in sterile double-distilled H_2O.

Antimicrobial Assay. A disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI)[25] was employed for the determination of the antimicrobial activity of the samples. All agar plates were prepared in 90 mm sterile Petri dishes (TPP, Trasadingen, Switzerland) with 22 mL of agar, giving a final depth of 4 mm. Inoculum suspension (100 μL) was spread on the solid media plates using the standard spread-plate technique. When testing the fungi, 100 μL of inoculum suspension was poured into the molten agar plates using a sterile micropipet, when the temperature reached around 40−45 °C, and homogenized thoroughly by mixing in a circular motion (pour-plate technique). Sterile assay paper disks (Schleicher & Schuell GmbH, Dassel, Germany; 6.0 mm in diameter) were impregnated with 40 μL of the samples, air-dried under the laminar air flow hood, and placed on inoculated plates. After allowing standing at 4 °C for 2 h, the plates were incubated at 37 °C for 24 h for bacteria or at 28 °C for 48 h for fungi. Three control sets were included. One control was the organism control and consisted of a seeded Petri dish with no sample. In the second control, samples were applied to unseeded Petri dishes to check for sterility. Finally, the solvent effect was controlled by a disk treated with 40 μL of HPLC grade MeOH or with 40 μL of sterile double-distilled H_2O run simultaneously. We used the standard antibiotics as reference standards in parallel to reveal the comparative antimicrobial efficacy of compounds **1**−**10** against the tested organisms. The diameters of the inhibition zones were measured in millimeters (to the nearest mm). Each test was repeated three times, and the mean values (±SD) were calculated.

Statistical Analysis. A one-way ANOVA test was applied in order to evaluate statistically any significant differences among mean values. The level of significance selected was 5% ($\alpha \leq 0.05$) in all tests. The mean values (±SD) followed by different superscripts within each column of Table 3 indicate their variance at a given probability level ($\alpha \leq 0.05$) according to the one-way ANOVA test. Furthermore, the mean values (±SD) followed by different subscripts within each column of Table 3 indicate that they were significantly different at that probability level.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR, ^{13}C NMR, ^{1}H,^{1}H COSY NMR, HMQC NMR, HMBC NMR, FT-APCI-MS, and FT-APCI-MS/MS of lachnoisoflavone A (**1**); ^{1}H NMR, ^{1}H,^{1}H COSY NMR, HMQC NMR, HMBC NMR, FT-APCI-MS, and FT-APCI-MS/MS of lachnoisoflavone B (**2**); computational details including Gaussian 98, Dalton 2.0, and GaussView03 references; electronic absorption and ECD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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