Isoprenoids and Flavonoids with Antimicrobial Activity from *Ficus conraui* WARBURG (Moraceae)

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A new triterpene, conrauidienol (**1**), and a new dihydroflavonol, conrauiflavonol (**2**), along with β-amyrin acetate (**3**), betulinic acid (**4**), ursolic acid (**5**), 6β-hydroxystigmasta-4,22-dien-3-one (**6**), 8-prenylapigenin (**7**), β-sitosterol glucoside (**8**), and 3,4′,5-trihydroxy-6″,6″-dimethylpyrano[2,3-g]flavone (**9**) were isolated from the stem barks of *Ficus conraui* WARBURG (Moraceae). Their structures were elucidated by spectroscopic analysis. The hexane, AcOEt, and MeOH extracts, as well as the new isolated compounds exhibited selective antimicrobial activities varying from weak to moderate.

Introduction. – As part of our continuing investigation of Cameroonian medicinal plants, we have examined the MeOH extract of the stem of *Ficus conraui* WARBURG (Moraceae). This plant is an epiphytic shrub, strangling or lianescent, occasionally developing into a small tree of *ca.* 3–4-m height, and occurring in Ivory Coast, Nigeria, Uganda, and Cameroon [1][2]. Its fruits are used as ingredient in some foods, and its foliage is ornamental [2]. The genus *Ficus* is known for its biological properties such as antimicrobial [3][4], antioxidant, antihepatotoxic [5], anti-HIV activity [6], *etc.* Previous phytochemical studies of this genus resulted in the isolation of flavonoids, coumarins, alkaloids, steroids, triterpenes, and ceramides [7–9]. To the best of our knowledge, no previous phytochemical study has been reported on this species.

Results and Discussion. – The hexane- and AcOEt-soluble fractions of the MeOH extract of the stem of *Ficus conraui* WARBURG was subjected, respectively, to repeated chromatography to afford a new triterpene, conrauidienol (**1**), and a new dihydroflavonol, conrauiflavonol (**2**), together with seven known compounds, β-amyrin acetate (**3**) [8], betulinic acid (**4**) [8][10], ursolic acid (**5**) [11], 6β-hydroxystigmasta-4,22-dien-3-one (**6**) [12], 8-prenylapigenin (**7**) [13], β-sitosterol glucoside (**8**) [14], and 3,4′,5-

trihydroxy-6″,6″-dimethylpyrano[2,3-*g*]flavone (**9**) [15]. Structures of the isolated compounds are presented in *Fig. 1*. Here, we describe the isolation and structure elucidation of the new compounds, as well as the evaluation of the antimicrobial activity of extracts and of some pure isolated compounds.

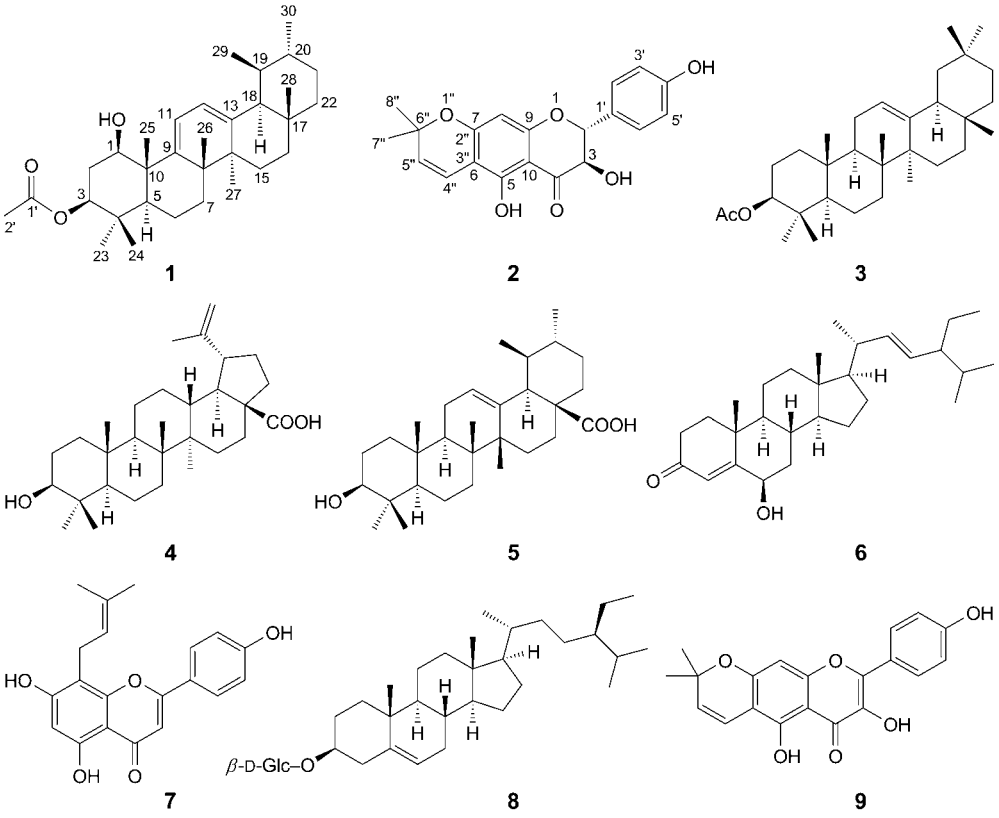

Fig. 1. *Chemical structures of compounds* **1**–**9**

Conrauidienol (**1**), isolated as a white amorphous powder, gave a positive test with *Liebermann–Burchard* reagent, characteristic of triterpenes. The molecular formula $C_{32}H_{50}O_3$ was assigned based on HR-EI-MS which showed the molecular-ion peak at 482.3853 (calc. 482.3819). The ^{1}H-NMR spectrum (*Table 1*) showed two vinyl H-atom signals as an *AB* pair of *doublet*s at δ(H) 5.50 and 6.53 ($J = 5.9$) coupled, respectively, in the HMQC with the C-atom signals at δ(C) 123.5 and 117.7. This ^{1}H-NMR also exhibited two *doublet* of *doublet*s at δ(H) 3.97 (*dd*, $J = 4.2, 12.0$, 1 H) and 4.58 (*dd*, $J =$ 4.2, 12.3, 1 H) coupled, respectively, in the HMQC with the resonances at δ(C) 77.9 and 75.6, assignable to two H-atoms at OH- and AcO-bearing C-atoms. The large coupling constant of these two CH H-atoms is consistent with β-orientations of the OH and AcO groups. Still in the ^{1}H-NMR, one sharp *singlet* at δ(H) 2.08 indicating the presence of one Ac group was observed, while the upfield region of the spectrum displayed signals for six tertiary (δ(H) 0.87, 0.89, 0.91, 0.92, 1.19, and 1.30) and two secondary (δ(H) 0.84

and 0.95) Me groups in the molecule. These data suggested an ursene-type skeleton [11]. The ^{13}C-NMR spectrum showed signals for 32 C-atoms due to nine Me, seven CH_2, eight CH (including two olefinic and two O-bearing CH) groups, and eight quaternary C-atoms according to DEPT experiments. The location of Ac and OH groups at C(1) and C(3), respectively was based on the HMBC between the H-atom signals at δ(H) 0.91 (Me(24)) and 1.30 (Me(25)), respectively, with those of the C-atoms at δ(C) 77.9 (C(3)), 48.8 (C(5)) on the one hand, and 75.6 (C(1)), 48.8 (C(5)), and 152.1 (C(9)), on the other. Therefore, compound **1** was determined as 3β-acetylursa-9(11),12-dien-1β-ol (= conrauidienol) by comparison of its spectral data with those of the structurally related ursa-9(11),12-dien-3β-ol acetate and 3β-acetylurs-14(15)-en-16-one [16–18]. Key HMBC and COSY correlations of **1** are presented in *Fig. 2*.

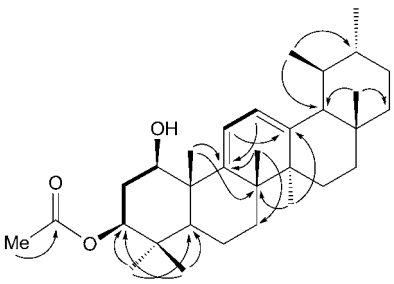

Fig. 2. *Key HMBC* (H → C) *and COSY* (—) *correlations of compound* **1**

Conrauiflavonol (**2**), isolated as a yellow amorphous powder, gave an intense purple-pink color when treated in MeOH solution with granular Mg and concentrated HCl (*Shinoda* test), indicative of a flavonoid derivative [19]. The HR-EI-MS of compound **2** exhibited exact molecular mass at *m*/*z* 353.9804, corresponding to the molecular formula $C_{20}H_{18}O_6$ (calc. 353.9801). The ^{1}H-NMR spectrum of **2** (*Table 2*) showed a typical *AB* system at δ(H) 4.70 and 5.13 (*d*, J = 11.4, each 1 H) characteristic for H–C(3) and H–C(2), respectively, in a dihydroflavonol skeleton with a *trans*-configuration [20–22]. This is confirmed by its UV spectrum with absorption maxima at 289 (2.63) and 310 (2.44) nm, and ^{13}C-NMR data (*Table 2*) with resonances at δ(C) 83.5 (C(2)) and 72.3 (C(3)). The ^{1}H-NMR spectrum of **2** also exhibited a signal at δ(H) 12.04 (chelated OH group at C(5), an *AA′BB′* system consisting of a pair of *doublet*s at δ(H) 6.99 (J = 8.7, 2 H) and 7.43 (J = 8.4, 2 H) correlating in the HMQC spectrum with C-atom signals at δ(C) 129.4 (C(2′/6′), 115.1 (C(3′/5′), respectively, assignable to a 1,4-disubstituted benzene ring [22]. The presence of a geminal-dimethyl-pyranyl ring was derived from the 6-H *singlet* at δ(H) 1.45 due to the geminal dimethyl group and the *AB* system at δ(H) 6.60 (*d*, J = 10.2, 1 H) and 5.66 (*d*, J = 10.2, 1 H). The remaining *singlet* at δ(H) 5.90 (1 H) assignable to a single aromatic *A*-ring H-atom, indicated that this ring is fused to the geminal-dimethyl-pyranyl moiety [23]. This was supported by the EI-TOF-MS (pos.-ion mode) data in which a *retro-Diels–Alder* (RDA) fragment-ion peak was observed at *m*/*z* 219 resulting from the ring *A*. This confirmed that ring *A* possesses a H-atom, a fused geminal-dimethyl-pyranyl moiety and a OH group [23]. The HMQC correlation between the H-atom signal at δ(H) 5.90

Table 1. *^1H- and ^{13}C-NMR* ($CDCl_3$) *Data of* **1**[a]). δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC (H → C)
1	3.97 (*dd*, J = 4.2, 12.0)	75.6	
2	1.79 (*dd*, J = 4.2, 12.0, H_{eq}–C(2), 1.97 (*dd*, J = 4.2, 12.3, H_{ax}–C(2)	34.5	
3	4.58 (*dd*, J = 4.2, 12.3)	77.9	
4		40.9	
5		48.8	
6		18.4	
7		31.0	
8		44.7	
9		152.1	
10		38.0	
11	6.53 (*d*, J = 5.9)	117.7	C(8), C(13), C(14)
12	5.50 (*d*, J = 5.9)	123.5	C(9), C(18), C(19)
13		141.7	
14		43.3	
15		28.3	
16		26.2	
17		33.7	
18		57.3	
19		39.4	
20		39.1	
21		31.2	
22		41.3	
23	0.89 (*s*)	27.7	
24	0.91 (*s*)	17.4	C(3), C(5)
25	1.30 (*s*)	17.8	C(1), C(5), C(9)
26	1.19 (*s*)	21.2	C(7), C(9), C(8)
27	0.92 (*s*)	23.0	C(8), C(13)
28	0.87 (*s*)	28.7	C(18), C(22)
29	0.84 (*d*, J = 6.9)	16.2	C(18), C(20)
30	0.95 (*d*, J = 7.0)	18.6	
*Me*COO	2.08 (*s*)	21.5	C=O
Me*C*OO		170.8	

[a]) All assignments are based on the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectral data.

and the upfield C-atom signal at δ(C) 95.9, and the comparison of these data with those of the corresponding angular compound [24] indicated a linear fusion of the geminal dimethyl unit to ring *A* with position C(8) being unsubstituted [20][25]. A positive $n \rightarrow \pi^*$ band CD maximum in the 330–340 nm wavelength region, and the similarity of the CD spectrum in the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ region to those of dihydroflavonols with established configuration indicated an absolute (*R*)-configuration at both C(2) and C(3) [23][26–29]. The Structure of compound **2**, conrauiflavonol, was, thus, elucidated as shown in *Fig. 1*.

The *in vitro* antimicrobial activities of hexane, AcOEt, MeOH, BuOH extracts, and of four isolated compounds from the stem of *Ficus conraui* were evaluated. The results (*Table 3*) revealed that all extracts and compounds exhibited varying levels of

Table 2. *^{1}H- and ^{13}C-NMR* ((D_6)acetone) *Data of* **2**[a]). δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1.*

Position	δ(H)	δ(C)	HMBC (H → C)
2	5.13 (*d*, *J* = 11.4)	83.5	C(3), C(1′), C(4)
3	4.70 (*d*, *J* = 11.4)	72.3	C(3), C(1′), C(6′)
4		198.0	
5		157.8	
6		103.0	
7		162.0	
8	5.90 (*s*)	95.9	C(6), C(7), C(9)
9		162.5	
10		101.0	
1′		128.1	
2′	7.43 (*d*, *J* = 8.4)	129.4	
3′	6.99 (*d*, *J* = 8.7)	115.1	C(3′), C(1′), C(4′)
4′		157.9	
5′	6.99 (*d*, *J* = 8.7)	115.1	
6′	7.43 (*d*, *J* = 8.4)	129.4	C(2), C(3′), C(4′), C(6′)
4″	6.60 (*d*, *J* = 10.2)	114.7	C(5), C(6), C(7), C(6″), C(5″)
5″	5.66 (*d*, *J* = 9.9)	126.7	C(6), C(6″), C(7″)
6″		78.2	
7″, 8″	1.45 (*s*)	27.6	C(5″), C(6″)
HO–C(3)	4.77 (br. *s*)		C(2′)
HO–C(5)	12.04 (br. *s*)		
HO–C(4′)	8.55 (br. *s*)		

[a]) All assignments are based on the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectral data.

antibacterial activities except the BuOH extract which did not show any inhibitory effect on bacterial growth. The hexane extract was the most active with *MIC* values ranging from 256 to 512 μg/ml. Antimicrobial activities of plant extracts can be classified as significant ($MIC < 100$ μg/ml), moderate ($100 < MIC \leq 625$ μg/ml), and weak ($MIC > 625$ μg/ml) [30]. According to this classification, the inhibition potential of the extract tested could be considered as weak-to-moderate. Concerning the pure isolated compounds, the lowest *MIC* value of 32 μg/ml was noted with **1** both on *Escherichia coli* ATCC8739 and *Enterobacter aerogenes* ATCC13048. *MIC* Values recorded for all the compounds tested were higher than that of the reference antibiotic (RA) chloramphenicol. Compared to the activity of the RA against the tested microbial species, the antibacterial activities of the tested compounds could be considered as weak. The whole data obtained from this study suggest that extracts and compounds from *Ficus conraui* exhibited selective antimicrobial activities varying from weak to moderate (see *Table 3*). Compounds with enough evidence for their antimicrobial activities such as betulinic acid (**4**) [31], β-sitosterol glucoside (**8**) [32], and β-amyrin acetate (**3**) [33] were not tested again in the present work. We are not aware of any previous report on the antimicrobial activities of 6β-hydroxystigmasta-4,22-dien-3-one (**6**) and 8-prenylapigenin (**7**). Nevertheless, apigenin [34] and apigenin -7-*O*-glucoside [35] are known to exhibit good antibacterial activities. The overall results of

Table 3. *Minimal Inhibitory Concentration* (*MIC*, [μg/ml]) of *Extracts and Compounds from* Ficus conraui *Stem Barks*

Tested samples	Bacterial strains				
	Escherichia coli ATCC8739	*Providencia stuartii* ATCC29916	*Enterobacter aerogenes* ATCC13048	*Escherichia coli* ATCC10536	*Klebsiella pneumoniae* ATCC11296
Extracts					
MeOH extract	>1024	1024	1024	512	512
Hexane extract	512	512	256	512	512
AcOEt extract	>1024	1024	>1024	1024	1024
BuOH extract	>1024	>1024	>1024	>1024	>1024
Compounds					
KFC6 (**1**)	32	64	32	64	64
KFC7 (**2**)	64	64	128	128	64
KFC12 (**9**)	>256	>256	256	256	128
KFC17 (**5**)	>256	128	128	64	64
Chloramphenicol	1	4	8	0.5	2

this study indicated that the antimicrobial activity of the crude extract from *F. conraui* could be due to the presence of antibacterial compounds in the extract.

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Experimental Part

General. Vacuum column chromatography (VCC): silica gel *60* (SiO_2; 0.04–0.063 mm). TLC: Pre-coated plates of SiO_2 GF_{254}; detection under UV light and spraying with anylsaldehyde/H_2SO_4 in EtOH or H_2O/H_2SO_4. Optical rotations: *Perkin–Elmer 241* polarimeter at 25°. UV Spectra: *Shidmadzu UV-210* PC UV/VIS spectrometer in acetone or $CHCl_3$ solns.; λ_{max} (log ε) in nm. CD Spectra: *JASCO J-600* spectropolarimeter in acetone or $CHCl_3$ at 25°. ^{1}H- and ^{13}C-NMR spectra: *Bruker* spectrometer operating at 300 and 75 MHz, resp.; in $CDCl_3$, (D_6)acetone, or (D_6)DMSO, with the residual solvent peak as internal references, δ in ppm, *J* in Hz. EI-TOF-MS (pos.): *Finnigan SSQ-7000* spectrometer by direct inlet (70 eV); in *m/z* (rel. %).

Plant Material. The stem of *Ficus conraui* WARBURG was collected in March 2006, in 'mont Kala', in the center region of Cameroon, and the plant was identified by Mr. *Nana Victor* at the National Herbarium, Yaounde, where a voucher specimen (No. 26999/SRF/Cam) is deposited.

Extraction and Isolation. The powdered stem of *Ficus conraui* WARBURG (3.8 kg) was extracted with MeOH at r.t. for 48 h. Evaporation of the solvent under reduced pressure provided a MeOH extract (80 g), which was re-dissolved in MeOH/H_2O 1:9 and partitioned with hexane (1 l), AcOEt (1 l), and BuOH (1 l) to give 23, 17, and 10 g of extracts, resp. The hexane extract (20 g) was subjected to VLC (SiO_2 (100 g, 0.04–0.063 mm); hexane/AcOEt of increasing polarity (1:0, *Frs. 1–8*; 19:1, *Frs. 9–19*; 9:1, *Frs. 20–28*; 4:1, *Frs. 29–35*; 7:3, *Frs. 36–41*; 3:2, *Frs. 42–47*; 1:1, *Frs. 48–53*; 1:3, *Frs. 54–59*, and 0:1, *Frs. 60–65*)). On the basis of anal. TLC, fractions were combined and further purified by CC (SiO_2; hexane/AcOEt (and CH_2Cl_2/MeOH) of increasing polarities). From *Frs. 1–10*, β-amyrin acetate (KFC1; **3**; 80 mg) was obtained after filtration and purification by recrystallization. *Frs. 18–28* yielded betulinic acid (KFC3; **4**; 15 mg). *Frs. 36–47* gave ursolic acid (KFC17; **5**; 15 mg), 6β-hydroxystigmasta-4,22-dien-3-one (KFC19; **6**; 10 mg), KFC6 (**1**; 7 mg), and KFC7 (**2**; 15 mg). *Frs. 48–53* yielded 8-prenylapigenin (KFC18; **7**; 8 mg), and *Frs. 54–65* gave mainly β-sistosterol glucoside (KFC5; **8**; 48 mg). The AcOEt

extract (15 g) was also subjected to VLC (SiO_2 (80 g, 0.04–0.063 mm); the same mixtures of solvent to afford fifty fractions: 9:1, *Frs.* 1–10; 3:1, *Frs.* 11–21; 3:2, *Frs. 22–28*; 1:1, *Frs. 29–36*; 1:3, *Frs. 37–44*; 0:1, *Frs. 45–50*). These fractions were combined and purified by CC (SiO_2; hexane/AcOEt (and CH_2Cl_2/MeOH). *Frs. 11–26* gave 3,4′,5-trihydroxy-6″,6″-dimethylpyrano-[2,3–*g*]flavone (KFC12; **9**; 12 mg) and KFC7 (**2**; 5 mg). *Frs. 37–50* gave mainly β-sistosterol glucoside (KFC5; **8**; 40 mg).

Conrauidienol (=*(1β,3β,18α)-1-Hydroxyursa-9(11),12-dien-3-yl Acetate*; **1**). White amorphous powder. $[\alpha]_D^{25} = +12.4$ ($c = 0.0014$, acetone). UV: 198 (3.44), 203 (3.45), 215 (3.36), 220 (3.32). CD ($c = 0.0014$, acetone [nm]): $[\theta]_{208} + 28560$, $[\theta]_{234} + 52536$, $[\theta]_{282} + 350298$. ^{1}H- (75 MHz, $CDCl_3$) and ^{13}C-NMR (75 MHz, $CDCl_3$): *Table 1*. EI-TOF-MS (pos.): 482 (100, M^+), 422 (60), 217 (38), 189 (13), 175 (24), 95 (21), 86 (52), 84 (80). HR-EI-MS: 482.3853 (M^+, $C_{32}H_{50}O_3^+$; calc. 482.3819).

Conrauiflavonol (=*(7*R*,8*R*)-7,8-Dihydro-5,7-dihydroxy-8-(4-hydroxyphenyl)-2,2-dimethyl-2*H*,6*H*-benzo[1,2-*b*:5,4-*b*′]dipyran-6-one*; **2**). Yellow amorphous powder. $[\alpha]_D^{25} = +2.55$ ($c = 0.003$, $CHCl_3$). UV ($CHCl_3$): 209 (2.82), 234 (2.90), 266 (2.96), 289 (2.63), 310 (2.44). CD ($c = 0.003$, $CHCl_3$ [nm]): $[\theta]_{354} + 55506$, $[\theta]_{316} - 38915$, $[\theta]_{284} + 26443$, $[\theta]_{258} + 33381$, $[\theta]_{239} - 27381$, $[\theta]_{225} + 28315$. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-TOF-MS (pos.): 354 (64, M^+), 339 (100), 219 (45), 203 (60), 177 (32). HR-EI-MS: 353.9804 (M^+, $C_{20}H_{18}O_6^+$; calc. 353.9801).

Antimicrobial Assays. Extracts and compounds were tested for their antimicrobial activities against five bacteria strains, *Escherichia coli* ATCC8739, *Providencia stuartii* ATCC29916, *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC10536, *Klebsiella pneumoniae* ATCC11296. Chloramphenicol was used as reference antibiotic (RA). This activity was evaluated by the determination of the minimal inhibitory concentration (*MIC*) using a rapid *p*-iodonitrotetrazolium violet (INT) method [36]. Samples to be tested and chloramphenicol were dissolved in DMSO-*Mueller Hinton* broth (DMSO-*MHB*). The soln. obtained was then added to *MHB* and serially diluted twofold in a 96-well microplate to give final concentrations ranging from 2 to 1024 μg/ml for extracts, and from 0.5–256 μg/ml for compounds and RA. One hundred μl of inoculums prepared in *MHB* at a concentration of 1.5×10^6 CFU/ml were then added. The plates were covered with a sterile plate sealer and then agitated with a shaker to mix the contents of the wells, and incubated at 37°. The final concentration of DMSO was less than 2.5%, and DMSO did not affect the microbial growth. Wells containing only *MHB*, 100 μl of inoculum and DMSO at a final concentration of 2.5% served as the neg. control. The *MIC* values of samples were detected after 18 h, following addition of 40 μl of INT (0.2 mg/ml) and incubation at 37° for 30 min. Viable bacteria reduced the yellow dye to pink color. The *MIC* value was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth. Each assay was repeated three times independently.

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