



HAL
open science

Semisynthetic Derivatives of Sissotrin Isolated from <i>Trifolium baccarinii</i> Chiov. (Fabaceae) and Evaluation of Their Antibacterial Activities

Donald Léonel Feugap Tsamo, Cyrille Ngoufack Tagousop, Arnaud Joseph Nguetse Dongmo, Laurence Voutquenne-Nazabadioko, David Ngnokam

► To cite this version:

Donald Léonel Feugap Tsamo, Cyrille Ngoufack Tagousop, Arnaud Joseph Nguetse Dongmo, Laurence Voutquenne-Nazabadioko, David Ngnokam. Semisynthetic Derivatives of Sissotrin Isolated from <i>Trifolium baccarinii</i> Chiov. (Fabaceae) and Evaluation of Their Antibacterial Activities. *Advances in Biological Chemistry*, 2024, 14 (01), pp.27-39. 10.4236/abc.2024.141003 . hal-04460130

HAL Id: hal-04460130

<https://hal.science/hal-04460130v1>

Submitted on 15 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/378148362>

Semisynthetic Derivatives of Sissotrin Isolated from *Trifolium baccarinii* Chiov. (Fabaceae) and Evaluation of Their Antibacterial Activities

Article in *Advances in Biological Chemistry* · February 2024

DOI: 10.4236/abc.2024.141003

CITATIONS

0

READS

25

7 authors, including:



Léonel Donald Tsamo Feugap

Université de Dschang

8 PUBLICATIONS 46 CITATIONS

SEE PROFILE



Cyrille Ngoufack Tagousop

University of Ngaoundere

11 PUBLICATIONS 211 CITATIONS

SEE PROFILE



Dongmo Nguetse Joseph Arnaud

Université de Dschang

3 PUBLICATIONS 1 CITATION

SEE PROFILE



Laurence Voutquenne

Université de Reims Champagne-Ardenne

193 PUBLICATIONS 2,289 CITATIONS

SEE PROFILE

Semisynthetic Derivatives of Sissotrin Isolated from *Trifolium baccarinii* Chiov. (Fabaceae) and Evaluation of Their Antibacterial Activities

Donald Léonel Feugap Tsamo^{1,2*}, Cyrille Ngoufack Tagousop³, Arnaud Joseph Nguetse Dongmo¹, Laurence Voutquenne-Nazabadioko⁴, David Ngnokam¹

¹Research Unit of Applied and Environmental Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, Dschang, Cameroon

²Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

³Department of Basic Scientific Studies, University Institute of Technology, University of Ngaoundéré, Ngaoundéré, Cameroon

⁴Groupe Isolement et Structure, Institut de Chimie Moléculaire de Reims (ICMR), Reims, France
Email: *tsamofeugap@yahoo.fr

How to cite this paper: Tsamo, D.L.F., Tagousop, C.N., Dongmo, A.J.N., Voutquenne-Nazabadioko, L. and Ngnokam, D. (2024) Semisynthetic Derivatives of Sissotrin Isolated from *Trifolium baccarinii* Chiov. (Fabaceae) and Evaluation of Their Antibacterial Activities. *Advances in Biological Chemistry*, **14**, 27-39.

<https://doi.org/10.4236/abc.2024.141003>

Received: December 8, 2023

Accepted: February 5, 2024

Published: February 8, 2024

Copyright © 2024 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



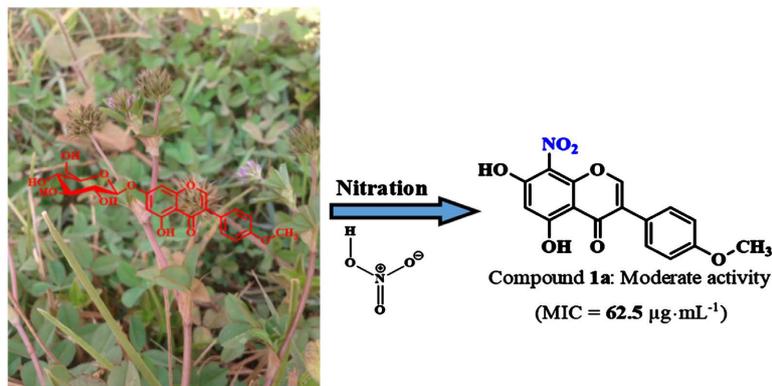
Open Access

Abstract

Starting from sissotrin (**1**), a natural isoflavonoid isolated from *Trifolium baccarinii* (Fabaceae), one new semisynthetic derivative, 6-nitrobiochanin A (**1b**) and two known derivatives, 8-nitrobiochanin A (**1a**) and 2",3",4",6"-tetraacetylsissotrin (**1c**) have been obtained after performing nitration and acetylation reactions. Their structures were assigned after interpretation of their spectrometric (HR-ESI-MS) and spectroscopic (NMR 1D and 2D) data and by comparison with those reported in the literature. The substrate as well as the semisynthetic derivatives were evaluated for their antibacterial activities against six strains. The results reveal that they are inactive or weakly active on the strains tested with the exception of 8-nitrobiochanin A (**1a**) which showed moderate activity (MIC = 62.5 µg·mL⁻¹) on *Staphylococcus aureus* ATCC 43300.

Keywords

Trifolium baccarinii, Isoflavonoid, Nitration, Acetylation, Antibacterial Activity



1. Introduction

Antibiotic resistance is one of the biggest threats to global health today [1]. In Cameroon, the work of Mouiche *et al.* (2019) shows the high prevalence of this phenomenon of resistance to antibiotics commonly used in humans [2]. So, more than ever, it becomes urgent and vital to find new effective drugs especially since it is estimated that if this is not done, there would be no more antibiotics capable to fight against deadly resistant pathogens by 2050 [3]. In this perspective, nitro and acetylated aromatic compounds are a promising avenue for the discovery of new antibiotic substances. Indeed, it has been demonstrated that aromatic compounds with one or more nitro groups directly attached to the aromatic nucleus exhibit pharmacological properties allowing them to be used as antibiotics [4]. Furthermore, it has been shown that the nitro group, present in antibiotic substances, plays a key role due to its physical and chemical properties such as its polarity, electronegativity, small size, redox properties as well as its ability to establish hydrogen bonds [5]. **Figure 1** below shows some structures of flavonoid derivatives obtained after the nitration reaction. It is also reported in the literature that the acetyl group increases the antimicrobial activity of certain compounds like flavonoid glycosides [6]. Therefore, in our continued search for new antibiotics compounds [7], we have undertaken the nitration and acetylation of a naturally occurring isoflavonoid, namely sissortrin previously isolated in our Research Unit from *Trifolium baccarinii* Chiov (Fabaceae) [8]. In this paper, we report the purification, structure elucidation as well as the evaluation of the antibacterial activity of the semisynthetic derivatives obtained after performing nitration and acetylation reactions.

2. Material and Methods

2.1. Extraction and Isolation of Substrate

Extraction as well as isolation and characterization of sissortrin (**1**) from *Trifolium baccarinii* were performed by a method previously described by Tsamo *et al.* (2021) [8].

2.2. Chromatography Methods

Column chromatography was carried out on silica gel 230 - 400 mesh, Merck

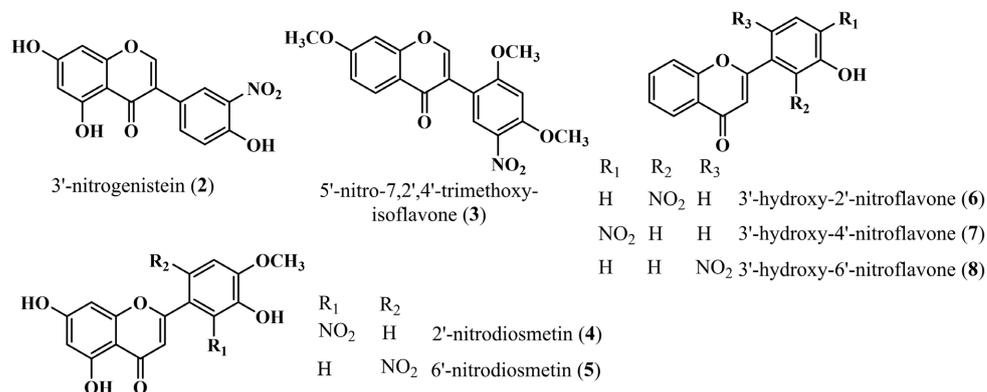


Figure 1. Some flavonoids derivatives obtained by nitration.

(Merck, Darmstadt, Germany), 70 - 230 mesh (Merck) (VWR, France). Thin-layer chromatography (TLC) was carried out on Merck pre-coated silica gel (60 F254) aluminium foil (Merck) and the spots were visualized by an UV lamp multiband UV-254/365 nm (Model UVGL-58, Upland, CA 91786, USA) followed by spraying with 50% H₂SO₄ followed by heating at 100°C.

2.3. Nuclear Magnetic Resonance (NMR) Analysis

The ¹H and ¹³C-NMR spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoplatform (¹H at 500 or 600 MHz and ¹³C at 125 or 150 MHz). 2D NMR experiments were performed using standard Bruker microprograms (Xwin-NMR version 2.1 software). All chemical shifts (δ) were reported in parts per million (ppm) using the residual solvent signals as secondary reference relatively to TMS ($\delta = 0$) as an internal standard, while the coupling constants (J) were given in hertz (Hz). Deuterated solvents, methanol (CD₃OD) and dimethyl sulfoxide (DMSO-*d*₆) were used as solvents for the NMR experiments.

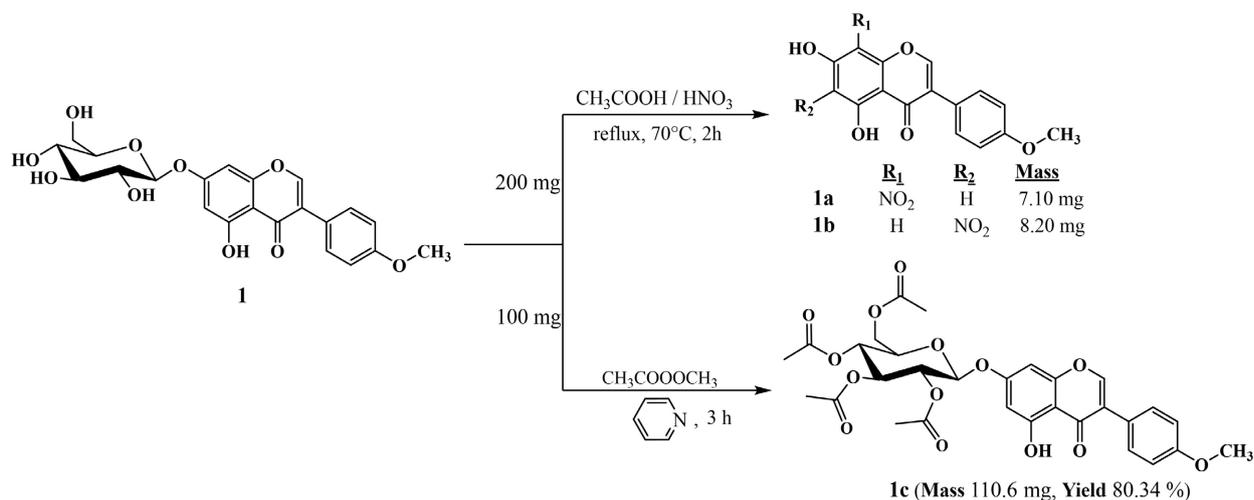
2.4. Spectrometric Analysis

High resolution mass spectra were obtained with QTOF (Quadrupole Time of Flight) Compact Spectrometer (Bruker, Germany) equipped with a HRESI source. The spectrometer was operated in positive and negative modes (mass range: 50 - 1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 0.4 ppm deviation using Na formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200°C. Nitrogen was used as sheath gas (4 L/min).

2.5. Semisynthesis and Purifications

2.5.1. Nitration of Sissotrin (1)

The reaction was carried out using the method previously described by Lewin *et al.* (2010) with slight modifications (**Scheme 1**) [9]. A mass of 200 mg (448.431 μ mol) of sissotrin (1) was dissolved in a 100 mL flask with 40 mL of acetic acid



Scheme 1. Nitration and acetylation of sissotrin (**1**).

(CH_3COOH). Then, 35 μL of fuming nitric acid (HNO_3 , 70%) was added to the solution obtained. The mixture was refluxed at 70°C and the reaction was monitored on a TLC plate. After 2 hours, the reaction medium underwent differential solubilization with *n*-BuOH (120 mL, H_2O ; 80 mL, *n*-BuOH, repeated 3 times). The organic phase obtained was evaporated on a rotary evaporator and under reduced pressure. The purification of the mixture of products obtained on silica gel column chromatography using *n*-hexane/ethyl acetate (50:50 \rightarrow 00:100, v/v) as eluent gave: 8-nitrobiochanin A (**1a**: 7.10 mg; *Rf*0.60) and 6-nitrobiochanin A (**1b**: 8.20 mg; *Rf*0.40) (**Scheme 1**).

8-nitrobiochanin A (**1a**): Yellow powder from *n*-hexane/ethyl acetate, (20:80, v/v); positive HRESIMS m/z : 330.0566 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{16}\text{H}_{12}\text{NO}_7$, 330.0608). ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data, see **Table 1** and **Table 2**.

6-nitrobiochanin A (**1b**): Yellow powder from *n*-hexane/ethyl acetate, (20:80, v/v); positive HRESIMS m/z : 330.0609 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{16}\text{H}_{12}\text{NO}_7$, 330.0608). ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data, see **Table 1** and **Table 2**.

2.5.2. Acetylation of Sissotrin (**1**)

In a 50 mL capacity Erlenmeyer flask containing the substrate (**1**, 100 mg, 2.2 mmol), we added 10 mL of pyridine in order to solubilize and then 1 mL of acetic anhydride. The mixture was stirred at room temperature. After 3 hours, the reaction medium was controlled by means of an analytical TLC which showed that the latter no longer contained any substrate. A little amount of water (90 mL) was added to this reaction medium and the solution obtained was subjected to differential solubilization in a separatory funnel (250 mL) with 100 mL of ethyl acetate until complete exhaustion. The organic phase obtained was purified after drying by column chromatography over silica gel (*n*-hexane/ethyl acetate 80:20, v/v) to yield to a known derivative identified as 2",3",4",6"-tetraacetylsissotrin (**1c**: 110.6 mg; Yield 80.34 %; *Rf*0.55) (**Scheme 1**).

Table 1. ¹H NMR data of compounds **1a**, **1b**, **1c** and **1**.

	1a (CD ₃ OD, 600 MHz)	1b (CD ₃ OD, 600 MHz)	1c (DMSO- <i>d</i> ₆ , 600 MHz)	1 (DMSO- <i>d</i> ₆ , 500 MHz)
Positions	$\delta^1\text{H}$ (<i>mult</i> , <i>f</i>)	$\delta^1\text{H}$ (<i>mult</i> , <i>f</i>)	$\delta^1\text{H}$ (<i>mult</i> , <i>f</i>)	$\delta^1\text{H}$ (<i>mult</i> , <i>f</i>)
2	8.22 (1H, <i>s</i>)	8.12 (1H, <i>s</i>)	8.51 (1H, <i>s</i>)	8.48 (1H, <i>s</i>)
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	6.30 (1H, <i>s</i>)	-	6.49 (1H, <i>d</i> , 2.1 Hz)	6.48 (1H, <i>d</i> , 2.2 Hz)
7	-	-	-	-
8	-	6.36 (1H, <i>s</i>)	6.72 (1H, <i>d</i> , 2.1 Hz)	6.73 (1H, <i>d</i> , 2.2 Hz)
9	-	-	-	-
10	-	-	-	-
1'	-	-	-	-
2'/6'	7.51 (2H, <i>d</i> , 8.6 Hz)	7.49 (2H, <i>d</i> , 8.4 Hz)	7.53 (2H, <i>d</i> , 8.8 Hz)	7.51 (2H, <i>d</i> , 8.8 Hz)
3'/5'	7.00 (2H, <i>d</i> , 8.6 Hz)	6.99 (2H, <i>d</i> , 8.4 Hz)	7.02 (2H, <i>d</i> , 8.8 Hz)	7.00 (2H, <i>d</i> , 8.8 Hz)
4'	-	-	-	-
-OCH ₃	3.79 (3H, <i>s</i>)	3.83 (3H, <i>s</i>)	3.80 (3H, <i>s</i>)	3.79 (3H, <i>s</i>)
5-OH	-	-	12.97 (1H, <i>s</i>)	-
1''	-	-	5.79 (1H, <i>d</i> , 8.0 Hz)	5.07 (1H, <i>d</i> , 7.3 Hz)
2''	-	-	5.11 (1H, <i>m</i>)	3.45 (1H, <i>m</i>)
3''	-	-	5.41 (1H, <i>m</i>)	3.28 (1H, <i>m</i>)
4''	-	-	5.03 (1H, <i>m</i>)	3.17 (1H, <i>m</i>)
5''	-	-	4.35 (1H, <i>m</i>)	3.30 (1H, <i>m</i>)
6''	-	-	4.20 (1H, <i>m</i>)	3.70 (1H, <i>m</i>)
			4.10 (1H, <i>m</i>)	3.47 (1H, <i>m</i>)
2''-OAc	-	-	2.04 (3H, <i>s</i>)	-
3''-OAc	-	-	1.97 (3H, <i>s</i>)	-
4''-OAc	-	-	2.03 (3H, <i>s</i>) ^a	-
6''-OAc	-	-	2.03 (3H, <i>s</i>) ^a	-

^aConfused on the spectrum.**Table 2.** ¹³C NMR data of compounds **1a**, **1b**, **1c** and **1**.

	1a (CD ₃ OD, 150 MHz)	1b (CD ₃ OD, 150 MHz)	1c (DMSO- <i>d</i> ₆ , 150 MHz)	1 (DMSO- <i>d</i> ₆ , 125 MHz)
Positions	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$
2	154.5	155.2	155.6	154.9

Continued

3	123.4	124.0	122.8	122.2
4	181.5	182.2	180.9	180.4
5	169.3	161.4	162.0	161.6
6	100.7	128.9	99.8	99.6
7	171.4	161.4	162.4	163.1
8	125.6	96.0	95.2	94.6
9	161.6	156.4	157.6	157.2
10	108.2	104.1	107.2	106.1
1'	123.6	124.3	123.8	122.7
2'/6'	131.4	131.3	130.7	130.2
3'/5'	114.9	114.9	114.2	113.8
4'	167.6	159.3	159.7	159.2
-OCH ₃	55.8	55.7	55.7	55.2
1''	-	-	96.6	99.8
2''	-	-	70.9	73.1
3''	-	-	72.3	77.2
4''	-	-	68.3	69.6
5''	-	-	71.6	76.4
6''	-	-	62.1	60.6
2''-OAc	-	-	169.6 20.9	-
3''-OAc	-	-	170.1 20.8	-
4''-OAc	-	-	169.8 20.9	-
6''-OAc	-	-	170.5 20.8	-

2'',3'',4'',6''-tetraacetylsissotrin (**1c**): Yellowish powder from *n*-hexane/ethyl acetate, (60:40, v/v); positive ESI-MS: *m/z*, 637.16 [M + Na]⁺. ¹H NMR (DMSO-*d*₆, 600 MHz) and ¹³C NMR (DMSO-*d*₆, 150 MHz) data, see **Table 1** and **Table 2**.

2.6. Antibacterial Assay

2.6.1. Bacterial Strains and Sample Preparation

The studied microorganisms consisted of six bacterial strains among which three

Gram-positive (*Staphylococcus aureus* ATCC 43300, Methicillin-resistant *Staphylococcus aureus* 33591 and *Staphylococcus aureus* CPC) and three Gram-negative (*Escherichia coli* ATCC 25922, *Shigella flexneri* CPC and *Shigella flexneri* NR 518) all previously stored in 80°C. Mueller Hinton Agar (MHA) was used to revive the bacterial strains 24 h before the experiment. They were obtained from our local stocks. The substrate as well as the semisynthetic derivatives were weighed and solubilized in dimethyl sulphoxide (DMSO) to obtain a final concentration of 20 µg·mL⁻¹. Ciprofloxacin (Sigma Aldrich, USA) used as reference drug was prepared in sterile distilled water at 1 µg·mL⁻¹.

2.6.2. Determination of the Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations of the samples were determined according to the microdilution method described by the Clinical and Laboratory Standards Institute in 2012, [10] using 96-well microtiter plates. Briefly, 190 µL of culture medium (Nutrient Broth) was introduced into the first line of the plate and 100 µL in the rest of the plate. Thereafter, 10 µL of test products were added exclusively in the wells of the first line to reach a final volume of 200 µL. After slight homogenization, serial two-fold dilutions of test compounds were made through a transfer of 100 µL of the mixture from the first well to the second and so on to the last. The final concentrations in the wells ranged from 500 - 3.906 µg·mL⁻¹ for the test compounds and 119 - 0.116 µg·mL⁻¹ for ciprofloxacin. Then 100 µL of bacteria inoculum standardized to 1.5 × 10⁶ CFU mL⁻¹ were introduced into each well. The sterility control consisted of the culture medium (Nutrient Broth) alone, while the negative control consisted of the medium and bacteria (Nutrient Broth + bacteria). Ciprofloxacin (Sigma-Aldrich, Germany) + bacteria was used as a positive control. The plates were incubated at 37°C for 24 hours and after this period, the turbidity was observed as an indication of growth. The MIC was considered as the lowest concentration with no visible growth of microorganisms (no turbidity). All tests were performed in triplicate.

3. Results and Discussion

3.1. Chemical Analysis

The nitration reaction of 200 mg of compound **1** led to two products, **1a** and **1b**, with masses of 7.10 mg and 8.20 mg respectively, while the 100 mg acetylation reaction of the same compound led to a single product, **1c** with a mass of 110.6 mg, giving a yield of 80.34%. A study of the literature reveals that the presence of a methoxyl group in 4' promotes nitration on the ring A, in particular in positions 6 and 8 of the 5,7-dihydroxylated flavonoids [11]. However, position 8 being the most favored [12] which was not the case for our reaction. The low yield obtained in the first reaction is justified by the fact that it proceeds in several steps, in particular in three steps (with loss of the osidic part) and it resulted in more than one product as presented in **Scheme 1**. In contrast, in the second reaction there is no loss of the osidic moiety.

Compound **1b** obtained from the nitration reaction was isolated as a yellow

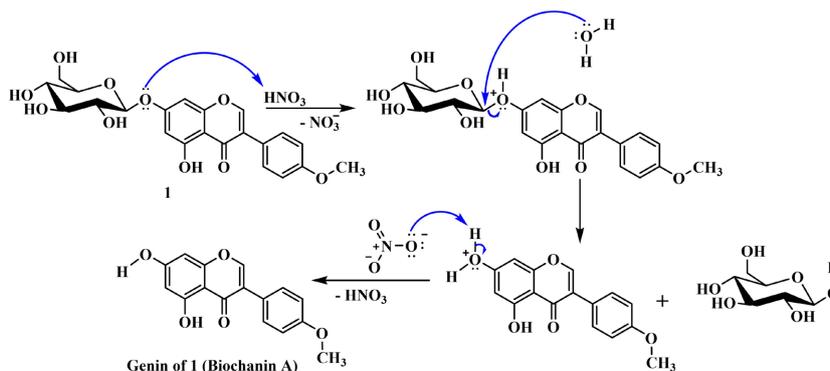
powder soluble in methanol. Its HR-ESI-MS shows the pseudomolecular ion peak $[M + H]^+$ at m/z 330.0609 *a.m.u* corresponding to the molecular formula $C_{16}H_{12}NO_7$ (calculated mass for $C_{16}H_{12}NO_7$: 330.0608 *a.m.u*) contain twelve degrees of unsaturation. The mass difference observed between **1b** and the genin of **1** (*i.e.* 284) is 45 *a.m.u*, which corresponds to the mass of a nitro group (46 *a.m.u*) minus one unit (corresponding to the substituted proton). This observation allows us to affirm that **1b** comes from the mono-nitration of **1** after hydrolysis. The 1H NMR data of this derivative is close to the substrate **1** but however with some dissimilarities (**Table 1**). The main difference is the disappearance of the signals attributable to the osidic protons in **1b**. We also observe the disappearance of the aromatic H-6 proton signal, resulting in the appearance of the H-8 proton signal as a singlet at δ_H 6.36 (1H, *s*).

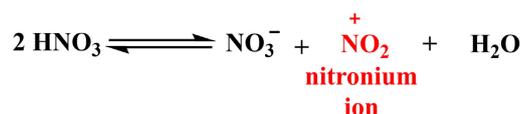
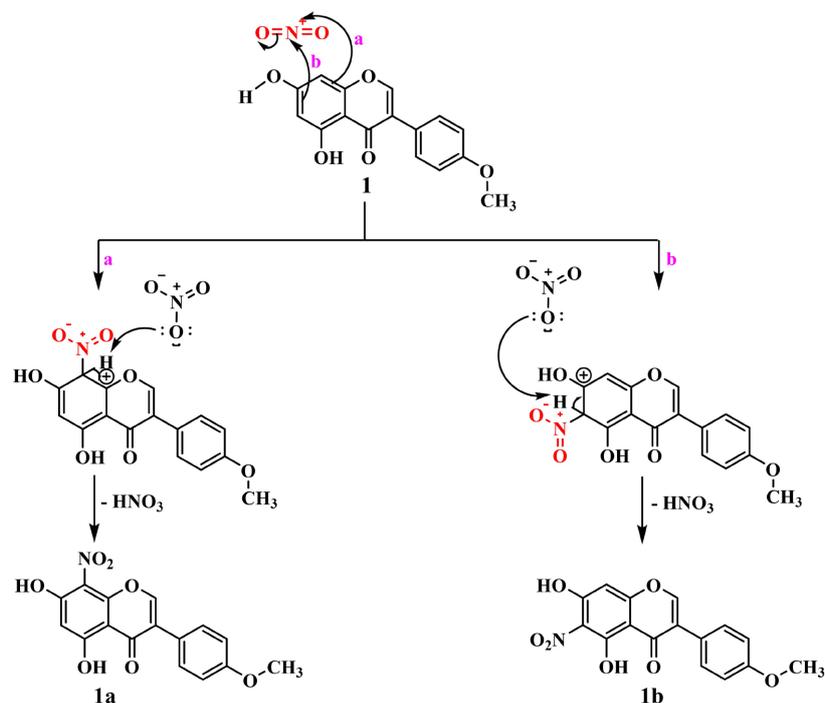
Similarly, a comparison of its ^{13}C NMR data with that of **1** (**Table 2**) further confirms the disappearance of the osidic part and the nitration of position 6. Indeed, a concomitant interpretation of the ^{13}C NMR and HSQC spectra of **1b** allows us to see that the single aromatic proton of ring A is carried by the carbon at δ_C 96.0 ppm characteristic of the carbon in position 8 of 5,7-dihydroxylated flavonoids [13] [14]. Indeed, it has been established that in these systems (5,7-dihydroxylated), the proton H-8 is more deshielded than H-6 while its carbon resonates at slightly stronger fields (around 98.0 ppm) than that of H-6 (generally around 100.0 ppm). In addition, the presence of the meta nitro group results in a slight shielding of the C-8 carbon which appears at slightly stronger fields (96.0 ppm). From the aforementioned data, the structure of compound **1b** was established as 6-nitrobiochanin A.

Compound **1a** obtained from the nitration reaction was isolated as a yellow powder soluble in methanol. Its HR-ESI-MS shows the pseudomolecular ion peak $[M + H]^+$ at m/z 330.0566 *a.m.u* corresponding to the molecular formula $C_{16}H_{12}NO_7$ (calculated mass for $C_{16}H_{12}NO_7$: 330.0608 *a.m.u*) contain twelve degrees of unsaturation. Its 1H and ^{13}C NMR data are identical to those of 8-nitrobiochanin A which was previously reported by Al-Maharik and Jaradat in 2020 [12].

Scheme 2 below shows the plausible mechanism by which derivatives **1a** and **1b** would have formed [15].

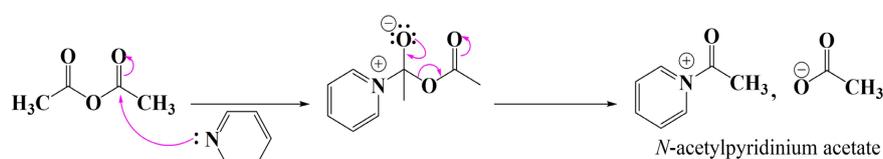
Step 1. Hydrolysis



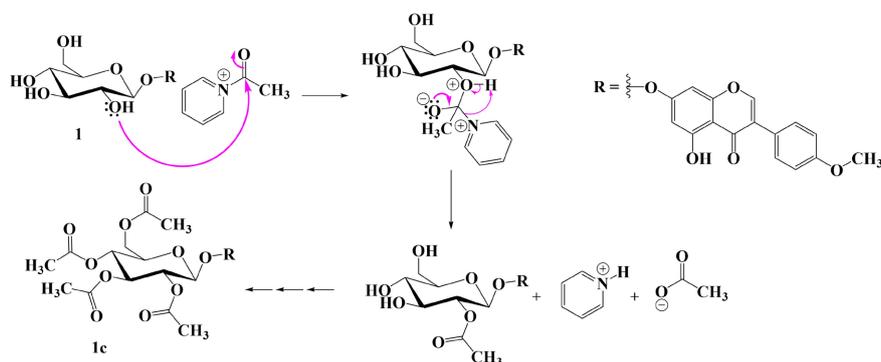
Step 2. Formation of nitronium ion**Step 3.** Electrophilic attack of nitronium ion and regeneration of nitric acid**Scheme 2.** Mechanistic steps leading to derivatives **1a** and **1b**.

Compound **1c** obtained from acetylation reaction was isolated as yellowish powder soluble in dimethyl sulfoxide. The comparison of its ^1H and ^{13}C NMR data with those of substrate **1** (**Table 1** and **Table 2**) shows that the main difference was observed in the sugar moiety. Indeed, in addition to the signal of sugar, we pick up the signals attributed to the proton and carbone of acetyl groups. Compound **1c** has been identified at 2'',3'',4'',6''-tetraacetylsissotrin previously synthesized by Ying-Jie and Yi-Cheng in 2009 through reaction between 5,7-dihydroxy-4'-methoxyisoflavone and acetobromoglucose [α -D-glucopyranosyl bromide tetraacetate] [16].

Scheme 3 below shows the mechanism of the acetylation reaction of **1**.

Step 1. Formation of acetylpyridinium acetate

Step 2. Nucleophilic attack



Scheme 3. Mechanistic steps leading to derivative **1c**.

3.2. Antibacterial Activity

The semisynthetic derivatives **1a**, **1b** and **1c** as well as substrate **1** were evaluated for their antibacterial activities (Table 3). This evaluation was carried out on six bacterial strains, three of which were Gram-positive (*Staphylococcus aureus* ATCC 43300, Methicillin-resistant *Staphylococcus aureus* 33591 and *Staphylococcus aureus* CPC) and three Gram-negative (*Escherichia coli* ATCC 25922, *Shighella flexineri* CPC and *Shighella flexineri* NR 518).

Antibacterial cutoff points of pure compounds have been defined as follows: highly active: MIC below $1 \mu\text{g}\cdot\text{mL}^{-1}$ (or $2.5 \mu\text{M}$), significantly active: $1 \leq \text{MIC} \leq 10 \mu\text{g}\cdot\text{mL}^{-1}$ (or $2.5 \leq \text{MIC} < 25 \mu\text{M}$), moderately active: $10 < \text{MIC} \leq 100 \mu\text{g}\cdot\text{mL}^{-1}$ (or $25 < \text{MIC} \leq 250 \mu\text{M}$), weakly active: $100 < \text{MIC} \leq 1000 \mu\text{g}\cdot\text{mL}^{-1}$ (or $250 < \text{MIC} \leq 2500 \mu\text{M}$) and not active: $\text{MIC} > 1000 \mu\text{g}\cdot\text{mL}^{-1}$ (or $>2500 \mu\text{M}$) [17].

Globally, the MIC values ranging from $62.5 \mu\text{g}\cdot\text{mL}^{-1}$ to more than $500 \mu\text{g}\cdot\text{mL}^{-1}$ depending on compounds and microorganisms. Concerning the substrate **1**, from all the strains tested, it shows weak antibacterial activity towards *Shighella flexineri* CPC (MIC: $250 \mu\text{g}\cdot\text{mL}^{-1}$) and *Shighella flexineri* NR 918 (MIC: $500 \mu\text{g}\cdot\text{mL}^{-1}$). It can be seen that derivative **1a** was active on all bacteria tested and the best activity (moderate) was observed on *Staphylococcus aureus* CPC (MIC: $62.5 \mu\text{g}\cdot\text{mL}^{-1}$). When tested against on *Staphylococcus aureus* ATCC 43300 (MIC: $500 \mu\text{g}\cdot\text{mL}^{-1}$); Methicillin-resistant *Staphylococcus aureus* 33591 (MIC: $500 \mu\text{g}\cdot\text{mL}^{-1}$); *Escherichia coli* ATCC 25922 (MIC: $500 \mu\text{g}\cdot\text{mL}^{-1}$); *Shighella flexineri* CPC (MIC: $125 \mu\text{g}\cdot\text{mL}^{-1}$) and *Shighella flexineri* NR 518 (MIC: $250 \mu\text{g}\cdot\text{mL}^{-1}$) compound **1a** exhibited a weak antibacterial activity and still remain the most active on these strains. All these results are in agreement with the literature which shows that some structural features such as the presence of the sugar moiety or the numbers of hydroxyl, methoxyl groups as well as nitro group had a great influence on their biophysical and biological properties [18] [19]. Unfortunately derivatives **1b** and **1c** were not active ($\text{MIC} > 500 \mu\text{g}\cdot\text{mL}^{-1}$) against all the strains tested. These observations allow us to state that the hydrolysis followed by the nitration of biochanin A tend to increase the antibacterial

Table 3. Antibacterial activity (MIC in $\mu\text{g}\cdot\text{mL}^{-1}$) of **1**, **1a**, **1b** and **1c** as well as ciprofloxacin.

Substrate/Semisynthetic derivatives	Parameters ($\mu\text{g}\cdot\text{mL}^{-1}$)	Bacterial strains					
		<i>S.a</i>	<i>MRSA 3391</i>	<i>E.c</i> ATCC 25922	<i>S.f</i> CPC	<i>S.f</i> NR 518	<i>S.a</i> CPC
Substrate							
1	MIC	>500	>500	>500	250	500	>500
Semisynthetic derivatives							
1a	MIC	500	500	500	125	250	62.5
1b	MIC	>500	>500	>500	/	/	/
1c	MIC	>500	>500	>500	>500	>500	>500
Ciprofloxacin	MIC	0.232 ± 000	0.116 ± 000	0.232 ± 000	1.859 ± 000	0.232 ± 000	3.718 ± 000

/: not determined; **S.a**: *Staphylococcus aureus* ATCC 43300; **MRSA3391**: Methicillin-resistant *Staphylococcus aureus* 33591; **E.c ATCC 25922**: *Escherichia coli* ATCC 25922; **S.fCPC**: *Shigella flexineri* CPC; **S.fNR 518**: *Shigella flexineri* NR 518; **S.a CPC**: *Staphylococcus aureus* CPC; **MIC**: Minimum Inhibitory Concentration; **Ciprofloxacin**: drug reference.

position 8 of the isoflavone. They also allow us to suggest that the hydroxyl activity. This increase is also depending on the position of the nitro group ($-\text{NO}_2$) because as it seems more pronounced when said nitration takes place at groups found on the osidic part would play a role in the antibacterial activity because their removal (by acetylation) led to a total loss of antibacterial activity ($\text{MIC} > 500 \mu\text{g}\cdot\text{mL}^{-1}$) compared to the substrate ($\text{MIC} = 250$ and $500 \mu\text{g}\cdot\text{mL}^{-1}$).

4. Conclusion

The present work describes the semisynthesis and structural elucidation of three derivatives (**1a**, **1b** and **1c**) obtained from an isoflavone, sissotrin (**1**) isolated from *Trifolium baccarinii* after nitration and acetylation reactions. The results of the evaluation of their antibacterial properties showed that only derivative **1a** exhibited moderate activity ($62.5 \mu\text{g}\cdot\text{mL}^{-1}$) on *Staphylococcus aureus* CPC. The substrate **1** showed only weak activity against *Shigella flexineri* CPC and *Shigella flexineri* NR 518. Based on these results, we can recommend that more such transformations on isoflavones be carried out in order to better understand and establish the structure-activity relationships.

Acknowledgements

The authors gratefully acknowledge Billy Toussie Tchegnitegni (University of Dschang), Pierre Mkounga (University of Yaounde I) and Augustin Ephrem Nkengfack (University of Yaounde I) to provide the facilities needed to carry out this work. The “Institut de Chimie Moléculaire de Reims” and the University of Dschang are gratefully acknowledged for administrative and technical support.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Melander, R.J. and Melander, C. (2017) The Challenge of Overcoming Antibiotic Resistance: An Adjuvant Approach? *ACS Infectious Diseases*, **3**, 559-563. <https://doi.org/10.1021/acsinfecdis.7b00071>
- [2] Mouiche, M.M.M., Moffo, F., Akoachere, J.-F.T.K., Okah-Nnane, N.H., Mapiefou, N.P., Ndze, V.N., Wade, A., Djuikwo-Teukeng, F.F., Toghoua, D.G.T., Zambou, H.R., Feussom, J.M.K., LeBreton, M. and Awah-Ndukum, J. (2019) Antimicrobial Resistance from a One Health Perspective in Cameroon: Systematic Review and Metaanalysis. *BMC Public Health*, **19**, 1135. <https://doi.org/10.1186/s12889-019-7450-5>
- [3] Bharadwaj, A., Rastogi, A., Pandey, S., Gupta, S. and Sohal, J.S. (2022) Multidrug-Resistant Bacteria: Their Mechanism of Action and Prophylaxis. *BioMed Research International*, **2022**, 17. <https://doi.org/10.1155/2022/5419874>
- [4] Ayoup, M.S., Rabee, A.R., Abdel-Hamid, H., Harras, M.F., Menofy, N.G.E. and Ismail, M.M.F. (2022) Exploration of Nitroaromatic Antibiotics *via* Sanger's Reagent: Synthesis, *In Silico*, and Antimicrobial Evaluation. *ACS Omega*, **7**, 5254-5263. <https://doi.org/10.1021/acsomega.1c06383>
- [5] Rice, A.M., Long, Y. and King, S.B. (2021) Nitroaromatic Antibiotics as Nitrogen Oxide Sources. *Biomolecules*, **11**, 267. <https://doi.org/10.3390/biom11020267>
- [6] Mellou, F., Lazari, D., Skaltsa, H., Tselepis, A.D., Kolisis, F.N. and Stamatis, H. (2005) Biocatalytic Preparation of Acylated Derivatives of Flavonoid Glycosides Enhances Their Antioxidant and Antimicrobial Activity. *Journal of Biotechnology*, **116**, 295-304. <https://doi.org/10.1016/j.jbiotec.2004.12.002>
- [7] Tagousop, N.C., Tamokou, J.-D.-D., Tsamo, F.L.D., Harakat, D., Voutquenne-Nazabadioko, L. and Ngnokam, D. (2021) New Hemisynthetic Oleanane Saponin with Antimicrobial Activities. *Advances in Biological Chemistry*, **11**, 1-11. <https://doi.org/10.4236/abc.2021.111001>
- [8] Tsamo, F.L.D., Tamokou, J.-D.-D., Kengne, I.C., Jouogo, N.D.C., Djamalladine, D.M., Voutquenne-Nazabadioko, L. and Ngnokam, D. (2021) Antimicrobial and Antioxidant Secondary Metabolites from *Trifolium baccarinii* Chiov. (Fabaceae) and Their Mechanisms of Antibacterial Action. *BioMed Research International*, **2021**, 15. <https://doi.org/10.1155/2021/3099428>
- [9] Lewin, G., Jullian, J.-C. and Rodrigo, J. (2010) Unexpected B-Ring Regioselective di-Nitration of Diosmetin, a *Citrus* flavonoid. *Tetrahedron Letters*, **51**, 1145-1148. <https://doi.org/10.1155/2021/3099428>
- [10] Clinical and Laboratory Standards Institute (2012) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard-Ninth Edition.
- [11] Quintin, J., Roullier, C., Thoret, S. and Lewin, G. (2006) Synthesis and Anti-Tubulin Evaluation of Chromone-Based Analogues of Combretastatins. *Tetrahedron*, **62**, 4038-4051. <https://doi.org/10.1016/j.tet.2006.02.024>
- [12] Al-Maharik, N. and Jaradat, N. (2020) Synthesis of Novel Aminoisoflavones and Their *L*-alanyl Conjugates, for Possible Use in Chromogenic Media for the Detection of Aminopeptidase. *Journal of Materials and Environmental Sciences*, **11**,

238-246.

- [13] Agrawal, P.K. (1989) Carbon-13 NMR of Flavonoids: Studies in Organic Chemistry. Elsevier, Amsterdam, New York, 580.
<https://doi.org/10.1016/B978-0-444-87449-8.50011-0>
- [14] Andersen, Ø.M. and Markham, K.R. (2006) FLAVONOIDS: Chemistry, Biochemistry and Applications. Taylor & Francis Group, Florida, 1212.
- [15] Andreozzi, R., Canterino, M., Caprio, V., Somma, I.D. and Sanchirico, R. (2006) Salicylic Acid Nitration by Means of Nitric Acid/Acetic Acid System: Chemical and Kinetic Characterization. *Organic Process Research & Development*, **10**, 1199-1204.
<https://doi.org/10.1021/op060148o>
- [16] Ying-Jie, L. and Yi-Cheng, Y. (2009) Synthesis of Isoflavone 7-*O*-Glucosides Catalyzed by Tris(3,6-dioxahexyl)amine. *Yingyong Huaxue* **23**, 306-310.
- [17] Tamokou, J.-D.-D., Mbaveng, A.T. and Kuete, V. (2017) Antimicrobial Activities of African Medicinal Spices and Vegetables. In *Medicinal Spices and Vegetables from Africa: Therapeutic Potential against Metabolic Inflammatory Infectious and Systemic Diseases*, Academic Press, Cambridge, 207-237.
<https://doi.org/10.1016/B978-0-12-809286-6.00008-X>
- [18] Mandalari, G., Bennett, R.N., Bisignano, G., Trombetta, D., Saija, A., Faulds, C.B., Gasson, M.J. and Narbad, A. (2007) Antimicrobial Activity of Flavonoids Extracted from Bergamot (*Citrus bergamia* Risso) Peel, a Byproduct of the Essential Oil Industry. *Journal of Applied Microbiology*, **103**, 2056-2064.
<https://doi.org/10.1111/j.1365-2672.2007.03456.x>
- [19] Noriega, S., Cardoso-Ortiz, J., López-Luna, A., Cuevas-Flores, M.D.R. and Flores De La Torre, J.A. (2022) The Diverse Biological Activity of Recently Synthesized Nitro Compounds. *Pharmaceuticals (Basel)*, **15**, 717. <https://doi.org/10.3390/ph15060717>