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Rauvolfianine, a new antimycobacterial glyceroglycolipid and other constituents from *Rauvolfia caffra*. Sond (Apocynaceae)

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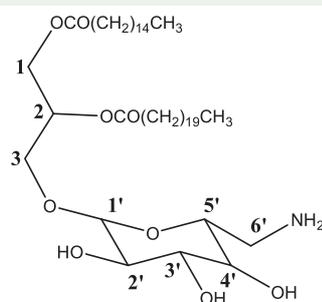
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ABSTRACT

The chemical investigation of the extract of the dried leaves of *Rauvolfia caffra* (Sond) (synonym *Rauvolfia macrophylla*) (Apocynaceae) led to isolation of a new glycoside derivative, rauvolfianine (**1**) as well as six known compounds: oleanolic acid (**2**), sitosterol-3-*O*- β -D-glucopyranoside (**3**), betulinic acid (**4**), vellosimine (**5**), sarpagine (**6**) and D-fructofuranosyl- β -(2 \rightarrow 1)- α -D-glucopyranoside (**7**). Compounds **1**, **2**, **3**, **4** and **7** were evaluated for antitubercular activity. Compounds **1** and **2** were the most active (MIC = 7.8125 and 31.25 μ g/mL) towards the Isoniazid resistant strain of *Mycobacterium tuberculosis* AC45. Their structures and relative stereochemistry were elucidated by spectroscopic methods.

KEYWORDS

Rauvolfia caffra;
Apocynaceae; Rauvolfianine;
glycerol ester; 1D and 2D
NMR; antitubercular activity



Rauvolfianine (**1**)

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1. Introduction

Tuberculosis is a chronic contagious disease caused by numerous species of *Mycobacterium*. The number of patients infected with the disease is rising worldwide. In addition, the highest mortality through co-infection with HIV/AIDS and the emergence of *bacilli* multi-resistant to antibiotics aggravates the impact of this disease especially in African continent (WHO 2014). Furthermore, the majority of *Mycobacterium* species are resistant to the most widely used therapeutic agents in the treatment of tuberculosis (isoniazid) (van Ingen 2015). Thus, there is an urgent need for new efficient antimycobacterial agents to replace those currently in use. Therefore, plant kingdom represents undoubtedly an untapped reservoir of novel drugs and alternate available medications or herbal remedies (Luo et al. 2011). *Rauvolfia caffra* which is a tree up to 25 m high, occurring in habitats of tropical forest zone of Cameroon and Nigeria (Burkill 1985). This species and others have been used in folk medicine to cure various ailments and infectious diseases (Mabberley 2008; Jain 2016; Kumar et al. 2016). The roots of *R. caffra* enter into treatment of intestinal worms, syphilis, as vermifuge and to cure venereal diseases (Walker-Raponda and Sillans 1961). Screening of literature revealed that, *R. caffra*, has been previously investigated for its chemical constituents. However, among the various phytochemicals identified to occur in this plant and other species of *Rauvolfia* genus, mainly alkaloids especially indole alkaloids along with phenols, flavonoids and tannins were detected in large amounts (Amer and Court 1981; O'Connor and Maresh 2006; Deshmukh et al. 2012; Jamkhande et al. 2013). *R. caffra* has not been investigated for antitubercular activity. Hence, this study was carried out to evaluate the potent bioactive constituents with antitubercular activity.

2. Results and discussion

By comparison of their spectral data with that reported in the literature compounds 2–7 (Figure 1) were identified as oleanolic acid (2) (Higuchi et al. 2008), sitosterol-3-O- β -D-glucopyranoside (3) (Ngono Bikobo et al. 2014), betulinic acid (4) (Sani et al. 2011), vellosimine (5), sarpagine (6) (Amer and Court 1981) and D-fructofuranosyl- β -(2 \rightarrow 1)- α -D-glucopyranoside (7) (Yamamori et al. 2017).

2.1. Identification of compound 1

Compound 1 was obtained as a white amorphous powder $[\alpha]_D^{25} = -5.1^\circ$ ($c = 0.10$, MeOH) and displayed a molecular ion at m/z 807.7 $[M + Li + H]^+$, 823.7 $[M + Na + H]^+$ and 839.7 $[M + K + H]^+$ (calcd for 807.69, 823.67 and 839.68, respectively) that was determined by ESI-MS (Figure S2) corresponding to the formula $C_{46}H_{89}NO_9$. The IR spectrum (Figure S3) showed bands at 3412 cm^{-1} due to amino group, 1728 and 1639 cm^{-1} for ester carbonyl absorptions and 1058 and 1039 cm^{-1} for C-O-C absorptions. Correlations on 2D-NMR (HMBC) spectra between numerous aliphatic carbons (δ_C 28.7 – 33.2) with common aliphatic protons (δ_H 1.24 – 2.32) indicated the presence of a long saturated alkyl chain (Table S1). The ^1H NMR spectrum (Figure S4) of 1 indicated the presence of two methyl signals at δ_H 0.84 (6H, t , $J = 6.5\text{ Hz}$, CH_3 -16''' and 21'') and methylene groups at δ_H 1.24 (58 x H, brs) and 1.48 (4H, m , $J = 6.70\text{ Hz}$). Besides these methylene groups, there was also signal originating from two methylene protons adjacent to carbonyl carbon atoms at δ_H 2.25 (2H, t , $J = 2.3; 7.3, 14.7\text{ Hz}$,

H-2''') and 2.29 (2H, *t*, $J = 2.3; 7.3$ Hz, H-2''). This was supported by the presence of two carbonyl carbon signals of the two fatty ester groups at δ_c 172.1 (C-1'') and 172.2 (C-1'''). The HMBC cross peaks of the latter with the carbon atoms corresponding to the carbonyl aforementioned allowed us to assume these two saturated alkyl chain moiety to be attached to the quaternary carbon atoms at δ_c 172.2 and 172.1, respectively. In addition, two primary alcohols were noticeable at δ_c 62.3 and 64.3 [linked, in HSQC (Figure S7) to the protons at δ_H 4.34/4.14 and 3.89/3.39, respectively] as well as a secondary one at δ_c 69.4 (δ_H 5.13, H-2); those data were in good agreement with a glyceroyl moiety substituted by saturated alkyl chain. Moreover, a glucopyranosyl moiety was indicated by the signal of the anomeric proton at δ_H 4.40 (*d*, $J = 3.9$ Hz, H-1') and in agreement with carbon signals at δ_c 98.0 (C-1'), 74.1 (C-4'), 72.6 (C-3'), 71.3 (C-2'), 68.2 (C-5') and 54.4 (C-6'). Therefore, compound **1** was deduced to be a glyceroglycolipid (He et al. 2006; Tebou et al. 2016). The ^{13}C NMR spectrum (Figure S5) revealed characteristic signals of aminosugar (He et al. 2006) in which the C-6' signal appeared in upfield shift at δ_c 54.4. The correlations between H-1 (δ_H 4.14) and H-2 (δ_H 5.13) to C-1''' (δ_c 172.2) and C-1'' (δ_c 172.1), respectively, observed in HMBC suggested the attachment of two fatty acid ester groups in position-1 and 2 of the glycerol moiety. Thus, **1** was determined to be a diacylglycerylaminoglucopyranoside. This was strengthened by the HMBC (Figure S8) cross peaks of the anomeric proton δ_H 4.40 (H-1') with carbon atom at 64.3 (C-3) indicating the aminosugar unit to be attached in position-3 of the glycerol moiety. The ^1H and ^{13}C NMR spectra of compound **1** were very similar to those observed for rouremin (He et al. 2006). The difference was provided by the presence of one more methylene group in **1**, observation supported by the ESI-MS data of **1** corresponding to 14 a.m.u. greater than rouremin. The length of two fatty acid ester chains of compound **1** were determined on the basis of ESI-MS spectrum and in comparison with literature data (He et al. 2006). Therefore, these two fatty acid chains were deduced to be a palmitoyl and heneicosanoyl groups which were attached at C-1 and C-2, respectively as described above. The relative configuration of carbon C-2 was determined from coupling constants of H-2, H-1 and H-3 protons and from NOESY results (Figure S9). The observation of 9.2, 10.0 and 10.6 Hz values of protons H-1 (δ_H 4.34), H-2 (δ_H 5.13) and H-3 (δ_H 3.89), respectively suggested the *trans*-relationships of these protons. The NOESY correlation observed between H-2 (δ_H 5.13) and H-3 (δ_H 3.39) and this latter and H'-1 (δ_H 4.40) indicated the common orientation on the same side of the molecule for these protons. The structure of **1** was, therefore, established as (2S*)-1-O-palmitoyl- β -O-heneicosanoyl-3-O-(6-amino-6-deoxy)- β -D-glucopyranosylglycerol named rauvolfinine. According to Cantrell et al. (2001), isolated compounds that display a MIC of 64 $\mu\text{g}/\text{mL}$ or lower are considered promising. For extracts, the MIC should be equal to or lower than 125 $\mu\text{g}/\text{mL}$. Thus, the values of 62.5 $\mu\text{g}/\text{mL}$ for compounds **3** and **4**, and of 31.25 $\mu\text{g}/\text{mL}$ as previously reported (Gu et al. 2004) for **2**, obtained here, are as good as a promising isolated compound. Compound **1** which had a minimal inhibitory concentration (MIC) of 7.8125 $\mu\text{g}/\text{mL}$, showed stronger antitubercular activity than other compounds. This might be explained by the susceptibility of less polar compounds to lipid hydrophobic cell of mycobacteria (Pauli et al. 2005; Chraïbi et al. 2016). Although the MIC values obtained here are larger than that of Ripamficin (0.976 $\mu\text{g}/\text{mL}$), these inhibitory concentrations are comparable to the MIC of pyrazinamide (another first-line antitubercular drug), 20–100 $\mu\text{g}/\text{mL}$ (Higuchi et al. 2008). Regarding the MBC values of all tested compounds (Table 2S) it seems that they could be similar to their MIC values against the isolate strain (AC45). According to Pankey and Sabath (2004) bacteriostatic

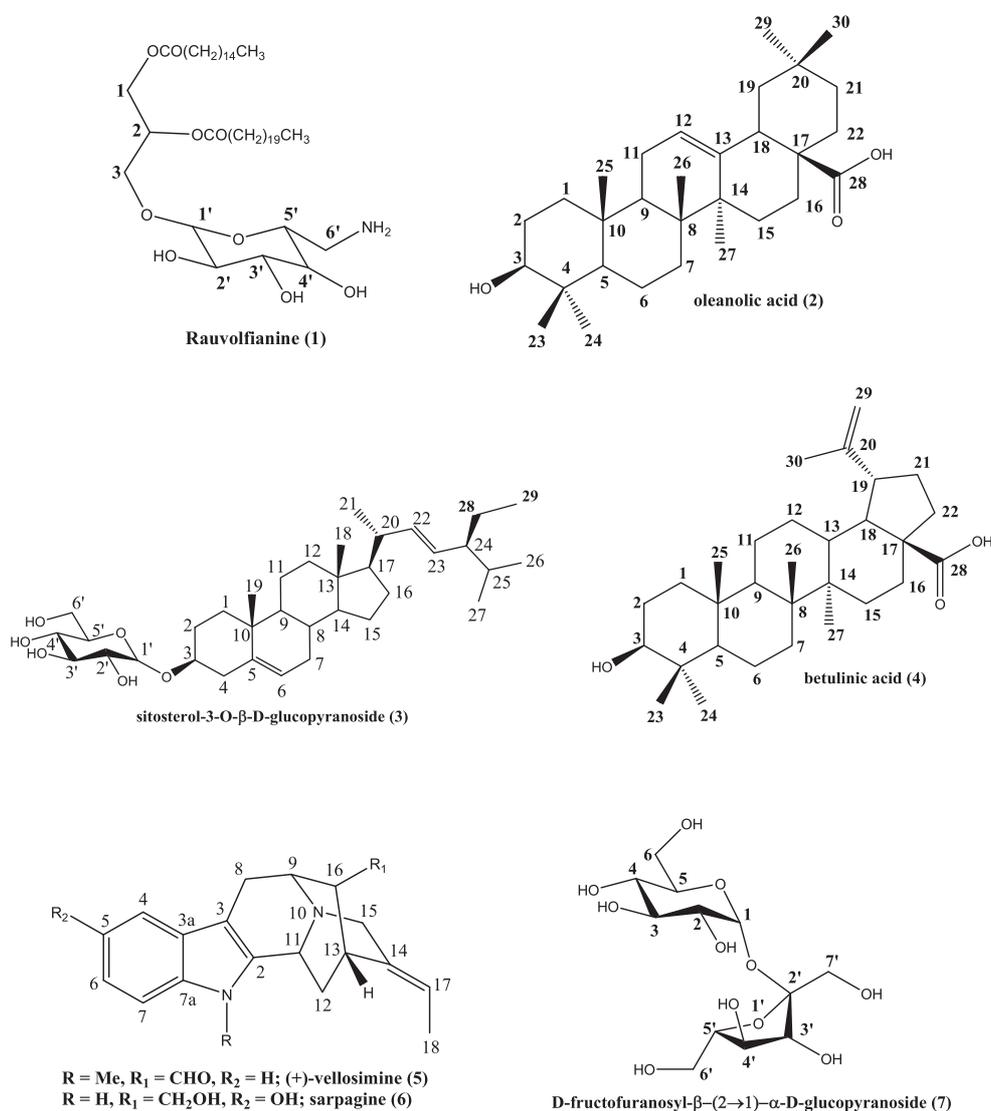


Figure 1. Compounds isolated from the leaves of *Rauvolfia caffra*.

activity has been defined as a ratio of MBC to MIC of > 4 . Thus, all tested compounds exhibited bactericidal action against the studied strain.

3. Experimental section

In supplemental data.

4. Conclusion

The species *R. caffra*, is known as abundant source of alkaloids especially indole alkaloids. Compounds **1**, **2**, **3** and **7** were isolated for the first time from this species. The bioactivity

study of the isolated compounds indicated that compounds **1** and **2** exhibited interesting antitubercular activity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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