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Bruceadysentoside A, a new pregnane glycoside and others secondary metabolites with cytotoxic activity from *brucea antidysenterica* J. F. Mill. (simaroubaceae)

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ABSTRACT

The chemical investigation of the root barks leaves and stem barks of Brucea antidysenterica J. F. Mill. (Simaroubaceae) led to the isolation of a new pregnane glycoside, named Bruceadysentoside A or 3-O-β-L-arabinopyranosyl-pregn-5-en-20-one (1) together with seventeen known compounds. Their structures were established from spectral data, mainly HRESIMS, 1 D and 2 D NMR and by comparison with literature data. Compounds 1, 2, 5, 6, 8, 10, 12 and 13 were tested in vitro for their effects on the viability of two different human cancer cell lines, namely prostate PC-3 adenocarcinoma cells and colorectal HT-29 adenocarcinoma cells. No substantial activities were recorded for 2, 10, 12 and 13 (up to 10 μM concentration). 1, 5 and 8 did not show strong anti-proliferative effects up to 100 μM, however, 6 exhibited a stronger anti-proliferative effect with IC50 values of ∼ 100 μM against PC-3 and ∼ 200 μM against HT-29.

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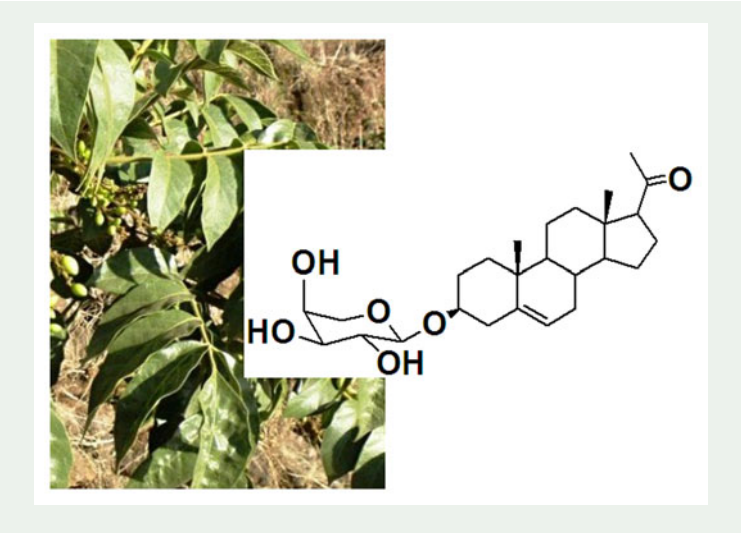

1. Introduction

Brucea antidysenterica J. F. Mill. (Simaroubaceae) is a deciduous, straight cylindrical, erect tree that grows to an average height of 7 m; exceptionally large trees may reach a height of 10-15 m. Leaves are alternate, usually crowded at the end of younger twigs. Fruits are composed of drupes-like ellipsoid mericarps. They are bitter and poisonous to farm animals (Dale and Greenway 1961; Burkill 2000). *B. antidysenterica* is native to Upper Guinea, Nigeria, Cameroon, Congo, Angola and to Eastern Africa from Ethiopia to Tanzania and Zimbabwe (Exell et al. 1963). It is used in folk medicine for the treatment of numerous affections including malaria, helminthic infections, diarrhea, dysentery, fever, asthma and other disorders (Watt and Breyer-Brandwijk 1962; Grace and Fowler 2008). Extracts prepared from the roots have been shown to be active against *Plasmodium gallinaceum* in chicks and hence there is the possibility of antimalarial activity (Spencer et al. 1947). Previous phytochemical studies on the genus Brucea have led to the isolation of quassinoids such as bruceins A-H and M with cytotoxic activities (Imamura et al. 1995; Bawn et al. 2008; Noorshahida et al. 2009; Su et al. 2013); 20-hydroxyyadanzigan with anticomplement activity (Zhan et al. 2019). Canthin-6-one alkaloids (Harris et al. 1985; Kitagawa et al. 1994) and triterpenoids (Liu et al. 2009) were also isolated from this genus. The present work describes the isolation of a new pregnane glycoside from the leaves of *Brucea antidysenterica* together with the evaluation of the cytotoxicity activities of some of the isolated compounds in two cancer cell lines: HT-29 and PC-3.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder soluble in pyridine. It showed positive Molish and Liebermann–Burchard reactions demonstrating its saponin nature (Zheng et al. 2010). Its molecular formula $C_{26}H_{40}O_6$ was deduced from its

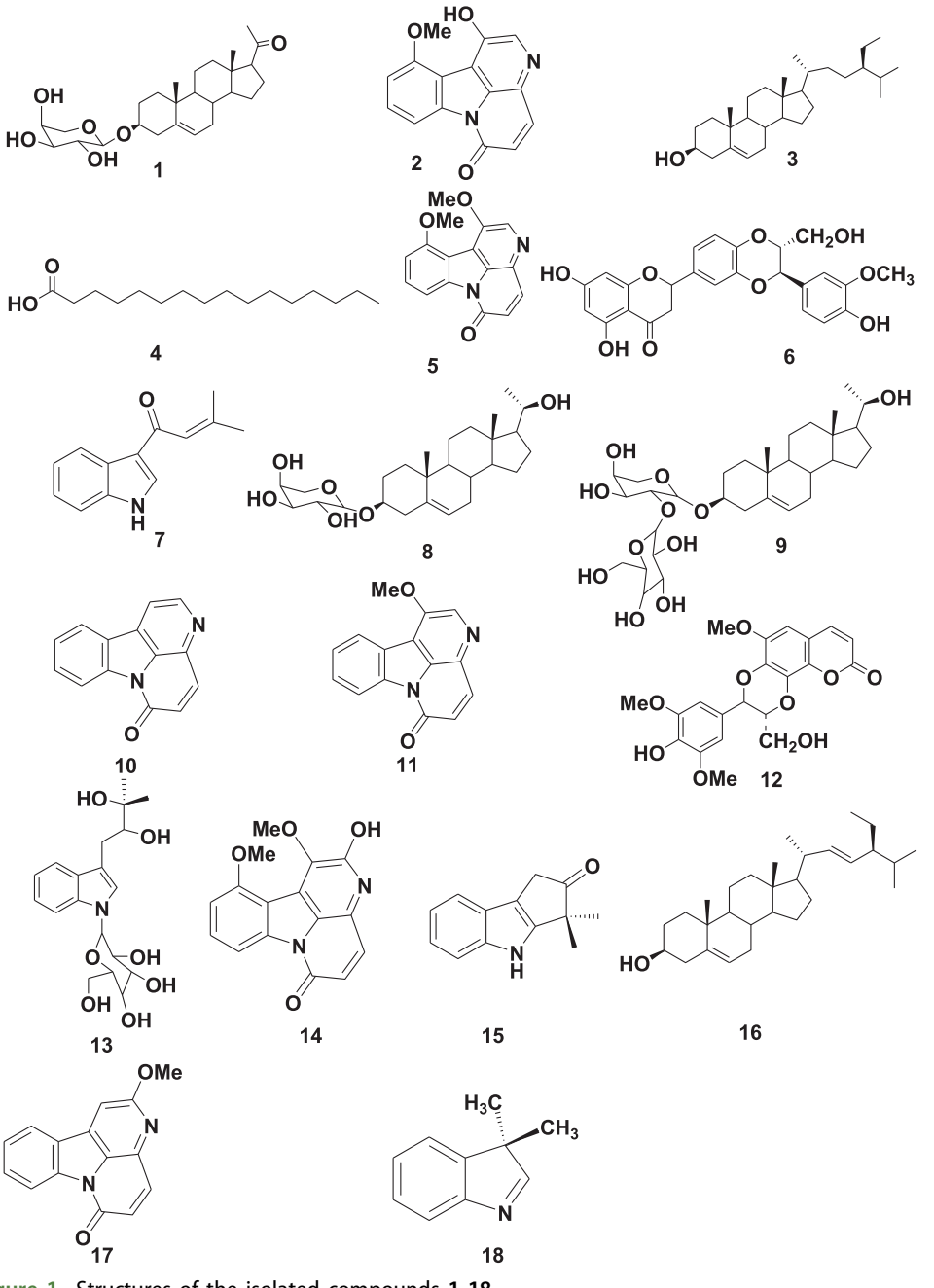

Figure 1. Structures of the isolated compounds **1-18**.

HRESIMS in positive-ion mode which exhibited a pseudo-molecular ion peak $[M + Na]^+$ at m/z 471.2827 (calcd 471.2723 for $C_{26}H_{40}O_6Na^+$). Its IR spectrum showed characteristic bands of hydroxyl, ketone and alkene respectively at 3307, 1690 and 1647 cm^{-1}. Compound **1** was hydrolyzed to give the monosaccharide L-arabinose. This was in accordance with the molecular formula of **1**, $C_{26}H_{40}O_6$ corresponding to a pregnane (C-21) linked to a pentose (C-5). This was confirmed by the ^1H and ^{13}C NMR data of **1**

coupled with its HSQC and DEPT spectra which displayed characteristic signals of a pregnane skeleton with two angular methyl groups at δ 0.58 (s, 3 H)/13.0 and δ 0.88 (s, 3 H)/19.1. However, the signal of the third methyl group C-21 didn't appear as doublet but was rather replaced by a characteristic signal of the methyl of an acetyl group at δ 2.07 (s, 3 H)/31.2. This information was confirmed by the presence of the signal of a ketone group at C-20 (δ 208.3) on the ^{13}C spectra. The anomeric proton of the L-arabinose was observed at δ 4.82 (1 H, d, $J = 7.1$ Hz, H-1')/102.9 and based on its high coupling constant, the configuration of the sugar was determined as β. The signal of one olefinic proton H-6 was observed at δ 5.32 (brs, 1 H)/121.4. The cross-analysis of the ^{13}C and DEPT spectra of **1** revealed (3 × CH_3, 8 ×CH_2, 6 ×CH, 3 ×C for the aglycone). This information suggested that compound **1** is likely to be a pregn-5-ene-3-ol-20-one bearing the L-arabinose sugar unit at C-3. This was in accordance with the HMBC spectrum of **1** in which a long-range correlation between H-3 (δ 3.85) and C-1' (ä 102.9), together with correlation of H-1' (δ 4.82) and C-3 (δ 77.7). On the HRESIMS of **1** the base peak $[M\text{-ara}]^+$ at m/z 299.2444 ($C_{21}H_{31}O^+$) was consistent with the loss of the sugar unit. Compound **1** was then univocally characterized as 3-O-β-L-arabinopyranosyl-pregn-5-en-20-one, an unreported glycosylated pregnane derivative to which the trivial name Bruceadysentoside A was given.

The structures of the known compounds were identified as: 1-hydroxy-11-méthoxycanthin-6-one (**2**) (Harris et al. 1985), β-sitosterol (**3**) (Chaturvedula and Prakash 2012), hexadecanoic acid (palmitic acid) (**4**) (Bulama et al. 2014), 1,11-dimethoxycanthin-6-one (**5**) (Narihiko et al. 1986), hydnocarpin (**6**) (Pan et al. 2009), (3-(3-methyl-1-oxo-2-butenyl))-1H-indole (**7**) (Vijaya et al. 1993), (20 R)-O-(3)-α-L-arabinopyranosylpregn-5-ene-3β,20-diol (**8**) (Liu et al. 2009) and (20 R)-O-(3)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-pregn-5-ene-3β,20-diol (**9**) (Liu et al. 2011). canthin-6-one (**10**) (Narihiko et al. 1986), 1-methoxycanthin-6-one (**11**) (Ohmoto et al. 1976), cleomiscosin C (**12**) (Ray et al. 1985), bruceolline F (**13**) (Yishan et al. 1994), 2-hydroxy-1, 11-dimethoxycanthin-6-one (**14**) (Harris et al. 1985), bruceolline D (**15**) (Yishan et al. 1994), mixture of stigmasterol and β-sitosterol (**16**) (Chaturvedula and Prakash 2012), 2-methoxycanthin-6-one (**17**) (Njar et al. 1993) and 3,3-dimethylindole (**18**) (National Center for Biotechnology Information) (Figure 1).

Bruceadysentoside A (**1**), 1-hydroxy-11-methoxycanthin-6-one (**2**), 1,11-dimethoxycanthin-6-one (**5**), hydnocarpin (**6**), (20 R)-O-(3)-α-L-arabinopyranosylpregn-5-en-3β,20-diol (**8**) canthin-6-one (**10**), cleomiscosin C (**12**) and bruceolline F (**13**) were tested for their effects on the viability of two different human cancer cell lines, namely prostate PC-3 adenocarcinoma cells and colorectal HT-29 adenocarcinoma cells. The cell viability and cytotoxicity assay was conducted by using the tetrazolium salt MTT and colorimetric read-out after 48 h cell treatment. The saponin digitonin (100 μM), a very potent permeabilizer of cell membranes, was used as positive control compromising the cells to yield 0% cell viability after 48 h. Initially, all compounds were fast screened with 10 μM, whereby the compounds **2**, **10**, **12** and **13** were detected to be inactive, whereas the other compounds showed some conflicting signs of activity (data not shown). Hence, the compounds **1**, **5**, **6** and **8** were subsequently tested with concentrations up to 100 μM (**1**, **8**) and 200 μM (**5**, **6**), respectively to determine IC_{50} values. Whereas the compounds **1**, **8** (data not shown) as well as compound **5** did not show

substantial anti-proliferative effects (IC_{50}s $>$ 100 μM for **1** and **8**; IC_{50} $>$ 200 μM for **5**), hydnocarpin (**6**) exhibited a stronger antiproliferative effect with IC_{50} values of ∼ 100 μM against PC-3 prostate cancer cells and ∼ 200 μM against HT-29 colon cancer cells (Figure 2).

3. Experimental (supplementary data)

3.1. Bruceadysentoside a or 3-β-L-arabinopyranosyl-pregn-5-ene-3-ol-20-one (1)

$C_{26}H_{40}O_6$, white amorphous powder; HRESI-MS m/z: 471.2827 $[M+Na]^+$ (cald for 471.2723); m/z 299.2444 $[M\text{-ara}]^+$ ($C_{21}H_{31}O^+$). ^{1}H NMR (600 MHz, pyridine-*d5*) δ: 0.58 (3H, s, 18-CH3), 0.88 (3H, s, 19-CH3), 0.89 (1H, m, H-9), 0.97 (1H, m, H-14), 1.01 (1H, m, H-1α), 1.07 (1H, m, H-15α), 1.23 (1H, m, H-8), 1.26 (1H, m, H-11α), 1.27 (1H, m, H-12α), 1.28 (1H, m, H-7α), 1.46 (1H, m, H-11β), 1.52 (1H, m, H-7β, 15β), 1.55 (1H, m, H-16α), 1.72 (1H, m, H-1β), 1.73 (2H, m, H-2), 1.92 (1H, m, H-12β), 2.07 (3H, s, 21-CH3), 2.29 (1H, m, H-16β), 2.39 (1H, m, H-4α), 2.44 (1H, t, $J = 9.1$ Hz, H-17), 2.65 (1H, m, H-4β), 3.77 (1H, t, $J = 10.7$ Hz, H-5′α), 3.85 (1H, m, H-3), 4.18 (1H, t, $J = 8.8$ Hz, H-3′), 4.29 (1H, d, m, H-4′), 4.31 (1H, m, H-5′β), 4.42 (1H, t, $J = 7.9$ Hz, H-2′), 4.82 (1H, d, $J = 7.1$ Hz, H-1′), 5.32 (1H, brs, H-6); ^{13}C NMR (150 MHz, pyridine-*d5*) δ: 13.0 (C-18), 19.1 (C-19), 21.1(C-11), 22.9 (C-16), 24.4 (C-15), 30.1 (C-2), 31.2 (C-21), 31.7 (C-8), 31.8 (C-7), 36.7 (C-10), 37.4 (C-1), 38.5 (C-12), 39.0 (C-4), 43.7 (C-13), 50.0 (C-9), 56.5 (C-14), 63.4 (C-17), 66.7 (C-5′), 69.3 (C-4′), 72.3 (C-2′), 74.4 (C-3′), 77.7 (C-3), 102.9 (C-1′), 121.4 (C-6), 140.8 (C-5), 208.3 (C-20).

4. Conclusion

The chemical investigation of the leaves, root and stem barks of *Brucea antidysenterica* J. F. Mill. (Simaroubaceae) afforded an unreported pregnane glycoside derivative together with seventeen known compounds including ten alkaloids, four steroids among which two glycosylated pregnane derivatives, a fatty acid, a coumarinolignan and a flavolignan. It is worth noting that quassinoids which are known to be present in several *Brucea* species were not isolated neither in leaves, nor stem and root barks of *Brucea antidysenterica*. However indole alkaloids were identified as principal constituents of this specie and can be considered as chemotaxonomic markers of the *Brucea* genus. One of them, identified as 3,3-dimethylindole is reported here for the first time from natural source. In addition the evaluation of the cytotoxic activities of some of the isolated compounds revealed that hypnocarpin, the only flavolignan isolated in this study, was the most active compound especially on prostate PC-3 adenocarcinoma cells and moderately on colorectal HT-29 adenocarcinoma cells compared to the coumarinolignan, cleomiscosin C which did not show any activity at the same concentration.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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