Cycloartanol and Sutherlandioside C peracetate from *Sutherlandia frutescens* and their immune potentiating effects

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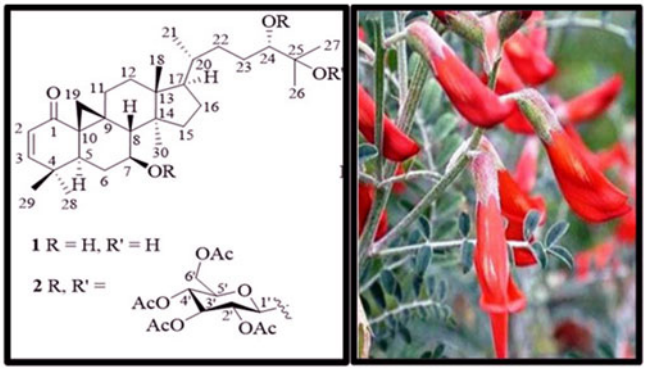
Abstract

A novel cycloartanol (1) and an acylated Sutherlandioside D (2) together with two known cycloartane derivatives, Sutherlandioside B (3) and Sutherlandioside A (4), were isolated from the aerial parts of Sutherlandia frutescens. The structures of these compounds were established by a combination of 1- and 2-D NMR techniques and further confirmed by high resolution ToF mass spectrometry (HRToFMS). Preliminary biological studies were also conducted to assess the activity of different plant extracts, fractions and compounds on cytokine expression. Compounds 1 and 2 prompted an increase in IL-6 expression while compound 4 showed a reduced IL-6 expression compared to the controls. Compound 1 is an effective suppressor of IL-10 expression. The plant compounds inhibited the expression of the two cytokines, IL-10 and TNFα. The results of the assays suggested that some components in the plant extract influence the immune system by suppressing the expression of IL-6, IL-10 and TNFα.

Keywords

Sutherlandia frutescens; cancer bush; cycloartenol; acetylated Sutherlandioside; cytokines



1. Introduction

Sutherlandia frutescens (L) R. Br (Fabaceae) is a medicinal plant used for a wide range of indications (Fernandes et al. 2004; Ojewole 2004; Van Wyk and Albrecht 2008). Reports have indicated that extracts of *S. frutescens* can be used in the treatment of HIV/AIDS (Harnett et al. 2005; Mills et al. 2005), cancer (Tai et al. 2004; Chinkwo 2005; Stander et al. 2007) as well as to treat diabetes (Sia 2004; Chadwick et al. 2007). It is distributed mainly in the Western Cape and Karoo regions of southern Africa and it has been known locally as 'cancer bush' since 1894 (Van Wyk and Albrecht 2008). Extracts of the plant have been shown to have antiviral, antibacterial, anti-oxidant, anti-inflammatory, analgesic, hypoglycaemic and anticonvulsant properties (Ojewole 2004; Katerere and Eloff 2005; Kundu et al. 2005). Extracts of *S. frutescens* have also been reported to alter antiretroviral metabolism through effects on cytochrome P450 and P-glycoprotein activity (Mills et al. 2005; Brown et al. 2008). Studies conducted in South Africa have shown that herbal remedies are good supplements for those undergoing antiretroviral therapy because of their immune boosting properties (Tshibangu et al. 2004). A study in western Uganda has reported that 38% of HIV-positive patients have used traditional medicines and antiretroviral drugs concurrently for the management of HIV infection (Langlois-Klassen et al. 2007). The major reasons given for this practice were the perceived additional efficacy offered by the natural material, improvement in quality of life, and a feeling of control over the disease.

From a phytochemical perspective, secondary bioactive metabolites identified in the aerial parts of *S. frutescens* include D-pinitol, L-canavanine, asparagine, methyl- and propyl-parabens, γ-amino butyric acid (Van Wyk 2008; Van Wyk and Albrecht 2008), multiple cycloartane glycosides (sutherlandiosides A-D; Fu et al. 2010) and four flavonols (sutherlandins A-D; Fu et al. 2008). The amino acids have been proposed to be responsible for the bioactivity. The sutherlandiosides are inhibitors of cytochrome P450 enzymes involved in adrenocorticosteroid metabolism; these thus could be responsible for the observed reduction in corticosterone levels in rats subjected to chronic immobilization stress (Prevoo et al. 2004) and for some of the claimed stress-reducing benefits in humans. The exact compounds responsible for the claimed benefits are, however, unknown. In previous isolation efforts, the sutherlandiosides were purified from the n-butanol soluble fraction of methanolic leaf extracts by silica and reverse-phase chromatography technologies (Katerere and Eloff 2005). Low yields due to irreversible adsorption and limited separation resulted. In this study, one novel cycloartanol, 7*S*,24*S*,25-trihydroxycycloart-2-en-1-one **(1)** was isolated in addition to two known members of the same family, namely 3*R*,7*S*,24*S*,25-tetrahydroxycycloartan-1-one 25-*O*-β-D-glucopyranoside **(3)** and 1*S*,3*R*,7*S*,10*S*,24*S*,25-tetrahydroxy-7,10-epoxy-9,10-*seco*-9,19-cyclolanost-9(11)-ene 25-*O*-β-D-glucopyranoside **(4)**. The tetra-acetylated derivative of sutherlandioside D **(2)** was also isolated and characterised (Figure 1).

2. Results and discussion

The major metabolites from the widely used South African medicinal plant, *S. frutescens*, are being investigated by many for biological activities. Studies conducted by

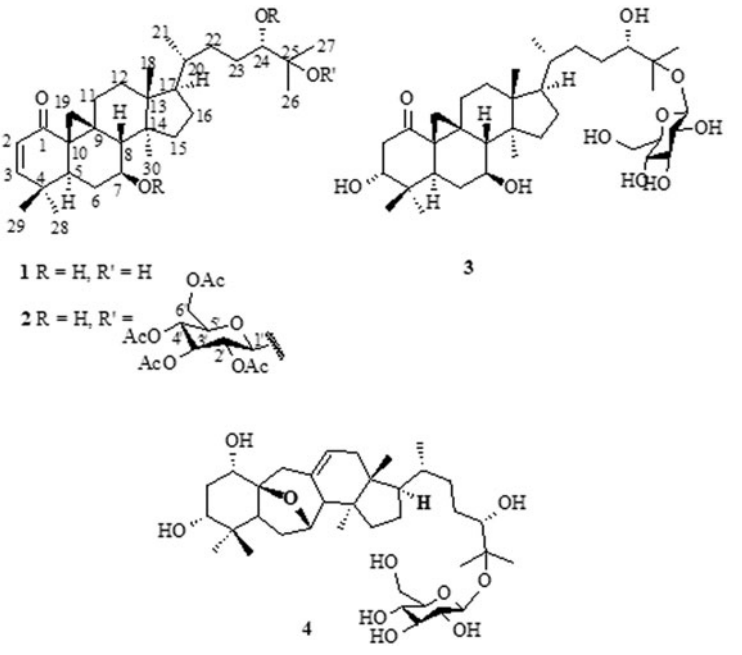


Figure 1. Structures of cycloartenol (**1**), acylated Sutherlandioside D (**2**), and Sutherlandiosides B (3) and -A (**4**).

Avula et al. (2010), using liquid chromatography mass spectrometry showed the presence of cycloartane-type triterpenoids and flavonoids. Four flavonol glycosides (sutherlandins A–D) and four cycloartanol glycosides (sutherlandiosides A–D) from the aerial parts were reported (Avula et al. 2010; Albrecht et al. 2012). The flavonol glycosides (sutherlandins A–D) were also isolated by Chen et al. (2017) using spiral countercurrent chromatography and a rapid quality control method was developed by Mavimbela et al. (2018) using vibrational spectroscopy in conjunction with chemometric data analysis. Extracts of this plant species were shown to consist of a complex mixture of constituents of which various are still unknown (Faleshini et al. 2013). In an attempt to identify other chemical markers, extracts of this plant were fractionated and further purified. TLC analysis of the ethanolic leaf extract of *S. frutescens* revealed the presence of the known cycloartane derivatives, Sutherlandioside A and Sutherlandioside B previously reported (Fu et al. 2008) as well other unknown compounds. Since the extract was very complex and the compounds quite polar, a portion of the extract was acetylated and further purified. Different chromatographic techniques were employed to isolate the compounds. One novel cycloartanol, 7S,24S,25-trihydroxycycloart-2-en-1-one **(1)** was isolated and characterised as well as the tetra-acetylated derivative of Sutherlandioside D **(2)**. Biological studies were also conducted to assess the activity of the plant extracts, fractions and compounds on cytokine expression.

2.1. 7S,24S,25-Trihydroxycycloart-2-en-1-one (1)

The HR ToFMS spectra of **1** (Figure 1) supported the molecular formula of $C_{30}H_{48}O_4$. The ESI^+ showed a pseudo molecular ion at *m/z* 473.363 (calculated for [$C_{30}H_{48}O_4$ +

H]$^+$) and a sodium adduct at *m/z* 495.345 (calculated for [$C_{30}H_{48}O_4$ + Na]$^+$). The ESI$^-$ showed a formic acid adduct at *m/z* 517.352 (calculated mass for [$C_{30}H_{48}O_4$ - H + HCOOH]$^-$) due to its presence in the source. A closer look at the ^{13}C NMR spectrum showed an oxygen-bearing quaternary carbon signal at δ 74.04 (C-25), a carbonyl signal at δ 203.49 (C-1) and two oxygen-bearing methine signals at δ 69.15 (C-7) and δ 79.89 (C-24). Also present were two olefinic carbon signals at δ 129.66 (C-2) and 161.31 (C-3). The ^{1}H NMR spectrum shows the presence of two olefin signals as doublets at δ 5.86 ($J = 9.9$ Hz) and δ 6.73 ($J = 9.9$ Hz), assigned to H-2 and H-3 of the triterpene moiety. It further revealed two protons on oxygen-bearing carbons at δ_H 3.70 (broad multiplet) and δ_H 3.31 (broad doublet) with a coupling constant of $J = 9.0$ Hz. Six tertiary methyl groups were present at δ 0.91 (s, H-30), 0.96 (s, H-28), 0.98 (s, H-18), 1.11 (s, H-26), 1.12 (s, H-29) and 1.14 (s, H-27). One secondary methyl at δ 0.89 (d, H-21, $J = 6.6$ Hz), and a cyclopropane methylene signal at δ 0.94 and 1.58 (each 1 H, $J = 4.8$ and $J = 4.2$, respectively) were also observed. These cyclopropane methylene signals are characteristic of a cycloartane-type structure. These observed correlations were further substantiated by long-range two-dimensional proton-carbon NMR correlations (HMBC) (Supplementary material).

2.2. 7S,24S,25-Cycloart-2-en-1-one hexa-acetate (2)

The molecular formula of this acetylated compound was determined to be $C_{48}H_{70}O_{15}$ by HR ToFMS. ESI + mode showed a pseudo molecular ion at *m/z* 887.479 (calculated for [$C_{48}H_{70}O_{15}$ + H]$^+$) and a sodium adduct at *m/z* 909.461 (calculated for [$C_{48}H_{70}O_{15}$ + Na]$^+$). The proton NMR spectrum revealed the presence of six acetate groups, a sugar moiety and a triterpene backbone. The six acetate methyl groups suggest that the compound contains four acetate groups in the sugar moiety and two acetate groups due to alcohols on the triterpene backbone. Two olefin signals were also present as doublets at δ 5.87 ($J = 9.8$ Hz) and 6.58 ($J = 9.8$ Hz), assignable to H-2 and H-3 of the triterpene moiety; two protons on oxygen-bearing carbons at δ 4.89 (multiplet) and 4.85 were also noted. Six tertiary methyl groups at δ 0.88 (s, H-30), 0.91 (s, H-18), 0.93 (s, H-29), 1.08 (s, H-28), 1.15 (s, H- 26) and 1.19 (s,H-27), one secondary methyl at δ 0.82 (d, H-21, $J = 6.4$ Hz), and cyclopropane methylene signals at δ 1.05 and 1.44 (each 1 H, $J = 4.4$ and $J = 4.8$) were observed. The presence of the cyclopropane methylene signals suggested that this compound might be a cycloartane tritterpene. Carbon NMR displayed 43 resonances with a characteristic oxygen-bearing quaternary carbon signal at δ 79.51 (C-25), along with a carbonyl signal at δ 200.58 (C-1) and two oxygen-bearing methine signals at δ 71.49 (C-7) and 78.62 (C-24). It also showed two olefinic carbon signals at δ 129.25 (C-2) and 158.62 (C-3). Acetate carbonyl signals were observed in the range of δ 169.23 to 170.79, with their corresponding methyl carbon signals in the range from δ 20.82 to 20.90 ppm. Significant here is the presence of a clear H-1′ to C-25 cross correlation, supporting the sugar/cycloartane linkage point. The position of the double bond at C2-C3 was supported by COSY correlation between H-2 and H-3. The cyclopropane methylene's presence was similarly supported by COSY correlation between the H-19a/H-19b protons and protons H-7 and H-8. For

the sugar moiety, typical H1′ to H2′, H2′ to H3′, H4′ to H5′ and H5′ to H6′ COSY correlations were observed.

2.3. Immunomodulating effects

Preliminary studies were conducted to assess any immune potentiation by *S. frutescens* extracts, fractions and compounds. Negative control (blood samples) showed unexpected *de novo* elevated circulating cytokine levels (pg/ml) of IL-6, IL-10 and TNF-α (Figure 2). Most of the samples showed little or no increase (immunopotentiation) or decrease (suppression) in IL-6 expression. However, compounds **1** and **2** prompted a relative increase in IL-6 expression while Fraction 1 and, to a lesser extent, compound **4** showed a reduced IL-6 expression compared to the controls. Fraction 1 and compound **1** are effective suppressors of IL-10 expression; Fraction Ac, compound **4** and compound **2** show intermediate suppression of IL-10 expression. In determining relative immunosuppressive effects, all the samples showed an immunosuppressive effect on TNFα. Emulsion extract, Fraction Ac, and Fraction 1 appeared to exert the greatest suppressive effect on TNFα expression while the remaining 4 samples showed an intermediate effect on TNFα suppression. The only consistent result was that fraction 1, and possibly compound **4**, inhibited the expression of all three of these cytokines. The elevation of TNF-α confirms an observation reported by Estcourt et al. (1997) where a comparison was made between healthy blood donors and HIV-infected patients, and where the healthy donors had high concentration of TNF-α. TNF-α and IL-6 had pro-inflammatory and pro-atherosclerotic actions that induced IL-10 to balance their action by inhibiting atherosclerosis factor as well as a pro-inflammatory inhibitor. This suggested that cytokines may be counterbalanced by inhibitors or other cytokines with opposing effects (Stenvinkel et al. 2005). The test subject in the current study tested negative for HIV.

3. Experimental section

3.1. 7S,24S,25-Trihydroxycycloart-2-en-1-one (1)

White powder; *m/z* 473.363 (calculated for $[C_{30}H_{48}O_4 + H]^+$), ^{1}H NMR (CD_3OD, 600 MHz) δ_H 5.86 (1H, d, $J = 9.9$ Hz, H-2), 6.73 (1H, d, $J = 9.9$ Hz, H-3), 2.29 (1H, dd, $J = 13.5$, 3.3 Hz, H-5), 1.95 (2H, m, H-6), 3.70 (1H, br m, H-7), 2.14 (1H, d, $J = 4.8$ Hz), 1.37 and 1.61 (2H, m, H-11), 1.50 (2H, m, H-12), 2.00 and 2.64 (2H, m, H-15), 1.24 and 1.92 (2H, m, H-16), 1.58 (1H, m, H-17), 0.94 and 1.58 (each 1H, d, $J = 4.8$ and $J = 4.2$, 19a and 19b), 1.47 (1H, m, H-20), 1.50 and 1.34 (2H, m, H-22), 1.27 (2H, m, H-23),3.31 (1H, br d, $J = 9.0$ Hz, H-24), 0.91(3H, s, H-30), 0.96 (3H, s, H-28), 0.98 (3H, s, H-18), 1.11 (3H, s, H-26), 1.12 (3H, s, H-29), 1.14 (3H, s, H-27), and 0.89 (3H, d, H-21, $J = 6.6$ Hz).

3.2. 7S,24S,25-Cycloarta-2-en-1-one peracetate (2)

Colourless oil; *m/z* 887.479 (calculated for $[C_{48}H_{70}O_{15} + H]^+$); ^{1}H NMR ($CD_3OD$, 600 MHz) δ_H 5.87 (1H, d, $J = 9.8$ Hz, H-2), 6.58 (1H, d, $J = 9.8$ Hz, H-3),2.33 (1H, dd, $J = 13.6$, 4.00, H-5), 4.89 (1H, m, H-7), 2.22 (1H, d, $J = 4.8$ Hz, H-8), 0.82 (1 H, d,

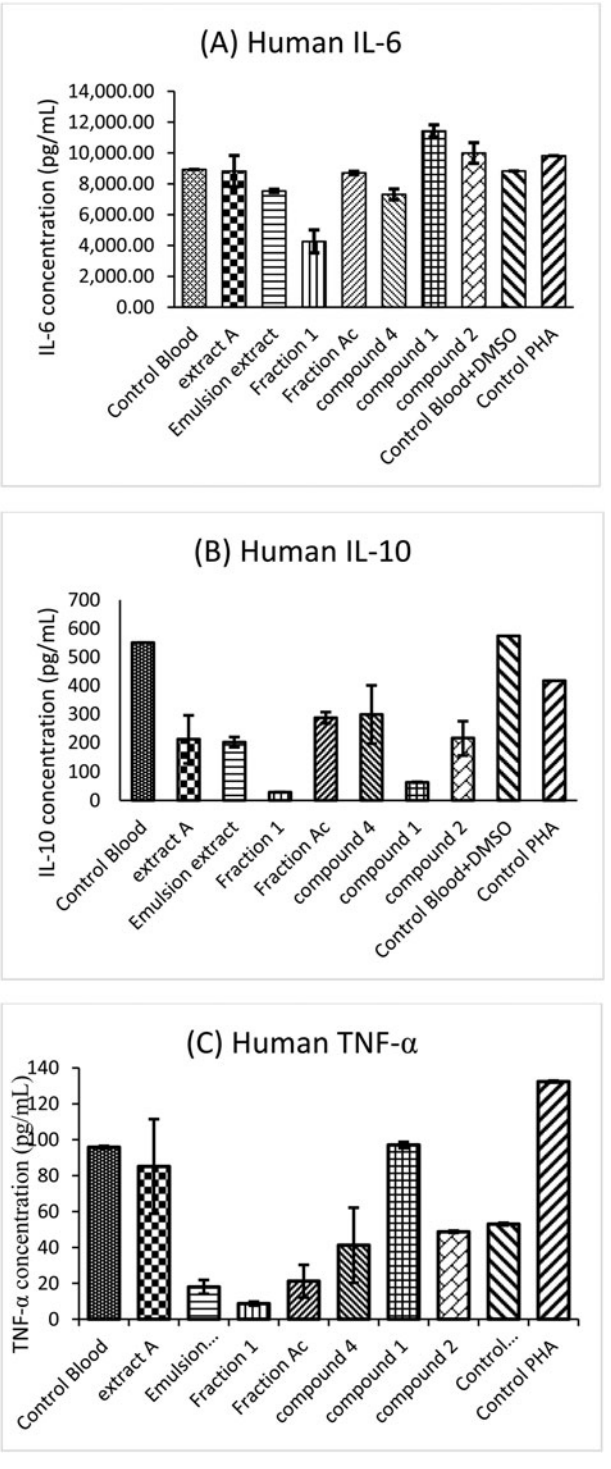


Figure 2. Flow cytometric evaluation of IL-6, IL-10 and TNF-α.

$J = 6.4$ Hz, H-21), 4.85 (1H, m, H-24), 0.88(3H, s, H-30), 0.91 (3H, s, H-18), 0.93 (3H, s, H-29), 1.08 (3H, s, H-28), δ_H 1.15 (3H, s, H- 26), 1.19 (3H, s,H-27), 0.82 (3H, d, H-21, $J = 6.4$ Hz), 1.05 and 1.44 (each 1 H, $J = 4.4$ Hz and $J = 4.8$ Hz, H-19a and 19b), 4.69 (1 H, d, H-1′, $J = 8.0$ Hz), 3.66 (1H, br m, H-2′), 5.19 (1H, t, $J = 9.4$ Hz, H-3′), 5.00 (1H, d, $J = 9.2$ Hz, H-4′), 4.93 (1H, m, H-5′), 4.09 and 4.16 (each 1H, dd, $J = 12.00$, 2.4 Hz and dd, $J = 12.00$, 5.6 Hz, H-6′a and 6′b).

4. Conclusion

The isolation of the novel compound, cycloartanol and the acylated Sutherlandioside D contributes to the phytochemistry profile of this valuable indigenous plant of South Africa. Preliminary studies conducted on the extracts, fractions and compounds of *S. frutescens* extracts showed immune potentiation activities. Compounds **1** and **2** prompted an increase in IL-6 expression while compound **4** showed a reduced IL-6 expression compared to the controls. Compound **1** is an effective suppressor of IL-10 expression. The plant compounds inhibited the expression of the two cytokines, IL-10 and TNFα. However, the study did not provide sufficient evidence to support immune potentiating effects associated with *de novo* expression of cytokines in the blood cells. Many possibilities may be the cause of the *de novo* elevation including stress and depression (Audet et al. 2014). An in-depth analysis of the three cytokines needs to be performed to assess any relationship between the plant extracts, fractions or compounds and the dynamics of cytokine expression/repression. The other confounding factor is the ability to correlate the results obtained in these *in vitro* experiments to immune-potentiating effects *in vivo*.

Disclosure statement

No conflict of interest reported by authors.

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