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Cardenolides from the stem bark of *Salacia staudtiana*

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ABSTRACT

Seven new cardenolides, staudtianoside A – F (**1** – **6**) and staudtianogenin A (**8**), were isolated along with six known compounds from the stem bark of the Cameroonian medicinal plant *Salacia staudtiana* Loes. ex Fritsch. The structures were elucidated by means of ESI-HRMS and NMR spectroscopic methods and by comparison with literature data. The relative configurations of the new compounds were determined by X-ray diffraction analysis, NOESY correlation and coupling constants. We evaluated the antibacterial efficacy of the isolated compounds against two commonly dispersed environmental strains of *Escherichia coli* and *Bacillus subtilis*, as well as against two human pathogenic clinical strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Compounds **1**, **2** and **8** exhibited marked antibacterial potencies against the clinically relevant *P. aeruginosa* that were comparable to the standard antibiotics. Compound **2** was also active against *S. aureus* and *E. coli*. Further, compounds **5** and **8** demonstrated efficacy against *E. coli* as well as *B. subtilis*. The structure-activity relationship of the tested compounds is discussed.

1. Introduction

The genus *Salacia*, belonging to the Celastraceae family, comprises of > 200 species that are widely distributed in tropical and subtropical regions around the world [1]. Many of these species are used indigenously as traditional medicines in the respective country of origin to treat different ailments such as rheumatism, gonorrhea, skin diseases, lumbar muscle strain, and asthenia [1] [2]. Chemical investigations carried out on some other species of the genus *Salacia* have led to the isolation of several types of triterpenes as the most widely encountered classes of compounds [3] [4]. Some of these compounds possess significant biological activities including antimalarial [5], antileishmanial [6], antileukemic [7] and cytotoxic activities [8] [9]. As a part of our ongoing research program on the phytochemical investigation of medicinal plants from Cameroon, a study was carried out on the bark of *S. staudtiana*.

Salacia staudtiana Loes. ex Fritsch is one of the species growing in Cameroon and has been commonly used by traditional healers for the treatment of microbial infections and obesity. To the best of our knowledge, this plant has not been investigated prior to this study. From the stem bark of this plant, we report herein the isolation of seven new compounds (**1**–**6**, **8**) along with six other known compounds. We evaluated the antibacterial efficacies of these compounds against the clinically important risk-group 2 (RG2) pathogenic bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as two environmental strains of *Escherichia coli* and *Bacillus subtilis*. The structure-activity relationship of the tested compounds based on their antibacterial efficacies is also discussed.

2. Results and discussion

The dichloromethane-methanol (1:1) extract of the stem bark of *Salacia staudtiana* was subjected to repeated silica gel column chromatography and further purifications by Sephadex LH-20 and preparative HPLC to give previously unreported compounds **1**–**6** and **8** (Fig. 1) along with six known compounds. By comparison with literature data, the known compounds were identified as elaeodendroside B (**7**) [10], D:B-friedoolean-5-ene-3α,29-diol (**10**) [11], salaspermic acid (**11**) [12], 2α,3β,14β-trihydroxy-3-*O*-(4-deoxy-3-*O*-(methyl-α-L-erythro-pentopyranosyl)-card-4,20(22)-dienolide (**12**) [13], betulin (**13**) [14] and desacetylelaeodendrogenin (**9**) [15]. The structural elucidation of the new compounds are described herein.

Compound **1** was isolated as white crystals and its molecular formula, $C_{29}H_{34}O_{12}$, was deduced from ESI-HRMS (*m/z* 575.2126

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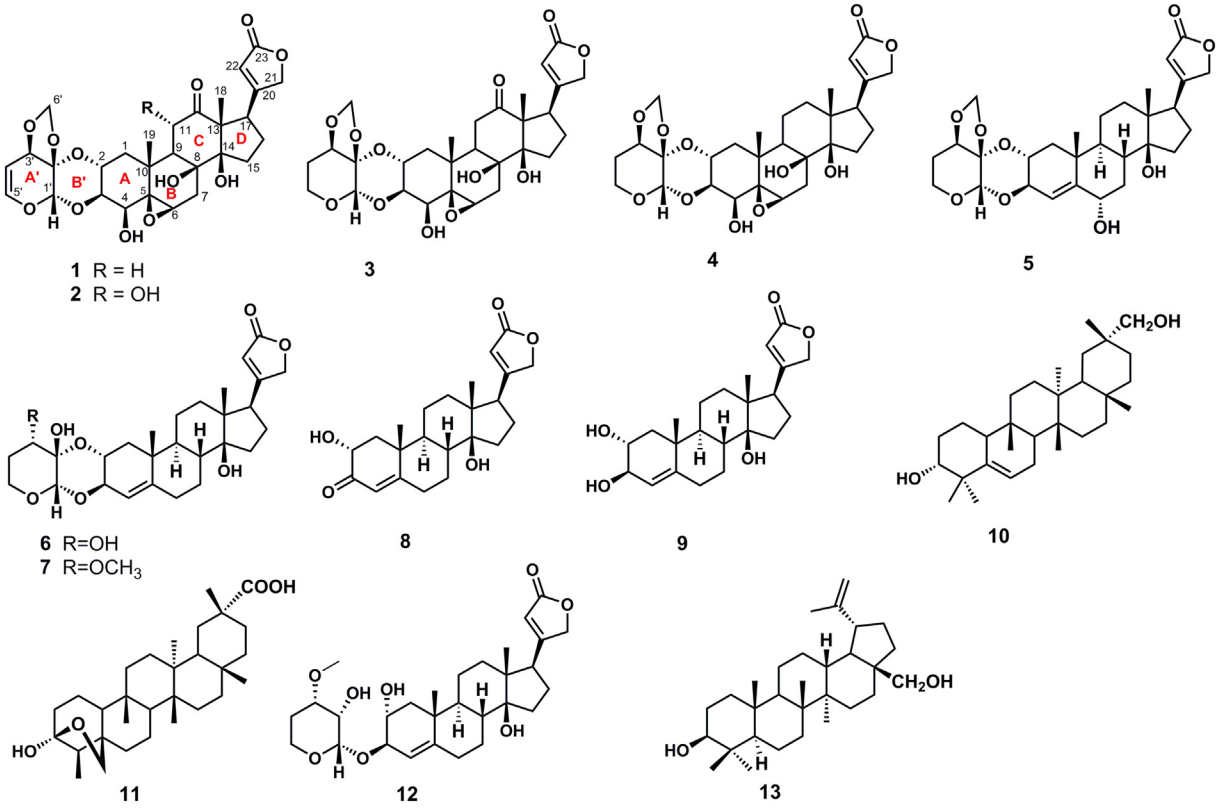

Fig. 1. Structures of compounds **1–13** isolated from *Salacia staudtiana*.

$[M + H]^+$, calcd for $C_{29}H_{35}O_{12}$, 575.2123) and NMR data. The ^1H NMR spectrum (Table 1) exhibited signals for two tertiary methyls [δ 1.17 and 1.50], a singlet olefinic proton (δ 5.97) and two olefinic protons with a *cis*-double bond [δ 5.21 (*dd*, J = 6.2, 4.0 Hz) and 6.51 (d, J = 6.2 Hz)]. In the ^{13}C NMR spectrum (Table 2) the signals for 29 carbon atoms were observed. From DEPT and HSQC experiments these signals corresponding to two methyls, seven methylenes, eleven methines (three olefinic), and nine quaternary carbons (a ketone carbonyl at δ 211.4 and a conjugated ester group at δ 174.2). All these data coupled with a literature survey indicated that compound **1** is an analogue of 4β,8β,11α,14β-tetrahydroxy-5β,6β-epoxy-12-oxo-2α-O,3β-O-[(2′β,3′β-methylendioxy-4′-desoxy-5′-dehydroxymethyl-hexosulose]-card-20(22)-enolide, a cardiac glycoside isolated from *Elaeodendron orientale* [16]. Comparison of their NMR data revealed that the two compounds share the same tetracyclic moiety and a characteristic α, β-unsaturated γ-lactone. Their ^1H and ^{13}C NMR data found to be similar except the presence of a double bond in compound **1**. This was confirmed from *cis*-coupled protons at δ 5.21 (*dd*, J = 6.2, 4.0 Hz) and 6.51 (d, J = 6.2 Hz) in the sugar ring. The position of this double bond to C-4′/C-5′ was determined from the HMBC spectrum: the methylendioxy protons H-6′ (δ 5.05 and 5.18) and the two olefinic protons [H-4′ (δ 5.2) and H-5′ (δ 6.5)] showed a strong correlations in the HMBC spectrum to carbon signal at δ 75.8 (C-3′). This correlations confirmed the double bond to C-4′/C-5′ rather than C-3′/C-4′. The presence of the characteristic α, β-unsaturated γ-lactone was evident from ^1H NMR signals which showed an olefinic singlet at δ 6.01(H-22) and two methylene protons at δ 4.78 and 4.88 (H-21). All of these protons showed HMBC correlations to C-20, C-21 and C-22. Moreover the connectivity of the α, β-unsaturated lactone to C-17 was deduced from the observed HMBC correlations of H-17 to C-20, C-21 and C-22.

The connectivity between C-1′ and C-3 through an oxygen bridge was confirmed from the observed HMBC correlation of H-1′ to C-3 and H-2 to C-2′, indicating the presence of the dioxane bridge. The relative configuration of the dioxane ring was established from the coupling constant $J_{2,3}$ (10.2 Hz) and suggested diaxial orientation for H-2 and H-3. Furthermore, the coupling constant $J_{3,4}$ (3.2 Hz) and the NOESY correlations of H-2/3H-19 suggested β-oriented position for the hydroxyl group at C-4 and H-2.

In order to determine the configuration and substitution patterns in **1**, the sample was re-crystallized from methanol-water and subjected to single-crystal X-ray diffraction analysis (Fig. 2). The result confirmed the structure of **1** with the relative configuration as shown in Fig. 1. Thus, on the basis of these observations compound **1** is reported for the first time as 4*β*,8*β*,14*β*-trihydroxy-5*β*,6*β*-epoxy-12-oxo-2*α*-*O*,3*β*-*O*-[(2′*β*,3′*β*-methylendioxy-4′-desoxy-5′-dehydroxymethyl-hexosulose]-card-4′,20(22)-dienolide and trivially named staudtianoside A.

Compound **2** was isolated as yellowish powder and its ESI-HRMS exhibited a quasi-molecular ion peak at *m/z* 591.2076 $[M + H]^+$ (calcd for $C_{29}H_{35}O_{13}$, 591.2072) compatible with a molecular formula $C_{29}H_{34}O_{13}$, in conjunction with NMR data. The ^1H NMR spectrum (Table 1) exhibited signals for two tertiary methyls [δ 1.20 and 1.78], a singlet olefinic proton (δ 6.01) and two olefinic protons with a *cis*-double bond [δ 5.2 (*dd*, J = 6.2, 4.0 Hz) and 6.51 (d, J = 6.2 Hz)]. In the ^{13}C NMR spectrum (Table 2) the signals for 29 carbon atoms were observed. DEPT and HSQC experiments revealed that these signals corresponded to a ketone carbonyl (δ 212.8), an ester carbonyl (δ 174.0), four sp^2 carbons (δ 99.6, 144.6, 171.9, and 119.3), two methyls (δ 18.7), six methylenes and twelve methines. The ^1H and ^{13}C NMR data of compounds **2** and **1** were nearly the same and the difference was just the presence of a hydroxylated carbon signal (δ_C 70.6) in the ^{13}C