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CHEMICAL CONSTITUENTS OF THE STEM OF *SALACIA IMPRESSIFOLIA* (MIERS) A. C. SMITH

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ABSTRACT

The plants of the genus Salacia are commonly used in the treatment of diabetes tipe-2, in several countries of the world. The phytochemical study of the stem of *Salacia impressifolia* led to the isolation and identification of seven compounds. The compounds include the steroids sitosterol and 3-*O*-β-D-glucopyranosyl sitosterol, of the coumarin isoscopoletin and of the triterpenes quinovic acid, 3-oxo-quinovic acid, 3-*O*-β-D-quinovopyranosyl quinovic acid, and 3-*O*-β-D-fucopyranosyl quinovic acid. The compounds were identified based on analysis of their NMR spectral data and comparison with literature data. This is the first report on the isolation and identification of quinovic acid and derivatives from species of the genus *Salacia*.

Keywords: Hippocrateaceae, *Salacia impressifolia*, quinovic acid, isoscopoletin, saponins, triterpenoids, NMR.

INTRODUCTION

Diabetes is one of the various diseases treated by the use of medicinal plants. The genus *Salacia*, with regard to treatment of diabetes type 2, has a long history in traditional medicine in Sri Lanka, India and Thailand (Tanabe *et al*., 2009). The use of these species in the treatment of diabetes is due to the presence of potent α-glucosidase inhibitors in their constitution, for example, salacinol, kotalanol and 13 MRT (Figure 1) (Muraoka *et al*., 2008; Oe and Ozaki, 2008). The inhibition of this enzyme promotes delayed absorption of glucose in the blood and suppresses postprandial hyperglycemia, resulting in glycemic control (Li *et al*., 2008). Several species of this genus have been studied in search of bioactive compounds and a better understanding of their chemical composition. The genus *Salacia* is rich in triterpenoids belonging to several series, including ursane and oleanane obtained from *Salacia amplifolia* (Wang *et al*., 2011), bisnortriterpenes obtained from *Salacia madagascariensis* (Thiem *et al*., 2005), quinonemethides from *Salacia campestris* (Carvalho *et al*. 2005), friedelanes and glycosides identified from *Salacia chinensis* (Morikawa *et al*., 2003; Nakamura *et al*., 2011), 1,3-diketofriedelane triterpene from *Salacia*

verrucosa (Somwong *et al*., 2011) and lupanes obtained from *Salacia cordata* (Tinto *et al*., 1992). This paper describes the isolation and identification of compounds **1**-**7** obtained from the extract of the stem bark of *Salacia impressifolia* (Miers) A. C. Smith (Hippocrateaceae).

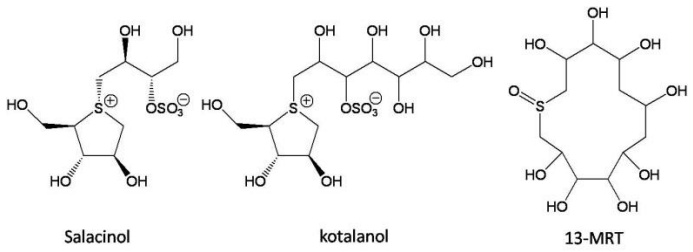

Figure 1: Structures of salacinol, kotalanol and 13 MRT.

MATERIALS AND METHODS

Materials

NMR spectra, including ^{1}H-^{1}H COSY, DEPT and HMBC experiments, were recorded on a Varian Mercury-300 spectrometer, operating at 300 MHz at ^{1}H and 75 MHz at ^{13}C (Varian, Palo Alto, CA, USA), $CDCl_3$, CD_3OD and pyridine-*d*5 were used as solvents (Sigma Aldrich, St. Louis, MO, USA). Column chromatography was performed on silica gel 60 (70–230 mesh, Macherey-Nagel, Düren, Germany).

Collection of Plant Material

The stem of *Salacia impressiflia* was collected in the Amazonas State, Brazil. A voucher specimen (No. 4321) was deposited in the Herbarium Embrapa/UFPA in Santarém, Pará State, Brazil.

Extraction and Isolation Procedure

The stem of *S. impressifolia* (2.0 kg) was air dried and extracted with MeOH at room temperature. After extraction, the methanolic solution was subjected to partition with hexane, yielding the concentrated methanolic phase (CMP) and concentrated hexane phase (CHP) after solvent evaporation in rotatory evaporator. CMP (20.0 g) was fractionated by chromatography on silica gel column using mixtures of hexane, EtOAc and MeOH as eluents in increasing order of polarity. Compound **1** (4.5 mg) was obtained from fraction eluted with hexane/EtOAc (9:1). The fraction hexane/EtOAc (7:3) was submitted to silica gel column eluted with mixtures of hexane and EtOAc in increasing order of polarity, and the resulting fraction eluted with hexane/EtOAc (8:2) furnished a mixture (30 mg) of compounds **2** and **3**. The CMP fraction eluted with EtOAc (100%) was also fractionated in silica gel column, eluted with mixtures of hexane, EtOAc and MeOH in increasing order of polarity and a mixture (55.7 mg) of compounds **4** and **5** was obtained from the subfraction eluted with hexane/EtOAc (4:6) after washing it with MeOH.

A second extraction from the stem of *Salacia impressifolia* (2.18 kg) was performed at room temperature successively with hexane and ethyl acetate, and concentrad under vacuum. The ethyl acetate extract (28.0 g) showed particles of a yellow solid. Part of this extract (25.0 g) was washed successively with a solution of hexane/CH_2Cl_2 (1:1) and ethyl acetatel. The fraction washed with hexane/CH_2Cl_2 1:1 (3.1 g) was fractionated by column chromatography (CC) with silica gel as stationary phase and mixtures of hexane/EtOAc in increasing order of polarity as mobile phase. Subfractions eluted with hexane/EtOAc (9:1) and EtOAc (100%) provided the compounds **6** (40 mg) and **7** (8.5 mg), respectively. The ethyl acetate fraction was also fractionated by column chromatography and from the fractions eluted with hexane/EtOAc (75:25) and hexane/EtOAc (15:85) the compounds **2** and **3** in mixture (1.2 g) and **4** and **5** also in mixture (2.3 g) were obtained, respectively.

RESULTS AND DISCUSSION

Methanolic extract of stem bark from *S. impresifolia*, after phytochemical procedures, gave one coumarin (**1**), four triterpenoids (**2**-**5**), and two steroids (**6** and **7**). The structural elucidations of all isolated compounds were performed by means of the comparison of their spectral data (^{1}H, ^{13}C and DEPT NMR) with those ones of the literature. The isolated compounds (**1**-**7**) were identified as: isoscopoletin (**1**) (Waight *et al*., 1987), quinovic acid (**2**) (Miana and Al-hazimi, 1986), 3-oxo-quinovic acid (**3**) (Adeoye and Waigh, 1983), 3-*O*-β-D-fucopyranosyl quinovic acid (**4**) (Ferrari *et al*., 1981), 3-*O*-β-D-quinovopyranosyl quinovic acid (**5**) (Frou *et al*., 2003), β-sitosterol (**6**) and 3-*O*-β-D-glucopyranosyl sitosterol (**7**) (Kojima, 1990). The structures of the compounds isolated are shown in figure 2.

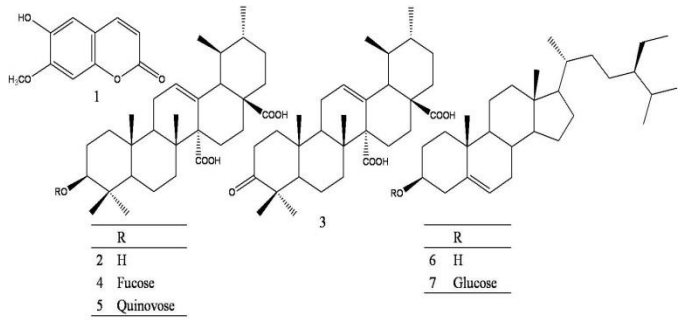

Figure 2: Structures of the compounds **1**-**7**.

The compounds 3-oxoquinovic acid (**2**), quinovic acid (**3**), and two glycosylated derivatives of quinovic acid (**4**) and (**5**) are not natural products obtained very often. This is the first report on the isolation and identification of the compounds quinovic acid (**2**), 3-oxo-quinovic acid (**3**), 3-*O*-β-D-fucopyranosyl quinovic acid (**4**) and 3-*O*-β-D-quinovopyranosyl quinovic acid (**5**) from species of *Salacia* genus. Quinovic acid and its glycosides derivatives quinovic acid 3*β*-O-*β*-D-glycopiranoside and quinovic acid-3*β*-O-*β*-D-glucopyranosyl-(28→1)-*β*-D-glucopyranosyl ester, isolated from *Fagonia cretica*, were reported as having anti-diabetic or DPP-4 inhibiting activity (Saleem *et al*., 2014). Based on this study, the quinovic acid derivatives **2**, **4** and **5** are compounds with potential anti-diabetic activity.

Spectroscopic data of Isoscopoletin (1), Quinovic acid (2) and derivatives (3-5)

*Isoscopoletin (**1**)*
Wellow crystals (hexane/EtOAc 9:1) 4.5 mg. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.95 (3H, *s*, OCH_3-7), 6.27 (1H, *d*, *J*=9.5 Hz, H-3), 6.84 (1H, *s*, H-8), 6.91 (1H, *s*, H-5), 7.60 (1H, *d*, *J*=9,5).

*Quinovic acid (**2**)*
White solid **(**hexane/EtOAc, 8:2) 30.0 mg. ^{13}C NMR (75 MHz, Pyridine) δ: 180.0 (C-28), 178.0 (C-27), 134.1 (C-13), 129.0 (C-12), 77.9 (C-3), 56.8 (C-14), 55.7 (C-5), 54.9 (C-18), 48.7 (C-9), 47.2 (C-17), 40.0 (C-8), 39.3 (C-19), 39.2 (C-1), 39.2 (C-4), 37.7 (C-7), 37.5 (C-20), 37.3 (C-10), 37.0 (C-22), 30.5 (C-21), 28.5 (C-15), 28.1 (C-23), 26.3 (C-2), 25.5 (C-16), 23.4 (C-11), 21.3 (C-30), 18.9 (C-6), 18.8 (C-29), 18.2 (C-26), 16.6 (C-25), 16.5 (C-24).

*3-oxo-quinovic acid (**3**)*
White solid **(**hexane/EtOAc, 8:2) 30.0 mg. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.77-1.23 twelve signals of twelve methyl groups (3H each, *s*, H-23, H-24, H-25, H-26, H-29 and H-30), 6.01 (1H, *s*, H-12). ^{13}C NMR (75 MHz, Pyridine) δ; 216.3 (C-3), 180.0 (C-28), 177.9 (C-27), 134.2 (C-13), 128.6 (C-12), 56.6 (C-14), 54.9 (C-18), 54.7 (C-5), 48.7 (C-17), 47.0 (C-4), 46.2 (C-9), 40.0 (C-7), 39.7 (C-1), 39.3 (C-20), 39.2 (C-8), 37.7 (C-19), 37.0 (C-10), 36.7 (C-22), 34.2 (C-2), 30.5 (C-21), 26.8 (C-23), 25.4 (C-15), 23.4 (C-16), 23.3 (C-11), 21.4 (C-25), 21.3 (C-30), 19.9 (C-6), 18.7 (C-26), 18.2 (C-29), 16.1 (C-24).

*3-O-β-D-fucopyranosyl quinovic acid (**4**)*
White solid **(**hexane/EtOAc, 4:6) 55.7 mg. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.82-1.00 six signals of six methyl groups (3H each, *s*, H-23, H-24, H-25, H-26, H-29 and H-30), 3.09 (1H, *dd*, *J*=11.5 and 4.3 Hz, H-3), 4.22 (1H, *d*, *J*=7.5 Hz, H-1'), 5.60 (1H, *m*, H-12). ^{13}C NMR (75 MHz, CD_3OD) δ: 181.6 (C-28), 179.1 (C-27), 133.8 (C-13), 130.4 (C-12), 107.1 (C-1'), 90.6 (C-3), 75.2 (C-3'), 73.1 (C-2'), 72.9 (C-4'), 71.6 (C-5'), 57.2 (C-14), 56.9 (C-5), 55.5 (C-18), 49.5 (C-17), 48.0 (C-9), 40.6 (C-8), 40.3 (C-4), 40.1 (C-19), 39.9 (C-1), 38.3 (C-20), 38.0 (C-10), 37.8 (C-7), 37.6 (C-22), 31.2 (C-21), 28.5 (C-24), 27.1(C-2), 26.4 (C-15), 25.7 (C-16), 23.8 (C-11), 21.5 (C-30), 19.2 (C-6), 19.1 (C-23), 18.2 (C-26), 17.1 (C-6'), 16.9 (C-25), 16.9 (C-29).

*3-O-β-D-quinovopyranosyl quinovic acid (**5**)*
White solid **(**hexane/EtOAc, 4:6) 55.7 mg. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.82-1.00 six signals of six methyl groups (3H each, *s*, H-23, H-24, H-25, H-26, H-29 and H-30), 3.09 (1H, *dd*, *J*=11.5 and 4.3 Hz, H-3), 4.27 (1H, *d*, *J*=7.8 Hz, H-1'), 5.60 (1H, *m*, H-12). ^{13}C NMR (75 MHz, CD_3OD) δ: 181.6 (C-28), 179.1 (C-27), 133.8 (C-13), 130.4 (C-12), 106.5 (C-1'), 90.7 (C-3), 77.9 (C-3'), 77.0 (C-5'), 75.8 (C-2'), 72.8 (C-4'), 57.2 (C-14), 56.9 (C-5), 55.5 (C-18), 49.5 (C-17), 48.0 (C-9), 40.6 (C-8), 40.3 (C-4), 40.1 (C-19), 39.9 (C-1), 38.3 (C-20), 38.0 (C-7), 37.8 (C-10), 37.6 (C-22), 31.2 (C-21), 28.5 (C-23), 27.1 (C-2), 26.4 (C-15), 25.7 (C-16), 23.8 (C-11), 21.5 (C-30), 19.2 (C-29), 19.1 (C-6), 18.2 (C-26), 18.2 (C-6'), 17.1 (C-25), 16.9 (C-24).

CONCLUSION

The identification of compounds Quinovic acid (**2**), 3-oxo-quinovic acid (**3**), 3-*O*-β-D-fucopyranosyl quinovic acid (**4**) and 3-*O*-β-D-quinovopyranosyl quinovic acid (**5**) belonging to the class of triterpenoids is consistent with the chemical composition of the family Hippocrateaceae and the genus *Salacia*. This is the first report on the isolation and identification of quinovic acid and derivatives from species of the genus *Salacia*. The paper contributes to knowledge of the chemical composition of species, genus and family in study.

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CONFLICT OF INTEREST

None declared.

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