Algerian Journal of Natural Products 8:1 **(2020)** 753 - 757

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products
www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

Leishmanicidal Activity of a Quinone Methide Triterpenoid from the roots of *Senna spectabilis var. excelsa* (Fabaceae)

J.A. Monjane [1,2,*], D. Capusiri [3], A. Giménez [3] and O. Sterner [2]

[1] *Chemistry Department, Eduardo Mondlane University, P.O. Box 257, Maputo, Mozambique*
[2] *Centre for Analysis and Synthesis, Lund University, P.O. Box 118, 22100, Lund, Sweden*
[3] *Instituto de Investigaciones Fármaco Bioquímicos, San Andrés University, La Paz, Bolivia*

**Corresponding Author: Phone: +258 82 8676 497; email:monjane.juliao@gmail.com*

Received: 2019 -10-03 /Accepted: 2020 -06-20

Abstract: From an extract of the roots of *Senna spectabilis* var. *excelsa (Fabaceae)*, eight known metabolites including the quinone methide triterpenoid 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**1**) and friedelin (**2**), a putative biosynthetic precursor of this class of metabolites, were isolated by applying several chromatographic methods. The structures of the isolated metabolites were established by High Resolution NMR and MS analysis, and confirmed by comparison of their experimental spectroscopic and physical data with those previously reported in the literature. The 2D structure and relative configuration of metabolite **1** were initially determined via analysis of NOESY experiment and confirmed by quantum mechanics-based ^{13}C NMR chemical shift calculations. Metabolite **1** showed a potent leishmanicidal activity against *Leishmania amazonensis* and *L. brazilensis*, with the IC_{50} values 9.6 and 6.3 µM, respectively, comparable with the positive control miltefosine (IC_{50} values 12.5 and 12.0 µM, respectively), a currently used agent for leishmaniosis treatment. The first example of the occurrence of a quinone methide triterpenoid in species belonging to the Fabaceae family and the first leishmanicidal activity of metabolite **1** are reported in this paper.

Keywords: Senna spectabilis (var. excels), Fabaceae, Quinone methide triterpenoids, Natural products, Chromatographic methods, Leishmanicidal activity

I. Introduction

Protozoan parasites are among the most common pathogens in the world. They are recognized as the causative agents of some of the most serious tropical diseases in humans and domestic animals. Malaria, trypanosomiasis, and leishmaniosis are among diseases caused by protozoan parasites that affect approximately 25 % of the world's population, mostly in developing countries. According to WHO, malaria, African trypanosomiasis, Chagas disease, and leishmaniosis are considered some of the most important tropical diseases [1–3].

Leishmaniosis is a tropical disease caused by a number of protozoan species belonging to the genus of *Leishmania*. This ailment affects around 12 million people in 80 countries around the world, and it has been estimated that there is about 2 to 3 million new cases each year. In addition, leishmaniosis is considered one of the most neglected diseases worldwide [2]. The first chemotherapy to treat leishmaniosis was based on the use of toxic heavy metals, particularly, compounds containing antimony. However, the emergence of resistant strains has limited their usefulness. The use of drugs, such as pentamidine isothionate, amphotericin B, miltefosine, and paramomycin, as alternatives for the treatment of leishmaniosis has some limitations due to their toxicity and high costs [4–6].

© Authors, 2020 Algerian Journal of Natural Products (Online ISSN: 2353-0391)
This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License

Currently, a number of alternative agents are being developed. However, up to date none of these has been demonstrated to be fully effective against *Leishmania* parasites [2].

In recent years, screening programs for secondary metabolites derived from medicinal plants used for the treatment of leishmaniosis have been carried out. These studies have confirmed the importance of many plant species as potential sources for the isolation of novel compounds with leishmanicidal activity, as highlighted in a review about plant secondary metabolites based on the above-mentioned activity published by Chan-Bacab and Peña-rodríguez [2]. The drug resistance developed by *Leishmania* strains requires the evaluation of alternatives to the therapeutically agents available, and their affordability and toxicity is also a problem [7]. In continuing search for interesting bioactive secondary metabolites with leishmanicidal activity from Mozambican plants, we investigated the roots extracts of *Senna spectabilis* for its chemical constituents.

Senna or *Cassia* is considered one of the most representative genus of the Fabaceae family. The genus is comprised by 350 plant species, and is widely distributed in tropical and subtropical areas of Africa, Asia, Australia, and Southern America. Several *Cassia* species are used in the traditional medicine to treat a wide range of ailments, as well as for ornamental purposes worldwide [8–10]. *Senna spectabilis* var. *excelsa* (*syn*. *Cassia spectabilis*) is a tree with 7 to 10m high [11]. The plant has been used traditionally in Mozambique for the treatment of diarrhoea, stomach-ache, tuberculosis, and asthma [12]. Previous phytochemical studies of *S. spectabilis* led to the isolation and characterization of several compounds, including alkaloids, in particular piperidine alkaloids, triterpenoids, and phenolic compounds [8, 9, 10, 13, 14]. The piperidine alkaloids present in the flowers were found to possesses leishmanicidal activity [15].

In this paper, we wish to report the isolation and the leishmanicidal activity of a quinone methide triterpenoid, 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**1**). Besides the isolation of compound **1**, friedelin (**2**), a putative biosynthetic precursor of this class of compounds [16], was also isolated, together with six known compounds from an extract of the roots of this plant. Despite the fact that compound **1** is a known compound previously isolated from *Salacia kraussii* and reported to possesses antimalarial activity [17], this is the first report describing the isolation of a quinone methide triterpenoid from a specie of the Fabaceae family, and the leishmanicidal activity of the compound **1**.

II. **Experimental Section**

II.1 ***General experimental procedures***

1D and 2D NMR spectra were recorded at room temperature with a Bruker Avance II 400 spectrometer. The chemical shifts (δ) are reported in ppm relative to solvent residual signals (δ_H 7.26 and δ_C 77.0 for $CDCl_3$). The HRESI-MS data were recorded with a Waters Q-TOF Micromass spectrometer, using H_3PO_4 for calibration and as internal standard. The IR spectra data were recorded on a Bruker Alpha-P ART-IR spectrometer. The melting point measurements were carried out on Gallenkamp instrument. The optical rotations were measured by a Perkin-Elmer Model 341 Polarimeter (T = 20 ^oC and D = 589 nm). The column chromatography (CC) was performed using silica gel 60 (230-400 mesh, Merck) and gel permeation on Sephadex LH-20 (GE-Healthcare). Analytical TLC plates were visualized under a UV lamp at 254 nm and by spraying with vanillin followed by heating. All solvents used were analytical grade.

II.2 ***Plant material***

The roots of *S. spectabilis (var. excelsa)* were collected in Moamba District, Maputo Province in Mozambique, in June 2013, identified locally by Mr. Francisco Mapanga, and were dried in the shade until use. A voucher specimen with number C/157 is kept in the herbarium of Departamento de Ciências Biológicas at Eduardo Mondlane University (Maputo, Mozambique).

© Authors, 2020 Algerian Journal of Natural Products (Online ISSN: 2353-0391)
This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License

II.3 *Extraction and isolation*

The air-dried roots (300g) of *S. spectabilis* (var. excelsa) were grinded into a fine power. The powder was extracted sequentially with heptane, chloroform, EtOAc, and mixture of MeOH:H_2O (7:3). The chloroform extract (500mg) was fractionated by column chromatography using a mixture of heptane:EtOAc (2:1 to 1:2) to yield three main fractions F1, F2, and F3. F1 (46.6mg) was subjected to Sephadex LH-20 MeOH:$CHCl_3$ (3:7) and three main fractions (F1A, F1B, and F1C) were collected. F1A (26.3mg) was then subjected to column chromatography over silica gel in a mixture of heptane:EtOAc (2:1) to yield compound **1** (12.5mg) as reddish solid. F2 (36mg) was fractionated in a silica gel column chromatography (eluted with heptane:EtOAc (2:1)) which led to the isolation of *β*-amyrin (**4**) (7.4mg) and lupeol (**6**, 6.1mg). F3 yielded pure octandronic acid (**3**, 15.3mg).

4.0g of the EtOAc extract were dissolved in a mixture of MeOH:H_2O (9:1) and then extracted with $CHCl_3$. The chloroform soluble part was concentrated and fractionated in a column chromatography over silica gel by elution with heptane:EtOAc (4:1 to 1:1) to yield friedelin (**2**, 24.6mg) and ursolic acid (**5**, 14.2mg). One of subfraction was subjected to PTLC using the eluent solvent heptane:EtOAc (4:1) to yield, chrysophanol (**7**, 7.2mg) and scopoletin (**8**, 5.2mg).

II.4 *Leishmanicidal assay*

The activity was measured on in vitro cultures of Leishmania parasite in promastigote forms of complex *L. amazonensis* (clon 1: Lma, MHOM/BR/76/LTB-012) and complex *L. braziliensis* (strand M2904 C192 RJA), cultivated at 26°C in Schneider medium (pH 6.8) supplemented with inactivated (56°C x 30min) calf bovine serum (10%). Parasites in logarithmic phase of growth, at a concentration of 1 x $\underline{10^6}$ parasites/mL, were distributed on 96 micro well plates and different concentrations of compound **1** (1.5, 3.1, 6.2, 12.5, 50, 100 µM) were added. The micro well plates were incubated for 72hrs at 26 °C after which a solution of XTT (1mg/mL) in PBS (pH 7.0 at 37°C) with PMS (Sigma Aldrich, 0,06mg/mL), was added (50µL/well) and the incubation continued for 4hrs at 26 °C. All assays were carried out as triplicates. DMSO (1%) and miltefosine were used as negative and positive control. The optical density of each well was determined with a Synergy HT microplate reader, at λ: 200-450nm. The IC_{50} values were calculated using the Gen5 program (BioTek) [18].

III. Results and Discussion

The roots extracts of *S. spectabilis* (var. excelsa) were subjected to several chromatographic fractionations to yield eight known compounds. Their chemical structures were determined by high resolution NMR and MS experiments, and confirmed by comparison of their spectroscopic data with those previously reported in the literature. The isolated compounds were determined to be 17-(methoxycarbonyl)-28-*nor*-isoiguesterin (**1**) [17], friedelin (**2**) [19], octandronic acid (**3**) [20], *β*-amyrin (**4**) [21], ursolic acid (**5**) [22], lupeol (**6**) [23], chrysophanol (**7**) [24], and scopoletin (**8**) [25]. Their structures are presented in the Figure 1.

The leishmanicidal activity of compound **1** was assayed against *L. amazonensis* and *L. braziliensis* strains. The results showed that compound **1** has the IC_{50} values 9.6 and 6.3 µM, respectively, and is more potent towards both strains tested than miltefosine (IC_{50} values 12.5 and 12.0 µM, respectively), a currently drug used for the treatment of leishmaniosis. The 20-*epi*-isoiguesterinol, another quinone methide triterpenoid isolated from *S. madagascariensis* was found to be more potent against *L. donovani* and *L. mexicana* when compared with amphotericin B, an agent also used to treat leishmaniosis [26]. Thus, the quinone methide triterpenoids could show a great potential for future development of drugs for the treatment of *Leishmaniosis*, mainly the isoguesterin derivatives. In addition, *S. spectabilis* extracts could be used for the treatment of leishmaniosis in folk medicine since other studies has been shown that piperidine alkaloids present in its flowers have leishmanicidal activity [14]. Quinone methide triterpenoids are class of secondary metabolites, which occur mainly in higher order plant families, such as Celastraceae and Hyppocrateaceae. This class of compounds

© Authors, 2020 Algerian Journal of Natural Products (Online ISSN: 2353-0391)
This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License

display a wide range of biological activities, including anti-inflammatory, antioxidant, antifungal, antitrypanosomal, antimicrobial, and antitumor properties [19].

.

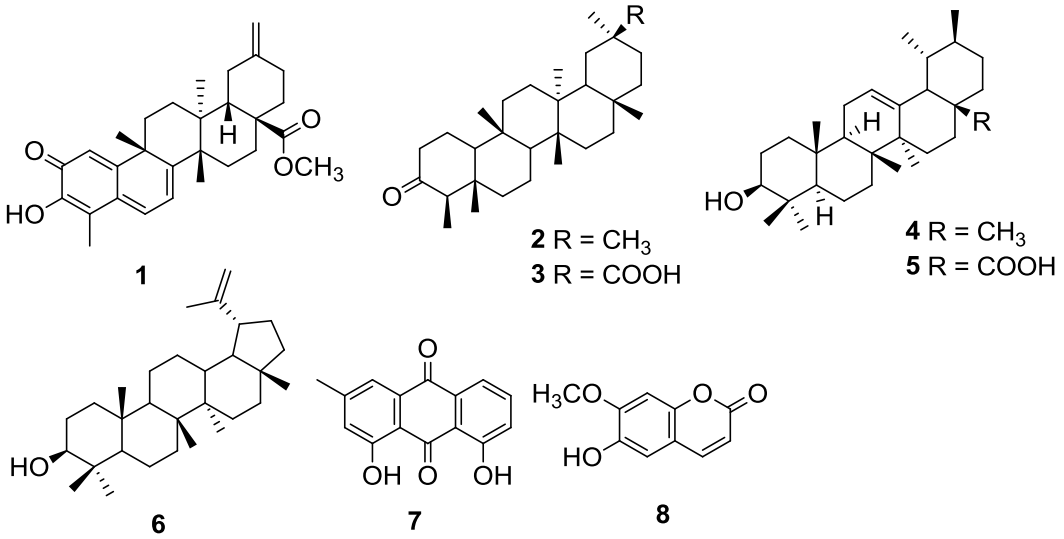

Figure 1. *Chemical structures of compounds (**1**-**8**) isolated from the roots of S. spectabilis (var. excelsa).*

17-(Methoxycarbonyl)-28-nor-isoiguesterin (**1**): redish solid; 12.5mg; m.p 193-195°C; $[\alpha]_D^{25}$ = +84.4 (*c* 1.0 $CHCl_3$); IR (film): 3367 (OH), 2947 and 2871 (aliphatic), 1724 (C=O), 1596 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): *δ* 7.04 (1H, *br s*, OH), 7.00 (1H, *d*, *J* = 6.8 Hz, H-6), 6.54 (1H, *s*, H-1), 6.30 (1H, *d*, *J* = 6.8 Hz, H-7), 4.61 (2H, *s*, H-29), 3.75 (3H, *s*, H-30), 2.62 (1H, *d*, *J* = 6.7 Hz, H-18), 2.49 (1H, *d*, *J* = 15.6 Hz, H-19), 2.30 - 2.06 (6H, *m*, H-11, H-16, H-19, H-21, H-22), 2.21 (3H, *s*, H-23), 2.04-1.94 (2H, *m*, H-11, H-22), 1.90-1.84 (2H, *m*, H-12), 1.82-1.72 (1H, *m*, H-15), 1.72-1.54 (2H, *m*, H-15, H-16), 1.48 (3H, *s*, H-25), 1.36 (3H, *s*, H-26), 0.74 (3H, *s*, H-27); ^{13}C NMR ($CDCl_3$, 100 MHz): *δ* 178.5 (C=O, C-2), 178.3 (C=O, C-28), 168.7 (C, C-8), 164.9 (C, C-10), 146.7 (C, C-3), 146.0 (C, C-20), 133.9 (CH, C-6), 127.8 (c, C-5), 119.7 (CH, C-1); 117.9 (CH, C-7), 117.3 (C, C-4), 108.9 (CH, C-29), 52.3 (CH_3O-, C-30), 45.8 (C, C-17), 44.4 (C, C-14), 43.0 (C, C-9), 40.5 (C, C-13), 39.4 (C, C-18), 39.0 (CH_3, C-25), 33.8 (CH_2, C-11), 32.9 (CH_2, C-22), 32.1 (CH_2, C-19), 31.9 (CH_2, C-21), 31.8 (CH_2, C-16), 29.4 (CH_2, C-12), 28.1 (CH_2, C-15), 21.1 (CH_3, C-26), 19.5 (CH_3, C-27), 10.3 (CH_3, C-23),; HRESIMS *m/z* 449.2692 (calc. for $C_{29}H_{37}O_4$).

IV. Conclusion

Compound **1** showed a potent leishmanicidal activity when compared with miltefone, a currently drug used in leishmaniosis treatment. These results could be attributed to the presence of many double bonds conjugated, which could react with tiols groups presents in protein as Michael acceptors. This could suggest that compound **1** as a promising structure for leishmaniosis treatment. Thus, the specie *S. spectabilis var. excelsa* could be used as a medicinal plant for the treatment of leishmaniosis in traditional medicine, and substitute anti-leishmaniosis drugs which are less efficient.

V. References

[1] Borst P, Quelette M. New mechanisms of drug resistance in parasitic protozoa, Ann. Rev. Microbiol. 49 (1995) 427–460.
[2] Chan-Bacab MJ, Peña-Rodríguez LM. Plant natural products with leishmanicidal activity, Nat. Prod. Rep. 18 (2001) 674–688.
[3] Gallagher R, Marx J, Hines P. Progress in parasitology, Science 264 (1994) 1827.
[4] Berman JD, Lee LS. Activity of Oral Drugs against *Leishmania Tropica* in Human Macrophages in Vitro, Am. J. Trop. Med. Hyg. 32 (1983) 947–951.

© Authors, 2020 Algerian Journal of Natural Products (Online ISSN: 2353-0391)
This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License

[5] Chawla B, Madhubala R. Drug targets in Leishmania, Am. J. Trop. Med. Hyg. 84 (2010) 1–13.
[6] Dos Santos VAFFM, Leite KM, Da Costa Siqueira M, Regasini LO, Martinez I, Nogueira CT. Galuppo MK, Stolf BS, Pereira MAS, Cicarelli RMB, Furlan M, Graminha MAS. Antiprotozoal activity of quinonemethide triterpenes from *Maytenus ilicifolia* root barks (Celastraceae), Molecules. 18 (2013) 1053–1062.
[7] Sen R, Chatterjee M. Plant derived therapeutics for the treatment of Leishmaniasis, Phytomedicine. 18 (2011) 1056–1069.
[8] Viegas C, Pivatto M, De Rezende A, Hamerski L, Silva DHS, Da Silva Bolzani V. 7-Hydroxycassine: A new 2,6-dialkylpiperidin-3-ol alkaloid and other constituents isolated from flowers and fruits of *Senna spectabilis* (Fabaceae), J. Braz. Chem. Soc. 24 (2013) 230–235. doi:10.5935/0103-5053.20130029.
[9] Viegas C, Da V, Bolzani S, Furlan M, Barreiro EJ, Young MCM, Tomazela D, Eberlin MN, Further bioactive piperidine alkaloids from the flowers and green fruits of *Cassia spectabilis*, J. Nat. Prod. 67 (2004) 908–910.
[10] Viegas C, Silva DHS, Pivatto M, De Rezende A, Castro-gambôa I, Bolzani VS. Lipoperoxidation and Cyclooxygenase Enzyme Inhibitory Piperidine Alkaloids from *Cassia spectabilis*, Green Fruits, J. Nat. Prod. 70 (2007) 2026–2028.
[11] Karau GM, Nyagah E, Njagi M, King A, Wangai LN. Phytonutrients , Minerals and in vitro Antioxidant Capacity of Leaf and Stem Bark Powders of *Senna spectabilis.* 2 (2013) 51–59.
[12] Bruschi P, Morganti M, Mancini M, Signorini, MA. Traditional healers and laypeople: A qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique), J. Ethnopharmacol. 138 (2011) 543–563.
[13] Christofidis I, Welter A, Jadot J. Spectalinine and iso-6-carnavaline, two unprecedented piperidine alkaloids from the seeds of *Cassia spectabilis*, Tetrahedron. 33 (1977) 3005–3006.
[14] De Albuquerque Melo GM, Silva MCR, Guimarães TP, Pinheiro KM, Da Matta CBB, De Queiroz AC, Pivatto M, Da Silva Bolzani V, Alexandre-Moreira MS, Viegas C.Leishmanicidal activity of the crude extract, fractions and major piperidine alkaloids from the flowers of *Senna spectabilis*, Phytomedicine. 21 (2014) 277–281.
[15] Muniz G, Melo DA, Campelo M, Silva R, Pereira T, Mantovani K, Barbosa C, Cavalcanti A, De Q, Pivatto M, Bolzani S, Alexandre-Moreira MS, Viegas C.Phytomedicine Leishmanicidal activity of the crude extract , fractions and major piperidine alkaloids from the flowers of *Senna spectabilis*, Eur. J. Integr. Med. 21 (2014) 277–281.
[16] Corsino J. Biosynthesis of friedelane and quinonemethide triterpenoids is compartmentalized in Maytenus aquifolium and Salacia campestris, Phytochemistry. 55 (2000) 741–748.
[17] Figueiredo JN, Räz B, Séquin U. Novel quinone methides from *Salacia kraussii* with in vitro antimalarial activity, J. Nat. Prod. 61 (1998) 718–723.
[18] Capusiri ES, Pinell GR, Huallpara JCT, Turba AG. Colorimetric method - XTT: as a tool for high performance evaluation of substances with leishmanicidal activity, Biofabro. 16 (2008) 21–27.
[19] Sousa GF, Duarte LP, Alcântara AFC, Silva GDF, Vieira-Filho SA, Silva RR, Oliveira DM. Takahashi JA. New triterpenes from *Maytenus robusta*: Structural elucidation based on NMR experimental data and theoretical calculations, Molecules. 17 (2012) 13439–13456.
[20] Yeboah EMO, Majinda RRT, Kadziola A, Muller, A. Dihydro-*β*-agarofuran sesquiterpenes and pentacyclic triterpenoids from the root bark of *Osyris lanceolata*, J. Nat. Prod. 73 (2010) 1151–1155.
[21] Abbas M, Disi A, Al-Khalil S. Isolation and identification of anti-ulcer components from *Anchusa strigosa* root, Jordan J. Pharm. Sci. 2 (2009) 131–139.
[22] Lai JS, Huang JY, Huang KF. Constituents from the Stems of Lantana camara, Chin. Pharm. J. 48 (1996) 451–458.
[23] Al-Musayeib NM, Mohamed GA, Ibrahim SRM, Ross SA. Lupeol-3-O-decanoate, a new triterpene ester from *Cadaba farinosa* Forssk. growing in Saudi Arabia, Med. Chem. Res. 22 (2013) 5297–5302.
[24] Zhang H, Guo Z, Wu N, Xu W, Han L, Li N, Han Y, Zhang H, Guo Z, Wu N, Xu W, Han L, Li N, Han Y. Two novel naphthalene glucosides and an anthraquinone isolated from *Rumex dentatus* and their antiproliferation activities in four cell lines, Molecules. 17 (2012) 843–850.
[25] Darmawan A, Kosela S, Kardono LBS, Syah YM. Scopoletin, a coumarin derivative compound isolated from *Macaranga gigantifolia* Merr, J. Appl. Pharm. Sci. 2 (2012) 175–177.
[26] Thiem DA, Sneden AT, Khan SI,.Tekwani BL. Bisnortriterpenes from *Salacia madagascariensis*, J. Nat. Prod. 68 (2005) 251–254.

© Authors, 2020 Algerian Journal of Natural Products (Online ISSN: 2353-0391)
This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License