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PENTACYCLIC TRITERPENES FROM S*arcostemma clausum*

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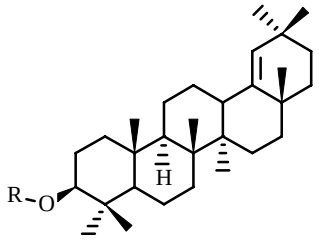
A homologous series of alkanoic acid (C_2–C_5) esters of germanicol was isolated from the methanolic extract of Sarcostemma clausum, *of which germanicol-3-propionate and 3-pentanoate have not been previously described in the literature. In addition, taraxasterol, multiflorenol , and bauerenol were also isolated for the first time from the genus* Sarcostemma. *Structures were elucidated by chemical and spectroscopic methods (NMR, IR, SM) and by comparison with literature data.*

Key words: *Sarcostemma clausum,* Asclepiadaceae, pentacyclic triterpenes, germanicol-3-propionate, germanicol-3-pentanoate.

The genus *Sarcostemma* (Asclepiadaceae) consists of about 100 species distributed in America, Africa and Australia. It is scarcely mentioned in the chemical literature. Indeed, only six species have been studied, namely *S. brevistigma*, *S. Viminale, S. stocksii*, *S. acidum, S. australe, S. brunourianum,* and *S. spp.* As part of our interest in the phytochemical study of *Asclepiadaceae* growing in the Andes, we decided to investigate the aerial part of *Sarcostemma clausum* (Jacq.) Roemer & Schultes. *S. clausum* is a laticiferous plant distributed from Florida to Argentine [1], also known as white twine vine (synonym: *Funastrum clausum* (Jacq.) Schltr). *S. clausum* is widely distributed in Venezuela, where it is known by the vernacular name "bejuco del Diablo" [2]. It has been described at several locations of the Orinoco and Botanamo rivers. In the Andes region *S. clausum* is found at several locations of Tachira State [2] and Merida State, where the material object of this study was collected [1]. No chemical investigation of *Sarcostemma clausum* has been reported up to now, except partial characterization of the latex proteases [3].

S. clausum is used in Jamaican folk medicine as a remedy for colds, while in Costa Rica and Guatemala, poultices of crushed leaves are used as larvicide against *Dermatobia hominis* [4]. A weak antimicrobial activity was found in the methanolic extract of *S. clausum* [5].

The crude methanolic extract of the aerial part was triturated in $CHCl_3$, leading to partial dissolution. Further trituration of the $CHCl_3$-soluble fraction in ethanol allowed precipitation of a solid (F1), which was separated from the EtOH-soluble part (F2).



1 - 4

R = $CH_3(CH_2)_nCO$,
n = 3 (**1**); n = 2 (**2**); n = 1 (**3**); n = 0 (**4**)

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F1 was chromatographed on silica gel columns using benzene/petroleum ether 5:1 as eluant. The resulting fraction yielded a sequence of four pentacyclic triterpene esters (**1–4**) along with taraxerol. GC analysis of the crude ester fraction led to the following proportions: **1** (0.9%), **2** (56.2%), **3** (8.4%), **4** (34.5%). Compounds **1–4** gave a positive Liebermann–Buchard test for a triterpene. They were identified as a homologous series of alkanoic acid (C_2–C_5) esters of germanicol on the basis of spectroscopic data (NMR, IR, SM) and chemical behavior. Germanicol-3-pentanoate (**1**) and 3-propionate (**3**) are new compounds, whereas 3-butyrate (**2**) and 3-acetate (**4**) were previously reported in *Sarcostemma spp*. [6]. Traces of germanicol-3-hexanoate (**5**) were also detected by GPC, but attempts to isolate a pure sample were unsuccessful. Compounds **1–5** led to germanicol after alkaline hydrolysis.

The HREIMS spectrum of **1** showed a $[M]^+$ at *m/z* 510.4429 consistent with the formula $C_{35}H_{58}O_2$. In the EIMS, the most abundant peak was at *m/z* 85 (C_5H_9O), suggesting a pentanoyl moiety, which was also evidenced by an IR absorption band at 1705 cm^{-1} and five signals in the ^{13}C NMR spectrum at 173.60, 34.70, 24.73, 22.22, and 13.62. The 30 additional signals observed in the ^{13}C NMR spectrum of **1** were in agreement with those described for germanicol [7].

The molecular formula of **3** was established as $C_{33}H_{54}O_2$ from the HREIMS. The most abundant fragment at *m/z* 57 (C_3H_7O) in the EIMS spectrum suggested a propionoyl moiety, which was confirmed by three signals at 174.21, 27.98, and 9.24 in the ^{13}C NMR spectrum in addition to signals pertaining to germanicol, and by an ester IR band at 1703 cm^{-1}.

The EtOH-soluble fraction F2 was also chromatographed on silica gel. The sequence of germanicol esters was isolated in the less polar fractions in mixture with alkanes. The most polar fractions are mixtures of triterpenoid alcohols and steroids. Repeated chromatographies of this mixture did not allow the isolation of pure compounds, except b-sitosterol and di-sitosterol. Spectroscopy data for the remaining mixture are consistent with triterpene alcohols. GC/MS analysis allowed identification of α-amiryn (44%), taraxasterol (24%), β-amiryn (10%), taraxerol (8%), germanicol (6%), lupeol (3%), bauerenol (3%), and unidentified compounds (2%). The fraction was acetylated and then chromatographed on 20% silver nitrate-impregnated silica gel. Height pure triterpene acetates were isolated and identified in order of elution as α-amiryn, β-amiryn, multiflorenol, bauerenol, germanicol (**4**), taraxerol, taraxasterol, and lupeol by comparison of their NMR spectra with published data [8–12]. Taraxasterol, multiflorenol, and bauerenol were found for the first time from the genus *Sarcostemma*.

EXPERIMENTAL

Melting points were determined on a Metler capillary apparatus and are uncorrected. IR spectra were recorded as KBr pellets on a Perkin Elmer FT instrument, Model Paragon series 1000 PC. NMR spectra were recorded with a Bruker DPX 300 spectrometer using $CDCl_3$ as solvent and internal standard. Mass spectra were recorded on an Autospec-Q apparatus at 70 eV and at a source temperature of 200°C. GC/MS analyses were performed on a Hewlett Packard 5973 mass spectrometer system equipped with a nonpolar bonded 50-m HP-5 capillary column (0.25 mm id, 0.25 mm film thickness). The ionization energy was 70 eV. Gas chromatography was performed isothermally at 300°C on a Shimadzu 14A fitted with a FID detector and a 5% phenylmethyl polysiloxane fused-silica column (VB-5, 30 m × 0.25 mm, film thickness 0.25 mm). Analytical thin-layer chromatography was performed on Macherey-Nagel and Fluka aluminum and plastic-backed silica gel foils (UV254). Phenol-sulfuric acid and Liebermann reagents were used for visualization. Column chromatography was performed on silica gel E. Merck grade 60, 230–400 mesh or 20% $AgNO_3$-impregnated silica gel. In all cases, elution was performed with freshly distilled solvents.

Plant Material. Aerial parts of *Sarcostemma clausum* were collected on March 2001 in San Rafael de Lagunillas, Merida State, Venezuela, at an elevation of 1000 m above sea level. The plant material was identified by Dr. Gilberto Morillo, from the Faculty of Forestry, University of Los Andes, where a voucher specimen is kept.

Extraction and Isolation of the Constituents from the Methanol Extract. The aerial parts (8 Kg) were dried at 45°C and ground to yield 1.1 Kg of dry material, which was extracted with MeOH at room temp. The methanolic extract was concentrated to dryness, yielding 168 g of a viscous material, which was triturated with $CHCl_3$ under sonication, rendering 49 g of a chloroform-soluble fraction. This material was suspended in boiling EtOH to yield a precipitate (F1, 4.5 g) and a solution (F2, 44.5 g), which were separated by filtration.

F1 was subjected to flash CC on silica gel using a mixture of benzene–petroleum ether (5:1) with the 90 eluted fractions (50 ml each) being combined to give 14 fractions (1–14). Repeated flash CC on fraction 2–5 using the same eluant afforded **1** (16 mg), **2** (654 mg), **4** (739 mg, 6.6%), and **5** (873 mg). Intermediate fractions (170 mg) were submitted to further flash

chromatography using petroleum ether–CH_2Cl_2 (1:1) followed by PTLC, leading to the isolation of **3** (18 mg). Repeated flash CC of fraction 8 led to taraxasterol (390 mg).

The ethanol-soluble fraction F2 (49 g) was flash chromatographied over silica gel using a gradient of CH_2Cl_2–Et_2O. The 36 eluted fractions (100 ml each) were combined to give 8 fractions (1–8). Repetitive flash chromatographies of fraction **7** (22.7 g) on silica gel did not allow isolation of pure compounds. Some fractions (700 mg) were gathered and acetylated with acetic anhydride–pyridine. Repeated chromatographies on 20% silver nitrate-impregnated silica gel eluting with a petroleum ether–CH_2Cl_2 mixture (1:1) yield the acetates of a-amiryn (70 mg), b-amiryn (50 mg), multiflorenol (3 mg), bauerenol (22 mg), germanicol (8 mg), taraxerol (25 mg), taraxasterol (80 mg), and lupeol (80 mg).

Germanicol-3-pentanoate (1). $C_{35}H_{58}O_2$, M^+ at *m/z* 510.4429 (calculated for $C_{35}H_{58}O_2$ 510.4437), R_f 0.78 (TLC Petroleum ether–CH_2Cl_2, 1:1), IR (KBr, cm^{-1}): 1705, ^{1}H NMR (300 MHz, $CDCl_3$, J/Hz): 0.75 (s, Me-27), 0.81 (s, Me-23, Me-25), 0.87 (s, t, Me-26, Me-5′), 0.90, 0.91 (2s, Me-29, Me-30), 0.98 (s, Me-28), 1.04 (s, Me-24), 2.26 (t, J = 7.5, H-2′, H-19), 4.45 (m, H-3), 4.82 (s, H-19).

^{13}C NMR (300 MHz, $CDCl_3$): 38.47 (C-1), 23.61 (C-2), 80.47 (C-3), 37.74 (C-4), 55.44 (C-5), 18.03 (C-6), 34.22 (C-7), 40.63 (C-8), 50.99 (C-9), 37.02 (C-10), 21.00 (C-11), 26.07 (C-12), 38.27 (C-13), 43.20 (C-14), 27.40 (C-15), 37.58 (C-16), 34.40 (C-17), 142.56 (C-18), 129.64 (C-19), 32.24 (C-20), 33.21 (C-21), 37.23 (C-22), 27.81 (C-23), 15.96 (C-24), 16.49 (C-25), 16.65 (C-26), 14.47 (C-27), 25.13 (C-28), 31.22 (C-29), 29.07 (C-30), 173.60 (C-1′), 34.70 (C-2′), 24.73 (C-3′), 22.22 (C-4′), 13.62 (C-5′).

Germanicol-3-butyrate (2): $C_{34}H_{56}O_2$, R_f 0.71 (TLC Petroleum ether–CH_2Cl_2, 1:1), ^{13}C NMR (300 MHz, $CDCl_3$): 38.97 (C-1), 24.11 (C-2), 80.94 (C-3), 38.1 (C-4), 55.94 (C-5), 18.52 (C-6), 34.89 (C-7), 41.13 (C-8), 51.48 (C-9), 37.50 (C-10), 21.49 (C-11), 26.56 (C-12), 38.76 (C-13), 43.69 (C-14), 27.89 (C-15), 38.08 (C-16), 34.71 (C-17), 143.04 (C-18), 130.13 (C-19), 32.73 (C-20), 33.70 (C-21), 37.75 (C-22), 28.30 (C-23), 14.94 (C-24), 16.46 (C-25), 16.94 (C-26), 14.12 (C-27), 25.64 (C-28), 31.74 (C-29), 29.56 (C-30), 173.85 (C-1′), 37.14 (C-2′), 19.02 (C-3′), 13.90 (C-4′).

Germanicol-3-propionate (3): $C_{33}H_{54}O_2$, M^+ at *m/z* 482.4115 (calculated for $C_{33}H_{54}O_2$ 482.4124), R_f 0.67 (TLC Petroleum ether–CH_2Cl_2, 1:1), IR (KBr, cm^{-1}): 1703, ^{1}H NMR (300 MHz, $CDCl_3$, J/Hz): 0.70 (s, Me-27), 0.81 (2s, Me-23, Me-25), 0.87 (s, Me-26), 0.90, 0.91 (s, Me-29, Me-30), 0.98 (s, Me-28), 1.04 (s, Me-24), 1.11 (t, J = 7.5, Me-3′), 2.28 (q, H-2′, J = 7.5), 2.22 (d, H-13), 4.45 (m, H-3), 4.83 (s, H-19).

^{13}C NMR (300 MHz, $CDCl_3$): 38.47 (C-1), 23.60 (C-2), 80.52 (C-3), 37.70 (C-4), 55.44 (C-5), 18.02 (C-6), 34.39 (C-7), 40.62 (C-8), 51.00 (C-9), 37.02 (C-10), 21.00 (C-11), 26.07 (C-12), 38.27 (C-13), 43.20 (C-14), 27.39 (C-15), 37.58 (C-16), 34.23 (C-17), 142.56 (C-18), 129.65 (C-19), 32.24 (C-20), 33.19 (C-21), 37.23 (C-22), 27.80 (C-23), 15.97 (C-24), 16.46 (C-25), 16.65 (C-26), 14.45 (C-27), 25.13 (C-28), 31.24 (C-29), 29.06 (C-30), 174.21 (C-1′), 27.98 (C-2′), 9.24 (C-3′).

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