S. Afr. J. Chem., 2009, **62**, 163–167,
<http://journals.sabinet.co.za/sajchem/>.

A New Sterol Diglycoside from *Conyza floribunda*

Sylvia A. Opiyo[a]*, Joseph A. Ogur[a], Lawrence O.A. Manguro[a], Lutz F. Tietze[b] and Heiko Schuster[b]

[a]*Department of Chemistry, Maseno University, P. O. Box 333, Maseno, Kenya.*

[b]*Institute for Organic and Biomolecular Chemistry, Georg-August University, Tamannstrasse 2, 37007-Göttingen, Germany.*

Received 7 November 2008, revised 24 July 2009, accepted 2 September 2009.

ABSTRACT

Chemical investigation of CH_2Cl_2 and MeOH extracts of *Conyza floribunda* have led to the isolation of (24S)-ethylcholesta-5, 22E, 25-trien-3-*O*-glucosyl (1″→ 4′)-rhamnoside (1), (24S)-ethylcholesta-5,25-dien-3-*O*-β-glucoside (2), cyasterone (3), stigmasta 5, 22-dien-3-acetate (4), stigmasta 5, 22-dien-3-ol (5), spinasta 7, 22-dien-3-ol (6), 3-oxofriedooleanane (7), 3-hydroxyfriedooleanane (8), 3-acetoxyfriedooleanane (9), betullinic acid (10), quercetin 3-*O*-β-glucoside (11), quercetin (12), myricetin (13) and kaempferol (14). Their structures were determined using spectroscopic and physical methods as well as comparison with data available in the literature.

KEYWORDS

***Conyza floribunda,* asteraceae, triterpenes, sterols, flavonols.**

1. Introduction

The genus *Conyza* (Asteraceae) comprises about fifty species, which are mainly found in the tropical and subtropical regions.[1,2] In Kenya, the genus is represented by twenty-four species, distributed countrywide and one such plant is *Conyza floribunda.* The plant grows up to 3 m tall when fully mature and is common in wet regions along the road edges, gardens and in disturbed soils at altitudes of 400–2000 m above sea level.[3] It is traditionally used for a variety of pharmacological applications including treatment of smallpox, chickenpox, sore throat, ringworm and other skin related diseases, toothache and to stop bleeding from injuries.[4] Previous phytochemical studies on the plant are scarce. However, studies on other related species have led to the isolation of secondary metabolites, some of which have been reported to exhibit biological activities including anti-inflammatory,[5–7] antitumour,[8,9] anti-oxidant[10,11] and antibacterial[12] activities. In the present study, CH_2Cl_2 and MeOH extracts of *C. floribunda* were subjected to phytochemical investigation, leading to the isolation of a new sterol glycoside (**1**). Together with it were known compounds (**2–14**).[13–15] This is the first phytochemical report on *C. floribunda* and the presence of compounds **1–3** is of interest because they have never been reported from any *Conyza* species. The NMR and mass spectra of the known compounds were in complete agreement with the published values and were deduced in a completely independent way using 2D experiments including COSY (homonuclear correlation spectroscopy) and gHSQC (gradient heteronuclear single quantum correlation spectroscopy), and 1D-TOCSY experiments with selective excitation.

2. Results and Discussion

Compound **1**, a colourless amorphous powder, was shown to have a molecular formula of $C_{41}H_{66}O_{10}$ as determined by ^{13}C NMR (41 distinct peaks) and DEPT, and the ESI-MS spectrum showed quasimolecular ion peaks at *m/z* 741.7 $[M+Na]^+$ and 763.8 $[M+HCOO^-]^+$, suggesting the molar mass of the compound to be 718 g mol^{-1}. The fragment ion peaks in the EIMS (70 eV) spectrum at *m/z* 410 $[M\text{-}C_{12}H_{20}O_9]^+$, 395 $[M\text{-}C_{12}H_{20}O_9\text{-}CH_3]^+$, 392 $[M\text{-}C_{12}H_{20}O_9\text{-}H_2O]^+$, 381 $[M\text{-}C_{12}H_{20}O_9\text{-}C_2H_5]^+$, 377 $[M\text{-}C_{12}H_{20}O_9\text{-}CH_3\text{-}H_2O]^+$, 363, 314, 300 $[M\text{-}C_{12}H_{20}O_9$ part of the side chain$]^+$, 271 $[M\text{-}C_{12}H_{20}O_9\text{-}C_{10}H_{19}\text{-}2H]^+$, 255 $[M\text{-}C_{12}H_{20}O_9\text{-}C_{10}H_{19}\text{-}H_2O]^+$, 213 $[M\text{-}C_{12}H_{20}O_9\text{-}H_2O\ C_{10}H_{19}$-part of ring D$]^+$ and the peaks at *m/z* 309 $[C_{12}H_{20}O_9]^+$, 163 $[C_6H_{11}O_5]^+$, 146 $[C_6H_{10}O_4]^+$, 127, 74 and 57 obtained from the sugar moieties suggested that compound **1** may be a diglycoside with a C_{29}-sterol (*m/z* 410) aglycone moiety.[16] In fact, the fragmentation pattern observed for the aglycone pointed out that **1** is a C_{29}-sterol with three double bonds, two in the C_{10} side chain and the other in the ring skeleton.[16] This was further supported by fragment ions at *m/z* 273, 231 and 213 which are typical of a Δ^5-3β-hydroxy sterol nucleus whereas the ions centered at *m/z* 326 and 310 are diagnostic of C-22 and C-25 unsaturated side chains of sterols.[16] The formation of the latter ions is probably due to electron impact induced rearrangement of the Δ^{25} compound to its Δ^{24} isomer, followed by a McLafferty rearrangement *via* a six- or seven-membered cyclic transition state. The foregoing evidence was supported by the ^1H NMR olefinic proton signals at δ 4.88 and 4.81 ppm, typical of an exomethylene group (CH_2-26). Similarly, the presence of two olefinic protons at δ 5.21 (dd, J = 15.4, 7.7 Hz) and 5.17 ppm (dd, J = 15.4, 7.8 Hz) conferred the configuration of the double bond as E,[18] a fact further supported by the IR peak at 963 cm^{-1} for a *trans*-disubstituted double bond.[19]

The configuration at C-24 was suggested to be 24S (24β) on the basis of the ^1H NMR chemical shifts and coupling constants, which agreed well with those of (24S)-ethylcholesta-5,22,25-triene-3 β-acetate.[16] This was further supported by comparative analysis of the ^1H and ^{13}C NMR signals of compound **1** side chain chemical shifts and coupling constants with an authentic sample (22E-stigma-4,22,25-trien-3-one) having confirmed 24β configuration.[19] The good compatibility of the side chains in terms of ^1H and ^{13}C NMR chemical shifts and coupling constants for the two compounds suggested that compound **1** also has 24β-ethyl configuration, which is consistent with previous observation for $\Delta^{5,22,25}$ sterols.[20]

Acid hydrolysis of the compound afforded glucose and rhamnose as the sugar residues, identified by TLC and PC co-chromatography with authentic samples. This was further supported by ESI-MS spectrum peaks at *m/z* 579.4 $[M+Na\text{-}162]^+$

* To whom correspondence should be addressed. E-mail: sylvopiyo@yahoo.com

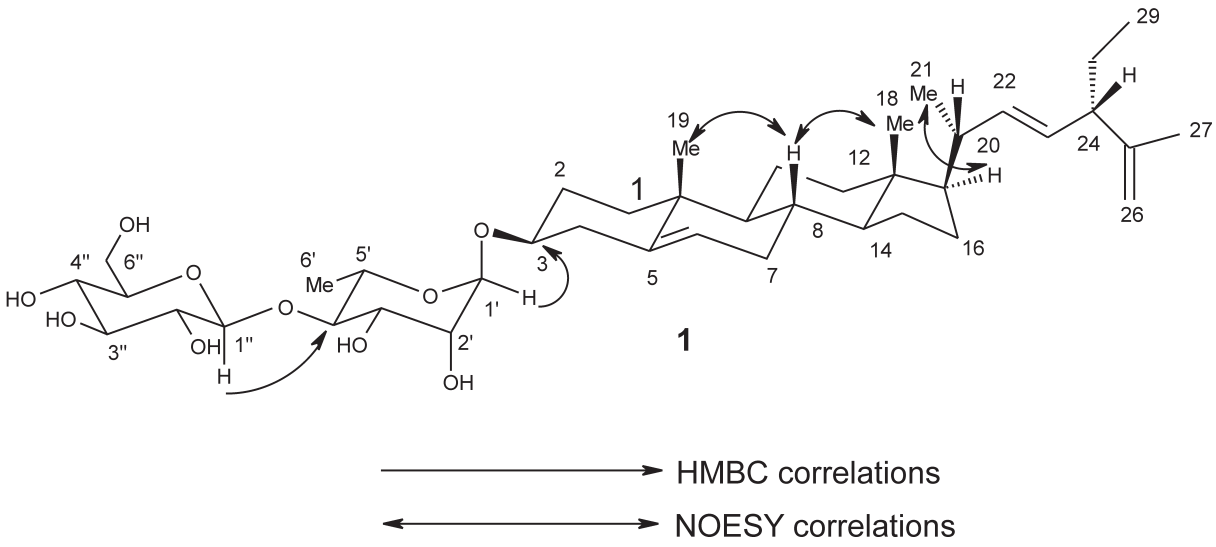


Figure 1 Key HMBC and NOESY correlations for compound **1**.

and 433.3 $[M+Na-162-146]^+$, which together with the characteristic ^{1}H NMR anomeric proton peaks at δ 4.75 (d, J = 1.2 Hz) and 4.46 ppm (d, J = 7.7 Hz) suggested that glucose was present as a terminal sugar.[21] The position of the glucosyl-rhamnosyl moiety on the aglycone was established by gHMBC experiments (Fig. 1), as was ascertained by the correlations between the peaks at δ 4.75 ppm (H-1′) and δ_c 74.6 ppm (C-3). Similarly, a cross-peak between H-1″ of glucose (δ 4.46 ppm) with δ_c 82.3 ppm (C-4′) of rhamnose indicated position C-4′ of rhamnose as the glycosidic linkage site, thus confirming a glucosyl (1″→4′)-rhamnose moiety as previously described for 3-*O*-substituted kaempferol derivatives[22] and triterpenoid saponins.[21] Therefore, on the basis of spectroscopic evidence, compound **1** was concluded to be (24S)-ethylcholesta-5,22E,25-trien-3-*O*-glucosyl (1″→ 4′)-rhamnoside.

Compound **2** showed UV absorption at λ_{max} 193.5 nm (log ε 0.197), suggesting lack of conjugation, while the IR absorption bands at ν_{max} 3406, 1644 and 1198–1023 cm^{-1} signified the presence of hydroxyl, double bond and ether linkages, respectively.

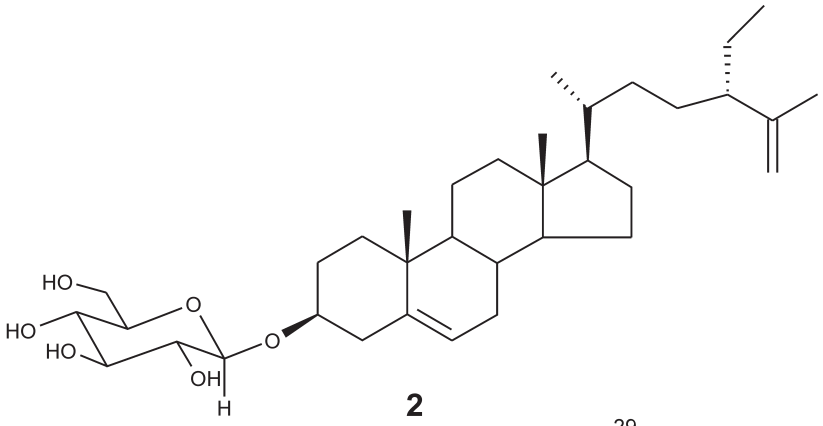
The NMR data of the aglycone of compound **2** were similar to those of clerosterol[23] except for the presence of a sugar unit, as evidenced by the doublet at δ 4.52 ppm (J = 7.5 Hz) representing the anomeric proton. Acid hydrolysis of **2** yielded glucose as the sugar residue, identified by TLC and PC co-chromatography with authentic samples and the large coupling constant of the anomeric proton indicated that the glucose unit was present in the β-configuration. This was supported by the ^{13}C NMR spectrum, which displayed a total of 35 carbons; their multiplicity assignments using DEPT established the presence of five methyls, thirteen methylenes, thirteen methines and four quaternary carbons. This, together with two quasimolecular peaks at m/z 619.4 $[M+HCOO^-]^+$ and 597.6 $[M+Na]^+$ in the ESI-MS spectrum suggested the molar mass to be 574 g mol^{-1}. Further insight into the structure of **2** was provided by close examination of both the ^{1}H NMR and the EIMS spectral data. The fragments at m/z 412 $[M\text{-glc}]^+$, 397 $[M\text{-glc-Me}]^+$, 394 $[M\text{-glc-H}_2O]^+$, 383 $[M\text{-glc-C}_2H_5]^+$, 271 $[M\text{-glc-C}_{10}H_{19}\text{-2H}]^+$ and 163 $[glc]^+$, together with ^{1}H NMR peaks at δ 5.32 (t, H-6), 4.73, 4.64 (br s × 2, H-26) and 3.46 ppm (m, H-3) indicated that the compound is $\Delta^{5,25}$-sterol glucoside,[18] with a sugar linked to the aglycone at C-3, as was ascertained by gHMBC correlation between C-1′ of glucose (δ 100.8 ppm) and H-3 (δ 3.46 ppm). On the basis of spectroscopic data as well as comparison with literature, compound **2** was concluded to be 24-ethylcholesta-5,25-diene-3-*O*-β-glucoside.

Compound **3,** a colourless amorphous powder, exhibited absorptions at λ_{max} 194.5 and 239.0 nm in the UV spectrum. Its IR ν_{max} showed the presence of an OH group (3405 cm^{-1}), a five-membered lactone ring (1741 cm^{-1}) and a conjugated carboxyl (1654 cm^{-1}).[24] The ESI-MS suggested a molar mass of 520 g mol^{-1}, corresponding to the formula $C_{29}H_{44}O_6$ due to quasimolecular ion peaks at m/z 543.2 $[M+Na]^+$ and 565.7 $[M+HCOO^-]^+$, respectively. The ^{1}H NMR spectrum of the compound afforded five methyl signals, of which three were tertiary (δ 1.18, 0.96 and 0.84 ppm) and the remaining two were secondary (δ 1.42 and 1.28 ppm, appearing as doublets). The fully decoupled ^{13}C NMR spectrum was consistent with a tetracyclic ring system of the ecdysterioid type of compound[25] and also further supported the presence of a five-membered lactone ring as evidenced by characteristic peaks at δ 81.9 ppm (C-28) and 181.7 ppm (C-26).[24] The presence of a carbon signal at δ 206.3 ppm suggested the presence of a conjugated carbonyl group at C-6,[26] a fact that was supported by the presence of olefinic carbon atoms at δ 122.3 (C-7) and 167.6 ppm (C-8); confirmed by the presence of a vinyl proton signal at δ 5.81 ppm in the ^{1}H NMR spectrum. In fact, the ^{1}H and ^{13}C NMR data closely resembled cyasterone previously isolated from *Ajuga reptans* var. Atropurpurca,[25] and supported by the carbon signals at δ 85.3, 81.9, 77.8, 75.2, 68.7 and 68.5 ppm, indicating the presence of six oxygenated carbon atoms, four of which were secondary and were correlated to protons at δ 4.20, 3.93, 3.82 and 3.52 ppm in the gHSQC spectrum of the compound. The structure of the compound was concluded by critically evaluating both the NMR (^{1}H, ^{13}C, DEPT and 2D NMR) and MS data and by comparing with the published data on cyasterone, which were found to be in complete agreement. Thus, based on spectroscopic data and comparative analysis with the literature data, compound **3** was concluded to be cyasterone.

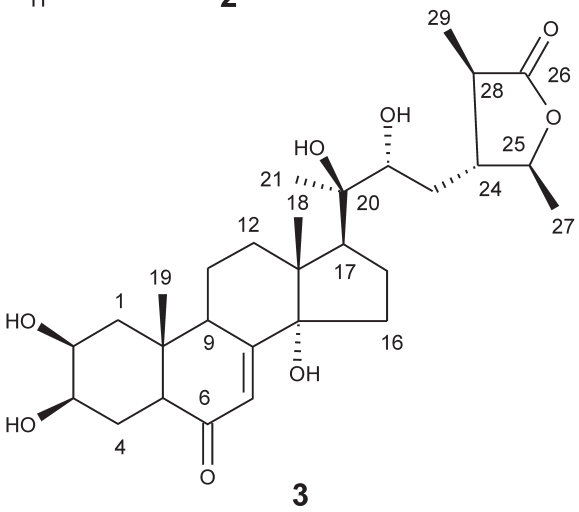
3. Experimental

3.1. General Experimental Procedure

Melting points were determined on a Gallenkamp (Loughborough, UK) melting point apparatus and are uncorrected. Distilled solvents were used for extraction and chromatographic separations. The UV spectra were run on Pye Unicam SP8-150 UV/visible spectrophotometer (Cambridge, UK) using acetonitrile. IR data were recorded on a Perkin-Elmer FTIR 600 series spectrophotometer (Waltham, MA, USA). The NMR data were



2



3

measured in $CDCl_3$ and $CDCl_3$-DMSO-d_6 on a Bruker NMR ultrashield TM (Darmstadt, Germany) operating at 500 and 125 MHz, respectively. The MS data were obtained on a Varian MAT 8200A instrument (Bremen, Germany). Preparative HPLC was performed using LC apparatus (Darmstadt, Germany) consisting of a L-6200 pump, an auto sampler spectral series AS 100, a variable wavelength 100 UV-visible detector set at 211 nm and a Hewlett-Packard integrator model HP 3396 A (Norwalk, CT, USA). A hypersil BDS C-18 5 μm (100 × 4.0 mm i.d.) column was used in the separation process and the mobile phase was acetonitrile:H_2O (35:65).

3.2. Plant Material

Authenticated *Conyza floribunda* whole plant was collected at Maseno University botanic garden in June 2005 and a voucher specimen deposited at the National Museum of Kenya (voucher deposit number: 2005/06/01/SAO/CHEMMK). The whole plant was air-dried in the open and reduced to a powder using a mill.

3.3. Extraction and Isolation of CH_2Cl_2 Extract

Powdered plant material (2 kg) was extracted with CH_2Cl_2 (3 L) by percolation for one week with occasional shaking, thereafter filtered and then concentrated *in vacuo* using a rotary evaporator to afford 80 g of a dark green semi-solid material. Approximately 75 g of the extract was dissolved in a small amount of CH_2Cl_2 and adsorbed onto silica gel for column chromatography. Fractionation of the extract using gradient of n-hexane:ethyl acetate, ethyl acetate and MeOH afforded 300 fractions (each 20 mL) whose compositions were monitored by TLC using n-hexane: EtOAc (9:1; 4:1 and 2:1) and CH_2Cl_2:MeOH (9:1 and 4:1) solvent systems respectively. Those showing similar TLC profiles were combined resulting in four pools (I–IV). Pool I (fractions 1–90, 15 g) was mainly composed of fats and waxes and was stored for GC-MS analysis. Fractions 91–170 constituted pool II (25 g), which was found to contain two major spots contaminated with impurities and was further purified using medium pressure chromatography, eluting with n-hexane:ethyl acetate (9:1 and 4:1) to give stigmasta-5,22-diene-3-acetate (**4**, 175 mg) and 3-acetoxyfriedooleanane (**9**, 95 mg). Pool III (fractions 171–250, 15 g) upon repeated fractionation using n-hexane:ethyl acetate (4:1 and 3:1) yielded spinasta-7,22-diene-3-ol (**6**, 75 mg) contaminated with **5**, 3-oxofriedooleanane (**7**, 55 mg) and 3-hydroxyfriedooleanane (**8**, 165 mg). Pool IV (11 g) gave stigmasta-5,22-dien-3-ol (**5**, 100 mg) and betulinic acid (**10**, 85 mg).

3.4. Extraction and Purification of MeOH Extract

The residue from CH_2Cl_2 extraction was further extracted with MeOH (3 L) in the cold for seven days, filtered and the solvent removed as usual using a rotary evaporator yielding a dark green material (45 g). The extract was pre-adsorbed onto silica gel and chromatographed with CH_2Cl_2:MeOH gradient to pure MeOH affording 120 fractions of 50 mL each. The compositions of the fractions were monitored by TLC using CH_2Cl_2:MeOH eluents (4:1, 3:2 and 1:1) and those that exhibited similar TLC profiles were combined to constitute three major pools (V–VII). Fractions 10–50 (Pool V, 12 g) was further purified by chromatography using CH_2Cl_2:MeOH (9:1) followed by the same solvent system in the ratio 4:1 to give kaempferol (**14**, 85 mg), cyasterone (**3**, 78 mg), quercetin (**12**, 105 mg) and 24-ethylcholesta-5,25-dien-3-*O*-β-glucoside (**2**, 93 mg). Pool VI (fractions 51–80, 15 g) was similarly isolated with CH_2Cl_2:MeOH (4:1) followed by 3:2 to afford myricetin (**13**, 55 mg) and quercetin 3-*O*-β-glucoside (**11**, 105 mg). The remaining fractions (300 mg dissolved in a minimum amount of acetonitrile) contained mainly compound

1 contaminated with impurities, which could not be obtained in pure form and was purified by preparative HPLC using acetonitrile:H_2O (35:65): mobile flow rate 10 mL min^{-1} and injecting 10 μL each time to afford 45 mg of pure compound.

(24S)-Ethylcholesta-5,22E,25-trien-3-O-glucosyl (1''→ 4')-rhamnoside (**1**)

Colourless amorphous powder from CH_2Cl_2:MeOH (9:1), m.p. > 250 °C; $[\alpha]_D^{25}$ +35 ° (MeOH, c 0.2); UV λ_{max} (CH_3CN) 201 nm; IR ν_{max} (KBr): 3550–3300 (OH), 3085 (=C-H), 2940, 2860 (C-H), 1640 (C=C), 1475, 1380, 1170, 1110, 1060, 1025, 963 (*trans*-disubstituted C=C), 810 (trisubstituted C=C), 720 cm^{-1}; ^{1}H NMR ($CDCl_3$+drop DMSO-d_6, 500 MHz): δ_H aglycone 5.34 (1H, d, *J* 5.5 Hz, H-6), 5.21 (1H, dd, *J* 15.4 and 7.7 Hz, H-22), 5.17 (1H, dd, *J* 15.4 and 7.8 Hz, H-23), 4.48 (1H, d, *J* 2.4 Hz, H-26$_a$), 4.81 (1H,d, *J* 1.4 Hz, H-26$_b$), 3.52 (1H, m, H-3), 2.52 (2H, m, CH_2-4), 2.40 (1H, m, H-12$_a$), 2.20 (1H, m, H-24), 2.18 (1H, m, H-12$_b$), 1.97 (1H, m, H-1$_b$), 1.96 (1H, m, H-2$_b$), 1.86 (1H, m, H-7$_b$), 1.66 (3H, s, CH_3-27), 1.63 (1H, m, H-8), 1.56 (1H, m, H-15$_a$), 1.52 (1H, m, H-11$_b$), 1.46 (1H, m, H-1$_a$), 1.41 (1H, m, H-2$_a$), 1.34 (1H, m, H-20), 1.32 (2H, m, CH_2-16), 1.30 (1H, m, H-11$_a$), 1.26 (1H, m, H-17), 1.25 (2H, m, CH_2-28), 1.20 (1H, m, H-15$_b$), 1.08 (1H, m, H-14), 1.07 (1H, m, H-7$_a$), 0.97 (3H, s, CH_3-19), 0.96 (1H, m, H-9), 0.85 (3H, t, CH_3-29), 0.70 ppm (3H, s, CH_3-18), rhamnose 4.75 (1H, d, *J* 1.2 Hz, H-1′), 3.64 (1H, m, H-3′), 3.38 (1H, br s, H-2′), 3.34 (1H, m, H-5′), 3.30 (1H, t, *J* 9.7 Hz, H-4′), 0.96 ppm (3H, d, *J* 6.4 Hz, CH_3-6′), glucose 4.46 (1H, d, *J* 7.7 Hz, H-1″), 3.67 (1H, dd, *J* 11.7 and 3.5 Hz, H-6$_b$″), 3.56 (1H, dd, *J* 9.6 and 2.4 Hz, H-4″), 3.46 (1H, m, H-5″), 3.41 (1H, dd, *J* 11.7 and 5.4 Hz, H-6$_a$″), 3.37 (1H, t, *J* 8.8 Hz, H-3″), 3.26 ppm (1H, t, 8.4 Hz, H-2″); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ_c aglycone 147.8 (C-25), 141.7 (C-5), 138.7 (C-22), 129.9 (C-23), 122.0 (C-6), 110.2 (C-26), 74.6 (C-3), 56.9 (C-14), 56.3 (C-17), 52.2 (C-24), 51.0 (C-9), 43.2 (C-13), 42.1 (C-4), 41.4 (C-20), 39.9 (C-12), 38.4 (C-1), 37.0 (C-10), 32.6 (C-8), 31.3 (C-7), 29.9 (C-2), 28.9 (C-16), 26.3 (C-28), 25.1 (C-15), 22.4 (C-21), 21.7 (C-11), 19.8 (C-27), 19.6 (C-19), 13.5 (C-29), 13.2 ppm (C-18), rhamnose 102.3 (C-1′), 82.3 (C-4′), 71.1 (C-5′), 70.5 (C-3′), 69.4 (C-2′), 17.8 ppm (C-6′), glucose 99.8 (C-1″), 78.1 (C-5″), 77.5 (C-3″), 74.4 (C-2″), 70.0 (C-4″), 62.2 ppm (C-6″); EIMS (injection probe, 70 eV) *m/z*: 410 [M-$C_{12}H_{20}O_9$]$^+$ (3), 395 [M-$C_{12}H_{20}O_9$-CH_3]$^+$ (11), 392 [M-$C_{12}H_{20}O_9$-H_2O]$^+$ (50), 381 [M-$C_{12}H_{20}O_9$-C_2H_5]$^+$ (5), 377 [M-$C_{12}H_{20}O_9$-CH_3-H_2O]$^+$ (7), 363 (9), 314 (3), 309 [$C_{12}H_{20}O_9$]$^+$ (3), 300 [M-$C_{12}H_{20}O_9$-part of side chain]$^+$ (6), 282 (6), 271 [M-$C_{12}H_{20}O_9$-$C_{10}H_{19}$-2H]$^+$ (14), 255 [M-$C_{12}H_{20}O_9$-$C_{10}H_{19}$-H_2O]$^+$ (45), 228 (60), 213 [M-$C_{12}H_{20}O_9$-H_2O-$C_{10}H_{19}$-part of ring D]$^+$ (100), 163 [$C_6H_{11}O_5$]$^+$ (13), 146 [$C_6H_{10}O_4$]$^+$ (3), 127, (23), 74 (51), 57 (85 %); ESI-MS *m/z*: 763.8 [M+HCOO-]$^+$ (negative ion mode); 741.7 [M+Na]$^+$ (positive ion mode), 579.4 [M+Na-162]$^+$, 433.8 [M+ Na-162-146]$^+$, HRMS: *m/z* 741.2644 ($C_{41}H_{66}O_{10}Na$, requires *m/z* 741.2637).

(24S)-Ethylcholesta-5,25-trien-3-O-β-glucoside (**2**)

Colourless amorphous powder from CH_2Cl_2:EtOAc (1:1), m.p. > 250 °C; $[\alpha]_D^{25}$ –46 ° (MeOH, c 0.5); UV λ_{max} (CH_3CN) 193.5 nm (log ε 0.197); IR ν_{max} (KBr): 3406 (OH), 3072 (=C-H), 2960, 2884 (C-H), 1644 (C=C), 1455, 1372, 1198–1023 cm^{-1} (glycosidic linkage); ^{1}H NMR ($CDCl_3$+drop DMSO-d_6, 500 MHz): δ aglycone 5.32 (1H, t, *J* 3.6 Hz, H-6), 4.73 (1H, br s, H-26$_a$), 4.64 (1H, br s, H-26$_b$), 3.46 (1H, m, H-3), 2.48 (2H, m, CH_2-4), 2.34 (1H, m, H-12$_a$), 2.12 (1H, m, H-12$_b$), 1.93 (1H, m, H-1$_b$), 1.92 (1H, m, H-2$_b$), 1.82 (1H, m, H-7$_b$), 1.80 (2H, m, H-22$_b$ and H-24), 1.61 (3H, s, CH_3-27), 1.54 (1H, m, H-15$_a$), 1.50 (1H, m, H-8), 1.46 (1H, m, H-11$_b$), 1.51 (1H, m, H-1$_a$), 1.38 (1H, m, H-20), 1.36 (1H, m, H-2$_a$), 1.28 (2H, m, CH_2-28), 1.24 (2H, m, H-16 and H-23$_a$), 1.22 (1H, m, H-15$_b$), 1.20 (1H, m, H-17), 1.02 (2H, m, H-14 and H-22$_a$), 1.00 (2H, m, H-7$_a$ and H-11$_a$), 0.96 (3H, s, CH_3-19), 0.94 (1H, m, H-9), 0.92 (1H, m, H-23$_b$), 0.88 (3H, d, *J* 6.6 Hz, CH_3-21), 0.78 (3H, t, *J* 7.2 Hz, CH_3-29), 0.65 ppm (3H, s, CH_3-18), glucose 4.52 (1H, d, *J* 7.5 Hz, H-1′), 3.65 (1H, dd, *J* 10.2 and 5.4 Hz, H-6$_b$′), 3.46 (1H, m, H-6′), 3.14 (1H, m, H-5′), 3.11 (1H, m, H-4′), 3.05 (1H, m, H-3′), 2.92 ppm (1H, m, H-2′); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ_c aglycone 146.8 (C-25), 140.4 (C-5), 121.1 (C-6), 111.4 (C-26), 71.9 (C-3), 56.1 (C-14), 55.4 (C-17), 49.6 (C-9), 48.7 (C-24), 41.8 (C-13), 40.3 (C-4), 38.7 (C-1), 38.3 (C-12), 36.1 (C-10), 34.8 (C-22), 34.7 (C-20), 33.1 (C-23), 31.4 (C-8), 31.3 (C-2), 29.3 (C-7), 28.6 (C-16), 25.9 (C-28), 24.7 (C-15), 20.5 (C-11), 18.9 (C-19), 17.6 (C-27), 12.8 (C-29), 11.6 ppm (C-18), glucose 100.8 (C-1′), 76.7 (C-5′), 76.6 (C-3′), 73.4 (C-2′), 70.1 (C-4′), 61.1 ppm (C-6′); EIMS (injection probe, 70 eV) *m/z*: 412 [M-$C_6H_{10}O_5$]$^+$ (8), 397 [M-$C_6H_{10}O_5$-CH_3]$^+$ (21), 394 [M-$C_6H_{10}O_5$-H_2O]$^+$ (25), 379 [M-$C_6H_{10}O_5$-CH_3-H_2O]$^+$ (33), 363 (9), 314 (20), 299 (7), 273 (41), 255 (66), 231 (19), 229 (45), 213 (100 %); ESI-MS m/z: 619.4 [M+HCOO$^-$]$^+$ (negative ion mode); 597.6 [M+Na]$^+$ (positive ion mode).

Cyasterone (***3***)

Colourless amorphous powder from n-hexane:EtOAc (3:2); m.p. 195–196 °C; UV λ_{max} (CH_3CN) 194.5 and 239.0 nm; IR ν_{max} (KBr): 3405 (OH), 1741 (lactone), 1654 (conjugated carbonyl), 1640, 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.81 (1H, t, *J* 2.1 Hz, H-7), 4.20 (1H, m, H-28), 3.93 (1H, d, *J* 15.0 Hz, H-3), 3.82 (1H, m, H-2), 3.52 (1H, d, *J* 20.5 Hz, H-22), 3.28 (1H, d, *J* 23.0 Hz, H-5), 3.14 (1H, m, H-9), 2.49 (1H, dq, *J* 11.4, 6.9 Hz, H-25), 2.40 (1H, t, 8.4 Hz, H-17), 2.32 (1H, m, H-24), 1.98 (2H, H-11$_a$ and H-15$_b$), 1.90 (1H, m, H-12$_b$), 1.78 (1H, m, H-1$_b$), 1.74 (2H, m, CH_2-4), 1.72 (1H, m, H-11$_b$), 1.61 (1H, m, H-15$_a$), 1.56 (1H, m, H-23$_b$), 1.45 (1H, m, H-23$_a$), 1.42 (3H, d, CH_3-29), 1.41 (1H, m, H-1$_a$), 1.29 (2H, m, CH_2-16), 1.28 (3H, d, J 6.5 Hz, CH_3-27), 1.18 (3H, s, CH_3-21), 0.96 (3H, s, CH_3-18), 0.87 (3H, s, CH_3-19), 0.84 ppm (1H, m, H-12$_a$); ^{13}C NMR ($CDCl_3$): δ 206.3 (C-6), 181.7 (C-26), 167.6 (C-8), 122.3 (C-7), 85.3 (C-14), 81.9 (C-28), 77.8 (C-20), 75.2 (C- 22), 68.7 (C-2), 68.5 (C-3), 51.8 (C-5), 50.5 (C-17), 50.0 (C-24), 48.8 (C-13), 43.5 (C-25), 39.4 (C-10), 37.5 (C-1), 35.2 (C-9), 34.9 (C-23), 32.6 (C-11), 31.9 (C-15), 30.7 (C-16), 24.5 (C-19), 21.6 (C-11), 20.6 (C-21), 19.7 (C-29), 18.0 (C-18), 15.8 ppm (C-27); ESI-MS *m/z*: 565.7 [M+HCOO$^-$]$^+$ (negative ion mode); 543.2 [M+Na]$^+$ (positive ion mode).

Acid Hydrolysis

Compounds **1** and **2**, each 15 mg in a mixture of 8 % HCl (2 mL) and MeOH were separately refluxed for 2 h. The reaction mixtures were reduced *in vacuo* to dryness, dissolved in H_2O (1 mL) and neutralized with NaOH. The neutralized products were subjected to TLC analysis (eluent EtOAc:MeOH:H_2O:HOAc, 6:2:1:1) and PC (eluent n-BuOH:HOAc:H_2O, 4:5:1 and C_6H_6:n-BuOH:H_2O:pyridine, 1:5:3:3). The chromatograms were sprayed with aniline hydrogen phthalate followed by heating. The sugars were identified after operating with authentic samples.

Acknowledgements

The authors are grateful to Maseno University Deans' Committee for partially providing funds for the research. The German Academic Exchange Service (DAAD) is sincerely thanked for the fellowship awarded to L.O.A. Manguro, which enabled most of the analyses to be done at Georg-August University, Göttingen, Germany. The National Museum of Kenya is acknowledged for identification of the plant.

References

1. A. Galal, E. Abdel-Sattar, F. S. El-Feraly, J. S. Mossa, M. R. Meselhy, S. Kodata and T. Namba, *Phytochemistry*, 1998, **48**, 159–163.

2 C. Zdero, F. Bohlman and G.M. Mungai, *Phytochemistry,* 1990, **29**, 2247–2252.

3 A.D.Q. Agnew and S. Agnew, *Kenya Upland Wild Flowers,* East African Natural History, Nairobi, Kenya, 1994, p. 169.

4 J.O. Kokwaro, *Medicinal Plants of East Africa,* East African Literature Bureau, Nairobi, Kenya, 1976, p. 62.

5 A.D. Pacciaroni, E. Mongelli, L.A. Espinar, A. Ramano, G. Ciccia and G.L. Silvia, *Planta Med.*, 2000, **66**, 720–723.

6 E.A. Asongalem, H.S. Foyet, J. Ngogang, G.N. Folefoc, T. Dimo and P. Kamtchouing, *J. Ethnopharmacol.*, 2004, **92**, 301–308.

7 Y. Su, K. Koike, T. Nikaido, J. Liu, J. Zheng and D. Guo, *J. Nat. Prod.*, 2003, **66**, 1593–1599.

8 I.M. Vilasenor and A.P. Domingo, *Teratog. Carcinog. Mutagen,* 2000, **20**, 99–105**.**

9 R. Mata, L. Aceveda, R. Bye, F. Calzada, S. Estrada, E. Linares, A. Rajas and I. Rajas, *Planta Med.*, 1997, **63**, 31–35.

10 J. Demytteenaere, S. Vervisch, J. Debenedetti, J. Coussio, D. Maes and N. De Kimpe, *Synthesis,* 2004, **11**, 1844–1848.

11 H. Masaki, T. Atsumi and H. Sakurai, *Biol. Pharm. Bull.*, 1995, **18**, 59–63.

12 A.H. Atta and S.M. Mouneir, *J. Ethnopharmacol.*, 2004, **92**, 303–309.

13 L.O.A. Manguro and L.A.D. Williams, *Planta Med.*, 1996, **62**, 178–179.

14 G. Topcu, G. Kokdil, Z. Turkmen, W. Voelter, E. Adou and D.G.I. Kingston, *Z. Naturforsch.*, 2004, **59b**, 584–588.

15 S.O. Opiyo, *Phytochemical, Anti-bacterial and Anti-fungal Studies of Conyza Floribunda,* MSc thesis, Maseno University, Maseno, Kenya, 2006.

16 T. Akihisa, W.C.M.C. Kokke, T. Tamura and T. Matsumoto, *Lipids,* 1991, **26**, 660–665.

17 T. Akihisa, W.C.M.C. Kokke, T. Tamura, T. Matsumoto, P. Ghosh and S. Thakur, *J. Chem. Soc., Perkin Trans.* **1**, 1990, 2213–2218.

18 P. Goswami, J. Kotoky, Z.N. Chen and Y. Lu, *Phytochemistry,* 1996, **41**, 279–281.

19 M.H. Chaves, N.F. Roque and M.C.J. Costa Ayres, *J. Braz. Chem. Soc.*, 2004, **15**, 600–613.

20 L.M. Bolger, H.H. Rees, E.L. Ghisalberti, L.J. Goad and T.W. Goodwin, *Biochem. J.*, 1970, **118**, 197–200.

21 H. Gao and Z. Wang, *Phytochemistry,* 2006, **67**, 2697–2705.

22 K.R. Markham, H. Geiger and H. Jaggy, *Phytochemistry,* 1992, **31**, 1009–1011.

23 G. Fontana, G Savoria, B. Rodriguez and M.C. de la Torrie, *Phytochemistry,* 1999, **50**, 283–285.

24 M. Nagakari, T. Kushiro, T. Matsumoto, N. Tanaka, K. Kakinuma and Y. Fujimoto, *Phytochemistry,* 1994, **36**, 907–910.

25 M.P. Calcagno, F. Camps, J. Coll, E. Mele and F. Sanchez-Baesa, *Tetrahedron,* 1995, **51**, 12119–12126.

26 F.M.M. Darwish and M.G. Reinecke, *Phytochemistry,* 2003, **62**, 1179–1184.