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A Diterpene, a Flavonol Glycoside, and a Phytosterol Glycoside from *Securidaca longipedunculata* and *Entada abyssinica*

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Summary. The MeOH extract of the stem bark of *Entada abyssinica* and of the leaves and stem bark of *Securidaca longipedunculata* yielded a diterpene, a flavonol glycoside, and a phytosterol glycoside. Their structures were established on basis of NMR spectroscopic analysis; the complete ^{13}C and ^{1}H assignment of the compounds was achieved by means of 2D NMR studies.

Keywords. *Securidaca longipedunculata*; *Entada abyssinica*; *Leguminosae*; *Polygalaceae*; Diterpene; Flavonol glycoside.

Introduction

Entada abyssinica Steud. Ex A. Rich (*Leguminosae* local name: Ambelt'a) is a tree about 10 m high, and *Securidaca longipedunculata* Fre (*Polygalaceae* locally known as Its'emenahe) is a flowering tree about 2–10 m high. Both are distributed in the south west lowlands of Ethiopia and used in the indigenous medical system for various ailments [1]. The recent bioassay guided investigation of the root bark of *E. abyssinica* has led to the isolation of a diterpene klovaol [2], whereas chemical studies of the bark and roots of *S. longipedunculata* have resulted in the isolation of alkaloids [3] and phenolic sucrose derivatives [4]. This paper describes the isolation, structural elucidation, and NMR spectroscopic investigation of compounds **1–3** which were isolated from the MeOH extract of the stem bark and roots of the above mentioned plants.

Results and Discussion

Solvent partition and repeated chromatographic purification over normal as well as reverse phase silica and Sephadex LH-20 of the MeOH extract of the stem bark of

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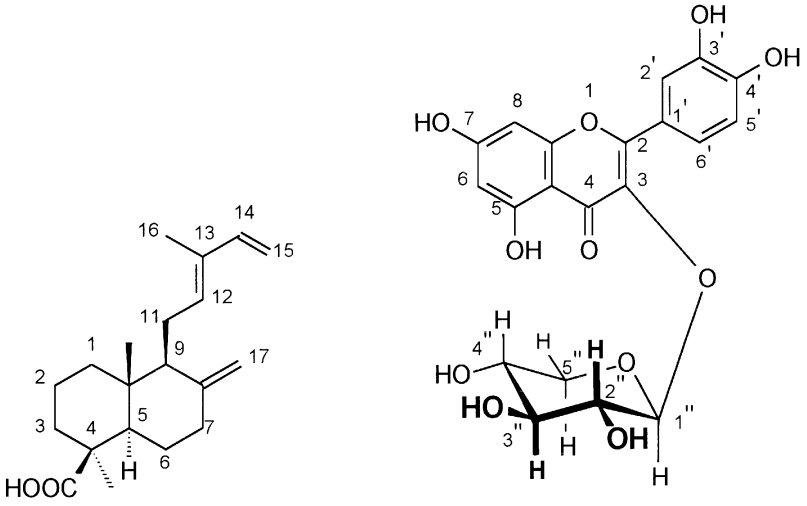
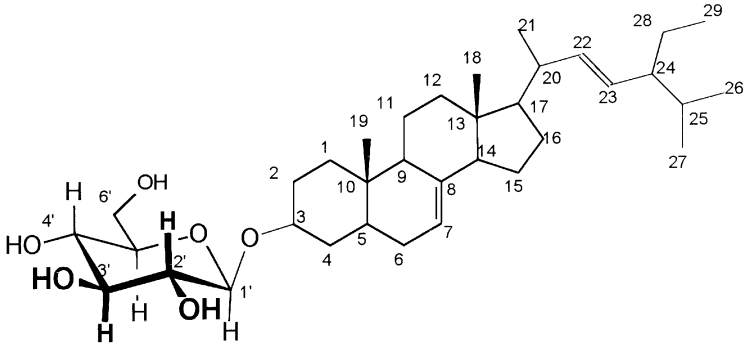
E. abyssinica and leaves and stem bark of *S. longipedunculata* afforded compounds **1**–**3**. The structure determination was mainly based on 1D (^{1}H, ^{13}C, ^{13}C-DEPT) and 2D NMR experiments (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-HMBC, ^{1}H,^{13}C-HSQC, ^{1}H, ^{1}H-ROESY). The ^{1}H and ^{13}C chemical shifts of **1** and **2** and the ^{13}C chemical shifts of **3** are given in Tables 1, 2, and 4.



1 **2**



3

Compound **1** was isolated as oil from the stem bark of *E. abyssinica*. The El-MS showed a strong ion peak at $m/z = 301$ suggesting a molecular formula of $C_{20}H_{30}O_2$. The ^{1}H NMR spectrum (Table 1) indicated the presence of two exocyclic olefinic methylene protons (4.49, 4.85 ppm) which were assigned to the protons at C-17. Signals at 4.87 and 5.05 ppm were due to the allylic protons at C-15. The ^{1}H NMR spectrum also included signals of three quaternary methyl protons (0.72, 1.23, and 1.76 ppm). Peaks at $m/z = 277$ and 153 in the EL-MS were due to the loss of methyl groups. The presence of a COOH group was revealed by IR absorptions at 1697 and 3424 cm^{-1} and a ^{13}C NMR signal at 181.3 ppm. The peak at $m/z = 183$ ($C_{11}H_{19}O_2$) originated from ring A by rupture of the C(6)-C(7)

and C(9)-C(10) bonds as also observed in compounds with related structures [5]. The peak at $m/z = 221$ was due to the loss of the side chain.

The *trans* (*E*) configuration of the $\Delta^{12,13}$ double bond in the side chain was deduced from the chemical shift of the proton at C-14 (6.31 ppm) appearing upfield as compared to the *cis* (*Z*) isomer (6.85 ppm, [6]). This conclusion was further confirmed by ROSEY correlation peaks between H-12 (5.41 ppm) and H-14 (6.31 ppm), H-14 (6.31 ppm) and one of the protons at C-15 (4.87 ppm), and H-16 (1.76 ppm) and the remaining proton at C-15 (5.05 ppm). *Trans* fusion of rings A and B and the configuration at C-4 were established by ROESY cross-peaks between the α-oriented H-5 (1.41 ppm) and H-9 (1.80 ppm), the β-oriented methyl protons at C-10 (0.72 ppm) and H-11β (2.44 ppm), H-1β (1.92 ppm) and H-11β (2.44 ppm), and the α-oriented methyl proton at C-4 (1.23 ppm and H-5 (1.41 ppm). The spectroscopic evidence presented above corresponds to *trans*-communic acid ((*E*)-Labda-8(17), 12, 14-trien-19-oic acid) that has been previously isolated from the bled resin of *Phyllocladus trichnomanoides* [7].

Compound **2** was isolated as partly solidified gum from the leaves of *S. longipedunculata*. The IR spectrum revealed an OH absorption band (3396 cm^{-1}) and a chelated carbonyl group (1654 cm^{-1}). The UV spectrum (Table 3) showed band-I and band-II absorption maxima at 358 nm and 256 nm (MeOH). A bathochromic shift with NaOAc(+15 nm), $AlCl_3$(+15 nm), and $AlCl_3$/HCl(+15 nm) shift reagents [8] indicated the presence of hydroxyl groups at positions 5 and 7 (ring A). The ^{1}H NMR spectrum (Table 2) also showed two aromatic doublets with a *meta*-coupling ($J = 2.2$ Hz) at 6.3 and 6.4 ppm for H-6 and H-8, respectively. The 3′,4′-dihydroxylation of ring B was evident from the proton resonances at 7.8 (d, $J = 2.2$ Hz, H-2′), 7.6 (d, $J = 8.6$ Hz and $J = 2.2$ Hz, H-6′), and 7.0 (d, $J = 8.6$ Hz, H-5′)ppm.

The above evidence was further supported by the UV spectrum. A bathochromic shift of the band-I absorption maximum in MeOH (358 nm) with NaOAc/H_3BO_3 as shift reagent (+19 nm) revealed *ortho*-dihydroxylation of ring B. The ^{1}H and ^{13}C NMR displayed the presence of one sugar moiety (anomeric proton and carbon: 5.2 (d, $J = 6.8$ Hz) and 105.5 ppm). The coupling constant is typical for an axial position of the anomeric proton. The point of attachment of the monosaccharide moiety was established by an HMBC experiment. A long-range correlation was observed between H-1 (5.2 ppm) of the sugar and C-3 (135.9) of the aglycone. Acid hydrolysis of **2** gave the aglycone quercetin which was identified by comparison of the ^{13}C chemical shifts reported in the literature [9]. From the aqueous hydrolysates, *D*-xylose was identified by TLC and GC-MS. The configuration was determined by capillary electrophoresis [10].

The presence of a pentose was also evident from the fragment ions appearing at $m/z = 303$ $[M\text{-}133]^+$, 133 (pentose), and 149 $[O\text{-pentose}]^+$ in the FAB^+ mass spectrum. Based on the above mentioned spectroscopic evidence, **2** was identified as quercetin-3-O-β-*D* -xyloside which has also been isolated from the leaves of *Reynoutria japonica* (*Polygonaceae*) [11].

Compound **3** was isolated from the stem bark of *S. longipedunculata*. It showed a $[M\text{-}1]^+$ peak at $m/z = 573$ in the FAB-MS, suggestive for a molecular formula of $C_{35}H_{56}O$. The ^{1}H and ^{13}C NMR spectra showed the presence of one monosaccharide, and the coupling constant indicated an axial position of the anomeric

proton (5.0 ppm, $J = 7.1$ Hz and (101.7 ppm). Acid hydrolysis gave the aglycone Δ^7-stigmasterol whose identity was confirmed by comparison with ^{13}C chemical shifts reported in the literature [12].

After neutralization and lyophilization, the aqueous hydrolysates indicated the presence of *D*-glucose (PC, GC-MS, capillary electrophoresis). The point of

Table 1. ^{1}H and ^{13}C chemical shifts (δ/ppm) of **1** in MeOH-d_4 at 30°C (*TMS* as internal standard)

Position	δ_H	δ_C
1	1.21, 1.92	40.6
2	1.55, 1.96	21.2
3	1.11, 2.18	39.3
4	–	45.1
5	1.41	57.3
6	1.92, 2.03	27.3
7	1.99, 2.43	39.6
8	–	134.6
9	1.80	57.3
10	–	41.4
11	2.20, 2.44	24.3
12	5.41	134.9
13	–	148.6
14	6.31	142.8
15	4.87, 5.05	110.1
16	1.76	12.0
17	4.49, 4.85	107.9
18	1.23	29.8
19	–	181.3
20	0.72	13.1

Table 2. ^{1}H and ^{13}C chemical shifts (δ/ppm) of **2** (CD_3OD, 40°C, *TMS* as internal standard)

Position	δ_H	δ_C	Position	δ_H	δ_C
2	–	159.0	1′	–	123.2
3	–	135.9	2′	7.8, d ($J = 2.2$ Hz)	117.8
4	–	179.2	3′	–	146.3
5	–	163.3	4′	–	150.3
6	6.3, d ($J = 2.2$ Hz)	105.1	5′	7.0, d ($J = 8.6$ Hz)	116.5
7	–	166.6	6′	7.6, dd ($J = 2.2$ and 8.6 Hz)	123.3
8	6.4, d ($J = 2.2$ Hz)	95.2			
9	–	158.7		Xylose	
10	–	106.0	1″	5.2 d ($J = 6.8$ Hz)	105.5
			2″	4.0	73.2
			3″	3.9	69.3
			4″	3.7	74.5
			5″	3.5, 3.5	67.7

attachment of the sugar was established by an HMBC correlation between H-1 (5.0 ppm) of glucose and C-3 (76.9 ppm) of the aglycone. Peaks at $m/z = 255$ and 433 in the FAB^+ mass spectrum were due to the loss of the side chain. Fragment ions at $m/z = 411$ $[M\text{-glc}]^+$, 395 $[M\text{-Oglc}]^+$, and 163 [Glc] in the FAB^+-MS also confirmed the presence of a hexose.

The position of the ring double bond is suggested to be located between C-7 and C-8 based on the observed significant difference in the ^{13}C NMR resonances of the olefinic carbons (Table 4; 117.5 (C-7) and 139.2 (C-8)ppm; Δ^5-stigmasterol: 140.7(C-5) and 121.7 (C-6)ppm) [13]. Furthermore, fragment ions at $m/z = 273$ and 306 from *retro-Diels-Alder* fragmentation of ring B also indicated that the position of the double bond was between C-7 and C-8. Based of this evidence, **3**,

Table 3. UV spectroscopic date of **2** in MeOH with and without shift reagents)

Solvent and shift reagents used	λ_{max}/nm (log ε)
CH_3OH	256 (1.462), 358 (1.052)
$AlCl_3$	271 (1.511), 443 (1.420)
$AlCl_3$/HCl	271 (1.411), 412 (0.995)
NaOAc	271 (1.343), 367 (0.900)
NaOAc/H_3BO_3	258 (1.585), 377 (1.123)
$NaOCH_3$	271 (1.609), 329 (0.604) (sh), 407 (1.314)

Table 4. ^{13}C NMR chemical shifts (δ/ppm) of **3** (pyridine-d_5, 40°C, *TMS* as internal standard)

Position	δ_C	Position	δ_C	
1	37.04	21	21.28	
2	29.71	22	138.25	
3	76.90	23	129.39	
4	34.24	24	51.10	
5	40.67	25	31.81	
6	29.71	26	20.89	
7	117.51	27	18.85	
8	139.23	28	25.30	
9	49.33	29	12.12	
10	34.45			
11	21.44			
12	39.34	Glucose	δ_H	δ_C
13	43.17	1′	5.0 ($J = 7.1$ Hz)	101.7
14	55.0	2′	4.03	75.0
15	23.0	3′	4.3	78.29
16	28.49	4′	4.24	71.56
17	55.81	5′	4.02	78.04
18	11.90	6′	4.26, 4.4	62.68
19	12.68			
20	39.92			

was identified as Δ-stigmasterol-3-O-*β*-*D*-glucoside which has been earlier isolated from *Cucurbita pepo* seeds [12].

Experimental

General

Melting points were determined in open capillaries in an electrothermal melting apparatus. El-MS were run at 70 eV and LSIMS at 5 kV neg. FAB-MS were recorded with a ZAB-E (VG analytical (micromass)); matrix: thioglycerol and ''magic bullet''. One- and two-dimensional NMR spectra were recorded as given in Ref. [14]. IR spectra were obtained with a Perkin Elmer 881 IR spectrometer. Optical rotations were measured with a Perkin Elmer 241 MC polarimeter. Gas liquid chromatography was run on a Hewlett-Packard 5890 (series 2 plus) equipped with a Hewlett Packard 5989B Mass spectrometer. UV spectra were obtained from a Shimadzu UV 160A spectrophotometer. Capillary electrophoresis was performed with a Prince Technologies device (the Emmen, Netherlands) equipped with an on-column UV detector (Bischoff, Germany). Paper partition chromatography (PPC) of sugars was conducted on Whatman No. 1 paper using a descending mode; detection was carried out with aniline/hydrogen phthalate as a spraying reagent. Column chromatography (CC) was carried out on Silicagel-60 (Merck, 230–240 μm), RP18 (Merck, 40–63 μm), and Sephadex LH-20 (Biochemica, 25–100 μm).

The homogeneity of fractions was tested on TLC (Silicagel, Merck) and (RP18, Merck). The spots were visualized by spraying with anisaldehyde-sulfuric acid reagent followed by heating at 100°C for 3 minutes. Unless otherwise noted, solvents used for TLC and CC were as follows: A: $CHCl_3$/MeOH/H_2O (20:1:0.1, 5:1:0.1), B: $CHCl_3$/MeOH/H_2O (70:30:4), C: $CHCl_3$/MeOH/H_2O (60:40:7), D:MeOH/H_2O (9:1), E: MeOH/H_2O (7:3), F: EtOAC/MeOH/H_2O/HOAc (13:3:3:4).

Plant material

The roots and leaves of *S. longipedunculata* and the stem bark of *E. abyssinica* were collected near Bedelle, a town 388 km south-west of Addis Ababa (Ethiopia) in August 1997 at an altitude of 1300 m and 1620 m, respectively. Both species were identified by Dr. *Dawit Abebe* of EHNRI. A voucher specimen representing *S. longipedunculata* (Herbarium No. 1121) and *E. abyssinica* (Herbarium No. 1120) was deposited at the Herbarium of the Department of Drug Research (EHNRI, Ethiopia) and at the Institute of Pharmaceutical Chemistry (Karl-Franzens University, Graz, Austria).

Extraction and isolation of compounds

Air dried and powdered stem bark (1 kg) of *E. abyssinica*, coarsely powdered roots (500 g), and finely powdered leaves (560 g) of *S. longipedunculata* were defatted with petroleum ether (40–60°C) in a percolator at room temperature. The solvent free powder was exhaustively extracted with methanol in a percolator to afford a gummy residue which was taken up in water and re-extracted with diethyl ether until all chlorophyll pigments were removed. The aqueous phase was then partitioned with *n*-butanol saturated with water ($3 \times 300\ cm^3$). After concentration under reduced pressure, the *n*-butanol extract yielded gummy residues of *E. abyssinca* stem bark (34 g), 25 g (leaves of *S. longipedunculata*) and 49 g (roots of *S. longipedunculata*). These were subjected to repeated CC on silicagel (solvents A, B, and C) and Sephadex LH-20 with methanol as eluting solvent followed by RP-18 silicagel with solvents D and E to afford compounds **1** (24 mg) from *E. abyssinca* and **2** (31 mg) and **3** (27 mg) from leaves and stem bark of *S. longipedunculata*.

(E)-Labda-8(17),12,14-triene-19-oic acid (trans-Communic acid) (**1**; $C_{20}H_{30}O_2$)

Oil; $[\alpha]_D^{23} = -11°$ ($c = 0.09$, MeOH); UV/Vis (MeOH); λ_{max} (log ε) = 250 (2.39) nm; IR (neat): $\nu = 3424$, 2931, 1697, 1466, 1381 cm^{-1}; El-MS (70 eV): *m/z* (rel. int) = 301 [M^-] (100), 291 (20), 277 (18), 221 (10), 183 (10), 153 (8), 97 (15), 91 (12); ^1H and ^{13}C NMR: see Table 1.

Quercetin-3-O-β-D-xyloside (**2**; $C_{20}H_{18}O_{11}$)

Partly solidified gum; $[\alpha]_D^{25} = -32.6°$ ($c = 0.031$, MeOH); UV/Vis (in MeOH and with shift reagents): see Table 3); IR (KBr): $\nu = 3396$, 2920, 1654, 1604, 1451, 1384, 1361, 1302 cm^{-1}; FAB-MS: *m/z* (rel. int.) = 434 $[M]^+$ (3), 303 [M-133]$^+$ (15), 149 [O-pentose]$^+$ (45), 133 (pentose) (25); ^1H and ^{13}C NMR: see Table 2.

Δ^7*Stigmasterol-3-O-β-D*-glucopyranoside (**3**; $C_{35}H_{58}O_6$)

White amorphous solid (MeOH); m.p.: 257.1–259.5°C (dec); $[\alpha]_D^{21} = \nu + 37.7°$ ($c = 0.13$, MeOH); UV/Vis (MeOH): λ_{max} (log ε) = 230 (0.89), 318 (SH) nm; IR (KBr): $\nu = 3390$, 2941, 2872, 1368, 1030, 802 cm^{-1}; FAB-MS: *m/z* (rel. int.) = 573 [M-1]$^+$ (30), 411 [M-glc]$^+$ (25), 395 [M-Oglc]$^+$ (90), 433 [M-side chain]$^+$ (15), 306 [RDA of ring B] (6), 273 [RDA of ring A] (6) 255 [M-glc-side chain-H2O]$^+$ (10), 163 [glc]$^+$ (10); ^{13}C NMR: see Table 4.

Acid hydrolysis

2 and **3** (10 mg each) were refluxed with 20 cm^3 7% HCl on a steam bath for 3 h. Extraction with $CHCl_3$ afforded the aglycone. The aglycone of **2** was identified as querecetin, whereas the aglycone of **3** was found to be identical with Δ^7-stigmasterol. The neutralized (Dowex basic anionic ion exchanger Cl^-) and lyophilized aqueous hydrolysates contained glucose for **3** and xylose for **2** (PPC, solvent system F, $R_f = 0.29$ (glucose), 0.36 (xylose)). GC-MS (column: 5% phenyl and 95% methyl silicone on ultra 2, 0.2×46 m, column temp.: 250°C, carrier gas: He (0.8 ml/min), sample: trimethylsilyl derivatives): t_R (min): 15.76 and 17.46 (**3**, glucose) 10.40 and 10.98 (**2**, xylose).

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