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Three New Furostanol Saponins from the Leaves of *Lycianthes synanthera* ("Chomte"), an Edible Mesoamerican Plant

ANNA LISA PICCINELLI,† JULIETA SALAZAR DE ARIZA,‡ RUBÈN VELÀSQUEZ MIRANDA,‡ SILVIA QUESADA MORA,§ RITA AQUINO,† AND LUCA RASTRELLI*,†

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte don Melillo, 84084 Fisciano (SA), Italy, Facultad de Ciencias Quìmicas y Farmacia, Universidad de San Carlos de Guatemala, Ciudad de Guatemala, and Departamento de Bioquimica, Escuela de Medicina, Universidad de Costa Rica, San Jose, Costa Rica

Three new furostanol oligoglycosides, 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-26-*O*-β-D-glucopyranosyl-22α-methoxy-25*R*-furost-5-ene-3β,17α,26-triol (**1**), 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-26-*O*-β-D-glucopyranosylfurost-5-ene-3β,17α,22α,25,26-pentol (**2**), and 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-26-*O*-β-D-glucopyranosylfurost-5-ene-3β,22α,25,26-tetrol (**3**), named lycianthosides A−C, together with known flavone glycosides were isolated from *Lycianthes synanthera* leaves, an edible plant of the Solanaceae family that grows naturally in Guatemala. The nutrient composition of the raw leaves was also evaluated.

KEYWORDS: ***Lycianthes synanthera*; Solanaceae; leaves; furostanol saponins; lycianthosides A−C, 1D and 2D NMR spectroscopy; flavone glycosides; nutrient composition**

INTRODUCTION

Lycianthes synanthera (Sendtn.) Bitter, commonly named "chomte", is an edible plant of the family Solanaceae that grows naturally in Guatemala from just above sea level to 900 m (*1*). The leaves of *L. synanthera* are used as food by people of the Q'eqchì ethnic group who live at Alta Verapaz, Guatemala. They boil and mix "chomte" with other foods, and make soups, stews, and other traditional dishes. The plant has a higher content in proteins than other vegetables (*2*).

Despite the wide use of this edible plant among the Mesoamerican native people, there are no data in the literature concerning the chemical composition and nutritional value of *L. synanthera* leaves. Because of our interest in Central American plants used as foods, in this study we investigated the phytochemical composition of a polar extract of the leaves of *L. synanthera*. The major constituents of the extract were isolated and their structures were established as flavone and steroidal glycosides. In addition, the nutrient composition of raw leaves has been evaluated to improve the understanding of its nutritional value.

Saponins play important roles in food and animal feedstuffs because of their biological properties (*3*−*5*). Recently, the hypocholesterolemic (*6*−*10*) and anticarcinogenic (*11*−*16*) activities of saponins, including those present in the diet, were reported, as well as the beneficial effects that some of them have on the efficiency of feed utilization and growth in ruminants.

Nevertheless, saponins possess membranolytic activity; they can damage intestinal mucosal cells by altering cell membrane permeability and interfering with active transport, an effect that is dependent upon the structure of the individual saponin molecule (*16*) and that might increase the uptake of antigens by the small intestine. It also has been found that some saponins impair the digestion of protein (*8*) and the uptake of vitamins and minerals (*6*) in the gut. The nutritional importance of flavonoids for the human diet is generally accepted (*5*); they are known to be effective free radical scavengers and their occurrence in food plants is desirable.

MATERIALS AND METHODS

General Experimental Procedures. Optical rotations were determined on a model 192 polarimeter (Perkin-Elmer, Norwalk, CT) equipped with a sodium lamp (589 nm) and a 10 cm microcell. UV spectra were recorded on a UV-2101PC UV/vis scanning spectrophotometer (Shimadzu Italia srl, Milan, Italy). A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for ^{1}H and at 150.86 MHz for ^{13}C, using the UXNMR software package was used for NMR experiments; chemical shifts are expressed in δ (parts per million) referring to the solvent peaks δ_H 3.34 and δ_C 49.0 for CD_3OD; coupling constants, *J*, are in hertz. Distortionless enhancement by polarization transfer (DEPT) ^{13}C, ^{1}H−^{1}H double quantum filtered corrrelation spectroscopy (DQF-COSY), ^{1}H−^{13}C heteronuclear single quantum coherence (HSQC),

* To whom correspondence should be addressed: phone 0039 89 964356; fax 0039 89 964356; e-mail rastrelli@unisa.it.
† Università di Salerno.
‡ Universidad de San Carlos de Guatemala.
§ Universidad de Costa Rica.

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and heteronuclear multiple bond coherence (HMBC) NMR experiments were carried out with the conventional pulse sequences as described in the literature.

A Napco 430 oven at 105 °C was used to quantify moisture, a Tecator 2020 digestor and Kjeltec 1030 autoanalyzer to determine crude protein; a Foss-Tecator 1010 fiber digestor to determine crude fiber; a Labconco 2020 goldfish apparatus for fat; and a Lindberg 51442 muffle furnace for total ash.

Electrospray-Ionization Mass Spectrometry. Electrospray-ionization mass spectrometry (ESIMS) was performed on a Finnigan LC-Q Deca instrument (Thermoquest, San Jose, CA) equipped with Xcalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump; the flow rate was 3 μL/min. The capillary voltage was set at 5 V, the spray voltage at 5 kV, and the tube lens offset at 35 V. The capillary temperature was 220 °C. Data were acquired in the MS1 scanning mode (*m*/*z* 150−1500). Exact masses were measured by a Q-Star Pulsar (Applied Biosystems) triple-quadrupole orthogonal time-of-flight (TOF) instrument. Electrospray ionization was used in TOF mode at 8.500 resolving power. Samples were dissolved in MeOH, mixed with the internal calibrant, and introduced directly into the ion source by direct infusion. Calibration was performed on the peaks of cesium iodide and synthetic peptide (TOF positive ion calibration solution, Bachem) at *m*/*z* 132.9054 and 829.5398, respectively.

High-Performance Liquid Chromatography. HPLC separations were performed on a Waters 590 series pumping system equipped with a W R401 refractive index detector and with a 300 × 7.8 mm i.d., 10 μm μ-Bondapak C_{18} column and a U6K injector.

Plant Material. Leaves of *Lycianthes synanthera* (Sendtn.) Bitter ("chomte") were collected near Cobàn, Alta Verapaz (200 km from Guatemala City) in May−July 2000 and identified by J. Castillo. A voucher sample (LS1, 2000) is deposited at the Herbario of the Facultad de Agronomia, Universidad de San Carlos de Guatemala.

Extraction and Isolation Procedure for Compounds 1−6. The dried and powdered leaves (700 g) were defatted at room temperature with petroleum ether and $CHCl_3$ and then extracted by maceration with MeOH to give 15 g of residue. This was partitioned between *n*-BuOH and H_2O to afford a *n*-BuOH-soluble portion (7 g). An aliquot (3 g) of the *n*-BuOH extract was chromatographed over a 1 m × 3 cm i.d Sephadex LH-20 column (Pharmacia, Uppsala, Sweden) with MeOH as eluent at a flow rate of 0.5 mL/min. Fractions (8 mL each) were collected and checked by TLC [Si-gel, *n*-BuOH−AcOH−H_2O (60:15:25), $CHCl_3$−MeOH−H_2O (7:3:0.3)] Fractions with similar R_f values were combined, giving six major fractions (I−VI) that were further purified by RP-HPLC on a 30 cm × 7.8 mm i.d. C_{18} μ-Bondapak column (flow rate of 2.0 mL/min). Fraction II (500 mg) was purified, with MeOH−H_2O (6:4) as the eluent, to yield pure saponins **1** (5.2 mg, t_R = 8.6 min), **2** (6.0 mg, t_R = 12.4 min), and **3** (5.7 mg, t_R = 30 min). Fraction IV (105 mg) was purified, with MeOH−H_2O (1:1), to yield flavone glycosides **4** (13.5 mg, t_R = 6.6 min) and **5** (6.3 mg, t_R = 8.0 min). Fractions V (84 mg) and VI (70 mg) afforded compound **6** (15.7 mg, t_R = 11 min) with MeOH−H_2O (1:1) as eluent.

To establish whether compound **1** was an artifact of MeOH extraction, part of the dried plant material (100 g) was extracted by maceration with EtOH. Compound **1** (1.4 mg) was isolated from the EtOH extract (1.2 g) following the same procedure described for the MeOH extract.

Lycianthoside A (1): white amorphous powder. $[\alpha]_D^{25} = -65.1°$ (c = 0.02, MeOH). HREIMS *m*/*z* 1078.5560, calcd for $C_{52}H_{86}O_{23}$ 1079.2254; ESIMS *m*/*z* 1079 $[M + H]^+$, *m*/*z* 1077 $[M - H]^-$, 931 $[(M - H) - 146]^-$, 785 $[(M - H) - (2 \times 146)]^-$, 623 $[(M - H) - 454]^-$. ^{1}H and ^{13}C NMR data, see **Tables 1** and **2**.

Lycianthoside B (2): white amorphous powder. $[\alpha]_D^{25} = -69.1°$ (c = 0.05, MeOH). HREIMS *m*/*z* 1080.5353, calcd for $C_{51}H_{84}O_{24}$ 1081.1983; ESIMS *m*/*z* 1081 $[M + H]^+$, *m*/*z* 1079 $[M - H]^-$, 933 $[(M - H) - 146]^-$, 787 $[(M - H) - (2 \times 146)]^-$, 625 $[(M - H) - 454]^-$. ^{1}H and ^{13}C NMR data, see **Tables 1** and **2**.

Lycianthoside C (3): white amorphous powder. $[\alpha]_D^{25} = -70.3°$ (c = 0.05, MeOH). HREIMS *m*/*z* 1064.5703, calcd for $C_{51}H_{84}O_{23}$ 1065.1989; ESIMS *m*/*z* 1065 $[M + H]^+$, *m*/*z* 1063 $[M - H]^-$, 917 $[(M - H) - 146]^-$, 771 $[(M - H) - (2 \times 146)]^-$, 609 $[(M - H) - 454]^-$. ^{1}H and ^{13}C NMR data, see **Tables 1** and **2**.

Apigenin-7-*O*-β-D-glucopyranoside (4): ^{1}H and ^{13}C NMR data were consistent with those previously reported (*17*). ESIMS *m*/*z* 433 $[M + H]^+$, *m*/*z* 431 $[M - H]^-$.

Apigenin-7-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside (5): ^{1}H and ^{13}C NMR data were consistent with those previously reported (*17*). ESIMS *m*/*z* 579 $[M + H]^+$, *m*/*z* 577 $[M - H]^-$.

Luteolin-7-*O*-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside (6): ^{1}H and ^{13}C NMR data were consistent with those previously reported (*18*). ESIMS *m*/*z* 595 $[M + H]^+$, *m*/*z* 593 $[M - H]^-$.

Chemical Analysis. The nutritional composition was determined according to procedures of AOAC (*18*). Crude protein content was calculated by use of the 6.25 conversion factor for Kjedahl N. Carbohydrates was calculated as nitrogen-free extract according to the formula carbohydrates = 100 − (% moisture + % protein + % crude fiber + % fat + % ash). Energy (kilocalories) was calculated according to the formula energy = 4(grams of protein) + 4(grams of carbohydrates) + 9(grams of fat) (*19*).

RESULTS AND DISCUSSION

The dried leaves of *L. synanthera* were defatted with petroleum ether and chloroform and then extracted with MeOH. Its *n*-BuOH-soluble fraction was subject to a purification by gel filtration on a Sephadex LH-20 column and RP-HPLC, giving compounds **1**−**6**.

The ESIMS in negative mode of compound **1** exhibited a quasi-molecular ion peak at *m*/*z* 1077 $[M - H]^-$ and a high-resolution measurement indicated the molecular formula, $C_{52}H_{86}O_{23}$, in accordance with ^{13}C NMR data. Major fragments at *m*/*z* 931, 785, and 623 were assigned to the loss of a deoxyhexose unit (146 amu), two deoxyhexose units (2 × 146), and the successive loss of an hexose unit (162 amu).

A bisdesmosidic furostanol saponin structure was indicated by ^{1}H and ^{13}C NMR analysis (*20*, *21*). ^{1}H NMR spectrum of **1** (**Tables 1** and **2**) exhibited the signals of six methyl groups, two singlets at δ 0.89 and 1.09, two doublets at δ 0.96 and 0.99 for the aglycon, and two doublets δ 1.27 and 1.29 for the sugar moiety; some overlapping signals from δ 1.10 to 2.48; and a number of signals between δ 3.21 and 5.24, attributable to protons on oxygen-bearing carbon. A further signal at δ 5.42 was ascribable to a proton on a sp^2 carbon. This pattern of protons and the ^{13}C NMR resonances of an -OMe (δ_H 3.21, δ_C 47.5), and 26 carbons, and a semiketal carbon signal at δ 114.7 (C-22) suggested the 22α-methoxyfurost-5-en-3β,26-diol nature of the aglycon (*22*). All the above proton resonances of **1** were associated unambiguously with the relevant carbon atom by use of the HSQC spectrum.

Inspection of the homonuclear Hartmann−Hahn (HOHAHA) spectrum of **1** allowed the detection of eight distinct spin systems, four belonging to the aglycon moiety and four to the saccharide units. The proton sequence within each spin system was elucidated by analysis of cross-peaks in the COSY spectrum, while data arising from HMBC spectra were used to interconnect the partial structures.

Concerning the aglycon moiety, the first spin system connecting C-1 to C-4 started from the oxygen-bearing carbon C-3 (δ_H 3.63, δ_C 79.3); the second one, starting from the sp^2 C-6 (δ_H 5.42, δ_C 122.6) connected all the protonated carbons from C-6 to C-12; the third connected C-16 (δ_H 3.99, δ_C 90.8), C-15, and C-14. The last spin system of the aglycon was constituted by protons of the side chain, from C-23 to the oxygen-bearing C-26 (δ_H 3.77 and δ_C 76.0).

Comparison of the resonances of **1** with those of methylprotodioscin (*20*) and other 3β-hydroxy-22α-methoxyfurost-5-ene 26-glucopyranoside models (*22*) indicated that **1** contains one

Table 1. ^{13}C NMR and ^{1}H NMR Spectroscopic Data[a] of the Aglycons of Compounds **1**–**3** in CD_3OD

	1		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.6	1.92 m	38.6	1.92 m	38.6	1.90 m
		1.11 m		1.10 m		1.09 m
2	30.8	1.95 m	30.8	1.96 m	30.8	1.95 m
		1.63[b]		1.64 m		1.64 m
3	79.3	3.63 br m	79.3	3.63 br m	79.3	3.62 br m
4	39.5	2.48 dd (12.0, 3.5)	39.5	2.48 dd (12.0, 3.5)	39.5	2.46 dd (12.0, 3.5)
		2.33 dd (12.0, 8.0)		2.33 dd (12.0, 8.0)		2.30 dd (12.0, 8.0)
5	141.9		142.0		141.8	
6	122.6	5.42 br d (3.8)	122.4	5.41 br d (3.8)	122.6	5.44 br d (3.8)
7	33.2	2.05 m	33.2	2.07 m	33.2	2.04 m
		1.60[b]		1.62[b]		1.60[b]
8	32.8	1.60[b]	32.8	1.61[b]	32.8	1.58[b]
9	51.5	1.05 m	51.5	1.00 m	51.7	1.02 m
10	38.0		38.0		38.0	
11	21.7	1.62[b]	21.7	1.62[b]	22.0	1.59[b]
		1.55 m		1.55 m		1.54 m
12	33.2	1.60[b]	33.2	1.60 m	41.0	1.83 m
		1.35 m		1.35 m		1.25 m
13	45.8		46.0		41.5	
14	53.9	1.74 m	53.8	1.74 m	57.7	1.18 m
15	32.1	2.02 m	32.1	2.02 m	33.6	1.98 m
		1.24 m		1.24 m		1.20 m
16	90.8	3.99 dd (6.5, 5.5)	90.5	4.06 dd (6.5, 5.5)	82.1	4.50 q (5.5)
17	91.6		90.7		63.2	1.80 dd (7.5, 6.5)
18	17.6	0.89 s	17.4	0.86 s	16.6	0.85 (s)
19	19.9	1.09 s	19.8	1.09 s	19.8	1.09 (s)
20	43.5	2.39 q (7.2)	42.1	2.39 q (7.2)	39.4	2.20 m
21	9.8	0.96 d (7.0)	9.4	0.94 d (7.0)	15.0	1.03 d (7.0)
22	114.7		111.8		111.0	
23	31.23	1.72 m	30.7	1.39 m	30.7	1.40 m
		1.63[b]		1.33 m		1.33 m
24	28.9	1.70 m	33.7	2.09 m	33.7	2.09 m
		1.26 m		1.71 m		1.69 m
25	35.0	1.80 m	85.6		85.2	
26	76.0	3.77 t (10.5)	77.8	3.89 d (11.0)	77.6	3.89 br d (11.0)
		3.45 dd (10.5, 3.9)		3.53 d (11.0)		3.51 d (11.0)
27	17.2	0.99 d (6.5)	24.2	1.28 s	24.2	1.29 s
OMe	47.5	3.21 s				

[a] *J* values in hertz are given in parentheses; assignments were from 2D COSY, HSQC, and HMBC experiments. [b] Overlapped signals.

more oxygenated carbon at C-17 (quaternary carbon, δ_C 91.6). The presence of an -OH substituent at C-17 was also indicated by the resonances and multiplicity of the atoms located near C-17 such as C-22 (δ_C 114.7 versus 114.1 in methylprotodioscin), C-20 (δ_C 43.5 instead of 41.2), Me-21 (δ_C 9.8 instead of 16.0), and C-16 (δ_C 90.8 instead of 80.6 in methylprotodioscin).

The total of this evidence allowed us to identify the aglycon moiety of **1** as the new compound 22α-methoxy-Δ-5-furostan-3*β*,17α,26-triol. The 25*R* stereochemistry was inferred from the assumption that the aglycon moiety possesses the same absolute configuration as found in all the furostane derivatives isolated to date (*20*, *21*, *24*).

The 600 MHz ^{1}H NMR spectrum (**Table 2**) of **1** showed signals for four anomeric hydrogens (δ 4.27, 4.54, 4.87, and 5.24, correlating to carbons at δ 104.6, 100.4, 103.0, and 102.3, respectively, in the HSQC spectrum) and two methyl doublet signals (δ 1.29 and 1.27, d, J = 6.5 Hz,) suggesting the occurrence of four sugar residues, among them two deoxyhexoses. The other proton sugar signals were overlapped in the region δ 3.21−3.95. Analysis of homonuclear 2D COSY and HOHAHA and 1D total correlation (TOCSY) spectra allowed the assignments of all the proton resonances, whereas evaluation of the coupling constants was used to elucidate the monosaccharide relative stereochemistry. The chemical shift of the anomeric proton signal at δ_H 4.27 (H-1‴, d, J = 7.7 Hz) correlated to the signal at δ_C 104.6 and suggested that this sugar moiety was attached to C-26 of the aglycon (*23*). The HMBC cross-peak of H_2-26 with the anomeric carbon at δ_C 104.6 allowed C-26 to be confirmed as one of the glycosidic linkage sites. Starting from H-1‴, a sequence of four oxymethines and a methylene was identified with large coupling constants showing axial−axial relationships. This information together with that obtained from the HSQC and HMBC spectra (**Table 2**) led to the identification of a terminal *β*-D-glucopyranosyl unit. The other sugars were determined to be a trisaccharide chain formed by a glucopyranosyl and two rhamnopyranosyls linked to C-3 of the aglycon by NMR experiments (Table 2). When the anomeric proton at δ 4.54 (H-1′) was used as starting point in the TOCSY and HOHAHA experiments, a sequence of four oxymethines and a methylene was identified. The COSY spectrum established the proton sequence within this monosaccharide as H-1′ to H_2-6′. The multiplicity and large coupling constants observed, in agreement with axial−axial relationships (**Table 2**) as well as ^{13}C NMR data, indicated the *β*-glucopyranosyl nature of this sugar. Similar observations on the second and third sugar residues, obtained by use, as starting points, of the TOCSY experiments methyl doublet signals at δ 1.27 and 1.29, allowed the identification of the H_3-6″/H-1″ and H_3-6‴/H-1‴ sequences. The axial−axial couplings H-3″/H-4″ and H-4″/H-5″, and H-3‴/H-4‴ and H-4‴/H-5‴, and the axial−equatorial relationship between H-2″/H-3″ and H-2‴/H-3‴ as well as the resonances of C-3 and C-5 led to the identification

Table 2. ^{13}C NMR and ^{1}H NMR Spectroscopic Data of Sugars for Compounds **1**–**3** in CD_3OD[a]

	1		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
glucose at C-3						
1′	100.4	4.54 (d, 7.7)	100.4	4.54 (d, 7.7)	100.4	4.56 (d, 7.7)
2′	80.1	3.55 (dd, 7.7, 9.0)	80.1	3.53 (dd, 7.7, 9.0)	80.0	3.52 (dd, 7.7, 9.0)
3′	78.1	3.60 (dd, 9.0, 9.0)	78.1	3.58 (dd, 9.0, 9.0)	78.2	3.59 (dd, 9.0, 9.0)
4′	79.3	3.40 (dd, 9.0, 9.0)	79.3	3.40 (dd, 9.0, 9.0)	79.2	3.40 (dd, 9.0, 9.0)
5′	76.6	3.35 (ddd, 2.5, 5.0, 9.0)	76.6	3.33 (ddd, 2.5, 5.0, 9.0)	76.6	3.34 (ddd, 2.5, 5.0, 9.0)
6′	62.0	3.68 (dd, 2.5, 12.0)	62.0	3.68 (dd, 2.5, 12.0)	62.1	3.67 (dd, 2.5, 12.0)
		3.80 (dd, 5.0, 12.0)		3.80 (dd, 5.0, 12.0)		3.79 (dd, 5.0, 12.0)
rhamnose at C-2						
1″	103.0	4.87 (d, 1.5)	103.0	4.87 (d, 1.5)	103.0	4.88 (d, 1.5)
2″	72.2	3.87 (dd, 1.5, 2.5)	72.2	3.87 (dd, 1.5, 2.5)	72.3	3.87 (dd, 1.5, 2.5)
3″	72.4	3.65 (dd, 2.5, 9.0)	72.4	3.65 (dd, 2.5, 9.0)	72.4	3.64 (dd, 2.5, 9.0)
4″	73.7	3.41 (t, 9.0)	73.7	3.41 (t, 9.0)	73.3	3.43 (t, 9.0)
5″	70.7	3.96 (m)	70.7	3.96 (m)	70.7	3.98 (m)
6″	17.9	1.29 (d, 6.5)	17.9	1.29 (d, 6.5)	17.8	1.28 (d, 6.5)
rhamnose at C-4						
1‴	102.3	5.24 (d, 1.5)	102.3	5.24 (d, 1.5)	102.2	5.25 (d, 1.5)
2‴	72.2	3.95 (dd, 1.5, 2.5)	72.2	3.95 (dd, 1.5, 2.5)	72.2	3.96 (dd, 1.5, 2.5)
3‴	72.5	3.69 (dd, 2.5, 9.0)	72.5	3.69 (dd, 2.5, 9.0)	72.5	3.70 (dd, 2.5, 9.0)
4‴	73.9	3.40 (t, 9.0)	73.9	3.40 (t, 9.0)	73.9	3.40 (t, 9.0)
5‴	69.8	4.16 (m)	69.8	4.16 (m)	69.9	4.14 (m)
6‴	18.0	1.27 (d, 6.5)	18.0	1.27 (d, 6.5)	17.9	1.26 (d, 6.5)
glucose at C-26						
1⁗	104.6	4.27 (d, 7.7)	105.0	4.34 (d, 7.7)	105.1	4.36 (d, 7.7)
2⁗	75.2	3.21 (dd, 7.7, 9.0)	75.3	3.25 (dd, 7.7, 9.0)	75.3	3.26 (dd, 7.7, 9.0)
3⁗	78.2	3.38 (dd, 9.0, 9.0)	77.7	3.39 (dd, 9.0, 9.0)	77.8	3.38 (dd, 9.0, 9.0)
4⁗	71.7	3.31 (dd, 9.0, 9.0)	71.7	3.32 (dd, 9.0, 9.0)	71.7	3.32 (dd, 9.0, 9.0)
5⁗	77.9	3.29 (ddd, 2.5, 5.0, 9.0)	78.0	3.29 (ddd, 2.5, 5.0, 9.0)	78.1	3.30 (ddd, 2.5, 5.0, 9.0)
6⁗	62.8	3.68 (dd, 2.5, 12.0)	62.8	3.68 (dd, 2.5, 12.0)	62.9	3.68 (dd, 2.5, 12.0)
		3.85 (dd, 5.0, 12.0)		3.85 (dd, 5.0, 12.0)		3.83 (dd, 5.0, 12.0)

[a] *J* values are given in hertz in parentheses; assignments were from 2D COSY, HSQC, and HMBC experiments.

of two L-rhamnopyranosyl units with α-configurations (*24*). The absence of any ^{13}C NMR glycosidation shift for the α-L-rhamnopyranosyl moieties suggested that these sugars were terminal units. Glycosidation shifts were observed for C-2′ (δ 80.1, +5 ppm by β-effect) and C-4′ (δ 79.3, + 7.5, β-effect) of the β-glucopyranosyl unit, demonstrating the (1→2) and (1→4) linkages between the rhamnosyl and glucosyl units.

The trisaccharide chain should be linked to C-3 of the aglycon as indicated by the HMBC correlations due to long-range couplings observed between the anomeric signal (H-1′, δ 4.54) of the glucosyl unit and C-3 (δ 79.3) of the aglycon. All the connectivity information inferred by the HMBC spectrum was compatible only with structure **1**. Accordingly, compound **1** (named lycianthoside A) was determined to be 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl}-26-*O*-β-D-glucopyranosyl-22α-methoxy-25*R*-furost-5-ene-3β,17α,26-triol. Because compound **1** was also isolated from an EtOH extract of *L. synanthera* leaves, it seems to be a native product and not an artifact formed by MeOH extraction.

The structure of compound **2** and **3** were elucidated on the basis of their similarity with **1** (**Figure 1**). The HREIMS of **2** and **3** indicated their molecular formulas were $C_{51}H_{84}O_{24}$ and $C_{51}H_{84}O_{23}$, respectively. The ^{1}H and ^{13}C NMR profiles showed close resemblances with corresponding spectra of **1** (**Tables 1** and **2**). In particular, the NMR spectra of **2** differed from those of **1** by the following: (i) lack of the signal at δ_H 3.21 and δ_C 47.5 (-OMe), and high-field shifts of C-22 (δ_C 111.8 in **2** vs δ_C 114.7 in **1**), C-20 (δ_C 42.1 in **2** vs δ_C 43.5 in **1**), and C-21 (δ_C 9.4 in **2** vs δ_C 9.8 in **1**), suggesting the loss of the -Me group at C-22; (ii) the presence of an additional quaternary oxygen-bearing carbon signal at δ_C 85.6, indicating an additional -OH substituent at C-25 that causes low-field shifts of C-27 (δ_C 24.2 in **2** vs 17.2 in **1**), C-26 (δ_C 77.8 in **2** vs 76.0 in **1**), and C-24 (δ_C 33.7 in **2** vs 28.9 in **1**) and significant shifts of resonances and multiplicity of key protons Me-27, H_2-24, and H_2-26 (**Table 1**).

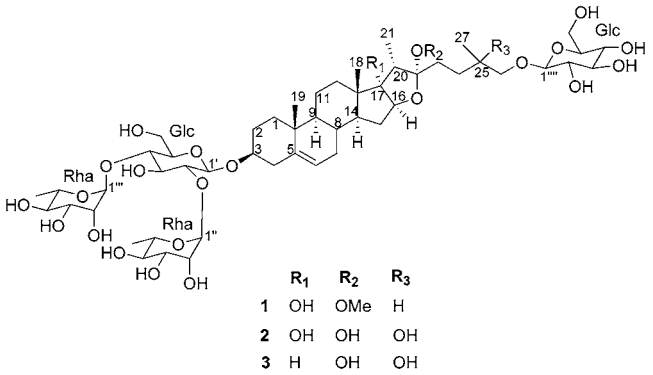
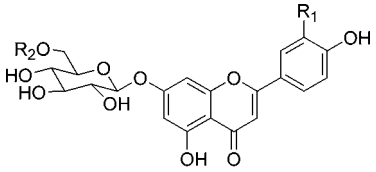


Figure 1. Lycianthosides A–C (**1**–**3**), furostanol oligoglycosides isolated from *Lycianthes synanthera* leaves.

Compound **3** differed from **2** in the absence of a -OH group at C-17 (δ_C 63.2 in **3** vs δ_C 90.7 in **2**), which causes high-field shift of C-16 (δ_C 82.1 in **3** vs 90.5 in **2**) and C-20 (δ_C 39.4 in **3** vs δ_C 42.1 in **2**), a low-field shift of C-21 (δ_C 15.0 in **3** vs δ_C 9.4 in **2**), and changes in the chemical shift and multiplicity of H-16 (δ_H 4.50, m in **3** vs δ_H 4.06, dd, J = 5.5 Hz in **2**), H-20 (δ_H 2.20, m in **3** vs δ_H 2.39, q, J = 7.2 Hz in **2**), and Me-21 (δ_H 1.03 in **3** vs δ_H 0.94 in **2**) whereas the resonances of protons and carbons of the side chain were superimposable on those of **2**.

The midfield region of the ^{1}H NMR spectra of **2** and **3** appeared almost the same as that of compound **1**, suggesting that these saponins must possess the same sugar units. Accord-



	R_1	R_2
4	H	H
5	H	Rha
6	OH	Rha

Figure 2. Flavone glycosides (**4**–**6**) isolated from *Lycianthes synanthera* leaves.

Table 3. Nutrient Composition of Raw Leaves of *Lycianthes synanthera*[a]

component	%
moisture	82.5 ± 2.4
protein	6.3 ± 0.7
fat	0.4 ± 0.1
carbohydrates	6.4 ± 1.2
crude fiber	2.7 ± 0.6
ash	1.7 ± 0.5
energy (kcal)	54 ± 2

[a] Data are expressed per 100 g of leaves and are means of four determinations ± SD.

ingly, the ^{13}C NMR resonances (**Table 2**) and results from 1D and 2D NMR spectra were closely comparable to those of **1** for the sugar moiety. All of these data led us to propose the structure 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranosyl}-26-*O*-*β*-D-glucopyranosyl-furost-5-ene 3*β*,17α,22α,25,26-pentol for **2** (lycianthoside B), and 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranosyl}-26-*O*-*β*-D-glucopyranosylfurost-5-ene-3*β*,22α,25,26-tetrol for **3** (lycianthoside C).

A steroidal sapogenin, neogitogenin, was isolated from *Lycianthes biflora* (*25*), whereas this is the first report on saponins from the genus *Lycianthes*. Bisdesmosidic furostanols are widely distributed in plants of Dioscoreaceae (*23*), Alliaceae (*24*), Solanaceae (*22*), Agavaceae (*26*), Taccaceae (*27*), and Liliaceae (*28*) families, but aglycons with oxygenated substituents at C-17 (like **1**), at C-17 and C-25 (like **2**), and at C-25 (like **3**) are reported here for the first time. Isolation of lycianthosides A−C represents the first finding of furostane saponins from a plant in the genus *Lycianthes*.

Generally leaves and vegetables are characterized by higher concentrations of flavonoids. Therefore, we have also accurately analyzed the flavonoid fraction of "chomte" and found that it is composed of high amounts of the flavones apigenin-7-*O*-*β*-glucopyranoside (**4**), apigenin-7-*O*-[α-L-rhamnopyranosyl(1→6)]-*β*-D-glucopyranoside (**5**) and luteolin-7-*O*-[α-L-rhamnopyranosyl-(1→6)]-*β*-D-glucopyranoside (**6**) (**Figure 2**). These compounds have been identified by ESIMS and NMR spectroscopic methods and by comparison with their spectroscopic data reported in the literature (*17*, *18*). Flavonoids are a class of natural plant products and common component in the human diet. Flavonoids demonstrate a wide range of biochemical and pharmacological effects including anti-oxidation, anti-inflammation, anti-platelet, anti-thrombotic action, and anti-allergic effects (*29*−*33*). They can inhibit enzymes such as prostaglandin synthase, lipoxygenase, and cyclooxygenase, closely related to inflammation and tumorigenesis (*34*, *35*), and induce detoxifying enzyme systems such as glutathione *S*-transferase (*36*). Their presence in the extract of *L. synanthera* increase the nutritrional interest in "chomte".

The nutrient composition of raw leaves of *L. synanthera* (reported in **Table 3**) has been evaluated to complete the knoledge on this plant and the understanding of its nutritional value. Chomte leaves were found to contain a substantially greater amount of protein (6.3%), carbohydrate (6.4%), and crude fiber (2.8%) than other commonly consumed edible leaves (*2*).

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