Sileneoside H, a New Phytoecdysteroid from *Silene brahuica*[1]

Z. T. Sadikov,[†] Z. Saatov,[†] Jean-Pierre Girault,[‡] and René Lafont*[,§]

Institute of Chemistry of Plant Substances, 700170 Tashkent, Uzbekistan, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, CNRS UMR 8601, Université René Descartes, 45 Rue des Saint-Pères, 75006 Paris, France, and Ecole Normale Supérieure et Université Pierre et Marie Curie, CNRS EP 2028, 46 Rue d'Ulm, 75230 Paris Cedex 05, France

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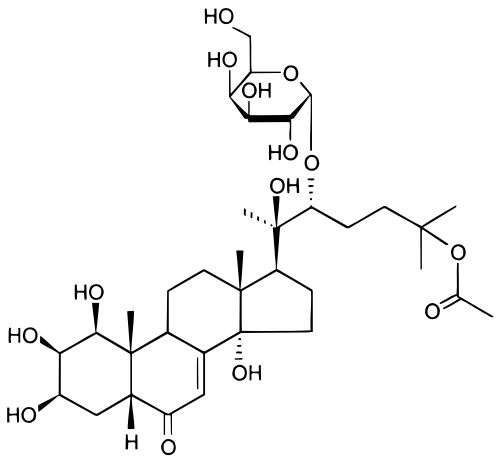
Sileneoside H (**1**), a new phytoecdysteroid, has been isolated from the roots of *Silene brahuica* and identified as 22-*O*-α-D-galactosylintegristerone A 25-acetate by MS and NMR analysis.

Phytoecdysteroids are steroid molecules related to insect molting hormones that comprise more than 200 representatives and are produced by many plant species.[2] They are thought to protect plants against non-adapted phytophagous insects.[3−6] In addition, these molecules possess anabolic properties in mammals and humans[7] and are currently used for that purpose by body-builders. Phytoecdysteroids are found in many species from the family Caryophyllaceae. *Silene brahuica* Boiss. is a species growing in the Kyrgyz Republic.[8] Although significant (ca. 0.3% of the dry weight in aerial parts), the ecdysteroid content of this plant is not so large, as concentrations of up to 2−3% of the dry weight have been reported for some other plant species. Contrary to most other phytoecdysteroid-containing plant species, which contain primarily 20-hydroxyecdysone together with a wide array of minor related molecules, *S. brahuica* contains a mixture of several compounds, including 2-deoxyecdysone, 2-deoxyecdysone 3-glucoside (sileneoside E), and in slightly lower amounts 20-hydroxyecdysone, as well as many minor ecdysteroids, among which are sileneosides A−G (i.e., sugar conjugates—glucosides, galactosides) and several acetates.[2,8] A further analysis of a root extract of *S. brahuica* has allowed the isolation of a new ecdysteroid glycoside, 22-*O*-α-D-galactosylintegristerone A 25-acetate (**1**).



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Compound **1** showed a UV absorption spectrum with a maximum value at 242 nm, which is characteristic of the 7-en-6-one moiety of ecdysteroids. HRCIMS using ammonia as reagent gas gave an ion at *m*/*z* 700.3893, a value consistent with $C_{35}H_{58}O_{13}N$ (700.3907), corresponding to a $[M - H_2O + NH_4]^+$ ion, establishing the molecular formula as $C_{35}H_{56}O_{14}$. Enzymatic hydrolysis yielded integristerone A 25-acetate, which was characterized by comparison with an authentic sample previously isolated from the same species.[9] Acid hydrolysis yielded a sugar identified by paper chromatography as D-galactopyranose.

The position of substitution of the sugar unit of **1** was performed using a combination of NMR procedures. 1D ^{1}H and ^{13}C spectra and 2D COSY, TOCSY, NOESY, PFG−HMQC, and PFG−HMBC NMR spectra allowed all the ^{1}H and ^{13}C NMR assignments to be made (Table 1). Proton and carbon signals of the steroidal ring system of **1** were identical to those of integristerone A, with the exception for the signals of the side chain. Moreover, the presence of new ^{1}H and ^{13}C NMR signals was evident, respectively, for the protons and carbons of an acetyl group, that is, methyl signals (^{1}H δ 2.04; ^{13}C, δ 23.1) and a carbonyl signal (^{13}C, δ 174.9). Location of the acetyl group at C-25 was confirmed by the shift of the C-26 and C-27 methyl signals (ca. + 0.2 ppm for protons and + 1.8−2.6 ppm for carbons, with respect to integristerone A). The presence of a hexose moiety was evident from ^{1}H NMR peaks in the region δ 3.3−5.1 ppm and from the ^{13}C NMR spectrum, where six additional oxygenated carbon signals were observed in the region δ 61.8−102.9 ppm. The C-22 signal (δ 90.6) was more deshielded (+ 12.3 ppm with respect to integristerone A) and thus suggested the attachment of the hexose unit at C-22, a conclusion confirmed from the ^{1}H−^{13}C long-range (^3J) correlations H-1′→C-22 and H-22→C-1′ observed in the PFG−HMBC spectrum. The identity of the sugar moiety as α-D-galactopyranose was conducted from the characteristic signal for the anomeric proton at δ 5.10 (d, J = 3.3 Hz)[10] and ^{1}H−^{1}H coupling patterns observed for H-4′ and H-5′ (confirmed with selective decoupling), and from ^{13}C NMR C-2′, C-3′, C-4′, and C-5′ chemical shifts.[11] A phase-sensitive NOESY experiment carried out for both integristerone A and **1** confirmed the 5β configuration from a strong NOE between H-5 and Me-19 observed for both compounds and between H-2 and H-9 for integristerone A. The OH-1β configuration was concluded from a medium NOE observed between H-1 and H-9, and the strong NOE between H-1 and H-11eq (1.80 ppm) for both compounds. For **1** the attachment of the hexose at C-22 was confirmed from the medium NOE between H-1′ and H-22, and the attachment of the acetyl group at C-25 confirmed from the NOE between MeCO and Me-27 and Me-26. The structure of **1** was therefore assigned unambiguously as 22-*O*-α-D-

* To whom all correspondence should be addressed. Tel.: 33-1-44323639. Fax: 33-1-44323910. E-mail: rlafont@biologie.ens.fr.
† Institute of Chemistry of Plant Substances.
‡ Université René Descartes.
§ Ecole Normale Supérieure.

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Table 1. ^{1}H and ^{13}C NMR Values for Sileneoside H (**1**) (125 and 500 MHz, D_2O)[a]

carbon	multiplicity	δ_C, ppm	δ_H, ppm, J (Hz)
aglycon			
1	CH	75.6	3.93 br s
2	CH	68.2	4.05 br t (3.1)
3	CH	70.0	4.15 br s ($W_{1/2}$ =12)
4	CH_2	32.7	1.79, 1.82
5	CH	46.5	2.62 dd br (11, 6)
6	C	ND[b]	
7	CH	122.1	5.99 br s ($W_{1/2}$ = 6)
8	C	ND[b]	
9	CH	35.4	3.05 m ($W_{1/2}$ = 23)
10	C	43.2	
11	CH_2	21.3	1.75, 1.80
12	CH_2	31.9	1.95, 1.75
13	C	48.4	
14	C	86.0	
15	CH_2	31.5	1.67, 2.06
16	CH_2	21.2	1.90, 1.75
17	CH	50.1	2.29 t (8.7)
18	CH_3	18.2	0.87 s
19	CH_3	20.0	1.09 s
20	C	80.6	
21	CH_3	21.8	1.30 s
22	CH	90.6	3.42 d (9.5)
23	CH_2	26.4	1.47, 1.59
24	CH_2	38.5	1.95, 2.06
25	C	85.9	
26	CH_3	26.5	1.42 s
27	CH_3	26.7	1.47 s
AcO−	CH_3	23.1	2.04 s
	CO	174.9	
sugar			
1′	CH	102.9	5.10 d (3.4)
2′	CH	70.9[c]	3.91 dd (10.3, 3.4 ABX)[d]
3′	CH	70.1[c]	3.94
4′	CH	70.3	4.04 dd (3, 1.2)[d]
5′	CH	72.2	4.11 dt (6.5, 1.2)[d]
6′	CH_2	61.8	3.74 dd (11.7, 5.8 ABX)[d], 3.76 dd (11.7, 6.7 ABX)[d]

[a] Multiplicity of signals: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad signal; $W_{1/2}$: width at half-height in hertz; δ in ppm. [b] ND: not determined. [c] Assignments could be interverted. [d] Values obtained from selective homonuclear decoupling and Gaussian resolution enhancement (GB = 0.4, LB = −2, SI = 32 K, 0.155 Hz/pt).

galactosylintegristerone A 25-acetate, and this new ecdysteroid has been termed sileneoside H.

Experimental Section

General Experimental Procedures. Thin-layer chromatography (TLC) was performed on Silufol plates (Si gel containing 10% calcium sulfate). Column chromatography was performed with Si gel, particle size 0.08−0.1 mm and 0.10−0.16 mm. Paper chromatography of sugars used FN-11 paper. IR spectra were obtained with a Perkin-Elmer 2000 FT−IR spectrophotometer as KBr pellets. MS were recorded on a JEOL JMS-700 spectrometer using a chemical ionization/desorption (CI/D) mode with ammonia as the reagent gas. NMR spectra were obtained using a Bruker AMX 500 spectrometer (500 MHz for ^{1}H) equipped with a Silicon Graphics workstation. Samples were run at 27 °C in D_2O. A presaturation of the solvent was used for all the 1D and homonuclear 2D ^{1}H experiments. The phase-sensitive NOESY experiment was carried out with a mixing time of 500 ms. The sample (7 mg) of **1** was dissolved in D_2O, lyophilized twice, and then redissolved in 1 mL D_2O prior to analysis by various NMR procedures (^{1}H, COSY, TOCSY, NOESY, HMBC, HMQC).[12] The errors on the chemical shifts are 0.01 ppm for ^{1}H and 0.1 ppm for ^{13}C. 3-(Trimethylsilyl)[2,2,3,3-d_4] propionic acid, sodium salt, was used as internal reference for the proton and carbon shifts.

Plant Material. *S. brahuica* Boiss. (Caryophyllaceae) was collected in the valley of the River Chonkemin, Kyrgyz Republic, in May 1982. A voucher sample is maintained at the Institute of Chemistry of Plant Substances of Tashkent.

Extraction and Isolation. Air-dried, powdered roots (5 kg dry wt) of *S. brahuica* were purified as described earlier.[13] Briefly, the roots were extracted first with MeOH (25 L). The extract was concentrated to 1 L, and 2.5 L of H_2O was added. The solution was extracted with $CHCl_3$, then EtOAc, and finally BuOH. The BuOH extract was evaporated to dryness, yielding 49 g of an ecdysteroid mixture, which was purified by column chromatography on Si gel, using a $CHCl_3$−MeOH−H_2O step-gradient system. The 70:23:3 fraction gave sileneoside B (142 mg, 0.003%) after recrystallization. Mother liquors accumulated during the isolation of sileneoside B were further purified by Si gel column chromatography using a mixture of $CHCl_3$−MeOH−H_2O (65:35:6) as eluent. This allowed the isolation of 25 mg of sileneoside H (**1**) (0.0005%).

Acid Hydrolysis. Compound **1** (5 mg) was used for acid hydrolysis and dissolved in 5 mL of 0.05% sulfuric acid and heated for 2 h at 100 °C. After neutralization with barium carbonate and centrifugation, the hydrolysate was concentrated in vacuo and analyzed by paper chromatography. Development used a *n*-BuOH−pyridine−H_2O (4:6:3) solvent system, and visualization was performed with aniline phthalate.

Enzymatic Hydrolysis. Compound **1** (10 mg) was used for enzymatic hydrolysis with 3 mL of an enzyme preparation from sweet almonds.[14] The mixture was kept at 38 °C for 30 days, then 7 mL of water was added, and the solution was extracted with *n*-BuOH. The *n*-BuOH extracts were evaporated to dryness and purified by column chromatography on Si gel, eluted with $CHCl_3$−MeOH (9:1). This allowed the isolation of a molecule identified as integristerone A 25-acetate by comparison with an authentic sample (R_f on TLC, IR, and NMR).[9]

Sileneoside H (1). Obtained as white powder, mp 210−212 °C; UV (EtOH) λ_{max} 242 nm (log ϵ 4.0); IR (KBr disk) ν_{max} 3398 (OH), 1707, 1655 (cyclohexenone), 1282 (OAc) cm^{-1}; MS (CI/D, positive mode) gave ions at *m/z* 700 [M^+], 682 [M^+ − 18], 664 [M^+ − 2 × 18], 640 [M^+ − 60], 623, 605, 587, 569, 479, 461, 443, and 180 (hexose); HRCIMS *m/z* 700.3893 (calcd for $C_{35}H_{58}O_{13}N$, 700.3907), corresponding to a [M − H_2O + NH_4]$^+$ ion.

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