EIMS *m/z* (rel. int.): 546 $[M]^+$ (1.1), 347 [Mclafferty rearrangement fragment]$^+$ (7.7), 306 [RDA fragment]$^+$ (31.2), 264 (32.0), 246 (28.4), 234 (99.1), 201 (58.1), 173 (100); ^{1}H NMR spectrum: see Table 2; ^{13}C NMR DEPT spectrum: see Table 1.

Esculentoside M (**2**). Amorphous powder; mp 219–221°; molecular formula $C_{48}H_{74}O_{22}$; IR ν^{KBr} cm^{-1}: 3400 (OH), 1740 (ester), 1660 (conjugated C=O), 1070, 1035 (C–O); FAB-MS *m/z*: 1025 $[M+Na]^+$, 1041 $[M+K]^+$, 863 $[(M+Na)-162]^+$, 547 $[aglycone+H]^+$; ^{1}H NMR spectrum: see Table 2; ^{13}C NMR spectrum: see Table 1.

Acid hydrolysis of compound **2**. Compound **2** (20 mg) in 5% HCl–EtOH soln (3 ml) were refluxed for 6 hr, and concd under red. pres. The residue was diluted with 5 ml H_2O. The resulting ppt. was collected by filtration and applied to a silica gel column, eluted with $CHCl_3$–MeOH (19:1), to afford **1** (3 mg), identified by direct comparison with an authentic sample (co-TLC, IR, EIMS). The filtrate was neutralized with 201 × 7 ion exchange resin and evapd to dryness. Glucose and xylose were detected by GLC according to the method reported by Li *et al.* [8].

Basic hydrolysis of compound **2**. Compound **2** (15 mg) in 1M NaOH (2 ml) was refluxed for 1 hr. The reaction mixture was neutralized with 1 M HCl and extracted with *n*-BuOH. Glucose in the H_2O layer was detected in the same way as the case of the acid hydrolysis of **2**. The *n*-BuOH layer was washed with H_2O, and evapd to dryness under red. pres. The alkaline hydrolysed saponin was purified on a silica gel column, eluted with $CHCl_3$–MeOH (4:1) to give **3** (4.5 mg): an amorphous powder; IR ν^{KBr} cm^{-1}: 3400 (OH), 1700 (CO_2H), 1660 (conjugated C=O), 1040 (C–O); FAB-MS *m/z*: 849 $[M+Na]^+$, 865 $[M+K]^+$.

Acetylation of compound **2**. Compound **2** (5 mg) was heated with Ac_2O (1 ml) and pyridine (2 ml) at 80° for 2 hr. After the addition of ice/H_2O, the resulting ppt. was filtered to yield an amorphous acetate (6 mg): mp 158–160°; IR ν^{KBr} cm^{-1}: 1735, 1240 (acetate); EIMS *m/z*: 331 [$(Glc)Ac_4$, terminal glucose], 547 [$(Xyl\text{-}Glc)Ac_6$].

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LEUCASIN, A TRITERPENE SAPONIN FROM *LEUCAS NUTANS*

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Key Word Index—*Leucas nutaus*; Labiatae; triterpenoidal saponin.

Abstract—A new saponin, leucasin, has been isolated from *Leucas nutans* and characterized on the basis of chemical investigation and spectroscopic studies as 3-*O*-[β-D-glucopyranosyl(1→2)β-D-glucopyranosyl]2α,3β-dihydroxylup-20(29)-ene. Lupeol palmitate, sitosterol and stigmasterol were also isolated.

INTRODUCTION

Leucas nutans Spreng (syn. *Leucas decurva* Bth.) is distributed in Sindh, Punjab and the North West Frontier provinces of Pakistan [1]. The medicinal properties attributed to several species of *Leucas* [2, 3] prompted us to investigate *Leucas nutans* for its chemical constituents. A literature survey revealed that no chemical work is reported on this species. We describe the isolation and characterization of a new triterpenoidal saponin named leucasin. Its structure was elucidated as 3-*O*-[β-D-glucopyranosyl]-(1→2)-β-D-glucopyranosyl]-2α,3β-di-

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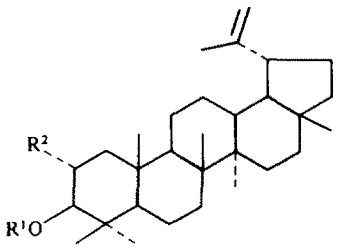

	R^1	R^2
1	Glc(1→2)Glc-	OH
2	Glc	OH
3	H	OH
4	$(CH_2)_{14}CO$-	H

hydroxy-lup-20(29)-ene. In addition lupeol palmitate, sitosterol and stigmasterol were isolated.

RESULTS AND DISCUSSION

Leucasin (**1**) was obtained as a crystalline material after purification through column chromatography as described in the Experimental. In the IR spectrum the compound showed peaks at 3400 (OH), 1635 (C=C) and 880 (C=CH_2) cm^{-1}. The negative ion FAB mass spectrum showed a $[M-H]^-$ peak at *m/z* 765 corresponding to a molecular formula $C_{42}H_{70}O_{12}$. In addition, the spectrum showed peaks at *m/z* 603 $[M-H-Glc]^-$ and 441 $[M-H-2\times Glc]^-$. The presence of two sugar moieties in **1** was also indicated by the ^1H NMR spectrum (CD_3OD). It showed two anomeric proton doublets at δ4.51 (*d*, *J* = 7.6 Hz) and 4.59 (*d*, *J* = 7.6 Hz). The coupling constant indicated that the two hexose units are present in the β-anomeric form. The spectrum further showed methyl singlets at δ0.88, 0.82, 0.91, 0.99, 1.02, 1.07 and 1.69, the last broad singlet resulted from the methyl group attached to an olefinic C atom. A doublet at δ3.08 (*J* = 9.7 Hz) resulted from H-3 showing diaxial coupling with one carbinylic proton. According to the COSY spectrum it is coupled with a multiplet at δ3.67 from H-2 which must also be axial. The hydroxy group at C-2 is, therefore, α-oriented. The ^1H NMR spectrum also showed signals at δ4.56 (*dd*, *J* = 1.4, 2.4 Hz) and 4.68 (*d*, *J* = 2.4 Hz) which result from the two C-29 protons of the aglycone (**3**).

The ^{13}C NMR spectrum of **1** in methanol-d_4 showed 42 carbon signals out of which 30 were due to the aglycone (Table 1). The chemical shifts of the two sugar units (Table 2) indicated that the disaccharide unit in **1** is sophorose. The glycosidation shifts of the internal glucose unit clearly indicate that the external glucose is attached to it at C-2′.

On mild acidic hydrolysis **1** yielded a mixture of the prosapogenin (**2**) through the removal of the external glucose unit, and the aglycone. They were separated by prep. TLC. The liberated sugar was identified by paper chromatography as glucose. Compound **2** shows, in the FAB mass spectrum (negative ion mode), the $[M-H]^-$ peak at *m/z* 603 corresponding to the molecular formula $C_{36}H_{60}O_7$. In the ^1H NMR spectrum (CD_3OD, 400 MHz) seven methyl singlets were present between δ0.81 and 1.68. The spectrum also showed the doublet at δ3.04 (*J* = 9.6 Hz, H-3), a multiplet at 3.64 (H-2′), and the doublet due the anomeric proton (*J* = 7.7 Hz) and two signals at 4.56 and 4.68 assigned to the two H-29 protons. These data clearly indicate that the structure of **2** is 3-*O*-[β-D-glucopyranosyl]-2α,3β-dihydroxy-lup-20(29)-ene. Compound **3** was identified as 2α,3β-dihydroxy-lup-20(29)-ene through comparison of its spectroscopic data with those given in the literature [4–6]. Thus **1** is 3-*O*-[β-D-glucopyranosyl(1→2)β-D-glucopyranosyl]2α,3β-dihydroxy-lup-20(29)-ene. In addition, several known compounds were isolated from this plant and identified through their spectral data as lupeol palmitate [7], sitosterol and stigmasterol [8–10].

Table 1. ^{13}C NMR spectral data of the aglycone of compound **1** (in methanol-d_4)

C	δ	C	δ
1	45.33	16	36.73
2	77.71	17	44.09
3	83.10	18	49.56
4	40.54	19	49.45
5	56.46	20	151.66
6	19.38	21	30.92
7	35.39	22	41.05
8	42.14	23	29.26
9	51.77	24	16.59
10	39.52	25	17.71
11	22.29	26	17.37
12	26.40	27	15.05
13	39.51	28	18.45
14	44.07	29	110.16
15	28.62	30	19.58

Table 2. ^{13}C NMR spectral data of sugar moieties (in methanol-d_4)

C	δ	C	δ
Glc g_1 (inner)		Glc g_2 (terminal)	
1	100.76	1	105.33
2	81.84	2	76.07
3	77.70	3	79.69
4	71.49	4	71.63
5	77.85	5	78.46
6	62.59	6	62.96

EXPERIMENTAL

Leucas nutans Spreng was collected from Quaid-i-Azam University campus, Islamabad, Pakistan during the autumn of 1988. It was identified by Dr Mir Ajab Khan (Quaid-i-Azam University). A voucher specimen is deposited in the Herbarium of the Department of Biology, Quaid-i-Azam University.

Extraction and isolation of leucasin. The whole air-dried and powdered plant (3.8 kg) was extracted with EtOH (90%) at room temp. several times. The combined ethanolic extract was evapd under red. pres. to afford a gummy residue (480 g) which was partitioned between EtOAc and H_2O. Both the layers were sepd and the aq. layer was further extracted with *n*-BuOH. The

butanolic extract was sepd and evapd (40 g) under red. pres. and chromatographed on a silica gel column using a gradient of MeOH in $CHCl_3$ as eluent. The fraction eluted with $CHCl_3$–MeOH (17:3) afforded a saponin leucasin (**1**) (0.2 g) together with some impurities. It was purified (0.15 g) by CC with $CHCl_3$–MeOH (9:1) as eluent system. Needles mp 190–192°; $[\alpha]_D^{25}$ −12° (MeOH; *c* 0.1) IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1635, 880; FABMS (−ve) *m/z* 765 $[M-H]^-$, 603 $[M-H-Glc]^-$, 441 $[M-H-2\times Glc]$; ^1H NMR (CD_3OD, 400 MHz): δ0.80 (*s*, Me), 1.07 (*s*, Me), 1.69 (*s*, Me), 3.08 (1H, *d*, *J* = 9.7 Hz, H-3), 3.67 (1H, *m*, H-2), 4.51 (1H, *d*, *J* = 7.6 Hz, H-1'), 4.56 (1H, *dd*, *J* = 1.4, 2.4 Hz, H-29B), 4.59 (1H, *d*, *J* = 7.6 Hz, H-1''), 4.68 (1H, *d*, *J* = 2.3 Hz, H-29A).[13]C NMR (see Tables 1 and 2).

Acid hydrolysis. Leucasin (**1**) (10 mg) was dissolved in MeOH (3 ml) and hydrolysed with 0.5 M HCl (7 ml) at 100° for 3 hr. When checked on TLC it revealed that some of the saponin (**1**) was partially and some of it was fully hydrolysed. After addition of water and removal of MeOH at red. pres., the hydrolysates were extracted with EtOAc. Thus, prosapogenin (**2**) (0.005 g) and the aglycone (**3**) (0.003 g) were sepd through prep. TLC using $CHCl_3$–MeOH (7:3) as the eluent system. The aq. layer was neutralized with alkali and concd at red. pres. The residue obtained was identified as glucose through comparison with an authentic sample by paper chromatography using *n*-BuOH–pyridine–H_2O (10:3:3) saturated with lower phase as the eluent system spraying with aniline pthalate sugar reagent.

Prosapogenin (**2**). Crystals, mp 222–225°, $[\alpha]_D^{25}$ −15.5° MeOH; *c* 0.09); FAB-MS (−ve) *m/z*: 603 $[M-H]^-$, 441 $[M-H-Glc]^-$. ^1H NMR (CD_3OD, 400 MHz): δ0.81 (*s*, Me), 1.07 (*s*, Me), 1.68 (*s*, Me), 3.04 (1H, *d*, *J* = 9.6 Hz, H-3), 3.64 (1H, *m*, H-2), 4.32 (1H, *d*, *J* = 7.7 Hz, H-1'), 4.56 (1H, *dd*, *J* = 1.4, 2.4 Hz, H-29B), 4.68 (1H, *d*, *J* = 2.3 Hz, H-29A).

2α,3β-Dihydroxylup-20(29)-ene (**3**). $[\alpha]_D^{25}$ +20° ($CHCl_3$; *c* 0.04), ^1H NMR ($CDCl_3$, 400 MHz): δ0.78, 0.80, 0.89, 0.94, 1.00, 1.02, 1.67, 2.96 (*d*, *J* = 9.5 Hz, H-3), 3.66 (*m*, H-2), 4.56 (*dd*, *J* = 1.3, 2.4 Hz, H-29B), 4.68 (*d*, *J* = 2.3 Hz, H-29A); EIMS *m/z*: 442 $[M]^+$, 427, 408, 218, 189, 121, 69.

Lupeol palmitate (**4**) was obtained by eluting the EtOAc extract (220 g) from the fractionation of the EtOH extract on silica with *n*-C_6H_{14}–$CHCl_3$ (7:3), purified (0.013 g) by TLC using *n*-C_6H_{14}–$CHCl_3$ (3:7).

Sitosterol (**5**) and stigmasterol (**6**) were obtained in the form of a mixture by eluting the main column with *n*-C_6H_{14}–$CHCl_3$ (2:3) and were purified (0.07 g) on a flash column using *n*-C_6H_{14}–$CHCl_3$ (1:3). Compounds **4**–**6** identified by comparison of their data with the published data.

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