Two New Spirostanol Glycosides from *Cestrum parqui*

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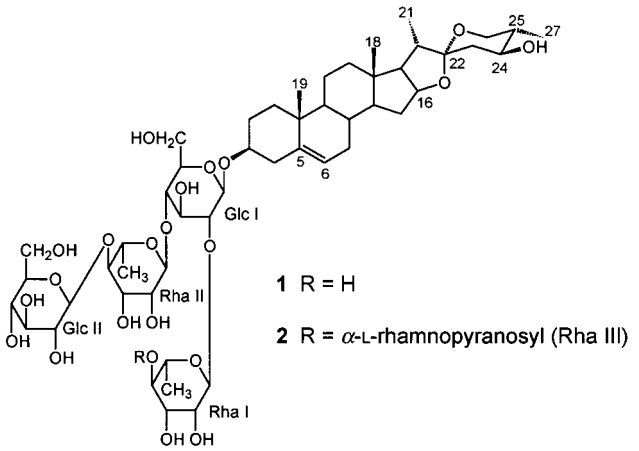
Two new steroidal glycosides, parquisoside A (**1**) and B (**2**) were isolated from the aerial parts of *Cestrum parqui* (family Solanaceae). Their common aglycone is a new steroid of the spirostane series, which we name parquigenin. It has the structure (3*β*,24*S*,25*S*)-spirost-5-ene-3,24-diol, *i.e.* a (24*S*,25*S*)-24-hydroxydiosgenin. The structures of parquisosides A and B were elucidated as (3*β*,24*S*,25*S*)-spirost-5-ene-3,24-diol 3-*O*-{[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)}-*β*-D-glucopyranoside (**1**) and (3*β*,24*S*,25*S*)-spirost-5-ene-3,24-diol 3-*O*-{[*α*-L-rhamnopyranosyl)-(1→4)-*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)}-*β*-D-glucopyranoside (**2**), respectively, on the basis of detailed spectroscopic studies and chemical analysis. The crude extract of *Cestrum parqui* showed inhibition of carrageenin-induced edema.

Introduction. – The plant *Cestrum parqui* L′-HERIT (Solanaceae) is commonly known as willow-leaved jasmine. It is a weed found in many parts of S. America and Australia [1]. In Pakistan, it has been introduced as an ornamental plant in the Abbottabad region [2]. It is used in Chile as a febrifuge. It is also known to be sudorific and is used for skin diseases such as allergies, herpes, and impetigo [3]. Two kaurene glycosides named carboxyparquin and parquin were isolated from the dried leaves. Carboxyparquin is toxic, whereas parquin is a relatively nontoxic cometabolite [1]. The anti-inflammatory action of the aerial parts of *C. parqui* has been reported [3]. The infusion as well as MeOH extract inhibits inflammation. Recently, we have reported that the wet MeOH extract of the aerial parts (Cp-50) in MeOH/H_2O 1:1 showed inhibition of carrageenin-induced edema. The aggregation of human-blood platelets induced by adenosine diphosphate (ADP) and platelet activation factor (PAF) was also inhibited (IC_{50}s were 3 and 2 mg/ml, resp.). However, the extract did not inhibit arachidonic acid (AA)-mediated platelet aggregation [4].

We now describe the isolation and structure elucidation of two new spirostanol glycosides, namely parquisoside A (**1**) and parquisoside B (**2**). The aglycones of both **1** and **2** are identical and represent a new steroid of the spirostane series. It is (24*S*,25*S*)-24-hydroxydiosgenin (=(3*β*,24*S*,25*S*)-spirost-5-ene-3,24-diol), and we name it parquigenin. The oligosaccharide chain is attached at C(3) of the aglycone in both glycosides.

Results and Discussion. – The aqueous MeOH extract of the air-dried aerial parts of *C. parqui* was distributed between hexane, AcOEt, BuOH, and H_2O. The BuOH extract was further fractionated by column chromatography (silica gel and *Sephadex LH-20*). Two monodesmosidic steroidal saponins, parquisoside A (**1**) and B (**2**), were finally purified by repeated column chromatography and preparative TLC on silica gel.

Parquisoside A (**1**) was obtained as a yellow gum. The negative-ion FAB-MS of **1** exhibits a pseudo-molecular ion peak at *m/z* 1045.5200 ($[M-H]^-$). The fragment ions at *m/z* 899 ($[M-H-146]^-$) and ($[M-H-162]^-$) show the loss of one deoxyhexose and one hexose from two terminal positions. The fragment ion *m/z* 727 ($[M-H-162-146]^-$) is consistent with the loss of an inner sugar moiety along with a terminal monosaccharide, or with a concomitant loss of one hexose and one deoxyhexose. A peak at *m/z* 591 ($[M-H-162-2(146)]^-$) confirms that the innermost sugar moiety is a hexose, and this fragment ion represents the aglycone with one monosaccharide moiety attached to it.



In the IR spectrum of **1**, in addition to the OH band at 3460 cm^{-1}, there are bands at 1019, 965, 920, 912, 899, and 865 cm^{-1}. These differ substantially from the pattern characteristic for the unsubstituted spiroketal F ring of a spirostanol. Thus, substitution is indicated in ring F due to the different 'finger-print' region [5–7]. It is known that when functional groups are present at ring F of a spirostanol, the nature of the absorption in the 800–1100 cm^{-1} region of the spectrum changes [8][9]. Complete acid hydrolysis of **1** was performed with a 20% HCl solution. Under these conditions, the aglycone was decomposed, probably because of the presence of OH–C(24) in the F ring. However, TLC of the hydrolysate and comparison with standards sugars allowed the identification of the monosaccharide units D-glucose and L-rhamnose. The ^{1}H- and ^{13}C-NMR (*Table 1*), ^{1}H,^{1}H-COSY, HMQC, and HMBC data of parquisoside A (**1**) suggested the structure 3*β*,24*S*,25*S*)-spirost-5-ene-3,24-diol 3-*O*-{[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-glucopyranosyl-(1 → 4)-*α*-L-rhamnopyranosyl-(1 → 4)]-*β*-D-glucopyranoside.

The ^{1}H-NMR spectrum (500 MHz, (D_5)pyridine) of **1** shows four anomeric signals at *δ* 4.78 (*d*, *J* = 7.5 Hz, H–C(1′) of Glc I), 4.92 (*d*, *J* = 7.0 Hz, H–C(1‴) of Glc II), 5.75 (br. *s*, H–C(1″) of Rha I), and 6.37 (*d*, *J* = 2.0, H–C(‴) of Rha II). The coupling constants indicate two *β*-linkages and two *α*-linkages. In addition, two tertiary Me signals at *δ* 0.87 (*s*, Me(18)) and 1.02 (*s*, Me(19)) and four secondary Me signals at *δ* 0.99 (*d*, *J* = 7.0 Hz, Me(27)), 1.31 (*d*, *J* = 7.0 Hz, Me(21)), 1.60 (*d*, *J* = 6.0 Hz, Me(6″) of Rha I), and 1.74 (*d*, *J* = 6.3 Hz, Me(6‴) of Rha II) are also observed. Two *m* at *δ* 3.61 and 3.91 are assigned to H–C(24) and H–C(3) of the aglycone, respectively. The signal at *δ* 4.90 (*q*-like) is due to H–C(16). The signals at *δ* 3.90 and 3.55 are ascribed to CH_2(26) and are indicative of *β* (axial) configuration of H–C(25) and, hence, *α* (equatorial) configuration of

Table 1. *^{13}C-(125 MHz) and ^{1}H-NMR (500 MHz) Spectral Data of Parquisoside A* (**1**) *in (D_5)Pyridine from 1D- and 2D-NMR Experiments.* δ in ppm, J in Hz.

	δ(C)	δ(H)(J)		δ(C)	δ(H)(J)
CH_2(1)	37.5	0.95 (H_α), 1.71 (H_β)	Me(27)	17.4	0.99 (d, J = 7.0)
CH_2(2)	29.9	1.82 (H_α), 1.50 (H_β)	Glc I: CH(1′)	104.9	4.78 (d, J = 7.5)
CH(3)	78.4	3.91 (m, H_α)	CH(2′)	79.7	4.81
CH_2(4)	38.9	2.69 (H_α), 2.78 (H_β)	CH(3′)	78.1	3.90[c]) (m)
C(5)	140.9	–	CH(4′)	78.9	4.35 (m)
CH(6)	121.9	5.3 (dist. t)	CH(5′)	75.2	3.99 (m)
CH_2(7)	32.4	1.88 (H_α), 1.85 (H_β)	CH_2(6′)	61.3	4.05, 4.15 (2m)
CH(8)	31.6	1.25 (H_β)	Rha I: CH(1″)	102.9	5.75 (br. s)
CH(9)	50.3	0.90 (H_α)	CH(2″)	72.7	4.60
C(10)	37.1	–	CH(3″)	71.7	4.85
CH_2(11)	21.1	1.43	CH(4″)	73.9	4.30 (m)
CH_2(12)	39.9	1.10 (H_α), 1.72 (H_β)	CH(5″)	70.4	3.61[b]) (m)
C(13)	40.8	–	Me(6″)	18.2	1.60 (d, J = 6.0)
CH(14)	56.6	1.05	Rha II: CH(1‴)	102.2	6.37 (d, J = 2.0)
CH_2(15)	31.9	2.01 (H_α), 1.45 (H_β)	CH(2‴)	72.5	4.80
CH(16)	81.1	4.90 (q-like, H_α)	CH(3‴)	70.8	4.55
CH(17)	63.8	1.91 (H_α)	CH(4‴)	78.7	4.32 (m)
Me(18)	16.7	0.87 (s)	CH(5‴)	69.3	3.57 (m)
Me(19)	19.4	1.02 (s)	Me(6‴)	18.6	1.74 (d, J = 6.3)
CH(20)	40.7	2.22 (H_β)	Glc II: CH(1⁗)	100.3	4.92 (d, J = 7.0)
Me(21)	16.4	1.31 (d, J = 7.0)	CH(2⁗)	74.1	4.27 (m)
C(22)	110.7	–	CH(3⁗)	77.9	4.20 (m)
CH_2(23)	34.1	1.90[a]) (H_α), 2.30 (H_β)	CH(4⁗)	72.8	4.50[d]) (m)
CH(24)	76.8	3.61[b]) (m, H_α)	CH(5⁗)	78.5	3.90[c]) (m)
CH(25)	34.3	1.90[a]) (H_β)	CH_2(6⁗)	62.8	4.33, 4.50[d]) (2m)
CH_2(26)	64.8	3.55 (H_α), 3.90[c]) (H_β)			

[a]), [b]), [c]), [d]) Repeated assignments.

Me(27). The downfield shift of the Me(27) d (δ 0.99) is due to the influence of the β-equatorial OH group. The distorted t at δ 5.30 is ascribed to the olefinic proton H–C(6). The HMQC experiment also reveals interaction between H–C(6) (δ 5.30) and C(6) at δ 121.9.

The ^{13}C-NMR (125 MHz, (D_5)pyridine) of **1** suggests that the aglycone is a spirostane-type steroid. It shows a total of 51 ^{13}C-resonances, of which 27 C-atoms belong to the aglycone and 24 C-atoms to the oligosaccharide moiety. The ^{13}C- and ^{13}C-DEPT spectra suggest that the 27 C-atoms of the aglycone moiety comprise 4 Me, 7 CH_2, 12 CH, and 4 quaternary C-atoms. When compared with published data [10], these 27 ^{13}C-resonances are in good agreement with those of diosgenin, except for the signals arising from ring F. The signals assignable to the spiroketal quaternary C(22) (δ 110.7), C(23) (δ 34.1), and C(25) (δ 34.3) show downfield shifts, and the resonance of C(26) (δ 64.8) shows an upfield shift. This is also observed when comparing yuccagenin to karatavigenin C [8] and pennogenin to 24-hydroxypennogenin [9]. In addition, the ^{13}C-signal of CH_2(24) at δ 29.2 in diosgenin [10] is replaced by the ^{13}C-signal of a CH at δ 76.8 in the DEPT spectrum of **1**. This indicates the presence of an OH group at C(24) of ring F. Moreover, the ^{13}C-signal at δ 17.4 for the secondary Me is attributed to Me(27), suggesting its α-equatorial orientation, hence C(25) has the (*S*) configuration [11]. The configuration at C(24) could not be determined by ^{1}H-NMR, because the H–C(24) signal of **1** is an unresolved m; however, the OH group is assumed to be β-oriented as the C(24) signal has nearly the same chemical shifts in both compounds **1** and **2**, and the H–C(24) signal of **2** is a dt, indicating its α-orientation in **2** (see *Fig.*).

The ^{13}C-NMR spectrum of **1** also shows four anomeric ^{13}C-signals at δ 100.3, 102.2, 102.9, and 104.9 and signals corresponding to two olefinic C-atoms at δ 121.9 and 140.9. The sugar moiety is attached at C(3) of the aglycon, and this is supported by the ^{13}C-NMR glycosidation shifts; indeed the C(3) signal of **1** is shifted downfield and to δ 78.4 compared to the corresponding signal in diosgenin (δ 71.5) [10]. In accordance with this,

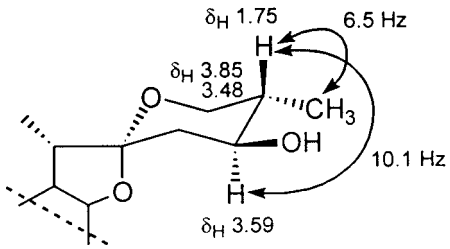


Figure. *Configuration of ring F of the aglycon of* **2**

an upfield shift is observed for the signals of C(2) (δ 29.9) and C(4) (δ 38.9), while in diosgenin, C(2) resonates at δ 31.6 and C(4) at δ 42.2, respectively [10].

The completion of the structure assignments of **1** required ascertaining the linkage points and sequence of the monosaccharide units. From the ^{13}C-assignments, sugar identities as well as branching sites are indicated by the characteristic downfield shifts (3–10 ppm) induced by the formation of each of the glycosidic linkage [12][13]. The values are assigned on the basis of one-bond ^{1}H,^{1}H COSY and HMQC experiments and further confirmed by the long-range HMBC experiment, which also indicates the linkage points in the tetrasaccharide moiety. The anomeric proton at δ 4.78 (H–C(1′) of Glc I) shows correlation with C(3) of the aglycone at δ 78.4. The anomeric proton at δ 5.75 (H–C(1″) of the terminal Rha I) manifests connectivity with C(2′) of the innermost Glc I at δ 79.7, whereas the anomeric proton of Rha II at δ 6.37 (H–C(1‴)) shows connectivity with C(4′) (δ 78.9) of the Glc I moiety. The anomeric proton at δ 4.92 (H–C(1⁗) of the terminal Glc II) exhibits correlations with C(4‴) of Rha II at δ 78.7.

The HMBC experiment also proved to be useful in confirming the values assigned to the aglycone, especially with reference to the chemical shifts exhibited by the ring-F protons and C-atoms. Thus, the Me(21) protons (δ 1.31) and H–C(20) (δ 2.22) exhibit long-range correlations with C(22) at δ 110.7. The H_α–C(23) at δ 2.30 manifests connectivity with C(20) (δ 40.7) and H–C(24) at δ 3.61 shows cross-peaks with C(23) at δ 34.1. The Me(27) protons at δ 0.99 exhibit connectivity with C(24) (δ 76.8), and this confirms the presence of an OH group at C(24) of ring F of **1**.

Parquisoside B **2** was also obtained as a yellow gum. Its negative-ion FAB-MS exhibits a pseudo-molecular ion peak at *m*/*z* 1191.4314 ($[M-H]^-$). The fragment ions appearing at *m*/*z* 1045 ($[M-H-146]^-$) and 1029 ($[M-H-162]^-$) indicate the simultaneous loss of a deoxyhexose and a hexose. Hence, terminal positions are assigned to the two different sugar moieties. The other fragment ions appear at *m*/*z* 899, 883, 737, and 591 ($[M-H-2(146)]^-$, $[M-H-146-162]^-$, $[M-H-2(146-162]^-$, and $[M-H-3(146)-162]^-$, resp.). The peak at *m*/*z* 591 represents the aglycone and the innermost monosaccharide moiety. The positive-ion FAB-MS of **2** shows pseudo-molecular ion peaks at *m*/*z* 1215 ($[M+Na]^+$) and 1069 $[M+Na-146]^+$. Acid hydrolysis of **2** with 20% HCl solution yielded D-glucose and L-rhamnose. The aglycone was destroyed under the hydrolysis conditions, and the organic phase showed a number of spots on TLC that could not be identified. Further spectral data (*Table 2*) and their comparison with those of **1** were in agreement with the structure (3β,24*S*,25*S*)-spirost-5-ene-3,24-diol 3-*O*-{[α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4)}-β-D-glucopyranoside for parquisoside B (**2**).

The ^{1}H-NMR (500 MHz, (D_5)pyridine + 2 drops of D_2O, 50°) of **2** shows 2 tertiary Me signals at δ 0.88 (*s*, Me(18)) and 0.98 (*s*, Me(19)) and 5 secondary Me signals at δ 0.97 (*d*, *J* = 7.0 Hz, Me(27)), 1.33 (*d*, *J* = 7.0 Hz,

Table 2. *^{13}C-(125 MHz) and ^{1}H-NMR (500 MHz) Spectral Data of Parquisoside B (**2**) in (D_5)Pyridine (+ 2 drops of D_2O) from 1D- and 2D-NMR Experiments.* δ in ppm, J in Hz.

	δ(C)	δ(H)(J)		δ(C)	δ(H)(J)
CH_2(1)	37.4	0.84 (H_α), 1.68 (H_β)	CH(3′)	75.3	3.95
CH_2(2)	29.6	1.80 (H_α), 1.49 (H_β)	CH(4′)	78.9	4.22[d])
CH(3)	78.2	3.88 (m, H_α)	CH(5′)	77.8	3.87
CH_2(4)	38.6	2.65 (H_α), 2.78 (H_β)	CH_2(6′)	61.2	4.05, 4.15 (2m)
C(5)	140.8	–	Rha I: CH(1″)	102.6	5.95 (d, J = 1.5)
CH(6)	121.6	5.28 (dist. t)	CH(2″)	72.3	4.85[a])
CH_2(7)	32.2	1.45 (H_α), 1.81 (H_β)	CH(3″)	70.2	4.58 (dd, J = 3.3, 9.0)
CH(8)	31.6	1.53 (H_α)	CH(4″)	78.2	4.22[d])
CH(9)	50.3	0.91 (H_α)	CH(5″)	68.4	4.30[e])
C(10)	36.9	–	Me(6″)	18.2	1.59 (d, J = 5.95)
CH_2(11)	20.8	1.41	Rha II: CH(1‴)	102.0	6.05 (br. s)
CH_2(12)	39.8	–	CH(2‴)	70.4	4.49
C(13)	40.7	–	CH(3‴)	71.1	4.60
CH(14)	56.4	1.05 (H_α)	CH(4‴)	78.5	4.35
CH_2(15)	31.8	1.28 (H_α), 1.98 (H_β)	CH(5‴)	69.3	3.90
CH(16)	81.1	4.85[a]) (q-like, H_α)	Me(6‴)	18.4	1.64 (d, J = 6.0)
CH(17)	63.3	1.87 (H_α)	Rha III: CH(1⁗)	101.8	6.02 (br. s)
Me(18)	16.2	0.88 (s)	CH(2⁗)	72.4	4.80
Me(19)	19.2	0.98 (s)	CH(3⁗)	71.7	–
CH(20)	40.4	2.22 (H_β)	CH(4⁗)	73.6	–
Me(21)	15.9	1.33 (d, J = 6.9)	CH(5⁗)	70.0	3.55
C(22)	110.3	–	Me(6⁗)	17.9	1.63 (d, J = 5.0)
CH_2(23)	33.8	2.10	Glc II: CH(1⁗′)	100.1	4.89 (d, J = 7.1)
CH(24)	76.4	3.59 (dt, J = 4.8, 10.1, H_α)	CH(2⁗′)	74.9	4.25
CH(25)	33.9	1.75 (dd, J = 10.1, 3.0, H_β)	CH(3⁗′)	77.9	–
CH_2(26)	64.2	3.48 (H_α), 3.85[e]) (H_β)	CH(4⁗′)	72.3	4.41
Me(27)	17.1	0.97 (d, J = 6.8)	CH(5⁗′)	77.7	3.85[e]) (m)
Glc I: CH(1′)	104.2	4.72 (overlapped)	CH_2(6⁗′)	62.4	4.30, 4.45 (2m)
CH(2′)	79.8	4.79			

[a]), [b]), [c]), [d]), [e]) Repeated assignments.

Me(21)), 1.59 (d, J = 5.9 Hz, Me(6″) of Rha I), 1.63 (d, J = 5.0 Hz, Me(6⁗) of Rha III), and 1.64 (d, J = 6.0 Hz, Me(6‴) of Rha II). A m at δ 3.88 is assigned to H–C(3) and the signal at δ 4.85 (q-like) to H–C(16) of the aglycone. The signals at δ 3.48 and 3.85 are ascribed to CH_2(26). The chemical shifts and coupling constants exhibited by H–C(25) at δ 1.75 (dd, J = 3.0, 10.1 Hz) are only possible because of its β-axial orientation and hence α-equatorial configuration of the Me group. The signal at δ 3.59 (dt, J = 4.8, 10.1 Hz) assigned to H–C(24) can only be explained if H–C(24) has the α-axial orientation. The observed coupling constants indicate that H–C(24) interacts diaxially with H–C(25), and also diaxially and axially-equatorially with the protons CH_2(23). Consequently, H–C(24) is α-axial and OH–C(24) is β-equatorial [9]. Thus, the aglycone moiety in both **1** and **2** is concluded to be (24*S*,25*S*)-24-hydroxydiosgenin, *i.e.* (3β,24*S*,25*S*)-spirost-5-ene-3,24-diol.

The ^{1}H-NMR spectrum of **2** also contains five anomeric signals at δ 4.72 (d, J = 7.2 Hz, H–C(1′) of Glc I), 4.89 (d, J = 7.1 Hz, H–C(1⁗′) of Glc II), 5.95 (d, J = 1.5 Hz, H–C(1″) of Rha I), 6.02 (br. s, H–C(1⁗) of Rha III), and 6.05 (br. s, H–C(1‴) of Rha II). The coupling constants indicate two β-linkages and three α-linkages. A distorted t at δ 5.28 is ascribed to the olefinic proton H–C(6). The HMQC experiment also reveals interaction between H–C(6) (δ 5.28) and C(6) at δ 121.6.

The ^{13}C-NMR (125 MHz, (D_5)pyridine + 2 drops of D_2O, 50°) of **2** exhibits 57 C-signals, 27 of which are due to the aglycone and the remaining 30 to the oligosaccharide moiety. The ^{13}C-DEPT spectra indicates that **2** contains 7 Me, 11 CH_2, and 35 CH, and, by difference from the broad-band spectrum, 4 quaternary C-atoms. The chemical shifts for the 4 quaternary C-atoms are δ 140.8, 110.3, 40.7, and 36.9; on comparison with parquisoside

A (**1**), these are assigned to C(5), C(22), C(20), and C(13), respectively. The two olefinic C-signals are located at δ 140.8 (C) and 121.6 (CH). Saponin **2** has the same aglycone as **1**, *i.e.* parquigenin (=(24*S*,25*S*)-24-hydroxydiosgenin), differing from **1** only by an additional deoxyhexose moiety. Thus ring F of the aglycone of **2** is also different from diosgenin. This is further confirmed by the absence of characteristic spiroketal bands in the IR spectrum of **2**. In the IR spectrum, absorptions are observed at 3480 (OH), 1400, 1380 (C=C), 1080 (OH), 1029, 965, 928, 912, 898, and 860 cm^{-1}, which differ significantly from the pattern characteristic for an unsubstituted (25*R*) or (25*S*) spiroketal ring-F structure.

In the ^{13}C-NMR, the presence of five anomeric ^{13}C-signals at δ 104.2, 102.6, 102.0, 101.8, and 100.1 confirm that saponin **2** contains a pentasaccharide moiety. The remaining 25 ^{13}C-signals comprise 3 Me, 2 CH_2 and 20 CH groups. In the sugar region, all the ^{1}H- and ^{13}C-signals are assigned on the basis of ^{1}H,^{1}H COSY, HMQC, and long-range homonuclear *Hartmann-Hahn* (HOHAHA) data, and by comparison with literature data. The values assigned by one-bond ^{1}H,^{1}H COSY and HMQC are further confirmed in the long-range HMBC experiment. In the oligosaccharide moiety, long-range HMBC correlations through the osidic bonds are observed. A long-range correlation between H–C(1′) of Glc I at δ 4.72 and C(3) (δ 78.2) of the aglycone is established. This confirms the linkage of the sugar moiety to C(3) of the aglycone. The remaining four anomeric protons H–C(1″) of Rha I, H–C(1‴) of Rha II, H–C(1⁗) of Rha III, and H–C(1⁗′) of Glc II at δ 5.95, 6.05, 6.02, and 4.89 exhibit cross-peaks with C(2′) of Glc I at δ 79.8 (C(2′) of the innermost hexose), C(4′) (δ 78.9) of Glc I, C(4″) (δ 78.2) of Rha I, and C(4⁗) (δ 78.5) of Rha II, respectively. These correlations help to ascertain the linkage positions and thus are very conclusive in establishing the sequence of the sugar units present in saponin **2**.

Experimental Part

General. Column chromatography (CC): silica gel *60* (35–70 mesh, *Merck*) and *Sephadex LH-20*. TLC: silica gel *60* F_{254} (*Merck*); prep. TLC on glass plates (*Merck*, article No. 5717). Optical rotations: *Jasco DIP-360* automatic digital polarimeter. IR Spectra: *Jasco IRA-1* spectrophotometer; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AM-500* spectrometers; C_5D_5N and $C_5D_5N + D_2O$ solns., with $SiMe_4$ as internal standard, δ in ppm, *J* in Hz; experiments: ^{1}H,^{1}H COSY, HMQC, 2D-J-RESOLVED, HOHAHA, and HMBC. FAB-MS: glycerol matrix; *Jeol JMS-HX-110* mass spectrometer; in *m*/*z*.

Plant Material. The aerial parts (leaves, stems, and flowers) of *Cestrum parqui* were collected from Abbottabad in May 1993 by Dr. *Muhammad Saeed*, Department of Pharmacy, University of Peshawar, Peshawar. The plant was identified by Dr. *Surayya Khatoon*, Department of Botany, University of Karachi, Karachi, Pakistan.

Extraction and Isolation. Air-dried aerial parts of the plant were crushed to a coarse powder and then exhaustively extracted with MeOH, MeOH/H_2O, and then with H_2O. The MeOH/H_2O extract was evaporated (145.5 g) and the residue suspended in H_2O (2 l). The suspension was extracted successively with hexane, AcOEt, and BuOH. Evaporation of the BuOH extract gave a dark brown gummy material (43.63 g), which was submitted to CC (silica gel). The fractions eluted with 20% MeOH/$CHCl_3$ and 25% MeOH/$CHCl_3$ were combined separately. The two eluates were successively subjected to *Sephadex LH-20* gel filtration with MeOH/H_2O 1:1. The fractions collected were dried and further purified by repeated CC and prep. TLC (BuOH/AcOH/H_2O 12:3:5). The less-polar *parquisoside A* (**1**) was obtained as a yellow gum (12.4 mg), which was eluated predominantly with 20% MeOH/$CHCl_3$. The more polar *parquisoside B* (**2**), also a yellow gum (19.8 mg), was predominantly present in the fractions obtained with 25% MeOH/$CHCl_3$.

Parquisoside A (=*(3β,24S,25S)-24-Hydroxyspirost-5-en-3-yl β-*D*-Glucopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-glucopyranoside*; **1**): $[\alpha]_D^{25} = -36.0$ ($c = 0.100$, MeOH). IR (KBr): 3460, 1019, 965, 920, 912, 899, 865. ^{1}H-NMR (500 MHz, (D_5)pyridine) and ^{13}C-NMR (125 MHz, (D_5)pyridine: *Table 1*. FAB-MS (neg.): 1045.5200 ($[M-H]^-$, $C_{51}H_{81}O_{22}^-$; calc. 1045.5219), 899 ($[M-H-146]^-$), 883 ($[M-H-162]^-$), 727 ($[M-H-162-146]^-$), 591 ($[M-H-162-2(146)]^-$).

Parquisoside B (=*(3β,24S,25S)-24-Hydroxyspirost-5-en-3-yl β-*D*-Glucopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→4)-[α-*L*-rhamnopyranosyl-(1→4)-α-*L*-rhamnopyranosyl-(1→2)]-β-*D*-glucopyranoside*; **2**). $[\alpha]_D^{25} = -95.2$ ($c = 0.018$, MeOH). IR (KBr): 3380 (OH), 1400, 1380 (C=C), 1080 (OH), 1029, 965, 928, 912, 898, 860. ^{1}H-NMR (500 MHz, (D_5)pyridine + 2 drops of D_2O, 50°) and ^{13}C-NMR (125 MHz, (D_5)pyridine + 2 drops of D_2O, 50°): *Table 2*. FAB-MS (neg.): 1191.4314 ($[M-H]^-$, $C_{57}H_{91}O_{26}^-$; calc. 1191.4242), 1045 ($[M-H-146]^-$), 1029 ($[M-H-162]^-$), 899 ($[N-H-2(146)]^-$), 883 ($[M-H-146-162]^-$), 737 ($[M-H-2(146)-162]^-$), 591 ($[M-H-3(146)-162]^-$). FAB-MS (pos.): 1215 ($[M+Na]^+$), 1069 ($[M+Na-146]^+$).

Acid Hydrolysis of Parquisoside A (**1**) *and B* (**2**). Pure parquisoside A (**1**, 9.16 mg) was dissolved in MeOH (20 ml), 20% HCl soln. (10 ml) was added, and the mixture was refluxed for 3 h. After evaporation, the mixture

was diluted with H_2O (10 ml) and extracted ($3\times$) with $CHCl_3$. The aq. layer was neutralized with Ag_2CO_3, filtered, and evaporated. The residue obtained was compared with standard sugars by TLC (*Merck*, article No. 5554, BuOH/AcOEt/i-PrOH/AcOH/H_2O 7:20:12:7:6; developed twice in the same direction). The sugars identified were D-glucose and L-rhamnose.

The $CHCl_3$ layer was washed with H_2O and then evaporated. TLC Analysis showed that the genin moiety of the saponin was decomposed.

Parquisoside B (**2**; 11.53 mg) was treated as described for **1**. From the aq. phase, the sugars identified were D-glucose and L-rhamnose. The org. phase showed several spots on TLC that could not be identified.

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