Phytochemistry, Vol. 27, No. 1, pp. 304–306, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

SAPONINS FROM *PRIMULA DENTICULATA*

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(*Revised received* 22 *April* 1987)

Key Word Index—*Primula denticulata*; Primulaceae; triterpenoid saponins; primulanin; saxifragifolin B.

Abstract—A new triterpenoid saponin, primulanin, isolated from the whole plant of *Primula denticulata* was characterized as 3-*O*[*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 4)-*α*-L-arabinopyranosyloxy]-16*α*-hydroxy-13*β*,28-epoxy-olean-30-al.

INTRODUCTION

Primula denticulata Sm. occurs as a common weed in the mountains of the North West Frontier Province of Pakistan [1]. Various other Primula species have been reported to have medicinal properties [2, 3] therefore a study of the saponins of *P. denticulata* has been undertaken. Previous work has identified five triterpenoid sapogenins pridentigenins A–E as constituents of the saponin fraction of *P. denticulata* [4–7]. We wish now to report the isolation and structure determination of a new saponin, primulanin (**2**), and saxifragifolin B (**1**) from this plant.

RESULTS AND DISCUSSION

Compounds **1** and **2** were isolated from the crude mixture of saponins by repeated chromatography on silica gel and by HPLC.

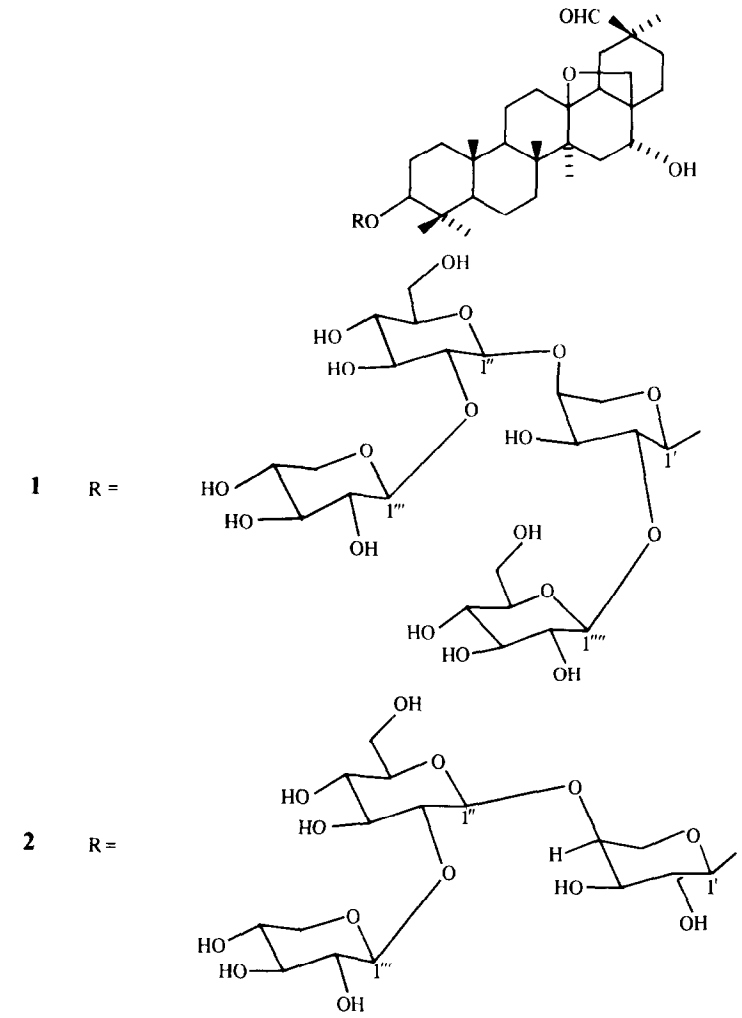
Compound **1** contained hydroxyl (3400–3200 cm^{-1}) and aldehyde (1720 cm^{-1}) groups. Its UV spectrum had only end absorption at 205 nm indicating the absence of double bonds. On acid hydrolysis, it yielded cyclamiretin A as the aglycone [8] and D-glucose, L-arabinose and D-xylose. The negative ion FABMS spectrum exhibited a molecular ion peak at *m/z* 1059 $[M-H]^-$ and fragment ions at *m/z* 927, 897 and 764 which were attributed to the loss of a terminal pentose, a terminal glucose and of a terminal glucose–pentose disaccharide or terminal pentose and terminal glucose unit respectively. There was no evidence of the loss of either a pentose–pentose or a glucose–glucose disaccharide.

The ^1H NMR spectrum of compound **1** in CD_3OD revealed the presence of six tertiary methyl groups through signals at *δ*0.85, 0.89, 0.97, 1.05, 1.13 and 1.27. In addition there were peaks at *δ*3.06 (1H, *d*, *J* = 7.6, H-28), 3.46 (*d*, *J* = 7.9 Hz, H-28), and multiplets at *δ*3.2 and 3.6 due to H-3 and H-16 respectively [overlapped due to the severe spectral crowding in the region *δ*2.5–4 characteristic of oligosaccharides]. Four anomeric proton signals were also observed at *δ*4.35 (*d*, *J* = 3 Hz, H-1′), 4.55 (*d*, *J* = 7.6 Hz, H-1″), 4.60 (*d*, *J* = 7.6 Hz, H-1‴) and 4.70 (*d*, *J* = 7.6 Hz, H-1⁗) supporting the *α*-configuration of L-arabinose, and the *β*-configurations of D-glucose and D-xylose. These assignments were also confirmed by means of 2D COSY-45, *J*-resolved, NOESY and hetero-COSY experiments.

The sequence and configuration of the sugar moieties were also verified by the ^{13}C NMR spectrum, in which four anomeric signals appeared at *δ*104.3, 104.7, 105.5 and 107.2, consistent with the presence of the *α*-L-arabinopyranosyl, *β*-D-glucopyranosyl and *β*-D-xylopyranosyl configurations in a 1:2:1 ratio. Comparison of the ^{13}C NMR spectrum (edited DEPT experiment) of **1** with those of related compound also helped in the assignments. It was confirmed from the ^{13}C NMR data, that cyclamiretin A was present with the sugar moieties attached at the C-3 position, as the C-3 signal of the aglycone appeared at *δ*90.8. On the basis of above findings, the structure of compound **1** was concluded to be 3-*O*[*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 4)-[*β*-D-glucopyranosyl-(1 → 2)]-*α*-L-arabinopyranosyloxy]-16*α*-hydroxy-13*β*,28-epoxy-olean-30-al. Glycosides of same structure as compound **1** have been isolated from *Androsace saxifragifolia* [9] and *Cyclamen europaeum* [10].

The UV and IR spectra of compound **2** were similar to those of compound **1**. Acid hydrolysis of **2** resulted in the formation of an aglycone characterized as cyclamiretin A [8] and D-glucose, D-xylose and L-arabinose. The positive FAB-MS exhibited peaks at *m/z* 921 $[M+Na]^+$ and 899 $[M+H]^+$ and a peak at *m/z* 767 due to the elimination of pentose. In the ^1H NMR spectrum, three anomeric proton signals were observed at *δ*4.48 (*d*, *J* = 7.6 Hz), 4.50 (*d*, *J* = 7.4 Hz) and 4.2 (*d*, *J* = 5.5 Hz) supporting the *β*-configurations of D-glucose and D-xylose and the *α*-configuration of L-arabinose.

Two-dimensional NMR measurements (COSY-45, NOESY, *J*-resolved, hetero-COSY) were also carried out to verify the assignments. A comparison of the ^{13}C NMR spectrum of **2** with that of **1** showed that the signals due to C-1, C-2 and C-3 of *α*-L-arabinopyranosyl moiety were shifted by +3.2, −5.9 and +0.6 respectively, while the other common signals were almost unchanged (Table 1) suggesting that the *β*-D-glucose attached to C-2 of the *α*-L-arabinopyranosyl moiety of **1** was absent in **2**. Based upon the above observations, the structure of the new saponin **2** was established as 3-*O*[*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucopyranosyl-(1 → 4)-*α*-L-arabinopyranosyloxy]-16-*α*-hydroxy-13,28-epoxy olean-30-al.



EXPERIMENTAL

Mps: uncorr. ^{1}H NMR and ^{13}C NMR: CD_3OD using TMS as int. standard. Analytical TLC: silica gel using *n*-BuOH–AcOH–H_2O (12:3:5) and cellulose using EtOAc–H_2O–MeOH–AcOH (13:3:3:4): HPLC: RP-18 column and Refracto Monitor III R.I. detector.

Plant material. P. denticulata (3.5 kg) was collected from Dongagali shade (north-west frontier province of Pakistan), air-dried then ground to a coarse powder and extracted with MeOH under reflux. The residue was shaken with *n*-BuOH and H_2O and the *n*-BuOH layer evaporated. The residue was dissolved in the minimum amount of MeOH and diluted with cold Et_2O to yield a cream coloured ppt. of crude saponins (10 g) 8 g of which was chromatographed on a silica gel column. The fractions obtained with $CHCl_3$–MeOH (9:1) contained compound **2** and (8.5:1.5) compound **1**, which were further purified by rechromatography on silica gel (230–400 mesh size) and by HPLC using as solvent systems MeOH–H_2O (17:3) and (4:1) (flow rate 5 ml/min).

Compound **1**. $C_{52}H_{84}O_{22}$, mp 238–239°, $[\alpha]_D$ −19.2°, (MeOH; *c* 0.052). UV λ_{max}^{MeOH} nm: 205; IR ν_{max}^{KBr} cm^{-1}: 3400–3200 (OH), 2900 (methylene), 1720 (CHO), 1040 and 890 (ether); ^{1}H NMR (CD_3OD, 300 MHz): δ0.85 (3H, *s*, H-25), 0.89 (3H, *s*, H-24), 0.97 (3H, *s*, H-29), 1.05 (3H, *s*, H-23), 1.13 (3H, *s*, H-26), 1.25 (1H, *dd*, *J* = 5.5, 13 Hz, H-18), 1.27 (3H, *s*, H-27), 3.06 (1H, *d*, *J* = 7.6 Hz, H-28), 3.20 (1H, *m*, H-3), 3.46 (1H, *d*, *J* = 7.9 Hz, H-28), 3.6 (1H, *m*, H-16), 3.2–4.0 (sugar protons), 4.35 (*d*, *J* = 3 Hz, H − 1′), 4.55 (*d*, *J* = 7.6 Hz, H-1″), 4.60 (*d*, *J* = 7.6 Hz, H-1‴), 4.70 (*d*, *J* = 7.6 Hz, H-1⁗), 9.40 (1H, *s*, H-30); Positive FABMS *m/z*: 1084 $[M+Na]^+$, 1061 $[M+H]^+$; negative FABMS *m/z*: 1059 $[M-H]^+$, 927 [M − pentose − H]$^+$, 897 [M − glucose − H]$^+$, 764 [M − glucose − pentose − H]$^+$.

Acid hydrolysis of compound **1**. Compound **1** (20 mg) was refluxed with 0.1 M HCl in aq. MeOH (5 ml) for 4 hr. The reaction mixture was then concentrated under red. pres. to remove MeOH. Addition of H_2O gave a white ppt. which was collected by filtration and identified as a mixture of two compounds, cyclamiretin A and D. The aq. filtrate was adjusted to pH 7 with Ag_2Co_3 and filtered. The supernatant was concd under red. pres. and compared with standard sugars on TLC (cellulose) The sugars were detected by spraying the plate with a satd soln of aniline phthalate in BuOH.

Compound **2**. $C_{46}H_{74}O_{17}$, mp 290° (dec.), $[\alpha]_D$ −39.2° (MeOH; *c* 0.055). UV λ_{max}^{MeOH} nm: 205; IR ν_{max}^{KBr}: 3300–3400 (OH), 2900 (methylene) 1718 (CHO), 1040 and 980 (CO); ^{1}H NMR (CD_3OD, 300 MHz): δ0.85 (3H, *s*, H-25), 0.89 (3H, *s*, H-24), 0.97 (3H, *s*, H-29), 1.05 (3H, *s*, H-23), 1.14 (3H, *s*, H-26), 1.25 (1H, *dd*, *J* = 6, 13 Hz, H-18), 1.27 (3H, *s*, H-27). 3.06 (1H, *d*, *J* = 7.6 Hz, H-28), 3.10 (1H, *m*, H-3), 3.46 (1H, *d*, *J* = 7.9 Hz, H-28), 3.60 (1H, *m*, H-16), 3.2–4.0 (sugar protons) 4.2 (*d*, *J* = 5.5 Hz, H-1′), 4.48 (*d*, *J*

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (75 MHz, CD_3OD)

C	1	2	C	1	2
1	40.2	40.2	1′	104.3	107.5
2	27.2	27.2	2′	80.1	74.2
3	91.3	90.8	3′	74.2	74.8
4	40.6	40.2	4′	75.8	75.6
5	56.6	56.6	5′	65.9	66.0
6	18.7	18.7	1″	104.7	105.2
7	32.8	32.8	2″	85.0	86.1
8	43.4	43.4	3″	77.5	77.5
9	53.9	53.9	4″	71.1	71.0
10	37.9	37.8	5″	78.0	77.8
11	19.8	19.8	6″	63.3	62.5
12	31.0	30.9	1‴	107.2	107.9
13	88.2	88.2	2‴	76.0	76.1
14	45.3	45.3	3‴	77.8	77.8
15	34.0	34.0	4‴	70.9	70.8
16	77.6	77.5	5‴	67.4	67.1
17	44.8	44.8	1⁗	105.5	—
18	51.3	51.3	2⁗	77.6	
19	37.0	37.0	3⁗	79.5	
20	48.2	48.2	4⁗	72.0	
21	35.1	35.1	5⁗	77.8	
22	33.2	33.2	6⁗	62.5	
23	28.4	28.4			
24	16.7	16.8			
25	16.7	16.8			
26	18.8	18.8			
27	20.1	20.1			
28	78.4	78.4			
29	24.3	24.3			
30	209.1	209.2			

= 7.6 Hz, H-1′), 4.50 (*d*, *J* = 7.4 Hz, 1″), 9.40 (1H, *s*, H-30); Positive FABMS *m/z*: 921 $[M + Na]^+$, 899 $[M + H]^+$, 767 $[M - \text{pentose} + H]^+$; Negative FABMS *m/z*: 898 $[M - H]^-$.

Acid hydrolysis of compound **2** was carried out by the same method as that described for compound **1**.

Acknowledgement We are sincerely thankful to Prof. David L. Smith, Department of Medicinal Chemistry and Pharmacognosy, West Lafayette, Indiana 47907, U.S.A. for performing negative FABMS on these compounds.

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