The extract obtained from the fourth band (lowest band of first prep. TLC) was treated over a neutral alumina column which was eluted with petrol, C_6H_6, $CHCl_3$. Elution with $CHCl_3$–MeOH (1:1) produced a residue which on recrystallization from MeOH yielded 95 mg of chlorogenin, mp 264–268°, identified by comparison with an authentic sample (TLC, mmp, IR, ^{13}C NMR).

Hydrolysis of compound **1a**. A soln of **1a** (50 mg) in MeOH was treated under reflux for 1 hr with NaOH. Upon cooling H_2O was added and the product extracted with $CHCl_3$. Crystallization from $CHCl_3$–MeOH yielded 38 mg of **1b**, mp 202–204°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1710 (CO), 900>920. ^1H NMR ($CDCl_3$): δ0.75 (6H, *s*, H-18 and H-19), 0.78 (3H, *d*, $J=7$ Hz, H-27), 0.94 (3H, *d*, $J=7$ Hz, H-21), 3.35 (1H, *m*, 16-H), 3.40 (1H, *s*, OH), 4.35 (1H, *m*, 3-H). ^{13}C NMR (see Table 1).

(25R)-5α-*Spirostane-3,6-dione (chlorogenone) from compound* **1b**. Kiliani's reagent was added drop by drop to a soln of 30 mg of **1b** in Me_2CO. After 30 min at room temp the reaction was complete and a few drops of MeOH were added to destroy excess reagent. The soln was diluted with H_2O and shaken with $CHCl_3$. The organic layer was washed with H_2O and taken to dryness. The product (28 mg) crystallized from Me_2CO, mp 233–236°. It was found to be identical to an authentic sample of chlorogenone (TLC, mmp, IR ^{13}C NMR).

Catalytic reduction of chlorogenone. Chlorogenone (100 mg) obtained from diosgenin as described by Marker *et al.* [7] dissolved in 50 ml of EtOH was mixed with 50 mg of pre-reduced PtO_2 and shaken for 1 hr in a Parr hydrogenator at 50 PSIG of H_2. The reaction product showed two spots (R_f, 0.40 and 0.15) on TLC (C_6H_6–EtOAc, 1:1). CC over silica gel with C_6H_6–EtOAc (20:1) eluted the less polar substance (R_f, 0.40, 64 mg), mp 202–204°, which was found to be identical to **1b** by TLC, mmp, IR and ^{13}C NMR.

Acknowledgement—The authors thank Mrs Emilia Benites de Rojas (Universidad Central, Maracay) for the botanical identification of the plant.

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Phytochemistry, Vol. 28, No. 9, pp. 2511–2513, 1989.
Printed in Great Britain.

0031–9422/89 $3.00+0.00

CLEMONTANOSIDE-A, A BISGLYCOSIDE FROM *CLEMATIS MONTANA*

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(*Received in revised form* 10 *February* 1989)

Key Word Index—*Clematis montana*; Ranunculaceae; saponins; triterpenic bisglycosides.

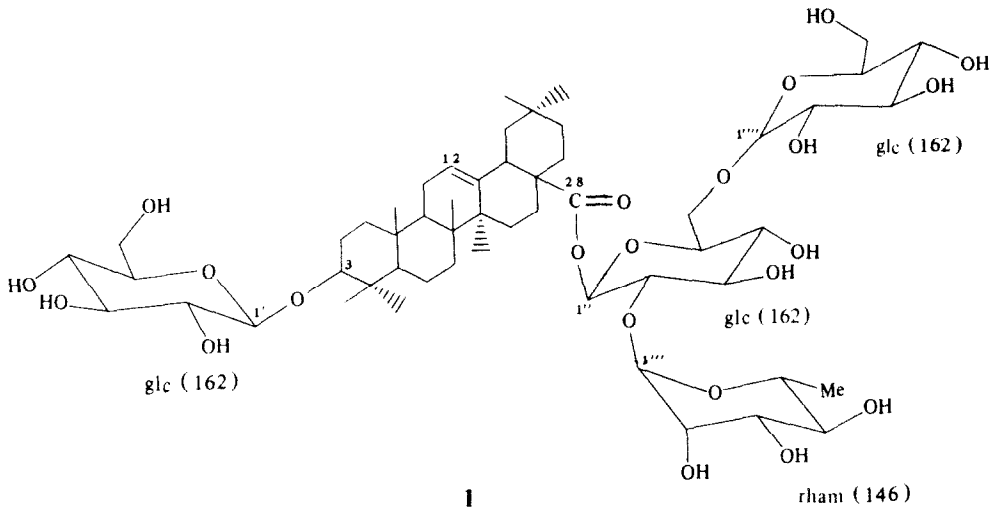
Abstract—Clemontanoside-A, a new triterpenic bisglycoside was isolated from the methanolic extract of the leaves of *Clematis montana* and its structure established.

INTRODUCTION

Clematis montana Buch.-Hem. (Ranunculaceae), a woody climber is distributed in temperate Himalaya up to an altitude of 4000 m [1]. The leaves of this plant are used in skin diseases and the seeds have purging properties [1, 2]. A survey of the literature revealed that no phytochemical work has been reported on this plant. This prompted us to investigate this plant for saponins. This paper describes the isolation and characterization of a new oleanolic acid based bisglycoside from the methanolic extract of the leaves of *Clematis montana*.

RESULTS AND DISCUSSION

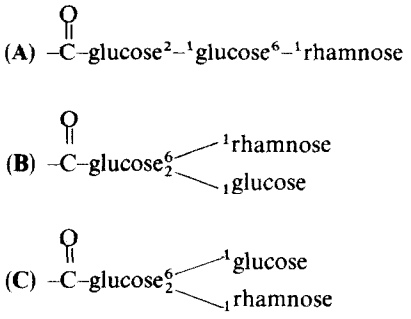
Column chromatography of the saponin mixture of the leaves of *Clematis montana* gave compound **1** (positive to the characteristic tests for triterpenic saponin). It also gave sugars (glucose and rhamnose (co-PC) on complete hydrolysis with 7% alcoholic sulphuric acid. Thus, the compound was anticipated to be a triterpenic saponin, which was later confirmed by spectral (^1H NMR, ^{13}C NMR, EIMS and FABMS) and hydrolytic studies. The ^1H NMR spectrum of **1** showed the presence of seven tertiary methyls (δ0.88, 0.96, 1.07 and 1.27), one second-



1

ary methyl (1.78, *d*, $J=6.2$ Hz), an olefinic proton (5.44), and four anomeric protons (4.92, *d*, $J=7.7$ Hz; 4.97, *d*, $J=7.7$ Hz; 6.09 *d*, $J=8.1$ Hz and 6.57, *s*). The EIMS of this compound exhibited intense peaks at *m/z* 456 ($C_{30}H_{48}O_3$), 248 and 203, thereby indicating that **1** could be a glycoside of oleanolic acid or ursolic acid [3]. The ^{13}C NMR spectrum exhibited the presence of 54 carbons, among which the peaks at δ122.6 (*d*) and 144.3 (*s*) suggested that the aglycone could be oleanolic acid [4]. Fast atom bombardment mass spectrometry exhibited a molecular peak at *m/z* 1112 $[M+Na+H]^+$, thereby indicating the molecular weight to be 1088 ($C_{54}H_{88}O_{22}$). Thus on the basis of hydrolytic and spectral data, **1** was an oleanolic acid tetraglycoside, the sugar moieties of which consist of one rhamnose (δ1.78 due to secondary methyl) and three glucoses. Alkaline hydrolysis (10% KOH) of **1** gave a product whose ^1H NMR spectrum revealed the presence of one anomeric proton at δ4.14 (*d*, $J=7.8$ Hz) but no secondary methyl protons were observed (rhamnose absent). Therefore the structure of the product was assumed to be 3-*O*-β-D-glucopyranosyl-oleanolic acid. Compound **1** was partially hydrolysed (1% MeOH–H_2SO_4) to lucyoside (3-*O*-β-D-glucopyranosyl-28-*O*-β-D-glucopyranoside of oleanolic acid) [5]. The ^{13}C NMR of **1** was very similar to that of lucyoside [6]. However, in the ^{13}C NMR spectrum of **1**, some additional signals assignable to sugar moieties attached to the C-28 carboxyl group were also observed. By substracting the chemical shifts due to the oleanolic acid substituted at C-3 by a glucose (δ106.9, 75.8, 78.8, 72.2, 78.2 and 63.1 corresponding to C-1′, C-2′, C-3′, C-4′, C-5′ and C-6′, respectively), the remaining 18 peaks were attributed to three sugars; two glucoses and a rhamnose. The signals at δ94.8 (*d*), 101.3 (*d*) and 105.3 could be assigned to anomeric carbons of a glucose esterified with the C-28 carboxyl of oleanolic acid, rhamnose and an esterified glucose respectively [5]. The signal at δ69.5 (*t*) indicated that one of the C-6 hydroxy groups of the glucose was etherified [7].

From the above argument and referring to the ^{13}C NMR chemical shifts of methyl glycosides [8], the three possible structures were suggested for **1** as shown below:



The possibility of structure **A** has been ruled out on the basis of FABMS (Fig. 1) and ^{13}C NMR spectral studies. The appearance of peaks at *m/z* 1112 $[M+Na+H]^+$, 1089 $[M+H]^+$, 1111 $[M+Na]^+$, 927 $[M+H-162]^+$, 943 $[M+H-146]^+$ and 642 $[M+Na+H-2\times162-146]^+$ in the FABMS of **1** confirmed that either **B** or **C** may be the possible structure of **1**.

Finally the structure **C** has been assigned to **1** on the basis of glycosidation shifts of the ^{13}C NMR signals (Table 1). In ^{13}C NMR spectrum of **1** the anomeric carbon of rhamnose was seen at δ101.3 and that of third glucose was at δ105.3. By calculating glycosidation shifts, it can be assumed that glucose should be the central sugar attached to the C-28 group of the aglycone with other sugars attached to the central sugar at the C-2″ and C-6″ positions, respectively. The anomeric carbon resonance of the rhamnose (δ101.3) seemed to be shifted upfield as compared to that of methylrhamnoside (δ102.4). These changes were caused by linkage of C-1‴ of the rhamnose to C-2″ of the central sugar and C-1⁗ of the glucose to C-6″ of the central sugar [9–11]. So **C** should be the final structure of **1**, a new bisglycoside, 3-*O*-β-D-glucopyranosyl-oleanolic acid-28-*O*-β-D-glucopyranosyl (1→6)-[α-L-rhamnopyranosyl (1→2)]-β-D-glucopyranoside, which we have named clemontanoside-A.

EXPERIMENTAL

The plant material was collected from Bhainsgaon (Chamoli Garhwal) U.P. India. The authentication of plant material was

Table 1. ^{13}C NMR chemical shifts (δppm) of clemontanoside (1)

C	1	C	1
1	38.9	g-1′	106.9 (+1.5)
2	26.6	g-2′	75.8 (+1.0)
3	88.9	g-3′	78.8
4	39.5	g-4′	72.2
5	56.0	g-5′	78.2
6	18.6	g-6′	63.1
7	32.3	g-1″	94.8 (−10.6)
8	40.0	g-2″	79.6 (+4.8)
9	48.1	g-3″	75.2
10	37.1	g-4″	71.3
11	23.5	g-5″	78.3
12	112.6	g-6″	69.5 (+7.0)
13	144.3	r-1‴	101.3 (−1.1)
14	42.4	r-2‴	71.9
15	28.8	r-3‴	72.6
16	23.8	r-4‴	73.9
17	47.3	r-5‴	69.7
18	42.0	r-6‴	18.7
19	46.5	g-1⁗	105.3
20	30.7	g-2⁗	75.2 (+0.40)
21	34.2	g-3⁗	78.4
22	33.3	g-4⁗	71.9
23	28.3	g-5⁗	77.7
24	17.0	g-6⁗	62.7
25	15.7		
26	17.5		
27	25.9		
28	176.5		
29	33.5		
30	23.8		

g, glucose; r, rhamnose; (+) downfield and (−) upfield.

made at the Botany Department, Garhwal University, Srinagar, U.P. A voucher specimen is available at the herbarium of the Botany Department. Mps: uncorr; ^{1}H NMR: 400 MHz and ^{13}C NMR: 100.533 MHz in pyridine-d_5 with TMS as int. standard. FABMS: with positive mode at an accelerating voltage 2.5 kV, gas: Xe. CC: silica gel (BDH, 60–120 mesh). TLC: Kieselgel 60G (Merck). The spots on TLC were visualized by spraying with 10% alcoholic H_2SO_4 followed by heating. PC: Whatman No. 1 paper using the descending mode and aniline hydrogen phthalate as the developer. The following solvent systems were used: (A) $CHCl_3$–MeOH (4:1); (B) $CHCl_3$–MeOH–H_2O (65:34:10); (C) C_6H_6–Me_2CO (4:1); (D) EtOAc–pyridine–H_2O (10:4:3) and (E) *n*-BuOH–EtOH–H_2O (5:1:4).

Isolation of saponin. The air-dried and powdered leaves (2 kg) were defatted with petrol in a Soxhlet. The solvent free leaves were exhaustively extracted with 90% MeOH until the extract become colourless. The concentrated mass was shaken with $CHCl_3$ and filtered. The residue was taken up in H_2O and extracted with *n*-BuOH (4 × 300 ml). The *n*-BuOH extracts after concentration under red. pres. yielded a saponin mixture (5 g) which was chromatographed to afford colourless crystals of **1** (200 mg), mp 230–232° (dec), $[\alpha]_D^{25}$ −100° ($CHCl_3$–MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 1990, ^{1}H NMR (pyridine-d_5): methyl protons: δ0.88 (9H, *s*), 0.96, 1.07, 1.27 (each 3H, *s*), rham-methyl protons: 1.78 (3H, *d*, J = 6.2 Hz), H-3: 3.36 (1H, *dd*, J = 4.2 and 11.5 Hz), H-12: 5.44 (1H, *br s*), H-18: 3.14 (1H, *br d*, J = 9.5 Hz), anomeric protons: 4.92 (1H, *d*, J = 7.7 Hz, C-1′-H of glucose), 6.09 (1H, *d*, J = 8.1 Hz, C-1″-H of glucose), 6.57 (1H, *br s*, C-1‴-H of rhamnose), 4.97 (1H, *d*, J = 7.7 Hz, C-1⁗-H of glucose); ^{13}C NMR (*cf* Table 1); FABMS (Fig. 1). Found C, 59.00; H, 8.10 required C, 59.56; H, 8.08%.

Acid hydrolysis of compound **1**. Clemontanoside (**1**) (25 mg) was hydrolysed with 7% H_2SO_4 in MeOH at 100° for 4 hr to afford a colourless compound identified as oleanolic acid 28-*O*-*β*-D-glucopyranoside [10]. The filterate from the hydrolysate was neutralized with Ag_2CO_3 and filtered. The filterate was concd under red. pres. and the residue tested for the presence of D-glucose and L-rhamnose (PC, solvent system *D*, R_f values 0.23 and 0.42, respectively.

Partial hydrolysis of compound **1**. Compound **1** (20 mg) was hydrolysed with 1% MeOH–H_2SO_4 to afford another compound identified as lucyoside [5].

Alkaline hydrolysis of **1**. Clemontanoside (**1**) (50 mg) was refluxed for 3 hr with 10% KOH in MeOH (15 ml). The reaction mixture was poured into H_2O and extracted with *n*-BuOH. The *n*-BuOH layer was purified by repeated PLC on silica gel, (eluent: $CHCl_3$–MeOH–H_2O = 13:7:2, lower layer) to give a product (6 mg) identified as 3-*O*-*β*-D-glucopyranosyl-oleanolic acid [10].

Acknowledgements—Our thanks are due to Prof. H. Furukawa (Meijo University) and Dr. Y. Hotta (Aichi Medical University) for measurement of NMR and FABMS. Financial assistance provided by CSIR is also gratefully acknowledged by the authors (RP and JS).

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