J. Mountain Res. Vol 2 pp.93-96, 2007

ISOLATION OF TRITERPENES FROM THE ROOTS OF *VIBURNUM MULLAHA*

P.P. Badoni[1] and S.C. Sati[2]

[1]Department of Chemistry H.N.B.Garhwal University, Campus, Pauri Garhwal

[2] Department of Chemistry H.N.B.Garhwal University, Campus,Srinagar, Garhwal

ABSTRACT

Oleanene type tri terpenes have been isolated from ethanolic extract of roots of *Viburnum mullaha* along with Oleanolic acid and their structures were elucidated as 6á hydroxy oleanolic acid and 2á,3 á, 19 á tri-hydroxy olean-12-ene-28 oic acid 28-O-α-D-glucopyranosyl (1-6)- α-D-glucopyranosyl with the help of chemical and spectral data.

Key words: Triterpenes, Roots, *Viburnum mullaha.*

INTRODUCTION

Viburnum mullaha, belonging to family *Caprifoliaceae,* is a large deciduous shrub, with lanceolate leaves. *Viburnum* species are used in folk medicine as uterotonic, chemostatic, sedative and diuretic drug. The aerial parts of *V. mullaha* showed anti protozonal activity and the seed oil used as a relief for burning [1].The alcoholic extract of the plant showed hypothermic and cardiovascular activity. Terpenoids, Iridoids, phenolic and Saponins are the main secondary metabolites of this genus.

MATERIAL AND METHODS

Collection of plant Materials

The roots of *V.mullaha* were collected from forests of Khirsu ,Pauri ,Garhwal Uttarakhand in the month of August. The identity of plant was confirmed from Ethano Botanical Laboratory, Department of Botany, H.N.B. Garhwal University Campus, Srinager Garhwal and the voucher specimen is available in Plant Identification Laboratory.

Extraction and Isolation

The air dried roots were finely powdered and defatted with light petroleum in soxhlet. The defatted mass was exhaustively extracted with 90% ethanol repeatedly until the extractive became colourless. All the extracts were mixed and concentrated under reduced pressure using rotatory vacuum evaporator.

The concentrated extract was adsorbed on silica gel and fractionated through column chromatography using chloroform-methanol (95:5) as eluent. The polarity of solvent was gradually increased by addition of methanol. The repeated CC afforded compound 1 and 2 along with known compound oleanolic acid (by direct comparison with authentic sample, mmp and co-TLC). Melting points were uncorrected. UV-spectra were taken in MeOH using TMS as internal standard and $CDCl_3$ and CD_3OD.

RESULTS AND DISCUSSION

The ethanolic extract of roots of *V. mullaha* on repeated CC over silica gel afforded compound 1 and 2 together with oleanolic acid.

Compound -1

M.P 237-240°C

Molecular Formula $C_{30}H_{48}O_4$

^{1}H-NMR(CD_3OD) δppm

0.80.0.85,0.87.0.89.0.96.1.01,1.12(3H,each s,Me),3.40(t,J=2.6Hz,H-3),3.60(dd ,J=10.0 and 4.4 Hz),5.24(t,J=3.6Hz,H-12)

^{13}C-NMR (CD_3OD) δppm

38.1 (C-1),29.3(C-2),76.1(C-3),38.4(C-4),56.7(C-5),69.5(C-6),36.2(C-7),40.0(C-8),46.8(C-9),35.7(C-10),24.4(C-11),126.2(C-12),141.5(C-13),42.8(C-14),29.2(C-15),25.4(C-16),48.3(C-17),54.4(C-18),34.2(C-19),39.2(C-20),31.8(C-21),36.5(C-22),29.3(C-23),21.6(C-24),17.6(C-25),17.5(C-26),24.7(C-27),182.0(C-28),33.5(C-29),24.1(C-30)

Compound 1 gave positive LB reagent[2] and $CeSO_4$ test for triterpenes.The IR spectrum showed the absorptions for an hydroxyl group at 4360 and 3600cm^{-1}, a carbonyl group at 3300 ,1700cm^{-1} and a tri substituted double bond at 1660 and 820 cm^{-1}. The ^{1}H-NMR spectrum exhibit signals for seven methyl groups at δ0 .80, 0.85,0.87, 0.89,0.96,1.01and 1.12.The signal at δ5.24(!H,J=3.6Hz)was due to an olefinic proton while the protons geminal to the hydroxyl groups were observed at δ3.40(t,J=2.6Hz) and 3.60(dd,J=10.0 and 4.4Hz).The ^{13}C-NMR spectrum showed the presence of thirty carbon atoms.

In the mass spectrum of compound the base peak arises at m/z 248 ,other peaks arises at m/z 223,202 and 132 were characteristic of amyrin skeleton.The presence of tertiary methyl groups indicated the olean-12-ene skeleton[].The carbinylic proton at ä3.60 showed connectivity to three protons limiting its presence to C-2 or C-6.Since it do not consume glycol splitting reagents,the hydroxyl group must be at C-6.The á and equatorial orientation could be Assigned to it on the basis of coupling constant and chemical shifts of methyl groups at C-24,C-25 and C-26 which did not show the downfield shift due to â hydroxyl group.Thus it was elucidated as 6 á hydroxy oleanolic acid

Compound 2

M.P. 278-279°C

Molecular Formula C42H68O15

^{1}H-NMR($CDCl_3$,δppm)

0.74,0.80,0.86,0.91,1.0,1.1,1.18(3H,each s,Me),6.30(1H,d,J=8.1Hz,C-1H of glucose) 5.02(1H,d,J=7.7Hz,C-1H of glucose) 4.25,3.72,3.51(each 1H,brs ,oxymethine)

^{13}C-NMR($CDCl_3$,δppm)

Carbons of aglycone

42.8(C-1), 66.3(C-2),79.6 (C-3),39.0 (C-4),49.0(C-5),18.8(C-6),33.2(C-7),40.6(C-8),48.4(C-9),39.0(C-10),24.4.(C-11),122.8(C-12),144.5(C-13),42.3(C-14),29.0(C-15),28.1(C-16),46.6(C-17),44.6(C-18),81.2(C-19),35.7(C-20),29.1(C-21),33.3(C-22),29.6(C-23),22.4(C-24),16.8(C-25),17.9(C-26),25.0(C-27),177.5(C-28),28.9(C-29)

Carbons of glycone

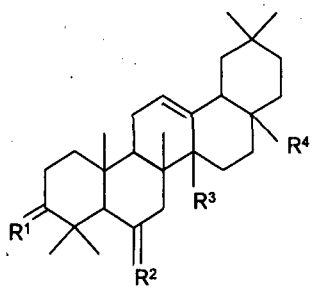
Glucose - 95.9(C-1'),74.0(C-2'),78.5(C-3'),71.0(C-4'),78.9(C-5'),69.5(C-6')

Glucose - 105.5(C-1"),75.1(C-2"),78.6(C-3"),71.6(C-4"),78.1(C-5"),62.8(C-6")

Compound 2 was isolated as amorphous solids and assigned the molecular formula $C_{42}H_{68}O_{15}$ as determined from mass spectra. Compound 2 exhibit the IR absorption of

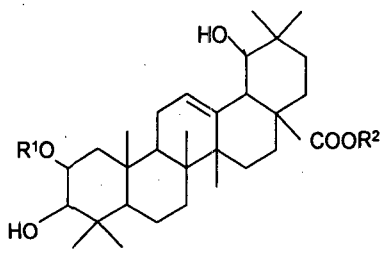
hydroxyl and ester function. On treatment with alkali it afford the aglycone. Acid hydrolysis of 2 afford glucose (by direct comparison with suga Profust and posfust Reliability of a network systemr). The ^{13}C-NMR Spectrum of compound showed 2 olefinic carbon signals at ä122.8, 144.5 which were in good agreement with those of C-12 and C-13 of olean-12-ene derivatives [3]. The chemical shift of one anomeric carbon atom at ä95.9 confirm the presence of an ester linked sugar unit. The ^{1}H and ^{13}C-NMR spectrum of compound displayed in addition to the tri-substituted double bond and seven tertiary methyl groups, characteristics of r^{12} oleanene skeleton. The signal of anomeric protons at δ6.30(d,J=8.1Hz)showed that glucose had â configuration. The exact interglycosidic points of linkages were confirmed by 13C-NMR studies. 13C-NMR chemical shift values of oleanolic acid and methyl pyranosides of â-D glucose are reported[4]

Thus on the basis of these observation compounds 2 was identified as 2á,3 á, 19 á tri-hydroxy olean-12-ene-28 oic acid 28-O-β-D-glucopyranosyl (1-6)- B-D-glucopyranosyl.



Compound -1

R^{1}-Alpha OH,Beta H
R^{2}-Alpha OH,Beta H
R^{3}-Me
R^{4}-COOH



Compound -2

R^{1}-H
R^{2}-Glu(1-6)Glu

REFERENCES

Gaur, R.D., 1999,"*Flora of District Garhwal*" Trans media, Srinagar, Garhwal, 547-548.

Liebermann, C.,1983; *Ber., Deusch. Chem. Gas.*, 18, 1804

Mahato, S.B., Kundu, A. P., 1994, *^{13}C NMR spectra of pentacyclic triterpenoids*, 37, 1517.

Kimata, H., Nakashi, T., Kokubun, S., Makayama, K., 1993, *Chem. Pharm. Bull.* 31, 1998