Int. J. Chem. Sci.: 10(3), 2012, 1271-1276
ISSN 0972-768X
www.sadgurupublications.com

OLEAN–12–ENE–16–β–OL–28–OIC–3–O–α–L RHAMNO–PYRANOSYL–(1→4)–O–β–D–GALACTOPYRANOSIDE FROM THE SEEDS OF *GRANGEA MADERASPATANA POIR*

SHAILENDRA BADAL*

Hindustan College of Science and Technology, Agra- Dehli Highway NH-2, Farah,
MATHURA – 281122 (U.P.) INDIA

ABSTRACT

The menthol-extract of the seeds of the plant *Grangea maderaspatana poir*, when worked up yielded a saponin which was identified as; Olean–12–ene–16–β–ol–28–oic–3–O–α–L–rhamnopyranosyl–(1→4) –O–β–D–galactopyranoside by chemical degradations and spectral analysis.

Key words: The saponin olean-12–ene–16–β–ol–28–oic–3–O–α–L–rhamnopyranosyl–(1 → 4) –O–β–D–galactopyranoside, Seeds of *Grangea maderaspatana*, *Poir*, Compositae.

INTRODUCTION

The plant *Grangea maderaspatana*[1] belonging to natural order compositae, is commonly known as Mustarn in hindi. It is found in throughout India. In literature the leaves of this plant are reported to be useful for curing stomachic and deodotruent. Being antiseptic, it is prescribed in infusion and electuary in cases of obstructed menses and hysteria. Literature also reports it to be used in preparing antiseptic and anodyne fomentation.

EXPERIMENTAL

The seeds (1 Kg) of the plant *Grangea maderaspatana poir* natural order composition were air dried and powered and then extracted with 95% rectified spirit and thereafter the rectified spirit extract was concentrated to a brown viscous mass. It was extract was benzene, chloroform and methanol. The methanol extract was concentrated and excess of solvent ether was added in it when a precipitate appeared which was separated by decantation.

*Author for correspondence; E-mail: badal70@rediffmail.com

On TLC analysis the above precipitate showed two spots and so it was subjected to column chromatography when a compound was obtained which responded to positive test of saponin[2]. It analysed for molecular formula $C_{42}H_{68}O_{13}$ m.p. 188-189°C and M^+ = 780 (CIMS). On acid hydrolysis with 7% dilute HCl, it yielded a sapogenin B-2(A) m.f. $C_{30}H_{48}O_4$ m.p. 278-279°C $M^{\bullet+}$ = 472 and sugar moieties as D- galactose and L-rhamnose (R_f 0.19 and 0.38). B-2(A) gave all characteristic colour tests of terpenoids.

Permethylation of B-2

The saponin B-2 (50 mg) was treated with methyl Iodide (6 mL) and Ag_2O (30 mL) in DMF (6 mL) in a 150 mL conical flask and left for 4 days at room temperature. The contents were filtered and the residue was washed with DMF. The filtrate was concentrated under reduced pressure to get a viscous mass, which on hydrolysis with dilute HCl gave sapogenin B-2 (A) and methylated sugars. The sapogenin B-2 (A) was separated and the aqueous hydrolysate was neutralised with $BaCO_3$ and $BaSO_4$ was filtered off and filtrate was concentrated under reduced pressure. Sugars were examined by paper chromatography[3] using Whatmann filter paper no. 1, solvent system used was B : A : W (4 : 1 : 5) and aniline hydrogen phthalate as spraying reagent as 2,3,6-tri-O-methyl galactose and 2,3,4-tri-O-methyl-rhamnose.

Periodate oxidation of B-2

The saponin B-2 (20 mg) was suspended in H_2O (15 mL), and was mixed $NaIO_4$ (200 mg). The solution was kept in dark for 48 hrs. Ethylene glycol was added to decompose excess of $NaIO_4$ and the solution was hydrolysed with 10% MeOH-HCl (45 min.), then it was filtered and filtere was neutrilised. It did not show the presence of any mono saccharide in it.

Enzymatic hydroysis of saponins B-2

The saponin B-2 (30 mg) was dissolved in MeOH and mixed with alomond emulsion (30 mL) in a 100 mL conical flask fitted with a stopper. The contents were allowed to stand at room temperature for 48 hours and then filtered. The concentrated hydrolysate was examined by paper chromatography for sugar moieties using Whatmann filter paper no. 1 and B : A : W (4 : 1 : 5) as solvent system. The sugars were identified as D-galactose and L-rhamnose.

The methanolic solution of the saponin (30 mg) was mixed with an equal volume of Takadiastase solution in a conical flask. The contents were allowed to stand for 2 days at

room temperature and filtered. The hydrolysate on paper chromatographic examination was found to contain L- rhamnose and D-galactose.

RESULTS AND DISCUSSION

The saponin, molecular formula $C_{42}H_{68}O_{13}$, m.p. = 188-189°C and M^+ = 780 (CIMS), showed characteristic IR bands at ν_{max}^{KBr} 3378 cm^{-1} (-OH group), 2938 cm^{-1} (CH_3 str. Band), 1530 cm^{-1} (C-H str. Vib.), 1235 cm^{-1} (C-H bending of CH_3 gp.), 1640 cm^{-1} (C = C str.) and 1065 cm^{-1} (triterpene nucleus.).

Compound B-2 on acid hydroysis yielded a sapogenin B-2 (A) molecular formula $C_{30}H_{48}O_4$, m.p. 278-279°C M^+ = 472 (CIMS) and sugar moieties as D-galactose and L-rhamnose (Co-PC and Co-TLC with authentic sample) B-2 (A) gave all characteristic colour tests of terpenoids.

Presence of double bond in B-2 was indicated, because solution of saponin in CCl_4 produced yellow colour with tetranitro methane, this was further confirmed by a band at 1410 cm^{-1} in the IR spectrum of B-2.

The permethylation by Kuhn[4] procedure followed by acid hydrolysis of B-2 yielded an aglycone B-2 (A) methylated sugars which were identified as 2,3,4-tri-O-methyl rhamnose and 2,3,6-tri-O-methyl galactose (Co-Pc and Co TLC)[5] showing the presence of D- galactose in pyranose form and also that C_4 of galactose was linked to C_1. OH group of rhamnose.

The enzymatic hydrolysis of the glycoside B-2 with Takadiastase gave prosapogenin and L-rhamnose by CoPc showing α- Linkage between the prosapogenin and L-rhamnose.

B-2 (A) was subsequently hydrolysis with almond emulsion when it yielded the aglycone B-2 (A) and D-galactose, B-2 on hydrolysis with Kiliani mixture liberated L-rhamnose first followed by D-galactose which suggested that L-rhamnose was attached in terminal position and D-galactose was attached to B-2 (A) aglaycone.

The fact that the aglycone B-2 (A) and sugars L-rhamnose and D-galactose were present in equimolar ratio was confirmed by sodium metaperiodate oxidation of B-2 which consumed 2.81 moles of periodate and liberated 1.03 moles of formic acid. By this it was also confirmed that both sugars were present in pyransose form in B-2.

Examination of IR spectrum of B-2 (A) showed a band at 3318 cm^{-1} indicating the presence of –OH groups (s) in it. B-2 (A) was found to form diacetyl derivative with m.f. $C_{34}H_{52}O_5$, [M^+] 498, m.p.- 200-201^oC. The estimation of acetyl group (210.0%) by Wiesenberger[6] method as described by the Belcher and Godbert[7] indicated the presence of two –OH group in B-2.

On Cr_2O_3/ pyridine oxidation[8,9], B-2 yielded a di-ketone m.f. $C_{30}H_{44}O_3$, [M^+] 452 and m.p. 215-217^oC. It gave positive Zimmerman test for Keto group and confirmed the presence of two –OH groups, one at C-3 and other at C-16 both of secondary in nature B-2.

Peaks in the (CIMS) spectrum at M^+ = 772, 626 and 465 indicated that it was C-3 (OH) group which was involved in glycosylation.

Further the characteristic band at ν_{max}^{KBr} 1625 and 1265 cm^{-1} in the IR spectrum of sapogenin B-2(A) indicated the presence of double bond in it. The sapogenin indicated the presence of double bond in B-2(A).

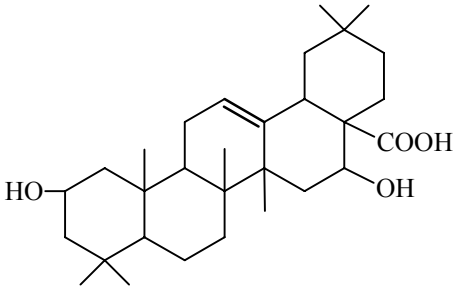
The sapogenin B-2(A) showed high terminal UV absorption, characteristic of C-12 and C-13 double bond in the triterpens of oleanane series.

The ^1H NMR spectrum of B-2 showed ethylene proton at 5.88 and 5.41 (d, J = 10 Hz), which confirmed double bond at C-12 and C-13 in the triterpene B-2(A).

The IR spectrum of B-(A) showed band at 2922 and 1453 cm^{-1} for angular methyl groups, which when estimated by Zeisels method (16.40 %) confirmed the presence of seven methyl groups in it. The chemical shifts in ^1H NMR spectrum of B-2 (A) gave singlet at 1.04, 0.95, 0.92, 0.80, 1.15, 0.70 and 0.88 showing the presence angular methyl groups at C-28, C-24, C-25, C-26, C-27, C-29, C-30 in B-2 (A).

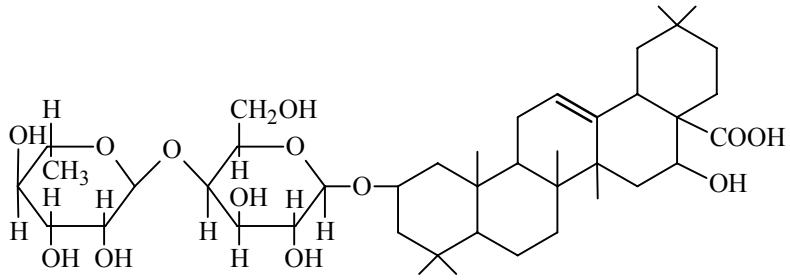
The IR spectrum of B-2 (A) displayed characteristic bands at 1740 cm^{-1} indicated the presence of –COOH groups, which was further confirmed by the fact that B-2 (A) gave effervescence with sodiums bicarbonate solution. Sapogenin B-2 (A) on treatment with CH_2N_2/AcOH yielded mono methyl ester of B-2 (A) there by indicating the presence of only one- COOH group in it.

Comparison of its properties identified it is Olean-12-ene-3α-16 β-diol-28-oic acids.



The monomethyl ester B-2 analysed for molecular formula $C_{31}H_{50}O_4$, m.p. 224-225°C and $[M^+] = 486$ (FABMS)

Various evidences describes above on compilation indicated that the structure of B-2 was Olean-12-ene-16-β-ol-28-oic-3-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-Galactopyranoside.



REFERENCES

1. R. N. Chopra, S. C. Nayar and I. C. Chopra, Glossary of Indian Medicine Plants,"CSIR Publication, New Dehli (1965) pp. 18.
2. C. Libermann, Ber. Deustch Chem. Soc. Ges., 1804 (1885).
3. E. Leaderer and M. Leaderer, Chromatography Elsevior Publication, New York (1957).
4. R. Kuhn, H. Trischmann and J. Low, J. Angew Chem., **67**, 32 (1995).
5. Egon Stahl, Thin Layer Chromatography, Academic Press Inc., Publisher, New York (London) (1965).
6. E. Weisenberger, Microchemie Ver Microchim Acta, 33-51 (1947).

7. R. Belcher and A. L. Godber, Semi-Micro Quantitative Organic Chemistry Longman Gree and Co., New York (1954).

8. R. M. Horowitz and L. Jurd, J. Org. Chem., **22**, 1618 (1957).

9. R. W. Doskotch and E. L. Nagger, N. Nat. Products, **42(1)**, 126 (1979).

Revised : 20.02.2012 *Accepted : 21.02.2012*