



## OLEANDRIGENIN-3-O- $\alpha$ -L RHAMNOPYRANOSIDE FROM THE SEEDS OF *OPHIORRHIZA MUNGOS* (LINN)

ALOK SAHAI\*, SUDHANSHU DWIVEDI<sup>a</sup> and NIDHI SAXENA

Chemistry Department, Government Girl's Post Graduate College of Excellence, SAGAR (M.P.) INDIA

<sup>a</sup>Chemistry Department, M.P. Bhoj Open University, Kolar Road, BHOPAL (M.P.) INDIA

### ABSTRACT

*Ophiorrhiza mungos* Linn. (No. Rubiaceae) which is generally known as Sarhati is indigenous to India and is reported to be associated with important medicinal properties. The ethyl acetate soluble fraction of the concentrated ethanolic extract of defatted seed of this plant was subjected to chromatographic analysis, when it yielded a compound, molecular formula  $C_{31}H_{46}O$ , m.p. 176-177°C,  $M^+ = 578$ , which gave positive test of cardenolide. Various characteristic reactions, chemical degradations and UV, IR <sup>1</sup>H NMR and mass spectroscopic analysis led to its identification as Oleandrigenin-3-O- $\alpha$ -L rhamnopyranoside.

**Key words:** Cardenolide, Oleandrigenin-3-O- $\alpha$ -L rhamnopyranoside *Ophiorrhiza mungos* (Linn).

### INTRODUCTION

The plant *Ophiorrhiza mungos* (Linn)<sup>1</sup> is known as Sarhati in Hindi and belongs to natural order Rubiaceae. It is distributed in the hills of Travancore and Andamans. Its root is bitter, tonic and it is reported to be a good remedy against snake bite and mad dog bites.

The ethyl acetate soluble fraction of concentrated ethanolic extract of defatted seeds of the plant *Ophiorrhiza mungos* (Linn) was concentrated under reduced pressure to get an yellow viscous mass, which was subjected to TLC and chromatographic analysis when it yielded a cardenolide, molecular formula  $C_{31}H_{46}O$ , m.p. 176-177°C,  $M^+ = 578$ . Various reactions and spectral analysis identified it as Oleandrigenin-3-O- $\alpha$ -L rhamnopyranoside.

### EXPERIMENTAL

The air dried, powdered and defatted seeds (1.8 Kg) of *Ophiorrhiza mungos* (Linn), were extracted with rectified spirit. The rectified spirit extract was concentrated under

---

\* Author for correspondence; E-mail: contacthasu@yahoo.co.in; Ph.: (O) 0285262358, Mo.: 09327770619

reduced pressure to get a light brown coloured viscous mass. The viscous mass was subjected to extraction with benzene, chloroform, acetone, ethyl acetate and methanol, respectively. The ethyl acetate soluble fraction was then concentrated to remove solvent to get yellow coloured mass, which was examined by TLC using butanol : acetic acid : water (4 : 1 : 5) as solvent system and vapours of iodine as visualizing agent, when it showed two spots. As such it was subjected to column chromatography with  $\text{CHCl}_3$  : EtOAc mixture in varying proportion as eluants. The fraction eluted with  $\text{CHCl}_3$  : EtOAc were in the ratio of 5 : 2, 5 : 3 and 5 : 4, respectively.

Eluates from  $\text{CHCl}_3$  : EtOAc (5 : 3) were collected and were found to have same  $R_f$  values and so combined together. On removal of the solvent, a yellow compound was obtained which was crystallized from methanol to give yellow crystals of compound NS-3 (0.068%). The compound NS-3 was found to be homogeneous on TLC examination using chloroform : acetone : water (7 : 4 : 3) as solvent system and vapours of iodine as visualizing agent.

The compound NS-3 was found to be soluble in chloroform, ethyl acetate, methanol solvent, ether and ethanol. It analysed for molecular formula  $\text{C}_{31}\text{H}_{46}\text{O}_{10}$ , m.p. 176-177°C and  $[M^+] = 578$  (FABMS). It responded positively to all the characteristic colour reaction of cardenolides<sup>2,3</sup>.

The IR spectrum of NS-3 displayed adsorption bands at  $\nu_{\text{max}}^{\text{KBr}}$  3488 (-OH), 2927 (C-H str.), 1795, 1736 ( $\alpha$ ,  $\beta$  unsaturated lactone), 1664 (C=C str.) 1460 (C-H bending) and 1076 (C-O str.). In  $^1\text{H}$  NMR signals were at  $\delta$  2.55 (d, j-3.1H, 1H, H-15),  $\delta$  5.46 (t, 1H, H-16),  $\delta$  2.81 (m, 1H, H-17),  $\delta$  4.86 (s, 2H, H-21),  $\delta$  5.81 (s, 1H, H-22),  $\delta$  5.55 (d, j-8.0 Hz, 1H, H-1')  $\delta$  1.34 (complex signal, 3H, -CH<sub>3</sub> rhamnose), and  $\delta$  2.42, (m, 4H, rhamnose protons).

### **The acid hydrolysis of the cardenolide NS-3**

300 mg of the cardenolide NS-3 was treated with 8 %  $\text{H}_2\text{SO}_4$  (75 mL) and refluxed in a 250 mL flask on water bath for 10 hrs. The content of the flask were cooled and then subsequently extracted with solvent ether. The aqueous layer which was separated and examined for the identification of the sugar, while the ethereal layer was washed with water and evaporated to dryness to get the cardiogenin NS-3 (A). The cardiogenin NS-3 (A), which analysed for m.f.  $\text{C}_{25}\text{H}_{36}\text{O}_6$ , m.p. 223-224°C and  $[M^+] = 432$  (FABMS), NS-3 (A), responded positively to all characteristic colour reaction of cardiogenin<sup>2,3</sup> and did not responded to the positive Molish test for glycoside. It was identified as Oleandrigenin by spectral data<sup>4,5</sup>, chemical degradations, along with superimposeable spectral analysis.

The aqueous hydrolysate thus obtained after acid hydrolysis of the cardenolide NS-3, was neutralized with BaCO<sub>3</sub> and BaSO<sub>4</sub> was filtered off. The filtrate was concentrated and subjected to paper chromatographic examination. The sugar present was identified as L-rhamnose. (R<sub>f</sub> - 0.36) and confirmed by CoPC and Co-TLC with authentic sample of L-rhamnose<sup>6</sup>.

### **The Position of attachment of sugar to the cardiogenin NS-3(A)**

The position of attachment of rhamnose to cardiogenin NS-3 (A) was confirmed to be at C-3-OH, because the cardiogenin responded positive to Zimmerman test<sup>7</sup>, whereas the cardenolide NS-3 did not responded to this test confirming attachment of rhamnose at position C-3 in the cardenolide NS-3.

### **The Permethylation and hydrolysis of cardenolide (NS-3)**

The cardenolide NS-3 (60 mg), 5 mg of methyl iodide and 25 mg of silver oxide in 6 mL of dimethyl formamide were refluxed on a water bath for 30 hrs at room temperature and thereafter worked up when the cardiogenin and methylated sugar were obtained, the methylated sugar was identified as 2, 3, 4-tri-O-methyl-L-rhamnose confirmed by (CoPC and CoTLC with authentic sample). This also confirmed that C1-OH of L-rhamnose was involved in glycosylation in the cardenolide NS-3.

### **The Periodate oxidation of the Cardenolide (NS-3)**

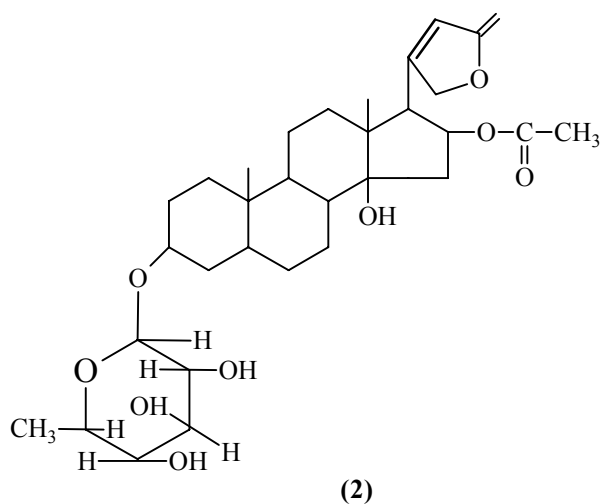
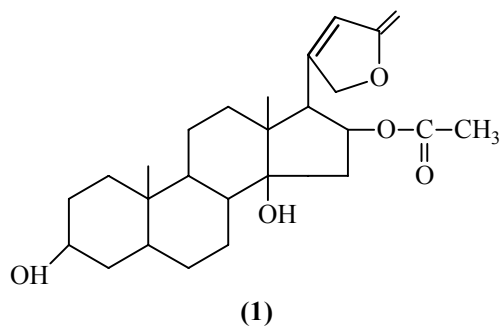
The cardenolide NS-3 (80 mg) was dissolved in 40 mL of methanol in a 250 mL conical flask, to which of 25 mg sodium meta periodate was added and the content of the flask were left for 45 hrs. A blank experiment was also run simultaneously following the same method. The amount of sodium meta periodate consumed and formic acid liberated were estimated by Jone's method<sup>8</sup> and it was found that the cardenolide NS-3 consumed 4.02 moles of sodium meta periodate and liberated 2.01 moles of formic acid, thus confirming that one molecule of sugar was attached to one molecule of cardiogenin and also confirmed that sugar was present in the pyranose form.

### **Enzymatic hydrolysis of cardenolide NS-3**

25 mg of the cardenolide NS-3 was dissolved in ethanol and treated with 30 mL of takadiastase. The contents were allowed to stand for five days at room temperature, when cardiogenin NS-3 (A) and L-rhamnose liberated out indicating  $\alpha$ -linkage between L-rhamnose and the cardiogenin.

## RESULT AND DISCUSSION

Based on above experimental facts along with critical examination of spectroscopic data, the cardiogenin and cardenolide were identified as; Oleandrigenin (1) and Oleandrigenin-3-O- $\alpha$ -L rhamnopyranoside (2) which were assigned as structure (1) and (2) respectively. Thus, the structure of the cardenolide NS-3 was established as; Oleandrigenin-3-O- $\alpha$ -L rhamnopyranoside (2).



## REFERENCES

1. R. N. Chopra, S. L. Nayer and I. C. Chopra, Glossary of Indian Medicinal Plants, CSIR, New Delhi (1968) pp. 181.
2. Cardiogenin C. Bose and A. Chakwaborty, Asian I. Chem., **14**, 1584-1586 (1988).
3. M. E. Deleca, A. M. Seldos, J. C. Obertic and E. G. Gross, Phytochemistry, G. M. Gabrera, **32** (1993) pp. 1253-1259.

4. F. Schimmain, An Introduction to Spectroscopy Method for the Identification of Organic Compounds Pergaman Press Oxford, Vol. I (1970).
5. M. Z. Robort, P. T. Frank and J. R. John, Application of Spectroscopy, **31(5)** (1984).
6. E. Lederer and M. Lederer, Chromatography 2<sup>nd</sup> Ed., Elsevier Publication, New York (1957).
7. D. H. R. Barton and P. D. Mayo, J. Chem. Soc., 1332 (1957).
8. A. Jones, I. A. Veliky and R. S. Ozubk, J. Nat. Products, **41**, 476 (1978).

*Revised : 19.12.2011*

*Accepted : 22.12.2011*