Int. J. Chem. Sci.: 8(3), 2010, 1669-1674

ISOLATION AND STUDY OF THE STEROIDAL; SAPONIN 24-α-ETHYL-20-ENE-7-HYDRO-STIGMAST-8β : 14β-Di-3-O-β-XYLOFURANOSIDE

ALOK SAHAI[a], SUDHANSHU DWIVEDI[a] and NIDHI SAXENA[*]

P.G. College of Excellence, SAGAR (M.P.) INDIA
[a]M. P. BOU, BHOPAL (M.P.) INDIA

ABSTRACT

A new steroidal saponin; 24-α-ethyl-20-ene-7-hydro-stigmast-8β : 14β-di-3-O-β-D-xylopyanoside; (NS-2) has been isolated from methanol soluble fraction of 95% ethanol extract of the roots of *Ophiorrhiza mungos* (Linn).

Key words: Steroidal, Isolation, *Ophiorrhiza mungos*.

INTRODUCTION

The plant *Ophiorrhiza mungos* (Linn)[1,2] is known as Sarahati in Hindi and belongs to natural order Rubiaceac. 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 log bites.

EXPERIMENTAL

About 5 kg of roots *Ophiorrhiza mungos* Linn were extracted with 95%, ethanol and the ethabol extract was concentrated under reduced pressure to get a brown viscous mass, which was successively extrated in turn with pet. ether, C_6H_6, $CHCl_3$, EtOAc, Me_2CO and MeOH.

Subsequently, the MeOH-soluble fraction was concentrated to a dark yellow viscous mass, which showed two spots on TLC examination [silica gel G plates, B. A. W. butanol : acetic acid: water and visualized by Kedde's reagent]. The fraction was subjected to column chromatography over silica gel and eluted with acetone : methanol (1 : 4). The eluents from

[*]Author for correspondence

9-17 were of the same R_f value and therefore, combined and removal of the solvent provided compound **NS-2** (2.16 g), white cream-coloured crystalline solid, m.p. 204°C, $C_{34}H_{58}O_6$; found (calcd.) % C = 72.48 (72.59), H = 10.31 (10.17), EI-MS $[M]^+$ 562; UV λ_{max} (MeOH) 217 and 294 nm; IR ν_{max}^{KBr} 3594, 3035, 2975, 2930, 1650, 1460, 1370, 1196, 1247, 1670, 968 and 884 cm^{-1}; ^1H NMR (60MHz, $CDCl_3$) of the diacetyl derivative of compound **NS-2**, 0.84 (1H, s, C_{19}-3H), 093 (3H, s, C_{18}-3H), 1.4-2.00 (polymethylene-CH_2 and –CH), 4.54 (1H, m, C_3-H), 4.71 (3H, s, C_{21}-3H), 2.16 (3H, s, C_3-OAc), 3.55 (1H, s, C_{14}-OH), 2.68 (1H, m, C_{17}-H), 5.55 (1H, dd, C_{21}-H vinylic proton), 4.27 (1H, d, 1'anomeric proton), 3.5-4.4 (9H, m, protons of sugar residue), 2.05 (3H, s, 2' –OAc) and 2.03 (3H, s, 3' –OAc); FABMS M/Z; 562 (1), 446 (21), 428 (24), 417 (20), 410 (32), 392 (42), 376 (46), 358 (33), 340 (58), 296 (65), 254 (71), 236 (59), 218 (78), 216 (82), 202 (89), 162 (91), 148 (87) and 134 (100).

Acid hydrolysis of NS-2

About 400 mg of **NS-2** was hydrolysed with 7.5 % of sulphuric acid (20 mL) by refluxing for 4 hrs on a water bath and then the acidic solution was cooled to give a solid white crystalline copound **NS-2 (G)**. The aqueous hydrolysate was neturalized with $BaCO_3$ and the resulting $BaSO_4$ was removed. The filtrate was concentrated to give a yellow golden mass showing the presence of D-xylose (R_f value = 0.28).

Study of NS-2 (G)

NS-2 (G) was obtained as white crystalline solid; m.p. 188-89°C, $C_{29}H_{50}O_3$, $[M]^+$ 446; $[\alpha]^{27}_D$ – 9.4 in $CHCl_3$, IR ν_{max}^{KBr}: 3565, 2936, 2900, 1442, 1372, 1351, 1316, 1247, 1070, 956, 800 cm^{-1}. ^1H NMR (60 MHz, $CDCl_3$) of monoacetyl derivative of compound δ = 0.88 (3H, s, C_{19} -3H), 0.94 (3H, s, C_{18}-3H), 3.00 (3H, s, C_8-OH), 3.55 (1H, s, C_{14}-OH), 2.16 (3H, s, C_3-OAc), 0.71 (3H, s, C_{21}-3H), 1.3-1.00 (2H, m. polymethylene CH_2 and CH), 4.54 (1H, t, J = 3.5 Hz, C_3-H), 5.56 (1H, dd, J = 4.7, C_{21}-H vinylic proton). FABMS : m/z 446 (1), 428 (19), 417 (22), 410 (31), 392 (36), 376 (25), 358 (41), 340 (46), 296 (58), 254 (54), 236 (68), 218 (70), 216 (61), 202 (78), 162 (86), 148 (91) and 134 (100).

Acetylation of compound NS-2 (G)

About 60 mg of compound **NS-2 (G)** was mixed with 4 mL of pyridine and 5 mL of acetic anhydiride in a R. B. flask and refluxed on a water bath for about 4 hrs. The mixture was filtered off after cooling and dried over anhydrous Na_2SO_4. Then it was recrystallized from acetone to yield monoacetyl derivative of **NS-2 (G)**, m.p. 166-67°C, $C_{31}H_{52}O_4$; found (calcd.) % C = 76.20 (76.22); H = 10.65 (10.65), EIMS : $[M]^+$ 488.

Anhydrosapogenin of NS-2 (G)

About 50 mg of compound **NS-2 (G)** in 50 % alcohol (10 mL) containing 7 N hydrochloric acid (0.6 mL) was boiled under reflux for 2 hrs. and the solution was diluted with 5 mL water and alcohol was removed by evaporation. There after, allowing the mixture to stand for some time, the semicrystalline deposit (60 mg) was separated and crystallized from acetone : ethylacetate (4 : 3) to get ahydro-sapogenin m.p. 180-80^{o}C, molecular formula $C_{29}H_{44}$: found (calcd) % C = 88.76 (88.77), H = 11.19 (11.22), EIMS : $[M]^{+}$ 392.

Preparation of ketone of NS-2 (G)

The solution of compound **NS-2 (G)** (60 mg) in pyridine (5 mL) was mixed with chromium trioxide (50 mg). It was refluxed on a sand bath at 135^{o}C and cooled, when a crystalline ketone of **NS-2 (G)** was obtained, m.p. 170-71^{o}C, molecular formula $C_{29}H_{49}O_3$; found (calcd.) % C = 78.18 (78.20), H = 11.02 (11.01), FABMS $[M]^{+}$ 445.

Preparation of isosapogenin of NS-2 (G)

About 70 mg of compound **NS-2 (G)** was dissolved in 10 mL 10 % solution of KOH in EtOH (4 mL) and kept for 20 minutes. Then the solution was diluted with water (10 mL) and acidified with 10% HCl (5 mL). After standing for 1 h, the solution was cooled and concentrated under reduced pressure when crystalline compound was obtained, m.p. 255-56^{o}C, molecular formula $C_{29}H_{50}O_3$: found (calcd.) % C = 78.10 (78.02), H = 11.17 (11.20), EIMS : $[M]^{+}$ 446

Preparation of dihydrosapogenin of NS-2 (G)

About 50 mg of compound **NS-2 (G)** was dissolved in 5 mL of 80 % EtOH and the mixture was shaken with Pd-back and hydrogen until no more gas was adsorbed. After 15 h, the crystalline deposit (80 mg) was separated and recrystallized with ethylacetate, m.p. 182-83^{o}C, molecular formula $C_{29}H_{52}O_3$; found (calcd.) % C 77.65 (77.76), H 10.72 (10.71), EIMS : $[M]^{+}$ 448.

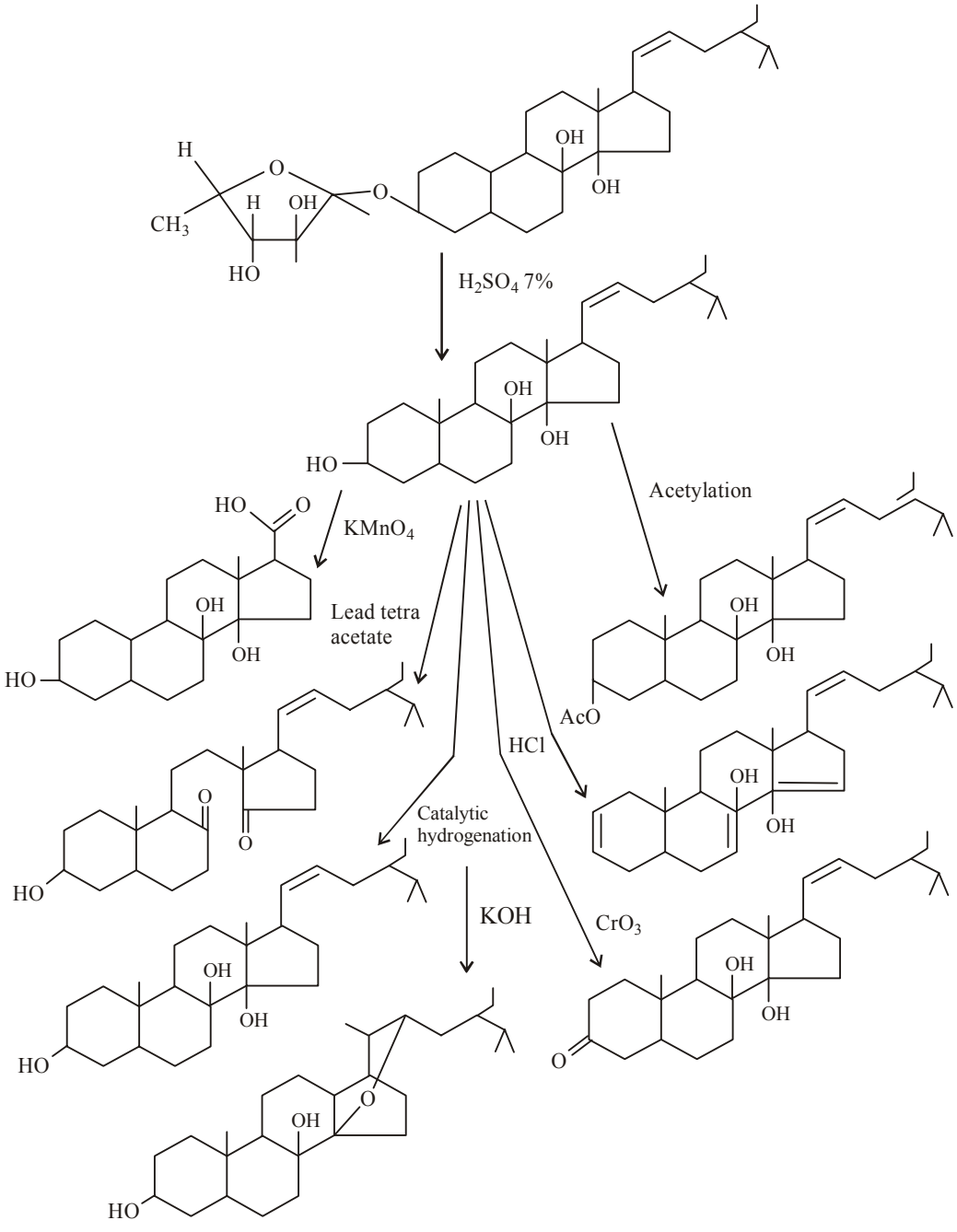
Periodate oxidation of NS-2

About 40 mg of **NS-2** was dissolved in MeOH and treated with $NaIO_4$ (20 mL of 0.1 N) for 2 days. The liberated HCOOH and consumed periodate were estimated by the Jones method.

Enzymatic hydrolysis of NS-2

About of the 60 mg compound in EtOH was treated with almond emulsion solution

(35 mL) in a conical flask and kept at room temperature for 72 h. Examination of the hydrosate on TLC using n-butanol : acetic acid : water (4 : 1 : 5) showed the presence of D-xylose.



RESULTS AND DISCUSSION

The MeOH soluble part of the 95 % EtOH extract of the roots of *Ophiorrhiza mungos* (Linn), when worked up gave the compound; **NS-2** (0.0624), m.p. 204-5°C, molecular formula, $C_{34}H_{58}O_6$, $[M]^+$ 562, and $[\alpha]^{27}_D = 18.5^o$ (in $CHCl_3$). **NS-2** responded to positive foam test[3], honey comb[4] and haemolytic test[5] indicating that it belongs to saponin class.

NS-2 gave the maximum absorption at 217 nm and 296 nm and responded to positive Molisch's test. On acid hydrolysis, **NS-2** yielded sapogenin **NS-2 (G)**, m.p. 189-90°C molecular formula $C_{29}H_{50}O_3$, $[M]^+$ 446 and $[\alpha]^{27}_D = 9.3$ in $CHCl_3$ and sugar moieties. **NS-2 (G)** responded to the positive colour reactions of the steroids[6,7].

Characteristic band at ν^{KBr}_{max} 3565 cm^{-1} in the IR spectrum of **NS-2 (G)** indicated the presence of OH group (s), which was further confirmed by ^1H NMR of the monoacetate derivative of **NS-2 (G)** m.p. 167-68°C, molecular formula $C_{31}H_{52}O_4$, $[M]^+$ 488, which showed singlets at δ 2.16 for – OAc and δ 3.14 for OH. The sapogenin **NS-2 (G)** on treatment with HCl formed a trianhydrosapogenin m.p. 180-81°C, molecular formula $C_{29}H_{44}$; confirming the presence of three OH group (s), in the sapogenin **NS-2 (G)**, out of which one-OH group was either primary or secondary and the remaining two-OH group (s) must be tertiary in the sapogenin **NS-2 (G)**.

CrO_3/pyridine oxidation of the compound **NS-2 (G)**, yielded a ketone, m.p. 170-71°C, molecular formula $C_{29}H_{49}O_3$ and $[M]^+$ 445, FABMS gave a positive Zimmermann test for C-3 keto group; thereby, confirming the presence of one –OH group at C-3 in the ketone. The KOH hydrolysis of compound **NS-2 (G)** gave an isosapogenin m.p. 254-56°C, molecular formula $C_{29}H_{49}O_3$, $[M]^+$ 445, which showed a quartet at δ 3.8, C-22 H and multiplet at δ 2.32, C-20 H indicating cleavage of double bond in between C-20 and C-22 and forming an epoxy-linkage, which was further confirmed by the presence of tertiary OH groups at C-14[8] in **NS-2 (G)**.

With lead tetra acetate, the sapogenin of **NS-2 (G)** formed a 8 : 14 dikotone, which indicated adjacent position of the tertiary OH group (s)[9].

The monoacetate of **NS-2 (G)**, on oxidation with $KMnO_4$ in acetone yielded another compound with molecular formula $C_{20}H_{32}O_4$, which showed absorption for carbonyl group in IR and was identified as; 3β : 8 β : 14 β-trihydroxy-etianic acid[10] (confirmed by mmp, Co-TLC and superimposable spectra); thus, establishing the position of the side chain at C-17.

Characteristic band in the IR spectrum at 652 cm^{-1} indicated the presence of unsaturation in it, which was further supported by the fact that **NS-2 (G)** on catalytic hydrogenation gave a dihydrosapogenin m.p. 181-82°C, molecular formula $C_{29}H_{52}O_3$, $[M]^+$ 448. The ^1H NMR showed an up field chemical shift at δ 4.23 ppm, for C-22 H and it also confirmed the presence of unsaturation in it. The position of attachment of sugar in the steroidal saponin **NS-2** was fixed at C-3 because **NS-2** did not respond to positive Zimmermann test, whereas the **NS-2 (G)** did this; thereby, confirming that C_3-OH group was free in the sapogenin but it was involved in the glycosylation in **NS-2**.

The periodate oxidation[11] of **NS-2** with HIO_4 indicated that D-xylose was present in furanose form and its hydrolysate showed the presence of 2 : 3 di-O-methyl-D-xylose (confirmed by Co-Pc and Co-TLC). It also suggested that C-1 of D-xylose was involved in glycosidic linkage, whereas hydrolysis with almond emulsion of **NS-2** yielded D-xylose indicating that the D-xylose was linked via β-linkage to the sapogenin. Thus, the sapogenin **NS-2** was finally identified as; 24-α-ethyl-20-ene-7-hydro-stigmast-8 β: 14-di-3-O-β-D-xylofuranoside.

REFERENCES

1. R. N. Chopra, S. L. Nayar and I. C. Chopra, Glossary of Indian Medicinal Plants, CSIR Publications, New Delhi, (1956) p. 160.
2. M. C. Pant and G. Sharma, Curr. Sci. (India), **57**, 1085 (1988).
3. C. H. Sannic, Anal. Biochem. Med., **9**, 175 (1948).
4. G. R. Vanatta and Guggotz, J. Agri. Food Chem., **6**, 649 (1958).
5. Kolvasapi Thes, E-Gyogyozrezet, **4**, 839 (1960).
6. C. R. Noller, J. Am. Chem. Soc., **54**, 3047 (1942).
7. E. Salkowski, Hopp. Seyl. Z., **57**, 521 (1908).
8. T. Reichstein, O. Schindlev and M. P. Khare, Helv. Chim. Acta., **45**, 1537 (1962).
9. T. Renz and A. Wartburg Von, Helv. Chim. Acta, **42**, 1620 (1959).
10. S. J. Smith, Chem. Soc., 2478 (1930).
11. E. L. Hirsh and J. K. N. Jones, Chem. Soc., 1959 (1959).

Accepted : 18.03.2010