

Antifertility principles from *Marsdenia tinctoria*: Pharmacological and phytochemical studies

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Abstract: *Marsdenia tinctoria*, a perennial climber has been used by the Shautal of Bangladesh to induce abortion. Bio-activity directed phytochemical investigation revealed that the plant contained tinctoramine, a new steroidal alkaloid and tinctoralactone, a novel steroid as the active constituents. Both the compounds showed oxytocic, antiimplantation and abortifacient activities in mice and rats. The alkaloidal fraction, which contained tinctoramine and tinctoralactone, did not have any adverse effect on SGOT, SGPT, BUN and serum albumin when administered to rats and mice. The structures of the compounds were elucidated by spectral studies (UV, IR, ^1H NMR and Mass) of these and related compounds isolated from plants of genus *Marsdenia*.

Marsdenia tinctoria (Family: Asclepiadaceae), a perennial climber has been used by the Shoutal, aboriginie tribe of Bangladesh, to induce abortion. Preliminary pharmacological investigation showed that the alcohol extract and the alkaloidal fraction of the plant each possessed oxytocic activity on sensitized uterine horns of rats (1). The alkaloidal fraction on thin layer chromatographic analysis revealed that it had two components, A_1 and A_2 having R_f values 0.58 and 0.32 respectively (Alumina G, solvent - chloroform : ethanol, 6 : 5). The component A_1 was found to be a new steroidal alkaloid and was named as Tinctoramine (1) (Fig. 1), (0.037%), m.p. 254-55 $^{\circ}\text{C}$; M^+ =511.256805, $\text{C}_{29}\text{H}_{37}\text{O}_7\text{N}$; UV $_{\text{max}}^{\text{CH}_3\text{OH}}$ 217 and 262 nm; IR $_{\text{max}}^{\text{CCl}_4}$ 3320, 1740, 1640, 1690 and 1250 cm^{-1} . ^1H NMR of selected protons of the compound were shown in Table 1. The structure was elucidated by spectral data and was described elsewhere (2). The Component A_2 was obtained in trace amount.

The alkaloidal fraction also yielded two polar novel steroids, ME_1 and ME_2 , having R_f values 0.60 and 0.55 respectively (Silica gel 60G; solvent-ethyl acetate: methanol, 9:1), which responded to steroidal spray reagents (but did not respond to alkaloidal spray reagents). The compounds ME_1 and ME_2 were named as Tinctoralactone (2) and Marsdenone (3) (Fig1) respectively.

Tinctoralactone (0.025%), m.p. 240-42 $^{\circ}\text{C}$, EIMS : 285 [$\text{M}-\text{C}_{12}\text{H}_{17}\text{O}_4$] $^+$, 168, 167, 150, 149, 141, 137, 101, 59; IR $_{\text{max}}^{\text{KBr}}$ 3390, 2925, 2860, 1720, 1680, 1448 cm^{-1} . ^1H NMR data of selected protons of Tinctoralactone and those of related compounds obtained from the plants of the genus *Marsdenia* were shown in Table 1.

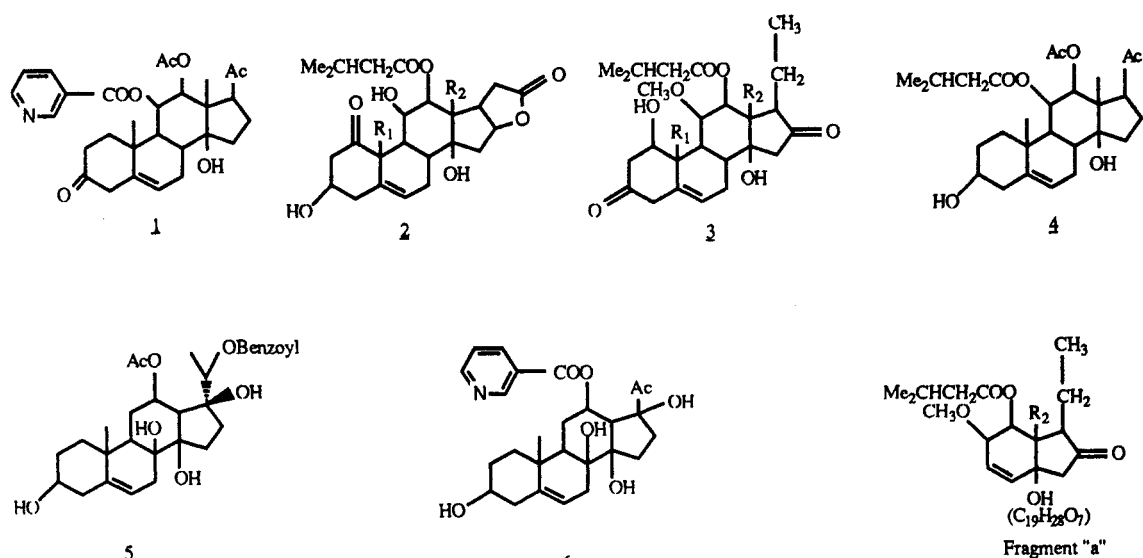


Fig. 1 Structures of the compounds isolated from the plants of genus *Marsdenia* and Fragment "a" ($R_1=CH_2OH$, $R_2=COOCH_3$).

The structures were proposed basing on the spectral data was presented elsewhere (3).

TABLE 1 : Comparative 1H NMR Spectral Data of Selected Protons of Tinctoramine, Tinctoralactone, Marsdenone, Rostratamine, O-acetyl-O-benzoyl sarcostine & Drevogenin Q

Compound	δ (ppm) values related to TMS (J in Hz)					
	H-3 α	H-6	H-12 α	Me-18	Me-19	Me-21
Tinctoramine (1)	3.50 <i>dddd</i> (12, 5)	5.49 <i>br. d</i> (5.5)	5.08 <i>d</i> (10)	1.19 <i>s</i>	1.15 <i>s</i>	2.10 <i>s</i>
Tinctoralactone (2)	3.58 <i>m</i>	5.30 <i>m</i>	5.35 <i>d</i> (9)	-	-	-
Marsdenone (3)	-	4.86 <i>dd</i> (3.2, 1.6)	4.84 <i>d</i> (8.3)	-	-	1.19 <i>t</i> (6.8)
Rostratamine (4)	3.37 <i>m</i>	5.35 <i>br. s</i>	4.90 <i>q</i> (11, 5)	1.60 <i>s</i>	1.15 <i>s</i>	2.09 <i>s</i>
O-acetyl-O-benzoyl sarcostine (5)	3.50 <i>m</i>	5.32 <i>m</i>	4.90	1.68 <i>d</i>	1.14 <i>s</i>	1.15 <i>d</i>
Drevogenin Q (6)	-	5.47 <i>m</i>	4.78 <i>d</i> (9.9)	1.09 <i>s</i>	1.03 <i>s</i>	-

Marsdenone m.p. 245-47 $^{\circ}C$; EIMS : 368 [$M - C_9H_{12}O_3$] $^+$ retro Diels Alder (fragment a; Fig 1), 308, 267, 237, 184, 168, 166, 162, 101, 74, 59; (1H NMR data of selected protons in Table1).

Hydrated alcohol extract (HAE), alkaloidal fraction (AF) peptide fraction and Tinctoramine showed oxytocic activity on isolated uterine horns of rats (Fig. 1 A & B). The activity compared well with that of oxytocin. Tinctoralactone also possessed oxytocic action but was not shown in the Fig. 1. The activity of the peptide fraction was comparatively less than the other agents mentioned above. HAE, AF, Tinctoramine and Tinctora-

lactone when tested for their anti-implantation activity in mice and rats by a method (4) showed prominent activity (Table 2). Each of these agents when administered to the animals at a dose of 400, 100, 80 and 0.1 mg/kg body weight respectively daily for seven consecutive days, starting from day 1 of pregnancy, inhibited the implantation of the fertilized ova in 100% cases ($P < 0.01$ to 0.001). Tinctoramine and Tinctoralactone each showed prominent activity in much smaller doses compared to those of the extract (HAE) and the fraction (AF) suggesting that the antifertility activity of the plant may be due to the presence of the steroidal moiety present in these compounds.

HAE, AF and Tinctoramine also showed significant abortifacient activity in mice and rats. Administration of single dose of any one of these agents (300, 200 or 100 mg/kg of body weight respectively) caused abortion in 100% rats and mice (Table 3). The time taken to abort was shorter when the agents were administered after 12 days of pregnancy. The interruption ratio of the experimental rats and mice was one. The oestrous cycles of the rats and mice which aborted under effect of the agents returned to normal within 6-10 days. The activity of Tinctoralactone and Marsdenone could not be ascertained due to paucity of the compounds.

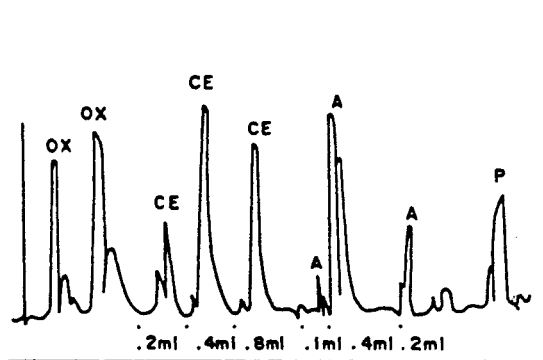


Fig 2A Effect of the Alcohol extract (CE) of *M. tinctoria*, its alkaloidal fraction (A), peptides (P), and oxytocin (OX) on isolated rat uterus.

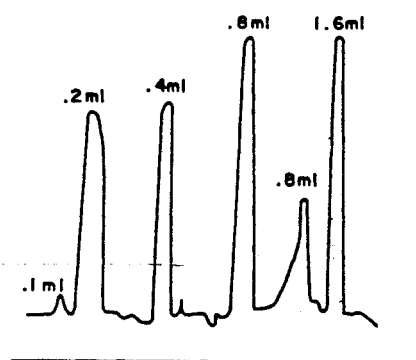


Fig 2B Effect of steroidal alkaloid Tinctoramine (A_1) on isolated rat uterus.

The alkaloidal fraction, which contained both Tinctoramine and Tinctoralactone in it, did not have any adverse effect on SGPT, SALP, SGOT, BUN, STP and Albumin when administered intraperitoneally, at a dose which elicited pharmacological action, for 20 days. The histopathology of the liver and kidney of the experimental rats and mice did not show any abnormality.

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TABLE 2: Antiimplantation activity of hydrated alcohol extract, alkaloidal fraction, tinctoramine and tinctoralactone isolated from *Marsdenia tinctoria*.

Agent, dose in mg/kg	Number of rats/mice failed to conceive	Number of rats/mice conceived (No. of implantation sites)	Remarks; significance of antiimplantation activity
^a Hydrated alcohol extract, (400)	10	0 (0)	a vs d; *** P < 0.001
^b Alkaloidal fraction, (300)	10	0 (0)	b vs d; *** p < 0.001
^c Tinctoramine, (80)	10	0 (0)	c vs d; *** p < 0.001
^d Vehicle only	0	10 (8.2)	control
^e Alkaloidal fraction, (100)	6	0 (0)	e vs h; ** p < 0.01
^f Alkaloidal fraction, (50)	4	2 (9.6)	f vs h; ** p < 0.025
^g Tinctoralactone 6 (0.1)		0 (0)	g vs h; ** p < 0.01
^h Vehicle only	0	6 (8)	control

Agents administered once daily for seven days; from day 1 to 7 of pregnancy to rats, per oral route in groups a-d; Agents administered intraperitoneally to mice in groups e-h following similar schedule. ***=Highly significant **= Significant activity.

TABLE 3: Abortifacient activity of hydrated alcohol extract, alkaloidal fraction and tinctoramine isolated from *Marsdenia tinctoria*.

Agent, dose; mg/kg (single dose)	State of Pregnancy in days (n=4)	Time taken to abort in hour	Average number of fetuses/rat	IR
Hydrated alcohol extract, (300)	9-12	36-40	3.0	1
"	12-14	18-20	5.0	1
Alkaloidal fraction (200)	12-14	14-20	3.5	1
Tinctoramine (100)	12-14	12-14	4.5	1
Vehicle; water+ cmc	12-14	-	-	0

N= Number of mice/rats per group; cmc= carboxymethylcellulose as suspending agent; IR= interruption ratio.

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