

From ethnobotanical uses of *Strychnos henningsii* to antiinflammatories, analgesics and antispasmodics

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Strychnos henningsii Gilg is used in African traditional medicine for the treatment of various ailments, including rheumatism, gastrointestinal complaints and snake bites. Different preliminary pharmacological experiments are described. The results show that some of the reported folk medicinal applications of *S. henningsii* can be at least partially explained by the presence of retuline-like alkaloids, whose use could lead to new antinociceptive (antiinflammatory and analgesic) and antispasmodic drugs.

Key words: *Strychnos henningsii*; indole alkaloids; (iso)-retuline; antinociceptive properties; therapeutic index.

Introduction

Regarding the pharmacology of *Strychnos*, emphasis in the past was on the investigation of the alkaloids present in the tetanizing or curarizing species. However, it is evident that the activity of other species as medicinal and useful plants is not a minor one. Detailed information has been published about the medicinal uses of twenty species of African *Strychnos* (Bisset, 1970).

On the basis of our interest for antirheumatic and antispasmodic drugs, we have selected *Strychnos henningsii* Gilg. This plant, a small evergreen tree or shrub like a ligustrum with leathery leaves and branches devoided of spines and tendrils, is one of the most widely distributed African species of *Strychnos* (Zaire, Angola, East and South Africa, Madagascar) (Leeuwenberg, 1969). *S. henningsii* is recommended for various therapeutic indications in traditional medicine such as: pain producing ailments (rheumatism,

gynaecological complaints); diseases of the digestive system (colic, nausea, worms, abdominal or gastrointestinal complaints); and snake bite treatment (Bisset, 1970; Hutchings, 1989).

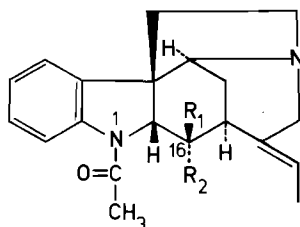
Indolinic alkaloids have been isolated from various *Strychnos henningsii* batches and a review has been previously published (Massiot and Delaude, 1988). Sufficient quantities of the main alkaloids present in *S. henningsii* collected in Zaire were firstly obtained by extraction (Angenot and Tits, 1981), secondly by semi-synthesis from strychnine, natural sources of which are abundant (*S. nux-vomica*, *S. icaja*, *S. ignatii*) or from strychnobiline (*S. variabilis*) (Tits, 1982). Isoretuline (16-epiretuline) and derivatives have also been prepared from the same sources (Angenot et al., 1988). Finally, the pharmacology of these products was considered in order to determine whether their presence may be at the basis of some reported medicinal uses.

Material and Methods

Chemicals

Retuline (1) and holstiine (3) have been isolated

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- (1) Retuline: $R_1 = H$, $R_2 = CH_2OH$.
 (2) Isoretuline: $R_1 = CH_2OH$, $R_2 = H$.

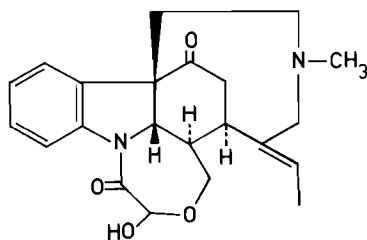
from *S. henningsii* bark as described before (Angenot and Tits, 1981).

Isoretuline (2) and derivatives have been prepared by semi-synthesis as related elsewhere (Wenkert and Sklar, 1966; Hymon et al., 1969; Szabo and Clauder, 1977; Tits, 1982, Angenot et al., 1988)

Lambda carrageenan, zymosan and arachidonic acid were purchased from Sigma and heparin from Leo. PGE_2 was obtained from Upjohn, indomethacin from Merck-Sharp and Dohme, methysergide from Sandoz, sodium pentobarbital from Sanofi, mepyramine maleate from Specia, aspirin and brucine from our collections. Arachidonic acid was dissolved in ether/methanol (1:1, vol/vol) and kept at $-25^\circ C$. Before use, the organic solution was evaporated under nitrogen and arachidonic acid dissolved in Tris-HCl (pH 7.5, 0.15 M). Indomethacin and aspirin were dissolved in Tris-HCl (0.15 M, pH 7.5), PGE_2 dissolved in ethanol was diluted in saline. All the other drugs were dissolved in saline.

Pharmacological

The pharmacological experiments were conducted using Swiss A mice (18–22 g) and Wistar rats of both sexes. These animals were used after



(3) Holstiine.

an acclimatisation period of at least 5 days to the laboratory environment. The animals were housed in standard metal cages and provided food and water ad libitum.

Antiinflammatory action (rat paw oedema)

Wistar rats of both sexes were used. The mean weight of the animals was 220 g. Foot volumes were measured by a water plethysmometer (Ugo Basile) immediately before the injection of lambda carrageenan (0.1 ml; 1%) or zymosan (0.1 ml; 1 mg) in the plantar region of the right hind paw, and at different intervals thereafter. A control injection of saline was given to the controlateral paw. The swelling was calculated, after subtracting the effect of saline injection, as a mean percentage increase in the volume of the injected paw compared to the initial control value. Each experiment was performed on groups of six animals.

Hypotensive action of arachidonic acid

Wistar rats of both sexes weighing about 290 g were used. Anaesthesia was secured by intraperitoneal sodium pentobarbital (35 mg/kg). A tracheotomy was performed and the mean arterial blood pressure was recorded from a carotid artery with a Harvard 50–8952 transducer connected to a Harvard Universal Oscillograph and coupled to a mercury manometer. After intravenous injection of heparin (250 U/kg), the hypotensive activity of arachidonic acid was recorded. Dose-response curves were initially established for arachidonic acid (0.4–1.6 mg/kg) and PGE_2 (0.25–2 $\mu g/kg$). Thereafter, retuline was administered by intravenous injection and 5 min after this injection dose-response curves were again recorded (Damas and Mousty, 1978).

Analgesic activity

Analgesic activity of alkaloids was tested as antinociceptive effect against chemical stimulus (phenylquinone writhing test). This was determined in groups of mice (12 in each) by noting the writhing responses produced by intraperitoneal administration of 0.25 ml of a 0.2% m/v aqueous solution of phenylquinone 30 min after intragastric administration of control vehicle or test material (isoretuline, acetylisoretuline or

desacetylisoetretuline). ED₅₀ is the dose which inhibits writhing for 50% of the animals (R. Menassé et al., 1978; Lewis, 1989).

Antispasmodic activity

Guinea-pigs were anaesthetized with sodium pentobarbital (50 mg/kg). The abdomen was opened and a piece (15 cm) of the terminal portion of the ileum was removed and washed in Tyrode solution. Three portions of 4 cm length of the ileum were separated and superfused with Tyrode solution (5 ml/min) at 37°C and aerated with 95% O₂ and 5% CO₂ (Vane, 1964). An initial tension of 1 g was applied and the organs were permitted to equilibrate for 30 min. Isometric contractions were recorded on a Watanabe multichannel recorder. Increasing amounts of the agonists, histamin or bradykinin, were injected into the stream of Tyrode solution at 5-min intervals and a dose-response curve was obtained simultaneously for each organ. Then the effect of various concentrations of isoretuline or *O*-acetylisoetretuline on these responses was investigated. The drug was added to the Tyrode solution and perfused the organs. A dose-response curve was again established 10 min after the addition of the drugs to the superfusing fluid.

Toxicity studies

For acute toxicity studies, groups of mice (10 in each) were given the test substance either i.v. or p.o. in different doses and mortality rates were observed after 24 h and every day for 14 days.

Results and Discussion

Antiinflammatory action

Carrageenan-induced paw oedema. The most widely used primary test to screen new non-steroidal antiinflammatory agents measures the ability of a compound to reduce local oedema induced in the rat paw by injection of the irritant lambda carrageenan (Winter et al., 1962). This oedema depends on the participation of kinins and of polymorphonuclear leucocytes with their pro-inflammatory factors including prostaglandins (Damas et al., 1986). Retuline and isoretuline demonstrated a significant antioedematogenic ac-

TABLE 1

EFFECT OF VARIOUS ALKALOIDS ON CARRAGEENAN-INDUCED OEDEMA IN THE RAT PAW (*n* = 6)

The inhibitory effect was determined 4 h after the injection of carrageenan.

Drug	Dose ^a (mg/kg)	% inhibition (mean ± S.E.M.)
Isoretuline	32	26 ± 7*
	40	35 ± 8*
Retuline	45	32 ± 6**
<i>O</i> -acetylisoetretuline	50	10 ± 16 NS
Holstiine	50	0
Brucine	50	0

^aDrugs were administered intraperitoneally 30 min prior to challenge with carrageenan.

P* < 0.05; *P* < 0.01.

tion while holstiine, acetylisoetretuline and brucine are devoid of activity at 50 mg/kg (Table 1). These findings show the importance of free hydroxymethylgroup at position 16 and acylsubstitution at nitrogen 1. Moreover, a structural change in the basic skeleton produces a loss of activity (holstiine and brucine in comparison with (iso)retuline).

Zymosan induced paw oedema. This oedema developed very rapidly as a consequence of the immediate release of mast cell amines. To minimize this effect, all the rats are treated with mepyramine and methysergide 30 min before the injection of zymosan (1 mg/paw). Isoretuline administered i.p. (40 mg/kg) exerts a statistically significant inhibitory effect (>50%) on the oedema in its early phase (Table 2). This oedema depends on the participation of complement with the involvement of mast cell amines (reduced under our conditions) and on the stimulation of leucocytes (Damas et al., 1986). It is also inhibited by aspirin and indomethacin (Table 3).

Hypotensive action of arachidonic acid

The hypotensive response to arachidonic acid in rats was taken as a measurement of the bioconver-

TABLE 2

EFFECT OF ISORETULINE ON ZYMOSAN-INDUCED OEDEMA IN RAT PAW ($n = 6$)

Each value of increase of paw volume represents the mean \pm S.E.M. in group of 6 rats.

Treatment	Dose ^a (mg/kg)	Increase after 1 h	% Inhibition	Increase after 3 h	% Inhibition
(Controls)					
Methysergide	2	28.3 \pm 7.2	—	44.8 \pm 5.7	—
+ mepyramine	2				
Methysergide	2				
+ mepyramine	2	12.5 \pm 2.8**	56	29.3 \pm 3.8*	35
+ isoretuline	40				

^aDrugs were administered intraperitoneally 30 min prior to challenge with zymosan.

* $P < 0.05$; ** $P < 0.005$.

sion in the cardiovascular system of arachidonic acid into prostaglandins (Damas and Mousty, 1978). This response was not modified by isoretuline (40 to 50 mg/kg i.v.), while it was suppressed by indomethacin and other cyclooxygenase inhibitors. These experiments suggest that isoretuline does not inhibit prostaglandins synthesis. From this we can deduce that the mechanism of action of the antiinflammatory effect of isoretuline seems altogether original.

In addition, the results show that isoretuline, at the used doses, is without influence on the mean blood pressure. There is a difference with diaboline, a closely related alkaloid exhibiting a significant hypotensive activity at rather similar dose (32 mg/kg i.v.) (K Kapoor et al., 1988).

Analgesic activity

Phenylquinone injected i.p. into mice causes a

writhing response characterized by repeated contractions of abdominal muscles and extension of the hind limbs. Various analgesics were shown to abolish the response. Isoretuline administered per os possesses this property at a dose of 20 mg/kg. *O*-acetyl isoretuline and *N*-desacetyl isoretuline are inactive at the same dose. Therefore, the combined effect of the acetamide in position 1 and the hydroxymethyl group in position 16 must be of major importance for the pharmacological effect.

Antispasmodic effect

Isoretuline inhibited the myostimulating effect of histamine and bradykinin on guinea-pig ileum. This inhibition occurred from 10^{-6} M and increased dose-dependently (Figs. 1 and 2). *O*-acetyl isoretuline (10^{-6} and 10^{-5} M) had no influence on the effect of histamine (Fig. 3).

TABLE 3

EFFECT OF SOME ANTI-INFLAMMATORY DRUGS ON OEDEMA IN THE RAT PAW ($n = 6$)

The inhibitory effect was determined 4 h after the injection of oedematogen. Each percentage of inhibition represents the mean \pm S.E.M.

Drug	Dose ^a (mg/kg)	% Inhibition zymosan-oedema	% Inhibition carrageenan-oedema
Aspirin	200		47 \pm 6.5**
	120	58 \pm 7**	
Indomethacin	4	38 \pm 12*	44 \pm 5.2**
Isoretuline	40	35 \pm 8*	35 \pm 8**

^aDrugs were administered intraperitoneally 30 min prior to challenge with oedematogen. * $P < 0.05$; ** $P < 0.01$.

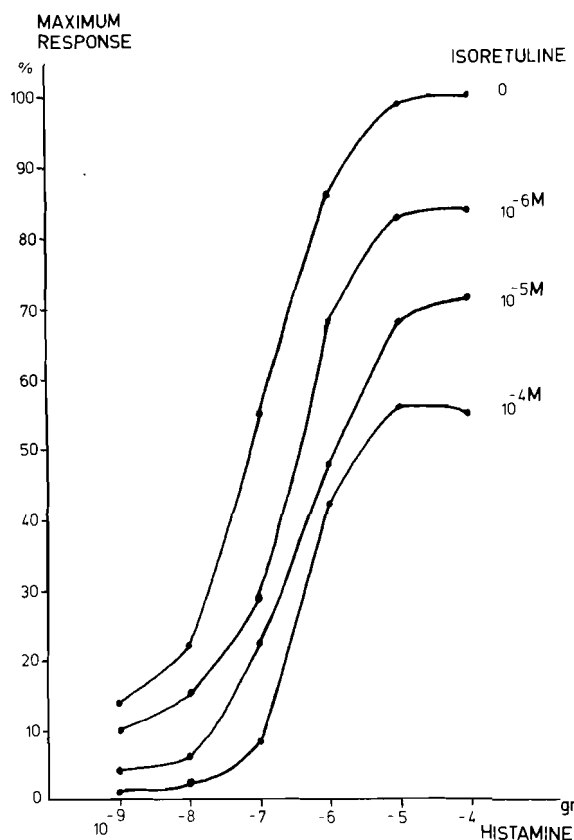


Fig. 1. Contractile responses of guinea-pig ileum to histamine in the presence of increasing concentrations of isoretuline. Each point is the mean of six determinations.

Toxicity studies

In mice, isoretuline administered intravenously possesses an LD_{50} of 70 mg/kg (55–87) and is 140-fold less toxic than strychnine, whose LD_{50} is 0.5 mg/kg (Sandberg and Kristianson, 1970). In the literature, it has been reported that the bark of *Strychnos henningsii* produces strychnine-like effects in rabbits, 20 to 50 g/kg by mouth being the minimum lethal dose (Watt and Breyer-Brandwijk, 1962). Bearing in mind that the bark contains approximately 1% of alkaloids, these latter could have a LD_{50} a hundred-fold less important than the bark. Our experimental data confirm this hypothesis, as LD_{50} of isoretuline and retuline administered per os in mice was about 325 mg/kg. Table 4 shows the comparative data of therapeutic

index (LD_{50}/ED_{50} writhing test) for some anti-inflammatory drugs and isoretuline. The therapeutic index of isoretuline in mice is favourable to this product. Moreover, animals treated with this molecule show no lesions at the gastrointestinal level. It is well established that the non-steroidal antiinflammatory agents are among the most prescribed drugs. The compounds most commonly used all contain an acidic or pseudo-acidic group, and a causal relationship has been established between the use of these compounds, and in particular, the risk of hemorrhagic gastrointestinal ulcer. Retuline-like compounds having an antinociceptive effect, lacking an acidic group and exerting few adverse side effects should hence be of considerable interest.

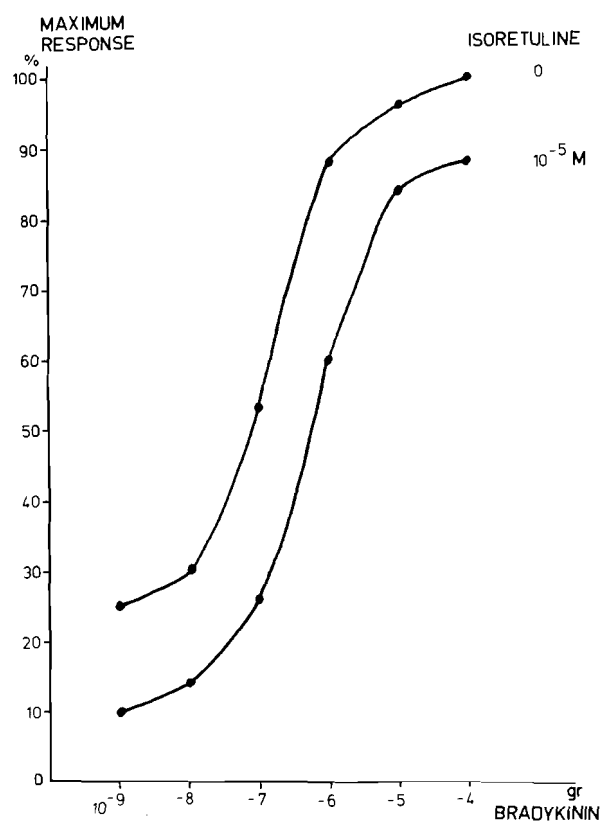


Fig. 2. Effect of isoretuline on the contractile responses of guinea-pig ileum to bradykinin. Each point is the mean of six determinations.

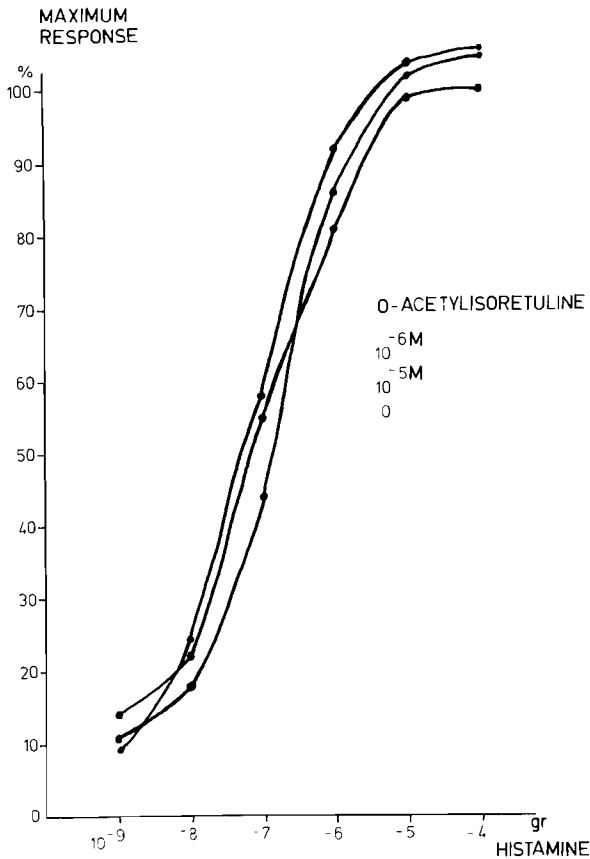


Fig. 3. Lack of effect of *O*-acetylisoetretuline on the contractile responses of guinea-pig ileum to histamine. Each point is the mean of three determinations.

Conclusions

It may be concluded from the above experiments that:

(1) Some reported folk medicinal applications of *Strychnos henningsii* can at least be partially explained by the presence of retuline-like alkaloids

(2) The therapeutic indications of the latter alkaloids could be

- (a) acute and chronic pains of any origin, especially
 - pains affecting the gastrointestinal tract, the bile ducts and the uro-genital system; dysmenorrhea of spasmodic origin; uterine cramps; spasmodic colitis
 - inflammatory rheumatism and periarticular conditions
- (b) treatment of inflammatory oedemas of any origin affecting the soft tissues

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TABLE 4

THERAPEUTIC INDEX OF SOME ANTIINFLAMMATORY DRUGS ADMINISTERED PER OS IN MICE

Drugs	LD ₅₀ (mg/kg)	ED ₅₀ (writhing test) (mg/kg)	Index
Aspirin	1650	165 ^a	10
Indomethacin	30	2.7 ^a	11
Isoretuline	325	20	16

^aMenassé et al. (1978).

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