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Sympathomimetic Effects of *Scoparia dulcis* L. and Catecholamines Isolated from Plant Extracts

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Abstract

The herb *Scoparia dulcis* L. is used in Brazilian folk medicine to treat bronchitis, gastric disorders, haemorrhoids, insect bites and skin wounds, and in oriental medicine to treat hypertension. A previous study has shown that extracts of *S. dulcis* have analgesic and anti-inflammatory properties; in this work the sympathomimetic activity of an ethanolic extract of *Scoparia dulcis* L. has been investigated in rodent preparations in-vivo and in-vitro.

Administration of the extract (0·5–2 mg kg^{-1}, i.v.) to anaesthetized rats produced dose-related hypertension blocked by the α-adrenoceptor antagonist prazosin (1 mg kg^{-1}). Partition of the extract in chloroform-water yielded an aqueous phase 20 times more potent than the extract; this produced hypertension in either reserpine-treated or pithed rats. In untreated and reserpine-treated rats the same fraction ($1–3 \times 10^3$ μg mL^{-1}) produced concentration-dependent contractions of the vas deferens musculature parallel to those obtained with noradrenaline (10^{-8}–10^{-4} M). Prazosin (10^{-7} M) reduced the maximum contractile effect of the aqueous fraction, and shifted the concentration-response curves for noradrenaline to the right. The aqueous fraction (25 and 50 μg mL^{-1}) increased the inotropism of electrically driven left atria of rats, the effect being blocked by propranolol (0·4 μg mL^{-1}). In preparations of guinea-pig tracheal rings the aqueous fraction ($1–3 \times 10^3$ μg mL^{-1}) relaxed the muscle contraction induced by histamine (10^{-4} M) in proportion to the concentration. The effect was antagonized competitively by propranolol (1·5 μM). High-performance liquid-chromatographic analysis of the aqueous fraction revealed the presence of both noradrenaline and adrenaline in the plant extract.

The results indicated that both catecholamines may account for the hypertensive and inotropic effects obtained after parenteral administration of *S. dulcis* extracts. This sympathomimetic activity is, however, unrelated to the previously reported analgesic and anti-inflammatory properties of the plant extract, but may explain its effectiveness upon topical application in the healing of mucosal and skin wounds.

Scoparia dulcis L (Scrophulariaceae) is a widespread tropical herb valued in Brazilian folk medicine for its analgesic and anti-pyretic properties and for its use to treat bronchitis and gastric disorders. The plant is used topically to treat haemorrhoids, insect bites and skin wounds (Coimbra 1958; Penna 1964; Cruz 1982; Freire et al 1993). In oriental medicine, it is also used to treat hypertension (Chow et al 1974).

In a previous study, extracts of *S. dulcis* were shown to have analgesic and anti-inflammatory properties related to the flavonoid and glutinol content of the plant (Freire et al 1993). It was also reported that intravenous injection of an aqueous extract, or an ethanolic extract or its hydrophilic contents to anaesthetized rats produced dose-related hypertension that was inhibited by α-adrenoceptor antagonists, but still obtained in animals pre-treated with reserpine. No pressor effects were, however, detected after oral administration of the plant extracts.

This study assessed the sympathomimetic activity of *S. dulcis* extracts and its relationship with polar vasoconstrictor constituents that mimic the effects of catecholamines. Two of these compounds were identified as noradrenaline and adrenaline by high-performance liquid-chromatographic analysis of the aqueous fraction.

The data presented indicate that these catecholamines do not account for and, apparently, do not influence the anti-inflammatory or analgesic activities of either the plant extract, or the non-polar fractions endowed with anti-inflammatory effects. The presence of catecholamines in the *S. dulcis* extract may, however, explain the efficacy of the plant in healing mucosal and skin wounds when applied topically.

Materials and Methods

High-performance liquid-chromatography (HPLC)

The plant extracts were prepared as detailed elsewhere (Freire et al 1993). In short, the powdered aerial parts were shaken with 90% ethanol and the extract (yield = 6·2% w/v) was dried under vacuum. The ethanolic extract obtained was partitioned in chloroform-water (100 mL:50 mL, four times) and the aqueous phase thus obtained was filtered, the pH was reduced to 3·5 with 0·1 M HCl and the solution kept frozen (−18°C).

Ion-pair HPLC analysis of the catecholamines in the aqueous fraction was performed with a Shimadzu LC-6A system equipped with an L-ECD-6A electrochemical detector set at +0·5 V, using Ag-AgCl electrodes for reference and glass electrodes (Guillemin et al 1988; Murai et al 1988). An analytical reversed-phase Shim-pack CLC-ODS column was eluted with a mobile phase comprising methanol (10%), 0·02 M

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citrate phosphate buffer pH 2·6, EDTA (0·12 mM) and heptanesulphonic acid (0·05%); the flow rate was 0·7 mL min^{-1}, pressure 44 kgf cm^{-2}. A sample of the aqueous fraction (0·15 mg) was mixed with acid alumina (90 mg), stirred for 10 min, and the supernatant was discarded. The alumina was washed with distilled water at pH 7 (3 × 2 mL) and extracted with perchloric acid (0·2 M; 1 mL). The acid extract was filtered through a 0.22-μm filter (Millipore) and then injected into the HPLC column.

Parallel extraction of noradrenaline and adrenaline was performed using dihydroxybenzylamine as internal standard. Extraction of a mixture of these compounds always preceded extraction of the aqueous fraction from alumina. In some experiments known amounts of noradrenaline or adrenaline were added to the fraction before extraction.

General pharmacological screening

Mice and rats were treated intraperitoneally (i.p.) or orally (p.o.) with the extract (10–2000 mg kg^{-1}) dispersed in Tween 80 (0·04 mL/100 mg extract) and diluted in saline to the concentration needed. Control animals were treated with the vehicle (Tween + saline). Changes in behaviour and other signs were recorded hourly for 6 h, then 12 and 24 h after injection.

Blood pressure recordings

The mean carotid blood pressure of Wistar rats (250–300 g), anaesthetized with urethane (0·8 g kg^{-1}) plus sodium pentobarbital (25 mg kg^{-1}) intraperitoneally, was recorded using a Statham P23AA transducer on a Beckman polygraph as detailed elsewhere (Carvalho & Lapa 1990). Some experiments were performed using pithed animals and rats pretreated with reserpine (10 mg kg^{-1}, i.p.) 24 and 3 h before the experiments. A similar procedure was used for blood pressure recordings from dogs (10–12 kg) anaesthetized with sodium pentobarbital (35 mg kg^{-1}, i.v.).

Rat vas deferens

Vasa deferentia were excised from male rats under ether anaesthesia, and stripped of connective tissue. Each organ was suspended in a 10 mL bath containing nutrient solution (in mM: NaCl 138; $NaHCO_3$ 15; KCl 5; $CaCl_2$ 1·8; NaH_2PO_4 0·36; glucose 5·5) at pH 7·6 and 30°C, and isotonic contractions were recorded under a load of 1 g. After 30 min stabilization, cumulative dose-response curves to noradrenaline (10^{-8}–10^{-4} M), the aqueous fraction (1–10^4 μg mL^{-1}) or tyramine (10^{-7}–10^{-3} M) were constructed. The EC50 (mean effective concentration) and pA_2 values were determined according to Van Rossum (1963).

Guinea-pig atria

Guinea-pigs (300–350 g) were stunned and exsanguinated. The left atria were dissected out and mounted in 10-mL organ baths containing physiological solution (in mM: NaCl 135; $NaHCO_3$ 15; KCl 5; $CaCl_2$ 2; $MgCl_2$ 1; NaH_2PO_4 1; and glucose 11) oxygenated with 95% O_2–5% CO_2 at pH 7·4 and 37°C. The tissues were electrically driven by square pulses (2 ms, 3 Hz, supramaximal voltage) through platinum electrodes placed in the bath. Contractions were isometrically recorded with a force transducer on a polygraph. After 20 min stabilization, concentration-response curves to the aqueous fraction (12·5–50 μg mL^{-1}) and isoproterenol (0·05–0·2 μg mL^{-1}) were obtained before and after 10 min exposure to propranolol (0·4 μg mL^{-1}). Separate preparations were used for each concentration of the agonists.

Guinea-pig tracheal chain

Preparations of five to six tracheal rings tied in a chain form were suspended in organ baths containing nutrient solution (in mM: NaCl 94·7; $NaHCO_3$ 25·2; KCl 4·7; $MgCl_2$ 2·4; KH_2PO_4 1·17; $CaCl_2$ 1·4; glucose 11) oxygenated with 95% O_2–5% CO_2 at pH 7·4 and 37°C. After 30 min stabilization, histamine (10^{-4} M) was added to the preparation and isometric contractions were recorded. When the maximum contraction had been reached, adrenaline (10^{-8}–10^{-4} M) or the aqueous fraction (1–10^4 μg mL^{-1}) were added accumulatively and concentration-relaxation curves were constructed in the absence and presence of propranolol ($1·5 \times 10^{-6}$ M).

Drugs

Drugs used were noradrenaline bitartrate, adrenaline bitartrate, isoproterenol hydrochloride, propranolol hydrochloride, tyramine chloride, histamine diphosphate, ethylenediaminetetraacetic acid, urethane (Sigma, USA); reserpine (Serpasil, Roche, BR), sodium pentobarbital (Abbott, BR), and prazosin (Pfizer, BR). All other reagents were of analytical grade (Merck, BR).

Statistics

Data were expressed as means ± s.e.m. The EC50 values were presented as geometric means and 95% confidence limits (CL). Statistical significance of the data was determined using one-way analysis of variance followed by the Tukey method (Sokal & Rohlf 1981). Data were considered different at the level of $P < 0·05$.

Results

General effects

Administration of the vehicle (Tween + saline) to rats and mice, intraperitoneally or orally, did not produce effects significantly different from those observed in control rats treated with equal volumes of saline. Oral treatment of rats with the extract (0·01–2 g kg^{-1}) did not change the animal behaviour nor did it induce detectable signs of toxicity. Injection of higher doses than 0·1 g kg^{-1}, intraperitoneally, produced piloerection and reduced spontaneous motor activity significantly, causing death of the animals after 6 h.

Blood pressure

The mean blood pressure of anaesthetized rats was 65 ± 7 mmHg (n = 5). Injection of 0·3 mL vehicle (Tween + saline, i.v.) produced hypertension of 6 ± 2 mmHg (n = 5) that was reversed within 2 min. The ethanolic extract (0·5, 1 and 2 mg kg^{-1}, i.v.) produced a dose-related hypertension that peaked in 30 s and lasted for 3–4 min. At these doses, the extract increased the mean blood pressure by 18 ± 3, 29 ± 6 and 36 ± 5 mmHg, respectively. In similar recordings the aqueous fraction was about 20 times more potent than the extract, but 100 times less active than noradrenaline. The hypertension produced by 0·05, 0·1 and 0·2 mg kg^{-1} of the aqueous fraction, intravenously, was respectively 26 ± 4, 39 ± 7 and 51 ± 6 mmHg. In these experiments, noradrenaline (0·5, 1 and 2 μg kg^{-1}, i.v.) increased the blood pressure by 22 ± 8, 42 ± 9

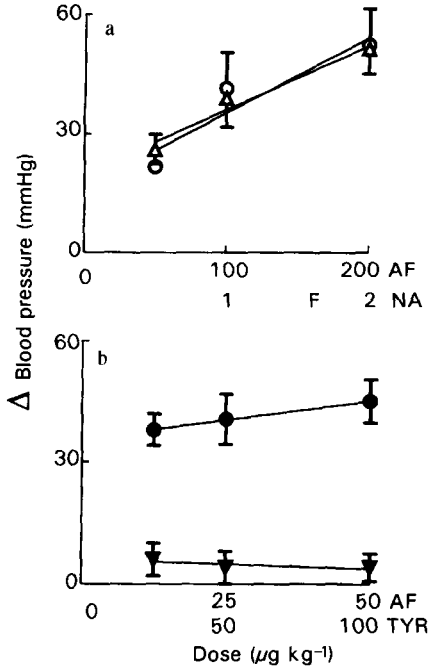

FIG. 1. Changes in the blood pressure of anaesthetized rats as a result of (a) intravenous injection of the aqueous fraction of the ethanolic extract of *Scoparia dulcis* L. (AF, ○) and noradrenaline (NA, △), and (b) pretreatment with reserpine (10 mg kg^{-1}) and then intravenous administration of the aqueous fraction of the ethanolic extract of *S. dulcis* L. (AF, ●) and tyramine (TYR, ▼). Symbols and vertical bars are means ± s.e.m. of four or five experiments.

and 52 ± 9 mmHg (n = 5), respectively (Fig. 1a). After administration of prazosin (1 mg kg^{-1}, i.v.), the effects of noradrenaline (1 μg kg^{-1}), the aqueous fraction (0·1 mg kg^{-1}) and the extract (1 mg kg^{-1}) were reduced by 87% to 88% of control.

The mean blood pressure (77 ± 4 mmHg; n = 5) of reserpine-treated rats (10 mg kg^{-1}, i.p., 24 and 3 h before the experiments) did not differ from that recorded from control untreated rats. Tyramine (25, 50 and 100 μg kg^{-1}, i.v.) did not alter the blood pressure of reserpine-treated rats. In these animals, however, the aqueous fraction (12·5, 25 and 50 μg kg^{-1}, i.v.) raised the blood pressure by 38 ± 4, 40 ± 6 and 45 ± 5 mmHg, respectively (n = 5) (Fig. 1b). Injections of equal doses of the aqueous fraction to pithed rats increased the basal blood pressure (32 ± 2 mmHg, n = 3) by 19 ± 2, 32 ± 5 and 47 ± 7 mmHg, respectively. In the same animals, noradrenaline (0·25, 0·5 and 1·0 μg kg^{-1}) induced hypertension of 14 ± 3, 23 ± 6 and 43 ± 14 mmHg, respectively.

Dogs anaesthetized with pentobarbital (35 mg kg^{-1}, i.v.) had a mean blood pressure of 112 ± 12 mmHg (n = 5). The vehicle (Tween + saline) did not alter the blood pressure, but injection of the extract (0·5, 1 and 2 mg kg^{-1}, i.v.) increased the blood pressure by 10 ± 1, 12 ± 1 and 15 ± 1 mmHg, respectively. In these animals the aqueous fraction (0·05, 0·1 and 0·2 mg kg^{-1}, i.v.) raised the blood pressure by 31 ± 6, 43 ± 6 and 56 ± 6 mmHg, respectively. Injection of prazosin (1 mg kg^{-1}, i.v.) before the aqueous fraction (0·1 mg kg^{-1}, i.v.) reduced the pressor response by 42%. In the same animals, the hypertensive effect of noradrenaline (0·5 μg kg^{-1}) was decreased by 46% after α-blockade.

Rat vas deferens

Successive addition of increasing concentrations of the aqueous fraction (1–3 × 103 μg mL^{-1}) or noradrenaline (10^{-8}–10^{-4} M) produced contractions of the rat vas deferens in proportion to the concentration added. In a same preparation the maximum responses to the aqueous fraction and noradrenaline were not different, both giving EC50 values of 183 μg mL^{-1} (CL: 135–249) and 1·5 × 10^{-6} M (CL: 1·3 × 10^{-6} – 2·1 × 10^{-6}) (n = 5), respectively. Previous incubation of prazosin (10^{-7} M) shifted the concentration-response curves for noradrenaline to the right without altering the maximum response ($pA_2 = 8·2 \pm 0·5$). In contrast, the α-adrenoceptor antagonist reduced the maximum contractile effect of the fraction by 86% (Fig. 2a).

In preparations from reserpine-treated rats, addition of the aqueous fraction (10–10^3 μg mL^{-1}) or noradrenaline (10^{-8}–10^{-4} M) induced contractions of the vas deferens in proportion to the concentration added, without changing the maximum response. The EC50 values of the aqueous fraction and noradrenaline in these experiments were 234 μg mL^{-1} (CL: 181–302) and 2·2 × 10^{-6} M (1·74 × 10^{-6} – 2·9 × 10^{-6}) (n = 7), respectively. Addition of tyramine (10^{-7}–3 × 10^{-4} M) after the aqueous fraction or noradrenaline produced responses that reached respectively 10% and 30% of the maximum contraction to noradrenaline.

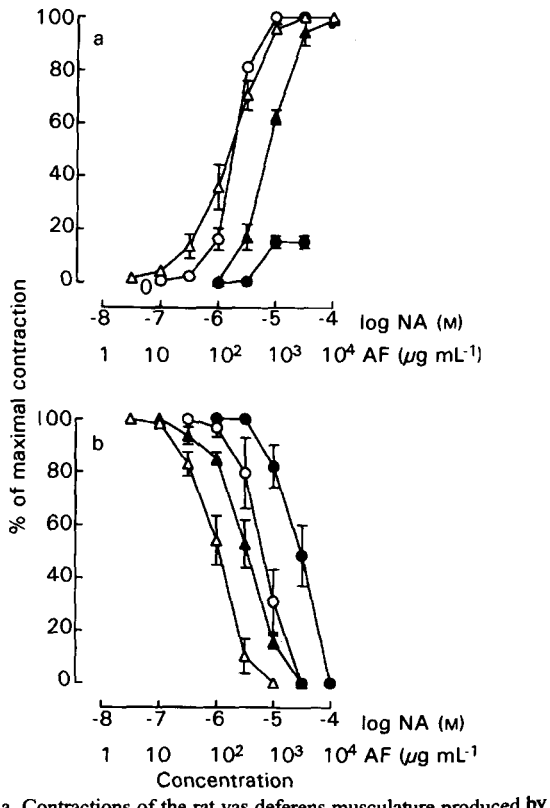

FIG. 2. a. Contractions of the rat vas deferens musculature produced by the aqueous fraction of the ethanolic extract of *S. dulcis* L. (AF, ○, ●) or noradrenaline (NA, △, ▲), in the absence (○, △) and presence (●, ▲) of prazosin (10^{-7} M). b. Relaxation of guinea-pig tracheal preparations produced by the aqueous fraction of the ethanolic extract of *S. dulcis* L. (AF, ○, ●) and adrenaline (AD, △, ▲) obtained in the absence (○, △) and presence (●, ▲) of propranolol (1·5 μM). The preparations were previously contracted with histamine (10^{-4} M). Symbols and vertical bars are means ± s.e.m. of four to seven experiments.

Rat left atria

At 25 and 50 $\mu g\ mL^{-1}$ the aqueous fraction increased the force of contraction of the electrically driven left atria ($8{\cdot}1 \pm 0{\cdot}5$ g; $n=29$) to respectively $147 \pm 10\%$ and $216 \pm 18\%$ of control after 3 min incubation. Lower concentrations of the fraction were ineffective. In the same preparations, isoproterenol ($0{\cdot}2\ \mu g\ mL^{-1}$) increased the atria contractions to $213 \pm 13\%$. The effects of both the aqueous fraction and isoproterenol were reduced in the presence of propranolol ($0{\cdot}4\ \mu g\ mL^{-1}$) by 82% and 86%, respectively.

Guinea-pig tracheal rings

The tonus of the tracheal rings induced by histamine (10^{-4} M) was reduced by addition of the aqueous fraction (10–$10^{3}\ \mu g\ mL^{-1}$) and adrenaline (10^{-8}–3×10^{-5} M) in a concentration-dependent manner. The EC50 values of the aqueous fraction and adrenaline in these experiments were $0{\cdot}74$ mg mL^{-1} (CL: 0·53–1·04; $n=7$) and $0{\cdot}99 \times 10^{-6}$ M (CL: $0{\cdot}77 \times 10^{-6}$–$1{\cdot}28 \times 10^{-6}$; $n=4$), respectively. Previous incubation of propranolol ($1{\cdot}5 \times 10^{-6}$ M) caused the concentration–response curves to shift to the right without changing the maximum response, increasing the EC50 values of both the aqueous fraction and adrenaline to $2{\cdot}6$ mg mL^{-1} (CL: 1·6–3·2; $n=7$) and $3{\cdot}2 \times 10^{-6}$ M (CL: $2{\cdot}2 \times 10^{-6}$–$4{\cdot}7 \times 10^{-6}$; $n=4$), respectively (Fig. 2b).

Extraction and identification of catecholamines

The retention times of noradrenaline, adrenaline and dihydroxybenzylamine were respectively 9·59, 11·85 and 14·02 min. Extraction of a mixture containing $0{\cdot}25\ \mu g\ mL^{-1}$ of each amine from alumina yielded 80·8%, 77·7% and 79·6% of the compounds, respectively.

After adsorption and extraction of 20 μL of the aqueous fraction (4 mg mL^{-1}) from alumina, the HPLC chromatogram showed major peaks with retention times of 6·66, 9·66 and 11·87 min corresponding to about 41% of the total area under the curve. The retention times of the last two peaks in the chromatogram were identical with those of noradrenaline and adrenaline, respectively (Fig. 3). When noradrenaline ($0{\cdot}25\ \mu g\ mL^{-1}$) was added to the aqueous fraction before extraction from alumina, the size of the peak with a retention time of 9·66 min was equivalent to an amount of noradrenaline equal to the amount of noradrenaline added plus that present in the fraction. A similar result was obtained when a mixture of adrenaline and the aqueous fraction was tested.

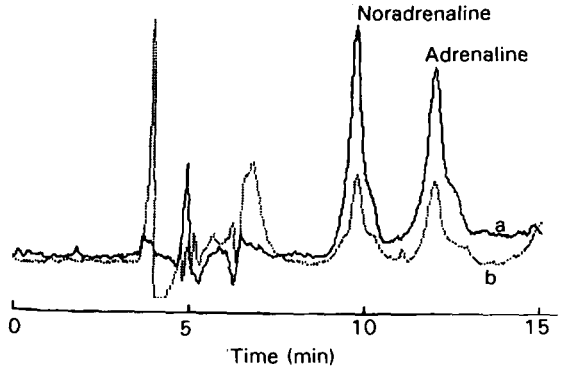

FIG. 3. Chromatograms obtained by injection of 20 μL of standards (a) of noradrenaline and adrenaline, and (b) the aqueous fraction of the ethanolic extract of *Scoparia dulcis* L.

Discussion

The ethanolic extract obtained from *Scoparia dulcis* L. has previously been shown to have analgesic and anti-inflammatory activity related to the presence of flavonoids and glutinol in the plant (Freire et al 1993). When administered intraperitoneally the extract induced exophthalmia and piloerection in rats, reflecting the presence in the plant of substances endowed with sympathomimetic activity.

Intravenous injection of the extract induced dose-related hypertension in anaesthetized rats and dogs. Oral administration of the extract did not, however, affect the blood pressure of either anaesthetized or conscious rats. Previous administration of α-adrenoceptor antagonists reduced the hypertensive effect of the extract, indicating a sympathomimetic action exerted either post-junctionally at the adrenergic receptors, or induced by reflex activation. Whichever the site of action, this sympathomimetic activity could explain the stress signs observed in animals treated with the plant extract.

Parallel chemical studies showed that the plant extract contained water-soluble vasoactive substances, and that partition of the extract between chloroform and water increased the specific activity of the hypertensive fraction by a factor of 20. The purified aqueous fraction thus obtained produced hypertension both in rats treated with reserpine and in pithed rats, indicating post-junctional interaction of polar substances with peripheral adrenoceptors. The dose–response curves obtained with these animals for the aqueous fraction and noradrenaline were, in fact, always parallel, and post-junctional blockade of α_1-adrenoceptors reduced both effects to the same extent. The fraction was, however, about 100 times less potent than noradrenaline.

In the isolated rat vas deferens the aqueous fraction gave concentration–response curves parallel to those obtained for noradrenaline. On a weight basis, however, the fraction was about 700 times less potent than noradrenaline. The α-adrenoceptor antagonist prazosin inhibited the responses to noradrenaline competitively, but it reduced the maximum effect produced by the fraction, indicating non-competitive interaction with post-junctional α-adrenoceptors. Alternatively, the decrease in maximum response to the fraction could be related to inhibitory actions of other substances prevailing at high concentrations of the fraction.

In vas deferens preparations from reserpine-treated rats the concentration–response curves obtained for the aqueous fraction and for noradrenaline were identical, indicating that the effect of the fraction did not depend on the release of transmitter from nerve terminals. In addition, tyramine did not produce significant effects after the concentration–response curves for the fraction had been obtained, indicating that the contractile substances in the fraction were not taken up by the nerve terminals.

The aqueous fraction also increased the inotropism of guinea-pig left atria and caused relaxation of the tracheal musculature. Both effects were blocked by propranolol indicating that the sympathomimetic components of the fraction also interacted with β_1- and β_2-adrenoceptors.

The results obtained in the pharmacological studies encour-

aged further chemical purification of the extract. Analysis of the aqueous fraction by HPLC revealed the presence, among other substances, of catecholamines eluting at the retention times of noradrenaline and adrenaline. When standards of these compounds were co-chromatographed with the fraction their peaks were superimposed on those obtained from the fraction, and increased the peak heights and the areas under the peaks in proportion to the concentrations of both noradrenaline and adrenaline added.

Pharmacologically active amines (Udenfriend et al 1959; Evans & Bell 1980) including catecholamines (Waalkes et al 1958; Feng et al 1961) have also been detected in extracts from other plants. Their presence in *Scoparia dulcis* L. may help to explain the folk medicinal use of the plant extracts in the treatment of mucosae and eroded skin, such as haemorrhoids, insect bites and open wounds. This use is not, however, inoffensive because the effects are comparable with those produced by other vasoconstrictor agents. On the other hand, the effects obtained by topical application of the plant extract may be additive to the concurrent general anti-inflammatory activity. This vasoconstrictor effect cannot, nevertheless, account for the analgesic and anti-inflammatory activities of the plant extracts because the polar vasoactive fraction given orally did not produce hypertension, nor did it inhibit the rat paw oedema induced by known phlogistic agents (Freire et al 1993).

In conclusion, the presence of noradrenaline and adrenaline in the polar extract of the aerial parts of *Scoparia dulcis* L. may explain the hypertensive effect of the plant extract obtained in rats and dogs in-vivo, contraction of the isolated vas deferens (α_1 response), relaxation of the tracheal smooth musculature (β_2 response), and the increase of inotropism in heart muscle (β_1 response). Both the vasoactive α_1 response and inotropic β_1 effect may account for the hypertensive effect of the plant extract.

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