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JUSTICIA PECTORALIS: A STUDY OF THE BASIS FOR ITS USE AS A HALLUCINOGENIC SNUFF INGREDIENT

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Summary

The use of *Justicia pectoralis* var. *stenophylla* as a *Virola* snuff admixture and also as the sole ingredient of a snuff was investigated. Extracts of the plant did not contain alkaloids, although the ubiquitous compound, betaine, was isolated because of its reaction with alkaloid reagents. Nor did extracts have any significant effect upon the gross behavioral effects, or increased spontaneous motor activity, elicited in mice by 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT), the primary psychotropic constituent of the *Virola* resin snuff. Coumarin and umbelliferone were identified because they are major constituents of the plant and because of their ability to relax smooth muscle.

Introduction

The widespread use of tryptamine based hallucinogenic snuffs in the Orinoco and northern Amazon drainage areas has been well documented during the past century (Wassén, 1967). Bark resin of species of *Virola* (Myristicaceae) was reported as one of the sources of these snuffs as early as 1909 (Koch-Grünberg, 1909) and their uses are now well established (Schultes and Holmstedt, 1968). Not until 1953, however, was it known that there existed other important constituents of *Virola* based snuffs. The missionary, Barker (1953), and the anthropologist, Zerries (1960), described the addition of a herbaceous additive to the pulverized resin snuff. This plant was later identified as *Justicia pectoralis* var. *stenophylla* (Acanthaceae) (Schultes and Holmstedt, 1968). The addition of its dry, pulverized leaves to *Virola* snuffs is now well established as a custom which is widespread amongst the tribes of the Yanomamö group (Seitz, 1967; Schultes and Holmstedt, 1968; Chagnon et al., 1971; Prance, 1972; Brewer-Carias and Steyermark, 1976).

The reason for the inclusion of *Justicia pectoralis* in *Virola* snuffs is not

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understood. Several Indian informants have suggested that it is added because of its aroma (Seitz, 1967; Schultes and Holmstedt, 1968; Prance, 1972). There is evidence, however, that it possesses other properties (Schultes and Holmstedt, 1968). The reports that *Justicia pectoralis* constitutes the sole ingredient of a snuff and that it produces a state of intoxication (Chagnon et al., 1971; Brewer-Carias and Steyermark, 1976; Prance, pers. commun.) are especially interesting.

The chemistry and pharmacology of the active constituents of *Virola* snuffs have been well studied (Holmstedt et al., 1980). In contrast, nothing is known of the chemistry of *Justicia pectoralis*. Moreover, no information on the chemical constituents of other American species of this genus appears to be available. This study of the possible physiological basis for the use of *Justicia pectoralis*, therefore, was undertaken.

Materials and methods

Plant material

The plant used in this study was collected in Pucallpa, Peru in 1981. It was identified as *Justicia pectoralis* var. *stenophylla* by Dr. Timothy Plowman of the Chicago Field Museum. Voucher specimens (D. McKenna No. 1) have been deposited at UBC herbarium, Chicago Field Museum, University of San Marcos herbarium, Lima and UNAP herbarium, Iquitos, Peru. It is the opinion of Prof. R.E. Schultes that the varietal name is used for a growth form of *Justicia pectoralis* (pers. commun.). The plant is hereon referred to, therefore, by only its species name. Plants were propagated vegetatively and grown under greenhouse conditions. Freshly cut leaves, flowers and stems of mature plants (1150 g) were washed, placed in boiling 100% ethanol and homogenized. The homogenates from four changes of ethanol were combined and concentrated by rotary evaporation in vacuo. Distilled water (500 ml) was added to the residue which was heated on a steam bath for 20 min and filtered through celite. The aqueous fraction was extracted continuously for 48 h with diethyl ether, then ethyl acetate. The diethyl ether, ethyl acetate and aqueous fractions accounted for 0.931, 0.478 and 42.3 g, respectively, of the original 1150 g fresh weight.

Chromatography and spectroscopy

Thin-layer chromatography (TLC) was carried out using Polygram Silica gel G UV_{254} (Brinkman) and cellulose (Eastman) precoated plates. High performance liquid chromatography (HPLC) separations were performed on a Varian MCH-10 reverse phase column and Varian Model 5000 HPLC with Varian Series 634 variable wavelength UV detector. Gas chromatography/mass spectrometry (GC/MS) data were obtained with a Finnigan 1020 GC/MS. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker

FT-100 instrument and infrared (IR) spectra were obtained using a Perkin Elmer 710B spectrophotometer.

Behavior experiments

Locomotor activity was measured in female Swiss mice using an adapted version of a "jiggle cage" (Robbins, 1977). An 18-cm cubic wire mesh cage was suspended from an isometric force transducer by elastic bands. The transducer signal was amplified 1000 times and monitored using a Spectrophysics SP 4100 integrator. A computer program which sampled the input voltage approximately every 100 ms, calculated the difference between successive signals and summed the absolute value of that difference over a 5-min period was written. This apparatus produced an average value for the degree of movement of the mouse, in arbitrary units, at 5-min intervals. It was sensitive to both locomotor activity and to fine body movements, although the former produced a proportionately larger signal.

Rat stomach strip experiments

The procedure used for evaluating the activity of the extracts on smooth muscle was based on that described by Vane (1957). Briefly, a male Wistar rat was killed by a blow to the head, the stomach was removed and the fundic region cut into a strip, 10—15 cm long by 1—2 mm wide, as described by Vane (1957). The stomach strip was suspended vertically in a 5-ml capacity bath containing Kreb's solution (composition in g/l: NaCl 6.9; KCl 0.35; $CaCl_2 \cdot 2H_2O$ 0.36; $MgSO_4 \cdot 7H_2O$ 0.29; KH_2PO_4 0.16; $NaHCO_3$ 2.1; dextrose 1.0) through which was bubbled continuously a mixture of 95% oxygen and 5% carbon dioxide. The lower end of the strip was fixed in position while the uppermost one was attached by a thread to the lever of a Harvard Isotonic Force Transducer (Model 363). A Harvard Apparatus Recorder (Model 350) and Harvard Chart Recorder (Model 480) were used to record the length of the muscle strip. The muscle preparations were perfused continuously from below at a flow rate of 2.5 ml/min.

Extracts, or compounds to be tested, were dissolved in Kreb's solution and used to perfuse the muscle strip for a period of 30 or 60 s. This was followed by a 5—10-min period of perfusion with Kreb's solution during which the muscle returned to a stable length prior to beginning the next assay.

Antimicrobial tests

A simple paper disc spot test was used to test for antimicrobial activity. Aqueous suspensions of bacteria or yeast were spread on Difco Bacto agar and Sabouraud's dextrose agar plates, respectively, using cotton swabs. Sterile filter paper discs, to which the extract to be tested had been applied,

were placed on the agar surface. The plates were examined for inhibition of growth around the filter paper discs after 24 and 48 h.

Results

Examination of Justicia pectoralis for alkaloids

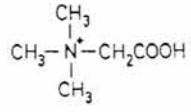
The reports that *J. pectoralis* is used, not only as an admixture to *Virola*, but as the sole ingredient of a snuff (Chagnon et al., 1971; Brewer-Carias and Steyermark, 1976; Prance, pers. commun.) suggest that this plant alone may have hallucinogenic activity. The known hallucinogens are alkaloids (Schultes, 1970). This fact, combined with preliminary evidence indicating that *J. pectoralis* may contain *N,N*-dimethyltryptamine (Schultes, 1970; 1972) indicated that the pharmacological activity of *J. pectoralis* may be attributable to the presence of indole or other classes of alkaloids.

The three *J. pectoralis* extracts were examined for alkaloids by TLC. Samples were applied to Silica gel G plates, developed with ethyl ether/2-butanone/6 N NH_4OH (5:4:1) (upper phase) and sprayed with Erlich's reagent (McKenna et al., 1984). *N,N*-Dimethyltryptamine (DMT), 5-OH-*N,N*-dimethyltryptamine and 5-MeODMT were included as standards. No Ehrlich's positive compound could be detected in any of the three extracts.

Each of the three extracts of *J. pectoralis* was tested for the presence of alkaloids using Valser's, Meyer's and Dragendorff's reagents for precipitation (Martello and Farnsworth, 1962). Only the aqueous fraction was positive, yielding a precipitate with each of the reagents. This fraction was chromatographed on cellulose thin-layer plates using *n*-propanol/0.3 N NH_4OH (19:1) as a developer. A single spot with R_f value 0.46 was observed after spraying with Dragendorff's reagent. It produced a distinct salmon-pink colour when sprayed with the Bregoff-Delwiche modification of Dragendorff's reagent which is specific for quaternary ammonium compounds (Stahl, 1969) and caused the deposition of platinum, forming a gray spot with iodoplatinate reagent for alkaloids (Stahl, 1969).

The compound (1) was purified by column chromatography on silica using *n*-propanol/0.3 N NH_4OH (3:1) as eluant and was crystallized from aqueous methanol. The following spectral data were obtained.

Compound 1 (betaine): UV λ_{max}^{MeOH} 215 nm; IR ν_{max}^{KBr} cm^{-1} 3250 br, 2900, 1650, 1480, 1400, 1330, 980, 930 and 880; ^{1}H-NMR (100 MHz, D_2O) δ 3.9 (2H, s), 3.31 (9H, s).



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The IR spectrum was identical to that reported for betaine (carboxymethyl-trimethylammonium hydroxide inner salt) (Poucert, 1981) and the 100 MHz ^{1}H-NMR spectrum matched that of an authentic sample (Sigma).

Compound 1 co-chromatographed with betaine both on cellulose TLC and by HPLC (retention time = 4.7 min using 2% acetonitrile/H_2O with a flow rate of 1 ml/min). On the basis of this evidence, it was concluded that compound 1 was betaine.

Behavioral effects of Justicia pectoralis

The first approach to the ethnopharmacology of *J. pectoralis* established that hallucinogenic alkaloids could not account for the use of this plant as a snuff. This did not eliminate the possibility that a non-alkaloid psychotomimetic compound might be present. To approach this question, behavioral changes in mice injected with *J. pectoralis* extracts were observed. A variety of behavioral changes are known to accompany the administration of psychotomimetic agents to rodents. Alterations in spontaneous locomotor activity are commonly observed (Hollister, 1982). The aqueous, ethyl acetate and diethyl ether extracts of *J. pectoralis* were administered intraperitoneally to mice at a dose of 250 mg/kg. Behavioral changes were observed and locomotor activity was recorded. The results of the spontaneous locomotor recording are presented in Table 1. Each extract caused a reduction in activity. Mice adopted a huddled posture, often with eyes closed, and displayed piloerection. The recording of activity level provides a quantitative estimate of the reduction in activity. The ethyl acetate fraction possessed the strongest of the relatively weak activities of the three extracts. None of the responses observed were indicative of the presence of psychotomimetic activity.

TABLE 1

EFFECT OF *JUSTICIA PECTORALIS* EXTRACTS ON SPONTANEOUS LOCOMOTOR ACTIVITY IN MICE

Extract injected[a]	% Normal activity level (min post-injection)											
	5	10	15	20	25	30	35	40	45	50	55	60
Aqueous	93	91	104	92	95	86	85	88	77	79	75	77
Ethyl acetate	96	107	101	64	52	61	58	44	53	57	51	59
Diethyl ether	92	94	98	91	94	87	83	88	77	82	75	88
25% Tween 80 (100 μl)	91	96	110	104	92	94	109	112	95	99	114	91

[a] Extracts were injected intraperitoneally at a dose of 250 mg/kg in 100 μl of 25% Tween 80.

Effect of Justicia pectoralis extracts on 5-MeODMT induced behavioral responses

There is extensive evidence that the use of *Virola* based snuffs is based on the hallucinogenic activity of its indole constituents. DMT and 5-MeODMT are, quantitatively, the most important indoles in most *Virola* barks (Agurell et al., 1969; Holmstedt et al., 1980). At least one of these two constituents is known from all of the *Virola* species used in the manufacture of snuffs and their presence in the snuffs themselves is well documented (Holmstedt and Lindgren, 1967; Agurell et al., 1969). 5-MeODMT is approximately ten times as potent a psychotomimetic as DMT (Shulgin, 1982). It would, therefore, be expected to account for most of the pharmacological effects of the snuffs. This tryptamine was chosen as a model compound for co-administration studies designed to test the hypothesis that some component of *J. pectoralis* was responsible for modulating the effects of the tryptamines.

5-MeODMT is known to cause a number of characteristic behavioral changes in rats injected intraperitoneally. Doses of 1 mg/kg produce excitation, hyperactivity, tremor and some salivation (Ahlborg et al., 1968; Grahame-Smith, 1971). The tremor produced by this compound is dose-dependent in the range 1—10 mg/kg (Ahlborg et al., 1968). Changes in activity levels produced in rats by 5-MeODMT (Ahlborg et al., 1968; Grahame-Smith, 1971) and in mice by DMT (Shah and Hedden, 1978) have been quantified in devices for measuring animal activity levels. The responses have been shown to be reproducible and to vary in a dose-dependent manner. This method was therefore chosen to examine the possibility that *J. pectoralis* contains some constituent that alters the behavioral response to 5-MeODMT.

Effect of 5-MeODMT on mouse activity

Solutions of 5-MeODMT (Sigma) were administered intraperitoneally using 2% aqueous Tween 80 (100 μl) as a vehicle. Activity levels were monitored at 5-min intervals for 60 min after injection. The results presented in Fig. 1 show that all doses of 5-MeODMT tested (0.31—12.5 mg/kg) produced an initial period of hyperactivity lasting 10—20 min. The degree of hyperactivity varied in a dose-dependent manner. In the case of a low (0.31 mg/kg) or a high (2.5 or 12.5 mg/kg) dose of 5-MeODMT, the activity levels returned almost to normal after 30 min post injection. Administration of intermediate doses (1.25 and 0.625 mg/kg; data not shown), in contrast, resulted in significant reductions in activity which persisted until at least 60 min post injection. Ahlborg et al. (1968) and Grahame-Smith (1971) observed very similar increases in activity in rats injected with 3 and 1 mg/kg of 5-MeODMT, respectively. The reduction in activity observed in the present study (50% normal activity level after 1.25 mg/kg and 70% normal activity level after 0.625 mg/kg 5-MeODMT; data not shown) have not previously

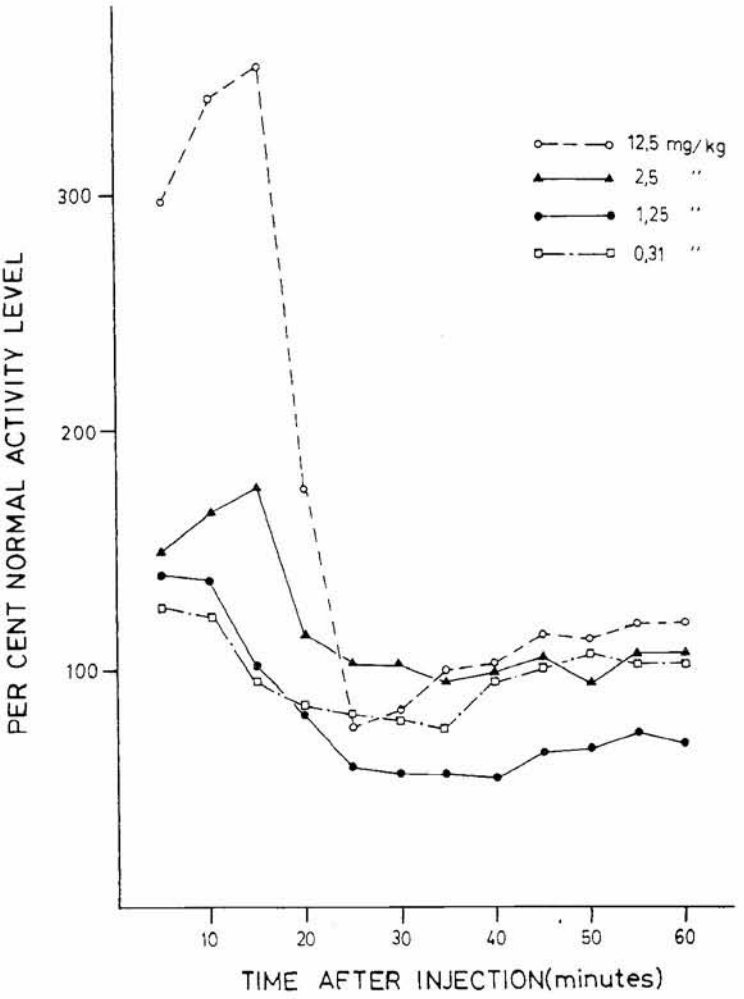


Fig. 1. Effect of 5-MeODMT on spontaneous locomotor activity of mice. Values are means, $N = 3$; S.E.M.s are not drawn but were all less than 5%.

been reported as an effect of 5-MeODMT administration. DMT, however, has been shown to produce a similar pattern of gradually decreasing activity levels following the administration of 2.5 and 25 mg/kg (Grahame-Smith, 1971). The reduction in activity was observed to last for approximately 60 and 120 minutes, respectively. The results presented here indicate that low doses of 5-MeODMT produce a similar reduction in motor activity but that, at high doses, activity levels increase markedly (3-fold at 12.5 mg/kg).

Gross behavioral effects of 5-MeODMT

Changes in behavior induced by 5-MeODMT correlate well with the effects

on motor activity presented in Fig. 1. A dose of 0.31 mg/kg resulted in an initial period of hyperactivity lasting approximately 10 min, followed by a period of roughly 60 min during which the animal lay quietly, displaying a flattened posture. As Shah and Hedden (1978) reported for DMT, fright responses were elicited when the animal was disturbed. At still higher doses (2.5 and 12.5 mg/kg), the mice adopted an extremely flattened posture with extension of the hindlegs and showed a series of multiple sudden backwards movements. This behavior was periodically and briefly interrupted when the animals crouched and pawed their noses. Gross locomotor activity was reduced but the number of fine body movements was markedly increased, compared to an untreated animal. Jerkiness, head twitching, rigidity and trembling, characteristically produced in mice by DMT (Shah and Hedden, 1978), were also observed. Approximately 20 min after injection, all of these behavioral effects gradually began to be reduced in frequency and severity. Activity levels slowly returned to normal.

Effect of co-injections of 5-MeODMT and J. pectoralis extracts

A dose of 5-MeODMT which would produce a marked elevation in activity level was selected to examine the *J. pectoralis* extracts for synergistic effects. Injections of 5 mg/kg 5-MeODMT, combined with 250 mg/kg of each of the three *Justicia* extracts, were made and the activity levels recorded. The results are presented in Table 2. Despite the fact that, alone, each extract caused slight reductions in activity level, there was no significant effect of any of the *Justicia* extracts upon 5-MeODMT induced hyperactivity. The

TABLE 2

EFFECT OF CO-ADMINISTRATION OF 5-MeODMT AND *JUSTICIA PECTORALIS* EXTRACT ON SPONTANEOUS MOTOR ACTIVITY OF MICE

Sample injected	Percent normal spontaneous locomotor activity (min post-injection)[a]					
	5	10	15	20	25	30
5 mg/kg 5-MeODMT + 250 mg/kg aqueous extract	176	204	204	128	108	105
5 mg/kg 5-MeODMT + 250 mg/kg ethyl acetate extract	155	194	204	126	101	95
5 mg/kg 5-MeODMT + 250 mg/kg ethyl ether extract	164	198	215	134	118	104
5 mg/kg 5-MeODMT	170	214	217	121	109	108
100 µl 25% Tween 80[b]	92	104	100	95	107	101

[a] Normal spontaneous locomotor activity was established for each mouse by measuring activity for 10 min prior to injection.
[b] Control.

observations of the gross behavioral responses were in agreement with the locomotor measurements. All of the previously mentioned behavioral effects of high doses of 5-MeODMT were observed after administration of 5 mg/kg 5-MeODMT, either alone, or in combination with any of the three *Justicia* extracts.

From this series of experiments it is concluded that the *J. pectoralis* extracts do not contain any constituent with a pharmacological activity that is comparable to that of the tryptamine hallucinogens. Nor do they contain compounds which are responsible for modulating the most obvious behavioral changes induced by 5-MeODMT. Constituents which inhibit the enzyme, monoamine oxidase (Bhattacharya et al., 1976; Holmstedt et al., 1980) and mixed function oxidases (Brattsten, 1979) are known to occur in higher plants. Although the existing data indicate that the former is the most important enzyme system for converting tryptamines to inactive metabolites (Grahame-Smith, 1971), there is evidence that the latter also plays a role (Ahlborg, 1968). Plant constituents with the ability to inhibit either enzyme system could possibly potentiate the physiological activity of tryptamines. The results obtained in the behavior experiments do not support this hypothesis for *J. pectoralis.* Any increase in the half-life of 5-MeODMT would be expected to be manifested in the behavioral responses observed. Neither the magnitude nor the duration of 5-MeODMT induced behavioral changes was affected by any of the three *J. pectoralis* extracts.

Effect of J. pectoralis extracts on smooth muscle

DMT and other tryptamine derivatives are known to antagonize the action of 5-hydroxytryptamine (serotonin) on smooth muscle (Barlow and Khan, 1959). This pharmacological activity correlates with hallucinogenic activity, suggesting that serotonin receptor affinity is central to the mechanism of action of these agents as hallucinogens (Glennon et al., 1980). The possibility that *J. pectoralis* could exert some subtle behavioral effect by either acting directly on the serotonin receptors or by competing for them with tryptamines, was considered. The rat stomach strip assay for serotonin receptors afforded a relatively easy means of testing this hypothesis.

A strip of smooth muscle prepared from the fundic region of a rat stomach responds to low concentrations of 5-hydroxytryptamine by contracting (Vane, 1957). Figure 2a shows a tracing of the length of such a rat stomach strip (RSS) exposed to 1 ng/ml 5-hydroxytryptamine (serotonin). The response was marked and reproducible. Muscle preparations were perfused with 1 ng/ml 5-hydroxytryptamine combined with one of the three *J. pectoralis* extracts at a concentration of 1 mg/ml.

The ether extract completely eliminated the 5-hydroxytryptamine induced contraction and produced a marked relaxation of the muscle. When the RSS was exposed to the ether extract alone, however, a similar relaxation was observed. In contrast, the aqueous extract alone elicited a strong and long

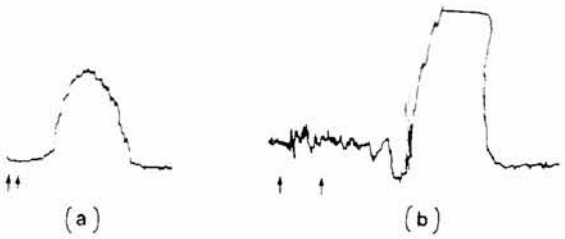


Fig. 2. Effect of (a) serotonin (1 ng/ml) and (b) betaine (50 μg/ml) on smooth muscle. Interval of perfusion of compound is delimited by arrows: chart speed 6 mm/min; amplification 16×.

lasting contraction at a concentration of 1 mg/ml. The spontaneous activities observed in the ether and aqueous fractions rendered an examination of these extracts for specific 5-hydroxytryptamine antagonism impractical. The ethyl acetate fraction produced no effect on the response of the RSS to 5-hydroxytryptamine.

A knowledge of the nature of the constituents responsible for the spontaneous activity on the smooth muscle preparations may shed light on the pharmacological activity of *J. pectoralis*. The aqueous and diethyl ether fractions were fractionated further in an attempt to isolate and identify the active compounds.

(i) Aqueous fraction

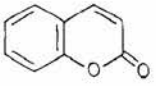
The aqueous fraction was fractionated as described in Materials and Methods. It was observed that contraction inducing activity was retained only by those fractions containing the quaternary ammonium compound, betaine, which had already been identified because of its reaction with reagents for alkaloid detection. A commercial sample of betaine hydrochloride (Sigma) was assayed for activity on the RSS. It was found to induce a strong and prolonged contraction. This effect was observed at relatively high concentrations (100 μg/ml) (Fig. 2b). Contractions were elicited at concentrations as low as 10 μg/ml, but at levels lower than this the contractions observed were slight and not reproducible. The concentration of betaine present in the aqueous extract of *J. pectoralis* was estimated, by comparison of TLC spot size with measured amounts of pure betaine, to be 2.4%. All of the smooth muscle contraction induced by the aqueous extract was attributable to the presence of this amount of betaine.

(ii) Ether fraction

An aliquot of the ether fraction was fractionated by column chromatography on silica using a chloroform/methanol gradient as the eluate. Smooth muscle relaxing (spasmolytic) activity was observed to be concentrated in two fractions. Crystalline compounds were obtained from these fractions. When applied to the RSS, each compound, at concentrations between 10 and 100 μg/ml, caused a marked increase in the length of the muscle. The

RSS rapidly returned to its resting length following the removal of the compounds from the medium (Fig. 3a and b). The following spectral data were obtained for the two compounds isolated from the diethyl ether fraction.

Compound 2 (coumarin): m.p. 67—69°C; UV λ_{max}^{MeOH} nm (log ϵ) 310 (3.70), 273 (3.70); MS *m/z* (rel. int.) 146[M$^+$] (72), 118 (100), 90 (52), 89 (53), 64 (12), 63 (41), 62 (16), 51 (13), 50 (12).



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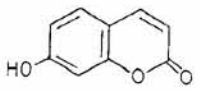
Compound 3 (umbelliferone): m.p. 223—225°C; UV λ_{max}^{MeOH} nm (log ϵ) 325 (4.22), 254 (sh), 242 (sh); IR ν_{max}^{KBr} cm^{-1} 3200 br, 1720, 1620, 1575, 1420, 1330, 1240, 1150, 910, 850; ^{1}H-NMR (100 MHz, acetone-D-6) δ 7.86 (1H, d, J = 12 Hz, H-4), 7.52 (1H, d, J = 8 Hz, H-5), 6.82 (2H, m, H-6 and H-8); MS *m/z* (rel. int.) 162[M$^+$] (67), 134 (74), 105 (39), 97 (18) 95 (14), 91 (14), 85 (13), 83 (14), 78 (35), 69 (38), 57 (32), 55 (40), 51 (32), 43 (100).



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On the basis of the melting points and the spectral data obtained, compounds 2 and 3 were identified as coumarin and 7-hydroxycoumarin (umbelliferone), respectively. This was confirmed by comparing their chromatographic behavior with that of authentic standards. TLC (toluene/acetone, 1 : 1 on silica gel) and high performance liquid chromatography (50% aqueous acetonitrile) were used.

A possible explanation for the inclusion of *J. pectoralis* in *Virola* snuff is that it contributes a desirable aroma (Seitz, 1967; Schultes and Holmstedt, 1968; Prance, 1972). A fragrant nature is indicated by one of its common names, "Jamaica garden balsam" (Schultes and Holmstedt, 1968). To establish the nature of the volatile constituents responsible for its aroma, samples of fresh and dried leaves and stems of *J. pectoralis* were subjected to steam distillation for 2 h. The distillate was analyzed by GC/MS. In the case of each sample, only a single constituent was present. This was identified, by its mass spectrum, as coumarin.

Quantitation of coumarins of J. pectoralis

The ether extract contained, as primary constituents, coumarin and umbelliferone. To provide quantitative information on the presence of these

Fig. 3. Effect of (a) coumarin (10 μg/ml) and (b) umbelliferone (10 μg/ml) on smooth muscle. Interval of perfusion of compounds is delimited by arrows: chart speed 6 mm/min; amplification 16×.

two important metabolites, various samples of *J. pectoralis* were analyzed by HPLC. Samples of young, green leaves, mature, pigmented leaves and flowers were collected, freeze dried and extracted exhaustively with methanol. A sample of leaves collected from the plant while it was growing in Peru was also analysed. The methanolic extracts were evaporated in vacuo and partitioned between water and dichloromethane. The latter fraction was evaporated, dissolved in methanol and analysed by HPLC. The sample was chromatographed using 50% aqueous methanol (flow rate = 1 ml/min) and the absorbance was monitored at 250 nm.

The leaf extracts were observed to consist primarily of two compounds, coumarin and umbelliferone (Fig. 4). Samples were quantified using peak height of authentic samples of coumarin (J.T. Baker) and umbelliferone (K and K Laboratories). The results are presented in Table 3. The importance of coumarin and umbelliferone as secondary metabolites of this species of *Justicia* is clearly evident. Not only are they present at concentrations several orders of magnitude higher than the other UV absorbing compounds of the extract, but they appear, from this limited sampling, to be sequestered in the plant and reach higher levels in more mature leaves.

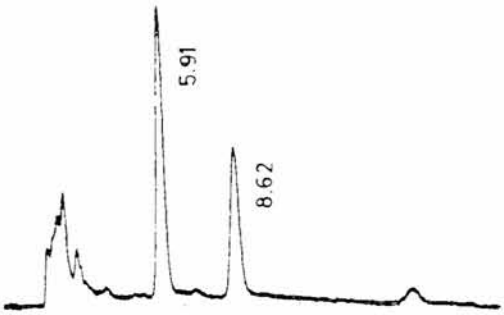

Fig. 4. HPLC chromatogram of the organic fraction of *Justicia pectoralis*. Flow rate 1 ml/min, isocratic, 50% MeOH, detection at 254 nm, chart speed 0.5 cm/min. Compound with R_t of 5.91 is umbelliferone; that with R_t of 8.62 is coumarin.

TABLE 3

LEVELS OF COUMARIN AND UMBELLIFERONE IN DIFFERENT SAMPLES OF *JUSTICIA PECTORALIS*

Sample analyzed	Concentration in percent dry weight[a]	
	Coumarin	Umbelliferone
Flowers	N.D.[b]	N.D.
Young leaves	0.42 (0.04)	0.30 (0.04)
Mature leaves	1.18 (0.08)	0.58 (0.05)
Leaves (Peru)	0.93 (0.05)	0.44 (0.04)

[a] Figures are the average of three determinations; standard deviation of mean in parentheses.
[b] N.D. = not detectable.

Examination of J. pectoralis for lignans

Of the seven species of *Justicia* that have previously been analysed chemically, six are known to contain lignans as major constituents (Ohta and Munakata, 1970; Okigawa et al., 1970; Ghosal and Banerjee, 1979; Ghosal et al., 1980; Olaniyi and Powell, 1980; Olaniyi, 1982). All but one of the 21 lignans identified from *Justicia* species contain the methylenedioxyphenyl moiety. To determine whether lignans containing this moiety were present in *J. pectoralis*, the chromotrophic acid spray reagent described by Beroza (1963) was used to examine TLC separations of the three *Justicia* fractions. Only one compound, present in the diethyl ether fraction, reacted with this reagent. It was isolated and its spectral data were obtained. Its melting point (132–135°C), ^{1}H-NMR, UV and mass spectrum were identical to those obtained for an authentic sample of β-sitosterol (Sigma). Furthermore, the behavior of the unknown compound on two chromatographic systems (TLC, R_f = 0.45 on Silica gel developed with chloroform/acetone (95:5) and HPLC, R_t = 6.10 min eluted with 25% aqueous methanol, 1 ml/min) was identical to that of an authentic sample of β-sitosterol.

The diethyl ether and ethyl acetate fractions of *J. pectoralis* were examined for the presence of justicidin B and diphyllin, lignans known from several other *Justicia* species. Samples were chromatographed on Silica gel G using toluene/acetone (1:1) as developer. Diphyllin and justicidin B had R_f values of 0.57 and 0.79, respectively. Both displayed a strong blue fluorescence and reacted with chromotrophic acid reagent. No evidence for the presence of either compound in the *J. pectoralis* extracts was obtained.

The chemistry of *J. pectoralis* appears to be quite different from that of any of the other species in this genus that have been examined. The apparent absence of lignans is of note. This difference may reflect phylogenetic distance since all of the species previously studied are native to Asia or Africa.

Screening for other biological activities

Although *J. pectoralis* is best known as a snuff admixture, there is considerable ethnobotanical information indicating that it has multiple uses. In the Colombian states of Vaupés and Amazonas, it is known to the Puinave Indians as *yakayú* and is used by them in the form of a decoction to treat pulmonary infections and pneumonia (Schultes, 1978). It has been said to be effective in the treatment of infections of the nasal cavity (N. Maxwell, pers. commun.). A herbarium specimen collected by José J. Triana is accompanied by a herbarium label stating that it is widely used in Meta, Colombia as a decoction to treat children afflicted with rickets (Schultes, 1978).

J. pectoralis is a relatively well known medicinal plant in Central America and the Caribbean (Morton 1977). It is used as a pectoral tea to relieve coughs (Wong, 1976), an expectorant (Roig y Mesa, 1945) and a wound poultice (Stehle and Stehle, 1962). It has also been reported to have sudorific and aphrodisiac effects (Morton, 1977).

It is possible that the use of *J. pectoralis* in the treatment of chest infections or wounds is dependent upon antimicrobial activity. This hypothesis was examined by testing extracts of the plant for inhibitory activity against a variety of microorganisms. The three fractions were tested against the gram-positive bacterium, *Staphylococcus aureus*, the gram-negative bacterium, *Escherichia coli*, the yeasts, *Candida albicans* and *Saccharomyces cerevisiae* and the dermatophytic fungi, *Microsporum canis*, *M. gypseum*, *M. fulvum* and *Trichophyton gallinae*. None of the extracts had any effect upon the growth of the bacteria or yeasts tested. The diethyl ether extract, however, exerted a strong inhibitory effect upon the growth of each of the dermatophytic fungi. Their growth was completely inhibited at a concentration of 1 μg/ml. (Neither coumarin nor umbelliferone was responsible for this activity.)

Discussion

Chemical and biological studies of *J. pectoralis* provided no evidence to support the hypothesis that the plant contains hallucinogenic constituents. Nor did *J. pectoralis* extracts have any detectable effect upon the behavioral responses of mice to the hallucinogen, 5-MeODMT. Three compounds with biological activity were isolated and identified: betaine, coumarin and umbelliferone.

Betaine is widely distributed in plants and animals (Guggenheim, 1951). Its only known function is as a source of methyl groups for the remethylation of homocysteine to methionine via the enzyme betaine-homocysteine methyl transferase (Shapiro and Schlenk, 1965). Few studies of a pharmacological nature have been published concerning betaine. The first report was that of Hunt and Renshaw (1929) who examined the effects of a series of derivatives of betaine on the autonomic nervous system. Most derivatives

were observed to have a muscarinic effect, i.e. to reduce blood pressure and heart rate. Their consideration of the parent compound, betaine, is brief and restricted to the statement that it is "practically without action on the autonomic nervous system." A fraction of an extract of the plant, *Pluchea lanceolata* (Compositae), containing betaine as a major constituent, has been reported to induce contractions in isolated smooth muscle preparations (Prasad et al., 1965; Dasgupta, 1967; Dasgupta et al., 1968). Furthermore, the extract potentiated barbiturate induced hypnosis in rats (Prasad et al., 1965) and possessed anti-inflammatory activity (Prasad et al., 1966). The smooth muscle contraction inducing effect of the crude extract is in close agreement with the results presented in the present study on pure betaine. To what extent the other properties of the crude extract can be attributed to betaine is not known. More recently, it has been reported that betaine is a non-specific anti-convulsive agent (Freed et al., 1979). At high doses, it was capable of blocking convulsions induced by electric shock as well as chemical convulsants.

Coumarin and umbelliferone, like betaine, are distributed widely. They are present in bacteria, fungi and a broad range of plants (Karrer, 1958). A smooth muscle relaxing, or spasmolytic, action has been reported previously for coumarin (Patyra, 1963a,b) as well as umbelliferone (Achterrath-Tuckermann et al., 1980) and this appears to be a feature that is common to many coumarin derivatives (Lee and Fujiwara, 1971; Ojewole and Adesina, 1983a,b). Because of their effect on the smooth muscle of the vascular system, some coumarins (e.g. coumarin and scopoletin), are responsible for a lowering of heart rate and blood pressure in animals (Ojewole and Adesina, 1983a,b). Coumarin, and some of its derivatives, possess the ability to reduce body temperature (Kitagawa and Iwaki, 1958). At doses approaching the toxic level, coumarin has sedative and hypnotic activity (Kreitmair, 1949; Ito et al., 1951; Kitagawa, 1956a,b). Both of these actions could possibly be related to the ability of these compounds to inhibit mitochondrial respiration (Kitagawa, 1956a; Pai et al., 1975). Coumarin is also effective as an anti-inflammatory agent, acting on macrophages to stimulate phagocytosis (Koh and Willoughby, 1979). Finally, coumarin is a volatile and sweetly aromatic compound and has many commercial applications as an aromatic additive (Kirk and Othmer, 1979). It is the sole ingredient responsible for the aroma of *J. pectoralis*.

The contribution of these varied biological activities to the overall effect of *J. pectoralis* snuff is not clear. The sedative and hypnotic activity observed for coumarin is interesting in this respect. Administered parenterally to small animals, it produces sedation and analgesia at doses of 50 mg/kg. Although it is unlikely that such high doses may be achieved during snuff taking, the possibility that localized regions of the head may be exposed to pharmacologically active levels as a result of its absorption across the nasal mucosa should be considered. Local analgesia or sedative/hypnotic effects could be induced at lower doses than if the compounds were administered parenterally.

The ability of scopoletin to reduce blood pressure (Ojewole and Adesina, 1983b) is also of interest. Tryptamine administration is often accompanied by transient increases in blood pressure (Szara, 1956). It is not known whether this effect is a direct one or whether it is mediated by psychological changes. The possibility that coumarin or umbelliferone could lower blood pressure and thereby reduce one of the stressful aspects of the tryptamines, is intriguing.

In conclusion, we found no evidence to support the belief that *Justicia pectoralis* is a hallucinogenic plant. Nor does it appear to directly affect the behavioral effects of 5-MeODMT, the hallucinogenic tryptamine of *Virola*, with which it is combined as a snuff. The possibility that courmarin and umbelliferone contribute to the pharmacological effects of the snuff has been raised.

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