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IDENTIFICATION OF TRYPTAMINE DERIVATIVES IN *RANUNCULUS SCELERATUS* L.

BY

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Ranunculus sceleratus L. (marsh-crowfoot) is an erect annual herb found on the banks of rivers in northern India. The leaves of this plant cause dermatitis, raise blisters on the skin and were formerly used by professional beggars to produce or maintain open sores and blisters to gain sympathy (Chopra, Chopra, Handa & Kapur, 1958). It was thus thought interesting to investigate this plant for pharmacologically active substances, namely histamine, acetylcholine, and 5-hydroxytryptamine, which are found in other plants that cause irritation (see Emmelin & Feldberg, 1947; Bowden, Brown & Batty, 1954; Collier & Chesher, 1956; Saxena, Kishor, Pant & Bhargava, 1964; Saxena, Kishor, Pant & Bhargava, 1965).

METHODS

The leaves and the stem of the fresh plants of *Ranunculus sceleratus*, obtained locally from the banks of the River Gomti, were stored at 0 to 4° C until extraction in acetone. The acetone was subsequently evaporated at room temperature (28 to 35° C) and the volume was made up with 0.9% saline to make a 100% w/v extract of the plant (*p*H 7.2). Some of this extract was boiled with strong hydrochloric acid to destroy pharmacologically active substances other than histamine (Gaddum, 1959). The acid was removed by distillation and the *p*H and the volume were adjusted to the original with dilute sodium hydroxide solution.

The acetone-extracted material of the plant, thus obtained, was tested on the blood pressure of cats (2.5 to 3.5 kg body weight) anaesthetized with pentobarbitone sodium (30 mg/kg, intraperitoneally), vagotomized and maintained on artificial ventilation. The blood pressure was recorded on smoked kymograph-paper by means of a mercury manometer from a common carotid artery. An indwelling polyethylene cannula was left in a femoral vein for injection.

The effects of the extracts were also studied on atropinized guinea-pig ileum, frog rectus-abdominis muscle and atropinized oestrus rat uterus preparations using a 10-ml. organ-bath containing appropriate bathing fluids.

Paper chromatography

The acetone-extracted material (100% w/v) was concentrated threefold *in vacuo*. Four chromatograms, each containing 0.5 ml. of the concentrated extract (on the left-hand side of the paper), and 10 μg of 5-hydroxytryptamine as a control (on the right-hand side of the paper), were subjected to unidimensional descending paper chromatography using upper phase obtained from *n*-butanol : acetic acid : water (4 : 1 : 5 v/v) as the solvent at 35° C. The chromatograms were scanned in ultraviolet light and the

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fluorescent spots were outlined by pencil. The colour of their fluorescence was noted and the bands were numbered from R_1 to R_7. The remaining region of the chromatograms, on the side of the extract, was again divided into several bands (R_8 to R_{12}) by drawing a pencil line transversely across the paper every 6 cm starting from the solvent front and working upwards. Thus, from the chromatogram of the extract twelve separate bands were obtained. Corresponding bands on the control side of the chromatogram were also marked, and were numbered from C_1 to C_{12}. The R_F values of the bands were then calculated.

The twenty-four bands (twelve on each side) of one of the four chromatographic strips were cut separately, eluted in 6 ml. of de Jalon fluid and subsequently tested on the oestrus rat uterus preparation. The three remaining chromatographic strips were treated with one of the following reagents: ninhydrin-acetic acid reagent (Jepson & Stevens, 1953); sulphanilic acid reagent (Smith, 1960); and Ehrlich's reagent (Jepson, 1960). The strip treated with the ninhydrin-acetic acid reagent was again scanned in ultraviolet light and the colour of the fluorescence was recorded.

The drugs used in the present study include 5-hydroxytryptamine creatinine sulphate, histamine acid phosphate, acetylcholine chloride, tryptamine hydrochloride, bromolysergic acid diethylamide (BOL, Sandoz) and atropine sulphate. The weights given are those of the salts.

RESULTS

Histamine and acetylcholine

The acetone-extracted material of *Ranunculus sceleratus* did not show appreciable effects on the cat blood pressure and the frog rectus abdominis muscle preparation, in amounts up to 3 ml. The same amount of acid-treated extract also failed to elicit a response of the guinea-pig isolated ileum.

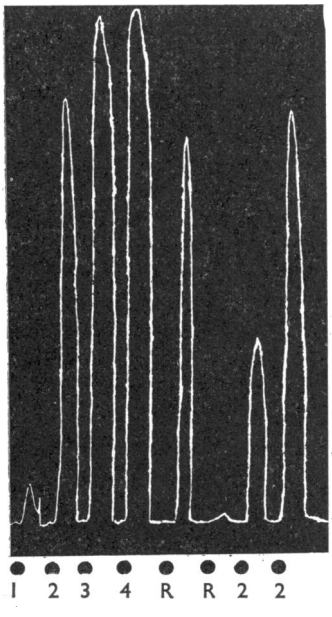

Fig. 1. Rat uterus suspended in 10 ml. of de Jalon solution containing atropine (10^{-8} g/ml.). The fluid in the bath was changed after every test response. 1 to 4 show the responses to 5-hydroxytryptamine (0.025, 0.05, 0.10 and 0.20 μg) added to the bath. At R, 1 ml. of acetone-extracted material of *Ranunculus sceleratus* was added in the bath. Subsequent addition of the same dose (1 ml.) of *Ranunculus* did not produce a contraction of the uterus. Partial blockade of the response to 5-hydroxy-tryptamine (0.05 μg) also occurred.

5-Hydroxytryptamine and other tryptamine analogues

The effects of the acetone-extract were observed on the oestrus rat uterus preparation. In Fig. 1 the numerals (1 to 4) show control responses of the rat uterus to 5-hydroxytryptamine (0.025 to 0.2 μg). At R, 1 ml. of the acetone-extracted material of the plant was added to the bath. A contraction of the uterus was produced by the extract but subsequently the same (1 ml.) dose of the extract was ineffective. The control response to 5-hydroxytryptamine (at 2) was also reduced, but recovery soon occurred.

The results of paper chromatography, summarized in Table 1, show the presence of several substances in the plant extract. The R_F values reported here are the arithmetical mean of three experiments.

TABLE 1

RESULTS OF CHROMATOGRAPHY WITH ACETONE-EXTRACTED *RANUNCULUS SCELERATUS* L.

Descending paper chromatography was performed with 0·5 ml. of concentrated acetone-extracted plant material (300% w/v) and control 5-hydroxytryptamine (10 μg) in *n*-butanol: acetic acid: water (4: 1: 5 v/v), as solvent, at 35° C. C_3 was the control 5-hydroxytryptamine band. Other control bands did not show any fluorescence or colour reaction

		Fluorescence in ultraviolet light		Colour reaction with		
Band	R_F	Without reagent	After ninhydrin-acetic acid reagent	Sulphanilic acid reagent	Ehrlich's reagent	Inference
C_3	0·66	Intense greenish-blue	Intense greenish-blue	Pink	Purple	5-Hydroxytryptamine
R_1	0·85	Blue	Intense blue	Nil	Nil	Tryptamine analogue
R_2	0·76	Blue	Intense blue	Nil	Nil	Tryptamine analogue
R_3	0·66	Greenish-blue	Greenish-blue	Light pink	Light purple	5-Hydroxytryptamine
R_4	0·55	Light blue	Blue	Cherry-red	Pink	Hydroxytryptamine analogue
R_5	0·43	Light blue	Blue	Cherry-red	Light pink	Hydroxytryptamine analogue
R_6	0·32	Greenish-blue	Greenish-blue	Yellowish-brown	Light pink	Hydroxytryptamine analogue
R_7	0·25	Pale green	Bluish green	Cherry-red	Light pink	Hydroxytryptamine analogue
R_8	0·95	Nil	Nil	Nil	Nil	Nil
R_9	0·50	Nil	Nil	Nil	Nil	Nil
R_{10}	0·16	Nil	Nil	Nil	Nil	Nil
R_{11}	0·10	Nil	Nil	Nil	Nil	Nil
R_{12}	0·03	Nil	Nil	Nil	Nil	Nil

The eluates from the chromatographic spots were tested on oestrus rat atropinized uterus. Fig. 2 shows the effects of 1.5 ml. of eluate of each of fluorescent bands R_1 to R_7. Only band R_1 elicited a contractile response which matched with 0.05 μg of 5-hydroxytryptamine (at HT). Changing the order of application of extracts did not modify the results. The responses to R_1 and to 5-hydroxytryptamine were completely blocked by bromolysergic acid diethylamide (1 μg) added to the bath (at the arrow). Seven bands of the paper (R_1 to R_7) fluoresced in ultraviolet light and five of these (R_3 to R_7) were coloured by sulphanilic acid reagent and by Ehrlich's reagent. A positive response on the rat uterus was obtained only with the eluate from band R_1. The activity of this band, however, could not be due to 5-hydroxytryptamine because the fluorescence characteristics, colour reactions

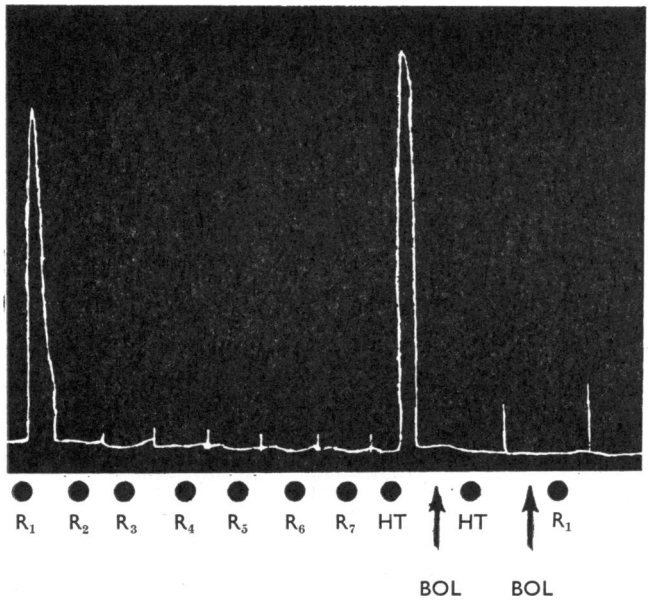

Fig. 2. Preparation as for Fig. 1. 1.5 ml. of eluates from the different fluorescent bands (R_1 to R_7) of the plant, *Ranunculus sceleratus*, were added to the bath at R_1 to R_7. At HT, 0.05 μg of 5-hydroxytryptamine and, at the arrows, 1 μg of bromolysergic acid diethylamide (BOL) were given. Only eluate from the band R_1 produced a contraction of the uterus which was blocked by bromolysergic acid diethylamide.

and R_F value were different from those of control 5-hydroxytryptamine experiments. The lack of a spasmogenic response of the rat uterus to the various indolic bands could be due to insufficient quantities of the active substance(s) in the eluates. The priming dose technique (Woolley & Campbell, 1962) was, therefore, used to test the presence of 5-hydroxytryptamine. Fig. 3 shows that the priming dose (P) of 5-hydroxytryptamine (0.025 μg) and 1 ml. of each of these eluates (R_1 to R_7), given separately, did not elicit an appreciable response. When the priming dose of 5-hydroxytryptamine was combined with the eluate of each of the bands they produced appreciable contractions (*a* to *g*) and could be compared with the contraction produced by a double priming dose of 5-hydroxytryptamine (*h*). However, this finding does not necessarily mean that the eluates contained subthreshold amounts of 5-hydroxytryptamine since, in another experiment, it was observed that 0.5 μg of tryptamine which was ineffective on the rat uterus preparation produced a contraction when primed with 5-hydroxytryptamine (0.025 μg). There was no antagonistic activity for 5-hydroxytryptamine in these bands.

In Fig. 4 are shown the submaximal and threshold responses to 5-hydroxytryptamine (0.05 μg at 1 and 0.025 μg at 2) on the oestrus rat uterus preparation. Eluate from band R_8 (2 ml.) was mixed with the threshold dose of 5-hydroxytryptamine (0.025 μg) and added to the bath at 3 and, similarly, the same amount of eluate from band R_8 was added at 4 in combination with 0.05 μg of 5-hydroxytryptamine. Instead of obtaining a priming effect, as with other bands (R_1 to R_7), an inhibitory effect was observed on the responses to 5-hydroxytryptamine (compare 2 with 3 and 1 with 4). Recovery of the control response

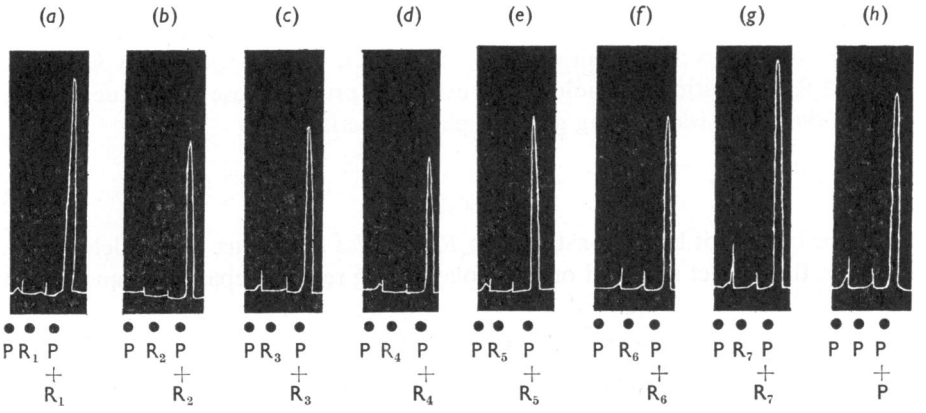

Fig. 3. Preparation as for Fig. 1. P, Priming dose of 5-hydroxytryptamine (0.025 μg); R_1 to R_7 indicate responses to 1 ml. of corresponding eluates from the chromatographic bands. The priming dose and R_1 to R_7, when added individually, do not elicit a response, but when the eluates are combined with P there is a response (*a* to *g*). Double the priming dose of 5-hydroxytryptamine (P+P) causes a contraction (*h*).

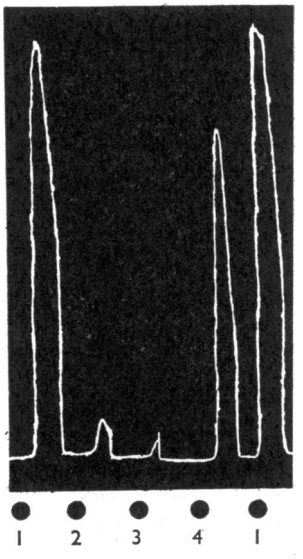

Fig. 4. Preparation as for Fig. 1. At 1, a submaximal response to 5-hydroxytryptamine (0.05 μg); at 2, a threshold response to 5-hydroxytryptamine (0.025 μg); at 3, 0.025 μg of 5-hydroxytryptamine mixed with 2 ml. of eluate from band R_8 of the chromatogram; at 4, 0.05 μg of 5-hydroxytryptamine with the same amount of R_8. Antagonism is apparent on comparing 3 with 2 and 4 with 1.

to 5-hydroxytryptamine was observed soon after washing the tissue. Similar results were obtained with band R_{12}.

The bands R_9 to R_{11} did not show any effect on the rat uterus by the priming dose method. The eluates from the corresponding bands on the control side of the chromatogram (C_1 to C_{12}) were also tested on the rat uterus preparation. Only the band C_3 elicited a spasmogenic response. This band also showed the characteristic fluorescence and colour

reactions of 5-hydroxytryptamine (see Table 1) and corresponded with the band R_3 obtained from the chromatogram of the plant extract. The 5-hydroxytryptamine content in the band R_3 was estimated biologically using the priming dose technique of Woolley & Campbell (1962) and was 0.16 μg/g of the plant material.

DISCUSSION

Histamine could not be demonstrated in *Ranunculus sceleratus*. Acetylcholine was also absent from the extract as tested on the isolated frog rectus preparation, and on cat blood pressure.

The crude acetone-extracted material of the plant contracted the rat uterus preparation but subsequent addition of the extract or 5-hydroxytryptamine did not show the spasmogenic response. This finding suggested the presence of one or more substances which stimulate the rat atropinized uterus and one or more substances which inhibit the response to 5-hydroxytryptamine. Another possibility is that the plant extract contains a tryptamine analogue which stimulates the rat uterus and also blocks it for a subsequent action of 5-hydroxytryptamine (Barlow & Khan, 1959). Hence paper chromatographic separation of the various constituents of the crude acetone-extracted material was necessary.

Chromatographic separation yielded seven bands of fluorescence which were clearer after treatment with ninhydrin-acetic acid reagent. This is a specific test for certain tryptamine derivatives which are not substituted at position 2, at the indolic nitrogen or on the nitrogen in the aliphatic side-chain (Jepson & Stevens, 1953). Thus chemically the fluorescent bands R_1 to R_7 were identified to be tryptamines. In order to establish further the nature of the constituents of the chromatographic bands, the colour reactions with sulphanilic acid reagent and Ehrlich's reagent were observed. The fluorescent bands R_1 and R_2 did not give the colour reaction to sulphanilic acid reagent and Ehrlich's reagent. Bands R_3 to R_7 gave positive reactions to these reagents and hence they must be hydroxyindoles. The negative colour reaction of bands R_1 and R_2 may be due to low sensitivity of the colour tests (Jepson & Stevens, 1953). The band R_3 of the extract corresponded with the control 5-hydroxytryptamine band (C_3) and also matched in other characteristics (see Table 1). Thus, band R_3 appears to be 5-hydroxytryptamine.

On testing the eluates from bands R_1 to R_7 on the oestrus rat uterus, only the eluate from band R_1 caused the contraction characteristically produced by 5-hydroxytryptamine. However, this band could not be 5-hydroxytryptamine because the R_F and fluorescence were quite different from control 5-hydroxytryptamine, yet this tryptamine derivative has a potent action on the receptors of 5-hydroxytryptamine in the rat uterus, as it is blocked by bromolysergic acid diethylamide. The tryptamines of the plant (bands R_1 to R_7) were further examined by the priming dose method of Woolley & Campbell (1962) which, according to the authors, is specific for 5-hydroxytryptamine. Our results show that several tryptamines contracted the rat uterus when primed with 5-hydroxytryptamine (0.025 μg). Obviously, all of these tryptamine analogues cannot be 5-hydroxytryptamine. Woolley & Campbell (1962) describe the priming dose of 5-hydroxytryptamine as the amount which just fails to contract the uterus and a subsequent addition of a small quantity of 5-hydroxytryptamine with the priming dose causes the uterine contraction. If we interpret this statement in terms of receptors, it could be assumed that the priming

dose is exciting a number of 5-hydroxytryptamine receptors in the rat uterus which is just short of that required to elicit a contraction of the uterus. The additional number of receptors are then excited by the small amount of 5-hydroxytryptamine which is subsequently added with the priming dose, and this brings about the effect. It is, therefore, reasonable to think that all substances which are able to act on the 5-hydroxytryptamine receptors in the rat uterus would also produce a contraction on being primed with 5-hydroxytryptamine. Many tryptamine analogues are already known to act on 5-hydroxytryptamine receptors (Barlow & Khan, 1959; Fastier, McDowall & Waal, 1959; Vane, 1959). The tryptamine analogues present in this plant seem to act similarly and, thus, were able to produce the contraction of the uterus when primed with 5-hydroxytryptamine. Moreover, tryptamine in a dose which alone did not contract the rat uterus, was able to do so on priming with 5-hydroxytryptamine. It may further be mentioned that neither acetylcholine nor oxytocin contract the rat uterus when similarly tested by the priming dose technique (unpublished observations). Thus, the priming dose method is not specific for 5-hydroxytryptamine, but the specificity may be for all substances which act on the 5-hydroxytryptamine receptors in the oestrus rat atropinized uterus preparation. Barlow & Khan (1959) observed a synergism between several tryptamine derivatives and 5-hydroxytryptamine which is comparable to the responses of the tryptamines observed by us with the priming dose method.

The eluates from the bands R_8 and R_{12} had an activity antagonistic to 5-hydroxytryptamine on the rat uterus. These substances may account for the antagonistic activity of the acetone-extracted material on rat uterus. The chemical nature of the anti-5-hydroxytryptamine substances cannot, however, be defined. If they are tryptamine analogues, they must be substituted at the nitrogen atoms or at position 2, since the fluorescence test of Jepson & Stevens (1953) was not positive with these substances.

The various tryptamine derivatives detected in *Ranunculus sceleratus* may represent substances involved in the synthesis and metabolism of 5-hydroxytryptamine in this plant and may have some physiological function.

SUMMARY

1. *Ranunculus sceleratus* L., a plant which causes dermatitis and raises blisters on the skin, has been shown to contain seven tryptamine derivatives; one of which is 5-hydroxytryptamine.

2. All of these tryptamines act on 5-hydroxytryptamine receptors in the rat uterus.

3. The plant has also two anti-5-hydroxytryptamine substances, the chemical nature of which is not known.

4. The priming dose technique of Woolley & Campbell (1962) does not appear to be specific for 5-hydroxytryptamine. Positive results are obtained with all the substances which act on the 5-hydroxytryptamine receptors in the rat uterus.

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