

Pharmacological Screening of Various Species of *Thalictrum* III

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Eleven different species of *Thalictrum* have been examined for hypotensive and smooth muscle relaxing effects. *Thalictrum minus* race B, *T. rochebrunianum*, and *T. rugosum* appeared to be the most promising and worthy of further investigation.

AS A continuation of a study of the genus *Thalictrum*, it was decided to screen extracts of 11 species for certain pharmacological actions which were described as characteristic of the genus in a previous paper (1). The intent of such a study was to determine which species merit more intensive investigation.

EXPERIMENTAL

Procurement of Plant Material.—The plants or seed were obtained as indicated in Table I. Plants of all the species, except those collected wild, are currently being cultivated in The Ohio State University College of Pharmacy Medicinal Plant Garden. Herbarium specimens have been made of each species.

Extraction Procedure.—Samples of powdered root, 34.5 Gm. each, were extracted to exhaustion with methanol. The methanol was evaporated off and the syrupy residue poured into dilute hydrochloric acid, pH 2. The insoluble residue was collected, redissolved in a small amount of methanol, concentrated, and again poured into dilute hydrochloric acid. This was repeated until it was evident that alkaloids were no longer being extracted by the acid solution as indicated by a negative test with Valser's T.S. The acid solutions of each sample were combined, made alkaline with ammonium hydroxide solution, and extracted with chloroform until a negative test was obtained with Valser's T.S. The chloroform was evaporated and the residue then extracted with a 5-ml. portion of dilute hydrochloric acid, pH 3, followed by three successive 4-ml. portions of the acid. The final volume of each was adjusted to 20 ml. by addition of dilute hydrochloric acid, pH 3.

Pharmacological Procedures and Results.—The extracts were tested for their ability to affect blood pressure in dogs, to relax the intestinal smooth muscle of the rabbit, and to depress the isolated rabbit heart.

Blood Pressure in Dogs.—Dogs of either sex, weighing 8 to 11 Kg., were anesthetized with 35 mg./Kg. of pentobarbital¹ i.p. The arterial blood pressure was recorded from the right carotid artery with the usual hemodynamic setup. The extracts were injected into the cannulated femoral vein in a dose of 0.2 ml./Kg. At least 2 dogs were used to test each extract.

T. aquilegifolium, *T. dioicum*, and *T. diptero-carpum* exerted no appreciable effect on the blood

pressure, but all other extracts produced some depressor response. *T. minus* race B, *T. rochebrunianum*, and *T. rugosum* appeared to be the most potent (Table II). In most cases tachyphylaxis was observed; that is, successive doses produced less blood pressure fall.

Effects on Rabbit Intestine In Vitro.—The classical Mangus method was used. Isolated pieces of intestine were suspended in a muscle bath in aerated Ringer's solution maintained at 37° and graded doses of the extracts added until a 50% reduction in muscle activity was obtained. The amount of extract required to produce this effect was considered a measure of potency (Table III).

Effects on the Isolated Rabbit Heart.—The isolated rabbit heart was perfused according to the method of Anderson and Craver (2) and 0.1 ml. of the extracts injected in the perfusion cannula. Most of the extracts, including *T. minus*, caused

TABLE I.—SOURCES OF *Thalictrum* SPECIES SCREENED

Species	Plant Part Supplied	Source
<i>T. aquilegifolium</i> L.	Plant	Wayside Gardens Mentor, Ohio
<i>T. dasycarpum</i> L.	Plant	Collected wild by Dr. Ervin M. Herrick, Twinsburg, Ohio
<i>T. dioicum</i> L.	Plant	Collected wild by Dr. Jack L. Beal, Columbus, Ohio
<i>T. diptero-carpum</i> Franch.	Plant	Sunbeam Farm Westlake, Ohio
<i>T. flavum</i> L.	Seed	Washington University College of Pharmacy, Seattle
<i>T. minus</i> L., race A ^a	Plant	The Ohio State University Department of Horticulture
<i>T. minus</i> L., race B ^a	Seed	Royal Botanic Garden Edinburgh, Scotland, through the University of Washington, College of Pharmacy, Seattle
<i>T. minus</i> var. <i>adiantifolium</i> Hort. (<i>T. adiantoides</i> Hort., <i>T. adiantifolium</i> , Bess.)	Plant	Sunbeam Farm Westlake, Ohio
<i>T. revolutum</i> L.	Plant	Collected wild by Dr. Jack L. Beal from banks of Scioto River, Delaware County, Ohio
<i>T. rochebrunianum</i> Franch.	Plant	Wayside Gardens Mentor, Ohio
<i>T. rugosum</i> Ait.	Plant	Wayside Gardens Mentor, Ohio

^a The identification was made by Dr. Bernard Boivin, Botanist, Central Experimental Farm, Plant Research Institute, Department of Agriculture, Ottawa Ontario, Canada.

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¹ Marketed as Nembutal by Abbott Laboratories, North Chicago, Ill.

TABLE II.—BLOOD PRESSURE EFFECTS IN THE DOG

Species	Dog No.	Dose, ^a ml./Kg.	Mean b.p., mm. Hg		Difference - Fall + Rise	Duration, Min.	Remarks
			Before	After			
<i>T. aquilegifolium</i>	B(3)	0.2	135	135	0	...	No apparent changes were observed
		0.4	140	140	0	...	
	B(11)	0.8	145	140	-5	2	
<i>T. dasycarpum</i>	B(6)	0.2	155	110	-45	3	
		0.2	145	100	-45	3	
	B(13)	0.2	155	120	-35	3	
<i>T. dioicum</i>	B(11)	0.2	115	124	+9	3	
	B(13)	0.4	155	165	+10	5	
<i>T. dipterocarpum</i>	B(4)	0.2	125	125	0	...	No apparent changes were observed
	B(5)	0.4	128	128	0	...	
<i>T. flavum</i>	B(12)	0.2	126	-98	-28	-2	Biphasic action
			+142	+16	+4		
	B(5)	0.2	126	-110	-16	-2	
			+146	+20	+5		
<i>T. minus</i> , race A	B(16)	0.2	126	56	-70	4	Subject to tachyphylaxis
	B(8)	0.2	150	87	-63	3	
<i>T. minus</i> , race B	B(14)	0.2	136	46	-90	20	Subject to tachyphylaxis
	B(10)	0.2	150	20	-130	42	
	B(8)	0.2	155	30	-125	35	
	B(9)	0.2	100	14	-86	15	
<i>T. minus</i> var. <i>adiantifolium</i>	B(1)	0.2	120	25	-95	5	
	B(2)	0.2	140	105	-35	6	
	B(16)	0.2	124	84	-40	5	
<i>T. revolutum</i>	B(4)	0.2	134	66	-68	4	Subject to tachyphylaxis
	B(17)	0.2	130	102	-28	3	
	B(1)	0.2	135	72	-63	6	
<i>T. rochebrunianum</i>	B(6)	0.2	130	56	-74	50	Subject to tachyphylaxis
	B(16)	0.2	125	48	-77	20	
	B(17)	0.2	145	20	-125	20	
<i>T. rugosum</i>	B(15)	0.2	155	20	-135	45	Subject to tachyphylaxis
	B(9)	0.2	128	50	-78	7	

^a Since all solutions were administered in an acid medium (pH 3), a control consisting of 0.2 ml./Kg. of pH 3 solution was injected into each dog. In every instance no appreciable effect on blood pressure was observed.

TABLE III.—EFFECTS ON RABBIT INTESTINE

Species	Tests, No.	Dose Required for 50% Inhibition, ml.	Approx. Relative Potency
<i>T. aquilegifolium</i>	4	Unattainable	0
<i>T. dasycarpum</i>	10	0.1	1
<i>T. dioicum</i>	4	1.0	1/10
<i>T. dipterocarpum</i>	4	Unattainable	0
<i>T. flavum</i>	5	0.1	1
<i>T. minus</i> , race A	4	0.1	1
<i>T. minus</i> , race B	3	0.2	1/2
<i>T. minus</i> var. <i>adiantifolium</i>	4	1.0	1/10
<i>T. revolutum</i>	4	0.2	1/2
<i>T. rochebrunianum</i>	4	0.1	1
<i>T. rugosum</i>	5	0.1	1

CONCLUSIONS

Extracts of *T. minus* race B, *T. rochebrunianum*, and *T. rugosum* showed considerable activity in all three pharmacological tests and seem to be the most promising candidates for further investigation. On the basis of these tests, *T. dipterocarpum*, *T. aquilegifolium*, and *T. dioicum* showed the least promise for further investigation. The authors were unable to confirm Ovsepyan's report (3) that *T. minus* causes cardiotoxic and pressor effect. In fact, our extracts produced just the opposite effect. Although *T. minus* race A and *T. minus* race B appear to be the same morphologically, there is a difference in the quantitative pharmacological effects of their extracts.

REFERENCES

a decrease in the heart rate and the amplitude of contraction but *T. dipterocarpum*, *T. aquilegifolium*, and *T. dioicum* produced no appreciable effect.

- (1) Patil, P. N., et al., *Lloydia*, **26**, 229(1963).
- (2) Anderson, F. P., and Craver, B. N., *J. Pharmacol. Exptl. Therap.*, **93**, 135(1948).
- (3) Ovsepyan, A. M., *Izvest. Akad. Nauk Armyan S.S.R.*, **9**, 56(1956); through *Chem. Abstr.*, **50**, 14111(1956).