

## STUDIES ON THE MEDICINAL PLANTS OF WEST PAKISTAN

Part I.—Chemical Investigation of *Inula Grantioides*

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A small, low, stout, woody shrub, known since ancient times for its poisonous properties, was collected from Thatta and Hyderabad where it grows wildly especially on small hillocks and sand dunes. In the month of December and January the plants flower with small yellow composite heads which fill the atmosphere with typical fragrance.

*Inula grantioides* first reported by Boiss<sup>5</sup> from Makran region (Fig.1) belongs to the family



Fig. 1.—*Inula grantioides*

*Compositae* and is widely distributed in Baluchistan, Multan, Ziarat, Sindh, Kuch, Waziristan and Karachi regions.<sup>7</sup> The plant has not an unpleasant balsamic odour and is 2-3 feet high, sometime may be several feet. The leaves are sessile or nearly so, 1-2", oblong, lobing, variable, toothed and succulent.

The plant bears numerous terminal corymb yellow heads  $\frac{1}{2}$ -1" in dia. with a solitary linear bract. Involucor, pubiscent, outer is short green and obtuse while inner involucor is long and acute.

Achenes are large and strongly ribbed silky, hisped (hairy) above and constricted below the tip.

The morphological studies were carried out when the crushed and powdered leaves were found as an unrecognizable adulterant for *Digitalis*. Especially *Inula conyza* and *Inula helenium*<sup>7</sup> are employed for this purpose.

## Experimental

The general experimental techniques were followed from Chopra<sup>1</sup> and Rosenthaler.<sup>6</sup> From the large quantities of the collections the flowers, leaves and stems were sorted out and treated separately for their essential oil contents. Both the steam distillation and solvent extraction (petroleum ether 60-80°C.) methods were employed. Percentage of oil in *Inula grantioides* parts was as under:

	Flowers	Leaves	Stems
<i>Fresh Parts</i>			
From Thatta ..	0.20	0.50	0.10
,, Hyderabad ..	0.30	0.41	0.07
<i>Dried Parts</i>			
From Thatta ..	0.001	0.20	tr.
,, Hyderabad ..	0.001	0.10	tr.

Thatta variety has been found to be slightly richer in this respect. The stems have been proved to be rather poor in oil contents and this too disappears as the wood is dried. The oil has very strong antibiotic properties and seems to be highly poisonous. *Drosophilla* when introduced in oil vapours died almost instantaneously, so were the silver fish, ants and mosquitoes. The local people keep the flowers and the twigs along with their clothes to be preserved for a long time. The oil has high refractive index i.e. 1.5870 at 20° C. and a low boiling point 90-92° C., does not solidify even at -10°C. The greenish yellow oil has a faint acidic reaction which may be due to the free acidic or phenolic components. The oil gives a negative test for sulphur and aldehydes and does not have any crystalline fraction even at -10°C. The empirical formula of the oil comes near to  $C_{18}H_{30-35}/O.2$

The leaves, stems and flowers were extracted by means of petroleum ether (60-80°C.) and concentrated under vacuum at a temperature not exceeding 60°C. The residue solidified to a dark brown gelatinous mass which gives a balsamic odour and is almost transparent.

The mass was again subjected to the action of various solvents, as under:

**Chloroform Fraction.**—Only a little of the portion, 2%, dissolves out from the crude mass giving a slightly pale brown solution which does not crystallize and appears to be homogeneous globules under the microscope. The mass has strong antibiotic properties and was studied by tube dilution method from an aqueous suspension of the resin in Czepak Dox liquid medium. The antibiotic properties upon the gram positive bacteria was found to be:—

Organism tested	Minimum inhibitory concentration (M.I.C.)
	μg./ml.
<i>Escherichia coli</i>	.. 0.5-0.7
<i>Streptococcus viridens</i>	.. 0.2-0.25
<i>Staphylococcus aureus</i>	.. 0.3
<i>Bacillus coli</i>	.. 0.2-0.3
<i>Bacillus subtilis</i>	.. 0.2-0.25
<i>Bacillus anthracococcus</i>	.. 0.3-0.5
<i>Bacillus diphtheria</i>	.. 0.4

The resin seems to have an empirical formula in which carbon varies from  $C_{26-28}$  and  $H_{50-60}$ , gives typical ketone reaction, precipitates with sodium hydrogen sulphite and reduces Fehling's solution. On heating with ammonia the resin condenses to a very hard mass.

**Petroleum Ether Fraction.**—White long needles separated from petroleum ether fraction which were found to be insoluble in almost all the organic solvents, viz., petroleum ether, chloroform, ether, acetone, benzene, butyl alcohol, pyridine and amyl alcohol. The crystals however are completely

soluble in aqueous alcohol and water, and do not seem to possess any antibacterial property, have a very high melting point, did not melt even above  $250^{\circ}C.$ , appear to be some inorganic compound in nature, give positive tests for sodium ion, but no acid radical has yet been confirmed, appear to be some silicate which is probably due to the xerophytic character of the plant.

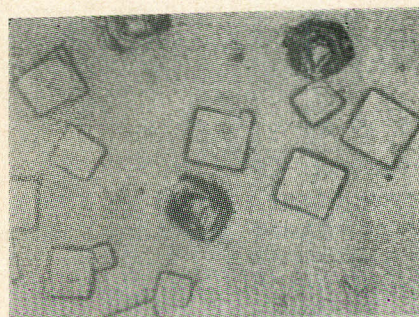


Fig. 2.—Translucent crystals.

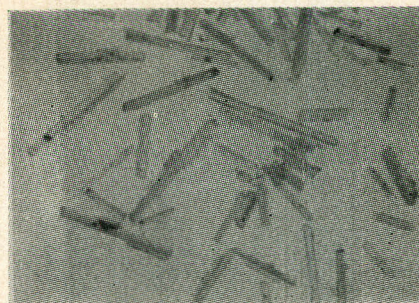
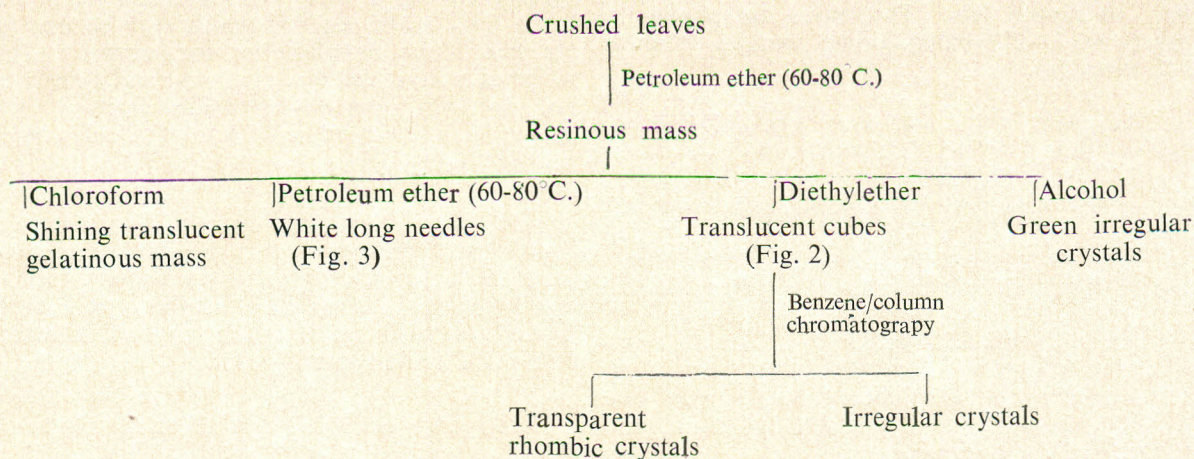


Fig. 3.—White long needles.



*Ether Fraction.*—Dull coloured cubes were found to be soluble in many of the organic solvents like benzene, petroleum ether, and butyl alcohol. The benzene solution was subjected to column chromatographic separation for any other possible fraction. The alumina column was soaked in benzene and after the solution was run, the column was developed by means of benzene at an approximate rate of 40 drops a minute. Two distinct fractions were obtained. A slightly greenish from which a few green irregular crystals were obtained. The other fraction, a comparatively bright solution gave rise to white rhombic crystals, with an approximate empirical formula  $C_8H_{15}NO_2$ , having a melting point of 207-210°C.

The crystals possess very strong antibiotic properties. These were recrystallized from methyl alcohol for further purification. These have a specific rotation of 165°. The antibiotic properties were studied both by tube dilution and streak methods.

The antibiotic property was first tested on agar plates. Several fungi and bacterial organisms were tested using cross streak tests in 10-cm. petri dishes containing about 20 ml. of glucose peptone agar medium. A solution of 10-20 µg./ml. strength was streaked by means of No. 2 camel hair brush upon set agar medium inoculated with the test micro-organism and the dish incubated for 72 hours at 30° ± 1°C. The zone of inhibition in case of the organism tested have been given below. The raising of the solution strength has a well-marked effect upon the antibiotic property.

Organism tested	Strength of the solution	
	10µg./ml.	20µg./ml
1. <i>Escherichia coli</i>	50 mm.	60 mm.
2. <i>Streptococcus viridens</i>	41 "	59 "
3. <i>Staphylococcus aureus</i>	45 "	50 "
4. <i>Bacillus coli</i>	40 "	58 "
5. <i>Bacillus subtilis</i>	43 "	58 "
6. <i>Bacillus anthracococcus</i>	40 "	53 "
7. <i>Bacillus diptheria</i>	48 "	49 "
8. <i>Aceto-bacillus casei</i>	48 "	49 "
9. <i>Streptomyces griseus</i>	35 "	40 "
10. <i>Aceto-bactor suboxy-dens</i>	35 "	40 "
11. <i>Mycobacterium tuberculosis</i>	35 "	39 "
12. <i>Clostridium acetobutylicum</i>	44 "	61 "

Boiling of the aqueous solution deteriorates much of this activity. After half an hour of

boiling, the antibacterial activity was not observed at all.

In view of the results obtained by the streak method, it was considered necessary to confirm by tube dilution method as well.

All the above mentioned micro-organisms were grown on the following liquid medium :

Glucose	..	1.5 g.
Peptone	..	0.4 "
Beef extract	..	0.2 "
NaCl	..	0.5 "
Agar	..	0.12 "
Water	..	100 ml.

The pH was adjusted to 7.2. Various dilutions were added to 10 ml. of the medium inoculated with the test micro-organisms and incubated for 72 hours at 30 ± 1°C.

Organism tested	M.I.C. of the compound µg./ml.
1. <i>Escherichia coli</i>	0.2-0.25
2. <i>Streptococcus viridens</i>	0.3-0.35
3. <i>Staphylococcus aureus</i>	0.2
4. <i>Bacillus coli</i>	0.15-0.3
5. <i>Bacillus subtilis</i>	0.1-0.2
6. <i>Bacillus anthracococcus</i>	0.4
7. <i>Bacillus diptheria</i>	0.3
8. <i>Lactobacillus casei</i>	—
9. <i>Acetobactor sub-oxydens</i>	—
10. <i>Streptomyces graseus</i>	0.25-0.4
11. <i>Myobacterium tuberculosis</i>	0.3-0.35
12. <i>Clostridium acetobutylicum</i>	0.2-0.25
13. <i>Saccharomyces cervisiae</i>	0.3-0.35

The M.I.C. did not increase to more than .4 µg./ml. in these cases.

*Alcohol Fraction.*—The greenish crystals appear to be of chlorophyll fraction or some carotenoid.

The stem and the flowers have also been subjected to similar solvent extraction treatments. The compounds isolated are under investigation.

It has been observed that the rabbits shirk the very odour of the plant, which is much poisonous to livestock and is not liked by any animal other than camels. The preparations from this plant have been reported by Nagai<sup>2</sup> in 1891 and Merz<sup>3</sup> in 1894 to be capable of paralysing both respiration and motor activities in animals.

### Summary

Various plant parts when subjected to steam distillation and solvent extraction gave essential oils and crystalline fractions. The oil has a refractive index of 1.5870, boiling point 90-92°C. and empirical formula comes out to be  $C_{18}H_{30-35}O_2$ . Petroleum ether (60-80°C.) extraction resulted in a resinous mass which on further fractionation by means of chloroform, petroleum ether (60-80°C.), ethyl ether and ethyl alcohol gives shining transparent gelatinous mass, white long needle-shaped crystals, translucent cubes and greenish irregular crystals respectively. The translucent crystals when subjected to column chromatography gave green irregular crystals resembling those of alcohol fraction and transparent rhombic crystals. The crystals have bacterio-static properties against almost all the avail-

able Gram positive and Gram negative bacteria.

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