THE DETECTION OF THE ALKALOIDS OF *LUPINUS TERMIS* IN VISCERA

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The seeds of the leguminous vegetable *Lupinus termis* (Family Papilionaceæ) form an important article of diet of the people of the Sudan and other Middle East countries, where they provide a valuable source of protein. The composition of the seeds has been shown to be as follows:—Moisture 3·2 per cent., oil 8·2 per cent., protein 38·9 per cent., carbohydrate 38·2 per cent., crude fibre 8·4 per cent., ash 3·1 per cent., providing a total calorific value of 392 kg. calories per 100 g. However, it has been shown by Clemo and Leitch[1] that these seeds contain the alkaloid lupanine, in the *d-* and *dl-* forms and according to Bamford[2] symptoms of poisoning occasionally arise which are attributed to the seeds when they are eaten without thorough washing. The fact that these seeds contain a bitter principle is of course well-known to those who include this material in their diet, but the alkaloid is readily soluble in water, and the bitter principle may easily be removed by soaking the seeds and washing thoroughly before cooking.

The properties of the lupin alkaloids, in particular lupanine, do not appear to have been very fully reported from the point of view of the toxicologist, and the purpose of the present investigation is to be able to detect its presence in post-mortem specimens, and to know exactly at what stage it appears when such specimens are subjected to the well-known Stas-Otto process and when the purified extracts are subsequently examined by the scheme for separation and identification for alkaloids described by Bamford[2].

The recorded colour tests for lupin alkaloids are as follows:—

Sparteine

(*a*) *Grant's Test*[3], as modified by Couch[4], is stated to be highly specific. A strip of filter-paper is moistened with a chloroform extract, allowed to dry, exposed to bromine vapour and then to ammonia vapour, and finally warmed. A bright pink colour is given in the presence of sparteine.

(*b*) *Jorissen's Test*[5] is said to be highly characteristic but requires about 10 mg., which is frequently impracticable in forensic work. However, a modification of this test is proposed which greatly increases its sensitivity. Take about 0·1 mg. of alkaloid in a small porcelain basin, and moisten with about 5 μl. of N sodium hydroxide. Add 2 ml. of ether and stir well with a small glass rod, add about 0·2 mg. of sulphur and again stir well. Pass hydrogen sulphide through a fine jet into the ether. A deep red turbidity is obtained in the presence of sparteine, and a red deposit remains when the ether volatilises.

(*c*) *Nascent Chlorine Test.* Bamford[2] classifies sparteine in Group VI in his scheme for identification of alkaloids using this test. When a little of the alkaloid is dissolved in a drop of concentrated hydrochloric acid with a trace of potassium chlorate, and evaporated to dryness, a "reddish purple" colour is stated to be obtained on exposing the residue to ammonia fumes. We were unable to obtain this reaction with either sparteine sulphate or with a sample of mixed alkaloids from *L. termis*, although both gave strongly positive reactions with Grant's test and with the modified Jorissen's test.

Lupinine

Chloranil Test[2]. An olive-green to brown residue is obtained in the presence of lupinine by mixing a little of the base dissolved in benzene, with a 1 per cent. solution of chloranil in benzene, and evaporating to dryness.

Preliminary Extraction and Separation of Lupin Alkaloids

120 g. of the seeds of *Lupinus termis* was ground and extracted with ethanol acidified with tartaric acid. The ethanol was removed by evaporating spontaneously at room temperature (25° C.), and the residue was extracted with acidulated water and filtered. The aqueous solution was then extracted in turn (*a*) with light petroleum to remove fatty material, (*b*) with ether, and (*c*) with chloroform after making ammoniacal. The ammoniacal chloroform extract was purified in the usual way, and the extracted alkaloids when tested gave the following results:—

General Alkaloidal Reagents

Wagner's reagent, Mayer's reagent, Marmé's reagent, Sonnenschein's reagent, Dragendorff's reagent, picric acid solution, gold chloride solution and mercuric chloride solution all gave strong reactions. Platinic chloride gave only a faint reaction.

Classification According to Bamford's Scheme

Group I—concentrated sulphuric acid—no colour.
Group II—Marquis' reagent—no colour.
Group III—Vitali's test—no colour.
Group IV—sulphuric acid-potassium dichromate—no colour.
Group V—ethanolic *p*-dimethylaminobenzaldehyde a brilliant crimson colour on warming. Residue is crimson after evaporation, turning deep orange with ammonia.

The alkaloids are therefore placed in Group V (*b*) together with nicotine, mescaline, and pilocarpine.

Further tests were made with the following results:—

Mecke's reagent—no colour.
Mandelin's reagent—no colour.
Fröhde's reagent—no colour.
Chloranil (1 per cent.)—deep brown to olive-green colour.
Nascent chlorine test—no colour.
Grant's test—positive.
Modified Jorissen's test—positive.

The acid-ether extract was also examined with the following results:—

Concentrated sulphuric acid—bright orange colour.

Marquis' reagent—strong purple-orange.

Mecke's reagent, Mandelin's reagent, Fröhde's reagent } as for sulphuric acid.

p-Dimethylaminobenzaldehyde—deep purple on taking to dryness on water bath. Brilliant violet on adding a drop of ethanol.

These results were confirmed on a larger scale by grinding and extracting 5 kg. of seed with 20 l. of acidified ethanol. The ethanolic extract was evaporated spontaneously at room temperature and the residue extracted with water. The extract was allowed to stand overnight in a separating funnel, when most of the oil (*c.* 100 ml.) separated to the surface. The lower layer was run off, and as it proved very difficult to filter, it was passed through muslin and was purified by treatment with basic and neutral lead acetate, allowed to stand, and filtered. The excess of lead was removed by treatment with hydrogen sulphide and filtration. The aqueous solution was evaporated to a small bulk by warming under a strong current of air. The aqueous solution was then given a preliminary extraction with ether, and then made alkaline to litmus with sodium hydroxide and extracted 5 times with chloroform, washing the extracts 3 times with water. The chloroform extracts were evaporated to dryness when 4·2 g. of crude alkaloids was obtained. The aqueous solution was then made alkaline to phenolphthalein and re-extracted with chloroform as before, when a further 0·07 g. of crude alkaloids was obtained.

The yellow colour remained in the aqueous (alkaline) layer, which was decolorised by treatment with animal charcoal and evaporated to small bulk. This solution gave no reactions for alkaloids.

Both extracts of crude alkaloids gave the same reactions with the general alkaloidal reagents and the colour reagents given above.

The mixed extracts were further purified by dissolving in acidulated water, extracting 3 times with chloroform, and rejecting the extracts, making alkaline to phenolphthalein with sodium hydroxide, and extracting 3 times with chloroform, and washing the combined chloroform extracts 3 times with water. The chloroform extracts were then shaken out 3 times with approximately 0·2 N hydrochloric acid. The aqueous solution was brown in colour, and was decolorised by adding 2 per cent. of animal charcoal, warming at 80° C. for 1 hour, and filtering, when a colourless solution was obtained. However, after making alkaline with sodium hydroxide, extracting with chloroform and removal of the solvent, the brown colour returned to the residue.

Partial Separation of the Mixed Alkaloids

A portion of the mixed alkaloids was extracted in turn with light petroleum (b.pt. 60° to 80° C.), benzene and acetone when the following fractions were obtained:—

Fraction I—soluble in light petroleum.

Fraction II—insoluble in light petroleum, soluble in benzene.

Fraction III—insoluble in benzene, soluble in acetone.
Fraction IV—insoluble in acetone.

Each fraction was re-extracted and redissolved with the appropriate solvents until fractions II, III, and IV were well separated. However, it was very difficult to obtain fraction I completely free from insoluble matter, even after 10 extractions.

Each fraction was found to give positive reactions with Wagner's and Dragendorff's reagents, and the following colour reactions were given:—

Fraction	I	II	III	IV
(a) Ethanolic *p*-dimethylaminobenzaldehyde evaporated to dryness	brilliant crimson	faint brown-pink	faint brown-pink	no colour
(b) Residue from above exposed to ammonia fumes	deep orange	light violet and yellow	light purple	no colour
(c) Nascent chlorine test	no colour	no colour	no colour	no colour
(d) Residue exposed to ammonia fumes	no colour	no colour	no colour	no colour
(e) Chloranil (1 per cent.)	deep brown to olive-green	no colour	no colour	no colour
(f) Modified Jorissen's test	red colour	no colour	no colour	no colour
(g) Grant's test	orange-red	no colour	no colour	no colour
(h) Solubility in water	insoluble	partially soluble	soluble	soluble

A further attempt to characterise these fractions was made by examining microscopically the precipitates formed by the general alkaloidal reagents. The results were as follows:—

Fraction		I	II	III	IV
Wagner's reagent	15 minutes	globules	amorphous	amorphous	amorphous
	1 day	globules and tufted crystals	amorphous	amorphous	amorphous
	4 days	some globules had become crystalline	amorphous	amorphous	amorphous
Dragendorff's reagent	15 minutes	amorphous	amorphous	amorphous	amorphous
	1 day	small globules	masses of minute crystals	masses of minute crystals	amorphous
	4 days	granular	masses of minute crystals	masses of minute crystals	amorphous
Mayer's reagent	15 minutes	amorphous	minute crystals	faint amorphous	faint amorphous
	1 day	globules	minute crystals	faint amorphous	faint amorphous
	4 days	globules, some crystallised	minute crystals	faint amorphous	faint amorphous
Marmé's reagent	15 minutes	amorphous	amorphous	faint amorphous	very faint amorphous
	1 day	small globules	amorphous	faint amorphous	very faint amorphous
	4 days	small globules	amorphous	faint amorphous	very faint amorphous
Picric acid	15 minutes	globules	amorphous	faint amorphous	no visible precipitate
	1 day	globules	masses of minute crystals	faint amorphous	no visible precipitate
	4 days	globules	masses of minute crystals	faint amorphous	no visible precipitate
Mercuric chloride	15 minutes	globules	minute crystals	faint amorphous	amorphous
	1 day	globules	minute crystals	globules	globules
	4 days	globules	minute crystals	globules	globules

Fraction		I	II	III	IV
Gold chloride	15 minutes	amorphous	amorphous	amorphous	faint amorphous
	1 day	small triangular crystals	amorphous	amorphous	faint amorphous
	4 days	small triangular crystals	amorphous	amorphous	faint amorphous

As far as is known, only one alkaloid has so far been described in *L. termis*[1], but it is apparent that there is a complex mixture of alkaloids present. In fraction I alone, besides the probability of the presence of sparteine, there is another alkaloid which gives the brilliant reaction with *p*-dimethylaminobenzaldehyde which does not hitherto seem to have been described. All the fractions failed to crystallise even after standing for several weeks, and, in view of their obvious complexity, no efforts were made to characterise them further. Clearly there is scope for investigation here, and it is hoped to attempt to identify the alkaloids present at a later date.

Summary and Conclusions

1. An investigation of the properties of the alkaloids of the seeds of *Lupinus termis*, a common article of food in the Middle East, with the intention of detecting them chemically in toxicological specimens is described.

2. The seeds are shown to contain a complex mixture of alkaloids. However, the colour reactions described are sufficiently specific to ensure identification in specimens, although their fate in the human body is unknown.

3. A new modification of Jorissen's test for sparteine is described, making it sensitive to 0·1 mg.

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References

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