THE TOXIC PRINCIPLE OF *ACACIA GEORGINAE*

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SUMMARY

Serious stock losses from *Acacia georginae* poisoning have occurred in north-western Queensland and the Northern Territory.

The toxic principle was isolated in a pure form from the seed of *A. georginae* by extractive and chromatographic procedures and identified as fluoroacetic acid. Confirmation of chemical composition was obtained by gas chromatography and infra-red absorption spectroscopy. A method of quantitative estimation was developed.

Both sheep and rats showed similar clinical symptoms and post-mortem findings when dosed either with an aqueous extract of the toxic principle from *A. georginae* or with an aqueous solution of authentic fluoroacetic acid of equivalent concentration.

I. INTRODUCTION

Acacia georginae was identified by Bell, Newton, Everist, and Legg (1955) as the cause of a disorder of cattle and sheep which had resulted in heavy mortalities in the Georgina River watershed in north-western Queensland since settlement of the land in the second half of the nineteenth century. The plant is a large shrub or small tree of irregular habit, generally with crooked branches and a dense leafy crown. If subjected to regular grazing it can remain as a low bushy shrub for many years. It can attain a height of 20 ft but is more often 10–15 ft high.

The plant was first described by Bailey (1896), while the first description of the disorder on record is that of H. O'Boyle (Unpublished report, Queensland Department of Agriculture and Stock files, 1910). In both sheep and cattle the disorder is characterized by suddenness of death. Excitement, exercise or fright may precipitate symptoms and increase the number of deaths. When driven, sheep become sluggish, tremble, stagger and fall or lie down. Breathing is embarrassed and visible mucous membranes show marked cyanosis. Affected cattle may lag behind a mob during mustering and may stop frequently. If forced along they may fall and die quickly, with or without struggling. The most striking feature in cattle is the suddenness of death. There are no pathognomonic lesions.

Bell *et al.* (1955) reported that *A. georginae* is distributed over an area approximately 150 miles long and 90 miles wide in the Georgina River basin. These authors also stated that the effect of this plant is the most important factor, except drought, limiting animal production in this area. The number of deaths and rate of mortality may reach spectacular proportions. Many instances of

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fatalities involving 300–500 animals have been recorded (Queensland Department of Agriculture and Stock files). Besides actual stock losses there is also great reduction in potential production because the risk involved makes it necessary to leave extensive areas unused. The problem also exists in adjacent areas of the Northern Territory (Barnes 1958).

Although both pods and suckers of *A. georginae* have been proven toxic, the plant is considered a valuable fodder in the Georgina River area, and as it is also widely spread, eradication would be both undesirable and impractical. In addition, a considerable variation in toxicity between localities and from season to season has been noted (Bell *et al.* 1955). To take advantage of this variation, and to use the areas involved to maximum advantage, it was necessary to have accurate information as to the level and duration of toxicity in such areas. To assemble such information it was necessary to determine the chemical nature of the toxin, and to devise a method for the quantitative estimation of the level present. In addition, any attempt to devise an antidote for the toxin must depend on knowledge of the structure of the toxin itself.

II. PREVIOUSLY RECORDED EXPERIMENTAL FINDINGS

The identification of *A. georginae* by Bell and co-workers (1955) as the plant responsible for Georgina River disease was confirmed by Barnes (1958) and was followed up with chemical work by Wluka in association with F. N. Lahey. Wluka (1955) reported the presence of pinitol, an inositol monomethyl ether, together with several amino acids in the seed of this plant. However, the toxic principle was not characterized.

Oelrichs and McEwen (1961) showed that the poisonous nature of *A. georginae* seeds was due to the monofluoroacetate radicle.

Murray, McConnell, and Whitten (1961) reported the suspected presence of fluoroacetate in this plant in the Northern Territory.

III. MATERIALS AND METHODS

(i) *Source of Material.*—The pods of *Acacia georginae* were collected from known toxic areas in north-western Queensland. The samples were identified by the Government Botanist and specimens were filed by him under the voucher number HERB BRI 025742. To obtain clean seed the pods were broken up, using a batch-type hammer-mill, and the seeds and shell were separated by winnowing. Small twigs and other debris were removed from the seed by hand. The seeds were then hammer-milled to facilitate extraction. Since early work indicated a higher level of toxicity in seed than in whole pod or leaf, initial studies were confined to the isolation of the toxic principle from seed.

(ii) *Experimental Animals.*—Both male and female albino rats of weight range 150–200 g were used in experimental toxicity testing. Merino ewes and wethers of weight range 90–110 lb were used in all large-animal toxicity testing.

Both rats and sheep were dosed by stomach tube with aqueous solutions which had been adjusted to pH 7. Sheep received doses of 1 l, while 10 ml of fluid was administered to rats. Appropriate dilutions of concentrated toxic solutions were made and findings related back to a known weight of seed.

(iii) *Specialised Equipment.*—

(1) Ion exchange resins—Amberlite IR–4B (OH−) and IRC–50(H+) analytical reagent grade resins. These were thoroughly regenerated and well washed to remove any soluble material; 250 g was used in columns of 5 cm internal diameter and 60 cm high.

(2) Micro-fractionating column—Craig (1937).

(3) Micro-fluorine analyses—Harvey (1952).

(4) Silicic acid column—Two procedures were used. Method 1 was essentially that defined by the Association of Official Agricultural Chemists (Horwitz 1960, p. 349). This was used to obtain a partial separation of fluoroacetic acid from acetic and other acids. Some residual acetic acid did not affect a quantitative gas chromatographic estimation of fluoroacetic acid. Method 2 was a modification of Method 1 to give a complete separation of fluoroacetic acid. Two mobile solvents were used, viz. (i) fractionated chloroform, and (ii) fractionated n-butyl alcohol (50 ml) added to fractionated chloroform (950 ml).

(5) Vapor fractometers. In the analysis of the butyl esters, the vapor fractometer at the Chemistry Department of the University of Queensland was used. This employed a heat of combustion detector, with a "Sunvic" 5 mV strip chart recorder. The metre column was packed with silane-treated "Embacel", the stationary phase being "Apiezon M". A Perkin Elmer vapor fractometer model 154 D with flame ionization detector, together with a "Kent" 2 mV strip chart recorder, was used in the analysis of free acids. The metre stainless steel column was packed with acid-washed "Embacel", the stationary phase being 20 per cent. by weight of "Tween 80" as stated by Emery and Koerner (1961).

(6) Infra-red spectrometer. The Perkin-Elmer model 21 at the C.S.I.R.O. Veterinary Parasitology Laboratory, Yeerongpilly, was used.

IV. EXPERIMENTAL AND RESULTS

(a) Toxicity Testing with Sheep and Rats

At all stages during the isolation of the toxic principle from *Acacia georginae,* toxicity testing with sheep and rats gave comparable results, although on a body-weight basis rats were more resistant than sheep to the toxin.

The first clinical symptom in sheep was a sharp rise in pulse rate and the onset of laboured respiration. This was followed by ataxia, marked excitability and convulsions which continued until death.

The time interval from dosing to onset of symptoms was less with rats than with sheep. Rats exhibited convulsions at more frequent intervals. However, unlike sheep, rats sometimes recovered after a number of convulsions.

The post-mortem and histological findings were similar for sheep and rats. The histological examination of sections of lung, liver, spleen and kidney confirmed the macroscopic changes which have been described previously in the characterization of *A. georginae* poisoning of cattle and sheep in Queensland. No abnormalities could be detected in the central nervous system of either sheep or rats. Degenerative changes in kidney tubules were evident in some treated sheep and rats but could not be regarded as typical.

In all cases similar clinical symptoms and autopsy findings were found in both sheep and rats dosed either with an aqueous solution of the toxic principle of *A. georginae* or with an aqueous solution of authentic fluoroacetic acid of equivalent concentration.

(b) Preliminary Studies

The aqueous extract of milled air-dried seed (1 kg) was concentrated to 1 l under reduced pressure at 39°C and proved toxic to a 100 lb sheep.

The dialysate from a similarly prepared concentrate was toxic to another sheep of equivalent weight. The pore size of the dialysis membrane was such that the dialysate contained all compounds with a molecular weight less than 1000.

A similarly prepared dialysate was passed through columns of IRC–50 (H^+) and IR–4B (OH^-) in series. On washing and eluting the columns the toxin was shown to be present in the anionic column eluate. After neutralization of the solutions, toxicity was determined, using both rats and sheep.

By neutralization and testing the toxin was shown to be stable in N sodium hydroxide and N hydrochloric acid aqueous solutions after standing for 24 hr or more at room temperature. However, upon drying the acid and alkaline solutions under reduced pressure at 30°C, the toxicity was found to be retained in the case of the alkaline residue, but lost completely in the acid. Toxicity was determined with rats after neutralization of the aqueous solutions of the residues.

A comparison was made of four different extractive procedures:

(1) Three extractions with warm water.

(2) Three extractions with warm 50 per cent. aqueous ethanol.

(3) Continuous extraction with methanol for 4 days.

(4) Continuous extraction with 95 per cent. ethanol for 4 days.

Of these, (4) gave a complete extraction of the toxin and a greater initial purification. Toxicity was determined with rats after removal of the organic solvents by concentration under reduced pressure at 30°C and dissolving the residues in water.

Further purification of the ethanol extract without apparent loss of toxicity was obtained by extraction with petroleum ether (b.p. 40–100°C). A reduction in toxicity occurred when sulphuric ether was used as an extractant. Upon increasing the acid strength of the solution and extracting several times with sulphuric ether, most of the toxicity was transferred to the ether phase. By using a continuous ether extraction technique this transfer was shown to be complete. Upon extraction of this ether solution with aqueous sodium bicarbonate, the toxin was transferred to the aqueous phase. By repeating this cycle, a greater degree of purification was effected. In all cases toxicity was determined using rats after neutralization and removal of organic solvents by concentration under reduced pressure at 30°C.

An ether extract of milled air-dried seed (1 kg), prepared as outlined above and dried over anhydrous sodium sulphate, was concentrated, transferred to a small distillation flask and distilled by gradually raising the temperature of an oil-bath to 220°C. By toxicity testing using rats, the aqueous solutions of both the distillate and the residues were shown to be toxic, but the larger proportion was present in the distillate.

A similarly prepared ether extract of milled air-dried seed (1·5 kg) was fractionated using a Craig micro-fractionating column. Seven fractions were collected with the temperature ranging from 35 to 200°C. Only Fraction 5 (oil-bath temperature 135–170°C) proved toxic to rats. The infra-red spectrum of a carbon tetrachloride solution of this fraction indicated the presence of a short-chain aliphatic molecule with an absorption in the carbon-halogen region. Analysis indicated the presence of an appreciable amount of fluorine in the toxic fraction.

A similarly prepared ether extract of milled air-dried seed (4 kg) was fractionated in a semi-micro fractionating column at oil-bath temperatures ranging from 35 to 140°C. Toxicity testing using rats showed that the major proportion of the toxin was still retained in the residue. An ether solution of the residue was neutralized with sodium bicarbonate and dried under reduced pressure at 30°C. An aliquot of the dried product was heated at 100°C for one hour in a sealed tube with an n-butanol-sulphuric acid mixture. The contents were washed with saturated aqueous sodium bicarbonate and dried with anhydrous sodium sulphate. Gas chromatographic analysis of this n-butanol solution compared with an authentic sample of the butyl ester of monofluoroacetic acid is shown in Figure 1. The n-butanol solution shows a peak with a retention time identical with that of butyl monofluoroacetate.

On the assumption that the toxic principle was fluoroacetic acid, the use of anisole (2·6 per cent.) as an internal standard and calculations based on heats of combustion indicate the recovery of 8·3 mg of sodium fluoroacetate from 1 kg of the milled air-dried seed by this preliminary extraction procedure.

(c) Quantitative Extraction Procedure

Milled air-dried seed (4 kg) was extracted with distilled, potassium-hydroxide treated, 95 per cent. ethanol in a Soxhlet-type apparatus for 4 days. The extract was neutralized with N sodium hydroxide and concentrated to a thick syrup under reduced pressure at 39°C. Excess water was added and water-insoluble materials were removed by filtration. The solution was extracted repetitively with petroleum ether (b.p. 40–100°C) until the petroleum ether extract remained colourless. Usually three or four extractions were sufficient.

The aqueous solution was again concentrated under reduced pressure at 39°C to remove the petroleum ether and diluted with water to 2 l. Enough dilute sulphuric acid (10 per cent.) was then added to give a total acid concentration equivalent to 1 per cent. sulphuric acid. The acidified solution was filtered, and extracted with sulphuric ether using a continuous liquid-liquid extraction apparatus. The ether extract was neutralized by shaking with N sodium bicarbonate solution in a separatory funnel and the aqueous phase removed, acidified and extracted with ether as outlined above. The procedure of repetitive re-extraction was continued until the ether solution, on neutralization, remained colourless. Usually about three extractions with ether were necessary.

The final ether extract was concentrated to approximately 100 ml, sodium sulphate (15 g) and activated carbon (5 g) were added, and the solution was allowed to stand for 5 hr before filtering. The dried ether solution was then concentrated to approximately 20 ml. Gas chromatographic analysis of this ether solution of free acids compared with an authentic sample of fluoroacetic acid is shown in Figure 2.

The ether concentrate was transferred to the flask of a semi-micro fractionating column. The flask was heated by means of a mechanically stirred oil-bath and the temperature raised gradually to 140°C. (The neutralized distillate had little toxicity to rats.) The residue was washed out of the flask with ether, neutralized by adding M aqueous sodium bicarbonate dropwise, and taken to dryness under reduced pressure at 30°C. The last traces of water were removed by drying in a vacuum desiccator over phosphorous pentoxide for 24 hr.

(d) Silicic Acid Chromatography

Using the modified method of silicic acid chromatography (Method 2), silicic acid (15 g) was placed in a mortar and 0·5 N sulphuric acid (7·5 ml) added in small quantities, grinding well with a pestle to obtain an even distribution of the stationary phase. Fractionated chloroform (100 ml) was added and the resultant slurry poured into the column. The slurry was mixed thoroughly, taking care to remove all air bubbles, and finally packed down under pressure (10 lb/sq. in.). A wad of powdered filter paper, 2 mm thick, was placed on top of the column.

A known quantity of the dried salts (0·77 g) from the quantitative extraction procedure was placed in a round-bottom flask, 18 N sulphuric acid (0·5 ml) added, and the stoppered flask gently swirled in an ice-bath till most of the solid was dissolved. To this solution anhydrous granular sodium sulphate (10 g) was added, followed by fractionated chloroform (10 ml). The resultant solution and sodium sulphate were transferred to the column, using standard techniques. Slight pressure was applied and approximately 100 ml of eluate was collected at the rate of 2–3 ml/min before the solvent level was allowed to reach the sodium sulphate layer. Elution was continued with the chloroform/butanol solvent at the same flow rate. Fractions of 4 ml were collected for examination by gas chromatography.

(e) Gas Chromatography

Gas chromatography was used in preliminary studies to detect the presence of an ester having a retention time identical with that of butyl fluoroacetate (Figure 1). Similarily, gas chromatography was used to detect the presence of an acid of identical retention time to fluoroacetic acid in an ether solution of free acids as outlined in the quantitative extraction procedures (Figure 2).

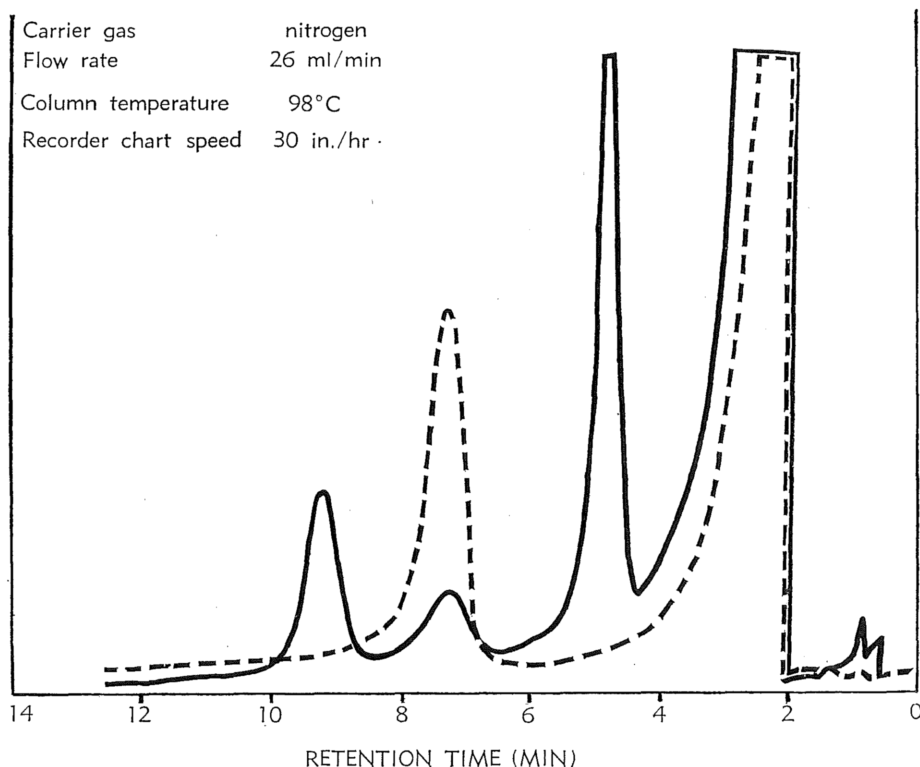

Fig. 1.—Separation of butyl esters by gas chromatography.
— — — — Butyl alcohol solution of the butyl ester of fluoroacetic acid.
———— Butyl alcohol solution of the butyl esters of acids from a concentrated fractionated ether extract from seed of *A. georginae*.

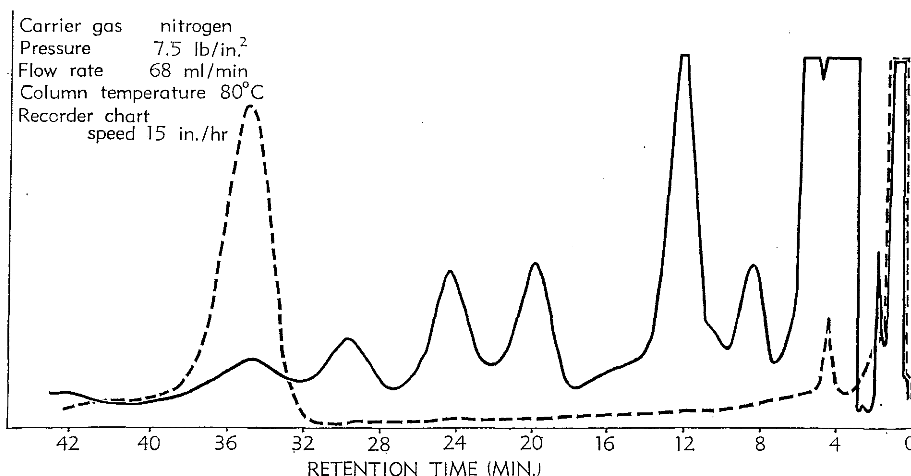

Fig. 2.—Separation of free acids by gas chromatography.

— — — — Ether solution of fluoroacetic acid.

———— Ether solution of acids from crude extract from seed of *A. georginae.*

Gas chromatography was used to identify which fractions from the silicic acid column eluate contained the acid of identical retention time to fluoroacetic acid. These fractions were combined, and the analysis of this butanol/chloroform solution, compared with an aqueous solution of authentic fluoroacetic acid, is shown in Figure 3. Assuming that the toxic principle is fluoroacetic acid, quantitative data were obtained on the basis that area under a curve is proportional to concentration. Calculations based on gas chromatographic analysis of the combined eluate from the silicic acid column indicate the recovery of 25 mg of sodium fluoroacetate from 1 kg of milled air-dried seed by the quantitative extraction procedure.

The isolation of a pure moisture-free sample of this acid was effected by gas chromatography. To the appropriate combined eluate fractions from the silicic acid column enough 0·1 N sodium hydroxide was added to neutralize the acid, and the solvents were removed at 30°C under reduced pressure. The dried salt was dissolved in water, acidified, and extracted with ether. This ether extract was concentrated to approximately 0·5 ml by cooling to —30°C and evaporating the ether slowly with a stream of dry nitrogen. Water condensation was prevented by using a heating coil above the flask containing the extract. Two successive injections (50 μ 1) were introduced into the gas flow and a U-tube of pyrex glass, 7 mm internal diameter, 5 in. high, filled with purified sand (0·04 in. mesh size), immersed in a freezing bath (—80°C) was connected to the column outlet at the appropriate predetermined times, to collect the sample. The sample was removed from the tube by rinsing three times with dried fractionated sulphuric ether (5 ml). Precautions were taken to avoid any evaporation of the sample and to

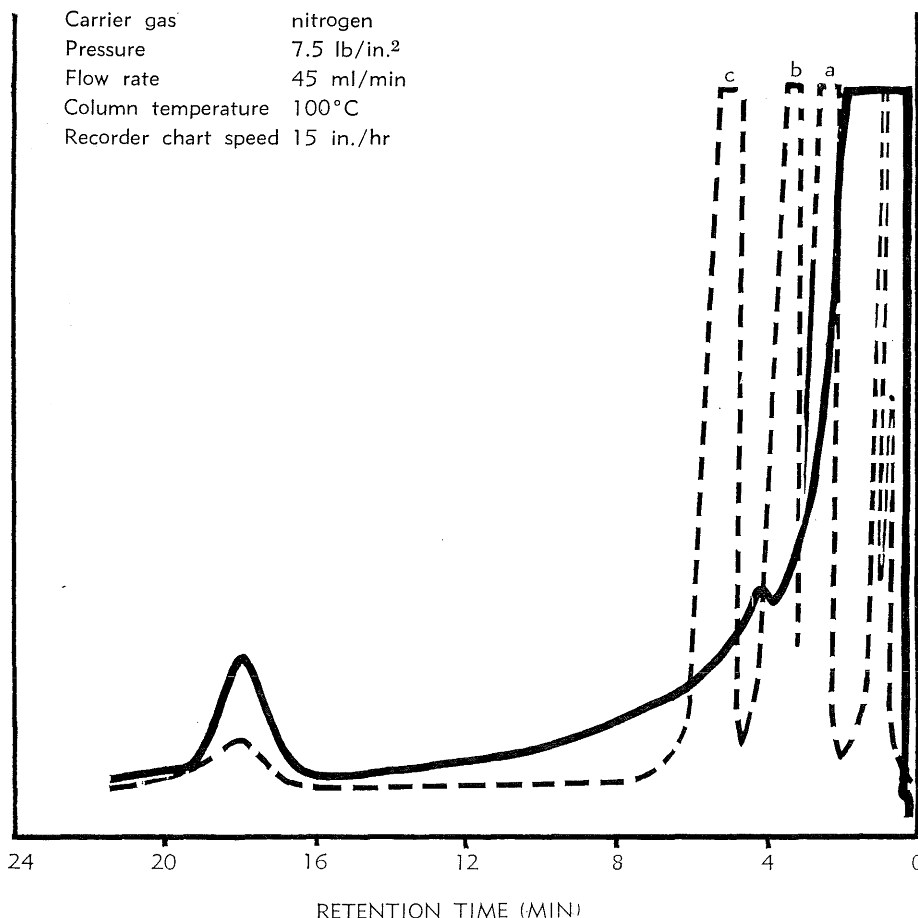

Fig. 3.—Separation of free acids by gas chromatography.

— — — — Aqueous solution of acetic (*a*), propionic (*b*), butyric (*c*) and fluoroacetic acid (heavy curve).

———— Chloroform-butyl alcohol solution of the combined eluate from the silicic acid column used in the isolation of the toxic principle from seed of *A. georginae*.

prevent any contamination with atmospheric moisture. The ether was evaporated from the sample, using the technique as outlined above, and the residue dissolved in moisture-free carbon tetrachloride (0·1 ml).

The column used for gas chromatographic analysis of this solution carried 30 per cent. by weight of "Tween 80" as stationary phase. Comparison with a carbon tetrachloride solution of authentic fluoroacetic acid is shown in Figure 4.

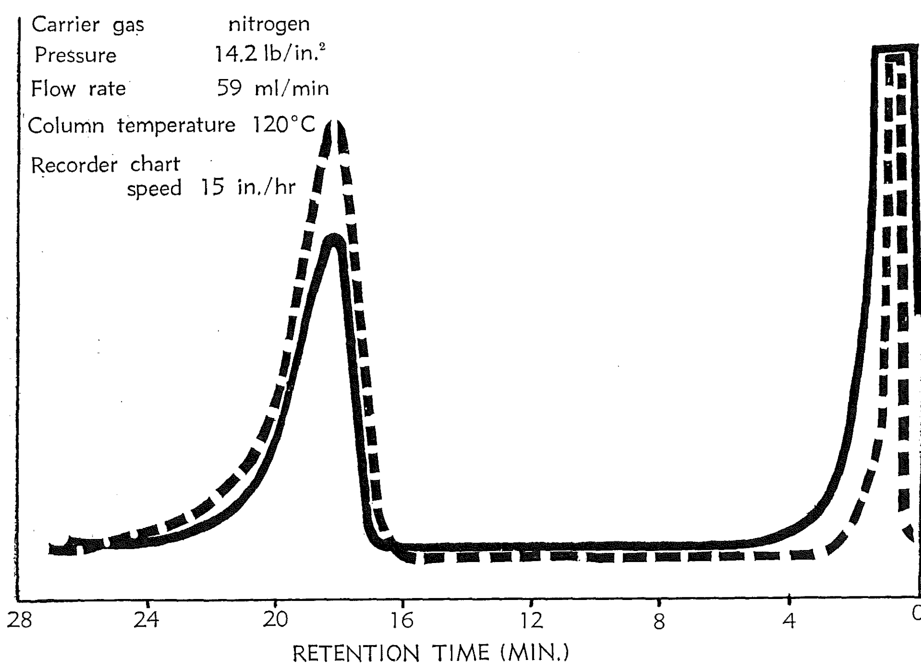

Fig. 4.—Separation of free acids by gas chromatography.

— — — — Ether solution of fluoroacetic acid.

———— Carbon tetrachloride solution of the pure toxic principle isolated from seed of *A. georginae*.

(f) Infra-red Studies

The infra-red spectra of carbon tetrachloride solutions of the sample collected after gas chromatographic fractionation and of an authentic sample of fluoroacetic acid are shown in Figure 5. Both spectra show the absorption bands characteristic of a short-chain aliphatic carboxylic acid together with a band at 1095 cm^{-1} characteristic of a carbon-fluorine stretching mode.

This evidence confirms the identification of the toxic principle as fluoroacetic acid.

(g) Quantitative Analytical Procedure

A quantitative procedure was developed for the routine estimation of fluoroacetic acid in milled air-dried seed, pod or leaf. A known weight (100 g) was extracted by the quantitative extraction procedure, but excluding the fractional distillation. Silicic acid chromatography (Method 1) was then used to give a satisfactory separation of fluoroacetic acid from other acids. Gas chromatography was used to identify the eluate fractions containing fluoroacetic acid. These were combined, neutralized, and the solvents removed under reduced pressure at 30°C. The residue was dissolved in water, acidified, and the free acid extracted in a small known volume of ether. Quantitative gas chromatography based on the use of a standard solution of fluoroacetic acid was used in the final analysis.

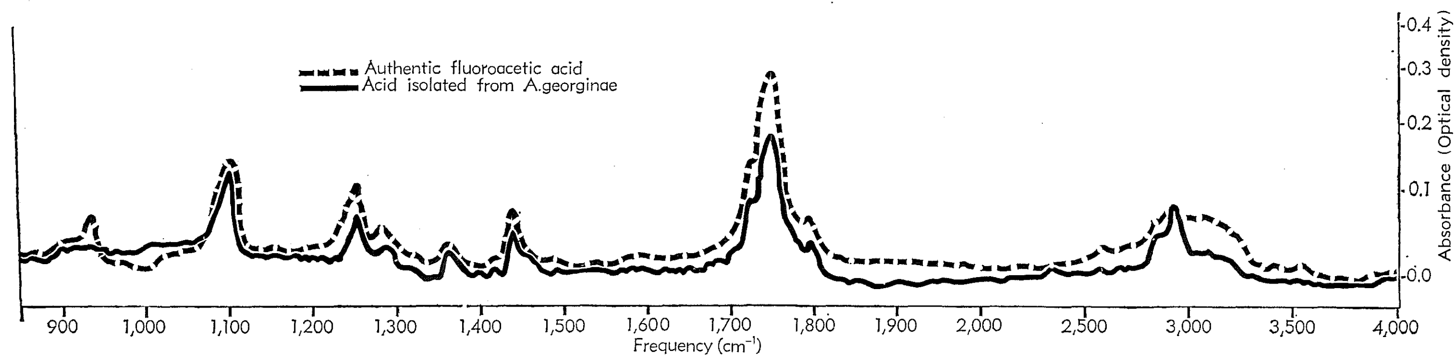

Fig. 5.—Infra-red absorption spectra of carbon tetrachloride solution of authentic fluoroacetic acid and the acid isolated from *A. georginae*.

V. DISCUSSION

From the preliminary studies a number of conclusions are drawn with regard to the toxic principle in the seed of *A. georginae*:

(1) The toxin is water-soluble.

(2) The toxin is a compound with a molecular weight less than 1000.

(3) The toxin is a strong acid.

(4) Continuous extraction with 95 per cent. ethyl alcohol is shown to be a superior method of obtaining the toxin in a crude form.

(5) The toxin is not appreciably soluble in petroleum ether (b.p. 40–100°C), but can be extracted with ether from an acidified aqueous solution using a continuous extractive procedure.

(6) The toxin is volatile in the free acid form.

(7) The toxin can be collected in a micro-fractionating column at a bath temperature of 135–170°C, and this fraction contains fluorine.

(8) A carbon tetrachloride solution of this fraction has an infra-red spectrum characteristic of a short-chain aliphatic acid with a carbon-halogen linkage. The impurity of this fraction precluded confirmation of chemical composition by direct comparison with the infra-red spectrum of an authentic sample of fluoroacetic acid.

(9) Gas chromatographic analysis of the butyl esters shows the presence of an ester the retention time of which is identical with that of an authentic sample of butyl fluoroacetate prepared from pure fluoroacetic acid. The method of Phillips (1957) was used to prepare crude fluoroacetic acid. This was fractionated in a 50 cm column and the middle fraction collected. The sample collected was shown to be pure by fluorine analyses, gas chromatography and acid titration.

(10) Assuming that the toxic principle is fluoroacetic acid, calculations indicate the recovery of 8·3 mg of sodium fluoroacetate from 1 Kg of milled air-dried seed.

Our quantitative extraction procedure enabled the removal of a large proportion of compounds not associated with toxicity. On fractionation in a semi-micro fractionating column, it was found that acetic acid (b.p. 118°C) constituted the bulk of the lower boiling point acids. Other acids with higher indeterminate boiling points were fractionated in smaller quantities. This fractionation procedure was used as a purification step prior to silicic acid chromatography and enabled the collection of a sufficient quantity of this acid for conclusive identification. On the other hand, the fractionation step was

omitted when gas chromatography was used solely as a quantitative analytical procedure. No detectable loss occurred when a known weight of an authentic sample of fluoroacetic acid was subjected to this treatment of extraction followed by chromatography.

A major difficulty was the collection of a pure moisture-free sample for infra-red analysis. Even traces of water prevented solubility of fluoroacetic acid in carbon tetrachloride. The combined eluate from the silicic acid column contained a pure sample of fluoroacetic acid, but owing to the volatile nature of this compound it was extremely difficult to remove the solvents and retain the sample. The ether extract, concentrated at −30°C, still contained an appreciable amount of water which could not be removed satisfactorily by drying agents. Preparative scale gas chromatography was found to be the only acceptable procedure. Even with this procedure precautions were necessary to remove the last traces of water from the nitrogen carrier gas. This was effected by placing in series two drying tubes (18 in. x 1 in. internal diameter) each containing a well-packed mixture of equal volumes of phosphorous pentoxide and powdered asbestos.

The pure moisture-free sample obtained from the final preparative scale gas chromatography was dissolved in a small volume (0·1 ml) of moisture-free carbon tetrachloride. A solution of approximately the same concentration was prepared from an authentic sample of fluoroacetic acid. The infra-red spectra of these samples confirmed the identification of the toxic principle as fluoroacetic acid. Minor differences between the spectra are evident in the frequency ranges 2400–3400 cm^{-1} and 850–1000 cm^{-1}. The findings of Bonner and Hofstadter (1938) and Colthup (1950) suggest that the differences are due to the degree of association of the acid molecules and small differences in the fluoroacetic acid concentration of the two solutions.

The only previous report of the occurrence of fluoroacetate in a plant poisonous to livestock would appear to be from South Africa. Serious stock losses due to poisoning by *Dichapetalum cymosum* (gifblaar) have been reported in that country by Rimington (1935), and the identification of the toxic principle as potassium fluoroacetate was reported by Marais (1943). Later work by Marais (1944) showed that the milled dried plant material contained 0·015 per cent. fluorine. Assuming all the fluorine to be present as sodium fluoroacetate, this would indicate the presence of 789 mg of sodium fluoroacetate per Kg of dried plant material. On the other hand, milled air-dried seed of *A. georginae* has been shown to contain 25 mg of sodium fluoroacetate per Kg. The figure 8·3 mg/Kg found in preliminary studies was not correct, and the discrepancy is most probably due to incomplete esterification.

It has been shown by Peters, Hall, and Ward (1960) that toxic fluorine-containing acids other than fluoroacetic are present in the seeds of *Dichapetalum toxicarium* (ratsbane). Although the concentration of fluoroacetate found in

A. georginae seed can fully account for stock losses, there is some evidence that other toxic fluorine-containing acids are present, and further work is in progress to determine their chemical nature.

As yet little work has been attempted with the leaf of *A. georginae,* but using gas chromatography fluoroacetic acid has been identified in young leaf. Indications are that fluoroacetic acid is present in leaf in even smaller quantities than in the seed. Further quantitative work is necessary also to define reported variations in toxicity of *A. georginae* in a number of areas in north-western Queensland.

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REFERENCES

ANON. (1952).—"Instruction Manual, Perkin-Elmer Infra-red Equipment. Vol. 1. Introduction to Infra-red Spectrometry" (Perkin-Elmer Corporation: Norwalk).

BAILEY, F. M. (1896).—Bot. Bull. Qd Dep. Agric. No. 13.

BARNES, J. E. (1958).—Georgina poisoning of cattle in the Northern Territory. *Aust. Vet. J.* 34:281.

BELL, A. T., NEWTON, L. G., EVERIST, S. L., and LEGG, J. (1955).—*Acacia georginae* poisoning of cattle and sheep. *Aust. Vet. J.* 31:249.

BONNER, L. G., and HOFSTADTER, R. (1938).—*J. Chem. Phys.* 6:531. Quoted by Jones, R. N., and Sandorfy, Camille (1956, p. 425).

COLTHUP, N. B. (1950).—Referred to by Anon. (1952). *J. Opt. Soc. Amer.* 40:6.

CRAIG, L. C. (1937).—A microfractionating column. *Industr. Engng Chem.* 9:441.

EMERY, E. M., and KOERNER, W. E. (1961).—Gas chromatographic determination of trace amounts of the lower fatty acids in water. *Anal. Chem.* 33:146.

HARVEY, J. M. (1952).—Chronic endemic fluorosis of Merino sheep in Queensland. *Qd J. Agric. Sci.* 9:47.

HORWITZ, W., Ed. (1960).—"Official Methods of Analysis of the Association of Official Agricultural Chemists." 9th Ed. (Association of Official Agricultural Chemists: Washington).

JONES, R. N., and SANDORFY, Camille (1956).—*In* "Technique of Organic Chemistry. Vol. IX. Chemical Applications of Spectroscopy". W. West, Ed. (Interscience Publishers: New York).

MARAIS, J. S. C. (1943).—The isolation of the toxic principle "potassium cymonate" from "Gifblaar," *Dichapetalum cymosum* (Hook.). Engl. *Onderstepoort J. Vet. Sci.* 18:203.

MARAIS, J. S. C. (1944).—Monofluoroacetic acid, the toxic principle of "Gifblaar," *Dichapetalum cymosum* (Hook.). Engl. *Onderstepoort J. Vet. Sci.* 20:67.

MURRAY, L. R., MCCONNELL, J. D., and WHITTEN, J. H. (1961).—Suspected presence of fluoroacetate in *Acacia georginae* F. M. Bailey. *Aust. J. Sci.* 24:41.

OELRICHS, P. B., and MCEWAN, T. (1961).—Isolation of the toxic principle in *Acacia georginae. Nature Lond.* 190:808.

PETERS, R. A., HALL, R. J., and WARD, P. F. V. (1960).—The chemical nature of the toxic compounds containing fluorine in the seeds of *Dichapetalum toxicarium. Biochem. J.* 77:17.

PHILLIPS, M. A. (1957).—Preparation of monofluoroacetic acid and of calcium, barium, and silver monofluoroacetates. *Mfg Chem.* 28:328.

RIMINGTON, C. (1935).—Chemical investigations of the "Gifblaar," *Dichapetalum cymosum* (Hook.) Engl. I. *Onderstepoort J. Vet. Sci.* 5:81.

WLUKA, D. J. (1955).—M.Sc. Thesis, University of Queensland.

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ADDENDUM

Since the completion of this manuscript, the quantitative analytical procedure has been used to determine the fluoroacetic acid content of samples of seed, pod, leaf and branch of *Acacia georginae* from three areas in north-western Queensland where stock losses have occurred. Results are as shown in the table:

Sample	Fluoroacetic acid mg/Kg					
	Area 1		Area 2		Area 3	
	Air-dry	Dry-matter	Air-dry	Dry-matter	Air-dry	Dry-matter
Seed—mature	9	10	38	42	..	..
immature	..	..	..	..	390	421
Pod—mature	11	12	12	13	..	..
immature	..	..	..	..	31	34
Leaf—mature	12	13	9	9	14	15
Branches (up to ¼ in. thick)	<1	<1	..	..	..	..

Tentative conclusions from these data are:—

(1) In the known toxic area all samples of seed, pod and leaf so far examined contained fluoroacetic acid.

(2) In any one area the order of magnitude of the fluoroacetic acid content is usually seed>pod>leaf>branch.

(3) The findings from Area 3 suggest that either this is an acutely toxic area or the fluoroacetic acid concentration is greater the more immature the seed and pod.

(4) The occurrence of this very high concentration in the seed from Area 3 is in keeping with field experiences of rapid deaths in livestock on some occasions after very limited exposure to a toxic area.

(5) If immature seed is more toxic than mature seed the presence of other fatty acids containing fluorine in mature seed requires investigation.

(6) There is an obvious need to obtain quantitative data on *Acacia georginae* from allegedly toxic and non-toxic areas in several seasons. Such a survey will be undertaken.