Onderstepoort Journal of Veterinary Science and Animal Industry, Volume I, Number 2, 1933.

Psilocaulon absimile N.E.Br. as a Stock Poison. I. Determination of Oxalic, Malic, Tartaric Acids, etc.

By CLAUDE RIMINGTON, M.A., PH.D., B.Sc., A.I.C., Research Fellow under the Empire Marketing Board, and
D. G. STEYN, B.Sc., Dr.Med.Vet., Research Officer, Onderstepoort.

Registered Number: Onderstepoort Spec. No. 626; 13/5/32. Nat. Herb. No. 11546.

Common Names: Asbos; loogbos.

Origin: Prieska.

State and Stage of Development: Fresh and in post-seeding stage.

ONE of the authors (D. G. S.) investigated mortality in Angora goats in the Willowmore District and strongly suspected a *Psilocaulon* sp. (Nat. Herb. No. 8819) of being the cause. The interesting observation was made that 50 grams of this *Psilocaulon* sp. collected on the farm, where the mortality occurred, caused death in rabbits, whilst 120 grams of specimens of this plant collected on the above farm and planted at Onderstepoort produced no ill-effects in rabbits. It was suggested that the plant grown at Onderstepoort had decreased in toxicity as there is a vast difference in the nature of the soil and climatic conditions in the Willowmore District and at Onderstepoort (Steyn, 1931). One hundred grams of fresh *Psilocaulon absimile* N.E. Br. administered to rabbits caused laboured respiration, tympanites, pronounced salivation, accelerated heart beat, symptoms of paralysis within an hour and death with symptoms of asphyxia within three hours after dosing. Fifty grams of this plant in the fresh state given on each of two consecutive days to rabbits produced no ill-effects.

Drenching experiments proved that the sun-dried plant had not decreased in toxicity.

The following lesions were present at autopsy: hyperaemia and oedema of the lungs; dilatation of the heart ventricles; marked hyperaemia and swelling of and haemorrhages in the gastric mucosa.

The results of chemical investigations revealed the fact that at least two toxic principles are present in the plant, one of which forms an insoluble lead salt and is probably an organic acid, whilst the second toxic factor passes through into the filtrate from the lead acetate precipitation, and is of an entirely different nature both chemically and pharmacologically. In the sample of *Psilocaulon* examined, both principles were present in such quantity as to cause death when administered separately in their respective proportions. No synergistic effect could be observed, but death in the field would appear to be due to the toxic principle, the isolation and chemical examination of which is described in the second communication of this series rather than to the organic acids present in the plant. The present paper deals only with the investigation of the organic acid fraction.

Preliminary experiments having shown that the plant contained a considerable quantity of oxalic acid together with lesser quantities of malic, tartaric acid, etc., a portion of the material was worked up in the following manner and quantitative determinations made of these constituents.

Since the fresh material dries only very slowly in the air, it was introduced into a large oven, the temperature of which was maintained at about 95°, and dried until practically constant in weight. The desiccated material was then ground in a coffee mill affording a light brown powder which was employed for the subsequent determination.

Moisture Content.

A 100 gm. sample of the fresh, green plant material upon drying until absolutely constant in weight, lost moisture corresponding to 67·75 per cent.

1 gm. portions of the larger bulk of ground material on drying to constant weight at 105° lost, on an average, moisture corresponding to 6·92 per cent. Determinations made upon the powder were in all cases corrected by this amount to relate them to percentages on the dry weight basis.

Free Acidity of the Aqueous Extract.

Although as mentioned later in the discussion, it is very doubtful whether the organic acids ever occur in the plant in the free state, the fact of their being polybasic and relatively strong acids allows of their occurrence as partially neutralized acid salts. Oxalic acid, for example, is found frequently in the form of potassium hydrogen oxalate. Watery extracts of the fresh plant were markedly acid to litmus and the plant itself on ingestion evidently exerts an irritating action upon the gastric mucosa, hence the fairly high titratable acidity which was found was not unexpected.

A 1 gram portion of the powdered material was extracted at the temperature of the boiling water bath with several successive portions of distilled water, the combined extracts (about 100 c.c.) were filtered and titrated by deci-normal sodium hydroxide until just alkaline to phenolphthalein.

Vol. of N/10 alkali required = 5·15 c.c. per gm. dry material.

Ash Left on Ignition.

1 gm. portions were incinerated in weighed platinum basins until no further loss in weight occurred. The results were as follows:—

No. 1 Wt. residue left 0·2288 g. = 24·58 per cent. on dry wt.

No. 2 Wt. residue left 0·2292 g. = 24·62 per cent. on dry wt.

Mean result 24·60 per cent.

Alkalinity, Total, Soluble and Insoluble of the Ash, also Acid-insoluble Residue (Silica, etc.).

Ash No. 1 was used for the determination of total alkalinity, whilst Ash No. 2 was treated in such a way that the water-soluble and acid-soluble fractions were titrated separately.

Ash No. 1 was moistened with water, 50 c.c. of deci-normal sulphuric acid added and the mixture warmed upon the water bath for some minutes, methyl orange was then added as indicator and the excess of acid titrated by decinormal sodium hydroxide.

In the second case, ash No. 2 was thoroughly extracted with hot water, added in successive small quantities, each portion being decanted through an ashless filter paper into a beaker where all was collected, methyl orange added and the liquid then titrated with decinormal sulphuric acid. The extractions were then repeated with small quantities of hot, diluted sulphuric acid, exactly 15 c.c. of a decinormal solution being added, and filtrate and washings titrated to methyl orange with standard alkali. Upon the completion of these operations, the neutralized solution from ash No. 1 was filtered and the two filter papers with their contents separately ignited and the weight of the acid-insoluble residue of sand, silica, etc., thus obtained. The results are collected below:—

No. 1 ash required 37·34 c.c. N/10 alkali = 40·12 c.c./gm. on dry wt.

No. 2 ash water-soluble fraction required 28·4 c.c. N/10 alkali = 30·51 c.c./gm. on dry wt.

acid-soluble fraction neutralized 9·6 c.c. N/10 alkali = 10·31 c.c./gm. on dry wt.

Total = 40·82 c.c./gm. on dry wt.

Wt. of acid-insoluble residues correspond to ... ... 1·02 per cent. on dry wt.

1·01 per cent. on dry wt.

Mean 1·02 per cent. on dry wt.

Determination of Calcium and Potassium in the Ash.

Qualitative examination of the ash showed the presence of Ca, K, Na in considerable quantities. Iron was present only in traces. The calcium content was determined by dissolving the ash derived from 1 gm. of the plant powder in warm dilute hydrochloric acid, filtering, adding ammonia until alkaline, again acidifying with acetic acid and adding a solution of ammonium oxalate. The mixture was kept on the water bath for some hours and then allowed to cool. The precipitate of calcium oxalate was filtered off, washed, dissolved in warm dilute sulphuric acid and titrated by potassium permanganate in the usual way.

Potassium was determined in the ash from a separate quantity (6 gm.) of the plant.* There was 5·2 per cent. calculated as K_2O.

Vol. of N/100 $KMnO_4$ required = 66·8 c.c.
= 0·01339 gm. Ca.

This represents 1·34 per cent. of Ca in the plant or, when expressed as CaO, 8·19 per cent. of the ash.

The high alkalinity of the ash of *Psilocaulon absimile* is in accordance with the fact that the plant is relatively rich in salts of organic acids. On ignition, the metals are left as carbonates or oxides.

In the districts of South Africa where it grows abundantly, the plant is used by the rural population for the manufacture of soap. By burning and extracting the residue with water a lye is obtained which is boiled with the oil or fat as in the familiar technical process. Locally the plant is known as " loog-as " signifying " lye-ash ".

Psilocaulon is closely related to the genus *Mesembryanthemum* comprising plants many of which have a high content of organic acids. Burtt-Davy (1912) describes the use of *Mesembryanthemum mahoni*, N.E. Br. by the natives as a fermenting agent in the preparation of an intoxicating liquor named " khadi ". It is said, however, that the root (the portion used) contains a poisonous principle which in time proves injurious to the khadi drinker. In the Bulletin of the Imperial Institute (1912) is summarized the report of an investigation of this root powder, in which it is stated that quantities of oxalates amounting to approximately 3 per cent. by weight of free oxalic acid were found. The poisonous effects upon natives are ascribed to this acid. That the fermentative activity of the roots of *M. mahoni* is in reality due to the mycelia of accompanying fungi, has been well established. The interesting observation was made that these fungi also are capable of producing oxalic acid when grown upon sugar solution (Bull. Imp. Inst. 1916). A *Mesembryanthemum* species, probably *bellidiflorum*, is used by the Hottentots for the softening of animal skins, the juice of the plant being worked into the tissues with the aid of a stone. *Mesembryanthemum crystallinum* is referred to by Dragendorff (1898) as a " soda-plant ".

Determination of Oxalic Acid.

The determination of the various organic acids when present together in plants is a problem of no little difficulty. In many respects the salts of oxalic, malic, tartaric, succinic and citric acids are closely similar. None of the methods so far proposed are capable of giving quantitative sharp separation. For purposes of identification the ester process devised by Franzen and his co-workers (1921-2) is convenient, but the yields are necessarily far from quantitative. As a more general method of procedure, the scheme proposed by Albahary (1912) wherein differences in the properties of the lead salts of the individual acids are exploited, may be recommended, provided great accuracy is not required.

* For the carrying out of this determination I am indebted to Mr. Holzapfel of this Laboratory.

In the present case, since widely different quantities of the different acids were present, it was found most practicable to concentrate upon the determination of one acid at a time and to adopt or elaborate methods capable of yielding the most reliable figures for this individual. The determination of each acid is, therefore, considered under a separate heading.

Oxalic acid is most readily separated from plant extracts by precipitation as calcium oxalate from a solution slightly acid with acetic acid. Some calcium tartrate may frequently be associated with the oxalate in this precipitate, but by careful reprecipitation, again from acid solution, a separation may be effected. 1 gm. of the finely-ground plant powder was extracted by two successive portions of 100 c.c. of 1 per cent. hydrochloric acid, the flask with its contents being on each occasion immersed in a boiling water bath for one hour. The fluid was decanted from the plant residue, centrifuged and finally filtered. It was then made alkaline by ammonia and the reaction brought once more to the acid side by the addition of a slight excess of acetic acid.

To the boiling liquid a solution of calcium acetate was then added and the mixture kept upon the water bath for about 2 hours. It was then placed in the ice-chest over night. The precipitate was filtered off, washed with water slightly acidified with acetic acid and finally dissolved in hot, dilute sulphuric acid, the volume of this solution of the crude oxalate precipitate being adjusted to 100 c.c. A titration was made upon an aliquot of this solution, using one-hundredth normal potassium permanganate. A further aliquot, or the remainder of the solution, was then subjected to precisely the same procedure. The end-point found when titrating this solution of the reprecipitated oxalate was invariably sharp, whilst some uncertainty attached to that with the solution of the crude precipitate. Any extra deposit separating out when the main filtrate from the calcium oxalate was kept for further periods in the ice-chest, or adhering to the sides of the flask was separately titrated as above.

Results :—

(*a*) Extract from 1 gm. powder (containing 6·92 per cent. of moisture).

Volume of solution of crude oxalate precipitate = 100 c.c.

5 c.c. aliquot required 9·4 c.c. of 0·009656 N $KMnO_4$.

90 c.c. reprecipitated and made up to 100 c.c.

5 c.c. aliquot of this solution required 8·1 c.c. $KMnO_4$ = 9·0 c.c. $KMnO_4$ in 5 c.c. of the original = 8·69 c.c. N/100.

$\therefore$ 7·82 gm. oxalic acid $KMnO_4$/100 gm. plant or 8·40 per cent. on dry wt. basis.

Further small deposit required 4·5 c.c. $KMnO_4$ = 4·345 c.c. N/100 $KMnO_4$.

or 0·21 per cent. oxalic acid on dry wt. basis.

Total oxalic acid found = 8·61 per cent.

(*b*) Extract from 1 gm. plant powder.
Volume of solution of crude oxalate precipitate = 100 c.c.
5 c.c. aliquot required 9·1 c.c. of $KMnO_4$ solution.
5 c.c. reprecipitated; precipitate required 9·0 c.c. $KMnO_4$ solution.
Small deposit on sides of flask required 6·4 c.c.
∴ 8·70 per cent. oxalic acid on dry wt. basis.
Mean of above determinations 8·66 per cent.

Determination of Tartaric Acid.

The filtrates from the two precipitations of calcium-oxalate described in (*a*) above, were combined, concentrated, made very slightly alkaline with ammonia and placed in the ice-chest for some days. The small precipitate of calcium salts which formed was filtered off, dissolved in a little dilute acetic acid and, after further concentration, two volumes of a 10 per cent. alcoholic solution of potassium acetate added. The mixture was vigorously stirred at intervals during the day and left in the ice-chest over night. The small precipitate of potassium bitartrate was filtered off, washed with alcohol until free from acid, dissolved in a little hot water and titrated with decinormal sodium hydroxide using phenolphthalein as indicator.

Volume of alkali required = 0·4 c.c. of N/10.
= 0·5 mg. tartaric acid.
∴ 0·064 per cent. in plant on dry wt. basis.

As a check upon this figure a determination was carried out as follows:—1 gm. of the plant powder was extracted with 1 per cent. hydrochloric acid in the usual way and the extract neutralized with ammonia. Lead acetate solution was then added in slight excess and the precipitated lead salts removed by centrifugation, washed with 50 per cent. alcohol, suspended in water and finally decomposed by passing hydrogen sulphide into the hot solution. The filtrate from the lead sulphide was concentrated to about 30 c.c., a few drops of acetic acid added and then 60 c.c. of 10 per cent. alcoholic potassium acetate. The precipitate of potassium hydrogen tartrate was treated as previously described.

Volume of alkali required = 0·45 c.c. of N/10.
= 0·675 mgm. tartaric acid.
∴ 0·072 per cent. in plant on dry wt. basis.
Mean of above determinations 0·068 per cent.

Determination of Citric Acid.

Citric acid is most conveniently determined by one or other of the methods depending upon its oxidation to acetone. The acetone formed may be determined by iodine titration (Kogan, 1930), conversion into penta-brom acetone (Hartmann & Hillig, 1930) or as the mercury compound. In our experience the method of Bleyer and Schwaibold (1925) is comparatively simple and capable of yielding good results provided no great quantity of tartaric acid is present. The neutralized solution of the acid is made up to 150 c.c. and

refluxed for 3 hours with 5 c.c. of the oxidation reagent for every 0·05 gm. citric acid. An excess of reagent causes no error. The precipitated mercury-acetone compound is filtered off whilst the solution is still warm and washed well with water. Concentrated nitric acid is then added (in the present investigation the paper and precipitate were returned to the reaction flask) followed by excess of a concentrated potassium permanganate solution, concentrated ferrous sulphate being then added in excess to remove unused permanganate and after addition of a few drops of iron ammonium alum as indicator the solution is titrated by decinormal ammonium thiocyanate until a permanent reddish brown coloration is produced.

1 c.c. of N/10 NH_4 CNS = 2·69 mgm. citric acid.

The accuracy of the method was tested upon solutions of pure citric acid.

Using the filtrate from the calcium oxalate precipitate obtained when working up an extract from 1 gm. of plant powder, no citric acid could be detected by this method. An extract of a larger quantity was therefore prepared in the following way:—

> 20 gm. plant powder was extracted by three successive portions of 300 c.c. each of 1 per cent. hydrochloric acid. The combined extracts were concentrated upon the water bath to about 100 c.c., a little decolorizing charcoal added and the solution filtered and made up to a volume of 150 c.c. This was then refluxed with 10 c.c. of reagent. The volume of ammonium thiocyanate required to produce a visible end point was one drop (slightly less than 0·1 c.c.). Citric acid is therefore absent.

Determination of Malic Acid.

Of all the commonly occurring plant acids, malic acid presents the greatest difficulty in the way of its quantitative determination. Its separation from the other oxy-acids requires much care and until recently there was no satisfactory way of evaluating the quantities present in such concentrates. Willard and Young (1930) have recently described a very elegant method for the determination of oxalic, malic, citric, tartaric and some other oxy-acids which is applicable to plant analysis once the difficulty of the separation of these individuals has been overcome. Acetic acid does not interfere, neither does succinic acid cause appreciable error under the conditions described. The process is a volumetric one, a solution of ceric sulphate being used as oxidising agent and the excess finally back titrated by means of a ferrous salt.

Since the method promises to gain in favour, a description of the preparation and standardization of the ceric sulphate solution is here briefly given. Commercial ceric oxide, containing relatively large quantities of the other rare earth metals was used. It was found that 24 gm. was sufficient to yield 500 c.c. of a decinormal ceric sulphate solution. This material was warmed in an evaporating basin with 100 c.c. of sulphuric acid solution of S.G. 1·5, small quantities being added at a time and the mixture continuously stirred. As the water evaporated off, the mass assumed first of all a rich red and then a deep yellow colour. This paste was again warmed with

25 c.c. of the acid and the stirring continued at as high a temperature as conveniently possible until no further lightening in the colour of the paste was observable. The whole operation occupied about 1½ hours. Water was then added to make a volume of about 450 c.c. and the solution kept at 75-80° for one hour after which it was cooled, filtered and adjusted to 500 c.c. Such a solution should be about normal in free sulphuric acid. For standardization a decinormal sodium oxalate solution was allowed to run into an aliquot of the ceric sulphate maintained at about 60°. The disappearance of the intense yellow colour of the ceric salt marks the end point, but as a check, or when titrating tinted solutions, a drop may be mixed externally on a spot plate with a few drops of a diphenylamine or diphenylbenzidine solution. A blue colour indicates the presence of free oxidising agent.

Tested upon citric acid, the method proved perfectly trustworthy. 20 c.c. citric acid solution (approx. 0·2 per cent.) was mixed with 75 c.c. of a 0·049 normal ceric sulphate solution (decinormal is to be preferred), 50 c.c. of sulphuric acid, S.G. 1·5 added, and water to 200 c.c. The mixture was boiled under the reflux condenser for 30 minutes, cooled and titrated with a freshly-prepared standardized ferrous sulphate solution (0·098 normal). The volume of ceric sulphate solution reduced was 64·6 c.c. whence (employing the factor 1 c.c. N/10 $Ce(SO_4)_4$ = ·001211 gm. citric acid) the original sample contained 0·1964 gm. per 100 c.c. The solution actually contained 0·1962 gm. citric acid per 100 c.c.

The determination of malic acid is carried out on precisely the same lines. Willard and Young find that 1 c.c. N/10 $Ce(SO_4)_4$ = ·001449 gm. malic acid. A preliminary experiment which afforded a rough indication of the quantity of malic acid present in the plant material was performed as follows: From a 1 per cent. hydrochloric acid extract, the lead salts of the organic acids were prepared by precipitation after neutralizing with ammonia. This lead precipitate was suspended in dilute acetic acid and the mixture kept at a temperature of 70° whilst being stirred for one hour. After centrifuging and washing, the acetic acid solution which contained the lead malate was made up to a volume of 250 c.c. An aliquot of this was treated with 2 volumes of alcohol and the precipitate filtered off, dried and weighed. Reckoned as lead malate it corresponded to slightly over 9 per cent. of malic acid in the plant powder. More accurate determinations were made by means of the ceric sulphate method, the solutions being first purified by taking advantage in the one case of the solubility of calcium malate in hot lime water and in the other the solubility of ammonium malate in 90 per cent. alcohol. The figures obtained by these two methods showed good agreement. A 1 per cent. hydrochloric acid extract of 2 gm. of the plant powder was precipitated by lead acetate and the lead salts decomposed by hydrogen sulphide, great care being taken to ensure complete decomposition. After filtration, the lead sulphide was again boiled out with a little ammonium sulphide and this filtrate added to the main bulk. After concentrating somewhat, an excess of hot lime water was added to the boiling solution and the precipitated calcium salts filtered off. Of the usually occurring acids all but malic acid are thus precipitated. The insoluble salts were boiled with

dilute acetic acid, when all but calcium oxalate dissolved, and determinations of oxalic acid and of tartaric acid made upon these two fractions are recorded below. Succinic acid was also looked for, but proved to be absent. The cooled solution of calcium malate in lime water was acidified with acetic acid and sufficient lead acetate added to precipitate the insoluble lead malate. This was removed, washed and decomposed with hydrogen sulphide by passing a stream of the gas through a suspension of the precipitate in hot water. After filtering off the lead sulphide and boiling vigorously to expel all traces of the gas, the solution was adjusted to a volume of 100 c.c. and an aliquot of 25 c.c. taken for determination by the cerium method.

25 c.c. solution, 40 c.c. decinormal ceric sulphate, 50 c.c. sulphuric acid S.G. 1·5 and 85 c.c. water were refluxed for 30 minutes.

Volume of N/10 ferrous sulphate used in back titration = 5·4 c.c.

∴ Volume of ceric sulphate reduced = 34·6 c.c.

Since 1 c.c. N/10 $Ce(SO_4)_4$ = ·001449 gm. malic acid, this represents 50·14 mgm. malic acid/25 c.c.

or 10·78 per cent. in plant on dry wt. basis.

In a second experiment, an extract was prepared from 1 gm. of the plant powder, treated as above, and made up to a final volume of 100 c.c. A 50 c.c. aliquot of this reduced 35·5 c.c. ceric sulphate solution.

∴ 51·44 mgm. malic acid/50 c.c.
or 11·06 per cent. in plant on dry wt. basis.

Although the agreement was satisfactory in these two determinations carried out upon different quantities of materials, confirmation of the above figures was afforded by the determinations made according to the second method when the ammonium salts of all of the organic acids except malic acid are precipitated by addition of 9 volumes of alcohol to their aqueous solution.

An extract from 2 gm. of plant powder was precipitated by lead acetate and the free acids regenerated by means of hydrogen sulphide. A slight excess of ammonia was then added and the liquid boiled down to a volume of 30 c.c. After cooling, 280 c.c. of 96 per cent. alcohol was added and the precipitate removed. Lead acetate solution in excess of the quantity required to transform the ammonium malate into insoluble lead malate was then added and the precipitate removed, washed and decomposed by hydrogen sulphide, taking the precautions noted above. After vigorous boiling, the resulting solution of malic acid was made up to a volume of 100 c.c. Upon 25 c.c. aliquots of this, determinations of malic acid were made by the cerium method. The amount corresponded to 11·21 per cent. in the plant on the dry weight basis.

It can safely be concluded from the results obtained by these two entirely different methods that, in spite of the difficulties of the problem, the procedures adopted were selective for malic acid.

The mean of the three determinations 10·78 per cent., 11·06 per cent. and 11·21 per cent. is 11·02 per cent.

DETERMINATION OF OXALIC AND TARTARIC ACIDS IN THE RESIDUES FROM THE MALIC ACID DETERMINATIONS.

The calcium salts insoluble in hot lime water were boiled with dilute acetic acid when calcium oxalate remained undissolved. It was filtered off, dissolved in 200 c.c. of hot, dilute sulphuric acid and an aliquot titrated with hundredth normal potassium permanganate.

5 c.c. required 9·4 c.c. N/100 $KMnO_4$.

5 c.c. after reprecipitation required 9·2 c.c. N/100 $KMnO_4$.

This corresponds to 8·59 per cent. in agreement with the figures previously found (mean 8·66 per cent.).

Tartaric acid was determined in the acetic acid solution by precipitation as potassium hydrogen tartrate as previously described. The precipitate required 0·9 c.c. of N/10 NaOH. This corresponds to 0·072 per cent. of tartaric acid agreeing with the figures of 0·064 per cent. and 0·072 per cent. previously found.

Succinic acid was tested for by making a solution of the free acids, regenerated from their lead salts, neutral to litmus and adding neutral ferric chloride solution. No precipitation of ferric succinate was formed indicating the absence of succinic acid.

RESUMÉ OF ANALYTICAL RESULTS.

For convenience, the figures found are here recapitulated. All percentages are upon the dry weight basis. The green plant contained 67·75 per cent. moisture.

Free titratable acidity of the aqueous extract	= 5·15 c.c. N/10 acid	per gm.
Ash left on ignition 24·58% / 24·62% Mean	= 24·60%	
Acid-insoluble residue (silica, etc.)		
1·02% / 1·01% Mean	= 1·02%	
Calcium in the ash as CaO	= 8·19%	
Total Ca in the plant	= 1·34%	
Potassium in the ash as K_2O	= 5·20%	
Alkalinity of the ash: water-soluble	= 30·51 c.c. N/10 alkali	per gm.
water-insoluble	= 10·31 c.c.	,,
Total 40·82 c.c. / 40·12 c.c. Mean	= 40·47 c.c.	,,
Oxalates as oxalic acid by—		
direct method 8·61% / 8·70% Mean	= 8·66%	
Indirectly *via* Pb salts during determination of malic acid 8·59%		

Malates as malic acid by—

Ca salt method 10·78%
11·06%
Mean = 11·02%
NH_4 salt method 11·21%

Tartrates as tartaric acid by—

direct method 0·064%
0·072%
Indirectly *via* Pb salts during determination of malic acid ... ... 0·072%
Mean = 0·069%

Citrates: absent.
Succinates: absent.

From the protocols of the feeding experiments, recorded in the appendix, it will be seen that the quantity of the fresh plant found to be lethal for rabbits is such as to contain approximately 3 gm. of oxalic acid. The minimum lethal dose of oxalic acid for the rabbit *per os* is given in the pharmacological literature as 2·4 gm. Malic acid is not toxic, in fact it fulfils an important rôle in certain physiological processes in animal metabolism. We were interested to see if any synergistic action was produced by the presence of such large quantities of malic acid along with the oxalic acid in *Psilocaulon absimile*, but such does not appear to be the case.

Interrelations of the Organic Acids and their Physiological Function in Plants.

There has been much speculation as to the rôle played by the organic acids, oxalic, malic, tartaric, citric, etc., in the economy of the plant, and several theories have been advanced.

In the first place it is noticeable that, although the acids concerned enjoy a fairly wide distribution in the vegetable kingdom, it is only in certain genera that they are met with in appreciably high concentration, and such plants are very frequently succulent or xerophytic types. Again it has always seemed perplexing that the quantities of oxalic and malic acids present seem often to be complementary, one rising whilst the other falls during the course of the season, or one being the main representative in a certain species, whilst in another closely allied member of the genus the relative proportions may be quite reversed.

Only recently has an acceptable theory of acid production, harmonizing with experimental data, been put forward. However, it is considered not out of place to give here a very brief summary of earlier views which have led up to the present position.

Mayer (1875, 1878) considered that the organic acids were products of plant respiration and that, under the influence of light, they suffered reduction to carbohydrates. A purely photochemical explanation of their disappearance was also advanced by Spoehr (1913), but, although the acids are sensitive to light of certain wavelengths, there can be no doubt that other factors more intimately connected with metabolic processes come into play in the living plant.

An enzyme with the power of decomposing oxalic acid has been demonstrated in *B. extorquens* by Bassalik (1913), and in the tissues of a wide variety of plants by Staehelin (1919). Light would seem to be capable of exerting a stimulating effect upon oxalate production in leaves.

Kraus (1886a, 1886b) regards the daily fluctuations in acid content of succulents and non-succulents to be a general phenomenon, the accumulation which takes place during the night being due to the incomplete oxidation of these products of respiration. With the return of daylight their oxidative removal is assisted by the higher tension of oxygen set free in the assimilation process and possibly also by the direct action of light itself. With this hypothesis Warburg (1886) is in general agreement. De Vries (1885) emphasized the importance of temperature in regulating the intensity of oxidation.

Oxalates have frequently been regarded as excretory products either incidental or subservient to a useful function by rendering the plant unpalatable and poisonous. They may also exert a regulatory function and some experimental evidence seems to bear out the accuracy of this view. Thus De Bary (1886) was able to show that oxalate production in the fungus *Peziza sclerotiorum* can be stimulated or depressed by including much or little calcium in the culture medium. Wehmer (1897, 1906, 1913) found that *Aspergillus* sp. produces little or no oxalate when grown upon a medium of sugar to which ammonium chloride has been added as a source of nitrogen, but considerable quantities of this acid when ammonium chloride is replaced by peptone. A similar finding is reported by Benecke (1903) who, working with *Zea mais*, found oxalates to be produced in much greater quantity when nitrates took the place of ammonium salts as the source of nitrogen. Amar (1903) showed many caryophyllaceous plants could be obtained oxalate-free by allowing the seeds to germinate and grow in a Ca-free medium. An association between incomplete carbohydrate oxidation and the production of oxalic acid has been postulated by many workers, among whom Duclaux (1883) and Wehmer (1891) may be particularly mentioned, and a great deal of experimentation has been directed towards a comparison of the quantities of oxalic acid produced by one or other of the lower organisms when growing upon different individual sugars as the sole source of carbon. It cannot be said that any very clear conclusions of a general nature have emerged from these experiments.

Ruhland and Wetzel (1926, 1927, 1929) have recently brought an entirely new light to bear upon the problem. These authors recognize two types of plants, the " amide " plants and the " ammonia " or " acid " plants. In the former, oxidative deamination of amino-acids leads to the formation of acid amides such as asparagine and glutamine and such plants are characterized by a relatively high content of amide nitrogen and correspondingly low ammonia nitrogen. Acid plants on the other hand differ in containing relatively much ammonia and little amide nitrogen, whilst they are also rich in organic acids. In such plants deamination proceeds so as to form simultaneously organic acids of the malic type and ammonia. The non-nitrogenous residues of deaminated amino-acids are considered to be the source of the oxalic, malic, tartaric and succinic acids found in the vegetable kingdom (c.f. Wetzel, 1927). Ruhland and Wetzel

find no correlation between the production of such acids and the degree of respiratory activity and therefore discard the theory that they are to be considered as products of respiratory activity. On the contrary, they were able to demonstrate a close relationship between the degree of deamination and the accumulation of organic acids and of ammonia in such typical acid plants as *Begonia semperflorens* and *Rheum hybridum* Hort. It is of interest that *Psilocaulon absimile* is also rich in ammonium compounds.

Following upon the acceptance of Ruhland and Wetzel's work, many of the earlier experimental findings can be viewed in a new light and many obscure points readily interpreted. The greater production of acids in young leaves during the night time is thus to be linked with the active deamination which is then known to proceed. Similarly the much greater production of oxalic acid by *Aspergillus niger* when peptone replaces sugar in the culture medium is attributable to the more active deamination proceeding in order to supply energy and nitrogen for the metabolic needs of the growing organism. The acid first formed is probably the α-ketonic acid pyruvic acid which Quastel (1925) has shown to occupy a central position with regard to the various lines of synthesis and degradation inherent in the life cycle of the normal bacterial cell. Pyruvic acid may be looked upon as the common exchange medium between the lines of carbohydrate, protein and fat metabolism. Its production from alanine and transformation into malic acid may be represented by the following scheme:—

$$CH_3\cdot CH(NH_2)\cdot COOH \xrightarrow[\text{deamination}]{-NH_3 + O,\ \text{oxidative}} CH_3\cdot CO\cdot COOH \xrightarrow[\text{reduction}]{+2H} CH_3\cdot CH(OH)\cdot COOH$$

alanine — pyruvic acid — malic acid

Oxalic acid probably arises from the oxidative deamination of glycine followed by further oxidation to the di-carboxylic acid or by degradation of the acids with longer carbon chains.

$$CH_2(NH_2)\cdot COOH \xrightarrow[\text{deamination}]{-NH_3 + O,\ \text{oxidative}} \begin{matrix} CHO \\ | \\ COOH \end{matrix} \xrightarrow[\text{oxidation}]{+O} \begin{matrix} COOH \\ | \\ COOH \end{matrix}$$

glycine — glyoxylic acid — oxalic acid

Oxalic acid is frequently deposited in various plant organs or specialized cells in the form of the sparingly soluble calcium oxalate, the quantity of which by slow accumulation may often come to represent an astonishingly large proportion of the whole plant. Thus in *Pilocereus senilis*, a member of the cactaceae, potassium oxalate may form between 80 per cent. and 90 per cent. of the total dry substance. *Mesembryanthemum cristallinum*, the lichens *Lecanora esculenta* (66 per cent. calcium oxalate) and *Chlorangium Jusuffii* (65 per cent. calcium oxalate), the bark of the Eucalyptus tree (16 per cent. calcium oxalate) and of Ceylon Cinnamon (6·6 per cent. oxalate) bear further evidence to this accumulation of oxalic acid. The soluble sodium salt occurs in *Salicornia* and *Salsola* species.

Citric and malic acids are frequently deposited as the sparingly soluble calcium salts in a manner analogous to oxalic acid. For example, 3·5 per cent. potassium malate in various *Rheum* species (Castoro, 1902); 8 per cent. expressed as malic acid in *Agave* leaves (Zellner, 1918). In this connection it is of interest to compare the various species of the genus *Mesembryanthemum* (Table I) which have been investigated, but it is also necessary to bear in mind the fact that marked fluctuations in the acid content may occur during the course of the seasons. This latter point is well illustrated by the table reproduced below (Table II) from the work of André (1905). From the toxicological standpoint this seasonal variation is also of direct importance, since such a plant may be highly poisonous at one season and practically innocuous at another. According to Berthelot and André (1886, 1887) the oxalate content of a large number of plants, including *Rumex*, *Amaranthus* and *Mesembryanthemum* species, tends to rise to a maximum in the summer months, declining again towards autumn.

TABLE I.

DISTRIBUTION OF THE ORGANIC ACIDS IN *Mesembryanthemum* SP.

	Oxalic Acid.	Malic Acid.	Citric Acid.	Other Constituents.
M. crystallinum L...	Much	Much	Possibly traces	Ash 30–50 % of which 50 % is K (André 1905–06).
M. tortuosum L....	—	—	Present in Raphides with Mg and H_3PO_4	Alkaloid " Mesembrin " also epidermal wax (Hartwich and Zwicky 1914).
M. expansum L.....				
" Channa " of the Hottentots				
M. edule L.....	None	Present	Present	(Berg and Gerber 1896.)
M. linguiforme L....	Little	Much	Little	
M. perfoliatum Mill..	Little	Little	Much	
Psilocaulon absimile..	8·6 %	11·02 %	None	Tartaric acid 0·07 %. (Present work).

TABLE II.

VARIATION OF CONTENT IN ORGANIC ACIDS DURING THE YEAR; AS PER CENT OF DRY SUBSTANCE (ANDRÉ, 1905).

	Soluble Oxalate.	Insoluble Oxalate.	Malic Acid.
Mesembryanthemum—			
May 26.....	10·53	11·92	3·67
June 13.....	6·16	9·68	4·40
July 1.....	5·29	5·50	10·71
July 22.....	4·86	4·79	—
August 17.....	1·90	2·56	13·83
Sedum azureum—			
May 25.....	0·15	1·67	7·62
June 17.....	0·23	0·25	8·73
June 21.....	0·45	1·62	8·42
July 8.....	Trace	0·74	10·13
July 29.....	Trace.	0·35	7·72

Toxicology of Oxalic Acid and Oxalates.

Oxalic acid is a relatively strong acid and its administration is therefore accompanied by some degree of gastric inflammation. The oxalate ion also possesses toxic properties, however, the effects of which are shown equally well by the soluble potassium and sodium salts as by the free acid; calcium oxalate, on account of its low solubility is poorly absorbed and is therefore quantitatively much less toxic. Chickens are said to be practically immune to oxalic acid poisoning, if administered orally, on account of the high proportion of calcium present in the contents of their intestines.

The toxic effects of oxalate ingestion may be summarized briefly as follows:—

(1) A local inflammation, present even when the acid is administered in dilute solution.

(2) Muscular twitching or tetany, accompanied by other nervous symptoms, due to the removal of calcium ions from the system and an upset of the base balance CaMg/NaK.

(3) Lowered coagulability of the blood owing to the decrease in calcium ions.

(4) Lesions in the excretory organs, kidney, etc., owing to the deposition in the cellular substance of hard, crystalline concretions of calcium oxalate.

In the experiments carried out upon rabbits during the course of the present work, it was found that a dose of 4·4 gm. of sodium oxalate (equivalent to 2·9 gm. oxalic acid) given per os to a 3 kilogram rabbit was sufficient to cause death in about 1 hour, the chief symptom being muscular weakness and tremor, followed by violent convulsions as death took place. Administration of a solution of the regenerated lead salts of the organic acids from 33 gm. dried *Psilocaulon absimile*, containing approximately 2·9 gm. oxalic acid, produced similar results, death taking place rather more quickly when the unneutralized fluid was given than when previously neutralized by sodium hydroxide.

Chronic oxalic acid poisoning through the ingestion of *Oxalis cernua* the " South African wood-sorrel " or " Soursobs " has been described by Bull (1929), who found farm animals in Australia to be affected by feeding upon this plant. A period of 6 to 8 weeks was required before the symptoms became serious or fatalities occurred.

Affected animals lost control of the fore-quarters or hind-quarters becoming unable to rise. Tonic spasms of the muscles of the forelegs and neck were noticed. In some cases death was sudden, whilst other animals lingered on for a greater or lesser period. On post-mortem examination the kidneys were found to be pale in colour, containing some fibrous tissue in the cortex; extensive degenerative changes were seen in the tubules, whilst scattered throughout the cortex and boundary zone numerous refractile deposits of crystalline calcium oxalate were found.

Continued ingestion of *Psilocaulon absimile* in sub-lethal doses would presumably lead eventually to the same result.

In the Willowmore cases of suspected subacute *Psilocaulon* poisoning in Angora goats the affected animals exhibited the following symptoms:—Cachexia, extreme weakness, anaemia, chronic diarrhoea and inappetence. The post-mortem revealed anaemia, hydroperitoneum, hydrothorax, hyperaemia of the lungs, liver and kidneys and a severe acute catarrhal gastro-enteritis.

REFERENCES.

ALBAHARY (1912). Ann. des Falsifications, Vol. 5, p. 147.

AMAR, M. (1903). Sur le rôle de l'oxalate de calcium dans la nutrition des végéteaux. *Comp. Rend.*, Vol. 136, p. 901.

ANDRÉ, G. (1905). *Compt. Rend.*, Vol. 140, p. 1708.

ANDRÉ, G. (1906). *Compt. Rend.*, Vol. 142, p. 902.

BASSALIK, K. (1913). Über die verarbeitung der Oxalsäure des Bacillus extorquens. *Jahrb. f. wiss. Bot.*, Vol. 53, p. 235.

BASSALIK, K. (1917). Über die Rolle der Oxalsäure bei den grünen Pflanzen. *Bull. Acad. Sci. de Cracovie S.B.*, 1917, p. 203.

BENECKE, W. (1903). Über Oxalsäurebildung in grünen Pflanzen. *Bot. Zeitg.*, Vol. 61, p. 79.

BERG AND GERBER (1896). *Bull. Soc. Chem.*, Series 3, Vol. 15, p. 1050.

BERTHELOT AND ANDRÉ (1886). *Compt. Rend.*, Vol. 102, pp. 995 and 1043.

BERTHELOT AND ANDRÉ (1887). *Ann. Chem. et Phys.*, Series 6, Vol. 10.

BLEYER, B., AND SCHWAIBOLD, J. (1925). *Milchwirtschaftl. Forsch.*, Vol. 2, p. 260.

BULL, L. (1929). Poisoning of sheep. Chronic oxalic acid poisoning. *Austr. Vet. Jnl.*, Vol. 5, No. 2, p. 60.

Bulletin of the Imperial Institute (1912), Vol. 10, p. 300.

Bulletin of the Imperial Institute (1916), Vol. 14, p. 34.

BURT-DAVY, J. (1912). Poisonous properties of *Mesembryanthemum Mahoni*, N.E.Br. *10th Report South African Ass. Adv. Sci.*, pp. 193-5.

CASTORO (1902). *Landw. Vers. stat.*, Vol. 55, p. 423.

DE BARY (1886). *Bot. Zeitg.*, 1886, p. 400.

DE VRIES (1885). Über die Periodizität in Säuregehalt der Fettpflanzen. *Verslagen en Mededeelingen der K. Akad. van Wetenschappen te Amsterdam* III. Reeks I, deel 58.

DRAGENDORFF, G. Die Heilpflanzen d. verschiedenen Völker u. Zeiten. Stuttgart, 1898.

DUCLAUX (1883). Chemie Biol. (Encyclop. chem. IX), p. 219.

FRANZEN, H., AND OSTERTAG, R. (1922). *Zeit. f. physiol. Chem.*, Vol. 122, 263, also Ber. Vol. 55, 2995 (1922).

FRANZEN, H., AND HELWERT, F. (1922). *Zeit. f. physiol. Chem.*, Vol. 122, p. 46.

FRANZEN, H., AND STERN, E. (1921). *Zeit. f. physiol. Chem.*, Vol. 115, p. 270.

FRANZEN, H., AND SCHUMACHER, E. (1921). *Zeit. f. physiol. Chem.*, Vol. 115, p. 9.

HARTMAN, B., AND HILLIG, F. (1930). *Jnl. Assn. Agric. Chemists*, Vol. 13, p. 103.

HARTWICH, C., AND ZWICKY, E. (1914). Channa, a delicacy of the Hottentots (*Mesembryanthemum expansum*, L. and *M. tortuosum*, L.), *Apoth. Zeitg.*, Vol. 29, p. 925.

KOGAN, A. (1930). *Zeit. f. Anal. Chemie*, Vol. 80, p. 112.

KRAUS, G. (1886*a*). Über die Wasserleitung in der Pflanze, IV. *Abhandl. d. Naturf. Gesellsch. zu Halle*, Vol. 16, p. 143.

KRAUS, G. (1886*b*). Über den Stoffwechsel der Crassulaseen. *Abhandl. d. Naturf. Gesellsch. zu Halle*, Vol. 16, p. 395.

MAYER, A. (1875). Über die Bedeutung der organischen Säuren in dem Pflanzen. *Landw. Vers-Stat.*, Vol. 18, p. 410.

QUASTEL, J. (1925). On a possible rôle of pyruvic acid in bacterial growth. *Biochem. Jnl.*, Vol. 19, p. 641-4.

RUHLAND, W., AND WETZEL, K. (1926). Zur Physiologie der organischen Säuren in grünen Pflanzen. I. *Planta*, Vol. 1, p. 558-564.

RUHLAND, W., AND WETZEL, K. (1927). Zur Physiologie der organischen Säuren in grünen Pflanzen III. *Planta*, Vol. 3, p. 765-769.

RUHLAND, W., AND WETZEL, K. (1929). Zur Physiologie der organischen Säuren in grünen Pflanzen V. *Planta*, Vol. 7, p. 503-507.

SPOEHR, H. (1913). Photochemische Vorgänge bei der diurnalen Entsäurung der succulenten. *Bioch. Zeit.*, Vol. 57, p. 99.

STAEHELIN, M. (1919). Die Rolle der Oxalsäure in der Pflanze. Enzymatischer Abbau des Oxalat-ions. *Biochem. Zeit.*, Vol. 96, pp. 1-49.

STEYN, D. (1931). Recent investigations into the toxicity of known and unknown poisonous plants in the Union of South Africa. *17th Rept. Dir. Vety. Serv. and Anim. Indust.*, August, 1931, pp. 708-9.

WARBURG, O. (1886). Über die Bedeutung der organischen Säuren in Lebensprozess der Pflanzen. *Unters. a.d. Bot. Inst., Tübingen*, Vol. 2, p. 53.

WEHMER, C. (1891). Entstehung u. physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. *Bot. Zeitung*, Vol. 49, p. 233.

WEHMER, C. (1897). *Zentr. Bakt.*, Series II, Vol. 3, p. 102.

WEHMER, C. (1906). *Ber. bot. Ges.*, Vol. 24, p. 381.

WEHMER, C. (1913). *Biochem. Zeit.*, Vol. 59, p. 63.

WETZEL, K. (1927). Zur Physiologie der organischen Säuren in grünen Pflanzen IV. *Planta*, Vol. 4, pp. 476-525.

WILLARD, H., AND YOUNG, P. (1930). *Jnl. Amer. Chem. Soc.*, Vol. 52, p. 134.

ZELLNER, J. (1918). *Zeit. f. physiol. Chem.*, Vol. 104, p. 2.