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The Occurrence of Cyanogenetic Glucosides in South African Species of Acacia I.

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DURING the recent drought many stock-owners asked for information in connection with the feeding-value of various species of *Acacia*, the pods of which are being used as a stock-feed, and which are also being extensively eaten by grazing stock. Several stock-owners had good reason to suspect that of these the *Transvaal kameeldoorn* (*Acacia giraffae* Willd.) had caused poisoning and death in their stock. These reports came as a great surprise, as the *kameeldoorn* pods had been and are still being extensively fed to stock with very good results.

The fact that the leaves of the *Natal kameeldoorn* (*Acacia lasiopetala* Oliv.), which is also considered an excellent stock feed, had undoubtedly killed a large number of sheep in the Klipriver District, Natal, at once directed our attention to the *Transvaal kameeldoorn* and other species of *Acacia*. The former *kameeldoorn* was found to contain a large amount of *prussic acid*.

It was, therefore, decided to examine all the obtainable species of *Acacia*, commonly known as *doringbome* (thorn trees, or Mimosa), for the presence of *prussic acid*, and this article is a preliminary report on this investigation, which is still proceeding.

Fig. 1.—*Acacia giraffae* Willd.

I. Acacia giraffae Willd.

Common names.—Afrikaans: Transvaal kameeldoorn, Transvaal kameeldoornboom, kameelboom. English: Camelthorn.

Origin.—(A) A specimen of ground mature pods (seed + shell) sent in from the Hoopstad District, O.F.S., was found to contain 25·6 mgm. of HCN per 100 gm. dry weight.

(B) The following specimens (O.P.H. No. 9262, 6.1.34) were collected by one of the authors with the kind assistance of Mr. C. A. Smith, botanist, from two trees growing near the Wonderboompoort, Pretoria North, and were found to contain the following amounts of *prussic acid*. The procedure was essentially that described in a previous communication (Rimington, 1932) maceration being continued for 36 hours at room temperature either with, or without, the addition of emulsin:—

(*a*) Fresh green leaves: 77·6 mgm. HCN per 100 gm. dry weight;

(*b*) Fresh immature pods: 72·6 mgm. HCN per 100 gm. dry weight.

(*c*) The shells of mature pods gathered under the tree contained the cyanogenetic glucoside, but not the enzyme necessary for its decomposition. The seeds contained in these pods, however, showed the presence of both the enzyme and the cyanogenetic glucoside. The shells of these pods were very moist and partially decomposed as heavy rains had recently fallen. No quantitative determination of the amount of *prussic acid* could be made as only a few pods were availalbe and these were used in the preliminary tests for the presence of *prussic acid* and the enzyme.

Fig. 2.—*Acacia karroo* Hayne.

II. Acacia karroo Hayne.

Common names.—Afrikaans: Soetdoorn, karodoring, suurdoring, witdoring. English: Karroothorn, white thorn, sweet thorn, Mimosa, gum arabic tree. Zulu: Umu Nga. Suto: Leoka.

Origin.—(A) Onderstepoort (O.P.H. No. 8087, 21.11.33) (N.H.P. No. 15748).

The fresh leaves, flowers, and immature pods collected from the same tree were repeatedly submitted to the *prussic acid* test (sodium picrate paper) with negative results. These tests were performed with the plant alone, and also with the addition of emulsin, or a few drops of chloroform, or in an acid environment (pH 6). Neither was *prussic acid* detectible in branches which had been picked and allowed to wilt in the sun.

(B) Leaves and flowers (O.P.H. Nos. 9802, 9803, 9804, 21.7.34) collected from three different trees on the Broederstroom Estates, Pietersburg, also yielded negative results. Subsequently the leaves and flowers of two further specimens collected in the Pietersburg District were examined. No *prussic acid* was detectable.

Fig. 3.—*Acacia lasiopetala* Oliv.

III. Acacia lasiopetala Oliv.

Common names.—Afrikaans: Natalse kameeldoring, Natalse kameeldoringboom. English: Natal camelthorn. Zulu: Um Khamba.

Origin: "Amberley", P.O. Matiwane, Klipriver District, Natal (O.P.H. No. 8038 17.11.33) (N.H.P. No. 15745).

Specimens of the flowering branches were submitted for investigation, as it was suspected of having caused mortality in sheep. A heavy hailstorm had passed over a camp in which there were many of the trees. The result was that numerous small branches were knocked off and these were eagerly eaten by the sheep, which were in a state of starvation owing to the then prevailing drought. Many of the sheep were found dead the following morning.

Five hundred grams of fairly fresh leaves and stems drenched to a full-grown sheep induced typical symptoms of *prussic acid* poisoning and death half-an-hour after administratioin. Another sheep, which had received 300 gm. of slightly wilted leaves and stems, recovered after having exhibited pronounced symptoms of *prussic acid* poisoning.

The sodium picrate paper test revealed the presence of a large amount of *prussic acid* both in the leaves and flowers. In all picrate paper tests referred to in this paper 20 gm. of fresh plant and 10 gm. of dry plant were used. Quantitative determinations yielded the following results:—

(*a*) Leaves: 166·0 mgm. HCN per 100 gm. dry weight.

(*b*) Flowers: 102·5 mgm. HCN per 100 gm. dry weight.

(*c*) Stems: 89·9 mgm. HCN per 100 gm. dry weight.

Subsequently seeds collected from mature pods also showed the presence of *prussic acid*. The amount was less than that found in the leaves, flowers, and stems.

IV. Acacia litakunensis Burch.

Common name.—Afrikaans: Haak-en-steek.

Origin.—Brits, Transvaal (O.P.H. No. A, 20.7.33).

The following sodium picrate paper tests were conducted with the mature pods:—

(1) Dry shells: Negative.

(2) Dry shells + emulsin: Negative.

(3) Dry shells + HCl (pH 6): Negative.

(4) Dry seeds: Definite, but slight, reddening of picrate paper.

(5) Dry seeds + emulsin: Definite, but slight, reddening of picrate paper.

(6) Dry seeds + HCl (pH 6): Definite, but slight, reddening of picrate paper.

It therefore appears that the shells are free from *prussic acid*, whilst the seeds yield a small quantity. No material was available for a quantitative determination of *prussic acid* in the the seeds.

V. Acacia permixta Burtt-Davy.

Common names.—Afrikaans: Doringboom. English: Mimosa, thorn tree.

Origin: Pietersburg (O.P.H. No. 9301, 10.1.34) (N.H.P. No. 15788).

The dry leaves tested alone, with the addition of a few drops of chloroform and with emulsin, failed to reveal the presence of *prussic acid.*

Fig. 4.—*Acacia robusta* Burch.

VI. Acacia robusta Burch.

Common names.—Afrikaans: Doringboom. English: Thorn tree.

Origin.—" Redlands ", P.O. Munnik, Pietersburg District. (O.P.H. No. 9301, 10.1.34) (N.H.P. No. 15788).

The following tests were conducted with sodium picrate paper:—

(a) Fresh Immature Pods.

(1) Pods alone: Negative.
(2) Pods + HCl (pH 6): Negative.
(3) Pods + chloroform: Negative.
(4) Pods + emulsin: Positive within half-an-hour.

Both the fresh and dry leaves yielded the same results as the fresh immature pods.

It therefore appears that the leaves and immature pods contain a cyanogenetic glucoside, but not the enzyme necessary for its decomposition.

Fig. 5.—(A) *Acacia litakunensis* Burch (seed); (B) *Acacia stolonifera* Burch.

VII. Acacia stolonifera Burch.

Common name.—Afrikaans: Trassiebos.

Origin.—Specimens were collected by one of the authors with the kind assistance of Mr. C. A. Smith, botanist, from a shrubby tree growing near the Wonderboompoort, Pretoria North. Both the fresh leaves and fresh immature pods were found to contain large amounts of *prussic acid* (O.P.H. No. 9263, 6.1.34).

Quantitative determination of Prussic Acid in leaves and immature pods.

(*a*) Leaves: 87·0 mgm. HCN per 100 gm. dry weight.

(*b*) Pods: 61·9 mgm. HCN per 100 gm. dry weight.

Discussion.

(*a*) *The Toxicity of Species of Acacia.*—From the results of the above investigation it is apparent that certain species of *Acacia* contain highly dangerous amounts of *prussic acid* (cyanogenetic glucoside), whilst others, for example *Acacia karroo*, do not contain this poison. The *prussic acid* is more concentrated in the leaves than in the mature pods. From the few specimens investigated it appears that immature pods may possibly be more dangerous than mature pods. *Acacia lasiopetala* contains a lethal amount of *prussic acid* in its leaves, flowers, and in the stems of the young shoots. The mature seeds were found to contain less cyanogenetic glucoside than the leaves and flowers. The leaves and immature and mature pods of *Acacia giraffae* contain dangerous amounts of *prussic acid*. The partly decomposed shells of the mature pods still showed the presence of the *cyanogenetic glucoside*, whilst the enzyme necessary for the liberation of *prusic acid* was absent. Dangerous amounts of *prussic acid* were also found in the leaves and immature pods of *Acacia stolonifera.*

In *Acacia robusta*, a cyanogenetic glucoside is present, whilst the enzyme necessary for its decomposition is absent. Other plants may, however, contain enzymes capable of decomposing this glucoside. If such plants are eaten together with *Acacia robusta*, poisoning may be expected to occur, as *prussic acid* will be rapidly liberated.

The seed of *Acacia litakunensis* contains a small amount of cyanogenetic glucoside and an enzyme capable of decomposing this glucoside. The amount of *prussic acid* evolved is, however, too small seriously to endanger stock eating the pods. It should be borne in mind that in many cases the amount of *prussic acid* varies to a considerable extent not only in the same species of plant growing in different localities, but also in the same plant at different times.

Seddon and King (1930) state that *fresh plants*, which contain 0·02 per cent. *prussic acid*, are capable of causing fatal effects in sheep if we assume that the animal eats an average of 500 gm. of the plant. They consider *dry plants* containing 0.05 per cent. *prussic acid* dangerous to stock.

Seddon and King (1930) found that 68·6 gm. of dry *Acacia glaucescens* killed a sheep (103 lb.) in fifty-five minutes. Almonds (6·86 gm.) were administered immediately after the plant in order to provide ample enzyme for the liberation of *prussic acid* from the cyanogenetic glucoside, *sambunigrin*.

(*b*) *Cyanogenetic glucosides in Species of Acacia.*—The only reference to the presence of cyanogenetic glucosides in species of *Acacia* which we have been able to find in the literature is that to the work of Finnemore and Gledhill (1928), who detected their presence in the four species *A. glaucescens*, *A. cheelii*, *A. doratoxylon*, and *A. Cunninghamii* out of sixty species examined, and the further paper by Finnemore and Cox (1930) in which the isolation of the glucoside is described from *A. glaucescens* and *A. cheelii* and its identification with *sambunigrin*, the cyanogenetic glucoside present in the black elder, *Sambucus nigra*.

It appeared to us that it would be a point of considerable interest to follow up our qualitative observations by isolating the glucoside present in the South African species of *Acacia* and comparing it with that found in the Australian species. The majority of the Australian spp. of the genus *Acacia* belong to a totally different group in the genus than do the South African spp.

The material used in this study was a consignment of *Acacia lasiopetala* from the Klipriver, Natal, and yielding 76·7 mgm. of HCN per 100 gm. dry weight.

At first the method described by Finnemore and Cox was used, in which, after a preliminary extraction with petroleum ether, the ground plant material was subjected to exhaustive extraction in a Soxhlet apparatus with ether. The Australian authors stated that *sambunigrin* crystallized out during the course of the extraction in a state approaching purity. *Acacia lasiopetala* yielded no such material; in fact, the ether solution contained only traces of glucoside. Direct extraction with ethyl acetate was then tried, a method which gave good results in the case of *Dimorphotheca spectabilis* and *D. cuneata* (Marais and Rimington, 1934). In the present instance, however, it was found unsatisfactory. Finally, the isolation of the glucoside was accomplished after a good deal of difficulty by following the usual method of procedure as described below.

1·5 Kilograms of the ground, dried plant, containing 76·7 mgm. HCN per 100 gm. or 1·15 gm. HCN in all, was dropped into 4 litres of boiling 92 per cent. alcohol, to which calcium carbonate had been added. After standing for some hours, the liquid was pressed off and the residue similarly treated with a further 4 litres of alcohol. The combined extracts were shaken with 20 gm. of decolourising charcoal which removed the chlorophyll and certain other troublesome substances, but did not remove any appreciable quantity of glucoside. After filtration and addition of solid calcium carbonate, the liquid was distilled under reduced pressure and most of the alcohol removed. The extract was diluted with water and filtered. The volume of the filtrate was 900 c.c. A determination of available HCN was carried out by adding 5 c.c. to 150 c.c. of citrate buffer mixture of pH 6·0 (Rimington, 1932), introducing some emulsin and leaving the stoppered flask at room temperature for three days. The HCN was then determined in the usual way by distillation and titration with silver nitrate.

5 c.c. extract yielded 6·3 mgm. HCN.
.. total HCN present in 900 c.c. = 1·13 gm.

The main extract was then concentrated on the water bath, a fan being used to assist evaporation and keep the temperature low. Calcium carbonate was present in excess.

The syrup so obtained was extracted exhaustively with hot 96 per cent. alcohol, ether added to this solution until precipitation of inorganic material, etc., ceased, and the clear filtrate then concentrated to dryness and the residue taken up in water, boiled with decolorising charcoal, filtered and again evaporated. The residue remaining was then extracted exhaustively with boiling ethyl acetate which had been saturated with water. After removing this solvent, the residue was extracted with dry ethyl acetate using a sufficient volume in successive portions to remove the bulk of the glucoside. Such ethyl acetate solutions deposited a crystalline material which was admixed with a good deal of syrupy residue, mostly uncrystallised glycoside; so after repeating the ethyl acetate crystallisation a few times, the crystalline residue was dissolved in hot absolute alcohol, about 6 volumes of dry ether added, and the mixture left in the ice-chest. The glucoside was deposited in finely crystalline form on the sides and bottom of the tube. To increase the yield, a little petroleum ether could be added when most of the glucoside had already separated out. After decanting the mother liquor, the crystals were washed well with ether and dried in vacuo. The glucoside was obtained in beautifully formed prisms (see Fig. 1), varying in character from short and stout to long and needle-like. It had M.P. 176–7° and yielded HCN rapidly when treated with emulsin. The optical rotatory power was determined in aqueous solution, using a 2-dm. tube.

Weight of glucoside: 0·1460 gm.
Volume of solution: 15 c.c.
Rotation observed: −0·70°.

$$\therefore [\alpha]_D^{22} = -\frac{0\cdot7 \times 100 \times 15}{2 \times 14\cdot6} = -35\cdot96^\circ.$$

Microanalysis yielded the following figures:—

	C	H	N
Found	50·94	6·68	5·45
$C_{11}H_{17}O_6N$ requires	50·78	6·59	5·43

The empirical formula for the glucoside is therefore $C_{11}H_{17}O_6N$.

The material isolated by us from *Acacia lasiopetala* corresponds to none of the known glucosides. We propose to call it *Acacipetalin*, a name indicative of its origin.

The constitution of *Acacipetalin* will be discussed in a separate article by one of the present authors.

It is of particular interest that the South African *Acacia lasiopetala* was found to contain a glucoside different from that present according to Finnemore and Cox in the Australian species *Acacia gluacescens* and *A. cheelii*. In view of this fact, it would seem to be of particular interest to ascertain what glucosides are present in the phyllodineous species of *Acacia* native to South Africa.

(*c*) *The Suitability of Species of Acacia as Stock Feeds.*—It is well known that the mature pods of *Acacia giraffae* and *Acacia lasiopetala* are extensively eaten by, and fed to, stock especially in times of drought and in winter, with excellent results. Several stock-owners, who had fed the pods of the former tree on an extensive scale in the recent drought, had reason to suspect that the ground pods had caused poisoning in some animals. In view of the fact that the pods of some species of *Acacia* contain *prussic acid* (*a cyanogenetic glucoside*) it is quite conceivable that stock will become poisoned if these pods are not fed with care or if large amounts are eaten in a short period of time. There is very little danger of poisoning in animals which pick up the mature pods in the veld, as ingestion is necessarily slow on account of their hard and woody character. Liberation of *prussic acid* in the gastro-intestinal tract is thus sufficiently slow to allow of its elimination.

The fresh leaves of species of *Acacia* containing *prussic acid* are more dangerous than the pods. As the trees are very thorny it is, however practically impossible for an animal to eat a sufficient quantity of fresh leaves within a short period of time to become poisoned. An illustration of what would happen if the fresh leaves removed from the trees are eaten by animals is given in the case that occurred in the Klipriver District, Natal, where the leaves of *Acacia lasiopetala* were knocked off by a hailstorm and eaten by sheep with fatal results. In winter when the leaves drop off and are dry they contain very little *prussic acid* and owing to the fact that they are scattered under the trees, they are eaten at a slow rate by animals, with the result that there is very little danger of poisoning.

Poisoning may occur in stock fed with the ground pods of species of *Acacia* containing *prussic acid* (cyanogenetic glucosides). The danger is greatest when large amounts of the ground pods are fed to hungry or starved animals, or even more so, when the ground pods are moistened and left standing for a while before being fed. The latter method of feeding the pods is adopted in some dairies *and is a most dangerous one, as lethal amounts of prussic acid are liberated in the moistened mass.*

Ground pods of species of *Acacia* containing prussic acid can be safely fed in the following ways:—

(1) *Feed small quantities at a time.*—It is difficult to state definite amounts of ground pods that can be fed with safety within a certain period if the amount of *prussic acid* present in the pods is unknown. The matter is further complicated by the fact that the *prussic acid* content of pods of the same species of *Acacia* may vary

considerably. The only safe procedure to be adopted by stock-owners desirous of feeding *Acacia* pods to their animals is to test each batch of ground pods on a few animals of low value before feeding it to large numbers of stock or to more valuable animals. The feeding of ground pods is rendered more safe if they are fed mixed with bran or mealie meal.

(2) *Ground pods should not be moistened with cold water.*—As explained above, there is rapid liberation of *prussic acid* in the moistened pods under these conditions.

(3) *If practicable the ground pods should be thoroughly soaked in boiling water or, better still, be made into a porridge and cooked for about ten minutes.*—In this way the enzymes responsible for the liberation of *prussic acid* from the cyanogenetic glucosides are destroyed. The acid present in the gastro-intestinal tract may cause the liberation of *prussic acid*, but only at such a slow rate that there is very little danger of poisoning. It is, however, possible that other plants, which contain enzymes capable of decomposing the cyanogenetic glucosides, may be present in the gastro-intestinal tract. In such cases *prussic acid* poisoning may occur.

(4) *Poisoning with foodstuffs which contain prussic acid may be successfully prevented by mixing them with sulphur or molasses, or by feeding licks containing these substances.*—The sulphur compounds present in the system combine with the *prussic acid* forming the harmless sulphocyanides, whilst the innocuous cyanhydrin is formed when sugar (molasses) and prussic acid are brought together.

If sulphur be mixed with the ground pods the mixture should be such that a full-grown beast does not ingest more than two table-spoonfuls (= 30 gm.) and a full-grown sheep not more than a heaped teaspoonful (=5 gm.) of sulphur per day.

If molasses be used, the ground pods should be moistened with it so as to give the mixture a dark brown colour.

The best method of administering sulphur to stock is to mix it in licks. At times when the ground pods are fed, or are extensively eaten by stock one pound of sulphur should be added to every twelve pounds of lick. This would suffice to prevent *prussic acid* poisoning to a large extent. Undoubtedly the most practicable method of preventing *prussic acid* poisoning is by the feeding of sulphur in licks.

SUMMARY.

(1) Cyanogenetic glucosides were found in five South African species of *Acacia* (*A. giraffae* Willd., *A. lasiopetala* Oliv., *A. litakunensis* Burch., *A. robusta Burch.*, and *A. stolonifera* Burch.), whilst repeated tests conducted with the fresh leaves, flowers, and immature pods of *Acacia karroo* Hayne failed to reveal the presence of such a glucoside. The glucoside in *A. robusta* was not accompanied by an enzyme capable of splitting it. Certain processes of decomposition appear to destroy the enzyme, but not the glucoside in the shells of the pods (*Acacia giraffae* Willd.).

(2) Methods are described of feeding the pods of species of *Acacia*, which contain cyanogenetic glucosides, to stock with safety.

(3) A cyanogenetic glucoside has been isolated from *Acacia lasiopetala* Oliv. Its formula is shown to be $C_{11}H_{17}O_6N$. It has M.P. 176–7°, and $[\alpha]_D^{22} = -35\cdot96°$, and does not correspond with any known cyanogenetic glucoside. The name *Acacipetalin* is suggested for this substance, the constitution of which will form the subject of a further communication.

REFERENCES.

FINNEMORE, H., AND COX, C. B. (1930). Cyanogenetic Glucosides in Australian Plants. *Jl. Proc. Roy. Soc., N.S. Wales*, 1930, Vol. 62, p. 369 (Sydney University Reprints, Series III, Vol. 1, 1927, pp. 369-378).

FINNEMORE, H., AND GLEDHILL, W. C. (1928). *Austr. Jnl. Pharm.*, 1928. N.S. 9, p. 174.

MARAIS, J. S. C., AND RIMINGTON, C. (1934). Isolation of the Poisonous Principles of *Dimorphotheca cuneata*, Less. *Onderstepoort Jnl.*, Vol. 3, No. 1, pp. 111-117.

RIMINGTON, C. (1932). Isolation and Chemical Examination of the Poisonous Principles of *Dimorphotheca spectabilis* Schltr. and *Dimorphotheca Zeyheri Sond. 18th Rept. Dir. Vet. Serv. and Anim. Ind.*, 1932, pp. 955-972.

SEDDON, H. R., AND KING, R. O. C. (1930). As to the Fatal Dose for Sheep of Cyanogenetic Plants containing Sambunigrin or Prunasin. *Jnl. Counc. Scientif. and Industr. Res., Australia*, Vol. 3, 1930, pp. 14-24.

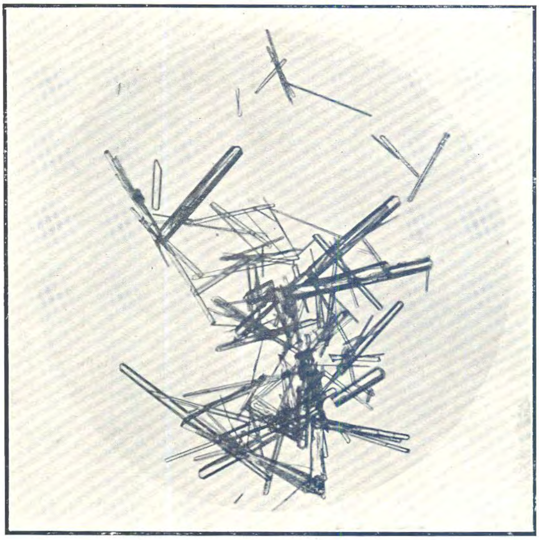

Fig. 6.—Glucoside *Acacipetalin* isolated from *Acacia lasiopetala* Oliv. × 65.