

Chemical Investigation of the Plant *Acalypha indica*. Isolation of Triacetoneamine, a Cyanogenetic Glucoside and Quebrachite.

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The plant *Acalypha indica* (Euphorbiaceae), system No. 2730; 7/7/36, suspected of causing death of stock in the Potgietersrust district, was examined at this laboratory by Dr. D. G. Steyn and its toxicity established. Rabbits drenched with 12.5 gm. of the dry, powdered plant, succumbed within about two hours of dosing, exhibiting symptoms of dyspnoea and convulsions. The blood was found on post mortem to be dirty brown in colour. Hyperaemia of the gastric and duodenal mucous membranes was present. Natives of the district in which the plant grows employ it as an eye medicine under the name of "Machelikoane" but are unaware of its poisonous properties.

Tests showed the plant to contain cyanogenetic substances. A determination of the hydrogen cyanide liberated on maceration in buffer solution at pH 6.0 for varying periods of time was therefore carried out in the usual way; four to six hours maceration liberated the maximum quantity which corresponded to 268.2 mgm. HCN per 100 gm. of the powdered material on a dry weight basis.

Loss of moisture on drying sample : 6.6 per cent.

Time of maceration in hours.	HCN liberated from 5 gm. samples as c.c. of N/50 AgNO ₃ .
2.0	9.0
2.0	9.1
2.5	10.4
3.0	10.8
3.0	10.8
4.0	11.6
4.0	11.8
6.0	11.6
6.0	11.5
12.0	10.0
12.0	10.7
18.0	7.6
72	6.8

Maximum liberated = $11.6 \times 1.08 \times 20$ mgm. HCN per cent.
= 250.5

On dry weight basis = 268.2

A determination of oxalate content was also carried out and afforded, in the mean, the figure of 3.14 gm. per 100 gm. dry material. Such a quantity could not account for the pathological symptoms of intoxication.

ISOLATION OF THE BASE TRIACETONAMINE.

During attempts to isolate the cyanogenetic glucoside, a substance was obtained which proved to be a salt of a base identified as triacetoneamine, $C_9H_{17}NO$. The exact procedure undertaken was as follows:

The powdered plant material was extracted by cold 96 per cent. alcohol, the deep green extract decolourised by shaking with adsorbant charcoal, and evaporated to a syrupy consistency in an open dish at room temperature.

The residue was taken up in water, sufficient basic lead acetate added to cause complete precipitation then about 30 gm. of cadmium nitrate dissolved in water, and ammonia until addition caused no further turbidity. The cadmium nitrate was added since a cyclose derivative was found to be present and this reagent had been found useful for removing Pinit, inositol monomethyl ether from *Acacia* spp. in a previous investigation (Rimington, 1936). The filtrate from the lead-ammonia clarification was freed from excess of lead by hydrogen sulphide gas and evaporated to a syrup. Decolourising charcoal was then stirred in and drying continued by spreading the material in a thin layer in a vacuum desiccator until it could be powdered and introduced into a soxhlet extraction thimble for extraction by boiling ethyl acetate. This solvent on evaporation left a mixture from which a material crystallising in shining colourless plates was eventually isolated. These were sparingly soluble in absolute alcohol, did not possess any melting point but gave positive alkaloidal reactions, the precipitates with phosphotungstic acid and picric acid being crystalline; they also gave a crystalline 2:4 dinitrophenylhydrazone under the proper conditions with Brady's reagent. Of all these derivatives, only the picrate was found to possess a melting point; melting and decomposition occurred at 196° . Upon analysis, the crystals isolated were found to be the nitrate of the base $C_9H_{17}NO$. They gave a blue colour with the diphenylamine-sulphuric acid reagent, and the analyses of the other salts were in agreement with the formula as recorded below.

Nitrate.

Long flat plates with approximately parallel sides but oblique ends (see Fig. 1). On heating, some darkening occurred at about 163° and some sublimation at a higher temperature but no melting point was observed up to 350° . Sparingly soluble in hot acetone and easily recrystallised from this solvent by addition of petroleum ether.

*Microanalysis.**

		C	H	N
	Found	49.56	8.35	12.15
		49.43	8.34	—
$C_9H_{17}NO \cdot HNO_3$	requires	49.53	8.26	12.84

Picrate.

By addition of aqueous picric acid and recrystallisation from hot dilute alcohol. Square-ended, flattened, golden prisms. M.P. 196° (see Fig. 2).

Microanalysis:

		C	H	N
	Found	47.14	5.51	14.68
$C_9H_{17}NO \cdot C_6H_3N_3O_7$	requires	46.86	5.25	14.59

Phosphotungstate.

By addition of aqueous phosphotungstic acid. White precipitate, even in very high dilution, which on stirring crystallises in characteristically shaped crystals (see Fig. 3).

Platinichloride.

By addition of aqueous or alcoholic $PtCl_4$ to alcoholic solution; orange-yellow crystals easily recrystallised from hot water. No melting point.



Fig. 1. Triacetoneamine nitrate ex *Acalypha indica*, $\times 330$.

* Microanalyses by Dr. Backeberg of the University of the Witwatersrand to whom we wish to express our thanks.

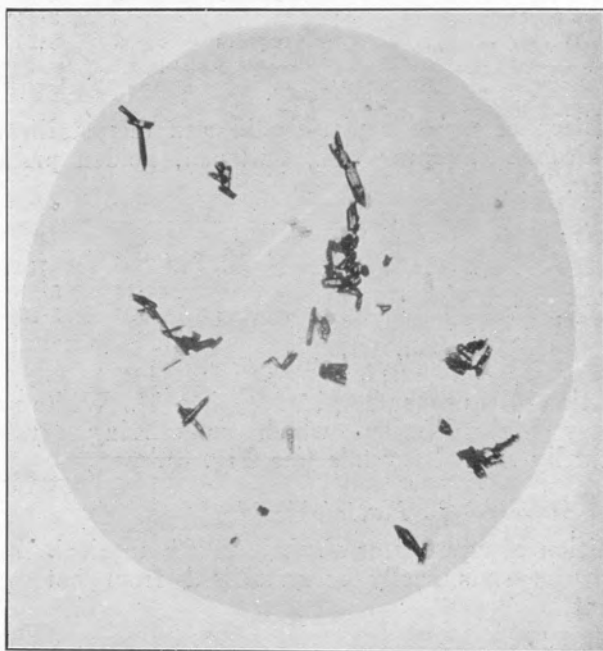


Fig. 2. Triacetoneamine picrate (*Acalypha indica*), $\times 30$.



Fig. 3. Triacetoneamine phosphotungstate, $\times 55$.



Fig. 4. Triacetonamine 2:4 dinitrophenylhydrazone, $\times 66$.

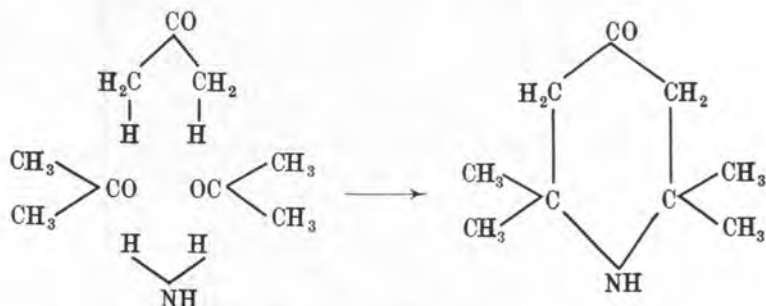
2:4 Dinitrophenylhydrazone.

By addition of Brady's reagent (solution of 2:4 dinitrophenylhydrazine in 2N hydrochloric acid) to solution of crystals ($B.HNO_3$) in 2N hydrochloric acid. Microcrystalline precipitate recrystallising from hot dilute alcohol in large yellow hexagonal tables usually resembling equilateral triangles with truncated apices (see Fig. 4). No melting point.

An examination of the literature suggested that the base closely resembled triacetonamine. This substance was therefore synthesised for comparison as described by Heinz (1874; 1875) by passing dry ammonia gas into boiling acetone for some hours, but the final isolation was accomplished not by way of the oxalate but by means of the well crystallised nitrate. The synthetic material was in every way identical with that isolated from *Acalypha indica*, and the salts had identical crystalline forms. It is unfortunate that of all the salts of triacetonamine recorded in the literature, none has a true melting point. The picrate does not appear to have been prepared but as stated above the picrate of the isolated base melted at 196° and that of the synthetic material also had M.P. 196° , without depression on mixing. The characteristic 2:4 dinitrophenylhydrazone was also prepared crystallising in the large hexagonal tables described. To render the identification still further complete the oximes of the bases were prepared according to Harries (1896) who gives the M.P. of triacetonamine oxime as $152-3^\circ$ (hexagonal prisms). 21.8 mgm. of the crystalline nitrate from *Acalypha indica* was dissolved in 0.3 c.c. of water, 6.9 mgm. of hydroxylamine hydrochloride and 0.2 c.c. of N sodium hydroxide added. The liquid was allowed to evaporate

slowly at room temperature when a crop of large, shining, colourless hexagonal prisms separated. They were separated and pressed between filter papers and then had M.P. 150° yield 13 mgm. The free base was also prepared from a small quantity of the nitrate and found to have M.P. 56° . That of free triacetonamine plus H_2O is given as 58° .

The occurrence of such a substance as triacetonamine in a plant might at first sight occasion surprise but when one considers its possible formation, biologically as well as in vitro, from such abundant materials as acetone and ammonia it is seen to provide a beautiful example of the biosynthesis of a cyclic base from the simplest raw products, thus:



It must be pointed out at this stage that attempts to demonstrate the presence of basic material in the dry plant powder by means of extraction with Prolius' solution were unsuccessful, hence it is possible that the base was formed from some precursor during the working up of the material. Being a ketone, there is a reasonable possibility that it might enter into the composition of the cyanogenetic glucoside, which was found to contain other nitrogen than that combined as hydrogen cyanide, but this possibility will be no more than mentioned provisionally whilst the constitution of the glucoside remains obscure.

THE CYANOGENETIC GLUCOSIDE.

The isolation of the glucoside presents considerable difficulty occasioned firstly by the great instability of the material and secondly by its sparing solubility in ethyl acetate, a solvent otherwise very convenient for the isolation of glucosides.

Extracts of the plant material were made by percolation with cold 96 per cent. alcohol in some cases before and in others after a preliminary extraction with hot ether. The alcoholic liquids were shaken with decolourising charcoal and the solvent removed by fanning. Repeated extraction of the sticky residues with boiling ethyl acetate removed some glucoside. On cooling, the hot liquors deposited a mass of colourless crystalline material. This was freed from glucosidal impurity, taking advantage of its almost complete insolubility in hot absolute alcohol, recrystallised several times and identified as Quebrachite, a monomethyl ether of *l*-inositol, isomeric with Pinit.

These figures would indicate a formula of either $C_{14}H_{22}N_2O_{10}$ or $C_{14}H_{20}N_2O_{10}$ but some uncertainty prevails owing to the fact that the analyses were not as strictly concordant as should have been the case had the different preparations been quite pure. The suggested molecular formulae are therefore put forward with all reserve but do suffice to show that the glucoside contains in its molecule more than one nitrogen atom. The high proportion of oxygen is also noteworthy.



Fig. 5. Glucoside from *Acalypha indica*, M.P. 108° , $\times 30$.

Hydrolysis experiments using emulsin or dilute sulphuric acid yielded hydrogen cyanide and dark, purplish coloured solutions from which no aglucone has as yet been isolated in a state of purity. It is noteworthy however that these hydrolysates give precipitates with alkaloidal reagents indicating the presence of some basic substance. The identification of this base must await the preparation of more pure starting material.

In one instance, Brady's reagent was added to a sulphuric acid hydrolysate in the hope of obtaining the dinitrophenylhydrazone of an aldehyde or ketone. A crystalline precipitate formed which when recrystallised from hot dilute alcohol had M.P. $187-9^{\circ}$. Analysis and mixed M.P. showed it to be identical with the 2:4 dinitrophenylhydrazide of acetic acid, the melting point of which is given as $187-190^{\circ}$. Both dissolved in aqueous sodium carbonate giving a brown solution.

Microanalysis:

		C	H	N
	Found	40.16	3.60	22.60
$C_8H_8O_2N_4$	requires	39.98	3.36	23.34
Mixed M.P. with synthetic material 187-9°				

The probability must be borne in mind, therefore, that one of the products of acid hydrolysis of the glucoside is acetic acid. This might conceivably arise from the hydrolysis of an N-acetyl group.

The above results are recorded with the object of putting on record the work already done and indicating the lines along which a more detailed investigation of the constitution of this unusual cyanogenetic glucoside might proceed.

SUMMARY.

(1) The plant *Acalypha indica* is cyanogenetic, containing approximately 270 mgm. HCN per 100 gm. dry weight of the dried powdered material.

(2) A base is present in extracts of the plant, being derived from some precursor, possibly the glucoside itself.

(3) This base has been identified as triacetoneamine, the picrate (M.P. 196°) and 2:4 dinitrophenylhydrazone of which have been prepared and described.

(4) From the plant, Quebrachite, *l*-inositol monomethyl ether, has also been isolated.

(5) The cyanogenetic glucoside appears to crystallise in two forms, thin hexagonal plates, M.P. 182-4° and fine, silky needles, M.P. 108°. The molecular formula $C_{14}H_{20}(22)N_2O_{10}$ is suggested. It yields basic material on hydrolysis.

(6) From an acid hydrolysate of the glucoside, tested with Brady's reagent, acetic acid 2:4 dinitrophenylhydrazide has been isolated. The possibility of the presence in the molecule of an N-acetyl group is indicated.

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