

Chemical Studies upon the Vermeerbos, *Geigeria Aspera* Harv. I. Isolation of a Bitter Principle "Geigerin."

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"VERMEERSIEKTE", or vomiting disease of sheep, has been known for some considerable time. According to Steyn (1934) the first record of the disease was due to Brandford in 1877, but it was not until 1928 that definite proof was forthcoming that its occurrence is due to ingestion of certain species of the composite *Geigeria*. In this year, du Toit (1928) reported that vomiting was produced by the experimental feeding of infusions of *Geigeria passerinoides* to a cow. In addition, experiments carried out at Donderbosfontein in 1923 had definitely shown that sheep grazing on veld cleared of everything but *Geigeria passerinoides* would contract the disease. A sufficient amount or "concentration" of feeding appears to be necessary; the individual susceptibility of animals also varies between wide limits.

Prior to this successful experiment, numerous negative results had been obtained by officers of the Research Division and by others (for details see Steyn, 1934).

Vermeersiekte has been responsible for very heavy losses of sheep in certain seasons. Thus in 1930, in a small area of Griqualand West, comprising about 20 farms, over fifteen thousand sheep were lost within three months from vermeersiekte (Steyn, 1934). The parts of the country most affected are the North-Western Cape Province, Griqualand West, Western Orange Free State and certain parts of the South-Eastern Transvaal, but heavy losses from vermeersiekte are also sustained in South West Africa. The plants concerned are all species of the Genus *Geigeria* and experimental work has shown that of these, *Geigeria aspera* Harv. is the most poisonous, *Geigeria passerinoides* Harv. only about one-tenth as harmful, whilst *Geigeria Zeyheri* Harv. and *Geigeria pectidea* Harv. occupy an intermediate position, although as previously mentioned, district and season appear to influence the toxicity of the plants. It has definitely been shown that toxicity is greatest in the pre-flowering stage and is much reduced after heavy rain has fallen. Concerning the stability of the toxin on storing, some doubt still remains.

Prior to the present work, nothing was known concerning the chemical nature of the active principle.

The action of the plant appears to vary somewhat and Steyn (1930) has divided vermeersiekte into four different forms, namely:—

- (a) The vomiting form.
- (b) The form in which hoven is the most outstanding symptom.
- (c) The stiff form,
- (d) The paralytic form.

Different species of animals are affected usually in different ways, but an animal may exhibit at the same time one or more of the above forms of the disease. The cause of death may be either—

- (a) asphyxia caused by aspiration into the lungs of large amounts of vomited ruminal contents,
- (b) paralysis of the centre of respiration,
- (c) exhaustion caused by the incessant vomiting and diarrhoea,
or
- (d) heart failure.

With regard to the cause of the vomiting, two possible explanations present themselves, namely that the affect is a central one, the toxic substance causing stimulation of the vomiting centre in the medulla oblongata or that the irritation is local upon the walls of the rumen and abomasum, the persistence of the stimulus eventually inducing vomiting. The undoubted nervous character of the stiff and paralytic forms of the disease lends support to the former hypothesis rather than to the latter. Post mortem, an acute catarrhal gastro-enteritis is nearly always to be observed.

Rotational grazing combined with eradication of the plant is the only preventive measure which has so far met with any success.

In pursuance of the programme of poison-plant research at present being carried out at this Laboratory, consignments of *Geigeria passerinoides* were obtained in 1932 from Boetsap in Griqualand West, but proved to be only slightly toxic. Of the dried, ground plant, 4 kilos were required to produce symptoms in a sheep. Rabbits were found to be unsatisfactory as test animals; however, by working with sheep it was definitely shown that the active principle is not extracted from the plant by cold water or by continuous extraction with hot petroleum. It is, however, removed by 96 per cent. alcohol and by exhaustive extraction of the plant with boiling ether.

In 1934, material of *Geigeria aspera* was obtained from Vereeniging and with it the work performed which is reported in the present paper. Since the quantity required for a single lethal dose was very considerable (in excess of 4 kilos for a sheep) the principle was adopted of purifying the toxic ether extract in which a substance was present giving a positive reaction with Wagner's reagent and consequently considered to be alkaloidal in type. This substance was isolated and shown to be a bitter principle. The yield is very small (average about 0.06 per cent. on weight of dried plant) and pharmacological tests have shown that it is not the substance producing vermeersiekte although it does not seem to be entirely devoid of

toxic properties. 15 gm. given to a sheep in three successive doses over 2 days was without effect but in tests upon cats it proved positive, the animals vomiting continuously for about 2 days, after having received doses of 1 gm. by mouth. These experiments are fully reported later in this paper, and would seem to indicate that the substance in question exerts to a slight degree the same pharmacological action as does the vermeersiekte-producing principle. Its isolation and chemical examination are reported in the present communication.

ISOLATION OF THE BITTER PRINCIPLE.

Working upon the hypothesis that the material giving the positive iodine reaction was a base, ether extracts of the plant were shaken repeatedly with dilute acid and then, after filtration and making alkaline by sodium hydroxide, carbonate or ammonia, the aqueous solution was again shaken with ether in the expectation of transferring the base to this solvent. The recovery, as judged by the intensity of the iodine reaction, was extremely poor. This suggested that the substance was labile towards alkalis.

In order to eliminate the use of alkali, an ether extract was then shaken with dilute hydrochloric acid, which took out the material fairly readily, and this solution then shaken with chloroform. The material being sought passed at once into the chloroform but not, as was expected, as a hydrochloride, since on testing the extract it was found to be chloride free. On evaporation of the chloroform, a pale yellow oil remained in which large rhombic crystals gradually appeared. The same crystals were deposited together with a little oil when an ether extract of the plant was decolorised by charcoal and allowed to evaporate to dryness at room temperature. By washing with benzene and then petroleum ether the bulk of the oil adhering to the crystals was removed and recrystallisation could then be effected by dissolving in chloroform, adding a slight excess of petroleum ether and setting the cloudy solution in the ice chest for some hours. Such crystals had a bitter taste and gave a positive reaction with Wagner's reagent but were free from nitrogen and were therefore not alkaloidal in character.

The method was gradually improved until finally the following technique was worked out which has been found to give consistently good results.

METHOD OF ISOLATION.

The dried, ground plant was extracted by ether in a Soxhlet apparatus until the solvent was no longer coloured. The extract was then concentrated until its volume represented about 1,400 c.c. per kilogram of plant material. This ethereal solution was shaken repeatedly in a large separatory funnel with 1 per cent. hydrochloric acid until the aqueous phase no longer gave a positive iodine reaction or only a very slight reaction. Through the acid solution, about 2.5 to 3 litres in volume, a stream of air was now drawn until the smell of ether could no longer be distinguished. A good decolorising charcoal (Kahlbaum) 5 to 7 grams per litre of solution, was then added,

the mixture shaken and filtered into a large separatory funnel. The water-clear aqueous phase was now shaken with small quantities of chloroform until this solvent no longer removed material giving a positive iodine reaction. The pale yellow chloroform extract was allowed to evaporate, either spontaneously or by blowing over it a stream of air until all the solvent was removed. There remained a pale, straw-coloured, viscous oil with a pleasantly aromatic smell. On standing, this generally crystallised, but a more convenient procedure was to boil up the oily residue with successive quantities of water, decant these and boil again with a pinch of charcoal and filter whilst hot into centrifuge tubes. Upon cooling, the liquid deposited a voluminous crop of beautifully formed large rhombic crystals. Crystallisation was completed in the ice-chest and the mother liquors centrifuged off. They contained a small quantity of non-crystallisable acidic oily material which was removed by chloroform extraction and reserved for further investigation. In all, about 150 to 200 c.c. of water was required to dissolve the chloroform residue from an original kilogram of plant material. A small quantity of tarry impurity insoluble in water was discarded.

The crystals were washed on the centrifuge by ice-cold water and dried in vacuo over sulphuric acid. The yield averaged 0.06 gm. per 100 gms. of dried *Geigeria aspera* material, but was found to vary somewhat in different batches. Thus, in one exceptional instance a yield of 0.248 per cent. was recorded, however, the figure was generally found to be between 0.05 and 0.10 per cent. There was some indication that the more toxic batches contained the larger proportion of this constituent.

PROPERTIES OF THE SUBSTANCE.

The material, as isolated, crystallised in large rhombs, often grouped together (see Fig. 1). It was colourless and odourless and exhibited a double melting point. At 78° partial liquefaction occurred but the material rapidly resolidified and when the temperature was raised, melted sharply at 189°. All melting points were observed on a Koffler electrically-heated microscope stage. There appeared to be loss of substance at the lower temperature and this was confirmed by weighing and elementary analysis. Apparently 1 molecule of water is lost at 78° but regained upon recrystallisation from aqueous or moist solvents.

The substance was readily soluble in chloroform and warm absolute alcohol, sparingly soluble in ether and insoluble in petroleum ether. In benzene and warm ethyl acetate it was slightly soluble.

Recrystallisation is most easily effected from water but was successfully accomplished from other solvents as described below. From determinations it appeared that the difference in solubility in water between 100° and 0° is 1.14 gms. per 100 c.c. and the order of solubility at room temperature is 0.3 gms. per 100 c.c. water. Solutions of the substance have an intensely bitter taste which is very persistent; its dust irritates the eyes and nasal mucous membranes.

Recrystallisation from chloroform was accomplished by dissolving the material in a small quantity of this solvent, adding petroleum ether until a slight turbidity persisted and setting the mixture aside in the ice chest. It sometimes happened that crystals formed having the melting points $65-70^{\circ}$ and 169° (β form) together with the typical form melting at 78° and 189° (α form). When recrystallisation was effected from hot ethyl acetate the β form predominated or appeared exclusively. Upon recrystallisation from water, it reverted into the α form melting at 189° . That this difference was not due to hydration in the aqueous solvent and separation of anhydrous substance from the ethyl acetate was clearly proved since,

- (1) the elementary analyses of the two products were identical,
- (2) the β form suffered the same loss of weight, (6.4 per cent.) as did the α form on heating at $85-90^{\circ}$. It seems, rather, to be a case of isomerism. The following investigations fully substantiate this view.

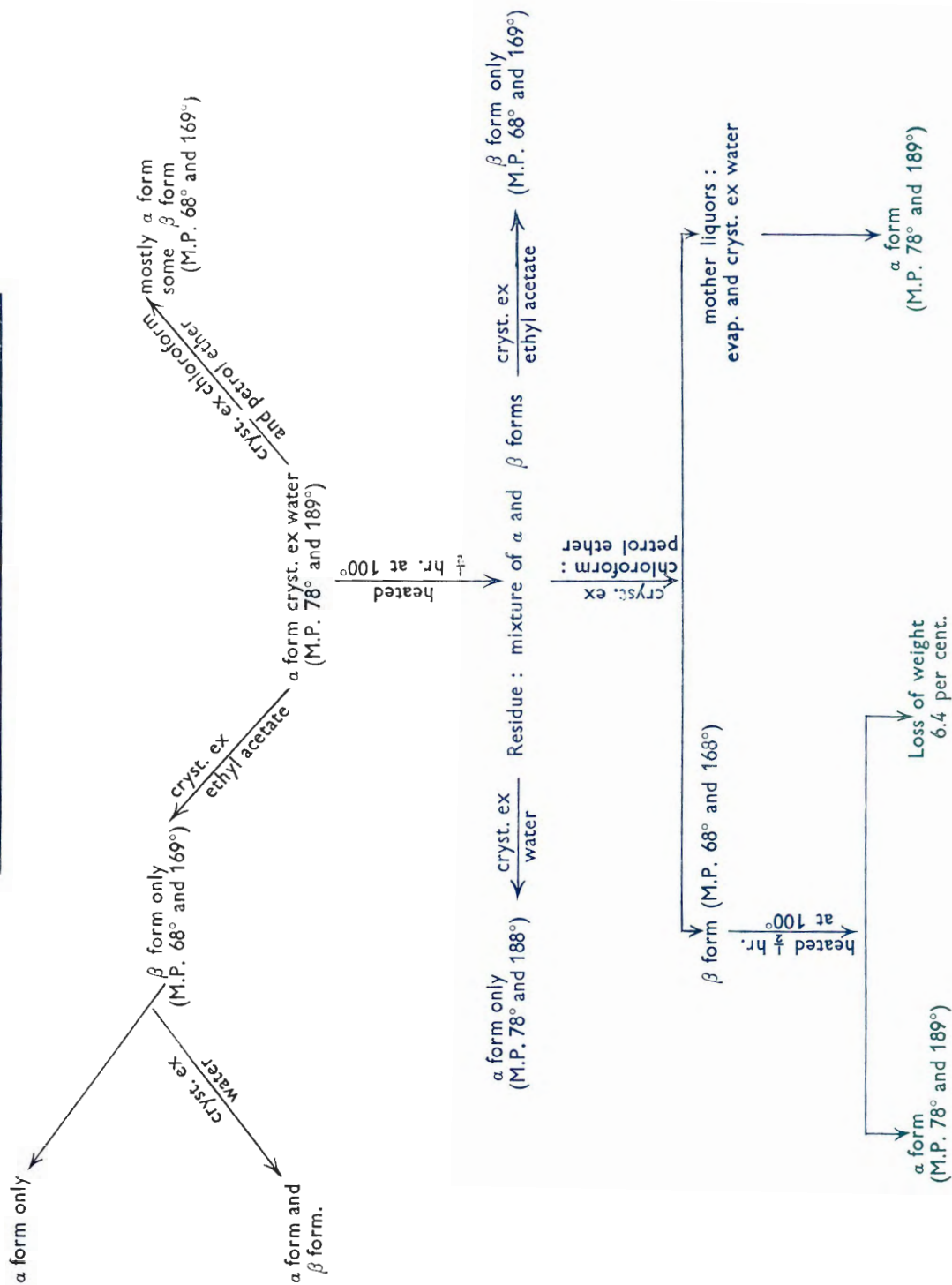


Fig. 1. Geigerin crystallised from water, $\times 63$.

ISOMERISM OF THE SUBSTANCE.

Upon heating some of the α form at 100° and taking samples at intervals for melting point determination, it was found that after the preliminary loss of approximately 6 per cent. of substance at 78° the residue melted sharply at 189° . One hour later, without further loss of weight, the melting point had altered, some crystals melting at 166° and some at 189° . Four hours later the weight was still constant but the melting point lay between 165° and 175° . In a similar manner the heating of the β form for periods of time extending from $\frac{1}{2}$ to 2 hours yielded mixtures of this and the α form. The α form seems to be that more easily assumed.

EXPERIMENTS ILLUSTRATING ISOMERISM OF GEIGERIN.



A difference in the optical rotatory power in different solvents, as recorded below, offers further evidence in favour of the hypothesis of isomerism. Before passing on to these figures, a summary is presented, in the chart above, of additional experiments carried out whilst investigating this isomerism. The organic solvents used were not specially dehydrated and there are suggestions that the very great affinity of the substance for the one molecule of water with which it crystallises enables it to assume this hydrated state even when crystallised, for example, from moist ethyl acetate.

A tendency is noticeable for the β form to separate from the organic solvents (this form also shows the double melting point, 68° and 169° , water being lost at the former temperature) although even from chloroform-petroleum ether, mixtures of α and β forms have been obtained and from alcohols of various strengths this is usually the case.

OPTICAL ROTATORY POWER.

This was determined in different solvents, using either α or β forms, in a Zeiss triple-field instrument with 2 dm. tube. The results may be summarised as follows:—

1. α form from water : solvent absolute alcohol.

$$W = 0.1010 \text{ gm.}$$

$$V = 15 \text{ c.c.}$$

$$\theta = -0.82^\circ.$$

$$\therefore \left[\alpha \right]_{\text{D}}^{22} = \frac{-0.82 \times 100 \times 15}{2 \times 10.1} \\ = -60.88^\circ$$

2. α form from water : solvent 5 per cent. alcohol.

$$W = 0.0508 \text{ gm.}$$

$$V = 16 \text{ c.c.}$$

$$\theta = -0.38^\circ.$$

$$\therefore \left[\alpha \right]_{\text{D}}^{19} = \frac{-0.38 \times 100 \times 16}{2 \times 5.08} \\ = -59.85^\circ.$$

3. β form from ethyl acetate : solvent absolute alcohol.

$$W = 0.0523 \text{ gm.}$$

$$V = 15 \text{ c.c.}$$

$$\theta = -0.42^\circ.$$

$$\therefore \left[\alpha \right]_{\text{D}}^{20} = \frac{-0.42 \times 100 \times 15}{2 \times 5.23} \\ = -60.23^\circ.$$

4. α form from water: solvent chloroform.

$$W = 0.0502 \text{ gm.}$$

$$V = 15 \text{ c.c.}$$

$$\theta = -0.285^\circ.$$

$$\therefore \left[\alpha \right]_{\text{D}}^{20} = \frac{-0.285 \times 100 \times 15}{2 \times 5.02}$$

$$= -42.58^\circ.$$

5. β form from ethyl acetate: solvent ethyl acetate.

$$W = 0.0508 \text{ gm.}$$

$$V = 15 \text{ c.c.}$$

$$\theta = -0.29^\circ.$$

$$\therefore \left[\alpha \right]_{\text{D}}^{20} = \frac{-0.29 \times 100 \times 15}{2 \times 5.08}$$

$$= -42.83^\circ.$$

It is clear from the above that no matter whether the starting material is the α or β form of the substance, it exhibits the same optical rotatory activity in the same solvent. Two constants were observed, for absolute or dilute alcohol, in the mean -60.32° and for chloroform or ethyl acetate in the mean -42.71° .

It is possible that this duality is related to the two isomeric configurations the substances may assume and may be an expression of keto-enol tautomerism (see discussion later). A similar irregularity of melting point was observed in the case of carolic acid (M.P. 112° and 132°) by Clutterbuck, Raistrick and Reuter (1935).

ELEMENTARY ANALYSIS AND MOLECULAR COMPOSITION.

Numerous analyses were performed upon specimens belonging to the α or β forms with consequently differing melting points but no difference whatever was observed in composition. All materials were nitrogen-free.

MICROANALYSIS.*

Material.	C.	H.	M. W.
α form crystallised from water, M.P. 78° and 189°	64.02	8.08	308
α form crystallised from water, M.P. 78° and 189°	63.99	7.47	298
β form crystallised from ethyl acetate, M.P. 68° and 169°	64.28	7.84	—
α form heated $1\frac{1}{2}$ hours then crystallised from ethyl acetate, M.P. 65° and 169°	64.00	7.82	—
α form heated $1\frac{1}{2}$ hours then crystallised from water, then recrystallised from chloroform: petroleum ether, M.P. 65° and 168°	64.08	7.90	—
$\text{C}_{15}\text{H}_{22}\text{O}_5$ requires.....	63.84	7.80	282
Residue after heating to constant weight at 100°	68.21	7.89	—
$\text{C}_{15}\text{H}_{20}\text{O}_4$ requires.....	68.19	7.61	—
Loss of weight..... 6.4 per cent.			
H_2O on $\text{C}_{15}\text{H}_{22}\text{O}_5$ requires 6.38 per cent.			

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

Water vapour evolved on heating was identified by its action in restoring the blue colour to anhydrous copper sulphate. The molecular formula of the substance is therefore $C_{13}H_{20}O_4 \cdot H_2O$. Tests carried out for methoxyl CH_3O groups and dioxy-methylene $\begin{matrix} O \\ | \\ CH_2 < \\ | \\ O \end{matrix}$ groups proved negative. The substance was found to give certain colour reactions; these and other properties are listed below. Since neither bromine water nor bromine in chloroform was decolorised nor permanganate reduced in the cold, the substance would appear to be fully saturated, that is to say no ethylenic linkages $C=C$ are present. It gave no colour with tetranitromethane.

Colour Tests and other Reactions.

Wagner's iodine reagent.....	Amorphous precipitate.
Acid potassium iodide.....	Nil.
Phosphotungstic acid.....	White amorphous precipitate.
Picric acid.....	Yellow amorphous precipitate.
Sulphuric acid, concentrated.....	Nil.
Sulphuric acid, 50 per cent. by volume	Cerise colouration; same absorption spectrum as in hydrochloric acid.
Sulphuric acid added to a solution of the substance in acetic anhydride (Liebermann-Storch)	Intense transitory crimson colour.
Nitric acid.....	Nil.
Hydrochloric acid.....	Bright reddish-mauve colouration appearing slowly at room temperature, rapidly on warming. This colour though faint, is very characteristic. The solution exhibits three absorption bands with centres at 543, 506, and 466 (see Fig. 2) and has a faint greenish fluorescence.
Diazobenzenesulphonic acid.....	Nil.
Ferric chloride.....	Nil.
Bromine in chloroform.....	No decolorisation.
Potassium permanganate.....	No decolorisation in the cold.
Molisch test.....	Negative.
Fehlings solution.....	Slight reduction on prolonged boiling; more marked when material first boiled with dilute hydrochloric acid and then tested (see later in text).
Schiff's reagent.....	Nil.
Ammoniacal silver nitrate (Tollens)....	No reduction.

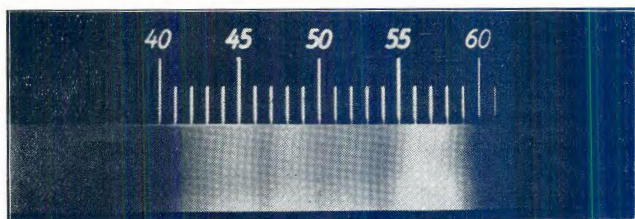


Fig. 2. Action of HCl upon Geigerin. Absorption spectrum of cherry-red solution. Bands at 543; 506; 466 $m\mu$.

PRESENCE OF A LACTONE GROUPING.

Although the substance is practically insoluble in cold water, it dissolves very slowly in boiling dilute alkali. The action of alcoholic alkali is more rapid and was used to demonstrate the presence of one lactone grouping in the molecule. Comparative resistance of a lactone to aqueous alkali but ready hydrolysis by alcoholic alkali is recorded by Hansen (1931) in the case of dihydroiso-alantolactone. 28.2 mgm. of material was dissolved in 5 c.c. of alcoholic potassium hydroxide (0.0804 N), a drop of phenol phthalein was added and the mixture immediately back titrated by decinormal hydrochloric acid. Alkali neutralised amounted to 0.25 c.c. There is thus no free carboxyl group present in the substance. A further 5 c.c. of alcoholic potash was added and the mixture back titrated after standing overnight at 37°.

5 c.c. alc. KOH is equivalent to 3.65 c.c. of 0.1086 N hydrochloric acid.

back titration required 3.0 c.c.

difference 0.65 c.c.

difference in preliminary titration 0.25 c.c.

∴ Amount of alkali neutralised by substance =

0.90 × 1.086 c.c. of N/10.

= 0.98 c.c.

Theory for 1 lactone group = 1.00 c.c.

In a second experiment, 28.2 mgm. of material dissolved in 5 c.c. of alcoholic potash was left for 1 hour at 37° and then back titrated. 1.08 c.c. n/10 was neutralised as against the theoretical value of 1.00 c.c.

A definite excess of acid was then added and after half an hour the mixture again titrated. No change was observed, indicating that no spontaneous reformation of the lactone ring takes place when the free acid is left in solution for this length of time.

OPTICAL ROTATORY POWER OF THE FREE ACID.

In preliminary experiments, the rotation was determined of the reaction mixture of the material and alcoholic potassium hydroxide before and after acidification. The same value was obtained in each case, thus:—

47.17 mgm. in 5 c.c. alc. potash for 3 hours at 37°.

Volume made up to 22 c.c. Rotation observed +0.48°.

$$\therefore \left[\alpha \right]_{\text{D}}^{21} = \frac{+0.48 \times 100 \times 22}{2 \times 4.717} = +111.9^{\circ}$$

In another case, 50 mgm. was left in 5 c.c. alc. potash overnight at 37°. The solution was diluted to 15 c.c. Rotation observed +0.70°. A drop of concentrated acid was now added to bring to acid reaction but the rotation remained unchanged.

$$\therefore \left[\alpha \right]_{\text{D}}^{20} = \frac{+0.70 \times 100 \times 15}{2 \times 5.0} = +105^\circ.$$

The free acid and its salts are therefore strongly dextrorotatory (see preparation of Geigeric acid, later).

Considering the abovementioned properties of the substance isolated from *Geigeria aspera*, it is considered that it belongs to the class of bitter principles. No substance with identical properties has been described in the literature and for this reason it is proposed to assign to it the name *Geigerin*.

There is, however, a record of the isolation of a crystalline substance from *Chrysanthemum cinerariaefolium* by Thoms in 1891 (see Klein, 1933) and from the data available it appeared to show certain points of similarity with Geigerin. Thus the melting point is recorded as 188-189° and the formula given as C₃₄H₄₄O₁₀, although with some reserve. Moreover the solubilities appear to be very similar to those of Geigerin and this substance, which Thoms designated "Pyrethrosin", also gave a reddish-violet colouration on warming with hydrochloric acid.

Unfortunately, attempts to obtain the journal in which Thoms' paper appeared were unsuccessful both in South Africa and by purchase from Europe and it would not have been possible to extend the comparison of the two substances any further had it not been for the kindness Professor Th. Sabalitschka, of the Pharmakologisches Institut, Berlin, who very kindly made a copy of Thoms' article (Berichte d. Deutsch. Pharmazeut. Ges. 1, 241, 1891) which he sent to one of us (C.R.). I wish to express my sincere thanks to Professor Sabalitschka.

Thoms does not record the optical activity of his substance Pyrethrosin, nor was a molecular weight determination carried out. From the general description, however, the colour reaction with hydrochloric acid and the production of a substance reducing Fehling's solution when the material is boiled with dilute acid, a substance which Thoms proves is not a sugar, there remains little doubt that "Pyrethrosin" and "Geigerin" are identical. The true molecular formula is C₁₅H₂₂O₅ not C₃₄H₄₄O₁₀. Thoms' Pyrethrosin may not have been quite pure. He used only organic solvents for crystallisation and it is difficult under these conditions to eliminate traces of sticky or tarry materials. The occurrence of one and the same bitter principle in *Chrysanthemum cinerariaefolium* and *Geigeria aspera* is of great interest. Thoms made no pharmacological test upon his material other than to show that it was devoid of insecticidal properties. Both plants belong to the family *Compositae* and it would be of interest to examine South African *Geigeria* species for the presence of the insecticidal pyrethrin oils.

ABSENCE OF HYDROXYL GROUPS - OH FROM THE GEIGERIN MOLECULE.

In order to establish the presence or absence of hydroxyl groups in Geigerin, attempts were made to acetylate the substance, as follows, but without success. In each case the material was recovered unchanged.

- (i) 50 mgm. substance was dissolved in 2 c.c. of chloroform, 0.2 c.c. of acetic anhydride added and the mixture left overnight. Petroleum ether was then added, causing the separation of a crystalline material in small stellate clusters of prisms. These proved to be unchanged Geigerin with M.P. 73° and 167.9°.
- (ii) 50 mgm. substance was dissolved in 1 c.c. of pyridine, 0.15 c.c. of acetic anhydride added and the mixture left for several days. On adding petroleum ether, unchanged Geigerin crystallised out, M.P. 76° and 168.9°.
- (iii) 50 mgm. of substance in 2 c.c. chloroform plus 0.5 c.c. acetic anhydride was refluxed for 8 hours. On addition of petroleum ether to the slightly yellow solution, unchanged Geigerin crystallised out, both α and β forms being present. M.P.'s. 74° and 166° also 77° and 187.8°.

Boiling with acetic anhydride and sodium acetate led to complete destruction and tar formation.

Since certain substances may contain hydroxyl groups which are difficultly acetylated but react smoothly with phenylisocyanate to form phenylurethanes, an experiment was conducted as follows.

- (iv) 50 mgm. of substance were dissolved in 2 c.c. of chloroform, 0.75 c.c. of phenylisocyanate added and the mixture set aside for several days. Some squarish crystals appeared but these proved to be diphenylurea with M.P. 258°. To the mother liquor, petroleum ether was added and the crystals which separated removed; they proved to be unchanged Geigerin M.P. 78° and 168° also some 187° (α form).

It may be concluded, therefore, that hydroxyl groups are absent from Geigerin.

DEMONSTRATION OF THE PRESENCE OF ONE KETONIC >CO GROUP.

To 30 mgm. of substance dissolved in 15 c.c. of hot water was added 3 c.c. of hydrochloric acid and then 1.5 c.c. of hot Brady's reagent—a solution of 0.5 gm. of 2:4 dinitrophenylhydrazine in 30 c.c. of normal hydrochloric acid. The colour deepened and almost at once the precipitation in microcrystalline form commenced of a deep orange-red substance. This was separated by centrifuging, washed with 2 normal hydrochloric acid and then with water and finally recrystallised from hot 60 per cent. alcohol. It separated in rosettes of red plates M.P. 250-5°.

Microanalysis.

	C.	H.	N.
Found.....	56.58	5.70	12.80
$C_{21}H_{24}N_4O_7$ requires.....	56.72	5.45	12.61

ABSENCE OF AN ISOPROPYL SIDE CHAIN.

In order to test for the presence of an isopropyl side-chain, a group occurring in several terpenes and bitter principles, 0.10 gm. Geigerin, dissolved in a little glacial acetic acid, was placed in a micro-kjeldahl distillation apparatus and a current of steam passed through whilst an oxidation mixture of chromic acid in glacial acetic acid was added slowly during the course of 2 hours.

The distillate was neutralised and again distilled, the first 20 c.c. being tested for acetone. Brady's reagent gave no turbidity and all colour tests were negative. The residual oxidation mixture was placed in the ice-chest when large colourless rhombic crystals (60 mgm.) separated. These were recrystallised from water and shown to be unchanged Geigerin. M.P. 78° and 168.9°.

$$\left[\alpha \right]_D^{21} = \frac{-0.40 \times 100 \times 16}{2 \times 5.03} = -63.63^\circ.$$

Alcoholic KOH neutralised by 15 c.c. of this solution during 12 hours at 37° = 1.76 c.c. N/10.

Theory requires 1.67 c.c.

The lactone grouping was therefore still intact and in every respect the substance proved to be identical with Geigerin. No other product could be isolated from the reaction mixture.

Geigerin, therefore, contains no isopropyl side chain and the whole molecule would appear to be fairly stable towards oxidising agents.

ZINC DUST DISTILLATION.

In preliminary experiments, Geigerin was distilled with zinc dust in a glass tube heated to about 400° and in presence of a current of hydrogen. The reaction products were dark, pleasant-smelling oils, dissolving in ether with a bluish fluorescence. Fractionation from absolute alcohol was attempted but insufficient material was obtained in a pure state for analysis.

It appeared that these oils contained hydro-aromatic hydrocarbons. They gave an intense red colour with vanilin-hydrochloric acid. A distillation was therefore carried out at a higher temperature, 700°, and the hydrogen stream stopped after all air had been displaced from the apparatus. There resulted a white crystalline distillate mixed with a little yellow oil. The crystals were easily drained and proved to be naphthalene. They sublimed easily, had M.P. 79°, yielded a picrate crystallising in needles with M.P. 150° and possessed the characteristic smell of naphthalene.

POTASH FUSION: PRELIMINARY EXPERIMENTS.

A potash fusion was carried out in a nickel crucible at 280°-300° for 3 hours using 1 gm. of Geigerin and 15 gm. of potassium hydroxide. The melt was almost completely soluble in water. It was acidified and steam-distilled, yielding an acid distillate possessing a rancid, butyric-like smell. The sodium salt prepared by neutralisation proved to be gelatinous whilst the acid itself could not be obtained in the solid state; the material was therefore converted into the insoluble, amorphous silver salt for analysis. Yield: 33 mgm.

Microanalysis.

	C.	H.	Ag.
Found	32.64	4.65	51.30

These figures agree most nearly with $C_6H_9O_2Ag$. Capronic acid, $C_6H_{12}O_2$, possesses an amorphous silver salt but a copper salt which crystallises in a characteristic manner. The acid obtained from Geigerin did not yield these crystals.

From the non-volatile fraction, other rancid-smelling oily acids were obtained but in no case could any individual be identified with certainty, hence the experiments were discontinued. The absence of any easily crystallised solid acid was noteworthy and since many of the materials isolated gave a cherry-red colour reaction with vanillin-hydrochloric acid, similar to that obtained with the naphthenic acids, the suspicion was engendered that the products might belong to the polymethylene series.

Only in one instance was a well-crystallised sodium salt obtained and this and the corresponding silver salt on analysis appeared to indicate a parent dibasic acid with the formula $C_7H_{12}O_8$. What structure such an acid might have it is impossible to hazard, but since the analytical figures agreed fairly well and these experiments are only of a preliminary nature the data will be recorded.

	C.	H.	Na.
Sodium salt. Found.....	30.85	4.02	17.10
$C_7H_{12}O_8Na_2$ requires.....	31.34	3.73	17.16
Silver salt. Ag. found.....	—	48.62	—
$C_7H_{12}O_8Ag_2$ requires.....	—	49.29	—

Geigeric Acid.

The free acid of which Geigerin is the lactone was prepared by dissolving 0.5 gm. Geigerin in 25 c.c. of approximately 0.1 normal alcoholic potassium hydroxide and after 24 hours at 37° acidifying the solution with the requisite quantity of hydrochloric acid. Upon shaking with ether, after dilution, the free acid passed over into the ether phase which was washed and evaporated to dryness, the residue being crystallised from hot petroleum ether in which it was sparingly soluble. The acid separated in beautiful, elongated flat

plates (see Fig. 3) and had M.P. 201–3°. It was only moderately soluble in chloroform, more soluble in absolute alcohol, which solvent was used for the determination of optical rotatory power. 30 mgm. substance in 15 c.c. absolute alcohol had rotation +0.42°.

$$\left[\alpha \right]_{\text{D}}^{28} = \frac{+0.42 \times 15 \times 100}{2 \times 3} = +105^{\circ}.$$

Micro-analysis.

	C.	H.
Found.....	63.79	7.92
C ₁₅ H ₂₂ O ₅ requires.....	63.84	7.80

It is proposed to designate this substance "Geigeric acid".



Fig. 3. Geigeric acid, M.P. 201–3°, ×70.

THE ACTION OF HYDROCHLORIC ACID UPON GEIGERIN.

The colour reaction given by Geigerin with hydrochloric acid has already been mentioned. There develops in the cold, more readily on warming, a faint cherry-red colour which is stable for a considerable period but very gradually assumes a browner tint. Solutions of hydrochloric acid more concentrated than 10 per cent. by weight yield the colour readily but with weaker solutions only a very faint pinkish-mauve colour is gradually formed. Chloroform does not extract this colour, but it is discharged at once by dilution with water and chloroform is then capable of extracting unchanged Geigerin from the solution. Similarly, if a concentrated solution of Geigerin in 20 per cent. hydrochloric acid be diluted largely and

placed in the ice chest, crystals of unchanged Geigerin slowly separate, but Geigerin is so much more easily soluble in dilute hydrochloric acid than in water that the yield is far from quantitative.

The cherry-red solution in 20 per cent. hydrochloric acid exhibits a well-marked absorption spectrum, the centres of the bands being at 543; 506 and 466 $m\mu$ respectively, the relative intensities being in descending order 543; 466; 506 (see Fig. 2). It is noteworthy that Vermeeric acid, the active principle of the Vermeerbos, to be described in the second paper of this series, gives the colour reaction very vividly, the positions of the bands being almost identical in the two cases, although the colour tint varies somewhat.

Prolonged action of hydrochloric acid undoubtedly causes some alteration in the structure of Geigerin. The following experiment was therefore carried out in an attempt to discover the nature of this change.

0.1 gm. of Geigerin was refluxed for 1 hour with 5 c.c. of 20 per cent. hydrochloric acid, the brown solution which possessed a pleasant aromatic smell was diluted, neutralised with sodium hydroxide and concentrated by distillation; the distillate gave no precipitate with Brady's reagent. The main solution was shaken with ether which extracted only a very small quantity of resinous material. It was then acidified and again extracted with ether which removed a pale yellow acidic oil. As all attempts to induce crystallisation failed, this material was esterified with diazomethane and the methyl ester crystallised from hot petroleum ether in which it was sparingly soluble. It was obtained in aggregates of very beautiful colourless, flattened prisms having pointed ends. The M.P. was 98.5°.

Micro-analysis.

	C.	H.	CH ₃ O.
Found.....	64.96	8.11	10.32
C ₁₅ H ₂₁ O ₄ (CH ₃ O) requires.....	64.82	8.17	10.47

The parent acid therefore possesses the same formula C₁₅H₂₂O₅ as Geigeric acid, showing that the lactone ring must have been opened during the reaction. Whether this acid and Geigeric acid are identical or isomeric, as is thought to be more probable, has not as yet been determined.

There is some evidence which suggests that the ketonic group yielding the 2:4 dinitrophenylhydrazone of Geigerin, described earlier, may form a part of some tautomeric grouping in the molecule, since the presence of hydrochloric acid appears to be essential for the formation of derivatives of this function. Thus, an attempt was made to prepare the dinitrophenylhydrazone by refluxing Geigerin with a slight excess of 2:4 dinitrophenylhydrazine in alcoholic solution but no reaction took place. Similarly an attempt to prepare a semicarbazone failed.

Should Geigerin exhibit keto-enol tautomerism, this might explain the two melting points observed with the pure substance, but the failure to detect an hydroxyl group by acetylation must be recalled. Comparison with the behaviour of carolic acid may prove significant in this connection (Clutterbuck, Raistrick and Reuter, 1935). On treatment with Brady's reagent, carolic acid gives an immediate precipitate of a mono-dinitrophenylhydrazone by virtue of the ketonic group in its side chain. The tetric acid ring structure, however, possesses, in addition, an oxygen atom which shows keto-enol tautomerism and this more slowly reacts with a further equivalent of the reagent to form a bisdinitrophenylhydrazone. Attempts to acetylate carolic acid in its enol form by boiling with acetyl chloride failed, only unchanged starting materials being recovered.

OXIDATIVE DEGRADATION OF GEIGERIN.

Geigerin does not decolourise cold permanganate solution but is fairly readily oxidised at the boiling temperature. 1 gm. Geigerin in 20 c.c. of water plus 1 gm. sodium carbonate required 82 c.c. of 2.5 per cent. potassium permanganate solution, added in small quantities at a time, before the reaction slowed up. The resultant mixture was filtered, affording a pale yellow liquid which gave a yellow precipitate with Brady's reagent. It was therefore concentrated by distillation (whilst still alkaline) and the distillate tested but with negative result; no substance reacting with Brady's reagent was present. The main solution was then extracted very thoroughly by ether, this solvent slowly but completely removing from the alkaline liquid the material giving the dinitrophenylhydrazone. Upon evaporation, the ether left a pale oil with a peculiar cucumber-like smell. It was fairly easily soluble in water and was found to give the following reactions:—

Schiff's reagent.....	Fairly rapidly +.
Fehling's solution.....	Only reduced on boiling for 1 minute.
Tollen's reagent ($\text{AgNO}_3 : \text{NH}_4\text{OH}$)..	—
Jean's test (Phenylhydrazine + Nitro- prusside)	— slowly brown.
m-dinitrobenzene + alkali.....	— yellow only.
Thiophene + H_2SO_4 test.....	+ cherry red.

Upon acidifying and adding Brady's reagent, a 2:4 dinitrophenylhydrazone precipitated and was separated off, washed and crystallised from hot dilute alcohol. It crystallised in yellow plates with M.P. 158-161° and proved to be identical with the derivative of acetaldehyde, thus:—

Micro-analysis.

	N.
Found.....	24.84
$\text{C}_8\text{H}_8\text{N}_4\text{O}_4$ requires.....	25.00

Mixed with acetaldehyde 2:4 dinitrophenylhydrazone of M.P. 159-161°, it had M.P. 159-161°.

This result was somewhat surprising, but it was noticed that of the colour tests listed above, only those were positive (and similar to acetaldehyde) which required the addition of acid to the solution. The substance isolated from the oxidation mixture clearly could not be identical with acetaldehyde since it could not be distilled but it was suspected that it was some material which affords acetaldehyde when treated with acids. (Compare the acetals which are stable to hot alkalies but yield aldehyde immediately on addition of cold dilute acids.)

This supposition was proved to be correct in the following way: To some of the substance was added 2 normal sulphuric acid and the mixture was gently distilled affording a distillate giving all the tests for acetaldehyde and from which acetaldehyde 2:4 dinitrophenylhydrazone was prepared.

Tests on Distillate.

Schiff's reagent.....	+
Fehling's solution.....	Reduced on boiling.
Tollen's solution.....	+
Jean's test.....	+ red.
m-dinitrobenzene + alkali.....	+ mauve.
Iodoform.....	+
Thiophene.....	+

Micro-analysis.

	N
2:4 dinitrophenylhydrazone M.P. 158-160°.	Found 25.33
Acetaldehyde dinitrophenylhydrazone requires	25.00

The mixed M.P. was 159-160°.

It was subsequently found, as reported in the following paper, that Vermeeric acid, on oxidation by means of alkaline permanganate, also yields a similar material which when treated with dilute acid liberates acetaldehyde.

The alkaline aqueous solution remaining after having removed, by ether extraction, all the material reacting with Brady's reagent, was acidified and again exhaustively extracted with ether which now removed a strongly acidic substance. This was converted into the sodium salt and the latter, after washing with acetone, dissolved in absolute alcohol and precipitated by ether. There was obtained 0.42 gm. of a nearly colourless amorphous powder. Attempts to crystallise the free acid from this small quantity failed; however an analysis was performed and after methylation with diazomethane in ethereal solution the methyl ester was obtained crystallising from hot petroleum ether, in which it is sparingly soluble, in short hexagonal prisms, M.P. 115-6° (see Fig. 4). On repetition, the acid itself was obtained crystallising in six-sided prisms, M.P. 200-1°,

$[\alpha]_D + 90^\circ$. Analysis indicated the formula $C_{10}H_{14}O_4$.

Micro-analysis.

	C.	H.
Methyl ester. Found.....	60.55	6.95
C ₁₀ H ₁₄ O ₄ requires.....	60.60	7.07

The substance appeared to react slowly with Brady's reagent forming a yellow precipitate.



Fig. 4. Methyl ester of acid obtained by alkaline permanganate oxidation of Geigerin, M.P. 115.6°, $\times 66$.

These experiments must be regarded as purely preliminary; further work awaits the accumulation of sufficient starting material. In general, the chemical work reported above supports the idea that Geigerin may be made up of hydroaromatic or polymethylene ring systems but certainty upon this point can only be obtained by the isolation of degradation products in sufficient quantity to allow of their transformation into known substances. The object of the present communication is to record the isolation of the lactone Geigerin from *Geigeria aspera* and to describe its reactions and properties. The question of its exact chemical structure will be dealt with in another communication at some future date.

PHARMACOLOGICAL EXPERIMENTS.

A cat (1,200 gm.) was given 0.1 gm. Geigerin dissolved in 3 c.c. of 30 per cent. alcohol subcutaneously. The animal looked ill next day and would only drink a little milk; however, complete recovery eventually took place.

About six months later the same animal was given 1 gm. of Geigerin suspended in water by stomach tube at 12.30 p.m. Symptoms developed as follows:—

12.47	p.m.	Vomited.
12.55	,,	Vomited again.
1.5	,,	Vomited again.
2.2	,,	A severe attack of retching, which brought up only a very small quantity of fluid together with a tape-worm about 5.5 inches in length
2.40	,,	Further retching.

The animal remained very ill throughout the day, but drank a little milk. Intense salivation and retching occurred at intervals. Next day it refused to eat and looked very ill. The following day another retching attack was observed at 11.30 a.m. and a considerable amount of fluid containing mucous was seen in the bottom tray of the cage suggesting that vomiting had occurred unobserved during the night. The animal was killed. The post mortem examination revealed a severe gastro-enteritis confined more particularly to the fundus region of the stomach. The trachea and glottis were normal. The liver showed slight fatty infiltration. The urine in the bladder was free from haemoglobin.

On a subsequent occasion, the same dose (1 gm.) was given in the same manner to a 2.1 kilo cat. It remained quiet and listless all day. Next morning vomited material was observed in the cage. The animal refused food and looked very ill. On the following day it appeared somewhat improved and was killed for post mortem examination. A slight mucous catarrh was seen in the stomach and small intestine, otherwise the appearance of all organs was normal.

A rabbit (1.9 kilos) which received 0.25 gm. Geigerin by stomach-tube exhibited no symptoms. A sheep was drenched with a total quantity of 15 gm. of Geigerin given in doses of 5 gm. at intervals over 2 days. It remained normal. These preliminary pharmacological tests suggest that cats are the most suitable test animals to be used. Geigerin would appear to have little or no action upon rabbits but to be not entirely devoid of vermeersiekte producing properties (gastric irritation) as judged by its effect when dosed to cats. It is hoped to continue this aspect of the work at some later date.

SUMMARY.

1. There has been isolated from toxic consignments of *Geigeria aspera*, Harv. a neutral lactonic bitter principle to which the name *Geigerin* has been assigned. This substance, though not the true toxic principle, would appear to be not altogether devoid of pharmacological properties.

2. Geigerin has the formula $C_{15}H_{20}O_4$ and crystallises from water or moist organic solvents with 1 molecule of water of crystallisation. It exhibits isomerism, the melting points of the two varieties, when anhydrous, being 169° and 189° but preliminary softening with liberation of water from the hydrated prisms is observed at about 68° and 75° respectively. No matter which isomer is used, the same optical activity is exhibited in the same solvent, the constants being

for absolute or dilute alcohol $\left[\alpha \right]_D^{20} = -60.32^\circ$ in the mean and

for chloroform or ethyl acetate $\left[\alpha \right]_D^{20} = -42.71^\circ$ in the mean.

3. There are some grounds for believing the isomerism to be due to keto-enol tautomerism, although no hydroxyl group could be detected by acetylation under various conditions.

4. Geigerin forms a 2:4 dinitrophenylhydrazone in the presence of hydrochloric acid, but attempts to prepare derivatives of the ketonic function in the absence of an acid failed.

5. Potassium permanganate is not decolorised by the substance in the cold neither can any acetone be recovered after the action of chromic acid under the proper conditions for the oxidation of isopropyl side-chains to acetone. Such a group is therefore not present in Geigerin.

6. Cold hydrochloric acid of concentration over 10 per cent. affords a very striking colour reaction with Geigerin. The substance dissolves easily, forming a cherry-red solution which shows absorption bands at 543, 506 and 466 $m\mu$. In time, the colour becomes more brown in tint.

7. Geigerin is a monolactone and the corresponding acid, *Geigeric acid*, has been prepared and found to crystallise in plates

with M.P. 201.3° and $\left[\alpha \right]_D^{28} = +105^\circ$.

8. When Geigerin is boiled for 1 hour with concentrated hydrochloric acid the cerise colour changes to brown and from the reaction mixture an acid was isolated in the form of its methyl ester melting at 98.5° .

9. From results reported in the following communication, it would appear that Geigerin is closely related, chemically, to the active principle of the plant which causes vermeersiekte. Preliminary degradation experiments indicate that on potash fusion a mixture of liquid rancid-smelling acids is produced, and certain reactions suggest that these may belong to the polymethylene series of compounds.

10. On distillation at low temperature with zinc dust, fluorescent, oily aromatic substances are produced. At 700° naphthalene is formed.

11. When Geigerin is oxidised by hot alkaline potassium permanganate, there arises an acidic fraction from which the methyl ester of an acid, probably in $C_{10}H_{13}O_3$ (OCH_3), was isolated in crystalline form, M.P. $115-6^\circ$, and also a neutral, non-volatile substance soluble in ether and slightly soluble in water, which on treatment with dilute mineral acid in the cold at once liberates acetaldehyde. The exact nature of this material awaits further investigation, but attention is drawn to the analagous behaviour of the acetals.

12. It would appear from the literature that Geigerin is in all probability identical with the substance extracted by Thoms from *Chrysanthemum cinerariaefolium* in 1891 and named by him "Pyrethrosin". His formula for this substance $C_{34}H_{44}O_{10}$ was put forward with some reserve.

13. Preliminary pharmacological experiments upon rabbits, sheep and cats are reported.

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