

On the Constitution of the Bitter Principle "Geigerin" I. The Isolation of Various Degradation Acids.

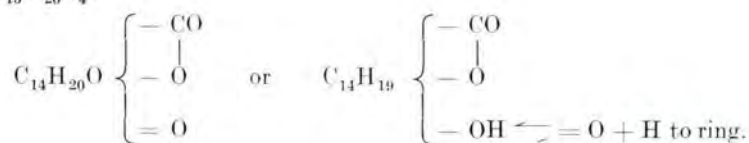
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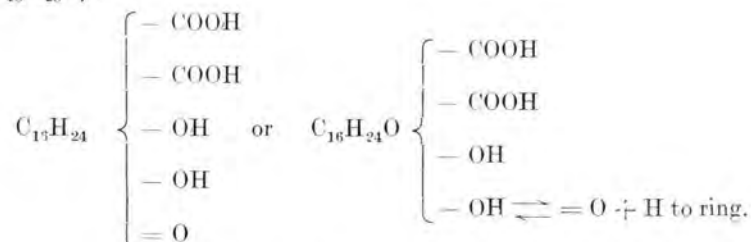
INTRODUCTION.

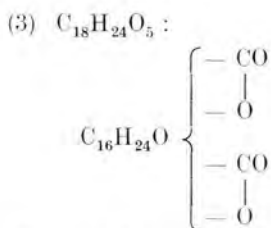
ACCORDING to the properties of the bitter principle Geigerin and the active principle Vermeeric acid of the "Vermeerbos" (*Geigeria aspera*, Harv.), described by Remington and Roets (1936) and Rimington, Roets and Steyn (1936), these substances would appear to be closely related with regard to their chemical structures. In summarizing the results already obtained, these authors suggest part formulae for Geigerin (1), Vermeeric acid (2) and its dilactone Vermeerin (3) as follows, giving an indication of the possible involvement of one atom of oxygen in a keto-enol tautomerism:

(1) $C_{15}H_{20}O_4$:



(2) $C_{18}H_{28}O_7$:





These substances being in all probability closely related to one another, constitutional work was begun on the chemical constitution of the bitter principle Geigerin. Should the structure of the latter be elucidated, then similar methods of constitutional investigation when applied to Vermeeric acid or Vermeerin, might be expected to lead successfully to the clearing up of their structures also. Meanwhile it may be pointed out that the bitter principles, although very interesting subjects for investigation have proved to be most difficult from the constitutional structural point of view. Thus Beth (1936) describing a few scores of bitter principles already isolated from different plants, especially the families Gentianaceae, Compositae and Labiateae points out that up till then not a single bitter principle's structure had been fully elucidated.

In this article we wish to record the first results already obtained on the constitution of Geigerin and to describe various degradation products obtained by oxidation or otherwise. These have been very encouraging. Until more starting material has however been prepared and until further results have been obtained, we do not wish to enter into a detailed discussion of its possible structural formula at this stage.

THE APPLICATION OF THE LEGAL TEST AS A METHOD FOR DETECTING ACETALDEHYDE IN GEIGERIN OXIDATION EXPERIMENTS.

According to Meyer (1931, p. 446) a characteristic intense colouration is obtained when to solutions of ketones or aldehydes preferably in water (also alcohol or ether) 0.5 to 1 c.c. of a freshly prepared 0.3 to 0.5 per cent. sodium nitroprusside solution is added followed by alkalification with concentrated potassium hydroxide (D-1-14).

In the aliphatic series this reaction is always positive when the group attached to the ketonic or aldehydic radicle (in α -position) consists of carbon and hydrogen only. Aldehyde or ketone groups directly bound to a ring or included in a ring structure do not give the reaction, neither do ortho-aldehydic acids.

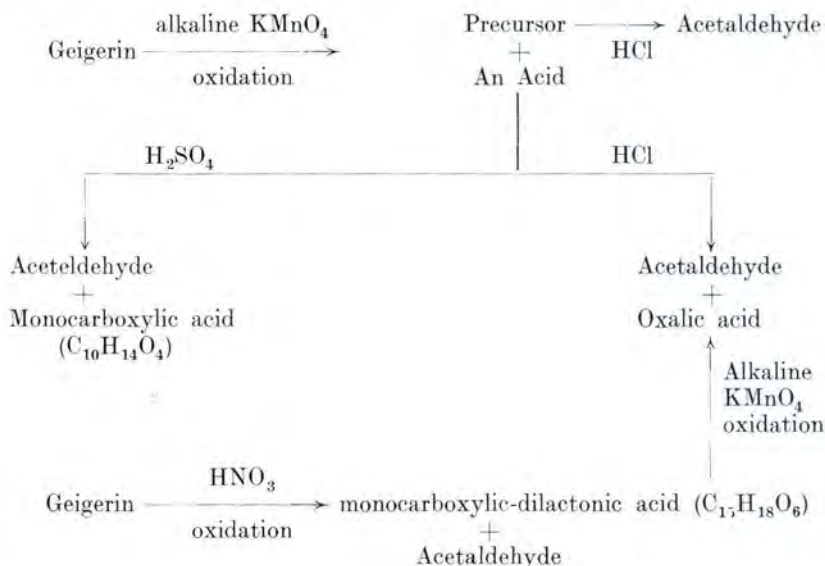
The colours produced vary from orange-red, red to violet-red, which on the addition of organic acids change from red to indigo-blue or from violet blue to blue-green e.g.

The solid α - or β -Geigerin is not a ketone, nor does it show aldehydic or ketonic properties in a neutral solution (it is presumably present in the enol-form) but in dilute hydrochloric acid solution it reacts as a ketone. The Legal test is therefore negative with Geigerin. When Geigerin is dissolved in a 10 per cent. hydrochloric

acid solution (ketonic-form), cooled down and then neutralized with 16 per cent. potassium hydroxide the immediate application of the Legal test yields a negative result. This shows either (1) that the enolic form is again obtained on neutralization or (2) that although Geigerin may still be present in the ketonic form, that the carbonyl-group is directly contained in a ring structure or immediately attached to it.

In the oxidation processes described below (see experimental part) acetaldehyde is very frequently formed as a byproduct during the oxidation. Under certain circumstances the acetaldehyde is most probably liberated from a pre-cursor formed during the oxidation and can thus only be detected after acidification. Under other conditions (ether extractions of acid mediums or acid oxidations) acetaldehyde accompanies the main oxidation product formed as a sticky oily material. When the latter is washed away with pure ether (only Merck's and Schering-Kahlbaum's absolute ether has been used in these extractions), the acetaldehyde may be easily and very conveniently detected by means of the Legal test. The ethereal solution or a watery or even an absolute alcoholic solution (when acetadehyde is present) will immediately give a positive test with the Legal reagent, the watery phase turning intense orange-red. The usual identification tests e.g. its pungent odour, its 2-4 dinitro-phenylhydrazone derivative with Brady's reagent and other tests will confirm its presence. Blank experiments with pure ether, absolute alcohol or water will give only the "blank" greenish-yellow colouration.

The following diagram will demonstrate the convenience of the Legal test for detecting the acetaldehyde also formed during the isolation of the various oxidation products (compare the experimental part):



Neither of the degradation acids, $C_{10}H_{14}O_4$ (M.P. 201°) and $C_{15}H_{18}O_6 \cdot H_2O$ (M.P. 280°) give the Legal test; thus in solution the application of the Legal test will quickly affirm the presence or absence of acetaldehyde.

EXPERIMENTAL.

Improved Geigerin Extraction.

About 75 Kilogram of dried ground "Vermeerbos" was extracted in the following way:

3.5 Kg. dried material was extracted with ether in a large soxhlet-type apparatus for 3 working days (i.e. about 22 hours). The ether was then drawn off, amounting to about 8 litres in volume. The ether solution was then divided into two equal portions and each part shaken with about 5 litres of 1 per cent. hydrochloric acid (in 5 or 6 portions), i.e. a total volume of 10 litres. Through this solution air was then drawn for 3 to 4 hours when the solution has a greenish-yellow tint with a pleasant ether-free fruity smell. The solution was then shaken with a little charcoal, filtered and now shaken with 8 lb. of chloroform (in successive portions). The chloroform solution was then washed with a one per cent. sodium carbonate solution, becoming nearly colourless, and again washed with distilled water.

The chloroform solution was then dried over sodium sulphate, concentrated at reduced pressure to about 500 c.c. of chloroform and left to evaporate in front of a fan. After 2 to 3 days the resulting crystalline residue was boiled out with 300 to 400 c.c. of distilled water and filtered into open dishes. Crystallization immediately set in on cooling and after recrystallization from water a yield (on the average) of 1.5 gm. of Geigerin was obtained, i.e. 0.43 gm. per 1 Kgm. dried plant material.

Oxidation of Geigerin with Nitric Acid to a monocarboxylic-dilactonic acid ($C_{15}H_{18}O_6 \cdot H_2O$).

Two different methods both yielding good results, are described below:—

(a) 1 gm. Geigerin and 50 c.c. nitric acid (1:1) were refluxed for 12 hours, during which time nitrogen peroxide fumes were evolved finally yielding a pale yellow solution. On cooling it was diluted with distilled water and then thoroughly shaken 3 times with chloroform, the chloroform solution washed once with water, dried over exsiccated sodium sulphate and then evaporated in front of a fan at room temperature. Crystals separated practically immediately. After the solution had been evaporated to dryness, the crystals were washed with ether and were obtained as colourless hexagonal prisms with a melting-point, crude, of about 250° and in a yield of 0.25 gm.

The ether washings evaporated at room temperature left acetaldehyde.

The original solution was again shaken 3 times with chloroform and after the same treatment yielded another 50 mgm. of the new product, thus increasing the yield to about 300 mgm. The material

was twice recrystallised from water. The crystals turned opaque* at 105° ; above 240° hexagonal prisms shot out which melted sharply at 280° (corr.) without decomposition.

The same oxidation product can be obtained by the oxidation of Geigerin with nitric acid (1 : 3 and 1 : 2), concentrated nitric acid ($D=1.4$) and fuming nitric acid. The following method is especially recommended:—

(b) Introduce into an open pyrex dish (capacity about 50 c.c.) 1.5 gm. Geigerin and cover the substance with about 20 c.c. of concentrated nitric acid ($D=1.4$) and warm on a boiling waterbath. The crystals easily dissolve. The oxidation is continued for about one day (8 hours). Continue the addition of concentrated nitric acid now and then and finally allow to concentrate to about 10 c.c. Allow to cool down (crystals frequently separating immediately) dilute with water to about 100 c.c. and leave to stand in an ice-chest overnight. Recrystallised from water 400 mgm. of the pure oxidation acid is obtained (M.P. 280° —see Fig. 1) (a byproduct of the oxidation is acetaldehyde).

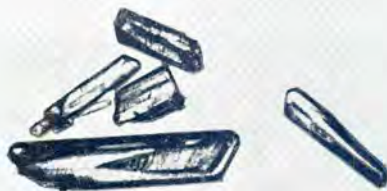


Fig. 1.—Acid obtained by the nitric acid oxidation of Geigerin. m.p. 280° ; $\times 25$.

Properties.—The substance—

- (1) is easily soluble in ammonia, alkalis and dilute sodium carbonate solution—is therefore an acid—and may be recovered on the addition of dilute acids;
- (2) dissolves slowly without colour in concentrated sulphuric acid but is practically insoluble in dilute mineral acids;
- (3) is insoluble in benzene, ether and petroleum ether;
- (4) is sparingly soluble in chloroform and ethyl acetate;
- (5) is soluble in water, methyl alcohol and ethyl alcohol;
- (6) is readily soluble in acetone;
- (7) does *not* react with Brady's reagent;
- (8) does *not* give a Legal test;
- (9) gives no colour with dilute or concentrated hydrochloric acid.

* Kofler micromelting point apparatus.

*Micro-analysis.**

- (1) Dried at room temperature in high vac. over P_2O_5 .
 (a) 5.208 mgm.; 10.960 mgm. CO_2 ; 3.060 mgm. H_2O .
 found C = 57.41; H = 6.58.
 Calc. C = 57.68; H = 6.46.
 $C_{15}H_{18}O_6 \cdot H_2O$
 (312.31)
- (b) 0.201 mgm. in 3.756 mgm. Camphor; $\Delta = 6.7$
 i.e. mol. weight = 319 (calcd. 312.31).
- (2) Dried at $110^\circ C$. in highvac.
 (a) 5.324 mgm.; 11.945 mgm. CO_2 ; 2.920 mgm. H_2O .
 (b) 4.539 mgm.; 10.230 mgm. CO_2 ; 2.550 mgm. H_2O .
 found (a) C = 61.22; H = 6.14.
 ,, (b) C = 61.49; H = 6.29.
 Calc. C = 61.22; H = 6.17.
 $C_{15}H_{18}O_6$.

Micro-titration.—16.6 mgm. of the acid dissolved in about 10 c.c. water required 0.65 c.c. 0.1 N NaOH, i.e. assuming one $-COOH$ the molecular weight is 299.

Rotation.—Weight = 59.8 mgm.

Volume = 15.0 c.c. abs. alc.

$$\Theta = + 1.45^\circ.$$

$$\text{Therefore } \left[\alpha \right]_D^{25} = \frac{+ 1.45 \times 100 \times 15}{2 \times 5.98}$$

$$= + 181.85^\circ.$$

Methyl-ester.

To a solution of 50 mgm. of the above oxidation acid dissolved in about 6 to 7 c.c. of acetone, an ethereal solution of diazomethane was slowly added (ice-cooling) until a slight excess of diazomethane was noticeable. The solution was then left to stand overnight well-stoppered. It was then allowed to evaporate and the crystalline residue (needles grouping in aggregates) was recrystallized from dilute methyl alcohol. After recrystallization, washed with 96 per cent. alcohol and then with ether, the colourless six-sided prismatic needles melted sharply without decomposition at $190-191^\circ$ (corr.).

Micro-analysis.

- 4.974 mgm.; 11.325 mgm. CO_2 ; 2.970 mgm. H_2O ; 0.010 mgm. res.
 found C = 62.25; H = 6.70.
 Calc. C = 62.33; H = 6.54.

$C_{11}H_{17}O_4COOCH_3$:

$(C_{16}H_{20}O_6)$

* All micro-analysis by Dr. Ing. A. Schoeller, Berlin.



Fig. 2.—Methyl ester of monocarboxylic dilactonic acid m.p. 190-191°; $\times 50$.

Presence of Lactone groupings in the "new" acid, m.p. 280°.

32.0 mg. of the acid was dissolved in the cold in 5 c.c. of 96 per cent. alcoholic potassium hydroxide ($F=0.097$) and after five minutes the solution back titrated with 0.1 N hydrochloric acid.

5 c.c. Alcoh. KOH ($F=0.097$)...	= 4.85 c.c. of 0.1 N KOH.
Back titration required	2.90 c.c. of 0.1 N HCl.
Therefore difference	= 1.95 c.c. 0.1 N KOH.
Now 31.23 (mol. wt. = 312.3) mgr. acid reqd.:	1 c.c. 0.1 N KOH
for one -COOH.	

Therefore 32.0 mgr. acid reqd.: 1.024 c.c. 0.1 N KOH for one -COOH.

i.e. theory for 2 carboxyles	= 2.05 c.c. 0.1 N KOH.
found	= 1.95 c.c. 0.1 N KOH.

To the above solution 4.85 c.c. 0.1 N alcoholic potassium hydroxide was again added and the solution left for 5 hours at 37° C.

Back titration required	3.95 c.c. 0.1 N HCl.
Therefore difference ...	= 0.90 c.c. 0.1 N KOH.
Theory for one -COOH	= 1.02 c.c. 0.1 N KOH.

Again 4.85 c.c. of 0.1 N alcoholic potass. hydroxide was added and the solution left for another 5 hours at 37°.

Back titration required 4.90 c.c. 0.1 N HCl.

Therefore no significant difference.

These figures indicate that after 5 minutes one lactone group is saponified to a carboxylic group and after 5 hours a second lactone group is saponified to a second carboxylic group. Finally we thus have a tribasic acid (the original acid being monocarboxylic) which was again proved by the relactonization.

Relactonization of the Tricarboxylic Acid.

Continuing with the above solution containing the tricarboxylic acid, experiments were conducted to determine whether any of the carboxyle groups could relactonise.

5 c.c. 0.1 N HCl was added and the solution left for 20 hours at 37° C;

Back titration required 2.80 c.c. 0.1 N KOH.

Therefore difference ... = 2.20 c.c. 0.1 N HCl.

Theory for 2 lactones ... = 2.05 c.c. 0.1 N HCl.

After the addition of another 2.0 c.c. 0.1 HCl and leaving the solution for 6 hours at 37°, it was back titrated and the back titration required exactly 2 c.c. of 0.1 N KOH; therefore no change.

Thus 2 of the 3 carboxyl groups were relactonized after 20 hours.

Duplicate Saponification and Re-lactonization.

32.0 mgr. of the dilactonic-monocarboxylic acid (M.P. 280°) was dissolved in 5 c.c. of 0.1 N alcoholic KOH at room temperature and back titrated with 0.1 N HCl after seven minutes.

Back titration required 2.80 c.c. 0.1 N HCl.

Therefore difference = 2.20 c.c. 0.1 N KOH.

After another hour (i.e. one hour and seven minutes) a back titration of 5 c.c. N alcoholic KOH with 0.1 N HCl gave a difference of 0.80 c.c. 0.1 N KOH.

Therefore total difference = 3.00 c.c. 0.1 N KOH.

Theory for 3 -COOH = 3.07 c.c. 0.1 N KOH.

After 18 hours the total difference was raised to 3.15 c.c. 0.1 N KOH, i.e. a practically complete saponification of the monobasic-dilactonic acid was accomplished after 67 minutes to a tribasic-acid.

Similarly relactonization of the above solution took place as follows:—

After 30 minutes difference = 0.80 c.c. 0.1 N HCl.

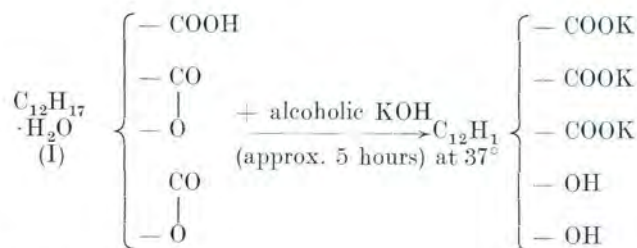
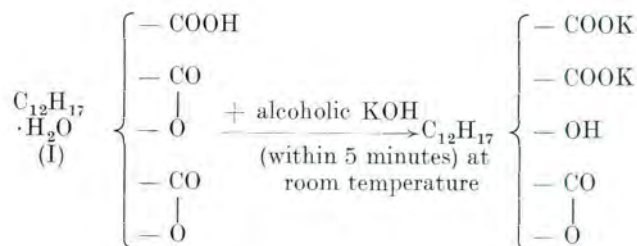
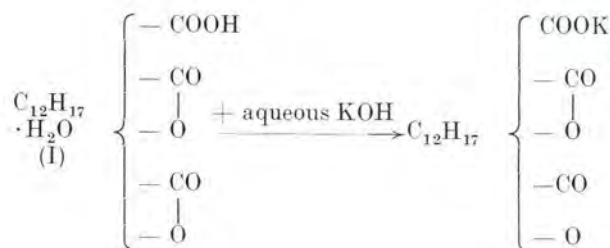
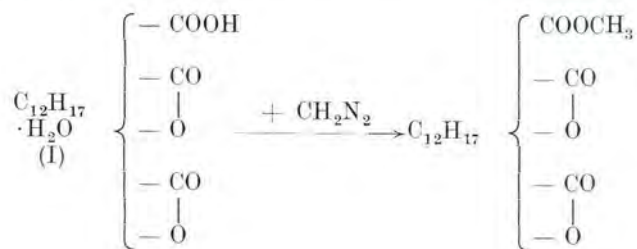
After 2 hours difference ... = 1.30 c.c. 0.1 N HCl.

After 7 hours difference ... = 1.60 c.c. 0.1 N HCl.

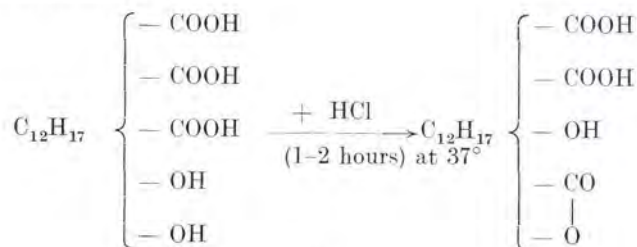
After 24 hours difference ... = 1.80 c.c. 0.1 N HCl.

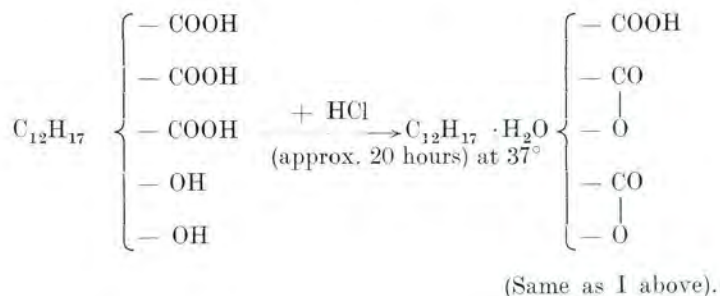
Theory for 2 lactone-groups = 2.05 c.c. 0.1 N HCl.

Summarising these results we have for the monocarboxylic-dilactonic-acid (M.P. 280°) an empirical formula of $C_{15}H_{18}O_6 \cdot H_2O$ constituted as follows:—



and the re-lactonization





Considering the ease with which one carboxyl group is relactonised in the presence of 0.1 N hydrochloric acid, it is interesting to compare this phenomenon with that of Vermeeric acid (see Rimington, Roets and Steyn, pp. 517-518). In the latter acid one carboxyl group similarly lactonises much more readily than the second (e.g. in the presence of the hydrochloric acid of the Brady's reagent when the 2.4-dinitrophenylhydrazone of Vermeeric acid is prepared). A possible structural explanation for this phenomenon is discussed further below.

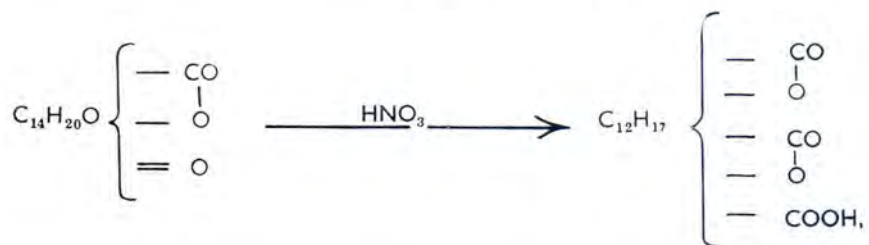
An attempt to prepare a semicarbazone from this new acid was negative. 50 mgm. acid was dissolved in 3 c.c. absolute alcohol and to this a solution of 50 mgm. semicarbazide-hydrochloride and 50 mgm. sodium acetate in 0.5 c.c. water was added. The mixture was refluxed for 8 hours. The original acid of M.P. 280° was regained quantitatively.

An acetylation attempt proved also the absence of free hydroxyl groups. 50 mgm. acid dissolved in 1 c.c. of pyridin was left to stand for several days with 0.2 c.c. of acetic anhydride. The original acid of M.P. 280° was again recovered.

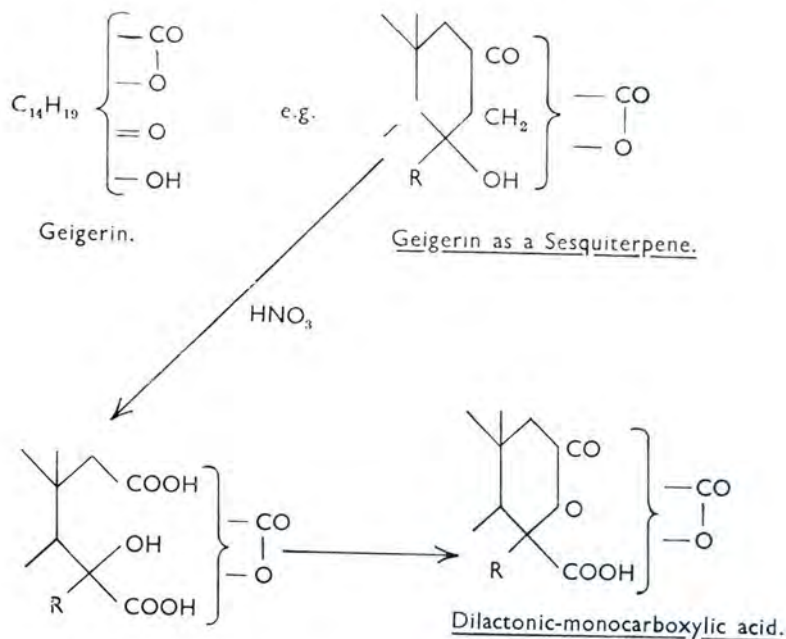
These negative experiments for a carbonyl- or hydroxyl radicle are in harmony with the saponification and relactonization findings above. The nature of the acid—a monobasic-dilactonic acid—accounts for the functions of all its six oxygen atoms. As this acid results from the oxidation of Geigerin ($\text{C}_{15}\text{H}_{20}\text{O}_4$), of which the function of the fourth oxygen atom (see page 395) is still unknown, it may prove very valuable for determining the function of that fourth oxygen atom. Also by comparing the two formulae of Geigerin ($\text{C}_{15}\text{H}_{20}\text{O}_4$) and of the monocarboxylic-dilactonic-acid ($\text{C}_{15}\text{H}_{18}\text{O}_6$) we find that the nitric acid oxidation led to a gain of two oxygen atoms accompanied by a loss of *two* hydrogen atoms only. A review of the literature showed that this acid is still unknown, and further experiments as regards its structure will have to be performed.

Meanwhile we could explain such an oxidation as follows:

The fact that Geigerin, which is a mono-lactone and which is in acid medium mono-ketonic, can be oxidised with nitric acid to a dilactonic-monobasic acid thus



seems to support the view that the fourth oxygen atom in Geigerin is not ether-bound or cyclic, but most likely present in a hydroxyl-group, which cannot be acetylated (steric hindrance). We could then explain such an oxidation by postulating a mechanism similar to that frequently encountered in the camphor group (terpenes). As an example the oxidation may be cited of camphor by nitric acid through camphoric acid to camphanic acid (Bredt). In an analogous manner the oxidation of Geigerin could be formulated as follows:



In the latter acid the lactone group (outside) may be easily saponified (same as in Geigerin where it gives Geigeric acid) and the new-formed lactone group (inside) may be more difficultly

saponified, i.e. as we have found, may be the lactone group which is only saponified after standing for 24 hours (at least) with alcoholic potassium hydroxide at 37°C.

Other facts which seem to coincide with a sesquiterpene structure for Geigerin are (1) the formation of naphthalene on distillation with zinc dust (compare Rimington and Roets p. 497) and (2) the formation (under proper conditions) of a $C_{10}H_{14}O_4$ acid, when Geigerin is oxidised with alkaline permanganate solution. We have again been able to confirm the formation of this acid (compare Rimington and Roets, p. 502) and its properties seem to point to a terpene-acid e.g. camphanic acid (which however still awaits identification).

Judging from the properties of the monocarboxylic-dilactonic acid (M.P. 280°), it appears to be a very stable compound as the following experiment will also serve to exemplify.

To 60 mgm. of the acid dissolved in 10 c.c. glacial acetic acid was added 2 c.c. concentrated sulphuric acid and the mixture warmed on a boiling waterbath (vertical tube) for 2 to 3 days. The acetic acid was distilled off under reduced pressure to a very small volume. The dark brown solution was diluted with a little water and when put into the ice-chest the original acid again crystallised out quantitatively. After recrystallization it had M.P. 280°

Permanganate Oxidation of the Monocarboxylic-dilactonic acid to oxalic acid.

100 mgm. of the above acid (M.P. 280°) and 1 gm. sodium carbonate were dissolved in 5 c.c. of distilled water and 25 c.c. of a 3 per cent. potassium permanganate solution slowly added. The solution was kept gently boiling under a " Normal-Schliff " reflux condenser for 2 hours. After having cooled down, the manganese dioxide of the completely decoloured solution was filtered off.

The still alkaline solution was thoroughly shaken with pure ether. On evaporation the ether left a very small amorphous residue, which on acidification with dilute hydrochloric acid yielded acetaldehyde (2, 4 dinitrophenyl-hydrazone derivative with Brady's reagent of M.P. 160-161 and other identification tests).

The above solution was then acidified with HCl and again thoroughly shaken with ether. On the evaporation of the ether a crystalline substance (shining plates) deposited, which darkened at 60-80°, and melted with sublimation and much evolution of gas at about 150°. The crystals were dissolved in a little cold water and the solution left to evaporate on a watch-glass. They darkened at 70-90°, sublimation occurred from 155° upwards and with gas-evolution the crystals melted at 165°. The substance dissolved with strong effervescence in dilute sodium carbonate solution and was found to be identical with the degradation acid obtained by the permanganate oxidation of Geigerin (see below) and both again identical with oxalic acid.

Degradation of Geigerin to Oxalic Acid.

To 1.5 gm. Geigerin dissolved in 150 c.c. of water was added 50 c.c. of a 10 per cent. sodium carbonate solution. The flask, containing this solution, was fitted with a "Normal-Schliff" reflux condenser, the top end of which again carried a "Normal-Schliff" dropping funnel. Through the dropping funnel 180 c.c. of a 2.5 per cent. potassium permanganate solution was dropped in, while the flask was held on a boiling waterbath for two hours. Boiling was then continued on a wire gauze for another hour, during which time another 70 c.c. of the 2.5 per cent. potassium permanganate solution was dropped in—in all therefore 250 c.c. of 2.5 per cent. KMnO_4 solution. At this stage decolourisation was very slow and the oxidation was interrupted after $3\frac{1}{4}$ hours.

When cool the solution was filtered and the faintly yellow clear solution extracted with pure ether in a bubble-extractor for 72 hours. (Except for a little acetaldehyde nothing else could be isolated from the faint residue obtained after the evaporation of the ether).

The solution was then acidified with hydrochloric acid and again extracted in a bubble-extractor with pure ether for several days until complete exhaustion.

After 24 hours extraction, the ether extract on evaporation deposited about 150 mgm. of degradation acid. After another 24 hours, another 80 mgm. were obtained. After one week's continued extraction no more of the degradation acid could be obtained and the total yield amounted to about 400 mgm.

On the evaporation of these ether extracts crystals separated which possessed the following properties:

- (1) Soluble without colour in dilute or concentrated HCl.
- (2) Soluble without colour in concentrated H_2SO_4 .
- (3) Soluble without colour in dilute or concentrated NH_4OH .
- (4) It dissolves with strong effervescence in $2\frac{1}{2}$ per cent. sodium carbonate solution.
- (5) No reaction with Brady's reagent.
- (6) Very readily soluble in water.
- (7) Easily soluble in absolute alcohol, dilute alcohol, methyl alcohol and acetic acid.
- (8) Soluble in ethyl acetate, ether, acetone and toluene.
- (9) Practically insoluble in benzene, petroleum ether and carbon tetrachloride.
- (10) Soluble in acetic anhydride and dioxan.

From a mixture of acetone and carbon tetrachloride colourless shining plates crystallised, which turned opaque (loss of water) at 75 to 80° , sublimation started at 140° and the crystals then melted at 180° .

Micro-analysis:

4.840 mg. dried at room temperature in h.v. lost 2.628 mg. in weight.

2.723 mg. dried at room temperature in h.v. lost 1.513 mg. in weight.

(1) 2.212 mgm. : 2.090 mgm. CO₂; 0.580 mgm. H₂O.

(2) 0.207 mgm. in 2.748 mgm. Camphor; Δ = 31.7, i.e. mol. weight = 95.

(3) 0.254 mgm. in 4.229 mgm. Camphor; Δ = 26.9, i.e. mol. weight = 89.

Found: C = 25.77 per cent.; H = 2.96 per cent.; mol. wgt. 89 and 95.

C₂H₂O₄; calculated: C = 26.67 per cent.; H = 2.22 per cent. mol. wgt. = 90.

The analysis and the properties proved that the degradation acid obtained above was identical with oxalic acid. Similarly the degradation acid obtained by the potassium permanganate oxidation of the monocarboxylic dilactonic acid (see p. 14) was identical with oxalic acid in all respects. Again these two degradation acids were fully identical between each other (no melting point depressions etc.).

Attempts to degrade Geigerin with concentrated sulphuric acid.

About 200 mgm. of Geigerin was dissolved in about 10 c.c. concentrated sulphuric acid. The solution was gently warmed to about 50° and the lemon-yellow solution was then slowly run into 100 c.c. of water under continuous stirring so that the temperature did not rise over 80° C (compare Pfeiffer and de Waal (1935)).

The pale yellow solution was then shaken with ether and on evaporation of the ether a big crop of colourless crystalline columns separated. The substance proved to be non-phenolic, non-acidic and was easily identified as β -Geigerin (compare Rimington and Roets page 489).

On repeating the above experiment with 1gm. of Geigerin and 40 c.c. of concentrated sulphuric acid run into about 400 c.c. of water (temperature 70-80°C) an ethereal extract of the solution gave a mixture of α - and β -Geigerin only. Repeated extraction with chloroform gave the same results.

Concentrated sulphuric acid is therefore incapable of decomposing Geigerin.

An attempt to oxidise 450 mgm. Geigerin dissolved in about 20 c.c. water and 10 c.c. 96 per cent. alcohol with 4 c.c. of 30 per cent. hydrogen peroxide and refluxing the mixture for 6 hours failed. Unchanged Geigerin was recovered quantitatively.

200 mgm. Geigerin was dissolved in about 20 c.c. glacial acetic acid; 2 c.c. of 48 per cent sulphuric acid was added and the solution heated on a boiling waterbath under a reflux condenser for 3 hours.

The acetic acid was then distilled off at reduced pressure leaving a final volume of about 3 c.c. (dark brown liquid). Then 40 c.c. water was stirred in yielding an oily precipitate which in the course of a fortnight slowly changed into a colourless group of crystals, which after recrystallization from water was found to be a mixture of α - and β -Geigerin.

Attempts to prepare acid amides of Geigerin and the monocarboxylic-dilactonic acid (M.P. 280°).

Hansen (1931) describes the preparation of Alantolic acid amide from the bitter principle Alanto-lactone by means of cold saturated alcoholic-ammonia solutions. Similar attempts to obtain the acid amide of the lactone, Geigerin, proved negative as the following experiments will illustrate:—

- (1) To 10 mgm. of Geigerin 1 c.c. of cold saturated methyl-alcoholic ammonia was added and the solution allowed to stand for one week at room temperature. Unchanged Geigerin of M.P. 189-190° was recovered (compare Abderhalden, 1936) where Alanto-acetamide crystallised out after standing for 2 hours).
- (2) To 10 mg. of Geigerin 1 c.c. of cold saturated ethyl-alcoholic ammonia was added and the solution, well stoppered, allowed to stand for 7 days. Unchanged α -Geigerin of M.P. 190°C was then quantitatively recovered.

(These experiments will be repeated with Geigeric acid).

- (3) To 10 mg. of the monocarboxylic-dilactonic acid (see p. 398) 1 c.c. of cold saturated methyl-alcoholic ammonia was added and the solution allowed to stand for one week. Unchanged acid of M.P. 280°C was again isolated.
- (4) A similar experiment as in (3) with ethyl-alcoholic ammonia gave the same negative results.

SUMMARY.

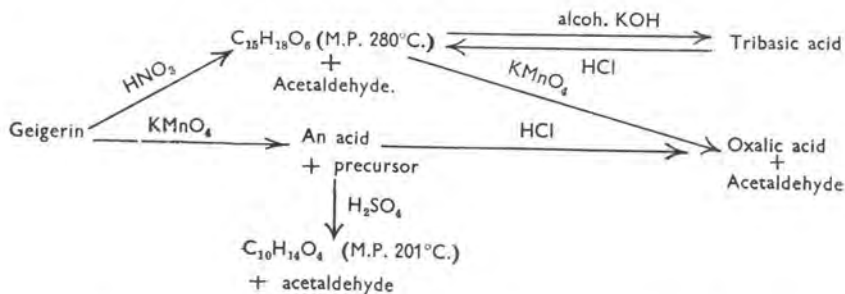
1. Based on the results already obtained on the constitution of the bitter principle Geigerin (*Geigeria aspera*, Harv.) a preliminary discussion as to its structure is put forward in this paper. A more detailed discussion as to the constitutions of Geigerin and the active principle Vermeeriac acid is reserved for a later date.

2. Nitric acid oxidation of Geigerin yields an acid $C_{15}H_{18}O_6 \cdot H_2O$ together with acetaldehyde.

3. The $C_{15}H_{18}O_6 \cdot H_2O$ (M.P. 280°C) acid is a mono-carboxylic-dilactonic acid, thus the functions of all six oxygen atoms are known. On saponification with alcoholic potassium hydroxide a tribasic acid results which may again be relactonized to the dilactonic-monobasic acid. It has $[\alpha]_D^{25} = +181.85^\circ$, is very stable but can be oxidised with alkaline potassium permanganate to oxalic acid.

4. Oxalic acid is also a direct oxidation product of Geigerin with potassium permanganate. It is interesting to note that quite a number of bitter principles e.g. Alanto-lactone, Artemisin and Pikroglobularin (Abderhalden, 1936) also yield oxalic acid when oxidised with potassium permanganate. A nitric acid oxidation of Podophyllotoxin (Abderhalden, 1936, p. 162) also led to oxalic acid.

5. These constitutional results may best be summarised as follows:



6. Basing on the information so far obtained the view is expressed, that the fourth oxygen atom of Geigerin may be constituted in a hydroxyl-group (instead of ether-bound or cyclic) and that Geigerin might belong to the sesquiterpene class of substances.

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