

Monofluoroacetic Acid, the Toxic Principle of "Gifblaar" *Dichapetalum cymosum* (Hook) Engl.

By J. S. C. MARAIS, Section of Pharmacology and Toxicology,
Onderstepoort.

In a recent article the author reported the isolation of the toxic principle of "Gifblaar", *Dichapetalum cymosum* (*Chailettia cymosa*). The potassium salt of the toxic principle was designated "potassium cymonate". Due to lack of plant material at that time, the determination of the chemical nature of the substance had to stand over until more material became available.

It has now been established that the toxic substance contains, except for the elements C, H and O, also the unexpected element fluorine. From the analysis of the potassium and calcium salts as well as of the p-nitrobenzyl and p-bromophenacyl esters, the formula $C_2H_3FO_2$ has been arrived at. Furthermore, the treatment of "potassium cymonate" with strong alkali yields hydroxy-acetic acid, thus establishing the identity of "potassium cymonate" as the potassium salt of monofluoroacetic acid.

EXPERIMENTAL.

The general method described previously (Marais, 1943) has been used for the preparation and purification of the potassium salt of the toxic substance. In the previous article it was stated that the lower boiling fractions of the ether extract contained comparatively large amounts of formic and acetic acids, whilst the higher fractions still contained some higher boiling unidentified acids. During the present investigation crystalline material was obtained from the fractions above $170^\circ C.$, when distilled under diminished pressure of 2 mm. and with the temperature rising to $185^\circ C.$ This crystalline material was rapidly washed with ether to free it from oily material and dried in the air. On refluxing with chloroform a part thereof remained insoluble, whilst from the concentrated chloroform solution, on cooling, crystals separated.

The chloroform insoluble material was crystallized from abs. alcohol. After purification, the crystals melted at $185^\circ C.$ (corr.). On investigation this substance proved to be identical with succinic acid. A mixed melting point with Merck's *pro analysi* succinic acid gave no depression of the melting point.

THE TOXIC PRINCIPLE OF "GIFBLAAR", *DICHAPETALUM CYMOSUM*.

The chloroform soluble substance after several recrystallizations from chloroform, melts at 120° C. (corr.). From analysis this substance was found to be succinic acid anhydride. A mixed melting point with prepared succinic acid anhydride gave no depression of the melting point. The succinic acid anhydride, as such, is probably not present in the plant, but has been derived from the succinic acid during the process of distillation.

The potassium salt of the toxic substance when crystallized from 96 per cent. alcohol separates in large irregular plates, but when crystallized from 96 per cent. alcohol-acetone mixture, it crystallizes in needles. After five recrystallizations from 96 per cent. alcohol, it melts at 212°-213° C. (corr.) with decomposition and a colour change from yellow to red, as seen under the microscope of the Kofler micro melting point apparatus.

Samples of the potassium salt for analysis were dried in an air oven at 110° C. The following results were obtained:—

	% C	% H	% K	% F
Found	20.49	2.12	33.80	15.79
Calculated for $\text{CH}_2\text{F.COOK}$...	20.68	1.74	33.67	16.36

PREPARATION OF THE CALCIUM SALT.

0.5 gm. of the potassium salt was dissolved in 10 c.c. N. H_2SO_4 . This was repeatedly shaken out with ether and the ether shakings neutralized by shaking with a saturated solution of $\text{Ca}(\text{OH})_2$, using phenolphthalein as an indicator. The calcium salt solution was evaporated to dryness on a water-bath. The dried salt was dissolved in 96 per cent. alcohol, the alcohol solution concentrated and acetone added until a slight turbidity formed. On standing, thin plate-like crystals separated. These were again recrystallized from 96 per cent. alcohol and acetone, and dried at 110° C. Melting point above 350° C.

	% C	% H	% Ca	% F
Found	23.91	3.05	20.52	16.10
Calculated for $(\text{CH}_2\text{F.COO})_2\text{Ca}$	24.74	2.08	20.64	19.57

PREPARATION OF THE P-NITROBENZYL ESTER.

The p-nitrobenzylbromide ($\text{O}_2\text{N.C}_6\text{H}_4.\text{CH}_2\text{Br}$) reagent was prepared according to the method of Brewster (1918), by the bromination of p-nitrotoluene. The p-nitrobenzyl ester was prepared by using the method of Reid (1917). 0.5 gm. of the potassium salt was dissolved in 5 c.c. water and 0.9 gm. p-nitrobenzylbromide, dissolved in 10 c.c. 96 per cent. alcohol, added. The mixture was refluxed for 2 hours. On cooling in an ice chest small leaflets separated. This was recrystallized several times from 64 per cent. alcohol. Samples for analysis were dried in high *vacuo* over H_2SO_4 . Melting point 73-74° C. (corr.).

Analysis:	% C	% H	% N	% F
Found	50.74	3.81	6.65	8.35
Calculated for $\text{C}_9\text{H}_8\text{NO}_4\text{F}$	50.71	3.78	6.57	8.91
Molecular weight: found		202.5		
Calculated		213.2		

PREPARATION OF THE P-BROMOPHENACYL ESTER.

The p-bromophenacylbromide ($\text{Br.C}_6\text{H}_4\text{CO.CH}_2\text{Br}$) reagent was prepared according to the method of Judefind and Reid (1920). For the preparation of the p-bromophenacyl ester 1.0 gm. of the potassium salt was dissolved in 5 c.c. water and 2.0 gm. p-bromophenacylbromide dissolved in 10 c.c. 96 per cent. alcohol was added. The mixture was refluxed for 2 hours. On cooling, leaflets separated. The mixture was left standing overnight in an ice chest. The crystalline material was separated and recrystallized four times from 96 per cent. alcohol. Samples for analysis were dried *in vacuo* over H_2SO_4 . Melting point 96°C . (corr.).

Analysis:	% C	% H	% Br	% F
Found	42.99	2.96	29.04	6.45
Calculated for $\text{C}_{10}\text{H}_8\text{O}_3\text{BrF}$	43.66	2.93	29.05	6.91
Molecular weight: found		244.5		
Calculated		275.1		

ON THE FORMATION OF GLYCOLLIC ACID ($\text{CH}_2\text{OH.COOH}$).

2.0 gm. of the potassium salt of the toxic substance was refluxed for 5 hours with 40 c.c. of a 30 per cent. KOH solution. After cooling, the solution was neutralized with 3N. H_2SO_4 , using phenolphthalein as an indicator. The solution was evaporated to dryness. The dried and powdered residue was repeatedly extracted with 96 per cent. alcohol. The insoluble residue consisted of K_2SO_4 and a small amount of KF. The 96 per cent. alcohol solution was evaporated to dryness. The residue was again taken up in 96 per cent. alcohol. The alcoholic solution was concentrated until crystallization took place. The separated crystalline potassium salt was repeatedly crystallized from 96 per cent. alcohol. After drying on filter paper at room temperature, the crystals melt at $110\text{--}115^\circ\text{C}$., probably with the loss of a molecule of H_2O . After drying for several days in high *vacuo* over H_2SO_4 , the crystals melt at $151\text{--}152^\circ\text{C}$. (corr.) with a slight change occurring at $114\text{--}116^\circ\text{C}$. This potassium salt proved on investigation, to be identical with potassium glycollate. A mixed melting point with prepared potassium glycollate gave no depression of the melting point. After corrections had been made for the percentage C figure, owing to the fact that the ash, after combustion, would consist of K_2CO_3 , the following results were obtained:—

	% C	% H	% K
Found	14.56	3.24	31.99
Calculated for $\text{CH}_2\text{OH.COOK} \cdot \frac{1}{2}\text{H}_2\text{O}$	14.63	3.27	31.75

PREPARATION OF CALCIUM GLYCOLLATE.

Using as starting material the potassium glycollate obtained in the previous experiment, calcium glycollate was prepared. 0.4 gm. of the potassium glycollate was acidified with 10 c.c. N. H_2SO_4 and repeatedly shaken out with ether. The ether solution was neutralized by shaking up with a saturated Ca(OH)_2 solution, using phenolphthalein as an indicator. The calcium salt solution was evaporated to dryness and then recrystallized from water. It crystallized in clusters of fine needles. It was dried at 110°C . Melting point above 350°C .

Analysis:	% C	% H	% Ca
Found	25.07	3.20	21.07
Calculated for $(\text{CH}_2\text{OH.COO})_2\text{Ca}$	25.26	3.18	21.08

PREPARATION OF THE P-NITROBENZYL ESTER OF GLYCOLLIC ACID.

Using the potassium glycolate, derived from the potassium salt of the toxic substance, the p-nitrobenzyl ester of glycollic acid was prepared. 0.5 gm. of the potassium glycolate was dissolved in 5 c.c. water and mixed with 0.5 gm. p-nitrobenzylbromide dissolved in 10 c.c. 96 per cent. alcohol. The mixture was refluxed for 3 hours. After cooling, water was added until slight turbidity showed. On further cooling in an ice chest, crystallization took place. The crystals were separated and recrystallized three times, by dissolving in hot 96 per cent. alcohol and adding water until turbidity occurred. It crystallized in thin plates. After drying in high *vacuo* over H₂SO₄ it melted at 109–110° C. (corr.). A mixed melting point with similarly prepared p-nitrobenzyl ester of glycollic acid, gave no depression of the melting point.

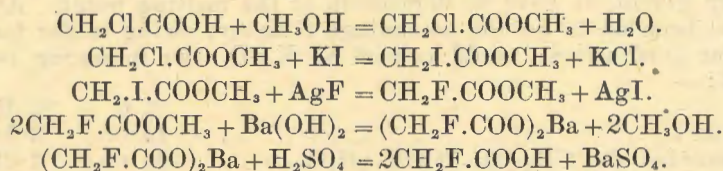
Analysis:	% C	% H	% N
Found	51.15	4.29	6.55
Calculated for C ₉ H ₉ NO ₃	51.19	4.30	6.63
Molecular weight: found	208.7		
Calculated	211.2		

From the above experimental results it could definitely be concluded that the toxic substance of "Gilblaar" is monofluoroacetic acid. Prof. S. Swarts (1896) was the first to synthesize monofluoro-acetic acid. Unfortunately access to the original article could not be obtained. The only other reference obtainable was an abstract. In this publication, however, no mention was made of the toxic properties of the substance. No other references could be obtained that monofluoroacetic acid has ever been isolated from plants.

The synthesis of monofluoroacetic acid was undertaken to compare its derivatives with those of the isolated toxic substance.

SYNTHESIS OF MONOFLUOROACETIC ACID.

The available starting material was monochloroacetic acid. From this the monofluoroacetic acid was prepared according to the following reactions:



THE PREPARATION OF CH₂Cl.COOCH₃.

The methyl ester of monochloroacetic acid is easily prepared by the methylation of monochloroacetic acid (Henry, 1873). 500 gm. monochloroacetic acid was dissolved in 500 gm. methyl alcohol. The mixture was saturated with HCl gas and kept saturated, whilst it was refluxed for two days. The excess methyl alcohol was distilled off on a waterbath. A small amount of ester distilled over, together with the methyl alcohol from which it could conveniently be recovered by shaking up the distillate with water and separating the insoluble methyl ester. The residue in the distillation flask was also washed with water and the methyl ester pooled. It was then dried over CaCl₂ and the methyl ester of monochloroacetic acid rectified by fractionation. The fraction B.P.₆₇₀124–126° C. was collected for further use.

THE PREPARATION OF $\text{CH}_2\text{I.COOCH}_3$.

The methyl ester of monoiodoacetic acid was prepared using the method of Aronstein and Kramps (1881). 200 gm. of the methyl ester of monochloroacetic acid was dissolved in 1,000 c.c. ethyl alcohol and 306 gm. finely powdered potassium iodide added. The mixture was refluxed for 7 hours, filtered and washed with ethyl alcohol. The ethyl alcohol was distilled off on a waterbath. The residue was washed with water by shaking up in a separating funnel. After separation, the ester was dried over CaCl_2 . The methyl ester of monoiodoacetic acid was fractionated and the fraction B.P.₆₆₇160–164° C. collected. The dissolved iodine was removed by shaking up with mercury. After filtering, a clear liquid which had a very disagreeable effect on the eyes, was obtained.

THE PREPARATION OF $\text{CH}_2\text{F.COOCH}_3$.

The methyl ester of monofluoroacetic acid was prepared by Swarts (1896) from the methyl ester of monoiodoacetic acid by refluxing with silverfluoride at 170° C. in a platinum apparatus. A platinum apparatus was not available, so a refluxing apparatus made out of lead was used. 75 gm. of the methyl ester of monoiodoacetic acid was thoroughly mixed with finely powdered 50 gm. of silverfluoride and refluxed in the lead refluxing apparatus for 4 hours. The content was then distilled over. The distillate was fractionated in an ordinary glass distillation flask. The fraction B.P.₆₆₇100–105° C. was collected for further use.

THE PREPARATION OF $(\text{CH}_2\text{F.COO})_2\text{Ba}$.

The saponification of the methyl ester of monofluoroacetic acid was carried out by refluxing with bariumhydroxide. 32 gm. of the methylmonofluoroacetate was dissolved in 160 c.c. water and refluxed with 55 gm. $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, the bariumhydroxide being introduced at the rate of a gram at a time and only after the reaction mixture reacted neutral against phenolphthalein. After the saponification had been completed, the boiling solution was filtered. The bariummonofluoroacetate was precipitated from the filtrate by the addition of 10 volumes of ethyl alcohol. The bariumfluoroacetate was purified by crystallization from water.

THE PREPARATION OF $\text{CH}_2\text{F.COOH}$.

The monofluoroacetic acid was liberated from the barium salt by acidifying with sulphuric acid and distilling the liberated acid. 20 gm. bariummonofluoroacetate was acidified with the equivalent amount of sulphuric acid and rubbed into a paste. The monofluoroacetic acid was distilled off by using a glycerine bath. The distillate was fractionated until the purified crystalline monofluoroacetic acid was obtained.

B.P.₆₆₇ 162–163° C. M.P. 33° C.

THE PREPARATION OF POTASSIUMMONOFLUOROACETATE.

The purified monofluoroacetic acid was neutralized with 2N. KOH using phenolphthalein as an indicator. The neutralized solution was evaporated to dryness on a waterbath. It was purified by several recrystallizations from 96 per cent. alcohol. From this potassiummonofluoroacetate, by means of the methods already described, the p-nitrobenzyl and p-bromophenacyl esters

were prepared. These exhibited the same properties and melting points as those derived from the toxic principle of " Gifblaar ". Furthermore mixed melting points of these substances gave no depression of the melting points, thus establishing the identity of the toxic substance of " Gifblaar " with that of monofluoroacetic acid.

SOME PROPERTIES OF MONOFLUOROACETIC ACID.

Monofluoroacetic acid slowly volatilizes in the air. It is only slightly volatile with steam and volatilizes in approximately the proportion 1:15,000

The following general qualitative tests for acetic acid are also positive for monofluoroacetic acid:—

- (1) Silver nitrate, when added to a strong neutral solution of monofluoroacetate produces a white crystalline precipitate, which is not reduced on boiling, but dissolves and recrystallizes on cooling. The silver salt, however, rapidly darkens on exposure to air.
- (2) Ferric chloride gives in neutral solution a deep red colouration, which is destroyed on addition of HCl. On boiling a brown precipitate is produced.
- (3) The lanthan nitrate and iodine spot test is also given by monofluoroacetic acid.

Monofluoroacetic acid can, however, easily be distinguished from acetic acid by:—

1. The indigo test reaction for acetone, with o-nitrobenzaldehyde reagent, which is naturally not given by monofluoroacetic acid.
2. The cacodyl oxide reaction. On mixing the dry monofluoroacetate with arsenious oxide and heating in a test tube irritating fumes are given off, which can, however, easily be distinguished from the nauseous odour of cacodyl oxide.

THE FLUORINE CONTENT OF " GIFBLAAR ".

Up to the present it has only been possible to estimate the toxicity of " Gifblaar " by means of biological dosing experiments. With the present knowledge of the nature of the toxic substance it will be possible to estimate the fluorine content of the plant and on the assumption that all the fluorine is present in the form of monofluoroacetic acid an approximate estimation of the plants toxicity can be carried out conveniently. Analysis of a sample of the dried and finely ground plant material collected in early spring, gave a fluorine content of 0.015 per cent. calculated on the basis of the dried plant. Since it has been found that 0.5-0.75 mgm. potassium monofluoroacetate per Kilogram bodyweight of the rabbit causes death; it follows, therefore, that from calculations 0.5-0.8 gm. of the dried plant material per Kilogram bodyweight should cause death. Dosing experiments showed that the lethal dose for this sample of plant material lies between 1-1.5 gm. per Kilogram bodyweight for the rabbit.

Since monofluoroacetic acid exhibits such toxic properties it would be of great interest to undertake the further study of other fluorine substitution products of acetic acid as well as of the innumerable other simple fluorine

substitution products of organic compounds. It seems quite possible that such research would lead to the discovery of valuable products for use as ordinary poisons and perhaps as insecticides.

SUMMARY.

The identity of the toxic substance of "Gifblaar", *Dichapetalum cymosum*, has been established to be monofluoroacetic acid.

The potassium and calcium salts as well as the p-nitrobenzyl and p-bromophenacyl esters of monofluoroacetic acid have been described.

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