Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 17, Numbers 1 and 2, July and October, 1941.

Printed in the Union of South Africa by the Government Printer, Pretoria.

South African Senecio Alkaloids. Part 6.—The Toxic Alkaloids of *Senecio sceleratus* sp. nov. Schweikerdt.

By H. L. DE WAAL, Section of Pharmacology and Toxicology, Onderstepoort, and T. P. PRETORIUS *, of the University College, Potchefstroom.

Senecio sceleratus is a new species first described by Schweickerdt (see Steyn and van der Walt, 1941) and occurs abundantly in the Eastern parts of Pietersburg District, Northern Transvaal. Toxicological experiments carried out at Onderstepoort, in which the plant was dosed *per os* to sheep, proved that *S. sceleratus* was extremely toxic. It may perhaps be considered our most active *Senecio* species for the production of typical " seneciosis " (liver-cirrhosis). The general appearance and growth of *S. sceleratus* closely resemble several members of the Paucifolii group in this respect, e.g. *S. retrorsus* D.C. and *S. latifolius* D.C. The plants generally grow about two feet in height (Pietersburg District) and have yellow flowers and alternate leaves (Fig. 1).

Alkaloidal Yield.

The plants used in this investigation were collected by Mr. P. D. Lance on his farm " The Downs ", Pietersburg, in the flowering stage in December, 1940, and sent to Onderstepoort. The chemical investigations began in January, 1941, and was continued during the months that followed. Three alkaloids have so far been isolated from this plant, viz., isatidine, retrorsine and a new alkaloid $C_{18}H_{27}O_7N$, m.p. 178° C., for which the name sceleratine is proposed. Mention of these alkaloids was made in the " Tydskrif vir Wetenskap en Kuns " (de Waal, 1941). *S. sceleratus* was found to contain about 0·17 per cent. of retrorsine, 0·11 per cent. of sceleratine and 0·05 per cent. of isatidine. Six months later is was found that the yield of sceleratine had considerably decreased in the same plants and that large amounts of its acid fission product could be extracted with ether from the alcoholic extract of the plant. It appeared that sceleratine had undergone a fairly rapid hydrolysis or decomposition in the dried and stored original plant material. The above yields were all calculated on the dried and ground plant material.

* Thesis presented in partial fulfilment of the requirements for the M.Sc. degree in the University of South Africa.

Method of Isolation.

The extraction of *S. sceleratus* with 96 per cent. ethyl alcohol in a large extraction apparatus was similar to the method already described by one of us (de Waal, 1939, 1941). The aqueous extract, after the alcohol had been removed, was rendered acid by the addition of citric acid. From the acid filtrate sceleranecic acid (see page 187) was extracted with ether. The purified acid filtrate was then basified with ammonium-hydroxide and thoroughly shaken with successive small volumes of chloroform. The first two chloroform solutions were combined and from this mostly retrorsine was isolated. The solutions of the successive further four shakings with chloroform were then again combined and from this mostly sceleratine and a little admixed retrorsine were isolated. Sceleratine could readily be separated from retrorsine by the conversion of the alkaloid mixture into its nitrate. The mixture of nitrates was dissolved in ethanol and from this sceleratine nitrate separates rapidly and quantitatively, being difficultly soluble in ethanol. Any admixed retrorsine nitrate could readily be decanted with ether from the heavier sceleratine nitrate solid deposit. More retrorsine nitrate could be recovered from the ethanol mother liquor upon the addition of a large volume of ether. Both nitrates are readily converted into their free alkaloids upon solution separately in a small volume of water and the addition of 10 per cent. ammonium hydroxide (retrorsine separates out whereas sceleratine remains in solution). More retrorsine may be shaken out with chloroform. Sceleratine is readily removed from the ammoniacal solution upon thorough shaking with ether.

It sometimes happened that the sixth or seventh chloroform shaking (above) dissolved a small quantity of isatidine. However this alkaloid generally remained in the ammoniacal solutions after retrorsine and sceleratine had both been removed with chloroform. It is then isolated from this solution either (*a*) according to the method described by one of us (de Waal, 1939) upon the evaporation of the mother liquors at room temperature or (*b*) by the treatment of the mother liquors with phosphotungstic acid and the decomposition of the dried precipitate with sodium carbonate. The yield in both instances is not quantitative but a better estimate of the yield of isatidine can be obtained with the first method.

Sceleratine.

Sceleratine is best obtained in a pure state over its nitrate. The free alkaloid can then be recrystallized either from water in which it is fairly soluble or from ethanol in which it is readily soluble. From water the alkaloid crystallizes in colourless prismatic columns (see Fig. 2) with one molecule of water of crystallization. From ethanol it crystallizes similarly but with apparently three molecules of ethanol of crystallization. The alkaloid readily loses some of its alcohol of crystallization when dried at room temperature and becomes brittle and opaque. The last alcohol of crystallization or the one molecule of water of crystallization can be removed when dried at 100° C. over P_2O_5 in high vacuum. Like isatidine but contrary to the rest of the large number of Senecio alkaloids sceleratine is fairly soluble in water and can be recrystallized from this solvent. It is dextro-rotatory, bitter and does not give a precipitate with Mayer's reagent. A very interesting phenomenon of the alkaloid is its extreme solubility in organic solvents including ether and acetone. The alkaloid can even be separated from admixed retrorsine by digesting it with ether which dissolves sceleratine and leaves retrorsine undissolved.

Sceleraneeic Acid.

Sceleratine upon hydrolysis with either alcoholic potassium-hydroxide or with barium-hydrate, yields the well-known necine base, retronecine and a new dilactonic acid, sceleranecic acid, $C_{10}H_{14}O_5$. The equation of hydrolysis is as follows:—

$$C_{18}H_{27}O_7N + H_2O = C_8H_{13}O_2N + (C_{10}H_{16}O_6)$$

sceleratine — retronecine — $\downarrow -H_2O$

$C_{10}H_{14}O_5$

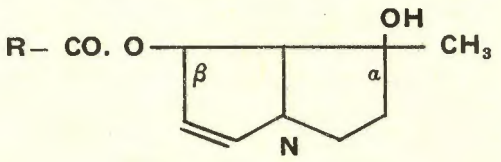
sceleranecic dilactone.

Sceleratine being unsaturated can be hydrogenated in the presence of platinum-dioxide as catalyst but the β-hydroxyl of retronecine thereby (N – HCl medium) readily hydrolyses (see formula for retronecine below) and the products of hydrogenolysis are retronecanol and unchanged sceleranecic dilactone.

$$C_{18}H_{27}O_7N + H_2 = C_8H_{13}ON + C_{10}H_{14}O_5 + H_2O.$$

sceleratine — retronecanol — sceleranecic acid.

Sceleranecic dilactonic acid is a saturated acid and crystallizes from water in hexagonal plates with a clear melting point at 156° C. (corr.). It contains one lactonic grouping which is very stable and it is assumed that sceleranecic acid is mono-esterified with retronecine in the alkaloid sceleratine thus:—



(R = sceleranecic acid.)

(Formula for retronecine by Adams and Rogers, 1941.)

Upon hydrolysis or hydrogenolysis of the alkaloid the free carboxyl group of sceleranecic acid is extremely readily converted into a lactone grouping (probably with a γ-hydroxyl).

Sceleranecic acid thus obtained as a dilactone does not taste acid but slightly bitter, it does not react with a cold 2½ per cent. sodium carbonate solution and does not decolourize a solution of potassium permanganate. Catalytic hydrogenation leads to the recovery of unchanged acid. The dilactone is very sparingly soluble in water but readily dissolves in organic solvents. The lactone group formed during hydrolysis behaves somewhat like a pseudo-acid group. It can be very slowly titrated with decinormal NaOH in the cold (phenolphthalein) either in watery solution (or suspension) or in alcoholic solution. The titration is stopped when one carboxyl group has been titrated (15 to 20 minutes—see experimental part). The stable lactone group can only be saponified upon boiling the acid for several minutes with an excess of decinormal NaOH or with alcoholic potash.

Sceleranecic acid appears to be present in the plant in fairly large quantities and existing as a mixture of two forms. The one form is that described above obtained upon the hydrolysis of the alkaloid. The other form is still less soluble

in water (practically insoluble) and crystallizes from dilute alcohol in thin hexagonal flakes with a m.p. of 213° C. It behaves exactly similar upon titration to the sceleranecic dilactone with m.p. 156° C.

Experimental Part.

Approximately 10 Kgm. of dried and ground *S. sceleratus* were extracted in a large extractor with 96 per cent. alcohol. The alcoholic extract was freed as far as possible of the solvent by evaporation on the steam bath under reduced pressure. Water was added and the remainder of the alcohol distilled off. The mixture was then acidified with citric acid, more water added, thoroughly stirred and allowed to stand 2-3 days.

The aqueous filtrate was then exhausted with ether and sceleranecic acid was isolated from the ether extract, purification being effected by recrystallization from (1) either water (low melting-point form), or (2) dilute alcohol (high melting-point form) and decolourization with charcoal.

The aqueous solution was then basified with 10 per cent. ammonium hydroxide and in some cases again exhausted with ether. This removed most of the sceleratine. In most of the cases, however, the alkaline solution was immediately exhausted with chloroform. The first two chloroform shakings were combined, evaporated and the residue again dissolved in citric acid solution. Further purification was effected by exhausting the solution with ether, basifying with ammonia and again extracting with ether or chloroform. Upon the evaporation of the solvent in front of a fan at room temperature the alkaloid(s) crystallized.

The crystalline residue was recrystallized from a small volume of ethanol or converted into the nitrate which was much more effective for the separation of retrorsine from sceleratine. The residue was therefore dissolved in decinormal nitric acid, the solution filtered and evaporated at room temperature. The crystalline residue was dissolved in hot ethanol from which sceleratine nitrate readily crystallized. Upon the addition of ether to the mother liquor retrorsine nitrate crystallized in fine thin needles. Both nitrates were then separately dissolved in a small volume of water, the solutions basified with 10 per cent. NH_4OH and shaken with chloroform. Upon the evaporation of the solvent retrorsine was obtained which could be purified by recrystallization from ethyl-acetate and sceleratine by recrystallization from either ethanol or water.

The rest of the chloroform shakings (four to five in succession) were combined and upon evaporation of the solvent the alkaloid(s) rapidly crystallized. This fraction consisted mainly of sceleratine which could be separated from any admixed retrorsine by solution in cold ether. Upon the evaporation of the ether the sceleratine was obtained and purified by recrystallization from either water or ethanol.

The ammoniacal liquors after the exhaustion with chloroform was (1) either allowed to evaporate in front of a fan at room temperature or (2) acidified and treated with phosphotungstic acid solution. In the first case the crystalline residue was washed with cold water to remove the inorganic salts leaving isatidine behind. In the second case the phosphotungstic precipitate was filtered, dried and ground with sodium carbonate. Isatidine was extracted from the reaction mixture with ethanol.

The above alkaloids, viz., retrorsine and isatidine are well-known *Senecio* alkaloids and were identified by comparison of their chemical properties as well as those of their fission products with the properties of authentic specimens of retrorsine and isatidine isolated from *S. retrorsus* and *S. isatideus* respectively.

Chemical Properies of Sceleratine.

Sceleratine is a new *Senecio* alkaloid. It crystallizes from water with one molecule of water of crystallization in colourless prismatic columns. The pure alkaloid melts at 178° C. to a clear yellowish liquid. It gives precipitates with the usual alkaloidal reagents except Mayer's reagent with which it gives no precipitate.

Note.—A very interesting phenomenon observed with some of the *Senecio* alkaloids investigated here is that those which are positive with Mayer's reagent, melt with strong brown-red decomposition and are extremely toxic *; e.g. retrorsine, senecionine and seneciphylline. Again the *Senecio* alkaloids that are negative with Mayer's reagent melt with less decomposition to a yellowish liquid and are far less toxic; e.g. isatidine (yellow melt at 138°), sceleratine (yellow melt at 178°) and rosmarinine (colourless melt at 209°).

Solubility.—The alkaloid is soluble in water and readily soluble in cold methanol, ethanol, ether, acetone, chloroform, ethyl-acetate, dioxane and acetic acid.

Specific Rotation.—(Dried at 100° C. over P_2O_5 in high vac.) weight = 101·8 mgm., vol. = 10·0 ml. ethanol, 1 dm. tube.

$\theta = +0{\cdot}55°$.

$$[\alpha]_D^{21} = \frac{0{\cdot}55 \times 10 \times 1000}{1 \times 101{\cdot}8}$$

$= +54{\cdot}0°$.

Micro-analysis.†—(Dried at 100° C. over P_2O_5 in high vac.)

(1) 3·537 mgm.: 7·524 mgm. CO_2 and 2·293 mgm. H_2O.

(2) 3·419 mgm.: 7·289 mgm. CO_2 and 2·148 mgm. H_2O.

2·953 mgm.: 0·150 ml. N_2 at 23° C. and 626·5 mm. Hg.

found—

(i): C = 58·02 per cent.; H = 7·25 per cent.

(ii): C = 58·14 per cent.; H = 7·03 per cent.; N = 4·8 per cent.

calculated $C_{18}H_{27}O_7N$: C = 58·54 per cent.; H = 7·32 per cent.; N = 3·8 per cent.

Since other investigators have also reported the difficulty of burning the *Senecio* alkaloids the value for carbon must be considered very reasonable. The nitrogen values found are usually too high.

∴ Formula is $C_{18}H_{27}O_7N$.

Determination of Water of Crystallization.

Sceleratine crystallized from water and dried at room temperature under atmospheric pressure was dried to constant weight at 100° C. over P_2O_5 in high vacuum.

Loss of weight 5 mgm. H_2O on 111 mgm. substance.

∴ Loss of weight per molecule = 17·4 gm. H_2O.

Theory for 1 H_2O = 18·0 gm. H_2O.

∴ $C_{18}H_{27}O_7N \cdot H_2O$.

* Details of toxicological experiments will be published later.

† All micro-analyses by Dr. O. G. Backeberg, of the Witwatersrand University, to whom we wish to express our thanks.

Sceleratine Nitrate.

This compound was prepared by dissolving sceleratine in the calculated quantity of decinormal nitric acid and allowing the solvent to evaporate in front of a fan at room temperature. The crystalline residue was crystallized from hot ethanol in which the nitrate is sparingly soluble. It crystallizes in large colourless transparent columns and completely decomposes at 250-255° C.

Micro-analysis.

(i) 3·598 mgm. : 6·623 mgm. CO_2 and 2·151 mgm. H_2O.
(ii) 3·528 mgm. : 6·468 mgm. CO_2 and 2·035 mgm. H_2O.
3·653 mgm. : 0·238 ml. N_2 at 24° C. and 621 mm. Hg.
found (i) : C = 50·20 per cent.; H = 6·69 per cent.
(ii) : C = 50·00 per cent.; H = 6·46 per cent.; N = 6·12 per cent.
Calculated $C_{18}H_{27}N_7ON.HNO_3$: C = 50·00 per cent.; H = 6·48 per cent.; N = 6·48 per cent.

Specific Rotation.—$[\alpha]_D^{24} = -10·0°$ (conc. 1·3 per cent. in H_2O).

Sceleratine-hydrochloride.

Sceleratine was dissolved in the calculated quantity of N-HCl, the solvent evaporated in front of a fan at room temperature and the crystalline residue crystallized from methanol. The colourless crystals melt with decomposition at 290° C.

Micro-analysis.

3·699 mgm. : 7·217 mgm. CO_2 and 2·222 mgm. H_2O.
found : C = 53·21 per cent.; H = 6·72 per cent.
Calc. $C_{18}H_{27}O_7N \cdot HCL$: C = 53·24 per cent.; H = 6·95 per cent.

Sceleratine-aurichloride.

Sceleratine-aurichloride was prepared by the addition of a 5 per cent. aqueous solution of gold trichloride to a solution of the alkaloid in normal hydrochloric acid. Crystals separated which were recrystallized from 2-Normal hydrochloric acid and ethanol. The aurichloride (golden yellow prisms) decomposed at 184-6° C.

Sceleratine-methiodide.

400 mgm. Alkaloid was dissolved in 6 ml. chloroform and a few drops of methanol and methyliodide was then added in excess to this solution. The mixture was warmed and on cooling a yellow oil separated on top. The chloroform was removed in front of a fan at room temperature and the resultant syrupy oil treated with acetone. Upon the careful addition of ether to the acetone solution sceleratine-methiodide crystallized. It is soluble in water, acetone, methanol and ethanol and melts with decomposition at 254° C.

Micro-analysis.

3·601 mgm. : 5·918 mgm. CO_2 and 1·848 mgm. H_2O.
found : C = 44·82 per cent.; H = 5·74 per cent.
Calc. $C_{18}H_{27}O_7N.CH_3J$: C = 44·62 per cent.; H = 5·87 per cent.

Sceleratine Picrate

To a solution of 200 mgm. sceleratine in 5 ml. ethanol was added a solution of 130 mgm. picric acid in 5 ml. ethanol. The picrate separated and was recrystallized from ethanol. It melts with decomposition at 216° C.

Constants of Isatidine, Retrorsine and Sceleratine.

	Isatidine.	Retrorsine.	Sceleratine.
m.p. °C. *	138 (145 dec.)	215–6	178
Spec. Rot.	− 24·1° (ethanol)	− 19·2° (ethanol)	+ 54·0° (ethanol)
m.p. °C. (nitrate)	130	110 (145 dec.)	250–5
Spec. Rot. (nitrate)	− 23·0 (H_2O)	− 36·1 (H_2O)	− 10·0° (H_2O)
m.p. °C. (picrate)	170	197	216
m.p. °C. (methiodide)	—	266	254
m.p. °C. (perchlorate)	205–210	250	—
m.p. °C. (aurichloride)	—	185	184–6

* All melting-points are corrected.

Hydrogenolysis of Sceleratine.

1·68 gm. Sceleratine was dissolved in 40 ml. normal hydrochloric acid, 50 mgm. of platinum dioxide was added and the solution hydrogenated under continuous mechanical shaking. The consumption of hydrogen began after thirty minutes and after another forty minutes the hydrogenation was completed. The isolation of retronecanol and unchanged sceleranecic acid proved that hydrolysis accompanied the process of hydrogenation. From the reaction mixture sceleranecic acid was directly isolated upon the evaporation of the solvent and exhaustion of the residue with ether.

The filtrate of the reaction mixture was refluxed with an excess of KOH for 15 minutes, the solvent evaporated and the residue extracted with acetone. After the evaporation of the acetone the residual oil was dissolved in ethanol and a solution of picric acid in ethanol added to this solution. Retronecanol picrate crystallized with m.p. 212° (corr.).

Hydrolysis of Sceleratine.

To a solution of 3 gm. of sceleratine in 30 ml. ethanol was added 1 gm. of potassium hydroxide and the mixture was then refluxed for 15 minutes. The solution was then evaporated to dryness on a waterbath and the dry residue extracted with acetone. From the acetone solution *retronecine crystallized* almost quantitatively.

Isolation of Sceleranecic Acid.

The residual residue after the base had been removed was dissolved in a small volume of water and the solution then titrated with sulphuric acid (1 : $4H_2O$) until it was acid to Congo red. Upon the concentration of the filtrate on the waterbath the acid crystallized. More acid was extracted from the dry residue with ether. Sceleranecic acid was obtained in an excellent yield and readily crystallized from water in which it is sparingly soluble. The colourlesss plates melted sharply at 156°. The same fission products were obtained when the alkaloid was hydrolyzed with barium-hydrate.

Solubility and Properties.

The acid dissolves well in organic solvents and is sparingly soluble in cold water. It does not react with sodium carbonate solution and does not decolourize potassium permanganate solution. Sceleranecic acid so obtained has no free carboxyl group but is a dilactone. One lactone group is slowly hydrolyzed when the dilactonic acid is dissolved in water or neutral aqueous alcoholic solution and titrated with decinormal caustic soda solution. The second lactone group is stable and can only be hydrolyzed when the acid is boiled with an excess of decinormal sodium hydroxide or alcoholic potassium hydroxide solution.

Specific Rotation.—Weight = 86·0.

Vol. = 8 ml. H_2O.

$\theta = -0·10°$.

$[\alpha]_D^{24} = -9·3°$.

Micro-analysis.

(i) 3·591 mgm. : 7·415 mgm. CO_2 and 1·987 mgm. H_2O.

(ii) 3·372 mgm. : 6·957 mgm. CO_2 and 1·914 mgm. H_2O.

found (i) : C = 56·32 per cent.; H = 6·19 per cent.

(ii) : C = 56·27 per cent.; H = 6·35 per cent.

Calc. $C_{10}H_{14}O_5$: C = 56·08 per cent.; H = 6·54 per cent.

$\therefore C_{10}H_{14}O_5$.

Titration.

105 mgm. Sceleranecic dilactone dissolved in 10 ml. 90 per cent. aqueous ethanol gradually neutralized 4·85 ml. $\frac{N}{40}$ NaOH after 15 minutes (phenolphthalein). The titration in the cold was then completed.

Theory for 1-COOH = 4·90 ml. $\frac{N}{10}$ NaOH. 5·0 ml. $\frac{N}{40}$ NaOH was then added and the solution refluxed for 5 minutes.

Back titration required 0·2 ml. $\frac{N}{10}$ HCl.

$\therefore$ Difference = 4·80 ml. NaOH.

Theory for 1-COOH = 4·90 ml. NaOH.

$\therefore$ The acid is a *dilactone*.

This acid together with an apparently more stable form of the same dilactone acid were also extracted from the ether solution of the acid filtrate of the plant alcoholic extract. This dilactone acid with a higher melting-point viz. 213° C. behaved exactly like the sceleranecic dilactonic acid above but was still less soluble in water. It was conveniently recrystallized from dilute ethanol.

Summary.

(1) *Senecio sceleratus* sp. nov. Schweickerdt is an extremely toxic *Senecio* species. It contains at least three more or less toxic alkaloids, viz., isatidine, retrorsine and a new alkaloid, *scleratine*.

(2) Scleratine, $C_{18}H_{27}O_7N$, crystallizes with one molecule of water of of crystallization from water and apparently three molecules of ethanol of crystallization from ethyl alcohol. M.P. 178° C, $[\alpha]_{21}^{D} = +54·0°$ (ethanol).

(3) Upon catalytic hydrogenation scleratine undergoes hydrogenolysis at the β-hydroxyl and retronecanol and sceleranecic dilactone acid are isolated.

(4) Hydrolysis of sceleratine leads to the isolation of the well-known necine base, retronecine and a new dilactone necic acid viz. sceleranecic acid.

(5) Sceleranecic acid has the formula $C_{10}H_{14}O_5$, the m.p. 156° C. (also 213° C.) and $[\alpha]_D^{24} = -9{\cdot}3$ (water).

(6) Sceleratine is bitter, fairly soluble in water and readily forms addition compounds with hydrochloric acid, nitric acid, picric acid, perchloric acid, methyl-iodide and aurichloride.

(7) Experments to determine the relative toxicity of the alkaloids mentioned are being carried out.

LITERATURE.

ADAMS, R., AND ROGERS, E. F. (1941). Structure of monocrotaline. VI. The structure of retronecine, platynecine and retronecanol. *J. Am. Chem. Soc.*, Vol. 63, pp. 537-541.

DE WAAL, H. L. (1939). The senecio alkaloids. Part 1. The isolation of isatidine from *S. isatideus*. *Onderst. J.*, Vol. 12, No. 1, pp. 155-153.

DE WAAL, H. L. (1940). The senecio alkaloids. Part 3. Chemical investigations upon the senecio species responsible for "Bread poisoning". The isolation of three alkaloids from *S. ilicifolius* Thunb. and a new alkaloid from *S. rosmarinifolius* Linn. *Ibid.*, Vol. 15, Nos. 1 and 2 pp.

DE WAAL, H. L. (1941). Suid-Afrikaanse senecio alkaloiede (oorsake van dunsiekte by diere en broodvergiftiging by mense). *Tydskr. Wet. Kuns* N.R.Bd. II, pp. 85-89. Bloemfontein.

STEYN, D. G., AND VAN DER WALT, S. J. (1941). Recent investigations into the toxicity of known and unknown poisonous plants in the Union of S.A. XI this journ.

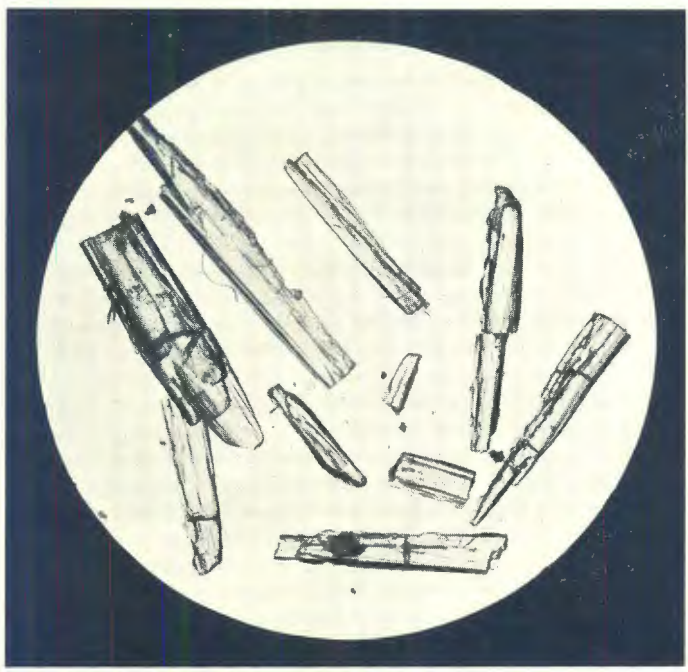


Fig. 2.—$C_{18}H_{27}O_7N$, m.p. 178° C.*×15.

* All melting-points are corrected (Kofler micro-melting point apparatus).

Fig. 1.—*Senecio Sceleratus* sp. nov. Schweick. Nat. Herb. No. 12954.