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The Senecio Alkaloids Part IV. Platyphylline, the Active Principle of *Senecio adnatus*, D.C.

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It is estimated to-day that approximately three hundred species of Senecio occur freely and often grow abundantly in South Africa. It is well known that many of these species, e.g., *S. retrorsus* D.C. and *S. isatideus* D.C. are the cause of acute or chronic seneciosis especially in horses, but also in cattle and sheep. *S. ilicifolius* Thunb. again is the cause of "bread poisoning" in human beings, whilst many other Senecio species are also suspected poisonous plants. Horse-breeding, especially, has suffered very severe losses in the past and many losses are still occurring in the Eastern Cape Province, in the Transkei and in Griqualand East. The following Senecio species, e.g., *S. retrorsus* D.C., *S. isatideus* D.C., *S. graminifolius* Jacq., *S. burleurioides* D.C., *S. pterophorus* D.C. and many others are definitely known to occur in these areas. The five species mentioned above, all contain toxic alkaloids (see de Waal, 1939, 1940 and in Press).

During an inspection tour through the above-mentioned areas in order to study the local Senecio species and the conditions of Senecio poisoning in stock, one of us and Dr. D. G. Steyn collected a few bags of another Senecio variety, viz., *S. adnatus* D.C. It grows in batches along water-streams and in sheltered mountain-valleys in the Griqualand East and Transkeian areas. Specimens of this plant were collected in the flowering stage on a farm, Slangfontein, about 7 miles from Kokstad, in the Mount Currie district. More specimens of the same species were collected at Sugarbush stream, which is about halfway between Mt. Ayliff and Mt. Frere on the main road from Kokstad to Umtata. The plant was identified by the Division of Botany and Plant Industry, Pretoria, as *S. adnatus* D.C.

S. adnatus is a vigorous grower under humid and sheltered conditions. It is a rigid, erect and herbaceous plant with elongate-lanceolate leaves and yellow flowers (see Fig. 1). The plants collected

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for chemical investigation were gathered at Sugarbush stream in November, 1939, in the flowering stage. They were approximately 3 to 5 feet high. Animal losses cannot with certainty be attributed to the eating of this plant, as many other species and even more toxic varieties occur abundantly in these areas.

In this paper the results of the chemical investigations of this plant and of its active principle are recorded. The isolated active principle is an alkaloid which proved to be identical with the alkaloid, platyphylline. Platyphylline was previously isolated from *S. platyphyllus* D.C. (Orechoff, 1935) an indigenous species of the Transcaucasian areas.

S. adnatus D.C. is a typical member of the Paucifolii group (sub-section: Lanigerosi: C. A. Smith, unpublished) but unlike the members of this group so far investigated, it does not contain either retrorsine or isatidine (de Waal, 1939, 1940 and in Press), but the alkaloid platyphylline.

While *S. platyphyllus* contains two alkaloids, platyphylline and seneciphylline, *Senecio adnatus* contains only the former alkaloid. In the light of these investigations *S. adnatus* D.C. must henceforth be considered a poisonous species of the genus Senecio.

Experimental Part.

Seven kilograms of dried and ground *S. adnatus* D.C. were introduced into a large extractor and thoroughly extracted with about 10 gallons of 96 per cent. alcohol. When the extraction was complete the alcohol was distilled off under reduced pressure. To facilitate the removal of the last traces of alcohol a little water was added in the final stages of the distillation. The volume of the watery residue was measured and four equal portions of 1 liter each were decanted and treated separately and differently to ascertain the best procedure for the isolation of the active principle. The residual fraction was then treated according to the method which yielded the best results.

The four methods tried were as follows:—

Method 1.—One litre of the watery extract was acidified with a concentrated solution of citric acid until the solution was distinctly acid to litmus, shaken and allowed to settle for several days. The supernatant was then filtered and the filtrate thoroughly shaken with ether, followed by two small portions of chloroform. Air was then bubbled through the clear tawny acid solution to remove any ether and chloroform which might be present. The acid solution was then alkalinified with a 5 per cent. ammonium hydroxide solution. The alkaline solution was thoroughly and repeatedly shaken with chloroform until no more substance was removed. The chloroform shakings were combined, washed once with water, concentrated on a steam-bath, dried over exsiccated sodium sulphate and allowed to evaporate in front of a fan at room temperature. The last traces of chloroform were removed by the addition of a small volume of methanol. The residue consisted of the alkaloid in crystalline form. Crude yield was 5·6 grams.

Method 2.—This method constituted exactly similar treatment of a second fraction with one exception only, namely, the alkalinification of the citric acid solution with a dilute potassium hydroxide solution.

The crude alkaloidal yield was 5·5 grams.

Method 3.—The watery extract of one litre was acidified with an equal volume of 5 per cent. hydrochloric acid solution, well shaken and allowed to settle. The purification of the acid solution was again effected in the same way as described in the first method. The pure acid solution was made alkaline with a 5 per cent. ammonium hydroxide solution and the alkaline solution extracted with chloroform as in the first method. The extraction with chloroform, however, resulted in the formation of very troublesome emulsions, which could only be overcome by laborious filtration. This also applies to the last method.

The yield was 3·4 grams of alkaloid.

Method 4.—This extraction of the alkaloid was again similar to the process described in the third method except that the hydrochloric acid solution was made alkaline with a dilute potassium hydroxide solution.

Alkaloidal yield was 3·5 grams.

The first method gave the best results, resulting in the quickest and purest isolation of the alkaloid and yielding the highest amount of active principle, namely 5·6 grams. The residual watery extract was therefore treated according to the first method.

A careful examination of the alkaline solutions (see de Waal, 1939) after the extraction with chloroform showed that no alkaloid was present in these alkaline liquors.

The examination of the chloroform solutions resulted in the isolation of one alkaloid only, which was later identified as platyphylline (see below). Calculated on the basis of the best extraction method (method 1) *S. adnatus* D.C. in the flowering stage (see Fig. 1) contains 0·5 per cent. platyphylline in the dried plant material.

Isolation of Platyphylline.

The dry alkaloidal residues, after the evaporation of the chloroform, were dissolved in a small volume of ethanol, the ethanol solution decolourized with a small amount of charcoal and the filtrate allowed to evaporate to dryness. Preliminary experiments showed that the colourless crystalline residue after the evaporation of the ethanol could be crystallized from a hot 20 per cent. alcohol solution. After several recrystallizations from this solvent, the substance melted sharply and without decomposition at 129° C. (corr.)*. The alkaloid crystallized in large rhombic prisms (see Fig. 2).

* All melting-points were recorded with the Kofler micro-melting point apparatus and are therefore corrected.

Micro-analysis†

3·289 mgm.: 7·800 mgm. CO_2 and 2·371 mgm. H_2O.

5·127 mgm.: 0·242 c.c. N_2 at 26° C. and 625 m.m. Hg.

found: C = 64·48 per cent.; H = 8·04 per cent.; N = 4·43 per cent.

Calculated for $C_{18}H_{27}O_5N$:

C 64·08 per cent.; H = 8·06 per cent.; N = 4·15 per cent.

A duplicate analysis confirmed the formula: $C_{18}H_{27}O_5N$.

Fig. 1.—*Senecio adnatus*, D.C.

Chemical Properties of the Alkaloid.

The isolated substance is an alkaloid and gave precipitates with Mayer's, Dragendorf's and Wagner's alkaloidal reagents and also with picric acid and phosphotungstic acid solutions. A solution of the alkaloid in 2½ cent. sodium carbonate solution readily decolourized potassium permanganate solution and the alkaloid also decolourized bromine water.

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

Solubility.

The solubility of the alkaloid in organic solvents was very marked. It readily dissolved in cold acetone, ether, chloroform, ethyl-acetate, methanol, ethanol, benzene, toluene, acetic acid and acetic acid anhydride. It was practically insoluble in cold water but dissolved slowly in boiling water. It readily dissolved in dilute mineral acids and was practically insoluble in alkalies. The alkaloid had a very bitter tase.

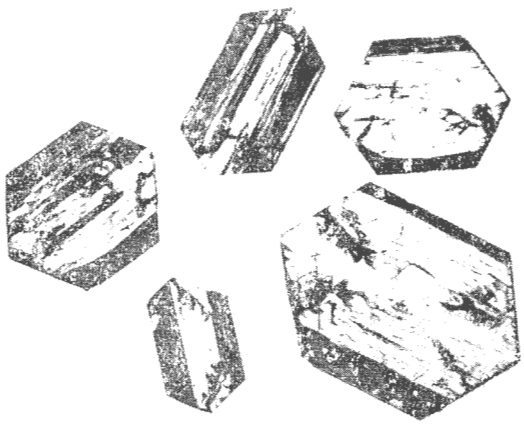

Fig. 2.—Platyhpylline X 10 m.p. 129°.

Specific Rotation.

$[\alpha]_D^{25} = -59{\cdot}0°$ (conc. 4 per cent. in ethanol).

$[\alpha]_D^{25} = -56{\cdot}4°$ (conc. 2 per cent. in chloroform).

Orechoff (1935) recorded for the impure alkaloid, platyphylline, an $[\alpha]^{D} = -45{\cdot}09°$ in chloroform. Later in the same year Orechoff and Konowalowa (1935 corrected the formula from $C_{17}H_{25}O_5N$ for the impure alkaloid to $C_{18}H_{27}O_5N$ for the pure alkaloid but failed to improve the specific rotation.

Picrate Derivative.

A solution of 400 mgm. picric acid in 3 c.c. 96 per cent. alcohol (hot) was added to a solution of 500 mg. of alkaloid in 2 c.c. 96 per cent. alcohol (hot) and the two solutions gently mixed. After a

while the cloudy precipitate settled to the bottom of the tube as a sticky oily syrup. The supernatant was decanted and the precipitate boiled out with about 10 c.c. of ethanol. The bulk of the precipitate became crystalline and remained undissolved. The crystalline residue was then transferred to a boiling flask and refluxed with enough ethanol to effect complete solution. From the filtrate the picrate crystallized in yellow shining plates, which, when pure, melted at 199-200° C. It dissolved in cold water, hot ethanol, chloroform, acetone, ethyl-acetate and was slightly soluble in ether.

Micro-analysis.

3·620 mgm.: 6·832 mgm. CO_2 and 1·700 mgm. H_2O.

2·817 mgm.: 0·306 c.c. N_2 at 24·5 C. and 625 m.m. Hg.

found: C = 51·47 per cent.; H = 5·26 per cent.; N = 9·90 per cent.

Calculated for $C_{18}H_{27}O_5N.C_6H_3N_3O_7$:

C = 50·90 per cent.; H = 5·33 per cent.; N = 9·90 per cent.

A duplicate analysis confirmed the formula of

$$C_{18}H_{27}O_5N.C_6H_3N_3O_7.$$

Perchlorate Derivative of the Alkaloid. (See Orechoff et al, 1935.)

200 mgm. Alkaloid was dissolved in three times the theoretical amount of normal hydrochloric acid. Again 90 mgm. (theoretical amount plus 10 per cent.) sodium perchlorate was dissolved in just enough water to obtain a saturated solution. The two solutions were mixed in the cold and the perchlorate derivative of the alkaloid immediately crystallized out. It was recrystallized from hot water. The m.p. was 244-245° C.

Micro-analysis.

(i) 3·588 mgm.: 6·563 mgm. CO_2 and 2·056 mgm. H_2O.

(ii) 3·659 mgm.: 6·676 mgm. CO_2 and 2·042 mgm. H_2O.

found (i): C = 49·89 per cent.; H = 6·41 per cent.

,, (ii): C = 49·75 per cent.; H = 6·24 per cent.

Calculated for $C_{18}H_{27}O_5N.HClO_4$:

C = 49·31 per cent.; H = 6·44 per cent.

Therefore formula is $C_{18}H_{27}O_5N.HClO_4$.

It is clear from the analysis and properties of the alkaloid, of its picrate and of its perchlorate that it has the formula $C_{18}H_{27}O_5N$ and is identical with the alkaloid platyphylline isolated from *S. platyphyllus* D.C. by Orechoff (1935). This was also established by the isolation of the basic and acidic products of the hydrolysed alkaloid and their analyses and properties. Henceforth our active principle isolated from *S. adnatus* D.C. will be referred to as platyphylline.

Attempts to hydrogenate platyphylline catalytically in the presence of PtO_2 did not lead to a quantitative hydrogen absorption. Apparently a complex arrangement in the molecule impedes this reaction.

Hydrolysis of Platyphylline.

To 7·5 gms. platyphylline dissolved in the minimum quantity of ethanol was added 3·4 gms. solid potassium hydroxide (about 1·3 mol.) and the solution refluxed for one hour. The hydrolysed solution was then evaporated on a boiling waterbath almost to dryness and the residue dried in a vacuum desiccator over concentrated sulphuric acid.

The dry residue was then repeatedly extracted with dry acetone until the acetone gave no precipitate when treated with petroleum-ether.

Isolation of Platynecine.

The acetone solution was evaporated slowly when the base crystallized. The dry crystalline residue was dissolved in ethyl-acetate from which the base crystallized in small colourless plates. After a few recrystallizations from ethyl-acetate pure platynecine was obtained with a constant melting-point of 149° C. A soda-alkaline solution of the base decolourised potassium permanganate solution immediately and the base readily dissolved in water, methanol and ethanol.

Micro-analysis.

(i) 3·390 mgm.: 7·611 mgm. CO_2 and 2·892 mgm. H_2O.
3·836 mgm.: 0·353 c.c. N_2 at 625 m.m. Hg and 20° C.
(ii) 3·320 mgm.: 7·484 mgm. CO_2 and 2·831 mgm. H_2O.
3·354 mgm.: 0·316 c.c. N_2 at 624 m.m. Hg and 23° C.

found—

(i) C = 61·23 per cent.; H = 9·54 per cent.; N = 8·92 per cent.
(ii) C = 61·48 per cent.; H = 9·54 per cent.; N = 8·92 per cent.

Calculated for $C_8H_{15}O_2N$:
C = 61·14 per cent.; H = 9·55 per cent.; N = 8·91 per cent.

Platynecine Picrate.

When the solution of the two components in absolute alcohol was mixed, the solution at first remained clear. The picrate was then precipitated with petroleum-ether and recrystallized with ethanol. The m.p. of the picrate was found to be 189-190° C. (corr.).

Micro-analysis.

Found: C = 43·95 per cent.; H = 4·91 per cent.

Calculated for $C_8H_{15}O_2N.C_6H_3N_3O_7$:
C = 43·53 per cent.; H = 4·70 per cent.

Isolation of Platynecic Acid.

The resultant dry residue after the extraction of the base with acetone (see above) was acidified with conc. HCl (1 : 1) until distinctly acid to Congo-red. The solution which was then concentrated on a boiling waterbath until the crystals were about to separate, was filtered and the precipitate on the filterpaper washed with a few millimetres of boiling water. The moment the water reached the filtrate crystals separated. When the crystallization ceased the crystals were filtered off. On evaporation of the mother liquor some more of the same crystals were obtained. The joint crystalline material was extracted with ethyl-acetate which removed the acid from insoluble potassium salt. The ethyl-acetate was again evaporated and the residual crystalline acid recrystallized from benzene, from which it was obtained in the form of long silky needles (see Fig. 3). The pure acid was found to have the same melting-point as recorded by Orechoff (1935) namely, 154°-155° C.

In alkaline solution it readily decolourized potassium permanganate solution.

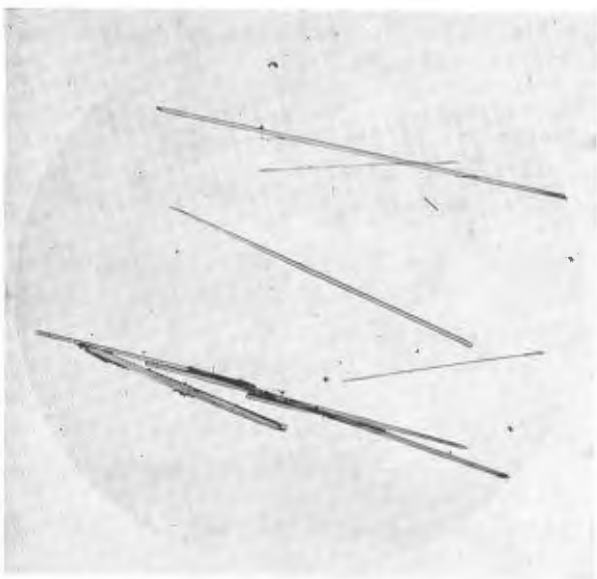

Fig. 3.—Platynecic acid X 14 m.p. 155°.

Micro-analysis.

Found: C = 60·41 per cent.; H = 7·02 per cent.

Calculated for $C_{10}H_{14}O_4$: C = 60·60 per cent.; H = 7·07 per cent.

The products of hydrolysis of platyphylline are therefore platynecine, $C_8H_{15}O_2N$, and platynecic acid $C_{10}H_{14}O_4$. The equation representing the hydrolysis is: $C_{18}H_{27}O_5N + H_2O = C_8H_{15}O_2N + C_{10}H_{14}O_4$.

These findings are in conformity with those of Orechoff (1935), whose method of hydrolysis was, however, different to ours. The $[\alpha]_D^{25}$ of platynecic acid is + 45·0° (Conc. 2 per cent. in ethanol), and not +37·9° as reported by Orechoff on the less pure acid. In a next article to appear in this journal the structural nature of this acid will be discussed. Meanwhile the view is expressed that platynecic acid and senecic acid (Barger and Blackie, 1936) are eventually one and the same acid, a monolactonic, monocarboxylic and acyclic acid (and not a hydroxy acid, Orechoff, 1935).

Summary.

1. In this article are described the isolation of the alkaloid, platyphylline from *Senecio adnatus* D.C. as well as the preparation of some of its derivatives.

2. The alkaloid has the formula $C_{18}H_{27}O_5N$ and its fission products are platynecine, $C_8H_{15}O_2N$ and the monobasic platynecic acid, $C_{10}H_{14}O_4$. These findings are in conformity with those found for platyphylline from *S. platyphyllus* D.C. (Orechoff, 1935).

3. The view is put forward with reserve that platynecic acid is identical with senecic acid and not a hydroxy acid as found by Orechoff, but that the acid is a monolactonic, monobasic and acyclic acid.

4. *Senecio adnatus* D.C. must henceforth be considered toxic to stock as it contains at least 0·5 per cent. (calculated on the dried and ground plant) of the active principle platyphylline.

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