

The Senecio Alkaloids, Part I.

The Isolation of Isatidine from *Senecio Retrorsus* and *Senecio Isatideus*.

By H. L. DE WAAL, Section of Pharmacology and Toxicology,
Onderstepoort.

INTRODUCTION.

ALTHOUGH Grandval and Lajoux (as far back as 1895) were the first authors to report on the isolation of two alkaloids from *Senecio vulgaris*, it was the discovery by Manske (1931) of an alkaloid retrorsine in *S. retrorsus*, which marked the beginning of a new era in the investigation of various alkaloids since then isolated from a large number of species of *Senecio*. The genus *Senecio* comprising over 1,250 different species, is the largest genus of flowering plants belonging to the natural order Compositae. The active ingredients of very many of these species attack the liver and when eaten in small amounts over prolonged periods cause cirrhosis of the liver. Species of *Senecio* are toxic to stock, especially to sheep, cattle and horses.

In Canada the disease is called "pictou", in New Zealand "Winton" disease and in South Africa "Molteno disease" or "dunsiekte". The English generally refer to this disease as "poisonous ragwort" and in Norway cattle die of a disease called "sirasyke", which has the characteristic symptoms of *Senecio* poisoning, attributed to the eating of *S. jacobaea*. The same species is responsible for the disease in Nova Scotia ("pictou") and in New Zealand ("Winton"), whereas *Senecio virellii* is held responsible for the "walking disease" of Northern Nebraska. In South Africa various spp. of *Senecio* are responsible for the Molteno sickness or straining disease in cattle and dunsiekte, stomach staggers or Molteno disease in horses. (See Steyn, D.G., 1934). In addition *Senecio ilicifolius* L., growing extensively as a weed on cultivated wheatlands in the south-western and southern Cape Province, is the cause of bread poisoning in these areas.

S. ISATIDEUS AND S. RETRORSUS.

In this paper only two alkaloids occurring in *S. retrorsus* and *S. isatideus* are briefly mentioned. Manske (1931) reported the isolation of retrorsine from *S. retrorsus* D.C. assigning to it the formula $C_{18}H_{25}O_5N$. This was confirmed by Barger and Blackie (1936). Retrorsine, however, was also found to be present in *S. latifolius* D.C. and *S. glaberrimus*, isolated from the former species by Barger and his associates (1935) and from the latter by Blackie (1937). Besides retrorsine, however, Manske (1931) and Barger and his associates (1935) could not isolate any other alkaloid from *S. retrorsus* contrary to the findings of the writer reported upon below.

Blackie (1937) reported the isolation of a new alkaloid from *S. isatideus* D.C. which he called *isatidine*. He found isatidine in very appreciable quantities (1·14 per cent.) in *S. isatideus* and also isolated retrorsine from this plant; this retrorsine proved to be identical with that isolated from *S. retrorsus*, but was present in the former in smaller quantities (0·15 per cent.). These findings by Blackie have been confirmed in numerous experiments dealing with the isolation of isatidine and retrorsine from *S. isatideus* by the writer, but in addition *isatidine* from *S. retrorsus* has also been isolated; both Manske (1931) and Barger and his associates (1935) have failed to do so. This isolation of isatidine from *S. retrorsus* reveals for the first time the very interesting facts, viz.:—

- (1) that retrorsine appears to occur together with isatidine in *S. retrorsus* D.C.
- (2) that isatidine is always accompanied by retrorsine in *S. isatideus* D.C., and
- (3) that when the same technique of isolation is applied to both *S. retrorsus* and *S. isatideus* then both alkaloids can be isolated from each of these two species.

Both *S. retrorsus* and *S. isatideus* occur widely in South Africa and are well known poisonous plants causing cirrhosis of the liver in sheep, cattle and horses. The writer was confronted with the task of preparing large quantities of isatidine and retrorsine from these species for feeding experiments to sheep and horses, with the object of studying the pharmacology and pathology of this disease by the respective sections of this Institute. The object of this communication is to bring to the notice of chemical research workers in this field the isolation of isatidine from *S. retrorsus* and *S. isatideus* and some of the results obtained on the constitution of isatidine.

ISOLATION OF ISATIDINE.

(a) From *S. isatideus* D.C.

Four five-litre flat-bottomed flasks, each containing 1 Kg. of dried and ground *S. isatideus* D.C. were filled with 95 per cent. alcohol (about 16 litres) and were allowed to digest for three days

under frequent shaking. The contents when filtered (suction) and washed with 96 per cent. alcohol afforded a deep green filtrate of approximately 20 litres. This was allowed to evaporate at first in front of a fan at room temperature and finally all the alcohol was distilled off under reduced pressure on a boiling water-bath. The dark green and tarry watery residue was well stirred with about an equal volume of 3 per cent. hydrochloric acid solution (about 1 litre) and allowed to settle overnight. The solution was then filtered and thoroughly shaken with ether until the ethereal layer was colourless. Air was then drawn through the solution to expel the ether and the dark tawny acid solution alkalified with concentrated ammonium hydrate (1:4). This solution was then thoroughly shaken with successive quantities of chloroform, which removes the retrorsine and the ammoniacal solution then allowed to evaporate in front of a fan at room temperature when isatidine was deposited in large quantities (yield 1.1-1.3 per cent.). About 50 gm. of isatidine was thus obtained which can be very conveniently purified by several recrystallisations from boiling water or absolute alcohol.

(b) *From S. retrorsus D.C.*

The operations for the isolation of isatidine from *S. retrorsus* were exactly similar to those described for the isolation of isatidine from *S. isatideus* D.C. (above) except for two slight variations: (1) The dilute hydrochloric acid solution of the residual tarry and watery extract should stand for at least two days in a refrigerator to allow for a proper settlement of all the tarry material, and (2) the final ammoniacal liquors, after thorough shaking with chloroform, must be evaporated to a very small volume in front of a fan at room temperature when a fair amount of isatidine crystallises out (about 0.3 per cent.).

The Isatidine obtained from *S. retrorsus* D.C. was perfectly identical (after purification) with the isatidine isolated from *S. isatideus* D.C. Their melting-points were the same and the mixed melting-points with each other showed no depressions. The chemical properties, optical activity and identification tests (see below) were identical. Again, on hydrolysis of both alkaloids the two separate acids obtained had the same melting-point, mixed melting-point between each other showed no depressions, the specific rotations and other properties were identical, the acids in each case being one and the same acid viz. isatineic acid.

Isatidine crystallized in rhombic shaped prisms (see Fig. 1). These crystals when heated darkened at 90-100° C. and melted to a clear liquid at 138° which decomposed to a red solution at 145°.*

Crystallographic Data of Isatidine.†

The crystals of Isatidine belong to the orthorhombic system, the axial ratio being $a:b:c = 0.692:1:0.432 (\pm 0.001)$.

* Kofler micromelting point apparatus.

† The crystal measurements were carried out by Professor B. V. Lombaard of the University of Pretoria to whom I express my sincere thanks.

The crystals are tabular to prismatic in habit and show the following crystal forms: brachypinacoid, the unit prism, the macrodome, the brachydome and sometimes a very small development of the base.



Fig. 1.—Isatidine $\times 10$.

The angular relationships of the faces may be summarised as follows:—

- (101) to (110) = $55\frac{1}{2}^{\circ}$.
 (101) to $(\bar{1}01)$ = $64^{\circ}11'$.
 (110) to $(\bar{1}\bar{1}0)$ = $69^{\circ}20'$.
 (011) to $(0\bar{1}1)$ = $46^{\circ}42'$.

CHEMICAL PROPERTIES OF ISATIDINE.

Isatidine dissolves without colour in concentrated H_2SO_4 , dilute and concentrated HCl , concentrated HNO_3 and is insoluble in alkalis.

Isatidine is readily soluble in cold and hot methyl alcohol; it dissolves in hot 60 per cent., 96 per cent. and in absolute alcohol; it is insoluble in acetone, chloroform, petroleum-ether, ether, ethyl-acetate, benzene and toluene. It dissolves with a brown colour in acetic acid and acetic anhydride. Its solubility in cold water is about 2 per cent. ($22^{\circ} C.$) and its solubility in boiling water is about 16.5 per cent., from which it can be crystallized very conveniently, and purified by washing with cold acetone and ether. It crystallizes from water with 2 molecules water of crystallization, which are very easily dispelled in a vacuum exsiccator over P_2O_5 or when

warmed to 100° C. Polymerization and great losses of weight occur when isatidine is dried to constant weight in a vacuum exsiccator over P₂O₅.

Isatidine gives positive alkaloidal reactions with Wagner's reagent, phosphotungstic acid, picric acid, and freshly prepared Dragendorff's reagent.

Isatidine decolourizes potassium permanganate solution instantaneously both in cold soda-alkaline and dilute sulphuric acid mediums; it also decolourizes bromine water, and it is negative towards aldehydic and ketonic reagents (Legal's test, Brady's reagent, m-Phenylendiamine and semicarbazone derivatives).

Micro-analysis:

(a) *Isatidine*, crystallized from water and dried at room temperature and atmospheric pressure.

4.845 mgm.: 9.540 mgm. CO₂; 3.180 mgm. H₂O.

3.387 mgm.: 0.109 c.c. N at 24.5° C. and 766 mm. Hg.

found: C = 53.70%; H = 7.34%; N = 3.72%

Calculated for

C₁₈H₂₃NO₇ · 2H₂O C = 53.59%; H = 7.25%; N = 3.47%.

(b) *Isatidine-picrate*, prepared by mixing 1 gm. Isatidine in 10 c.c. absolute alcohol and 1 gm. picric acid dissolved in 8 c.c. absolute alcohol. Fine yellowish needles soluble in hot water, hot acetone, hot alcohol, hot ethyl-acetate and cold methanol.

4.725 mgm.: 8.430 mgm. CO₂; 2.040 mgm. H₂O.

found: C = 48.67%; H = 4.83%

Calculated for

C₁₈H₂₃NO₇ · C₆H₃N₃O₇ C = 48.32%; H = 4.70%.

Specific rotation:

Isatidine from *S. isatidens*

weight = 200 mgm.

volume = 15 c.c. H₂O

$\theta = -0.22^\circ$

$[\alpha]_D^{22} = -8.25^\circ$

Isatidine from *S. retrorsus*.

weight = 200 mgm.

volume = 15 c.c. H₂O

$\theta = -0.22^\circ$

$[\alpha]_D^{24} = -8.25^\circ$

There was no change in the optical rotation immediately after solution and after three days.

Hydrogenation of Isatidine :

(a) 3 gm. Isatidine dissolved in 120 c.c. water was hydrogenated at 22° C. and 665 mm. pressure in the presence of 100mgm. PTO_2 as catalyst for four hours when the consumption of hydrogen had completely stopped. 820 c.c. Hydrogen utilized reduced to N.T.P. = 644.5 c.c. H_2 . Theoretically *four* double bonds require 640 c.c. H_2 .

(b) Similarly 3 gm. isatidine in 150 c.c. H_2O in the presence of 150 mgm. PTO_2 was hydrogenated for 4 hours. The consumption of hydrogen had then stopped. After another continuation for 4 hours, also in the presence of 50 mgm. fresh PTO_2 catalyst, no more hydrogen was taken up.

Gas absorbed at N.T.P. = 631.4 c.c. H_2 .

Theory for 4 Mols. H_2 = 640.0 c.c. H_2 .

The hydrogenated solutions *still* decolourised soda-alkaline and dilute sulphuric acid potassium permanganate solutions immediately. This phenomenon cannot be explained at the moment. More information may be available when the nature of the hydrogenated substance has been studied.

ISATINECIC ACID.*

After preliminary hydrolysis experiments had been carried out after the methods described by Manske (1931) for retrorsine, Barger and Blackie (1936) for retrorsine and Orechhoff and Konowalowa (1935) for platyphyllin the following procedure was adopted:

To 20 gm. of isatidine dissolved in 200 c.c. of 0.5 N alcoholic potassium hydroxide was added 6 gm. of potassium hydroxide (so that the solution was about normal) and this solution then refluxed for one day. The solution immediately showed a green-yellow fluorescence and after hydrolysis was allowed to stand overnight well stoppered.

The hydrolysed solution was then evaporated on a boiling water-bath to dryness and the residue washed with hot chloroform followed by hot absolute alcohol.

The dry residue was then acidified by an excess of concentrated hydrochloric acid (1:1), the solution filtered from some undissolved potassium chloride and the filtrate allowed to evaporate in front of a fan and finally to dryness on a boiling water-bath.

The dry residue was then refluxed with ethyl-acetate until all the isatinecic acid had been removed (a Bayer's test on the residue will show the absence or presence of isatinecic acid as the latter spontaneously decolourises potassium permanganate solution). The ethyl-acetate solution was dried over calcium chloride, if necessary

* While this article was in the press it was found that the hydrolysis of isatidine may lead to the formation of more than one acid fission product. This will be discussed in the next article of this series in a subsequent publication of this *journal*.

a pinch of charcoal was added, the solution heated to boiling-point and filtered. Petroleum-ether was then added until the solution was distinctly turbid. Isatineic acid then crystallised out immediately in prismatic needles.

The crystallization was complete after 24 hours. The supernatant liquid was decanted, the crystals washed with petroleum ether followed by ether and recrystallized from ethyl-acetate and petroleum-ether. Isatineic acid (see fig. 2) melts to a clear liquid at 178-180° C. (corr.).

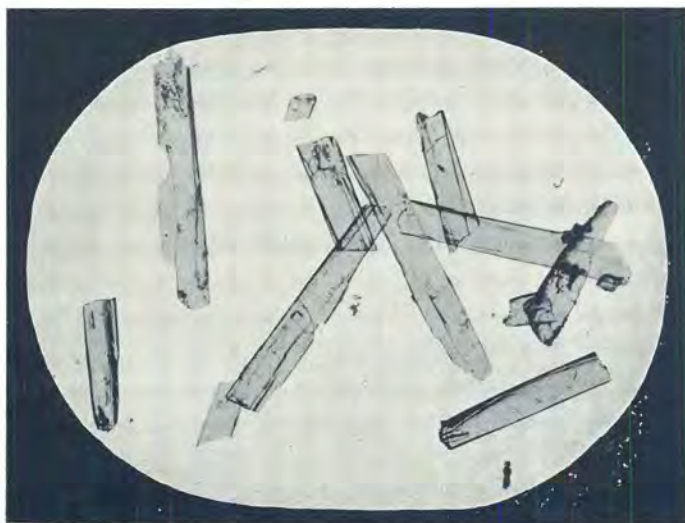


Fig. 2.—Isatineic acid, m.p. 178-180°, $\times 15$.

Micro-analysis for isatineic acid:*

When isatineic acid is dried at room temperature in highvac. over P_2O_5 no loss of weight occurs.

4.872 mgm.: 9.245 mgm. CO_2 ; 3.100 mgm. H_2O .

Found: C = 51.66%; H = 7.11%.

Calculated for: C = 51.72%; H = 6.94%.

$C_{10}H_{16}O_6$.

Isatineic acid dissolves in $2\frac{1}{2}$ per cent. sodium carbonate solution with effervescence, readily dissolves in dilute ammonium-hydroxide and is practically insoluble in concentrated mineral acids.

It readily decolorizes potassium permanganate solution and bromine-water due to the presence of one double band (see hydrogenation experiment below). It is readily soluble in water which may perhaps be due to the presence of hydroxyl groups. It is insoluble in ether, petroleum-ether and soluble in absolute alcohol, methyl-alcohol, acetone, ethyl-acetate and in hot chloroform.

* All micro-analyses by Dr. A. Schoeller, Berlin.

Specific Rotation.

weight = 100 mgm.

volume = 7 c.c. H₂O $\theta = +0.80$

$$\left[\alpha \right]_{\text{D}}^{22} = +56^{\circ}$$

Titration:

100 mgm. of isatineic acid dissolved in 15 c.c. cold water required 8.5 c.c. 0.1 N NaOH.

Now 23.2 mgm. (mol. wgt. = 232) isatineic acid requires 1.0 c.c. of 0.1 N NaOH for one carboxyl-group, therefore 100 mgm. requires

4.3 c.c. for 1-COOH

i.e. 8.6 c.c. for 2-COOH

Found 8.5 c.c.

Therefore Isatineic acid is a dicarboxylic acid.

Hydrogenation:

Using platinum dioxide as catalyst a solution of 2 gm. of isatineic acid was hydrogenated until the hydrogen-level in the gasometer remained absolutely constant. A correction for blank experiments was made and it was found that 280 c.c. of hydrogen had been used up at 24° C. and 663 mm. pressure.

Hydrogen reduced to N.T.P. = 217 c.c.

Theory for *one* double bond = 193 c.c.

Therefore Isatineic acid has *one* double bond.

The hydrogenated solution *did* not decolourize potassium permanganate solution.

OXIDATION OF ISATIDINE TO OXALIC ACID.

To a solution of 3 gm. of isatidine in 50 c.c. of hot water was added a solution of 3 gm. sodium carbonate in 10 c.c. of water. A 2 per cent. potassium permanganate solution was then run in (the flask being fitted with a Normal-Schliff reflux condenser, the top end of which carried a dropping funnel) and during two hours 650 c.c. was completely decolourized, the temperature having been raised to boiling-point (see de Waal, 1938).

The oxidation was then interrupted, the solution filtered and the yellowish filtrate shaken with ether, which left no residue. It was then shaken with chloroform which on evaporation left a crystalline substance to be reported upon in a later publication and which had a crude m.p. of about 110° C. (clear).

The above solution was then acidified with 5 per cent. hydrochloric acid and extracted with pure ether in a bubble-extractor for several days. The extracted residue was positive for acetaldehyde

and deposited oxalic acid crystals (compare the potassium permanganate oxidation of Geigerin, de Waal, 1938). Melting-point, analysis and other identification tests proved that the substance was oxalic acid. The identification test for oxalic acid by Paget and Berger (1938) proved to be not only an excellent test but also very useful to identify non-crystalline material, containing oxalic acid. (Reports on nitric acid oxidations, other potassium permanganate oxidations etc. are reserved for a later publication).

SUMMARY.

1. Isatidine has been isolated not only from *S. isatideus* D.C. but also from *S. retrorsus* D.C., not hitherto observed by other workers.

2. *S. retrorsus* contains as its main alkaloid retrorsine, accompanied by isatidine in lesser quantities. *S. isatideus* contains as its main alkaloid isatidine, accompanied by very small quantities of retrorsine.

3. The chemical properties of isatidine and isatinecic acid are described, as well as the hydrolysis of isatidine to isatinecic acid and the oxidation of isatidine to oxalic acid.

4. The pharmacology, pathology and further structural results with isatidine will be reported in due course.

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