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The Senecio Alkaloids. Part 2: Hydrogenation, Hydrolysis and Structural Results of Isatidine.

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In the first paper of this series (de Waal, 1939) the isolation and chemical properties as well as the results of the preliminary hydrolysis and of the hydrogenation of the alkaloid isatidine were recorded. This alkaloid is the active principle particularly of *Senecio isatideus* but was also found to be present in very much smaller quantities in *S. retrorsus* D.C. It was then found that when isatidine was hydrogenated in the presence of platinum dioxide four molecules of hydrogen were consumed, which at the time could not be explained. Continuous efforts have since led to the repeated and facilitated isolation of the reduced compound in the crystalline form, and this as well as some other structural results on isatidine are now reported upon.

The Nature of the Hydrogenated Substance.

The hydrogenation of isatidine (PtO_2) in half-normal or normal-hydrochloric acid solution leads to the consumption of four molecules of hydrogen for one molecule of the alkaloid. The resulting hydrogenated product has been isolated both as the free base and as its hydrochloride. Both are laevo-rotatory, crystalline substances and the analysis revealed a striking phenomenon. Isatidine, $C_{18}H_{25}NO_7$ took up 4 molecules of hydrogen, then split off one molecule of water, so that the resulting base has the formula $C_{18}H_{31}O_6N$ or $C_{18}H_{31}O_6N.HCl$ for its hydrochloride, thus:

$$C_{18}H_{25}NO_7 + 4H_2 = [C_{18}H_{33}NO_7]$$
$$[C_{18}H_{33}NO_7] - H_2O = C_{18}H_{31}NO_6.$$

Octahydro-anhydro-isatidine.

Hydrolysis of Octahydro-anhydro-isatidine.

When octahydro-anhydro-isatidine was hydrolysed with barium-hydroxide, the split in the molecule occurred at the same place as is the case with isatidine, i.e. a basic fission product containing 8 carbon

atoms was produced, viz. $C_8H_{17}O_3N$, a tetrahydro-derivative of isatinecine ($C_8H_{13}O_3N$). Barger and his associates (1932) hydrogenated retrorsine (as its diacetyl derivative) and found that only two molecules of hydrogen were used. They failed to isolate the hydrogenated substance but isolated from the hydrolysis mixture a base $C_8H_{15}ON$ (retronecine has the formula $C_8H_{13}O_2N$), which they named retronecanol. Our base, $C_8H_{17}O_3N$, contributes new information towards the chemistry of the Senecio alkaloids, a hydrogenated base having been obtained without any loss of oxygen atoms. The hydrogen has therefore saturated most likely ethylenic or benzylenic double bonds only and the possibility of an enolic-CO-CH_2-grouping as suggested for retrorsine must be ruled out for isatidine.

The Function of the Oxygen Atoms in Isatinecine, Isatinecic Acid and Isatidine.

It must be accepted meanwhile that isatinecine $C_8H_{13}O_3N$, has three reactive hydrogen atoms although this could not be acetylated or determined (Zerewitinoff), in the latter case due to its insolubility in either pyridine or anisole.

Isatinecic acid $C_{10}H_{16}O_6$, is a dihydroxy, dicarboxylic acid and the function of all its six oxygen atoms is therefore known.

Isatidine, $C_{18}H_{25}NO_7$, according to Blackie (1937) may have three reactive hydrogen atoms (i.e. hydroxyl-groups), and the remaining four oxygen atoms must then be contained either in two ester-linkages or in one ester-linkage and one lactone group.

Isatinecic acid so readily forms a monolactone (see experimental part), that the slightest deviation from the optimum conditions for the formation of the dibasic acid results in the isolation of the monobasic monolactonic acid. The equation for the hydrolysis of the alkaloid may therefore be either:

I. $C_{18}H_{25}NO_7 + 2\ H_2O = C_8H_{13}O_3N + C_{10}H_{16}O_6$
isatidine — isatinecine — isatinecic acid or the 'new' acid (see p. 445).

or

II. $C_{18}H_{25}NO_7 + H_2O = C_8H_{13}O_3N + C_{10}H_{14}O_5$
isatidine — isatinecine — isatinecic monolactone.

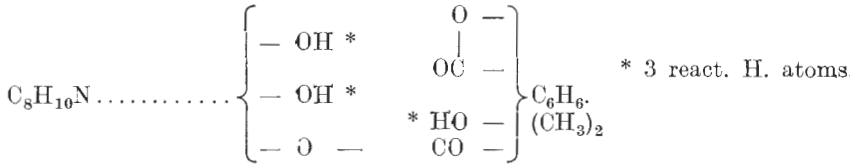
Reversely the alkaloid can therefore either be constituted by the combination of the dibasic acid, $C_{10}H_{16}O_6$, with the base isatinecine, $C_8H_{13}O_3N$, by two ester-linkages, e.g.

$C_8H_{10}N$.....{ — OH *, * HO —, * HO — } $C_6H_6(CH_3)_2$ { — O — CO —, — O — CO — }

* 3 react. H. atoms.

$C_{18}H_{25}NO_7$ (isatidine)—see equation I.

Or the alkaloid may be a combination by one ester-linking of isatinecine with the monolactonic acid as follows:—



$C_{18}H_{15}NO_7$ (isatidine)—see equation II.

The Nature of the Natural and Hydrogenated Isatinecic Acids.

As is described in the experimental part isatidine yields two different acids on hydrolysis, depending upon the method used. With alcoholic KOH a dibasic acid, $C_{10}H_{16}O_6$, with melting-point 181·5° C.* and dextro-rotatory is obtained (see de Waal, 1939); this acid as has now been found also forms monobasic monolactonic acid. Using $Ba(OH)_2$ for the hydrolysis an isomeric dihydroxy-dibasic acid is obtained, with melting-point 148·5° C. and either inactive or dextro-rotatory. Its corresponding monolactone has now also been isolated for the first time with an $[\alpha]_D^{20} = +108{\cdot}8(H_2O)$ and a melting-point of 197-8°C. In a private communication to the author, Dr. J. J. Blackie of Edinburgh suggested the name "isatinecic acid" to the $Ba(OH)_2$ hydrolysis acid of m.p. 148·5° C. which we now will retain in future, and the KOH-hydrolysis acid of melting-point 181·5° C. will meanwhile be termed the "new" acid until its identification has been completed and a suitable name, if necessary, suggested.

Both "isatinecic" acid and the "new" acid as well as their monolactones (which incidentally prove that in each case one hydroxyl-group must be situated in the γ-position to one carboxyl-group) can be very readily hydrogenated in the presence of PtO_2. In each case 2 molecules of hydrogen are consumed for one molecule of the acid. As these hydrogenated acids persist to be of a syrupy nature it is hoped that it will be possible to isolate ester-derivatives in a crystalline form.

Now octahydro-anhydro-isatidine, $C_{18}H_{31}O_6N$, on hydrolysis yields a basic fraction $C_8H_{17}O_3N$, thus accounting for two molecules or four atoms of hydrogen. Again, octahydro-anhydro-isatidine shows a nett increase of six hydrogen atoms only and a loss of one oxygen atom (although 8 atoms of hydrogen had been taken up during the hydrogenation). Therefore the loss of two hydrogen atoms and one oxygen atom *as one molecule of water must have occurred in the acidic moiety* of the hydrogenated substance, $C_{18}H_{31}O_6N$. If such an intramolecular elimination of one molecule of water had taken place in the acidic fraction of the molecule, then it is expected that the hydrolysis of octahydro-anhydro-isatidine will lead to the isolation of a $C_{10}H_{20}O_6$ dibasic acid which had lost one molecule of water, i.e. a $C_{10}H_{18}O_5$ dibasic acid. Should the hydrolysis lead to the isolation of a monolactonic acid, then similarly its formula will be $C_{10}H_{16}O_4$ (i.e. $C_{10}H_{18}O_5 - H_2O$).

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

It is also perfectly clear from the two part formulae for isatidine (see page 434) that the loss of one molecule of water in the acidific moiety of the hydogenated substance cannot be due to a lactone formation in that part of the molecule. It can only be accounted for by the reaction of one hydroxyl-group with one other hydrogen atom to eliminate one molecule of water in the acidic fraction of the molecule.

It is hoped that this obscure problem will be clarified in our next contribution on the subject after the number of the reactive H atoms in octahydro-anhydro-isatidine has been determined and the hydrogenated acidic fraction has been isolated. Similarly it will be interesting to know whether the same phenomenon of water elimination takes place during the hydrogenation of isatinecic acid itself as well as its monolactone.

Experimental Part.

Catalytic Hydrogenation and Reduction of Isatidine and the Isolation of Octahydro-anhydro-isatidine.

10 Gms. Isatidine dissolved in 70 c.c. N hydrochloric acid was hydrogenated under continuous mechanical shaking (Gattermann and Wieland, 1936) using 200 mgm. platinum-dioxide as catalyst. The hydrogen consumption advanced as follows:—

The first molecule of hydrogen was taken up after 100 minutes, i.e. at the rate of 420 c.c. H_2 per hour.

The second molecule of hydrogen was taken up after a further 110 minutes, i.e. 390 c.c. H_2 per hour.

The third molecule of hydrogen was taken up after a further 145 minutes, i.e. at about 290 c.c. H_2 per hour.

The fourth molecule of hydrogen was taken up after approximately 250 minutes, i.e. at about 170 c.c. per hour.

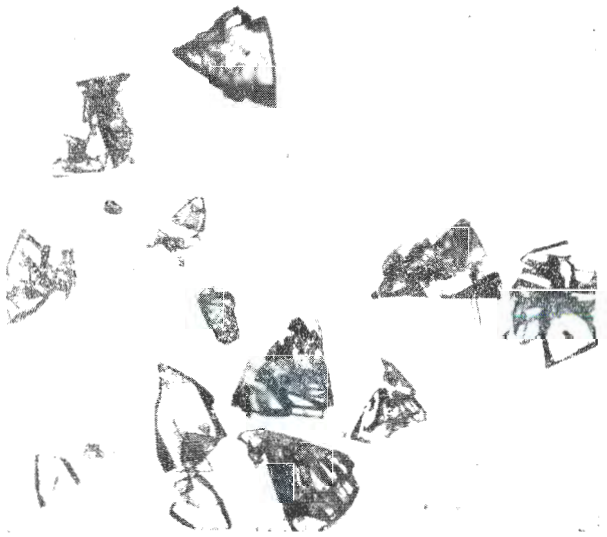
Various independent hydrogenations with 3 gms., 12 gms. and 20 gms. of isatidine and 100 mgm., 200 mgm. and 500 mgm. platinum-dioxide respectively in water, half-normal and normal hydrochloric acid solutions all proved that the first two molecules of hydrogenation were absorbed at practically the same rate but that the consumption of the third molecule of hydrogen was much slower, whereas the fourth molecule of hydrogen was consumed at a still more reduced rate.

The hydrogenated acid solution (above) of 10 gms. of isatidine was very unstable towards acid or soda-alkaline permanganate solutions and gave strong precipitates with Mayer's, Wagner's and Dragendorf's reagents and with phospho-tungstic acid. Thorough shakings of this acid solution with ether or with chloroform removed nothing. The solution was then alkalinified with concentrated ammonium hydroxide (1 : $3H_2O$), allowed to evaporate and finally dried in a vacuous desiccator over concentrated sulphuric acid. The residue was then extracted first with acetone (twice) and then with chloroform followed by absolute alcohol.

The acetone solution deposited a good crop of crystals (about 2 gms.) and the chloroform solution on evaporation left about 5 gms. of an oily substance.

The purified crystals (see Fig. 1) from the acetone solution was dissolved in a little absolute alcohol from which it crystallized in clusters of needles on the addition of a small volume of dry ether. From the oily residue of the chloroform extract the same substance was isolated after repeated treatment of the oil with acetone. The acetone washings deposited the same base as was isolated above. This base exhibited a double melting point. It melted at 115 to 120°, resolidified and finally melted to a clear solution at 183 to 184° C.

FIG. 1.



Octa-hydro-anhydro-isatidine × 35.

Micro-analysis.

5·347 mgm.: 11·565 mgm. CO_2; 4·170 mgm. H_2O.

3·144 mgm.: 0·104 c.c. N at 22·5° C. and 766 m.m.

Calculated for $C_{18}H_{31}O_6N$:

C = 60·48 per cent.; H = 8·74 per cent.; N = 3·92 per cent.

found:

C = 59·01 per cent.; H = 8·73 per cent.; N = 3·85 per cent.

(See confirmation of this formula from the analysis of its hydrochloride, p. 439).

The substance had a bitter taste; it readily dissolved in water, methanol and acetic acid; it was soluble in ethanol and chloroform; it was sparingly soluble in acetone, benzol and ethyl-acetate and

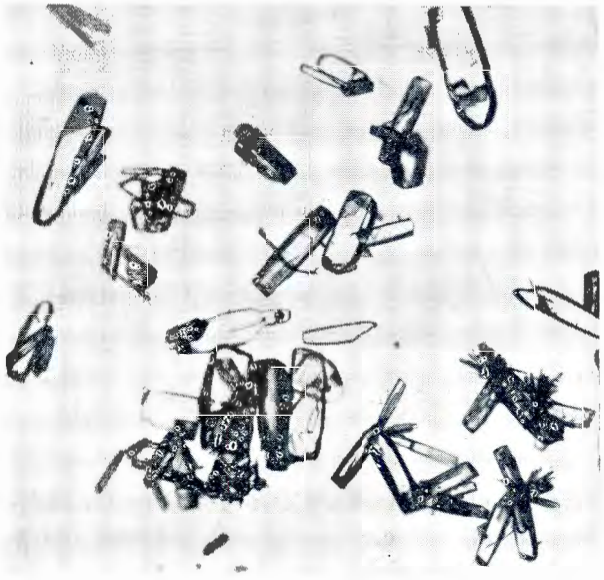
was insoluble in ether and petroleum-ether. A solution of octahydro-anhydro-isatidine in twice-normal hydrochloric acid gave strong precipitates with phospho-tungstic acid and with Mayer's, Wagner's and Dragendorf's alkaloidal reagents.

The formula $C_{18}H_{31}O_6N$ was definitely established by the preparation of the hydrochloride from this base and the isolation of the hydrochloride from the hydrogenated acid solution.

Isolation of Octahydro-anhydro-isatidine-hydrochloride.

This compound was very readily obtained when isatidine was catalytically reduced in a normal hydrochloric acid solution (see above) and the filtrate after the hydrogenation allowed to evaporate in front of a fan at room temperature. Crystals rapidly began to separate in the form of stout prismatic columns. The liquid was finally evaporated to dryness on a water-bath. The crystals were dried, washed with acetone followed by ether and recrystallized from boiling ethanol. After two recrystallizations the hydrochloride (see Fig. 2) melted sharply at 218° with strong evolution of gas to a clear melt.

FIG. 2.



Octa-hydro-anhydro-isatidine-hydrochloride × 35.

Optical Rotation.

Wht..... = 50·0 mgm.

Vol..... = 7·5 c.c. H_2O.

θ..... = — 0·35.

$$\left[\alpha\right]_{D}^{26} = \frac{-0{\cdot}35 \times 7{\cdot}5 \times 1{,}000}{1 \times 50} = -52{\cdot}5°.$$

Micro-analysis:

5·407 mgm.: 10·875 mgm. CO_2: 3·870 mgm. H_2O.

2·914 mgm.: 0·090 c.c. N at 23°C and 766 m.m. Hg.

13·420 mgm.: 4·940 mgm. AgCl.

Calculated for $C_{18}H_{31}O_6$ N.HCl	C. = 54·88 per cent.:	H = 8·19 per cent.
	N = 3·55 per cent.:	Cl = 9·00 per cent.
Found.....	C. = 54·87 per cent.:	H = 8·01 per cent.
	N. = 3·59 per cent.:	Cl = 9·10 per cent.
Therefore.....	$C_{18}H_{31}O_6N.NCl$.	

The substance was readily soluble in water, methanol and acetic acid; it was sparingly soluble in ethanol, ethyl-acetate and chloroform and it was insoluble in ether, acetone and petroleum ether.

Preparation of Octahydro-anhydro-isatidine (free base) from the above hydrochloride.

The pure hydrochloride (m.p.218°) was dissolved in a small volume of water and the solution made alkaline with a concentrated ammonium hydrate solution (1:3 H_2O). It was then allowed to evaporate in front of a fan. The residue, which had the consistency of a syrup, was stirred with dry acetone when it became crystalline. Purification was effected by recrystallization from ethanol on the addition of a small volume of pure ether. The base had the same double melting-point and showed no depression when mixed with the free base directly isolated from the isatidine-hydrogenated solution (see page 436).

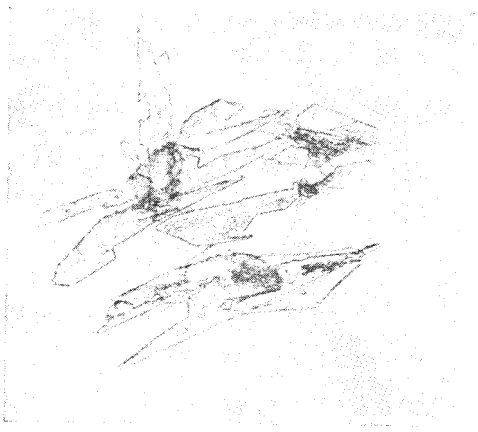
Hydrolysis of Octahydro-anhydro-isatidine and the Isolation of Tetrahydro-isatinecine.

The hydrolysis of Octahydro-anhydro-isatidine can be effected in two ways: (1) immediately after the hydrogenation (PtO_2) of isatidine was completed, i.e., with the base still in normal hydrochloric acid solution, or (2) with the crystalline hydrochloride after its isolation. In the first case the filtrate, after 4 molecules of H_2 had been taken up by the isatidine in N-HCl solution, was shaken with ether. The ether was removed and the acid solution neutralized with concentrated ammonium hydrate (1:3 H_2O) and then 1·2 mol. of solid barium hydrate were added. In the second case the crystalline tetrahydro-anhydro-isatidine hydrochloride was dissolved in a small volume of water and a small excess of barium oxide-hydrate was then added.

The solution (in either case)with the barium hydrate was then refluxed for about one hour [e.g., 10 gms. octahydro-anhydro-isatidine and 12 gms. of $Ba(OH)_2 \cdot 8H_2O$]. It was then filtered. The filtrate was titrated with concentrated sulphuric acid (1:4 H_2O) until just acid to phenolphthalein. The $BaSO_4$ was centrifuged off. The clear supernatant was allowed to evaporate on a waterbath to dryness and the dry residue extracted with hot absolute alcohol. The alcoholic solution was then allowed to evaporate on a waterbath and the syrupy residue stirred with dry acetone. The base crystallized.

This substance was then repeatedly refluxed with acetone which removed the base and on the concentration of the acetone and the addition of a small volume of ether readily crystallized (see Fig. 3). After one or two similar recrystallizations this base tetrahydro-isatinecine, had a constant melting-point (sharp) of 174·5°. It is very hygroscopic.

FIG. 3.



Tetrahydro-isatinecine, m.p. 175×10.

Micro-analysis :

5·201 mgm.: 10·380 mgm.CO_2; 4·440 mgm. H_2O.

3·021 mgm.: 0·210 c.c.N at 25·5° C. and 754 m.m. Hg.

Calculated for $C_8H_{17}O_3N$:

C = 54·83 per cent.; H = 9·78 per cent.; N = 7·99 per cent.

found:

C = 54·43 per cent.; H = 9·55 per cent.; N = 7·90 per cent.

Optical rotation.

The mean value of a solution of 50·0 mgm. in 8·0 c.c. distilled H_2O was found to be as follows: —

$$\alpha = -0{\cdot}55°.$$

Therefore $\left[\ \alpha\ \right]_D^{20} = -88{\cdot}0°$.

Chemical Properties:

This substance (tetrahydro-isatinecine) was easily soluble in cold water, ethanol, methanol and chloroform.

It readily dissolved in hot acetone; it was sparingly soluble in ethyl-acetate and practically insoluble in ether and petroleum-ether.

A solution of tetrahydro-isatinecine in two normal HCl gave strong precipitates with phosphotungstic acid and with Wagner's,

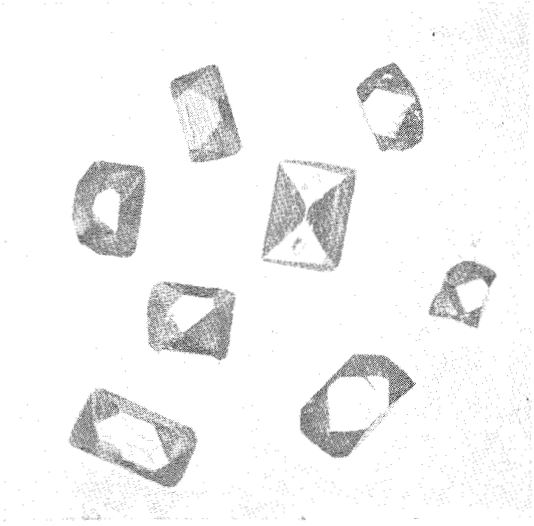
Dragendorf's and Mayer's reagents. With Mayer's reagent lemon-yellow crystalline flakes were obtained with a crude melting-point of 117°. Tetrahydro-isatinecine was unstable towards soda-alkaline potassium-permanganate solution.

... Barium-hydroxide hydrolosis of Isatidine. The isolation of... isatinecine, isatinecic acid and isatinecic monolactonic acid.*

To a solution of 20 gms. of isatidine in 200 c.c. of water was added 20 gms. of solid barium-oxide-hydrate (1·2 mol.) and was then refluxed for 40 minutes. The filtrate was titrated with concentrated sulphuric acid (1:4 H_2O) until just acid to phenolphthalein and the $BaSO_4$ precipitate centrifuged off. The supernatant was decanted and evaporated on a waterbath under reduced pressure. The dry residue was then twice extracted with hot ethanol which readily removed the base isatinecine.

On concentration of the alcohol and the addition of acetone isatinecine crystallized out in a very good yield of about 8 gms. Thus recrystallized the basic fission product (see Fig. 4) decomposed at 212-215°.

FIG. 4.



Isatinecine, m.p. 212-5°, ×10.

Micro-analysis.

4·729 mgm. dried at room temperature in high vacuum over P_2O_5 lost 0·058 mgm. in weight.

(*a*) 4·671 mgm.: 9·655 mgm. CO_2; 3·160 mgm. H_2O.

* In a private communication Dr. J. J. Blackie of Edinburgh suggested the hydrolysis with $Ba(OH)_2$ for which we wish to express our sincere thanks.

(*b*) 2·865 mgm.: 0·214 c.c. N at 25° C. and 764 m.m. Hg. found: C = 56·41 per cent.; H = 7·57 per cent.; N = 8·6 per cent.

Calculated for $C_8H_{13}O_3N$: C = 56·12 per cent.; H = 7·65 per cent.; N = 8·2 per cent.

(*c*) Active hydrogen could not be determined due to the insolubility of the substance in either pyridine or anisole.

(*d*) Negative for C-methyl groups.

(*e*) Negative for N-methyl groups.

Optical Rotation.

$$\left[\alpha\right]_D^{20} = +22{\cdot}4^\circ \text{ (50·0 mgm. in 8 c.c. } H_2O)$$

Properties.

Isatinecine gave strong precipitates with phosphotungstic acid, Wagner's and Dragendorf's reagents, but nil with Mayer's reagent.

It is soluble in water, methanol, ethanol and acetic acid.

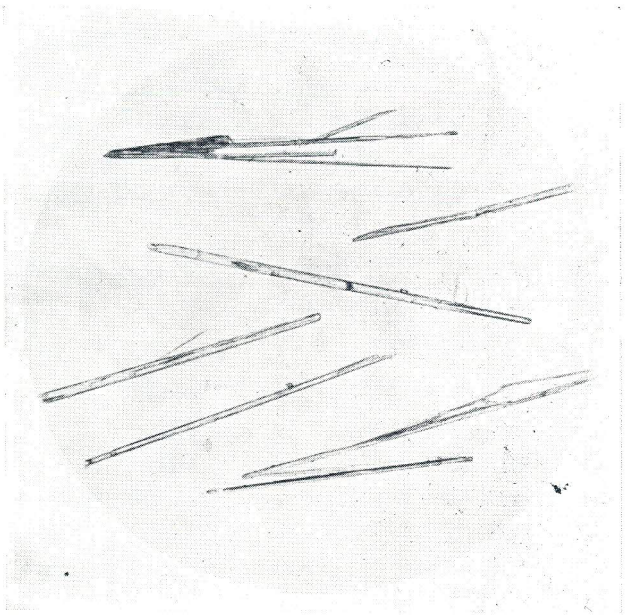
It is sparingly soluble in acetone and ethyl-acetone and practically insoluble in ether, petroleum-ether and chloroform.

Isolation of Isatinecic Acid and Isatinecic Monolactonic Acid.

The residue after the extraction of the base with ethanol (above) was then dissolved in a small volume of water, titrated with concentrated sulphuric acid (1 : $4H_2O$) until the solution was this time just acid to congo red. The $BaSO_4$ was again centrifuged off and the supernatant evaporated to dryness as already stated for the base above. The dry residue was then refluxed with ethyl-acetate for two to three minutes which removed the acid, the ethyl-acetate solution was dried over exsiccated Na_2SO_4 and if necessary decolourised by the addition of a pinch of charcoal. On the addition of a little petroleum-ether to the filtrate isatinecic acid crystallized in needles. After a similar recrystallization the acid crystallized in fairly large beautiful colourless needles (see Fig. 5) with a clear constant melting-point of 148·5°.

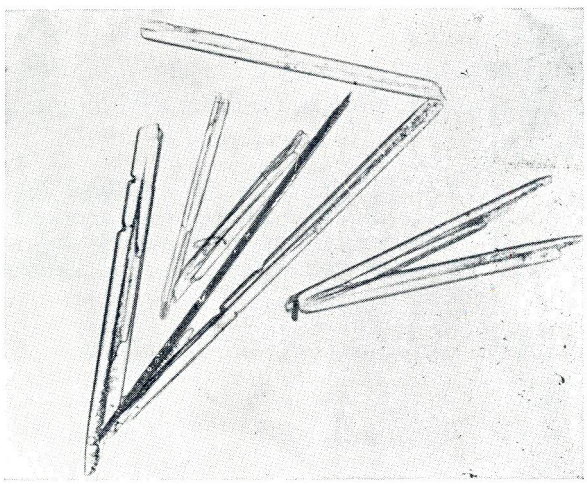
When similar hydrolysis experiments of isatidine with barium-hydroxide were carried out under slightly excessive heat, the hydrolysis invariably resulted in the isolation of the isatinecic monolactonic acid. This monolactonic monobasic acid readily crystallizes into beautiful large crystals (see Fig. 6) from pure ethyl-acetate only. After the third recrystallization the substance was pure with a sharp melting-point at 197-8° C.

FIG. 5.



Isatinecic acid, m.p. 148·5, ×5.

FIG. 6.



Isatinecic monolactonic acid, m.p. 197-8, ×10.

Micro-analysis of Isatinecic Acid.

(*a*) The inactive acid and (*b*) the active acid $\left[\alpha\right]_{D}^{20} = +86(H_2O)$

(*a*) 4·260 mgm.: 8·100 mgm. CO_2; 2·640 mgm. H_2O.

(*b*) 5·476 mgm.: 10·410 mgm. CO_2; 3·410 mgm. H_2O.
found (*a*): C = 51·88 per cent.; H = 6·94 per cent.
found (*b*): C = 51·87 per cent.; H = 6·97 per cent.
Calculated for $C_{10}H_{16}O_6$: C = 51·72 per cent.; H = 6·94 per cent.

(*c*) 0·201 mgm. in 3·027 mgm. camphor; $\triangle = 11·2°$.
therefore mol. weight = 238.
$C_{10}H_{16}O_6 = 232·23$.

(*d*) *Active H Determination* (*Zerewitinoff*).

(1) 6·210 mgm.: Vo = 1·23 c.c. CH_4.
(2) 6·301 mgm.: Vo = 1·25 c.c. CH_4.
found (1) = 0·88 per cent. reactive H atoms.
found (2) = 0·88 per cent. reactive H atoms.
Calculated = 0·86 per cent. for two reactive H atoms.
therefore 2 – OH groups.

(*e*) Micro-titration.

54·0 mgm. of the dibasic acid dissolved in about 5 c.c. H_2O required 4·60 c.c. N NaOH.

Now 23·2 mgm. (mol.wht.232) required 2·0 c.c. N NaOH for 2-COOH.

Therefore 54 mgm. required 4·66 c.c. NaOH for 2-COOH.

Therefore 2 carboxyl-groups.

No lactonic groups were found to be present.

(*f*) *Solubility.*

Isatinecic acid immediately dissolved in cold water, cold methanol, cold ethanol, cold acetone and cold acetic acid. It was soluble in ethyl-acetate and practically insoluble in ether, petroleum-ether and chloroform.

Chemical Properties of Isatinecic Monolactonic Acid.

1. *Micro-analysis.*

5·311 mgm.: 10·945 mgm. CO_2; 3·142 mgm. H_2O.
found: C = 56·22 per cent.; H = 6·63 per cent.
Calculated for $C_{10}H_{14}O_5$: C = 56·08 per cent.; H = 6·59 per cent.

(2) *Micro-titration.*

50·0 mgm. acid dissolved in about 5 c.c. H_2O. required 2·30 c.c. N NaOH.

Now 21·4 mgm. (mol.wht.214) required 1·0 c.c. N NaOH for 1-COOH.

Therefore 50·0 mgm. required 2·32 c.c. NaOH for 1-COOH.

Therefore one carboxyl group.

(3) *Saponification.*

4 c.c. $\frac{N}{10}$ NaOH was added to the titrated solution (2), and this solution then refluxed for 30 minutes.

Back titration required 1·85 c.c. $\frac{N}{10}$ HCl.

Therefore difference = 2·15 c.c. $\frac{N}{10}$ NaOH.

Therefore monobasic-monolactonic-acid.

Theory for one lactone-group = 2·32 NaOH.

(4) *Specific Rotation* (mean of several determinations).

Weight = 50·0 mgm.

Volume = 8·0 c.c. H_2O

$$\alpha = +0{\cdot}68^\circ$$

$$\left[\alpha\right]_D^{20} = \frac{+\ 0{\cdot}68 \times 1000 \times 8}{1 \times 50}$$

$$= +108{\cdot}8^\circ.$$

(5) *Solubility.*

The isatinecic monolactonic acid dissolved in cold water, cold methanol, cold ethanol and cold acetone, but not so readily as the dihydroxy-dibasic isatinecic acid. It dissolved in ethyl-acetate and acetic acid, but was practically insoluble in ether, petroleum-ether and chloroform.

Alcoholic KOH Hydrolysis of Isatidine.

The Isolation of the "New" Isomeric Acid, $C_{10}H_{16}O_6$.

In the first paper of this series (de Waal, 1939), the hydrolysis of isatidine with alcoholic potassium hydroxide had been recorded and the isolation of the acid fission product only had been described. The following improved hydrolysis led to the isolation of both the base and the acid.

To 10 gms. of isatidine dissolved in 80 c.c. ethanol was added 4 gms. of solid KOH (= 1·3 mol.) and then boiled under a reflux condenser. (The addition at this stage in another experiment of 2 c.c. of water led to the same hydrolysis results.) Within 5 minutes crystals separated and after 10 minutes the contents of the flask was one mass of crystals. The hydrolysis was stopped and the crystals

were filtered off, thoroughly washed with ethanol and dried. This substance was the dibasic potassium salt of the inactive acid. [From the alcoholic filtrate isatinecine was isolated adopting the same procedure as described further above for the $Ba(OH)_2$ hydrolysis.]

Isolation of the Acid.

The potassium crystalline salt was dissolved in about 20 c.c. of cold water and the solution divided into two equal portions. The one portion was neutralized with concentrated sulphuric acid (1:4 H_2O) until just acid to congo red and the other portion neutralized with concentrated hydrochloric acid (1:3 H_2O) again until just acid to Congo red. (The object was to test whether H_2SO_4 would lead to the isolation of a dibasic acid and HCl to the isolation of a monobasic monolactonic acid.) From both filtrates on evaporation crystals separated. The crystallization was more rapid and complete from the H_2SO_4 neutralized solution. In both instances one and the same dibasic acid crystallized, identical with the dibasic acid already described in the first publication (1939). With solid KOH the isolated acid was found to be inactive, whereas formerly with twice normal alcoholic potassium hydroxide the optically active dibasic acid was isolated. The melting of this acid is 181·5° corrected (not 178-180° as was reported previously).

Summary.

1. The principle alkaloid of *Senecio isatideus* D.C. isatidine, $C_{18}H_{25}NO_7$, on hydrogenation in the presence of platinum-dioxide, took up 8 atoms of hydrogen with the elimination of one molecule of water. The formula is $C_{18}H_{31}O_6N$, m.p. 183-184° C.

2. The hydrogenated crystalline substance, octahydro-anhydro-isatidine, readily forms a hydrochloride m.p. 218° C., $[\alpha]_D^{20} = -52{\cdot}5(H_2O)$, and can easily be converted into the free base.

3. Octahydro-anhydro-isatidine or its hydrochloride yields on hydrolysis with $Ba(OH)_2$ a new basic fission product with the formula $C_8H_{17}O_3N$. Isatinecine has the formula $C_8H_{13}O_3N$. It is therefore a tetrahydro-isatinecine.

4. Tetrahydro-isatinecine, m.p. 175° C. and $[\alpha]_D^{20} = -88°$; is unstable towards potassium-permanganate and gives positive reactions with alkaloidal reagents.

5. Isatidine hydrolysed with $Ba(OH)_2$ yields isatinecine and the dihydroxy-dibasic isatinecic acid formulae $C_8H_{13}O_3N$ and $C_{10}H_{16}O_6$ respectively.

6. When isatidine is hydrolysed with alcoholic KOH the same base isatinecine, but a different dibasic acid is obtained, isomeric with isatinecic acid. It has m.p. 181·5° C.

7. Isatinecic acid readily forms a monobasic monolactonic acid, $C_{10}H_{14}O_5$, m.p. 197-8° and $[\alpha]_D^{20} = +108{\cdot}8°$ (H_2O).

8. Both isatinecic acid and its "new" isomeric acid each take up four atoms of hydrogen on hydrogenation in the presence of PtO_2.

9. From the hydrolysis results with octahydro-anhydro-isatidine and the isolation of the basic fission product tetrahydro-isatinecine, $C_8H_{17}O_3N$, it is concluded, that it must be the acidic fraction of the hydrogenated molecule which eliminates one molecule of water as follows: —

$$C_{10}H_{16}O_6 + 4H_2 = C_{10}H_{20}O_6.$$
$$C_{10}H_{20}O_6 - H_2O = C_{10}H_{18}O_5.$$
$$C_8H_{17}O_3N + C_{10}H_{18}O_5 = C_{18}H_{31}O_6 + 2H_2O.$$

10. The work is being continued with the view to furnish a further contribution towards the structure of the Senecio alkaloids.

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Author's Note.

After this article had been submitted to the Press it was discovered that isatinecic acid is a per acid, i.e., has one —R.CO.O.OH group and that this is ester-linked in isatidine. Therefore both isatidine and isatinecic acid have each one per-oxygen atom, accounting for one excess hydrogen molecule during their hydrogenations above those required for the saturation of olefine double-bonds. (A more detailed article will appear in a subsequent issue of this journal.)

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