364. *The* Senecio *Alkaloids. Part III. The Structure of Retrorsine and Isatidine, and the Isomerism of Retronecic Acid and Isatinecic Acid.*

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Retronecic and isatinecic acids, previously obtained by hydrolysis of retrorsine and isatidine, respectively, have now each been obtained from both these alkaloids; retronecic acid is identical with " new " isatinecic acid obtained by de Waal (*Onderstepoort J. Vet. Sci. Animal Husb.*, 1939, **12**, 155; 1940, **14**, 445) from isatidine.

Isatinecic and retronecic acids are shown to contain an ethylidene group and are, respectively, *cis*- and *trans*-$\alpha\beta$-unsaturated dihydroxy-dicarboxylic acids which on reduction give identical dihydro-acids. The *cis*- is readily converted into the *trans*-isomer and it is in the *cis*-form in which the acid occurs in both retrorsine and isatidine.

Retrorsine and isatidine are formulated as the esters of isatinecic acid with retronecine and retronecine *N*-oxide, respectively. On this basis the reduction of isatidine to octahydroanhydroisatidine is explained.

Manske (*Canad. J. Res.*, 1931, **5**, 651) and Barger, Seshadri, Watt, and Yabuta (*J.*, 1935, 11) hydrolysed retrorsine, $C_{18}H_{25}O_6N$, with sodium hydroxide to retronecine, $C_8H_{13}O_2N$, and retronecic acid, $C_{10}H_{16}O_6$, m. p. 177°. Blackie [*Pharm. J.*, 1937, **138**, (iv), 84] hydrolysed isatidine, $C_{18}H_{25}O_7N$, with barium hydroxide to isatinecine, $C_8H_{13}O_3N$, and isatinecic acid, $C_{10}H_{16}O_6$, m. p. 143°, whilst with alcoholic potash de Waal (*Onderstepoort J. Vet. Sci. Animal Husb.*, 1939, **12**, 155; 1940, **14**, 445) obtained a " new " isatinecic acid, m. p. 181·5°, having the same formula.

The two alkaloids, retrorsine and isatidine, have been isolated from *S. isatideus* D.C. (Manske, *loc. cit.*) and from *S. retrorsus* D.C. (de Waal, 1939, *loc. cit.*). Barger *et al.* (*loc. cit.*) found only retrorsine in the latter, and de Waal (1939, *loc. cit.*) states that isatidine is always accompanied by retrorsine. In a large number of extractions in these laboratories we have found varying quantities of the two alkaloids from the same sample of *S. isatideus* from which yields as high as 3% of isatidine were obtained when extraction was effected in glass vessels, whilst the quantity of isatidine decreased and that of retrorsine increased to about 1% when extractions were carried out in tinned copper vessels.

Since isatinecine has recently been shown by Leisegang and Warren (Part II) to be retronecine *N*-oxide, it seemed likely that the two alkaloids themselves were closely related. Oxidation and reduction experiments, similar to those previously effected with the " necine " bases, were carried out, and it was found that isatidine was quantitatively reduced by zinc and dilute sulphuric acid to retrorsine which in turn was readily oxidised to isatidine by hydrogen peroxide. It seemed possible that the acid portions were also involved in the oxidation, because de Waal (1940, *loc. cit.*) reports isatinecic acid as a per-acid. On the other hand, the chemical characteristics of the two acids as reported in the literature are different. Barger *et al.* (*loc. cit.*) state that retronecic acid is a dihydroxy-dicarboxylic acid containing two methyl groups, and, since they recovered it unchanged from the hydrogenolysis of retrorsine, Barger and Blackie (*J.*, 1937, 584) assumed it was saturated and must therefore be a cyclic compound. Isatinecic acid, however, is unsaturated and contains one or two double bonds (de Waal, *loc. cit.*).

Hydrolysis of retrorsine by sodium hydroxide under the conditions described by Barger and co-workers (1935, *loc. cit.*) gave isatinecic acid, and not retronecic acid as recorded by these authors. When the same method was repeated with alcohol as the solvent, retronecic acid was found with a melting point and rotation identical with those recorded by de Waal (*loc. cit.*) for his " new " isatinecic acid, obtained by hydrolysis of isatidine with alcoholic potash. Retronecic acid and the " new " acid gave no melting point depression on admixture. Furthermore the brucine salts of the two acids were identical, sintering at 142° and melting at 148°, and differed from the *brucine* salt, $C_{33}H_{42}O_{10}N_2,1\frac{1}{2}H_2O$, m. p. 160—161°, of isatinecic acid.

Isatidine on hydrolysis with aqueous barium or sodium hydroxide gave an impure acid, m. p. 143°, from which was obtained isatinecic acid, m. p. 148°, and a lactone, m. p. *ca.* 186°. The same lactone was obtained by boiling isatinecic acid with alcoholic potash and acidifying to Congo-red, by heating retronecic acid (Barger *et al., loc. cit.*) or isatinecic acid with anhydrous oxalic acid, or by evaporating retronecic acid with concentrated hydrochloric acid. We could not isolate isatinecic acid with a measurable rotation or as its lactone, m. p. 197—198° (de Waal, 1940, *loc. cit.*). In our experiment lactonisation of isatinecic acid gave retronecic acid lactone.

The results are summarised in the table in which are also set out the properties previously recorded.

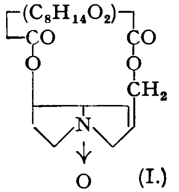
	M. p.	$[\alpha]_D$.	Equiv. wt.
Retronecic acid, $C_{10}H_{16}O_6$, equiv. wt. 116.			
Barger (1935), ex retrorsine	177°	−11·4°	117
de Waal (1940), " new " isatinecic acid	181·5	+56, 0	117·9
Hydrolysis of isatidine by alc. KOH or NaOH	178—180 (*a*)	+28	—
Hydrolysis of retrorsine by alc. KOH	180—181·5 (*b*)	+58	122·7
Isatinecic acid, $C_{10}H_{16}O_6$, equiv. wt. 116.			
de Waal (1940)	148·5	0, +86	117·4
Hydrolysis of isatidine with $Ba(OH)_2$	148·5	0	116·7
Hydrolysis of retrorsine with aq. NaOH	148	0	116
Retronecic acid lactone, $C_{10}H_{14}O_5$, equiv. wt. 214.			
Manske (1931)	186	—	—
Barger (1935)	181—183	—	217
Retronecic acid, evap. with HCl	184—185 (*x*)	*ca.* +66	215
Isatinecic acid lactone, $C_{10}H_{14}O_5$, equiv. wt. 214.			
de Waal (1940)	197—198	+108	217·4
Isatinecic acid with anhyd. oxalic acid	185—186 (*y*)	—	215·6
Isatinecic acid with aq. KOH at 100°	185—186	—	—

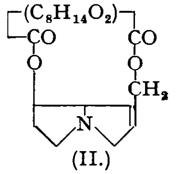
Mixed melting point (*a*) and (*b*), 180—181°.
Mixed melting point (*x*) and (*y*), 185—186°.

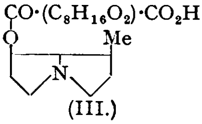
In short, hydrolysis of either retrorsine or isatidine with aqueous barium or sodium hydroxide gave isatinecic acid, whilst with alcoholic sodium or potassium hydroxide or concentrated potassium hydroxide retronecic acid was obtained. A partial interconversion of isatinecic or retronecic acid into retronecic acid lactone was obtained when concentrated mineral acids were used in working up the hydrolysis solutions. A satisfactory procedure for obtaining isatinecic acid was to hydrolyse the alkaloid with barium hydroxide and precipitate the excess of alkali as carbonate. The solution was then evaporated and the basic " necine " extracted to leave pure hydrated *barium isatinecate,* $C_{10}H_{16}O_6Ba,2H_2O$, which readily lost its water of crystallisation at 100°, and from which isatinecic acid was liberated by addition of the exact quantity of dilute sulphuric acid.

A study of the catalytic hydrogenation of both acids showed that they contained one ethylenic linkage. The oily reduction products failed to crystallise and the bis-*p*-bromophenacyl esters could not be induced to solidify, whilst the dibenziminazole (cf. Link, *J. Biol. Chem.*, 1942, **143**, 551) failed to melt below 300°. With *p*-phenylphenacyl bromide the reduction products gave *bis-*p*-phenylphenacyl dihydroisatinecate,* m. p. 117—118°, and *dihydroretronecate,* $C_{38}H_{36}O_8$, m. p. 118—120°, which showed no depression on admixture.

Light-extinction curves for isatinecic acid ($\lambda_{max.}$ at 218 mμ., $\varepsilon_{max.}$ 4720) and retronecic acid ($\lambda_{max.}$ at 218 mμ., $\varepsilon_{max.}$ 9400) reveal these two acids as αβ-unsaturated acids (cf. Andrews, Cristol, Lindenbaum, and Young, *J. Amer. Chem. Soc.*, 1945, **67**, 716).


(I.)


(II.)


(III.)

Ozonolysis of both acids gave acetaldehyde, so that the ethylenic linkages are present as ethylidene groups. It follows, therefore, that isatinecic and retronecic acids are geometrical isomers. Finally, since isatinecic acid has a lower melting point and greater solubility in water and is converted into retronecic acid monolactone on heating with alkali and acid (cf. Auwers and Wissenbach, *Ber.*, 1923, **56**, 715; Fittig, *Annalen*, 1894, **283**, 107), isatinecic acid and retronecic acid are assigned *cis-* and *trans-*configurations, corresponding to angelic and tiglic acids, respectively. It is the *cis-*form in which the acid is attached in the two alkaloids, and the *trans-*form is obtained by isomerisation.

Retrorsine (II) and isatidine (I) may be considered as the *cis*-retronecic (*i.e.*, isatinecic) esters of retronecine and retronecine *N*-oxide, respectively. This relation between the two alkaloids is exactly similar to that found previously by Richardson and Warren (*J.*, 1943, 452) between two other *Senecio* alkaloids, platyphylline and rosmarinine, which are esters of senecic acid with platynecine and hydroxyplatynecine, respectively.

The structure of isatidine is in agreement with de Waal's observation (1940, *loc. cit.*), which we have confirmed, that catalytic reduction gives octahydroanhydroisatidine which may now be formulated as the dihydro-*cis*-retronecic ester (III) of retronecanol.

EXPERIMENTAL.

Interconversion of Retrorsine and Isatinecine.—(*a*) Retrorsine, m. p. 212—214° (corr.) (1 g., 1 mol.), in hot alcohol (10 ml.) was treated with 30% hydrogen peroxide (0·31 ml., 1 mol.), and the mixture maintained at 60° for 6 hours. The solvent was removed under reduced pressure, and the oily residue dissolved in water (20 ml.) and extracted with chloroform to remove unchanged retrorsine. Evaporation of the aqueous solution gave isatidine which, crystallised from ethanol, had m. p. 140·5—141·5°, undepressed on admixture with authentic specimen.

(*b*) Isatidine (5 g., 1 mol.) in 2N-sulphuric acid (50 ml., 7 equivs.) and a few drops of copper sulphate solution was allowed to react with zinc dust for 2 hours at room temperature. The filtered solution was made strongly alkaline with ammonia; the chloroform extract gave an oil which crystallised from chloroform to give retrorsine, m. p. 212—214° (corr.) (darkens at 200°), undepressed with authentic specimen, $[\alpha]_D^{18°}$ −17·6° (*c*, 2·1 in ethanol). Barger *et al.* (*loc. cit.*) give $[\alpha]_D^{18°}$ −17·6°.

Isatinecic Acid.—Isatidine (1 mol.), barium hydroxide (1 mol.), and water (120 mols.) were heated under reflux for 40 minutes, after which carbon dioxide was passed into the hot solution, the barium carbonate was filtered off, and the filtrate and washings were evaporated to dryness in a vacuum. The isatinecine was extracted by hot ethanol to leave pure ***barium isatinecate*** [Found (after air-drying): Ba, 33·94; (after drying at 100°) Ba, 36·18. $C_{10}H_{14}O_6Ba,2H_2O$ and $C_{10}H_{14}O_6Ba$ require Ba, 34·05 and 37·38%, respectively]. The air-dried salt was treated with the theoretical quantity of dilute sulphuric acid, the barium sulphate filtered off, and the filtrate evaporated in a vacuum, to leave a solid, m. p. 138—139°, raised to 143° after three crystallisations from ethyl acetate. The acid was lixiviated with ethyl acetate to leave a small residue of lactone, m. p. *ca.* 188° (Found: Equiv. Wt., 210). The ethyl acetate gave pure isatinecic acid, m. p. 148° (Found: Equiv. Wt., 116·7). The ***brucine*** salt formed short needles, m. p. 160—161°, from absolute ethanol (Found: C, 60·60; H, 6·95. $C_{33}H_{42}O_{10}N_2,1\frac{1}{2}H_2O$ requires C, 60·61; H, 6·94%).

Retronecic Acid Lactone from Isatinecic Acid.—Isatinecic acid (3 g.) and potassium hydroxide (1·86 g.) in ethanol (37 ml.) were boiled under reflux for 15 minutes. The potassium salt, which filled the whole liquid with a mass of crystals, was filtered off, washed with absolute alcohol, dissolved in a little water, and acidified to Congo-red. The solution was evaporated on a water-bath, and the residue extracted with ethyl acetate, from which retronecic acid lactone, m. p. 185—186°, crystallised. Repetition with concentrated aqueous potassium hydroxide gave similar results.

Dihydroisatinecic Acid.—Isatinecic acid (1·00 g.) in water (150 ml.) was hydrogenated with Adams's catalyst (0·2 g.), reduced immediately before this hydrogenation in the presence of isatinecic acid; absorption was complete in 200 minutes (observed: 92·7 ml. at S.T.P. Calc. for $C_{10}H_{16}O_6$, 1 mol. = 96·5 ml.). Evaporation of the solvent gave an oily acid, the ***bis-p-phenylphenacyl*** ester of which crystallised from benzene in nodules, m. p. 117—118° (Found: C, 72·80; H, 6·28. $C_{38}H_{36}O_8$ requires C, 73·28; H, 6·16%).

Dihydroretronecic Acid.—Retronecic acid (0·891 g.) was reduced as above at the same rate (observed: 92·7 ml. at S.T.P. Calc.: 86·0) to give an oily acid, the bis-*p*-phenylphenacyl ester of which crystallised from benzene in nodules, m. p. 118—120°, undepressed on admixture with the dihydroisatinecate (Found: C, 73·07; H, 6·10%).

Ozonolysis of Isatinecic and Retronecic Acid.—(*a*) Isatinecic acid (1 g.) in ethyl acetate was ozonised at 0°. The solvent was removed in a vacuum, the ozonide warmed with water, carbon dioxide passed through the solution and then into aqueous dimedon. The flocculent precipitate had m. p. 140°, which was undepressed on admixture with acetaldehyde-dimedon. (*b*) Ozonisation of retronecic acid gave similar results.

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