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Dicrotaline: The Toxic Alkaloid from *Crotalaria dura* (Wood and Evans) and *Crotalaria globifera* (E. Mey).

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THE ingestion of both *Crotalaria dura* (Wood and Evans) and *Crotalaria globifera* (E. Mey) causes the so-called " Jaagsiekte " in horses (*Crotalariosis equorum*). Sir Arnold Theiler (1918) proved *C. dura* poisonous to horses, whilst Marais (Allerton Laboratory Experiment 141, 27.1.27) produced typical " Jaagsiekte " in horses by feeding fresh *C. globifera* in the flowering and seeding stage at the rate of 90 to 180 gm. daily, over a period of 116 days. Death occurred on the 128th day. Later investigations by Steyn (1931) and (1937) supported the results of Sir Arnold Theiler, as " Jaagsiekte " was caused in horses by feeding *C. dura* in a dry state. In sheep a very similar disease to " Jaagsiekte " in horses was produced by Steyn and de Kock (1932) by drenching sheep with *C. dura*. An experiment conducted by Steyn (1937) on a horse which received 15 Kg. of *C. globifera* failed to produce symptoms of poisoning, the experiment being terminated, owing to insufficient plant material being available. It is, however, stated by Steyn that it is quite possible, that larger quantities of the plant than fed to the horse are required to produce poisoning. This seems quite probable, since, according to Steyn (1934), the least amount of *C. dura* which produces " Jaagsiekte " in a horse was 20·5 Kg. fed in the course of 23 days.

Several species of *Crotalaria* are known to be poisonous. A review on the subject is given by Becker, Neal, Arnold and Shealy (1935). The presence of an alkaloid in the seeds of *Crotalaria retusa* and *C. striata* was demonstrated by Greshoff (1890). The isolation of the alkaloid monocrotaline from *Crotalaria spectabilis* was reported by Neal, Rusoff and Ahmann (1935). They also suggested that succeeding alkaloids from this genus should be named dicrotaline, tricrotaline, etc. The chemical nature of the alkaloid monocrotaline was more fully investigated by Adams and Rogers (1939). They were the first to draw attention to the chemical similarity between monocrotaline and a number of the *Senecio* alkaloids. In *Crotalaria* poisoning it is usually the lungs that are affected, whilst in *Senecio* poisoning it is the liver. In this respect it is, however, noteworthy to refer to the results of Sir Arnold Theiler (1918) with *C. dura* on one of the bovines, which showed cirrhosis of the liver extending to all parts of the labule " somewhat resembling the early stages in the cirrhosis of ' dunsiekte ' (Seneciosis) in horses ". Steyn and de Kock (1932) also observed

slight cirrhosis in the liver of sheep drenched with *C. dura*. Regressive changes and cirrhosis of the liver in the horse poisoned with *C. dura* have also been observed by Steyn (1937).

The chemical investigation of *C. dura* and *C. globifera* led to the isolation of the same alkaloid from both plants. This alkaloid, however, differs from monocrotaline and the name, therefore, suggested is dicrotaline. Dicrotaline shows the same resemblance to the *Senecio* alkaloids as monocrotaline, in that on alkaline hydrolysis it also yields retronecine ($C_8H_{13}O_2N$) as the basic cleavage product. The acidic cleavage product, dicrotalic acid is a dibasic acid ($C_6H_{10}O_5$) differing from either monocrotalic or monocrotic acid.

Both plants when collected were in the flowering and seeding stage. *C. dura* was kindly supplied by Mr. Gray of Ixopo, Natal whilst *C. globifera* was collected near Pietermaritzburg, Natal. I am indebted to Mr. C. A. Smith of the *Natal Witness*, Pietermaritzburg for his able assistance in identifying and help in the collection of *C. globifera*.

Experimental.

The procedure adopted for the extraction of the alkaloid from both plants was the same. 10 Kilograms of the dried and finely ground plant material were continuously extracted for 24 to 36 hours, with 96 per cent. alcohol in a large soxhlet extraction apparatus. The alcohol extract was distilled under diminished pressure on a waterbath in order to remove all the alcohol. The residue was taken up in about 4 litres of 2 per cent. citric or hydrochloric acid and left to stand for a couple of days to allow the insoluble material to settle. The solution was filtered, using a large Buchner funnel and applying suction. The filtrate had a deep dark brown colour. This acid filtrate was repeatedly shaken out with ether until all the ether soluble acids were extracted. (This ether extract was worked up and from it dicrotalic acid was isolated.) After shaking the acid filtrate with ether it was shaken with chloroform, until the chloroform shakings were not too deeply coloured. The acid filtrate was then made distinctly alkaline with ammonia. The alkaloid was now shaken out with either chloroform or ether. The chloroform or ether extract was concentrated by distilling off the greater part of the solvent under diminished pressure on a steambath. The last traces of the solvent were removed by evaporating in front of a fan. A brown syrupy mass, which showed no inclination to crystallize was obtained. All further attempts to induce crystallization by further purifications failed. The yield of the crude alkaloid from *C. dura* was 0·27 per cent. whilst *C. globifera* gave only 0·18 per cent.

Preparation of Dicrotaline-hydrochloride.

Since all the attempts to crystallize the free alkaloid failed, the hydrochloride was tried. The alkaloid extract was neutralized against N. hydrochloric acid, using congo red as an external indicator. The neutralized solution was filtered and allowed to evaporate to dryness in front of a fan. From this on concentration, crystals separated. The crystalline alkaloid hydrochloride was purified by crystallization from 96 per cent. alcohol. After several recrystallizations the pure alkaloid hydrochloride was obtained. M.P. 258°–260° (corr.) with decomposition. (With regard to the melting point of the alkaloid hydrochloride it was observed that the initial alkaloid

hydrochloride obtained in a fairly pure state melted at 200° (corr.) with decomposition. From this experience it would appear that the alkaloid exists in two modifications, the higher melting form being probably the most stable. Later attempts to obtain the 200° melting form again, failed.)

Dicrotaline hydrochloride crystallizes from 96 per cent. alcohol either in needles or prisms. It has even been observed that it would crystallize in needles and on standing a day or two, the needles would redissolve and prisms would separate in their place.

Rotation: 0·2062 gm. dicrotaline hydrochloride made up to 10 c.c. in water at 20° C.

$\alpha_D = + 0{\cdot}53 \quad l, 1; \quad [\alpha]_D^{20^\circ} = + 25{\cdot}7^\circ.$

Analysis:	% C	% H	% N
Found	53·10	6·99	4·52
Calculated for $C_{14}H_{19}O_5N.HCl$	52·91	6·34	4·41

Preparation of Dicrotaline-Picrate.

From the syrupy alkaloid obtained from the chloroform or ether extract the picrate was prepared by dissolving 1·0 gm. of the alkaloid syrup in 2 c.c. abs. alcohol and adding 20 c.c. of a saturated solution of picric acid in abs. alcohol. A yellow precipitate was obtained. The precipitate was washed with ether and dissolved in methyl alcohol. From the concentrated methyl alcohol solution the picrate separated in fine needles. The picrate was recrystallized several times from methyl alcohol.

M.P. 238°–240° (corr.) with decomposition.

Analysis:	% C	% H	% N
Found	47·39	4·31	10·30
Calculated for $C_{14}H_{19}O_5N.C_6H_3N_3O_7$	47·06	4·35	10·98

Preparation of the Free Alkaloid.

Since all the attempts to induce the crude alkaloid obtained from the chloroform or ether extract, to crystallize failed, the free alkaloid was prepared from the purified alkaloid hydrochloride, by dissolving in dilute ammonia and shaking out with chloroform. From this chloroform solution on slow evaporation, after several weeks, the free crystalline alkaloid was obtained. It is easily soluble in most organic solvents. After various attempts it was successfully crystallized from acetone-petroleum ether (B.P. 40–60°) mixture on keeping several weeks in an ice chest. M.P. $\pm$170° with decomposition. Due to the fact that it is so difficult to induce crystallization no further attempts have been made to purify the free alkaloid. It has also been observed that on keeping it decomposes and turns black.

Alkaline Hydrolysis of Dicrotaline-Hydrochloride.

For the hydrolysis, 3·0 gm. dicrotaline hydrochloride was dissolved in 50 c.c. water and refluxed with 9·0 gm. $Ba(OH)_2 \cdot 8H_2O$ for 2 hours. After cooling, the solution was neutralized with 1:4 H_2SO_4, using phenolphthalein as indicator. The $BaSO_4$ precipitate was centrifuged and the clear solution evaporated to dryness on a waterbath.

I. On the Basic Cleavage Product.

The above dried residue was extracted several times with 96 per cent. alcohol. On concentrating the alcohol solution, crystals separated. M.P. 162° C. (corr.). On further recrystallization, no change in the melting point was obtained. A mixed melting point with authentic retronecine hydrochloride, obtained from retrorsine, showed no depression.

Analysis:	% C	% H	% N
Found	50·31	6·89	7·61
Calculated for $C_8H_{13}O_2N.HCl$	50·13	7·36	7·31

Preparation of the Retronecine.

1·033 gm. Retronecine hydrochloride, obtained by the hydrolysis of dicrotaline hydrochloride, was dissolved in 5·3 c.c. N. NaOH, then evaporated to dryness in front of a fan and further dried in high *vacuo* over H_2SO_4. The dried material was repeatedly extracted with dry acetone. On slow evaporation of the acetone solution crystals separated. After recrystallization from acetone, it melted at 121° C. (corr.). A mixed melting point with authentic retronecine gave no depression of the melting point.

Rotation: 0·4044 gm. made up to 10 c.c. in abs. ethyl alcohol at 22° C.

$$\alpha_D = + 2{\cdot}07 \quad l, 1; \quad [\alpha]_D^{22^\circ} = + 51{\cdot}2^\circ.$$

This leaves no doubt that the basic cleavage product of dicrotaline on alkaline hydrolysis is retronecine.

II. On the Acidic Cleavage Product of Dicrotaline.—Dicrotalic Acid.

The dried residue of the hydrolysate, after it had been extracted with 96 per cent. alcohol, was dissolved in a small amount of water and acidified with dilute H_2SO_4 using congo red as an indicator. The precipitated $BaSO_4$ was centrifuged and the clear solution, which gave no further precipitation of Ba SO_4 on the addition of a drop of dilute H_2SO_4, was evaporated to dryness on a waterbath. The residue was taken up in ethyl acetate and dried over anhydrous Na_2SO_4. After filtering, the ethyl acetate solution was concentrated. Petroleum ether (B.P. 40–60°) was added until slight turbidity occurred. On standing, crystals separated in thin plates. After several recrystallizations from ethyl acetate-petroleum ether mixture, it melted at 109° C. (corr.).

Analysis:	% C	% H
Found	44·69	6·14
Calculated for $C_6H_{10}O_5$	44·44	6·22

Titrations with $\frac{N}{10}$ KOH proved dicrotalic acid to be dibasic. An acid identical with dicrotalic acid was isolated from the ether shakings of the acidified extract from both plants. A more detailed study on the chemical nature of dicrotalic acid will be published at a later date.

Summary.

The isolation of the alkaloid dicrotaline from *Crotalaria dura* and *Crotalaria globifera* has been described.

On alkaline hydrolysis dicrotaline yields retronecine and an unknown dibasic acid, dicrotalic acid.

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