LXXVII. EXPERIMENTS ON THE SYNTHESIS OF HYOSCYAMINE IN *ATROPA BELLADONNA*

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The recent observations of Klein & Linser [1932; 1933, 1, 2] on the betaines stachydrine and trigonelline in *Stachys palustris* and *Trigonella Foenum-graecum* point to the fact that these substances are intimately connected with protein metabolism. These workers showed that proline and ornithine when injected into the plant caused increases in the concentrations of stachydrine and trigonelline. Other amino-acids were without effect. They also found that when stems of *Nicotiana* were placed in a solution of proline an increase in nicotine content took place. Furthermore, as the result of experiments on the trigonelline content of various plants throughout their life history, Klein & Linser conclude that the fluctuations which were recorded indicated utilization of the alkaloid as a nitrogenous reserve. In a previous publication by the present writer [Cromwell, 1933] an account was given of a series of experiments made on *Berberis Darwinii* with the object of investigating the problem of the synthesis and function of the alkaloid berberine. The results of this work appeared to show that in *Berberis Darwinii* the alkaloid does not function as a nitrogenous reserve and that synthesis is dependent on condensation of sugar residues with protein intermediates. Although *Berberis Darwinii* provides satisfactory material for stem and root experiments on alkaloid content it is not an ideal plant for experiments on leaves for two reasons. Firstly, the half-leaf method of sampling cannot be adopted on account of the smallness of the leaves, and secondly the leaves contain large amounts of colouring matter and resins which create difficulties in analysis. It was therefore considered desirable to carry out experiments on an alkaloid-producing plant the leaves of which did not possess the disadvantages of *Berberis Darwinii*. Accordingly *Atropa Belladonna*, which possesses large bilaterally symmetrical leaves, was chosen with a view to extending and testing the theory that the alkaloid molecule is synthesized partly from sugar residues and partly from protein residues. All parts of *Atropa Belladonna* contain hyoscyamine which in the process of extraction is largely converted into its racemic modification atropine. Experiments have been carried out to determine the effects on the hyoscyamine content of (*a*) withdrawal of certain essential elements from the culture medium, (*b*) presentation of specific organic sources of nitrogen, (*c*) presentation of certain inorganic sources of nitrogen, (*d*) feeding with a combined solution of glucose and potassium nitrate, (*e*) etiolation, involving both feeding and starvation experiments.

Experimental

Material. The plants used throughout these experiments were raised from seed obtained from vigorous plants growing wild on the chalk outcrops at Drewton on the south-western slopes of the Yorkshire Wolds. The seeds were germinated in pans and the seedlings transferred to an open bed where they

remained until required for experiment. The experiments were divided into two groups: (I) experiments on whole plants and (II) experiments on leaves. Plants used in (I) were potted in coarse river sand which had been thoroughly washed free from salts and were grown throughout the experimental period in a greenhouse. Flower buds which appeared were removed. Plants used for (II) remained in the open bed and leaves were removed as required.

Methods of sampling and preparation of material. In the first series of experiments on whole plants samples of main roots, fibrous roots, stems and leaves were taken immediately prior to potting. The roots were washed free from sand in tap water and allowed to dry in the air. Stems and roots were then cut into small pieces, and 50 g. each of the main and fibrous roots and 25 g. each of the stems and leaves were weighed out and frozen in solid carbon dioxide for three hours. The tissues were allowed to thaw out and the sap expressed in a strong hand press. The residue was washed with $N/20$ sulphuric acid until the washings were free from alkaloid. In practice it was found that after four successive washings (with 10–15 ml. sulphuric acid) followed by expression the residue was free from hyoscyamine by Vitali's test. In the second series of experiments on leaves, the half-leaf method of sampling was adopted. Halves of the leaf blades immediately bordering on the midribs were removed for control experiments and the remaining halves placed with their petioles in distilled water or culture solution. The half leaves were frozen for two to three hours in solid carbon dioxide and ground to a fine powder in a mortar, the temperature of which was kept at approximately that of the frozen tissue. The leaf powder was next transferred to a fine-mesh silk bag, allowed to thaw out and the sap expressed. The cell residue was washed with $N/20$ sulphuric acid as in the first series of experiments. Leaf area was therefore the basis for the calculation of the alkaloid value.

Estimation of total alkaloids. The acid extract was made up to 100 ml. and transferred to a separating funnel. 20 ml. of 5 % ammonia solution were added and the alkaloids shaken out with 400 ml. of a mixture of four volumes of ether and one volume of chloroform to which two or three drops of octyl alcohol had been added to minimize the risk of emulsification. The extraction was repeated with a further 400 ml. of the mixture and the ether and chloroform distilled off on a water-bath. The residue was heated on a water-bath for 10 min. to drive off any traces of free ammonia present and a micro-Kjeldahl determination carried out after digestion by the phosphoric-sulphuric-persulphate method of Van Slyke [1927]. The alkaloid value in terms of hyoscyamine was calculated from the total nitrogen present and expressed as mg./100 ml. extract.

I. Cultural experiments on whole plants

The effects of withdrawal of essential elements

Plants were watered with culture solutions lacking (1) potassium and (2) nitrogen, for a period of 4 months from May to the end of August.

The plants starved of potassium showed very little active growth and towards the end of the experimental period the leaves showed a tendency to turn yellow and wither at the margins. The figures in Table I show that deprivation of potassium has virtually little effect on the alkaloid concentration of the various organs, and in most cases a slight decline is evident. The present experiments on *Atropa* bear out the results obtained from previous experiments on potassium deficiency in *Berberis Darwinii* where the alkaloid value remained substantially unaffected by deprivation of this element.

Table I. *Culture experiments with potassium-free and nitrogen-free solutions*

mg. alkaloid/100 ml. extract

Solution	Plant	Age years	Control samples: Main root	Control samples: Fibrous root	Control samples: Stems	Control samples: Leaves	After experiment: Main root	After experiment: Fibrous root	After experiment: Stems	After experiment: Leaves
1. No potassium	(*a*)	2	123·2	76·6	—	30·8	122·6	74·9	—	31·2
	(*b*)	2	136·2	97·0	37·2	22·3	134·1	96·1	31·2	22·3
	(*c*)	3	183·7	148·6	49·6	62·0	178·4	149·9	44·3	62·5
2. No nitrogen	(*a*)	3	173·5	48·7	22·6	44·8	173·4	49·6	—	—
	(*b*)	3	155·6	80·9	27·8	40·4	156·9	82·6	—	—
	(*c*)	3	162·6	65·8	31·9	39·2	167·1	66·1	—	—

Nitrogen starvation quickly checked the growth of plants. Leaves were cast and eventually the stems withered and died. Later, short shoots appeared from the stem bases, but made comparatively little growth. Roots alone remained for analysis. The results of analyses show that in spite of acute nitrogen starvation the concentration of alkaloid in the sap continued to increase. Qualitative tests revealed the presence of starch and absence of nitrate-nitrogen in the roots throughout the period of experiment. It would appear that the alkaloid does not act as a mobile nitrogenous reserve but that in its formation nitrogen is actually being removed from tissues where its presence would be essential for any growth to take place.

Culture experiments with organic nitrogenous substances

Plants of 2 and 3 years of age were watered with culture solutions containing (*a*) asparagine, (*b*) hexamine and (*c*) urea, as the sole source of nitrogen. The experiments were conducted over a period of 4 months from May to the end of August. In this set of experiments growth was in all instances remarkably pronounced, leaves, stems and roots showing abnormally strong development and throughout the period the plants remained vigorous and healthy. Qualitative tests showed the presence of nitrate in the saps of the three groups.

Table II. *Culture experiments with organic nitrogenous substances*

mg. alkaloid/100 ml. extract

Solution	Plant	Age years	Control samples: Main root	Control samples: Fibrous root	Control samples: Stems	Control samples: Leaves	After experiment: Main root	After experiment: Fibrous root	After experiment: Stems	After experiment: Leaves
1. Asparagine	(*a*)	2	127·2	89·1	21·8	31·7	150·7	104·9	25·3	48·8
	(*b*)	2	140·8	70·5	17·2	25·2	196·2	110·6	15·1	38·8
2. Hexamine	(*a*)	2	120·4	66·7	28·1	35·7	169·3	78·4	28·9	41·3
	(*b*)	2	136·2	58·4	12·9	20·3	140·4	61·9	14·5	25·9
3. Urea	(*a*)	2	144·5	99·2	18·2	41·2	142·9	98·3	19·4	41·0
	(*b*)	3	206·5	156·9	28·9	55·7	208·1	154·2	27·8	56·3
	(*c*)*	2	—	—	—	56·2	—	—	—	54·5

* Half-leaf method.

The effect of both asparagine and hexamine was to cause a definite rise in alkaloid values in all organs. The figures for urea are somewhat contradictory but show for the greater part that the alkaloid concentration after experiment tends to fall below that of the control values.

Culture experiments with inorganic nitrogenous substances

Plants of 2 and 3 years of age were watered with culture solutions containing (*a*) ammonium sulphate, (*b*) potassium and calcium nitrates (1 g. of each in 2 litres of solution) as the sources of nitrogen, for a period of 4 months, extending from June to October. In both sets of experiments growth was active throughout the period, being more pronounced in the ammonium sulphate than in the nitrate cultures. Nitrates were present in abundance in the sap of the plants receiving ammonium sulphate, while the sand in which they were growing showed traces only.

Table III. *Culture experiments with inorganic nitrogenous substances*

mg. alkaloid/100 ml. extract

			Control samples				After experiment			
Solution	Plant	Age years	Main root	Fibrous root	Stem	Leaves	Main root	Fibrous root	Stem	Leaves
1. Ammonium sulphate	(*a*)	2	140·1	93·2	12·2	10·2	165·2	107·3	16·5	24·7
	(*b*)	3	191·8	118·2	14·9	35·2	210·6	123·9	16·5	45·4
2. Potassium and calcium nitrates	(*a*)	2	131·8	120·7	20·4	39·6	132·1	119·7	20·7	39·2
	(*b*)	3	210·8	133·7	62·7	49·1	213·1	132·2	64·0	49·5
	(*c*)	3	215·5	116·1	26·4	38·9	218·9	113·2	24·7	39·2

Presentation of ammonium-nitrogen brought about an increase in concentration of alkaloid in all organs, whereas application of potassium and calcium nitrates resulted in little or no change in concentration, the tendency being in the direction of loss.

Culture experiments with combined glucose and inorganic nitrate solution in absence of light

Two plants (*a*) and (*b*) were placed in darkness and watered with a full culture solution containing 2 % glucose with potassium nitrate as the source of nitrogen for a period of 1 month. The plants possessed all the characteristic appearances of etiolation and many of the leaves were cast.

Table IV. *Culture experiments with glucose and inorganic nitrate solutions. Plants grown in dark*

mg. alkaloid/100 ml. extract

			Control samples				After experiment			
Solution	Plant	Age years	Main root	Fibrous root	Stem	Leaves	Main root	Fibrous root	Stem	Leaves
Glucose 2 % plus nitrate	(*a*)	2	127·4	86·1	30·6	31·9	190·4	101·1	28·2	45·3
	(*b*)	3	180·5	115·6	32·9	40·6	219·2	130·8	38·1	53·1

Both plants during the experimental period showed accumulation of alkaloid in all organs and the tissues contained an abundance of nitrate.

II. Experiments on leaves

Culture experiments on detached leaves in darkness

Groups (*a*) and (*b*) were placed with their petioles in a 2 % solution of glucose, and groups (*c*), (*d*) and (*e*) in a 0·2 % solution of potassium nitrate. Samples (*a*), (*b*), (*c*) and (*d*) were removed from the plants in the morning and sample (*e*) in the late afternoon.

Table V. *Culture experiments on detached leaves in darkness. Half leaves placed with petioles in culture solution*

mg. alkaloid/100 ml. extract

Culture solution	No. of leaves	Duration of exp. days	Time of day sample taken	Control halves	Experimental halves
(*a*) Glucose	42	3	Morning	19·4	20·3
(*b*) Glucose	42	5	,,	40·5	81·3
(*c*) Potassium nitrate	70	7	,,	34·5	23·2
(*d*) Potassium nitrate	42	7	,,	18·2	18·2
(*e*) Potassium nitrate	42	7	Afternoon	24·7	27·7

Glucose feeding in one group of leaves (*b*) was responsible for a marked increase in alkaloidal content, but in group (*a*) the rise was very slight. Only in one group (*e*) of nitrate feeding experiments was an increase of alkaloid recorded, and it is significant that this group of leaves was gathered and sampled late in the afternoon when the carbohydrate content would be high.

Starvation experiments on detached leaves

In the first series of experiments recorded in Table VI, detached leaves were placed in distilled water in darkness, thus being deprived of all sources of food supply. In the second series (Table VII), whole plants were placed in darkness after half-leaf samples had been removed. The synthesis of fresh carbohydrate was therefore inhibited, but translocation of reserve carbohydrate from the stems to the leaves and movement of salts to and from the leaves would still be possible to a certain extent.

Table VI. *Starvation experiments on detached leaves in darkness. Half leaves placed with petioles in distilled water*

mg. alkaloid/100 ml. extract

Exp.	No. of leaves	Duration of exp. days	Time of day sample taken	Control halves	Experimental halves
(*a*)	60	7	Morning	54·4	54·4
(*b*)	60	5	Afternoon	13·2	35·5
(*c*)	60	7	Morning	23·5	27·3

Table VII. *Starvation experiments on leaves attached to plants. Half-leaf samples were removed and plants placed in darkness*

mg. alkaloid/100 ml. extract

Exp.	No. of leaves	Duration of exp. (weeks)	Control halves	Experimental halves
(*a*)	70	2	49·6	56·1
(*b*)	60	2	38·8	50·9

The experiments on detached leaves showed that complete starvation resulted in an increase of alkaloid in two groups of leaves, the rise being especially marked in group (*b*) in which the leaves had been removed from the plants in the afternoon. In both experiments on attached leaves the alkaloid value was increased by etiolation.

Discussion

Theoretically the synthesis of alkaloids in the plant admits of two possibilities, namely, a primary or up-grade synthesis and a secondary or down-grade synthesis. Although much work has been carried out in an endeavour to obtain knowledge of the reactions which lead to the formation of alkaloids, little of a positive nature is yet known. The trend of opinion would seem to be that the alkaloid molecule is built up from products of protein breakdown and carbohydrate intermediates, i.e. the synthesis is secondary. Our very imperfect knowledge of the constitution and metabolism of proteins has greatly impeded progress in the elucidation of problems relating to the synthesis of secondary nitrogenous plant products. The experiments on *Atropa Belladonna* described in this paper point to the conclusion that intermediates or breakdown products of proteins are the essential building stones of the alkaloid molecule, and that carbohydrates or their derivatives play an important part in the synthesis. At present it is impossible to decide whether the carbohydrate residues or intermediates actually enter into the constitution of the alkaloid molecule, or whether the carbohydrate is essential merely for the purpose of supplying energy for the synthetic reactions leading to alkaloid formation. The experiments involving the withdrawal of potassium and nitrogen showed similar results to those obtained in previous experiments on *Berberis Darwinii* [Cromwell, 1933]. Potassium withdrawal for a period of 4 months was practically without effect on the alkaloid concentration. Recent work on the role of potassium in plant metabolism has shown that carbohydrate synthesis and translocation are greatly impaired by lack of this element [James, 1930; James & Cattle, 1933; Janssen & Bartholomew, 1930; Penston, 1931; Richards, 1932; Tincker & Darbishire, 1933], and the water content of the tissues of potassium-starved plants has in many instances been shown to be low [James, 1931; Morse, 1927]. Furthermore, various workers have reported an accumulation of soluble nitrogen and starch in potassium-deficient plants [Janssen & Bartholomew, 1929; Burrell, 1926; Nightingale *et al.* 1930] and James [1930] has shown that potassium acts as a catalyst in hydrolytic reactions. Thus, by favouring starch accumulation, low water content and paralysis of hydrolytic activity potassium deficiency would tend to lower the concentration of active carbohydrate residues. The importance of potassium in relation to protein synthesis is not well understood, but the evidence of accumulation of soluble nitrogen points to the fact that synthesis of protein is inhibited in the absence of potassium. It would appear, therefore, that potassium deficiency is unfavourable to alkaloid formation as the result of its inhibiting effect on protein synthesis and its disorganizing influence on carbohydrate metabolism and translocation. The experiments on withdrawal of nitrogen indicated clearly that lack of this element does not check the synthesis of alkaloid, and one is justified in assuming that increase of alkaloid under these conditions must necessarily be due to synthesis from products of protein breakdown, which would take place to a great extent in nitrogen-starved plants. Mothes [1928] has stated that conditions unfavourable to the growth of tobacco plants result in continued production of nicotine. The cultural experiments in which asparagine, hexamine and urea were fed to the plants appeared to show that when certain nitrogenous substances are presented, alkaloid synthesis may result from direct combination of these substances or their derivatives with other active intermediates or residues. On the other hand asparagine feeding may lead to increased protein synthesis, and rise in alkaloid value may be referred to subsequent breakdown of the excess protein. In the tissues hexamine may yield amines

which may play the part of alkaloid intermediates. The action of urea in stimulating the growth of the plants to which it was fed was well marked, but apparently urea feeding does not stimulate alkaloid formation. The abundance of nitrate in the sap of the urea-fed plants indicates that urea, possibly by the action of urease, undergoes decomposition with the formation of ammonia. The plants, however, were not tested for this enzyme and further experiments are necessary before any conclusions on the behaviour of urea can be reached. If, as is supposed, urea undergoes rapid decomposition followed by oxidation of the ammonia to nitrate the application of urea would lead to conditions approximating to those of nitrate feeding. Calcium and potassium nitrates, when presented as the sole sources of nitrogen, were virtually without effect on the alkaloid concentration, as the slight rise recorded would presumably take place in the growth of the plants under normal nutritional conditions. A high concentration of inorganic nitrate therefore is not conducive to alkaloid synthesis. In this connexion Mothes [1928] found that in tobacco plants where abundance of inorganic nitrogen was present nicotine synthesis was inhibited. The experiments on ammonium sulphate feeding showed that this compound when presented as the sole source of nitrogen caused an increase in alkaloid production. It is difficult to reconcile this result with the results of urea feeding where it is presumed ammonia would be present as the result of decomposition of urea, and further work is necessary to throw more light on the fate of the ammonium radicle in these experiments. Feeding with glucose in conjunction with inorganic nitrate in darkness brought about a definite accumulation of alkaloid in the tissues. Under these conditions it would be expected that protein degradation would tend to be checked by the presence of ample respiratory material in the form of glucose. Examination of the soluble nitrogen showed that amino-acids and asparagine were present in large amounts, but the ammonia value was negligible (unpublished data). The presence of glucose appears to prevent the accumulation of ammonia, if protein breakdown has proceeded to this extent, by facilitating the production of asparagine. Alternatively the presence of glucose may prevent complete breakdown of the protein, and amino-acids would therefore accumulate. From these experiments the inference is drawn that circumstances which lead to the accumulation of protein intermediates provide the optimum conditions for alkaloid synthesis provided that carbohydrate is present. The importance of carbohydrate is clearly demonstrated by the experiments on detached leaves, which when kept in darkness gave increased alkaloid yield only in the presence of carbohydrate. Leaves fed with glucose showed an increase in alkaloid, whereas leaves fed with nitrate showed an increase only when a certain amount of carbohydrate was available. Leaves gathered in the early part of the morning would possess a low carbohydrate value, and nitrate feeding was responsible for little or no stimulation of alkaloid production. On the other hand, leaves which were gathered in late afternoon would possess a much higher carbohydrate content, and nitrate feeding of these leaves brought about an increased yield of alkaloid. Starvation experiments on detached leaves also support the view that carbohydrate is essential to alkaloid synthesis, for leaves detached in the early part of the morning and placed in distilled water in darkness revealed a slight increase of alkaloid only, whereas in leaves gathered in late afternoon a considerable increase occurred. Again, starvation experiments on leaves attached to the plants, the roots and stems of which contained reserves of carbohydrate capable of translocation to the leaves, gave indication of a consistent rise in alkaloid content.

The scope of the experiments carried out on *Atropa Belladonna* being some-

what restricted, it is not possible to make assumptions regarding the mode of synthesis of alkaloid, but the evidence obtained points to the fact that carbohydrate and protein residues are of primary importance. The suggestion is put forward that the function of alkaloids may be analogous to that suggested for asparagine by Prianischnikoff [1924, 1, 2] and Chibnall [1924], who consider that asparagine acts as a means of combining free ammonia which if present in the tissues in large amounts would prove toxic. These authors suggest that the ammonia bound as asparagine may be utilized in protein synthesis. The formation of alkaloids likewise may remove from the tissues certain residues of protein breakdown which if present in any degree of concentration would be toxic. Tryptophan, proline, pyrrolidine and amines such as tetramethylenediamine and pentamethylenediamine may possibly be substances of this nature, and it is noteworthy that the nuclei of these compounds enter into the constitution of certain groups of alkaloids. Furthermore, injection of certain of these compounds e.g. proline, results in increased alkaloid production [Klein & Linser, 1933, 2]. The work of Klein & Bartosch [1928] on the behaviour of ricinine in germinating seeds of *Ricinus* suggests that ricinine is synthesized at the growing points of the young shoot from degradation products of the reserve protein of the seed, and Mothes [1928] has shown that nicotine is present to a greater extent in old than in young leaves of tobacco plants, i.e. where protein breakdown is more pronounced. Etiolation, a condition favouring protein breakdown, also leads to increased alkaloid synthesis [Cromwell, 1933; Klein & Linser, 1932; 1933, 1; Weevers, 1932–33]. It is known that reserve proteins of plants differ in constitution from protoplasmic proteins, and it may be suggested that as the result of this difference certain residues remain in the tissues when storage protein is broken down and re-synthesized to protoplasmic protein. These residues present in the free state may be toxic to the plant and accordingly combination with carbohydrate or other active residues may take place resulting eventually in the formation of alkaloids. Moreover, fluctuations in alkaloid content of various organs may in many instances be due to the transformation of one form of protein into the other, certain portions of the alkaloid nucleus taking part in the transformation. An explanation of the restricted distribution of alkaloids may be forthcoming when it is considered that proteins of different plants vary in constitution; only certain reserve proteins therefore possessing a potential alkaloid nucleus would contribute to the synthesis.

Further work is being carried out with a view to obtaining more detailed knowledge of the intermediates involved in alkaloid synthesis and their relation to the general metabolism.

Summary

1. Culture experiments have been carried out on plants of *Atropa Belladonna* to determine the effects of withdrawal of essential elements and of presenting nitrogen in different forms. Potassium withdrawal was found to be without effect on the alkaloid content. During the period of nitrogen withdrawal formation of alkaloid continued. Presentation of nitrogen in the forms of asparagine, hexamine and ammonium sulphate brought about a rise in alkaloid value, while presentation in the forms of calcium and potassium nitrates and urea caused little or no alteration.

2. Plants grown in darkness and fed with a solution containing potassium nitrate and glucose showed increased alkaloid production.

3. Detached leaves in darkness were placed with their petioles in (*a*) glucose solution, and (*b*) potassium nitrate solution. Increase of alkaloid resulted in

leaves fed with glucose, but leaves fed with potassium nitrate showed a rise only if a reserve of carbohydrate was present.

4. Detached leaves placed in darkness with their petioles in distilled water gave a high yield of alkaloid when carbohydrate reserves were available.

5. Starvation experiments on leaves attached to the plant showed an increase in alkaloid.

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REFERENCES

Burrell (1926). *Bot. Gaz.* **82**, 320.
Chibnall (1924). *Biochem. J.* **18**, 395.
Cromwell (1933). *Biochem. J.* **27**, 860.
James (1930). *Ann. Bot., Lond.*, **44**, 173.
—— (1931). *Ann. Bot., Lond.*, **45**, 425.
—— & Cattle (1933). *Biochem. J.* **27**, 1805.
Janssen & Bartholomew (1929). *J. agric. Res.* **38**, 447.
—— —— (1930). *J. agric. Res.* **40**, 243.
Klein & Bartosch (1928). *Östr. bot. Z.* **77**, 241.
—— & Linser (1932). *Hoppe-Seyl. Z.* **209**, 75.
—— —— (1933, 1). *Planta*, **19**, 366.
—— —— (1933, 2). *Planta*, **20**, 470.
Morse (1927). *J. agric. Res.* **35**, 939.
Mothes (1928). *Planta*, **5**, 563.
Nightingale, Schermerhorn & Robbins (1930). *Bull. N.J. agric. Exp. Sta.* No. 499.
Penston (1931). *Ann. Bot.* **45**, 673.
Prianischnikoff (1924, 1). *Rev. gén. bot.* **36**, 108.
—— (1924, 2). *Biochem. Z.* **150**, 407.
Richards (1932). *Ann. Bot.* **46**, 367.
Tincker & Darbishire (1933). *Ann. Bot.* **47**, 27.
Van Slyke (1927). *J. biol. Chem.* **71**, 235.
Weevers (1932–33). Die Pflanzenalkaloide. Extrait du *Rec. Trav. bot. néerland.* **30**.