THE INACTIVITY OF 1-DOCOSANOL IN SOME PLANT GROWTH TESTS IN RELATION TO THE AUXIN OF MARYLAND MAMMOTH TOBACCO

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Summary

In extracts of shoots of *Nicotiana tabacum* cv. Maryland Mammoth, an auxin was detected with the same chromatographic properties as 3-indolylacetic acid (IAA). This auxin promoted cell extension in *Avena* coleoptile and first internode sections and was active in the *Avena* curvature test.

The long-chain fatty alcohols 1-docosanol and 1-octadecanol were found to be inactive in *Avena* coleoptile extension and *Avena* coleoptile curvature tests. 1-Docosanol was inconsistently active in promoting the extension of *Avena* first internode sections and the increase in wet weight of isolated tobacco stem pith.

It is argued that an auxin can be extracted from Maryland Mammoth plants, which is probably IAA and not a long-chain fatty compound. The claim in the literature that IAA is absent from this tobacco and that the auxin activity detected in extracts is due to substances similar to 1-docosanol must be reconsidered.

I. INTRODUCTION

The nature of the native auxins of plants is a subject actively argued in the literature (Bentley 1958). The substances originally proposed, namely auxins *a* and *b*, have not been found in plants. 3-Indolylacetic acid (IAA) was next proposed as the native auxin (Bonner and Bandurski 1952). But, recently, the universality of IAA as the native auxin has been challenged on two counts: (i) many substances exhibiting auxin activity, other than IAA, have been detected in plant extracts and some of these are not indole derivatives; (ii) IAA has been claimed to be absent from a number of plant tissues (Bentley 1958).

A plant which is involved in the current controversy on both counts is the Maryland Mammoth variety of tobacco. First Vlitos, Meudt, and Beimler (1956*a*, 1956*b*) reported their inability to detect IAA on paper chromatograms of extracts of leaf, stem, root, or apical tissue. Then growth activity at the same R_F value as IAA was detected in extracts of leaves and stems by Kefford and Helms (1957) and in ovaries by Lund (1956). Vlitos and Meudt (1957) then argued that the activity so detected was not due to IAA, but to a non-indole auxin. Subsequently Crosby and Vlitos (1959, 1961) found part of the non-indole auxin activity in Maryland Mammoth extracts to be due to 1-docosanol. Another active substance has yet to be definitely characterized, but it is also a long-chain fatty compound.

In this paper the activities of 1-docosanol, the related alcohol 1-octadecanol, and an extract of Maryland Mammoth tobacco shoots are compared in three *Avena* growth tests. In case 1-docosanol has some specificity towards the species from which it was extracted, its activity in tobacco pith growth is also investigated.

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II. Materials and Methods

(*a*) *Extraction and Chromatography of the Tobacco Auxin*

Nicotiana tabacum L. cv. Maryland Mammoth plants were grown in soil in a glasshouse for 7 weeks until they were approximately 9 in. high and were entering the phase of rapid stem elongation. The entire tops were harvested, and acidic, ether-soluble substances were extracted by the method of Kefford (1955). These substances were chromatographed on a large scale on sheets of Whatman No. 3MM paper using

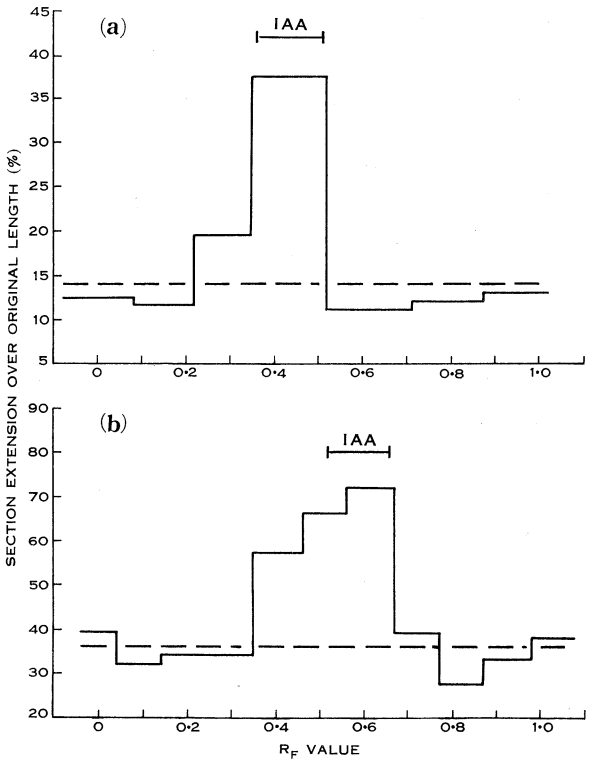

Fig. 1.—Activities in *Avena* first internode extension (*a*) and *Avena* coleoptile extension (*b*), shown as histograms, of squares cut from chromatograms of extracts of Maryland Mammoth tobacco equivalent to 400 g (*a*) and 1000 g (*b*) of fresh tissue. Chromatographic solvents were isopropanol–ammonia (*a*) and water (*b*). The broken lines represent the extension of controls. The positions of the IAA marker spots are shown.

isopropanol, water, and concentrated ammonia (10 : 1 : 1 v/v) as solvent. The portions of these chromatograms corresponding in R_F value with IAA were eluted with boiling ether. The substances eluted were rechromatographed on Whatman No. 1 paper in one of the following solvents: water, petroleum ether saturated with water, or t-butanol–concentrated ammonia (10 : 1 v/v). Portions of these chromatograms were assayed directly or were eluted with ether and the eluted substances were assayed.

(*b*) *Dispersion of Long-chain Alcohols in Water*

Samples of the long-chain fatty alcohols 1-docosanol and 1-octadecanol were supplied by Dr. D. G. Crosby, Union Carbide Chemicals Company, U.S.A. Emulsions of the alcohols were produced by the methods of Crosby and Vlitos (1959) or Stowe (1960).

(*c*) *Biological Assays*

For the *Avena* tests, *Avena sativa* L. cv. Siegeshafer from Svalöf was used and for the tobacco pith test, *N. tabacum* cv. Wisconsin Havana 38 was used. The *Avena* coleoptile section extension test and the direct biological assay of chromatograms with this test were done as described by Kefford (1955) except that the

TABLE 1

AVENA COLEOPTILE CURVATURE PRODUCED BY ELUATES OF PORTIONS OF CHROMATOGRAMS OF MARYLAND MAMMOTH TOBACCO EXTRACT

Extracts equivalent to 120 g (test 1) and 250 g (test 2) of fresh tissue. Chromatographic solvents: test 1, isopropanol–ammonia, IAA R_F range 0·35–0·40; test 2, t-butanol–ammonia, IAA R_F range 0·0–0·13

Test No.	R_F Range Eluted	Curvature of Eluate (degrees)	Curvature of Control (degrees)	Curvature of IAA at Concentration of $7{\cdot}5 \times 10^{-8}$ g/ml (degrees)
1	0·30–0·35	−0·9	−0·6	−30·2
	0·35–0·40 (IAA)	−7·9	−0·6	−30·2
	0·40–0·50	−0·0	−0·6	−30·2
2	0·0–0·13 (IAA)	−15·4	−1·4	

section growth medium contained 2% sucrose, 10^{-3}M phosphate–citrate buffer of pH 5, and 10^{-5}M $CoCl_2$ after Nitsch and Nitsch (1956). The *Avena* first internode section extension test and the *Avena* coleoptile curvature test were done as described by Nitsch and Nitsch (1956) and Zwar and Rijven (1956) respectively. The tobacco stem pith assay was done under sterile conditions. Pieces of pith 2 by 5 by 10 mm were supported on filter paper in a test tube containing 1 ml of a basal medium of macro- and micro-salts, vitamins, and sucrose. The addenda to the basal medium included 1-docosanol at three or four concentrations, 10^{-5}M IAA, and a kinin from apple fruitlets described by Goldacre and Bottomley (1959). Increase in wet weight of the pith blocks was measured after 21 days.

III. RESULTS

(*a*) *Growth Activity of the Auxin in Maryland Mammoth Tobacco Corresponding in* R_F *Value with IAA*

On paper chromatograms of the acidic, ether-soluble substances extracted from Maryland Mammoth tobacco leaves and stems, the region of greatest promotion of *Avena* coleoptile section extension (Kefford and Helms 1957) and *Avena* first internode

section extension (Fig. 1(*a*)) corresponded in R_F value with IAA. This region of chromatograms (e.g. R_F 0·35–0·52 in Fig. 1(*a*)) was eluted and the eluted substances were rechromatographed in three solvents. These chromatograms were assayed directly with *Avena* coleoptile sections or substances eluted from portions were assayed in the *Avena* coleoptile curvature test. For the three solvent systems the only region of promotion of coleoptile section extension corresponded in R_F value with IAA. This fact is illustrated in Figure 1(*b*) where water was the solvent and a large amount of eluate was applied to the chromatogram, in an attempt to expose minor regions of growth activity. On this chromatogram some tailing of the growth activity occurred. Activity in the *Avena* coleoptile curvature test also occurred on chromatograms at R_F values corresponding with IAA (Table 1).

TABLE 2

AVENA COLEOPTILE SECTION EXTENSION IN SOLUTIONS OF 1-DOCOSANOL OR 1-OCTADECANOL WITH OR WITHOUT IAA

Increments in section length are expressed as a percentage of the increment in buffer plus sucrose or buffer plus sucrose and IAA. The increment in buffer plus sucrose was approximately 10% of the original length (6 mm). The increment in the presence of IAA was approximately 500% of the increment in the absence of IAA

Alcohol Tested	Concentration (p.p.m.)	Buffer plus Sucrose	Buffer plus Sucrose plus 10^{-5}M IAA
1-Docosanol	0·1	86	
	1·0	92	88
	10·0	100	81
	100·0	72	77
1-Octadecanol	0·1	105	
	1·0	81	93
	10·0	100	91
	100·0	100	84

(*b*) *Growth Activity of the Long-chain Alcohols*

The activities of emulsions of 1-docosanol and 1-octadecanol were tested, in the presence and absence of IAA, in the *Avena* coleoptile section, first internode section, and coleoptile curvature tests. The results of some experiments are shown in Tables 2, 3, and 4. Growth promotion was detected only on one occasion with 100 p.p.m. of 1-docosanol in the absence of IAA using the *Avena* first internode test (Table 3). The effect of 1-docosanol upon the wet weight of tobacco pith blocks in the absence and presence of IAA and in the presence of IAA plus the kinin from the apple fruitlets is shown in Table 5. The results were variable. 1-Docosanol promoted wet weight increase in the absence and presence of IAA in experiment 1, but not in experiment 2; and promoted in the presence of IAA plus kinin in experiment 2 but not in experiment 1.

IV. Discussion

An auxin has been detected in Maryland Mammoth tobacco plants which is active in promoting the extension of *Avena* coleoptile and first internodes sections and is active in the curvature of decapitated *Avena* coleoptiles. To obtain "deep" curvatures of *Avena* coleoptiles, as were obtained with the present tobacco auxin, a substance must be moved down the coleoptile by the auxin transport system. The auxin transport system has a much higher chemical specificity than the auxin growth reaction (Zwar and Rijven 1956), and so far IAA is the only natural auxin

Table 3

Avena first internode section extension in solutions of 1-docosanol or 1-octadecanol with or without IAA

Increments in section length are expressed as a percentage of the increment in buffer plus sucrose or buffer plus sucrose and IAA. The increment in buffer plus sucrose was approximately 10% of the original length (6 mm). The increment in the presence of IAA was approximately 400% of the increment without IAA

Alcohol Tested	Concentration (p.p.m.)	Experiment 1		Experiment 2	
		Buffer plus Sucrose	Buffer plus Sucrose plus 10^{-6}M IAA	Buffer plus Sucrose	Buffer plus Sucrose plus 10^{-7}M IAA
1-Docosanol	0·1	49			
	1·0	98	100		
	10·0	100	140		90
	50·0			90	
	100·0	465	100	90	100
	200·0			95	
	400·0			85	
1-Octadecanol	0·1	98			
	1·0	100	105		
	10·0	115	115		100
	50·0				97
	100·0	82	140	90	83
	200·0			95	
	400·0			90	

known to be transported. There is thus strong biological evidence for the auxin from Maryland Mammoth tobacco being IAA. Further evidence is found in the correspondence of R_F value between IAA and the tobacco auxin in a number of solvent systems and after double chromatography.

It has been suggested, on the basis of activity in the *Avena* first internode test, that 1-docosanol is a Maryland Mammoth tobacco auxin (Vlitos and Crosby 1959; Crosby and Vlitos 1959, 1961). We have been unable to demonstrate consistent growth promotion in this test by 1-docosanol. Crosby and Vlitos (personal

communications) also report that the response of *Avena* first internodes to fatty alcohols is variable. We have found the effect of 1-docosanol upon tobacco pith sections to be variable. The effect upon *Avena* coleoptiles in a number of section extension and curvature tests was nil. The most obvious source of variability in the biological assays is the plant material. It would appear that small differences in the mode of preparation of a tissue can make it self-sufficient or not for the growth factor or process stimulated by long-chain fatty compounds.

TABLE 4

EFFECTS UPON AVENA COLEOPTILE CURVATURE OF THE ADDITION OF 1-DOCOSANOL OR 1-OCTADECANOL, WITH OR WITHOUT IAA, TO THE UNILATERALLY PLACED AGAR BLOCK

Alcohol Tested	Concentration (p.p.m.)	Curvature (degrees)	
		Agar Alone	Agar plus 5×10^{-7}M IAA
1-Docosanol	1·0	+0·6	
	10·0	+0·8	−20·3
	100·0	+0·1	
	200·0	+0·8	
1-Octadecanol	10·0	+0·8	
	100·0	+0·4	
Control		−0·4	−19·9

This paper presents no evidence for the absence of long-chain fatty compounds in the present extracts of Maryland Mammoth tobacco; nor is there any doubt that substances of the long-chain type can promote cell enlargement under certain conditions. Stowe (1960) has shown that a variety of such compounds, and particularly fatty acid esters, promote the extension of pea stem sections, but only in the presence of IAA. On the other hand, no evidence has been obtained which could suggest that the present Maryland Mammoth tobacco auxin with R_F value corresponding with IAA is a compound of the same class as 1-docosanol.

Since it is clear that the claimed absence of IAA from Maryland Mammoth tobacco tissues must be reconsidered, other reports of the absence of IAA might also be re-examined.

In a review of natural auxins, Bentley (1958) reported the absence of IAA from 29 different tissues including the tobacco tissues discussed above. Andreae (1959) pointed out that for the tissues in this list with which he was associated (viz. oat, maize, barley coleoptiles; pea, sunflower, cucumber, buckwheat hypocotyls; potato sprouts; pea, tomato, cabbage stems) too little material was assayed to expect to detect IAA (Good, Andreae, and van Ysselstein 1956). It is possible that

too little tissue was assayed also in the case of soybean, spinach, barley, and tomato leaf tissue, investigated by Vlitos and Meudt (1953, 1954). For four tissues listed by Bentley, namely blackcurrant berries, cabbage, Brussels sprouts, and apple fruits, there is evidence for the presence of IAA (Bentley 1958; von Bargen 1960). Thus these claims of absence must be considered unproven. The claim of the absence of IAA from *Ustilago* tumors and healthy tissues of maize has been withdrawn (Turian and Hamilton 1960). There remain then only six tissues (maize coleoptiles and roots; apple leaves; tomato fruits and roots; Jerusalem artichoke gall tissues) of the original 29 for which there is, as yet, no reason to doubt a claim of the absence of IAA. Hence, the doubt about the universality of IAA as native auxin, on the grounds of its absence from a number of tissues, is not strongly founded.

TABLE 5

INCREASE IN WET WEIGHT OF TOBACCO PITH BLOCKS IN SOLUTIONS OF 1-DOCOSANOL WITHOUT OR WITH 10^{-5}M IAA OR AN EXTRACT OF APPLE KININ

Increments in wet weight are expressed as a percentage of the increment on basal medium, basal medium plus IAA, or basal medium plus IAA and kinin extract. The increment in basal medium was approximately 25% of the original wet weight (150–200 mg). The increment in the presence of IAA or of IAA plus kinin was approximately 500% of the increment in the absence of addenda to the basal medium. The period of growth was 21 days

Concentration of 1-Docosanol (p.p.m.)	Experiment 1			Experiment 2		
	Basal	Basal + IAA	Basal + IAA + Kinin	Basal	Basal + IAA	Basal + IAA + Kinin
1	260*	90	130	70	50	130*
3	160	150**	110	90	40	110
10	160	160**	110	70	70	140**
30	250*	120*	40			

* Difference from control without 1-docosanol significant at $P = 0{\cdot}05$.

** Difference from control without 1-docosanol significant at $P = 0{\cdot}01$.

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