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HORMONAL CONTROL OF STEROL BIOSYNTHESIS IN *PHASEOLUS AUREUS*

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Abstract—Naphthalene acetic acid increased the sterol content of mung bean hypocotyl sections mainly in the zone of elongation growth. The increased sterol synthesis can be explained by a stimulated conversion rate of cycloartenol into sterols. During the 20-hr incubation period the stigmasterol: sitosterol ratio increased considerably.

INTRODUCTION

HITHERTO few authors have studied the regulatory effects of plant hormones on the biosynthesis or metabolism of steroids.[1–4] Mercer and Pughe[1] found no inhibitory effect of abscisic acid (±)-ABA on sterol synthesis in maize leaf tissue. Heble *et al.*,[2] studying the hormonal control of steroid synthesis in *Solanum xanthocarpum* tissue cultures, found a reduction of sitosterol content on treatment with indole acetic acid and indole butyric acid. Recently we reported some investigations on the variation in sterol levels and composition of etiolated mung bean plants.[5] Sterol synthesis occurred in growing tissues, and in older tissues a much lower rate of synthesis was found, associated with an increase in the stigmasterol: sitosterol ratio. In the present study the influence of α-naphthalene acetic acid-(NAA) on sterol biosynthesis in conjunction with its promoting effects on growth processes has been investigated in the 1-, 3- and 5-cm of etiolated mung bean hypocotyls.

RESULTS AND DISCUSSION

Table 1 shows the fresh and dry weights of the different hypocotyl sections. NAA stimulated the elongation growth of the 1-cm by about 45% and increased the fresh wt by about 70% (results not published). In the 3- and 5-cm there was no increase in length, and little increase in fresh wt. There was also pronounced stimulation (56%) of the free sterol fraction by NAA in the 1-cm (zone of elongation growth), but no significant stimulation in the older tissues of the hypocotyl (3- and 5-cm).

Table 2 shows that the ratio stigmasterol: sitosterol increases after an incubation period of 20 hr. In the 1-cm the ratio changes from 0·30 to 1·23 in the control sections and to 1·00 in the sections treated with NAA, in the 3-cm from 0·87 to 1·89 and 1·62 and in the 5-cm from 1·00 to 1·75 and 1·66, in the control sections and those treated with

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[2] HEBLE, M. R., NARAYANASWAMI, S. and CHADHA, M. S. (1971) *Phytochemistry* **10**, 2393.
[3] HARDMAN, R. and WOOD, C. N. (1971) *Phytochemistry* **10**, 757.
[4] KOPCEWICZ, J. (1972) *Biol. Plantarum (Praha)* **14**(3), 223.
[5] GEUNS, J. M. C. (1973) *Phytochemistry* **12**, 103.

NAA respectively. There is only a minor influence of NAA on the stigmasterol : sitosterol ratio. Treatment with NAA also results in a higher yield of 28-isofucosterol. The latter effect is probably due to the enhanced sterol synthesis, in which 28-isofucosterol is transformed into sitosterol, and ultimately is converted into stigmasterol.[6] [9] These results confirm our first observations concerning the increasing stigmasterol : sitosterol ratio in the older tissues of mung bean hypocotyls.[5] So far we have no explanation for this increasing ratio.

TABLE 1. FRESH AND DRY WEIGHTS OF THE VARIOUS SECTIONS OF ETIOLATED *Phaseolus aureus* HYPOCOTYLS

Zone (cm)	Treatment	fr. wt (g)	Dry wt (g)	μg sterols/g dry wt
1	Control	8·6	0·44	1820
	NAA 2 ppm	9·9	0·39	2890
3	Control	9·8	0·48	1640
	NAA 2 ppm	10·6	0·50	1740
5	Control	12·8	0·65	1380
	NAA 2 ppm	11·8	0·66	1330
1	Before incubation	5·1	0·42	1360
3	Before incubation	9·2	0·60	970
5	Before incubation	12·4	0·71	840

The amounts of free sterols were determined by GLC.

The stimulatory effect of NAA on sterol synthesis could also be demonstrated in incorporation experiments with [2-^{14}C]-MVA. From Table 3 it is seen that NAA has a stimulatory effect on incorporation of label in the sterol fraction. Since in all cases the 4,4-dimethylsterols are equally labelled, the rate limiting step in sterol synthesis is not the synthesis of 4,4-dimethylsterols, but the conversion rate of the 4,4-dimethylsterols into sterols. The older the tissues (3- and 5-cm), the less the conversion of 4,4-dimethylsterols into sterols takes place. The ratio cpm sterols : cpm 4,4-dimethylsterols is a good measure for this conversion rate. From Table 3 we see then that NAA stimulated the conversion of 4,4-dimethylsterols into sterols, by which it can stimulate total sterol synthesis. The suggestion of the stimulation of the conversion rate is supported by the finding that the production of $^{14}CO_2$ is stimulated by NAA (Table 3). Rees *et al.*[10] studied the mechanism of cycloartenol formation and found that the ^{14}C of [2-^{14}C]-MVA was localized at C_1, C_7, C_{15}, C_{22}, C_{26} and 4 α-Me. Therefore the $^{14}CO_2$ produced probably comes from demethylation at C_4. The enhanced $^{14}CO_2$ production by NAA thus reflects the stimulated conversion of cycloartenol into sterols. NAA has little effect on the stigmasterol : sitosterol ratio, but enhances the total sterol fraction (Table 2). This also suggests that a common step before the formation of sitosterol and stigmasterol is influenced by NAA. This view is further supported by the distribution of radioactivity in the different sterols, where there is no difference between the labelling of sterols from the

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[7] ALCAIDE, A., DEVYS, M. and BARBIER, M. (1970) *Phytochemistry* **9**, 1553.
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[9] KNAPP, F. F. and NICHOLAS, H. J. (1971) *Phytochemistry* **10**, 85.
[10] REES, H. H., GOAD, L. J. and GOODWIN, T. W. (1968) *Biochem. J.* **107**, 417.

control sections or from the sections treated with NAA (Table 4). However, between the different experiments there may exist differences which are probably due to a differing physiological state of the plant material.

TABLE 2. PERCENTAGE OF EACH STEROL IN THE STEROL FRACTIONS OF THE VARIOUS HYPOCOTYL SECTIONS

Zone (cm)	Treatment	Cholesterol	Campesterol	Stigmasterol	Sitosterol	28-Isofucosterol	Stigmasterol/ Sitosterol
1	Control	4	14	42	34	3	1·23
	NAA 2 ppm	3	12	37	37	9	1·00
3	Control	3	13	53	28	1	1·89
	NAA 2 ppm	3	13	47	29	5	1·62
5	Control	2	15	51	29	Trace	1·75
	NAA 2 ppm	3	14	50	30	1	1·66
1	Before Incubation	3	13	16	52	14	0·30
3	Before Incubation	3	14	34	39	8	0·87
5	Before Incubation	4	14	39	39	3	1·00

These results suggest that NAA stimulates sterol synthesis by increasing the conversion rate of cycloartenol into sterols. In a recent study, Hartmann and Benveniste[11] found that the rate limiting step in sterol biosynthesis lies in a step between cycloartenol and the sterols. Like Baisted[12] they suggest that the activity of *S*-adenosyl-methionine-Δ^{24} sterol methyl transferase could regulate sterol synthesis. This suggestion has still to be proven experimentally.

TABLE 3. RADIOACTIVITY RECOVERED IN THE ELUATES OF THE VARIOUS ZONES OF TLC

Zone	1-cm		3-cm		5-cm	
Treatment	Control	NAA	Control	NAA	Control	NAA
Sterols	100 ± 7	154 ± 10	43 ± 5	71 ± 5	19 ± 2	30 ± 4
4,4-Dimethylsterols	33 ± 5	32 ± 6	42 ± 4	55 ± 5	38 ± 3	38 ± 6
Squalene	29 ± 5	27 ± 3	23 ± 4	15 ± 4	24 ± 4	21 ± 5
Total incorporation in "neutral fraction"	271 ± 17	323 ± 17	263 ± 16	314 ± 13	225 ± 26	255 ± 18
Ratio: cpm sterols/cpm 4,4-dimethylsterols	3·0	4·7	1·0	1·3	0·5	0·8
$^{14}CO_2$ produced	19	29	6	10	11	17

The growth medium before each incorporation experiment contained 4 μM [2-^{14}C]-MVA (1680×10^3 cpm). After the incubation period of 20 hr about 60% of radioactivity was taken up by the tissues from each medium. The values are given in cpm $\times 10^{-3}$.

EXPERIMENTAL

Plant material. For each treatment, 200 5-day-old etiolated mung bean seedlings (*Phaseolus aureus* Roxb.) with a hypocotyl length of 6 cm were selected. The 1-, 3- and 5-cm from the top of the hypocotyls were cut out in green light and soaked in dist. H_2O for 1 hr. The dried sections were weighed before and after the incubation period of 20 hr. To determine the sterol composition before the incorporation experiments, 200 sections from each zone were extracted without incubation.

[11] HARTMANN, M.-A. and BENVENISTE, P. (1973) *Compt. Rend.* **276D**, 3143.
[12] BAISTED, D. J. (1971) *Biochem. J.* **124**, 375.

TABLE 4. DISTRIBUTION OF RADIOACTIVITY FROM [2-^{14}C]-MVA BETWEEN THE STEROLS OF THE DIFFERENT STEROL FRACTIONS

Zone (cm)	Treatment	Cholesterol		Stigmasterol + campesterol		Sitosterol + 28-isofucosterol	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
1	Control	5	2	38	53	56	44
	NAA 2 ppm	1	2	40	55	58	42
3	Control	13	5	31	47	55	47
	NAA 2 ppm	8	4	34	42	56	52
5	Control	23	16	23	28	53	55
	NAA 2 ppm	19	11	24	26	55	62

The incorporation in each sterol is given as a percentage of the total radioactivity recovered. Because of the lesser resolution in preparative GLC, campesterol and stigmasterol, respectively sitosterol and 28-isofucosterol were trapped together in the capillary tubes.

Incorporation experiments. The various hypocotyl sections were incubated in 30 ml [2-^{14}C]-MVA soln, sp. act. 0·25 mCi/mmol (4 μmol; dark, 23°) for 20 hr with continuous shaking with or without 2 ppm NAA in the presence of Streptomycin (10 mg/l). The $^{14}CO_2$ produced was collected on a strip of filter paper impregnated with 0·1 ml 4 N NaOH. After incubation, the sections were rinsed several times with dist. H_2O and the vol. made up to 100 ml. Aliquots were counted in a liquid scintillator.

Sterol extraction and analysis. 1 μCi ^{3}H-cholesterol was added to the homogenized plant materials to make corrections for losses during extraction procedure. The homogenates were Soxhlet extracted with Me_2CO for 20 hr. The extracts were fractionated between a satd. $NaHCO_3$ soln. and freshly dist. Et_2O. The Et_2O fraction contained, besides the free sterols, the steryl esters, but these were not further examined because of the very low incorporation of label into them. For further details see Ref. 5. For the quantitation by GLC we added to the eluted sterol band 0·2 ml of a cholestanylformate soln. (3 mg/10 ml) as GLC standard. Calibration curves of sterols were made using this standard; its retention time lies between that of cholesterol and that of campesterol. A sample of each sterol mixture was counted in a liquid scintillator to calculate the extraction efficiency by the channel ratio method. To measure the radioactivity incorporated in the different sterols, preparative GLC was carried out on a 3% OV17 column fitted with an effluent splitter (± 1/50), and compounds were collected as they were eluted from the column in glass capillary tubes at room temp. The eluates of these tubes were counted in a liquid scintillator. The values given in Tables 1–4 are the mean of at least three experiments.

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