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VARIATION IN THE ALLITOL CONTENT OF *ITEA* PLANTS DURING PHOTOSYNTHESIS

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Abstract—The allitol content of *Itea* leaves increases considerably during photosynthesis whereas the converse is true during metabolism in the dark. Allitol is thought to function in a reserve capacity.

DURING investigations of the D-glucitol content of various species of green leaves, Plouvier[1] isolated from acetone extracts of *I. ilicifilia* and *I. virginica* a crystalline polyol subsequently characterized as allitol. The yield of allitol from leaves was about 1·6 per cent and that from the stems 0·5–1·2 per cent (on a dry weight basis). As a preliminary to biosynthetic studies, we isolated allitol from alcoholic extracts of leaves of *I. ilicifolia* and *I. yunnanensis* and yields >6 per cent were obtained.[2] As these yields were much higher than those quoted by Plouvier, suggesting diurnal or seasonal variations, we have investigated the allitol content of *Itea* leaves in conjunction with a related study to be described later of the incorporation of $^{14}CO_2$ into allitol and D-allulose during photosynthesis.

Several leafy spurs of *I. ilicifolia*, collected in April, 1963, and previously kept in the dark for 15 hr, were allowed to photosynthesise in an atmosphere containing 0·5% v/v $^{14}CO_2$ for 1 hr followed by photosynthesis for further periods of up to 47 hr in a normal atmosphere. One of the samples was placed in the dark for 23 hr after the initial incorporation of $^{14}CO_2$. Crystalline allitol was isolated from both the alcoholic extracts of the leaves and of the stems. As was evident from melting point determinations and chromatograms, the purity of the almost colourless, initial product was high and hence the following discussion is based upon yields of this non-recrystallized product. D-Allulose was also isolated, as the crystalline di-*O*-isopropylidene derivative from selected extracts.

The yields of allitol from alcoholic leaf extracts showed a rapid increase during the first two hours of photosynthesis followed by a more gradual increase to 48 hr, while the yield from the "dark" experiment was very much lower than even the initial sample (Table 1). In contrast, the proportion of D-allulose in the leaves appeared to decrease with time of photosynthesis, the yield of di-*O*-isopropylidene D-allulose being greatest in the case of the "dark" experiment (the true allulose content of the leaves may well be 100 per cent or more higher than the values calculated from the yield of the di-*O*-isopropylidene derivative owing to losses incurred in isolation). The yields of allitol from stem extracts did not show a regular increase with time as did those of allitol from leaf extracts but the general trend was for the yield to increase with time of photosynthesis (Table 2). This data indicates that during prolonged photosynthesis, anabolism of allitol is favoured at the expense of D-allulose whilst the converse is true in the absence of sunlight.

[1] V. PLOUVIER, *Compt. Rend.* **249**, 2828 (1959).
[2] L. HOUGH and B. E. STACEY, *Phytochem.* **2**, 315 (1963).

In another experiment, leafy spurs collected in August, 1963, and previously kept in the dark for 15 hr, were allowed to photosynthesise for various periods of up to 2 hr. The allitol contents of the leaves once again increased with time of photosynthesis but the yields were

TABLE 1. YIELDS OF ALLITOL AND D-ALLULOSE ISOLATED FROM LEAVES OF *I. ilicifolia*

Duration of photosynthesis (hr)	Wt of alcohol-insoluble leaf residue (g)	Initial yield of allitol (mg)	Initial yield of allitol (mg per g of alcohol-insoluble leaf residue)	Initial yield of di-*O*-isopropylidene D-allulose (mg)	Initial yield of di-*O*-isopropylidene D-allulose (mg of D-allulose per g of alcohol-insoluble leaf residue)
1	1·308	42	33	17	9
2	1·345	92	71	5·6	3
6	1·306	61	49	1·6	0·9
8	1·272	104	85		
24	1·164	119	106	–	–
48	1·482	173	122	—	—
1 + 23 in dark	1·185	10	9	18·7	11

TABLE 2. YIELDS OF ALLITOL ISOLATED FROM STEMS OF *I. ilicifolia*

Duration of photosynthesis (hr)	Wt of alcohol-insoluble stem residue (mg)	Initial yield of allitol (mg)	Initial yield of allitol (mg per g of alcohol-insoluble stem residue)
1	335	10	33
2	373	22	66
6	361	15	46
8	311	15	54
24	441	15	38
48	290	16·5	63
1 + 23 in dark	323	5·4	19

much higher (Table 3) than those obtained in the previous experiment. This would seem to indicate that the leaves are more metabolically active in August than in April and recalls the wide seasonal variation in the mannitol content of fronds of the brown algae with a maximum about mid-summer.[3] In young fronds, as photosynthesis proceeds, the mannitol content rises from 5 to as much as 36 per cent of the dry weight of *Laminari cloustonii*. The concentration of mannitol within the frond is greatest at depths when the light intensity is optimal for photosynthesis. In contrast to the seaweeds, the mannitol content of olive trees increases during the Winter.[4] The leaves of *Cardenial jarmoides* contain no mannitol in Summer but accumulate the polyol during the Winter. Leaves detached from the plant in Summer synthesise and accumulate mannitol when placed in a cold environment: light has no apparent effect on the

[3] W. A. P. BLACK, *Marine Biol. Assoc. United Kingdom*, **33**, 49 (1954).
[4] R. NUCCORINI, *Ann. Chim. Appl.* **20**, 535 (1930).

process. The D-glucitol content of plum leaves has been investigated by Anderson *et al.*[5] who found that, under natural conditions, there was no diurnal variation, the D-glucitol content remaining constant at about 4·5 per cent. Thus, no generalization regarding the variation in hexitol content of leaves seems to be possible.

The marked decrease in allitol content of *Itea* leaves when kept in the dark was investigated further. Four leafy spurs of *I. ilicifolia* were allowed to photosynthesise for 16 hr and then placed in the dark. The yields of allitol, isolated from the leaves, showed a marked decrease

TABLE 3. YIELDS OF ALLITOL ISOLATED FROM LEAVES OF *I. ilicifolia*

Duration of photosynthesis (min)	Wt of alcohol-insoluble leaf residue (mg)	Initial yield of allitol (mg)	Initial yield of allitol (mg per g of alcohol-insoluble leaf residue)
15	912	40	46
30	745	50	69
45	931	62	69
60	781	61	81
120	452	52	120

TABLE 4. VARIATION IN ALLITOL AND ALLULOSE CONTENTS OF *Itea* LEAVES DURING METABOLISM IN THE DARK

Duration of metabolism (hr)	Wt of alcohol-insoluble leaf residue (g)	Initial yield of allitol (mg)	Initial yield of allitol (mg per g of alcohol insoluble leaf residue)	Yield of allulose-containing syrup (mg)	Yield of allulose-containing syrup (mg per g of alcohol-insoluble leaf residue)
0	1·23	191	155	74	60
4	0·935	132	141	114	120
8	1·36	138	101	126	93
24	0·94	63	67	189	200

with time of metabolism in the dark, the value after 24 hr being less than half the initial value (Table 4). These results serve to confirm the previous findings and indicate that allitol acts in a storage capacity, accumulating in the plant as an end product of photosynthesis and being metabolised during the hours of darkness.

EXPERIMENTAL

Photosynthetic Experiments

The apparatus was similar to that described by Folkes *et al.*[6] Steady illumination was provided by twelve 150 W tungsten filament lamps mounted between a reflecting surface

[5] J. D. ANDERSON, P. ANDREWS and L. HOUGH, *Biochem. J.* **81**, 149 (1961).
[6] B. F. FOLKES, A. J. WILLIS and E. W. YEMM, *New Phytol.* **51**, 31 (1953).

above and a glass-bottomed water tank below which was fitted with a constant level device and a steady flow of cold water to serve as a heat filter.[7]

Experiment I

Eight selected spurs, collected April, 1963, each bearing 8–9 leaves, were placed with cut ends in water and kept in the dark overnight (15 hr). The spurs were then transferred to a desiccator (4 l.) in which $^{14}CO_2$ was subsequently generated by the action of lactic acid (~ 25 ml; 80% v/v) on $Ba^{14}CO_3$ (145 mg; 1790 μc). After photosynthesis had been allowed to take place for 1 hr, one spur was immediately extracted as described below whilst another spur, with its cut end still under water, was kept for 23 hr in the dark. The remaining six spurs, their cut ends under water, were replaced under the light battery for different periods of time (Table 1).

After each period of photosynthesis, the leaves were rapidly detached from the stems; both were cut into small pieces and separately extracted with boiling ethanol (~ 75 ml and 40 ml respectively; 15 min). The residues were than exhaustively extracted with boiling methanol and each combined alcoholic extract made up to a known volume with methanol (leaves, 250 ml; stems 100 ml). The final alcohol-insoluble residues were dried at 60 . to constant weight (alcohol-insoluble leaf residue, C,44 per cent; alcohol-insoluble stem residue C,45 per cent).

Isolation of allitol and D-*allulose from leaf extracts.* A portion (240 ml; 96 per cent of total) was concentrated to a syrup and shaken with water (~ 20 ml) for 15- 30 min. After centrifugation, the supernatant was passed slowly through a pad of activated charcoal/Hyflo Supercell to give an almost colourless solution which on concentration yielded crystalline allitol.

The mother liquor from the crystallization of (crude) allitol was evaporated to a dry syrup and shaken with a mixture of anhydrous acetone (9 ml), anhydrous copper sulphate (1 g) and concentrated sulphuric acid (0·02 ml) for 48 hr. Crystalline di-*O*-isopropylidene D-allulose was eventually isolated from the leaves which had photosynthesised for 1, 2 and 6 hr respectively, and also from the leaves which had been allowed to metabolise in the dark for 23 hr but no crystalline derivative could be obtained from the remaining samples. The di-*O*-isopropylidene D-allulose was recrystallized from light petroleum (40-60°). The yields of allitol and di-*O*-isopropylidene D-allulose samples are given in Table 1. (Note—with extract of leaves which had photosynthesised for 6 hr, treatment with activated charcoal was not carried out until after isolation of crude allitol which in this case was light brown in colour in contrast to the almost white product obtained from other extracts.)

Isolation of allitol from stem extracts. A 90 ml-aliquot (i.e. 90 per cent of total) was concentrated to a syrup and crystalline allitol isolated as described above. Yields are recorded in Table 2.

Experiment II

Five leaf spurs (collected August, 1963) with ~ 10 leaves on each were treated in a manner similar to that described for Experiment I except that incorporation of $^{14}CO_2$ was terminated after 15 min. The spurs were finally extracted with alcohol after total photosynthetic periods of 15, 30, 45, 60 and 120 min respectively, and allitol isolated as before. Yields are recorded in Table 3.

Variation in allitol content of Itea *leaves during metabolism in the dark.* Four leafy spurs of

[7] P. ANDREWS and L. HOUGH, *J. Chem. Soc.* 4483 (1958).

I. ilicifolia (collected August, 1963) were allowed to photosynthesise under the light battery for 16 hr after which time one specimen was immediately extracted with boiling ethanol while the others were placed in the dark for various periods of time (4, 8 and 24 hr respectively). Allitol was isolated from the alcoholic extracts of the leaves in the usual way whilst the mother liquors (after removal of allitol by crystallization) were concentrated to a dry syrup to give some indication of the amount of allulose present. Paper chromatography of the extracts prior to crystallization of allitol gave a general indication that the proportion of allulose increases with respect to the allitol during metabolism in the dark. The yields of crystalline allitol and allulose-containing syrups are recorded in Table 4.

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