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# THE INFLUENCE OF PHOTOSYNTHESIS AND SKF INHIBITORS ON CANNABINOID PRODUCTION IN *CANNABIS SATIVA*

W. MARY L. CROMBIE

Department of Chemistry, The University of Nottingham, Nottingham, NG7 2RD, U.K.

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**Key Word Index**—*Cannabis sativa*; Cannabinaceae; chlorophyll; photosynthesis; SKF inhibitors.

**Abstract**—Experiments using darkened shoots, and a *Cannabis sativa* sport having completely green and completely white shoots, show that cannabinoid production continues in green tissue in the dark, and in chlorophyll-free white tissue. Using high $\Delta^1$-THC-containing Thai-strain plants, inhibitors SKF 7732-$A_3$ and 7997-$A_3$ restrict growth and cannabinoid synthesis by all the criteria employed. On the other hand the inhibitors SKF 525-A and 3301-A cause diminished growth and diminished total cannabinoid production relative to an untreated shoot, whilst production of cannabinoids per unit weight of fresh or dry tissue is enhanced by their chemical stress. This behaviour is compared with effects on the fibrous high cannabidiol-containing Kew-strain plants.

Cannabinoids have a general association with the photosynthetic parts of *Cannabis sativa*, being normally present only in traces in non-green parts such as roots and seeds, [1] although pollen grain has recently been reported to be a rich source [2]. In this paper, the relationship between cannabinoid formation and the presence of chlorophyll is examined in various ways: in addition, some experiments on the effect of terpenoid/steroid-biosynthesis inhibitors on cannabinoid formation are reported.

Dark-grown (7 days from germination) etiolated seedlings of a Thailand strain of *C. sativa* contained 0.42 μg cannabinoids (after decarboxylation), whilst light-grown seedlings contained 0.78 μg/seedling (averaged from 50 seedlings): the corresponding figures on a dry-weight basis were 46 μg and 94 μg/g dry tissue, respectively. When the dark-grown (7 day) seedlings were exposed to light for 7 days the cannabinoid content rose to 2.54 μg/seedling (203 μg/g dry tissue) whereas in the reverse experiment, in which light-grown (7 day) seedlings were kept in the dark for 7 days a figure of 0.71 μg/seedling (89 μg/g dry tissue) was obtained. Cannabinoid content in this second 7 day dark period is thus fairly static at this stage of plant growth: the cannabinoid composition was generally similar to that of the mature plant (see later).

For experiments on more mature plants, a 'paired shoot' technique was used. Removal of the main stem above the second internode of 5–6 week-old Thailand-strain plants resulted, after 1 week, in the development of paired side-shoots in the axils of the uppermost pair of leaves. Observations on a large number of plants prepared in this way, over a considerable period (up to flowering), showed a marked parallelism of growth between the two shoots and no establishment of dominance. These paired shoots can be used to form an experimental subject and its control.

Fairbairn [3] has reported that *Cannabis* plants grown in the light show a parallel increase of dry weight and cannabinoid content relative to those found in similar plants grown for the same period in the dark. Table 1 tends to support this relationship, where a dry weight increase of 179% is matched by a cannabinoid increase of 210%. The relationship does not hold well where the darkened shoot (ventilated dark box: shoot wilted) is compared with its paired shoot, on the same plant, main-

Table 1. Effect of light and dark on 'paired' shoots of *C. sativa*

| | Mean length cm | Fresh wt g per shoot | Dry wt g per shoot | Total cannabinoids‡ mg per shoot |
|---|---|---|---|---|
| Paired shoots at start† | 5.84 (0.76)* | 0.79 | 0.16 | 0.07 |
| | 5.59 (0.76) | 0.87 | 0.17 | |
| Paired shoots† both 6 days light | 9.91 (1.27) | 1.44 | 0.33 | 0.59 |
| | 9.91 (1.27) | 1.48 | 0.34 | |
| Paired shoots† both 6 days dark | 5.33 (1.27) | 0.27 | 0.13 | 0.19 |
| | 5.08 (1.27) | 0.25 | 0.11 | |
| Paired shoots† one 6 days light, one 6 days dark | 13.72 (2.03) | 2.68 | 0.47 | 0.61 |
| | 10.92 (2.03) | 1.03 | 0.19 | 0.14 |

* Standard deviation in parentheses. † six plants in each set. ‡ decarboxylated: GLC estimation.

tained under normal light conditions. Here a dry weight increase of 137% is matched by a cannabinoid increase of 336% and translocation has disturbed the relationship. The experiments in Table 1 show that cannabinoids continue to be formed in growing shoots in the dark, though at a much diminished rate relative to a normal photosynthesising shoot.

A unique opportunity to look at the matter in a different way arose when a sport appeared in our collection of Thailand-strain plants. This contained completely white branches, totally denuded of chlorophyll along with fully normal green branches and branches with part white and part green leaves: it proved possible to propagate the plant as cuttings. The white leaves were generally thinner and less robust than the green, but possessed typical cannabinoid-secreting glandular hairs in the normal way. Analyses for the green and the white leaves are shown, at different seasons, in Table 2, together with the cannabinoid content of green and of white flower bracts.

The quantitative composition of the cannabinoid mixture for white and green organs does not vary importantly, and is the pattern we usually find for this Thailand strain. White leaves (and bracts) contain a substantial, though diminished, cannabinoid content relative to the corresponding green parts when measured on a fresh tissue weight basis. The difference is reduced further on a dry-weight basis because the white organs of the plant have uniformly higher water-content than the green. Here therefore, there is cannabinoid production in light on a substantial scale without any local connexion with chlorophyll and photosynthesis. The production of cannabinoids in these white tissues must be dependent on translocated biosynthetic intermediates from the green areas of the plant.

The 'paired shoot' technique encouraged an examination of the effect of terpenoid-biosynthesis inhibitors of the SKF series. Four were employed, and results presented are in each experiment averaged for eight plant samples. Two of the inhibitors SKF 525-A and 3301-A are considered to inhibit isopentenyl pyrophosphate isomerase [4] and probably affect more than one stage of terpenoid synthesis [5] (a recent study however [4], indicates that they do not prevent incorporation of labelled mevalonic acid into geraniol in cell-free systems from *Tanacetum vulgare*). SKF 7732-$A_3$ and 7997-$A_3$ are considered to act on later stages in steroid biosynthesis [5] and might affect cannabinoid synthesis in intact *C. sativa* in indirect ways. Earlier work [5] suggests that there is little movement of these inhibitors from treated leaves, and in the present experiments comparisons with other untreated whole *Cannabis* plants gave no indications that the 'paired' control shoots were being affected by the proximate treated shoot.

Paired shoots of the Thailand strain which were to be treated were dipped into aqueous Tween 20 (0.1%) to facilitate penetration, and allowed to dry: the shoot was then immersed in the appropriate inhibitor solution (at the concentration given in Table 3) containing Tween 20 (0.1%) at pH 5–6. The concentrations used were the maximum acceptable from the point of view of phytotoxicity and there was some damage as indicated by leaf-browning during the growing-on period. Table 3 shows the situation at the end of a 9-day period of growth after treatment, under normal greenhouse conditions. All four inhibitors had roughly halved the growth of the treated shoot (in terms of length) relative to the twinned control. In the same way the fresh weight of tissue produced by treated shoots was drastically reduced, especially in the case of SKF 3301-A. For three of the inhibitors the water content of the treated tissues is notably less than their untreated controls, as indicated by the 'dry weight as % of fresh weight' column.

Table 3 shows that all four inhibitors at high concentrations cause substantial diminution of cannabinoid production on a 'per shoot' basis, though this of course is associated with the general diminution in growth. Expressed as cannabinoid production per unit of fresh tissue weight however, the production is notably increased relative to the control in the case of the SKF 525-A and 3301-A treatments. In three of the experiments, despite the lower water-content of the treated tissues, increased production is also indicated by the 'cannabinoids as a % of dry weight' criterion. Such a situation is not found for the SKF 7732-$A_3$ and SKF 7997-$A_3$ inhibitors, which were also less phytotoxic. Here, assessed either on a fresh or dry weight criterion, cannabinoid production is lowered by the inhibitors. On treatment with any of the four inhibitors the composition of the mixture of cannabinoids being produced is not seriously upset although there appears to be some increased production of cannabichromen (CC-$C_5$) under toxic stress.

In summary, the SKF inhibitors fall into two groups as regards Thai-strain plants. SKF 525-A and 3301-A cause diminished growth (shoot length or weight) and production of less cannabinoid in terms of total weight than

Table 2. Cannabinoid content of white and green leaves of a *C. sativa* male sport

| | Total cannabinoids %* | | Cannabinoid composition %* | | |
|---|---|---|---|---|---|
| | Fresh leaf | Dried leaf | $\Delta^1$-THC-$C_3$ | $\Delta^1$-THC-$C_5$ | CC-$C_5$ |
| White leaves (31/10/75) | 0.15 | 0.91 | 3 | 81 | 16 |
| Green leaves (31/10/75) | 0.23 | 1.03 | 1 | 87 | 12 |
| White leaves (4/5/76) | 0.18 | 0.68 | trace | 91 | 9 |
| Green leaves (4/5/76) | 0.52 | 1.58 | trace | 93 | 7 |
| White leaves (11/6/76) | 0.09 | 0.50 | 2 | 91 | 7 |
| Green leaves (11/6/76) | 0.28 | 1.03 | 3 | 90 | 7 |
| White bracts (4/5/76) | 0.27 | 1.14 | 2 | 87 | 11 |
| Green bracts (4/5/76) | 0.59 | 1.63 | 1 | 87 | 12 |

* By GLC after decarboxylation and trimethylsilylation (SCOT OV-225 column, 15 m × 0.05 cm) and by on-column decarboxylation (OV-17, 2.74 m × 0.64 cm).

Table 3. Effects of SKF terpenoid-inhibitors on 'paired' *C. sativa* (Thai variety) shoots after 9 days*

| | Shoot-length increase cm† | Leaf-tissue‡ fresh wt g per shoot | Leaf tissue‡ dry wt g per shoot | Leaf tissue dry wt as % of fresh wt | Total cannabinoids mg formed by shoot | Cannabinoids as % of fresh wt | Cannabinoids as % of dry wt | Cannabinoid composition %§ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | $\Delta^1$-THC-$C_5$ | $\Delta^1$-THC-$C_3$ | CC-$C_5$ |
| SKF 525-A (1 mg/ml) | 9.9 (2.8)‖ | 0.60 | 0.18 | 30 | 1.16 | 0.19 | 0.64 | 1 | 87 | 12 |
| Untreated twin | 17.0 (2.5) | 1 79 | 0.39 | 22 | 1.47 | 0.08 | 0.38 | 4 | 88 | 8 |
| SKF 525- A 2 mg/ml) | 8.4 (1.3) | 0.48 | 0.15 | 31 | 0.81 | 0.17 | 0.54 | 3 | 85 | 12 |
| Untreated twin | 17.0 (1.5) | 1.62 | 0.35 | 22 | 1.43 | 0.09 | 0.41 | 2 | 90 | 8 |
| SKF 3301-A (1 mg/ml) | 9.1 (3.3) | 0.35 | 0.14 | 40 | 0.75 | 0.21 | 0.54 | 4 | 89 | 7 |
| Untreated twin | 17.3 (4.1) | 1.70 | 0.38 | 22 | 1.48 | 0.09 | 0.39 | 5 | 86 | 9 |
| SKF 3301-A (2 mg/ml) | 6.9 (5.3) | 0.21 | 0.10 | 48 | 0.32 | 0 15 | 0.32 | 7 | 73 | 20 |
| Untreated twin | 17.0 (6.1) | 1.52 | 0.34 | 22 | 1.14 | 0.08 | 0.34 | 3 | 83 | 14 |
| SKF 7732-$A_3$ (5 mg/ml) | 9.1 (2.5) | 1.17 | 0.30 | 26 | 0.85 | 0.07 | 0.28 | 3 | 84 | 13 |
| Untreated twin | 13.5 (2.8) | 1.59 | 0.38 | 24 | 1.63 | 0 10 | 0.43 | 9 | 85 | 6 |
| SKF 7997-$A_3$ (5 mg/ml) | 8.4 (1.8) | 0.73 | 0.22 | 30 | 0.40 | 0.05 | 0.18 | 3 | 79 | 18 |
| Untreated twin | 14.2 (1.3) | 1.92 | 0.45 | 23 | 1.64 | 0.09 | 0.36 | 3 | 82 | 15 |

* Results are the average of eight plant samples in each group, taken 9 days after treatment with the SKF inhibitor. † Average length at start of 9 day period 3.1 cm (0.3). ‡ Stalks and midrib removed. § After decarboxylation (by GLC as Table 2). ‖ Standard deviation in parentheses.

Table 4. Effects of SKF terpenoid-inhibitors on 'paired' *C. sativa* (Kew variety) shoots after 13 days*

| | Shoot length increase cm† | Leaf tissue‡ fresh weight g per shoot | Leaf tissue dry wt g per shoot | Leaf tissue dry wt as % of fresh wt | Total cannabinoids mg formed by shoot | Cannabinoids as % of fresh wt | Cannabinoids as % of dry wt | Cannabinoid composition %§ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | CD-$C_5$ | $\Delta^1$-THC-$C_5$ | CC-$C_5$ |
| SKF 525-A (1 mg/ml) | 9 1 (6.9)‖ | 0.37 | 0.09 | 24 | 0.34 | 0.09 | 0.38 | 87 | 5 | 8 |
| Untreated twin | 20.8 (8.9) | 1.28 | 0.29 | 23 | 1.33 | 0.10 | 0.44 | 90 | 4 | 6 |
| SKF 3301-A (1 mg/ml) | 10.4 (6.4) | 0.45 | 0.11 | 24 | 0.34 | 0.08 | 0.32 | 86 | 5 | 9 |
| Untreated twin | 20.3 (6.1) | 1.11 | 0.27 | 24 | 0.64 | 0.06 | 0.24 | 89 | 4 | 7 |
| SKF 7732-$A_3$ (5 mg/ml) | 13.7 (11.2) | 0.95 | 0.23 | 24 | 0.41 | 0.04 | 0.18 | 85 | 6 | 9 |
| Untreated twin | 18.3 (11.9) | 1.07 | 0.26 | 24 | 0.94 | 0.08 | 0.36 | 86 | 6 | 8 |
| SKF 7997-$A_3$ (5 mg/ml) | 13.2 (8.4) | 0.79 | 0.18 | 23 | 0.65 | 0.08 | 0.36 | 84 | 6 | 10 |
| Untreated twin | 15.2 (8.6) | 0.88 | 0.20 | 23 | 0.98 | 0.11 | 0.48 | 89 | 5 | 6 |

* Results are the average of eight plant samples in each group, taken 13 days after treatment with the SKF inhibitor. † Average length at start of 13 day period 3.8 cm (2.8). ‡ Stalks and midrib removed. § After decarboxylation (by GLC as Table 2). ‖ Standard deviation in parentheses.

occurs in a normal shoot. Associated with this, the cannabinoid produced by a given weight of fresh tissue is higher under the toxic stress, and in some cases this is also so on a dry tissue weight basis. It is of interest to note that water stress, nutrient deficiency, and plant competition, have earlier been reported to stimulate cannabinoid production (dry wt basis) in *C. sativa* [6]. By all criteria, production of total weight of cannabinoids per shoot, or per unit of fresh or dry tissue, SKF 7732-$A_3$ and 7997-$A_3$ diminish cannabinoid production.

A set of experiments was also carried out on the tall, fibrous, high-cannabidiol Kew-strain [7] of *Cannabis sativa* and although they refer to a 13 day period they form a useful comparison. Plants of this strain show more variability than the Thai (high THC) variety in terms of shoot length, because of the differences between male and female plants: they are also more sensitive to the phytotoxic effects of SKF 525-A and 3301-A. These latter inhibitors (Table 4) brought about reductions in fresh- and dry-weight of tissue/shoot as before, but SKF 7732-$A_3$ and 7997-$A_3$ had more limited effects than those experienced on Thai-strain plants. As in the Thai-strain, treatment of Kew-strain plants with SKF 3301 produces a higher percentage of cannabinoids on either a fresh- or dry-weight of tissue basis: however, in this variety, the effect of SKF 525 resembles that of SKF 7997. In sharp contrast to Thai-strain plants, the water-content of treated shoots of Kew-strain is largely unaffected by treatment with any of the four inhibitors.

It is clear that the effects of the SKF inhibitors on whole *Cannabis* plants are complex. Interference with the terpenoid biosynthesis of cannabinoids cannot be isolated from the biosynthesis of regulatory and similar systems, and different strains may show differences in response.

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