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CHANGES IN THE STEROIDAL ALKALOID SOLASODINE DURING DEVELOPMENT OF *SOLANUM NIGRUM* AND *SOLANUM INCANUM*

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Key Word Index—*Solanum nigrum*; *Solanum incanum*; Solanaceae; development; steroidal alkaloid; solasodine.

Abstract—All tested organs of *Solanum nigrum* and *Solanum incanum* elaborated solasodine, but the levels varied widely. In both species, the smallest leaves showed the highest alkaloid concentration. Maximum levels in *S. incanum* leaves were greater than twice those in *S. nigrum*. The absolute amount of alkaloid per leaf increased during leaf development whereas the concentration declined. The pattern of change in leaf alkaloid concentration with increasing age of the plant was different in the two species. The concentration of alkaloid in roots was higher than in the stem in *S. nigrum*, but initially similar to stem levels in *S. incanum*. Both organs showed alkaloid accumulation with time, although root levels inexplicably fell in *S. incanum* after 20 weeks. Small unripe fruits of *S. nigrum* had a high concentration of solasodine, but both the concentration and the absolute amount per fruit decreased with fruit maturation. The concentration and absolute amount of alkaloid also diminished in fruits of all developmental stages with increasing age of the plant. *S. incanum* plants did not produce fruits during the study period. © 1997 Elsevier Science Ltd

INTRODUCTION

Steroidal glycoalkaloids found in the genus *Solanum* (family Solanaceae) are an important group of secondary compounds both ecologically and commercially. In probably the majority of plants that elaborate glycoalkaloids, the principal aglycone is solasodine (Fig. 1) and the most common glycosides are its triosides solasonine and solamargine [1, 2]. The biological activity of only a relatively small number of glycoalkaloids has been examined, but available evidence indicates that they can be toxic or inhibitory to a wide range of organisms, from microorganisms to mammals [3]. Most notable effects are the disruption of strategic membranes [4] and the inhibition of acetylcholinesterase, a key enzyme in nerve impulse transmission [5]. Glycoalkaloids are widely regarded as defensive allelochemicals against a number of pathogens and predators [6]. Information on the biological activity of the solasodine glycosides is very limited [see 7–9]. Interest in these compounds has focused instead on their use as a starting material for the industrial production of pharmaceutical steroids [10]. Recently, mixtures of solasodine glycosides have been successfully used for the treatment of certain human skin carcinomas [11–12].

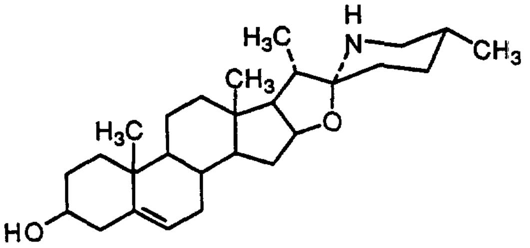

Fig. 1. Structure of solasodine.

The steroidal sapogenin diosgenin from tubers of *Dioscorea* spp. still remains the most important steroid raw material for the industrial production of corticoids, especially the 16-substituted ones (Mann, J. D., personal communication), but the growth in demand for these pharmacologically-active steroids and problems with the supply of diosgenin have led to searches for alternative raw materials. For a number of years, solasodine, which is a nitrogen analogue of diosgenin and can be similarly converted to the starter compound 16-dehydropregnenolone [13], has been used for the semi-synthetic production of pharmaceutical and contraceptive steroids in many parts of the world, especially the former U.S.S.R. and Eastern Europe, India and China [10], and it continues to be a potentially important source in many other countries

[14]. In contrast to the *Dioscorea* yams, solasodine-containing *Solanum* species are relatively easy to cultivate.

The genus *Solanum* is a very large group of about 1400 species found throughout the temperate and tropical regions of the world. Phytochemical surveys of *Solanum* species for solasodine have been conducted in many countries [see 1, 2, 15, 16] and have identified the vegetative parts of *S. laciniatum* and *S. aviculare* (indigenous to New Zealand) and the fruits of *S. khasianum* (from the Indian subcontinent) and *S. marginatum* (from Ecuador) as the highest yielding sources. About 20 *Solanum* species are recorded from Arabia [17]. In Oman, seven species of *Solanum* are known, viz. *S. cordatum*, *S. incanum*, *S. melongena*, *S. nigrum*, *S. surattense*, *S. tuberosum*, and *S. villosum* [18] although no studies have been made in Oman of their alkaloid content. Most surveys of solasodine levels in plants tend to be restricted to a particular stage of plant development. The aim of this study was to compare changes in the whole-plant solasodine profile during development in the two species *S. nigrum* and *S. incanum*. Solasodine glycosides have been reported in both species [1, 2, 19–22]. Other steroidal alkaloids/glycoalkaloids may also be present in these species, especially in the roots, but were not identified or studied here.

RESULTS

S. nigrum. Eight-week-old *S. nigrum* plants did not produce older leaves or fruits; these appeared at 12 weeks and 16 weeks, respectively. At all stages of development, all the organs present contained solasodine, with the highest concentration consistently in the small leaves (Fig. 2). As leaves developed, levels declined and concentrations in mature leaves were only about 25% of those in small leaves (Fig. 2). In contrast, the highest absolute amount of solasodine per leaf was in the mature leaves, with least (*ca* 70% less) in the small leaves (Fig. 3). All leaves (especially small and young leaves) showed a similar pattern of increase in alkaloid concentration with the age of the plant up till 16 weeks, after which levels progressively diminished (Fig. 2). However, this was not consistently mirrored in changes in the absolute amount of solasodine, as seen in young and mature leaves where the amount progressively increased (Fig. 3).

Overall, the lowest solasodine concentration in the tested organs was in the stem; levels in the root were around three times that of the stem (Fig. 2). Concentrations increased steadily with age in both organs, although more markedly in the root (Fig. 2). The absolute amount of alkaloid in these organs showed a similar pattern of change (Fig. 3), although the large increases in solasodine per stem and per root between 8 and 12 weeks (*ca* × 4 and × 3, respectively) were not reflected in similar concentration changes, which were modest by comparison (Fig. 2).

At 16 weeks, plants bore fruits at all four stages of development (small green, young green, breaker and ripe) and all contained solasodine. Maximal concentrations were about twice those of roots but half those of small leaves (Fig. 2). Small green fruits had the highest alkaloid level on both a concentration (Fig. 2) and a per fruit basis (Fig. 3), with both parameters diminishing as the fruits matured. Ripe fruits had *ca* 3% and 9% of the concentration and absolute amount, respectively, of solasodine in small fruits. Both alkaloid concentration and absolute amount in fruits of all stages also progressively declined with the age of the plant. At 24 weeks, *S. nigrum* plants appeared to be approaching the end of their life and many fruits were produced, but levels and quantities

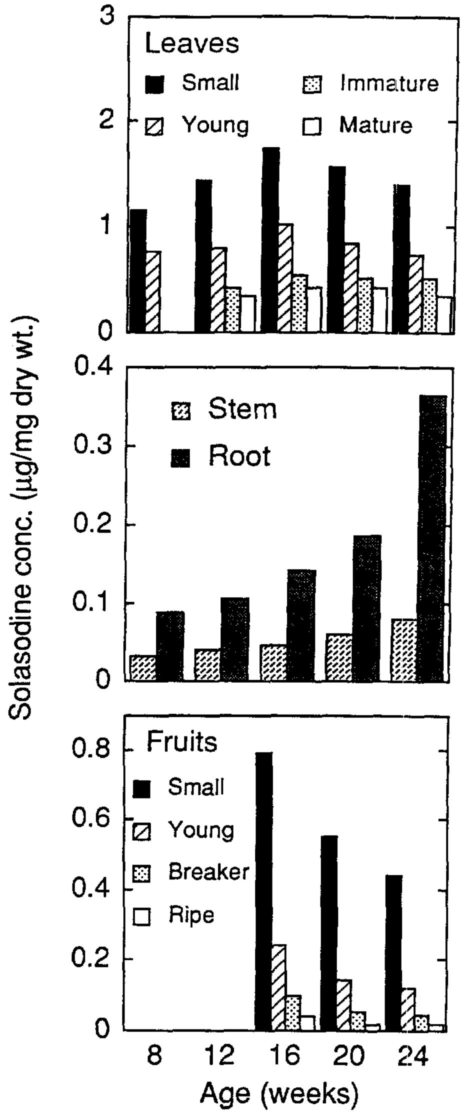

Fig. 2. Changes in the concentration of solasodine in vegetative and reproductive organs of *S. nigrum* at different stages of development. Each data point is the mean of three replicate determinations. Criteria governing the different categories of leaves and fruits are detailed in the Experimental section.

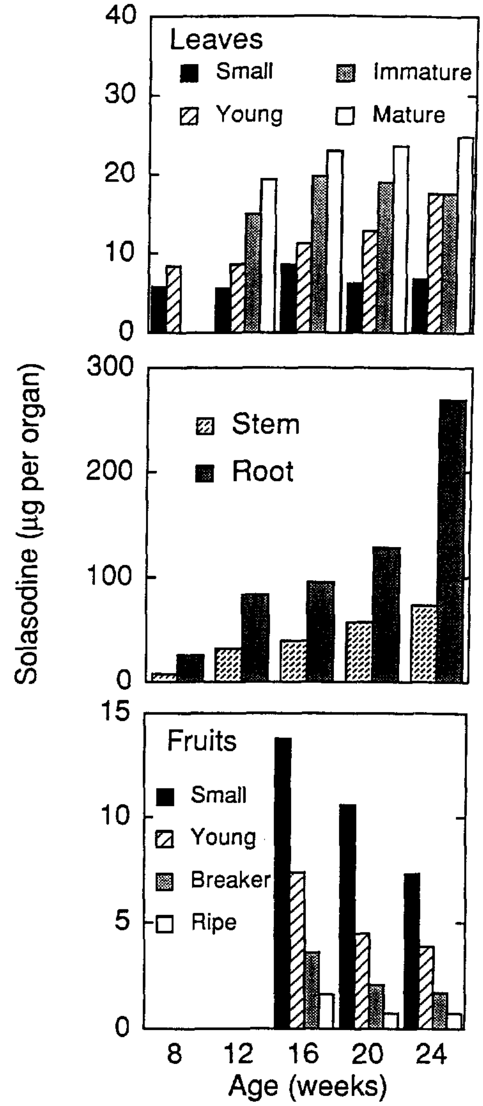

Fig. 3. Changes in the absolute amount of solasodine in vegetative and reproductive organs of *S. nigrum* at different stages of development. Each data point is the mean of three replicate determinations. Data for leaves and fruits are mean values for representative individual organs; those for roots and stems relate to the entire organ. Criteria governing the different categories of leaves and fruits are detailed in the Experimental section.

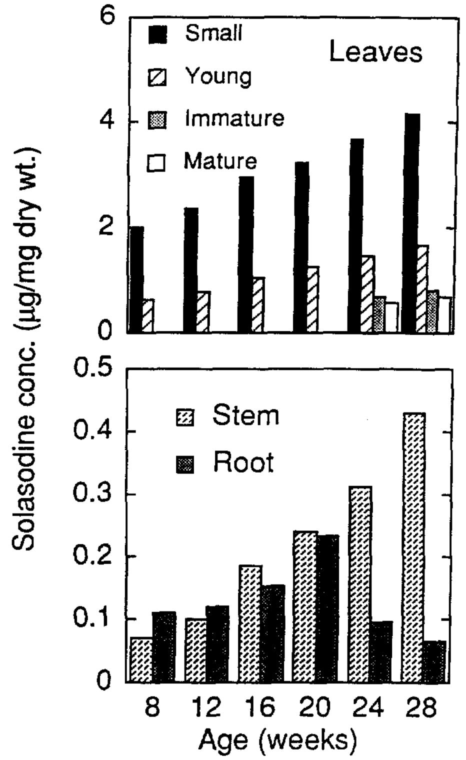

Fig. 4. Changes in the concentration of solasodine in vegetative and reproductive organs of *S. incanum* at different stages of development. Each data point is the mean of three replicate determinations. Criteria governing the different categories of leaves are detailed in the Experimental section.

of solasodine in fruits of all stages were only about half those in younger plants.

S. incanum. Although plants of this species started to flower by 28 weeks, no fruits were available for analysis within the duration of the experiment. Also, the two categories of older leaves (immature and mature) were not produced till 24 weeks, compared with 12 weeks in *S. nigrum*. As in *S. nigrum*, the highest solasodine concentration occurred in the small leaves and the lowest in the mature leaves (Fig. 4), with the situation being reversed for the absolute amount of alkaloid per leaf (Fig. 5). However, leaf alkaloid concentration showed a continuous increase with plant age (Fig. 4) which paralleled changes in absolute amount (Fig. 5), unlike *S. nigrum* where reductions in concentration occurred after 16 weeks (Fig. 2) which were not closely reflected in changes in absolute amounts (Fig. 3). At 24 weeks, the solasodine concentration in small leaves was more than twice that in comparable leaves of *S. nigrum*, while the quantity of alkaloid per leaf in mature leaves was more than × 6 that in *S. nigrum*.

Up to 20 weeks, concentrations and absolute amounts of solasodine in roots of *S. incanum* (Figs 4 and 5) were broadly similar to those in *S. nigrum* (Figs 2 and 3), but thereafter fell sharply by about 65%. Between 24 and 28 weeks, root concentration declined by about one third even though the absolute amount of solasodine showed no significant change. On the other hand, stem alkaloid concentrations were consistently higher than those in *S. nigrum*, by a factor of *ca* 2 at week 8 rising to *ca* 4 at week 24 (Figs 2 and 4). The absolute amount of alkaloid per stem was likewise greater in *S. incanum* than *S. nigrum*—by a factor of *ca* 2.5 at week 24 (Figs 3 and 5). Thus, whereas in *S. nigrum* concentrations and absolute amounts of solasodine in roots were consistently greater than those in stems (Figs 2 and 3), in *S. incanum* the differences between root and stem were minimal to negligible up to 20 weeks, after which the stem values far exceeded those in roots (Figs 4 and 5).

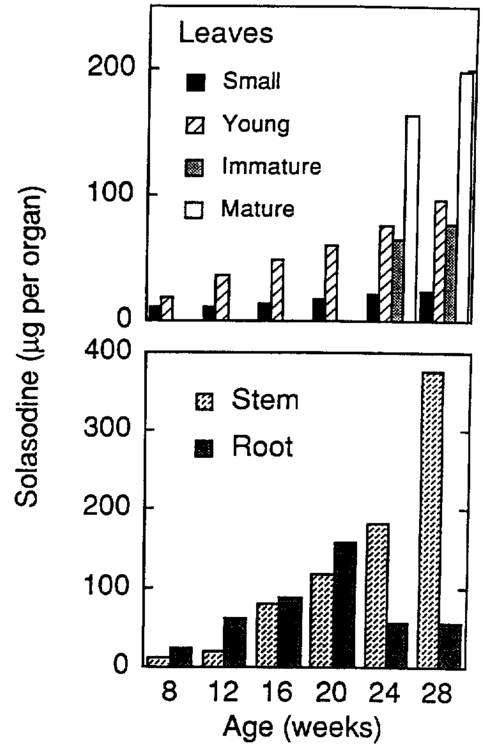

Fig. 5. Changes in the absolute amount of solasodine in vegetative and reproductive organs of *S. incanum* at different stages of development. Each data point is the mean of three replicate determinations. Data for leaves are mean values for representative individual organs; those for roots and stems relate to the entire organ. Criteria governing the different categories of leaves are detailed in the Experimental section.

DISCUSSION

The high concentration of solasodine in leaves of *S. nigrum* and *S. incanum* suggests this organ to be a major site of alkaloid synthesis in these species. Maximum values of *ca* 0.17% in leaves of *S. nigrum* are in keeping with those reported elsewhere for this species [19–21] while values of 0.42% in *S. incanum* are greater than the traces reported by Lin *et al.* [22]. The highest solasodine concentration in the youngest ('small') leaves contrasts with *S. laciniatum* where this was observed in the fully expanded leaves [23]. The inverse relationship between alkaloid concentration and leaf maturity could reflect a reduction in biosynthetic activity with age, but this requires confirmation. However, the fact that in both species the absolute amount of solasodine per leaf continues to increase with leaf maturity suggests that dilution due to a proportionately greater accumulation of dry matter may be a major contributory factor. Other differences in the pattern of changes in the concentration and absolute amount of alkaloid confirm that solasodine elaboration is not necessarily or consistently closely associated with dry matter accumulation. The age of the plant when leaves develop can influence the solasodine content, and differently in different species as shown by the increasing (then decreasing) alkaloid concentration in (all) leaves of *S. nigrum* and the continually increasing concentration in (all) *S. incanum* leaves (Figs 2 and 4). The pattern of change in leaf concentration in *S. nigrum* has also been observed in *S. khasianum* leaves [24] and in mature (but not immature) leaves of *S. laciniatum* [25].

In contrast, stems and roots would appear to be less active areas of solasodine biosynthesis. In *S. incanum*, the sudden reduction in root solasodine between 20–24 weeks (Figs 4 and 5) is difficult to explain and possibly anomalous. Infection by *Fusarium solani* (of which there was evidence in the soil) is an unlikely cause in view of the restriction of the effect to the root. Also, there is as yet no evidence that this fungus produces enzymes which can degrade solasodine.

The solasodine concentration in small fruits of *S. nigrum* (*ca* 0.08%) is at the lower end of a wide range of values reported by Khanna and Rathore [26]. This variability is probably due to *S. nigrum* existing as a genetic complex. The reduction in the solasodine concentration in *S. nigrum* fruits with increasing maturity is possibly partly a dilution effect from accumulating dry matter, but is primarily due to a drastic decline in the absolute amount of solasodine per fruit. This, in turn, is most likely a result of enzymic degradation, although solasodine disappearance from fruits of this species (and most other *Solanum* species) has not been investigated in detail. Biosynthetic studies using tomato fruits [27] and grafted tomato/potato plants [28] have established that tomatine is enzymically degraded in developing fruits and that no large-scale or long-distance translocation of tomato or potato glycoalkaloids occurs. Thus the marked increases in solasodine levels in stems and roots with age probably do not result from mobilised fruit alkaloid in transit through stems and imported into roots, but rather from synthesis and accumulation in these organs. A suggestion that solasodine accumulation in roots might be due to an influx of alkaloid from leaves as levels there declined [25] is not supported by our findings.

Of the two species investigated, *S. incanum* is the better source of solasodine, firstly because concentrations in above-ground vegetative parts are significantly higher (> × 2) and secondly because it is a perennial plant (*S. nigrum* is an annual). Although the smallest leaves have the highest solasodine concentration (in both species), they constitute only a small proportion of the plant biomass whereas immature and mature leaves, even with lower concentrations, give a greater overall yield of solasodine by virtue of their greater biomass. *S. nigrum* fruits are not a promising source of the alkaloid because of their small size and declining alkaloid content. Other workers have reported solasodine in *S. incanum* fruits at levels ranging from traces [22] to 0.031% [29] on a fresh weight basis in mature fruits.

The highest leaf values found in *S. incanum* are only *ca* 10% of those in *S. laciniatum* [10]. Our plants, which were pot-grown indoors under controlled conditions, probably produced a lower biomass and alkaloid yield than they would had they been field-grown, but it still remains doubtful whether the solasodine content of *S. incanum* leaves, even in field-grown plants, would be able to match that of *S. laciniatum.* The aqueous hydrolysis method employed [25] was claimed to give total recovery of solasodine. However, there are indications [10] that this method can suffer losses due to solasoda-3,5-diene production and the possibility that this might have affected the values cannot be ruled out. Nevertheless, our findings confirm the variability between species and organs in solasodine accumulation and highlight the importance of the developmental stage of particular organs and the overall age of the plant when harvesting for this alkaloid. Leaf extracts of *S. nigrum* and *S. incanum* are used in traditional medicine in the Middle East [30] and for contraceptive purposes in India [21], but whether their efficacy is related to their solasodine content is not known.

EXPERIMENTAL

Plant material. Solanum incanum L. and *S. nigrum* L. seeds were obtained from the Royal Botanic Gardens, Kew, England and grown in the Biology Department, Sultan Qaboos University. Germination (on damp filter paper in Petri dishes at 25°, 16 hr light, 70% RH) occurred within one week after which seedlings were transplanted into 15 cm pots containing a clay soil, peat moss and sand compost (2:1:1) and grown in a laboratory at 22–28°, 1.5 m below a bank of six 500 W tungsten-halogen floodlamps. Plants were routinely watered × 3 per week. Sampling commenced 8 weeks after germination. Three plants were randomly selected for analysis every 4 weeks for 24–28 weeks.

Each plant was divided into 10 different parts, according to a modified scheme proposed by Lancaster and Mann [25], as follows: 'Small' leaves, $\leqslant 3.4$ cm in length; 'Young' leaves, 3.4–7.4 cm in length; 'Immature' leaves, 7.4–11.00 cm in length; 'Mature' leaves, >11.00 cm in length: 'Small' fruits, green, $\leqslant 3.0$ mm diam; 'Young' fruits, green, *ca* 6.0 mm diam; 'Breaker' fruits, pale green/light brown; 'Ripe' fruits, dark orange (*S. incanum*) or black (*S. nigrum*): Stem, whole stem used: Root, whole root used. After measuring, plant material was weighed fresh, dried in an oven at 85° to constant wt (*ca* 2 days) and reweighed.

Extraction and purification of solasodine. The method for the extraction and purification of solasodine was based on that of Lancaster and Mann [25] as follows. 1 g portions of dried, powdered tissue were gently shaken for 30 min in 50 ml of 2% aq. oxalic acid in an automatic tilting apparatus. Suspensions were vacuum filtered through Whatman no. 1 filter paper with only the first 10–20 ml of clear extract being collected. Tubes were gently heated to 75°, 1 ml of 60% NaOH was added, and after heating for a further 5 min (to aid pptn) the tubes were kept at room temp. overnight. Tubes were centrifuged at 20° for 10 min at 3000 *g*. The supernatants were decanted and the pellets dissolved in 5 ml of 0.5 M HCl and hydrolysed by refluxing at 100° for 90 min. Samples were cooled briefly and made alkaline with 1 ml 60% NaOH. Heating to 100° was resumed for 10 min to complete the formation of the insoluble solasodine base.

Colorimetric determination of solasodine. Solasodine was dissolved in 10 ml alcohol-free $CHCl_3$. Aliquots of the $CHCl_3$ solns (0.2–2.0 ml) were transferred to 10 ml capped graduated polythene centrifuge tubes and made to 5.0 ml with alcohol-free $CHCl_3$. A 2.5 ml aliquot of 200 μM bromothymol blue in borate buffer pH 8.0 was added to each tube and mixed for about 10 sec with a vortex stirrer. Formation of an emulsion was carefully avoided. The upper aq. phase was removed, and the lower $CHCl_3$ phase adjusted to 4.0 ml by the removal of excess $CHCl_3$. Any droplets of aq. dye still adhering to the walls of the tube were removed by swabbing with a cotton wool bud. The dye which had been carried into the $CHCl_3$ solution as a complex with solasodine, was then converted to a more strongly coloured ion by adding 0.1 ml of methanolic 0.01 M NaOH to the $CHCl_3$. The absorbance of the soln was determined at 610 nm. A standard curve was constructed using authentic solasodine (Sigma, St. Louis) in alcohol-free $CHCl_3$. The graph was linear within the selected range (0–80 μg solasodine ml^{-1}) and the correlation coefficient (r) of the regression line ($y = 0.0023 + 0.0229x$) was 0.92 ($p < 0.001$).

Experimental design and statistical analysis. All experiments were replicated three times and s.e. of the means calcd. With the exception of only two values, all s.e. were between 2 and 13% of the corresponding mean. No s.e. value exceeded 25% of the mean. Where appropriate, a completely randomised design single factor ANOVA was used to analyse the data.

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