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HIGH LEVEL OF GLYCOSYLATED STEROLS IN SPECIES OF *SOLANUM* AND STEROL CHANGES DURING THE DEVELOPMENT OF THE TOMATO*

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Key Word Index—*Solanum*; Solanaceae; sterols; sterylglycosides; acylated sterylglycosides; development; leaves; stem; roots; senescence.

Abstract—In the Solanaceae, leaves from various *Solanum* species show an exceptional abundance of glycosylated sterols essentially due to a high acylated steryl glycoside content. Changes in sterol composition during the development of tomato indicated a low proportion of glycosylated sterols in the ungerminated seeds with these compounds becoming progressively predominant compared with other sterols during the course of germination. This biochemical characteristic could be observed throughout the development of all parts of this plant. However, the high glycosidic sterol content tends to decrease when the organs become senescent.

INTRODUCTION

Steryl glycosides (SG) and acylated steryl glycosides (ASG) are present with free sterols (FS) and esterified sterols (ES) probably in all higher plants. Lower plants, algae and fungi also contain these glycosylated forms [1]. The amount of glycosides found in higher plants varies considerably, but in most cases, the proportion they comprise of total sterols (TS) is rather low, rarely above 25–35% of TS. However, much higher percentages of glycosylated sterols have been found in a few plants. Thus, in *Solanum tuberosum* tuber, SG and ASG together make up 70–75% of TS [2, 3]. High percentages have also been found in several organs from *Solanum dulcamara* [4]. The abundance of these glycosylated sterols may suggest the existence of a particular sterol metabolism in these two plants, both of which belong to the family Solanaceae. Thus, the aim of the present work was to determine: (a) whether the predominance of glycosylated sterols compared with other sterol forms also occurs in the other species of the Solanaceae; (b) whether the exceptionally high glycoside content, observed in one particular species, is found in the various organs of the plant and throughout its development.

In order to answer these questions, two series of studies were carried out: (a) Identification and quantitative estimation of sterol compounds in the leaves of 15 species belonging to the five subfamilies comprising the Solanaceae (Nicandreae, Solaneae, Datureae, Cestreae, Salpiglossideae [5]), and, for comparison, in the leaves of a few plants of families more or less closely related to the Solanaceae (Convolvulaceae, Scrophulariaceae, Boraginaceae, Labiatae); (b) Identification and quantitative estimation of sterol compounds in the various vegetative organs, and throughout the development of the tomato (*Solanum lycopersicum* L. = *Lycopersicon esculentum* Mill.), a plant in which glycosidic forms of sterols were found to be particularly abundant.

*A preliminary and partial account of this work was presented in *C. R. Acad. Sci. Paris* **296**, 239 (1983).

RESULTS

Sterol composition of leaves: a comparison of Solanaceae with more or less closely related families

The total sterol content of leaves, collected at flowering, ranges between 0.1 and 0.2% (dry wt) in all the plants investigated in this work (Table 1). The proportion of glycosylated sterols varies with the species. However, in plants of the genus *Solanum*, the steryl glycosidic forms (SG + ASG) are much more abundant than the other sterol forms (FS and ES). Thus, in the leaves of tomato, the two classes of glycosylated sterols represent 83% of total sterols (TS). Plants of other genera of the Solanaceae, as well as those of the other families studied in this work show a definitely lower percentage of total glycosylated sterols. However, it should be noted that *Cestrum parquii* (Solanaceae, subfamily: Cestreae) shows a fairly high glycosylated sterol content, though somewhat lower than that found in plants of the genus *Solanum*. The high glycosylated sterol content in plants of the genus *Solanum* is due mainly to the abundance of acylated steryl glycosides.

In most species (Table 2), the glycosides contain the same sterols as those present as free or esterified forms, but the relative proportions of the various sterols from glycosides may be different from those encountered in the free or esterified forms. So, in *Salpichroa origanifolia* (Solanaceae, subfamily Solaneae) the predominant sterol in free or esterified forms is isofucosterol whereas sitosterol is more abundant in glycosylated sterols.

Lastly, it should be stressed that the predominance of glycosylated sterols in leaves from plants of the genus *Solanum* does not depend on the culture medium of the plant. In the case of tomato, the high glycosylated sterol percentages (80–85%) found in the leaves are present in plants grown in a kitchen-garden as well as in those grown

Table 1. Sterol content of leaves from plants of the family Solanaceae and families more or less closely related to Solanaceae

Species	TS (% dry wt)	Composition (% of TS)			
		FS	ES	SG	ASG
Family SOLANACEAE					
Subfamily NICANDREAE					
Nicandra physalodes L.	0.11	54	15	22	9
Subfamily SOLANEAE					
Solanum tuberosum L.	0.20	12	13	27	48
Solanum dulcamara L.	0.12	28	7	27	38
Solanum melongena L.	0.19	22	11	22	45
Solanum nigrum L.	0.15	38	3	9	50
Solanum lycopersicum L.	0.16	10	8	26	56
Solanum pseudocapsicum L.	0.18	25	8	28	39
Lycium barbarum L.	0.20	73	18	7	2
Physalis alkekengi L.	0.17	44	50	3	3
Hyoscyamus albus L.	0.12	57	9	15	19
Salpichroa origanifolia Lmk.	0.20	65	33	1	1
Subfamily DATUREAE					
Datura arborea L.	0.14	65	9	11	15
Subfamily CESTREAE					
Cestrum parquii L'Hérit.	0.10	37	17	16	30
Nicotiana tabacum L.	0.11	50	34	10	6
Subfamily SALPIGLOSSIDEAE					
Schizanthus pinnatus Ruiz-Pavone	0.19	70	16	6	8
Families more or less closely related to SOLANACEAE					
CONVOLVULACEAE					
Convolvulus arvensis L.	0.15	74	12	10	4
SCROPHULARIACEAE					
Linaria cymbalaria L.	0.16	88	4	4	4
Veronica chamaedrys L.	0.16	61	13	14	12
BORAGINACEAE					
Myosotis sp.	0.18	75	10	7	8
LABIATAE					
Lamium purpureum L.	0.16	80	14	3	3

TS = total sterols; FS = free sterols; ES = sterols from esters; SG = sterols from sterylglycosides; ASG = sterols from acylated sterylglycosides. The leaves were collected at the flowering stage.

under controlled conditions in constant environment chambers.

Changes in the relative percentage of different classes of sterol compounds during the development of tomato

The above results show that the leaves of tomato have a particularly high conjugated sterol content. We therefore studied the changes in the different classes of sterol compounds in the various vegetative organs of that plant throughout the course of its development (Fig. 1).

First of all, in ungerminated seeds, the total sterol content reaches a fairly high value (0.26% dry wt). Concerning the changes in the percentage of TS during development, a fast decrease in the cotyledonary leaves was noted while the other leaves maintained a fairly constant concentration of TS. A high percentage of TS was observed in the stem of young seedlings, but the level of TS in this organ decreases rapidly and thereafter remains approximatively stable in the stem of the adult plant. Similar observations were made in the roots.

On the other hand, if we consider the distribution of the four classes of sterol compounds, the seeds differ considerably from the vegetative organs of the growing plant: the ungerminated seeds contain essentially ES and FS, but relatively few glycosylated sterols (mainly SG). It is during germination, and subsequently, that glycosylated sterols become predominant. Thus, in the 7-day-old seedlings there is a predominance of glycosylated forms of sterols but only in the stem and the roots. At this developmental stage, the cotyledon leaves are the only leaves of the seedlings, and these organs do not show a high level of SG and ASG, ES being the most abundant form of sterol compounds. Later, the glycosylated sterols become predominant in all organs, including the cotyledonary leaves which are still present. This abundance of glycosidic forms is essentially due to the increase of ASG. It is rather marked in the leaves. Finally, in old plants, when the leaves become senescent, the predominance of glycosylated sterols tends to decrease in all organs while, in contrast, the proportion of FS and ES increases. However, in the roots only, the percentage of FS increases.

It should be noted that SG and ASG of tomato are monoglycosides of sterols (characteristic separation on TLC according to the method described by Fujino [6]). Acid hydrolysis of these substances (SG and ASG)

Table 2. Principal sterols in each class of sterol compounds of leaves from plants of the family Solanaceae and families more or less closely related to Solanaceae

Species	Principal sterols			
	FS	ES	SG	ASG
Family SOLANACEAE				
Subfamily NICANDREAE				
Nicandra physalodes L.	S, Ca, St, Ch	Ca, S, Ch, St	S, Ca, Ch, St	S, Ca, Ch, St
Subfamily SOLANEAE				
Solanum tuberosum L.	S, IF, St, Ch	S, Ch, St, MC	S, Ch, St, MC	S, St, Ch, IF
Solanum dulcamara L.	S, St, Ch, Ca	S, Ch, Ca, St	S, St, Ch, B	S, St, Ch, Ca
Solanum melongena L.	S, St, Ch, Ca	S, St, Ch, IF	S, St, Ch, Ca	S, St, Ch, Ca
Solanum nigrum L.	S, IF, Ch, Ca	S, Ch, IF, Ca	S, Ch, Ca, St	S, Ch, Ca, St
Solanum lycopersicum L.	S, St, Ch, Ca	S, Ch, Ca, MC	S, Ch, St, MC	S, St, Ch, Ca
Solanum pseudocapsicum L.	S, St, Ch, Ca	S, Ca, Ch, St	S, St, Ch, Ca	S, St, Ch, Ca
Lycium barbarum L.	S, IF, Ca, St	S, IF, Ch, Ca	S, Ch, Ca, St	S, Ch, Ca, St
Physalis alkekengi L.	S, Ca, St, Ch	IF, S, Ca, St	S, Ca, Ch, St	S, Ch, Ca, St
Hyoscyamus albus L.	S, Ch, Ca, St	S, Ch, Ca, St	S, Ca, Ch, St	S, Ca, Ch, St
Salpichroa origanifolia Lmk.	IF, Ca, S, Ch	IF, S, Ca, Ch	S, Ca, Ch, St	S, Ca, Ch, St
Subfamily DATUREAE				
Datura arborea L.	St, S, Ca, Ch	S, Ca, St, Ch	S, Ch, Ca, St	S, Ch, Ca, St
Subfamily CESTREAE				
Cestrum parquii L'Hérit.	S, St, Ca, Ch	Ch, S, Ca, St	S, Ca, St, Ch	S, St, Ca, Ch
Nicotiania tabacum L.	S, St, Ca, Ch	S, Ca, St, IF	S, St, Ca, Ch	S, Ch, Ca, St
Subfamily SALPIGLOSSIDEAE				
Schizanthus pinnatus Ruiz-Pavone	S, Ca, Ch, St	S, Ca, IF, Ch	S, Ca, Ch, St	S, Ca, Ch, St
Families more or less closely related to SOLANACEAE				
CONVOLVULACEAE				
Convolvulus arvensis L.	S, Ca, St, Ch	S, Ca, Ch, St	S, Ch, Ca, St	S, Ch, Ca, St
SCROPHULARIACEAE				
Linaria cymbalaria L.	S, Ca, St, Ch	S, Ch, Ca, St	S, Ch, Ca, St	S, Ch, Ca, St
Veronica chamaedrys L.	S, Ca, St, Ch	S, Ch, Ca, St	S, Ch, Ca, St	S, Ch, Ca, St
BORAGINACEAE				
Myosotis sp.	S, Ca, Ch, St	S, Ca, Ch, St	S, Ca, Ch, St	S, Ch, Ca, St
LABIATAE				
Lamium purpureum L.	S, St, Ca, Ch	S, Ch, St, Ca	S, Ch, Ca, St	S, Ch, Ca, St

FS = free sterols; ES = sterols from esters; SG = sterols from sterylglycosides; ASG = sterols from acylated sterylglycosides. S = sitosterol; Ca = campesterol; IF = isofucosterol; Ch = cholesterol; St = stigmasterol; MC = 24-methylenecholesterol; B = brassicasterol. The sterols are listed in order of decreasing concentration.

releases galactose and glucose. The constituent sterol is mainly sitosterol; smaller quantities of cholesterol, stigmasterol, campesterol, 24-methylene cholesterol and brassicasterol, are also freed by hydrolysis (see Table 2). In the ASG, the fatty acid is mainly palmitic acid.

DISCUSSION

Contrary to most other plants, the species of *Solanum* studied here contain sterol compounds predominantly in the glycosylated form and more especially as ASG. These glycosylated sterols become progressively predominant in the course of germination, at least in tomato. This tends to imply that in such plants the glycosylation processes affecting sterol molecules must be particularly active. These observations should be considered together with the fact that many species of *Solanum* contain steroidal saponins, some of which are neutral (aglycone = tigogenin, digitogenin, etc . . .), and others basic (steroidal alkaloids: aglycone = solanidine, tomatidine, etc . . .) [7].

Thus, these plants show two characteristics relating to their metabolism of steroids: the first being a wide diversity in the steroidal molecules synthesized, the second residing in the capacity of these plants for glycosylating these molecules. The latter property might, in particular, have consequences for the physiology of the cell. In particular, FS and glycosylated sterols are known to be located in membranes [8–12] and FS would affect membrane fluidity while steryl glycosides would not [13–15]. As a consequence, in the *Solanum* species examined, the high level of glycosylated sterol together with the low free sterol content in membranes might give rise to special permeability properties. This highly speculative hypothesis would require further testing.

On the other hand, it should be pointed out that the increasing ES content observed in this work in several organs of tomato as they become senescent is consistent with similar observations we have previously made in other senescent organs (*Solanum tuberosum* tubers [16], *Parthenocissus* leaves [17], *Phaseolus* cotyledons [18]). These various observations could lead one to assume that

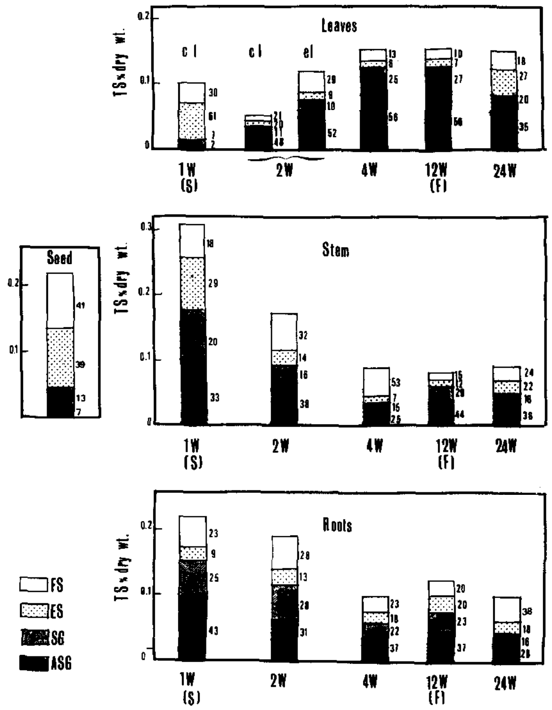

Fig. 1. Changes in total sterol content and proportions of different classes of sterol compounds in the different vegetative organs during the development of *Solanum lycopersicum*. cl = cotyledonary leaves; el = early leaves; S = seedlings; F = flowering stage. 1,2,4 . . . . W = number of weeks of development. TS = total sterols; FS = free sterols; ES = sterols from esters; SG = sterols from glycosides; ASG = sterols from acylated steryl glycosides. Numbers beside blocks represent % of TS.

when cellular structures become disorganized as a consequence of senescence, the sterols (free or conjugated such as steryl glycosides) might be freed from membrane structures, and then converted into esterified forms. The latter, at least in part, might then constitute translocatable forms of sterol molecules. Such a hypothesis has already been proposed [19].

EXPERIMENTAL

Plant materials. Most species were collected in the Botanical Garden of the Museum d'Histoire Naturelle of Paris, and the Botanical Garden of the University of Tours, Faculty of Pharmacy. Several plants (*Solanum lycopersicum, Solanum tuberosum, Solanum melongena, Physalis alkekengi* etc.), were grown in a kitchen-garden. Studies on the changes in sterol composition during the growth of *Solanum lycopersicum* were made with plants growing in a constant environment chamber (20°; light period: 16 hr; light energy: 45 000 ergs/cm^2/sec; light supplied by Mazda fluorescent tubes 'Blanc industrie' supplemented with incandescent lamps).

Qualitative and quantitative analysis of sterols. After collecting, the plant material was killed in boiling EtOH. The total lipids were extracted by $CHCl_3$–MeOH (2:1). The separation of FS, ES, SG, ASG was performed by successive TLC. Identification and estimations of FS, sterols from ES (after saponification) and sterols of SG and ASG (after acid hydrolysis) were carried out using GC and GC/MS. The sugar from SG and ASG, as well as the fatty acids from ASG, were identified by GC. These procedures have been described in a previous work [1].

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