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REVIEW

BIOGENESIS OF STEROIDS IN SOLANACEAE*†

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Key Word Index—Solanaceae; steroid; sapogenins; alkaloids; biosynthesis; metabolism; review.

Abstract—The biogenetic pathways leading to the characteristic steroids of the Solanaceae are reviewed. The experimental data from feeding experiments on members of the Solanaceae are described and, where necessary, pertinent experiments on other plants, such as *Veratrum* spp., are mentioned. The review covers the simple sterols, the steroidal sapogenins and the steroidal alkaloids found in these plants, and their metabolism as well as biosynthesis are considered.

INTRODUCTION

Members of the Nightshade family differ somewhat from other plants in their steroid composition. Some genera contain classes of steroids that are not found in other genera (e.g. glycoalkaloids, withanolides and physalins) or they are richer in certain steroids (e.g. vitamin D and steroidal saponins). Some of these steroids are important starting materials for the partial synthesis of hormones [1–6] and some are toxic substances [7–10], which threaten us and other plant predators or parasites. In view of their economic and ecological importance, much research has gone into determining the distribution of steroids in various species, varieties and organs of plants belonging to the Solanaceae under different physiological conditions. Obviously, the observed changes in steroid content are the result of biosynthetic and metabolic activity in the plants. However, while we know a lot about the biochemistry of plant steroids in general [11–29], the biogenesis of steroids in Solanaceae is still largely unexplored. Because experimental data are inadequate or lacking in many instances, data obtained from other plants are used where necessary in order to present a fairly coherent account.

One of the benefits of studying biosynthesis with radioactive tracers is that intermediates having a fleeting existence and low concentration become labeled and, thus, detectable. In 1963, while attempting to trace the conversion of radioactive mevalonic acid to solanidine in potato plants, we observed intense radioactivity in a sterol fraction [30]. Purification of the sterol to constant specific activity showed unequivocally that we had isolated cholesterol. Subsequent experiments on many other plants and in many other laboratories have led to the conclusion that cholesterol is probably present in all plants [29]. This is not surprising, since cholesterol is known to be an essential constituent of cell membranes [31, 32]. Moreover, cholesterol or one of its precursors is now recognized to be the starting material from which living organisms make all the other steroids they contain [11–14, 17, 18, 20–23, 25, 26, 29, 33]. Thus, it behoves us to review the biosynthesis of cholesterol and related compounds with special reference to experiments in Solanaceae.

BIOSYNTHESIS OF STEROLS

As the biosynthesis of sterols in plants has been repeatedly reviewed [11, 13, 16, 21, 24, 27, 29, 34–39], I shall be rather brief. Figure 1 recapitulates the first steps in the biosynthesis of isopentenoids, pieced together from the results of experiments with many different organisms. Acetate (C_2) condenses to form mevalonic acid, the

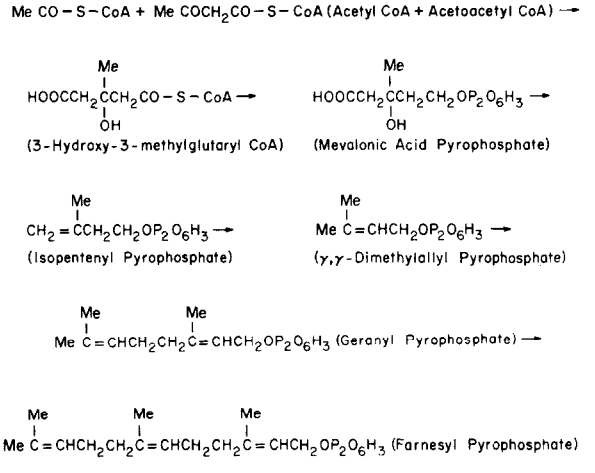

Fig. 1. Biogenesis of a sesquiterpene (C_{15}).

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†Dedicated to Professor Edgar Lederer in honour of his 75th birthday, 5 June 1983.

precursor of the isoprene unit (C_5), isopentenyl pyrophosphate. In 1961, in one of the first experiments on the biosynthesis of sterols in solanaceous plants, we injected tomato fruits with radioactive mevalonic acid and demonstrated its conversion to stigmasterol [40]. Administration of labeled mevalonate to growing potato plants subsequently also provided evidence of conversion to sterols in that species [30, 41]. *In vitro* experiments with tobacco tissue cultures have confirmed the biosynthesis of sterols from labeled acetate [42].

Two C_5 units then combine head-to-tail to form the monoterpene (C_{10}) geranyl pyrophosphate, which is converted to the sesquiterpene (C_{15}) farnesyl pyrophosphate by addition of another C_5 unit. Tail-to-tail condensation of two C_{15} units produces the acyclic triterpene (C_{30}) squalene (Fig. 2). A tissue culture of tobacco cells was used to show that acetate is converted to squalene [43], and a cell-free system from tobacco tissues was used to demonstrate the conversion of mevalonic acid to farnesol and squalene [44, 45]. Similarly, tomato plastids have been shown to incorporate [2-^{14}C]mevalonic acid and farnesyl [4,8,12-^{14}C]pyrophosphate into squalene *in vitro* [46]. The epoxidation of squalene to 2,3-oxidosqualene (Fig. 2) was observed in tissue cultures of tobacco cells incubated with radioactive acetate [47]. When tobacco leaf slices were incubated with radioactive mevalonate in the presence of SKF-7997, a known inhibitor of sterol biosynthesis, accumulation of 2,3-oxidosqualene was easily detected [48]. *In vivo*, the formation of 2,3-oxidosqualene from mevalonate was demonstrated in tomato plants treated with CCC [49], in tobacco plants treated with AMO-1618 and other growth inhibitors [50, 51], and *in vitro* in inhibitor-treated tobacco tissues [52] and cell-free preparations [53]. Growth retardation and sterol inhibition not only went hand-in-hand [54], but it was possible to reverse the growth inhibition by the application of exogenous stigmasterol [55].

The first cyclization product of 2,3-oxidosqualene in animals is lanosterol (Fig. 2) [13], but in plants cycloartenol (Fig. 2) is usually isolated instead [56]. Thus, tobacco plants have been used to show that radioactive acetate *in vitro* [57–59] and radioactive mevalonate both *in vivo* and *in vitro* [60] form cycloartenol. Similarly, chopped potato leaves converted labeled mevalonate to cycloartenol [61]. Furthermore, tissue cultures of tobacco cyclized 2,3-oxidosqualene to cycloartenol [62], and the same reaction could be demonstrated with microsomes extracted from tobacco plants [63]. According to Nes [24, 34, 36], all photosynthetic organisms synthesize sterols via cycloartenol, whereas in nonphotosynthetic organisms lanosterol is the intermediate. If this is correct, the same sterol, e.g. cholesterol, would be synthesized by different routes in different plants. One of the arguments in favor of this hypothesis—the isolation of lanosterol from some plants and the isolation of cycloartenol from others—can be dismissed, because the detectable amount of actively metabolized intermediates depends, of course, on supply and demand. A more convincing argument would be the utilization of either lanosterol or cycloartenol as sterol precursor, but evidence for the obligatory role of cycloartenol as a sterol precursor is lacking. While it is true that conversion of cycloartenol to cholesterol has not been observed in nonphotosynthetic organisms, both lanosterol and cycloartenol act as precursors of sterols in photosynthetic organisms, e.g. in tobacco tissue cultures [64].

Lanosterol has been isolated from several photosynthetic organisms [24, 27, 29, 39], including *Capsicum annuum* [65] and the seeds of other Solanaceae [66]. Perhaps cycloartenol is just a stable precursor of lanosterol. At least the latex of *Euphorbia lathyris*, which contains both of these triterpenes [67], was found to convert [25-^{14}C]cycloartenol to labeled lanosterol, but not vice versa [68]. Whether or not there is a bifurcation in the biosynthetic route at this stage, many avenues appear to lead to the sterols found in higher plants, such as the Solanaceae. The sequence in which the 'extra' methyl groups at C-4 and C-14 are removed, the cyclopropane ring is opened, and the side chain is alkylated seems to be quite variable [34, 69].

The alkylation of the side chain has been reviewed elsewhere [35, 37, 70]. In Fig. 3, we consider the changes in the side chain of the completely demethylated sterol,

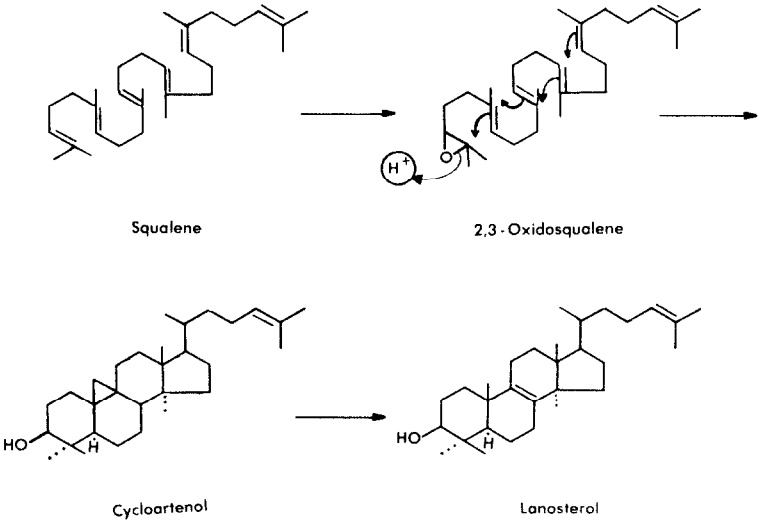

Fig. 2. Cyclization of squalene (C_{30}).

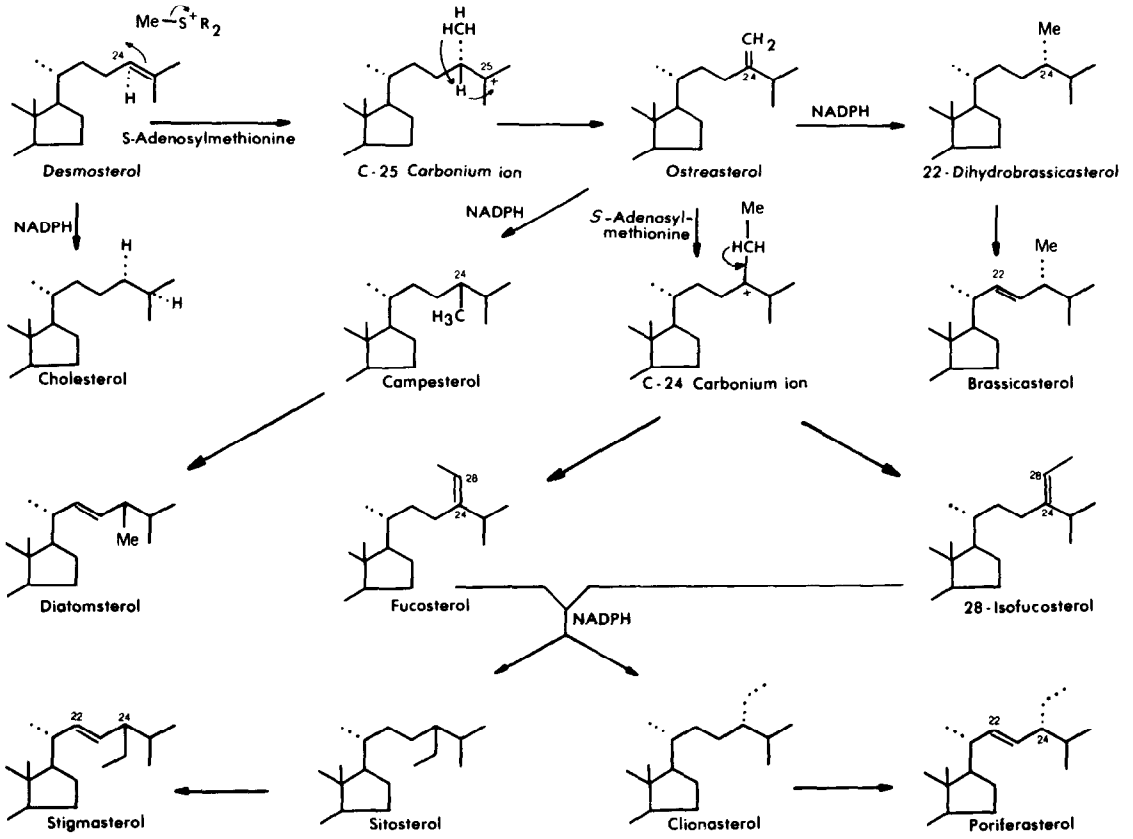

Fig. 3. Biogenesis of sterols (C_{27-29}).

desmosterol (24-dehydrocholesterol). Because the introduction of an alkyl group at C-24 creates a new asymmetric center, two optical isomers are possible, which we designate as α- or β-isomers, according to Fieser and Fieser [71]. When the side chain is drawn extended to the right, the methyl group at C-20 points towards the rear of the molecule, is designated as β-oriented, and drawn either above the side chain or attached by a dotted line (we use all three conventions). Substituents located on the same side as that methyl group are also designated as β-oriented and represented in the same manner. Substituents located on the opposite side of that methyl group are designated as α-oriented and drawn below the side chain or attached by a full line (or both). The α- and β-designations are less confusing than the *S*- and *R*-nomenclature, but it must be remembered that their meaning differs from that of the α- and β-designations used for nuclear substituents. Nuclear substituents attached to the rear of the molecule are designated as α-oriented and connected with the nucleus by a dotted line, whereas the β-oriented substituents are represented by a full line. The α-, β- and γ-designations for sitosterol, etc., often seen in the older literature, are meaningless and no longer in use [72].

Introduction of a methyl group at the C-24 position of a Δ^{24}-unsaturated precursor by transmethylation with *S*-adenosylmethionine produces a C-24 carbonium ion and homocysteine. Rearrangement then leads to ostreasterol (24-methylenecholesterol), which may undergo either reduction or further alkylation. Reduction by NADPH gives rise to either 22-dihydrobrassicasterol [24β(*S*)-methylcholesterol] or campesterol [24α(*R*)-methylcholesterol]. Alkylation by another molecule of *S*-adenosylmethionine produces a C-24 carbonium ion, which may undergo rearrangement to either one of two geometric isomers, best named by *cis*- and *trans*-, rather than *E*- and *Z*-designations. Either of the two isomers, fucosterol [24,28-*cis*(24*E*)-ethylidenecholesterol] and 28-isofucosterol [24,28-*trans*(24*Z*)-ethylidenecholesterol] may then undergo reduction to one of the two optical isomers, sitosterol [24α(*R*)-ethylcholesterol] or clionasterol [24β(*S*)-ethylcholesterol].

Reduction of desmosterol yields cholesterol. That Δ^{22}-sterols are produced by dehydrogenation of saturated side chains was first discovered in potato plants [41, 73]. Thus, 22-dihydrobrassicasterol is desaturated to brassicasterol [*trans*-Δ^{22}-24β(*R*)-methylcholesterol] and campesterol to diatomsterol [*trans*-Δ^{22}-24α(*S*)-methylcholesterol]. Similarly, clionasterol is desaturated to poriferasterol [*trans*-Δ^{22}-24β(*R*)-ethylcholesterol] and sitosterol is desaturated to stigmasterol [*trans*-Δ^{22}-24α(*S*)-ethylcholesterol]. Some of the reactions just described have been observed in the Solanaceae. Thus, Alcaide *et al.* [74] found that tobacco plants convert [3-^{3}H]desmosterol administered by foliar application, to tritiated cholesterol and campesterol.

Figures 4 and 5 are partly hypothetical schemes, outlining the fate of cycloartenol and lanosterol, respectively. They are based on reactions observed in solanaceous and other plants [11–14, 16–18, 20, 21, 24, 25, 27–29,

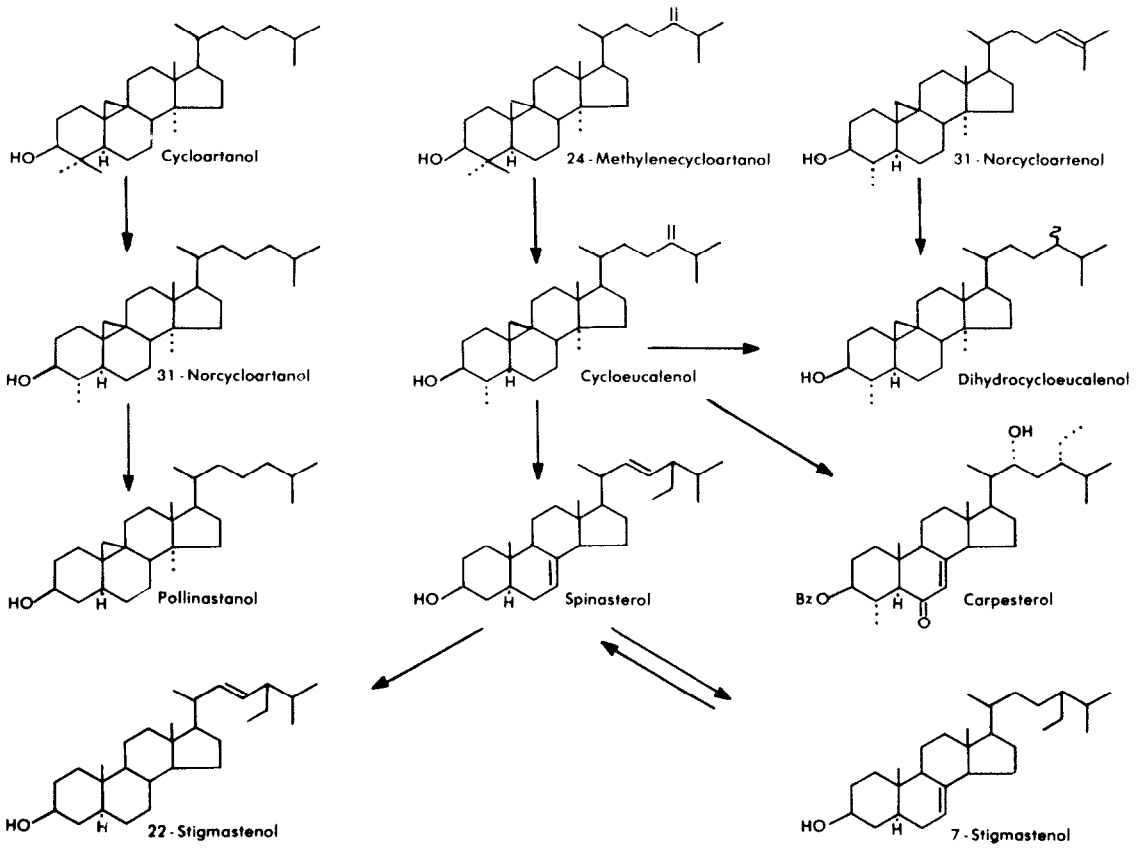

Fig. 4. Metabolism of cycloartenol.

35–37, 39, 75] and on the occurrence in Solanaceae of the intermediates shown in the schemes. The sterols in Fig. 3 have been isolated so frequently from solanaceous plants that it is safe to say that they occur in all of them [15, 24, 36, 76–78] and that their distribution has no taxonomic significance. However, analysis of tobacco genotypes has shown that the relative amounts of individual sterols are hereditary [79–81]. When tobacco scions were grafted on tomato stocks, their sterol composition remained unchanged, but their total sterol content decreased [82]. As for the intermediates in Figs. 4 and 5, Itoh *et al.* have identified six 4, 4-dimethylsterols [66] and 17 4-monomethylsterols [83, 84], besides 13 4-desmethylsterols [77], in the seeds of various Solanaceae.

Cycloartenol (Fig. 2) may undergo reduction to cycloartanol (Fig. 4), which was identified in, e.g. the fruits of *Solanum xanthocarpum* [85]. Alternatively, cycloartenol may be alkylated to 24-methylenecycloartanol (Fig. 4), which occurs in the leaves of *S. tuberosum* [86], *S. demissum* [87], *S. polyadenium* [87] and *S. dulcamara* [88], and in tobacco tissues [89]. Doubly-labeled mevalonate, incubated with tobacco tissue cultures, gave rise to labeled cycloartenol (Fig. 2) and 24-methylenecycloartenol (Fig. 4) [90, 91]. However, potato tuber slices incorporated labeled acetate into cycloartenol and not into 24-methylenecycloartenol unless they were first aged for a few hr [92]. Demethylation of cycloartenol leads to 31-norcycloartenol (Fig. 4) [83] and opening of the cyclopropane ring of cycloartenol produces lanosterol (Fig. 2) [66].

Analogous reactions of lanosterol are shown in Fig. 5. Reduction of the double bond at C-24 produces 8-lanosterol [66], alkylation yields 24-methylenedihydrolanosterol [66], and demethylation gives 31-norlanosterol, which was first isolated from potato leaves [93]. Each of these metabolites may undergo further demethylation and the cyclopropane ring may open at any stage to produce the corresponding Δ^8-analog. Thus, e.g. obtusifoliol (Fig. 5), which occurs in *S. dulcamara* [88], among other Solanaceae, may be produced by demethylation of 24-methylenedihydrolanosterol, as shown in Fig. 5, but it may also arise by ring opening from cycloeucalenol (Fig. 4), found in the same plant [88]. After a short incubation of tobacco tissues with [1-^{14}C]acetate cycloeucalenol and obtusifoliol were isolated in labeled form [94].

Shifting of the double bond from C-8 to C-7 converts 8-lanostenol to 7-lanostenol (Fig. 5) and (with the loss of a methyl group at C-14) obtusifoliol to gramisterol (Fig. 5), which was first isolated from potato leaves [95]. The Δ^7-sterol, spinasterol, is shown in Fig. 4 only in order to conserve space. Its biogenesis from cycloeucalenol, while theoretically possible, would require not only the opening of the cyclopropane ring and shift of the Δ^8-double bond to C-7, but also the loss of two methyl groups, alkylation at C-28 and dehydrogenation at C-22. Carpesterol (Fig. 4), isolated from *S. xanthocarpum* [96] together with related compounds [97], would require similar transformations, in addition to oxygenation at C-6 and C-22 and benzoylation at C-3.

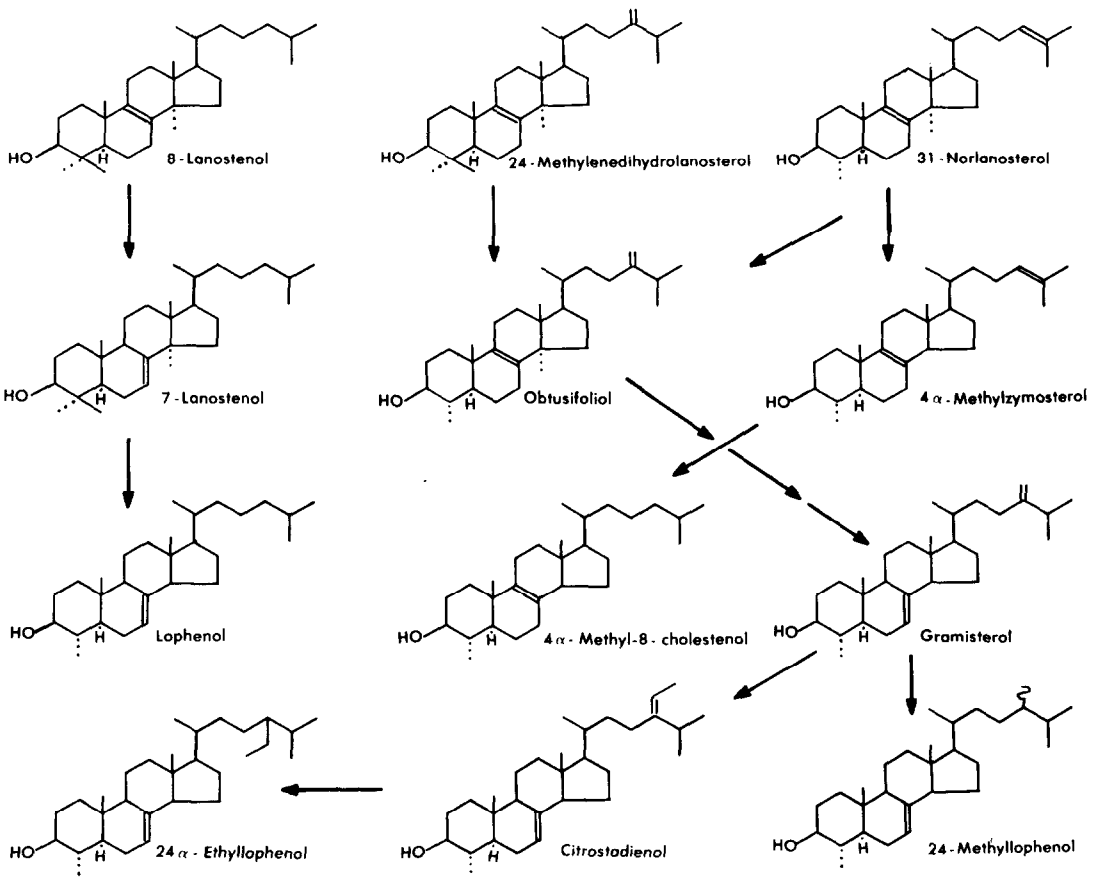

Fig. 5. Metabolism of lanosterol.

Foliar application of cycloartanol (Fig. 4) [98] and pollinastanol (Fig. 4) [99] to growing tobacco plants showed that they are converted to cholesterol. Similarly, 24-methylenedihydrolanosterol (Fig. 5) was converted to sitosterol (Fig. 3), while 24-methylenecholesterol (Fig. 3) was hydrogenated to campesterol (Fig. 3) [100]. The relative amounts of sterols in the leaves of *S. andigena* were found to be under photoperiodic control [101]. Change from long- to short-day conditions caused a rise in cholesterol and a simultaneous drop in sitosterol and cycloartenol levels. The conversion of spinasterol (Fig. 4) [102] and 7-stigmastenol (Fig. 4) [103] to sitosterol was observed by foliar application to tobacco plants, and additional evidence for the dehydrogenation of sitosterol to stigmasterol (Fig. 3) was also obtained [102].

During the ripening of tomatoes, the incorporation of precursors into sterols declined [104] and their stigmasterol content increased in proportion to a decrease in sitosterol content [105]. Studies on different tobacco cultivars have shown that the sum of sitosterol and stigmasterol is fairly constant, while their relative proportions may vary [80, 81]. The sterol content of tobacco leaves increased with age, mainly as a result of increasing concentrations of stigmasterol [106] or sitosterol [107]. The uppermost tobacco leaves contain more sitosterol and less stigmasterol than the lower leaves [107, 108]. However, because stigmasterol predominates in shaded leaves, basal leaves tend to be richer in stigmasterol, and the apical leaves, which are exposed to higher light intensities, tend to be richer in sitosterol [109, 110].

METABOLISM OF STEROLS

One of the most common transformations sterols undergo in plants is conjugation [21, 28, 29, 75, 111–113]. They are esterified with fatty acids or conjugated with sugars, which are often also acylated. Cholesteryl esters have been isolated from tobacco leaves [114], acylated steryl glycosides from potato tubers [115], and all three forms of conjugates from the leaves of *Solanum dulcamara* [116]. Because the esterification of sterols is a reversible process, the theory has been advanced that this is a mechanism for modulating membrane fluidity [113]. The formation of steryl glycosides in tobacco has been studied by Grunwald *et al.* [117, 118]. Although their experiments indicate that acyl glycosides can be formed by conjugation of sterols with acylated sugars, the more common pathway [29] leads from the sterols to steryl glycosides and thence to the acylated steryl glycosides. The intracellular distribution of sterols in tobacco leaves reflects their function as membrane components [119]. The acylated steryl glycosides constitute the main sterol fraction of microsomes [120, 121] and mitochondria [122] from potato tubers. In the roots of *S. dulcamara* the free sterols constitute 75% of the total sterol fraction, while in the leaves 86% of the sterols are conjugated [123].

A transport function has been postulated for conjugated sterols, but experiments with labelled cholesterol on growing *Nicotiana tabacum* [124] and *Solanum khasianum* [125] plants indicate that it is translocated in the free form. Dupéron *et al.* have observed that potato tubers lost some of their acylated steryl glycosides during storage in the dark [126] while esterified sterols increased [121]. When they germinated, they synthesized sterols from stored precursors rather than from administered acetate [127]. Experiments involving the incorporation of labeled mevalonate into the sterols of *S. andigena* have led Bae and Mercer [128] to the conclusion that a substance related to sterols may participate in tuber growth. During germination of tobacco seeds the esterified sterols increased while the steryl glycosides decreased [129]. Light stimulated the incorporation of labeled mevalonate into cholesteryl and stigmasteryl esters of tobacco seedlings for a few hr [130].

In the course of sterol metabolism in animals [13], the Δ^8-double bond of lanosterol shifts to C-7 and then to C-5. Cholecalciferol is produced from 7-dehydrocholesterol by a photochemical reaction involving the scission of the bond between C-9 and C-10 (Fig. 6). Plants belonging to the Solanaceae have been found to be rich in vitamin D activity [131–134]: *S. glaucophyllum* (*S. malacoxylon*) [135–142], *S. torvum* [143] and *Cestrum diurnum* [144–146]. Analysis indicates that the active principle is a glycoside or glycosides [142] of 1,25-dihydroxycholecalciferol [137, 138, 146]. No biosynthetic experiments have been reported so far, but it seems likely that the biogenesis of cholecalciferol in plants will turn out to be due to solar irradiation [147] of 7-dehydrocholesterol, as in animals. The occurrence of vitamin D in plants may be significant as it has been shown to stimulate rhizogenesis [148].

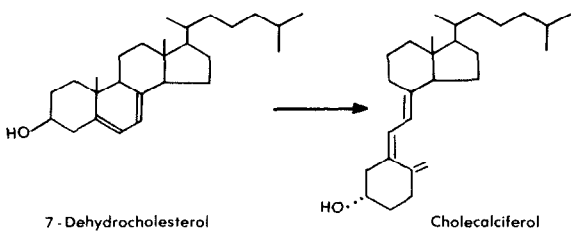

Fig. 6. Biogenesis of cholecalciferol.

Another transformation of cholesterol and other Δ^5-sterols which is analogous to previously observed animal metabolism is the reduction at C-5 via oxidation at C-3 (Fig. 7). The oxidation of cholesterol to 4-cholesten-3-one and its reduction to 5α-cholestan-3β-ol and 5α-cholestan-3α-ol have been demonstrated with chopped potato leaves [149]. The *in vivo* administration of [4-^{14}C]cholesterol to potato plants resulted in the isolation of two radioactive metabolites: 4-cholesten-3-one and 26-hydroxycholesterol (Fig. 8) [150].

BIOSYNTHESIS OF STEROIDAL SAPOGENINS

The biosynthesis of steroidal sapogenins has been reviewed earlier [11, 72, 151–155]. Steroidal saponins, the glycosides of the sapogenins, are widely distributed in the plant kingdom [156, 157], occurring in at least three genera of the Solanaceae [158]: *Solanum*, *Lycopersicon* and *Cestrum*. The best commercial source of sapogenins is *Dioscorea*, Dioscoreaceae [2]. However, in countries where high-yielding *Dioscorea* species are unavailable, *Solanum* species and methods for increasing their sapogenin yield are being explored [159, 160]. Because most of the work on the biogenesis of sapogenins has been done on Dioscoreaceae and there is no reason to assume that a different process operates in Solanaceae, I will draw mainly on the *Dioscorea* literature.

The steroidal sapogenins are spiroketals having the same configuration at C-22, but stereoisomerism at C-25. In Fig. 8 the sapogenins are drawn in full projection rather than half projection and half conformation. Thus, the oxygen atom in the F ring, which is actually at the back, is shown on top of the pyrane ring and the axial methyl group, which actually points upward, is shown as pointing to the front.

For the first experiments on the biosynthesis of sapogenins in 1961 we used homogenates of *Dioscorea* tubers [161]. Radioacetate was incorporated into diosgenin (Fig. 8), but the incorporation of mevalonate could not be observed. In 1965 we succeeded in demonstrating that [4-^{14}C]cholesterol, applied to the leaves of *D. spiculiflora*, is a precursor of diosgenin [162]. This was confirmed in 1966 by Tschesche and Hulpke [163], who administered cholesteryl glucoside to *Digitalis* plants. 26(25*R*)-[26-

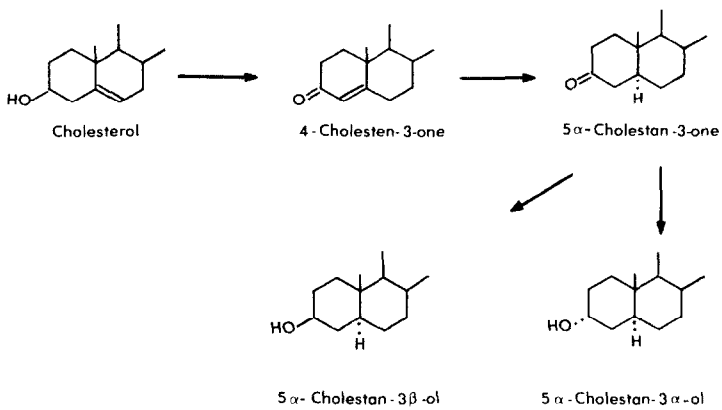

Fig. 7. Oxidation and reduction of cholesterol.

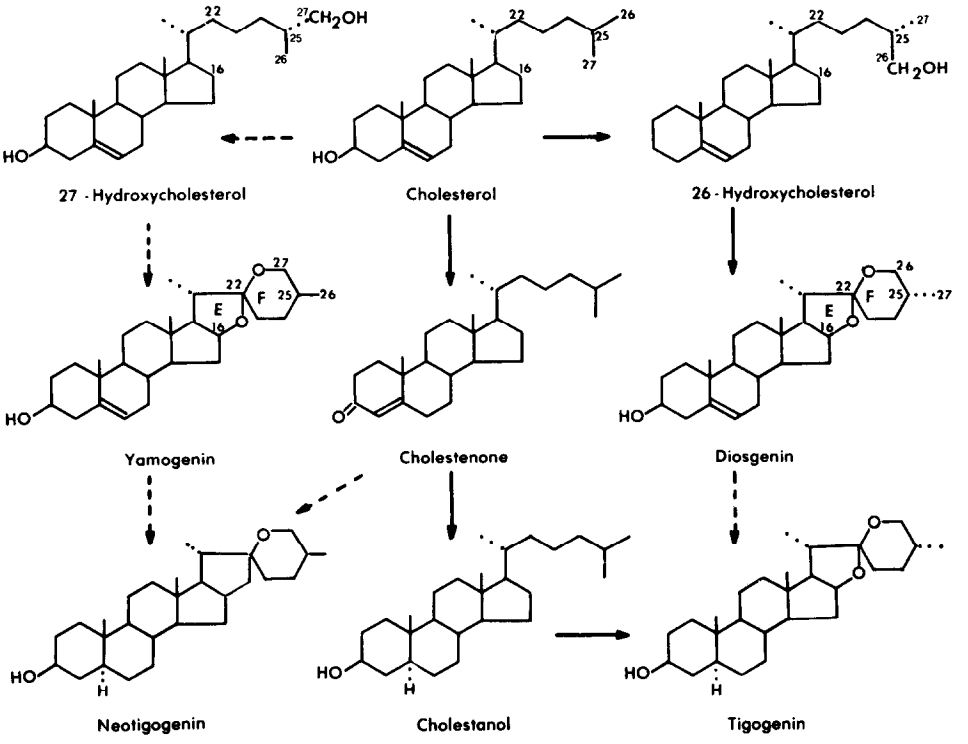

Fig. 8. Biosynthesis of C_{27} sapogenins.

^{14}C]Hydroxycholesterol (Fig. 8) was shown to be the first intermediate in this process by foliar administration of this compound to *Dioscorea* plants [164]. It was converted to diosgenin, but not to yamogenin (Fig. 8). However, when we administered cholesterol to *Lycopersicon pimpinellifolium*, we obtained neotigogenin (Fig. 8) [165]. Subsequently, Ronchetti *et al.* [166] were able to show that 5α-cholestane-3β,27 (25*S*)-diol was the key intermediate in that reaction. Because C-25 in cholesterol is a prochiral center, it depends on the introduction of the hydroxyl group at either C-26 (25*R*) or C-27 (25-*S*) whether the methyl group at C-25 of the resulting sapogenin will be equatorial (25α) or axial (25β). Earlier speculations that the cholesterol side chain is either degraded or dehydrogenated at C-24 were disproved when diosgenin, produced by *Dioscorea* [167] or *Digitalis* [168] plants showed the same $^{3}H/^{14}C$ ratio as the administered [4-^{14}C,25-^{3}H]cholesterol.

Formation of the E and F rings requires, besides hydroxylation at C-26(27), functionalization of C-16 and C-22. In experiments with *Digitalis* plants, Tschesche *et al.* found that 16β-hydroxycholestanol [169], 20α-hydroxycholesterol [170] and 16β,26-dihydroxycholesterol [171] were incorporated into sapogenins, while 22-hydroxycholesterol and 22-ketocholesterol were not [172]. The reduction at C-5 occurs before ring closures via 4-cholesten-3-one [150, 172], 5α-cholestan-3-one and 5α-cholestan-3β-ol (cf. Fig. 7), all of which have been shown to be precursors of tigogenin (Fig. 8) [173], although direct reduction of Δ^5-sapogenins is theoretically possible.

The occurrence of saponins lacking the F ring (furostanols) in plants [174] is of some practical importance. It explains the frequently reported observation that saponin yields increase when plant homogenates are 'fermented' in water [175–181]. After treatment of *Dioscorea* plants with [4-^{14}C]cholesterol, we isolated a furostanol, protodioscin (Fig. 9) [182]. Incubation of this product with a leaf homogenate from *Dioscorea* hydrolysed the 26-glucoside, permitting the F ring to close spontaneously with the formation of dioscin [183]. Similarly, (25*S*) 5α-furostane-3β,27-diol was converted to neotigogenin by *Lycopersicon pimpinellifolium* [166]. Among the solanaceous plant sources in which furostanols have been found are the seeds of tomatoes [184] and red peppers [185], the stems of *Solanum lyratum* [186], and the roots of *S. paniculatum* [187].

Several reports have been published on the *in vitro* production of sapogenins by tissue cultures of Solanaceae. Thus, diosgenin was obtained from *S. xanthocarpum* [188–190], *S. aviculare* [190], *S. laciniatum* [191], *S. nigrum* [192] and *S. jasminoides* [193].

It is reasonable to assume that all plants belonging to the genus *Solanum* contain steroidal sapogenins, as they have been isolated from so many of them, e.g.: diosgenin from *S. vespertilio* [194], *S. sodomeum* [195], *S. aviculare* [196], *S. laciniatum* [196], *S. lyratum* [186], *S. nigrum* [197] and *S. eleagnifolium* [198]; tigogenin from *S. curtipes* [199] and *S. nigrum* [200]; yamogenin from *S. dulcamara* [201]; hispigenin [202], solaspigenin [203] and neosolaspigenin [203] from *S. hispidum*; nuatigenin from *S. sisymbriifolium* [204] and *S. abutiloides* [205]; andesgenin from *S. hypomalacophyllum* [206]; barogenin from *S. tuberosum* [207]; paniculogenin from *S. paniculatum* [208] and *S. torvum* [209], together with jurubidine.

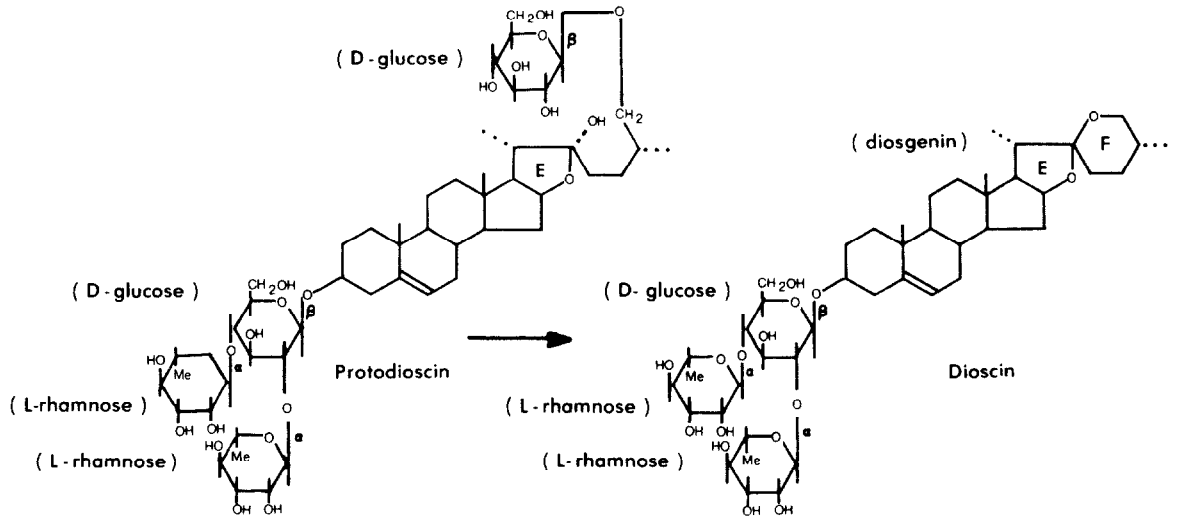

Fig. 9. Biosynthesis of dioscin.

BIOSYNTHESIS OF STEROIDAL ALKALOIDS

The organic chemistry of the steroidal alkaloids has been reviewed by Schreiber [210–212], their distribution in Solanaceae by Wojciechowska and Pasciak [213, 214] and by Mahmood and Thakur [215], and biochemical aspects by Heftmann [11, 72, 155], Schreiber [216, 217] and Roddick [218]. Special reviews have been devoted to the alkaloids of *S. tuberosum* [219–221] and *S. dulcamara* [222, 223], to tomatine [224] and solasodine [4], and to alkaloids in food plants in general [7–10, 225].

The steroidal alkaloids occur in plants as glycosides, the so-called glycoalkaloids, accompanied by analogous steroidal sapogenins, in the form of saponins, both glycosides containing the same sugars. So far *ca* 250 species of *Solanum* and related genera have yielded steroidal alkaloids and, thus, it is quite possible that they occur in all representatives of these genera. However, this does not mean that the so-called *Solanum* alkaloids are characteristic for the Solanaceae, because they also occur in Asclepiadaceae and Liliaceae. There is some fuzziness in the literature concerning the distribution of glycoalkaloids due to the fact that work on the isolation and organic chemistry is far ahead of biochemical studies. It is gradually becoming clear that they are synthesized in certain parts of plants under certain conditions and then degraded. Thus, we cannot expect to find the same amount of a certain alkaloid in all parts all the time. Moreover, knowing how, e.g. *Veratrum* alkaloids are synthesized, we are not surprised to find the *Solanum* alkaloid, solanine, in *Veratrum*, because it is somehow involved in the biosynthesis of *Veratrum* alkaloids.

In the first biosynthetic experiment with radioactive tracers on solanaceous plants, Sander and Grisebach [226] demonstrated the incorporation of acetate into tomatine by tomato seedlings in 1958. Guseva *et al.* soon afterwards reported the utilization of radioactive acetate [227] and mevalonate [228] for the synthesis of glycoalkaloids in potato sprouts and in leaves of *S. laciniatum* [229, 230]. Similar experiments were later carried out with potato slices [231] and isolated potato chloroplasts [232].

The next step in the biosynthesis of steroidal alkaloids that has been established experimentally is the conversion of both [26,27-^{14}C]cycloartenol and 26,27-^{14}C]lanosterol to tomatidine by intact. *L. pimpinellifolium*, to solanidine by *S. chacoense*, and to solanocapsine by *S. pseudocapsicum* (Fig. 10) [233]. By administering labeled cholesterol it was shown, furthermore, that it is a precursor of tomatidine in *L. esculentum* [234] and *L. pimpinellifolium* [235, 236] and of solanidine in *S. tuberosum* [236, 237] and *S. andigena* [238]. 20-Hydroxycholesterol was also utilized in the biosynthesis of solasodine by *S. laciniatum* [170].

In contrast to the sapogenins, which are isomeric at C-25 but have the same stereochemical configuration at C-22, the 25-methyl group in the steroidal alkaloids is always equatorial whereas the nitrogen in spirosolanes may be either in front, as in tomatidine (Fig. 10), or at the back, as in solasodine (Fig. 11). Thus, (25*S*)5α-cholestane-3β,27-diol was converted to tomatidine [166], while (25*R*)5α-cholestane-3β,26-diol was converted to soladulcidine (Fig. 11) by *L. pimpinellifolium* [239]. One of the differences between the biosynthesis of the sapogenins and alkaloids is that the alkaloids require the introduction of a nitrogen atom into the molecule. Tschesche *et al.* [239] have found that it is introduced through a simple replacement of the terminal hydroxyl group by an amino group, an exchange reaction we had observed earlier in the biosynthesis of C_{21} alkaloids [240]. The donor molecule is an amino acid, e.g. glycine and alanine in *S. tuberosum* [241], or arginine in *V. grandiflorum* [242].

Although conversion of (25*R*)26-aminocholesterol and (25*R*)26-amino-16β-hydroxycholesterol (Fig. 11) [243] and of 26-aminodihydrodiosgenin (Fig. 11) [170] to solasodine by *S. laciniatum* indicate that the formation of the E ring can, in principle, precede the closure of the F ring [170], the occurrence in plants of many alkaloids lacking an E ring (cf. Figs. 11 and 12) supports Schreiber's hypothesis [210] that in steroidal alkaloids the formation of the F ring precedes the closure of the E ring. This is also borne out by experimental evidence, because solacongestidine and dihydrosolacongestidine were converted to soladulcidine (Fig. 11) and some analogous Δ^5-steroids to solasodine by *S. laciniatum* [244].

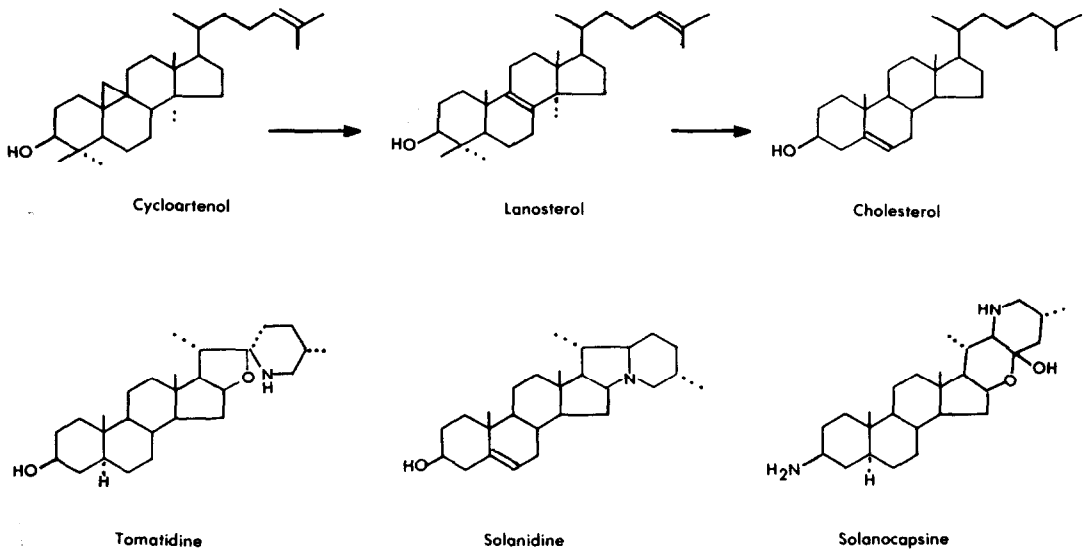

Fig. 10. C_{27} alkaloids from cholesterol.

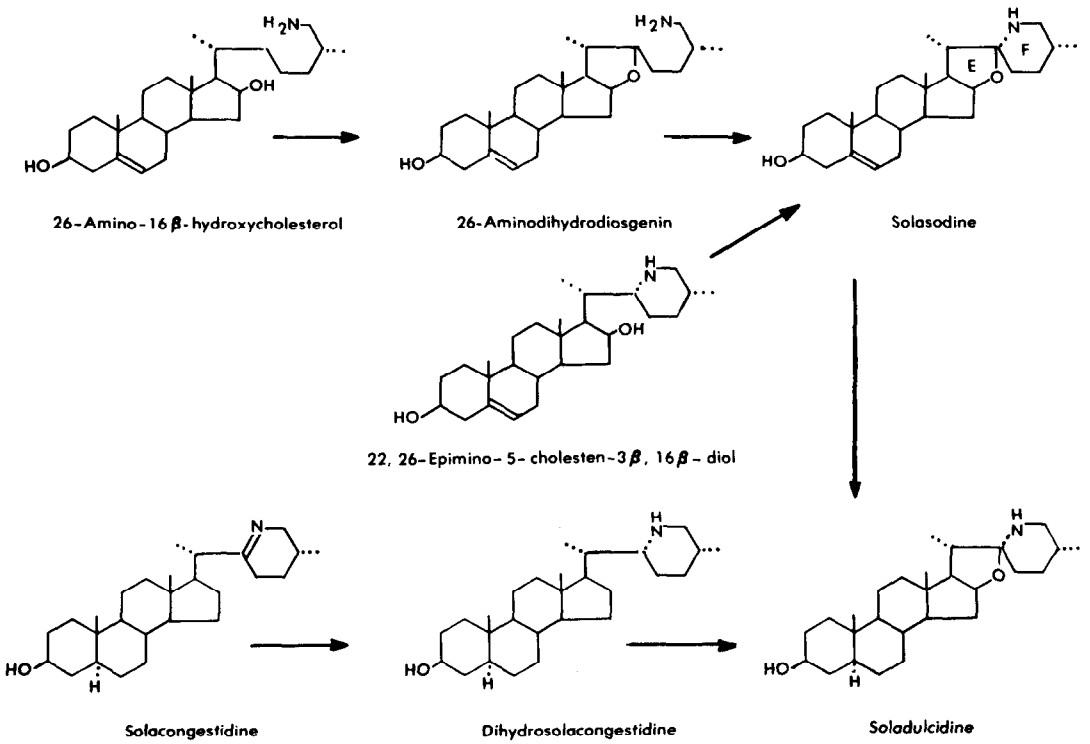

Fig. 11. Biogenesis of *Solanum* alkaloids.

Figure 12 represents a biosynthetic scheme derived from work with *Veratrum grandiflorum* [245], but it may, nevertheless, shed some light on intermediates in the biogenesis of alkaloids in *Solanum* species, particularly the origin of the condensed ring system in solanidine. Cholesterol, dormantinol and etioline were identified in budding *V. grandiflorum* [246]. Verazine, which was also present, is a logical intermediate in the biosynthesis of solanidine in the leaves and of rubijervine in the rhizomes. This was actually demonstrated when labeled verazine, but not etioline, was converted to hakurirodine and rubijervine (Fig. 12) by dormant rhizome slices [245]. After budding, solanidine was formed in the leaves, probably via etioline (Fig. 12).

Like other plant steroids, solanidine is glycosylated by UDP-glucose in the presence of a glucosyltransferase in enzyme preparations from *S. tuberosum* sprouts [247] or tubers [248, 249], or *S. laciniatum* stems [250]. According

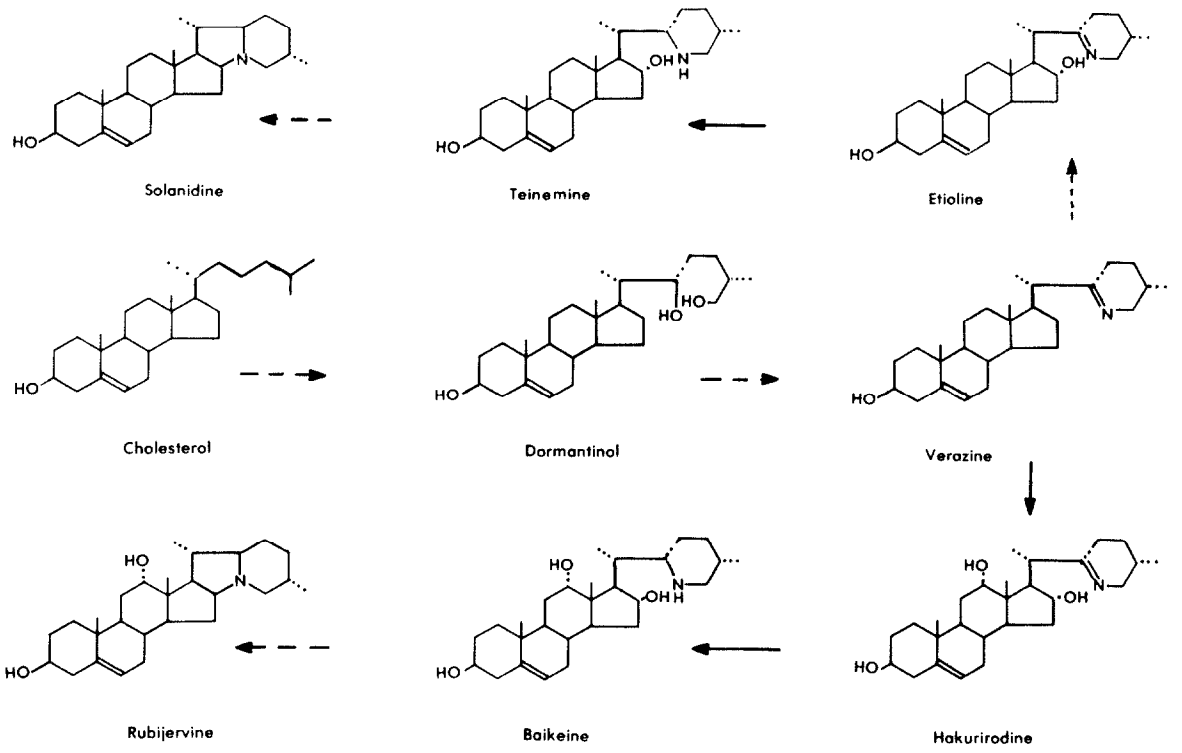

Fig. 12. Biogenesis of C_{27} alkaloids.

to Petrochenko [251], potato sprouts contain an enzyme, solaninase, which hydrolyses solanine, but not tomatine or demissine, whereas tomato leaves contain tomatinase [252], an enzyme capable of hydrolysing tomatine and demissine, but not solanine.

Plant tissue cultures are useful for studying details of steroid biosynthesis and metabolism, provided care is taken in extrapolating observations on callus tissue to biochemical processes in intact plants [253–256]. Much work has been done in this area with the aim of producing useful alkaloids [257, 258]. While Vágújfalvi *et al.* [191] and Supniewska and Dohnal [259] found no alkaloids in their tissue cultures of *S. laciniatum*, Hosoda *et al.* [260, 261] established the proper conditions for the production of not only solasodine but also diosgenin and other steroids by callus tissues of that species. Solasodine synthesis by *S. xanthocarpum* tissues [262] was stimulated, e.g. by the addition of cholesterol [263]. Other plant tissues yielding solasodine were *S. acculeatissimum* [264], *S. khasianum* [265, 266] and *S. verbascifolium* [267]. The glycoalkaloid production of *S. khasianum* tissues started when rootlets began to form [268]. Root cultures of *S. chacoense* produced solanidine glycosides [269] and tomato root cultures produced tomatine [270, 271]. A reactor containing immobilized cells of *S. aviculare* was designed for the continuous production of glycoalkaloids [272].

Although the nature and proportions of glycoalkaloids in plants are genetically determined [273–277], the total concentrations are modified by ecological factors [278–287]. The biosynthesis of glycoalkaloids by solanaceous plants is an important subject when toxicity in food plants must be minimized [288] or the productivity of plants raised for the pharmaceutical industry must be maximized [4, 289–293]. It was observed as early as 1929 that the production of glycoalkaloids is associated with growth and intensified metabolic activity [294]. Grafting experiments showed that the glycoalkaloids are synthesized in the scion and that, in contrast to many other alkaloids, the alkaloids occurring in the roots or tubers are not transported upwards [295, 296]. Furthermore, no transport of alkaloids from shoots to fruits was observed in *S. dulcamara* [297]. The analysis of various organs during development may shed some light on the biosynthetic activity in them. However, some caution in the interpretation of data on steroid 'dynamics' is indicated, as the amount of a metabolite found at any moment represents the balance of anabolic, metabolic, catabolic and translocation processes.

No saponins or glycoalkaloids were found in dormant tomato seeds [298], only traces of solasonine in *S. laciniatum* seeds [299], but high concentrations of glycoalkaloids in *S. acculeatissimum* seeds [300]. The alkaloid synthesis begins during germination and reaches a peak during the flowering period in *S. laciniatum* [299, 301, 302], *S. persicum* [303] and *L. esculentum* [304]. However, in *L. glandulosum*, a short-day plant, the tomatidine content was about five times higher when it was kept vegetative than when it was flowering [305, 306]. In addition to seasonal variations [307–312], a diurnal rhythm in glycoalkaloid content, which is probably temperature-related, has been noted by many authors [302, 313–316]. Generally, the leaves attain a maximum glycoalkaloid concentration first [317–320], followed by an even higher concentration in unripe fruits [299, 310–303, 307, 309, 321–323]. The flowers have the highest glyco-alkaloid concentration in tomato [304, 324, 325] and potato [326, 327] plants.

As detached leaves wilt, their glycoalkaloid content increases [328–330]. However, when either attached or detached fruits ripen, their glycoalkaloids decrease and finally disappear. This has been observed not only in ripening tomatoes [323, 331–335] but also in the berries of *S. dulcamara* [336, 337], *S. khasianum* [338–340], *S. laciniatum* [341] and *S. marginatum* [342]. The significance of these observations is discussed below.

The solanidine glycosides in potato tubers are also subject to seasonal variations [312, 343]. As the plants mature, their concentration generally decreases [312, 326, 327, 344]. Fertilization with nitrogen and potassium may increase the glycoalkaloid content of the epigeous portion of the potato plants but decrease it in the tubers [312, 345, 346]. In isolated tubers the biosynthetic activity continues and increases the glycoalkaloid concentration [347]. It is greatest around the eyes and developing shoots [327, 348–351]. Exposure of potato tubers to light [352–357] and heat [355, 357, 358] stimulates glycoalkaloid synthesis, and treatments [359] or agents [354, 357, 360] that inhibit sprouting also inhibit biosynthesis. Mechanical injury, such as bruising or slicing of potatoes [358, 361–363], stimulates glycoalkaloid synthesis [364, 365] and can also be inhibited by sprout inhibitors [366] or γ-irradiation [367]. Accumulation of rishitin in potato tubers appears to be correlated to the suppression of glycoalkaloid production by injured potato tubers [368]. Postharvest glycoalkaloid biosynthesis can be suppressed by any treatment that inhibits respiration of potatoes [369, 370], e.g. dipping them in oil [371, 372], paraffin [373], or water [374–376], or by spraying them with lecithin [377].

METABOLISM OF STEROIDAL SAPOGENINS AND ALKALOIDS

The disappearance of tomatine from ripening tomato fruits was first investigated by Sander and Angermann in 1961 [331] and, subsequently, by Bennett *et al.* [165]. Injection of radioactive tomatidine into *Lycopersicon esculentum* and immature *L. pimpinellifolium* fruits showed extensive metabolism, but none of the metabolites could be identified. However, we observed in 1967 [165] that the administration of [4-^{14}C]cholesterol to *L. pimpinellifolium* plants produced not only labeled neotigogenin but also labeled 3β-hydroxy-5α-pregn-16-en-20-one (allopregnenolone) (Fig. 13). This C_{21} steroid had previously been isolated from the same source by Schreiber and Aurich[378]. In 1972 we injected labeled tomatine into ripe tomato fruits and succeeded in demonstrating its conversion to labeled allopregnenolone [379].

This compound, or rather its Δ^5-analog, is the key intermediate in the industrial preparation of progesterone. The Δ^{16}-double bond marks it as a dehydration product of 16-hydroxy steroids. It has been identified in various sapogenin- and alkaloid-containing plants [29]. Adam *et al.* have isolated 3β-hydroxy-5α-pregnan-16-one [380] and 4-solasoden-3-one [381, 382] from *Solanum hainanense* (Fig. 13). These steroids could easily be metabolites of solasodine, as it is known that diosgenone is degraded to 20α-hydroxy-4-pregnene-3,16-dione by *Verticillium theobromae* [383] and to 1,4-androstadiene-3,16-dione by *Fusarium solani* [384] (Fig. 13).

Many higher and lower plants have the ability to

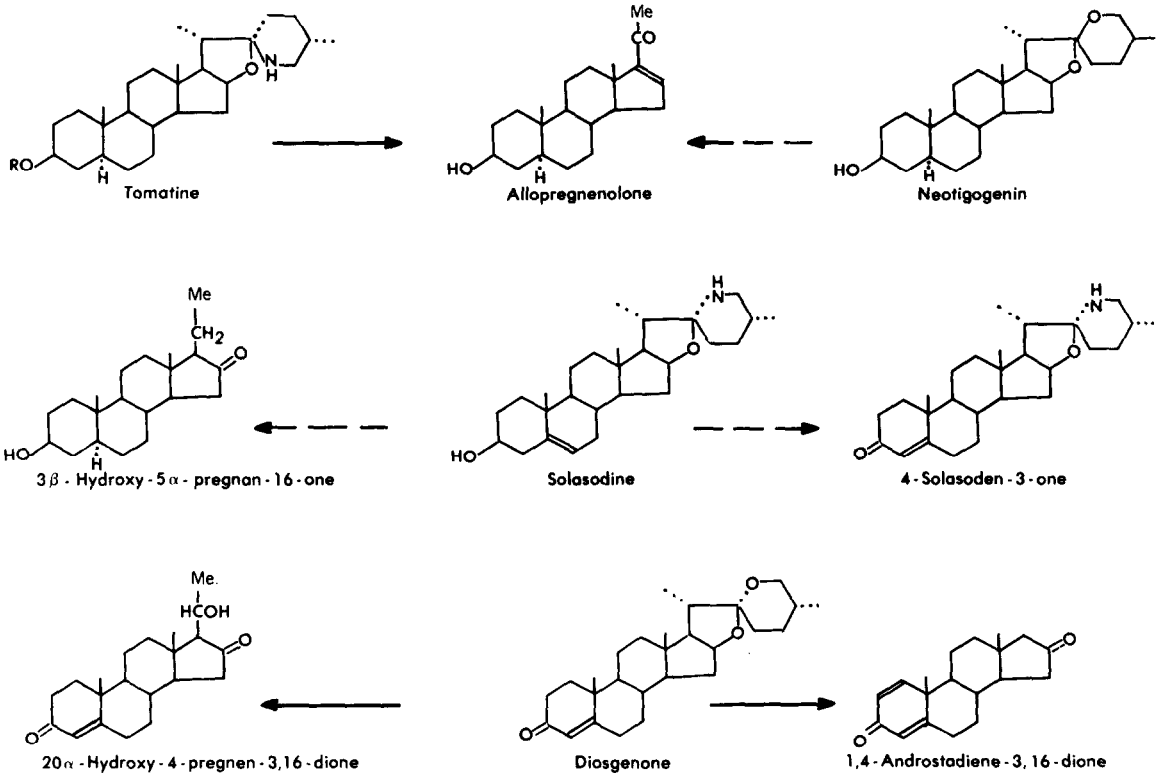

Fig. 13. Degradation of alkaloids and sapogenins.

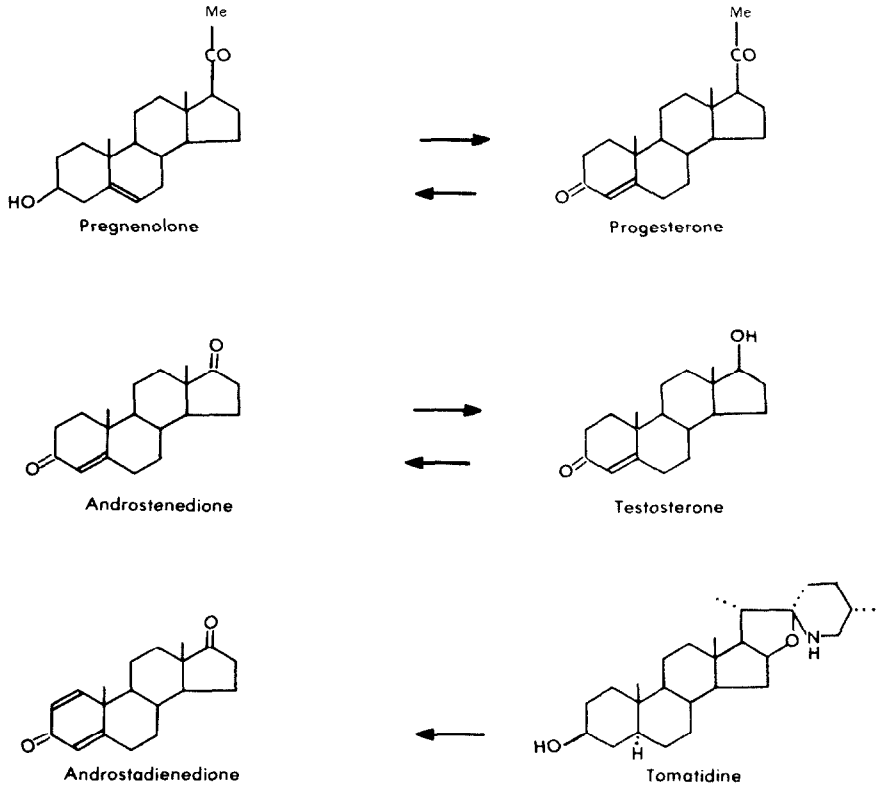

Fig. 14. Biogenesis of C_{21} and C_{19} hormones.

degrade sterols to pregnenolone and to convert the latter to progesterone [385] (Fig. 14), but the side-chain cleavage, known to occur in other plants [29], has not yet been demonstrated in intact solanaceous plants. Tissue culture experiments [255, 256] have resulted mainly in hydroxylation of steroids or reversible oxidation/ reduction reactions, including the interconversion of pregnenolone and progesterone [386, 387] and of androstenedione and testosterone [388] (Fig. 14) by *Nicotiana tabacum*. The conversion of solanidine (Fig. 12) to the jerveratrum alkaloids [389] has not been observed in solanaceous plants.

The microbiological transformations of steroidal sapogenins [390] and alkaloids [391, 392], which may serve as models of enzymatic reactions in higher plants, have recently been reviewed. Maas *et al.* [393] have claimed that *Phytophthora infestans* synthesized glycoalkaloids. Microbial conjugation of tomatine [394] and hydrolysis of glycoalkaloids [395, 396] have been demonstrated; e.g. *Aspergillus japonicus* converts solaradixin to solasonine [397]. In addition to various hydroxylation [398–402], dehydrogenation [403–408] and epimerization [409] reactions, Belič *et al.* [410] have demonstrated side-chain cleavage of tomatidine to 1,4-androstadiene-3,17-dione (Fig. 14) by cholesterol-treated *Arthrobacter symplex*.

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