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REVIEW

SESQUITERPENOID STRESS COMPOUNDS OF THE SOLANACEAE

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Key Word Index—*Solanaceae*; sesquiterpenes; ^{1}H NMR spectra; ^{13}C NMR spectra; biogenesis; stress compounds; phytoalexins.

Abstract—The sesquiterpenes isolated from species of the Solanaceae under various conditions of stress are reviewed, with brief references to other solanaceous stress metabolites. The chemistry and selected physical properties of the sesquiterpenoidal compounds are summarized with special emphasis on their ^{1}H and ^{13}C NMR spectra. The close biogenetic relations between the compounds are discussed, and their possible biological function as antifungal agents ("phytoalexins") is considered.

INTRODUCTION: STRESS COMPOUNDS AND PHYTOALEXINS

Injury to a plant typically leads to profound alterations in the metabolism of the affected cells. Frequently, the changes that occur are made manifest in the production of substances which may conveniently be referred to as stress compounds [1]. These may be primary or secondary metabolites [2] which accumulate to substantially higher levels in damaged than in healthy tissue; oxidation and polymerization products derived from them; aglucones liberated from glycosides, sometimes with subsequent rearrangement; or secondary metabolites which have no known immediate precursors in the plant but appear to be synthesized *de novo* from the usual, elementary building blocks. Stress compounds, especially those of the last group, have been attracting increasing attention during recent years. One reason is that several have been implicated in the toxicity of diseased food plants to humans and livestock. Another and more frequent source of interest in the compounds is their relevance to the mechanisms of plant disease and their possible function, in some instances, as natural defensive agents [3–7].

Many different types of injury or stress can bring about their formation. For herbaceous plants, the most important are infection with fungi, bacteria, and viruses. Mechanical wounding, irradiation with UV light, dehydration, cold, or treatment of plant tissue with phytotoxic compounds can also lead to the production of stress metabolites. In trees, normal heartwood constituents are often formed during the transformation of living sapwood into dead tissue. Such compounds probably should also be regarded as stress metabolites, particularly since they include several which can be induced in living tissue, of the same or related plant species, by fungal or equivalent damage.

A sub-group of the stress compounds which is attracting particular interest at the present time consists of the phytoalexins. According to the classical paper by Müller and Börger [8] in which the term was coined, phytoalexins are compounds, formed or activated in a living plant cell through contact with a fungus, which are inhibitory to fungi at large and thereby prevent the full development of the incipient disease. The hypothesis, which postulated yet further criteria, was restated in a modified form in an important article by Cruickshank [9]. A detailed treatment is outside the scope of this review but the point must be made that both these statements of the phytoalexin hypothesis incorporated postulates which are only incompletely supported by the experimental facts which have since emerged. As a consequence, the literature now contains a number of other and, in part, mutually exclusive definitions [10–14]. In practice, however, and subject to certain, essentially conventional exceptions, the current tendency is to apply the term phytoalexin to any antifungal compound that is synthesized by a plant in greatly increased amounts after fungal infection. The niceties of a rigorous definition are often disregarded, with sometimes anomalous results.

The confusion is symptomatic of deeper issues than terminology. Some of the compounds now regarded as phytoalexins may be induced, in the same plant and by the same stimulus, side by side with chemically and biogenetically closely related but as far as tested, antifungally inactive stress metabolites. On the other hand, phytoalexins from different plant families usually differ from one another profoundly in chemical structures and biosynthetic derivation, as well as in other characteristics. On these counts, and regardless of the rigour or laxity of the definition which is employed, phytoalexins cannot be regarded as members of a homogeneous class, nor does their antifungal activity necessarily constitute a common raison d'être. It would therefore be wrong to view them as similar or equivalent to the antibodies of

‡ The review of the literature was completed on 1.12.75.

higher animals. Antibodies have a common basic structure [15] which is infinitely variable in detail and thus was ideally suited for their selection for a common evolutionary purpose. No such design is apparent in the phytoalexins.

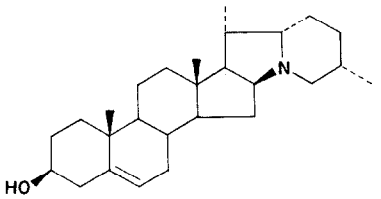
Diversity of structure is not, of course, a feature of phytoalexins alone but is shown by stress metabolites in general although some, for instance certain cinnamic acid derivatives, are found throughout the plant kingdom. Others belong to structural groups that are highly characteristic for one or for only a few families. Conversely, probably no plant is ever restricted to the production of only one type of stress compound. A particularly well studied case which illustrates such associations is that of the Leguminosae. Many species in this family are characterized by the post-infectional production of isoflavonoid-like compounds amongst which pterocarpans (antifungal) and coumestans (mostly inactive) are the most prominent [16,17]. With a single exception, elaboration of these compounds appears to be restricted to the Leguminosae and almost exclusively to members of the sub-family Lotoideae. However, flavonoid derivatives, as distinct from isoflavonoids, also occur as stress compounds in the Leguminosae [18], as well as in other plant families. At least one species of the legumes, *Vicia faba*, produces acetylenic phytoalexins [19] which are totally unrelated to the flavonoids in structure and biogenetic origin. The same species may produce pterocarpanoid phytoalexins [9] also, but no well defined compound has been reported as yet.

A similar situation has emerged for the Solanaceae. Some members of the family have been known for a considerable time to produce a variety of compounds in response to stress conditions, including several widely distributed aromatic acids, coumarins, and other phenols. In addition, highly characteristic alkaloids are produced as stress compounds by several species. The more important aspects of these compounds will be briefly discussed but the primary subject of this review are the bicyclic sesquiterpenes which have been isolated as stress metabolites from several members of the Solanaceae during the last few years. More of these compounds can be expected to be discovered in the future. For this reason, concise accounts will be given of the circumstances of their formation and their chemical and biological properties.

DISTRIBUTION AND INDUCTION

Potatoes

Because of their great economic importance, tubers of the cultivated potato (mainly *S. tuberosum* and certain of its hybrids [20]) have been the subject of intensive chemical, biochemical, and plant pathological investigations for a very long time. Much of the older literature [3–7] on stress conditions has been concerned with the role of phenolics and this interest is also evident in more recent studies [21–23]. Individual substances that have received particular notice include chlorogenic and other hydroxycinnamic acids, their conjugates and oxidation products, and the coumarins scopolin, aesculin, and umbelliferone. Non-phenolics have also attracted attention. Thus, pipecolic acid has been reported [24,25] as present in diseased but not in healthy tissues. Sterol production in cut tubers has also been studied



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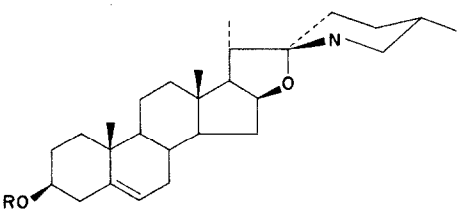
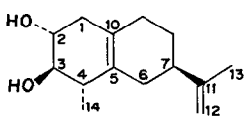


(2) R = H
(10) 5,6-dihydro-2 (R = lycotetraosyl)

[26]. The formation of the steroidal alkaloid solanidine (**1**), mostly in the form of its glycosides solanine and chaconine, is greatly stimulated in tubers by mechanical injury and ageing [27–30]. Infection with certain fungi has also been reported [29,30] to lead to an increase in these alkaloids. More recently, glycosides of tomatidenol (**2**) were induced, as variety-specific metabolites, by the slicing of Kennebec tubers [31]. The formation of these steroidal compounds is of particular interest because of their biogenetic relationships to the sesquiterpenoidal stress metabolites to which we now turn our attention.

The classical work [8] which led to the phytoalexin hypothesis was conducted with potatoes, but it was almost 30 years before rishitin (**3**) was isolated, as the first well-defined compound from this plant, with properties which approximated those postulated for phytoalexins [32]. Its isolation was followed in quick succession by those of the antifungal stress compounds lubimin (**4**) [33] and phytuberin (**5**) [34]. Rishitinol (**6**) [35], hydroxylubimin (**7**) [36], anhydro-β-rotunol (**8**) [37], and solavetivone (**9**) [37] have since also been isolated as stress metabolites of potato. They clearly belong to the same group biogenetically as the first three but little or nothing has yet been reported about their possible implication in disease resistance.

None of the compounds ever appears to be induced alone but not all of them are produced in detectable amounts in any given situation. The relative proportions in which they are formed appear to be a function of incubation time and potato cultivar [34] and of the fungal species [38] used as inducers. Preparative separation of the compounds from one another is generally accomplished by column and preparative TLC on silica. Liquid and gas chromatography were employed [37] for the separation of the vetispiranes **8** and **9** but details have not yet been reported. Repeated chromatography, over silica and alumina, was required to separate rishitinol from rishitin [35]. In our own laboratories, substantially pure lubimin (**4**), hydroxylubimin (**7**), and rishitin (**3**) were obtained by careful chromatography of a potato extract over a single, though large column of silica (TLC type, Camag DF5, unactivated, with 5% methanol in



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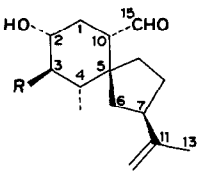
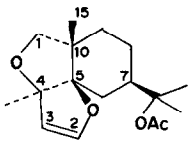


(4) R = H
(7) R = OH



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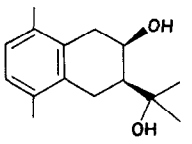

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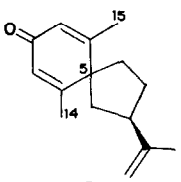

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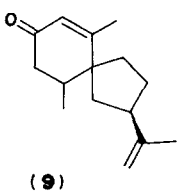

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ether as solvent). Typical data on analytical, as opposed to preparative separations are given in Tables 1 and 2.

The variety of stresses which lead to the formation of the compounds has been studied in some detail, particularly in the case of rishitin. This compound will also serve, as a representative example, for a fuller discussion of isolation procedures and related aspects.

Rishitin (**3**) was obtained originally [32] from sliced potato tubers (*S. solanum* × *S. demissum*, var. Rishiri)

Table 1. R_f values of sesquiterpenoids on TLC

Compound	R_f values*†		
	A	B	C
3	0.22	0.46	0.24
4	0.41	0.53	0.33
5	0.69	0.71	0.62
7	0.18	0.30	0.13
8‡	0.62	0.55	0.40
9‡	0.68	0.68	0.60
11	0.43	0.48	0.31
12	0.58	0.68	0.58
13	0.46	0.61	0.48
14	0.37	0.61	0.46
15	0.17	0.38	0.19
16	0.40	0.55	0.35
17	0.24	0.48	0.30
23§	0.62	0.65	0.55

* Determined at 23° on 0.3 mm layers of silica gel (Camag DF 5), prewashed with solvent, in filterpaper lined tanks pre-equilibrated with solvent; with the samples (2 μl of 0.25% solutions in alcohol) applied as single spots 1.5 cm from the lower edge of the chromatograms; origin to front *ca* 16 cm; development with phosphomolybdic acid (5% in alcohol) or vanillin (1% in 50% aq phosphoric acid) at 110°.

† Solvents: A, MeOH–$CHCl_3$ (1:19); B, MeOH–Et_2O (1:19); C, Et_2O.

‡ We thank Dr. R. F. Curtis for supplying these compounds.

§ We thank Dr. R. S. Burden for this substance.

Table 2. Retention times of sesquiterpenoids on GLC

Compound	Retention time*	
	Free alcohol	TMSi derivative
3	197	305
4	258	336
7	d†	468
11	272	nd‡
15	265	326
16	295	nd
17	198	464
23	168	nd

* In seconds, determined using 180 cm × 1.5 mm column, packed with 3% SE 30 on Gas Chrom Q 80–100. Column temperature 160°, detector temperature 250°, N_2 flow rate 38 ml/min, f.i.d.

† Decomposes.

‡ Not determined.

which had been inoculated with an incompatible race of *Phytophthora infestans*, the causal organism of late blight of potatoes. After 2 days' incubation, the slices were extracted with methanolic chloroform and rishitin was isolated by chromatography of the hexane-soluble fraction over silica, with 8% acetone in hexane as solvent. Yields of 40 μg/g tissue were realized. Crystalline rishitin, mp 65–67°, was obtained after further purification via the *bis*-3,5-dinitro-benzoate (mp 172–173°). In analytical experiments, healthy tuber tissue was found to contain no detectable rishitin, tubers that had been cut but not inoculated contained traces, while tubers that had been inoculated with a compatible and incompatible race of *P. infestans* were found to contain 0.44 and up to 120 μg/g tissue respectively. *Fusarium solani* f. sp. *phaseoli*, a non-pathogen of potatoes, was also found to be an effective inducer. Since then, work in the same [39] and in other laboratories [33,34,38,40,41] established that the formation of rishitin, in the tubers of all of the several potato cultivars tested, is a general response to inoculation with non-pathogenic or avirulent fungi. The situation holds for other parts of the plant except for one report of rishitin and phytuberin production in shoots in a compatible interaction [40].

Rishitin and phytuberin (**5**) are also formed in potatoes inoculated with a bacterium [42] or treated with cell-free, boiled extracts and sonicates of both compatible (virulent) and incompatible (avirulent) races of *P. infestans* [40]. Fungal proteins, when applied to potato slices, induce rishitin and lubimin (**4**) formation [43] and fungal lipids appear to evoke a similar response [44]. Most interestingly, a DNA fraction from a resistant potato hybrid was found to lead to rishitin formation when applied to a susceptible cultivar [45]. Mercuric chloride was reported as an inducer of rishitin by Tomiyama's group [46] and the nematocide, 1,2-dibromo-3-chloropropane, was so described by Komai and Sato [47]. Yet other organic and inorganic compounds have been described as inducers by Metlitskii and coworkers [43]. However, several of these compounds were amongst those examined as potential inducers by Kuć and colleagues [40], who reported that all the substances they tested were ineffective, except material of fungal origin. Similarly sodium fluoride, described earlier as a rishitin inducer [43], was reported recently [48] to stimulate only the production of solanidine (**1**). Yet another group

[49,50] found that the addition of chloramphenicol, which gave a positive response when tested by the Russian workers [43], led to rishitin formation in the interaction of potatoes with a virulent race of *P. infestans*, but that neither chloramphenicol nor the fungus would induce rishitin formation when tested on their own. Similar results were obtained with streptomycin. The contradictions between some of these results may reflect no more than differences between experimental techniques. The overall picture that emerges is that a variety of causes can lead to rishitin formation and that the potential to induce it, is neither a specific attribute of particular fungi nor of fungi in general. A general disturbance of plant metabolism might well suffice. Further evidence for this view can perhaps be seen in the induction of certain sesquiterpenes in tobacco by viral infection (see below), which precludes the intermediacy of metabolites exogenous to the plant other than the nucleic acid and protein components of the virus itself.

A related question is why rishitin, and its congeners, are not produced in some compatible interactions. Evidence has been presented [51] for the view that races of *P. infestans* possess the specific ability to suppress both the resistance response, that is the syndrome known as hypersensitivity, and the production of rishitin (**3**) and phytuberin (**5**) in certain potato cultivars, to which they are pathogenic by virtue of this property. Attractive as this hypothesis is, it fails to account for the formation of the compounds in one isolated example of a compatible interaction of *P. infestans* with potato shoots [40]. One explanation of this last observation would indicate that rishitin and phytuberin formation is not necessarily connected causally with either resistance or the hypersensitive reaction. According to arguments advanced by Király *et al.* [49], both the hypersensitive reaction of potatoes and rishitin production are indeed a consequence rather than a cause of resistance. However, these views have been disputed by other authors [52,53] and the matter is discussed more fully in the section on plant pathology.

Hydroxylubimin (**7**) production has been induced in potato tubers by a variety of fungi [36,38]. Only an incompatible race of *P. infestans* has so far been reported [35] as an inducer of rishitinol (**6**). Both anhydro-β-rotunol (**8**) [37] and solavetivone (**9**) are formed in potato tubers in response to inoculation with *P. infestans* races. The latter of the two compounds was also isolated after inoculation with bacteria, together with rishitin, phytuberin, and desacetylphytuberin [54] which had not been reported previously as naturally occurring.

Tomatoes (Lycopersicon esculentum)

These have not been investigated as thoroughly as potatoes but the situation appears to be very similar. Infection with various fungi leads to much increased levels of often unspecified phenols (e.g. [55]), chlorogenic and other cinnamic acid derivatives, together with scopolin and a number of flavonoids [56]. Certain cinnamic acid derivatives accumulate also after infection with a bacterium while others decrease [57]. The concentration of the amino-acid asparagine rises substantially after infection with fungi [58]. The antifungal steroid alkaloid tomatine (**10**), which was discussed during several decades as a possible defensive agent of those tomato tissues in which it occurs normally [59], recently has also been identified, tentatively, as an inhibitor which accumulates in tomato stems and roots after infection with fungi [60]. Tomatine has also been found to increase in resistant but not in susceptible varieties of *Lycopersicon pimpinellum* on bacterial infection [61].

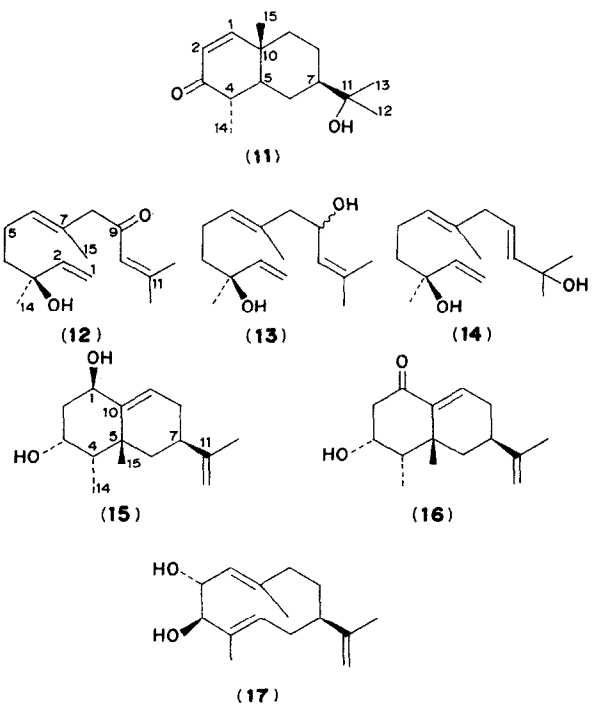
The only identified terpenoidal stress compound so far reported from tomatoes is rishitin (**3**). Its induction by fungi has been described by Japanese [62] and English [63] workers and it has also been isolated from tomato fruit inoculated with *Monilinia fructicola* in our laboratories (unpublished). The tomato extracts from which rishitin can be separated, are complex and one may anticipate that other sesquiterpenes will be isolated from them in due course.

Eggplant (Solanum melongena)

Hydroxytryptamine and indolepyruvic acid have been found in root-knot affected eggplants but not in healthy tissue [64]. No other characterized stress metabolites of eggplants appear to be discussed in the literature prior to the isolation [65] and chemical characterization [66] of lubimin (**4**) and the enone **11**, together with the acyclic sesquiterpenes **12**, **13**, and **14**. These compounds cannot be detected with certainty in cut but otherwise healthy fruit tissue but they are produced in substantial amounts after its inoculation with any one of the five fungal species that were tested [65]. Other means of inducing their formation have not yet been investigated. The separation of the compounds was accomplished by column and repeated TLC on silica.

Sweet peppers (Capsicum frutescens)

Various phenolic compounds accumulate in peppers after both fungal [67] and viral [68] infection. Phenols accumulate also as a consequence of chilling injury [69]. Carbohydrates and amino-acids have been found in much increased concentrations after inoculation of peppers with a bacterium [70].



A number of monoterpenes have been reported as normal metabolites of peppers [71] but only one terpene has as yet been described as a stress compound. This is the sesquiterpene capsidiol (**15**), which is formed after inoculation with many fungi [72,73] and at least one bacterium [74]. The induction of the compound by other means is still to be explored. Capsenone (**16**), which can be found in peppers infected with certain fungi, is formed from capsidiol (**15**) by fungal agency [73].

Capsidiol accounts for about 50% of the weight of the material which is extracted by ether from the aqueous diffusates from inoculated peppers [72] and also, as will be discussed below in greater detail, for most of the radioactivity which is incorporated into this material from acetate and mevalonate in feeding experiments [75]. The pepper diffusates are therefore much less complex than those obtained from any of the other Solanaceae, probably because no mechanical damage is done to the fruit tissue during the inoculation. This, of course, is a consequence of the morphology of the pepper fruit which renders them an almost ideal substrate for host-parasite studies [72]. Capsidiol can often be isolated by direct crystallization of the crude ether extracts of the diffusates. It is obtained in concentrations of up to 0.75 mM in diffusates [72] and of 0.35 mM in tissue [76].

Datura stramonium

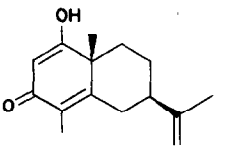
Inoculation of the opened and de-seeded fruit capsules of this plant, with *M. fructicola* or other fungi [77], leads to the production of appreciable amounts of lubimin (**4**), hydroxylubimin (**7**), and a compound which was identified as the germacrenediol **17** [78]. Very small amounts of capsidiol (**15**) are also formed.

Nicotiana *spp.*

Because it includes the species which are used for the manufacture of tobacco, the genus has received a great deal of phytochemical and plant pathological attention. The more recent literature reports on increased concentrations of coumarins and cinnamic acids and esters following treatment of *Nicotiana* spp. with fungi [79,80], bacteria [81], viruses [82,83], and UV light [84]. Flavonoids [82], ferulic acid amides [85], and pipecolic acid [24] have also been found in injured *Nicotiana* spp.

The genus has been a rich source of mono-, sesqui-, and di-terpenes, many of them isolated from cured *N. tabacum*. The compounds are not necessarily stress metabolites and many are probably artefacts of the curing and isolation processes. In general, they cannot be discussed here but papers by Roberts [86], by Demole and Berthet [87], and by the group led by Aasen and Enzell [88] may be consulted for leading references. However, one of these compounds, 1-keto-α-cyperone (**18**), must be singled out for mention. It was isolated in very small amount from burley tobacco [86]; there is no evidence that it is a stress metabolite but on account of its structure, it may be relevant to the biogenesis of several of those that are.

Quiesone was isolated in extremely small amount from tobacco infected with *Peronospora tabacina*. The compound, for which structure **19** was suggested on spectroscopic evidence [89], may be a degraded sesquiterpene or, perhaps more probably, a degraded carotene. It is an exceedingly active germination inhibitor of the fungus but it is not yet certain whether it is of fungal or plant origin. Some of the evidence favours the former possibility [89] but the close structural relationships to the ketone **20**, β-ionone **21**, and related compounds which are known tobacco metabolites [87], should not be overlooked. Quiesone is, of course, structurally close also to abscisic acid **22** which, in turn, is probably identical with a growth inhibitor which increases in tobacco following infection with a bacterium [90].



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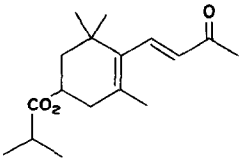

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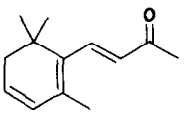

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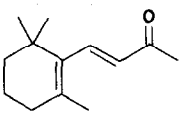

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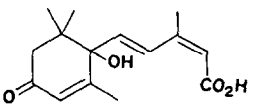

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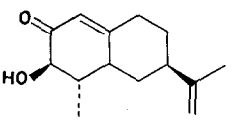


(23)

Two bicyclic sesquiterpenes are at present unambiguously established as stress metabolites of *Nicotiana* species. One of them is capsidiol (**15**) which was isolated recently from *N. tabacum* and *N. clevelandii* that had been infected with tobacco necrosis virus [91]. Its induction in tobacco by fungi is being investigated by Cruickshank [92]. The second compound, glutinosone (**23**), was isolated from leaves of *N. glutinosa* infected with tobacco mosaic virus [93]. Column and thin layer chromatography on silica was employed for its purification. Neither capsidiol nor glutinosone could be detected in healthy tissues of their progenitors.

PHYSICAL AND CHEMICAL PROPERTIES

This section gives an account of those physical and chemical properties which are relevant to the recognition and structure elucidation of the compounds under discussion. Proton and ^{13}C NMR spectroscopy, which has been of the utmost utility in the structure determination of almost all of the compounds, is treated separately in the next section. Thus far, mass spectroscopy has been of value in this series of compounds mainly in establishing molecular constitutions and in confirming the loss of certain functional groups; it has not yet been exploited in the structure elucidations of these compounds to any great extent and certainly merits more attention.

Physical constants for the compounds are given in Tables 1–3. In order to render the TLC and GLC data useful, those quoted were determined under standard conditions in our laboratories. References to other TLC and GLC systems can be found in the literature but these usually give data for only one or two compounds under a given set of conditions and may be correspondingly

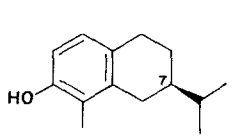
Table 3. Physical constants of sesquiterpenoids

Compound	mp°	$[\alpha]_D$	λ_{max} nm(log ϵ)	ν_{max} cm^{-1}	refs
3	65–7	−29(EtOH)	—	1640, 890 (liq)	[94]
4	oil	+ 27(EtOH) (+ 39(EtOH))	—	2740, 1715, 1640, 890 (CCl_4)	[66,98]
5	oil	?	—	1735, 1250, 1620, 690	[99]
				1728, 1260 (CCl_4)	[100]
6	128–9	+47($CHCl_3$)	263(2.45)	3070, 1600, 810, 804 (oil)	[102]
7	96–8	+55(EtOH)	293(1.48)	2745, 1725, 900 ($CHCl_3$)	[78]
8	44–44.5	+57(EtOH)	247(4.32)	1660, 1620, 1606, 892	[37]
9	oil	−119 (EtOH)	243(4.11)	1669, 1650, 1616, 893	[37]
11	65–8	~0	227(3.94)	1667, 920, 827 (liq)	[105]
		($[\alpha]_{365}$ −7.6(EtOH))			
12	oil	+24(CCl_4)	238(4.08)	3140, 1690, 1625, 923 (liq)	[105]
13	oil	~0	—	3130, 922 (liq)	[105]
14	oil	+14(CCl_4)	—	3120, 978, 926, 692 (liq)	[105]
15	152–3°	+21($CHCl_3$)	—	1651, 900, 720 (KBr)	[107]
	(also 167–9)				
16	oil	?	238(3.80)	1692, 1633, 896 ($CHCl_3$)	[107]
17	oil	103(EtOH)	—	1670, 1650, 890, 842 (liq)	[78]
18	76–76.5	−154($CHCl_3$)	259(4.23)	1670(w), 1600, 900, 870, 787	[86]
23	oil	?	237(4.19)	1685, 1630, 895 ($CHCl_3$)	[93]

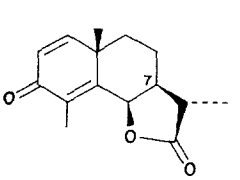
less useful than the data in Table 1 and 2 in screening extracts of unknown composition.

Rishitin (**3**) was characterized [94] as a vicinal diol which furnished diesters on acylation and a dialdehyde with periodic acid. On hydrogenation, it gave dihydro- and tetrahydrorishitin according to conditions. Selenium dehydrogenation of dihydrorishitin afforded a 60% yield of eudalene. Oxidation of dihydrorishitin with chromic acid furnished a phenol **24**, as one of several products, which was also obtainable from a santonin derivative by a series of straightforward reactions. This structural correlation of rishitin with santonin (**25**) also served to establish the absolute configuration at C-7. The relative configurations at C-2 to C-4 were indicated by a comparison of the appropriate ^{1}H-NMR spectra [94]. The absolute configuration of the diol system, and hence also of the C-4 methyl group, was established by two independent studies. One of these utilized the fact that both the hydroxyl groups are equatorial, as shown by the ^{1}H-NMR studies, and hence capable of forming a chelate complex in cuprammonium solution; the CD spectrum of the solution indicated a *k'* conformation [95] for the complex and hence, the absolute configuration shown in **3** for rishitin [96]. The same conclusion was reached in the other study (which enunciated the generally valid dibenzoate chirality rule [97]) in which the chiroptical properties of rishitin dibenzoate, as one of a series of compounds, were considered.

Lubimin (**4**) was characterized as a monohydric alcohol by the preparation of an acetate, and as an aldehyde by the preparation of a 2,4-dinitrophenylhydrazone [98]. By a sequence [36] of sodium borohydride reduction, hydrogenation, monobenzoylation, and oxidation, with all intermediates isolated and characterized, it furnished



(**24**)



(**25**)

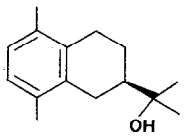
the 15-benzoyloxy-2-keto derivative, in which two methylene groups were shown to flank the keto group by deuteriation studies. A 15-monobrosylate of the dihydroglycol rearranged spontaneously to the 2→15 ether [36]. These facts led to the part structure

$$-CH_2CH(OH)-CH_2CH(CHO)-$$

but it may be noted that this and additional information was also and more easily accessible by the NMR studies which are discussed in the next section.

Phytuberin (**5**) contains a tertiary acetoxyl group which is resistant to hydrolysis but which was reduced by lithium aluminium hydride, providing desacetylphytuberin. Acid catalysed hydration of phytuberin afforded an epimeric pair of lactols which could be oxidized to a ketone. Hydrogenation gave dihydrophytuberin, mp 62° [99,100]. The last compound was suitable for X-ray analysis [101] which gave the correct structure **5**, replacing several earlier proposals. Nevertheless, it may be pointed out that the published NMR data [100], when considered in conjunction with the biogenetic proposals outlined in a subsequent section, would suffice to lead to the correct solution.

Rishitinol (**6**), a diol which resisted acetylation and oxidation with perbenzoic acid, was clearly a hydroxyoccidol on the basis of the spectral data [102]. The non-equivalence in the ^{1}H-NMR spectra of the methyls of the oxyisopropyl group was ascribed to steric hindrance to free rotation, and the additional hydroxyl group was thereby located most probably at either C-6 or C-8 but the non-equivalence of the methyl protons is inherent since these are diastereotopic groups. Several hydroxyoccidols were synthesized, and 8-*cis*-hydroxyoccidol proved to be the racemate of **6**. The synthesis, achieved by the hydroboration of 5,8-dimethyl-3-oxyisopropyl-1,2-dihydronaphthalene which was itself elaborated from ethyl-5,8-dimethyl-4-oxo-1,2,3,4-tetrahydro-2-naphthroate in three steps, also furnished 8-*trans*-hydroxy occidol; both epimers being available, the relative stereochemistries could be assigned without ambiguity from a comparison of the methine absorption region of the ^{1}H-NMR spectra. The absolute configuration was



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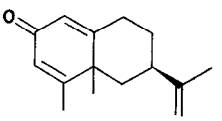

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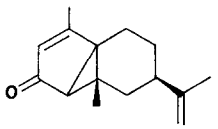

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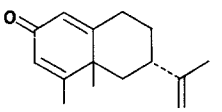

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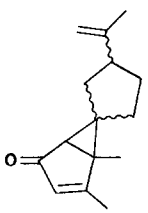

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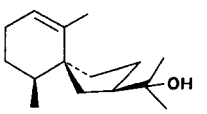

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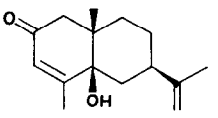


(32)

assigned by analogy with that of rishitin (**3**) and occidol (**26**).

The potato metabolite **8** was identical in all respects, including that of absolute stereochemistry [37], with anhydro-β-rotunol which had very shortly before been reported [103] as a rearrangement product formed from dehydronootkatone (**27**). That route involved photolysis of **27**, in dioxan, to the cyclopropylenone **28** which, on further photolysis in dilute acetic acid, gave **8**. Irradiation of **8** in the latter solvent regenerated **27** together with **29**, as an alternate rearrangement product. With dioxan as solvent, photolysis of **8** gave the four possible diastereomers represented by **30**.

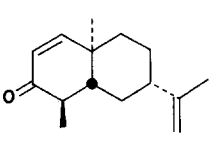
In an even earlier study [104], anhydro-β-rotunol had been obtained from hinesol (**31**) by oxidation to the dienone (with *t*-butylchromate and dichlorodicyanoquinone (DDQ) in separate steps) and dehydration; and also by a phosphorusoxychloride-mediated cationic rearrangement of β-rotunol (**32**). The structure and stereochemistry of **8** was established by its correlation with hinesol. These and also the other reactions leading to **8** are of considerable interest as possible analogues for its biosynthesis.

Solavetivone (**9**), characterisable in the form of a semicarbazone and the *syn*- and *anti*-2,4-dinitrophenylhydrazones, gave a fully saturated, bicyclic alcohol on catalytic reduction. Oxidation of the alcohol to the ketone followed by base-catalysed deuteriation established the absence of substitution α to the carbonyl. The complete structure and the absolute configuration at C-7 followed from the dehydrogenation of **9** to **8** with DDQ [37].

The chemical behaviour of the eggplant metabolite **11** has not been explored since its structure followed very readily [105] from spectroscopic data and biogenetic theory (see below). Its absolute configuration became apparent from a comparison of its ORD data with those for the synthetic enone **33**, whose relevant structural moiety is enantiomerically related to that of **11**.

The acyclic ketone **12**, identical with 9-oxonerolidol from camphortree leaf oil [106], gave the 9-hydroxynerolidol (**13**) on sodium borohydride reduction as a 1:1 mixture of the 9-epimers. When isolated from eggplant [105], the alcohol **13** was also a mixture of the epimers but with a ratio of about 4:1 (no assignments of the configuration at C-9 have been made). This indicates that **13** may be interconvertible, either in the plant or during isolation, with its allylic isomer **14** which was isolated from eggplants in the same experiments. Hydrogenation of **12** gave the expected saturated hydroxyketone, together with the saturated ketone formed by hydrogenolysis of the allylic hydroxyl group [106].

Capsidiol (**15**) readily furnished crystalline 11,12-dihydrocapsidiol, the 9,10-double bond resisting hydrogenation under the conditions used. Acetylation of **15** to an oily diacetate confirmed the presence of two secondary hydroxyls. Oxidation with chromic acid under mild conditions afforded capsenone (**16**) (which is more efficiently prepared by fungal oxidation [73]) and the non-conjugated ketone **34**. The latter underwent an extremely facile, base-catalysed elimination to capsidienone (**35**). Presumably because of severe steric hindrance, the 1-hydroxyl group in capsidiol did not undergo allylic oxidation with manganese dioxide; instead, a large excess of reagent furnished capsidienone in a slow reaction in which **34** was detected as an intermediate. Capsenone **16** has two α-protons exchangeable for deuterium under basic conditions. On treatment with very dilute mineral acid, it very readily gave isocapsidienone (**36**). The relevant features of all these products were easily recognizable by spectroscopic means and, together with full ^{1}H-NMR analyses, led to the proposal of the eremophilane-type structure **15** [107]. The ^{1}H-NMR data, and an assessment of the possible conformations suggested the stereochemistry as shown, with a *trans* relation between the methyls attached to C-4 and C-5. The number of naturally occurring eremophilanes now known is of the order, 100, and with the exception of **15**, all of these have the methyls in a *cis* relation. The uniqueness of the *trans* configuration in **15** prompted a rigorous verification, achieved by both a full X-ray



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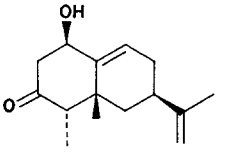

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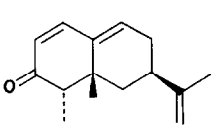

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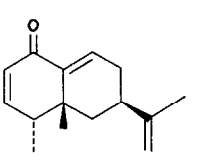

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analysis and a detailed study of the ^{13}C-NMR spectra [108], as outlined below.*

Glutinosone is an α-ketol which formed an acetate and which was reduced to the *vic*-diol by sodium borohydride. On treatment with hydrochloric in acetic acid, it furnished a phenol formulated as 7-hydroxy-3-isopropylidene-5-methyl-1,2,3,4-tetrahydronaphthalene. The structure **23** proposed for glutinosone [93] is the only formulation which is consistent with these and the spectral data and biogenetic considerations.

The structures proposed for hydroxylubimin (**7**) [36,78] and the germacrenediol **17** [78] until recently rested completely on spectral data and biogenetic considerations but they have now been confirmed by ^{13}C incorporation studies which are described in the next section.† It may be noted that hydroxylubimin (**7**) co-occurs with the 3-desoxy-compound **4** in two of the Solanaceae so far examined and similar Cotton effects imply the same absolute configuration at C-10 in the two compounds.

NMR SPECTROSCOPY

As already indicated, the structural elucidation of each member of this series has benefited from the information provided by high-resolution NMR spectroscopy. In all cases, specific fragments or partial structures were identified and, in some cases, the complete structure could be deduced from the NMR data. With the exception of the first few examples (**3**, **6**, and **18**) for which only ^{1}H spectra were recorded initially, ^{13}C spectra have been utilized to advantage. For example, the ^{13}C NMR data for lubimin showed unequivocally that the original structural proposal was untenable and led directly to the revised constitution **4**. The ^{13}C spectra of capsidiol (**15**) and some related model compounds led to the complete stereostructure of **15** which was confirmed by an X-ray examination. Also, ^{13}C spectra have been uniquely useful in biosynthetic studies of **15** and several other compounds.

In this section the specific features of ^{1}H and ^{13}C spectra which have been of greatest utility in these studies are discussed. All chemical shift data are given in ppm from internal tetramethylsilane (TMS) with coupling constants in Hz.

^{1}H Spectra

Most of the compounds contain an isopropenyl group which exhibits characteristic signals for the olefinic methyl (1.7–1.9 ppm) and the terminal olefinic protons (4.6–4.9 ppm). In many cases, the olefinic protons are nearly equivalent and the absorption is a broadened singlet, having a half-width of a few Hz and lacking distinctive fine structure. Spin decoupling can be used to reveal the weak coupling with the methyl protons by the sharpening of each band upon simultaneous irradiation of the other band. Additional methyl absorptions (0.9–1.6 ppm) are present in each case and their doublet or singlet nature readily distinguishes between those on tertiary or quaternary carbon, respectively, while methyl signals in the range of 1.9–2.3 ppm indicate attachment to sp^2 carbons. For the compounds having an oxyisopropyl group, the diastereotopic methyl protons are expected to be nonequivalent but the difference may be small and the signals unresolved, as in **11** for which a singlet at 1.23 ppm was found [105]. In contrast, these methyl protons appeared at 1.35 and 1.46 ppm for **6** [102].

The olefinic absorption for **8**, **9**, **11** and **16** in the range 5.7–6.8 ppm arose from the α,β-unsaturated carbonyl functions [37,105,108]. The mutually coupled ($J \sim 10$ Hz) doublets at 5.86 and 6.77 ppm for **11** showed that the γ-carbon was fully substituted, i.e. ⩾C–CH=CH–CO–. Similarly, the pair of doublets ($J \sim 3$ Hz) at 4.67 and 6.44 ppm in the spectrum of **5** [100] gave strong evidence for a dihydrofuran moiety fully-substituted at the 4-position. For **9**, the weak coupling of 1.2 Hz for the olefinic proton at 5.72 ppm, also apparent in the methyl signal at 1.89 ppm, showed that these protons were associated with the conjugated carbonyl function. The two-proton singlet at 6.9 ppm exhibited by **6** showed that the aryl ring was tetra-substituted in a symmetrical manner.

As an illustration of the utility of proton spectroscopy in these systems, the spectrum of **17** in the 3.5–5.5 ppm region is reproduced in Fig. 1a. The six patterns in this region arise from four olefinic and two carbinyl protons. The lowest field doublet, 4.97 ppm, $J \sim 10$ Hz, was shown to be coupled to the "triplet" at 4.35 ppm by spin-decoupling and can be assigned to an olefinic proton of a trisubstituted double bond. The two bands centred at 4.58 and 4.67 ppm arise from the olefinic protons of the isopropenyl group whose methyl signal appeared at 1.73 ppm. The coupling with the methyl protons is discernible in the 4.58 ppm pattern. The rather ill-defined multiplet centred at 4.83 ppm collapsed to a broadened singlet upon irradiation at 2.1 ppm (Fig. 1b) and, thus, was assigned to an olefinic proton coupled to a methylene group. As noted, the "triplet" at 4.35 ppm represents a single proton coupled to the olefinic proton at 4.97 ppm; the second coupling is to the doublet at 3.92 ppm, since simultaneous irradiation at 4.35 ppm collapsed the doublets at 3.92 and 4.97 ppm to singlets. With the assignment of the 3.92 and 4.35 ppm patterns to carbinyl protons, the presence of two hydroxyl protons having been established by deuterium exchange, it was possible to write

$$-CH_2-CH=C(Me)-CH(OH)-CH(OH)-CH=C(Me)-$$

as a partial structure. This fragment together with the isopropenyl group accounted for twelve of the fifteen carbon atoms in **17**.

The carbinyl absorptions in the spectra of this series have been very informative. As examples, the carbinyl protons in **4** and **7** gave rise to multiplets at 3.6 and 3.4 ppm, respectively, each with half-widths of >20 Hz clearly revealing an equatorial hydroxyl group since the carbinyl proton must be axial with two axial neighbors. The remaining carbinyl pattern for **7** at 2.98 ppm appeared as a doublet of doublets with $J \sim 9$ and ~ 10 Hz. Spin decoupling showed that one of these couplings was an axial-axial vicinal interaction with the carbinyl proton at 3.4 ppm while the second was that with the methine proton mutually coupled to the methyl

* In passing it may be noted that l-epicapsidiol, prepared for this study by borohydride reduction of **16** [108], has a melting point (151–2°) and specific rotation ($[\alpha]_D$ + 24($CHCl_3$)) very close to those of capsidiol; a reliable distinction between the two compounds is best achieved by reference to their NMR spectra.

† In the case of **7**, the structure and full relative stereochemistry have also been established by an X-ray analysis [119].

group at 1.06 ppm. This evidence not only revealed the presence of the fragment:

(*quat*)C–CH(Me)–CH(OH)–CH(OH)–CH_2–

but also established that the methyl and the two hydroxyl groups were each equatorial. In a similar manner, it was established that capsidiol (**15**) contained the sequence:

(*quat*)C–CH(Me)–CH(OH)–CH_2–CH(OH)–C=CH–CH_2–

as well as an angular methyl and an isopropenyl group; thirteen of the fifteen carbons were thereby characterized.

The presence of an aldehyde function in **4** and **7** was immediately apparent from the signals at 9.74 and 9.78 ppm, respectively and, since each was a doublet with $J \sim 3$ Hz, the aldehyde group was attached to a carbon bearing a single proton. Spin decoupling was used to locate this methine absorption at 2.26 and 2.33 ppm, respectively, for **4** and **7** from which it was apparent that, in both cases, the methine proton was coupled by 8–10 Hz to a second and, less strongly, to a third proton. The larger coupling indicated an axial orientation for the methine proton which means the aldehyde function is equatorial. The absorptions of the second and third protons were located by spin decoupling which showed that these were the methylene protons coupled to one of the carbinyl protons. Thus. the partial structure for 7 could be extended to

(*quat*)C–CH(Me)–CH(OH)–CH(OH)–CH_2CH(CHO)–C(*quat*),

in which all substituents are equatorial, and which, combined with the isopropenyl group, accounted for ten of the fifteen carbon atoms.

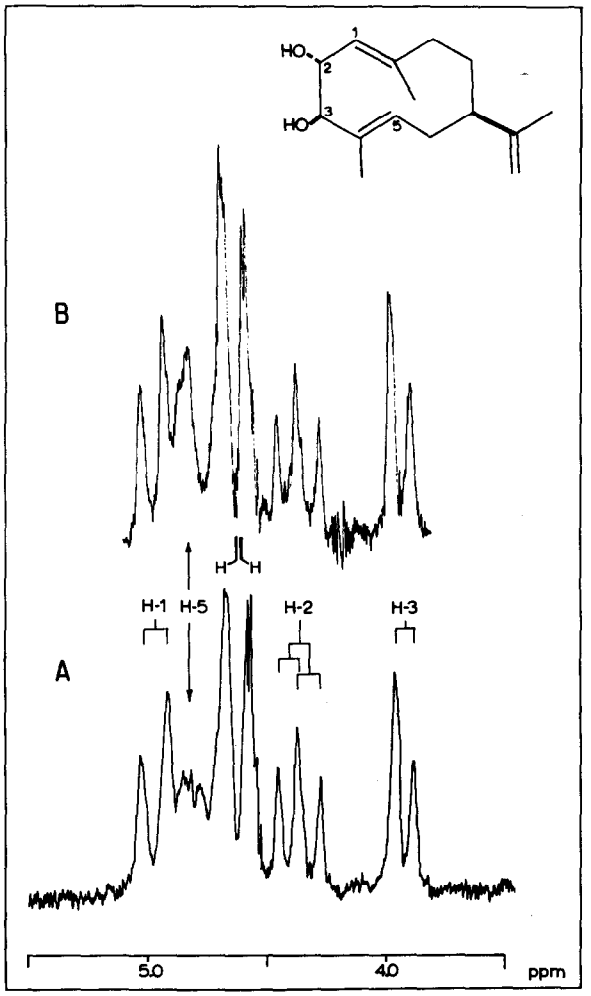


Fig. 1. Low field region (3.5–5.5 ppm) of the 100 MHz proton spectrum of 2,3-dihydroxygermacrene in $CDCl_3$.

In recent years, the use of lanthanide shift reagents to aid the analysis of proton spectra has become very common [109]. The increased chemical shift dispersion produced upon the addition of a shift reagent often is sufficient to permit identification of the absorptions of otherwise nearly equivalent nuclei and the technique has been helpful in the studies of some members of this series, e.g. **4**, **5** and **9**. Through the use of the europium shift reagent, Eu(fod$_3$-d_{27})(fod = 1,1,1,2,3,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione), the seven individual protons on the cyclohexane moiety of desacetylphytuberin were identified to establish the 1,3,3,4,4-pentasubstituted nature of this portion of the molecule [100]. In the absence of the shift reagent, these ring protons absorb in a single uninterpretable band near 1.6 ppm. The diastereotopic methyl groups of the oxyisopropyl group were also resolved with the shift reagent although these were equivalent in the normal spectrum. Clearly, shift reagents can provide valuable structural information.

^{13}C *Spectra*

Although the low natural abundance (1.1%) and smaller magnetic moment of ^{13}C relative to ^{1}H renders its detection more difficult by a factor of ~6000, the development of Fourier transform techniques [110] has made it possible to obtain ^{13}C spectra routinely and compendia of data for a wide variety of organic compounds are available [111]. Thus ^{13}C spectra can be employed readily for identification, structural elucidation and in many cases, stereochemical assignments [112]. Compared to that of protons, the ^{13}C chemical shift range is large, ~220 ppm for neutral organic species and, normally, spin couplings to protons are eliminated by broad-band irradiation of the ^{1}H resonances through noise decoupling. This decoupling operation increases the signal-to-noise ratio not only by collapse of multiplets to single lines but also through the nuclear Overhauser effect which can provide an enhancement of up to 3-fold [113]. In practice, ^{13}C spectra are readily recorded with 0.1–1 M solutions; hence, for sesquiterpenes, quantities in the range 20–50 mg are sufficient for structural purposes but one can work with smaller amounts. Sesquiterpenes generally give rise to spectra having separate lines for each carbon and the specific carbon types are readily identified by off-resonance decoupling [111]. Selective decoupling utilizes the knowledge of specific proton chemical shifts to identify individual carbon signals [111].

In all cases for this series, the ^{13}C spectrum immediately revealed carbonyl, olefinic (aromatic), carbinyl and alkyl carbons from the positions of the signals. Ketonic carbonyl carbons absorb near 200 ppm and are distinguishable from aldehydes by off-resonance decoupling although the relative intensity qualitatively provides the same distinction since aldehyde carbonyls benefit from

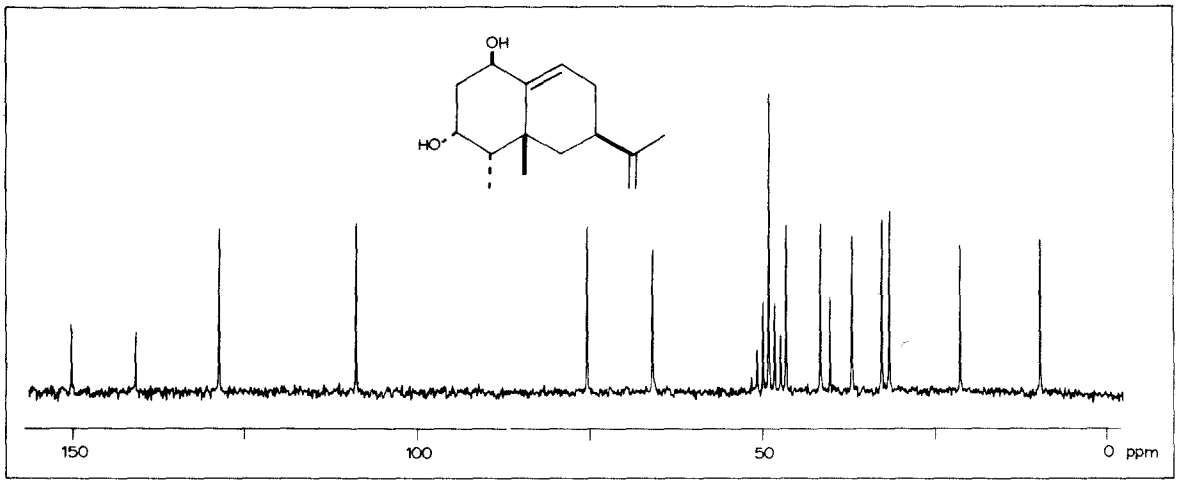


Fig. 2. 25.2 MHz spectrum of capsidiol (7 mg in 50 μl CD_3OD) with proton noise-decoupling.

the Overhauser effect of the directly bonded proton. Ester carbonyl carbons appear near 170 ppm. Olefinic and aromatic carbons absorb in the region, 100–150 ppm, with the specific positions depending on the polarity of the system. Signals in the range 60–80 ppm generally identify carbinyl carbons bonded to ethereal or hydroxylic oxygen, although methoxyl carbons appear near 50 ppm. Carbon-bonded sp^3 carbons absorb at highest field, in the range 0–50 ppm.

Several of these features are illustrated in Fig. 2, the ^{13}C spectrum of capsidiol (**15**) recorded for a CD_3OD solution (7 mg in 50 μl). The solvent signals appear as a septet in a 1:3:6:7:6:3:1 pattern because of ^{13}C-D spin coupling but solute signals coincide with the central peak and with the highest field component of the multiplet. Thus, fifteen signals arising from **15** are apparent. The four at lowest field are olefinic signals with the fully substituted pair evident from their much lower intensities. Similarly, one of the high field peaks (40.0 ppm) has a relatively low intensity, identifying it as a quaternary signal. The remaining absorptions have comparable relative intensities since the Overhauser enhancement is independent of the number of directly bonded protons. Offresonance decoupling showed that the 9.5, 21.1 and 32.4 ppm signals arise from methyl carbons and those at 41.4, 48.9, 65.8, 75.4 and 128.9 ppm from carbons with one directly bonded proton. Thus, the methylene carbons absorb at 31.4, 36.8, 46.4 and 109.0 ppm; the last of these is clearly the *exo*-methylene carbon of the isopropenyl group, while its partner is responsible for the signal at 150.3 ppm. Throughout this series of compounds, the three signals near 21, 109 and 150 ppm characterize the isopropenyl group. From the ^{13}C data, **15** was shown to have three methyl, three methylene, two –CHOH–, two methine and one quaternary carbon, a trisubstituted double bond and the isopropenyl group, accounting for the molecular formula $C_{15}H_{24}O_2$. From a detailed consideration of the methyl carbon shieldings, together with those of several model compounds it was possible to substantiate the earlier proposals [107] for the structure and stereochemistry of **15**. The conclusions reached from the ^{13}C NMR study were confirmed in every aspect by X-ray analysis [108].

The ^{13}C spectrum of lubimin (**4**) [66] contained a quaternary carbon signal at 46.9 ppm which immediately ruled out the original structural proposal for this molecule. In addition to the signals typical of the isopropenyl group (21.2, 108.8 and 147.3 ppm), the spectrum contained absorptions for an aldehyde (204.9 ppm), a –CH(OH)– (69.3 ppm), a secondary methyl (16.4 ppm) as well as five methylene (25.9, 32.6, 33.3, 40.3 and 41.8 ppm) and three methine carbons (41.8, 47.4 and 58.4 ppm). It may be noted that this spectrum contained two accidentally equivalent signals at 41.8 ppm, which were easily distinguished, however, by off-resonance decoupling. Upon combination of these data with the proton results and biogenetic considerations, the vetispirane skeleton fitted the observations for **4** admirably. A similar conclusion was drawn by others [36] from a shift reagent study of the proton spectrum. The corresponding spirocarbons in **7**–**9** were similarly identified directly by ^{13}C NMR [37,78]. It may be mentioned that the carbonyl signals at 186.3 and 198.4 ppm in the spectra of **8** and **9**, respectively, give clear indications of the cross-conjugated and conjugated nature of these two groupings, and this is supported by the olefinic signals near 165 ppm for the β-carbons. These could be placed in six-membered rings with some confidence since nonconjugated carbonyl carbons in six or larger membered rings and conjugated carbonyl carbons in five-membered rings tend to absorb below 210 ppm. Analogous deductions followed from the spectra of **11** and **16** with regard to the nature of the α,β-unsaturated carbonyl groupings [105,108].

While the methyl groups of the hydroxyisopropyl moiety in this series are diastereotopic, the methyl protons were generally unresolved although in principle the two methyl environments are non-equivalent. In the ^{13}C spectra, however, the two methyl carbon signals are resolved, as a result of the greater shift dispersion of ^{13}C nuclei, although the shielding differences are not large (<1 ppm). This illustrates how ^{13}C spectra may "amplify" a more subtle structural feature. In the examination of the acyclic sesquiterpenes **12**–**14** isolated from eggplant, it was found that the proton spectrum of 9-hydroxynerolidol (**13**) was not so clean as expected, presumably owing to the presence of another closely similar component, inseparable by TLC but suspected to be a

diastereomer. The ^{13}C spectrum contained fifteen major signals, as expected, but seven of these were accompanied by much smaller signals lying nearby. The spectrum of **13** obtained by sodium borohydride reduction of **12**, however, contained the same fifteen major signals found for natural **13** but the seven lines had components of equal intensity at the positions of the smaller peaks in the spectrum of natural **13**. This finding constituted good evidence that the sample of natural **13** contained approximately 20% of a diastereomer, presumably arising from the interconversion of **13**⇌**14**, either in the plant or during the isolation. The synthetic sample of **13**, obtained by reduction of **12** was expected to be essentially a 50:50 mixture of diastereomers as shown by the ^{13}C spectrum.

Since the first report in 1970 [114], the use of ^{13}C-enriched precursors and ^{13}C NMR detection of the labelled positions in the products obtained by biosynthetic incorporation has received considerable attention as a means to establish the origin and mode of formation of a variety of acetogenins, terpenes and other natural products; this approach has been reviewed recently [115]. While valuable information is provided through incorporation of singly-labelled precursors, there are added benefits derivable from working with doubly-labelled material which are appropriate for brief mention here. For compounds containing ^{13}C in natural abundance, the probability of two adjacent ^{13}C nuclei is very low ($\sim 10^{-4}$) and consequently the ^{13}C spectra are not complicated by ^{13}C–^{13}C couplings. If, however, a labelled compound contains two enriched centres directly bonded (or nonadjacent but spin-coupled) the ^{13}C–^{13}C coupling interactions will be evident. The absorption for the enriched carbons will consist of three lines, with a new doublet essentially centred on the original signal. The separation of this doublet, whose components are termed satellites, is the ^{13}C–^{13}C coupling constant. Thus, the observation of ^{13}C–^{13}C satellites in a product formed from a doubly-labelled precursor such as $^{13}Me^{13}COO^-Na^+$ is compelling evidence that at least part of the precursor has been incorporated as a unit. Absence of coupling with enrichment proves that bond cleavage has occurred while a change in coupling, such as ^{13}C–$^{13}C \rightarrow {}^{13}C$–C–$^{13}C$ or ^{13}C–C–C–^{13}C, may be indicative of a rearrangement. The relative intensities of the satellites provide a reliable quantitative measure of the level of enrichment [116].

In the genesis of sesquiterpenes, the parent skeleton of farnesyl pyrophosphate forms from three mevalonic acid units each of which arises from three acetate units. With double-labelled acetate as the source, each mevalonic acid unit gives an isopentenyl pyrophosphate moiety containing two intact ^{13}C–^{13}C units and one unique carbon (originally C-2 of mevalonic acid) which has lost its partner upon decarboxylation of mevalonic acid. A sesquiterpene derived therefrom will contain six intact C_2 units and three individually labelled centres *if* its formations does not involve a rearrangement. The ^{13}C spectrum of such a product will therefore exhibit three signals, enhanced due to the ^{13}C enrichment but without satellites, and the remaining twelve signals will be flanked by satellites. Since each pair of doublets for a given C_2 unit will exhibit the same ^{13}C–^{13}C coupling constant it is, in principle, possible to determine precisely which pairs of ^{13}C patterns arise from adjacent carbons. This is straightforward if the coupling constants differ for the various C–C bonds but may require homonuclear decoupling for cases having identical ^{13}C–^{13}C coupling constants [117]. In any event, the analysis of the ^{13}C spectrum in this manner constitutes an additional structure proof for the compound.

The biosynthesis of capsidiol (**15**) has been investigated using doubly-labelled acetate as the source for ^{13}C enrichment [118]. The ^{13}C spectrum of **15**–^{13}C contained five signals lacking satellites of appreciable intensity while the remaining ten signals were flanked by satellites approximately 25% as intense as the central peaks corresponding to the signals in the natural abundance spectrum. The absence of ^{13}C satellites for the angular methyl (C-15) and one of the fully-substituted olefinic carbons (C-10), in addition to the signals for C-3, -9 and -13, provided strong support for the migration of a methyl group from C-10 to C-5 during the formation of **15**. C-3, C-9 and C-13 are the centres arising from C-2 of mevalonic acid and consequently were not expected to exhibit ^{13}C satellites.

A biosynthetic investigation of the stress metabolites of *Datura stramonium* has recently been completed [119] also using doubly-labelled acetate as the precursor, with some $Me^{14}COO^-Na^+$ included to permit monitoring the isolation and purification stages by radioactivity measurements. The product contained enriched lubimin (**4**), hydroxylubimin (**7**) and 2,3-dihydroxygermacrene (**17**) as the major components. ^{13}C spectra of these after their isolation readily showed that each contained six intact ^{13}C–^{13}C units and three enriched carbons lacking ^{13}C neighbors from the same acetate units, i.e. three centres arising from C-2 mevalonic acid. While the detailed analysis of the biosynthetic routes to these materials is discussed in the following section, it may be noted that the ^{13}C spectra of the enriched metabolites confirmed the structural proposals for each conclusively. Alternative structures which had been rejected as biogenetically implausible earlier were now rigorously excluded by the bonding patterns of the labelled carbon atoms. In addition, the results allowed some unequivocal assignments for specific carbons which were not otherwise uniquely distinguished. For example, C-6 and C-8 in **17**–^{13}C were found to absorb at 34.4 and 34.2 ppm, respectively, as clearly shown by the ^{13}C–^{13}C coupling constants of 44 and 34 Hz, with C-5 and C-9, respectively. This finding uniquely distinguished C-1 (J_{CC} = 52 Hz with C-2) from C-5. In a similar manner, certain assignments for **4** and **7** were substantiated. It may be noted that these experiments were performed on a relatively small scale such that samples of ~10 mg, before final purification, were obtained. Nevertheless ^{13}C spectroscopy was gainfully employed for their examination.

The ^{13}C spectrum of the enriched sample of **7**, after careful purification, is shown in Fig. 3. Because of the relatively high level of enrichment (*ca* 7%), the patterns arising from most of the carbons exhibit satellites which are more intense than the parent signals. However, it is readily apparent that this is not the case for the signals at 21.2, 26.8 and 76.8 ppm, each of which has relatively weak satellites. Hence, these carbons were derived from C-2 of mevalonic acid and the others from intact acetate units. The carbon skeleton, therefore, was generated from a farnesol with the expected rearrangement. The three "unique" signals arise from C-13, C-9 and C-3, respectively. It may be noted that the satellites for the latter two have approximately twice the intensity of those for

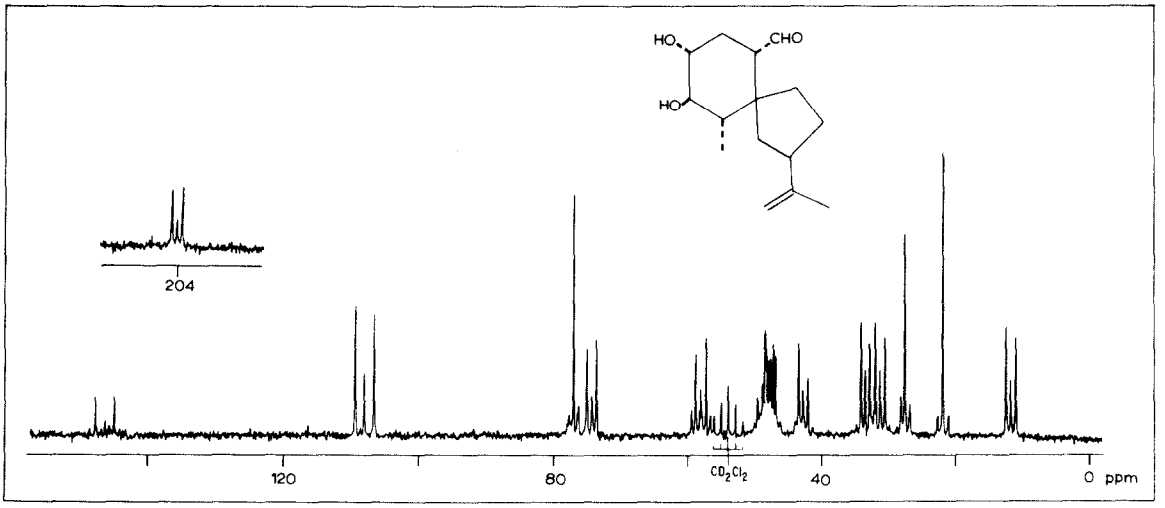


Fig. 3. 25.2 MHz proton noise decoupled spectrum of hydroxylubimin—$^{13}C_x$ (2.5 mg in 50 μl CD_2Cl_2); inset of the region near 204 ppm to show the carbonyl pattern.

C-13 because their probability of having a labelled neighbor is twice that of C-13. For a similar reason, the central peak in the pattern for C-11 (147 ppm) is barely discernible while that for C-12 (109 ppm) is clearly evident.

BIOGENETIC RELATIONSHIPS

The first compound of the series whose biosynthesis was studied was rishitin (**3**), in experiments in which ^{14}C-acetate and -mevalonate (MVA) were fed to potato tuber slices inoculated with *P. infestans* [120]. In both cases, radioactive rishitin was isolated in chromatographically homogeneous form. Labelling patterns and dilution values were not ascertained but the high degree of incorporation of MVA (8.5% of the racemate fed) leaves little doubt that it was incorporated directly. Labelling patterns were clearly revealed in more recent experiments in which acetate, doubly labelled with ^{13}C, was incorporated, also in a remarkably high degree, into capsidiol (**15**) from peppers [118], and into lubimin (**4**), hydroxylubimin (**7**), and 2,3-dihydroxygermacrene (**17**) from *D. stramonium* [119]. Details of these experiments are discussed in the preceding section. The results which were obtained accord with the currently accepted concepts of sesquiterpene biogenesis [121–123] in general. In particular, they are consistent with the cyclization of germacrene precursors to eudesmanes and the rearrangement of the latter, to capsidiol by migration of a methyl group from C-10 to C-5, and to lubimin and hydroxylubimin by migration of an electron pair from C-9–C-10 to C-9–C-5.

There can be no doubt that the other compounds in Table 3 are similarly sesquiterpenes (or norsesquiterpenes) which are linked by several common features. These may be summarized* as follows:

(a) The bicyclic sesquiterpenoidal stress compounds now known from the Solanaceae are eudesmanes or can be derived formally from eudesmanes by plausible rearrangements.

(b) Except in the case of rishitinol, these rearrangements have C-5 as a migration terminus.

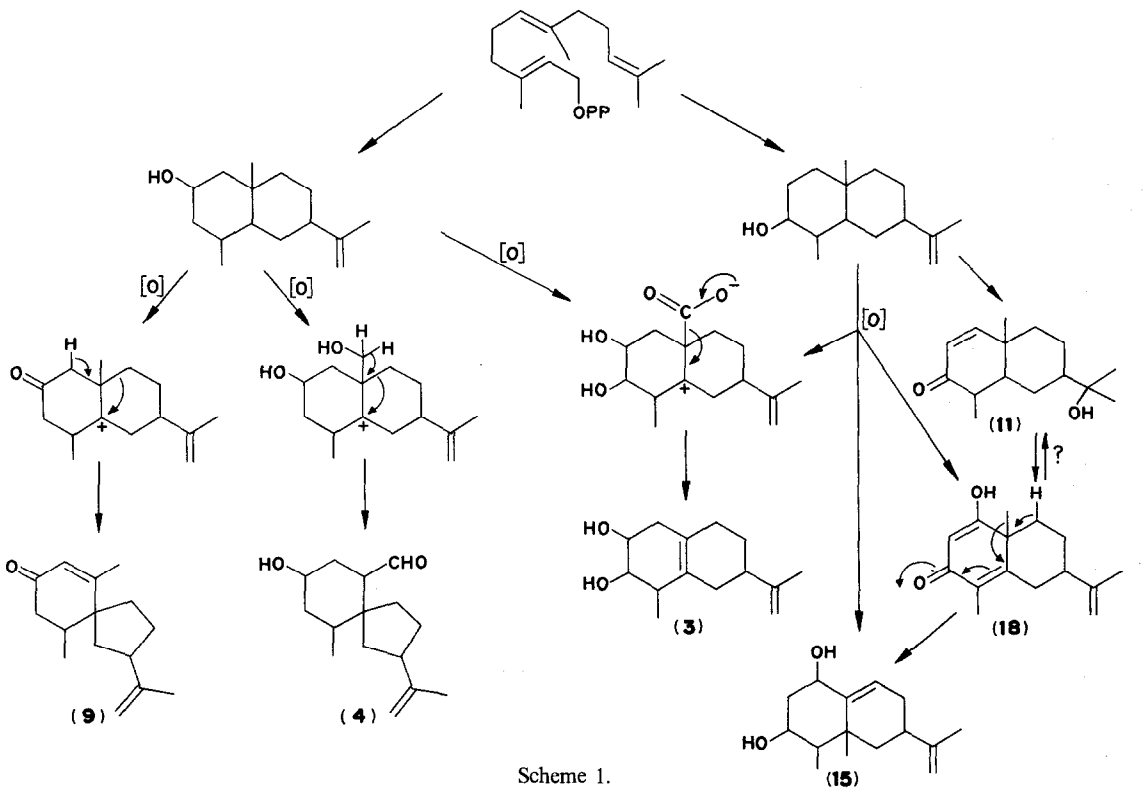
(c) Except for rishitinol, the bicyclic compounds carry oxygen on one or more of carbon atoms 1–4 but none on carbon atoms 6–9.

The first two generalizations imply that for the synthesis of these compounds, different Solanaceae utilize the same or closely related enzyme systems and precursors. As discussed by Geissman for the precursors of sesquiterpene lactones [124], the components of the biosynthetic apparatus will be at a comparatively advanced level of elaboration, thereby accounting for the production of family-characteristic metabolites. It may be expected that both known and new members of the series will be found in Solanaceae which have not yet been investigated. If used with the appropriate caution, the first two generalizations can be, and have been of value as guides in structural studies on newly isolated metabolites. However, the propositions do not imply that all of the bicyclic sesquiterpenoidal stress compounds which remain to be discovered in the Solanaceae will be eudesmanes or rearranged eudesmanes.

The third generalization is conceivably based on only coincidental data and therefore may be transitory in character. However, it is noteworthy that rishitinol (**6**), the odd member of the bicyclic series, is oxygenated at a carbon atom (C-8) which also is in an oxidized state in the acyclic sesquiterpenes **12** to **14** (oxygen or double bond at C-9, farnesane numbering). The oxidation patterns of the "typical" members of the bicyclic series on the one hand, and of rishitinol and the acyclics on the other, thus may have significance, in reflecting two different biosynthetic pathways.

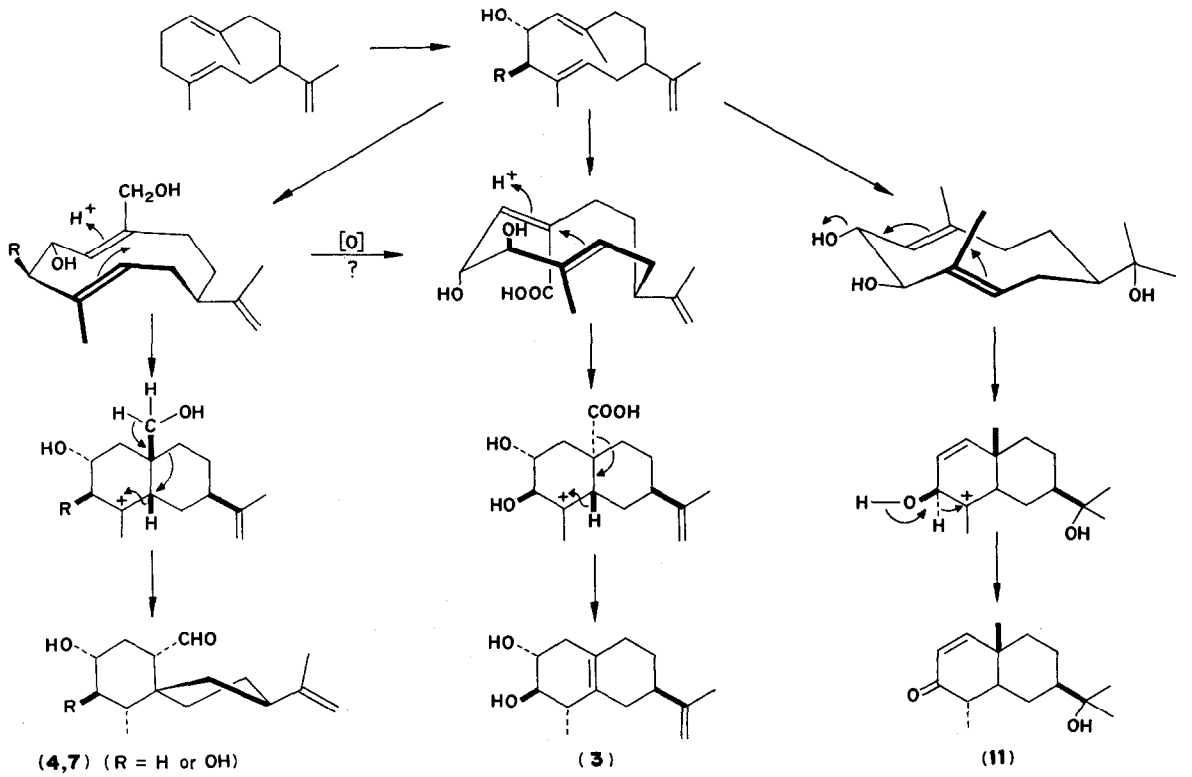
The details of the biosynthetic routes to the bicyclic compounds have not yet been studied experimentally but a number of different though essentially equivalent, speculative schemes can be considered and have at least illustrative value. The first to be proposed [66] was Scheme 1, which suggested links between five of the com-

* These concepts were presented previously at the *Symposium on Current Topics in Plant Pathology*, Budapest, June 24–27, 1975.

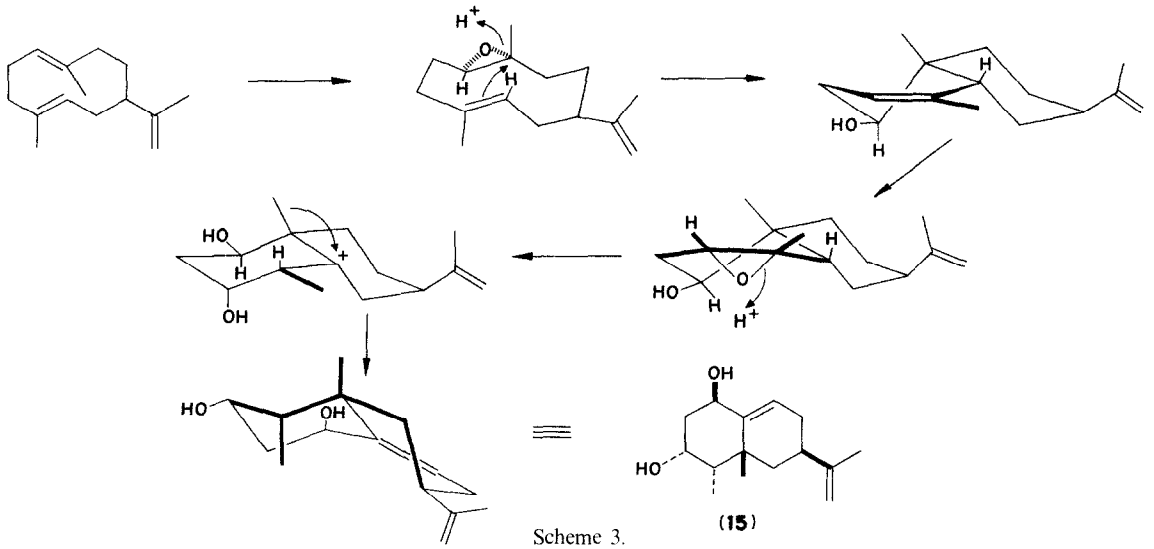


Scheme 1.

pounds. As indicated in the modified form given here, this scheme can also accommodate the recently discovered solavetivone (**9**) and, by an obvious extension, anhydro-β-rotunol (**8**). Subsequently also, hydroxylubimin (7) and the germacrenediol **17** were isolated from *Datura stramonium*, together with lubimin (**4**) and capsidiol (**15**). Molecular models of **17** are very flexible, indicating that this compound can adopt several conformations. When these are considered, it is seen that **17** could function as an almost direct precursor of **4** and **7**, as



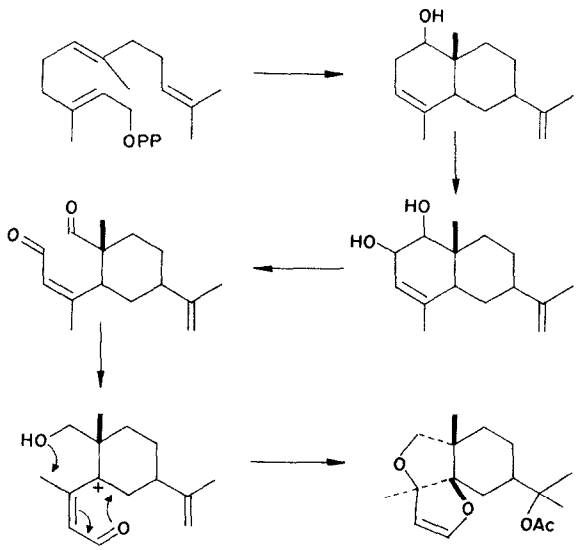
Scheme 2.



Scheme 3. (15)

well as of rishitin (**3**) and the eggplant enone **11**, as in Scheme 2. It will be remembered that **3**, **4**, and **7** co-occur in the potato, together with **8** and **9** which could be generated by very similar mechanisms. Rishitin (**3**), perhaps as an enzyme-bound intermediate, could give rise to glutinosone (**23**) by an obvious, brief sequence of oxidation at C-2 and double bond isomerization. Capsidiol (**15**) could be generated from enone **11**, formed as in Scheme 2, by further oxidation to 1-keto-α-cyperone (**18**) and subsequent reduction as in Scheme 1. An attractive feature of this possibility is the fact that both **15** and **18** have been isolated from tobacco, albeit under different conditions. However, an alternative and mechanistically perhaps more attractive pathway to capsidiol (**15**) is indicated in Scheme 3. Finally, Scheme 4 shows that a plausible biogenetic route to phytuberin (**5**) conforms to the pattern.

Two groups of workers have observed that the accumulation of steroid alkaloids derived from solanidine (**1**), which takes place in cut or otherwise wounded potato tuber tissue, is diminished when the cut tissue is inoculated with certain fungi or treated with products of their metabolism [125,126]. They found that rishitin (**3**) is produced under these conditions in amounts which are largest when the reduction of alkaloid accumulation is most marked [125]. It has been suggested [120,126] that the observations indicate a diversion of the steroidal alkaloid to sesquiterpene biosynthesis, with the branching point at farnesylpyrophosphate [126] or at some point beyond MVA [120]. However, one of the groups, in a more thorough study of the situation, reversed its opinion when finding that solanine accumulation, which significantly takes place over a much wider area of tissue than rishitin formation, may be a function of wound periderm formation. The biosyntheses of solanine and rishitin are thus regarded as under separate and independent control [127].



Scheme 4. (5)

PLANT PATHOLOGICAL SIGNIFICANCE

The question of the biological significance of many of the compounds discussed in this review is of importance to current research in plant disease resistance. Produced either *de novo* or in greatly enhanced amounts during abortive infections or other stresses which generally cause death of plant cells [34,91,93,128,133] they could be dismissed simply as the arbitrary products of cellular disorganization. However, the possibility that they play a role in the defence of plants against attack by disease-causing fungi forms the basis of the phytoalexin concept (lit. "plant warding off" substance) first formulated under that term by Müller and Börger [8] in relation to disease resistance in potatoes. The phenomenon was actually described much earlier from studies of orchid mycorrhizae [134–136] and it was from orchids that the first phytoalexin, orchinol, was isolated and characterized [137–139]. The term phytoalexin was originally limited to antifungal compounds but under certain conditions phytoalexins may also be antibacterial [140].

The wide range of factors that stimulate phytoalexin production suggests that the response is quite unspecific. Specificity exists, nevertheless, in the resistance of host cells to avirulent races of pathogens, which may differ

from virulent races in a single gene, and to nonpathogenic fungi. Such incompatible interactions are generally manifested in a hypersensitive response which involves the rapid death of the invaded cells and often the associated production of phytoalexins. Evidence has been obtained both from peppers [129–132] and potatoes [128] that phytoalexin production is a secondary event, following the hypersensitive response by several hours. It seems reasonable that the determination of specificity should be associated with the earlier hypersensitive stage, or some other as yet unrecognized primary response. Electron microscope studies of pepper cells indicate that a compatible (susceptible) response can be distinguished from a hypersensitive response 4 hr after inoculation with *P. capsici*, before the fungus has penetrated the cell wall and made contact with the host cytoplasm [129,141]. This suggests that specificity is determined by substances diffusing from the invading hyphae or by the interaction of hyphal and host cell walls. Arguments [49] that the hypersensitive response as well as phytoalexin production are merely the consequence of the release of toxins from fungal hyphae killed by the resistant host cell have been effectively answered by electron microscope studies. In peppers [129,130], potatoes [53], and also in lettuce [52], hypersensitive death of host cells clearly precedes death of the infection structures they contain. In both potatoes and peppers, however, ultrastructural changes reminiscent of those described [142] for oat root cells treated with the phytotoxin, victorin, were observed, and toxins released by the living fungus may well play a role in incompatible interactions. Hypotheses have been proposed in which polypeptides [143,144] and carbohydrates [145] are envisaged as elicitors of the resistant response in incompatible interactions in the Leguminosae. The difficulty with these views is that an essentially infinite number of incompatible combinations must be accounted for [146]. It would certainly be more credible if specificity were associated with the rare compatible interactions. The ability of virulent races of *P. infestans* to suppress the hypersensitive response and phytoalexin production in potatoes would then be accommodated more easily also [34,147].

To provide protection against invading fungi, phytoalexins must not only be fungitoxic but accumulate sufficiently rapidly to inhibitory concentrations. Relatively low activity in itself need not disqualify a compound if it is compensated for by high concentrations. In practice a complete evaluation is complicated by the technical difficulties of determining concentrations in the immediate vicinity of the host parasite interaction, by the rate of accumulation in relation to the progress of infection and by the fact that in most examples the production of several compounds must be considered.

Several of the compounds isolated from potatoes cannot be regarded at the present as phytoalexins as no evidence of fungitoxicity has been reported. Thus rishitinol (**6**), solavetivone (**9**), and anhydro-β-rotunol (**8**) have not been shown to be fungitoxic. Hydroxylubimin (**7**) was stated to be antifungal, without supportive evidence, by Katsui *et al* [36] but we found it to have relatively low activity against *P. infestans* and *M. fructicola* in spore germination assays [77]. Details of the fungitoxicity of glutinosone (**23**) from tobacco have not yet been published and several of the compounds from eggplant are of low activity against the test fungi used [65].

The simplest situation appears to prevail in peppers where the bulk of the antifungal activity accumulating in incompatible interactions resides in capsidiol (**15**). Tested against a range of pathogenic and nonpathogenic fungi, it was active between approximately 5×10^{-4} and 10^{-5} M (ED_{50} values *in vitro* tests), being more active than twenty structurally related compounds [148]. The concentrations that accumulate *in vivo* are very high when related to individual reacting cells in leaves [133]. In fruit, parallel capsidiol determinations and ultrastructural studies of incompatible interactions demonstrated that the rate and amount of capsidiol accumulation is more than sufficient to halt infection [129–132] provided *in vivo* toxicity is similar to that *in vitro*. The ability of some weak parasites to grow slowly in fruit tissue correlated with their ability either to tolerate high concentrations of the compound [149] or to carry out a measure of detoxification [73,150]. Evidence discussed in more detail elsewhere in this review clearly demonstrates that capsidiol is rapidly formed from acetate following infection and not by simple conversion of an accumulated non-active precursor [118].

In contrast to this, potatoes present a much more complex picture. The three compounds, rishitin (**3**), lubimin (**4**) and phytuberin (**5**) have received greatest attention but unfortunately no one investigation has considered all three at the same time, and hence it is impossible to assess the contribution they, and possibly the other compounds produced by potatoes, make to the overall fungitoxicity developing in an incompatible interaction. Phytuberin would appear to accumulate more slowly than rishitin [34], quite possibly too slowly to influence the progress of disease [151]. Detailed studies, by Tomiyama and his colleagues, of rishitin accumulation in potato tubers in response to compatible and incompatible races of *Phytophthora infestans* indicate that the concentration reaches a level almost sufficient to inhibit growth completely at the time when lesion development is about to cease [128,152,153]. The fungitoxicity of rishitin [154] is of the same order as that of capsidiol [148] and similarly is non-selective with respect to the pathogenicity of fungi or the virulence of races of *P. infestans*. Like capsidiol also, rishitin is synthesized *de novo* from acetate following infection [120]. It has been isolated from a number of different varieties of potato [33,34,62] and from various parts of the plant [40,128,155]. Rishitin is also produced following bacterial infection of tubers and may be sufficiently antibacterial to provide a measure of resistance [42,54,140].

The role of lubimin (**4**) has been studied much less extensively. It was first described by Ozeretskovskaya *et al.* [33] as occurring together with, and providing a similar level of fungitoxicity as, rishitin in tubers. *In vitro* tests of fungitoxicity indicate it to be rather less active than rishitin [65]. However, it was not found by Lyon *et al.* [54] in tubers inoculated with *Erwinia carotovora* which yielded rishitin and phytuberin, nor by Varns *et al.* [40] in interactions with fungi which yielded these two compounds. The possibility that differences in technique or other experimental factors may lead to the production of differing spectra of compounds is an interesting one, suggesting that similar variations may occur in nature. Evidence that different fungi may stimulate the production of different combinations was provided by studies of eggplant [65] and in unpublished experiments with potatoes and *Datura stramonium*. Lubimin, as discussed elsewhere, occurs in several Solanaceae and Met-

litskii *et al.* [155] provided evidence for its formation in potato leaves as well as tubers. Critical studies of the rate of accumulation of fungitoxic concentrations in relation to the progress of infection have not yet been reported.

Rishitin has been demonstrated in roots of both resistant and susceptible tomato varieties [156]. Again further work is required to establish a role for rishitin as a phytoalexin in tomato. Very probably the situation will be found to be similar to that in potato and it is of interest that several unidentified antifungal compounds are produced together with rishitin in plants inoculated with *Verticillium albo-atrum* [63,156].

Studies of phytoalexins in *Datura stramonium* [77], eggplant [65], and tobacco [91,93] have gone little beyond the stage of demonstrating the production of antifungal sesquiterpenes, as already described. In both eggplant and *D. stramonium*, the mixture of compounds produced contained lubimin as a major constituent. It was demonstrated that the diffusates became totally inhibitory to *Monilinia fructicola* and *P. infestans* within 48 hr but the contribution of each of the individual compounds and the rates of their production and inhibition of the infection process still have to be examined.

In summary then, there is good evidence that capsidiol and rishitin may function as phytoalexins in peppers and potatoes. Further detailed study is required to establish whether many of the other compounds produced postinfectionally serve in the same way, whether the production of an array of compounds is a purely arbitrary phenomenon or whether it is an indication of a series of definable sequences which benefit the host more than the production of larger quantities of a single fungitoxic compound.

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