predicted from Gödel's special solution of Einstein's equations, which admits of a general rotation of the universe as a whole. If this prediction happens to be correct, the rate of rotation can actually be evaluated by finding the shear coefficients of the anisotropy.

In recent years the optical identification of radio sources has been extremely fruitful. Now that thousands of accurate radio positions are becoming available through the work of the Australian radio astronomers with the 210-foot dish at Parkes, powerful optical telescopes are more than ever needed in the Southern Hemisphere for such identification and analysis.

Quasi-stellar objects in large numbers are awaiting identification and investigation with powerful optical equipment. The latest results indicate that the number of these objects, including both radio-emitting and radio-quiet types, may exceed 100,000 over the entire sky to an optical (blue) limit of magnitude 19.7.

The excellent observing conditions that prevail at the best locations in the Southern Hemisphere constitute yet another reason for erecting telescopes there—one that was not anticipated before the recent site-testing operations were undertaken. Extensive observatory-site surveys have been conducted by ESO in Africa, by AURA (following a beginning by the Yerkes Observatory) in South America, and by the Carnegie Institution in Australia and in Chile, in addition to the work in Australia, mentioned above, of the Mount Stromlo Observatory group and of the University of California. Site surveys have also been made in New Zealand. The consensus is that, while good conditions may be found in Africa and in Australia, truly excellent and probably unequaled sites are available in Chile, where long tests with recording photoelectric monitors have shown that the seeing is unsurpassed and that more than 65 percent of all nights are completely free of clouds for 6 hours or more. Such evidence strengthens very importantly the arguments for locating there the largest optical tools that astronomers can command. I believe that American astronomers are generally of the opinion that the Whitford report was too conservative. At least one 200-inch telescope should be built in Chile, and two major instruments there would not be excessive. Furthermore, the recommended design study for a telescope even larger than 200 inches should be implemented.

It is clear that observational astronomy is at a crucial stage, and that unparalleled opportunities are now at hand which, if grasped, will ensure for decades to come the future of this, the oldest of all the sciences.

References

1. I. S. Bowen, *Telescopes*, vol. 1 *of Stars and Stellar Systems*, G. P. Kuiper and B. M. Middlehurst, Eds. (Univ. of Chicago Press, Chicago, 1961), p. 1.
2. "Ground-Based Astronomy, a Ten-Year Program," *Nat. Acad. Sci.–Nat. Res. Council Pub. No. 1234* (Washington, D.C., 1964).
3. J. H. Oort, *ICSU (Intern. Council Sci. Unions) Rev. World Sci.* **3**, No. 1, 30 (1964).
4. L. H. Aller, "Why Go South?," *Astron. Soc. Pacific Leaflet, No. 406* (1963); S. C. B. Gascoigne, *Nature* **197**, 1240 (1963).

Leaf Epicuticular Waxes

The waxy outer surfaces of most plants display a wide diversity of fine structure and chemical constituents.

Geoffrey Eglinton and Richard J. Hamilton

In a plant, a surface which is exposed to the atmosphere usually has a layer which contains wax and is called the cuticle (*1*). Stems, fruits, petals, and leaves may all be covered with wax, though leaf waxes have received most attention. As Fig. 1 shows, the cuticle itself, which is a layer of cutin composed of cross-linked hydroxy fatty acids, is generally bounded by a layer of wax. Next, usually, comes a layer of pectin, and then the cellulose cell wall. Some workers believe that intercellular cytoplasmic bridges sometimes appear in the cutin, having extended through the pectinaceous layer. The amount of epicuticular wax varies greatly with species but can be sizable —for instance, it may be up to 4 percent of the green weight of the leaf and up to 15 percent of the dry weight. These figures might represent a wax layer of up to 50 micrograms per square centimeter of leaf surface.

The wax, when present, undoubtedly serves to preserve the water balance of the plant, and its other protective functions may include minimizing mechanical damage to leaf cells and inhibiting fungal and insect attack. Thus, it may be that the light-scattering character of the rough-textured wax layer and the light-absorbing powers of trace constituents, such as polyphenolics, in the cutin shield the plant from excessive ultraviolet radiation. Agricultural sprays must come in contact with the cuticle if they are to be effective, and the presence or absence of wax (as well as its composition and fine structure) seems to govern the wettability of leaves and the penetration of the spray chemicals.

It has been suggested that the physical arrangement, the morphology, of the epicuticular waxy covering is all-important, but the chemical constituents must also determine its role to a very considerable extent. Interest in epicuticular wax is by no means a new phenomenon. De Bary (*2*) established, as early as 1871, that the waxy covering of plants is made up of closely packed minute plates or rods which are clearly visible under the light microscope. Chibnall and his colleagues (*3*), in the period 1930 to 1950, examined the chemical composition of the waxes by means of fractional crystallizations, precision melting point determinations, and x-ray powder diagrams. Academic study of the waxes is now probing more deeply, with the help of newer techniques and analytical

Dr. Eglinton is senior lecturer in the chemistry department, University of Glasgow, Glasgow, Scotland; Dr. Hamilton is lecturer in the department of chemistry and biology, Regional College of Technology, Liverpool, England.

procedures. Similar studies of other protective coverings, such as coverings of pollen grains (*4*), mammalian skin [sheep (*5*) and man (*6*)], and arthropodal exoskeleton cuticle [for example, cockroach (*7*)], are being pursued and are bringing to light interesting similarities and contrasts. Man's use of plant waxes extends far back into prehistory; interesting recent examples include the clean-burning wax from the bayberry, *Myrica* spp., much used by the early American settlers (*8*), and the high-melting polishing wax of the carnauba palm, *Copernicia cerifera,* long commercially exploited in Brazil (*9*).

The cuticle with its wax is effective in minimizing the damage to the epidermal cells caused by mechanical abrasive action, such as that occasioned by leaf rubbing brought about by winds. Wax brushed off is often renewed, provided the leaf is still in an expanding stage of development.

Again, Barber (*10*) has shown that "the clines in glaucousness [of *Eucalyptus* species] are correlated with changes in frost activity, the more glaucous populations occurring in the more frosty localities."

The relationship between xerophytic character and quantity of wax has aroused some controversy in the literature. Schieferstein and Loomis (*11*) believe that there is no correlation between xeromorphic adaptation and the amount of surface wax, yet Hall and Jones (*12*) have shown that removal of the surface wax of Moroccan red clover (*Trifolium repens*) increases the rate of transpiration, especially when the stomata are closed—the cuticular phase. Daly (*13*) has shown, with populations of *Poa colensoi,* that those growing in regions of high precipitation are uniformly green while those growing in semiarid or summer-dry habitats are uniformly glaucous. He summarized his findings by concluding that there are high negative correlations between leaf-surface wax of *P. colensoi* and precipitation and that there is a slight positive correlation between leaf wax and mean temperature.

Pines also show a marked resistance to desiccation by drought, possibly due to inhibition of cuticular transpiration by the thick waxy covering over the cuticle of the needles. It had been suggested that pines could absorb water through the leaves, but it seemed unlikely that this could be absorption through the stomata. Leyton and Juniper (*14*) have shown by electron microscopy that the surface of the needles of the Scots pine, *Pinus sylvestris,* is covered by two morphologically different types of wax. Waxy projections cover the exposed surface, while the enclosed portion of the needle inside the sheath completely lacks these projections. It would seem that water can be absorbed through the surface inside the basal sheath, where the contact angle made by water droplets is very low.

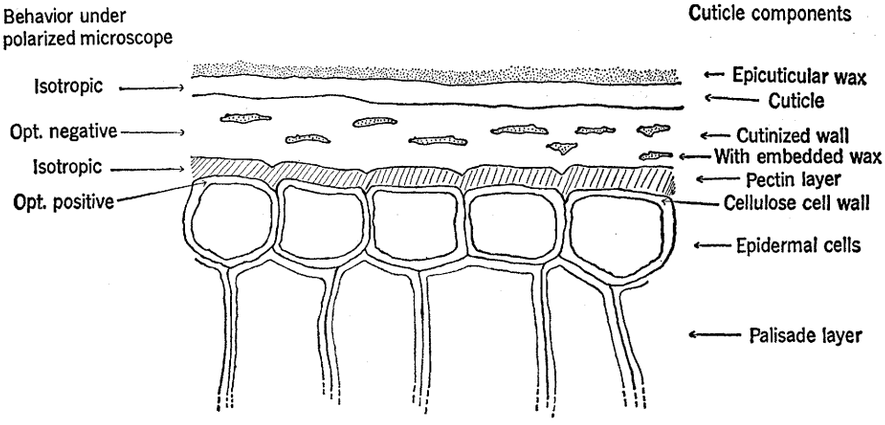


Fig. 1. Diagrammatic representation of the epicuticle of the plant, seen in cross-section. The lines dividing the layers above the epidermal cells indicate regions of major change in the construction of components rather than sharp boundaries. Individual plant species may depart greatly from this general arrangement. [B. E. Juniper, Botany Schools, Oxford University]

Attempts have been made to produce xeromorphy (the possession of characters associated with plants of dry habitats) by artifically increasing the wax on leaf surfaces. Cetyl alcohol, acting as a transpiration suppressor, can increase tolerance of drought, but it also reduces growth. It has been suggested (*15*) that small quantities of pure petroleum hydrocarbons have definite herbicidal properties.

Since the surface wax helps to control the water balance of a plant, it is obviously important in relation to the efficacy of agricultural sprays. The active ingredient of a systemic spray is effective only if it can penetrate to the inner portions of the plants. The ease with which a plant surface can deflect a water droplet is affected by the type and amount of wax; thus, measuring the contact angle has become a way of estimating the likely ease of deposition of sprayed chemicals. When a resistant plant is treated with trichloroacetate (which is applied to soil as a grass killer), wax formation is suppressed; peas and brassicas no longer deflect water droplets, and application of herbicides is much more effective. Juniper (*16*) has shown that trichloroacetate causes a reduction in the wax bloom on pea leaves (*Pisum sativum*).

The prevailing intensity of light also can affect the amount of wax produced by a plant. Juniper (*17*) has shown that peas grown in the dark have a leaf surface reminiscent of dicotyledons which do not normally exhibit projections of surface wax. This is in agreement with the finding of Dorschner and Buchholtz (*18*), who used artificial shade to show that a reduction in available sunlight influenced the morphological development of lucerne (*Medicago sativa*) growing among oats (*Avena sativa*) and increased the wetting capacity of applied chemical sprays.

The epicuticular wax may also confer, on the leaf, resistance to fungal, bacterial, and insect attack. It certainly assists in the capture of insects by the pitcher plant *Nepenthes.* Such features surely represent specific evolutionary adaptations to the processes involved in wax biogenesis. The behavior of aphids preparing to insert their stylets into a leaf surface is suggestive of some preference for a particular surface and a particular location on it. Both chemical and morphological aspects of the waxy layer may play some part in this choice; the aphid stylet is about 5 microns in diameter, as compared to a diameter of about 0.5 micron for a typical waxy projection.

Relatively little attention has been given as yet to the fate of the leaf waxes subsequent to the natural decay and interment of the plant debris. Waxes, presumably derived from the leaf cover, are, however, present in substantial amounts in most soils (*19*) and must contribute to soil fertility.

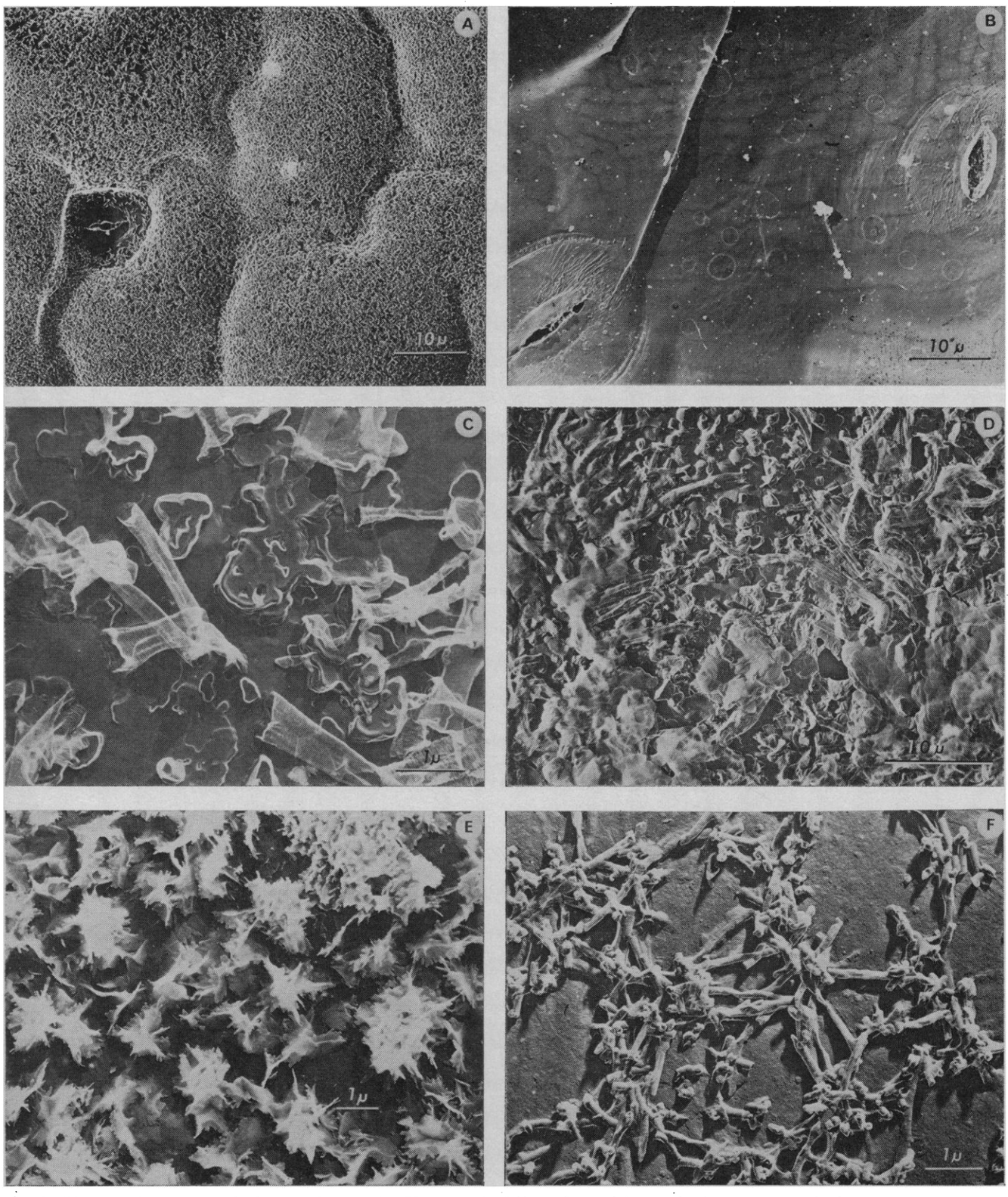


Fig. 2. Electronmicrographs of plant surfaces. (A) Upper (adaxial) surface of incompletely grown pea leaf, *Pisum sativum*, var. Alaska. The picture covers the area of cuticle above several epidermal cells whose boundaries correspond to the curved "valleys." The circular dark area is a partially open stoma; the absence of waxy excrescences on its surface is marked. The waxy platelets are approximately 600 millimicrons long. (B) Upper leaf surface of beet leaf, *Beta vulgaris*. Waxy excrescences are largely absent, but it is likely that the cuticle is covered with a thin, smooth layer of wax. The diagonal white line is a fold in the cuticle which occurred in the replication process. (C) Upper leaf surface of the cabbage, *Brassica oleracea*, var. January King. Note the tubelike growths emerging from the surface. (D) Upper surface of *Cotyledon orbicularis* (Crass). Again, tubelike growths of wax are visible. (E) Upper leaf surface of *Eucalyptus loeziana*. (F) Surface of the needle of the spruce, *Picea abies*. [B. E. Juniper, Botany Schools, Oxford University]

The long-chain constituents of leaf waxes are relatively stable compounds, but the soil microflora do possess the enzymes required to hydrolyze the cutin (*20*) and to metabolize the leaf waxes albeit rather slowly. Thus, long-chain methyl ketones have been found in soils (*21*), and several classes of organisms, including some *Pseudomonas* species, are known (*22*) to grow on media containing hydrocarbons as the sole source of carbon, producing octanoic acid from octane, for example (*23*). Their persistence is typified by the presence of some of the original leaf wax constituents in tobacco smoke (*24*) and in the excreta of cows fed on clover (*Medicago arabica*) (*25*) and of the caterpillar of the emperor gum moth (*Antherea eucalypti*) fed on *Eucalyptus perinìana* (*26*). Again, the waxes extracted from brown coal of Miocene age (about 25×10^6 years old) and from the Green River shale (Eocene, about 50×10^6 years old) contain large proportions of long-chain compounds presumably derived from the leaf waxes of the plant cover of those times (*27*).

Table 1. Long-chain constituents of leaf waxes. In the natural, unsaponified wax the alcohol and carboxylic acid functions are often present as esters. Olefinic and branched constituents have been omitted.

Dominant carbon number*	
Even	Odd†
$CH_3(CH_2)_nCO_2H$	$CH_3(CH_2)_nCH_3$
$CH_3(CH_2)_nOH$	$CH_3(CH_2)_nCHOH(CH_2)_mCH_3$
$CH_3(CH_2)_nCHO$	$CH_3(CH_2)_nCO(CH_2)_mCH_3$
$HO(CH_2)_nOH$	$CH_3(CH_2)_nCX(CH_2)_4CX(CH_2)_mCH_3$
$HO(CH_2)_nCO_2H$	$CH_3(CH_2)_nCOCH_2CO(CH_2)_mCH_3$
$HO_2C(CH_2)_nCO_2H$	

* The *n* and *m* are appropriately odd or even, the chain length being in the range C_{20} to C_{37}, generally being from C_{29} or C_{30}. † CX is $>C=O$ or $>CHOH$.

Fine Structure (Physical Appearance)

Even to the naked eye it is evident that plant leaf surfaces differ one from another; some are green, while others have a bloom or glaucousness. Barber (*10*) suggests that there are two forms of bloom in eucalypts; the first is bluey gray and stable to the touch (he calls this structural glaucousness), while the second consists of a thick white deposit of brilliant white wax, easly removed by rubbing.

De Bary's early light-microscope studies (*2*) led him to propose four distinctive types of waxy coating: (i) heaped wax layers; (ii) single granulated layers; (iii) rodlet layers, and (iv) membranaceous or crusty wax layers. Plants which he fitted into these groups were, for example, (group i) *Eucalyptus* and *Secale* species; (group ii) *Allium* and *Saccharum* species; (group iii) *Musa* and *Canna* species; (group iv) *Sempervivum* and *Euphorbia* species.

Kreger and Schamhart (*28*) have studied the waxy coatings by means of x-ray crystallography, which makes it possible to determine not only the angle the projections make to the leaf surface but also the structure of some major components and the orientation of their molecules within the projections.

It is with the advent of the electron microscope and the work of Loomis (*29*), Juniper and Bradley (*30*), and Dewey *et al.* (*31*) that the detailed fine structure of leaf surfaces has become accessible. Under the electron microscope the surface of a young pea plant, *Pisum sativum* (Fig. 2A), with its forest of waxy projections (which incidentally must occasion an "ultramicroclimate" at the cuticular surface) is in strong contrast to the lower surface of a beet leaf, *Beta vulgaris* (Fig. 2B), which has little or no apparent surface wax.

The replication technique commonly used for obtaining such electron micrographs of a leaf surface involves vacuum deposition of a carbon film and formation of a plastic coating to back this deposit, followed by the mechanical stripping of the hardened replica and final shadowing with gold or palladium (*32*). This technique involves the use of high vacuum, but Juniper has shown that the surface is not disrupted. Some people believe that the wax projections exude from pores 60 to 70 angstroms in diameter (*33*), but Juniper holds that there are no internal pressure gradients to account for such a phenomenon (*34*). Rather, he is of the opinion that the process involves "the independent migration of the most volatile constituents through the less volatile," and he cites the gray bloom found on shoe polish or beeswax left in a desiccating atmosphere.

The crystalline shapes formed are visualized as resulting partly from the distances that have to be traveled by the diffusing constituents and partly from the nature and relative amounts of the constituent compounds. That is, the wax reaches the surface dissolved in a "solvent" which has a relatively low boiling point and which slowly volatilizes when in contact with the air. A distant analogy can be drawn with the offensive fluid ejected by stink insects (Pentatomidae), where irritating quinones are dissolved in *n*-tridecane, $C_{13}H_{28}$ (*35*). An alternative mechanism, involving "a stable, liquid-crystalline state of wax in an aqueous solvent, which changes to a solid in contact with a seeding nucleus from the air," has been suggested by Edwards (*36*) to account for the supercooling of cornicle wax of aphids. Juniper points out that the spiral shape of the wax projections on cabbage leaf (Fig. 2C) may be caused by the exudation of wax into cylindrical form and the subsequent expansion, as the leaf expands, of the aperture through which the wax flows, a process which gives a cylinder wider at the bottom than at the top.

The great variety in the shape of these wax projections may be seen in Fig. 2, where six different genera—*Pisum, Beta, Brassica, Cotyledon, Eucalyptus,* and *Picea*—are represented. Though the shape and density of these waxy projections may be of some taxonomic value, it is possible for a plant to have more than one form of wax surface. For example, the pitcher plant *Nepenthes benecti* (*37*), which traps flies and other insects, has two different wax surfaces within the pitcher. The wax scales on one area (Fig. 3A) are easily detached and stick to the fly's footpad, thereby preventing the insect from getting a grip on other surfaces. (Isolated scales are shown in Fig. 3B.) For most plants the abaxial leaf surface (the surface away from stem) and the adaxial leaf surface appear different under the electron microscope. Baker *et al.* (*38*) have shown that more wax can be extracted from the lower (abaxial) surface of apple leaves. There is some evidence that the region

on the surface above an epidermal cell has a higher density of wax projections than the region over the boundaries between the cells (see Fig. 2A). This may arise simply from the fact that the diffusion pathways from the synthesizing cells may be easier below this region than along the anticlinal walls.

The plant has the ability to regenerate a wax layer which has been brushed off. Two of the electron micrographs of Fig. 3 (C and D) show that the half-grown wax has small projections as compared to the fully recovered leaf surface. That these projections represent the developing wax layer is easily shown, since the structures collapse when the temperature of the leaf is raised to the melting point of the wax; at least this is the case with Barber's nonstructural glaucous types (*10*).

One major role of the epicuticular waxes is that of preventing wetting of some leaves. The very fine projections on the leaf surface help to prevent water droplets from coming into contact with the cutin beneath. Since the cutin is composed of polymerized and interesterified hydroxy acids, "the cutin acids," it should be hydrophilic and should form hydrogen bonds with the water droplets, thus lowering the contact angle. One might reasonably expect a positive correlation between the melting point of a leaf wax and the highest leaf temperature commonly experienced by the species in question, for collapse of the fine structure would considerably reduce the contact angle.

Chemical Constitution

Early studies revealed that leaf and petal waxes were generally mixtures which were difficult to separate by the methods then available. The newer chromatographic and spectroscopic procedures have resulted in rapid advances. The surface waxes are now known to be complex mixtures (Table 1) of long-chain alkanes, alcohols, ketones, aldehydes, acetals, esters, and acids, but the picture is complicated by the positioning and number of functional groups, the degree of chain branching and unsaturation, and the increasing number of other known types of constituent. However, a few waxes have been examined in detail sufficient to reveal most of their chemi-

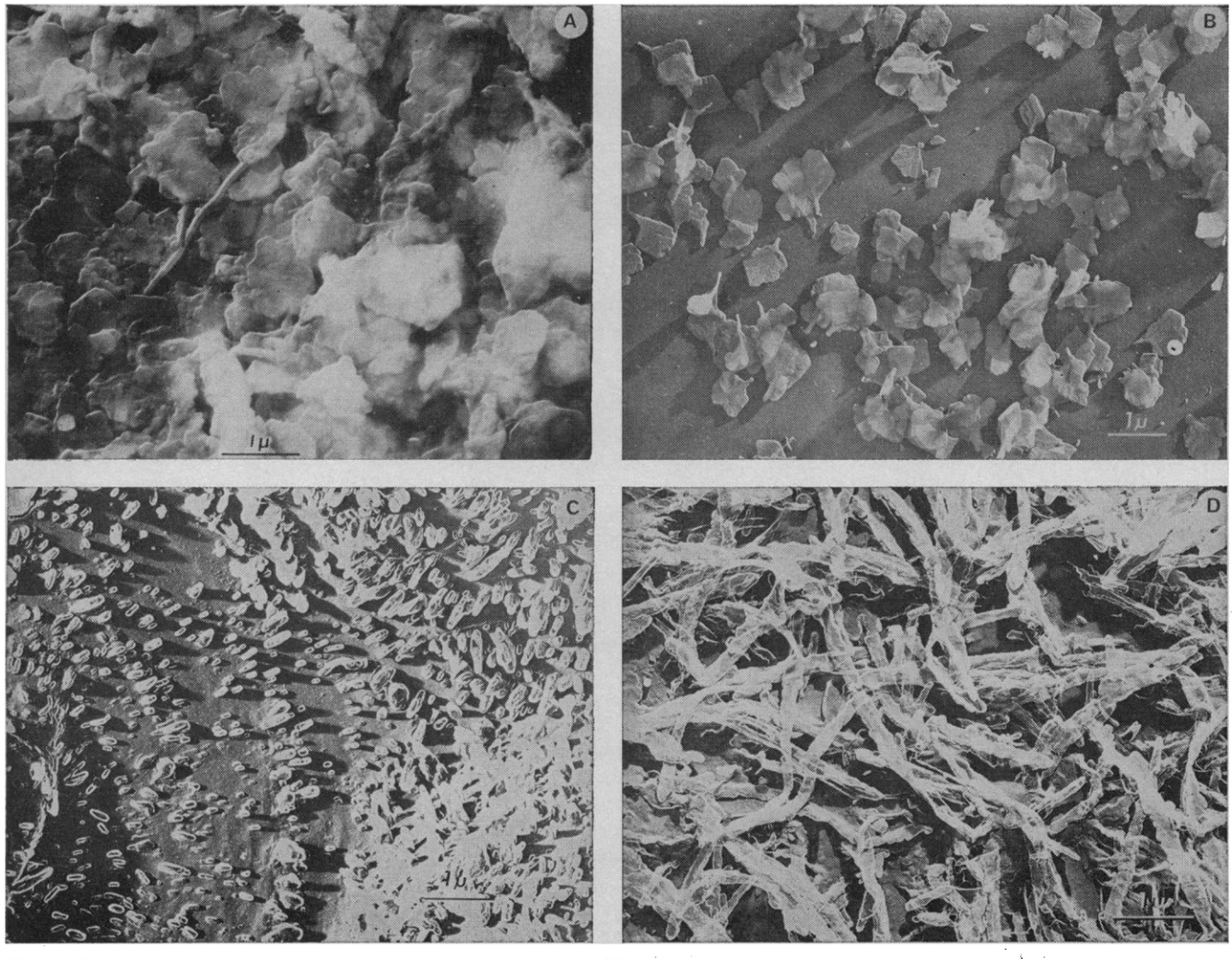


Fig. 3. Electronmicrographs of replicas of plant surfaces. (A) The undamaged, internal surface of the pitcher of the pitcher plant *Nepenthes benecti*. (B) Individual scales from the inner surface of the pitcher of the pitcher plant. Each scale is about 1200 millimicrons long. Many display angular corners characteristic of crystal growth. The narrow "stem" is suggestive of growth from a "pore" of about 100-millimicron diameter. These scales readily break off at the stem and stick to the feet of flies invading the trap of the pitcher plant. (C) Lower (abaxial) leaf surface of a pea plant, *Pisum sativum*, var. Alaska, 4 days after transfer from darkness to light. (D) The similar lower surface of a mature pea leaf. This older surface displays what appears to be a tangle of collapsed wax tubes. [B. E. Juniper, Botany Schools, Oxford University]

cal constituents; these waxes include those of the cabbage [*Brassica oleracea* (*39*)], the apple [*Pyrus malus* (*40*)], the carnauba palm [*Copernicia cerifera* (*41*)], sugar cane [*Saccharum officinarum* (*42*)], two *Eucalyptus* species (*26*), and the grape [*Vitis vinifera* (*43*)].

The compounds listed in Table 1 belong to a number of chemical classes, each of which is often present as a homologous series. The chain length of the homologs is usually from C_{21} to C_{37}, though some homologous series extend down to very low carbon numbers.

The *n*-alkanes, mono- and di-ketones, and secondary alcohols, though mixtures, commonly have the odd-carbon-numbered members predominating over the even-carbon-numbered members, while the carboxylic acids, the primary alcohols, the aldehydes, the α,ω-diols, the hydroxy acids, and the dicarboxylic acids have chiefly, though not exclusively, even-carbon-numbered chains. The acids and alcohols may be present either free or as esters—usually long-chain acid with long-chain alcohol, though some workers (*8*) report having found glycerides. The proportion of any one component in the wax differs for different plant species; for example, sugar-cane cuticle wax contains only a small amount of alkane (10 percent), whereas the leaf wax of *Cotyledon orbicularis* is almost entirely alkane.

The composition of these fascinating mixtures must certainly influence the fine structure of the deposits on the leaf surfaces (Figs. 2 and 3). There is even one report of a seleniferous leaf wax (*44*).

Less common long-chain constituents such as alkenes and branched-chain alkanes have been omitted from Table 1, as their odd-even predominance is still in doubt. The alkenes have been found in the waxes of rye pollen [*Secale cereale* (*4*)], sugar cane (*45*), and rose petals (*45*), where they constitute 60, 20, and 12 percent, respectively, of the total hydrocarbon content. In rye pollen the chain length varies from C_{23} to C_{33}, but the positions of the double bonds are not known. The olefines from sugar-cane wax include *cis*-Δ^{10}-alkenes—chain lengths, C_{15} to C_{33}—with C_{31} and C_{33} the major components; Δ^1-alkenes—chain lengths, C_{20} to C_{33}—with the odd-even ratio greater than unity; and some *trans*-Δ^2-alkenes. In rose-petal wax there are *cis*-Δ^3-alkenes in the chain-length range C_{17} to C_{33} and *trans*-alkenes in the range C_{19} to C_{33}. A report of several aromatic hydrocarbons in banana leaf, *Musa sapientum*, has been published recently (*46*).

The branched-chain hydrocarbons may occur less frequently, but there are a number of well-authenticated examples known. Thus, tobacco wax contains 0.4 percent (by weight) of hydrocarbons, of which 17 percent are isoalkanes (the methyl branch being on the penultimate carbon of the chain) and 19 percent are anteisoalkanes [the methyl branch being on the second-from-the-last carbon of the chain (*47*)]. The leaf wax of *Aeonium lindleyi* (Crass) contains isoalkanes (*48*). Hop oil has been examined by Sörm and his colleagues (*49*), who report monomethyl alkanes (either iso- or anteiso-) of chain length C_{13} to C_{33} and a further series of dimethyl alkanes (or, possibly, monomethyl alkanes with the branch near the middle of the chain). The same authors report branched-chain alkanes in the waxes from sugar cane and from rose petals. Branched-chain acids are also suspected in plant waxes and may be related biosynthetically to the branched alkanes. Kaneda (*50*) has just shown that branched-chain hydrocarbons of the tobacco plant become radioactively labeled when the plant is fed radioactive short-chain (*n*-C_4 to *n*-C_6) fatty

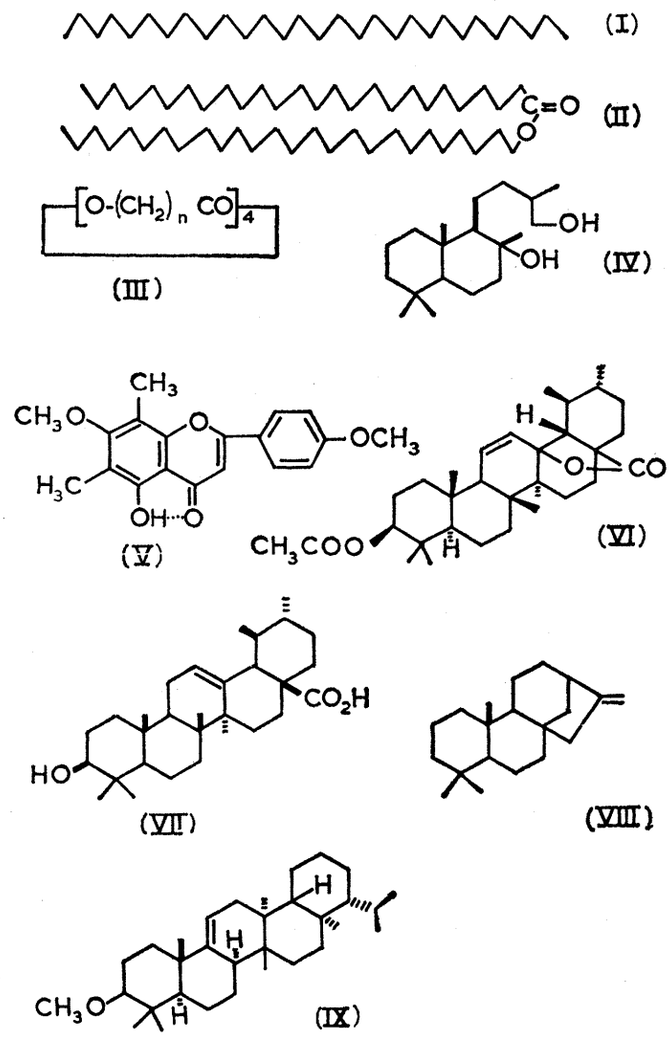


Fig. 4. Some constituents of plant waxes: (I) *n*-nonacosane, $C_{29}H_{60}$, from *Brassica oleracea* (*39*); (II) *n*-hexacosanyl hexacosanoate $C_{52}H_{104}O_2$, from *Leptospermum scoparium* (*86*); (III) a series of estolides from *Picea pungens* (*55*); (IV) labdane-8α,15-diol from *Aeonium lindleyi* (*59*); (V) eucalyptin from *Eucalyptus globulus* (*56*); (VI) 11,12-dehydro-ursolic lactone acetate from *E. urnigera* (*61*); (VII) ursolic acid from *Pyrus malus* (*57*); (VIII) phyllocladene from *Podocarpus nivalis* (*58*); (IX) β-amyrin methyl ether from *Cortaderia toe toe* (*60*). (Most reports describing "plant waxes" relate to extracts from whole plants or leaves—"epicuticular waxes," specifically, are not often distinguished.)

acids. These results, he declares, suggest that a condensation mechanism is operating.

Incidentally, a very-long-chain alkane, n-$C_{62}H_{126}$, has been reported in the grass *Leptochloa digitata* (*51*). Since most standard analytical procedures would miss double-chain-length compounds, it is possible that they are more widespread than is believed at present.

The likelihood that other types of long-chain constituent are yet to be found in plant waxes is indicated by the relatively recent discovery of the long-chain aldehydes (*52*), the β-diketones (*53*) and hydroxy β-diketones (*54*), and the cyclic estolides (Fig. 4, structure III) (*55*). Other classes of constituent include phenols and other aromatic compounds such as the flavone eucalyptin (structure V), present in *Eucalyptus globulus* (56). Terpenoid compounds are sometimes

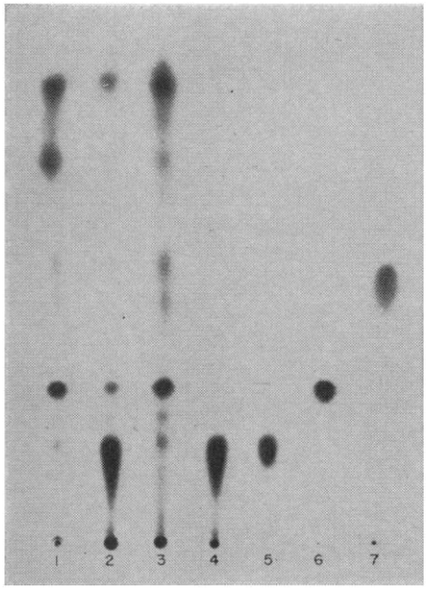


Fig. 5. Thin-layer chromatogram of wax from grapes, on silica gel activated at 90°C for 30 minutes. Solvent: light petroleum, ether, and acetic acid (70:30:1.5). Spray reagents: 5 percent $K_2Cr_2O_7$ in 40 percent H_2SO_4. Left to right: (1) 100 micrograms of wax from fresh sultana grapes, extracted with light petroleum vapor; (2) 100 micrograms of whole wax from dried sultana grapes, extracted with chloroform; (3) 100 micrograms of the light-petroleum-soluble part of wax from dried sultanas ("soft wax"); (4) 50 micrograms of the light-petroleum-insoluble part of wax from dried sultanas ("hard wax"); (5) 20 micrograms of oleanolic acid; (6) 20 micrograms of a mixture of the n-alcohols C_{24} and C_{28}; (7) 40 micrograms of docosanoic acid. [From Radler and Horn (*43*), reprinted from the *Australian Journal of Chemistry*, with permission]

major components of waxes—for example, ursolic acid (structure VII) in the fruit and leaf waxes of apple (*57*). Mazliak (*40*) has shown that microscopic crystals of ursolic acid are embedded in apple cuticle. Diterpene hydrocarbons, such as phyllocladene (structure VIII), have been found in *Podocarpus nivalis* (*58*), and a diterpene diol, lindleyol (structure IV), has been found in *Aeonium lindleyi* (*59*). Triterpenoid methyl ethers such as β-amyrin methyl ether (structure IX) have been isolated from the New Zealand grass *Cortaderia toe toe* (*60*), and 11,12-dehydro-ursolic lactone acetate (structure VI), from *Eucalyptus urnigera* (*61*). Some of the constituents of cutin are given in Table 2.

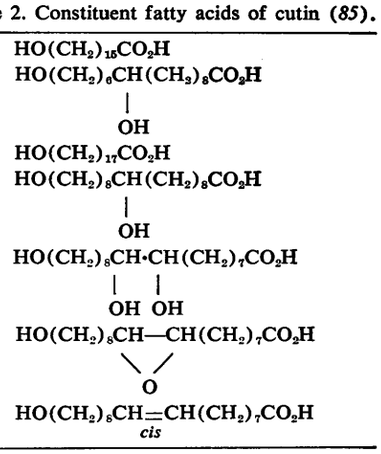
The epicuticular leaf wax can be obtained in several ways. Roberts *et al.* (*62*) favor three dippings of the leaf in fresh solvent. Purdy and Truter (*39*) went on to demonstrate that, with ether, there was no component in the sixth extract which was not present in the first. They also showed that, of the material which could be extracted in 12 dippings, 80 percent was extracted in the first three, but it seems inevitable that some extraction of cuticular (embedded) and cytoplasmic waxes must occur.

Spectroscopic examination provides some information about the lipid extract. Thus, conjugated unsaturation, such as is found in the enolic forms of β-diketones or flavonoid-type compounds, might be noted from the ultraviolet spectrum. The infrared spectrum might indicate hydroxy compounds, ketones, ethers, and lactones, as well as the more common esters and hydrocarbons. Thin-layer chromatography of the total lipid can demonstrate the complexity of the waxy mixture; for example, in Fig. 5 the presence of a large quantity of oleanolic acid is evident.

The total extracted wax was formerly fractionated into a "hard" and a "soft" wax by precipitation of the hard wax out of a light petroleum solution through the addition of acetone. Even partitioning of the wax between light petrol and methanol, though it leads to enrichment of the fractions, does not produce pure components (*63*).

Chromatography (thin-layer or column) would seem to be the most promising separation technique. Hydrocarbons, esters, alcohols, and free fatty acids can all be obtained by alumina or silicic acid chromatography, but aldehydes are destroyed by alumina and must be chromatographed on silica gel (*43*, *63a*). Infrared spectroscopy can be used to make sure that there is no carry-over of one class of component into another. Thus, in Fig. 6, the infrared spectrum shows that the hydrocarbon fraction is contaminated with ethers; these were subsequently shown to be triterpenoid in nature (*60*, *64*).

Table 2. Constituent fatty acids of cutin (*85*).



The hydrocarbon fraction is the easiest to isolate from the total lipid, being eluted in hexane from an alumina or silicic acid column. Chromatography of the hydrocarbon fraction on silica gel impregnated with silver nitrate will separate alkenes from alkanes, since the former are selectively held to the silver ions by π bonding. Alternatively, the alkenes can be converted to alkanes by hydrogenation; addition of bromine is also an effective means of differentiation. The branched and cyclic hydrocarbons can be separated from the straight-chain compounds by clathration of the latter either with a Linde sieve of 5-angstrom mesh or with urea. The alkane fractions can then be further separated into homologous series by gas-liquid chromatography, either isothermally or by temperature-programmed operation (see Fig. 7). The large molecular weights of the alkanes necessitate high temperatures for gas-liquid chromatography analysis; this requirement limits the choice of stationary phases to rather stable ones, such as silicone gum, seven-ring polyphenyl ether, Apiezon L vacuum grease, and a silicone nitrile polymer phase, all at low (1 to 5 percent) loadings. The high resolution afforded by long capillary columns is desirable.

Further structural evidence can be obtained from the breakdown pattern

of the mass spectrum. As shown in Fig. 8 (top left), the n-C_{32} alkane shows the parent peak, M, = 450 mass-to-charge units (m/e), and a series of peaks of increasing intensity identifiable as M-C_2H_5; M-C_3H_7; M-C_4H_9; and so on. The isoalkane (iso-C_{33}) exhibits a parent peak at M = 464 m/e in the mass spectrum, but there is also a definite peak at M-CH_3 = 449 m/e. However, the peak at M-C_2H_5 is fairly small and that at M-C_3H_7 (421 m/e units) is large because of the ease of cleavage at the bond between the carbon containing the branch and the remainder of the straight chain. It can be clearly seen that the iso-C_{33} hydrocarbon isolated from tobacco wax (Fig. 8, bottom right) shows a larger peak at M-C_2H_5 than does the synthetic iso-C_{33} (Fig. 8, top right); this can be explained by the presence of anteiso-C_{33} alkane (about 5 percent) as impurity. The combined gas-chromatographic and mass-spectrometric units now commercially available should speed up analyses of complex mixtures of this type. Nuclear-magnetic-resonance spectra are of less value in the study of these long-chain compounds than they are in some other fields, since there are so many similar protons. However, they can be useful in determining the presence of impurities.

The carboxylic acid fraction can be analyzed by gas-liquid chromatography and mass spectrometry, either as the corresponding methyl esters or after reduction to the saturated hydrocarbons

$$RCOOH \xrightarrow{LiAlH_4} RCH_2OH \longrightarrow RCH_2X \xrightarrow{LiAlH_4} RCH_3$$

(X is bromine, iodine, or p-toluenesulfonyloxy.)

This latter route, which has been well studied by the Commonwealth Scientific and Industrial Research Organization group in Australia (*5*), has the added advantage that the resulting mixture of alkanes can be cleanly separated into straight- and branched-chain alkanes through use of the Linde sieve. The alcohol fraction can be analyzed by gas-liquid chromatography, either as the free alcohols or as their acetates, but it seems likely that the best derivatives will prove to be the trimethyl-silyl ethers, $ROSi(CH_3)_3$, since they are easily chromatographed and display characteristic mass-spectrometric fragmentation patterns (*65*).

The complexity of most plant waxes has discouraged attempts at complete analyses of the constituents of individual waxes. However, such studies are important, for the relative proportions of the different homologs in the various classes of compound must reflect the underlying biochemistry. Purdy and Truter (*39*) have examined the leaf wax of *Brassica oleracea* and report that several compounds with 29 carbon atoms in the chain are present and together make up 60 percent of the wax. These compounds range from nonacosane to the oxygenated derivatives, $CH_3(CH_2)_8CX(CH_2)_4CY(CH_2)_{13}CH_3$, where CX or CY, or both, can be CH_2, CHOH, or C=O, though neither diols nor diketones were present. They also found unusual polymodal-carbon-number patterns for the acids and alcohols.

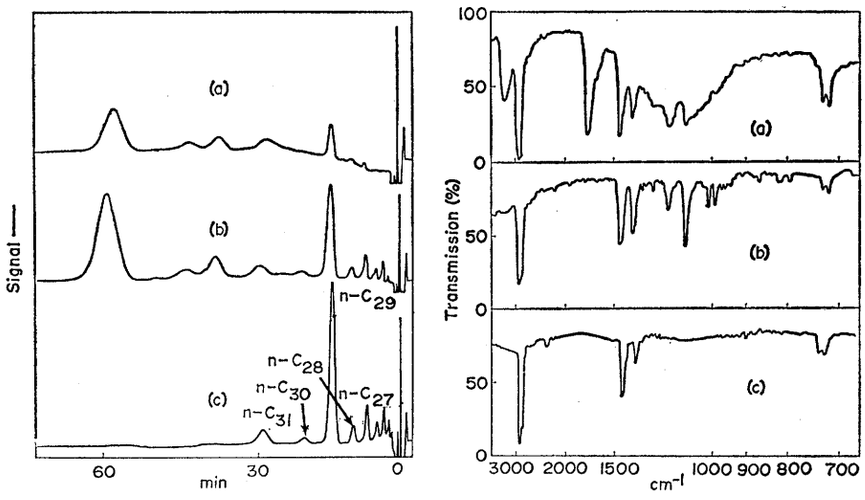


Fig. 6. Isolation of the hydrocarbon fraction from the plant wax of *Cortaderia toe toe* Zotov. (a) The crude plant wax as extracted from the dried plant. (b) The wax remaining after successive treatments with alcoholic sodium hydroxide and 2,4-dinitrophenylhydrazine reagent, followed by chromatography over alumina. (c) The wax remaining after treatment of fraction (b) with hot concentrated sulfuric acid. (Left) Gas-liquid chromatograms. Load, approximately 5 micrograms of solid wax; column (130 centimeters × 0.4 centimeter), 0.5 percent Apiezon L on Embacel, 80–100 mesh at 225°C; gas flow, 45 milliliters of argon per minute; detector voltage, 1750 volts; attenuation × 10. (Right) Infrared spectra; solid films. [Reprinted from Eglinton, Hamilton, Martin-Smith (*87*)]

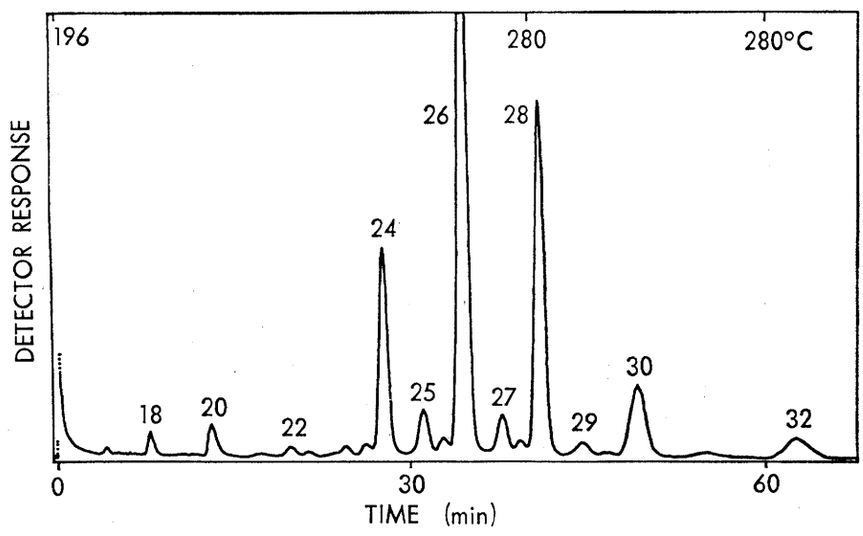


Fig. 7. Separation of the n-aldehydes of wax from fresh sultana grapes by temperature-programmed gas chromatography. The numbers at the peaks indicate the chain-length of the corresponding aldehyde. Column, 15 percent silicone grease on Chromosorb W (80–100 mesh); column temperature, 196°C; linear temperature programming at 2.1°C to 280°C per minute; nitrogen flow rate, 52 milliliters per minute. [From Radler and Horn (*43*), reprinted from the *Australian Journal of Chemistry*, with permission]

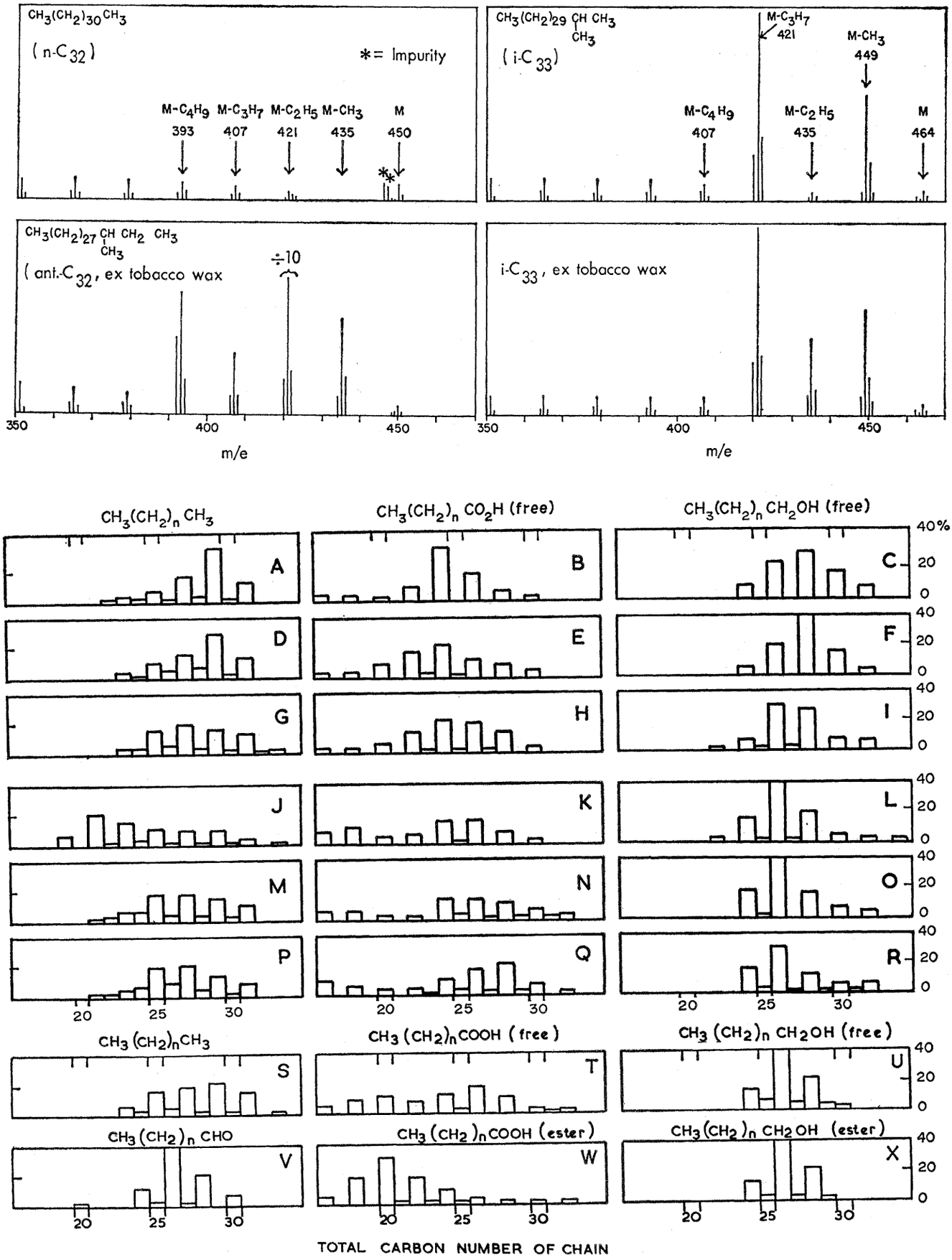


Fig. 9. Histogrammatic presentation of the percentage composition of certain plant-surface waxes. The histograms represent the percentage composition relative to the total carbon number of the chain for the *n*-alkanes, free fatty acids, and free normal alcohols, as shown by analysis of the sultana grape plant and fruit (*43*). (A–C) Young leaf; (D–F) mature leaf; (G–I) stem; (J–L) young fruit; (M–O) mature fruit; (P–R) dried fruit; (S–X) components extracted from fresh fruit; (S) hydrocarbons; (T) free acids; (U) free alcohols; (V) aldehydes; (W) ester acids; (X) ester alcohols.

Fig. 8 (left). Mass spectra of synthetic dotriacontane (n-C_{32}) and 2-methyldotriacontane (iso-C_{33}) and of 3-methylhentriacontane (anteiso-C_{32}) and 2-methyldotriacontane (iso-C_{33}) from tobacco wax.

The primary alcohols, both free and from the ester, have a maximum in their distribution curve at C_{26}. The free acids, however, have a maximum at C_{20}. More surprisingly, the acids derived by hydrolysis of the ester fraction exhibit equal proportions of myristic acid (C_{14}) and stearic acid (C_{18}) but no palmitic acid (C_{16}).

Another extensively studied wax is that of the apple fruit (*40, 57*). The cuticle of apples is thicker than that of most leaves and bears a proportionate quantity of rather oily wax. The composition of the wax depends on the developmental stage reached by the fruit; there would seem to be a greater amount of the oily unsaturated short-chain constituents at maturity and during senescence.

The Australian group of workers (*26*) has examined several *Eucalyptus* waxes and has identified β-diketones. However, unlike the ketones in cabbage wax, there is no relationship between the chain length of the hydrocarbons and the chain length of the β-diketones; for example, *E. globulus* has C_{29} as major hydrocarbon, but the major β-diketone has 33 carbons in the chain.

Eucalyptus risdoni has a maximum at C_{27} in the distribution curve of the hydrocarbon fraction, but the maxima for the primary and secondary alcohols derived from the esters are at C_{26} and at C_{11}, respectively. The free acids have two maxima, at C_{16} and at C_{26}, while acids from the esters have a maximum at C_{16}. Once again, the β-diketone maximum is quite different, being at C_{29}. Horn and his co-workers (*26*) believe this suggests that the biosynthetic pathways to the long-chain ketones and to the β-diketones are different.

The cuticle of the fruit of the sultana grape, *Vitis vinifera*, has been extracted (*43*), and the components have been examined by gas-liquid chromatography, with results shown in Fig. 9 (J–X). It is interesting to note the similarity in the patterns of free alcohols (Fig. 9, U), ester alcohols (X), and aldehydes (V), all of which have C_{26} as the major component, and to contrast this distribution with distributions for the acids, which show quite different patterns for free (T) and esterified (W) portions. Radler (*66*) has extended this work to a study of the components from different parts of the plant. The leaf-wax hydrocarbons (A) have a maximum at C_{29}, whereas the stem-wax hydrocarbons (G) have almost equal amounts of C_{25}, C_{27}, C_{29}, and C_{31}. The hydrocarbons (J) from the young fruit are quite different and have a maximum at C_{21}, a finding similar to findings of Herbin (*67*). It is, however, interesting to note that the dried-grape hydrocarbon pattern (P) is reminiscent of the stem hydrocarbon (G). The pattern for the free fatty acid of leaf wax (B) is quite distinct from that of stem wax (H), but patterns for wax of the young (K) and mature (N) fruit are similar to one another and similar to that of stem wax (H). The distribution patterns for free alcohol in wax of the young (L), mature (O), and dried (R) fruit are similar; all have C_{26} as the major component. The leaf wax (C) has C_{28} as the major alcohol; it is only in the stem that the free primary alcohol pattern shows n-C_{26} as the principal component. These results show that the distribution patterns for each class of compound differ markedly in different parts of the plant. Further, for this single plant species, there is no simple carbon-number correspondence between the classes of compound examined; for example, the major acid does not possess one carbon atom more than the major hydrocarbon.

Chemotaxonomy

Plant-wax constituents have received some study from the standpoint of chemotaxonomy. Their use in plant taxonomy would seem advantageous in view of the almost universal occurrence of these coatings; the species variation in wax composition; the fact that the wax is extracellular and almost certainly an end product insulated from the regular, essential metabolic functions of the plant; the simplicity of sampling; and the present-day availability of precise and rapid microanalytical tools. Because of its speed and simplicity, even direct thin-layer chromatography has been used to provide patterns which might be used taxonomically (*68*).

An understanding of the biogenetic pathways involved is essential if leaf-wax constituents are to be successfully used as taxonomic guides—for the recognition and validation of hybrids, for example, and the detection of chemical parallelisms and convergences in evolution, as adumbrated by Alston (*69*) for polyphenolics. Erdtman (*70*) has pointed out that the most valuable substances taxonomically are not those which are involved in primary metabolic processes but those which are relatively stable by-products in their biological environment. The plant-wax hydrocarbon fraction meets this requirement quite well, while its very complexity is a positive advantage in that it provides a taxonomic fingerprint (*71–73*).

Attempting to test this approach, Eglinton and his colleagues (*68, 73*) examined the leaf waxes of a compact grouping of closely related genera of the subfamily Sempervivoideae (Crassulaceae), endemic to the Canary Islands, which had already been extensively studied botanically by Uhl (*74*). Lems (*75*) has stated that, from the standpoint of evolution, *Aeonium* species present "a situation comparable in many ways to the finches of the Galapagos." The conclusions drawn from the alkane carbon-number patterns were that, in *Aeonium* species, such comparisons could serve to confirm relationships between closely related species but that the differences between related genera were often insufficiently discriminating. Moreover, even similar species sometimes had widely differing patterns, and there was only a rough parallelism of hydrocarbon pattern and botanical classification. One interesting finding was the high content of branched alkanes in some species.

The tobacco plant *Nicotiana tabacum* (Solanaceae) also has a substantial proportion of branched alkanes, and Mold and his associates (*47*) have shown that closely similar alkane distributions are found for three different varieties. The alkane fractions from South African species of *Aloe* (Liliaceae) have been examined recently by Herbin (*67*). Petal waxes and leaf waxes for each species were separately studied. The n-C_{31} alkane constituted more than 80 percent of the petal alkane in 11 species and is the major constituent of the remaining four species studied. There was no such regularity in the corresponding leaf alkanes; n-C_{31} was the major constituent of leaf alkane in ten species, and either n-C_{29} or n-C_{27} was the major constituent in the remainder. In at least five species the petal- and leaf-wax patterns for the same species differed considerably, and Herbin remarks that this may be generally the case. The botanical sections, subsections, and

groups showed little or no correlation with the leaf-alkane distribution. In another study, Borges del Castillo *et al.* (*71*) examined representatives of the families Podocarpaceae (33 species), Araucariaceae (six species), and Cupressaceae (seven species). Here there did appear to be some correlation between carbon-number distribution and botanical classification. For example, in the Cupressaceae n-C_{33} and n-C_{35} were dominant, while in the *Podocarpus* species (Podocarpaceae) n-C_{29} and n-C_{31} were dominant. The *Dacrydium* species (Podocarpaceae) had unusual distributions spaced over ten carbon numbers, with odd and even numbers in roughly equal amounts.

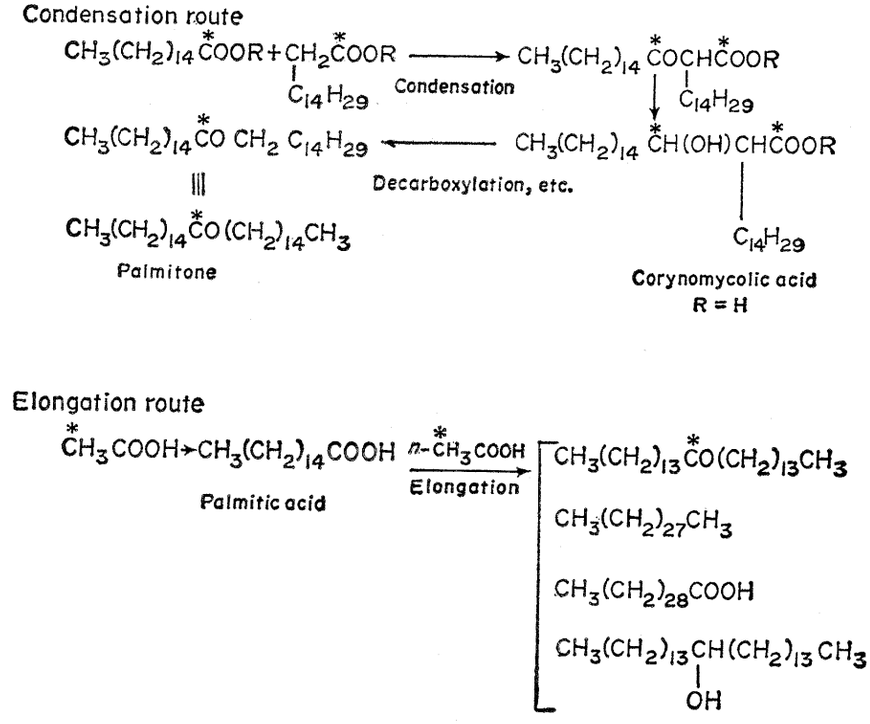


Fig. 10. Postulated biosynthetic routes to long-chain compounds. (The asterisk indicates the radioactive carbon.) (Top) Biosynthesis of corynomycolic acid in *Mycobacterium tuberculosis* (*81*), illustrating the condensation route, involving "head-to-head" condensations of two long-chain acids or derivatives. (Middle) Elongation route, involving the further addition of C_2 units to a previously formed long-chain acid starter, as suggested by Kolattukudy (*78*) from labeling evidence in *Brassica oleracea*. (Bottom) Compartmentalization of the elongation route. [Scheme suggested by Kolattukudy (*78*) to represent the separate sites of *de novo* synthesis and elongation in the plant.] The terms *head* and *tail* are commonly applied to the ends of the long-chain carboxylic acids which bear the carboxylic ($-CO_2H$) and alkyl (CH_3-) groups, respectively. "Head-to-head" condensation implies carbon-carbon bond formation, involving the "head" ends of two such molecules.

It remains to be seen how valid this type of chemotaxonomic approach is, in which comparisons are made of the relative distribution of homologous series of compounds of virtually universal occurrence. Of course, the other well-established approach is that based on the presence or absence of single complex compounds which in turn indicate the presence or absence of enzyme systems responsible for their elaboration.

Biosynthesis

Although a number of possible biosynthetic routes to wax constituents have been suggested in the past, by Chibnall and Piper, by Channon and Chibnall, and by Kreger, Wanless, and Warth (for reviews see *72*, *76*), only two, the condensation and elongation mechanisms, are being considered seriously at present. Most work is directed toward understanding the biosynthetic origin of the hydrocarbons, probably because more is known about the distribution of plant alkanes. In 1962 Matsuda showed (*77*) that wax components are derived from acetate units, and this conclusion is now widely accepted.

The "condensation" and the "elongation" mechanisms postulated for the two different routes are illustrated in Fig. 10. Kolattukudy (*78*) has shown that cabbage leaves (*Brassica oleracea*) and broccoli leaves (*B. oleracea*) will incorporate administered radioactive acetate into all wax constituents. By considering the specific activities of the C_{29} compounds, which make up 60 percent of the wax, he found that "there may not be any precursor-product relationship among the three C_{29} compounds" (nonacosan-15-one, nonacosan-15-ol, nonacosane). He has shown that the carbon bearing the oxygen atom in nonacosan-15-one is derived from the methyl carbon of administered acetate. The condensation route to this ketone would require two molecules of pentadecanoic acid, but the observed labeling pattern would rule out the generation of the C_{15} acid through the action of a propionate "starter" (*79*). The labeling pattern would not rule out formation of the C_{15} acid by α-oxidation of the C_{16} acid (*80*). That α-oxidation is not involved is suggested by the fact that pentadecanoic acid-1-^{14}C was incorporated into the C_{29} compounds less

rapidly than uniformly labeled palmitic acid-^{14}C. According to Martin and Stumpf, equal amounts of radioactivity were incorporated into *n*-nonacosane of broccoli leaves from palmitic acid-1-^{14}C, uniformly labeled palmitic acid-^{14}C, and palmitic acid-9,10-^{3}H. These findings and the fact that palmitic acid-1-^{14}C was found to be as efficient as uniformly labeled palmitic acid-^{14}C in labeling the other C_{29} compounds of broccoli leaf, nonacosane and nonacosanol, are taken by Kolattukudy to mean that palmitic acid is incorporated as a unit, presumably by an elongation route. A condensation reaction would require the loss of one radioactive carboxyl carbon, with the consequence that uniformly labeled palmitic acid-^{14}C would be more efficient than palmitic acid-1-^{14}C.

The findings of Kaneda (*50*) with intact specimens of the tobacco plant (*Nicotiana tabacum*), which has an unusually large proportion of branched-chain alkanes (especially isoalkanes), present a somewhat different picture. Administration to tobacco leaves of short-chain (*n*-C_4 to *n*-C_8), carboxyl-labeled fatty acids resulted in good incorporation of the radioactivity into both straight- and branched-chain hydrocarbons. Kaneda suggests that "head-to-head" condensation (see Fig. 10) of fatty acids of chain length C_{14} to C_{18}, some of them formed in the glyceride pool from the added short-chain acids, would account for this incorporation, whereas continued lengthening of the starter acids (chain lengths, C_4 to C_8) by the elongation mechanism would lead to labeled straight-chain hydrocarbons only. This suggestion would be invalid if extensive scrambling of the label had occurred, as by degradation of the administered labeled acids to acetate, followed by incorporation into the wax components.

In a second experiment, Kaneda administered labeled amino acids (L-valine, L-isoleucine, and L-leucine) to the tobacco plant. Most of the radioactivity appeared in the branched-chain hydrocarbons (Fig. 11). Horning *et al.* (*79*) had previously suggested that valine and isoleucine might act as precursors of the iso- and anteiso- fatty acids of the glycerides in adipose tissue. Kaneda postulates that this process is again followed and that the iso- fatty acids so formed would then condense with straight-chain fatty acids to give the monomethyl branched hydrocarbon (iso-C_{29} and anteiso-C_{32}) following the

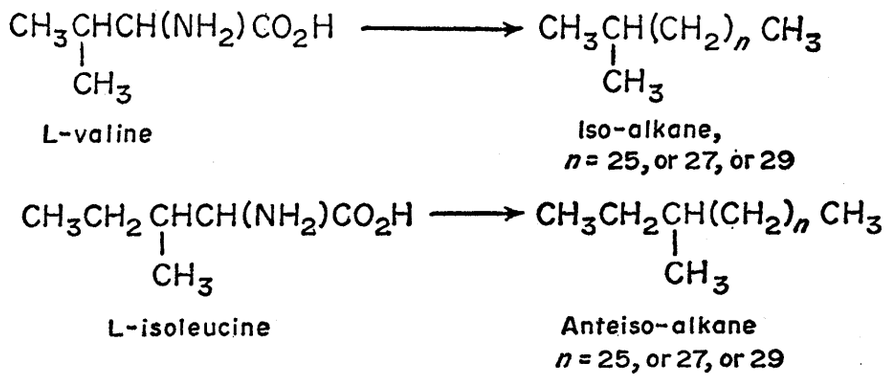


Fig. 11. Postulated incorporation of L-valine and L-isoleucine during biosynthesis of branched alkanes (*50*).

"head-to-head" condensation operating in the biosynthesis of corynomycolic acid (*81*), (see Fig. 10). However, from the available labeling evidence, the elongation procedure would seem equally possible, especially if diterminal dimethyl alkanes cannot be found in this plant wax. Kaneda also suggests that the branched hydrocarbons are produced when there is an excess of amino acids in the plant.

Work by Mazliak (*40*) on the cuticle of apple (*Pyrus malus*), where the radioactive label was incorporated into the acids and alcohols but not into the hydrocarbons, and by Matsuda (*77*) on the wax of candelilla (*Euphorbia antisyphilitica* Zucc.) provide supporting evidence for the belief that the wax constituents (alcohols, acids, hydrocarbons, and ketones) are end products of the plant metabolism (*78*). Matsuda believes that there is no precursor-product relationship between any two of these components. If this is correct, the validity of an alternative way to consider biosynthetic relationships will be open to question. Histograms for wax constituents, such as the histograms of Fig. 9, representing abundance relative to carbon number, usually take the form of smooth unimodal distribution curves with a simple maximum. If there is a simple decarboxylation precursor-product relationship between the acids and the hydrocarbons—without an intermediary pool of acids or their derivatives—one might expect the maximum for the smooth distribution curve of the hydrocarbons to occur at a chain length one carbon less than the maximum for the acids. *Eucalyptus globulus*, however, has C_{20} as the major acid and C_{29} as the major alkane (*26*). In the case of the fruit cuticle of the sultana grape (*Vitis vinifera*), the free acids have two maxima in the distribution curve—the one at C_{26} being in good agreement with the maxima for alcohols and aldehydes—but the hydrocarbons have their maximum at C_{29}, not at C_{25}. In the work on South African species of *Aloe* (*67*), one of the most interesting results has been the emergence, for the acids, of a distribution maximum several carbons less than that for the hydrocarbon components. Stumpf (*82*) has suggested that the α-oxidation enzyme system found in plant leaves may be involved in the route to hydrocarbons, perhaps by the scheme shown in Fig. 12.

The incorporation of labeled acetate into *Brassica* plants treated with trichloroacetate has been examined by Kolattukudy (*78*). The biosynthesis of wax was inhibited, but inhibition was most severe in the case of the hydrocarbons, ketones, and secondary alcohols and much less severe for primary alcohols, acids, and esters. [Juniper (*16*) observed that low concentrations of trichloroacetate changed the shape

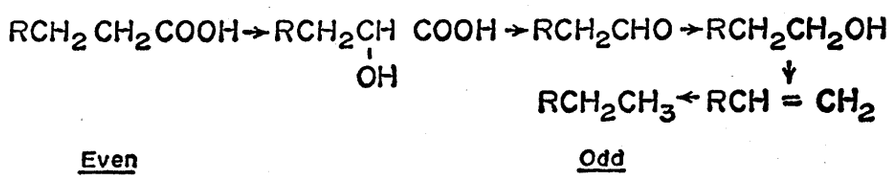


Fig. 12. Postulated oxidative biosynthetic route to the alkanes (*82*).

of the growing crystals, while higher concentrations inhibited their formation altogether.] Hence, Kolattukudy assumes that there is compartmentalization in the biosynthesis of these two groups of wax constituents. If such compartmentalization is widespread, it would account for the lack of success in finding agreement in the carbon number comparisons mentioned above. Compartmentalization, and the presence of rather slowly changing pools of intermediates, would be an alternative to the view, expressed by Horn *et al.* (*26*), that the biogenetic origins of long-chain β-diketones and long-chain ketones are different (different, that is, in pathway, not in site). Such compartmentalization would not be surprising, and presumably it would take place in the subcuticular cells. Little wax is found in the internal tissues of avocado pears (*Peresea americana*) after the surface wax has been removed, and it has been shown (*83*) that spinach chloroplasts lack the ability to synthesize spinach leaf waxes.

A variation of the comparative approach to biosynthetic studies has been developed by workers in New Zealand and Australia (*84*). They compared the chemical composition and the fine structure of waxes evolved by green and glaucous variants of *Eucalyptus urnigera, Poa colensoi, Brassica oleracea,* and *Pisum sativum*. Their results on the infraspecific variation in waxes show that the quantity of wax can be altered by gene action, and in most cases chemical differences were noted also. *Eucalyptus urnigera* specimens growing at 1000 meters and 600 meters were described as glaucous and green, respectively. The waxes had fine structures which were quite distinct, and the plant surfaces had contact angles of 141 and 100 degrees, respectively. The difference in composition of the two waxes was considerable; the β-diketone content was 52 percent in glaucous plants and only 7 to 8 percent in green plants, while the hydrocarbon contents were 3 and 25 percent, with a principal hydrocarbon of n-C_{29} and n-C_{27}, respectively. Such differences are difficult to explain on the basis of action of a single gene, and several genes may be active in determining the differences in leaf surface. It is clear that such infraspecific variations, if widespread, will greatly complicate chemotaxonomic studies based on plant-wax constituents.

Summary and Conclusions

The external surface of the higher plants comprises a cuticular layer covered by a waxy deposit. This deposit is believed to play a major part in such phenomena as the water balance of plants and the behavior of agricultural sprays. The wax contains a wide range of organic compounds. These complex mixtures are amenable to modern microchromatographic and microspectrometric analytical procedures. The few surveys which have been made of the species distribution of certain classes of constituents indicate that such distribution may be of limited taxonomic value; however, the wax composition of a species may differ for different parts of the same plant and may vary with season, locale, and the age of the plant.

This fascinating subject, in which the disciplines of botany, biochemistry, chemistry, and physics overlap and interact, is still in a very active state. Much remains to be learned about the composition and fine structure of the wax deposits, and, for this, experimental study of wax crystallization and permeation through artificial membranes will be required. Enzymic studies, radiolabeling, and electron microscopy will be needed to reveal the mode of biogenesis of the wax constituents and their site of formation and subsequent pathway through the cuticle to the leaf surface.

References and Notes

1. J. Van Overbeek, *Ann. Rev. Plant Physiol.* **7**, 355 (1956).
2. A. De Bary, *Botan. Ztg.*, **14**, 128 (1871); *ibid.*, p. 145.
3. A. C. Chibnall, S. H. Piper, A. Pollard, R. F. Williams, P. N. Sahai, *Biochem. J.* **28**, 2189 (1934); A. C. Chibnall and S. H. Piper, *ibid.*, p. 2209.
4. B. Hallgren and S. Larsson, *Acta Chem. Scand.* **17**, 1822 (1963); G. Shaw and A. Yeadon, *J. Chem. Soc.* **1966**, C16 (1966).
5. D. T. Downing, Z. H. Kranz, K. E. Murray, *Australian J. Chem.* **13**, 80 (1960); J. D. Mold, R. E. Means, R. K. Stevens, J. M. Ruth, *Biochemistry* **5**, 455 (1966).
6. E. O. A. Haahti and E. C. Horning, *Acta Chem. Scand.* **15**, 930 (1961); N. Nicolaides and R. E. Kellum, *J. Amer. Oil Chem. Soc.* **43**, 54 (1966).
7. G. L. Baker, H. E. Vroman, J. Padmore, *Biochem. Biophys. Res. Commun.* **13**, 360 (1963).
8. L. O. Williams, *Econ. Botany* **12**, 103 (1958).
9. A. H. Warth, *Chemistry and Technology of Waxes* (Reinhold, New York, ed. 2, 1956).
10. H. N. Barber, *Evolution* **9**, 1 (1955).
11. R. H. Schieferstein and W. E. Loomis, *Plant Physiol.* **31**, 240 (1956).
12. D. M. Hall and R. L. Jones, *Nature* **191**, 95 (1961).
13. G. T. Daly, *J. Exp. Botany* **15**, 160 (1964).
14. L. Leyton and B. E. Juniper, *Nature* **198**, 770 (1963).
15. J. R. Havis, *Agr. Exp. Sta. Mem. 298* (1950), p. 20.
16. B. E. Juniper, *New Phytologist* **58**, 1 (1959).
17. ———, *J. Linnean. Soc. (Botany)* **56**, 413 (1960).
18. K. P. Dorschner and K. P. Buchholtz, *Agronomy* **48**, 59 (1956).
19. F. J. Stevenson, *J. Amer. Oil Chem. Soc.* **43**, 203 (1966).
20. W. Heinen, *Acta Botan. Neerl.* **9**, 167 (1960); *ibid.* **10**, 171 (1961).
21. R. I. Morrison and W. Bick, *Chem. Ind. London* **1966**, 596 (1966).
22. J. W. Foster, *J. Antonie van Leeuwenhoek* **28**, 241 (1962).
23. J. N. Baptist, R. K. Gholson, M. J. Coon, *Biochim. Biophys. Acta* **69**, 40 (1963).
24. R. A. W. Johnstone and J. R. Plimmer, *Chem. Rev.* **59**, 885 (1959).
25. J. Oró, D. W. Nooner, S. A. Wikström, *J. Gas Chromatog.* **3**, 105 (1965).
26. D. H. S. Horn, Z. H. Kranz, J. A. Lamberton, *Australian J. Chem.* **17**, 464 (1964).
27. G. Eglinton, *Geol. Rundschau* **55**, 551 (1965).
28. D. R. Kreger and C. Schamhart, *Biochim. Biophys. Acta* **19**, 22 (1956).
29. L. E. Mueller, P. H. Carr, W. E. Loomis *Amer. J. Botany* **41**, 593 (1954).
30. B. E. Juniper and D. E. Bradley, *J. Ultrastruct. Res.* **2**, 16 (1958).
31. O. R. Dewey, G. S. Hartley, J. W. G. MacLauchlan, *Proc. Roy. Soc. London Ser. B* **155**, 532 (1962).
32. D. H. Kay, Ed., *Techniques in Electron Microscopy* (Blackwell, Oxford, ed. 2, 1965).
33. D. M. Hall and L. A. Donaldson, *Nature* **194**, 1196 (1962).
34. B. E. Juniper, unpublished work.
35. M. S. Blum, J. G. Traynham, J. B. Chidester, J. D. Boggus, *Science* **132**, 1480 (1960).
36. J. S. Edwards, *Nature* **211**, 73 (1966).
37. B. E. Juniper and J. K. Burras, *New Sci.* **13**, 75 (1962).
38. E. A. Baker, R. F. Batt, A. M. Silva Fernandes, J. T. Martin, *Ann. Rep. Long Ashton Res. Sta. Bristol* (1963), p. 106.
39. S. J. Purdy and E. V. Truter, *Proc. Roy. Soc. London Ser. B* **158**, 536 (1963); *ibid.*, p. 544; *ibid.*, p. 553.
40. P. Mazliak, thesis, Univ. of Paris, 1963; *Ann. Biol.* **2**, 35 (1963); in *First International Congress of Food Science and Technology* (Gordon and Breach, New York, 1964).
41. ———, *Phytochem.* **1**, 79 (1962); D. T. Downing, Z. H. Kranz, K. E. Murray, *Australian J. Chem.* **14**, 619 (1961).
42. J. A. Lamberton and A. H. Redcliffe, *Australian J. Chem.* **13**, 261 (1960).
43. F. Radler and D. H. S. Horn, *ibid.* **18**, 1059 (1965).
44. R. J. McCulloch, J. W. Hamilton, S. K. Brown, *Biochem. Biophys. Res. Commun.* **11**, 7 (1963).
45. F. Sörm, V. Wollrab, P. Jarolimek, M. Streibl, *Chem. Ind. London* **1964**, 1833 (1964); B. Stoianova-Iranova, D. Nikolova, V. Izvetkova, *Compt. Rend. Acad. Bulgare Sci.* **18**, No. 2, 161 (1965).
46. B. Nagy, V. Modzeleski, M. T. J. Murphy, *Phytochemistry* **4**, 945 (1965).
47. J. D. Mold, R. K. Stevens, R. E. Means, J. M. Ruth, *Biochemistry* **2**, 605 (1963).
48. G. Eglinton, R. J. Hamilton, W. B. Kelly, R. I. Reed, *Phytochemistry* **5**, 1349 (1966).
49. V. Wollrab, M. Streibl, F. Sörm, *Collection Czech. Chem. Commun.* **30**, 1670 (1965).
50. T. Kaneda, Canadian Institute of Chemistry annual meeting (1966).
51. Z. H. Kranz, J. A. Lamberton, K. E. Murray, A. H. Redcliffe, *Australian J. Chem.* **14**, 264 (1961).
52. J. A. Lamberton and A. H. Redcliffe, *ibid.* **13**, 498 (1960).
53. D. H. S. Horn and J. A. Lamberton, *Chem. Ind. London* **1962**, 2036 (1962).
54. A. P. Tulloch and R. O. Weenink, *Chem. Commun. No. 2* (1965), p. 225.
55. E. von Rudloff, *Can. J. Chem.* **37**, 1038 (1959).
56. D. H. S. Horn and J. A. Lamberton, *Chem. Ind. London* **1963**, 691 (1963).
57. A. M. Silva Fernandes, E. A. Baker, J. T. Martin, *Ann. Appl. Biol.* **53**, 43 (1964).
58. R. T. Aplin, R. C. Cambie, P. S. Rutledge, *Phytochemistry* **2**, 205 (1963).
59. A. J. Baker, G. Eglinton, A. G. Gonzalez, R. J. Hamilton, R. A. Raphael, *J. Chem. Soc.* **1962**, 4705 (1962).
60. G. Eglinton, R. J. Hamilton, M. Martin-Smith, S. J. Smith, G. Subramanian, *Tetrahedron Letters* **1964**, 2323 (1964).
61. D. H. S. Horn and J. A. Lamberton, *Australian J. Chem.* **17**, 477 (1964).

62. M. F. Roberts, R. F. Batt, J. T. Martin, *Ann. Appl. Biol.* **47**, 573 (1959).
63. E. A. Baker, R. F. Batt, A. M. Silva Fernandes, J. T. Martin, *Ann. Rep. Long Ashton Res. Sta. Bristol* (1962), p. 69.
63a. P. J. Holloway and J. B. Challen, *J. Chromatog.* **25**, 336 (1966).
64. K. Nishimoto, M. Ito, S. Natori. T. Ohmoto, *Tetrahedron Letters* **1965**, 2245 (1965).
65. P. Duperon, W. Vetter, M. Barbier, *Phytochemistry* **3**, 89 (1964).
66. F. Radler, *Australian J. Biol. Sci.* **18**, 1045 (1965).
67. G. A. Herbin, *Proc. East African Acad.* **2**, 11 (1964).
68. S. J. Purdy and E. V. Truter, *Nature* **190**, 554 (1961).
69. R. E. Alston, in *Chemistry of Phenolic Compounds*, J. B. Harborne, Ed. (Academic Press, New York, 1962).
70. H. Erdtman, in *Prespectives in Organic Chemistry*, A. Todd, Ed. (Interscience, New York, 1956), p. 473.
71. J. Borges del Castillo, C. J. W. Brooks, R. C. Cambie, G. Eglinton, R. J. Hamilton, P. Pellitt, *Phytochemistry* **6**, 391 (1967).
72. G. Eglinton and R. J. Hamilton, in *Chemical Plant Taxonomy*, T. Swain, Ed. (Academic Press, New York, 1963), p. 187; A. G. Douglas and G. Eglinton, in *Comparative Phytochemistry*, T. Swain, Ed. (Academic Press, New York, 1965), p. 57.
73. G. Eglinton, A. G. Gonzalez, R. J. Hamilton, R. A. Raphael, *Phytochemistry* **1**, 89 (1962).
74. C. H. Uhl, *Amer. J. Botany* **48**, 114 (1961).
75. K. Lems, *Ecology* **41**, 1 (1960).
76. D. R. Kreger in *Handbuch der Pflanzenphysiologie*, W. Ruhland, Ed. (Springer, Berlin, 1958), p. 249.
77. K. Matsuda, thesis, Univ. of Michigan, 1962.
78. P. E. Kolattukudy, *Biochemistry* **4**, 1844 (1965); *ibid.* **5**, 2265 (1966).
79. M. J. Horning, D. B. Martin, A. Karman, P. Vagelos, *J. Biol. Chem.* **236**, 669 (1961).
80. R. O. Martin and P. K. Stumpf, *ibid.* **234**, 2548 (1959).
81. M. Gastambide Odier and E. Lederer, *Nature* **184**, 1563 (1959).
82. P. K. Stumpf, in *Plant Biochemistry*, J. Bonner and J. E. Varner, Eds. (Academic Press, New York, 1965), p. 322.
83. L. P. Zill and E. A. Harmon, *Biochim. Biophys. Acta.* **57**, 573 (1962).
84. D. M. Hall, A. I. Matus, J. A. Lamberton, H. N. Barber, *Australian J. Biol. Sci.* **18**, 323 (1965).
85. C. H. Brieskorn and J. Boss, *Fette Seifen Anstrichmittel* **16**, 925 (1964); E. A. Baker and J. T. Martin, *Nature* **199**, 1268 (1963); J. T. Martin, *Ann Rev. Phytopathol.* **2**, 81 (1964); ——— and D. J. Fisher, *Ann. Rep. Long Ashton Res. Sta. Bristol* (1965), p. 251; E. A. Baker, R. F. Batt, J. T. Martin, *Ann. Appl. Biol.* **53**, 59 (1964); A. M. Silva Fernandes, *ibid.* **56**, 297 (1965); ———, *ibid.*, p. 305.
86. R. E. Corbett, M. A. McDowall, S. G. Wyllie, *J. Chem. Soc.* **1964**, 1283 (1964).
87. G. Eglinton, R. J. Hamilton, M. M. Smith, *Phytochemistry* **1**, 137 (1962).
88. We are deeply indebted to Dr. B. E. Juniper, Botany Schools, Oxford, for many helpful comments and suggestions and for the beautiful photomicrographs. We also thank Dr. D. E. Bradley, department of zoology, Edinburgh University, for a photomicrograph and Dr. J. T. Martin, Long Ashton Research Station, Bristol University, for advice. We are indebted to Academic Press and to the editors of the following publications for permission to reproduce diagrams and photographs: *Journal of the Linnean Society, Australian Journal of Chemistry, Endeavour*, and *Phytochemistry*.

Michael Faraday and the Physics of 100 Years Ago

L. Pearce Williams

Michael Faraday was born on 22 September 1791, in Newington, Surrey, near London. His father, a journeyman blacksmith, had left the North-country to try to better his lot in the metropolis as a depression gradually settled over England. His mother had worked as a maidservant before her marriage to James Faraday and had already borne her husband a daughter, Elizabeth, and a son, Robert. A second daughter, Margaret, soon followed Michael. The family was desperately poor. James Faraday was in almost constant ill health and could work only sporadically. As prices rose as a result of England's involvement in the French Revolutionary Wars, simple subsistence became a major problem. In later years Faraday told of having been given a single loaf of bread which was to serve him as his main course for a week.

What sustained the Faradays throughout their hardships was a simple but extraordinarily powerful religious faith. James Faraday was a Sandemanian. The Sandemanian Church rejected what it considered to be all the false trappings of the Church of England and sought to recapture both the letter and the spirit of the early Church. The congregation was a true brotherhood in which all helped one another both materially and spiritually. Life within the Sandemanian community was often hard, but it was never desperate. With the exception of the influence of his family, about which we know very little, the Sandemanian Church was the most important factor in Michael Faraday's education. It was at a church "school" that he learned the three R's—the total extent of his formal education. More importantly, the Sandemanian religion provided him with two convictions that were essential elements of his later scientific career. According to Judeo-Christian tradition, the universe was, literally, made for man. The philosophical result of this view is the belief in final causes which, as innumerable science texts assure us, was banished from science by the Scientific Revolution. They were not banished from Faraday's mind. He had a deep conviction of the ultimate harmony of the world which led him onward in his physical pursuits. This harmony, he also believed, was designed for man's well-being. Thus he could state at the end of a lecture on ozone in 1859 (*1*, p. 103):

> These are the glimmerings we have of what we are pleased to call the *second causes* by which the *one Great Cause* works his wonders and governs this earth. We flattered ourselves we knew what air was composed of, and now we discover a *new* property which is imponderable, and invisible, except through its *effects* which I shewed you in the last experiment; but while it fades the ribbon, it gives the glow of health to the cheek, and is just as necessary for the good of mankind, as the other parts of which air is composed.

From his religion Faraday also drew a deep and profound sense of his own (and everyone else's) fallibility. He *knew* that he must err and accepted this as a simple fact. He would do his utmost to minimize his errors—he would repeat his experiments hundreds of times; he would scrutinize them with the most critical eye; he would check and recheck his arguments. But he would not insist upon them, once pub-

The author is professor of the history of science at Cornell University, Ithaca, New York. This article is based upon his *Michael Faraday, A Biography* (Basic Books, New York, 1965).