BIOCHEMICAL PHASES OF OLEORESIN PRODUCTION

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(WITH FOUR FIGURES)

The key position of naval stores production in the profitable utilization of the more than 50,000,000 acres of longleaf and slash pine lands of the southeastern United States puts a premium upon exact and comprehensive knowledge of the biochemical facts which underlie successful oleoresin production. Oleoresin, or gum, the source of naval stores, exudes from cuts on living pines, from their earliest seedling stage. A cut into a young sapling, for example, illustrates the abundance of the exudation. The exudate is not the sap of the tree in the ordinary sense of the term, however, nor does it come from the ordinary sap-conducting tissues as does the watery solution of the exuding maple sap.

The exudation of oleoresin comes from areas of parenchyma cells in the outer sapwood next to the bark (fig. 1). These parenchyma cells contain active functioning protoplasm for a longer period than the associated fibrous cells which conduct the watery sap. The parenchyma cells also manufacture, transform, translocate, and store many different compounds, including sugars, tannins, and starch. Resin passages, the so-called resin ducts, are spaces which arise in the midst of clusters of parenchyma cells by a separation or splitting apart of the cells (schizogenous not lysigenous origin). It is from these that the oleoresin collected in turpentinng wells out in drops (fig. 2, A). The chipping practiced by the turpentine operator is a freshening of the wound or cut made in the sapwood, which causes a continued and even augmented exudation of oleoresin.

Resin passages are an outstanding characteristic of all pine wood, but they reach an optimum development in slash (Pinus caribaea) and longleaf (P. palustris) pines. They extend both vertically and horizontally in the tree. The two systems frequently connect with each other and with the rays (also parenchymatous in nature) at different levels, thus forming an extensively anastomosing system which is also connected with the parenchyma tissues of the phloem or inner bark through which compounds elaborated in the leaves are translocated (fig. 1).

Resin passages are more numerous than is often realized. Recent microscopical investigations at the Forest Products Laboratory have shown (7)

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FIGS. 1–4. Fig. 1: Microscopical view of cross section of pine wood showing vertical resin passages (p) at center of groups of parenchyma cells; rays (r), including a wide ray with a horizontal resin passage connecting a vertical resin passage with the inner bark or phloem (b) rich in stored material; and the wood tracheids or fibers (f) which function in water conduction. Fig. 2: A, heavy exudation next bark obtained from the increased number of wound-induced resin passages; B, wood above the face or wound on a turpentined pine; b, normal wood formed before turpentining; a, two years of growth during turpentining showing great increase in number of resin passages. Fig. 3: A, cross section of normal southern pine wood (slash pine); a, portion of one annual growth ring; b, springwood; d, summerwood; e, resin passages; B, wood formed by same tree over face during turpentining, containing greatly increased number of resin passages; a, portion of one annual growth ring. Fig. 4: Diagrammatic drawing of pine wood structure showing horizontal resin passages (hrd) and vertical resin passages (vrd) and the connections frequently found between them. Also springwood fibers (sp), summerwood fibers (sm), and rays (wr, fwr).
that in slash pine on the average more than 450 horizontal resin passages were present per square inch of tangential surface (fig. 4). The number present in various specimens ranged from about 250 to 800 per square inch. The exudation from these horizontal resin passages is more directly utilized in the French method of turpentining than in the American, because a relatively greater area of tangential surface is regularly freshened owing to the shape of the French wound or face.

The vertical resin passages, which are much larger than the horizontal passages, number about 200 per square inch of cross section in normal wood. This number ranges from about 70 to 400 per square inch. In wood formed above the face during turpentining the number of vertical resin passages may be increased in varying amounts up to more than tenfold the normal number (fig. 3). The induced increase of resiniferous tissues by wounding is of considerable importance since a large proportion of the yield obtained after the first year of turpentining comes from it (fig. 2, A). The effect of the wound may extend vertically for 10 to 20 feet above the face and is clearly registered in the structure of the wood formed above the face. The wound response is limited to a few inches circumferentially, however, although characteristic traumatic structures are apparent in the rolls of healing tissue formed at the sides of the face. They often offer a rich source of oleoresin when trees are back-cupped during successive workings.

Investigations other than chemical have dealt to date with anatomical and physiological studies of the normal character of resiniferous tissue in virgin and second-growth trees, and with the responses induced by different types of wounding. The work now in progress is a joint attack, using in addition cytological, microchemical, and biochemical techniques to determine the character and sequence of changes in the compounds which underlie oleoresin formation.

Oleoressins are biological materials, products of the living protoplasm, and therefore referable to the original products of photosynthesis for their ultimate chemical derivation. It must be said that here, however, as with most other complex substances of vegetable origin, not one step is yet known with certainty in the complex processes by which the pine tree elaborates oleoresin. But if empirical experimentation in forest management and turpentining is to be firmly grounded, all possible information must be obtained concerning the biochemistry of oleoresin formation.

Although technologically pine oleoresin has usually been considered as merely a solution of resin acids in a mixture of terpenes, such a concept does not suffice in an investigation of this kind. As a product of vital processes, oleoresin might be expected to contain small quantities of materials not yet completely elaborated, the careful examination of which may throw considerable light on the processes immediately preceding the excretion of the oleo-
resinous mixture. A part of this investigation therefore involves the thorough examination of substances in the oleoresin other than terpenes and resin acids, particularly such materials as are soluble in water.

The place of excretion of the completed oleoresin is known, as has been indicated (fig. 2), although the mechanism of the process is unknown. Similarly the processes by which assumed complex oleoresin precursors are translocated are unknown, as well as the seat of formation within the tree of these assumed complexes. A second phase of this investigation, therefore, involves the chemical examination of the tissues of the pine which are apparently concerned in oleoresin formation. A definite search is being made for substances which may be defined as oleoresin in process of formation.

Such a concept is by no means original, although comparatively little evidence has hitherto been brought forward in support of it. Köhler (8) found certain resinous secretions in *Picea excelsa*, which were subject to rapid and spontaneous change upon exposure to the air. He postulated an aldehydic or camphoraceous precursor, C16H30O, which was secreted as such, and changed to the mixture of resin acids and terpenes outside of the living cells.

Dupont (4) developed an ingenious formulation, *viz.*,

\[ 3 \text{C}_{16}\text{H}_{30}\text{O} = \text{C}_{16}\text{H}_{16} + \text{C}_{29}\text{H}_{39}\text{O}_2 + \text{H}_2\text{O}. \]

The elaboration of this equation explains very satisfactorily the proportions of resin acids and terpenes found in the oleoresins from *Pinus maritima* and *P. halepensis*, in which the ratio of resin to turpentine approximates closely the 69 : 31 ratio required by Dupont's equation. However, numerous pines do not approximate this composition in their oleoresins; slash and long-leaf oleoresins run more nearly a ratio of 80 : 20 (1). It is also true that there is appreciable variation in turpentine content between individual trees. Further, it must be considered that any such mechanism must also explain the fact of the existence of n-heptane as the volatile ingredient of oleoresins from *P. jeffreyi* and *P. sabiniana*, as well as the large proportions of sesquiterpene in the oleoresin of *P. longifolia*. In short, although the assumption of some common precursor of volatile and non-volatile components of oleoresin has a logical basis, it does not seem that so simple a balance as indicated by Dupont can explain the facts of oleoresin composition throughout the genus *Pinus*.

Devaux and Bargues (3) showed that the oleoresinous materials were not entirely localized in the resin passages of the wood and in the cells bordering them. The living parenchyma cells of the wood and phloem are connected with the resin passages through the rays (fig. 2) which allow translocation of the materials which are concerned in oleoresin production. Note-worthy is the statement of Devaux and Bargues that the secretion is acidic
only in the canals and generally neutral in the living cells. The change is said to occur when the resin reaches the canal, although it may occur prematurely.

Glitchitch and Naves (5) call attention to the difference in sesquiterpene content of ylang-ylang oils obtained by steam distillation and petroleum ether extraction. The very low content of sesquiterpenes in the latter leads them to suggest that the sesquiterpenes may arise during the distillation process from substances insoluble in petroleum ether and non-volatile with steam.

There is further the evidence of Bridel (2) concerning the existence of glucosides of geraniol, which gives a clue to the possible nature of the mechanism by which substances insoluble in cell sap are held in solution by means of glucosidal combinations.

A recent theoretical study (6) showed the probable derivation of the terpenes from underlying hydroxylated bodies. A similar type of generalization doubtless holds for the resin acids although sufficient examples of known structure are not yet available to warrant their classification. Such assumptions seem more warranted than the well known isoprene polymerization in that nothing has been observed indicating the existence of isoprene in nature, nor has it been formed in vitro by means analogous to those of biological reactions.

From these considerations, the search for oleoresin precursors both in the oleoresin and in the tissues of the tree has been primarily for glucosidal materials, water-soluble and resolvable into oleoresinous constituents and sugars. The difficulties of this work are apparent, since it involves the isolation of very small quantities of delicate compounds from very large quantities of starting material by means sufficiently gentle to avoid decomposition. While no single phase of the problem may be regarded as completed, considerable progress has been made which is briefly reported here.

The oleoresin itself contains small quantities of highly complex glucosidal material which is soluble in water and can be obtained by treatment of the oleoresin with water. The original clear, light, aqueous extract soon deposits a black, amorphous precipitate which is now insoluble in water but soluble in alcohol. This substance is easily hydrolyzed, yielding a large proportion of acidic bodies of a resinous nature. While these substances cannot yet be identified, since they have been available to the extent of only a few grams, their color reactions are those of the acids of rosin, and it is extremely probable that they belong to this class of substances.

The solution from which this dark precipitate has been obtained still contains a considerable quantity of material in solution. By extraction with ether, a substance is obtained which is instantly hydrolyzed by very dilute acid. It yields a resin acid, a volatile oil, and a water-soluble body. The
last may be broken up further into a sugar and a resin acid. The sugar is remarkable in that it reduces Fehling’s solution in the cold, and reacts with phenylhydrazine very quickly in the cold, yet it is not an uronic acid. It is highly reactive and tends to polymerize to unworkable complexes on very slight provocation.

Complete interpretation of these results is still far distant, and must await considerably more material and effort. However, they suggest very definitely the presence in the oleoresinous exudate of glucosidal complexes of resin acids, volatile oil, and reactive sugars, very easily broken up and very sensitive to slight changes in environment.

Investigation of slash pine tissues has so far been confined to the phloem, since, as was pointed out, this seems to constitute a rich reserve tissue (fig. 2), physiologically closely related to that immediately concerned with production of the oleoresin in the resin passages of the wood or xylem. While a rather complete phytochemical survey of this material has been made, it will suffice in this connection to point out only the occurrence in the phloem of glucosidal complexes of exactly similar nature to those obtained from the oleoresin itself; that is, a glucoside soluble in both water and ether, yielding to very mild acidic hydrolysis in the cold, resin acid, volatile oil, and the same resin acid-sugar complex as obtained from the oleoresin.

It is regretted that further data on the constitution of these substances cannot be given at this time, but their extremely complex nature makes necessary the preparation of further quantities of material for more complete investigation. It is believed, however, that the attack thus far has been justified and has yielded considerable chemical substantiation of the anatomical evidence of functional connection between resiniferous tissues of both phloem and xylem, and thrown considerable light on the processes preceding the excretion of oleoresin at the wounded surface.

At present the picture presented is of a large complex of resin acids and terpenes combined with sugars, the whole being thus soluble in the elaborated sap of the parenchyma cells and translocated as such to the neighborhood of the area of excretion, where it is resolved by a mechanism still obscure.

In conclusion, it may be pointed out that the demonstrated presence of these highly reactive, water-soluble bodies in the oleoresin contributes in no small degree to the variations in color of manufactured rosins, and is thus of considerable technological importance.

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LITERATURE CITED


