Availability of organic compounds in higher plants

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Introduction

Substances that bring about changes in living organisms are said to be biologically active. This property of chemical compounds depends as a rule upon a large number of factors, so that the relationship with the molecular structure is usually highly complex.

Attempts to analyse this relationship are based on a very simplified model (fig. 1). The biological system is represented by a membrane-enclosed space (compartment) which contains a receptor for the biologically active substance, the "drug". This receptor may be regarded as a small part of a biological macromolecule (an enzyme for example) possessing a very specific spatial structure on which the biochemical processes essential to the living organism take place. Interaction between the drug and the receptor can produce a biological effect by affecting or inhibiting the processes that normally take place [1].

In order to produce such an interaction the substance has to penetrate to the receptor; the concentration of the drug at the site of the receptor is called the availability of the drug. On the basis of the foregoing the biological activity of a substance could be defined as the product of availability and specific biological activity. By specific biological activity we mean the biological activity of unit concentration at the location of the receptor.

The availability at the site of the receptor is affected by two groups of processes. In the first place, the drug has to penetrate the cellular or sub-cellular barriers enclosing the compartment and then move to the receptor (permeation and transport). Secondly, in the biological organism, it is subject to various breakdown processes, and part of the permeated substance may sometimes be prevented from reaching the receptor by other causes, such as adsorption and evaporation (from plants).

In actual research for biologically active compounds systems are encountered which are far more complex than the system of the model; a single cell alone is already more complicated than the model. Drugs administered to humans, animals and plants generally have to pass through a large number of "biological compartments" with their associated barriers, transport paths and metabolic processes, before reaching the receptor. In parasite control permeation in the parasite will often have to be taken into account as well as the availability of drugs in the host. In principle however the considerations applicable to the model remain valid.

Research on availability is important in the development of drugs. The object of this research is to learn something about the relations between the molecular structure or physical properties of chemical compounds and their availability in biological organisms. The application of these relations can be of value in the selection of classes of chemical compounds for drug research and can increase the efficiency of the search for optimum compounds in those classes in which biological activity has been found.

Research in this field has been carried out since 1900, particularly in connection with cell permeability [2], but the amount of research work has increased considerably in the last ten years. One of the motivations has undoubtedly been the need for safety in pharmaceutical application which makes it highly desirable to find out as much as possible about what happens to these substances in humans, animals and plants. Apart from this, it is now realized more clearly than in the past that a deeper insight into the availability of chemical compounds is of importance in the development of medicinal substances.

[1] The receptor concept was first put forward at the beginning of this century by Ehrlich and others. It has been elaborated upon in recent years by Ariens and associates; see E. J. Ariens, Molecular pharmacology, Vol. 1, Academic Press, London 1964.
ment of new drugs\(^3\). All this work is however still largely in an initial phase, in which attempts are being made to compile systematic data on the availability of these substances in various organisms\(^4\).

Primarily with the object of illustrating the approach to the problems in this field, we shall discuss in this article two processes which affect the availability of biologically active compounds in plants.

**Plant processes which affect the availability of chemical compounds**

The classical methods of controlling plant diseases were concerned solely with destroying parasites outside the plant, e.g. in the soil or on the leaves. In about 1945 the idea was conceived of trying to develop pesticides which, by analogy with the combating of disease in humans and animals, would be taken up and transported in the plant and thus provide it with internal protection against its invaders. These substances were referred to as *systemic* pesticides. The first indications that this protection method is in principle possible were obtained with organic phosphorus compounds developed in Germany during the second world war and which were found to possess systemic insecticidal activity\(^5\). Since then various other systemic pesticides have been developed. For the development of such systemic agents, and also of agents for controlling weeds (herbicides) it is essential to gain some understanding of their availability in plants\(^6\). In higher plants there are two important pathways by which the substances are translocated: the pathway via the parts above the soil (i.e. the leaves) and the pathway via the roots (and the soil). In the following we shall confine our attention to the second pathway. Through its roots the plant takes up water containing dissolved inorganic salts and other food materials. These are translocated through the transport ducts in the stems (the xylem) to the leaves. This "transpiration process" is maintained principally by the suction force exerted by the leaves as water evaporates from them.

The biologically active compounds which enter the plant by this pathway, have to negotiate various "barriers". First of all, there are *adsorption* and *absorption* effects. Compounds of an apolar character are often strongly absorbed by the humus in the soil and by parts of the plants that contain a large amount of lipids. Ionized substances with a positive charge are often adsorbed on soil constituents and parts of plants (e.g. the walls of the xylem) which contain negative groups. Secondly the *permeation processes* play an important rôle: in order for a substance to enter the transport system in the plant it must be able to permeate through the roots, and for a good distribution (e.g. in the leaves) it must pass through a large number of cell membranes. Thirdly, many substances are subject to various *conversions* during the transport process. These include microbiological conversions in the soil and enzymatic processes in roots, stems and leaves. A final aspect, presumably unimportant in animals, but which can have a considerable effect on the availability of biologically active compounds in plants, is the *evaporation* from the leaves of substances taken up in the plant.

Each of these processes will generally have its own characteristic relationship with the molecular properties of the relevant compounds. There will therefore be a greater chance of learning more about these processes if the experiments are designed so as to permit separate study of the individual barriers in the various parts of the plant which a molecule has to traverse in penetrating from the outside to the receptor. The effects of permeation and transport on one hand and stability on the other should also be separately evaluated. By way of example we shall describe some experiments on root permeability and stability in the plant, making use of results obtained with the aromatic sulphones of the type

\[
R_1\text{SO}_2R_3
\]

where \(R_1\) and \(R_3\) are arbitrary ortho-, meta- or para-substituents and \(R_2\) is an alkyl group.

**Root permeation**

**Method**

Investigations on root permeation are carried out with an experimental arrangement making use of the exudation from tomato plant roots. Exudation is a physiological process in which the xylem in the roots continuously gives out water which can easily be collected (see fig. 2). We use roots from tomato plants which have been grown in identical conditions of illumination,
temperature, etc. This standardization is necessary to obtain reproducible experimental results.

The roots are placed in a solution in water of the substance under investigation. The substance is taken up from this "external solution" and is metabolized in the root tissues, and the metabolites are then exuded into the external solution. After some time the percentage conversion in the external solution will be approximately equal to that in the roots, unless the substance is particularly unstable in the roots.

The stability of the substance in the roots is determined by measuring the concentration in the external solution at the end of the experiment (after six days), and expressing this concentration as a percentage of the initial concentration after correcting for the amount that has permeated into the roots. The possibility that the conversions may have other causes such as chemical instability in solution, bacterial conversions, etc., is checked by means of control solutions (with no roots present).

The permeation is determined by measuring the concentration of this substance in the exuded sap as a function of time. Some examples are shown in fig. 3.

**Nature of the permeation process**

According to the literature, the permeation of substances in biological systems may be active or passive in character. In active permeation the metabolism has a direct effect on the permeation mechanism. In passive permeation the passage of the substance through the biological membrane is determined entirely by physico-chemical factors.

In the literature various mechanisms have been described which are thought to play a rôle in active permeation. In many instances the carrier concept is used. A carrier is a natural substance occurring on the outside of the membrane, which can form specific bonds with an entering molecule. According to one theory the complex thus formed diffuses passively through the membrane along a concentration gradient, and then splits up again on the inside of the membrane. This
splitting process, and possibly the biosynthesis of the carrier, is governed by the metabolism of the biological object.

Rosenberg and Wilbrandt have proposed various criteria for the recognition of active permeation [7]. These include:

a) A constant rate of permeation above a critical saturation concentration, as all the available carrier molecules are then occupied. In root permeation experiments with a number of biochemically stable aromatic sulphones we found a practically linear relationship between external concentration and rate of permeation in the concentration range between 0.03 and 4 mmole/l. This shows that there are no saturation phenomena in this case.

b) Inhibition of the permeation process by inhibitors, which stop the active process by disturbing the processes supplying energy. Using the compound p-aminophenylmethylsulphone an investigation was made into the effect of the inhibitors NaF, NaN₃, 2,4-dinitrophenol and KCN on permeation, and also into the effect of the supply of O₂ in the root system. Although the rate of exudation of water (which is also dependent on the metabolism for its energy supply) may indeed be affected by these factors, no direct effect at all on the permeation of the sulphone was found. On the other hand, no direct effect on the permeation was found after the addition of salts (e.g. KNO₃) which strongly stimulate the exudation.

c) High structural specificity of the permeating substances, since they must "fit" the carrier molecules. Permeation through tomato roots was investigated for about 150 neutral organic compounds. Compounds were chosen which are stable enough in the relevant biological system to allow the permeability to be determined with reasonable accuracy. No marked structural specificity was found, although there were some indications of a gradual change in the permeation properties following structural variations which led to a change in the physico-chemical properties of the relevant compounds.

d) High temperature coefficient of the permeation constant. Here also, there were no indications pointing to an active process in root permeation.

It may be concluded from the foregoing that root permeation of the organic compounds which we have investigated is a passive process. This contrasts with the root permeation of inorganic ions, whose active character has been fairly clearly established by other investigators.

In view of the non-specific character of passive permeation it seems reasonable to assume that many other types of organic compounds can also permeate the roots of plants in the same way. This is a vitally important point, because only for this type of permeation process does it seem possible to find rules which are generally applicable.

Relation between physico-chemical properties and root permeation

The hydrophilic/lipophilic balance of a molecule (HLB) was as early as 1900 described in literature as a physico-chemical quantity which has a very marked effect on the permeability in plant cells. This HLB may be defined as the ratio of the affinity of a substance for a more polar medium (e.g. water) to its affinity for a more apolar medium (e.g. ether or olive oil). A measure of this ratio is the distribution coefficient between, say, water and ether. In order to permeate into a cell the substances have to pass through the cell membrane. The observed effect of HLB on permeation led at the time to the assumption that the cell membranes must contain large amounts of lipophilic substances, in this case lipids. This hypothesis was later confirmed by analysis of the cell membranes.

Fig. 4 shows the permeation of neutral aromatic sulphones of the type

![SO₂R₂]_

in the exudate, plotted against a measure of the HLB. No relationship is found whatsoever. However, when the results of those compounds which are not biochemically stable are omitted, a rough relationship can be seen (fig. 5). This illustrates how necessary it is to keep stability and permeation data separate when interpreting experimental results.

Just as with separate plant cells, the HLB of the molecules obviously plays an essential rôle in permeation in the much more complex systems of tomato roots. With other groups of substances and for the root systems of other plants similar relations have been found to exist between permeation and HLB parameters. These results lead to the conclusion that it is possible to formulate physico-chemical rules governing the permeability of (neutral) organic compounds.

Biochemical stability in plants

Method

We have already seen how the stability of a substance in the roots of a plant can be determined. To determine the stability of a substance in the plant as a whole a

different method is employed, which we shall illustrate by describing experiments carried out with broad bean plants. The experimental arrangement is shown in fig. 6. The plants are placed with their roots in a solution in water of the substance under investigation; the uptake of the substance is governed by the transpiration mechanism previously described. To obtain comparable results all experiments were performed in identical conditions of temperature, relative humidity and illumination, since these experimental conditions affect the evaporation of water from the leaves, and therefore the amount of water and substance taken up. The conditions are chosen in such a way that about half of the external solution is taken up during the course of the experiment, giving the best situation for obtaining quantitative results. By extraction of roots, stems and leaves, and analysis of the extracts, it is possible to determine the stability of the relevant substance in the

Fig. 4. Permeation of aromatic sulphones through tomato plant roots. The logarithm of the distribution coefficient between a polar and an apolar solvent is shown on the horizontal axis; the vertical axis indicates the saturation concentration in the exuded sap divided by the initial concentration in the external solution.

Fig. 5. As fig. 4, but excluding the biochemically unstable compounds.

Fig. 6. Experimental arrangement for determining the stability of the chemical substances in plants (broad bean). When the plant has taken up about half of the external solution, roots, stems and leaves are extracted and analysed. The stability of the substance in the plant is expressed as the percentage ratio of the amount of substance found in the plant to the amount of substance taken up by the plant.
plant. This is generally defined as the ratio of the quantity of substance found in the plant to the quantity of substance taken up by it. The accuracy achieved is between 5 and 10%.

Stability of basic structure and substituents

In systematic research on a particular class of organic chemical compounds structural changes in the molecule are generally introduced only in one place at a time. In aromatic and heterocyclic compounds, for example, the ring system and all substituents except one are left unchanged; this part of the molecule might be called the basic structure. This basic structure may show considerable instability in some types of compounds. For instance, in all compounds investigated of the type R-O-CSNH₂ (thiobenzamides) the stability in the external solution in the exuded sap experiments proved to be less than 50%, and it is even lower in the intact plants. Further investigation has shown that this is due to the marked instability of the thioamide group, which is found in even simpler molecules such as CH₃CSNH₂ (methyl thioamide).

In various other classes of organic compounds the basic structure is stable, so that experiments with substituted compounds can give information on the stability of the substituents. If conversion takes place the nature of the products formed must then be known, for it is of course also possible that the activating effect of substituents can make the basic structure itself unstable.

As an example we shall discuss the group of aromatic sulphones of the type R-O-SO₂CH₃. Table I shows the stabilities of a number of substituted compounds.

<table>
<thead>
<tr>
<th>Substituent R</th>
<th>Concentration of external soln.</th>
<th>Stability in plant (in %)</th>
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<tbody>
<tr>
<td>H</td>
<td>0.25 mmole/l 0.25 mmole/l</td>
<td>95 95</td>
</tr>
<tr>
<td>CH₃</td>
<td>2 mmole/l 2 mmole/l</td>
<td>100 100</td>
</tr>
<tr>
<td>OCH₃</td>
<td></td>
<td>90 90</td>
</tr>
<tr>
<td>Cl</td>
<td></td>
<td>80 80</td>
</tr>
<tr>
<td>CN</td>
<td></td>
<td>90 90</td>
</tr>
<tr>
<td>NH₃</td>
<td></td>
<td>70 70</td>
</tr>
<tr>
<td>NH-CH₃</td>
<td></td>
<td>60 60</td>
</tr>
<tr>
<td>NO₂</td>
<td></td>
<td>70 70</td>
</tr>
<tr>
<td>NH-CO-CH₃</td>
<td></td>
<td>90 90</td>
</tr>
<tr>
<td>NH-PO-[N(CH₃)₂]₂</td>
<td>100 mmole/l 0.25 mmole/l</td>
<td>35 35</td>
</tr>
<tr>
<td>NH-glucosyl</td>
<td></td>
<td>20 20</td>
</tr>
<tr>
<td>OH</td>
<td></td>
<td>0 0</td>
</tr>
<tr>
<td>H₃C = O</td>
<td></td>
<td>0 0</td>
</tr>
<tr>
<td>NO₂</td>
<td></td>
<td>0 0</td>
</tr>
</tbody>
</table>

As can be seen, a few substituents are converted to a considerable extent. These are:
the NO₂ group, which in the first instance is reduced to NH₃;
the (H₃C = O)-group, in which there is presumably conversion to COOH;
the OH-group, where it is assumed, on the basis of similar results in the literature, that there is a conversion into a glycoside;
the NH-glucosyl group, where hydrolysis to the free NH₃ compound has been demonstrated.

On the other hand, with various other substituents such as CH₃, OCH₃, Cl and CN, the stability in the plant is very high.

Interesting behaviour is shown by the NH₂ substituted compound, in which it was found that the stability was strongly dependent on the concentration of the external solution. Upon substitution of the NH₂ group it is found in some cases (α-alkylation, acetylation, phosphorylation), that this dependence can be completely neutralized but in others this result is not found (mono-alkylation, glucosidation).

In order to find out more about the background to these phenomena, an investigation was made into the behaviour of the compound H₂N-O-SO₂CH₃, radioactively labelled with ³⁵S, in broad bean and tomato plants. It was found that about 10% of the substance taken up is converted into glucosyl and acetyl derivatives soluble in water. Another part, however, is bound to polymeric constituents of the plant (not soluble in water) and the extent to which this occurs depends strongly on the concentration used in the experiment. At a concentration of 2 mmole/l in the external solution about 10% is converted in this way, but at 0.25 mmole/l the percentage has risen to about 60%. The latter quantitatively much greater conversion is therefore responsible for the dependence on concentration shown by the stability of the free NH₂ compound. This effect of the concentration is presumably due to the presence of a limited number of active sites at the plant polymers.

On the basis of results obtained and from model experiments it is assumed that the polymeric bonding also involves two types of reaction, one being the formation of a glycoside bond and the other being the formation of an acyl bond (see fig. 8).

When the plants are extracted with boiling water, the free NH₂ compound and the glucosyl and acetyl derivatives are quantitatively extracted. The same applies to the substance bound to the plant polymers by a glucoside bond, because in these conditions quantitative hydrolysis of this bond takes place. The p-aminophenyl-methylsulphone which is bound to the polymer by a
bond of the type RNHCO-polymer, can only however be isolated after extraction using 0.5 mole/l HCl at 100 °C. This provides a neat method of determining the approximate location of this latter bond in the plant by making autoradiographs of the leaves before and after extraction with boiling water. It can be seen from the results presented in fig. 7a and b that this bond is located at or near the walls of the xylem vessels.

With the information obtained in this way it is therefore easy to explain the effect of the various N substituents. Thus, di-alkylation removes the possibility of the indicated reaction, whereas mono-alkylation does not; acetylation, phosphorylation and glucosidation also protect the NH₂ group, but the glucoside is not stable in the plants.

In all cases investigated the basic structure -SO₂CH₃ is found to be unaffected, so that in this case the stability of the para-substituents has in fact been determined. A summary of the results is presented in fig. 8.

The question arises as to whether such stability results found for substituents with the aid of a particular basic structure might possess more general validity.

In organic chemistry it is quite common for the reactivity of a particular substituent group to be affected by the rest of the molecule; on a priori grounds one might expect a similar effect on the reactivity of substituents towards enzymatic systems. Investigations using other stable basic structures have shown that this is in fact the case; the substrate-enzyme interaction or the reactivity of the substrate in the substrate-enzyme complex can be changed by altering electron density or the spatial situation of the substituent.

In addition there is another factor which can affect the stability of a substituent. An example of this is shown in Table II.

![Fig. 7. Autoradiographs of a leaf of a broad bean plant which has taken up H₂N-○-SO₂CH₃ radioactively labelled with ³⁵S, a) before and b) after extraction with water at 100 °C. This extraction removes all the radioactive compounds, except those which are bound by an acyl bond to polymeric constituents of the plant. This enabled the acyl bond to be localized: it appears to occur at or near the walls of the xylem vessels.](image)

![Fig. 8. Possible conversions in broad bean plants of the substituents R in the compounds R-○-SO₂CH₃. If no indication is shown behind a substituent it is stable.](image)
Table II. Effect of the hydrophilic/lipophilic balance (HLB) of the total molecule on the biochemical conversion of the CH₃O-group into a hydroxyl group.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Distribution coefficient water/ether</th>
<th>Stability in broad bean plants after 14 days (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃O-(\text{-SO₂CH₃})</td>
<td>0.27</td>
<td>90</td>
</tr>
<tr>
<td>CH₃O-(\text{-SO₂C₄H₉})</td>
<td>0.02</td>
<td>10</td>
</tr>
</tbody>
</table>

Whereas the electron density and the spatial situation of the CH₃O-group are found here to be virtually equal, the stability is seen to differ very considerably. There is however a great difference in the HLB of the two molecules, which is illustrated by the distribution coefficients given in the table. This effect on the stability of substituents is probably due to subcellular localization of the enzymes that cause the relevant conversions in a part of the cell with high lipid content.

Further research will have to show whether it is possible to obtain a sufficient understanding of the effect of factors we have mentioned to make it possible to predict the stability of substituents in potential pesticides.

Summary. The availability of chemical substances in a living organism is defined and discussed as one of the factors determining their biological action. The approach to the problems in this field is illustrated by considering certain processes that affect the availability of chemical substances in plants. These processes, namely root permeation and biochemical conversion, are discussed with reference to experimental results obtained with aromatic sulphones. The nature of the root permeation process and a relationship found with certain physico-chemical properties of the investigated compounds are dealt with in more detail. It is concluded that it should be possible to draw up general rules for this process. The effect of biochemical stability is discussed in terms of results obtained on the stability of various substituents in phenylmethylsulphone. It is shown that this stability of substituents does not necessarily have the same value in other classes of organic compounds since it is affected by the rest of the molecule.