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PROBLEMS INVOLVED IN STRUCTURE DETERMINATION OF ACTIVE PRINCIPLES OF PLANTS USED IN TRADITIONAL MEDICINE: EXTRACTION, SEPARATION AND DETERMINATION OF CHARACTERISTICS OF ACTIVE PRINCIPLES*

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Summary

A number of examples, mainly from the author's personal researches, give a realistic picture of the complicated methodology and approach to the chemical study of medicinal plants.

Introduction

Extracts of plants are used in traditional medicine, and investigations on their constituents have attracted the attention of many generations of chemists working in the field of organic chemistry of natural products.

There have been remarkable advances in this area during the last two decades and many new biologically active compounds have been discovered.

With modern advances in the techniques for isolation and structure determination of active principles, even minute amounts of them can be isolated and their structure determined.

This paper is concerned with the problems involved in structure determination of active principles of plants used in traditional medicine, and I intend to illustrate the theme, amongst others, with examples of my own research.

When one works in the field of organic chemistry of natural products, the choice of a research project tends to be concentrated on biologically active components in plant or other material, the structure of the active principle(s) in most cases being unknown. The properties of these active principles are, of course, unknown too, apart from facts obtained by hearsay!

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Successful research on such a topic requires in the first place the isolation of the active principle(s), guided by an appropriate bioassay, which in general is laborious. Each step of the isolation and purification procedures has to be followed by biological screening. Such a test will usually be concentrated on *one* of the biological activities.

I. In 1949 I started to work on the isolation of muscarine from the fly agaric (*Amanita muscaria* L), the component which at that time was considered to be the most important active principle in the mushroom.

From previous research (dating from 1811!), the following facts were known:

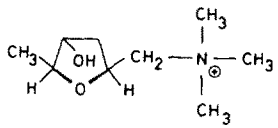
(1) the fly agaric, a source of muscarine, grows in a very irregular manner, its growth depending to a great extent on the climatological conditions, and its collection being restricted to a very short period of the year;

(2) the isolation procedure was guided by testing the fractions during the isolation and purification steps on an isolated frog heart, measuring the reduction of the heart amplitude after injection of a sample of a newly obtained fraction.

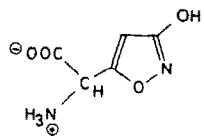
One of the complications of such a test was not only the difficulty of obtaining frogs during the whole year, but especially the changing sensitivity of the frog heart, depending on the period of the year.

A more general point to be mentioned here is how to start (or not to start) the isolation procedures. Very often one is inclined to follow the pathway(s) of other researchers, when work has already been done on the topic.

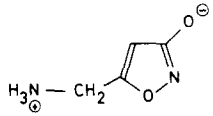
So we started to collect the fly agaric mushroom, putting the specimens immediately in ethanol — as has been done before — and thereafter treating the ethanol/water extract with ether, which was followed by many other purification steps. We managed to work out a reasonable pathway for the isolation of muscarine, but if we had started instead to extract the mushrooms with water, we would have isolated the more important biological factors (see references), ibotenic acid and its derivative muscimol:



(+)-(2S, 3R, 5S)-muscarine;



ibotenic acid;



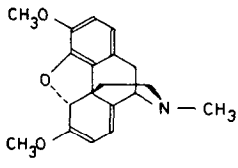
muscimol.

If the structure type of the active principle(s) is not known, one should take all precautions to avoid loss of activity due to structural deterioriation and try to find out under which conditions it will be possible to work: in acid or basic surroundings, under the influence of heat, oxygen or light, *etc.*

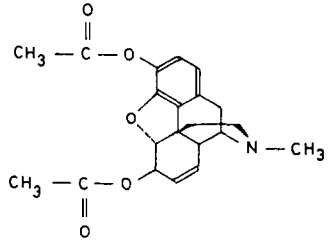
II. Research on drugs — in our case on *Papaver somniferum*, *Papaver bracteatum* Lindley and *Cannabis sativa* L. — involves, as well as the above-mentioned problems, several other ones.

Our start in the *Papaver* field originated from a project initiated by the Department of Narcotics of the United Nations in Geneva in December 1972. The proposed idea was to replace *P. somniferum* — the main source of morphine from which codeine should be made — by *P. bracteatum*, a newly (re)-discovered plant growing wild in the mountains north of Teheran in Iran. *P. bracteatum* did not produce the alkaloid morphine but the related alkaloid thebaine.

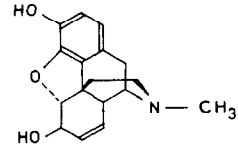
The hope was that, through culturing *P. bracteatum* instead of *P. somniferum*, one could decrease or avoid, for obvious reasons, the easy preparation of heroin from morphine, as it is hardly possible to synthesize heroine from thebaine.



Morphine



Heroine



Thebaine

When we started with this research project in 1973 it was my honest opinion that most of the chemistry of the opium alkaloids, since the isolation of morphine in 1817 by Sertürner, was already known, but to my surprise this was not true.

The main reason for this — and it applies to any other similar research field — is that the physical methods of structure determination only became available after 1940. In the last twenty years, however, they have virtually supplanted the chemical approach.

Physical methods have several advantages. Observations can be carried out on very small samples (~ 1 mg or even smaller) of material and these

are in general not affected by the observations, as they are in chemical reactions, and hence may be re-used. Furthermore, these observations and their interpretations take far less time than the pure chemical degradation reactions during the structure elucidation.

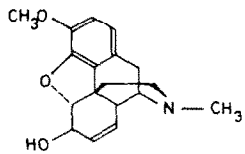
In the nineteenth century considerable effort was expended in tabulating physical constants of known compounds in the hope that such lists might be rewarded by some insight into correlations with structural features. Such attempts were made with all physical measurements available, such as melting point, density, index of refraction, *etc.*, and even with the tabulation of crystal facet angles.

However, these searches all failed to yield significant structural correlations since the physical datum in each case was a single number, which encompassed a complex mixture of responses to many structural features at once and these could not be unravelled.

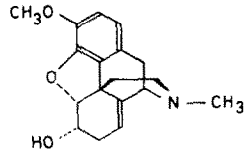
Structure correlation became possible only with methods that produced a rich spectrum of separate responses from a single compound such that each particular response might be caused by a single structural feature. The contemporary physical methods of this kind are the following:

ultraviolet and visible light spectroscopy (UV and VIS), the oldest methods, already used before 1940; infrared spectroscopy (IR); nuclear magnetic resonance spectroscopy (NMR); and mass spectrometry (MS).

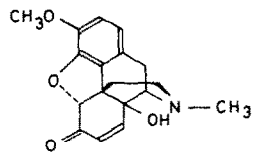
With the application of these new methods in the *Papaver* field, combined with refined modern isolation procedures, we were able to isolate several new alkaloids from *P. bracteatum* and elucidate their structures (one should keep in mind that this will be possible in other older research fields). We found the following alkaloids, present in minute quantities:



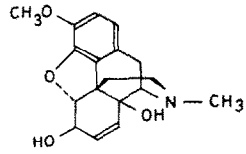
Codeine



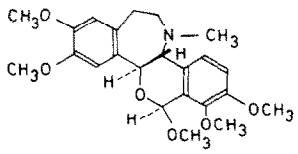
Neopine



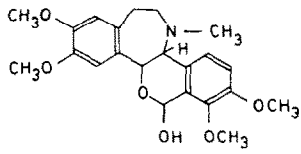
14-β-Hydroxycodeinone



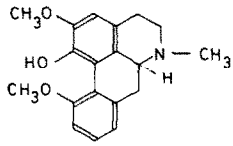
14-β-Hydroxycodeine



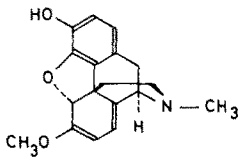
Alpinine



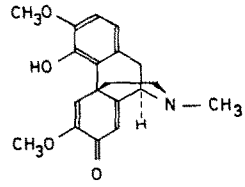
Alpiniginine



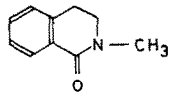
Isothebaine



Oripavine



Salutaridine



N-Methylcorydaldine

Several other alkaloids have been isolated and their structures are under discussion.

The isolation of the above-mentioned minor alkaloids was preceded by the difficulty of cultivating the wild *Papaver bracteatum*, which, however, is successful if the young plants survive the first year.

An extra goal during our research was to find out if it is possible to increase the alkaloidal content of the plant.

The isolation problems were the following:

(1) the very small quantities of alkaloids to isolate;

(2) the fluctuating quantities of those compounds during the day and night cycle — it is usually not known at what time one should collect the plant material in order to obtain as much of the alkaloids as possible.

As the structures of minor alkaloids could be established only by spectroscopic methods, it was necessary to obtain a definite structure proof by synthesizing reference compounds. This had the advantage that larger quantities of the material became available for pharmacological research.

III. Isolating components from material which is biologically active — according to folklore — has to be guided by bioassays. But what kind of activity is one looking for? It is my strong belief that too many aspects of biological activity are overlooked; as we remember, for instance, all those substances which have been recommended for this or that therapeutic effect and which prove after all to be carcinogenic!

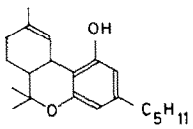
Too often scientists are working in too narrow a field, overlooking or not willing to see facts which would not be ignored if a multidisciplinary approach is chosen.

Let me illustrate this with the present *Cannabis* problems.

The chemical research in the *Cannabis* field presents special aspects owing to the psychotropic activity of the material! It can be divided into three periods.

The first period can be characterized as the one in which pharmacological research was executed without knowledge of the chemical composition of the *Cannabis* material. This explains the confusion with regard to the biological properties as *Cannabis* material is extremely variable.

After the isolation of the main psychotropic component Δ1(2) tetrahydrocannabinol,

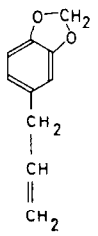


its structure elucidation and the synthesis thereof, the second period started, in which practically all the scientific research was concentrated on this component!

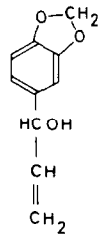
The third period, which is not yet ten years old, promoted the research on the interaction of the cannabinoids and the analyses of *Cannabis* smoke, as in general the material is smoked! The researcher is confronted with a very large number of components, many of them in trace quantities, the isolation of which requires refined analytical equipment and great skill.

The use of *Cannabis* should be reevaluated because one fact has been completely neglected: *Cannabis* smoke is a complex mixture of several hundreds of components, each of which can possess a specific biological activity of great potential benefit for the medical sciences.

IV. Other special methods and accompanying difficulties can be mentioned, depending on the type of research field on plants in traditional medicine. We have been working on the isolation of phytotoxins, where a real bioassay could only be performed on living trees during six weeks of the year, a not very attractive situation, which illustrates that one has to look for other test possibilities. The isolation and structure determination of an active plant component, followed by a thorough research on its pharmacological properties, also includes the identification of possible metabolites in the human body which might have more dangerous properties than the mother substance, as has been shown recently, for example, with one of the metabolites of safrole, 1′-hydroxysafrole (see references), which is much more hepatotoxic than safrole itself.



Safrole



1′-Hydroxysafrole

Conclusions

Finally, I should like to summarize the following aspects and problems:

(1) The present refined equipment and techniques make it possible to analyze the chemical composition of plant material. With regard to biologically active plants, this should be guided by proper bioassays (Table 1).

(2) Collaboration of scientists of different disciplines is necessary, in order to obtain better and more elaborate results in the field of plants used in traditional medicine (Table 2).

(3) It should be kept in mind that the results of well-known topics could be enlarged by newer methods and techniques.

(4) Research on the relation between structure and biological activity should be promoted.

TABLE 1

Chemical research on higher plants

1. Taxonomical identification
2. Literature survey on chemistry and medicinal use
3. Phytochemical investigation
Analysis of (a) alkaloids
(b) glycosides or saponines and their aglycones
(c) bitter principles
(d) volatile oils (terpenes)
(e) fixed oils, fats and waxes
(f) low molecular weight proteins (amino acids)
(g) other constituents
combined with
pharmacological testing in as many directions as possible

TABLE 2

International organization

Co-ordination of all activities of research institutes
Organization of meetings of specialists
Promotion of a card index of a detailed bibliography of research on plant drugs and their applications
Investigation and promotion of applied and directed research for immediate and long-term application

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