THE IMPACT OF STUDIES OF NATURAL PRODUCTS ON CHEMICAL INDUSTRY

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I regard it as a great honour to be able to speak to you at this opening ceremony of the Symposium on The Chemistry of Natural Products. I would like to express my sincere and heartfelt thanks to the Australian Academy of Science, and especially to the Organizing Committee, for the invitation which they extended to me and which I accepted with great pleasure.

This is my first visit to Australia, and I am delighted to have the opportunity of becoming personally acquainted with your country, for I have heard so many pleasant and charming things about Australia and its people.

Until recently, Australian economic life was confined mainly to agriculture. Now it has entered a new phase, and far-reaching industrialization is developing rapidly. That may well be one of the reasons why the Organizing Committee invited me, as a man who has studied natural products for fifty years and who has been manufacturing some of them on an industrial scale for forty years, to illustrate the chemistry of natural products more from the practical, that is, from the industrial side. In this short address I can give only a few examples from an immensely large field, for almost the entire organic chemical industry originated in the study of natural products.

One of the oldest branches of this industry, dating back one hundred years, is the dyestuffs industry. Until the middle of the nineteenth century, indigo and alizarin, both of vegetable origin, were the most important materials used for the dyeing of textile fibres. Baeyer worked for decades to elucidate the chemical constitution of indigo. In 1878 he achieved the first synthesis of indigo, but we know from another synthesis which he carried out subsequently that the process involved was wasteful. In 1890, Heumann of Zurich performed the first technically usable synthesis of indigo, based on the work of Baeyer.

A similar course of events was followed in the case of the other important natural dyestuff, alizarin, the chemical constitution of which was elucidated by two pupils of Baeyer in 1868. These two young chemists, Graebe and Liebermann, also succeeded in synthesizing alizarin.

Even the first artificial dyestuff, mauveine, which was prepared in 1856 by the extremely gifted chemist Perkin when he was seventeen years old, owes its origin indirectly to a natural substance. Perkin's teacher, Hoffmann, of the Royal College of Chemistry, suggested to him that he should attempt to synthesize quinine, which was highly valued because of its beneficial effects on malaria. In his preliminary studies Perkin discovered mauveine. This provided a basis for the world-wide development of the dyestuffs

industry; the greatest stimulus was, of course, given to the so-called " benzol chemistry ", and this engaged the interest of most chemists until well into the twentieth century.

At that time, investigations on natural products played a minor rôle, even though the profound investigations of Fischer and his school on proteins and the amino-acids from which proteins are built up, and on carbohydrates, were of great scientific importance. These investigations did not find any direct industrial application. They must be regarded, together with investigations on natural rubber, as important preliminary work that has since become of major significance to industry, namely for the manufacture of high polymers in the synthetic fibre and plastics industries of today, which have developed on such a colossal scale.

A new era in the study of natural substances was initiated early in this century by the investigations of Willstätter and his school on chlorophyll. Although chlorophyll, the colouring matter of leaves, had long been known to research workers, and its fundamental importance in the conversion of carbon dioxide in the air into organic substances under the influence of light was realized, the investigations on this widespread and important colouring matter did not lead to clear-cut results until Willstätter began his studies, for the usual chemical methods of investigations produced profound changes in the chlorophyll molecule. As long as the chlorophyll in leaves (like the blood pigment in haemoglobin) is bound to high-molecular-weight substances such as proteins, it is perfectly stable and resistant to light and to weak acids, but these can easily destroy the dyestuff dissolved out from leaves. Alkalis, oxygen from the air, and even enzymes induce changes in the chlorophyll molecule.

Chlorophyll plays a very minor rôle in industry, but it was from chlorophyll that we learned and developed many of the methods necessary for the preparation of highly sensitive substances, for chemical changes in chlorophyll could be easily observed with the naked eye or with the spectroscope. A further complication in the investigations on chlorophyll was the fact that the leaf colouring matter is not a uniform substance but consists of two yellow components, carotene and xanthophyll, and two green components, chlorophyll *a* and chlorophyll *b*. The two chlorophylls are very similar in composition and properties. It was not possible to use chemical methods to separate them because of the risk of decomposition. Only with the aid of physical methods, *e.g.* chromatography—as used first in 1912 by Tswett—or separation between non-miscible organic solvents, was it possible to obtain the pure components, chlorophyll *a* and chlorophyll *b*; the latter procedure was later developed systematically and led to Craig's counter-current apparatus.

From this example of chlorophyll, we learned how to purify and isolate sensitive natural products by means of physical methods and without using chemicals, not only on a laboratory scale but also on an industrial scale. The studies on chlorophyll revealed that enzymes may occur in living cells which break down, to some extent, the natural products which one is looking for. Thus chlorophyll is broken down by chlorophyllase, an enzyme which splits off the phytol ester group if chlorophyll is slowly extracted with an organic solvent.

The observations made during the study of chlorophyll proved of great assistance to us in the isolation of the cardiac glycosides. We were able to recognize that enzymes were splitting off sugars from the native glycosides during the extraction processes. This cleavage could be prevented if we wanted to isolate the glycosides in their native state. Alternatively, it enabled us to subject the latter to controlled, step-wise enzymatic degradation.

In his investigations on chlorophyll, and later on anthocyanins and enzymes, Willstätter followed a principle of his teacher, Baeyer. He writes about this in his autobiography[1] and reports that Baeyer said: "What are the distinguishing features of the great scientist? He should not govern but listen, he should adapt himself to what is heard and re-model himself on it. . . . This is what the old empiricists did: they lent an ear to Nature. The modern scientist does the same and I have also tried to do it. A very special influence is exerted on men when they approach nature. They develop quite differently from someone who approaches nature with a preconceived idea." Willstätter himself remarked: "In these words Baeyer associates himself with Francis Bacon's view that Nature is not to be governed except through obeying her."

The chemistry of natural products and its industrial exploitation underwent an expansion that could hardly have been foreseen, following the research work carried out on hormones, vitamins and antibiotics in the nineteen-thirties and nineteen-forties.

Hormones are the products of internal secretion glands, and are carried in the blood to the effector organs where they exert the most diverse effects. With the exception of a few very simple hormones such as adrenalin and serotonin, hormones are generally complicated substances. The great majority, such as the adrenocortical hormones and the sex hormones, are derived from steroids and can be prepared, on a technical scale, from readily available substances by chemical treatment, by partial or total synthesis.

Many other hormones such as insulin, oxytocin and vasopressin are polypeptides. The composition and the structure of insulin, the anti-diabetic hormone, have been elucidated by the brilliant work of Sanger. The total synthesis of insulin, which consists of fifty-one amino-acids arranged in two parallel chains, has not yet been effected. In preparing insulin today we are still dependent on the pancreas for our starting material. Nevertheless, it has proved possible to synthesize on an industrial scale simpler polypeptide hormones, such as oxytocin and vasopressin, which consist of eight amino-acids arranged in a ring.

The chemistry of the vitamins forms one comprehensive chapter within that of the natural products. Vitamins are so-called additives to nutrition, which are indispensable factors for growth and the normal metabolism of human and animal organs. As long ago as the beginning of the twentieth century they were considered to be of vital importance, and were therefore given the name vitamins in 1911 by Funk. However, twenty years elapsed before they were isolated and their constitutional formulae were elucidated. In 1931, Karrer obtained a highly effective concentrate of Factor A and was able to determine the constitution of Vitamin A. This was the first determination of the chemical structure of any vitamin. In 1933, Haworth

and Reichstein synthesized Vitamin C. In 1935, Kuhn and Karrer synthesized lactoflavin (Vitamin B_2). In 1936, Williams synthesized aneurin (Vitamin B_1). In the same year, Brockmann isolated Factor D, the anti-rachitic factor, and ascertained that it was identical with a cholesterol derivative obtained by Windaus at almost the same time.

Subsequently, whole series of vitamins were elucidated. Kuhn succeeded in synthesizing lactoflavin-5-phosphoric acid, which combined with Warburg's protein components to form the fully effective " yellow enzyme ". This provided proof that a vitamin acts as a prosthetic group of an enzyme.

Only in the last decade has it proved possible to elucidate the structure of the important anti-anaemic principle, Vitamin B_{12}. This was prepared in a crystalline form almost simultaneously in England and in the United States in 1948. More detailed investigations were then carried out by the Folkers group in the Merck organization and by Todd and his associates in the University of Cambridge. The main portion of the molecule has a porphyrin-like structure with cobalt, built in a complex bond. The formula was elucidated with the aid of X-ray studies performed in Hodgkin's laboratory at Oxford.

In view of the great importance of vitamins to human beings and to animals, it is not surprising that, with the exception of B_{12}, they are prepared on a large scale by total synthesis. The value of production of vitamins for the United States in 1958, announced recently by the Census Bureau, was 300 million dollars at manufacturers' level[2].

Production figures are even higher for the antibiotics. Biologists, biochemists and industrial chemists have been feverishly active in the last decades, an activity that has not previously been experienced in the field of organic chemistry. You know the story of the discovery of penicillin, the first antibiotic, by Fleming in 1929, and its isolation, associated with the first clinical trials, by Florey and Chain in Oxford some ten years later. The sulphonamides, which derive from purely synthetic dyestuffs chemistry, had already been successfully employed in the treatment of many infectious diseases, but with the coming of penicillin, a new era began in this important branch of medicine. A search was then made for further antibiotics, countless soil samples being studied for strains of fungi and bacteria. As a result, a great number of substances inhibiting the growth of pathogens were isolated. However, only a relatively small number met the requirements of high potency against pathogens and relative absence of toxicity. Some examples, in addition to penicillin, are streptomycin, its dihydro-derivative, and tetracycline.

The chemical structure of most antibiotics has been elucidated. They have been found to be relatively low-molecular-weight compounds, although in some instances they have a rather complicated structure. Consequently, even in those instances where synthesis has been effected on a laboratory scale, no attempts have been made to synthesize them on an industrial scale. This work is left to the skilful micro-organisms; a suitable composition of the nutrient substrates and favourable physical conditions, such as temperature and ventilation of the cultures, leads to optimum development of the antibiotics. The growth of the micro-organisms takes place in culture

tanks containing up to 400,000 litres, such as are used in the United States. The antibiotics are isolated in various ways from the cultures and then prepared in crystalline form.

The antibiotic sales of many pharmaceutical firms amount to as much as 50 per cent of the annual turnover. The United States production of antibiotics for human use in 1958—announced by the Census Bureau—amounted to 362 million dollars at manufacturers' level[2].

Antibiotics, vitamins and hormones are products of research in recent decades. By contrast, the use of medicinal plants is much older. These have been used by man for some thousands of years. Until the middle of the nineteenth century they were, with few exceptions, the only material available for treatment and prevention of diseases. Throughout the middle ages the *materia medica* of plant origin seemed to be far more important and more respectable than, for instance, surgery. They were subject to the very strict regulations of the medical faculties.

It was, therefore, a natural development at the beginning of the nineteenth century, when modern chemistry and pharmacy began to develop, to study medicinal plants. Sertürner recognized and isolated morphine, the hypnotic and anaesthetic principle of opium, in 1805. Subsequently a whole series of other plants was investigated to detect similar products. Some of them were found to be complicated organic compounds of a basic nature. In view of their alkaline properties, which are due to their nitrogen content, they were called alkaloids. Many of them were shown to be the actual therapeutic agents, *e.g.* quinine from *Cinchona* bark used in the treatment of malaria, atropine from *Atropa belladonna*, and many others.

In more recent times, reserpine has been obtained from *Rauwolfia serpentina*, and the curare alkaloids have been isolated from South American poisonous plants or plant juices which the natives use as arrow poisons. Not all alkaloids, and there are some hundreds of them, have proved to be useful drugs, but many have become so well established in the medical armoury that they are used every day by doctors.

Until well into the nineteenth century, the preparation of drugs from plants or animal organs was exclusively the responsibility of the pharmacist. At first, extracts, and later pure substances, were prepared on an ever-increasing scale as the demand grew; finally, they were prepared in small factories, and thus began research on the rational preparation and chemical elucidation of the active principles. It was realized that the active contents varied greatly with the quality, particularly with respect to the origin and storage of the raw material. Some active principles decompose on storage, so that the only way to provide the doctor with a reliable drug is to isolate the pure substance so that it can be dosed according to weight, thus excluding variations in effectiveness. Only when the pure material is available is it possible to carry out chemical analysis and elucidate the constitution; then synthesis can be attempted.

Many of the plant alkaloids which are of importance in medicine today are obtained from hundreds of tons of plant material, either growing wild or, more recently, specially cultivated. The beautiful, crystalline, pure substances are then processed to make tablets, oral solutions, syrups, ampoule solutions, *etc.*

The vegetable kingdom provides us not only with alkaloids but also with many other highly active substances, for example, the cardiac glycosides obtained from *Digitalis* species, the therapeutic effect of which was first recognized by the English doctor Withering in 1785. It was not until 1859 that the French pharmacist Nativelle succeeded in isolating one of these glycosides, " digitaline cristallisée ". Today there are a great number of such cardiac glycosides of plant origin. However, it was shown that all the cardiac glycosides obtained in crystalline form up to 1930 were actually products obtained by enzymatic cleavage of the native glycosides. Today, the original, native glycosides are prepared on a large scale in industry. Some are subjected to enzymatic cleavage. In view of the increasing importance of cardiovascular diseases, these preparations are becoming indispensable. A change-over has, therefore, been made to selecting plant strains which are rich in glycosides, and cultivating them on a large scale so that we are no longer dependent on the wild plants as a source of raw material. Earlier, similar attempts at cultivation were made on a large scale in Indonesia with respect to *Cinchona*. For instance, various species were crossed in order to obtain bark which was rich in quinine.

The preparation of drugs is therefore not only of industrial but also of agronomical interest.

The vegetable kingdom of Australia[3] and the neighbouring islands, particularly those in the tropics, certainly includes as yet unknown natural products which may be used for therapeutic and other purposes. Australian research workers have already been active, and with success, in this field. The lectures by Dr Price and by Dr Mathieson will certainly provide us with interesting data on this work.

In this connection, I would like to refer to the recent working-up of species of *Rauwolfia*. It was *Rauwolfia serpentina*, which is found particularly in India, that was used for centuries in folk medicine " for all the ills of man ".

It was used for epilepsy, insomnia and insanity.

It was prescribed for dysentery, diarrhoea and cholera.

It was considered valuable in headaches and blindness.

It was a standard treatment for a wide variety of fevers.

It was applied as an antidote for insect bites and snake bites.

This last indication, " an antidote for snake bites " was mentioned to me as late as 1952 in Madras. It may well be that the popular belief that *Rauwolfia* was a panacea was responsible for the medical schools' refraining from using this plant for such a long time. Nevertheless, in the 1930's, Indian doctors were able to establish beyond doubt that the powdered root of *Rauwolfia serpentina* can be used successfully in states of excitement and in hypertension. Attempts were made to find the active substances and a great number of alkaloids were isolated from the various species of *Rauwolfia*. Unfortunately, the first alkaloids isolated some thirty years ago by Siddiqui and Siddiqui: ajmaline, ajamalinine and ajmalicine, as well as serpentine

and serpentinine, possessed no interesting pharmacological properties. Some twenty years, therefore, elapsed before Schlittler and his associates succeeded in isolating three highly potent alkaloids. One of these, reserpine, is effective in states of excitement and high blood pressure in human beings when given in fractions of a milligram. The action of reserpine is hypotensive and sedative. Schlittler also succeeded in elucidating the chemical structure of reserpine and other *Rauwolfia* alkaloids. The quite recently reported synthesis of reserpine by Woodward is a brilliant achievement. Nevertheless, it is still more economical to obtain the alkaloids from the roots of *Rauwolfia* than to obtain them by synthesis. It is estimated that the value of the annual production of reserpine is 20 million dollars. Use is made not only of *Rauwolfia serpentina* from India but especially of *Rauwolfia vomitoria* from Africa and *Rauwolfia tetraphylla* from Central America.

To conclude my address, I would like to outline my personal experience of investigations in one particular field and its industrial exploitation.

When rye is flowering, the naked seed buds can be infected by filamentous spores from a fungus. Instead of the grains of rye you then have proliferations, which, when the rye is mature, project from the ears as dark-brown horn-shaped sclerotia. These are the mycelia of the fungus *Claviceps purpurea*, the form in which the fungus spends the winter in the ground. In the spring the sclerotia germinate and spores are formed which infect the rye.

The brown horn-shaped sclerotia are best collected before the rye is harvested. For centuries they were used in popular medicine under the name " Secale cornutum ". They were mentioned for the treatment of post-partum haemorrhage by the Frankfurt doctor Lonicerus in his herbal book published as long ago as 1582. It was not until Stearns, an American doctor, published in 1808 a paper entitled " Account of the Pulvis Parturiens, a Remedy for Quickening Child Birth ", that the drug was used in medical schools. The chemists then attempted to isolate the active principles but were unsuccessful for a long time. In 1875, the French pharmacist Tanret was able to isolate the first alkaloidal preparation, " ergotinine cristallisée ".

At the beginning of this century, Barger and Carr, and the Swiss pharmacist Kraft isolated ergotoxin, but this product was scarcely used in medicine. One reason for this was, as I shall show later, that the ergotoxin preparations were not uniform, their composition varying greatly. On the other hand it was known that the effectiveness of extracts from ergot was subject to extraordinary variations, depending on the origin and the age of the raw material and the decomposition of the active principles during storage. The use of weak or inactive ergot preparations could be disastrous in post-partum uterine atony. The first task, therefore, was to isolate the active principle in a pure form so that it could be dosed according to weight. This task was fundamentally completed in 1918, when it proved possible to isolate, in crystalline form, ergotamine, a highly active alkaloid. Soon the alkaloid was prepared on a technical scale. The experience gained in the studies of chlorophyll facilitated the isolation of the highly sensitive substance ergotamine. Subcutaneous injections of $\frac{1}{4}$ mg of ergotamine elicit a rapid and long-lasting contraction of the uterus, thus stopping life-endangering haemorrhage.

When the pure substance was available, it was possible to analyse it chemically and elucidate its structure, and to attempt synthesis. Actually no total synthesis has yet proved successful. We still have to rely on the production of the raw material by a fungus, as we did at a time when we knew nothing of the production of antibiotics by fungi.

When the pure substance ergotamine was available, it was possible to carry out reliable pharmacological and clinical trials. It was found that ergotamine exhibited to a marked degree the antagonism to adrenalin which Dale had detected with ergotoxi preparations at the beginning of this century. Ergotamine not only exerts a constrictor effect on smooth muscle fibres, *e.g.* of the uterus, but it also exerts a marked effect on the autonomic nervous system, a so-called sympathicolytic effect. In the course of time, it has been widely used in internal medicine and neurology, for instance in migraine, in gastro-intestinal atony, in thyrotoxicosis, nervous tachycardia and, in combination with belladonna and phenobarbitone, in the treatment of autonomic imbalance, *etc.*

Later, a whole series of active principles similar to ergotamine were isolated from ergot. For instance, ergosine was isolated by Smith and Timmis in 1936, and then alkaloids of the ergotoxin group, ergocristine, ergokryptine and ergocornine, thus showing that ergotoxin was a variable mixture. In 1935, a more simple, water-soluble alkaloid, ergometrine, was discovered in four laboratories in England, the United States and Switzerland. Ergometrine exerts a rapid but somewhat transient contractile effect on the uterus but does not have any significant effect on the autonomic nervous system.

Within the scope of this short address, it is not possible to go into detail on the investigations, which stretched over some decades, on the structural elucidation and partial synthesis of the ergot alkaloids. However, I must mention something that is also of industrial importance: by making modifications in the molecule it is possible to obtain substances with new pharmacological properties. In this connection I am thinking of a passage from Sir Alexander Todd's Nobel Prize address: " At Oxford I had the privilege of working on the anthocyanins and other natural colouring matters with Robert Robinson. It was from him that I learnt that synthesis is not merely a means of confirming a structure arrived at by degradative studies. Synthesis and degradation are complementary tools in structural work, and, properly used, synthesis can serve not only in the elucidation of structure, but can in many instances open up wider vistas regarding the significance and function of biologically important compounds."

The heterocyclic ring system of lysergic acid, which is common to all ergot alkaloids, contains a readily hydrogenated double bond. When hydrogen is added to this double bond by catalytic hydrogenation, all the ergot alkaloids lose almost entirely their contractile effects on smooth muscle. Ergometrine becomes inactive, but the alkaloids of the ergotamine and ergotoxin type, in which lysergic acid is associated with a polypeptide residue, exhibit more marked and pure effects on the autonomic nervous system. The sympathicolytic action is enhanced. The vasoconstrictor action is replaced by a vasodilator action which is of beneficial effect in peripheral and cerebral vascular disorders. One consequence is subjective

improvement in hypertension due to increased cerebral oxygen uptake, which may or may not be accompanied by a fall in blood pressure. This property is most pronounced in the case of the hydrogenated alkaloids of the ergotoxin group.

The structure of ergometrine is simpler than that of the other alkaloids. In ergometrine there is an amide-like linkage of lysergic acid with an amino-alcohol, aminopropanol. If the amino-alcohol is replaced by a diethylamide group we obtain one of the most potent substances known. Even one-thirtieth of a milligram taken by mouth elicits, after thirty minutes, marked mental changes, such as occur in schizophrenia, accompanied by hallucinations, *etc.* The extraordinarily violent effect of lysergic acid diethylamide (LSD-25) is an obstacle to the more widespread use of this substance in therapeutics. At the moment its use is confined to psychoanalysis.

The aminopropanol group in ergometrine can be replaced by aminobutanol. This leads to a methyl homologue of ergometrine with a markedly enhanced and longer-lasting contractile effect on the smooth muscle of the uterus.

On the other hand, if the hydrogen on the indole nitrogen of the lysergic acid moiety of ergometrine is replaced by a methyl group, we obtain the most potent antagonist of serotonin, a neurohumour normally occurring in the body. The therapeutic possibilities of this synthetic derivative have not yet been fully elucidated. Nevertheless, it has proved in some cases to be more potent than adrenocorticoid hormones.

The marked widening of the range in which the ergot alkaloids and their derivatives are used resulted in a significantly greater demand for the raw material, so much so that supplies were inadequate. A change-over therefore had to be made to the artificial infection of rye fields. This was initially done by hand, and later by special machines. In his book[4], Barger estimated the world consumption of ergot to be 100 tons. The requirements today for pure preparations are many times as great, even though we use raw materials with a higher alkaloid content and protect the sensitive substances against degradation.

In previous centuries and even until quite recently, ergot, appearing as a poisonous impurity in cereals, was often the cause of serious large-scale poisoning of the population. By means of exemplary co-operation between chemical, pharmacological and medical research, ergot has evolved as the essential basic material for the production of important drugs, and today it must be cultivated in order to satisfy the needs of the industry and medicine. An enemy of man has been transformed into a friend.

Studies of natural substances not only increase and deepen our scientific knowledge, but also provide a basis for a highly developed industry which provides countless people with their daily bread, and helps to raise living standards and to treat or prevent disease. In this way excellent services are rendered to humanity. One of the most noteworthy endeavours of scientific research is to give the benefits of its splendid achievements to ever wider circles of the world's population. The Symposium which begins here today will also assist these endeavours handsomely.

References

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