

Chromatography of Natural Substances: An Introduction

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Chromatography of natural substances is an obvious subject for a special issue: after all, chromatography started with the investigation of complex natural substances, well before their chemical composition was known: conversely, without the use of chromatography the complex nature of these substances and the existence of large number of closely related compounds of similar composition could have taken much longer to discover. In this introductory article I shall briefly survey the most important fields where the symbiosis of chromatography and the investigation of natural substances resulted in significant achievements.

Plant Pigments

As our readers are aware, one hundred years ago, the aim of Mikhail S. Tswett, the young Russian botanist, was the study of chlorophyll in its natural form, as it exists in living plants, and not in the chemist's way, by breaking it to fragments and groups. In fact he has also stated that some of the seemingly pure forms shown by other scientists are actually artifacts. Tswett then developed chromatography in order to be able to isolate the native chlorophyll, separated from other substances present in leaves and algae. He also demonstrated unequivocally the existence of two major types of chlorophyll (a and b), and he also indicated the possible existence of a third green pigment that we call today chlorophyll c. This achievement would have been impossible without his newly developed method. Thus, the birth of chromatography and the start of the serious study of complex natural substances are closely related (1,2).

Tswett also pioneered in the study of the other important plant pigments, the carotenoids. At his time, the general knowledge of these substances was ruddy: they were aware of only two types, a hydrocarbon ("carotene") and one containing oxygen in its molecule ("xanthophyll"), but no further information was available. It was Tswett who, based on chromatography, expressed the opinion that neither of these is a single compound, but a mixture of similar compounds. Then, in the early 1930s, through the extensive chromatographic investigations of Richard Kuhn, Edgar Lederer, Paul Karrer, and László Zechmeister (just to name a few) the multiplicity of this compound group was finally

revealed, and scores of individual substances separated. In fact, the rebirth of chromatography is closely associated with this work (3,4).

Amino Acids

Amino acids are the keystones of life and thus, their studies represent an important part of science. The story of the fundamental work of Archer J.P. Martin and Richard L.M. Synge in 1940–1941, developing partition chromatography and demonstrating the possibility of amino acid separation, and then a few years later the development of paper chromatography by Martin's group, revolutionizing biochemical investigations, is well known (5). However, Synge's subsequent investigations leading to the structure elucidation of Gramicidin S, a cyclic decapeptide, are mostly forgotten, although this was the first successful attempt to describe the structure of a polypeptide molecule (6). Synge's chromatographic method served as the basis of the technique used a few years later by Frederick Sanger in revealing the full amino acid sequence of the molecule of bovine insulin, consisting of 51 amino acids (7).

In the 1950s the study of the amino acid composition of proteins was further improved by the introduction of ion-exchange chromatography, at the Rockefeller Institute for Medical Research (now Rockefeller University) in New York City, by Stanford Moore and William H. Stein. Their painstaking work finally resulted in the first true instrument designed for chromatographic separation (8,9). Today's breakthroughs in the field of proteins would be impossible without the achievements of chromatography.

Essential Oils

Another field where advances would have been impossible without chromatography is the investigation of essential oils and fragrances. These contain a very large variety of highly volatile substances, mostly terpenes, and the separation and identification of the individual compounds represent a very difficult task. Pioneering work in this field had been carried out in the second part of the 1950s by Erwin sz. Kováts, at the Federal Technical

University in Zurich, Switzerland, and the need for a reliable way to identify the individual constituents led him to the development of the retention index system. The most complex oil investigated by Kováts was the Bulgarian rose oil (10) (the publication of his results was delayed over 15 years due to patent considerations by the sponsor.)

Traditional Chinese Medicines

In China the use of traditional medicines of plant origin goes back to thousands of years and even today, these represent a special segment of health care: the Chinese pharmacopoeia lists over 11,000 medical plants. These plants contain a wide variety of volatile compounds but until recently very little was known about their actual composition. Finally, chromatography permitted their detailed investigation and today, one cannot open an issue of the chromatographic journals without finding a paper from this field, revealing the very complex nature of these medicines, each consisting of scores of compounds. As a typical example I want to cite a recent paper investigating the Traditional Chinese Medicine (T.C.M.) prepared from the leaves of the *Toona* tree grown in Shandong province and used as an anti-tumor agent: in it 34 compounds, mostly terpenes, aldehydes and olefinic hydrocarbons, were identified by GC-MS (11). Such traditional medicines are in use also in other geographic areas of this region: for example, a recent paper reported on the investigation of a traditional medicine of Tibetan origin, based on *Gentiana* herbs, grown at high altitudes, above 7500 ft: researchers could identify all C₁-C₃₀ free fatty acids in the roots of the plant (12). Such investigations would have been impossible before the advent of high-resolution chromatography.

Petroleum

One of the most complex natural substances is petroleum, containing hundreds if not thousands of hydrocarbons and other more-or-less volatile substances. For decades the aim of chemists had been to learn more about its composition, and in the first part of the 20th century extensive investigations have been carried out to separate, identify and isolate the constituents of commercial petroleum fractions. Gradually liquid adsorption chromatography had been introduced to these investigations, but the real breakthrough came in the 1950s with the advent of gas chromatography, particularly the introduction of capillary columns for separation. In fact, one-day work with these high-efficiency columns yielded more results than decades of painstaking investigations with other methods (13).

Life Tests

Finally, I want to mention a very interesting use of chromatography in the study of natural substances, where the technique not only helped in separating the individual compounds, but also served as the media for "life tests" for the identification of the active compound. These earlier applications are now mostly forgotten although they indicated important possibilities and would have warranted further follow-up. The first example utilized paper chromatography, while the second gas chromatography, and both have been described in the 1950s.

Gerhard Hesse, professor at the University of Erlangen, in Germany, was asked in 1955 to find out what particular com-

pound present in the bark of spruce trees lures the beetle *Hylobius abietis*, damaging, and even destroying, the young trees. To solve the problem he prepared an extract of the bark and then separated the compounds present by paper chromatography. Next he placed the insect on the paper strip with the separated spots: it searched on the paper and, after little hesitation, stopped at one particular spot and started to eat it. In this way the insect identified the particular compound (14,15).

The other example is related to the pheromones, the sex attractants emitted in very small quantities by female animals, to lure from great distances the male counterparts to mate. Such studies are difficult for a number of reasons. These compounds are usually present together with often closely related other substances, thus have to be separated for further study. Their concentration in the atmosphere is very low (e.g., the male silk moth responds to amounts as little as 10⁻¹¹ gram) and the amount available in the female's gland is small, thus very large number of species would have to be sacrificed to obtain a pure substance in appreciable amount. The high sensitivity and resolution of chromatography make the technique eminently suitable for such investigations. But even if separated and detected, one problem remains: how to establish unequivocally which particular compound in the mixture actually serves as the sex attractant.

In the second part of the 1950s Adolf Butenandt, the famous German organic chemist who, in 1939, received the Chemistry Nobel Prize for his work on sex hormones, studied the pheromone of the silk moth (*Bombyx mori*). He described the characteristic ways how the male moths react when sensing even traces of the female's sex attractant in the air, and prepared extracts of the female's glands. However, the question remained open, which compound in the extract actually serves as the pheromone. This question was answered in a unique way by Ernst Bayer, then the head of the Department of Biochemistry at the German Government Research Institute in Geilweilerhof. He constructed a special gas chromatograph where a small cell was attached to the column outlet, placed a male moth in this cell, and chromatographed the female's gland extract. When most of the peaks eluted from the column into the cell, the male moth remained quiet; however, for one particular peak he suddenly became excited, showing the peculiar wing movement and a whirling dance, indicating that that compound must serve as the pheromone (16). Based on this finding Butenandt could then establish the chemistry of the sex attractant, describe it as 10-trans-12-cis-hexadecadiene-1-ol, and named it Bombykol.

Almost simultaneously with this work researchers at the Entomology Research Division of the U.S.D.A. Agricultural Research Service, in Beltsville, Maryland, were trying to find a way to reduce the devastating effect of the caterpillars of the gypsy moth (*Lymantria dispar*) on trees and bushes, stripping them clean of leaves. They also extracted the abdominal segments of the female moth and chromatographed the extract in the same way as done by Bayer, placing a male moth directly in the column effluent and observing its reaction to the individually eluting compounds (17,18). Subsequent painstaking work permitted the chemical identification of the pheromone as cis-7,8-epoxy-2-methyloctadecane (Disparlure), and finally, its synthesis (19). With the availability of the pheromone, traps were constructed as the bait for the male moth (20). Today, tens of thou-

sands of such traps are used in the United States.

These few examples demonstrate the close relationship between chromatography and the investigation of complex natural substances. I used some typical examples from the past; the papers in this special issue prove that the investigation of natural substances with chromatographic methods continues to be an important subject.

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