

REPORT

MODERN INSTRUMENTAL AND CHEMICAL APPROACHES TO PHYTOCHEMISTRY

(Received 1 November 1967)

A SYMPOSIUM on "Modern Instrumental and Chemical Approaches to Phytochemistry" at the University of Wisconsin, 24-26 August, 1967, marked the seventh annual meeting of the Phytochemical Society of North America (formerly the Plant Phenolics Group of North America, organized in 1961). The symposium aimed to explore some of the newer or less commonly used methods now applied to phytochemical problems and to present their advantages and limitations. Both biological and chemical types were included.

The meeting opened with a paper by professor Donald A. Larson, Department of Botany, University of Texas, on "Cellular Analysis of Micro- and Macromolecules". He emphasized the wide variety of instruments and techniques now available to the cytologist in locating the exact site of many types of organic compounds including enzymes within the cells, their concentration, and the changes in concentration with cell growth. Instruments include not only the electron microscope, but microspectrophotometers (which can discriminate down to one micron), electron probes, electron diffraction, and electron reflecting microscopes. Specific examples included localization of alkaloids, cellular changes preceding crystallization of calcium oxalate, study of acid phosphatase reactions in corn pollen, and structural studies of pyruvate dehydrogenase.

The second symposium lecture dealt with the "Nuclear Magnetic Resonance Studies of Plant Biosynthesis" by J. J. Katz of the Argonne National Laboratories. The technique comprises growing organisms on D_2O or mixtures of it with H_2O with or without any other hydrogen or deuterium substrate and then determining the proton magnetic resonance spectrum of the products of biosynthesis. The isotope fractionation reveals something of the paths of biosynthesis, which at present seems to be more complex (less unique) than originally thought. The method is limited by the fact that only lower micro-organisms (algae, yeasts, molds, bacteria) grow in high concentrations of D_2O and by the care necessary in obtaining accurate integration for determining hydrogen/deuterium ratios. The technique was illustrated by a discussion of the biosynthesis of chlorophyll and bacteriochlorophyll.

J. M. Harkin of the Forest Products Laboratory, Madison, opened the second session of the symposium with a paper on "Methods of Attacking the Problem of Lignin Structure". After summarizing the older, more vigorous chemical methods of isolation, analysis, and degradation, he showed how milder chemical methods are leading to the isolation of larger and more significant degradation products of this complex molecule. The use of enzymes in isolation work and in studying the model biosynthesis of artificial lignin from coniferyl alcohol and theories of the free radical formation were emphasized. Modern instrumental techniques have aided certain aspects of the work, but the complete structure of the macromolecule as it exists in the plant is still a matter of hypothesis.

"Tissue Culture as a Tool for the Phytochemist", presented by Professor E. J. Staba of the College of Pharmacy, University of Nebraska, was the fourth paper of the symposium. The technique itself was outlined in some detail since it is not widely used. Examples of its use in studying biochemical problems and in producing secondary plant products of value included the production of alkaloids, steroids and furanochromones. Often biosynthesis in tissue cultures does not parallel that in the intact plants from which the tissues were derived, and many problems remain to be solved before industrial production becomes a reality.

The symposium closed with two papers on modern instrumental techniques. A. M. Duffield of Stanford University discussed "Utilization of Mass Spectrometry in Natural Product Chemistry". After a brief introduction on the limitation of the technique to volatile compounds and on the use of high resolution mass spectrometers for determining molecular formulae on less than a milligram of material, the determination of the structure of unsaturated steroidal side-chains was detailed in order to illustrate the possibilities of the method and the theories advanced to explain fragmentation patterns. A second example was concerned with the elucidation of the structure of two new triterpenes.

"Scope and Limitations of Gas-Liquid Chromatography of Terpenes in Chemosystematic Studies" by Dr. E. von Rudloff of National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, described the application of this widely used technique to a specific type of problem and one of considerable interest nowadays to phytochemists. A valid taxonomic fingerprint requires the identification and quantitative analysis of a large number of similar components in related species. The seasonal variation and the variation among individual plants must be considered in collection of samples. Details were given for chromatographic conditions suitable for analyzing mixtures of terpenes containing mono- and sesquiterpenes and their oxygenated derivatives. The direct injection technique is of value for qualitatively studying small samples of individual tissues and for very volatile components, but it is unreliable for quantitative chemosystematic work. Characteristic and reproducible terpene distribution patterns were obtained for the leaf oils of some *Picea* and *Juniperus* species, and their use in studying hybridization and introgression was outlined.

The banquet speaker, Professor S. Morris Kupchan, University of Wisconsin, in his talk on "Recent Advances in the Chemistry of Tumor Inhibitors of Plant Origin" gave good illustrations of two other modern methods available to the phytochemist. Every step in his isolation of active natural products is followed by screening against two standard carcinomas (at the Cancer Chemotherapy National Service Center). Structural work on the various different chemical types (alkaloids, steroids, and terpenoids) of new active principles finally isolated has been speeded up immeasurably by recourse to X-ray crystallography.

The symposium papers will be published as the second volume in the series "Recent Advances in Phytochemistry", Appleton-Century-Crofts, New York, 1968, editors M. K. Seikel and V. C. Runeckles.

In 1968 the Phytochemical Society of North America will hold its annual meeting on 6-8 June, 1968 at the University of Arizona under the auspices of the Departments of Chemistry, Biology and Plant Pathology. Under the chairmanship of Professor Cornelius Steelink, a symposium on "Environmental Influences on Plant Enzyme Action" is being arranged.

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