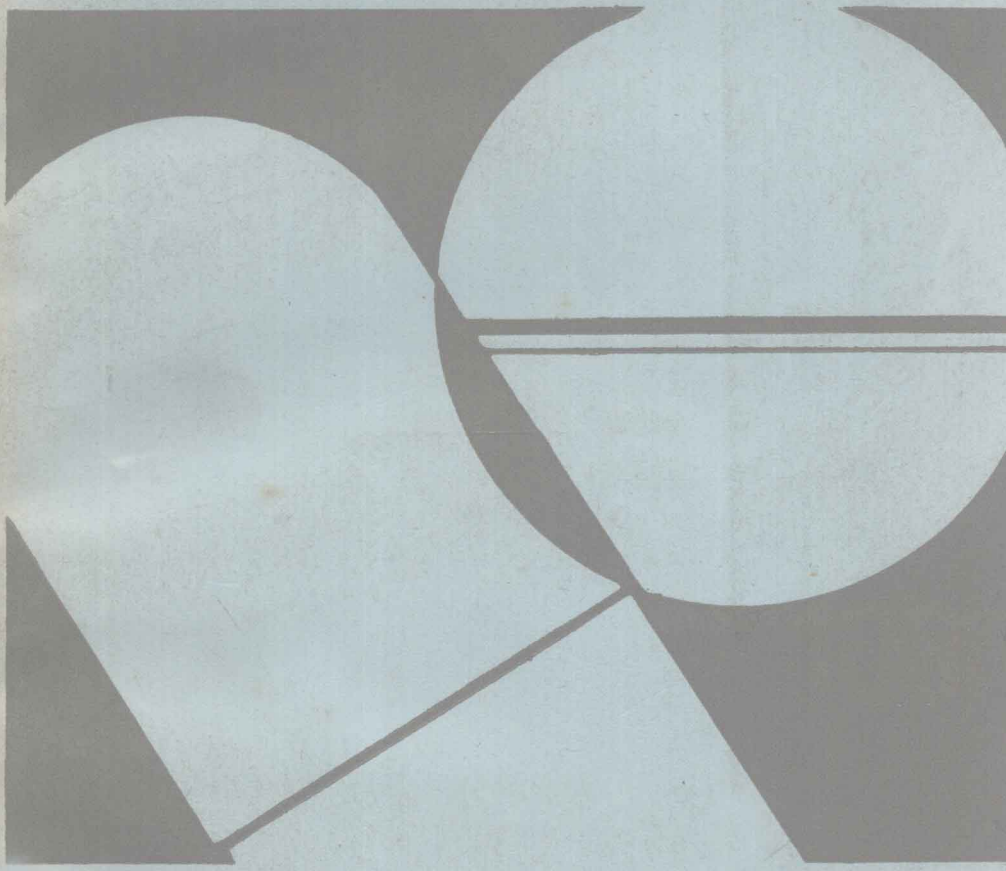
Drug Discovery

Science and Development in a Changing Society



ADVANCES IN CHEMISTRY SERIES 108

Drug Discovery

Science and Development in a Changing Society

Two symposia sponsored by the Division of Medicinal Chemistry at the 160th Meeting of the American Chemical Society, Chicago, Ill., Sept. 15-16, 1970.

Barry Bloom and Glenn E. Ullyot,

nposia Chairmen

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FOREWORD

ADVANCES IN CHEMISTRY SERIES was founded in 1949 by the American Chemical Society as an outlet for symposia and collections of data in special areas of topical interest that could not be accommodated in the Society's journals. It provides a medium for symposia that would otherwise be fragmented, their papers distributed among several journals or not published at all. Papers are refereed critically according to ACS editorial standards and receive the careful attention and processing characteristic of ACS publications. Papers published in ADVANCES IN CHEMISTRY SERIES are original contributions not published elsewhere in whole or major part and include reports of research as well as reviews since symposia may embrace both types of presentation.

PREFACE

"The world is a scene of changes, and to be constant in nature were inconstancy." No one takes issue with this expression from the pen of Abraham Cowley, but too often we fail to recognize the direction of change and adjust our way of life to accommodate it. Thus we become trapped and bogged down in outmoded institutions, obsolescent methods, and unproductive traditions.

Several years ago, the Public Affairs Committee of the ACS Division of Medicinal Chemistry began to reflect upon the profound changes taking place in the world about us. How were these changes going to affect the coalition of industry, universities, and government involved in the drug discovery process? This was the concern.

Many of the changes were taking place in the science of drug discovery itself. We had witnessed the beginning of the application of computer technology to the synthesis of chemical compounds. Chromatographic devices and spectroscopic instruments had begun to revolutionize the art of structure determination. The probing of biological processes at the molecular level was beginning to unravel some of the mysteries of disease states. These developments, among many others, were already affecting the drug discovery process. Even more significant changes could be expected in the future.

Society itself was also in the throes of fundamental changes, and the effect was certain to influence the system for drug discovery and development. It had become national policy that health care is the right of everyone. Drugs are an important part of the care system, and although they represent only 20% of the total health care cost, their price had come under heavy attack and criticism. The movement toward increased government regulation of the drug industry was another facet of the kaleidoscopic environment. Altogether such factors were fast becoming determinants in the economics of drug discovery. Indeed, had the research and development process reached the point where it was no longer paying for itself?

The Public Affairs Committee concluded that a critical look at the process of change and the influence of such change on future drug development would be constructive. From this nebula arose the two

symposia "The Science of Drug Discovery" and "Drug Discovery and Development in a Changing Society," which were organized under the able leadership of Barry Bloom and Glenn Ullyot, respectively. The papers from these symposia comprise the substance of this volume.

Abbott Laboratories
North Chicago, Ill.
August 1971

WARREN J. CLOSE

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Drugs from Natural Products—Plant Sources

S. MORRIS KUPCHAN

University of Virginia, Charlottesville, Va. 22901

The plant kingdom has served as one of man's oldest sources of useful drugs. The history of classic plant-derived medicinals, such as morphine and quinine, illustrates the origin of the older medicinals as major and relatively easily isolated constituents of folk remedies. More recently discovered agents, such as reserpine and vincaleukoblastine, have been minor constituents of complex mixtures, whose isolation was guided by pharmacological assay. A model for future searches for plant derived medicinals is illustrated by the isolation and characterization of the tumor inhibitors vernolepin and jatrophone. Screening of many hundreds of crude extracts yielded a significant number of active extracts, and fractionations guided by biological assays have yielded a fascinating array of novel biologically active plant products.

The use in medicine of drugs derived from plants goes back to antiquity. When one considers the therapeutic impact of morphine, quinine, digitalis, ergot, atropine, cocaine, reserpine, and vincaleukoblastine, to name but a few, it is evident how great is the debt of medicine to plant-derived drugs even today. If one adds the synthetic derivatives and variants of plant-derived products, the role of natural products from plant sources has been most impressive.

The most important plant-derived drugs were developed between 1800 and 1950. The past few decades have witnessed an unquestionable diminution in the number of such compounds introduced into medicine. These facts have led to suggestions that the intensive investigations of the past century have nearly exhausted the plant kingdom as a potential source for new drugs and that future work in this area is unlikely to be rewarding. I address myself to the contrary thesis—*viz.*, that the plant kingdom continues to offer a rich and virtually inexhaustible supply of new potential drugs. However, the success in tapping this source will depend upon the extent to which newer approaches to the study of biologically-active plant constituents are used.

The Past: Morphine and Quinine

As we look to the past, morphine and quinine represent classic examples of early plant-derived medicinals. Opium, the sun-dried latex of the unripe fruit of *Papaver somniferum*, is believed to have been used before history was recorded. The first undisputed reference to poppy juice is found in the writings of Theophrastus in the third century B. C. Dioscorides in the first century A. D. was fully acquainted with the method for collecting and preparing opium, and his directions for preparing syrup of poppy are essentially unchanged in modern pharmacopeias. Arabian physicians were well versed in the uses of opium. This drug was introduced to the Orient and China by Arabian traders. The spread of the opium habit throughout China did not occur until the latter part of the eighteenth century when the Portuguese and later the English started to exploit the natives in this regard. The war against opium has continued in the Orient and elsewhere ever since.

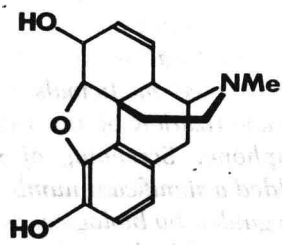


Figure 1. Morphine

From the sixteenth century and well into the nineteenth century the uses of opium for its analgesic and hypnotic properties were fairly well understood in Europe. In 1805 a young German pharmacist in Hanover named Sertürner isolated and described morphine. This epochal finding went unnoticed until his later publication in 1816. Sertürner almost lost his life by experimenting with morphine on himself. The discovery of other alkaloids in opium quickly followed that of morphine, and the use of pure alkaloids rather than crude preparations soon spread throughout the medical world. Extensive structural studies led to elucidation of morphine's structure by Gulland and Robinson in 1925 (*1*), and total syntheses by Gates and Tschudi (*2*) and by Elad and Ginsburg (*3*) confirmed completely the structure and stereochemistry of the molecule (Figure 1). It is noteworthy that morphine is the major alkaloid of opium; in a good grade of opium it averages 10%, although samples containing over 20% have been reported.

Quinine is the chief alkaloid of cinchona, the bark of the cinchona tree indigenous to certain regions of South America. The first written

record of the use of cinchona occurs in a religious book written in 1633 and published in Spain in 1639. A variety of colorful and fanciful versions of the discovery of the fever bark exist. A popular and persistent version is that the bark was used in 1638 to treat Countess Anna del Chinchon, wife of the viceroy to Peru, and that her miraculous cure resulted in the introduction of cinchona into Spain in 1639 for the treatment of ague. By 1640, the drug was being used for fevers in Europe. The term "cinchona" was chosen by Linné (who accidentally misspelled it) for the species of plants yielding the drug. Jesuit priests were the main importers and distributors of cinchona in Europe, and the name "Jesuit bark" soon became attached to the drug.

For almost two centuries, the bark was used in medicine as a powder, extract, or infusion. In 1820 Pelletier and Caventou isolated quinine and cinchonine from cinchona, and the use of the alkaloids as such gained favor rapidly. Extensive and classic studies led to elucidation of the structure of quinine (Figure 2) (*4*) and to its total synthesis in 1944 (*5*). Cinchona contains 25 closely related alkaloids, of which the most important are quinine, quinidine, cinchonine, and cinchonidine. The average yield of alkaloid is about 6–7%, of which one-half to two-thirds is quinine. It has been said that quinine owes its dominant position in the treatment of malaria only to the fact that it was the first alkaloid isolated from cinchona, and that there is little among the four major alkaloids to choose from in treating this disease (*6*).

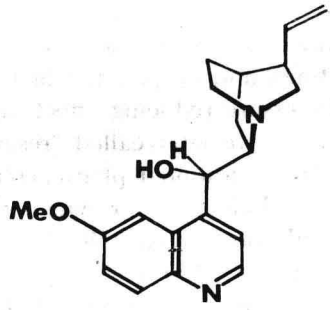


Figure 2. Quinine

The history of morphine and quinine, like that of most classic plant-derived medicinals, reveals that the compounds represented major and relatively easily isolated plant constituents. The ready accessibility of the compounds played a major role in their characterization as the active principles of the plants.

The Present: Reserpine and Vincaleukoblastine

Reserpine and vincaleukoblastine represent the most important plant-derived medicinals introduced into medicine by our generation, and it is instructive to compare their history with those of morphine and quinine. Descriptions of the use of extracts of plants resembling *Rauwolfia* may be traced back to ancient Hindu ayurvedic writings. They were used in primitive Hindu medicine for a variety of diseases, including snake bite, hypertension, insomnia, and insanity. The early remedies were used for various other purposes, but it seems clear now that our present day application of *Rauwolfia* alkaloids in treating hypertension and mental disease was foreshadowed in the folk medicine of the Eastern peoples.

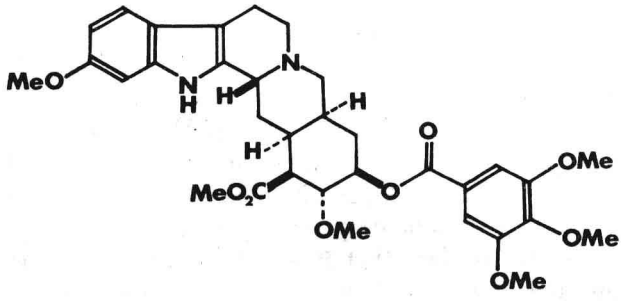


Figure 3. Reserpine

Although *Rauwolfia* was investigated in the nineteenth century and the presence of alkaloids indicated, a systematic investigation of *Rauwolfia* was only started by Siddiqui and Siddiqui in 1931 (*7*). Five alkaloids were isolated at that time, and despite the fact that one alkaloid (serpentine) had a blood-pressure reducing effect, none of the five showed the characteristics which were later called "reserpinelike" (*8*). Chopra and others concluded that additional pharmacologically active material must be present in the whole root for which the crystalline alkaloids available at the time could not account (*9, 10*). The *Rauwolfia* problem received a great stimulus from the 1949 paper by Vakil in the *British Heart Journal* on the antihypertensive effects of *Rauwolfia* extracts in man (*11*). In the newer studies, systematic fractionation and isolation were coupled with pharmacological evaluation, and it became apparent that the hypotensive and alkaloidal material was concentrated into the "oleoresin" fraction. Reserpine, the most important *Rauwolfia* alkaloid, was isolated from the "oleoresin" fraction in 1952 (*12*), and shortly afterward it was shown to be responsible for most of the tranquilizing and hypotensive effects of *Rauwolfia* extracts. The elucidation of its structure (Figure 3) (*13*) and an elegant total synthesis (*14, 15*) constituted major

achievements in alkaloid chemistry. Reserpine is one of over 50 alkaloids isolated from various *Rauwolfia* species.

The beneficial properties of the periwinkle plant, *Vinca rosea* Linn., have been described in medicinal folklore for many years in various parts of the world. An alleged activity as an oral hypoglycemic agent prompted its phytochemical examination in two different laboratories independently. While neither group could substantiate this reported activity in either normal or experimentally-induced hyperglycemic rabbits, the Canadian group of Noble, Beer, and Cutts observed a peripheral granulocytopenia and bone marrow depression in rats associated with certain fractions (*16*). These effects guided the extraction and purification of an active alkaloid, termed vincaleukoblastine. The Lilly group, which included Johnson, Svoboda, and others, demonstrated that certain alkaloidal fractions inhibited the growth of an acute lymphocytic leukemia in mice. Fractionation, followed by assay in the leukemic mice, yielded vincaleukoblastine, vincristine, and two other active dimeric alkaloids (*17, 18*). Vincaleukoblastine and vincristine are now among the most important drugs for the treatment of acute leukemia of childhood and other neoplasms (*19*). The molecular structures of vincaleukoblastine and vincristine were determined by chemical studies in 1964 (Figure 4) (*20*), and the complete stereochemistry and absolute configuration were elucidated by x-ray crystallographic analysis in 1965 (*21*). Vincaleukoblastine is one of more than 50 alkaloids isolated from *Vinca rosea.*

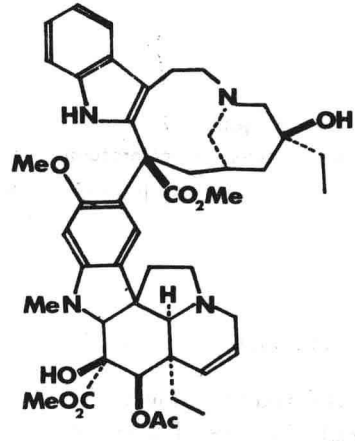


Figure 4. Vincaleukoblastine

The review of the history of reserpine and vincaleukoblastine reveals that each alkaloid was a minor constituent of a complex mixture and that its isolation from the mixture was guided in each case by assay for characteristic pharmacological properties. It is likely that, had the investiga-

tions of *Rauwolfia serpentina* and *Vinca rosea* proceeded along classical phytochemical lines, without pharmacological guidance, the discovery of reserpine and of vincaleukoblastine would have been postponed by many years.

The Future: Vernolepin and Jatrophone

The past and present states of any field are far simpler to comment upon than the future. On the other hand, the absence of clear-cut guidelines provides the writer considerable latitude in discussing the future.

Below I outline briefly some recent findings in my laboratory in a program directed at tumor inhibitors of plant origin. This program, which has already led to the isolation of the active principles of more than 80 tumor-inhibitory extracts, has been the subject of two recent reviews (*22*). For this discussion of the future of plant-derived drugs, the stories of vernolepin and jatrophone will exemplify one important approach.

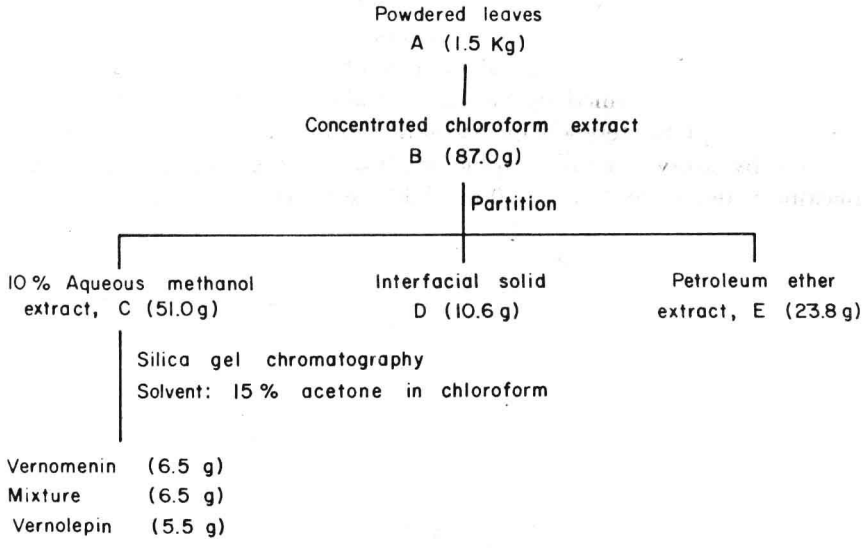


Figure 5. Fractionation of tumor-inhibitory extract from Vernonia hymenolepis

In our program, the fractionation and isolation studies are guided at every stage by biological assays. The systematic fractionation has made possible the isolation of important minor constituents which would most probably have been missed in the classical approach. During the screening program sponsored by the Cancer Chemotherapy National Service Center, an extract of *Vernonia hymenolepis, A. rich,* was found to show significant and reproducible cytotoxicity against the KB tissue culture

of human carcinoma of the nasopharynx. Figure 5 summarizes the fractionation procedure that led to the isolation of the cytotoxic principles, vernolepin, and vernomenin. Although the compounds were concentrated and isolated solely on the basis of *in vitro* cytotoxicity, vernolepin was subsequently found to show significant *in vivo* tumor inhibitory activity against the Walker 256 carcinosarcoma in the rat. Vernolepin and its isomer, vernomenin, were interrelated by conversion to a common methanol adduct. A combination of degradative, spectral, and x-ray crystallographic studies resulted in assignment of the biogenetically novel, elemanolide dilactone structures shown in Figure 6 (*23, 24*).

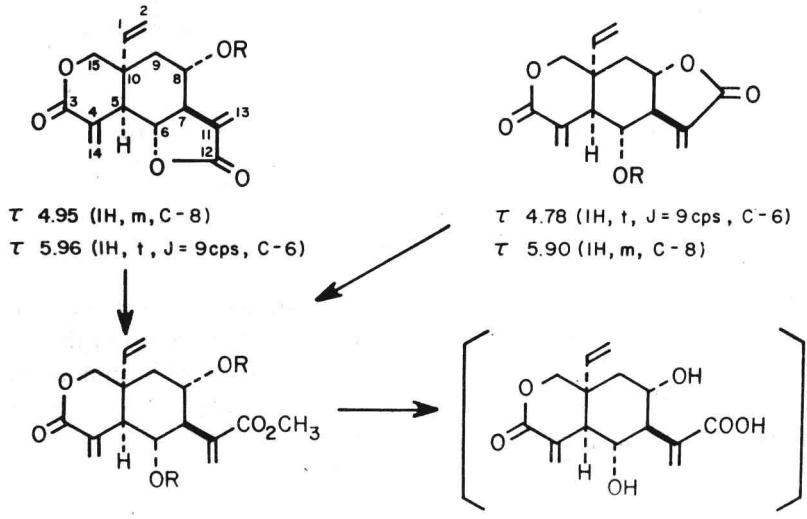
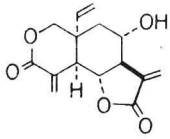
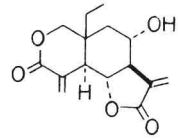


Figure 6. Structures of vernolepin (upper left, R=H) and vernomenin (upper right, R=H)

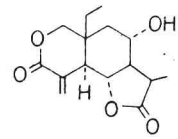
Several recent observations have focused attention on the importance of the conjugated α-methylene lactone function for the biological activity of vernolepin and other sesquiterpene lactones. Furthermore, the results support the view that the α-methylene lactones may exert their effects on cells by interacting with sulfhydryl enzymes that regulate cellular growth. For instance, vernolepin is a potent inhibitor of the extension growth of wheat coleoptile sections (*25*); this inhibitory effect is blocked completely by adding sulfhydryl compounds such as mercaptoethanol to the medium. Second, vernolepin and other sesquiterpene lactones can inhibit phosphofructokinase by reacting with the enzyme's sulfhydryl groups (*26*). Third, as shown in Figure 7, the cytotoxicity of vernolepin derivatives appears to be related directly to the presence of free conjugated α-methylene lactone functions. Thus, selective reduction of the ethylidene



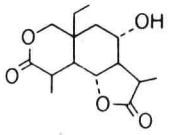
VERNOLEPIN
ED_{50} 2.0 γ



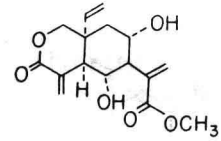
DIHYDROVERNOLEPIN
2.0 γ



TETRAHYDROVERNOLEPIN
19.0 γ



HEXAHYDROVERNOLEPIN
> 100 γ



ACIDIC METHANOLYSIS
PRODUCT
26 γ

Figure 7. Cytotoxicity of vernolepin derivatives

double bond does not appear to affect the cytotoxicity. However, modification of the α-methylene-γ-lactone (by trans-esterification to the methanol adduct or by hydrogenation) results in a 10-fold diminution in cytotoxicity. Modification of both α-methylene lactone systems, as in hexahydrovernolepin, leads to a derivative which is essentially inactive. [The synthesis of dihydrovernolepin exemplifies a new blocking sequence for the protection of the highly reactive conjugated α-methylene groups of lactones (Figure 8). Vernolepin was treated with excess *n*-propylthiol at pH 9.2 to give a bisthiol adduct. Hydrogenation of the bisthiol adduct (with one mole equivalent of hydrogen), followed by methyl iodide methylation and sodium bicarbonate-catalyzed elimination, gave dihydrovernolepin (*27*).] Recently we studied the reactions of several conjugated α-methylene lactones with model biological nucleophiles, such as cysteine, lysine, and guanine (*28*). Thiols such as cysteine were the most reactive, and the rate of reaction was of the same order as that of cysteine with iodoacetate, a commonly used sulfhydryl reagent (Figure 9). The biscysteine adducts, in accord with expectations, were essentially inactive.

Extracts of *Jatropha gossypiifolia* L. and related species have been used for many years in Costa Rica to treat cancerous growths. An alcoholic extract of the roots of *Jatropha gossypiifolia* (supplied by J. A. Saenz Renauld of the University of Costa Rica) was found by CCNSC to show inhibitory activity against four standard animal tumor systems (sarcoma 180, Lewis lung carcinoma, and lymphocytic leukemia P-388 in the mouse, and the Walker 256 intramuscular carcinosarcoma in the rat) and *in vitro*