Biosynthetic Products for Cancer Chemotherapy

Volume 2

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To Julius A. Rippel,
A pioneering advocate
of biosynthetic products
for cancer treatment and cure

Preface

An overall view of the cancer problem and development of cancer chemotherapeutic biosynthetic products to February 1976 was presented in Volume 1.[231] In the short time that has elapsed since the preparation of Volume 1, several very stimulating advances in application of biosynthetic cancer chemotherapeutic drugs in cancer treatment have been reported. At the May 1976 meeting (in Toronto) of the American Association for Cancer Research, a Sloan-Kettering research group summarized an improved treatment of human neuroblastoma using a combination of vincristine, cytoxan, trifluorothymidine, and papaverine. In the same period other clinical groups described significant advances in the cancer chemotherapeutic treatment of human breast cancer and oat cell carcinoma of the lung. Each of these newer advances in cancer treatment was based on combinations of biosynthetic and synthetic cancer chemotherapeutic drugs. Certainly, further examination of the antineoplastic biosynthetic agents summarized in this volume and the vast number yet to be discovered will eventually provide the means for controlling and/or curing the various types of human cancer.

The main purpose of the present volume is to provide a summary of all the better known naturally occurring anticancer and cytotoxic substances that have appeared in the literature to April 1976. Volume 3 now in preparation will bring the summary to November 1977. The survey of plant and animal antineoplastic constituents was conceived as a means of providing ready access to this field by both chemists and biologists. The biosynthetic anticancer and cytotoxic agents have been summarized in broad groups based on chemical classification and biological origins. In each such group the substances have been arranged according to increasing carbon atom content. Wherever known a summary of the antineoplastic and/or cytotoxic activity, principal physical measurements, and the botanical or zoological source has been included. It is hoped this arrangement will prove exceptionally useful to a cross section of scientists interested in antineoplastic natural products and especially to those bioorganic chemists and biologists actively engaged in discovery and development of cancer chemotherapeutic drugs.

Doubtlessly, some important compounds were inadvertently overlooked and

some errors have not been eliminated from the pages that follow. In both cases we extend our apologies to those affected by such omissions and oversights.

In the final preparation of this volume grateful acknowledgment is extended to Mrs. Christine H. Duplissa for very valuable and expert assistance, to Mrs. Marie D. Baughman for very helpful contributions, and to Mss. Sally J. Keehl, Melinda A. Duplissa, and Robin K. Pettit for their assistance.

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Contents

Introduction

From substantial (and indisputable) evidence already outlined in the previous volume, at least 2–4% of plant species and 8–10% of animal species synthesize antineoplastic and/or cytotoxic substances. The potential of these figures for treatment of human cancer truly staggers the imagination and offers great promise of many curative approaches to the cancer problem. For some perspective one need only to consider that the world's flora may number up to 800,000 and the more conspicuous members of our terrestrial vegetation, the angiosperms, may number from 300,000 to some 500,000[131,231] Further, enormous numbers of microorganism species appear to be available. In the animal segment of life the marine invertebrates alone number over 1,000,000 species, and with marine vertebrates the fishes comprise over 25,000 species. In the arthropod area the class insecta alone includes over 1,000,000 species. Since only a few percent of the known plants and less than 0.5% of the known animals have been evaluated for anticancer or cytotoxic constituents, it is apparent that we have just about reached the end of the beginning in our search for biosynthetic cancer chemotherapeutic drugs.

Most of the better known biosynthetic anticancer and cytotoxic substances mentioned in literature available to April 1976 have been collected, organized, and summarized in the survey of this volume. So far, the higher and lower (microorganisms) plants have been most extensively studied and this biological source accounts for a majority of the biosynthetic products covered in the survey. More specifically, 265 of such agents from plants, 103 from microorganisms, and 35 from animals have been listed. These represent some 145 plant species and 45 animal species. Obviously a great number of new cancer chemotherapeutic drugs of biosynthetic origin await discovery.

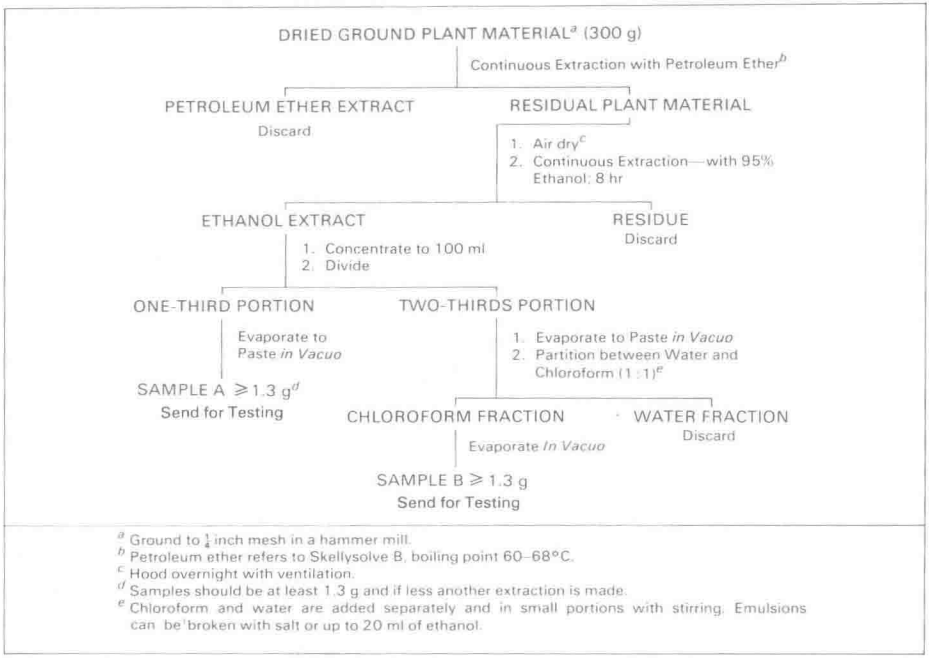
The plant and animal antineoplastic and/or cytotoxic agents have been grouped according to natural products chemistry classification and biosynthetic origin. For example, in Chapter 1, all of the higher plant terpenoids have been grouped together by empirical formula based on increasing carbon content. Similarly, the fungi and other lower plant biosynthetic products appear in Chapter 6 while higher animal biosynthetic products are grouped together in Chapter 8. The surveys include, where known, a structure, a common name, the system and

results of antineoplastic screening and/or cytotoxicity evaluations, a melting point and optical rotation value, whether certain spectral data have been reported, and finally the organism of origin and reference. The listings were prepared to expedite characterization of a known anticancer or cytotoxic compound and to provide an overall assessment of the current chemistry and biology for these important natural products. Unfortunately for some of the newer and/or lesser known anticancer and cytotoxic biosynthetic products, few or no biological screening data have been recorded in the technical literature. Hence, the brief notations under the heading "bioactivity" should be considered only preliminary results and not usually the net result of a comprehensive study involving at least several tumor systems. Generally the most significant biological data have been provided by the U.S. National Cancer Institute, and the key systems used in this program have been emphasized whenever possible.

As was noted in Chapter 1 of Volume 1, the National Cancer Institute's lymphoid leukemia L1210 (LE), lymphocytic leukemia P388 (PS or P388), Walker carcinosarcoma 256 (WA subcutaneous, WM intramuscular), B-16 melanoma (B1), and Lewis lung carcinoma (LL) have been selected as especially valuable tumor systems for selecting compounds potentially effective against human cancer.[322] About five years ago the Walker carcinosarcoma 256 was deemphasized and more recently discontinued in favor of the PS, B1, and LL systems. The KB cell line has been used for many years and has been augmented recently by the P388 cell line.[238] Over 260 experimental tumor systems in animals have been employed in various parts of the world to assess naturally occurring compounds. Many laboratories have a specific preference among these systems and employ them for routine screening. Some of the more widely used tumor systems have been summarized in the Appendix with the National Cancer Institute's abbreviation. A summary of the National Cancer Institute's key systems has also been presented in the Appendix.

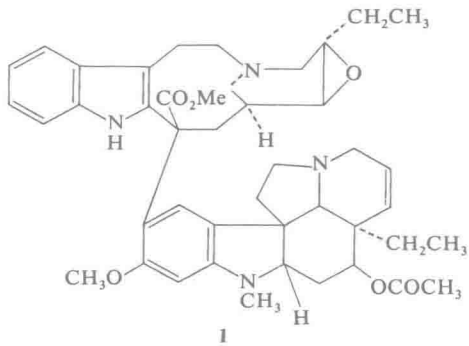
The actual selection of a plant or animal for detailed chemical investigation is usually based on initial screening of a solvent extract or series of solvent extracts. If one or more such mixtures displays a confirmed level of antineoplastic or cytotoxic activity then the extensive chemical and physical manipulations (guided by bioassay) needed for isolation of the active constituent(s) are undertaken. In laboratories collaborating with the U.S. National Cancer Institute, the initial testing involves the P388 and KB systems. A confirmed level of activity (see Appendix) in either one or both systems justifies further investigation. All of the separation techniques common to bioorganic chemistry and biochemistry are then applied to isolating the antineoplastic constituents. Generally these techniques begin with solvent fractionation of the crude extracts followed by application of various chromatographic procedures. By way of illustration, the preliminary fractionation procedure employed in the National Cancer Institute's programs for initial screening of plant products has been outlined in Scheme I. For this procedure at least 1 kg (dried weight) of plant material should be collected to cover initial biological evaluation and where appropriate subsequent confirmatory screening.

SCHEME I



[a] Ground to ¼ inch mesh in a hammer mill.
[b] Petroleum ether refers to Skellysolve B, boiling point 60–68°C.
[c] Hood overnight with ventilation.
[d] Samples should be at least 1.3 g and if less another extraction is made.
[e] Chloroform and water are added separately and in small portions with stirring. Emulsions can be broken with salt or up to 20 ml of ethanol.

Once a confirmed active extract has been selected for separation the initial solvent fractionation is guided by bioassay using either the P388 or KB systems. Here it should be emphasized that many unknown events can intervene to complicate the problem when using biological evaluation as a guide to fractionation. Frequently activity is lost during fractionation and this can be due to one or a combination of events including synergistic effects, chemical changes, and the canceling of activity by certain concentrations of substances. For example, in the isolation of leurosine (**1**) the crude alkaloid fraction showed no activity against the P1534 *in vitro* screening system but the pure alkaloid showed marked P1534 cytotoxicity.[56] Also, crude fractions may contain substances with



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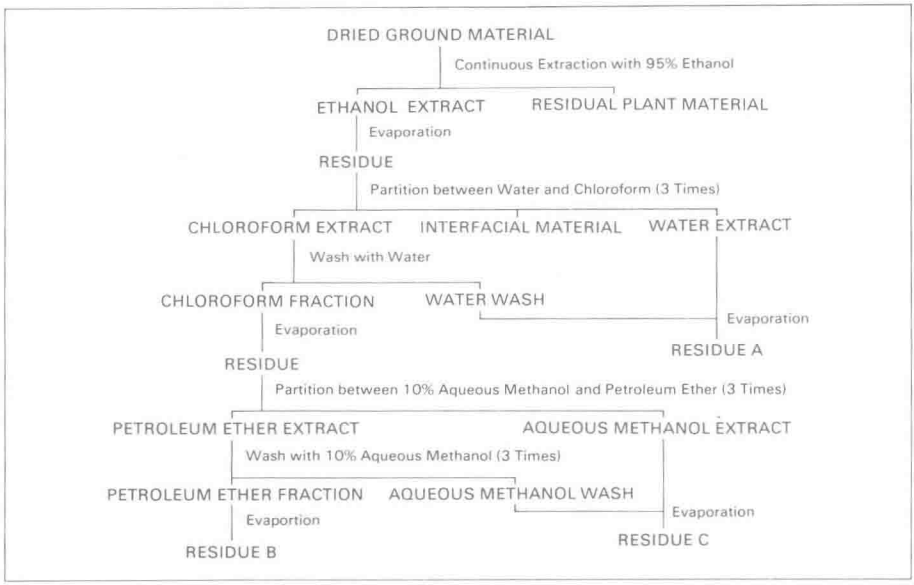
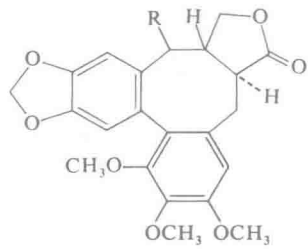
SCHEME II

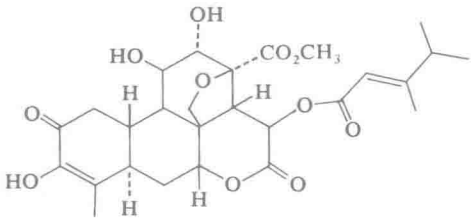


delayed toxicity causing the test animal to die at about the same time as the control animals.[56]

Several solvent fractionation procedures have been developed and some examples follow in Schemes II–VI. Scheme II has been applied to the isolation of alkaloids,[135] cardenolides,[48] and sesquiterpene lactones.[161] Once the active solvent fraction has been located further solvent partitioning can be very useful. For example, in Scheme II further partitioning of the 10% aqueous methanol fraction designated residue C between 20% aqueous methanol and carbon tetrachloride led to isolation of the lignan lactones, steganacin (**2a**), and steganangin (**2b**) from *Steganotaenia araliacea*.[137] For isolation of the simaroubolide, bruceantin (**3**) from *Brucea antidysenterica* the aqueous methanolic fraction was further partitioned between 40% aqueous methanol and chloroform.[138]



2a, R = $OCOCH_3$
b, R = $OCOC(CH_3)=CHCH_3$(Trans)



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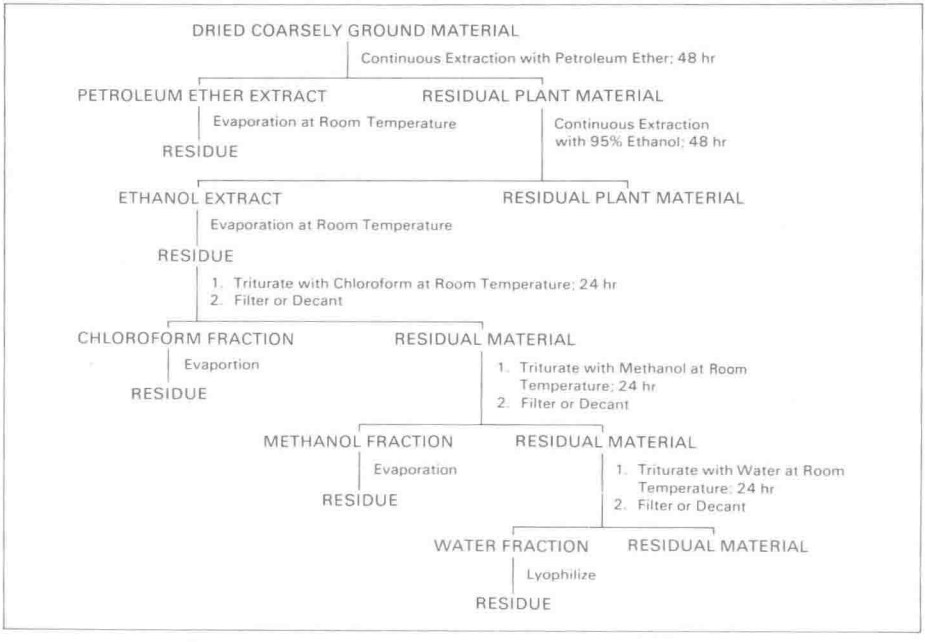
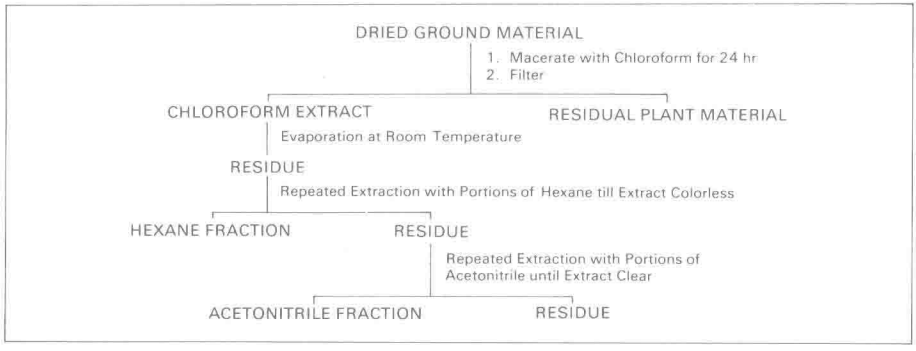
Our group has employed similar fractionation procedures and one of these is illustrated in Scheme III. The above procedure has been applied to isolation of sesquiterpene lactones[233] as well as to the fractionation of insect[240] and marine animal extracts.[249] A very useful alternative to this general solvent fractionation procedure is to dissolve the ethanol extract in 9:1 methanol–water. Next the methanol–water solution is successively extracted with ligroin, carbon tetrachloride, and chloroform while diluting the original solution to 4:1 methanol–water and then to 3:2 methanol–water.[138] The ligroin, carbon tetrachloride, chloroform, and 3:2 water–methanol fractions are sent for biological evaluation.

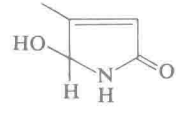
A solvent fractionation procedure frequently used by Cole and co-workers involves initial extraction of the plant with chloroform and is illustrated by Scheme IV. By this means jatropham (**4**) was isolated from *Jatropha*

SCHEME III



SCHEME IV





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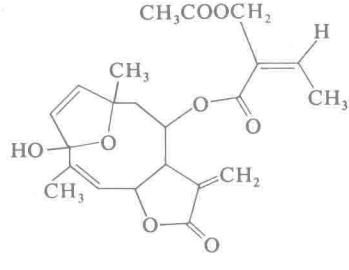
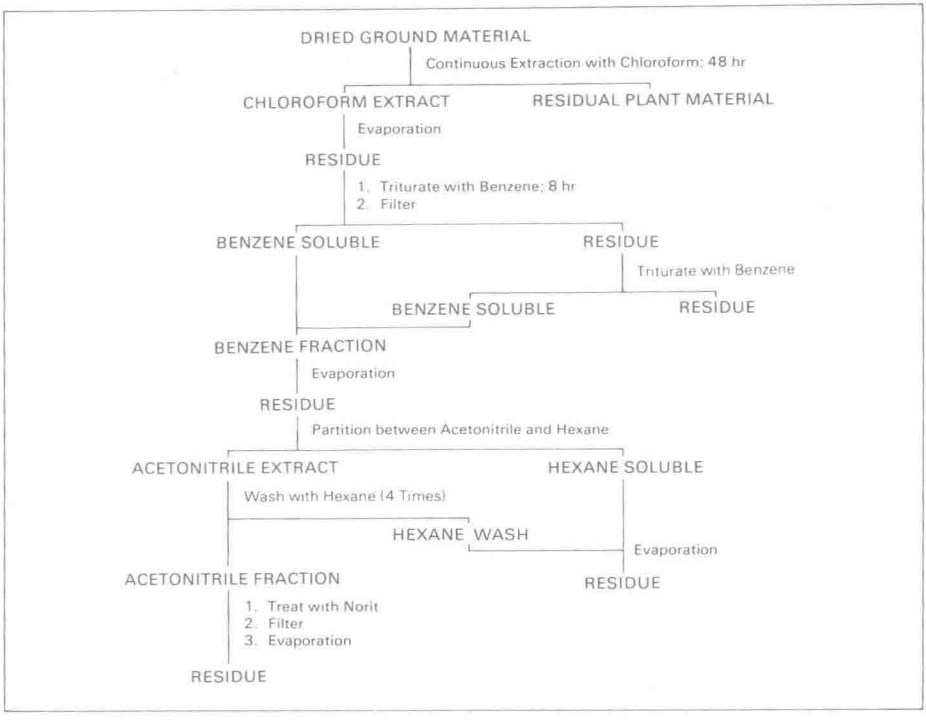
macrorhize.[334] Lactam (**4**) was obtained in crystalline form upon evaporation of the acetonitrile fraction. Application of a similar procedure to the sesquiterpene lactones of *Liatris chapmanii* by Kupchan and colleagues has been used to isolate Liatrin (**5**), Scheme V.[142]



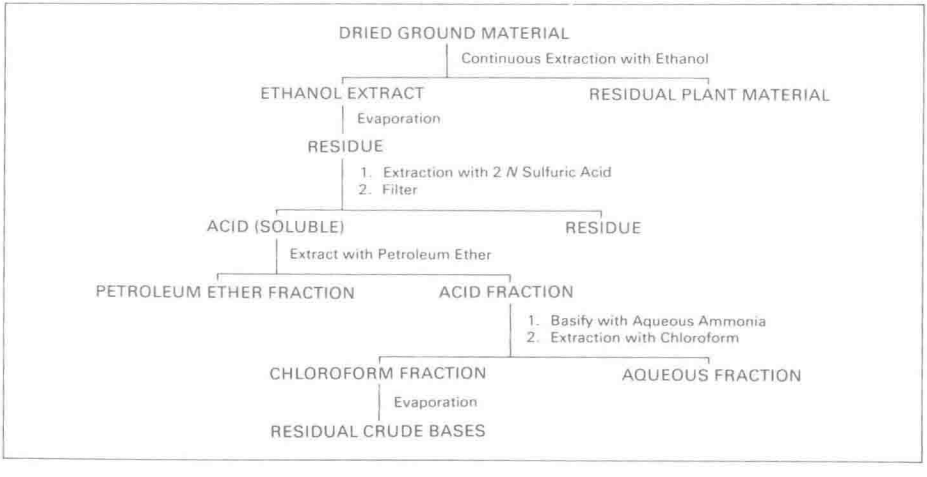
5

As already noted, Scheme II has been applied to the isolation of alkaloids but usually such substances are obtained by employing extraction with dilute aqueous acid as the key step. Two such procedures have been outlined in Schemes VI and VII. As an illustration, Scheme VI has been applied to the isolation of bisbenzylisoquinoline alkaloids from *Pycnarrhena ozantha*.[185] The procedure presented in Scheme VII has been applied to the isolation of related alkaloids from *Cyclea peltata*.[160,174] By means of Scheme VII fractional basification of citric acid fractions A and B with aqueous ammonia, followed by chloroform extraction, ion exchange chromatography, column chromatography (on basic and neutral alumina), and thin-layer chromatography (on alumina and silica gel plates) led to five bisbenzyltetrahydroisoquinoline alkaloids and three artifacts.[160,174]

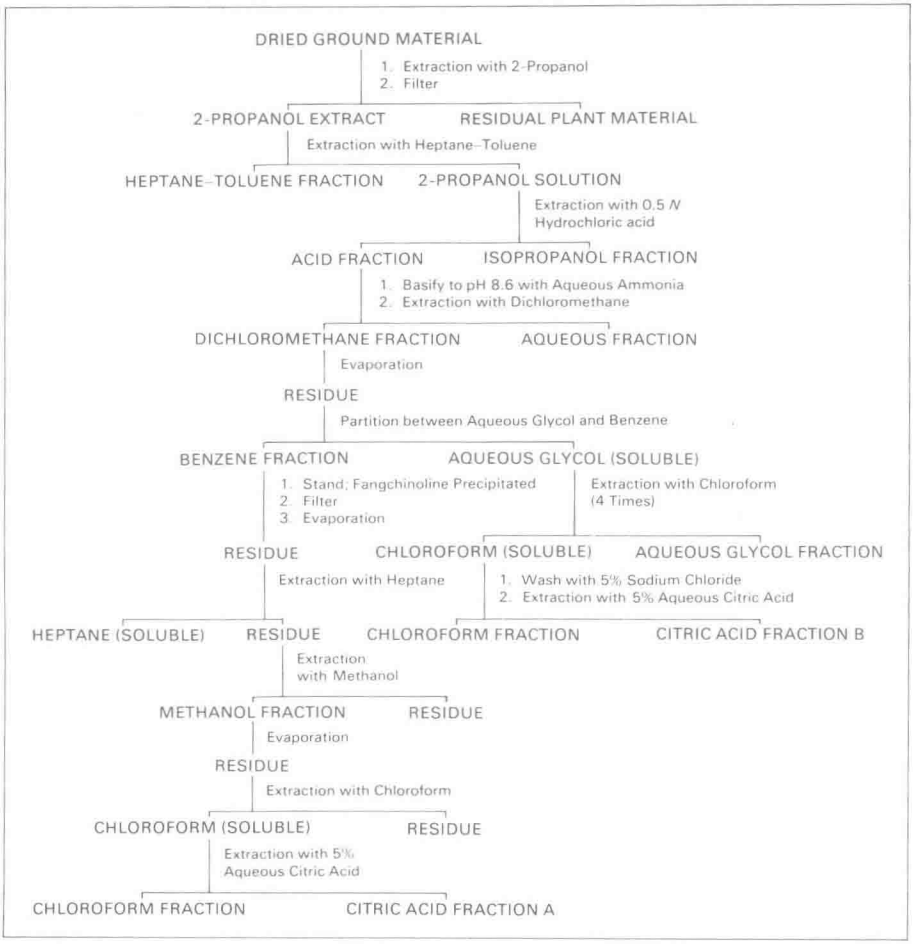
SCHEME V



SCHEME VI



SCHEME VII



When the practical limits of solvent partitioning have been reached, the next step generally involves selection and extensive application of one or more column, preparative-layer, and thin-layer chromatographic procedures. Such techniques range from proper orchestration of alumina and silica gel chromatographic adsorption techniques to gel permeation chromatography on Sephadex LH-20, the Sephadex G-10 to G-200 series, and the Sepharose series to 2B. Also, the various ion exchange resins ranging from the well-known cation and anion exchanges to the newer macroreticular resins of the XAD series may need to be utilized. In our group's isolation of antineoplastic agents from marine animals, arthropods, and plants, we have had to rely on many of the chromatographic procedures common in organic chemistry and biochemistry laboratories and devise improvements.[113,222,230,233,238,246,247,250]

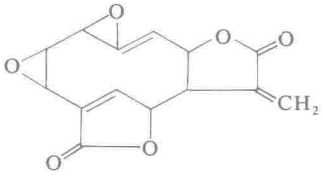
The actual isolation of a naturally occurring antineoplastic agent is nearly

always fraught with difficulties and every step requires expert judgment, improvision, and discovery. On the happy occasion when the isolated antineoplastic agent is a new substance the organic chemical problems begin in earnest. At this point purity must be assessed with great care as nature has a marvelous facility for producing very closely related substances in a particular species. Unless great care is exercised a mixture of two or more compounds may seem to be a pure substance. Here, various thin-layer chromatographic and physical measurements (such as infrared, proton magnetic resonance, and mass spectral) must be carefully interpreted. Establishment of the purity is followed by detailed antineoplastic evaluation and structural determination. The latter usually presents a new and challenging problem requiring all the best resources of instrumental (particularly x-ray crystallographic) and chemical methods of structural elucidation. This stage and subsequent research directed at total synthesis is one of great intellectual excitement and challenge for the chemist and is the starting point for further advances in biology and medicine. Both observations are splendidly illustrated in the following chapters and this is only the beginning.

Chapter 1

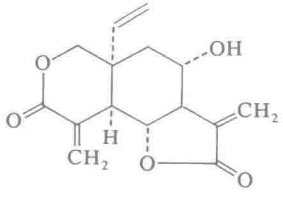
Higher Plant Terpenoids

$C_{15}H_{14}O_6$ Mikanolide



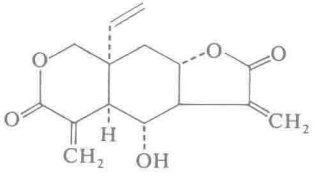
MOL. WT.: 290
BIOACTIVITY: KB: ED_{50}, <1 μg/ml
MELTING POINT: 230–233°C
$[\alpha]_D$: 53.4 SOLVENT: Di
SPECTRAL DATA: UV, IR, PMR
ORGANISM: *Mikania scandens* (Compositae)
REFERENCE: 93, 80

$C_{15}H_{16}O_5$ Vernolepin



MOL. WT.: 276
BIOACTIVITY: KB: ED_{50}, 2.0 μg/ml
WA: T/C, 32
MELTING POINT: 179–180°C
$[\alpha]_D$: +72 SOLVENT: An
SPECTRAL DATA: UV, IR, PMR, Mass Spec
ORGANISM: *Vernonia hymenolepis* A. Rich. (Compositae)
LOCATION: Ethiopia
REFERENCE: 153

$C_{15}H_{16}O_5$ Vernomenin



MOL. WT.: 276
BIOACTIVITY: KB: ED_{50}, 20 μg/ml
WA: T/C, 63 (5–8 mg/kg)
$[\alpha]_D$: −62 SOLVENT: An
SPECTRAL DATA: UV, IR, PMR, Mass Spec
ORGANISM: *Vernonia hymenolepis* A. Rich. (Compositae)
LOCATION: Ethiopia
REFERENCE: 153