Distribution of Anticancer Activity in Higher Plants

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SUMMARY

The systematic distribution in higher plants of general anticancer activity, high-interest anticancer activity, and high-interest compounds is presented. Special emphasis is placed on those taxonomic groups offering, by virtue of their high-interest activity and/or compounds, the greatest potential as sources of useful anticancer agents.

[Cancer Treat Rep 60:1081–1113, 1976]

The search for higher plant sources of anticancer drugs began in 1957, initiated by the Cancer Chemotherapy National Service Center, now the Drug Research and Development Program (DR&DP), of the National Cancer Institute (NCI). Three years later, botanists of the Agricultural Research Service, US Department of Agriculture, were asked to participate in this research program by procuring plant materials for screening. This liaison was propitious because it brought botanical expertise, methodology, and philosophy into the screening program at an early stage of its development.

The objectives of the screening program are to test a broad spectrum of biologically active plant constituents in a group of selected, experimental, in vivo and in vitro tumor systems and to identify those of potential value for the treatment of cancer in man. For reasons described in other papers in this symposium and elsewhere (1–3), it was decided that a worldwide, random procurement program would best suit the objectives of the current large-scale, long-term search for plant sources of anticancer drugs. With few exceptions, broad screening, completely at random, has been the established *modus operandi.*

In keeping with the screening objectives, the botanists' primary role is the procurement of plant samples. The botanical input, however, does not end with the collection, documentation, and identification of these samples. It was realized from the outset that the systematic distribution of anticancer activity and active agents among higher plants could provide a scientific basis for selective plant procurement, once enough screening data were available. The purpose of this presentation is to define the state of the art, as of June 1975, in attaining this objective.

DEFINITIONS AND LIMITATIONS

In the context of this paper, "higher plants" are those that have developed vascular systems: the pteridophytes, or ferns and their allies, and the spermatophytes, or seed plants, which include the gymnosperms and the angiosperms. The data on anticancer activity in higher plants come from the files of the DR&DP, NCI, and are limited to experimental tumor systems. Frequent reference will be made to "active genera," those containing one or more "active species." "Distribution of anticancer activity" means the distribution of activity in recognized taxonomic groups such as genera, families, orders, and subclasses.

METHODS

Since its inception, the screening program has generated a wealth of data, positive and negative, on anticancer activity in higher plants and the types of agents responsible. It has passed through many stages of evolution, as outlined in other papers in this symposium. Not only has the screen continually evolved, but the preparation of crude extracts for testing has changed also—the most recent during the last year when a preliminary fractionation procedure was adopted to concentrate

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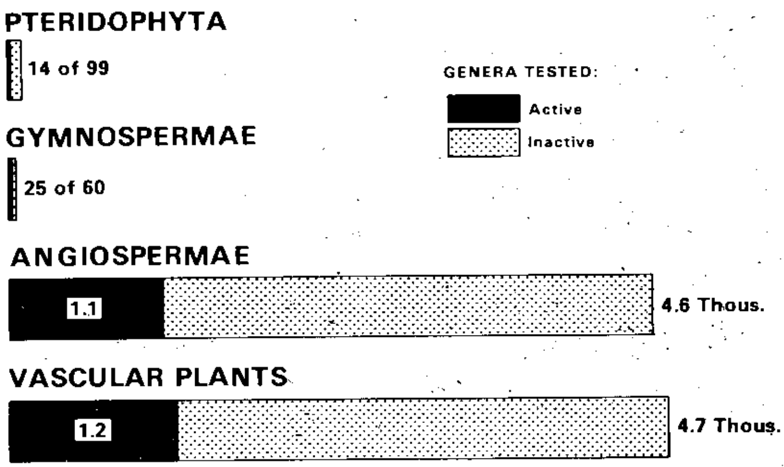

FIGURE 1.—Nos. of genera screened and active in the 3 major divisions of higher plants and in the higher (vascular) plants as a whole.

active agents before testing (4). All of these changes have a profound impact on the yield of anticancer activity and active agents.

As screening methods evolved, so did the significance of anticancer activity. Concomitantly, the accuracy of the screen in predicting potential sources of anticancer drugs increased. Earlier reviews of screening (3,5) revealed not only high concentrations of activity in certain plant families, but activity diffused among many families. It is now evident that this broad spectrum is due to the great chemical diversity of plant products capable of inhibiting cancer growth in laboratory animals. Furthermore, a high percentage of these plants owed their efficacy to tannins and phytosterols, nearly ubiquitous in higher plants, which have no real value as anticancer agents. The primary screen, a result of much experimentation, now consists of two tumor systems insensitive to tannins and phytosterols: a cell culture of human epidermoid carcinoma of the nasopharynx (KB) and mouse P388 lymphocytic leukemia (P388).

As used in this paper, anticancer activity includes effectiveness against all tumor systems, both current and obsolete. With general activity being so variable, it is evident that merely documenting its distribution in higher plants will not provide solid leads to sources of potentially useful anticancer agents. However, valuable clues can be obtained if close attention is given to activity against the in vivo tumor systems most likely to be predictive for clinical activity in man: namely, P388 and mouse L1210 lymphoid leukemia (L1210). When these data are combined with what is known about the nature of active compounds isolated from plants and incorporated into a reliable botanical classification, true

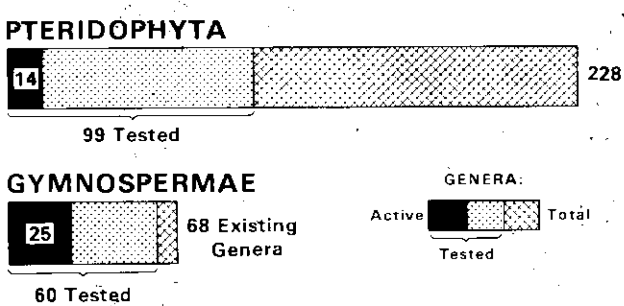

FIGURE 2.—Nos. of active, tested, and total genera in the Pteridophyta and Gymnospermae.

correlations emerge among activity, compounds, and related plant groups.

This paper will first discuss the distribution of anticancer activity in the major categories of higher plants, and will then narrow down to the most promising classes, subclasses, orders, and families. To bring the most important sources of potential anticancer agents into bold relief, three high-interest criteria were selected: (*a*) the five most important classes of chemical compounds;[2] (*b*) activity against L1210; and (*c*) high T/Cs in P388.[3] The

[2]Many varieties of compounds with anticancer activity have been isolated from higher plants. In this symposium, Hartwell (1) has identified those compounds belonging to five chemical classes as being of greater interest than the others: diterpenes, lignans, quassinoids, ansa macrolides, and alkaloids.

[3]In the two leukemia systems, no distinction is made between marginal and high activity except in the case of P388. The degree of activity in P388 is determined by the T/C or ratio of average survival of treated animals in days compared to that of controls × 100. While a T/C of 125% is an indication of activity, a figure of 175% is usually required for a priority high enough to warrant advanced screening (1). In the context of this paper, a high T/C in P388 is ≥ 175%.

resulting systematic distribution of these criteria among higher plants can provide guidance to productive plant procurement and screening.

SCOPE AND IMPLICATIONS OF DATA

Overall Extent of Anticancer Screening and Yield of Anticancer Activity in Higher Plants

Any comprehensive consideration of the distribution of anticancer activity in higher plants should take into account the proportion of plants screened to those not screened. More than 67,500 plant extracts have been screened, representing 20,525 species in 4716 genera and 317 families. Based on recent estimates,[4] about 9% of the species, 39% of the genera, and 83% of the families of higher plants have been screened.

Of the 67,500 extracts tested, 2787 have shown confirmed activity against one or more of the tumor systems used for screening since the inception of the program. The active extracts represent 2127 species in 1225 genera and 189 families. About 4% of the extracts, 10% of the species, and 26% of the genera submitted for screening were active.

Comparison of Anticancer Activity in the Major Groups of Higher Plants[5]

Figure 1 illustrates the number of genera screened and found to be active in the higher (vascular) plants as a whole and compares the number of genera screened and determined to be active in the pteridophytes, gymnosperms, and angiosperms. The Angiospermae far outweigh the Pteridophyta and Gymnospermae in numbers of tested and active genera. This is due to the sizes of the three groups; together, the pteridophytes and gymnosperms contain an estimated 296 genera, whereas the angiosperms contain 11,680 genera.

The number of tested, active, and total genera in the pteridophytes and gymnosperms is indicated in figure 2. In the pteridophytes, only 14% of the genera tested were active, whereas in the gymnosperms, 42% of the genera tested were active. The percentage of active genera in the gymnosperms is well above the 26% yield in the higher plants as a whole and three times greater than that of the pteridophytes.

[4]In this paper, the numbers of species and genera in the families, orders, subclasses, classes, and divisions of the higher plants are based on estimates from several sources (6–9).

[5]Throughout this paper, genera are used to illustrate comparative anticancer activity, since they, more than species, indicate the degree of morphologic and chemical diversity within taxonomic groups of plants at or above the family level.

So far, the pteridophytes have yielded practically no activity against the tumor systems currently in use. Only one fern, *Jamesonia imbricata* var. *glutinosa* (Karst.) A. Tryon, has shown activity against P388. The remaining active pteridophytes were active only in the obsolete, tannin-sensitive tumor systems. Indeed, the only active agents isolated from the pteridophytes are tannins. Hence, the ferns and their allies are eliminated from further consideration here. The more sophisticated preliminary fractionation prior to screening (4) may reveal promising activity in the pteridophytes, but this remains to be seen.

Among higher plants, truly promising activity and compounds have been found only in the spermatophytes. Therefore, the remainder of this presentation will concentrate on this group which constitutes the majority of dominant land plants: the gymnosperms and the angiosperms.

In terms of total genera and species available for screening, the gymnosperms are only a fraction the size of the angiosperms: 759 species in 68 genera and 12 families versus 220,000 species in 11,680 genera and 340 families. Nevertheless, the gymnosperms (table 1) are nearly twice as productive as the angiosperms in the yield of active extracts, species, and genera. However, the activity (table 1)

TABLE 1.—SCREENING AND ACTIVITY IN SPERMATOPHYTES

	Gymnospermae	Angiospermae
Extracts		
No. tested	2,164	64,634
No. active	166	2,602
% activity	8%	4%
Species		
No. tested	393	19,796
No. active	88	2,022
% activity	22%	10%
Genera		
No. tested	60	4,557
No. active	25	1,066
% activity	42%	23%
Families		
No. tested	11	285
No. active	8	175
% activity	73%	61%

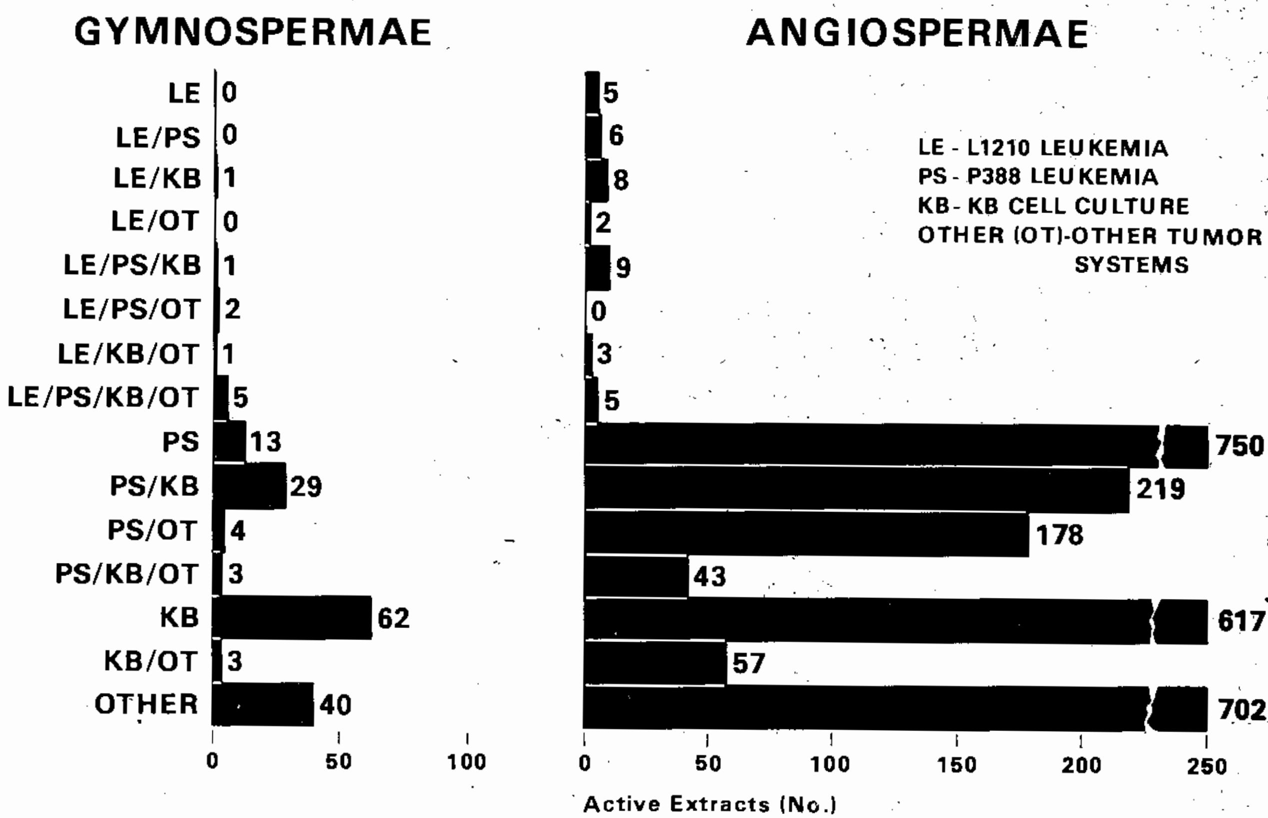

FIGURE 3.—"Activity profiles" for the Angiospermae and Gymnospermae. *See* text for further details.

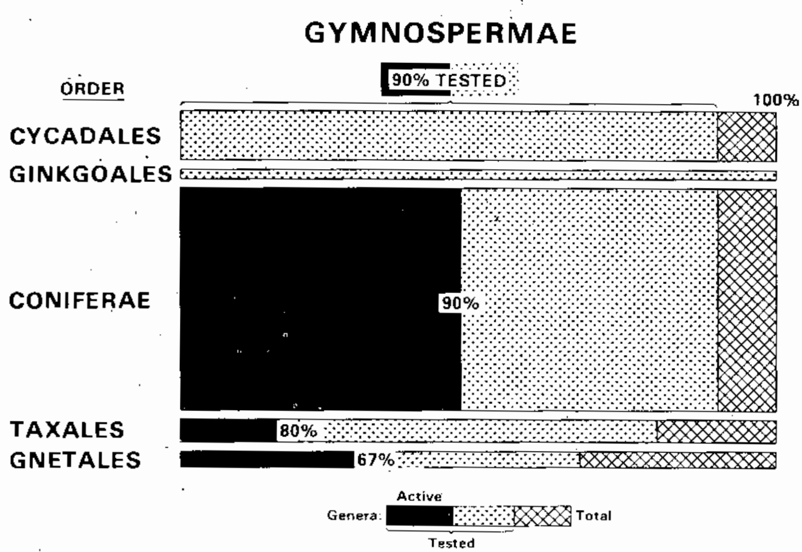

FIGURE 4.—Extent of screening and comparative yields of anticancer activity in orders of Gymnospermae. The 5 orders of Gymnospermae are represented by horizontal bars. The depth of each bar, determined by the number of genera in the orders, is a measure of their relative size and diversity. The length represents 100% of the genera in each order. The combined solid and stippled portions indicate the percentage of genera tested in the orders, whereas the crosshatched portion indicates the percentage of genera not yet screened. The solid portion of the bar represents only the percentage of activity in the genera tested, not a percentage of the whole.

is the general activity accrued against all tumor systems, current and obsolete.

To gain a perspective of the amount of potentially useful activity in the gymnosperms and angiosperms, it is worthwhile to examine the distribution of activity in the tumor systems used in the screen, with major emphasis placed on those currently in use. For this purpose, "activity profiles" were prepared (*see* fig 3) for both gymnosperms and angiosperms. In these, the obsolete systems discussed in other papers of this symposium (1,10) appear in the "other" category. As shown in figure 3, the screen consists of KB, P388, L1210, and other. There are 15 possible combinations of these. By definition, an "active extract" must be effective against one or more of the systems represented.

There is one striking difference between the two "activity profiles" (fig 3). In the Gymnospermae, activity is proportionally highest in KB, while in the Angiospermae, activity is highest in P388. The high KB activity of the gymnosperms is probably due to the presence of cytotoxic lignans, to which KB is sensitive.

In both the Gymnospermae and Angiospermae, there are significant numbers of extracts active in both KB and P388, and the chances are good that many of these will prove to be active against L1210. Active compounds isolated from these plants will later be evaluated in the slow-growing solid tumors: mouse B16 melanoma and the new mouse Lewis lung carcinoma (LL). Those that are active in B16 or LL will become strong candidates for preclinical evaluation.

Anticancer Activity in Orders and Families of the Gymnospermae

Figures 4 and 5 illustrate the extent of screening and comparative yields of activity in the orders and families of the Gymnospermae as recognized in the 12th edition of *A. Engler's Syllabus der Pflanzenfamilien* (8).

Figure 4 depicts the relative sizes of the five orders of Gymnospermae. The percentage of genera tested is numerically indicated and the solid portion represents the percentage of these found active.

Only three orders are of significance. One of these, the Gnetales, has little activity in the important tumor systems.

Figure 5 narrows the field to families of the two orders having significant activity: (*a*) the Coniferae containing the families Pinaceae, Taxodiaceae, Cupressaceae, Podocarpaceae, Cephalotaxaceae, and Araucariaceae; and (*b*) the Taxales containing the single family Taxaceae. The left bar graph illustrates the number of active, tested, and total genera in each family. These families have been intensively screened and each contains active genera.

The right bar graph shows the percent of active extracts, a good indicator of promising groups. This points up three attractive families: the Cupressaceae, Cephalotaxaceae, and Taxaceae. In fact, these are the only families in the Gymnospermae having L1210 activity (table 2).

Distribution of High-Interest Activity and Compounds in Families of the Gymnospermae

In this section, the high-interest activity and compound criteria referred to earlier will be applied to further narrow the field to those families with the greatest potential as sources of anticancer agents.

In the Gymnospermae, high-interest compounds have been isolated from 15 species in eight genera and five families (table 2). Among these compounds are three alkaloids included in Hartwell's list (1) of the 21 most interesting compounds isolated from plants: harringtonine, homoharringtonine, and taxol. Also, ten species in five genera and three families are active against L1210 and seven species in five genera and four families have high T/Cs in P388 (table 3).

These selected criteria identify at least three families of special interest: the Cephalotaxaceae, Cupressaceae, and Taxaceae. Though of less interest, the Podocarpaceae and Taxodiaceae are promising families worthy of further exploration.

Although the Gymnosperms have been an important source of potentially valuable compounds, the possibilities of locating new active compounds would appear to be low because few genera remain to be screened (cf, figs 4 and 5). Nevertheless, the new extraction procedure (4) may reveal additional novel compounds of key interest in those genera already tested and found inactive.

Roughly 88% of the genera and 52% of the species of Gymnospermae have been screened. In comparison, only 39% of the genera and 9% of the species of Angiospermae have been screened. The Angiospermae contain, by far, the greatest number of active genera and species and have been the source of most of the high-interest compounds. They are clearly the more promising group for future screening.

Extent of Screening and Activity in the Classes, Subclasses, and Orders of the Angiospermae

The remainder of this paper will focus on the distribution of anticancer activity and high-interest

TABLE 2.—HIGH-INTEREST COMPOUNDS IN GYMNOPERMAE

COMPOUND	GENUS AND SPECIES	FAMILY
Alkaloids		
Harringtonine	Cephalotaxus harringtonia (Knight ex Forbes) K. Koch	Cephalotaxaceae
Homoharringtonine	" " " " " " "	"
Isoharringtonine	" " " " " " "	"
Methoxyharringtonine	" " " " " " "	"
Taxol	Taxus brevifolia Nutt.	Taxaceae
	T. canadensis Marsh.	"
	T. cuspidata Sieb. & Zucc.	"
	T. X media Rehd.	"
a Tetraol	T. brevifolia	"
Diterpenes		
Podolide	Podocarpus gracilior Pilg.	Podocarpaceae
Taxodione	Taxodium distichum (L.) Rich.	Taxodiaceae
Taxodone	" " " "	"
Lignans*		
Deoxypodophyllotoxin	Callitris columellaris F. Muell.	Cupressaceae
	Calocedrus decurrens (Torr.) Florin	"
	Juniperus communis L.	"
	Thuja occidentalis L.	"
Podophyllotoxin	Juniperus chinensis L.	"
	J. virginiana L.	"
Podophyllotoxin glucoside	Callitris drummondii (Parl.) F. Muell.	"

*Until recently, the lignans were considered to be low interest types of compounds; podophyllotoxin and several related natural products proved to be of little clinical interest. However, their status has been changed to the high interest category because several semi-synthetic derivatives have shown some clinical activity against brain tumors, lymphosarcomas and Hodgkin's disease (1).

TABLE 3.—HIGH-INTEREST ACTIVITY IN GYMNOSPERMAE

FAMILY, GENUS AND SPECIES	T/C in P388 = 175%	L1210 Activity
Cupressaceae		
Chamaecyparis lawsoniana (A. Murr.) Parl.		+
Cupressus lusitanica Mill.	+	
Juniperus chinensis L.	+	+
J. communis L.		+
J. scopulorum Sarg.		+
Thuja occidentalis L.		+
Podocarpaceae		
Podocarpus gracilior Pilg.	+	
P. milanjianus Rendle	+	
Cephalotaxaceae		
Cephalotaxus harringtonia (Knight ex Forbes) K. Koch	+	+
Cephalotaxus wilsoniana Hayata	+	
Taxaceae		
Taxus baccata L.		+
T. brevifolia Nutt.		+
T. canadensis Marsh.		+
T. cuspidata Sieb. & Zucc.		+
T. X media Rehd.	+	

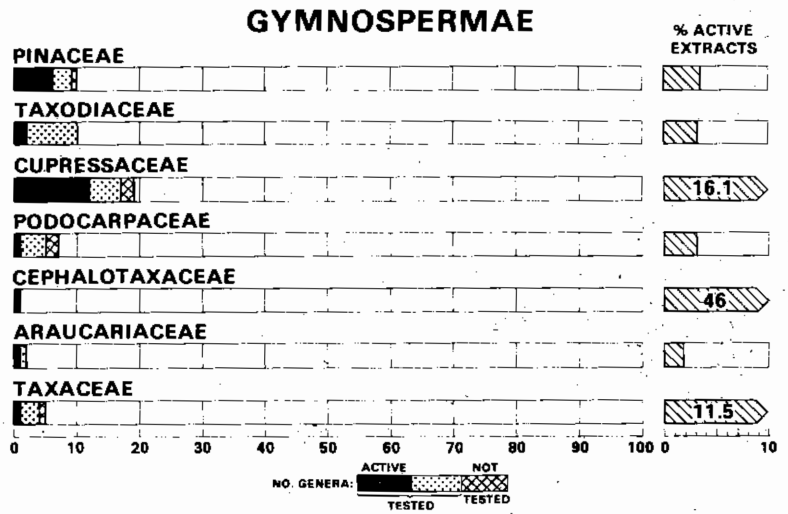

FIGURE 5.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in families of Coniferae and Taxales.

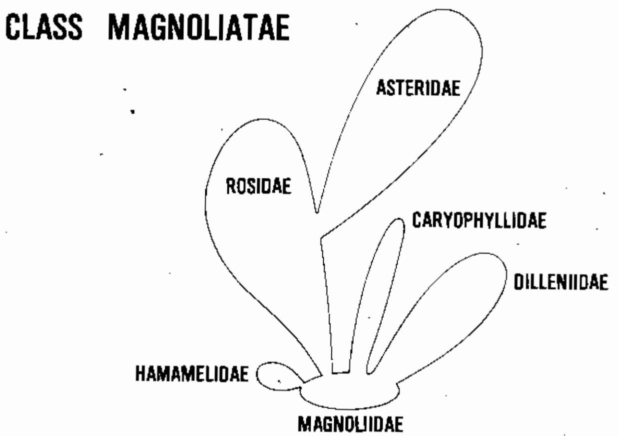

FIGURE 6.—Phyletic diagram illustrating Cronquist's (7) conception of the probable relationships among the 6 subclasses of Magnoliatae. The size of each balloon is proportional to the number of species in each subclass. [Redrawn with slight modifications from Cronquist (7)].

TABLE 4.—SCREENING AND ACTIVITY IN CLASSES OF ANGIOSPERMAE

	Magnoliatae	Liliatae
Extracts		
No. tested	59,640	4,994
No. active	2,366	236
% activity	4%	5%
Species		
No. tested	17,829	1,940
No. active	1,837	185
% activity	10%	10%
Genera		
No. tested	3,950	607
No. active	956	110
% activity	24%	18%
Families		
No. tested	242	43
No. active	157	18
% activity	65%	42%

compounds in the Angiospermae. This, and the following discussions of the Angiospermae, will draw heavily on the system of classification outlined by Arthur Cronquist in his book, *The Evolution and Classification of Flowering Plants* (7).

In Cronquist's system of classification, the Magnoliophyta or Angiospermae are divided into two classes: the Magnoliatae or dicots, and the Liliatae or monocots. According to his estimates, the Liliatae are only a third the size of the Magnoliatae: 55,000 species *versus* 165,000 species. The majority of the Angiospermae screened belong to the Magnoliatae (table 4). The two classes are nearly equal in percentages of active extracts and species, but the percentages of active genera and families are significantly higher in the Magnoliatae. The reasons for this will be apparent later.

Figures 6 and 7 illustrate Cronquist's concepts of the probable relationships among the subclasses of the Magnoliatae and Liliatae. In both figures, the

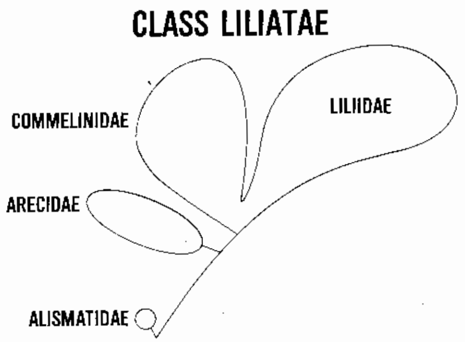

FIGURE 7.—Phyletic diagram illustrating Cronquist's (7) conception of the probable relationships among the 4 subclasses of Liliatae. The size of each balloon is proportional to the number of species in each subclass. [Redrawn with slight modifications from Cronquist (7)].

size of each "balloon" is proportional to the number of species in the subclass. It is not apparent from these diagrams, but according to Cronquist, "The Magnoliidae are the basal complex from which all other angiosperms have been derived."

Figures 8–17 show the relative sizes of the principal orders of the ten subclasses. The percentage of genera tested is numerically indicated and the solid portion represents the percentage of these found active. The orders and their sequence in the graphs follow Cronquist's system of classification. In some of the graphs, one or more small orders have been excluded.

Class Magnoliatae

Subclass Magnoliidae (11,000 species in 473 gen-

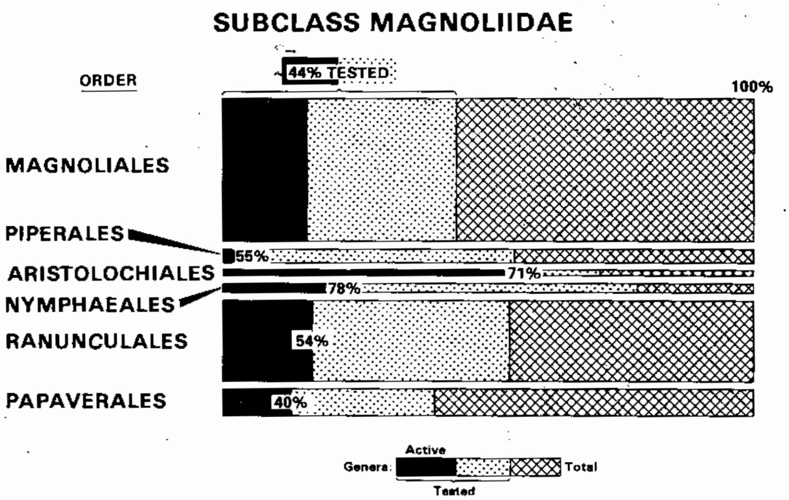

FIGURE 8.—Extent of screening and comparative yields of anticancer activity in the 6 orders of Magnoliidae. *See* fig 4 for explanation and interpretation.

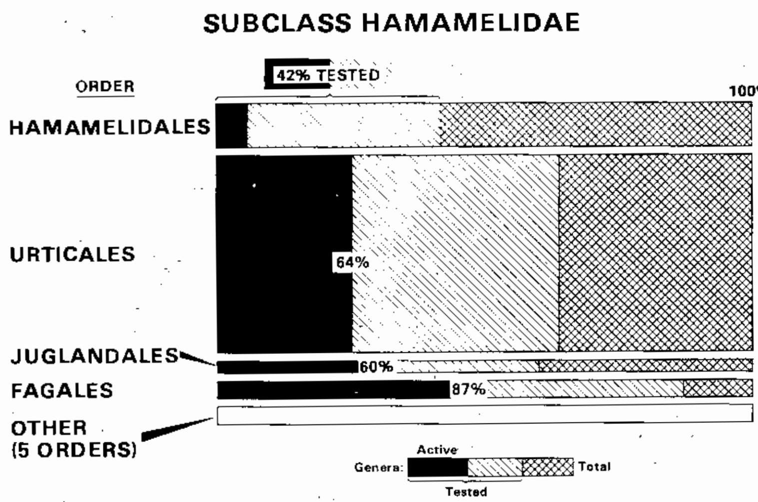

FIGURE 9.—Extent of screening and comparative yields of anticancer activity in 4 of the 9 orders of Hamamelidae. *See* fig 4 for explanation and interpretation.

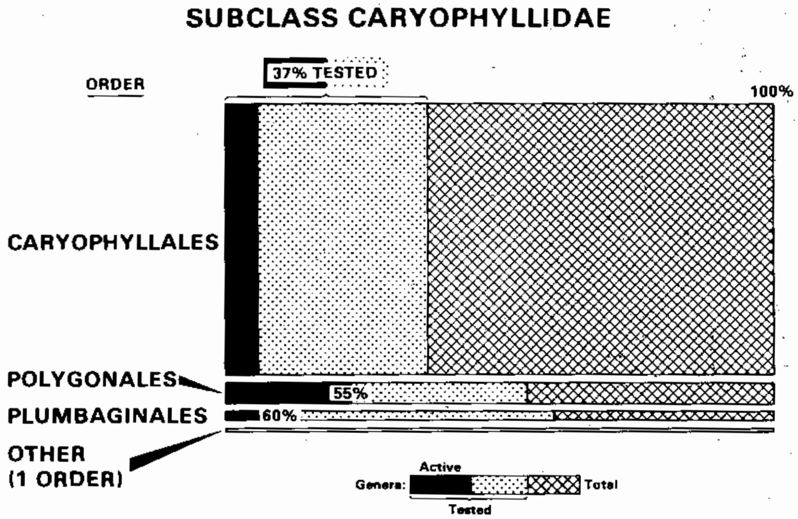

FIGURE 10.—Extent of screening and comparative yields of anticancer activity in 3 of the 4 orders of Caryophyllidae. *See* fig 4 for explanation and interpretation.

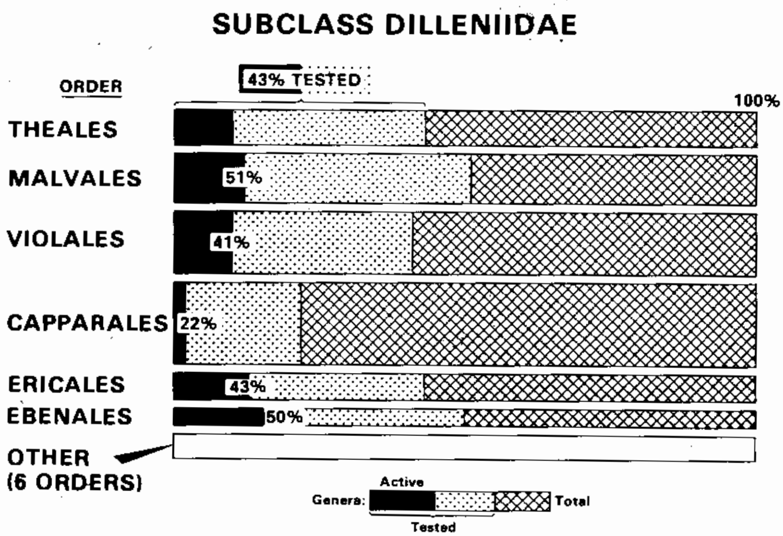

FIGURE 11.—Extent of screening and comparative yields of anticancer activity in 6 of the 12 orders of Dilleniidae. *See* fig 4 for explanation and interpretation.

era, 34 families, and six orders).—All orders of this primitive subclass have been screened (fig 8) and activity has been found in each. With the exception of the Piperales, the percentage of active genera in these orders is above the 26% yield for active genera in the higher plants as a whole: 36% for the Magnoliales, 80% for the Aristolochiales, 29% for the Nympheales, 33% for the Ranunculales, and 32% for the Papaverales. Also, high-interest compounds and activity have been found in four of the six orders.

Subclass Hamamelidae (3400 species in 184 genera, 21 families, and nine orders).—In this smallest subclass of the Magnoliatae, representatives of six of the nine orders screened were found active. Two of the inactive orders, the Eucommiales and Leitneriales, are monotypic, and the third, the Trochodendrales, contains only two species. The more important orders of the subclass are included in figure 9. The orders in this subclass have been broadly screened, and a substantial number of genera are active. However, the only valuable activity and compounds are concentrated in the Urticales. This order contains nearly 70% of the genera in the subclass.

Subclass Caryophyllidae (11,000 species in 554 genera, 17 families, and four orders).—Although activity occurs in three of the four orders tested (fig

10), the active compounds were found to be tannins, phytosterols, saponins, and proteins, all of little interest. Although an active alkaloid was isolated from one cactus species, this relatively small subclass seems to offer little promise.

Subclass Dilleniidae (24,000 species in 1437 genera, 72 families, and 12 orders).—The principal orders in this subclass are represented in figure 11. Activity was found in all but the Diapensiales, but the active compounds isolated so far are mainly tannins, phytosterols, and cucurbitacins which now are of no interest.

Subclass Rosidae (60,000 species in 3140 genera, 100 families, and 16 orders).—The most important orders in this largest subclass of the Magnoliatae are included in figure 12. Two of the smallest, the Podostemonales and Rafflesiales, have not yet been screened, but of the 14 tested, activity was found in all but the Haloragales. Although the orders in figure 12 appear to have activity similar to that shown in the preceding figures, these orders are much more promising because of the types of active compounds isolated.

Subclass Asteridae (56,000 species in 3325 genera, 43 families, and nine orders).—Anticancer activity has been found in all nine orders (fig 13). In contrast to the other subclasses of the Magnoliatae, the Asteridae, at the evolutionary pinnacle of the dicots, are derived from the Rosidae (*see* fig 6). Like the Rosidae, they are a rich source of high-interest compounds and activity.

It is evident that anticancer activity is nearly ubiquitous among the dicots: 54 of the 56 orders have been screened and anticancer activity has been found in all but six, each of which is very small.

Class Liliatae

Subclass Alismatidae (500 species in 55 genera, ten families, and four orders).—Although one order remains to be screened, no activity has been found in the chiefly aquatic Alismatidae (fig 14).

Subclass Commelinidae (19,000 species in 960 genera, 20 families, and eight orders).—The five principal orders in this subclass are depicted in figure 15. With the exception of the Eriocaulales, activity was found in all orders. However, the percentage of active genera in the subclass is only 13%, with most of the activity attributable to tannins. Because they contain the grass family, the Commelinidae are one of the most important groups of economic plants. However, they appear to offer little hope as sources of anticancer drugs. Botanists involved in the plant screening program commonly refer to a promising plant family as a FOSI, or *family of special interest;* those of no interest are described by the acronym FONI, or *family of no interest*. Past screening experience relegates the grass family to the FONI category. In fact, the grasses show so little promise that they were the first family so designated. The orders of the Commelinidae follow the pattern of the grass family.

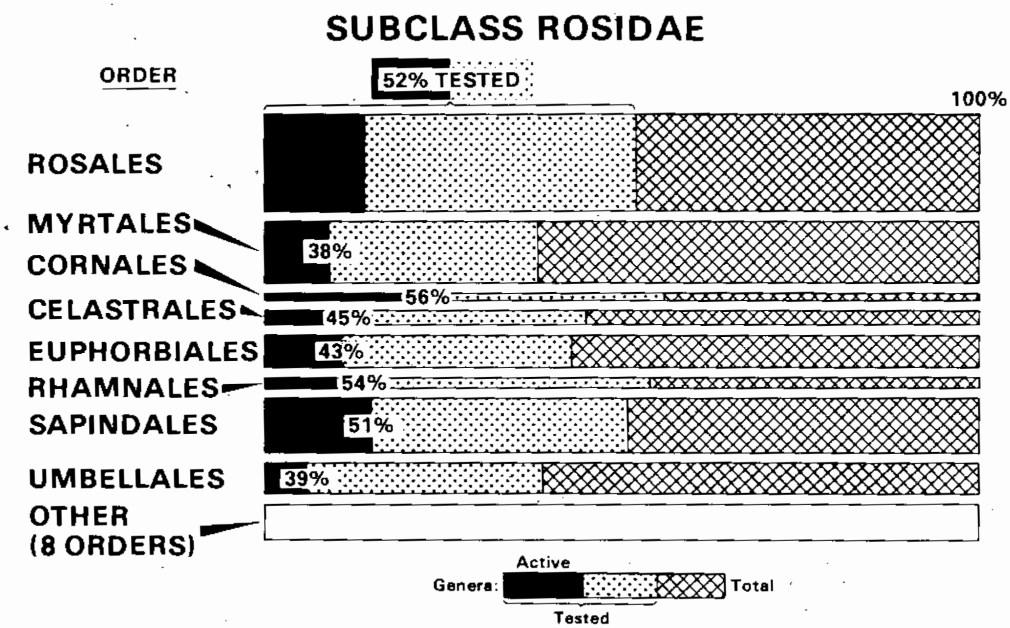

FIGURE 12.—Extent of screening and comparative yields of anticancer activity in 8 of the 16 orders of Rosidae. *See* fig 4 for explanation and interpretation.

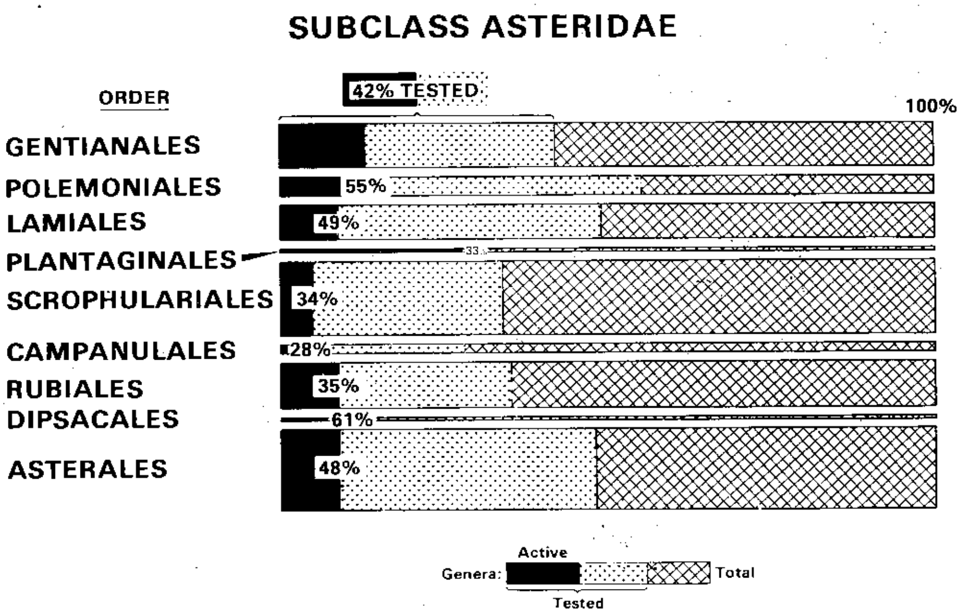

FIGURE 13.—Extent of screening and comparative yields of anticancer activity in the 9 orders of Asteridae. *See* fig 4 for explanation and interpretation.

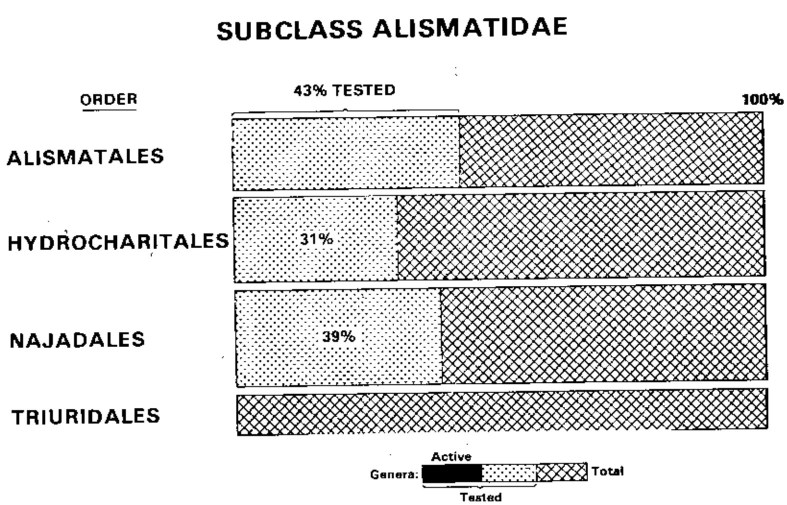

FIGURE 14.—Extent of screening and comparative yields of anticancer activity in the 4 orders of Alismatidae. *See* fig 4 for explanation and interpretation.

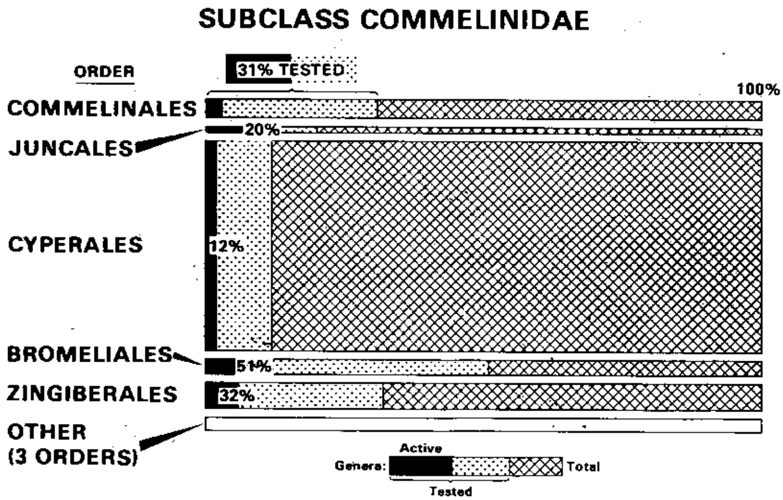

FIGURE 15.—Extent of screening and comparative yields of anticancer activity in 5 of the 8 orders of Commelinidae. *See* fig 4 for explanation and interpretation.

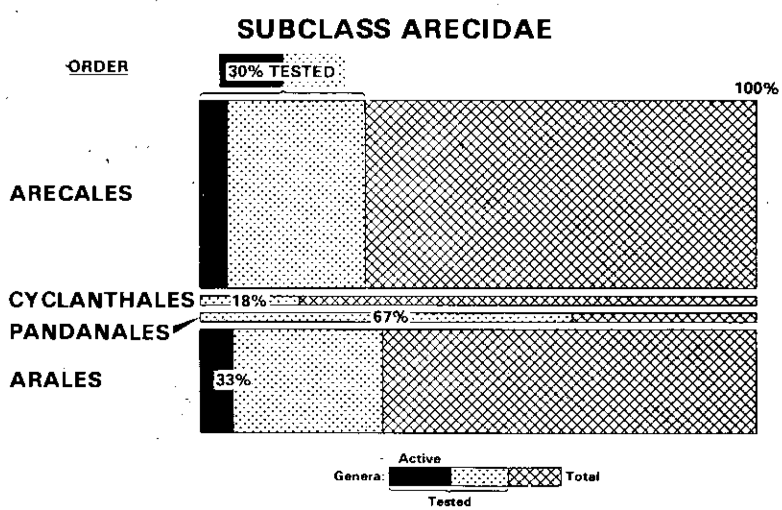

FIGURE 16.—Extent of screening and comparative yields of anticancer activity in the 4 orders of Arecidae. *See* fig 4 for explanation and interpretation.

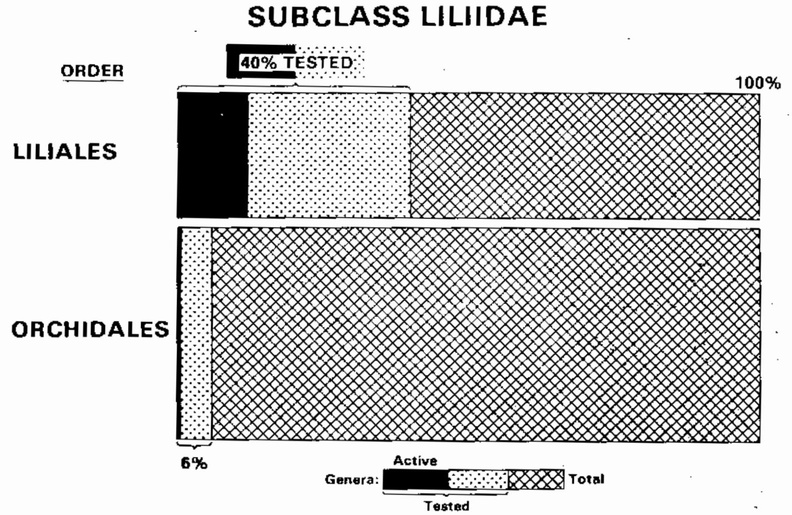

FIGURE 17.—Extent of screening and comparative yields of anticancer activity in the 2 orders of Liliidae. *See* fig 4 for explanation and interpretation.

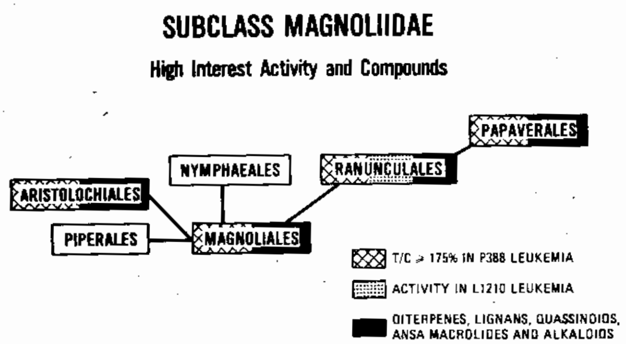

FIGURE 18.—Distribution of high-interest activity and compounds among the orders of Magnoliidae. Here, and in the following 4 figs, the phyletic diagrams illustrate Cronquist's (7) concepts of the probable relationships among the orders of the subclasses. Also, according to Cronquist, "the length of the line between two orders is governed by the requirements of two-dimensional representation and does not indicate the closeness of the relationship." [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

Subclass Arecidae (6400 species in 352 genera, five families, and four orders).—This subclass includes the palms, aroids, and screwpines (fig 16). Activity is present in two of the four orders, but the only active compounds isolated from the entire group are tannins.

Subclass Liliidae (28,000 species in 1200 genera, 18 families, and two orders).—The family Orchidaceae, containing an estimated 17,000 species in 735 genera, makes the Orchidales the larger of the two orders (fig 17). Although they are one of the largest families of flowering plants, rarely is any one species abundant enough to provide adequate samples for screening. Consequently, the Liliales have received the greatest attention, proving to be an excellent source of high-interest compounds and promising activity. Among the monocots, they hold the greatest promise for future screening.

Distribution of High-Interest Activity and Compounds in the Subclasses and Orders of the Angiospermae

It is apparent from the preceding discussion and figures that general anticancer activity, without additional criteria, will not provide leads to the most promising sources of anticancer drugs. Subclasses appearing to have a fair amount of activity can be of low interest because the plants tested lack activity against the current in vivo tumor systems, or because the active compounds isolated are not of interest.

Obviously, the potential of taxonomic groups will rise or fall on the basis of the compounds they provide. However, the agents responsible for the activity in many plants are unknown. Consequently, program guidance must also rely on high-interest activity.

In this section, the three high-interest criteria previously applied to the gymnosperms will again be applied to identify those subclasses and orders with the most potential. A combination of these criteria will reinforce those taxonomic groups already known to be important sources of compounds, and should indicate where to look for new sources of novel, high-interest compounds.

The following discussion and figures will continue to draw heavily on Cronquist's system of classification (7). The phyletic diagrams used in the following figures were designed by him to express his concepts of probable relationships among the orders of the subclasses. These have proved particularly adaptable to the illustration of the distribution of high-interest compounds and activity.

The discussion following this one will narrow down to the families in orders identified by the three criteria.

Class Magnoliatae

Subclass Magnoliidae.—Figure 18 illustrates the distribution of high-interest compounds and activity among the orders. Two important types of compounds have been isolated, lignans and alkaloids; their occurrence in orders of the Magnoliidae is shown in table 5. Lignans occur in three related orders, including two that also occur in the gymnosperms: podophyllotoxin and deoxypodophyllotoxin. Active alkaloids have also been isolated from members of three orders following the chain of evolution at approximately two o'clock in the diagram (fig 18). They include thalicarpine and liriodendine in the Magnoliales, thalicarpine and berberine in the Ranunculales, and berberine in the Papaverales. Species with high T/Cs in P388 occur in four orders, and two species in the Ranunculales have activity against L1210. The bioassay criteria have identified no new orders of interest and the Piperales and Nymphaeales appear to offer little promise for the future.

Subclass Hamamelidae.—There is a broad spectrum of activity among the orders of this subclass (*see* fig 9), due almost entirely to tannins, occurring here in great abundance, and phytosterols. Only in the order Urticales is there any promise for the future (fig 19): the active alkaloid cryptopleurine has been isolated from one species, another species has activity against L1210, and two species have high T/Cs in P388.

Subclass Caryophyllidae.—Only one active alkaloid, pilocereine, has been isolated from a cactus species (*Lophocereus schottii* [Engelm.] N. L. Britt. & Rose). Hence, this subclass is eliminated from further discussion.

Subclass Dilleniidae.—This subclass constitutes a fairly large element of the dicots (fig 6). Intensive screening has not uncovered any promising compounds (fig 20), although the activity data suggest that a closer examination of the Theales, Malvales, Violales, and Ebenales may be worthwhile.

Subclass Rosidae.—This subclass is the largest in the angiosperms and the richest in high-interest compounds and activity. Of the 16 orders in figure

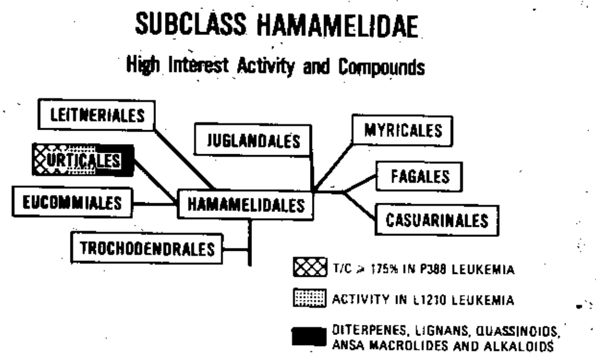

FIGURE 19.—Distribution of high-interest activity and compounds among the orders of Hamamelidae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

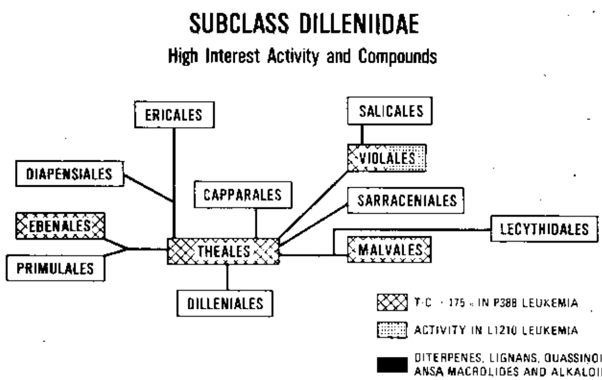

FIGURE 20.—Distribution of high-interest activity and compounds among the orders of Dilleniidae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

TABLE 5.—HIGH-INTEREST COMPOUNDS IN ORDERS OF MAGNOLIIDAE

	Chemical Classes				
Orders	Diterpenes	Lignans	Quassinoids	Ansa macrolides	Alkaloids
Magnoliales		+			+
Aristolochiales		+			
Ranunculales		+			+
Papaverales					+

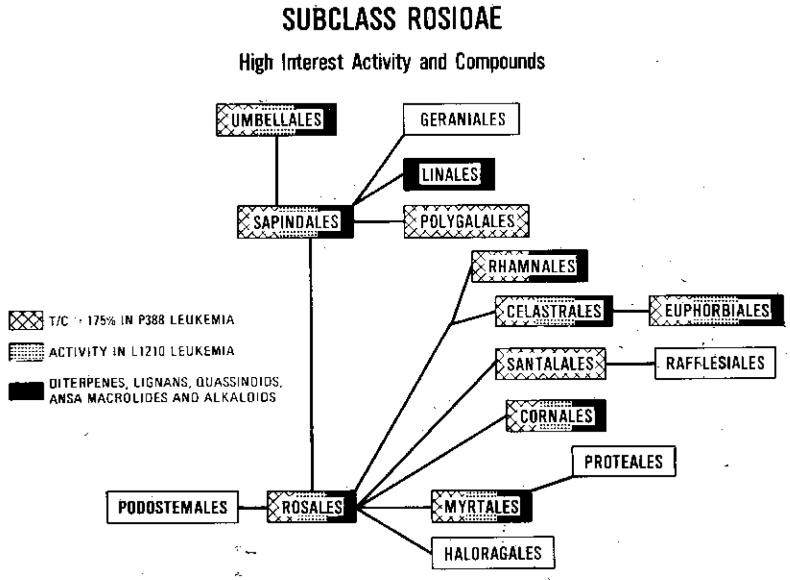

FIGURE 21.—Distribution of high-interest activity and compounds among the orders of Rosidae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

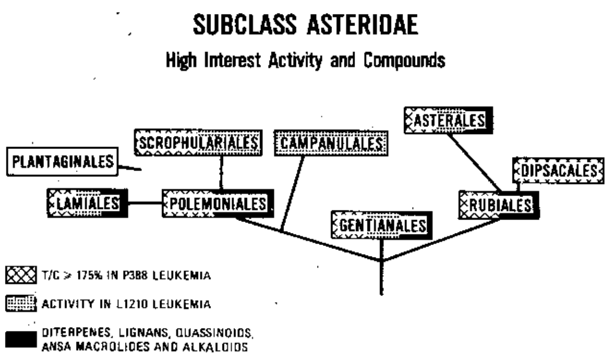

FIGURE 22.—Distribution of high-interest activity and compounds among the orders of Asteridae. *See* fig 18 and text for explanation and interpretation. [Phyletic diagram redrawn with slight modifications from Cronquist (7)].

21, nine have high-interest compounds, seven have L1210 activity, and ten have high T/Cs in P388. Active compounds belonging to all five of the important chemical classes have been isolated from members of the Rosidae (table 6). These include seven of the 21 plant-derived compounds of greatest interest (1): the diterpenes—tripdiolide and triptolide; the quassinoid—bruceantin; the ansa macrolide—maytansine; and the alkaloids—camptothecin, fagaronine, and nitidine. This subclass also includes two compounds not belonging to the five important chemical classes but selected for preclinical pharmacology and possible clinical trial: Acer saponin P and the protein, cesalin. Although these compounds are inactive in the leukemias and slow-growing tumors (B16 and LL), they represent the best of two large chemical classes.

Some interesting chemotaxonomic relationships are revealed by an examination of table 6, where the sequence of orders follows Cronquist's classification (7), and figure 21, showing the probable relationships among the orders of the subclass.

Diterpenes.—Active diterpenes were isolated from plants in the orders Myrtales, Celastrales, and Euphorbiales.

Lignans.—Lignans are concentrated in three related orders: the Sapindales, Umbellales, and Linales.

Quassinoids.—This unique class of compounds is found in one family of the Sapindales.

Ansa macrolides.—These compounds were isolated from two species in closely related orders with a common origin: the Celastrales and Rhamnales. Ansa compounds were originally found as microbial products, and their occurrence in higher plants was once suspected to be due to microorganisms associated with the plant sources. However, the close relationship of the orders suggests that ansa macrolides may also be bona fide higher-plant products.

Alkaloids.—With the exception of the Euphorbiales, all of the orders containing alkaloids stem directly from the Rosales. Among the alkaloids isolated is camptothecin which occurs in both the Cornales and Celastrales.

Present knowledge indicates that members of the

TABLE 6.—HIGH-INTEREST COMPOUNDS IN ORDERS OF ROSIDAE

Orders	Chemical Classes: Diterpenes	Lignans	Quassinoids	Ansa macrolides	Alkaloids
Rosales					+
Myrtales	+				+
Cornales					+
Celastrales	+			+	+
Euphorbiales	+				+
Rhamnales				+	
Sapindales		+	+		+
Linales		+			
Umbellales		+			

TABLE 7.—HIGH-INTEREST COMPOUNDS IN ORDERS OF ASTERIDAE

Orders	Chemical Classes: Diterpenes	Lignans	Quassinoids	Ansa macrolides	Alkaloids
Gentianales					+
Polemoniales					+
Lamiales					+
Rubiales					+
Asterales					+

subclass Rosidae will play a major role in the future search for plant sources of anticancer drugs.

The Myrtales have not yet produced a compound destined for preclinical evaluation. However, on the basis of the diterpenes isolated from one family, the Thymelaeaceae, it is likely that this order will yield one or more such compounds. And, from data to be discussed later, the Euphorbiales are beginning to show real potential.

The Rosidae also appear promising from the activity data: 15 species in 13 genera and 11 families are active against L1210, and 95 species in 56 genera and 16 families have high T/Cs in P388. The T/C data identify two additional orders that deserve closer inspection: the Santalales and the Polygalales.

Subclass Asteridae.—This subclass, derived from the Rosidae, also has high potential. The only drugs from higher plants fully established in the treatment of cancer come from a member of this subclass: the vinca alkaloids from *Catharanthus roseus* (L.) G. Don in the family Apocynaceae of the order Gentianales. The occurrence of high-interest compounds and activity in the orders of subclass Asteridae is reflected in figure 22. In contrast to the

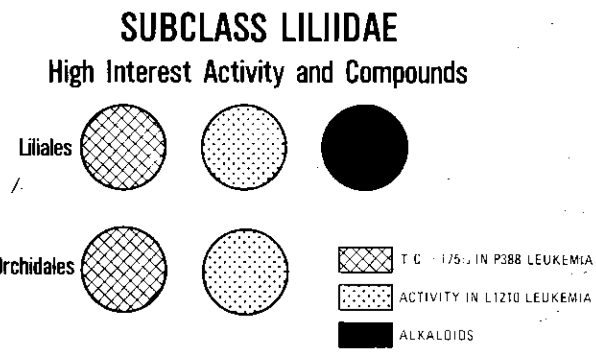

FIGURE 23.—Distribution of high-interest activity and compounds among the orders of Liliidae.

Rosidae, all of the high-interest compounds are alkaloids (table 7), including ellipticine, 9-methoxyellipticine, emetine, indicine-*N*-oxide, and tylocrebrine. This subclass also includes a compound on Hartwell's list not belonging to the five chemical classes: the quinone lapachol.

The activity data for the Asteridae are also highly promising: six species in six genera and five families

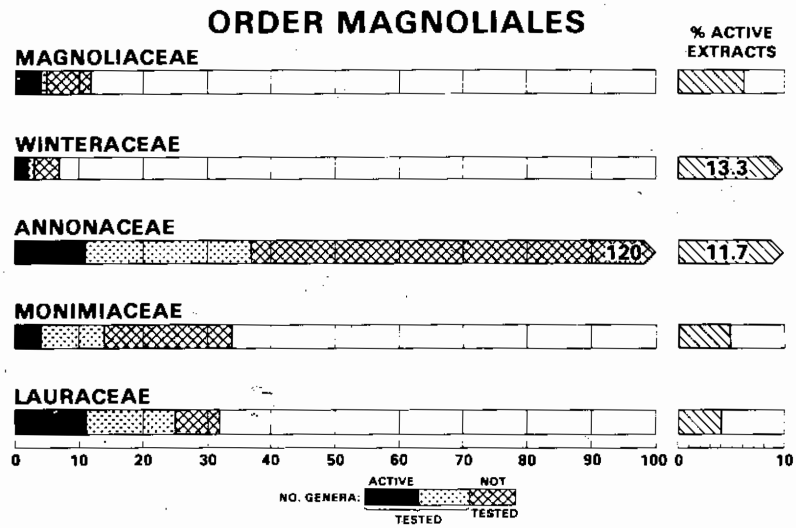

FIGURE 24.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 5 families of Magnoliales.

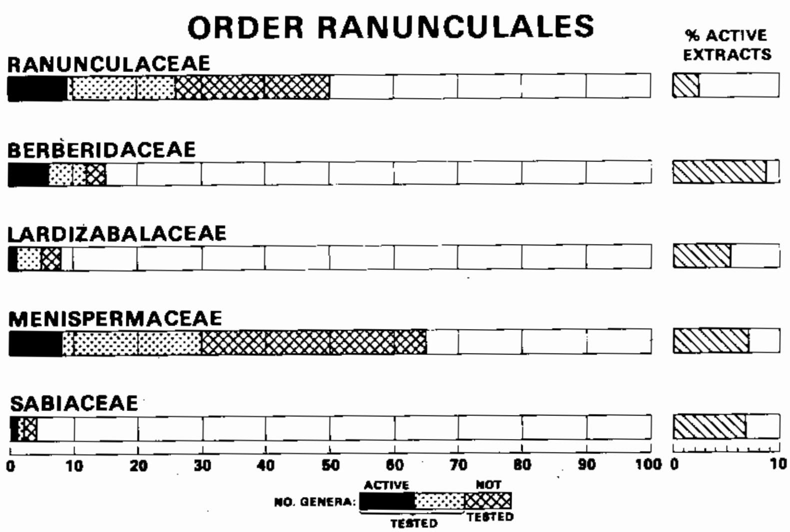

FIGURE 25.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 5 families of Ranunculales.

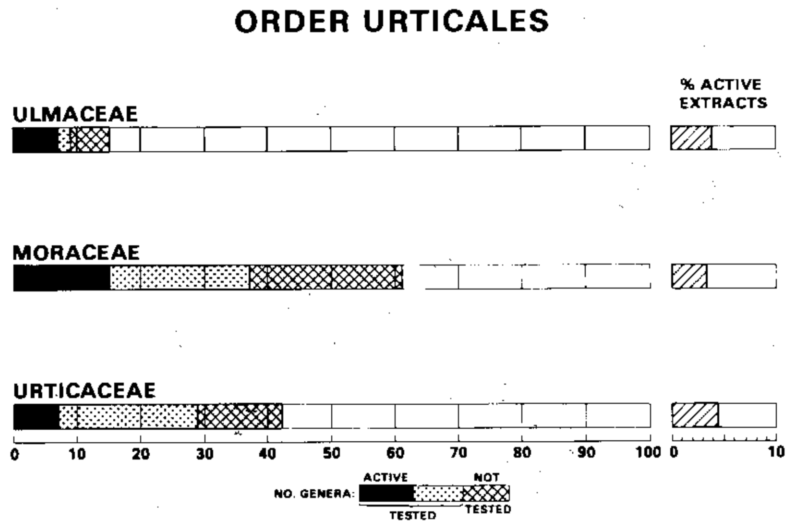

FIGURE 26.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in the families of Urticales.

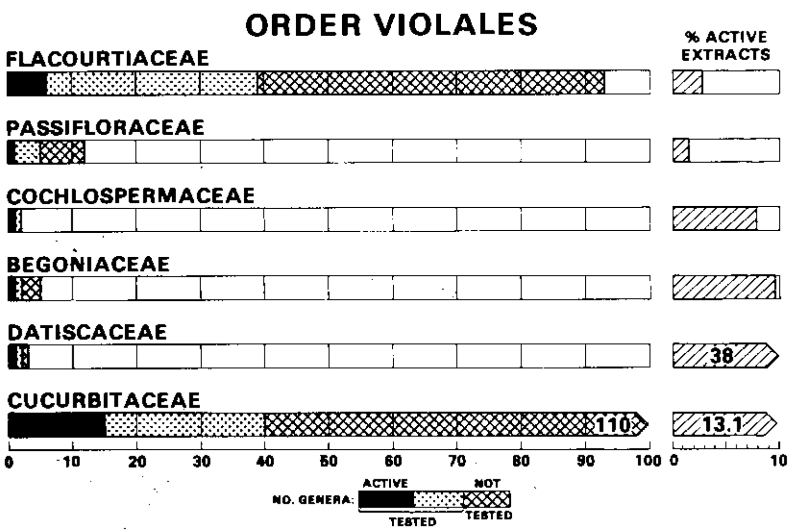

FIGURE 27.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Violales.

have activity against L1210 and 40 species in 34 genera and 12 families have high T/Cs in P388. The T/C data also indicate two orders which may merit special attention: the Scrophulariales and the Dipsacales.

The Asterales consist of a single family, the Asteraceae. About 12% of all species and 30% of all genera under consideration belong to this family. Yet, only nine species have high T/Cs in P388 and only one species has L1210 activity. Most of the activity in the Asteraceae is due to sesquiterpene lactones and other low-priority compounds. This family qualifies as a FONI, even though two active alkaloids have been isolated from a member of the genus *Senecio*.

Class Liliatae

Subclass Liliidae.—The only real potential in the monocots or Liliatae resides in this subclass which contains two orders: the Liliales and the Orchidales. As shown in figure 23, the high-interest compounds isolated from members of the Liliales are alkaloids, one of which is 3-desmethylcolchicine. Also in the Liliales, three species in two genera and two families have L1210 activity, and nine species in seven genera and four families have high T/Cs in P388.

Distribution of High-Interest Activity and Compounds in the Orders and Families of the Angiospermae[6]

By applying high-interest activity and compound criteria it was possible, in the preceding section, to narrow the field of subclasses and orders to those having the greatest potential as sources of anticancer agents (table 8). The same criteria will be applied to identify the most promising families in these groups.

Order Magnoliales (5600 species in 243 genera and 19 families).—Screened: 438/107/16. Active: 74/39/10. The three families not screened contain only one or two species each. Figure 24 illustrates the number of active, tested, and total genera and the percentage of active extracts in five of the most promising families. The yield of active extracts in the Winteraceae and Annonaceae is well above the 4% yield for the higher plants as a whole. Activity against

TABLE 8.—HIGH-INTEREST ACTIVITY AND COMPOUNDS IN ORDERS OF ANGIOSPERMAE

CLASS, SUBCLASS, AND ORDER	T/C in P388 = 175%	L1210 Activity	HIGH INTEREST COMPOUNDS*
Class Magnoliatae			
Subclass Magnoliidae			
Order Magnoliales	+		+
Order Aristolochiales	+		+
Order Ranunculales	+	+	+
Order Papaverales	+		+
Subclass Hamamelidae			
Order Urticales	+	+	+
Subclass Dilleniidae			
Order Theales	+		
Order Malvales	+		
Order Violales	+	+	
Order Ebenales	+		
Subclass Rosidae			
Order Rosales	+	+	+
Order Myrtales	+	+	+
Order Cornales	+	+	+
Order Santalales	+		
Order Celastrales	+	+	+
Order Euphorbiales	+	+	+
Order Rhamnales	+		+
Order Sapindales	+	+	+
Order Linales			+
Order Polygalales	+		
Order Umbellales	+	+	+
Subclass Asteridae			
Order Gentianales	+	+	+
Order Polemoniales	+		+
Order Lamiales	+	+	+
Order Scrophulariales	+	+	
Order Rubiales	+		+
Order Dipsacales	+		
Order Asterales	+	+	+
Class Liliatae			
Subclass Liliidae			
Order Liliales	+	+	+
Order Orchidales	+	+	

*Presence of diterpenes, lignans, quassinoids, ansa macrolides or alkaloids

[6] Chemists under contract with the DR&DP, NCI, are working to isolate and characterize the active agents from many angiosperms with promising anticancer activity. To protect their interests in rights of discovery and publication, such plants are referred to by generic name only. As the active agents become known, they and their source plants are reported in the chemical literature.

both KB and P388 is well represented in the active families of this order, and one species of the Monimiaceae, *Siparuna* sp., has a T/C in P388 > 200%. Active alkaloids have been isolated from four species in four families: (-)-dicentrine from *Magnolia virginiana* L. of the Magnoliaceae, liriodendine from *Annona glabra* L. of the Annonaceae, cryptowolline iodide from *Cryptocarya laevigata* var. *bowiei* (Hook.) Kosterm. of the Lauraceae, and thalicarpine from *Hernandia ovigera* L. of the Hernandiaceae. Also, the active lignan, deoxypodophyllotoxin, has been isolated from *H. ovigera*. The overall screening results encourage continued sampling in this order.

Order Aristolochiales (600 species in seven genera and one family).—Screened: 40/5/1. Active: 8/4/1. Both high-interest compounds and activity occur in this family: *Asarum* sp. and *Aristolochia* sp. have high T/Cs in P388, and an active lignan, α-peltatin, has been isolated from *Asarum canadense* L.

Order Ranunculales (3200 species in 145 genera and eight families).—Screened: 321/78/8. Active: 42/26/6. The five most important active families are depicted in figure 25. The Ranunculaceae, Berberidaceae, and Menispermaceae have an excellent representation of high-interest types of compounds (table 9), one of which is the alkaloid thalicarpine. In addition, four species of the Berberidaceae have high-interest activity: two *Berberis* spp. with T/Cs in P388 of 200% or more, and sp. *Diphylleia* and *Podophyllum* sp. with activity against L1210. Although the Sabiaceae are, as yet, relatively unexplored, 100% of the extracts tested are active in P388, and one species of *Meliosma* has a T/C in P388 of ≥ 200%. The screening results indicate that at least three families of the Ranunculales are actual or potential FOSIs: the Ranunculaceae, Berberidaceae, and Menispermaceae.

Order Papaverales (600 species in 47 genera and

TABLE 9.—HIGH-INTEREST COMPOUNDS IN RANUNCULALES

COMPOUND	GENUS AND SPECIES	FAMILY
Alkaloids		
Berberine sulfate	Coptis teeta Wall.	Ranunculaceae
	Hydrastis canadensis L.	"
	Thalictrum rugosum Ait.	"
	Berberis asiatica Roxb. ex DC.	Berberidaceae
Cocsulinine	Cocculus pendulus (Forst.) Diels	Menispermaceae
Cycleadrine	Cyclea peltata Hook. fil. & Thoms.	"
Cycleanorine	" " " " " "	"
Cycleapeltatin	" " " " " "	"
Isochondrodendrine	" " " " " "	"
Obamegin	Xanthorrhiza simplicissima Marsh.	Ranunculaceae
Oxyacanthine	" " "	"
	Berberis asiatica	Berberidaceae
epi-Stephanine	Stephania hernandifolia (Willd.) Walp.	Menispermaceae
Stephavanine	S. abyssinica (Dillon & A. Rich.) Walp.	"
Thalicarpine	Thalictrum dasycarpum Fisch., C.A. Mey. & Ave.-Lall.	Ranunculaceae
Thalidasine	" " " " " " "	"
Lignans		
α-Peltatin	Diphylleia cymosa Michx.	Berberidaceae
β-Peltatin	" " "	"
Deoxypodophyllotoxin	Podophyllum pleianthum Hance	"
Podophyllotoxin	" " "	"

one family).—Screened: 58/19/1. Active: 10/6/1. Both high-interest activity and active compounds occur in the Papaveraceae (including the Fumariaceae). Four active alkaloids have been isolated from two species: berberine sulfate from *Argemone mexicana* L., and chelidimerine, coptisine chloride, and sanguidimerine from *Chelidonium majus* L. Moreover, one species of *Hunnemannia* has a T/C in P388 of ≥ 200%. Screening should continue; this family is known to be rich in alkaloids.

Order Urticales (2400 species in 118 genera and three families).—Screened: 141/75/3. Active: 58/29/3. Of the nine orders comprising the subclass Hamamelidae (fig 19), only the Urticales show any real potential; of the three families in this order (fig 26), only the Urticaceae or nettle family have both high-interest activity and compounds. The alkaloid cryptopleurine was isolated from *Boehmeria cylindrica* (L.) Sw., L1210 activity occurs in *Cypholophus* sp., and *Urera* sp. has a high T/C in P388. Aside from the Urticaceae, one species of *Ficus* in the Moraceae has a high T/C. Although activity is not spectacular in the order as a whole, it should be screened further.

Order Theales (3000 species in 155 genera and 15 families).—Screened: 257/66/9. Active: 28/16/5. Among the families comprising the order, high-interest activity occurs only in one species of *Calophyllum* and one of *Psorospermum* in the family Clusiaceae; both have T/Cs in P388 of ≥ 200%.

Order Malvales (3500 species in 239 genera and six families).—Screened: 519/123/5. Active: 40/30/5. Here, high-interest activity occurs in two families; *Sloanea* sp. in the Elaeocarpaceae and *Theobroma* sp. in the Sterculiaceae have high T/Cs in P388.

Order Violales (5200 species in 304 genera and 22 families).—Screened: 436/125/17. Active: 55/31/10. Six of the active families are shown in figure 27. The exceptionally high percentage of active extracts in the last three families—the Begoniaceae, Datiscaceae, and Cucurbitaceae—is now known to be due to the cucurbitacins, a group of highly toxic triterpenes of little interest to this program. In Cronquist's system (7), these families follow one another in a sequential numerical order indicating close relationship. All activity in this order is not necessarily due to cucurbitacins; since high T/Cs in P388 occur in two species of Flacourtiaceae (*Xylosma* sp. and *Zuelania* sp.), one species of Passifloraceae (*Passiflora* sp.), and one species of Cochlospermaceae (*Cochlospermum* sp.).

Order Ebenales (1700 species in 70 genera and six families).—Screened: 239/35/4. Active: 24/13/4. Although this order has yet to yield any high-interest compounds, species with high T/Cs in P388 occur in two families, *Manilkara* sp. in the Sapotaceae and *Styrax* sp. in the Styracaceae.

Order Rosales (20,000 species in 882 genera and 16 families).—Screened: 2576/457/11. Active: 256/124/6. The six active families comprise figure 28. With 12,000 species in 600 genera, the Fabaceae or legumes far outweigh the other families of this order in size. Of the 316 genera tested, 85 are active. Most of the activity, however, is due to tannins, sterols, quinones, rotenoids, and proteins (including cesalin from *Caesalpinia gilliesii* [Hook.] D. Dietr.). An active alkaloid, monocrotaline, was isolated from *Cro-*

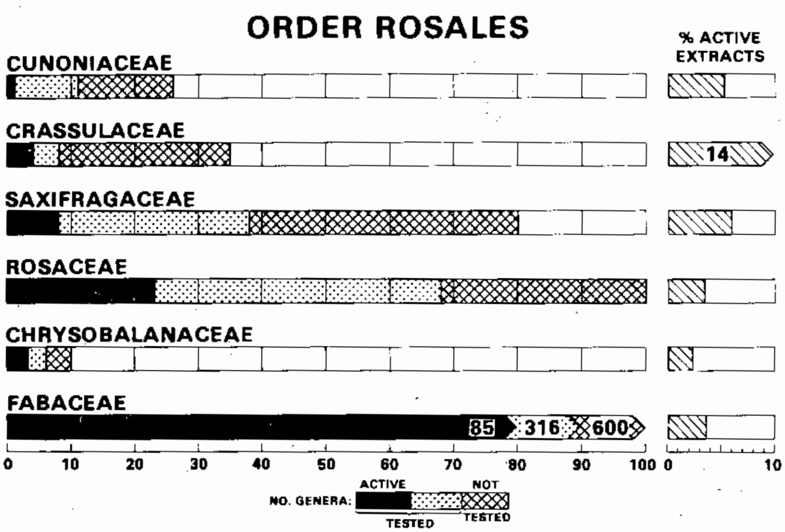

FIGURE 28.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Rosales.

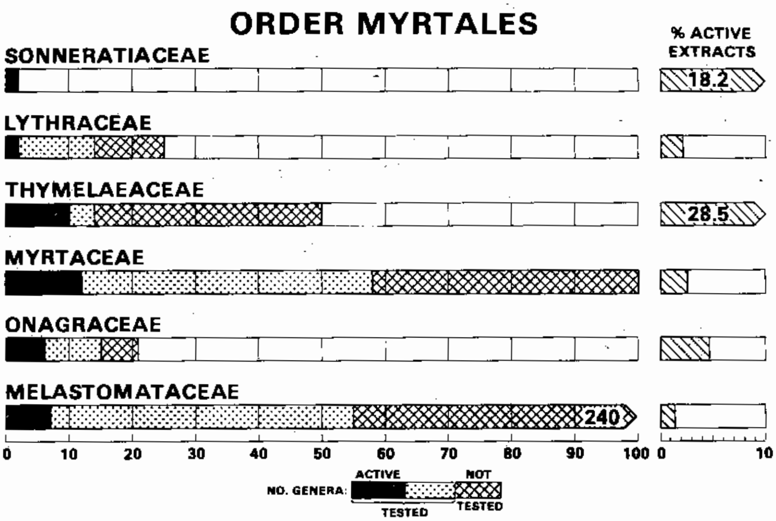

FIGURE 29.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Myrtales.

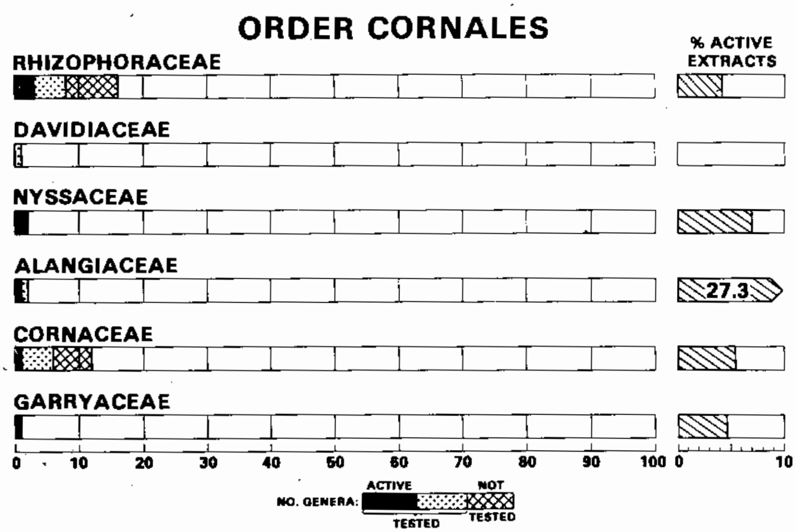

FIGURE 30.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in families of Cornales.

talaria spectabilis Roth. No extracts were active against L1210, and many of the extracts tested were active only against the obsolete tumor systems. Nevertheless, species in two closely related genera have T/Cs in P388 of ≥ 200%: two *Sesbania* spp. and *Glottidium* sp. Also, four legume species have high T/Cs in P388: *Astragalus* sp., *Calliandra* sp., *Cytisus* sp., and *Diphysa* sp. These may prove to be exceptions to the rule for the family.

The family Crassulaceae has a high number of active extracts and L1210 activity in one species of *Kalanchoe*. In the Rosaceae, *Horkelia* sp. and *Prunus* sp. have high T/Cs in P388, and *Vauquelinia* sp. has a T/C of ≥ 200%. The Chrysobalanaceae also have one species of *Parinari* with a T/C of ≥ 200%. Furthermore, active alkaloids have been isolated from two members of the Saxifragaceae: anopterine from *Anopterus macleayanus* F. Muell. and an unnamed alkaloid from *Dichroa febrifuga* Lour. All of these families need further exploration.

Order Myrtales (9000 species in 469 genera and 13 families).—Screened: 909/177/11. Active: 98/45/8. Figure 29 includes six of the active families. The unusual feature of this order is that the family Thymelaeaceae, a superb FOSI, is surrounded by a group of apparent FONIs. The only high-interest activity

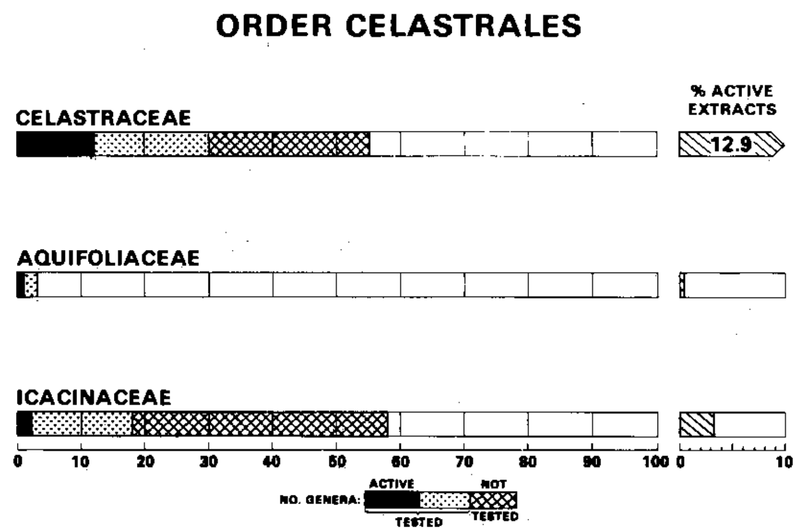

FIGURE 31.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 3 families of Celastrales.

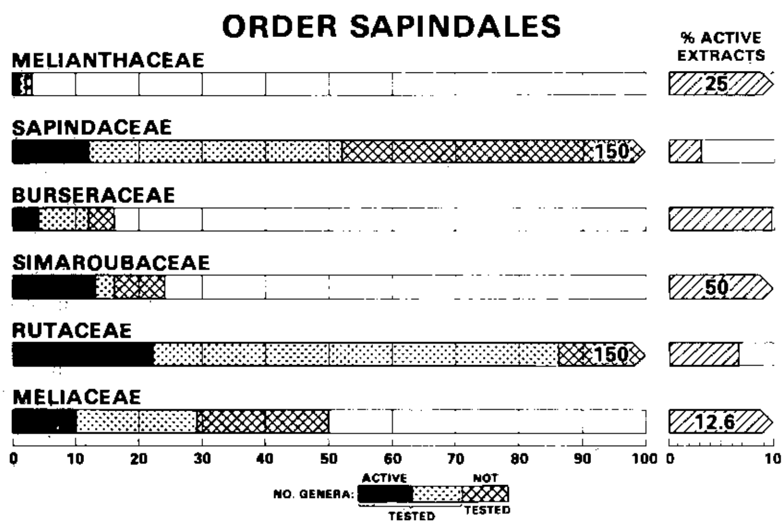

FIGURE 32.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Sapindales.

in the Myrtales is found in this family: *Daphne* sp. has L1210 activity and 14 species in six genera have high T/Cs in P388: five *Gnidia* spp., three *Wikstroemia* spp., two *Daphne* spp., two *Daphnopsis* spp., *Dais* sp., and *Pimelia* sp. In addition, potentially valuable diterpenes have been isolated from two species: 12-hydroxydaphnetoxin and mezerein from *Daphne mezereum* L., and gnidicin, gnididin, and gniditrin from *Gnidia laprantha* Gilg. The future prospects for the Thymelaeaceae are excellent. The very small family Sonneratiaceae has an excellent percentage of active extracts but, as yet, nothing is known of the chemistry.

Order Cornales (250 species in 34 genera and six families).—Screened: 74/19/6. Active: 15/8/5. All six families in this order are shown in figure 30. The Cornales produced camptothecin, one of the first compounds from higher plants to undergo clinical trial. This alkaloid comes from *Camptotheca acuminata* Decne. (Nyssaceae). The discovery of camptothecin prompted screening in closely related families, which led to the discovery of L1210 activity in

Alangium salviifolium (L. fil.) Wanger. and its active alkaloid, tubulosine. With the exception of the Alangiaceae, little promising activity has been found in the related families.

Order Santalales (2200 species in 134 genera and ten families).—Screened: 187/62/7. Active: 14/9/4. Although this order has not yet produced any high-interest compounds, all of the activity is against P388 and KB and two species have high T/Cs in P388: *Phoradendron* sp. of the Loranthaceae and *Schoepfia* sp. of the Olacaceae. Further screening may be profitable here.

Order Celastrales (2300 species in 128 genera and eight families).—Screened: 216/58/7. Active: 40/15/3. The three active families are represented in figure 31. Without a doubt, the Celastraceae (including the Hippocrateaceae) are one of the super FOSIs. The high-interest compounds isolated from members of this family (table 10) include three of the 21 most interesting compounds (1): tripdiolide and triptolide, diterpenes from *Tripterygium wilfordii* Hook. fil., and maytansine, an ansa macrolide from several species of *Maytenus*. In addition, 16 species in seven genera have high T/Cs in P388: nine *Maytenus* spp., two *Putterlickia* spp., *Euonymus* sp., *Hippocratea* sp., *Salacia* sp., *Wimmeria* sp., and *Tripterygium* sp.

A related family, the Icacinaceae, is also proving to be a promising source of high-interest compounds and activity. The species *Nothapodytes foetida* (Wight) Sleumer, an L1210 active, is a new source of camptothecin, as well as a derivative, 9-methoxycamptothecin, not found in *Camptotheca*. In addition, a species of *Apodytes* has a T/C in P388 of 200% or more. Although the percentage of active extracts in this family has not been high, there is sufficient reason to screen further in the Icacinaceae.

By association, one might expect the Aquifoliaceae to be a promising source of activity, but this family, though widely screened, has produced little or nothing of interest.

Order Euphorbiales (8000 species in 301 genera and five families).—Screened: 674/128/5. Active: 89/33/2. There is good reason to believe that the Euphorbiales, an order derived from the Celastrales, may also prove to be a promising source of anticancer agents. This is especially true of the family Euphorbiaceae which, with 7500 species in 290 gen-

TABLE 10.—HIGH-INTEREST COMPOUNDS IN CELASTRACEAE

COMPOUND	GENUS AND SPECIES						
	Maytenus buchananii (Loes.) Wilczek	Maytenus heterophylla (Eckl. & Zeyh.) N. Robs.	Maytenus serrata (Hochst. ex A. Rich.) Wilczek	Maytenus wightiana Babu	Putterlickia pyracantha (L.) Endl.	Putterlickia verrucosa (E. Mey. ex Sond.) Szyszyl.	Tripterygium wilfordii Hook. fil.
Ansa macrolides							
Maysenine	+						
Maysine	+						
Maytanacine	+					+	
Maytanbutine	+	+	+	+		+	
Maytanprine	+	+	+	+	+	+	
Maytansine	+	+	+	+	+	+	
Maytansinol	+						
Maytanvaline	+			+			
Diterpenes							
Tripdiolide							+
Triptolide							+
Triptonide							+

TABLE 11.—HIGH-INTEREST COMPOUNDS IN SIMAROUBACEAE

COMPOUND	GENUS AND SPECIES					
	Brucea antidysenterica J.F. Mill.	*Brucea guineensis* G. Don	*Holacantha emoryi* Gray	*Picrasma excelsa* (Sw.) Planch.	*Pierreodendron kerstingii* Little	*Simarouba glauca* DC.
Quassinoids						
2'-Acetylglaucarubinone				+	+	
Ailanthinone					+	
Bruceantarin	+	+				
Bruceantin	+	+				
Bruceantinol	+	+				
Bruceine B	+	+				
Dehydroailanthinone					+	
Dehydrobruceantarin	+					
Dehydrobruceantin	+	+				
Dehydrobruceantol	+					
Glaucarubinone				+	+	+
Glaucarubolone					+	+
Holacanthone			+			
Lignans						
Dehydroanhydropicropodophyllin	+	+				

era, dwarfs the other families of the order. Of the two active families in this order, the Buxaceae and Euphorbiaceae, only the latter has high-interest activity: a species of *Garcia* is active against L1210 and 22 species in 13 genera have high T/Cs in P388: seven *Euphorbia* spp., two *Croton* spp., two *Jatropha* spp., two *Pycnocoma* spp., *Codiaeum* sp., *Garcia* sp., *Mallotus* sp., *Maprounea* sp., *Pausandra* sp., *Pedilanthus* sp., *Phyllanthus* sp., *Sapium* sp., and *Vernicia* sp. In addition, high-interest compounds have been isolated from two species: the diterpene, jatrophone, from *Jatropha macrorhiza* Benth., and the lignan, justicidin B, from *Phyllanthus brasiliensis* (Aubl.) Poir. The family Euphorbiaceae, also known to be rich in alkaloids, is definitely worthy of close attention. The other active family in the Euphorbiales, the Buxaceae, has yielded the active alkaloid, cycloprotobuxine, from *Buxus sempervirens* L.

Order Rhamnales (1670 species in 71 genera and three families).—Screened: 245/38/3. Active: 18/10/2. In this order, representatives of the Rhamnaceae and Vitaceae were active. However, high-interest activity and compounds occurred only in the family

TABLE 12.—HIGH-INTEREST COMPOUNDS IN RUTACEAE

COMPOUND	GENUS AND SPECIES
Berberine sulfate	*Zanthoxylum monophyllum* (Lam.) P. Wilson
Fagarone	*Fagara zanthoxyloides* Lam.
Lunasine chloride	*Lunasia amara* Blanco
	L. quercifolia (Warb.) Lauterb. & K. Schum.
5-Methoxycanthin-6-one	*Pentaceras australis* (F. Muell.) Hook. fil. ex Benth.
Methoxydihydronitidine	*Fagara leprieurii* (Guillem., Perr. & A. Rich.) Engl.
	F. macrophylla (Oliv.) Engl.
O-Methylfagaronine	*F. zanthoxyloides*
4-Methylthiocanthin-6-one	*Pentaceras australis*
Nitidine chloride	*Fagara chalybea* (Engl.) Engl.
	F. leprieurii
	F. macrophylla
	F. rubescens (Planch. ex Hook.) Engl.
	F. usambariensis Engl.
	Toddalia asiatica Lam.
	Zanthoxylum monophyllum
Oxynitidine	*Fagara macrophylla*

Rhamnaceae. Three species of the genus *Colubrina* have high T/Cs in P388 and three ansa macrolides have been isolated from *C. texensis* (Torr. & Gray) Gray: colubrinol, colubrinol acetate, and maytanbutine. It is interesting that maytanbutine has also been isolated from four species of the genus *Maytenus* (Celastraceae) of the order Celastrales.

The distribution of ansa macrolides may be of special phylogenetic significance. Among higher plants, ansa macrolides have appeared only in the Celastrales and Rhamnales, two orders which, according to Cronquist (7), have evolved from a common ancestor (*see* fig 21). The occurrence of ansa macrolides in only these orders corroborates Cronquist's hypothesis based on comparative morphology.

Because of the potential importance of ansa macrolides in anticancer research, the Rhamnaceae must be regarded as a FOSI. Furthermore, the closely related families Vitaceae and Leeaceae should receive special consideration.

Order Sapindales (6700 species in 517 genera and 16 families).—Screened: 903/265/14. Active: 155/80/11. Of the six active families (fig 32), two are definitely FOSIs (the Simaroubaceae and the Rutaceae), and at least two are very promising (the Burseraceae and the Meliaceae). The five active families not shown have neither high-interest compounds nor activity.

The small family Melianthaceae has excellent activity, but the active compounds isolated are primarily bufadienolides, which are of relatively low interest. On the more positive side, the active lignan, (+)-dimethylisolariciresinol-2 α-xyloside, has been isolated from *Bersama abyssinica* Fresen.

The least promising of the families (fig 32) appears to be the Sapindaceae because the activity is due to tannins.

In contrast to the Sapindaceae, the Burseraceae show considerable promise. A species of *Commiphora* has activity against L1210, and lignans have been isolated from three species of *Bursera:* deoxypodophyllotoxin, 5′-desmethoxy-β-peltatin A methyl ether, and β-peltatin A methyl ether from *B. fagaroides* (H.B.K.) Engl.; burseran and deoxypodophyllotoxin from *B. microphylla* Gray; and deoxypodophyllotoxin from *B. morelensis* Ramirez.

The active agents of the Simaroubaceae are the quassinoids, so far found only in this family. The high-interest compounds isolated from members of this family (table 11) include two of the 21 most interesting compounds (1): bruceantin from *Brucea antidysenterica* J. F. Mill. and *B. guineensis* G. Don and holacanthone from *Holacantha emoryi* Gray. Bruceantin is a strong candidate for preclinical toxicology. In addition, two species of *Brucea* are active against L1210 and 11 species in seven genera have high T/Cs in P388: three *Brucea* spp., two *Hannoa* spp., two *Simarouba* spp., *Picrasma* sp., *Picrolemma* sp., *Pierreodendron* sp., and *Quassia* sp.

The family Rutaceae with its promising alkaloids was early recognized as a FOSI. Nine active alkaloids have been isolated from 11 species in five genera (table 12). The active alkaloids include two strong candidates for preclinical toxicology (1): fagaronine and nitidine. In addition, two species of *Fagara* have activity against L1210 and the genera *Fagara* and *Zanthoxylum* each have three species with high T/Cs in P388.

The family Meliaceae merits special attention because not only does it have a good percentage of active extracts, but a species of *Guarea* has activity against L1210. Furthermore, three species in two genera have high T/Cs in P388: two *Aglaia* spp. and *Guarea* sp. With this percentage of active extracts and type of activity, the Meliaceae must be considered a FOSI, though nothing is known of the compounds responsible for the activity.

The family Aceraceae, not shown in figure 32, is noteworthy only because the previously mentioned Acer saponin P was isolated from *Acer negundo* L.

Order Linales (700 species in 29 genera and two families).—Screened: 45/9/2. Active: 10/2/2. Although the order has yet to produce high-interest activity, two active lignans have been isolated from *Linum album* Kotschy ex Boiss.: 3′-demethylpodophyllotoxin and podophyllotoxin.

Order Polygalales (1900 species in 87 genera and six families).—Screened: 122/41/6. Active: 14/11/6. So far, only tannins and phytosterols have been isolated from members of this order. However, one species of *Polygala* (family Polygalaceae) has a high T/C in P388.

Order Umbellales (3700 species in 330 genera and two families).—Screened: 332/129/2. Active: 22/19/2. The Araliaceae and Apiaceae, two closely related families, have promising species. In the Araliaceae, a species of *Schefflera* has activity against L1210 and a T/C in P388 of ≥ 200%, and a species of *Oplopanax* has a high T/C. In the Apiaceae, four active lignans have been isolated from *Steganotaenia araliacea* Hochst.: steganacin, steganangin, steganol, and steganone. Further screening in both of these families should be profitable.

Order Gentianales (5600 species in 408 genera and four families).—Screened: 600/171/4. Active: 117/52/3. The four families in this order (fig 33) run the gamut from super FOSI to complete FONI.

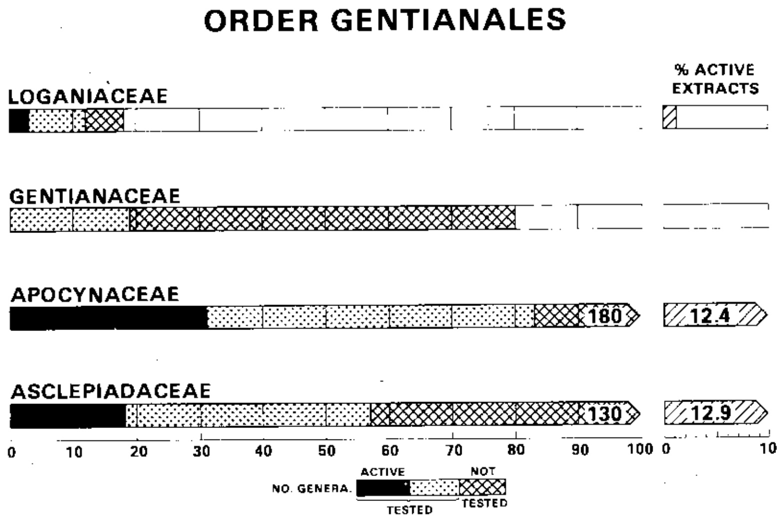

FIGURE 33.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in the families of Gentianales.

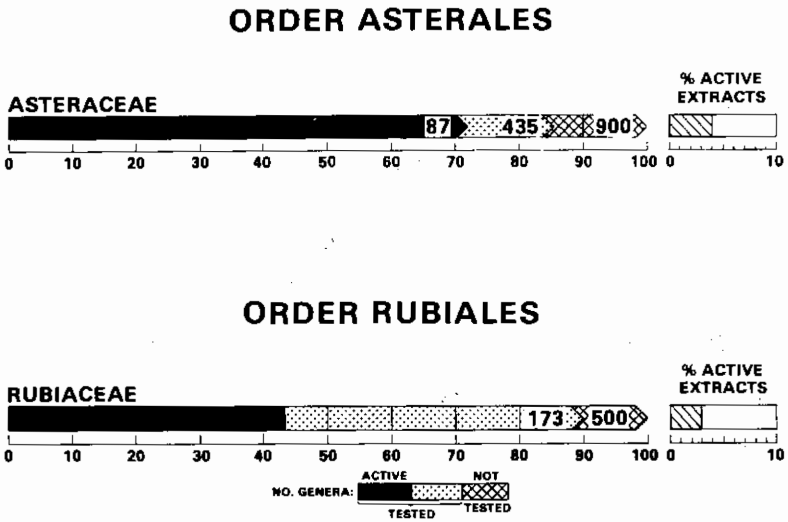

FIGURE 34.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in the Rubiales and Asterales.

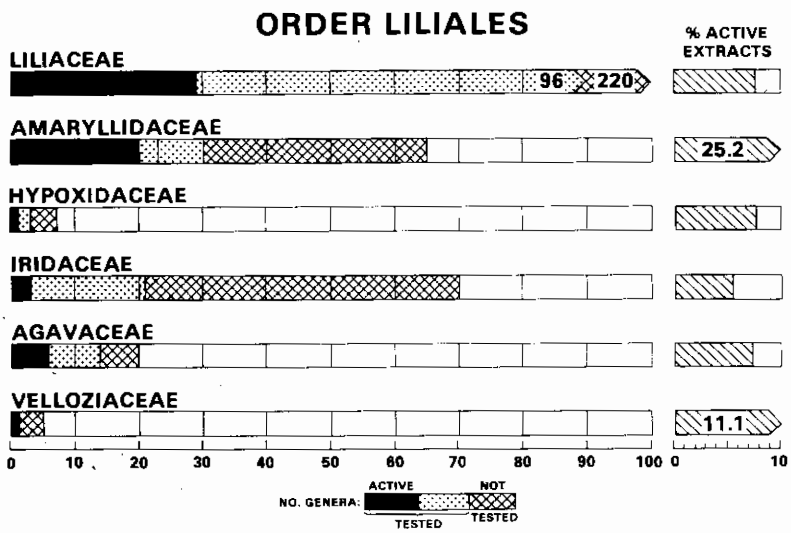

FIGURE 35.—Nos. of active, tested, and total genera, and percentage of activity in extracts tested in 6 families of Liliales.

The family Apocynaceae is the sole source of higher plant compounds used in the treatment of cancer; these are the vinca alkaloids from *Catharanthus roseus*. Furthermore, seven active alkaloids have been isolated from eight species in six genera (table 13).

Ellipticine and 9-methoxyellipticine are among the 21 most interesting compounds (1). In addition, ten species in six genera have high T/Cs in P388: five *Tabernaemontana* spp., *Alstonia* sp., *Catharanthus* sp., *Ervatamia* sp., *Schizozygia* sp., and *Stemmadenia* sp. Continued intensive screening is definitely indicated for the Apocynaceae.

The Asclepiadaceae, closely related to the Apocynaceae, must be regarded as a potential FOSI. As in the Apocynaceae, much of the activity here is due to cardenolides and the best activity is due to alkaloids. Five active alkaloids have been isolated from four species of the genus *Tylophora* (table 14), including one, tylocrebrine, on the list of the 21 most interesting compounds (1).

The family Gentianaceae is a classic example of a FONI. After screening 52 species in 19 genera, not a single active species has materialized. Although the Loganiaceae contain three active species, the family is lacking in high-interest activity or compounds and is only of marginal interest.

Order Polemoniales (4600 species in 187 genera

TABLE 13.—HIGH-INTEREST COMPOUNDS IN APOCYNACEAE

COMPOUND	GENUS AND SPECIES
Conessine hydrochloride	*Holarrhena antidysenterica* (L.) Wall. ex A. DC.
Ellipticine	*Excavatia coccinea* (Teysm. & Binnend.) Markgraf
	Ochrosia moorei (F. Muell.) F. Muell.
Leurosine	*Catharanthus lanceus* (Boj. ex A. DC.) Pinchon
	C. pusillus (Murr.) G. Don
9-Methoxyellipticine	*Excavatia coccinea*
	Ochrosia moorei
Reserpine	*Excavatia coccinea*
Voacamine	*Tabernaemontana johnstonii* (Stapf) Pinchon
	T. ventricosa Hochst. ex A. DC.
	Voacanga africana Stapf ex G. Ell.
Voacorine	*V. africana*

TABLE 14.—ACTIVE ALKALOIDS IN *TYLOPHORA*

COMPOUND	GENUS AND SPECIES
Desmethyltylophorinine	*T. dalzellii* Hook. fil.
	T. indica (Burm. fil.) Merr.
Oxytylocrebrine	*T. crebriflora* S. T. Blake
	T. hirsuta (Wall.) Wight
Tylocrebrine	*T. crebriflora*
	T. hirsuta
Tylophorine	*T. crebriflora*
	T. hirsuta
	T. indica
Tylophorinine	*T. crebriflora*
	T. indica

and seven families).—Screened: 575/103/7. Active: 63/27/4. In this order, high-interest compounds have been isolated only from members of the Solanaceae, and they too, are alkaloids: solaplumbin, solasodine base, solasodine hydrochloride, and solasodine rhamnoside (*Nicotiana plumbaginifolia* Viv.); β-solamarine (Solanum dulcamara L.); and solapalmatenine and solapalmatine (*Solanum tripartitum* Dun.). High-interest activity has been detected in only one species of the Convolvulaceae: *Argyreia* sp., with a high T/C in P388.

Order Lamiales (7800 species in 395 genera and five families).—Screened: 973/194/5. Active: 60/37/3. All three active families appear to be promising. The family Boraginaceae is the source of indicine-*N*-oxide, an alkaloid isolated from *Heliotropium indicum* L., and included among the 21 most interesting compounds (1). Among the species in the Verbenaceae, *Citharexylum* sp. is active against L1210 and *Cornutia* sp. has a high T/C in P388. Also, three species of the Lamiaceae have high T/Cs in P388: *Hyptis* sp., *Roylea* sp., and *Lepechinia* sp. Nothing is known of the compounds responsible for this activity in the Verbenaceae and Lamiaceae.

Order Scrophulariales (10,000 species in 795 genera and 13 families).—Screened: 924/268/12. Active: 60/37/6. In the context of this paper, no high-interest compounds have been isolated from members of this order. However, it should be noted that the quinone, lapachol, from *Stereospermum suaveolens* (Roxb.) DC. of the Bignoniaceae, is included on the list of the 21 most interesting compounds (1). High-interest activity is found in three families. *Digitalis* sp. of the Scrophulariaceae has activity against L1210, and three species have high T/Cs in P388: *Stereospermum* sp. of the Bignoniaceae, *Schrebera* sp. of the Oleaceae, and *Penstemon* sp. of the Scrophulariaceae.

Order Rubiales (6500 species in 500 genera and one family).—Screened: 591/173/1. Active: 62/43/1. The single family Rubiaceae (fig 34) is a source of both high-interest compounds and activity. The alkaloid, emetine, from *Cephaelis ipecacuanha* (Brot.) A. Rich. is among the 21 most interesting compounds (1). In addition, six species in six genera have high T/Cs in P388. The genera are *Bouvardia, Feretia, Genipa, Pogonopus, Psychotria*, and *Rubia*. This family is known to be rich in alkaloids and is definitely worthy of further investigation.

Order Dipsacales (1100 species in 44 genera and five families).—Screened: 152/27/4. Active: 15/5/2. Although the active families, Caprifoliaceae and Valerianaceae, have yet to produce a high-interest compound, both have representatives with high T/Cs in P388: *Lonicera* sp., *Sambucus* sp., and *Viburnum* sp. of the Caprifoliaceae and *Valeriana* sp. of the Valerianaceae.

Order Asterales (19,000 species in 900 genera and one family).—Screened: 2484/435/1. Active: 177/87/1. This order consists of the single family Asteraceae, one of the largest families of flowering plants. Considering the extent to which this family has been screened, the yield of high-interest compounds has been practically nil. So far, only the alkaloids senecionine and senecionine-*N*-oxide, from *Senecio triangularis* Hook., are high-interest types. The active compounds isolated are mainly sesquiterpene lactones and other low-interest compounds such as phytosterols and saponins. Also, the yield of high-interest activity has been poor: only a species of *Oligochaeta* has activity against L1210, and nine species in eight genera have high T/Cs in P388: two *Helenium* spp., *Baccharis* sp., *Carthamnus* sp., *Eriophyllum* sp., *Hymenoclea* sp., *Senecio* sp., *Xanthocephalum* sp., and *Zaluzania* sp. Based on past screening experience, it would be reasonable to expect that most of this activity is due to sesquiterpene lactones, and it is likely that the L1210 active will turn out to be a false-positive. The bar graphs in figure 34 provide a good comparison of the general activity in two very large families, the Rubiaceae and Asteraceae. Of these two families, the Asteraceae would appear to be the more promising source of anticancer agents but, as has been seen in similar cases, the activity in the Asteraceae is largely useless due to the nature of the responsible agents. And, for the same reason, the activity in the Rubiaceae is potentially valuable.

Order Liliales (7700 species in 445 genera and 14 families).—Screened: 666/176/13. Active: 117/54/6. Among the monocot orders, both high-interest compounds and activity occur only in the Liliales. Of the six active families depicted in figure 35, two are definitely FOSIs—the Liliaceae and Amaryllidaceae; the family Agavaceae is an apparent FONI.

The high-interest compounds in the Liliaceae are alkaloids: five have been isolated from *Colchicum speciosum* Stev. and one from *Urginea altissima* (L. f.) J. G. Baker (table 15). One of the alkaloids, 3-desmethylcolchicine, is included on the list of the 21 most interesting compounds (1). In addition, two *Colchicum* spp. have activity against L1210, and five species in three genera have high T/Cs in P388: two *Colchicum* spp., two *Merendera* spp., and *Urginea* sp.

The Amaryllidaceae are also a promising source of high-interest compounds and activity. Here again, the high-interest compounds are alkaloids: two have been isolated from *Crinum macrantherum* Engl. and one from *Hymenocallis latifolia* (Mill.) M. J. Roem. (table 15). A species of *Pancratium* has

TABLE 15.— HIGH-INTEREST COMPOUNDS IN LILIALES

COMPOUNDS	FAMILY, GENUS AND SPECIES			
	LILIACEAE		AMARYLLIDACEAE	
	Colchicum speciosum Stev.	Urginea altissima (L. fil.) J.G. Baker	Crinum macrantherum Engl.	Hymenocallis latifolia (Mill.) M.J. Roem.
Alkaloids:				
Colchicine	+			
Crinamine			+	
Demecolcine	+			
3-Desmethylcolchicine	+			
N-Formyldesacetylcolchicine	+			
Lycorine			+	+
Scilliglaucosidin		+		

activity against L1210 and two species have high T/Cs in P388: *Amaryllis* sp. and *Pancratium* sp. Also, the percentage of active extracts is exceptionally high in this family.

The family Agavaceae, thought to hold great promise early in the program, now appears to be a FONI. The activity is overwhelmingly due to saponins, but other unimportant compounds like tannins and cucurbitacins have also been isolated. However, one species of *Cordyline* has a high T/C in P388.

Little is known about the remaining families. However, there are promising indications: 100% of the extracts tested from members of Hypoxidaceae and Velloziaceae are active against P388, and one species of the Iridaceae (*Iris* sp.) has a high T/C in P388. All three families are worthy of further exploration.

COMMENTS AND CONCLUSIONS

In this symposium, Hartwell (1) demonstrated that a wide variety of chemical structures are active against tumors in laboratory animals. Under the present screening criteria, relatively few of these compounds appear to have potential as anticancer agents. Nevertheless, the activity against all tumor systems, current and obsolete, represents a wealth of information on biologic activity of higher plant products, information which transcends the immediate search for plant sources of anticancer drugs.

Thanks to the random nature of procurement and screening, the more than 20,000 species encompassed in this investigation represent a broad spectrum of the higher plants. Throughout this paper, the numbers or percentages of active, tested, and total genera illustrate the extent of screening and anticancer activity in the major divisions of the vascular plants, in the orders of the gymnosperms, and in the major orders comprising the ten subclasses of the angiosperms. These generic data, as well as the percentage yields of active extracts, are shown for selected families belonging to orders of the gymnosperms and angiosperms known to be sources of high-interest compounds and/or activity.

The distribution of the overall positive and negative screening results in taxonomic groups of higher plants shows their relative yields of biologic activity, but not necessarily their yields of useful anticancer activity or agents. Some of the reasons for the inadequacy of the general, broad-spectrum activity to identify useful anticancer agents are brought out.

The continuing search for plant sources of anticancer drugs should take into account the systematic distribution in plants of chemical compounds producing activity against those test systems most predictive for clinical efficacy. The three high-interest criteria selected for the present taxonomic analysis are: (*a*) the five most important classes of chemical compounds: diterpenes, lignans, quassinoids, ansa macrolides, and alkaloids; (*b*) activity against L1210; and (*c*) high T/Cs in P388. The screening criteria selected were shown in this study to emphasize those taxonomic groups already known to be important sources of compounds. The application of these criteria should indicate where to locate new sources of known high-interest compounds and provide leads to sources of related or novel high-interest compounds.

The above criteria serve not only to narrow the field of higher plants to those taxonomic groups with the most potential, but also to identify those groups with little or no potential.

The high-interest compounds with the plant species from which they were isolated and the genera with high-interest activity are cited under the orders and families in which they occur.

Whether a given plant family is a FOSI or a FONI or lies somewhere between these extremes depends on the extent of screening, the yield of general activity, and the presence or absence of high-interest compounds and/or activity. Certain special cases of clear-cut FOSIs and FONIs are brought out. This same arbitrary ranking can apply to taxonomic groups above and below the family level. Taxonomists, however, for mnemonic and didactic reasons, tend to think in terms of the family unit. Hence, the FOSI/FONI concept evolved.

The new procedure involving preliminary fractionation to concentrate active agents before screening (4) may significantly alter the FOSI/FONI concept. It has already increased the yields of plants active against P388 and KB. It remains to be seen whether the new procedure will redefine those families now believed to be FONIs.

Other papers in this symposium (1,11) suggest that folk uses of plants may provide valid guidelines for selective procurement and screening. Although such guidelines could be established, limiting the search to these plants would certainly reduce the opportunities for chance discoveries offered by random screening.

In 1969, Perdue and Hartwell (3) concluded that "the search for plant sources of anticancer drugs must be a long-term program," and the results of the present investigation emphasize that the same holds true today. There are few shortcuts to the successful exploration, discovery, and evaluation of anticancer agents in higher plants. The strongest evidence supporting this is the fact that compounds of potential value in cancer chemotherapy are widely distributed among many groups of the higher plants.

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