UNITED STATES DEPARTMENT OF AGRICULTURE
SCIENCE AND EDUCATION ADMINISTRATION

AGRICULTURAL RESEARCH
NORTHEASTERN REGION
BELTSVILLE AGRICULTURAL RESEARCH CENTER
BELTSVILLE, MARYLAND 20705

November 6, 1979

SUBJECT: Activity in Plant Parts as Related to Vegetation and Climate

TO: A. S. Barclay

As you know, I have been sorting our program data according to vegetation or climatic types to determine if a correlation exists with antitumor activity.

Five years ago I noted a concentration of U.S. actives in southwestern deserts. This led to reviewing collections of plants from different climatic regions in U.S. and Africa; higher percentages of active plants were found with increasingly drier climates. Lately, I am refining this on the basis of: woody plants vs. nonwoody plants, eliminating duplication, plant parts, criteria to define climate, limiting data to current tumor systems used in the preliminary screen with comparisons to past and present extraction proceedures. Of immediate value to procurement are data dealing with plant parts.

Sampling parts for screening has been reviewed by Perdue (Cancer Treatment Reports, 1976). His conclusions led to the two sample guideline that we have applied until recently - SB (or ws-sb) and TW.

The following table shows frequencies of activity in woody plant parts according to vegetation (reflecting climate). Data are based on samples collected in Africa and U.S. from 1969 - 1972 and were assumed to have been screened in both PS and KB tumors.

	RT	SB	TW	LF	TW-LF
RAIN FORESTS					
Cool	5.8%	5.7%	1.1%	1.2%	
Warm	3.2% (rb only)	5.8%	-	-	1.9%
DRY FORESTS					
Cool	3.7%	8.7%	0.5%	1.2%	
Warm	5.1%	2.4%	2.1%	3.4%	
GRASSLANDS & WOODLANDS	6.8%	2.6%	2.8%	0	
DESERTS	INSUFFICIENT DATA				2.4%

For herbs, a histogram is included and illustrates activity frequencies in species rather than plant parts. Percentages of active species for both past and present extraction procedures (in PS & KB) are side by side respectively for each vegetation type with one (desert herbs) projected at 13.3% activity (dotted line). It should be noted that these comparisons are based on the same samples (PR-Numbers) screened in 1972-1974 and again in 1976-1978. The prediction for desert herbs will have to await the results on other collections such as those recently from California, Nevada, Arizona, Texas, and Mexico (PR-Numbers - 49852-956, 50000, 50535-602, 50981-997, 51101-51221, 51425-452, 52005-056, 81225-239, 81297-326, 81257-8, 81375-81); it is believed that any remaining plant material from earlier screening will not be enough to meet the current weight requirements.

If ratios of old and new frequencies in herb activity are compared, an interesting trend is seen. A three-fold increase in rain forest herbs gradually lowers to 1.8 for those from grasslands; the reverse occurs with stembark samples (discussed later). Not enough herbs were recorded from the warm rain forest to be included; nevertheless, a textbook correlation between antitumor activity and increasingly drier climates is certainly evident.

ROOTS (TREES AND SHRUBS)

Roots are often survival (also storage) organs in grassland and semi-desert vegetation. Shoots regenerate from perennial roots following rains, fires in warm grasslands or freezing winter temperatures in cold deserts and grasslands; thus, greater root activity here, in comparison to forests, seems reasonable.

R. E. Perdue suspected that rootbark was superior to other vegetative parts but also a costly sample to obtain. I agree that removing the bark from roots is impractical, but is it necessary? Of 63 species in Ghana rain forest where both RB and SB were collected, SB activity was favored 5.8% to 3.2%. In a cool dry forest as at Mufindi Tanzania, RB activity was higher than SB; however, root samples of Croton sylvaticus, Gnidia glauca, Bridelia brideliifolia and Fagara sp. had activity in samples labeled "RB, "RT, and "WR". In some species of Gnidia and Tabernaemontana, WR samples have been the preferred recollection. Dr. Suffness has mentioned that they have recently completed screening a series of RB samples from Kenya and only about 1% were active.

Retesting the same root samples under the new extraction procedures has identified a few more active species in each vegetation type except the warm dry forest. The overall increase is from 20%-40% (1.2-1.4 times).

STEMS

In contrast to roots, stembark seems to have less activity in grassland species. It follows, then, that in dry vegetation types, such as warm dry forests and grasslands, activity is more likely to be detected in samples of roots than in stembark. But retesting of SB samples has yielded some interesting data. The incidence of SB activity increased as follows:

RAIN FORESTS		DRY FORESTS		GRASSLANDS - WOODLANDS
Cool	Warm	Cool	Warm	
up 30% (1.3)	up 60% (1.6)	up 100% (2.0)	up 300% (4.0)	up 400% (5.0)

Although active agents are often concentrated in the roots of grassland species, these same chemicals were also present in the stembark in lower concentration but not detected until the new extraction procedure (?).

Stem samples without bark (WS) have been regarded to yield little activity (cf. Perdue 1976). Values of 6.5% and 10% were found for dry forest samples but no activity was recorded for rain forest and grasslands. As with roots, the wood may be important in dry forests. On the other hand, stems with bark (WS-SB) were less active in dry forests (2.3%) and more active in rain forests (4.3% & 4.7%). Retesting has identified new activity in only stems with bark (WS-SB).

Thus, with respect to stem samples, bark is preferred but one might save the wood in dry forests*. More interesting, however, is that WS-SB samples are superior to twigs and/or leaves (in almost all cases) and WS was superior to WS-SB in dry forests. Finally, the new extraction procedure, will have the greatest impact on finding new leads with woody plants (SB activity) in warm (lowland) dry forests, woodlands or savannas and probably deserts.

TWIGS AND LEAVES

These are the most unyielding samples in activity. Leaves usually contain more water than twigs; thus, requires more than twice as much twigs to obtain a separate leaf sample. Separating the twigs and leaves can identify more active species, as in dry or sclerophyllous forests and scrub. No leaf activity was recorded for grassland trees and shrubs which were mostly deciduous species. Retesting resulted in two-three fold increase in dry forests, 1.5 increase in warm rain forest and no change in grasslands and cool rain forests.

*More comparative retesting data is needed here on activity in WS vs. RT and in both WS and RT. Species with WS activity may also have RT activity; thus, if one collects RT, WS would be unnecessary?

FRUITS

Fruits were not listed in the table because a meaningful sample size was lacking. In lumping all vegetation types, there were 35 fruit samples. Four (11%) were initially active and retesting added two more for a total of 6 (17%).

SUFFRUTESCENTS AND HERBS

Suffruticose or semi-woody plants were included with herbs because, in the field, the woody portion has often been left behind; or, if collected, it may have been lumped with the herbaceous parts and was not designated on the collector's shipping list. Exceptions are evident with Perdue's samples from Africa. When he separated the woody stems from "RT" and "ST-LF-FL", he designated these as "WS-SB" (ex. - Paullinia pinnata, Brillantaisia curatricosa, Coleus barbatus, Pseuderanthemum ludovicianum, Vernonia amygdalina, V. glabra, and V. hymenolepis), "ST" (ex. - Crotalaria clemifolia) or "TW" (ex. - Dissotis brazzaei). In other situations, he combined the woody stems with other parts which were evident as in the following examples:

Eupatorium africanum	rt-st, tw-lf
Spermacocca dibrachiata	rt-st, st-lf-fl
Gnidia mollis	rt-st, lf-fr
Gecium sp	rt-ws, st-lf

Most collectors, like myself, have not clearly identified the woody portion in their samples of suffruticose plants. One problem is that our guidelines lack an appropriate designation like 'WB' (woody base). Combinations like WB (rt) or WB (st-lf-fl) could indicate that the bulk of the sample consists of the woody base but with other parts shown in parenthesis, like if (fl-fr).

The need for collecting and separating a WB sample should be evident from data on trees and shrubs. The softer parts, like stems or twigs and leaves, have considerably less activity. In my recent field work, emphasis on 'WB', which I labeled as "RT", was given as a result of reviewing medicinal folklore.

Most herb data represent samples of annuals or short-lived perennials. Separating roots from the above parts has about the same effect as separating the leaves from twigs in woody plants. More active species are discovered but preference for plant parts was not evident among the vegetation types. Samples of corms, bulbs and tubers were few and, like fruits, these seem to have a high incidence of activity.

The evident correlation between herbs from increasingly drier climates and antitumor activity may be related to an increasingly higher fruit or seed content in samples. Many herbs from Eastern U.S. and Turkey have been divided into stems and leaf-fruit samples and stem samples are rarely, if ever, active. Additionally, the medicinal folklore often mentions leaves

and fruits of herbs, even though from a procurement standpoint such samples are impractical to collect in bulk. Since botanists generally recognize and collect herbs only when they are fertile, most herb samples screened will have had flowers or fruits present. Plants from arid regions appear to bear a greater concentration of fruits than those in humid areas.

COMBINING SAMPLES

One or more samples from a single collection frequently have been combined either because a sample weight was below the new extraction requirements or because more than two samples per collection were supplied as in the Sri Lanka samples - PR-47427-47688. For herbs, this usually means combining above and below ground parts.

With woody plants, there are sometimes many combinations or choices but the most reoccuring ones are "RT-TW" or "SB-TW". This, to me, is like trying to combine the blood of an AB doner with an A recipient. There is a common element between the two but they don't mix. Twigs and leaves or RB and SB go together. Even RT and WS or SB fit better than RT-TW or SB-TW. I have had to ignore data concerning combined active samples of woody plants. Thus, where mention was made to retesting, the increases or percentages are actually higher than what I indicated. Also, does combining active bark with inactive twigs have any effect on T/C values?

DESERT PLANTS

Whether the desert or semi-desert vegetation has the highest yield in active plants depends not only on the frequency of activity during one point in time but also on the yield in a cumulative sense. The more pronounced the dry season, the greater the extent that activity could vary according to seasonal differences. I believe our guidelines have strongly underestimated this with respect to recollections.

Some clear examples where activity depends on timing were described in my memorandum of April 15, 1976, "Patterns of Anticancer Activity in Vascular Plants". Statistical data can not be directly obtained from our records. Indirect clues are the realization that greater species duplication has accumulated with collecting in humid and mesic U.S. forests but percentages of active species are higher in the more arid regions. For example, in Western U.S., 19% of all KB and PS actives are from coniferous forests, whereas 55% are from the deserts (in 1974).

Regardless of how the percentages of active species are distributed, the insufficient data indicated in the table suggests we have yet to really screen desert plants. The University of Arizona has been the largest supplier. Their dried samples have primarily consisted of "px" (all except root) or "pl" parts. Root, bark and fruit samples were better represented in the "fresh" samples. Most samples were extracted using procedures that differed from WARF and screened in outmoded tumor systems. A lot of extracts and samples give an impression of species duplication, but how much, if any, of this is comparable to the present. To what extent have species from Arizona been tested against PS?

From an empirical sense, I lean towards the deserts for field work. A close encounter with desert plants imprints a strange kind of diversity upon you. Life forms vary from grotesque to attractive, or even appear to have come from another world. Upon direct contact, one can certainly feel the differences: spines that stab, hairs that sting or leaves that cling. More relevant, however, is that the desert, at times, reeks with chemical diversity produced by the many odiferous plants, which I find inescapable of asking - Are our chances of finding novel anticancer drugs greater in the desert than in other regions?

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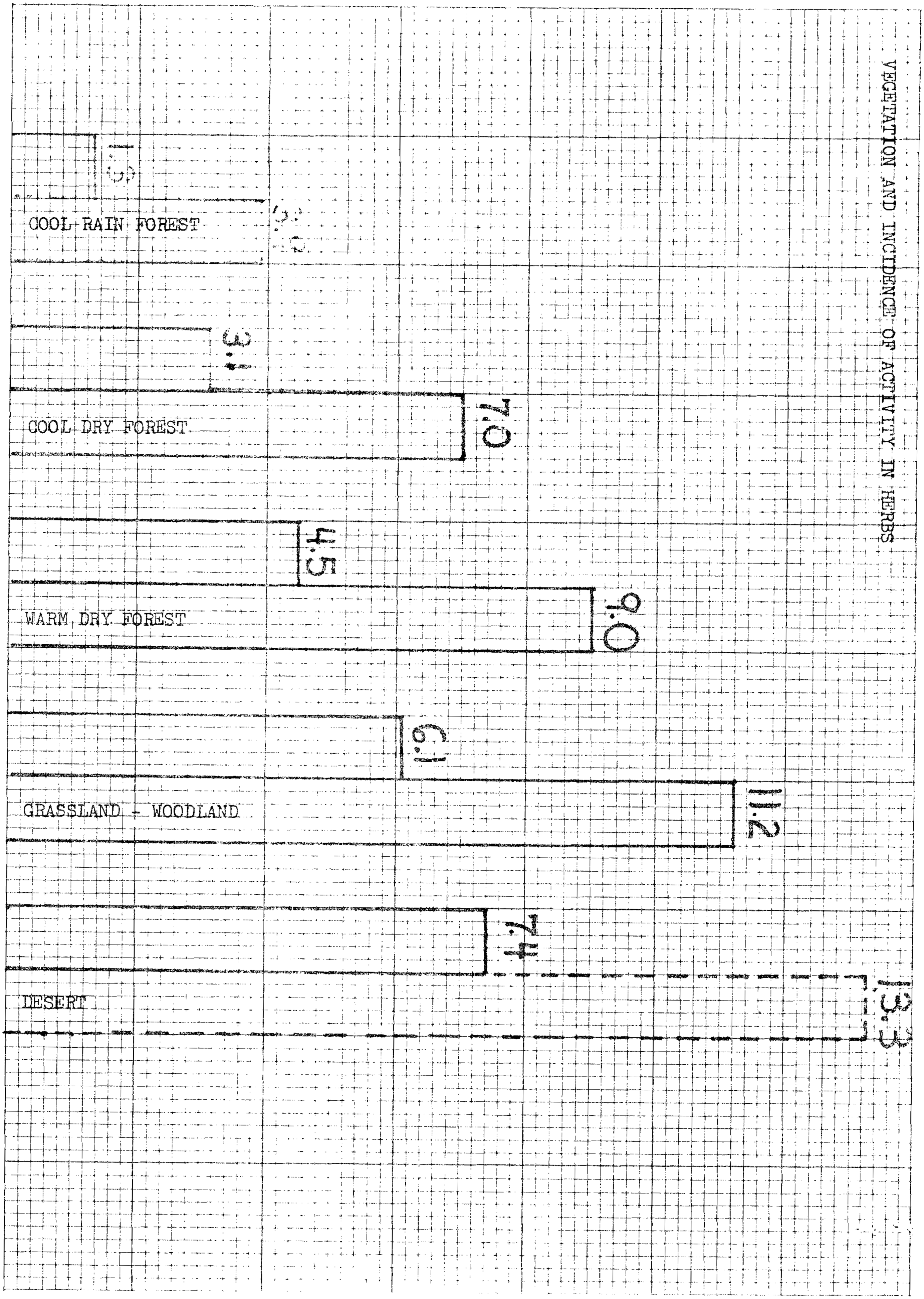
VEGETATION AND INCIDENCE OF ACTIVITY IN HERBS
COOL RAIN FOREST
3.1
COOL DRY FOREST
7.0
4.5
WARM DRY FOREST
9.0
6.1
GRASSLAND - WOODLAND
11.2
7.4
DESERT
13.3