Phytochemistry, 1980, Vol. 19, pp. 403–408. Pergamon Press Ltd. Printed in England.

# ALKALOID DISTRIBUTION AND CATABOLISM IN *CEPHALOTAXUS HARRINGTONIA*

NORMAN E. DELFEL

Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604, U.S.A.

(*Received* 3 *April* 1979)

**Key Word Index**—*Cephalotaxus harringtonia*; Cephalotaxaceae; Japanese plumyew; alkaloid distribution; catabolism.

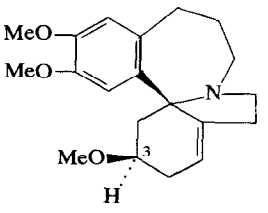
**Abstract**—*Cephalotaxus harringtonia* produces a variety of antitumor alkaloids that are distributed throughout the tree. In a young plant grown in a controlled environment, the concentration of free alkaloids (homoerythrina alkaloids and cephalotaxine) did not increase with age, whereas the concentration of cephalotaxine esters (harringtonine, deoxyharringtonine, isoharringtonine and homoharringtonine) increased roughly 5-fold. Total alkaloid concentrations increased in the older leaves of the plant and decreased in the older stems. Physiological stress (pruning) causes hydrolysis of part of the stored alkaloid esters to free cephalotaxine within one week. In 4 out of 5 field-grown trees environmental factors caused complete ester hydrolysis and, in addition, the oxidation of cephalotaxine to 11-hydroxycephalotaxine and drupacine and of homoerythrina alkaloid to its epoxy derivative. This shows that the alkaloids in this perennial tree are not inert storage products, but are under metabolic control.

## INTRODUCTION

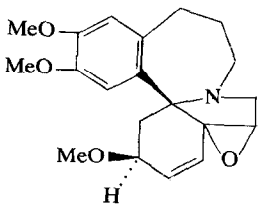
Extracts of Japanese plumyew, *Cephalotaxus harringtonia* (Forbes) K. Koch, were active in the antitumor screening program of the National Cancer Institute. Powell and associates at this laboratory have subsequently isolated and identified two groups of alkaloids in this tree (see review by Smith *et al.* [1]) that appear to arise from a common precursor via similar pathways [2]. One group is comprised of the homoerythrina alkaloids (for example **1**) [2] and epoxyhomoerythrina alkaloids (for example wilsonine (**2**)) [3], which differ from one another in having either methoxy-, dimethoxy- or methylenedioxy-substituents on the aromatic ring or in being epimeric at the C-3 position. These compounds are also found in *Schelhammera pedunculata*, *S. multiflora* and *Phelline comosa* and constitute only a minor fraction of the *C. harringtonia* alkaloids. The second group of compounds is unique to the genus *Cephalotaxus*. These are derivatives of cephalotaxine (**3**), the major component, and include small amounts (1–5%) of 11-hydroxycephalotaxine (**4**) and drupacine (**5**). The active antiumor substances in this tree are all cephalotaxine esters: deoxyharringtonine (**6**), harringtonine (**7**), isoharringtonine (**8**) and homoharringtonine (**9**) [4]. These esters typically constitute *ca* 30% of the alkaloid mixture. In addition, a fifth ester, homodeoxyharringtonine (**10**) was found in tissue cultures of this species [5].

In this country, trials of the active antitumor esters have been delayed by a lack of plant material for testing [6] and are still at the preclinical stage. However, in China, where *Cephalotaxus* grows wild, clinical trials with 41 leukemia patients [7, 8] showed partial remission in 63% of the cases, plus complete remission in 12%. More over, patients who had become resistant to treatment with other chemotherapeutic drugs responded to treatment with the cephalotaxine esters.

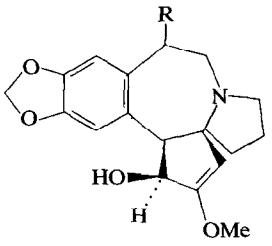
We have initiated studies on the production of these antitumor alkaloids via *C. harringtonia* tissue culture [5]. Such cultures synthesized the same alkaloids as the intact plant although at much lower concentrations. The pattern of alkaloid biosynthesis, however, resembled that of field-grown trees [2, 9] rather than that of the laboratory-grown tree from which the



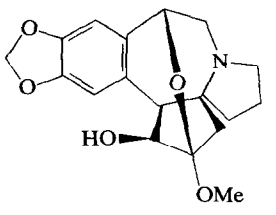

**1**



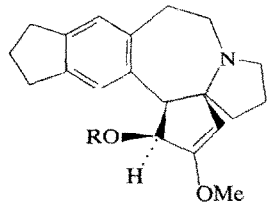

**2**

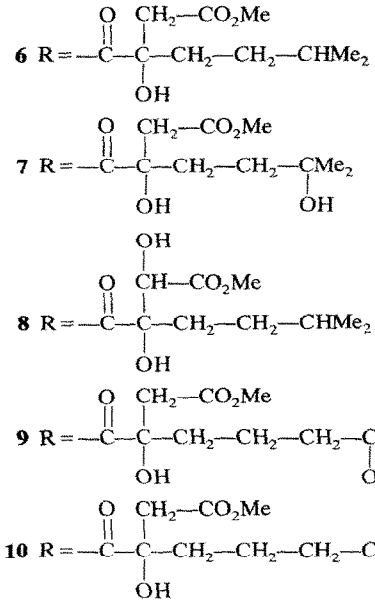



**3** R = —H
**4** R = —OH




**5**





cultures were derived. The callus cultures and field plants contained inactive cephalotaxine as the major alkaloid; the laboratory plant contained mainly the highly active ester, homoharringtonine.

Understanding the reason for the apparent metabolic differences between the laboratory-grown tree and either field-grown trees or tissue cultures is important for two reasons. Firstly, Zenk *et al.* [10] have shown that significant increases in the yield of secondary metabolites in tissue culture can be made by selecting high yielding plants for culture initiation. Factors affecting the alkaloid yield of *C. harringtonia* must be understood before trees grown under different conditions can be compared and superior plants selected. Secondly, qualitative metabolic differences between cultured cells and their parent plants, also noted by Mok *et al.* [11], Ikuta *et al.* [12], Chenieux *et al.* [13] and others, have serious consequences. They adversely affect the prospects of tissue culture for the production of secondary metabolites, and they prevent the use of tissue culture for the selection of cells from which to regenerate metabolically superior plants.

The differences in alkaloid composition between cultured *C. harringtonia* tissues and their parent laboratory-grown tree could have been due to either altered metabolism in tissue culture or altered metabolism in the tree growing in an artifical environment. Since the callus tissues resembled field-grown trees in their alkaloid pattern, the reason why the laboratory tree did not was explored first. Several possible explanations were apparent: (i) age (the laboratory plant was much younger than the field material), (ii) portion of plant samples (top portion of laboratory tree vs entire tree in field studies), (iii) treatment of sample after harvest (immediate oven drying vs slow drying at ambient temperatures), (iv) environmental differences between laboratory and field and (v) possible genetic differences in plant material. Each of these possibilities was explored in turn. Since *C. harringtonia* cannot be grown outdoors in this area, initial studies were on laboratory material and were later extended to the field. The results provide information on the metabolism and storage of alkaloids in *C. harringtonia* and show the extent to which alkaloids concentrations in this slow-growing tree are affected by environmental factors.

## RESULTS

### *Plant age*

The effect of age on the alkaloid composition of a young *C. harringtonia* plant grown under constant conditions of light and temperature (16-hr day, *ca* 25°) was studied by comparing two samples of the upper branches taken 9 months apart. During this time the alkaloid concentration increased 5-fold as a result of higher levels of each of the alkaloid esters **6–9**; the level of homoerythrina alkaloid (**1**) and unesterified cephalotaxine (**3**) remained the same (Table 1). The final concentrations of deoxyharringtonine, harringtonine and isoharringtonine were comparable to values from field-grown trees reported earlier [2] (24, 64 and 98 μg/g, respectively), but the levels of homoerythrina alkaloids and cephalotaxine were lower (8 vs 51, and 20 vs 860 μg/g, respectively) and the level of homoharringtonine was much higher (1690 vs 61 μg/g). Rather than being minimized, the differences between the laboratory and field plants were accentuated.

Table 1. Alkaloid increase with time in a laboratory-grown *Cephalotaxus harringtonia* tree

| Alkaloid | Alkaloid concentration (μg/g) Initially | 9 months later |
|---|---|---|
| Homoerythrina alkaloids (**1**) | 12 | 8 |
| Cephalotaxine (**3**) | 17 | 20 |
| Deoxyharringtonine (**6**) | tr | 28 |
| Harringtonine (**7**) | 25 | 160 |
| Isoharringtonine (**8**) | 34 | 94 |
| Homoharringtonine (**9**) | 298 | 1690 |
| Total alkaloids | 386 | 2000 |

Table 2. Alkaloid distribution in laboratory-grown *Cephalotaxus harringtonia* tree

| Alkaloid | Plant part | Alkaloid concn. (μg/g) in branch tier Upper | 2nd | 3rd | Lower |
|---|---|---|---|---|---|
| Homoerythrina alkaloids (**1**) | Leaf | 0 | 0 | 10 | 35 |
| | Stem | 26 | 16 | 10 | 16 |
| | Whole branch | 8 | 5 | 10 | 27 |
| Cephalotaxine (**3**) | Leaf | 13 | 8 | 17 | 41 |
| | Stem | 33 | 25 | 9 | 20 |
| | Whole branch | 20 | 13 | 14 | 32 |
| Deoxyharringtonine (**6**) | Leaf | 10 | 2 | 20 | 5 |
| | Stem | 65 | 49 | 36 | 9 |
| | Whole branch | 28 | 17 | 26 | 7 |
| Harringtonine (**7**) | Leaf | 198 | 158 | 222 | 280 |
| | Stem | 82 | 65 | 35 | 33 |
| | Whole branch | 160 | 128 | 152 | 176 |
| Isoharringtonine (**8**) | Leaf | 105 | 69 | 118 | 182 |
| | Stem | 71 | 71 | 48 | 43 |
| | Whole branch | 94 | 70 | 92 | 123 |
| Homoharringtonine (**9**) | Leaf | 2030 | 1500 | 2430 | 3190 |
| | Stem | 958 | 575 | 687 | 797 |
| | Whole branch | 1690 | 1200 | 1780 | 2180 |
| Total alkaloids | Leaf | 2360 | 1740 | 2820 | 3730 |
| | Stem | 1240 | 800 | 825 | 920 |
| | Whole branch | 2000 | 1430 | 2080 | 2540 |

*Alkaloid distribution*

The distribution of alkaloids in the upper portion of the laboratory plant was studied next to determine the extent to which alkaloid level and composition might be affected by the portion of the plant sampled or the relative amounts of leaf or stem in the sample. The amount of total alkaloid in the tree tended to be fairly constant (Table 2), being already high in the youngest tissues (upper tier of branches) and increasing little, if any, in the older branches. Within the branch, however, there was a definite tendency for the alkaloid concentrations to increase in the leaves of the lower branches and to decrease in stems. Hence, the ratio of total alkaloids in leaves vs stems was 1.9, 2.2, 3.4 and 4.1 in tiers 1 through 4, respectively. Deoxyharringtonine was more abundant in the stem than the leaf in all 4 samples; the homoerythrina alkaloids and cephalotaxine were more abundant in the stem in the upper two samples only, whereas the remaining alkaloids were more abundant in the leaves in all 4 samples.

In spite of the increase in alkaloid concentration in the leaf with age of tissue or position on plant, the relative composition of leaf alkaloids was remarkably constant (Table 3). The stem alkaloid composition, however, varied somewhat from branch to branch, tending to be higher in deoxyharringtonine and harringtonine in the upper branches than in the lower. Compared to the leaf composition, the stem alkaloid mixture contained higher percentages of homoerythrina alkaloids, cephalotaxine, and deoxyharringtonine. These differences between samples from the same tree are minor, however, compared to differences between trees.

Table 3. Alkaloid composition of leaf and stem extracts of laboratory-grown *Cephalotaxus harringtonia* tree

| Plant part | Alkaloid | Composition (%)* in branch tier Upper | 2nd | 3rd | Lower |
|---|---|---|---|---|---|
| Leaf | Homoerythrina alkaloids (**1**) | 0 | 0 | <1 | 1 |
| | Cephalotaxine (**3**) | 1 | <1 | 1 | 1 |
| | Deoxyharringtonine (**6**) | <1 | <1 | 1 | <1 |
| | Harringtonine (**7**) | 8 | 9 | 8 | 8 |
| | Isoharringtonine (**8**) | 4 | 4 | 4 | 5 |
| | Homoharringtonine (**9**) | 86 | 86 | 86 | 85 |
| Stem | Homoerythrina alkaloids (**1**) | 2 | 2 | 1 | 2 |
| | Cephalotaxine (**3**) | 3 | 3 | 1 | 2 |
| | Deoxyharringtonine (**6**) | 5 | 6 | 4 | 1 |
| | Harringtonine (**7**) | 7 | 8 | 4 | 4 |
| | Isoharringtonine (**8**) | 6 | 9 | 6 | 5 |
| | Homoharringtonine (**9**) | 77 | 72 | 83 | 87 |

*Total homoerythrina and cephalotaxine-type alkaloids = 100%.

*Plant stress*

The effect of stress on alkaloid composition was studied to see whether the stored alkaloids in *C. harringtonia* are metabolically inert or are influenced by the physiological state of the plant. For this purpose the top of the laboratory tree was removed and 1 week later the second tier of branches was taken for analysis. The previous data in Tables 2 and 3 showed that although the alkaloid levels differed to some extent from one part of the tree to another, the relative amounts of the various alkaloids in the extracts were quite constant. There was, however, a considerable change in alkaloid composition within 1 week after pruning. The concentration of the oxidized alkaloids (epoxyhomoerythrina alkaloid (**2**), 11-hydroxycephalotaxine (**4**) and drupacine (**5**)) varied little in this experiment, but the relative amounts of isoharringtonine and homoharringtonine decreased markedly, and the levels of homoerythrina alkaloids and especially cephalotaxine increased (Table 4). The higher homoerythrina alkaloid value can be accounted for by the tendency of these alkaloids to increase in concentration in the lower branches, which were sampled at 1 week (cf. Table 2); for cephalotaxine, however, the differences noted between branches were smaller and do not adequately explain the 5-fold increase in concentration for the free alkaloid following pruning. This increase could conceivably have been due to *de novo* synthesis, but more likely it resulted from the hydrolysis of esters **6–9**. Had the percentage increase of cephalotaxine been due to new synthesis, the amount of total alkaloid would have increased by 29%, rather than decreasing by 22% as found. Moreover, ester hydrolysis in response to stress and regrowth is consistent with the next set of data.

*Field samples*

The hydrolysis of alkaloid esters to free cephalotaxine in response to plant stress suggested that the same process might have occurred during the slow drying of whole trees at ambient temperature in previous studies [2, 9] to produce the large amounts of free cephalotaxine typical of the field results. To minimize any such effect, field samples were oven-dried immediately after harvesting.

Five *Cephalotaxus* trees of various ages from two locations in the eastern United States were sampled in early April during the period of rapid growth. Only one of the 5 trees (tree 1, Table 5) had any alkaloid esters at all, and the relative amount of its alkaloids was similar to previous field results. The absence of harringtonine from this tree is reminiscent of certain callus tissues from our tissue culture studies [5] in which this particular ester was also lacking. The other 4 trees in Table 5 showed no alkaloid esters, but instead very high levels of the oxidized alkaloids, epoxyhomoerythrina alkaloid (**2**), 11-hydroxycephalotaxine (**4**) and drupacine (**5**), together with lower percentages of the free alkaloids, homoerythrina alkaloid (**1**) and cephalotaxine (**3**). At other times of the year, these trees were known to contain esters **6–9**, in accordance with the findings of Powell and coworkers (e.g. late summer [14], fall [2] and winter [9]). In tree 2, the branches with inflorescences had a lower cephalotaxine level and higher 11-hydroxycephalotaxine level than those without (sample 2a vs 2b), although the total concentration of cephalotaxine-type alkaloids was the same in both (2720 vs 2820 $\mu$g/g). The present data are not extensive enough to ascribe differences between trees to sex, age or growing location, but it is interesting that the young male (tree 4) resembled its male parent (tree 2) much more than the female parent (tree 3). Differences between varieties may, however, be an important factor in explaining the presence of esters in tree 1 (var. *fastigiatus*), and the high drupacine content of tree 5 (var. *drupacea*), a variety reported also by Powell and coworkers [15] to contain high levels of drupacine.

## DISCUSSION

*Cephalotaxus* was once classified in the family Taxaceae and resembles members of that family in growth and appearance. As is the case with *Taxus*,

Table 4. Effect of pruning laboratory *Cephalotaxus harringtonia* tree on alkaloid composition

| Alkaloid | Composition (%)* Before pruning | 1 week after |
|---|---|---|
| Homoerythrina alkaloids (**1**) | 3 | 8 |
| Epoxyhomoerythrina alkaloids (**2**) | <1 | <1 |
| Cephalotaxine (**3**) | 4 | 26 |
| 11-Hydroxycephalotaxine (**4**) | 7 | 3 |
| Drupacine (**5**) | <1 | 3 |
| Deoxyharringtonine (**6**) | <1 | <1 |
| Harringtonine (**7**) | 6 | 6 |
| Isoharringtonine (**8**) | 8 | 4 |
| Homoharringtonine (**9**) | 71 | 51 |
| Total free cephalotaxine alkaloids (**3–5**) | 11 | 32 |
| Total cephalotaxine esters (**6–9**) | 85 | 61 |

*Total homoerythrina and cephalotaxine-type alkaloids = 100%.

Table 5. Alkaloid composition in branches of field-grown *Cephalotaxus harringtonia* trees

| Alkaloid | Composition (%)* Spring† 1 | 2a | 2b | 3 | 4 | 5 | Fall‡ |
|---|---|---|---|---|---|---|---|
| Homoerythrina alkaloids (**1**) | 8 | <1 | <1 | 1 | 1 | <1 | 5 |
| Epoxyhomoerythrina alkaloids (**2**) | 0 | 12 | 11 | 1 | 11 | <1 | — |
| Cephalotaxine (**3**) | 66 | 6 | 3 | 30 | 6 | 5 | 50 |
| 11-Hydroxycephalotaxine (**4**) | 4 | 20 | 27 | 24 | 24 | 5 | 4 |
| Drupacine (**5**) | 0 | 62 | 59 | 44 | 58 | 90 | 8 |
| Deoxyharringtonine (**6**) | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Harringtonine (**7**) | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Isoharringtonine (**8**) | 17 | 0 | 0 | 0 | 0 | 0 | 20 |
| Homoharringtonine (**9**) | 5 | 0 | 0 | 0 | 0 | 0 | 6 |

*Total homoerythrina and cephalotaxine-type alkaloids = 100%.

†Sex, approximate age, location, and variety of *C. harringtonia* harvested on 4–11 April: (1) unknown, 4 yr, Maryland, var. *fastigiatus*; (2) male, 45 yr, Pennsylvania, var. *harringtonia*, (a) branches without inflorescences, (b) branches with inflorescences; (3) female, 45 yr, Pennsylvania, var. *harringtonia*; (4) male, unknown, Pennsylvania, var. *harringtonia* (volunteer seedling from tree 2 × tree 3); (5) female, 18 yr, Maryland, var. *drupacea*.

‡Typical pooled sample of *C. harringtonia* var. *harringtonia* from Maryland [25].

*Cephalotaxus* undergoes a period of rapid growth in early spring when longer days and warmer weather break its winter dormancy. The disappearence, then, of cephalotaxine esters in trees 2–5 appears to be attributable to the period of rapid growth during which they were sampled. This conclusion is supported by the similar alkaloid pattern induced in the laboratory plant when pruning stimulated regrowth. The inverse relationship between growth and secondary metabolism has been noted in many plant cell and tissue culture studies, for example by Phillips and Henshaw in the case of phenolic biosynthesis in *Acer pseudoplatanus* [16]. Presumably tree 1 was either late in entering the growth cycle or early in recovering from its effects because of the varietal differences.

Both homoerythrina alkaloids and cephalotaxine apparently are degraded by similar, yet distinct pathways:

**1 → 2**
**6–9 ⇄ 3 → 4 → 5**

These pathways are different from the proposed biosynthetic routes [2, 17] and are probably not reversible; however, the metabolic fate of the epoxyalkaloids is currently unknown and no further catabolic products were found in the GLC chromatograms in spite of the large quantities of drupacine present.

The catabolism of homoerythrina alkaloid and cephalotaxine is apparently not directly controlled by the same factor that promotes ester hydrolysis, since in the stress experiment ester hydrolysis was not followed by alkaloid oxidation. Presumably, it is the level of free cephalotaxine in the cell that initiates the oxidation reaction. In the stressed laboratory plant the cephalotaxine concentration, although elevated, was still much lower than in the field plants having a 'normal' alkaloid pattern (87 μg/g compared to 419 μg/g in tree 1 (Table 5) or 860 μg/g in a fall sample [2]). In trees 2–5, showing active alkaloid oxidation, the total cephalotaxine-type alkaloid concentrations (**3** + **4** + **5**) were very much higher, ranging from 1820 to 4850 μg/g.

It is apparent that neither age of plant, portion of plant sampled nor treatment of sample after harvesting affect the alkaloid composition of *C. harringtonia* to the same extent as environmental factors. It now seems likely that the differences between laboratory- and field-grown trees noted earlier [5] can be ascribed mainly to the influence of the environment on plant growth. In the laboratory, with long daylength and constant temperature, growth is slow and continuous; in the field, growth is very rapid in spring and much slower at other times. Significantly, when growth was stimulated by pruning the laboratory tree, its alkaloid pattern rapidly shifted toward that previously found in field studies.

Secondary metabolites have sometimes been considered waste products of plant metabolism, but there is accumulating evidence that this is not so (see review by Robinson [18]). The present data show that alkaloids in *C. harringtonia* are not inert storage products; on the contrary, they are under the active metabolic control of the tree. Alkaloid catabolism or turnover has been noted previously in various herbaceous annual or perennial plants; the present study shows this to occur also in a woody perennial. Whether alkaloid catabolism occurs only during periods of rapid growth, or whether there is a constant turnover, as for example in *Catharanthus roseus* [19] or *Papaver bracteatum* [20], remains to be seen.

The following hypothesis is consistent with available facts and will be tested in further experiments: *C. harringtonia* alkaloids are as toxic to the plant itself as to other organisms. (The alkaloid esters are known to be potent inhibitors of eukaryotic protein synthesis, inhibiting either initiation [21, 22] or elongation [23].

Such inhibitors characteristically have a broad range of activity [24].) Since the alkaloid esters are present in the tissues of the tree at above toxic levels [21, 22], they must be stored in vacuoles. When the plant requires energy for surges of growth, the vacuoles in some or all of the cells are lysed and the alkaloids are liberated. (Presumably, the actual energy reserves in the vacuoles are something other than the alkaloids themselves, which constitute only 0.1% of the dry wt of the plant.) To prevent autotoxicity, the liberated alkaloid esters are promptly hydrolysed to free cephalotaxine (which is only 1/1000 as toxic as its esters [21, 22]), and when the cephalotaxine levels thus tend to rise too high, further catabolism occurs.

## EXPERIMENTAL

*Plant material.* The young laboratory-grown *C. harringtonia* var. *harringtonia* tree and the environmental conditions were the same as in the previous study [5]. In sampling, the main stem between two tiers of branches was included with the upper tier of branches. Except for the pruning expt, all sampling was spaced at least 9 months apart to avoid physiological after effects of pruning. The sex, age, growing location (Gladwyne, Pennsylvania, or Glenn Dale, Maryland) and variety of the field-grown trees are given in Table 5. Samples of the outer portions of the branches were taken on 4–11 April. All samples were chopped and immediately dried 6–8 hr at 80°.

*Alkaloid analysis.* The alkaloids were extracted and partially purified by the abbreviated version of the procedure of ref. [9] used earlier [2]. Individual alkaloids were identified and quantitated by the GC–MS method of ref. [25], using both 3% Dexsil 300 and 3% OV-101, as before. All results in the text are based on dry wt of plant. Homodeoxyharringtonine (**10**) has been found in the culture medium of *C. harringtonia* callus tissue, but not in trees. In this study a peak having a GLC $R_t$ equal to that of homodeoxyharringtonine on OV-101 columns was detected whenever deoxyharringtonine was present, but the MS data necessary to confirm its identity were not obtained. Since it was not fully identified and since the amount present was generally too low for accurate quantitation (*ca.* 20% that of deoxyharringtonine), values for homodeoxyharringtonine are not reported.

*Acknowledgements*—I thank Miss Josephine DeN. Henry, Henry Foundation for Botanical Research, Gladwyne, Pennsylvania, and H. T. Jayne, U.S. Plant Introduction Station, Glenn Dale, Maryland, for plant samples.

## REFERENCES

1. Smith, C. R., Jr., Powell, R. G. and Mikolajczak, K. L. (1976) *Cancer Treat. Rep.* **60**, 1157.
2. Powell, R. G. (1972) *Phytochemistry* **11**, 1467.
3. Powell, R. G., Mikolajczak, K. L., Weisleder, D. and Smith, C. R., Jr. (1972) *Phytochemistry* **11**, 3317.
4. Powell, R. G., Weisleder, D., and Smith, C. R., Jr. (1972) *J. Pharm. Sci.* **61**, 1227.
5. Delfel, N. E. and Rothfus, J. A. (1977) *Phytochemistry* **16**, 1595.
6. Perdue, R. E., Jr., Spetzman, L. A. and Powell, R. G. (Winter, 1970) *Am. Hortic. Mag.* 19.
7. Anon. (1976) *Chin. Med. J.* **2**, 263.
8. Anon. (1977) *Chin. Med. J.* **3**, 319.
9. Powell, R. G., Rogovin, S. P. and Smith, C. R., Jr. (1974) *Ind. Eng. Chem., Prod. Res. Dev.* **13**, 129.
10. Zenk, M. H., El-Shagi, H., Arens, H., Stöckigt, J., Weiler, E. W. and Deus, B. (1977) in *Plant Tissue Culture and Its Biotechnological Application*, p. 27. Springer, Berlin.
11. Mok, M. C., Gabelman, W. H. and Skoog, F. (1976) *J. Am. Soc. Hortic. Sci.* **101**, 442.
12. Ikuta, A., Syōno, K. and Furuya, T. (1974) *Phytochemistry* **13**, 2175.
13. Chenieux, J.-C., Rideau, M. and Gerard, M. (1977) *Bull. Soc. Bot. Fr.* **124**, 265.
14. Powell, R. G. and Mikolajczak, K. L. (1973) *Phytochemistry* **12**, 2987.
15. Powell, R. G., Madrigal, R. V., Smith, C. R., Jr. and Mikolajczak, K. L. (1974) *J. Org. Chem* **39**, 676.
16. Phillips, R. and Henshaw, G. G. (1977) *J. Exp. Botany* **28**, 785.
17. Fitzgerald, J. S., Johns, S. R., Lamberton, J. A. and Sioumis, A. A. (1969) *Aust. J. Chem.* **22**, 2187.
18. Robinson, T. (1974) *Science* **184**, 430.
19. Daddona, P. E., Wright, J. L. and Hutchinson, C. R. (1976) *Phytochemistry* **15**, 941.
20. Hodges, C. C., Horn, J. S. and Rapoport, H. (1977) *Phytochemistry* **16**, 1939.
21. Huang, M.-T. (1975) *Mol. Pharmacol.* **11**, 511.
22. Tscherne, J. S. and Pestka, S. (1975) *Antimicrob. Agents Chemother.* **8**, 479.
23. Fresno, M., Jimenez, A. and Vazquez, D. (1977) *Eur. J. Biochem.* **72**, 323.
24. Pestka, S. (1977) in *Molecular Mechanisms of Protein Biosynthesis*, p. 468. Academic Press, New York.
25. Spencer, G. F., Plattner, R. D. and Powell, R. G. (1976) *J. Chromatogr.* **120**, 335.