15 to 30 min increased the amount of LAL.

The production of LAL in proteins by alkali treatment was reported to parallel a decrease in the content of lysine (Bohak, 1964; De Groot and Slump, 1969). In the present study, however, the lysine contents of alkali-treated samples were slightly higher than that of the control. A recent report by Katz and coworkers (1974) notes that in the lime-prepared tortillas a reduction occurs in the essential amino acids, with the exception of lysine. In view of the much smaller amounts of LAL produced (i.e., micrograms/gram) as compared with the quantities of lysine present (milligrams/gram), it is evident that a corresponding fall in lysine would not necessarily be obvious. In addition, changes in the lysine content, unrelated to the LAL reaction, may result from preferential effects of alkalization on digestibility by acid hydrolysis or on losses during the washing process.

The formation of very low amounts of LAL in the case of the treatments with lime or $Ca(OH)_2$ as compared with NaOH or KOH is intriguing. Perhaps the amount of $Ca(OH)_2$, a weak alkali, which can penetrate into the corn kernel is relatively low. However, preliminary semiquantitative experiments with NH_4OH resulted in the production of amounts of LAL approximately equal to those produced by KOH. It may be possible that calcium ions interfere with the mechanism of LAL formation by binding to certain portions of the amino acid sequence or blocking of certain function groups in the side chains of proteins.

It is interesting to note that lime has been traditionally used in Mesoamerica and the southwestern United States as the source of alkali in the preparation of "tortilla". Ashes and lye have been used elsewhere.

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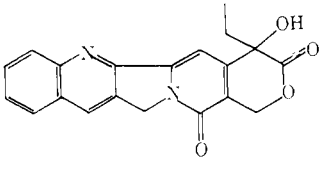
Camptothecin, a Selective Plant Growth Regulator

Camptothecin has been identified as a phytochemically active component of *Camptotheca acuminata*. Selective growth inhibition was found among the plant species tested. Tobacco and corn were retarded while no effect was noted on beans and sorghum when a 1×10^{-4} M emulsion was applied as a spray. Growth inhibition appeared to be confined to the meristematic portions of the test plants.

In a search for new naturally occurring plant growth regulants, an ethanolic extract of *Camptotheca acuminata* (Nyssaceae) was found to effectively control axillary bud growth of topped Xanthi tobacco plants. However, no effect was noted using a Greenpod bean assay. This activity was discovered in a screening program using extracts of plants collected as part of a search for tumor inhibiting compounds from plant sources.

Camptothecin, a novel alkaloid isolated from *Camptotheca*, has been intensively studied as an antitumor



CAMPTOTHECIN

reagent (Wall et al., 1966), but apparently has not been tested for plant growth regulating activity. Several extracts of *Camptotheca* have been fractionated to isolate the component affecting plant growth and to ascertain whether this component might be camptothecin. The effect of the plant growth regulating component was investigated using several species of mono- and dicotyledonous plants.

MATERIALS AND METHODS

Air-dried *Camptotheca* stem wood was ground in a Wiley mill and then exhaustively extracted with 95% EtOH. The initial sample for bioassay was fractionated via an 8-tube Craig countercurrent distribution (Tin-Wa et al., 1971) using $CHCl_3$–MeOH–H_2O (2:2:1). The phytochemically active fraction was located via bioassay on tobacco using a 10% concentration in lanolin (Marth and Mitchell, 1964). The active fraction was separated on TLC using silica gel H with acetone–$CHCl_3$ (1:1). The TLC plates were divided into sections and eluted with $CHCl_3$ and the isolated materials bioassayed using the tobacco seed germination assay (Mitchell and Livingston, 1968).

Camptothecin was isolated by silica column chromatography followed by recrystallization (Wall et al., 1966).

Camptothecin: mp 260–264 °C dec; λ_{max} (EtOH) 219, 253, 290, and 370 nm (ϵ 38000, 29000, 5000, 19000, respectively); ν_{max} (mull) 3440, 1750, 1660, 1610, 1570 cm^{-1}; δ 0.91 (3 H, triplet), 1.90 (2 H, multiplet), 5.26 (2 H, multiplet), 5.50 (2 H, multiplet); aromatic proton signals were not well resolved.

The NMR spectrum was obtained on a Model XL-100 spectrometer (Varian Associates) using Me_2SO-d_6 as a solvent. The purity of the isolated alkaloid was established by high-pressure liquid chromatography (HPLC). The HPLC analysis was performed on a Spectra Physics 3500 B liquid chromatograph [5 μm Spherisorb silica column (0.25 m × 4 mm), $CHCl_3$–EtOAc (70:30)].

Stable emulsions for spray application to test plants were prepared by dissolving camptothecin in tetrahydrofuran, addition of surfactant, Tween 80, and dilution to desired volume.

Table I. Inhibition of Growth by Camptothecin

Plant	Concn × 10^{-4} M	% inhibition
Tobacco	1.0	100[a]
Tobacco	0.1	46
Tobacco	1.0[c]	71
Beans	1.0	0
Corn	1.0	57[b]
Sorghum	1.0	0

[a] Percentage inhibition by weight of axillary buds of topped plants after 14 days. [b] Percentage inhibition by weight of above-ground portion after 14 days. [c] Camptothecin applied as diethanolamine salt. Percentage of inhibition expressed is (control minus test/control) × 100.

Tobacco (*Nicotiana tabacum* Xanthi-nc), beans (*Phaseolus vulgaris* C.V. Greenpod), and corn (*Zea mays* C.V. Golden Bantam) were used in the assay procedures described earlier (Buta, 1975). Ten plants were used per treatment and each treatment was replicated at least three times. An additional species, *Sorghum bicolor* var. Rio, was treated as seedlings or as adventitious shoots when they were emerging from older nodes. The emulsions and lanolin test preparations were applied to leaves, stems, and meristems.

RESULTS AND DISCUSSION

An ethanolic extract of *Camptotheca* was fractionated by countercurrent solvent partition and the eight fractions bioassayed on Xanthi tobacco. Fractions containing the active compound (7 and 8) were separated further by TLC. The various bands visible under uv (365 nm) as well as the intervals between were eluted and assayed with a tobacco seed germination assay. The inhibition of germination only occurred with the extract from a bright blue fluorescent band (R_f 0.60). This fluorescence along with the λ_{max} 253 and 360 nm suggested that the growth regulating compound might be camptothecin. A large scale isolation based on Wall's procedure was used to obtain sufficient camptothecin for chemical identification studies. The isolated alkaloid was chromatographically and spectrally identical with the plant growth inhibitor obtained from TLC.

Camptothecin was applied to the test plants mainly as an emulsified spray at several rates (Table I). On tobacco a concentration of 1×10^{-4} M is sufficient to completely inhibit axillary bud growth of decapitated test plants. The diethanolamine salt formed by opening the lactone ring was somewhat less effective than the lactone itself. No growth regulating activity was observed with applications to bean, the other dicotyledonous plant tested. The effect of camptothecin on monocotyledonous plants was investigated using corn and sorghum. Again selectivity in growth inhibition was observed in that the growth of corn (Figure 1) was retarded, but no effect was noted on sorghum.

The camptothecin-induced growth inhibition appeared to be confined to meristematic areas of test plants with no visible effects on adjacent portions. No chlorosis or other loss of pigmentation was noticed. The inhibition was characterized by a lack of local necrosis and toxicity which would result from use of a contact type of growth regulator or herbicide.

Camptothecin in a lanolin carrier applied to young tobacco buds maintained the inhibition for at least 1 month, the duration of the experiment. Preliminary microscopic observations indicate very few cells in the meristematic area of tobacco were affected, while no obvious effects were seen in the meristematic areas of inhibited corn plants. Camptothecin in mammalian

Figure 1. Corn treated with 1×10^{-4} M camptothecin (right) compared to control plants (left).

systems was reported to selectively inhibit the biosynthesis of ribosomal and messenger RNAs (Abelson and Penman, 1972). Although we do not know the mode of action in plant tissues, our available evidence would not disagree with this proposal.

Extensive screening programs recently have uncovered many naturally occurring plant constituents which possess plant growth regulating activity (Gross, 1975). Camptothecin, while obtained from a plant source, apparently has not been reported to be a regulant in plant systems. This may have been due in part to its selectivity. Many plant growth regulants have not shown the species specificity of inhibition that camptothecin has thus far shown. Further investigations are underway into the effects of camptothecin on plants other than the limited number of species reported here. This should allow a better understanding of the apparent selectivity observed.

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