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Note

Growth-regulating substances from *Viburnum trilobum* Marsh. separated on Sephadex G-15

Extracts of seeds and/or seed coverings have frequently been studied in an attempt to determine the presence of substances inhibitory to seed germination. KNOWLES AND ZALIK[1] noted the presence of water-soluble substances inhibitory to its germination in seed testa extracts of *Viburnum trilobum* Marsh., a native deciduous shrub. In addition to this, endocarp and testa extracts were found to be inhibitory to root growth of both *Triticum vulgare* L. and *Viburnum trilobum*. Although the presence of plant growth-regulating substances in seed coverings of *Viburnum trilobum* was recognized[1], no previous attempt has been made to determine the number or nature of such substances.

The purpose of this paper is two-fold; first, to show that gel filtration is a practical means of separating low-molecular-weight compounds in plant extracts for further study of growth regulation and second, that crude testa and endocarp extracts of *Viburnum trilobum* are complex integrations of numerous water-soluble compounds. Individually, after gel filtration, these compounds of the testa water extract show varied degrees of inhibition or stimulation activity[2], whereas a crude extract shows only inhibition unless highly diluted[1] before applying to bioassay material.

KLINGSTRÖM[3] has shown some success in separating growth-regulating substances occurring in *Pinus silvestris* L. shoot tips on Sephadex LH-20 (for organic solvents). Certain indoles and auxins have recently been purified and separated by Sephadex column chromatography[4], however, RAJ AND HUTZINGER[4] noted that closely related acidic and neutral indoles could not be separated satisfactorily on either Sephadex G-10, G-15 or LH-20. In both of the above cases, elution by organic solvents posed interferences. REYNOLDS[5] has, however, described a separation method avoiding organic solvents for chromatography of gibberellins on Sephadex G-15.

To determine the number of separate components in the crude testa extract originally described by KNOWLES AND ZALIK[1] and to determine their growth-regulatory activity on bioassay material, gel filtration of the testa extract of *Viburnum trilobum* was attempted.

Experimental

Aqueous extracts of *Viburnum trilobum* were prepared by intermittent shaking, for a period of 5 h, of samples of two hundred decorticated seeds in 50 ml of distilled water. The resulting pink-coloured extract was decanted and evaporated almost to dryness under vacuum at 40°. The residue was taken up in 2 ml of distilled water and drawn through a 0.4-μ microporous filter to remove bacteria and fungal spores. The filtered sample was then loaded onto a 90 × 1.5 cm I.D. column of Sephadex G-15 (Pharmacia Fine Chemicals, Uppsala) and eluted with 0.1 *M* phosphate buffer (pH 7.0) at a flow-rate of 5.5 ml/h. Fractions of 3.1 ml were collected at room temperature using an LKB model 7000 fraction collector (LKB Produkter, Stockholm-Bromma). Optical activity of the collected fractions was determined at 253 and 280 nm using a Beckman DK-1 spectrophotometer (Beckman-Spinco Instruments, Palo Alto).

From the ultraviolet (UV) analysis, an absorption pattern resolving seven distinct peaks over the 90 tubes collected was observed. Six millilitres of effluent from each individual peak were used for bioassay. Each bioassay was performed on twenty-five *Triticum vulgare* seeds laid on Whatman No. 2 filter paper in a petri dish. A control using 6 ml of eluting buffer was run simultaneously. Seedling response was measured after 7 days in the dark at 20° and recorded in terms of germination percentage and extension of growth of root and shoot.

Results and discussion

The void volume, 62 ml, was collected and discarded. After chromatography and UV monitoring, products were found to occur in seven peaks: peak 1 centered at 80 ml, peak 2 at 112 ml, peak 3 at 127 ml, peak 4 at 136 ml, peak 5 at 167 ml, peak 6 at 208 ml and peak 7 at 220 ml. In addition, a pink pigmented product remained fixed to the upper portion of the gel column. Of these seven dinstinct fractions, numbers 2 and 3 stimulated germination, shoot and root elongation of *Triticum vulgare*. Numbers 1 and 4 inhibited germination and shoot and root elongation. The remainder of the fractions were similar to the control. Root initiation was enhanced in all fractions as compared to the control.

Molecular-weight estimations of the substances could not be determined due to the undetermined adsorption interaction of the gel and the pink-pigmented material retained on the column. GELOTTE[6] has noted that such interferences have unpredictable retarding properties on sequential elution patterns and further that estimation of molecular weights may not conform to prepared standard curves. A number of buffers were used in an attempt to elute the pink material from the column but to no avail[2].

These studies indicate that the testa extract shown by KNOWLES AND ZALIK[1] to inhibit root growth of *Triticum vulgare* contains, not one, but seven or more water-soluble substances which are individually capable of affecting germination and as well, stimulating or inhibiting growth of the seedling. It is suggested that compounds present in *Viburnum trilobum* seed testa, being of a water-soluble nature, need not be looked upon as typical growth-regulating substances since the latter generally have low solubility in water. Furthermore, gel filtration holds potential for separating constituents of the water-soluble extract of *Viburnum trilobum* seed, or of other seed coats for that matter, and coupled with purifying procedures could lead to characterization of the active compounds.

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1 R. H. KNOWLES, AND S. ZALIK., *Can. J. Bot.*, 36 (1958) 561.
2 P. FEDEC, *M. Sc. Thesis*, 1970.
3 A. KLINGSTRÖM, *J. Chromatogr.*, 30 (1967) 605.
4 R. K. RAJ AND O. HUTZINGER., *Anal. Biochem.*, 33 (1970) 471.
5 T. REYNOLDS, *J. Exp. Bot.*, 21 (1970) 702.
6 B. GELOTTE, *J. Chromatogr.*, 3 (1960) 330.

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