

# Derivatives of (+)-Limonene. Effect of Chain Length in *n*-Alkyl Quaternary Ammonium Derivatives on Plant Growth-Retardant Activity

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The growth-retardant activity of 11 *n*-alkyl quaternary ammonium compounds was determined by measuring and comparing with experimental controls the second internode of young bean plants that were treated with 1% concentrations of test compound. For each addition of a carbon atom to the alkyl chain from ethyl to heptyl, the activity increased. However, progressive increases of chain

length beyond heptyl decreased the activity of the compounds. The hexyl, heptyl, and octyl quaternary ammonium compounds were rated biologically as highly active. Statistical analysis showed that the heptyl compound was more active as a growth retardant on young bean plants than Alar, and the hexyl and octyl compounds had equal activity to this commercial growth retardant.

The growth-retardant activity of a number of quaternary ammonium compounds synthesized from (+)-limonene was reported previously (Newhall and Pieringer, 1966). Several benzyl quaternary ammonium derivatives had about the same degree of activity as Alar (succinic acid 2,2-dimethylhydrazide) when applied in lanolin to the stems of Black Valentine bean plants. As a continuation of this work, the effects of chemical structure on growth retardant activity were investigated. This paper describes the synthesis of 11 new, saturated, *n*-alkyl derivatives of (+)-limonene and their growth retardant effect on Black Valentine bean plants.

## METHODS AND PROCEDURES

The *n*-alkyl quaternary ammonium compounds were prepared by quaternization of the mixed *trans* isomers of 2-dimethylamino-1-*p*-menthanol (Newhall, 1959) with the appropriate alkyl halide. As in the case of the previously reported quaternary ammonium compounds (Newhall and Pieringer, 1966), these *n*-alkyl derivatives are also mixtures of the possible *trans* isomers. Since the procedures for preparing these compounds were similar, they are summarized in Table I with the yields and elemental nitrogen analyses. Although the yields were rather low in most cases, extensive work to obtain optimum yields was not considered necessary at this time. All these compounds are ethanol soluble and readily soluble in water except the dodecyl and octadecyl derivatives which are only sparingly water-soluble.

Test compounds were applied to 7-day-old Black Valentine bean plants at a concentration of 1% in lanolin containing 2.5% Tween-80 (Newhall and Pieringer, 1966). This mixture was applied as a 3- to 5-mm. band around the stem below the second node (Krewson *et al.*, 1959; Newhall and Pieringer, 1966). Treated plants were grown for 7 days, and the lengths of the second internodes were recorded.

A complete test series consisted of the following 13 treatments, each comprising eight bean plants: 11 *n*-

alkyl test compounds, a lanolin-treated control, and an Alar reference. The entire test series was repeated four times at weekly intervals. Growth-retardant activity, determined for each treatment in each series, was statistically analyzed using Duncan's multiple range test to express significance of differences.

## RESULTS AND DISCUSSION

Among the 11 *n*-alkyl compounds studied, growth-retardant effects varied from slightly active to highly active. However, all treatments except one had a highly significant retarding effect on the growth of the second internode, the exception being the octadecyl derivative which was only significant at the 5% level when compared

Table I. Preparation of *n*-Alkyl Quaternary Ammonium Compounds<sup>a</sup>

| Compound   | Yield,<br>% | Nitrogen, <sup>b</sup> % |       |
|--|-------------|--------------------------|-------|
|  |             | Calcd.                   | Found |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, ethyl bromides     | 62          | 4.54                     | 4.18  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, propyl bromides    | 64          | 4.35                     | 4.25  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, butyl bromides     | 60          | 4.17                     | 4.17  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, pentyl bromides    | 50          | 4.00                     | 3.86  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, hexyl bromides     | 56          | 3.84                     | 3.88  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, heptyl bromides    | 50          | 3.70                     | 3.69  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, octyl bromides     | 53          | 3.57                     | 3.55  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, nonyl bromides     | 50          | 3.45                     | 3.65  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, decyl bromides     | 48          | 3.33                     | 3.53  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, dodecyl bromides   | 45          | 3.22                     | 3.29  |
| Mixed <i>trans</i> -1- <i>p</i> -menthanol, 2-dimethylamino-, octadecyl bromides | 41          | 3.12                     | 3.31  |

<sup>a</sup> Each quaternary ammonium compound was prepared by refluxing 5 grams of 2-dimethylamino-1-*p*-menthanol with 5 grams of the appropriate alkyl halide in 5 ml. of methyl ethyl ketone (Newhall and Pieringer, 1966).

<sup>b</sup> Nitrogen analyses were made using a semimicro-Kjeldahl-Gunning procedure.

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Table II. Growth-Retardant Effects of *n*-Alkyl Quaternary Compounds

| Carbon No. | Activity <sup>a</sup> |          |          |          |                       | Statistical Analysis          |                           |    |
|------------|-----------------------|----------|----------|----------|-----------------------|-------------------------------|---------------------------|----|
|            | Series 1              | Series 2 | Series 3 | Series 4 | Over-all <sup>b</sup> | Mean growth, mm. <sup>b</sup> | Significance <sup>c</sup> |    |
|            |                       |          |          |          |                       |                               | 5%                        | 1% |
| Ethyl      | -39                   | -20      | -17      | -6       | -21                   | 84.31                         | c                         | c  |
| Propyl     | -29                   | -39      | -31      | -23      | -30                   | 74.13                         | e                         | d  |
| Butyl      | -51                   | -51      | -55      | -40      | -50                   | 53.56                         | f                         | e  |
| Pentyl     | -56                   | -70      | -65      | -54      | -62                   | 40.44                         | g                         | f  |
| Hexyl      | -77                   | -84      | -76      | -70      | -77                   | 24.31                         | hi                        | g  |
| Heptyl     | -78                   | -85      | -82      | -73      | -80                   | 21.31                         | i                         | g  |
| Octyl      | -70                   | -81      | -78      | -65      | -70                   | 28.03                         | h                         | g  |
| Nonyl      | -44                   | -59      | -52      | -37      | -49                   | 54.84                         | f                         | e  |
| Decyl      | -35                   | -25      | -19      | -22      | -25                   | 79.41                         | d                         | cd |
| Dodecyl    | -1                    | -8       | -22      | +2       | -17                   | 98.31                         | b                         | b  |
| Octadecyl  | -4                    | -12      | -8       | +3       | -5                    | 101.03                        | b                         | ab |
| Alar       | -72                   | -76      | -79      | -71      | -75                   | 26.75                         | h                         | g  |
| Control    | ...                   | ...      | ...      | ...      | ...                   | 106.50                        | a                         | a  |

<sup>a</sup> Expressed as average per cent reduction (-) or increase (+) in growth as compared to controls.

<sup>b</sup> Expressed as average of 32 measurements.

<sup>c</sup> Mean growth values followed by the same letter or letters under the respective level of significance are not statistically different from each other by Duncan's multiple range test.

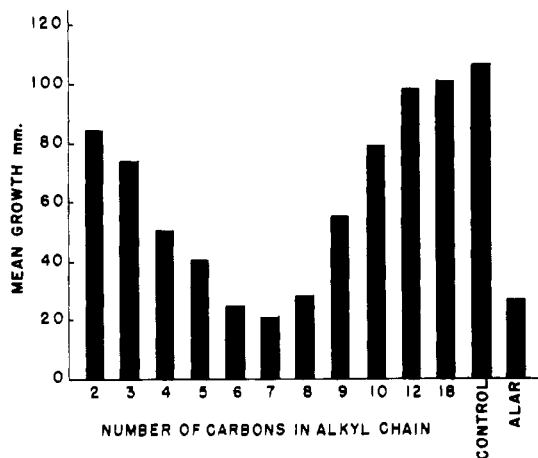


Figure 1. Average growth of second internode of 32 bean plants treated with *n*-alkyl quaternary ammonium compounds having different carbon chain lengths

with the control. The effect of increasing length of the *n*-alkyl chain on plant growth is depicted in Figure 1. Starting with ethyl and continuing through heptyl, each successive addition of a carbon atom caused an increase in growth-retardant activity. Progressive increases of carbon chain length beyond heptyl decreased activity. The relative position of each compound with regard to activity conformed with this order in the over-all analysis of the experiment and, with only one exception, within each of the four test series (Table II). In terms of growth response, differences between successive compounds were generally significant at both the 5 and 1% level (Table II). Statistically significant differences in growth did not occur, however, between the hexyl, heptyl, and octyl compounds, the most active of the series. The activity of these three quaternary compounds was similar to Alar at the 1%

level, but the heptyl consistently exhibited a greater retarding effect than Alar. This difference was evident at the 5% level of significance (Table II). Within this series of 11 derivatives, high growth-retardant activity was limited to the hexyl, heptyl, and octyl compounds. This limited range of high activity conforms with what has been found in the case of quaternary, medicinal germicides for which the following generalization has been made: "In any given quaternary ammonium series, there is a small zone of peak performance; the lengths of the long alkyl groups of compounds that fall within this zone differ from one another by 1 to 4 carbon atoms" (De Benneville, 1956).

In addition to the shortened internodes, treated plants exhibited a darker green coloration than control plants. Distorted growth and toxicity symptoms were not observed.

As in the case of other plant-growth retardants, the exact mode of action of these *n*-alkyl compounds in plants is unknown. The activities of these compounds are undoubtedly dependent on the factors involved with movement of the test compound from the point of application to the site of activity, but under the conditions of this study, molecular structure seems to be the principal factor affecting biological activity.

#### LITERATURE CITED

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