

Isomerism in Quaternary Ammonium Derivatives of (+)-Limonene and Its Effect on Plant Growth

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Three similar mixtures of quaternary ammonium alcohol derivatives of *p*-menthane prepared separately from (+)-limonene and (-)-limonene were tested for growth-retardant activity on young bean plants. Optical activity had no apparent influence on growth of the second internode of test plants and

dextro, levo, and racemic mixtures proved equally effective. Three pure geometrically isomeric benzyl quaternary ammonium alcohol derivatives of $\Delta^{8(9)}$ -*p*-menthane prepared from (+)-limonene had different growth-retardant activities which were additive in their mixtures.

Previous papers have described the synthesis of quaternary ammonium plant-growth retardants from (+)-limonene (Newhall and Pieringer, 1966), their translocation in bean and grapefruit plants (Newhall and Pieringer, 1967), and some effects of chemical structure on their growth-retardant activity (Pieringer and Newhall, 1968). Since biological activity is often dependent upon optical and/or geometrical isomerism, a study was made of the contribution of these molecular properties to the plant growth-retardant activity of quaternary ammonium derivatives of (+)-limonene.

Whether or not optical activity is essential to growth-retardant activity is of particular economic importance to any future commercial production of these compounds, since racemic limonene can be synthesized from isoprene recovered from natural gas. Considerable progress has also been made in recent years by the naval stores industry toward the preparation of racemic limonene of sufficient purity for synthetic work.

METHODS AND PROCEDURES

To determine the effect of optical rotation on growth-retardant activity, three similar mixtures of quaternary ammonium derivatives of *p*-menthane were prepared separately from (+)-limonene and (-)-limonene. In each case, this involved hydrogenation of the 8(9) exocyclic double bond (Newhall, 1958) and peracetic acid oxidation of the resulting Δ' -*p*-menthane to give the mixed cis-trans isomers of *p*-menthane-1,2-epoxide (Newhall, 1959). Reaction of the mixed epoxides with dimethylamine gave a mixture of *trans*-dimethylaminomenthans (Figure 1, I) (Newhall, 1959). The functional groups of I are enclosed in parentheses to indicate that a mixture of three 1,2-*trans* and position isomers is represented. Quaternization of the amino alcohol mixtures from (+)-limonene and (-)-limonene with α -chloro-*p*-xylene, α ,3,4-trichlorotoluene, and 1-bromoheptane gave the dextro and levo mixtures, respectively, of the corresponding quaternary ammonium derivatives (Figure 1, II, III, and IV). Mixture IV from (+)-limonene has been reported previously (Pieringer and Newhall, 1968). The physical constants of (-)-limonene and its derivatives are compared with those of (+)-limo-

nene in Table I for characterization purposes, since the levo derivatives have not been previously synthesized. Quaternary ammonium derivatives or mixtures not previously reported are also included with their elemental nitrogen analyses and optical rotations.

To determine the contribution of geometrical isomerism to plant growth-retardant activity, the pure *trans* amino alcohol isomers (Figure 2, V, VI, and VII) were isolated from the reaction product of (+)- $\Delta^{8(9)}$ -*p*-menthane-1,2-epoxide (limonene oxide) and dimethylamine (Newhall, 1964). These isomers comprise approximately 50, 30, and 20%, respectively, of the reaction product (Kuczynski and Zabza, 1963). Isomer V was removed completely from the mixture by crystallization of its picrate (m.p. 158°). Isomer VI was then separated by vacuum distillation. The isolation and purification of the least abundant isomer VII were facilitated by recrystallization of its picrate (yellow prisms, m.p. 117–21°) from benzene-methanol solution. This picrate has not been previously reported. Each geometrical amino alcohol isomer was converted to its corresponding benzyl quaternary ammonium chloride derivative (Figure 2, VIII, IX, and X) by reaction with benzyl chloride as described previously (Newhall and Pieringer, 1966). The optical rotations and elemental nitrogen analyses for these $\Delta^{8(9)}$ -*p*-menthane derivatives

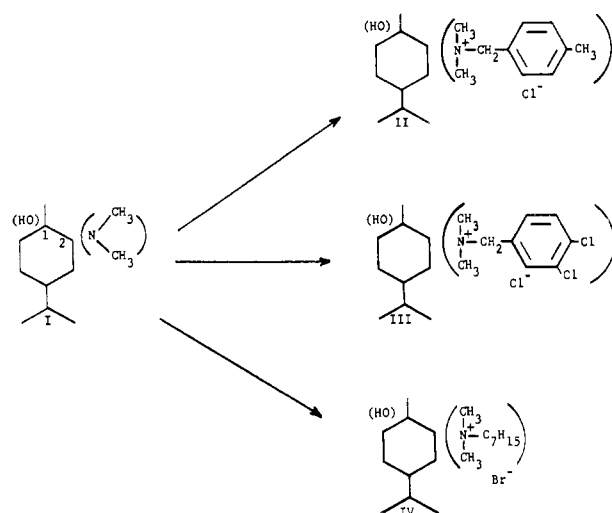


Figure 1. Synthesis of (+) and (-) quaternary ammonium derivatives

are included in Table I. Compounds VIII and IX are both amorphous solids, whereas X crystallizes as colorless prisms from acetone, (m.p. 169–71°). The infrared absorption maxima for these derivatives are consistent with the indicated structural formulas (Figure 2).

Table I. Physical Constants and Elemental Nitrogen Analyses of Limonene Derivatives

Compound	n_D^{25}	$[\alpha]_D^{25}$	Nitrogen, %	
			Calcd.	Found
(+)-Limonene	1.4722	+120°		
(-)-Limonene	1.4700	-109°		
(+)- Δ' - <i>p</i> -Menthene	1.4557	+109°		
(-)- Δ' - <i>p</i> -Menthene	1.4545	-101°		
(+)- <i>p</i> -Menthane-1,2-epoxides	1.4493	+57.9°		
(-)- <i>p</i> -Menthane-1,2-epoxides	1.4497	-53.7°		
(+)-Dimethylamino-menthanols (I)	1.4722	+36.0°		
(-)-Dimethylamino-menthanols (I)	1.4722	-31.6°		
(+)- <i>p</i> -Methylbenzyl quat. (II)		+4.2° ^b	4.12	4.28
(-)- <i>p</i> -Methylbenzyl quat. (II)		-3.2° ^b	4.12	4.31
(+)-3,4-Dichlorobenzyl quat. (III)		+2.0° ^b	3.57	3.40
(-)-3,4-Dichlorobenzyl quat. (III)		-1.7° ^b	3.57	3.53
(+)-Heptyl quat. (IV)		+2.7° ^b		
(-)-Heptyl quat. (IV)		-2.2° ^b	3.70	3.56
Benzyl quat. VIII		-5.5° ^c	4.33	4.31
Benzyl quat. IX		0° ^c	4.33	4.58
Benzyl quat. X		-15.8° ^c	4.33	4.10

^a Nitrogen analyses made using semimicro-Kjeldahl-Gunning procedure.

^b 50% ethanol solution. This high concentration was necessary to obtain measurable rotations.

^c 30% ethanol solution.

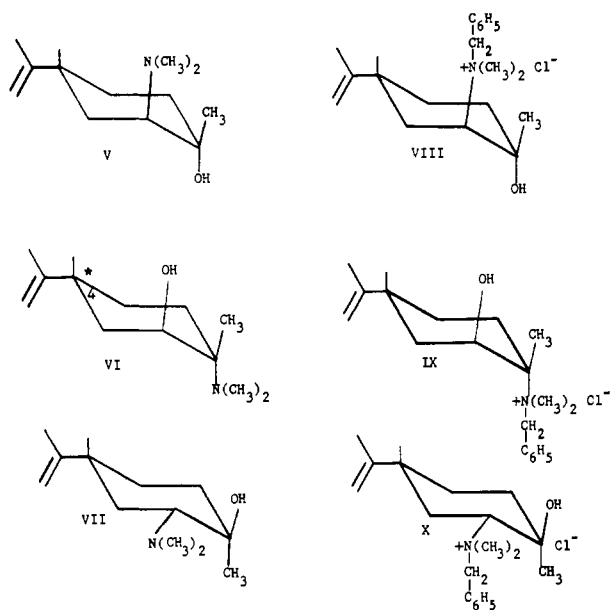


Figure 2. Synthesis of pure quaternary ammonium geometrical isomers

VIII. λ (cap. film max.), 2.95, 3.37, 6.09, 6.73, 6.88, 8.76, 9.22, 9.51, 9.88, 10.02, 10.90, and 11.4 microns.

IX. λ (cap. film max.), 2.95, 3.37, 3.70, 6.09, 6.73, 6.88, 8.48, 9.23, 10.03, 10.97, and 11.4 microns.

X. λ (KBr, max.), 3.07, 3.36, 6.05, 6.70, 7.06, 8.76, 9.14, 10.00, 10.87, 11.16, and 11.52 microns.

The presence of the tertiary hydroxyl at C-1 in both VIII and X is evident from the strong absorption in both compounds at 8.76 microns. The secondary hydroxyl in IX causes less strong absorption at 8.48 microns.

Each test compound was applied below the second node of 7-day-old Black Valentine bean plants at a concentration of 1% in lanolin containing 2.5% Tween 80 (Newhall and Pieringer, 1966; Pieringer and Newhall, 1968). The lengths of the second internodes were recorded 7 days after treatment, and activities were calculated as average per cent reduction in length of internode compared to control plants. Racemic mixtures were prepared by mixing equal weights of the dextro and levo forms of a given compound.

To determine the effect of optical activity on growth retardation, each of the following 11 treatments was applied to eight bean plants: the dextro (+), levo (-), and racemic (\pm) forms of II, III, and IV, a lanolin-treated control, and an Alar (succinic acid 2,2-dimethylhydrazide) reference. This test series was repeated four times at weekly intervals (Table II). Two experiments were also made to determine the growth effects of various concentrations in lanolin of the (+) and (-) forms of IV (Table III). Eight plants were used per treatment plus eight lanolin-treated controls.

The growth-retardant activities of the pure geometrical isomers VIII, IX, and X were determined by applying each one in lanolin to eight bean plants. Six such experiments were completed and the activities relative to the lanolin-treated control were calculated (Table IV). Also, in Experiments 5 and 6, equal weights of 1% lanolin pastes of

Table II. Results of Screening Tests on Optically Active Quaternary Ammonium Compounds

Compound	Activity, %			
	Expt. 1	Expt. 2	Expt. 3	Expt. 4
(+)-II	72	64	91	60
(-)-II	74	63	90	62
(\pm)-II	80	66	91	53
(+)-III	38	31	69	26
(-)-III	31	5 ^a	42	9 ^a
(\pm)-III	32	23	65	17
(+)-IV	89	86	94	90
(-)-IV	86	82	94	81
(\pm)-IV	89	85	94	87
Alar	88	84	91	86

^a These two activities are excessively low, possibly because of experimental error.

Table III. Effect of Concentration of (+) and (-) IV in Lanolin on Growth Retardant Activity

Isomer	Activity, % at				
	2%	1%	0.5%	0.25%	0.125%
(+) (Expt. 1)	83	81	63	64	62
(-) (Expt. 1)	79	75	69	47	47
(+) (Expt. 2)	93	91	91	91	89
(-) (Expt. 2)	92	91	88	87	89

Table IV. Results of Screening Tests on Pure Geometrical Isomers of Quaternary Ammonium Compounds

Compound	Activity, %					
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6
VIII	76	75	79	20	49	23
IX	24	48	29	4	18	0
X	79	81	84	25	60	30
VIII + IX					44(34) ^a	5(12)
VIII + X					63(55)	21(26)
IX + X					37(39)	14(15)
VIII + IX + X					41(42)	14(18)

^a Numbers in parentheses are average activities calculated on assumption that isomers contributions are additive.

VIII and IX, VIII and X, IX and X, and VIII, IX, and X were mixed and each mixture was applied to eight bean plants.

RESULTS AND DISCUSSION

The data presented in Table II show that growth-retardant activity is not dependent on optical activity. Only two activities for (–) III are excessively low, and these can be considered as experimental error. In fact, the results obtained for the more active compounds II and IV show a striking similarity between the activities of the dextro, levo, and racemic forms. The growth-retardant activities of various concentrations in lanolin of the enantiomers of the most active retardant (IV) were determined to see if any differences could be detected at lower concentrations. The results in Table III show no marked differences in activities of the dextro and levo forms of IV even after dilution to 0.125%. Therefore, racemic limonene (dipentene) could serve just as well as a starting material for the preparation of quaternary ammonium growth retardants as (+)-limonene from citrus.

The growth-retardant activities of the pure, geometrical isomers VIII, IX, X, and their mixtures are summarized in Table IV. In all six experiments, the order of activity is X > VIII > IX. The respective optical activities (Table I)

are in this same order—i.e., $-15.8^\circ > -5.5^\circ > 0$. Therefore, for these geometrical isomers, there may be a relationship between optical activity and growth retardation. However, molecular geometry is probably more of a determining factor. The greater activity of the two normal trans isomers VIII and X is consistent and the position isomerism of the oxygen and nitrogen substituents in IX results in decreased plant growth-retardant activity. The absence of optical activity in IX (Figure 2) can be explained by base-catalyzed racemization at C-4 through the 2-keto form of VI. This would occur during the prolonged heating of the amino alcohol (VI) with benzyl chloride and would be favored by the high degree of conjugation in the keto form. Such keto-enol racemization is not possible in V or VII because of the tertiary hydroxyl at C-1. The data presented for the various mixtures of the three geometrical isomers indicate that their growth-retardant activity is additive. Thus, the separation of the isomers in any of the quaternary ammonium mixtures which have been discussed would not be advantageous and would certainly not be economic in any commercial production of these derivatives.

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