in acetic anhydride,[17] and tetranitromethane[18] were similar to those previously reported. In all cases, tars plus small amounts of acidic material were isolated. With tetranitromethane, an amorphous red solid was obtained but this rapidly decomposed.

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Characterization of Several *n*-Alkyl Esters of Gibberellin A_3 and Their Comparative Biological Activity[1,2]

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The gibberellins, metabolic products of the fungus *Gibberella fujikuroi*, some of which are also native to higher plants,[3,4] have been shown to alter markedly plant behavior.[5,6] As with other growth regulators, some derivatives are biologically active. Takahashi, *et al.*[7] reported that the methyl ester of gibberellin A_3 (gibberellic acid) was inactive but did not specify the bioassay used. Esterification of the hydroxyl group of gibberellin A_3 had no significant effect on biological activity, while esterification of the carboxyl group resulted in compounds which were inactive when applied to the leaves but were slightly active when applied to the root medium.[8] The response from root treatment may have resulted from hydrolysis of the ester in the culture solution.

These results prompted further study of the *n*-alkyl esters of gibberellin A_3. The synthesis, physical, and chemical properties, and biological activity of the methyl through the *n*-decyl esters are described in this report.

EXPERIMENTAL

Esterification of the carboxyl group of gibberellin A_3 was accomplished with the appropriate alkyl iodide similar to the procedure described in the Australian patent of Imperial Chemical Industries Limited.[8] A mixture of 1.5 g. of gibberellin A_3, 6.5 g. of anhydrous potassium carbonate, 3.5 ml. of allkyl iodide, and 60 ml. of dry acetone was refluxed at 62° for 48 hr. with mechanical stirring. After esterification the acetone was removed by distillation *in vacuo* and the residue washed with water. The esters were further purified by recrystallization from a mixture of ethyl acetate and benzene. All esters were recrystallized to a constant melting point and subjected to carbon and hydrogen analysis. The chemical and physical properties are given in Table I.

BIOLOGICAL ACTIVITY

The comparative biological activities of the esters of gibberellin A_3 in stimulating germination of lettuce seed in the dark, parthenocarpic growth of tomato ovaries, and stem elongation of the bean were determined.

Solutions were prepared by dissolving the appropriate ester in a few drops of ethanol and diluting to the desired volume with distilled water. One hundred lettuce seeds (var. Grand Rapids) were placed in a Petri dish on Whatman No. 1 filter paper, and 5 ml. of the ester solution (3×10^{-5} *M*) were added to each dish. Seeds similarly treated with five milliliters of distilled water and a comparable concentration of ethanol were used as a control. Seeds were germinated in an incubator at 26 ± 0.5° for 96 hr. Each treatment was replicated five times and the experiment performed twice. In parthenocarpic fruit growth the esters were applied in lanolin paste in concentrations of 3×10^{-3} to 3×10^{-6} *M* directly to emasculated ovaries of the Michigan-Ohio Hybrid tomato variety. The diameter of the ovaries was measured 5 days after the treatment. Comparative stimulation of stem elongation was determined with Blue Lake beans. Ten ml. of a 3×10^{-5} or 3×10^{-6} *M* solution was applied to the epicotyl apex and stem elongation (distance from cotyledon to epicotyl apex) was determined after 48 hr.

Methyl, ethyl, *n*-propyl, *n*-butyl, *n*-amyl, *n*-hexyl, and *n*-heptyl gibberellates significantly increased the per cent of lettuce seed that germinated in the dark (Table I) with the methyl, ethyl, *n*-propyl, *n*-butyl, and *n*-amyl gibberellates equal to or approaching the activity of gibberellin A_3. Germination was not significantly enhanced by the *n*-octyl, *n*-nonyl, or *n*-decyl gibberellates. Parthenocarpic development of tomato ovaries and stem elongation in the bean were not significantly stimulated by any of the esters of the carboxyl grouping of gibberellin A_3. The promotive responses obtained from methyl, ethyl, *n*-propyl, *n*-butyl, *n*-amyl, *n*-hexyl, and *n*-heptyl gibberellates in lettuce seed germination in

(1) Journal Article No. 2462 from the Michigan Agricultural Experiment Station, East Lansing.
(2) This research was supported by the Horace H. Rackham Research Endowment.
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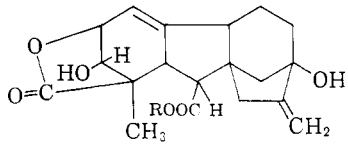
TABLE I

CHEMICAL AND PHYSICAL PROPERTIES OF SEVERAL *n*-ALKYL ESTERS OF GIBBERELLIN A_3 AND THEIR COMPARATIVE EFFECT ON GERMINATION OF LETTUCE SEED IN THE DARK



R	Empirical Formula	Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	M.P., °C.	Germination of Lettuce Seed, %
Control							41.2[b]
H (Gibberellin A_3)							80.6
Methyl	$C_{20}H_{24}O_6$	66.6[a]	66.2	6.7	6.6	202	63.5
Ethyl	$C_{21}H_{26}O_6$	67.4[a]	67.7	7.0	7.1	155	78.4
n-Propyl	$C_{22}H_{28}O_6$	68.0	67.9	7.3	7.5	138	68.9
n-Butyl	$C_{23}H_{30}O_6$	68.6[a]	68.5	7.5	7.6	145	83.7
n-Amyl	$C_{24}H_{32}O_6$	69.2	69.0	7.7	7.7	165–66	79.1
n-Hexyl	$C_{25}H_{34}O_6$	69.7	69.5	7.9	7.9	188–89	56.8
n-Heptyl	$C_{26}H_{36}O_6$	70.2	70.1	8.2	8.2	181–82	57.2
n-Octyl	$C_{27}H_{38}O_6$	70.7[a]	70.6	8.4	8.4	157–58	48.0
n-Nonyl	$C_{28}H_{40}O_6$	71.2	71.5	8.5	8.9	131–32	46.7
n-Decyl	$C_{29}H_{42}O_6$	71.6	71.6	8.7	8.8	102.5–108.5	40.0

[a] *Cf.* ref. (8). [b] L.S.D. at p. 05: 11.7. L.S.D. at p. 01: 16.4.

the dark may have resulted from hydrolysis of the esters to the acid (gibberellin A_3) in the aqueous germinating medium or hydrolysis within the seed or seedling after imbibition.

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Chloromethylation of 1,2,4-Trimethylbenzene

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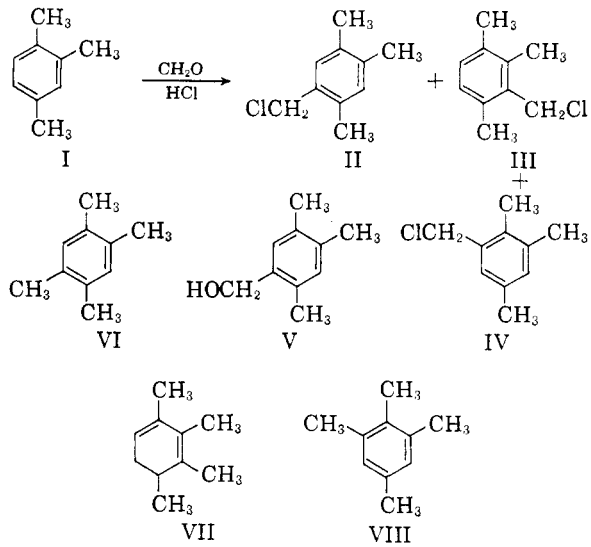
Previous investigators[1,2] of the chloromethylation of 1,2,4-trimethylbenzene (I) reported the product to be 2,4,5-trimethylbenzyl chloride (II). We have found the product, obtained in 78% yield, to be a mixture of 75% of 2,4,5-trimethylbenzyl chloride (II), 22% of 2,3,6-trimethylbenzyl chloride (III) and 2% of 2,3,5-trimethylbenzyl chloride (IV). The mixture boiled over a range of 3° and all fractions showed essentially identical refractive indices and infrared spectra. The mixture was nonseparable by vapor phase chromatography.



From the hydrolyzed chloride mixture was isolated 2,4,5-trimethylbenzyl alcohol (V, m.p. 81–82°) whose identity was confirmed by reduction to 1,2,4,5-tetramethylbenzene (VI). Authentic 2,4,5-trimethylbenzyl chloride (II) obtained from 2,4,5-trimethylbenzyl alcohol (V) showed a refractive index which was similar, but an infrared spectrum which was different, from that of the original mixture. Using authentic II as standard, the infrared spectrum of the original mixture indicated the presence of 74% of II.

The chloride mixture was further characterized by catalytic reduction to a mixture of tetramethylbenzenes whose infrared spectrum showed the presence of 76% of 1,2,4,5-tetramethylbenzene (VI), 22% of 1,2,3,4-tetramethylbenzene (VII) and 2% of 1,2,3,5-tetramethylbenzene (VIII). The analytical value for VI was obtained by direct com-

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(2)(a) G. Vavon and J. Bolle, *Compt. rend.*, **204**, 1826 (1937); *Bull. soc. chim.* (5), **6**, 1025 (1939). (b) W. John and P. Günther, *Ber.*, **74B**, 879 (1941) report that chloromethylation of pseudocumene under conditions similar to our own, gives in addition to the main product (b.p. 98–108°/1 mm.), a by-product, $C_{11}H_{14}Cl_2$, b.p. 120–130°/1 mm., m.p. 99–101°. The latter is presumably an isomeric α,α'-dichloropentamethylbenzene.