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# Studies on Plant-Growth Regulators. IV.[1] Structure of Growth Retardants and Syntheses of Related Compounds

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The structure of Crystals E, F, and G were determined as methyl (*p*-methylcarbamoyloxy)cinnamate, methyl (*p*-methylcarbamoyloxy)hydrocinnamate, and methyl *p*-methoxycinnamate, respectively. Crystals E and F were confirmed to be the artifacts resulting from the methylation with diazomethane–ether. *p*-Hydroxycinnamic acid was proved to be a plant-growth retardant existing in Moyashi (etiolated seedlings of a bean, *Phaseolus mungo*). Several growth retardants were synthesized.

In the previous paper, separation of methylated acid fraction (Me-F-2) of Moyashi and the isolation of three crystalline compounds were reported. These three compounds, crystal E, F, and G showed a weak growth-inhibiting activity as shown in Table I. This paper deals with the structures of these three compounds and the syntheses of their related compounds.

Table I. Biological Activity of Crystals E, F, and G in Avena Straight Growth Test

| | Concn. (ppm) | Length (mm) | | Concn. (ppm) | Length (mm) |
|---|---|---|---|---|---|
| Crystal E | 10 | 6.2 | Crystal E+IAA | 10 +1 | 8.5 |
| | 1 | 6.6 | | 1 +1 | 9.3 |
| | 0.1 | 6.6 | | 0.1+1 | 9.6 |
| Crystal F | 100 | 6.0 | Crystal F+IAA | 100 +1 | 8.1 |
| | 10 | 6.3 | | 10 +1 | 8.6 |
| | 1 | 6.4 | | 1 +1 | 9.1 |
| Crystal G | 10 | 6.2 | Crystal G+IAA | 10 +1 | 9.2 |
| | 1 | 6.2 | | 1 +1 | 9.1 |
| | 0.1 | 6.6 | | 0.1+1 | 9.3 |
| Control | | 6.7 | | | |
| IAA | 1 | 9.4 | | | |

Crystal G, mp 88—89° (from hexane), $C_{11}H_{12}O_3$, has infrared and nuclear magnetic resonance (NMR) spectra as shown in Fig. 1, which were identical to those of authentic methyl *trans-p*-methoxycinnamate and the mixed melting point test exhibited no depression.

Crystal E, mp 137.5—141.0°, had a molecular formula, $C_{12}H_{13}O_4N$. Its NMR and infrared spectra are presented in Fig. 2. The NMR spectrum of crystal E is very similar to that of crystal G in the region of aromatic and olefinic protons, which suggests the crystal E being a *p*-substituted derivative of methyl cinnamate. The four protons in the benzene ring

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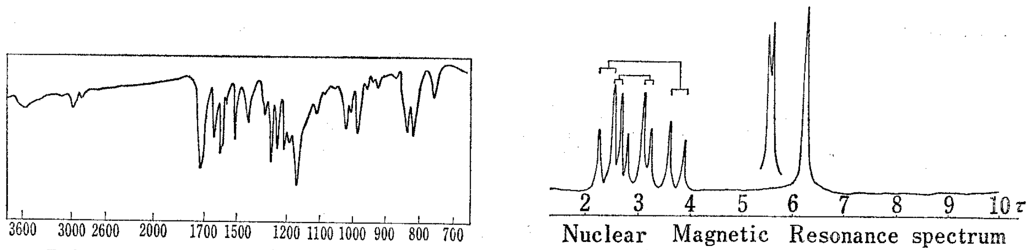
[1] Part III: T. Okamoto, Y. Isogai, T. Koizumi, H. Fujishiro, and Y. Sato: *Chem. Pharm. Bull.* (Tokyo), **15**, 163 (1967).

[2] Location: *a) Hongo 7-3-1, Bunkyo-ku, Tokyo, 113.; b) Komaba-cho, Meguro-ku, Tokyo, 153.*

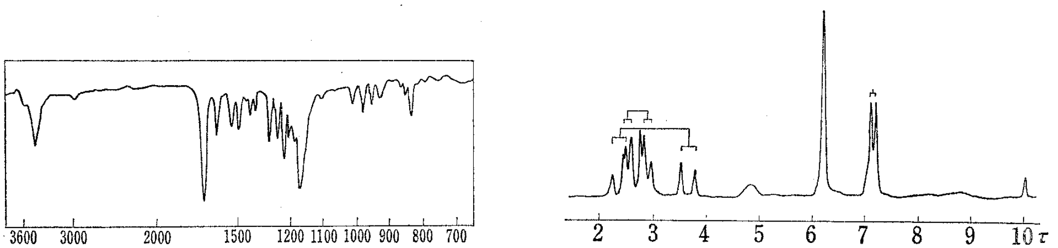


Infrared spectrum (KBr tablet)

Nuclear Magnetic Resonance spectrum ($CDCl_3$ solution)

Fig. 1 Infrared and Nuclear Magnetic Resonance Spectra of Crystal G

gave $A_2B_2$ quartet centered at 2.55 $\tau$[3] and 2.88 $\tau$ ($J$=8.5 cps). The former signals presumably arise from the *ortho*-protons of the CH=CHCOOCH$_3$ group. Another AB quartet centered at 2.38 and 3.65 $\tau$ ($J$=15.5 cps) would be the signals of the olefinic protons in the side chain. The $J$ value of this quartet suggested that the two protons are *trans* to each other. The methylester absorption occurs as a singlet at 6.23 $\tau$. Infrared absorption at 840 cm$^{-1}$ supported also *trans*-olefin structure. The presence of a methylcarbamoyloxy group ($-OCONHCH_3$) was confirmed by the following data: (a) Infrared (IR) absorption at 1720 cm$^{-1}$ (ester carbonyl and urethan carbonyl), 1550 cm$^{-1}$ (amide II band), and 1323 cm$^{-1}$ (amide III band), (b) the NMR spectrum having a broad signal at 4.9 $\tau$ due to amide-NH proton, (c) the sharp doublet at 7.15 $\tau$ ($J$=5 cps) due to N–CH$_3$ protons, coupled with the proton of NH. From these results, the structure of crystal E was assigned as methyl *trans*-*p*-(methylcarbamoyloxy)-cinnamate.



Infrared spectrum (KBr tablet)

Nuclear Magnetic Resonance spectrum ($CDCl_3$ solution)

Fig. 2. Infrared and Nuclear Magnetic Resonance Spectra of Crystal E

In the ultraviolet spectrum of crystal E, absorption maxima were observed at 218 and 284 mμ, which shifted to 243, 312, and 365 mμ upon addition of one drop of dilute sodium

Table II. Ultraviolet Spectral Data of Crystals E and F

| | Crystal E, mμ (ε) | Crystal F, mμ (ε) |
|---|---|---|
| In EtOH | 218 (13600)<br>284 (22600) | 215 (8450)<br>266 (1350)<br>275 (1510)<br>285 (1410) |
| $OH^-$ addition | 243 (10900)<br>312 (shoulder)<br>365 (30600) | 243 (11800)<br>297 (289)<br>365 (1890) |
| $H^+$ addition after $OH^-$ addition | 230 (9600)<br>313 (17400) | 223 (—)<br>280 (—)<br>313 (—) |

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[3] The NMR spectra were run in $CDCl_3$ solution with JNB-3H-60 high resolution instrument of Japan Electronic optics Co., Ltd., tetramethylsilane serving as the internal standard.

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hydroxide solution. This shift is not reversible when acidified with dilute hydrochloric acid. This change of absorption bands suggested that the methylcarbamoyloxy group of crystal E was hydrolyzed by a alkaline solution to produce phenolate anion. The ultraviolet spectral data of crystal E and F are shown in Table II.

Crystal F, mp 114.3—115.8°, had a molecular formula, $C_{12}H_{15}O_4N$. The infrared spectrum of crystal F is shown in Fig. 3. Comparison of the infrared spectrum of crystal F with that of crystal E revealed that (1) the absorption at 1740 $cm^{-1}$ (ester carbonyl) occurred in crystal F, (2) 1640 $cm^{-1}$ band dissappeared in crystal F, (3) 1720 $cm^{-1}$ (urethan-carbonyl), 1555 and 1284 $cm^{-1}$ (amide II and III band) also appeared in the spectrum of crystal F. These data and the molecular formula, permitted structural assignment of crystal F as methyl (*p*-methylcarbamoyloxy)hydrocinnamate, that is a dihydro form of crystal E. Synthesis of the authentic samples of crystal E and F, were carried out as follows;

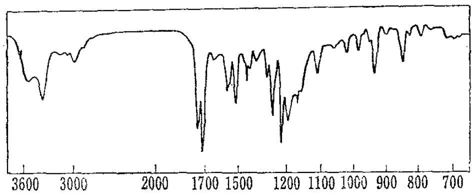

Fig. 3 Infrared Spectrum (KBr tablet) of Crystal F

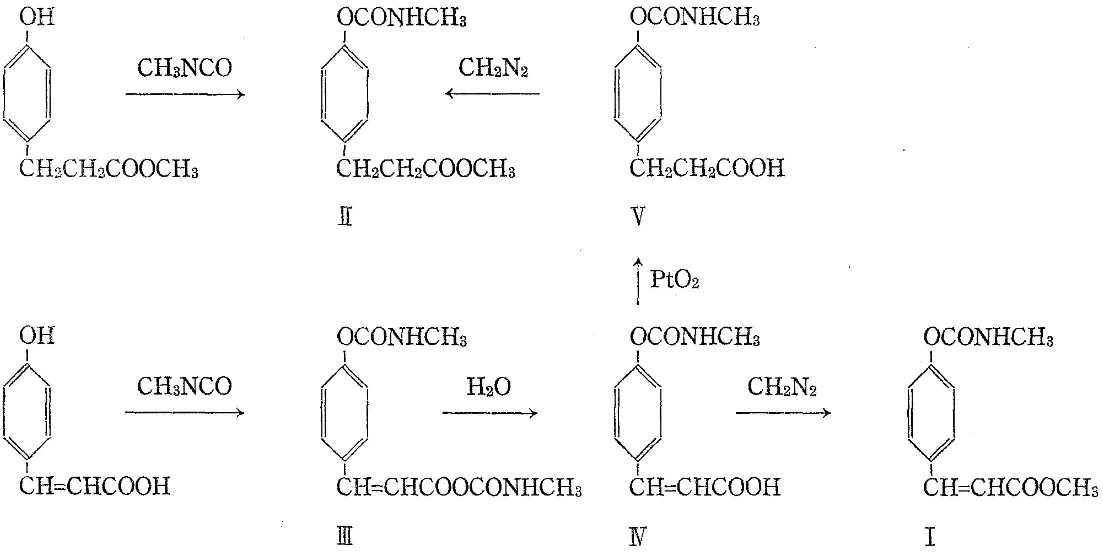

Chart 1

Treatment of *p*-hydroxycinnamic acid with methylisocyanate[4] in anhydrous tetrahydrofuran, afforded *p*-methylcarbamoyloxycinnamic methylcarbamic anhydride (III) whose anhydride structure was confirmed by the presence of infrared absorptions at 1770 and 1715 $cm^{-1}$, and by the reaction of III with water to give IV. The structure of IV was established by infrared absorptions at 1715 (urethan-carbonyl), 1692, 1635 (C=C), and 3375 $cm^{-1}$ (urethan-NH). The compound (IV) was methylated with diazomethane-ether to give a product (I), which was identified with crystal E by the infrared spectrum and admixture test.

Reaction of methyl *p*-hydroxyhydrocinnamate with methyl isocyanate in anhydrous ether gave a crystalline methyl *p*-methylcarbamoyloxyhydrocinnamate (II). This product was identified with crystal F by the infrared spectrum and admixture. The catalytic hydroganation of IV with Adams catalyst furnished *p*-methylcarbamoyloxyhydrocinnamic acid (V), which was then methylated with diazomethane-ether to give methyl *p*-methylcarbamoyloxyhydrocinnamate (II).

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[4] K.H. Slotta and R. Tscheshe, *Ber.*, **298**, 60 (1925).

Since it was likely that crystal E and F might be artifacts resulting from the methylation with diazomethane,[5] the methylation of *p*-hydroxycinnamic acid with diazomethane was tested. Crystal E was isolated as a minor product[6] together with main 3-carbomethoxy-4-(*p*-methoxyphenyl)-2-pyrazoline, using the chromatographic separation over alumina. This experiment established that crystals E and F were artifacts formed during methylation with diazomethane, and that *p*-hydroxycinnamic acid and *p*-hydroxyhydrocinnamic acid were contained in Moyashi. Further, the growth-inhibiting activity of the acid fraction from Moyashi might be attributed to the action of *p*-hydroxycinnamic acid, the well-known growth retardant.[7]

In order to obtain new plant growth retardants, the following *p*-hydroxycinnamic acid derivatives listed in Chart 2 were synthesized.

Chart 2

The physical properties of these compounds and the results of bioassay are outlined in Table III.

Table III. Physical Properties of Synthsized Acid Derivatives and Results of Bioassay (Avena. Straight Growth Test)

| | Molecular formula | mp (°C) | ppm | Length (mm) | ppm | Length |
|---|---|---|---|---|---|---|
| | | | Control | 8.0 | IAA1 | 11.1 |
| *p*-Hydroxy cinnamic acid | $C_9H_8O_3$ | 198 —199 | 50 | 7.1 | 50+IAA1 | 9.6 |
| | | | 10 | 8.0 | 10+1 | 9.9 |
| | | | 1 | 7.2 | 1+1 | 11.1 |
| 3,5-Dichloro-4-OH-cinnamic acid | $C_9H_6O_3Cl_2$ | 218 —219 | 25 | 7.3 | 25+1 | 9.9 |
| | | | 10 | 7.4 | 10+1 | 10.3 |
| | | | 1 | 7.4 | 1+1 | 11.0 |
| α-Ethyl-*p*-OH-cinnamic acid | $C_{11}H_{12}O_3$ | 147 —148 | 50 | 7.4 | 50+1 | 10.7 |
| | | | 10 | 7.7 | 10+1 | 10.4 |
| | | | 1 | 7.4 | 1+1 | 10.2 |
| α-Cyano-4-OH-cinnamic acid | $C_{11}H_{10}O_3Cl_2$ | 208 —211 | 50 | 7.4 | 50+1 | 10.5 |
| | | | 10 | 7.6 | 10+1 | 11.0 |
| | | | 1 | 7.8 | 1+1 | 11.3 |
| α-Et-3,5-diCl-4-OH-cinnamic acid | $C_{10}H_7O_3N$ | 225 —226 | 50 | 7.0 | 50+1 | 9.3 |
| | | | 10 | 7.7 | 10+1 | 9.7 |
| | | | 1 | 8.1 | 1+1 | 10.4 |
| α-Cyano-3,5-diCl-4-OH-cinnamic acid | $C_{10}H_5O_3Cl_2N$ | 196.5—197.5 | 50 | 7.0 | 50+1 | 9.4 |
| | | | 10 | 6.7 | 10+1 | 9.9 |
| | | | 1 | 7.6 | 1+1 | 10.4 |

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5) Methyl isocyanate was evolved by the heating of nitrosomethylurea (F. Arndt, L. Loewe, and S. Avan, *Ber.*, **73**, 606 (1940).

6) Methyl *p*-methylcarbamoyloxy-α-ethylcinnamate was obtained by the methylation of α-ethyl-*p*-hydroxycinnamic acid with freshly prepared diazomethane-ether as a main product (see experimental).

7) M. Tomaszewski, *Colloq. Intern. Centre. Natl. Rech. Sci.* (Paris), **123**, 335 (1963).

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The synthesized compounds showed inhibiting activity as strong as *p*-hydroxycinnamic acid (see, Table III). The syntheses of more effective *p*-hydroxycinnamic acid type growth retardants is now in progress.

## Experimental

***p*-Methylcarbamoyloxycinnamic Methylcarbamic Anhydride (III)**——To a solution of 1.1 g of *p*-hydroxycinnamic acid in 12 ml of dry THF, freshly prepared methyl isocynate was added and the reaction mixture was kept in a refrigerator for 5 days to precipitate 971 mg of III, colorless prisms mp 207—209°. *Anal.* Calcd. for $C_{13}H_{14}O_5N_2$: C, 56.11; H, 5.07; N, 10.07. Found: C, 56.09; H, 5.29; N, 10.35. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3362, 1770, 1715, 1640, 1550, 1275, 1120, 840.

***p*-Methylcarbamoyloxycinnamic Acid (IV)**——To a solution of 128 mg of III dissolved in 5 ml of MeOH, 1 ml of water was added and the mixture was kept on a steam bath for 1 min. Methyl isocyanate gas evolved with separation of crystalline substance. Recrystallization from EtOH gave 70 mg of IV as colorless needles, mp 198—199°. *Anal.* Calcd. for $C_{11}H_{11}O_4N$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.59; H, 4.62; N, 6.52. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3375, 1715, 1692, 1635, 1500, 1230, 840.

**Synthesis of I**——To a solution of 256 mg of IV in 50 ml of MeOH, freshly prepared $CH_2N_2$–ether solution was added under ice cooling and the reaction mixture was kept overnight in a refrigerator. After evaporation of the solvent, the residue was recrystallized from ether to give colorless prisms, mp138—141°. *Anal.* Calcd. for $C_{12}H_{13}O_4N$: C, 61.27; H,5.57; N, 5.96. Found: C, 61.38; H, 5.55; N, 5.81. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1720 (broad), 1640, 1550, 1320, 840.

***p*-Methylcarbamoyloxyhydrocinnamic Acid (V)**——A solution of 10 mg of IV in 10 ml of MeOH was hydrogenated with 5 mg of $PtO_2$ at an atmospheric pressure for 30 min. The colorless residue was recrystallized from benzene, giving 7 mg of V, colorless needles, mp 160—165°. *Anal.* Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28. Found: C, 58.82; H, 5.78; N, 6.43. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1710 (broad), 1545, 1280, 1230, 840.

**Methyl (*p*-Methylcarbamoyloxy)hydrocinnamate (II)**——a) To a solution of 200 mg of *p*-hydroxyhydrocinnamic acid in 20 ml of ether freshly prepared diazomethane–ether was added dropwise until the solution colored yellow and the solvent was distilled off to give 220 mg of yellow oily residue. The solution of 100 mg of the methylated residue in 5 ml of anhyd. ether was treated with methyl isocyanate under cooling and the reaction mixture was kept overnight in a refrigerator. The precipitate of trimethyl isocyanurate (mp 173—174°) was filtered off and the filtrate was evaporated to dryness. The residue was chromatographed over 10 g of silica gel (Kanto Chemical Co.) using ether–hexane (1:2) and 90 mg of II was obtained from fraction No. 11—17. Recrystallization from ether gave 70 mg of pure II, as colorless prisms, mp 112—115°. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1740, 1718, 1555, 1283, 1230, 848.

b) To a solution of 5 mg of V in 2 ml of MeOH diazomethane–ether was added dropwise until the solution colored yellow. After 5 min the solvent was evaporated off to give yellowish crystals, which were recrystallized from ether afording 6 mg of II, colorless prisms, mp 112—115°. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1740, 1718, 1555, 1283, 1230, 850, which was identical with the sample obtained in a) by the infrared spectrum and admixture test.

**Methylation of *p*-Hydroxycinnamic Acid**——To a solution of 413 mg of *p*-hydroxycinnamic acid in 10 ml of MeOH, an excess of freshly prepared diazomethane–ether was added and the mixture was kept in a refrigerator for 2 days. The solvent was evaporated to give an amorphous solid which was chromatographed over silica gel using hexane–ether (2:3) as an eluent, giving about 500 mg of a crystalline product in fraction No. 3—8. The product appeared to consist of two different crystalline forms, which were separated manually into 300 mg of a main product and 10 mg of a minor product. The minor product, mp 138—139°, was identified with methyl (methylcarbamoyloxy)cinnamate (II) by a mixed melting point test and comparison of IR spectrum. The main product 3-carbomethoxy-4-(*p*-methoxyphenyl)-2-pyrazoline, mp 105—107°. *Anal.* Calcd. for $C_{12}H_{14}O_3N_2$: C, 61.52; H, 6.02; N, 11.96. Found: C, 61.55; H, 5.96; N, 11.78. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3440, 1690, 1620; $\nu_{max}^{CHCl_3}$: 1730. UV $\lambda_{max}^{EtOH}$ mμ (ε): 290 (9660). NMR: 2.65 τ (2Hd $J=9$ cps), 2.95 τ (2Hd $J=9$ cps), 6.15 τ (3H, s), 6.1 τ (3H), 5.5—6.5 τ (unresolved multiplet). The main product (48 mg) was acetylated with pyridine–acetic anhydride method giving 11 mg of a product, after recrystallization from ether–benzene to give mp 120.5—121.0°. *Anal.* Calcd. for $C_{14}H_{16}O_4N_2$: C, 60.86; H, 5.84; N, 10.14. Found: C, 61.01; H, 5.90; N, 10.24. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1720, 1670, 1610. UV $\lambda_{max}^{EtOH}$ mμ (ε): 280 (19800). NMR: 7.5 τ (3H), 6.20 τ (6H), 6.0—5.5 τ (unresolved multiplet), 3.01 τ (2H doublet, $J=9$ cps), 2.83 τ (2H, doublet $J=9$ cps).

**3,5-Dichloro-4-hydroxycinnamic Acid**——A mixtur of 10 g of 3,5-dichloro-4-hydroxybenzaldehyde and 12 g of freshly fused AcONa was refluxed in 30 ml of $Ac_2O$ at 190° for 14 hr. The cooled reaction mixture was made alkaline with NaOH solution and the resinous substance was removed by filtration. The aq. alkaline solution was heated at 185—190° for 2 hr, filtered through a filter paper, and conc. HCl was added to the well-cooled filtrate. The precipitate was collected by suctional filtration, washed with $H_2O$, and recrystallized from MeOH, to give 4.2 g fo 3,5-dichloro-4-hydroxycinnamic acid, mp 218—219° (decomp.).

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*Anal.* Calcd. for $C_9H_6O_3Cl_2$: C, 46.39; H, 2.60. Found: C, 46.16; H, 2.68. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1683, 1640, 1299, 980.

**$\alpha$-Ethyl-*p*-hydroxycinnamic Acid**——A mixture of 5 g of *p*-hydroxybenzaldehyde, 14 g of butyric anhydride, and 4 g of freshly fused AcONa was refluxed at 140—145° for 38 hr. The cooled mixture was made alkaline with NaOH solution and the resinous substance was removed. The aq. alkaline solution was heated at 140° for 2 hr, filtered, and conc. HCl was added to the cooled filtrate. The reddish oil was extracted with $CH_2Cl_2$ and the solvent was distilled off. The residue was recrystallized from $H_2O$ to yield 0.5 g of $\alpha$-ethyl-*p*-hydroxycinnamic acid, mp 147—148°. *Anal.* Calcd. for $C_{11}H_{12}O_3$: C, 68.73; H, 6.29. Found: C, 68.41; H, 6.44. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1683, 1640, 1299, 980.

**Methylation of $\alpha$-Ethyl-*p*-hydroxycinnamic Acid with $CH_2N_2$**——To the solution of 300 mg of $\alpha$-ethyl-*p*-hydroxycinnamic acid in MeOH, diazomethane–ether was added and the reaction mixture was kept overnight in a refrigerator. The solvent was evaporated to dryness and 400 mg of the residue was purified by silica gel column chromatography with benzene–$CHCl_3$. The eluate from fraction No. 11—14 (benzene: $CHCl_3=1:1$) was evaporated and the residue was recrystallized from ether to give 60 mg of methyl (*p*-methylcarbamolyloxy)-$\alpha$-ethylcinnamate, mp 110°. *Anal.* Calcd. for $C_{14}H_{17}O_4N$: C, 63.84; H, 6.46; N, 5.32. Found: C, 64.09; H, 6.27, N, 5.25. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3360, 1720. NMR: 8.82 $\tau$ (3H, triplet $J=7.5$ cps), 7.37 $\tau$ (2H, quartet $J=7.5$ cps), 7.02 $\tau$ (3H, doublet $J=5.0$). 6.05 $\tau$ (3H, singlet), 4.7 $\tau$ (1H, broad), 2.63 $\tau$ (2H, doublet $J=9$), 2.41 $\tau$ (2H, doublet $J=9$), 2.2 $\tau$ (1H, singlet). UV $\lambda_{max}^{EtOH}$m$\mu$: 277, 222; $\lambda_{max}^{EtOH}$ m$\mu$ 3N NaOH: 352, 245.

**$\alpha$-Ethyl-3,5-dichloro-4-hydroxycinnamic Acid**——A mixture of 2 g of 3,5-dichloro-4-hydroxybenzaldehyde, 7 g of butyric anhydride, and 1 g of freshly fused AcONa was refluxed at 135—145° for 42 hr. The cooled mixture was made alkaline by the excess of NaOH soln. and the resinous substance was removed. The aq. solution was heated at 140° for 2 hr., filtered, and *conc.* HCl was added to the well-cooled filtrate. The precipitate was recrystallized from MeOH to give 0.9 g of $\alpha$-ethyl-3,5-dichloro-4-hydroxycinnamic acid as colorless prisms, mp 208—211°. *Anal.* Calcd. for $C_{11}H_{10}O_3Cl_2$: C, 50.62; H, 3.86. Found: C, 50.44; H, 4.04. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3460, 1679, 873, 795.

**$\alpha$-Cyano-3,5-dichloro-4-hydroxycinnamic Acid**——A solution of 0.1 g of NaOH in 4 ml of $H_2O$ was added to 6 ml of sodium cyanoacetate solution, the resulting alkaline solution warmed to 40° was shaken vigorously with 1 g of 3,5-dichlorobenzaldehyde for 1 min and then mixed with NaOH solution. After 3 days, the mixture was acidified with conc. HCl, with yellow precipitate which was recrystallized from MeOH and $H_2O$ to give 449 mg of $\alpha$-cyano-3,5-dichloro-4-hydroxycinnamic acid as yellow needles, mp 196.5—197.5°. *Anal.* Calcd. for $C_{10}H_5O_3NCl_2$: C, 46.55; H, 1.95; N, 5.40. Found: C, 46.75; H, 2.13; N, 5.14. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2220, 1770, 1583, 873.

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