be improved by more thorough washing of the catalyst and K_2CO_3. The specific activity of these compounds can be optimized to ~90 Ci/mmol by using tritium gas of high isotopic purity and abundance and also by proper preexchange of the catalyst surface with the tritium gas prior to the reaction. Benzoylarylureas of high specific activity synthesized as described here may serve as sensitive probes in understanding their metabolism and mode of action.

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Registry No. 1, 35367-38-5; [^{3}H]-1, 111409-69-9; 2, 71422-67-8; 3, 83121-18-0; 4, 86479-06-3; 5, 111409-67-7; 6, 111468-43-0; 8, 111409-68-8; 9, 111409-70-2; 10, 111409-71-3; 11, 111409-72-4; p-ClC_6H_4NCO, 104-12-1; 3,5-dichloro-2,4-difluoroaniline, 83121-15-7; 2,6-difluorobenzoic acid, 385-00-2; 3,5-dichloro-4-[[3-chloro-5-(trifluoromethyl)pyridin-2-yl]oxy]aniline, 73265-15-3; 3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)aniline, 104147-32-2.

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Identification of Alcohol-Incorporated Byproducts of the Plant Growth Regulator 1-(3-Chlorophthalimido)cyclohexanecarboxamide (AC 94,377)

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The experimental plant growth regulator 1-(3-chlorophthalimido)cyclohexanecarboxamide (AC 94,377) was found to be transformed into two isomeric byproducts upon dissolution in methanol under basic conditions. In this study, the isomeric alcohol-incorporated byproducts derived from methanol, ethanol, and propanol were identified by spectroscopic methods, and alcohol addition was shown to occur at either carbonyl of the phthalimide moiety of AC 94,377. Dissolution in dimethyl sulfoxide or dimethylformamide caused the byproducts to decompose back to AC 94,377. A biological test for gibberillin-like activity was conducted on the alcohol-incorporated byproducts, and the results were negative.

A series of phthalimide plant growth regulators were synthesized by American Cyanamid Co. (1976, 1977). These substituted phthalimides have been shown to mimic the growth-regulating activity of gibberellins (Los et al., 1980a; Devlin, 1981). The most promising plant growth regulator (PGR) in the phthalimide series is 1-(3-chlorophthalimido)cyclohexanecarboxamide (1) (Figure 1). Upon treatment of 28 different crop plants with the substituted phthalimide 1, 93% of the treated plants demonstrated moderate to very responsive PGR effects (Los et al., 1980b). Testing nine species of dormant weed seeds for germination activity, 1 actively promoted germination of five of the nine species tested (Metzger, 1983). On a weight-to-weight basis, 1 demonstrated seed germination activity either equal to or greater than that of gibberellic

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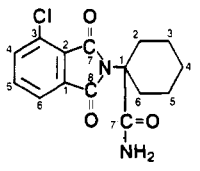
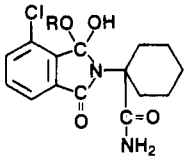


AC 94,337, 1

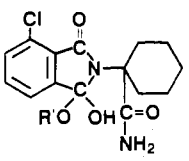


Insoluble Isomer, 2

2a, R = $-\overset{9}{C}H_3$

2b, R = $-CH_2\overset{10}{C}H_3$

2c, R = $-CH_2CH_2\overset{11}{C}H_3$



Soluble Isomer, 3

3a, R' = $-CH_3$

3b, R' = $-CH_2CH_3$

3c, R' = $-CH_2CH_2CH_3$

Figure 1. Structure of AC 94,377 and the two isomeric byproducts formed by reaction with alcohol under basic condition.

acid, GA_3. Surface application of 1 onto buried dormant wild mustard seeds at 1.3-, 2.5-, and 3.8-cm depths showed enhanced germination over controls (Donald and Hoerauf, 1985). These data suggest that 1 can move from the soil surface to the depth of dormant seed planting. To estimate the extent of its biological activity, 1 was subjected to a series of bioassay tests normally employed for the assessment of PGR activity (Suttle and Schreiner, 1982). In the bioassay tests, no cytokinin or ethylene induction activity was observed; however, gibberellin-like activity was clearly demonstrated.

In order to examine 1 under field plot conditions, it was necessary to synthesize several kilograms of this material. Because methanol was found to be the most effective solvent for 1, the crude product of synthesis was recrystallized from methanol. Upon recrystallization from methanol, however, 1 was transformed into an unknown byproduct in essentially quantitative yield. This study investigates the cause of transformation and the identification of the transformation products.

EXPERIMENTAL SECTION

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are reported uncorrected. High-performance liquid chromatography (HPLC) was performed with a Beckman HPLC unit equipped with two Model 112 high-pressure pumps, a Model 421 solvent program controller, a Hitachi Model 100-10 variable-wavelength spectrophotometric detector, and a Spectra-Physics Model SP4270 integrator. HPLC separations were accomplished with an Ultrasphere ODS C_{18} column (4.6 mm (i.d.) × 15 cm) packed with 5-μm spherical particles. Electron ionization mass spectrometry (EI-MS) was conducted with a Varian MAT 112S spectrometer. Fast atom bombardment mass spectrometry (FAB-MS) was performed with a Varian MAT CH5-DF spectrometer equipped with an Ion Tech saddle-field gun. Samples for FAB-MS were inserted into the spectrometer on a copper-tipped probe in a glycerol matrix, and xenon was used as the ionizing gas. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were taken on a JEOL FX-90Q Fourier transform spectrometer, and tetramethylsilane was employed as the internal standard. Infrared (IR) spectra were obtained with a Perkin-Elmer Model 399B spectrophotometer. IR analyses were performed with use of KBr pellets (13-mm diameter) prepared with 1.5 mg of sample in 400 mg of anhydrous KBr.

Synthesis of AC-94,377 (1). From 3-nitrophthalic acid (Eastman Kodak Co.), the anhydride was prepared by treatment with acetic anhydride (Nicolet and Bender, 1961). Reaction of the 3-nitrophthalic anhydride with chlorine at 240 °C gave 3-chlorophthalic anhydride as product (Newman and Scheurer, 1956). The 1-(3-chlorophthalimido)cyclohexanecarboxylic acid was prepared by reaction of 3-chlorophthalic anhydride with 1-aminocyclohexanecarboxylic acid (Aldrich Chemical Co.) by the method of Bose (1973). The chlorophthalimidocyclohexanecarboxylic acid was converted to the acid chloride with thionyl chloride (Kent and McElvain, 1955), and reaction of the acid chloride with 28% ammonium hydroxide (Kent and McElvain, 1955) afforded the AC 94,377. The product was decolorized with activated charcoal and recrystallized from acetonitrile: mp 194.5–195 °C [lit. mp 194–195 °C (American Cyanamid Co., 1977)]; EI-MS m/z (relative intensity) 306 (M^+, 1), 288 (12), 262 (78), 194 (24), 182 (100), 164 (37), 138 (17), 110 (15), 81 (80), 44 (9); ^1H NMR (Me_2SO-d_6) δ 1.1–3.0 (m, 10 H, cyclohexyl), 7.08 (s, 1 H, N*H*), 7.46 (s, 1 H, N*H*), 7.80 (s, 3 H, ArH); ^{13}C NMR (Me_2SO-d_6) δ 20.34 (C-3′,-5′), 23.70 (C-4′), 29.60 (C-2′,-6′), 65.63 (C-1′), 119.61 (C-6), 125.99 (C-3), 127.11 (C-2), 132.09 (C-1), 133.55 (C-4,-5), 164.66 (C-7), 165.63 (C-8), 171.68 (C-7′). Assignments of the cyclohexane portion of the ^{13}C NMR spectrum were based on cyclohexylamine and cyclohexanecarboxylic acid chloride spectra (Breitmaier et al., 1979), and the phthalimide portion was based on the spectrum of 3-chlorophthalic anhydride (Fifolt et al., 1982).

General Synthesis of Alcohol Byproducts. To 50 mL of alcohol [MeOH (a), EtOH (b), *n*-PrOH (c)] was added 1 g of 1, and the mixture was heated until all material dissolved. To the cooled solution was added about 10 drops of 28% ammonium hydroxide, and after stirring, the solution was allowed to stand for about 2 h. The precipitated product that was isolated by filtration was mainly isomer 2 (87% **2a**, 80% **2b**). The material in the filtrate was composed mainly of isomer 3 (79% **3a**, 78% **3b**). The percentage distribution for the *n*-PrOH isomers (**2c** and **3c**) was not determined owing to difficulties in HPLC separation.

Purification of Alcohol Byproducts. The insoluble MeOH product **2a** was obtained in about 99.5% purity after three successive recrystallizations from methanol. The more soluble **3a** was purified by dissolving the filtrate residue in 2-propanol and removing the more insoluble **2a** as it crystallized from solution. When crystals of **2a** no longer formed, the solution was concentrated and **3a** was allowed to crystallize from solution to yield isomer **3a** in about 98% purity. The insoluble EtOH product **2b** was recrystallized from ethanol and washed with acetone to yield a product of approximately 99% purity. The isomer **3b** was purified by thin-layer chromatography (TLC) on Anasil HF plates with benzene–acetone (2:1, v/v) as the developing solvent. TLC purification gave **3b** in essentially 100% purity. The insoluble *n*-PrOH product **2c** was recrystallized from 2-propanol to yield a product of about 100% purity. Isomer **3c** was recrystallized from 2-propanol in the same manner as **3a** was purified from methanol solvent (vida supra) to afford a product of 97% purity. The estimation of isomer purity was accomplished by HPLC analysis.

HPLC Analysis of Alcohol Byproducts. With a two-reservoir system, solvent A was composed of 10% CH_3CN in degassed, distilled water and solvent B was CH_3CN. In the description of the eluting solvent given below, the quantity of solvent B is provided and solvent

A makes up the remainder to equal 100%.

For separation of **2a** from **3a**, elution was started with 10% solvent B for 1 min. Then solvent B was increased to 20% over a 25-min period. Upon reaching 20% solvent B, elution was continued at this percentage for an additional 24 min. Retention times for **2a** and **3a** were 43.5 and 39.5 min, respectively. With the same HPLC solvent program used for MeOH byproduct separation, the EtOH byproducts **2b** and **3b** gave retention times of 45.2 and 42.5 min, respectively.

For HPLC separation of the isomeric byproducts of *n*-PrOH, solvent A was made up of 10% solvent B in distilled water and solvent B was composed of 20% 1-propanol in CH_3CN. Elution was started with 10% solvent B for 1 min followed by a gradient that increased the amount of solvent B to 30% over a 25-min period. Elution was continued for an additional 24 min after solvent B reached 30%. The retention times of **2c** and **3c** were 37.0 and 34.9 min, respectively.

AC 94,377–MeOH (2a): EI-MS, *m/z* (relative intensity) 338 (M^+, 0), 294 (19), 288 (38), 262 (48), 207 (24), 197 (100), 182 (69), 164 (47), 138 (19), 110 (11), 81 (68), 44 (8); FAB-MS, *m/z* (relative intensity) 339 (MH^+, 5), 332 (4), 307 ($MH^+ - CH_3OH$, 86), 291 (26), 290 (51), 262 (100); ^1H NMR (pyridine-d_5) δ 1.2–2.9 (m, 10 H, cyclohexyl), 3.96 (s, 3 H, OCH_3), 7.22 (t, J = 7.4 Hz, 1 H, Ar*H*-5), 7.45 (dd, J = 7.4, 1.5 Hz, 1 H, Ar*H*-6), 7.72 (br s, 1 H, N*H*), 7.80 (dd, J = 7.4, 1.5 Hz, 1 H, Ar*H*-4), 8.13 (br s, 1 H, N*H*), 9.20 (s, 1 H, O*H*); ^{13}C NMR (pyridine-d_5) δ 22.23 (C-3′,-5′), 25.94 (C-4′), 32.86 (C-2′,-6′), 52.95 (C-9), 61.73 (C-1′), 126.67, 130.96, 131.64, 131.93, 138.76, 167.33, 167.72, 177.08 (C-7′).

AC 94,377–MeOH (3a): EI-MS, *m/z* (relative intensity) 338 (M^+, 0), 294 (37), 288 (2), 262 (10), 197 (100), 182 (17), 164 (14), 154 (12), 139 (15), 110 (12), 81 (18), 44 (31); FAB-MS, *m/z* (relative intensity) 677 ($2M + H^+$, 1), 339 (MH^+, 8), 322 (25), 294 (32), 262 (3), 197 (100); ^1H NMR (CH_3CN-d_3) δ 1.1–2.4 (m, 10 H, cyclohexyl), 3.89 (s, 3 H, OCH_3), 5.71 (br s, 1 H, N*H*), 6.91 (s, 1 H, O*H*), 7.40 (br s, 1 H, N*H*), 7.48 (t, J = 7.7 Hz, Ar*H*-5), 7.70 (dd, J = 7.7, 1.5 Hz, 1 H, Ar*H*-6), 7.95 (dd, J = 7.7, 1.5 Hz, 1 H, Ar*H*-4); ^{13}C NMR (MeOH-d_4) δ 22.47 (C-3′,-5′), 26.28 (C-4′), 33.20 (C-2′,-6′), 53.48 (C-9), 62.55 (C-1′), 130.13, 130.61, 131.20, 133.73, 135.30, 138.90, 167.88, 168.16, 179.86 (C-7′).

AC 94,377–EtOH (2b): EI-MS, *m/z* (relative intensity) 352 (M^+, 0), 308 (10), 288 (13), 262 (42), 211 (43), 194 (21), 183 (80), 182 (100), 164 (49), 138 (23), 110 (17), 81 (55), 45 (20); FAB-MS, *m/z* (relative intensity) 353 (MH^+, 32), 336 (44), 308 (21), 307 (35), 262 (41), 211 (100); ^1H NMR (pyridine-d_5) δ 1.30 (t, J = 7.0 Hz, 3 H, CH_3), 1.7–2.9 (br m, 10 H, cyclohexyl), 4.49 (q, J = 7.0 Hz, 2 H, OCH_2CH_3), 7.22 (t, J = 7.4 Hz, 1 H, Ar*H*-5), 7.45 (dd, J = 7.4, 1.5 Hz, 1 H, Ar*H*-6), 7.64 (br s, 1 H, N*H*), 7.79 (dd, J = 7.4, 1.5 Hz, 1 H, Ar*H*-4), 8.10 (br s, 1 H, N*H*), 9.18 (s, 1 H, O*H*); ^{13}C NMR (MeOH-d_4 δ 15.26 (C-10), 23.55 (C-3′,-5′), 27.35 (C-4′), 33.98 (C-2′,-6′), 62.94 (C-9), 64.31 (C-1′), 128.18, 132.45, 133.74 (some aromatic and carbonyl peaks were not detected due to low solubility).

AC 94,377–EtOH (3b): EI-MS, *m/z* (relative intensity) 352 (M^+, 0), 308 (14), 288 (7), 262 (28), 211 (76), 194 (23), 183 (100), 182 (78), 164 (45), 138 (17), 110 (14), 81 (79); FAB-MS, *m/z* (relative intensity) 705 ($2M + H^+$, 3), 445 (MH^+ + glycerol, 2), 353 (MH^+, 52), 336 (72), 308 (40), 211 (100); ^1H NMR (MeOH-d_4) δ 1.2–2.4 (br m, 10 H, cyclohexyl), 1.39 (t, J = 7.0 Hz, 3 H, CH_3), 4.39 (q, J = 7.0 Hz, 2 H, CH_2CH_3), 7.51 (t, J = 7.9 Hz, 1 H, Ar*H*-5), 7.73 (dd, J = 7.9, 1.5 Hz, 1 H, Ar*H*-6), 8.02 (dd, J = 7.9, 1.5 Hz, 1 H, Ar*H*-4); ^{13}C NMR (MeOH-d_4) δ 14.38 (C-10), 22.38 (C-3′,-5′), 26.28 (C-4′), 33.20 (C-2′,-6′), 62.65 (C-9), 63.24 (C-1′), 129.94, 130.91, 131.20, 133.74, 135.20, 138.91, 166.89, 168.16, 179.96 (C-7′).

AC 94,377–PrOH (2c): EI-MS, *m/z* (relative intensity) 366 (M^+, 0), 322 (17), 288 (4), 262 (13), 225 (35), 183 (100), 139 (9), 110 (3), 81 (12); FAB-MS, *m/z* (relative intensity) 367 (MH^+, 12), 350 (12), 333 (7), 316 (6), 225 (20), 185 ((2 gly + H)$^+$, 100).

AC 94,377–PrOH (3c): EI-MS, *m/z* (relative intensity) 366 (M^+, 0), 322 (3), 288 (35), 262 (100), 194 (21), 182 (89), 164 (32), 138 (16), 110 (11), 81 (63); FAB-MS, *m/z* (relative intensity) 733 ((2 M + H)$^+$, 0.2), 643 ((MH + 3 gly)$^+$, 0.1), 551 ((MH + 2 gly)$^+$, 0.3), 459 ((MH + gly)$^+$, 1), 367 (MH^+, 6), 350 (6), 185 ((2 gly + H)$^+$, 100).

Nuclear Overhauser Effect (NOE). The soluble isomer **3a** was dissolved in CH_3CN-d_3 in a 5-mm NMR tube, and the sample was purged with N_2. The spectrometer was set in the HOMO decoupling mode with radiofrequency power at 3. The spectra were obtained with an acquisition time of 2.3 s and a pulse delay of 8 s. Selective irradiation of the ArH-6 proton at δ 7.70 showed no NOE effect on the OCH_3 group. Irradiation of the OH proton at δ 6.91 gave an NOE effect of 8% for the ArH-6 proton, and saturation of the OCH_3 group at δ 3.89 gave an intramolecular NOE effect of 15% for the ArH-6 proton.

The NOE effect could not be measured for **2a** because of its extreme insolubility in acetonitrile, methanol, and acetone, its instability in dimethyl sulfoxide and dimethylformamide, and the interfering solvent peaks in the aromatic region of pyridine.

RESULTS AND DISCUSSION

In the purification of crude synthetic **1**, this material was transformed in approximate quantitative yield into an unknown byproduct during recrystallization in methanol. Final purification of **1** was therefore accomplished by recrystallization from acetonitrile. However, upon attempting to prepare additional unknown by heating pure **1** in methanol, no byproduct could be obtained. Employing an acid catalyst in methanol solutions of purified **1** gave less than 5% yield of unknown byproduct. Examination of the synthetic pathway revealed that ammonium hydroxide was the last reagent used in the bulk synthesis of **1**, and this reagent, still present in the crude product, was apparently catalyzing the rapid transformation of **1** into the unknown byproduct. Addition of ammonium hydroxide to methanol solutions of purified **1** verified that this reagent was indeed responsible for catalytic conversion of **1** into the observed byproduct.

The NMR spectra of **1** were obtained in Me_2SO-d_6 because of its ease of solubility in this solvent. Consequently, the initial NMR spectra of the isolated byproduct of **1** (**2a**–**3a** mixture) were also determined in Me_2SO-d_6. The proton integral of the unknown showed that methanol and **1** were at a 1:1 ratio in the unknown byproduct. The protons of the OCH_3 group were observed as a doublet (δ 3.16), and the OH group was observed as a quartet (δ 4.11), which was the same as that of MeOH dissolved in Me_2SO-d_6. Examination of the ^{13}C NMR spectrum of the unknown in Me_2SO-d_6 showed that all the carbon chemical shifts of the unknown were exactly the same as those of standard **1** and the chemical shift of the OCH_3 carbon (δ 48.0) was the same as that reported for methanol (Breitmaier et al., 1979). Further examination by HPLC verified that reversal of reaction of the unknown back to **1** and MeOH was indeed occurring after dissolution in Me_2SO.

To examine the reverse reaction, solutions of the **2a**–**3a** mixture were allowed to stand at room temperature for 2 days; conversion of the unknown back to **1** and MeOH was

estimated to be 91% complete in Me_2SO and 41% complete in dimethylformamide (DMF). Upon determination that isomers were present in the unknown by HPLC, the stability of the individual isomers was then examined in DMF solution because transformation back to 1 was much slower in this solvent. When DMF solutions of **2a**, **2b**, **3a**, and **3b** were allowed to stand for 3 days at ambient temperature, it was found that 80% of **2a** was transformed back to 1, **3a** showed no conversion to 1, 22% of **2b** was transformed back to 1, and 5% of **3b** was converted back to 1. Thus, factors that appear to influence the rate of reversal of alcohol byproduct back to 1 are steric effects (position and size of OR or OR′ group), solvents of high dielectric constant, and high solution temperatures.

Melting point determinations were attempted with the alcohol-incorporated byproducts. However, the melting temperatures were very inconsistent and depended primarily upon heating rates. HPLC verified that melting of the alcohol byproducts resulted in transformation of the material back to 1. Thus, if heating was too slow, the incorporated alcohol was slowly released and the product melted at the melting temperature of 1. On the other hand, if heating was too rapid, the incorporated alcohol was quickly released and premature melting of the sample would occur. Due to the unreliability of these data, melting points were not reported for the alcohol byproducts.

Characterization of Alcohol Byproducts of 1. Since the unknown byproducts could be converted back to 1 by dissolution in Me_2SO, there was a possibility that these products might be tightly held complexes rather than new products. The spectroscopic data given below for the unknown byproducts resolve this question.

The EI-MS of **2a** and **3a** both gave a strong ion fragment at m/z 294, which results from loss of the carboxamide neutral fragment from the molecular ion. Both the m/z 294 peak and the base peak (m/z 197) still had the methoxy group attached. Similarly for **2b** and **3b**, the ion fragments at m/z 308 and 211 possessed the ethoxy group but were 14 mass units higher than the same ion fragments of **2a** and **3a**. The FAB-MS spectra gave pseudomolecular ions (MH^+) for **2a** and **3a** at m/z 339 and for **2b** and **3b** at m/z 353 to verify the molecular mass of each byproduct.

The infrared spectra show the OH stretching vibrations at 3292–3276 cm^{-1} for the **2** isomers and at 3340–3320 cm^{-1} for the **3** isomers. The intense absorption of all the unknown byproducts at the 1300–1275-cm^{-1} region due to the C–O–C stretching vibrations verifies formation of the ether linkage in the new structure. The ^1H NMR spectra of **2a** and **3a** indicated three protons with appropriate coupling constants remained on the aromatic ring while the chemical shift of the OCH_3 group was shifted slightly downfield and the OH group was shifted strongly downfield from where dissolved methanol should be observed. In the ^{13}C NMR spectra of **2a** and **3a**, the OCH_3 peak of the unknowns was shifted about 5 ppm downfield from where dissolved methanol would be expected to appear and all the carbon chemical shifts were different from those observed for the phthalimide portion of 1.

In summary, the evidence to verify that new products were being produced by reaction of alcohols with 1 rather than alcohols being associated with 1 in tightly held complexes are as follows: (a) the presence of pseudomolecular ions for the byproducts by FAB-MS; (b) the observation of major ion fragments possessing the bonded alkoxyl group by EI-MS; (c) the presence of the hydroxyl and ether absorption bands by IR spectroscopy; (d) the exhibition of ^1H and ^{13}C chemical shifts different from those of 1 by NMR spectrometry.

The EI-MS ion fragments at m/z 197 (**2a**, **3a**) and m/z 211 (**2b**, **3b**) suggest that no change was taking place on the cyclohexanecarboxamide portion of 1 after reaction with alcohol. The presence of three aromatic protons in the ^1H NMR spectra verifies that no change was taking place on the aromatic ring of 1 in formation of the alcohol byproducts. Therefore, alcohol must be reacting with the two carbonyls associated with the phthalimide portion of 1 to yield the two isomeric byproducts observed with each alcohol (Figure 1).

Identification of the Alcohol Byproduct Isomers. The **2** isomers were relatively insoluble in most solvents, and **3** isomers were fairly soluble in solvents of moderate polarity (Figure 1). The **2** isomers were also less stable than **3** isomers, ans steric strain was apparently responsible for the greater instability. However, to verify the true identity of the two isomeric structures, additional spectroscopic evidence was required.

The application of the intramolecular nuclear Overhauser effect (NOE) has been a useful technique for structural determination in organic chemistry (Bachers and Schaefer, 1971). Therefore, if the protons of the methoxy group were to be at such a distance as to be involved in the relaxation process of the ArH-6 proton of **3a**, a positive NOE effect would be observed in the integrated signal of the ArH-6 proton upon irradiation of the OCH_3 group. Using **3a** in the NOE experiment, saturation of the OH group resulted in a NOE effect of 8% while irradiation of the OCH_3 group resulted in a 15% NOE effect for the ArH-6 proton.

The internuclear distance between the ArH-6 proton and the irradiated group (OCH_3 OH) was calculated to be about 3 Å by means of the equation $NOE = 1/Ar^6$ where A is a constant and r is the distance between the two nuclei (Bachers and Schaefer, 1971). In agreement with calculated results, an internuclear distance of about 3 Å was also obtained when measurements were taken with calipers reading directly in angstrom units on a molecular model of **3a** constructed from the space-filling Courtauld atomic models (The Ealing Corp., Cambridge, MA).

It was unfortunate that the NOE experiment could not be conducted with **2a** owing to its low solubility in any suitable NMR solvent. However, an NOE effect would not be expected to be observed with **2a** because the internuclear distances between the methoxy group and the aromatic protons appear to be too great for any interaction to take place.

In the ^{13}C NMR spectra, the chemical shifts of the alkoxy group of **2a** and **3a** in pyridine-d_5 were δ 52.95 and 52.40, respectively, and those for **2b** and **3b** in methanol-d_4 were δ 62.94 and 62.65, respectively. The observed downfield shift of the alkoxy group in **2a**,**b** in comparison with **3a**,**b** apparently resulted from hydrogen bonding of the alkoxyl protons with chlorine (Fifolt et al., 1982).

In the FAB-MS of **2a** and **3a**, the OCH_3 group was lost in the base peak of **2a** (m/z 262) but remained present in the base peak of **3a** (m/z 197). The loss of OCH_3 in **2a** was apparently encouraged by steric interaction of the methoxy group with the bulky chlorine on the aromatic ring. Furthermore, the formation of a glycerol adduct (MH + glycerol)$^+$ at m/z 445 in the FAB-MS of **3b** may be an indication that the hydroxy group of **3b** is less hindered than that of **2b** and can more readily solvate with glycerol.

In the infrared spectra of aromatic compounds, the C–H stretching vibrations are observed between 3100 and 3000 cm^{-1} (Jones and Sandorfy, 1956). Therefore, isomers having ROH reacting with the C-7 carbonyl of 1 should have no effect on the aromatic C–H stretching vibrations

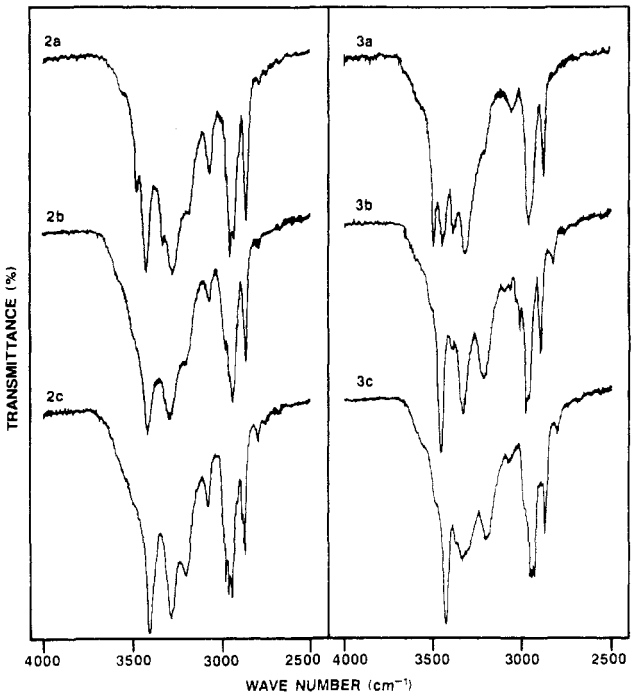


Figure 2. Infrared spectra showing the steric effects of the OCH_3 (**2a**, **3a**), OCH_2CH_3 (**2b**, **3b**), and $OCH_2CH_2CH_3$ (**2c**, **3c**) on the aromatic C–H stretching vibrations between 3100 and 3000 cm^{-1}.

while ROH reacting with the C-8 carbonyl would be expected to show some interaction between the alkoxy group and the ArH-6 proton in the IR spectra. The IR spectra given in Figure 2 show the absorption of the C–H stretching vibrations of the aromatic protons between 3100 and 3000 cm^{-1}. For the aromatic protons of **2a–c**, moderately sharp absorption bands can be observed, while on the other hand the absorption bands for the aromatic protons of **3a–c** all show significant band deformation. Therefore, to cause deformation of the IR absorption bands of the aromatic protons, the alkoxy groups **3a–c** must be in close proximity with the ArH-6 proton.

The assignments of structure for isomers **2** and **3** are based on the following data: (a) The instability of **2a–c** is caused by the desire for steric relief from the bulky chlorine group. (b) The positive NOE effect observed in the NMR spectrum of **3a** shows that the methoxy group is very close to the ArH-6 proton. (c) The chemical shifts of the alkoxy groups in the ^{13}C NMR spectra of **2a,b** are shifted further downfield than those of **3a,b** due to hydrogen bonding with chlorine. (d) The IR spectra of **3a–c** show that the alkoxy group is hindering the C–H stretching vibrations of the ArH-6 proton. Therefore, the structural assignments of **2a–c** and **3a–c** as given in Figure 1 are in agreement with their spectroscopic data.

Biological Activity of Byproducts. Since these alcohol byproducts are new compounds, there is an apparent need to test these compounds for biological activity. The lettuce hypocotyl elongation test has been shown to demonstrate significant response with **1** (Suttle and Schreiner, 1982); therefore, this assay was employed to test **2a,b** and **3a,b** for biological activity. The lettuce hypocotyl assay demonstrated no biological activity with **2b** and **3a,b** while **2a** showed only a trace quantity of activity. Since **2a** has proved to be slightly unstable in solution, apparently a small quantity of **2a** was decomposing back to **1**, in turn eliciting the observed biological response. Because a biological response was not observed with these analogues of **1**, additional analogues are being prepared with a variety of alcohols to hopefully obtain a product with desired solubility and stability properties to yield **1** at a consistent rate over a suitable time period after field application.

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Registry No. **1**, 51971-67-6; **2a**, 111435-66-6; **2b**, 111435-68-8; **2c**, 111435-70-2; **3a**, 111435-67-7; **3b**, 111435-69-9; **3c**, 111468-25-8.

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