Radiosynthesis of [*benzoyl*-3,4,5-^{3}H]Diflubenzuron by a Route Applicable to Other High-Potency Insect Growth Regulators

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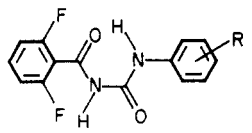
2,6-Difluoro-3,4,5-tribromobenzoic acid, from reaction of 2,6-difluorobenzoic acid and dibromoisocyanuric acid in concentrated sulfuric acid, is used to prepare difluorotribromobenzoyl analogues of diflubenzuron, chlorflurazuron, teflubenzuron, and XRD-473, which on catalytic reductive debromination are converted to the corresponding potent insect growth regulators. The applicability of this reaction sequence for radiosynthesis using tritium gas is illustrated by the preparation of [*benzoyl*-3,4,5-^{3}H]diflubenzuron at 60 Ci/mmol.

Diflubenzuron (**1**, Dimilin, TH 6040) is a potent insect growth regulator that acts by interfering with the synthesis or deposition of chitin (van Daalen et al., 1972; Verloop and Ferrell, 1977; Hajjar, 1985; Mitsui, 1985). Structure–activity studies on thousands of analogues have ultimately focused on chlorflurazuron (**2**, CGA 112913) (Haga et al., 1982), teflubenzuron (**3**; CME 134) (Sagenmueller and Rose, 1986), and XRD-473 (**4**) (Sbragia et al., 1983). These three "second-generation" insect growth regulators and chitin synthesis inhibitors are at least 1 order of magnitude more potent than 1 on one or more important pests. Compounds **1–4** are each 1-(2,6-difluorobenzoyl)-3-(substituted phenyl)ureas, differing only in the anilino substituents.



1 R = 4-Cl

2 R = 3,5-Cl_2; 4-O-(2-Cl, 5-CF_3-pyridin-2-yl)

3 R = 3,5-Cl_2; 2,4-F_2

4 R = 3,5-Cl_2; 4-OCF_2CHF_2

Radiolabeled preparations have contributed importantly to an understanding of benzoylarylurea insecticide distribution and metabolism, i.e. [^{3}H]- and [^{14}C]**1** (Metcalf et al., 1975; Verloop and Ferrell, 1977), [^{14}C]**2** (Neumann and Guyer, 1983), and [^{14}C]**3** (Eichler and Schlüter, 1986). The specific activity of [^{3}H]**1** used in these studies, i.e. 23 mCi/mmol, is insufficient for some types of metabolism and mode of action investigations where at least 1000-fold higher specific activity is required. We therefore developed a simple method to prepare 1 at 60 Ci/mmol, which is also applicable to **2–4**.

MATERIALS AND METHODS

Spectroscopy. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker WM-300 spectrometer at 300 MHz in dimethyl-d_6 sulfoxide. Residual ^{1}H from the solvent was used as the internal standard and chemical shifts are reported as δ. Infrared (IR) spectra were obtained in paraffin oil with a Perkin-Elmer 457 grating IR spectrophotometer. Absorption frequencies (ν_{max}) are given in reciprocal centimeters. Mass spectrometry (MS) was carried out with a Hewlett-Packard 5985B instrument by either chemical ionization (CI) (methane, 0.8 Torr, 230 eV) or electron impact (EI, 70 eV). Negative-ion fast atom bombardment (FAB) MS was accomplished in tetraethylene glycol dimethyl ether with a Kratos MS-50 instrument, which was also used for high-resolution mass spectrometry (HRMS) in the EI mode. The reported values (m/e, relative intensity) correspond to the isotope of lowest mass in each case.

Analyses. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) involved silica gel 60 F_{254} chromatoplates (0.25-mm thickness) (Merck, Darmstadt, Germany). High-pressure liquid chromatography (HPLC) utilized a Beckman 344 gradient liquid chromatograph equipped with a UV detector (λ_{max} 258 nm). The columns of μBondapak C_{18} (Waters Associates, Milford, MA) were 3.9 or 7.8 mm × 30 cm for separations of 100 or 200 μg, respectively, of benzoylarylurea per run. Elution (isocratic conditions) used methanol–water (4:1) at a flow rate of 1 mL/min.

Chemicals. Sources for the chemicals were as follows: **1** from the Environmental Protection Agency (Research Triangle Park, NC); 3,5-dichloro-4-[(3-chloro-5-(trifluoromethyl)-2-pyridinyl)oxy]aniline and **2** from Ciba-Geigy Corp. (Greensboro, NC); **3** from Celamerck Gmbh and Co. (Ingelheim, Germany); 3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)aniline from Dow Chemical Co. (Walnut Creek, CA); 4-chlorophenyl isocyanate from Aldrich Chemical Co. (Milwaukee, WI). All solvents were distilled and stored over molecular sieves (4 Å).

3,5-Dichloro-2,4-difluoroaniline was obtained on photolysis (Ruzo et al., 1974) of **3** (400 mg, 1 mmol) in anhydrous tetrahydrofuran (THF) (200 mL) at 254 nm for 20 h. The reaction mixture was concentrated, redissolved in ether (100 mL), and washed with 2 N HCl (4 × 50 mL). The combined aqueous phase was washed with ether (3 × 20 mL) and basified with 2 N NaOH. It was then reextracted with ether (4 × 30 mL); the extracts were combined, washed with brine, dried ($MgSO_4$), and concentrated to yield 150 mg (73%) of 3,5-dichloro-2,4-difluoroaniline: EI–MS, 197 (M^+, 2 Cl, 100), 179 (5), 162 (10), 126 (15).

Catalytic reductive debromination with tritium gas was accomplished at the National Tritium Labeling Facility (University of California, Berkeley, CA).

SYNTHESES

Synthesis routes for appropriate intermediates **5–7** and the (difluorotribromobenzoyl)arylureas **8–11** are shown in Figure 1.

2,6-Difluoro-3,4,5-tribromobenzoic Acid (5). A solution of dibromoisocyanuric acid (Gottardi, 1967) (10 g, 30 mmol) in concentrated H_2SO_4 (50 mL) was added over 10 min to a stirred solution of 2,6-difluorobenzoic acid (3 g, 18 mmol) in concentrated H_2SO_4 (20 mL). After the mixture was stirred for 2 h at 25 °C, the temperature was raised to 100 °C and the stirring was continued overnight; the elevated temperature was required or only mono- and

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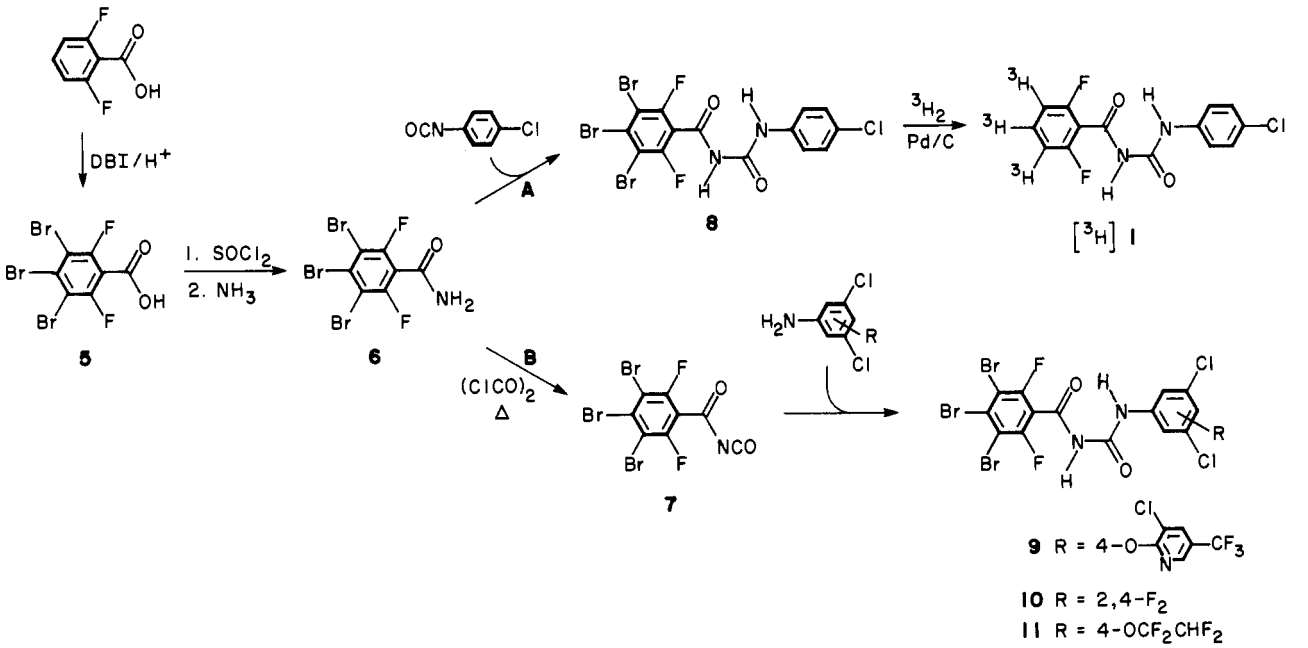

Figure 1. Routes of synthesis for (2,6-difluoro-3,4,5-tribromobenzoyl)arylureas 8–11 and radiosynthesis of [^{3}H]1 by catalytic reductive debromination of 8 with tritium gas. DBI = dibromoisocyanuric acid.

dibromo derivatives were obtained. The mixture was then poured on ice, and the resulting precipitate was filtered and resuspended in saturated $NaHCO_3$ solution (200 mL). The undissolved solid (cyanuric acid) was filtered off, rinsed with a small portion of aqueous $NaHCO_3$, and discarded. The filtrate was extracted with ether (3 × 25 mL) and acidified (20% H_2SO_4) with cooling. The resulting precipitate was extracted with ether (5 × 25 mL); the extracts were combined, washed with brine, dried ($MgSO_4$), and concentrated to yield 4.5 g (60%) of **5**. Recrystallization from aqueous ethanol gave needlelike crystals: mp 195–197 °C; IR 3500, 1715, 1600; CI–MS, 377 ($MH^+ - H_2O$, 3 Br, 6), 349 ($MH^+ - CO_2$, 35); HRMS calcd for $C_7HO_2Br_3F_2$ 391.7513, found 391.7509.

2,6-Difluoro-3,4,5-tribromobenzamide (6). A solution of **5** (3 g, 7 mmol) in excess thionyl chloride (10 mL) was refluxed for 1 h, the volatile portion distilled off, and the residual mixture cooled on ice. Saturated NH_4OH (ice cooled, 10 mL) was added cautiously and the mixture stirred (10 min). The resulting precipitate was filtered and washed with saturated NH_4OH. The crude reaction product was recrystallized from aqueous ethanol (1:1) to give needlelike crystals: mp 228–230 °C; yield 2.7 g (90%); IR 3420, 3300, 1700, 1675, 1600; CI–MS, 392 (MH^+, 3 Br, 34); HRMS calcd for $C_7H_2NOBr_3F_2$ 390.7654, found 390.7653.

(2,6-Difluoro-3,4,5-tribromobenzoyl)arylureas 8–11. Method A illustrated with 8 is based on Wellinga et al. (1973), and method B shown for **9–11** is modified from Speziale and Smith (1962).

Method A. A solution of **6** (0.5 g, 1.28 mmol) and 4-chlorophenyl isocyanate (0.3 g, 1.96 mmol) in dry xylene (5 mL) was refluxed for 20 h. The crystals formed on cooling were filtered, washed with xylene and petroleum ether, and dried to give **8**: mp 225–227 °C; 0.59 g (85%); IR 1720, 1685, 1590, 1565; ^{1}H NMR δ 7.5 (2 H, d, J = 9 Hz), 7.6 (2 H, d, J = 9 Hz), 10.0 (1 H, br s, C(O)NH), 11.5 (1 H, br s, C(O)NH); EI–MS, 544 (M^+, 3 Br, 30), 528 (11), 485 (16), 468 (19), 448 (11), 421 (19), 377 (23), 270 (11), 153 (100), 127 (21), 110 (12); HRMS calcd for $C_{14}H_6N_2O_2Br_3ClF_2$ 543.7636, found 543.7618.

Method B. A suspension of **6** (0.5 g, 1.28 mmol) in dry 1,2-dichloroethane (20 mL) was treated with excess pure oxalyl chloride (3 mL) and the mixture refluxed for 20 h. The solvent and excess oxalyl chloride were distilled off at reduced pressure. A solution of the residue in dry benzene (3 mL) was added with stirring to the appropriate substituted aniline (1 mmol) in dry benzene (2 mL). After standing (2 h, 25 °C), the crystals formed were filtered, washed with benzene, and dried. Pure samples were obtained by TLC purification (petroleum ether–THF, 4:1). The following compounds were prepared in this manner.

9: yield 0.67 g (85%); mp 198–200 °C; ^{1}H NMR δ 7.9 (s, 2 H, Ar H), 8.5 (1 H, s, Ar H), 8.7 (1 H, s, Ar H), 10.2 (1 H, br s, C(O)N*H*), 11.7 (1 H, br s, C(O)N*H*); FAB-MS, 773 ($M^{\bullet-}$, 40), 694 (M – Br, 100), 675 (70), 596 (20); HRMS calcd for $C_{20}H_6N_3O_3F_4Cl_3Br_2$ (M – Br – F) 639.8089, found 639.8092.

10: yield 0.5 g (80%); mp 213–215 °C; ^{1}H NMR δ 8.2 (1 H, t, J_{HF} = 7 Hz, Ar H), 10.3 (1 H, br s, C(O)NH), 11.8 (1 H, br s, C(O)NH); EI-MS, 535 (M – Br, 20), 516 (18), 481 (25); HRMS calcd for $C_{14}H_4N_2O_2F_4Cl_2Br_2$ (M – Br) 535.7952, found 535.7950.

11: yield 0.5 g (90%); mp 203–205 °C; ^{1}H NMR δ 6.9 (1 H, t, J_{HF} = 53 Hz), 7.9 (2 H, s, Ar H), 10.2 (1 H, br s, C(O)NH), 11.7 (1 H, br s, C(O)NH); FAB-MS, 694 ($M^{\bullet-}$, 50), 614 (M – Br, 100), 594 (85); HRMS calcd for $C_{16}H_6N_2O_3F_6Cl_2Br_2$ (M – Br) 615.8050, found 615.8053.

Catalytic Hydrogenation of (2,6-Difluoro-3,4,5-tribromobenzoyl)arylureas. The difluorotribromo ureas 8–11 (10 mg) in anhydrous THF (5 mL) were hydrogenated at room temperature (1 atm) with 10% palladium on carbon (Pd/C) (5 mg) and anhydrous K_2CO_3 (10 mg) with stirring. The K_2CO_3 serves to trap the HBr liberated on catalytic debromination. On completion of the reaction (2 h), the catalyst was filtered off, the solvent evaporated, and the product purified by HPLC. Yields were over 90% in preparing each of **1–4**.

The same procedure was used for converting **8** to [^{3}H]**1** with tritium gas as an example of its general applicability for radiosynthesis. Catalytic reductive debromination was carried out on 10 mg of **8** for 4 h; the longer reaction time was utilized due to the kinetic isotope effect. Radiochemically pure (100%) **1** was isolated at 60 Ci/mmol on purification by HPLC in a yield of 400 mCi. No attempt was made to optimize the radiochemical yield, which might

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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