Isotopically Labelled Compounds for Hazardous Waste Site Cleanup Investigations: Part I. Synthesis of [phenyl-U-^{14}C] labelled 2,4-dinitro-6-*sec*-butylphenol (dinoseb) and [phenyl-U-^{14}C] labelled 4-n-propylphenol†

Stefan Goszczynski[1‡] and Ronald L. Crawford[1,2*]

Department of Bacteriology and Biochemistry[1]
University of Idaho
Moscow, Idaho 83843
208 (885-7966)

Institute for Molecular and Agricultural Genetic Engineering[2]
and
Center for Hazardous Waste Remediation Research[2]
University of Idaho
Moscow, Idaho 83843
208 (885-6580)

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* Author to whom correspondence should be addressed

‡ On leave from Poznan Technical University, Poznan, Poland

SUMMARY

The Fries rearrangement of [phenyl-U-^{14}C] propionate forming a mixture of *ortho* and *para* hydroxypropiophenones was followed by steam distillation to separate the isomeric products. A Grignard addition to the *ortho* isomer and elimination of water from the intermediate carbinol, followed by hydrogenation and nitration, yielded [phenyl-U-^{14}C] labelled dinoseb. A Wolff-Kishner reduction of the *para* isomer furnished [phenyl-U-^{14}C] 4-n-propylphenol.

Keywords: [^{14}C]-2,4-dinitro-6-*sec*-butylphenol, [^{14}C]-dinoseb, [^{14}C]-4-n-propylphenol, [^{14}C]-hydroxypropiophenones

INTRODUCTION

Alkylphenols are common industrial chemicals that are precursors of numerous agricultural compounds, including herbicides. Alkylphenols and their derivatives have

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thus become common environmental contaminants. New biotechnological approaches to cleanup of hazardous chemicals in the environment include the use of microorganisms to degrade the chemical contaminants to innocuous products. However, to establish that true degradation has occurred, radiolabelled compounds are generally required. This allows confirmation that a labelled compound is mineralized to labelled products such as CO_2. For studies of biological cleanup of soils contaminated by alkylphenols, we required ^{14}C-labelled dinoseb (2,4-dinitro-6-*sec*-butylphenol) and 4-n-propylphenol. Routes for radiosynthesis of these compounds are described here.

Radioactively labelled 2-butylphenol and several of its derivatives have been prepared and described (1-4). The introduction of *tert*-butyl or *iso*-butyl substituents into the phenol moiety (3, 4) has not presented difficulties regarding the isomeric homogeneity of the products. Synthesis of the pure *sec*-butyl isomer, however, is not straightforward. Preparation of 2-*sec*-butylphenol by the Claisen rearrangement, although regioselective, is not an acceptable route to a homogeneous product. The allyl rearrangement generates an equilibrium mixture of any crotyl halide (5, 6) and causes isomerization during subsequent nucleophilic substitutions (7, 8). These problems were discussed by Oettmeier and Masson (4) and have been confirmed in our laboratory. In our hands, the synthetic approach through the Claisen rearrangement resulted in the formation after hydrogenation of significant amounts of the 2-n-butyl-phenol isomer. The separation of the *sec*-butyl and n-butyl isomers, although possible (9), does not appear to be applicable in microscale synthesis for preparation of isotopically labelled compounds such as dinoseb.

We felt that the multistep reaction pathway shown below presented a synthetic approach that might assure the needed structure of the alkyl group if we were to prepare ^{14}C-dinoseb:

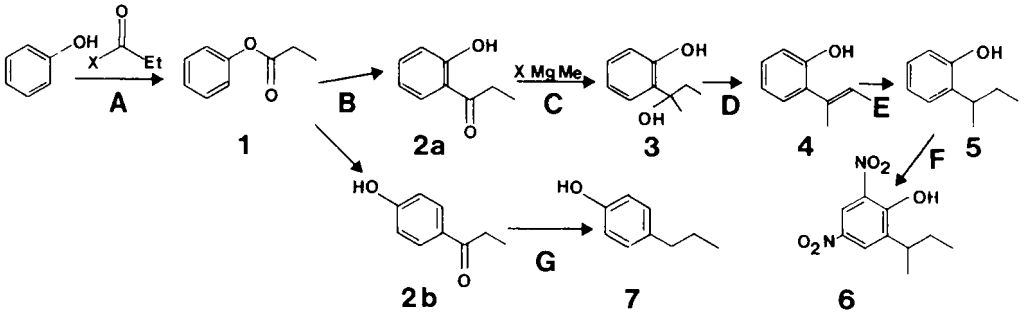

Figure 1. Synthetic route to dinoseb

Successful attempts in steps C to E have been reported (10-12), although the instability of carbinol (Fig. 1, product 3) (12) and poor yield of its dehydration product (10) were noted. We found the separation of the isomeric hydroxypropiophenones (Fig. 1, products 2a and 2b) reasonably easy, but a microscale procedure had to be developed. The nitration (step F) has recently been performed in a heterogenous medium (2), and we have confirmed an earlier report (9) that prior sulfonation is necessary for high yield and good quality of the final dinitro derivative. The carbonyl reduction to a methylene group (step G) to prepare the useful compound 4-n-propylphenol has usually been performed using a Clemmensen or Wolff-Kishner reaction. A microscale procedure for production of a radiolabelled product, however, was needed.

RESULTS AND DISCUSSION

O-acylation of phenol (step A) by propionyl chloride was easily accomplished with high yield. The removal of trace amounts of unreacted phenol by extraction with alkali was necessary to obtain high-purity dinoseb.

The Fries rearrangement (step B) catalyzed by aluminum chloride was carried out without solvent. Control over temperature in the first vigorous reaction period was achieved by applying the proper particle size (20 mesh) of the granulated catalyst. Thermostabilization of the reaction mixture is of great importance. The temperature-dependent *ortho* to *para* product ratio, which favors the *ortho* isomer at higher temperatures, may encourage the use of an excessively elevated temperature to obtain a higher yield of the *ortho* isomer. The disadvantage of such an approach is the formation of polymeric by-products, deteriorating or even destroying the quality of the *para* isomer. Since the *para* isomer is a useful product for other synthetic reactions, we optimized conditions for the Fries rearrangement.

The separation of 2- and 4-hydroxypropiophenones was accomplished by steam distillation. Because the volatility of the *para* isomer is remarkably low, it could be readily freed from the *ortho* isomer. However, isolation of the pure *ortho* isomer could not be achieved by a single distillation. The repeated steam distillation with limited distillate collection made it possible to obtain the *ortho* isomer with only trace-level ($< 0.5\%$) contamination by the *para* isomer.

We confirmed the lability of carbinol (Fig. 1, 3) (12), especially in acidic media.

The carbinol readily formed polymeric products, resulting in low yields of the desired elimination product (10, 11). Because of these problems, we employed a one-pot procedure for the C → D reaction sequence. Our relatively high yield of compound 4 (Fig. 1) was probably a result of several factors. Most important seemed to be the low solubility of the carbinol in diluted sulfuric acid and the immediate removal of the unsaturated intermediate compound from the acidic medium by steam distillation.

The hydrogenation product (step E) was purified by alkaline extraction to remove minute amounts of alkylcyclohexanol. Oxidation of this trace contaminant to 2-*sec*-butylcyclohexanone by nitric acid in the next reaction stage made the purification of the final product much more tedious.

The nitration (step F) in homogeneous medium of sulfuric acid proved to be best in regard to high yield and purity of the final product. The preliminary sulfonation prevented formation of dark-colored admixtures, as observed in comparative experiments without sulfuric acid.

The reduction (step G) of 2b to 7 (Fig. 1) was accomplished most satisfactorily by a Wolff-Kishner reaction when the hydrazone formation was performed separately with anhydrous hydrazine. The base-catalyzed elimination stage then proceeded at relatively low temperature without using a solvent.

EXPERIMENTAL

Materials and Equipment

The following chemicals were used: UL-^{14}C-phenol, 213.3 mCi/mmol (Sigma Chemical Co., St. Louis, Mo.); redistilled phenol, propionyl chloride, methylmagnesium bromide 3M solution in diethyl ether, palladium on barium sulfate 5%, and anhydrous hydrazine (Aldrich Chemical Co., Milwaukee, Wisc.); and aluminum chloride anhydrous (Fluka Chemical Co., Ronkonkoma, N.Y.) All solvents, solutions, and auxiliary chemicals were of the appropriate grade for our purposes.

^{1}H NMR spectra were acquired using a Varian 360 NMR spectrometer. HPLC assays were performed on a Hewlett Packard 1090 liquid chromatograph equipped with a Spherex 5 C18 (250× 2.0 mm) column. Radioactivity measurements were carried out by standard liquid scintillation-counting techniques. Thin layer chromatographic analyses were run on Merck silica gel F254 plates. All TLC R_f values and HPLC retention times

of labelled compounds matched those of synthetic samples of the respective unlabelled compounds.

Preparative procedures

A. Phenyl propionate

Phenol (0.94 g, 10 mmol, 250 μCi) was treated with propionyl chloride (1.3 ml, 15 mmol), warmed, and kept under reflux for 15 min. Ether was added to the cooled solution, which was then washed with water, extracted with 5% sodium hydroxide and washed with water until neutral. The solution was dried over $MgSO_4$ and evaporated *in vacuo* to give 1 as a colorless oil (1.39 g, 9.3 mol, 93%, 232 μCi).

B. 2- and 4-hydroxypropiophenones

Phenyl propionate (1.39 g, 9.3 mmol) was treated in a 50-ml round-bottomed flask with one portion of granulated Flucka reagent (20-mesh) aluminum chloride (1.9 g, 14 mmol) and mixed by swirling until a spontaneous reaction started. The vessel was then rotated in an oil bath at 110°C for 30 min. After the vessel was removed from the bath, a vacuum was applied immediately, but carefully, to the hot reaction mixture to produce a sponge-like glassy mass within the entire flask. When the material had cooled to room temperature, it was easily crushed to small particles. Concentrated hydrochloric acid (1.5 ml) and ice (10 g) were added. Mechanical stirring was applied and ether added. When the separation of two phases was sharp, the ether solution was washed several times with water and then extracted with 5% sodium hydroxide. The aqueous solution was acidified with 10% hydrochloric acid and extracted with ether. After evaporation of the combined ether phases *in vacuo*, a pale yellow oil remained which quickly solidified. Steam distillation was applied to the solid and 80 ml of distillate was collected. After cooling, the solid nonvolatile residue was filtered off and dried, giving yellow, thick crystals of the *para* isomer (2b) (0.49 g, 3.6 mmol, 39%, 90 μCi, m.p. 147-149°C).

The distillate was extracted with ether, and after evaporation of the ether *in vacuo* the oil was steam distilled once again, and 20 ml of distillate was collected, extracted with ether, and dried ($MgSO_4$). Evaporation left the *ortho* isomer (2a) as a colorless oil (0.61 g, 4.5 mmol, 48%, 112 μCi).

C and D. 2-(1-methyl-1-propenyl)-phenol

Methylmagnesium bromide (4.5 ml, 13.5 mmol) was treated dropwise under stir-

ring with the solution of 2-hydroxypropiophenone (0.61 g, 4.5 mmol) in 10 ml of ether. The reaction mixture was then kept at a gentle boil under an argon blanket for 15 min. The ether was removed by applying vacuum in a steady stream of argon. The solid grey material was treated with 5 g of crushed ice, and after gas evolution subsided, 25% sulfuric acid (8 ml) was added in one portion. When decomposition of the complex was complete (shown by the color changing from violet to yellow), a small amount of hydroquinone (0.01 g) was added and steam distillation was immediately applied and continued until 130 ml of distillate had been collected. The distillate was extracted with ether and the organic solution dried over $MgSO_4$. After evaporation *in vacuo*, the product (4) was obtained as a colorless oil (0.48 g, 3.3 mmol, 71%, 80 μCi).

E. 2-(1-methyl-1-propyl)-phenol

A solution of 2-(1-methyl-1-propenyl)-phenol (0.48 g) in ethanol (5 ml) was hydrogenated over a palladium catalyst (0.2 g) until gas absorption ceased. By the usual work-up a colorless oil was obtained (0.49 g). Dissolution of the oil in 5% sodium hydroxide, extraction with ether, acidification, and reextraction with ether resulted in pure (98% by HPLC) material (5) as a colorless oil (0.46 g, 3.1 mmol, 96%, 77 μCi).

F. 2,4-dinitro-6-sec-butylphenol

2-*sec*-butylphenol (0.46 g, 3.1 mmol) was dissolved in concentrated sulfuric acid (0.46 ml, 8.6 mmol) and the reaction mixture was heated at 95°C for 30 min. After cooling, water (2.1 ml) was added, and the mixture was cooled again to room temperature. Nitric acid (0.46 ml, 6.6 mmol) was introduced, forming a homogeneous solution. The solution was heated in an oil bath at 60°C until the solution became turbid. The temperature was then raised to 95°C, and heating continued for 30 min. After cooling and dilution with water, the nitration product was extracted with ether. The ether solution was washed with water until washings were neutral, and then dried over $MgSO_4$. Evaporation of the ether *in vacuo* yielded a yellow oil that solidified on cooling to crystals (6) (m.p. 39-41°C) (0.63 g, 2.6 mmol, 84%, 65 μCi). The overall yield from phenol was 26%. Characterizations gave:

λ_{max}nm (lg ϵ), in 0.01 M HCl in methanol, 263 (4.21); 0.01 M NaOH in methanol, 373 (4.16), 413 (4.11).

^{1}H NMR ($CDCl_3$): 0.91 (t, 3H), 1.28 (d, 3H), 1.67 (m, 2H), 3.30 (m, 1H), 8.33

(d,1H, 3-ArH), 8.92 (d, 1H, 5-ArH), 11.32 (s, 1H, OH).

MS (70eV); m/z (relative intensity): 240 M^+ (18), 211 (95), 163 (67), 147 (53), 117 (68), 89 (63), 77 (78), 63 (74), 53 (74), 39 (100).

Theoretical composition (%) for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.04; N, 11.66.

Found: C, 50.18; H, 5.04, N, 11.59.

The radiochemical purity was > 95% as judged by TLC (toluene : hexane, 1 : 1, R_f = 0.36) and > 97% (toluene : 2-butanone, 4 : 1, R_f = 0.85).

HPLC assays gave purities of:

97.2% (isocratic elution, 0.25 M triethylammonium phosphate buffer, pH 2.5 : water : methanol, 3 : 7 : 12 at flow rate 1 ml/min).

98.1% (linear gradient elution of 10% THF in methanol [A] and 1% acetic acid in water [B] from 60% A + 40% B to 100% A over 15 min and hold at 100% A for 5 min, 0.5 ml/min).

TLC spots and HPLC peaks were collected and their radioactivity quantified by standard liquid scintillation counting procedures.

G. 4-n-propylphenol

4-hydroxypropiophenone (0.49 g, 3.6 mmol, 90 μCi) and anhydrous hydrazine (0.8 ml, 25 mmol) were heated under reflux for 30 min. Powdered potassium hydroxide (90.8 g, 12 mmol) was added, and the mixture heated under reflux in an oil bath for 10 hrs. The reaction mixture then was dissolved in water, acidified, and extracted with ether. After drying ($MgSO_4$), and evaporating *in vacuo*, a colorless oil (7) (0.41 g, 3.0 mmol, 83%, 75 μCi) was obtained. The overall yield from phenol was 30%. Characterizations gave:

λ_{max}nm (lg ϵ) in 0.01 M HCl in methanol, 276 (3.20); 0.01 M NaOH in methanol, 296 (3.43).

^{1}H NMR ($CDCl_3$): 0.92 (t, 3H), 1.62 (m, 2H), 2.54 (t, 2H), 6.77 (m, 2H, 2,6-ArH), 7.04 (m, 2H, 3,5-ArH).

MS (70eV); m/z (relative intensity): 136 M^+ (42), 121 (100), 107 (22), 103 (12), 91 (32), 77 (20).

Theoretical composition (%) for $C_9H_{12}O$: C, 79.37; H, 8.88.

Found: C, 79.31; H, 8.92.

The radiochemical purity was 94% as determined by TLC (1-bromo-butane : ethyl acetate, 10 : 1, R_f = 0.46), and 96% (hexane : acetone, 2 : 1, R_f = 0.64).

HPLC assays gave:

97.7% (isocratic elution, 0.25 M triethylammonium phosphate buffer, pH 2.5 : water : acetonitrile, 6 : 14 : 5, 1 ml/min).

95.9% (linear gradient elution from 20% to 60% methanol/water over 20 min, flow rate 1 ml/min).

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