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6-Aroyl and 6-Aryl-2-naphthols[1]

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Intra-cellular localization of tissue enzymes may be accomplished by use of appropriate synthetic chromogenic substrates. Such methods were first evolved for the histochemical demonstration of acid and alkaline phosphatase.[2,3] The calcium phosphate esters of alpha and beta naphthol were converted by enzymatic hydrolysis in tissue sections to the respective naphthols, which immediately coupled with an appropriate diazonium salt present in the solution to yield an insoluble azo dye at the site of enzymatic activity. In order to improve the method for the histochemical demonstration of acid phosphatase,[3] and to evolve satisfactory methods for the histochemical demonstration of sulfatase and of β-glucuronidase, the appropriate esters and the glucuronide of various water-insoluble naphthols were prepared and the rate of their hydrolysis by these tissue enzymes was determined.[4] Although derivatives of β-naphthol itself readily underwent enzymatic hydrolysis, β-naphthol was diffusible and gave good histochemical localization only when a diazonium salt was present during the incubation with the substrate, so that immediate production of an azo dye would result in sufficient insolubility to maintain sharp localization in the tissues.[2,3,5] In the case of sulfatase and β-glucuronidase long incubation (24 hours) at 37° was necessary at a *p*H which was not favorable for coupling. Under these conditions diazonium salts were not sufficiently stable. By selecting a sufficiently insoluble naphthol and preparing the soluble derivative it was hoped that enzymatic hydrolysis could be conducted for the required time, at the optimum *p*H and temperature in the absence of the diazonium salt. The azo dye then could be produced by exposure of the tissue sections to the diazonium salt for 2–3 minutes at *p*H appropriate for coupling. Satisfactory methods for acid phosphatase,[6] sulfatase,[6] and β-glucuronidase[4] were evolved from derivatives of 6-bromo-2-naphthol. Various other naphthols were studied. Among these were aryl and aroyl derivatives. The sulfuric ester of 2-hydroxy-6-naphthyl phenyl ketone (V) proved to be even more readily hydrolyzed enzymatically[7] and yielded a superior pigment with tetrazotized diorthoanisidine than did the sulfate of 6-bromo-2-naphthol. The phosphate of V,[4] on the other hand was poorly hydrolyzed by the tissue phosphatases. Aryl naphthols VII, XI and XIII and the aroyl naphthol IX were also prepared. However, attempts to obtain exclusively mono phosphate and mono sulfate esters of these naphthols were unsuccessful presumably because of the reaction of phosphorus oxychloride and chlorosulfonic acid in pyridine with the active hydrogen in the methylene groups and because of addition to the ethylenic linkage in XIII.[8] Enzymatic hydrolysis of such esters, which contained an additional acid radical, was very poor.

Contrary to the claim of Desai and Waravdekar,[9] 2-methoxynaphthalene reacts with benzoyl chloride and zinc chloride in nitrobenzene to give the 1-benzoyl and not the 6-benzoyl derivative (IV). The same product was obtained when aluminum chloride was used, when the Perrier modification[10] was used or when the reaction was conducted in carbon disulfide. Following demethylation, this naphthol failed to couple with a diazonium compound, and melted at 142–143° (reported melting point 145–146°).[9] The melting point of 2-hydroxy-1-naphthyl phenyl ketone is reported at 141°[11] and 142°.[12] The melting point of 2-hydroxy-6-naphthyl phenyl ketone (V) is reported to be 158–159°.[13] It was prepared from 6-bromo-2-methoxynaphthalene (II), magnesium and phenyl nitrile.[13] We prepared V (m. p. 161–162°) by conversion of 6-bromo-2-naphthol (I) to 2-methoxy-6-naphthonitrile (III), which was reacted with phenyl magnesium bromide. This was followed by hydrolysis of the ketimine and demethylation. The product depressed the melting point of the benzoyl naphthol obtained from the Friedel and Crafts reaction. 6-Benzyl-2-naphthol (VII) was obtained from IV by a Huang-Minlon modification[14] of the Wolff–Kishner reduction, followed by demethylation of the crude product. The reduction gave better yields with the methyl ether (IV) than when the naphthol (V) was used.

The Friedel and Crafts reaction conducted in nitrobenzene with phenacetyl chloride gave 6-substitution into 2-methoxynaphthalene as has

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(2) Menten, Junge and Green, *J. Biol. Chem.*, **153**, 471 (1944).

(3) (a) Manheimer and Seligman, *J. Natl. Cancer Inst.*, **9**, 181 (1948); (b) Seligman and Manheimer, *ibid.*, **9**, 427 (1949).

(4) Seligman and Nachlas, unpublished data.

(5) Nachlas and Seligman, *J. Natl. Cancer Inst.*, **9**, 415 (1949).

(6) Seligman, Nachlas, Manheimer, Friedman and Wolf, *Ann. Surg.*, **130**, 333 (1949).

(7) Seligman and Cohen, unpublished data.

(8) Seligman, unpublished data.

(9) Desai and Waravdekar, *Proc. Indian Acad. Sciences*, **24A**, 382 (1946).

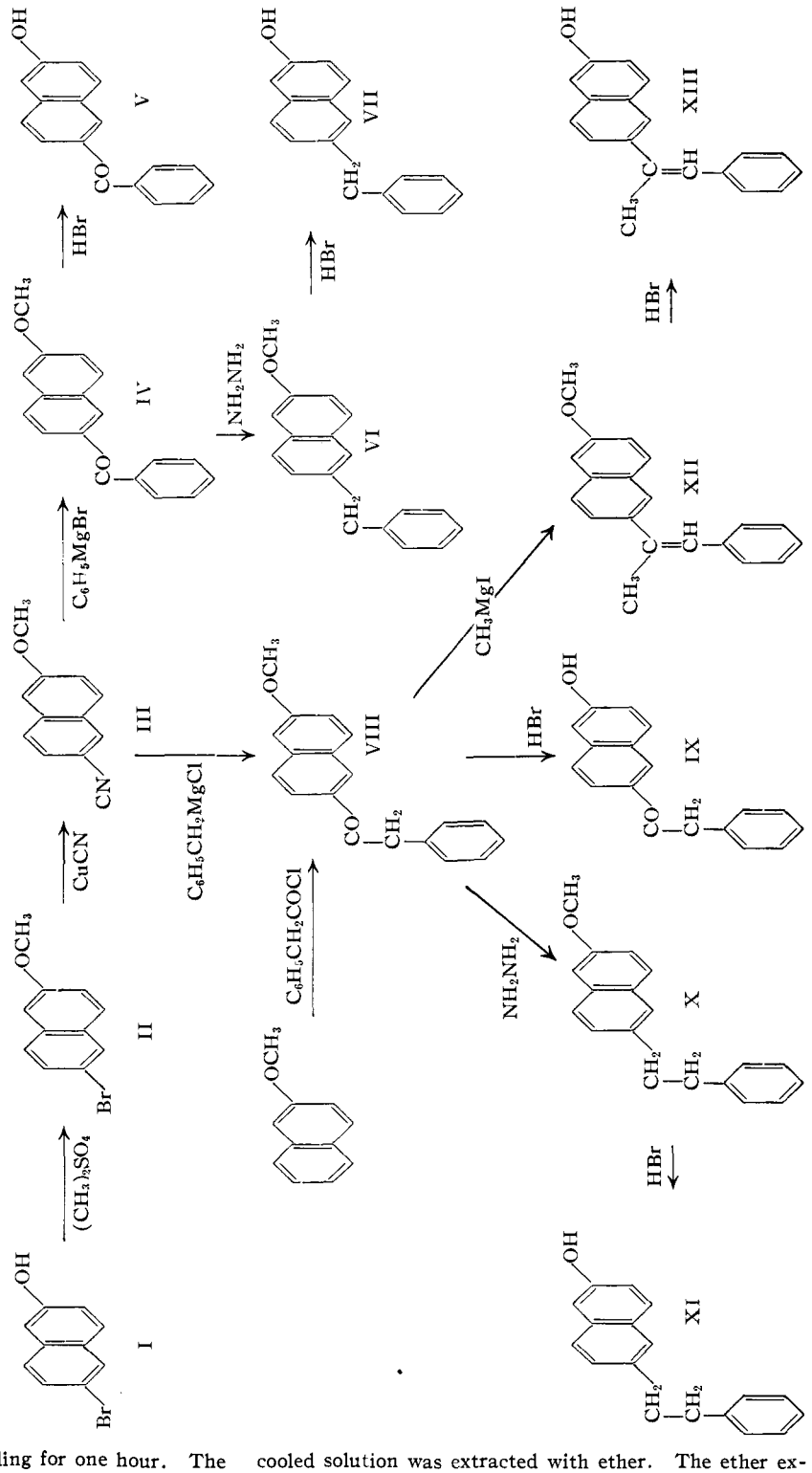
(10) Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., New York, N. Y., 1941.

(11) Perrier, *Compt. rend.*, **116**, 1140 (1893).

(12) Schönberg and Mustafa, *J. Chem. Soc.*, 642 (1946).

(13) Anderson and Thomas, THIS JOURNAL, **65**, 234 (1943).

(14) Huang-Minlon, *ibid.*, **68**, 2487 (1946).



been reported for acetyl chloride.[15] 2-Methoxy-6-naphthyl benzyl ketone (VIII) was also obtained from 2-methoxy-6-naphthonitrile (III) and benzylmagnesium chloride. Conversion to the naphthols IX and XI was accomplished as in the phenyl series. The propylene derivative (XIII) was prepared from the ketone (VIII) with methylmagnesium iodide followed by demethylation.

Experimental Part[16]

2-Methoxy-6-naphthonitrile (III).[17]—6-Bromo-2-naphthol[18] was methylated with dimethyl sulfate and aqueous alkali.[10] A quantitative conversion was obtained by alternately adding the reagents to the warmed mixture until a drop of the solution failed to test for naphthol with a diazonium salt. The methyl ether was washed and dried. A mixture of 90 g. of the product and 60 g. of cuprous cyanide was heated for one hour at 240°. The dark brown melt was poured onto a cold surface and pulverized. The powder was warmed in a solution of 300 cc. of concentrated ammonia in one liter of water. The mixture was filtered and the residue was dried, extracted with hot ethyl acetate (500 cc.), and the extract was distilled at reduced pressure. The nitrile was obtained in 75% yield, b. p. 205–208°, 14 mm.; m. p. 103°.

2-Methoxy-6-naphthyl Phenyl Ketone (IV).—The nitrile (90 g.) in dry benzene (400 cc.) was added slowly (45 minutes) with shaking to a gently refluxing ethereal solution (400 cc.) of phenylmagnesium bromide prepared from 78 cc. of bromobenzene and 18 g. of magnesium. The mixture was refluxed for one hour longer, cooled and poured into a stirred mixture of ice and 10% sulfuric acid (2500 cc.). The yellow ketimine was collected, suspended in two liters of dilute hydrochloric acid (15%) and hydrolyzed by boiling for two hours. The crude ketone was collected from the cooled solution and crystallized from ethanol. Additional product was obtained from the sulfuric acid filtrate by heating to remove ether and benzene, and boiling for one hour. The cooled solution was extracted with ether. The ether extract was evaporated and the ketone crystallized from ethanol; yield 111 g. (87%); m. p. 84–86°. Two crystallizations gave colorless needles melting at 86–87° (reported m. p. 81–82°).[18]

Anal. Calcd. for $C_{18}H_{14}O_2$: C, 82.42; H, 5.38. Found: C, 82.28; H, 5.34.

(15) Haworth and Sheldrick, *J. Chem. Soc.*, 864 (1934).

(16) Microanalyses by Mrs. Shirley Golden; melting points are uncorrected.

(17) Seligman, Friedman and Herz, *Endocrinology*, **44**, 584 (1949).

(18) Koelsch, "Organic Syntheses," **20**, 18 (1940).

2-Hydroxy-6-naphthyl Phenyl Ketone (V).—Demethylation of IV (50 g.) was accomplished in quantitative yield by refluxing a solution in 100 cc. of hydrobromic acid (48%) and 200 cc. of glacial acetic acid for four hours. The mixture was cooled and poured into 2000 cc. of water. The yellow oil, which separated, later solidified into a white crystalline product. This was dissolved in dilute alkali (1 *N*) filtered, and reprecipitated with acid; yield 47 g.; m. p. 157–158°. Two crystallizations from ethanol gave white prisms; m. p. 161–162°.

Anal. Calcd. for $C_{17}H_{12}O_2$: C, 82.23; H, 4.87. Found: C, 82.07; H, 5.10.

2-Hydroxy-6-benzylnaphthalene (VII).—The methoxy ketone (IV) was reduced by the Huang-Minlon modification[14] of the Wolff–Kishner reduction as follows: a mixture of the ketone (10 g.), hydrazine hydrate (5.2 cc.), potassium hydroxide (7.2 g.), and triethylene glycol (52 cc.) was refluxed for 1.5 hours. The water was then drained from the condenser and the temperature was allowed to rise to 195°, when refluxing was continued for two hours at 185–200°. The cooled solution was diluted with 60 cc. of water and poured slowly into 35 cc. of 6 *N* hydrochloric acid. The crude 2-methoxy-6-benzylnaphthalene (VI) was extracted with ether and the residue obtained after evaporation was demethylated by refluxing four hours with 20 cc. of hydrobromic acid (48%) and 40 cc. of glacial acetic acid. The cooled mixture was poured into 300 cc. of water. An oil separated and was extracted with ether. After evaporation, the residue was dissolved in warm dilute alkali, treated with norite, filtered, and precipitated as a gummy solid by acidification. It was extracted with ether, evaporated, and distilled *in vacuo*. The yellow, solid distillate crystallized from benzene–ligroin in pale tan needles; m. p. 89.5–92°; yield 5 g. (56%). A sample for analysis was prepared by three crystallizations from benzene–ligroin, m. p. 100–101°.

Anal. Calcd. for $C_{17}H_{14}O$: C, 87.15; H, 6.02. Found: C, 87.39; H, 6.16.

Clemmensen reduction of the methoxy ketone (IV) was not successful. When the reduction carried out above was conducted on the hydroxy ketone (V), the yield was poor.

2-Methoxy-6-naphthyl Benzyl Ketone (VIII).—2-Methoxy-6-naphthonitrile (III) reacted with benzylmagnesium chloride[19] as described for the phenyl derivative. The crude ketone, obtained after hydrolysis crystallized from ethanol in colorless plates; m. p. 115–116°; yield 7.9 g. (72%).

The same product was obtained by a Friedel and Crafts reaction as follows. 2-Methoxynaphthalene (16 g.) and phenacetyl chloride (15.5 g.) were dissolved in 70 cc. of nitrobenzene. The solution was cooled to 0° and 20 g. of aluminum chloride was added in portions with vigorous shaking. After standing in the cold for two hours, the mixture was allowed to stand at room temperature for twenty-four hours. The dark green solution was added to 300 cc. of water and 20 cc. of concentrated hydrochloric acid. The nitrobenzene was separated, washed, and combined with an ether extract of the aqueous portion. The ether was evaporated on the steam-bath, the nitrobenzene was distilled at the water pump, and the ketone was distilled at 3–5 mm. The yellow oil solidified rapidly and crystallized from ethanol in white platelets; m. p. 115–116°; yield 24 g. (87%). After recrystallization the melting point was 117–118°.

Anal. Calcd. for $C_{19}H_{16}O_2$: C, 82.58; H, 5.84. Found: C, 82.67; H, 5.87.

(19) Gilman and Catlin, "Organic Syntheses," **1**, 471 (1941).

A Friedel and Crafts reaction in nitrobenzene with benzoyl chloride and 2-methoxynaphthalene gave a ketone melting at 124–125°, and depressed the melting point of 2-methoxy-6-naphthyl phenyl ketone (IV) to 82–90°. After demethylation, the naphthol failed to couple with diazonium salts, indicating benzoyl substitution in the 1-position.

2-Hydroxy-6-naphthyl Benzyl Ketone (IX).—Demethylation was conducted exactly as described for the preparation of the phenyl derivative (V); white prisms, m. p. 197–199°; yield quantitative. After two recrystallizations, the m. p. was 202–203°.

Anal. Calcd. for $C_{18}H_{14}O_2$: C, 82.42; H, 5.34. Found: C, 82.33; H, 5.36.

1-Phenyl-2-(2-hydroxy-6-naphthyl)-ethane (XI).—Reduction and demethylation was conducted as described for the preparation of the phenyl derivative (VII). The crude hydroxy derivative was too insoluble to purify by solution in alkali. It crystallized from ether–petroleum ether in white prisms; m. p. 143–145°; yield 52%. A sample for analysis was prepared by three recrystallizations from ether–petroleum ether; m. p. 149–150°.

Anal. Calcd. for $C_{18}H_{16}O$: C, 87.06; H, 6.49. Found: C, 86.81; H, 6.40.

1-Phenyl-2-(2-methoxy-6-naphthyl)-propylene-1 (XII).—2-Methoxy-6-naphthyl benzyl ketone (VIII) (25 g.) in 300 cc. of dry benzene and 500 cc. of ether was added slowly to methylmagnesium iodide prepared from 22 g. of methyl iodide and 3.5 g. of magnesium. The mixture was refluxed for two hours. The ether was evaporated, and the benzene solution was added to ice-water. The solution was acidified with hydrochloric acid, and extracted with ether. The ether was evaporated and the benzene solution was concentrated to a volume of 200 cc. when crystals separated. After cooling 23 g. (93%) was collected, m. p. 176–178°. A sample crystallized from alcohol in white plates and prisms, m. p. 177–178°.

Anal. Calcd. for $C_{20}H_{16}O$: C, 87.55; H, 6.61. Found: C, 87.63; H, 6.68.

1-Phenyl-2-(2-hydroxy-6-naphthyl)-propylene-1 (XIII).—The methyl ether (XII) (22 g.) was demethylated by refluxing with 48% hydrobromic acid (75 cc.) and acetic acid (200 cc.) for four hours. The solution became wine colored. It was cooled and poured into 1 liter of water. The rose-colored precipitate was collected and purified by solution in alkali and precipitation with acid; 20 g. (96%). A specimen crystallized three times from ether–ligroin gave tan crystals; discolored at 115° and melted at 145–148°.

Anal. Calcd. for $C_{19}H_{16}O$: C, 87.66; H, 6.16. Found: C, 87.76; H, 6.30.

Summary

In a search for insoluble β-naphthols from which to prepare soluble substrates for the histochemical demonstration of intracellular enzymes, 6-substituted aroyl and aryl derivatives of β-naphthol were prepared. The syntheses of 2-hydroxy-6-naphthyl phenyl ketone, 2-hydroxy-6-naphthyl benzyl ketone, 2-hydroxy-6-benzylnaphthalene, 1-phenyl-2-(2-hydroxy-6-naphthyl)-ethane, and 1-phenyl-2-(2-hydroxy-6-naphthyl)-propylene-1 are described.

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