Organic Syntheses

A Publication
of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

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These paragraphs were added in September 2014. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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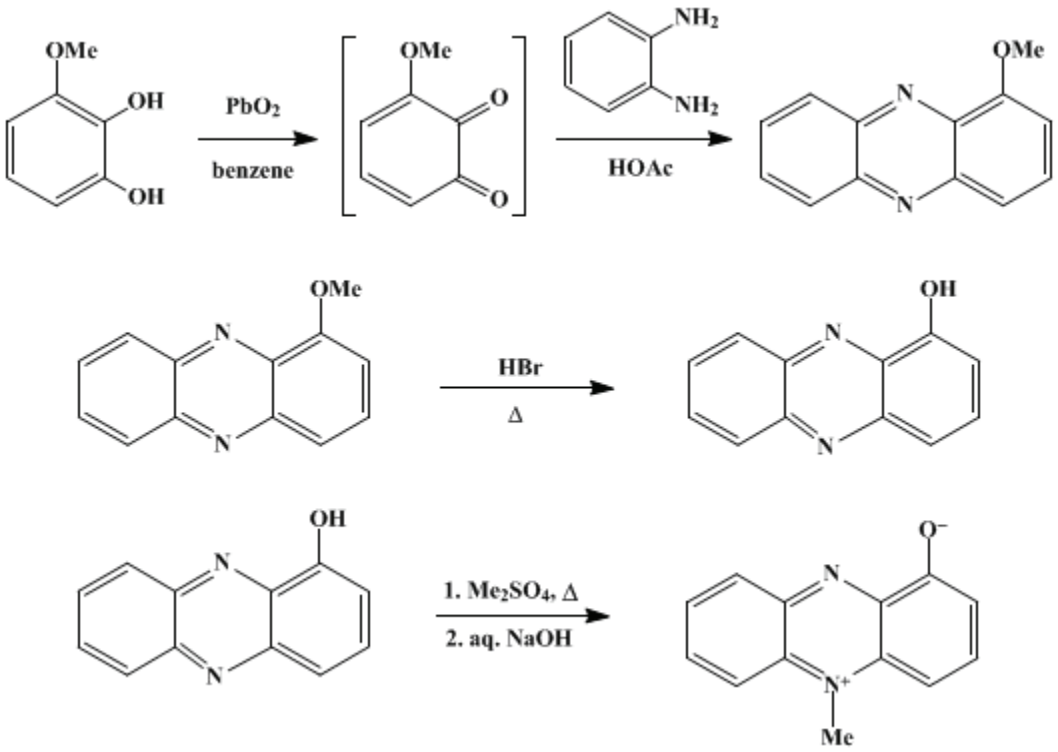
PYOCYANINE



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1. Procedure

A. α-*Methoxyphenazine* (*condensation*). Two hundred grams (0.42 mole) of powdered lead dioxide (Note 1) is added to a solution of 10 g. (0.07 mole) of pyrogallol monomethyl ether (p. 759) in 3 l. of dry benzene in a 1-gal. narrow-necked acid bottle. The bottle and contents are placed in a shaking machine and shaken for 10–20 minutes (Note 2). The reddish brown solid is filtered through an 11-cm. Büchner funnel, and the filter cake is washed once with 400 ml. of benzene. To this filtrate there is added, immediately and with mechanical stirring, a solution of 6 g. (0.06 mole) of *o*-phenylenediamine (Note 3) in a mixture of 80 ml. of glacial acetic acid and 200 ml. of benzene. The solution, which becomes dark brown, is allowed to stand at room temperature for 1.5 hours; it is then divided into two portions and each portion is washed, in a 3-l. separatory funnel, three times with water, twice with 5% sodium hydroxide solution, and finally twice with water, 100-ml. portions being taken each time. Each of the benzene solutions is shaken with 50 g. of anhydrous sodium carbonate and 5 g. of Norit and filtered through an 11-cm. Büchner funnel. Each filtrate is stirred with 50–60 g. of activated alumina (Note 4) until a filtered sample shows a light-yellow color. The alumina is filtered on a folded filter and washed with benzene until the washings are almost colorless. The benzene is removed from the combined filtrates by distillation under reduced pressure on a water bath at 40–50°. The residual light-yellow solid (Note 5) is recrystallized by dissolving it in the least possible amount of hot pyridine, adding water to the point of incipient precipitation, and cooling. The light-yellow crystals are filtered on a 7-cm. Büchner funnel, washed with water, and air-dried. The yield is about 5 g. (33%) of a product that melts at 167–169° (Note 6).

B. α-*Hydroxyphenazine* (*demethylation*). A solution of 4.2 g. (0.02 mole) of α-methoxyphenazine, from A above, in 125 ml. of 55% hydrobromic acid (Note 7) is placed in a 250-ml. round-bottomed flask fitted with a reflux condenser. The flask is immersed in an oil bath, and the solution is heated to 110–120° for 5 hours; the evolved gases are absorbed with water in a trap. The reaction mixture is

cooled to room temperature, diluted with about 125 ml. of water, almost neutralized with sodium hydroxide (Note 8), and extracted six times with 30- to 40-ml. portions of ether. The combined ether extracts are extracted with 25-ml. portions of 10% sodium hydroxide solution (Note 9) until no more purple sodium salt is removed from the ether. The aqueous extracts are combined, made acid to litmus with dilute acetic acid, and re-extracted four times with 50-ml. portions of ether. The combined ether extracts are dried over anhydrous sodium sulfate, and the ether is removed by distillation on a steam bath. The residue is recrystallized as follows: It is dissolved in the least possible amount of hot ethanol, water is added to the point of incipient precipitation, then 0.5 g. of Norit is added, and the hot solution is filtered. The filtrate is cooled in ice water, and the orange solid is filtered on a 7-cm. Büchner funnel, washed with water, and dried in an oven at 100°. The yield is 2.7 g. (70%) of a product that melts at 153–155°.

C. *Pyocyanine* (*alkylation*). A solution of 2 g. (0.011 mole) of α-hydroxyphenazine in 13.4 g. (10 ml., 0.1 mole) of methyl sulfate (Note 10) is placed in a 250-ml. Erlenmeyer flask fitted with a calcium chloride drying tube and heated at 100° (oil bath) for 10 minutes. The solution is allowed to cool to room temperature, and about 75 ml. of dry ether is added. The dark brown solid is filtered on a 7-cm. Büchner funnel and washed with about 150 ml. of dry ether in several portions (Note 11).

The dry methosulfate, dissolved in about 30 ml. of water, is made alkaline with 2–3 ml. of 10% sodium hydroxide, and the solution is then extracted *exhaustively* with successive 15-ml. portions of chloroform until no more blue substance is removed from the aqueous solution (Note 12). The combined chloroform solutions are extracted three times with 20-ml. portions of 5% hydrochloric acid. The combined acid extracts are made alkaline to phenolphthalein with 10% sodium hydroxide and re-extracted *exhaustively* with 25-ml. portions of chloroform until no more blue substance is removed from the aqueous solution (Note 12). The combined chloroform solutions are dried over anhydrous sodium sulfate and decanted, and the chloroform is removed by distillation under reduced pressure. The blue crystalline residue is recrystallized by dissolving it in the least possible amount of water at 60° and then cooling the solution in an ice bath. The product is filtered on a 5-cm. Büchner funnel and dried in the dark in a vacuum desiccator over calcium chloride. The yield is 1.35 g. (58%) of dark blue needles that melt at 133° (Note 13).

2. Notes

1. Lead peroxide "analytical reagent" of low manganese content was used.
2. On a scale twelve times as large, a "Lightnin" stirrer was used for 20 minutes.
3. *o*-Phenylenediamine, m.p. 99–101°, from Eastman Kodak Company was used.
4. The amount of alumina needed depends upon the color of the benzene solution. Sufficient alumina (activated alumina, 80 mesh, from Aluminum Corporation of America) is taken so that the filtrate is only light yellow in color. On the larger scale only 100–200 g. of alumina was needed.
5. The residue, without recrystallization, is pure enough for the preparation of α-hydroxyphenazine.
6. The submitter reports yields somewhat higher (33–40%) and states that, on a larger scale, the yields are slightly better than 40%.
7. The concentration of hydrobromic acid used ranged from 50% to 55%.
8. Concentrated sodium hydroxide solution (about 100 ml. of 35%) is used at the beginning of the neutralization, and dilute sodium hydroxide solution is added towards the end. The reaction mixture should be just faintly acid to litmus.
9. If any solid sodium salt separates during the extraction, it can be redissolved by adding water.
10. The methyl sulfate should be freshly distilled under reduced pressure.
11. The yield of methosulfate is practically quantitative.
12. The solution must be extracted *exhaustively*; the checkers found that thirty such extractions were required to remove all the product. A continuous extractor might be used at this point and certainly would be necessary for large-scale runs.
13. The same percentage yield was obtained with double the amounts specified above. The pyocyanine thus obtained can be stored in a vacuum desiccator in the dark for several weeks without appreciable decomposition. It slowly decomposes on longer standing to give the yellow α-hydroxyphenazine.

3. Discussion

This series of reactions is essentially the one described by Wrede and Strack.[1] Pyocyanine can also be prepared by the photochemical oxidation of phenazine methosulfate.[2]

A different synthesis of 1-hydroxyphenazine, starting with 2,6-dinitroaniline and *o*-iodonitrobenzene, has also been described.[3]

References and Notes

1. Wrede and Strack, *Ber.*, **62**, 2053, 2054 (1929); *Z. physiol. Chem.*, **74**, 181, 184, 185 (1929).
2. McIlwain, *J. Chem. Soc.*, **1937**, 1708.
3. Hegedüs, *Helv. Chim. Acta*, **33**, 766 (1950).

Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

alumina

Pyocyanine

ethanol (64-17-5)

calcium chloride (10043-52-4)

hydrochloric acid (7647-01-0)

acetic acid (64-19-7)

Benzene (71-43-2)

ether (60-29-7)

sodium hydroxide (1310-73-2)

chloroform (67-66-3)

HYDROBROMIC ACID (10035-10-6)

sodium carbonate (497-19-8)

sodium sulfate (7757-82-6)

Norit (7782-42-5)

pyridine (110-86-1)

methyl sulfate (75-93-4)

phenolphthalein (77-09-8)

manganese (7439-96-5)

α-Methoxyphenazine (2876-17-7)

pyrogallol monomethyl ether (934-00-9)

1-hydroxyphenazine,
α-Hydroxyphenazine (528-71-2)

phenazine methosulfate

2,6-Dinitroaniline (606-22-4)

o-Phenylenediamine (95-54-5)

o-iodonitrobenzene (609-73-4)

Lead peroxide