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CHEMICAL STUDIES ON ENVIRONMENTAL POLLUTANTS

II SYNTHESIS OF THE ISOMERIC 3-METHOXY-4,4'-AMINO ACETAMIDOBIPHENYLS

Souheil Laham and Ganti Venkat Rao
Environmental Health Directorate
Health Protection Branch
Department of National Health and Welfare

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During studies on biological activity and biotransformation of carcinogenic aromatic amines such as benzidine, 3-methoxybenzidine and 4-amino-4'-nitrobiphenyl, it was found necessary to prepare a substantial amount of these compounds. The preparation of 3-methoxybenzidine from 4,4'-diamino-3-hydroxy-5-sulfonic acid has been reported by SCIARINI (5) in connection with the synthesis of 3-hydroxybenzidine. The partial reduction of 4,4'-dinitrobiphenyl by sodium sulfide has been described by SCHULTZ (3), SHERWOOD and CALVIN (4). However, the modifications introduced respectively by these authors were not satisfactory for the synthesis of substantial amounts of these carcinogens.

In addition to the preparation of these aromatic amines, synthesis of some possible metabolites such as the ester sulphate, the methoxy and acetyl derivatives was also carried out. Both isomeric monoacetyl derivatives of 3-methoxybenzidine were synthesized. Direct acetylation afforded the diacetyl and a monoacetyl derivative. The latter was different from that obtained by catalytic reduction of 4-acetamido-3-methoxy-4'-nitrobiphenyl. Therefore, in the partial acetylation of 3-methoxybenzidine, the fixation of the CH_3-CO group occurs in the 4' position, that is on the unhindered amino group. A detailed chart (Figure 1) shows the synthetic routes used for the preparation of acetyl derivatives of 3-methoxybenzidine.

EXPERIMENTAL

Chemicals

All starting materials were either C.P. chemicals or purified before use. In all cases, paper* and thin-layer chromatography were used to check their purity. The sulfate ester 3

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* Solvent 1: n-butanol-dist. H_2O (1:1 by vol)
Solvent 2: n-butanol-acetic acid-dist. H_2O (4:1:5 by vol)
Solvent 3: n-butanol-N-ammonia (2:1 by vol)

which has a low yield was compared (by infrared spectroscopy) to an authentic sample kindly supplied by Professor D.B. Clayson.

Melting Point All melting points (uncorrected) were taken on a Reichert hot stage microscope.

Microanalyses They were carried out by Galbraith Laboratories, Knoxville, Tennessee, U.S.A.

4-amino-4'-nitrobiphenyl 2

Sulphur (10 g.) was suspended in 125 ml. of a solution of sodium sulphide (40 g. Na_2S). The aqueous solution was warmed on a steam bath until all the sulphur dissolved. The sodium polysulphide solution thus obtained was added dropwise during one-half hour to a boiling solution of 4,4'-dinitrobiphenyl (C.P. grade, Eastman Kodak) in ethanol (0.05 mole, 12.5 g. in 600 ml. 95% ethanol). After all the polysulphide solution was added, the reaction mixture was refluxed for another hour. The alcohol was removed by evaporation under vacuum at a bath temperature of 45-50°. The residue was boiled with 300 ml. of water and filtered off at the pump. This process was repeated twice to remove as much of the inorganic material as possible. The yellow-brown residue was then extracted with 300 ml. of boiling hydrochloric acid (1:1) and filtered. The filtrate on cooling deposited orange-red crystals. The hydrochloride salt was filtered off, suspended in dilute ammonia (1:4) and the liberated free base was extracted with ethyl ether. The ether solution, on evaporation and recrystallization from ethyl alcohol, yielded 4-amino-4'-nitrobiphenyl (3.5 g., 29%) melting at 202-204°.

4-acetamido-4'-nitrobiphenyl

Acetic anhydride (5 ml.) and 4-amino-4'-nitrobiphenyl (500 mg.) was heated on a hot plate until complete solution occured, during which time the bright orange-red colour gradually faded to straw yellow. After standing for one-half hour in solution, the reaction mixture was poured on crushed ice, and after all the acid anhydride had decomposed, the crystalline derivative was filtered off and recrystallized from ethanol to give 450 mg. (80%) melting at 246-247° with slight softening at 240°.

Anal. Calcd. for $C_{14}H_{12}N_2O_3$: C, 65.63; H, 4.72; N, 10.93
Found : C, 65.90; H, 4.79: N, 10.64

Persulphate oxidation product of 4-amino-4'-nitrobiphenyl 3

A solution of potassium persulphate (5.54 g., 0.022 mole) in the minimum amount of water was added dropwise with continuous stirring during the course of six hours to a solution of 4-amino-4'-nitrobiphenyl (4.28 g., 0.022 mole in 200 ml. of water), 20 ml. of 2 N potassium hydroxide and sufficient pyridine to keep the aminonitrobiphenyl in solution (approx. 250 ml.).

The reaction mixture was allowed to stand overnight at room temperature. The insoluble material was filtered off, and the filtrate concentrated in vacuo to 100 ml. on a rotary evaporator at a bath temperature not exceeding 45°. Unreacted starting material and pyridine were removed by extraction with ether (three 50 ml. portions). The aqueous alkaline solution was made distinctly acid to Congo Red using 2 N sulphuric acid. The solution was filtered from tarry impurities, then extracted with six portions of n-butanol (100 ml. each time). The butanol extracts were pooled and rendered alkaline with a slight excess of potassium hydroxide solution (2 N KOH). The alkaline butanol solution was then evaporated to dryness under vacuum, and the deep red-brown residue was extracted with hot 95% ethanol (4 x 100 ml.) and the combined alcoholic extracts were evaporated to dryness. The potassium salt 3 separated on standing at 0° for several hours and was recrystallized from fresh ethanol (approx. 15-20 ml.) to give a dark red-brown crystalline mass. The yield was 600 mg. (6%). Mild HCl hydrolysis of this compound gave the 3-hydroxy derivative melting at 226-227° (1,2).

4-amino-3-hydroxy-4'-nitrobiphenyl 4

Concentrated hydrochloric acid (3 ml.) was added to suspension of the above described potassium salt (375 mg., 0.001 mole) in 10 ml. of water and the mixture was heated on a steam bath for one-half hour. Water (50 ml.) was then added to the mixture and refluxing was continued for another half-hour. Filtration of the hot mixture then removed resinous impurities. The acidic solution was cautiously neutralized with saturated sodium bicarbonate solution. The liberated phenol was extracted with ethyl acetate (150 ml.), washed with water, and the ethyl acetate removed by evaporation under vacuum. Recrystallization from ethanol gave 120 mg. (52%) of pure material, m.p. 226-228°.

Anal. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.61; H, 4.38; N, 12.17
Found : C, 61.82; H, 4.49; N, 12.33

Acetylation of 4-amino-3-hydroxy-4'-nitrobiphenyl, with excess acetic anhydride yielded diacetyl derivative melting at 188-190° (75%). Bradshaw and Clayson reported the same melting point (2).

4-acetamido-3-hydroxy-4'-nitrobiphenyl 5

Acetic anhydride (0.2 ml., 0.002 mole) was added to a solution of 4 (460 mg. 0.002 mole) in 10 ml. of acetic acid + 5 ml. water and the mixture was warmed on a boiling water bath for 20 minutes, then cooled and diluted with water. The crystalline product was recrystallized from hot ethanol to give 300 mg. (60%) of material, m.p. 274-276°.

Anal. Calcd. for $C_{14}H_{12}N_2O_4$: C, 61.70; H, 4.42; N, 10.30
Found : C, 61.78; H, 4.53; N, 10.23

Catalytic reduction of 4-acetamido-3-hydroxy-4'-nitrobiphenyl

A solution of the above compound (150 mg. in 50 ml. ethanol) was hydrogenated at room temperature, using 250 mg. of Raney nickel catalyst (Parr Hydrogenator) under 3 atmospheres pressure. The catalyst was filtered off, washed several times with ethanol, and the alcoholic solution on evaporation yielded a crystalline residue (60 mg., 50%) which was difficult to purify. Several recrystallizations from ethyl acetate-petroleum ether mixture yielded a material melting at 204-206°.

Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.39; H, 5.82; N, 11.57
Found : C, 69.30; H, 5.91; N, 11.47

4-acetamido-3-methoxy-4'-nitrobiphenyl 6

A solution of 5 (100 mg.) in 40 ml. of diglyme* was treated with an excess of diazomethane in ether, and allowed to stand at room temperature for 4 hours. The ether and diazomethane were removed by warming on a water bath and the Diglyme evaporated under vacuum at a bath temperature of 90°. The brilliant yellow residue was recrystallized from dilute alcohol to yield 70 mg. (60%) of feathery yellow needles which melted at 210-212° with slight sintering at 206°.

Anal. Calcd. for $C_{15}H_{14}N_2O_4$: C, 62.92; H, 4.93; N, 9.79
Found : C, 62.47; H, 4.89; N, 9.82

4-acetamido-3-methoxy-4'-aminobiphenyl 7

A mixture of 6 (140 mg.) and Raney Nickel (200 mg.) in ethanol (50 ml.) was hydrogenated for 2 hours at room temperature and 3 atmospheres pressure. The catalyst was filtered off and the solvent removed under reduced pressure. The residue crystallized from dilute ethanol as shining elongated plates melting at 161-163° (70 mg., 50%).

Anal. Calcd. for $C_{15}H_{16}N_2O_2$: C, 70.31; H, 6.29; N, 10.93
Found : C, 69.93; H, 6.18; N, 10.87

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* Diglyme = dimethylether of diethylene glycol

The NMR spectra of both acetyl derivatives of 3-methoxybenzidine were run on a HA-100 Varian spectrometer. The samples were dissolved in DMSO-d_6. Chemical shifts in value, indicated below with TMS as internal reference, (s=singlet, m=multiplet)* show clearly that both compounds were different in structure. Furthermore, 4-amino-3-methoxy-4'-acetamidobiphenyl obtained by partial acetylation of 3-methoxybenzidine, melts at 151-152°.

2-methoxyazobenzene 8

Glacial acetic acid (2.5 ml.) was added to a solution of freshly prepared nitrosobenzene (27.5 g., 0.25 mole) in 100 ml. of ethanol. A solution of o-anisidine (30.6 g., 0.25 mole) in 50 ml. of ethanol was then added with swirling, and the mixture was allowed to stand at room temperature for 48 hours. The solvent was removed under vacuum at a bath temperature not exceeding 45° and the dark brown residue steam distilled to remove unreacted nitrobenzene. The residue was then extracted with petroleum ether (b.p. 30-60°, approx. 2 liters), washed several times with water, and the organic extract was dried over anhydrous sodium sulfate. On evaporation, a dark red-brown oily residue was obtained**. The residue was redissolved in low-boiling petroleum ether and the solution was filtered from resinous impurities, and chilled overnight in the ice box (0-5°). Crystals of the azo compound, m.p. 40-41°, were obtained. SCIARINI (5) reported the same melting point.

2-methoxyhydrazobenzene 9

Reduction of 2-methoxyazobenzene with zinc was carried out as described earlier (5). A 75% yield of pure compound melting at 83-84° was obtained.

3-methoxybenzidine 10

To 2-methoxyhydrazobenzene (17 g., 0.08 mole) placed in 600 ml. beaker was added anhydrous ether (125 ml.) and the mixture chilled in an ice-bath. A small amount of the hydrazobenzene remained undissolved. An ice-cold solution of 10 ml. of concentrated hydrochloric acid in 30 ml. of absolute ethanol was added to the cold ethereal solution with stirring. The temperature increased to 20° and, after standing for five minutes, a viscous precipitate of the hydrochloride salt of the rearranged material appeared. The supernatant solution was decanted, and the viscous material triturated with more ether (25 ml.). The somewhat hardened gum was then treated with diluted ammonia (1 part concentrated ammonia, 4 parts water). The free base was

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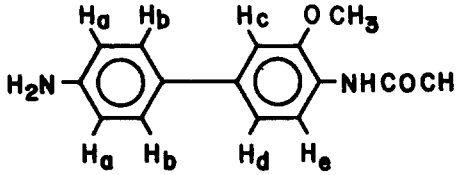
* See Table 1

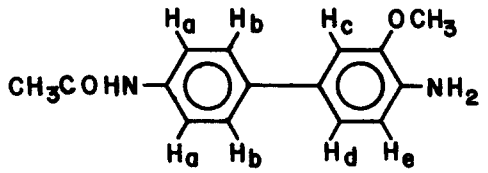
** Because of the high boiling point of the azo compound, this procedure was more convenient than the prolonged steam-distillation used by SCIARINI (5).

4-Acetamido-3-methoxy-4'-aminobiphenyl



2.06 ppm (3 H, $\underline{CH_3}CO-$, s)
3.86 " (3 H, $\underline{CH_3}-O-$, s)
6.99 " (4 H, $\underline{H_a}$ and $\underline{H_b}$, A_2B_2 system)
7.03 and 7.85 ppm (3 H, $\underline{H_c}$, $\underline{H_d}$ and $\underline{H_e}$, m)
9.06 ppm (1 H, $N\underline{H}$ $COCH_3$, m)

4-Amino-3-methoxy-4'-Acetamidobiphenyl



2.01 ppm (3 H, $\underline{CH_3}-CO$, s)
3.79 " (3 H, $\underline{CH_3}-O-$, s)
6.82 " (2 H, $\underline{H_d}$ and $\underline{H_e}$, AB quartet, J_{AB} = 8 HZ)
7.02 " (1 H, $\underline{H_c}$, s)
7.52 " (4 H, $\underline{H_a}$ and $\underline{H_b}$, A_2B_2 system)
9.88 " (1 H, $N\underline{H}$ $COCH_3$, s)

TABLE 1. Chemical shifts observed for monoacetyl isomers of 3-methoxybenzidine.

obtained initially as an oil chich hardened on standing to a dirty grey solid. Recrystallization from ethanol yielded the crystalline base (7.25 g., 40%) melting at 90-91°.

Partial acetylation of 3-methoxybenzidine 12

A solution of 3-methoxybenzidine (1.0 g., 0.005 mole) in 80% ethanol was treated with acetic anhydride (0.5 g., 0.005 mole) and then gently warmed on a steam bath for one-half hour. The reaction proceeded rapidly. The mixture was cooled and the insoluble crystalline material collected. The filtrate, when diluted with water and allowed to stand for some time at room temperature, yielded a glistening crystalline material which was collected and recrystallized from aqueous methanol to give 0.25 g. (20%) of monoacetyl-3-methoxybenzidine, m.p. 151-152°.

Anal. Calcd. for $C_{15}H_{16}N_2O_2$: C, 70.31; H, 6.29; N, 10.93
Found : C, 70.75; H, 6.43; N, 11.13

Diacetyl-3-methoxybenzidine 11

A mixture of 3-methoxybenzidine (1. g., 0.005 mole) and acetic anhydride (5 ml., excess) was warmed on the steam bath for one-half hour, then poured on crushed ice (50 g.) and allowed to stand until all the unreacted acetic anhydride was decomposed. The diacetyl-3-methoxybenzidine obtained was filtered off and recrystallized from ethanol to give 1 g. (66%) of the diacetate, m.p. 224-226°.

Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.45; H, 6.08; N, 9.40
Found : C, 68.65; H, 6.18; N, 9.40

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* Deceased

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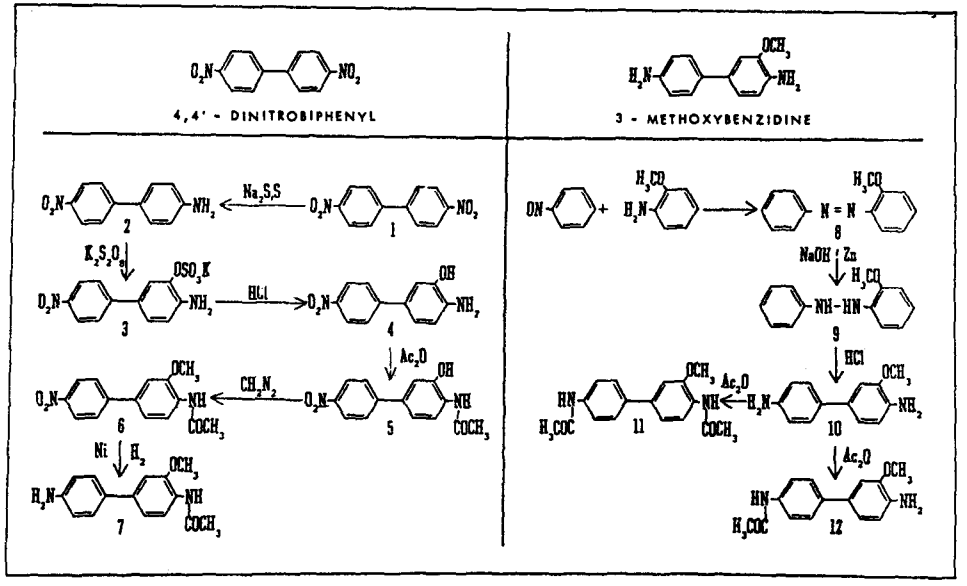

FIG. 1. Synthetic routes used for the preparation of acetyl derivatives of 3-methoxybenzidine.