Identification of Toxic Impurities in Commercial Diphenylamine

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Introduction

Diphenylamine (DPA) is widely used as an industrial antioxidant, dye mordant and reagent and is also employed in agriculture as a fungicide and antihelmintic. A number of reports have indicated that samples of DPA fed to rats induce a polycystic kidney disease (THOMAS et al, 1970; KIME et al, 1962; SAFOUN et al, 1970; CROCKER and VERNER, 1970). Recent studies have shown that the toxicity of DPA was dependent on the quality (i.e. degree of purity) and the source of the chemical and it was concluded that the toxicological properties were associated with impurities in commercial DPA (CROCKER et al, 1970, 1972). This investigation was initiated to isolate and identify the impurities in a number of commercial DPA samples and to evaluate their potential biological properties.

Methods and Materials

Cleanup of crude DPA: Crude DPA (50g.) was dissolved in benzene (100ml), silicic acid (50g) was added and the solvent removed "in vacuo". The solid was applied to the top of a glass column packed with silicic acid (200g) in petroleum spirit (b.p.30-60°). The column was eluted with the same solvent (4 l.) until most of the DPA had been removed; the column was then eluted with petroleum spirit: benzene mixtures (9:1, 500ml; 8:2, 500ml; 6:4, 500ml; 4:6, 500ml; 2:8, 500ml) and benzene (500ml). The fractions were combined, concentrated and the residue purified by preparative thin-layer chromatography (tlc) in hexane:ether (96:4) to remove the remaining traces of DPA from the non-polar fraction. A polar fraction was obtained by electron of the column with ether (500ml).

Gas chromatographic analysis: The non polar DPA-free fraction was analyzed by gas chromatography on a 6'x1/8" glass column packed with 1.5% OV-17 (Applied Science) on 100/120 Gas Chrom Q (Applied Science). The column was operated at 130° with a flame ionization detector; Helium gas flow, 40ml/min; hydrogen flow, 44ml/min; inlet and detector temperature, 290°. The gas chromatograms obtained from the non polar fractions exhibited 5 major peaks (R_T 25.3, 27.0, 28.0, 35.3 and 36.3 min) which varied according to the commercial source of the DPA. Preliminary gas-chromatography mass spectrometric (Varian MAT CH-7 mass spectrometer) analysis showed that the peaks exhibited molecular ions at m/e 173, 169, 175, 169 and 175 respectively although there was considerable overlap of the spectra since the R_T 27.0 and 28.0 min, R_T 35.3 and 36.3 min were not fully resolved.

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Purification and identification of non polar DPA impurities. The non polar impurities were separated and isolated by tlc on silica gel using hexane:ether (96:4) as solvent to give 5 bands (bands 1-5 in order of increasing polarity). The least polar band 1 corresponded to the R_T 25.3 glc component and the mass spectrum of this impurity gave a molecular ion at m/e 173. The infrared spectrum of band 1 exhibited 2 broad bands between 3500-3000cm^{-1} and the 220 MH_z nuclear magnetic resonance spectrum gave signals at δ 7.0 (1H, dt, J=8.2, 2.2 Hz), 6.83 (1H, q, J=8.2, 2.2Hz), 6.62 (1H, dt, J=8.2, 2.2Hz) and 6.55 ppm (1H, q, J=8.2, 2.2Hz) as well as a series of complex unresolved multiplets at high field (3.0-1.0 ppm).

Band 2 corresponded to the R_T 27.0 glc peak and gave a molecular ion at m/e 169. The glc retention time, tlc behavior, mass spectrum and infrared spectrum of band 2 were identical in all respects to the corresponding data obtained for an authentic sample of o-biphenylamine (Aldrich).

Band 3 corresponded to the R_T 28.0 glc peak (M^+ 173) and the infrared spectrum of this component also gave 2 bands in the 3500-3000 cm^{-1} region. The 220 MH_z nmr spectrum gave δ 7.12 (1H, q, J=8.2, 2.2Hz), 7.02 (1H, dt, J=8.2, 2.2 Hz), 6.80 (1H, dt, J=8.2, 2.2 Hz), 6.71 (1H, q, J=8.2, 2.2 Hz), 2.64 - 1.20 ppm (11H, m).

Band 4 corresponded to the R_T 36.3 glc peak (M^+ 169) and the glc retention time, tlc R_F value, mass spectrum and infrared spectrum were identical to the corresponding data obtained for an authentic sample of p-biphenylamine (Aldrich).

Band 5 corresponded to the R_T 35.3 glc peak (M^+ 175) and the infrared spectrum of this component gave 2 bands in the 3500-3000 cm^{-1} region of the spectrum. The 220 MH_z nmr spectrum gave δ 7.01 (2H, d, J=8.2Hz), 6.64 (2H, d, J=8.2Hz), 2.60 - 1.20 ppm (11H, m) and crystallization of the residue gave needles (m.p. 46-47°).

Synthesis of ortho and para-cyclohexylaniline: Commercial grade cyclohexylaniline (1.0g, Burdick and Jackson Chemicals) was added to a mixture of acetic anhydride (5.0ml) and concentrated nitric acid (0.3ml) at 20° and stirred continuously for 10 hours. The resulting suspension was poured into ice-water and the crude mononitration products isolated by ether extraction. The product (1.25g) exhibited a single molecular ion (m/e 205) in the mass spectrum and glc analysis gave only 2 peaks in a 3:1 ratio. The crude nitrated cyclohexyl-benzene was dissolved in methanol (50ml) and hydrogenated over 10% palladium on charcoal catalyst. After the uptake of hydrogen had ceased the solution was filtered to remove the catalyst and glc analysis showed only 2 peaks with retention times (R_T280 and R_T35.3) identical to those observed for bands 3 and 5 respectively. The two synthetic cyclohexylaniline isomers were separated by preparative tlc and the chromatographic and spectral properties of the less polar product (310mg) were identical to that of the non-polar DPA impurity band 3. The chromatographic and spectral properties of the more polar product (620mg m.p. 46-47°) were identical to the R_T35.3 DPA impurity (bands 5) and a mixture melting point of the two compounds was not depressed (m.p. 46-47°).

Results and Discussion

The mass spectra of bands 3 and 5 gave molecular ions at m/e 175 which was equivalent to a hexahydro diphenylamine molecular composition. The infrared spectrum suggested the presence of a primary aromatic amine functionality for both impurities and this was confirmed by their respective 220 MH_Z nmr spectra. The spectrum of band 3 exhibited 2 quartets and 2 double triplets and this was consistent with an ABCD tetrasubstituted benzene ring and the o-cyclohexylaniline structure. The spectrum of band 5 showed 2 doublets which suggested a symmetrical AA'BB' benzenoid ring substitution pattern and was consistent with the p-cyclohexylaniline. The structures of both DPA-impurities were confirmed by unambiguous synthesis of these isomers by nitration of cyclohexylbenzene and reduction of the cyclohexylnitrobenzene product to give the ortho and para-cyclohexylanilines which were readily separated and purified by preparative tlc.

Bands 2 and 4 exhibited a molecular ion peak at m/e 169 and by comparison with authentic standards these 2 impurities were identified as o-biphenylamine and p-biphenylamine respectively. The least polar impurity (band 1) gave a molecular ion peak at m/e 173 which was equivalent to a tetrahydrodiphenylamine molecular structure. The 220 MH_Z nmr spectrum exhibited an ABCD benzenoid ring pattern and suggested the structure of an ortho-substituted aniline. The high field part of the spectrum was highly complex and the structure of this DPA impurity has not been precisely determined. This compound was found to be remarkably unstable to heat, light and oxygen and rapidly polymerized to give a deep purple insoluble resinous material.

Analyses of 6 commercial brands of DPA is summarized in the Table and indicates a wide variety in both the levels of these impurities and the number of impurities in the samples. Only 1 impurity, o-cyclohexylaniline was detected in all the commercial DPA samples whereas p-cyclohexylaniline was detected in only one sample. It was also noted that p-biphenylamine, a known carcinogen was identified in 4 commercial DPA preparations.

Preliminary testing of the 5 DPA impurities with pregnant rats indicates that several of these chemicals induce a type of polycystic kidney disease previously noted for crude commercial DPA (CROCKER et al, 1972). In addition the components E, F and G identified in the previous study were comparable to band 1, band 3 (o-cyclohexylaniline) and band 5 (p-cyclohexylaniline) respectively.

TABLE

Toxic Impurities in Commercial Diphenylamine Preparations *

Commercial DPA	Band 1 (ppm)	Band 2 o-biphenyl-amine (ppm)	Band 3 o-cyclohexyl-aniline (ppm)	Band 4 p-biphenyl-amine (ppm)
Brand 1	50	-	93	-
Brand 2	45	32	22	94
Brand 3	12	22	17	27
Brand 4	9	3.2	5	17
Brand 5	-	-	48	6.9
Brand 6	-	-	12	-

* Brand 1 contains an additional impurity (i.e. Band 5) identified as p-cyclohexylaniline

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