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Short Communications

Direct *ortho*-Substitution on Hexafluorobenzene with Anilides

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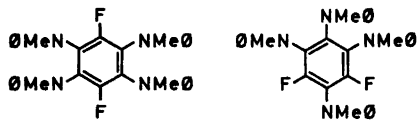
A recent paper by Vasilevskaya *et al.*[1] prompts me to report some results on nucleophilic replacement reactions on hexafluorobenzene with amines.

To the best of my knowledge a direct *ortho*-substitution on hexafluorobenzene with anilides has not previously been described. I have found that lithium anilide in the presence of lithium amide reacted with hexafluorobenzene in a mixture of tetrahydrofuran and hexamethylphosphoric triamide, to give 3,4,5,6-tetrafluoro-*N*,*N*′-diphenyl-1,2-phenylenediamine in good yield. When the reaction was run in tetrahydrofuran only, a low yield of 2,3,5,6-tetrafluoro-*N*,*N*′-diphenyl-1,4-phenylenediamine was obtained. This is similar to the results obtained from reactions run between sodium anilide and hexafluorobenzene in dioxan.[2] The presence of the *ortho*-substituted compound was demonstrated by ^{19}F NMR spectrum in chloroform-*d*, which showed two different multiplets of fluorine peaks.

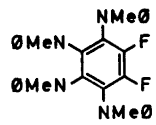
Reactions run between *N*-lithium-*N*-methyl-anilide and hexafluorobenzene (2:1) in a mixture of tetrahydrofuran and hexamethylphosphoric triamide afforded a tetra-substituted compound. ^{19}F NMR spectra in chloroform-*d* showed only the one peak. This would be consistent with compound I, II or III.

An NMR spectrum run in chloroform-*d* showed only one peak at $\delta = 2.96$ ppm for the methyl protons. This peak only broadened by expansion when the spectrum was run in chloroform-*d* or hexadeuterated benzene. These results are only consistent with structure I where all methyl groups are identical.



I

II



III

Experimental. All reactions were carried out under an atmosphere of dry oxygen-free nitrogen. Tetrahydrofuran was dried and distilled prior to use from sodium benzophenone ketyl. Hexamethylphosphoric triamide (HMPT) was first stored on 5 Å molecular sieves and then on calcium hydride. All melting points are uncorrected. IR, NMR, and MS spectra were determined employing Perkin-Elmer 457-IR, Varian 100 MHZ, NMR, and AEI-MS 902 spectrometers, respectively.

3,4,5,6-Tetrafluoro-N,N′-diphenyl-1,2-phenylenediamine. To a mixture of $LiNH_2$ (5.1 g) in THF (25 ml) at 0°, aniline (9.3 g, 0.1 mol) in THF (25 ml) was added over 30 min. After an additional 20 min hexafluorobenzene (9.3 g, 0.05 mol) was added slowly over 30 min to give a reddish mixture, whereupon HMPT (30 ml) was added. The colour turned dark red. The cooling bath was removed and the temperature raised slowly to 65°. The mixture was stirred overnight at 65°, cooled to 0° and hydrolyzed with 5 % HCl to acid reaction after the addition of ether (50 ml) to the mixture. The organic layer was separated and dried over granular, anhydrous sodium sulphate, whereafter the solvent was stripped off leaving a bluish-violet oil which slowly crystallized. The crystallized mixture was treated with cold petroleum ether (40–65°). The undissolved

material was sublimed and recrystallized from petroleum ether (40–65°) to give (11.9 g, 72.3 %) 3,4,5,6-tetrafluoro-*N*,*N'*-diphenyl-1,2-phenylenediamine (m.p. 95.5–97.5°). Characteristic infrared absorption bands in nujol were 3365(w), 3345(w), 1597(m), 1512(s), 1492(s), 1310(w), 1250(w), 1230(w), 1075(w), 1013(m), 980(s), 750(s), 695(m). (Found: C 65.24; H 4.02; mol.wt. 332. Calc. for $C_{18}H_{12}F_4N_2$: C 65.01; H 3.66; mol.wt. 332.3).

1,4-Difluorotetrakis (N-methylanilino)benzene. To a suspension of $LiNH_2$ (2.6 g) in THF (25 ml) at 0°, *N*-methylaniline (10.7 g, 0.1 mol) in THF (25 ml) was added over 50 min. 5 min later HMPT (30 ml) was added. Hexafluorobenzene (9.3 g, 0.05 mol) was added slowly over 25 min as an exothermic reaction took place. 35 min thereafter the 0° bath was removed. 1 h 45 min later the light brown mixture was cooled to 0° and hydrolyzed with 5 % HCl to acid reaction. A white precipitate which was present, was filtered off. The filtrate was separated, and the water layer treated twice with ether (30 ml). The collected organic layers were washed with water, dried with sodium sulphate, and concentrated under reduced pressure. The resultant solid was identical with the white precipitate, which was filtered off. Recrystallization from chloroform/petroleum ether (40–65°) gave (9.9 g, 74 %*) of 1,4-difluorotetrakis(*N*-methylanilino)benzene (m.p. 184–186°). Characteristic infrared absorption bands in nujol were 1590(s), 1568(w), 1489(s), 1475(s), 1352(m), 1292(w), 1278(m), 1199(w), 1181(w), 1150(m), 1105(m), 1026(w), 990(w), 969(m), 753(s), 738(w), 691(s). (Found: C 76.36; H 6.11; N 10.62; mol.wt. 534. Calc. for $C_{34}H_{32}F_2N_4$: C 76.38; H 6.03; N 10.48; mol.wt. 534.6).

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1. Vasilevskaya, T. N., Baturina, I. I., Kollegova, M. I., Gerasimova, T. N. and Barkhash, V. A. *Zh. Org. Khim.* **7** (1971) 1230.
2. Burdon, J., Castaner, J. and Tatlow, J. C. *J. Chem. Soc.* **1964** 5017.

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* Calculated on consumed *N*-methylaniline.

The C-Terminal Amino Acid Sequence of Bovine Pepsin

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Previous studies have shown that the primary structure of calf chymosin (also called rennin), EC.3.4.4.3, shows a high degree of homology with the primary structure of gastric proteases from other species.[1–3] This has prompted us to start investigations on the primary structure of bovine pepsin in order to compare two acidic proteases produced in the same species, but on different stages of ontogeny. In this communication we report a C-terminal sequence of 19 amino acid residues from bovine pepsin. 10 of the residues are identical with similar residues in calf chymosin, but the C-terminal sequence of bovine pepsin seems more closely related to those of human and porcine pepsins than to that of calf chymosin.

Partly purified bovine pepsin was obtained as a gift from Chr. Hansen's Laboratory, Copenhagen. The enzyme was further purified, first by gel filtration on a column of Sephadex G-100, using 0.05 M sodium phosphate buffer of pH 5.5 as eluent, subsequently the fractions which contained milk clotting activity were pooled and subjected to ion exchange chromatography on a column of DEAE cellulose equilibrated with 0.05 M phosphate buffer of pH 5.5. Elution took place with a linear gradient of sodium chloride in the equilibration buffer. Under such conditions the pepsin was eluted at salt concentrations between 0.4 to 0.5 M sodium chloride. After dialysis the preparation was freeze-dried. 150 mg of the freeze-dried preparation was denatured as described by Tang and Hartley.[4] The enzyme was suspended in 3 ml of acetone and 3 M ammonia was added dropwise under constant stirring, until the total volume was about 12 ml. After stirring for 30 min at room temperature the solution was dialysed against 3 × 1 l of distilled water for a total of 24 h and subsequently freeze-dried. Digestion took place with 1.5 mg trypsin at pH 8.5, maintained constant by addition of 0.1 M NaOH. The digestion was brought to an end by lowering pH to 6 with addition of 0.1 M HCl. The peptide mixture was