SYNTHESIS OF ORTHO DEUTERATED PHENYLISOCYANATE UNDER MILD CONDITIONS

SUMMARY

A new method has been developed for the synthesis of phenylisocyanate 2-^{2}H through the use of azido (tris dimethylamino) phosphonium hexafluorophosphate and benzoic acid 2-^{2}H. The product prepared is obtained with a high percentage of isotopic substitution, specificity of label and appreciable yield.

I - INTRODUCTION

The method of Bergmann et al. (1) was used by Abderhalden and Brockmann (2) and by Jensen and Evans (3) to determine the N-terminal group of a peptide or a protein by removal of only one residue. The identification of the N-terminal amino-acid was determined through the use of phenylisocyanate as the coupling reagent. Phenylisocyanate reacts with the alpha amino groups at pH=8 and 0°C. The phenylcarbamyl peptide obtained undergoes a cyclization at 100°C for one hour in the presence of methanol or hydrochloric acid.

The application of Bergmann's method to low quantities of proteins and peptides is possible if labeled phenylisocyanate is used. Such an application led to the synthesis of the ortho deuterated phenylisocyanate (PhNCO 2-^{2}H)

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The non-selectivity of the deuteration of aniline (4) and the redistribution of deuterium on the aromatic ring (5) during the phosgenation of this amine (6) does not allow the recovery of the expected isocyanate with a sufficiently high percentage of deuteration and appreciable yield. Similarly, the preparation of the isocyanate by the reaction of lead tetraacetate with deuterated benzamide (7) was unsuccessful . It was decided, therefore, to apply the CURTIUS rearrangement on the benzoyl azide 2-^{2}H. However, the classical method - benzoyl chloride and sodium azide - used to obtain benzoyl azide 2-^{2}H could not be used. In effect, the preparation of benzoyl chloride 2-^{2}H, starting with benzoic acid 2-^{2}H and thionyl chloride, leads to an extensive loss of label (8). The interest in the method presented here, lies in obtaining benzoyl azide 2-^{2}H in one step under mild conditions, starting with benzoic acid 2-^{2}H and the phosphorylated reactant : $N_3\overset{+}{P}(NMe_2)_3$, PF_6^- - azido (tris dimethylamino) phosphonium hexafluorophosphate (9,10; noted 5 in figure 1). In this way, easy access to phenylisocyanate 2-^{2}H is obtained with a high percentage of isotopic substitution, a high specificity of label and an appreciable yield.

II - Method and materials.

The reaction scheme is shown schematically in fig. 1.

Benzoic acid 2-^{2}H can be easily obtained with a high degree of purity through the process described by Jones et al. (11). The selectivity of the deuteration is due to the formation of the bicyclic compound 2 during treatment of the dimethylbenzylamine (DMBA) by n-butyllithium in anhydrous ether. The hydrolysis of 2 with D_2O, giving the DMBA 2-^{2}H (bp $_{20}$= 67°C), is followed by permanganic oxidation in alkaline solution and leads to benzoic acid 2-^{2}H.

Compound 5, a stable reactant (12), with a structure similar to that of diphenylphosphoryl azide (DPPA) (13), allows easy access to arylic acyl azides. To our knowledge DPPA has not been used for this purpose, although the intermediate acyl azide has been suggested by Fieser and Fieser (13). The reaction of benzoic acid 2-^{2}H with 5 in acetone, yielded compound 6 . The acetone was removed under vacuum, the residue dissolved in cold ether and the solution washed with cold water. The etheral phase was dried over magnesium sulphate in the cold and evaporated under low pressure at a temperature below 30° C . The thermolysis of compound 6 was carried out on the crude product in anhydrous benzene. The evolution of the reaction was follo-

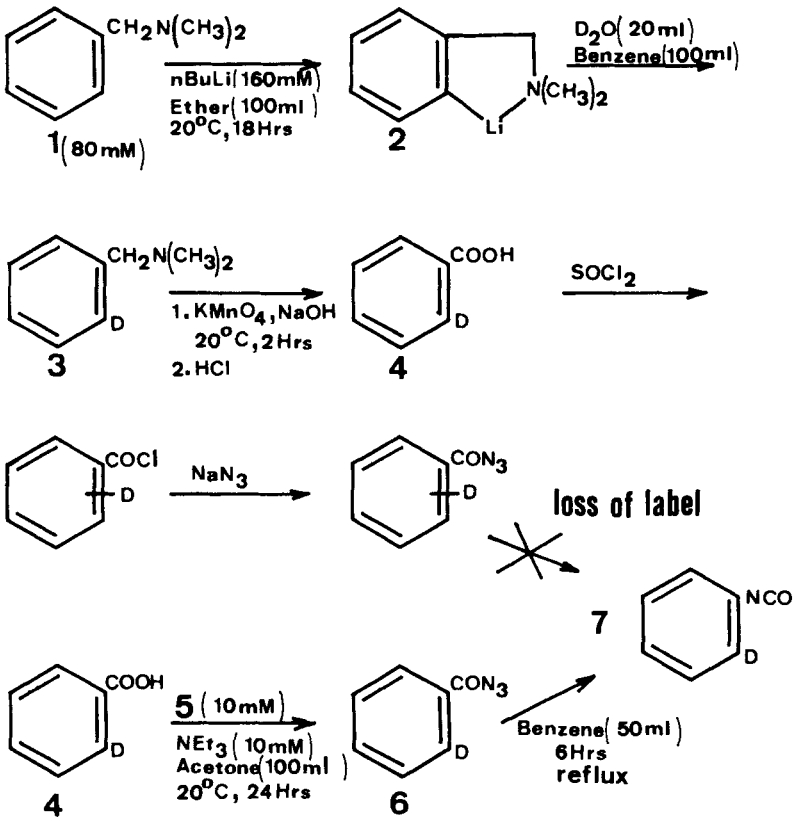


fig 1

SYNTHESIS OF PHENYLISOCYANATE 2-^{2}H

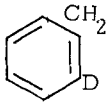
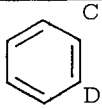
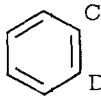
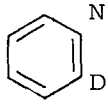
wed by infra-red spectroscopy and the characteristic bands of the isocyanate progressively replaced those of the azide group as the reaction progressed.

Phenylisocyanate 2-^{2}H was isolated in good yield by distillation under low pressure (bp_{20} = 62 - 63 ° C)

III - RESULTS

The spectral data, yield and deuterium analysis of each intermediate are given in Table 1.

Table 1

	Compound	Spectral data	a Yield (%)	Deuterium analysis D atom/molecule
3		IR [11]: 847, 777, 734, 696 cm^{-1}	98	0.93
4		IR [14]: 779, 863, 857, 800, 709, 628 cm^{-1}	55	0.93 Mass : m/e = 123 (M)
6		IR [15]: 1693, 2134, 2147 cm^{-1}	81	-
7		IR [16]: 2260 2278 cm^{-1} MW b	78	0.91 Mass: m/e = 120 (M)

a) isolated yield

b) The specificity of the deuteration on the ortho position has been checked by microwave spectroscopy.

IV - CONCLUSIONS

The use of the phosphorylated reactant 5 allows easy access to phenylicocyanate 2-^{2}H, via benzoyl azide 2-^{2}H, with a high percentage of isotopic substitution, a specificity of label and an appreciable yield. The method presented in this communication offers the essential advantage of avoiding any redistribution of deuterium on the aromatic ring, which is the case with the traditional synthetic route (15).

Redistribution of deuterium on the aromatic ring was not observed when phenylisocyanate was used as the coupling reagent with alpha amino groups. Therefore , the compound prepared should be of interest in the determination of the N-terminal amino acid of low amounts of peptides or proteins, by coupling the latter with the tritiated homologue.

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