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Mass Spectra of two Deuterated Isothiocyanates

ERIK BACH, ANDERS KJÆR* and R. H. SHAPIRO**

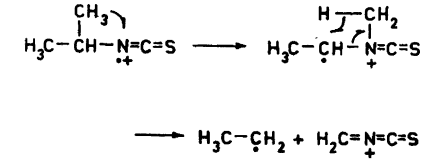
Organic Chemistry Department, Royal Veterinary and Agricultural College, Copenhagen, Denmark

CARL DJERASSI***

Department of Chemistry, Stanford University, Stanford, California, USA

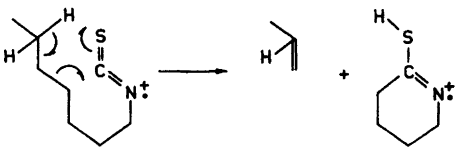
In a previous paper [1] the mass spectral fragmentation of about forty isothiocyanates was studied and tentative identifications of some of the observed ions were presented. In the present note evidence based on deuteration experiments is adduced to substantiate some of the previous suggestions.

The two α-branched alkyl compounds studied, 2-propyl and 2-butyl isothiocyanate, both exhibited a peak at *m/e* 72, attributable to the ion CH_2NCS^+, possibly formed by an intramolecular rearrangement involving the transfer of a methyl group:



In order to test this possibility 2-*d*-2-propyl isothiocyanate was prepared from 2-*d*-2-propylamine, which, in turn, was obtained by reduction of acetoxime with lithium aluminium deuteride. The mass spectrum of the α-deuterated isothiocyanate exhibited a very strong molecular ion peak (*m/e* 102), the expected signal at *m/e* 87 (M−15), and, most significantly, a peak at *m/e* 72 of about the same relative intensity as the *m/e* 72 signal in the non-deuterated species, indicating that the α-carbon atom does not contribute to the formation of the CH_2NCS^+-ion. Hence, the previously suggested migration of a methyl group, followed by breakage of the original α-bond, appears to be correct.*

In the series of straight-chain isothiocyanates a strong peak at *m/e* 115 was consistently observed in all species containing six or more carbon atoms in the side chain. Tentatively the following rearrangement was proposed, [1] where the required deviation from linearity of the functional group in the molecular ion could be brought about by 'unpairing' of π-electrons from one of the double bonds.



* Part LIV of a series of papers on isothiocyanates. Part LIII: *Acta Chem. Scand.* **19** (1965) 1989.

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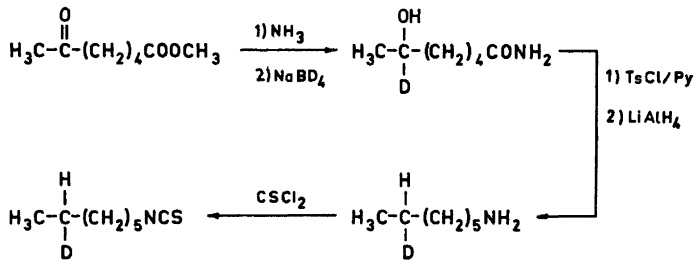
* Until recently alkyl migrations in the mass spectrometer have been considered to be relatively rare (*cf.* Ref. 2). Lately, however, several such cases have been encountered.[3,4]



The correctness of this assumption has now been proved by mass spectral study of *6-d*$_1$-heptyl isothiocyanate, synthesized according to the scheme above.

6-Oxoheptanamide was produced by ammonolysis of methyl 6-oxoheptanoate and converted into *6-d*-6-hydroxy-heptanamide with sodium borodeuteride. O-Tosylation of the hydroxy-amide, followed by hydrogenolysis with lithium aluminium hydride afforded *6-d*$_1$-heptylamine which was, in turn, converted into *6-d*$_1$-heptyl isothiocyanate upon reaction with thiocarbonyl chloride.

Mass spectra were recorded of the non-labelled and the *6-d*$_1$-heptyl isothiocyanates under identical conditions. The relative abundances as per cent of the base peak are quoted in Table 1. It is clearly brought out that a substantial transfer of hydrogen positioned at C-6 occurs, in keeping with the above scheme for the rearrangement. The qualitative discrepancy in the shift to *m/e* 116 may be attributable to an isotope effect. It cannot be excluded, however, that a slight contamination of the unlabelled species also contributes to the relative abundance of the *m/e* 115-ion.

Table 1. Relative abundance of ions, as per cent of the base peak (*m/e* 115), produced from heptyl isothiocyanate and its *6-d*$_1$-derivative on electron impact.

Ion	Unlabelled		*6-d*$_1$-Series	
	mass	%	mass	%
M^+	157	2	158	4
M− 1	156	2	157	4
M−15	142	4	143	5
M−33	124	34	125	44
M−42	115	100	116	78
M−43			115	100

Experimental. 2-d-*2-Propyl isothiocyanate.* A solution of acetoxime (2 g) and lithium aluminium deuteride (0.5 g) in ether (20 ml) was heated under reflux for 4.5 h. Water was cautiously added to the ice-cooled reaction mixture which was then distilled until water started coming over. The distillate was collected in cold, excess 1 N HCl. The ethereal phase was discarded, and the aqueous layer concentrated to a semi-solid residue of the hygroscopic amine hydrochloride. Without further purification this was dissolved in chloroform (7 ml) and treated with thiocarbonyl chloride (1.5 g) and triethylamine (3.5 ml) for 2.5 h. The solution was washed with 1 N HCl, 1 N NaOH, and water. After drying, the solvent was removed through a short column, and the dark oily residue was further purified by vapor phase chromatography at 100° on a column (300 × 0.5 cm), packed with 2 % Dowfax 9N9 on kieselguhr. The homogeneity of the resulting *2-d*-2-propyl isothiocyanate, obtained in an over-all yield of about 10 %, was confirmed by v.p.c.

6-Oxoheptanamide. Methyl 6-oxoheptanoate (16 g),[5] prepared according to a general procedure,[6] was subjected to ammonolysis by treatment with 13 N methanolic ammonia (100 ml) in a closed vessel at 35° for 4 days. After removal of excess ammonia and methanol, the residue was extracted with ethyl acetate to give 11.6 g of impure amide, which was further extracted with petroleum ether. The residue was recrystallized twice from ethyl acetate:petroleum ether to give the slightly yellow amide (4.6 g), m.p. 74—75°. An analytical specimen was produced by two additional recrystallizations from the same solvent mixture, m.p. 80°. (Found: C 58.70; H 9.22; N 9.73. Calc. for $C_7H_{13}NO_2$: C 58.71; H 9.15; N 9.78). The IR-spectrum possessed the expected bands.

6-d$_1$-*Heptyl isothiocyanate.* To an ice-cold solution of 6-oxoheptanamide (400 mg) in D_2O (3 ml), sodium borodeuteride (188 mg) was added in one portion. The solution was kept overnight at room temperature, then

made slightly acidic with dilute hydrochloric acid, and extracted continuously with ethyl acetate for 15 h. The solvent was removed, and a semisolid mass resulted after removal of water traces by distillation with small amounts of toluene.

Without further purification the hydroxyamide was dissolved in pyridine (3 ml), and *p*-toluenesulphonyl chloride (1 g) was added at 5°. After 48 h's standing at 5°, water (5 ml) and ether were added, and the solution was extracted with 2 N HCl, saturated $NaHCO_3$-solution, and water. After drying and removal of the solvent, 255 mg of the oily tosylate resulted.

The crude material (250 mg) was treated in boiling ether solution (10 ml) for 5 h with lithium aluminium hydride (250 mg). Then saturated Na_2SO_4-solution and thereafter solid Na_2SO_4 were added. The suspension was filtered, the filter cake washed thoroughly with ether, and the ether phases combined and dried. Anhydrous HCl was passed into the solution, and after removal of the solvent, a semicrystalline, hygroscopic hydrochloride resulted.

Without further purification this was dissolved in water (1 ml), and chloroform (3 ml) was added, followed by thiocarbonyl chloride (250 mg). The two-phase system was stirred vigorously, and triethylamine (2 ml) was added dropwise. The solution was stirred for another 2 h and then worked up as described above for 2-propyl isothiocyanate.

The crude isothiocyanate was purified by v.p.c. on the above described column (isothermally, 150°, 40 ml N_2/min), and a homogeneous fraction with a retention time of 5.6 min (the same as authentic heptyl isothiocyanate) was collected for mass spectrometry.

Mass spectrometry. The mass spectra were obtained by Mr. John Smith with a Consolidated Electrodynamics Corporation 21—103 C mass spectrometer fitted with an all-glass heated inlet system, maintained at 200°. An ionizing current of 50 μA and an ionizing potential of 70 eV were used.

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The Inactivation of Trypsin by Diethyl Pyrocarbonate

L. HULLÁN, T. SZONTAGH, I. TURTÓCZKY and I. FEDORCSÁK*

Institute of Genetics, Eötvös Loránd University, Budapest, Hungary

and

Institute of Biochemistry, University of Stockholm, Stockholm, Sweden

In connection with the testing of the mutagenic effect of different chemicals, going on in these laboratories, we found that the autolysis is completely inhibited in bacteria or in the cells of other microorganisms killed by diethyl pyrocarbonate (DEP). This observation prompted a further study of the interaction of DEP and different proteolytic enzymes. In the present communication we report briefly on the interaction of DEP and trypsin.

The tryptic activity was determined by the conventional casein digestion method according to Kunitz.[1] The trypsin used was commercial lyophilised product from Richter, and the DEP was a commercial product from Bayer Ltd., called Baycovin.[2]

The result of the experiments are presented in Table 1. From the data it

* Present address: Institute of Biochemistry, University of Stockholm.