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.

Table 1. Action of DEP on trypsin and on casein.

DEP incubation: in phosphate buffer (0.1 M, pH=7.6) in a final volume of 1 ml; 0.02 units of trypsin, or 10 mg of casein substrate, or only the buffer (without trypsin and casein) was incubated in the presence of DEP for 4 days at 0°C. The samples were then placed in a water bath at 35° and the enzymatic reaction was started by adding the 10 mg of casein, or the 0.02 units of trypsin, or both these protein components, respectively, dissolved in 1 ml of 0.1 M phosphate buffer pH = 7.6. After 20 min the enzymatic reaction was stopped by the addition of 3 ml TCA and after centrifugation the optical density of the supernatant was read at 280 mμ. The readings were corrected for the value of the actual blank in which the enzymatic reaction was stopped by TCA at 0 min. The 100 % tryptic activity is equal to an increase of 0.3 extinction units at 280 mμ.

Conditions for DEP incubation	Tryptic activity after treatment with DEP; Concentration of DEP (mole/l) during the DEP incubation.							
	0.000	0.00003	0.00015	0.0003	0.0015	0.003	0.015	0.03
Trypsin treated with DEP for 4 days	100	100	87	83	33	11	0	0
Casein treated with DEP for 4 days	100	100	98	97	84	67	4	0
Phosphate buffer treated with DEP for 4 days	100	—	—	—	—	100	—	100

may be concluded that the tryptic activity is inactivated by DEP but that DEP may also react with the casein making it an undigestible substrate for trypsin. Comparing the figures for the trypsin inactivation and for the "casein inactivation" we see that the inhibition rates are practically the same. This fact is interesting particularly because the protein concentration in the case of casein was about 1000 times higher than that of the trypsin. This circumstance may indicate some kind of general denaturation reaction to occur between DEP and proteins.

It is known that DEP in water solution decomposes rather quickly to CO_2 and alcohol (half-life at 0° is about 5 h):

$$H_2O + C_2H_5-O-CO-O-CO-O-C_2H_5 \rightarrow 2C_2H_5OH + 2CO_2$$

It seems clear from our control experiment (*cf.* Table 1) that this decomposition is quantitative and that the decomposition products have no effect on the activity of trypsin.

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NMR Spectra of Atropic Acids

KURT NILSSON

Department of Organic Chemistry, University of Lund, Lund, Sweden

and S. STERNHELL

Department of Organic Chemistry, The University of Sydney, Sydney, Australia

One of us [1] has recently established the configuration of a number of alkyl substituted atropic acids by means of ultraviolet spectroscopy. We report here a confirmation of these assignments by means of NMR spectroscopy.

It is well established [2-4] that in olefins the carboxyl groups deshield both protons and alkyl groups situated *cis* to them rela-