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 µ. 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 % trypsic activity is equal to an increase of 0.3 extinction units at 280 µ.

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

may be concluded that the trypsic 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 CO₂ and alcohol (half-life at 0° is about 5 h):

\[
\text{H}_2\text{O} + \text{C}_2\text{H}_4\text{O} = \text{O} - \text{CO} - \text{O} - \text{CO} - \text{O} - \text{C}_2\text{H}_4 \rightarrow 2\text{C}_2\text{H}_5\text{OH} + 2\text{CO}_2
\]

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

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One of us 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 that in olefins the carboxyl groups deshield both protons and alkyl groups situated cis to them rela-
**SHORT COMMUNICATIONS**

Table 1. 60 Me/s NMR spectra \(^a\)\(^b\) of atropic acids.

<table>
<thead>
<tr>
<th>R(_1)</th>
<th>R(_2)</th>
<th>R(_1)</th>
<th>R(_2)</th>
<th>Ph</th>
<th>Coupling Constants (c/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>d 359</td>
<td>d 391</td>
<td>n m 446</td>
<td>(J_{H,H} = 1.15)</td>
</tr>
<tr>
<td>Me</td>
<td>H</td>
<td>d 92</td>
<td></td>
<td>b m 410—430</td>
<td>(J_{H,CH_3} = 7.3)</td>
</tr>
<tr>
<td>H</td>
<td>Me</td>
<td>q 384</td>
<td>d 130</td>
<td>n m 436</td>
<td>(J_{H,CH_3} = 7.3)</td>
</tr>
<tr>
<td>Et</td>
<td>H</td>
<td>CH(_3) quint.129</td>
<td>CH(_3) t 62.5</td>
<td>b m 430</td>
<td>(J_{H,CH_3} \sim J_{CH_3CH_3} \sim 7)</td>
</tr>
<tr>
<td>H</td>
<td>Et</td>
<td>t 375</td>
<td>CH(_3) quint.157</td>
<td>CH(_3) t 68.5</td>
<td>n m 437</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(J_{CH_3CH_3} = 7.5)</td>
</tr>
</tbody>
</table>

\(^a\) The NMR spectra were obtained for 1—2 % solutions in carbon tetrachloride on a Varian model A60 spectrometer. The line positions were reproducible to \(\pm 1\) c/s, all signals were of the expected relative intensity. Multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, m = multiplet, n = narrow, b = broad.

\(^b\) Signals assigned to the -COOH protons occurred at 610—690 c/s in all compounds studied and were strongly concentration and temperature dependent.

\(^c\) Overlapped by the signal due to aromatic protons.

...to those situated trans to them. Recent work \(^4\) shows that in \(\alpha,\beta\)-substituted styrenes the \(\beta\)-alkyl groups cis to the benzene ring are shielded relatively to those trans while the opposite is true for the \(\beta\)-protons. However, even in this case, the deshielding effect of the phenyl groups is smaller \(^5\) than that of the carboxyl group. It is probable that these effects are not significantly changed by the conjugation between the phenyl and carboxyl groups, and thus an unambiguous result could be expected for the series of compounds here investigated. It could thus be confidently predicted that both alkyl groups and protons cis to the carboxylic acid groups should resonate downfield of those cis to the phenyl group.

NMR data summarized in Table 1, show that the assignments based on ultraviolet studies are in fact correct. It is also interesting to note that even the protons of the methyl groups in ethyl atropic acids experience a differential shielding (approximately 6 c/s) although this is (not unexpectedly) much smaller than the 28 c/s and 38 c/s shown for the methylene groups in the same compounds and the methyl group in methyl atropic acids respectively. Characteristic changes can also be observed in the appearance of the signals due to the aromatic protons; the broadening in the case of cis phenyl-alkyl configuration may be due to a sterically induced \(^1\) distortion which results in an altered average conformation of the phenyl group \(^a\) toward the magnetically anisotropic carboxyl group.


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