ACYLATION OF N-ACETYLENEURAMINIC ACID

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Neuraminic acid is a widely distributed monomer of numerous oligo- and polysaccharides, glycopeptides, glycolipids, and mucopolysaccharides which is responsible for the specific biological action of the compounds mentioned [1]. In view of this, the systematic study of the chemistry of neuraminic acid is of great importance. In the present communication we give the results of an investigation of the acetylation and benzylation of N-acetyleneuraminic acid (I) (cf. [2]).

A study of the direct acetylation of (I) by the action of acetic anhydride in pyridine under the conditions described by Meindl and Tuppy [3] showed that in spite of what was reported, the reaction takes place ambiguously even under mild conditions and, judging from the results of chromatographic analysis, leads to the formation of at least two acetylated derivatives of the acid (I). Their preparative separation by means of chromatography on silica gel enabled us to obtain the chromatographically homogeneous acetates (II) (13%) and (III) (61%).

The structure of the acetate (II) was established on the basis of the following results. Its IR spectrum had a strong absorption band at 1752 cm⁻¹ indicating the presence of a γ-lactone grouping in the compound. It gave an intense coloration with ferric chloride and, consequently, contained an enol function.

Thus, one of the acetates formed by the direct acetylation of the acid (I) can be assigned the structure (II). Finally, we confirmed this by synthesizing (II) independently from the tert-butoxycarbonyl lactone (IV), an intermediate in the synthesis of N-acetyleneuraminic acid by Kuhn and Baschang [4] starting from N-acetyl-D-mannosamine (V):

The two samples of the lactone (II) proved to be identical in their chromatographic behavior and had identical IR and UV spectra (figure). Since 5-substituted 3-hydroxy-3-(4)-butenolides have not previously been studied spectroscopically, we specially synthesized 5-phenyl- (VII) and 5-ethyl-3-hydroxy-3-(4)-butenolides (VI).
A comparison of the UV spectra of the lactones (II), (VI), and (VII') definitively proved the presence in compound (II) of an unsaturated γ-lactone grouping (see figure).

The second substance obtained in the direct acetylation of the acid (I), the acetate (III), coincided in its chromatographic behavior, constants, and IR and UV spectra with an authentic sample of 2, 4, 7, 8, 9-penta-O-acetyl-N-acetyleneuraminic acid (III) synthesized from the benzhydryl ester of N-acetyleneuraminic acid (VIII), which we have described previously [5]:

\[
\begin{align*}
\text{H COH} & \quad \text{OH} \\
\text{H} & \quad \text{C} \quad \text{O} \\
\text{H} & \quad \text{C} \quad \text{H}_2 \text{O} \\
\end{align*}
\]

It is important to stress that the elimination of the benzhydryl protection in the acetate (IX) takes place smoothly, under mild conditions, and with practically quantitative yield.

Just like the acetylation, the direct benzoylation of N-acetyleneuraminic acid (I) by benzoyl chloride in pyridine takes place ambiguously. The nonhomogeneity of the benzoate formed in the benzoylation of (I) can be shown chromatographically. This fact was confirmed by comparing the UV spectra of the product of direct benzoylation of the acid (I) and 2, 4, 7, 8, 9-penta-O-benzoyl-N-acetyleneuraminic acid (X) obtained from the benzhydryl ester (X) (see figure).

The IR spectrum of the product of the direct benzoylation of the acid (I) [a mixture of (XI) and (XII)] is similar to the spectrum of the pentabenzolate (XI) but differs from the latter by the presence of an absorption band at 1770 cm\(^{-1}\) which again shows the presence of the γ-lactone (XII) in the mixture.

Since the UV spectrum of a mixture of (XI) and (XII) has absorption bands at 255 and 305 mp characteristic, as has been shown above, for 5-substituted 3-hydroxy-3(4)-butenolides and completely absent from the spectrum of the pentabenzolate (XI), this lactone must be assigned the structure (XII).

**Experimental**

The paper chromatography, descending, on Whatman No. 3 chromatographic paper was carried out on the following solvent systems (by volume): 1) n-propanol - 0.1 N hydrochloric acid - 1-butanol (2:1:1); 2) ethyl acetate - acetic acid - water (9:2:2). Chromatography in a thin fixed layer of silica gel (KSK, 150-200 mesh) was carried out in the following systems: 3) n-propanol - water (7:3); 4) ethyl acetate - ethanol (1:1); 5) chloroform-methanol (7:3). The spots on the chromatograms were revealed with Svennerholm’s resorcinol reagent [6] and Ehrlich’s reagent.

**Di-tert-butyl oxalate.** A suspension of 360 g of anhydrous oxalic acid in 800 ml of dry ether in a thick-walled flask was cooled to -10 °C, 1500 ml of liquid isobutylene was added, and the flask was hermetically sealed and shaken at room temperature for 3 days. The mixture was cooled to -10 °C, the flask was carefully opened, the homogeneous yellowish liquid was poured into a solution of 350 g of caustic soda in 2000 ml of water and 500 g of ice, the ethereal layer was separated off, the aqueous layer was extracted with ether (4 × 500 ml) and the extracts were combined with the main portion of the reaction product, dried with calcium chloride, and evaporated in vacuum to dryness. Recrystallization of the residue from petroleum ether yielded 517 g (65%) of di-tert-butyl oxalate, mp 73 °C.

Found, %: C 59.70; H 8.51. Calculated for C\(_{10}\)H\(_{16}\)O\(_4\), %: C 59.42; H 8.97.

**N-Acetylneuraminic acid (I).** The acid was obtained by Kuhn and Baschang’s method [4] from N-acetyl-D-mannosamine (V); yield 29%, mp 179°-181 °C, [a]\(_D\) +32.2° (c 1.0; water), chromatographically homogeneous, Rf 0.4 (system 1), 0.12 (system 2), 0.17 (system 3). Literature data: mp 180°-182 °C, [a]\(_D\) +32.4° (c 0.9; water) [4].

The tert-butoxycarbonyl lactone (IV). The substance was synthesized by Kuhn and Baschang’s method [4] and purified by chromatography on silica gel with methanol → water gradient elution; colorless amorphous powder chroma-