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ACYLATION OF N-ACETYLNEURAMINIC ACID

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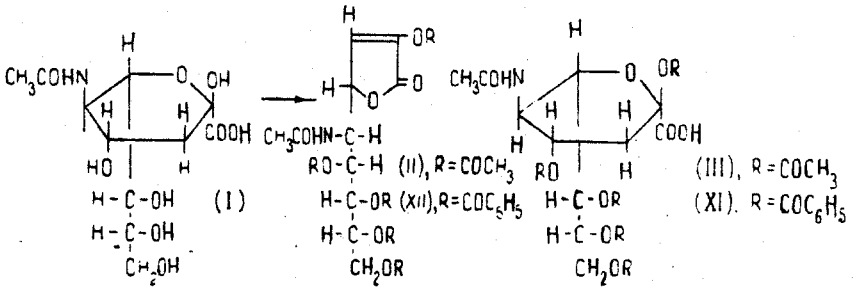
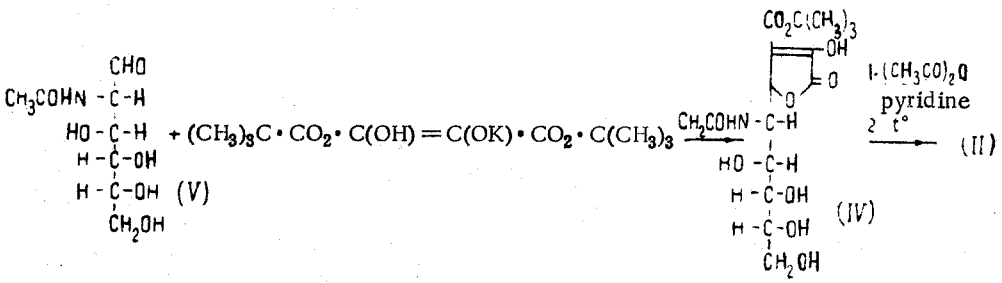
Neuraminic acid is a widely distributed monomer of numerous oligo- and polysaccharides, glycopeptides, glycolipids, and mucopolysaccharides which is responsible for the specific biological action of the compounds mentioned [1]. In view of this, the systematic study of the chemistry of neuraminic acid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

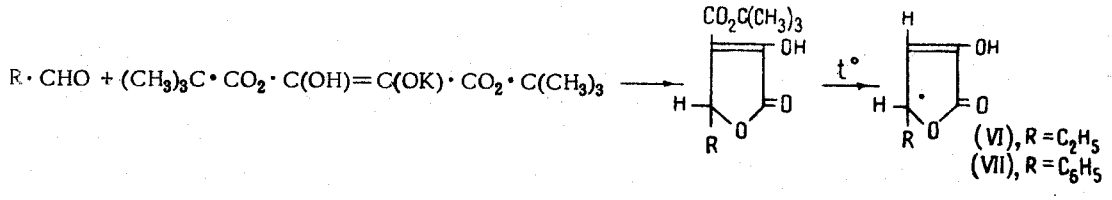


The structure of the acetate (II) was established on the basis of the following results. Its IR spectrum had a strong absorption band at 1752 cm^{-1} indicating the presence of a γ-lactone grouping in the compound. It gave an intense coloration with ferric chloride and, consequently, contained an enol 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-acetylneuraminic acid by Kuhn and Baschang [4] starting from N-acetyl-D-mannosamine (V):

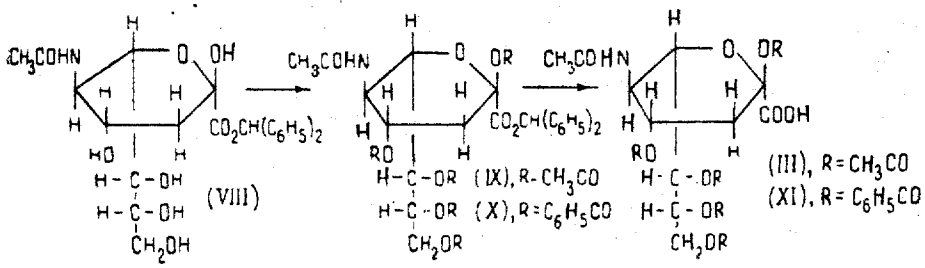


The two samples of the lactone (II) proved to be identical in their chromatographic behavior and had identical IR and UV spectra (figure). Since 5-substituted 3-hydroxy-3(4)-butenolides have not previously been studied spectroscopically, we specially synthesized 5-phenyl-(VII) and 5-ethyl-3-hydroxy-3(4)-butenolides (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).

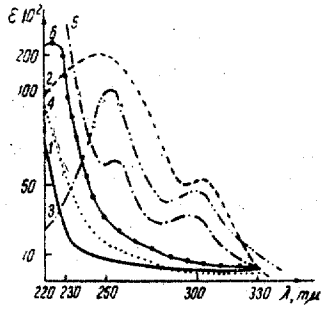
The second substance obtained in the direct acetylation of the acid (I), the acetate (III), coincided in its chromatographic behavior, constants, and IR and UV spectra with an authentic sample of 2, 4, 7, 8, 9-penta-O-acetyl-N-acetylneuraminic acid (III) synthesized from the benzhydryl ester of N-acetylneuraminic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



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-acetylneuraminic 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-acetylneuraminic acid (XI) 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 pentabenzoate (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 mμ characteristic, as has been shown above, for 5-substituted 3-hydroxy-3 (4)-butenolides and completely absent from the spectrum of the pentabenzoate (XI), this lactone must be assigned the structure (XII).



UV spectra of the following compounds. 1) N-Acetylneuraminic acid; 2) acetate of the lactone (II); 3) 5-phenyl-3-hydroxy-3 (4)-butenolide (VII); 4, 2, 4, 7, 8, 9-penta-O-acetyl-N-acetylneuraminic acid (III); 5) product of the direct benzoylation of the acid (I) – a mixture of (XI) and (XII); 6) 2, 4, 7, 8, 9-penta-O-benzoyl-N-acetylneuraminic acid (XI).

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 $C_{10}H_{18}O_4$, %: 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, $[\alpha]_D^{20}$ −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, $[\alpha]_D^{20}$ −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-

tographically homogeneous, R_f 0.6 (system 2), 0.5 (system 4), $[\alpha]_D^{20}$ −7.1° (c 1.0; water). Literature data: $[\alpha]_D^{23}$ −6° (c 2.5; water) [4].

Acetate of the lactone (II). 100 mg of the lactone (IV) was acetylated with acetic anhydride (5.2 mg) in pyridine (5 ml) at 20° C for 24 hr, and then the reaction mixture was poured on to ice, the aqueous solution was decolorized with carbon and passed through a column (2 × 20 cm) of Amberlite IR-120 (H^+ form) and eluted with methanol, the water–methanol eluate was evaporated in vacuum to dryness, and the residue was dried in vacuum over phosphorus pentoxide. The substance was chromatographically homogeneous, R_f 0.8 (system 3), yield 101.2 mg (67%).

A mixture of 100 mg of this acetate and 20 ml of methanol–toluene (1:1) mixture was boiled for 20 min, after which the evolution of gas had ceased, and the solution was decolorized with carbon and evaporated in vacuum to dryness. Substance (II) was dried in vacuum over phosphorus pentoxide and paraffin wax; yield 60 mg (73.5%) of a colorless powder decomposing at about 129° C, $[\alpha]_D^{20}$ −8° (c 0.5; methanol), chromatographically homogeneous, R_f 0.75 (system 3).

Found, %: C 50.32; H 5.30; N 2.66. Calculated for $C_{21}H_{27}O_{13}N$, %: C 50.29; H 5.42; N 2.79.

IR spectrum: 1375, 1450 cm^{-1} (−$OCOCH_3$), 1545, 1665 (−CONHR), 1752 cm^{-1} (γ-lactone). UV spectrum: $\lambda_{max}^{ethanol}$ 250 and 305 mμ (log ε 4.24, 3.75, respectively).

Benzhydryl ester of 2, 4, 7, 8, 9-penta-O-acetyl-N-acetylneuraminic acid (IX). At 0° C, with stirring, 4 ml of acetic anhydride was added to a solution of 200 mg of benzhydryl N-acetylneuraminate (VIII) [5] in 5 ml of pyridine. The mixture was left at 0° C for 30 min and at 20° C for 2 days and was then poured onto ice and treated with 10 ml of methanol; the solution was passed through a column of Amberlite IR-120 (H^+ form) and eluted with methanol, the eluate was evaporated in vacuum, and the residue was dried in vacuum over phosphorus pentoxide. Yield 218.4 mg (76%), decomposing at 169°–171° C, $[\alpha]_D^{20}$ − 12.2° (c 0.6; pyridine), $[\alpha]_D^{20}$ − 9.0° (c 0.2; methanol). The substance was chromatographically homogeneous, R_f 0.85 (system 3).

Found, %: C 59.80; H 5.93; N 2.48. Calculated for $C_{34}H_{39}O_{14}N$, %: C 59.55; H 5.73; N 2.04.

Direct acetylation of N-acetylneuraminic acid (I). 200 mg of (I) was acetylated with acetic anhydride (5.5 ml) in pyridine (6 ml) (30 min at 0° C and a day at 20° C), 10 ml of methanol was added, the mixture was poured onto ice, the resulting solution was decolorized with carbon and extracted with chloroform, and the extracts were washed with 0.5% hydrochloric acid, 5% sodium hydrogen carbonate solution, and water, dried with magnesium sulfate, and evaporated in vacuum to dryness. This gave 40 mg of the acetate of the lactone (II) (13%) as a colorless powder, $[\alpha]_D^{20}$ −7.5° (c 0.9; methanol), chromatographically homogeneous and identical with the (II) described previously, R_f 0.75 (system 3). IR spectrum: 1375, 1450; 1550, 1663, 1750 cm^{-1}. UV spectrum: $\lambda_{max}^{ethanol}$ 252, 305 mμ (log ε 4.25, 3.75).

Evaporation of the subsequent fractions gave 205 mg (61%) of the acetate (III) as a colorless powder, decomposing about 148° C, $[\alpha]_D^{10}$ −10.8° (c 0.23; water), R_f 0.61 (system 3).

Found, %: C 46.66; H 5.77; N 2.66. Calculated for $C_{21}H_{29}O_{14}N \cdot H_2O$, %: C 46.93; H 5.81; N 2.61.

IR spectrum: 1380, 1450 cm^{-1} ($OCOCH_3$); 1550, 1662 (CONHR); 1740 (−COOC); 2600 cm^{-1} (COOH).

2, 4, 7, 8, 9-Penta-O-acetyl-N-acetylneuraminic acid (III). 100 mg of benzhydryl 2, 4, 7, 8, 9-penta-O-acetyl-N-acetylneuraminate (X) was hydrogenated in 20 ml of methanol over 240 mg of $Pd/BaSO_4$ at room temperature for 3 hr, and then the catalyst was filtered off and washed with water, the combined filtrates were concentrated in vacuum at 35° C, and the residue was lyophilized. The yield of (III) was 70 mg (93%); white amorphous powder, decomposing about 145° C, chromatographically homogeneous, R_f 0.62 (system 3), $[\alpha]_D^{20}$ −10.3° (c 0.3; water).

Found, %: C 46.49; H 6.04; N 2.40. Calculated for $C_{21}H_{29}O_{14}N \cdot H_2O$, %: C 46.93; H 5.81; N 2.61.

IR spectrum: 1380, 1452, 1550, 1665, 1739, 2620 cm^{-1}.

Direct benzoylation of N-acetylneuraminic acid (I). In drops, 1 ml of benzoyl chloride was added to a stirred suspension of 100 mg of (I) in 5 ml of pyridine at 0° C. After 30 minutes at 0° C, 1 hr at 10° C, and 3 hr at 20° C, the mixture was poured onto ice and extracted with chloroform (3 × 50 ml), and the extracts were washed with 10% sodium hydrogen carbonate solution and water, dried with magnesium sulfate, and evaporated in vacuum; the residue was twice crystallized from ethanol. This gave 210 mg of material [a mixture of (XI) and (XII)], decomposing about 158° C, $[\alpha]_D^{19}$ −5.3° (0.6; chloroform), R_f 0.58 (system 5).

Found, %: C 67.12; H 5.27; N 1.29. Calculated for $C_{48}H_{39}O_{14}N$, %: C 66.70; H 4.72; N 1.68.

IR spectrum: 1450, 1500, 1583, 1601 (aromatic nucleus), 1670 (CONHR), 1738 (COOC), 1770 (weak, γ-lactone), 2600 cm^{-1} (COOH). The UV spectrum is given in the figure.

Benzhydryl 2, 4, 7, 8, 9-penta-O-benzoyl-N-acetylneuraminate (X). 500 mg of the benzhydryl ester (VIII) was

benzoylated with benzoyl chloride (3 ml) in 15 ml of pyridine (for a day, 0–10° C), and the reaction mixture was poured into a mixture of ice and a saturated solution of sodium hydrogen carbonate and extracted with ether (3 × 50 ml); the extracts were washed with water, dried with magnesium sulfate, and evaporated, and the residue was twice crystallized from methanol. This gave 450 mg (43%) of (X), decomposing at about 165° C, $[\alpha]_D^{21}$ −9.8° (c 1.2; chloroform), R_f 0.91 (system 5).

Found, %: C 71.60; H 4.94; N 1.24. Calculated for $C_{59}H_{49}O_{14}N$, %: C 71.16; H 4.95; N 1.40.

2,4,7,8,9-Penta-O-benzoyl-N-acetylneuraminic acid (XI). 150 mg of (X) was hydrogenated in 15 ml of ether over 200 mg of $Pd/BaSO_4$ for 2 hr, and then the catalyst was filtered off and washed with ether, the filtrates were evaporated in vacuum, and the residue was crystallized from methanol. This gave 101 mg (81%) of (XI), decomposing at about 160° C, $[\alpha]_D^{20}$ −6.1° (c 0.5; chloroform), R_f 0.58 (system 5).

Found, %: C 66.92; H 4.67; N 1.49. Calculated for $C_{46}H_{39}O_{14}N$, %: C 66.70; H 4.72; N 1.68.

IR spectrum: 1450, 1500, 1585, 1603, 1670, 1735, 2600 cm^{-1}.

5-Ethyl- (VI) and 5-phenyl-3-hydroxy-3(4)-butenolides (VII). A mixture of 0.04 mole of the potassium derivative of di-tert-butyl oxaloacetate, 0.04 mole of propionaldehyde (or benzaldehyde), and 120 ml of dioxane was boiled for 2 hr after which the homogeneous solution was evaporated to 1/3 of its original volume, diluted with a fourfold volume of water, acidified with 1 N sulfuric acid to pH 4.5, and extracted with ether (3 × 25 ml); the extracts were washed with 5% sodium hydrogen carbonate solution and with water and were dried with sodium sulfate and evaporated, and the residue was dissolved in 20 ml of toluene and the solution was boiled for 20 min, after which the evolution of gas had ceased, and was then cooled, washed with water (3 × 10 ml), and evaporated in vacuum. The residue was crystallized from ether—petroleum ether. Yield of (VI) 37.5%, mp 49.5°–50.5° C.

Found, %: C 56.15; H 6.91. Calculated for $C_6H_8O_3$, %: C 56.24; H 6.29.

IR spectrum: 1380, 1460, 1670 (enol), 1780 cm^{-1} (γ-lactone).

The synthesis of (VI) and (VII) was carried out with the participation of L. Gaile.

Anilide of 2-oxo-4-hydroxycaproic acid. The substance had mp 99°–101° C.

Found, %: C 64.13; H 6.93; N 6.56. Calculated for $C_{21}H_{15}O_3N$, %: C 64.10; H 6.21; N 6.68.

Yield of (VII) 46%, mp 126°–127° C.

Found, %: C 67.99; H 4.92. Calculated for $C_{10}H_8O_3$, %: C 68.18; H 4.57.

IR spectrum: 1450, 1550 (aromatic nucleus), 1660 (enol), 1782 cm^{-1} (γ-lactone). UV spectrum: $\lambda_{max}^{ethanol}$ 255, 305 mμ (log ε 4.02, 3.66).

Summary

The direct acylation of N-acetylneuraminic acid takes place ambiguously and leads to two reaction products: the acylated γ-lactone and the acylated derivative of the pyranose form of the acid.

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