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Shigeru Takanashi, Tsutomu Kawaguchi, and Minoru Kawada : N-Acetylation of Hyalobiuronic Acid and Chondrosine.

(*Research Laboratories, Chugai Pharmaceutical Co., Ltd.*[*1])

Hyalobiuronic acid (2-amino-2-deoxy-3-O-β-D-glucopyranuronosyl-D-glucopyranose) and chondrosine (2-amino-2-deoxy-3-O-β-D-glucopyranuronosyl-D-galactopyranose) is the repeating unit of hyaluronate and of chondroitin sulfate respectively, whose amino groups are acetylated. N-Acetylated hyalobiuronic acid and chondrosine are required for studying their biochemical properties.

N-Acetylhyalobiuronic acid can be prepared by enzymatic hydrolysis of hyaluronate,[1] however N-acetylation of hyalobiuronic acid is preferable in order to obtain pure material. N-Acetylation of hyalobiuronic acid using ketene has been described by Weissmann, *et al.*[2] and the product was obtained as amorphous state.

Several reports on selective N-acetylation of amino sugars involving acetic anhydride and aqueous alkali have appeared. The method for N-acetylation by Roseman, *et al.*[3] using acetic anhydride and an anion-exchanger (carbonate form) was reported to give excellent results but attempted application of this method to the N-acetylation of hyalobiuronic acid and chondrosine resulted in unsatisfactory yield because of absorption of the products on the anion-exchanger even after eluting them with acetic acid.

The present report is concerned with the experiments in which the N-acetylation of hyalobiuronic acid and chondrosine was carried out with acetic anhydride in the mixed solution of sodium hydrogencarbonate and sodiumcarbonate and the products were isolated as crystalline barium salts for the first time in good yield.

Experimental

Hyalobiuronic Acid and Chondrosine——Hyalobiuronic acid was synthesized as described previously.[4,5] Chondrosine was obtained by synthesis or acid hydrolysis of chondroitin sulfate.

N-Acetylhyalobiuronic Acid (2-acetoamido-2-deoxy-3-O-β-D-glucopyranuronosyl-D-glucopyranose)——Hyalobiuronic acid (142 mg.) was dissolved in 2.4 ml. of a mixed solution of 1*M* $NaHCO_3$ and 0.5*M* Na_2CO_3 (1:1, pH 9.4). The solution was cooled to 0~5° with ice-water and acetic anhydride (0.08 ml.) was added under stirring. Stirring was continued for 60 minutes at 0~5° and then for 30 minutes at room temperature, after which 2 ml. (settled volume) of Dowex-50 (H form) was added to the reaction mixture. The mixture was filtered and the filtrate and washings were concentrated to a syrup *in vacuo* under 50°. The concentration procedure was repeated after addition of water in order to remove acetic acid.

The syrupy residue, which gave a positive ninhydrin test, was dissolved in water and was passed through a column containing 2 ml. of Dowex-50 (H form). The acidic effluent giving a negative ninhydrin test showed a single spot on thin-layer of silica gel G using *n*-BuOH-AcOH-H_2O (4:1:2) as solvent. The effluent and washings were passed again through a column containing 2 ml. of Amberite IRC-50 (Ba form). The neutral effluent and washings were concentrated to a small portion *in vacuo* and filtered. The filtrate and washings were concentrated to dryness *in vacuo* and the residue was crystallized from aqueous EtOH to give 192 mg. of the barium salt, m.p. 154~156° (decomp.), $[\alpha]_D^{20}$ −25°(c=1, H_2O). *Anal.* Calcd. for $C_{14}H_{22}O_{12}NBa_{1/2}$: N, 3.01. Found : N, 2.90.

*1 Takadaminami-cho, Toshima-ku, Tokyo (高梨 茂, 川口 勉, 川田 稔).

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N-Acetylchondrosine (2-acetoamido-2-deoxy-3-O-β-D-glucopyranuronosyl-D-galactopyranose)—Chondrosine (142 mg.) was treated as described above giving fine prisms of the barium salt (178 mg.), m.p. 155~157°(decomp.), $[\alpha]_D^{20}$ −10°(c=1, H_2O). *Anal.* Calcd. for $C_{14}H_{22}O_{12}NBa_{1/2}$: N, 3.01. Found : N, 3.02.

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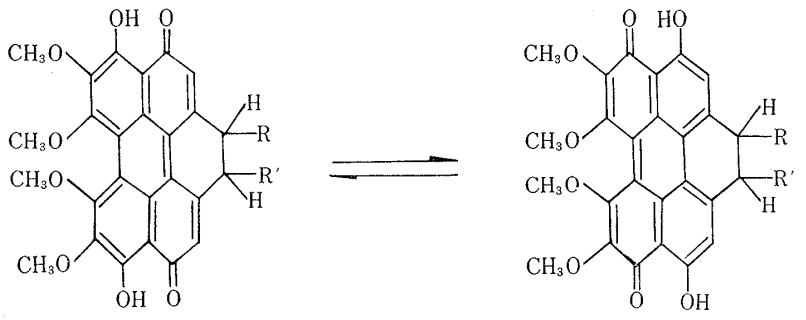
Ching-Tan Chen, Koji Nakanishi,*1 and Shinsaku Natori*2 : Biosynthesis of Elsinochrome A, the Perylenequinone from *Elsinoë* spp. I.

(*Department of Chemistry, Tohoku University,*1 *and National Institute of Hygienic Sciences*2)

The formation of red pigments by the fungus *Elsinoë* (*Myrianginales, Ascomycetes*) and its conidial stage *Sphaceloma* had been noticed for a long time. Weiss, *et al.*[1,2] isolated the pigments, designated elsinochromes, and proposed the rapidly interconverting tautomeric structures (Ia) for the major pigment, elsinochrome A, chiefly from spectral evidences.



Ia : R, R′=CH_3CO
Ib : R=CH_3CO, R′=$CH_3CH(OH)$
Ic : R, R′=$CH_3CH(OH)$

The same pigment was also studied by Hackeng, *et al.*,[3] and by collaboration with Weiss' group, they established the structures of the minor pigments, elsinochrome B and C as Ib and Ic.[4]

Formation of dimeric phenolics through the oxidative coupling of two identical units[5] is now a widely accepted concept. As regards the binaphthyls, binaphthoquinonyls, and

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*1 Katahira, Sendai (陳 鏡潭, 中西香爾).
*2 Tamagawayoga, Setagaya-ku, Tokyo (名取信策).

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