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Note

A facile procedure for the isolation of *N*-acetylneuramic acid from edible bird's-nest

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Sialic acids are commonly found as the nonreducing sugar residue in a variety of glycoproteins, mucins, and gangliosides which appear to play an important role in the plasma membrane of eucaryotic cells. *N*-Acetylneuraminic acid (**1**) is the best characterized sialic acid, and it can be prepared either by two tedious synthetic routes[1,2] or by isolation[3–5] from natural products available to laboratories with access to medical or veterinary sources. More recently, Schauer and Buscher[6] reported the isolation, for radiolabeling purposes of **1** from "edible bird's-nest". This procedure involved methanolysis, followed by ion-exchange chromatography which provided the methyl β-glycoside; **1** was then obtained by *N*-reacetylation followed by hydrolysis of the glycosidic linkage. When a hot-water extract of "edible bird's-nest" was studied as an inducer for the synthesis of neuraminidase from an *Arthrobacter* isolate[7], it was observed that the extract from this commercial source contains large quantities of free sialic acids. We report, on this basis, a facile isolation procedure which reproducibly yields gram quantities of **1**. The steps in the isolation procedure include a hot-water extraction, followed by two ultrafiltrations, and finally ion-exchange chromatography.

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler hot-stage, and correspond to corrected values. Optical rotations were measured with a Beckman 145 polarimeter. ^{13}C-N.m.r. spectra were recorded with a Varian XL-100-15 spectrophotometer operating at 25.16 MHz in the pulsed, Fourier-transform mode with complete proton-decoupling. Thin-layer chromatography (t.l.c.) was performed on cellulose plates (Eastman Kodak Co., Rochester, NY 14650, U. S. A.) with butanol–*n*-propanol–0.1M HCl (1:2:1, v/v) development; spots were located by spraying the plates with the Ehrlich reagent[8] followed by heating at ~110°. Alternatively, t.l.c. was performed with Silica Gel F 254 (Woelm, ICN Pharmaceuticals, 3340 Eschwege,

West Germany) developed with *n*-propanol–water (7:3, v/v); spots were located by spraying the plates with 10% H_2SO_4 and heating at ~110°.

Analysis of sugars. — The direct Ehrlich reaction was performed as described by Gottschalk[9]. Sialic acids were quantitatively determined with the thiobarbituric assay as described by Warren[10].

Isolation of N-*acetylneuraminic acid.* — Pulverized "edible bird's-nest" (300 g) was added to boiling water (6 l), and the solution was boiled under reflux for 5 h. The solids were allowed to settle, and the supernatant (~5 l) was obtained by suction filtration. The hot-water bird's-nest extract was concentrated to ~750 ml by partial freeze-drying. The solution (12.6 g of sialic acids) was subjected to a two-step ultrafiltration (N_2), first through a Diaflo XM-50 filter (Amicon Corporation, Lexington MA 02173) followed by two washings with water and then through a Diaflo UM-10 filter followed by repeated water washings until free of sialic acids. Purification of **1** was achieved by ion-exchange chromatography of the combined filtrate and washings on a column of Dowex 1-X4, as shown in Fig. 1. The eluate from the column with formic acid was rapidly screened for sialic acids by the direct Ehrlich[8] assay, which

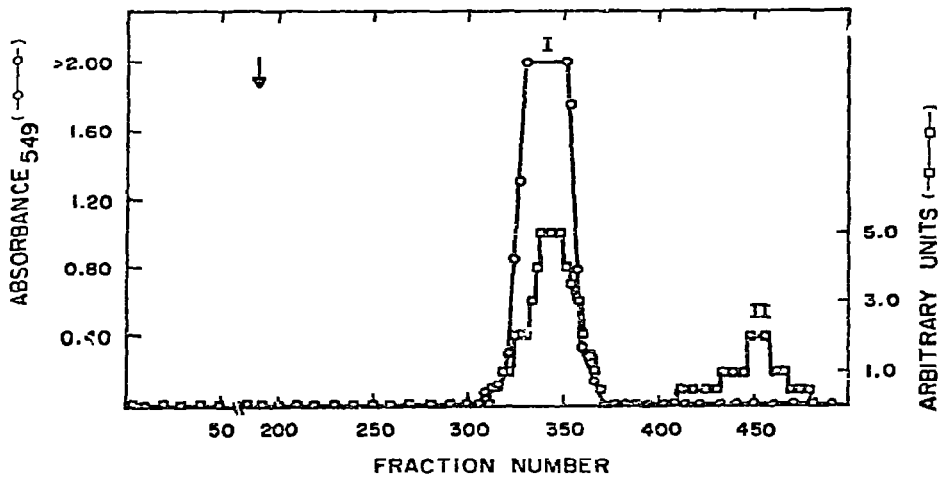

Fig. 1. Purification of **1** by ion-exchange chromatography. A column (4.5 × 70 cm) of Dowex 1-X4 (HCO_3^-) anion-exchange resin was prepared essentially as described by the distributor (BioRad, Richmond, CA 94804, U. S. A.). The UM-10 ultrafiltrate was added, followed by washing with water (~2 l). At the indicated point (arrow), a linear formic acid gradient (4 l, 0–2M) was applied. The flow rate was 120 ml/h, and 20-ml fractions were collected. From every third fraction, a sample (5 µl) was examined by Ehrlich[8] (—□—) and Warren[10] (—○—) tests. For the former assay, the arbitrary units refer to relative color intensities.

revealed two peaks. Only Peak I consisted of material giving a positive Warren reaction[10]. The latter fractions were combined, and the formic acid was removed by flash evaporation at a bath temperature <40°. The resulting residue was dissolved in water, and crude **1** was obtained by lyophilization. When necessary, final purification was accomplished by a second passage of the redissolved material through the

same column under identical conditions. The pooled, lyophilized material was obtained in a final yield of 6.5 g. It proved to be homogeneous by t.l.c. on cellulose and Silica Gel F, and migrated at the same speed as a standard of **1** (synthetic, Sigma Chemical Co., St. Louis, MI 63178); $[\alpha]_D^{20}$ −31.7 (*c* 2.0, water); lit.[5]: $[\alpha]_D^{20}$ −32°. The lyophilized material was recrystallized from water–glacial acetic acid (1:10), m.p. 187–189° (dec.); lit.[5]: m.p. 187° (dec). The methyl ester of **1**, homogeneous by t.l.c., was prepared as described by Kuhn *et al.*[11], m.p. 182–184°, $[\alpha]_D^{20}$ −28.3° (*c*, 1.8, water); lit.[11]: m.p. 179–180°, $[\alpha]_D^{20}$ −27°; ^{13}C-n.m.r. spectrum identical to that reported by Bhattacharjee *et al.*[12].

In a separate experiment, the amount of **1** present in the hot-water extract, that remained glycosically bound was determined: Hydrolysis with 25mM H_2SO_4 for 1 h at 80° released only 0.46 μmol of additional **1** per ml. In addition, the high-molecular-weight material obtained from the XM-50 concentrate, which should have been similar to the Collocalia mucoid prepared by the method of Howe *et al.*[13], was treated with neuraminidase in order to determine whether the material still had any bound sialic acid. No free **1** was detected under a variety of assay conditions. Therefore, it can be concluded that, in the hot-water extract, more than 95% of **1** is present as the free sugar. The chemical nature of Peak II from the ion-exchange chromatography remains to be established; however, no Warren positive material was detected before or after acid hydrolysis (see conditions just described), and the R_F (on t.l.c.) of this material was less than that of **1**.

This simplified isolation procedure at a minimal cost and with few manipulations provides substantial quantities of **1** which is useful for further synthetic purposes.

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