Isolation of 2-Hydroxyethanesulfonic Acid (Isethionic Acid) from Dog Heart*

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(Received for publication, October 23, 1961)

Koechlin (1) reported that the axoplasm of the giant nerve fiber of the squid contains a high concentration of taurine (2-aminoethanesulfonic acid). He also showed that the deaminated analogue of taurine, isethionic acid (2-hydroxyethanesulfonic acid), is present as the major anion and suggested that isethionic acid influences the irritability of the axon by associating with cations and dominating the ion distribution across the membrane.

It is a consistent observation that heart tissue contains a high concentration of taurine (2). As part of a study to define the function of taurine in the heart, we wished to explore the possibility that taurine, by serving as the precursor of isethionic acid, has a role in regulating the irritability of cardiac tissue.

The present report describes the isolation and identification of isethionic acid as a component of heart tissue.

EXPERIMENTAL PROCEDURE

According to Awapara (3), taurine is eluted with water from a column of Dowex 50W-X8 (4 x 1 cm), 200 to 400 mesh, acid form, in the first 5 ml. In our hands, taurine was eluted from a Dowex column in tubes 2 through 8 when collecting 1 ml fractions. This slight difference is due to technique, for we included the washout volume as tubes 1 and 2. To determine whether isethionic acid could be eluted from a Dowex column as a specific fraction separate from taurine, a 1 ml (25 μmoles) sample of sodium isethionate was placed on a Dowex 50 column (4 x 1 cm) and eluted with water. One milliliter fractions of the eluate were collected and analyzed separately for isethionic acid. The analysis consisted of converting the sulfonate to sulfate, which was precipitated as barium sulfate (4). The results of 10 separate runs showed that isethionic acid was eluted from the column in the 9 through 18 ml fraction with the peak recovery occurring in the 11 ml fraction. The total recovery was 99.7%.

Having determined the fraction in which isethionic acid was eluted, we used this procedure to isolate the compound from dog heart. An extract of ventricular tissue from adult male mongrel dogs was prepared according to Awapara (5). Isethionic acid was separated from the extract by chromatography on Dowex columns. The first 8 ml from each column, the taurine fraction, were discarded. The next 12 ml, the isethionic acid fraction, were collected from each column and combined. The combined eluate was evaporated to 10 ml, and the pH was adjusted to 7.0 with NaOH. The sodium isethionate was precipitated by adding absolute ethanol until a distinct precipitate formed. This mixture was gently warmed until solution was complete and allowed to cool slowly, during which time crystals began to form. After the solution reached room temperature, it was refrigerated at 5°C for 24 hours. The crystalline precipitate was harvested on Whatman No. 42 filter paper. Further addition of absolute ethanol to the filtrate, warming, and cooling yielded a second crop of crystals. After they were dried in a vacuum desiccator for 48 hours, the yield was 12.9 mg/100 g of tissue. We have also used this procedure for isolation of the compound from rat heart from which a greater yield was obtained, amounting to 42.6 mg/100 g of ventricular tissue.

RESULTS

The titration curve of the material crystallized from heart extract indicated a monovalent acidic function with a pK at 2.3. Samples of the compound were oxidized with nitric acid and assayed for liberation of free sulfate. From 14.819 mg of the crystallized material, 23.33 mg were recovered as the barium salt, indicating a sulfur content of 21.62%, theory 21.64%. Hydrolysis of the compound with N HCl or NaOH for 1 hour at 100°C failed to liberate any detectable amount of free sulfate indicating the presence of a sulfonic acid rather than a sulfate ester.

Further identification of the crystalline material obtained from heart tissue as the sodium salt of isethionic acid was accomplished by paper chromatography, x-ray diffraction, and infrared absorption.

Paper Chromatography—Ascending paper chromatography was run for 17 hours on Whatman No. 1 filter paper with n-butanol-acetic acid-water (45:25:30) as solvent. The strips were developed with spraying with bromphenol blue, 0.05% in ethanol at pH 2.8. The paper chromatogram of the material crystallized from heart extract showed an Rf value of 0.42, similar to that of a known sample of isethionate (recrystallized from a product of the K & K Laboratories, Jamaica, New York). The chromatogram showed only one spot. This gave us confidence that heart tissue homogenate could be fractionated by the described procedure, and that the eluate from the Dowex column in the 9 through 20 ml fraction contained only one component, tentatively identified as isethionic acid.

X-ray Diffraction—The crystallized material obtained from the heart extract was compared with a known sample of sodium isethionate. X-ray powder diffraction patterns were run with the General Electric XRD-1 equipment on Kodak type KK...
Fig. 1. X-ray powder diagrams of the crystallized material from heart and a sample of known sodium isethionate. The patterns were run for 2 hours at a peak voltage of 28 kv with a tube current of 15 ma.

Infrared Absorption Spectra—The crystallized material obtained from heart was subjected to infrared absorption analysis. The spectrum of the crystallized material from heart was in complete agreement with the spectrum of a known sample of sodium isethionate (Fig. 2). Four major absorption peaks occur at wave numbers 752, 1065, 1200, and 3430 cm⁻¹. With the exception of the absorption band at 752 cm⁻¹, none of the bands were sharp. According to Colthup (6), the two bands at 1065 and 1200 cm⁻¹ indicate the sulfonate group. The band at 3430 cm⁻¹ indicates a primary hydroxyl group and smaller peaks at 1400 to 1475 and 850 cm⁻¹ suggest the ethyl structure.

Further minor bands common to both preparations occur at 805, 955, 1010, 1325, and 1637 cm⁻¹. We feel that the data offered constitute strong support for the identification of the material from heart muscle as isethionic acid crystallized as the sodium salt.

Fig. 2. Infrared spectra in KBr of: A, sample of known sodium isethionate; B, crystallized material from dog heart extract.

Fig. 3. Wavelengths of absorbed light in microns.

Discussion

The high concentration of taurine in heart muscle suggested that it might serve as the precursor of its deaminated product, isethionic acid. The present report describes the isolation and identification of isethionic acid from heart tissue. Further work in progress is concerned with the metabolic relation of taurine to isethionic acid and to the irritability of ventricular tissue.

Summary

The compound, 2-hydroxyethanesulfonic acid (isethionic acid) was isolated from ventricular tissue of dog heart.

References


1 The infrared spectra were run by the Anderson Physical Laboratory, Champaign, Illinois.

Industrial x-ray film in a powder camera 14.32 cm in diameter. Radiation from a General Electric CA-6 copper anode was filtered through nickel foil to yield relatively pure Ka radiation.

Fig. 1 shows the x-ray powder diagram of the crystallized material from heart compared with a sample of known sodium isethionate. The two yield similar x-ray diffraction patterns. The main diffractions occur at spacings (in order of decreasing intensity) of 8.96, 4.45, 4.29, 2.99, and 3.55 Å in both specimens, although it is not easy to distinguish differences in the intensity of the three lines at spacings of 4.94, 4.29, and 3.55 Å in the crystallized material from heart. The pattern of sodium taurinate was run for comparison and was quite different, with main diffraction lines (in order of decreasing intensity) at spacings of 3.87, 9.47, 5.68, and 2.99 Å.
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