Preparation of [2-^{14}C]- and [^{35}S]-5-(2-Chloroethyl)-4-methylthiazole

ULF HENRIK LINDBERG

Development Laboratories, AB Astra, Södertälje, Sweden

In order to study the metabolic fate of the sedative, hypnotic and anticonvulsant drug di-[5-(2-chloroethyl)-4-methylthiazolium] ethane-1,2-disulfonate (clomethiazole * ethanedisulfonate, Hemineurine ®),[1,2] this compound was labelled in the thiazole ring with ^{14}C and ^{35}S. The syntheses of labelled 5-(2-hydroxyethyl)-4-methylthiazoles followed essentially the method described by Williams and Ronzio [3] in their preparation of ^{14}C-thiamine, starting with ^{14}C- or ^{35}S-thiourea.

[2-^{14}C]-Clomethiazole ethanedisulfonate. A mixture of ^{14}C-thiourea (339 μC/mg; 191.6 mg; 2.52 mmole) and 3-chloro-5-hydroxy-2-pentanone (445.2 mg; 3.26 mmole) was refluxed in 3 ml of water for 2.5 h. The solution was then washed with ether (2 × 2.5 ml) and evaporated to dryness *in vacuo*. The residual crude product was washed with cold acetone (5 × 2 ml) yielding 2-amino-5-(2-hydroxyethyl)-4-methyl-[2-^{14}C]-thiazole hydrochloride, (432.0 mg; 88.5 %) m.p. 154–155°C, identified by mixed melting point determination and infrared spectroscopy.

To the aminothiazole hydrochloride (432.0 mg; 2.22 mmole) in 11.0 ml of concentrated hydrochloric acid was added at −10°C 3.4 ml of 1 N sodium nitrite solution during 15 min under stirring. The mixture was stirred for a further half hour, the precipitated diazonium salt was redissolved by the addition of 11 ml of water, and then reduced at −10°C by the dropwise addition of 5.0 ml (24.2 mmole) of 32 % hypophosphorous acid during 15 min. Evolution of nitrogen began immediately and was completed overnight at about −5°C. After addition of 15.6 ml of 10 N sodium hydroxide the alkaline reaction mixture was saturated with sodium chloride and extracted with ether (3 × 65 ml + 5 × 25 ml). The ether was dried over sodium sulfate and evaporated *in vacuo*, affording a light brown oil (300.0 mg; 94.4 %) which was identified by infrared spectroscopy as 5-(2-hydroxyethyl)-4-methyl-[2-^{14}C]-thiazole. The crude thiazole derivative was then transferred into the chloride according to the method of Sawa and Ishida.[4]

To a stirred solution of 5-(2-hydroxyethyl)-4-methyl-[2-^{14}C]-thiazole (300.0 mg; 2.10 mmole) in 10 ml of dry chloroform, a solution of thionyl chloride (710.0 mg; 5.96 mmole) in 10 ml of dry chloroform was added during 10 min, the temperature being kept at about 20°C by external cooling. A brown oil, possibly the chlorosulfurous ester of the thiazole alcohol, separated from the solution during addition of the thionyl chloride.

After a further 10 min at room temperature, the reaction mixture was heated under reflux (bath temperature about 75°C) for 2 h, whereupon the oil dissolved and sulfur dioxide evolved. After distilling off the chloroform, excess thionyl chloride was decomposed with absolute ethanol (2 × 30 ml). Evaporation *in vacuo* afforded a brown oil which crystallized shortly after. The product was washed with cold acetone (2 × 2 ml) yielding 232.5 mg (56.9 %) of 5-(2-chloroethyl)-4-methyl-[2-^{14}C]-thiazole hydrochloride, m.p. 131–134°C, identified by mixed melting point and infrared spectrum.

The hydrochloride was dissolved in 8 ml of distilled water and the solution made alkaline with 125 mg of sodium hydrogen carbonate. The liberated base was then extracted with ether (4 × 10 ml), the ethereal solution dried over sodium sulfate and evaporated *in vacuo*. To the residual brown oil (156.6 mg; 0.970 mmole) was added dropwise a solution of ethane-1,2-disulfonic acid (92.2 mg; 0.485 mmole) in absolute alcohol (0.54 ml) under stirring and cooling. The crude salt that crystallized within 5 min was washed with cold acetone (5 × 2 ml) and recrystallized once from 1.0 ml of a mixture of absolute ethanol and absolute ether (1:1) and twice from 1.0 ml of absolute ethanol and acetone (1:1). The white crystalline product (154.1 mg; 50.4 % yield) melted at 127–128°C and was identified by mixed melting point and infrared spectrum as di-[5-(2-chloroethyl)-4-methyl-[2-^{14}C]-thiazolium] ethane-1,2-disulfonate.[2] The overall yield from ^{14}C-thiourea was 23.9 %.

The specific activity of the end-product was determined in a liquid scintillation spectrometer; the mean value of three different measurements was 100 μC/mg (51.3 mc/mmole), which gives a radio-

* pINN

active yield of 23.7 %, in agreement with the chemical yield. A radiochromatogram of the product [*tert.*-butanol-glacial acetic acid-water (16:1:3)] gave a single peak, demonstrating the substance to be radiochemically uniform.

[^{35}S]-*Clomethiazole ethanedisulfonate.* The synthesis of the ^{35}S-labelled thiazole derivative followed the same procedure as outlined above for the [2-^{14}C]-clomethiazole ethanedisulfonate. Starting with ^{35}S-thiourea (72.4 μC/mg; 79.6 mg; 1.05 mmole), 60.0 mg of di-[5-(2-chloroethyl)-4-methyl-^{35}S-thiazolium] ethane-1,2-disulfonate (21.5 μC/mg; 11.0 mC/mmole) was obtained. The overall yield was 22.4 % and the total radioactive yield was 22.4 %. The product was identified by melting point (127–128°C), mixed melting point and infrared spectrum. The purity and radiochemical uniformity of the product was demonstrated by a single peak on a radiochromatogram [*tert.*-butanol-glacial acetic acid-water (16:1:3)].

My thanks are due to Mr. A. Telč for the radiochemical measurements. I am indebted to Dr. P. Moses for linguistic criticism of the manuscript.

1. Allgén, L.-G., Lindberg, U. H. and Ullberg, S. *Nord. Psykiatr. Tidskr.* **17** (1963) 13.
2. Charonnat, S., Charonnat, A.-M. J., Lechat, P., Chareton, J. and Boime, A. *French Patent* 1.273.865 (1961).
3. Williams, D. L. and Ronzio, A. R. *J. Am. Chem. Soc.* **74** (1952) 2409.
4. Sawa, Y. and Ishida, T. *J. Pharm. Soc. Japan* **76** (1956) 337.

Received March 23, 1966.

Differential Spectrophotometry on Humic Acids

INGVAR LINDQVIST and BERTIL BERGMAN

Department of Agricultural Chemistry I, College of Agriculture, Uppsala 7, Sweden

It is well known that all humic acids are characterized by a steadily increasing light absorption from the visible to the ultraviolet range.[1,2] The lack of details in the spectra has seriously limited the possibilities to use light absorption studies for the characterization of *different* humic acid fractions. The most promising suggestion has been to determine the varying gradient of the increasing absorption in different spectral ranges,[3-5] which probably gives some idea about the degree of humification.

We have now applied differential spectrophotometry to the study of humic acids, and the results seem to indicate an improved characterization of different fractions. The acidity has been varied and the difference spectra between neutral and acid solutions and between basic and neutral solutions will be presented. This corresponds partly to the $\Delta\varepsilon$-procedure used by Aulin-Erdtman [6,7] in her studies of lignin and model compounds of lignin. In this short communication some results obtained with brown humic acids from different soils will be presented, only in order to show the possibilities of the method. We are continuing with more detailed studies of the titration curves.

Experimental. Brown humic acids have been prepared by Springer's method.[3] All solutions have been aqueous and pH has been approximately controlled to 2, 7, and 11 (with HCl and NaOH). Time-dependent changes take place slowly but all measurements have been made immediately after the preparation of the solutions with different pH. (A detailed study of the time-dependent changes would probably also be of interest for the characterization.) All measurements have been made on a Zeiss PMQ II Spectrophotometer. Stray light and light scattering have been shown to be negligible. Below 250 nm the light absorption of the inorganic ions influence the ΔA-spectra.

In the figure the difference spectra A(pH = 7)−A(pH = 2) are drawn as full lines and the difference spectra A(pH = 11)−A(pH = 7) are drawn as broken lines. Only some examples are given, all other soils studied give intermediate values. For comparison, a lake humic acid and an oxidation product of catechol have also been included.

The basic solutions have all an absorbancy of 2.0 at 250 nm.

Discussion of results. Many features differ in these ΔA-spectra and more penetrating studies might reveal interesting details. The curves are, however, rather similar in two respects. The titrations from pH = 2 to pH = 7 give rise to maxima near 270−280 nm and the further titrations from pH = 7 to pH = 11 to other maxima (or inflexion points) near 340−360 nm.