Journal of Labelled Compounds — October-December — Vol. IV, No. 4

The preparation of uniformly labeled cyclohexylamine and cyclamate

A. ALTER and J. C. FORMAN

Departments of Pharmacology and Biological Development Abbott Laboratories, North Chicago, Illinois, U. S. A. 60064

Received on 3rd June 1968

SUMMARY

Aniline uniformly labeled with ^{14}C was reduced, in the presence of Rhodium on Alumina catalyst, to cyclohexylamine. The cyclohexylamine was converted to uniformly labeled sodium cyclamate. The overall radiochemical yield was 34 %.

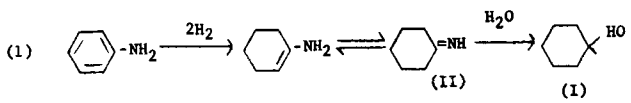
INTRODUCTION

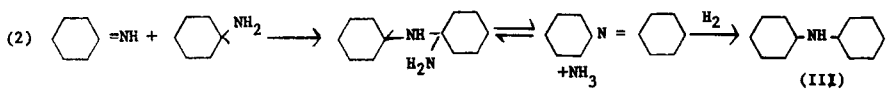
The use of cyclamates as artificial sweetening agents has increased greatly in the last two decades. With the increased use of cyclamates the question of the metabolic fate of these substances became an important one. In 1951 Taylor *et al.* [1] and in 1953 Schoenberger *et al.* [2], using ^{35}S labeled cyclamate showed that the excretion was essentially complete. The question still remained, however, as to whether or not the cyclohexyl portion of the structure underwent a metabolic change. It was for this reason that cyclamate uniformly labeled with ^{14}C in the ring was prepared in these laboratories.

Cyclamate may be prepared by the reaction of aniline and sulfamic acid and subsequent reduction of the phenyl sulfamic acid so formed [3] or by the reaction of cyclohexylamine and sulfamic acid. It can also be prepared by the action of chlorosulfonic acid on cyclohexylamine [4] or by the action of SO_3 on cyclohexylamine. For the preparation of ^{14}C labeled material, the latter procedure was used, modified for reaction on a small scale and under conditions which did not require an excess of cyclohexylamine.

Cyclohexylamine, uniformly labeled with ^{14}C, was prepared by the catalytic hydrogenation of uniformly labeled aniline using Rhodium on Alumina as a catalyst. In the reduction of aniline the major side products which might be expected are cyclohexanol and dicyclohexylamine. Under various conditions both of these compounds were found.

When aniline hydrochloride was hydrogenated in 95 % ethanol solution much dicyclohexylamine was found to be present, along with some cyclohexanol. On the other hand, reduction of the sulfate salt in water produced little or no dicyclohexylamine but much cyclohexanol. The presence of dicyclohexylamine could easily be demonstrated by GLC and by TLC. Cyclohexanol could not easily be demonstrated by TLC because of the rapid evaporation from the plate, despite its high boiling point. It could, however, be easily detected by GLC. No unchanged aniline could be detected in the reduction product of either the hydrochloride salt or the sulfate salt. The conditions under which the two salts are reduced to form cyclohexylamine, cyclohexanol, and dicyclohexylamine were not thoroughly investigated. Cyclohexanol (I) is probably formed by the hydrolysis of the intermediate imine (II) (Eq. 1) while dicyclohexylamine (III) is probably formed by the reaction of the imine with cyclohexylamine (Eq. 2). In water it might be expected that





more cyclohexanol would be formed than in ethanol. When aniline hydrochloride was hydrogenated in water instead of ethanol, much cyclohexanol was indeed found, but also at least 10 % dicyclohexylamine. Aniline sulfate is only slightly soluble in ethanol. Indications are that if aniline sulfate was reduced in ethanol solution no dicyclohexylamine would be formed, and possibly less cyclohexanol. In order to keep the volume down to reasonable size, however, reduction was carried out in water as the solvent. In a few cases two or three per cent dicyclohexylamine was observed, but in other "cold" runs, no dicyclohexylamine was found. Apparently the conditions of pH, amount of catalyst, possibly temperature and pressure, are critical. In the present work, aniline sulfate was reduced rather than aniline hydrochloride because the by product, cyclohexanol, is easier to separate than is dicyclohexylamine, the by product of the hydrochloride reduction.

Aniline has been reduced to cyclohexylamine by the use of various catalysts and conditions [5, 6, 7]. In each case some dicyclohexylamine plus various other side products are obtained. The conditions used in the present work appear to be well adapted to reaction on a 10 mM scale.

Experimental

GLC was done on an 8′ 10 % carbowax 20 M-3 % KOH column on a Barber-Colman series 5 000 instrument equipped with a combustion furnace and a proportional counter for detection of $^{14}CO_2$.

TLC was done on silica gel plates.

Preparation of cyclohexylamine U-^{14}C.

Aniline hydrochloride U-^{14}C [8], 1 620 mg = 12.5 mM, 49.2 mC^{14}C activity, was dissolved in water, 5 ml 10 N NaOH added and the free aniline steam distilled into 4.33 ml of 3 N H_2SO_4. Water was added to 100 ml volume and the solution was hydrogenated in a Parr shaker bottle with 100 mg 5 % Rh on Al_2O_3 at 40 to 35 psig. The reduction was essentially complete in 5.5 hours. The reduction mixture was filtered, washed with water and the cyclohexanol removed by continuous extraction with ether. There was obtained 15.6 mC (32.5 %) cyclohexanol in the extract.

TLC of the cyclohexylamine sulfate solution in the following solvent systems : (A) EtOH-1N NH_4OH(95-5) and (B) *n*-BuOH-HAc-H_2O (50-25-25) indicated only cyclohexylamine by spray (iodine in chloroform) and by radioactive scanning of the TLC plate on a Vanguard plate scanner. Solvent system (A) affords a wide separation between cyclohexylamine and dicyclohexylamine (Rf's 0.18 and 0.45 respectively). Sequential ½ cm portions of the silica gel from this plate were scraped into counting vials and counted for ^{14}C activity. Activity in the dicyclohexylamine region amounted to 0.1 % of the cyclohexylamine activity.

The cyclohexylamine sulfate solution was made basic by the addition of NaOH and extracted with ether. The ether extract was first dried over Na_2SO_4, then the last trace of water was removed by addition of CaH_2. GLC indicated only cyclohexylamine. The ether extract was reduced in volume to about 5 ml by distilling off ether through a long column. 164 μC cyclohexylamine was carried over with the distillate.

Preparation of Triethylamine-Sulfur Trioxide Complex.

0.865 ml (13 mM) redistilled chlorosulfonic acid was dissolved in 5 ml dry dichloroethylene. This was added drop-wise to an ice-cooled, stirred solution of triethylamine in 25 ml chloroform over a 15-20 minute period, then stirred 15 minutes longer in an ice bath.

Five ml dry chloroform was added to the ether solution of the U-^{14}C cyclohexylamine prepared above and this solution was added over a 15 minute period to the ice-cold triethylamine-SO_3 complex solution with stirring. The reaction mixture was allowed to slowly warm to room temperature and stirred another 8 hours.

The clear solution was evaporated almost to dryness under reduced pressure. The distillate contained no ^{14}C activity. The residue was dissolved in water, 1.5 ml 10 N NaOH added and the solution was extracted continuously with ether for 8 hours. The ether extract contained only a little ^{14}C activity. The aqueous phase was evaporated almost to dryness under reduced pressure, dissolved in hot 80 % acetone and filtered from an insoluble solid. On cooling, crystals deposited which were filtered and dried in a vacuum desiccator. 445.2 mg was obtained. Two more fractions were obtained by successively evaporating the filtrates and recrystallizing from hot 80 % acetone. The second and third fractions weighed 170.5 and 313.1 mg.

ASSAY AND PURITY DETERMINATIONS.

From the specific activity of the starting aniline hydrochloride, the expected specific activity was 4.0 mC/mM or 19.9 μC/mg. The three fractions were assayed by liquid scintillation counting against a benzoic acid standard and found to be :

Fraction I	19.7 μC/mg (8.78 mC)
Fraction II	18.25 μC/mg (3.15 mC)
Fraction III	15.8 μC/mg (4.95 mC)

TLC of each of the three fractions in the following solvent systems showed only one spot corresponding to authentic cyclamate, and only one radioactive peak on scanning of the TLC plate. These solvent systems are : (A) anhyd EtOH-100 %, (B) 95 % EtOH-100 %, (C) 95 % isoPrOH-100 % and (D) CH_3CN-NH_4OH-H_2O-60-3-10.

Visualization of the cyclamate spot was best accomplished by spraying plate first with 1 : 20 Chlorox solution, allowing the plate to stand 15 minutes, then spraying with EtOH to remove excess hypochlorite, drying and finally spraying with fresh starch-iodide solution. A purple color develops [9].

It appears, therefore, that Fraction I is radiochemically and chemically pure. Fractions II and III appear to be contaminated with a non-radioactive impurity, possibly sodium sulfate. At this time no special effort has been made to identify this impurity or to further purify these two fractions.

REFERENCES

1. TAYLOR, J. D., RICHARDS, R. K. and DAVIN, J. C. — *Proc. Soc. Exp. Biol. Med.*, **78** : 530-33 (1951).
2. SCHOENBERGER, J. A., RIX, D. M., SAKAMOTO, A., TAYLOR, J. D. and KARK, R. M. — *Am. J. Med. Sci.*, **225** : 551 (1953).
3. FREIFELDER, M., MELTSNER, B., ILLICH, G. M. and ROBINSON, R. M. — *Brit. Pat.*, **882** : 952 (Nov. 22, 1961).

4. AUDREITH, L. F. and SVEDA, M. — *J. Org. Chem.*, **9** : 89 (1944).
5. SKITA, A. and BERENDT, W. — *Chem. Ber.*, **52B** : 1519 (1919).
6. RYLANDER, P. N. and STEELE, D. R. — *Engelhard Ind. Tech. Bull.*, **3** : 19 (1962).
7. ROBINSON, R. M. — U. S. Patent 3,196,179 (July 20, 1965).
8. New England Nuclear Corporation, Boston, Massachusetts.
9. RYDON, H. N. and SMITH, P. W. G. — *Nature*, **169** : 922 (1952).