SYNTHESIS OF 2,4-DIAMINO-6-PIPERIDINYLPYRIMIDINE-3-OXIDE-3',4',5'-^{3}H(N) TRITIATED MINOXIDIL

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SUMMARY

Minoxidil-3',4',5'-^{3}H(N), 25.6 Ci/mM, has been synthesized by the reaction of piperidine-3,4,5-^{3}H(N) with 2,4-diamino-6-chloropyrimidine-3-oxide. Purification was by paper chromatography. Minoxidil prepared in this way was shown to be suitable for radioimmunoassay and was stable for six months stored in MeOH at 4° C.

Key Words: 2,4-diamino-6-piperidinylpyrimidine-3-oxide, minoxidil, tritium, piperidine, 2,4-diamino-6-chloropyrimidine-3-oxide, radioimmunoassay

INTRODUCTION

The compound 2,4-diamino-6-piperidinylpyrimidine-3-oxide, minoxidil, is a potent hypotensive agent (1, 2) and has been therapeutically used in daily doses as low as 2.5 mg. A radioimmunoassay was required for bioavailability and pharmacokinetic studies. A high specific activity (10 Ci/mM) tritium labeled form of the drug was necessary for the development of such an assay.

The current methods for using piperidine-3,4,5-^{3}H(N) in the synthesis of minoxidil have several drawbacks. In one method (3), the piperidine was introduced in the first step of a four-step synthesis; in the other method (4), a threefold excess of piperidine in an organic solvent was required. We examined methods that allowed the use of one equivalent of piperidine·HCl in aqueous media.

RESULTS

Maggiolo and Phillips (5) have reported that piperidine reacts with 2-amino-4-chloro-6-methylpyrimidine in aqueous sodium acetate buffer. In our case,

this buffer was found to be insufficiently basic to give adequate liberation of piperidine from piperidine·HCl. After investigating several bases, NH_4OH was chosen because it was a strong enough base and easily removed. However, the reaction still did not go to completion with one equivalent of piperidine in the usual open reaction apparatus. A sealed tube at 80-90° C was therefore used apparently increasing the amount of dissolved piperidine by increasing the pressure and resulting in yields of 80-82%.

Purification systems involving chromatography on silica gel were found to be unsatisfactory because the product was contaminated with silica gel which interferes with the radioimmunoassay. A system of descending chromatography on Whatman No. 2 paper was found to be satisfactory.

The product was characterized by its coincidence with minoxidil in three TLC systems and was further characterized and quantitated by ultraviolet analysis (u.v.). Material from similar cold runs had mass spectra identical to an authentic sample.

The ^3H-minoxidil was tested for ability to bind and be displaced from rabbit-antiminoxidil-BSA antiserum and found to be satisfactory.

The ^3H-minoxidil was stored in vials containing 2 mCi in 2 ml of MeOH at 4° C. Analysis by TLC in two solvent systems showed less than 2% decomposition had occurred in six months of storage.

EXPERIMENTAL

Materials. Piperidine-3,4,5-^3H(N)·HCl was prepared by New England Nuclear by a custom reduction of 1,2,3,6 tetrahydropyridine (8.3 mg, 0.1 mM). The material was received in an HCl solution containing black particles assumed to be catalyst. The material was used as received.

2,4-diamino-6-chloropyrimidine-3-oxide was prepared from 4-chloro-2,6 diamino-pyrimidine (Aldrich) (4).

TLC Systems. The following solvent systems were used with silica gel plates to evaluate the purity of minoxidil:

benzene/MeOH/NH_4OH, 50/50/0.5	Rf = 0.48
piperidine/H_2O, 2/98	Rf = 0.40
ethylacetate/acetone/H_2O, 5/5/1	Rf = 0.17
	Rf = 0.56*

*paper

Minoxidil decomposes rapidly when exposed to air on silica gel. Therefore, polar material at the origin can only be avoided by quickly transferring freshly spotted plates to the solvent chambers.

Minoxidil-3',4',5'-^{3}H(N). 2,4-diamino-6-chloropyrimidine-3-oxide, 1.0 mg (6.2 μM), was dissolved in abs. EtOH and transferred to a necked down 1 x 17-cm glass tube. The alcohol was removed by vacuum and gentle heating. Unlabeled piperidine·HCl, 0.425 mg (3.5 μM) in 0.1 ml H_2O, and ^{3}H-piperidine·HCl solution, 0.5 ml (∿3.0 μM), were added to the bottom of the tube along with concentrated NH_4OH, 0.5 ml, and the solution mixed. The pH was checked with pH paper to make sure it was 10 or above. The contents of the tube were frozen in dry ice acetone, and the tube was sealed. The sealed tube was placed in 1/8-inch thick capped steel pipe and heated in an oven in a hood at 80-90° C for 48 h. The tube was removed and cooled. The contents were frozen and the tube opened. The reaction mixture was transferred with 2 x 1-ml washings and freeze dried. The residue was dissolved in 500 μl of MeOH. Half of the solution, 250 μl, was applied to a 15 x 56-cm strip of Whatman No. 2 paper. The paper was developed in a descending manner with ethylacetate/acetone/H_2O, 5/5/1. The minoxidil was located by u.v. and eluted into MeOH. The radioactivity corresponded to the location of minoxidil standards in the three TLC systems described. The u.v. spectra in methanol, λmax 230, 262 and 286 (ϵ_{286} = 12,250), was identical to that of an authentic sample. The specific activity was 25.6 Ci/mM. The yield was 15% by weight and 12% radiochemical. The low yields are attributed to the poor quality of the commercial ^{3}H-piperidine. Cold yields were always 80%, and a run containing only 0.1 ml ^{3}H-piperidine solution gave a 49% yield.

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