Tritiated Guanethidine of High Specific Activity

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Summary

     Due to the desire for biological assay techniques of greater sensitivity
and specificity than presently exist for the antihypertensive drug guanethidine,
tritium labeled guanethidine of high specific activity was prepared for use in
radioimmunoassay procedures. This drug has been labeled in other positions than
described here resulting in much lower specific activity and less than optimal
sensitivity in RIA work. The work described here involves the preparation,
deuteration and tritiation of an olefinic precursor, N-(2-aminoethyl)-Δ^4-
hexahydroazocine which was converted in one subsequent step to tritiated gua-
nethidine with a specific activity of 72 Ci/mmole (145 mCi/mg) for the sulfate
salt and 36 Ci/mmole (182 mCi/mg) for the free base.

Key Words: guanethidine, N-(2-aminoethyl)-Δ^4-hexahydroazocine, N-(2-amino-
               ethyl)octahydroazocine, catalytic reduction, deuterium, tritium.

Introduction

     The antihypertensive guanidino compound, guanethidine (Ismelin®), has

enjoyed widespread clinical application for near two decades. Although very

effective in reducing even severe hypertension, its use has been complicated by

side effects which stem from its pharmacologic action. Data regarding absorption

of the drug, its distribution or elimination which could be of use in enhancing

therapeutic uses or in minimizing adverse effects is conflicting.[1-8] In general,

a sensitive assay for guanethidine in plasma or urine has been lacking and as a

result has compromised satisfactory evaluation of the relationship of guanethi-

dine in plasma to blood pressure reduction or toxicity. The most sensitive assay

technique for guanethidine in plasma in the absence of radiolabeled drug utilizes

gas chromatography—mass spectrometry.[9] Although quantities as low as 1 ng/ml may

be detected, the complexity of the preliminary extraction procedure and limited

access of most clinical labs to the required analytical equipment argues in favor

of the development of a simpler but equally sensitive and specific technique.

Loeffler and Pittman[10] have reported a technique involving radioimmunoassay in which nanogram sensitivity and good specificity in the presence of metabolites have been achieved. Initially, tritium labeled guanethidine (62 mCi/mmole, labeled at C-2 of ethyl group) was prepared by lithium aluminum tritide reduction of a nitrile precursor[11]. Tritium labeled guanethidine (300 mCi/mmole) was also prepared by rhodium catalyzed exchange with tritium gas[a]. Both labeled drugs were used in performing the assay. While much greater sensitivity and specificity was obtained than with non-labeled techniques, still greater sensitivity was desired, especially for the conduct of pharmacokinetic studies. Therefore, labeled guanethidine of specific activity equal to or greater than 10 Ci/mmole was indicated.

It was felt that guanethidine of much higher specific activity could be obtained by catalytic reduction of an unsaturated precursor with carrier free tritium gas. A suitable precursor appeared to be N-(2-aminoethyl)-Δ^4-hexahydroazocine (9). Reduction of this compound with tritium gas should afford a theoretical specific activity of 58 Ci/mmole followed by a one step conversion to guanethidine (12) (Figure 1).

<div align="center">Discussion</div>

The synthesis of compound 9 is shown in Figure 1. Initially, it was hoped that the double bond of 9 could be reduced with deuterium and tritium gas in the presence of the homogeneous catalyst tris(triphenylphosphine)rhodium chloride [$(\phi_3P)_3RhCl$] as use of this catalyst is known to be accompanied by minimal label scrambling[12,13]. Upon attempting to carry out this reduction under 1.0 atm of deuterium gas (model for tritiation), it was found that the eight membered unsaturated ring was extremely resistant to reduction with this catalyst after a reaction time of 24 h. Therefore, the reduction was carried out using 10% Pd on charcoal and went to completion in 2 h to afford 10. Mass spectral analysis of the product indicated that some scrambling had occurred as expected (see experi-

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Figure 1
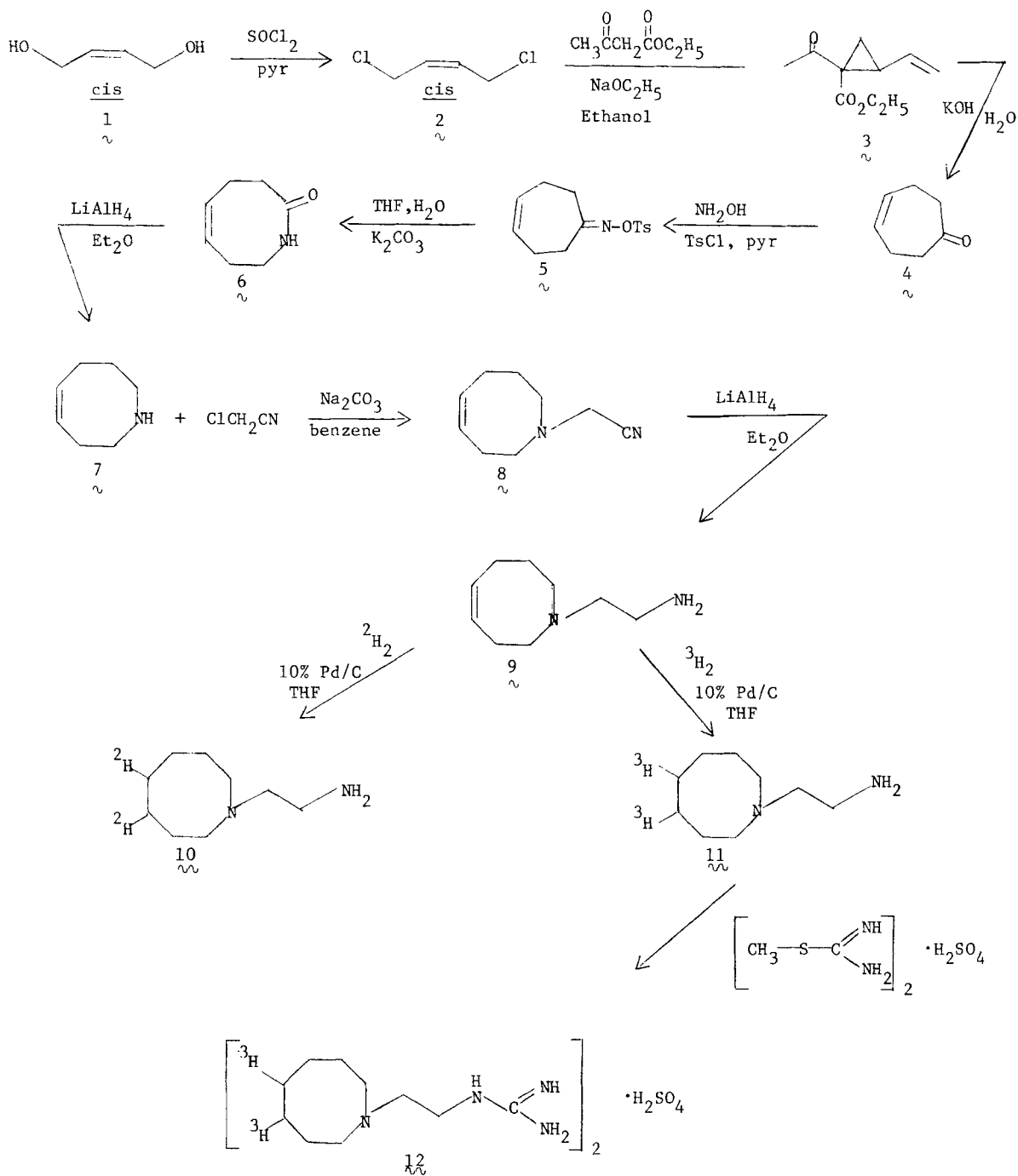
mental methods) but this presents no problem in the future radioimmunoassay procedure. Actual deuterium incorporation predicted a theoretical specific activity for 11 of ~40 Ci/mmole upon tritiation. Reduction of 9 with tritium gas under identical conditions afforded 11 with a specific activity of 36 Ci/mmole (221 mCi/mg). Conversion of 11 to 12 afforded guanethidine with specific activity of 36 Ci/mmole (182 mCi/mg) for the free base or 72 Ci/mmole (145 mCi/mg) for the sulfate salt.

Experimental Procedures

All chemicals were used as obtained from the manufacturer. Melting points were obtained on a Thomas Hoover Melting Point Apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a JOEL FX-60 60 MHz FT spectrometer using CDCl$_3$ (TMS) as solvent unless otherwise noted. Mass spectral analyses were carried out using an AEI MS-902 mass spectrometer. Gas-liquid chromatography was performed using a Shimadzu GC-8A (FID) chromatograph (2.0 m glass column, 2% OV-17 on Supelcoport, 30 ml/min). Radiopurity was determined using an Autochron LB-2722 radioscanner. Tritium was counted using a Packard Liquid Scintillation Counter Model 3255 (internal standard) with Scintiverse® (Fisher) counting solution. Silica gel plates (iodine) were used for TLC analysis. Elemental compositions of all novel compounds were determined by high resolution mass spectrometry. Authentic guanethidine sulfate (Ismelin®) was obtained from Ciba-Geigy Corp., Summit, NJ 07901.

cis-1,4-Dichloro-2-butene (2). This compound was prepared from the diol 1 by treatment with thionyl chloride according to the procedure of Amundsen[14]. The yield was 64% of a colorless liquid which boiled at 153°C/760 mm Hg (lit.[14] 152.5°C/760 mm Hg) (Figure 2).

Cyclohept-4-en-1-one (4). Bahurel, et al.[15] prepared this unsaturated cyclic ketone by treatment of 2 with ethyl acetoacetate sodium salt to afford the cyclo-propyl intermediate 3 followed by saponification, decarboxylation and rearrangement of 3 to afford the product 4. Using this procedure, we obtained the

product in 14% yield (based on cis-1,4-dichloro-2-butene) (Figure 1) as a light

yellow liquid; GC: 120°C, retention time = 1.5 min. ^{1}H-NMR (δ) 5.82 [m, 2H, HC =

CH], 2.45 [m, 8H, -CH$_2$CH=CHCH$_2$-, -CH$_2$COCH$_2$-].

Δ^4-Hexahydroazocine (7). The procedure of Wilson, et al.[16,17] was used to prepare

this unsaturated heterocycle from 4. The oxime tosylate 5 was obtained as a

crude crystalline solid in 31% yield from 4 (mp = 73-75°C, lit.[16] 83-84°C).

Beckmann rearrangement of 5 in aqueous THF to the lactam 6 was accomplished in

71% yield (mp = 80-82°C, lit.[16] 82-83°C). Lithium aluminum hydride reduction of

6 afforded the amine 7 in 45% yield (bp = 85-90°C/15 mm Hg, lit.[16] 90-100°C/20

mm Hg). ^{1}H-NMR (δ) 5.75 [m, 2H, HC=CH)], 2.81 [m, 4H, CH$_2$NHCH$_2$], 2.21 [m, 4H,

CH$_2$CH=CHCH$_2$], 1.75 [m, 2H, CH$_2$CH$_2$NH-].

N-Cyanomethyl-Δ^4-hexahydroazocine (8). The procedure of Mull, et al.[18] was used.

Chloroacetonitrile (1.3 g, 17.1 mmole), sodium carbonate (0.9 g, 8.55 mmole) and

25 ml of benzene were stirred under reflux under N$_2$. A solution of 7 (1.9 g,

17.1 mmole) in 10 ml of benzene was added dropwise followed by stirring under

reflux for 3 h. The reaction was cooled, filtered and evaporated in vacuo to

afford 2.3 g (90%) of a light yellow liquid which was approximately 90% pure by

GC (150°C, retention time = 4.0 min). ^{1}H-NMR (δ) 5.73 [m, 2H, HC = CH], 3.55 [s,

2H, CH$_2$CN], 2.70 [t, 4H, CH$_2$NCH$_2$], 2.25 [m, 4H, CH$_2$CH=CHCH$_2$], 1.52 (m, 2H,

CH$_2$CH$_2$N-]; m/e 150.1159 (C$_9$H$_{14}$N$_2$ requires 150.1157).

N-(2-Aminoethyl)-Δ^4-hexahydroazocine (9). The procedure of Mull, et al.[18] was

used. A solution of 8 (2.3 g, 15.3 mmole) in 20 ml of anhydrous ether was added

dropwise with stirring to a suspension of lithium aluminum hydride (0.82 g, 22

mmole) in 30 ml of anhydrous ether. The reaction was stirred under reflux (N$_2$)

for 2 h at which time GC indicated completion of reaction. The excess LAH was

decomposed by dropwise addition of cold water and the resulting suspension fil-

tered. The organic phase was separated from the filtrate, dried (Na$_2$SO$_4$) and

evaporated in vacuo to afford a light yellow oil. Distillation (125°C/50 mm Hg)

afforded 1.4 g (60%) of product as a colorless oil; GC: 150°C, retention time =

2.6 min. ^{1}H-NMR (δ) 5.7 [m, 2H, HC=CH], 2.68 [m, 6H, (CH$_2$)$_3$N], 2.22 [m, 4H, CH$_2$CH=CHCH$_2$], 1.58 (m, 4H, CH$_2$CH$_2$N, -NH$_2$]; m/e 154.1471 (C$_9$H$_{18}$N$_2$ requires 154.1470).

N-(2-Aminoethyl)-[4,5-[2]H$_2$]octahydroazacine (10). A solution of 9 (100 mg, 0.65 mmole) in 3 ml of THF was stirred at room temperature for 2h with 10% Pd/C (10 mg) under 1.0 atm of deuterium gas. ^{1}H-NMR of an aliquot indicated complete reduction. The reaction suspension was filtered through a small Celite column and the THF evaporated under a stream of N$_2$. Ethanol (1.0 ml) was added to the residue and evaporated under a stream of N$_2$ to afford 85 mg (84%) of a colorless oil which was pure by GC. ^{1}H-NMR and GC agreed with that of N-(2-aminoethyl)-octahydroazocine[18]; GC: 150°C, retention time = 2.8 min. ^{1}H-NMR (δ) 2.60 [m, 8H, (CH$_2$)$_4$N], 1.61 [m, 10H, (CDH)$_2$, (CH$_2$)$_3$, NH$_2$]; mass spec indicates: d$_0$=20.96%, d$_1$=37.27%, d$_2$=24.89%, d$_3$=11.44%, d$_4$=4.35%, d$_5$=1.09%.

Guanethidine Sulfate. The procedure of Mull et al.[18] was used, but the purification procedure was modified to accommodate a much smaller scale. N-(2-aminoethyl)octahydroazocine (18 mg, 0.115 mole) was refluxed with a solution of S-methylisothiourea sulfate (16 mg, 0.058 mmole) in 2 ml of water for 1.5 h. The cooled reaction solution was then chromatographed on a column of 10 g of Sephadex G-10 (H$_2$O) to afford 15.0 mg (44%) of colorless solid whose melting point and ^{1}H-NMR agreed with that of authentic guanethidine sulfate (Ismelin®); mp = 272-276°C, Ismelin=276-281°C. ^{1}H-NMR (δ) D$_2$O, 3.45 [m, 8H, (CH$_2$)$_4$N], 1.70 [m, 10H, (CH$_2$)$_5$].

Tritiated Guanethidine Sulfate (12). A solution of 9 (10.7 mg, 0.07 mmole) in 1.0 ml of THF was stirred for 3 h at room temperature with 5.0 mg of 10% Pd/C under 5.0 Ci (0.086 mmole) of carrier free tritium gas. The reaction suspension was filtered through a small Celite/Na$_2$SO$_4$ column and the filtrate evaporated in vacuo. Absolute ethanol (20 ml) was added to dissolve the residue and the solution was evaporated in vacuo to afford 2.318 Ci. The residue was dissolved in 5.0 ml of CH$_2$Cl$_2$, quantitated by GC using β-phenylethanol as internal standard,

and found to contain 10.5 mg of pure 11 (specific activity = 36 Ci/mmole, 221

mCi/mg). The CH_2Cl_2 was evaporated *in vacuo* and a solution of S—methyliso-

thiourea sulfate (9.2 mg, 0.033 mmole) in 4.0 ml of H_2O was added and the reac-

tion was stirred under reflux for 1.5 h followed by dilution with 250 ml of

absolute ethanol. Approximately 55% decomposition of the labeled compound was

indicated (TLC—radioscan) as a result of heating the reaction mixture to 100° C

at this high concentration (0.58 Ci/ml) which is not surprising for compounds of

comparably high specific activity. A 50 ml portion was taken, concentrated and

applied to a column of 10 g of Sephadex G-10 but recovery was extremely poor

(< 3.0 mCi). A 125 ml portion was then taken, concentrated and applied to a

column of 10 g of Bio Rad AG 50W-8X H^+ form resin. The column was first eluted

with 200 ml of H_2O, then 500 ml of 5.0 N HCl. The acidic eluent contained three

20 ml fractions of > 95% pure guanethidine sulfate (TLC—radioscan, n—BuOH—HoAc—

H_2O 10:4:6). These fractions were combined, concentrated *in vacuo* to half volume

and diluted to 500 ml with absolute ethanol to afford 90 mCi of product. This

relatively low yield may be attributed to the above mentioned decomposition as

well as to the difficulty encountered in separating the product from the

impurities formed during the final reaction step.

Acknowledgements

References

1. Rahn K.H. and Goldberg L.T. - Clin. Pharmacol. Ther. 10: 858 (1969).

2. McMartin D. and Simpson P. - ibid. 12: 73 (1970).

3. McMartin C., Rondell R.K., Vinter J., Allan B.R., Humberstone P.M., Leishman
   A.W.D., Sandler G. and Thirkettle J.L. - ibid. 11: 423 (1970).

4. Rahn K.H. - Clin. Nephrol. 1: 14 (1973).

5. Rahn K.H. and Rupp W. - Prog. Nephrol. Proc. Sympos. Ges. Nephrol., 7th, 23-
    25: 55 (1971).

6. Dollery C.T., Emslie-Smith D. and Milne M.D. - Lancet 2: 381 (1976).

7. Oates J.A., Mitchell J.R., Feagen O.T., Kaufman J.S. and Shand D.G. - Ann.
    N.Y. Acad. Sci. 179: 302 (1971).

8. Shand, D.G., Nies A.S., McAllister, R.G. and Oates J.A. - Clin. Pharmacol.
    Ther. 18: 139 (1975).

9. Walter I.E., Khandelwal J., Faulkner F. and Nies A.S. - Clin. Pharmacol.
    Ther. 18: 571 (1975).

10. Loeffler L.J. and Pittman A.W. - J. Pharm. Sci. 68: 1419 (1979).

11. Abramson F.B., Furst C.I., McMartin C. and Wade R. - Biochem. J. 113: 143
    (1969).

12. James B.R. - Homogeneous Hydrogenation, J. Wiley and Sons, 1973, p. 219.

13. Seltzman H.H., Wyrick S.D., Pitt C.G. - J. Lab. Compds. XVIII: 1365
    (1981).

14. Amundsen L.H., Mayer R.H., Pitts L.S. and Malentacchi L.A. - J. Amer. Chem.
    Soc. 73: 2118 (1951).

15. Bahurel Y., Colonges F., Menet A., Pautet F., Poncet A. and Descotes G. -
    Bull. Soc. Chem. France: 2203 (1971).

16. Wilson S.R. and Sawicki R.A. - J. Chem. Soc.: 431 (1977).

17. Wilson S.R., Sawicki R.A. and Huffman J.C. - J. Org. Chem. 46: 3887 (1981).

18. Mull R.P., Egbert M.E. and Dapero M.R. - J. Org. Chem. 25: 1953 (1960).