PREPARATION OF ^{3}H-LABELLED APOMORPHINE, N-n-PROPYLNORAPOMORPHINE, AND O, O-DIPIVALOYLAPOMORPHINE

William H. Soine, Peter Salgo and Robert V. Smith
Drug Dynamics Institute, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712 (U.S.A.).

SUMMARY

Apomorphine and N-n-propylnorapomorphine were tritiated using trifluoroacetic acid- tritiated water mixtures. This provides a route to the synthesis of tritiated O,O-dipivaloylapomorphine. Deuterium exchange and NMR studies indicate that exchange only involves protons at the C-8 and C-9 positions.

Key Words: Apomorphine, N-n-propylnorapomorphine, O,O-dipivaloylapomorphine, tritium

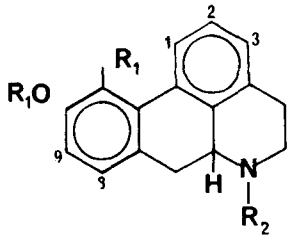
INTRODUCTION

Apomorphine and N-n-propylnorapomorphine are dopaminergic agonists with potential use in a variety of clinical diseases in which subnormal dopaminergic transmission occurs or has been implicated[1-7].

As part of ongoing studies on the metabolism of aporphines, radioactively labelled 1 and 2 were required to study the bioavailability and metabolism of these compounds. Synthetic routes were desired that would also allow preparation of aporphine diesters, such as 3, which have been shown to prolong the half-life of the parent aporphine[8-10]. Tritiated apomorphine is reportedly preparable by the use of $^{3}H_3PO_4$[11] and is available commercially[12], however, use of the published procedures in the synthesis of labelled prodrugs is limited, primarily due to the presence of aqueous media. Because of the excellent stability of apomorphine in strong acid, trifluoroacetic acid-tritiated water mixtures were studied as means of preparing the tritiated aporphines 1, 2, and 3.

0362-4803/79/0416-0597$01.00

Received August 17, 1978
Revised October 13, 1978



	R_1	R_2
1	H	CH_3
2	H	$CH_2CH_2CH_3$
3	$C-C(CH_3)_3$ (O)	CH_3

RESULTS AND DISCUSSION

The tritiation of the aporphines goes smoothly and in reasonable yield due to their stability in strong acids. The use of trifluoroacetic acid, in particular, eliminates the problem of oxidation of apomorphine and opening of the hydropyridine ring that is commonly seen with aporphines[14]. [^{3}H]-N-n-Propylnorapomorphine was obtained in higher specific activity than [^{3}H]-apomorphine

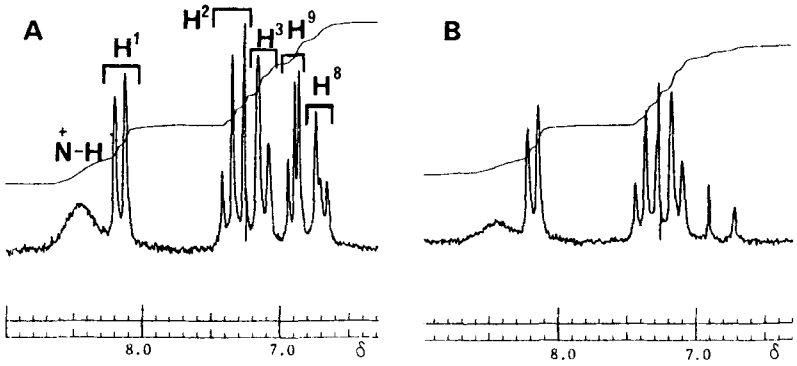


Figure 1. The 100-MHz pmr spectrum of the aromatic protons of apomorphine·HCl in CF_3CO_2D after A) 0.5 hr, B) 72 hr.

which is probably due to the latter's increased solubility in trifluoroacetic acid. In our studies on the bioavailability and metabolism of aporphines[15], compounds of relatively low specific activity were satisfactory, however, the described procedures are potentially suitable for obtaining compounds of much higher specific activity by using more T_2O or CF_3CO_2T. The location of tritium exchange was determined based on a deuterium exchange study using NMR. The pmr-bands of the aromatic protons of 1 had been previously assigned[11] for solution in DMSO-d_6. The spectrum of 1 in CF_3CO_2D is slightly modified. Interestingly, however, the protons on C-8 and C-9 exchange so rapidly in CF_3CO_2D that the expected doublets at 6.72δ and 6.92δ begin to collapse to singlets before the pmr spectrum is run (see Figure 1). The increasing exchange of the C-8 and C-9

protons with time and the unaltered nature of the remaining pmr spectrum when 1 is allowed to remain in CF_3CO_2D for 72 hours suggests that the C-8 and C-9 positions are the exclusive sites of tritiation in the compounds described herein.

The tritiated aporphines 1, 2, and 3 are stored as their HCl salts because of their rapid decomposition at neutral and basic pH[16]. These labelled HCl's are quite stable and appear to remain chemically pure for several weeks when stored at -20°C. In summary, this method provides a simple, rapid, and inexpensive route to aporphines labelled with isotopes of hydrogen.

EXPERIMENTAL

R-(-)-Apomorphine hydrochloride hemihydrate (MacFarland Smith, Ltd., Edinburg, Scotland), trifluoroacetic acid (Matheson, Coleman and Bell, Norwood, OH) and tritiated water, 5 Ci/ml (Amersham/Searle Corporation, Arlington Heights, IL) were used as purchased. N-n-Propylnorapomorphine was a gift from Sterling Winthrop Research Institute (Rensselaer, N.Y.). All other solvents and reagents were analytical reagent grade. UV spectra were obtained with Aminco DW-2 spectrophotometer. Radioactive samples were counted in a Beckman LS8000 liquid scintillation counter at 30-40% efficiency.

[^{3}H]-R-(-)-Apomorphine·HCl

R-(-)-Apomorphine·HCl·1/2 H_2O (100 mg, 0.33 mmol) was placed in a 16x100 mm teflon-sealed screwcap culture tube and dissolved in 0.5 ml (6.73 mmol) trifluoroacetic acid. After dissolution, 10 µl (50 mCi) T_2O was added and the reaction kept in the dark and at room temperature for a minimum of 72 hrs. The trifluoroacetic acid was then removed under a stream of nitrogen leaving a pale brown oil. The oil was treated with a saturated solution of sodium bicarbonate and the mixture extracted with ether (4 x 5 ml). The ether extract was evaporated to dryness under reduced pressure and redissolved in dry ether (15 ml). The HCl salt was obtained by bubbling gaseous HCl through the ether solution, filtering and drying at 65°C to give 60 mg (60-80% yields are usually obtained) of an off white to pale green powder. The tritiated apomorphine had a specific activity of 10.5 mCi/mmol, with chemical purity greater than 99%; chemical purity was

determined by UV spectrophotometry at 273 nm as compared to standard apomorphine ($\lambda_{max}^{H_2O}$ = 273 nm, ε = 17,300). The UV spectrum of ^{3}H-apomorphine·HCl and the glc retention time of the O,O-diheptafluorobutyrate[13] were identical to that obtained with authentic apomorphine.

[^{3}H]-N-n-Propylnorapomorphine·HCl

The desired compound was synthesized the same as [^{3}H]-apomorphine, except that N-n-propylnorapomorphine was dissolved in only 0.25 ml trifluoroacetic acid; 91 mg (91% theoretical yield) of an off white to pale green powder with a specific activity of 17 mCi/mmol and chemical purity greater than 99% was obtained. The chemical purity was determined by UV spectrophotometry at 273 nm as compared to standard ($\lambda_{max}^{H_2O}$ = 273 nm, ε = 17,000). The UV spectrum of [^{3}H]-N-n-propylnorapomorphine·HCl and the glc retention time of the O,O-diheptofluorobutyrate[13] were identical to that observed with authentic N-n-propylnorapomorphine.

[^{3}H]-O,O-Dipivaloylapomorphine·HCl

Apomorphine was tritiated as outlined above but, instead of removing the excess CF_3CO_2H under a stream of N_2, the mixture was treated with 1.5 ml of pivaloyl chloride, and the reaction was heated at 100°C for 1 hour (a slight modification of the procedure by Borgman, et al[8].). The reaction mixture was concentrated to a thick oil under a stream of N_2, made alkaline with a saturated solution of sodium bicarbonate and extracted with ether (3 x 10 ml). The ether extract was evaporated to dryness, redissolved in dry ether, and the HCl salt was generated with gaseous HCl. The [^{3}H]-O,O-dipivaloylapomorphine·HCl was filtered and dried to give 143 mg of an off white powder, which upon TLC (alumina, $CHCl_3$) appeared to contain better than 85% desired product. The powder was dissolved in a saturated solution of $NaHCO_3$, extracted with ether, concentrated to an oil and passed over a column (50 g; 25 mm i.d.) of neutral alumina ($CHCl_3$). The fractions corresponding to the desired product were pooled and the HCl salt generated to give 30 mg (.07 mmol, 20% theoretical yield) with a specific activity of 10.0 mCi/mmol. The chemical purity was determined to be 97% by TLC of the free base (alumina, $CHCl_3$).

ACKNOWLEDGEMENTS

This work was supported in part by grant NS-12259 from the National Institute of Neurological and Communicative Disorders and Stroke. The authors are grateful to the Sterling-Winthrop Research Institute for a generous supply of N-n-propylnorapomorphine.

REFERENCES

1) Cotzias, G.C., Papavasiliou, P.S., Tolosa, E.S., Mendez, J.S., and Bell-Midura, M.- New Engl. J. Med. 294: 567 (1976).

2) Schwab, R.S., Amador, L.V., and Littvin, J.Y. - Trans. Amer. Neurol. Assoc. 76: 251 (1951).

3) Tolosa, E.S. and Sparber, S.B. - Life Sci. 15: 1371 (1974).

4) Gessa, R., Tagliamonte, A., and Gessa, G.L. - Lancet 11-4: 981 (1972).

5) Cotzias, G.C., Lawrence, W.H., Papavasiliou, P.S., Duby, S.E., Ginos, J.Z., and Mena, I., - Trans. Amer. Neurol. Assoc. 97: 156 (1972).

6) Castaigne, P., Laplane, D., and Dordain, G. - Res. Commun. Chem. Path. Pharmacol. 2: 154 (1971).

7) Tamminga, C.A., Schaffer, M.H., Smith, R.C., and Davis, J.H. - Science 200: 567 (1978).

8) Borgman, R.J., Baldessarini, R.J., and Walton, K.G. - J. Med. Chem. 19: 717 (1976).

9) Baldessarini, R.J., Walton, K.G., and Borgman, R.J. - Neuropharmacol. 15: 471 (1976).

10) Worms, P. and Scatton, B. - J. Pharmacol. 45: 395 (1977).

11) Ginos, J.Z., LeMonte, A., Cotzias, G.C., Bose, A.K., and Brambilla, R.J.- J. Amer. Chem. Soc. 95: 2991 (1973).

12) [^{3}H(G)]-R-(-)-Apomorphine, packaged in 0.03 N ascorbic acid under nitrogen, New England Nuclear, Boston, Massachusetts.

13) Baaske, D.M., Keiser, J.E., and Smith, R.V. - J. Chromatogr. 140: 57 (1977).

14) Borgman, R.J., Smith, R.V., and Keiser, J.E.-Synthesis: 249 (1975).

15) Riffee, W.J., Wilcox, R.E., Soine, W.H., and Smith, R.V. - Pharmacologist 20: 184 (1978).

16) Kaul, P.N., Brochmann-Hanssen, E., and Way, E.L. - J. Am. Pharm. Assoc. 48: 638 (1959).