PREPARATION OF ^{3}H-, and ^{14}C-LABELED ETHYLMORPHINE

Yul Yost and Jordan L. Holtzman
Research Laboratories, Veterans Administration Medical Center, Minneapolis, Minnesota 55417; and Departments of Medicine and Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455

SUMMARY

7,8-Didehydro-4,5-epoxy-3-ethoxy-17-methyl(^{3}H)--, and --17-methyl(^{14}C)morphinan-6-ol were prepared by substituting the N-methyl group with a carbphenoxy group which was then either reduced with $LiAl^{3}H_4$ or removed to remethylate the N-atom with $^{14}CH_3I$.

Key Words: Morphine N-demethylation, tritium-, and ^{14}C-labeled ethylmorphine

INTRODUCTION

We have previously reported a sensitive, convenient, radiometric assay for microsomal N-demethylation of 7,8-didehydro-4,5-epoxy-3-ethoxy-17-methyl(^{3}H)-morphinan-6-ol (ethylmorphine, EM) (1). This assay depends on the enzymatic formation of $^{3}H_2CO$ and $^{3}H_2O$ from tritiated EM. Established methods for labeling the N-methyl group of the morphine involved the initial N-demethylation of the parent drug to 2 followed by remethylation of 2 with either labeled CH_3I or $C^{3}H_2O$ (2). Isotopic CH_3I has also been used for studies of the effect of deuteration of the methyl group on the N-demethylation (3). Abdel-Monem and Portoghese (4) subsequently reported the preparation of the carbamate of normorphine and suggested that the reduction of the carbphenoxy group with $LiAl^{3}H_4$ would be a convenient way to tritiate morphine. In our hands the N-demethylation of EM with phenyl chloroformate gave inconsistent results apparently because of the formation of undesirable by-products instead of the expected 1 and chloromethane (5).

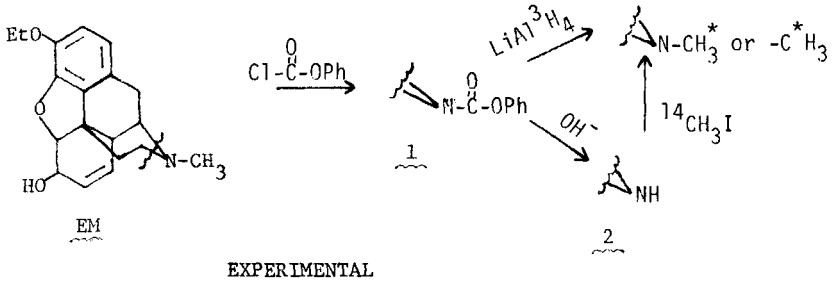
Recently we have simplified the procedure for preparing 1 and identified the major by-product, the diphenyl carbonate. Further, we have found that the product can be purified without column chromatography as previously reported (4). The

0362-4803/81/121793-04$01.00

Received December 15, 1980
Revised February 5, 1981

diphenyl carbonate apparently results in part from the hydrolysis of the phenyl chloroformate by adventitious water to give phenol. This then reacts with the remaining formate to give diphenyl carbonate. Thus liberated HCl forms morphine salts which are unreactive towards electrophilic chloroformate. It is for this reason that, unless anhydrous conditions are maintained, erratic yields result. This method has also proven to be applicable in the preparation of a series of homologous 3-O-alkyl derivatives of morphine. EM of high specific activity was obtained by reduction of the carboxy group of 1 with $LiAL^3H_4$. For this reduction the active (alcoholic) H was first removed with $LiAlH_4$. $LiAl^3H_4$ was then added, affording good incorporation of tritium. The product has since then been used in numerous radiometric studies of EM N-demethylase (6).

The carbphenoxy group of 1 was readily removed by base (4). The resulting norethylmorphine, 2, was remethylated (7) with $^{14}CH_3I$ to afford EM with specific activity of 8.67 mCi/mmole.



EXPERIMENTAL

7,8-Didehydro-17-carbophenoxy-4,5-epoxy-3-ethoxymorphinan-3-ol. EM (1.50 g, 4.59 mmole), $KHCO_3$ (9 g), and phenyl chloroformate (6 g, 38 mmole) were refluxed in $CHCl_3$ (120 ml) for 30 hrs under an N_2 atmosphere. The EM had been consumed after 23 hrs of reflux as shown by TLC (silica gel GF developed in benzene:ethanol, 8:1, where the product has an R_f of 0.75 and EM 0.15). The mixture was filtered and the filtrate was concentrated under vacuum. A small portion of the residue was chromatographen by TLC in benzene. The band, R_f 0.80, was diphenyl carbonate as shown by IR spectrum. The rest of the oily residue was stirred in an 8% solution of methanolic KOH (40 ml) for 20 min, thus hydrolyzing the phenyl carbonates. The solution was concentrated at reduced pressure and the residue was suspended in water (60 ml). The product was extracted with $CHCl_3$ (3 x 20 ml) and the organic

phase was concentrated. The oily residue was dissolved in hot EtOH (12 ml); hot water (12 ml) was then added. Upon cooling, the product crystallized out (3.13 g, 90%), m.p. 169°, lit. 170° (4), IR: (KBr) 3560 $_{(OH)}$ and 1705 $_{(C=O)}$ cm^{-1}.

The basic aqueous phase was acidified and washed with ether. The ether phase gave phenol (31.2 mmole, 93%) as determined spectrophotometrically (8).

Ethylmorphine ($NC^{3}H_{3}$). 1 (100 mg, 0.240 mmole) was dissolved in cold anh. tetrahydrofuran (8 ml) under N_2. $LiAlH_4$ (4.00 mg 0.105 mmole) was added in small portions. After the mixture had been stirred for 1 hr at 20°, $LiAl^{3}H_4$ (1.4 mg, 5.00 mCi, 135 mCi/mmole) was added and the mixture refluxed for 8 hrs. More $LiAlH_4$ (16 mg, 0.422 mmole) was added and the reflux was continued for 3 hrs. EtOAc (10 drops) was added, followed in an hour by water (4 ml). The reaction mixture was brought to near dryness at reduced pressure. The residue was then washed with EtOH until little radioactivity was obtained by washing (about 7 x 3 ml). The EtOH solution was dried ($MgSO_4$) and chromatographed by TLC on silica gel with MeOH:NH_4OH (25:1). The second of the three bands, R_f 0.46, yielded 2.10 mCi (41%). The material was by UV absorption (λ_{max} 286 nm; ε, 1776) and MS (313 M^+) shown to be ethylmorphine; spec. act. 34 mCi/mmole. The faint band, R_f0.85 gave 1, MS 419 (M^+); and band, R_f0.35, 2.

Ethylmorphine(N-$^{14}CH_3$). 2 (36 mg, 0.12 mmole), a cold 6.15 mM ethanolic solution of CH_3I (4.15 ml, 0.025 mmole) and a magnetic stirring bar were added to a 10 ml ampule (ampule A). The ampule, while being cooled with dry ice, was sealed to one end of an inverted U-shaped glass tube. To the other end of the tube was attached the glass ampule (ampule B) which contained $^{14}CH_3I$ (0.025 mmole, 1 mCi, 40 mCi/mmole) and a 5 mm diameter iron slug. A stopcock was attached at the bend of the U-shaped vessel. Ampule A was cooled (Dry-Ice), the system was evacuated and the seal to $^{14}CH_3I$ ampule was broken with the iron slug. To effect the transfer, ampule B was then warmed while ampule A was kept cold. Ampule A was then flame -sealed detached and kept at room temperature for 12 hrs, and at 60° for 1 hr. The solvent was removed under a flow of N_2 and the residue was chromatographed as above. The radiochromatogram showed about 2% activity at the origin. The rest of the activity co-chromatographed with EM. This band was eluted with MeOH (50 ml) yielding the desired product (15%) with specific activity of 8,67 mCi/mmole as shown spectrophotometrically. The band, R_f 0.35, gave unreacted norethylmorphine, 2.

REFERENCES

1. Thompson, J. and Holtzman, J.L. - Pharmacol. Exp. Therap. 186:640 (1973)
2. Andersen, K.S. and Woods, L.A. - Org. Chem. 24:274 (1959); Werner, G. and von der Heyde, O. - J. Label. Comp. 7:233 (1971)
3. Elison, C., Elliott, H.W., Look, M. and Rapport, H. - J. Med. Chem. 6:237 (1963)
4. Abdel-Monem, M.M. and Portoghese, P.S. - J. Med. Chem. 15:208 (1972)
5. Flynn, H.E., Murphy, H.W., and McMahon, R.D. - J. Am. Chem. Soc. 77:3104 (1955)
6. Erickson, R.R. and Holtzman, J.S. - Fed. Proc. 36:960 (1977); Erickson, R.R. and Holtzman, J.L. - Biochem. Pharmacol. 25:1501 (1975); Erickson, R.R., Yu-Drent, P. and Holtzman, J.L. - Pharmacologist 22:263 (1980); Erickson, R.R., Yu-Drent, P. and Holtzman, J.L. - J. Pharmacol. Exp. Therap. (submitted)
7. Kloster, G., Roder, E. and Machulla, Y. - J. Label. Comp. 16:440 (1979)
8. Yost, Y., Gutmann, H.R. and Muscoplat, C. - J. Chem. Soc. C:2119 (1971)

ACKNOWLEDGEMENT

This work was supported by grants from the Veterans Administration and NIH grant #GM-24535. We thank O. Hamerston for recording the spectra and Sue Elg for typing of the manuscript.