NOTE

OXIDATION AND RADIOLABELLING OF ETHYLMORPHINE

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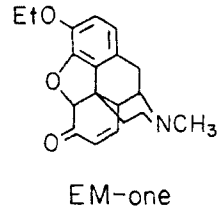
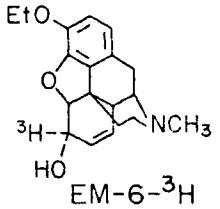
Over a number of years we have been using radiolabelled 7,8-didehydro-4,5-epoxy-3-ethoxy-17-methyl-morphinan-6-ol (ethylmorphine, EM) as a substrate in the study of mixed function oxidase (1). We have previously reported the preparation of EM, labelled with ^{3}H and ^{14}C on the N-Me group (2). Moreover, Fishman *et al.*, have labelled morphine at C-6 with ^{3}H and shown that the ^{3}H at C-6 is metabolically stable (3). Based on their method, we report similarly labelled EM. The preparation involves oxidizing the EM with MnO_2 to ethylmorphinone (EM-one), and then reducing the latter with $NaB^{3}H_4$, thus regenerating the EM. Inexplicably, we found the procedure reported for oxidation of codeine (3) inapplicable for EM; and have, therefore, studied the time course of the oxidation with radioactive EM and also identified one by-product.

Oxidizing EM with MnO_2 for longer than four hours resulted in increasing amounts of at least three other, less polar products, while some starting material remained even after two days. This was shown as follows: $[^{14}C]N\text{-}CH_3$-EM was oxidized with MnO_2. Aliquots of the mixture (unfiltered) were then chromatographed at 1,2,3,4,6 and 22 hours of oxidation. All radiochromatograms showed activity at the origin, as well as that due to EM, EM-one and also due to a less polar by-product, R_f 0.70. This by-product was devoid of the hydrogen at C-6, as shown again by



EM-one



EM-6-^{3}H

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oxidizing EM-6-^{3}H (2), except that now no radioactivity was found at Rf 0.70, where there was a UV-light absorbing substance clearly visible. This material gave m/e of (327 M^+), 311 (M^+-16), and no peak at 310 (M^+-OH); the substance is thus not an alcohol. Moreover, since it is less polar than EM, it appears to be EM-one-N-oxide. Its relative amount increases with time at the expense of EM-one, so it apparently derives from it. The last radiochromatogram also showed UV-light-absorbent spots with R_f 0.70, 0.80 and a yellow one with R_f 0.85, m/e 200 (M^+) and 205 (P^+) (see Figure). No further identification tests were made. Moreover, as evident from the radiochromatogram, about a third of the radioactivity remained along with "MnO_2" at the point of application. This activity was associated with the MnO_2, as shown with chromatograms where the "MnO_2" had been filtered. The collected solids and the filtrate were then chromatographed separately; and, unlike the radiochromatogram of the solids, that of the filtrate showed no activity at the origin.

During the reduction, either an excess of the hydride or reduction times longer than about two hours brought about some reduction of the double bond at C-7. This is conveniently prevented by using an excess of the EM-one; this excess also insures a better use of the labelling reagent.

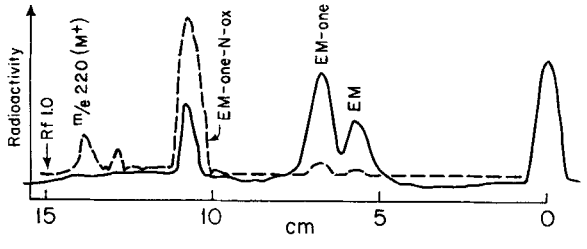

Figure: Thin layer radiochromatogram of oxidation of ethylmorphine (EM) with MnO_2 at 6hrs (——) and 22hrs (– – –).

Crude mixtures after the oxidation and the reduction consist mainly of EM and EM-one. The separation is laborious because the two compounds have similar R_f's. The chromatography of the oxidation products is avoidable by treating the crude residue (in MeOH) with NaB^3H_4; the presence of EM-one-N-oxide is of no consequence, because the N-oxide is also reduced to EM. Concomitant dilution of radioactivity by (unoxidized) EM is a minor inconvenience; in view of the NaB^3H_4 being available in high specific activity (about 80 Ci/mmole) (3). The activity of EM can then be determined spectrophotometrically.

EXPERIMENTAL

Ethylmorphine-6-^{3}H

Activated MnO_2 (Alpha Products), (500 mg) in $CHCl_3$ (20 ml) and EM (200 mg, 0.60 mmol)were stirred at 25° for two hours. The mixture was filtered and the filtrate was concentrated. The residue was chromatographed by tlc (two silica gel, 1 mm 20 x 20 cm plates) in $CHCl_3$:MeOH:NH_4OH - 80:20:1. The EM-one from the major band, R_f 0.43 (visualized by UV light), was eluted with THF; 25% yield; m/e 311 (M^+). The less intense band, R_f 0.35, gave the starting material.*

The NaB^3H_4, 25 mCi (sp. act 592 mCi/mmol) was washed out of the supplier's (New England Nuclear) ampule with MeOH (5 x 0.3 ml). The washings were put into a one-dram vial which contained EM-one (62 mg, 0.20 mmol). The solution was stirred in the capped vial for two hours at 25°. Ammonium chloride (2 mg) was added and the mixture was reduced in volume with a stream of N_2 and then chromatographed as above (one 1 mm 20 x 20 cm plate). Radiochromatogram showed activity

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*The oxidation mixture can be applied to tlc plates without first filtering off the "MnO_2". Tetrahydrofuran (THF) was used for elution; MeOH, though a better eluent, leaches the adsorbent, SiO_2.

at the solvent front, at R_f 0.35 (the product) and at the point of application, with areas in a ratio of 30:50:20. The desired band yielded the prodcut in 33% yield with a sp. act. of 123 mCi/mmol; with the concentration having been determined in EtOH at 283 nm (ε 1776) and the activity by liquid scintillation.

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