SYNTHESIS OF HIGH SPECIFIC ACTIVITY [15,16-$^3H_2$]BUPRENORPHINE

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SUMMARY

Tritium labelling of buprenorphine, a mixed agonist-antagonist opioid ligand, was performed with a specific activity of 2.35 TBq/mmol (63.6 Ci/mmol) starting with 15,16-didehydrobuprenorphine. Labels at positions 15 and 16 of the morphine skeleton proved to be sufficiently stable under strong acidic or basic conditions.

Key words: tritiation, buprenorphine, 15,16-didehydrobuprenorphine, opioid ligand

INTRODUCTION

Buprenorphine (= [5α,7α(S)]-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-etheno-morphinan-7-methanol), a potent analgesic drug is a partial mixed opioid (μ, δ, κ) receptor agonist with low dependence liability and toxicity (1, 2) and κ opioid receptor antagonist (3, 4). Recently controlled trials have been made for the clinical treatment of heroin (1, 2, 5) and/or cocaine addicts (6, 7) with buprenorphine, instead of methadone and/or naltrexone. Better results with buprenorphine respective to the agonist methadone and the antagonist naltrexone may arise from the mixed μ agonistic and κ antagonistic characters of buprenorphine (8, 9). In binding studies, **2** binds almost equally well to μ, δ, and κ opioid receptors (8, 10). Further clarification of the interaction of buprenorphine with various opioid receptor types and subtypes awaits additional systematic competitive binding assays with selective ligands in

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different tissues and cell lines. For this purpose we synthesized tritiated buprenorphine using the method described by Lewis *et al.* (11) for etorphine and its derivatives, and Rance *et al.* (12) for deuterated buprenorphine.

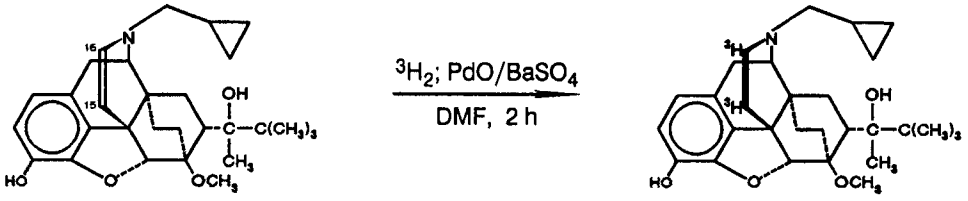
RESULTS AND DISCUSSION

[15,16-$^3H_2$]Buprenorphine (**2**) was prepared from 15,16-didehydrobuprenorphine (**1**) by catalytic saturation of the 15-16 double bond using $PdO/BaSO_4$ catalyst and $^3H_2$ in DMF solution (Scheme 1). Tritium labelling at positions 15 and 16 was described earlier for naltrexone and naloxone (13), and for oripavine series (11, 14). Use of Pd/C in catalytic saturation of the 15-16 double bond with $^3H_2$ (13) resulted in 0.566 TBq/mmol (15.3 Ci/mmol) of specific activity, while combining with exchange by ^3H_2O (11) an estimated 0.4 TBq/mmol (11 Ci/mmol) was obtained. Other experiments performed with exchange followed by tritiated sodium borohydride or sodium cyanoborohydride reduction gave lower specific activity (11, 13, 14).

As $PdO/BaSO_4$ is known to have higher catalytic activity to saturate double bond and higher capability for isotope exchange reactions than other supported palladium catalysts (*e. g.* Pd/C) or Pd without support (15), it was used in our experiments resulting in a specific activity of 2.35 TBq/mmol (63.6 Ci/mmol). Morever, DMF was used as a solvent because it lacks mobile protons. Specific activity, higher than expected theoretically, 2.18 TBq/mmol (59 Ci/mmol) may be due to isotope exchange at the carbon 15 with ^3H_2O (11) produced by reduction of the catalyst and/or at the benzylic carbon of the morphinan skeleton. Exchange reaction was proved by TLC showing a radioactive peak with R_f identical to that of **1** on the radiochromatogram of the crude product. The positions of ^3H labelling as well as the quantitative proportion of ^3H on positions 15 and 16 were not determined, because ^3H-NMR was not available.

High reactivity or exchange capability of hydrogens on carbon 15 of **2** is not known contrary to **1** (11, 14). Investigation on the stability of labels was carried out treating **2** with HCl and NaOH solutions. 30 min acid treatment (0.2 M HCl) caused no change in specific activity of **2** neither at room temperature nor at 70 °C. 30 min basic treatment (0.2 M and 1 M NaOH) at room temperature caused no change, while at 70 °C 38 % (0.2 M) and 56 % (1 M) loss of specific activity was shown.

Scheme 1



1 2

EXPERIMENTAL

General. - $^3H_2$ gas was purchased from Technabexport, Russia, and contained at least 98 % $^3H_2$. All materials were analytical grade, but DMF was repurified by vacuum destillation and dried over molecular sieves prior to use. PdO/$BaSO_4$ catalyst (10 % Pd) was from Merck. Purity controls were performed by TLC on precoated Silica gel 60 F_{254} plates (Merck, 0.25 mm) visualizing by UV light (254 nm) or iodine vapor. Radiochemical purity was checked with a Berthold Radiochromatogram Tracemaster. The amount of tritiated material was measured by UV detection on a Shimadzu-160 spectrophotometer. Radioactivity was counted in Liquidfluor scintillant (BDH, England) with a Searle-Delta-300 liquid scintillation counter. ^{1}H-NNMR spectra were recorded on a Bruker AM-200 spectrometer in $CDCl_3$. Chemical shift standard was Me_4Si. Mass spectra were obtained with a VG Trio-2 mass spectrometer under EI mode (70 eV).

[5α,7α(S)]-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-etheno-morphinan-7-methanol (Buprenorphine). - The compound was prepared from thebaine using the procedure of Bentley (16). Analytical data were the same than those of authentic buprenorphine.

[5α,7α(S)]-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-etheno-15,16-didehydromorphinan-7-methanol (1, 15,16-didehydrobuprenorphine). - Compound (1) was prepared by the method of Haddlesey et al. (14). A solution of buprenorphine (3.1 g, 6.6 mmol) and yellow mercury(II)-oxide (8.0 g, 37.0 mmol) in 1.5 M acetic acid was boiled for 1 h, cooled,

saturated with hydrogen sulphide, and filtered. Neutralization of the filtrate with aqueous ammonia solution gave the desired compound (2.4 g, yield: 77.8 %). M.p.: 218-220 °C (after recrystallization from ethanol). $C_{29}H_{39}NO_4$, m.w. 456.61. ^{1}H-NMR ($CDCl_3$): 0.1-0.9 (m, 5 H, cyclopropyl); 1.0 (s, 9 H, *t*-Bu); 1.4 s, 3 H, Me-C(19)); 3.6 s, 3 H, MeO-C(6)); 4.3 (d, H-C(15)); 4.8 (s, βH-C(5)); 5.1 (s, HO-C(3)); 5.9 (m, 2 H, HO-C(19) and H-C(16)); 6.7 (ABq, 2 H, H-C(1) and H-C(2)). MS: 465 (60, M^+), 433 (40), 408 (100), 376 (90).

[5α,7α(S)]-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-etheno-)-[15,16-$^3H_2$]morphinan-7-methanol (2, [15,16-$^3H_2$]Buprenorphine). To a solution of 1 (2.8 mg, 5.99 μmol) in DMF (0.8 ml) $PdO/BaSO_4$ (17 mg) was added and stirred for 2 h in the presence of 555 GBq (15 Ci) of $^3H_2$ gas in a closed vacuum manifold (17). The excess of $^3H_2$ gas was removed by absorption on pyrophoric uranium. The catalyst was filtered off using Whatman-GF/C glass-fiber filter. Labile ^3H was removed by repeated evaporation with $EtOH/H_2O$ 1:1. The radioactivity of the crude material was 12.1 GBq (326 mCi), then purifying of 4.21 GBq (114 mCi) of 12.1 GBq (326 mCi) by TLC (silica gel 60 F_{254} plate (Merck), acetonitrile/*n*-butanol/water solvent 20:6:1), 2.790 GBq (75.4 mCi, 56.6 % based on the purified portion) were recovered. TLC plates used for preparative purification were prewashed by the method described by Stanley et al. (18). The purity of 2 was > 95 % by TLC (acetonitrile/*n*-butanol/water solvent 20:6:1, R_f 0.41 for 2 and 0.59 for 1; *n*-butanol/acetic acid/water 20:5:8 (12) R_f 0.55 for 2 and 0.43 for 1; acetonitrile/methanol/water 4:1:1 R_f 0.37 for 2 and 0.52 for 1). Specific radioactivity was 2.35 TBq/mmol (63.6 Ci/mmol).

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