157. Tritium Labelling of Naltrindole, a δ-Receptor-Selective Opioid Antagonist *via* 1-Bromonaltrexone[1])

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The synthesis of [1,5′-$^3H_2$]naltrindole (**9**) with labels at both the morphine skeleton and the indole moiety was carried out by catalytic tritiodehalogenation of 1,5′-dibromonaltrindole (**8**) resulting in a specific activity of 46.1 Ci/mmol (1705 GBq/mmol). The brominated precursor was prepared by the *Fischer* indole synthesis starting from 1-bromonaltrexone (**7**) and (4-bromophenyl)hydrazine.

Introduction. – The existence of three major types of opioid receptors, μ, δ, and κ, is generally revealed. Detailed studies of these receptor types requires radiolabelled ligands with high receptor-binding affinity, high selectivity, and high specific radioactivity. Naltrindole, (= (−)-17-(cyclopropylmethyl)-4,5α-epoxy-1′*H*-indolo[2′,3′:6,7]morphinan-3,14-diol), a δ opioid receptor antagonist, is a very useful tool to study the interaction of ligand and the δ opioid receptor [2]. Naltrindole has higher affinity toward δ opioid receptors compared to the linear peptide antagonists (ICI 174864 [3] and TIPP[2]) [4]). Recently, naltrindole was labelled with ^3H by two different groups. In our laboratory, [5′-^3H]naltrindole was synthesized by catalytic dehalogenation from 5′-bromonaltrindole [5] [6] with a specific radioactivity of 20.5 Ci/mmol (758 GBq/mmol). *Dorn et al.* [7] prepared [5′,7′-$^3H_2$]naltrindole with a specific activity of 39.5 Ci/mmol (1460 GBq/mmol). The binding characterization of [^3H]naltrindole using rat-brain membrane [6], mouse-brain membrane, and also with mouse vas deferens [8] revealed that this ligand is a very active one and very selective for δ opioid receptors.

Here we describe a new radiolabelling of naltrindole starting from 1,5′-dibromonaltrindole (**8**). The precursor was synthesized from 1-bromonaltrexone (= 1-bromo-17-(cyclopropylmethyl)-3,14-dihydroxy-4,5α-epoxymorphinan-6-one; **7**) and (4-bromophenyl)hydrazine by the *Fischer* indole method. ^3H-Labels in the thus obtained naltrindole **9** are in positions 1 and 5′, and the specific activity is 46.1 Ci/mmol (1705 GBq/mmol).

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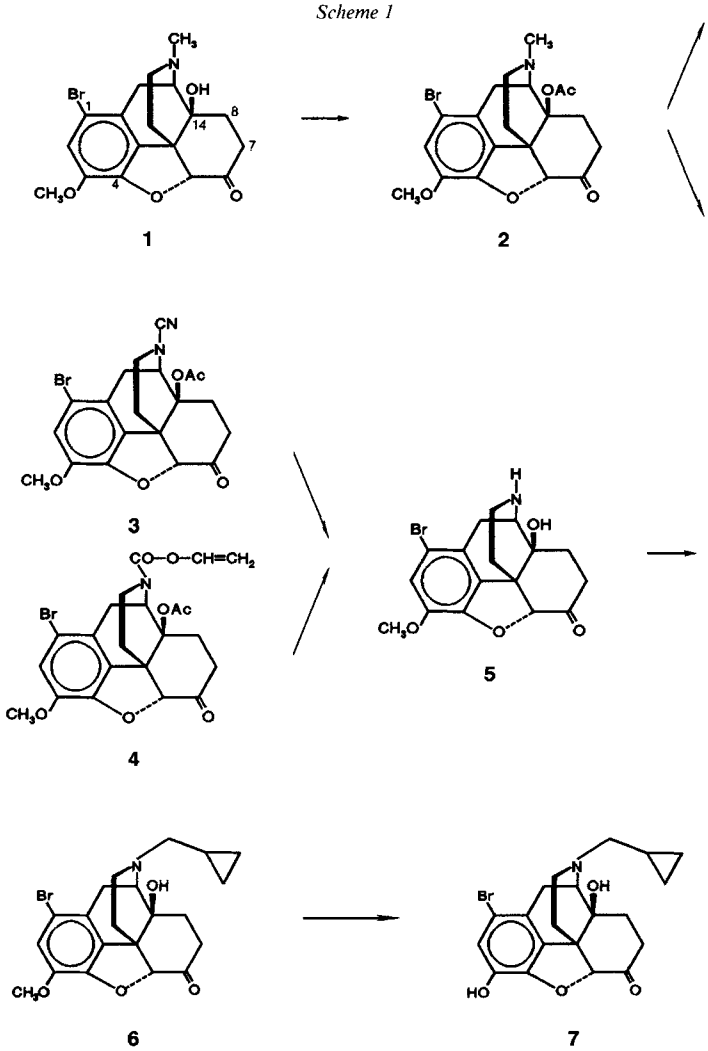
[1]) This work was partly presented earlier [1].

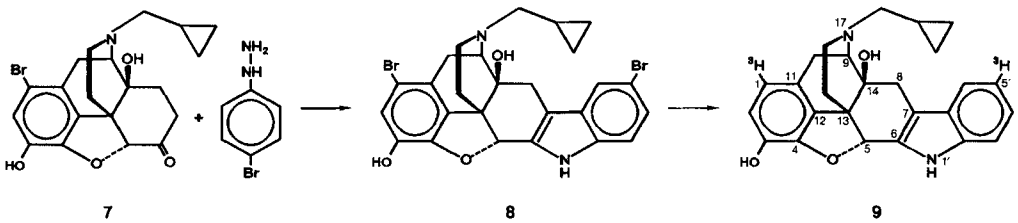
[2]) TIPP: Tyr-Tic-Phe-Phe, where Tic is (*S*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.

Chemistry. – The 7,8-dihydro-14-hydroxycodeinone hydrobromide was brominated with Br_2 in H_2O to obtain 1-bromo-7,8-dihydro-14-hydroxycodeinone (**1**). Compound **1** was acetylated (*Scheme 1*) according to *Speyer* and *Sarre* [9]. The acetyl derivative **2** was *N*-demethylated with either cyanogen bromide [9] or vinyl chloroformate [10] to give **3** or **4**, respectively, which were subsequently hydrolyzed by 10% HCl/H_2O resulting in 1-bromo-17-demethyl-7,8-dihydro-14-hydroxycodeinone (**5**). The reaction between **5** and cyclopropylmethyl bromide in DMF resulted in the 3-methyl ether derivative **6** of 1-bromonaltrexone. The desired 1-bromonaltrexone (**7**) was obtained by *O*-demethylation of **6** with BBr_3. Noteworthy is that **7** bound to the μ opioid binding site with an affinity of 1/3 compared to naltrexone (unpublished results).

Scheme 1



Scheme 2



7 8 9

Preparation of 1,5′-dibromonaltrindole (**8**; *Scheme 2*) was carried out by acid-catalyzed *Fischer* indole synthesis in HCl/MeOH starting with 1-bromonaltrexone (**7**) and (4-bromophenyl)hydrazine. Dehalogenation of **8** was performed with $^3H_2$ gas using $PdO/BaSO_4$ as catalyst in DMF. After purification by TLC, compound **9** had a specific activity of 46.1 Ci/mmol (1705 GBq/mmol). It is interesting to note that our former labelling [6] at the 5′-position of the indole moiety resulted in a specific activity of 20.5 Ci/mmol (758 GBq/mmol), suggesting a distribution of ^3H between positions 1 and 5′ of 25.6 Ci/mmol (947 GBq/mmol) and 20.5 Ci/mmol (758 GBq/mmol), respectively.

These results are in harmony with the result of *Dorn et al.* [7] who reported a specific activity of 39.5 Ci/mmol (1460 GBq/mmol) for tritiated naltrindole obtained from 5′,7′-dibromonaltrindole. Their investigations with ^3H-NMR showed an equal distribution of ^3H between the positions 5′ and 7′, *e.g.* 19.75 Ci/mmol (730 GBq/mmol). Thus, it can be assumed that tritiodehalogenation of the brominated naltrindole derivatives would be performed with higher efficiency, if the Br-atom was present at the aromatic ring A of the molecule rather than in the indole moiety.

Biological studies performed with **9** are in progress, and the preliminary results of the receptor-binding properties of this radioligand reveal a similar trend of binding affinity and selectivity for δ opioid receptor than [5′-^3H]naltrindole. The results will be published elsewhere.

Experimental Part

General. $^3H_2$ Gas was purchased from *Technabexport*, USSR, and contained at least 98% $^3H_2$. All materials were anal. grade, but DMF and Et_3N were repurified by vacuum destillation and dried over molecular sieves prior to use. The amount of tritiated material was measured by UV detection on a *Shimadzu-160* spectrophotometer. Tritiated samples were counted in *Liquidfluor* scintillant (*BDH*, England) with a *Searle-Delta-300* liquid scintillation counter. Radiochemical purity was checked with a *Berthold Radiochromatogram Tracemaster*. Anal. purity of compounds were controlled by TLC (silica gel *60* F_{254} (*Merck*, Art. No. 5554) plates, $CHCl_3$/MeOH 9:1, benzene/MeOH 8:2, and $CHCl_3$/acetone/Et_2NH 5:4:1 (*v/v*), unless otherwise stated, detection by UV light). Column chromatography (CC): silica gel *60 M* (0.063–0.2 mm, *Reanal*). M.p.: *Kofler* melting-point microscope and *Electrothermal* (8103) digital instrument; uncorrected. ^1H-NMR: 400 MHz: *Bruker-AM-400* spectrometer, measured by the NMR Laboratory of the Szeged Regional Center for Scientific Instruments, Szeged, Hungary; 200 MHz: *Bruker-AM-200* spectrometer; measured at the Analytical Laboratory of *Alkaloida Chemical Factory Ltd.*; δ in ppm, J (apparent) in Hz. Mass spectra (EI, 70 eV): *VG Trio-2* mass spectrometer; measured at the Analytical Laboratory of *Alkaloida Chemical Factory Ltd.* All compounds gave satisfactory elemental analyses (C ± 0.3%, H ± 0.2%, N ± 0.3%). Tritiation was performed in a glass manifold described earlier [11].

1-Bromo-4,5α-epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one (**1**) was prepared according to [9]. Yield 90%. M.p. 181–183° (EtOH; [9]: 182°). ^{1}H-NMR ($CDCl_3$): 2.4 (*s*, Me–N(17)); 3.95 (*s*, MeO–C(3)); 4.7 (*s*, H–C(5)); 6.95 (*s*, H–C(2)). MS: 398 (90, M^+), 336 (14), 308 (32).

14-Acetoxy-1-bromo-4,5α-epoxy-3-methoxy-17-methylmorphinan-6-one (**2**). Yield 60%. M.p. 215–217° (EtOH; [9]: 217°). ^{1}H-NMR ($CDCl_3$): 2.25 (*s*, AcO–C(14)); 3.9 (*s*, MeO–C(3)); 4.25 (*d*, H–C(9)); 4.7 (*s*, H–C(5)); 6.9 (*s*, H–C(2)). MS: 435 (32, M^+).

14-Acetoxy-1-bromo-17-cyano-4,5α-epoxy-3-methoxymorphinan-6-one (**3**). To a soln. of **2** (8.0 g, 18.3 mmol) in dried $CHCl_3$ (50 ml), cyanogen bromide (4.1 g, 39 mmol) in $CHCl_3$ (40 ml) was added. The mixture was refluxed for 8 h, then more cyanogen bromide (4.1 g) added, and boiling continued for 8 h (TLC: reaction complete). After evaporation, crystallization from EtOH yielded 4.0 g (49%). M.p. 277–280°. ^{1}H-NMR ($CDCl_3$): 2.25 (*s*, AcO–C(14)); 3.9 (*s*, MeO–C(3)); 4.7 (*s*, H–C(5)); 4.85 (*d*, H–C(9)); 7.0 (*s*, H–C(2)). MS: 447 (32, M^+).

14-Acetoxy-1-bromo-4,5α-epoxy-3-methoxy-17-(vinyloxycarbonyl)morphinan-6-one (**4**). To a soln. of 14-acetoxy-1-bromo-7,8-dihydrocodeinone (5.5 g, 12.5 mmol) in dried 1,2-dichloroethane (70 ml), $NaHCO_3$ (2.5 g) and vinyl chloroformate (5.0 ml, 59 mmol) were added. After 8 h stirring and refluxing, another portion of vinyl chloroformate (2.5 ml) was added and refluxed for 8 h again (TLC: reaction complete). The inorg. salt was filtered off, the filtrate evaporated, and the residue dissolved in $CHCl_3$, then washed with H_2O and dried. Compound **4** was crystallized from MeOH: 4.12 g (66%). M.p. 202–204°. ^{1}H-NMR ($CDCl_3$): 2.15 (*d*, AcO–C(14)); 3.9 (*s*, MeO–C(3)); 4.5 (*m*, 1 H); 4.7 (*s*, H–C(5)); 5.0–4.8 (*m*, 1 H); 5.8–5.6 (*m*, 1 H); 6.95 (*s*, H–C(2)); 7.2–7.1 (*m*, 1 H). MS: 491 (3, M^+).

1-Bromo-4,5α-epoxy-14-hydroxy-3-methoxymorphinan-6-one (**5**). *From* **3**. A suspension of **3** (4.0 g, 9 mmol) in 10% aq. HCl soln. (50 ml) was refluxed for 15 h under N_2. On cooling, **5**·HCl precipitated. It was filtered off, washed with cold H_2O, and then transformed to **5** by NH_4OH: 2.0 g (59%). TLC: homogeneous. M.p. 212–214° (EtOH). ^{1}H-NMR ($CDCl_3$): 3.9 (*s*, MeO–C(3)); 4.7 (*s*, H–C(5)); 6.9 (*s*, H–C(2)). MS: 379 (100, M^+), 294 (35).

From **4**. A soln. of **4** (4.0 g, 8.1 mmol) in dried 1,2-dichloromethane (80 ml) was stirred and cooled while passing through dried HCl gas. The solvent was evaporated and the residue dissolved in abs. MeOH (100 ml) and refluxed for 2 h. MeOH was evaporated and the residue refluxed in 10% HCl/H_2O (70 ml) at 100° for 4 h. As described above, **5**·HCl was precipitated and transformed into **5**: 2.0 g (65%). ^{1}H-NMR and MS: identical with those of **5** obtained from **3**.

1-Bromo-17-(cyclopropylmethyl)-4,5α-epoxy-14-hydroxy-3-methoxymorphinan-6-one (**6**). To a soln. of **5** (1.0 g, 2.6 mmol) in abs. DMF (30 ml), $NaHCO_3$ (0.5 g) and cyclopropylmethyl bromide (0.4 ml) were added. The mixture was stirred at 90° for 20 h. The inorg. salt was filtered off, the filtrate evaporated, the residue dissolved in H_2O and then extracted with $CHCl_3$, the $CHCl_3$ soln. evaporated, and the crude colorless oil purified by CC (silica gel, $CHCl_3$/MeOH 9:1). The pure product fractions (0.9 g, 79%) were triturated with Et_2O to crystallize. M.p. 116–119°. ^{1}H-NMR ($CDCl_3$): 1.0–0.2 (*m*, 5 H, cyclopropyl); 3.9 (*s*, MeO–C(3)); 4.7 (*s*, H–C(5)); 6.9 (*s*, H–C(2)). MS (thermospray method): 433 (M^+).

1-Bromo-17-(cyclopropylmethyl)-3,14-dihydroxy-4,5α-epoxymorphinan-6-one (= *1-Bromonaltrexone*; **7**). A soln. of **6** (0.9 g, 2 mmol) in $CHCl_3$ (30 ml) was added dropwise to BBr_3 (2.0 ml, 21 mmol) in dried $CHCl_3$ (30 ml) within 20 min, while passing N_2 and cooling with ice. The mixture was stirred for 1 h in the ice-bath, then for 1 h at r.t. The mixture was poured onto ice, the pH adjusted to 9 by 10% NH_4OH/H_2O, the aq. phase extracted with $CHCl_3$ (3 × 50 ml), the combined org. phase dried (Na_2SO_4) then evaporated, and the crude product purified twice by CC (silica gel, benzene/MeOH 9:1): 0.3 g (34%) of pure **7** which slowly crystallized from acetone. M.p. 186–188°. ^{1}H-NMR ($CDCl_3$): 6.92 (*s*, H–C(2)); 4.68 (*s*, H–C(5)); 1.0–0.2 (*m*, 5 H, cyclopropyl). MS: 419 (100, M^+).

1,5'-Dibromo-17-(cyclopropylmethyl)-4,5α-epoxy-1'H-indolo[2',3':6,7]morphinan-3,14-diol Hydrochloride (= *1,5-Dibromonaltrindole Hydrochloride*; **8**·HCl). A soln. of **7** (0.3 g, 0.52 mmol) and (4-bromophenyl)hydrazine (0.17 g, 0.75 mmol) in MeOH sat. with HCl was refluxed for 4 h. The crude material was purified by CC (silica gel, $CHCl_3$/MeOH 9:1): 0.15 g (48%). M.p. 265–272° (dec.). ^{1}H-NMR ((D_6)DMSO): 7.49 (*s*, H–C(4')); 7.33 (*d*, J = 8.5, H–C(7')); 7.21 (*d*, J = 8.5, H–C(6')); 6.92 (*s*, H–C(2)); 5.74 (*s*, H–C(5)). MS: 572 (76, M^+), 531 (43).

17-(Cyclopropylmethyl)-4,5α-epoxy-1'H-[1,5'-$^3H_2$]indolo[2',3':6,7]morphinan Hydrochloride (= *[1,5'-$^3H_2$]Naltrindole Hydrochloride*; **9**·HCl). To a soln. of **8** (2 μmol) in DMF (1 ml), PdO/$BaSO_4$ catalyst (10 mg) and Et_3N (5 μl) were added and stirred for 60 min in the presence of 555 GBq (15 Ci) of $^3H_2$ gas in a closed vacuum manifold [11]. The excess of $^3H_2$ gas was removed by absorption on pyrophoric uranium. The catalyst was filtered off using *Whatman-GE/C* glass-fiber filter. Labile ^{3}H was removed by repeated evaporation with EtOH/H_2O 1:1. The radioactivity of the crude material was 106 mCi (3.92 GBq), and after purification by TLC (silica gel *60* F_{254} plate (*Merck*), $CHCl_3$/MeOH/NH_3 soln. 90:10:0.5), 10.7 mCi (396 MBq, 11.6%) were recovered. The purity of **9** was > 95% by TLC ($CHCl_3$/MeOH/NH_3 soln. 90:10:0.5, R_f 0.47; MeOH/AcOH/H_2O 1:1:8, R_f 0.35). Specific radioactivity was 46.1 Ci/mmol (1705 GBq).

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REFERENCES

[1] G. Tóth, F. Ötvös, S. Hosztafi, H. Schmidhammer, B. Búzás, A. Borsodi, 'Synthesis and Binding Characteristics of New μ and δ Opioid Receptor Selective Tritiated Antagonists', 'International Narcotics Research Conference', Poster 140; Keystone, Colorado, U.S.A., June 23–27, 1992.

[2] P.S. Portoghese, M. Sultana, H. Nagase, A.E. Takemori, *J. Med. Chem.* **1988,** *31,* 281; P.S. Portoghese, M. Sultana, H. Nagase, A.E. Takemori, *Recent Adv. Receptor Chem.* **1988,** 307; P.S. Portoghese, M. Sultana, A.E. Takemori, *J. Med. Chem.* **1990,** *33,* 1714; H. Rogers, A.G. Hayes, P.J. Birch, J.R. Traynor, A.J. Lawrence, *J. Pharm. Pharmacol.* **1990,** *42,* 358.

[3] R. Cotton, M.G. Giles, L. Miller, J.S. Shaw, D. Timms, *Eur. J. Pharmacol.* **1984,** *97,* 331.

[4] P.W. Schiller, T.M.-D. Nguyen, G. Weltrowska, B.C. Wilkes, B.J. Marsden, C. Lemieux, N.N. Chung, *Proc. Natl. Acad. Sci. U.S.A.* **1992,** *89,* 11871.

[5] G. Tóth, F. Ötvös, B. Búzás, A. Borsodi, V.J. Hruby, 'Proceedings of the 4th International Symposium – Toronto (September 91)', in 'Synthesis and Applications of Isotopically Labelled Compounds 1991', Eds. E. Buncel and G.W. Kabalka, Elsevier, Amsterdam, 1992, p. 490.

[6] M.S. Yamamura, R. Horvath, G. Toth, F. Otvos, E. Malatynska, R.J. Knapp, F. Porreca, V.J. Hruby, H.I. Yamamura, *Life Sci.* **1992,** *50,* PL119.

[7] C.R. Dorn, C.S. Markos, M.S. Dappen, B.S. Pitzele, *J. Labelled Compd. Radiopharm.* **1992,** *31,* 375.

[8] R.J. Knapp, H.I. Yamamura, *Biochem. Pharmacol.* **1992,** *44,* 1687.

[9] E. Speyer, K. Sarre, *Ber. Dtsch. Chem. Ges.* **1924,** *57,* 1409.

[10] R.A. Olofson, J.P. Pepe, *Tetrahedron Lett.* **1977,** *18,* 1575.

[11] G. Tóth, F. Sirokmán, *Izotóptechnika (Budapest)* **1981,** *24,* 259.