Deoxymorphines: Role of the Phenolic Hydroxyl in Antinociception and Opiate Receptor Interactions

Jürgen Reden,[1] Marvin F. Reich, Kenner C. Rice, Arthur E. Jacobson, Arnold Brossi,*

Section on Medicinal Chemistry, Laboratory of Chemistry, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014

Richard A. Streaty, and Werner A. Klee

Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20014. *Received October 30, 1978*

Several 3-deoxy opioids and 3,6-dideoxydihydromorphine were synthesized to ascertain the effect of the phenolic hydroxyl group on antinociceptive potency and receptor binding affinity. Catalytic reduction of the 3-tetrazolyl ether derivatives of dihydromorphine provided the entry into the 3-deoxydihydro series. The prototype, 3-deoxymorphine, was prepared by lithium aluminum hydride reduction of 3-deoxy-*N*-carbethoxymorphinone, obtained via its 7-(phenylseleno) derivative. 3-Deoxydihydromorphinone and 3,6-dideoxydihydromorphine were found to be about as potent as, or more potent than, morphine in standard antinociceptive assays. Each of them, however. was less potent than the comparable 3-hydroxy analogue, and their binding affinity to the opiate receptor was substantially decreased. The epoxy ring in 3,6-dideoxydihydromorphine was found to increase the antinociceptive potency of the compound.

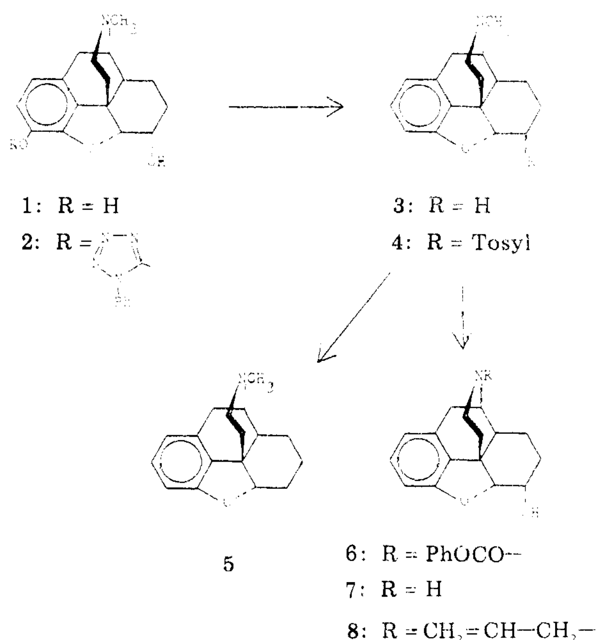
We have extended our investigations concerning the removal of phenolic functions in the aporphine series[2] to the morphine group of alkaloids by the synthesis of 3-deoxymorphine (**13**), 3,6-dideoxydihydromorphine (**5**), and a variety of 3-deoxydihydro opioids.[3] The synthesis of 3,6-dideoxydihydromorphine enabled us to determine the effect of the oxide bridge in morphine-like compounds on antinociception and receptor binding affinity.

The phenolic group in morphine has been assumed by many workers to be very important to the biological activity of the compound and its ability to bind to the opiate receptor.[4,5] This assumption probably derived from the finding that (±)-3-hydroxy-*N*-methylmorphinan has about 15 times the potency of (±)-*N*-methylmorphinan,[6] but the assumption was never proven in the morphine series. It is believed that the tyrosine hydroxyl is important to the biological activity of the synthetic opiate peptides.[7] It has been noted, however, that an exceptional deoxy-*endo*-ethenooripavine derivative (3-deoxy-6,14-*endo*-etheno-6,7,8,14-tetrahydrooripavine) was somewhat more potent, in vivo, than the corresponding phenol.[8]

Chemistry. It seemed likely to us that 3-deoxydihydromorphine (**3**) could serve as a precursor for the 3-deoxy compounds in the 7,8-dihydro series (Scheme I), as well as for 3-deoxymorphine (**13**) itself (Scheme II). Accordingly, conversion of dihydromorphine (**1**) to the 3-(1-phenyl-1*H*-5-tetrazolyl) derivative **2**, followed by catalytic hydrogenolysis of **2** in AcOH over 5% Pd on C, readily afforded (84%) pure **3** which had previously[3] been obtained by hydrogenolysis of the corresponding tetrazolyl ether of morphine. Lithium aluminum hydride reduction of tosylate **4**, prepared from **3**, provided 3,6-dideoxydihydromorphine (**5**) which represents the "naked" carbon–nitrogen–oxygen pentacyclic skeleton of dihydromorphine. N-Demethylation of **3** to 3-deoxydihydronormorphine (**7**) was easily accomplished via the *N*-carbophenoxy derivative **6** (not isolated) and hydrazinolysis, using our improved method[9] for this transformation. N-Alkylation of **7**, using the standard method,[10] then gave the *N*-allyl derivative **8**.

Synthesis of **13** was accomplished by the sequence outlined in Scheme II and paralleled the first[11] of two routes[11,12] we recently described for the synthesis of (+)-morphine. Oppenauer oxidation[13] of **3** easily afforded (95% yield) the required 3-deoxydihydromorphinone (**9**), which was of sufficient purity for direct conversion to **10**. Conversion of basic **9** into neutral carbamate **10** was ac-

Scheme I



complished by treatment of **9** with excess ethyl chloroformate and was necessary to prevent interference[11,14] of the amino group of **9** in the subsequent selenation step. Acid-catalyzed reaction[15] of phenylselenenyl chloride with **10** introduced the 7-(phenylseleno) substituent in **11**, which was assigned the axial configuration by analogy with the corresponding 3-methoxy derivative[11] in the (+) series, whose absolute stereochemistry was determined by X-ray analysis.

This assignment was supported by the coupling constants (J = 2.8 and 2.8 Hz) observed in the NMR spectrum for the equatorial C-7 H coupled with the C-8 protons in **11** and by the downfield shift ($\Delta\delta$ 0.7 ppm) of C-5 H [due to axial–axial 1,3 interaction with the 7-(phenylseleno) substituent] relative to the corresponding absorption in unsubstituted carbamate **10**.

Oxidation of **11** with excess $NaIO_4$ afforded 3-deoxy-*N*-carbethoxynormorphinone (**12**) which, on reduction with LAH in THF, gave crystalline 3-deoxymorphine (**13**) directly. The structural assignment of **13** was supported by spectral data [and by the observation[11] that the corresponding enantiomeric 3-methoxy derivative gave (+)-

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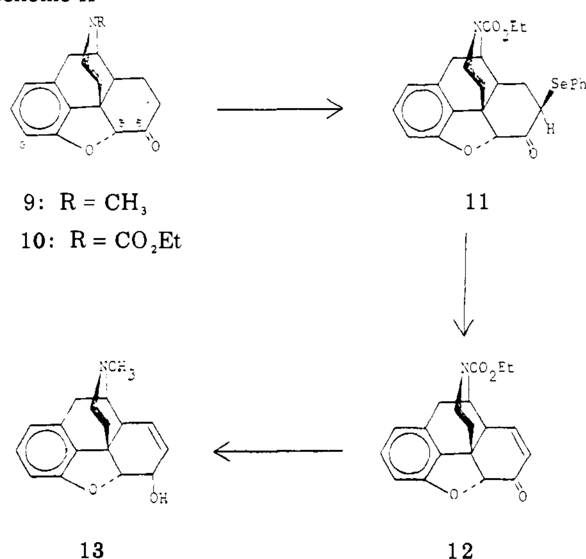
Scheme II



codeine] and was secured by catalytic hydrogenation to the dihydro derivative, which proved to be identical with **3**, prepared from authentic dihydromorphine (**1**).

Results and Discussion

The effect of the removal of the phenolic hydroxy group can be seen by comparison of the antinociceptive activities and receptor binding affinities of the related compounds, shown in Table I. Antinociceptive activity was determined by the hot-plate assay[16-18] (in mice, sc injection) and the Nilsen assay[19] (in mice, sc injection). The affinities of the compounds for the opiate receptor from rat brain homogenates were measured under standard conditions (i.e., 0.32 M sucrose–0.01 M Tris, pH 8.0, at 37 °C).[20]

3-Deoxymorphine (**13**) had one-eighth the in vivo potency and one-thirtieth the binding affinity of morphine. However, 3-deoxydihydromorphine (**3**) retained almost all of the antinociceptive activity but only about one-thirtieth the binding affinity of dihydromorphine. Another deoxy compound which was as potent as, or more potent than, morphine was the 3,6-dideoxydihydromorphine (**5**). Even this compound, when compared with its phenolic relative 6-deoxydihydromorphine, showed a large decrease in in vivo potency. The degree of loss in antinociceptive activity was variable, depending on the particular compound, but receptor binding affinity decreased substantially in all of the 3-deoxy opioids.

Although it has been theorized that the effect of the oxide bridge in the morphine series was, at best, not harmful to in vivo activity[21] and, perhaps, caused a loss in potency,[22] it is apparent that the oxide bridge actually causes an increase in antinociceptive potency. The two compounds from which this observation was made were the 3,6-dideoxydihydromorphine (**5**) and the *N*-methylmorphinan. Further, the 3,6-dideoxy compound binds relatively well to the opiate receptor, for a rigid multicyclic opioid which lacks a phenolic hydroxyl group. Evidently, binding to the opiate receptor need not be via hydrogen bonding with the phenolic hydroxyl, although that obviously does aid in the binding, but perhaps through some sort of interaction with the aromatic π-electron system as one point of attachment. The 6α-hydroxyl group apparently hinders binding to the receptor and causes a loss in in vivo activity. A direct, quantitative, relationship does not exist between the antinociceptive potency of these compounds and their receptor binding affinities. Some of these compounds will be further investigated to determine whether the opioid pattern of side effects exists

Table I. Antinociceptive Activities and Binding Affinity of Deoxy Opioids and Related Compounds

compd[a]	ED_{50}[b,c]	ED_{50}[c,d]	EC_{50}[e]
7	45.3 (35.7–57.9)	67.8 (41.4–110.6)	60
3	4.2 (2.8–6.4)	8.1 (5.8–11.4)	90
13[f]	8.6 (6.7–11.2)	30.9 (22.3–42.3)	100
9	1.8 (1.3–2.6)	2.8 (2.0–3.9)	20.0
8	44.0 (33.5–58.0)	59.9 (40.0–90.6)	100
5	1.3 (0.89–1.85)	4.4 (3.8–5.1)	10.0
morphine[g]	3.3 (2.5–4.4)	4.1 (2.9–5.6)	3.0
6-deoxydihydromorphine[g]	0.14 (0.11–0.20)		0.6
dihydromorphine	2.5 (1.5–3.4)		3.0
dl-*N*-methylmorphinan[h]	~16.5[i]		
levorphanol[j]	0.5 (0.3–0.7)	0.5 (0.4–0.7)	0.7
dihydromorphinone	0.4 (0.3–0.5)	0.7 (0.5–0.9)	1.0
nalorphine	104.6 (78.1–140.3)	13.8 (7.8–24.5)	1.5

[a] Hydrochloride salt, unless otherwise specified. [b] Hot-plate assay,[16-18] subcutaneous injection, in mice, μmol/kg. [c] Parenthesized numbers are 95% SE limits as obtained from probit analysis. [d] Nilsen assay,[19] subcutaneous injection, in mice, μmol/kg. [e] Binding affinity to rat brain homogenate,[20] nmol. [f] Base. [g] Sulfate. [h] Phosphate. [i] Adjusted to accord with data from newer NIH general-purpose mice.[17] [j] Tartrate.

in the 3-deoxy and 3,6-dideoxy series.

Thus, the phenolic hydroxyl group generally has a greater effect on binding to the opiate receptor than on antinociception. It appears to greatly increase the binding affinity of 6α-hydroxy-substituted morphine-like compounds for the opiate receptor but generally causes a more variable, and less pronounced, effect on antinociceptive activity. We have unequivocally demonstrated that the phenolic group in these morphine-like compounds is not essential for effective antinociception. In view of this observation, this readily available class of antinociceptive agents appears to be worthy of further study. The presence of the phenolic group in the morphines, which allows better binding to the opiate receptor, could influence other biochemical parameters, such as bioavailability, biotransformation, excretion, and metabolism and could conceivably turn out to be a disadvantage for its use as an analgesic in man.

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover melting point apparatus and are corrected. Elemental analyses, listed in Table I, were performed by the Section on Microanalytical Services and Instrumentation of this Laboratory. IR and mass spectra were obtained on a Perkin-Elmer 257 and Hitachi Perkin-Elmer RMU-6E spectrometer, respectively. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. NMR spectra were obtained using a Varian A-60 or HR 220 spectrometer with tetramethylsilane (δ 0.0) as internal reference. Organic extracts were dried using anhydrous Na_2SO_4.

3-(1-Phenyl-1*H*-5-tetrazolyl)dihydromorphine (2). A mixture of 14.4 g (50 mmol) of dihydromorphine (**1**), 10.8 g (60 mmol) of 5-chloro-1-phenyl-1*H*-tetrazole, and 13.5 g (98 mmol) of anhydrous K_2CO_3 in 250 mL of DMF was stirred for 24 h at room temperature. The mixture was diluted with water, and the

gum which separated was extracted into Et_2O. After washing with 5% NaOH, the Et_2O solution was extracted with dilute HCl and the ether was discarded. The aqueous acidic solution was basified with NH_4OH and the gum which separated was extracted into Et_2O. The Et_2O solution was dried and evaporated to give 19.4 g (90%) of **2** as a white solid which could not be recrystallized. Purification of **2** was accomplished by formation of the oxalate salt (mp 140–145 °C from EtOH). Basification of an aqueous solution of the salt gave pure **2** as a white solid: mp 98–100 °C; MS M^+ 431; $[\alpha]^{21}_D$ 170° (c 0.5, $CHCl_3$). Anal. ($C_{24}H_{25}N_5O_3$) C, H, N.

3-Deoxydihydromorphine (3). A mixture of 4.00 g (9.26 mmol) of **2** and 2.5 g of 5% Pd on C in 250 mL of AcOH was hydrogenated (60 psig, 25 °C) until TLC ($CHCl_3$–MeOH–NH_4OH, 89:10:1; silica gel GF) indicated that **2** had completely reacted (24 h). The catalyst was filtered and washed with AcOH. The combined filtrate and washings were evaporated to a residue which was partitioned between Et_2O and excess 5% NaOH. The Et_2O was separated, dried, and evaporated to give 2.3 g (92%) of **3** as a viscous, nearly colorless oil which was converted to the oxalate, mp 220–220.5 °C (from EtOH–H_2O, 10:1) (lit.[3] mp 216–217 °C). Conversion of the above oxalate to the base gave a colorless oil, which was crystallized from hexane–Et_2O to give 2.10 g (84%) of **3**: mp 107–108.5 °C; $[\alpha]^{21}_D$ −191.5° (c 0.5, $CHCl_3$) [lit.[3] mp 102–104 °C; $[\alpha]^{21}_D$ −188° (c 0.5, $CHCl_3$)]; MS M^+ 271. Anal. ($C_{17}H_{21}NO_2$) C, H, N.

Treatment of an acetone solution of **3** with HCl gas gave **3**·HCl as a white crystalline solid, mp 291 °C dec. Anal. ($C_{17}H_{21}NO_2$·HCl) Cl.

3-Deoxy-6-*O*-(*p*-tolylsulfonyl)dihydromorphine (4). To a cooled solution of 4.30 g (15.9 mmol) of **3** in 25 mL of dry pyridine was added 4.0 g (21.0 mmol) of *p*-toluenesulfonyl chloride in portions. The red mixture was stirred for 3 days at 25 °C, diluted with 170 mL of cold H_2O, and treated with excess $NaHCO_3$. The mixture was extracted throughly with Et_2O, and the combined extracts were backwashed with H_2O and dried. Evaporation left a red residue, which gave 4.5 g (67%) of pure **4** after repeated recrystallization from hexane–benzene: mp 123 °C; $[\alpha]^{21}_D$ −216° (c 0.5, $CHCl_3$). Anal. ($C_{24}H_{27}NO_4S$) C, H, N.

3,6-Dideoxydihydromorphine (5). A solution of 0.30 g (8 mmol) of LAH in 100 mL of dry THF was added to a solution of 3.1 g (7.3 mmol) of **4** in 100 mL of dry THF. The mixture was refluxed for 6 h and then stirred overnight at 25 °C. After the addition of 100 mL of Et_2O, several milliliters of H_2O was added. The insoluble material was filtered and washed with 3 × 200 mL of Et_2O, and the combined filtrate and washings were extracted with 3 × 25 mL of 3 N HCl. The aqueous extract was rendered alkaline with 10% NaOH, and after several minutes white crystalline material separated, which was filtered, washed with H_2O, and dried to give 746 mg (40%) of essentially pure **5** which was sublimed at 110 °C (0.5 mmHg) to give analytically pure material: MS M^+ 255; mp 132–133 °C; $[\alpha]^{21}_D$ −140° (c 0.5, $CHCl_3$). Anal. ($C_{17}H_{21}NO$) C, H, N.

Treatment of **5** in acetone with HCl gas gave **5**·HCl (78%), mp 254–255 °C dec. Anal. ($C_{17}H_{21}NO$·HCl) Cl.

3-Deoxydihydronormorphine (7). Using the method previously described[9] in the 3-substituted series, a mixture of 4.4 g (1.63 mmol) of **3**, 18.5 g (130 mmol) of $KHCO_3$, and 16.3 g (100 mmol) of phenyl chloroformate in 280 mL of $CHCl_3$ was refluxed, with stirring, for 20 h. Filtration and evaporation of the $CHCl_3$ in vacuo left a liquid mixture which was deaerated with nitrogen for 10 min and then treated with 18 mL of 64% hydrazine plus 25 mL of 95% hydrazine. While continuing to pass nitrogen through the solution, it was refluxed for 8.5 h and then treated with 15 mL of H_2O. The mixture was evaporated to dryness, and the residue was dissolved in $CHCl_3$ and washed with 5% NaOH and saturated NaCl solution. Evaporation of the dried $CHCl_3$ solution yielded 3.54 g (84%) of nearly pure **7**, mp 180–181 °C. Recrystallization from EtOAc yielded 3.1 g (74%) of **7**: mp 185–186 °C; $[\alpha]^{21}_D$ −190° (c 0.5, $CHCl_3$); MS M^+ 257. Anal. ($C_{16}H_{19}NO_2$) C, H, N.

Treatment of an acetone solution of **7** with HCl gas gave 89% of **7**·HCl·H_2O: mp 280–281 °C. Anal. ($C_{16}H_{19}NO_2$·HCl·H_2O) C, H, N.

***N*-Allyl-3-deoxydihydronormorphine (8).** A mixture of 2.32 g (9 mmol) of 3-deoxydihydronormorphine (**7**), 1.13 g (9.3 mmol) of allyl bromide, and 3.0 g (21.7 mmol) of K_2CO_3 in 30 mL of DMF was heated and stirred at 100 °C for 2 h. After cooling the mixture, the inorganic material was filtered and washed with $CHCl_3$, and the combined filtrate and washings were evaporated. The residue was dissolved in $CHCl_3$, washed with water, and dried. Evaporation of the solvent left a yellow residue, which was dissolved in boiling hexane, decanted from insoluble material, and evaporated to yield 2.04 g (77%) of **8** as a nearly colorless oil: MS M^+ 297; $[\alpha]^{21}_D$ −193.2° (c 0.5, $CHCl_3$). Treatment of an acetone solution of oily **8** with HCl gas gave 83% of **8**·HCl·H_2O as a white crystalline solid: mp 217–218 °C. Anal. ($C_{19}H_{23}NO_2$·HCl·H_2O) Cl.

3-Deoxydihydromorphinone (9). A solution of 11.9 g (43.9 mmol) of **3** and 80.0 g (439 mmol) of benzophenone in 120 mL of dry benzene was added dropwise, with stirring, over 15 min to a slurry of 14.8 g (132 mmol) of potassium *tert*-butoxide in 480 mL of benzene under N_2.[13] After heating at 85–90 °C for 50 min, the mixture was cooled and acidified with 250 mL of 3 M HCl. The aqueous layer was removed and combined with two acidic (3 M HCl) washes of the organic layer. The aqueous solution was washed with two portions of ether (discarded), chilled in ice, and basified with concentrated NaOH. The insoluble material was extracted repeatedly with EtOAc, and the combined extracts were washed with saturated NaCl, dried, and evaporated to give 11.2 g (95%) of **9** as pale yellow crystals. Recrystallization (EtOAc) provided an analytical sample: mp 241.5–245 °C dec (lit.[23] mp 246–247 °C); MS M^+ 269; $[\alpha]^{21}_D$ −250.6° (c 1.06, MeOH); IR (Nujol) 1715 cm^{-1} (C=O). Anal. ($C_{17}H_{19}NO_2$) C, H, N.

3-Deoxy-*N*-carbethoxydihydronormorphinone (10). A solution of 13.6 g (125 mmol) of ethyl chloroformate in 45 mL of benzene was added dropwise to a boiling solution of 11.2 g (41.6 mmol) of **9** in 225 mL of benzene. After refluxing the solution for 8 h, an additional 6.81 g (62.8 mmol) of ethyl chloroformate was added and heating continued for 8 h. The cooled reaction mixture was then washed with 10% HCl (two times), H_2O, saturated $NaHCO_3$, and saturated NaCl, dried, and evaporated. Recrystallization (EtOAc) yielded 7.5 g of **10** as cream-colored crystals: mp 135–136.6 °C; MS M^+ 327; NMR ($CDCl_3$) δ 1.28 (t, 3 H, J = 8 Hz, CH_3), 4.16 (q, 2 H, J = 8 Hz, CO_2CH_2), 4.64 (s, 1 H, C-5 H), 6.76 (m, 2 H, C-1, C-2 H), 7.11 (m, 1 H, C-3 H); IR (Nujol) 1714 (C=O), and 1685 (>NCO–) cm^{-1}. Anal. ($C_{19}H_{21}NO_4$) C, H, N.

An additional 3.7 g of product (mp 134.5–136.5 °C) was obtained by evaporating the mother liquors to a small volume and adding Et_2O (total yield 82%).

3-Deoxy-*N*-carbethoxy-7-(phenylseleno)dihydronormorphinone (11). A solution of 7.00 g (21.4 mmol) of **10**, 4.93 g (25.7 mmol) of C_6H_5SeCl, and 0.40 mL of 37% HCl in 110 mL of EtOAc was stirred at 25 °C.[15] After 5 h, the reaction mixture was washed with H_2O, saturated $NaHCO_3$, and saturated NaCl, dried, and evaporated. Recrystallization (EtOH/octane) yielded 8.9 g (86%) of **11** as pale yellow crystals. An analytical sample was obtained by filtration of this material through silica gel (60% Et_2O/petroleum ether) and recrystallization (EtOH/octane): mp 151.5–154.5 °C; MS M^+ 483; NMR ($CDCl_3$) δ 1.27 (t, 3 H, J = 8 Hz, CH_3), 3.77 (dd, 1 H, J = 2.8 and 2.8 Hz, H-7), 4.15 (q, 2 H, J = 8 Hz, CO_2CH_2–), 5.34 (s, 1 H, C-5 H), 6.70 (m, 2 H, C-1 and C-2 H), 7.07 (m, 1 H, C-3 H), 7.30 and 7.44 (2 m, 5 H, SeC_6H_5); IR (Nujol) 1704 (>C=O) and 1682 (>NHCO) cm^{-1}. Anal. ($C_{25}H_{25}NO_4Se$) C, H, N.

3-Deoxy-*N*-carbethoxynormorphinone (12). A solution of 8.90 g (18.4 mmol) of **11** in 175 mL of EtOAc was mixed with a slurry of 39.3 g (184 mmol) of $NaIO_4$ in 215 mL of H_2O and stirred vigorously at 25 °C for 2 h. Water was added, the organic layer was separated, and the aqueous layer was extracted with 100 mL of EtOAc. The combined organic material was washed with H_2O, 10% $NaHCO_3$ and saturated NaCl, dried, and evaporated. Recrystallization (EtOAc) yielded 2.20 g of **12** as white crystals. An additional 0.9 g of **12** was obtained from the mother liquor (total yield 52%). The analytical sample was prepared by preparative TLC (silica gel, 40% EtOAc/hexane, three developments), followed by Darco treatment, filtration through a short silica column (50% EtOAc/hexane), and recrystallization from 50% EtOAc/hexane. The desired α,β-unsaturated ketone was obtained as white crystals: mp 164–167 °C; MS M^+ 325; NMR ($CDCl_3$) δ 1.28 (t, 3 H, J = 8 Hz, CH_3), 4.16 (q, 2 H, J = 8 Hz,

CO_2CH_2–), 4.68 (s, 1 H, C-5), 6.10 (dd, 1 H, $J_{7,8}$ = 10 Hz, $J_{7,14}$ = 3 Hz, C-7 H), 6.67 (m, 3 H, C-1, C-2, C-8 H), 7.06 (m, 1 H, C-3 H); IR (Nujol) 1685 (both >CO) cm^{-1}. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

3-Deoxymorphine (13). A solution of 3.10 g (9.54 mmol) of 12 in 30 mL of dry THF was added dropwise, with stirring, to a slurry of 3.63 g (95.4 mmol) of $LiAlH_4$ in 90 mL of THF under argon. After heating the solution at reflux for 24 h, the reaction was chilled in ice and quenched by the careful addition of 3.60 mL of H_2O, 3.60 mL of 15% NaOH, and 10.8 mL of H_2O. The solid which separated was removed by filtration and washed well with THF, and the combined filtrate and washings were evaporated. Column chromatography (silica gel, $CHCl_3$–MeOH–NH_4OH, 89:10:1/$CHCl_3$, 60:40) followed by recrystallization (EtOH) provided 1.54 g (60%) of **13** as white crystals: mp 227.5–229 °C; MS M^+ 269; $[\alpha]^{21}_D$ –218° (*c* 1.00, MeOH); NMR ($CDCl_3$) δ 2.45 (s, 3 H, NCH_3), 4.16 (br s, 1 H, C-6 H), 4.83 (d, 1 H, *J* = 7 Hz, C-5 H), 5.28 (m, 1 H, C-8 H), 5.66 (d, 1 H, *J* = 8 Hz, C-7 H), 6.57 (m, 2 H, C-1, C-2 H), 6.98 (m, 1 H, C-3 H); IR (Nujol) 3555 cm^{-1} (OH). Anal. ($C_{17}H_{19}NO_2$) C, H, N.

The hydrochloride of **13** had mp 269–273 °C dec. Anal. ($C_{17}H_{20}ClNO_2$) C, H, N, Cl.

References and Notes

(1) Guest scientist from the Pharmaceutical Division of Hoechst AG., Frankfurt, Federal Republic of Germany.
(2) A. Brossi, M. F. Rahman, K. C. Rice, M. Gerecke, R. Borer, J. P. O'Brien, and S. Teitel, *Heterocycles*, 7, 277 (1977), and references therein.
(3) The first preparation of 3-deoxydihydromorphine, without mention of its remarkable analgesic activity, was reported by R. Bognâr, Gy. Gaâl, P. Kerekes, G. Horvâth, and M. T. Kovacs, *Org. Prep. Proced. Int.*, 6, 305 (1974).
(4) A. K. Reynolds and L. O. Randall, "Morphine and Allied Drugs", University of Toronto Press, Canada, 1957, p 6.
(5) N. B. Eddy and E. L. May, *Science*, **192**, 410 (1973).
(6) E. L. May and L. S. Sargent, "Analgetics", Monograph on Medicinal Chemistry, Vol. 5, G. de Stevens, Ed., Academic Press, New York, 1965, p 142.
(7) G. D. Smith and J. F. Griffin, *Science*, **199**, 1214 (1978).
(8) J. W. Lewis and M. J. Readhead, *J. Med. Chem.*, **13**, 525 (1970).
(9) K. C. Rice and E. L. May, *J. Heterocycl. Chem.*, **14**, 665 (1977).
(10) I. Iijima, J. Minamikawa, A. E. Jacobson, A. Brossi, and K. C. Rice, *J. Med. Chem.*, **21**, 398 (1978).
(11) I. Iijima, K. C. Rice, and J. V. Silverton, *Heterocycles*, **6**, 1157 (1977).
(12) I. Iijima, J. Minamikawa, A. E. Jacobson, A. Brossi, and K. C. Rice, *J. Org. Chem.*, **43**, 1462 (1978).
(13) H. Rapoport, R. Naumann, E. R. Bissell, and R. M. Benner, *J. Org. Chem.*, **15**, 1103 (1950).
(14) D. D. Weller and H. Rapoport, *J. Med. Chem.*, **19**, 1171 (1976).
(15) H. J. Reich, J. M. Renga, and I. L. Reich, *J. Am. Chem. Soc.*, **97**, 5434 (1975).
(16) N. B. Eddy and D. Leimbach, *J. Pharmacol. Exp. Ther.*, **107**, 385 (1953).
(17) A. E. Jacobson and E. L. May, *J. Med. Chem.*, **8**, 563 (1965); see ref 9 therein.
(18) L. Atwell and A. E. Jacobson, *Lab Anim.*, **7**, 42 (1978).
(19) T. D. Perrine, L. Atwell, I. B. Tice, A. E. Jacobson, and E. L. May, *J. Pharm. Sci.*, **61**, 86 (1972).
(20) W. A. Klee and R. A. Streaty, *Nature (London)*, **248**, 61 (1974).
(21) A. E. Jacobson, *Handb. Psychopharmacol.*, **12**, 47 (1978).
(22) L. F. Small, N. B. Eddy, E. Mosettig, and C. K. Himmelsbach, *Public Health Rep.*, **138**, 25 (1938).
(23) Y. Sawa, R. Maeda, and J. Irisawa, U.S. Patent 3 707 470, Dec. 26, 1972.

Metabolic Oxidation of Nicotine to Chemically Reactive Intermediates

Trong-Lang Nguyen, Larry D. Gruenke, and Neal Castagnoli, Jr.*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.

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Studies on the metabolism of nicotine by rabbit liver microsomal fractions in the presence of 0.01 M sodium cyanide have led to the characterization of two isomeric cyanonicotine compounds. The locations of the cyano groups were established by GC–EIMS analyses of the deuterium-labeled products obtained from the specifically deuterium-labeled substrates (*S*)-nicotine-5′,5′-d_2, (*R*,*S*)-nicotine-2′,5′,5′-d_3, and (*R*,*S*)-nicotine-*N-methyl*-d_3. One cyano adduct was shown to be 5′-cyanonicotine, a product previously isolated from similar microsomal preparations. The second cyano adduct was shown to be *N*-(cyanomethyl)nornicotine; this structure assignment was confirmed by synthesis. Formation of *N*-(cyanomethyl)nornicotine appears to occur, at least in part, without prior nitrogen–carbon bond cleavage, implicating the in situ generation of the *N*-methyleniminium species during the course of metabolic oxidative N-demethylation of nicotine.

Many of the biotransformation processes observed in the metabolism of xenobiotics are mediated by hepatic mixed-function oxidases with cytochrome P-450 as the terminal oxidase. Metabolic oxidations of aliphatic amines often occur at carbon atoms bonded to nitrogen, presumably with the initial formation of chemically unstable carbinolamines which may undergo spontaneous carbon–nitrogen bond cleavage to the corresponding dealkylated amines and aldehydes or ketones.[2] The resulting aldehydes may then suffer a further two-electron oxidation to yield carboxylic acids, or in the case of cyclic amines, lactams.[3] According to the proposed pathway, the three unique carbon atoms of nicotine (**1**) bonded to the pyrrolidine nitrogen atom should form the carbinolamines **2–4** as the initial metabolic products (Scheme I). Cotinine (**5**) and nornicotine (**6**), major and minor mammalian metabolites of nicotine,[4] presumably arise via the carbinolamines **2** and **3**, respectively. To date there have been no reports of mammalian metabolites arising from initial hydroxylation at the 2′ position of nicotine.

Evidence for the intermediacy of 5′-hydroxynicotine (**2**) in the biotransformation of **1** to **5** has been reported. In 1960, Hucker et al.[5] showed that rabbit liver microsomal preparations convert **1** to **5** and proposed the carbinolamine **2** as an intermediate which is further metabolized by a soluble aldehyde oxidase to **5**. By blocking the aldehyde oxidase system with cyanide ion, these workers claimed evidence of an equilibrium mixture of **2** and the open-chain amino aldehyde **7** which could be trapped as its 2,4-DNP derivative.

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