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Preparation of Spin-Labeled Opiates: Morphine and Codeine

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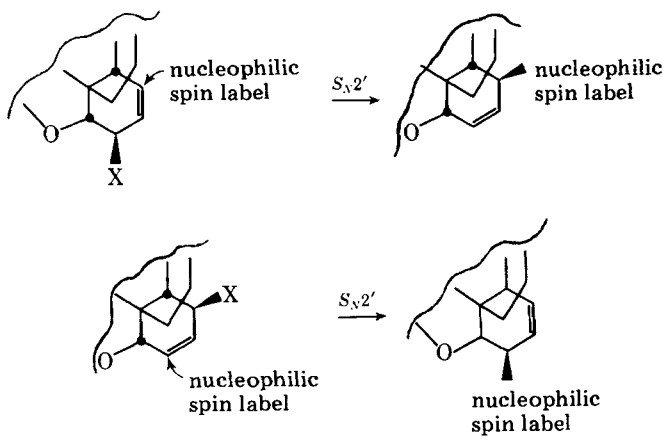
Abstract □ The preparation of 6-spin-labeled codeine and morphine is described. Treatment of either 6-chlorocodide or 8-bromocodide with 4-amino-2,2,6,6-tetramethylpiperidino-1-oxyl free radical in dimethylformamide afforded 6-spin-labeled codeine. Similar treatment of 6-chloromorphide afforded 6-spin-labeled morphine. Exclusive formation of the 6-isomer in these reactions is explained by halide-ion-catalyzed isomerization of the 6-halo opiate to the 8-halo isomer followed by a normal S_N2' displacement of the halogen. Both spin-labeled compounds displayed weak *in vivo* analgesic activity and did not bind appreciably to receptors in brain homogenate.

Keyphrases □ Spin-labeled opiates—morphine and codeine labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Morphine—spin labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Codeine—spin labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Opiates—morphine and codeine, spin labeled in 6-position, effect on binding to brain receptors and analgesic activity □ Analgesics, narcotic—morphine and codeine, spin labeled in 6-position, effect on binding to brain receptors and analgesic activity

In numerous attempts to identify and characterize the opiate receptor (1–4), the binding characteristics of pharmacologically active and inactive isomers were compared or the reduced agonist binding in the presence of antagonists was noted using radiolabeled opiates with high specific activity. The spin label method (5, 6) is an attractive alternative to the radioisotope method for studying this drug–receptor interaction *in vitro*, since it obviates the need for separating bound from unbound drug. A preliminary report demonstrated the feasibility of this method (7).

DISCUSSION

Essential to the success of this approach is the requirement that the nitroxide label not constitute a significant perturbation to the interaction of the drug with its receptor. As such, the label should be attached to a pharmacologically unimportant position in the drug molecule. For purposes of stability, the label should be covalently attached to the drug *via* a linkage resistant to hydrolytic enzymes and inert to aqueous media at physiological pH. Finally, it is desirable, although not essential, that the labeled drug possess biological activity similar to that of the parent drug.



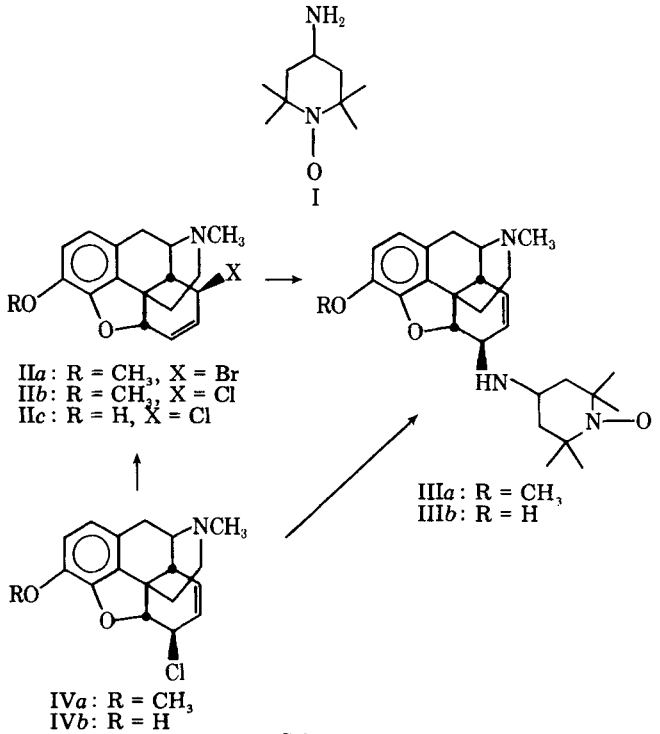
Scheme I

With these requirements in mind, the preparation of spin-labeled derivatives of morphine and codeine suitable for the *in vitro* study of the interaction of these drugs with possible receptors was attempted.

The hydroxyl group at the 6-position of morphine and codeine appeared to offer a suitable point for attachment for the spin label, particularly in view of the relative unimportance of this position in the pharmacological activity of these drugs (8, 9). However, the steric inaccessibility of this alcohol (10, 11) precluded all attempts to alkylate at this position[1]. Consequently, the mode of reaction was reversed by using a nucleophilic spin label to displace halogen from a 6- or 8-halo opiate (Scheme I). In the isomeric halocodides and morphides, the halogen atom has a beta configuration (*syn* to the ethylamino bridge) and is displaced by nucleophiles exclusively from the beta side in an S_N2' fashion (13). Following this precedent, the reaction of the isomeric halocodides and morphides with 4-amino-2,2,6,6-tetramethylpiperidino-1-oxyl free radical (I) as a route to spin-labeled morphine and codeine was investigated.

Reaction of I with 8-bromocodide (IIa) in dimethylformamide afforded the expected 6-spin-labeled codeine (IIIa) in 23% yield (Scheme II).

[1] A recent report (12) described the selective methylation of morphine at the 6-position *via* its dipotassium salt. A similar approach to introduce a spin label at the 6-position was attempted; however, with alkylating agents much larger than methyl, reaction occurred exclusively at the 3-position.



Scheme II

Similar reaction of I with 6-chlorocodide (IV*a*) produced a spin-labeled codeine in 10% yield identical in all respects to III*a* (see *Experimental*). This apparently anomalous result is accounted for by the fact that isomerization of IV*a* to the more stable 8-chlorocodide (II*b*) occurred more rapidly than did the reaction with the spin label[2]. While IV*a* did not isomerize to II*b* under the reaction conditions in the absence of added nucleophile, addition of a small amount of chloride ion to the mixture resulted in rapid conversion of IV*a* to II*b*. Thus, chloride ion generated from the initial reaction of I with IV*a* catalyzed the isomerization of the halogen to the 8-position. The 8-chlorocodide thus produced underwent a normal S_N2' reaction to produce III*a*.

In an analogous fashion, 6-chloromorphide (IV*b*) reacted with I to yield 22% of 6-spin-labeled morphine (III*b*). The structure of III*b* was confirmed by elemental and spectral analyses (see *Experimental*) and its conversion to III*a* on methylation with diazomethane. Interestingly, the chloromorphide recovered from this reaction was exclusively the 8-isomer, II*c*, verifying that halogen migration occurred during the reaction.

The low yields of spin-labeled products were due mostly to low conversions (yields based on recovered starting materials were substantially higher). An evident side reaction was the elimination of hydrogen halide from the halo opiates to produce dehydromorphine and codeine derivatives. No attempt was made to ascertain whether the dehydro derivatives were the 5,7- or 6,8-diene isomers.

The spin-labeled derivatives prepared in this study showed only marginal *in vivo* analgesic activity in the Eddy hot plate test (14, 15) and displayed weak *in vitro* binding to receptors in brain homogenate (P_2 fraction) by the method of Klee and Streaty (16). There was a small but noticeably greater potency of the labeled morphine over the labeled codeine[3]. These results, while disappointing, do not preclude the use of these compounds for studying *in vitro* binding to receptors in brain homogenate or subfractions thereof by the spin label method.

EXPERIMENTAL[4]

General—When reaction mixtures were to be separated and analyzed, a 1.9-m (6-ft), 2-mm i.d. glass column packed with 3% OV-17 on 60–80-mesh Gas Chrom Q[5] was operated in the 200–240° range with a helium carrier gas flow of 25 ml/min. The gas chromatograph was interfaced to the mass spectrometer by a permeable silicone rubber membrane separator maintained at 240°. Samples that were pure or not amenable to GLC–mass spectrometry were introduced directly into the mass spectrometer ion source *via* a direct insertion probe with a sample heater, which was varied from ambient to 250° depending on the compound.

For electron-impact ionization, the following conditions were employed: ion source, 180°; ionizing current, 0.15 mamp; and electron energy, 70 ev. Chemical-ionization mass spectra were obtained with an ion source pressure of 0.35 torr methane[6] (instrument grade, 99.7%), an ionizing current of 0.15 mamp, and an electron energy of 75 ev.

4-Amino-2,2,6,6-tetramethylpiperidino-1-oxyl (I) was obtained commercially[7]. 6-Chlorocodide, 8-bromocodide, and 6-chloromorphide were prepared as described previously (17, 18).

Preparation of 6-Spin-Labeled Codeine (III*a*)—*Reaction of I with II*a—A mixture of 2.8 g (7.8 mmoles) of II*a*, 1.5 g (9.0 mmoles) of I, and 1.1 g (8.0 mmoles) of solid potassium carbonate in dimethylformamide (20 ml) was heated in an oil bath at 90–95° for 4.5 hr. The cooled mixture was diluted with an equal volume of water, and the resulting suspension was extracted with 3 × 50 ml of ether. The combined ether extracts were washed with brine and then dried (sodium sulfate). Evaporation of solvent afforded 2.2 g of a residue, which was chromatographed on 75 g of silica gel[8] (100–200 mesh) and eluted with a chloroform–methanol gradient. Fractions were monitored by TLC [silica gel, chloroform–methanol (10:1)].

The first component eluted was a dehydrocodeine derivative; mass spectrum: *m/e* 281 (M^+, 100), 280 (67.3), 266 (45.0), 264 (11.9), 238 (14.9), 223 (11.4), 220 (11.9), 165 (17.8), 152 (19.3), and 41 (13.4). The second component eluted was III*a*, which was obtained as an oil. The oil crystallized from ether–petroleum ether as a tan solid, 970 mg (27%), mp 66–70°; mass spectrum: *m/e* 150, 453 (M + H[9], 15.3), 452 (M, 1.0), 437 (2.9), 420 (2.3), 379 (51.0), 366 (9.3), 323 (7.9), 308 (15.0), 282 (100), 266 (10.9), 192 (36.7), and 154 (25.4); methane chemical-ionization mass spectrum: *m/e* 250, 454 (M + 2H, 68.5), 453 (M + H, 100), 438 (8.3), 436 (13.4), 379 (13.2), and 282 (10.9). The electron spin resonance spectrum of III*a* exhibited the usual three-line pattern for a nitroxide derived from tetramethylpiperidine.

Anal.—Calc. for $C_{27}H_{38}N_3O_3$: C, 71.65; H, 8.46; N, 9.28. Found: C, 71.44; H, 8.51; N, 9.31.

*Reaction of I with IV*a—A mixture of 2.5 g (7.8 mmoles) of IV*a*, 1.5 g (9.0 mmoles) of I, and 1.1 g (8.0 mmoles) of potassium carbonate in dimethylformamide (20 ml) was heated in an oil bath at 110° for 8 hr. By using the same workup and chromatographic procedures described in the preceding experiment, 383 mg (10%) of III*a* was obtained as a tan solid, mp 65–68°. That this substance was indeed III*a* was ascertained by the following comparisons. The IR and mass spectra were superimposable on the spectra obtained for III*a*. TLC on silica gel using six different solvent systems demonstrated identical R_f values for this substance and III*a*. Finally, the melting point of this substance was not depressed on admixture with III*a*.

Isomerization of IV*a* to II*b*—A solution of 50 mg of IV*a* in 1 ml of dimethylformamide was heated at 90° for 1.5 hr. The IR spectrum of the recovered material was identical to that of the starting material. This experiment was repeated except that 20 mg of sodium chloride was added to the mixture. The IR spectrum of the recovered material indicated that 50% of IV*a* had isomerized to II*b*.

Preparation of 6-Spin-Labeled Morphine (III*b*)—A solution of 700 mg (2.4 mmoles) of IV*b* and 855 mg (5 mmoles) of I in dimethylformamide (10 ml) was stirred in an oil bath at 90–100° for 20 hr. The cooled solution was diluted with 25 ml of chloroform, and the resultant solution was extracted with 3 × 25 ml of 3 *N* NaOH. The combined aqueous extracts were adjusted to pH 8.5 by the slow addition of cold, concentrated hydrochloric acid, and the resultant slurry was extracted with 3 × 50 ml of chloroform. The combined chloroform extracts were dried (sodium sulfate), and the solvent was removed to afford 2.6 g of a residue containing dimethylformamide.

The residue was chromatographed on 75 g of silica gel (100–200 mesh) using a chloroform–methanol gradient. Fractions were monitored by TLC

[2] Piperidine displaces halide ion from the haloopiates much more rapidly than I (13).

[3] In the *in vitro* assay (16), III*a* was about 1/500th and III*b* about 1/350th as potent as morphine.

[4] Melting points were determined on a Thomas-Hoover melting-point apparatus. IR spectra were recorded on a Beckman IR-5A spectrophotometer using potassium chloride pellets or sodium chloride solution cells. Mass spectra were obtained on a Hewlett-Packard 5982 A gas chromatograph–mass spectrometer system. Electron spin resonance spectra were recorded on a Varian E-9 spectrometer.

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[8] W. R. Grace.

[9] The much more intense than expected M + 1 peak was probably due to hydrogen radical abstraction from water adsorbed in the ion source or glass capillary sample holder by the even electron molecular ion species (19, 20).

[silica gel, chloroform–methanol (4:1)]. Compound III*b*, 960 mg (22%), was obtained as tan flakes from ether–petroleum ether, mp 212–215° dec.; mass spectrum: *m/e* >150, 439 (M + H, 18.9), 438 (M^+, 1.8), 406 (3.8), 365 (74.8), 352 (27.9), 309 (15.3), 268 (100), 266 (12.8), 178 (33.5), and 154 (27.3); methane chemical-ionization mass spectrum: *m/e* >250, 440 (M + 2H, 100), 439 (M + H, 71.1), 424 (27.1), 422 (22.6), 365 (8.3), and 268 (24.0). The electron spin resonance spectrum of III*b* exhibited the usual three-line pattern for a nitroxide derived from tetramethylpiperidine.

Anal.—Calc. for $C_{26}H_{37}N_3O_3$: C, 71.20; H, 8.27; N, 9.58. Found; C, 71.12; H, 8.11; N, 9.81.

A small portion of this material was treated with excess ethereal diazomethane. Evaporation of solvent and excess diazomethane afforded a crystalline residue identical to III*a* in all respects (melting point, IR, TLC, and mass spectral data).

Also isolated from column chromatography of this reaction mixture was a component having an R_f similar to that of IV*b*. This substance was identified as II*c* by its melting point and IR and mass spectra. No IV*b* was eluted from the column.

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Radiochemical GLC Assay for Nortriptyline in Human Plasma

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Abstract □ A novel method was developed for the assay of nortriptyline in plasma. After nortriptyline was extracted, it was acetylated with ^{3}H-acetic anhydride; the quantity of ^{3}H-acetylnortriptyline in the extract was determined by radiochemical GLC. The method is capable of assaying 5 ng of nortriptyline/ml of plasma. The instrumentation was assembled from commercially available components.

Keyphrases □ Nortriptyline—radiochemical GLC analysis, human plasma □ Radiochemical GLC- -analysis, nortriptyline in human plasma □ GLC, radiochemical—analysis, nortriptyline in human plasma □ Antidepressants—nortriptyline, radiochemical GLC analysis in human plasma

The assay of nortriptyline in plasma is important because its clinical activity is related to plasma levels (1, 2), which vary remarkably among individuals even at steady state (3, 4). GLC methods for nortriptyline assay (5–7), although capable of detecting 10–20 ng of compound/ml of plasma, are frequently inadequately sensitive for measuring the levels attained after a single dose. A recently published TLC assay is considerably less sensitive, having a lower limit of 50 ng/ml (8).

Zuleski *et al.* (9) developed a nortriptyline assay method which involved extracting the compound from plasma by a modification of the method of Hammer and Brodie (10), acetylating it with ^{14}C-acetic anhydride, separating acetylnortriptyline by TLC, locating this derivative by radiochromatogram scanning, removing it from the plate, and counting it by scintillation spectrometry. The present approach was more direct. Radiochemical GLC was performed directly following derivatization with ^{3}H-acetic anhydride. Thus, ^{3}H-acetylnortriptyline was resolved and counted simultaneously. Despite the wide applicability of radiochemical GLC to drug metabolism studies, this method has been developed only in a few laboratories (11–13). The present investigation shows that the method can be developed using commercially available components.

EXPERIMENTAL

Reagents—^{3}H-Acetic anhydride (50 mCi/4.36 mg in 5 ml of dry benzene) was received[1] in a sealed ampul. A 0.05-ml aliquot of this so-

[1] New England Nuclear, Boston, Mass.