Extraction of Morphine Enhanced by Addition of *N*-Methyl-3-piperidyl-(*N'N'*)-diphenylcarbamate

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Abstract □ The extractibility of morphine (N-$^{14}CH_3$) into chloroform from aqueous solution was enhanced to completeness by adding *N*-methyl-3-piperidyl-(*N'N'*)-diphenylcarbamate under the following conditions: ratio of *N*-methyl-3-piperidyl-(*N'N'*)-diphenylcarbamate–morphine between 40:1 and 200:1, with morphine concentration no greater than 30 mcg./ml. and the pH between 6.8 and 10. This high extractibility was decreased by concentration of morphine greater than 30 mcg./ml. and by addition of dihydromorphinone, apomorphine, nalorphine, cyclazocine, and naloxone. Thebaine, codeine, methadone, meperidine, levorphanol, dextrophan, and levallorphan did not affect the extractibility. The same qualitative relationships were obtained by using carbon tetrachloride instead of chloroform as the solvent.

Keyphrases □ Morphine (N-$^{14}CH_3$) extraction enhancement—*N*-methyl-3-piperidyl-(*N'N'*)-diphenylcarbamate □ *N*-Methyl-3-piperidyl-(*N'N'*)-diphenylcarbamate—morphine (N-$^{14}CH_3$) interaction □ Countercurrent distribution—analysis □ TLC—analysis □ Scintillometry—analysis

The effect of SK&F 525A and *N*-methyl-3-piperidyl-(*N'N'*)-diphenylcarbamate (MPDC) on the renal metabolism and tubular transport of morphine in the chicken has been recently reported (1). The present investigation concerns the unusual chemical interaction that occurs between morphine and MPDC. The rationale for embarking on this study is found in the preliminary observation that when MPDC was infused along with radioactive morphine into the saphenous vein of the chicken, the free morphine excreted in the urine was more extractible into chloroform than when no MPDC was given (1). Since addition of MPDC directly to urine containing radioactive morphine produced the same increase in extractibility, it was evident that a chemical interaction was occurring between MPDC and morphine. In the present study, the authors examined and characterized the factors involved in producing this unusual interaction between the two bases. Other narcotic analgesics and antagonists also were tested. A preliminary report on these studies has appeared (2).

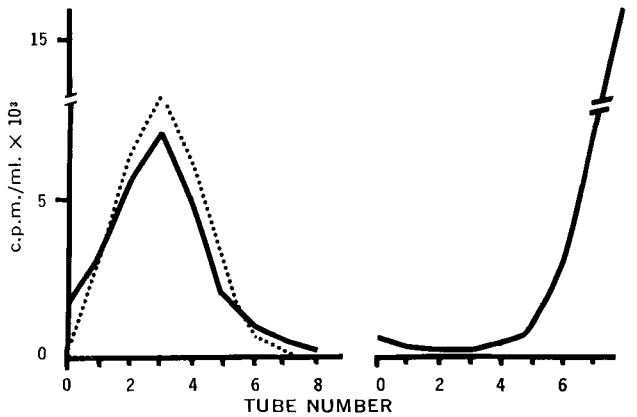

Figure 1—*Left panel is the countercurrent distribution analysis of ^{14}C-morphine. Key: ——, experimental; and . . ., theoretical. Right panel is the countercurrent distribution analysis of ^{14}C-morphine with 10 mcg./ml. MPDC added. Stationary phase, pH 8.5 buffer; mobile phase, chloroform; volume of each phase, 3 ml.*

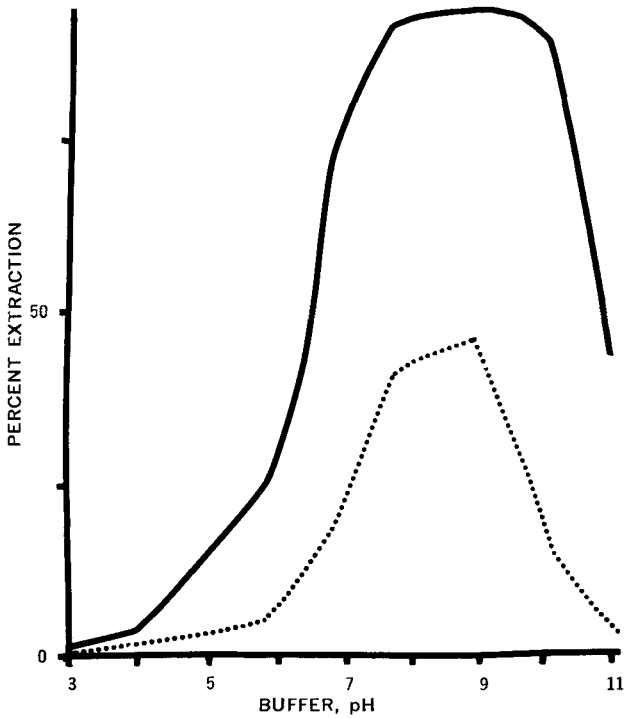

Figure 2—*Extraction of ^{14}C-morphine into chloroform from buffers at different pH's. Key: . . . ., ^{14}C-morphine extraction in absence of MPDC; and ——, ^{14}C-morphine extraction in presence of 5 mcg./ml. of MPDC. Volume of each phase was 3 ml.*

EXPERIMENTAL

Materials—Morphine-N-$^{14}CH_3$ hydrochloride,[1] specific activity 17 mc./mmole, was dissolved in distilled water to make a 0.01 μc./ml. solution. MPDC HCl[2] was used. The solvents used, chloroform, carbon tetrachloride, methylene chloride, ethylene dichloride, ethyl acetate, *n*-butanol, and ethyl ether, were reagent grade (Baker analyzed).

Countercurrent Distribution Analysis—Characterization of ^{14}C-morphine and separation of the ^{14}C-morphine MPDC reaction products were accomplished by using the solvent system previously used for separation of morphine from morphine metabolites in urine samples (1, 3, 4). The solvent system consisted of a sodium bicarbonate (2 g./l.) buffer, pH 8.3–8.5, equilibrated with chloroform. The ^{14}C-morphine, along with MPDC, was added to the system in the buffer phase. Equal volumes of buffer-to-chloroform phases were used; chloroform, the mobile phase, was transferred manually with a syringe equipped with a long needle. One milliliter of each phase was plated and counted in a Tracerlab low background counting system (efficiency of counting was 10% at infinite thinness). Countercurrent analyses were also done with buffers at pH 3; in these instances, sodium phosphate (3 g./l.) buffers were used.

[1] Purchased from Amersham/Searle.
[2] Lakeside Laboratories.

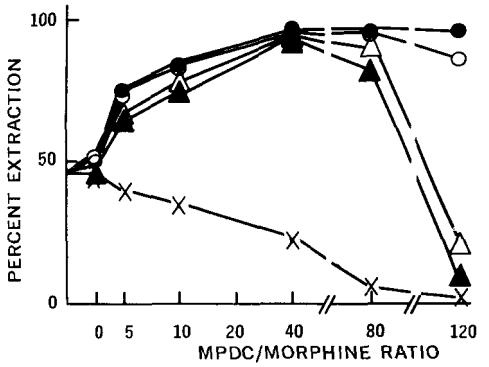

Figure 3—*The extraction of ^{14}C-morphine into chloroform from pH 8.3–8.8 buffer to which 6 (●), 12 (○), 18 (△), 30 (▲), and 80 (×) mcg./ml. morphine and 0–120 times these concentrations of MPDC were added.*

Chromatographic Studies—TLC on Gelman silica gel fiberglass sheets (TLC type SG) was performed with a solvent system consisting of benzene–dioxane–ammonium hydroxide, 5:5:0.1 (v/v/v). The nonradioactive compounds were visualized either by spraying the chromatogram with iodoplatinate reagent (5) or by spraying with sulfuric acid followed by charring the TLC on a hot plate. Radioactive components on the TLC were detected by passing the chromatogram through a 4-pi scanner (Tracerlab).

RESULTS AND DISCUSSION

Countercurrent Distribution of ^{14}C-Morphine with and without MPDC Added—In Fig. 1 the countercurrent analyses performed at pH 8.5 for ^{14}C-morphine are shown to be markedly affected when 10 mcg./ml. of MPDC was added. This addition of MPDC shifted the peak for ^{14}C-morphine from Tube 3 to Tube 8, demonstrating that an increase in the extractibility of ^{14}C-morphine occurred. Although the preliminary observation was that MPDC added to urine containing ^{14}C-morphine altered the extraction of the morphine (1), the present experiment demonstrated that the effect could be obtained in solutions without the presence of urine.

Extractibility of ^{14}C-Morphine with and without Addition of MPDC—*Effect of pH*—A single-extraction procedure was used to expedite the study of several parameters. The extraction of ^{14}C-morphine into chloroform (Fig. 2) was maximal at pH 8.3–9; outside this pH range, extraction decreased. Chloroform in the absence of MPDC, even under optimal pH conditions, did not extract over 50% of the ^{14}C-morphine. These data on extraction of morphine with chloroform agree with expectations from the literature (6). Addition of MPDC increased the extraction of ^{14}C-morphine to beyond 90% in the pH range 7.5–10.

Effect of Concentration of MPDC and Morphine—To study the stoichiometric relationship, a series of experiments was done by adding different concentrations of MPDC and carrier morphine to a constant amount of ^{14}C-morphine. For example, in Fig. 3 if the curve corresponding to 6 mcg./ml. of morphine is examined, the ^{14}C-morphine extraction was less than 50% initially in the absence of MPDC. When the MPDC concentration was increased to between 40 and 120 times the morphine concentration of 6 mcg./ml., the ^{14}C-morphine extraction was greater than 96%. Each curve in the figures should be examined in this manner, since the figures contain a large mass of data. In the range of 6–30 mcg. of carrier morphine, addition of up to 40 times as much MPDC generally increased the extraction. Increasing the relative concentration of MPDC beyond 40 had a detrimental effect on extraction of ^{14}C-morphine, especially at the 18- and 30-mcg. concentrations of morphine. At 80 mcg./ml. of carrier morphine, the extraction decreased from a control value of 46% (with no MPDC) down to 2% when MPDC was 120 times this morphine concentration. At no time was the extraction better than the control value in this situation. This decrease in extraction, as well as the increase in extraction effected by MPDC at lower concentrations of carrier morphine, indicated that chemical interaction was occurring between MPDC and morphine.

Effect of Different Solvents—As shown in Table I, a number of solvents were tested for ability to extract ^{14}C-morphine in the presence and absence of MPDC and carrier morphine. For these situations, no change in extraction of ^{14}C-morphine was seen when the solvents were ethyl acetate, hexane, methylene chloride, ethylene chloride, and ethyl ether. Butanol appeared to be a special case in which extraction was decreased by adding MPDC and carrier morphine. This case was not studied any further. On the other hand, carbon tetrachloride showed an increase in extraction of ^{14}C-mor-

Table I—Extraction of ^{14}C-Morphine from a pH 8.5 Buffer (5 ml.) with Different Organic Solvents (5 ml.)

Organic Solvent	% Extraction of ^{14}C-Morphine into Solvent: No MPDC	MPDC, 400 mcg./ml.	Morphine, 10 mcg./ml.; MPDC, 400 mcg./ml.
Ethyl acetate	53	52	52
Carbon tetrachloride	8	87	35
Hexane	10	10	10
n-Butanol	26	20	13
Methylene chloride	36	37	39
Ethylene dichloride	26	28	30
Ethyl ether	2	2	2
Ethylene dichloride, chloroform[a]	60	97	
Ethylene dichloride, carbon tetrachloride[a]	19	66	

[a] A mixture containing 2.5 ml. of each organic solvent.

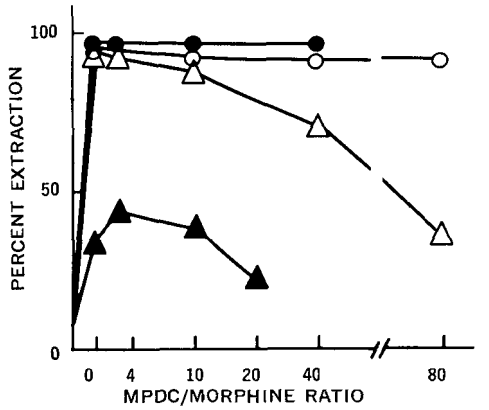

Figure 4—*The extraction of ^{14}C-morphine into carbon tetrachloride from pH 8.3–8.8 buffer to which 6 (●), 12 (○), 18 (△), and 30 (▲) mcg./ml. morphine and 0–80 times these concentrations of MPDC were added.*

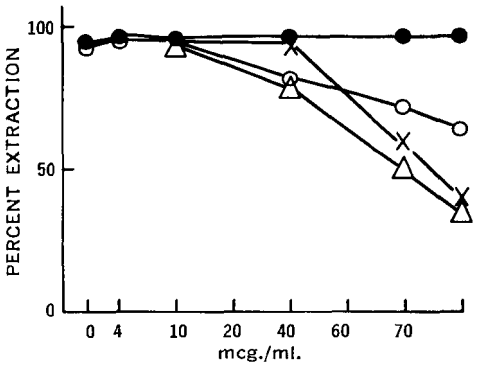

Figure 5—*The effect of various narcotics and antagonists on the extraction of ^{14}C-morphine into 5 ml. chloroform from 5 ml. of pH 8.5 buffer in the presence of MPDC. The concentration of morphine (○), dihydromorphinone (×), nalorphine (△), and thebaine (●) is read from the abscissa. The concentration of MPDC was always 40 times the drug concentrations. Codeine, methadone, meperidine, dextrophan, and levallorphan gave the same results as thebaine.*

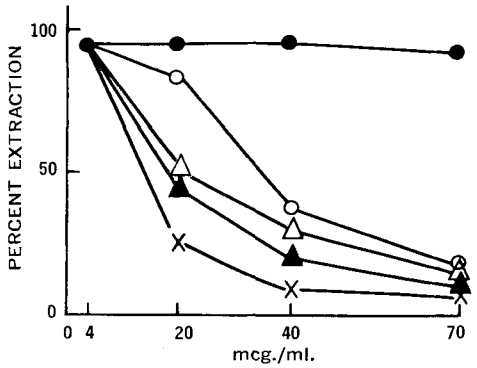

Figure 6—*The effect of various narcotics and antagonists on the extraction of ^{14}C-morphine into 5 ml. carbon tetrachloride from 5 ml. of pH 8.5 buffer in the presence of MPDC. The MPDC concentration was always 20 times that of the drugs. The other designations were as in Fig. 5:* ○, *morphine;* ×, *dihydromorphinone;* △, *nalorphine;* ▲, *naloxone; and* ●, *thebaine, codeine, methadone, meperidine, dextrophan, and levallorphan.*

phine from 8 to 87% by addition of MPDC. But, addition of 10 mcg./ml. of morphine caused the extraction to drop to 35%. Thus, carbon tetrachloride was similar in a qualitative sense to the studies with chloroform as the solvent. Chloroform or carbon tetrachloride added to an equal volume of ethylene dichloride, a solvent that showed no effect to addition of MPDC and morphine, still produced the same phenomenon. Considerable increases in extraction occurred beyond that of ethylene dichloride alone.

The extraction of ^{14}C-morphine from sodium bicarbonate buffer, pH 8.5, was examined (Fig. 4) with carbon tetrachloride used in place of chloroform (Fig. 3). The extraction of ^{14}C-morphine into carbon tetrachloride was almost complete (over 90%) for solutions containing 6 and 12 mcg./ml. of carrier morphine and up to 80 times these respective concentrations of MPDC. Beyond these concentrations of morphine and MPDC, extraction of ^{14}C-morphine decreased. The general conclusions were the same as for the results given in Fig. 3 with chloroform as the solvent. Namely, MPDC was interacting with morphine.

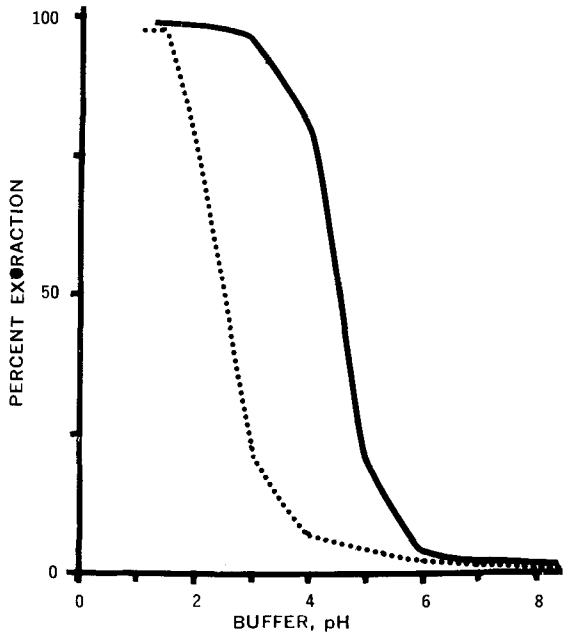

Figure 7—*Reextraction of radioactive product (—) and MPDC (. . .) from chloroform into buffers of different pH's. ^{14}C-Morphine and MPDC (in 1:40 molar ratio) were extracted from pH 8.5 buffer into chloroform. This chloroform was then shaken with an equal volume (5 ml.) of buffer. The percent extraction was calculated for this last step.*

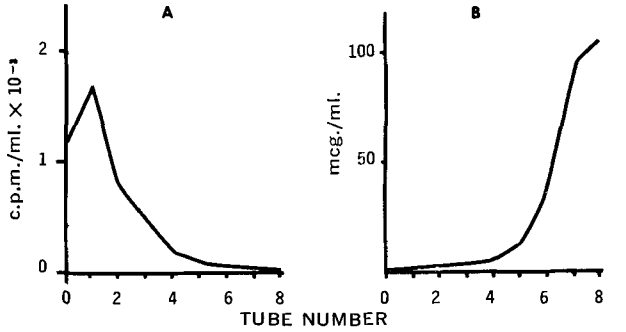

Figure 8—*Countercurrent distribution at pH 3 of material reextracted from chloroform and analyzed for (A) ^{14}C-activity and (B) MPDC. MPDC was determined by UV absorption spectrometry.*

Effect of Other Drugs in Extraction of ^{14}C-Morphine—Investigations of the effects of other narcotic analgesics and antagonists on this interaction of morphine with MPDC are shown in Figs. 5 and 6. There were two types of effects. One group of drugs had no effect; this group included thebaine, codeine, methadone, meperidine, dextrophan, and levallorphan. The other group depressed ^{14}C-morphine extraction and included morphine itself, nalorphine, and dihydromorphinone. Apomorphine at 50 mcg./ml. also belonged in the latter group, but only this one concentration was tested. The same kind of grouping into two types is seen in Fig. 6, where carbon tetrachloride was used as the solvent instead of chloroform. The first group consisted of the same drugs in which no effect was found. The second group consisted again of morphine, nalorphine, dihydromorphinone, apomorphine (one concentration of 20 mcg./ml., not shown), and, in addition, naloxone. It is evident from the experiments (Figs. 5 and 6) that certain drugs affect the interaction of MPDC with ^{14}C-morphine, and some structural specificity exists in this interaction. Dihydromorphinone, morphine, nalorphine, and naloxone all possess the phenolic OH and either an alcoholic OH or keto group at position 6. It is interesting to note that two were agonists (morphine and dihydromorphinone) and two were antagonists (nalorphine and naloxone), but there appeared to be little relationship to pharmacologic potency. At first, it appeared that the morphine–MPDC system would provide an *in vitro* approach for preliminary screening for narcotic analgesics and antagonists. However, some important compounds known to have pharmacologic activity would have been missed.

Reextraction of ^{14}C-Morphine and MPDC from Chloroform into Buffers of Different pH's—The chloroform phase containing the ^{14}C and MPDC was extracted with buffers of different pH's. Figure 7 shows that the radioactive material presumably present as a complex with MPDC was extractible back into acid buffers. This curve, which might be thought of as an inverse plot of the extraction of ^{14}C-

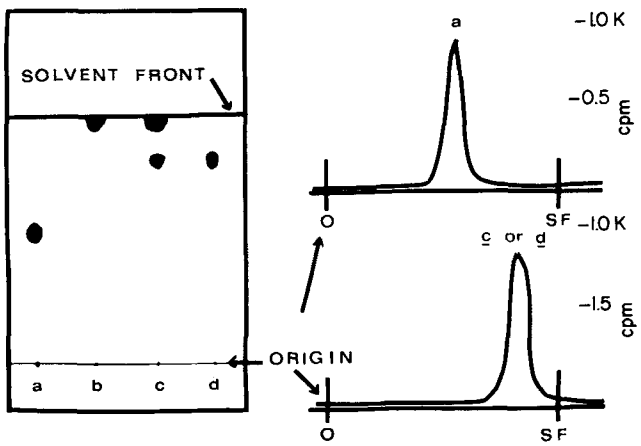

Figure 9—*TLC on Gelman silica gel fiberglass sheets. On the left the chromatogram shows (a) morphine, (b) MPDC, (c) chloroform extract at pH 8.5 containing morphine and MPDC, and (d) Tube 0 pH 3 buffer phase of countercurrent distribution analysis performed in Fig. 8.*

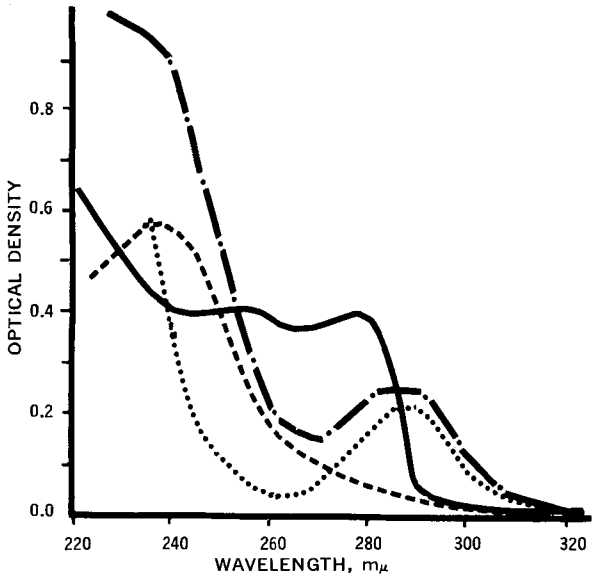

Figure 10—*UV spectrum of the ^{14}C-morphine–MPDC product (—) from Tube 0 of the countercurrent distribution at pH 3.0 (Fig. 8 and d in Fig. 9); morphine (. . .) at pH 3, MPDC (- - -) at pH 3, and morphine and MPDC together (–·–) in pH 3 buffer where no chloroform extraction was involved.*

morphine in the presence of MPDC from buffer into chloroform, was not identical to the results derivable from Fig. 2. For the experiment shown in Fig. 7, transfer of radioactive material from chloroform back to buffer required lower pH's than the transfer of ^{14}C-morphine from buffer to chloroform (Fig. 2). Extraction of ^{14}C-morphine in the absence of MPDC into chloroform and reextraction of this morphine from chloroform into the buffer showed the same type of hysteresis. Occurrence of such hysteresis militated against a clearcut interpretation of these results from the reextraction experiments. Figure 7 includes a further experiment, in which the reextraction into the buffer of the MPDC portion from the morphine–MPDC chloroform solution was measured. Since the amount of MPDC was large relative to morphine (40:1 molar ratio), it was possible to measure MPDC by UV spectrometry. The data show that at a 2–6 pH range, MPDC was not extracted from chloroform into buffer to the same proportion that the radioactive material was extracted.

The practical consequence of the information derived from Fig. 7 was that it was possible to separate the radioactive material from the excess MPDC in the chloroform by adjusting the aqueous phase to pH 3. These conditions were employed in performing the countercurrent distribution analysis given in Fig. 8. As expected, the radioactive material that peaked in Tube 1 was clearly separated from the MPDC that peaked in Tube 8. Since free ^{14}C-morphine itself would have peaked in Tube 0, the peak for the radioactive material in Tube 1 of the present experiment indicated that the radioactive material was not free ^{14}C-morphine. Therefore, attempts were made to characterize this material further.

TLC of the material from the aqueous phase of Tubes 0 and 1 of the countercurrent fractionation is shown in Fig. 9. The results indicated that this material was a single component with an R_f of 0.83, both by iodoplatinate reaction and by radioactivity. It was different from MPDC or morphine. The TLC for *c* is for a 40:1 mixture of MPDC–morphine in pH 8.5 buffer extracted with chloroform and the chloroform spotted. In this case, both the material corresponding to *d* and excess MPDC were present as expected.

UV absorption spectra were obtained from these same materials (Fig. 10). The spectrum of the material in Tube 0 of the countercurrent distribution analysis was not characteristic of either morphine or MPDC. Also, this spectrum was different from a spectrum obtained by simple addition of morphine and MPDC spectra. A spectrum obtained experimentally by having both MPDC and morphine together in a buffer at pH 3 given in the figure was also different from that for the material in Tube 0. Thus, the results from the TLC and the UV spectral studies supported the idea that some sort of "complex" was formed between morphine and MPDC. This complex acted as a single entity with its own characteristics. Efforts to crystallize this material have not been successful.

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