

On the Characterization of 2-Nitromorphine

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2-Nitromorphine, which has been stated to be either a nitroso or a nitro derivative, is found to be, in fact, 2-nitromorphine. The elementary analyses and the titanometric titration were in agreement with a nitro derivative. Four moles of hydrogen were used for reduction. If the compound had been a nitroso derivative the uptake of 4 moles of hydrogen could only have been explained through a reductive splitting of the oxygen bridge in the molecule. That was not the case as it was possible to isolate dihydromorphine after diazotization of the reduced compound and replacement of the diazo group with hydrogen. Furthermore, the infrared spectrum supports the concept that a nitro group is present *ortho* to the phenolic hydroxyl group.

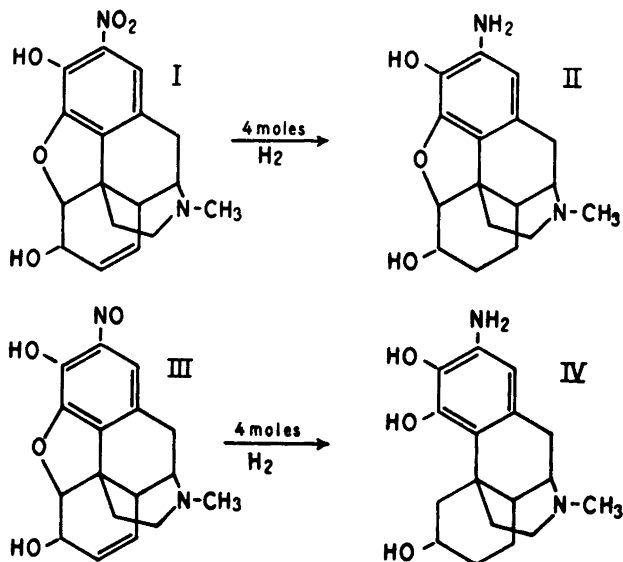
In 1918 Wieland and Kappelmeier¹ prepared a derivative of morphine, called 2-nitrosomorphine, by reacting an aqueous suspension of morphine hydrochloride with nitrous fumes at -2°C to -3°C . This compound has in alkaline solution a distinct red colour and has been used in colourimetric methods for determination of morphine in the presence of other alkaloids.

Baggesgaard Rasmussen *et al.*² have developed a method for determination of morphine based on the polarographic determination of 2-nitrosomorphine. The elementary analyses given in this paper for the latter compound were in agreement with a nitroso derivative.

However, two Japanese workers, Ochiai and Nakamura³, have shown that the compound formed by the procedure of Wieland and Kappelmeier is not 2-nitrosomorphine but 2-nitromorphine. None of the numerous papers published on the determination of morphine by means of "2-nitrosomorphine" seem to have taken notice of the Japanese publication, as all papers mention the "2-nitrosomorphine method".

The purpose of this publication is to confirm the results obtained by Ochiai and Nakamura.

The 2-nitromorphine was prepared following the procedure given by Wieland and Kappelmeier. In the course of the investigation several batches of the compound were prepared and each time the experimental conditions mentioned by the latter authors were followed as closely as possible. The compound was extremely difficult to purify by recrystallization just as it



was difficult to reproduce the elementary analyses, especially the Dumas nitrogen determinations. This is apparently due to the fact that the compound is difficultly combustible. Column chromatography on alumina gave a pure compound and left a fairly large amount of side products adsorbed on the alumina. This is in agreement with the findings of Wieland and Kappelmeier that the reaction of nitrous acid with morphine affords different products and is highly dependent on temperature and time of reaction.

The resulting chromatographically pure 2-nitromorphine hydrochloride was titrated with titanium trichloride and gave an equivalent weight of 67. If the compound had been a nitroso derivative it would have required 4 equivalents of titanium trichloride for reduction and would have given a molecular weight of 268, whereas a nitro derivative requires 6 equivalents of titanium trichloride. The equivalent weight multiplied by 6 gives a molecular weight of 402, a value which is in agreement with the calculated value for 2-nitromorphine hydrochloride with 2 1/2 moles water of crystallization. The elementary analyses found and the analyses given by Ochiai and Nakamura are also in agreement with a hydrate of this composition.

By catalytic reduction of 2-nitromorphine the uptake of hydrogen was 4 moles. If the compound had been a nitroso derivative(III) this fact could only have been explained by an additional reductive splitting of the oxygen bridge. The reaction product would then be a monoaminotrihydroxy derivative(IV). This did not seem to be the case as it was possible to convert the reduced amino compound(II) to dihydromorphine by means of diazotization and a following replacement of the diazo group with hydrogen.

The absorption curves for 2-nitromorphine hydrochloride and the potassium salt 2-nitromorphine recorded for the ultraviolet part of the spectrum

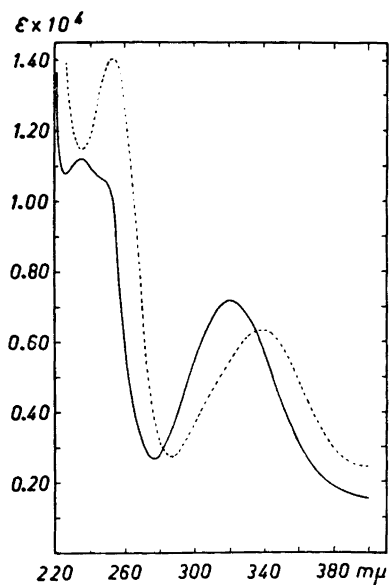


Fig. 1. The ultraviolet absorption spectra of 2-nitromorphine hydrochloride (full line) and the potassium salt of 2-nitromorphine (broken line).

are given in Fig. 1 and are similar to curves given earlier for "2-nitrosomorphine hydrochloride" and the potassium salt of "2-nitrosomorphine"².

Ochiai and Nakamura³ observed that the compound did not give Liebermann's nitroso reaction or liberate iodine of an acidified potassium iodide solution. This was confirmed by this investigation just as the compound was found not to reduce Fehling's or Tollens' reagents. These negative results are, of course, in agreement with the concept that the compound is a nitro compound. On the other hand, we have tried the same reactions on 1-nitroso-2-naphthol and even in this case found them negative.

In the infrared part of the spectrum a nitroso compound ($\text{N} \leftrightarrow \text{O}$) is characterized by only one absorption band whereas a nitro compound due to both symmetrical stretching $\left(\begin{array}{c} \swarrow \text{O} \\ -\text{N} \rightarrow \\ \nwarrow \text{O} \end{array} \right)$ and unsymmetrical stretching $\left(\begin{array}{c} \swarrow \text{O} \\ -\text{N} \rightarrow \\ \nwarrow \text{O} \end{array} \right)$

is characterized by two absorption bands. All these bands have to be strong. The spectrum of the compound considered to be 2-nitromorphine hydrochloride has indeed such two bands at 1 525 and 1 350 cm^{-1} . These frequencies correspond closely to the frequencies expected for a nitro compound. A nitroso compound, on the other hand, should have given only one band at 1 525 cm^{-1} .

It has been shown by Amstutz *et al.*⁴ that the fundamental band of hydroxyl absorption in the infrared part of the spectrum is lacking for 1-nitroso-2-naphthol, whereas 1-nitro-2-naphthol exhibits a definite, although weak band at approximately 3 250 cm^{-1} . This has been confirmed by one of us⁵, who also

Table 1. Fundamental bands of hydroxyl and amine absorption in the infrared part of the spectrum.

Compound	Hydroxyl and amine band (cm ⁻¹)				Interpretation
	solid (KBr)		in CHCl ₃		
2-Nitromorphine hydrochloride	3 450	(NH)	3 620	(NH)	intermolecular H-bond intermolecular H-bond intramolecular H-bond
	3 360	(OH)	3 540	(OH)	
	3 220	(OH)	3 220	(OH)	
Morphine hydrochloride	3 450	(NH)	3 620	(NH)	intermolecular H-bond intermolecular H-bond
	3 360	(OH)	3 540	(OH)	

showed that 4-nitrosoresorcinol did not give the fundamental band of hydroxyl absorption contrary to 4-nitrosoresorcinol*, which gave a distinct band.

2-Nitromorphine hydrochloride exhibits the same type of band as 1-nitro-2-naphthol and 4-nitrosoresorcinol at approximately 3 220 cm⁻¹ and the band is not shifted to a higher frequency by dissolving the compound indicating an intramolecular type of bonding. This observation that an intramolecular hydrogen bond is present, even in solution, appears to give further evidence for the compound being a nitro derivative. The presence of this intramolecular type of hydrogen bonding seems also to support the concept that the nitro group is *ortho* to the phenolic hydroxyl group.

EXPERIMENTAL

The melting points are corrected and have been determined with the hot stage microscope essentially according to Kofler.

Potassium salt of 2-nitromorphine. The compound was prepared according to Wieland and Kappelmeier¹ and purified by column chromatography in the following manner:

3.5 g of the crude potassium salt of 2-nitromorphine dissolved in 100 ml of methanol was poured onto a column of wet-packed alumina (Alcoa) and eluted with 90 % methanol. After collection of a 200 ml fraction, the eluant was changed to 70 % methanol and 120 ml had to be collected before the strongly red-coloured salt appeared in the eluate. On the alumina remained a very dark red-coloured band which only could be eluted with dilute hydrochloric acid.

Table 2. Fractionation of the potassium salt of 2-nitromorphine by column chromatography on alumina.

Fraction	Eluant	Volume (ml)	Colour	Weight of residue
I	effluent	100	colourless	0.004 g
II	90 % CH ₃ OH	200	weak yellow	0.012 g
III	70 % CH ₃ OH	120	weak yellow	0.037 g
IV	70 % CH ₃ OH	375	red	2.71 g

* It has to be mentioned that the hydroxyl group placed in the *para*-position to the nitro or nitroso group in 4-nitrosoresorcinol and 4-nitrosoresorcinol, respectively, showed intermolecular type of hydrogen bonding.

The salt which appeared as the residue after evaporation of fraction IV to dryness was recrystallized twice from 96 % ethanol. The needle-shaped, dark red crystals were dried in the air. When tested by paper chromatography the crystals gave only one spot. The crystals were chromatographed by descending chromatography at 25°C on Whatman No. 1 paper using water-saturated 1-butanol as developing reagent. The compound was visible as a red spot with an R_F -value = 0.42. In ultraviolet light no other spots could be detected. (Found: C 47.15; H 6.00; H₂O 13.40. Calc. for C₁₇H₁₈O₃N(NO₂)K · 3 1/2 H₂O (mol.wt. 432.5): C 47.33; H 5.84; H₂O 14.61).

2-Nitromorphine hydrochloride. To a suspension of 2 g potassium salt in 10 ml of water was at one time and under stirring added 4 ml of 4 N hydrochloric acid. The salt dissolved and the yellow hydrochloride precipitated shortly after. After standing the hydrochloride was collected by filtration and washed with N hydrochloric acid. The yellow crystals were recrystallized from N hydrochloric acid and air-dried. (Found: C 49.40; H 5.77; H₂O 10.05. Calc. for C₁₇H₁₈O₃N(NO₂) · HCl · 2 1/2 H₂O (mol.wt. 411.8): C 49.59; H 5.87; H₂O 10.94.)

The uptake of hydrogen was determined quantitatively by means of the microhydrogenation apparatus described by Clauson-Kaas and Limborg⁶. Uptake: 4.03 moles of H₂ (Calc. for C₁₇H₁₈O₃N(NO₂) · HCl · 2 1/2 H₂O: 4.00 moles of H₂).

After drying 2-nitromorphine hydrochloride *in vacuo* over phosphorus pentoxide the values were as follows: Found: C 53.25; H 5.57. Calc. for C₁₇H₁₈O₃N(NO₂) · HCl · H₂O: C 63.08; H 5.50.

Silver salt of 2-nitromorphine. The salt was prepared according to the procedure given by Wieland and Kappelmeier¹. The salt precipitated as an insoluble voluminous red compound, which after drying changed to a nearly black powder. (Found: Ag 24.80. Calc. for C₁₇H₁₈O₃N(NO₂)Ag: Ag 24.61.)

2-Aminodihydromorphine hydrochloride. 3 g of the potassium salt of 2-nitromorphine was dissolved in 70 ml of water, 2 equivalents of hydrochloric acid were added and the compound was catalytically reduced using palladium carbon (5 %). After reduction the catalyst was removed by filtration and the filtrate evaporated under reduced pressure. The oily residue was dissolved in a little methanol and evaporated again. Addition of ethanol to the residue gave sandy crystals which after recrystallization from ethanol-methanol melted at 320–325°C (Lit. value⁸ 325°C).

Transformation of 2-aminodihydromorphine to dihydromorphine. The aminomorphine was diazotized following the procedure of Wieland and Kappelmeier¹. To 1.5 g of the hydrochloride suspended in 30 ml absolute ethanol was added under cooling 2.25 g of ethyl nitrite (prepared after Wallach and Otto⁷) and finally 3 ml of 19.5 % ethanolic hydrochloric acid.

The diazo compound was precipitated by addition of ethyl ether, removed by filtration, washed with more ethyl ether and immediately dissolved in 5 ml of absolute ethanol. Now 0.5 g of copper powder was added and the suspension was carefully heated to beginning nitrogen evolution. After cooling the suspension was filtered and the filtrate evaporated to dryness and then extracted with ethyl ether. The residue was now dissolved in a little water and sodium carbonate was added to precipitate the alkaloid. An amorphous and dark-coloured compound precipitated. Recrystallization from ethanol-water gave no purification. Therefore, it was purified on Amberlite IR-120. 100 mg of the crude product dissolved in 10 ml of methanol was chromatographed on the resin (H⁺ form, 9 cm × 1.6 cm), washed with 30 ml methanol and eluted with 0.5 N methanolic ammonia water. Only a few mg of product was obtained after evaporation of solvent. After recrystallization from methanol the crystals melted at 154–156°C (Lit. value⁸ 155–157°C) and mixed with authentic dihydromorphine the melting point did not show any depression. Furthermore, the infrared spectrum of the isolated compound was identical with the spectrum of dihydromorphine.

Titanometric titration of the potassium salt and the hydrochloride. To a weighed sample (about 50 mg) in 10 ml glacial acetic acid was added 30 ml 2 N sodium acetate solution. During the titration carbon dioxide was led through the solution. After 5 min of carbon dioxide flow, a measured excess of titanium trichloride solution kept in a carbon dioxide atmosphere was added. After another 5 min, 20 ml of concentrated hydrochloric acid was added and the excess of titanium trichloride was titrated with 0.06 N ammonium thiocyanate using ferric ammonium sulphate as an indicator. The actual amount of titanium trichloride used for reduction was determined by titration of a blank (Table 3).

Table 3. Titanometric titration of two nitromorphine derivatives.

Compound	Equiv. wt. found	Mol. wt. of nitroso cpd.	Mol. wt. of nitro cpd.
Potassium salt of 2-nitromorphine	68	272	408
	68.5	274	411
2-Nitromorphine hydrochloride	67	268	402
	67	268	402

The infrared spectra of 2-nitromorphine hydrochloride, morphine hydrochloride and dihydromorphine were obtained on a Perkin-Elmer Spectrophotometer Model 21. Besides recording the spectra of the compounds in pressed potassium bromide discs, the spectra of the two first compounds were recorded as saturated solutions in chloroform.

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