Stability of Morphine in Aqueous Solution IV: Isolation of Morphine and Sodium Bisulfite Interaction Product

S. Y. YEH* and JOHN L. LACH

Abstract □ The interaction product of morphine and sodium bisulfite was isolated and characterized as 8-sulfonic acid-dihydromorphinone based on UV and IR spectra and elemental analysis.

Keyphrases □ Morphine–sodium bisulfite interaction product—isolation of 8-sulfonic acid-dihydromorphinone □ 8-Sulfonic acid-dihydromorphinone—identification, isolation as morphine–sodium bisulfite interaction product □ IR—structure, identification □ UV—structure, identification

Previous studies on the stability of morphine in aqueous solution showed that morphine undergoes interaction with sodium bisulfite (1). This communication describes the nature and isolation of the interaction product.

EXPERIMENTAL

Materials—The following were used: morphine base USP, m.p. 255°; morphine sulfate USP; dihydromorphine HCl; and sodium bisulfite A.R.

Solubility Analysis—An excess quantity of morphine base was placed in one set of 125-ml. glass-stoppered bottles containing 25 ml. of distilled water with varying amounts of sodium bisulfite. The bottles were placed in a mechanical shaker in a constant-temperature bath at 30° and equilibrated for 20 hr. Aliquots were removed and assayed spectrophotometrically at wavelength 286 nm. for their morphine content.

In the analysis for the stoichiometric ratio of morphine and sodium bisulfite in the interaction product, 0.4 g. of morphine base was placed in ground-glass-stoppered bottles containing 25 ml. of distilled water and 0.15 g. of sodium bisulfite. The bottles were filled with N_2 and equilibrated at 30° for 4 hr. Aliquot samples were removed and assayed for morphine content spectrophotometrically at wavelength 286 nm. and for free bisulfite content by titration with iodine in acid solution.

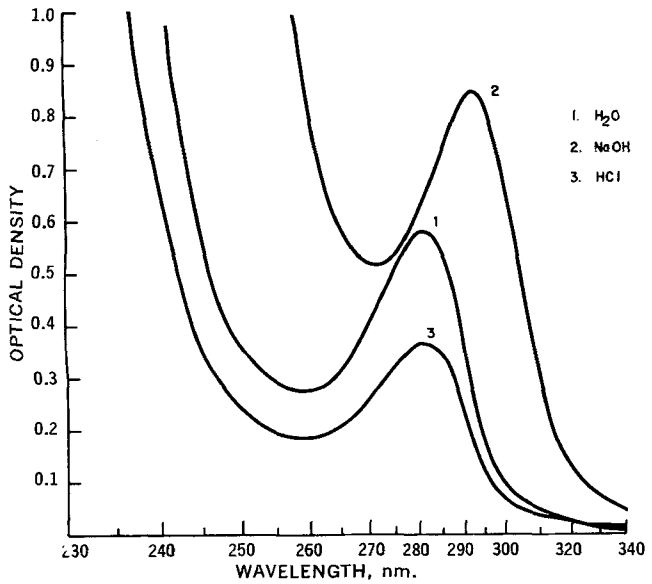


Figure 1—*UV spectrum of 8-sulfonic acid-dihydromorphinone.*

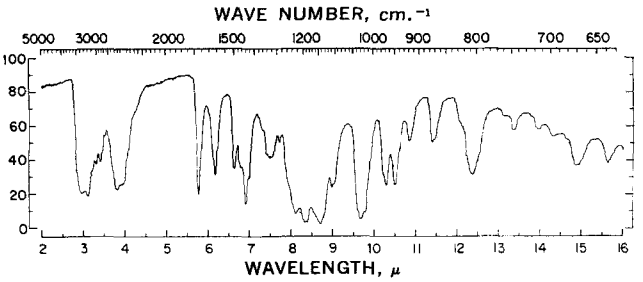


Figure 2—*IR spectrum of 8-sulfonic acid-dihydromorphinone.*

Isolation of Morphine–Sodium Bisulfite Interaction Product—Morphine sulfate (2.1 g.) with tracer amounts of ^{14}C-morphine (as indicator for chromatographic separation) and 7 g. of sodium bisulfite were dissolved in 700 ml. of distilled water, sealed in 20-ml. samples, and heated at 105° for 4 days, the time for the reaction to reach maximum (1). Two procedures were used to isolate the morphine–sodium bisulfite interaction product.

Procedure A—The solution (250 ml.) was chromatographed on an activated charcoal (50–200 mesh)[1] column (1 × 10 cm.). The column was washed with 75 ml. of water and eluted with 50% of acetic acid in water. The effluent was collected in 10-ml. fractions for 250 ml. Morphine and morphine–sodium bisulfite interaction product were completely adsorbed on the column and eluted from the column as indicated by counting 0.5-ml. aliquots of the effluent with 10 ml. of Bray's solution (2). The effluent was concentrated under reduced pressure to near dryness; the residue was suspended in 10 ml. of water, filtered, washed with methanol, recrystallized from boiling water, and dried in vacuum to yield 70 mg. (12% of morphine added) of white prisms, m.p. > 300°.

Anal.—Calcd. for $C_{17}H_{19}NO_6S$: C, 55.72; H, 5.24; N, 3.82; S, 8.73. Found: C, 55.28, 55.71; H, 5.10, 5.17; N, 3.66, 3.82; S, 8.72.

The UV spectrum of this compound in water and HCl medium showed a minimum at 258 nm. and a maximum at 280 nm.; in NaOH solution, the minimum was at 270 nm. and maximum at 292 nm. (Fig. 1). The IR spectrum showed strong absorption bands at 2600 cm.$^{-1}$ (R_3N^+), 1725 cm.$^{-1}$ (C=O), 1000–1200 cm.$^{-1}$ (RSO_3H), and 800 cm.$^{-1}$ (C—S—) (Fig. 2).

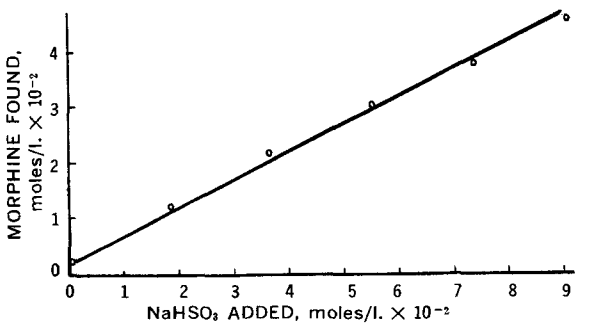


Figure 3—*Effects of sodium bisulfite on the solubility of morphine alkaloid in aqueous solution.*

[1] Fisher Scientific Co.

Table I—Stoichiometric Determination of Morphine Sodium Bisulfite Interaction in Water at 30°

Bottle Number	Free $NaHSO_3$ Found, moles/l. × 10^{-2}	Interacted $NaHSO_3$[a], moles/l. × 10^{-2}	Morphine Found[b], moles/l. × 10^{-2}	Ratio, Morphine / $NaHSO_3$
1	4.5387 (average)	—	—	—
2	3.345	1.194	1.265	1.06
	3.383	1.155		1.09
3	3.154	1.385	1.425	1.03
	3.365	1.174		1.21
4	3.358	1.481	1.458	1.00
	3.153	1.386		1.07

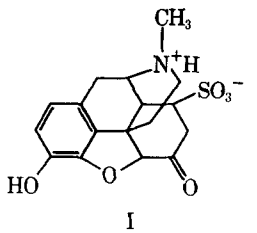
[a] The sodium bisulfite undergoing interaction was determined by subtracting the blank containing only sodium bisulfite from the bisulfite present in the other bottles. [b] The morphine concentrations were corrected for the free morphine in aqueous solution (0.1201 × 10^{-2} mole/l.).

Procedure B—Isolation with Amberlite XAD-2 Resins—The morphine–sodium bisulfite solution (120 ml.) was chromatographed on Amberlite XAD-2 resins[2] in a column 2.2 × 41 cm. (0.87 × 16 in.) previously washed with acetone, methanol, and water. The column was washed with 100 ml. of water and eluted with 160 ml. of methanol. The eluate collected from 60–160 ml. was concentrated to near dryness under a stream of N_2. The crystals were washed with methanol and dried (70 mg., 24% of morphine added). The crystals thus obtained from several batches were recrystallized from boiling water and dried in vacuum at 60°. The results of elemental analysis and the IR and UV spectra of these crystals were identical to those isolated from the charcoal column.

RESULTS AND DISCUSSION

The results obtained from solubility studies indicate that the amount of morphine dissolved in the solution increases with increased sodium bisulfite concentration (Fig. 3). The ratio of morphine to sodium bisulfite in the solution is 1 : 2. One part of the sodium bisulfite, presumably salt formation with morphine, is titratable with iodine solution. The other part of sodium bisulfite formed a stable interaction product with morphine and is untitratable with iodine solution. The stoichiometric ratio of morphine to sodium bisulfite in the interaction product was found to be 1 : 1 (Table I).

The isolated interaction product of morphine–sodium bisulfite is insoluble in water, in dilute HCl, and in common organic solvents. The compound contains no sodium ion as detected with flame analysis. The UV and IR spectra reveal that the compound contains a phenol, a carbonyl, and a sulfonic acid group. The tertiary amine is protonized, suggesting that the compound exists as an inner salt or zwitterion. The UV and IR spectra of this compound are quite different from that of morphine-3-ethereal sulfate (3).



I

Based on the elemental analyses and UV and IR spectra, the structure of this compound was assigned as 8-sulfonic acid-dihydromorphinone (I).

Critchfield and Johnson (4) indicated that sodium bisulfite reacts with alpha-beta unsaturated compounds having a strong electron-attracting group on the alpha-position to saturate the double bond and to form a stable sulfonic acid. Since morphine has a similar structure, *i.e.*, with a hydroxyl group at the 6-position and an unsaturated double bond at the 7–8-position, it is probable that the (—SO_3) attaches on the 8-position. When the double bond of morphine was saturated, *e.g.*, dihydromorphine, no such reaction was observed under similar condition[3] whereas codeine does undergo this type of interaction[4]. It can be assumed that similar chemicals and pharmaceuticals with alpha-beta unsaturated compounds having a strong electron-attracting group on the alpha-position will react with bisulfite if the concentration of bisulfite ions is high enough. Some pharmaceutical preparations such as epinephrine (6) and steroids (7) containing bisulfite undergo such a bisulfite interaction.

The interaction of morphine and sodium bisulfite takes place very rapidly. Five minutes after addition of sodium bisulfite (1%) to the morphine solution at room temperature, 20% of the morphine (3 mg./ml.) underwent this type of reaction (1). The interaction of morphine and sodium bisulfite probably also takes place in commercial preparations, although the amount of the bisulfite preservative (0.5%) is usually small. Analgesic and toxicity aspects of this interaction product have not yet been studied.

REFERENCES

(1) S. Y. Yeh and J. L. Lach, *J. Pharm. Sci.*, **50**, 35(1961).

(2) G. A. Bray, *Anal. Biochem.*, **1**, 279(1960).

(3) S. Y. Yeh, H. I. Chernov, and L. A. Woods, *J. Pharm. Sci.*, **60**, 469(1971).

(4) F. E. Critchfield and J. B. Johnson, *Anal. Chem.*, **28**, 73 (1956).

(5) S. Y. Yeh and J. L. Lach, *J. Pharm. Sci.*, **50**, 30(1961).

(6) L. S. Schroeter, T. Higuchi, and E. E. Schuler, *J. Amer. Pharm. Ass., Sci. Ed.*, **47**, 723(1958).

(7) W. F. Charnicki and R. E. King, U. S. pat. 2,970,944 (1961).

(8) W. J. Leanza and E. F. Rogers, U. S. pat. 2,720,944 (1955).

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* Present address: Department of Psychiatry, College of Medicine, University of Kentucky, Lexington, KY 40508

[2] Rohm & Haas Co., Philadelphia, Pa.

[3] The column partition chromatography used for determination of free morphine (5) had to be modified for determination of unreacted dihydromorphine. The modification was made as follows: 5 g. of diatomaceous earth buffered with 5 ml. of 1.0 *M* of K_2HPO_4, pH 9.2, as supporter. Three milliliters of dihydromorphine HCl (0.3%) with or without 1% of sodium bisulfite, 1.4 ml. of 5% of K_3PO_4 solution, and 0.7 ml. of 1 *M* of K_2HPO_4 solution were mixed thoroughly with 5 g. of diatomaceous earth. The mixture was made slush with chloroform and packed in the column on the supporter. The column was eluted with chloroform. The first 25-ml. fraction of the effluent was discarded, and the optical density of the effluent collected from the 25–125-ml. fraction was read at wavelength 286 nm.

[4] Codeine was determined as described for morphine (5), except that chloroform instead of chloroform containing 15% of *n*-butyl alcohol was used as eluent and the effluent was collected from 0–100 ml.