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# $O^3$-MONOACETYLMORPHINE

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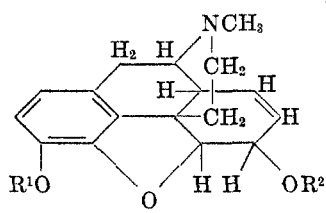
It is theoretically possible to obtain from morphine (I) two O-monoacetyl derivatives, $O^3$-monoacetylmorphine (II) and $O^6$-monoacetylmorphine (III).[1] The $O^6$-monoacetate is most conveniently prepared by the partial deacetylation of $O^3$, $O^6$-diacetylmorphine (IV) (2–6) and is one of the products formed directly from morphine by the action of acetic acid or anhydride (2, 3, 7). Whether or not II has ever been isolated can not be decided definitely on the basis of evidence in the literature. By reacting acetic anhydride and morphine in equimolar proportions at 100°, Beckett and Wright (7) obtained three products which afforded the analyses expected of a monoacetylmorphine and which were designated $\alpha$-, $\beta$-, and $\gamma$-acetylmorphine.[2] The $\alpha$-product corresponds to III. The $\beta$-




I, $R^1 = R^2 = H$
II, $R^1 = CH_3CO$, $R^2 = H$
III, $R^1 = H$, $R^2 = CH_3CO$
IV, $R^1 = R^2 = CH_3CO$

base, which was the most abundant of the three products,[3] was amorphous and yielded no crystalline derivatives. The $\gamma$-base and its ethiodide were crystalline, and its hydrochloride "crystallizable, but with difficulty." Since Beckett and Wright (7) reported their $\beta$- and $\gamma$-products, when pure, gave no color with ferric chloride, it is possible that one of the two represented II. However, since these workers were unable to obtain a color reaction from this reagent in the presence of III, it is evident that in their hands the test gave unreliable results.[4]

Recent work in this laboratory has shown that in the presence of acetic anhydride and aqueous bicarbonate selective acetylation of phenolic hydroxyl

[1] The system of numbering the carbon skeleton is that of Cahn and Robinson (1).

[2] As a matter of fact, the products were described as "diacetylmorphines," since a $C_{34}H_{38}N_2O_6$ molecular formula was then accepted for morphine. See Danckwort (3).

[3] Beckett and Wright (7) do not give percentage yields of their monoacetylmorphines, but state that of the three the $\beta$-product was "formed in the largest quantity," whereas the $\gamma$- and $\alpha$-products respectively comprised "about 25% of the whole" and "2 or 3% of the whole."

[4] Danckwort (3) confirmed the observations of Wright (2) and Beckett and Wright (7) in applying the ferric chloride test to the $\alpha$- and $\gamma$-products. See, however, Merck (4).

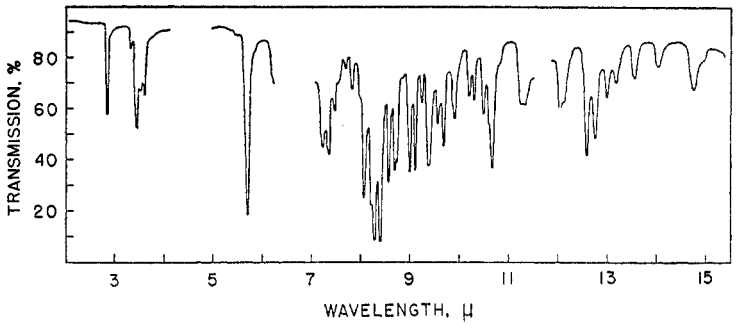



FIG. 1. INFRARED ABSORPTION SPECTRUM OF $O^3$-MONOACETYLMORPHINE BASE TAKEN in carbon disulfide (1% soln.) with a model 21 Perkin-Elmer recording spectrophotometer (0.5-mm. cell).

groups occurs in a number of substances having, as does morphine, both phenolic and secondary alcoholic functions (8). When morphine was acetylated under these conditions there resulted a quantitative yield of an amorphous base which as such or as its crystalline sulfamic acid salt gave the analytical data expected of a monoacetylmorphine. Its infrared absorption spectrum (Fig. 1) showed the presence of a hydroxyl group (absorption peak at 2.85 $\mu$) which other tests conclusively demonstrated to be non-phenolic. On acetylation with acetic anhydride-pyridine it quantitatively yielded IV. These data permit identification of the substance as II.

With benzoic acid, II combines to form a salt which crystallizes from chloroform-carbon tetrachloride with the composition of two moles of acid per mole of base.[5] From ethyl acetate-ether the benzoate crystallizes with the composition 3 base•5 acid•1 ether. The two crystalline substances exhibit different melting-point characteristics, but their optical crystallographic properties are so nearly alike as to render them indistinguishable under the polarizing microscope.

Attempts to prepare other crystalline salts of II, including the hydrochloride, were unsuccessful.

The formation of II in the reaction between equimolar quantities of morphine and acetic anhydride was demonstrated by repeating the procedure of Beckett and Wright (7). The reaction was not clean-cut, and the product contained considerable unreacted morphine and IV. However, the experimental results indicated that II was the principal monoacetylation product. This evidence and the amorphous nature of the base and its hydrochloride strongly indicate that II corresponds to the product designated "$\beta$" by Beckett and Wright (7).

The status of the $\gamma$-product, the formation of which Danckwort (3) was unable to confirm, remains completely unclarified. The substance may have been a derivative of an isomorphine,[6] since it is not inconceivable that isomerization of morphine may occur under the experimental conditions.

[5] It appears to be not unusual for benzoic acid to form compounds of this type with nitrogenous bases (9).

[6] Emde (5) has referred to unpublished evidence indicating that the $\beta$-product cor-

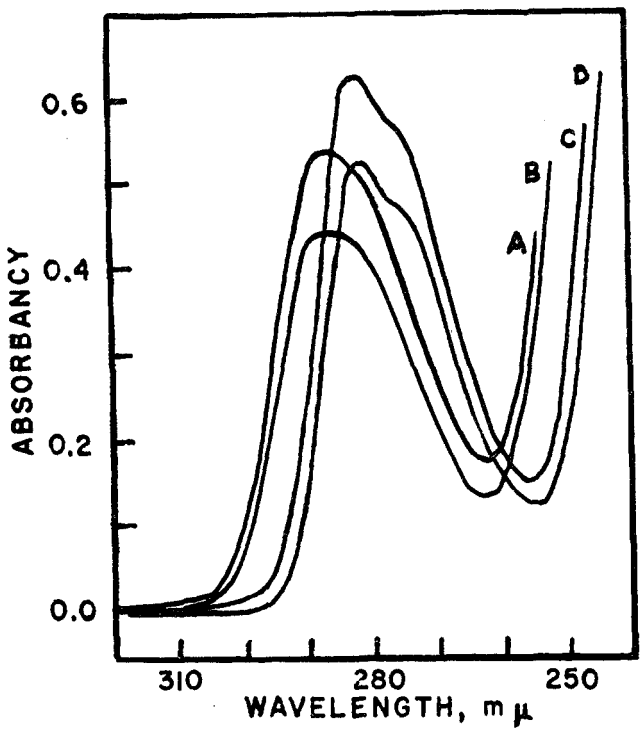



FIG. 2. ULTRAVIOLET ABSORPTION SPECTRA OF MORPHINE AND ITS ACETYLATION PRODUCTS taken in 95% ethanolic solution (100 mg./l.) with a model 11 Carey spectrophotometer (1-cm. cell): A, morphine, $\lambda_{max}$ 287 mμ, $\epsilon_{287}$ 1540; B, $O^6$-monoacetate, $\lambda_{max}$ 287 mμ, $\epsilon_{287}$ 1460; C, $O^3$-monoacetate, $\lambda_{max}$ 282 mμ, $\epsilon_{282}$ 2050; D, $O^3$,$O^6$-diacetate, $\lambda_{max}$ 281 mμ, $\epsilon_{281}$ 1940.

In Fig. 2 are recorded the ultraviolet absorption spectra of morphine and its acetylation products in ethanolic solution. It may be seen that in the transition I → II, the wavelength of the absorption peak, $\lambda_{max}$, is shifted from 287 mμ to 282 mμ, and the molecular absorbancy, $\epsilon$, is increased from 1540 to 2050. In the analogous transition, III → IV, $\lambda_{max}$ is shifted from 287 mμ to 281 mμ while $\epsilon$ is increased from 1460 to 1940. Acetylation also modifies the shape of the curve by introducing a shoulder in the direction of the shorter wavelengths. The decrease in $\lambda_{max}$ associated with acetylation of the phenolic hydroxyl group in this series of compounds is in qualitative agreement with the effect which has been observed in the acetylation of other phenols. However, the increase of 33% in $\epsilon$ which accompanies the transitions I → II and III → IV contrasts

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responds to $O^6$-monoacetyl-α-isomorphine. It should be noted, however, that the physical properties recorded for the hydrochloride of the β-product (amorphous, hygroscopic, extremely soluble in water) (2, 3, 7) differ greatly from those of $O^6$-monoacetyl-α-isomorphine hydrochloride as observed by L. F. Small and reported in the publication of Sumwalt, Oswald, and Lusk (10).

sharply with the large *decrease* associated with the acetylation of other phenolic substances.[7]

## EXPERIMENTAL

Melting points are corrected.

*Acetylation of morphine* (I → II). In effecting the reaction, 100 cc. of water, 5.00 cc. (53.0 millimoles) of reagent grade acetic anhydride, and 10.00 g. (119 millimoles) of sodium bicarbonate were used per gram (3.51 millimoles) of finely powdered morphine base. The technique and quantities of materials employed depended on the use intended for the acetylation product.

For the determination of the quantitative aspects of the reaction and the neutralization equivalent, specific rotation, and absorption spectra of acetylmorphine base (II), 100-mg. samples of morphine were acetylated. The reaction was carried out in a 125-cc. separatory-funnel according to the technique employed in a study of the acetylation of ephedrine (8c); separation and isolation of the product were effected as described in a method for the determination of ephedrine as its acetyl derivative (8b), except that acidification of the reaction mixture and washing of chloroform extracts with aqueous bicarbonate were omitted. By this means it was possible to secure accurately weighed *ca.* 120-mg. specimens of amorphous II. Material so obtained consistently weighed about 1% more than the theoretical, apparently because of the presence of occluded solvent, and showed a neutralization equivalent of 330 to 332 (Calc'd 327.4) and $[\alpha]_D^{22}$ −195° to −197° (*c*, 1 in U.S.P. $CHCl_3$; *l*, 2). It contained substantially less than 1% morphine or the $O^6$-monoacetate. This was demonstrated by adding to its ethanolic solution the calculated amount of aqueous sulfamic acid, applying the ferricyanide-ferric chloride test (described in the succeeding section) to a dilution of the resulting solution of sulfamate, and comparing the reaction with that given by a morphine standard.

Prior to heating 30 minutes in an oven at 105° (8b), II appeared as a practically colorless transparent resin. Although the heating period caused it to acquire a brownish tint, the extent of chemical change was evidently small, since such discolored material in carbon disulfide solution exhibited an infrared spectrum indistinguishable from that shown by practically colorless material. The ultraviolet spectrum of the former did differ slightly from that of the latter because of a "background" effect. For this reason the curve in Fig. 2 was obtained from material which had not been subjected to the heating period; the weight factor, which enters into the calculation of $\epsilon$, was determined on the basis of the weight of the sample of morphine subjected to the acetylation procedure and the established quantitative nature of the procedure.

The free base was also prepared quantitatively from the recrystallized sulfamate by dissolving 130 mg. of the salt in 5 cc. of 5% aqueous sodium bicarbonate, extracting the base into chloroform, and isolating it as previously described. Material obtained in this manner was indistinguishable from that provided by the direct acetylation of morphine as regards absorption spectra, neutralization equivalent, specific rotation, and response to the ferricyanide-ferric chloride reagent.

In acetylating morphine in quantities of the order of 3 g., it was convenient to carry out the reaction in a 1-l. Erlenmeyer flask equipped with a motor-driven wire stirrer. The anhydride was added in three *ca.* equal portions to the vigorously stirred suspension of

[7] For example, in ethanolic solution, $\lambda_{max}$ estrone, 280 mμ; $\lambda_{max}$ estrone acetate, 265 mμ; $\epsilon$ acetate/$\epsilon$ estrone, 0.53 (11); $\gamma_{max}$ *m*-cresol, 275 mμ; $\lambda_{max}$ *m*-cresyl acetate, 270 mμ; $\epsilon$ acetate/$\epsilon$ cresol, 0.41 (12). In ethereal solution, $\lambda_{max}$ phenol, 282 mμ; $\lambda_{max}$ phenyl acetate, 258 mμ; $\epsilon$ acetate/$\epsilon$ phenol, 0.14 (11). The author has been unable to determine whether these examples illustrate a general relationship between the absorption spectra of phenols and their acetylation products. A statement by Fieser and Fieser (13), however, suggests that they do.

morphine in aqueous bicarbonate. Violent foaming was controlled by the addition of small amounts of ether. After extracting the product into chloroform, the extract was treated with Drierite and filtered. The bulk of the solvent was distilled off, and all but a small amount of the remainder was removed by repeatedly heating the residue on the steam-bath and allowing it to cool under diminished pressure. The II so obtained was used in preparing the sulfamate and benzoate.

*$O^3$-Monoacetylmorphine* (II) *sulfamate.* The acetylation product from 3 g. of morphine was dissolved in 20 cc. of isopropyl alcohol and to the solution was added the calculated amount (1.02 g.) of sulfamic acid in 20 cc. of water. After adding an additional 180 cc. of isopropyl alcohol, crystallization was initiated, the system was chilled, and the product was filtered off, washed with acetone, and air-dried. The crystals so obtained (3.46 g., 78%) were recrystallized by dissolving them in 5 cc. of water, adding 50 cc. of absolute ethanol, and chilling. Two similar recrystallizations from water (5 cc.) plus acetone (50 cc.) yielded 1.87 g. of sulfamate after it had been dried over calcium chloride under diminished pressure then heated two hours at 78° in a high vacuum.

*Anal.* Calc'd for $C_{19}H_{21}NO_4 \cdot NH_3SO_3$: C, 53.76; H, 5.70; N, 6.60; Acetyl, 10.14.
Found: C, 53.84, 53.74; H, 5.4, 5.3; N (Kjeldahl), 6.54; Acetyl, 10.02.

Under the microscope, the sulfamate consisted of talc-like thin plates and plates on edge giving a fibrous appearance; *elongation*, negative (shown by plates on edge); *polarization color*, first order gray; *refractive indices*, $\alpha$, 1.559; $\beta$, 1.605; $\gamma$, 1.622 (all $\pm 0.003$).

The substance showed $[\alpha]_D^{21}$ −66.4° (*c*, 2 in $H_2O$; *l*, 2). A capillary tube specimen, placed in a bath at 200° and heated at a rate of 1° per minute, began to brown and soften at 203°; decomposition was virtually complete at 209°.

To a solution of 22 mg. of the sulfamate in 2 cc. of water was added 3 drops of reagent prepared by mixing 10 cc. of 10% potassium ferricyanide and 10 drops of 9% ferric chloride.[8] After a few seconds the solution acquired a greenish tint which, as the solution was allowed to stand, slowly deepened to a blue-green (hydrolysis of the acetoxy group). When the test was applied to a solution containing the equivalent of 0.10 mg. of morphine sulfamate in 2 cc. a blue-green color developed immediately. The tests showed that the salt contained no more than a trace of morphine or its $O^6$-monoacetate.

*$O^3$-Monoacetylmorphine* (II) *benzoate. A.* The acetylation product from 2.00 g. of morphine was dissolved in 80 cc. of carbon tetrachloride, and a solution of 1.71 g. (2 equivalents) of benzoic acid in 10 cc. of chloroform was added. After crystallization was essentially complete at room temperature, the system was chilled two hours in a freezing unit, and the product was filtered off, washed first with carbon tetrachloride, then with petroleum ether, and was air-dried two hours at room temperature.

The benzoate thus obtained (3.61 g., 90% yield) exhibited the following optical crystallographic properties under the polarizing microscope; *habit*, platy prisms, often six-sided in outline; *extinction*, parallel; *elongation*, negative; *refractive indices*, $\alpha$, 1.580; $\beta$, 1.595; $\gamma$ (shown by plates on edge), 1.624; (all $\pm 0.003$); *optic sign*, positive; *axial angle* (2 *V*), moderate; *interference colors*, mostly first order gray and white. Acute bisectrix interference figures were frequently observed.

Capillary tube specimens placed in a bath at 140° and heated at a rate of 1° per minute began to sinter and discolor at 151°, adhered to the tube at 153.5°, and formed a clear reddish-brown melt at 154.5°.

When gravimetrically assayed for its basic component, II, by a procedure essentially that used to isolate the base from its sulfamate, the salt was found to contain 57.5% of II.

The salt was assayed spectrophotometrically for its acidic component by suspending a sample in a small amount of water, acidifying the system with sulfuric acid, and quantitatively extracting the benzoic acid with chloroform. After filtering the extracts and

[8] This reagent, which is employed in U.S.P. color tests (14) for morphine and dihydromorphinone, is much more sensitive than ferric chloride alone in detecting the phenolic hydroxyl group in this series of compounds.

diluting with a suitable volume of chloroform, the absorbancy of the dilution was determined at the 275 mμ maximum and compared with the absorbancy of a standard solution of benzoic acid in chloroform. The benzoic acid content so determined was 43.6%.

A salt of the composition 1 base•2 acid theoretically contains 57.3% and 42.7% of the respective components.

*B.* The benzoate obtained in *A* was dissolved in hot ethyl acetate (5 cc./g.) and, after cooling somewhat, the solution was diluted with 3 volumes of ether. Crystallization was completed in a freezing unit, and the product was filtered off, washed first with ether, then with petroleum ether, and was air-dried two hours: recovery, *ca.* 80%.

The product was odorless in the open air, but the odor of ether became detectable after storing it in a closed container or on treating it with water. A 180-mg. sample underwent an apparent loss of 0.1 mg. on standing in the open for 24 hours. It assayed 59.1% base, 36.5% acid. A composition of 3 base•5 acid•1 ether should contain 58.9% base, 36.6% acid.

When the melting-point behavior was observed under the conditions described in *A*, the substance immediately sintered when placed in the bath, soon softened and discolored, adhered to the wall of the tube at *ca.* 144°, and formed a clear, reddish brown melt at 152°.

The optical crystallographic properties of the substance were indistinguishable from those recorded in *A*. On recrystallizing from chloroform-carbon tetrachloride it reverted to the composition found in *A*, assaying 57.9% base, 43.0% acid.

*Other salts of* II, the preparation of which was attempted, but which could not be obtained in a crystalline condition, were the normal benzoate (1 base•1 acid), *o*-methoxybenzoate, phenylacetate, glycollate, D- and L-mandelates, cinnamate, and hydrochloride.

*Acetylation of $O^3$-monoacetylmorphine* (II → IV). To a solution of 120 mg. of II in 0.50 cc. of pyridine was added 0.50 cc. of acetic anhydride. After standing one week at room temperature, the reaction mixture was dissolved in water, an excess of sodium bicarbonate was added, and the solution was extracted several times with chloroform. Evaporation of the extracts to dryness provided a quantitative yield (0.135 g.) of somewhat discolored IV; m.p. 167.5–172.5°, raised to 172–173° after recrystallization from ethyl acetate.

*Acetylation of morphine according to Beckett and Wright* (7). To 1.00 g. of anhydrous morphine base in a small test tube was added 0.33 cc. (1 equivalent) of acetic anhydride. The mixture became warm and formed a mass the viscous nature of which prevented thorough mixing. The tube was closed with a glass stopper and heated at 100° for one hour. After being cooled, the purple reaction mixture, the odor of which indicated the absence of anhydride, was dissolved in 25 cc. of water, and the solution was basified with 1 g. of sodium bicarbonate and extracted with three 20-cc. portions of chloroform. Unreacted morphine (150 mg.) precipitated during the extraction process. The filtered extracts were concentrated to an oil which was dissolved in 20 cc.of benzene. In order to remove additional morphine and its $O^6$-monoacetate, the solution was extracted with 10 cc. then 5 cc. of 4% sodium hydroxide. After washing with water, the now almost colorless benzene solution was filtered and concentrated to an oil which was dissolved in 3 cc. of ethyl acetate. The solution was seeded with diacetylmorphine base and left in a freezing unit for 12 hours. Addition of petroleum ether caused the deposition of an oil most of which was taken back into solution by the addition of ethyl acetate. The supernatant liquid was decanted from the magma (100 mg.) rich in diacetylmorphine and evaporated to dryness. The resin of crude base, II, thus obtained (540 mg., 61% based on morphine unrecovered as such or as crude diacetate), was dissolved in 1 cc. of ethanol and to the solution was added 150 mg. (94% of the calculated amount) of sulfamic acid in 1.5 cc. of water. The crystalline product obtained after diluting the system with 20 cc. of acetone and chilling was recrystallized in a similar manner from 0.5 cc. of water and 10 cc. of acetone. The resulting salt (310 mg.) had optical crystallographic properties identical with those of pure II sulfamate. The base obtained from the salt showed an infrared absorption spectrum which agreed very closely with that of pure II. The major difference between the spectra was at 8.3 μ, at which wavelength the pure preparation showed a much greater minimum than did the base obtained by the Beckett and Wright procedure. Since diacetylmorphine

base, IV, exhibits a strong absorption maximum at 8.3 $\mu$, the discrepancy may be ascribed to the presence of IV in the Beckett and Wright base. The data supported the conclusion that the amount was no more than 5%.

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SUMMARY

$O^3$-Monoacetylmorphine has been prepared by the action of acetic anhydride on morphine in the presence of aqueous bicarbonate, and characteristics of the amorphous base and its crystalline sulfamate and benzoate have been determined.

Evidence has been presented which supports the conclusion that $O^3$-monoacetylmorphine corresponds to the $\beta$-acetylmorphine previously reported in the literature.

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