Application of $\Delta\epsilon$-Analysis to Pharmaceuticals II

Determination of Morphine

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Morphine exhibits a bathochromic displacement typical for phenols of its U.V. absorption spectrum in alkaline solution as compared to its spectrum in acid solution. Subtracting the absorbances of the U.V. spectrum in acid solution from those of the U.V. spectrum in alkaline solution results in a characteristic $\Delta\epsilon$-spectrum (differential spectrum). This difference spectrum exhibits a maximum at 298 mμ of sufficient magnitude to be suitable for quantitative determinations in a manner analogous to the use of the usual U.V. absorption spectra but with added specificity. The method has been applied to the determination of morphine in tablets, tincture of opium, and camphorated tincture of opium.

THE application of $\Delta\epsilon$-analysis (differential analysis) for the determination of eugenol in pharmaceuticals has been described in a previous paper from these laboratories (1). Williams (2, 3) has suggested a similar procedure for the analysis of barbiturates and salicylates. It is the purpose of this investigation to use $\Delta\epsilon$-analysis for the assay of morphine in some representative dosage forms.

Morphine exhibits a bathochromic displacement, typical for phenols, of its U.V. absorption spectrum in alkaline solution as compared to its spectrum in acid solution (4, 5). Subtracting the absorbances of the U.V. spectrum in acid solution from those of the U.V. spectrum in alkaline solution results in a characteristic $\Delta\epsilon$-spectrum (6). This difference spectrum exhibits maxima of sufficient magnitude to be suitable for quantitative determinations in a manner analogous to the use of the usual U.V. absorption spectra.

The difference in absorbance of a sample of morphine was determined at a difference maximum and this value served as the basis for the determination of morphine in tablets, tincture of opium, and camphorated tincture of opium.

EXPERIMENTAL

Ultraviolet absorption measurements were made with a Beckman, model DU spectrophotometer. A Beckman Zeromatic meter was used to determine pH.

Materials.—Morphine monohydrate (Mallinckrodt) was purified by chromatography (7) followed by two recrystallizations from alcohol-water. All reagents were of C.P. grade and were used without additional purification.

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Following the acceptance of this paper, a similar procedure has been described by Milos, C., THIS JOURNAL, **50**, 837 (1961).

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Determination of Morphine Spectra.—Samples of about 80 mg. of morphine, accurately weighed, were dissolved in alcohol in 100-ml. volumetric flasks. Ten-milliliter aliquots were transferred to a second set of 100-ml. volumetric flasks and diluted to volume with a series of solutions prepared from standard solutions of sodium hydroxide and sulfuric acid. In this manner the U.V. spectra for 12 solutions of morphine ranging in pH from 3.0 to 12.0 were determined.

Determination of $\Delta\epsilon$ for Morphine.—Samples of about 80 mg. of morphine were accurately weighed, transferred to a 100-ml. volumetric flask, and dissolved by adding alcohol to volume. One 10-ml. aliquot of this solution was diluted to volume with water and 1.00 ml. of 1.000 *N* sodium hydroxide in a 100-ml. volumetric flask. A second 10-ml. aliquot of the alcoholic morphine solution was transferred to a 100-ml. volumetric flask and diluted to volume with water and 1.00 ml. of 0.100 *N* sulfuric acid. The absorbance of the alkaline solution was determined at 298 mμ relative to the acid solution in the reference cell. Blank solutions were run under the same conditions in order to correct for any contribution due to solvents and reagents. A total of 18 determinations were made in this manner. The alkaline solutions were recorded with a pH of 12.0 $\pm$0.1 and the acid solutions with a pH of 3.0 $\pm$0.1.

Assay of Morphine Sulfate Tablets.—Twenty 1/4 gr. tablets of morphine sulfate were weighed and powdered. A portion of this powder, about 90 mg., was accurately weighed, transferred to a 100-ml. volumetric flask, and dissolved by adding water to volume. Two 10-ml. aliquots of this solution were diluted, buffered, and the relative absorbance at 298 mμ was determined as described under the determination of $\Delta\epsilon$ for morphine.

Assay of Tincture of Opium.—A 5-ml. portion of the tincture was transferred to a beaker by pipet, mixed with 5 ml. of water, and reconcentrated on a steam bath to about 5 ml. To insure the removal of alcohol, a second 5-ml. portion of water was added and the mixture reconcentrated to about 5 ml. After cooling, 0.5 ml. of ammonia solution was mixed with the opium extract followed by 2 Gm. of Celite 545. This mixture was quantitatively transferred to the top of a column containing 5 Gm. of Celite 545. A total of 150 ml. of benzene was used to complete the transfer of the Celite-opium residue to the column and to develop the chromatogram. The

elution with benzene was followed by a mixture of chloroform-absolute ethanol (3:1). The material eluted before this change in solvents was discarded, while the eluate after the change was collected to volume in a 100-ml. volumetric flask. A 10-ml. aliquot of this eluate was transferred to a 100-ml. volumetric flask, diluted with 50 ml. of alcohol, 0.50 ml. of 1.000 *N* sodium hydroxide, and water to volume. In a like manner a second 10-ml. aliquot was diluted with 50 ml. of alcohol, 2.00 ml. of 0.100 *N* sulfuric acid, and water to volume in a 100-ml. flask. The differences in absorbances of these two solutions and a set of blank solutions were determined at 298 mμ.

Assay of Camphorated Tincture of Opium.—A 100-ml. portion of camphorated tincture of opium was evaporated to constant volume with the aid of a steam bath and a jet of air. The residue was mixed with 10 ml. of water, reconcentrated to constant volume, treated with ammonia, and chromatographed as described under the assay of tincture of opium. However, 250 ml. of the chloroform-alcohol mixture was required for the complete elution of the morphine. Therefore, a 25-ml. aliquot of this eluate was then transferred to a 100-ml. volumetric flask and brought to volume with alcohol and 0.50 ml. of 1.000 *N* sodium hydroxide. A second 25-ml. aliquot was diluted to volume with alcohol and 2.00 ml. of 0.100 *N* sulfuric acid in a 100-ml. flask. The differences in absorbances of these two solutions and a set of blank solutions were again determined at 298 mμ.

Table I.—Analysis of Morphine Sulfate Pentahydrate in Tablets

	Labeled Concentration	16.2 Gm./Tab.
Found	1	16.3
	2	16.3
	3	16.5
	4	16.2
	5	16.3
	6	16.1
	7	16.9
	8	16.9
	9	17.0
	10	17.0
Av.		16.6

Table II.—Analysis of Anhydrous Morphine in Opium Tinctures

Theoretical Amount		Tincture of Opium 1.00 Gm./100 ml.	Camphorated Opium Tincture 0.0432 Gm./100 ml.
Found	1	1.09	0.0448
	2	1.08	0.0468
	3	1.05	0.0451
	4	1.05	0.0462
	5	1.08	0.0477
	6	1.10	0.0441
	7	1.08	...
	8	1.09	...
	9	1.08	...
	10	1.09	...
Av.		1.08	0.0458

RESULTS AND DISCUSSION

A Δ-absorbance curve for morphine, that is the curve obtained by subtracting the absorbances of the U.V. spectrum in acid solution from those of the U.V. spectrum in alkaline solution, shows maxima at 256 and 298 mμ. The maxima at 298 mμ, even though it is of lower intensity, was utilized because of its stability over larger pH ranges. The difference at 298 mμ was found to be constant provided that the acid solution was at a pH of 5.0 or less and the basic solution was greater than 11.4. To serve as the basis for quantitative determinations of morphine solutions, the difference in molar absorptivity, $\Delta\epsilon$, at this maximum was determined for solutions of known concentrations of anhydrous morphine. The average of 18 determinations resulted in a value of 2,346 for $\Delta\epsilon$, or the difference in absorbance at 298 mμ for molar solutions of morphine at a pH of 12 ±0.1 as compared to molar solutions at a pH of 3.0 ±0.1.

Based upon this value for $\Delta\epsilon$, the assay of various dosage forms of morphine was undertaken. The assay of morphine sulfate in tablets involves the solution of the tablets and the selection of aliquots of this solution so that the difference in absorbance, ΔA, in a basic solution as compared to an acid solution is in the order of 0.3. The amount of morphine sulfate pentahydrate in the aliquot is then calculated by the formula: X Gm./100 ml. = $379.4\ \Delta A/(10\ \Delta\epsilon)$. Table I contains a summary of the results of this analysis of morphine sulfate pentahydrate in 1/4 gr. tablets.

The assay of opium preparations for their morphine content by this procedure still requires preliminary separation. For example, the opium alkaloids narceine, narcotine, and papaverine, as well as some of nonalkaloidal constituents of opium, exhibit differences in U.V. absorption in acid and basic media at 298 mμ.

Grosfeld-Nir, *et al.* (7), have described a chromatographic separation for the determination of morphine in opium. While this procedure is efficient for the separation of morphine from the other opium alkaloids, its application to opium depends upon the careful control of the original extraction of the drug. Under such controlled conditions it is possible to assay for morphine by alkalimetry following chromatographic separation. Tincture of opium or camphorated tincture of opium assayed by this procedure did not give quantitative results for morphine. However, the chromatographic procedure in combination with $\Delta\epsilon$-analysis did give satisfactory results.

Table II is a summary of the results of the analysis of these tinctures by chromatography followed by $\Delta\epsilon$-analysis. The labeled concentration of the commercial tincture of opium was 1 Gm. per 100 ml. The camphorated opium tincture was prepared from the tincture of opium. On the basis of our analysis of 1.08 Gm. of morphine per 100 ml. for this tincture of opium, the camphorated tincture was calculated to contain 0.0432 Gm. of morphine per liter.

CONCLUSION

It is concluded from the results of this investigation that $\Delta\epsilon$-analysis has value for the determi-

nation of morphine in dosage forms and has the advantage of greater specificity as compared to direct U.V. measurements.

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Evaluation and Mechanisms of Action of Several Experimental Hypotensive Agents

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The hypotensive activity of [1-methyl-3-(2'5'6'-trimethyl-1'-cyclohexenyl)propyl] [3-morpholinopropyl] dimethyl ammonium bromide methobromide (RO 2-7832), (+) - 6 - methyl - 9 - diethylaminomethyl - 10 - hydroxy - 1,2,3,3a,5,6,6a,7,11b,11c-decahydro-4H-dibenzo [de,g] quinoline dihydrobromide (RO 2-9618), and 8,8'-bis(2-dimethylaminoethoxy)-6,6'-bithiachroman-4,4'-dione dihydrochloride (RO 2-9811) were investigated in anesthetized rats and dogs. Central and peripheral hypotensive activities were studied utilizing dog cross circulation preparations; and included trimethidinium methosulfate and beta-dimethyl-aminoethyl-N-methylpipecolinate dimethobromide (JB-591) as well as the previously mentioned compounds. RO 2-7832 and trimethidinium appeared to act as potent ganglionic blockers. The hypotensive activity of RO 2-9618 was due to both ganglionic blockade and mild adrenolytic activity. JB-591 was the only compound that demonstrated any central hypotensive activity.

ALTHOUGH the etiology of essential hypertension is still not fully understood, many investigators agree that the reduction in arterial blood pressure is beneficial to the hypertensive patient and will, in many instances, prolong life (1–4). Even though there are many hypotensive compounds available, there is still a need for safer therapeutic agents possessing a minimum of side effects, thereby permitting the patient to lead a useful, productive life. The mechanism of action of the compound should be understood so that the drugs can be intelligently used by the physician in the control of hypertensive cardiovascular disease. This present report is mainly concerned with the evaluation of the hypotensive activity of RO 2-7832,[1] RO 2-9618,[1] and RO 2-9811[1] (Fig. 1), and the mechansim of action of these compounds, and trimethidinium[2] (5) and JB-591[3] (6–8).

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[1] Kindly supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.
[2] Kindly supplied as WY-1395 (Ostensin) by Wyeth Laboratories, Philadelphia, Pa.
[3] Kindly supplied by Lakeside Laboratories, Inc., Milwaukee, Wis., (beta-dimethylaminoethyl-N-methylpipecolinate dimethobromide).

EXPERIMENTAL

Hypotensive Activity in Rats.—The RO compounds were screened and evaluated in anesthetized Wistar rats (urethan, 1.25 Gm./Kg., i.p.) utilizing the method described in a previous paper (9). Direct blood pressure was recorded via the left common carotid artery onto a slowly moving kymograph. The compounds were dissolved prior to use in distilled water and administered via a femoral vein. Hexamethonium, 5 mg./Kg., was utilized as a control compound in this phase of the study.

The oral activity of the three RO compounds was investigated in: (*a*) anesthetized normotensive albino rats, (*b*) unanesthetized normotensive rats, and (*c*) unanesthetized hypertensive rats.

The animals in (*a*) were fasted for 24 hours prior to their use. They were anesthetized with urethan, 1.25 Gm./Kg., i.p., and prepared for direct blood pressure recording. The proximal end of the trachea was cannulated with polyethylene tubing (2 mm. o.d. and 1.5 mm. i.d.). Fresh solutions of the compounds were administered via gastric intubation utilizing a Davol No. 8 French rubber catheter, and each dose was diluted with distilled water to a volume of 2 ml.

The blood pressures of unanesthetized normotensive rats (*b*) were obtained utilizing the photoelectric tensometer (10). Albino Wistar rats were trained for a period of one week prior to the initiation of the study. The animals were fasted for 24 hours, after which fresh solutions of the compounds were administered via gastric intubation as previously described. Blood pressures were obtained periodically prior to and after drug administration.