Determination of Radioactive-Labeled Codeine, Morphine, Dihydromorphine, and Their Metabolites in Biological Materials

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Abstract □ Recoveries of ^{14}C-labeled morphine from aqueous solutions containing 1 to 500 ng. of drug (containing no ammonium ions) by extraction with three volumes of ethylene chloride containing 10, 20, or 30% of *n*-amyl alcohol were 82.9, 91, and 96%, respectively. Dihydromorphine was 79.1% extracted by ethylene chloride containing 30% *n*-amyl alcohol, and codeine, 97%, with all the above mentioned solvent mixtures. Complete hydrolysis of conjugated metabolites of morphine, codeine, or dihydromorphine was achieved by autoclaving samples in a portable autoclave at 18–20 lb. pressure for 2 hr. in a solution of 1.1 *N* HCl, or for 1 hr. in 2.2 *N* HCl, or 0.5 hr. in 3.3 *N* HCl. The conjugated metabolites were also completely hydrolyzed by treatment in a steam-jacketed autoclave at 20 lb. pressure for 0.5 hr. in 2.2 *N* HCl or 1 hr. in 1.1 *N* HCl. A procedure is described for the estimation of codeine and its metabolites, including morphine, in the same samples.

Keyphrases □ Codeine, metabolites—determination, biological samples □ Morphine, metabolites—determination, biological samples □ Dihydromorphine, metabolites—determination, biological samples □ Radioctive labeling—codeine, morphine, dihydromorphine, metabolites □ Scintillometry, liquid—analysis

Methods for determining codeine metabolites in biological samples have been described (1, 2). Johannesson and Woods (3) modified the procedure for estimating radioactive morphine (4, 5) to quantitate codeine metabolites in biological samples. Low recoveries of codeine metabolites, especially conjugated codeine and conjugated morphine, were obtained when using the hydrolysis procedure of Chernov and Woods (5) for biological samples. Incomplete hydrolysis of conjugated morphine in the early studies has been noted (6). The present paper describes conditions for complete hydrolysis of conjugated morphine, conjugated codeine, and conjugated dihydromorphine, and maximum extraction of codeine, morphine, and dihydromorphine in biological materials.

EXPERIMENTAL

Reagents—All reagents used were analytical grade. Toluene counting solution: 100 mg. of 1,4-bis-2(5-phenyloxazolyl)benzene (POPOP), and 3 g. of 2,5-diphenyloxazole (PPO) per liter of toluene; phenethylamine–methanol–toluene counting solution: 100 mg. of 1,4-bis-2(5-phenyloxazolyl)benzene (POPOP), 3 g. of 2,5-diphenyloxazole (PPO), 270 ml. of redistilled colorless phenethylamine (stored in an amber bottle less than 1 month after redistillation), 270 ml. of absolute methanol, and toluene to make 1 l.; naphthalene–dioxane counting solution (7): 60 g. of naphthalene, 4 g. of PPO, 200 mg. of POPOP, 20 ml. of ethylene glycol, 100 ml. of methanol, and dioxane to make 1 l.; NaOH solutions: 1.0–10.0 *N;* morphine carrier, 1 mg./ml. of morphine sulfate in distilled H_2O; codeine carrier, 1 mg./ml. of codeine phosphate in distilled H_2O; dihydromorphine carrier, 1 mg./ml. of dihydromorphine HCl in distilled H_2O; *N*-$^{14}CH_3$-morphine (5) solution in 0.01 *N* HCl; *N*-$^{14}CH_3$-codeine (8) in 0.01 *N* HCl; *N*-$^{14}CH_3$-dihydromorphine (9) in 0.01 *N* HCl; 40 and 4% anhydrous K_2HPO_4 solutions.

Determination of Free and Conjugated Morphine—*Estimation of Free Morphine*—Duplicates of 1-ml. aliquots of biological samples were placed in 40-ml. centrifuge tubes containing 1 ml. of H_2O and 0.5 ml. of nonlabeled morphine carrier. The samples were buffered with 2 ml. of 40% K_2HPO_4 solution, shaken with 15 ml. of ethylene chloride containing 30% *n*-amyl alcohol in an International shaker at 280–300 oscillations per minute for 30 min., and centrifuged at 1700 r.p.m. for 15 min. The aqueous phase was removed by aspiration.[1] The organic layer was shaken by hand for 30 sec. with 5 ml. of 4% K_2HPO_4 solution. After the mixture was centrifuged for 15 min., the aqueous phase was removed by aspiration.

Ten-milliliter aliquots of the organic phase from each sample were transferred to 20-ml. scintillation-counting vials and evaporated to dryness on a Fisher slide-warmer at 55°. The residue was dissolved in 0.8 ml. *n*-amyl alcohol, 10 ml. of toluene counting solution was added, and the radioactivity of each sample was determined in a Nuclear-Chicago model 720 series liquid scintillator spectrometer for 3 × 10 min. Controls for background and known amounts of labeled morphine were run concurrently to serve as a check on the technique and the performance of the counter.

Known concentrations of 1–500 ng. of *N*-$^{14}CH_3$-morphine were carried through the described extraction procedure and a linear relationship was obtained with the curve passing through the origin when net counts per minute (c.p.m.) or disintegrations per minute (d.p.m.) were plotted against the concentration. From the graph a factor converting c.p.m. or d.p.m. values to concentration was obtained for use in calculation of the drug present in biological materials.

Estimation of Total (Free plus Conjugated) Morphine—Duplicates of 1-ml. aliquots of biological samples, obtained after administration of morphine, were placed in 40-ml. centrifuge tubes containing 0.5 ml. of nonlabeled morphine. The mixture was autoclaved 1 hr. in 2.2 *N* HCl (final normality) at 18–20 lb. pressure in a portable autoclave (All-American portable pressure sterilizer, No. 25X, Wisconsin Aluminum Foundry Co.) and remained in the autoclave until the pressure dropped to 1–2 lb. The hydrolyzed samples were first carefully adjusted to pH 9 by saturating with $NaHCO_3$ powder and then buffered with 2 ml. of 40% K_2HPO_4 solution. The procedure for the determination of free morphine was then followed.

Conjugated morphine was calculated from the difference between the autoclaved and nonautoclaved biological samples.

Determination of Free Dihydromorphine and Conjugated Dihydromorphine—Free and conjugated dihydromorphine in biological specimens were determined according to the above procedure except using 0.5 ml. of nonlabeled dihydromorphine carrier instead of morphine carrier.

Determination of Codeine and Its Metabolites—*Estimation of Free Codeine plus Free Morphine*—Free codeine and the free morphine, metabolite of codeine in the biological samples, were estimated according to the procedure for determination of free morphine except that 0.5 ml. of nonlabeled codeine carrier instead of morphine carrier was used.

Estimation of Free Codeine—Free codeine in the biological samples was determined by modifying slightly the method for determination of free morphine and free codeine described above. Two milliliters of 0.5 *N* NaOH solution, instead of 2 ml. of 40% K_2HPO_4 solution, was added to the sample. The apparent morphine remaining in the aqueous phase as sodium morphinate was removed by aspiration. The organic phase was transferred to another tube and shaken with 4 ml. of 0.1 *N* NaOH solution instead of 4 ml. of 4% K_2HPO_4 solution. The counting procedure for determination of morphine was then followed.

Estimation of Total (Free plus Conjugated) Codeine and Total Morphine—Duplicates of 1-ml. aliquots of the biological samples were placed in 40-ml. centrifuge tubes containing 0.5 ml. nonlabeled

[1] If radioactivity remaining in the aqueous was to be estimated, this phase was completely separated and 0.5-ml. aliquots were counted with 10 ml. naphthalene–dioxane or phenethylamine–methanol–toluene solution.

Table I—Recovery of N-$^{14}CH_3$-morphine, N-$^{14}CH_3$-dihydromorphine, and N-$^{14}CH_3$-codeine in Aqueous Solution with and without NH_4OH by Extraction with Ethylene Chloride Containing Varying Amounts of *n*-Amyl Alcohol[a]

Solvents Containing *n*-Amyl Alcohol	Percentage of Added Radioactivity, Mean ± *SD* Extracted into the Organic Phase	Remained in the Aqueous Phase
I. Samples contained no NH_4OH		
A. Morphine		
10%	82.9 ± 3.3	11.4 ± 0.5
20%	91.0 ± 3.3	5.2 ± 0.3
30%	96.0 ± 1.8	4.8 ± 0.3
B. Dihydromorphine		
30%	79.1 ± 0.8	9.0 ± 0.2
C. Codeine		
30%[b]	99.8–103.2	2.9–3.1
II. Samples contained 1 ml. of 1.8 *N* NH_4OH		
A. Morphine		
10%	67.9 ± 1.8	17.8 ± 0.2
20%	88.2 ± 2.9	9.3 ± 0.5
30%	93.8 ± 1.0	8.1 ± 0.5
B. Dihydromorphine		
30%	78.3 ± 0.6	9.0 ± 0.2
III. Samples contained 1 ml. of 3.6 *N* NH_4OH		
A. Morphine		
10%[c]	33.5 ± 4.6	45.1 ± 1.6[d]
20%	56.5 ± 0.9	24.4 ± 0.3
30%	66.4 ± 0.7	17.0 ± 0.1
B. Dihydromorphine		
30%	74.9 ± 1.6	11.3 ± 0.5
C. Codeine		
10%	96.9 ± 1.9	3.7 ± 0.4
20%	96.3 ± 0.7	3.0 ± 0.5
30%	97.5 ± 2.2	3.8 ± 0.1

[a] Samples contained 1 ml. of N-$^{14}CH_3$-morphine (5080 ± 107 d.p.m.) or N-$^{14}CH_3$-dihydromorphine (8191 ± 156 d.p.m.), or N-$^{14}CH_3$-codeine (4778 ± 79 d.p.m.) and 0.5 ml. nonlabeled drug carrier (1 mg./ml.), 2 ml. of 40% K_2HPO_4 solution and 1 ml. of distilled water or ammonium hydroxide solution as indicated. Quadruplicate samples were used for each experiment. [b] Duplicate samples were analyzed. [c] Four experiments (16 samples) were carried out. [d] Radioactivity in the aqueous phase of 4 out of 16 samples was measured in duplicate.

codeine carrier. The mixture was autoclaved in 2.6 *N* HCl at 18–20 lb. pressure in a portable autoclave for 1 hr. The hydrolyzed samples were carefully adjusted to pH 9.0 by saturating with $NaHCO_3$ powder and buffered with 2 ml. of 40% K_2HPO_4 solution. The procedure for estimation of free codeine and free morphine was then followed.

Estimation of Total Codeine—Total codeine in samples, which had been autoclaved and the pH adjusted to above 7.0, was estimated as free codeine.

Calculation of Codeine Metabolites—Free codeine was calculated from a standard curve prepared from known concentrations of N-$^{14}CH_3$-codeine. Conjugated codeine was calculated from the difference between the samples determined for total codeine and for free codeine. Free morphine was estimated from the difference between samples extracted for free morphine plus free codeine and that for free codeine only. Conjugated morphine was obtained from the difference between samples analyzed for total morphine plus total codeine and for total codeine only, followed by the subtraction of the calculated amount of free morphine.

Estimation of Total Radioactivity—Total radioactivity in the sample was determined by counting 0.5-ml. aliquots with 10 ml. of naphthalene–dioxane counting solution or 10 ml. of phenethylamine–methanol–toluene solution. Toluene-^{14}C was used as the internal standard. Disintegrations per minute (d.p.m.) of each sample were determined with the FORTRAN IV computer (10).

Statistical Evaluation—Duncan's new multiple-range test (11) was used for comparison of all the means.

RESULTS

Recoveries of Morphine, Dihydromorphine, and Codeine—The recoveries of morphine, dihydromorphine, and codeine from aqueous solution containing 0.5 mg. alkaloids and 1–500 ng. of radioactive-labeled drug by the described procedures were 96.0 ± 1.8, 79.1 ± 0.8, and 99.8–103.2%, respectively (Table I). The mean recoveries of morphine from aqueous solution containing 2.5 to 10.0 mg. of morphine were 94.0 ± 1.5%. The results indicate that the solvent mixture is very efficient for extraction of these alkaloids.

The recovery of morphine from aqueous solution improved as the proportion of *n*-amyl alcohol was increased in the solvent mixture. Eighty-three percent of morphine was recovered with ethylene chloride containing 10% of *n*-amyl alcohol (Table I). One hundred-percent recovery of morphine with the same solvent mixture has also been reported (4). The higher recovery presumably was due to the salting-out effect of Na_2HPO_4 (5). The effect of saturation of NaCl or $(NH_4)_2SO_4$ on extraction of alkaloids from acidic solution has also been described (12). The presence of ammonium ions was found to decrease the extraction of morphine (Table I).

Separation of Codeine from Morphine—Codeine was extracted into the organic phase from an alkaline aqueous solution containing both codeine and morphine. The effect of alkalinity of the aqueous phase on the removal of morphine by the organic solvent mixture was such that it was necessary to add 2 ml. of 0.5 *N* NaOH solution and to transfer the organic phase to another tube to be washed with 4 ml. of 0.1 *N* NaOH solution.

Recoveries of morphine and codeine, or codeine alone, from samples containing known amounts of both codeine and morphine indicated that both codeine and morphine were quantitatively extracted into the organic phase when the pH of samples was adjusted to 9.0, and only codeine was extracted when the pH was adjusted to 12–13.

Hydrolysis of Conjugated Morphine, Dihydromorphine, or Codeine—The plasma, bile, and urine of dogs collected after s.c. injection of N-$^{14}CH_3$-morphine and urine of rats after s.c. injection of N-$^{14}CH_3$-codeine and N-$^{14}CH_3$-dihydromorphine were used for hydrolysis studies. Autoclaving samples in 1.1 *N* HCl at 18–20 lb. pressure in a portable sterilizer for 0.5 hr. gave 60–70% hydrolysis of conjugated metabolites in biological samples. By increasing either the acid strength or the autoclaving time, hydrolysis of conjugated metabolites of each drug was increased (Tables II and III).

Complete hydrolysis of the morphine conjugate was achieved by autoclaving samples (*a*) in a portable autoclave at 18–20 lb. pressure in 3.3 *N* HCl for 0.5 hr., in 2.2 or 3.3 *N* HCl for 1.0 hr., or with 1.1, 2.2, or 3.3 *N* HCl for 2.0 hr. (Table II), or (*b*) in a steam-jacketed autoclave (American Sterilizer Co.) at 20 lb. pressure with 2.2 or 3.3 *N* HCl for 0.5 and 1.0 hr. or with 1.1 *N* HCl for 1.5 hr. (Table

Table II—Comparison of the Effect of Acidic Strength and the Autoclaving Time on Hydrolysis of Morphine, Dihydromorphine, and Codeine Conjugated Metabolites[a] in Biological Samples Using a Portable Autoclave[b]

	Maximal Mean, d.p.m.[c]	Percentage of Hydrolyzed Conjugates in the Biological Specimens, Mean ± *SD*[d]								
Time of autoclaving, hr.		0.5			1.0			2.0		
Normality of HCl solution		1.1	2.2	3.3	1.1	2.2	3.3	1.1	2.2	3.3
Plasma of dog after morphine	927	64.1 ± 3.1*	96.1 ± 6.0	101.0 ± 1.4	76.2 ± 1.9*	97.2 ± 1.4	100.5 ± 2.4	100.8 ± 4.9	100.5 ± 4.1	101.6 ± 1.9
Bile of dog after morphine	17972	69.7 ± 7.5*	84.4 ± 16.1	104.3 ± 1.9	89.7 ± 5.7*	100.9 ± 5.0	105.0 ± 10.0	91.0 ± 14.2	92.4 ± 8.9	100.5 ± 6.4
Urine of dog after morphine	1627	63.6 ± 1.5*	86.4 ± 6.6	99.6 ± 0.9	91.6 ± 4.8*	98.7 ± 0.4	96.7 ± 3.6	101.5 ± 1.8	97.8 ± 4.0	100.6 ± 3.5
Urine of rat after codeine	2023	60.3 ± 5.7*	89.4 ± 7.3*	96.3 ± 5.5	91.4 ± 10.3*	98.4 ± 3.6	97.6 ± 2.6	98.8 ± 4.8	104.0 ± 3.6	99.2 ± 2.6
Urine of rat after dihydromorphine	3867	73.4 ± 8.4*[e]	91.8 ± 3.1*[e]	104.1 ± 1.4[e]	89.9 ± 5.4*	104.9 ± 11.4	101.7 ± 3.4	94.4 ± 3.4	95.7 ± 3.6	99.1 ± 3.8

[a] Quadruplicate samples were analyzed in each experiment. [b] All-American portable pressure sterilizer, No. 25X, Wisconsin Aluminum Foundry Co. Inc., Manitowoc, Wis. [c] The maximal mean was calculated from the means of the conjugate obtained upon autoclaving the sample in a portable autocalve at 17–20 lb. pressure with 3.3 *N* HCl for 0.5, 1.0, and 2.0 hr.; 2.2 and 3.3 *N* HCl for 1.0 and 2.0 hr., 1.1, 2.2 and 3.3 *N* HCl for 2 hr., and in a steam-jacketed autoclave at 20 lb. pressure with 2.2 and 3.3 *N* HCl for 0.5 and 1.0 hr., and 1.1 *N* HCl for 1.5 hr. [d] The * indicates a significant difference from the maximal mean at the 0.05 level. [e] Samples after autoclaving were left overnight in the sterilizer.

Table III—Comparison of the Effect of Acidic Strength and the Autoclaving Time on Hydrolysis of Morphine, Dihydromorphine, and Codeine Conjugated Metabolites[a] in Biological Samples Using a Steam-Jacketed Autoclave[b]

	Maximal Mean, d.p.m.[c]	Percentage of Hydrolyzed Conjugates in the Biological Specimens, Mean ± *SD*[d]								
Time of autoclaving, hr.		0.5			1.0			2.0		
Normality of HCl solution		1.1	2.2	3.3	1.1	2.2	3.3	1.1	2.2	3.3
Plasma of dog after morphine	927	93.5 ± 3.6	101.4 ± 1.9	100.8 ± 0.9	101.8 ± 2.3	99.2 ± 2.3	96.5 ± 5.8	98.3 ± 2.8	96.2 ± 1.2	88.1 ± 8.2
Bile of dog after morphine	17972	101.3 ± 2	100.3 ± 4.8	104.6 ± 4.8	103.0 ± 2.1	102.0 ± 2.1	99.0 ± 0.8	100.2 ± 3.3	101.1 ± 1.8	94.4 ± 3.2
Urine of dog after morphine	1627	97.5 ± 2.5	100.5 ± 1.8	99.5 ± 2.0	102.9 ± 1.5	100.1 ± 1.2	102.1 ± 0.8	99.7 ± 1.0	100.4 ± 1.5	97.2 ± 3.1
Urine of rat after codeine	2023	84.9 ± 5.7*	101.1 ± 5.1	102.6 ± 3.7	89.6 ± 4.8	103.9 ± 4.2	98.4 ± 4.7	91.7 ± 1.7	91.7 ± 1.7	79.2 ± 2.0

[a] Quadruplicate samples were analyzed in each experiment. [b] American Sterilizer Company, Erie, Pa., [c] The maximal mean was calculated from the means of the conjugate obtained upon autoclaving the sample in a portable autoclave at 17–20 lb. pressure with 3.3 *N* HCl for 0.5, 1.0, and 2.0 hr.; 2.2 and 3.3 *N* HCl for 1.0 and 2.0 hr., 1.1, 2.2, and 3.3 *N* HCl for 2 hr., and in a steam-jacketed autoclave at 20 lb. pressure with 2.2 and 3.3 *N* HCl for 0.5 and 1.0 hr., and 1.1 *N* HCl for 1.5 hr. [d] The * indicates a significant difference from the maximal mean at the 0.05 level.

Table IV—Hydrolysis of Pure Drug Conjugates: Codeine-6-glucuronide, Morphine-3-glucuronide, and Morphine-3-ethereal Sulfate[a]

Sample	HCl, *N*	Autoclaving Time, hr.	Liberated Drug, mcg., Mean ± *SE*	Conjugate Hydrolyzed, %
Codeine-6-glucuronide dihydrate, 105 mcg.	1.1	0.5[b]	33.5 ± 0.2	57.6
		2.0	31.7	54.5
	2.6	0.5	36.6	62.9
		1.0[b]	55.1 ± 0.8	94.7
		2.0	54.5	93.7
	5.3	0.5	52.5	90.2
		1.0	53.1	91.3
Morphine-3-glucuronide dihydrate, 70 mcg.	2.2	1.0	41.4	98.3
Morphine-3-ethereal sulfate, 210 mcg.	2.2	1.0	150.5	92.3

[a] Quadruplicate samples were autoclaved with HCl in a portable autoclave at 18–20 lb. pressure. The liberated drug was determined with the modified methyl orange dye method (16). [b] Three experiments with four samples each were done.

III). The recovery of morphine was lower than maximum when the samples were autoclaved with 2.2 or 3.3 *N* HCl in a steam-jacketed autoclave at 20 lb. pressure for 1.5 hr. and allowed to remain in the autoclave for 2 hr. (Table III). After extraction, the radioactivity remaining in the aqueous phase was approximately 10% of the total in the biological samples. This radioactivity may represent morphine altered chemically by the conditions of autoclaving.

Complete hydrolysis of isolated pure morphine-3-glucuronide, the major metabolite of codeine in rats or of morphine in rats, dogs, and man, and that of isolated pure morphine-3-ethereal sulfate, the major metabolite of morphine in cats, is accomplished under similar conditions as described above (Table IV).

Codeine-6-glucuronide, the major metabolite of the drug isolated from the urine of dogs (13), is hydrolyzed completely in 2.6 *N* HCl at 18–20 lb. pressure in a portable autoclave for 1 hr., or in 5.3 *N* HCl for 0.5 hr. (Table IV).

Determination of Radioactivity in Aqueous Samples—Total radioactivity was determined successfully by mixing 0.5-ml. aliquots of aqueous solutions, plasma, bile, urine, or fecal homogenate with either 10 ml. of dioxane–naphthalene solution or 10 ml. phenethylamine–methanol–toluene solution.

The results with the described procedure for analysis of *N*-$^{14}CH_3$-codeine metabolites in the bile, urine, and fecal homogenate of rats have previously been reported (8).

DISCUSSION

The recovery of total morphine, dihydromorphine, or codeine by extraction with ethylene chloride containing *n*-amyl alcohol is dependent on complete hydrolysis of conjugated drug and the proper adjustment of pH and salt concentration in the hydrolyzed samples. Using NaOH solutions to adjust the hydrolyzed samples to pH 9–10 was somewhat tedious and laborious, involving some loss of samples due to testing the solution with pH paper. Ammonium hydroxide, instead of NaOH solution, had been used in these experiments to bring the hydrolyzed samples to pH 9–10. However, lower and inconsistent recovery of total morphine was observed, presumably due to the formation of ammonium morphinate salt which remained in the aqueous phase (Table I). The adjustment of autoclaved biological samples to pH 9 by saturating with $NaHCO_3$ was found to give most satisfactory results. The recovery of codeine was not affected by either the presence of NH_4OH ions in the system or by the amount of *n*-amyl alcohol.

Conditions used in previous studies for hydrolysis of conjugated morphine and its analogs varied considerably. The original work on hydrolysis of conjugated narcotics reported by Gross and Thompson (14) stated that 35–92% of the injected morphine could be recovered in the urine and feces of the dog after the biological samples had been autoclaved in 1.1 *N* HCl at 15 lb. pressure for 0.5 hr. Longer hydrolysis or use of stronger acid (up to 5.5 *N*) did not change the yield. The autoclave used by Gross and Thompson was presumed to be a steam-jacketed one. Identical conditions were followed by most subsequent investigators (1, 15). Data have also been reported on hydrolysis of morphine conjugate in 1.1 *N* HCl in a steam-jacketed autoclave at 18 lb. pressure for 0.5 hr. (2, 16) or 25 min. (4); in 0.8 *N* HCl at 15–18 lb. pressure in a portable autoclave for 20 min. (5, 17); and in 4.1 *N* HCl on a boiling water bath for 0.5 hr. (18). The different results are probably due to the variablility of hydrolysis conditions. The elevated temperature is prolonged in a steam-jacketed autoclave which has been switched off, while the temperature in a portable autoclave drops quickly to room temperature. Furthermore, the kinetics of degradation of morphine in aqueous solution have been shown to be dependent on the morphine concentration, on the concentration of oxygen, and on temperature (19).

The strength of the alkaline solution used by Johannesson and Woods (3) for extraction of codeine from aqueous solution containing codeine and morphine was found inadequate to remove morphine completely. In addition, with their procedure, 0.5 to 1.0 ml. of the alkaline solution residue remained on the top of the organic phase. Ten to twenty-five percent of the apparent morphine was extracted back to the organic phase when it was shaken with 4 ml. of 4% K_2HPO_4 solution. Conjugated morphine [45% of codeine injected in the rat (8)], a major metabolite of codeine, therefore would be mistakenly estimated as conjugated codeine.

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Complexes of Ergot Alkaloids and Derivatives III: Interaction of Dihydroergocristine with Xanthine Analogs in Aqueous Media

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Abstract □ The capacity of the poorly water-soluble ergot derivative, dihydroergocristine methanesulfonate, to form intermolecular complexes with caffeine, theophylline, and 7-β-hydroxypropyltheophylline was studied. Upon inclusion of xanthine, dihydroergocristine exhibited elevated solubility at pH 6.65, a larger dissolution rate constant in 0.1 *N* HCl, and a change in partitioning-rate constants for transfer of the alkaloid from aqueous to organic phases. These alterations of physicochemical properties appear to be a consequence of mutual interaction between the two components in solution. The effect of dihydroergocristine was enhanced on enteral administration with each of the three complexing agents as measured on α-adrenergic blockade in cats. When tritiated dihydroergocristine was given orally to humans along with 7-β-hydroxypropyl-theophylline, blood levels went higher and stayed higher than when the alkaloid was administered alone. The same situation was true of total urinary excretion of tritium.

Keyphrases □ Ergot alkaloids—physicochemical analyses □ Dihydroergocristine, interacting—xanthine analogs □ Xanthine-dihydroergocristine complex formation—solubility effect □ Biological activity, dihydroergocristine—xanthines, effect □ Partitioning rates—dihydroergocristine-xanthine complex □ Colorimetric analysis—spectrophotometer

Previous studies in this area (1, 2) have attempted to correlate *in vitro* data with physiological responses observed on addition of caffeine and other xanthines to several ergot alkaloids and their congeners (3–5).

The present report is concerned with interactions occurring in solution between three complexing agents—caffeine, theophylline, and the soluble derivative 7-β-hydroxypropyl-theophylline—and dihydroergocristine methanesulfonate. Dihydroergocristine is one of the hydrogenated alkaloids of the ergotoxine group (6).

This work points to a good accord between physicochemical data derived from solubility, dissolution rate, and partitioning-rate studies and pharmacological results from human and animal investigations. The evidence appears to indicate complex formation leading to increased absorption rate, as well as the amount of absorption of many ergot derivatives in the presence of xanthines.

EXPERIMENTAL

Materials—Dihydroergocristine methanesulfonate[1] (mol. wt. 707.8) showed only traces of contaminants when subjected to thin-layer chromatography.

The various xanthines utilized were: 7-β-hydroxypropyl-theophylline,[2] m.p. 135–138°; theophylline,[3] m.p. 272–274°; and caffeine anhydrous powder USP,[4] m.p. 238°.

Melting points are uncorrected. Reagent grade chloroform (Mallinckrodt Chemical Works) was employed in the partitioning studies. A pH 6.65 buffer was made by dissolving 13.6 g. KH_2PO_4 in 500 ml. water, adjusting the pH with concentrated KOH, and diluting to 1 l. (ionic strength, 0.2).

The pH measurements were taken on a Metrohm pH meter and spectrophotometric data were obtained from a Cary model 14 spectrophotometer.

Partitioning Studies—A solution was prepared by placing dihydroergocristine methanesulfonate (150 mg.) in 950 ml. of pH 6.65 phosphate buffer, stirring magnetically for 30 min. to 1 hr, followed by filtration (Whatman No. 1 filter paper) into a flask immersed in a water bath maintained at 30°, and finally addition of pH 6.65 buffer to make 1 l. of solution.

This solution was immediately analyzed for dihydroergocristine (7) and read at 585 mμ (absorbance of 0.902 equivalent to 0.1 mg./ml.). The usual concentrations of alkaloid obtained in this manner were in the range of 0.06 to 0.09 mg./ml.; 500 ml. (half) of this solution of known concentration was kept and 500 ml. had xanthine added to it.

Fifteen milliliters of the freshly prepared aqueous phase—either with or without xanthine—was added carefully to 15 ml. chloroform in screw-capped vials (50 ml.). The vials were sealed and rotated at 6 r.p.m. in a 30° (±0.1°) water bath. Five-milliliter samples were taken at 3, 5, 7, 9, 11, 13, and 15 min. from the aqueous phase and analyzed for dihydroergocristine by the Van Urk method (7).

Solubility Studies—Dihydroergocristine (50 mg.) was placed in watertight, amber, screw-capped vials (50 ml.) containing exactly 10 ml. of pH 6.65 phosphate buffer and varying quantities of the three xanthines being considered. The vials were clamped onto the edge of metal disks (15-cm. diameter) mounted on a motor-driven shaft and rotated vertically at 6 r.p.m. in a constant-temperature bath, 30 ± 0.1°. After 24 hr., samples were taken using pipets with filters attached and analyzed for dihydroergocristine by the Van Urk method (7).

[1] Sandoz, A.-G., Basel, Switzerland.
[2] Ganes Chemical Works, Inc., New York, N. Y.
[3] Matheson, Coleman and Bell.
[4] Chas. Pfizer Co.