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

H. V. MAULDING and M. A. ZOGLIO

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.

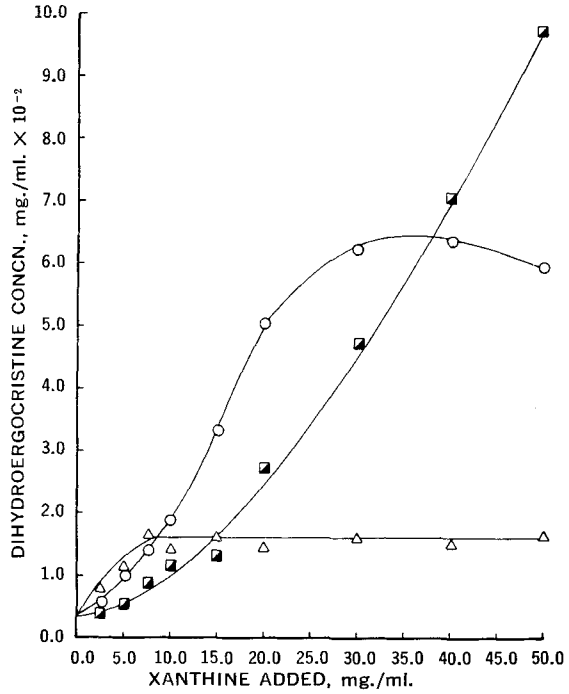

Figure 1—*Solubilizing action of xanthine on dihydroergocristine in phosphate buffer (pH 6.65) at 30° for 24 hr. Key: ◪, 7-β-hydroxypropyl-theophylline; ○, caffeine; and △, theophylline.*

Dissolution Rates—A 25-r.p.m. stirrer motor fitted with a 2.54-cm. propeller blade placed 4 cm. from the bottom of an 800-ml. beaker containing 500 ml. 0.1 *N* HCl solution was employed for these determinations. Dihydroergocristine (50 mg.) or the alkaloid in combination with xanthine, prepared by mixing 50 mg. alkaloid with 5 g. xanthine in a mortar, was placed into the stirred solution from a height of about 1.5 cm. The temperature was maintained at 37 ± 0.1° by immersing the beaker in a constant-temperature bath. Samples were withdrawn periodically for dihydroergocristine analysis in the usual manner (7).

RESULTS AND DISCUSSION

The solubility of dihydroergocristine methanesulfonate is elevated to some degree by all three xanthines examined at pH 6.65 as may be seen from Fig. 1. It is apparent (Fig. 1) that 7-β-hydroxypropyl-theophylline ultimately allows a greater solubilization of the alkaloid than caffeine or theophylline; however, this was not shown in concentrations greater than 50 mg./ml. xanthine because extremely large amounts are of little practical consequence. Caffeine leads to greater solubilization of the alkaloid than theophylline as it elevates the amount of dihydroergocristine in solution at this pH and temperature (30°) by almost 12 times, while theophylline increases the amount about 3 times. This was previously shown to be the situation with dihydroergotoxine (2). Curves as seen in Fig. 1 do not readily allow analysis by elegant treatments as those of Connors *et al.* (8, 9), thus leaving the exact nature of the complex in doubt.

It is recognized that dissolution rates of some solids may be the rate-limiting step in their absorption (10, 11). When ergot derivatives were subjected to dissolution-rate studies with and without the presence of xanthines, the rate constant was found to be increased nearly threefold in the first instance (1, 2, 5). The authors have previously found HCl (500 ml., 0.1 *N*) allows approximation of a first-order process with these substances. Figure 2 gives an example where a 100:1 ratio of 7-β-hydroxypropyl-theophylline to dihydroergocristine was utilized in the dissolution rate at a stirrer speed of 25 r.p.m. and 37°. The rate constants vary by a factor of 2.5:1 (0.053 to 0.138 $min.^{-1}$), which is in agreement with those previously reported (1, 2). Caffeine and theophylline gave almost the same ratios and results as those reported in Fig. 2 relative to the alkaloid, although they are not reported here. Therefore, for the conditions employed, there is evidence of a significant change in dissolution-rate constant on addition of xanthine to dihydroergocristine.

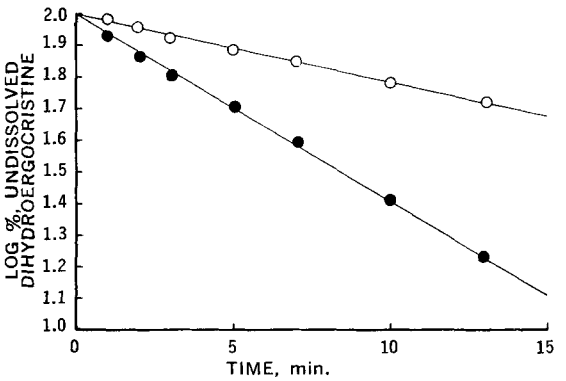

Figure 2—*Effect of 7-β-hydroxypropyl-theophylline on the dissolution rate of dihydroergocristine in 0.1 N HCl (500 ml.) at 37° and 25 r.p.m. Key: ○, the dissolution of dihydroergocristine, 50 mg.; and ●, the dissolution of dihydroergocristine, 50 mg., and 7-β-hydroxypropyl-theophylline, 5 g.*

It was previously observed that xanthines had the effect of increasing the partitioning rate of ergot derivatives from an aqueous to an organic phase under certain conditions, although the equilibrium distribution was not disturbed (1, 2). Figure 3 gives an example of the action of 7-β-hydroxypropyl-theophylline in altering the partitioning rate of dihydroergotoxine methanesulfonate of which dihydroergocristine methanesulfonate is one of the three components (2). This phenomenon was noted with dihydroergocristine methanesulfonate itself in the presence of 7-β-hydroxypropyl-theophylline in a weight–weight ratio of 100:1 (xanthine–alkaloid) and the results may be seen in Fig. 4. The results in Fig. 4 show slopes of 2.5×10^{-2} $min.^{-1}$ and 5.1×10^{-2} $min.^{-1}$ for the alkaloid alone and with xanthine, respectively. Similar results were obtained with caffeine and theophylline and are not reported here. In 0.1 *N* HCl the rate was reversed as was found to be the case with ergotamine tartrate (1).

An investigation was made of the effect of increasing 7-β-hydroxypropyl-theophylline concentration on the partitioning-rate constants of dihydroergocristine methanesulfonate as the amount of xanthine goes from 0 to 500 times the weight of the ergot derivative

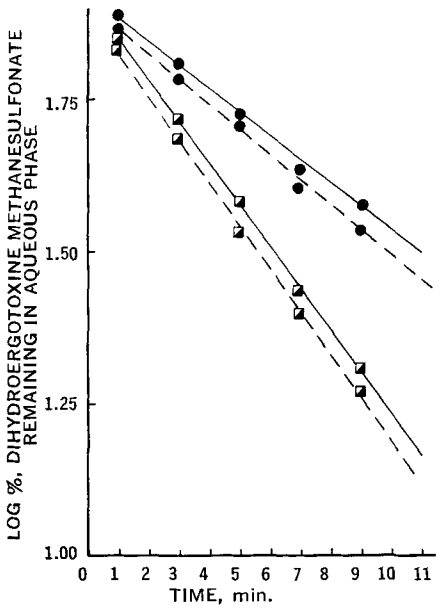

Figure 3—*Effect of 7-β-hydroxypropyl-theophylline on the partitioning rate of dihydroergotoxine methanesulfonate from an aqueous (phosphate buffer, pH 6.65) to an organic phase (chloroform). Key: ●, dihydroergotoxine, 0.1 mg./ml.; ◪, dihydroergotoxine, 0.1 mg./ml., and 7-β-hydroxypropyl-theophylline, 10.0 mg./ml.; - - -, experimental run No. 1; and ——, experimental run No. 2.*

Table I—First-Order Partitioning Rate Constants Showing Partitioning of Dihydroergocristine Methanesulfonate from pH 6.65 Phosphate Buffer into Chloroform at 30°[a]

Dihydroergocristine methanesulfonate[b]	1	1	1	1	1	1
7-β-Hydroxypropyl-theophylline[c]	0	10	50	100	200	500
Rate constant × 10^{-2} $min.^{-1}$[d]	2.5	3.0	4.4	5.1	5.8	6.6

[a] All experiments carried out as mentioned in *Experimental* section with xanthine added after assay to determine alkaloid present. Concentrations of dihydroergocristine varied from 0.06 mg./ml. as mentioned. [b,c] Quantities given are measured on a weight to weight basis, *i.e.*, 1:50 is 1 mg. dihydroergocristine and 50 mg. 7-β-hydroxypropyl-theophylline. [d] Rate constants and slopes thereof obtained by linear regression analysis from four experiments utilizing each ratio and run consecutively. Values were measured between 3 and 15 min.

in solution. The results are listed in Table I and show a general trend upward for the rate constants as the amount of xanthine is increased. A dose–effect relationship of this same type was encountered on enteral administration of dihydroergocristine to cats with a general increase in effect up to a 50:1 ratio (w/w) of xanthine to alkaloid followed by leveling up to a ratio of 200:1 (5).

Biological—The α-adrenergic blockade of dihydroergocristine methanesulfonate in cats was studied with and without xanthine present and this activity was enhanced on enteral administration of the alkaloid together with caffeine, theophylline, and 7-β-hydroxypropyl-theophylline. Significant increases in blocking action were noted at 7-β-hydroxypropyl-theophylline to dihydroergocristine ratios of 12.5:1 (w/w), the maximum effect being at 50:1 with no further increases on addition of excess complexing agent. This phenomenon was characterized by: (*a*) faster onset, (*b*) higher maximum, and (*c*) longer duration of action when the alkaloid and xanthine were administered concurrently. These differences were not encountered on i.v. dosing and are a result of increased dihydroergocristine absorption from the gut (5).

Tritiated dihydroergocristine was administered orally to five patients along with 7-β-hydroxypropyl-theophylline (1 mg. alkaloid and 100 mg. xanthine) and plasma levels were found to go higher and stay higher longer than when dihydroergocristine (1 mg.) was given alone. Total urinary tritium excretion was appreciably higher in the combination than in the case of the alkaloid itself; however, not much appears to be unchanged dihydroergocristine (5).

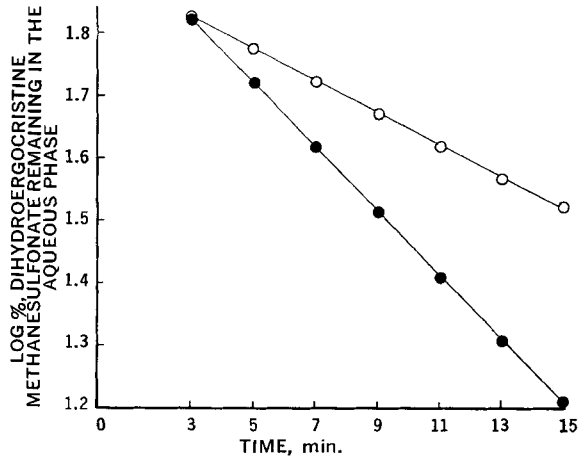

Figure 4—*Effect of 7-β-hydroxypropyl-theophylline on the partitioning rate of dihydroergocristine from an aqueous (phosphate buffer, pH 6.65, 30°, 6 r.p.m.) to an organic phase (chloroform). Key:* ○, *dihydroergocristine, 0.072 mg./ml.; and* ●, *dihydroergocristine, 0.072 mg./ml., and 7-β-hydroxypropyl-theophylline, 7.2 mg./ml.*

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