Complexes of Ergot Alkaloids and Derivatives IV: Investigations into the Nature of Ergot Alkaloid–Xanthine Complexes

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Abstract □ Studies of the effect of xanthines on the solubility of molecules related to the proteinaceous ergot alkaloids indicate the indole moiety to be a primary site for complexation. The tendency of indole and several analogs to form intermolecular complexes with caffeine was surveyed. This property leads to considerable solubility elevation for many of the compounds examined. Apparent equilibrium or association constants are calculated based on an assumed 1:1 complex between the indole substrate and xanthine ligand. Experimental evidence with several simple indoles and caffeine indicates general adherence to this principle. These relatively uncomplex compounds and even larger more complicated molecules such as methysergide exhibit this property of increased solubility through interaction. These substances may be treated in a somewhat rigorous manner contrary to the intricate proteinaceous alkaloids previously examined.

Keyphrases □ Ergot alkaloid–xanthine complexes—solubility effect □ Xanthine–ergot alkaloid complexes—solubility effect □ Spectrophotometry—analysis

The oral administration of ergotamine tartrate with caffeine has been reported to bring about an enhancement of the clinical activity of the alkaloid against migraine headaches (1, 2).

In the course of investigating the physicochemical nature of the combination, several ergot alkaloids and their analogs, such as ergotamine, 9,10-dihydroergocristine, and methysergide, have been observed to possess the capacity for interaction with caffeine and other xanthines in aqueous solution (3–6). This mutual attraction is exemplified by: (*a*) increased solubility; (*b*) increased dissolution-rate constant, and (*c*) altered partitioning-rate constants depending on pH. These changes are noted on inclusion of xanthines as compared to the alkaloids alone (3–6). Recent biological results (7) were found to be in good agreement with the postulation that complex formation leads to increased enteral absorption of ergotamine and ergot alkaloids.

A series of papers in 1957 reported that caffeine and related compounds complex with a wide variety of organic compounds (8–10) as well as with themselves (11). This behavior of caffeine and other xanthines has been studied by means of solubility (8, 11, 12), spectral (13), and kinetic (9, 10, 14) techniques.

It was believed that an investigation of simpler indoles might give some clue regarding the nature of the complex present in complicated ergot alkaloids. A project was initiated to examine indole and several congeners to determine structural factors affecting interaction and delineation of structure–activity relationships. It was hoped this approach would help in the eventual unraveling of the nature and stoichiometry of the species comprising the ergot–xanthine complex, the ultimate aim being application of these and other data to formulation of more suitable medicinal agents.

EXPERIMENTAL

Watertight, screw-capped vials (18-ml. capacity) containing exactly 10 ml. of solvent, 100 mg. of substrate (indole compound), and varying amounts of caffeine were clamped onto the edge of metal disks, 15.24 cm. (6 in.) in diameter, mounted on a motor-driven shaft and rotated vertically at 6 r.p.m. in a constant-temperature bath at 30 ± 0.2°. After exactly 24 hr., samples were withdrawn using pipets with filters attached and analyzed for the indole compound by the Van Urk method (3). Each sample was run at minimum in triplicate.

RESULTS AND DISCUSSION

The absence of observable spectral changes precluded utilization of this technique in these experiments. The kinetic method was not employed because of the absence of a suitable reaction for study, thus leaving solubility analysis. This mode has previously been found to be practicable in the study of proteinaceous ergot derivatives, although certain problems have arisen from their erratic solubility properties (3–6).

The usual experimental operation involves addition of an equal weight (in excess of its normal solubility) of the slightly soluble substrate into each of several vials containing a fixed amount of solvent. Varying amounts of xanthine were placed in the vials, which were rotated 24 hr. in a constant-temperature bath. The solution phase was withdrawn by means of a filter pipet with care taken not to include undissolved indole. Assays were performed by the Van Urk (15) method, which lends itself to most molecules containing the indole nucleus. The colors formed absorbed at various wavelengths, depending on the compound in question (Table I).

Data obtained by the solubility analysis scheme may be treated in the following manner, as summarized by Connors and Mollica (16) and using their symbolism. The term S in Eq. 1 represents substrate, indole compound, and L represents ligand, xanthine:

$$mS + nL \rightleftarrows S_mL_n \qquad \text{(Eq. 1)}$$

The strength of the complex is given in terms of K_{mn}, which is the stability or association constant:

$$K_{mn} = \frac{[S_mL_n]}{[S]^m[L]^n} \qquad \text{(Eq. 2)}$$

Table I—Absorptivity and Wavelengths of Maximum Absorbance of Indole Compounds

Compound	ϵ^a	λ, mμ^b
1. Indole	4217	400
2. 1-Methylindole	5245	400
3. 2-Methylindole	4810	380
4. 3-Methylindole	3142	410
5. 5-Methylindole	4810	410
6. 7-Methylindole	4198	425
7. 5-Methoxyindole	5151	400
8. 6-Methoxyindole	3287	400
9. 7-Methoxyindole	2362	450
10. 5-Fluoroindole	5575	400
11. 5-Chloroindole	6216	400
12. 5-Bromoindole	6240	400
13. Methysergide[c]	6905	322

[a] Molar absorptivity. [b] Compound 13, methysergide, measured in the UV region; the remainder in the visible, using Van Urk reagent (13). [c] Measured as bimaleate salt, pH 6.65, 25°.

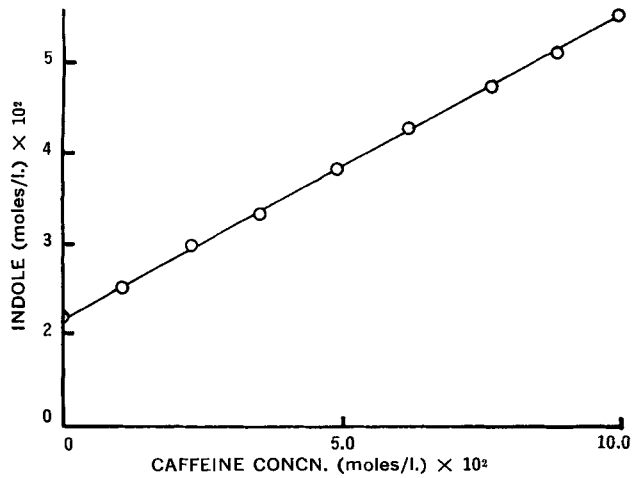

Figure 1—*Solubility phase diagram for the indole–caffeine system at 30° in pH 6.65 phosphate buffer.*

The parsimonious approach stipulates assumption, in lieu of conflicting information, of a 1:1 stoichiometry brought about by a single complex to be responsible for the results. Thus the values for m and n (Eqs. 1 and 2) are 1. Making proper substitutions in these equations, one obtains the following (16):

$$S_t = \frac{K_{11}S_0L_t}{1 + K_{11}S_0} + S_0 \qquad \text{(Eq. 3)}$$

In Eq. 3 the quantities S_t and S_0 are the total concentrations (moles/l.) of indole or alkaloid in solution at various finite and zero xanthine concentrations, respectively. K_{11} is the association constant as previously stated, and L_t is xanthine concentration in moles/liter.

A plot of L_t against S_t gives a straight line with a slope of

$$\frac{K_{11}S_0}{1 + K_{11}S_0}$$

and a y-intercept of S_0 or the solubility at zero ligand concentration. If a single 1:1 complex is present, the apparent K_{11}, written K'_{11} may be written (16):

$$K'_{11} = \frac{\text{slope}}{y\text{-intercept}\ (1 - \text{slope})} \qquad \text{(Eq. 4)}$$

The compounds considered in this work were run at pH 6.65 in phosphate buffer. The simpler substances such as indole, 1-methylindole, and 5-methoxyindole all have pKa values of 2 or less. Therefore, they are essentially present in only the neutral species at the hydrogen-ion concentrations employed. The same is true for caffeine. As a result of negligible protonation of both molecules (substrate and ligand) at these pH values, the result should be binding between two neutral species. Methysergide possesses a pKa of 6.62 ± 0.02 (6), which indicates any study concerning the molecule at pH 6.65 is at the point of half-ionization, *i.e.*, pH = pKa.

Caffeine was chosen as the best candidate for the ligand for several reasons. It is more soluble than the naturally occurring xanthines such as theophylline and theobromine and offers the best basis for comparison. Much of the earlier work was done with this compound, even though it does complex to an appreciable extent with itself in aqueous solutions (8–14). Caffeine exhibits about 2% solubility at 30°. For this reason, caffeine (ligand) concentrations of greater than 20 mg./ml. were not generally examined.

When the concentration of proteinaceous alkaloid is plotted *versus* caffeine concentration, a strictly linear function is not generated (3). Positive and negative deviations from this linearity take place more or less at random, depending on the alkaloid, xanthine, and solvent in some unknown manner (4, 5). With simple

Table II—Values Obtained from Phase Diagrams of Indole and Analogs in the Presence of Caffeine[a]

Compound[b]	Slope[c]	$S_0 \times 10^{2}$[d]	K'_{11}[e]
1. Indole	0.34	2.11	24.1
2. 1-Methylindole	0.13	0.30	50.0
3. 2-Methylindole	0.19	0.41	59.4
4. 3-Methylindole	0.31	0.55	83.8
5. 5-Methylindole	0.35	0.48	112.0
6. 7-Methylindole	0.23	0.50	59.7
7. 5-Methoxyindole	0.47	1.30	64.1
8. 6-Methoxyindole	0.16	0.41	47.1
9. 7-Methoxyindole	0.30	0.85	52.9
10. 5-Fluoroindole	0.19	1.52	15.8
11. 5-Chloroindole	0.06	0.28	23.0
12. 5-Bromoindole	0.07	0.13	58.3
13. Methysergide	0.26	1.60	22.0

[a] Measurements made at 30 ± 0.2° in pH 6.65 phosphate buffer. [b] Compound 2 secured from K & K, Inc., Plainview, N. Y.; Compounds 6 and 8 from Chemical Procurement, College Point, N. Y.; and the remainder from Aldrich Chemical Co., Milwaukee, Wis. [c] For calculations, see text and figures. [d] Intrinsic solubility of substrate, 30°, and zero xanthine concentration (moles/l.). [e] K'_{11} = slope/(1 − slope)S_0.

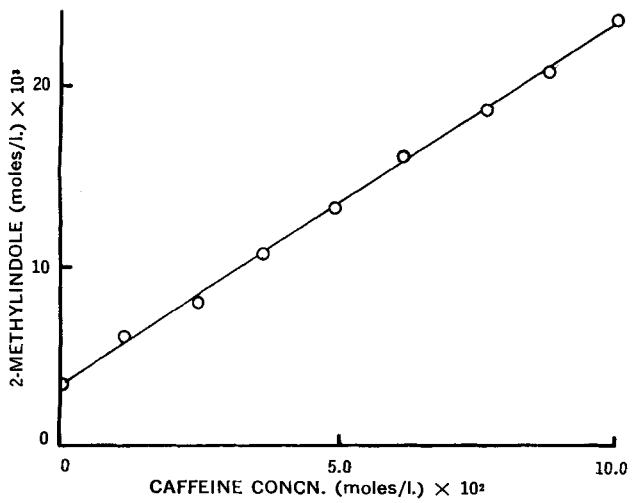

Figure 2—*Phase solubility diagram for the 2-methylindole–caffeine system at 30° in pH 6.65 phosphate buffer.*

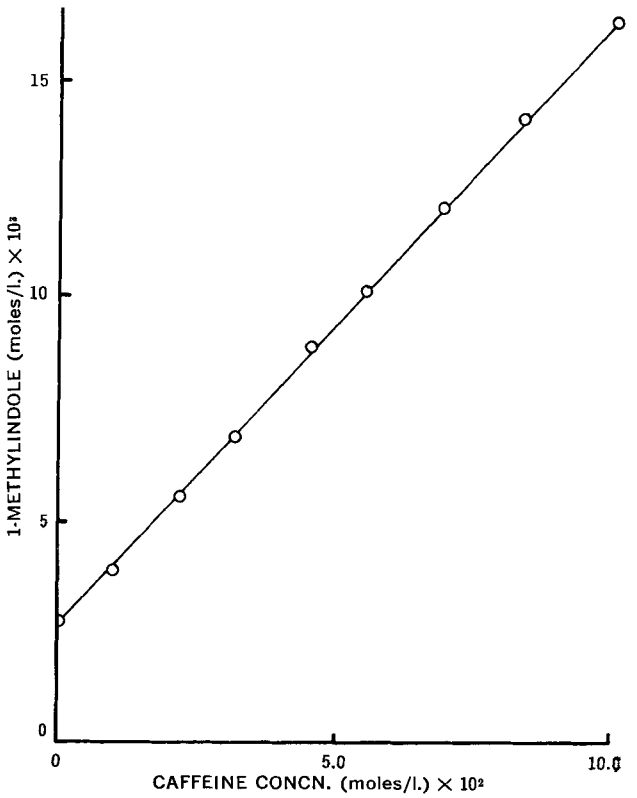

Figure 3—*Phase solubility diagram for the 1-methylindole–f system at 30° in pH 6.65 phosphate buffer.*

indoles, this is not the situation, as may be seen in Figs. 1–3. These substances have K'_{11} values; other pertinent information is listed in Table II.

CONCLUSION

The stoichiometry of the complex has not been delineated, but slopes of less than one as encountered in Figs. 1–3 imply a 1:1 complex. This does not prove the absence of other complex species (16).

Table II illustrates that all K'_{11} values, including that of methysergide, are of approximately the same magnitude. This indicates that a good portion of the capacity for complexation of the high molecular weight ergot alkaloids lies within the indole moiety of the molecule. The role the cyclic tripeptide portion plays in the mutual attraction of caffeine and related xanthines for these proteinaceous alkaloids has yet to be spelled out; however, it is likely that these protein substituents may alter binding in some way.

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Absolute Configuration of (+)-*trans*-2-*o*-Tolylcyclohexanol by X-Ray Crystallography

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Abstract □ The determination of the absolute configuration of the 3-nitro-4-bromobenzoate ester of (+)-*trans*-2-*o*-tolylcyclohexanol by X-ray crystallographic analysis is reported. The results give unequivocal proof that the original assignments of (1S,2R)-(+)-*trans*-2-*o*-tolylcyclohexanol, (1R,2R)-(−)-*cis*-2-*o*-tolylcyclohexanol, and (2R)-(+)-2-*o*-tolylcyclohexanone are correct.

Keyphrases □ X-ray crystallography—configuration, (+)-*trans*-2-*o*-tolylcyclohexanol □ (+)-*trans*-2-*o*-Tolylcyclohexanol—configuration confirmation, X-ray crystallography

The absolute configurations of (+)-*trans*-2-*o*-tolylcyclohexanol (I) and (−)-*cis*-2-*o*-tolylcyclohexanol (II) were reported in an earlier communication (1) on the basis of the positive Cotton effect of the carbonyl chromophore of the (+)-2-*o*-tolylcyclohexanone (III) obtained from the oxidation of I and II. Compounds I and II are two of several key reference compounds currently used in this laboratory in a study associated with Cotton effects of aromatic chromophores. Although the original assignment of absolute configurations of I and II was considered reliable, an unquestionable proof was desired. Unequivocal proof is now given from X-ray crystallographic analysis of the 3-nitro-4-bromobenzoate ester of I that the initial assignments of (1S,2R)-(+)-*trans*-2-*o*-tolylcyclohexanol for I, (1R,2R)-(−)-*cis*-3-*o*-tolylcyclohexanol for II, and (2R)-(+)-2-*o*-tolylcyclohexanone for III are correct.

Figure 1 shows perspective drawings of the three-dimensional structure and correct absolute configuration of the 3-nitro-4-bromobenzoate ester of (+)-*trans*-2-*o*-tolylcyclohexanol.

EXPERIMENTAL

(1S,2R)-(+)-*trans*-2-*o*-Tolylcyclohexyl 3-nitro-4-bromobenzoate was prepared by reaction of the known (+)-*trans*-2-*o*-tolylcyclohexanol (I) with 3-nitro-4-bromobenzoyl chloride in pyridine. The ester was purified by chromatography on silica gel, using a 50:50 benzene–hexane mixture, and recrystallized from hexane, m.p. 64.5–65.5°, IR (KBr) 1720 cm.$^{-1}$ (C=O), 1536, 1352 (NO_2), $[\alpha]_D^{27}$ + 100° (c 1.0, methanol).

The compound crystallizes in space group $P2_12_12$ with the following crystal data:

a = 7.922 ± 0.002 $\alpha = \beta = \gamma = 90°$
b = 26.342 ± 0.008 Dm = 1.4 (flotation in CsCl solution)
c = 18.694 ± 0.006 $D_{calc.}$ 1.426 Z = 8 molecules/unit cell

X-ray intensities were measured on a crystal approximately 0.13 × 0.38 × 0.53 mm. to $2\theta = 45°$, corresponding to an interplanar spacing of 0.93 Å, on a computer-controlled, four-circle diffractometer. The $\omega/2\theta$ scan technique using Nb-filtered Mo radiation was employed. A total of 2928 independent reflections were measured, of which 1852 had intensities greater than twice the standard deviation of their measurement. Absorption corrections were applied using the method of De Meulenaer and Tompa (2), and struc-