Structural Studies on Complexes II

Crystal and Molecular Structure of a 1:1 Caffeine and 5-Chlorosalicyclic Acid Complex

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Crystals of a 1:1 complex of caffeine and 5-chlorosalicylic acid are orthorhombic with space group P *bcn*. The unit cell parameters are a = 7.952 Å., b = 16.303 Å., and c = 25.491 Å. The structure was solved by a symbolic addition procedure. The positional and thermal parameters were refined by least squares to an R value of 0.077. The caffeine molecule shows significant deviations from planarity. The molecules are held together in the lattice by O—H...N and C—H...O intermolecular hydrogen bonds. Polarization bonding also seems to play a dominant role in the stacking of the molecules in the lattice.

THE ABILITY of xanthine and purine derivatives to form association complexes with a wide variety of biologically important molecules is well documented in the literature. It has been shown that caffeine, through its complexing behavior, can influence the rates of hydrolysis of various molecules (1, 2) and also inhibit salivary amylase (3). To date there appears to be very little information reported as to the exact mode of the interaction in these "complexes." Recent structural studies on complexes of tetramethyluric acid with a variety of aromatic hydrocarbons (4–6) have indicated that polarization bonding is of primary importance. The structure analysis of the 1:1 complex of caffeine and 5-chlorosalicylic acid was undertaken as part of a continuing study to gain further insight into the nature of the forces that are responsible for intermolecular complexes between xanthines and purines with substituted aromatic compounds of biological and pharmaceutical importance.

EXPERIMENTAL

Orthorhombic prisms of the complex were obtained from an aqueous solution saturated with the two respective molecules. The following crystallographic data were measured for these crystals:

a = 7.952 ± 0.003 Å. $\alpha = \beta = \gamma = 90.0°$
b = 16.303 ± 0.003 Space group $P\ bcn$ (uniquely defined)
c = 25.491 ± 0.004 ρ measured = 1.47 ± 0.01 Gm./cm.^{3}
ρ calcd. (assuming 8 molecules of a 1:1 complex) = 1.462 Gm./cm.^{3}

The density was determined in a mixture of chloroform and methylene chloride. The unit-cell parameters were established by means of least-squares refinement of 30 general *hkl* reflections measured on a GE XRD-6 diffractometer. The temperature during these measurements and the subsequent intensity collection was approximately 22 ± 4°.

The intensity data were measured by the stationary crystal-stationary counter technique with balanced nickel and cobalt filters. The size of the crystal utilized was approximately 0.9 mm. × 0.3 mm. × 0.2 mm. These data were converted to structure factor amplitudes, $|F|$s, by applying α_1-α_2 splitting corrections, Lorentz-polarization factors, and an absorption factor based on the anisotropy of transmission of the X-rays as a function of the angle ϕ. The $|F|$'s were put on an absolute scale by use of Wilson's statistics (7), and then converted to their respective normalized structure factors, $|E|$'s. In the sphere of the data collection (0°–125° in 2 θ) 2519 reflections out of a possible number of 2,646 had values greater than twice the standard deviation of their measurement.

A trial structure was obtained using the symbolic addition procedure. The phases of 180 normalized structure factors, all having values of $|E|$ greater than 1.75, were determined using the Sayre equation (8), which is the same as the Σ_2 formula of Hauptman and Karle (9). A program written by Long was utilized for this purpose (10). Three linearly independent reflections (2.1.4, 2.2.1, 1.1.14) each having a large number of interactions were assigned positive signs in order to specify the origin. Aside from the origin-determining planes four other structure factors having large $|E|$ values (4.1.8, 1.2.10, 3.1.6, and 3.1.10) were allowed to take up each of the 16 possible phase combinations. The program in an interactive manner generated the phases for the other 173 reflections for the 16 sets of phases. A consistency index, C,

$$C = \frac{\langle |E_A \Sigma_{A=B+C} E_B E_C| \rangle}{\langle |E_A| \Sigma_{A=B+C} |E_B|\,|E_C| \rangle}$$

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A list of the calculated and observed structure factors has been deposited in the Health Sciences Laboratory at the State University of New York at Buffalo.

where the sums are overall pairs of reflections B and C for which $B + C = A$. $\langle\rangle$ means the average overall values of A. A high value for this index usually indicates a consistent set of phases. A E-Fourier map was calculated from the solution with the highest C value (0.70). The whole molecule was not recognizable from this map, but the xanthine moiety clearly stood out. The positions of these atoms were then utilized to calculate an electron-density Fourier synthesis from which the positions of all the nonhydrogen atoms were located. The reason for the ambiguous nature of the E-map was due to the fact that a number of the phases were incorrectly assigned by the program.

The positional and thermal parameters were refined by least squares using a block-diagonal approximation to the normal equations. A modified version of the ACA program 317 (Gantzel, Sparks, and Trueblood, unpublished) was utilized. After anisotropic temperature factors were introduced and the usual reliability index (R value) was 0.10, the positions of the hydrogen atoms were found from a three-dimensional Fourier difference synthesis. These atoms were included in the final cycles of least squares with fixed isotropic B's of 5.0 Å.^{2}. The refinement was concluded when the parameter shifts were less than a third of their calculated e.s.d.'s (estimated standard deviations). In the last stages of least squares the weighing scheme utilized was $1/w = [(|F_0| - 15)/30]^2 + 1$, such that $\langle w\Delta^2\rangle$ was constant over the whole range of $|F_0|$s. The unobserved data were given zero weight in the last cycles. The final R index for the observed reflections is 0.077 and the goodness of fit, $(\Sigma w|F_0 - F_0^2|/m - n)$, is 1.23.

The nonhydrogen atomic parameters and their e.s.d.'s are given in Table I, and those for the hydrogen atoms in Table II. The e.s.d.'s were calculated from the inverses of the full normal equation blocks.

Throughout the above calculations the atomic scattering factors used were those of Cromer and Waber (11), except for hydrogen which was taken from the *International Tables for X-ray Crystallography* (12).

TABLE II—REFINED POSITIONAL PARAMETERS AND THEIR E.S.D.'S OF THE HYDROGENS × 10^3[a]

Name	x/a	y/b	z/c
H1	533(6)	240(3)	186(2)
H2	521(6)	288(3)	157(2)
H3	681(6)	235(3)	165(2)
H4	278(6)	210(3)	−30(2)
H5	156(6)	173(3)	−17(2)
H6	216(6)	253(3)	− 1(2)
H7	448(6)	−137(3)	112(2)
H8	633(6)	− 87(3)	112(2)
H9	495(6)	− 73(3)	154(2)
H10	302(6)	− 94(3)	33(2)
H11	163(6)	225(3)	218(2)
H12	272(6)	76(3)	257(2)
H13	59(6)	− 65(3)	149(2)
H14	− 38(6)	191(3)	103(2)
H15	−160(6)	− 25(3)	36(2)

[a] E.s.d.'s of the hydrogens × 10^3 in parentheses. Isotropic temperature factors (B's) were fixed at 5.0 Å.^{2}.

The results of a rigid body analysis of the temperature factors for the two residues of the complex are shown in Table III. The method of Schomaker and Trueblood (13, 14) was utilized to calculate the librational and translational tensors. The root mean-square difference between the observed and calculated Uij's were 0.0033 and 0.0041 for the caffeine and 5-chlorosalicylic acid molecules, respectively. The caffeine has a substantially smaller thermal motion than the salicylic acid moiety, which is consistent with the intermolecular bonding in the lattice (see below). Neither the librational nor the translational motions of the two molecules are significantly anisotropic.

DISCUSSION OF RESULTS

The intramolecular bond distances and angles of the two residues composing the complex are shown

TABLE I—LEAST-SQUARES PARAMETERS AND THEIR ESTIMATED STANDARD DEVIATIONS × 10^4[a]

Name	x/a	y/b	z/c	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
CN1	4939(4)	1911(2)	1177(1)	159(5)	34(1)	15(1)	− 3(5)	−11(3)	− 7(1)
CC2	3955(4)	2261(2)	786(1)	148(6)	35(1)	15(1)	−18(5)	15(3)	1(2)
CN3	3278(4)	1723(2)	422(1)	153(5)	31(1)	15(1)	5(4)	−18(3)	5(1)
CC4	3480(4)	896(2)	486(1)	136(6)	30(1)	12(1)	− 8(5)	− 1(3)	− 1(1)
CC5	4403(4)	579(2)	883(1)	154(6)	28(1)	12(1)	7(5)	− 1(3)	1(1)
CC6	5258(4)	1073(2)	1264(1)	132(6)	38(1)	12(1)	− 3(5)	− 5(3)	2(1)
CN7	4282(4)	−264(2)	835(1)	164(5)	28(1)	16(1)	2(4)	− 2(3)	6(1)
CC8	3305(5)	−393(2)	417(2)	172(7)	29(1)	19(1)	−17(5)	1(4)	− 4(2)
CN9	2782(4)	298(2)	186(1)	159(6)	34(1)	15(1)	−25(4)	−12(3)	0(1)
CO10	6124(4)	840(2)	1621(1)	218(6)	53(1)	16(1)	16(5)	−45(3)	4(1)
CC11	5728(6)	2493(3)	1546(2)	273(10)	53(2)	25(1)	− 4(8)	−46(5)	−27(2)
CO12	3708(3)	2991(1)	761(1)	224(6)	25(1)	26(1)	1(4)	− 4(3)	3(1)
CC13	2164(6)	2055(3)	12(2)	262(10)	45(2)	21(1)	7(7)	−40(5)	19(2)
CC14	5005(6)	−891(2)	1179(2)	231(8)	39(2)	25(1)	12(7)	− 6(5)	22(2)
C1	2391(2)	−936(1)	2388(1)	314(3)	108(1)	21(0)	76(3)	−41(2)	23(1)
SC1	208(5)	565(2)	1345(1)	151(6)	38(1)	14(1)	−29(6)	7(3)	− 4(2)
SC2	602(5)	1343(3)	1544(2)	177(8)	47(2)	19(1)	−41(6)	31(4)	− 9(2)
SC3	1560(6)	1417(3)	1999(2)	224(9)	75(3)	19(1)	−71(9)	11(5)	−18(2)
SC4	2115(6)	731(4)	2250(2)	185(9)	109(3)	15(1)	−72(9)	−10(4)	−24(3)
SC5	1718(5)	− 57(3)	2054(2)	170(7)	76(3)	15(1)	25(8)	− 5(4)	4(2)
SC6	795(5)	−133(2)	1606(1)	158(7)	54(2)	14(1)	−12(6)	0(4)	− 2(4)
SC7	− 773(4)	501(2)	859(1)	129(6)	41(1)	16(1)	−10(5)	7(3)	4(2)
SO8	−1084(3)	−249(1)	704(1)	222(5)	37(1)	18(1)	−22(4)	−36(3)	− 3(1)
SO9	−1289(3)	1106(2)	619(1)	236(6)	39(1)	20(5)	8(4)	−18(3)	9(1)
SO10	94(5)	2038(2)	1312(1)	326(8)	38(1)	30(1)	−32(5)	15(4)	− 9(2)

[a] Thermal parameters B_{ij} are defined by the temperature factor = exp − $(h^2B_{11} + k^2B_{22} + l^2B_{33} + hkB_{12} + hlB_{13} + klB_{23})$.
[b] E.s.d.'s in parentheses.

TABLE III—RESULTS OF RIGID-BODY ANALYSIS OF THERMAL PARAMETERS[a]

	Eigenvalues Cl-S.A.	Eigenvalues Caff.	Eigenvectors (10^4)[b] Cl-S.A.			Eigenvectors (10^4)[b] Caff.		
Librational tensor, **L**	31.4 (°)²	24.9	3465	−0696	9355	−1112	9910	0747
	17.6	15.8	9371	−0185	−3484	−9887	−1027	−1096
	13.8	9.6	0413	9974	0590	−1008	−0861	9912
Translational tensor, **T**	Å.²							
	0.068	0.041	−3254	8965	−3005	−2974	8921	3402
	0.046	0.039	−4206	−4219	−8032	−8518	−0869	−5167
	0.036	0.033	−8469	−1349	5144	−4314	−4434	7857
			Cl-S.A.			Caff.		
Symmetrized screw tensor, **S**		206	−277	−183	139	64	− 4	× 10^5 rad. Å.
			−131	361		−111	8	
				− 75			−28	

[a] Calculated by the method of Schomaker and Trueblood (13, 14). [b] These are the direction cosines of the eigenvectors relative to *a*, *b*, and *c*, respectively. **L** is independent of the origin, **T** and **S** depend upon it, and here they are given relative to the unique origin which symmetrizes **S**.

in Figs. 1 and 2. These distances and angles were corrected for the effects of molecular libration by the method of Cruickshank (15). The distances and angles for the nonhydrogen atoms have average e.s.d.'s of 0.007 Å. and 0.3°. The distances and angles for the hydrogens have an average uncertainty of approximately 0.07 Å. and 2°. These e.s.d.'s include an assumed e.s.d. of 0.002 Å. for the uncertainty in intramolecular bond distances and angles corrected for librational motion.

In general the bond distances and angles of the caffeine moiety of the complex agree fairly well with

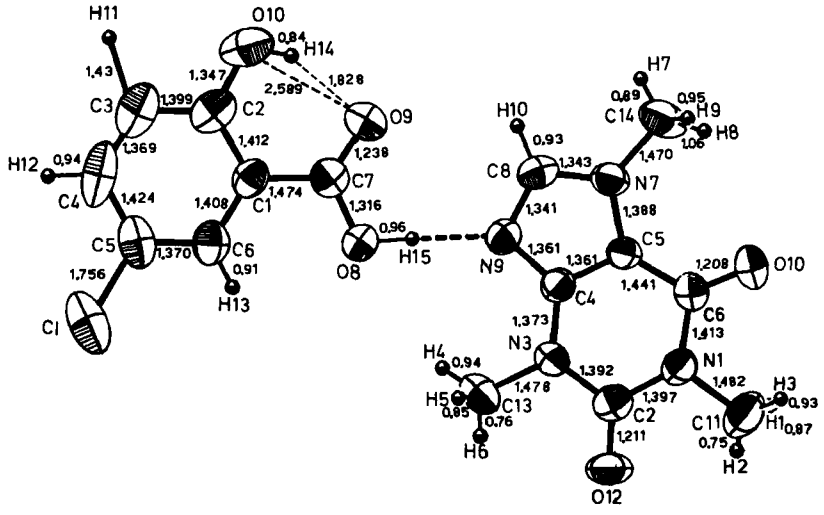

Fig. 1—Thermally corrected intramolecular distances. The thermal ellipsoids in this figure, and the others, enclose a probability density of 0.50 (30), for the nonhydrogen atoms.

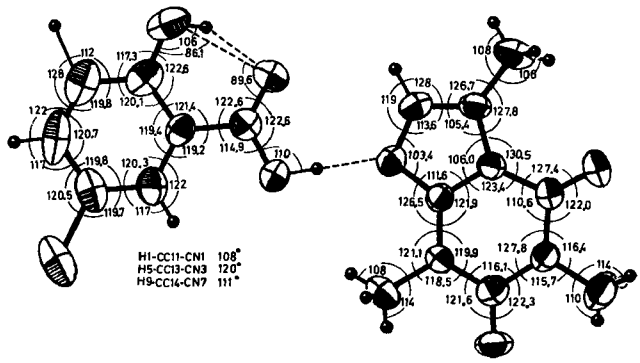

Fig. 2—Thermally corrected intramolecular angles.

those found in the structure of caffeine hydrate (16). There are however some differences worth noting; these are between the C6—O10, C6—N1, N3—C4, C4—N9,and C4—C5 bonds, whose values were found to be 1.26 Å., 1.36 Å., 1.42 Å., 1.31 Å., and 1.32 Å., respectively, in the caffeine structure. These discrepancies may reflect the higher uncertainty in the X-ray study of Sutor (av. e.s.d. ~ 0.02 Å. in bond lengths), or possibly the effect of intermolecular polarization bonding. The former of these reasons appears to be the more likely as the size of the differences in the intramolecular xanthine bonds are much smaller when these results are compared to the theophylline structure (17); *i.e.*, they all fall within 2.5 times the e.s.d.'s of that structure.

The atoms comprising the purine ring show a significant degree of puckering (see Table IV). These deviations most likely reflect the contribution of some sp^3 character to valence state of the nitrogens. This finding is not unusual as a variety of pyrimidine and purine structures have been observed to be significantly nonplanar (19).

There is a remarkably good similarity between the precise structure determination of salicylic acid (20) and the 5-chloro derivative in this complex. Only two bond lengths, the C5—C4 and C1—C7, differ by more than 2 e.s.d.'s The values for these bonds are 1.384 Å. and 1.457 Å., respectively, in the salicylic acid structure. The substitution of the electronegative chlorine atom in the five position of the ring, undoubtedly is a major factor for these observed differences. As already pointed out by Cochran (21) and Sundaralingam and Jensen (20), there appears to be a substantial contribution of the quinoid structure to the possible valence states of the molecule.

TABLE IV—LEAST-SQUARES PLANES[a]

Atoms Comprising L.S. plane	Deviation Å.	Other Atoms	Deviation Å.
SC1	−0.002	SC7	0.024
SC2	−0.001	SO8	0.017
SC3	0.001	SO9	0.031
SC4	0.003	SO10	0.002
SC5	−0.006	C1	−0.045
SC6	0.006	H11	−0.19
		H12	−0.04
		H13	0.03
		H14	0.07
		H15	0.15
CN1	0.013	CO10	−0.047
CC2	0.052	CC11	0.030
CN3	−0.030	CO12	0.144
CC4	−0.031	CC13	0.065
CC5	−0.023	CC14	0.091
CC6	−0.028	H10	0.10
CN7	0.019		
CC8	0.033		
CN9	−0.005		

[a] Calculated by the method of Schomaker *et al.* (18).

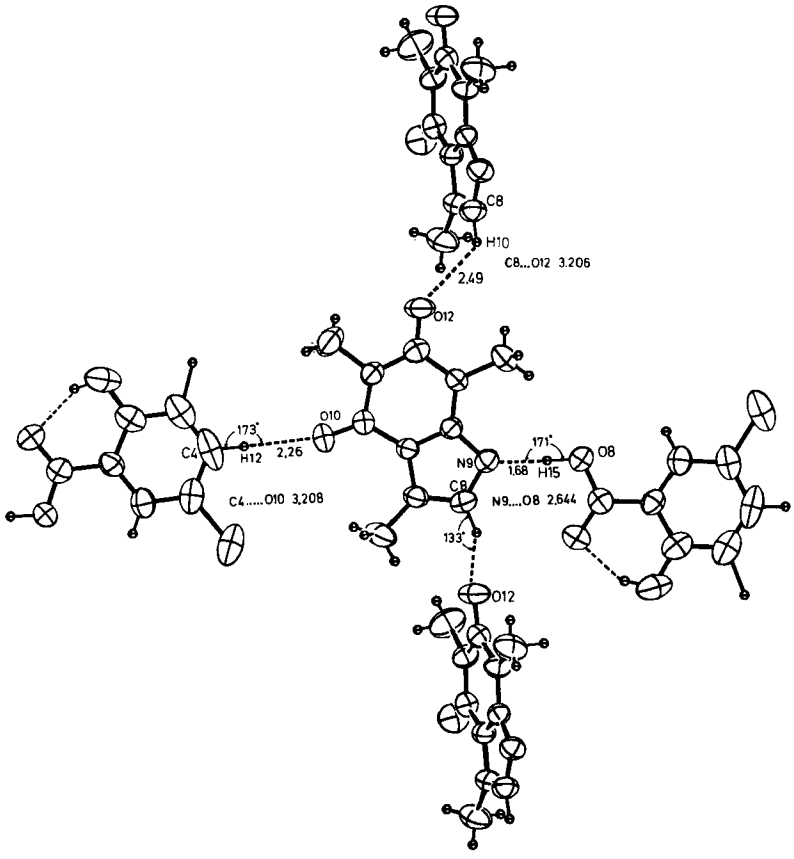

Fig. 3—A general view of the packing, showing the short intermolecular contacts.

The phenyl ring atoms are coplanar (see Table IV), within experimental error. The equation for this plane is $-0.8358\ X - 0.0142\ Y + 0.5488\ Z = 1.7274$ Å., where X, Y, and Z are in Å. measured along a, b, and c. The chlorine atom and the carboxyl group displacements from this plane are significant. In regard to the chlorine atom, it is most interesting to note that the displacement of the hydrogen in the five position of the salicylic acid structure is also significantly displaced from the ring. In the present instance the chlorine does not appear to have any short intermolecular contacts to it which might cause this distortion. The displacement of the carboxyl group from the least-squares plane is not unreasonable, as in both the structures of salicylic acid and 2-amino-3-methyl benzoic acid (22) a similar effect is observed.

The most dominant intermolecular bond observed in the complex is the N9...H—O8 interaction (see Fig. 3). The 2.644 Å. N...O length together with the 1.68 Å. H...N9 distance indicate this to be a relatively strong interaction. In general O—H...N bonds found in structures where the dominating proton comes from an acidic group, the hydrogen bond lengths are usually low. In the structures of nicotinic acid (23), hydroxy-L-proline (24), and 1,4-piperazine-α,α^1-dibutyric acid (25) the N...H—O lengths are 2.66, 2.67, and 2.60 Å., respectively. In most other types of O—H...N bridges the lengths are considerably longer as evidenced by the average value of 2.80 ± 0.09 Å. tabulated by Pimental and McClellan (26).

There are two other short intermolecular interactions that appear to be joining the molecules in chains throughout the crystal lattice (see Fig. 3). These are between SC4—H...O10 and CC8—H...O12. The hydrogen to oxygen distance in these two bridges are 2.26 and 2.49 Å., respectively, which are much less than the sum of the van der Waals radii of the atoms involved (2.6 Å.). There are numerous examples of this type of bonding in the recent literature. In the structures of both caffeine hydrate and theophylline hydrate the C8 hydrogen was also found (27) to be participating in a hydrogen bond with a carbonyl oxygen. The resonance in the purine system is probably responsible for the acidic nature of this proton. The acidic character of the SC4 hydrogen possibly results from the inductive effect of the chlorine atom. It is of interest to point out while on the subject of C—H hydrogen bonds, that spectroscopic evidence shows that C—H...O=C bonding plays a significant role in the stability of polypeptides (28).

The general features of the stacking of the molecules are shown in Fig. 4. Some of the distances between the stacked planes are listed in Table V. Though none of these lengths are shorter than the sum of the van der Waals radii of the atoms concerned, the general arrangement of the stacked molecules tends to indicate polarization bonding between layers. This idea has been discussed in a previous communication on this structure (29). The greatest degree of stacking overlap occurs between the salicylic acid moiety at $-x, -y, -z$ (drawn with the thickest bonds in Fig. 4) and the caffeine at x, y, z. Only the carboxyl group overlaps the caffeine on the underside, *i.e.*, at $1 - x, -y, -z$ (thinnest lines in Fig. 4). Similar observations have been reported by Damiani *et al.* (4–6) in the complexes of tetramethyluric acid and some aromatic hydrocarbons. The perpendicular separations between planes in the latter structures were 3.45 Å.

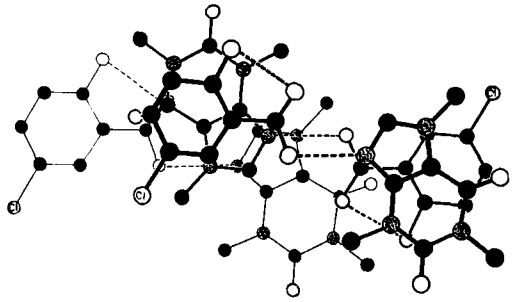

Fig. 4—The stacking arrangement viewed in the direction perpendicular to the molecular plane of caffeine. Broadest bonds indicate molecules at −x, −y, −z, *next broadest bonds indicate molecules at* x, y, z, *and the thinnest represents the molecules at* 1 − x, −y, −z. *Key:* ●, *C;* ⊛, *N;* ○, *O.*

TABLE V—INTERMOLECULAR DISTANCES LESS THAN 3.45 Å.[a]

Atom	to Atom	Equivalent Position	Distance, Å.
CC6	SC7	$1 - x, -y, -z$	3.350
CC6	SO9	$1 - x, -y, -z$	3.202
CC2	SO10	$-x, -y, -z$	3.370
CN3	SO10	$-x, -y, -z$	3.438
CC5	SC1	$-x, -y, -z$	3.443
CN7	SC6	$-x, -y, -z$	3.405
CO10	SC1	$1 - x, -y, -z$	3.354
CO10	SC7	$1 - x, -y, -z$	3.189
CO10	SO9	$1 - x, -y, -z$	3.309

[a] Excluding those shown in Fig. 3.

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Keyphrases

Complexes—structural studies
Caffeine-5-chlorosalicylic acid complex
Crystal structure
Molecular structure

Comparative Study of a New Fluorometric Assay Mestranol Ether in Tablets with GLC and Colorimetric Assays

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Mestranol (17α-ethinylestradiol-3-methyl ether) has been found to react with methanol–sulfuric acid mixture of a critical composition to give a stable fluorophor of high intensity. This fluorescence permits the analysis of mestranol in the submicrogram range. The use of varying proportions of methanol and sulfuric acid enables the analyst to measure the fluorescence at several different wavelengths. The analyst can choose the reagent which gives him the selectivity necessary for his analytical problems. Colorimetric, GLC, and TLC methods for the determination of mestranol are also discussed and compared with the fluorometric method. The described method is satisfactory for both control and stability measurements of mestranol.

THE ANALYTICAL DETERMINATION of the estrogenic component of oral contraceptive tablets is of the utmost interest to the pharmaceutical industry. Control of manufacturing processes, quality control of the finished product, and stability measurements of the active ingredients require accurate and sensitive methods of analysis. The amounts of estrogen formulated into these tablets require analytical methods which are highly sensitive and selective in the microgram range. Other applications, such as the analysis of biological fluids, may require sensitivity in the submicrogram range.

Mestranol and ethinyl estradiol have been determined by colorimetric measurements for many years. Ganshirt and Polderman (1) developed a chromophor with mestranol using an aqueous sulfuric acid reagent. Shroff and Huettemann (2) used a phenol–sulfuric acid reagent to measure mestranol. The colorimetric determination of ethinyl estradiol by Tsilifonis and Chafetz (3) made use of a methanol–sulfuric acid reagent, which has been used in the determination of mestranol in this laboratory for several years.

The determination of mestranol by UV was described by Bastow (4). Shroff and Grodsky compared UV and GLC procedures (5), and an assay which was a combination of UV and TLC was compared to a GLC procedure by Schulz (6). France and Knox (7) assayed ethinyl estradiol by GLC without derivative formation, and Umbreit and Wisniewski (8) reported that mestranol could be assayed by GLC without thermal degradation.

Mestranol was determined fluorometrically by Hüttenrauch and Keiner (9) with antimony trichloride in glacial acetic acid. Bates and Cohen (10) heated steroids with aqueous sulfuric acid to develop fluorescence. Phosphoric acid was used by Boscott (11) to develop specific colors and fluorescence with several steroids. Comer *et al.* (12) and Cali and Khoury (13) have reported automated methods for mestranol.

The fluorometric procedure described by the authors is quite simple. The reagent develops a fluorophor at room temperature, which is highly intense and stable for at least 30 min. This

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