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Structural Studies on Complexes. VI. X-Ray Investigation of A 2:1 5-Chlorosalicylic Acid: Theobromine Complex[1]

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The dominant force of attraction in the 2:1 5-chlorosalicylic acid: theobromine crystalline complex is hydrogen bonding. There are three relatively strong hydrogen bonds, all involving a carboxyl group of the salicylate. A substantial degree of π overlap exists between the phenyl ring of the acid and the xanthine nucleus.

The quantity of investigations reported in the pharmaceutical literature on molecular association complexes clearly testifies to their relative importance. Properties such as chemical stability and bioavailability of a drug can be dramatically influenced by the addition of a complexing agent to a formulation. Even with the availability of theoretical and experimental data on many association complexes, their unique properties are still not understood on a molecular level in most instances.

Purine analogues have been shown to form complexes with a variety of unsaturated ring systems with varying degrees of stability. The studies of Higuchi and co-workers[3,4] on an assortment of salicylate complexes indicated that in aqueous systems, molecular forces other than hydrogen bonding are responsible for their formation. Others have proposed donor-acceptor mechanisms[5-7] to account for similar associations. X-ray diffraction studies on a series of salicylate–xanthine complexes were initiated to obtain definitive structural data on these complexes. The initial structural analyses[8,9] intimated that at least in the solid state both polarization bonding and hydrogen bonding are operative in these complexes. To further clarify the situation a crystallographic study was recently completed on a 2:1 5-chlorosalicylic acid: theobromine complex; the results of which are presented here.

Experimental

Small needles of the complex were obtained by slow evaporation of an alcoholic solution containing theobromine and 5-chlorosalicylic acid. The triclinic crystals so derived have the following cell dimensions.

a= 18.448 (2)	b= 9.057 (2)	c= 7.258 (2)Å
α=108.12 (3)	β=86.12 (3)	γ=105.72 (2)°

and a unit cell volume of 1109.3 A^3. Å statistical analysis of the diffraction data[10] suggested that crystals are centrosymmetric and the space group is P$\bar{1}$; this choice was further verified by the successful structure

1) Paper No. V: E. Shefter and P. Sackman, *J. Pharm. Sci.*, **60**, 282 (1971). Supported in part by a U.S.P.H.S. research grant (CA-10104) from the National Cancer Institute. We acknowledge the Computing Center of SUNY/B for their assistance, and Prof. Roy Lackman for some of his interesting dialogues.

2) Location: *Buffalo, New York 14214 USA.*

3) T. Higuchi and J. L. Lach, *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 349 (1954).
4) T. Higuchi and D. A. Zuck, *J. Am. Pharm. Assoc. Sci. Ed.*, **41**, 10 (1952); **42**, 132 (1953).
5) T. Eckert, *Arch. Pharm.*, **295**, 233 (1962).
6) M. Dombrow and Z. A. Jan, *J. Pharm. Sci.*, **54**, 1707 (1965).
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8) E. Shefter, *J. Pharm. Sci.*, **57**, 1163 (1968).
9) E. Shefter, *J. Pharm. Sci.*, **58**, 710 (1969).
10) I.L. Karle, K.S. Dragoneete, and S.A. Brenner, *Acta Cryst.*, **19**, 713 (1965).

analysis and refinement. The measured density (by flotation) of 1.59 gm. cm^{-3} agreed well with the calculated density (assuming a 2:1 complex of 5-chlorosalicylic acid and theobromine) of 1.595 gm. cm^{-3} for two complexes per unit cell.

The intensity data were collected on a GE XRD-6 diffractometer equipped with a single crystal orienter. The intensities were measured by the stationary counter-stationary crystal technique[11] using balanced filters for the CuK^{α} radiation. The dimensions of the crystal used for data collection were 0.40 × 0.08 × 0.02 mm. In the range of data measurement ($2\theta \leq 100°$) 1593 reflections of the 2278 unique data had peak intensities significantly greater than background. An empirical absorption correction, based on the anisotropy of transmission of the x-rays as a function of diffractometer angle Phi was applied. The absorption corrected intensities were converted to structure factor amplitudes, |Fo|'s, and normalized structure factors, |E|'s, in the usual manner.

The phases of 217 reflections having |E| values greater than 1.65 were determined by means of the Sayre relationship[12], using a program written by Long.[13] An E-map[14] calculated with the most consistent set of signs determined for these 217 data revealed the positions of all 37 non-hydrogen atoms. The structure was refined by the least squares method using a block diagonal approximation of the normal equations. The hydrogen atoms were located in a difference electron density synthesis and included in the final cycles of least squares. The weighting scheme in the final stages of refinement was

$$w^{-1} = 1.444 - 0.089|Fo| + 0.004|Fo|^2,$$

designed such that $\langle w\Delta^2 \rangle$ is constant over the whole range of Fo values. The 'unobserved' data were given zero weight. The final R value (usual reliability index) is 0.057 for the observed data. A list of the calculated and observed structure factors may be obtained from the authors.

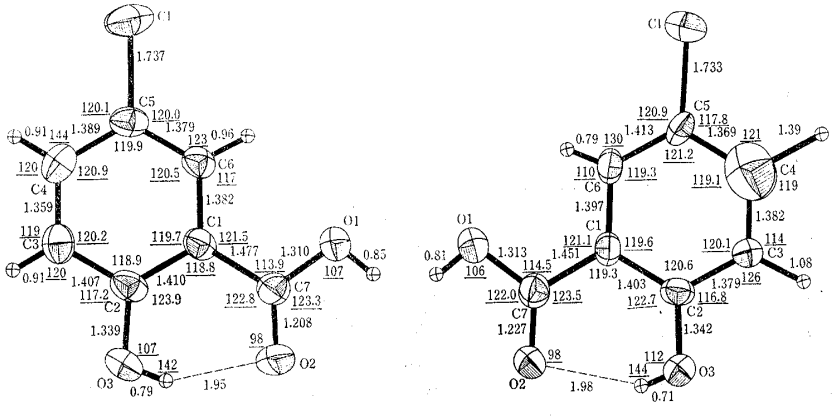
The final atomic positional and thermal parameters together with their estimated standard deviations (e.s.d.s) are tabulated in Table I. The e.s.d.s in the atomic positions result in e.s.d.s for the bond lengths and angles between non-hydrogen atoms of on the average 0.007A and 0.4°. The corresponding e.s.d.s. for bonds involving hydrogen atoms are 0.07 A and 2.0°.

With the exception of hydrogen, the atomic form factors used throughout the above calculations are given in the International Tables for Crystallography.[15] The hydrogen scattering factors used were those published by Stewart, *et al.*[16]

Result and Discussion

Intramolecular Bonding

There are two unique 5-chlorosalicylic acid molecules associated with theobromine in the asymmetric unit. The two independent salicylates show small differences in their bonding



1 2

Fig. 1. The Intramolecular Bonding Parameters found for the Two Unique 5-Chlorosalicylic Acid Molecules. Thermal Ellipsoilds are drawn at the 0.50 Probability Level for the Non-hydrogen Atoms in This Drawing and All Other Figures

11) T.F. Furnas and D. Harker, *Rev. Sci. Instr.*, **26**, 449 (1955).
12) D. Sayre, *Acta Cryst.*, **5**, 60, (1952).
13) R.E. Long and Ph.D. Thesis, University of California, Los Angeles, 1965.
14) I.L. Karle, H. Hauptman, J. Karle, and A.B. Wing, *Acta Cryst.*, **11**, 257 (1958).
15) International Tables for X-ray Crystallography, Vol. III, Kynoch Press, Birmingham, England, 1965, p. 202.
16) R.F. Stewart, E.R. Davidson, and W.T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).

TABLE I. Positional and Thermal Parameters with Their Estimated Standard Deviations in Parentheses (All Times 10^4 except for Biso)[a)]

Atom	x	y	z	b_{11}/Biso	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
				Theobromine					
N(1)	−377(3)	−2988(7)	2204(9)	17(2)	120(10)	271(18)	10(7)	−9(10)	166(22)
C(2)	−420(4)	−1492(9)	2183(11)	25(3)	151(13)	215(22)	6(10)	−9(12)	158(28)
N(3)	−1123(3)	−1411(6)	1716(8)	21(2)	82(9)	230(16)	21(7)	7(9)	114(19)
C(4)	−1703(4)	−2812(8)	1225(9)	21(2)	107(11)	137(17)	18(8)	−4(10)	81(22)
C(5)	−1603(3)	−4257(8)	1171(9)	15(2)	101(11)	151(18)	6(8)	−13(10)	96(22)
C(6)	−914(4)	−4441(8)	1716(10)	20(2)	105(11)	150(18)	12(9)	−1(10)	50(22)
N(7)	−2289(3)	−5366(6)	616(8)	18(2)	110(9)	164(14)	29(7)	−8(8)	95(19)
C(8)	−2743(4)	−4519(9)	373(10)	20(2)	157(13)	152(18)	21(9)	−5(11)	100(25)
N(9)	−2415(3)	−2949(6)	745(8)	18(2)	127(10)	187(15)	29(7)	0(9)	136(20)
O(10)	−800(2)	−5692(5)	1782(7)	23(2)	100(8)	295(15)	21(6)	−41(8)	127(17)
O(11)	119(3)	−332(6)	2560(9)	25(2)	137(9)	435(20)	−20(7)	−34(10)	202(22)
C(12)	−1206(4)	127(9)	1651(13)	35(3)	136(14)	340(26)	41(11)	32(15)	225(31)
C(13)	−2467(4)	−7068(8)	343(11)	27(3)	113(12)	228(21)	11(9)	−14(12)	151(26)
H(N1)	25(36)	−2942(76)	2505(91)	3.8(1.5)Å^2					
H(C8)	−3260(31)	−4891(67)	203(85)	2.9(1.3)					
H(C12)1	−1195(46)	133(95)	296(118)	7.1(2.1)					
H(C12)2	−1577(47)	388(97)	2112(119)	6.8(2.0)					
H(C12)3	−948(71)	1227(150)	2852(180)	14.6(3.8)					
H(C13)1	−2033(47)	−7535(97)	−617(120)	7.3(2.2)					
H(C13)2	−2464(43)	−7243(92)	1665(111)	6.3(2.0)					
H(C13)3	−2985(37)	−7614(79)	−220(96)	4.5(1.6)					
				5-Chlorosalicylic acid molecule 1					
C1	5281(1)	3544(2)	2566(3)	44(1)	95(3)	301(6)	36(2)	−54(3)	119(6)
C(1)	5401(3)	−886(7)	2141(9)	17(2)	99(11)	165(17)	7(8)	−24(10)	123(22)
C(2)	4659(4)	−1637(8)	2551(10)	24(3)	105(11)	157(18)	9(9)	−8(11)	144(23)
C(3)	4125(4)	−733(8)	3011(10)	20(2)	133(12)	161(18)	26(9)	−6(11)	74(24)
C(4)	4320(4)	827(8)	3015(10)	28(3)	137(13)	187(19)	60(10)	−6(12)	90(25)
C(5)	5046(4)	1554(7)	2567(9)	26(3)	83(11)	148(17)	7(8)	−37(11)	88(22)
C(6)	5584(3)	702(7)	2162(9)	18(2)	92(11)	156(17)	5(8)	−22(10)	86(22)
C(7)	5964(4)	−1831(1)	1682(10)	20(2)	109(11)	225(20)	25(9)	−18(11)	171(24)
O(1)	6623(2)	−1047(5)	1226(8)	19(2)	128(8)	377(17)	29(6)	25(9)	233(20)
O(2)	5830(3)	−3192(6)	1758(9)	29(2)	112(8)	464(20)	33(6)	1(10)	269(21)
O(3)	4429(3)	−3170(5)	2555(8)	25(2)	107(8)	323(16)	1(6)	29(8)	193(18)
H(C3)	3676(32)	−1131(66)	3499(80)	2.4(1.2)Å					
H(C4)	3976(37)	1407(80)	3330(96)	4.4(1.6)					
H(C6)	6084(29)	1141(63)	1784(78)	2.1(1.2)					
H(O1)	6913(42)	−1666(89)	1056(108)	5.8(1.9)					
H(O3)	4745(39)	−3593(82)	2059(101)	4.7(1.7)					
				Molecule 2					
C1	2227(1)	610(2)	3935(3)	37(1)	113(3)	374(7)	54(2)	−4(3)	171(7)
C(1)	1615(3)	4481(7)	3790(9)	14(2)	94(11)	130(17)	11(8)	−12(10)	70(21)
C(2)	2288(4)	5695(7)	4347(10)	24(2)	76(10)	165(18)	27(9)	6(10)	108(19)
C(3)	2927(4)	5338(7)	4764(10)	23(2)	75(11)	214(20)	11(8)	−28(11)	96(23)
C(4)	2907(4)	3778(9)	4659(10)	55(3)	293(15)	397(22)	88(11)	5(13)	193(29)
C(5)	2229(3)	2556(7)	4078(9)	17(2)	90(11)	134(17)	−16(8)	−14(10)	78(22)
C(6)	1593(3)	2896(8)	3653(10)	13(2)	113(11)	167(18)	2(8)	−19(10)	106(23)
C(7)	956(3)	4888(8)	3323(10)	18(2)	112(11)	164(18)	12(8)	−5(10)	120(23)
O(1)	361(2)	3661(6)	2774(8)	21(2)	127(8)	343(16)	16(6)	−39(8)	194(19)
O(2)	952(2)	6254(5)	3384(7)	22(2)	114(8)	290(15)	23(6)	−24(8)	176(18)
O(3)	2345(2)	7243(5)	4534(8)	21(2)	108(8)	326(16)	4(6)	−27(8)	182(18)
H(C3)	3461(33)	6201(69)	5267(88)	3.2(1.3)Å^2					
H(C4)	3520(39)	3478(82)	5368(100)	5.4(1.8)					
H(C6)	1202(38)	2313(82)	3206(100)	4.9(1.7)					
H(O1)	29(35)	4030(73)	2603(89)	3.7(1.5)					
H(O3)	2000(37)	7361(78)	4324(96)	4.2(1.5)					

a) anisotropic temperature factor of the form $\exp -(h^2b_{11}+k^2b_{22}+l^2b_{33}+hkb_{12}+hlb_{13}+klb_{23})$

parameters (See Figure 1), none of which are considered to be significant at the $p=0.001$ level. Similarly their bond lengths and angles are in reasonably good agreement with the intramolecular parameters of salicylic acid,[17] and of 5-chlorosalicylic acid when complexed with theophylline[9] and caffeine.[8] The differences in bond order between molecules 1 and 2 (C(3)-C(4), C(5)-C(6), C(2)-C(3), and C(1)-C(7) bond are significant at $p=0.01$ level) could possibly result from the contrasting hydrogen bonds emanating from the two unique acid molecules which might influence the stability of the various resonance forms of the molecules. There is also a possibility that stacking forces may result in the small differences in the bond lengths (packing arrangements about the two salicylates are distinct).

Table II. Least Squares Planes[a]

Atoms Comprising L.S. plane	Displacement (Å)	Othher atoms	Displacement (Å)
	Theobromine-pyrimidine ring		
N(1)	0.018	N(7)	−0.017
C(2)	−0.025	C(8)	0.017
N(3)	0.009	N(9)	0.052
C(4)	0.015	O(10)	0.035
C(5)	−0.023	O(11)	−0.079
C(6)	0.006	C(12)	−0.044
		H(N1)	0.02
Eqn. $-0.2699X-0.1142Y+0.9561Z-1.7493$Å$=0$			
	Imidazole ring		
C(4)	−0.003	N(1)	0.066
C(5)	0.000	C(2)	−0.017
N(7)	0.003	N(3)	−0.018
C(8)	−0.005	C(6)	−0.067
N(9)	−0.005	C(13)	0.000
		H(C8)	0.14
Eqn. $-0.2481X-0.1378Y+0.9589-1.7814$Å$=0$			
	5-Chlorosalicylic acid molecule 1		
C(1)	0.004	Cl	−0.023
C(2)	−0.011	C(7)	0.004
C(3)	0.006	O(1)	−0.053
C(4)	0.005	O(2)	0.075
C(5)	−0.012	O(3)	−0.022
C(6)	0.007	H(C3)	0.16
		H(C4)	0.03
		H(C6)	−0.05
Eqn. $0.2467X+0.0018Y+0.9691Z-3.9625$Å$=0$			
	Molecule 2		
C(1)	−0.004	Cl	0.006
C(2)	0.000	C(7)	0.017
C(3)	0.006	O(1)	0.045
C(4)	−0.007	O(2)	0.039
C(5)	0.003	O(3)	−0.026
C(6)	0.002	H(C3)	−0.04
		H(C4)	−0.21
		H(C6)	0.09
Eqn. $-0.2590X-0.1277Y+0.9574Z+1.5722$Å$=0$			

a) Calculated in the manner described by Schomaker, V., Waser, J., Marsh, R.E., and Bergman, G., *Acta Cryst.*, **12**, 600 (1959).

17) M. Sundaralingam and L.H. Jensen, *Acta Cryst.*, **18**, 1053 (1965).

The least squares planes calculated through the phenyl rings of the 5-chlorosalicylic acid molecules (in Table II) show that the atoms comprising these two molecules are coplanar with the exception of the oxygens, and the chlorine in molecule 1. The carboxyl group is twisted slightly about the C(1)-C(7) bond and O(3) is displaced from the phenyl ring in order to accomodate the intramolecular hydrogen bond between O(2) and O(3) with the minimum amount of strain. Similar distortions are found in all the salicylate structures. The small displacement of the Cl of molecule 1 from the plane of the phenyl ring is in all probability due to non-bonded intermolecular forces (the packing arrangement shows H(C3)-molecule 2 in close proximity to this chlorine −2.81Å).

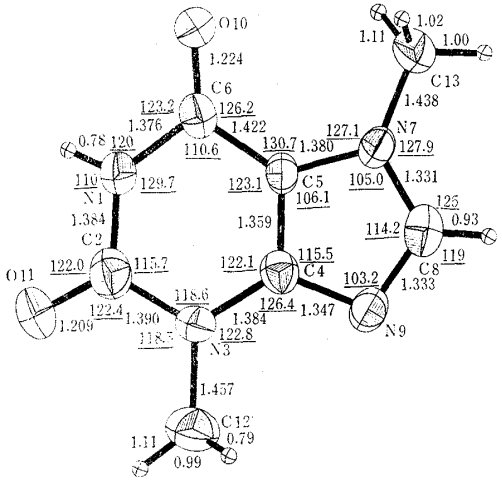


Fig. 2. Intramolecular Bonding Parameters for Theobromine

The bond distances and angles calculated for theobromine are shown in Figure 2. Although there has been no previous determination of this molecule, it is of interest to compare its molecular parameters with those of other xanthines and with purine. Such a compilation is presented in Table III. The greatest variations between the methylated xanthines exist about the substituted nitrogens, but none of these are highly significant (p= 0.001 level). In general, methylation in the 1,3, and 7 positions appears to have a very minor effect on the bond orders of the xanthine backbone. A significant alteration of the purine backbone occurs when carbonyl groups are added; purine to xanthine to uric acid.

While the atoms comprising the imidazole portion of theobromine are planar (See Table II), the pyrimidine ring is significantly puckered. It is not uncommon to find puckering in

TABLE III. Bond Lengths (Å) in Various Xanthine Derivatives and Purine

Bond	Theobromine[a]	Caffeine[b]	Theophylline[c]	Theophylline[a,d]	Purine,[a,e]	Uric acid[a,f]
N(1)-C(2)	1.384(7)[g]	1.397(7)	1.404(8)	1.40(1)	1.349(8)	1.367(5)
C(2)-N(3)	1.390	1.392	1.359	1.35	1.332	1.382
N(3)-C(4)	1.384	1.373	1.376	1.37	1.337	1.356
C(4)-C(5)	1.359	1.361	1.370	1.37	1.403	1.360
C(5)-C(6)	1.422	1.441	1.421	1.41	1.389	1.411
C(6)-N(1)	1.376	1.413	1.387	1.38	1.330	1.397
C(5)-N(7)	1.380	1.388	1.373	1.34	1.374	1.387
N(7)-C(8)	1.331	1.343	1.332	1.31	1.330	1.359
C(8)-N(9)	1.333	1.341	1.334	1.31	1.312	1.376
N(9)-C(4)	1.347	1.361	1.354	1.33	1.374	1.360
C(6)-O	1.224	1.208	1.217	1.22		1.233
C(2)-O	1.209	1.211	1.215	1.19	1.223	
N(1)-CH_3		1.482	1.468	1.48		
N(3)-CH_3	1.457	1.478	1.473	1.46		
N(7)-CH_3	1.438	1.470				
C(8)-O						1.241

a) uncorrected for thermal motion
b) complexed with 5-chlorosalicylic acid; ref. 8
c) complexed with 5-chlorosalicylic acid; ref. 9
d) monohydrate and D.J. Sutor, *Acta Cryst.*, **11**, 83 (1958)
e) D.G. Watson, R.M. Sweet, and R.E. Marsh, *Acta Cryst.*, **19**, 573 (1965).
f) H. Ringertz, *Acta Cryst.*, **20**, 397 (1966).
g) Average standard deviation in parentheses.

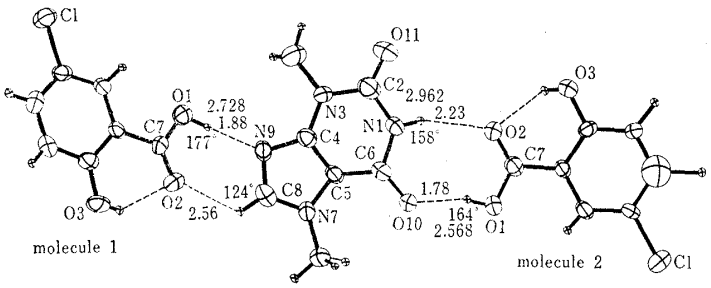


Fig. 3. Intermolecular Hydrogen Bonding Scheme in the 2:1 Complex

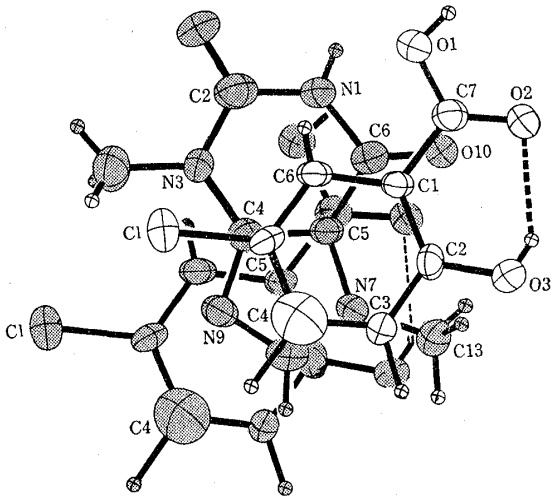


Fig. 4. A View of the Stacking about Theobromine in this Complex

pyrimidine and purine derivatives.[18] The reason behind this phenomenon has not been clearly defined, but could result from a combination of intermolecular forces (*e.g.*, hydrogen bonding) and the contribution of some sp^3 character to the valence state of the nitrogens. The least squares planes through the 5 and 6-membered rings are tilted from each other by 1.6° about the C(4)-C(5) bond. The caffeine and theophylline molecules in the 5-chlorosalicylic acid complexes are similarly bent, but to a greater degree, 3.2°and 3.7°, respectively. A comparison of a number of purine analogues revealed[19] that the dihedral angle between the pyrimidine and imidozole ring usually ranges between 0.30° to 1.26°. This small amount of bending requires little energy and is probably due to intermolecular packing forces.

Intermolecular Bonding

The dominant intermolecular force operating in this complex is that of hydrogen bonding. Within each 2: 1 complex unit, there are three rather strong hydrogen bonds (Figure 3), each of which is a distinct type; O(1)–H···N(9), N(1)–H···O(2) and O(1)–H···O(10). The donor to acceptor atom distances for these interactions are all in the lower range of distances compiled from crystallographic data by Pimental and McClellan[20] for the three types of hydrogen bonds.

A comparison of the three 5-chlorosalicylic acid: xanthine (theobromine, theophylline, and caffeine) hydrogen bonding schemes reveals several common features. 1) In each complex N(9) participates in a relatively strong hydrogen bond with the carboxyl proton of the acid. The N(9) to O(1) lengths for theobromine, theophylline, and caffeine complexes, which are 2.728 Å, 2.682 Å, and 2.644 Å, respectively, suggests that the basicity of N(9) in the crystalline complexes varies as follows: caffeine>theophylline>theobromine. 2) The portion attached to C(8) of the xanthine is always involved in a short contact with an oxygen. The H···O distances are always greater than upper value accepted for a hydrogen bond (2.40 Å)[21] but less than the sum of the van der Waals radii of the two atoms (2.60 Å). 3) The C(2) carbonyl oxygen in all three xanthines complexes does not participate in a hydrogen bond. The basic character of this oxygen is no doubt much less than that of the C(6) carbonyl oxygen. 4) Unlike caffeine which has the 1,3, and 7 positions methylated, theobromine and theophylline

18) W.M. Macintyre, *Biophys. J.*, **4**, 495 (1964).
19) J. Sletten and L.H. Jensen, *Acta Cryst.*, **25**, 1608 (1969).
20) G.C. Pimental and A.L. McClellan, "The Hydrogen Bond," W.H. Freeman and Co., San Francisco, Calif., 1960, p. 289.
21) W. C. Hamilton in "Structural Chemistry and Molecular Biology," Edts. A. Rich and N. Davidson, W. H. Freeman and Co., San Francisco, Calif., 1968, p. 468.

have a proton attached to N(1) and N(7) respectively. These protons are in close proximity to O(10) which enables these molecules to form cyclic hydrogen bridges with neighboring molecules. Theobromine and the carboxyl group of 5-chlorosalicylic acid (molecule 2) are in such an arrangement, while in the theophylline complex the cyclic scheme occurs between inversion related theophylline molecules.

The fact that theobromine contains two basic proton accepting positions on opposite ends of the xanthine residue and that the carboxyl groups are the most acidic hydrogen bond donors, explains why these molecules cocrystallize in a 2: 1 complex.

There is a relatively short contact between the Cl of 5-chlorosalicylic acid (molecule 1) and the hydrogen attached to C(3) (molecule 2) of a neighboring molecule. The 2.81 Å H to Cl distance is shorter than the sum of van der Waals radii of these two atoms (3.2 Å) but not in the range for a hydrogen bond.[21] This van der Waals contact is very likely responsible for displacing the chlorine from the plane of the phenyl ring to which it is attached.

The stacking about theobromine in this complex is shown in Figure 4. The xanthine is sandwiched between two 5-chlorosalicylic acid molecules (both molecule 2) which are related by a unit translation along the *c* axis. The xanthine and phenyl rings are almost parallel, distortion is only 0.6°. There is extensive overlap between the phenyl π-systems and the xanthine nucleus, but not to the same degree found in the caffeine and theophylline complexes.[8,9] In the regions of overlap, the separation between the planes ranges from 3.32 Å to 3.45 Å. It thus appears that though the degree of polarization bonding[22] is substantially less than that found in, for example, the caffeine: 5-chlorosalicylic acid complex, there is a significant amount of stacking interaction present in the crystalline complex.

22) E. Shefter, *J. Pharm. Sci.*, **57**, 350 (1968).