Interaction of Theobromine with Sodium Benzoate

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Abstract □ The interaction of theobromine with sodium benzoate was investigated by PMR spectroscopy. The interaction of theobromine with pentadeuterated benzoic acid (benzoic acid-d_5) was examined in the same manner but to a lesser degree. Chemical shifts of theobromine protons were determined as a function of sodium benzoate concentration in deuterium oxide at 30 and 15°. Signals of both methyl groups of theobromine underwent significant upfield shifts when sodium benzoate was added to a theobromine solution. This fact suggests that a complex is formed by vertical stacking or plane-to-plane stacking. The same results were obtained for benzoic acid-d_5.

Keyphrases □ Theobromine—interaction with sodium benzoate □ Sodium benzoate—interaction with theobromine □ Diuretics—theobromine, interaction with sodium benzoate

Xanthine derivatives such as theophylline and caffeine form a 1:1 complex with sodium benzoate in aqueous solution. The physicochemical properties relating to this complex formation were studied by various methods including calorimetry (1), a solubility method (2), and by PMR spectroscopy (^{1}H-NMR) (3, 4). In the present study, the interaction of theobromine(I), a xanthine derivative, with either sodium benzoate(II) or pentadeuterated benzoic acid (benzoic acid-d_5)(III) was studied by ^{1}H-NMR in deuterium oxide.

EXPERIMENTAL

Materials—Reagent grade theobromine[1] and sodium benzoate JP were used without further purification. Benzoic acid-d_5 (>99.0% pure) and deuterium oxide (99.8%) were used as received[2].

Methods—To examine whether self-association of theobromine takes place, 1.9×10^{-4}–2.6×10^{-3} *M* theobromine was prepared. The concentration of theobromine was maintained constant while that of benzoic acid-d_5 was varied from 1.0×10^{-3} to 3.0×10^{-2} *M* and that of sodium benzoate varied from zero to 1 *M*. ^{1}H-NMR spectra were recorded[3] in duplicate at probe temperatures of 30 ± 0.5° or 15 ± 0.5°. External tetramethylsilane was used as the reference and as the source of a lock signal[4].

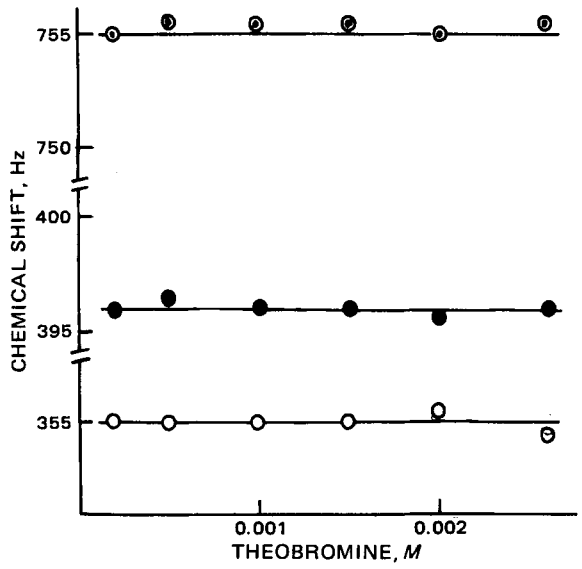
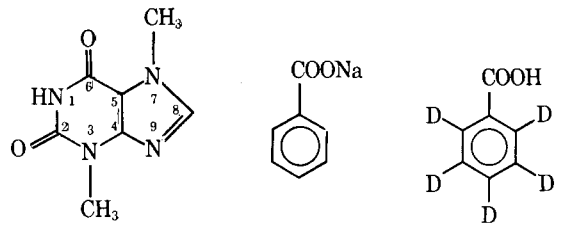


Figure 1—*Concentration dependence of theobromine proton chemical shifts. Key:* ⊙, *8-H;* ●, *7-CH_3; and* ○, *3-CH_3.*



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Bulk susceptibility corrections were tried, but it was found that they were not necessary. In the theobromine-sodium benzoate system, the corrections were too small in comparison with the large induced chemical shift changes due to complexation (4) and in the theobromine-benzoic acid-d_5 system and the study of theobromine self-asasociation, the volume magnetic susceptibilities of the samples were equal to deuterium oxide for the low concentrations of theobromine and benzoic acid-d_5. Chemical shifts were reproducible to better than 0.5 Hz.

RESULTS AND DISCUSSION

The ^{1}H-NMR spectrum of the 1.5×10^{-3} *M* deuterium oxide solution of theobromine has three signals of relative intensities 1:3:3, at 8.36, 4.40, and 3.94 ppm (from external tetramethylsilane), corresponding to the proton at C-8 and to the 7- and 3-methyl protons, respectively, at 30°. The self-associations of theophylline (5) and caffeine (6) in aqueous solution is known, but theobromine self-association has not been reported.

Initially, ^{1}H-NMR spectra of theobromine in deuterium oxide were recorded at 30°. The results (Fig. 1), show that none of the theobromine proton chemical shifts changed regardless of the theobromine concentration and, therefore, no theobromine self-association appears to occur

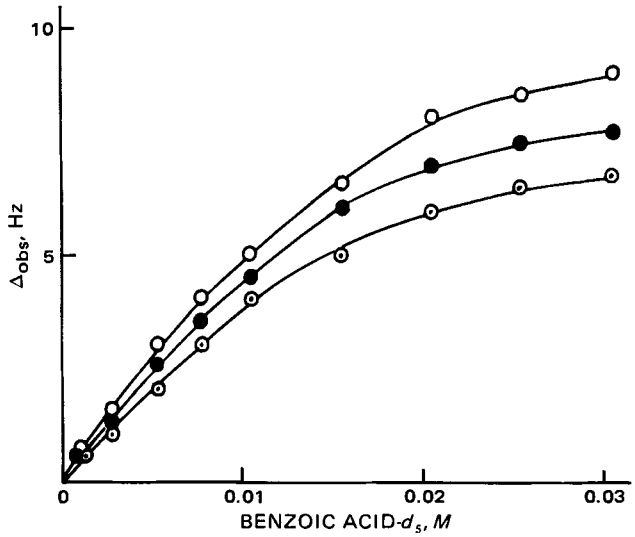

Figure 2—*Induced chemical shift changes for theobromine protons as a function of benzoic acid-*d_5. *Key:* ○, *3-CH_3;* ●, *7-CH_3; and* ⊙, *8-H.*

[1] Tokyo Kasei Kogyo Co., Ltd.
[2] Merck, West Germany.
[3] Nichiden-Varian type NV-21 spectrometer, 90 MHz, FT mode.

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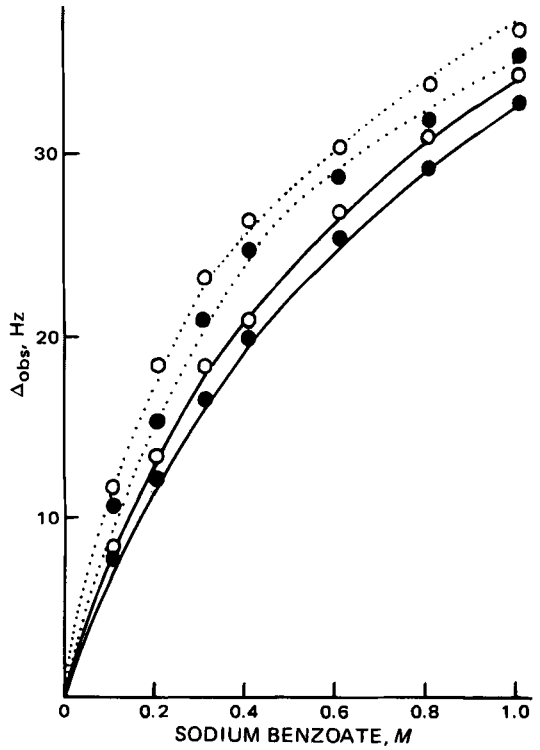

Figure 3—*Induced chemical shift changes for theobromine protons as a function of sodium benzoate concentration. Key: —, 30°; . . ., 15°; ○, 3-CH_3; and ●, 7-CH_3.*

within the concentration range examined. Because of poor theobromine solubility in water, it is thought that theobromine molecules exist mostly in a monomer state.

^{1}H-NMR spectra of the solution containing both theobromine in a fixed concentration of 1.5×10^{-3} *M* benzoic acid-d_5 at various concentrations were recorded at 30°. Signals for the proton at C-8, and the 7- and 3-methyl protons of theobromine, shifted upfield with an increase in the benzoic acid-d_5 concentration. The extent of the upfield shift of the 3-methyl protons was larger than that of the 7-methyl protons and that of the 7-methyl protons was larger than that of the proton at C-8 (Fig. 2).

The upfield shifts of the 3- and 7-methyl signal, and the proton signal at C-8 in the presence of benzoic acid-d_5 suggest that theobromine and benzoic acid form a complex in which the 3- and 7-methyl groups, and the C-8 proton in the theobromine molecule are located over the benzene ring of benzoic acid. The relative small upfield shift of the C-8 proton signal may be caused by compensation with a downfield shift, resulting from the addition of a proton arising from benzoic acid-d_5 to nitrogen at the 9 position of theobromine. However, the addition of a proton seems to occur very sparingly because benzoic acid is weakly acidic with a pKa of 4.19 (7) at 25°. The pKa assigned to nitrogen at the 9-position of theobromine is 0.45 (8) at 18°.

To confirm this theory, the pH of an aqueous solution of 1.5×10^{-3} *M* theobromine was measured at 18° in the presence of 2.0×10^{-2} *M* benzoic acid. Using this value (pH 3.05), theobromine and protonated theobromine were in a molar ratio of 500:1 and the theory was proven valid. Consequently, the 3-methyl protons are nearer the axis of the benzene ring than the 7-methyl protons, which in turn are nearer the axis than the C-8 proton.

^{1}H-NMR spectra of the solution containing both 1.5×10^{-3} *M* theobromine and sodium benzoate at various concentrations were measured (Fig. 3). The signals of the 3- and 7-methyl protons shifted upfield with an increase in the sodium benzoate concentration, the extent of the upfield shift of the 3-methyl protons being slightly larger than that of the 7-methyl protons. The signal of the proton at C-8 could not be measured because of the overlap with the signals of benzene protons of sodium benzoate. These results suggest that the formation of a complex between theoxyline and benzoic acid or sodium benzoate involves vertical stacking or plane-to-plane stacking. It was similarly reported (4) that theobromine interacts with sodium benzoate to form a complex by vertical stacking or plane-to-plane stacking.

Table I—Apparent Thermodynamic Parameters for Complex Formation of Theobromine with Sodium Benzoate in Deuterium Oxide

Parameter	15°		30°
K, M^{-1}	2.6[a]		1.4[a]
ΔG, kcal/mole	−0.55		−0.20
ΔH, kcal/mole		−7.1	
ΔS, kcal/mole degree	0.023		0.023

[a] Average value calculated from 3-CH_3 and 7-CH_3 of theobromine.

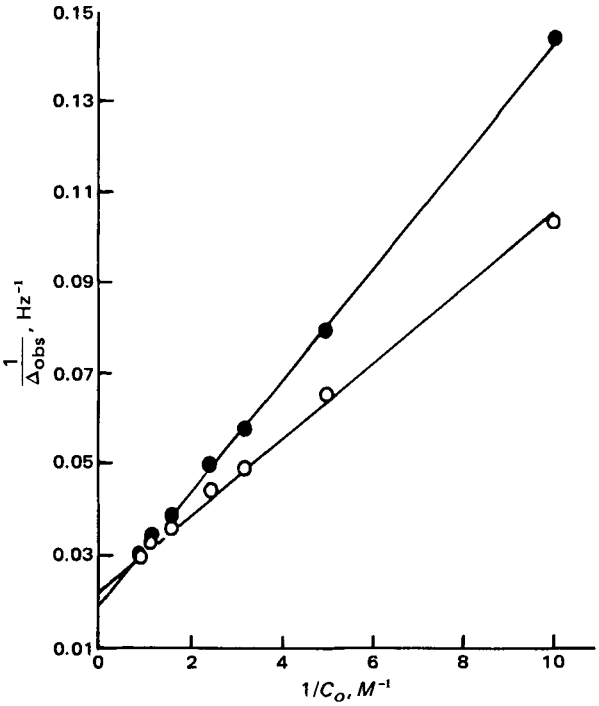

Figure 4—*Plots of theobromine 7-CH_3 versus $C_b{}^{-1}$ of sodium benzoate. Key: ●, 30°; and ○, 15°.*

Following an earlier procedure (9), the apparent formation constant *K* was calculated using Eq. 1, assuming a 1:1 complex:

When $C_b \gg C_a$

$$\frac{1}{\Delta_{obs}} = \frac{1}{K(\delta_c - \delta_a)} + \frac{1}{\delta_c - \delta_a} \qquad \text{(Eq. 1)}$$

where:

K = apparent formation constant
C_a = theobromine concentration
C_b = sodium benzoate concentration
δ_c = chemical shift of theobromine proton in the complex form
δ_a = chemical shift of theobromine proton in the uncomplex form
Δ_{obs} = the difference between the observed chemical shift and δa

The results are shown in Fig. 4 and the apparent formation constant is listed in Table I. The results of the interaction of theobromine with sodium benzoate at 15 and 30° are shown in Figs. 3 and 4 and Table I. As the apparent formation constant *K* was varied depending on temperature, the enthalpy change of complex formation (ΔH), the free energy change (ΔG), and the entropy change (ΔS) were calculated (Table I).

It was concluded that the complex formation of theobromine with sodium benzoate is an exothermic reaction accompanied by reduction of entropy.

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Antileukemic Activity of Tetrazole Analogs of Phenylalanine Derivatives

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Received February 18, 1981, from the *Auburn University School of Pharmacy, Department of Pharmacal Sciences, Auburn, AL 36849.* Accepted for publication June 9, 1981.

Abstract □ Eleven tetrazole analogs of substituted phenylalanines were prepared and tested for antitumor activity using P-388 lymphocytic leukemia cells in mice. None of the compounds exhibited significant activity (T/C %, < 125).

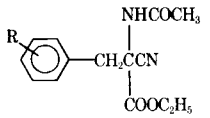
Keyphrases □ Antileukemic activity—tetrazole analogs of substituted phenylalanines against P-388 lymphocytic leukemia □ Phenylalanines, substituted—tetrazole analogs, preparation and screening for antileukemic activity □ Tetrazole—analogs of substituted phenylalanines, preparation and screening for antileukemic activity

The demand for nutrients differs between tumor cells and normal cells (1). Since rapidly proliferating cancer cells take up nutrients more rapidly, they may be selectively "starved" by substituting nonfunctional amino acid derivatives for the normal substrate. *N*-Chloroacetyl derivatives of *para*-substituted phenylalanines were reported (2) to have significant growth inhibitory activity in a microbial antitumor prescreen.

Recent reviews (3, 4) indicated that no reports have appeared for testing tetrazole analogs of amino acids for antineoplastic activity. Studies of the biological activity of 5-substituted tetrazoles have been prompted by a close similarity between the acidity of the tetrazole group and the carboxylic acid group, and the fact that the tetrazole function appears to be metabolically more stable (3).

The chemically similar tetrazole ring system (5) has been used to replace the carboxyl group in several amino acids (6–8). These *in vitro* studies suggested that the tetrazole analog may serve as substrate inhibitor of the respective amino acid in certain enzymatic reactions. The purpose of this study was to determine whether tetrazole analogs of certain phenylalanine derivatives would exhibit antileukemic action in mice.

Table I—Aryl-Substituted Ethyl 2-Acetamido-2-cyano-3-phenylpropanoates



Compound	R	Melting Point	Yield, %	Formula	Analysis, %	Calc.	Found
					C	60.43	61.07
I	2F	94–96°	65.8	$C_{14}H_{15}FN_2O_3$	H	5.43	5.51
					N	10.07	10.07
					C	58.35	58.46
II[a]	2Cl	145–8°	28.6	$C_{15}H_{17}ClN_2O_3$	H	5.55	5.61
					N	9.07	9.04
					C	49.57	49.63
III	2Br	150–152°	68.7	$C_{14}H_{15}BrN_2O_3$	H	4.46	4.51
					N	8.26	8.24
					C	43.54	43.61
IV	2I	174–176°	67.5	$C_{14}H_{15}IN_2O_3$	H	3.92	3.96
					N	7.25	7.23
					C	62.06	62.12
V	$2OCH_3$	142–144°	92.8	$C_{15}H_{18}N_2O_4$	H	6.25	6.31
					N	9.65	9.67
					C	62.06	62.01
VI	$4OCH_3$	164–167°	57.1	$C_{15}H_{18}N_2O_4$	H	6.25	6.29
					N	9.65	9.63
					C	49.57	49.41
VII	4Br	165–168°	62.7	$C_{14}H_{15}BrN_2O_3$	H	4.46	4.52
					N	8.26	8.24

[a] Isopropyl ester (the analytical sample was inadvertently recrystallized from isopropyl alcohol resulting in transesterification).

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