Complexation of Theophylline with Sodium Benzoate: An NMR Study

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Abstract □ The interaction of theophylline with sodium benzoate was examined by NMR to understand the mode of interaction. Chemical shifts of theophylline protons were determined as a function of sodium benzoate concentration in D_2O at 30°. Several different concentrations of theophylline were used. Signals of both methyl groups undergo significant upfield shifts, indicating vertical or plane-to-plane stacking.

Keyphrases □ Theophylline–sodium benzoate complexation—studied by NMR □ Sodium benzoate–theophylline complexation—studied by NMR □ Interactions—theophylline–sodium benzoate complexation, studied by NMR □ NMR—analysis, theophylline–sodium benzoate complexation, mode of interaction

Although complexes of theophylline, caffeine, and other xanthines with drug molecules have been studied extensively, the mode of association for such complexes has remained largely a matter of speculation. For crystalline complexes, direct information has been provided regarding the geometry of the components in complexes of caffeine and theophylline with 5-chlorosalicylic acid (1, 2). On the basis of a linear correlation between the standard unitary free energy change and the planar area of neutral as well as ionic interactants for several xanthine complexes, Connors and coworkers (3, 4) suggested that the interacting species have a plane-to-plane arrangement. Similar views were advocated by other workers (5, 6) as well; however, direct experimental evidence that might aid in understanding the mode of interaction and the geometry of the interacting species *in aqueous solution* has been lacking.

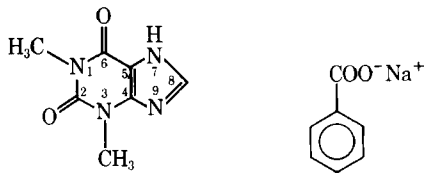
This paper concerns the interaction between theophylline (I) and sodium benzoate (II) in aqueous solution as examined by NMR spectroscopy. This system was chosen as a model because of its simplicity and because of conflicting reports on whether these two components interact (7, 8).

EXPERIMENTAL

Materials—Theophylline NF, sodium benzoate USP, and deuterium oxide (99.7 mole %)[1] were used as received.

Methods—Several sets of solutions were prepared; within each set the concentration of theophylline was maintained constant while that of sodium benzoate was varied from 0 to 1.0 *M*. The following concentrations of theophylline were used: 0.004, 0.010, 0.015, and 0.020 *M*. NMR spectra were recorded[2] in duplicate at a probe temperature of 30 ± 1°. External tetramethylsilane was used as the reference and as the source of a lock signal. Bulk susceptibility corrections were not made. Approximate calculations indicated that these corrections are too small in comparison with the large induced chemical shift changes due to complexation. Accuracy of the observed chemical shifts is estimated to be better than ±0.005 ppm.

[1] Merck Sharp and Dohme of Canada Ltd., Montreal, Canada.
[2] Varian HA-100.



I

II

RESULTS AND DISCUSSION

The NMR spectrum of a dilute (0.004 *M*) D_2O solution of theophylline consists of three signals of relative intensities, 1:3:3, at about 8.46, 4.01, and 3.80 ppm (from external tetramethylsilane) corresponding to the proton at C-8 and the 3- and 1-methyl protons, respectively. Signal assignments are based upon a comparison with the spectrum of caffeine (9). Theophylline, like caffeine and other xanthines, undergoes self-association in aqueous solution, and this behavior is reflected in the NMR spectrum by the upfield shift of all three signals as the concentration of theophylline is increased (10). In the present study, the concentration of theophylline was kept rather low to minimize the possibility of interactions of the self-associated species.

When sodium benzoate is added to a solution of theophylline, all three signals in the NMR spectrum move upfield. Stamm (11)

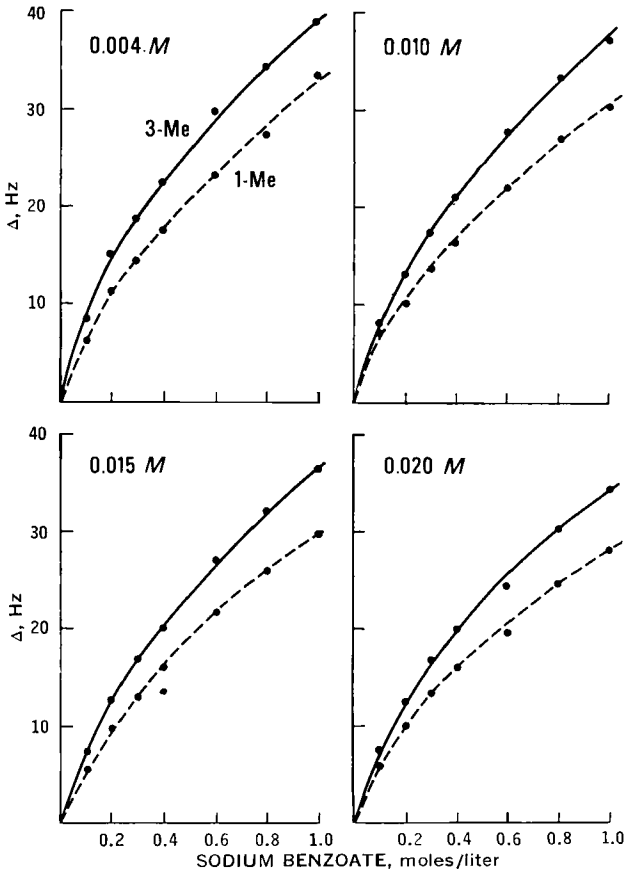


Figure 1—*Induced chemical shift changes for the methyl protons of theophylline as functions of sodium benzoate concentration.*

Table I—Formation Constants (K) and Complexation Shifts (Δ_0) for the Interaction of Theophylline with Sodium Benzoate at 30°

Theophylline, M	K, M^{-1}						Δ_0, Hz					
	Method I[a]		Method II[b]		Method III[c]		Method I[a]		Method II[b]		Method III[c]	
	1-CH_3	3-CH_3	1-CH_3	3-CH_3	1-CH_3	3-CH_3	1-CH_3	3-CH_3	1-CH_3	3-CH_3	1-CH_3	3-CH_3
0.004	1.00	1.26	1.00	1.38	1.05	1.44	64	69	63	66	65	92
0.010	1.00	1.07	1.20	1.20	1.34	1.25	60	72	54	68	68	82
0.015	1.00	1.11	1.01	1.18	1.05	1.22	58	68	58	66	59	78
0.020	1.14	1.24	1.35	1.42	1.42	1.47	52	61	48	57	65	81

[a] Method of Johnston *et al.* (12). [b] Method of Nakano *et al.* (13). [c] Method of Foster and Fyfe (14).

observed a similar effect upon the interaction of sodium benzoate with caffeine. Figure 1 shows the induced chemical shift change for the two methyl groups of theophylline as a function of sodium benzoate concentration. A similar plot could not be made for the C-8 proton because the signal appeared in the same spectral region as the signals due to the aromatic protons of benzoate and it could not always be distinguished. As the figure shows, both methyl signals move appreciably upfield.

The upfield changes in chemical shift suggest that the complex is formed in such a way that the two methyl groups of theophylline come under the shielding region associated with the anisotropic benzoate moiety. Since the conical shielding regions of the aromatic ring are perpendicular to and above and below the plane of the aromatic ring, it follows that the complex is formed by vertical stacking. If the opposite, horizontal (side-by-side or end-to-end) arrangement were the case, then chemical shift changes in the opposite direction would have been observed. The larger value of the upfield shift of the 3-methyl group (0.71 ppm) as compared with that of the 1-methyl group (0.60 ppm) provides information about the time-averaged, relative orientation of theophylline and benzoate; the 3-methyl group spends somewhat more of its time nearer the axis of the phenyl ring than does the 1-methyl group.

Apparently, three types of interactions are involved in the formation of the theophylline–benzoate complexes. Hydrophobic interactions tend to localize theophylline and benzoate molecules in the neighborhood of each other in such a manner as to minimize *effectively the surface exposed* to water. Maximum overlap of the two molecules would result in minimum exposure to water. A plane-to-plane arrangement, as in vertical stacking, would provide such a condition. Interactions between the π-electron systems would reinforce the plane-to-plane arrangement.

The hydrophobic and π interactions by themselves would permit free rotation of the two molecules about a common axis. If such were the case, one would not expect differences in the shielding experienced by the two methyl groups of theophylline. Specific interactions between the carboxyl ion of the benzoate and the imino group of theophylline would tend to hinder rotation of the molecules. The specific interaction would tend also to reduce the parallelism of the two molecules. The observed chemical shift difference indicates that the time-averaged position of the 3-methyl group is nearer the axis of the phenyl ring than is that of the 1-methyl group.

The chemical shift data were treated by three different methods to obtain the apparent formation constant K. All of the methods assume a 1:1 stoichiometry. Two of the methods, those of Johnston *et al.* (12) and Nakano *et al.* (13), apply over all concentration ranges. The third method, given in different but mathematically equivalent forms by Foster and Fyfe (14) and by Hanna and Ashbaugh (15), imposes the restriction that the molar concentration of one reactant be much larger than that of the reactant giving rise to the signals.

According to Johnston *et al.* (12):

$$\Delta = \frac{[1 + A_0K + B_0K] - [(1 + A_0K + B_0K)^2 - 4K^2A_0B_0]^{1/2}}{2KA_0}(\Delta_0) \qquad \text{(Eq. 1)}$$

where Δ is the difference between the observed chemical shift, δ_{obs}, and the chemical shift, δ_A, of a given signal of A in the absence of any B; Δ_0 is the chemical shift of that signal in the fully complexed A; A_0 and B_0 are the initial molar concentrations of theophylline and sodium benzoate (in this case), respectively; and K is the equilibrium constant in terms of M^{-1}.

A nonlinear least-squares computer program was used to fit the data (δ_{obs}, A_0, and B_0) to Eq. 1 and to compute the values of K and Δ_0. The computer[3] printout included a curve calculated from the computed values of K and Δ_0 along with the actual experimental values.

Nakano *et al.* (13) derived a general expression in which the equilibrium concentration of the complex, C, appears as a parameter:

$$\frac{B_0}{\Delta} = \left(\frac{1}{\Delta_0}\right)(A_0 + B_0 - C) + \frac{1}{K\Delta_0} \qquad \text{(Eq. 2)}$$

This expression can be solved graphically by successive approximations, plotting $B_0\Delta^{-1}$ *versus* $A_0 + B_0$, the first slope being approximately Δ_0^{-1}. This value for Δ_0 is utilized in:

$$C = \left(\frac{\Delta}{\Delta_0}\right)A_0 \qquad \text{(Eq. 3)}$$

to gain successive, convergent values for C. When the Nakano expression is solved with a computer, Eqs. 2 and 3 are solved simultaneously, and the method is essentially equivalent to the Johnston approach.

When $B_0 \gg A_0$, Eq. 2 reduces to Hanna and Ashbaugh's (15) equation:

$$\frac{B_0}{\Delta} = \frac{B_0}{\Delta_0} + \frac{1}{K\Delta_0} \qquad \text{(Eq. 4)}$$

or to Foster and Fyfe's (14) equivalent form:

$$\frac{\Delta}{B_0} = -\Delta K + \Delta_0 K \qquad \text{(Eq. 5)}$$

In concentration regions where $B_0 \gg A_0$, plots of ΔB_0^{-1} *versus* Δ should be linear with a slope of $-K$ and an intercept of Δ_0K. In each experiment reported here, $B_0 \gg A_0$.

The values of K and Δ_0, calculated by the three methods, are shown in Table I. The average value of K, 1.20 ± 0.13 M^{-1}, demonstrates that a significant interaction does take place between theophylline and sodium benzoate. The absolute value of K should be regarded with reservation, however, since the ionic strength was not maintained constant in each determination. The formation constant is only approximate. The reproducibility in the Δ_0 values indicates the validity and reliability of the NMR method.

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Tumor-Inhibitory Agent from *Zaluzania robinsonii* (Compositae)

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Abstract □ The chloroform extract of *Zaluzania robinsonii* Sharp has shown activity against the P-388 lymphocytic leukemia test system. The constituent responsible for this activity was a guaianolide, identified as zaluzanin C ($C_{15}H_{18}O_3$). The identity was proven by melting point, mixed melting point, elemental analysis, IR, PMR, mass spectroscopy, and preparation of derivatives.

Keyphrases □ *Zaluzania robinsonii* (Compositae)—isolation and identification of zaluzanin C as tumor-inhibiting agent □ Zaluzanin C—isolation and identification from *Z. robinsonii*, tested for tumor-inhibitory activity □ Antitumor agents, potential—isolation, identification, and screening of zaluzanin C from *Z. robinsonii*

As a result of the continuing search for plants having tumor-inhibiting constituents, the ethanol extract of the leaves, stems, flowers, and roots of *Zaluzania robinsonii* Sharp (Compositae)[1] was found to have inhibitory activity toward the P-388 lymphocytic leukemia test system (3PS)[2].

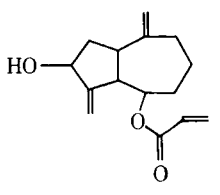
DISCUSSION

The chloroform extract, obtained from an ethanol extract by partition between chloroform and water, was subjected to systematic fractionation employing partition and solvent extraction followed by column chromatography on silica gel. The sequence of steps in the fractionation procedure leading to the isolation of the guaianolide is outlined in Scheme I. The guaianolide was identified as zaluzanin C (I) by means of its melting point, elemental analysis, mass spectroscopy, and comparison of IR and PMR spectra with authentic sample spectra. In addition, no depression in a mixed melting point and a superimposable IR spectrum of the acetyl derivative with an authentic sample and identical physical data of the dehydro derivative further proved the identification of the guaianolide as zaluzanin C. The compound had been previously isolated from two closely related species, *Z. augusta* (lag) Schultz, Bip. (1) and *Z. triloba* Pers. (2).

This compound demonstrated an activity of 161% test/control (T/C) at 150 mg/kg in the 3PS test system. Activity in the 3PS system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ≥ 125% (3).

EXPERIMENTAL[3]

Isolation Procedure—The roots, stems, leaves, and flowers (8.5 kg) of *Z. robinsonii* were ground and extracted exhaustively in a Lloyd-type extractor with ethanol. After removal of the solvent in air, the residue was subjected to three-funnel partition in three lots between benzene–methanol–water (8:5:1) using 1700 ml of each phase. All upper phases were combined, the solvent was removed in air, and the residue was stirred mechanically twice with ethyl acetate and filtered. The combined ethyl acetate-soluble fraction yielded a residue weighing 46 g. The lower phases (second and third) obtained from these partitions were similarly combined and the solvent was removed. The residue was stirred twice with *n*-hexane and filtered. The hexane-insoluble residue, weighing 8.5 g, was then mixed with the ethyl acetate-soluble residue (46 g). A portion of this mixture (39 g) was subjected to silica gel (1700 g) column (10 × 152 cm) chromatography. The column was eluted



I

[1] Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resources Laboratory, Plant Genetics and Germ Plasm Institute, Beltsville, Md. A reference specimen was deposited in that herbarium. The plant was collected in San Luis Potosi, Mexico, in August 1970.

[2] Of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md.

[3] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. The melting points were determined on a Kofler hot-stage apparatus and are uncorrected.