the basic physical systems involved in the formulations under study. A few shrewd observations early in the game will often save a great deal of trouble and effort later on.

(*c*) When using heat to accelerate a physical aging process in a heterogeneous preparation, it should be remembered that the system at elevated temperatures is different from the system at room temperature; consequently, extrapolation to room temperature stability must be done with care and caution.

(*d*) The analytical method employed to study disperse systems (suspensions, etc.) must not be too harsh because the disturbed system may have little or no relationship to the system as it sits on the shelf.

(*e*) A change in pH can often have a profound effect on physical stability; consequently, all physical stability data should include periodic measurement of pH whenever possible.

(*f*) One of the most important aspects of physical stability—physiological availability of the active ingredient—is often the most neglected. All good stability studies should include *in vitro* tests (dissolution rate, etc.) that will help detect when changes in *in vivo* absorption characteristics are likely to occur.

(*g*) Before final approval is given to the physical stability of any product, it should be taken home and used just as a patient would use it (without actually taking the medication). This "home trial" often reveals physical stability problems that never arise in the laboratory.

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Notes

Assignment of the *N*-Methyl Hydrogen NMR Peaks of Caffeine

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The NMR spectrum of caffeine exhibits a separate peak for each of the three *N*-methyl groups. Assignment of each peak to a specific *N*-methyl group was made by comparison of the spectrum of caffeine with those of two caffeine homologs, in each of which one of the *N*-methyl groups is replaced with an *N*-ethyl group.

IN THE nuclear magnetic resonance spectrum of caffeine published by Bhacca, *et al.* (1), *N*-methyl hydrogen peaks are shown at 6.60, 6.41, and 5.99 p.p.m. However, there is no specific assignment of these to the 1, 3, and 7 positions. We undertook this study to make such assignments.

The *N*-methyl hydrogen peaks of caffeine can be selectively removed from the spectrum by (*a*) the substitution of the methyl hydrogen atoms with deuterium atoms, (*b*) the substitution of methyl groups with ethyl groups, or (*c*) the substitution of methyl groups with hydrogen atoms. The last technique might result in a significant difference in the chemical shift of the remaining *N*-methyl hydrogen peaks from the corresponding ones of caffeine, since a hydrogen atom would have considerably less inductive effect upon the rings than a methyl group. Because it was more convenient to synthesize two of caffeine's higher homologs than to prepare the partially deuterated caffeines, the second approach was used. The homologs were synthesized by treating theobromine and theophylline with ethylating agents. Samples of these compounds and of caffeine were dissolved in deuterated chloroform and their NMR spectra were obtained (Fig. 1).[1] Tetramethylsilane was used as an internal reference standard.

Caffeine—(1,3,7-Trimethylxanthine).—Eastman's white label was used.

Ethyltheobromine—(3,7-Dimethyl-1-ethylxanthine).—The sample was prepared by the method of Rodionov (2). When recrystallized from water, the product melted at 161–163°.

Ethyltheophylline—(1,3-Dimethyl-7-ethylxanthine).—The sample was prepared by a modification of Schmidt's method (3). One gram of theophylline and 2 ml. of ethyl sulfate were refluxed in 20 ml. of 5% NaOH for several hours. The solution was kept basic during the refluxing by the addition of 5% NaOH. The product, when extracted with chloroform and recrystallized from water, melted at 150–151°. (Schmidt reported a value of 154°.)

As anticipated, the spectra of each of the caffeine homologs consisted of two sharp *N*-methyl peaks, a triplet, a quartet, and a single olefinic hydrogen

Received August 20, 1963, from the Division of Pharmaceutical Chemistry, Bureau of Biological and Physical Sciences, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.
Accepted for publication December 2, 1963.

[1] A model A-60 Varian NMR spectrometer was used in these studies.

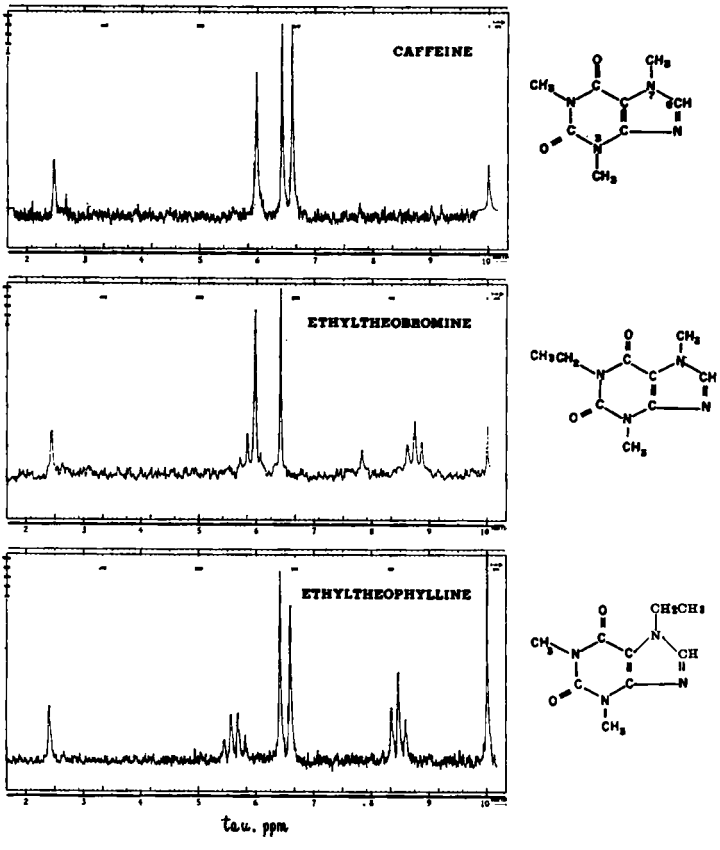

Fig. 1.—NMR spectra.

TABLE I.—ASSIGNED CHEMICAL SHIFTS IN TAU UNITS

	Caffeine			
	Reported	Observed	Ethyltheobromine	Ethyltheophylline
N-Methyl hydrogens at 1	...[a]	6.60	...	6.58
N-Methyl hydrogens at 3	...[a]	6.41	6.42	6.40
N-Methyl hydrogens at 7	...[a]	5.99	5.98	...
N-Ethyl hydrogens at 1				
Methyl triplet	...	...	8.74	...
Methylene quartet	...	...	5.89	...
N-Ethyl hydrogens at 7				
Methyl triplet	...	...	...	8.46
Methylene quartet	...	...	...	5.61
Olefinic hydrogens	2.40	2.44	2.44	2.38

[a] Unassigned *N*-methyl hydrogen peaks are shown at 5.99, 6.41, and 6.60 p.p.m. (1).

peak. The triplets and quartets result from the spin-spin interaction of the methyl and methylene hydrogens of the *N*-ethyl groups. The introduction of the methylene groups into the molecule causes very little change in the observed chemical shifts of the remaining *N*-methyl hydrogens. Thus, the technique chosen proved to be satisfactory for the interpretation of the caffeine spectrum, and it may prove valuable in similar problems of interpretation.[2]

Common to all three spectra was a sharp line between 6.40 and 6.42 p.p.m. This was assigned to position 3 since there is an *N*-methyl group at this position in each of the compounds. The other assignments are presented in Table I.

It is particularly interesting to note that the *N*-methyl hydrogens of position 1 resonate at a higher field strength than do those at positions 3 and 7. Also, the *N*-ethyl hydrogens of ethyltheobromine resonate at higher field strengths than those of ethyltheophylline. There appears to be a greater degree of magnetic shielding for those groups at position 1 than for those at position 7.

[2] The work of Graham and Rogers (4) and of Brey and Jones (5) shows that this technique would not work in the spectra interpretation of derivatives of cyclopropane and pyrazoline.

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