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SPECTROSCOPIC STUDIES ON CAFFEINE AND ISOCAFFEINE

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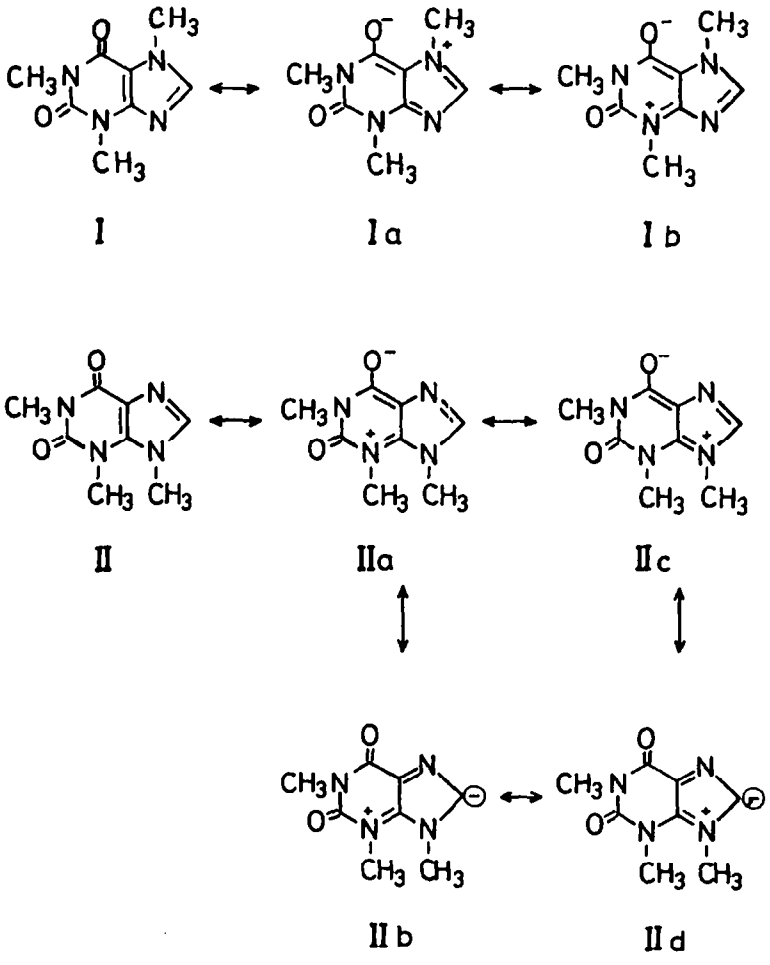
Abstract - UV and NMR spectra of caffeine and isocaffeine were measured in three solvents of different polarity. The information so obtained revealed unique differences in the electronic structure of 7- and 9- alkyl substituted xanthines. 9-Methyl derivatives bear a "soft" nitrogen at position 3; in 7-methylxanthines this nitrogen is rather "hard". This characteristic distinction is explained on the basis of orbital interactions, which are also responsible for self-association. In the process of self-association, isocaffeine and caffeine share a hydrophobic effect, but in caffeine water bridges play a decisive role in molecular aggregation. In contrast, in isocaffeine high polarisability makes the most important contribution to self-association. Steric interference between 3- and 9-alkyl substituents or between a 9-methyl and a 3-NH group, proposed earlier, does not explain satisfactorily the properties of these xanthines.

In previous publications it was reported that in a variety of 3,9-dimethylated purines, both NMR methyl signals are shifted downfield by 0.2-0.4 ppm, relative to the monomethylated derivatives.[1-4] It was assumed that this phenomenon results from steric interference, forcing the peri-methyl groups to spread apart within the plane of the purine ring.[5] Similarly, interference of a 9-methyl substituent with a 3-NH group was used to explain the much lower pK_a value of the 3-NH, as compared with the dissociation constant of the same group in xanthines and 6-thioxanthines, lacking a 9-alkyl substituent.[1,2] Likewise, the tendency of xanthines without an N-substituent in the imidazole moiety, to assume the 7-NH tautomeric form was interpreted as avoidance of the simultaneous presence of 3- and 9-NH groups.[1,2] These assumptions also accorded with the observation of Pfleiderer and Nübel that in xanthine and its 1-methyl derivative, anion formation at N-3 is followed by spontaneous tautomerisation of the imidazole moiety from its 7- to the 9-NH form.[6]

However, steric interference cannot be the only factor explaining the special properties of 9-methylxanthines, as will become clear in the following discussion. In the present study, we attempt to explain the downfield shifts of the methyl signals in 3,9-dimethylxanthines by the specific electronic structure of these compounds. This will be especially exemplified by comparison of the physical properties of caffeine (=1,3,7-trimethylxanthine: I, scheme 1) and isocaffeine (1,3,9-trimethylxanthine: II, scheme 1).

SCHEME 1



1) The melting point of isocaffeine (284-286°) differs markedly from that of caffeine (230°), indicating easier dissociation of the molecules from the crystal lattice of caffeine.

2) When run on TLC, alumina-chloroform, caffeine migrates appreciably, while isocaffeine remains at the origin, thus reflecting its high polarisability. These differences will be interpreted below.

3) UV spectra

a) Caffeine - In the literature, UV spectra for caffeine are reported only in water.[7] We have used chloroform, methanol and water. In chloroform, caffeine exhibits two absorption maxima at 276 and 229 nm, with higher extinction at the former (Fig. 1, curve A)*. In water, the first peak is moved slightly to a shorter wave length (273 nm), while in place of the second peak a shoulder appears at about 230 nm. A strong band is now observed at 205nm (Fig. 1, curve B). However, the spectrum in water becomes similar to that in chloroform, if small concentrations of acetic acid are added (Fig. 2, curves C-E). Instead of the shoulder, a well-defined band appears now at 230 nm, after the strong extinction at 205 nm has been cut off by comparing with the solvent blank**. A similar behaviour is observed for methanolic solutions (spectra not shown). The intensity of the long-wave band is always higher than that of the band near 230 nm.

As stated before, the transition from chloroform to water is accompanied by a hypsochromic shift of λ_{max} of 3nm, but the corresponding value in methanol lies even below that in water (Table 1).

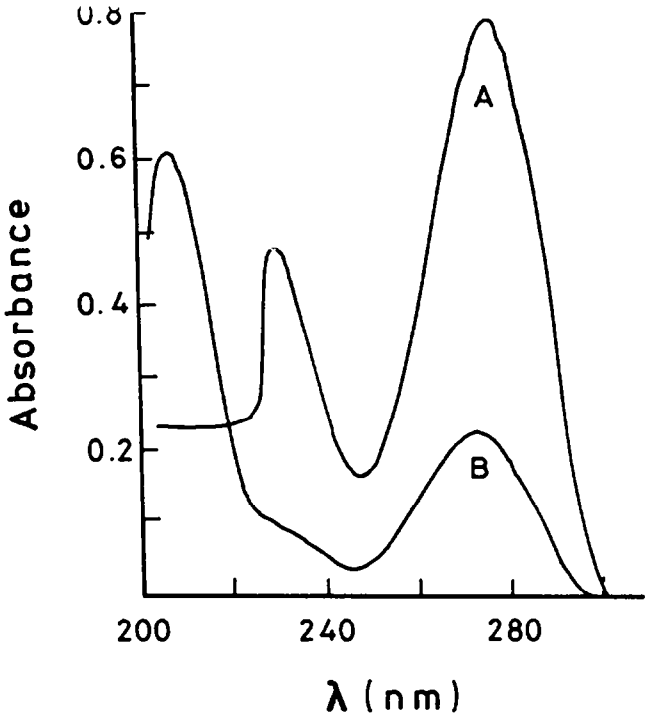


Fig. 1. UV spectra of caffeine.
A, 200 μg/ml in chloroform;
B, 50 μg/ml in water.

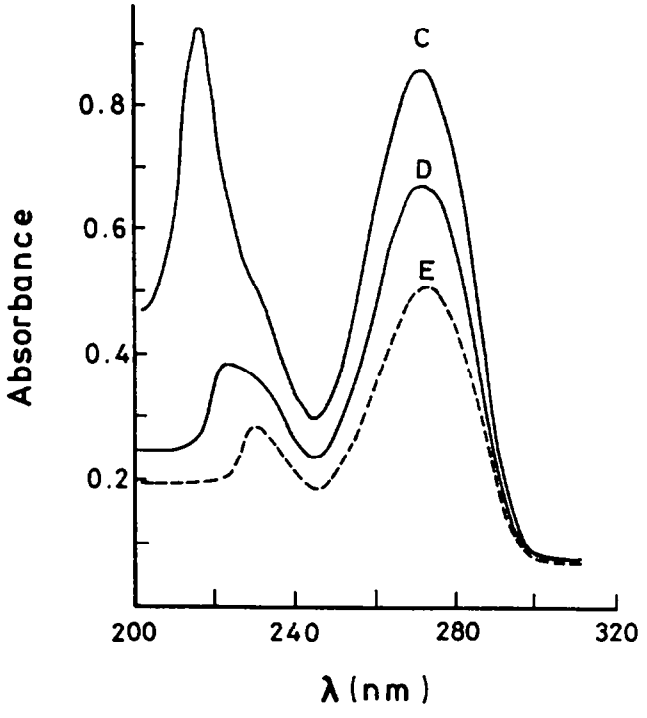


Fig. 2. UV spectra of caffeine in aqueous acetic acid. C, 0.5% acetic acid; D, 1% and E, 2% acetic acid. Note that increasing concentrations of acetic acid bring the UV spectrum in water (Fig. 1, curve B) close to that in chloroform (Fig. 1, curve A).

TABLE 1 Absorption bands in three solvents

Solvent	λ max in nm (ε mol)	
Isocaffeine		
H_2O	268 (1.1×10^4);	237 8.7×10^3)
CH_3OH	266 (1.0×10^4);	237 (9.7×10^4)
$CHCl_3$	265 (9.9×10^3);	242 (1.0×10^4)
Caffeine		
H_2O	273 (7.7×10^3)	
CH_3OH	271 (7.7×10^3)	
$CHCl_3$	276 (7.7×10^3)	229 (4.8×10^3)

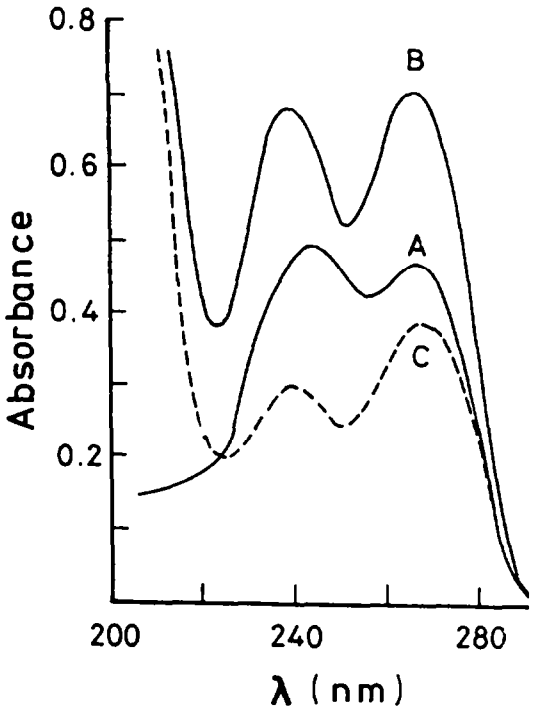


Fig. 3. UV spectra of isocaffeine. A, 170 μg/ml in chloroform; B, 250 μg/ml in methanol; C (——), 140 μg/ml in water.

b) Isocaffeine - Isocaffeine exhibits two maxima in all solvents (Fig. 3). The long- wave band is shifted by 5-9 nm towards the blue, relative to its position in caffeine. Scheme I shows that more resonance structures contribute to the molecule of isocaffeine than of caffeine, i.e., more C=N groups participate in the electronic structure of isocaffeine. It is known that such groups stabilise the ground state relative to the excited state and thus cause hypsochromic shifts of λ_{max} in conjugated systems.[8] The reverse is observed for the second maximum of II: In chloroform this band is displaced by 13 nm to the red, compared with its position in caffeine. It is also important that the relative intensities of the two maxima of isocaffeine change with solvent polarity: In chloroform, absorption at the shortwave band is stronger, while the reverse is true in water.

In order to explain the spectral differences between I and II, we shall discuss first the pK_a values for 3-NH in 1,7-dimethylxanthine (III) and its 1,9-dimethyl isomer (IV). For cpd. III, the value is 8.6, while in IV it is 6.3.[1] This difference expresses the stronger conjugation of the p-electrons of N-3 in IV with the π-system of the molecule, thus reflecting the softness of N-3.[9] Applying this result to cpds. I and II, we may conclude that there exists stronger conjugation of the p-electrons of N-3 with the high-lying orbitals of isocaffeine. This conjugation is responsible for the formation of raised and lowered energy levels of the high-lying orbitals and thus for the presence of two absorption maxima which change their relative intensity according to the polarity of the solvent (Fig. 3).

In chloroform, the lone electrons at N-3 cause the greatest hypsochromic shift of λ_{max} in the low energy transition of II, relative to I. In polar solvents, the shift is less marked because of the smaller contribution of the polar resonance structures in scheme 1. The λ_{max} values of caffeine are in the opposite order because of the "hard" character of N-3, stabilising the ground state relative to the excited state.

As stated above, for caffeine a second maximum (above 205 nm) is observed only in chloroform (Fig. 1). Pullman et al. who measured only in water, could not explain why caffeine - in contrast to isocaffeine - shows only a single peak at long wave lengths.[7] However, these authors noted already that surprisingly the UV spectrum of 8-decylthio-1,9-dimethylxanthine in ethanol is

TABLE 2 UV absorption spectra of neutral and anionic forms of xanthines

		λ_{max} (nm) ($\varepsilon_{mol} \times 10^4$)	
Compound	Solvent	Neutral form	Anion
Theobromine	water	273 (1.1)	274 (1.1)
1,9-Dimethyl-xanthine (IV)	water	265 (0.9)	278 (0.8)
		236 (0.6)	250 (0.8)
	methanol	260 (0.9)	277 (0.8)
		236 (0.8)	241 (1.0)

identical with that of similarly 8-substituted 3,9-dimethylxanthines, although in the former steric hindrance cannot play any role. Our own measurements with 1,9-dimethylxanthine in methanol and water also reveal two absorption maxima (Table 2 and Fig. 4, curve A), like those found for isocaffeine (Table 1 and Fig. 3, curve B). Thus this property is independent of steric interferances between 3- and 9-alkyl substituents in xanthines.

The spectral characteristics of I and II demonstrate a profound difference in the general behaviour of these two isomers. Isocaffeine is more polarisable, while caffeine - due to the hard character of its N-3 - is more highly solvated.

In methanol, caffeine exhibits the shortest λ_{max} (Table 1). This may be explained by the dual character of solvation by methanol. By means of hydrogen bonding of the hydroxyl group of the solvent to the polar groups of caffeine, the ground state is stabilised, similar to the effect of solvation by water. But in addition, methanol also causes hydrophobic solvation via its methyl group. This effect also contributes to stabilisation of the ground state.

Ionisation of the 1-NH group in xanthines causes only a slight bathochromic shift (see theobromine in Table 2). In contrast, dissociation of the 3-NH group (as in III and IV) (see scheme 2) is accompanied by large red shifts of the λ_{max} of 21 and 13 nm, respectively (ref. 1 and Table 2) It is evident that the lone electrons of N-1 are not incorporated into the high-lying molecular orbitals, while the negative charge at N-3 contributes strongly to the main chromophore. Thus curve B in Fig 4 represents evidence for the marked contribution of the lone electrons of N-3 to the chromophore of the anion of IV.

Protonation of caffeine and isocaffeine leads to the same λ_{max},[1] i.e., similar chromophores are formed by attachment of a proton to the amidine moiety in the imidazole ring (see scheme 3). The pK of acetamidine is 12.1,[10] while for the cations of I and II the values are 0.5 and 0.6, respectively.[1] Evidently both these xanthines are very weak bases.

Since the excited states of molecules, which contain both electron donating and withdrawing groups, are destabilised by protonation, the cations of I and II likewise undergo a hypsochromic shift.[1] The change of the absorption peak is greater for caffeine, because its neutral form is stabilised more in the excited state and thus exhibits a λ_{max} at longer wave lengths than does isocaffeine.[1]

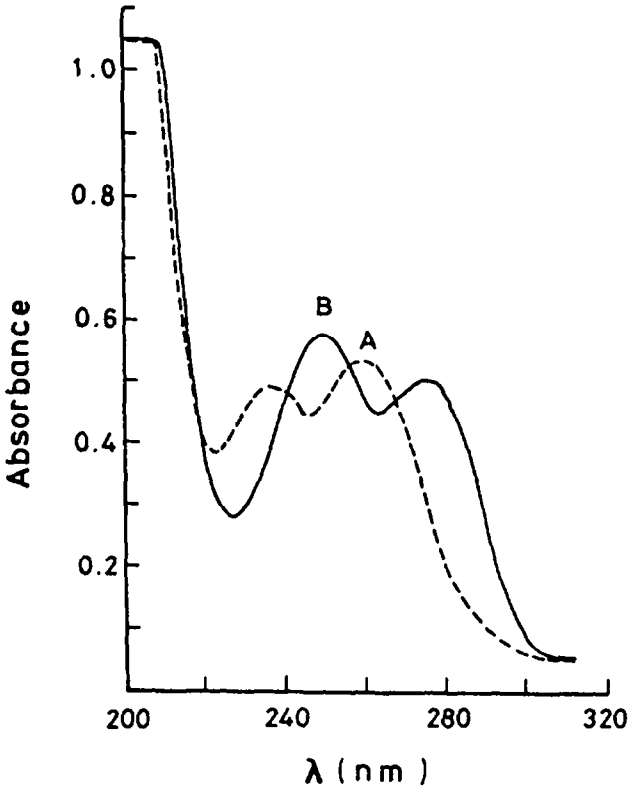


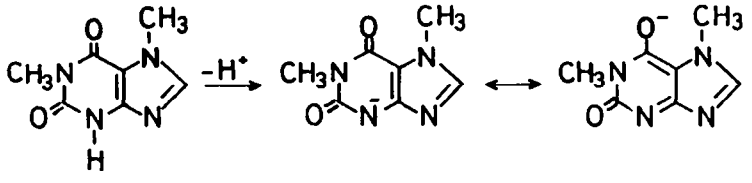
Fig. 4. UV spectra of 1,9-dimethylxanthine IV. A, in methanol; B, in methanol, containing a slight excess of the KOH equivalent of IV.

4. NMR spectra

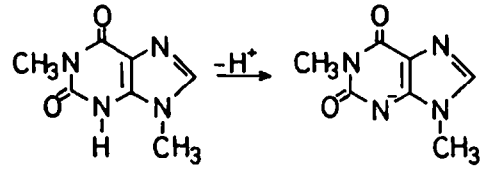
A. Cations

The chemical shifts in D_2O are recorded in Table 3. For interpretation of these data, we shall make use of the following physical properties: a) charge density; b) solvation; and c) ring current.

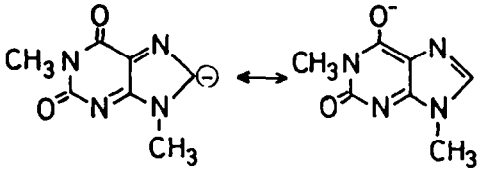
SCHEME 2



III IIIa IIIb



IV IVa



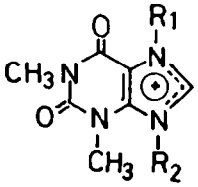
IVc IVb

TABLE 3 NMR spectra (in D_2O) of neutral and cationic forms of caffeine and isocaffeine

	1-methyl	3-methyl	7- or 9-methyl	8-H
Caffeine (I)				
Neutral form	3.32	3.50	3.95	7.88
Cation	3.38	3.57	4.06	8.31
Δδ	0.06	0.07	0.11	0.43
Isocaffeine (II)				
Neutral form	3.35	3.76	3.99	7.62
Cation	3.42	3.85	4.25	8.82
Δδ	0.07	0.09	0.26	1.20

Quoted from the Ph.D. thesis of D. Lichtenberg, Jerusalem, 1972

SCHEME 3



Cation of

Caffeine : $R_1 = Me$; $R_2 = H$

Isocaffeine: $R_1 = H$; $R_2 = Me$

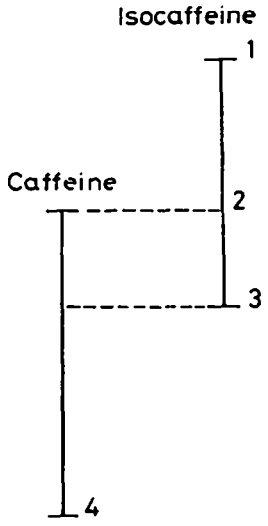


Fig. 5. Diagram comparing the solvation capacities of caffeine and isocaffeine in water. Between level 1 and 2, the δ_{8H} value of isocaffeine corresponds to 0.195 ppm; the difference between level 2 and 3 represents 0.125 ppm and between level 3 and 4, 0.265 ppm (see Table 5).

Charge density - The value of the 8-H signal between cationic and neutral forms is nearly three times larger for isocaffeine than for caffeine (Table 3). Furthermore, the downfield shift of δ_{9Me} in cpd. II is more than double the displacement of δ_{7Me} in caffeine. When a proton is attached to isocaffeine, the positive charge abolishes spreading of the N-3 electrons into the imidazole ring (see structures IIb and d in scheme 1).

Solvation - When a proton is attached to nitrogen bearing a methyl substituent, the methyl signal should shift downfield by 0.5-1.0 ppm.[11] Table 3 indicates that in the cations of I and II, the paramagnetic shift of the Me signals is one third to one quarter or less of these values. We propose that solvation plays an important role in determining the δ-values of the cations. As shown in Fig. 5, hydration of II is much smaller than that of I (see below). Therefore, a proton can approach the molecule of isocaffeine much closer and thus is more effective in charge fixation, i.e. in the enhancement of charge density. In contrast, the caffeine molecule is protected by a larger layer of water and the increase of charge density in the cation of I is much weaker.

Ring current - Ring current may also play a role in the chemical shifts of the components of the aromatic imidazole ring. In the cations of I and II, the ring current is weakened, thus producing a diamagnetic shift. This may explain in part the unexpectedly small displacement of the 8-H and methyl signals to lower field.

TABLE 4 NMR spectra in solvents of different polarity
Concentration: 5 mg/ml; 25°

Solvent	Caffeine				Isocaffeine			
	1-Me	3-Me	7-Me	8-H	1-Me	3-Me	9-Me	8-H
$CDCl_3$	3.41	3.60	4.00	7.51	3.44	3.78	3.96	7.32
CD_3OD	3.36	3.52	3.97	7.87	3.36	3.78	4.00	7.62
D_2O	3.32	3.49	3.94	7.90	3.34	3.78	4.00	7.64

B. NMR spectra in 3 solvents

The data in Table 4 reflect the important role of solvent polarity. Upon passage from a non-polar to a polar solvent, the aromatic 8-H signal both in I and II suffers a marked paramagnetic shift. Simultaneously, δ_{1Me} undergoes a small diamagnetic displacement of similar magnitude in either of the two isomers.

The solvent-dependent shift of the 7-Me band in caffeine has been discussed in a previous paper.[12] Here we shall concern ourselves with the 3- and 9-Me signals in isocaffeine. When a 3-methyl group is added to a 9-Me-xanthine, a positive charge is placed both on N-3 and N-9, causing the methyl bands to move to lower field (see scheme 1, II a-d).

The data in Table 5A clearly demonstrate the incorporation of the lone electron pair at N-3 into the molecular orbitals of the main isocaffeine chromophore. The paramagnetic shift of δ_{3Me} is accompanied by a similar diamagnetic displacement of δ_{8H}, both relative to caffeine. The latter effect is shared by all 9-methylxanthines,[1] independently of any possible steric interference. The opposite shift of δ_{3Me} and δ_{8H} in Table 5A appears in all three solvents, but the magnitude of the changes increases with solvent polarity. These observations shed light on the source of these nearly equal, but opposing displacements. Both originate from the soft character of the N-3, which is responsible for the high electron density at C-8 (scheme 1, IIb and d). The nearly equal absolute size of the shifts presented in Table 5A for the 3-Me and 8-H signals, together with the unique UV spectrum of isocaffeine, cannot be readily explained by the alternative hypothesis of steric interference.[1] This conclusion is also supported by the above interpretation of the low pK_a of IV.

When passing from chloroform to water, similar small differences are found for the 1-Me signals in both isomers (Table 5B, column 3). The values in this column imply that the 1-Me and 2-CO groups are very little involved in the main chromophore of these two xanthines. In caffeine the 3-Me signal behaves very similar to δ_{1Me} (Table 5B). In contrast, $\Delta\delta_{3Me}$ of isocaffeine is only 1/13 of the corresponding value in caffeine. This demonstrates again the different character of N-3 in the two isomers.

The similar shift of δ_{1Me} in cpds. I and II, when passing from chloroform to water (Table 5B, solvent pair 1) indicates a similar degree of association for both cpds. in water. For the pair chloroform-methanol (pair 2 in Table 5B), the shift of δ_{1Me} in isocaffeine is not much different from the displacement in the solvent pair 1, reflecting a high degree of association also in methanol. In contrast, the δ-value for 1Me in caffeine is nearly one half for solvent pair 2, relative to solvent pair 1. Thus caffeine shows reduced association in methanol. Evidently the degree of association of I is determined by the hydrophobic effect and by water bridges,[12] while in II polarisability is the dominant factor for association. Polarisability thus plays a decisive role in bringing the molecules of isocaffeine into close contact with each other. This is also reflected in the high m.p. of II and its very small R_f values.

The different factors, responsible for association of caffeine or isocaffeine molecules, are illustrated in the diagram of Fig. 5. Taking the δ_{8H} signal of isocaffeine in chloroform as base line (= level 1 in the diagram), we may in a theoretical experiment perform stepwise hydration until level 2 is reached, when the 8-H band has shifted downfield by 0.195 ppm, corresponding to the level of caffeine in chloroform. The total change of δ_{8H} in isocaffeine for solvent pair 1 (i.e. 0.195 + 0.125 = 0.320; see Table 5B), is achieved by attaching the maximal possible number of water molecules (= level 3). This value however corresponds to only 1/3 of the saturation level of caffeine in aqueous solution (=0.390; level 4). Thus a correlation exists between the $n-\pi$ conjugation liabilities of the two isomers and their hydration capacities: the higher the former, the less is the maximal extent of hydration (see Fig. 5).

TABLE 5

A. $\Delta\delta$ (isocaffeine-caffeine); values in $CDCl_3$, CD_3OD and D_2O

Proton	$CDCl_3$	CD_3OD	D_2O
N-1 - CH_3	+0.030	-0.004	-0.022
N-3 - CH_3	+0.183	+0.258	+0.285
C-8 - H	-0.195	-0.254	-0.265

B. $\Delta\delta$ values between pairs of solvents

Compound	Proton	($CDCl_3$-D_2O)	($CDCl_3$-CD_3OD)	(CD_3OD-D_2O)
		1	2	3
Caffeine	1-CH_3	+0.090	+0.050	+0.040
	3-CH_3	+0.110	+0.080	+0.030
	8-H	-0.390	-0.360	-0.030
Isocaffeine	1-CH_3	+0.097	+0.083	+0.014
	3-CH_3	+0.008	+0.008	0
	8-H	-0.320	-0.301	-0.019

The proposal of different degrees of hydration finds further support in the following observation: At a temperature of 50°, the NMR spectrum of caffeine reveals marked splitting of the signals, especially of the methyl bands (Fig. 6), while for isocaffeine splitting is only very slightly indicated (Fig. 7). Since water bridges are more important for the association of caffeine molecules, thermal breaking of these bridges gives a pronounced effect. Splitting is much less manifest for isocaffeine, because of its lesser hydration.

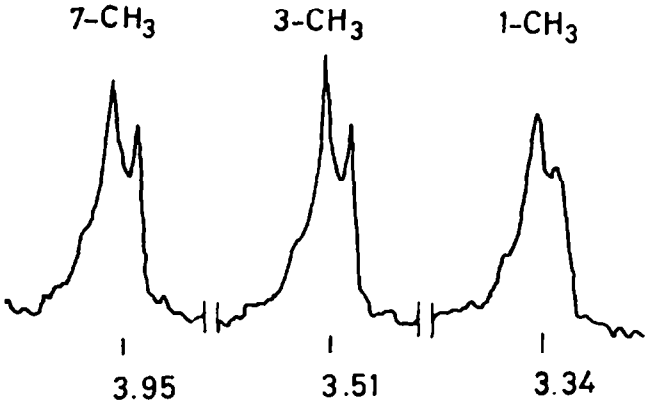


Fig. 6. Expanded NMR spectra of caffeine, 5 μg/ml in D_2O. Numbers indicate position of signals (in ppm). Note marked splitting of the three methyl bands.

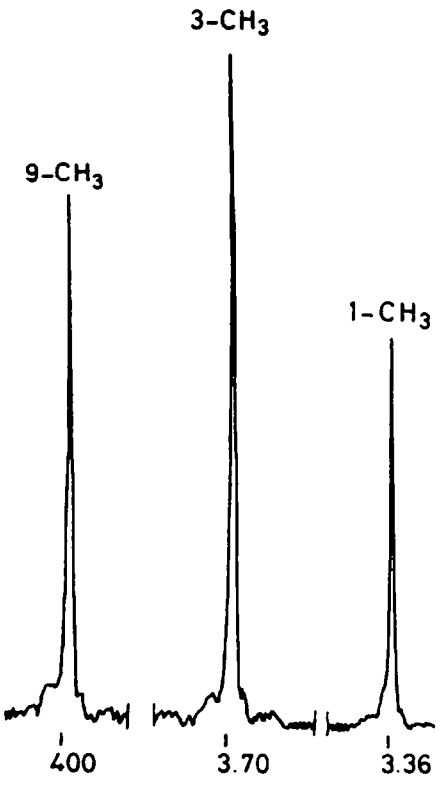


Fig. 7. Expanded NMR spectra of isocaffeine. 5 µg/ml in D_2O. Note the lack of measurable splitting of the methyl signals.

Discussion

The data of this study shed light on the main factors responsible for the differences in the electronic structure and in the association tendency of caffeine and isocaffeine.

1) The reduced affinity of isocaffeine to water originates from the "soft" character of the electrons of the nitrogen at position 3.

2) Incorporation of the lone electron pair of N-3 into the main chromophore is responsible for the high polarisability of isocaffeine and other 9-substituted xanthines.

3) In caffeine, association is mainly dependent on the hydrophobic effect and on the role of water bridges in the molecular stacks. In isocaffeine the polarisability of the molecule plays a decisive role in the process of aggregation.

4) The paramagnetic shift of the methyl signals in 3,9-disubstituted xanthines results from the strong contribution of the polarised forms, placing a negative charge at the 6-carbonyl and leaving both 3- and 9-N positively charged (see scheme 1).

On this basis we may now explain the observations of Pfleiderer and Nübel.[6] In xanthine and its 1-methyl derivative, the 7-NH tautomer is present in aqueous solution, presenting its extended conjugated system. Ionisation of the 3-NH group opens up two possibilities: a) Fixation of the negative charge at N-3 would make this a hard nitrogen and would leave the caffeine-like conjugated system untouched; b) Isomerization to the 9-NH form makes N-3 soft and thus permits dispersal of the negative charge over the whole chromophore.

Similar considerations exclude the possibility that in xanthine and its 1-methyl derivative, 7-NH could be the first ionising group. The UV spectra give evidence that dissociation of the

7-NH group, e.g. in 3-methylxanthine or in theophylline, produces a much smaller bathochromic shift ($\Delta\lambda_{max}$=3nm) than does ionisation of the 3-NH group in 7-methyl- or in 1,7-dimethylxanthine respectively.[1] In the latter two compounds, anion formation leads to bathochromic shifts of about 20nm. Ionisation of 7-NH clearly shows that the 7-nitrogen is hard, as compared to 3-N.

Protons of peri-methyl groups, attached either to carbon or nitrogen, undergo pronounced deshielding, a phenomenon that has been attributed to proximity effects.[13,14] However, the paramagnetic shifts of the 3- and 9-Me signals of isocaffeine in three solvents are accompanied by additional spectral characteristics:

1) The appearance of a second long-wave peak in the UV spectrum in all solvents.

2) Deshielding of the peri-methyl groups is accompanied by shielding of the 8-H signal of almost equal magnitude.

3) The 8-H band of isocaffeine resonates at higher field than the 8-H signal of caffeine, a property shared by all 9-methylated xanthines. Evidently all these three phenomena and the low pK_a values of 3-NH in 9-substituted xanthines cannot be rationalised only on the assumption of proximity effects. The soft character of the 3-nitrogen in 9-substituted xanthines affords a better explanation, since it accounts for all the various phenomena observed.

Experimental

UV spectra were measured on a Uvicon 810 spectrophotometer. NMR spectra were obtained with a 300 MHz NMR spectrometer (Bruker W.H. 300), with an automatic recorder. 2,2,3,3-Tetradeutero-3-(trimethylsilyl) propionic acid sodium salt (=TSP, Merck A.G., Darmstadt, West Germany) in deuterium oxide and tetramethylsilane in deuterochloroform or deuteromethanol served as internal standards. Thin layer chromatography was carried out on alumina with chloroform as solvent.

Anhydrous caffeine was B.P. standard; isocaffeine, 1,7- and 1,9-dimethylxanthine were prepared according to ref. 1. All solvents were of analytical grade.

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References

1. D. Lichtenberg, F. Bergmann and Z. Neiman, J. Chem. Soc. 1676 (1971)
2. D. Lichtenberg, F. Bergmann and Z. Neiman, J. Chem. Soc. 1676 (1972)
3. F. Bergmann, M. Rahat and D. Lichtenberg, J. Chem. Soc. Perkin I, 1225 (1973)
4. M. Rahat, F. Bergmann and I. Tamir, J. Chem. Soc. Perkin I, 2229 (1974)
5. F. Bergmann, I. Tamir, A. Frank and W. Pfleiderer, J. Chem. Soc. Perkin II, 35 (1979)
6. W. Pfleiderer and G. Nubel, Annalen 647, 155 (1961)
7. B. Pullman, E.D. Bergmann, H. Weiler-Feilchenfeld and Z. Neiman, Intern. J. Quantum Chem. III S, 103 (1969)
8. S.C. Schulman, Chapter 6 in Acid-base chemistry of excited singlet states. "Modern Fluorescence Spectroscopy", p. 239-275, E.L. Wehry, Editor; Plenum Press, New York (1976)
9. I. Fleming, Frontier Orbitals and Organic Chemical Reactions, John Wiley and Sons (1976)
10. The Merck Index, 9. Edition p. 5, No. 32 (1976)
11. L.M. Jackman and S. Sternhell, Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, 2nd Edition p. 180, Pergamon Press, Oxford (1969)
12. Y. Yanuka, J. Zahalka and M. Donbrow, J. Chem. Soc. Perkin Trans. II, 911 (July 1986)
13. F. Gerson, B. Weidman and E. Heilbronner, Helv. Chim. Acta 47, 1951 (1964)
14. C. MacLean and E.L. Mackor, Mol. Phys. 3, 223 (1960)

Footnotes

* Absorption of chloroform near 200nm is so strong that after setting solvent extinction to zero, the spectrum of the solute cannot be measured in this region.

** See above remark on measurements in chloroform. A similar behaviour is observed for methanolic solutions (spectra not shown).