^{13}C-Nuclear Magnetic Resonance Investigations of Xanthine and Some of its N-Methylated Derivatives

Claude Nicolau and Knut Hildenbrand

Institut für Strahlenchemie im Max-Planck-Institut für Kohlenforschung, Mülheim a. d. Ruhr

(Z. Naturforsch. **29 c**, 475–478 [1974] ; received May 22/June 18, 1974)

^{13}C-NMR, Xanthines, N-Methylation Shifts, Protonation Shifts

The ^{13}C-Nuclear Magnetic Resonance spectra of xanthine, 1,3-dimethyl-xanthine (theophylline), 3,7-dimethyl xanthine (theobromine) and 1,3,7-trimethylxanthine (caffeine) were obtained and the lines assigned. Protonation- and N-Methylation parameters are derived by comparison of the ^{13}C-chemical shifts of the protonated cations with those of the neutral molecules and also with those of the xanthine cation. The shifts are discussed in terms of variations in the shielding at the different C-atoms induced by N-methylation and protonation. Approximate correlations are found between the ^{13}C-chemical shifts and the π-electron densities at the C-atoms.

Introduction

Of the eleven possible N-methyl-xanthines seven have been identified in living matter [1]. Recently there has been increased interest in some of these compounds as they show interesting biological properties. Extensive ^{13}C-NMR studies of purines and of their monomethylated derivates permit the prediction of protonation parameters when protons are bonded to nitrogen lone-pairs electrons [2–4]. It further emerged that N-methylation shifts in purines are comparable to protonation shifts in azines [2].

We wish to report here the ^{13}C-NMR investigations of xanthine, theobromine, theophylline and caffeine, and of their protonated cations.

Material and Methods

Sample preparations

Theobromine (3,7-dimethyl xanthine), theophylline (1,3-dimethyl xanthine) and caffeine (1,3,7-trimethyl xanthine) were reagent grade (Merck) and used without further purifications. The protonated species were prepared by simple acid-base reactions. We examined only the protonated cations and the neutral molecules of these compounds. All measurements were carried out at 50 °C, using saturated solutions in D_2O. The sodium salt of 3-trimethylsilylpropane-1-sulfonic acid (TMSS) was used as internal standard in the neutral solutions. The protonated species were compared with TMS as external standard.

Requests for reprints should be sent to Prof. Dr. Claude Nicolau, Institut für Strahlenchemie im Max-Planck-Institut für Kohlenforschung, *D-4330 Mülheim a. d. Ruhr*, Stiftstrasse 34–36, Germany.

Equipment

The proton-coupled and decoupled ^{13}C-NMR spectra were obtained with a Bruker WH 90 spectrometer operating in the FT Mode at 22, 63 MHz, equipped with a Nicolet B-NC 12 computer with 8K data memory.

Spectroscopic details

The chemical shifts of the neutral molecules were measured against TMSS and calculated against TMS and benzene by using the following values: $\delta_{TMSS} = 0$ ppm; $\delta_{TMS} = 0$ ppm; $\delta_{C_6\dot{H}_6} = 128.5$ ppm.

Results

The chemical shifts obtained are listed in Table I for all compounds. The data are presented relative to TMS and to benzene (positive values are downfield from benzene).

Fig. 1 shows the ^{13}C-NMR spectra, proton-coupled (a) and proton-decoupled (b) of the xanthine protonated cation.

In the case of theophylline and caffeine solutions it was possible to obtain the ^{13}C-NMR spectra of both neutral molecule and protonated cations. This permitted the measurement of the protonation shifts in the two N-methylated species.

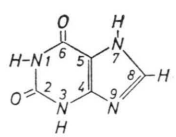
The methylation shifts in the protonated cations were measured relative to those of the xanthine cation. The results are listed in Table III.

Table I. ^{13}Carbon chemical shifts of xanthine and its N-methylderivates.

Compound	Position	^{13}C [ppm] [a]	^{13}C [ppm] [b]
Xanthine cation	2	156.4	27.9
	4	140.6	12.1
	5	108.9	−19.6
	6	152.3	23.2
	8	137.4	8.9
Theobromine cation	CH_3	32.8	−95.7
	CH_3	37.4	−90.7
	2	156.0	27.5
	4	143.5	15.0
	5	110.0	−18.5
	6	152.3	23.8
	8	138.9	10.4
Theophylline cation	CH_3	30.4	−98.1
	CH_3	33.4	−95.1
	2	157.0	28.5
	4	143.1	14.6
	5	109.2	−19.3
	6	153.7	25.2
	8	138.9	10.4
Theophylline	CH_3	30.8	−97.7
	CH_3	32.8	−95.7
	2	158.7	30.2
	4	150.7	22.2
	5	110.1	−18.4
	6	155.2	26.7
	8	144.0	15.5
Caffeine cation	CH_3	29.9	−98.6
	CH_3	32.0	−96.5
	CH_3	35.7	−92.8
	2	157.5	29.0
	4	148.6	20.1
	5	109.3	−19.2
	6	154.7	26.2
	8	144.3	15.8
Caffeine	CH_3	30.4	−98.1
	CH_3	32.3	−96.2
	CH_3	35.8	−92.7
	2	158.5	30.0
	4	151.3	22.8
	5	110.3	−18.2
	6	155.0	26.5
	8	146.5	18.0

[a] Taken with respect to internal TMSS and in the case of the cations to external TMS.

[b] Taken with respect to benzene ($\delta_{C_6H_6}$ = 128.5 ppm, negative sign means upfield from benzene).



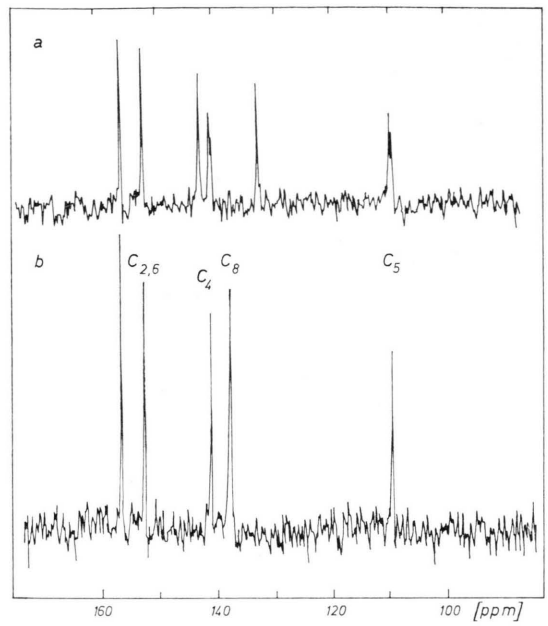


Fig. 1. ^{13}C-NMR spectra of the xanthine-cation; saturated D_2O solution; pH ~ 1.0. a. Proton-coupled; b. proton-decoupled. 6000 accumulations; pulse angle 70°; pulse rate 5 seconds; τ-scale is taken with respect to τ_{TMSS} = 0 ppm.

Table II. ^{13}C-Protonation shifts of theophylline and caffeine.

Compound	Position	$\Delta\delta$ ^{13}C [a]
Theophylline	2	−1.7
	4	−7.6
	5	−0.9
	6	−1.5
	8	−5.1
Caffeine	2	−1.0
	4	−2.7
	5	−1.0
	6	−0.3
	8	−2.2

[a] $\Delta\delta = \delta\,^{13}C_{cation} - \delta\,^{13}C_{parent}$.

Table III. ^{13}C-methylation shifts in the protonated N-methylated xanthines.

Position	$\Delta\delta$ ^{13}C [ppm] [a] Theobromine$^+$	Theophylline$^+$	Caffeine$^+$
2	−0.4	+0.6	+1.1
4	+2.9	+2.5	+8.0
5	+1.1	+0.3	+0.4
6	0.0	+1.4	+2.4
8	+1.5	+1.5	+6.9

[a] Taken with respect to the xanthine cation.

Discussion

Assignment of the spectra

The peaks in the spectra were assigned by comparison with the data of Pugmire and Grant [3] and by comparing the proton-coupled and decoupled spectra. The methyl groups in the methylated xanthines are easily recognized as they appear between 29.9 and 37.4 ppm downfield from TMSS for the neutral molecules and the protonated cations in all derivatives.

In all derivatives, spin coupled spectra allowed unambiguous assignment of the C (8) resonance due to coupling to the proton. It appears at 137.4 ppm in the protonated xanthine cation and in theophylline and theobromine it is shifted downfield to 138.9 ppm. In the caffeine cation the shift is significantly larger, 144.3 ppm. C(4) and C(5) in the protonated xanthine cation could be assigned due to their coupling over 3 bonds (of $\sim$7 cps) with the proton C(8) – H (see Fig. 1 a). The C(4) resonance occurs in the xanthine cation at 140.6 ppm, 17.5 ppm upfield from the purine cation. Introduction of the two carbonyls at positions 2 and 6 causes a strong shielding at this bridgehead carbon. As compared to purine, the C(5) resonance shows a very strong upfield shift in all the species we investigated. It correlates well with the high π-electron density on this atom. No coupling of C with protons on *N* was observed in xanthine cation suggesting as a cause that either the equilibration of the different tautomers or the establishment of the protonation equilibrium are very rapid on the NMR-time-scale.

Protonation shifts

Protonation shifts could only be given for theophylline and caffeine, since spectra of both neutral molecules and protonated cations could be measured only for these two compounds. The shift of the ^{13}C-resonance peaks of these two protonated cations show an upfield tendency as compared to the resonances of the parent molecules. The upfield shift in theophylline at C(8) is higher than in caffeine (-5.1 ppm as compared to -2.2 ppm) and this could be evidence for the tautomeric averaging leading to equivalence of the N(7) and N(9) as protonation sites in the theophylline cation. In caffeine protonation may occur only at N(9). The C(4) is strongly influenced by protonation at N(9) or at N(7). In theophylline the protonation shift is -7.6 ppm and in caffeine -2.7 ppm. Upon protonation there is a strong decrease of the negative charge at N(7) and/or N(9) which should induce considerable π-electron density shifts in the cation, predominantly at the C(8) and C(4) atoms. The neighbouring CH_3 groups substituted at N(3) and at N(7) in caffeine, with their electron withdrawing effect should increase the deshielding of C(4) and C(8) in caffeine, which we actually observed. The C(5) atom is slightly or not at all affected by protonation in caffeine or theophylline; this might be due to the neighbouring carbonyl group.

Methylation shifts

The methylation shifts in the methylated xanthine cations taken with respect to the xanthine cation are opposite to the protonation shifts. In each N-methylated derivatives we observe strong shifts at C(4) and at C(8). The greatest methylation shifts are observed in caffeine. At C(4) we measured $+8.0$ ppm and at C(8) $+6.9$ ppm. In theobromine and theophylline we measured at C(4) $+2.9$ ppm and 2.5 ppm and at C(8) $+1.5$ ppm. The C(8) methylation shift is almost the same for these compounds and quite different from caffeine. In the case of caffeine, where three out of four nitrogen atoms are methylated, the protonation site is N(9). This becomes the predominant factor and thus we can account for the strong deshielding at C(8) and C(4). A strong shift is observed at C(4) upon methylation in this compound indicating considerable deshielding in the cation (taken with respect to the xanthine cation) due to the presence of the methyl groups at N(1), N(3) and N(7).

In theophylline and theobromine only two nitrogen atoms are methylated (1 and 3, 3 and 7 respectively) which induces decreased deshielding at C(4) and C(8) as compared to caffeine. The availability for protonation of N(7) and N(9) in theophylline and of N(1) and N(9) in theobromine further decreases the deshielding and thus we can account for the lower methylation shifts in these compounds as compared to caffeine.

Claude Nicolau gratefully acknowledges the hospitality granted to him in the Institut für Strahlenchemie im Max-Planck-Institut für Kohlenforschung by Prof. Dr. D. Schulte-Frohlinde. We thank Dr. G. Olbrich for the π-electron density-calculations in the xanthine-derivatives.

[1] J. H. Lister, Heterocyclic compounds — Fused Pyrimidines, D. J. Brown, ed., **Vol. II**, p. 253, Wiley-Interscience, New York, London 1971.
[2] R. J. Pugmire and D. M. Grant, J. Amer. Chem. Soc. **90**, 4232 [1968].
[3] R. J. Pugmire and D. M. Grant, J. Amer. Chem. Soc. **93**, 1880 [1971].
[4] R. J. Pugmire, D. M. Grant, L. B. Townsend, and R. K. Robins, J. Amer. Chem. Soc. **95**, 2791 [1973].