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PURINE N-OXIDES—XXXIX
N-ACETOXY DERIVATIVES OF N-HYDROXYXANTHINES‡

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Abstract—N-Acetoxyderivatives of 3-hydroxyxanthine and several of its methyl derivatives of 1-hydroxyxanthine, and of 8-aza-3-hydroxyxanthine are described. The UV spectra of the 7 or 9-acetyl derivatives of 1- and 3-N-acetoxyxanthines indicate the presence of intramolecular H-bonding which influences the tautomeric structures.

PRELIMINARY COMMUNICATIONS have reported[1] that 3-acetoxyxanthine (**2a**) can undergo an apparent rearrangement in water to yield uric acid (**3a**) and, with other nucleophiles, substitution at C-8 to yield 8-substituted xanthines. A similarly reactive derivative of 3-hydroxyxanthine (**1a**) must be formed metabolically *in vivo* since rats convert **1a**, in part, to 8-chloro- and 8-methylmercaptoxanthine,[2] products which are also formed *in vitro* from **2a** by reactions with chloride ion or methionine.[1] These chemical reactivities may be implicated[2] in the induction of tumors[3] by 3-hydroxyxanthine. Several N-acetoxyxanthines have now been synthesized to permit studies of the details of their structures and of their chemical reactivities in the complex 8-substitution reaction.

The assignment of the 3-acetoxyxanthine structure (**2a**) is supported by the following facts. The compound shows a delayed $FeCl_3$ test through partial hydrolysis to **1a**. Its IR peak at 1.820 cm^{-1} indicates an N–O–COMe rather than an N–COMe structure,[4] and the NMR spectrum is consistent with that structure, as is the UV spectrum as interpreted below.

By mild acetylation procedures. 3-acetoxy-7-methylxanthine (**2b**), 3-acetoxy-1-methylxanthine (**2c**) and 3-acetoxy-1-benzylxanthine (**2d**) were prepared from **1b**, **c** and **d**. In hot Ac_2O **2b** and **c**, as well as **1b** and **c**, yield the corresponding uric acids **3b** and **3c**. No 3-acetoxy-9-methylxanthine (**5**) could be isolated after acetylations of 3-hydroxy-9-methylxanthine (**4**); only unchanged starting material and 9-methyluric acid (**6**) were obtained. 7,9-Dimethyl-3-hydroxyxanthine can similarly be rearranged to 7,9-dimethyluric acid[6] but the 3-acetoxy intermediate has not been isolated. Steric hindrance from the 9-Me group must impede acetylation at the 3-position, although once **5** has formed the same steric factors enhance cleavage of the N—O bond and lead to a rapid conversion to 9-methyluric acid.

The acetylation of an isomeric N-hydroxyxanthine, 1-hydroxyxanthine,[6] afforded a mixture of two diacetyl derivatives. For each of these an IR peak at 1,820 cm^{-1}, an

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TABLE 1. CHEMICAL SHIFTS (τ) OF GROUPS AT THE POSITIONS INDICATED

Compound	1	3	7 or 9	8	2	6
2a	−1·60	7·55	−3·8	1·90	—	—
2b	−1·56	7·58	6·11	1·99	—	—
2c	6·61	7·57	−3·7	1·89		—
2d	4·93[a]					
	2·72[b]	7·59	−3·9	1·88	—	—
8	7·63	−2·7	7·22	1·30	—	—
9	7·64	—[c]	7·30	1·57	—	—
12	−1·80	7·53	7·13	1·30	—	—
13	−1·80	7·51	7·19	—	—	—
10	7·62	−2·9[d]	−2·9[d]	1·90	—	—
14	−1·70	7·57	−3·7	—	—	—
purine	—	—	—[e]	1·25	0·98[9]	0·77[9]
7-acetyl-purine	—	—	7·17	0·64[f]	0·90	0·57[f]
9-acetyl-purine	—	—	7·03	0·96	0·90	0·73
6-methyl-purine	—	—	—[e]	1·40	1·17	7·28
9-acetyl-6-methyl-purine	—	—	7·04	1·08	1·08	7·25

[a] CH_2 of Bz group; [b] Ph of Bz group; [c] not observed;
[d] coalesced; [e] not determined; [f] assignment not definite.

NMR peak at $\tau \sim 7{\cdot}6$ and a delayed $FeCl_3$ test, indicates the presence of an N–O–COMe moiety.[4] The second acetylation must have taken place in either of the possible positions in the imidazole ring[7,8] to give **8** and **9**. Either isomer could be methanolyzed to 1-acetoxyxanthine (**10**).

Assignment of specific structures to these and other 7-or 9-acetyl derivatives was possible through comparisons of NMR data with that from model compounds, The acetylation of purine gives two isomers, 7- and 9-acetyl purine, 6-Methylpurine, presumably because of steric hindrance, yields only one isomer, 9-acetyl-6-methyl-purine.[8] The NMR spectra of these three compounds in Me_2SO-d_6 indicate that a 7-acetyl groups shifts the 8-H signal τ 0·6 downfield and a 9-acetyl group moves it down by τ 0·3 (Table 1).[9]

One of the isomeric 1-acetoxy-acetylxanthines has its 8-H shifted τ 0·6 downfield from that of **10**, and is the 7-acetyl-, (**8**); the other has a downfield shift of τ 0·3 and is the 9-acetyl-, (**9**).

TABLE 2. COMPARISONS OF APPARENT pK_a'S OF ACETOXYXANTHINES AND XANTHINES

Acetoxyxanthine		Xanthine[11]	
10	5·2 ± 0·1	1-Methyl-	7·70 ± 0·1
2b	7·7 ± 0·1	3.7-Dimethyl-	10·00 ± 0·05
2a	6·8 ± 0·5[a]	3-Methyl-	8·45 ± 0·03
2c	6·5 ± 0·5[a]	1.3-Dimethyl	8·68 ± 0·04

[a] Because of the extremely rapid rate of decomposition. $t_{\frac{1}{2}} = <20$ secs at pH 7·0. the pK was estimated to be 1·5 units higher than the pH at which the absorption. extrapolated to time zero. started to change. The slower changes in absorption of compounds **10** and **2b** were also extrapolated to $t = 0$.

TABLE 3. COMPARISON OF THE UV SPECTRA OF ACETYLATED XANTHINES AND METHYLXANTHINES[a]

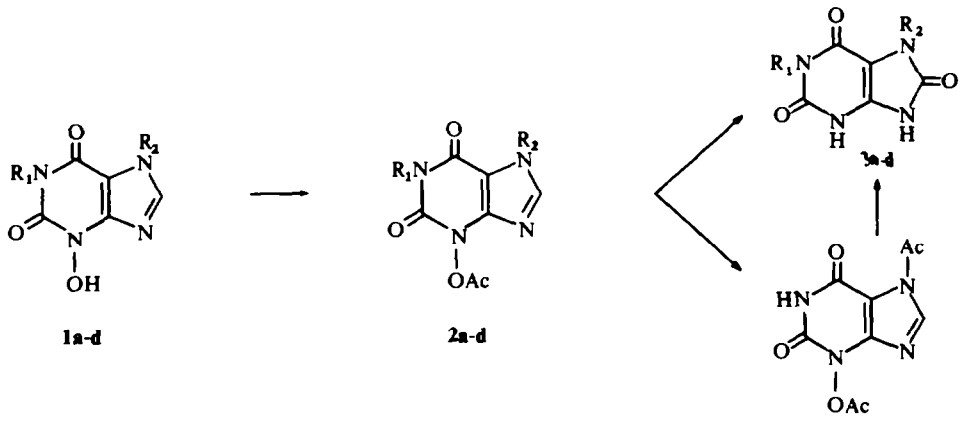
Compound	Neutral molecule	Monoanion
1-Methylxanthine	267	242. 276
1-Acetoxyxanthine. **10**	267·5	241. 279
3-Methylxanthine	270	274
3-Acetoxyxanthine. **2a**	267·5	dec.
1.3-Dimethylxanthine	270	274
3-Acetoxy-1-methylxanthine. **2c**	268	dec.
1.7-Dimethylxanthine	268	233. 288
1-Acetoxy-7-acetylxanthine. **8**	237. 283[b]	—
1.9-Dimethylxanthine	238. 263	248. 276
1-Acetoxy-9-acetylxanthine. **9**	250. 273[b]	—
3.7-Dimethylxanthine	271	234. 273
	276[c]	244. 282[d]
3-Acetoxy-7-methylxanthine. **2b**	269	237. 274[e]
3-Acetoxy-7-acetylxanthine. **12**	237. 290[e]	dec.

[a] Values for the methylxanthines in water are from Pfleiderer and Nübel.[11] In several examples the UV spectra of N-Me. N-COMe and N-OCOMe xanthines are quite similar. [b] In EtOH. [c] In dioxane. since 12. and also **2a**. react in a complex manner with alcohols. [d] In dioxane with tetrabutylammonium hydroxide. [e] Decomposes slowly.

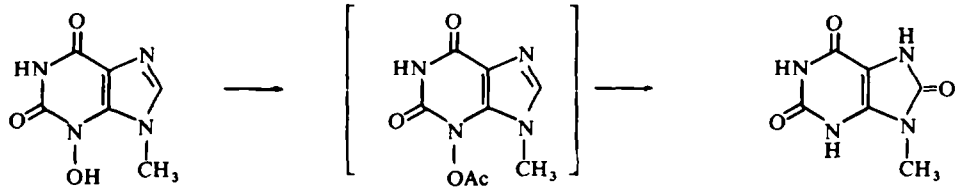
3-Hydroxyxanthine (**1a**) and 3-hydroxy-8-azaxanthine (**11**) also form diacetyl derivatives, **12** and **13**. respectively. Steric factors would suggest that N-acetylation of **2a** should take place at the 7 position. This is confirmed by the observed shift of the 8-H signal of **12** downfield by τ 0·6 relative to **2a**, NMR data on **13** indicate that the triazole proton was replaced by an acetyl, and methanolysis of **13** yields 3-acetoxy-8-azaxanthine (**14**). The positioning of the acetyl in formula **13** is based upon analogy with structure **12** and the NMR value of 7·19 for the N-acetyl (Table 1). Those acetyls assigned to the 7 position all have consistent Me τ values of 7·18 $\pm$ 0·05. while the single example of the Me of a 9-acetyl group is at τ 7·30 (Table 1). The N–O–COMe Me protons appear at slightly higher τ values, 7·51 to 7·64. The assignment of the N-Me protons is in agreement with those observed for the corresponding N-methylated xanthines,[10] but the exchangeable protons appear at lower fields than those for the corresponding methylated xanthines which range as follows: 1–NH τ −0·67 to −1·07; 3–NH τ −1·4 to −1·5; 7,9–HN τ −2·9 to −3·6. The electron-withdrawing effects of the N–O–COMe are also manifested. by a decrease of about 2 pH units in the *pK*'s of the first ionizations of **2a**, **b** and **c**, and of **10** relative to those of the methyl xanthines (Table 2). They are similar to the decreases previously noted[12] in N-oxides or N-hydroxy derivatives of purines.

The UV spectra also support the structural assignments. From the similarity of the spectra of the neutral molecules of the first three pairs of compounds in Table 3, it is evident that the N–O–COMe group is spectrally equivalent to an N–Me in either the 1 or 3 positions. From the similarity of the spectra of the monoanions, the first ionizations occur at the same position, N-3,[13] in the first pair.

When an imidazole nitrogen is also acetylated, the spectrum of the neutral species of the diacetyl derivative differs from that of the neutral species of the respective dimethylxanthine. It does resemble that of the monoanion, as shown for the pairs



1a-d 2a-d 3a-d 12



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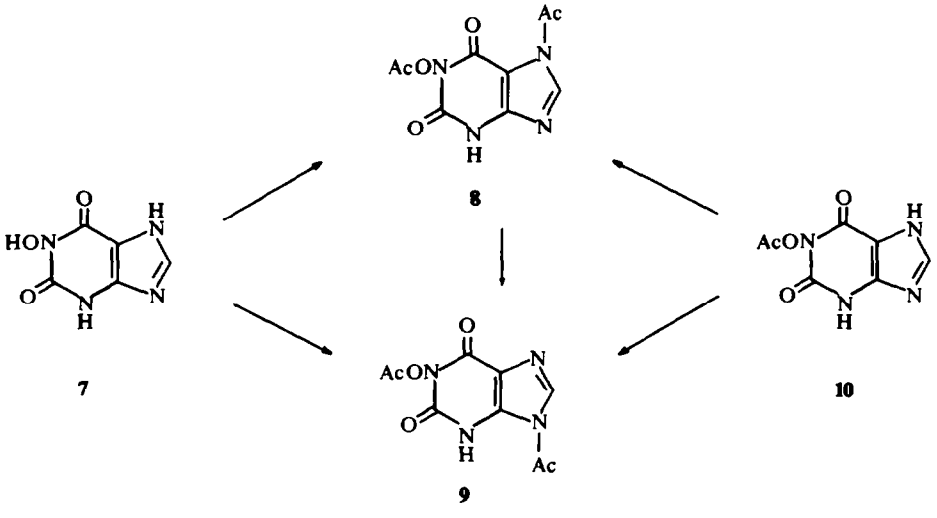
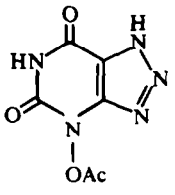


7 8 9 10

11

13



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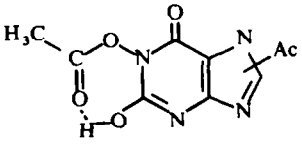

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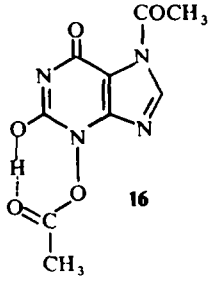

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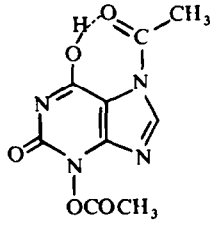

17

	R_1	R_2
a =	H	H
b =	H	Me
c =	Me	H
d =	Bz	H

of 1,7- and 1,9-derivatives in Table 3. The 1,7- and the 1,9-dimethylxanthines each ionize at N-3, and must exist largely as enolate anions with the negative charge localized on the 2-oxygen. The resemblance of the neutral species of **8** and **9** to those monoanions can be rationalized if **8** and **9** are stabilized in an enol form by H-bonding, as depicted in the partial formula **15**.

A 3-acetoxy derivative can also be influenced by acetylation of the imidazole ring. While the spectra of the neutral molecule and monoanion of 3-acetoxy-7-methylxanthine, **2b**, are both comparable to those of the corresponding species of 3,7-dimethyl-, that of the neutral molecule of the 3-acetoxy-7-acetyl (**12**) most closely resembles that of the monoanion of 3,7-dimethyl-, again a suggestion that the presence of the 7-acetyl- results in a stabilized enol form such as **16** or **17**.‡ Since the spectrum of the neutral molecule of **10** does correspond to that of the neutral molecule of 1-methylxanthine, it is obvious that **10** is not stabilized in an enol form, and thus that an electron withdrawing N-acetyl group on the imidazole ring together with an acetyl group suitably located for H-bonding is essential for the formation of the H-bond-stabilization enol structure.

‡ The possibility of ionization of **16** and **17** in ethanolic solution is eliminated by the observation that their UV spectrum changes very little upon addition of HCl.

EXPERIMENTAL

Analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich., and by Galbraith Laboratories, Inc., Knoxville, Tenn. on samples dried *in vacuo* over P_2O_5 at room temp. M.ps were obtained on a Meltemp apparatus and are uncorrected. The UV spectra were determined with Unicam SP800A and Beckman DU spectrophotometers, the IR data with a Perkin–Elmer Model 137B Infracord spectrophotometer (KBr pellet), and the NMR spectra at room temp with a Varian A60 spectrometer (Me_2SO-d_6). The pK_a values were determined spectrophotometrically with 0·01 M buffers.[14,15]

Acetylation of 3-hydroxyxanthine (**1a**). Finely ground 3-hydroxyxanthine‡ (20·0 g) was stirred in AcOH and Ac_2O (600 ml; 1:1) for 6 days at room temp in the dark. It slowly dissolved and new crystals separated. These were collected, washed with a little glacial AcOH and dried *in vacuo* over P_2O_5 and KOH, first at room temp and then at 80° to give 3-acetoxyxanthine (2a) (14·5 g, 58%). (Calc. for $C_7H_6N_4O_4$: C, 40·01; H, 2·87; N, 26·66. Found: C, 39·80; H, 2·87; N, 26·43%). The samples have a slight odor of AcOH. Assayed by immediate application in water to a 9 × 150 mm column of Dowex-50, 8-X (H^+), 200–400 mesh, and elution with 0·05 N HCl at 60 ml per hr, 3-acetoxyxanthine, 95% is eluted at 33 ml and 3-hydroxyxanthine, 5%, at 85 ml. The latter may well arise by hydrolysis prior to adsorption on the resin. After a year at room temp, samples yielded the same chromatographic results. 3-Acetoxy-7- acetylxanthine is eluted at 16 ml from this column.

Evaporation of the filtrate from the 3-acetoxyxanthine to 30 ml and cooling to 0° yielded crystals of 3-acetoxy-7-acetyl-xanthine (**13**) (3·5 g, 12%). It was dried at 80° over P_2O_5 *in vacuo*. (Calc. for $C_9H_8N_4O_5$: C, 42·86; H, 3·20; N, 22·22. Found: C, 42·62; H, 3·03; N, 22·25%).

3-Acetoxy-1-methyl-, 7-methyl-, and 1-benzylxanthines. 3-Hydroxy-1-methylxanthine[16] (50 mg), Ac_2O (0·5 ml) and AcOH (1·5 ml) were stirred at room temp for 7 days. The addition of ether (50 ml) to the solution gave a white precipitate of 3-acetoxy-1-methylxanthine (15 mg, 28%). (Calc. for $C_8H_8N_4O_4$: C, 42·86; H, 3·60; N, 24·99. Found: C, 42·60; H, 3·63; N, 24·84%). Similar acetylation of 50 mg of 3-hydroxy-7-methylxanthine[16] gave 3-acetoxy-7-methylxanthine (**2b**) (40 mg, 75%). (Calc. for $C_8H_8N_4O_4$: C, 42·86; H, 3·60; N, 24·99. Found: C, 42·87; H, 3·62; N, 24·90%).

1-Benzyl-3-hydroxyxanthine[16] (100 mg), Ac_2O (2 ml) and AcOH (1 ml), stirred 5 days and precipitated with ether (25 ml) gave 3-acetoxy-1-benzylxanthine (50 mg, 43%), m.p. 170 dec. (Calc. for $C_{14}N_{12}N_4O_4$: C, 56·00; H, 4·03; N, 18·66. Found: C, 56·27; H, 4·00; N, 18·79%).

Attempted preparation of 3-acetoxy-9-methylxanthine (**4**). (a) 3-Hydroxy-9-methylxanthine[16] (200 mg) was stirred for 5 days in AcOH (5 ml) and Ac_2O (2·5 ml). Filtration gave unchanged starting material (180 mg), and evaporation of filtrate gave a white solid (25 mg) that showed no 1,820 cm^{-1} peak in the IR, and proved, chromatographically, to be a mixture of starting material and 9-methyluric acid (**6**).

(b) 3-Hydroxy-9-methylxanthine (100 mg) was dissolved in hot AcOH (200 ml), cooled and Ac_2O (100 ml) added. After 18 hr at room temp, a slight precipitate of **4** was collected and the filtrate evaporated to dryness *in vacuo* at 40° to give 9-methyluric acid, **6**, (95 mg, 95%), which was identified by comparison of its IR spectrum and chromatographic properties with those of an authentic sample.[17]

Acetylation of 1-hydroxyxanthine (**7**). 1-Hydroxyxanthine[6] (1·00 g) was heated to 100° in Ac_2O and AcOH (37 ml 3:1) for 1·5 hr. The clear solution was evaporated to dryness to give a white solid which, as estimated from an NMR analysis, contained ~50% 1-acetoxy-7-acetylxanthine (**8**), 30% 1-acetoxy-9-acetylxanthine (**9**) and 20% 1-acetoxyxanthine (**10**). This solid, stirred with Et_2O (2 × 500 ml), gave, after filtration and evaporation, 1-acetoxy-7-acetylxanthine (**8**) (680 mg, 45%). (Calc. for $C_9H_8N_4O_5$: C, 42·86; H, 3·20; N, 22·22. Found: C, 43·11; H, 3·04; N, 22·68%). The residue after filtration was recrystallized from Ac_2O and AcOH 3:1 to yield 1-acetoxy-9-acetylxanthine (**9**) (500 mg, 33%). (Calc. for $C_9H_8N_4O_5$: C, 42·86; H, 3·20; N, 22·22. Found: C, 42·87; H, 2·94; N, 21·56%). Each of these diacetyl compounds was unstable at room temp; **8** could be converted to **9** by boiling for 10 min in Ac_2O and AcOH.

1-Acetoxyxanthine (**10**). After 1-acetoxy-9-acetylxanthine (**9**) (1·05 g) was stirred in boiling MeOH for 15 min, and cooled to 0°, crystals of 1-acetoxy-xanthine precipitated (710 mg, 81%). (Calc. for $C_7H_6N_4O_4$: C, 40·01; H, 2·88; N, 26·66. Found: C, 39·65; H, 2·78; N, 26·20%). Similar treatment of 1-acetoxy-7-acetylxanthine yields the same product.

Acetylation of 3-hydroxy-8-azaxanthine (**11**). 3-Hydroxy-8-azaxanthine monohydrate[18] (500 mg) in Ac_2O (5 ml) was stirred for 40 min at 40°. The clear solution was diluted with an equal volume of Et_2O

‡ The N-oxides of guanine and xanthine originally designated 7-N-oxides (T. J. Delia, G. B. Brown, *J. Org. Chem.* **31**, 178 (1966) were later proven to be 3-N-oxides (U. Wölcke, G. B. Brown, *Ibid.* **34**, 978 (1969)).

and cooled to 0° to yield 3-acetoxy-7-acetyl-8-azaxanthine (**13**) as colourless crystals (250 mg, 39%). This compound is unstable and a satisfactory analysis was not obtained, but the NMR, IR, and UV spectra of fresh samples were in accord with a diacetyl derivative.

3-Acetoxy-8-azaxanthine (**14**). 3-Acetoxy-7-acetyl-8-azaxanthine (**13**) (150 mg) was dissolved in MeOH (10 ml) at room temp. The methanolysis, monitored by the change in the UV spectrum, was complete in 1 hr and the solution was evaporated to give 3-acetoxy-8-azaxanthine (**14**) as a white powder (125 mg, 97%). (Calc. for $C_6H_5N_5O_4$: C, 34·13; H, 2·39; N, 33·17. Found: C, 34·45; H, 2·47; N, 33·05%).

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REFERENCES

[1] U. Wölcke, N. J. M. Birdsall and G. B. Brown, *Tetrahedron Letters* 785 (1969); N. J. M. Birdsall, U. Wölcke and G. B. Brown, 157th Meeting American Chemical Society, Minneapolis, April Abstract 0–172 (1969)
[2] G. Stöhrer and G. B. Brown, *Science* **167**, 1622 (1970)
[3] G. B. Brown, K. Sugiura and R. M. Cresswell, *Cancer Res.* **25**, 986 (1965); K. Sugiura, M. N. Teller, J. C. Parham and G. B. Brown, *Ibid.* **30**, 184 (1970)
[4] J. A. Montgomery *J. Am. Chem. Soc.* **78**, 1928 (1956); T. Koenig and T. Barklow, *Tetrahedron* **25**, 4857 (1969)
[5] U. Wölcke, W. Pfleiderer, T. J. Delia and G. B. Brown, *J. Org. Chem.* **34**, 981 (1969)
[6] J. C. Parham, J. Fissekis and G. B. Brown, *Ibid.* **32**, 1151 (1967)
[7] J. H. Lister, *Advan. Heterocycl. Chem.* **6**, 1 (1966)
[8] G. S. Reddy, L. Mandel and J. H. Goldstein *J. Chem. Soc.* 1414 (1963)
[9] M. P. Schweizer, S. I. Chan, G. K. Helmkamp and P. O. P. Ts'O, *J. Am. Chem. Soc.* **86**, 696 (1964)
[10] D. Lichtenberg, F. Bergmann, Z. Neiman and I. Ringel, *Suppl., Proc. Israel Chem. Soc.* **8**, 17 /1970)
[11] W. Pfleiderer and G. Nübel, *Ann.* **647**, 155 (1961)
[12] J. C. Parham, T. G. Winn and G. B. Brown, *J. Org. Chem.* **36**, 2639 (1971)
[13] L. F. Cavalieri, J. J. Fox, A. Stone and N. Chang, *J. Am. Chem. Soc.* **76**, 1119 (1954)
[14] A. Albert and E. P. Serjeant, *Ionization Constants of Acids and Bases*, John Wiley & Sons, New York. (1962)
[15] D. D. Perrin, *Aust. J. Chem.* **16**, 572 (1963)
[16] N. J. M. Birdsall, T-C. Lee, T. J. Delia and J. C. Parham, *J. Org. Chem.* **36**, 2635 (1971)
[17] For which we are indebted to W. Pfleiderer of Universität Konstanz, Germany
[18] R. M. Cresswell, H. K. Maurer, T. Strauss and G. B. Brown, *Ibid.* **30**, 408 (1965)