# Synthesis and Properties of 7-Hydroxy-8-phenylxanthine and its Derivatives. Disproportionation to Derivatives of 8-Phenylxanthine and 6-Amino-5-nitrosouracil

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8-Phenyl, 8-(*p*-methoxyphenyl) and 8-(*p*-chlorophenyl)-7-hydroxyxanthine were synthesized by ring closure of 6-amino-5-nitrosouracil with benzaldehyde, *p*-methoxybenzaldehyde and *p*-chlorobenzaldehyde, respectively. The disproportionation of these products to the corresponding 8-phenylxanthines and 6-amino-5-nitrosouracil was studied. The dependence of the rate of the reaction on the various substituents was studied. The disproportionation reaction is inhibited by the polarophilic ethyl acetylenedicarboxylate and enhanced by phosphate.

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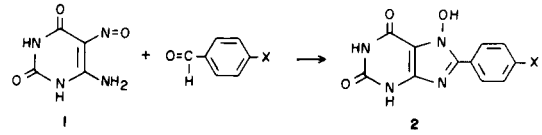
Purine *N*-oxides exhibit various biological activities (1,5). For compounds where the predominant tautomer is the *N*-hydroxy form, the name *N*-hydroxy is preferred. The compound reported in 1966 as 7-hydroxyxanthine (6) was shown later to be 3-hydroxyxanthine (7). 7-Hydroxyxanthine and its 8-alkyl derivatives were prepared from 6-amino-5-nitrosouracil by reaction with aliphatic aldehydes (8,9). Disproportionation of the 8-alkyl derivatives to 8-alkylxanthines were recently described (10). 8-Phenyl derivatives of theophylline were shown to be antagonists in the formation of cyclic AMP in human fibroblasts (11).

8-Aryltheophylline derivatives which were reported (12) in 1964 were prepared by the reaction of 6-amino-1,3-dimethyl-5-nitrosouracil with aromatic aldehydes. The authors have described the isolation of a light sensitive intermediate, *eg*, 8-phenyltheophylline 7-oxide, which was claimed to undergo reduction by dimethylformamide to 8-phenyltheophylline. In 1970 the same products were prepared (13) by using aldehyde hydrazones instead of aldehydes. In this article (13) no attempt was made to explain the deoxygenation of the purine 7-oxide. We have repeated the work done by Taylor and Garcia (12) and observed that 8-phenyltheophylline 7-oxide is a relatively stable product and is not light sensitive. Moreover, we could show that the formation of 8-phenyltheophylline occurs also upon heating in dimethylsulfoxide.

The aim of the present work was the preparation of the corresponding xanthine *N*-oxide derivatives *eg* without substitution at $N^1$ and $N^3$ and the study of their disproportionation to 8-arylxanthines. The *N*-hydroxy group in these compounds is quite stable. However, under certain conditions they undergo an unusual disproportionation which was investigated here. The ring closure which led to the formation of these *N*-hydroxyxanthines was carried out in dimethylsulfoxide at 145-150°. While ring closure with aliphatic aldehydes took place (8) at 120-125°, with aromatic aldehydes the reaction did not start under 145°. At temperatures higher than 150° or on prolonged heating the yields were very poor due to decomposition of the *N*-hydroxyxanthine derivatives. During the preparation and work-up some of the 8-aryl-7-hydroxyxanthines underwent disproportionation and were purified from the resulting 8-arylxanthines either by repeated recrystallization from dioxane or by ion exchange chromatography on Dowox-50 (eluted with warm dioxane or ethanol). The overall yields of the reaction with aromatic aldehydes were fair (30-40%).

Scheme I



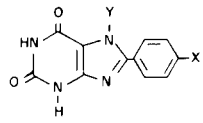
1 2

Pure samples of 8-aryl-7-hydroxyxanthines showed three separate nmr signals for the three exchangeable protons, whereas even slightly impure products gave a broad diffused absorption. A similar behaviour was observed in the reduced form (*eg*, 8-arylxanthines). A comparison of these signals is given in Table I. The proton of the *N*-hydroxy group absorbs at about 1 ppm higher field (δ 12.5-12.65) than the NH proton at the same position in the reduced form (δ 13.3-13.7). The *ortho* protons of the phenyl group are shifted downfield by the adjacent heterocycle, resulting in AB and AX systems for the *p*-chloro and *p*-methoxy derivatives, respectively.

Whereas alkyl groups at position 8 have a negligible effect on the uv spectra as compared to the unsubstituted 7-hydroxyxanthine (8), a considerable red shift of about 30-40 nm was observed in the aromatic derivatives (see Table II). The N-OH group is a weak acid with a $pK_a$ of about 5.0 for the phenyl and anisyl derivatives and 4.6 for the *p*-chlorophenyl derivative. At higher *p*H values a dianion is formed with $pK_a$ values from 9.5 to 8.25 for the various derivatives (see Table II). The second ionization is probably at the pyrimidine ring.

Table I

PMR Spectra of 8-Aryl-7-hydroxyxanthines and 8-Arylxanthines

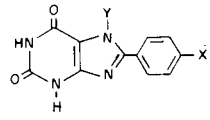


| X | Y | Aromatic Protons (a) | 1-NH | 3-NH | 7-NOH or NH |
|---|---|---|---|---|---|
| H | OH | 7.8 (m), 7.3 (m) | 10.86 (s) | 11.65 (s) | 12.65 (broad s) |
| $OCH_3$ | OH | 6.8-7.9 (AX System) | 10.83 (s) | 11.50 (s) | 12.50 (broad s) |
| Cl | OH | 7.7-8.2 (AB System) | 10.75 (s) | 11.50 (s) | 12.60 (broad s) |
| H | H | 8.0 (m), 7.5 (m) | 10.75 (s) | 11.50 (s) | 13.60 (broad s) |
| $OCH_3$ | H | 6.8-7.9 (AX System) | 10.80 (s) | 11.50 )s) | 13.60 (broad s) |
| Cl | H | 7.7-8.2 (AB System) | 10.78 (s) | 11.50 (s) | 13.30 (broad s) |

(a) In derivatives where X = $NO_2$ which were obtained in an impure form and in low yields, the aromatic protons gave a singlet indicating the deshielding effect of the heterocyclic system.

Table II

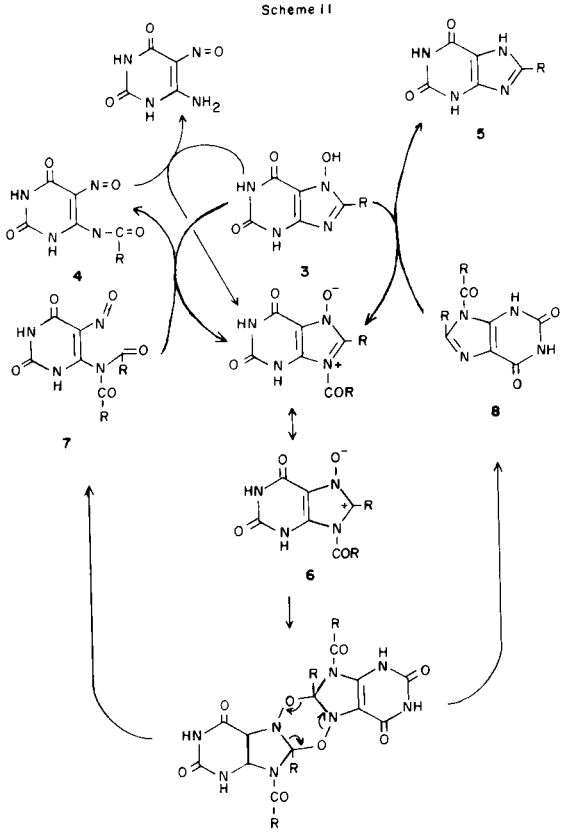
Dissociation Constants and UV Absorptions of 8-Aryl-7-hydroxyxanthines and 8-Arylxanthines



| X | Y | Species | *p*H | $\lambda_{max}$/nm | ($\epsilon \times 10^{-3}$) | $\lambda_{min}$/nm | ($\epsilon \times 10^{-3}$) | *p*Ka |
|---|---|---|---|---|---|---|---|---|
| H | OH | Neutral | 3.0 | 296 (17.9) | 232 (16.7) | 255 (4.5) | | |
| | | Monoanion | 7.0 | 309 (11.7) | 250 (15.6) | 286 (9.1) | | 5.0 |
| | | Dianion | 12.0 | 325 (9.5) | 251 (19.7) | 292 (5.4) | | 9.4 |
| $OCH_3$ | OH | Neutral | 3.0 | 308 (24.0) | 250 (17.3) | 269 (8.7) | 237 (9.1) | |
| | | Monoanion | 7.0 | 315 (14.3) | 260 (16.5) | 292 (7.4) | 227 (9.1) | 5.0 |
| | | Dianion | 13.0 | 325 (12.2) | 259 (24.7) | 294 (8.2) | | 9.5 |
| Cl | OH | Cation | 12.5 | 301 (14.5) | 242 (8.0) | 251 (4.1) | 221 (3.5) | 10.4 |
| | | Neutral | 2.5 | 304 (11.1) | 241 (7.1) | 261 (2.7) | 230 (4.9) | |
| | | Monoanion | 6.6 | 312 (12.2) | 237 (13.1) | 280 (7.1) | | 4.6 |
| | | Dianion | 13.0 | 328 (10.9) | 252 (14.9) | 288 (5.9) | | 8.25 |
| H | H | Cation | 12.0 | 297 (21.3) | 236 (9.6) | 258 (6.9) | 228 (15.8) | 0.9 |
| | | Neutral | 5.0 | 298 (18.9) | 224 (16.4) | 256 (4.5) | | |
| | | Monoanion | 9.6 | 307 (17.2) | 227 (15.9) | 250 (7.9) | | 8.3 |
| | | Dianion | 13.0 | 318 (15.3) | 240 (18.0) | 279 (6.7) | | 10.8 |
| $OCH_3$ | H | Neutral | 5.0 | 319 (23.1) | 248 (14.2) | 270 (9.9) | | |
| | | Monoanion | 10.5 | 316 (18.3) | 260 (15.2) | 282 (12.6) | | 8.2 |
| Cl | H | Neutral | 4.0 | 313 (11.4) | 240 (9.8) | 272 (6.7) | | |
| | | Monoanion | 8.5 | 300 (9.5 | | 252 (7.8) | | 6.5 |
| | | Dianion | 13.0 | 331 (12.8) | | 270 (6.7) | | 10.5 |
| $NO_2$ | H | Neutral | 0.5 | 347 (7.0) | 263 (8.3) | 292 (6.4) | | |
| | | Monoanion | 5.0 | 380 (7.4) | 233 (11.9) | 321 (4.0) | | 2.5 |
| | | Dianion | 13.0 | 409 (12.0) | 292 (11.7) | 338 (5.2) | 273 (10.0) | 9.8 |
| | | | | 227 (20.18) | | | | |

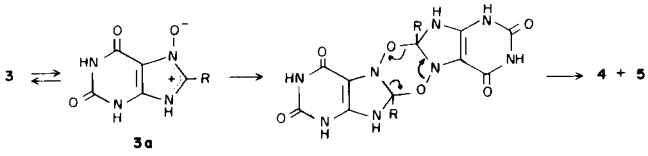
The spontaneous disproportionation of 8-aryl-7-hydroxyxanthines to 8-arylxanthines and 6-amino-5-nitrouracil took place upon heating in various solvents, *eg*, in dimethylsulfoxide or dimethylformamide as well as in water. The more the solution of 8-aryl-7-hydroxyxanthine is concentrated the faster is the disproportionation. It is also enhanced considerably by buffers. For comparison aqueous dimethyl sulfoxide containing buffers was used. The spontaneous disproporationation was shown to be autocatalytic (10) and the induction period could range between several minutes and a few days depending on the conditions or on the type of substituent (see Table III). The mechanism of this autocatalytic disproportionation is best represented in Scheme II and is more complete than that shown earlier (10). The initiation (Scheme III) involves the formation of an acylating species (**4**). In the aromatic series the lag period is shorter than that in the aliphatic series. It was also found that in the *p*-methoxyphenyl derivative (**3**, R = $CH_3OC_6H_4$) this period is four times longer than in the phenyl derivative (**3**, R = $C_6H_5$), whereas in the *p*-chlorophenyl derivative (**3**, R = Cl-$C_6H_4$), it is 10 times shorter. In the absence of a buffer at 42° the

Scheme II



appearance of color was not observed even after several days. The considerable effect of *p*H on the length of the initiation period is probably due to its effect on tautomeric equilibrium that leads to the formation of tautomer **(3a)** which is responsible for the initiation process (Scheme III). Additional evidence for such a mechanism was de-

Scheme III



rived from the experiments run in the presence of ethyl acetylenedicarboxylate as 1,3-dipole scavenger. In the presence of ethyl acetylenedicarboxylate no color developed and no disproportionation took place. There was some evidence from nmr of the reaction mixture that a cycloaddition of diethyl acetylenedicarboxylate to the *N*-oxide **(3)** might have occurred.

The disproportionation becomes fast when acylation reagents such as **4**, **7** and **8** are available (Scheme II). 6-Acylamino-5-nitrosouracil was shown previously to be a reactive acylation reagent (14). Compound **8** has to be an active acylating species as a derivative of *N*-acylimidazole.

Table III

Lag Period and Rate Constants of the Disproportionation of 8-Substituted 7-Hydroxyxanthines ($6.4 \times 10^{-3}$ *M* in 77% Aqueous DMSO at 42° with $1.6 \times 10^{-2}$ *M* Phosphate Buffer)

| Substituent at position 8 | *p*H of added phosphate | Lag time minutes | $k_{SAD}$ $min^{-1}$ $M^{-1}$ |
|---|---|---|---|
| Phenyl | 4 | 20 | 21.8 |
| Phenyl | 5 | 30 | 16.8 |
| Phenyl | 6 | 130 | 15.1 |
| Phenyl | No phosphate | >3760 | |
| *p*-Methoxyphenyl | 4 | 80 | 10.2 |
| *p*-Methoxyphenyl | Acetate *p*H 4 | 90 | 3.6 |
| *p*-Methoxyphenyl | 5 | 160 | 6.2 |
| *p*-Methoxyphenyl | 6 | 320 | 4.5 |
| *p*-Chlorophenyl | 4 | 3 | 52.0 |
| *p*-Chlorophenyl | 5 | 6 | 36.8 |
| Propyl | 4 | 120 | 5.6 |
| Propyl | 5 | 120 | 5.4 |
| Propyl | 6 | 180 | 4.8 |

The influence of the type of buffer and the *p*H is shown in Table III. The apparent rate constant with acetate buffer was 3 times smaller than that with phosphate buffer. The specific phosphate catalysis arises probably from its ability to catalyse the transacylation reaction. The substituent on the phenyl group has a pronounced effect on the rate of the disproporationation in the autocatalytic stage (Table III). The order of magnitude of the rate constant ($k_{SAD}$) is $Cl < H < OCH_3$ and it was possible to show that Hammett's $\varrho$ has a positive value (1.4-1.6). This is in agreement with the assumption that the transacylation is the rate determining step. The unsubstituted 7-hydroxyxanthine **(3**, R = H) undergoes neither a spontaneous nor an induced disproportionation. In the presence of acetic anhydride, conditions in which the 8-substituted derivatives disproportionate instantaneously, the unsubstituted 7-hydroxyxanthine undergoes a rearrangement to uric acid (9).

## EXPERIMENTAL

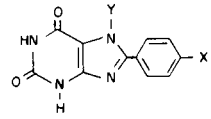
The uv measurements were carried out in a "Varian Techtrone" spectrophotometer Model 650. Adjustments of *p*H were done with a "Radiometer Copenhagen" *p*H-meter 29. Buffered solutions were prepared by *p*H adjustment of disodium hydrogen phosphate or sodium acetate solutions with concentrated hydrochloric acid. The nmr spectra were taken with a "Varian" Model T-60. Dowex 50-X8 of 200-400 mesh in the H-form was used as ion exchange resin in column chromatography. Elution of columns was carried out by water, aqueous dioxane (8) or dioxane-hydrochloric acid mixtures. Dimethylsulfoxide was dried by azeotropic distillation with benzene.

8-Aryl-7-hydroxyxanthines **(2)**.

Dry DMSO (375 ml) was heated to 120° and 6-amino-5-nitrosouracil (6.25 g) was introduced portionwise during 10 minutes. After the solid dissolved, an aromatic aldehyde was added (2 g) and the temperature was raised to 145°. More of the aldehyde (2 g) was added and the temperature kept at 145-150°. An additional three portions of the aldehyde (2 g each) were added at intervals of 5 minutes. The reaction mixture was kept at

Table IV

Experimental Data for Derivatives of 8-Phenyl-7-hydroxyxanthine and 8-Phenylxanthine



| X | Y | % Yield | Mp | Formula | Found % C | % H | % N | % Cl |
|---|---|---|---|---|---|---|---|---|
| H | OH | 37 | >300 | $C_{11}H_8N_4O_3 \cdot H_2O$ | 50.44 | 4.15 | 20.85 | |
| | | | | | (50.38 | 3.84 | 21.31) | |
| $OCH_3$ | OH | 30 | >300 | $C_{12}H_{10}N_4O_4$ | 52.75 | 3.95 | 20.85 | |
| | | | | | (52.56 | 3.68 | 20.43) | |
| Cl | OH | 19 | >300 | $C_{11}H_7N_4O_3Cl$ | 47.52 | 2.65 | 19.94 | 12.75 |
| | | | | | (47.44 | 2.55 | 20.10 | 12.70) |
| H | H | 50 | >300 | $C_{11}H_8N_4O_2$ | 57.54 | 3.81 | 24.15 | |
| | | | | | (57.89 | 3.53 | 24.55) | |
| $OCH_3$ | H | 65 | >300 | $C_{12}H_{10}N_4O_3$ | 54.75 | 4.15 | 22.20 | |
| | | | | | (55.81 | 3.90 | 21.70) | |
| Cl | H | 70 | >300 | $C_{11}H_7N_4O_2Cl \cdot \frac{1}{2}H_2O$ | 48.45 | 3.25 | 20.70 | 12.60 |
| | | | | | (48.65 | 3.04 | 20.61 | 13.05) |
| $NO_2$ | H | 9 | >300 | $C_{11}H_7N_5O_4 \cdot 2H_2O$ | 42.64 | 3.35 | 22.15 | |
| | | | | | (42.72 | 3.59 | 22.65) | |

the same temperature for an additional 5 minutes and cooled to 75°. The volume of the solvent was decreased to 37.5 ml by evaporation of the solvent at 75°. Chloroform (130 ml) was added and any solid precipitated within 1-2 minutes was filtered off. Hydrochloric acid (0.5*N*, 60 ml) was added and the layers were shaken for 1 minute and left for three days at room temperature. The precipitate which formed was collected by filtration and dried in the air. The crude product contained the desired 8-aryl-7-hydroxyxanthine contaminated with varying amounts of 8-arylxanthines which were formed by the disproportionation of **2**. The crude prcipitate was boiled for 4 minutes in dioxane and filtered hot. On cooling overnight the pure 8-aryl-7-hydroxyxanthine precipitated. Additional crops could be isolated by more extractions with boiling dioxane. Yields and analyses are given in Table IV.

8-Arylxanthines.

8-Aryl-7-hydroxyxanthine (**2**, 1 g) either in the pure form or the crude precipitate (see above) was suspended in water (80 ml). A 0.5 *M* solution of phosphate buffer of *p*H 5 (20 ml) was added and the mixture boiled for 3 hours. After cooling to room temperature concentrated hydrochloric acid (10 ml) was added and the mixture was stirred overnight at room temperature. The precipitated 8-arylxanthine was collected by filtration and washed with boiling ethanol. Yields and analytical results are given in Table IV.

7-Hydroxy-8-phenyltheophylline.

1,3-Dimethyl-4-amino-5-nitrosouracil (3.7 g) was heated in boiling DMF (50 ml) and benzaldehyde (8 g) was added during 15 minutes. The solution was refluxed until color started to fade (5 minutes). The mixture was concentrated *in vacuo* to 20 ml at 75° and some 8-phenyltheophylline which precipitated was filtered off. The filtrate was treated with 40 ml of chloroform and 20 ml of 1*N* hydrochloric acid and kept overnight at room temperature. The precipitate (1.85 g, 34%) which melted (12) at 187° was otherwise identical with that reported earlier (12). On boiling in DMF it gave 8-phenyltheophylline as well as dimethylamine. It underwent a slow disproporationation induced by acetic anhydride (9) to 8-phenyltheophylline and 6-amino-5-nitrosouracil. The crystals were stable in light. In solution it underwent a very slow spontaneous disproportionation to 8-phenyltheophylline and 6-amino-5-nitrosouracil.

Determination of $pK_a$.

Standard solutions were prepared: Solution (a) consisted of the measurement substance ($1 \times 10^{-4}$ *M*), sodium dihydrophosphate (0.1 *M*) and sodium hydroxide (enough to maintain *p*H 13). Solution (b) contained the same concentration of the substantce ($1 \times 10^{-4}$ *M*), sodium monohydrophosphate (0.1 *M*) and hydrochloric acid (enough to maintain *p*H 1). The extinction coefficient ($\epsilon$) of the substance was measured at *p*H values corresponding to the range from 15% to 85% ionization. The *p*H was varied by mixing solution (a) with (b) in different proportions (measuring with a *p*H-meter). Dissociation constants were determined from these data by the appropriate equations (15). Solutions of *p*H = 0 and lower were prepared according to Hammett's procedure from mixtures of water and sulfuric acid (16). Results are summarized in Table II.

Following of the Autocatalytic Disproportionation of 8-Aryl-7-hydroxyxanthines.

8-Aryl-7-hydroxyxanthines which were tested were purified by repeated crystallizations from dioxane. 8-Propyl-6-hydroxyxanthine and the unsubstituted 7-hydroxyxanthine whcih was used for comparison were prepared by an earlier procedure (8) and purified by ion exchange column chromatography. The studied substance (0.02 mmole) was dissolved in dimethylsulfoxide (2.4 ml) in a quartz cell (light path 10 mm). Water (0.6 ml) and 0.5 *M* buffer solution (0.1 ml) were added and the reaction was followed at 42° by the change of absorption at 535 nm. The rate constant was elucidated (10) from the plot of $\ln OD/OD_\infty - OD$ against time, where OD is the absorption at 535 nm during the autocatalytic stage of the reaction and $OD_\infty$ is the absorption at the same wavelength at the end of this stage. Results are summarized in Table III.

Inhibition of the Spontaneous Disproportionation by Ethyl Acetylenedicarboxylate.

8-Aryl-6-hydroxyxanthines (0.06 mmole) were dissolved in hexadeuterio DMSO (0.6 ml) in the nmr tube. Ethyl acetylenedicarboxylate (0.02 g ) was added and the tube kept at 50°. The nmr was determined at intervals of 12-24 hours. The ethyl protons showed the formation of 2 nonequivalent carbethoxy groups and the pattern of phenyl protons changed as a result of the conversion of the benzylic position into a tetrahedral carbon. In the *p*-$OCH_3$ derivative (**2**, X = $OCH_3$) it changed from an AX into an AB system. In the *p*-Cl derivative as well as in the unsubstituted phenyl a broad singlet was formed instead of a split system. No formation of a vinyl proton was observed. The solution remained colorless and no disproportionation occurred. Unsubstituted 7-hydroxyxanthine remained unchanged under the same conditions. Reference tubes with the

same concentration of 8-aryl-7-hydroxyxanthines not containing ethyl acetylenedicarboxylate underwent disproportionation accompanied by coloration.

Determination of the Value of $\varrho$ of the Spontaneous Autocatalytic Disporportionation of 8-Aryl-7-hydroxyxanthines.

Hammett's substituent constants ($\sigma$) which were used are those based on the ionization of benzoic acids (17). The values are 0.227 and -0.268 for *para*-Cl and *para*-$OCH_3$ respectively. $Ln(k/k_o)$ was plotted against $\sigma$, then $\varrho$ of the disporportionation was taken from the slope.

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