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N-ACETOXY-4-METHOXYANILINE, A MODEL COMPOUND FOR THE ULTIMATE CARCINOGEN OF THE PHENACETIN RELATED 4-ETHOXYANILINE

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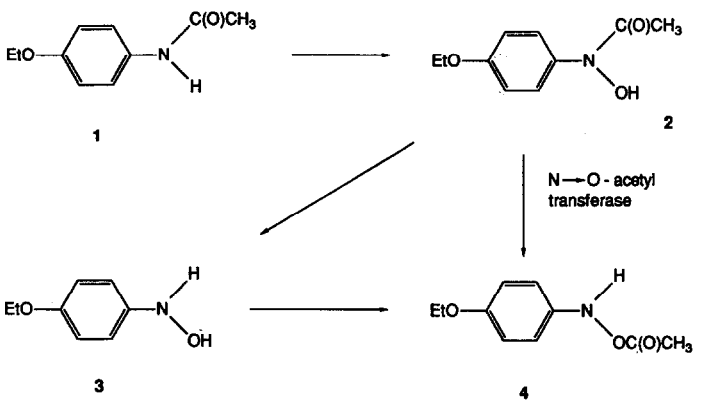
Summary: We prepared and trapped in situ with N-methylaniline and deoxyguanosine (dG) the three N-acyloxy-4-methoxyanilines **7a-c**. Furthermore, we synthesized N-acetoxy-4-methoxyaniline **7a** and reacted it with dG. Especially **7a** is a model for the ultimate carcinogen of phenacetin **1**, namely N-acetoxy-4-ethoxyaniline **4**.

Phenacetin **1**[1] was introduced as a drug in 1898 because of its analgetic and antipyretic effects, and its low acute toxicity[2]. Abuse, however, led to damages[3]: phenacetin **1** reduces the lifetimes of erythrocytes[4], and causes anaemia[5] and methaemoglobinaemia[6]. An important side-effect is the formation of nephritis[7]. Furthermore, in some cases **1** led to kidney necrosis[8], kidney carcinoms[9], bladder cancer[10] and tumors in the urinary system[11]. The carcinogenicity of **1** was also demonstrated with animals. When male and female Sprague-Dawley rats were fed with diet containing 2.5 (1.5)% phenacetin **1**, 96.3 (90.9)% of the male and 77.8 (76.9)% of the female rats showed neoplasmas[12]. Because of these non-negligible side-effects phenacetin **1** was forbidden as part of mixed analgetics in the Federal Republic of Germany in 1986[13,14].

Scheme I



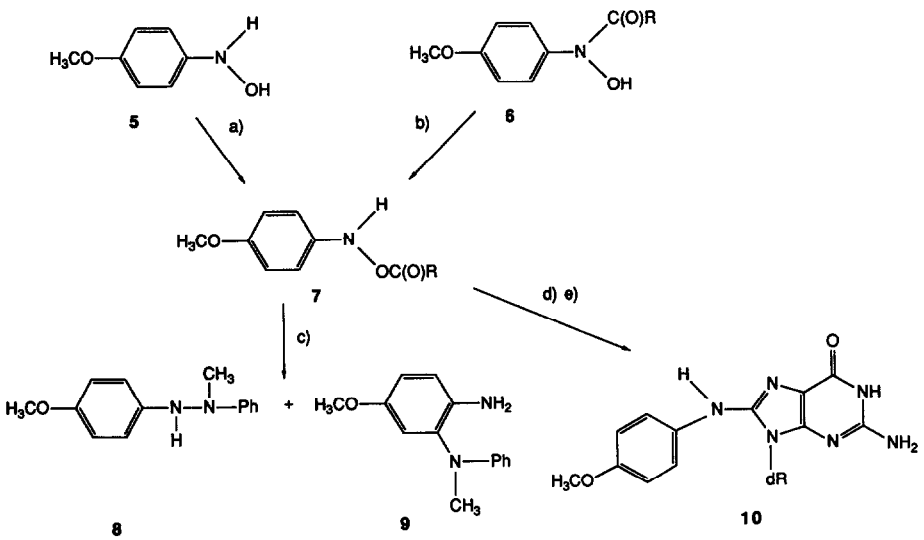
1 **2** **3** **4**

Among the investigations of the pathways leading to toxic, mutagenic and carcinogenic metabolites of **1** the following are relevant to the work reported in this communication. As in the case of other mutagenic and carcinogenic aromatic amines[15], N-oxidation of **1** by means of microsomal mixed function oxidases leading to N-hydroxyphenacetin **2** is a first important step[16]. The transformation of **2** in mutagen (Ames test) and nucleic acid binding metabolites by N -> O acetyltransferase suggests the intermediate formation of N-acetoxy-4-ethoxy-aniline **4** as a reactive metabolite[17]. **4** might also result from O-acetylation of N-hydroxy-4-ethoxyaniline **3**[15] (Scheme I). So far, however, there is no report in the literature on the synthesis of **4** or the characterization of a (bio)nucleophile adduct with this metabolite of **1**.

In the following we report on model compounds of **4**: the intermediate formation of the N-acyloxy-4-methoxyanilines **7a-c**, their in situ trapping reactions with the nucleophile N-methylaniline to give the adducts **8** and **9** and with the bionucleophile deoxyguanosine (dG) to give the adduct **10**, as well as the synthesis of the extremely labile N-acetoxy-4-methoxyaniline **7a** and its reaction with dG to give **10**.

We have chosen these model compounds and model reactions for the following reasons: (1) p-methoxyphenylhydroxylamine **5** as a starting compound is more easily accessible and more stable than p-ethoxyphenylhydroxylamine **3**; at the same time the donor quality of the p-methoxy substituent is essentially the same as that of the p-ethoxy substituent which is responsible for the pharmacological and toxical properties of phenacetin **1**; (2) it is generally assumed that adduct formation of the ultimate carcinogen of an aromatic amine with the DNA base deoxyguanosine finally leads to cancer[15,18]. It is therefore important to know whether a putative ultimate carcinogen like **7** reacts with deoxyguanosine; (3) N-methylaniline has shown to be a successful nucleophile in testing the electrophilic character of nitrogen electrophiles like **7**[15,18,20].

Scheme II



a: R = CH_3; **b**: R = tBu; **c**: R = $CH(CH_3)NHCH_2Ph$

Reaction conditions: a) $CH_3C(O)CN$, THF, -78°C, 5 min; b) NEt_3, EtOH:$CHCl_3$:H_2O = 7:4:3, 85°C, 3 d; c) from **6**: b) plus N-methylaniline; d) from **6**: b) plus dG; e) **7a** in THF (cooled to -78°) added to dG in H_2O, 20°C

In situ trapping of **7a-c**. As shown earlier[15,18] we can model in vitro the in vivo N -> O transacylation leading from N-acyl-N-arylhydroxamic acids to N-acyloxy-N-arylamines by means of an amine catalyst. Applying this methodology to the hydroxamic acids **6a-c** and triethylamine (route b) in Scheme II), trapping of **7a-c** with N-methylaniline (route c) in Scheme II) gave the hydrazine **8** and the "ortho adduct" **9**[19], see Table 1. It is noteworthy that ortho adducts of the type **9** are only formed in the case of reactive N-acyloxy-N-arylamines derived from amines with carcinogenic potential like 4-aminobiphenyl[20a], 2-naphthylamine[20b], 2-aminofluorene[20c] or p-toluidine[15]. In similar trapping reactions of **7a-c** with deoxyguanosine (dG) (route d) in Scheme II) the dG-adduct **10** was formed, see Table 1. The yields of **10** are higher by a factor of two if compared, e.g. with the yields in the trapping reactions of N-acyloxy-N-4-methylanilines with dG[15]. This indicates that the N-acyloxy-4-methoxyaniline intermediates **7a-c** are very reactive due to the p-methoxy donor substituent.

Table 1. Yields [%] of adducts **8** and **9** (reactions with N-methylaniline) and adduct **10** (reactions with deoxyguanosine dG) starting from the p-methoxyphenylhydroxyl amine derived hydroxamic acids **6a-c** and N-acetoxy-p-methoxyaniline **7a**, respectively.

	8	**9**	**10**
6a	28	30	5.2
6b	21	28	6.3
6c	28	30	6.3
7a	0	0	4.2

7a: *Synthesis and reactions*. N-acetoxy-4-methoxyaniline **7a** was prepared according to a procedure first introduced by Lobo et al.[21] and successfully applied to other labile N-acetoxy-N-arylamines by us[15,18,20]: reaction of the hydroxylamine **5** with acetylcyanide in THF (route a) in Scheme II). Because of the extreme thermal lability of **7a** this reaction had to be performed at -78°C within 5 min. All attempts to isolate **7a** failed because of decomposition, however, when **7a** was prepared in THF-D_8 it could be characterized by means of its ^{1}H-nmr spectrum[22]. From the reactions of **7a** with N-methylaniline we were unable to isolate either **8** or **9**. The reaction with dG, however, led to the C8 adduct **10** (route e) in Scheme II). When both nucleophiles, N-methylaniline and dG, reacted simultaneously with **7a**, again only the dG-adduct **10** was isolated. Although we have no explanation for these interesting observations at the moment, the formation of **10** from **7a** and dG proves that **7a** is also formed from **6a** via transacylation. Judging from the yields of the adducts **8**, **9** and **10**, the N-acyloxy-4-methoxyanilines **7b** and **7c** should similarly result from the hydroxamic acids **6b** and **6c**, respectively, by N -> O transacylation, and react with N-methylaniline and dG, respectively.

The model N-acyloxy-4-methoxyanilines **7a-c**, prepared from the hydroxamic acids **6a-c** and trapped in situ by N-methylaniline to give **8** and **9**, and by dG to give **10**, in high yields thus indicate the strong electrophilic character of the phenacetin **1** related ultimate carcinogen N-acetoxy-4-ethoxyaniline **4**. The extreme lability of N-acetoxy-4-methoxyaniline **7a**, prepared at -78°C, is in accord with these observations.

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[22] ^{1}H nmr (400 MHz; THF-D_8; ^{3}J[Hz]: δ = 2.10 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 6.82 (d, 2H, $H^{2,6}$, 8.94), 6.98 (d, 2H, $H^{3,5}$, 8.80), 9.57 (s, broad, 1 H, NH).

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