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N-ACETOXY-N-ALKOXYAMIDES - A NEW CLASS OF NITRENIUM ION PRECURSORS WHICH ARE MUTAGENIC

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Summary: N-chloro-O-alkylbenzohydroxamates react with silver acetate in ether giving N-acetoxy-N-alkoxybenzamides which, by analogy with N,O-diacyl-N-arylhydroxylamines, have been shown to be mutagenic in the Ames test.

Contemporary theories of chemical carcinogenesis have focussed upon the electrophilicity of known carcinogens or their metabolites.[1,2] In particular, the class of aromatic amines (1) are deemed to undergo successive metabolic activation to N-hydroxy-N-arylamides (2), N-acetoxy-N-arylamides (3) or the sulphate esters (4) which are all precursors to the 'ultimate' carcinogens, the N-aryl-N-acylnitrenium ions (5). [2] Much endeavour is currently centred on whether these reactive intermediates are actually formed in vivo [3] and how they interact biologically. [2,4] It is evident that arylamines(1) are less carcinogenic than N-hydroxy-N-arylamides(2) or their acetyl or sulphate derivatives [2] and while the case for nitrenium ion intermediacy is yet to be proven, (3), (4) and the conjugate acid of (2) are potential sources of such electrophilic ions or are susceptible to nucleophilic attack at nitrogen because of the leaving group at that centre.

$ArNH_2$ (1)

$ArNH(OX)COCH_3$
(2) X=H
(3) X=Ac
(4) $X=SO_3^-$

ArN^+COCH_3 (5)

$RCON(Cl)OR'$ (6)

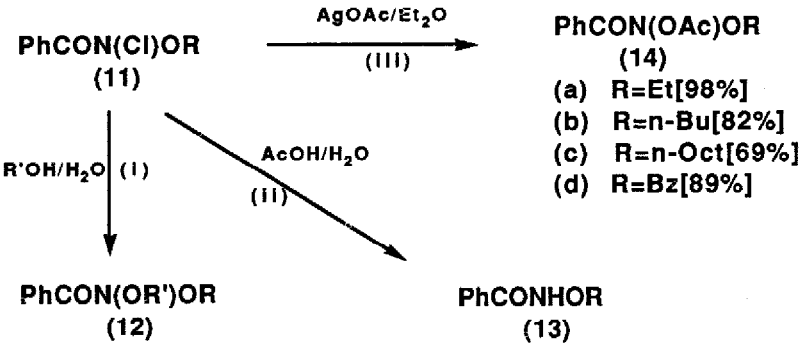
$RCO\overset{+}{N}OR'$ (7)

$HCO\overset{+}{N}R$
(8) R=OH
(9) R=Ph

$RCON(OAc)OR'$ (10)

Recent studies on the reactions of N-chloro-N-alkoxyamides(6) have shown that these substrates react with Lewis acids such as silver(I) to generate N-alkoxy-N-acylnitrenium ions (7) which add intramolecularly and intermolecularly to aromatic rings. [5,6,7] Theoretical computations at the MNDO level have now shown that the stabilisation imparted by an N-alkoxy substituent on a nitrenium ion centre parallels that of an N-aryl substituent. [8] For instance the N=OH and $N=C_{ipso}$ π bond orders in (8) and (9) are 0.9 and 0.895 respectively; heterolyses of their N-chloro precursors are respectively 294 and 410 $kJmol^{-1}$ less endothermic than heterolysis of N-chloroformamide; the SOMO energies (a reflection of the ease of oxidation) in the corresponding amidyl radicals are -5.66 and -5.3eV. [9] It was therefore of importance to ascertain whether potential precursors to such nitrenium ions (7), N-acetoxy-N-alkoxyamides (10) could be synthesised and whether they, as well as the N-chloro alkoxyamides (6), are mutagenic by analogy with the N-acetoxy-N-arylamides(3).

Scheme 1



We have recently found that solvolysis of N-chloro-N-alkoxybenzamides (11) in aqueous alcohols yields novel N,N-dialkoxyamides (12) in good yields (Scheme 1,(i)).[10] However solvolysis of (11) in aqueous acetic acid resulted predictably in regeneration of the parent hydroxamic ester (13) instead of the acetoxy compound (10,R=Ph) (Scheme1,(ii)). On the other hand treatment of (11) with equimolar amounts of silver acetate in anhydrous ether affords N-acetoxy-N-alkoxybenzamides (14) in excellent yields (Scheme 1,(iii)) .The reactions were monitored by reverse-phase hplc and were generally complete within five hours. The compounds were isolated as relatively clean oils by filtration and concentration under reduced pressure at room temperature. They were purified by flash chromatography on silica gel without decomposition. This new class of N,N-geminally substituted amides were characterised fully by ^{1}H and ^{13}C nmr which, in addition to the resonances of the parent residues, indicated acetoxy methyl resonances at δ2.0 and δ18.5 respectively. Each showed highly characteristic carbonyl absorptions close to 1800 and 1730 cm^{-1} in the infrared as well as an absence of NH stretch (Table 1).

Table1. Nmr and infrared data for N-acetoxy-N-alkoxybenzamides (14)

R	$^{1}H(\delta)$	^{13}C (δ)	$\nu_{max}(cm^{-1})$
Et	1.27(t,3H), 2.09(s,3h), 4.2(q,2H),7.29(t,2H-*m*), 7.42(t,1H-*p*),7.76(d,2H-*o*)	13.15(q),18.36(q),70.91(t), 127.9(d),128.65(d),131.36(s), 132.5(d),167.9(s),173.9(s)	1795(s),1732(s), 1608(w),1455(m), 1378(m),1270(s)1190(s)
Bu	0.88(t,3H),1.34(sextet,2H), 1.618(t,2H),2.08(s,3H), 4.18(t,2H),7.4(t,2H-*m*),7.52, (t,1H-*p*),7.76(d,2H-*o*)	13.44(q),18.42(q),18.72(t), 29.79(t),75.06(t),128.03(d), 128.73(d),131.56 (s),132.48(d), 167.84(s)173.91(s)	1795(s),1732(s), 1608(w),1458(m), 1378(m),1272(s), 1190(s)
Oct	0.867(t,3H),1.2-1.4(m,10H), 1.63(quintet,2H),2.109(s,3H), 4.17(t,2H),7.42(t,2H-*m*), 7.54(t,1H-*p*),7.77(d,2H-*o*)	14.08(q),18.80(q),22.62(t),25.75(t), 28.0(t),29.12(t),29.23(t),31.74(t), 75.71(t),128.25(d),129.0(d),131.74(s) 132.71(d),168.21(s),174.29(s)	1798(s),1728(s), 1608(w),1458(m), 1378(m), 1265(s), 1190(s)
Bz	1.91(s,3H),5.06(s,2H),7.2-7.35(m,7H),7.4(t,1H), 7.65(d,2H-*o*)	18.62(q),77.5(t),128.34(d),128.52(d), 128.75(d)129.08(d),129.22(d), 131.65(s),132.84(d),134.75(s), 168.08(s),174.12(s)	1798(s), 1728(s), 1608(w),1460(m), 1378(m),1275(s), 1190(s)

The compounds were relatively stable at room temperature and could be stored at low temperature for several days without noticeable deterioration. (14a) was stirred in 80% acetonitrile/water for 24h without decomposition although it appears to decompose rapidly in basic solution. [10]

Fig. 1 Mutagenicities of (14) for strain TA100

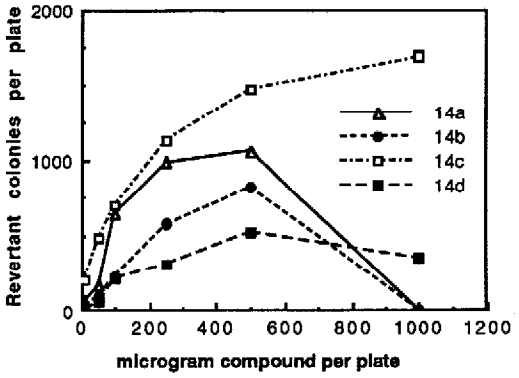
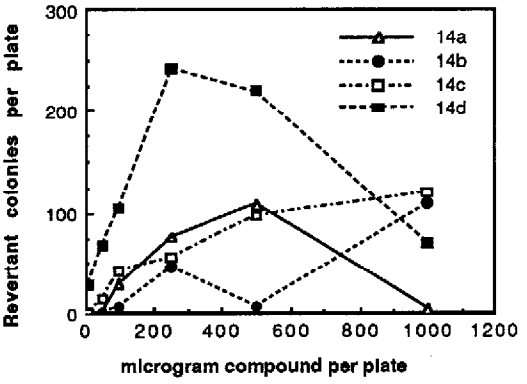


Fig.2 Mutagenicities of (14) for strain TA98



In the Ames test, [11] the compounds were found to be significantly mutagenic towards both the TA100 and TA98 strains of salmonella without metabolic activation. Dose-response curves for the four compounds (14 a-d) with each strain are shown in Figures 1 and 2. [12] N-acetoxy-N-octyloxybenzamide (14 c) was clearly more mutagenic to TA100 than (14 a,b or d) while in TA98 the benzyloxy compound (14 d) was most active. A linear dose response curve with TA100 was also demonstrated for compound (14 c) in the 50-200μg range. With either strain, metabolic activation with rat liver enzymes did not result in markedly higher rates of induced revertants at low concentrations but consistently increased activity at high concentrations. Representatives of the classes of N-chloro-N-alkoxyamides(6), N,N-dialkoxyamides (12), and the hydroxamic esters (13) afforded negative results with and without metabolic activation. Detailed results of mutagenic studies and their significance will be presented elsewhere.

It is clear from these studies that the title compounds are mutagenic and may therefore behave as such in humans. It is equally significant that the underlying feature of these molecules is their propensity to form and stabilize a partial or well developed positive charge on the nitrogen atom thus facilitating nucleophilic attack at that center. [13] This, together with the theoretical analogy between N-acyl-N-alkoxy- and N-acyl-N-arylnitrenium ions [8] adds weight to the nitrenium ion theory of carcinogenesis of aromatic amines. [2,3,4] Investigations into the nature of the biological action of (14) and studies of their solvolytic behaviour are presently underway in these laboratories.

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12. A spontaneous revertant rate of *ca*160 and 35 for TA100 and TA98 respectively have been subtracted from the respective counts. Compounds are considered mutagenic with an induced revertant count of twice these levels at 500 µg/plate.
13. Mutagenic aromatic amides bind to nucleic acids through such mechanisms. See references1,2 and 4 above

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