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POTENTIAL METABOLITES OF CARCINOGENIC AZA AROMATIC HYDROCARBONS SYNTHESIS OF K-REGION OXIDE, PHENOL AND DIHYDRODIOLS OF 7-METHYLBENZ[*c*]ACRIDINE

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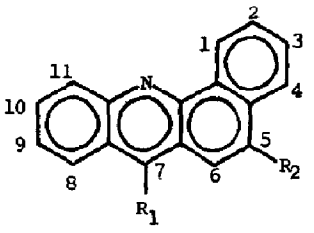
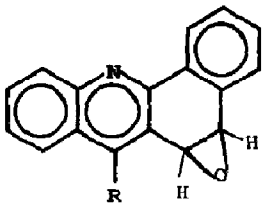
The potential K-region metabolites, trans- and cis-5,6-dihydroxy-7-methyl-5,6-dihydrobenz[*c*]acridine, 5,6-epoxy-7-methyl-5,6-dihydrobenz[*c*]acridine and 5-hydroxy-7-methylbenz[*c*]acridine, of 7-methylbenz[*c*]acridine have been synthesised.

Extensive studies with polycyclic aromatic hydrocarbons (PAH), such as benzo[*a*]pyrene, have indicated that mammalian metabolites which bind to DNA are probably responsible for the mutagenicity and carcinogenicity of the parent hydrocarbon.[1] In contrast, the aza aromatic hydrocarbons are little studied despite their occurrence as environmental pollutants,[2] and the carcinogenicity of examples such as 7,9- and 7,10-dimethylbenz[*c*]acridine.[3] A notable exception is the work of Okuda et al. on aza pentacyclic compounds.[4]

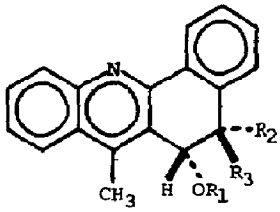
The carcinogen 7-methylbenz[*c*]acridine (1a) is an aza analogue of the carcinogenic hydrocarbon 7-methylbenzanthracene. We report here the preparation of several of its K-region oxygenated derivatives for mutagenicity testing and for comparison with in vivo and in vitro metabolites of labelled 7-methylbenz[*c*]-acridine.[5] These are the 5,6-oxide (2a), the trans- and cis-dihydrodiols (3a) and (3c), the diacetates (3b) and (3d), and the 5-phenol (1c).



	R_1	R_2
(1a)	CH_3	H
(1b)	CHO	H
(1c)	CH_3	OH



(2a) R = CH_3
(2b) R = H
(2c) R = CH_2OH



	R_1	R_2	R_3
(3a)	H	H	OH
(3b)	Ac	H	OAc
(3c)	H	OH	H
(3d)	Ac	OAc	H

Standard synthetic routes to a K-region arene oxide all commence by osmium tetroxide addition to the K-region of the hydrocarbon.[6] The cis-dihydrodiol formed is then transformed into the ring-opened di-aldehyde,[7] or the trans-dihydrodiol,[8] or the trans-halohydrin acetate,[9] each of which may be cyclised to an arene oxide.[10] Instead we prepared the K-region oxide by direct oxidation of 7-methylbenz[*c*]acridine by the hypohalite phase-transfer system, a method developed by Krishnan et al.[11] Thus 5,6-epoxy-7-methyl-5,6-dihydrobenz[*c*]-acridine (2a), $C_{18}H_{13}NO$,[12] m.p. 148-149°, was obtained in 49% yield when

7-methylbenz[*c*]acridine in chloroform was oxidised by 0.6 M aqueous sodium hypochlorite containing 0.8M sodium phosphate buffer (pH 8.5), in the presence of tetrabutylammonium hydrogen sulphate (0.45 equiv.). The location of the epoxide at position 5,6 was established from the ^{1}H NMR spectrum ($CDCl_3$). An AB quartet signal (J_{AB} 4.2 Hz) is due to the two vicinal protons on the 5,6-epoxide ring. The higher field proton H-5 showed W-type coupling (0.6Hz) to the characteristic ally low-field bay-region proton H-1 at δ8.95, while the other (H-6) exhibited a nuclear Overhauser effect (NOE) when the 7-methyl, which is peri to it, was irradiated (enhancement relative to H-5, 24%).

When the reaction was carried out without the buffer,[11] the epoxide product (2a) was contaminated with the 7-nor-analogue (2b) formed probably via oxidation of the 7-methyl group[13] and decarboxylation. The ^{1}H NMR spectrum of the 7-nor-epoxide (2b) is characterised by a singlet in the aromatic region due to H-7 (δ8.3), and a doublet signal for H-6 (δ4.6) which is 0.25 p.p.m. upfield of that of the 7-methyl-5,6-epoxide (2a). This difference in chemical shifts reflects the deshielding effect of the peri 7-methyl group. Likewise in the ^{13}C NMR spectrum, C-6 in the nor-epoxide (2b) (which is no longer shielded by a methyl γ-gauche to it) resonated 3.0 p.p.m. lower-field than in the 7-methyl-5,6-epoxide (2a) (55.6 p.p.m. *vs.* 52.6 p.p.m.).

To prepare the trans-5,6-dihydro-5,6-diol we used a one-step process instead of the conventional route from the cis-dihydrodiol via a quinone. 7-Methylbenz[*c*]acridine was oxidised with trifluoroperacetic acid under conditions described by Evans *et al.* for the N-oxidation of a weakly basic N-heteroaromatic compound.[14] The major non-polar product, trans-5,6-dihydroxy-7-methyl-5,6-dihydrobenz[*c*]acridine (3a), $C_{18}H_{15}NO_2$ (*m/e* 277.110), m.p. 241-243°, showed an AB quartet NMR signal (*J* 3.2 Hz) at δ4.9 and 5.4 (in CD_3OD) for protons 5 and 6. For comparison an authentic sample of the corresponding cis-5,6-diol (3c), $C_{18}H_{15}NO_2$, m.p. 248-249°, was synthesised by oxidation of 7-methylbenz[*c*]acridin with osmium tetroxide in pyridine-chloroform.[15] The two dihydrodiols are not identical, nor are the corresponding diacetates [cis-diacetate (3d), $C_{22}H_{19}NO_4$, m.p. 351-353°; trans-diacetate (3b), $C_{22}H_{19}NO_4$, m.p. 253-255°].

The trans-dihydrodiol (3a) was likely formed on ring-opening of the intermediate 5,6-epoxide (2a) by trifluoroacetic acid. The solvolysis of phenanthrene-9,10-oxide in acidic aqueous dioxane had been shown to yield predominantly the trans-9,10-dihydrodiol and the 9-phenol.[16] We have studied the solvolysis of th epoxide (2a) in acetic acid. The products were silylated [$NH(SiMe_3)_2$ and $SiMe_3C$ in pyridine at 70°] and analysed by gas-chromatography chemical ionisation mass spectrometry (using OV17 column and methane). The reconstructed chromatogram an limited mass scans revealed the presence of phenol(s) and at least three dihydro diol monoacetates (MH^+ of silylated species, 392). The trans- and cis-dihydrodiols (3a) and (3c) were then individually partially acetylated using perdeuterated acetic anhydride in pyridine. Analysis showed that (besides the d_6-diacetate

and the unreacted diol) the trans-dihydrodiol (3a) formed two d_3-monoacetates (MH^+ 395), while the cis-dihydrodiol (3c) formed either only one d_3-monoacetate (MH^+ 395) or an inseparable mixture of two d_3-monoacetates. These d_3-monoacetates (MH^+ 395) co-chromatographed with the monoacetates (MH^+ 392) formed in the epoxide solvolysis.

To prepare the K-region phenol(s), the epoxide (2a) was treated overnight with 0.2% sulphuric acid in acetic acid whereupon 5-hydroxy-7-methylbenz[*c*]-acridine (1c), $C_{18}H_{13}NO$, m.p. 230-232°, was isolated. At the high-field end (δ 7.3) of the aromatic region of the ^1H NMR spectrum (20% CD_3SOCD_3 in $CDCl_3$) is a one-proton singlet which showed 20% NOE upon saturation of the 7-methyl signal, and is thus assigned to H-6. A similar enhancement was exhibited by the H-8 signal (δ 8.2; J 8.0, 1.7, 0.6).

The conformational preference of the 5,6-disubstituted 1,3-cyclohexadiene system in the two pairs of K-region dihydrodiols and diacetates (3a) - (3d) may be deduced from the ^1H NMR data.[18] For the trans-dihydrodiol (3a) and its diacetate (3b), the low magnitute of $J_{5,6}$ (3.2 Hz) shows that the two substituents are pseudoaxial. The pseudoequatorial H-6 in the diol (3a) yields a large NOE (32% relative to H-5) upon irradiation of the 7-methyl group peri to it.

In the case of the cis-diacetate (3d), the extreme chemical shift values of the acetate groups (δ 1.93 for pseudoaxial acetate, δ 2.29 for pseudoequatorial one) indicates that one of the two pseudoaxial-pseudoequatorial conformers predominates. A large NOE for H-6 at δ6.3 (37% relative to H-5 at δ6.9) on irradiation of the 7-methyl is evidence that the preferred conformer is the 5-pseudoequatorial-6-pseudoaxial one (with smaller CH_3/OAc non-bonded interaction). The cis-dihydrodiol (with $J_{5,6}$ < 6Hz) may have the same preferred conformation as the cis-diacetate (3d) (with $J_{5,6}$ = 3.6 Hz) since a significant NOE (23%) was observed for H-6.

An examination of the literature[8,19] shows that *^1H shielding of acetate as indicator of conformational preference is of general applicability*[20] *for K-region and non-K-region dihydrodiol mono- or di-acetates of a large number of PAH*. To our knowledge this has not been pointed out by earlier workers.

The mutagenicity of the two dihydrodiols, the epoxide and the 5-phenol in the Ames test have been examined.[5] Only the 5-phenol and the 5,6-epoxide are active, the latter both in the presence and absence of activating liver supernatant. Only weak activity was observed compared with 7-methylbenz[*c*]acridine.

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20. To illustrate, the pseudoaxial acetates of the trans-dihydrodiol diacetates of 7-methylbenz[c]acridine [viz. (3b)] and of 7,12-dimethylbenzanthracene[8] resonate within the narrow range of $\delta 1.91 \pm 0.07$ in $CDCl_3$.

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