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SHORT COMMUNICATION

The *in vitro* metabolic activation of the 11-trifluoromethyl analogue of the potent carcinogen 15,16-dihydro-11-methyl-cyclopenta[a]-phenanthren-17-one to mutagens

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A strongly electronegative, bay-region analogue of the potent carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one, namely 15,16-dihydro-11-trifluoromethylcyclopenta[a]phenanthren-17-one, is mutagenic to *Salmonella typhimurium* TA100. Also it is metabolized at the 1,2- and 3,4-positions in the A-ring as well as C-15 in the D-ring to give 3,4-dihydroxy-3,4,15,16-tetrahydro-11-trifluoromethyl-cyclopenta[a]phenanthren-17-one as the only mutagenic metabolite. In these respects its behaviour is closely similar to that of the 11-methyl compound, suggesting that the electronic nature of the bay-region substituent is rather less critical than its spatial configuration in influencing metabolism to genotoxic intermediates. It remains to be seen, however, whether the trifluoromethyl compound is also a carcinogen.

15,16-Dihydrocyclopenta[a]phenanthren-17-one (**1**, cpp-17-one*) (Figure 1), the parent 17-ketone of the 15,16-dihydrocyclopenta[a]phenanthrene (cpp) series, is devoid of intrinsic carcinogenicity (1); however, substitution of a methyl group at C-11 (Figure 1) confers considerable carcinogenic activity on the molecule (2). This 11-methyl derivative (**2**) has been found to induce skin tumours in a number of mouse strains (1–5) as well as a variety of other soft tissue tumours following s.c. injection in rats and mice (2,3,6). More recently it has been observed to increase the rate of myeloid leukaemia in male Sprague–Dawley rats; in addition the compound was found to act as a potent initiator of silica-induced mesothelioma (7). The carcinogenicity of cpps is believed to be mediated through metabolic activation to bay-region diolepoxides (2,8). It was therefore of interest to examine whether it is the steric or electronic effects of the 11-methyl group that influence the activation of the compound to reactive intermediates. It was decided to synthesize an analogue of 11-CH_3-cpp-17-one (**2**) possessing a group at C-11 which was spatially similar to methyl, yet electronically very different. The most appropriate candidate for this replacement was considered to be trifluoromethyl, due to the similarity in size of hydrogen and fluorine (van der Waals radii H = 1.2 Å; F = 1.35 Å), but their very different affinities for electrons (H = 0.8 eV; F = 3.448 eV, in the gas phase at 0K) (9). Consequently the 11-trifluoromethyl derivative (**3**) was synthesized (10), and its *in vitro* metabolism has now been examined and the identities of the principal metabolites determined. Moreover the compound and its metabolites have been tested for activity in the Ames mutagenicity assay

The 11-trifluoro-17-ketone (**3**) was metabolized and the metabolites separated by HPLC as previously described (11), using hepatic microsomal preparations from rats pretreated with Aroclor 1254 (prepared as outlined in ref. 12). The principal *in vitro* metabolites were tentatively identified on the basis of retention times on HPLC coupled with UV absorption spectra. Previous experience (2,13) has shown that the positions of saturation in the aromatic rings (and hence the positions of the hydroxyl groups) can be unambiguously assigned from their UV chromophores, especially since in each case simple *in situ* reduction of the carbonyl group produces a second, highly

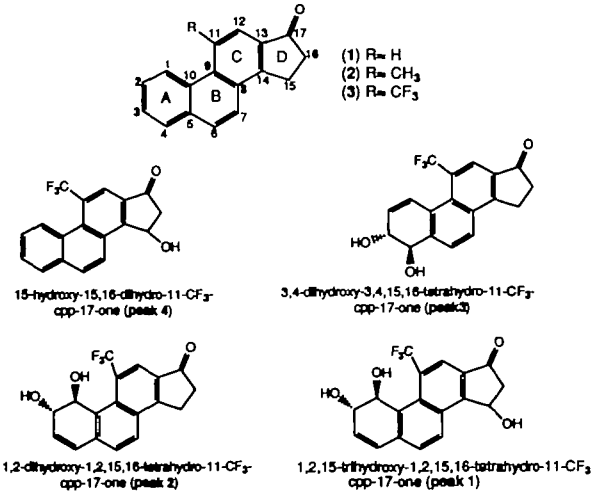

Fig. 1. Structures of compounds **1–3**, and the major metabolites of the 11-trifluoromethyl-17-ketone (**3**).

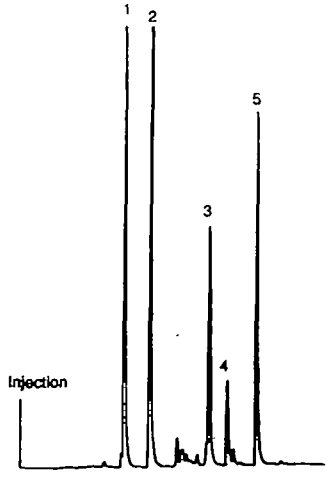

Fig. 2. HPLC profile of the *in vitro* metabolites of 15,16-dihydro-11-trifluoromethylcyclopenta[*a*]phenanthren-17-one. Metabolism was carried out as previously described. Trace monitored by UV absorption at 254 nm.

*Abbreviations: cpp-17-one, 15,16-dihydrocyclopenta[*a*]phenanthren-17-one. Other derivatives of 15,16-dihydrocyclopenta[*a*]phenanthrene (cpp) are similarly abbreviated.

Table I. Mass spectral data of peaks 1–5

Peak	Predicted mol. wt	*m/z* (% relative abundance) and probable fragment(s) lost	*m/z* of molecular ion of TMS derivative
5	$C_{18}H_{11}OF_3$ M, 300	300 (100), M; 272 (7), M-CO; 231 (11) M-CF_3; 203 (91), M-CO-CF_3	–
4	$C_{18}H_{11}O_2F_3$ M, 316	316 (100), M; 298 (45), M-H_2O; 288 (11), M-CO; 270 (47), M-H_2O-CO; 251 (50), M-H_2O-CO-F; 247 (11), M-CF_3; 229 (16), M-H_2O-CF_3; 219 (18), M-CO-CF_3	388 (one OH group)
3	$C_{18}H_{13}O_3F_3$ M, 334	334 (13), M; 316 (100), M-H_2O; 288 (80), M-H_2O-CO; 287 (12), M-H_2O-CHO; 259 (13), M-H_2O-CO-CHO; 219 (52), M-H_2O-CO-CF_3	478 (two OH groups)
2	$C_{18}H_{13}O_3F_3$ M, 334	334 (19), M; 316 (43), M-H_2O; 288 (100), M-H_2O-CO; 287 (9), M-H_2O-CHO; 259 (11), M-H_2O-CO-CHO; 219 (34), M-H_2O-CO-CF_3	478 (two OH groups)
1	$C_{18}H_{13}O_4F_3$ M, 350	350 (16), M; 332 (30), M-H_2O; 314 (31), M-2H_2O; 304 (49), M-H_2O-CO; 286 (43), M-2H_2O-CO; 258 (21), M-2H_2O-2CO; 189 (100), M-2H_2O-2CO-CF_3	566 (three OH groups)

characteristic chromophore. These identities were then verified by 70 eV electron impact mass spectrometry using a VG Quattro instrument; the metabolites were dissolved in ethyl acetate for direct insertion, and later derivatized by addition of trimethylsilyl imidazole (Aldrich, product no. 15 358–3) to the solution for a few minutes at room temperature before reinsertion. Mutagenic potential was determined in the Ames test (14) using *Salmonella typhimurium* TA100 as previously described (11)

Incubation of 11-CF_3-cpp-17-one (**3**) with microsomal preparations from Aroclor 1254-treated rats revealed the presence of five major peaks which were absent from blank microsomal preparations (Figure 2). Peak 5, being the least polar compound present, was unchanged 11-trifluoromethyl-cpp-17-one (**3**) identified by its retention time and UV absorption spectrum (λ_{max} *271*, 300, 354, 371; after BH_4^- *259* nm—italics indicate peaks of highest intensity). Peak 4 was assigned the structure of a D-ring mono-ol since it was observed to be somewhat more polar than the parent compound (peak 5), and its UV spectrum was very similar (λ_{max} *275*, 300, 357, 374; after BH_4^- *258* nm) as expected, since they possess the same chromophore. It is thought likely that the compound was in fact the 15-hydroxy derivative since 15-ols have been found as major metabolites of other cpp-17-ones (2). Peak 3 was found to inhabit the region of the chromatograph usually occupied by a diequatorial 3,4-dihydrodiol and this was confirmed by its UV absorption characteristics (λmax *268*, 331; after BH_4^- *246*, 323 nm). Peaks 1 and 2 were observed to elute at HPLC areas usually inhabited by the diaxial 1,2-dihydrodiols and 1,2,15-triols, and their structures were again established by their ultraviolet spectra (peak 1, λ_{max} 234, *272*, 334; after BH_4^- *257*, *265*, 311 nm; peak 2, λ_{max} 233, *265*, 331; after BH_4^- *258*, *266*, 310 nm). Since peak 1 was rather more polar than peak 2 it was considered likely that peak 2 was the 1,2-dihydrodiol, while peak 1 was the 1,2,15-trihydroxy derivative found as a major metabolite of other cpp-17-ones (2). All these retention times were closely similar to those of the corresponding metabolites derived from the 11-methyl compound, in these cases known to be *trans*. These tentative identifications were confirmed by MS (Table I) which showed that the metabolites' mol. wts and major fragments were consistent with the proposed structures (see Figure 1). Moreover,

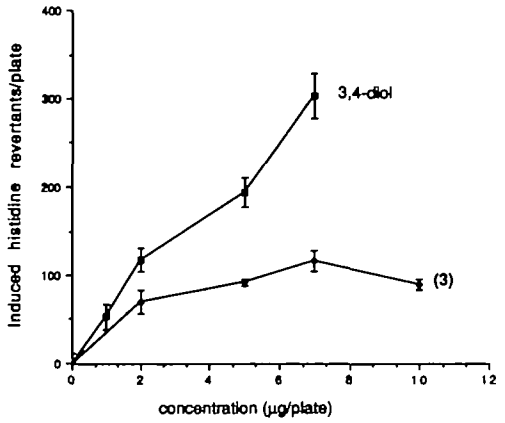

Fig. 3. Activation of 11-CF_3-cpp-17-one (3) and 3,4-dihydroxy-11-CF_3-cpp-17-one to mutagens by hepatic microsomal preparations from rats pretreated with Aroclor 1254. Spontaneous reversion rate was 81 ± 12. Figures are presented as mean ± SD. Neither compound was mutagenic in the absence of an hepatic microsomal activation system.

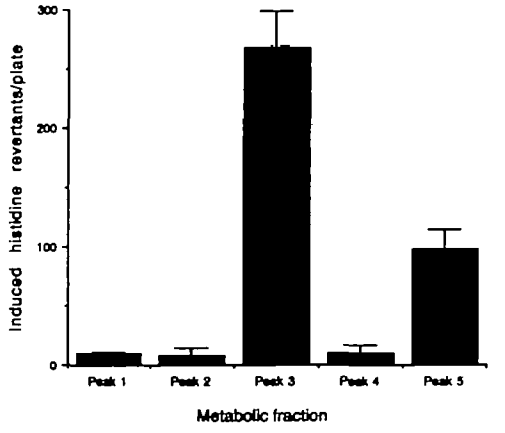

Fig. 4. Mutagenicity of the principal compounds separated by HPLC following *in vitro* metabolism of 11-CF_3-cpp-17-one. All compounds tested at 7 µg/plate using a 10% (v/v) microsomal activation system prepared from the livers of rats pretreated with Aroclor 1254. Spontaneous reversion rate was 87 ± 9. Figures are presented as mean ± SD.

after treatment with trimethylsilyl imidazole in order to convert hydroxyl residues to their trimethylsilyl ethers, in each case the molecular ion was in accord with the expected number of OH groups.

The 11-trifluoro-17-ketone (**3**) was examined in the Ames test using *Salmonella typhimurium* TA100 and a 10% (v/v) microsomal activation system utilizing microsomes from rats pretreated with Aroclor 1254 (Figure 3). Since the compound was found to be mutagenic only after metabolic activation, it was decided to ascertain which of the *in vitro* metabolites were involved in its mutagenicity. The five principal peaks present after *in vitro* metabolism were collected and put into solution in DMSO at 100 μg/ml. Each was tested, with metabolic activation, at 7 μg/plate, the most mutagenic concentration of 11-CF_3-cpp-17-one (Figure 3). Only peak 5 [11-CF_3- cpp-17-one (**3**)] and peak 3 (the 3,4-dihydrodiol) were found to be mutagenic (Figure 4). To confirm the mutagenicity of the 3,4-dihydrodiol (peak 3) the assay was repeated at four concentrations of the compound (Figure 3), giving a good dose–response relationship.

Thus the *in vitro* metabolism of 15,16-dihydro-11-trifluoromethylcyclopenta[a]phenanthren-17-one (**3**) by hepatic microsomal preparations resulted in the production of four principal metabolites (Figure 1) identified as 15,16-dihydro-15-hydroxy-11-trifluoromethylcyclopenta[a]phenanthren-17-one (peak 4), 3,4-dihydroxy-3,4,15,16-tetrahydro-11-trifluoromethylcyclopenta[a]phenanthren-17-one (peak 3), 1,2-dihydroxy-1,2,15,16-tetrahydro-11-trifluoromethylcyclopenta[a]phenanthren-17-one (peak 2) and 1,2,15,16-tetrahydro-1,2,15-trihydroxy-11-trifluoromethylcyclopenta[a]phenanthren-17-one (peak 1). Thus the 11-trifluoromethyl compound (**3**) is metabolized in a qualitatively similar manner to its 11-methyl analogue (**2**) (15) in that metabolism occurs at two sites on the A-ring and at one of two positions on the D-ring. Although the metabolism proceeds in a qualitatively similar manner, in terms of the major metabolites produced, to that of 11-CH_3-cpp-17-one, the overall metabolic profile of the 11-trifluoromethyl compound (**3**) is simpler (Figure 2). Metabolism of the 11-methyl compound (**2**) produces a more complex pattern of minor metabolites such as the 16-ol and 11-hydroxymethyl compound and their A-ring derivatives (2) which are largely absent in the metabolism of its 11-CF_3 analogue.

The activation of 11-CF_3-cpp-17-one (**3**) to genotoxic intermediates appears to proceed via the 3,4-dihydrodiol since this was the only metabolite found to be mutagenic (Figures 3 and 4) and moreover was observed to be a more potent mutagen than the parent compound. The cpp-17-ones are considered to exert their observed genotoxicity through a bay-region 3,4-diol-1,2-epoxide (2) and it is known that this reactive intermediate is generated from the parent compound via the 3,4-dihydrodiol which is more mutagenic than the parent compound (2,11). Thus it appears that 11-CF_3-cpp-17-one (**3**) is activated to genotoxic species in an entirely analogous manner to its 11-methyl analogue (**2**). The electronic nature of the -CF_3 substituent at C-11 is radically different from that of the -CH_3 group that it mimics, yet qualitatively similar metabolism occurs especially in the apparent activation of the compound to genotoxins. Given the apparent similarity of the metabolism and activation of 11-CH_3-cpp-17-one (**2**) and its 11-CF_3 analogue (**3**), it would appear that the electronic nature of the substituent at C-11 is not vital in determining whether or not the compound is activated to genotoxic intermediates; consequently the more important effect of the substituent at C-11 would appear to be steric. Whether the electronic nature of the substituent at C-11 affects the carcinogenicity of the molecule is a question that cannot be addressed through the medium of these metabolic studies and must therefore await the results of longer term tumorigenicity experiments.

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