Synthesis of the Ultimate Hepatocarcinogen, 2-Acetylaminofluorene *N*-Sulphate

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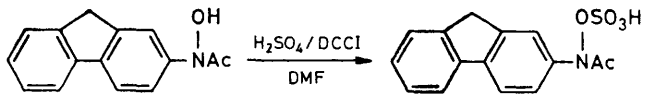
Reaction of *N*-hydroxy-2-acetylaminofluorene with dicyclohexylcarbodi-imide and H_2SO_4 yields 2-acetylaminofluorene *N*-sulphate, the presumed ultimate form of the hepatocarcinogen 2-acetylaminofluorene.

The hepatocarcinogen 2-acetylaminofluorene (2-AAF) is metabolically activated to an electrophile through sequential *N*-oxidation and *O*-esterification.[1,2] A specific ester, 2-AAF *N*-sulphate, has been implicated in the carcinogenic action of this arylamide.[3,4] Metabolic inhibition of 2-AAF *N*-sulphate formation results in decreases in macromolecular binding,[5] specific DNA adducts,[6] glutathione conjugates,[7] and a prevention of hepatotoxicity.[8] The synthesis of 2-AAF *N*-sulphate has been described but the product was not fully characterized.[9] We now report a simple preparation of this compound through the use of dicyclohexylcarbodi-imide (DCCI) and H_2SO_4.[10,11]

To *N*-hydroxy-2-AAF (**1**) (100 mg) were added a 5-fold molar excess of DCCI and dimethylformamide (DMF; 20 ml;



(1) (2)

previously dried over 3 Å molecular sieves). The mixture was stirred, concentrated H_2SO_4 (26 μl; 1.1 molar excess) was added, and the reaction was continued for 1.5 h during which time a white precipitate formed. The solution was filtered and the precipitate was rinsed with DMF (10 ml). The combined DMF fractions, which were beige in colour, were purged with a rapid stream of NH_3 gas for 3 min to neutralize residual acid and then evaporated under reduced pressure to yield 2-AAF *N*-sulphate (**2**). Further purification was not attempted, owing to the lability of the compound.

The u.v. spectrum of (**2**) had λ_{max} 276 nm (ethanol) with a shoulder at 302 nm. Its i.r. spectrum showed strong S–O bond stretching at 1200—1300 cm^{-1}. Its 500 MHz ^1H n.m.r. spectrum in CD_3SOCD_3 exhibited all the expected resonances which were assigned by nuclear Overhauser effect and homonuclear decoupling experiments[12] [δ 7.66 (d, 1H, H-1, $J_{1,3}$ 1.84 Hz), 7.47 (dd, 1H, H-3, $J_{3,4}$ 8.46 Hz), 7.83 (d, 1H, H-4), 7.87 (d, 1H, H-5, $J_{5,6}$ 7.35 Hz), 7.38 (dd, 1H, H-6, $J_{6,7}$ 7.35 Hz), 7.30 (dd, 1H, H-7, $J_{7,8}$ 7.35 Hz), 7.58 (d, 1H, H-8), 3.92 (s, 2H, CH_2), and 2.32 (s, 3H, CH_3)]. There were additional aliphatic signals, due to residual dicyclohexylurea. Fast atom bombardment mass spectrometry on the sodium salt of (**2**) gave ions at *m/z* 364 and 319 which represent the disodium complex of the sulphate anion and the (M −1) ion of (**2**), respectively.

Incubation of (**2**) with deoxyguanosine resulted in the formation of *N*-(deoxyguanosin-8-yl)-2-AAF, the same compound obtained from the reaction of the model ultimate carcinogen, *N*-acetoxy-2-AAF, with deoxyguanosine.[13] Reaction with glutathione yielded four conjugates which have been previously identified as 1-, 3-, 4-, and 7-(glutathion-*S*-yl)-2-AAF.[7] The *N*-sulphate underwent rapid solvolysis in 100 mM potassium phosphate buffer, pH 7.4, to give at least three products as indicated by reversed-phase high pressure liquid chromatography that as yet have not been identified.

This simple synthesis should be applicable to other sulphate esters of carcinogenic aromatic amines and amides.[14]

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