

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

Frederick A. Beland,* Dwight W. Miller, and Ronald K. Mitchum

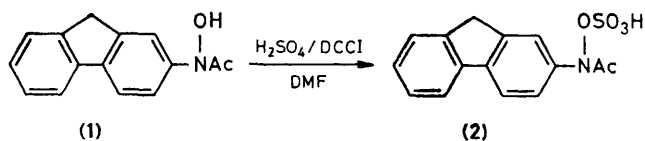
National Center for Toxicological Research, Jefferson, Arkansas 72079, U.S.A.

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;



previously dried over 3 Å molecular sieves). The mixture was stirred, concentrated H_2SO_4 ($26 \mu\text{l}$; 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 $1200\text{--}1300 \text{ cm}^{-1}$. 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 experiments¹² [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

Received, 1st October 1982; Com. 1160

References

- 1 E. Kriek and J. G. Westra, in 'Chemical Carcinogens and DNA,' ed. P. L. Glover, CRC Press, Boca Raton, FL, 1979, Vol. 2, pp. 1—28.
- 2 E. C. Miller, *Cancer Res.*, 1978, **38**, 1479.
- 3 J. R. DeBaun, E. C. Miller, and J. A. Miller, *Cancer Res.*, 1970, **30**, 577.
- 4 J. H. Weisburger, R. S. Yamamoto, G. M. Williams, P. H. Grantham, T. Matsushima, and E. K. Weisburger, *Cancer Res.*, 1972, **32**, 491.
- 5 J. H. N. Meerman, A. B. D. van Doorn, and G. J. Mulder, *Cancer Res.*, 1980, **40**, 3772.
- 6 J. H. N. Meerman, F. A. Beland, and G. J. Mulder, *Carcinogenesis*, 1981, **2**, 413.
- 7 J. H. N. Meerman, F. A. Beland, B. Ketterer, S. K. S. Srail, A. P. Bruins, and G. J. Mulder, *Chem. Biol. Interact.*, 1982, **39**, 149.
- 8 J. H. N. Meerman and G. J. Mulder, *Life Sci.*, 1981, **28**, 2361.
- 9 V. M. Maher, E. C. Miller, J. A. Miller, and W. Szybalski, *Mol. Pharmacol.*, 1968, **4**, 411.
- 10 V. P. Hoiberg and R. O. Mumma, *J. Am. Chem. Soc.*, 1969, **91**, 4273.
- 11 D. B. Johnson and R. T. Thissen, *J. Chem. Soc., Chem. Commun.*, 1980, 598.
- 12 F. A. Beland, W. T. Allaben, and F. E. Evans, *Cancer Res.*, 1980, **40**, 834.
- 13 E. Kriek, J. A. Miller, U. Juhl, and E. C. Miller, *Biochemistry*, 1967, **6**, 177.
- 14 G. J. Mulder, in 'Sulfation of Drugs and Related Compounds,' ed. G. J. Mulder, CRC Press, Boca Raton, FL, 1981, pp. 213—226.