Synthesis and Stability of Sulphate Ester Conjugates of Monohydroxybenzo[*a*]-pyrenes in relation to Possible Benzo[*a*]pyrene Detoxification Mechanisms

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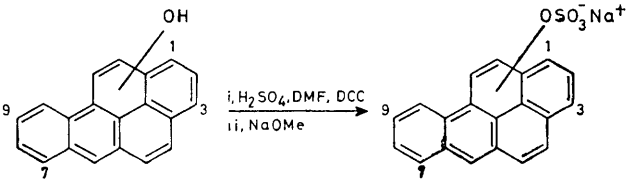
Summary The reaction of 1-, 3-, 7-, and 9-monohydroxy benzo[*a*]pyrenes with dicyclohexylcarbodi-imide (DCC) and H_2SO_4 in dimethylformamide yields the corresponding sulphate esters, which are stable in a pH range of 2 to 14 at 37 °C.

THE sulphate ester conjugates of monohydroxybenzo[*a*]-pyrenes have been reported as major water-soluble metabolites in cell culture.[1] Also, Cohen[2] and Autrup[3] have shown that the sulphate esters are major metabolites in short-term cultures of human lung and human colon, respectively. Therefore, the sulphates of the known monohydroxy metabolites[4] of benzo[*a*]pyrene were prepared and their stability toward hydrolysis was examined.

Sulphation of the 3-hydroxybenzo[*a*]pyrene[2] with SO_3–pyridine or sulphonyl chloride gave ring substitution and poor yields of the sulphate. However, the 3-hydroxy-benzo[*a*]pyrene was smoothly converted into the benzo-[*a*]pyrene-3-sulphate ester with dicyclohexylcarbodi-imide and 2 equiv. of H_2SO_4 in dimethylformamide (DMF) at 0 °C.[5] The sulphate ester was purified by applying the reaction mixture to a DEAE cellulose column,[6] pretreated with 1 N NaOMe in methanol. After eluting with two column volumes of methanol, the sulphate ester was eluted with 1 M NaOMe in methanol. The yield of sodium benzo-[*a*]pyrene-3-sulphate was 85%, after recrystallization from methanol–water. The sulphate esters of 1-, 7-, and 9-hydroxybenzo[*a*]pyrenes were prepared in a similar manner, to give 80%, 90%, and 70% yields, respectively.

The spectral data were consistent with the structure of the four benzo[*a*]pyrene sulphates.† The i.r. spectra of the sulphates are characterized by broad, strong S–O bands



between 1220 and 1280 cm^{-1}. Also, the u.v. spectra of the sulphate esters have the same general line shape as the parent compounds. However, many of the peaks are shifted to shorter wavelengths and decreased in absorption intensity. Furthermore, the u.v. spectrum of each sulphate was identical in either ethanol or ethanolic sodium hydroxide, which eliminates the possibility of ring rather than hydroxy substitution.

The enzymic hydrolysis and stability of the sulphates were studied with sulphatase, from *Helix pomatia*, and buffered aqueous solutions, respectively. The sulphatase rapidly hydrolysed the 1-, 3-, and 9-benzo[*a*]pyrene sulphate esters to their parent phenols. However, the 7-benzo[*a*]-pyrene sulphate ester was refractory to sulphatase hydrolysis, which may be attributed to steric hindrance.‡ All of the benzo[*a*]pyrene sulphates were stable in a pH range of 2 to 14 for 24 h at 37 °C. Also, all of the sulphates were stable for 4 h in 0·5 M H_3PO_4 at 37 °C. Because of the resistance of benzo[*a*]pyrene sulphates to non-enzymic hydrolysis, they may play a key role in the detoxification of benzo[*a*]pyrene.

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† Correct combustion analyses were obtained for the 1-, 3-, 7-, and 9-sulphate esters.

‡ The non-bonded interaction of the hydrogen in the 6-position with the 7-sulphate ester may prevent enzyme hydrolysis.

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