Interaction of 5α,6α-Cholesterol Oxide with DNA and Other Nucleophiles

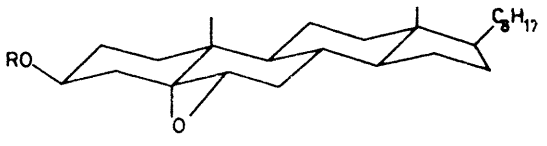
By G. MICHAEL BLACKBURN,* ABDUL RASHID, and MICHAEL H. THOMPSON†
(*Department of Chemistry, The University, Sheffield* S3 7HF *and*
†*Bacterial Metabolism Research Unit, PHLS, Colindale Hospital, London* NW9 5DX)

Summary 5α,6α-Cholesterol oxide forms a strong physical complex with DNA which, on incubation, leads to a significant amount of covalent attachment of steroid to DNA; both this oxide and its 3-acetate give adducts with neutral thiols and heterocyclic amines, the former alone responding to acidic catalysis.

THE potential carcinogenicity of certain oestrogenic steroids has led to investigations of their covalent bonding to DNA *in vitro*[1,2] and *in vivo*[3] in association with their metabolism by oxygenase enzymes. Colon cancer is thought to be associated with faecal steroid levels[4] and a particular correlation has been established in the case of cholesta-3β,5α,6β-triol, a known metabolite of cholesterol[5] which can be produced *in vivo* from exogenous 5α,6α-cholesterol oxide.[6] This oxirane can be formed photochemically in skin *in vivo*,[7] detected in human serum,[8] and shown capable of tumour initiation in animals.[9] We now report studies of its interaction with DNA *in vitro* and with a variety of nucleophiles.

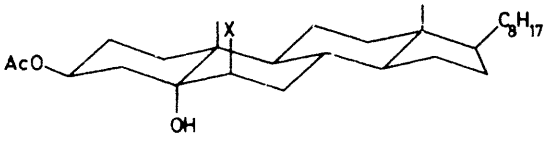
14-Carbon-labelled oxide (**1a**) was prepared[10] from [4-^{14}C]cholesterol and equilibrated with calf thymus DNA at pH 6·8. The physical complex formed was analysed by both caesium chloride density centrifugation and by gel-filtration on Sephadex G-200. In both cases the DNA-containing fraction showed a coincidence of u.v. absorption and of radioactivity. The extent of steroid association was in excess of one molecule per hundred DNA base-pairs. Much lower levels of physical association were observed under identical conditions for cholesterol, oestradiol, and progesterone. The physical complexes were readily disrupted by extraction of the steroid into organic solvents.

Prolonged incubation of a physical complex between DNA and the oxide (**1a**) led to extensive covalent attachment of the steroid to the DNA (300 μmol per mole base-pairs). This bonding survived enzymatic degradation of



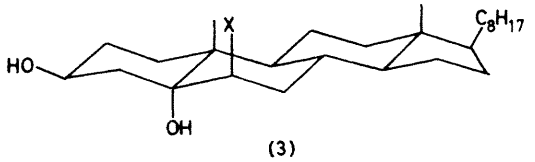
(1)

a, R = H; b, R = MeCO



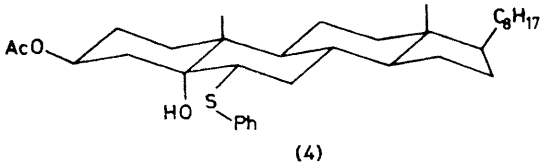
(2)

a, X = *p*-$MeC_6H_4SO_3$; b, X = CF_3CO_2
c, X = PhS; d, X =



(3)

a, X = *p*-$MeC_6H_4SO_3$; b, X = CF_3CO_2
c, X = PhS; d, X = 



(4)

the DNA,[11] the products of which gave a principal radioactive peak on LH-20 gel chromatograms similar in elution profile to fragments resulting from the covalent binding of benzo[*a*]pyrene epoxides to DNA.[12]

Since such covalent interaction with DNA must involve neutral nucleophiles with, possibly, general acid catalysis, we have explored the influence of various acids on the addition of neutral nucleophiles to (**1a**) and to its 3β-acetate (**1b**)

Imidazole adds to (**1b**) under neutral conditions at 80 °C to yield the 6β-imidazolyl derivative‡ (**2d**) while wet[13] piperidine opens the epoxide with concomitant deacylation to give (**3d**). Neither of these amines showed any benefit from the presence of phosphoric or other acids in their reaction with (**1b**).

Ethanethiol and benzenethiol proved unreactive towards (**1a**) and (**1b**) at room temperature but, in the presence of a catalytic amount of phosphoric acid, benzenethiol with (**1b**) gave (**2c**) in good yield, though only traces of an adduct were obtained using ethanethiol. Neither *p*-toluenesulphonic acid nor trifluoroacetic acid promoted the addition of these thiols to (**1a**) or (**1b**). Instead, even in the presence of a large excess of thiol, the tosylates (**2a**) and (**3a**) were formed by addition of *p*-toluenesulphonic acid, and the known[14] compounds (**2b**) and (**3b**) by addition of trifluoroacetic acid. Deacetylation of (**2c**) gave (**3c**), also formed from (**1a**) by the action of sodium benzenethiolate *via* normal transdiaxial ring-opening of the epoxide. The tosylate (**2a**) gave an isomer of (**2c**) with sodium benzenethiolate, provisionally assigned structure (**4**).

The 5α,6α-cholesterol oxide ring is thus seen to exhibit relatively weak electrophilic activity under neutral and mild acidic conditions and thus its effective bonding to DNA must be attributed in some measure to the strong physical affinity of these two species reported here. The biological importance of this phenomenon was explored by subjecting the oxide (**1a**) to assay by means of the Ames' Test,[15] which showed it to be sufficiently cytotoxic to preclude evaluation of its mutagenicity.

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‡ All new compounds have been fully characterised by elemental and spectroscopic analyses.

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