Since total microsomal protein and glucose-6-phosphatase activity were not significantly changed, the cytochrome synthesis was probably inhibited. The liver cytochromes are principally located in the hepatocytes in which the endoplasmic reticulum is much more developed than in the Kuppfer cells.[10] On the other hand, the majority of the carbon particles accumulate in the latter cells.[4] Thus the decrease in microsomal cytochromes is an indirect effect showing that very close biochemical relations exist between the two principal types of liver cells. The cause of the inhibition of cytochrome synthesis could be a lack of iron in the liver. We have found no variation in the total liver iron, but this does not exclude a perturbation in the exchange of this metal between the Kuppfer cells and the hepatocytes.

Loading of the liver reticuloendothelial cells with carbon particles is therefore a non-toxic method useful for the study of microsomal cytochrome turnover and function. It supplements the information obtained from the induction of cytochromes by phenobarbital or methylcholantrene. Using this method we have shown that a decrease in the level of P-450 could explain the protective effect of carbon particle injection against carbon tetrachloride injury,[4,11] since the hepatotoxicity of this compound is thought to be due to its activation as a free radical by cytochrome P-450.[12]

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Steric hindrance to hydroxyl-group assisted acyl group migration in 2′(3′)-*O*-acylribonucleosides: a reassessment and correction

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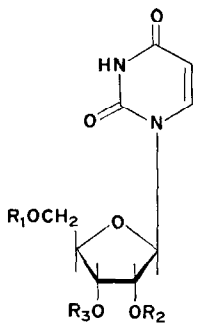
WE REPORTED earlier[1] on aminoacylribonucleosides, prepared as potential antitumour agents and derived from the tumour inhibitor, 1-aminocyclopentanecarboxylic acid. It was suggested that migration of the aminoacyl group between the 2′- and 3′-positions in compounds (I) and (II) (and their adenosine analogues) might be inhibited by steric factors. More recent studies, however, have led us to revise this view.

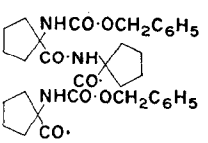
When 3′,5′-di-*O*-benzyluridine[2] was fused with 1-(benzyloxycarbonylamino)cyclopentanecarboxylic anhydride (reaction in solvents, e.g. pyridine, dimethylformamide, was slower and more incomplete)

two nucleosidic products were formed. On the basis of elemental analysis and NMR spectroscopy, which served to define the number and site of protons in the products, structures (III) and (IV) were assigned to these materials. The formation of the peptide derivative (III) had not been anticipated. Catalytic hydrogenolysis of compound (III) gave a product negative towards the *cis*-diol[3] and ninhydrin reagents, as expected for structure (V) (it is known[1] that the free dipeptide (VI) is ninhydrin-negative on TLC plates). Hydrogenolysis of compound (IV) led to a product which did not respond to the *cis*-diol test but gave a purple colour with ninhydrin, findings consistent with structure (II). Since hydrogenolysis of the valyl analogue of compound (IV) was accompanied by equilibration to give a mixture of 2′- and 3′-*O*-valyluridine,[2] it is probable that equilibration of the products from compounds (III) and (IV) also occurs, although the products are formally depicted herein as 2′-isomers.

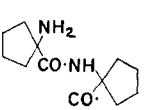


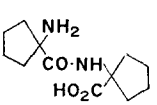
(I) $R_1 = R_2 = H$, $R_3 =$ 

(II) $R_1 = R_3 = H$, $R_2 =$ 

(III) $R_1 = R_3 = C_6H_5CH_2$, $R_2 =$ 

(IV) $R_1 = R_3 = C_6H_5CH_2$, $R_2 =$

(V) $R_1 = R_3 = H$, $R_2 =$ 

(VI) 

Derivatives (II) and (V) were respectively identical in their chromatographic and hydrolytic behaviour to the two products designated "slow" and "fast", obtained[1] via the reaction between 5′-*O*-trityluridine and 1-(benzyloxycarbonylamino)cyclopentanecarboxylic anhydride.

Our earlier assignment[1] of structures (I) and (II) to the "fast" and "slow" compounds respectively was therefore wrong. It was based on elemental analytical data, but these figures do not in fact discriminate adequately between the protected or free amino-acyl nucleoside and a mixture of this and the dipeptidyl analogue.

The acylated trityluridines (and, by analogy, the acylated ditrityladenosines) used in the earlier series of reactions[1] must therefore have been mixtures of benzyloxycarbonyl-aminoacyl and -dipeptidyl derivatives.

In the original work,[1] a product chromatographically and chemically similar to the dipeptide (VI) was found in basic and neutral hydrolysates of the "fast" component (uridine or adenosine series). We now ascribe the formation of this dipeptide to the direct cleavage of the ester (V) and its 3′-isomer (or the analogous adenosine derivatives).

The present studies thus remove the basis for the postulate that steric hindrance may inhibit the migration of bulky acyl groups in nucleoside derivatives. Indeed, the similarly-hindered pivaloyl derivatives analogous to compounds (I) and (II) have been shown to interconvert,[2] as have the corresponding valyl derivatives,[2] under conditions like those prevailing during the hydrogenolysis of intermediates (III) and (IV).

A precisely analogous series of reactions to those reported above occurred[4] when 2′,5′-di-*O*-benzyluridine[2] was the starting material.

Experimental. 3′,5′-Di-O-benzyluridine[2] (300 mg) and 1-(benzyloxycarbonylamino)cyclopentanecarboxylic anhydride (600 mg) were heated at 110° for 16 hr. Two nucleosidic products were detected (TLC, Merck Kieselgel GF_{254}, chloroform–methanol, 47:3), R_f 0·55 and 0·59. The cooled melt was subjected to preparative TLC in the same solvent (20 × 20 cm plates; Merck Kieselgel). Subsequent elution with chloroform–methanol (9:1) gave (a) 2′-*O*-[1-(1-benzyloxycarbonylaminocyclopentanecarbonylamino)cyclopentanecarbonyl]-3′,5′-di-*O*-benzyluridine (III, R_f 0·55), a white solid (100 mg) of indefinite m.p. (Found: C, 66·0; H, 6·2; N, 7·0%. Calc. for $C_{43}H_{48}N_4O_{10}$: C, 66·1; H, 6·2; N, 7·2%), τ (10% w/v in CCl_4) 2·39 (1H, d, $J_{5,6}$ 8·5 Hz, H-6), 2·72 (15H, m, aromatic H), 3·96 (1H, d, $J_{1',2'}$

3·5 Hz, H-1′), 4·50 (1H, d, H-5), 4·93 (2H,s) and 5·57 (4H,s), both benzyl CH_2; 8·21 (16H, m, cyclopentane H); (b) 2′-*O*-(1-benzyloxycarbonylaminocyclopentanecarbonyl)-3′,5′-di-*O*-benzyluridine (IV, R_f 0·59), a white solid (30 mg) of indefinite m.p. (Found: C, 66·0; H, 5·5; N, 6·1%. Calc. for $C_{37}H_{39}N_3O_9$; C, 66·4; H, 5·9; N, 6·3%), τ (10% w/v in CCl_4) 1·91 (1H, d, $J_{5,6}$ 7·0 Hz, H-6), 2·78 (15H, m, aromatic H), 4·18 (1H, d, $J_{1',2'}$ 5·0 Hz, H-1′), 4·56 (1H, d, H-5), 8·34 (8H, m, cyclopentane H); benzyl-CH_2 signals overlap those of ribose protons.

Compounds (III) and (IV) (mg quantities) were separately hydrogenolyzed over a 5% palladium–charcoal catalyst in acetic acid–water (4:1). In each case, a unique final product was formed. Compound (III) gave a product of R_f (TLC, 144 LS/254 Avicel, *n*-butanol–acetic acid–water, 5:2:3) 0·73 (negative *cis*-diol and ninhydrin tests); (IV) afforded a compound of R_f 0·63 (negative *cis*-diol test, positive ninhydrin reaction). These products were respectively identical both chromatographically and hydrolytically to the "fast" and the "slow" derivatives of uridine described previously and using the systems and methods reported for these substances.[1]

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