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AMINOACYL NUCLEOSIDES DERIVED FROM THE TUMOUR INHIBITOR, 1-AMINOCYCLOPENTANE-CARBOXYLIC ACID

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Abstract—The 2′(3′)-*O*-adenosine and -uridine esters of 1-aminocyclopentanecarboxylic acid have been prepared. They had no significant effect against an experimental plasma cell tumour in mice, nor did they inhibit protein synthesis *in vitro*. Each aminoacyl derivative was separated into its two components which were characterized by n.m.r. spectroscopy. No interconversion between the 2′- and 3′-substituted nucleosides occurred, although base catalyzed hydrolysis proceeded at a rate comparable with that of other aminoacyl nucleosides. The possible implications of these findings in protein biosynthesis are discussed.

Some related compounds derived from 6-(methylthio) purine are described.

1-AMINOCYCLOPENTANECARBOXYLIC acid (ACPA; Ia) has been shown to inhibit the growth of a number of experimental tumours.[1] In general, marked tumour inhibition was obtained only at doses showing toxicity. Most clinical reports[2,3] indicated that ACPA was ineffective in human cancer, although objective responses were claimed in some instances[3,4] and there may be a case for further clinical studies.[5]

The mechanism of antitumour action of ACPA in mammals has not been established, but may be related to interference with cellular uptake of natural amino acids.[6–8] The analogue inhibited the incorporation of valine into rat tissues,[9] apparently by suppressing the formation of the valyl ester of the corresponding transfer RNA (tRNA). The relevance of this finding to the mechanism of carcinostasis is not clear. Although ACPA also inhibited the incorporation of amino acids into Ehrlich ascites tumour cells, the effect is probably a secondary one arising from transport inhibition.[6,8] ACPA is not itself incorporated to a significant extent into mammalian proteins, either *in vitro*[6] or *in vivo*,[9,10] although the enzymic activation of ACPA is known to occur.[11]

It has been reported[12] that synthetic 2′(3′)-*O*-leucyl adenosine and a mixture of phenylalanyl- and tyrosyl-adenosines isolated from tRNA inhibited *E.coli* mRNA-mediated protein synthesis ,although the potency of these compounds was 10 or 100 times less than that of puromycin. This work suggested that the analogous ACPA derivative might inhibit protein synthesis and show tumour-inhibitory effects greater than, or different from, those of ACPA itself. It was also thought remotely possible that an ACPA adenosine ester (II) might become incorporated into the terminal

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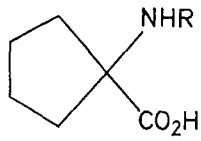
position of a tumour tRNA molecule, to give an aminoacyl tRNA. The corresponding uridine derivatives (III a,b) were prepared for comparative purposes, and an attempt was also made to synthesise the analogous derivatives (IV a,b) of 6-(methylthio)purine riboside.

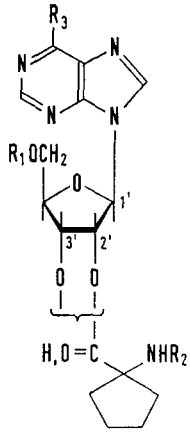
CHEMICAL METHODS AND RESULTS

The basic synthetic method was the condensation of *N*-benzyloxycarbonyl-ACPA (Ib) with 5′-*O*-trityl- or N^6, $O^{5'}$-ditritylnucleoside and the subsequent removal of the protecting groups. An attempt to condense *N*-benzyloxycarbonyl-ACPA directly with 5′-*O*-tritylladenosine by the use of dicyclohexylcarbodiimide in pyridine was unsuccessful; only the anhydride (X) was formed. This result contrasted with earlier work[13] in which benzyloxycarbonylphenylalanine was successfully used. The required



Ia; R = H

Ib; R = $C_6H_5CH_2OCO$



II, $R_1 = R_2 = H$; $R_3 = NH_2$

IV; $R_1 = R_2 = H$; $R_3 = CH_3S$

V; $R_1 = (C_6H_5)_3C$; $R_2 = C_6H_5CH_2OCO$; $R_3 = NH_2$

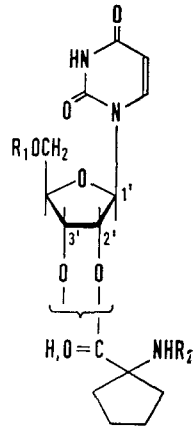
VI; $R_1 = H$; $R_2 = C_6H_5CH_2OCO$; $R_3 = NH_2$

VII; $R_1 = (C_6H_5)_3C$; $R_2 = C_6H_5CH_2OCO$; $R_3 = (C_6H_5)_3CNH$

VIII; $R_1 = H$; $R_2 = C_6H_5CH_2OCO$; $R_3 = CH_3S$

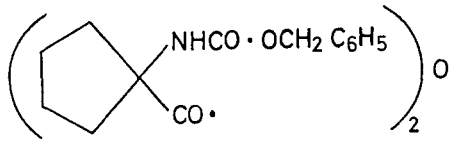
IX; $R_1 = (C_6H_5)_3C$; $R_2 = C_6H_5CH_2OCO$; $R_3 = CH_3S$
(a, 2′-isomer; b, 3′-isomer)

2′(3′)-*O*-acyl-5′-*O*-trityladenosine (Va,b) was, however, obtained by reaction of the anhydride (X) with 5′-*O*-trityladenosine. Detritylation with hot acetic acid gave 2′(3′)-*O*-(benzyloxycarbonyl-aminocyclopentanecarbonyl)adenosine (VIa,b). That the 6-amino group of the adenosine was not involved was shown by the fact that compound (VIa,b) was also obtained by detritylation of the corresponding acyl-N^6,$O^{5'}$-ditrityl-adenosine (VII). In the final step, the benzyloxycarbonyl group was removed by catalytic hydrogenolysis in aqueous acetic acid; it is known [14] that 2′(3′)-*O*-acylnucleosides



III; $R_1 = R_2 = H$

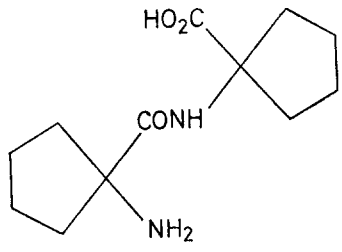
XIII; $R = H$; $R_2 = C_6H_5CH_2OCO$
(a, 2′-isomer; b, 3′-isomer)



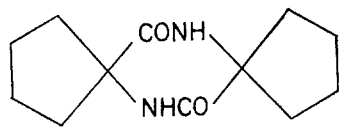
X

are relatively stable under mildly acid conditions. Elemental analysis of the product indicated the diacetate salt of the required 2′(3′)-*O*-(aminocyclopentanecarbonyl) adenosine (IIa,b). The material was chromatographically separable into two components, "slow" (lower R_f) and "fast" (higher R_f), presumably the 2′- and 3′-substituted nucleosides (IIa and IIb, not necessarily respectively). These two compounds had similar electrophoretic mobilities. The monoacetate salt of the corresponding uridine derivative (IIIa,b) was prepared in a similar way; it, too, was separable into two components. At room temperature the half-life of each of the four compounds at pH 7 was approximately 1 hr; and that of each of the adenosine derivatives at pH 7 and 37° was about 20 min. Alkaline hydrolysis of each mixture (IIa,b; IIIa,b) gave, as expected, free nucleoside and ACPA. None of the four compounds gave a *cis*-diol reaction. Unexpectedly, only the two slow components were ninhydrin-positive.

Hydrolysis studies later enabled us to rationalize this apparent anomaly. The fast and slow components were extracted separately with ethanol from paper chromatograms. The hydrolysing medium was usually aqueous 0·05M phosphate buffer of pH 7·0, but 0·1N aqueous sodium hydroxide was used in some experiments. There were two unexpected results. Firstly, neither the fast nor the slow components displayed acyl migration; mobility of the aminoacyl group would have been expected from earlier work with 2′- and 3′-acylnucleosides.[14,15] Secondly, while both slow components gave, on hydrolysis at pH 7 or pH 13, free nucleosides plus ACPA, the fast components gave free nucleoside but no ACPA nor any ninhydrin-positive product detectable on chromatograms. The same result was obtained when N aqueous ammonium hydroxide was the hydrolysing medium. Nevertheless, vigorous acid hydrolysis of the products of the alkaline hydrolysis of the fast aminoacyl adenosine gave ACPA in amounts comparable with that from the slow isomer. This established that the basic units of both fast and slow compounds were ACPA and nucleoside. It now seemed likely that the absence of ACPA from the products of the neutral or alkaline hydrolysis of the fast components arose from the formation of ninhydrin-negative peptidic material, and this view was confirmed. The peptides contained materials with R_fs comparable with those of the known[16] linear dipeptide (XI) and of the diketopiperazine (XII)



XI



XII

neither of which gives a colour with ninhydrin. The diketopiperazine was also isolated from a sample of the aminoacyladenosine (II; diacetate salt) which had been stored in a desiccator. In summary then, neutral or alkaline hydrolysis of the fast derivatives gave free nucleoside and peptidic products of unknown complexity; while hydrolysis of the slow components gave free nucleoside and amino acid. At the dilutions employed (*ca.* 0·1M with respect to aminoacylnucleoside), these reactions are almost, but not quite, mutually exclusive. Hydrolysis of either slow component at pH7 gave a trace of peptidic material with the same R_f (thin layer chromatography; TLC) as that of the diketopiperazine (XII). When the concentration of hydroxide ion was increased by using 0·1N or N sodium hydroxide as hydrolysing medium some ACPA was detected on paper electrophoretograms well loaded with the hydrolysates of the fast component of the aminoacyluridine (III). It is likely that the fast adenosine derivative would behave similarly.

Although the negative *cis*-diol test confirmed that the aminoacyl group was attached at the 2′ or 3′ position, we did not, at this stage, know the orientation of the fast and the slow products. To establish this we used nuclear magnetic resonance (n.m.r.) spectroscopy, which is known[17] to afford a means of differentiating between isomeric ribonucleoside derivatives. The diacetate salt of the freshly prepared aminoacyladenosine (IIa,b) was separated into its fast and slow components by silicic acid column chromatography at 0°. Substantial decomposition to adenosine occurred during removal of eluant and subsequent drying of each component, and the results are therefore not completely unambiguous. The spectrum of the slow component showed an H(1′) (anomeric) proton signal at lower field than the corresponding signal for adenosine. The 2′-*O*-aminoacyl structure (IIa) was therefore assigned to this compound. The fast component showed no anomeric proton separated from that of adenosine, a finding consistent[17] with its being the 3′-isomer (IIb). Both components exhibited an H(2) signal at slightly higher field than that of adenosine, a feature observed in the spectra of other 2′- and 3′-*O*-acyladenosines.[17] The pair of doublets ascribable to the anomeric protons in the spectrum of the benzyloxycarbonyl compound (VIa,b) was additional evidence of a mixture of 2′- and 3′-*O*-acyl derivatives and the intensities indicated that the isomers were in approximately equal proportions. The results are summarized in Table 1. The overlapping of the H(1′) and H(5) proton signals in the

TABLE 1. N.M.R. SPECTRA

Compound	Solvent	Chemical shifts* H(2)	H(8)	H(1′)	$J_{1',2'}$	Isomer
VIII	D^6-acetone	1·32	1·44	3·67	6	2′
		1·32	1·44	3·87	7	3′
VI	$CDCl_3$	2·02	2·15	4·05	7	2′
		2·02	2·15	4·37	8	3′
IIa	DMSO†	1·57	1·75	3·92	6	2′ ('slow')
IIb	DMSO	1·57	1·75	3·98	7	3′ ('fast')
Adenosine	DMSO	1·55	1·75	3·98	7	

* In ppm on τ scale; $J_{1',2'}$ in c/s.
† The dimethylsulphoxide (DMSO) solutions contained D_2O and acetic acid (see Experimental).

uridine compounds renders any interpretation based on n.m.r. uncertain. The chemical similarities have, however, enabled us to assign the 2′- and 3′-*O*-aminoacyluridine structures (IIIa, IIIb) to the slow and the fast components respectively.

In an attempt to make the analogous aminoacyl derivative (IV) of 6-methylthiopurine riboside, the benzyloxycarbonyl compound (VIII) was synthesised, starting with 6-methylthio-5′-*O*-trityl-β-D-ribofuranosylpurine. The n.m.r. spectrum indicated that the 2′- and 3′-substituted nucleosides were present in approximately equal proportions. Hydrogenolysis over palladium-barium sulphate,[18] however, appeared to cause cleavage of the methylthio group rather than removal of the benzyloxycarbonyl residue.

EXPERIMENTAL

Melting points were determined on a Köfler block and are corrected. Thin layer chromatograms were run on plates (7·6 × 2·5 cm, 8 × 8 cm, or 20 × 5 cm) coated with silicic acid (Merck Kieselgel G) or cellulose powder (Avicel No. 144). Paper chromatograms were run on Whatman No. 1 paper. The eluting solvents were: A, benzene–acetone (9:1); B, ethyl acetate–methanol (19:1); C, *n*-butanol–acetic acid–water (5:2:3); or as indicated. Spots were detected by (a) spraying with concentrated sulphuric acid, and heating at 100° for a few minutes (silicic acid plates); (b) spraying with ninhydrin (aerosol in butanol) (mainly for ACPA); (c) examining under ultraviolet (u.v.) light (nucleosides); (d) the method of Viscontini *et al.*[19] (cis-diols); (e) the method of Pan and Dutcher,[20] but with undiluted "Milton" hypochlorite solution instead of diluted "Clorox" (peptides); it was important to allow sufficient time to elapse (overnight drying was best), after removal of plates from solvent C, to ensure complete evaporation of the acetic acid before spraying with hypochlorite. Electrophoresis was carried out on Whatman No. 4 paper (10v/cm; pH 1·85 formic–acetic buffer[21]) with a Shandon Universal Electrophoresis Apparatus and Vokam power unit; n.m.r. spectra were measured on a 60 MHz Perkin–Elmer R10 spectrometer; tetramethylsilane was used as internal standard. Ultraviolet absorption measurements were made with a Unicam SP800 spectrophotometer.

1-(*Benzyloxycarbonylamino*)*cyclopentanecarboxylic anhydride* (*X*). Rammler and Khorana's procedure[22] was followed (0·1M scale), except that the starting material was 1-(benzyloxycarbonylamino)cyclopentane carboxylic acid,[16,23] and the *product* was extracted with chloroform and finally crystallized from ethyl acetate–ether (81% yield, mp 130–131°). (Found: C, 65·7%; H, 6·5%; N, 5·6%; Calc. for $C_{28}H_{32}N_2O_7$: C, 66·1%; H, 6·3% N, 5·5%).

2′(3′)-O-(1-*Benzyloxycarbonylaminocyclopentanecarbonyl*)-5-′O-*trityladenosine*(V). The anhydride (X; 21·0g; 30 m-mole) was added in three portions during 4 hr to a stirred solution of 5′-*O*-tritylad enosine[24] (15·3g; 30 m-moles) in 1:1 dry pyridinedimethylformamide (120 ml) at 105°. The cooled solution was poured on ice and the precipitate washed with water and dried. The yellow solid (26·5g) was dissolved in benzene (400 ml) and set aside overnight. Precipitated trityladenosine (3g) was removed, the filtrate evaporated to dryness, and the residue chromatographed on alumina (500g), first with ethyl acetate (1l.) then with ethanol (100 ml fractions). The major component (TLC, silicic acid ethyl acetate) occurred in fraction 6 to 40 inclusive. The less pure fractions were rechromatographed. The solid obtained by evaporation was dissolved in benzene and petrol added. The precipitated *product*, chromatographically homogeneous, was obtained as the hemihydrate (16g; 70%; mp 98–115°) (Found: C, 67·3%; H, 5·9%; N, 11·0%. Calc. for $C_{43}H_{42}N_6O_7$ 0·5 H_2O: C, 67·65%; H, 5·7%; N, 11·0%).

2′(3′)-O-(1-*Benzyloxycarbonylaminocyclopentanecarbonyl*)-N,^{6}O$^{5'}$-*ditrityladenosine* (*VII*). The anhydride (X; 5·6g; 11 m-moles) and N^6, $O^{5'}$-ditrityladenosine[25] (7·5g; 10 m-moles) were heated in dry pyridine (50 ml) for 7 hr at 105°. The reaction mixture was poured on ice, the washed and dried precipitate extracted into ether, the filtered solution washed (N H_2SO_4, $NaHCO_3$, and water successively), dried (Na_2SO_4), and evaporated. The solid residue (9·2g) consisted largely of one compound (TLC, silicic acid, solvent A). Part (0·5g) was chromatographed on silicic acid (25g) with ether (50 ml), then ether containing 1, 5, and 10% methanol (50 ml of each). Evaporation of the first 150 ml of eluant, and addition of a solution of the residue in benzene (2 ml) to 1:1

ether-petrol (20 ml) gave the *ester* (0·32g), mp 118–123°. (Found: C, 74·4%; H, 6·0%; N, 8·25%. Calc. for $C_{62}H_{56}N_6O_7$: C, 74·7%; H, 5·7%; N, 8·4%.)

2′(3′)-O-(1-*Benzyloxycarbonylaminocyclopentanecarbonyl*)-*adenosine* (*VI*). A solution of the 5′-*O*-trityl compound (V; 1·5g) in 4:1 glacial acetic acid–water (7·5 ml) was heated at 105° for 30 min. The solution was cooled, the precipitated tritanol filtered off, and the filtrate evaporated. The residue was dried by evaporation with benzene, taken up in ethyl acetate (5 ml), and the solution poured into 1:1 ether–petrol (100 ml). The precipitate was chromatographed on alumina (50 g), the eluting solvents being ethyl acetate (100 ml), then methanol. The methanol fractions contained the *product* (0·75 g; 74%; mp 108–111°; homogeneous on silicic acid TLC, solvent B). (Found: C, 56·2%; H, 5·8%; N, 16·0%. Calc. for $C_{24}H_{28}N_6O_7$: C, 56·2%; H, 5·5%; N, 16·4%.) Detritylation of the ditrityl derivative (VII) by the same procedure gave an identical (TLC) product.

2′(3′)-O-(1-*Aminocyclopentanecarbonyl*)-*adenosine* (*II*). The benzyloxycarbonyl compound (VI; 1·2g) was hydrogenolysed over a 5% palladium-on-charcoal catalyst (1·2g) in 4:1 glacial acetic acid–water (20 ml). Reduction was complete within 1·5 hr (TLC, silicic acid, 1:2 ethylacetate–ethanol). The filtered solution was freeze dried, the residue dissolved in a little water and the solution again freeze-dried. The ester (II) was thus obtained quantitatively as its amorphous diacetate salt, hygroscopic and of indefinite mp (from 90°). (Found: C, 48·1%; H, 6·2%; N,16·65%. Calc. for $C_{16}H_{22}N_6O_5$. 2 $C_2H_4O_2$: C, 48·2%; H, 6·1%; N, 16·9%.)

A sample (0·7g) of the compound (II) which had been standing 1 month in a desiccator (H_2SO_4) was no longer completely soluble in water. The insoluble residue (0·17g) was identical (elemental analysis; mp) with the diketopiperazine (XII) described below.

Chromatography separated the product (II) into two components whose R_fs (paper, solvent C) were 0·62 ("slow" component) and 0·70 ("fast" component) respectively (adenosine, 0·55). The order of R_f values was the same in other systems (TLC; silicic acid, cellulose). Only the slow component was ninhydrin positive. Neither the fast nor the slow component gave a positive cis-diol reaction on the thin layer plate (cellulose or silica), although the area of the slow component slowly bleached on standing.

The electrophoretic mobilities of the fast and slow components were 6·0 and 6·5 cm respectively (adenosine, 4·8 cm; 1 hr run).

2′(3′)-O-(1-*Benzyloxycarbonylaminocyclopentanecarbonyl*)-*uridine* (*XIII*). 5′-*O*-trityluridine was prepared on a 10 m-moles scale from the anhydride (X) and 5′-*O*-trityluridine[26] (15 hr in pyridine at 105°; poured on ice; extracted with chloroform). The crude product was detritylated by the method used for the adenosine analogue.

Chromatography on alumina (500g) with elution with ethyl acetate (500 ml) then methanol (1l.), recovery of the major product from the methanol fraction and addition of an ethyl acetate solution of the product to 1:1 ether–petrol afforded the *acyl compound* (XIII) in 75% yield and substantially pure (TLC, silicic acid, solvent B), but of indefinite mp (softening from 70°). (Found: C, 56·2%; H, 5·8%; N, 8·7%. Calc. for $C_{23}H_{27}N_3O_9$: C, 56·4%; H, 5·6%, N, 8·6%).

2′(3′)-O-(1-*Aminocyclopentanecarbonyl*)-*uridine* (*III*). The benzyloxycarbonyl derivative (XIII) was hydrogenolysed and the product isolated, exactly as for the adenosine analogue. The *ester* was obtained quantitatively as the amorphous, hygroscopic

mono-acetate salt of indefinite mp. (Found: C, 49·5%; H, 6·3%; N, 9·9%. Calc. for $C_{15}H_{21}N_3O_7$. $C_2H_4O_2$: C, 49·2%; H, 6·1%; N, 10·1%.) The compound was resolvable by chromatography (paper or TLC) into two components, "slow" and "fast" (R_fs, paper, solvent C: 0·60 and 0·72 respectively; uridine, 0·50). Neither component gave a *cis*-diol reaction and only the slow component was ninhydrin-positive.

6-*Methylthio*-5′-O-*trityl*-9-β-D-*ribofuranosylpurine*. Trityl chloride (9·2g; 33 m-moles) was added to a solution of 6-methylthio-9-β-D-ribofuranosylpurine[27] (8·2g; 28 m-moles) in dry pyridine (40 ml) and the stoppered mixture set aside for 2 days at room temperature. The solution was poured on ice, the solid product extracted with chloroform, and the extract washed successively with N H_2SO_4, water, $NaHCO_3$, and water. The dried (Na_2SO_4) solution was evaporated to dryness, the residue taken up in ether, and the solution allowed to evaporate. The resulting crystalline product was recrystallized from benzene, giving the *trityl compound* (11·2 g; 76%), mp 108–111°. (Found: C, 66·0%; H, 5·2%; N, 10·65%; S, 5·9%. Calc. for $C_{30}H_{28}N_4O_4S$: C, 66·6%; H, 5·2%; N, 10·4%; S, 5·9%.)

2′ (3′)-O-(1-*Benzyloxycarbonylaminocyclopentanecarbonyl*)-6-*methylthio*-5′-O-*trityl*-9-β-D-*ribofuranosylpurine* (IX). The above trityl derivative (5·4g; 10 m-moles) and the anhydride (X; 6·1g; 12 m-moles) were heated in dry pyridine (50 ml) for 7 hr. The solution was evaporated to dryness, the residue taken up in chloroform and the solution washed and dried in the usual way and the solvent removed. The residual syrup was passed through a column of alumina (500 g), the eluants being ethyl acetate (200 ml), then ethanol (500 ml). The combined eluates were taken to dryness, the residue was dissolved in ethyl acetate (5 ml) and the solution poured into petrol (bp 40–60°; 200 ml). The precipitated *ester* (5·2g; 66%), mp 93–95°, was substantially homogenous (TCL, silicic acid, solvent A). (Found: C, 66·9%; H, 5·6%; N, 9·15%; S, 4·5%. Calc. for $C_{44}H_{43}N_5O_7S$: C, 67·2%; H, 5·5%; N, 8·9%; S, 4·1%.)

2′(3′)-O-(1-*Benzyloxycarbonylaminocyclopentanecarbonyl*)-6-*methylthio*-9-β-D-*ribofuranosylpurine* (*VIII*). Detritylation of compound (IX; 0·79g) and the subsequent isolation procedure were exactly as for the corresponding adenosine derivative (VI). Chromatography of the crude product on alumina (50g) with ethyl acetate (100 ml), then methanol (100 ml) as eluants gave the *esters* (0·35g; 66%) mp 94–7°; it was chromatographically homogenous (TLC, silicic acid, 3:1 benzene acetone). (Found: C, 55·7%; H, 5·8%; N, 12·9%; S, 5·8%. Calc. for $C_{25}H_{29}N_5O_7S$: C, 55·2%; H, 5·4%; N, 12·9%; S, 5·9%.)

3,6-*Di*(*spirocyclopentane*)-*piperazine*-1,-5-*dione* (*XII*). *N*-(1-Benzyloxycarbonylaminocyclopentanecarbonyl)-1-aminocyclopentanecarboxylic acid methyl ester[16] (0·35 g) was hydrogenolysed in ethanol (10 ml) with a 5% palladium-charcoal catalyst (0·2 g). The filtered solution was taken to dryness and the free, oily dipeptide ester was dissolved in ice-cold saturated methanolic ammonia (30 ml). The *diketopiperazine* deposited as colourless needles (0·14g; 70%) mp > 300°. (Found. C, 64·9%; H, 8·1%; N, 12·7%. Calc. for $C_{12}H_{18}N_2O_2$: C, 64·8%; H, 8·2%; N, 12·6%.) The compound was probably also isolated as a reaction by-product by Connors.[28]

Hydrolytic experiments with 'fast' and 'slow' aminocyclopentanecarbonyl-adenosines (IIa,b) and -uridines (IIIa,b)

Solutions, approximately 0·1 M, of the 2′(3′)-*O*-amino-acyl compounds in acetic acid–water (4:1) were prepared by hydrogenolysis of the benzyloxycarbonyl deriva-

tives (100 mg) as described above. In each case the solution was streaked along a line parallel to the shorter edge of a sheet (57 × 23 cm) of Whatman No. 1 paper, and the chromatograms run overnight (ascending) in solvent C. The paper was allowed to dry, and the fast and slow components were located by u.v. The 'fast' strip was cut out, then divided into small pieces, extracted with ethanol (5 ml × 2; each 2 min shaking) and the combined, filtered extracts taken rapidly to dryness under oil-pump vacuum (cold water bath). No significant decomposition occurred under these conditions. A small reference sample was removed from the ethanol solution towards the end of the evaporation and dropped into a roughly equal volume of glacial acetic acid. The residue was taken up in 0·1 M pH 7·0 phosphate buffer (0·1 ml), giving a solution of the order of 0·1 M with respect to substrate, and set aside at room temperature. Small samples (*ca.* 10 μl) were removed by capillary tube at intervals and each was dropped into glacial acetic acid (*ca.* 5 μl) to terminate hydrolysis. These acidified samples were spotted on to cellulose plates and run in solvent C. The effects of NaOH or aq. NH_3 were determined in a similar way. Exactly the same procedure was employed for the slow components. The plates were examined by u.v. or after spraying with the appropriate reagent. The pH 7 hydrolysates of the slow components showed parent nucleoside and ACPA and a trace of u.v.-negative ninhydrin-negative peptide material (R_f *ca* 0·9). The pH 7 hydrolysates of the fast components showed parent nucleoside and two u.v.-negative ninhydrin-negative elongated peptidic spots [R_fs *ca.* 0·9 and 0·6; similar to diketopiperazine (XII) and dipeptide (XI)[19] respectively]. Hydrolysis with NaOH or aq. NH_3 (N or 0·1 N) gave similar results with respect to products detectable by u.v. and ninhydrin; the hydrolysates were not examined for peptidic products. A well-loaded electrophoretogram of acidified hydrolysates from the action of 0·1 and 1·0 N NaOH (5 min) upon the fast component of the aminoacyluridine (III) revealed traces of ACPA (ninhydrin).

Half-life of fast and slow components of compound (II). The components separated by paper chromatography as described above and on a similar scale, were separately dissolved in 0·1 M pH7 phosphate buffer (0·1 ml) and the stoppered solutions immersed in a bath at 37°. Samples (each 25 μl) were taken at intervals (fast: 0, 10, 15 min; slow: 0, 15, 20 min), immediately added to glacial acetic acid (*ca.* 5 μl), and transferred to Whatman No. 1 paper (whole of each sample applied with repeated spotted and drying). The chromatograms were run overnight in solvent C, and the nucleosidic spots located by u.v. They were separately cut out, eluted with N HCl, and the optical densities (OD) determined (initial sample, whose adenosine content was negligible, as blank). The OD values for the starting material (and the derived adenosine) respectively at the three times were as follows:

Fast: 0 min, 0·202 (0·000); 10 min, 0·135 (0·060); 15 min, 0·120 (0·080).

Slow: 0 min, 0·122 (0·000); 15 min, 0·075 (0·050); 20 min, 0·080 (0·090).

Assuming first order kinetics, the half-life of both the fast and slow components was calculated as being approximately 20 min under the conditions used.

Acid hydrolysis of alkaline hydrolysates of 'fast' and 'slow' adenosine derivatives (II). Excess 10 N HCl (50 μl) was added to each (0·1 N NaOH) hydrolysate solution (*ca.* 10–15 μl) obtained as described above. Each solution was heated in a sealed tube at 150° for 3 days, then evaporated to dryness and taken up in aq. NH_3 (*ca.* 10 μl), spotted on a cellulose plate and run in solvent C. Subsequent ninhydrin treatment gave purple spots of approximately equal intensity and corresponding to ACPA.

Nuclear magnetic resonance spectroscopy

Aminoacyladenosine (IIa,b) freshly prepared as the diacetate salt from the benzyloxycarbonyl precursor (VI a,b; 250 mg), was dissolved in ethanol (2 ml), the solution diluted with ethyl acetate (2 ml), and then applied to a silicic acid (Merck Kieselgel G; 0·05–0·2 mm) column (25 × 3 cm^2). The chromatography was carried out at 0°. Elution with 1:1 ethanol–ethyl acetate (10 ml fractions) gave, in order, adenosine (trace, fractions 11–14), fast component (80 mg, 16–26), and slow component (63 mg, 30–42). The dried components each contained adenosine (*ca.* 50%; estimated by u.v. absorption of spots eluted from paper chromatogram) showing that considerable decomposition had occurred during evaporation *in vacuo* and storage overnight over P_2O_5 *in vacuo*). The spectra were recorded in dimethylsulphoxide (0·4 ml) containing D_2O (0·05 ml) to exchange OH and NH protons, and acetic acid (0·05 ml) to stabilize the compound. Spectral features attributable to the aminoacyladenosines were discerned in spite of the contaminating adenosine (Chemical Discussion and Table 1).

The hydrogenolysis product from the uridine derivative (XIII; 250 mg) was similarly chromatographed and examined. Elution gave uridine (trace, fractions 6–8), fast component (35 mg, 9–11), and slow component (70 mg, 13–16). Decomposition occurred in this case also, prior to the n.m.r. measurements. The H(1′) and H(5) signals of the uridine derivatives overlapped.

BIOLOGICAL METHODS AND RESULTS

The inhibitory effects of ACPA and its 2′(3′)-adenosine and -uridine esters (IIa,b and IIIa,b) were assessed, using the 10-day ADJ/PC6 plasma cell tumour in Balb/C⁻ mice. ACPA appeared to show slight antitumour activity in this system at a dose of 50 mg/kg/day (i.p.) × 4. The adenosine and uridine esters were inactive or almost inactive at 200 mg/kg/day × 2. Doubling the doses caused marked body weight loss.

Neither of the esters (IIa,b and IIIa,b) had an observable effect, at a concentration of 10^{-3} M, upon the incorporation of ^{14}C-L-valine or ^{3}H-L-leucine into protein *in vitro*; the *Escherichia coli* S30 system of Nirenberg and Matthaei[32] was used. The polyuridylic acid–phenylalanine system of the same authors was also unresponsive.

GENERAL DISCUSSION

In contrast to the corresponding adenosine derivatives[12] of natural amino acids referred to above, the 2′(3′)-adenosine and -uridine esters (IIa,b and IIIa,b) of ACPA did not inhibit protein synthesis *in vitro* at 10^{-3} M. Nor were these products more active against an experimental plasma cell tumour on a molar basis than ACPA itself, perhaps because of rapid hydrolysis to the latter.

The unexpected failure of the supposed 2′- and 3′-*O*-aminoacyl nucleosides to interconvert was referred to earlier. However, the isolation has been claimed[30] of a mixture of aminoacyladenosines, containing over 90% of the 3′-isomers, derived enzymically from aminoacyl-tRNAs under conditions (18 min at pH 7) which would, from a consideration of comparable acyl[14] or acylaminoacyl[15] systems, be expected to lead to complete equilibration. Although the validity of the German work[30] has been questioned,[14] we are aware of no direct evidence that migration does occur in 2′(3′)-*O*-aminoacyl-adenosines or -uridines. Because of the different mechanisms of cis vicinal hydroxyl group-assisted acyl migration and hydrolysis,[31] however, the relative rates of these processes might depend markedly on the structure of the acyl group.

Space-filling molecular models of esters (IIa,b) showed that the carbonyl carbon of the bulky aminoacyl group cannot readily make contact with the oxygen atom of the neighbouring hydroxyl group. The absence of migration in this case may thus be due to steric factors.

If our structural assignment is correct, the hydrolytic behaviour of the 2′- and 3′-*O*-aminocyclopentanecarbonyl-adenosine (and -uridine) may be relevant to the process of protein biosynthesis. Inhibition by puromycin implies the involvement of 3′-*O*-aminoacyladenosine in this process,[32] and the work[30] referred to above supports this view. The hydrolytic studies described here suggest that 3′-aminoacyl substitution is more conducive to peptide bond formation than is 2′ substitution, even in the absence of enzymic control. The apparent absence of intermediate peptidyl nucleosides from the chromatograms of the the hydrolysates of the 3′-*O*-aminoacylnucleosides presents a difficulty, unless one invokes the rapid hydrolysis of such an intermediate; however, polyphenylalanyl-t-RNA hydrolyses seven times more slowly than phenylalanyl-tRNA.[33] Diketopiperazine formation (which may predominate) need not involve the formation of peptidyl nucleoside intermediates.

Re-examination of acyl migration and hydrolysis using pure 2′- and 3′-*O*-aminoacyl-adenosine and -uridine derived from natural amino acids now seems a desirable extension of the present work.

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