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REACTION OF NUCLEOBASES WITH α-ACETYLENIC ESTERS, POTENTIALLY USEFUL FOR CHEMICAL MODIFICATION OF NUCLEIC ACIDS

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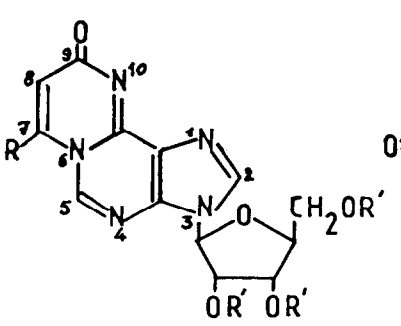
Summary : The NH_2 group and the adjacent ring nitrogen of adenosine and cytidine react with α-acetylenic esters by addition across the triple bond and formation of a lactam with the ester group.

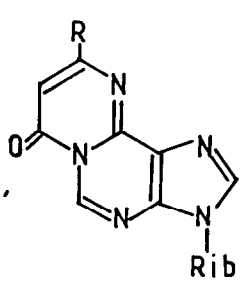
We have recently shown that chlorotetrolic (4-chloro-2-butynoic) esters[1], $ClCH_2$-C≡C-COOR, react under mild conditions with nucleophiles analogous to those present in proteins[2]. Tests with small model molecules and with proteins have demonstrated the alkylating ability of methyl chlorotetrolate, which easily undergoes substitution of chlorine and addition across the triple bond ; this product thus appears as a potential bifunctional protein reagent.

We have found that these small, highly functionalized molecules are also able to react with nucleic acid bases such as adenine or cytosine. When adenosine was treated for a few days at room temperature with methyl or ethyl chlorotetrolate in hydroalcoholic solution of apparent pH 4.5 - 5, or with p-nitrophenyl chlorotetrolate in dimethylformamide, product 1a, 3-β-D-ribofuranosyl-9-oxo-7-chloromethyl-8,9-dihydropyrimido [1,2-c] purine, mp 230°C (decomp.), was isolated in 82% yield. If the apparent pH of the hydroalcoholic reaction medium is 7, 31% of the isomeric compound 2a, 3-β-D-ribofuranosyl-7-oxo-9-chloromethyl-6,7-dihydropyrimido [1,2-c] purine, mp 170°C, is obtained. The two isomers have distinctly different properties. As a rule, product 1a is less soluble and, besides DMSO and DMF, it dissolves slightly only in water, while compound 2a is more soluble in water and is also slightly soluble in some organic solvents (methanol, acetone).

The electronic spectra were useful in establishing the structures of the two isomers. According to Allen *et al.*, the absorption bands of fused pyrimidones fall into three regions a, b and c in the order of increasing λ_{max}, with $\varepsilon_c/\varepsilon_b > 1$ in compounds having the carbonyl group connected to the ring nitrogen, while this ratio never exceeds 1/3 or sometimes the c band is not observed at all in isomers in which the carbonyl group is connected to the exocyclic nitrogen atom. The shapes of the UV spectra[4] of products 1a and 2a



1



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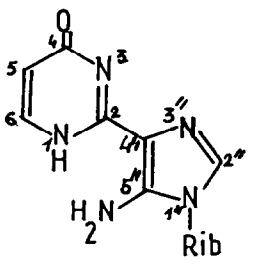

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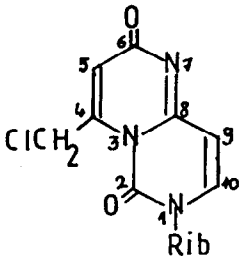


4

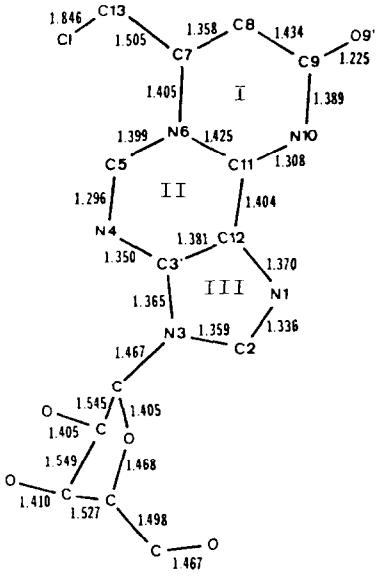
a, R = $ClCH_2$, R' = H
b, R = $ClCH_2$, R' = Ac
c, R = R' = H

a, R = $ClCH_2$
b^5, R = ICH_2

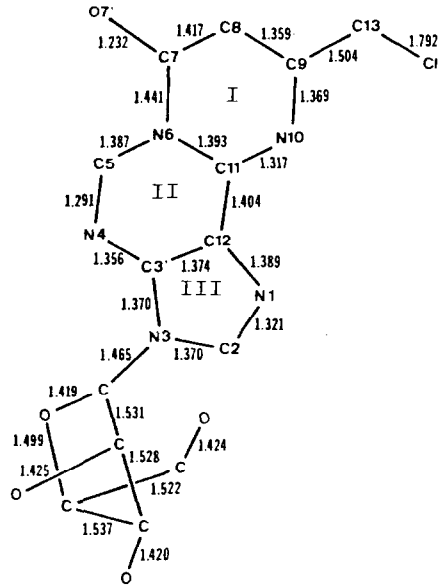
are consistent with these rules, showing for 1a : λ_a 240 nm (ε 39 000), λ_b 297 nm (ε 14 000) and for 2a : λ_a 240 nm (ε 10 950), λ_b 307 nm (ε 6700) and λ_c 346 nm (ε 12 050). The IR spectrum of 1a has a band at 1670 cm^{-1}, while in 2a this band appears at 1710 cm^{-1}, which again confirms[3] the structures ascribed to these compounds. The ^1H NMR spectrum of 1a shows, in addition to the expected signals of the ribose moiety, the following signals : δ 5.25 (s, 2H, CH_2Cl), 6.67 (s, 1H, H_8), 8.66 (s, 1H, H_2), 8.95 (s, 1H, H_5) ; nuclear Overhauser effect (nOe) on irradiation of CH_2Cl : 42% for H_5 and 26% for H_8, the H_2 proton remaining unchanged. In the case of 2a the CH_2Cl singulet appears at δ 4.73, the H_8 and H_2 peaks (6.61 and 8.78 respectively) being close to those found for 1a. However, the H_5 signal in 2a is strongly deshielded (9.54) and shows practically no nOe on irradiation of CH_2Cl. The mass spectrum of 1a does not show the molecular peak but a peak appears at m/z 235 (rel. int. 100), corresponding to the loss of ribose, and at 132 (13) (ribose) ; other peaks are due to fragmentation of the base moiety : 207 (29), 172 (61) and 119 (62). Product 2a has a similar mass spectrum.

The crystal structures of molecules 1a and 2a were established by X-ray diffraction using direct methods[6,7] (see next Fig.). The three rings of the dihydropyrimido-purine moiety are quasi coplanar in the 2a molecule (dihedral angles between mean planes : I and II, 1.3° ; II and III, 0.7°) but are slightly distorted in the 1a isomer (I and II, 6.2° ; II and III, 5.5°). The torsion angles are : 1a, N(6)-C(7)-C(13)-Cl : -60.3° ; 2a, N(10)-C(9)-C(13)-Cl : -67.9°.

Thus the value of the earlier methods of determining the structure of fused pyrimidones, based on their spectral characteristics, can be fully confirmed.



1a



2a

Bond lengths and main structural features in the crystal structures of 1a and 2a. The maximum values of e.s.d.'s are 0.020 and 0.008 Å respectively for 1a and 2a.

The chloromethyl group of 1a proved more reactive than the one of 2a, as shown by preliminary tests with thiols and amines and with protein SH groups.

Reaction of 2',3',5'-triacetyladenosine (obtained by treatment of adenosine with acetic anhydride in the presence of catalytic amounts of 4-dimethylaminopyridine) with methyl chlorotetrolate leads to product 1b, mp 142°C (yield 50%), having UV, NMR and IR characteristics consistent with the structure represented above. However, its mass spectrum shows the triacetylribose rather than the base moiety fragmentation peaks : 259 (0.7) and 258 (0.8) (triacetyl-ribose), 198 (16), exact mass 198.0526 (ribose - AcOH ; for base - HCl calcd mass 198.0415), 156 (53), 139 (10), 114 (51), 97 (40), 60 (21), 43 (100).

Reaction of adenosine with ethyl propiolate in hydroethanolic solution of apparent pH 4.5 or 6 yields 60% of derivative 1c, 3-β-D-ribofuranosyl-9-oxo-8,9-dihydropyrimido[1,2-c]purine, mp 243-244°C (decomp.), which has all the spectral characteristics of the isomer of type 1 : UV ε_{236} 42 600, ε_{291} 16 300 ; IR 1660 cm^{-1} ; NMR δ 6.42 (d, J = 8 Hz, 1H, H_8), 8.41 (d, J = 8 Hz, 1H, H_7), 8.63 (s, 1H, H_2), 8.86 (s, 1H, H_5) ; mass 187 (29) (base moiety), 132 (18) (ribose). When the reaction was carried out under reflux (75 hours, pH about 6), compound 3, 3,4-dihydro-4-oxo-2-(1"-β-D-ribofuranosyl-5"-amino-4"-imidazolyl)-pyrimidine, mp 197-198°C was formed in 90% yield.

UV : ε_{214} 17 100, ε_{240} 10 400, ε_{318} 16 700 ; IR : 1677 cm^{-1}. The mass spectrum shows i.a. the molar peak at m/z 309 (6) and a peak at 177 (90) resulting from the loss of ribose. ^{1}H and ^{13}C NMR spectra confirm the structure of product 3, in particular the absence of the CH group lost by the purine ring of the original adenine nucleus. As a side product, 1,3,5-triethoxycarbonylbenzene, mp 133°C, was also formed in the latter case by trimerization of ethyl propiolate. Heating of 1c in water for 2 days at 80°C converts this compound to derivative 3.

Among other nucleobases, cytosine also reacts with α-acetylenic esters. Thus, treatment of cytidine with chlorotetrolic ester yields 50% of product 4, mp about 150°C (decomp.). UV : ε_{249} 20 200, ε_{307} 9600. NMR (Jeol FX 90Q, $(CD_3)_2SO$), δ 5.184 (s, 2H, CH_2Cl), 6.263 (d, J = 8 Hz, 1H, H_9), 6.525 (s, 1H, H_5), 8.188 (d, J = 8 Hz, 1H, H_{10}). IR : 1730 cm^{-1}.

Guanosine does not react with acetylenic esters, at least under the above conditions.

These new derivatizations of nucleobases are now being extended to nucleotides and nucleic acids.

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References and Notes

1. M. Olomucki, J.Y. Le Gall, I. Barrand, J. Chem. Soc., Chem. Commun. 1290 (1982).
2. J. Diopoh, M. Olomucki, Bioorg. Chem. 11, 463 (1982).
3. C.F.H. Allen, H.R. Beilfuss, D.M. Burness, G.A. Reynolds, J.F. Tinke, J.A. van Allan, J. Org. Chem. 24, 779 (1958).
4. All the new products gave satisfactory elemental analyses. The UV spectra were recorded in water, pH 7, using a Zeiss PMQ II or Beckman 34 instrument. IR spectra (KBr discs) were measured on a Perkin Elmer 720 spectrometer, ^{1}H and ^{13}C NMR spectra were taken in $(CD_3)_2SO$ at 80 and 20 MHz respectively on a Varian FT 80A apparatus, unless otherwise stated, and mass spectra (70 eV) on AEI MS-30 or MS-50 instruments.
5. Obtained from 2a by the Finkelstein reaction.
6. 1a : orthorhombic space group $P2_12_12_1$; a = 12.082, b = 17.943, c = 6.826 Å, α = β = γ = 90° ; R = 6.05. 2a : $P2_12_12_1$; a = 11.922, b = 24.931, c = 5.471 Å, α = β = γ = 90° ; R = 3.56.
7. The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 IEW, UK. Any request should be accompanied by the full litterature citation for this communication.

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