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STEREOCHEMISTRY OF THE CYCLOPENTENE SIDE CHAIN IN THE NUCLEOSIDE Q OBTAINED FROM *ESCHERICHIA COLI* tRNA

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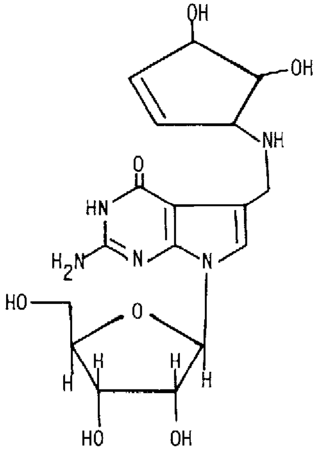
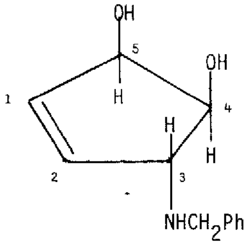
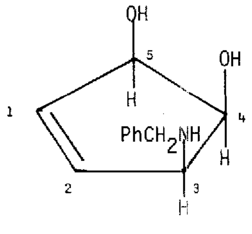
The nucleoside Q is found in the first position of the anticodon of *Escherichia coli* tRNA^{Tyr}, tRNA^{His}, tRNA^{Asn}, and tRNA^{Asp}.[1] As reported in the previous paper,[2] extensive analysis of mass and ^{1}H nmr spectra has disclosed that its structure (I) is very unique in having a 4,5-dihydroxycyclopent-1-en-3-ylaminomethyl group at position 7 of 7-deazaguanosine. Although all of the chemical shifts and coupling constants of the protons were analyzed, assignment of stereochemistry of the substituents on the side chain was not possible since coupling constant in five-membered rings cannot be unambiguously used for assignment of the configurations.[3] It was suggested that the vicinal diol in the side chain has a *cis* configuration since Q gave a bis-*O*-isopropylidene derivative[2] and was cleaved rapidly with periodate (vide infra).



Nucleoside Q (I)



3,4-*trans*;4,5-*cis* (II)



3,4-*cis*;4,5-*cis* (III)

To unambiguously establish the stereochemistry of the side chain we synthesized 3,4-trans; 4,5-cis- (II) and 3,4-cis;4,5-cis-3-benzylamino-4,5-dihydroxycyclopent-1-ene (III) and their ^1H nmr spectra were compared with that of Q. Although both isomers show patterns very similar to that of the side chain of Q, a detailed comparison of their spectra established that the former has a configuration identical with that of the corresponding part of Q (Tables 1 and 2). Thus, large differences in chemical shifts of H-3 and H-4 signals are observed between the spectra of the cis;cis compound (III) and Q (I), whereas the chemical shifts and coupling constants of the trans;cis compound (II) and the corresponding values of Q are practically identical Unexpectedly, $J_{3,4}$ as well as most of other vicinal coupling constants is identical to the trans;cis (II) and cis;cis (III) isomers, and hence it cannot be used for diagnostic purposes.

Table 1. Chemical Shifts (in ppm)

	δ_1	δ_2	δ_3	δ_4	δ_5
Q (I)[a]	6.11	6.29	4.29	4.39	4.70
trans;cis (II)[b]	6.09	6.28	4.30[c]	4.40[c]	4.70[d]
cis;cis (III)[b]	5.98	6.22	4.24	4.58	?

a) Reported values in Ref. 2; measured in D_2O (pD 7.0) at 220 MHz and analyzed by comparison with a simulated spectrum. The signal of int. $Me_4N^+Cl^-$ is taken as 3.20 ppm. The spectrum is not changed between pD 3 and 7.

b) Measured in D_2O (pD ca 5) at 100 MHz; the signal of int. $Me_4N^+Cl^-$ is taken as 3.20 ppm (actually $Me_4N^+Cl^-$ shows a signal at 3.16 ppm from int. 3-trimethylsilylpropionate-2,2,3,3-d_4).

c) These signals are overlapped with the $-NCH_2Ph$ signal. Measurement was carried out using $-NCD_2Ph$ derivative prepared from XIII and $PhCD_2Br$; a triplet (J=5.2) corresponding to H-4 being observed clearly.

d) This signal is overlapped with the signal of water, but was observed by addition of trifluoroacetic acid, which removed the signal of water downfield.

Table 2. Coupling Constants (in Hz)

	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{1,3}$	$J_{1,5}$	$J_{2,5}$
Q (I)	6.5	2.5	5.2	5.2	ca 0.6	1.5	1.7
trans;cis (II)	6.5	2.5	5.2[a]	5.2[a]	ca 0.5	1.6	1.8
cis;cis (III)	6.5[a]	2.5[a]	5.2[a]	5.2[a]	1.3[a]	2.1[a]	1.8[a]

a) Confirmed by spin decoupling technique.

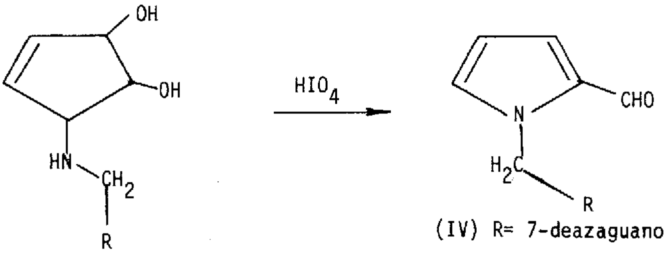
A large change in chemical shifts in the ^1H nmr spectrum of Q has been observed with increasing pD from 7 to 11, because of the presence of an amino group. Comparison of the chemical shifts of Q and the synthetic compounds measured at above pD 11 also indicates the structural similarity between the trans;cis isomer (II) and Q.

Table 3. Chemical Shifts at pD above 11

	δ_1	δ_2	δ_3	δ_4	δ_5
Q (I)[a]	6.01	5.98	3.78	4.02	4.65
trans;cis (II)	6.00	6.00	3.78	4.02	4.64
cis;cis (III)	5.90	5.90	3.68	4.40	?

a) Reported values in Ref. 2.

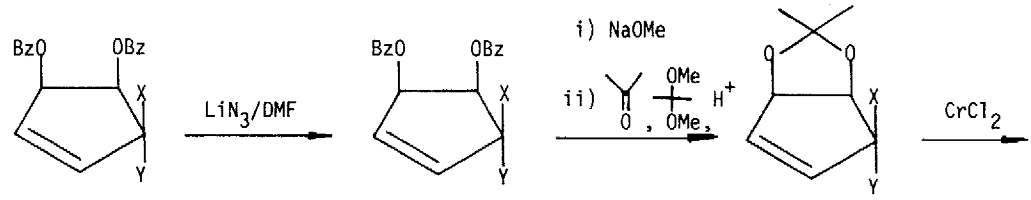
Periodate oxidation of Q (2 mol. $NaIO_4$, 2 min at r.t.) gave a compound whose structure has been suggested as (IV) from the analysis of its mass and uv spectra.[2] Similar treatment of the two synthetic isomers, (II) and (III), gave the expected product (V), the structure of which was confirmed by nmr.[4] Comparison of the uv spectra of (IV) and (V) supports the conclusion that (IV) contains the pyrrole-2-aldehyde chromophore.



(IV) R= 7-deazaguanosine

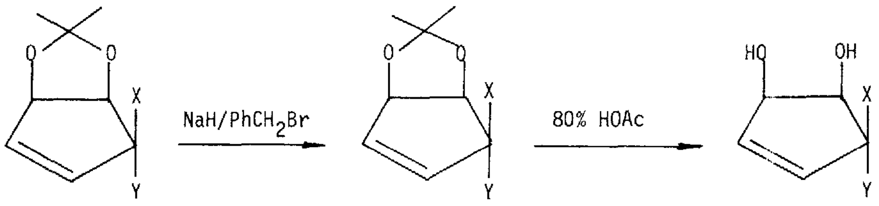
(V) R= Ph

Synthesis of the two isomers was carried out according to the scheme shown below.[5]



(VI)[6] X=H, Y=Br .... (VIII) $X=N_3$, Y=H .... (X) $X=N_3$, Y=H ....

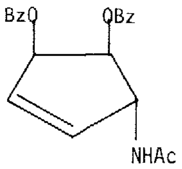
(VII)[6] X=Br, Y=H .... (IX) X=H, $Y=N_3$ .... (XI) X=H, $Y=N_3$ ....



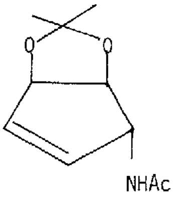
(XII) X=NH$_2$, Y=H .... (XIV) X=NHCH$_2$Ph, Y=H .... (III) X=NHCH$_2$Ph, Y=H

(XIII) X=H, Y=NH$_2$ .... (XV) X=H, Y=NHCH$_2$Ph .... (II) X=H, Y=NHCH$_2$Ph

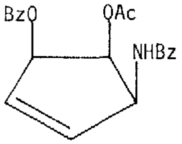
Assignment of the configurations was done as follows: Reduction of the trans-azidodibenzoate (IX) with CrCl$_2$ followed by acetylation gave the normal product (XVI), whereas the cis isomer (VIII) gave a product (XVIII) in which a benzoyl group was migrated intramolecularly to the amino group. These products were further characterized by transforming them to the corresponding amidoacetonides, (XVII) and (XIX). These results confirm the stereochemistry of the final products, (II) and (III), unambiguously.



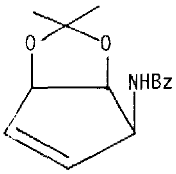
(XVI)



(XVII)



(XVIII)



(XIX)

Determination of absolute configuration of the side chain in Q is under investigation.

REFERENCES and FOOTNOTES

1) See references cited in Ref. 2.
2) H. Kasai, Z. Ohashi, F. Harada, S. Nishimura, N. J. Oppenheimer, P. F. Crain, J. G. Liehr, D. L. von Minden, J. A. McCloskey, Biochemistry, **14**, 4198 (1975).
3) A. A. Bothner-By, Advances in Magnetic Resonance, **1**, 195 (1965).
4) Physical data of (V): nmr (ppm in CDCl$_3$) δ5.60 (2H,s), 6.28 (t,J=3.5, H-4), 7.00 (2H,d,J=3.5, H-3 & H-5), 7.30 (5H, Ph), 9.64 (1H,s, CHO); ir (CHCl$_3$) 1660 cm^{-1} ; mass (m/e) 185 (M$^+$), 184, 156; uv (MeOH) 260 nm (sh) (ε 7200), 288 (ε 13200) (not changed with pH).
5) All of the synthesized compounds were characterized by spectroscopic methods. The following derivatives gave satisfactory elemental analyses: (V) 2,4-dinitrophenylhydrazone, mp 201° (dec); N-benzoyl-(XIII), mp 170-1°; N-benzoyl-(XIV), mp 124-5°; N-benzoyl-(XV), mp 136-7°; (XVI), mp 158-9°; (XVII), mp 93-5°; (XVIII), mp 149-50°; (XIX), mp 87°. Field desorption mass spectra of (II) and (III) gave their molecular peak [M$^+$: base peak; M+1: (II) 40%, (III) 93%]. We thank JEOL Co. Ltd. for measurement of fd mass spectra.
6) G. Molczunowicz, F. G. Cocu and Th. Posternak, Helv. Chim. Acta, **53**, 2275 (1970).