^{13}C and ^{1}H NMR Analysis of the Nitration Products of 2-Amino-4-oxo-(3*H*)-5-trifluoromethylquinazoline and 2-Amino-4-oxo-(3*H*)-5-fluoroquinazoline

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Nitration of 2-amino-4-oxo-(3*H*)-5-trifluoromethylquinazoline is shown to occur exclusively at C^6 as determined from an analysis of long range ^{1}H and ^{19}F scalar couplings to ring carbons. Nitration of 2-amino-4-oxo-(3*H*)-5-fluoroquinazoline is found to occur both at C^6 and C^8 as evident from an analysis of the ^{19}F and ^{1}H couplings of the ring protons.

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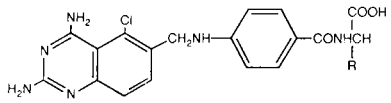
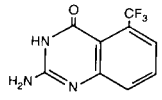
For the past twenty years this laboratory has been involved with the synthesis of quinazoline (5,8-dideaza) analogues of folic acid. Of particular interest has been the introduction of small non-polar substituents at position 5 of the quinazoline ring. The presence of such substituents often has an enhancing effect upon biological activity. For example, 5-chloro-5,8-dideazaaminopterin (**1a**) is the most efficient substrate for mammalian folylpolyglutamate synthetase known. As measured by V max/K_m it is 26-fold better than its counterpart possessing a hydrogen atom at position five and 22-fold superior to the natural substrate, (6*S*)-5,6,7,8-tetrahydrofolic acid [1,2]. On the other hand, its analogue in which the L-glutamate residue is replaced by an L-ornithine moiety (N^{α}-4-amino-4-deoxy-5,8-dideazapteroyl-L-ornithine) (**1b**) is the most potent inhibitor of folylpolyglutamate synthetase reported to date [2]. During efforts to prepare similar analogues possessing 5-trifluoro-



1a R = $(CH_2)_2COOH$
1b R = $(CH_2)_3NH_2$

methyl and 5-fluoro groups, the nitrations of 2-amino-4-oxo-(3*H*)-5-trifluoromethylquinazoline (**2**) and 2-amino-4-oxo-(3*H*)-5-fluoroquinazoline (**4**) were conducted. Nitration of the 5-trifluoromethyl derivative, **2**, yielded a single product as adjudged by thin layer chromatography [3]. By analogy with earlier work, it was assumed that the 6-nitro isomer had formed [4]. However, this study was conducted in order to establish unequivocally that the nitration product was 2-amino-4-oxo-(3*H*)-6-nitro-5-trifluoromethylquinazoline (**3**). Interestingly the nitration of the 5-fluoro analogue, **4**, resulted in the formation of two products [5] which were shown to be 6- and 8-nitro derivatives **5a** and **5b** respectively, in this study.



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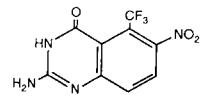

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Figure 1 shows expansions of the ^{1}H-coupled ^{13}C nmr spectra of 2-amino-4-oxo-(3*H*)-5-trifluoromethylquinazoline (**2**, Figure 1A) and its nitration product (**3**, Figure 1B);

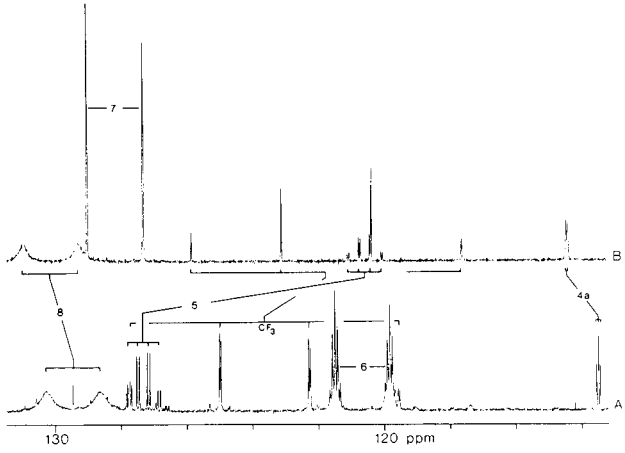


Figure 1

their ^{13}C chemical shifts and heteronuclear scalar coupling constants are listed in Table 1. Of the three possible nitration products, the C^7 isomer could be eliminated immediately: the C^{4a} resonance in **2**, split into a triplet by two equivalent $^{4}J_{CH}$ couplings, appeared in the product as a doublet indicating that either C^6-H or C^8-H was lost. This conclusion is supported by C^5's retention of a 6.3 Hz coupling ($^{3}J_{CH}$) and the appearance of a 1.7 Hz coupling

($^4J_{CH}$) in the CF_3 carbon resonance, possible only if C^7-H is present. Because the CH resonance at 120.7 ppm in the carbon spectrum of **2** showed a 7.3 Hz coupling to ^{19}F ($^3J_{CF}$) it was assigned to C^6. In the spectrum of the nitrated product, **3**, this resonance shifted downfield to 144.1 ppm and lost its $^1J_{CH}$ coupling indicating that nitration had occurred exclusively at C^6. This conclusion was supported by the disappearance of a 4.8 Hz coupling ($^3J_{CH}$) from the CF_3 multiplet resonance.

The ^1H nmr study of the nitration product of 2-amino-4-oxo-(3*H*)-5-fluoroquinazoline (**4**) using chemical shift values, coupling constants and integrations for the aromatic protons, allowed the unequivocal assignment of the structure and the exact determination of the ratio of the formed isomers. The ^{19}F-coupled ^1H nmr spectrum of the nitration product of **4**, expanded in the region from 6.8 to 8.3 ppm, is shown in Figure 2. The chemical shift values

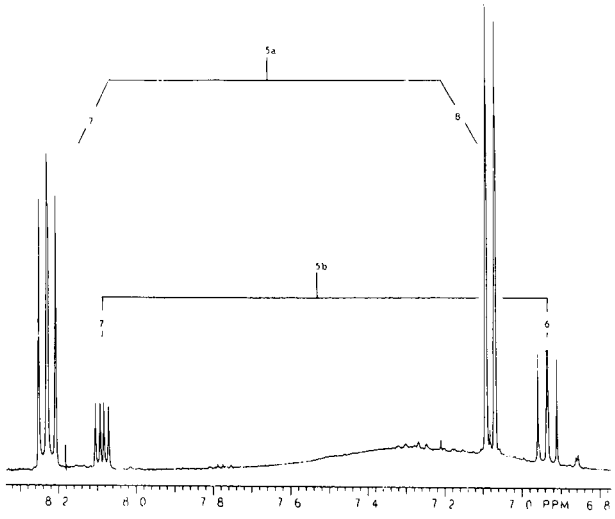
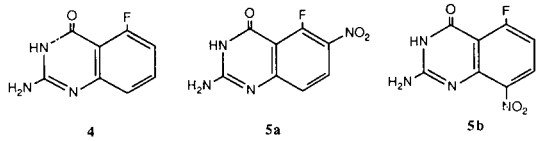


Figure 2

and coupling constants for the four doublets of doublets obtained are summarized in Table 2. The splitting patterns of the peaks disprove that nitration occurs at position C^7. The 7-nitro analogue would be expected to display a doublet of a doublet for C^6-H, with coupling constants of $J_{6,F}$ = approx. 10 Hz and $J_{6,8}$ = 1-3 Hz, in addition to a doublet of a doublet for C^8-H with coupling constants of $J_{8,F}$ = approx. 1 Hz and $J_{6,8}$ = 1-3 Hz. No peaks matching these requirements were seen. Instead, the peak patterns exactly fit the structures of the 6-nitro product **5a** with coupling frequencies of $J_{7,8}$ = 9.28-9.31 Hz, $J_{7,F}$ = 8.19 Hz



4 5a 5b

Table 1. ^{13}C chemical shifts (ppm) and heteronuclear coupling constants (Hz) of 2-amino-4-oxo(3H)-5-trifluoromethylquinazoline (2) and its C^6-nitration product (3)[a]

^{13}C	δ		J_{CH}[b]	J_{CF}[b]
2	152.7	**153.7**		
4	159.7	**159.3**		
4a	113.5	**114.5**	6.2(**5.5**)^3	
5	127.3	**120.6**	8.1(**6.3**)^3, **1.3**^{4}	31.9(**34.3**)^2
6	120.7	**144.1**	167.5^1	7.3(**3.0**)^3
7	133.0	**128.2**	165.7(**171.0**)^1, **1.7**^{2}	
8	129.5	**130.2**	165.7(**169.5**)^1	
8a	153.8	**155.2**		
CF_3	123.6	**121.8**	4.8^3,**1.7**^{4}	275.3(**273.0**)^1

[a]Values for the C^6-nitro isomer, **3**, are shown in boldcase; [b]The bond numbers of the scalar couplings are shown in superscripted italic

Table 2. ^1H and ^{19}F chemical shifts (ppm) and coupling constants (Hz) of the nitration product of 2-amino-4-oxo(3H)-5-fluoroquinazoline (4)[a]

^1H	δ		J_{HH}		J_{HF}	
NH_2	**7.30**	7.30				
6		6.93		8.87		10.43
7	**8.23**	8.08	**9.28**	8.84	**8.19**	5.05
8	**7.08**		**9.31**		**1.00**	

^{19}F	δ		$J_{7,F}$
5	**-40.11**	-27.83	**8.05**

[a]Values for the 6-nitro isomer, **5a**, are shown in boldcase while the values for the 8-nitro isomer, **5b**, are in plain text.

and $J_{8,F}$ = 1.00 Hz and the 8-nitro product **5b** with coupling frequencies of $J_{6,7}$ = 8.84-8.87 Hz, $J_{6,F}$ = 10.43 Hz and $J_{7,F}$ = 5.05 Hz. Shift values, coupling constants, and integration of the corresponding peak areas on the ^{19}F nmr spectrum supported the assignments and established a 4:1 ratio for 6-nitro/8-nitro product distribution.

EXPERIMENTAL

The 100.6 MHz ^{13}C nmr spectra of samples **2** and **3** prepared in DMSO-d_6 (300 mg/3 ml) were acquired at 25° using a Varian VXR400 spectrometer (9.4 Tesla). Following a 45° (15 μs) observe pulse, an acquisition time of 1s was used to collect 30K complex points defining a 15000 Hz spectral window. Broadband WALTZ-modulated ^1H decoupling was gated on during the 1s relaxation delay that preceeded each pulse of the 4K transients collected per sample. The resultant free induction decays, multiplied by both resolution enhancement and apodization functions having

250 ms and 400 ms time constants, respectively, were Fourier transformed to 64K complex points. Resonances were assigned based on one-bond and long-range scalar couplings to both ^{1}H and ^{19}F as well as on published data for similar compounds. Chemical shifts are reported relative to the center line of the DMSO-d_6 resonance taken as 39.5 ppm. The 400 MHz ^{1}H and ^{19}F nmr spectra of the nitration product of **4** were acquired in DMSO-d_6 (5.00 mg/0.70 ml) at 25° on a Varian VXR400 spectrometer using tetramethylsilane and trifluoroacetic acid as respective internal standards.

REFERENCES AND NOTES

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