BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 52 (1), 259—260 (1979)

Allylation of Nucleic Acid-Bases with Triallyl Phosphate

Toshizumi TANABE,* Kiyoshi YAMAUCHI, and Masayoshi KINOSHITA
Department of Applied Chemistry, Osaka City University, Sumiyoshi-ku, Osaka 558

(Received May 12, 1978)

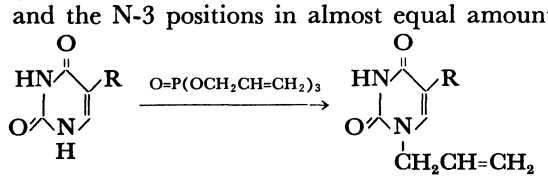
Synopsis. Triallyl phosphate alkylated uracil (N-1), thymine (N-1), cytosine (N-1), adenine (N-3 and N-9), and guanine (presumably N-1 or N-7) in a water solution at 60 °C (pH values were 10—10.5 for uracil, thymine, cytosine, and adenine, and 12 for guanine), giving the corresponding allyl derivatives in considerable yields.

Many modified nucleosides are present in various tRNAs and have been considered to play important roles in their biogenesis.[1] Since most of the modified nucleosides are methylated at nitrogen atoms of base moieties, methylation studies of nucleic acid-bases or nucleosides have been carried out using various alkylating agents, such as dimethyl sulfate,[2-4] diazomethane,[5] and methyl iodide.[6,7] Previously, we reported methylation[8] and ethylation[9] of nucleic acid-bases in an aqueous phase by means of trimethyl and triethyl phosphate (TMP and TEP).

In this paper, we wish to describe the reactions of nucleic acid-bases such as uracil (**1**), thymine (**2**), cytosine (**3**), adenine (**7**), and guanine (**10**) with triallyl phosphate (TAP) in an aqueous phase. These reactions may be interest since there is a class of nucleic acid components which are substituted by allyl or isopentenyl groups like triacanthine.[10] Moreover, the present reactions are considered to resemble the biosynthesis of cytokinin which involves a transfer of an allylic group from Δ^2-isopentenyl pyrophosphate to the 6-amino group of adenine ring.[11]

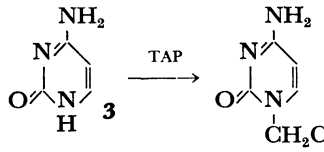
The reactions were carried out at 60 °C by stirring a mixture of a base and 2 molar excess of TAP in water. (The pH values were maintained at 10—10.5 for **1**, **2**, **3**, and **7**, and 12 for **10** throughout the reactions by the occasional addition of 4 M sodium hydroxide.)

Generally, **1** and **2** have been known to be alkylated with alkyl halides to give mainly 1-alkyl derivatives.[12,13] In the present reactions of **1** and **2** with TAP, 1-allyluracil (**4**) and 1-allylthymine (**5**) were obtained in 12 and 13% yields, respectively. The preference for the alkylation at the N-1 position was also observed in the ethylation of **1** and **2** with TEP,[9] while TMP methylated these two pyrimidine bases at both the N-1 and the N-3 positions in almost equal amounts.[8]



1: R=H
2: R=Me
4: R=H 12%
5: R=Me 13%

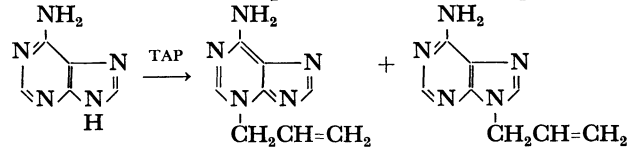
In cytosine (**3**), alkylation has been reported to take place mainly at the N-3 position in the reaction with dimethyl sulfate in DMF,[2] while TMP and TEP alkylated **3** at the N-1 position predominantly.[8,9] The present reaction of **3** with TAP occurred at the N-1 position to provide 1-allyl derivative (**6**) in 15% yield.



3 **6** 15%

Thus, triallyl phosphate was found to alkylate the above pyrimidines (**1**, **2**, and **3**) mainly at the N-1 position to give 1-allyl derivatives. The difficulty in alkylation at the N-3 position is attributed to the steric hindrance around the N-3 position of **1**, **2**, and **3** by two adjacent carbonyl or amino groups.

Meanwhile, adenine (**7**) was alkylated by TAP at the N-3 and the N-9 positions to afford 3-allyladenine (**8**) and 9-allyladenine (**9**) in 35 and 31% yields. Although **7** is generally known to be alkylated at the N-3 position under neutral conditions[3,4,14,15] and at the N-9 position under alkaline conditions,[7] alkylations with trialkyl phosphates such as TMP,[8] TEP,[9] and TAP in alkaline aqueous phase tend to take place at the N-3 and the N-9 positions in almost equal ratios.



7 **8** 35% **9** 31%

Most of guanine (**10**), on the other hand, remained unchanged in the reaction with TAP, because of the sparing solubility of **10** and TAP in water. (More than 70% of **10** was recovered.) But the reaction mixture, which was obtained after removing the unchanged **10**, showed eight product-spots on its thin-layer chromatography. Identification of these products, however, remained unsolved; the spectra of the isolated compounds did not resemble those of any known alkylated guanines.

Based on the above experiments, TAP was found to alkylate all five main nucleic acid-bases at various positions. This rather strong alkylating property of TAP may originate from its conjugated structure. The reactivity order of nitrogen atoms in each nucleic acid-base except **10**[16] toward TAP in weakly alkaline aqueous phase was found as follows; uracil, thymine, and cytosine: N-1>N-3; adenine:N-9, N-3>N-7, N-1.

Experimental

Melting points are uncorrected. The UV spectra were measured with a Hitachi EPS-3T spectrometer. The NMR spectra were recorded on a Hitachi 3T spectrometer, with a dilute solution in deuterium oxide, deuteriochloroform, or dimethyl-d_6 sulfoxide. Tetramethylsilane was used as the internal and outside standard. Mass spectra were obtained using a JEOL 01SG-2 spectrometer. Column chromatography was carried out using silica gel (Merck, Art. 7734, 70—230 mesh).

Commercially available uracil, thymine, cytosine, adenine,

and guanine were used without further purification. Triallyl phosphate was prepared by the procedure of Toy and Costello.[17]

*1-Allyluracil (**4**).* A mixture of **1** (1.23 g, 1.1 mmol) and TAP (5.10 g, 30.0 mmol) in water (15 ml) was stirred at 60 °C. The suspension was maintained at pH 9—10 throughout the reaction by the occasional addition of 4 M sodium hydroxide. After stirring for 48 h, the reaction mixture was neutralized with concentrated hydrochloric acid and extracted with chloroform. Unchanged **1** was recovered from the water layer (0.76 g, 63%). The organic layer was concentrated to give a residue which afforded crystalline 1-allyluracil (**4**, 0.20 g, 12%) by the addition of hexane; mp 106—108 °C; NMR ($CDCl_3$) δ=4.40 (2H, complex m, $-CH_2-CH=CH_2$), 5.08—5.57 (2H, complex m, $-CH_2-CH=CH_2$), 5.60—6.40 (1H, complex m, $-CH_2-CH=CH_2$), 5.78 (1H, d, J=7 Hz, **H**^{5}), 7.28 (1H, d, J=7 Hz, **H**^{6}), and 10.48 (1H, bs, N^3-**H**); UV, λ_{max} (H_2O) nm: pH 1, 266.0 (log ε 4.05), pH 7, 266.0 (log ε 4.00), pH 13, 265.0 (log ε 3.84); Found: C, 55.35; H, 5.04; N, 18.62%. Calcd for $C_7H_8N_2O_2$: C, 55.25; H, 5.30; N, 18.41%.

*1-Allylthymine (**5**).* A reaction of **2** (1.20 g, 10.0 mmol) with TAP (5.10 g, 30.0 mmol) in water (15 ml) at 60 °C for 48 h afforded **5** (0.20 g, 13%) after a procedure similar to that described above; mp 96—97 °C (from methanol); NMR ($CDCl_3$) δ=1.94 (3H, d, J=1.2 Hz, $-CH_3$), 4.35 (2H, complex m, $-CH_2-CH=CH_2$), 5.02—5.50 (2H, complex m, $-CH_2-CH=CH_2$), 5.50—6.30 (1H, complex m, $-CH_2-CH=CH_2$), 7.02 (1H, d, J=1.2 Hz, **H**^{6}), and 9.80 (1H, bs, N^3-**H**); UV, λ_{max} (H_2O) nm: pH 1, 272.0 (log ε 3.96), pH 7, 272.0 (log ε 3.95), pH 13, 272.0 (log ε 3.82); Found: C, 57.76; H, 5.98; N, 16.75%. Calcd for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.86%. Unchanged **2** was recovered in 67% yield (0.80 g).

*1-Allylcytosine (**6**).* A mixture of **3** (1.00 g, 0.9 mmol) and TAP (5.10 g, 30.0 mmol) was stirred in water (10 ml, pH 9—10, NaOH) at 60 °C for 48 h. The reaction mixture was then neutralized by concentrated hydrochloric acid and extracted with chloroform to remove the unchanged TAP. The water layer was concentrated to give a residue, which was then separated by silica gel chromatography (2×50 cm). Elution with chloroform–methanol (5:2) provided the salt of **6** with diallyl hydrogen phosphate (0.46 g) and unchanged **3** diallyl hydrogen phosphate (1.52 g, 59%). The salt of **6** was subsequently treated with an anionic exchange resin (Dowex 1×8, 200—400 mesh, OH form). Elution with water gave the free form of **6** (0.20 g, 15%); mp 245—247 °C (sublime); NMR (D_2O) δ=4.37 (2H, complex m, $-CH_2-CH=CH_2$), 4.85—5.48 (2H, complex m, $-CH_2-CH=CH_2$), 5.52—6.30 (1H, complex m, $-CH_2-CH=CH_2$), 5.99 (1H, d, J=7 Hz, **H**^{5}), and 7.48 (1H, d, J=7 Hz, **H**^{6}); UV, λ_{max} (H_2O) nm: pH 1, 282.0 (log ε 4.19), pH 7, 274.0 (log ε 4.01), pH 13, 274.0 (log ε 4.00); Found: C, 54.38; H, 5.76; N, 27.21%. Calcd for $C_7H_9N_3O\cdot 0.2H_2O$: C, 54.33; H, 6.08; N, 27.13%.

*3- and 9-Allyladenines (**8** and **9**).* Compound **7** (1.35 g, 10.0 mmol) and TAP (5.20 g, 31.0 mmol) was stirred in water (10 ml, pH 10, NaOH) at 60 °C for 48 h. After the reaction mixture was neutralized by hydrochloric acid, the resulting solution was concentrated to give a residue which was subsequently mixed with ethanol and separated from undissolved substances. The residue which was obtained after concentrating the ethanolic solution was then purified with a silica gel column (3×50 cm). Elution with chloroform–methanol (10:1) provided **9** (0.57 g, 31%); mp 162 °C (from benzene) (lit,[18] 163 °C); NMR ($CDCl_3$) δ=4.75—5.00 (2H, complex m, $-CH_2-CH=CH_2$), 5.05—5.53 (2H, complex m, $-CH_2-CH=CH_2$), 5.75—6.55 (1H, complex m, $-CH_2-CH=CH_2$), 6.32 (2H, bs, $-NH_2$), 7.88 (1H, s, **H**^{2}), and 8.46 (1H, s, **H**^{8}); UV, λ_{max} (H_2O) nm: pH 1, 259.5 (log ε 4.11), pH 7, 261.0 (log ε 4.11), pH 13, 261.0 (log ε 4.11). The subsequent elution with the same solvent afforded crude **8** contaminated with some phosphate, which was then treated with an anionic exchange resin (Dowex 1×8, 200—400 mesh, OH form). Elution with water gave **8** (0.64 g, 35%); mp 207—210 °C (from methanol) (lit,[15] 199—201 °C); NMR (D_2O) δ=4.40—5.06 (2H, complex m, $-CH_2-CH=CH_2$), 5.06—5.50 (2H, complex m, $-CH_2-CH=CH_2$), 5.58—6.50 (1H, complex m, $-CH_2-CH=CH_2$), 7.80 (1H, s, **H**^{2}), and 8.06 (1H, s, **H**^{8}); UV, λ_{max} (H_2O) nm: pH 1, 274.0 (log ε 4.22), pH 7, 274.0 (log ε 4.10), pH 13, 274.0 (log ε 4.06).

*Allylation of Guanine (**10**).* Compounds **10** (2.50 g, 16.6 mmol) was suspended in a mixture of TAP (10.2 g, 60.0 mmol) and water (100 ml, pH 12, NaOH) at 60 °C. After stirring for 48 h, undissolved **10** (1.83 g, 73%) was recovered by filtration and the resulting solution was extracted with toluene to remove unchanged TAP. The water layer was developed on silica gel [GF_{254} (type 60), Merck] thin-layer chromatography using chloroform–methanol (5:1), and showed eight unidentified spots. After concentrating the water solution, the residue was mixed with ethanol and separated from the undissolved substances. The residue which was obtained after concentrating the alcoholic solution was purified by silica gel chromoatography (3×55 cm). Two kinds of effluents were obtained, using chloroform–methanol (8:1) as the developing solvent. One of them showed a single spot on silica gel TLC, which turned out to be monoallylguanine from its mass spectrum (0.03 g, 1%); *m/e*: 191; UV λ_{max} (H_2O) nm: pH 1, 251, 275(s), pH 13, 280.0. The other effluent (0.01 g) seemed to be a mixture of monoallyl- and diallylguanines, since there were two spots on its silica gel TLC and its mass spectrum showed *m/e* 231 and 191.

References

1) J. A. McClosky and S. Nishimura, *Acc. Chem. Res.*, **10**, 403 (1977).
2) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, **1962**, 1348.
3) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **84**, 1914 (1962).
4) C. B. Pal, *Biochemistry*, **1**, 558 (1962).
5) J. A. Haines, C. B. Reese, and L. Todd, *J. Chem. Soc.*, **1962**, 5281.
6) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
7) M. Rasmussen and H. -S. Chan, *Aust. J. Chem.*, **28**, 1031 (1975).
8) K. Yamauchi, T. Tanabe, and M. Kinoshita, *J. Org. Chem.*, **41**, 3691 (1976).
9) T. Tanabe, K. Yamauchi, and M. Kinoshita, *Bull. Chem. Soc. Jpn.*, **50**, 3021 (1977).
10) N. J. Leonard and J. A. Deyrup, *J. Am. Chem. Soc.*, **84**, 2148 (1962).
11) Y. Taya, Y. Tanaka, and S. Nishimura, *Nature*, **271**, 545 (1978).
12) G. E. Hilbert and J. B. Johnson, *J. Am. Chem. Soc.*, **52**, 2001 (1930).
13) E. Wittenburg, *Chem. Ber.*, **99**, 2380 (1966).
14) N. J. Leonard and R. A. Lauren, *J. Am. Chem. Soc.*, **85**, 2026 (1963).
15) N. J. Leonard and T. Fujii, *J. Am. Chem. Soc.*, **85**, 3719 (1963).
16) The reactivity of nitrogen atoms in guanine was uncertain due to the low extent of allylation reaction, but methylation by TMP and ethylation by TEP have suggested the following order: N-1>N-7>N-3>N-9.
17) A. D. Toy and J. R. Costello, U. S. Patent 2754315 (1950).
18) K. Kondo, K. Kuwata, and K. Takemoto, *Makromol. Chem.*, **160**, 341 (1972).