Reactions with Deoxyguanosine of 4-(Carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone, a Model Compound for α-Hydroxylation of Tobacco-Specific Nitrosamines[1,2]

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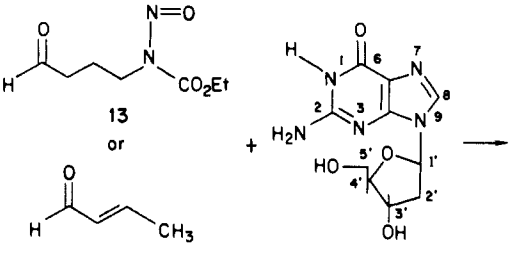
Abstract: 4-(3-Pyridyl)-4-oxobutanediazohydroxide (**10**) is a likely product of metabolic α-hydroxylation of the tobacco nitrosamines *N'*-nitrosonornicotine (**2**) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (**1**). The reactions of 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (**12**), a stable precursor to **10**, with deoxyguanosine and H_2O at pH 8 were investigated. Three products of the reaction of **12** with deoxyguanosine were characterized as 2'-deoxy-*N*-[1-methyl-3-oxo-3-(3-pyridyl)propyl]guanosine (**18**), 2'-deoxyguanosine 3'-(ethyl carbonate), and 2'-deoxyguanosine 5'-(ethyl carbonate). The adduct **18** was also formed upon reaction of 1-(3-pyridyl)-2-buten-1-one (**17**) with deoxyguanosine. Whereas the branched chain adduct **18** was the major product formed upon reaction of **12** with deoxyguanosine, the solvolysis of **12** gave mainly the nonrearranged keto alcohol 4-hydroxy-1-(3-pyridyl)-1-butanone (**21**), with lesser amounts of 3-hydroxy-1-(3-pyridyl)-1-butanone (**22**) and **17**. Possible mechanisms for the formation of **18** are discussed.

Nicotine can be nitrosated to form 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, **1**) and *N'*-nitrosonornicotine (NNN, **2**).[3] These nitrosamines are present in tobacco and tobacco smoke in relatively high concentrations and are strong carcinogens in mice, rats, and hamsters.[4] They are likely to be causative factors in tobacco-related cancer in man.[4-6]

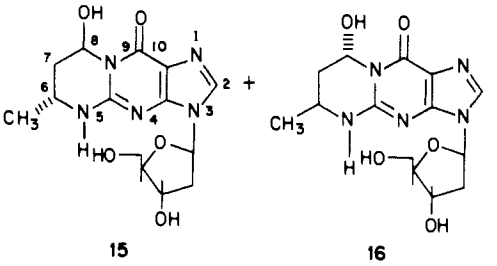
Modification of DNA is considered to be important in initiation of carcinogenesis. Compounds **1** and **2** do not react directly with DNA but are metabolized in vitro and in vivo to intermediates which do react with DNA.[7,8] Metabolic methylene hydroxylation of **1** gives the unstable α-hydroxynitrosamine **3**, which spontaneously decomposes yielding keto aldehyde **7** and the unstable electrophile methanediazohydroxide (**8**, Scheme I).[4,8] Methanediazohydroxide reacts with DNA, yielding 7-methylguanine and O^6-methylguanine which have been detected in the liver, lung, and nasal mucosa of rats treated with **1**.[9] The analogous pathways of α-hydroxylation at the methyl carbon of **1** or the 2'- or 5'-carbons of **2** would yield α-hydroxynitrosamines **4-6**. These, in turn, are expected to give diazohydroxides **10** and **11**. Solvolyses of the acetates of **5** and **6** support this assumption.[10] The structures of the adducts formed upon reaction of **10** and **11** with DNA are not known. Such adducts should be formed in animals or humans exposed to **1** and **2**. Therefore, we have carried out studies aimed at elucidating their structures. Our initial investigations have focused on diazohydroxide **10** since it can be formed from either **1** or **2**. We have used 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (**12**) as a stable precursor to **10**. Since deoxyguanosine is often the most reactive of the DNA bases toward electrophiles, we have studied its reaction with **10** under mildly basic conditions.

Results and Discussion

The nitrosourethane **12** was allowed to react with [^{14}C]-deoxyguanosine for 4 days at 37 °C in pH 8 phosphate buffer. Analysis by TLC showed that **12** had reacted completely. Analysis of the mixture by HPLC-scintillation counting gave the radiochromatogram illustrated in Figure 1. In addition to unreacted [^{14}C]deoxyguanosine which eluted at 32 min, three major radioactive peaks, I-III, were detected. Their UV spectra are illustrated in Figure 2. These spectra were similar to those of the diastereomeric adducts **15** and **16** which we had previously characterized as the major products formed upon reaction of nitrosourethane **13** or crotonaldehyde (**14**) with deoxyguanosine.[11] The spectra suggested a 1- and/or N^2-substitution pattern and excluded reaction at the 7- or O^6-position of deoxyguanosine.

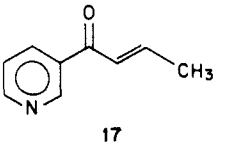


13 or 14



15 16

Therefore, we suspected that they might have similar structures to those of **15** and **16** and that larger quantities, sufficient for further characterization, might be obtained by reaction of ketone **17** with deoxyguanosine. The unstable ketone **17** was synthesized



17

by reaction of pyridine-3-carboxaldehyde with allylmagnesium

(1) Paper 91 in the series "A Study of Chemical Carcinogenesis".
(2) Supported by NCI Grant CA-21393.
(3) Hecht, S. S.; Chen, C. B.; Ornaf, R. M.; Jacobs, E.; Adams, J. D.; Hoffmann, D. *J. Org. Chem.* **1978**, *43*, 72.
(4) Hoffmann, D.; Hecht, S. S. *Cancer Res.* **1985**, *45*, 935.
(5) Craddock, V. M. *Nature (London)* **1983**, *306*, 638.
(6) Bartsch, H.; Montesano, R. *Carcinogenesis* **1984**, *5*, 1381.
(7) Chen, C. B.; Hecht, S. S.; Hoffmann, D. *Cancer Res.* **1978**, *38*, 3639.
(8) Hecht, S. S.; Young, R.; Chen, C. B. *Cancer Res.* **1980**, *40*, 4144.
(9) Hecht, S. S.; Trushin, N.; Castonguay, A.; Rivenson, A. *Cancer Res.* **1986**, in press.
(10) Hecht, S. S.; Chen, C. B. *J. Org. Chem.* **1979**, *44*, 1563.
(11) Chung, F. L.; Hecht, S. S. *Cancer Res.* **1983**, *43*, 1230.

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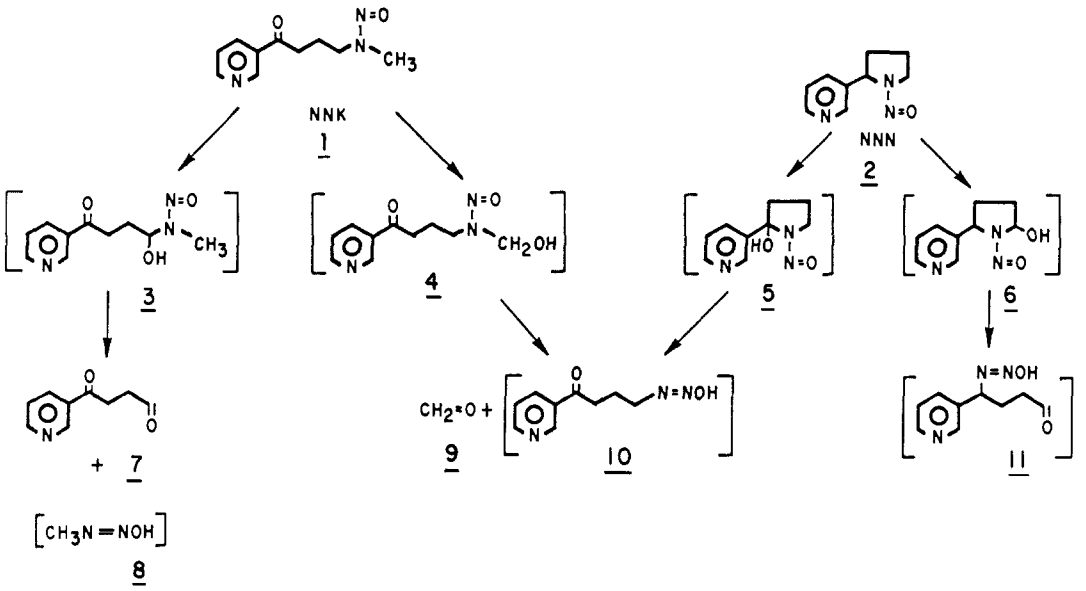
Scheme I



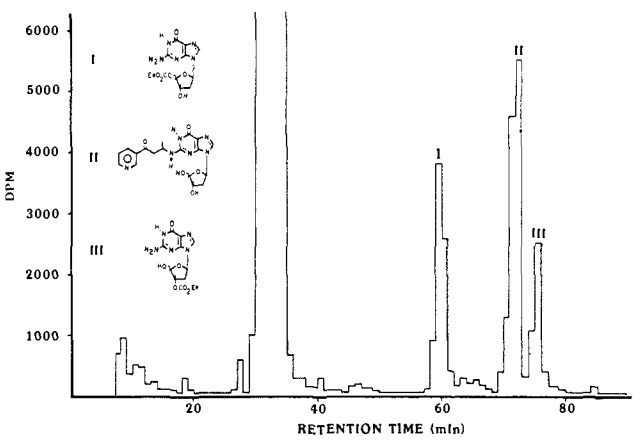


Figure 1. Analysis by HPLC of the products formed in the reaction of [^{14}C]deoxyguanosine with **12**.

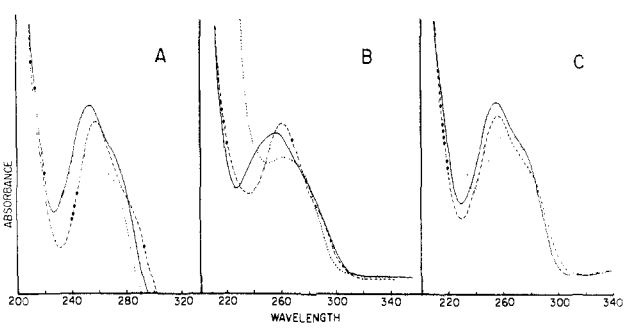


Figure 2. UV spectra of A, peak I, B, peak II, and C, peak III, of Figure 1 at pH 7 (–), 1 (– – –), and 13 (···).

bromide, followed by oxidation with CrO_3 in H_2SO_4. It was purified and identified by its NMR and MS. It is presumed to be predominantly trans. Compound **17** was allowed to react with [^{14}C]deoxyguanosine under conditions identical with those used for reaction of **12** with [^{14}C]deoxyguanosine. Only one product other than [^{14}C]deoxyguanosine was detected, and it eluted at the same position as peak II of Figure 1. Its UV spectrum was identical with that of peak II.

The 500-MHz PMR spectrum of the product together with the UV data cited above provides strong evidence that the structure of the adduct is 2′-deoxy-*N*-[1-methyl-3-oxo-3-(3-pyridyl)-propyl]guanosine (**18**), as a mixture of two diastereomers. Several features of the PMR spectrum are noteworthy. The methyl resonances appear as two doublets at 1.27 and 1.28 ppm, corresponding to the two diastereomers; the diastereomeric 1′-protons and C-8 protons are also resolved. The exchangeable N^2–H and 1-NH protons appeared at 6.6 and 10.5 ppm, respectively, which is in agreement with the proposed N^2-substitution pattern. These data alone would not exclude a cyclic 1,N^2-adduct analogous to **15** and **16**. However the position of the 2-hydrogen of the pyridine ring at 9.13 ppm is consistent with **18** rather than the cyclic structure. Pyridines substituted at the 3-position with a carbonyl group show a downfield shift of the 2-hydrogen from 8.5–8.7 ppm, where it is observed when the 3-position is substituted by –CHOH or $-CH_2$, to 9.1–9.4 ppm, as in **18**. The relatively downfield position, 3.2 ppm, of the protons on the carbon adjacent to the carbonyl are also in agreement with structure **18**. The protons at position 7 of **15** or **16** are observed at 1.4 and 2.0 ppm.

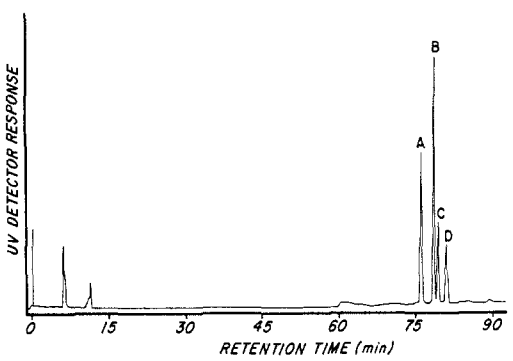
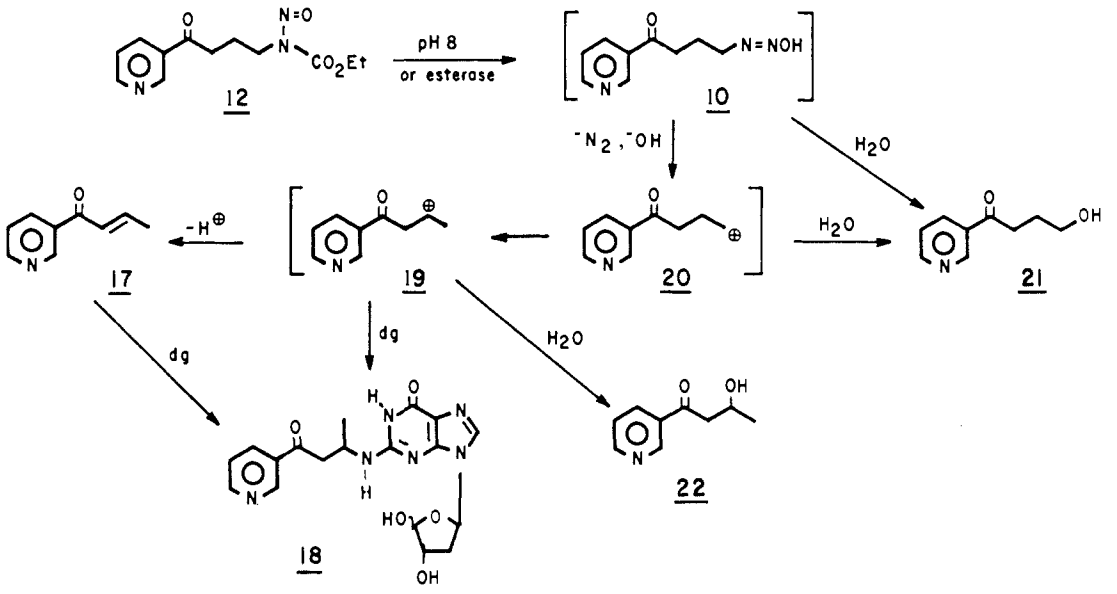


Figure 3. Analysis by HPLC of the products formed by reduction of **18** with $NaBH_4$.

The FAB spectrum of **18** showed an M + 1 peak at *m/e* 415 and a peak at 437.155 93, corresponding to $C_{19}H_{22}N_6O_5Na$ (calculated, 437.15496).

We then sought further evidence for the identity of peak II of Figure 1, formed in the reaction of **12** with deoxyguanosine, with the identified diastereomers **18**, formed in the reaction of **17** with deoxyguanosine. HPLC with UV detection allowed partial separation of the two diastereomers. The retention times of **18** formed in the two reactions were identical, but the ratios of the two peaks were slightly different: 48:52 from **12** and 54:46 from **17**. Reduction of **18** with $NaBH_4$ would be expected to give four diastereomeric alcohols since a new asymmetric center is created. As illustrated in Figure 3, four peaks were observed upon reduction of the diastereomers obtained from **17**. From the ratios of the peak heights, it is probable that peaks A and C of Figure 3 are formed from the minor diastereomer and peaks B and D from the major diastereomer of **18**, as obtained from reaction of **17** with deoxyguanosine. Reduction of **18**, obtained from the reaction of

Scheme II



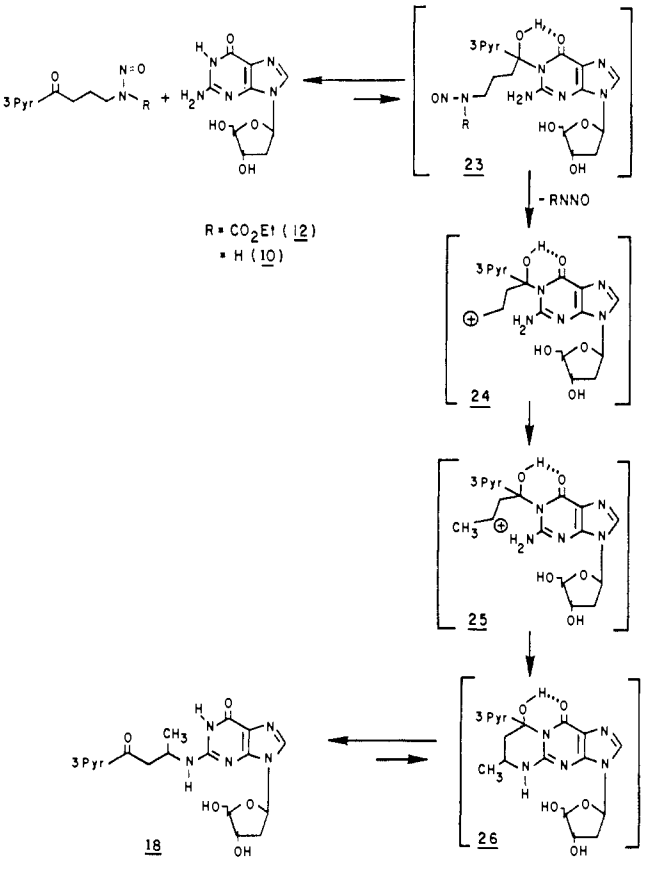
12 with deoxyguanosine, gave the same four peaks as illustrated in Figure 3 except that the relative proportions of the peaks corresponded to the 48:52 ratio of the diastereomers of **18** in this mixture. These results provide further confirmation of the identities of the diastereomeric adducts **18** formed by reaction of deoxyguanosine with either **12** or **17**. Inspection of space-filling models suggests that approach of borohydride from one side of each of the diastereomers is hindered. This could explain the preferential formation of peaks A and B from each of the two diastereomers.

The NMR spectra of peaks I and III of Figure 1 showed no pyridine protons but did display resonances corresponding to $-CH_3$ and $-CH_2$ of a $-CO_2CH_2CH_3$ group. By comparison of their spectra to that of deoxyguanosine, the structures of peaks I and III were tentatively assigned as 2′-deoxyguanosine 5′-(ethyl carbonate) and 2′-deoxyguanosine 3′-(ethyl carbonate), respectively. Their identities were not further investigated.

Thus, the major pyridine-containing adduct produced by reaction of **12** with deoxyguanosine at pH 8 was **18**. To examine the basis for formation of this rearranged product, we reinvestigated the solvolysis products of **12**, formed in the same reaction from which **18** was isolated. As in our previous study,[10] the major product was the nonrearranged keto alcohol **21** (66%). The rearranged keto alcohol **22** was produced in 7% yield and the ketone **17** in 2% yield. The formation of these products is rationalized in Scheme II. The nonrearranged keto alcohol **21** can be formed either by solvolysis of diazohydroxide **10** or via carbonium ion **20**; the latter can rearrange to **19** which reacts with solvent, yielding **22**, or eliminates to give **17**. The formation of the nonrearranged keto alcohol **21** as a major product in the solvolysis of **12** contrasts to the formation of the rearranged adduct **18** in the reaction of **12** with deoxyguanosine. Although **18** appears to be the major deoxyguanosine adduct, we cannot exclude the possibility that some nonrearranged adduct is also produced.

The simplest explanation for the apparent preferential formation of the rearranged adduct **18** is that deoxyguanosine reacted with the small amount of **17** produced in solvolysis of **12** (Scheme II). However, considering the 2% yield of **17** from **12**, and the 30% yield in reaction of **17** with deoxyguanosine, this may not account for **18**, which was formed in 7% yield from **12**. Alternatively, **18** could be produced by selective reaction of the carbonium ion **19** with deoxyguanosine. However, preferential reaction of deoxyguanosine with **19**, as opposed to **20**, seems unlikely. Another proposal for selective formation of the rearranged adduct **18** is presented in Scheme III. The carbonyl group of diazohydroxide **10** or nitrosourethane **12** may react with N-1 of deoxyguanosine, yielding intermediate **23**. This postulate is based on our studies with nitrosourethane **13**, in which the aldehyde carbonyl group

Scheme III



has a strong directing effect, resulting in preferential formation of N–1,N^2 cyclic adducts, rather than the 7-alkylated products which are generally formed from alkanediazohydroxides. In the case of **10** or **12**, the carbonyl group is a ketone and is less reactive than in **13**. Thus, we expect that the equilibrium would not favor **23**. Hydrolysis of **23** (R = CO_2Et) or spontaneous decomposition (R = H) would give **24**. Rearrangement to **25** would be favored for production of the six-membered ring in **26**. Compound **26** is analogous to the adducts **15** and **16** formed upon reaction of **13** with deoxyguanosine. As in **23**, the ring-opened tautomer **18** would be favored due to the relative stability of the carbonyl group of **18**. Scheme III would suggest that some **26** might be present in the reaction mixture; we did not detect it in this study.

We are presently using **18** as a marker for studies of in vivo DNA adduct formation from tritiated NNN and NNK, with the label in the pyridine ring.

Experimental Section

PMR spectra (90MHz) were determined with a Jeol Model FX90Q spectrometer; 500-MHz spectra were kindly provided by Dr. Dwight Miller of the National Center for Toxicological Research, Jefferson, AR. All spectra are reported as parts per million (ppm) downfield from Me_4Si as the internal reference. UV spectra were determined with a Cary Model 118 instrument. MS were run with a Hewlett-Packard Model 5982A dual-source instrument. High-resolution FAB MS were kindly provided by Dr. Pamela Crain, Department of Medicinal Chemistry, University of Utah, using a Varian MAT 731 instrument operated at 8 kV with fast-atom bombardment, 6 keV of Xe. Conventional high-resolution MS were obtained at the Rockefeller University Mass Spectrometric Biotechnology Resource. HPLC was carried out with a Waters Assoc. Model ALC/GPC-204 high-speed liquid chromatograph equipped with a Model 660 solvent programmer and a Model LC-25 UV/visible detector. Scintillation counting was performed with a Beckman LS-9000 Liquid Scintillation System. GC was performed with a Hewlett-Packard Model 5830A instrument.

4-(Carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (**12**) and 4-hydroxy-1-(3-pyridyl)-1-butanone (**21**) were prepared as described previously.[10,12] [^{14}C]Deoxyguanosine (56 mCi/mmol) was procured from Moravek Biochemicals, Brea, CA. Deoxyguanosine, 3-pyridinecarboxaldehyde, allylmagnesium bromide, 3-acetylpyridine, and acetaldehyde were obtained from Aldrich Chemical Co., Milwaukee, WI. Regisil RC-2 [bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane] was obtained from Regis Chemical Co., Morton Grove, IL.

1-(3-Pyridyl)-2-buten-1-one (17). To a solution of 3-pyridinecarboxaldehyde (5.35 g, 0.05 mol) in 100 mL of anhydrous ether was added allylmagnesium bromide (0.05 mol, 1 M in ether). The addition was carried out under a stream of N_2, dropwise at −5 °C over a 20-min period. After the addition was complete, 20 mL of saturated NH_4Cl was gradually added while the temperature was maintained at 0 °C. After 20 min of stirring, the mixture was extracted 4 times with 40-mL portions of ether. The combined ether extracts were dried (Na_2SO_4) and concentrated, giving 5.2 g of a brownish-red oil. The crude product was purified by chromatography on a column of basic alumina with elution by 1.5% MeOH in CH_2Cl_2, yielding a yellow oil, 3.1 g, 42%: NMR ($CDCl_3$) δ 8.8 (m, 2, pyr-2H, 6 H), 7.7 (d, pyr-4H), 7.2 (m, pyr-5H), 5.7 (m, 1, CH_2—*CH*=CH_2), 5.1 (d, 2, =*CH_2*), 4.7 (t, 1, C*H*OH), 4.6 (s, 1, CHO*H*), 2.5 (dd, 2, —*CH_2*—); MS, *m/e* (rel intensity) 108 (100), 85 (37), 83 (59), 80 (46). This material (2 g, 0.013 mol) was used without further purification. It was dissolved in 19 mL of acetone, and 3 g (0.03 mol) of CrO_3 in 63 mL of 10 N H_2SO_4 was added dropwise at 0 °C. After stirring at 0 °C for 3.5 h, the mixture was washed with hexane, neutralized with $NaHCO_3$, and extracted with ether (3 × 25 mL) and CH_2Cl_2 (4 × 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give 1.6 g of crude product containing **17**. It was purified by column chromatography on silica gel with elution by 0.5% MeOH in $CHCl_3$, giving 0.7 g (10%) of **17**, which darkens rapidly on standing in air: NMR ($CDCl_3$) δ 9.4–9.0 (br s, 1, pyr-2H), 9.0–8.7 (m, 1, pyr-6H), 8.25 (d, 1, pyr-4H), 7.7–7.0 (m, 3, pyr-5H, C*H*=C*H*), 2.05 (d, 3, CH_3); MS, *m/e* (rel intensity) 147 (32), 146 (39), 145 (21), 132 (15), 118 (18), 106 (28), 89 (15), 78 (100), 52 (25), 51 (45), 50 (37); high-resolution MS calcd for C_9H_9NO 147.0684, found 147.0710.

3-Hydroxy-1-(3-pyridyl)-1-butanone (22). A mixture of 3-acetylpyridine (5.0 g, 0.04 mol) and acetaldehyde (1.8 g, 0.04 mol) was added at 0 °C to a stirred suspension of $NaOCH_3$ (2.3 g, 0.04 mol) in 50 mL of toluene. After 3 min, the ice bath was removed and solid formed. Stirring was continued for 30 min at room temperature. After addition of H_2O, the mixture was extracted twice with Et_2O and twice with CH_2Cl_2. The combined extracts were dried and concentrated, giving a residue which was purified by silica gel chromatography with elution by 0.5% MeOH in CH_2Cl_2. Fractions containing **22** were detected by TLC on silica (R_f = 0.28, 15/1; $CHCl_3$/MeOH) and were repurified by preparative TLC. The R_f of **21** under these conditions is 0.20: NMR ($CDCl_3$) δ 9.05 (br s, 1, pyr-2H), 8.68 (m, 1, pyr-6H), 8.12 (m, 1, pyr-4H), 7.32 (m, 1, pyr-5H), 4.35 (sextuplet, 1, CH_2*CH*(OH)CH_3), 3.44 (brs, 1, OH), 3.03 (d, 2, *CH_2*CH(OH)CH_3), 1.21 (d, 3, CH_2CH(OH)*CH_3*); MS, *m/e* (rel intensity) 165 (M^+, 5.9), 150 (17.5), 148 (11.1), 121 (29.1), 106 (100), 78 (37.9); high-resolution MS calcd for $C_9H_{11}NO_2$ 165.0786, found 165.0789.

Reaction of 4-(Carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (12) with Deoxyguanosine. A mixture of **12** (0.7 g, 2.9 mmol), [^{14}C]deoxyguanosine (5 μCi, 0.27 g, 1 mmol) and pH 8 sodium phosphate buffer (53 mL, 0.1 M) was incubated in a shaking water bath at 37 °C for 4 days. The mixture was analyzed by HPLC using two Waters μ-Bondapak-C_{18} columns in series with a gradient from 0 to 50% MeOH/H_2O in 100 min at 1 mL/min. Fractions were collected for scintillation counting; the radiochromatogram is illustrated in Figure 1. Yields of peaks I–III (percent of deoxyguanosine) were I, 4, II, 7, III, 2. The reaction mixture was concentrated under reduced pressure and the residue partially purified by column chromatography on silica gel with elution by 5–10% MeOH in CH_2Cl_2. Peaks I–III of Figure 1 were then isolated by HPLC and characterized by their spectral properties. These are described in the text and below for peak II (**18**): PMR (90 MHz) of peak I (Me_2SO-d_6) δ 10.6 (s, 1, 1-NH), 7.80 (s, 1, 8-H), 6.4 (s, 2, NH_2), 6.15 (t, 1, 1′-H), 5.45 (d, 1, 3′-OH), 4.4–3.9 (m, 7, $CO_2CH_2CH_3$ + 5′-CH_2 + 4′-H + 3′-H), 2.8–2.2 (m, 2, 2′-H), 1.20 (t, 3, CO_2CH_2,*CH_3*); PMR (500 MHz) of peak III (Me_2SO-d_6) δ 10.65 (s, 1, 1-NH), 7.95 (s, 1, 8-H), 6.48 (s, 2, NH_2), 5.25 (d, 1, 3′-H), 5.16 (t, 1, 5′-OH), 4.18 (q, 2, $CO_2CH_2CH_3$), 4.07 (t, 1, 4′-H), 3.60 (m, 2, 5′-CH_2), 2.81 (m, 1, 2′-βH), 2.5 (m, 1, 2′-αH), 1.25 (t, 3, CO_2CH_2, *CH_3*).

In a separate experiment, **12** was allowed to react with deoxyguanosine under conditions identical with those described above. The mixture was analyzed for **17** by HPLC using the conditions described above. The retention time of **17** was 104 min. The structure of **17** was confirmed by GC–MS of the collected HPLC peak, using a 25 m × 0.25 mm SE-54 fused silica capillary column, programmed from 30 to 200 °C at 4 °C/min in the splitless mode, at a flow rate of 1 mL/min of He. The retention time of **17** was 25.8 min. For quantitation of **21** and **22**, the reaction mixture was extracted 4 times with EtOAc. An aliquot was dried and concentrated and the residue silylated with Regisil-RC-2. The resulting mixture was analyzed by GC using a 10 m × 0.25 mm cross-linked methylsilicone-fused silica capillary column, programmed from 80 to 220 °C at 4 °C/min. The flow rate was 1 mL/min of He, and the split ratio was 20:1. Retention times of the trimethylsilyl ethers of **21** and **22** were 17.48 and 14.56 min, respectively. Their structures were confirmed by GC–MS.

Reaction of 1-(3-Pyridyl)-2-buten-1-one (17) with Deoxyguanosine. A mixture of **17** (0.12 g, 0.8 mmol) in 0.2 mL of Me_2SO and [^{14}C]-deoxyguanosine (0.5 μCi, 0.22 g, 0.8 mmol) in pH 8 sodium phosphate buffer (27 mL, 0.1 M) was incubated with shaking for 4 days at 37 °C. The mixture was extracted with CH_2Cl_2 (5 × 30 mL), and the aqueous layer was concentrated. HPLC analysis of the aqueous layer showed that the yield of **18** was 30% based on deoxyguanosine. The residue was passed through a column of silica gel with elution by 5–10% MeOH in CH_2Cl_2 to remove most of the deoxyguanosine. The adduct **18** was isolated by HPLC on a Whatman Magnum 9, ODS-3 column with elution by a 0–50% MeOH/H_2O gradient in 100 min at 4 mL/min: PMR (500 MHz) (Me_2SO-d_6) δ 10.5 (brs, 1, 1-NH), 9.13 (m, 1, pyr-2H), 8.78 (m, 1, pyr-6H), 8.31 (m, 1, pyr-4H), 7.91 (s, 0.6, C-8H), 7.90 (s, 0.4, C-8H), 7.56 (m, 1, pyr-5H), 6.6 (brs, 1, N^2–H), 6.11 (dd, J = 7, 7 Hz, 0.5, 1′-H), 6.07 (dd, J = 8, 7 Hz, 0.5, 1′-H), 5.27 (brs, 1, —OH), 4.85 (brs, 1, OH), 4.45 (m, 1, CH_3C*H*), 4.10 (m, 1, 3′-H), 3.79 (m, 1, 4′-H), 3.48 (m, 1, 5′-H_2), 3.23 (m, 2, CH_2C=O), 2.56 (m, 1, 2′-H), 2.18 (m, 1, 2′-H), 1.28 (d, J = 6.6 Hz, 1.5, C*H*$_3$CH), 1.27 (d, J = 6.6 Hz, 1.5, C*H*$_3$CH).

Registry No. 12, 68743-68-0; **17**, 100021-45-2; **18** (diastereomer 1), 100021-48-5; **18** (diastereomer 2), 100021-49-6; **21**, 59578-62-0; **22**, 100021-46-3; 3-pyridinecarboxaldehyde, 500-22-1; allyl bromide, 106-95-6; 3-acetylpyridine, 350-03-8; acetaldehyde, 75-07-0; deoxyguanosine, 961-07-9; 2′-deoxyguanosine 5′-(ethyl carbonate), 100021-47-4; 2′-deoxyguanosine 3′-(ethyl carbonate), 100044-90-4.

(12) Hecht, S. S.; Chen, C. B.; Dong, M.; Ornaf, R.; Hoffmann, D.; Tso, T. C. *Beitr. Tabakforsch.* **1977**, *9*, 1.