Acknowledgment. This investigation was supported by Grants CA13592 and CA23263 from the National Cancer Institute, Department of Health, Education, and Welfare.

Registry No. 1, 61865-50-7; **2c**, 65941-40-4; **2d**, 72301-31-6; **3b**, 72345-98-3; **3c**, 24587-83-5; **6**, 72345-99-4; **8**, 72346-00-0; **10**, 72346-01-1.

Aralkylation of Guanosine by the Carcinogen *N*-Nitroso-*N*-benzylurea

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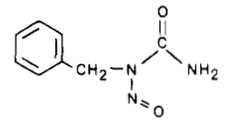
Our recent investigations[1] of the solvent effects on sites of reaction on adenosine and guanosine with benzyl bromide, benzyl chloride, and benzyl tosylate led us to suggest that alkylation and aralkylation of exocyclic sites on nucleoside purine residues (i.e., N^6 of adenosine as well as N^2 and O^6 of guanosine) are favored by changes in substrate structure or reaction medium which advance carbon-leaving group bond breakage and thus reduce the sensitivity of the substrate to the nucleophilicity of ring nitrogen sites (e.g., N-1 of adenosine and N-7 of guanosine). In addition, the positive charge density or "hardness"[2] of the reaction center determines the ratio of reaction at the O^6 and N^2 sites on guanosine: when the center is "soft", reaction with the exocyclic amino group is favored. Higher charge density, creating a "harder" center, directs a greater portion of the total reaction to exocyclic oxygen.

N-Nitroso-*N*-benzylurea (1) has recently been shown to be a potent, locally acting carcinogen.[3] As an aralkyl nitrosourea its reaction with nucleosides would be expected to result in the transfer of a benzyl moiety through an intermediate benzyldiazonium ion.[4] This should render this benzylating agent less susceptible to the nucleophilicity of ring nitrogen sites than the agents examined previously[1] and should, in addition, impose the highest positive charge density on the benzylic carbon reaction center. Consequently, under similar solvent conditions, we would expect that, of the four benzylating agents, the reactions of **1** with guanosine and adenosine would yield the highest ratio of exocyclic-substituted/ring-substituted products and the highest ratio of O^6/N^2 substitution with guanosine. The data we present here demonstrate that these expectations are met. Furthermore, the type and distribution of nucleoside adducts resulting from reaction with **1** should be common to reactions of other carcinogens (e.g., the unsymmetrical alkylbenzylnitrosamines)[5] whose interactions with nucleic acid components could proceed through a benzyldiazonium ion intermediate.



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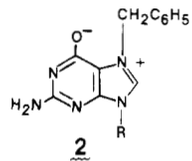

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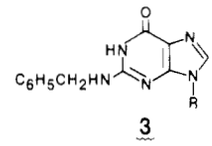

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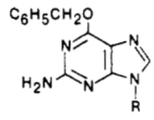

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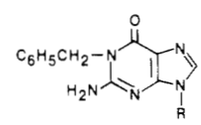

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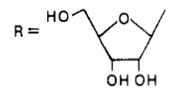

Reactions of guanosine with **1** yielded four products, 7-benzylguanosine (**2**), N^2-benzylguanosine (**3**), O^6-benzylguanosine (**4**), and 1-benzylguanosine (**5**). Product distributions as a function of solvent composition are presented in Table I. These data, presented as percentage of radioactive guanosine converted to benzylated product, were collected after 24-h reaction incubations. Since the half-life of *N*-nitroso-*N*-benzylurea is far less than 24 h under all solvent conditions employed here, these percentages (Table I) represent the extent of nucleoside benzylation at reaction completion. Most of **1** reacts with the solvent under these conditions.

The data of Table I show that for the reaction of *N*-nitroso-*N*-benzylurea with guanosine in 99% aqueous solution, the major sites of reaction are N-7 and *both* the exocyclic amino and oxo group of the purine residue, i.e., the N^2 and O^6 positions. Furthermore, reaction at these exocyclic sites is significantly more extensive than reaction at ring nitrogen. Changes in solvent composition from 1% *N*,*N*-dimethylformamide (DMF) in water to either 60% DMF or 60% ethanol (EtOH) in water caused a decrease in the yield of benzylated products, but these same solvent changes had little effect on product ratios presumably because the neutral leaving group in this case, N_2, is little affected by the electrophilic (anionic solvating) properties of the solvent. These results differ significantly from those obtained previously with benzylating agents bearing anionic leaving groups.[1] With these latter agents, increases in the water content of the solvent mixtures dramatically increased the exocyclic-substitution/ring-substitution ratios.

Reactions of guanosine with **1** in the four least aqueous solvents afforded substantially different product distributions. In either 80% DMF or 80% EtOH in water as well as absolute DMF and EtOH, benzylation at the N-1 position of guanosine was observed and reaction at this site was accompanied by an increased extent of reaction at the O^6 position. Indeed, the greatest amounts of **4** and **5** were obtained in dry DMF solution. Substitution at the exocyclic amino group was not observed in DMF, and, although it was detectable in EtOH, the yield of **3** in this solvent was the lowest observed in the EtOH–water series. The observation that reaction at O^6 is accompanied by reaction at N-1 in the least aqueous solutions points to the involvement of guanosine anion under these conditions while reaction at O^6 in the more aqueous solvents (where it is not accompanied by N-1 substitution) probably results

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(3) S. Ivankovic, *Z. Krebsforsch.*, **91**, 63 (1978).
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Table I. Percentage of Guanosine Benzylated at N-7 (2), N^2 (3), O^6 (4), and N-1 (5)[a]

reacn medium[b]	2	3	4	5
1% DMF in H_2O	0.11	0.26	0.22	nd[c]
20% DMF in H_2O	0.03	0.13	0.13	nd
40% DMF in H_2O	0.02	0.09	0.09	nd
60% DMF in H_2O	0.03	0.08	0.08	nd
80% DMF in H_2O	0.04	0.04	0.28	0.04
DMF	0.14	nd	1.53	0.26[d]
20% EtOH in H_2O	0.05	0.20	0.20	nd
40% EtOH in H_2O	0.02	0.05	0.05	nd[e]
60% EtOH in H_2O	0.03	0.08	0.13	nd
80% EtOH in H_2O	0.02	0.09	0.21	0.08
EtOH	0.34	0.05	0.20	0.09[d]

[a] Results are percentages of guanosine converted to benzylated product after 24 h. Reactions were carried out in 0.056 M $NaHCO_3$ buffer, pH 6.8–7.4, and at 25 °C. [Guanosine-5′-^3H] = 5×10^{-7} M (specific radioactivity 21 Ci/mmol), [*N*-nitroso-*N*-benzylurea] = 1.1×10^{-2} M. [b] All solvents were prepared v/v. [c] nd denotes not detected. [d] Reaction carried out in unbuffered solution. [e] [*N*-Nitroso-*N*-benzylurea] = 4.3×10^{-3} M.

from direct attack at oxygen of neutral guanosine.

In similar studies with adenosine, 1-benzyladenosine and N^6-benzyladenosine were detected as products of reaction with 1. In 99% aqueous solution 0.24% of radioactive adenosine was converted to the N^6-substituted product and 0.03% to 1-benzyladenosine, showing again with this nucleoside that reactions with 1 occur predominantly with exocyclic sites.

The extension of our previous studies to include examination of the reaction of *N*-nitroso-*N*-benzylurea with guanosine and adenosine has enabled us to document a gradual change in the site selectivity of nucleoside benzylation as a function of leaving group. In neutral aqueous solution, the ratio of exocyclic-substituted/ring-substituted products for guanosine reactions increases in the leaving group sequence Br (0.46) < Cl (0.52) < OTs (2.1) < nitrosourea (4.4). The corresponding ratios for adenosine reactions are Br (1.3) < Cl (1.9) < OTs (5.7) < nitrosourea (8.0). In addition, the O^6/N^2 ratio for guanosine reactions increases in the same order, i.e., Br (0.10) < Cl (0.15) < OTs (0.58) < nitrosourea (0.85). Thus, changes in leaving group which advance carbon–leaving group bond breakage (i.e., increase S_N1 character) favor reaction at exocyclic sites. Furthermore, the greater the positive charge density at the reaction center (i.e., the "harder" the center), the more extensive is reaction at exocyclic oxygen.

The reactivity of 1 toward nucleosides is unique among the alkylating and aralkylating agent classes of carcinogens in that it is capable of modifying both the amino and oxo group of nucleoside purine residues with equal facility. The ability to alkylate the exocyclic oxygen of guanine residues is a common feature of carcinogenic alkylating agents (e.g., the *N*-alkyl-*N*-nitrosoureas),[6-11] but with these carcinogens, reaction with the exocyclic amino group of guanine has not been observed. In contrast, polycyclic aromatic hydrocarbon aralkylation occurs predominantly on the amino groups of the DNA bases in aqueous solution.[12-16] The ability of 1 to modify both these sites on guanine residues indicates that this agent exhibits chemical properties common to both groups of carcinogens and that the chemical reactivity of the carcinogenic alkylating agents and of reactive metabolites of the polycyclic aromatic hydrocarbons differs primarily in the greater S_N1 character and ability to delocalize charge in the latter case. *N*-Nitroso-*N*-benzylurea will no doubt prove to be a useful tool in evaluating the relative biological potency of O^6 and N^2 substitution on guanine residues in nucleic acids.

Experimental Section

Samples of *N*-nitroso-*N*-benzylurea (1) were provided by Dr. W. Lijinsky, Frederick Cancer Research Center, and Dr. G. Eisenbrand, Deutsches Krebsforschungzentrum, Heidelberg. [G-^3H]Adenosine (specific radioactivity 9 Ci/mmol), [5′-^3H]guanosine (specific radioactivity 21 Ci/mmol), and N^6-benzyladenosine were obtained commercially. 1-Benzyladenosine and 7-benzylguanosine (2) were prepared by the method of Brookes et al.[17] O^6-Benzylguanosine (4) was prepared by the method of Gerster and Robins.[18] N^2-Benzylguanosine (3) and 1-benzylguanosine (5) were prepared as before.[1]

Tritiated nucleosides were purified before use by column chromatography on a 0.72 × 30 cm Aminex A-5 column (ammonium ion form) at 40 °C, using aqueous 0.05 M ammonium formate buffer (pH 4.5) as eluting solvent. Fractions containing the labeled nucleosides were lyophilized and the salt-free solid residue after lyophilization was redissolved in water and stored at 2 °C. Chromatography of this recovered radioactive material under the chromatographic conditions used to separate the benzylated nucleoside products (see below) indicated that the labeled nucleosides were at least 97% radiochemically pure and that no contaminating radioactive material cochromatographed with markers for the benzylated products.

Reactions with nucleosides were carried out by adding a 10-μL aliquot of an aqueous stock solution of either labeled nucleoside ([adenosine] = 1.1×10^{-4} M, [guanosine] = 5×10^{-5} M) to 1 mL of reaction solution (Table I). A 10-μL aliquot of a freshly prepared 1.1 M solution of 1 in either DMF or EtOH was added and the solutions were incubated at 25 °C. After incubation for 24 h, aliquots of reaction solutions were withdrawn and mixed with a solution containing markers for the benzylated nucleoside products. Guanosine reaction solutions were loaded on a 0.72 × 30 cm Aminex A-5 column (ammonium ion form). The column was initially eluted with 1 M ammonium formate in DMF/H_2O (1:9) (pH 4.2) at 40 °C (flow rate 0.5 mL/min). Fractions (1.0 mL) were collected for scintillation counting. Unmodified guanosine eluted in fractions 15–17, 5 eluted in fractions 33–38, 3 eluted in fractions 45–53, and 4 eluted in fractions 58–67. When 75 mL of solvent had passed through the column, the eluting solvent was changed to 1 M ammonium formate in DMF/H_2O (3:7) (pH 7) and elution was carried out at 50 °C. 7-Benzylguanosine (2) eluted in fractions 100–105. Adenosine reaction solutions were loaded on a 0.72 × 18 cm Aminex A-6 column (ammonium ion form). The column was initially eluted with 0.1 M ammonium formate (pH 4.5) in MeOH/H_2O (3:7) at 40 °C (flow rate 0.3 mL/min). Unmodified adenosine eluted in fractions (1.0 mL) 20–23; N^6-benzyladenosine eluted in fractions 36–40. When 48 mL of initial buffer had passed through the column, elution was carried out at 60 °C using 1.0 M ammonium formate (pH 4.5) in MeOH/H_2O (3:7). 1-Benzyladenosine eluted in fractions 75–77.

Acknowledgment. We are grateful to Dr. W. Lijinsky of the Frederick Cancer Research Center and Dr. G. Ei-

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senbrand, Deutsches Krebsforschungszentrum, Heidelberg, for providing us with samples of *N*-nitroso-*N*-benzylurea. This work was supported by Contract No. N01-CO-75380 with the National Cancer Institute, NIH, Bethesda, Md. 20014.

Registry No. 1, 775-11-1; 2, 72360-76-0; 3, 71171-58-9; 4, 4552-61-8; 5, 55043-75-9; N^6-benzyladenosine, 4294-16-0; 1-benzyladenosine, 4294-16-0; guanosine, 118-00-3; adenosine, 58-61-7.

Structure of Deacetylviguiestenin (Tagitinin E). An Addendum[1]

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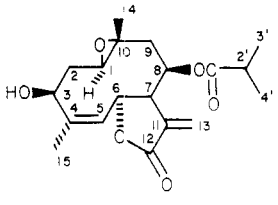
In a recent article[2] we presented complete stereochemical expressions for several sesquiterpene lactones from *Tithonia diversifolia* Hemsl., viz., tagitinin A, B (=deacetylwoodhousin), C, D (=tirotundin), and E,[3,4] as well as for some other related compounds. Since formulation of tagitinin E as the heliangolide **1** was based entirely on



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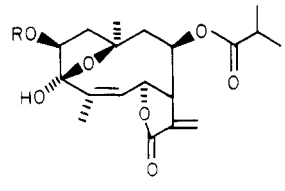

3a, R = H
b, R = Ac

NMR analysis of a very small authentic sample, it was deemed desirable to adduce additional chemical proof. This has now been accomplished by relating tagitinin E to tagitinin C (**2**). The latter has been correlated previously[3] with deacetylwoodhousin (**3a**) whose structure is securely based on an X-ray analysis of **3b** (woodhousin).[5]

The correlation is shown in Scheme I. Zinc–acetic acid reduction of tagitinin C afforded **4**[6] which was epoxidized to **5**. The oxirane stereochemistry of **5** which is the same as that of several naturally occurring 1,10-epoxyheliangolides of confirmed structure[7] is dictated by the preferred "outside" approach of epoxidizing agent observed in at least two other thoroughly documented instances.[8] Protection of the α-methylene-γ-lactone function by way of the morpholine adduct **6**[9] followed by $NaBH_4$ reduction furnished two alcohols, **7** and **8**.[10] The major product, **7**, was converted to a substance identical in all respects with tagitinin E. The minor product, **8**, afforded **9** whose stereochemistry at C-4 remains undefined. That at C-3 is probably the same as that of **1**.

In view of our formulation of tagitinin E as **1**, we concluded earlier[2] that deacetylviguiestenin, an apparently isomeric substance from *Viguiera stenoloba*,[11] might be the C-8 epimer of **1**. However, on reexamination of the evidence in the original report,[11] it seemed possible that the two substances might be identical, and, in fact, Romo de Vivar and co-workers have proposed formula **1** for deacetylviguiestenin in a second, more recent publication.[12] Since then, direct comparison has shown that deacetylviguiestenin and tagitinin E are indeed identical.[13] The name tagitinin E should therefore be stricken from the literature.

Experimental Section

Reduction of Tagitinin C. A solution of 0.10 g of **2** in 2.5 mL of acetic acid and 1.5 mL of H_2O was kept at 100 °C while 0.2 g of Zn dust was added over a 15-min period. After an additional hour at 100 °C (TLC monitoring) the mixture was neutralized with 10% aqueous $NaHCO_3$ and extracted with ether. The washed and dried extract was evaporated and the residue purified by preparative TLC (silica gel, solvent ethyl acetate–benzene, 9:1). The main fraction, **4**, was recrystallized from ethyl acetate–petroleum ether (bp 60–80 °C): mp 135–137 °C; yield 0.025 g; IR ($CHCl_3$) 1760, 1730, 1695, 1660, 1140, 860 cm^{-1}; NMR (60 MHz, $CDCl_3$) δ 6.35 and 5.78 (2 d, J = 2 Hz, H-13), 5.1–5.5 (c, H-1, H-5, H-6, and H-8), 1.98 (H-14), 1.82 (H-15), 1.15 (d, J = 7 Hz, H-3′ and H-4′); mass spectrum m/e 332 (M^+), 262, 244, 71 (base peak).

Anal. Calcd for $C_{19}H_{24}O_5$: C, 68.66; H, 7.28. Found: C, 68.34; H, 7.18.

Epoxidation of 4. To 25 mg of **4** in 2 mL of $CHCl_3$ was added at 0 °C 0.5 mL of 8% perbenzoic acid in $CHCl_3$. After 12 h at 0 °C, the mixture was diluted with $CHCl_3$, washed with 10% aqueous $NaHCO_3$ and water, dried, and evaporated. Purification of the residue by preparative TLC (ethyl acetate–benzene, 4:1) gave 10 mg of **5** as a gum which exhibited the following: IR 1765, 1730, 1660, 1130, 1000 cm^{-1}; NMR 6.30 and 5.78 (2 d, J = 2 Hz, H-13), 5.2–5.5 (c, H-5, H-6, and H-8), 3.50 (m, H-7), 1.98 (br, H-15), 1.25 (H-14), 1.08 (d, J = 7 Hz, H-3′ and H-4′); mass spectrum m/e 348 (M^+), 278, 260. A minor fraction appeared to be the epimeric epoxide but could not be purified satisfactorily.

Anal. Calcd for $C_{19}H_{24}O_6$: C, 65.50; H, 6.94. Found: C, 65.41; H, 7.12.

Preparation of 1 and 9. To 25 mg of **5** in 0.5 mL of EtOH at 0 °C was added 0.2 g of morpholine. After 12 h at 0 °C, the mixture was diluted with ice-cold water and extracted with $CHCl_3$. On evaporation of the washed and dried extract, 25 mg of adduct **6** was obtained as a gum which had the following: IR 5.2–5.6 (c, H-5, H-6, and H-8), 1.80 (br, H-15), 1.15 (H-14), 1.05 (d, J = 7 Hz, H-3′ and H-4′); mass spectrum m/e 435 (M^+), 417, 364, 348, 260, 100, 87.

Anal. Calcd for $C_{23}H_{33}O_7N$: mol wt 435.225 50. Found: mol wt 435.225 38.

To 25 mg of **6** in 0.6 mL of isopropyl alcohol was added 20 mg of $NaBH_4$. After the solution was stirred at −15 °C for 4 h, TLC

(1) Work at The Florida State University was supported in part by a U.S. Public Health Service grant (CA-13121) through the National Cancer Institute.

(2) Barua, N. C.; Sharma, R. P.; Madhusudanan, K. P.; Thyagarajan, G.; Herz, W.; Murari, R. *J. Org. Chem.* **1979**, *44*, 1831. Formula **2** of this paper was mislabeled tagitinin D instead of tagitinin B, and the NMR data in the last column of Table II are those of tagitinin E (**19a**), not **20a**.

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(5) Herz, W.; Blount, J. F. *J. Org. Chem.* **1978**, *43*, 4887.

(6) The low yield (25%) of **4** can probably be attributed to the competing reduction of the α,β-unsaturated lactone system.

(7) Several lactones of this type have been correlated with dihydroheliangin whose structure was established by X-ray crystallography (Nishikawa, M.; Kamiya, K.; Takabatake, A.; Oshio, H. *Tetrahedron* **1966**, *22*, 3601), without disturbing the epoxide function.

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(13) Private communication from Dr. Romo de Vivar.

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