Light- and γ-Ray-Induced Reactions of Purines and Purine Nucleosides with Alcohols

H. STEINMAUS,[1] I. ROSENTHAL, AND D. ELAD*

Department of Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Received February 12, 1971

Photochemical- and γ-ray-induced reactions of purines and purine nucleosides with alcohols are described. The reactions of 6-substituted and 2,6-disubstituted purines resulted in substitution of an alcohol moiety for the hydrogen atom at C-8, while with 2-aminopurine a primary attack took place at the C-6 position of the purine system. Yields of up to 80% were obtained. A free-radical mechanism is proposed for the reactions, and the following order of reactivity of the various sites in the purine system toward the alcohol-free radicals has been found: C-6 > C-8 > C-2.

The photochemical reactions of nucleic acids and their constituents have been the subject of an extensive investigation in recent years.[2] While the photoreactions of the pyrimidine bases and their products have been examined in detail, those of purine derivatives remain to be studied. The purines form part of the light-absorbing system in nucleic acids, but this apparently does not result in their photochemical alteration, and the absorbed light may well be transferred to other moieties in the molecule.[3]

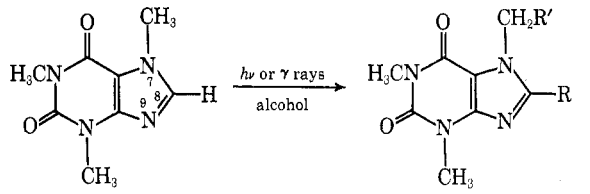
Substituents on the purine moiety are known to affect its reactivities in chemical reactions,[4] and apparently such effects are also observed in photochemical- and γ-ray-induced reactions of purine derivatives. Thus, while ultraviolet-light-induced reactions of purine itself and purine riboside with a variety of alcohols resulted in the addition of the alcohol across the 1,6 double bond,[5] similar reactions of substituted purines led to substitution at the C-8 position.[6]

The aim of the present study is to investigate the reactions of purines and purine nucleosides with various substrates under ultraviolet and γ radiation. These serve as model reactions for the investigation of the photochemical reactions of purine moieties in nucleic acids. The eventual development of a photochemical procedure which will lead to a purine modified DNA or RNA is also hoped for in the course of this project.[7] The present publication includes a full description of the photochemical- and γ-ray-induced reactions of some alcohols with a variety of purines, including those from nucleic acids.

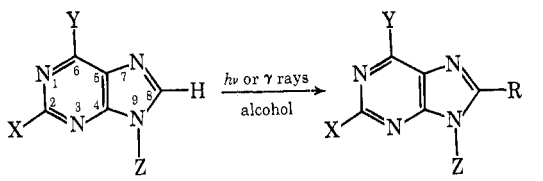
Results and Discussion

It has been found that purines and purine nucleosides, such as caffeine, adenine, 2-aminopurine, or guanosine, undergo photoreactions when irradiated in the appropriate alcohol with ultraviolet light or when exposed to γ radiation. In all purines and purine nucleosides studied the reactions resulted in substitution at the C-8 position, except for 2-aminopurine which underwent a primary attack at C-6. The resulting substituent depended on the alcohol employed and was usually the corresponding hydroxyalkyl group; however, in some reactions of caffeine and primary alcohols the newly introduced substituent at C-8 was an alkyl group. The reactions can be presented as shown in Scheme I.

SCHEME I



1, R = CH_3CHOH; R′ = H
2, R = $(CH_3)_2COH$; R′ = H
3, R = $CH_3CH_2CH(OH)CH_3$; R′ = H
4, R = CH_3CH_2; R′ = H
5, R = $CH_3CH_2CH_2$; R′ = H
6, R = R′ = $(CH_3)_2COH$



X = H; Y = NH_2; Z = H (adenine) — **7**, R = $(CH_3)_2COH$
X = H; Y = NH_2; Z = ribose (adenosine) — **8**, R = $(CH_3)_2COH$
9, R = $CHOHCH_3$
X = NH_2; Y = OH; Z = ribose (guanosine) — **10**, R = $(CH_3)_2COH$
X = NH_2; Y = OH; Z = 2′-deoxyribose — **11**, R = $(CH_3)_2COH$
X = H; Y = OH; Z = H (hypoxanthine) — **12**, R = $(CH_3)_2COH$
X = H; Y = EtO; Z = H — **13**, R = $(CH_3)_2COH$
14, R = X = $(CH_3)_2COH$
X = NH_2; Y = H; Z = H — **15**, R = H; Y = $(CH_3)_2COH$
16, R = Y = $(CH_3)_2COH$

The primary product of the reaction of 2-aminopurine and 2-propanol was the N-1,C-6 addition product (1,6-dihydropurine type),[5] as shown by the gradual disappearance of the maximum at 310 nm in the ultraviolet spectrum of the reaction mixture. This band reappeared upon exposure of the reaction mixture to air, and work-up led to the isolation of **15** which resulted from oxidation of the primary product. In the other purines and purine nucleosides the alcohol moiety substituted at C-8 leading to the N-7,C-8 addition products

(1) Stiftung-Volkswagenwerk Fellow, 1968–1970.

(2) For reviews, see (a) K. C. Smith, *Radiat. Res., Suppl.*, **6**, 54 (1966); (b) J. K. Setlow, *ibid.*, **6**, 141 (1966); (c) J. C. Burr, *Advan. Photochem.*, **6**, 193 (1968); (d) E. Fahr, *Angew. Chem., Int. Ed. Engl.*, **8**, 578 (1969).

(3) C. Helene, P. Douzou, and A. M. Michelson, *Proc. Nat. Acad. Sci. U. S.*, **55**, 376 (1966); R. O. Rahn, R. G. Shulman, and J. W. Longworth, *ibid.*, **53**, 893 (1965).

(4) For a summary, see R. K. Robins, *Heterocycl. Compounds*, **8**, 162 (1967).

(5) H. Linschitz and J. S. Connolly, *J. Amer. Chem. Soc.*, **90**, 297 (1968); J. S. Connolly and H. Linschitz, *Photochem. Photobiol.*, **7**, 791 (1968); B. Evans and R. Wolfenden, *J. Amer. Chem. Soc.*, **92**, 4751 (1970).

(6) D. Elad, I. Rosenthal, and H. Steinmaus, *Chem. Commun.*, 305 (1969); H. Steinmaus, I. Rosenthal, and D. Elad, *J. Amer. Chem. Soc.*, **91**, 4921 (1969).

(7) H. Steinmaus, D. Elad, and R. Ben-Ishai, *Biochem. Biophys. Res. Commun.*, **40**, 1021 (1970).

TABLE I

ULTRAVIOLET- AND γ-RADIATION-INDUCED REACTIONS OF PURINES AND PURINE NUCLEOSIDES WITH ALCOHOLS

Purine or purine nucleoside	Alcohol	Product (yield, %)[a]	Source of radiation	Radiation time, hr
Caffeine	Ethanol	**4** (11)	Uv[b]	72
		4 (17) **1** (14)	γ rays[c]	19
Caffeine	Ethanol[d]	**2** (35) **1** (41)	γ rays	24
Caffeine	1-Propanol	**5** (14)	Uv[b]	140
Caffeine	2-Propanol	**2** (35)	Uv[b]	72
		2 (37)	γ rays	25
Caffeine	2-Propanol[d]	**2** (78) **6** (10)	Uv[e]	24
		2 (67)	γ rays	30
Caffeine	2-Butanol	**3** (44)	Uv[b]	72
Adenine	2-Propanol	**7** (59)	Uv[b]	94
Adenine	2-Propanol[d]	**7** (69)	Uv[e]	14
		7 (81)	γ rays	19
Adenosine	2-Propanol	**8** (62)	Uv[b]	70
Adenosine	2-Propanol[d]	**8** (50)	Uv[e]	11
		8 (54)	γ rays	14
Adenosine	Ethanol	**9** (28)	Uv[b]	125
Guanosine	2-Propanol[d]	**10** (45)	Uv[e]	15
		10 (68)	γ rays	15
2′-Deoxyguanosine	2-Propanol[d]	**11** (25)	Uv[e]	18
		11 (41)	γ rays	14
Hypoxanthine	2-Propanol[d]	**12** (27)	Uv[e]	10
6-Ethoxypurine	2-Propanol[d]	**13** (41) **14** (25)	Uv[e]	17
		13 (47) **14** (19)	γ rays	30
2-Aminopurine	2-Propanol[d]	**15** (30) **16** (34)	Uv[e]	8

[a] Based on total amount of starting purine. [b] Hanovia 450-W high-pressure mercury vapor lamp (Corex filter). [c] ^{60}Co γ source, Gamma cell 220 (Atomic Energy of Canada Ltd., Ottawa, Canada). Dose rate = 1.27×10^{18} eV ml^{-1} min^{-1}. [d] With acetone as sensitizer; alcohol–acetone 1:1 (v/v). [e] Pyrex filter.

(C-8,N-9 addition products in caffeine), which were very sensitive to oxygen. These, upon work-up, gave the corresponding C-8 substituted purines. The addition of the alcohol to the 7,8 (or 8,9) double bond could be observed through the changes in the ultraviolet spectra of the appropriate reaction mixtures. The spectra were characterized by reduction in the intensity at the 260-nm maximum (reintensified upon exposure to air). These spectral changes could be observed only in properly degassed mixtures. When oxygen was not thoroughly removed, *e.g.*, while working on a preparative scale, these changes were not significant, since the absorption spectra of the final products are similar to those of the starting purines. The primary substitution at C-6 in 2-aminopurine was followed by one at C-8, while with 6-ethoxypurine the primary substitution at C-8 was followed by one at C-2. In both cases the secondary attacks were of a slower rate than the primary ones.

The ultraviolet-induced reactions of caffeine with ethanol and 1-propanol led to 8-ethylcaffeine (**4**) and 8-propylcaffeine (**5**), respectively, while the γ-ray-induced reaction of caffeine and ethanol led to 8α-hydroxyethylcaffeine (**1**) and **4** in a 1:1 ratio. **1** could be transformed to **4** when irradiated in methanol, ethanol, or 2-propanol but not in dioxane.

The use of acetone as a photosensitizer for the reactions of 2-propanol led to higher yields of the corresponding purine C-8 substituted products. Sensitization with acetone of the γ-ray-induced reaction of caffeine and ethanol led to the formation of **2** in addition to **1**. The reactions studied are summarized in Table I.

Products of the caffeine, 6-ethoxypurine, and 2-aminopurine reactions were isolated by column chromatography on silica gel, while the photoproducts of the other purines and purine nucleosides were isolated by preparative paper chromatography. The progress of the reactions was followed by thin layer chromatography. Characterization of the products was achieved by elemental analyses as well as by nmr and mass spectra. The caffeine photoproducts were compared with authentic samples. Longer irradiation periods of the acetone-sensitized caffeine–2-propanol reaction led to some substitution at the N-7-CH_3 group.

The structures of compounds **1**–**12** have been discussed previously[6] and were derived from elemental analyses, mass spectra, nmr data, and deuterium exchange experiments. The latter are based on the observation that H-8 in the purines studied exchanges for deuterium with D_2O at 105°.[8] The nmr spectrum of 6-ethoxypurine showed, among others, bands at τ (DMSO-d_6; DSS, internal) 1.6 and 1.48. The absorption at τ 1.6 was reduced by 50% after treatment with C_2H_5OD at 100° for 4 hr and thus belongs to the H-8

(8) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'O, *J. Amer. Chem. Soc.*, **86**, 696 (1964); J. M. Rice and G. O. Dudek, *ibid.*, **89**, 2719 (1967).

proton.[9] This band was absent in the spectrum of the 6-ethoxypurine–2-propanol photoadduct, indicating that substitution took place at C-8. The 2-aminopurine–2-propanol photoproduct has an exchangeable H-8 purine proton, as does 2-amino purine. Thus, the primary substitution in 2-aminopurine occurred at C-6.

It is noteworthy that the sugar moiety remained intact during these photochemical- and γ-ray-induced reactions,[10] as proved by mild acid hydrolysis of the appropriate nucleoside photoproducts which gave ribose or 2′-deoxyribose (tlc). However, the absorption bands in the nmr spectra of the sugar protons in the C-8 substituted nucleosides indicate some conformational differences in the molecule as compared to the starting purine nucleosides.[11]

The mass spectra of the photoadducts confirmed the proposed structures for these compounds. In the case of caffeine photoproducts all showed the appropriate molecular ion peaks. The adenine and the hypoxanthine–2-propanol photoproducts behaved similarly and showed the appropriate molecular ion peaks. The adenosine–ethanol and –2-propanol products exhibited the appropriate molecular peaks as well as the typical fragmentation pattern of ribose nucleosides, *i.e.*, peaks of B + H, B + 24, B + 30 and M − 89 (B presents the mass of the free base with the C-8 side chain minus one).[12] Thus, the mass spectra supply additional proof for the preservation of the ribose moiety in the photoproducts. The guanosine photoproduct did not show a molecular ion peak; however, the mass spectra of guanosine did not show a molecular ion peak either under the same conditions of recording.

The reported reactions could be induced by light of wavelength of λ >260 nm (Corex filter) or λ >290 nm (Pyrex filter) in the presence of acetone. In the former case light is absorbed exclusively by the purine, since the alcohols absorb at shorter wavelengths. The possible initiation of the reactions with peroxides at elevated temperature (see Experimental Section) as well as esr studies[13] suggest the existence of free-radical intermediates. The alcohol free radicals are formed probably through the abstraction of a hydrogen atom from the alcohol by the excited purine, due to the abstraction ability of a C=N group in the excited state, which might be compared to that of a carbonyl.[14,15] The subsequent step involves the attack of an alcohol free radical on the carbon end of a C=N group of a ground state purine molecule[16] leading to a radical[17] which by further hydrogen atom abstraction from the solvent yields the N-1,C-6 or N-7,C-8 adducts ("dihydro" type). These are oxidized during work-up to the appropriate substituted purines.

In the sensitized reactions acetone absorbs most of the incident light (λ >290 nm), as shown from spectral data. The excited acetone may transfer the excitation energy to the purine,[18] which initiates the reaction as described above. On the other hand, excited acetone may also abstract a hydrogen atom from the alcohol which serves as solvent. Hence, two routes for the generation of alcohol free radicals may operate in the sensitized reactions. In the γ-ray-induced reactions most of the radiation energy is absorbed by the alcohol, which serves as solvent, and the excited alcohol molecules then fragment to produce the corresponding free radicals.[19]

The reactivities of the various sites of the purine nucleus toward alcohol free radicals have been derived. The reactions involve two types of attack: (1) at C-6, and (2) at C-8. Purine itself,[5] 2-aminopurine, and purine-9-riboside[5] belong to the first group, while the 6-substituted and 2,6-disubstituted purines and purine nucleosides belong to the second. The preliminary substitution at C-6 in purine and 2-aminopurine indicates that this site is more reactive than C-8 or C-2, while the primary attack at C-8 in adenine and 6-ethoxypurine indicates that C-8 is more reactive than C-2. Thus, current results, which are based on product analysis, suggest the following order of reactivity toward alcohol free radicals in the purine systems studied: C-6 > C-8 > C-2.[20]

Experimental Section

Kieselgel (0.05–0.20 mm; Merck) was used for chromatography. Petroleum ether refers to the fraction bp 60–80°. Ascending tlc was performed with cellulose CE F (Riedel-de-Haen) except for the caffeine products where Kieselgel SI F (Riedel-de-Haen) was used. Mixtures of 1-butanol–water–concentrated ammonia (86:9:5 v/v) or 2-propanol–water–concentrated ammonia (68:14:1.5 c/c) (for the guanosine derivative) eluted the products from cellulose, while mixtures of 2-propanol and petroleum ether were used as eluents for the Kieselgel. Spots were detected with a Mineralight lamp. Preparative ascending paper chromatography was performed with Whatman No. 17 paper in mixtures similar to those used for tlc. The product zone was detected with a Mineralight lamp and cut; the strips were rolled and placed into a glass tube where they were washed with meth-

(9) 6-Ethoxypurine was the only 6-substituted purine studied by us which had the H-8 proton absorbing at higher field than H-2. *Cf.* W. C. Coburn, M. C. Thorpe, J. A. Montgomery, and K. Hewson, *J. Org. Chem.*, **30**, 1114 (1965), for a similar effect in 6-methoxypurine.

(10) *Cf.* K. Keck, *Z. Naturforsch., B*, **23**, 1034 (1968).

(11) The most pronounced changes in the nmr spectrum of the adenosine photoadduct are a strong downfield shift of the H-1′ proton (anomeric) and a weaker one of H-2′ in the same direction. Thus, while H-1′ in adenosine absorbs at τ 3.97, it appears at τ 3.77 in C-8 $CH(OH)CH_3$ and 3.04 in C-8 $C(CH_3)_2OH$. The differences in the shifts of the other C–H proton in the sugar moiety are relatively small as compared to those of the starting nucleoside. The pronounced downfield shift (0.93 ppm) in the absorption of the anomeric proton of the adenosine–2-propanol adduct indicates that it is probably forced into the plane of the purine ring and is being deshielded by the ring current. The two possible conformations, anti or syn [J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, **2**, 363 (1960); A. E. V. Haschemeyer and A. Rich, *ibid.* **27**, 369 (1967)] of the relative orientation of the planar purine with respect to the sugar ring in the photoproduct cannot be distinguished using these data. Examination with models indicates that in the anti conformation there is a strong interaction between the ribose moiety and the side chain at C-8. On the other hand, this interaction does not exist in the syn conformation. We, therefore, assume that the adenosine–2-propanol photoproduct adapts the more favorable syn conformation, as opposed to adenosine for which an anti conformation has been proposed [F. Jordan and B. Pullman, *Theor. Chim. Acta.*, **9**, 242 (1968)]. A recent publication by D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Inskeep, and H. Eyring, *J. Amer. Chem. Soc.*, **93**, 1600 (1971), confirms our proposal.

(12) K. Biemann and J. A. McCloskey, *ibid.*, **84**, 2005 (1962); S. Hanessian, D. C. DeJongh, and J. A. McCloskey, *Biochim. Biophys. Acta*, **117**, 480 (1966); S. H. Eggers, S. I. Biedron, and A. O. Hawtrey, *Tetrahedron Lett.*, 3271 (1966).

(13) C. Helene, R. Santus, and P. Douzou, *Photochem. Photobiol.*, **5**, 127 (1966).

(14) F. R. Stermitz, C. C. Wei, and C. M. O'Donnell, *J. Amer. Chem. Soc.*, **92**, 2745 (1970).

(15) Helene, *et al.*,[13] observed the formation of alcohol free radicals in irradiated frozen alcoholic solutions of adenosine or guanosine and proposed an energy transfer process involving a higher excited state, populated *via* a biphotonic absorption, for the generation of these radicals. We, however, consider the hydrogen atom abstraction process to be more plausible.

(16) E. C. Taylor, Y. Maki, and B. E. Evans, *J. Amer. Chem. Soc.*, **91**, 5181 (1969), and references cited therein.

(17) *Cf.* W. Gordy, *Ann. N. Y. Acad. Sci.*, **158**, 1, 100 (1969).

(18) *Cf.* A. A. Lamola, *Photochem. Photobiol.*, **7**, 619 (1968).

(19) J. W. T. Slinks and R. J. Woods, "An Introduction to Radiation Chemistry," Wiley, New York, N. Y., 1964, p 129.

(20) *Cf.* ref 4, pp 270–275.

anol to elute the product. Infrared spectra were obtained with a Perkin-Elmer Infracord Model 137. Ultraviolet spectra were recorded on a Cary 14 spectrophotometer. Nmr spectra were determined with a Varian A-60 instrument in the appropriate organic solvent using TMS as internal standard. Spectra were taken in D_2O or DMSO-d_6 employing 1% (w/w) DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate, Merck Sharp Dohme of Canada, Ltd., Montreal) as an internal reference. Absorptions are reported in τ values. Mass spectra were recorded with an MAT-Atlas CH4 instrument. ORD spectra were determined on a Jasco Model ORD/UV-5 instrument.

Experiments were carried out at room temperature in an immersion apparatus with Hanovia 450-W high-pressure mercury vapor lamps which were cooled internally with running water. Corex filters (λ <260 nm) were employed except for the reactions with acetone where Pyrex filters (λ >290 nm) were used. The reaction apparatus was flushed with nitrogen for 15 min before irradiation. Some typical experiments are described in detail. Other experiments were conducted under similar conditions and are summarized in the tables. Progress of the reactions was followed by tlc. The γ-ray-induced reactions were performed in glass tubes which were placed into the ^{60}Co γ source.

Reaction of Caffeine and 2-Propanol with Ultraviolet Light.—A mixture of caffeine (1.5 g), 2-propanol (145 ml), and water (55 ml) was irradiated for 62 hr. Excess reagents were removed under reduced pressure and the residue was chromatographed on silica gel. Acetone–petroleum ether (3:17) eluted **2** (0.69 g, 35%): mp 199–200° (from 2-propanol); ir 3350 cm^{-1} (OH); nmr ($CDCl_3$) τ 5.8 (s, 3 H, N-7-CH_3), 6.46 (s, 3 H, N-3-CH_3), 6.6 (s, 3 H, N-1-CH_3), 6.83 (broad s, 1 H, OH), and 8.3 [s, 6 H, C(CH_3)$_2$OH].

Anal. Calcd for $C_{11}H_{16}N_4O_3$: C, 52.37; H, 6.39; N, 22.21; mol wt, 252. Found: C, 52.55; H, 6.47; N, 22.40; mol wt, 252 (mass spectrum).

The product was found to be identical with an authentic sample prepared from 8-acetylcaffeine[21] and CH_3MgJ. Further elution with the same solvent mixture (1:4) gave unreacted caffeine.

Reaction of Caffeine, 2-Propanol, and Acetone with Ultraviolet Light.—A mixture of caffeine (1.5 g), 2-propanol (100 ml), and acetone (100 ml) was irradiated for 24 hr. The usual work-up as above and chromatography on silica gel led to **6** [0.23 g, 10%: eluted with 2-propanol–petroleum ether (1:19)]: mp 206–207° (from ethanol); nmr τ 5.1 (s, 2 H, N-7-CH_2), 6.45 (s, 3 H, N-3-CH_3), 6.63 (s, 3 H, N-1-CH_3), 8.33 [s, 6 H, C(CH_3)$_2$OH], and 8.7 [s, 6 H, N-7-CH_2C(CH_3)$_2$OH].

Anal. Calcd for $C_{14}H_{22}N_4O_4$: C, 53.83; H, 7.74; N, 17.94; mol wt, 310. Found: C, 54.06; H, 7.50; N, 17.88; mol wt, 310 (mass spectrum).

Further elution with the same solvent mixture (3:7) gave **2** (1.5 g, 77.5%) followed by unreacted caffeine (0.29 g). Longer irradiation periods led to higher yields of the 2:1 adduct **6**. For example, after 48 hr of irradiation 1.07 g (45%) of this product was obtained.

Reaction of Caffeine and 2-Propanol with γ Rays.—A solution of caffeine (1.5 g) in 2-propanol (200 ml) was exposed to γ rays for 25 hr. The usual work-up led to **2** (0.63 g, 37%).

The reaction in the presence of acetone was carried out with caffeine (1.5 g), 2-propanol (100 ml), and acetone (100 ml) and was exposed to γ rays for 30 hr. The usual work-up gave **2** (1.3 g, 67%) and traces of **6**. Other reactions of caffeine and alcohols were conduced under similar conditions and are summarized in Tables I and II.

Reaction of Adenosine, 2-Propanol, and Acetone with Ultraviolet Light.—A mixture of adenosine (0.75 g), water (50 ml), 2-propanol (80 ml), and acetone (80 ml) was irradiated at room temperature for 11 hr. Solvents were removed under reduced pressure and the residue was chromatographed on paper in a mixture of 1-butanol–water–concentrated ammonia (86:9:5 v/v) leading to **8** (0.50 g, 50%): mp 227–230° (from water); nmr (0.2 *M* solution in D_2O, DSS as internal reference); τ 1.9 (s, 1 H, C-2-H), 3.04 (d, $J_{1',2'}$ = 7.5 Hz, C-1′-H), 4.77 (dd, $J_{2',1'}$ = 7.5 Hz, $J_{2',3'}$ = 5.5 Hz, 1 H, C-2′-H), 6 (an apparent d, 2 H, C-5′-H_2), and 8.15 [s, 6 H, C(CH_3)$_2$OH].

Anal. Calcd for $C_{13}H_{19}N_5O_5 \cdot 2H_2O$: C, 43.21; H, 6.42; N, 19.38; mol wt, 361. Found: C, 42.83; H, 6.35; N, 19.8; mol wt, 325 (mass spectrum).

(21) G. Ehrhart and I. Hennig, *Arch. Pharm.* (*Weinheim*), **289**, 453 (1956).

TABLE II

PRODUCTS OF REACTIONS OF CAFFEINE WITH ALCOHOLS

Alcohol[a]	Product	Mp, °C	Elution mixture
Ethanol	**4**[b]	184–186	Acetone–petroleum ether (3:7)
Ethanol	**1**[c]	178	2-Propanol–petroleum ether (3:17)
1-Propanol	**5**[b]	111–112	Acetone–petroleum ether (3:17)
2-Butanol	**3**[d]	138–139	2-Propanol–petroleum ether (1:9)

[a] Reactions were carried out in 30% aqueous–alcoholic mixtures. [b] *Cf.* E. S. Golovchinskaya and E. S. Chaman, *Zh. Obshch. Khim.*, **22**, 2220 (1952). [c] An authentic sample has been prepared through $NaBH_4$ reduction of 8-acetylcaffeine. *Cf.* ref 21. [d] Nmr ($CDCl_3$) τ 5.83 (s, 3 H, N-7-CH_3), 6.43 (s, 1 H OH), 6.5 (s, 3 H, N-3-CH_3), 6.65 (s, 3 H, N-1-CH_3), 8 (q, J = 7.5 Hz, 2 H, CH_2CH_3), 8.33 [s, 3 H, C(CH_3)OH], and 9.11 (t, J = 7.5 Hz, 3 H, CH_2CH_3). *Anal.* Calcd for $C_{12}H_{18}N_4O_3$: C, 54.12; H, 6.81; N, 21.04; mol wt, 266. Found: C, 54.08; H, 7.08; N, 21.05; mol wt, 266 (mass spectrum).

Reaction of Adenosine and Ethanol with Ultraviolet Light.—A mixture of adenosine (0.6 g), ethanol (120 ml), and water (30 ml) was irradiated for 125 hr. The usual work-up and preparative paper chromatography (twice) led to a heavy oil which was dissolved in water (8 ml), filtered, and left in the refrigerator to deposit a solid which was pure **9** (0.2 g, 28%): mp 150°; nmr (0.2 *M* solution in D_2O, DSS as internal standard) τ 2.18 (s, 1 H, C-2-H), 3.8 (d, $J_{1,2}$ = 7.5 Hz, 1 H, C-1′-H), 4.79 [q, J = 6.5 Hz, 1 H, CH(OH)CH_3], 5 (dd, $J_{2',1'}$ = 7.5 Hz, $J_{2',3'}$ = 5.5 Hz, 1 H, C-2′-H), 5.54 (dd, $J_{3',2'}$ = 5.5 Hz, $J_{3',4'}$ = 2.0 Hz, 1 H, C-3′-H), 5.7 (m, 1 H, C-4′-H), signal at 6.12 (2 H, C-5′-H_2), and 8.36 [d, J = 6.5 Hz, 3 H, CH(OH)CH_3].

Anal. Calcd for $C_{12}H_{17}N_5O_5$: C, 46.30; H, 5.50; N, 22.50; mol wt, 311. Found: C, 45.95; H, 6.10; N, 22.83; mol wt, 311 (mass spectrum).

Other reactions of purines and purine nucleosides with 2-propanol were conducted under similar conditions and are summarized in Tables I and III.

Reaction of 6-Ethoxypurine, 2-Propanol, and Acetone with Ultraviolet Light.—A mixture of 6-ethoxypurine (0.475 g), 2-propanol (80 ml), and acetone (80 ml) was irradiated 17 hr. The usual work-up and chromatography on kieselgel (100 g) led to the 2,8-disubstituted product **14** [0.205 g, 25%; eluted with acetone–petroleum ether (1:3)]: mp 200–201° (from acetone–petroleum ether); nmr (DMSO-d_6 + D_2O DSS as internal standard) τ 5.37 (q, J = 7 Hz, 2 H, =OCH_2CH$_3$), 8.39 [s, 6 H, C(CH_3)$_2$OH], 8.45 [s, 6 H, C(CH_3)$_2$OH], and 8.53 (t, J = 7 Hz, 3 H, OCH_2CH_3).

Anal. Calcd for $C_{13}H_{20}N_4O_3$: C, 55.70; H, 7.19; N, 19.99; mol wt, 280. Found: C, 55.72; H, 7.48; N, 20.10; mol wt, 280 (mass spectrum).

Further elution with acetone–petroleum ether (7:13) gave **13** (0.265 g, 41%): mp 201–202° (from acetone–petroleum ether); nmr (DMSO-d_6 + D_2O, DSS as internal standard) τ 1.5 (s, 1 H, C-2-H), 5.38 (q, J = 7 Hz, 2 H, OCH_2CH$_3$), 8.35 [2, 6 H, C(CH_3)$_2$OH), and 8.54 (t, J = 7 Hz, 3 H, OCH_2CH_3).

Anal. Calcd for $C_{10}H_{14}N_4O_2$: C, 54.04; H, 6.35; N, 25.21; mol wt, 222. Found: C, 53.98; H, 6.41; N, 25.10; mol wt, 222 (mass spectrum).

While exposing the same mixture to γ rays for 30 hr, **13** and **14** were obtained in 47 and 19% yield, respectively.

Reaction of 2-Aminopurine and 2-Propanol with Ultraviolet Light.—A suspension of 2-aminopurine (0.4 g) in 2-propanol (90 ml) and acetone (80 ml) was irradiated until the solid dissolved (8 hr). The usual work-up and chromatography on kieselgel led to the 6,8-disubstituted product **16** [0.17 g, 30%; eluted with 2-propanol–petroleum ether (1:3)]: mp 245–246° dec (from 2-propanol); nmr (DMSO-d_6–D_2O, TMS external) τ 8.4 [s, 6 H, C(CH_3)$_2$OH], 8.44 [s, 6 H, C(CH_3)$_2$OH].

Anal. Calcd for $C_{11}H_{17}N_5O_2$: C, 52.57; H, 6.82; N, 27.87; mol wt, 251. Found: C, 52.35; H, 7.00; N, 27.80; mol wt, 251 (mass spectrum).

Further elution with 2-propanol–petroleum ether (1:2) gave **15** (0.25 g, 34%), which was recrystallized successively from acetone and water and showed mp 205°; nmr (DMSO-d_6–D_2O, TMS external) τ 1.8 (s, 1 H, H-8), 8.4 [s, 6 H, C(CH_3)$_2$OH].

TABLE III
PRODUCTS OF THE REACTIONS OF PURINES AND PURINE NUCLEOSIDES WITH 2-PROPANOL

Purine or purine nucleoside	Product formula[a]	Mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
Adenine	$C_5H_{11}N_5O \cdot H_2O$	249–251	45.49	6.20	33.16	45.19	6.21	33.19
Hypoxanthine	$C_5H_{10}N_4O_2 \cdot CH_3OH$		47.78	6.24	24.77	47.46	6.19	24.46
Guanosine	$C_{13}H_{19}N_5O_6 \cdot CH_3OH$	211–213	45.03	6.21	18.76	45.35	6.10	19.04
2′-Deoxyguanosine	$C_{13}H_{19}N_5O_5 \cdot 2CH_3OH$		46.26	6.99	17.99	46.03	6.94	18.50

[a] In some experiments purification of the product presented some difficulties since the crystalline product contained solvent of crystallization. It was necessary, therefore, to employ a pure solvent in the final recrystallization.

TABLE IV
REACTIONS OF CAFFEINE AND ALCOHOLS INDUCED BY DI-*tert*-BUTYL PEROXIDE

Alcohol	Product (8-substituted caffeine)	Yield, %
Methanol	CH_2OH[a]	24
Ethanol	$CH(OH)CH_3$	15
1-Propanol	$CH(OH)CH_2CH_3$[b]	6.2
	CH_2CH_3	6.7

[a] H. Bredereck, E. Sugel, and B. Foehlisch, *Chem. Ber.*, **95**, 403 (1962). [b] Mp 154–155°; nmr ($CDCl_3$) τ 5.3 [t, J = 7 Hz, 1 H, CH(OH)], 6.05 (s, 3 H, N-7-CH_3), signal at 6.48 (1 H, OH), 6.58 (s, 3 H, N-3-CH_3), 4.7 (s, 3 H, N-1-CH_3), 8.07 (quintet J = 7 Hz, 2 H, CH_2CH_3), 8.98 (t, J = 7 Hz, CH_2CH_3). *Anal.* Calcd for $C_{11}H_{16}N_4O_3$: C, 52.37; H, 6.39; N, 22.21; mol wt, 252. Found: C, 52.49; H, 6.58; N, 22.21; mol wt, 252 (mass spectrum).

Anal. Calcd for $C_5H_{11}N_5O \cdot H_2O$: C, 45.49; H, 6.20; N, 33.16; mol wt, 211. Found: C, 45.64; H, 6.38; N, 33.20; mol wt, 193 (without H_2O, mass spectrum).

Reaction of 2-Propanol and Caffeine Induced by Di-*tert*-Butyl-peroxide.—A mixture of caffeine (0.7 g), 2-propanol (100 ml), and di-*tert*-butyl peroxide (1.5 g) was heated in a sealed tube at 130–140° for 35 hr. The usual work-up led to **2** (0.2 g, 22%). The other reactions of caffeine and alcohols induced by di-*tert*-butyl peroxide are described in Table IV.

Registry No.—**2**, 22439-97-0; **3**, 31326-94-0; **6**, 31385-42-9; **7**, 23865-41-0; **8**, 23844-14-6; **9**, 31326-97-3; **10**·CH_3OH, 31428-81-6; **13**, 31326-98-4; **14**, 31326-99-5; **15**, 31327-00-1; **16**, 31327-01-2; caffeine 8 substitutent $CHOHCH_2CH_3$, 31327-02-3; caffeine, 58-08-2; adenine, 73-24-5; adenosine, 58-61-7; guanosine, 118-00-3; 2′-deoxyguanosine, 961-07-9; hypoxanthine, 68-94-0; 6-ethoxypurine, 17861-06-2; 2-aminopurine, 26730-59-6; ethanol, 64-17-5; 1-propanol, 71-23-8; 2-propanol, 67-63-0; 2-butanol, 78-92-2.

The Mechanism of Formation of Some Pentofuranosyl Halides

C. P. J. GLAUDEMANS AND HEWITT G. FLETCHER, JR.*

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare, Bethesda, Maryland 20014

Received April 6, 1971

The behavior of the anomeric 2,3,5-tri-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-arabinofuranoses (**10** and **11**, Scheme I) with hydrogen chloride in dichloromethane solution has been studied. 2-*O*-Benzyl-1,3,5-tri-*O*-*p*-nitrobenzoyl-β-D-arabinofuranose (**15**, Scheme II) has been prepared through the action of silver *p*-nitrobenzoate on 2-*O*-benzyl-3,5-di-*O*-*p*-nitrobenzoyl-α-D-arabinofuranosyl chloride (**3**, Chart I); the reaction of **15** and of its previously known anomer **14** with hydrogen bromide in dichloromethane solution has been examined. In the presence of added chloride ions, 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**1**) shows a levomutarotation in dichloromethane solution; the same phenomenon is shown by 2-*O*-nitro-3,5-di-*O*-*p*-nitrobenzoyl-α-D-arabinofuranosyl bromide in the presence of bromide ions. All observations appear to be consistent with the view that the first step in the formation of the pentofuranosyl halides of the type studied is under kinetic control and leads to the α-D-arabinofuranosyl halide; partial anomerization to the corresponding β-D-arabinofuranosyl halides then follows but at a slower rate. A mechanism designed to rationalize these facts is discussed.

In 1965[1] we described studies of the methanolysis of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride (**1**, Chart I), 2,3-di-*O*-benzyl-5-*O*-*p*-nitrobenzoyl-α-D-arabinofuranosyl chloride (**2**), and 2-*O*-benzyl-3,5-di-*O*-*p*-nitrobenzoyl-α-D-arabinofuranosyl chloride (**3**). Although the steric features of the methanolyses were virtually identical (methyl β-D-arabinofuranoside derivatives preponderating in each case), the rates of methanolysis contrasted sharply, standing in the order, respectively, of 106:13:1. Thus, replacement of the benzyl group at C-5 in **1** by a *p*-nitrobenzoyl group reduced the rate of methanolysis by a factor of 8 and the replacement of the benzyl group at C-3 by a second *p*-nitrobenzoyl group caused a further reduction in the methanolysis rate by a factor of 13. In order to see whether exchange of benzyl by *p*-nitrobenzoyl groups exerts a similar stabilizing effect on aldopyranosyl halides, a subsequent study[2] was directed to the methanolysis of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl bromide (**4**), 2,3,4-tri-*O*-benzyl-6-*O*-*p*-nitrobenzoyl-D-glucopyranosyl bromide (**5**), 2,3-di-*O*-benzyl-4,6-di-*O*-*p*-nitrobenzoyl-β-D-glucopyranosyl bromide (**6**), and of the two anomeric forms of 2-*O*-benzyl-3,4,6-tri-*O*-*p*-nitrobenzoyl-D-glucopyranosyl bromide (**7**). Although the picture in the glucopyranose series is somewhat complicated by the anomeric effect, it was abundantly clear that this exchange of groups appeared to have a stabilizing effect on the C-1–halogen bond and that this effect is cumulative,

(1) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Amer. Chem. Soc.*, **87**, 4636 (1965).

(2) T. Ishikawa and H. G. Fletcher, Jr., *J. Org. Chem.*, **34**, 563 (1969).