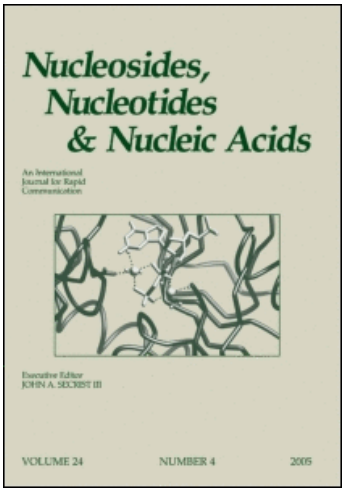

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CONDENSATION OF TRIFORMYLMETHANE WITH GUANOSINE

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ABSTRACT

Guanosine has been reacted with triformylmethane (TFM) in refluxing pyridine. Four different products, **4**–**7**, were isolated by preparative RP-HPLC, and characterized by ^{1}H and ^{13}C NMR and UV spectroscopy and mass spectrometry. One of the products, the cyclic 1:1 adduct **4**, is a stable cyclic carbinolamine formed probably by cyclization of the expected aminomethylene derivative **3**. Compound **4** then undergoes reversible dehydration to the fully conjugated adduct **5**. The appearance of the additional adducts, **6** and **7**, suggests that TFM is prone to transformations resulting in the formation of methylenemalonaldehyde (**9**) and 1,1,3,3-tetraformylpropane (**11**).

INTRODUCTION

α,β-Unsaturated carbonyl and 1,3-dicarbonyl compounds are known to generate adducts with nucleic acid bases. In this field malonaldehyde (MDA), the major carbonyl product of lipid peroxidation, is of primary importance[1]. As to the substituted malonaldehydes, only few reports exist. Moschel and

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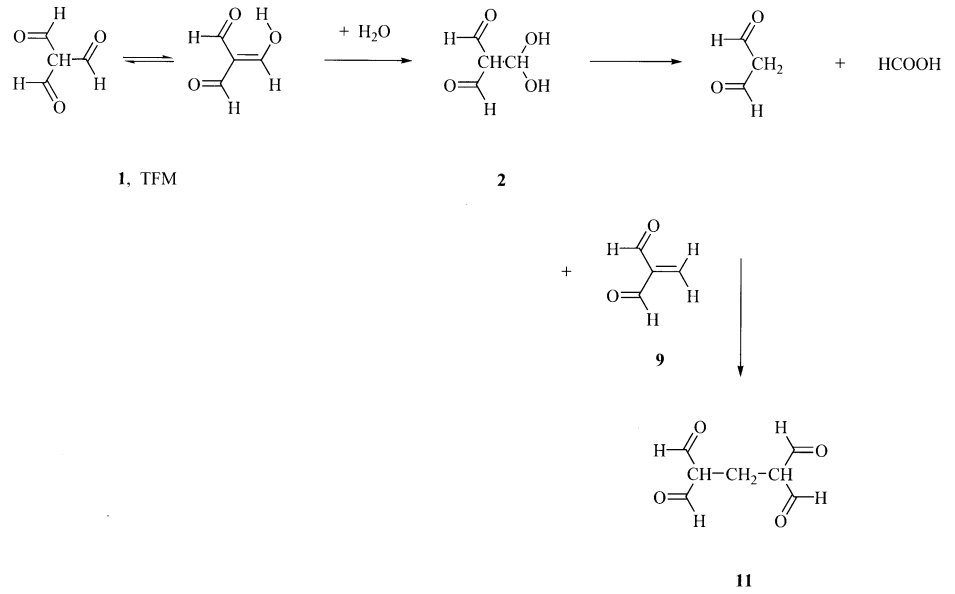


Scheme 1.

Leonard used alkyl, alkoxy or aryl substituted malonaldehydes for the preparation of fluorescent derivatives of guanine[2]. Furthermore, the kinetics and mechanisms of the reactions of adenosine and guanosine with chloro- and bromomalonaldehydes have been studied[3].

Triformylmethane (TFM, **1**), first prepared by Arnold and Žemlička[4], may also be classified as a 2-substituted malonaldehyde. Owing to keto-enol tautomerism (Scheme 1), this compound undergoes reactions that are typical for either aldehydes or enols. Although a potential mutagen, the data on the reactions of TFM with nucleosides, nucleotides or nucleic acids is still scarce. In fact, only the reactions with 1-substituted cytosines have been described[5]. The present work is focused on the reactions of TFM with guanosine.

RESULTS AND DISCUSSION

The reaction between TFM and guanosine was studied applying different reaction times, temperatures and solvents (pyridine, dioxane-water and aq. phosphate buffer, pH = 7.4). The reactions were monitored by HPLC. The best crude reaction mixture for separation was obtained at reflux in pyridine for 3 h. Four different products, **4**–**7**, were isolated by preparative HPLC and characterized by ^{1}H and ^{13}C NMR and UV spectroscopy and mass spectrometry. The NMR spectroscopic data are recorded in Tables 1 and 2, while the UV spectroscopic and mass spectrometric results are given in Experimental Section. The formation of these products may be rationalized

Table 1. ^{1}H NMR Chemical Shifts, δ_H in [D_6]DMSO and Coupling Constants (in Hz)

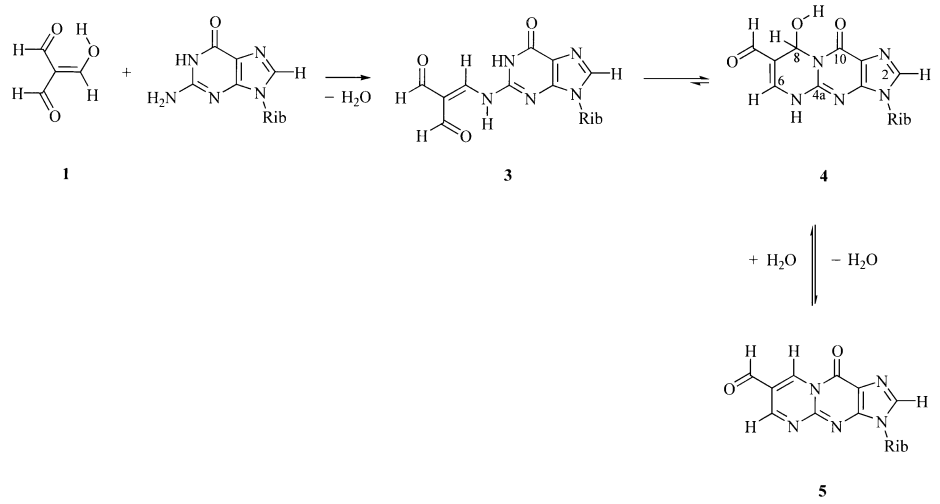
	4	**5**	**6**	**7a**	**7b**
2-H	8.20(s)	8.59(s)	8.15(s)		
5-H	11.50(d)[b]				
6-H	7.76(d)[b]	9.97(d)[f]	7.50(s)		
6-OH				7.25(s, br)	7.14(s,br)
8-H	6.902[c]; 6.899[d]	9.28(d)[f]	4.57[g]; 4.54[g]	7.88(s)	7.93(s)
8-OH	6.869[c]; 6.865[d]				
a-H				8.51(s)	8.96(s)
c-H				2.96(s)	7.43(s)
e-H				8.51(s)	4.77(s)
CHO	9.40(s)	10.16(s)	9.29(s)	9.49(s)	9.50(s)[i]; 9.24(s)[j]
1′-H[a]	5.78(d, J = 5.9)	6.02(d, J = 5.7)	5.72(d, J = 6.0)	5.79(d, J = 5.8)	5.81(d, J = 6.1)
2′-H	4.46(m)	4.57(q, J = 5.5)	4.43(t, J = 5.1)	4.58(t, J = 6.6)	4.55(m)
3′-H	4.12(m)	4.18(m)	4.12(t, J = 3.8)	4.16(q, J = 3.9)	4.12(q, J = 4.0)
4′-H	3.90(q, J = 4.0)	3.99(q, J = 3.7)	3.89(q, J = 3.9)	3.88(q, J = 4.0)	3.89(q, J = 4.0)
5′-H	3.63(m)[e]	3.70(m)[e]	3.66[h](dd, J = 11.5; 2.7)	3.65(m)[e]	3.62(m)[e]
5″-H	3.54(m)[e]	3.59(m)[e]	3.48 [h](dd, J = 11.5; 3.3)	3.54(m)[e]	3.53(m) [e]
2′-OH	5.55(dd, J = 9.8; 5.9)	5.53(d, J = 6.1)	5.48(s)	5.35(d, J = 5.6)	5.36(d, J = 6.9)
3′-OH	5.15(d, J = 4.6)	5.25(d, J = 5.1)	5.21(s)	5.13(s)	5.12(s)
5′-OH	4.99(q, J = 5.5)	5.11(t, J = 5.5)	5.03(s)	4.93(t, J = 5.2)	4.91(t, J = 6.4)

[a]1′-H – 5″-H and 2′-OH, 3′-OH and 5′-OH protons in the ribosyl unit; the assignment of signals of the sugar moiety was based on the connectivities in the H–H COSY spectra. –[b] ^{3}J = 5.6 Hz. –[c]An AB system, ^{2}J = 7.04 Hz; c and d refer to the different diastereomers exhibiting mole fractions 0.53 and 0.47, respectively. Assignment of their C*HOH* protons is based on the variable temperature NMR measurements and on addition of D_2O in [D_6]DMSO used as the NMR solvent. In both experiments, the upfield portion of the AB system corresponding to the hydroxyl proton disappears because of the fast exchange reaction. –[d]An AB system, ^{2}J = 6.97 Hz. –[e]An AB system further split into multiplets. –[f] ^{4}J = 2.6 Hz. –[g] An AB system, ^{2}J = 16.0 Hz. –[h] An AB system further split into doublets. –[i] d-CHO. –[j] b-CHO.

Table 2. ^{13}C NMR Chemical Shifts, δ_C in $[D_6]$DMSO

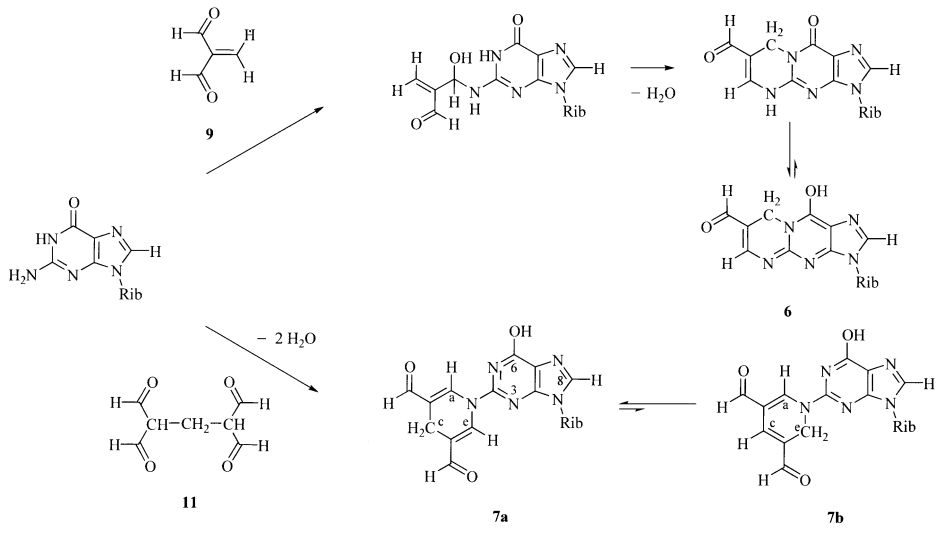
	4[b]		**5**[c]	**5**[d]	**6**[e]
C-2	137.97	137.93	141.23	140.52	137.73
C-3a	148.13	148.05	149.52	149.11	147.80
C-4a	145.62	145.59	148.94	148.32	146.11
C-6	143.06	143.06	144.86	143.27	143.25
C-7	119.11	119.08	119.20	118.83	119.40
C-8	66.78	66.74	157.84	157.38	
C-10	154.99	154.99	152.18	151.51	155.94
C-10a	114.97	114.97	118.45	118.02	110.80
CHO	187.33	187.33	189.02	187.66	187.24
C-1′[a]	86.63	86.63	87.41	87.62	86.56
C-2′	73.98	73.81	74.14	73.81	73.89
C-3′	70.38	70.37	70.31	69.90	70.32
C-4′	85.52	85.45	85.79	85.41	85.42
C-5′	61.30	61.30	61.24	60.96	61.27

[a] C-1′–C-5′ carbons in the ribosyl unit. –[b] Compound **4** appears as a roughly equimolar mixture of two diastereomers possessing closely related ^{13}C chemical shifts. Therefore no mutual assignment of ^{13}C chemical shifts of the different isomers was possible. –[c] at 30 °C. –[d] at 120 °C. –[e]C-8 shift could not be observed due to overlapping with the solvent signal.



Scheme 2.

by the mechanisms depicted in Schemes 2 and 3. The initial condensation product **4** (Scheme 2) is actually a cyclic carbinolamine which in $[D_6]$DMSO solution is equilibrated by dehydration with the fully conjugated adduct **5**. The proportion of the corresponding open-chain form **3**, in turn, remains at a



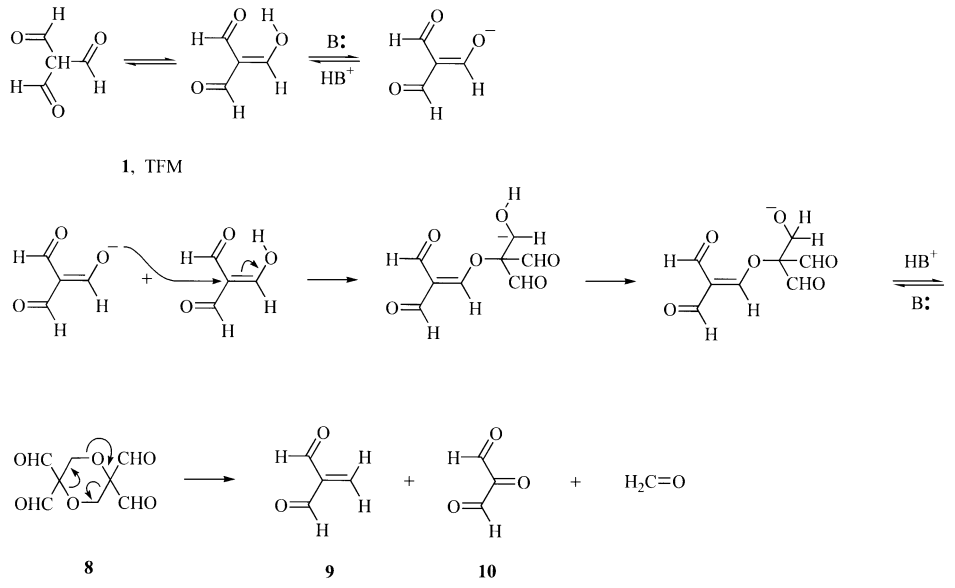
Scheme 3.

low level. The effects that changing the temperature and addition of water in [D_6]DMSO had on the equilibrium was studied by NMR spectroscopy.

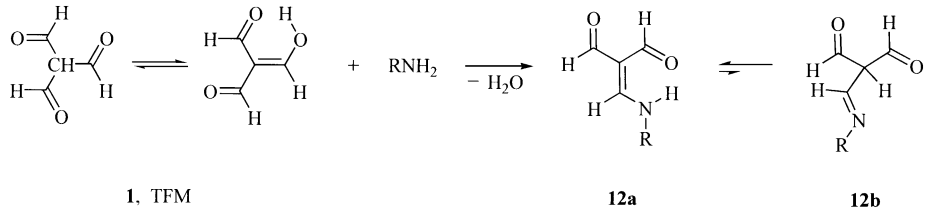
To explain the appearance of the two other adducts, *viz*. the 1:1 adduct **6** and 2:1 adduct **7** (Scheme 3), occurrence of secondary processes must be assumed. Hydrolysis of TFM to malonaldehyde (see Scheme 1), and conversion to methylenemalonaldehyde **9**, possibly *via* dimerization to **8** (Scheme 4), may be assumed to produce the required starting materials **9** and **11**. It is worth noting that the formation of **6** could be mimicked by treating guanosine with a mixture of malonaldehyde and formaldehyde.

1:1 TFM-guanosine Adduct

Condensation of TFM with various primary amines, including those derived from aromatic heterocyclic compounds, is known to yield aminomethylene derivatives, *i.e*. enamino tautomers (**12a**), instead of the imino tautomers (**12b**), which are the expected products of an addition-elimination reaction with the formyl group[6]. The driving force for the formation of enamino tautomers is in all likelihood the thermodynamic stabilization that conjugation of the C=C double bond with the C=O double bonds of the formyl groups results in. With aminopyrimidinones, such as the nucleic acid base cytosine[5], cyclization of the aminomethylene derivatives *via* a ring-chain process has been shown to occur. When the reaction of TFM with guanosine was followed in the present study by NMR spectroscopy, the presence of the aminomethylene form (**3**) could not be detected, but the formation of its cyclization product **4** as the major product was verified. A tentative



Scheme 4.



Scheme 5.

explanation for the predominance of the ring-form over the aminomethylene form is the stabilization of **4** by intramolecular hydrogen bond formation between the carbinolamine hydroxyl group and the guanine carbonyl oxygen. In fact, competition between the two possible intramolecular hydrogen bonds, *viz*. the one with the carbonyl oxygen of guanine and the other with that of the formyl group, undoubtedly occurs. The scrutiny of the ^{1}H and ^{13}C NMR spectra indicates that, in fact, **4** is a mixture of two different closely related species (exhibiting mole fractions 0.53 and 0.47). This is revealed in the ^{1}H NMR spectrum by the presence of two closely situated AB systems for the C*HOH* protons. Their low-field counterparts showed a longe-range H–H correlation (long-range COSY) both with the 6-H and the formyl proton signal. The ^{13}C NMR spectrum, however, is more sensitive. For eight carbons from the total of fourteen two different signals are observed. The NMR chemical shift differences are too small, however, to allow any reliable

assignment of the isomers. Because C-8 (see Scheme 2) is a chiral center, appearance of two diastereomers is a plausible explanation. The presence of two different diastereomers can also be considered as a further proof for the structure given to **4**. The diastereomers are in thermodynamic equilibrium, because the equilibration is possible through the open-chain form **3**.

In addition to ring-chain tautomerism, **4** may also undergo reversible dehydration to the fully conjugated adduct **5**. The occurrence of this process was observed by NMR spectroscopy. Especially informative is the difference between the 6-H shifts. The low field value, 9.97 ppm, referring to **5** is due to the anisotropy of the neighbouring formyl group. An analogous behaviour is known, for example, with the $1,N^6$-formylethenoadenosine[7]. The high-field shift, 7.76 ppm, referring to **4** is, in turn, consistent with the enamino structure. Accordingly, the covalent hydration seems to be well-established[8]. The equilibrium nature of the covalent hydration in question was further verified by studying the effects of temperature and concentration of D_2O in [D_6]DMSO on the equilibrium. The increase of temperature had a marked effect on the equilibrium constant. Near room temperature (30°C), the mole fraction of the dehydrated form (**5**) was 0.2 and at 120 °C 0.9. At the latter temperature, the OH proton signal in **4** had completely disappeared due to fast exchange and 8-H appears as a somewhat broadened singlet. It should be emphasized that the equilibrium was reached as a function of temperature in a completely reversible manner. By addition of known amounts of D_2O in [D_6]DMSO, the equilibrium could be gradually shifted towards **4**.

Adducts Formed by Decomposition of TFM

Besides the expected 1:1 TFM-adduct of guanosine, two additional adducts could be isolated. One of them, compound **6**, showed according to ^{1}H NMR spectroscopy structural similarity to **4**. The proton chemical shifts of its 2-H, 6-H and the CHO group were close to those of the corresponding protons of **4**. However, the ^{1}H NMR spectrum clearly displayed an AB quartet centered at $\delta = 4.56$ ppm, indicating the presence of a CH_2 fragment. The ^{1}H NMR chemical shifts suggest a methylene group adjacent to a nitrogen atom. Because of this and the plausible mechanism of the formation of **6**, *viz*. the assumed role of the primary amino group of guanosine as the initial nucleophile, the alternative structure where the methylene group is situated at position 6 appears less probable. The assignment is supported by comparison with the ^{13}C NMR chemical shifts of the corresponding 7-oxalo derivative[9]. The ^{13}C NMR chemical shifts of these two compounds are in good agreement. The high field value $\delta = 115.3$ ppm for the C-6 carbon of the carboxy derivative can be explained by operation of the high field γ-effect of the electronegative hydroxyl group. The one-proton signals at $\delta = 9.29$ ppm and 7.50 ppm showed a H–H correlation (long-range COSY) with the CH_2

signals, in agreement with the proposed assignment. In the positive ion electrospray mass spectrum of compound **6**, the protonated molecular ion was observed at m/z 350 at a relative abundance of 90%. The most abundant ion corresponding to the loss of the ribosyl unit was present at m/z 218.

For the other secondary adduct, **7**, a dihydropyridine structure was assigned. Interestingly, according to the ^{1}H NMR spectrum, compound **7** appears as a mixture of two tautomeric forms. The major tautomer (exhibiting mole fraction 0.7) clearly exhibits a symmetric 1,4-dihydropyridine system, as can easily be seen by comparing the ^{1}H NMR integral ratios. The methylene proton signal at $\delta = 2.96$ ppm appears as a singlet. No signal assignable to the NH proton (at $\delta = 10.60$ ppm in guanosine) could be detected in the ^{1}H NMR spectrum of **7a**. Instead, a broad one proton signal was detected at $\delta = 7.25$ ppm. This fact suggests the predominance of the enol tautomer **7a** given in Scheme 3. Both the a-H/e-H and the formyl signals showed a H–H correlation (long-range COSY) with the CH_2 signal, as would be expected by consideration of the coupling pathways in question. In the former case a favourable planar W pathway occurs. The ^{1}H NMR data of the minor tautomer are consistent with the 1,2-dihydropyridine structure of **7b** presented in Scheme 3. The ^{1}H NMR shift of one formyl proton ($\delta = 9.50$ ppm) is close to that of the formyl protons in **7a**. This corresponds to d-CHO which is adjacent to a methylene group and one methine proton analogously to the formyl groups in **7a**. The d-C*HO* proton showed a H–H correlation (long-range COSY) with the methylene signal, in agreement of the proposed assignment. The c-H signal ($\delta = 7.43$ ppm) showed a H–H long-range correlation with the a-H, the methylene proton and the b-C*HO* proton signals.

The unexpected appearance of the methylene derivatives **6** and **7** among the condensation products of TFM with guanosine suggests that decomposition/self-condensation of TFM must take place under the conditions used. As presented in Scheme 1, hydrolysis of TFM can result in formation of MDA and formic acid. Formation of methylenemalonaldehyde **9**, the probable source of the methylene group, is more difficult to explain. It should be noted that in the present work dry pyridine was used as a medium. There are only few remarks in literature that concern the decomposition or self-condensation reactions of TFM. Žemlička et al. reported that in water TFM undergoes self-condensation affording 1,3,5-triformylbenzene[10]. This process is probably initiated by intermolecular addition of the methine group to a formyl group resulting in formation of a new carbon – carbon bond. The net result is cyclization between three molecules of TFM. The cleavage of formic acid from the cyclic intermediate results in aromatization. Arnold and Buděšínský[11] reported formation of a compound from two molecules of TFM by extrusion of one molecule of water, described originally as 2,6-diformyl-4-oxa-2,5-heptadienedial. This process is attributed to enol-tautomer of TFM through addition of the enolic hydroxyl group to a formyl group and resulting loss of water. In other words, this process results in formation of a new

carbon – oxygen bond. Later, however, it was shown that the compound possesses a bicyclic propeller-like structure because of consecutive cycloaddition[12]. It has been shown that TFM is a strong acid ($pK_a = 2.0$ in water)[12]. In the anion, the negative charge is distributed between the carbon atom bearing the formyl groups and the oxygen atoms. In a basic medium, such as pyridine, TFM exists in the anionic form. The enolate oxygen can attack on C-2 of a neutral molecule, and a similar intramolecular attack then results in cyclization to **8** (see Scheme 4). This cyclic dimer may decompose as indicated to a mixture of formaldehyde, methylenemalonaldehyde (**9**) and oxomalonaldehyde (**10**)[13]. Condensation of **9** with guanosine then gives **6**, as indicated in Scheme 3. Addition of MDA to **9** gives the symmetric tetra-aldehyde **11**, and this when condensed with guanosine gives **7a** and its minor tautomer **7b**.

CONCLUSION

The condensation of TFM with guanosine affords a cyclic 1:1 adduct **4**, probably through the initial formation of the expected aminomethylene derivative **3**. Compound **4** is a stable covalent hydrate of the fully conjugated compound **5**, which presence could also be observed. Interestingly, two more adducts, **6** and **7**, could be isolated and characterized. Their appearance suggests that TFM is prone to transformations resulting in the formation of methylenemalonaldehyde (**9**) and 1,1,3,3-tetraformylpropane (**11**).

Franzén et al. have postulated that TFM is an intermediate of the decomposition of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone, a very potent mutagen and carcinogen present in chlorine-disinfected tap water, and that TFM reacts with guanosine giving a polycyclic quinone type adduct[5]. The role of TFM in this process may, however, be questioned, as indicated by the results of the present study.

EXPERIMENTAL SECTION

Caution

TFM is a new reagent for nucleic acid bases. It has not yet been determined whether facile chemical alteration of structure is accompanied by mutagenicity in living organisms. Nevertheless, caution should be taken in the handling and disposal of the compound.

Materials

The starting materials employed were commercial products of Sigma Chemical Co. (St Louis, Mo) or Aldrich Chemical Co. (Milwaukee, WI), and

they were used without further purification. Dioxane was of spectroscopical grade, and the solvents otherwise of analytical grade. Water was purified by reverse osmosis followed by ion exchange. Pyridine was dried by refluxing on calcium hydride and subsequent distillation. The solvents for HPLC were of commercial HPLC grade. Crude TFM[4b] was purified by sublimation (Büchi GKR-50 glass tube oven; 45 °C, 1 mm Hg). The average yield in sublimation was 88%, the amount of about 0.6 g crude TFM being conveniently handled. The white crystalline solid obtained melted at 105 °C [lit.[4b] 104–106 °C].

Spectroscopic and Spectrometric Methods

NMR spectra were acquired on a JEOL Alpha 500 NMR spectrometer (Tokyo, Japan) equipped with either a 5 mm normal configuration tuneable probe or a 5 mm inverse z-axis field-gradient probe operating at 500.16 MHz for ^{1}H, and 125.78 MHz for ^{13}C. The deuterium resonance of the solvent was used as a lock signal. The spectra were recorded at 30 °C in [D_6]DMSO (if not otherwise stated); ^{1}H and ^{13}C spectra were referenced internally to tetramethylsilane (both 0 ppm).

1D proton spectra were acquired with single-pulse excitation, 45° flip angle, pulse recycle time of 9 sec and with spectral widths of 8 kHz consisting of 65 k data points (digital resolution 0.11 Hz/pt), zero-filled to 128 k prior to Fourier transformation. 0.02 Hz of exponential weighting was usually applied prior to Fourier transformation. FG DQF COSY spectra were acquired in phase-sensitive mode with spectral widths appropriately optimized from the 1D spectra, and processed with zero-filling ($\times 2$, $\times 4$) and exponential weighting (1 Hz) applied in both dimensions prior to Fourier transformation.

1D carbon spectra were acquired with single-pulse excitation, 45° flip angle, pulse recycle time of 3.5 sec, and with spectral widths of 34 kHz consisting of 64 k data points (digital resolution 0.52 Hz/pt), zero-filled to 128 k and with 1 Hz exponential weighting applied prior to Fourier transformation. DEPT 135° spectra were acquired with similar spectral windows and with a pulse delay time of 3 secs. CHSHF (with partial homonuclear decoupling in f1) experiments were acquired in magnitude mode with spectral widths appropriately optimized from the 1D spectra and processed with zero-filling ($\times 2$, $\times 4$), a $2\pi/3$-shifted sinebell function, and exponential weighting applied in both dimensions prior to Fourier transformation. The CHSHF spectra utilized a $^{1}J_{HC}$ coupling of 145 Hz.

The positive ion electrospray ionization mass spectra (ESI^{+}-MS) were recorded on a Fisons ZabSpec-oaTOF instrument (Manchester, U.K.). Ionization was carried out using nitrogen as both the nebulizing and bath gas. A potential of 8.0 kV was applied to the ESI needle. The temperature of the pepperpot counter electrode was 90 °C. The samples were introduced by loop

injection at a flow rate of 20 μL/min (80/20/1 H_2O/CH_3CN/acetic acid). The mass spectrometer had a resolution of 7000.

The UV spectra of the isolated compounds were recorded with the diode-array detector as the peaks eluted from the HPLC column. The column was coupled to a Shimadzu HPLC system which consisted of two Shimadzu LC-9A pumps and a variable-wavelength Shimadzu SPD-6A UV spectrophotometric detector.

Chromatographic Methods

Analytical HPLC was performed on a Kontron Instruments liquid chromatographic system consisting of a model 322 pump, a 440 diode-array detector (UV), a JASCO FP-920 fluoresence detector, and a KromaSystem 2000 data handling program (Kontron Instruments S. P. A., Milan, Italy) using a 5 μm, 4 mm × 125 mm reversed phase C18 analytical column (Spherisorb ODS2, phase separation; Hewlett-Packard, Espoo/Esbo, Finland). The column was eluataed isocratically for 5 min with 0.01 M phosphate buffer (pH 7.1) and then with a gradient from 0 to 30% acetonitrile in 25 min at a flow rate of 1 mL/min. The compounds were isolated from the crude reaction mixtures by column chromatography on a reversed phase LiChroprep RP-18 column (40–63 μm, 37 mm × 440 mm, Merck KGaA). The column was coupled to a Pharmacia LKB liquid chromatographic system (GradiFrac System) consisting of the following units: Pharmacia LKB Pump P1, Control Unit UV-1, Optical Unit UV-1 and GradiFrac fraction collector. The collected fractions were evaporated to dryness. The fractions which according to the ^1H NMR spectroscopy contained modified nucleosides were dissolved in 5 mL of water and then further purified on a semipreparative reversed phase C18 column (5 μm, 10 mm × 250 mm, LiChrospher RP-18). The column was coupled to a Perkin Elmer HPLC system which consisted of a Perkin Elmer Isocratic LC 250 pump and a variable wavelength Perkin Elmer LC 290 UV/VIS spectrophotometric detector. The nucleosides were eluated as described below at a flow rate of 4 mL/min at 254 nm.

Preparation and Isolation of TFM-guanosine Adducts (4–7)

Guanosine (0.085 g, 0.30 mmol) and TFM (0.045 g, 0.45 mmol) in pyridine (10 mL) were stirred and refluxed for 3 hours. The resulting solution was cooled to room temperature and then evaporated to dryness at 40 °C in rotary evaporator under reduced pressure. The amount of the remaining pyridine was reduced by adding toluene into the residue and evaporating the mixture to dryness under reduced pressure. The treatment was repeated three times. The crude was dried overnight in a vacuum desiccator. The reaction was monitored by HPLC (*cf*. Fig. 1). The products were isolated from the

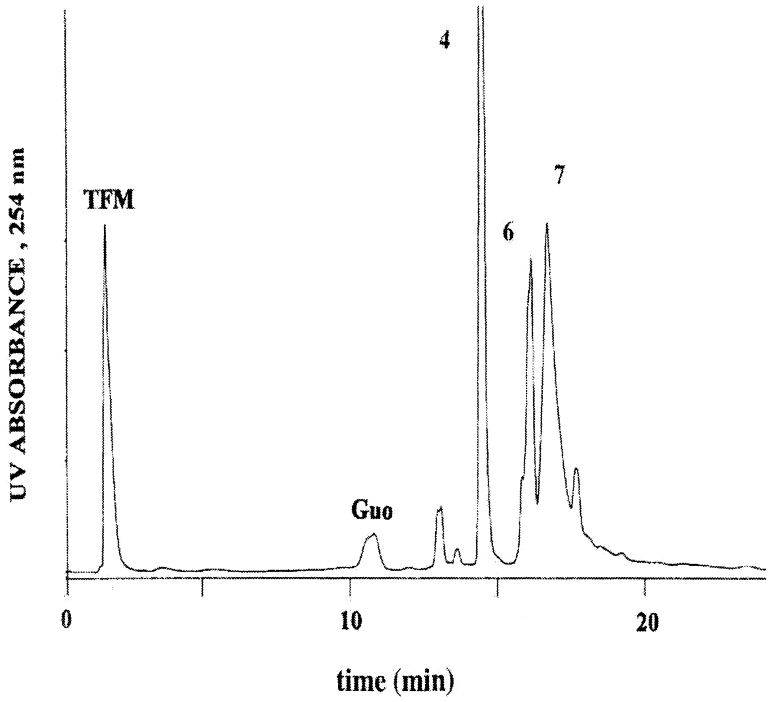


Figure 1. Reversed phase C18 analytical column HPLC chromatogram of the reaction mixture of TFM and guanosine after 3 h at reflux.

reaction mixture by the reversed phase column indicated above using 8% aqueous acetonitrile as the eluent. Alternatively, semipreparative HPLC was used by applying 300 mL of 0% (7 min), 2,5% (7 min), 5% (7 min), 7% (10 min) , 10% (10 min), and 15% (20 min) aqueous acetonitrile successively. Compounds **5**, **4**, **6**, and **7** were eluted from the column with 0%, 7%, 15% and 15% solutions, respectively. The fractions containing the products were evaporated to dryness and dissolved in 5 ml of water for the final purification with 6% aqueous acetonitrile on an RP-18 semipreparative column. The products were received as glassy solids.

7-Formyl-8-hydroxy-3-(β-D-ribofuranosyl)pyrimido[1,2-*a*]purin-10(3*H*, 5*H*,8*H*)-one (4). UV (H_2O): λ_{max} = 204 nm, 268, 328; λ_{min} = 235 nm, 295. – ESI^+–MS: m/z 366 (100, $[M+H]^+$), 348 (10, $[M (H_2O + H]^+$), 234 (60, $[M (C_5H_8O_4 + H]^+$), 216 (40, $[M [–C_5H_8O_4 - H_2O + H]^+$). - As to the ^1H and ^{13}C NMR spectroscopic data see Tables 1 and 2.

7-Formyl-3-(β-D-ribofuranosyl)pyrimido[1,2-*a*]purin-10(3*H*)-one (5). UV H_2O): λ_{max} = 204 nm, 255; λ_{min} = 222 nm. – ESI^+-MS: m/z 347 (100, $[M]^+$), 215 (10, $[M – C_5H_8O_4]^+$).

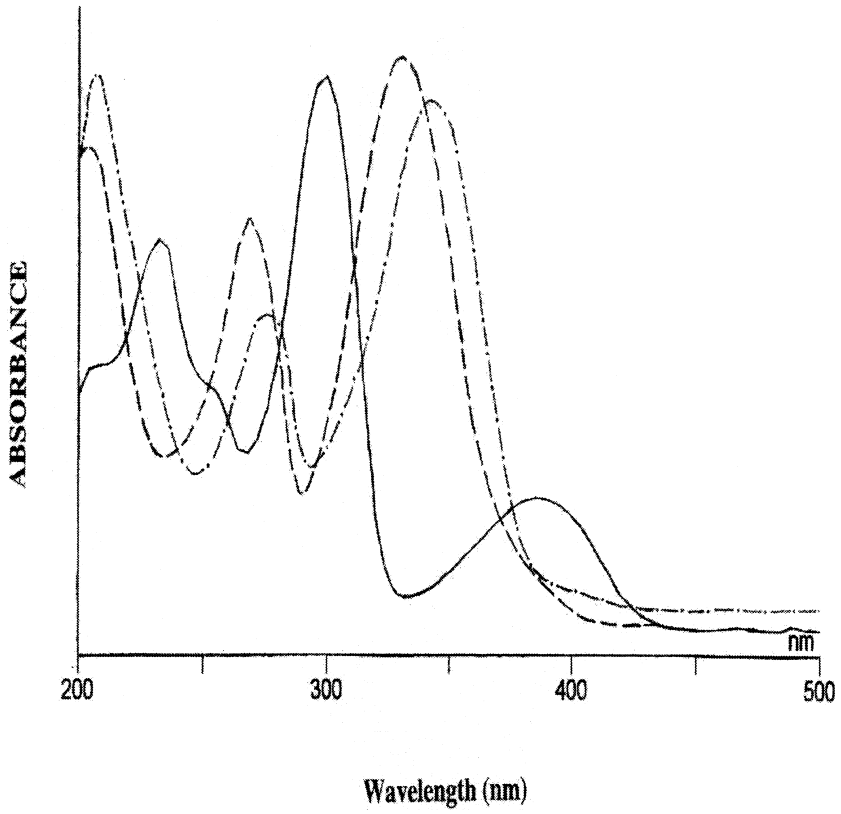


Figure 2. UV absorbance spectra of the adducts (dashed line for compound **4**, dashed-dotted line for compound **6** and solid line for compound 7); the UV spectra were recorded with the diode-array detector as the compounds eluted from the HPLC column.

7-Formyl-3-(β-D-ribofuranosyl)pyrimido[1,2-*a*]purin-10(3*H*,5*H*,8*H*)-one (6). UV (H_2O): λ_{max} = 210 nm, 270, 340; λ_{min} = 250 nm, 290. – ESI^+-MS: m/z 350 (90, $[M + H]^+$), 218 (100, $[M - C_5H_8O_4 + H]^+$).

2-(3,5-Diformyl-1,4-dihydropyrid-1-yl)-6-hydroxy-9-(β-D-ribofuranosyl)-purine (7). UV (H_2O): λ_{max} = 232 nm, 300, 388; λ_{min} = 268 nm, 332. – ESI^+–MS: m/z 404 (57, $[M + H]^+$), 272 (100, $[M - C_5H_8O_4 + H]^+$).

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