Compounds Relating to Dibenzoyladenine Riboside. The Choice between the N^6,1-Dibenzoyl and N^6,N^6-Dibenzoyl Structures

Kentaro ANZAI and Masanao MATSUI

The Institute of Physical and Chemical Research, Wako-shi, Saitama 351

(Received January 29, 1973)

It was clarified, by NMR analysis and on the grounds of synthetic work, that dibenzoyladenine riboside and its analogues have the N^6,N^6-dibenzoyl structure rather than the N^6,1-dibenzoyl structure, which has customarily been used to describe the benzoyl derivatives of adenosine.

5′-*O*-Trityl-tetrabenzoyladenosine and its detritylated product were tentatively identified by Khorana and his colleagues[1] as N^6,1,2′-*O*,3′-*O*-tetrabenzoates (I and II), but without strictly excluding another possible assignment, N^6,N^6,2′-*O*-3′-*O*-tetrabenzoates (III and IV). Polybenzoylated adenosine residues in protected oligonucleotides have also been described in the N^6,1-dibenzoyl structure.[2] The perbenzoylated product of 2′,3′-*O*-ethoxymethylideneadenosine was registered by Sŏrm and his colleagues[3] as N^6,1,5′-*O*-tribenzoyl-2′,3′-*O*-ethoxymethylideneadenosine (V). The reaction product with benzoyl chloride of 6-benzamido-9-(5-deoxy-2, 3-*O*-isopropylidene-β-D-*erythro*-pent-4-enofuranosyl)purine was described by Moffatt and his colleagues[4] as the N^6,1-dibenzoate (VI).

We also met a similar situation when we synthesized derivatives of tubercidin[5] (7-deazaadenosine),[6] where two benzoyl groups were introduced into the pyrrolo-[2,3-*d*]pyrimidine moiety.[7–10] The perbenzoylated product of 2′,3′-*O*-isopropylidenetubercidin has been ambiguously described as *N*,*N*,5′-*O*-benzoyl-2′,3′-*O*-isopropylidenetubercidin.[11]

Thus, compounds relating to dibenzoyladenine riboside have been described as having the N^6,1-dibenzoyl structure, but without any reliable grounds. It is the purpose of this paper to present proof that they are actually N^6,N^6-dibenzoyl compounds.

The tetrabenzoyl derivatives of adenosine (III[1] and IV[1]) and the tribenzoyl derivatives of tubercidin (VII and VIII) show complex NMR patterns in the aromatic region; these patterns do not give any good information about the position of benzoylation. In the case of a dibenzoylated derivative (IX)[8] of adenosine and those of tubercidin (X,[8] XI,[7] and XII[7]) signals of four *ortho* protons of benzoyl groups have been observed to separate well from the signals of the other aromatic protons, and these four protons have been shown to be equivalent; IX δ 7.84 J_{ortho}=7 Hz, X δ 7.82 J_{ortho}=7 Hz, XI δ 7.84 J_{ortho}=8 Hz, XII δ 7.83 J_{ortho}=7 Hz.

To obtain more certain information about the position of benzoylation, we prepared several toluyl derivatives of adenosine and observed the signals of the methyl protons. The results are shown in Table 1. In every case the signals corresponding to two methyls of toluyl groups, which are introduced in the adenine moiety, are found never to split, exhibiting a sharp singlet. Moreover, the NMR spectrum of the ditoluyl compound (XVI) (Fig. 1) exhibits a pair of doublets (δ 7.13 and 7.74, J_{ortho}=8 Hz) assignable to aromatic protons of toluyl groups; this also supports the N^6,N^6-ditoluyl structure. In the case of a tubercidin derivative the NMR spectrum of the tritoluyl compound (XVIII) exhibited a sharp singlet at δ 2.34 corresponding to two methyls of toluyl groups introduced in the pyrrolo-[2,3-*d*]pyrimidine moiety. The spectrum of XIII was taken at lower temperatures (Fig. 2), for we expected that a signal at δ 2.28 corresponding to two methyls might split by the restricted rotation of the bond between N^6 and C-6. However, only a broadening of the signal was observed, so we devised another proof for the N^6,N^6-diacyl structure.

9-Methyladenine (XIX) was converted to its benzoyl *p*-toluyl derivative *via* two routes; thus, the benzoylated product of 9-methyl-N^6-*p*-toluyladenine and the toluylated product of N^6-benzoyl-9-methyladenine were prepared, and these two products were compared exhaustively. If they are the same compound, both acyl groups should be bound at N^6.

The reaction of 9-methyladenine (XIX) with excess *p*-toluyl chloride afforded N^6,N^6-di-*p*-toluyl-9-methyl-

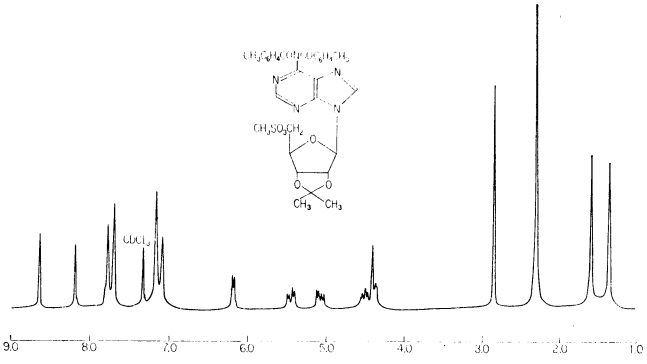


Fig. 1. NMR spectrum of N^6,N^6-di-*p*-toluyl-2′,3′-*O*-isopropylidene-5′-*O*-mesyladenosine (XVI). (100 MHz, $CDCl_3$).

1) M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Amer. Chem. Soc.*, **84**, 430 (1962).

2) K. Lohrman, D. Soell, H. Hayatsu, E. Otsuka, and H. G. Khorana, *ibid.*, **88**, 819 (1966).

3) S. Chládek, J. Zemlika, and F. Sŏrm, *Coll. Czech. Chem. Commun.*, **31**, 1785 (1966).

4) I. D. Jenkins, J. P. H. Verheyden, and J. G. Moffatt, *J. Amer. Chem. Soc.*, **93**, 4323 (1972).

5) K. Anzai, G. Nakamura, and S. Suzuki, *J. Antibiot.* (Tokyo), *Ser A*, **10**, 201 (1957).

6) S. Suzuki and S. Marumo, *ibid.*, **13**, 360 (1960); **14**, 34 (1961); Y. Mizuno, M. Ikehara, K. A. Watanabe, S. Suzaki, and T. Itoh, *J. Org. Chem.*, **28**, 3329 (1963).

7) K. Anzai and M. Matsui, This Bulletin, **46**, 618 (1973).

8) K. Anzai and M. Matsui, *Agr. Biol. Chem.* (Tokyo), **37**, 301 (1973).

9) K. Anzai and M. Matsui, *ibid.*, **37**, 345 (1973).

10) K. Anzai and M. Matsui, *ibid.*, **37**, 921 (1973).

11) Upjohn Co., Netn. 6606669 (1966); *Chem. Abstr.*, **67**, P 82375w (1967).

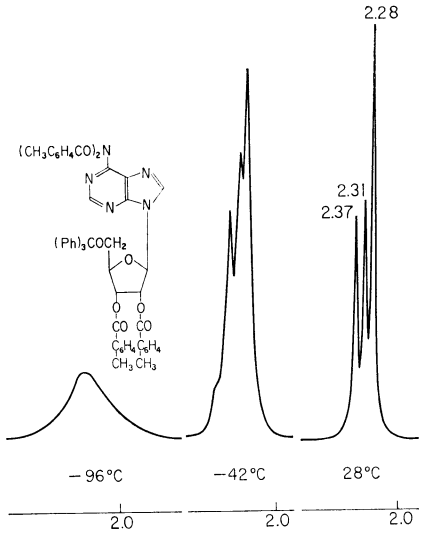


Fig. 2. NMR spectrum of N^6,N^6,2′-*O*, 3′-*O*-tetra-*p*-toluyl-5′-*O*-trityladenosine (XIII) at 28, −42, and −96 °C. (100 MHz, CS_2).

adenine (XX). The NMR spectrum of XX shows a sharp singlet at δ 2.31 corresponding to two methyls of *p*-toluyl groups. Similarly, N^6,N^6-di-*o*-toluyl-9-methyladenine (XXI) was prepared. The spectrum of XXI exhibits a six-proton singlet at δ 2.53. Attempts to prepare the monotoluyl compound (XXII), on the hydrolysis of XX, failed. In aqueous acetic acid, in a solution of sodium bicarbonate, or even in ethanol the only product obtained was XIX under the conditions where XX was partially hydrolysed. When XIX was treated with an equimolar amount of *p*-toluyl chloride, half of the starting material was converted to XX.

Compound XX was treated with lithium aluminum hydride in refluxing tetrahydrofuran to give 9-methyl-N^6-*p*-methylbenzyladenine (XXIII). However, when this reaction was carried out at room temperature, 9-methyl-N^6-*p*-toluyladenine (XXII) was obtained in a moderate yield. The benzoylation of XXII afforded N^6-benzoyl-9-methyl-N^6-*p*-toluyladenine (XXIV). Similarly, N^6,N^6-dibenzoyl-9-methyladenine (XXV) was treated with lithium aluminum hydride, and the product, N^6-benzoyl-9-methyladenine (XXVI), was treated with *p*-toluyl chloride. Thus, two samples of the benzoyl *p*-toluyl compound were prepared, and their NMR, IR (Fig. 3), UV, and Mass spectra were compared. It was shown that all of the spectral data were identical, strongly supporting the idea that dibenzoyladenine riboside and its derivatives should be described as having the N^6,N^6-dibenzoyl structure.

Preparation. The benzylidene benzoate (VIII) was prepared by the treatment of 2′,3′-*O*-benzylidenetubercidin (XXVII) with excess benzoyl chloride. The tetra-*p*-toluyl trityl ether (XIII) was prepared by the treatment of 5′-*O*-trityladenosine with excess *p*-toluyl chloride. The treatment of XIII in hot aqueous acetic acid for a short period afforded the detritylated compound (XIV). On the prolonged heating of XIII, the glycosidic bond was cloven and the isolated products were N^6,N^6-di-*p*-toluyladenine (XXVIII), N^6-*p*-toluyladenine (XXIX), and 2′,3′-*O*-di-*p*-toluylribose (XXX). The isopropylidene tri-*p*-toluyl compound (XV) was

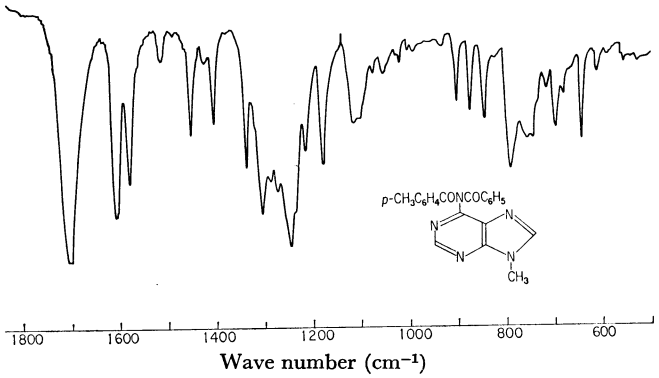
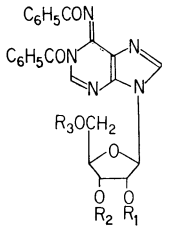


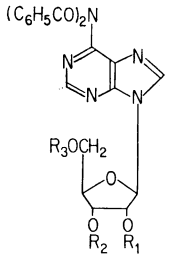
Fig. 3. IR spectrum of N^6-benzoyl-9-methyl-N^6-*p*-toluyladenine (XXIV). (KBr).

prepared by the treatment of 2′,3′-*O*-isopropylideneadenosine (XXXI) with excess *p*-toluyl chloride. Similarly, XVIII was prepared from 2′,3′-*O*-isopropylidenetubercidin (XXXII).[11] Compound XXXI was treated with ethyl vinyl ether in the presence of trifluoroacetic acid to give the acetal (XXXIII), which was then converted to the di-*p*-toluyl compound (XVII).

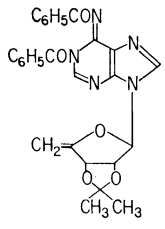
The treatment of XXXII with acetyl chloride afforded N^6,5′-*O*-diacetyl-2′,3′-*O*-isopropylidenetubercidin (XXXIV), and a triacetate corresponding to VII and XVIII was not obtained.



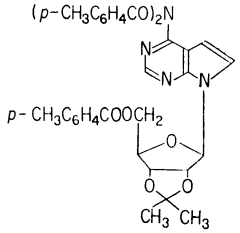
I R_1,R_2=C_6H_5CO, R_3=$(C_6H_5)_3C$
II R_1,R_2=C_6H_5CO, R_3=H
V R_1R_2=C_2H_5OCH<, R_3=C_6H_5CO



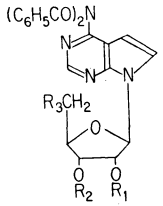
III R_1,R_2=C_6H_5CO, R_3=$(C_6H_5)_3C$
IV R_1,R_2=C_6H_5CO, R_3=H
IX R_1R_2=$(CH_3)_2C$<, R_3=CH_3SO_2



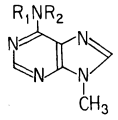
VI



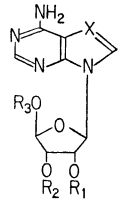
XVIII



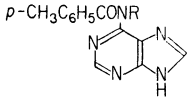
VII $R_1R_2=(CH_3)_2C<$, $R_3=C_6H_5COO$
VIII $R_1R_2=C_6H_5CH<$, $R_3=C_6H_5COO$
X $R_1R_2=(CH_3)_2C<$, $R_3=CH_3SO_3$
XI $R_1R_2=(CH_3)_2C<$, $R_3=I$
XII $R_1R_2=(CH_3)_2C<$, $R_3=H$



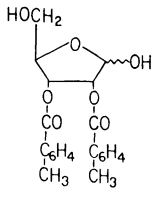
XIX $R_1,R_2=H$
XX $R_1,R_2=p\text{-}CH_3C_6H_4CO$
XXI $R_1,R_2=o\text{-}CH_3C_6H_4CO$
XXII $R_1=H$, $R_2=p\text{-}CH_3C_6H_4CO$
XXIII $R_1=H$, $R_2=p\text{-}CH_3C_6H_4CH_2$
XXIV $R_1=p\text{-}CH_3C_6H_4CO$, $R_2=C_6H_5CO$
XXV R_1, $R_2=C_6H_5CO$
XXVI $R_1=H$, $R_2=C_6H_5CO$



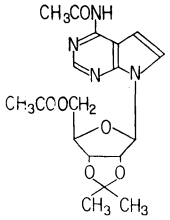
XXVII $R_1R_2=C_6H_5CH<$, $R_3=H$, X=CH
XXXI $R_1R_2=(CH_3)_2C<$, $R_3=H$, X=N
XXXII $R_1R_2=(CH_3)_2C<$, $R_3=H$, X=CH
XXXIII $R_1R_2=(CH_3)_2C<$, $R_3=CH_3CH(OC_2H_5)$, X=N



XXVIII R=*p*-$CH_3C_6H_5CO$
XXIX R=H



XXX



XXXIV

Experimental

2′,3′-O-Isopropylidenetubercidin (XXXII). The reported method[11] was improved to result in an increase in the yield. Tubercidin (5 g) was treated with tosyl chloride (37.5 g) in a mixture of acetone (250 ml) and acetone dimethyl acetal (25 ml) with stirring at room temperature. Within 2 hr the tubercidin was dissolved completely. The reaction mixture was poured into a cold solution of sodium bicarbonate (0.5 M, 1 liter), and the acetone was evaporated under reduced pressure. After the product had been extracted with chloroform, the organic layer was repeatedly washed with water and concentrated to dryness to afford a syrup (5.24 g, 92%), which was used without furthur purification for the succeeding processes. When acetone dimethyl acetal was excluded from this system, as has been reported previously,[11] the yield was 80%.

2′,3′-O-Benzylidenetubercidin (XXVII). A mixture of tubercidin (500 mg), zinc chloride (500 mg), and benzaldehyde (10 ml) was stirred at room temperature overnight and then poured into ice water. The product was extracted with chloroform, and the organic layer was washed with a cold solution of sodium bicarbonate and then with water. The chloroform was then evaporated, and petroleum ether was added to the residue. A syrupy sample of XXVII was separated by decantation and repeated precipitation from chloroform and petroleum ether; yield, 600 mg. An analytical sample was obtained by chromatography on silica gel (Kieselgel 0.05—0.2 mm, Merck), the developing being done with a mixture of ethyl acetate and methanol (9: 1); λ_{max}^{MeOH} mμ(ε) 270 (12000).

Found: C, 61.21; H, 5.27; N, 15.74%. Calcd for $C_{18}H_{18}O_4N_4$: C, 61.01; H, 5.12; N, 15.81%.

N⁶,N⁶,5′-O-Tribenzoyl-2′,3′-O-benzylidenetubercidin (VIII). Into a stirred solution of XXVII (5.1 g) in pyridine (50 ml), benzoyl chloride (10 g) was added dropwise keeping the temperature at −10 °C. The solution was then left at 0 °C for additional 4 hr and was then poured into an ice-cooled solution of sodium bicarbonate. The product was extracted with chloroform, and the solvent was evaporated to dryness. The residue was crystallized from ethyl acetate; yield, 6.9 g; mp 203 °C; $[\alpha]_D^{23}$ −87.5° (*c* 2.69, DMSO); λ_{max}^{MeOH} mμ(ε) 280 (sh, 12000), 224 (47000).

Found: C, 70.20; H, 4.55; N, 8.40%. Calcd for $C_{39}H_{30}O_7N_4$: C, 70.26; H, 4.54; N, 8.40%.

N⁶,N⁶,2′-O,3′-O-Tetra-p-toluyl-5′-O-trityladenosine (XIII). 5′-*O*-Trityladenosine[1] (2.6 g) was suspended in pyridine (20 ml), and then *p*-toluyl chloride (5 ml) was added at once. After stirring at room temperature for 18 hr, a clear solution was obtained. The reaction mixture was poured into an ice-cooled solution of sodium bicarbonate, and the product was extracted with chloroform. The solution was concentrated to dryness, and the residue was chromatographed on silica gel; development with a mixture of benzene and ethyl acetate (9: 1) resulted in the separation of XIII from triphenyl carbinol, the latter being eluted earlier. A crystalline mass melting at 132—138 °C was obtained from benzene and ligroin; yield, 5.1 g; $[\alpha]_D^{25}$ −71.7° (*c* 1.51, $CHCl_3$).

Found: C, 75.00; H, 5.43; N, 7.13%. Calcd for $C_{61}H_{51}O_8N_5$: C, 74.60; H, 5.24; N, 7.13%.

N⁶,N⁶,2′-O,3′-O-Tetra-p-toluyladenosine (XIV). A solution of XIII (1 g) in 70% aqueous acetic acid (50 ml) was heated on a water bath for 10 min. Several products were detected on tlc; among them one was found to be predominant; $R_{f\ major\ product}$: 1.3, 1.0, 0.3, 0 (benzene and ethyl acetate 4: 1). The major product was separated by chromatography on silica gel, developing with a mixture of benzene and ethyl acetate (4: 1) and increasing the content of the latter gradually; yield, 270 mg (36%). An analytical sample was obtained as crystals from benzene and ligroin; mp 173—174 °C; $[\alpha]_D^{25}$ −236° (*c* 1.73, $CHCl_3$).

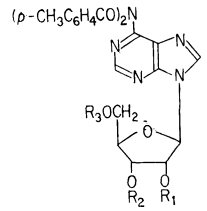
Found: C, 68.23; H, 5.16; N, 9.35%. Calcd for $C_{42}H_{37}O_8N_5$: C, 68.19; H, 5.04; N, 9.47%.

Glycosidic Bond Cleavage of XIII. A solution of XIII (1 g) in 70% aqueous acetic acid (50 ml) was heated on a water bath for 2 hr. The formation of three products was shown on tlc (benzene and ethyl acetate 1: 1), and they

Table 1. NMR and UV data of ditoluyladenine ribosides

	Chemical shift (δ)[a]				λ_{max}^{MeOH} mμ(ε)
	$(C\underline{H}_3C_6H_4CO)_2N$-	H-2	H-8	Other signals of methyl protons	
XIII	2.31	8.54	8.28	2.35, 2.40 ($2CH_3C_6H_4CO\cdot O$-)	270 (sh,[b] 28800), 242 (44200)
XIV	2.30	8.16	8.17	2.32, 2.36 ($2CH_3C_6H_4CO\cdot O$-)	270 (sh, 35400), 244 (51700)
XV	2.34	8.60	8.16	1.42, 1.65 ($(CH_3)_2C<$)	270 (sh, 32000), 247 (36400)
XVI[9]	2.32	8.64	8.20	1.38, 1.60 ($(CH_3)_2C<$)	270 (sh, 26800), 260 (28000)
XVII	2.34	8.62	8.16	1.38, 1.64 ($(CH_3)_2C<$) 2.17 (J=3Hz) ($C\underline{H}_3CH(OC_2H_5)$-)	270 (sh, 27400), 262 (28000)

a) NMR spectra were taken in $CDCl_3$ at 100 MHz with TMS standard.
b) shoulder



XIII R_1, R_2=p-$CH_3C_6H_4CO$, R_3=$(C_6H_5)_3C$
XIV R_1, R_2=p-$CH_3C_6H_4CO$, R_3=H
XV R_1R_2=$(CH_3)_2C<$, R_3=p-$CH_3C_6H_4CO$
XVI R_1R_2=$(CH_3)_2C<$, R_3=CH_3SO_2
XVII R_1R_2=$(CH_3)_2C<$, R_3=$CH_3CH(OC_2H_5)$

were separated by chromatography on silica gel. The least polar one was eluted with a mixture of benzene and ethyl acetate (1: 1) and was obtained as a syrup from benzene and petroleum ether; yield, 340 mg. It was identified as 2′,3′-*O*-di-*p*-toluylribose (XXX), $[\alpha]_D^{25}$ +14.8° (*c* 1.03, $CHCl_3$); λ_{max}^{MeOH} mμ(ε) 236 (21300); NMR (100 MHz, $CDCl_3$): δ 2.34 and 2.39 (6H, two singlets, $2C\underline{H}_3C_6H_4CO$); M^+ 386.

Found: C, 65.50; H, 5.70%. Calcd for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74%.

After the column had been washed with ethyl acetate, a mixture of ethyl acetate and methanol (9: 1) was used for elution. Two products were obtained. The less polar one, which was identified as N^6,N^6-ditoluyladenine (XXVIII), was crystallized from chloroform and benzene; yield, 83 mg; mp 231 °C; λ_{max}^{MeOH} mμ(ε) 290 (sh, 6500), 240 (33400); NMR (100 MHz, DMSO-*d*): δ 2.32 (6H, s, $2C\underline{H}_3C_6H_4CO$), 7.24 and 7.84 (8H, two doublets, J=8 Hz, $2CH_3C_6\underline{H}_4CO$).

Found: C, 64.70; H, 5.23; N, 18.11%. Calcd for $C_{21}H_{17}N_5O_2\cdot H_2O$: C, 64.77; H, 4.92; N, 17.99%.

The most polar compound, N^6-toluyladenine (XXIX), was crystallized from chloroform and benzene; yield, 110 mg; mp 224—225 °C; λ_{max}^{MeOH} mμ(ε) 287 (23400), 252 (16200); NMR (100 MHz, DMSO-*d*): δ 2.40 (3H, s, $C\underline{H}_3C_6H_4CO$), 7.35 and 8.04 (4H, two doublets, J=8 Hz, $CH_3C_6\underline{H}_4CO$); M^+ 253.

Found: C, 61.45; H, 4.31; N, 27.95%. Calcd for $C_{13}H_{11}ON_5$: C, 61.65; H, 4.38; N, 27.66%.

N^6,N^6,*5′*-O-p-*Toluyl-2′,3′*-O-*isopropylideneadenosine (XV).* To a solution of XXXI (614 mg) in pyridine (50 ml), *p*-toluyl chloride (1.6 g) was added, drop by drop, at −10 °C. The solution was then kept in a refrigerator overnight and subsequently poured into an ice-cooled solution of sodium bicarbonate. The product was extracted with chloroform, and the chloroform layer was concentrated to dryness. Tlc showed the presence of two UV-positive compounds, which were separated by silica gel chromatography, developing with a mixture of benzene and ethyl acetate (8: 1), the content of the latter being gradually increased. The less polar one was identified as *p*-toluic anhydride. The more polar compound, identified as XV, was crystallized from benzene and ligroin; yield, 550 mg; mp 170 °C, $[\alpha]_D^{25}$ −11.0° (*c* 1.69, $CHCl_3$).

Found: C, 67.15; H, 5.32; N, 10.55%. Calcd for $C_{37}H_{35}O_7N_5$: C, 67.16; H, 5.33; N, 10.59%.

5′-O-*(1-Ethoxyethyl)-2′,3′*-O-*isopropylideneadenosine (XXXIII).* To a solution of XXXI (1 g) and ethyl vinyl ether (3 ml) in dimethylformamide (50 ml) cooled at −15——20 °C, trifluoroacetic acid (3 ml) was added. After 3 hr the reaction mixture was poured into a cold solution of sodium bicarbonate (0.5 M, 500 ml). The desired product was extracted with chloroform and was separated by silica gel chromatography, being eluted with a mixture of ethyl acetate and methanol (95: 5), the content of the latter being gradually increased. The eluate was concentrated to dryness, affording a syrup; yield, 785 mg; $[\alpha]_D^{25}$ −35.7° (*c* 0.32, $CHCl_3$); λ_{max}^{MeOH} mμ(ε) 260 (13000); Mass *m/e*: 379 (M^+), 364 (M^+-CH_3), 350 ($M^+-C_2H_5$), 334 ($M^+-C_2H_5O$).

Found: C, 54.32; H, 6.77; N, 18.07%. Calcd for $C_{17}H_{25}O_5N_5$: C, 53.81; H, 6.64; N, 18.46%.

N^6,N^6-*Di*-p-*toluyl-5′*-O-*(1-ethoxyethyl)-2′,3′*-O-*isopropylideneadenosine (XVII).* To an ice-cooled and stirred solution of XXXIII (758 mg) in pyridine (5 ml), *p*-toluyl chloride (1 g) was added; it was then kept standing in a refrigerator overnight. The reaction mixture was poured onto a cold solution of sodium bicarbonate, and the product was extracted with chloroform. Silica gel chromatography, on developing with a mixture of benzene and ethyl acetate (4: 1), afforded 580 mg of a syrup; this was repeatedly precipitated from carbon tetrachloride and *n*-hexane; $[\alpha]_D^{25}$ −52.6° (*c* 0.79, $CHCl_3$).

Found: C, 64.11; H, 5.92; N, 10.91%. Calcd for $C_{33}H_{37}O_7N_5$: C, 64.37; H, 6.06; N, 11.38%.

N^6, N^6, *5′*-O-*Tri*-p-*toluyl-2′, 3′*-O-*isopropylidenetubercidin (XVIII).* To an ice-cooled and stirred soltion of XXXII (306 mg) in pyridine (30 ml), *p*-toluyl chloride (800 mg) was added drop by drop. After it had then been

kept standing in a refrigerator overnight, the reaction mixture was poured into a cold solution of sodium bicarbonate and the product was extracted with chloroform. The solution was concentrated to dryness, and the residue was crystallized from ethyl acetate and ligroin; yield, 460 mg (70%); mp 204—207 °C; $[\alpha]_D^{25}$ −47.5° (*c* 2.51, $CHCl_3$); λ_{max}^{MeOH} mμ(ε) 240 (47500); NMR (100 MHz, $CDCl_3$): δ 1.39 and 1.64 (6H, two singlets, isopropylidene methyls), 2.34 (6H, s, $(C\underline{H}_3C_6H_4CO)_2N$), 2.39 (3H, s, $C\underline{H}_3C_6H_4COO$).

Found: C, 68.55; H, 5.54; N, 8.34%. Calcd for $C_{38}H_{36}O_7N_4$: C, 69.08; H, 5.49; N, 8.48%.

N⁶,N⁶-*Di*-p-*toluyl-9-methyladenine (XX).* 9-Methyladenine (826 mg) was suspended in pyridine (50 ml), and then *p*-toluyl chloride (3 ml) was added at once. After the solution had been stirred at room temperature overnight, it was poured into a cold solution of sodium bicarbonate and the product was extracted with chloroform. *p*-Toluic anhydride formed was separated by silica gel chromatography, developing with a mixture of benzene and ethyl acetate (2: 1); thus, 1.37 g of XX were isolated. It was crystallized from benzene and *n*-hexane; mp 216—218 °C; λ_{max}^{MeOH} mμ(ε) 262 (30000); NMR (100 MHz, $CDCl_3$): δ 2.31 (6H, s, $(C\underline{H}_3C_6H_4CO)_2N$), 3.82 (3H, s, $CH_3N\langle$), 7.12 and 7.76 (8H, two doublets, J=8 Hz, $(CH_3C_6\underline{H}_4CO)_2N$), 8.20 (1H, s, H-8), 8.64 (1H, s, H-3).

Found: C, 68.49; H, 4.99; N, 18.17%. Calcd for $C_{22}H_{19}O_2N_5$: C, 68.56; H, 4.97; N, 18.17%.

N⁶,N⁶-*Di*-o-*toluyl-9-methyladenine (XXI).* 9-Methyladenine (92 mg) was treated with *o*-toluyl chloride (300 mg), as has been described in the preceeding section. The isolated product, XXI, was crystallized from carbon tetrachloride; yield, 160 mg; mp 184—185 °C; λ_{max}^{MeOH} mμ(ε) 270 (18400), 252 (20000); NMR (60 MHz, $CDCl_3$): δ 2.53 (6H, s, $2C\underline{H}_3C_6H_4CO$), 3.97 (3H, s, $CH_3N\langle$).

Found: C, 67.26; H, 4.90; N, 17.87%. Calcd for $C_{22}H_{19}O_2N_5\cdot 1/2H_2O$: C, 67.00; H, 5.11; N, 17.76%.

N⁶,N⁶-*Dibenzoyl-9-methyladenine (XXV).* 9-Methyladenine (1 g) was treated with benzoyl chloride (3 ml), as has been described in the preceeding section. Crystals of XXV were obtained from ethyl acetate and carbon tetrachloride; yield, 1.07 g; mp 182—184 °C; λ_{max}^{MeOH} mμ(ε) 270 (sh, 20000); 249 (26000).

Found: C, 65.96; H, 4.18; N, 19.42%. Calcd for $C_{20}H_{15}O_2N_5\cdot 1/2H_2O$: C, 65.56; H, 4.40; N, 19.12%.

9-Methyl-N⁶-p-*methylbenzyladenine (XXIII).* Compound XX (165 mg) was treated with lithium aluminum hydride (95 mg) in refluxing tetrahydrofuran (70 mg) for 17 hr. After the excess lithium aluminum hydride had been destroyed with water, the product, which was shown to be homogeneous on tlc, was extracted with chloroform. The solvent was evaporated, and the residue was crystallized from carbon tetrachloride; yield, 120 mg; mp 154 °C; λ_{max}^{MeOH} mμ(ε) 270 (18200); NMR (100 MHz, $CDCl_3$): δ 2.34 (3H, s, $C\underline{H}_3C_6H_4CH_2$), 3.82 (3H, s, $CH_3N\langle$), 3.90 (2H, broad d, J=4 Hz, $CH_3C_6H_4C\underline{H}_2$).

Found: C, 65.74; H, 5.94; N, 27.86%. Calcd for $C_{14}H_{15}N_5$: C, 65.38; H, 5.97; N, 27.65%.

9-Methyl-N⁶-p-*toluyladenine (XXII).* Compound XX (180 mg) was treated with lithium aluminum hydride (68 mg) in tetrahydrofuran (100 ml) at room temperature for 4 days. After the excess reagent had been destroyed with water, the products were extracted with chloroform. Tlc exhibited several UV-positive spots; among them, two major products were isolated by silica gel chromatography, developing with a mixture of ethyl acetate and methanol (9: 1), the content of the latter being gradually increased. The less polar one was identified as XXIII; yield, 15 mg. The more polar one, identified as XXII, was crystallized from ethyl acetate; yield 75 mg; mp 176—177 °C; λ_{max}^{MeOH} mμ(ε) 282 (15400); NMR (100 MHz, $CDCl_3$): δ 2.42 (3H, s, $C\underline{H}_3C_6H_4CO$), 3.90 (3H, s, $CH_3N\langle$).

Found: C, 60.82; H, 5.02; N, 25.73%. Calcd for $C_{14}H_{13}ON_5\cdot 1/2H_2O$: C, 60.85; H, 5.11; N, 25.35%.

N⁶-*Benzoyl-9-methyladenine (XXVI).* Compound XXV (730 mg) was treated with lithium aluminum hydride (250 mg) in tetrahydrofuran (100 ml) at room temperature for 6 days. After the excess reagent had then been destroyed with water, the products were extracted with chloroform and chromatographed on silica gel, developing with a mixture of ethyl acetate and methanol (9: 1), the content of the latter being gradually increased. The less polar one (yield, 107 mg) was found to be N^6-benzyl-9-methyladenine, as had already been reported by some workers.[12] The more polar one, which was identified as XXVI, was crystallized from ethyl acetate; yield, 133 mg; mp 189—191 °C; λ_{max}^{MeOH} mμ(ε) 280 (18300), 230 (sh, 14500).

Found: C, 61.65; H, 4.38; N, 27.96%. Calcd for $C_{13}H_{11}ON_5$: C, 61.47; H, 4.37; N, 28.38%.

N⁶-*Benzoyl-9-methyl*-N⁶-p-*toluyladenine (XXIV).* Compound XXII (125 mg) was treated with benzoyl chloride (108 mg) in pyridine (5 ml) at 0 °C for 1 hr; then the reaction mixture was poured into a cold solution of sodium bicarbonate. The product was extracted with chloroform and chromatographed on silica gel developing with ethyl acetate. From carbon tetrachloride, 34 mg of a glassy substance, which was shown to be homogeneous on tlc, were obtained. The analytical data showed that the solvent was difficult to exclude even on prolonged heating *in vacuo*; *e.g.*, after heating at 56 °C for 4 hr, a 2/3 equivalent of carbon tetrachloride was found to remain, and a 1/3 equivalent still remained after it had been treated at 80 °C for 8 hr. The same compound was prepared from XXVI (85 mg) and *p*-toluyl chloride (150 mg); yield, 40 mg; λ_{max}^{MeOH} mμ(ε) 270 (sh, 18700), 255 (20000); NMR (60 MHz, $CDCl_3$): δ 2.20 (3H, s, $C\underline{H}_3C_6H_4CO$), 4.00 (3H, s, $CH_3N\langle$); Mass *m/e*: 370 (M^+), 342 (M^+-CO), 266 ($M^+-C_6H_5CO+H$), 252 ($M^+-CH_3C_6H_4CO+H$).

N⁶,*5'*-O-*Diacetyl-2',3'*-O-*isopropylidenetubercidin (XXXIV).* To an ice-cooled solution of XXXII (306 mg, 1 mml) in pyridine, acetyl chloride (800 mg, 10 mml) was added drop by drop. After 2 hr, the reaction mixture was poured into a cold solution of sodium bicarbonate. The product was extracted with chloroform and chromatographed on silica gel, developing with a mixture of benzene and ethyl acetate (2: 1), the content of the latter being gradually increased. Precipitation from ethyl acetate and ligroin afforded 183 mg of a syrup; $[\alpha]_D^{25}$ −20.0° (*c* 2.04, $CHCl_3$); λ_{max}^{MeOH} mμ(ε) 287 (7800).

Found: C, 55.69; H, 5.57; N, 14.04%. Calcd for $C_{18}H_{22}O_6N_4$: C, 55.55; H, 5.59; N, 14.35%.

12) Shell Internationale Research Maatschappij N. V., Brit. 953897 (1964); *Chem. Abstr.*, **62**, P 6494a (1965). Shell Internationale Research Matschappij, Ger. 1132784, (1962); *Chem. Abstr.*, **57**, P 13776g (1962). Johannes von Overbeek, U. S. 3013885, (1960); *Chem. Abstr.* **56**, P 14306e (1962).