Purines. LXXVIII.[1] An Alternative Synthesis of the Sea Anemone Purine Alkaloid Caissarone

Taisuke ITAYA,* Yasutaka TAKADA, Tae KANAI, and Tozo FUJII

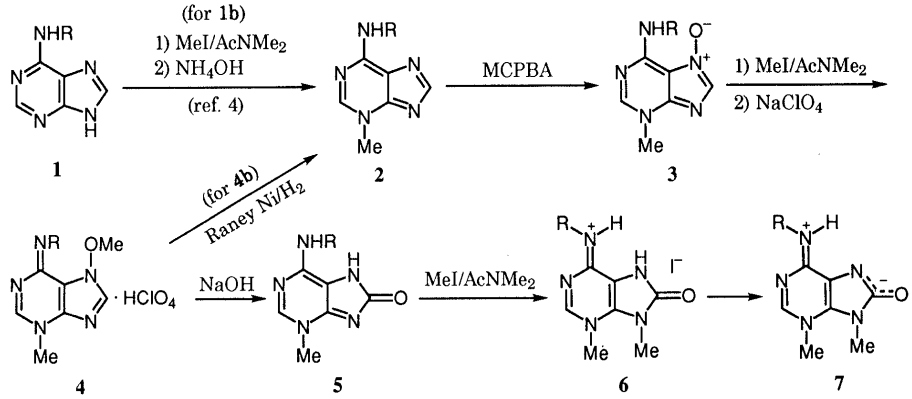
Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.
Received May 28, 1997; accepted July 7, 1997

An alternative synthesis of N^6,3,9-trimethyl-8-oxoadenine (caissarone) (7b) has been accomplished by regioselective methylation of N^6,3-dimethyl-8-oxoadenine (9-demethylcaissarone) (5b), which was obtained by N(7)-oxidation of N^6,3-dimethyladenine (2b) with *m*-chloroperoxybenzoic acid followed by *O*-methylation with MeI and subsequent treatment with aqueous NaOH. The UV spectral data for the dimethylated 8-oxoadenine 5b and eight of its regioisomers, among which the O^8,9-dimethyl isomer 9 was prepared for the first time in the present work, are summarized.

Key words caissarone synthesis; N^6,3-dimethyladenine *N*-oxidation; N^6,3-dimethyl-8-oxoadenine methylation; N^6,3-dimethyl-7-methoxyadenine; N^x,N^y (or O^8)-dimethyl-8-oxoadenine UV spectrum

We have already reported the synthesis of caissarone (**7b**), a biologically active 8-oxoadenine derivative isolated as the hydrochloride salt from the sea anemone *Bunodosoma caissarum* CORREA 1964,[2] through regioselective N(3)-methylation of N^6,9-dimethyl-8-oxoadenine, which was obtained from 9-methyladenine in four steps.[3] Another synthetic approach to **7b**, starting from 3-methyladenine (**2a**) and proceeding through N(7)-oxidation, *O*-methylation, nucleophilic substitution, and N(9)-methylation, led to our synthesis of 3,9-dimethyl-8-oxoadenine (N^6-demethylcaissarone) (**7a**) for the first time (Chart 1).[4] However, **7a** proved inert to further methylation,[4] failing to give the desired alkaloid **7b**. We considered that an N^6-methyl version of this reaction sequence starting from N^6,3-dimethyladenine (**2b**) might represent an alternative route to **7b**. Now we report the synthesis of caissarone (**7b**) accomplished along this line.

Treatment of N^6,3-dimethyladenine (**2b**),[5] which was readily accessible from N^6-methyladenine (**1b**) by regioselective methylation, with *m*-chloroperoxybenzoic acid (MCPBA) in a mixture of MeOH and phosphate buffer (pH 6.5) at 30 °C for 20 h afforded the N(7)-oxide **3b** in 48% yield, together with 30% recovery of **2b**. The N(7)-oxide structure was assignable to **3b** on the basis of its identity with a sample[6] prepared in 13% yield by methylation of 3-methyladenine 7-oxide (**3a**) with dimethyl sulfate in 0.1 N aqueous NaOH. Treatment of **3b** with MeI in $AcNMe_2$ at 30 °C for 20 h and subsequent anion exchange produced the 7-methoxy compound as the perchlorate **4b** in 93% yield. Hydrogenolysis of **4b** with H_2 and Raney Ni catalyst gave **2b** in 92% yield, verifying the correctness of the *O*-methyl structure of **4b**. Compound **4b** was then treated with boiling 0.1 N aqueous NaOH for 1 h, and the 8-oxo compound was isolated as the hydrochloride **5b**·HCl[7] in 50% yield. The correctness of the structure of **5b**·HCl was supported by its UV spectral similarity to **5a**,[6] with an expected bathochromic shift of the maxima due to N^6-methylation. Methylation of **5b**·HCl with MeI in $AcNMe_2$ in the presence of 0.5 mol eq of K_2CO_3 at 40 °C for 48 h provided caissarone hydriodide (**6b**), which was identical with an authentic specimen,[3] in 51% yield (11% overall yield from **2b**). The free base **7b**[3] was obtained in 76% yield by treatment of an aqueous solution of **6b** with Amberlite IRA-402 (HCO_3^-). Although the overall yield of **6b** from **2b** is inferior to that from 9-methyladenine [*via* regioselective N(3)-methylation of the key intermediate N^6,9-dimethyl-8-oxoadenine] in the previous synthesis, the method for introducing an oxo function into C(8) of **2b** and the regioselective N(9)-methylation of the key intermediate **5b** are important



a: R = H
b: R = Me

Chart 1

* To whom correspondence should be addressed.

features of the present alternative synthesis of caissarone (**7b**).

Table 1 assembles the UV spectral data for N^6,3-dimethyl-8-oxoadenine (9-demethylcaissarone) (**5b**) and eight of its positional isomers. Of the five possible 9-methyl-8-oxoadenine derivatives further methylated at a hetero atom, N^6,9-,[8] 1,9-,[8] 3,9-,[1,4] and 7,9-dimethyl-8-oxoadenines[4] have already been synthesized by us. As shown in Chart 2, the remaining positional isomer, 8-methoxy-9-methyladenine (**9**), was prepared from 8-bromo-9-methyladenine (**8**) in the present study according to the procedure reported for the synthesis of 8-methoxyadenosine,[9] and the correctness of its structure was supported by its UV spectral similarity to this nucleoside. The syntheses of 3,7-dimethyl-8-oxoadenine,[4] 8-methoxy-3-methyladenine,[6] and 8-methoxy-N^6-methyladenine[10] have also been reported. Although seven other isomers [N^6,N^6-, N^6,1-, N^6,7-, 1,3-, and 1,7-dimethyl-8-oxoadenines and 8-methoxyadenines monomethylated at N(1) and N(7)] are still unknown, the regioisomers included in Table 1 can be easily discriminated UV spectrophotometrically from each other. Thus, the data given in Table 1 will be useful for determining the structures of disubstituted 8-oxoadenines, especially for identifying the position of monosubstitution on the 9-substituted 8-oxoadenine ring.

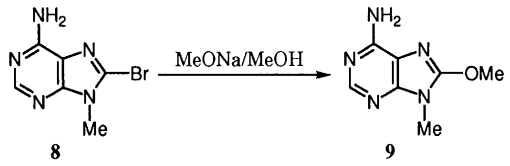


Chart 2

Experimental

General Notes All melting points were determined by using a Yamato MP-1 or a Büchi model 530 capillary melting point apparatus and values are corrected. Spectra reported herein were recorded on a JEOL JMS-SX102A mass spectrometer, a Hitachi model 320 UV spectrophotometer [for solutions in 95% aqueous EtOH, 0.1 N aqueous HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous NaOH (pH 13)], a Shimadzu FTIR-8100 IR spectrophotometer, or a JEOL JNM-EX-270 NMR spectrometer [measured at 25 °C in $(CD_3)_2SO$ with Me_4Si as an internal standard]. Elemental analyses and MS measurements were performed by Mr. Y. Itatani, Dr. M. Takani, and their associates at Kanazawa University. Flash chromatography was performed according to the reported procedure.[11] The following abbreviations are used: br = broad, d = doublet, q = quartet, s = singlet, sh = shoulder.

N^6,3-Dimethyladenine 7-Oxide Monohydrate (3b·H_2O) A mixture of **2b**[5] (490 mg, 3 mmol), MCPBA (of *ca.* 70% purity) (1.48 g, 6 mmol), 1 M phosphate buffer (pH 6.5) (20 ml), H_2O (30 ml), and MeOH (30 ml) was stirred at 30 °C for 20 h and then concentrated *in vacuo*. The residue was partitioned between 10% aqueous HCl (20 ml) and Et_2O (30 ml). The aqueous layer was separated from the ethereal layer, washed with Et_2O (4 × 30 ml), neutralized with 10% aqueous Na_2CO_3, and concentrated *in vacuo*. The residual solid was extracted with hot MeOH (4 × 50 ml). Silica gel (4 g) was added to the combined methanolic extracts, and the mixture was concentrated *in vacuo*. The resulting solid mixture was subjected to flash chromatography [$CHCl_3$–MeOH–concentrated aqueous NH_3 (40 : 7 : 1, v/v)], affording **2b** (149 mg, 30% recovery) and **3b**·H_2O (287 mg, 48%), mp 225—227 °C. The latter product was purified by precipitation from a mixture of MeOH and Me_2CO (1 : 1, v/v), dried over P_2O_5 at 2 mmHg and 80 °C for 15 h, and exposed to air at room temperature until a constant weight was reached, providing an analytical sample of **3b**·H_2O as colorless prisms, mp 227—229 °C (dec.). *Anal.* Calcd for $C_7H_9N_5O \cdot H_2O$: C, 42.64; H, 5.62; N, 35.51. Found: C, 42.39; H, 5.57; N, 35.49. This sample was identical (by comparison of the MS, UV, IR, and ^{1}H-NMR spectra and TLC mobility) with authentic **3b**·H_2O.[6]

7-Methoxy-N^6,3-dimethyladenine Perchlorate (4b) A mixture of **3b**·H_2O (406 mg, 2.06 mmol), MeI (1.46 g, 10.3 mmol), and $AcNMe_2$ (20 ml) was stirred at 30 °C for 20 h and then concentrated *in vacuo*. The residue was washed with Et_2O (10 ml) and dissolved in a mixture of H_2O (3 ml) and MeOH (3 ml). This solution was mixed with a solution of $NaClO_4$·H_2O (376 mg, 2.68 mmol) in H_2O (1 ml) and cooled in an ice bath. The precipitate that deposited was collected by filtration, washed with H_2O (2 × 1 ml), and dried to provide **4b** (562 mg, 93%), mp 191—193 °C.

Table 1. UV Spectral Data for Three Demethylcaissarones and Six of Their Positional Isomers Bearing Methyl Groups at Hetero Atoms

Compound	UV spectra in H_2O					
	pH 1		pH 7		pH 13	
	$\lambda_{max}^{H_2O}$ (nm)	$\varepsilon \times 10^{-3}$	$\lambda_{max}^{H_2O}$ (nm)	$\varepsilon \times 10^{-3}$	$\lambda_{max}^{H_2O}$ (nm)	$\varepsilon \times 10^{-3}$
N^6,9-Dimethyl-8-oxoadenine (3-demethylcaissarone)[a]	275	14.1	274	17.0	284	18.0
1,9-Dimethyl-8-oxoadenine[a]	221	28.0	220	24.5	280	14.6
	278	10.4	285	12.0	310 (sh)	4.8
3,9-Dimethyl-8-oxoadenine (N^6-demethylcaissarone) (**7a**)[b]	222	21.3	300	15.4	305	16.6
	291	20.0				
7,9-Dimethyl-8-oxoadenine[b]	220	25.5	213	35.4	273	13.3
	279	10.6	273	13.2		
8-Methoxy-9-methyladenine (**9**)	210	25.2	264	13.9	264	13.8
	265	13.4				
N^6,3-Dimethyl-8-oxoadenine (9-demethylcaissarone) (**5b**)[c]	221	19.7	219 (sh)	14.8	314	14.6
	292	20.1	233	19.9		
			298	20.3		
3,7-Dimethyl-8-oxoadenine[b]	219	21.9	217	19.1	230	17.0
	290	18.3	230	16.9	296	18.5
			296	18.4		
8-Methoxy-3-methyladenine[d]	210	16.8	210	19.6	224 (sh)	13.1
	222	18.6	225 (sh)	13.2	284	14.8
	279	20.4	284	14.8		
8-Methoxy-N^6-methyladenine[e]	280	11.1	—	—	280[f]	15.8[f]

a) Taken from ref. 8. *b*) Taken from ref. 4. *c*) As a monohydrate of the hydrochloride. *d*) Taken from ref. 6. *e*) Taken from ref. 10. *f*) Measured at pH 11.

Recrystallization of this sample from 90% (v/v) aqueous MeOH afforded an analytical sample of **4b** as colorless plates, mp 201.5—202.5 °C; UV $\lambda_{max}^{95\%\ EtOH}$ 288 nm (ε 18200); $\lambda_{max}^{H_2O}$ (pH 1) 286 (18100); $\lambda_{max}^{H_2O}$ (pH 7) 286 (18200); $\lambda_{max}^{H_2O}$ (pH 13) unstable; IR ν_{max}^{Nujol} cm^{-1}: 3360, 3112 (NH), 1667 (C=N); ^{1}H-NMR δ:[12] 3.16 (3H, d, J=5 Hz, MeNH), 3.96 [3H, s, N(3)-Me], 4.25 (3H, s, OMe), 8.86 [1H, s, C(2)-H], 8.94 (1H, br q, J=5 Hz, MeNH), 9.10 [1H, s, C(8)-H]. *Anal.* Calcd for $C_8H_{11}N_5O \cdot HClO_4$: C, 32.72; H, 4.12; N, 23.85. Found: C, 32.65; H, 4.13; N, 23.93.

N^6,3-Dimethyl-8-oxoadenine Hydrochloride Monohydrate (5b·HCl·H_2O) A solution of **4b** (294 mg, 1 mmol) in 0.1 N aqueous NaOH (20 ml) was heated under reflux for 1 h, neutralized with 10% aqueous HCl, and concentrated *in vacuo*. The residue was recrystallized from 5% aqueous HCl (decolorized by activated charcoal powder) to give **5b**·HCl·H_2O (116 mg, 50%), mp 287—288 °C (dec.). This sample was further recrystallized from 5% aqueous HCl, dried over P_2O_5 at 2 mmHg and 100 °C for 6 h, and exposed to air at room temperature until a constant weight was reached, providing an analytical sample of **5b**·HCl·H_2O as colorless pillars, mp 293—296 °C (dec.); UV $\lambda_{max}^{95\%\ EtOH}$ 224 nm (ε 17100), 299 (18600); $\lambda_{max}^{H_2O}$ (Table 1); IR ν_{max}^{Nujol} cm^{-1}: 3362, 3179, 3052 (NH), 1730 (C=O), 1682 (C=N); ^{1}H-NMR δ: 3.03 (3H, d, J=5 Hz, MeNH), 3.81 [3H, s, N(3)-Me], 8.54 [1H, s, C(2)-H], 8.82 (1H, br q, J=5 Hz, MeNH), 11.40 (br s) and 13.0 (br) (1H each, two NH's). *Anal.* Calcd for $C_7H_9N_5O \cdot HCl \cdot H_2O$: C, 35.98; H, 5.18; N, 29.97. Found: C, 36.13; H, 5.28; N, 30.17.

N^6,3,9-Trimethyl-8-oxoadenine Hydriodide (Caissarone Hydriodide) (6b) A mixture of **5b**·HCl·H_2O (350 mg, 1.5 mmol), anhydrous K_2CO_3 (104 mg, 0.752 mmol), and $AcNMe_2$ (20 ml) was stirred at 40 °C for 1 h and then stirred at this temperature for a further 48 h after addition of MeI (2.13 g, 15 mmol). The resulting suspension was mixed with Et_2O (15 ml) and cooled in an ice bath. The insoluble solid was collected by filtration, washed with Et_2O (3 ml), and recrystallized from 90% (v/v) aqueous MeOH to yield **6b** (246 mg, 51%), mp 263—265 °C (dec.). Further recrystallization of this sample provided an analytical sample of **6b** as colorless prisms, mp 267—270 °C (dec.) [lit.[3] mp 266—267 °C (dec.)]. *Anal.* Calcd for $C_8H_{11}N_5O \cdot HI$: C, 29.92; H, 3.77; N, 21.81. Found: C, 29.81; H, 3.81; N, 21.68. This sample was identical (by comparison of the UV, IR, and ^{1}H-NMR spectra and TLC mobility) with authentic **6b**.[3]

Caissarone (N^6,3,9-Trimethyl-8-oxoadenine) (7b) A solution of **6b** (250 mg, 0.779 mmol) in H_2O (15 ml) was passed through a column packed with Amberlite IRA-402 (HCO_3^-) (2 ml), and the column was eluted with H_2O (100 ml). The eluate was concentrated *in vacuo*, and the residue was recrystallized from MeOH to give **7b** (114 mg, 76%) as colorless prisms, mp 254—259 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **7b**.[3]

Hydrogenolysis of 4b Leading to 2b A solution of **4b** (46 mg, 0.16 mmol) in H_2O (17 ml) was shaken with Raney Ni W-2 catalyst[13] (0.1 ml) under H_2 at atmospheric pressure and 40 °C for 2.5 h. The catalyst was filtered off and washed with hot H_2O (5×10 ml). The filtrate and washings were combined and passed through a column of Amberlite IRA-410 (HCO_3^-) (1 ml), and the column was eluted with H_2O (30 ml). The eluate was concentrated *in vacuo* to afford **2b** (24 mg, 92%) as colorless needles, mp 277—286 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **2b**.[5]

8-Methoxy-9-methyladenine (9) A suspension of 8-bromo-9-methyladenine (**8**)[8] (228 mg, 1 mmol) in a 1 M solution (5 ml) of MeONa in MeOH was heated under reflux for 2 h, cooled to room temperature, and neutralized with 10% aqueous HCl. The precipitate that resulted was collected by filtration, washed with H_2O (2×1 ml), and dried to afford **9** (149 mg, 83%), mp 221—223 °C (dec.). Recrystallization of this sample from 5% aqueous NH_3 afforded an analytical sample as colorless plates, mp 230.5—231.5 °C (dec.); MS m/z: 179 (M^+); UV $\lambda_{max}^{95\%\ EtOH}$ 261 nm (ε 13800); $\lambda_{max}^{H_2O}$ (Table 1); IR ν_{max}^{Nujol} cm^{-1}: 3380, 3324 (NH), 1647 (C=N); ^{1}H-NMR δ: 3.45 and 4.10 (3H each, s, two Me's), 6.77 (2H, br, NH_2), 8.04 [1H, s, C(2)-H]. *Anal.* Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.09. Found: C, 46.72; H, 5.00; N, 38.95.

References and Notes

1) Paper LXXVII in this series, Itaya T., Kanai T., Shimada M., Nishikawa T., Takada Y., Hozumi Y., Mori S., Saito T., Fujii T., *Chem. Pharm. Bull.*, **45**, 1601—1607 (1997).
2) *a*) Zelnik R., Haraguchi M., Matida A. K., Lavie D., Frolow F., Weis A. L., *J. Chem. Soc., Perkin Trans. 1*, **1986**, 2051—2053; *b*) de Feritas J. C., Sawaya M. I., *Toxicon*, **24**, 751—755 (1986); *c*) *Idem, ibid.*, **28**, 1029—1037 (1990); *d*) Cooper R. A., de Feritas J. C., Porreca F., Eisenhour C. M., Lukas R., Huxtable R. J., *ibid.*, **33**, 1025—1031 (1995).
3) Saito T., Mori S., Chikazawa J., Kanai T., Fujii T., *Chem. Pharm. Bull.*, **41**, 1746—1752 (1993).
4) Itaya T., Takada Y., Kanai T., Kaneko M., Fujii T., *Chem. Pharm. Bull.*, **45**, 1582—1588 (1997).
5) Fujii T., Saito T., Muramoto T., *Chem. Pharm. Bull.*, **31**, 4270—4276 (1983).
6) Itaya T., Takada Y., Fujii T., *Chem. Pharm. Bull.*, **44**, 2025—2032 (1996).
7) The N^6-benzyl analogue of **5b** has been prepared from 8-hydroxy-3-methyl-6-methylsulfonylpurine by reaction with benzylamine: Diller D., Neiman Z., Bergmann F., *J. Chem. Soc.* (*C*), **1968**, 878—881.
8) Fujii T., Saito T., Mori S., *Chem. Pharm. Bull.*, **38**, 2146—2150 (1990).
9) Holmes R. E., Robins R. K., *J. Am. Chem. Soc.*, **87**, 1772—1776 (1965).
10) Robins R. K., *J. Am. Chem. Soc.*, **80**, 6671—6679 (1958).
11) Still W. C., Kahn M., Mitra A., *J. Org. Chem.*, **43**, 2923—2925 (1978).
12) Assigned by comparison of the ^{1}H-NMR spectrum with that of 7-methoxy-3-methyladenine perchlorate (**4a**).[6]
13) Mozingo R., "Organic Syntheses," Coll. Vol. III, ed. by Horning E. C., John Wiley and Sons, New York, 1955, pp. 181—183.