Purines. LXVIII.¹⁾ Trifluoroperoxyacetic Acid Oxidation of *N*⁶-Benzyladenine: Formation of the *N*(3)-Oxide and *N*(7)-Oxide, and Their Cytokinin Activities

Tozo FUJII,^{*,a} Yasutaka TAKADA,^a Kazuo OGAWA,^a Taisuke ITAYA,^a and Satoshi MATSUBARA^b

Faculty of Pharmaceutical Sciences, Kanazawa University,^a Takara-machi, Kanazawa 920, Japan and Laboratory of Applied Biology, Kyoto Prefectural University,^b Shimogamo Hangi-cho, Sakyo-ku, Kyoto 606, Japan.

Received September 7, 1994; accepted October 25, 1994

Treatment of *N*⁶-benzyladenine (**2**) with 15% aqueous H₂O₂ in trifluoroacetic acid at 65–70°C for 1 h was found to give the *N*(3)-oxide (**3**) and the *N*(7)-oxide (**4**) in 4% and 4% yields, respectively. The structure of **3** was established by its identity with a sample prepared from 6-chloropurine 3-oxide (**6**) and benzylamine, and the structure of **4** by its identity with a sample obtained from 1-benzyladenine 7-oxide (**8**) by Dimroth rearrangement. The *N*-oxides **3** and **4**, together with previously reported *N*⁶-benzyladenine 1-oxide (**1**), were tested for cytokinin activity in the tobacco callus bioassay. Each of the three *N*-oxides was active at 4 μM concentration, being less active than the parent base **2** by a factor of 40.

Key words *N*⁶-benzyladenine; trifluoroperoxyacetic acid oxidation; *N*(7)-oxide; *N*(3)-oxide; Dimroth rearrangement; 1-benzyladenine 7-oxide

Direct *N*-oxidation of *N*⁶-benzyladenine (6-benzylaminopurine) (**2**), a typical potent synthetic cytokinin,²⁾ was first reported by Nasyr *et al.*³⁾ in 1982. On treatment of **2** with 15% aqueous H₂O₂ in trifluoroacetic acid (TFA) at 65–70°C for 1 h or at 20°C for 17 h, they obtained an *N*-oxide [mp 222–224°C (dec.) (from EtOH)] in 33% yield.³⁾ The *N*-oxide was found to exhibit a cytokinin activity similar to that of **2** or kinetin (*N*⁶-furfuryl-adenine),^{3,4)} but its chemical and spectroscopic characterization was insufficient to specify the location of the *N*-oxide function. On the other hand, we recently found that the main product obtained from the *m*-chloroperoxybenzoic acid (MCPBA) oxidation of **2** in MeOH (30°C, 20 h) was the *N*(1)-oxide (**1**) (mp 201–202°C).⁵⁾ The difference in melting point between both *N*-oxides therefore suggested that the one obtained by the Russian research group³⁾ might be a positional isomer rather than the *N*(1)-oxide **1**. This led us to re-examine the Russians' results in the present work.

Thus, oxidation of **2** with 15% aqueous H₂O₂ in TFA at 65–70°C for 1 h and work-up of the reaction mixture were carefully carried out according to the literature.³⁾ Purification of the resulting product fraction by flash chromatography [silica gel, CHCl₃–MeOH (4:1, v/v)] and subsequent trituration with MeOH afforded a hardly separable 52:48 mixture of the *N*(3)-oxide **3** and the *N*(7)-oxide **4** in 14% yield. Trituration of the mixture with CHCl₃–MeOH (1:2, v/v) and recrystallization of the insoluble component from 50% (v/v) aqueous EtOH furnished **3** in 4% yield. The other component, soluble in these solvents, was isolated in the form of the hydrochloride salt and the salt gave, after neutralization, the free base **4** in 4% yield (from **2**).

The structure of **3** was determined by its identity with a sample synthesized, according to the literature procedure, from 6-chloropurine (**5**) by MCPBA oxidation to form the *N*(3)-oxide **6**⁶⁾ and subsequent amination with benzylamine.⁷⁾ The structure of **4**⁸⁾ was established

Chart 1

* To whom correspondence should be addressed.

TABLE I. Cytokinin Activity of *N*⁶-Benzyladenine (**2**) and Its *N*-Oxides in the Tobacco Callus Bioassay

Compound	Average fresh weight of tobacco callus (mg)									
	Concentration of test compound (μM)									
	0	0.001	0.004	0.01	0.04	0.1	0.4	1	4	10
<i>N</i> ⁶ -Benzyladenine 1-oxide (1)	27	—	—	28	24	31	252	1165	1651	723
<i>N</i> ⁶ -Benzyladenine 3-oxide (3)	24	—	—	23	33	33	195	1195	1540	584
<i>N</i> ⁶ -Benzyladenine 7-oxide (4)	22	—	—	28	84	208	952	1214	1559	478
<i>N</i> ⁶ -Benzyladenine (2)	23	23	75	157	880	1636	1388	—	—	—

by its identity with a sample prepared in 77% yield by means of the Dimroth rearrangement^{9,10} (0.1 N aqueous NaOH, reflux, 3 h) of 1-benzyladenine 7-oxide (**8**), which was obtainable¹¹ from 1-benzyladenine (**7**) by MCPBA oxidation.

Interestingly, the above trifluoroperoxyacetic acid oxidation of **2** has thus been proved to produce the *N*(3)-oxide **3** as well as the *N*(7)-oxide **4**, and we were unable to isolate the expected *N*(1)-oxide **1** from the reaction mixture (if it were present in any quantity). This offers a sharp contrast with the case of our previous MCPBA oxidation of **2** in MeOH, where the *N*(1)-oxide **1** was the main product.⁵ At present, however, the factor(s) influencing regioselectivity in the *N*-oxidation of **2** remain(s) uncertain.

Finally, the three *N*-oxides (**1**, **3**, and **4**) of *N*⁶-benzyladenine (**2**) were tested for cytokinin activity in the tobacco callus bioassay. It may be seen from Table I that the maximal yield of the callus was obtained with **1**, **3**, and **4** at 4 μM, whereas the maximal yield was obtained with the parent base **2** at 0.1 μM. This indicates that the introduction of an *N*-oxide function into the typical synthetic cytokinin **2** at the 1-, 3-, or 7-position lowers the cytokinin activity by a factor of 40.

In conclusion, the above results reveal that the trifluoroperoxyacetic acid oxidation of *N*⁶-benzyladenine (**2**) produces both the *N*(3)-oxide **3** and the *N*(7)-oxide **4**, but only in poor yields. In view of this fact and the weakened cytokinin activities (relative to **2**) of **3** and **4**, we consider that the sample (with a strong cytokinin activity) obtained from **2** by the Russian research group³ according to the same oxidation procedure was probably impure; there is a fair possibility that it was a mixture of small amounts of **3** and **4** contaminated with a large amount of the starting cytokinin **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 are corrected. Chromatography and measurements of spectra were carried out as described previously.¹¹ Elemental analyses and MS measurements were performed by Mr. Y. Itatani and his associates at Kanazawa University. The following abbreviations are used: br = broad, d = doublet, m = multiplet, s = singlet, sh = shoulder, t = triplet.

Oxidation of *N*⁶-Benzyladenine (2**) Leading to *N*⁶-Benzyladenine 3-Oxide (**3**) and *N*⁶-Benzyladenine 7-Oxide (**4**)** A solution of **2** (2.25 g, 9.99 mmol) in trifluoroacetic acid (TFA) (22.6 ml) was stirred at room temperature, and 15% aqueous H₂O₂ (11.3 ml) was added dropwise over a period of 15 min. The resulting mixture was stirred at 65–70 °C for 1 h and then concentrated *in vacuo* to a volume of ca. 10 ml. The residual orange solution was poured onto ice (60 g), and the aqueous mixture was triturated. The insoluble solid that resulted was collected

by filtration, washed with H₂O (10 ml), and triturated with 5% aqueous Na₂CO₃ (25 ml). The insoluble crystals were removed from the alkaline aqueous mixture by filtration, washed with H₂O (10 ml), and recrystallized from 50% (v/v) aqueous EtOH to recover **2** (209 mg, 9%), mp 227.5–229.5 °C (dec.). This sample was identical (by comparison of the IR spectrum and TLC mobility) with authentic **2**. The alkaline filtrate and aqueous washings were combined, brought to pH 5 by addition of 10% aqueous HCl, and cooled in a refrigerator overnight. The pale yellow solid (679 mg) that deposited was filtered off, dried, and subjected to flash chromatography¹² [silica gel, CHCl₃–MeOH (4:1, v/v)] to give a pale yellow solid (506 mg), which was triturated with MeOH. The resulting insoluble solid was filtered off and dried to furnish a 52:48 mixture¹³ (358 mg) of **3** and **4** as a pale yellowish solid. The mixture was triturated with a mixture of MeOH (10 ml) and CHCl₃ (5 ml), and the resulting insoluble solid was filtered off, washed with MeOH (3 ml), and recrystallized three times from 50% (v/v) aqueous EtOH (15 ml) to afford **3** (96 mg, 4%) as colorless needles, mp 224–225 °C (dec.). Recrystallization from H₂O yielded an analytical sample of **3** as colorless needles, mp 224–225 °C (dec.); p*K*_a (in H₂O at 30 °C and ionic strength 1.0): 2.86 ± 0.03 (basic), 7.27 ± 0.05 (acidic); MS *m/z*: 241 (M⁺), 225 (M⁺ – 16); UV λ_{max}^{95% aq. EtOH} 229 nm (sh) (ε 14700), 304 (16100); λ_{H₂O}^{max} (pH 1) 288 (21700); λ_{H₂O}^{max} (pH 7) 231 (17500), 300 (17800); λ_{H₂O}^{max} (pH 13) 233 (21000), 298 (16800); ¹H-NMR (Me₂SO-*d*₆) δ: 4.76 (2H, br, NHCH₂Ph), 7.15–7.41 (5H, m, NHCH₂Ph), 8.11 (1H, dull s) and 8.47 (1H, s) (purine protons), 8.57 (1H, br t, NHCH₂Ph). *Anal.* Calcd for C₁₂H₁₁N₅O: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.71; H, 4.60; N, 28.88. This sample was identical (by comparison of the mass, UV, IR, and ¹H-NMR spectra and TLC mobility) with the one synthesized from **6** and benzylamine (*vide infra*).

The filtrate and washings, obtained when the above 52:48 mixture of **3** and **4** was triturated with MeOH–CHCl₃, and the mother liquors of recrystallizations of the crude **3** were combined and concentrated *in vacuo*. The residue was dissolved in hot EtOH (20 ml). The ethanolic solution was cooled to room temperature, and 10% ethanolic HCl (1 g) and ether (5 ml) were added in that order. The colorless needles that deposited were collected by filtration, washed with EtOH (3 ml), and then dissolved in H₂O (10 ml). The aqueous solution was brought to pH ca. 7, and the colorless needles that deposited were filtered off, washed with H₂O (3 ml), and dried to give **4** (85 mg, 4%), mp 197–198 °C (dec.). Recrystallization from MeOH furnished an analytical sample of **4** as colorless needles, mp 198–200 °C (dec.); p*K*_a (in H₂O at 30 °C and ionic strength 1.0): 3.07 ± 0.05 (basic), 5.80 ± 0.05 (acidic). *Anal.* Calcd for C₁₂H₁₁N₅O: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.83; H, 4.66; N, 28.77. This sample was identical (by comparison of the UV, IR, and ¹H-NMR spectra and TLC mobility) with the one prepared by the Dimroth rearrangement of **8** (*vide infra*).

6-Chloropurine 3-Oxide (6**)** 6-Chloropurine (**5**) (2.00 g, 12.9 mmol) was treated with MCPBA (of ca. 70% purity) (12.7 g, 51.5 mmol) in ether (150 ml) according to the literature procedure,⁶ giving **6** (1.62 g, 73%) as colorless needles, mp 156–159 °C (dec.); MS *m/z*: 170, 172 (M⁺); UV λ_{max}^{95% aq. EtOH} 234 nm (ε 24500), 311 (8600); λ_{H₂O}^{max} (pH 1) 229 (21800), 300 (9000); λ_{H₂O}^{max} (pH 7) 230 (28100), 303 (7800); λ_{H₂O}^{max} (pH 13) 230 (28600), 303 (7800); ¹H-NMR (Me₂SO-*d*₆) δ: 7.20 (br, NH), 8.33 (1H, dull s) and 8.66 (1H, s) (purine protons). The UV spectra of this sample at pH 1 and 13 were similar to those reported¹⁴ for the NH₃ salt of **6**.

Conversion of **6 into *N*⁶-Benzyladenine 3-Oxide (**3**)** For the preparation of **3**, the literature procedure⁷ was slightly modified. A stirred mixture of **6** (100 mg, 0.586 mmol) and 50% aqueous benzylamine (5 ml) was heated at 100 °C for 2 h. After cooling, the reaction mixture was combined with 2 N aqueous NH₃ (30 ml) and extracted with ether

(10 × 10 ml). The aqueous layer was concentrated *in vacuo* to leave a solid, which was dissolved in hot 50% (v/v) aqueous EtOH (20 ml) after the pH of the mixture had been adjusted to *ca.* 4 by addition of a few drops of AcOH. The resulting solution was cooled in a refrigerator overnight, and the colorless needles that deposited were filtered off, washed with EtOH (1 ml), and dried to yield a first crop (102 mg, 72%) of **3**, mp 225–226 °C (dec.). The filtrate and washings were combined and concentrated *in vacuo* to leave a colorless solid, which was recrystallized from 50% (v/v) aqueous EtOH to give a second crop (5 mg, 4%) of **3**, mp 218–219 °C (dec.). The total yield of **3** was 107 mg (76%). For analysis, the crude **3** was recrystallized from 50% (v/v) aqueous EtOH to afford a pure sample as colorless needles, mp 225–226 °C (dec.) [lit.⁷⁾ mp 224–225 °C (dec.)]; MS *m/z*: 241 (M^+), 225 ($M^+ - 16$); UV $\lambda_{\max}^{95\% \text{ aq. EtOH}}$ 232 nm (sh) (ϵ 14000), 305 (16400); $\lambda_{\max}^{H_2O}$ (pH 1) 288 (22700); $\lambda_{\max}^{H_2O}$ (pH 7) 232 (17400), 300 (18200); $\lambda_{\max}^{H_2O}$ (pH 13) 233 (21600), 298 (17200); ¹H-NMR (Me_2SO-d_6) δ : 4.76 (2H, br, $NHCH_2Ph$), 7.19–7.40 (5H, m, $NHCH_2Ph$), 8.12 (1H, dull s) and 8.47 (1H, s) (purine protons), 8.59 (1H, br t, $NHCH_2Ph$). *Anal.* Calcd for $C_{12}H_{11}N_5O$: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.99; H, 4.65; N, 29.01.

Conversion of 1-Benzyladenine 7-Oxide (8) into N⁶-Benzyladenine 7-Oxide (4) A stirred solution of **8** · H_2O ¹⁾ (200 mg, 0.771 mmol) in 0.1 N aqueous NaOH (10 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was brought to pH 4 with 10% aqueous HCl under ice-cooling. The colorless crystals that deposited were collected by filtration, washed with MeOH (1 ml), and dried to afford **4** (144 mg, 77%), mp 195.5–196.5 °C (dec.). Recrystallization from MeOH gave a pure sample of **4** as colorless needles, mp 199–201 °C (dec.); MS *m/z*: 241 (M^+), 225 ($M^+ - 16$); UV $\lambda_{\max}^{95\% \text{ aq. EtOH}}$ 280 nm (ϵ 13800); $\lambda_{\max}^{H_2O}$ (pH 1) 285 (18700); $\lambda_{\max}^{H_2O}$ (pH 7) 244 (12900), 288 (9400); $\lambda_{\max}^{H_2O}$ (pH 13) 244 (13100), 288 (9300); ¹H-NMR (Me_2SO-d_6) δ : 4.74 (2H, d, $J=6$ Hz, $NHCH_2Ph$), 7.17–7.43 (5H, m, $NHCH_2Ph$), 7.66 (1H, br, $NHCH_2Ph$), 8.23 and 8.37 (1H each, s, purine protons), 12.55 (br, NH). *Anal.* Calcd for $C_{12}H_{11}N_5O$: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.76; H, 4.70; N, 28.81. This sample was identical (by comparison of the UV, IR, and ¹H-NMR spectra and TLC mobility) with the one obtained by the N-oxidation of **2** (*vide supra*).

N⁶-Benzyladenine Hydrochloride (2 · HCl) N⁶-Benzyladenine (**2**) (302 mg, 1.34 mmol) was dissolved in hot EtOH (12 ml), and 10% ethanolic HCl (760 mg, 2.08 mmol) was added. The resulting mixture was cooled in an ice bath, and the colorless needles that deposited were collected by filtration, washed with EtOH (5 ml), and dried to give **2** · HCl (270 mg, 77%), mp 218–219 °C (dec.). Recrystallization from EtOH yielded an analytical sample as colorless needles, mp 218–219 °C (dec.); pK_a (in H_2O at 30 °C and ionic strength 1.0): 4.09 ± 0.03 (basic), 10.02 ± 0.04 (acidic); UV $\lambda_{\max}^{95\% \text{ aq. EtOH}}$ 270 nm (ϵ 19600); $\lambda_{\max}^{H_2O}$ (pH 1) 275 (18700); $\lambda_{\max}^{H_2O}$ (pH 7) 269 (19400); $\lambda_{\max}^{H_2O}$ (pH 13) 275 (18900); ¹H-NMR (Me_2SO-d_6) δ : 4.88 (2H, br, $NHCH_2Ph$), 7.21–7.50 (5H, m, $NHCH_2Ph$), 8.59 and 8.64 (1H each, s, purine protons), 10.14 (1H, br, NH). *Anal.* Calcd for $C_{12}H_{11}N_5 \cdot HCl$: C, 55.07; H, 4.62; N, 26.76. Found: C, 55.27; H, 4.64; N, 26.71.

Spectrometric Determination of Acid Dissociation Constants of 2, 3, and 4 The pK_a values in H_2O at 30 °C and ionic strength 1.0 were determined in a manner similar to that described previously,¹⁵⁾ by using samples of **2** · HCl, **3**, and **4**. The results are described under the names of the individual compounds (*vide supra*).

Bioassay Procedure The cytokinin activities of **1**,⁵⁾ **2**, **3**, and **4** were tested in the tobacco callus bioassay in a manner similar to that¹⁶⁾ described previously. However, the following slight modification was

made for the preparation of aqueous solutions of the test compounds because of their low solubilities in H_2O . A solution of **2** (0.01 mmol) in Me_2SO (0.5 ml) was diluted with H_2O to a volume of 100 ml, and solutions of **1**, **3**, and **4** (0.01 mmol each) in EtOH–1 N aqueous NaOH– H_2O (16:1:8, v/v) (0.5 ml) were first diluted with H_2O (*ca.* 90 ml) and then further diluted with H_2O to a volume of 100 ml after adjusting the pH of the solutions to 5.6. These solutions were filter-sterilized, diluted with H_2O to appropriate concentrations, and then added to autoclaved basal culture media. Table I summarizes the results.

Acknowledgment This work was supported by a Grant-in-Aid for Scientific Research (C) (No. 06672093) from the Ministry of Education, Science and Culture, Japan. We are grateful to Drs. E. V. Grishin (Russian Academy of Sciences, Moscow), G. B. Elyakov (Russian Academy of Sciences, Vladivostok), and Marek Pietraszkiewicz (Polish Academy of Sciences, Warsaw) for translation of the Russian literature into English. Our thanks are also due to Messrs. Kado Ikeda, Katsuhiro Ikeda, and Fujio Nohara (Ikeda Mohando Co.) for their interest and encouragement.

References and Notes

- 1) Paper LXVII in this series, T. Fujii, K. Ogawa, T. Saito, T. Itaya, T. Date, K. Okamura, *Chem. Pharm. Bull.*, **43**, 321 (1995).
- 2) For reviews on cytokinins, see a) K. Koshimizu, H. Iwamura, *Nippon Nogeikagaku Kaishi*, **52**, R49 (1978); b) S. Matsubara, *Phytochemistry*, **19**, 2239 (1980); c) D. S. Letham, L. M. S. Palni, *Annu. Rev. Plant Physiol.*, **34**, 163 (1983); d) S. Matsubara, *Crit. Rev. Plant Sci.*, **9**, 17 (1990).
- 3) I. A. Nasyr, G. P. Mozgovaya, Yu. V. Karabanov, I. V. Boldyrev, V. P. Borisenko, V. M. Cherkasov, *Fiziol. Akt. Veshchestva*, **14**, 91 (1982) [*Chem. Abstr.*, **99**, 48877n (1983)].
- 4) G. P. Mozgovaya, V. S. Petrenko, I. V. Boldyrev, V. M. Cherkasov, *Fiziol. Akt. Veshchestva*, **16**, 57 (1984) [*Chem. Abstr.*, **103**, 2068x (1985)].
- 5) T. Fujii, K. Ogawa, T. Itaya, *Heterocycles*, **37**, 219 (1994).
- 6) A. Giner-Sorolla, *J. Heterocycl. Chem.*, **8**, 651 (1971).
- 7) H. Kawashima, I. Kumashiro, Japan. Patent 7028783 (1970) [*Chem. Abstr.*, **74**, P42375j (1971)].
- 8) Until the tautomeric structure is established, we prefer to use the N(7)-oxide structure for convenience.
- 9) For reviews, see a) D. J. Brown, "Mechanisms of Molecular Migrations," Vol. 1, ed. by B. S. Thyagarajan, Interscience Publishers, New York, 1968, pp. 209–245; b) J. H. Lister, "Fused Pyrimidines. Part II. Purines," ed. by D. J. Brown, Wiley-Interscience, New York, 1971, pp. 313–315; c) T. Fujii, T. Itaya, T. Saito, *Yuki Gosei Kagaku Kyokai Shi*, **41**, 1193 (1983).
- 10) a) T. Fujii, T. Saito, H. Hisata, K. Shinbo, *Chem. Pharm. Bull.*, **38**, 3326 (1990), and references cited therein; b) T. Fujii, T. Saito, R. Ii, T. Suzuki, *ibid.*, **42**, 382 (1994).
- 11) T. Fujii, K. Ogawa, T. Saito, K. Kobayashi, T. Itaya, T. Date, K. Okamura, *Chem. Pharm. Bull.*, **43**, 53 (1995).
- 12) W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).
- 13) Determined by the usual UV spectrophotometric analysis.
- 14) H. Kawashima, I. Kumashiro, *Bull. Chem. Soc. Jpn.*, **42**, 750 (1969).
- 15) T. Fujii, T. Itaya, T. Saito, *Chem. Pharm. Bull.*, **23**, 54 (1975).
- 16) T. Fujii, T. Itaya, S. Matsubara, *Chem. Pharm. Bull.*, **37**, 1758 (1989).