by DHA. Nevertheless, on treatment of GSH with DHA, no decrease of SH contents was observed, and on treatment of papain (non-activated) with DHA, the increase of SH contents took place, as compared with control. From this results, it may be thought that inhibitory effect of ALX depends on the nature of polycarbonyl compound to react with SH group, but that the polycarbonyl group in DHA does not react with SH group.

DHA and AA for papain activated by cyanide was non-competitive inhibitors, and the mode of the inhibitory effects of DHA and AA was diverse from that of ALX (competitive inhibitor[12] for papain activated by cyanide). And non-activated papain had 0.18 mole of SH group per mole of papain and cyanide-activated papain had 0.52 mole of SH group (Table II). Morihara reffered it in his studies[13] that the active site is composed of both SH and aldehyde groups which act on each other (thiohemiacetal) in an equilibrium state and that the activators combine with the active site (thiohemiacetal) to convert it into an activated form. But the presence of the aldehyde group in papain has not been directly identified as yet. Recently, Klein, *et at.* also refered it in their studies[14] that the inactive form of papain prepared by the method of Kimmel and Smith[7] is that containing a mixed disulfide between the SH group at the active site of papain and free cysteine. The results in this paper and in the previous paper[8] support the idea that the active site is composed both SH and a certain groups which act on each other in an equilibrium state. A certain group in papain is now under investigation.

Acknowledgement The authors express their gratitude to the hononary Prof. Masashichi Yoshioka of Kyoto College of Pharmacy for his helpful suggestions throughout this study.

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[Chem. Pharm. Bull. 21(8)1835—1838(1973)] UDC 547.857.04 : 542.941

Purines. XII.[1] Catalytic Hydrogenolysis of Alkoxyaminopurines and Related Derivatives

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(Received December 13, 1972)

In the course of our synthesis of N-alkoxyadenosines (type Ib,d)[1] and their 9-alkyl analogues (type Ia,c),[1,3] we became interested in studying the selective hydrogenolytic cleavage of the N-benzyloxy group at the C–O bond from which an alternative synthetic route to N-hydroxyadenosine (IIIb), an antileukemic substance[4] synthesized first by Giner-

1) Paper XI in this series, T. Fujii, C.C. Wu, T. Itaya, S. Moro, and T. Saito, *Chem. Pharm. Bull.* (Tokyo), **21**, 1676 (1973).
2) Location: *13-1 Takara-machi, Kanazawa, 920, Japan*; *a*) To whom correspondence should be addressed at Kanazawa University; *b*) *3-1 Hongo-7-chome, Bunkyo-ku, Tokyo, 113, Japan.*
3) *a*) T. Fujii, T. Itaya, C.C. Wu, and S. Yamada, *Chem. Ind.* (London), **1966**, 1967; *b*) T. Fujii, T. Itaya, C.C. Wu, and F. Tanaka, *Tetrahedron*, **27**, 2415 (1971).
4) J.H. Burchenal, M. Dollinger, J. Butterbaugh, D. Stoll, and A. Giner-Sorolla, *Biochem. Pharmacol.*, **16**, 423 (1967).

Sorolla, *et al.*[5] from 6-chloro-9-β-D-ribofuranosylpurine (IVb), was expected. This paper describes the details of our investigation on the catalytic hydrogenolysis of 9-substituted N-alkoxyadenine derivatives (Ia—d) and the related derivative (V).

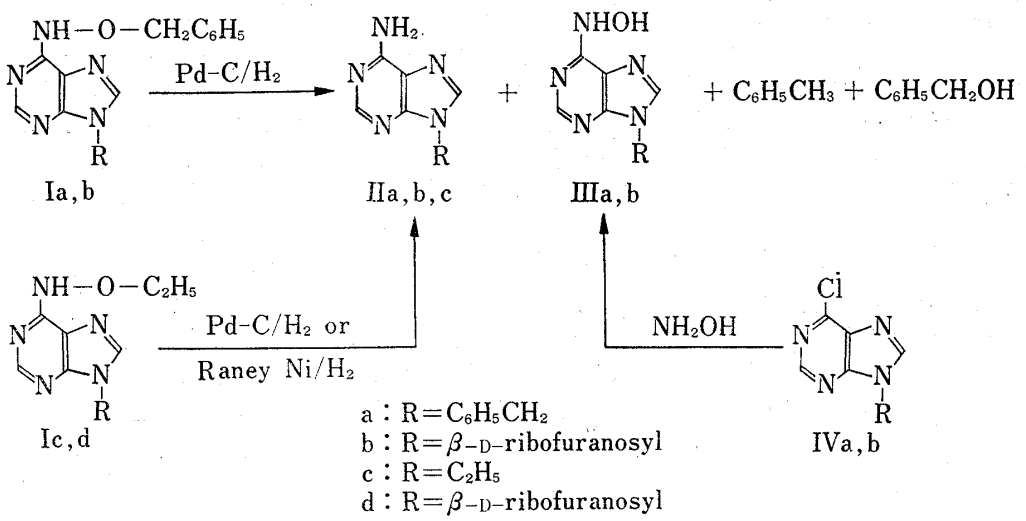

Chart 1

In a model experiment, N-benzyloxy-9-benzyladenine (Ia)[3b] was hydrogenated in ethanol over palladium-on-charcoal (Pd-C) at atmospheric pressure and room temperature. Instead of the expected debenzylation product (IIIa), 9-benzyladenine (IIa) was obtained as the main product (91% yield). For the formation of IIa from Ia, two modes of pathways may warrant consideration: (a) direct hydrogenolytic cleavage of the N—O bond; (b) debenzylation of Ia to give IIIa, followed by hydrogenolysis of the generated IIIa at the N—O bond. Evidence for that such two mechanisms are competitively operative was provided by the following facts: (a) during the course of the hydrogenation, both IIa and IIIa were simultaneously detected by means of paper chromatography (PPC). (An authentic sample of IIIa was prepared by condensing 6-chloro-9-benzylpurine (IVa)[6] with hydroxylamine); (b) benzyl alcohol could be isolated from the reaction mixture and was identified by converting it into benzyl N-phenylcarbamate; (c) the analogous N—O bond of IIIb suffers hydrogenolysis under similar reaction conditions.[5] The lack of selectivity in the hydrogenolysis of the N-benzyloxy group described above presents a striking contrast to the almost complete selectivity of the debenzylation of 1-benzyloxy-9-benzyladenine leading to 9-benzyladenine 1-oxide, which has been found to proceed very rapidly.[7]

Likewise, the hydrogenolysis of N-benzyloxyadenosine (Ib)[1] using Pd-C and hydrogen yielded adenosine (IIb) (88%) along with isolable amounts of benzyl alcohol and toluene. The N-hydroxy derivative (IIIb) was also detectable in an incomplete reaction mixture. Replacement of the palladium catalyst by Raney nickel in this hydrogenation caused only the N—O bond to split off.

In order to compare the capability of Pd-C in cleaving the N—O bond with that of Raney nickel, both catalysts were separately tested in the hydrogenation of N-ethoxy-9-ethyladenine (Ic)[3] at atmospheric pressure and 15° for 7 hr. In either case, the reaction took place smoothly to produce 9-ethyladenine (IIc) in 97% yield. Similar treatment of N-ethoxyadenosine (Id)[1] with hydrogen and Raney nickel furnished IIb in a good yield. Next, in the hope of finding a possible route to IIIb, N'-benzyloxy-1-β-D-ribofuranosyl-5-formamidoimidazole-4-

5) A. Giner-Sorolla, L. Medrek, and A. Bendich, *J. Med. Chem.*, **9**, 143 (1966).
6) J.A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).
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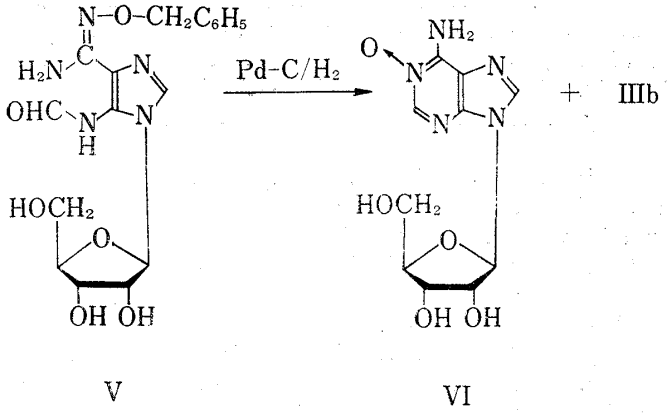

V VI

Chart 2

carboxamidine (V)[1] was hydrogenated in ethanol with Pd-C. However, the main product was found to be adenosine 1-oxide (VI)[8] (53% yield), wihch probably resulted from ring-closure of the debenzylated monocyclic derivative once formed, in general agreement with the recently reported results.[9] Concomitant formation of a small amount of the desired product (IIIb) was indicated by PPC and thin-layer chromatography (TLC) of another fraction of the reaction mixture.

The findings described above suggest that the ease with which the hydrogenolytic fission of the C—O bond of the N-benzyloxy group occurs may depend upon the nature of the nitrogen atom.

Experimental[10]

N-Hydroxy-9-benzyladenine (IIIa)——Hydroxylamine hydrochloride (2.40 g, 34.5 mmoles) was dissolved in boiling ethanol (40 ml), and a solution of KOH (85% purity, 2.24 g, 33.9 mmoles) in hot ethanol (8 ml) was added. The precipitates (KCl) that resulted were removed by filtration to give an ethanolic solution of NH_2OH. To a portion (35 ml) of the ethanolic solution was added 6-chloro-9-benzylpurine (IVa)[7] (240 mg, 0.981 mmole) and the mixture was stirred at 60° for 3 hr. The colorless needles that separated out were filtered off and dried to furnish IIIa (190 mg, 80%), mp 218° (decomp.). Recrystallization from methanol yielded an analytical sample as colorless needles, mp 219° (decomp.); UV $\lambda_{max}^{95\%\ aq.\ ethanol}$ 268 nm (ε 12800); $\lambda_{max}^{H_2O}$ (pH 1)[11] 266 (16900); $\lambda_{max}^{H_2O}$ (pH 7)[12] 267 (13000); $\lambda_{max}^{H_2O}$ (pH 13)[13] 257 (8500), 306 (6100). *Anal.* Calcd. for $C_{12}H_{11}ON_5$: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.89; H, 4.61; N, 29.31.

Hydrogenolysis of N-Benzyloxy-9-benzyladenine (Ia)——A solution of Ia[3b] (500 mg, 1.51 mmoles) in 95% (v/v) aq. ethanol (220 ml) was hydrogenated over 10% Pd-C (500 mg) at atmospheric pressure and room temp.; one equivalent mole of H_2 was taken up within 5.5 hr. The catalyst was removed by filtration and the filtrate was distilled at ordinary pressure to yield a small volume of fore-run. The ultraviolet (UV) spectrum of the fore-run indicated the presence of toluene. The residual main solution was then evaporated *in vacuo* to dryness. Trituration of the resulting residue with ether (50 ml) and filtration of an insoluble solid gave IIa (310 mg, 91%) as almost colorless needles, mp 233—234°, identical (by mixed melting-point test, TLC, and infrared (IR) spectrum) with an authentic sample.[7] The ethereal filtrate was washed successively with 10% aq. HCl, saturated aq. $NaHCO_3$, and H_2O, dried, and evaporated to leave a brown oil (70 mg, 43%) whose IR spectrum was virtually superimposable on that of authentic benzyl alcohol. For further identification, the oil was allowed to react with phenyl isocyanate in the usual way, yielding benzyl N-phenylcarbamate as colorless needles, mp 76—77°, identical (by mixed melting-point test and IR spectrum) to an authentic specimen.

In a separate hydrogenation experiment, small samples were withdrawn at intervals from the reaction mixture. TLC [Merck silica gel GF_{254}, chloroform–ethanol (8: 1, v/v)] of the sample solutions revealed that the starting material (Ia), IIa, and IIIa were coexistent in the reaction mixture before completion of the hydrogenolysis.

Hydrogenolysis of N-Benzyloxyadenosine (Ib)——A solution of Ib·H_2O[1] (860 mg, 2.2 mmoles) in 50% (v/v) aq. ethanol (120 ml) was hydrogenated over 10% Pd-C (860 mg) at atmospheric pressure and room

8) *a*) M.A. Stevens, D.I. Magrath, H.W. Smith, and G.B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958); *b*) T. Fujii, C.C. Wu, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1368 (1971).
9) J.A. Montgomery and H.J. Thomas, *J. Med. Chem.*, **15**, 182 (1972).
10) All melting points are corrected. Paper chromatographies were developed as described previously.[9b] We are grateful to Dr. E. Kimura and his associates at University of Tokyo for microanalyses.
11) Determined in 0.1N aq. HCl.
12) Determined in 0.005M phosphate buffer.
13) Measured in 0.1N aq. NaOH. The ε recorded may not be accurate because of the instability of this compound in alkali.

temp. for 6 hr, taking up approximately one equivalent mole of H_2. Work-up as described above for Ia resulted in separation of three fractions: (A) an ethanolic fore-run containing toluene; (B) an ether-insoluble solid (IIb); (C) a brown oil (100 mg, 42%) (benzyl alcohol) as a neutral component. The solid of fraction B was recrystallized from ethanol (30 ml) to give adenosine sesquihydrate (IIb·1.5 H_2O) (570 mg, 88%) as colorless needles, mp 233° (decomp.), identical (by PPC, TLC, and IR spectrum) with an authentic sample.

Coexistence of Ib, IIb, and IIIb in a reaction mixture obtained from incomplete hydrogenolysis was confirmed in a manner similar to that described above for Ia.

When Ib (10 mg) was hydrogenated in ethanol (2 ml) with Raney Ni W-2 catalyst (0.1 ml) at atmospheric pressure and room temp., smooth formation of IIb was recognized by means of chromatography. However, IIIb could not be detected.

Hydrogenolysis of N-Ethoxy-9-ethyladenine (Ic)——i) With Pd-C: A solution of Ic[4] (500 mg, 2.41 mmoles) in ethanol (40 ml) was hydrogenated over 10% Pd-C (500 mg) at atmospheric pressure and 15° for 7 hr. Hydrogen uptake was about one equivalent mole. Removal of the catalyst by filtration and evaporation of the filtrate gave a residue, which was dissolved in benzene (50 ml). The benzene solution was evaporated *in vacuo* to dryness to leave IIc (380 mg, 97%). Recrystallization from benzene produced colorless needles, mp 193°, identical (by mixed melting-point test and IR spectrum) with authentic IIc.[7]

ii) With Raney Ni: A solution of Ic[4] (500 mg, 2.41 mmoles) in ethanol (40 ml) was hydrogenated over Raney Ni W-2 catalyst (1 ml) under the same comditions as in method-(i). Yield of IIc was 97%.

Hydrogenolysis of N-Ethoxyadenosine (Id)——A solution of Id·0.5 H_2O[1] (500 mg, 1.56 mmoles) in 50% (v/v) aq. ethanol (60 ml) was hydrogenated over Raney Ni W-2 catalyst (1 ml) at atmospheric pressure and room temp. for 7 hr. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo* to dryness to leave colorless needles (360 mg, 78%), mp 233° (decomp.), which were identical (by mixed melting-point test and IR spectrum) with authentic adenosine sesquihydrate (IIb·1.5 H_2O).

Hydrogenolysis of N′-Benzyloxy-1-β-D-ribofuranosyl-5-formamidoimidazole-4-carboxamidine (V)——A solution of V[1] (391 mg, 1 mmole) in 90% (v/v) aq. ethanol (50 ml) was hydrogenated over 10% Pd-C (250 mg) at atmospheric pressure and room temperature for 4 hr, absorbing one equivalent mole of H_2. The catalyst was filtered off and washed with hot 90% (v/v) aq. ethanol (70 ml). The filtrate and washings were combined and concentrated to a volume of *ca.* 5 ml. The precipitates that resulted were collected by filtration, washed with 90% (v/v) aq. ethanol (5 ml), and dried to give VI·H_2O[9] (112 mg, 37%) as colorless needles, mp 224—225° (decomp.), shown to be identical with authentic adenosine 1-oxide monohydrate[9b] by means of IR spectrum. The filtrate and washings of VI·H_2O were combined and evaporated to dryness and the residue was subjected to column chromatography [silica gel (11 g), ethanol–ethyl acetate (1: 6, v/v)]. From the first UV-absorbing fraction was obtained a crystalline solid (86 mg), which was presumed, from its PPC and TLC, to be a mixture of IIIb and an unidentified substance. Recrystallization of the mixture from methanol, however, failed to give a pure sample of IIIb. Further elution of the column with the same solvent system as above afforded an additional amount (44 mg, 16%) of anhydrous VI, total yield 53%.

When treated with 10% Pd-C alone in 90% (v/v) aq. ethanol in the absence of H_2 under the same conditions as those used in the hydrogenolysis, V was found to be quite stable; neither cyclization to Ib nor cyclization to 1-benzyloxyadenosine was observed.

Acknowledgement Support of this work by a Grant-in-Aid for Cancer Research (92058, to Professor D. Mizuno) from the Ministry of Education, Japan, is gratefully acknowledged.