the interaction energy was calculated to be -45.9 kcal/mol. Since the interaction energy between NH_4^+ and SCN^- was -47.5 kcal/mol, the effects of these ether oxygens were very small. Even when the four carbonyl oxygens (O^{17}, O^{22}, O^{67} and O^{72}) were included, the effects were still very small. Accordingly, the interaction energy between SCN^- and the tetranactin-NH_4^+ complex is almost entirely due to the direct electrostatic interaction between NH_4^+ and SCN^-.

When NH_4^+ is inserted into tetranactin, the interaction energy between NH_4^+ and the tetranactin model was -109.0 kcal/mol.[4] Since the interaction energy between SCN^- and the tetranactin-NH_4^+ complex model was -49.4 kcal/mol in Table II, the interaction energy on ternary complex formation will be over -158.4 kcal/mol. Therefore the enthalpy of ternary complex formation in a nonpolar solvent will be over -158.4 kcal/mol.

Ternary complexes among tetranactin, NH_4^+ and an uncoupler such as picric acid or SF6847 have been reported.[10] The factors involved in the formation of these ternary complexes should be similar to those discussed here.

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[10] A. Yamaguchi, Y. Anraku, and S. Ikegami, *Biochim. Biophys. Acta*, **50**, 150 (1978).

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UDC 547.854.4.04 : 547.335.2.04

A New Synthesis of Pyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*)diones (5-Deazaalloxazines) by Oxidative Cyclization of Aryl-bis(6-amino-1,3-dimethyluracil-5-yl)-methanes with Diethyl Azodicarboxylate

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Treatment of aryl-bis(6-amino-1,3-dimethyluracil-5-yl)methanes, which were prepared by the condensation of 6-amino-1,3-dimethyluracil with aryl aldehydes, with diethyl azodicarboxylate in the presence of sulfolane led to the formation of the corresponding 1,3-dimethylpyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*)diones (1,3-dimethyl-5-deazaalloxazines).

Keywords——5-deazaalloxazine; pyrimido[4,5-*b*]quinoline; oxidative cyclization; diethyl azodicarboxylate; bis(6-amino-1,3-dimethyluracil-5-yl)methane

Diethyl azodicarboxylate (DAD) is a strong hydrogen acceptor and has been shown to be a useful reagent for the syntheses of various heterocycles.[2] The present paper describes a new synthesis of 1,3-dimethylpyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*)diones (1,3-dimethyl-5-deazaalloxazines) by the oxidative cyclization of aryl-bis(6-amino-1,3-dimethyluracil-5-yl)-methanes with DAD. Previously it was reported that the treatment of 6-anilino-1,3-dimethyluracils with dimethylformamide dimethylacetal afforded the corresponding 1,3-dimethylpyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*)diones (1,3-dimethyl-5-deazaalloxazines).[3] Although this

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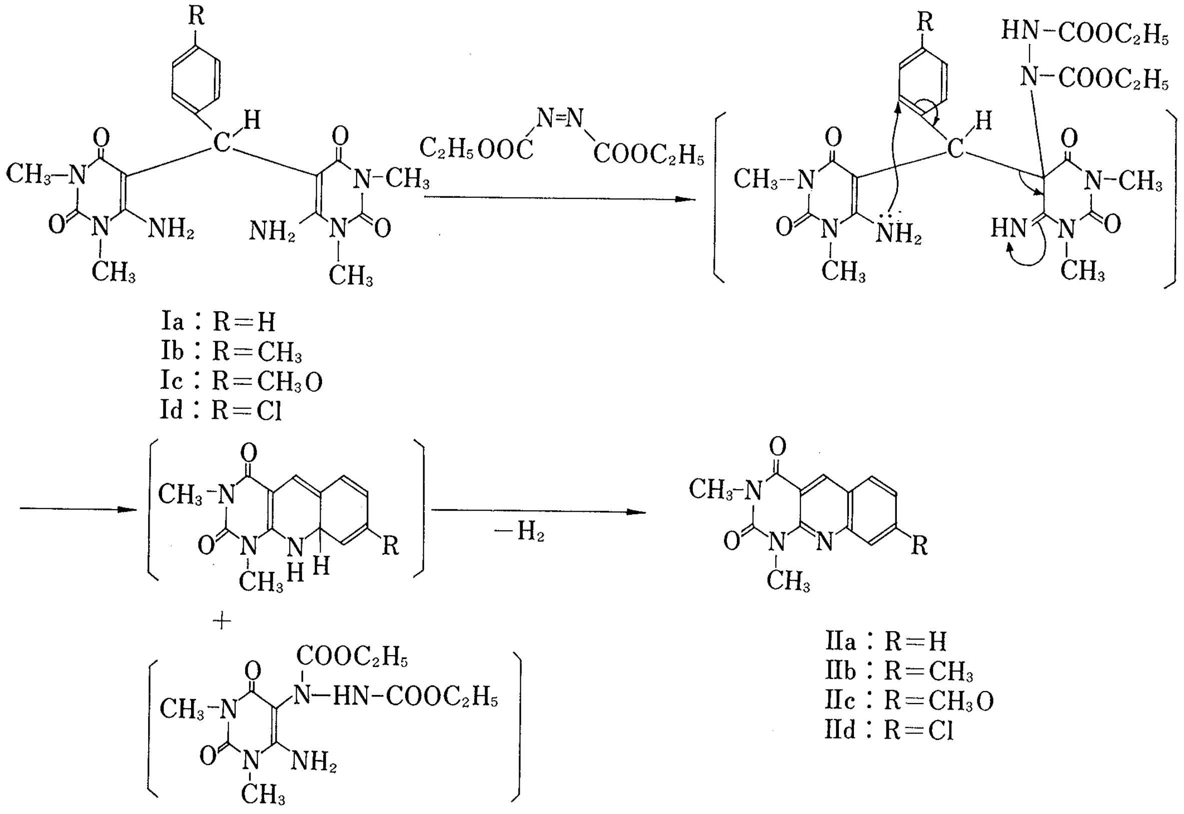
[1] Location: *a) Oe-honmachi, Kumamoto 862, Japan*; *b) Kogushi, Ube 755, Japan.*

[2] F. Yoneda, M. Higuchi, K. Mori, K. Senga, Y. Kanamori, K. Shimizu, and S. Nishigaki, *Chem. Pharm. Bull.* (Tokyo), **26**, 2905 (1978).

[3] K. Senga, K. Shimizu, S. Nishigaki, and F. Yoneda, *Heterocycles*, **6**, 1361 (1977).

is a convenient method, there is a possibility of obtaining a mixture of 8- and 6-substituted 5-deazaalloxazines in the case of the cyclization of 6-(*m*-substituted-anilino)uracils. However, the new route shows excellent selectivity in the synthesis of 8-substituted 5-deazaalloxazines and is applicable to a wide variety of aryl groups.



Ia : R=H
Ib : R=CH_3
Ic : R=CH_3O
Id : R=Cl

IIa : R=H
IIb : R=CH_3
IIc : R=CH_3O
IId : R=Cl

Chart 1

TABLE I. Aryl-bis(6-amino-1,3-dimethyluracil-5-yl)methanes

Compd. No.	R	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
Ia[3)]	H	288	75	$C_{19}H_{20}N_6O_4$	57.57	5.09	21.20	57.68	5.33	20.95
Ib	CH_3	277	83	$C_{20}H_{22}N_6O_4$	58.53	5.40	20.48	58.29	5.64	20.17
Ic	CH_3O	271	73	$C_{20}H_{22}N_6O_5$	56.33	5.20	19.71	56.78	5.37	19.42
Id	Cl	312	91	$C_{19}H_{19}ClN_6O_4$	52.96	4.45	19.51	52.99	4.55	19.20

TABLE II. 1,3-Dimethyl-5-deazaalloxazines

Compd. No.	R	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
IIa[2)]	H	212	84	$C_{13}H_{11}N_3O_2$	64.72	4.60	17.42	64.78	4.43	17.09
IIb	CH_3	224	95	$C_{14}H_{13}N_3O_2$	65.87	5.13	16.46	65.61	5.08	16.45
IIc	CH_3O	258	93	$C_{14}H_{13}N_3O_3$	61.98	4.83	15.49	62.29	4.90	15.20
IId	Cl	232	90	$C_{13}H_{10}ClN_3O_2$	56.63	3.66	15.24	56.86	3.37	15.08

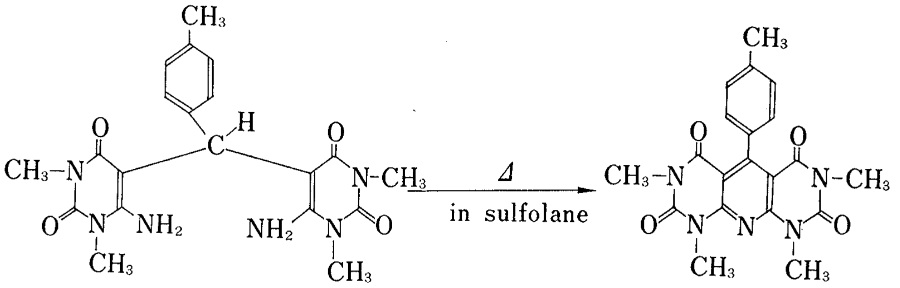
Heating of 6-amino-1,3-dimethyluracil with aryl aldehydes in ethanol or acetic acid gave the corresponding aryl-bis(6-amino-1,3-dimethyluracil-5-yl)methanes (Ia—d), as previously reported by Pfleiderer and co-workers.[4]

Heating of compound Ia with excess DAD in the presence of sulfolane[5] at 180° afforded 1,3-dimethyl-5-deazaalloxazine (IIa). The reaction is equally applicable to compounds (Ib—d) to give the corresponding 5-deazaalloxazines (IIb—d) (see Table II). The structures of (IIa—d) were confirmed by the analytical and spectral data, particularly by the presence of the characteristic 5-C proton signal in the NMR (Table III). Furthermore, compound IIa was identical with an authentic sample prepared by the known method.[3]

TABLE III. NMR Data for 1,3-Dimethyl-5-deazaalloxazines

Compd. No.	δ (CF_3COOH)						
	N_1-CH_3	N_3-CH_3	C_5-H	C_6-H	C_7-H	C_9-H	8-Substituent
IIa	4.08 (s, 3H)	3.69 (s, 3H)	9.80 (s, 1H)		7.83—8.53 (m, 4H, Ar)		—
IIb	4.10 (s, 3H)	3.72 (s, 3H)	9.74 (s, 1H)	8.28 (d, 1H, $J=9$)	7.85 (d, 1H, $J=9$)	8.14 (s, 1H)	C_8-CH_3: 2.79 (s, 3H)
IIc	4.06 (s, 3H)	3.67 (s, 3H)	9.58 (s, 1H)	8.25 (d, 1H, $J=9$)	7.24 (d, 1H, $J=9$)	7.64 (s, 1H)	C_8-CH_3O: 4.15 (s, 3H)
IId	4.06 (s, 3H)	3.68 (s, 3H)	9.75 (s, 1H)	8.33 (d, 1H, $J=9$)	7.93 (d, 1H, $J=9$)	8.36 (s. 1H)	—

Control experiments, in which DAD was ommited, did not give 5-deazaalloxazines, but yielded self-cyclized products, 5-aryl-1,3,7,9-tetramethylpyrido[2,3-*d*: 6,5-*d*′]dipyrimidine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)tetrones (III).



Ib

III

Chart 2

As depicted in Chart 1, this 5-deazaalloxazine synthesis is presumably initiated by the addition of DAD to I. The Michael-type adduct could undergo intramolecular cyclization with concomitant elimination of 6-amino-5-(1,2-bisethoxycarbonylhydrazino)uracil[6] to provide the dihydro-5-deazaalloxazine. This could be dehydrogenated by excess DAD or by air to give the final 5-deazaalloxazine (II).

Experimental[7]

Aryl-bis(6-amino-1,3-dimethyluracil-5-yl)methanes (Ia—d)——A mixture of 6-amino-1,3-dimethyluracil (5 g, 0.032 mol) and aryl aldehyde (0.04 mol) was heated in ethanol or acetic acid (200 ml) at 90° for 3 hr, then allowed to stand overnight to precipitate a colorless powder, which was recrystallized from ethanol (Table I).

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[4] W. Pfleiderer, F. Sági, and L. Crözinger, *Chem. Ber.*, **99**, 3530 (1966).

[5] Sulfolane was used as an inert, high-boiling solvent.

[6] This compound would be thermally decomposed to unidentified compounds.

[7] All melting points are uncorrected. NMR spectra were determined with a JEOL-PMX 60 spectrometer (with tetramethylsilane as an internal standard). The identity of compounds was confirmed by comparison of infrared spectra (Nujol mulls) using a JASCO IR-1A spectrometer.

1,3-Dimethylpyrimido[4,5-*b*]quinoline-2,4(1*H*,3*H*)diones (1,3-Dimethyl-5-deazaalloxazines) (IIa—d)——A mixture of the adduct (I) (0.02 mol) and DAD (0.06 mol) in sulfolane (5 ml) was heated at 180° for 30 min. After cooling, the crystals which separated were collected by filtration and recrystallized from acetic acid to give pale yellow prisms (Table II).

5-(*p*-Tolyl)-1,3,7,9-tetramethylpyrido[2,3-*d*: 6,5-*d'*]dipyrimidine-2,4,6,8(1*H*,3*H*,7*H*,9*H*)tetrone) (III)——Compound Ib (0.4 g, 0.01 mol) was heated in sulfolane (4 ml) at 200° for 2 hr. The reaction mixture was diluted with water and allowed to stand overnight. The resulting crystals were collected by filtration and dried. Recrystallization from ethanol gave colorless plates (0.25 g, 63.6%), mp 335°, MS *m/e*: 393 (M^+). *Anal.* Calcd. for $C_{20}H_{19}N_5O_4$: C, 61.06; H, 4.87; N, 17.80. Found: C, 60.88; H, 4.71; N, 17.65.

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Guanylate Cyclase in Carrageenin Granuloma Tissue of Rat[1]

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Guanylate cyclase activity was examined in inflammatory granuloma tissue induced by subcutaneous injection of carrageenin solution in the rat. Enzymological properties of guanylate cyclase in this tissue, such as pH optimum, metal ion requirement, *etc.*, were examined, with special reference to the intracellular distribution of the enzyme. Differences were found between guanylate cyclase in the soluble fraction and that in the particulate fraction in the response to Mn^{2+} and Triton X-100 in this tissue.

Keywords——guanylate cyclase; carrageenin granuloma; inflammation; Triton X-100; Mn^{2+}; subcellular fractionation

It has been reported that guanosine 3',5'-monophosphate (cyclic GMP) is involved in inflammatory processes, such as the release of lysosomal enzymes,[3] chemotaxis,[4] *etc.* Guanylate cyclase (GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2) is responsible for the production of cyclic GMP, and the presence of two types of guanylate cyclase, soluble and particulate types, which differ from each other in molecular weight, metal ion requirement, and antigenicity, has been reported in several tissues.[5] However, few studies have been carried out on guanylate cyclase in inflammatory tissues, and seldom on the two types of this enzyme.

In this paper, we describe the activity of guanylate cyclase in an experimental inflammatory granuloma tissue induced by subcutaneous injection of carrageenin solution in the rat. The presence and properties of soluble and particulate types of this enzyme in the granuloma tissue were also studied.

Experimental

Materials——GTP-8-^{3}H and cyclic GMP-8-^{14}C were purchased from the Radiochemical Centre; creatine kinase, phosphocreatine, and neutral alumina (type WN 3) were from Sigma Chemical Co.; cyclic GMP from

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[1] A part of this work was presented at the 51st Annual Meeting of the Japanese Biochemical Society, Kyoto, November, 1978.

[2] Location: *1-2-3, Kasumi, Hiroshima.*

[3] L.J. Ignarro, *Nature New Biology*, **245**, 151 (1973).

[4] H.R. Hill, R.D. Estensen, P.G. Quie, N.A. Hogan, and N.D. Goldberg, *Metabolism*, **24**, 447 (1975).

[5] *a*) H. Kimura and F. Murad, *Metabolism*, **24**, 439 (1975); *b*) J.C. Bloser and S.H. Appel, *J. Biol. Chem.*, **253**, 3088 (1978); *c*) W.E. Criss, F. Murad, and H. Kimura, *J. Cyclic Nucleotide Res.*, **2**, 11 (1976); *d*) J.D. Chrisman, D.L. Garbers, M.A. Parks, and J.G. Hardman, *J. Biol. Chem.*, **250**, 374 (1975); *e*) D.L. Garbers, *J. Biol. Chem.*, **253**, 1898 (1978).