UDC 615.778〔547.859.3〕-092 : 576.852.24

184. Tadashi Okabayashi and Yasuo Makisumi : On the Action of Substituted Azaindolizines on Microörganisms. III.[*1] Action of Aminated Azaindolizines on Lactic Acid Bacteria.

(*Research Laboratory, Shionogi & Co., Ltd.*[*2])

In a previous paper of this series, substituted azaindolizines were tentatively divided into three groups according to their modes of action on lactic acid bacteria.[1] It has also been shown that halogenated azaindolizines (Group 3 compounds) inhibited the growth of *Escherichia coli* and that this inhibition was reversed by various amino acids.[*1] In the present work, the actions of Group 2 compounds, the aminated azaindolizines (I, II, III in Chart 1) were compared with those of some diaminopyrimidine compounds (IV, V, VI, VII) and the mode of action of these compounds was deduced.

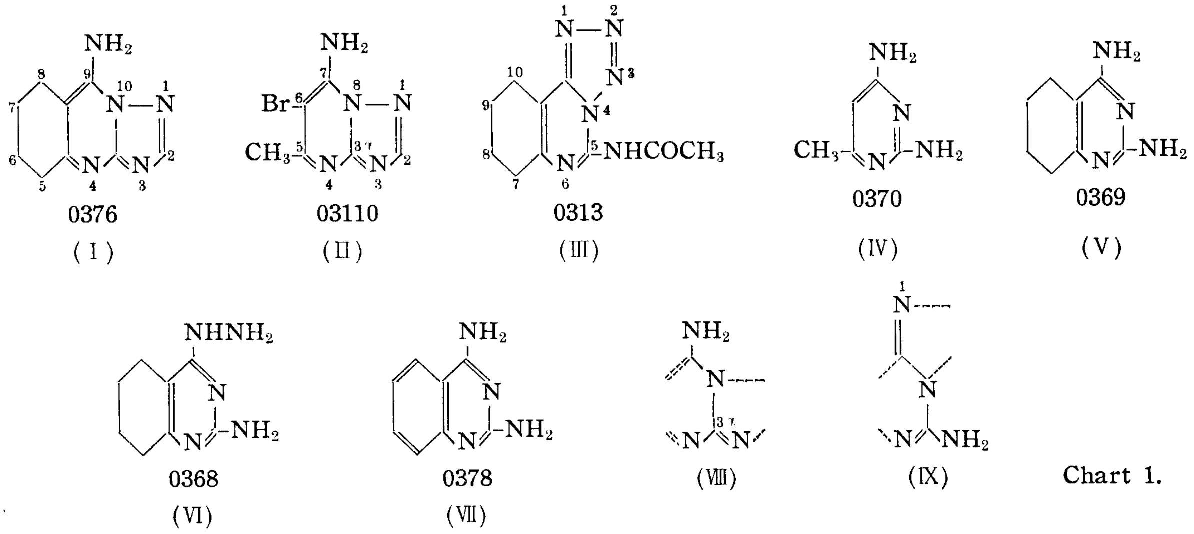

Chart 1.

Data presented in Table I show that all compounds tested exhibited a more pronounced effect on *Streptococcus faecalis* than on *Lactobacillus casei* and that their actions were reversed by folic acid, leucovorin, and thymine. When *St. faecalis* was used, thymine was the most effective with regard to reversible properties, and in the presence of 1 γ/cc. of thymine, no compounds, with the exception of 0368, exhibited growth inhibition at the level of 100 γ/cc. The addition of 100 mγ/cc. of leucovorin also caused a marked decrease in inhibitory activity. Folic acid was least effective among these metabolites and some agents (0313, 0369, and 0367) caused no decrease in their inhibitory activity, when their respective concentration reached a certain level in the presence of 100 mγ/cc. of folic acid.

Data obtained with *L. casei* demonstrate that there are no remarkable differences between growth inhibitory activity of these compounds in PFA and OFA media. This may imply that the effect of adenine, if any, is not so significant. A separate experiment, the results of which are not presented in this paper, showed that other purines such as guanine, xanthine, and hypoxanthine also have only slight effects.

Effect of folic acid on the action of 0313 and 2,4-diaminoquinazoline (0378) is indicated

---

[*1] Part II : This Bulletin, 8, 162(1960).

[*2] 192 Imafuku, Amagasaki, Hyôgo-ken (岡林 直, 牧角徳夫).

1) T. Okabayashi, H. Kano, Y. Makisumi : This Bulletin, 8, 157(1960).

Table I. Action of Aminated Azaindolizines and Diaminopyrimidines on *L. casei* and *St. faecalis*

Substance	Amt. (γ/cc.)	*St. faecalis*					*L. casei*		
		Folic acid		Leucovorin		Thymine			
		1 mγ/cc.	100 mγ/cc.	1 mγ/cc.	100 mγ/cc.	1 γ/cc.	OFA	PFA	FA
0376	100	−97	—	—	—	0	−56	−20	0
	20	−78	—	—	—	0	−14	0	0
	4	−32	—	—	—	0	−13	0	0
03110	100	−98	0	−51	0	0	−70	−50	0
	20	−90	0	−50	0	0	−35	0	0
	4	−66	0	−30	0	0	0	0	0
	0.8	−22	0	0	0	0	0	0	0
0313	100	−99	−98	−99	0	−30	−70	−96	0
	20	−99	−66	−72	0	−20	−52	−77	0
	4	−98	0	−33	0	0	−23	0	0
	0.8	−94	0	−12	0	0	0	0	0
	0.16	−16	0	−10	0	0	0	0	0
0370	100	−97	0	0	0	0	0	0	0
	20	−97	0	0	0	0	0	0	0
	4	−40	0	0	0	0	0	0	0
0368	100	−98	−97	−95	−91	−89	−80	−80	−91
	20	−98	−97	−87	0	0	−60	−50	−70
	4	−98	0	−17	0	0	−10	0	0
	0.8	−30	0	0	0	0	−10	0	0
0369	100	−98	−98	−99	−95	−25	−97	−97	−99
	20	−98	−98	−99	0	−25	−97	−97	−79
	4	−98	−97	−98	0	0	−96	−96	0
	0.8	−98	0	−95	0	0	−63	0	0
	0.16	−88	0	0	0	0	0	0	0
0378	100	−99	−99	−99	−99	−27	−92	−94	−98
	20	−99	−99	−99	0	−27	−92	−94	−57
	4	−99	−99	−99	0	−18	−91	−92	0
	0.8	−99	−51	−34	0	0	−71	−88	0
	0.16	−99	0	−15	0	0	0	0	0

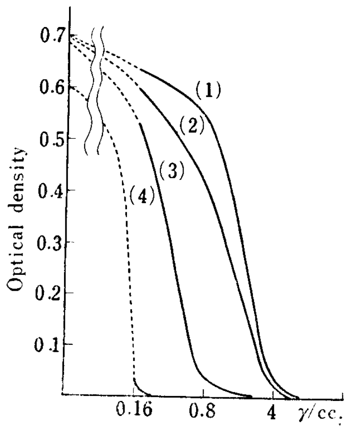

Fig. 1. Effect of Folic Acid on Inhibition of 0313

Folic acid (1) 625 mγ/cc. (3) 25 mγ/cc.
(2) 125 mγ/cc. (4) 5 mγ/cc.

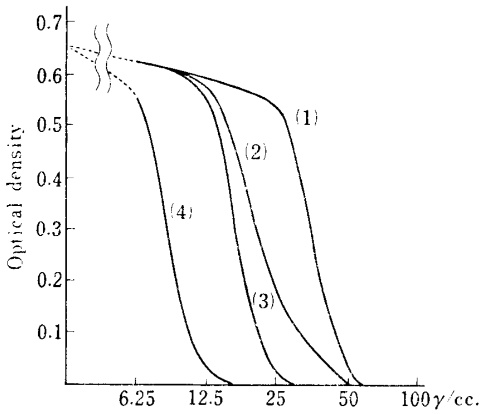

Fig. 2. 2,4-Diaminoquinazoline (0378) vs. Folic Acid

Folic acid (1) 3125 mγ/cc. (3) 25 mγ/cc.
(2) 125 mγ/cc. (4) 5 mγ/cc.

in Figs. 1 and 2. The curves in both cases show that folic acid overcomes the effect of relatively low concentrations of these compounds in a non-competitive manner and thus, 625 mγ/cc. of folic acid does not affect the activity of above 100 γ/cc. of 0313 or 4 γ/cc. of 0378. Figs. 3 and 4 illustrate the effect of leucovorin on 0313 and 0378. Leucovorin overcomes the inhibition of both agents more effectively than folic acid. Mathematical analysis of Fig. 4 revealed that the relation between leucovorin and 0378 was non-competitive. Fig. 3 illustrates the relation between 0313 and leucovorin. It can be seen that 0313, which is less harmful to *St. faecalis* than 0378, exhibited no growth inhibition at the concentration of 160 γ/cc., in the presence of 25 mγ/cc. leucovorin. Owing to the insolubility of 0313, further attempts to determine the I/S ratio were unsuccessful. As shown in Figs. 5 and 6, the effect of thymine was most prominent and the pressence of 1 γ/cc. of thymine almost completely suppressed the inhibition of both agents.

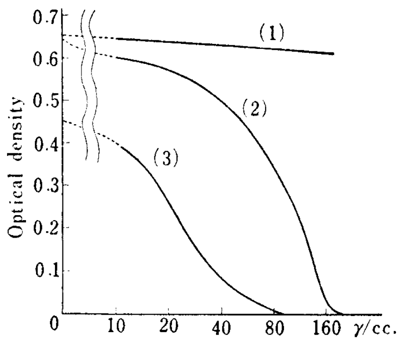

Fig. 3. Effect of Leucovorin on Inhibition of 0313
Leucovorin (1) 25 mγ/cc. (3) 1 mγ/cc.
(2) 5 mγ/cc.

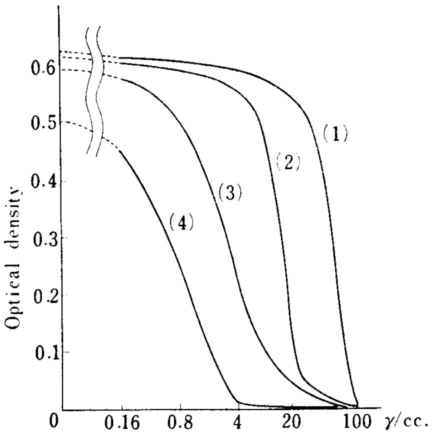

Fig. 4. 2,4-Diaminoquinazoline (0378) vs. Leucovorin
Leucovorin (1) 125 mγ/cc. (3) 5 mγ/cc.
(2) 25 mγ/cc. (4) 1 mγ/cc.

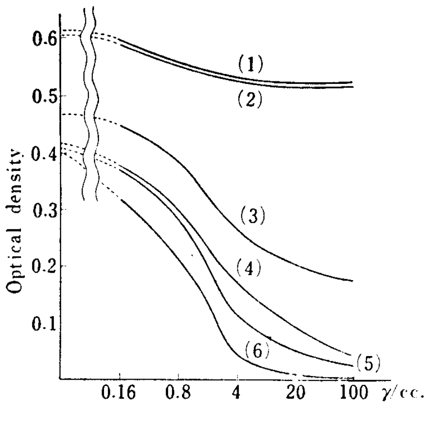

Fig. 5. Effect of Thymine on Inhibition of 0313
Thymine (1) 1 γ/cc. (4) 0.125 γ/cc.
(2) 0.5 γ/cc. (5) 0.06 γ/cc.
(3) 0,25 γ/cc. (6) 0.03 γ/cc.

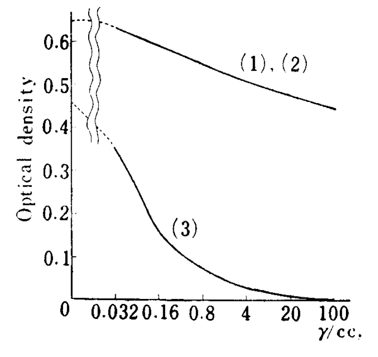

Fig. 6. 2,4-Diaminoquinazoline (0378) vs. Thymine
Thymine (1) 10 γ/cc.
(2) 1 γ/cc.
(3) 0.1 γ/cc.

From the data presented in these tables and figures, azaindolizines which have an amino or closely related group in 5-position seem to have an effect on the lactic acid bacteria similar to that of 2,4-diaminopyrimidines tested. This seems to imply that the C=N bond between 1- and 3a-positions of aminated 1-azaindolizine ring may exert an effect similar to that of NH_2 groups in 2,4-diaminopyrimidines on the lactic acid bacteria requiring external supply of folic acid or related compounds for their growth (See (Ⅷ) and (Ⅸ) in Chart 1).

The results obtained in this experiment are consistent with the fact that 2,4-diaminopyrimidine moiety acts as an antagonist of folic-folinic system.[2,3] The fact that growth inhibitory activity of azaindolizines and the reversible effect of metabolites on these compounds were much weaker than those of the corresponding diaminopyrimidines (for instance, 0313 or 0369 vs. folic acid, leucovorin, and thymine) may possibly be explained by distant structural relation of azaindolizines to these metabolites.

The effectiveness of the metabolites with regard to reversal was in the decreasing order of thymine, leucovorin, and folic acid. The fact that leucovorin was overwhelmingly effective in its reversal effect seems to offer a clue for predicting the position of blockage in metabolic sequence caused by aminated azaindolizines. However, owing to the lack of competitive nature between metabolites and inhibitors, and to the scarcity of other evidence that supports this prediction, many points remain to be investigated for elucidation of the mode of action of aminated azaindolizines.

Experimental

Azaindolizines used in this experiment were 9-amino-5,6,7,8-tetrahydro-*s*-triazolo[3,2-*b*]quinazoline (I, designated as 0376), 7-amino-6-bromo-5-methyl-*s*-triazolo[2,3-*a*]pyrimidine[4] (Ⅱ, 03110), and 5-acetamido-7,8,9,10-tetrahydrotriazolo[*c*]quinazoline (Ⅲ, 0313). Diaminopyrimidines used for comparison of their action with those of aminated azaindolizines were 2,4-diamino-6-methylpyrimidine[5] (Ⅳ, 0370), 2,4-diamino-5,6,7,8-tetrahydroquinazoline[6] (Ⅴ, 0369), 2-amino-4-hydrazino-5,6,7,8-tetrahydroquinazoline (Ⅵ, 0368), and 2,4-diaminoquinozoline[7] (Ⅶ, 0378). Compound (Ⅴ) was prepared by the reaction of 2-amino-4-chloro-5,6,7,8-tetrahydroquinazoline[8] with $EtOH-NH_3$ at 150∼160° in a good yield. Compounds (I), (Ⅵ), and (Ⅲ) were prepared in the following way.

9-Amino-5,6,7,8-tetrahydro-*s*-triazolo[3,2-*b*]quinazoline (I)—A mixture of 5 g. of 9-chloro-5,6,7,8-tetrahydro-*s*-triazolo[3,2-*b*]quinazoline[9] and 25 cc. of dehyd. EtOH saturated with dry NH_3 at 0° was heated at 110∼120° for 7 hr. in a sealed tube. The reaction mixture was evaporated and the residue was dissolved in 10% HCl. The acid solution was basified with 10% NaOH and the resulting precipitate was recrystallized from EtOH to 3.7 g. of colorless needles, m.p. 286∼287°. *Anal.* Calcd. for $C_9H_{11}N_5$: C, 57.14; H, 5.82; N, 37.03. Found: C, 57.25; H, 6.05; N, 37.28.

2-Amino-4-hydrazino-5,6,7,8-tetrahydroquinazoline (Ⅵ)—A mixture of 3.6 g. of 2-amino-4-chloro-5,6,7,8-tetrahydroquinazoline[8] in 40 cc. of EtOH and 4 cc. of 80% $NH_2NH_2\cdot H_2O$ was refluxed for 3 hr. After the volatile portion was removed, the residue was extracted with hot benzene. The extract was cooled and the separated crystalline residue (3.5 g.) was recrystallized from MeOH to slight yellow prisms, m.p. 190∼191°. *Anal.* Calcd. for $C_8H_{13}N_5$: C, 53.61; H, 7.31; N, 39.09. Found: C, 53.80; H, 7.43; N, 39.36.

5-Acetamido-7,8,9,10-tetrahydrotetrazolo[*c*]quinazoline (Ⅲ)—To a solution of 1.8 g. of (Ⅵ) dissolved in 20 cc. 10% AcOH, 7.5 cc. of 10% $NaNO_2$ solution was added dropwise at below 10° with stirring. After stirring for several hr., the separated crystals were collected and recrystallized from EtOH to 1.9 g. of white needles, m.p. 239∼240° (decomp.). *Anal.* calcd. for $C_8H_{10}N_6$ (5-Amino-7,8,9,10-tetrahydrotetrazolo[*c*]quinazoline): C, 50.52; H, 5.30; N, 44.19. Found: C, 50.88; H, 5.82; N, 43.56.

A mixture of 0.5 g. of this compound and 1.5 cc. of Ac_2O was refluxed for 1 hr. After cool, the reaction mixture was poured into ice-water, the resulting crystals were collected, and recrystallized from hydr. EtOH to 0.5 g. of colorless needles, m.p. 157∼158°. *Anal.* Calcd. for $C_{10}H_{12}ON_6$: C, 51.72;

---

2) G.H. Hitchings, E.A. Falco, H. Vanderwerff, P.B. Russell, G.B. Elion: J. Biol. Chem., **199**, 43 (1952).
3) M. Kawashima: This Bulletin, **7**, 13, 17(1959).
4) H. Kano, Y. Makisumi, S. Takahashi, M. Ogata: This Bulletin, **7**, 903(1959).
5) S. Gabriel, J. Colman: Ber., **34**, 1253(1901).
6) B.H. Chase, J. Walker: J. Chem. Soc., **1953**, 3518.
7) Edw. Vopicka, N.A. Lange: J. Am. Chem. Soc., **57**, 1068(1935).
8) R. Hull, B.J. Louell, H.T. Openshaw, L.C. Payman, A.R. Todd: J. Chem. Soc., **1946**, 357.
9) J.W. Cook, R.P. Gentles, S.H. Tukee: Rec. trav. chim., **69**, 343(1950); K. Shirakawa: Yakugaku Zasshi, **79**, 1487(1959).

H, 5.22; N, 36.20. Found: C, 51.44; H, 5.54; N, 36.07.

The action of the compounds on lactic acid bacteria was measured as described earlier.[1] In this experiment three different media, OFA, PFA, and FA, were used for *L. casei*. The content of folic acid in FA medium increased to 10 $m\gamma$/cc.

The authors are deeply grateful to Dr. Ken'ichi Takeda, Director of this Laboratory, Dr. Eitaro Masuo, Assistant Director, and Dr. Hideo Kano for their valuable advice and encouragement throughout this work. They are also greatly indebted to Dr. Kanzo Iwai, Assistant Professor of Research Institute for Food Science, University of Kyoto, for the gift of leucovorin.

Summary

Action of aminated azaindolizines on *Lactobacillus casei* and *Streptococcus faecalis* was compared with that of several diaminopyrimidine compounds. All compounds exhibited more pronounced effect on *St. faecalis* than on *L. casei*. Growth inhition caused by these compounds was reversed in the decreasing order by thymine, leucovorin, and folic acid. These results suggested that C=N bond between 1- and 8a-positions in 1-azaindolizine molecule might have an effect similar to amino group on the lactic acid bacteria.

(Received April 16, 1960)

UDC 547.92.02

185. **Kaname Hamamoto**: Studies on the Steroidal Components of Domestic Plants. XXIII.[1] Structure of Metagenin. (3).[1]

(*Research Laboratory, Shionogi & Co., Ltd.*[*1])

In the previous papers[1,2] from this laboratory, metagenin was assigned the structure of 5β,25D-spirostane-2β,3β,x-triol ($x=7$ or 11) (I) and the location of the third hydroxyl group had remained still undetermined. The present paper is concerned with the elucidation of the properties and conformation of the undecided hydroxyl group.

Metagenone (IIa), prepared[1] with chromium trioxide oxidation of metagenin 2,3-acetonide[2] (Va) or 2,3-diacetate,[1] afforded a new triol (IIIa), epimetagenin, m.p. 134∼136°, when it was treated with sodium borohydride, while metagenin (I) was regenerated in a good yield with sodium and isopropanol. In the case of reduction with lithium aluminium hydride, metagenone diacetate (IIb) gave epimetagenin as a main product together with a small amount of metagenin.

The activities of the unknown hydroxyl groups of both isomers (I) and (IIIa) were compared by acylation reactions. Epimetagenin gave only a diacetate (IIIb), m.p. 175°, by the action of acetic anhydride and pyridine at room temperature. Epimetagenin acetonide (IV), m.p. 218∼221°, prepared from epimetagenin, was not affected by cathylation reaction[3] with ethyl chlorocarbonate. On the other hand, metagenin acetonide (Va) readily gave an acetonide cathylate (Vb), m.p. 159°, which turned into metagenin monocathylate (VIa), m.p.

[*1] 192 Imafuku, Amagasaki, Hyogo-ken (浜元 要).

1) Part XXII, Part (2): K. Takeda, K. Hamamoto: This Bulletin, 8, 1004(1960).

2) Part (1): K. Takeda, T. Okanishi, K. Hamamoto, A. Shimaoka, N. Maezono: Yakugaku Zasshi, **77**, 175(1957).

3) L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romers, T. Utne: J. Am. Chem. Soc., **74**, 3309(1952).