deriv.), 90672-24-5; **32**, 90671-39-9; **32**·2HCl, 90671-40-2; **32** (carbinol deriv.), 90672-25-6; **33**, 90671-41-3; **33**·2HCl, 90671-42-4; **34**, 90671-43-5; **34**·2HCl, 90671-44-6; **35**, 90671-45-7; **35**·HCl, 90671-46-8; **36**, 90671-47-9; **36**·HCl, 90671-48-0; **37**, 90671-49-1; **37**·HCl, 90671-50-4; **38**, 90671-51-5; **38**·2HBr, 90671-52-6; **39**, 90671-53-7; **39**·2HCl, 90671-54-8; **40**, 90671-55-9; **40**·2HCl, 90671-56-0; **40** (carbinol deriv.), 90672-84-7; **41**, 90671-57-1; **41**·2HCl, 90671-58-2; **41** (carbinol deriv.), 90672-85-8; **42**, 90671-59-3; **42**·2HCl, 90671-60-6; **42** (carbinol deriv.), 90672-86-9; **43**, 90671-61-7; **43**·2HCl, 90671-62-8; **43** (carbinol deriv.), 90672-87-0; **44**, 90671-63-9; **44**·2HCl, 90671-64-0; 44 (carbinol deriv.), 90672-88-1; **45**, 90671-65-1; **45**·2HCl, 90671-65-1; **45** (carbinol deriv.), 90672-89-2; **46**, 90671-67-3; **46**·2HCl, 90671-68-4; **46** (carbinol deriv.), 90672-90-5; **47**, 90671-69-5; **47**·2HCl, 90671-70-8; **47** (carbinol deriv.), 90672-91-6; **48**, 90671-71-9; **48**·2HCl, 90671-72-0; **48** (carbinol deriv.), 90672-92-7; **49**, 90671-73-1; **49**·2HCl, 90671-74-2; **49** (carbinol deriv.), 90672-93-8; **50**, 90671-75-3; **50**·2HCl, 90671-76-4; **51**, 90671-77-5; **51**·2HCl, 88247-59-0; **52**, 90671-78-6; **52** (carbinol deriv.), 90672-94-9; **52**·2HCl, 90671-79-7; **53**, 90671-80-0; **53**·2HCl, 90671-81-1; **53** (carbinol deriv.), 90672-95-0; **54**, 90671-82-2; **54**·2HCl, 90671-83-3; **54** (carbinol deriv.), 90672-96-1; **55**, 90671-84-4; **55**·2HCl, 90671-85-5; **55** (carbinol deriv.), 90672-97-2; **56**, 90671-86-6; **56**·2HCl, 90671-87-7; **56** (carbinol deriv.), 90672-98-3; **57**, 90671-88-8; **57**·2HCl, 90671-89-9; **58**, 90671-90-2; **58**·2HCl, 90671-91-3; **59**, 90671-92-4; **59**·2HCl, 90671-93-5; **60**, 90671-94-6; **60**·2HCl, 90671-95-7; **60** (carbinol deriv.), 90672-99-4; **61**, 90671-96-8; **61**·2HCl, 90671-97-9; **61** (carbinol deriv.), 90673-00-0; **62**, 90672-26-7; **62**·HCl, 90672-27-8; **63**, 90672-29-0; **63**·HCl, 90672-30-3; **64**, 90672-31-4; **65**, 90672-32-5; **65**·2HCl, 90672-33-6; **67**, 90672-34-7; **67**·HCl, 90672-35-8; **68**, 90672-36-9; **68**·2HCl, 90672-37-0; **69**, 90672-38-1; **69**·2HCl, 90672-39-2; **70**, 90672-40-5; **71**, 90672-41-6; **71**·oxalate, 90672-42-7; **72**, 90672-43-8; **73**, 90672-44-9; **74**, 90672-45-0; **74**·2HCl, 90672-46-1; **74** (carbinol deriv.), 90672-47-2; **75**, 90672-48-3; **75**·2HCl, 88247-58-9; **75** (carbinol deriv.), 90672-49-4; **76**, 90672-50-7; **76**·2HCl, 90672-51-8; **76** (carbinol deriv.), 90672-52-9; **77**, 90672-53-0; **77**·2HCl, 90672-54-1; **78**, 90672-55-2; **78**·2HCl, 90672-56-3; **79**, 90672-57-4; **79**·2HCl, 90672-58-5; **80**, 90672-59-6; **80**·HCl, 90672-60-9; **81**, 90672-61-0; **81**·2HCL, 90672-62-1; **81** (carbinol deriv.), 31796-72-2; **82**, 90672-63-2; **82**·HCl, 90672-64-3; **82** (carbinol deriv.), 90672-65-4; **83**, 90672-66-5; **83**·2HCl, 90672-67-6; **83** (carbinol deriv.), 90672-68-7; **84**, 90672-69-8; **84**·2HCl, 90672-70-1; **84** (carbinol deriv.), 90672-71-2; **85**, 90672-72-3; **85**·HCl, 90672-73-4; **86**, 90672-74-5; **86**·HCl, 90672-75-6; **87**·I, 90672-76-7; **88**, 90672-77-8; **88**·2HCl, 90672-78-9; **89**, 90672-79-0; **89**·2HCl, 90695-85-5; benzaldehyde, 100-52-7; 4-chlorobenzaldehyde, 104-88-1; 2-methylbenzaldehyde, 529-20-4; 3-methylbenzaldehyde, 620-23-5; 4-methylbenzaldehyde, 104-87-0; 2-ethylbenzaldehyde, 22927-13-5; 2-isopropylbenzaldehyde, 6502-22-3; 4-isopropylbenzaldehyde, 122-03-2; 4-butylbenzaldehyde, 1200-14-2; 4-*sec*-butylbenzaldehyde, 28293-43-8; 4-*tert*-butylbenzaldehyde, 939-97-9; 4-cyclohexylbenzaldehyde, 27634-89-5; 2-methoxybenzaldehyde, 135-02-4; 3-methoxybenzaldehyde, 591-31-1; 4-methoxybenzaldehyde, 123-11-5; 2-ethoxybenzaldehyde, 613-69-4; 2-isopropoxybenzaldehyde, 22921-58-0; 4-isopropoxybenzaldehyde, 18962-05-5; 2-butoxybenzaldehyde, 7091-13-6; 4-phenoxybenzaldehyde, 67-36-7; 4-benzyloxybenzaldehyde, 4397-53-9; 3-nitrobenzaldehyde, 99-61-6; 3-(trifluoromethyl)benzaldehyde, 454-89-7; 2-chlorobenzaldehyde, 89-98-5; 3-chlorobenzaldehyde, 587-04-2; 2-fluorobenzaldehyde, 446-52-6; 4-fluorobenzaldehyde, 459-57-4; 2,4-dimethylbenzaldehyde, 15764-16-6; 3,4-dimethylbenzaldehyde, 5973-71-7; 3,4-dimethoxybenzaldehyde, 120-14-9; 1-methylpiperazine, 109-01-3; 1-(2-hydroxyethyl)piperazine, 103-76-4; ethyl 1-piperzinecarboxylate, 120-43-4; 1-(methylsulfonyl)piperazine, 55276-43-2; 1-benzoylpiperazine, 13754-38-6; piperazine, 110-85-0; morpholine, 110-91-8; piperidine, 110-89-4; 3-pyridinamine, 462-08-8; 2,6-dimethylpiperazine, 108-49-6; 2-(*N*,*N*-dimethylamino)ethanethiol, 108-02-1; 1-pyrrolidineethanol, 2955-88-6; 2-(*N*,*N*-dimethylamino)ethanol, 108-01-0; 4-iodo-3,5-dimethylisoxazole, 10557-85-4; 4-bromo-2,5-dimethylthiazole, 90672-80-3; 1-methylimidazole, 616-47-7; 4-(1-methylethyl)phenyl 3,5-dimethylisoxazoyl-4-yl ketone, 90672-81-4; 4-(1-methylethyl)phenyl 3-methylisothiazol-5-yl ketone, 90695-86-6; 4-chloro-1-methylpiperidine, 5570-77-4; 4-(1-methylethyl)-phenyl ketone, 21192-57-4; [4-(1-methylethyl)phenyl](3-methylisothiazol-5-yl)carbinol, 90672-82-5; 4-(1-methylethyl)-phenyl 3-methylisothiazol-5-yl ketoxime, 90672-83-6.

Nitrogen Bridgehead Compounds. 44.[1] New Antiallergic 4*H*-Pyrido[1,2-*a*]pyrimidin-4-ones. 4[2]

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The weak antiallergic activity of 6-methyl-4-oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid (1) in the rat reaginic passive cutaneous anaphylaxis test was enhanced by the introduction of an (arylamino)methylene moiety into position 9 of the pyridopyrimidine ring. Compound **34**, (+)-6(*S*)-methyl-9-[(*m*-methylphenyl)-hydrazono]-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidine-3-carboxylic acid, displayed about 10 000 times the activity of the starting compound 1. A structure–activity relationship study of 9-[(arylamino)methylene]tetrahydropyrido-pyrimidine-3-carboxylic acids resulted in conclusions similar to those found for the 9-(arylhydrazono)tetrahydro- and 9-(arylamino)dihydropyridopyrimidine series. Replacement of the 3-carboxy group of 9-(phenylhydrazono)-tetrahydropyridopyrimidin-4-ones with an acrylic acid moiety caused slight increases in potency. In the 6-methyl-substituted series, a high stereospecificity was observed between the enantiomers with 6*S* and 6*R* absolute configurations, the former being responsible for the antiallergic activity. The effects of some 9-[(arylamino)-methylene]tetrahydropyridopyrimidine-3-carboxylic acids on the rat passive peritoneal anaphylaxis test were also investigated.

We recently reported[2-4] that the weak antiallergic effect of the tetrahydropyridopyrimidinecarboxylic acid (1) on the rat reaginic passive cutaneous anaphylaxis (PCA) test could be enhanced by the introduction of certain substituents[2] into the reactive methylene group[5] in position 9 of the pyridopyrimidine ring system, resulting in compounds with higher potencies than that of the reference disodium cromoglycate (DSCG). The structure–activity relationships within the 9-amino-6,7-dihydro-4*H*-pyrido-

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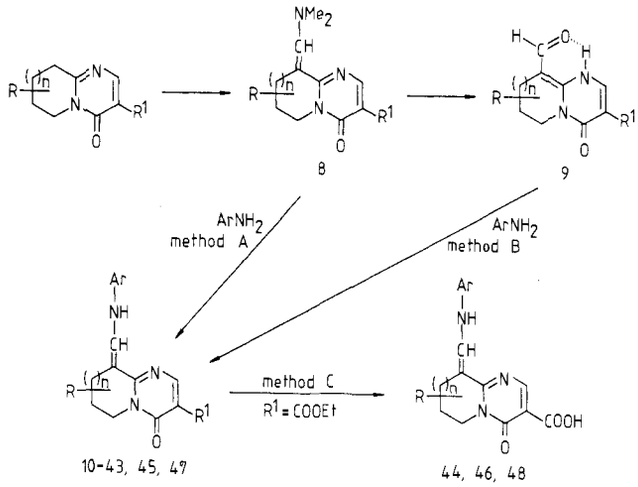
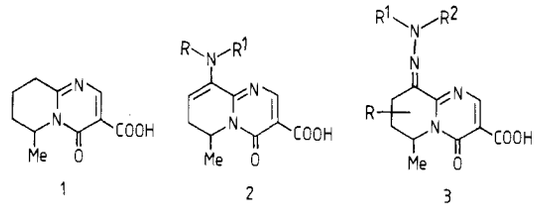
(1) Part 43. Balogh, M.; Hermecz, I.; Mészáros, Z. *Synthesis*, in press.
(2) Hermecz, I.; Breining, T.; Vasvári-Debreczy, L.; Horváth, Á.; Mészáros, Z.; Bitter, I.; De Vos, C.; Rodriguez, L. *J. Med. Chem.* **1983**, *26*, 1494.
(3) Hermecz, I.; Breining, T.; Mészáros, Z.; Horváth, Á.; Vasvári-Debreczy, L.; Dessy, F.; De Vos, C.; Rodriguez, L. *J. Med. Chem.* **1982**, *25*, 1140.

0022-2623/84/1827-1253$01.50/0

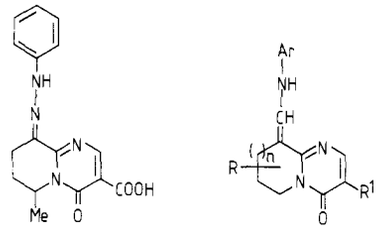
Scheme I



[1,2-*a*]pyrimidines (**2**) and the hydrazono-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidines (**3**) were studied in detail.[2-4] The 6-*S* enantiomer of 9-(phenylhydrazono)-



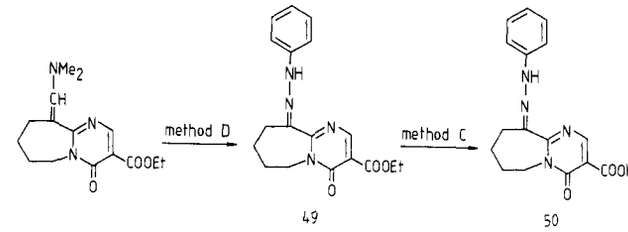
tetrahydropyridopyrimidine-3-carboxylic acid (**5**; Chinoin-1045; UCB L140), which also displays activity when administered orally, was selected for further detailed pharmacological[6] and clinical investigation.



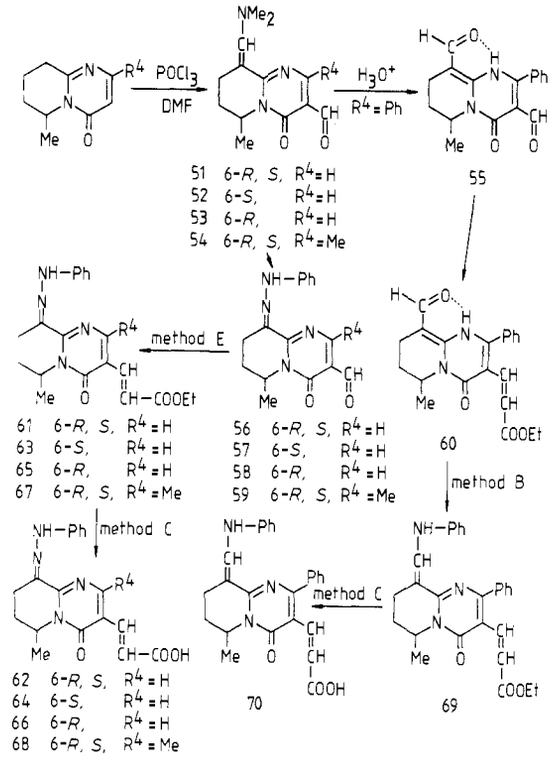
Our structure–activity study has now been extended to the 9-(aminomethylene)tetrahydropyridopyrimidines (**7**; $n = 1$), which can be considered as isosteric derivatives of the hydrazonotetrahydropyridopyrimidines (**3**), obtained by –CH= for –N= isosteric replacement, to their homologues in the piperidine ring (**7**; $n = 0, 2$), and to some 9-(phenylhydrazono)tetrahydropyridopyrimidine-3-acrylates, which can be considered as the vinylogues of **3**. In this paper we report the preparation and pharmacological investigation of these compounds.

Chemistry. The 9-[(arylamino)methylene]pyridopyrimidines **10–42** are easily formed by reacting the 9-(dimethylamino)methylene derivatives 8 (method A) or the

Scheme II



Scheme III



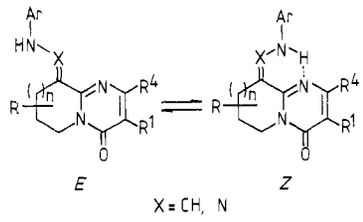
9-formyl derivatives **9** (method B) with aniline derivatives in acetic acid. Compounds **8** and **9** are products of the Vilsmeier–Haack formylation of the 6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-ones.[7] The homologous (phenylamino)methylene 3-carboxylic ester derivatives ($n = 0, 2$; **43**, **45**, and **47**) are prepared from the corresponding aminomethylene derivatives[8] in a similar manner, and they can be transformed into the corresponding 3-carboxylic acids **44**, **46**, and **48** by hydrolysis in aqueous potassium hydroxide solution (method C, see Scheme I).

The (phenylhydrazono)azepino[1,2-*a*]pyrimidine-3-carboxylic ester **49** was obtained from ethyl 10-[(dimethylamino)methylene]-4-oxo-4,6,7,8,9,10-hexahydroazepino[1,2-*a*]pyrimidine-3-carboxylate[8] in the Japp–Klingemann reaction[9] with phenyldiazonium chloride (method D). Ester **49** was hydrolyzed to the azepino-[1,2-*a*]pyrimidine-3-carboxylic acid **50** by method C (see Scheme II).

In the preparation of the 9-(phenylhydrazono)tetrahydropyridopyrimidine-3-acrylic acids **62**, **64**, **66**, and **68**, the 9-[(dimethylamino)methylene]-3-formylpyridopyrimidines **51–54**[7] were reacted with phenyldiazonium

(4) Hermecz, I.; Breining, T.; Mészáros, Z.; Kökösi, J.; Mészáros, Z.; Dessy, F.; De Vos, C. *J. Med. Chem.* **1983**, *26*, 1126.
(5) (a) Náray-Szabó, G.; Hermecz, I.; Mészáros, Z. *J. Chem. Soc., Perkin Trans. 1*, **1974**, 1753. (b) Kökösi, J.; Hermecz, I.; Szász, Gy.; Mészáros, Z.; Tóth, G.; Pongor-Csákvári, M. *J. Heterocycl. Chem.* **1982**, *19*, 909.
(6) De Vos, C.; Dessy, F.; Hermecz, I.; Breining, T.; Mészáros, Z. *Int. Arch. Allergy Appl. Immunol.* **1982**, *67*, 362.
(7) Horváth, Á.; Hermecz, I.; Vasvári-Debreczy, L.; Simon, K.; Pongor-Csákvári, M.; Mészáros, Z. *J. Chem. Soc., Perkin Trans. 1*, **1983**, 369.
(8) Hermecz, I.; Horváth, Á.; Mészáros, Z.; Tóth, G.; Szöllosy, Á, manuscript to be published.
(9) Phillips, R. R. *Org. React.* **1959**, *10*, 2.

Scheme IV



chloride and the resulting 9-(phenylhydrazono)-3-formylpyridopyrimidines were then subjected to the Wittig reaction with (carbethoxymethylene)triphenylphosphorane[10] to obtain the ethyl 9-(phenylhydrazono)-tetrahydropyridopyrimidine-3-acrylates **61**, **63**, **65**, and **67** (method E).

To prepare the 9-[(phenylamino)methylene]tetrahydropyridopyrimidine-3-acrylic acid **70**, the 3,9-diformyl-2-phenylpyridopyrimidine **55**[7] was transformed in the Wittig reaction with (carbethoxymethylene)triphenylphosphorane into the ethyl 9-formylpyridopyrimidine-3-acrylate **60** and the latter was treated with aniline in acetic acid. The ethyl pyridopyrimidine-3-acrylates **61**, **63**, **65**, **67**, and **69** were hydrolyzed into the corresponding pyridopyrimidine-3-acrylic acids **62**, **64**, **66** **68**, and **70** (see Scheme III).

The products of the Wittig reaction consist of mixtures of *Z* and *E* 3-acrylic side-chain isomers. Following crystallization, however, the pure *E* isomers were obtained. The *E* geometric arrangement around the double bond of the side chain in position 3 is proved by the coupling constant of 12–18 Hz in the ^{1}H NMR spectrum taken in deuteriochloroform.

The (arylamino)methylene and the phenylhydrazono derivatives were *E*–*Z* isomeric mixtures (see Scheme IV). The interconversion of these *E*–*Z* geometric isomers requires low activation energy, and the ratio of the isomers is dependent on the solvent used. Solvent-dependent *E*–*Z* isomerizations of related systems were thoroughly investigated previously.[11]

For the purpose of study of the structure–activity relation, some optically active pyridopyrimidine derivatives (**34**, **35**, andd **63**–**66**) were also prepared. In these syntheses we used optically active starting materials.[12]

Biological Results and Discussion

The pharmacological data obtained on the new derivatives in the rat PCA test are presented in Tables I–III. The 9-[(arylamino)methylene]pyridopyrimidines are in general less soluble than the 9-arylhydrazono derivatives, and in some cases it was not possible to test them by the iv route.

Comparing the data on the PCA activities of the 9-[(arylamino)methylene]pyridopyrimidines, we may draw conclusions similar to those obtained for the 9-(arylamino)-6,7-dihydropyridopyrimidines[2] and the 9-(arylhydrazono)tetrahydropyridopyrimidines.[3,4]

The PCA-active compounds were found among the derivatives containing a carboxy group in position 3 of the pyridopyrimidine ring (see Table I). Derivatives **10** and **11** bearing the carboxy group only on the phenyl ring were inactive. The most potent carboxylic acids (i.e., **19**, **29**, and **33**) contained a methyl group in position 6 of the pyridopyrimidine ring. The absence of the methyl group (**18**) or the change of its position to 7 (**20**) or 8 (**21**) also resulted in a decrease in effectiveness.

The 9-[(phenylamino)methylene]pyridopyrimidinecarboxylic acid **16** could not be tested by the iv route because of its insolubility. Introduction of a fluoro atom or carboxy, methyl, hydroxy, trifluoromethyl, ethoxy, acetyl, or carboxymethyl substituents into the phenyl group or replacement of the phenyl ring by a 3-pyridyl ring resulted in compounds with enhanced solubilities in 5% aqueous sodium carbonate solution. Compounds with an *o*-carboxy (**19**), a *p*-hydroxy (**29**), a *m*-methyl (**33**), a *p*-methyl (**36**), or a *p*-acetyl (**39**) substituent on the phenyl ring and the 9-[(3-pyridylamino)methylene] derivative (**42**) displayed similar iv activities to that of DSCG. Only compounds **33** and **39** had intravenous activity similar to that of the carboxylic acid **4**, but they had no oral activity.

For compound **33** the optically active enantiomers **34** and **35** were synthesized and investigated. There was a marked difference in activity between the enantiomers **34** and **35** in the rat PCA screen, a finding that parallels that seen with the 9-(arylamino)dihydropyridopyrimidinecarboxylic acids[2] and 9-(arylhydrazono)tetrahydropyridopyrimidinecarboxylic acids.[3] The enantiomer with the 6*S* absolute configuration (**34**) was active, whereas the enantiomer with the 6*R* absolute configuration (**35**) was practically inactive.[13] Compound **34** displayed an activity about 10 000 times higher than that of the starting carboxylic acid **1**.

Some five- and seven-membered ring homologues (**43**–**50**) of the piperidine derivatives were also prepared (Table II). Ring homologues **44**, **46**, **48**, and **50** exhibited slight activities when administered iv.

Nohara and co-workers observed an enhanced antiallergic activity in the 4-oxobenzopyran series if the 3-carboxy group was replaced by a *trans*-acrylic acid moiety.[14] This enhanced activity of the acrylic acid derivatives was explained by the increased acidities of the compounds as compared with the hydrogen-bonded 3-carboxylic acids. We also investigated some 3-acrylic acids (see Table III). 9-(Phenylhydrazono)tetrahydropyridopyrimidine-3-acrylic acid (**62**) showed about twice the activity of the 3-carboxylic acid **4**. In this case, too, the enantiomer with the 6*S* absolute configuration (**64**) was responsible for the antiallergic activity.

Earlier we reported that the introduction of an alkyl group into position 2 of 9-(arylhydrazono)tetrahydropyridopyrimidine-3-carboxylic acid resulted in completely inactive compounds.[3,4] The substituent in position 2 presumably sterically prevents the biofunctional 3-carboxylic acid moiety from interacting with the active site of the receptor. When the carboxy group and the bicycle were separated by a *trans*-vinyl moiety (**68**), the methyl group in position 2 could not exert a steric effect on the carboxy group, and thus the PCA activity appeared again. 9-[(Phenylamino)methylene]-2-phenyltetrahydropyridopyrimidine-3-acrylic acid (**70**) was insoluble and therefore could not be tested by the iv route.

None of the carboxylic acids and acrylates showed any activity when they were administered by the oral route.

(10) Isler, O.; Gutmann, H.; Montavon, M.; Röegg, R.; Ryses, G.; Zeller, P. *Helv. Chim. Acta* **1957**, *40*, 1242.

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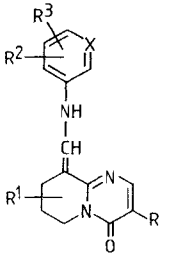
(13) The levorotatory isomer **35** showed about 3300 times less iv PCA activity than the dextrorotatory isomer **34**. This activity can be produced by less than 0.1% of **34** as an impurity in **35**.

(14) Nohara, A.; Kuriki, H.; Saijo, T.; Okawa, K.; Murata, T.; Kanno, M.; Sanno, Y. *J. Med. Chem.* **1975**, *18*, 34.

Table I. 9-[(Phenylamino)methylene]-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-ones

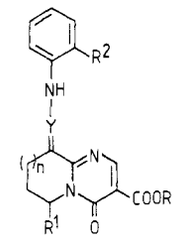


10-42

compd	R	R^1	R^2	R^3	X	method	yield, %	mp, °C	recrystn solvent	formula	rat PCA: ID_{50},[a] μmol/kg iv
DSCG											1.0
1											240.0
4											0.6
5[b]		(CHINOIN-1045)									0.3
6[c]											54.8
10	H	6-Me	3'-COOH	H	CH	B	66	261	EtOH	$C_{17}H_{17}N_3O_3$	>100
11	H	6-Me	4'-COOH	H	CH	B	77	245	EtOH	$C_{17}H_{17}N_3O_3$	>100
12	COOEt	6-Me	H	H	CH	A	83	181-182	EtOH	$C_{19}H_{21}N_3O_3$	insol
13	COOEt	6-Me	2'-COOH	H	CH	A	97	196	EtOH	$C_{20}H_{21}N_3O_5$	24.8
14	COOEt	6-Me	3'-COOH	H	CH	A	83	160-162	EtOH	$C_{20}H_{21}N_3O_5$	100
15	COOEt	6-Me	4'-COOH	H	CH	A	95	265	EtOH	$C_{20}H_{21}N_3O_5$	67.1
16	COOH	6-Me	H	H	CH	A	90	262-263	MeCN	$C_{17}H_{17}N_3O_3$[4]	insol
17	COOH	8-Me	H	H	CH	B	75	290	MeCN	$C_{17}H_{17}N_3O_3$	insol
18	COOH	H	2'-COOH	H	CH	A	90	268	DMF	$C_{17}H_{15}N_3O_5$	22.9
19	COOH	6-Me	2'-COOH	H	CH	A	77	240-242	EtOH[d]	$C_{18}H_{17}N_3O_5$	1.11
20	COOH	7-Me	2'-COOH	H	CH	A	73	250	EtOH[d]	$C_{18}H_{17}N_3O_5$	48.1
21	COOH	8-Me	2'-COOH	H	CH	B	73	252	EtOH[d]	$C_{18}H_{17}N_3O_5$	100
22	COOH	6-Me	2'-F	H	CH	A	70	220-222	EtOH	$C_{17}H_{16}N_3O_3F$	14.5
23	COOH	6-Me	4'-F	H	CH	A	87	217-218	EtOH	$C_{17}H_{16}N_3O_3F$	10.6
24	COOH	6-Me	3'-Cl	H	CH	A	75	239-240	MeCN	$C_{17}H_{16}N_3O_3Cl$	insol
25	COOH	6-Me	4'-Cl	H	CH	A	98	240-242	AcOH	$C_{17}H_{16}N_3O_3Cl$	insol
26	COOH	6-Me	3'-Br	H	CH	A	64	245-247	DMF	$C_{17}H_{16}N_3O_3Br$	insol
27	COOH	6-Me	4'-Br	H	CH	A	87	235-237	DMF	$C_{17}H_{16}N_3O_3Br$	insol
28	COOH	6-Me	2'-OH	H	CH	A	95	245	AcOH	$C_{17}H_{17}N_3O_4$	4.11
29	COOH	6-Me	4'-OH	H	CH	A	93	243-244	AcOH	$C_{17}H_{17}N_3O_4$	1.2
30	COOH	6-Me	4'-OMe	H	CH	A	91	240-242	AcOH	$C_{18}H_{19}N_3O_4$	insol
31	COOH	6-Me	3'-OEt	H	CH	A	90	218-220	MeCN	$C_{19}H_{21}N_3O_4$	insol
32	COOH	6-Me	4'-OEt	H	CH	A	89	221-223	MeCN	$C_{19}H_{21}N_3O_4$	8.96
33	COOH	6-Me	3'-Me	H	CH	A	89	242-244	DMF	$C_{18}H_{19}N_3O_3$	0.8
34[b,e]	COOH	6-Me	3'-Me	H	CH	A	85	218-219	EtOH	$C_{18}H_{19}N_3O_3$	0.02
35[c,f]	COOH	6-Me	3'-Me	H	CH	A	89	218	EtOH	$C_{18}H_{19}N_3O_3$	66.9
36	COOH	6-Me	4'-Me	H	CH	A	92	235-236	AcOH	$C_{18}H_{19}N_3O_3$	3.6
37	COOH	6-Me	4-CF_3	H	CH	A	75	240	MeCN	$C_{18}H_{16}N_3O_3F_3$	>100
38	COOH	6-Me	4'-NO_2	H	CH	A	69	260	DMF	$C_{17}H_{16}N_4O_5$	insol
39	COOH	6-Me	4'-COMe	H	CH	A	68	238	AcOH	$C_{19}H_{19}N_3O_4$	0.75
40	COOH	6-Me	4'-CH_2COOH	H	CH	A	89	238-240	AcOH	$C_{19}H_{19}N_3O_5$	5.8
41	COOH	6-Me	3',4'-$(CH{=}CH)_2$		CH	A	90	250-251	AcOH	$C_{21}H_{19}N_3O_3$	insol
42	COOH	6-Me	H	H	N	A	56	240-242	AcOH	$C_{16}H_{16}N_4O_3$	2.4

[a] All data are considered significant at $p \geq 0.05$ as determined by Student's *t* test. [b] Dextrorotatory isomer with 6*S* absolute configuration. [c] Levorotatory isomer with 6*R* absolute configuration. [d] Refluxed in the solvent given. [e] $[\alpha]^{20}_D$ +200° (*c* 0.5, DMF). [f] $[\alpha]^{20}_D$ −200° (*c* 0.5, DMF).

Table II. Pyrrolo[1,2-*a*]pyrimidines and Azepino[1,2-*a*]pyrimidines

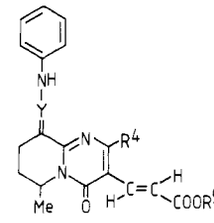


43-50

compd	R	R^1	R^2	Y	*n*	method	yield, %	mp, °C	recrystn solvent	formula	rat PCA: ID_{50},[a] μmol/kg iv
43	Et	H	H	CH	0	A	86	260–263	AcOH	$C_{17}H_{17}N_3O_3$	
44	H	H	H	CH	0	C	81	260–262	AcOH	$C_{15}H_{13}N_3O_2 \cdot AcOH$	15.4
45	Et	Me	H	CH	0	A	88	236–238	EtOH	$C_{18}H_{19}N_3O$	
46	H	Me	H	CH	0	C	97	257–258	AcOH	$C_{16}H_{15}N_3O_3$	20.2
47	Et	H	2′-COOH	CH	2	A	78	235–237	EtOH	$C_{20}H_{21}N_3O_5$	
48	H	H	2′-COOH	CH	2	C	82	240	AcOH	$C_{18}H_{17}N_3O_5$	68.7
49	Et	H	H	N	2	D	90	155–156	AcOEt	$C_{18}H_{20}N_4O_3$	
50	H	H	H	N	2	C	91	270–273	AcOH	$C_{16}H_{16}N_4O_3$	14.4

[a] All data are considered significant at $p \geq 0.05$ as determined by Student's *t* test.

Table III. 4-Oxo-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-3-acrylic Acid Derivatives



61-70

compd	R^4	R^5	Y	method	yield, %	mp, °C	recrystn solvent	formula	rat PCA: ID_{50},[a] μmol/kg iv
61	H	Et	N	E	42	182	EtOH	$C_{20}H_{22}N_4O_3$	
62	H	H	N	C	91	255	MeOH[b]	$C_{18}H_{18}N_4O_3$	0.27
63[c,d]	H	Et	N	E	52	170–172	EtOH	$C_{20}H_{22}N_4O_3$	
64[c,e]	H	H	N	C	89	258	EtOH	$C_{18}H_{18}N_4O_3$	0.15
65[f,g]	H	Et	N	E	52	170–172	EtOH	$C_{20}H_{22}N_4O_3$	
66[f,h]	H	H	N	C	78	258	EtOH	$C_{18}H_{18}N_4O_3$	>100
67	Me	Et	N	E	53	241–242	MeCN	$C_{21}H_{24}N_4O_3$	
68	Me	H	N	C	90	270	MeOH[b]	$C_{19}H_{20}N_4O_3$	0.29
69	Ph	Et	CH	B	94	186–188	EtOH	$C_{27}H_{27}N_3O_3$	
70	Ph	H	CH	C	93	255–257	AcOH	$C_{25}H_{23}N_3O_3$	insol

[a] All data are considered significant at $p \geq 0.05$ as determined by Student's *t* test. [b] Refluxed in the solvent given. [c] Dextrorotatory isomer with 6*S* absolute configuration. [d] $[\alpha]^{20}_D$ +550° (*c* 0.1, MeOH). [e] $[\alpha]^{20}_D$ +588° (*c* 0.2, DMF). [f] Levorotatory isomer with 6*R* absolute configuration. [g] $[\alpha]^{20}_D$ −552° (*c* 0.1, MeOH). [h] $[\alpha]^{20}_D$ −590° (*c* 0.2, DMF).

The effects of some 9-(aminomethylene)tetrahydropyridopyrimidine-3-carboxylic acids on the rat passive peritoneal anaphylaxis (PPA) test were investigated after intraperitoneal administration and were compared with those of DSCG and Chinoin-1045 (compound **5**). The results in Table IV indicate that the 9-aminomethylene derivatives **19**, **33**, **40**, and **42** are very active inhibitors of histamine release and Evans blue extravasation. The most active compound, 9-[[(*m*-methylphenyl)amino]-methylene]tetrahydropyridopyrimidine-3-carboxylic acid (**33**), had 8 times the activity of the reference DSCG in the inhibition of histamine release, but it did not surpass the activity of Chinoin-1045 (**5**).

Table IV. Inhibitory Effect on PPA in Rats

compd	inhib of Evans blue extravasation, ID_{50}, nmol	inhib of histamine release, ID_{50}, nmol
DSCG	55	16
5[a]	5	1
19	140	10
33	130	2
40	280	110
42	360	100

[a] Chinoin-1045.

Experimental Section

Melting points were not corrected. Combustion analyses for C, H, N, and halogen gave results within 0.4% of theory. The procedures for the preparation of the reported compounds, methods A–E, may be considered as general methods for preparation. Yields were not maximized. Spectra of the products (UV, Pye Unicam SP 8-200; IR, Zeiss UR 20; ^{1}H NMR, Bruker WP 80) are in full accord with the proposed structures. Optical rotations were determined by use of a Zeiss polarimeter.

Preparation of the Enantiomers 52 and 53. The enantiomers **52** and **53** of 6-methyl-9-[(dimethylamino)methylene]-

3-formyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one were prepared from the respective enantiomers of 6-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one[12] with $POCl_3$/DMF, analogously to the preparation of the racemic pyridopyrimidin-4-one[7] **51**. Enantiomer **52** with 6*S* absolute configuration was obtained in 68% yield, mp 190–191 °C from ethanol, $[\alpha]^{20}_D$ −510° (*c* 0.2, MeOH). Anal. ($C_{13}H_{17}N_3O_2$) C, H, N. Enantiomer **53** with 6*R* absolute configuration was obtained in 66% yield, mp 191 °C from ethanol, $[\alpha]^{20}_D$ +512° (*c* 0.2, MeOH). Anal. ($C_{13}H_{17}N_3O_2$) C, H, N.

Ethyl 9-Formyl-6-methyl-2-phenyl-4-oxo-1,6,7,8-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidine-3-acrylate (60). 3,9-Diformyl-6-methyl-2-phenyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]-pyrimidin-4-one[7] (**55**; 8.88 g, 30 mmol) and (carbethoxymethylene)triphenylphosphorane[10] (10.45 g, 30 mmol) were stirred in dimethyl sulfoxide (40 mL) at ambient temperature for 24 h. The precipitated crystals were filtered off, washed with ethanol, dried, and crystallized from ethanol to give the title compound **60** (7.4 g, 67%), mp 205–207 °C. Anal. ($C_{21}H_{22}N_2O_2$) C, H, N. ^{1}H NMR ($CDCl_3$) δ 1.32 (3 H, d, 6-Me), 1.27 (3 H, t, CH_3), 1.60–2.20 (2 H, m, H_2-7), 2.40–2.60 (2 H, m, H_2-8), 4.19 (2 H, q, OCH_2), 4.90–5.30 (2 H, m, H_2-6), 7.28 (1 H, d, 3-CH=, J = 16 Hz), 7.53 (1 H, d, =CHCO, J = 16 Hz), 7.53 (5 H, s, 2-Ph), 8.85 (1 H, d, 9-CHO, ^3J = 2 Hz), 15.97 (1 H, br, NH).

Diazonium Coupling. To a solution of sodium acetate trihydrate (12 g) and phenyldiazonium chloride, prepared from aniline (1.86 g, 20 mmol) in 5.5 M hydrochloric acid (10 mL) with a solution of sodium nitrite (1.44 g, 20 mmol) in water (10 mL) at 0 °C by the usual procedure,[15] was added dropwise a solution of the requisite 9-[(dimethylamino)methylene]-3-formylpyridopyrimidinone (**51–54**; 20 mmol) in 75% acetic acid (60 mL) at 0 °C. The mixture was stirred at 0 °C for 3 h and was then poured into water (100 mL). The precipitated crystals were filtered off, washed with water, dried, and crystallized to give 3-formyl-9-(phenylhydrazono)pyridopyrimidin-4-one (**56–59**). Compound **56**: yield 97%; mp 166–167 °C from acetonitrile. Anal. ($C_{16}H_{16}N_4O_2$) C, H, N. Compound **57** with 6*S* absolute configuration: yield 95%; mp 198 °C from ethanol, $[\alpha]^{20}_D$ +428° (*c* 0.2, DMF). Anal. ($C_{16}H_{16}N_4O_2$) C, H, N. Compound **58** with 6*R* absolute configuration: yield 92%; mp 196–197 °C from ethanol, $[\alpha]^{20}_D$ −425° (*c* 0.2, DMF). Anal. ($C_{16}H_{16}N_4O_2$) C, H, N. Compound **59**: yield 82%; mp 197 °C from methanol. Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

Method A. A mixture of the (dimethylamino)methylene derivative **8**[7] (10 mmol) and the requisite amine (10 mmol) was stirred in glacial acetic acid (30 mL) at ambient temperature for 24 h. The reaction mixture was poured into water (100 mL) and the precipitated (arylamino)methylene derivative was filtered off, washed with water, dried, and crystallized.

Method B. This process was carried out as described under method A, but instead of the (dimethylamino)methylene derivative **8**, the appropriate formyl derivative **9**[7] was applied.

Method C. The requisite carboxylate or acrylate (10 mmol) was hydrolyzed in a mixture of water (40 mL) and ethanol (10 mL) in the presence of potassium hydroxide (1.68 g, 30 mmol). The applied temperatures and reaction periods were as follows: 55 °C and 1.5 h for compounds **43**, **45**, and **49**; 25 °C and 3 h for compound **47**; and reflux temperature and 1 h for compounds **61**, **63**, **65**, **67**, and **69**. The pH of the reaction mixture was adjusted to 3 with acetic acid. The precipitated carboxylic acid or acrylic acid was filtered off, washed with water, dried, and crystallized.

Method D. To a solution of sodium acetate trihydrate (4.7 g) and phenyldiazonium chloride, prepared from aniline (7.8 mmol) as above, in 5.5 M hydrochloric acid, was added dropwise a solution of ethyl 10-[(dimethylamino)methylene]-4-oxo-4,6,7,8,9,10-hexahydroazepino[1,2-*a*]pyrimidine-3-carboxylate[8] (2.27 g, 7.8 mmol) in methanol (20 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 4 h and was then poured into water (80 mL). The precipitated crystals were filtered off, washed with water, dried, and crystallized from ethyl acetate to give the azepinopyrimidinecarboxylate **49** (yield 2.4 g, 90%), mp 155–156 °C. Anal. ($C_{18}H_{20}N_4O_3$) C, H, N.

Method E. A mixture of the requisite 3-formyl-9-(phenylhydrazono)pyridopyrimidin-4-one (**56–59**; 20 mmol) and (carbethoxymethylene)triphenylphosphorane[10] (6.97 g, 20 mmol) was stirred in dimethyl sulfoxide (60 mL) at ambient temperature for 24 h. The reaction mixture was poured into water (240 mL). The aqueous phase was decented off and the residue crystallized to give the appropriate acrylate.

Passive Cutaneous Anaphylaxis (PCA) Test. Adult female Sprague–Dawley rats (200 g, five rats per group) were sensitized at two sites with an intradermal injection (0.05 mL) of rat serum containing reaginic antibodies to chicken ovalbumin. After a 48-h latency period, the animals were challenged with 20 mg/kg of chicken ovalbumin, together with 40 mg/kg of Evans blue. Thirty minutes later, the rats were sacrificed and skinned. The area of the dermal bluing that occurred at the sites of sensitization was measured (100 mm^2 spot in the control rats), and the results were used to calculate the drug-induced percentage inhibition of this effect. For iv administration, the test compounds (32, 3.2, and 0.32 μmol/kg) were injected at the same times as the antigen challenge. At least three doses and five animals for each dose (i.e., 10 spots) were used to obtain a dose–inhibition relationship. The dose that inhibited the PCA by 50% (ID_{50}) was determined from a dose–response regression curve for each compound. The statistical significance of the results was determined by the Student's t test ($p \leq 0.05$).

Passive Peritoneal Anaphylaxis (PPA) Test. The following method was derived from that described by Ross et al.[16] Adult male Sprague–Dawley rats (~250 g, six rats per group) were passively sensitized by intraperitoneal injection of 1 mL of anti-ovalbumin reaginic serum; 4 h later, the rats received 0.3 mL of Evans blue (5% solution in isotonic saline) intravenously, followed by an intraperitoneal injection of the antigen (1 mg/rat) dissolved in 5 mL of a buffered solution (Tris, 3.75 g; NaCl, 6.95 g; KCl, 0.370 g; $CaCl_2 \cdot 2H_2O$, 0.090 g; $MgCl_2 \cdot 6H_2O$, 0.23 g; HCl to adjust pH to 7.4 in a volume of 1 L), which was heparinized (1 mL of Liquemine Roche for 1 L of buffered solution).

For intraperitoneal administration, the test compounds (32, 3.2, and 0.32 nmol/kg) were injected 30 s before the anaphylaxis.

Exactly 3 min after the allergen was administered, the animals were decapitated, and peritoneal fluid was collected. Supernatant was obtained after centrifugation at 150*g* and the histamine content was determined by flourometric method as described by Sheard,[17] and adapted to Technicon autoanalysis by Evans.[18]

Extravased Evans blue was measured photometrically at 615 nm by using a Uvikon spectrophotometer. A negative control group consisted of animals that received neither the antigen nor the inhibitor. A positive control group contained animals that received only antigen without inhibitor, while the remaining groups received the antigen preceded by increasing dosages of the inhibitor. At least three doses and six animals for each doses were used to obtain a dose–inhibition relationship. The ID_{50} dose was determined from a dose–response regression curve for each compound. The statistical significance of the results was determined by the Student's t test ($p \leq 0.05$).

Registry No. **10**, 91111-49-8; **11**, 91111-50-1; **12**, 71165-72-5; **13**, 91111-51-2; **14**, 91111-52-3; **15**, 91111-53-4; **16**, 71165-95-2; **17**, 91111-54-5; **18**, 91111-55-6; **19**, 91111-56-7; **20**, 91111-57-8; **21**, 91111-58-9; **22**, 91111-59-0; **23**, 91111-60-3; **24**, 91111-61-4; **25**, 91111-62-5; **26**, 91111-63-6; **27**, 91111-64-7; **28**, 91111-65-8; **29**, 91111-66-9; **30**, 91111-67-0; **31**, 91111-68-1; **32**, 91111-69-2; **33**, 91111-70-5; **34**, 91111-71-6; **35**, 91111-72-7; **36**, 91111-73-8; **37**, 91111-74-9; **38**, 91111-75-0; **39**, 91111-76-1; **40**, 91111-77-2; **41**, 91111-78-3; **42**, 91111-79-4; **43**, 91111-80-7; **44**, 91111-81-8; **45**, 91111-82-9; **46**, 91111-83-0; **47**, 91111-84-1; **48**, 91111-85-2; **49**, 91129-66-7; **50**, 91111-86-3; **51**, 91177-80-9; **52**, 91177-81-0; **53**, 91177-82-1; **54**, 91177-83-2; **55**, 85808-59-9; **56**, 91111-96-5; **57**, 91177-84-3; **58**, 91177-85-4; **59**, 91111-97-6; **60**, 91111-98-7; **61**, 91111-87-4; **62**, 91129-67-8; **63**, 91111-88-5; **64**, 91111-89-6; **65**, 91111-90-9; **66**, 91111-91-0; **67**, 91111-92-1; **68**, 91111-93-2; **69**,

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91111-94-3; **70**, 91111-95-4; (6*S*)-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, 71165-90-7; (6*R*)-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, 88243-65-6; (carbethoxymethylene)triphenylphosphorane, 1099-45-2; phenyldiazonium chloride, 100-34-5; ethyl 10-[(dimethylamino)methylene]-4-oxo-4,6,7,8,9,10-hexahydroazepino[1,2-*a*]pyrimidine-3-carboxylate, 87932-12-5; 4*H*-pyrido[1,2-*a*]pyrimidin-4-one, 23443-10-9.

Interaction of N^4-Hydroxy-2′-deoxycytidylic Acid with Thymidylate Synthetase

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The interaction of dTMP synthetase with N^4-hydroxy-2′-deoxycytidylate (N^4-HOdCMP) has been investigated. With use of standard assay conditions, N^4-HOdCMP is a competitive inhibitor with an apparent K_i of 8.0 μM. Incubation of N^4-HOdCMP with dTMP synthetase in the presence of 5,10-methylenetetrahydrofolate (CH_2-H_4folate) resulted in a rapid time-dependent inactivation of the enzyme which was not first order and the formation of complexes which could be isolated on nitrocellulose filter membranes. With use of radioactive ligands, the isolable native complex was shown to possess 2 mol of N^4-HOdCMP and 2 mol of CH_2-H_4folate/mol of dimeric enzyme; the apparent dissociation constant of N^4-HOdCMP was 1.0 μM. Ultraviolet difference spectroscopy of the ternary complex showed a loss of the pyrimidine chromophore which did not reappear upon denaturation with $NaDodSO_4$. The rate of dissociation of N^4-HOdCMP from the ternary complex was biphasic in which one-half of the initially bound ligand dissociated with $t_{1/2} \simeq 2.3$ min and the remainder with $t_{1/2} \simeq 13$ min. When the N^4-HOdCMP–CH_2-H_4folate–enzyme complex was denatured, one-half of the CH_2-H_4folate dissociated whereas all of the N^4-HOdCMP remained bound to the enzyme. Taken together, our results indicate that N^4-HOdCMP forms a covalent bond with dTMP synthetase and reveal an unusual asymmetry in the two subunits of the N^4-HOdCMP–CH_2-H_4folate–enzyme complex. It appears that one subunit is covalently bound to N^4-HOdCMP, which, in turn, is covalently linked to CH_2-H_4folate whereas the other subunit is covalently bound to N^4-HOdCMP but CH_2-H_4folate is bound by noncovalent interactions.

dTMP synthetase (EC 2.1.1.45) catalyzes the reductive methylation of dUMP to dTMP with concomitant conversion of CH_2-H_4folate to H_2folate. Because this enzyme is essential for de novo synthesis of dTMP, much effort has been invested in the development and studies of inhibitors (see ref 3–5). Thus far, the most successful inhibitors have been 5-substituted dUMPs, which, in general, bind reasonably well to the enzyme. further, some 5-substituted dUMPs act as potent mechanism-based inhibitors; such inhibitors reversibly bind to the enzyme and undergo events in a manner analogous to the normal catalytic reaction, ultimately leading to stable covalent complexes involving covalent bond formation between the catalytic thiol of the enzyme and the 6-position of the heterocycle. Although other analogues of dUMP have been examined, modifications at positions other than the 5-carbon generally lead to substantial decreases in affinity for the enzyme.[5] One interesting exception to this is N^4-HOdCMP, which reversibly binds to dTMP synthetase about as well as the substrate, dUMP.[6,7] In addition, it has been reported that incubation of N^4-HOdCMP and CH_2-H_4folate with the enzyme from chick embryo results in time-dependent inactivation of the enzyme and a resultant change from competitive to noncompetitive inhibition kinetics.[7] In this report, we describe studies of the interaction of N^4-HOdCMP with dTMP synthetase from *Lactobacillus casei* which demonstrate that it is a mechanism-based inhibitor of the enzyme and reveal an unusual asymmetry in binding of the analogue to the subunits of the dimeric enzyme.

Results

By use of the conventional spectrophotometric assay for *L. casei* dTMP synthetase and initiation of the reaction with enzyme, N^4-HOdCMP was a competitive inhibitor with respect to dUMP with an apparent K_i of 8.0 μM. When N^4-HOdCMP was incubated with the enzyme in the absence of CH_2-H_4folate, there was no loss of activity for as long as 60 min. As previously reported for the enzyme from chick embryo,[7] when the cofactor was included in the incubation buffer, there was a time-dependent loss of activity. For example, with use of 15 nM dTMP synthetase, 0.15 mM CH_2-H_4folate, and 80 μM N^4-HOdCMP, 50% loss of activity occurred at about 20 s. However, experimental difficulties did not permit a kinetic evaluation of the time-dependent inactivation. First, there were considerable uncertainties in determinations of remaining enzyme activities by initial velocity measurements; when excess dUMP was added to aliquots of the incubation mixture, the initial velocity increased with time ultimately approaching that of the control. As described below, this phenomenon is attributable to dissociation of the enzyme–inhibitor complex during the period of assay. Second, the loss of activity did not appear to be a first-order process and classical analysis of time-dependent inhibition was not possible.

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(2) Abbreviations used are: N^4-HOdCMP, N^4-hydroxy-2′-deoxycytidylic acid; 5-CH_3-N^4-HOdCMP, 5-methyl-N^4-hydroxy-2′-deoxycytidylic acid; FdUMP, 5-fluoro-2′-deoxyuridylic acid, CH_2-H_4folate, (6*RS*)-L-5,10-methylenetetrahydrofolic acid; $(n\text{-}Bu)_4N^+HSO_4^-$, tetrabutylammonium hydrogen sulfate; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid; $NaDodSO_4$, sodium dodecyl sulfate. All other abbreviations are as suggested by IUPAC.
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