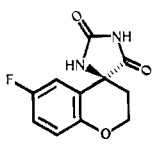
Spiro Oxazolidinedione Aldose Reductase Inhibitors

Rodney C. Schnur,* Reinhard Sarges, and Michael J. Peterson

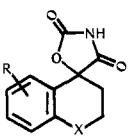
Central Research, Pfizer Inc., Groton, Connecticut 06340. *Received May 3, 1982*

Spiro oxazolidinediones (**2**) derived from five- and six-membered ring aralkyl ketones are potent aldose reductase inhibitors in vitro and in vivo. Their novel and general synthesis has been devised with α-hydroxyimidates (**5**) and 4-alkoxy-2-oxo-3-oxazolines (**6**) as key intermediates, since traditional synthetic routes through α-hydroxy amides (**8**) usually led to α,β-unsaturated amides (**9**). Resolution with cinchonidine afforded optically active spiro oxazolidinediones. Optimum biological activity resided in (4*S*)-6-chlorospiro[4*H*-2,3-dihydrobenzopyran-4,5′-oxazolidine]-2′,4′-dione (**21**) and its 6,8-dichloro congener (**23**).

Increased flux of glucose through the "polyol pathway" has been implicated in the etiology of diabetic complications, including accelerated cataract formation and possibly neuropathies and retinopathy.[1] Since a key step in this pathway is the aldose reductase catalyzed formation of sorbitol, it has been postulated that inhibition of this enzyme would ameliorate or retard the development of these diabetic complications. The search for inhibitors of aldose reductase has been ongoing for several years in these and other laboratories.[2] We have found that the spiro hydantoin (**1**) is a moderately potent inhibitor of calf lens



1

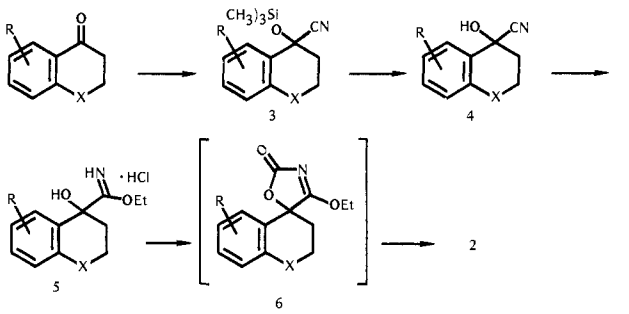


2

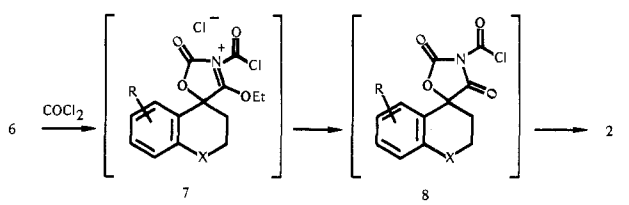
aldose reductase[3] in vitro and is very effective in preventing sorbitol accumulation in the sciatic nerve in streptozotocin-diabetic rats in vivo.[4] These encouraging findings prompted us to investigate the role of hydantoin ring modification on aldose reductase inhibition. This article describes the chemistry and pharmacology of spiro oxazolidinediones (**2**) as one example of non-hydantoin aldose reductase inhibitors.

Chemistry. A chemistry review[5] of 2,4-oxazolidinediones indicated that the most promising reaction scheme for such compounds was that originally described by Stoughton.[6] This involved in our case the conversion of

Scheme I



Scheme II



cyanohydrin **4** to an intermediate α-hydroxy amide, **10**, with concentrated HCl, followed by cyclization to **2** with alkyl carbonate and alkoxide.

Compounds **11**, **14**, **15**, and **17** could be made by this method. However, attempts to prepare, for example, **12**, **16**, and **18** by this route failed, leading to negligible generation of the key intermediate **10**. In contrast, the preparation of all spiro oxazolidinediones could be accomplished by the following general route: conversion of ketones to cyanohydrins **4**, followed by treatment with anhydrous ethanolic HCl to give α-hydroxy imidates **5**, the key intermediates, and transformation of these into oxazolidinediones by a variety of reagents, the most efficient being phosgene and carbonyl diimidazole (Scheme I).

The cyanohydrins **4** were formed expediently in situ from trimethylsilyl cyanohydrins **3**, which in turn were conveniently and quantitatively prepared from ketones with trimethylsilyl cyanide and zinc iodide in ether.[7] This procedure obviated the need to prepare **4** by the more hazardous and cumbersome liquid HCN procedure described by Stoughton.[6] In practice, the moisture-sensitive **4** was usually not isolated; rather **3** was treated with ethanolic HCl to give imidates **5** directly in good yield. The water-soluble α-hydroxy imidate hydrochloride could be purified either by aqueous extraction, followed by liberation of the free imidate with basification, or by trituration of the HCl salt of **5** with ether. The oxazolidinediones (**2**)

(1) For recent reviews of polyol pathway hypothesis, see Jin H. Kinoshita, Peter Kador, and Manuel Catiles, *J. Am. Med. Assoc.*, **246**, 257 (1981); R. S. Clements, Jr. *Diabetes*, **28**, 604 (1979); D. Dvornik, *Annu. Rep. Med. Chem.*, **13**, 159 (1978); J. W. Anderson, *Am. J. Clin. Nutr.*, **28**, 273 (1975); K. Gabbay, *N. Engl. J. Med.*, **288**, 831 (1973); R. van Heyningen, *Nature (London)*, **184**, 194 (1959); J. H. Kinoshita, *Invest. Ophth.*, 4, 786 (1965); K. H. Gabbay and J. B. O'Sullivan, *Diabetes*, 17, 239 (1968); P. Beaumont, F. C. Hollows, P. J. Schofield, J. F. Williams, and W. Steinbeck, *Lancet*, 1, 579 (1971).

(2) R. Sarges, U.S. Patent 4 130 714 (1978); 4 147 795 (1979); R. Sarges, J. L. Belletire, R. C. Schnur, and M. J. Peterson, "Abstracts of Papers", 177th National Meeting of the American Chemical Society, Honolulu, HI, Apr 1–6, 1979, American Chemical Society, Washington, DC, 1979. L. D. Waterbury, J. M. Mahoney, and J. R. Pfister, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **38**, 254 (1979); M. E. Maragoudakis, J. Wasvery, P. Gargiulo, and H. Hankin, *ibid.*, **38**, 255 (1979); F. Fauran, C. Fenion, J. Mosser, and G. Prat, *Eur. J. Med. Chem.*, **13**, 503 (1978); D. Dvornik, "Abstracts of Papers", 174th National National Meeting of the American Chemical Society, Chicago, IL, 1977, American Chemical Society, Washington, DC, 1977.

(3) S. Hayman and J. H. Kinoshita, *J. Biol. Chem.*, **240**, 877 (1965).

(4) M. J. Peterson, R. Sarges, C. E. Aldinger, and D. P. McDonald, *Metabolism*, **28**(Suppl. 1), 456 (1979).

(5) J. W. Clark-Lewis, *Chem. Rev.*, **58**, 63 (1958).

(6) (a) R. W. Stoughton, *J. Am. Chem. Soc.*, **63**, 2376 (1941); (b) V. H. Wallingford, M. A. Thorpe, and R. W. Stoughton, *ibid.*, **67**, 522 (1945).

(7) (a) D. A. Evans, L. K. Truesdale, and G. L. Carroll. *J. Chem. Soc., Chem. Commun.* 53 (1973); (b) D. A. Evans, G. L. Carroll, and L. K. Truesdale, *J. Org. Chem.*, **39**, 914 (1974).

0022-2623/82/1825-1451$01.25/0

Table I. Aldose Reductase Inhibiting Oxazolidinediones 2

no.	R	X	mp	formula[a]	yield, %	method[b]	IC_{50},[c] M	ED_{50},[d] mg/kg
10	H	O	168-170	$C_{11}H_9NO_4$	57	P	10^{-5}	
11	6-Cl	O	196-198	$C_{11}H_8ClNO_4$	79	P	$10^{-6}/10^{-7}$	>1.5 (28)
12	6-F	O	177.5-180	$C_{11}H_8FNO_4 \cdot {}^1/_6H_2O$	43	I	$10^{-6}/10^{-7}$	>1.5 (8)
13	6-Br	O	188-191	$C_{11}H_8BrNO_4$	38	I	10^{-7}	>1.5 (35)
14	6,8-Cl_2	O	193-195	$C_{11}H_7Cl_2NO_4$	57	A	$10^{-7}/10^{-8}$	>1.5 (44)
15	6-Cl, 8-CH_3	O	185.5-187	$C_{12}H_{10}ClNO_4$	22	A	$10^{-6}/10^{-7}$	>1.5 (33)
16	H	S	165-167	$C_{11}H_9NO_3S$	45	I	10^{-6}	25 (16)
17	6-Cl	S	213-216	$C_{11}H_8ClNO_3S$	38	A	$10^{-6}/10^{-7}$	>1.5 (14)
18	6-F	S	193-194.5	$C_{11}H_8FNO_3S$	41	I	10^{-6}	25
19	H	CH_2	170-172	$C_{12}H_{11}NO_3$	28	I	10^{-5}	*e*
20	H		163-164.5	$C_{11}H_9NO_3$	36	I	10^{-5}	>25 (22)
21	6-Cl	O	200-200.5	(−)$C_{11}H_8ClNO_4$	60		10^{-7}	1.5-2.5
22	6-Cl	O	200-201.5	(+) $C_{11}H_8ClNO_4$	40		$10^{-4}/10^{-5}$	
23	6,8-Cl_2	O	221-223	(−) $C_{11}H_7Cl_2NO_4$	49		$10^{-7}/10^{-8}$	1.5-2.5
24	6,8-Cl_2	O	223-224	(+) $C_{11}H_7Cl_2NO_4$	33		10^{-5}	

[a] All new compounds analyzed for C, H, and N. [b] Compounds prepared via Scheme I (see text) are denoted P (phosgene) or I (1,1-carbonyldiimidazole); compounds prepared via Scheme III are denoted A; selected preparative procedures are found under Experimental Section, while complete procedures of all intermediates and final products may be found in U.S. Patent 4 200 642 (1980). [c] Concentration that causes a 50% inhibition of partially purified calf lens aldose reductase using glyceraldehyde as a substrate. [d] Oral dose given t.i.d. which causes a 50% reduction in sorbitol levels of streptozotocin-diabetic rat sciatic nerve, numbers in parentheses denote % decrease in nerve sorbitol levels at stated dose. [e] Inactive at 50 mg/kg.

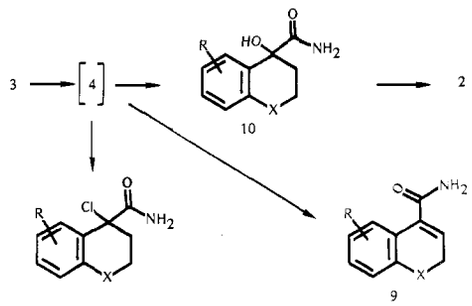
were prepared from **5** by treatment with 1,1′-carbonyldiimidazole (method I) or optimally with triethylamine and excess phosgene (method P). With the latter procedure, the imidate hydrochloride could be used directly by employing an additional equivalent or triethylamine. The use of ethyl chloroformate in place of phosgene gave **2** in lower yield.

Oxazoline **6** was an isolable intermediate of these reactions and could be converted to **2** by heating with imidazole or by treatment with a slight excess of potassium carbonate in aqueous THF. When imidazole was used, *N*-ethylimidazole was formed as a byproduct, probably via an S_N2 process. Although no other intermediates could be isolated in the transformation of **6** to **2** using phosgene, it is likely that **6** is acylated with excess phosgene to give an *N*-acyl iminium ion **7**. This loses the ethyl group as ethyl chloride (or possibly ethylene), yielding **8** (Scheme II). Intermediate **8** would be expected to decompose upon aqueous workup to give the oxazolidinedione. Compound **7** should be highly reactive and is analogous to the *N*-acyl ammonium ions formed during tertiary amine dealkylations with chloroformates[8] where chloride serves as a nucleophile to decompose those intermediates.

This mechanism may also explain why the reaction of ethyl chloroformate with **5** as free base or as hydrochloride with triethylamine present did not go to completion at 0 °C or above. Addition of excess amine and/or ethyl chloroformate did not increase the yield, since triethylamine probably competes successfully with imidate as a nucleophile, consuming the chloroformate in a catalytic manner.[9]

In a number of cases where the original Stoughton procedure failed to generate α-hydroxy amides from cyanohydrins, the products obtained were either α,β-unsaturated amides **9** (e.g., 6-fluorochromanone) or mixtures of **9**,**10**, and α-chloro amides, Scheme III (e.g., 6-chlorochromanone). Reaction of **9** with $Hg(OAc)_2$, $NaBH_4$, and

Scheme III



NaOH according to the method of Brown and Geoghegan[10] failed to give the desired α-hydroxy amide **10** but yielded only starting material. Other attempts to convert these byproducts to oxazolidinediones were also unsuccessful.

Spiro oxazolidinediones **2** are racemates, two examples of which, **11** and **14**,. have been resolved.[11] While brucine formed a racemic adduct with both **11** and **14**, resolution of both was accomplished by making the cinchonidine adduct. In both instances, (+) isomers formed adducts with the alkaloid. The mother liquor afforded a mixture greatly enriched with (−) isomer, which after several recrystallizations gave the pure (−) enantiomer, proven to be 4*S* for **21** by X-ray analysis.[12] Alternatively, addition of *d*-amphetamine to this mother liquor afforded a pure adduct with the (−) isomer of **11**, in high yield. Amphetamine also effected direct resolution of **11**, though this base was not as efficient, requiring multiple recrystallizations. Thus, both enantiomers were available to test the stereospecificity of enzyme inhibition.

Biological Results and Discussion

The spiro oxazolidinediones were examined for aldose reductase inhibitory activity in vitro and in vivo. The in vitro assay was performed in the manner of Hayman and

(8) (a) R. A. Olofson, R. C. Schnur, L. Bunes, and J. Pepe, *Tetrahedron Lett.*, 1567 (1977); (b) R. A. Olofson and R. C. Schnur, *ibid.*, 1571 (1977) and references therein.

(9) Ethyl chloroformate is known to react with triethylamine to give CO_2, ethyl chloride, and triethylamine as products in addition to some triethyl carbamate: J. A. Campbell, *J. Org. Chem.*, **22**, 1259 (1957).

(10) H. C. Brown, and P. Geoghegan, Jr., *J. Am. Chem. Soc.*, **89**, 1522 (1967).

(11) The only previous example of a chemical resolution of an oxazolidinedione is that of Butler using brucine for resolution of the anticonvulsant metabolite 5-ethyl-5-methyl-2,4-oxazolidinedione: T. C. Butler, *J. Pharmacol. Exp. Ther.*, **113**, 178 (1955).

(12) R. C. Schnur and J. Bordner, unpublished results.

Kinoshita[3] with partially purified calf lens aldose reductase, while the inhibition of sorbitol accumulation in rat sciatic nerves of diabetic rat, as described by Peterson et al., [4] provided the in vivo assay. The biological results are summarized in Table I. In vitro activity was enhanced by 6-halo substituents relative to unsubstituted compounds in both the chromans, **11** vs. **10**, and the thiochromans, **17** vs. **16**. In vivo the thiochroman derivatives were slightly less efficacious than chromans (**18** vs. **12**), while indan (**20**) and tetralin (**19**) oxazolidinediones were substantially less active. Aldose reductase inhibition resided in the (-) isomer of the resolved spiro oxazolidinediones **21** and **23**, which have the *S* configuration. Thus, the structure–activity relationships of spiro oxazolidinediones generally parallel that of the spiro hydandoins,[2] but the spiro oxazolidinediones proved to be generally less effective in vivo in the rat sciatic nerve model.

Experimental Section

All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by the Pfizer Central Research microanalysis laboratory, and results obtained for specified elements are within ±0.4% of the theoretical values unless otherwise denoted. IR spectra were obtained on a Perkin-Elmer Model 21 spectrophotometer using the stipulated solvents and are reported in reciprocal centimeters. ^{1}H NMR spectra of $CDCl_3$, CD_3OD, or $(CD_3)_2SO$ solutions [$(CH_3)_4Si$, δ 0] were recorded on a Varian A-60 or Perkin-Elmer T-60 spectrometer. High-resolution mass spectral data were recorded on an A.E.I. MS-30. Low-resolution mass spectral data were recorded on an Hitachi RMU6-E spectrometer.

Method P. 6-Chlorospiro[4*H*-2,3-dihydrobenzopyran-4,5′-oxazolidine]-2′,4′-dione (11). A 1-L, four-neck, round-bottom flask fitted with an overhead stirrer, a thermometer, a gas inlet tube, and a gas outlet tube leading to traps containing $PhNH_2$ in EtOH and then KOH in H_2O was charged with 10.0 g (0.039 mol) of ethyl 6-chloro-4-hydroxy-4*H*-2,3-dihydro-4-benzopyran-4-carboximidate (**5**, R = 6-Cl; X = O), 7.8 g (0.078 mol) of triethylamine, and 300 mL of THF. This cooled (0 °C) homogeneous solution was perfused gently with phosgene gas (**caution**: Toxic!) for 20 min. The resultant heterogeneous solution was stirred for 16 h at 20 °C and then poured into 300 mL of ice (**caution**: gas evolution!) and basified to pH 8.5 with 6 N NaOH. After the solution was diluted with 100 mL of 5% $NaHCO_3$ and stirred for 48 h, the separated organic layer was diluted with 100 mL of Et_2O and extracted with 2 × 100 mL of 5% $NaHCO_3$. The combined basic phases were acidified to pH 2 and extracted with 3 × 250 mL of EtOAc. The combined EtOAc extracts were washed with 50 mL of brine, dried (Na_2SO_4), and filtered, and the filtrate was evaporated in vacuo to a white solid: yield 7.2 g (78%). Recrystallization from toluene gave material of mp 196–198 °C; IR (KBr) 1818, 1735 cm^{-1}; NMR (CD_3OD) δ 2.2–2.7 (m, 2 H, CH_2), 4.1–4.7 (m, 2 H, CH_2), 6.84–7.36 (ABX, J_{AB} = 9 Hz, J_{AX} = 3 Hz, J_{BX} = 0 Hz, 3 H × centered at 7.10); mass spectrum, *m/e* 253 (P), 184 (P - CONHCO). Anal. ($C_{11}H_8ClNO_4$) C, H, N. Compound **11** was also prepared by method I (25%) and by method A (27%). Compound **11** (1.00 g, 3.95 mmol) and cinchonidine (1.16 g, 3.95 mmol) were dissolved in 10 mL of warm EtOH. The mother liquor obtained after filtration of the cooled reaction mixture was partitioned between 100 mL of EtOAc and 100 mL of 1 N HCl. The organic phase was washed with 100 mL each of 1 N HCl and brine, dried ($MgSO_4$), and evaporated in vacuo to a solid, which was recrystallized twice from hot toluene to give the (-) isomer, **21**: yield 143 mg (29%); $[\alpha]^{25}_D$ (EtOH) -61.7°. The (+) isomer, **22**, was obtained from the initial EtOH-insoluble material by a similar liberation/extraction procedure: yield 200 mg (40%); $[\alpha]^{25}_D$ (EtOH) +60.6°.

Method I. Spiro[4*H*-2,3-dihydrobenzothiopyran-4,5′-oxazolidine]-2′,4′-dione (16). A mixture of 0.475 g (2.00 mmol) of ethyl 4 hydroxy-4*H*-2,3-dihydrobenzothiopyran-4-carboximidate (**5**, X = S) and 0.356 g (2.20 mmol) of 1,1′-carbonyldiimidazole (Aldrich Chemical Co.) was heated at 100 °C for 7 h. The mixture was diluted with 100 mL of EtOAc, washed with 100 mL of 1 N HCl, and extracted with 2 × 100 mL of saturated $NaHCO_3$. The basic phase was acidified with 6 N HCl and extracted with 3 × 100 mL of Et_2O. The pooled Et_2O phases were washed with 2 × 50 mL of brine, dried ($MgSO_4$), and filtered, and the filtrate was evaporated in vacuo to a residue, which was crystallized from toluene: yield 254 mg (45%); mp 165–167 °C; IR (KBr) 1820, 1745 cm^{-1}; NMR [$CDCl_3$/$(CD_3)_2SO$] δ 2.4–2.7 (m, 2 H, CH_2), 3.1–3.3 (m, 2 H, CH_2), 7.0–7.3 (m, 4 H, arom); mass spectrum, *m/e* 235 (P), 164 (P - CONHCO). Anal. ($C_{11}H_9NO_3S$) C, H, N.

Method A. 6,8-Dichlorospiro[4*H*-2,3-dihydrobenzopyran-4,5′-oxazolidine]-2′,4′-dione (14). A mixture of 0.524 g (2.00 mmol) of 6,8-dichloro-4-hydroxy-4*H*-2,3-dihydrobenzopyran-4-carboxamide (**10**, R = 6,8-Cl_2; X = O), 0.53 g (4.50 mmol) of ethyl carbonate, 0.396 g (2.67 mmol) of potassium *tert*-butoxide (Aldrich Chemical Co.), and 1.80 mL of 1-butanol was heated at reflux for 64 h. The reaction mixture was quenched with 100 mL of 1 N H_2SO_4 and 100 mL of EtOAc. The aqueous phase was washed with another 100 mL of EtOAc, and the combined organic layers were then extracted twice with 50 mL of 5% $NaHCO_3$. The basic phase was acidified with 6 N HCl and extracted with 2 × 75 mL of EtOAc. This latter combined organic phase was washed with 50 mL of brine, dried ($MgSO_4$), and filtered, and the filtrate was evaporated in vacuo to a solid, 330 mg (57%), which was recrystallized from toluene: mp 193–195 °C; IR (KBr) 1815, 1735 cm^{-1}; NMR [$CDCl_3$/$(CD_3)_2SO$] δ 2.3–2.6 (m, 2 H, CH_2), 4.2–4.9 (m, 2 H, CH_2) 7.10 (d, *J* = 2 Hz, 1 H, arom), 7.45 (d, *J* = 2 Hz, 1 H, arom); mass spectrum, *m/e* 287 (P), 216 (P - CONHCO). Anal. ($C_{11}H_7Cl_2NO_4$) C, H, N.

Ethyl 6-Chloro-4-hydroxy-4*H*-2,3-dihydrobenzopyran-4-carboximidate (5, R = 6-Cl; X = O). To a cooled, 0–8 °C, solution of EtOH, which had been saturated with anhydrous HCl, was added 5.0 g (1.77 mmol) of 6-chloro-4-cyano-4-[(trimethylsilyl)oxy]-4*H*-2,3-dihydrobenzopyran (**3**, R = 6-Cl; X = O) [prepared by the method of Evans[7] and from Me_3SiCN, ZnI_2, and 6-chlorochromanone (Aldrich Chemical Co.) in Et_2O at 20 °C]. After 16 h at 0 °C, the volatiles were evaporated in vacuo leaving a residue which was triturated with ~300 mL of Et_2O and then partitioned between 100 mL of $CHCl_3$ and 60 mL of saturated $NaHCO_3$. The $CHCl_3$ phase was dried ($MgSO_4$) and filtered, and the filtrate was concentrated to a solid residue: yield 4.35 g (96%). Analytically pure imidate **5** (R = 6-Cl; X = O) could be obtained by multiple trituration with Et_2O: 90% yield; mp 124–126 °C; IR (CH_2Cl_2) 1653; NMR [$(CD_3)_2SO$] 1.09 (t, *J* = 7 Hz, 3 H, CH_3), 1.6–2.7 (br d m, 2 H, CH_2), 3.9–4.4 (m, 2 H, CH_2), 4.14 (q, *J* = 7 Hz, 2 H, CH_2), 6.55 (br d s, 1 H, OH), 6.6–7.4 (m, 3 H, arom), 8.40 (br d s, 1 H, NH); mass spectrum, *m/e* 255 (P), 228 (P - CH_2CH_3). Anal. ($C_{12}H_{14}ClNO_3$) C, H, N.

6,8-Dichloro-4-hydroxy-4*H*-2,3-dihydrobenzopyran-4-carboxamide (10, R = 6,8-Cl_2; X = O). A solution of 1.90 g (6.00 mmol) of 4-cyano-6,8-dichloro-4-[(trimethylsilyl)oxy]-4*H*-2,3-dihydrobenzopyran (**3**, R = 6,8-Cl_2; X = O, prepared from the ketone as above) in 1.8 mL of Et_2O at 0 °C was combined with 1.8 mL of concentrated HCl at 0 °C (after Stoughton[6]). The mixture was perfused with HCl gas for 10 min and stoppered. After warming to 20 °C and stirring for 16 h, the mixture was poured into ~40 mL of ice–H_2O and dried in vacuo: yield 1.31 g (83%); mp 200.0–200.5 °C; IR (KBr) 1664 cm^{-1}; NMR [$CDCl_3$/$(CD_3)_2SO$] δ 1.9–2.6 (m, 2 H, CH_2), 4.0–4.8 (m, 2 H, CH_2), 7.23 (d, *J* = 3 Hz, 1 H, arom), 7.52 (d, *J* = 3 Hz, 1 H, arom); mass spectrum *m/e* 261 (P), 217 (*p*-$CONH_2$).

6-Chloro-4′-ethoxyspiro[4*H*-2,3-dihydrobenzopyran-4,5′-oxazolin]-2′-one (6, R = 6-Cl; X = O). A solution of ethyl 6-chloro-4-hydroxy-4*H*-2,3-dihydropyran-4-carboximidate (1.15 g, 400 mmol) in 60 mL of THF was cooled to 0 °C and stirred while phosgene was infused for 5 min. After 30 min, thin-layer chromatography analysis of the reaction showed a new spot at R_f 0.57 (1:1 chloroform/ethyl acetate on silica gel) with no starting imidate, R_f 0.29, present. The mixture was poured onto 90 mL of ice/H_2O and extracted twice with 50 mL of EtOAc. The organic layer was washed twice with 30 mL of 5% $NaHCO_3$, dried ($MgSO_4$), and filtered, and the filtrate was evaporated in vacuo to an oil (0.651 g), which was crystallized at low temperature from Et_2O/hexane: yield 0.350 g (31%); mp 108–110 °C; IR (oil) 1800, 1618 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (t, *J* = 7 Hz, 3 H, CH_3), 2.0–2.5 (m, 2 H, CH_2), 4.1–4.8 (m, 2 H, CH_2), 4.67 (q, *J* = 7 Hz, 2 H, CH_2), 6.7–7.5 (m, 3 H, arom); mass spectrum, *m/e* 281 (P), 253 (P - C_2H_4).

Hydrolysis of 6 (R = 6-Cl; X = O). A solution of 88 mg (0.7 mmol) of $Na_2CO_3 \cdot H_2O$ in 1 mL of H_2O was added to a solution 100 mg (0.36 mmol) of **6** (R = 6-Cl; X = O) in 1 mL of THF. After stirring at 20 °C for 16 h, the mixture was diluted with 10 mL each of H_2O and EtOAc. The aqueous phase was washed with 10 mL of EtOAc and then acidified to pH 1 and extracted with 2 × 15 mL of EtOAc. These latter-combined EtOAc phases were dried ($MgSO_4$) and filtered, the filtrate was and vacuum evaporated to give **11**: yield 63 mg (70%); mp 192–195 °C.

Preparation of 11 Using Ethyl Chloroformate and Imidazole. A mixture of 0.500 g (1.96 mmol) of the imidate **5** (R = 6-Cl; X = O) and 0.228 g (3.35 mmol) of imidazole was combined with 0.254 g (2.35 mmol) of ethyl chloroformate and heated at 120 °C for 15 min. After removal of the heat, the mixture was diluted with 100 mL of EtOAc, washed with 2 × 25 mL of 1 N HCl and 25 mL of brine, dried ($MgSO_4$), and filtered, and the filtrate was evaporated in vacuo to a residue, **11**, which was crystallized from toluene: yield 360 mg (72%). The acidic washes were basified and extracted with Et_2O. A mixture of imidazole and *N*-ethylimidazole (*m/e* 96) was obtained from the concentrated Et_2O extracts.

Preparation of α,β-Unsaturated Amide 9 (R = 6-F; X = S). The Stroughton[6] procedure was used. Ice-cold concentrated HCl (1 mL) was added to a solution of 1.00 g (3.60 mmol) of 4-cyano-6-fluoro-4-hydroxy-4*H*-2,3-dihydrobenzothiopyran in 1.0 mL of Et_2O at 0 °C. The mixture was perfused with HCl gas for 2 min and then stoppered and kept 16 h at 20 °C. The amide was obtained after quenching the reaction with 60 mL of ice/H_2O and filtering. The solid was washed with H_2O and dried: yield 0.63 g (83%); mp 221–223 °C dec ($MeOH/H_2O$); IR (KBr) cm^{-1}; NMR ($CDCl_3$) δ 3.50 (d, *J* = 6 Hz, 2 H, CH_2), 6.60 (t, *J* = 6 Hz, 1 H, = CH), 6.9–7.6 (m, 3 H, arom); mass spectrum, *m/e* 209 (P). Anal. ($C_{10}H_8FNOS$) C, H, N.

Preparation of α-Chloro Amide (R = 6-Cl; X = O). A solution of 1.40 g (5.00 mmol) of 6-chloro-4-cyano-4-[(trimethylsilyl)oxy]-4*H*-2,3-dihydrobenzopyran in 1.5 mL of Et_2O at 0 °C was combined with 1.5 mL of concentrated HCl at 0 °C, perfused with HCl gas for 10 min, and then allowed to stand at room temperature for 16 h. The oil obtained from the concentrated extract of the quenched reaction mixture was column chromatographed on silica gel with Et_2O and gave four fractions, the first (R_f 0.82) was 6-chloro-4-chromanone, the second was α-chloroamide [R_f 0.44; yield 0.44 g (36%); mp 118–120 °C dec; IR (KBr) 1692 cm^{-1}; NMR ($CDCl_3$) δ 2.3–2.6 (m, 1 H, CH_2), 2.9–3.3 (m, 1 H, CH_2), 4.3–4.6 (m, 2 H, CH_2), 6.6–7.4 (m, 3 H, arom, and 2 H NH_2), mass spectrum *m/e* calcd, 246.9981; found, 246.9981], the third was α-hydroxy amide [R_f 0.144; yield 0.071 g (6%); mp 168–169 °C; IR (KBr) 1672 cm^{-1}; NMR [$(CD_3)_2SO$] δ 1.6–2.5 (m, 2 H, CH_3), 3.8–4.7 (m, 2 H, CH_2), 6.7–7.7 (m, 3 arom); mass spectrum *m/e* 227], and the fourth was a mixed fraction [R_f 0.15; mass spectrum, *m/e* 227 and 209].[13]

Acknowledgment. The authors are indebted to H. R. Howard, D. MacDonald, and C. aldinger for their technical assistance.

(13) The material with *m/e* 209 peak was shown to be the α,β-unsaturated amide by NMR, see above.

Methotrexate Analogues. 16. Importance of the Side-Chain Amide Carbonyl Group as a Structural Determinant of Biological Activity[1]

A. Rosowsky* and R. Forsch

The Sidney Farber Cancer Institute and Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115.
Received June 1, 1982

N-[[[(2,4-Diaminopteridin-6-yl)methyl]amino]benzyl]-L-glutamic acid ("deoxoaminopterin", **1**), a new aminopterin analogue containing a CH_2 group in the side chain in place of the amide C=O, was synthesized by condensation of 2,4-diamino-6-(bromomethyl)pteridine with diethyl *N*-(*p*-aminobenzyl)-L-glutamate, followed by saponification with a stoichiometric amount of barium hydroxide in 50% ethanol. The apparent importance of the amide C=O group as a structural determinant of biological activity was indicated by the finding that **1** has 10- to 20-fold lower affinity for bacterial and mammalian dihydrofolate reductase than aminopterin, is not toxic to L1210 murine leukemia cells in culture at a concentration of up to 1.0 μM, and shows no antitumor effect in L1210 leukemic mice at doses as high as 240 mg/kg (q3d × 3).

As part of an ongoing research effort aimed at correlating structure and biological activity in folate antagonists,[2-8] it was of interest to assess the possible importance of the C=O group in the amide bond separating the pteroyl and glutamate moieties of classical antifols such as aminopterin and methotrexate. The presence of the amide C=O in these compounds might be expected a priori to have several possible consequences. First, the extent of dissociation of the neighboring α-COOH might be affected, since *N*-acylamino acids are known to be less acidic than amino acids themselves. Secondly, the amide resonance of the conjugated CONH bond should restrict rotation, which might confer on the side chain of the molecule a certain amount of conformational rigidity. Thirdly, the C=O oxygen is potentially a hydrogen bond acceptor, and this might contribute to binding of the molecule to the active site of dihydrofolate reductase. Apart from these effects, there is also the possibility that the amide C=O could influence binding to the membrane-associated proteins that mediate active transport of folates and folate analogues into cells and that activity might be affected at the pharmacological level as a result of differences in plasma protein binding, tissue distribution, or renal/hepatobiliary excretion.

With these considerations in mind, we prepared and investigated some biological properties of *N*-[[[(2,4-diaminopteridin-6-yl)methyl]amino]benzyl]-L-glutamic acid

(1) For paper 15 in this series, see Rosowsky, A.; Wright, J. E.; Ginty, C. E.; Uren, J. *J. Med. Chem.* **1982**, *25*, 960.
(2) Rosowsky, A.; Chen, K. K. N. *J. Med. Chem.* **1974**, *17*, 1308 (and earlier papers cited therein).
(3) Rosowsky, A.; Chen, K. K. N.; Papathanasopoulos, N. *J. Heterocycl. Chem.* **1976**, *13*, 727.
(4) Rosowsky, A.; Ensminger, W. D.; Lazarus, H.; Yu, C.-S. *J. Med. Chem.* **1977**, *20*, 925.
(5) Rosowsky, A.; Yu, C.-S. *J. Med. Chem.* **1978**, *21*, 170.
(6) Rosowsky, A.; Beardsley, G. P.; Ensminger, W. D.; Lazarus, H.; Yu, C.-S. *J. Med. Chem.* **1978**, *21*, 380.
(7) Rosowsky, A.; Yu, C.-S.; Uren, J.; Lazarus, H.; Wick, M. *J. Med. Chem.* **1981**, *24*, 559 (paper 13 in this series).
(8) Rosowsky, A.; Forsch, R.; Uren, J.; Wick, M. *J. Med. Chem.* **1981**, *24*, 1450 (paper 14 in this series).

0022-2623/82/1825-1454$01.25/0