Apyrogenic, Adjuvant-Active *N*-Acetylmuramyl-dipeptides[1]

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New analogues of *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) have been synthesized in which the D-isoglutaminyl residue has been replaced by D-glutamine alkyl esters. They are all adjuvant active; however, their intravenous injection does not induce a febrile response in rabbits at a dose of 10 mg/kg, in contrast to MDP in which the minimal pyrogenic dose is 25 μg/kg.

A chemically well-defined, synthetic, nontoxic compound able to amplify specific and/or nonspecific immune responses would be of paramount therapeutic value. Such an agent might be, among other candidates, *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP, **1**), which has been identified as the minimal structure capable of replacing whole myobacterial cells in complete Freund's adjuvant for enhancing the level of circulating antibodies and producing delayed hypersensitivity against a given antigen.[2,3] MDP was found to be adjuvant active in saline[4] even when administered by the oral route.[5] Moreover, it was shown to increase nonspecific resistance to bacterial infections.[6] Since its first synthesis,[7] more than 200 papers on the chemistry and biological properties of MDP and its derivatives have been published; for reviews see, ref 8–11. Nevertheless, its potential clinical applications have become somewhat questionable, since it was reported that MDP elicits a febrile response in rabbits[12–14] and the release of endogenous pyrogen.[15]

This unexpected untoward effect was first thought to be correlated with its adjuvant properties.[12] In previous studies on the relationship between the glycopeptide structure and immunostimulant activity, we had synthesized adjuvant-active, structurally related compounds,[16–18] which were found to be only weakly pyrogenic, such as MurNAc-L-Ala-D-Glu (**2**)[13] and MurNAc-L-Ala-D-Gln (**3**), or almost apyrogenic, such as MurNAc-L-Ala-D-Glu(OMe)-OMe (**4**)[15] and MurNAc-L-Ala-D-Gln-NH_2 (**5**) (Table I). The above results suggested that the immunostimulant activity could be more or less dissociated from the pyrogenic effect by chemical modification of the functionality of the D-glutamyl residue. The favorable properties of **5** compared to **3** and **4** in particular lead us to study various structural modifications of the D-glutamyl residue and to expect that MurNAc-L-Ala-D-Gln-OMe (**6**) would be still less pyrogenic.

This paper presents the detailed synthesis of **6** and of some higher alkyl ester homologues (Table I, **6–12**) and reports on their lack of pyrogenicity in the rabbit and on their adjuvant and antiinfectious activities.

Synthesis. The glycopeptides **6–12** were prepared according to the previously reported procedure for the synthesis of muramyl-dipeptides.[16–18] The desired protected dipeptides were prepared by conventional peptide synthesis techniques (see Experimental Section). Then they were freed of their amino-protective group and coupled with 1-α-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetylmuramic acid, using the mixed anhydride method. The resulting protected *N*-acetylmuramyl-peptides were hydrogenated to give the free glycopeptides, which were purified by ion exchange or silica gel chromatography.

Biological Results and Discussion

Table I shows the febrile response induced by the previously described compounds (**1–5**) and the new compounds (**6–12**). It can be seen that none of the latter elicit a fever response in rabbits at a dose of 10 mg/kg, the minimal pyrogenic dose of MDP being 25 μg/kg.[19] Adjuvant activity of the compounds given in PBS was tested

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Table I. Febrile Responses Induced by the Previously Described Compounds (1-5) and the New Compounds (6-12)

no.	glycopeptides	ΔT,[a] °C dose: 1 mg/kg	3 mg/kg	5 mg/kg	10 mg/kg
1	MurNAc-L-Ala-D-isoGln[b]	0.8, 0.9, 0.6 (0.76)			
2	MurNAc-L-Ala-D-Glu-OH	0.0, 0.9, 0.2 (0.37)	1.1, 0.9, 0.3 (0.8)		
		0.1, 0.5, 1.0 (0.5)			
3	MurNAc-L-Ala-D-Gln	0.0, 0.1, 0.0 (0.03)	0.5, 1.2, 1.0 (0.9)		
4	MurNAc-L-Ala-D-Glu(OCH_3)-OCH_3	0.0, 0.2, 0.8 (0.33)	1.0, 0.2, 0.0 (0.4)	1.1, 1.0, 1.2 (1.1)	
		0.3, 0.1, 0.0 (0.13)	0.0, 0.0, 0.5 (0.17)	1.3, 0.6, 0.8 (0.9)	
5	MurNAc-L-Ala-D-Gln-NH_2	0.1, 0.0, 0.2 (0.1)	0.3, 0.3, 0.0 (0.2)	0.7, 0.9, 0.4 (0.67)	
6	MurNAc-L-Ala-D-Gln-OCH_3	0.0, 0.2, 0.1 (0.1)	0.4, 0.0, 0.3 (0.23)		0.2, 0.1, 0.0 (0.1)
7	MurNAc-L-Ala-D-Gln-OC_2H_5	0.2, 0.2, 0.3 (0.23)	0.0, 0.0, 0.1 (0.03)		0.7, 0.6, 0.2 (0.47)
					0.0, 0.2, 0.2 (0.32)
8	MurNAc-L-Ala-D-Gln-O-*n*-C_3H_7	0.0, 0.4, 0.2 (0.20)	0.5, 0.7, 0.1 (0.43)		0.0, 0.0, 0.2 (0.07)
			0.0, 0.0, 0.1 (0.23)		
9	MurNAc-L-Ala-D-Gln-O-*n*-C_4H_9	0.1, 0.0, 0.0 (0.03)	0.5, 0.1, 0.2 (0.26)		0.2, 0.6, 0.1 (0.3)
			0.4, 0.3, 0.1 (0.26)		0.1, 0.2, 0.4 (0.23)
10	MurNAc-L-Ala-D-Gln-O-*n*-C_5H_{11}	0.0, 0.0, 0.3 (0.1)	0.0, 0.0, 0.1 (0.03)		0.2, 0.1, 0.1 (0.13)
11	MurNAc-L-Ala-D-Gln-O-*n*-C_6H_{13}	0.1, 0.2, 0.1 (0.13)	0.2, 0.1, 0.3 (0.2)		0.1, 0.1, 0.2 (0.13)
12	MurNAc-L-Ala-D-Gln-O-*n*-$C_{10}H_{21}$	0.4, 0.2, 0.0 (0.2)	0.1, 0.3, 0.1 (0.17)		0.1, 0.1, 0.5 (0.23)

[a] Pyrogenicity (mean values in parentheses). [b] MDP.

Table II. Adjuvant Activity of MDP, MurNAc-L-Ala-D-Gln, and Its Derivatives When Administered Subcutaneously in PBS to Mice with BSA

no.	adjuvants	primary response[a]	secondary response[a]
	controls	<1.64	3.57 ± 1.78
1	MurNAc-L-Ala-D-isoGln (MDP)	3.64	8.34 ± 1.34[b]
	MurNAc-L-Ala-D-Gln	2.97	7.35 ± 1.21[b]
6	MurNAc-L-Ala-D-Gln-OCH_3	3.85	8.72 ± 1.23[b]
7	MurNAc-L-Ala-D-Gln-OC_2H_5	4.64	9.53 ± 0.93[b]
8	MurNAc-L-Ala-D-Gln-OC_3H_7	4.64	9.52 ± 0.5[b]
9	MurNAc-L-Ala-D-Gln-O-*n*-C_4H_9	4.21	8.43 ± 1.52[b]
10	MurNAc-L-Ala-D-Gln-O-*n*-C_5H_{11}	1.64	7.80 ± 1.39[b]
11	MurNAc-L-Ala-D-Gln-O-*n*-$C_{10}H_{13}$	2.75	7.71 ± 1.67[b]
12	MurNAc-L-Ala-D-Gln-O-*n*-$C_{10}H_{21}$	3.64	9.1 ± 1.66[b]

[a] Titers are given as log, of the highest dilution of serum able to agglutinate BSA-coated SRBC. [b] Significantly different from control ($p < 0.01$) as calculated by Student's *t* test.

Table III. Protective Activity of Synthetic Glycopeptides Administered Before an Intramuscular Infection with 10^4 *K. pneumoniae*

no.	treatment[a]	survivors *N*/total	protection,[b] %
	controls	1/24	
1	MurNAc-L-Ala-D-isoGln (MDP)	15/24	58
6	MurNAc-L-Ala-D-Gln-OCH_3	3/24	8
7	MurNAc-L-Ala-D-Gln-OC_2H_5	0/24	0
8	MurNAc-L-Ala-D-Gln-OC_3H_7	7/24	25
9	MurNAc-L-Ala-D-Gln-O-*n*-C_4H_9	16/24	63
10	MurNAc-L-Ala-D-Gln-O-*n*-C_5H_{11}	10/24	37
11	MurNAc-L-Ala-D-Gln-O-*n*-$C_{10}H_{13}$	8/24	29
12	MurNAc-L-Ala-D-Gln-O-*n*-$C_{10}H_{21}$	4/24	12

[a] 100 μg intravenously, 24 h before infection. [b] Protection (%) = difference between percentages of survivors in treated group and its respective control group at day +15.

in mice. Data reported in Table II show that these glycopeptides highly enhanced the humoral response to a protein antigen during both the primary and the secondary responses. Results were comparable to those obtained with MDP. In the MurNAc-L-Ala-D-Gln alkyl ester series (**6–12**), the level of the protective effect against a *Klebsiella pneumoniae* challenge is dependent on the length of the alkyl chain (Table III), **9** being the most active compound.[20] These results clearly show that simple chemical modifications of the MDP molecule can dissociate its pyrogenic and adjuvant activities. Besides its action on the production of leucocyte pyrogen, MDP very recently has been found to interact directly with the thermoregulatory center, since an intense pyretic effect could be obtained after its intracerebroventricular injection at a dose of 0.13 ng/kg.[19] In contrast, the most interesting compound among the new analogues, i.e., *N*-acetylmuramyl-L-alanyl-D-glutamine *n*-butyl ester (**9**), does not elicit any febrile response by intracerebroventricular injection; moreover, it does not produce endogenous pyrogen by intravenous administration.[21] It is a good candidate for clinical application.

Experimental Section

Melting points were determined with a capillary melting point apparatus (Büchi, Flawil, Switzerland) and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Elemental analyses were performed with a Perkin-Elmer 240 elemental analyzer. Samples for elemental analyses were dried for 20 h at 25 °C (0.1 torr). Compounds are sometimes formulated as containing water on the basis of elemental analysis. This retention may be due to the necessary mild drying conditions. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 plates (0.25 mm) using the following solvent systems: chloroform–methanol, from 10:1 to 20:1 (v/v); ethyl acetate–pyridine–acetic acid–water, from 6:2:0.6:1 to 30:2:0.6:1 (v/v), (for protected peptides); chloroform–methanol, from 5:1 to 10:1 (v/v), chloroform–methanol–benzene, 85:10:5 (v/v) (for protected glycopeptides), 1-butanol–pyridine–acetic acid–water, 30:20:6:24 (v/v); 1-butanol–acetic acid–water 4:1:5 (v/v upper phase); ethyl acetate–pyridine–acetic acid–water 6:2:0.6:1 (v/v) (for free glycopeptides). Detection was carried out with ninhydrine and with

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the chlorine–*O*-tolidine reagent; all compounds described were homogeneous on TLC.

Standard Hydrogenation Procedure. The totally protected *N*-acetylmuramyl-dipeptides were dissolved in acetic acid and hydrogenated for 40 h over 5% palladium on charcoal at atmospheric pressure. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. After purification, the free glycopeptides were obtained as a lyophilized powder.

The following abbreviations were used: Cbz, benzyloxycarbonyl; Bzl, benzyl; Boc, *tert*-butyloxycarbonyl; DMF, dimethylformamide; EtOAc, ethyl acetate; HOAc, acetic acid; THF, tetrahydrofuran.

Cbz-L-Ala-D-Gln (13). To a mixture of D-glutamine (2.4 g, 16.4 mmol) and triethylamine (2.3 mL, 16.4 mmol) in 35 mL of H_2O was added a solution of *N*-(benzyloxycarbonyl)-L-alanine succinimide ester[22] (4.6 g, 14.4 mmol) in 65 mL of anhydrous THF. After 24 h at 4 °C, dimethylaminopropylamine (0.5 mL, 3.85 mmol) was added. The reaction mixture was stirred for 1 h, diluted with 65 mL of H_2O, and acidified to pH 2.5 with 1 N HCl. Evaporation of THF in vacuo yielded a precipitate, which was filtered and washed with a cold mixture of THF–H_2O (1:1, v/v): yield 2.86 g (62%); mp 179–182 °C; $[\alpha]^{20}_D$ −13.3° (*c* 1.0, methanol). Anal. ($C_{16}H_{21}N_3O_6$) C, H, N.

Cbz-L-Ala-D-Gln-OCH$_3$ (14). To a solution of **13** (2.8 g, 8 mmol) in 150 mL of methanol–THF (2:1, v/v) was added an ethereal solution of diazomethane at room temperature until the yellow color persisted. After 10 min, a few drops of acetic acid were added, and the mixture was evaporated in vacuo. The residue was crystallized from methanol: yield 2.0 g (69%); mp 183–4 °C; $[\alpha]^{20}_D$ −11.7° (*c* 1.0, HOAc). Anal. ($C_{17}H_{23}N_3O_6$) C, H, N.

Cbz-L-Ala-D-Gln-OC$_2$H$_5$ (15). **13** (700 mg, 2 mmol) was dissolved in 150 mL of THF and 5 mL of H_2O. After a 20% solution of Cs_2CO_3 (1 mmol) was added, the water was evaporated in vacuo. The residue was dissolved once in methanol (25 mL) and then twice in DMF (25 mL), the mixture being concentrated each time to dryness. To the solution of the ensuing dried residue in 50 mL of DMF was added bromoethane (0.33 mL, 4.4 mmol). The reaction mixture was stirred for 48 h at room temperature and then concentrated in vacuo. A precipitate was obtained by adding H_2O, which was crystallized from ethanol: yield 465 mg (61%); mp 167–170 °C; $[\alpha]^{20}_D$ −13° (*c* 0.5, HOAc). Anal. ($C_{18}H_{26}N_3O_6$) C, H, N.

Cbz-L-Ala-D-Gln-*O*-*n*-C$_3$H$_7$ (16). The title compound was prepared as described for **15**: yield 65%; mp 150 °C; $[\alpha]^{20}_D$ −3° (*c* 0.5, HOAc). Anal. ($C_{19}H_{27}N_3O_6$) C, H, N.

Cbz-L-Ala-D-Gln-*O*-*n*-C$_4$H$_9$ (17). The title compound was prepared as described for **15**: yield 81%; mp 148–150 °C; $[\alpha]^{20}_D$ +14.7° (*c* 1.0, DMF). Anal. ($C_{20}H_{29}N_3O_6$) C, H, N.

Cbz-L-Ala-D-Gln-*O*-*n*-C$_5$H$_{11}$ (18). The title compound was prepared as described for **15**: yield 72%; mp 147–149 °C; $[\alpha]^{20}_D$ −8.6° (*c* 0.5, HOAc). Anal. ($C_{21}H_{31}N_3O_6$) C, H, N.

Cbz-L-Ala-D-Gln-*O*-*n*-C$_6$H$_{13}$ (19). The title compound was prepared as described for **15**: yield 74%; mp 160–162 °C; $[\alpha]^{20}_D$ −8.5° (*c* 0.5, HOAc). Anal. ($C_{22}H_{33}N_3O_6$) C, H, N.

Cbz-L-Ala-D-Gln-*O*-*n*-C$_{10}$H$_{21}$ (20). The title compound was prepared as described for **15**: yield 70%; mp 118 °C; $[\alpha]^{20}_D$ +7.3° (*c* 1.0, DMF). Anal. ($C_{26}H_{41}N_3O_6$) C, H, N.

1-α-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-alanyl-D-glutamine Methyl Ester (21). **14** (1.1 g, 3 mmol) dissolved in 40 mL of HOAc containing 3 mL of 1 N HCl was hydrogenated for 2 h over 5% palladium on charcoal at atmospheric pressure. The catalyst was filtered off, and the filtrate was evaporated in vacuo to dryness. The ensuing residue was dissolved in 20 mL of DMF containing *N*-methylmorpholine (0.33 mL, 3 mmol) and added to a solution, cooled at −15 °C, of 1-α-*O*-benzyl-4,6-*O*-benzylidene-*N*-acetylmuramic acid (**23**; 1.4 g, 3 mmol), *N*-methylmorpholine (0.33 mL, 3 mmol), and isobutyl chloroformate (0.39 mL, 3 mmol) in 20 mL of DMF. The reaction was performed overnight at −15 °C, and then a 2.5 M $KHCO_3$ solution (2 mL) was added at 0 °C. After 30 min an oily residue was precipitated by adding water and then extracted with EtOAc. The organic phase was washed successively with 10% citric acid, water, and 1 M $KHCO_3$, dried on $MgSO_4$, and evaporated in vacuo to dryness to give a residue which was crystallized from EtOAc–petroleum ether: yield 1.7 g (82%); mp 250 °C; $[\alpha]^{20}_D$ +94° (*c* 0.5, DMF). Anal. ($C_{34}H_{44}N_4O_{11}\cdot0.5H_2O$) C, H, N, O.

1-α-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-alanyl-D-glutamine Ethyl Ester (22). Starting from **15**, compound **22** was prepared as described for **21**: yield 85%; mp 228–232 °C; $[\alpha]^{20}_D$ +75° (*c* 0.5, HOAc). Anal. ($C_{35}H_{46}N_4O_{11}\cdot0.6H_2O$) C, H, N.

1-α-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-alanyl-D-glutamine *n*-Propyl Ester (23). Starting from **16**, compound **23** was prepared as described for **21**: yield 94%; mp 240–242 °C; $[\alpha]^{20}_D$ +75.2° (*c* 0.5, HOAc). Anal. ($C_{36}H_{48}N_4O_{11}$) C, H, N.

1-α-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-alanyl-D-glutamine *n*-Butyl Ester (24). Starting from **17**, compound **24** was prepared as described for **21**: yield 80%; mp 230 °C; $[\alpha]^{20}_D$ +82.6° (*c* 1.0, HOAc). Anal. ($C_{37}H_{50}N_4O_{11}$) C, H, N.

1-α-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-alanyl-D-glutamine *n*-Pentyl Ester (25). Starting from **18**, compound **25** was prepared as described for **21**: yield 63%; mp 210–225 °C; $[\alpha]^{20}_D$ +68.5° (*c* 0.5, HOAc). Anal. ($C_{38}H_{52}N_4O_{11}$) C, H, N.

1-α-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-alanyl-D-glutamine *n*-Hexyl Ester (26). Starting from **19**, compound **26** was prepared as described for **21**: yield 96%; mp 220–222 °C; $[\alpha]^{20}_D$ +69.3° (*c* 0.5, HOAc). Anal. ($C_{39}H_{54}N_4O_{11}\cdot0.5H_2O$) C, H, N.

1-α-*O*-Benzyl-4,6-*O*-benzylidene-*N*-acetylmuramyl-L-alanyl-D-glutamine *n*-Decyl Ester (27). Starting from **20**, compound **27** was prepared as described for **21**: yield 95%; mp 221 °C; $[\alpha]^{20}_D$ +69.8° (*c* 1.0, DMF). Anal. ($C_{43}H_{62}N_4O_{11}$) C, H, N.

***N*-Acetylmuramyl-L-alanyl-D-glutamine Methyl Ester (6).** **21** was hydrogenated according to the standard procedure. **6** was chromatographed on a silica gel column eluted with chloroform–methanol–HOAc (50:20:5, v/v): yield 70%; $[\alpha]^{20}_D$ +36° (*c* 1.0, HOAc). Anal. ($C_{20}H_{34}N_4O_{11}\cdot H_2O$) C, H, N, O.

***N*-Acetylmuramyl-L-alanyl-D-glutamine Ethyl Ester (7).** **22** was hydrogenated according to the standard procedure. **7** was chromatographed on a silica gel column eluted with EtOAc–pyridine–HOAc–H_2O (6:2:0.6:1, v/v) and on an AG50WX2 (OH^-) column eluted with 0.1 M HOAc: yield 60%; $[\alpha]^{20}_D$ +34° (*c* 0.5, HOAc). Anal. ($C_{21}H_{36}N_4O_{11}\cdot1.25H_2O$) C, H, N, O.

***N*-Acetylmuramyl-L-alanyl-D-glutamine *n*-Propyl Ester (8).** **23** was hydrogenated according to the standard procedure. **8** was chromatographed on a silica gel column eluted with chloroform–methanol–HOAc (50:15:5, v/v): yield 80%; $[\alpha]^{20}_D$ +32.3° (*c* 1.0, HOAc). Anal. ($C_{25}H_{44}N_4O_{11}\cdot0.5H_2O$) C, H, N, O.

***N*-Acetylmuramyl-L-alanyl-D-glutamine *n*-Butyl Ester (9).** **24** was hydrogenated according to the standard procedure. **9** was purified as described for **8**: yield 80%; $[\alpha]^{20}_D$ +35° (*c* 1.0, HOAc). Anal. ($C_{23}H_{40}N_4O_{11}\cdot0.5H_2O$) C, H, N, O.

***N*-Acetylmuramyl-L-alanyl-D-glutamine *n*-Pentyl Ester (10).** **25** was hydrogenated according to the standard procedure. **10** was purified as described for **7**: yield 65%; $[\alpha]^{20}_D$ +36.5° (*c* 1.0, HOAc). Anal. ($C_{24}H_{42}N_4O_{11}\cdot1.25H_2O$) C, H, N, O.

***N*-Acetylmuramyl-L-alanyl-D-glutamine *n*-Hexyl Ester (11).** **26** was hydrogenated according to the standard procedure. **11** was purified as described for **7**: yield 63%; $[\alpha]^{20}_D$ +31.6° (*c* 0.5, HOAc). Anal. ($C_{25}H_{44}N_4O_{11}\cdot1H_2O$) C, H, N, O.

***N*-Acetylmuramyl-L-alanyl-D-glutamine *n*-Decyl Ester (12).** **27** was hydrogenated according to the standard procedure. **12** was purified as described for **8**: yield 60%; $[\alpha]^{20}_D$ +30° (*c* 1.0, HOAc). Anal. ($C_{29}H_{52}N_4O_{11}\cdot0.5H_2O$) C, H, N, O.

Adjuvant Activity. Adjuvant activity was tested in 5- to 6-week-old female Swiss mice. The antigen, bovine serum albumin (Miles Laboratories), was administered subcutaneously in phosphate-buffered saline (PBS) at the dosage of 0.5 mg for priming and 0.1 mg a month later as a boost. In experimental groups, 0.1 mg of glycopeptides was given with the primary injection. Mice were bled, and their sera were pooled on day 21; on day 37 they were titrated separately. Antibodies are measured by passive hemagglutination of BSA-coated sheep red blood cells (SRBC) according to the method previously described.[24]

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Infectious Challenge. Antiinfectious activity was tested using 24 mice per group. On day 0 they received an intravenous injection of 100 μg of compound. On day +1 they were infected by an intramuscular injection of 10^4 *K. pneumoniae*. Mortality was observed from day 1 to day 15.

Test of Pyrogenicity. This test was carried out as described in the French as well as in the European pharmacopoeia. Each specimen was dissolved in pyrogen-free isotonic saline solution and injected intravenously at a dose of 1 mg/kg into rabbits (strain HYLA, weighing 3 to 3.5 kg). The body temperature was measured per rectum continuously and automatically. When none of the three rabbits treated showed a rise in temperature above 0.5 °C, the specimen was judged to be nonpyrogenic; in that case, doses of 3, 5, and 10 mg/kg were tested.

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Hypocholesterolemic and Antiaggregatory Properties of 2-Hydroxytetronic Acid Redox Analogues and Their Relationship to Clofibric Acid[1]

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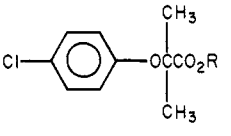
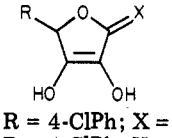
A rationale is presented for investigating *aci*-reductone 2-hydroxytetronic acids as antilipidemic drugs. These compounds are lipophilic Brönsted acids capable of forming water-soluble anions having biologically relevant redox potentials. The inhibitory effects of 4-(4-chlorophenyl)-2-hydroxytetronic acid (**2a**) on human platelet aggregation and [^{14}C]serotonin secretion were compared with clofibric acid (**1b**), the hydrolysis product of clofibrate (**1a**). In cholesterol-fed rats, this analogue was superior to clofibrate as a hypocholesterolemic drug and modifier of heparin-$MnCl_2$ precipitated lipoprotein cholesterol to α-lipoprotein cholesterol ratios. Whereas clofibrate (**1a**) produced hepatomegaly, this effect was not observed for the tetronic acid **2a**.

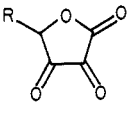
For several years we have investigated the synthesis,[2] effects on lipid and lipoprotein parameters,[3] modes of action,[2,3] and metabolism[4] of clofibrate and related analogues. To provide leads for drugs which may demonstrate different modes of action and pharmacokinetic parameters, we now report certain "clofibrate-related" compounds which possess the *aci*-reductone moiety —C(OH)=C-(OH)—C=O.[5-7] Thus, the hydroxyvinylene carboxylic acid homologue 4-(4-chlorophenyl)-2-hydroxytetronic acid (**2a**) and clofibric acid (**1b**) have similar pK_a values (4.46[8] for **1a**; 5.1 for **2a**) and molecular weights differing only by 12. However, **2a** has a redox potential (0.15 V) similar to L-ascorbic acid (**2c**) (0.127 V)[9] and may interfere with redox enzyme systems involved in lipogenesis [e.g., 3-hydroxy-3-methylglutaric acid (HMG)-CoA reductase; EC 1.1.1.34].[10] In this note we compare the antilipidemic activity of **1a** with **2a**, its tetronimide **2b** (which likely exists as the enamine tautomer),[6] and its dehydro form **3a** in cholesterol (CH) fed rats. Also, **1b**[11] was compared with **2a** for antiaggregatory activity in human platelets in vitro.



1a, R = Et
b, R = H



2a, R = 4-ClPh; X = O
b, R = 4-ClPh; X = NH
c, R = $HOCH_2CH(OH)$; X = O (L isomer)



3a, R = 4-ClPh
b, R = $HOCH_2CH(OH)$ (L isomer)

Chemistry. 4-Aryl-2-hydroxtetronic acids and their synthetic tetronimide precursors and dehydro forms first were prepared in the middle 1950's.[12-15] L-Ascorbic acid syntheses first appeared in the early 1930's,[16,17] but, except for L-ascorbic acid, chemical-biological interactions of 2-hydroxytetronic acids have been neglected.[18-20] Ana-

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