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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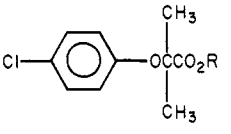
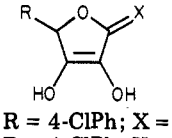
A rationale is presented for investigating *aci*-reductone 2-hydroxytetronic acids as antilipidemic drugs. These compounds are lipophilic Brönsted acids capable of forming water-soluble anions having biologically relevant redox potentials. The inhibitory effects of 4-(4-chlorophenyl)-2-hydroxytetronic 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**.

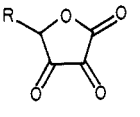
For several years we have investigated the synthesis,[2] effects on lipid and lipoprotein parameters,[3] modes of action,[2,3] and metabolism[4] of clofibrate and related analogues. To provide leads for drugs which may demonstrate different modes of action and pharmacokinetic parameters, we now report 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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Table I. Effects of Clofibrate (1a), 2-Hydroxytetronic Acids 2a,b, and 2,3-Diketobutyrolactone 3a on Serum Total Triglycerides of Cholesterol-Fed Rats[a]

compd[b]	day −1	day +4	day +7	day +10	day +14
control	243 ± 36.1	212 ± 52.5	170 ± 39.4	207 ± 59.1	127 ± 33.4
1a (0.4)	266 ± 75.9	141 ± 27.1	169 ± 43.3	196 ± 47.3	155 ± 30.6
2a (0.2)	313 ± 108	251 ± 75.5	204 ± 64.4	193 ± 52.3	148 ± 38.1
2a (0.4)	193 ± 31.9	167 ± 54.7	147 ± 45.7	139 ± 61.1	87.8 ± 28.9
2b (0.4)	179 ± 21.7	147 ± 26.1	128 ± 26.1	158 ± 33.2	119 ± 18.9
3a (0.4)	268 ± 110	198 ± 49.8	164 ± 45.5	151 ± 47.4	105 ± 29.7

[a] Expressed as mg/dL ± SD. [b] Dose (in mmol/kg) in parentheses.

Table II. Effects of Clofibrate (1a), 2-Hydroxytetronic Acids 2a,b, and 2,3-Diketobutyrolactone 3a on Serum Total Cholesterol of Cholesterol-Fed Rats[a]

compd[b]	day −1	day +4	day +7	day +10	day +14
control	210 ± 83.6	150 ± 55.6	229 ± 90.7	338 ± 160	211 ± 95.1
1a (0.4)	202 ± 54.3	127 ± 25.9	209 ± 57.6	316 ± 65.0	302 ± 45.6
2a (0.2)	276 ± 127	195 ± 70.7	204 ± 90.7	161 ± 57.1[c]	145 ± 41.7[c]
2a (0.4)	196 ± 42.2	154 ± 30.2	125 ± 17.3	135 ± 18.1[c]	138 ± 39.9[c]
2b (0.4)	193 ± 34.7	138 ± 33.9	252 ± 65.0	344 ± 98.1	278 ± 105
3a (0.4)	250 ± 75.2	205 ± 38.6	377 ± 127[d]	356 ± 115	217 ± 79.4

[a] Expressed as mg/dL ± SD. [b] Dose (in mmol/kg) in parentheses. [c] Significantly lower than control values at $p < 0.05$. [d] Significantly higher than control values at $p < 0.05$.

Table III. Effects of Clofibrate (1a), 2-Hydroxytetronic Acids 2a,b, and 2,3-Diketobutyrolactone 3a on α-Lipoprotein Cholesterol of Cholesterol-Fed Rats[a]

compd[b]	day −1	day +4	day +7	day +10	day +14
control	42.2 ± 2.5	35.0 ± 7.0	37.7 ± 7.8	39.8 ± 6.4	29.7 ± 4.4
1a (0.4)	44.4 ± 8.3	38.5 ± 10.5	52.7 ± 10.0[c]	51.7 ± 9.6	55.0 ± 12.8[c]
2a (0.2)	40.8 ± 10.9	43.8 ± 13.7	42.5 ± 6.8	38.8 ± 8.8	48.5 ± 10.4[c]
2a (0.4)	39.7 ± 5.6	41.8 ± 8.4	46.0 ± 5.6	52.2 ± 15.1[c]	61.0 ± 24.3[c]
2b (0.4)	43.8 ± 7.5	33.2 ± 7.7	43.0 ± 11.3	43.7 ± 8.1	38.8 ± 2.9[c]
3a (0.4)	40.3 ± 8.4	32.0 ± 5.2	30.5 ± 6.4	29.0 ± 5.2	34.2 ± 4.4

[a] Expressed as mg/dL ± SD. [b] Dose (in mmol/kg) in parentheses. [c] Significantly higher than control values at $p < 0.05$.

Table IV. Effects of Clofibrate (1a), 2-Hydroxytetronic Acids 2a,b, and 2,3-Diketobutyrolactone 3a on the Heparin-$MnCl_2$ Precipitated Lipoprotein Cholesterol to α-Lipoprotein Cholesterol Ratios of Cholesterol-Fed Rats[a]

compd[b]	day −1	day +4	day +7	day +10	day +14
control	4.06 ± 2.28	3.68 ± 2.62	5.58 ± 3.60	7.63 ± 4.47	6.73 ± 4.97
1a (0.4)	3.81 ± 1.70	2.54 ± 1.28	3.12 ± 1.47	4.89 ± 2.14	4.76 ± 1.55
2a (0.2)	6.03 ± 3.01	3.72 ± 1.94	4.03 ± 2.54	3.33 ± 1.75[c]	2.20 ± 1.53[c]
2a (0.4)	3.96 ± 0.99	2.79 ± 0.92	1.74 ± 0.38	1.75 ± 0.76[c]	1.39 ± 0.74[c]
2b (0.4)	3.65 ± 1.31	3.43 ± 1.73	5.59 ± 2.08	7.13 ± 2.76	6.31 ± 2.92
3a (0.4)	5.76 ± 3.35	5.63 ± 1.67	12.62 ± 6.77[d]	11.9 ± 5.6[d]	5.75 ± 3.26

[a] Expressed as a ratio ± SD. [b] Dose (in mmol/kg) in parentheses. [c] Significantly lower than control values at $p < 0.05$. [d] Significantly higher than control values at $p < 0.05$.

logues **2b**[12,20] and **3a**[20] and the 2-chlorophenyl isomer of **2a**, **2b**, and **3a** are known[12,13] and were prepared by published methods.[15,20] For **2b** and **3a**, physical properties were in accord with published data,[13,20] and **2a**, prepared[15] from **3a** in 70% yield, exhibited mp 167–168 °C dec (ether/chloroform). Anal ($C_{10}H_7O_4Cl$) C, H, Cl.[21] The pK_a for **2a** (0.02% in 5% Me_2SO in double-distilled H_2O) was determined potentiometrically by titration with 0.01 N NaOH solution. The redox potential for **2a** (0.02% in 5% Me_2SO in double-distilled H_2O) was determined by titrating with 0.002 N $K_3Fe(CN)_6$ using a standard Pt and calomel electrode.[22]

Biological Results and Discussion

The effects of compounds **1a**, **2a,b**, and **3a** following 14 days of treatment using CH-fed (7 + 14 days) male Sprague–Dawley rats on serum total triglycerides (TG), serum total CH, serum α-lipoprotein (LP) CH, serum heparin-$MnCl_2$ precipitated LP CH to α-LP CH ratios, and liver to body weight ratios and liver lipids are found in Tables I–V, respectively. Like clofibrate (**1a**) none of

Table V. Effects of Clofibrate (1a), 2-Hydroxytetronic Acids 2a,b, and 2,3-Diketobutyrolactone 3a on Liver to Body Weight Ratio and Liver Lipid Levels of Cholesterol-Fed Rats[a]

compd[b]	liver/body wt, wt × 100[a]	liver TG levels[c]	liver CH levels[d]
control	4.1 ± 0.8	4.30 ± 1.75	4.45 ± 1.28
1a (0.4)	6.2 ± 0.3[e]	1.84 ± 0.43[f]	3.58 ± 0.64
2a (0.2)	3.8 ± 0.3	4.82 ± 1.76	3.28 ± 0.74
2a (0.4)	3.6 ± 0.2	5.08 ± 1.79	2.93 ± 0.43[f]
2b (0.4)	4.1 ± 0.3	2.80 ± 0.51	4.03 ± 1.12
3a (0.4)	4.0 ± 0.3	3.76 ± 1.21	4.90 ± 0.52

[a] Expressed as a ratio ± SD. [b] Dose (in mmol/kg) in parentheses. [c] Grams of triglyceride/100 g of wet liver ± SD. [d] Grams of cholesterol/100 g of wet liver ± SD. [e] Significantly higher than control values at $p < 0.05$. [f] Significantly lower than control values at $p < 0.05$.

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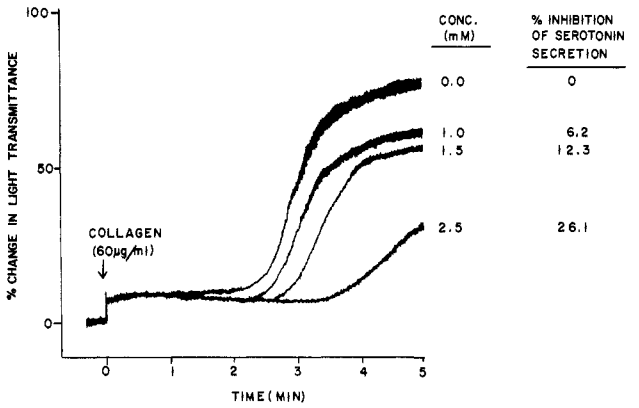

Figure 1. Inhibitory effects of 4-(4-chlorophenyl)-2-hydroxytetronic acid (**2a**) on collagen-induced aggregation and [^{14}C]serotonin secretion in human platelets in vitro. Serotonin secretion data were expressed as the percent inhibition of net ^{14}C released in the presence of collagen (60 µg/mL) alone.

the analogues (**2a,b** and **3a**) were effective in lowering serum TG concentrations in these rats which were fasted 18 h prior to sample collection (Table I). Clofibrate (**1a**) and analogues **2b** and **3a** were ineffective as hypocholesterolemic drugs, but 2-hydroxytetronic acid (**2a**) significantly lowered serum total CH by day +10 at both doses (0.2 and 0.4 mmol/kg) studied (Table II). Compared to control values **3a** increased serum total CH concentration at day +7, and the ratio of heparin–$MnCl_2$ precipitated LP CH to α-LP CH was elevated at days +7 and +10 (Table II). Whereas clofibrate (**1a**) had no effect on serum on serum total CH concentrations, this drug significantly increased α-LP CH concentrations by day +7, and these concentrations remained elevated until day +14. Both **2a** and **2b** also significantly increased α-LP CH concentrations by day +14 (Table III).

Human serum low-density lipoprotein (β-LP) CH increases and high-density lipoprotein (α-LP) CH decreases risk for coronary heart disease (CHD).[23] Thus, drugs which favorable modify this ratio in patients may reduce CHD risk. Interestingly, only 2-hydroxytetronic acid (**2a**) decreased this ratio at both doses (0.2 and 0.4 mmol/kg) by day +10 in CH-fed rats (Table IV). On this basis, **2a** would appear to be more efficacious than clofibrate (**1a**), but further work using other animal models is desired to support this proposal. We chose this CH-fed rat model for our initial studies, since CH-feeding reduces the catabolism of chylomicrons in rabbits[24] and VLDL in rats,[25] thus generating remnants of these LP. Such LP in rabbits induce severe atheromatosis,[26] and it has been shown that these LP are taken up by macrophages which contribute to arterial lipid accumulation.[27,28]

Unlike clofibrate (**1a**), **2a** caused no hepatomegaly (Table V), adding further support to its potential clinical utility. Whereas **1a** decreased liver TG levels at 0.4 mmol/kg and had no effect on liver CH levels, **2a** exhibited a CH-lowering effect without any significant effect on liver TG levels (Table V), suggesting different modes of action for these two compounds.

The results of clofibric acid (**1b**) and 2-hydroxytetronic acid (**2a**) on human platelet aggregation in vitro are shown in Figure 1. Such studies are significant, since blood platelets also play an important role in the genesis of atherosclerosis[29] and **1b** previously was shown to have antiplatelet effects.[30] Analogues **2b** and **3a** were not assessed for these effects in vitro owing to their lack of solubility in physiological buffers. Both **1b**[30] and **2a** (Figure 1) inhibiting collagen-induced platelet aggregation and secretion of [^{14}C]serotonin in a concentration-dependent manner at equivalent doses. Arachidonic acid induced platelet aggregation was not affected by either of these agents. These preliminary results indicate that **2a** may be inhibiting platelet function by a mechanism similar to the one proposed for clofibric acid (**1a**), not involving prostaglandin synthesis but rather inhibiting arachidonic acid release.[30]

Clearly, additional studies are required to define the potential usefulness of 4-aryl-2-hydroxytetronic acids in CHD and hyperlipoproteinemia, their biochemical modes of action, and structural requirements for maximum potency and reduced toxicity. However, it is also encouraging that neither **2a,b** nor its oxidized form **3a** are toxic to P-388 cells or to the host mouse at 150 mg/kg.[31] Whereas **2a** is structurally related to L-ascorbic acid (**2c**), **2a** should not be construed as a "lipophilic vitamin C analogue". Foremost, dehydroascorbic acid (**3b**) is a biological equivalent of **2c** in antiscorbutic assays,[32] unsubstituted 2-hydroxytetronic acids have no antiscorbutic activity,[33] and dehydro analogue **3a** has effects different from those obtained with compound **2a**. Avitaminosis C, however, may be a risk factor in atherosclerosis,[34,35] but in L-ascorbic acid deficient guinea pigs the increase in liver CH has been attributed to reduced transformation of CH to bile acids.[36] The transient increase in CH levels observed at day +7 for dehydro analogue **3a** is difficult to explain and would require further evaluation. We anticipate that analogue **2a**, the first in this series of lipophilic Brönsted acids capable of forming water-soluble anions, will be shown to have selective antilipidemic properties not related either to clofibric acid (**1b**) or L-ascrobic acid (**2c**), owing to the influence of the biologically relevant redox function influencing enzymatic processes in cells, particularly their membranes. This may not be the case for the inhibitory effects of **1b** and **2a** on human platelet functions.

Experimental Section

The chemical experimental methodology has been adequately defined by reference to the literature. Compounds were characterized in part by use of Varian A-60 NMR and Beckman IR 4230 spectrometers and Altex Model 4500 digital and Leeds and Northrup pH meters. Platelet aggregation methodologies were identical with those previously described.[30]

Antilipidemic Studies. Male Sprague-Dawley rats (180–210 g) were maintained on a high CH diet (dextrin, 48%; casein, 18%; sucrose, 11%; coconut oil, 10%; USP XVII, 4%; Celufil, 2%; cholesterol USP, 1%; cholic acid, 0.5%; liver concentrate NF, 0.4%; choline chloride, 0.2%; D-methionine, 0.2%; vitamin sup-

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plements, 4%;) for a period of 1 week prior to drug or vehicle treatment. Rats were maintained on this diet for the remainder of the experiment (14 days). Groups (n = 6) of rats received various doses of **1a**, **2a,b**, and **3a** [0.2 or 0.4 (mmol/kg)/day in 0.25% methylcellulose] or 0.25% methylcellulose orally twice daily for 14 consecutive days. In all experiments, drugs were given in a total volume of 1.0 mL/100 g of body weight. All rats were fasted 16–18 h before blood collection. Blood was drawn (orbital plexus) from rats under light ether anesthesia one day before (day-1) and 4, 7, and 10 days (day +4, +7, +10, respectively) after drug treatment. After 14 days (day +14) of drug treatment, blood was collected by exsanguination from the abdominal aorta of rats, and livers were excised, rinsed in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 (containing 1.15%, w/v, KCl), blotted, weighed, minced, and homogenized in the same buffer using a glass homogenizer equipped with a Teflon pestle.

Liver Assays. CH and TG's were extracted from the liver homogenates (25%, w/v) by the method of Abell et al.[37] and analyzed by the method of Parekh and Jung[38] and Soloni,[39] respectively.

Serum Assays. All blood samples were placed on ice, and after clotting, serum was separated by centrifugation at 2000g for 10 min. The serum samples were kept at 4 °C and analyzed within 48 h. Serum total CH was measured by the enzymatic method of Allain et al.[40] with A-Gent cholesterol reagent (Abbott Laboratories, Diagnostics Division, Chicago, IL) on an Abbott Bichromatic Analyzer (ABA-100) equipped with a 1:51 syringe-plate. Serum TG concentrations were measured with A-Gent triglyceride reagent (Abbott Laboratories Diagnostics Division, Chicago, IL) on an ABA-100 according to the method of Sampson et al.[41] α-LP CH was estimated by the polyanionic precipitation method[42] using a modification described by Steele et al.[43] Heparin-$MnCl_2$ precipitable LP CH was determined by subtracting α-LP CH values from serum total CH values.

Statistical Evaluation. Data obtained was statistically evaluated by an analysis of variance with repeated measures using GLM of SAS.[44] Differences between groups were determined using the Duncan's multiple range procedure.[44]

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L-[4-^{11}C]Aspartic Acid: Enzymatic Synthesis, Myocardial Uptake, and Metabolism

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Sterile, pyrogen-free L-[4-^{11}C]aspartic acid was prepared from $^{11}CO_2$ using phosphoenolpyruvate carboxylase and glutamic/oxaloacetic acid transaminase immobilized on Sepharose supports to determine if it is a useful indicator for in vivo, noninvasive determination of myocardial metabolism. An intracoronary bolus injection of L-[4-^{11}C]aspartic acid into dog myocardium showed a triexponential clearance curve with maximal production of $^{11}CO_2$ 100 s after injection. Inactivation of myocardial transaminase activity modified the tracer clearance and inhibited the production of $^{11}CO_2$. Positron-computed tomography imaging showed that the ^{11}C activities retained in rhesus monkey myocardium are higher than those observed in dog heart after intravenous injection of L-[4-^{11}C]aspartic acid. These findings demonstrated the rapid incorporation of the carbon skeleton of L-aspartic acid into the tricarboxylic acid cycle after enzymatic transamination in myocardium and suggested that L-[4-^{11}C]aspartic acid could be of value for in vivo, noninvasive assessment of local myocardial metabolism.

Amino acids play a central role in myocardial energy metabolism, specifically in the coordination of mitochondrial and cytosolic biochemical processes. This knowledge has been derived from in vitro studies by using, for example, ^{14}C- or ^{15}N-labeled compounds. These nuclides, however, are not adequate in the application of the principles of tracer kinetics to animals and man for the assessment of local biochemical and physiological processes. Positron-emitting radiopharmaceuticals, e.g., labeled with ^{13}N or ^{11}C, are ideally suited for this purpose. ^{11}C and ^{13}N are short-lived radionuclides (20.38 and 9.96 min half-lives, respectively) which decay by positron emission, and their in vivo usage is based on the visualization of the two 511-KeV γ annihilation photons emitted per decay event.[2] In a recent paper we discussed the enzymatic synthesis of ^{13}N-labeled L-amino acids and their utilization for in vivo assessment of local myocardial metabolism.[3] Evidence obtained from a variety of studies[4] suggests that regulation of oxidative metabolism of carbohydrates requires the malate–aspartate cycle, by which carbon skeletons from amino acids can be shuttled into the tricarboxylic acid cycle. Therefore, it seems reasonable to expect that the types of labels in the amino acid, e.g., ^{13}N or ^{11}C, might produce different in vivo tissue distribution and kinetic patterns of positron activity. Prompted by the above considerations, we designed the present study to determine if ^{11}C-labeled L-aspartic acid is a useful indicator for in vivo, noninvasive determination of myocardial metabolism.

Chemistry. We describe herein the covalent immobilization of phosphoenolpyruvate carboxylase[5] and glutamic/oxaloacetic acid transaminase[6] to Sepharose by the cyanogen bromide activation method. This work represents the first example of the use of this technique for the

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