JOURNAL OF
MEDICINAL
CHEMISTRY

© Copyright 1997 by the American Chemical Society

Volume 40, Number 2 January 17, 1997

Expedited Articles

Chlorogenic Acid and Synthetic Chlorogenic Acid Derivatives: Novel Inhibitors of Hepatic Glucose-6-phosphate Translocase

Horst Hemmerle,* Hans-Joerg Burger, Peter Below, Gerrit Schubert, Robert Rippel, Peter W. Schindler, Erich Paulus, and Andreas W. Herling

Hoechst AG, Hoechst Marion Roussel, 65926 Frankfurt am Main, Germany

Received October 21, 1996[⊗]

The enzyme system glucose-6-phosphatase (EC 3.1.3.9) plays a major role in the homeostatic regulation of blood glucose. It is responsible for the formation of endogenous glucose originating from gluconeogenesis and glycogenolysis. Recently, chlorogenic acid was identified as a specific inhibitor of the glucose-6-phosphate translocase component (Gl-6-P translocase) of this enzyme system in microsomes of rat liver. Glucose 6-phosphate hydrolysis was determined in the presence of chlorogenic acid or of new synthesized derivatives in intact rat liver microsomes in order to assess the inhibitory potency of the compounds on the translocase component. Variation in the 3-position of chlorogenic acid had only poor effects on inhibitory potency. Introduction of lipohilic side chain in the 1-position led to 100-fold more potent inhibitors. Functional assays on isolated perfused rat liver with compound **29i**, a representative of the more potent derivatives, showed a dose-dependent inhibition of gluconeogenesis and glycogenolyosis, suggesting glucose-6-phosphatase as the locus of interference of the compound for inhibition of hepatic glucose production also in the isolated organ model. Gl-6-P translocase inhibitors may be useful for the reduction of inappropriately high rates of hepatic glucose output often found in non-insulin-dependent diabetes.

Introduction

The enzyme system glucose-6-phosphatase (Gl-6-Pase) (EC 3.1.3.9) is known to play a major role in the homeostatic regulation of blood glucose.[1,2] It is responsible for the formation of endogenous glucose originating from gluconeogenesis *and* glycogenolysis. Gl-6-Pase is localized within the membranes of the endoplasmic reticulum. Upon disruption of membranes of microsomal vesicles from rat liver with optimal concentrations of detergents, higher activity is found compared to untreated vesicles. In order to explain the molecular basis of this latent activity, and the role of the membrane for Gl-6-Pase function, two major hypotheses have been put forward during the last two decades.[3,4]

The conformational model, suggested first by the groups of Nordlie[5] and Schulze,[6] and its most recent version, termed the flexibility−substrate transport model by Berteloot et al.,[7] postulates that Gl-6-Pase is a membrane-spanning pore-forming protein with the catalytic site located within a water-filled pore accessible to the substrate from the cytoplasmic surface of the membrane. According to this model, Gl-6-Pase activity in intact microsomes is constrained by the membrane, and translocation of glucose 6-phosphate into the lumen of the endoplasmic reticulum is not required. However, Countaway et al., investigating the catalytic turnover number for the enzyme, did not find any evidence for a constrained activity of Gl-6-Pase in intact microsomes.[8]

Alternative to the conformational model, the substrate transport model proposed by Arion and co-workers[9,10] and supported by the results of numerous kinetic studies from the same group[8,11,12] postulates a catalytic component with its active site oriented toward the lumen of the endoplasmic reticulum, a glucose-6-

[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1997.

S0022-2623(96)00736-4 CCC: $14.00 © 1997 American Chemical Society

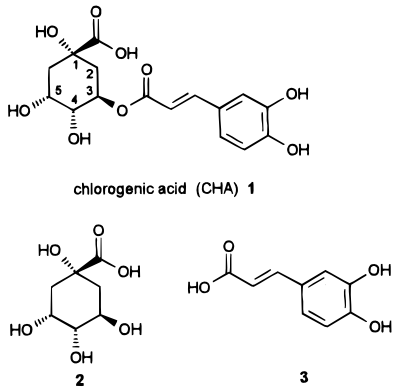


Figure 1. Structural formulas of chlorogenic acid (CHA, **1**), (−)-quinic acid (**2**), and 3,4-dihydroxycinnamic acid (**3**).

phosphate translocase (Gl-6-P translocase) T1, facilitating the movement of glucose 6-phosphate through the membrane, Pi released at the luminal surface is believed to equilibrate via a second translocase T2. While the catalytic component[13,14] and the phosphate transporter T2[15] have been recently isolated and/or cloned, evidence for the Gl-6-P translocase is still circumstantial. However, recent findings that the Gl-6-Pase gene of a glycogen storage disease type Ib patient is normal,[16] and light-scattering experiments by Fulceri et al.,[17] which provided further evidence for the translocation of glucose 6-phosphate into the lumen of the ER, are more consistent with the substrate transport model.

Using the nomenclature of the substrate transport model, the catalytic unit of disrupted microsomes has been shown to be a relatively nonspecific hydrolase of a variety of organic phosphate esters, whereas glucose 6-phosphate is the only physiological sugar phosphate known to be transported by the Gl-6-P translocase T1,[18] which makes this transporter an interesting and logical pharmacological target.

Recently, we identified chlorogenic acid **1** (CHA, Figure 1) as a new inhibitor of microsomal Gl-6-Pase activity.[19] CHA, first described in 1920,[20] plays an important role in plant metabolism.[21,22] In the literature, mainly antioxidant properties have been described for CHA, which presumably can be ascribed to its polyhydroxyphenylic nature.[23,24]

Detailed kinetic studies of the interaction of CHA with the microsomal Gl-6-Pase system of rat liver suggested Gl-6-P translocase as the targeted function,[12] whereas the catalytic unit and the phosphate translocase were not affected even at millimolar concentrations of the compound. Thus, CHA is the first specific inhibitor of Gl-6-P translocase. Other described inhibitors of Gl-6-P translocase (e.g. Hg^{2+}, pyridoxal phosphate, NEM, certain sulfhydryl poisons, diazobenzene sulfonate, tosyllysine, chloromethyl ketone, and diethyl pyrocarbonate; for a recent review and literature references of translocase inhibitors, see ref 12), in addition to inhibiting Gl-6-Pase activity of intact microsomes, show some degree of inhibition of the phosphohydrolase in detergent disrupted microsomes and hence are not well suited for specific pharmacological targeting of the Gl-6-P translocase activity.

In non-insulin-dependent diabetes mellitus (NIDDM), excessive endogenous hepatic glucose production has been described to contribute to fasting hyperglycemia.[25,26] Although, this contention has recently been challenged based on methodological grounds,[27] even "normal" rates of hepatic glucose production are evidence for an abnormal response of the liver in the face of the elevated fasting plasma glucose concentrations generally found in overt NIDDM.

Thus, hepatic glucose production might be an attractive target for therapeutic intervention in order to reduce fasting blood glucose in NIDDM. Recently published results suggest that even intensive pharmacological interventions with insulin or sulfonylureas in NIDDM do not reach the goal of obtaining near normal glycemia,[28] clearly justifying the search for additional therapeutical approaches.

Previous attempts to alter hepatic glucose output and fasting plasma glucose levels in patients with NIDDM by inhibiting specifically the gluconeogenic pathway have failed,[29] presumably due to the existence of a functioning autoregulatory mechanism in the liver involving compensatory glycogenolysis.[30]

Therefore, we reasoned that simultaneous targeting of gluconeogenesis *and* glycogenolysis with an inhibitor of Gl-6-P translocase should result in a reduction of hepatic glucose production. Complete absence of functional Gl-6-Pase activity, as evidenced in human glycogen storage disease type I, results in severe hypoglycemia.[2,31]

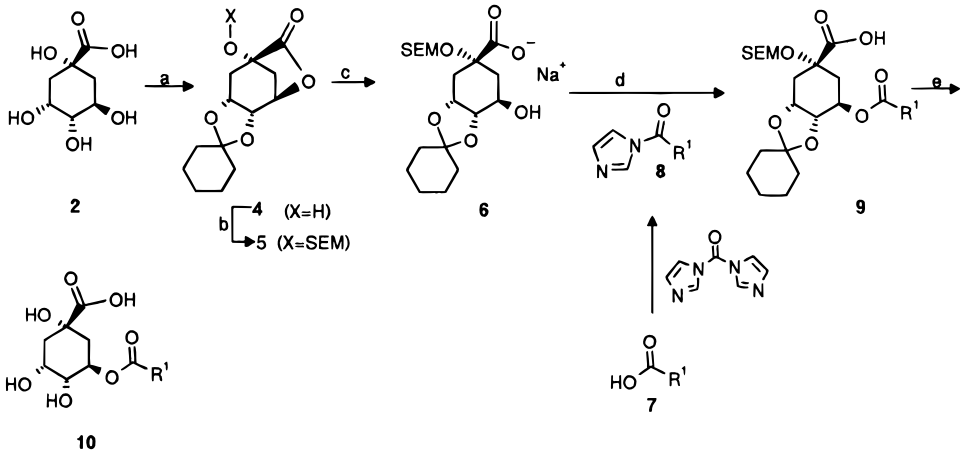
Our aim was to find more active derivatives of CHA which inhibit microsomal Gl-6-P translocase and, furthermore, are active in functional assays of hepatic glucose production. In this study, we report the first structure−activity relationships for inhibition of Gl-6-P translocase by synthetic analogues of this natural product. In addition, inhibition of glucose production by a synthesized CHA analogue was evaluated in functional assays on the isolated perfused rat liver.

Chemistry

Chlorogenic acid **1** (CHA, Figure 1) is the ester product of (−)-quinic acid (**2**) and 3,4-dihydroxycinnamic acid (**3**). Our first aim was to find an efficient and flexible way to synthesize analogues of **1**. For variation of the cinnamic moiety, we used the readily available 3,4-*O*-cyclohexylidene lactone **4**, which could be synthesized in one step by acidic catalysis starting from quinic acid (**2**) and cyclohexanone.[32] In compound **4** all functional groups are protected except the hydroxy group in position 1. After transformation of this alcohol to SEM ether **5**, the lactone moiety was hydrolyzed with an equimolar amount of sodium hydroxide to **6**. The sodium salt **6** was the key intermediate for all variations in position 3 of (−)-quinic acid. Most of the examined acylation conditions yielded the lactone **5** as the main product. This side reaction can be explained by transfer of the activated acyl group to the carboxylate of **6** resulting in a mixed anhydride, which can readily cyclize. The exceptions are acyltriazolides or acylimidazolides. Activation of carboxylic acids **7** was achieved by carbonylditriazole or carbonyldiimidazole, and these derivatives reacted rapidly at 5 °C with the sodium salt of **6**. The ester **9** was obtained in 40−85% yield after purification on silica gel. Cleavage of the cyclohexylidene and SEM protecting groups **9** was performed with 2 N hydrochloric acid in dioxane and yielded **10** in 70−90% (Scheme 1).

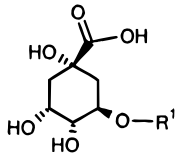
When R^1 in acid **7** contained one or more hydroxy groups, these were protected as SEM ether. Table 1

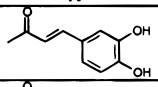
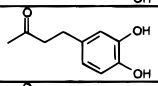
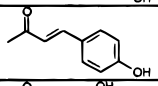
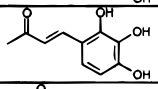
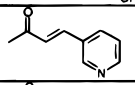
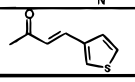
Scheme 1[a]



[a] (a) Cyclohexanone, H_2SO_4, 75%; (b) [(trimetylsilyl)ethoxy]methyl chloride (SEM-Cl), diisopropylethylamine, 98%; (c) NaOH, dioxane, 95%; (d) NaH, DMF, 40−85%; (e) aqueous hydrochloric acid, dioxane, 70−90%.

Table 1. Variation of Cinnamic Moiety of Chlorogenic Acid



compound	R¹	IC_{50}[μM]
1		230
10a		680
10b		250
10c		310
10d		>1000
10e		380

lists some examples synthesized according to the described procedure.

Analysis of the structure−activity relationship for inhibition of glucose-6-phosphate hydrolysis in intact microsomes shows that a single hydroxy group in the para position of the phenyl ring in CHA (**1**) is sufficient for activity. Hence, further studies were performed using the *p*-hydroxyphenyl side chain of **10b**.

Similar to building block **4**, we used the known shikimic acid derivative **11a**[33] (Table 2) to synthesize the dehydrated CHA analogue **11**.[34] The precursor **12a** of **12** was obtained through radical deoxygenation of **4**.[35] For hydrolytically stable analogues of **11** we used the known building block **13a**.[36] Reaction of this amine **13a** at 60 °C with the imidazolide **8**, and removal of the protecting groups as described in Scheme 1 produced the desired amide **13**.

To investigate the influence of the carboxylic group in CHA (**1**), we esterified this compound with diazomethane to yield the ester **14**.[37] As further examples, we synthesized **15**−**17** from the already published precursors **15a**−**17a**.[38,39] Diisobutylaluminum hydride reduction of lactone **5** followed by Horner−Emmons reaction with triethyl phosphonoacetate led to **19**, which was hydrogenated under normal pressure in the presence of rhodium on alumina to yield **20** (Scheme 2). Subsequent use of the same reaction sequence illustrated in Scheme 1 resulted in homologue **21**. We also synthesized the shorter homologue **27**. In this case we used the known ketone **22**[40] in which we protected the hydroxy group with dihydropyran and then added the lithium enolate of *tert*-butyl acetate at −78 °C (Scheme 3). The stereoselectivity of this addition was 3:1 isomer **24** vs isomer **25**, respectively. The configuration of **24** was confirmed by X-ray crystallography.[46]

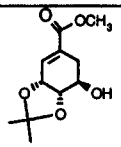
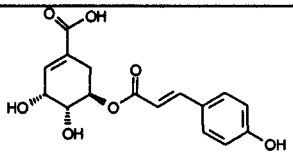
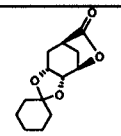
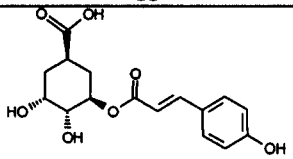
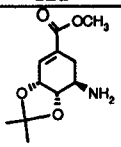
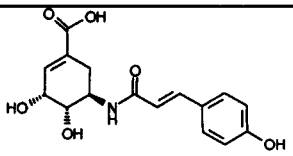
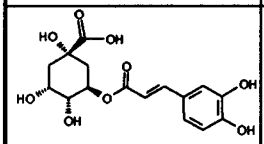
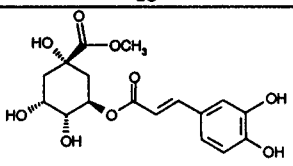
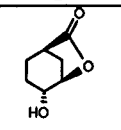
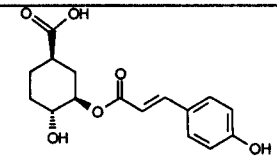
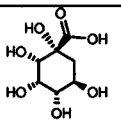
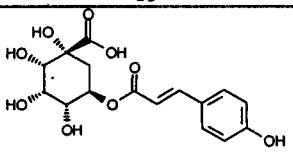
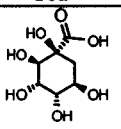
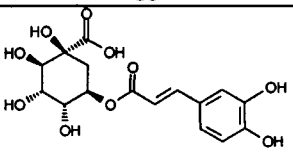
We selected the hydroxy group in position 1 of CHA for further variations of the lead structure. From a synthetic chemical point of view, this position can be modified easily by alkylation of the unprotected tertiary hydroxy group in lactone **4**. Thus, we deprotonated **4** with sodium hydride in dimethylformamide and added an activated alkyl halide. Table 3 contains a selection of the obtained products. Sterically hindered or unactivated alkyl halides gave very poor yields of **29** or no reaction at all.

With the 4-chlorophenylpropyl side chain in position 1, considerable variation of the ester side chain in position 3 was tolerated, as shown by the examples in Table 4.

Biological Results and Discussion

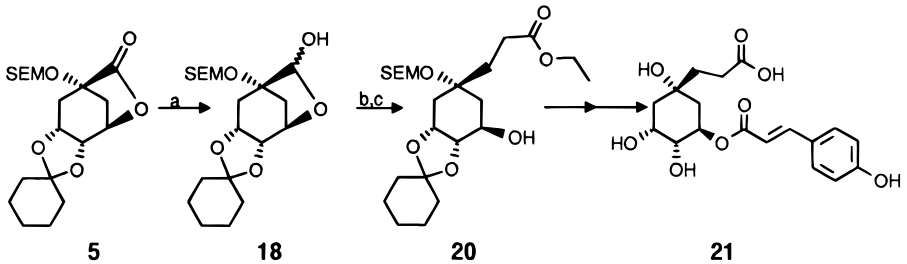
Effect of Chlorogenic Acid and Chlorogenic Acid Derivatives on Microsomal Glucose-6-phosphatase Activity. The inhibitory activity of CHA and synthesized CHA analogues was evaluated by their ability to inhibit glucose 6-phosphate hydrolysis in highly intact microsomal preparations obtained from livers of 20-h fasted rats. Gl-6-P translocase imposes significant rate limitations on the Gl-6-Pase activity of intact microsomes, whereas under these conditions other translocase components do not play a significant role for the steady state hydrolysis of glucose 6-phosphate by the system.[10] Measuring of the effects of test compounds on the Gl-6-Pase activity of intact microsomes can thus be conveniently used to assess their potency as inhibitors of the Gl-6-P translocase component.

Table 2. Variations of the Cyclohexyl Moiety of CHA

starting material	compound	Percent inhibition of gl-6-p-hydrolysis
 11a [a]	 11	50%@0.25 mM
 12a [b]	 12	50%@0.23 mM
 13a [c]	 13	15%@1mM
 1	 14	50%@1mM
 15a [d]	 15	20%@1 mM
 16a [e]	 16	30%@1 mM
 17a [e]	 17	10%@1 mM

[a] Reference 33. [b] Reference 35. [c] Reference 36. [d] Reference 38. [e] Reference 39.

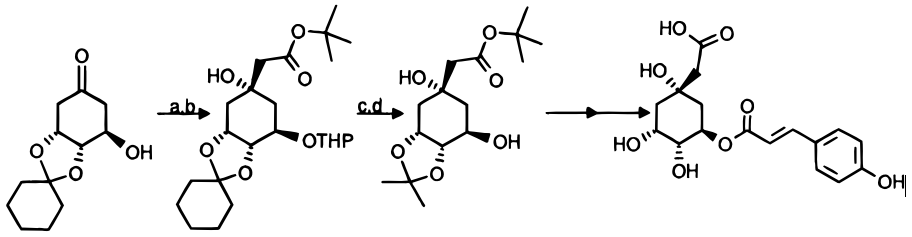
Scheme 2[a]



[a] (a) 1.1 equiv of 1.2 M DIBAH solution in toluene, 85%; (b) triethyl phosphonoacetate, NaH, THF, 82%; (c) H_2, Rh/Al_2O_3, ethyl acetate, 94%; further steps as described in Scheme 1.

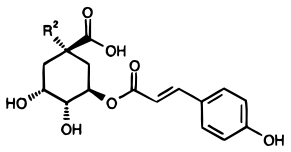
Cinnamic acid **3** and quinic acid (**2**) alone did not inhibit the Gl-6-Pase activity of intact microsomes (data not shown). Variation of the ester residue in position 3 of CHA (**1**) had little effect on inhibitory activity. Compound **11** had nearly the same inhibitory activity as CHA (Table 2), demonstrating that the OH group in position 1 is also not essential for inhibition. This result could also be confirmed by the deoxygenated CHA

Scheme 3[a]

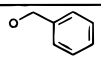
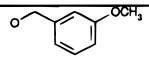
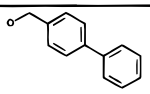
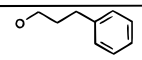
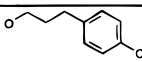
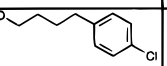


[a] (a) DHP, PPTS, CH_2Cl_2, 95%; (b) LDA, *tert*-butyl acetate, THF, 55%; (c) PPTS, MeOH; (d) 2,2-dimethoxypropane, PPTS; further steps as described in Scheme 1.

Table 3. Alkylated Derivatives of CHA

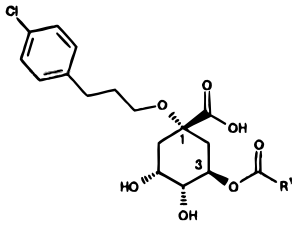


29

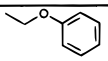
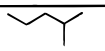
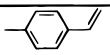
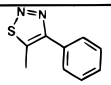
compound 29	R^2	IC_{50} [μM]
a	O-Met	280
b	O-Et	38%@1mM
c		230
d		190
e		110
f		47
g		21
h		31
i		2.5
j		85

analogue **12** (IC_{50} = 230 μM). The examples **15**−**17** illustrate the role of hydroxy groups in **1**. Compounds **21** and **27** were only weak inhibitors of glucose 6-phosphate hydrolysis with apparent IC_{50} values above 1 mM. The examples given in Table 2 and Schemes 2 and 3 illustrate the importance of the carboxylic acid and ring hydroxy groups in CHA (**1**) for the inhibitory activity. The methyl ether **29a** had nearly the same activity as the free alcohol **10b**, but the corresponding ethyl analogue **29b** showed only little effect on glucose 6-phosphate hydrolysis. Introduction of aromatic substituents into the side chain increased inhibitory activity, with an optimal chain length of four atoms between the aromatic and the cyclohexyl ring. Variations of substituents on the aromatic ring resulted in more potent inhibitors. Introduction of the 4-chlorophenylpropyl side chain led to compound **29i**, which was 100 times more potent than **1**.

Table 4. Variation of Ester Moiety of CHA Analogue



30

compound 30	R^1	IC_{50} [μM]
a		12.0
b		41
c		1.3
d		8.9
e		4.5

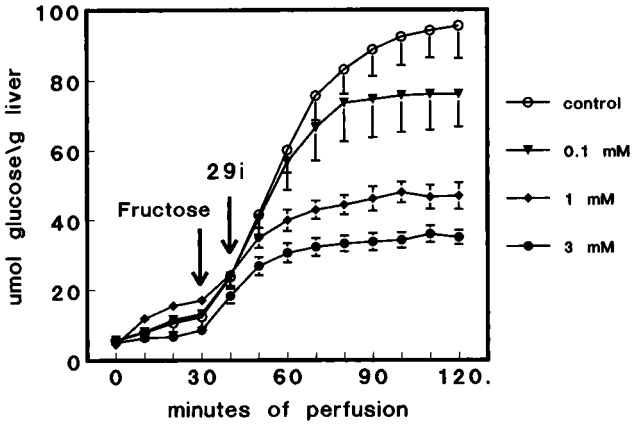


Figure 2. Inhibitory effect of **29i** on fructose-induced hepatic glucose output (gluconeogenesis) in isolated perfused livers from starved rats. Values are mean ± SEM, n = 4−8.

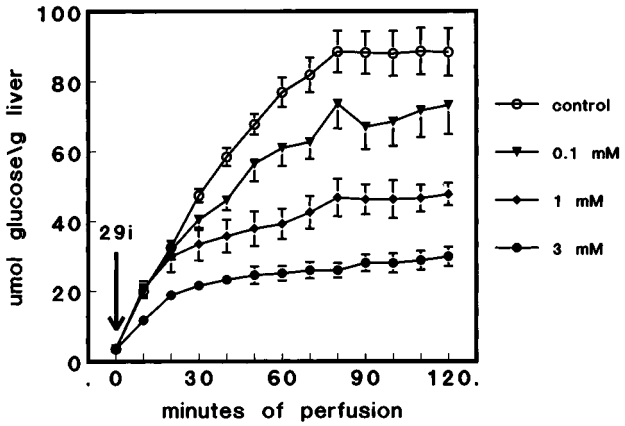


Figure 3. Inhibitory effect of **29i** on hepatic glucose ouput (glycogenolysis) in isolated perfused livers from fed rats. Values are mean ± SEM, $n = 4-8$.

Gl-6-P translocase specific inhibitors have to fulfill the additional general criteria established previously[9] in that they inhibit glucose 6-phosphate hydrolysis in intact microsomes, but not in detergent-disrupted microsomal vesicles. This has been convincingly demonstrated for CHA (**1**).[12] At a concentration of 1 mM, none of the CHA derivatives inhibiting glucose 6-phosphate hydrolysis in intact microsomes to various extents (Tables 1−4) inhibited glucose 6-phosphate hydrolysis in fully disrupted microsomes obtained by short treatment of the vesicles with an optimal concentration of detergent (data not shown), conditions under which the hydrolysis of glucose 6-phosphate is not restricted by substrate transport. Thus, the more active derivatives of CHA, like CHA itself, appear to be specific inhibitors of the translocase component of the Gl-6-Pase system; albeit definite evidence of specificity for Gl-6-P translocase will require the exclusion of an effect of the analogues on the pyrophosphatase activity in intact microsomes as has been already demonstrated for CHA,[12] experimental conditions where only T2, but not T1 exerts a rate limiting influcence on the hydrolysis of pyrophosphate. These new compounds could have a significant potential for the elucidation of the structure and function of the Gl-6-Pase system.

Effects of a Chlorogenic Acid Derivative on Glucose Production in Isolated Perfused Rat Livers. An expected and important consequence of blocking Gl-6-P translocase by derivatives of CHA would be the inhibition of hepatic glucose production.

The results of liver perfusion experiments with glycogen-depleted rat livers perfused with glucose-free medium containing 10 mM fructose, and glycogen-rich livers with glucose-free medium without any additives are presented in Figures 2 and 3, respectively. With glycogen-depleted livers, perfusate glucose formation was significantly stimulated in the 60 min following addition of fructose to the system (Figure 2). Addition of 0.1, 1, and 3 mM compound **29i**, as a representative example for a highly active CHA derivative with a lipophilic side chain at position 1, caused a concentration-dependent decrease of the hepatic glucose output (Figure 2), confirming preliminary findings in isolated hepatocytes which suggested an inhibitory effect on substrate-stimulated glucose production (H.-J. Burger, unpublished data).

Perfusion experiments with glycogen-rich livers showed net glucose production for the first 80 min (Figure 3). In those livers with 0.1, 1, and 3 mM compound **29i** included, glucose production decreased significantly, again in a concentration-dependent manner (Figure 3), also indicating that compound **29i** is able to reach the target enzyme system in a complex in vitro system with an intact intra-organ architecture. Similar series of experiments with addition of CHA to the perfusate did not show any effect of the compound on perfusate glucose formation (data not shown). The more lipophilic character of compound **29i** compared to that of CHA might be beneficial with respect to the uptake of this organic anion into the hepatocytes. On the other hand, concentrations of CHA sufficient for inhibition might not be reached at the intracellular target site, perhaps due to an inability of the more hydrophilic CHA to cross the cytoplasmic membrane.

The observations described above for compound **29i** are consistent with an inhibitory activity of this CHA derivative at the level of Gl-6-Pase also in the intact organ model. Concomitant inhibition of glycogenolysis and fructose-stimulated gluconeogenesis by the CHA derivative **29i** strongly supports inhibition of Gl-6-Pase via T1 over other possible sites of inhibition of hepatic glucose output. The availability of inhibitors of Gl-6-P translocase active in an intact organ model will allow the study of metabolic directive effects of specific inhibition of Gl-6-Pase activity under numerous experimental conditions. Possibilities for further optimization of the lead structure **29i** are currently being investigated.

Experimental Section

General Methods. Reactions with materials sensitive to air or moisture were run in dry-glass apparatus under an argon atmosphere with dry solvents. Melting points were determined on a Büchi capillary melting point apparatus (according to Dr. Tottoli) and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM 270 spectrometer. Significant ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constant(s) in hertz. FAB mass spectra were obtained on a Kratos MS 902 in a 3-nitrobenzylic alcohol matrix (optionally in the presence of LiCl) using xenon as the target gas. DCI mass spectra were determined on a Kratos MS 80 RFA using isobutane as reagent gas. High-resolution FAB MS: mass spectrometer ZAB2-SEQ (VG Analytical) with data system 11-250J, equipped with the standard VG FAB ion source and a cesium ion gun operated at 35 kV. A methanolic sample solution was added to a 10% solution of polyethylene glycol 1000 or PEG 1000 dimethyl ether, respectively, in 3-nitrobenzyl alcohol. Resolution $M/\Delta M = 5000$, 30 s linear voltage scan over a mass range of 100 Da, raw data accumulation (MCA) of 15−17 scans. Mass determination by software interpolation between two PEG reference signals; reproducibility better than 0.002 Da ($n = 4$). Flash chromatography was carried out on E. Merck silica gel 60 (0.04−0.063 mm). Thin-layer chromatography was performed on silica gel F_{254} plates from E. Merck. Visualization was done using phosphomolybdic acid/cerium(IV) sulfate/H_2SO_4 and heating on a 100 °C plate. Elemental analyses were performed by the Analytical Laboratories, Hoechst AG.

Syntheses of Compounds. (A) Preparation of 4 from 2. A 163.3 g (0.85 mol) portion of compound **2** was suspended in 186 mL (1.8 mol) of cyclohexanone, and 0.5 mL of concentrated sulfuric acid was added. The mixture was then heated slowly to a heating bath temperature of 200 °C, and a water/cyclohexanone azeotrope was removed by distillation. After the azeotrope no longer distilled over, the light brown reaction solution was stirred at a bath temperature of 200 °C for a further 2 h. The reaction solution was then allowed to cool to 70 °C, and 10 g of sodium hydrogen carbonate was added. The mixture was then treated with 700 mL of ethyl acetate, and

the organic phase was washed with water and saturated sodium chloride solution. The organic phase was then concentrated in vacuo. The light yellow residue was crystallized from 2-propanol/water, 1:1. A yield of 142.1 g (75%) of lactone **4** was obtained as colorless crystals.

(B) Preparation of 5 from 4. A 38.14 g (0.15 mol) portion of hydroxy lactone **4** was dissolved in 180 mL of dichloromethane, 53.0 mL (0.3 mol) of diisopropylamine was added, and 45.0 mL (0.254 mol) of [(trimethylsilyl)ethoxy]methyl chloride was added dropwise at room temperature to this solution, and it was stirred at reflux temperature for 6 h. The reaction solution was then added to a saturated ammonium chloride solution and extracted with ethyl acetate. The combined organic phases were extracted using cold 1 N potassium hydrogen sulfate solution at about 6 °C and dried using sodium sulfate. After concentration in vacuo, a light yellow residue was obtained which was crystallized from heptane/ethyl acetate, 6:1. A 57.0 g (98%) yield of **5** was obtained.

(C) Preparation of 6 from 5. A 1.38 g (3.6 mmol) portion of **5** was dissolved in 8 mL of dioxane, 3.8 mL of 1 N sodium hydroxide solution was added at room temperature, and the resulting reaction mixture was stirred and after 2 h concentrated in vacuo. A 1.2 g yield of **6** as an amorphous solid was obtained.

(D) Preparation of 8 from 7 (Common Procedure). A 27 mmol portion of carboxylic acid **7** was dissolved in 35 mL of anhydrous dimethylformamide. A solution of 27 mmol of carbonyldiimidazole (or carbonylditriazole) dissolved in 35 mL of anhydrous dimethylformamide was added dropwise at room temperature. After 2 h this solution was ready for use in the following reaction.

(E) Preparation of 9 from 6 and 8 (Common Procedure). A 25 mmol portion of sodium hydride was added at room temperature to a solution of 21 mmol of sodium salt **6** in 50 mL of anhydrous dimethylformamide. This suspension was stirred for 1 h at room temperature before the solution of imidazolide **8** was added at 0−5 °C. After 2 h at 0−5 °C the reaction mixture was added to saturated ammonium chloride solution. The mixture was acidified to pH 4 by addition of 1 N potassium hydrogen sulfate and extracted using ethyl acetate. The combined organic phases were washed successively with saturated ammonium chloride solution, water, and saturated sodium chloride. The organic phase was dried using sodium sulfate and concentrated in vacuo. The oily residue was purified on silica gel.

(F) Preparation of 10 from 9 (Common Procedure). A 52 mmol portion of cyclohexylidene ketal **9** was dissolved in 130 mL of dioxane and treated with 95 mL (0.19 mol) of 2 N hydrochloric acid at room temperature with stirring. The mixture was stirred at room temperature for 20 h. Then the clear solution was adjusted to pH 3−4 using 2 N sodium hydroxide and concentrated in vacuo. The solid residue was suspended in ethyl acetate/methanol, 3:1, and the insoluble sodium chloride was filtered off. The filtrate was concentrated again, and the residue was purified on silica gel.

According to these procedures we synthesized the compounds **10a**−**e**, which were characterized by NMR, MS, and C, H, N analyses. Yields: (**6** → **10a**) 54%, (**6** → **10b**) 61%, (**6** → **10c**) 43%, (**6** → **10d**) 48%, (**6** → **10e**) 57%.

Compounds **11**−**17** were prepared according to the steps d and e (Scheme 1) starting from the known building blocks **11a**−**17a**. Yields: (**11a** → **11**) 77%, (**12a** → **12**) 65%, (**13a** → **13**) 49%, (**15a** → **15**) 81%, (**16a** → **16**) 34%, (**17a** → **17**) 23%.

(G) Preparation of 18 from 5. A 15.0 g (39 mmol) portion of **5** was dissolved in 200 mL of anhydrous toluene, and 38 mL (43 mmol) of 1.2 M diisobutylaluminum hydride solution in hexane was added dropwise at −70 °C. The reaction mixture was allowed to warm to 0 °C in the course of 2 h and was hydrolyzed using 10 mL of saturated sodium hydrogen carbonate solution, and 10 mL of 1 N sodium hydroxide solution and 10 mL of water were added successively. The reaction mixture was vigorously stirred at room temperature for 30 min, and finally the solution was concentrated. A 12.9 g (85%) yield of **18** was as a colorless oil which crystallized at 0 °C was obtained. Mp: 20−25 °C.

(H) Preparation of 19 from 18 (Step b, Scheme 2). A 7.5 g (33.5 mmol) portion of triethyl phosphonoacetate was added dropwise at 0 °C under argon atmosphere to a suspension of 0.9 g (29.9 mmol) of 80% sodium hydride in 200 mL of anhydrous tetrahydrofuran. The reaction mixture was slowly allowed to warm to room temperature, and then 7.7 g (19.9 mmol) of **18** dissolved in 20 mL of anhydrous tetrahydrofuran was added dropwise at −30 °C. This solution was stirred at −20 to −30 °C for 24 h and then treated with 100 mL of saturated ammonium chloride solution. It was necessary to maintain the low temperature, since at higher temperatures, intramolecular Michael addition of the free hydroxy group occurred which resulted in the formation of a cyclic ether. The combined organic phases were washed with saturated sodium chloride solution and dried using magnesium sulfate. After concentration in vacuo, the residue was purified on silca gel (eluent: ethyl acetate/*n*-heptane, 1:1), and 7.5 g (82%) of **19** was obtained as a colorless oil.

(I) Preparation of 20 from 19. To a solution of 1.0 g (2.2 mmol) of **19** in 50 mL of ethyl acetate was added 100 mg of Rh/Al_2O_3 (5% Rh). The mixture was shaken at 25 °C and normal pressure under a hydrogen atmosphere for 3 h. The catalyst was filtered off, and the filtrate was concentrated in vacuo. A 0.95 g (94%) yield of **20** as a colorless solid was obtained.

(J) Preparation of 21 from 20. 21 was prepared from **20** according the synthesis procedures from **5** to **10** (yield: 37%).

(K) Preparation of 23 from 22. A 20.0 g (84.4 mmol) portion of compound **22** was dissolved in 200 mL of anhydrous dichloromethane and treated at 25 °C with 14.9 g (176.8 mmol) of dihydropyran and 200 mg of pyridinium *p*-toluenesulfonate. This solution was stirred for 12 h, 500 mL of ethyl acetate was then added, and the organic phase was washed with sodium chloride solution. The organic phase was dried using magnesium sulfate and concentrated in vacuo. A 26.0 g (95%) yield of **23** as a colorless solid was obtained.

(L) Preparation of 24 and 25 from 23. A 3.66 g (36.0 mmol) portion of diisopropylamine was dissolved in 100 mL of anhydrous tetrahydrofuran, and 25 mL of 1.5 M *n*-butyllithium solution in hexane was added dropwise at −20 °C under an argon atmosphere. The reaction solution was allowed to warm to 0 °C and was then cooled again to −60 °C. A 4.1 g (35.3 mmol) portion of *tert*-butyl acetate dissolved in 20 mL of anhydrous tetrahydrofuran was slowly added dropwise at this temperature. The solution was stirred at −60 °C for 30 min, and subsequently 10.0 g (32.2 mmol) of **23** dissolved in 30 mL of anhydrous tetrahydrofuran was added dropwise at −60 °C. After being stirred for 1 h at the same temperature, the reaction mixture was hydrolyzed using saturated sodium hydrogen carbonate solution. The mixture was extracted using ethyl acetate. The combined organic phases were washed with saturated sodium chloride solution and dried using magnesium sulfate. After concentration, 11.9 g (87%) of **24** and **25** was obtained in a ratio of 3:1 as a light brown oil. The separation of the isomers was achieved by chromatography on silica gel (eluent: ethyl acetate/heptane, 1:2). We obtained **24** in 55% yield.

For proofing the relative configuration of the new stereocenter in **24**, we cleaved the protecting groups by treatment with *p*-toluenesulfonic acid in methanol and acylated the deprotected intermediate with acetic anhydride in dichloromethane with triethylamine and 4-(dimethylamino)pyridine as bases to obtain the tetraacetate **24a** as colorless crystals.

(M) Preparation of 26 from 24. A 11.9 g (27.9 mmol) portion of **24** was dissolved in 200 mL of methanol, and 1.8 g of pyridinium *p*-toluenesulfonate was added. The mixture was heated at reflux temperature for 1 h, and the reaction mixture was then concentrated in vacuo. The residue was dissolved in 200 mL of anhydrous dichloromethane, and 8.6 g (93.5 mmol) of dimethoxypropane was added. After 72 h at room temperature, the solution was concentrated in vacuo again and the residue was purified by chromatography on silica gel (eluent: ethyl acetate/*n*-heptane, 1:1). A 6.6 g (82%) yield of **26** was obtained.

(N) Preparation of 27 from 26. According to the synthesis of **10** from **6** the methylene-prolonged CHA analogue **27**

could be obtained with the only modification that aqueous trifluoracetic acid (95%) was used to cleave the protecting groups, especially the *tert*-butyl ester moiety, to yield **27** in 81%.

(O) Preparation of 28 from 4. The introduction of the side chains **28a**−**i** into hydroxy lactone **4** was achieved by deprotonation with 1.5 equiv of sodium hydride in anhydrous dimethylformamide and subsequent treatment with the corresponding alkyl halides at 0−10 °C. The alkylated products **28a**−**i** were transformed to **29** according to the procedure described in Scheme 1. Yields: (**4** → **29a**) 23%, (**4** → **29b**) 27%, (**4** → **29c**) 17%, (**4** → **29d**) 27%, (**4** → **29e**) 19%, (**4** → **29f**) 24%, (**4** → **29g**) 17%, (**4** → **29h**) 13%, (**4** → **29i**) 26%, (**4** → **29j**) 11%.

(P) Preparation of 30. According to the synthesis of **29** we obtained the esters **30a**−**f**. Yields: (**4** → **30a**) 18%, (**4** → **30b**) 21%, (**4** → **30c**) 15%, (**4** → **30d**) 19%, (**4** → **30e**) 13%.

Microsomal Gl-6-Pase Activity Assay. Microsomes were prepared by differential centrifugation from 10% (w/v) liver homogenates obtained from 20-h-fasted male Wistar rats (160−180 g body weight, Hattersheim, FRG) as has been reported in detail previously.[41] Protein concentration of the microsomal fraction was determined with the bicinchoninic acid method.[42] Intactness of the preparations, assessed by measuring the hydrolysis of 1 mM mannose 6-phosphate,[43] was usually above 97%. Optimally detergent disrupted microsomes were prepared according to published procedures[43] by exposing thawed microsomes to concentrations of detergent Triton X-100 which resulted in the maximal release of latent activity assessed by determination of the intactness of the vesicles.[43] Glucose 6-phosphate hydrolysis was determined in untreated and optimally detergent disrupted microsomes at 22 °C using a colorimetric assay described elsewhere[43] with some modifications for microtiter plates. Briefly, 100 μg of microsomal protein were incubated for 10 min at 22 °C in a total of 100 μL of assay buffer (250 mM sucrose, 50 mM HEPES, pH 7.0) containing 1 mM glucose 6-phosphate in the presence or absence of test compound (0.1−1000 μM). The reaction was started by the addition of microsomes. Plates for untreated and disrupted microsomes were run in parallel. The reaction was stopped by addition of 200 μL of phosphate color reagent,[43] and the formation of inorganic phosphate was quantified colorimetrically after incubation at 37 °C for at least 30 min by reading the extinction at 630 nm. For each concentration of test compound, background extinction was determined in parallel incubations by adding phosphate color reagent before addition of the microsomes. After correction for background extinction, the extinction obtained in the presence of a given concentration of inhibitor was compared to the extinction of control incubations with only vehicle to determine the percent inhibition. Stock solutions of test compounds were prepared in MeOH and diluted with assay buffer. The resulting maximal methanol concentration of 1.25% (v/v) was without any effect on the intactness of microsomes or the phosphatase activity (data not shown).

IC_{50} values of test compounds were routinely determined, where appropriate, by nonlinear least-squares analysis of the inhibition values. In case 50% inhibition was not reached at a 1 mM concentration of test compound, activities are listed as percent inhibition at 1 mM. IC_{50} values and inhibition values are representative values of at least two independently performed experiments with a variability of the inhibition values between experiments of less than 10%.

Glucose Output of Isolated Perfused Rat Livers. The procedure was performed as described previously[44] with the following specifications: Male Sprague−Dawley rats (Moellegard, Denmark; 250−300 g body weight) were anaesthetized with pentobarbital sodium (60 mg/kg ip). The liver was exposed by longitudinal midline and transverse subcostal incisions, and the portal vein was cannulated with a venous cannula. The liver was infused immediately with oxygenated saline containing heparin (70 units/ml) at 37 °C. The vena cava caudalis was opened to allow a continuous flow of the saline/heparin solution for about 2 min. Then the liver was transferred into a heated (37 °C) perfusion chamber and perfused via the portal vein in a recirculating manner at a constant flow of 35 mL/min with continuously oxygenated Krebs−Ringer−Bicarbonate buffer (100 mL total volume). The buffer consists of 137 mM NaCl, 2.7 mM KCl, 11.9 mM $NaHCO_3$, 0.72 mM NaH_2PO_4, 1.8 mM $CaCl_2$, and 0.5 mM $MgCl_2$ without glucose and was supplemented with 30% (v/v) washed bovine erythrocytes and 1.6% (w/v) bovine serum albumin. Routinely, isolated perfused livers from four rats were prepared for one experiment. Samples of the perfusate were taken in 10 min intervals for the determination of glucose and in 30 min intervals for the determination of lactate dehydrogenase activity using standard enzymatic procedures.[45] The measured values for glucose are cumulative values during a perfusion of up to 2 h.

To study the effect of a test compound on the gluconeogenetic process, livers from rats starved for 24 h were used, assuming that under these conditions there is only a neglectable content of glycogen. Hepatic glucose production derived from gluconeogenesis was induced by adding fructose at 10 mM to the perfusate 30 min after the start of perfusion. Ten minutes later the test compound (**29i**) was added to the perfusate with concentrations ranging from 0.1 to 3 mM.

To study the effect of a test compound on the glycogenolytic process, livers from rats with access to food ad libitum prior to the beginning of the experiment were used, assuming that under these conditions liver glycogen stores are full. The test compound was added to the perfusate at the start of the perfusion experiment at concentrations ranging from 0.1 to 3 mmol/L. Perfusate lactate dehydrogenase activities measured for the estimation of the integrity of the livers during the perfusion period did not significantly differ between livers perfused in the absence of or with test compound (data not shown).

Acknowledgment. We would like to express our thanks to the collegues of the Hoechst Marion Roussel research divisions who contributed to the work. We also would like to thank Prof. William J. Arion for his critical reading of the manuscript.

Supporting Information Available: Analytical data for structure confirmation and purity criteria (20 pages). Ordering information is given on any current masthead page.

References

(1) Cori, G. T.; Cori, C. F. Glucose-6-Phosphatase of the liver in glycogen storage disease. *J. Biol. Chem.* **1952**, *199*, 661−667.
(2) Ashmore, J.; Weber, G. The role of hepatic glucose-6-phosphatase in the regulation of carbohydrate metabolism. *Vitamins Hormones* **1959**, *17*, 91−132.
(3) Nordlie, R. C.; Sukalski, K. A. In *The Enzymes of Biological Membranes*; Martonosi, A., Ed.; Plenum Publishing Corp.: New York, 1985; pp 349−398.
(4) Burchell, A.; Waddell, I. D. The molecular basis of the hepatic microsomal glucose-6-phosphatase system. *Biochim. Biophys. Acta* **1991**, *1092*, 129−137.
(5) Nordlie, R. C. Metabolic regulation by multifunctional glucose-6-phosphatase. *Curr. Top. Cell. Regul.* **1974**, *8*, 33−117.
(6) Schulze, H. U.; Speth, M. Investigations on the possible involvement of phospholipids in the glucose-6-phosphate transport system of rat-liver microsomal glucose-6-phosphatase. *Eur. J. Biochem.* **1980**, *106*, 505−514.
(7) Berteloot, A.; St Denis, J. F.; Van de Werve, G. Evidence for a membrane exchangeable glucose pool in the functioning of rat liver glucose-6-phosphatase. *J. Biol. Chem.* **1995**, *270*, 21098−21102.
(8) Countaway, J. L.; Waddell, I. D.; Burchell, A.; Arion, W. J. The phosphohydrolase component of the hepatic microsomal glucose-6-phosphatase system is a 36.5-kilodalton polypeptide. *J. Biol. Chem.* **1988**, *263*, 2673−2678.
(9) Arion, W. J.; Wallin, B. K.; Lange, A. J.; Ballas, L. M. On the involvement of a glucose 6-phosphate transport system in the function of microsomal glucose 6-phosphatase. *Mol. Cell. Biochem.* **1975**, *6*, 75−83.
(10) Arion, W. J.; Lange, A. J.; Walls, H. E.; Ballas, L. M. Evidence for the participation of independent translocation for phosphate and glucose 6-phosphate in the microsomal glucose-6-phosphatase system. Interactions of the system with orthophosphate, inorganic pyrophosphate, and carbamyl phosphate. *J. Biol. Chem.* **1980**, *255*, 10396−10406.
(11) Arion, W. J.; Ballas, L. M.; Lange, A. J.; Wallin, B. K. Microsomal membrane permeability and the hepatic glucose-6-phosphatase system. Interactions of the system with D-mannose 6-phosphate and D-mannose. *J. Biol. Chem.* **1976**, *251*, 4891−4897.

(12) Arion, W. J.; Canfield, W. K.; Ramos, F. C.; Schindler, P. W.; Burger, H.-J.; Hemmerle, H.; Schubert, G.; Below, P.; Herling, A. W. Chlorogenic Acid and Hydroxynitrobenzaldehyde: New Inhibitors of Hepatic Glucose-6-Phosphatase. Submitted for publication in *Arch. Biochem. Biophys.*

(13) Speth, M.; Schulze, H. U. The purification of a detergent-soluble glucose-6-phosphatase from rat liver. *Eur. J. Biochem.* **1992**, *208*, 643−650.

(14) Shelly, L. L.; Lei, K.-J.; Pan, C.-J.; Sakata, S. F.; Ruppert, S.; Schutz, G.; Chou, J. Y. Isolation of the gene for murine glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1A. *J. Biol. Chem.* **1993**, *268*, 21482−21485.

(15) Waddell, I. D.; Lindsay, J. G.; Burchell, A. The identification of T2; the phosphate/pyrophosphate transport protein of the hepatic microsomal glucose-6-phosphatase system. *FEBS Lett.* **1988**, *229*, 179−182.

(16) Lei, K.; Shelly, L. L.; Lin, B.; Sidbury, J. B.; Chen, Y.; Nordlie, R. C.; Chou, J. Y. Mutations in the glucose-6-phosphatase gene are associated with glycogen storage disease types 1a and 1aSP but not 1b. *J. Clin. Invest.* **1995**, *95*, 234−240.

(17) Fulceri, R.; Bellomo, G.; Gamberucci, A.; Scott, H. M.; Burchell, A.; Benedetti, A. Permeability of rat liver microsomal membrane to glucose 6-phosphate. *Biochem. J.* **1992**, *286*, 813−817.

(18) Arion, W. J.; Wallin, B. K.; Carlson, P. W.; Lange, A. J. The specificity of glucose 6-phosphatase of intact liver microsomes. *J. Biol. Chem.* **1972**, *247*, 2558−2565.

(19) Schindler, P. W.; Below, P.; Hemmerle, H.; Burger, H.-J.; Arion, W. J.; Efendic, S. Identification of two new inhibitors of hepatic glucose-6-phosphate translocase. *Diabetologia* **1994**, *37* [Suppl. 1], A134.

(20) Freudenberger, K. Über Gerbstoffe; III Chlorogensäure, der gerbstoffartige Bestandteil der Kaffeebohnen. (About tannines: chlorogenic acid, a tannine like component of coffee beans.) *Ber.* **1920**, *53*, 237.

(21) Gamborg, O. L. The biosynthesis of chlorogenic acid and lignin in potato cell cultures. *Can. J. Biochem. Cell. Biol.* **1967**, *45*, 1451−1457.

(22) Oszmianski, J.; Lee, C. Y. Enzymic oxidative reaction of catechin and chlorogenic acid in a model system. *J. Agric. Food. Chem.* **1990**, *38*, 1202−1204.

(23) Ohnishi, M.; Morishita, H.; Iwahashi, H.; Toda, S.; Shirataki, Y.; Kimura, M.; Kido, R. Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. *Phytochemistry* **1994**, *36*, 579−583.

(24) Iwahashi, H.; Negoro, Y.; Ikeda, A.; Morishita, H.; Kido, R. Inhibition by chlorogenic acid of haematin-catalysed retinoic acid 5,6-epoxidation. *Biochem. J.* **1986**, *239*, 641−646.

(25) DeFronzo, R. A. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* **1988**, *37*, 667−687.

(26) Best, J. D.; Judzewitsch, R. G.; Pfeifer, M. A.; Beard, J. C.; Halter, J. B.; Porte, D. The effect of chronic sulfonylurea therapy on hepatic glucose production in non-insulin-dependent diabetes. *Diabetes* **1982**, *31*, 333−338.

(27) Hother Nielsen, O.; Beck Nielsen, H. On the determination of basal glucose production rate in patients with type 2 (non-insulin-dependent) diabetes mellitus using primed-continuous 3−3H-glucose infusion. *Diabetologia* **1990**, *33*, 603−610.

(28) Turner, R.; Cull, C.; Holman, R. United Kingdom Prospective Diabetes Study 17: a 9-year update of a randomized, controlled trial on the effect of improved metabolic control on complications in non-insulin-dependent diabetes mellitus. *Ann. Intern. Med.* **1996**, *124*, 136−145.

(29) Yki-Jarvinen, H. The liver as a target for therapy in non-insulin dependent diabetes mellitus. *Diab. Nutr. Metab.* **1994**, *7*, 109−119.

(30) Jenssen, T.; Nurjhan, N.; Consoli, A.; Gerich, J. E. Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. Demonstration of hepatic autoregulation without a change in plasma glucose concentration. *J. Clin. Invest.* **1990**, *86*, 489−497.

(31) Burchell, A.; Waddell, I. D. The molecular basis of the genetic deficiencies of five of the components of the glucose-6-phosphatase system: Improved diagnosis. *Eur. J. Pediatr.* **1993**, *152*, Suppl. 1, S18−S21.

(32) Mercier, D.; Leboul, J.; Cleophax, J.; Gero, S. D. Ring-opening reactions of a diepoxide derived from (-)-quinic acid. *Carbohydr. Res.* **1971**, *20*, 299−304.

(33) Campbell, M. M.; Kaye, A. D.; Sainsbury, M.; Yavarzadeh, R. Brief syntheses of (±)-methyl shikimate, (±)-methyl epishikimate and structural variants. *Tetrahedron* **1984**, *40*, 2461−2470.

(34) Ulbrich, B.; Zenk, M. H. Partial purification and properties of p-hydroxycinnamoyl-CoA: shikimate-p-hydroxycinnamoyl transferase from higher plants. *Phytochemistry* **1980**, *19*, 1625−1629.

(35) Mills, S.; Desmond, R.; Reamer, R. A.; Volante, R. P.; Shinkai, I. Diastereospecific, non-racemic synthesis of the C-20 to C-34 segment of the novel immunosuppressant FK-506. *Tetrahedron Lett.* **1988**, *29*, 281−284.

(36) McGowan, D. A.; Berchtold, G. A. (-)-Methyl cis-3-hydroxy-4,5-oxycyclohex-1-enecarboxylate: stereospecific formation from and conversion to (-)-methyl shikimate; complex formation with bis(carbomethoxy)hydrazine. *J. Org. Chem.* **1981**, *46*, 2381−2383.

(37) Kimura, Y.; Okuda, H.; Okuda, T.; Hatano, T.; Agata, I.; Arichi, S. Studies on the activities of tannins and related compounds; V. Inhibitory effects on lipid peroxidation in mitochondria and microsomes of liver. *Planta Med.* **1984**, *50*, 473−477.

(38) Hanessian, S.; Sakito, Y.; Dhanoa, D. Synthesis of (+)-palitantin. *Tetrahedron* **1989**, *45*, 6623−6630.

(39) Adlersberg, M.; Bondinell, W. E.; Sprinson, D. B. Synthesis of 2-hydroxyquinic and 2-hydroxyepiquinic acids from shikimic acid. *J. Am. Chem. Soc.* **1973**, *95*, 887−896.

(40) Barriere, J. C.; Cleophax, J.; Gero, S. D.; Vuilhorgne, M. Synthesis of enantiomerically pure substituted cyclopentenes from (-)-quinic acid. *Helv. Chim. Acta* **1983**, *66*, 296−307.

(41) Nordlie, R. C.; Arion, W. J. *Methods Enzymol.* **1966**, *9*, 619−625.

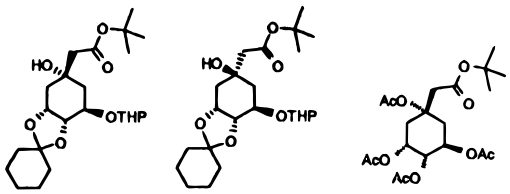
(42) Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **1985**, *150*, 76−85.

(43) Arion, W. J. Measurement of intactness of rat liver endoplasmic reticulum. *Methods Enzymol.* **1989**, *174*, 58−67.

(44) Wolkoff, A. W.; Johansen, K. L.; Goeser T. The isolated perfused rat liver: preparation and application. *Anal. Biochem.* **1987**, *167*, 1−14.

(45) Bergmeyer, H. U.; Bernt, E. In *Methoden der enzymatischen Analyse*, Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1974; pp 607−623.

(46) For the Xray analysis we deprotected **24** with p-toluolsulfonic acid in methanol to the tetrol which was subsequently transformed to the well crystallizing tetraacetate **24a**.



24 **25** **24a**

JM9607360