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Communications to the Editor

11-Ketotigogenin Cellobioside (Pamaqueside): A Potent Cholesterol Absorption Inhibitor in the Hamster[1]

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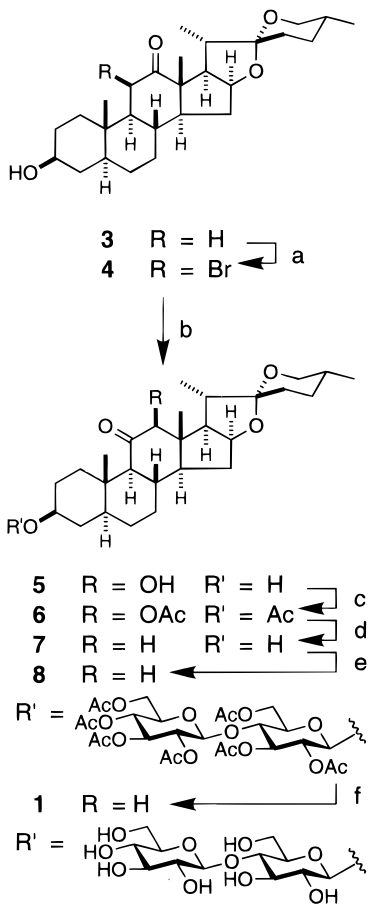
Heart attacks are a major cause of death in Western industrialized countries. In the United States alone, over 500 000 people die of heart attacks each year. The underlying disease, coronary heart disease, is estimated to cost the U.S. economy more than $60 billion each year. There is a straightforward relationship between coronary heart disease death and serum cholesterol: the higher the serum cholesterol level, the higher the death rate.[2] Further studies have shown that dietary or pharmacological reduction in serum cholesterol levels reduces coronary heart disease mortality[3] and total mortality.[4]

In recent years, HMG-CoA reductase inhibitors such as lovastatin, pravastatin, simvastatin, and fluvastatin have been introduced to combat elevated serum cholesterol. These agents have proven to be efficacious, convenient, and, for the most part, safe; however, safety concerns still exist.[5] For these reasons, there is need for new cholesterol-lowering agents. Ideally, a new agent should have a safety profile superior to that of the HMG-CoA reductase inhibitors. This may alter the benefit−risk analysis in mild hypercholesterolemics in favor of treatment. A new agent should also have a complementary mechanism-of-action and safety profile to allow for combination therapy with LDL-lowering agents (HMG-CoA reductase inhibitors) and HDL-elevating agents (fibrates and niacin). Herein we communicate our preclinical data on pamaqueside (**1**, CP-148,623), an agent which may demonstrate this profile.

Scheme 1[a]



[a] (a) Br_2, HCl, MeOH, CH_2Cl_2, 59%; (b) NaOH, *t*-BuOH, H_2O; (c) Ac_2O, pyridine; H_2O, 93% from **4**; (d) Ca, NH_3, THF; bromobenzene, H_2O; THF, MeOH, H_2O, 87%; (e) heptaacetylcellobiosyl bromide, ZnF_2, CH_3CN; CH_2Cl_2, 93%; (f) NaOMe, MeOH, THF, 57%.

Alfalfa saponins provided the genesis for our approach. Many lines of research have shown that certain alfalfa saponin subfractions inhibit cholesterol absorption in animals,[6] probably through a nonsystemic mech-

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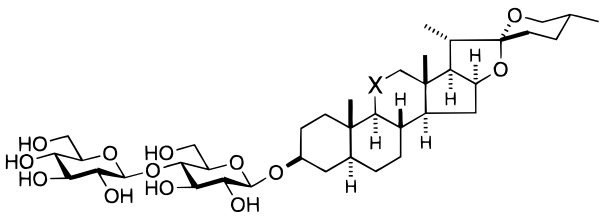
anism of action.[7] Tiqueside (**2**, CP-88,818) is a synthetic saponin with a structure based upon naturally-occurring saponins. As summarized elsewhere, **2** is an efficacious inhibitor of cholesterol absorption in several species.[8] In humans dosed 4 g/day for 12 weeks, **2** was well-tolerated and lowered LDL cholesterol levels by more than 20%.[9] These effects were associated with a decrease in fractional cholesterol absorption and increased excretion of fecal neutral sterols. The bioavailability of **2** in dogs was low (1.7%), but not insignificant.[10] Although these results were encouraging, **2** appeared limited by its weak potency and its higher than necessary bioavailability.



1 X = C=O
2 X = CH_2

Through exploration of the structure−activity relationship of **2**, we came to focus on C-ring-oxygenated spirostane glycosides such as **1**. The synthesis of **1** (Scheme 1) begins with a precedented[11] four-step ketone transposition to convert hecogenin (**3**) into 11-ketotigogenin (**7**).[12] Bromination of **3** under carefully controlled conditions provides **4** without contamination of the 11,23-dibromo side product.[11a] The 11-bromide of **4** is hydrolyzed, and the resulting ketol rearranges under the reaction conditions to the more stable ketol represented by structure **5**. Acetylation of the C-3 and C-12 hydroxy groups provides **6**. This material must be scrupulously dried prior to calcium and ammonia treatment, or else the desired reduction product **7** is contaminated with a hydrolysis product **5**. We have investigated a variety of methods for glycosidic coupling.[13] One of the better procedures involves a zinc fluoride[14] coupling of **7** with cellobiosyl bromide[15] which provides high β-selectivity. Deprotection of **8** gives compound **1**.

We tested the pharmacological effects of these agents in the hamster. Hamsters have a more human-like lipoprotein profile than other rodents and respond to dietary cholesterol and fat in a manner similar to humans. To assess the ability of these agents to acutely inhibit cholesterol absorption, we used the procedure of Harwood.[8] Briefly, cholesterol-fed hamsters are adminstered a bolus of radiolabeled cholesterol in liquid diet with or without test compound. Twenty-four hours later, the animals are euthanized and cholesterol absorption is estimated by comparing liver counts with those of control animals. In this model, **1** was found to be approximately 25 times more potent than **2** (Figure 1).

Next, we wanted to demonstrate that the cholesterol absorption inhibition potency advantage would translate into a similar potency advantage for lowering hepatic and plasma cholesterol levels. These parameters were evaluated in a 4-day dosing protocol in cholesterol-fed hamsters. In this subchronic model, similar potency

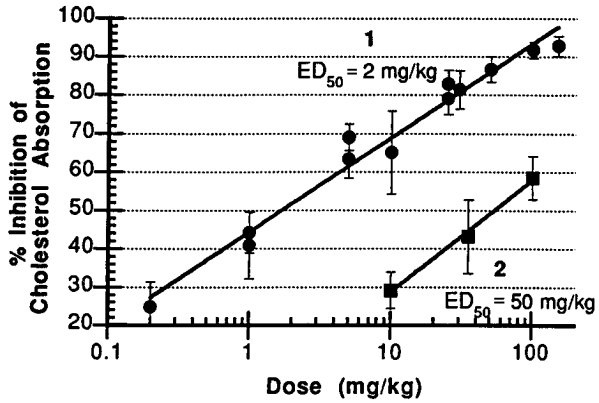


Figure 1. Hamsters (n = 4/dose group) were pre-fed a high-cholesterol diet for 3 d and then fasted overnight. They were dosed with drug followed immediately by a liquid diet bolus containing [^{3}H]cholesterol. Cholesterol absorption was determined by hepatic ^{3}H content 24 h later. Data on **1** are a compilation of three different dose−response experiments and are given as the mean ± SD for each dose group. Data on **2** are given as the mean ± SD of eight experiments.

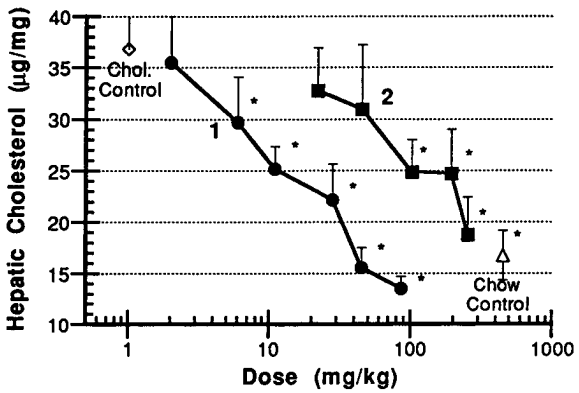


Figure 2. Hamsters (n = 6/group) were fed a 0.2% cholesterol, 0.1% cholic acid diet or a cholesterol-free diet for 4 days with **1** or **2** mixed in the chow. Liver was homogenized prior to extraction, and data was expressed per milligram of protein. Values are the mean ± SD. *p < 0.05 versus cholesterol-fed controls (Student−Newman Keuls Test).

advantages for **1** were indeed observed for both hepatic cholesterol accumulation (Figure 2) and plasma non-HDL cholesterol levels (Figure 3). Given the close structural relationship of these two compounds, the increased potency is remarkable.

Since these agents are believed to act in the intestinal lumen, absorption of any drug poses an unnecessary burden on the patient. For this reason, the bioavailability of **2**, although quite low, could stand improvement. From our experience with **2**, we found that the dog was a good pharmacokinetic model for humans, so we chose to compare **1** and **2** in the dog. As shown in Table 1, even at a higher dose, **1** demonstrated dramatically lower systemic exposure than **2**. **1** demonstrated a 4-fold lower volume of distribution and 2-fold higher clearance, resulting in a 10-fold shorter half-life than **2**. The bioavailability of **1** was more than 40-fold lower than that of **2**. The lower bioavailability of **1** coupled with its improved potency should lead to a much lower systemic drug burden to the patient.

In summary, compared with **2**, **1** is 10−30-fold more potent and substantially less bioavailable. On the basis of these data, **1** offers a new mechanistic class of hypocholesterolemic agents with the potential for an

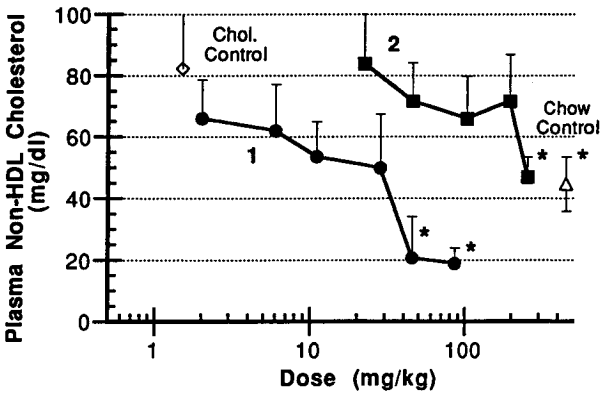


Figure 3. Hamsters ($n = 6$/group) were fed a 0.2% cholesterol, 0.1% cholic acid diet for 4 days with **1** or **2** mixed in the chow. Non-HDL values are calculated by difference (TPC minus HDL cholesterol). Values are the mean ± SD. *$p < 0.05$ versus cholesterol-fed controls (Student−Newman Keuls Test).

Table 1. Comparison of the Pharmacokinetics of **1** and **2** in Fed Beagle Dogs

parameter	1	2
dose, mg/kg	500	375
C_{max}, g/mL	0.11 ± 0.04	2.93 ± 1.39
AUC, ghr/mL	2.15 ± 1.03	186 ± 100
bioavailability, %	0.04 ± 0.02	1.7 ± 0.8
half-life,[a,b] h	4.5	37.1
volume$_{ss}$,[a] L/kg	0.5 ± 0.1	2.1± 0.6
clearance,[a] mL/min/kg	1.4 ± 0.5	0.6 ± 0.1

[a] Calculated following iv administration of 1.5 mg/kg. [b] Harmonic mean; 0.693/mean Kel.

improved safety profile by virtue of a non-systemic mechanism of action. **1** has been advanced to clinical evaluation, and further research on this new mechanistic class has been pursued. Further results will be reported in due course.

Supporting Information Available: Experimental procedures and spectral data for the preparation of compound **1** and details of the pharmacology and pharmacokinetic studies (10 pages). Ordering information is given on any current masthead page.

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