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# Influence of Sapogenins on Cholesterol Metabolism in Rats (40403)

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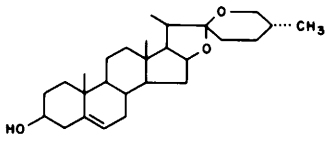
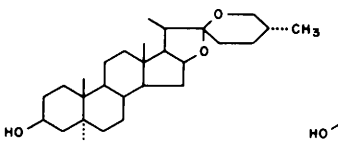
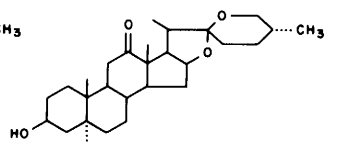
Diosgenin (25$\alpha$-spirost-5-en, 3$\beta$-ol) (Fig. 1) has been shown to inhibit cholestermia in cholesterol-fed chickens and rabbits (1). This sapogenin was tested because its nuclear structure resembled that of cholesterol and because another compound that resembled cholesterol, sitosterol (Fig. 2), had been shown to inhibit cholesteremia in cholesterol-fed chickens (2) and rabbits (3). Both diosgenin (4) and $\beta$-sitosterol (5) inhibit cholesterol absorption. Since dihydrocholesterol is also hypocholesteremic (6), we investigated the possibility that tigogenin (25$\alpha$-spirostan-3$\beta$-ol) (Fig. 1), a sapogenin whose nuclear structure resembles that of dihydrocholesterol, might also possess hypocholesteremic properties. We also tested hecogenin (12 keto, 25$\alpha$-spirostan-3$\beta$-ol) (Fig. 1), a sapogenin that in nature is often a companion of tigogenin.



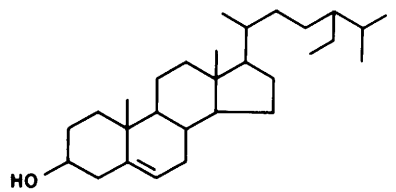

DIOSGENIN




TIGOGENIN




HECOGENIN




SITOSTEROL

Peterson *et al.* (7) reported that sitosterol decanoate is not hypocholesteremic. Mattson *et al.* (8), however, found that sitosteryl acetate, decanoate, oleate and succinate inhibited cholesterol absorption in rats. We tested the acetates of all three sapogenins to ascertain whether the acetate esters were as active as the free alcohols. For purposes of comparison, we tested the hypocholesteremic properties of $\beta$-sitosterol and its acetate as well.

*Materials and methods.* Ten groups of six male Wistar rats (avg. weight 430 g; range 380–470) were used. The basal diet (B) contained: 60% sucrose; 10% corn oil; 24% casein; 5% salt mix USP XIV; and 1% vitamin mix. One group was fed the basal diet to which 1% of cholesterol (BC) was added at the expense of sucrose. Each of the other groups was fed diet BC plus 1% of a test sapogenin or its acetate, sitosterol and sitosterol acetate. These additions were also made at the expense of sucrose. Four days before termination of the experiment, each rat was given an oral dose of 0.5 $\mu$Ci of [4-$^{14}$C]cholesterol in 0.2 ml of propylene glycol. The animals were killed after 4 weeks on the diet. The serum total cholesterol was assayed by the method of Pearson *et al.* (9), and serum triglycerides were assayed by the method of Levy and Keyloun (10). Livers were homogenized in chloroform–methanol 2:1, and aliquots were taken for analysis of total cholesterol (9) and triglycerides (10). The level of cholesterol radioactivity in serum and liver was determined by liquid scintillation spectroscopy of the digitonides which were prepared by the method of Sperry and Webb (11).

The test compounds were purchased from Steraloids, Inc., Wilton, NH. A sample of diosgenin was also made available to us by Ayerst Laboratories, Montreal, Canada.

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The radioactive cholesterol was purchased from the New England Nuclear Corporation, Boston, MA and purified by thin-layer chromatography prior to use.

*Results and discussion.* The effects of the various compounds on serum and liver lipids are summarized in Table I. The only major difference in weight gain was observed in the rats fed diosgenin. These animals gained significantly less weight than those fed diosgenin acetate or hecogenin acetate ($P < 0.05$), sitosterol or the basal–cholesterol diet ($P < 0.01$). The daily intake of foods by all the rats was between 20 and 25 g. Thus, this effect may be attributable to the test compound. The liver weights, as percentages of body weight, were similar in all groups except in the one fed hecogenin acetate. Liver weight as percentage of body weight in the hecogenin acetate-fed group was significantly lower than it was among rats fed the basal–cholesterol diet ($P < 0.01$).

The addition of cholesterol to the basal diet did not generally affect serum cholesterol levels. The group fed diosgenin had relatively high serum cholesterol levels, which were significantly elevated compared to the groups fed basal diet ($P < 0.01$), sitosterol ($P < 0.001$), hecogenin, tigogenin or diosgenin acetate (all $P < 0.05$) (t test).

Addition of cholesterol to the basal diet resulted in a gross elevation in liver cholesterol levels ($P < 0.001$). Every test compound used significantly inhibited the cholesterol-induced liver cholesterol increase. However, only sitosterol acetate completely inhibited the increase in liver cholesterol, the levels in all the other groups being significantly higher than those observed in the group fed the basal diet. These results confirm earlier reports that indicate sterols do not affect serum cholesterol levels of rats (12, 13) but decrease liver and total body cholesterol (13, 14).

Calculating on the basis that the serum volume of a rat is 3% of its body weight (15, 16), we computed the content of the serum plus liver cholesterol pool. The pool size of the group fed the basal diet plus cholesterol was significantly larger than that of any other group. The control group's serum plus liver cholesterol pool was significantly smaller than that of all other groups except those fed

TABLE I. INFLUENCE OF STEROIDS ON SERUM AND LIVER LIPIDS OF RATS[a] (± SEM).

| | | | | Cholesterol | | | Triglyceride | | |
|---|---|---|---|---|---|---|---|---|---|
| Group[b] | Wt. gain (g) | Liver wt. (g) | Liver wt. % body wt. | Serum, (mg/dl) | Liver, (mg/g) | Serum-liver (pool mg) | Serum, (mg/dl) | Liver, (mg/g) | Serum-liver (pool mg) |
| Diosgenin (D) | 8 | 12.7 | 2.90 | 185 | 7.2 | 115.5 | 44 | 9.4 | 125.8 |
| | ±8 | ±0.8 | ±0.13 | ±8 | ±1.0 | ±11.1 | ±2 | ±1.6 | ±19.4 |
| D-Acetate | 32 | 12.1 | 2.63 | 153 | 11.5 | 159.3 | 53 | 14.4 | 179.8 |
| | ±7 | ±0.6 | ±0.14 | ±11 | ±1.4 | ±15.9 | ±3 | ±3.4 | ±42.6 |
| Tigogenin (T) | 34 | 13.2 | 2.85 | 153 | 12.2 | 184.1 | 45 | 19.1 | 256.7 |
| | ±18 | ±0.5 | ±0.07 | ±9 | ±1.0 | ±20.5 | ±2 | ±3.9 | ±48.0 |
| T-Acetate | 25 | 13.3 | 2.90 | 167 | 7.3 | 118.1 | 38 | 9.2 | 127.3 |
| | ±6 | ±0.7 | ±0.12 | ±9 | ±0.5 | ±6.8 | ±3 | ±1.3 | ±19.0 |
| Hecogenin (H) | 28 | 12.0 | 2.65 | 151 | 13.3 | 180.8 | 42 | 22.5 | 281.3 |
| | ±13 | ±0.6 | ±0.06 | ±11 | ±0.8 | ±18.5 | ±3 | ±5.4 | ±71.2 |
| H-Acetate | 58 | 11.6 | 2.37 | 162 | 6.0 | 93.7 | 52 | 11.5 | 155.1 |
| | ±17 | ±0.7 | ±0.10 | ±9 | ±0.4 | ±8.6 | ±3 | ±1.5 | ±31.4 |
| Sitosterol (S) | 45 | 12.6 | 2.64 | 142 | 6.5 | 101.1 | 48 | 17.8 | 220.1 |
| | ±6 | ±0.5 | ±0.06 | ±4 | ±0.8 | ±8.7 | ±5 | ±4.4 | ±59.6 |
| S-Acetate | 47 | 12.7 | 2.64 | 170 | 4.4 | 80.5 | 49 | 10.9 | 149.3 |
| | ±18 | ±0.7 | ±0.09 | ±6 | ±0.3 | ±6.8 | ±4 | ±1.5 | ±26.7 |
| Basal + Cholesterol | 42 | 13.7 | 2.84 | 169 | 24.6 | 353.2 | 49 | 35.7 | 511.5 |
| | ±6 | ±0.7 | ±0.08 | ±12 | ±3.6 | ±61.8 | ±3 | ±3.0 | ±57.5 |
| Basal | 28 | 11.8 | 2.58 | 155 | 4.3 | 71.8 | 45 | 13.5 | 170.8 |
| | ±6 | ±0.4 | ±0.09 | ±5 | ±0.4 | ±6.0 | ±3 | ±3.5 | ±41.7 |

[a] Basal diet contained: sucrose, 60; corn oil, 10; casein, 24; salt mix XIV, 5; and vitamin mix, 1. Basal–cholesterol contained 1% cholesterol added at expense of sucrose. All other diets contained 1% cholesterol and 1% steroid. All diets were fed for 4 weeks.

[b] Groups T-Acetate and H had five rats, all others had six.

the acetates of hecogenin and sitosterol. Among the test groups, the ones with largest serum plus liver cholesterol pools were those fed diosgenin acetate, tigogenin and decogenin. Nevertheless, all the compounds tested significantly lowered the serum plus liver cholesterol pool compared to rats fed 1% cholesterol.

Serum triglyceride levels of the rats fed hecogenin acetate were significantly higher than those of rats fed diosgenin ($P < 0.05$), tigogenin acetate ($P < 0.01$) or hecogenin ($P < 0.05$). Addition of cholesterol to the basal diet significantly elevated liver triglyceride levels ($P < 0.001$). Every compound used inhibited the cholesterol-induced increase in liver triglyceride levels. Diosgenin, tigogenin acetate, hecogenin acetate and sitosterol acetate inhibited the cholesterol-induced increase in liver triglycerides.

Serum plus liver triglyceride pools were also calculated and it was found that the pool of the group fed cholesterol alone was significantly larger than that of any of the test groups or of the control group.

The effects of the steroids on serum and liver cholesterol levels are confirmed by the radioactivity data (Table II). Addition of cholesterol to the basal diet resulted in a large increase in liver cholesterol, and much of the single radioactive dose may have been trapped here. Other significant differences in serum [$^{14}$C] levels are shown in Table II.

TABLE II. ACCUMULATION OF [4-$^{14}$C]CHOLESTEROL IN SERUM AND LIVER OF RATS FED VARIOUS STEROIDS[a, b].

| Group | Total radioactivity (dpm) ± SEM[c] | |
|---|---|---|
| | Serum × $10^{-3}$ | Liver × $10^{-5}$ |
| Diosgenin (D) | 5.79 ± 0.69 | 1.56 ± 0.14 |
| D-Acetate | 5.11 ± 0.77 | 2.68 ± 0.28 |
| Tigogenin (T) | 5.82 ± 0.25 | 3.32 ± 0.34 |
| T-Acetate | 4.34 ± 0.33 | 1.69 ± 0.16 |
| Hecogenin (H) | 4.44 ± 0.41 | 2.68 ± 0.37 |
| H-Acetate | 6.86 ± 0.43 | 1.67 ± 0.24 |
| Sitosterol (S) | 4.02 ± 0.59 | 1.76 ± 0.21 |
| S-Acetate | 4.51 ± 0.34 | 1.33 ± 0.2 |
| Basal + Cholesterol | 4.60 ± 0.41 | 3.49 ± 0.29 |
| Basal | 4.65 ± 1.15 | 1.73 ± 0.16 |

[a] Rats fed 0.5 μCi of [4-$^{14}$C]cholesterol 4 days before autopsy.

[b] See Table I for description of diets.

[c] Analysis of variance shows that the results obtained in the eight experimental groups (for both serum and liver) are significantly different from those obtained in group fed basal diet plus cholesterol ($F_{8,41} = 10.276$, $P < 0.01$).

Liver radioactivity was highest in the rats fed basal diet plus cholesterol as was the sum of serum and liver radioactivity. The next highest recoveries were in the rats fed tigogenin, diosgenin acetate and hecogenin. Liver radioactivity in rats fed the basal diet plus cholesterol was 102% higher than that in rats fed only basal diet. Addition of diosgenin, tigogenin acetate, hecogenin acetate or sitosterol acetate to the basal–cholesterol diet inhibited the uptake of radioactive cholesterol. Liver radioactivity in the rats fed diosgenin acetate, tigogenin or hecogenin was higher than that observed in the group fed basal diet but lower than that in the rats fed cholesterol. Our earlier studies (17) had shown that soy sterols reduced liver deposition of [$^{14}$C] cholesterol and increased its excretion.

The data show that the three sapogenins and their acetates, like sitosterol and sitosteryl acetate, inhibit the accumulation of cholesterol and triglycerides which usually results when cholesterol is added to a fiber-free, high carbohydrate diet (18, 19). Diosgenin was more effective than diosgenin acetate in preventing the increase in liver lipids, but the acetates of tigogenin, hecogenin and sitosterol were more effective than the free sterols. The finding that several of the steroid acetates were more effective than the corresponding unesterified steroid is noteworthy. Mattson *et al.* (8) had demonstrated that plant sterol esters were effective in decreasing cholesterol absorption but did not provide data on cholesterol levels in serum and liver.

Addition of 1% cholesterol to the basal diet resulted in a fivefold increase in the size of the serum plus liver cholesterol pool. Addition of 1% sitosteryl acetate to the diet nearly returned the serum-liver cholesterol pool to its normal level. The serum plus liver cholesterol pool of the rats fed basal diet was 20% of that observed when 1% cholesterol was added to the diet. Serum plus liver cholesterol pools of the test groups were all lower than that of the group fed 1% cholesterol. The percentage by which they were lower (as compared with the basal plus cholesterol group) was: diosgenin, 67%; diosgenin acetate, 55%; tigogenin, 48%; tigogenin acetate, 67%; hecogenin, 49%; hecogenin acetate, 73%; sitosterol, 71% and sitosteryl acetate, 77%.

The effect of the test compounds on the serum plus liver triglyceride pool was similar to their effect on the cholesterol pool. Percentage reduction in serum plus liver triglyceride pool (compared to the cholesterol-fed control) was: diosgenin, 75%; deosgenin acetate, 65%; tigogenin, 50%; tigogenin acetate, 67%; hecogenin, 49%; hecogenin acetate, 73%; sitosterol, 57%; and sitosteryl acetate, 71%. Diosgenin and the acetates of tigogenin, hecogenin and sitosterol all reduced liver triglyceride accumulation to lower than normal values.

This work not only confirms some of the earlier data on diosgenin (1, 4, 20), but further indicates that other sapogenins such as tigogenin and hecogenin also inhibit cholesterol absorption.

*Summary.* Rats were fed for four weeks on a basal fiber-free diet (B) or the same diet augmented with 1% cholesterol (BC). Diosgenin, tigogenin, hecogenin, β-sitosterol and their acetates (1%) were added to diet BC. Liver cholesterol and triglyceride levels of rats fed BC were significantly elevated compared to rats fed diet B (472 and 165%, respectively). The increases in liver cholesterol and triglyceride levels were significantly inhibited by all eight test compounds. When all rats were fed a single dose of [4-$^{14}$C]cholesterol, appearance of isotope in serum and liver of animals fed the various steroids was up to 61% less than in rats fed diet BC. The acetates of hecogenin, tigogenin and β-sitosterol were more effective than the unesterified steroids in inhibiting cholesterol accumulation in liver.

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