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Antihypercholesterolemic Studies with Sterols: β-Sitosterol and Stigmasterol

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Abstract □ Stigmasterol, which differs from β-sitosterol by unsaturation at C_{22}, was tested for antihypercholesterolemic activity under an experimental protocol that gave the results expected with β-sitosterol and cholestyramine. In terms of serum cholesterol, stigmasterol had a barely significant antihypercholesterolemic effect while exhibiting no obvious effect on the heart or liver. It was concluded that saturation of the side chain, at least at C_{22}, is important in conferring antihypercholesterolemic activity on a sterol.

Keyphrases □ β-Sitosterol—antihypercholesterolemic activity evaluated in chickens □ Stigmasterol—antihypercholesterolemic activity evaluated in chickens □ Antihypercholesterolemic activity—β-sitosterol and stigmasterol evaluated in chickens □ Structure–activity relationships—β-sitosterol and stigmasterol evaluated for antihypercholesterolemic activity in chickens

There is no conclusive evidence that lowering plasma cholesterol levels can either reverse or prevent certain cardiovascular diseases. Nevertheless, many antiatherosclerotic studies have been performed with compounds that reduce plasma cholesterol.

Currently, cholestyramine is the only antihypercholesterolemic agent that is relatively free of adverse reactions and reasonably effective in reducing plasma cholesterol levels in type II hyperlipemia (1–3). Unfortunately, it is not always effective (4); therefore, more toxic agents must often be employed.

Niacin and clofibrate, the agents most widely used, have been generally considered effective in reducing cholesterol and triglyceride levels (5). Although their effect on blood triglyceride levels is pronounced, they produce only modest reductions in blood cholesterol levels (6). Both are associated with unpleasant or hazardous side effects.

β-Sitosterol also lowers cholesterol levels in humans (1, 7–11). Reductions in plasma cholesterol levels of the same order of magnitude as those with cholestyramine have been observed (7, 12–14); however, the drug has little effect on plasma triglycerides (10). β-Sitosterol is free of detectable toxicity, and there is no evidence that it accumulates in tissues (2, 15, 16).

The value of these nontoxic agents in the treatment and prevention of such diseases as atherosclerosis, myocardial infarction, and stroke could be significant. Consequently, it was decided to study the structure–activity relationships of sterols related to cholesterol for their antihypercholesterolemic activity. The selection of the White Leghorn cockerel as the animal model was discussed previously (17), as was the use of GLC for sterol analysis (18).

EXPERIMENTAL

General—Day-old White Leghorn cockerels were fed *ad libitum* for 14 days. They were then weighed, banded, and randomly assigned to one of five groups: pretest group, sacrificed on the 1st day of the experiment; Group A, basic diet; and Groups B, C, and D, basic diet supplemented with 1% cholesterol, 1% cholesterol plus 1% test compound, and 1% test compound, respectively. Drinking water was always present.

Serum cholesterol levels were determined (blood collected *via* the brachial vein) at least once during the 4-week experiment. On the final day of the experiment, the chicks were weighed and killed; the hearts and livers were weighed, photographed, and frozen until analyzed. Serum samples also were collected.

Assays were performed by GLC with cholestane as an internal standard (18). Separation of β-sitosterol, campesterol, and stigmasterol from cholesterol was accomplished on columns packed with 3% SE-30 or 3% OV-17 on 100–120-mesh Gas Chrom Q (18). Commercially available laboratory control samples were used routinely to ensure day-to-day reproducibility (18).

Cholestyramine Experiment—In this experiment, the "test compound" was the anion-exchange resin cholestyramine. Only 35 chicks were employed (seven per group); otherwise, the details followed the general procedure described.

β-Sitosterol Experiment—Day-old chicks (White Leghorn cockerels) were fed *ad libitum* for 25 days. On Day 25, blood samples were collected from each chick in the four groups (11 chicks each) of weighed chicks. The chicks were then put on the special diets for 19 days; during this time, blood was collected every 4th day. On Day 19, they were killed.

Stigmasterol Experiment—In this experiment, 44 chicks were used (nine per group, except the pretest group which consisted of eight). The experiment was terminated on Day 24, following the general procedure.

RESULTS AND DISCUSSION

The selection of agents for these experiments was based largely on the knowledge that β-sitosterol and cholestyramine are antihypercholesterolemic agents. These agents were used as standards for comparison of relative activities. Since stigmasterol differs from β-sitosterol only by unsaturation at C_{22}, the results of these experiments should allow the influence of that function on antihypercholesterolemic activity to be determined.

In all experiments, the chicks tolerated their diets well, as indicated by their healthy appearance and consistent weight gain (Table I). The

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Table I—Summary of the Antihypercholesterolemic Studies with Cholestyramine, Stigmasterol, and β-Sitosterol

Group[a]	Number in Group	Body: Final Weight, g	Body: Weight Gain, g	Liver: Final Weight, g	Liver: Cholesterol, mg/100 g of lipid	Heart: Final Weight, g	Heart: Cholesterol, mg/100 g of lipid	Serum Cholesterol, mg/100 ml
				Cholestyramine				
A	7	399 ± 17	296 ± 18	13.37 ± 0.79	4894 ± 652	2.84 ± 0.16	926 ± 248	138 ± 16[b]
B	7	462 ± 22	363 ± 16	14.99 ± 0.71	14852 ± 1087[c]	3.12 ± 0.13	2462 ± 416	331 ± 40[c]
C	7	437 ± 18	333 ± 16	12.75 ± 0.29	8368 ± 487[c,d]	2.79 ± 0.10	1938 ± 411	114 ± 5[b,d]
D	7	457 ± 17	352 ± 17	13.64 ± 0.91	7633 ± 464[c]	2.96 ± 0.11	1990 ± 463	98 ± 7[d]
				Stigmasterol				
A	9	511 ± 25	366 ± 20	12.74 ± 0.73	7522 ± 425	3.21 ± 0.13	1232 ± 145	122 ± 6
B	9	504 ± 14	355 ± 12	13.69 ± 0.23[b]	11909 ± 960[b,c]	3.35 ± 0.21	1656 ± 282	538 ± 76[c]
C	9	515 ± 17	373 ± 14	13.48 ± 0.55	10820 ± 844[c]	3.52 ± 0.15	1501 ± 149	343 ± 36[c,d]
D	9	535 ± 17	395 ± 16	13.85 ± 0.61	6822 ± 383[c]	3.25 ± 0.13	1186 ± 80	134 ± 13[d]
				β-Sitosterol				
A	11	353 ± 12	187 ± 9	9.28 ± 0.42[b]	6082 ± 526[b]	1.94 ± 0.13[b]	833 ± 67[b]	108 ± 6[b]
B	11	350 ± 15	190 ± 12	10.10 ± 0.47[b]	16231 ± 726[b,c]	1.92 ± 0.10[e]	1459 ± 258[e]	349 ± 24[c,e]
C	11	401 ± 11	219 ± 9	11.60 ± 0.61[b,c]	8360 ± 746[b,d]	2.02 ± 0.07[b]	1257 ± 184[b]	150 ± 5[d,e]
D	11	376 ± 13	211 ± 14	11.42 ± 0.24[b,c]	6560 ± 908[b,d]	2.18 ± 0.08[b]	767 ± 88[b,d]	126 ± 8[d,e]

[a] Group mean values ± *SE*. [b] Number in group is one less than stated value. [c] Significant at the 95% confidence level when compared to Group A by analysis of variance. [d] Significant at the 95% confidence level when compared to Group B by analysis of variance. [e] Number in group is two less than stated value.

final mean weights and mean weight gains were not significantly different among the groups in any experiment.

Serum collected from the chicks during the experiments showed a significant increase in the cholesterol level in Group B animals (Table I and Figs. 1–3). Group C animals in the stigmasterol experiment, but not the others, also exhibited significantly elevated serum cholesterol levels, although the mean value was still significantly lower than that of Group B as in the other experiments. Neither of the test sterols was detected in any of the tissues studied.

Macroscopic examination of the hearts revealed no apparent differences among the four groups in any experiment. Differences in mean weights, percentage lipid, and mean cholesterol levels of the hearts were not significant, except in the β-sitosterol experiment where the Group D mean cholesterol level was significantly lower than that of Group B.

The livers of all chicks in Group B and in the stigmasterol experiment were a distinct yellow, especially when compared to the healthy red color of the remaining groups. There were no significant differences in percentage lipid and mean liver weights, except for Groups C and D in the β-sitosterol experiment in which the mean weights of the livers were significantly greater than those of Group A. This observation, however, is in keeping with the greater mean body weight of these two groups.

Analysis of the livers for cholesterol demonstrated the highly significant hypercholesterolemia induced in Group B (Table I).

In the experiment with β-sitosterol, the mean liver cholesterol was significantly greater in Group B than in the other groups, which did not differ significantly from each other. With cholestyramine, levels were significantly greater in Group B than in all other groups. Group C was significantly lower than Group B. These results clearly show the antihypercholesterolemic effect of the two compounds on liver cholesterol content.

In the stigmasterol experiment, however, the mean liver cholesterol levels of Groups B and C, although significantly greater than the control level, were not different from each other (Table I). This result shows the relative ineffectiveness of stigmasterol as an antihypercholesterolemic agent.

SUMMARY AND CONCLUSIONS

In summary, the chicks in all experiments tolerated their diets well and gained weight steadily. All Group B animals showed significantly elevated

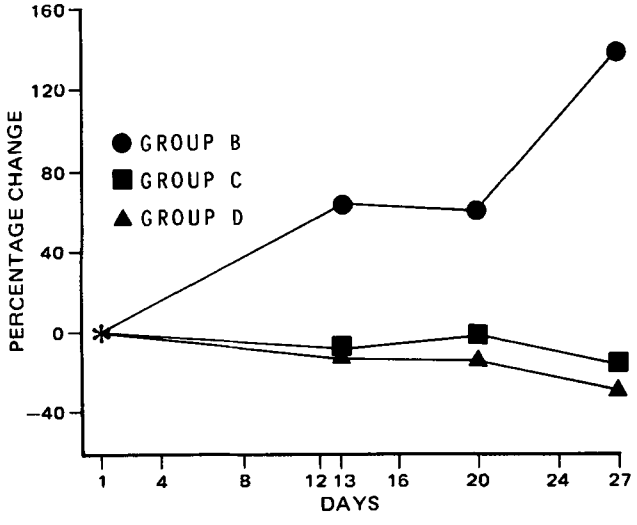

Figure 1—*Percentage change from Group A in mean serum cholesterol of chicks in cholestyramine experiment.*

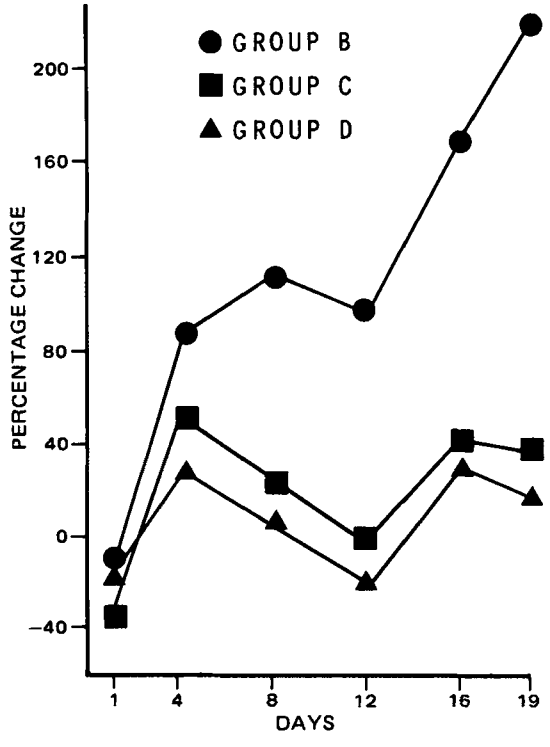

Figure 2—*Percentage change from Group A in mean serum cholesterol of chicks in β-sitosterol experiment.*

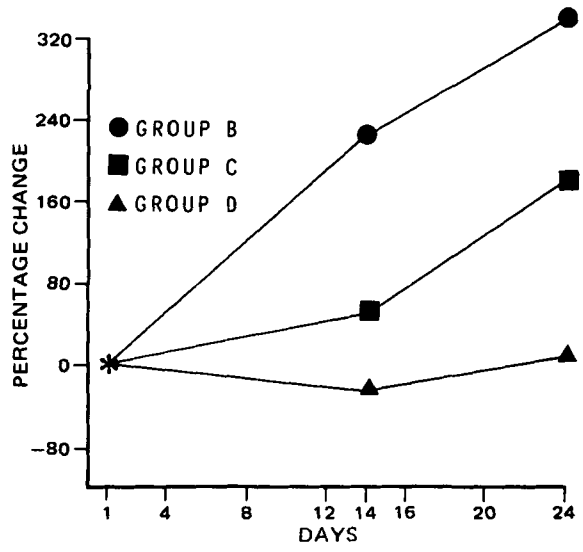

Figure 3—*Percentage change from Group A in mean serum cholesterol of chicks in stigmasterol experiment.*

serum and liver cholesterol values. The coloration of livers closely paralleled the serum cholesterol concentration.

Consistent with the literature (1–3, 7–11), the results of these experiments illustrate the potent antihypercholesterolemic activity of cholestyramine and β-sitosterol. Furthermore, the antihypercholesterolemic activity of these two compounds appears to be of the same order of magnitude, as reported previously (7, 12–14).

Although present in the diet of Groups C and D, neither β-sitosterol nor campesterol (which represents about 40% of the β-sitosterol NF employed) could be detected in the serum. This finding supports the theory that their action is due to their competitive inhibition of cholesterol absorption.

In contrast to the literature (19), the results indicate that stigmasterol, while apparently not exhibiting any deleterious effects, is ineffective as an antihypercholesterolemic agent. It appears, therefore, that unsaturation at C_{22} (the only structural difference between β-sitosterol and stigmasterol) is an undesirable factor in effecting antihypercholesterolemic activity.

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Chemical Constituents of *Echites hirsuta* (Apocynaceae)

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Abstract □ A phytochemical investigation of an ethanolic extract of the whole plant of *Echites hirsuta* (Apocynaceae) resulted in the isolation and identification of the flavonoids naringenin, aromadendrin (dihydrokaempferol), and kaempferol; the coumarin fraxetin; the triterpene ursolic acid; and the sterol glycoside sitosteryl glucoside.

Keyphrases □ *Echites hirsuta*—whole plant ethanolic extract, various flavonoids and sterol glycoside isolated and identified □ Flavonoids, various—isolated and identified in whole plant ethanolic extract of *Echites hirsuta* □ Sitosteryl glucoside—isolated from whole plant ethanolic extract of *Echites hirsuta*

Echites hirsuta (Apocynaceae) (1), also known as *Mandevilla hirsuta* Malme (2), is a woody vine indigenous to the subtropics and tropics (3). Although no medicinal uses have been reported for this genus, the total absence of any phytochemical studies on *Echites* species and the established toxicity of many Apocynaceous plants prompted a phytochemical investigation of this plant.

DISCUSSION

The plant material was extracted by percolation with ethanol to ex-

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