102. THE METABOLISM OF β -SITOSTEROL

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THE views held on the metabolism of sitosterol have undergone considerable modification since the time when phytosterols were recognized as distinct from cholesterol, the characteristic sterol of animal organs and tissues. The earlier investigators assumed that the plant sterols, when taken up with the food by the animal organism, were converted into cholesterol, which itself was considered to be of exogenous origin. Modern opinion, as summed up in leading monographs, however, holds that all phytosterols, including sitosterol, are treated as 'ballast' by the animal organism and excreted unchanged. Without entering into a detailed discussion of the extensive literature, it may be said that much of the evidence on which this view is based is unconvincing and indirect. Moreover as far as sitosterol is concerned, the experimental work has been carried out with what are now known to have been mixtures of various sterols, such as the sitosterols of wheat or soya beans. The only component of these mixtures of which the constitution has recently been definitely established, is β -sitosterol, the main sterol of cottonseed oil [Wallis & Chakravorty, 1937] and of coniferous woods [Sandqvist & Hök, 1930; Sandqvist & Bengtsson, 1931]. This phytosterol differs from cholesterol merely by the presence of an additional C_2H_5 group at C_{24} of the side chain. Its close relationship to stigmasterol, the best known phytosterol, was suggested by the work of Bengtsson [1935], and subsequently Bernstein & Wallis [1937] succeeded in selectively hydrogenating stigmasterol to β -sitosterol, thus justifying the designation of the latter as 22-dihydrostigmasterol.

The recognition that a dietary factor plays an essential role in cholesterol metabolism [Rosenheim & Webster, 1941], made it desirable to investigate the behaviour of a pure sitosterol under controlled conditions on a standard diet and in the presence of the dietary factor. We found that β -sitosterol was excreted unchanged on a sterol-free synthetic diet, just like cholesterol under the same conditions. When administered, however, together with cholesterol-free brain powder or with Thudichum's phrenosin, β -sitosterol is converted into an isomeride of β -sitostanol, for which we propose the name *copro*-sitostanol. In contrast to the original sitosterol, and by analogy with coprosterol, this excretory phytosterol derivative is dextrorotatory. It was characterized by the preparation of various esters and by its oxidation product *copro*-sitostanone.

An interesting relationship was found between the chemical structure of sterols and their metabolism in the presence of the factor. The substitution of a hydrogen atom at C_{24} in the side chain of cholesterol, converting it into β -sitosterol, evidently does not interfere with its metabolism. The introduction of an ethylenic linkage at C_{22} , however, prevents it, as shown by the fact that stigmasterol is excreted unchanged. Ergosterol, containing the same ethylenic linkage, as well as an additional one at C_7 in the ring system, behaves in the same way. It will be recalled that changes in the structure of the side chain exert a profound influence on the conversion of various steroids into antirachitic vitamins.

EXPERIMENTAL

The technique of the animal experiments and the analytical methods used were identical with those described in the preceding communication [Rosenheim & Webster, 1941]. The feeding tests were carried out with a specimen of β -sitosterol from tall oil, M.P. 136–137° (acetate, M.P. 124–125°) as well as with typical sitosterol mixtures obtained from soya beans and wheat germ respectively. The soya bean 'sitosterol', M.P. 138–139°, freed from stigmasterol by bromination of the acetates [Windaus & Hauth, 1906], consisted mainly of γ -sitosterol with a considerable percentage of β -sitosterol, the α -sitosterols having been removed by the preliminary bromination. The wheat germ 'sitosterol', M.P. 138–139°, gave an intense green Tortelli-Jaffé reaction, indicating a high percentage of α -sitosterols in the usual mixture of β - and γ -sitosterols. A 'concentrate' of β -sitosterol was prepared from another specimen of wheat germ sitosterol by means of extensive fractionation and crystallization. The crude sterol mixture (1 kg.) was first crystallized 10 times from absolute alcohol, in batches of 500 g. each, and separated into three main fractions by combining the crystallizations obtained at 36°, 15° and –4° respectively. The top and bottom fractions, containing the γ - and α -sitosterols respectively, were discarded. The middle fractions were acetylated and fractionally recrystallized from benzene-alcohol (1:4) as above. After hydrolysis and final recrystallization of the middle fractions, the 'sitosterol', M.P. 138–139°, free from dihydrositosterol and the α -sitosterols, was rich in β -sitosterol but presumably still contained some γ -sitosterol (as indicated by the melting point of the acetate and its optical activity). Specimens of stigmasterol, M.P. 170–171°, and ergosterol, M.P. 162–163°, were also tested. The preparation of cholesterol-free brain powder and 'white matter' has previously been described [Rosenheim & Webster, 1941]. The results of the feeding experiments are given in Table 1.

Table 1

Sterol	Supplement	Sterol excretion									
		Per rat, per day g.	No. of rats	Days	Sterol intake		Total		Saturated		
					Total g.	Per rat, per day mg.	g.	Per rat, per day mg.	g.	Per rat, per day mg.	% of total
β -Sitosterol (tall oil)	Brain powder	1.6	3	7	3.0	143	2.40	114	2.23	106	93
β -Sitosterol (tall oil)	Thudichum's phrenosin	0.3	3	7	3.0	143	2.30	110	1.88	90	82
'Sitosterol' (wheat germ)	Brain powder	1.6	6	7	7.0	167	3.40	95	2.12	59	62
'Sitosterol' (wheat germ)	'White matter'	0.3	3	7	3.4	162	2.81	134	1.78	85	63
β -Sitosterol concentrate (wheat germ)	Brain powder	1.6	6	7	6.0	143	3.79	90	2.76	66	73
β -Sitosterol concentrate (wheat germ)	None	—	6	7	7.0	167	3.47	83	0.55	13	16
'Sitosterol' (soya bean)	Thudichum's phrenosin	0.25	3	7	3.4	162	2.36	112	1.05	50	45
'Sitosterol' (soya bean)	None	—	3	7	3.5	167	2.62	120	0.45	21	18
Stigmasterol	Brain powder	1.6	3	7	2.5	119	1.67	80	0.29	14	18
Ergosterol	Brain powder	1.6	6	7	5.5	132	2.59	60	0.42	10	17

It is evident from these results that hydrogenation of β -sitosterol occurs to the same extent as that of cholesterol, when the diet contains either cholesterol-free brain powder or Thudichum's phrenosin. It may be assumed that the partial conversion into saturated sterols of the sitosterols of soya beans and wheat germ, runs parallel with their content of β -sitosterol, since the fraction of wheat germ sterols, enriched in β -sitosterol by extensive fractionation, shows a markedly increased conversion (73 % of the total faecal sterols) when administered together with brain powder. Further, the saturated sterols obtained in this case yielded the same *copro-sitostanol* as the saturated sterols excreted after feeding with pure β -sitosterol. The remarkable inertness of stigmasterol and ergosterol, both sterols being characterized by the presence of an unsaturated linkage in the side chain, contrasts strongly with the behaviour of β -sitosterol and cholesterol, in which the side chain is saturated.

Copro-sitostanol. (a) *From β -sitosterol.* The alcoholic solution of the unsaponifiable fraction of the faecal extract was precipitated with a 1 % solution of digitonin after bromination according to Schoenheimer's [1930] method. The saturated digitonides (8 g.) obtained from one of the feeding tests were dissolved in pyridine (80 ml.) and the digitonin precipitated with ether (800 ml.). The ethereal solution of the sterol was freed from pyridine by washing with dilute HCl and water, dried over anhydrous Na_2SO_4 and the crystalline ether residue fractionally recrystallized from 60 vols. of methyl alcohol. The fractions obtained at room temperature and at -4° varied in melting point from 120 to 125° . The higher-melting fractions were combined and yielded on further recrystallization uniform rhombic plates, m.p. 126 – 127° , $[\alpha]_D^{20} + 24.6^\circ$; $[\alpha]_{5461}^{20} + 28.4^\circ$ ($c = 1.178$ in chloroform, $l = 4$ dm.), $\alpha_{5461}/\alpha_D = 1.16$. (Found: C, 83.1; H, 12.4; $\text{C}_{29}\text{H}_{52}\text{O}$ requires C, 83.5; H, 12.8 %.) The melting point and optical activity of the substance remained constant on further recrystallization. From the final mother liquors a small amount (8 mg.) of crystals was obtained, melting indefinitely from 99° to the clearing point 140° , which may have been a mixture of coprosterol and sitostanol (or cholestanol).

In another experiment the alcoholic solution of the 'unsaponifiable' was kept at -4° for some days and deposited sphaerocrystals (m.p. 85 – 86° , lignoceryl sphingosine?), which adhered firmly to the sides of the flask and were easily separated from the colourless floating crystals of *copro-sitostanol*, m.p. 124 – 125° . The filtrate was brominated and yielded 6.5 g. of saturated digitonides, from which *copro-sitostanol* was obtained as above.

(b) *From wheat germ sitosterol 'concentrate'.* The saturated sterol recovered from the digitonide (1.5 g.) gave *copro-sitostanol*, m.p. 125 – 126° , $[\alpha]_D^{20} + 24.0^\circ$, melting points of the sterol and its acetate showing no depression when mixed with the specimens obtained from β -sitosterol.

Copro-sitostanol acetate, prepared by refluxing *copro-sitostanol* (100 mg.) with acetic anhydride and evaporation of the solvent *in vacuo*, crystallized in shiny leaflets from methyl alcohol, m.p. 88 – 89° . (Found: C, 80.8; H, 11.5; $\text{C}_{31}\text{H}_{54}\text{O}_2$ requires C, 81.2; H, 11.8 %.) A mixed melting point with coprosterol acetate, m.p. 89 – 90° , gave a depression of 18 – 20° .

Copro-sitostanol benzoate was obtained by adding benzoyl chloride (0.06 ml.) to a solution of *copro-sitostanol* (100 mg.) in pyridine (1.5 ml.) and pouring the solution into water after 24 hr. The product was recrystallized from alcohol, from which it separated as a mass of needles, m.p. 103 – 104° . (Found: C, 82.6; H, 10.5; $\text{C}_{36}\text{H}_{56}\text{O}_2$ requires C, 83.0; H, 10.8 %.)

Copro-sitostanone. A solution of *copro-sitostanol* (200 mg.) in glacial acetic acid (10 ml.) was mixed with a solution of chromic anhydride (50 mg.) in 90 %

acetic acid (0.5 ml.) and kept at room temperature for 24 hr. On the addition of water crystals deposited which were extracted with ether. The ethereal solution was washed with dilute KOH and water, the ether removed after drying over anhydrous Na_2SO_4 and the crystalline residue (190 mg.) recrystallized from methyl alcohol. The ketone crystallized in thin plates, m.p. 113–114°. (Found: C, 83.3; H, 11.8; $\text{C}_{29}\text{H}_{50}\text{O}$ requires C, 83.5; H, 12.2%) and was characterized by its *o*-tolylsemicarbazone. This derivative was obtained by dissolving the ketone (20 mg.) and *o*-tolylsemicarbazide (20 mg.) in alcohol (2 ml.) containing a drop of acetic acid. Copro-sitostanone-*o*-tolylsemicarbazone crystallizes in colourless needles, m.p. 198–199°, from benzene alcohol.

After the isolation of copro-sitostanol from rats' faeces (early in 1937) a communication appeared by Marker & Wittle [1937], in which the preparation of 24-ethylcoprostanol, m.p. 127°, from β -sitostenone by the usual chemical methods was described. It may be assumed that this substance is identical with the natural excretory product, for which the name copro-sitostanol seems more appropriate.

In the control feeding experiments in which β -sitosterol was added to the standard diet without supplements of brain powder etc. the alcoholic solutions of the 'unsaponifiable' deposited the bulk of the sterols on cooling to -4° in characteristic crystals. A single recrystallization sufficed to yield β -sitosterol, m.p. 136–137°, a mixed melting point with the original sterol showing no depression. In these cases, as well as in the experiments with 'sitosterols' of soya beans and wheat germ, we were unable to isolate any saturated sterols by crystallization, although the analytical figures indicated the presence of traces of saturated sterols.

Stigmasterol, m.p. 170–171°, and ergosterol, m.p. 162–163°, not depressed in admixture with respective authentic specimens, were obtained by direct crystallization as above from the faeces after feeding the sterols mixed with the standard diet together with, or in the absence of, brain powder.

SUMMARY

The metabolism and excretion of β -sitosterol is influenced by the diet and dependent on the presence of a dietary factor.

When administered together with cholesterol-free brain powder or Thudichum's phrenosin, β -sitosterol is hydrogenated and excreted as copro-sitostanol, which has been characterized by various derivatives.

The relationship between chemical structure of sterols and their absorption and excretion is discussed.

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