CLXXIX. THE ABSORPTION SPECTRA OF STEROLS FROM VARIOUS NATURAL SOURCES WITH PARTICULAR REFERENCE TO ERGOSTEROL AND OTHER VITAMIN D PRECURSORS.

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THE fact that most of the animal and vegetable sterols can be rendered antirachitic by suitable irradiation with ultraviolet rays was, until recently, attributed to the presence of small quantities of ergosterol in the crude sterol. Support is lent to this view by the definite isolation of ergosterol from a number of its natural sources, e.g. fungi [Tanret, 1908], yeast [Windaus & Grosskopf, 1922], etc. In cases where it has not actually been isolated ergosterol has usually been detected by means of its characteristic absorption spectrum, consisting of a system of four narrow bands at 293.5, 281.5, 270 and 261 m μ in alcoholic solution [Pohl, 1926; Heilbron *et al.* 1927; Rosenheim & Webster, 1927; Windaus & Hess, 1927; Bills *et al.* 1928; Heilbron *et al.* 1928]. On the other hand, the preparation of 7-dehydrocholesterol, 22-dihydroergosterol [Windaus & Langer, 1933; Windaus *et al.* 1935], 7-dehydrocholestene and ergostatriene [Dimroth and Trautmann, 1936], all of which possess the same absorption spectrum as ergosterol, indicates that absorption spectra data alone cannot be accepted as sole proof of the presence of this compound, the more so as it has been demonstrated that 22-dihydroergosterol and 7-dehydrocholesterol also produce antirachitic products on irradiation [Windaus *et al.* 1935].

Moreover, cholesterol itself can apparently be rendered antirachitic [Bills *et al.* 1928; Koch *et al.* 1929; Waddell, 1934; Hathaway & Lobb, 1936], and the position caused by this acknowledged plurality of substances capable of activation is still further complicated by the slowly accumulating evidence tending to show that natural vitamin D is different from the artificially prepared calciferol [Steenbock *et al.* 1932; Ender, 1933].

With these points in mind and as part of a larger investigation we are endeavouring to isolate the selectively absorbing substance (or substances) which occurs in the sterol fraction derived from various animal products, the ultimate object being to ascertain whether the selective light absorption is in fact due to ergosterol or to some related body. As a necessary preliminary to this project, we have made an examination of the absorption spectra of a number of samples of crude and fractionated sterols, from diverse natural sources, with a view to finding the most suitable raw material for subsequent detailed examination of the absorbing compounds. The present paper records the absorption spectra data only (Table I).

The sterols derived from the lugworm (*Arenicola marina*), in particular, have been found to exhibit in addition to the ergosterol-like maxima, a triplet of bands at 346, 328 and 316 m μ . These maxima are closely comparable with those

Table I.

Source of sterol	Melting point °C.	Ergosterol absorption bands	Gross value of $E_{1\text{cm}}^{1\%}$ (281 m μ)	Estimated % of active substance as ergosterol	Comments and some relevant literature references
Ergosterol pure	162-164		300	—	[Bacharach <i>et al.</i> 1933; Tanret, 1908]
22-Dihydroergosterol	152-153	293.5, 281.5, 271 (261) m μ	(300)	—	} Data by Windaus and Langer [1933; Windaus <i>et al.</i> 1935]
7-Dehydrocholesterol	142-143.5		(300)	—	
7-Dehydrocholestone	88-89	None	—	—	} Data by Dimroth <i>et al.</i> [1936]
Ergostatriene	99-100		—	—	
Cholesterol pure	147	None	—	—	—
Cholesterol coml. "pure" <i>ex</i> fish liver oil	148	Bands plus much general absorption	0.33	Order 0-10	—
Cocksfoot grass	138	Very clear	2.43	0.81	} [Gillam <i>et al.</i> 1933; Pollard, 1936]
Perennial rye grass	137	Very clear	4.45	1.48	
Butter (mixed sample)	—	Very ill-defined	0.71	Order 0-12	} [Hentschel & Bachmann, 1930]
Colostrum butter	—	Clear	0.21	0.07	
Egg yolk	147	Very clear	0.66	0.22	[Bischoff, 1931]
Blood serum (cow)	144-145	Clear	0.44	0.15	—
Brain (cow)	146	Bands plus general absorption	0.11	Order 0-01	Solid unsap. = 22% on dry wt.
Brain (sheep)	145-146	Bands plus general absorption	0.25	Order 0-04	Solid unsap. = 10.8% on dry wt.
Brain (source unknown)	145-146	Selective absorption not detectable	0.07	<0.01	[Page, 1930]
Yeast	93-101	Very clear	59.1	19.7	Crude unsap. matter = 0.85% dry wt. of yeast [Drummond <i>et al.</i> 1935]
Wheat germ oil	—	Fairly clear	2.4	0.8	—
Halibut liver oil, 1	146	Very clear	0.30	0.10	—
Halibut liver oil, 2	—	Not present	—	None detectable	—
Japanese cod liver oil	146	Very clear	0.28	0.09	—
Whale liver oil	146	Clear	0.035	0.012	—
Cod roe (female)	147	Very weakly defined	3.49	Order 0-55	Solid unsap. = 8.0% on dry wt.
Herring roe (female)	145	Bands plus general absorption	1.56	Order 0-35	Unsap. matter = 2.1% on dry wt.
Plankton (<i>Munidia banffica</i>)	—	Plus general absorption	1.65	Order 0-35	Acetate (not free sterol) examined
Ragworm (<i>Nereis</i>)	148	Bands plus much general absorption	0.94	Order 0-20	—
Lugworm (<i>Arenicola marina</i>):					
(a)	135-136	Clear	35.9	12.0	—
(b)	143-144	Clear	27.2	9.0	—
(c)	—	Clear	13.9	4.6	—
Sea Mat (<i>Flustra securifrons</i>):					
(a)	142	Clear	10.3	3.43	Purified sterols (a)
(b)	132-135	Very clear	32.3	10.7	Crude sterols, another batch (b)
Seaweed (<i>Fucus vesiculosus</i>)	—	Rather ill-defined	0.23	0.08	Purified sterol
Oyster (a)	—	Very indefinite	10.7	Order 1-8	(a) Acetate, not free sterol examined
(b)	—	Very clear bands	16.5	5.5	(b) Crude sterol; another batch
"Ostreasteryl acetate" <i>ex</i> <i>Flustra securifrons</i>	—	Very clear	5.15	1.7	} Oyster sterols [Bergmann, 1934]. Absorbing substance present as impurity
"Ostreasteryl acetate" <i>ex</i> <i>Pennatula quadrangularis</i>	142-143	Very clear	12.5	4.2	
<i>Actinia equina</i>	145	Very clear	15.0	5.0	} Sea anemones [Heilbron <i>et al.</i> 1935; Klenk and Diebold, 1935]
<i>Actinoloba dianthus</i> (a)	134-138	Clear	11.5	3.8	
(b)	—	Clear	13.5	4.5	
(c)	—	Very clear	6.18	2.03	
(d)	—	Clear	31.9	10.6	} Fraction only of sterols
<i>Anemonia sulcata</i>	—	Clear	5.02	1.67	

characteristic of the chromophoric grouping of dehydroergosterol (Heilbron *et al.* 1929); the intensities of the bands in the lugworm sterols vary between $E_{1\text{cm}}^{1\%}$ 0.7 and 4.9 (equivalent to dehydroergosterol of the order 0.2-1.6%). As however, identity of absorption maxima, in itself, is not conclusive proof of the presence of a particular compound we are endeavouring to isolate this absorbing

substance also, with a view to examining its chemical and biological properties. Sterol fractions having absorption maxima similar to those of dehydroergosterol have also been obtained from wheat germ oil [Edisbury, 1933].

EXPERIMENTAL.

Preparation of the sterols. In the examination of the raw materials recorded in this work the main object has been to obtain the crude sterols with the minimum of manipulation since it has been found that the usual methods of purification tend to reduce the intensity of selective absorption of the "ergosterol" type. The sterols have, with few exceptions, been obtained by hot saponification of the raw biological material with aqueous or alcoholic potash followed by ether extraction, the examination generally being made on the solid portion of the unsaponifiable matter after one recrystallization from alcohol.

Determination of the absorption spectra. All the photometric measurements recorded were made on a Hilger E₃ Quartz spectrograph attached to a Spekker photometer, the light source being a tungsten steel spark for use when determining intensities. A hydrogen tube was also used occasionally to render more certain the detection of absorption bands when these were obscured by irrelevant absorption. The solvent used in all cases has been pure ethyl alcohol. No claim is made that the spectroscopic properties recorded for these gross sterol fractions are typical of the source from which they were derived, as only one or two samples of each kind have been examined. That two different samples of sterol from the same biological source may differ is shown by the variation between the two samples of halibut liver oil sterol and between the various samples of lugworm sterol (cf. Table I).

SUMMARY.

As part of a larger investigation into the apparent difference in properties between natural vitamin D and calciferol a survey of the absorption spectra of sterols from various animal sources has been made, with the ultimate idea of isolating the absorbing substances for subsequent examination. It has been found that certain marine animal sterols, notably those from lugworms (*Arenicola marina*), sea anemones and oysters, exhibit absorption bands identical with those of ergosterol and, calculated as such, indicate the presence of 5–12% of the absorbing substance. The lugworm sterols exhibit, in addition, maxima at 346, 328 and 316 μ , closely comparable with those of dehydroergosterol.

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