XIV. CONTRIBUTIONS TO OUR KNOWLEDGE OF THE PLANT STEROLS. PART II. THE OCCURRENCE OF PHYTOSTEROL IN SOME OF THE LOWER PLANTS.

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(Received March 4th, 1918.)

IN the first paper of this series [1918], an account of the phytosterols occurring in the lower plants is given. These are represented chiefly by two substances occurring in the fungi: (1) ergosterol, first isolated by Tanret [1889], and (2) fongisterol, which have been obtained by Tanret [1908] and other workers. Gerard [1892, 1895] puts forward the theory that the sterols may be divided into two groups: (1) the Phytosterols occurring in the higher plants, and (2) the Ergosterols found only in the lower plants. To obtain more knowledge on this matter, several species, chosen from different groups belonging to the lower plants, were examined for the occurrence of a phytosterol.

The following were dealt with:

- (1) Musci, *Sphagnum*.
- (2) Algae, *Laminaria*.
- (3) Fungi, (i) *Polyporus nigricans*.
(ii) *Polyporus betulinus*.
(iii) *Agaricus rubescens*.
(iv) *Lactarius subdulcis*.

Method of Experiment.

The plant material is dried, and then ground up very finely in order to render all the tissues permeable.

In the case of the juicy fungi, the material is ground up in a mortar with coarse sand, adding plaster of Paris until the mixture hardens. When it is dry, the mixture is coarsely powdered.

The dried material in either case is then subjected to prolonged extraction with ether in a Soxhlet apparatus. The ether extract so obtained is saponified

with a large excess of alcoholic sodium ethylate, according to the method of Kossel and Obermüller. The saponified liquid is always allowed to stand overnight to insure complete saponification. The precipitate of soap is separated by filtration, and washed with ether to remove all the ether-soluble substances. The filtrate is shaken up with water several times to free it from the excess of alkali. Finally the washed ether extract is evaporated to dryness, the residue dried, and examined. Further details will be given for each experiment.

1. *Musci. Sphagnum.* Plants were gathered in the autumn and dried. The dry weight was 622 g.

After saponifying and drying, the ethereal extract showed no sign of crystalline matter, consisting mainly of a brown oil. This oil, however, gave the cholesterol colour reactions.

2. *Algae. Laminaria.* The seaweed was dried in air and "stalks" and fronds were extracted separately. The dry weight of the fronds was 7.132 kilos., and of the "stalks" 1.516 kilos. They were extracted with ether, and the ether extract saponified as usual. Incidentally it was observed that while the ethereal extract from the "stalks" was brown, that from the fronds was green and fluorescent. This points to further modifications in the direction of "stem" and leaf function than the mere external appearance. The pseudophloem found in the stalk gives histological support to such a suggestion.

The Fronds. The unsaponifiable extract from the fronds weighed 18 g. It consists mostly of a dark brown oil with a curious penetrating odour. A very small amount of crystalline matter was separated by recrystallisation from alcohol in sheaves of needle crystals, melting at 105°-110°. These gradually turned brown in colour as they were heated from 50°. On further purification with alcohol they melted at 125°.

On standing for some time these crystals seemed to change, lost their form and broke down to an amorphous powder, but the amount was so small that it was impossible to investigate this phenomenon further.

The Stalks. The unsaponifiable extract from the stalks weighed 3.6 g. It consisted almost entirely of a brown oil, only a trace of crystalline matter being separated. This resembled the needle crystals from the fronds.

The bulk of the unsaponifiable matter in both cases consisted of a brown oil which gave the cholesterol colour reactions. This was kept, and from time to time attempts were made to obtain crystalline matter from it from solution in various solvents. It did not apparently change on standing, however, or deposit crystalline matter, and it was thought possible that this oil itself

might be a form of sterol. The oil was therefore distilled in steam, when it came over as a pale yellow liquid which darkened very quickly on exposure. This oil gave the cholesterol colour reactions distinctly.

This evidently opens out fresh fields of investigation. Windaus was able to separate the various isomeric forms of dihydrocholesterol by means of digitonin, as they do not all form a compound with this substance, and it is thought that this fact gives a suggestion in regard to the separation of these plant sterols.

The oils from this and other material are being kept. Their examination and identification obviously entail somewhat lengthy investigation, but it may lead to important discoveries in regard to the occurrence of sterols.

3. *Fungi*. (i) *Polyporus nigricans*. The *Polyporus* was gathered in the autumn. The total dry weight of fungi was 4.1 kilos., which gave an unsaponifiable ether extract of about 40 g.

The fungi were extracted in two portions. The first portion of 1050 g. gave an unsaponifiable ether extract weighing 9.6 g. This residue consisted of a dark brown oil, mixed with crystalline matter. The whole was dissolved in 95 % alcohol. (1) The first crop of pure white crystals melted at 248°–249°; on further recrystallisation the m.p. was 250° and this remained constant. The substance was obtained from alcohol in beautiful sheaves of needle crystals. They did not give the cholesterol colour reactions, and were not investigated further at the time. The mother liquors yield a little more of this substance, not quite pure.

(2) The bulk of the more soluble substance, however, melted from 158°–167° and finally a substance giving a constant melting point of 163° was obtained. This was converted into the acetate which melted at 158°. This proved to be a mixture, however, and the author succeeded in separating the constituents by their different solubility in acetone. The less soluble substance melted at 185° after several recrystallisations, and was identified as ergosteryl acetate; this yielded ergosterol, m.p. 165°, on saponification. The acetate of fongisterol, m.p. 158.5°, remained in solution, and was obtained from the mother liquors. Fongisterol itself melts at 144°.

These substances were further identified by a colour test. Ergosterol and strong sulphuric acid give no colour at the end of a minute, whereas fongisterol colours red, then red-violet at the end of a few seconds, so that the crystalline sterols in this *Polyporus* were identified as a mixture of ergosterol and fongisterol—described by Tanret in other fungi.

A quantity of heavy brown oil was left and for some time no more crystal-

line matter could be extracted. On standing, however, for a long period, it was found that the residues yielded more crystalline matter, similar to the mixture described above, when dissolved in glacial acetic acid. Alcohol, acetone and ethyl acetate were not found to produce the same result.

The residue consists of a heavy, almost clear brown oil, which gives the cholesterol colour reactions, and is being kept for future investigation.

In the case of the second portion of the fungi, the unsaponifiable residue was a sticky, gummy, yellow substance. This dissolved in ether, alcohol, acetone, ethyl acetate, and acetic acid, but came out of solution in the same form and no crystalline matter was obtained.

For some time, all efforts to crystallise this substance failed, but, after it had stood for a considerable period, a further attempt was successful, and the solution in acetone deposited crystalline matter identical with that already described, a less soluble substance melting at 250° and the more soluble mixture of ergosterol and fongisterol.

This seems to give an indication that these bodies may change on standing, from one form to another, or that some process of oxidation occurs on standing.

(ii) *Polyporus betulinus*. The fungi were gathered in the autumn, dried, ground up, and extracted with ether. The weight of fungi was 950 g. which gave an unsaponifiable ether extract weighing 5.48 g.

The extract contains a very little crystalline matter, which melts at 157° and gives a colour test with strong sulphuric acid resembling that of fongisterol, blood-red, then red-violet at the end of a few seconds. It is probably a mixture like that occurring in the other fungi, but the amount was too small for separation of the constituents.

The main bulk of the unsaponifiable ether extract consists of a heavy brown oil, which gives the cholesterol colour reactions. It did not yield any further crystalline matter after standing a considerable time.

(iii) *Agaricus rubescens* (5.5 kilos.) and (iv) *Lactarius subdulcis* (3.5 kilos.) were also examined. The material, consisting of young plants, was ground up with sand and plaster of Paris and allowed to dry. The ethereal extract was saponified and the unsaponifiable ether extract obtained in the usual way. This showed no sign of crystalline matter in the case of either fungus. The residues consisted of brown oils which, however, gave the cholesterol colour reactions.

SUMMARY OF RESULTS.

(1) From the fungus, *Polyporus nigricans*, and probably also from *Polyporus betulinus*, a mixture of two sterols previously known to occur in the fungi—ergosterol and fongisterol—was isolated.

(2) There is a certain amount of evidence that in the lower plants at least, the sterols may occur in a non-crystalline form, as in the alga—*Laminaria*—an oil was obtained by distillation in steam, which gave the cholesterol colour reactions, and the unsaponifiable ether extracts from *Sphagnum* (Musci), *Agaricus rubescens* and *Lactarius subdulcis* (Fungi) consist of brown oils, giving these tests. Also on standing the substances seem to change from one form to another. In *Polyporus nigricans* the unsaponifiable ether extract was at first gummy and sticky and no crystalline matter could be isolated. After standing for some time, however, crystals were obtained from solution in acetone.

In conclusion the author would like to express her grateful thanks to Professor Waller, and particularly to Mr J. A. Gardner, for the facilities afforded.

The work was carried out partly with the aid of a Government grant, and thanks are due to the Royal Society's Committee for this help.

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