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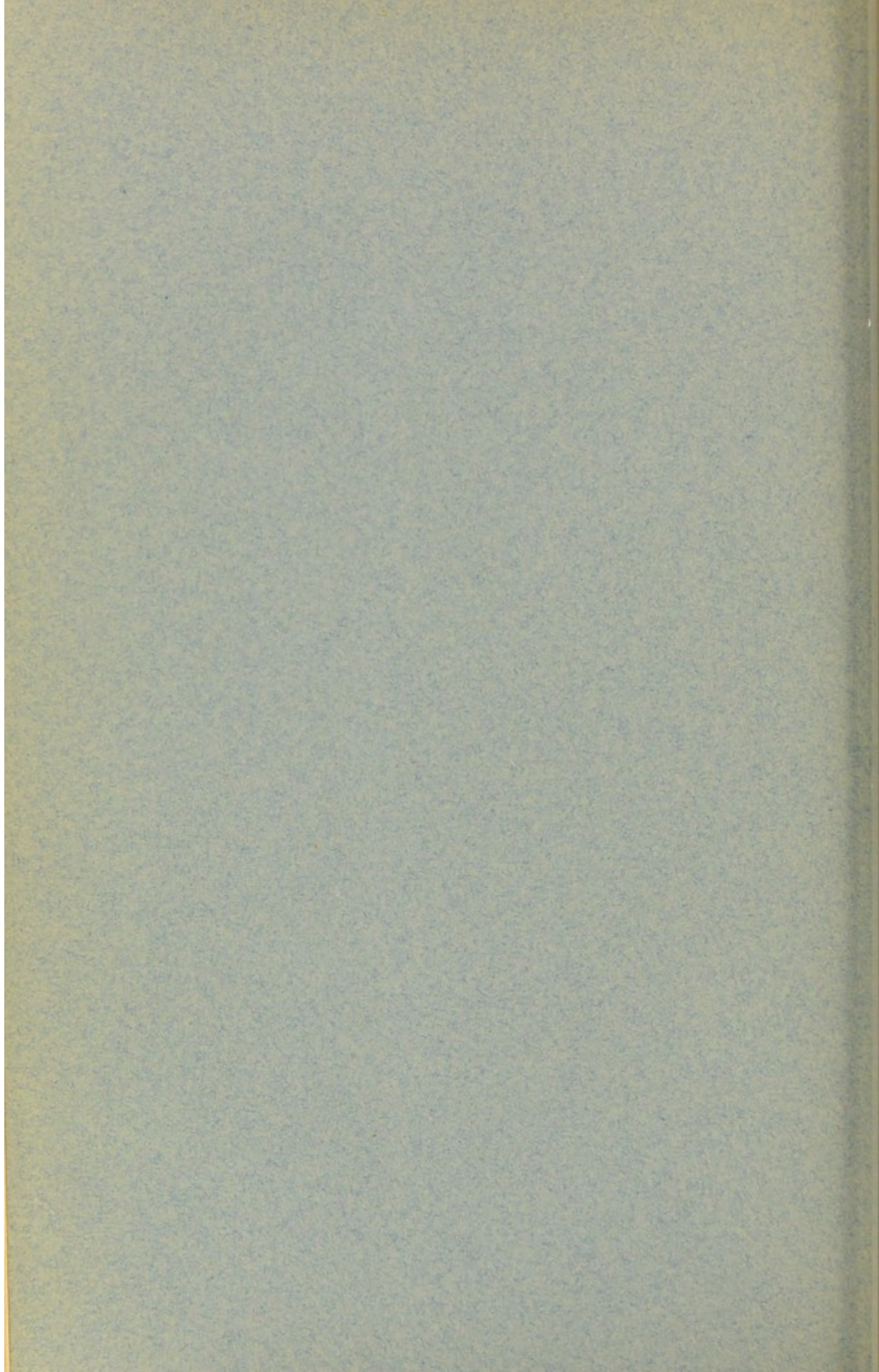
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## CHEMICAL EXAMINATION OF WHEAT GERM.\*

BY

FREDERICK B. POWER AND ARTHUR H. SALWAY.

The material known as wheat germ was formerly a waste product of the flour mills, or used only as fodder, but in recent years it has been utilised for its dietetic value as a constituent of certain kinds of bread, and in various other forms for food purposes. In distinction from ordinary wheat flour, the germ of wheat appears to be particularly characterised by its high percentage of fat and high nitrogen content.

The present investigation of wheat germ was not undertaken primarily for its inherent interest, but was the indirect sequence of some other researches for which a considerable quantity of the respective material was required. In the first place, it may be recalled that a number of compounds which have been isolated from plants during the past few years in these laboratories, and were regarded as dihydric alcohols, such as ipuranol, citrullol, trifolialol, ipurganol, bryonol, etc., have recently been shown by the authors to consist of phytosterol glucosides (*J. Chem. Soc.*, 1913, 103, 399). As such glucosides, now collectively designated as phytosterolins, represented a new class of compounds, which had previously not been known to occur in nature, it was deemed desirable to effect their synthesis. For this purpose a considerable quantity of a definite phytosterol was required, and the best source of such a substance was evidently the so-called wheat

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germ, which is known to contain a comparatively large proportion of sitosterol,  $C_{27}H_{46}O$ . This compound was, in fact, first isolated from the fatty oil of wheat germ by Burian (*Monatsh.*, 1897, 18, 551), and completely characterised by him. The synthetical preparation of a sitosterol-*d*-glucoside,  $C_{33}H_{56}O_6$ , has now been accomplished by one of the present authors (compare Salway, *J. Chem. Soc.*, 1913, 103, 1,022), and has been found to agree in its characters with the naturally occurring compound which was first designated as ipuranol.

Apart from the isolation of sitosterol, the wheat germ has previously been the subject of several independent investigations, the results of which, however, as recorded in the literature, are quite disconnected. As a considerable quantity of material was available for the purpose mentioned, it seemed desirable that the opportunity should be utilised for subjecting it to a more complete and systematic examination. In connection therewith consideration has been taken, so far as possible, of the present state of knowledge on the subject.

Under the title of "wheat oil," as distinguished from "wheat meal oil," or the oil from wheat flour, Lewkowitsch ('*Chemical Technology and Analysis of Oils, Fats, and Waxes*,' third edition, Vol. II., p. 520) has recorded the physical and chemical constants of the oil from wheat germ and of the mixed fatty acids obtained therefrom (compare also Frankforter and Harding, *J. Amer. Chem. Soc.*, 1899, 9, 758).

Richardson and Crampton (*Ber.*, 1886, 19, 1,180) found the wheat germ to contain, besides fatty oil, about 15 to 18 per cent. of sugar. The latter, although consisting chiefly of cane sugar, was observed to be accompanied by another form of sugar, which possessed a high dextrorotatory power, and was not fermentable. This was presumed to be raffinose, although they did not succeed in separating it. The same authors also isolated allantoin, and noted the presence of "a wax-like, unsaponifiable fat," as also of several albuminous substances.

Schulze and Frankfurt (*Ber.*, 1893, 26, 2,151) have conclusively shown the presence of both choline and betaine in wheat germ, the yield of betaine chloride having been about 0.16 to 0.2 per cent., while that of choline chloride was considerably less. The same authors (*Ber.*, 1894, 27, 64) subsequently established the presence of raffinose.

Frankfurt (*J. Chem. Soc. Abst.*, 1897, 72, ii., 67), in a summary of the constituents of wheat germ, has also indicated, in addition to the above-mentioned compounds, the occurrence of asparagine, lecithin, glucose, and a ferment which vigorously inverts cane sugar.

#### EXPERIMENTAL.

The material employed for this investigation consisted of a good quality of wheat germ, which was obtained directly from one of the large flour mills near London.

A small portion (10 grammes) of the material was first subjected to a preliminary test for an alkaloid by extraction with Prollius' fluid. The reactions obtained with the usual alkaloid reagents were, however, very slight.

Another portion (25 grammes) of the material was extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained :

Petroleum (b.p. 35-50°)	extracted 2.08 grammes	=	8.32 per cent.
Ether	0.14	=	0.56 " "
Chloroform	0.31	=	1.24 " "
Ethyl Acetate	0.19	=	0.76 " "
Alcohol	6.20	=	24.80 " "
Total	8.92 grammes	=	35.68 per cent.

For the purpose of a complete examination 50.8 kilogrammes of the wheat germ were extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, 22.75 kilogrammes of a light brown, viscid extract were obtained. The whole of this extract, in convenient portions, was mixed with water, and the mixture distilled in a current of steam, but it yielded no appreciable amount of volatile oil. After this treatment the distillation flask contained an aqueous liquid and a considerable quantity of a fatty oil, together with a small quantity of resinous material, the whole forming a thick emulsion, which did not separate on keeping. A separation was effected, however, by agitating the mixture with hot amyl alcohol, which dissolved the fatty oil, while the resinous material remained suspended in the aqueous liquid. The aqueous liquid was then filtered and the resin collected, when, after being washed and dried, it amounted to 22 grammes. This resinous material was separately examined, but no definite substance could be isolated from it.

### EXAMINATION OF THE AQUEOUS LIQUID.

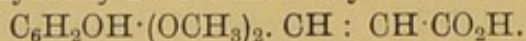
The aqueous liquid from which the fatty oil and resin had been separated, as above described, was extracted many times with ether. This solvent removed about 3 grammes of viscid, fatty material, which was evidently similar in character to the main portion of fatty oil.

The aqueous liquid was next shaken repeatedly with hot amyl alcohol. The amyl alcohol liquids were then united, washed with a little water, and the solvent removed by distillation under diminished pressure, when a quantity (10 grammes) of a dark brown syrup was obtained. This was dissolved in alcohol and the solution kept for some time, when a small quantity of a crystalline solid separated. The latter was collected and dissolved in hot dilute alcohol, from which it was deposited in slender, colourless needles, melting and decomposing at about  $240^{\circ}$ . The same compound was subsequently isolated in larger amount from the aqueous liquid, as described below, and was then completely identified as allantoin.

The alcoholic liquid from which the small amount of allantoin had first separated, as above noted, was evaporated, and the residue divided into two portions. One portion was heated for some time with dilute sulphuric acid, when a small amount of a sugar was formed, which yielded an osazone melting at  $210^{\circ}$ , but no other definite hydrolytic product was obtained. It was thus evident, however, that the material was glucosidic in character.

### ISOLATION OF SINAPIC ACID, $C_{11}H_{12}O_5$

(4-Hydroxy-3: 5-dimethoxycinnamic Acid)



The remaining portion of the residue from the above-mentioned alcoholic liquid was heated for a few minutes with an excess of aqueous potassium hydroxide, the mixture then acidified with dilute sulphuric acid, and subsequently extracted with ether. After washing and drying the ethereal liquid, the solvent was removed, when about 0.5 gramme of a brown, crystalline solid remained. This was redissolved in ether, the solution shaken with aqueous ammonium carbonate, and the alkaline liquid acidified and extracted with ether, when a crystalline substance was obtained, which melted and decomposed at about  $186^{\circ}$ . After recrystallisation from dilute alcohol, it separated in nearly

colourless needles, which melted and decomposed at 190-192°. The substance was freely soluble in alcohol, but only moderately soluble in water. In alcoholic solution it gave with ferric chloride a deep red colour, whilst in aqueous solution with the same reagent a red precipitate was produced. The substance was analysed, and titrated with a decinormal solution of sodium hydroxide, with the following results:—

0.0958 gave 0.2068 CO<sub>2</sub> and 0.0458 H<sub>2</sub>O. C=58.9; H=5.3.

0.1091 required for neutralisation 5.05 C.c. N/10 NaOH.

M.W. (for a monobasic acid)=216

C<sub>11</sub>H<sub>12</sub>O<sub>5</sub> requires C=58.9; H=5.4 per cent. M.W.=224

It is evident from these results and from the properties of the above-described compound that the latter is identical with sinapic acid (compare Gadamer, *Arch. Pharm.*, 1897, 235, 92, 570). In order to confirm its identity it was converted by methylation into 3:4:5-trimethoxycinnamic acid, C<sub>6</sub>H<sub>2</sub>(OCH<sub>3</sub>)<sub>3</sub>·CH:CH·CO<sub>2</sub>H, which has been shown by Gadamer (*loc. cit.*, p. 110) to melt at 123-124°. This was accomplished by dissolving the substance in an excess of aqueous potassium hydroxide, and warming the solution with methyl sulphate, after which the mixture was acidified and extracted with ether. The ethereal liquid, on evaporation, yielded a crystalline acid which melted at 123-124°, and was identical in its properties with trimethoxycinnamic acid.

The isolation of sinapic acid as a product of alkaline hydrolysis indicates that it was originally present in some form of combination. Inasmuch as it has been shown that the sinapine contained in black mustard seed consists of a choline ester of sinapic acid (*Arch. Pharm.*, 1897, 235, 101), and as choline is also a constituent of wheat germ, it seems highly probable that the sinapic acid obtained from the latter source was originally present as sinapine.

#### ISOLATION OF RAFFINOSE, C<sub>18</sub>H<sub>32</sub>O<sub>16</sub>, 5H<sub>2</sub>O.

The original aqueous liquid which had been extracted with amyl alcohol, as described above, was set aside for a considerable time, when a quantity of a colourless, crystalline solid slowly separated. This was collected, and re-crystallised from very dilute alcohol, when it separated in slender, elongated needles, containing water of crystallisation. The air-dried substance was found to melt at 85°, whilst the anhydrous solid melted at 135-140°. The substance was

analysed, and its optical rotatory power determined, with the following results:—

0.4645 air-dried substance, heated at  $100^{\circ}$ , lost  $0.0682 \text{ H}_2\text{O}$ .  
 $\text{H}_2\text{O} = 14.7$

0.1116 anhydrous substance gave  $0.1745 \text{ CO}_2$  and  $0.0648 \text{ H}_2\text{O}$ .  
 $\text{C} = 42.6$ ;  $\text{H} = 6.5$

$\text{C}_{18}\text{H}_{32}\text{O}_{16}$ ,  $5\text{H}_2\text{O}$  requires  $\text{H}_2\text{O} = 15.2$  per cent.

$\text{C}_{18}\text{H}_{32}\text{O}_{16}$  requires  $\text{C} = 42.9$ ;  $\text{H} = 6.3$  per cent.

1.9854 anhydrous substance, made up to 20 C.c. with distilled water, gave  $\alpha_D +23^{\circ} 30'$  in a 2-Dcm. tube, whence  $[\alpha]_D +118.4^{\circ}$

These results indicated the above-described substance to be raffinose, which had previously been obtained from wheat germ by Schulze and Frankfurt (*Ber.*, 1894, 27, 64). It has, however, been recorded in the literature that raffinose, in the anhydrous state, melts at  $118-119^{\circ}$ , and in a 10 per cent. solution has a specific optical rotation of  $+104.4^{\circ}$ . The somewhat higher melting point and rotatory power observed in the present instance are doubtless to be attributed to a greater degree of purity of the substance, since the raffinose obtained by previous investigators might easily have been contaminated with a little cane sugar.

After the extraction of the original aqueous liquid with amyl alcohol and the separation of the raffinose, as described above, it was treated with a solution of basic lead acetate, when a comparatively small amount of a pale yellow precipitate was produced. This was collected, washed with water, and then suspended in water and decomposed by hydrogen sulphide. The mixture was subsequently filtered, and the filtered liquid concentrated under diminished pressure to a thin syrup. It gave a reddish-brown colouration with ferric chloride, but no precipitate with gelatin, and therefore contained no tannin. As nothing separated from the liquid on keeping for some time, it was heated for a few minutes with aqueous potassium hydroxide, after which it was acidified with sulphuric acid and extracted with ether. The ethereal liquid yielded only a further small amount of the previously described sinapic acid (m.p.  $190-192^{\circ}$ ).

#### ISOLATION OF CANE SUGAR, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ .

The filtrate from the above-mentioned basic lead acetate precipitate, after treatment with hydrogen sulphide for the re-

removal of the excess of lead, was concentrated under diminished pressure to the consistency of a syrup. The latter contained a considerable quantity of a reducing sugar, since it readily yielded *d*-phenylglucosazone (m.p.  $210^{\circ}$ ). As it was observed many years ago by Richardson and Crampton (*Ber.*, 1886, 19, 1180) that the sugar of wheat germ consists to a large extent of cane sugar, it was deemed desirable also to ascertain the presence of the latter in the material under examination. A portion of the syrup was therefore digested with hot alcohol, the alcoholic liquid decanted while still hot, and a little ethyl acetate added until a slight cloudiness appeared. On keeping this mixture for some time, colourless, prismatic crystals were deposited, which melted at  $184^{\circ}$ . An analysis and a determination of the optical rotatory power of this substance gave the following results:—

0.1264 gave 0.1950  $\text{CO}_2$  and 0.0745  $\text{H}_2\text{O}$ .  $\text{C} = 42.1$ ;  $\text{H} = 6.5$ .

$\text{C}_{12}\text{H}_{22}\text{O}_{11}$  requires  $\text{C} = 42.1$ ;  $\text{H} = 6.4$  per cent.

0.8560, made up to 20 C.c. with distilled water, gave  $\alpha_D + 5^{\circ} 39'$  in a 2-Dcm. tube, whence  $[\alpha]_D + 66.0^{\circ}$ .

These results proved the identity of the substance with cane sugar.

#### ISOLATION OF ALLANTOIN, $\text{C}_4\text{H}_6\text{O}_3\text{N}_2$ .

The occurrence of allantoin in wheat germ was recorded several years ago by Richardson and Crampton (*Ber.*, 1886, 19, 1180), who found the amount present to be somewhat less than 0.5 per cent. of the weight of the germ. It was also noted by those investigators that allantoin had only once previously been found in plants, but it is now known to be quite widely distributed in the vegetable kingdom (compare *P.J.*, 1913, 90, 550).

In order to confirm the presence of allantoin in the material under examination, a portion of the above-mentioned syrupy liquid was employed, which had been purified by means of basic lead acetate. To this liquid an aqueous solution of mercuric nitrate was added until no further precipitate was produced, the precipitate being then collected, washed with water, and subsequently suspended in water and decomposed by hydrogen sulphide. The mixture was then filtered, and the filtrate, after removing the excess of hydrogen sulphide, exactly neutralised with ammonia, after which it was evaporated to a small volume under diminished pressure. On keeping the liquid for some time, it deposited colourless,

rhombohedral prisms, which decomposed at about  $238^{\circ}$ . This substance possessed all the characters of allantoin, and its identity was confirmed by an analysis.

0.1477 gave 0.1654  $\text{CO}_2$  and 0.0514  $\text{H}_2\text{O}$ .  $\text{C} = 30.5$ ;  $\text{H} = 3.9$   
 $\text{C}_4\text{H}_6\text{O}_3\text{N}_4$  requires  $\text{C} = 30.4$ ;  $\text{H} = 3.8$  per cent.

ISOLATION OF BETAINÉ,  $\text{C}_5\text{H}_{11}\text{O}_2\text{N}$ , AND CHOLINE,  $\text{C}_5\text{H}_{15}\text{O}_2\text{N}$ .

Although the occurrence of both betaine and choline in wheat germ had been definitely established by Schulze and Frankfurt (*Ber.*, 1893, 26, 2151), it was deemed desirable, for the sake of completeness, to confirm their presence in the material under investigation. For this purpose a portion of the syrupy liquid was employed which had been purified by means of basic lead acetate, as already described. To this liquid an aqueous solution of phosphotungstic acid was added, when a voluminous precipitate was produced, which was collected, and washed with water. The precipitate was then intimately mixed, in the cold, with aqueous barium hydroxide, the mixture filtered, and the excess of baryta removed from the filtrate by means of carbon dioxide. After being again filtered, the liquid was rendered faintly acid by the addition of a little hydrochloric acid, and then concentrated under diminished pressure to the consistency of a syrup. The latter was digested with hot alcohol, and to the filtered liquid an alcoholic solution of mercuric chloride was added. On keeping the mixture for several days, a crystalline double mercuric salt separated, which was collected, dissolved in hot water, and decomposed by hydrogen sulphide. The filtrate from the mercuric sulphide was concentrated under diminished pressure, when a residue was obtained which quickly crystallised. This product was treated with cold absolute alcohol, when a portion, which was very sparingly soluble in the latter liquid, remained undissolved. The concentrated aqueous solution of this sparingly soluble salt, on the addition of gold chloride, yielded an aurichloride which slowly separated in glistening laminae, and was analysed.

0.1287 gave on ignition 0.0555 Au.  $\text{Au} = 43.1$ .

$\text{C}_5\text{H}_{12}\text{O}_2\text{N} \cdot \text{AuCl}_4$  requires  $\text{Au} = 43.2$  per cent.

The presence of betaine in wheat germ was thus confirmed.

The portion of the above-mentioned crystalline product which was readily soluble in alcohol was recovered by the

evaporation of the solvent, and the residue again treated with cold absolute alcohol. This alcoholic liquid, on the addition of a solution of platonic chloride, yielded a platini-chloride, which was analysed.

0.0915, dried at  $110^{\circ}$ , gave on ignition 0.0287 Pt. Pt = 31.4.

$(C_5H_{14}ON)_2PtCl_6$  requires Pt = 31.7 per cent.

This result afforded confirmation of the presence of choline.

### Examination of the Fatty Oil.

As previously noted, the mixture resulting from the distillation of the original alcoholic extract with steam was shaken with hot amyl alcohol in order to effect the separation of the fatty oil. The latter, after the removal of the solvent, amounted to 3600 grammes, which is equivalent to about 7 per cent. of the weight of wheat germ employed.

#### ISOLATION OF SITOSTEROL, $C_{27}H_{46}O$ .

A portion (300 grammes) of the fatty oil was hydrolysed by heating with an alcoholic solution of potassium hydroxide, after which the greater part of the alcohol was removed, water added, and the alkaline liquid repeatedly extracted with ether. The pale yellow, ethereal liquid was washed, dried, and the ether removed, when 20 grammes of a crystalline residue were obtained. On dissolving the latter in hot ethyl acetate, it yielded 11 grammes of a pure crystalline substance, which separated in colourless needles, melting at  $138^{\circ}$ . An analysis and a determination of its optical rotatory power gave the following results:—

0.6198 of air-dried substance, on heating at  $110^{\circ}$ , lost 0.0306  $H_2O$   
 $H_2O = 4.9$ .

0.1115 of anhydrous substance gave 0.3428  $CO_2$  and 0.1190  $H_2O$ .  
 C = 83.8 ; H = 11.9.

$C_{27}H_{46}O$ ,  $H_2O$  requires  $H_2O = 4.5$  per cent.

$C_{27}H_{46}O$  requires C = 83.9 ; H = 11.9 per cent.

0.5892 of anhydrous substance, made up to 20 C.c. with chloroform, gave  $\alpha_D - 1^{\circ} 54'$  in a 2-Decm. tube, whence  $[\alpha]_D - 32.2^{\circ}$ .

The above described substance was thus shown to consist of pure sitosterol. The amount obtained from 300 grammes of the fatty oil indicates that sitosterol is contained in the wheat germ to the extent of about 0.26 per cent.

The ethyl acetate mother-liquor remaining after the

removal of the sitosterol had a deep yellow colour, and contained a considerable amount of uncrystallisable material. The solvent was therefore removed, and the residue distilled under diminished pressure, when two fractions were collected, which passed over at 80 to 250°/20 Mm. and above 250°/20 Mm. respectively. The first fraction was a limpid, yellow oil, which possessed a strong, somewhat aromatic odour, but did not yield the characteristic phytosterol reaction. When dissolved in chloroform with a little acetic anhydride, and a drop of concentrated sulphuric acid subsequently added, only a brown colouration was produced. The fraction boiling above 250°/20 Mm. yielded a little sitosterol, melting at 138°.

#### IDENTIFICATION OF THE FATTY ACIDS.

The alkaline liquid resulting from the hydrolysis of the fatty oil, which had been extracted with ether for the removal of the sitosterol, as described above, was next acidified with sulphuric acid, and again extracted with ether. This ethereal solution was washed, dried, and the ether removed, when about 230 grammes of fatty acid were obtained. As the product evidently consisted of a mixture of saturated and unsaturated acids, it was first separated into solid and liquid portions by means of the lead salts and treatment of the latter with ether.

*The Solid Acids.*—The portion of the lead salt which was insoluble in ether, when decomposed by hydrochloric acid, yielded 50 grammes of solid acid. For the examination of the latter, it was converted into the methyl ester, which was then distilled under diminished pressure. Although the entire amount passed over at 193-195°/15 Mm., it was collected in three portions, which, however, were found to be identical in character. They all formed colourless solids, melting at 29-30°, and having a saponification value of 205°, while each of them yielded a fatty acid melting at 63-64°. The middle portion of the methyl esters was analysed.

0.1190 gave 0.3312 CO<sub>2</sub> and 0.1350 H<sub>2</sub>O. C = 75.9; H = 12.6.  
 C<sub>15</sub>H<sub>31</sub>·CO<sub>2</sub>CH<sub>3</sub> requires C = 75.6; H = 12.6 per cent. S.V. = 208.  
 C<sub>17</sub>H<sub>35</sub>·CO<sub>2</sub>CH<sub>3</sub> requires C = 76.5; H = 12.8 per cent. S.V. = 188.

It is evident from these results that the solid acids consisted entirely of a mixture of palmitic and stearic acids, and apparently in about equal proportions.

*The Liquid Acids.*—The portion of lead salt which was soluble in ether, when decomposed by hydrochloric acid, yielded about 150 grammes of liquid acid. When distilled under diminished pressure, practically all of this product passed over at 245°/25 Mm. as a pale yellow oil. This was analysed, and its neutralisation and iodine values determined, with the following results:—

0.1218 gave 0.3444 CO<sub>2</sub> and 0.1284 H<sub>2</sub>O. C = 77.1; H = 11.7.

0.2297 required 8.1 C.c. N/10 KOH for neutralisation. N.V. = 198.

0.1302 absorbed 0.2181 Iodine. Iodine value = 167.

C<sub>18</sub>H<sub>32</sub>O<sub>2</sub> requires C = 77.1; H = 11.4 per cent,

Neutralisation value = 200.4; Iodine value = 181.3.

These results indicate that the liquid acid consisted of practically pure linolic acid.

### Summary.

The so-called wheat germ, as already noted, is the best known source of the typical phytosterol which has been designated sitosterol. As a considerable quantity of this compound was required for the purpose of the synthetical preparation of its glucoside (Salway, *J. Chem. Soc.*, 1913, 103, 1022), it was deemed desirable, incidentally, to subject the wheat germ to a complete chemical examination. The amount of wheat germ thus employed was 50.8 kilogrammes.

In addition to the above-mentioned sitosterol, it has been shown by previous investigators that wheat germ contains the following substances:—Choline, betaine, allantoin, cane sugar, dextrose, and raffinose. In the course of the present investigation the occurrence of all these substances has been confirmed, but no evidence was obtained of the presence of asparagine, which has been recorded by Frankfurt (*J. Chem. Soc. Abst.*, 1897, 72, ii. 67) as a constituent of wheat germ.

The amount of fatty oil obtained in the present instance, which was separated from an alcoholic extract of the wheat germ, was equivalent to about 7 per cent. of the weight of material employed. The physical and chemical constants of this oil, as obtained by a more direct process of extraction, and also of the mixed fatty acids therefrom, have previously been recorded (compare Lewkowitsch, 'Chemical Technology and Analysis of Oils, Fats, and Waxes,' third edition, Vol. II., p. 520). So far as known to us, the nature of the fatty acids, which are chiefly contained in the oil as glycerides,

has, however, not hitherto been determined. These have now been found to consist of palmitic, stearic, and linolic acids, the amount of the latter being about three times as much as that of the total solid acids. The amount of resinous material contained in the wheat germ is exceedingly small, representing only about 0.04 per cent. of its weight. A small amount of amorphous glucosidic material was also obtained.

It has now been ascertained that wheat germ contains a very small amount of sinapic acid,  $C_{11}H_{12}O_5$ , which is present in some form of combination. This fact is of special interest, inasmuch as the respective acid has hitherto only been known to occur in mustard seed, or at least in the family of *Cruciferae*. As it exists in black mustard seed in the form of sinapine, which is a choline ester of sinapic acid, it is highly probable that the latter occurs in the same form of combination in wheat germ.

