ensure acidity and then sufficient water to dissolve the sodium iodide and to cause a separation of the ether layer. The ether layer was removed and the aqueous solution extracted with fresh ether. The combined ether solutions were dried and the ether distilled. The residue was fractionally distilled under reduced pressure. The alkylphenyl derivatives given under II in the table were prepared in this way.

III. $(R)(C_6H_5)\overline{CCONHCONHC}{=}NH$, **5,5-Alkylphenyl-4-iminobarbituric Acids.**—The condensation of the cyanoalkylphenylacetates with urea, with the formation of 5,5-alkyl-4-iminobarbituric acids, was by the method of Conrad.[10] Sodium ethylate, prepared from 13 g. of sodium wire in 200 g. of absolute ethyl alcohol, was placed in the previously described three-necked flask and to it were added 65 g. of ethyl cyanoethylphenylacetate, or an equivalent amount of one of the other cyanoalkylphenylacetates, and 20 g. of urea. The mixture was heated, stirred and refluxed for eight hours. The alcohol was then distilled off and the residue dissolved in about 800 cc. of water. The unchanged acetate was removed by extraction of the water solution with ether. The aqueous solution was then acidified with a slight excess of concentrated hydrochloric acid. Pure white precipitates were obtained, filtered off and dried as crude products. The compounds prepared are given under III in the table.

IV. $(R)(C_6H_5)\overline{CCONHCONHCO}$, **5,5-Alkylphenylbarbituric Acids.**—The hydrolysis of the iminobarbituric acids was easily accomplished by boiling about 10 g. for a short time in about 500 cc. of 3.3 *N* hydrochloric acid On cooling the barbituric acid separates as a white crystalline product usually in the theoretical yield from the imino acid. The acids prepared are given under IV in the table

Conclusions

In the syntheses described sodamide was a most efficient condensing agent. Absolutely anhydrous reagents and conditions, continued stirring and efficient refluxing are essential. Five 5,5-alkylphenylbarbituric acids have been synthesized successfully, four of them hitherto unsynthesized by this method. In most cases the general properties and physical constants have been determined for the cyanoalkylphenylacetates, the 5,5-alkylphenyl-4-iminobarbituric acids and the 5,5-alkylphenylbarbituric acids. The pharmacological evaluation of the final barbituric acids has not yet been made.

AMHERST, MASS. RECEIVED OCTOBER 29, 1934

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

The Chemistry of Mold Tissue. VII. The Lipids of Penicillium Aurantio-Brunneum[1]

BY E. H. KROEKER, F. M. STRONG AND W. H. PETERSON

As an extension of the previously reported study of the lipids of *Aspergillus sydowi*,[2] it seemed desirable to examine at least one representative of the *Penicillium* species of mold fungi. *Penicillium aurantio-brunneum* was selected for this purpose because it grew well on a synthetic medium and contained a fairly large amount of lipoidal material.

While the present investigation was in progress, a paper appeared by Ward and Jamieson[3] concerning the fat from *Penicillium javanicum*. The composition of this fat appears to be roughly similar to that of *A. sydowi*, and *P. aurantio-brunneum*.

Experimental Part

Extraction and Preliminary Fractionation of Crude Lipids.—The mold used was grown for ten days on a glucose–inorganic salts medium in large sterilized incubators.[4] The pads were then killed by steaming, dried at 65° and finely ground. The ground mycelium (4110 g.) was extracted with alcohol–ether (1:1), and the extracts worked up as previously described.[2] The yield of crude lipids was 475 g. or 11.6% of the mycelium.

When the crude lipids were poured slowly into three liters of ice-cold acetone, eight grams of an amorphous precipitate separated. This substance contained 3.3% phosphorus and 1.48% nitrogen, the P:N ratio, therefore, being 1:1.01. This behavior is in marked contrast to that of the *A. sydowi* lipids, which contained only a trace of acetone-insoluble phospholipids. Upon further cooling of the acetone solution in an ice–salt bath 78 g. of a dark, viscous oil separated as a distinct layer, and was drawn off (Fraction A). Still further standing of the acetone solution in the ice–salt bath resulted in the precipitation of 35 g. of a light colored solid, which was filtered off (Fraction B). The lipids remaining in the acetone still contained 0.12% phosphorus, which was not precipitable by alcoholic magnesium chloride. Of various other salts tried strontium chloride proved most efficient. The ice-cold acetone solution was, therefore, treated with 25 cc. of saturated alcoholic strontium chloride, and the precipitate (10 g.) separated after several hours of standing in the cold. It contained 2.29% phosphorus and 1.21% nitrogen; P:N ratio, 1:1.17. The remaining acetone solution on concentration yielded 375 g. of "simple lipids" containing only 0.04% phosphorus.

(1) This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

(2) Strong and Peterson, THIS JOURNAL, **56**, 952 (1934).

(3) Ward and Jamieson, *ibid*, **56**, 973 (1934).

(4) Peterson, Pruess, Gorcica and Greene, *Ind. Eng. Chem.*, **25**, 213 (1933).

Examination of Fractions A and B.—Saponification of Fraction A gave 1.93% unsaponifiable matter, and 89.6% acids, of which 40.5% was insoluble in petroleum ether, and had a neutral equivalent of 236. A sample of this insoluble acid was esterified, but the ester was not distillable at 3 mm. pressure. Approximately one-fourth of the original fraction was also insoluble in petroleum ether. This material showed no optical activity in chloroform solution, and had an acetyl value[5] of 51. Attempts to isolate a definite substance from this fraction were unsuccessful.

Fraction B gave on saponification 7.15% unsaponifiable matter, and 87.2% fatty acids. Since a preliminary examination of these acids and of the petroleum ether-soluble acids from Fraction A showed them to be essentially similar to those of the simple lipids, they were not further investigated.

Examination of the Simple Lipids.—The acetone-soluble material obtained as above was saponified with alcoholic potassium hydroxide, and the fatty acid, unsaponifiable, and water soluble fractions separated in the usual manner. From the water-soluble fraction, following the procedure previously described,[2] 11.7 g. of glycerol was isolated in the form of triacetin, b. p. 136–137° (9 mm.), saponification equivalent 70.9 (calcd. 72.7). Further identification of the glycerol was effected through preparation of the tribenzoate, which melted at 75–76° both alone and when mixed with an authentic sample.

The fatty acids (320 g.) were separated by the standard lead salt–ether procedure into saturated (16.4%) and unsaturated (83.6%) fractions. The saturated fraction gave an iodine number (Hanus) of 5.2, and the unsaturated fraction 129.7.

Saturated Acids.—The methyl esters of 38 g. of the saturated acids were prepared, and fractionated under diminished pressure. Seven fractions boiling over the range 147–210° (3 mm.) were collected. A small sample of each was saponified, and the neutral equivalent of the free acid determined. Fractions of similar neutral equivalents were then combined and redistilled with the following results.

Fraction	Weight, grams	B. p., °C. (3 mm.)	N. e. of corresponding acid
I	3.40	143–151	262
II	9.84	151–160	261–263
III	12.73	165–180	276–280
IV	2.5	180–182	284

From Fractions I and IV, palmitic and stearic acids, respectively, were isolated by saponification and fractional crystallization of the free acids from acetone, and were identified by neutral equivalents, melting points and mixed melting points with authentic samples. The constants observed were: palmitic acid, m. p. 62.5–63°, n. e. 256 (calcd. 256.2); stearic acid, m. p. 68–69°, n. e. 286 (calcd. 284.3). The absence of any fraction distillable above 182° in the small residue indicated that not more than a trace of acids higher than C_{18} could have been present. In this respect the fatty acids differ from those of *A. sydowi*[2] which contained small amounts of a C_{24} acid, *n*-tetracosanic acid.

Unsaturated Acids.—The methyl esters of 147 g. of the crude unsaturated acids were prepared, and extracted twice with dilute sodium hydroxide, which removed a brown resinous substance. The esters were then separated by distillation at 3 mm. pressure into a forerun weighing 11.8 g., b. p. 144–158°, and a main fraction of 123.2 g., b. p. 159–160°. A portion of the latter was saponified, and the free acids found to have an I no. of 131.7, and a neutral equivalent of 285. Catalytic reduction of a small sample resulted in a 96% yield of stearic acid, m. p. 68–69°, neutral equivalent, 285.1 (calcd. 284.3). Consequently this fraction consisted almost entirely of straight chain, C_{18}, acids.

A sample of the above acids was also oxidized with alkaline permanganate, and the product worked up as previously described.[2] The chloroform-insoluble fraction after purification melted at 169–170°, indicating the presence of linoleic acid. The chloroform-soluble portion melted at 129°, and was shown by mixed melting point to be identical with 9,10-dihydroxystearic acid. These data establish the presence of oleic acid in the unsaturated acids.

The unsaturated acids were further examined by bromination at −10° in petroleum ether solution. From 19 g. there were obtained 10.65 g. of petroleum ether-insoluble tetrabromides, and 23.55 g. of soluble "dibromides," which, however, contained some tetrabromides as was indicated by a bromine content of 40.1% (calcd. for dibromostearic, 36.15%). The insoluble material after three recrystallizations from hot ligroin, b. p. 60–80°, melted at 116–116.5°, and gave no depression when mixed with known tetrabromostearic acid. Calcd. for $C_{18}H_{32}O_2Br_4$: Br, 53.28. Found: Br, 54.1. Only traces of ether-insoluble hexabromides were present in this fraction. The unsaturated acids, therefore, contained linoleic but no linolenic or more highly unsaturated acids.

Unsaponifiable Matter.—When the ether extract containing the unsaponifiable matter was concentrated, a yellow-brown solid separated from the light brown oil. This solid weighed 17 g., and represented 3.7% of the crude fat. Repeated crystallization from alcohol–benzene (2:1) produced a white product, m. p. 159–160°, $[\alpha]_D^{25}$ −130°. A mixed melting point with a purified sample of ergosterol showed no depression. The melting point and rotation reported by Windaus for ergosterol are 163° and −130°, respectively.[6]

Quantitative Composition of the Simple Lipids.—The approximate composition of the simple lipids is given in the following table.

APPROXIMATE COMPOSITION OF THE SIMPLE LIPIDS

	%		%
Total fatty acids	85.4	Stearic[b]	5.3
Oleic[a]	40.2	Unsaponifiable	4.5
Linoleic[a]	31.2	Ergosterol	1.9
Palmitic[b]	8.6	Glycerol	3.1

[a] Calculated from the weight and iodine number of the unsaturated acids. [b] Calculated from the weight and neutral equivalent of the crude saturated acids.

Summary

1. The simple lipids of *P. aurantio-brunneum*

(5) West, Hoagland and Curtis, *J. Biol. Chem.*, **104**, 627 (1934).

(6) Windaus and Borgeaud, *Ann.*, **460**, 236 (1928).

have been shown to consist essentially of glycerides of palmitic, stearic, oleic and linoleic acids.

2. Ergosterol was isolated from the unsaponifiable matter.

MADISON, WISCONSIN RECEIVED NOVEMBER 14, 1934

[CONTRIBUTION FROM THE COCONUT RESEARCH SCHEME, LUNUWILA, CEYLON]

The Seed Oil of Aegle Marmelos, Corr.

BY REGINALD CHILD

The fruit of the Bael or Beli fruit tree, *Aegle Marmelos*, Corr., is commonly used in India and Ceylon for the treatment of dysentery. A chemical investigation of the plant was commenced by the late Professor J. P. C. Chandrasena, of the University College, Colombo, at whose request the author examined the seed oil. The analytical constants and approximate composition of this oil have been thought worth recording since few examples of seed oils of the *Rutaceae* are to be found in the literature.

Dikshit and Dutt[1] obtained from the seeds an oil (11.94%) having d^{20} 0.914, saponification value 195.2, Hehner value 91.3, and iodine value 126.1. The constants recorded for the present sample differ somewhat from the foregoing.

The fruits resemble oranges in size; they have a hard gray-green pericarp and the seeds (twenty or more in a fruit) are embedded in a thick gummy pulp. The latter are roughly of the shape shown (Fig. 1) and average in dimensions 6.6 × 5.6 × 3.6 mm.

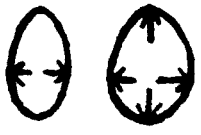

Fig. 1.—(×2).

Two samples were examined, 200 seeds from 9 ripe fruits, and 250 seeds from 11 fruits just short of complete ripeness. The leaves, fruits and roots of the trees from which the samples were taken were kindly identified by Dr. J. C. Haigh, Economic Botanist, Department of Agriculture, Ceylon.

In each case the shells were removed, the kernels ground, dried and extracted with light petroleum, b. p. 40–60°.

	Sample 1	Sample 2
Av. wt. of shell, g.	0.026 (23.5%)	0.023 (25.3%)
Av. wt. of kernel, g.	.084 (76.5%)	.068 (74.7%)
Av. wt. of seed, g.	.11	.091
Moisture of kernels, %	8.35	25.3
Oil of kernels, wet basis, %	45.0	35.1
Oil of kernels, dry basis, %	49.1	44.0
Oil of whole seeds, %	34.4	25.3

(1) Dikshit and Dutt, *J. Indian Chem Soc.*, **7**, 759 (1930).

The oil was clear and had a faint odor resembling linseed oil; Sample 1 was practically colorless, while Sample 2 had a pale yellow tinge. The usual constants were as follows.

	Sample 1	Sample 2
Density d_4^{30}	0.918	.....
Refractive index n_D^{40}	1.4647	1.4647
Dispersive power ω	0.0202	0.0202
Free fatty acid (oleic %)	.42	1.26
Saponification value	193.6	196.8
Iodine value (Wijs)	108.0	107.1

Sample 1 only was examined further. Unsaponifiable matter was determined by the standard method described in the *Analyst*, **58**, 203 (1933). For the thiocyanogen value, 0.4 g. samples and 50 cc. of reagent were employed as recommended by Wiley and Gill,[2] titrations being carried out after twenty-four hours. Saturated acids were determined by Twitchell's method, and had a mean molecular weight of approximately 266.

Thiocyanogen value (24 hours)	70.4
Hehner number	93.7
Unsaponifiable matter, %	1.58
Satd. acids, % (corr.)	23.9

Calculation from the iodine value, thiocyanogen value and percentage of saturated acids in the usual way gives approximately the following composition for the oil (Sample 1). It should be emphasized, however, that on account of the limited amount of material available, these figures are not claimed to be more than approximate.

Palmitic acid	15.6	
Stearic acid	8.3	23.9%
Oleic acid	28.7	
Linoleic acid	33.8	
Linolenic acid	7.6	70.1%
Unsaponifiable matter		1.6%
Glyceryl $C_3H_2\equiv$		4.4%
		100.0%

(2) Wiley and Gill, *Ind. Eng. Chem.*, **6**, 298 (1934).