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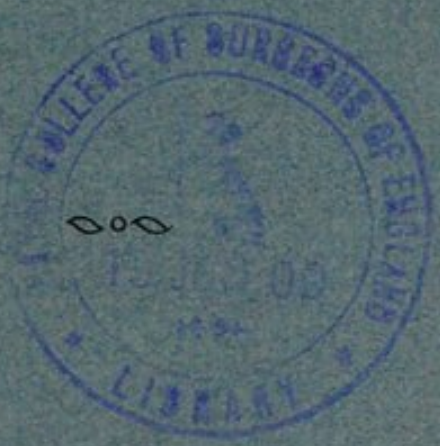

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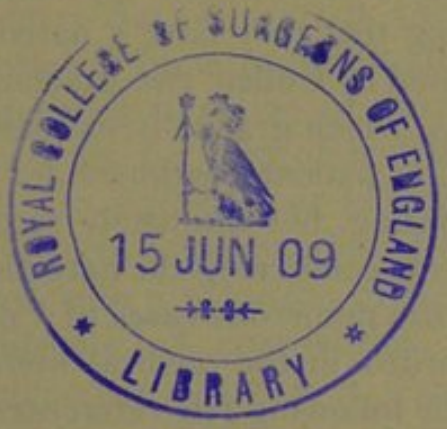

CHEMICAL EXAMINATION AND PHYSIOLOGICAL ACTION OF NUTMEG.

By Frederick B. Power and Arthur H. Salway.

A Contribution from the Wellcome Chemical Research Laboratories, London.

The nutmeg, although considerably used as a condiment or flavoring agent, and to some extent medicinally as an aromatic stimulant, has long been known to possess a decided narcotic action when administered in any appreciable amount. The general recognition of this property is evident from the fact that it is recorded in many of the standard works descriptive of the *materia medica,* as the following few abstracts will indicate.

The "United States Dispensatory," nineteenth edition, p. 799, makes the following statement: "Nutmeg unites to the medicinal properties of the ordinary aromatics considerable narcotic power. In the quantity of two or three drachms (7.7 or 11.6 grammes), it has been known to produce stupor and delirium, and dangerous if not fatal consequences are said to have followed its free use in India." The "National Standard Dispensatory," p. 990, remarks as follows: "Nutmeg possesses aromatic, narcotic, and intoxicating properties. Given in overdose it produces stupor, decreased reflex excitability, slowness of respiration, and slight cardiac sedation." The "Pharmacographia Indica," Vol. III, p. 193, records the following information: "Mahometan doctors describe nutmegs and mace as stimulating, narcotic, digestive, tonic, and aphrodisiac." Also *Ibid.*, p. 196: "The narcotic effects of nutmegs noticed by the old

Mahometan physicians have been confirmed by Bontius, Rumphius, Lobel, Schmid, and Cullen, and more recent experiments upon man and animals agree in showing that they have a narcotic and intoxicating action. In a case related by Cullen, two drachms of powdered nutmeg produced drowsiness, which gradually increased to complete stupor and insensibility. The patient continued for several hours alternately delirious and sleeping, but ultimately recovered."

The above general statements concerning the narcotic action of nutmeg are fully confirmed by the numerous cases of "nutmeg poisoning" which have been recorded in the medical literature of more recent times, among which the following few references may be cited: *The Lancet,* April 12, 1902, p. 1035; Squibb's *Ephemeris of Materia Medica, etc.,* Vol. VII, 1904, p. 243; *The British Medical Journal,* 1906, pp. 539, 778, 900, 984; *Chem. Zeit. Rep.,* Feb. 12, 1908, p. 79, from *Deutsch. med. Wochenschrift,* 1907, Bd. 33, p. 2001; Cushny, in *Proceedings of the Royal Society of Medicine, Therapeutical and Pharmacological Section,* 1908, Vol. I, pp. 39–44.

With regard to the constituent of the nutmeg to which its narcotic effects may be attributed, the following statement in the "United States Dispensatory," nineteenth edition, p. 799, is of interest: "Dr. H. C. Wood found in experiments upon the lower animals that the oil of nutmeg is a powerful narcotic, with very much less sedative influence upon the heart than is possessed by most volatile oils. Injected into the circulation of the dog, it caused profound sleep, with slowing of the respiration, and, if the dose had been large enough, loss of reflex activity."

In the *Bericht* of Schimmel & Co., Leipzig, April, 1904, pp. 159–165, special consideration was given to the subject of nutmeg poisoning by a contribution from Dr. Fritz Jürss, Assistant at the Pharmacological Institute of the University of Rostock, entitled: "On Myristicin and some closely related substances." This comprised an account of the action of myristicin, $C_{11}H_{12}O_3$, a constituent of the essential oil of nutmeg, on frogs, fish, birds, and mammals, especially the guinea pig and rabbit. It was noted by this investigator (*loc. cit.,* p. 159) that "the oils of nutmeg and mace only cause fatal poisoning in a rabbit in doses of 10.0 to 12.0 grammes, whereas a single nutmeg (4.0 to 5.0 grammes) is capable of producing in man serious effects," and the conclusion was therefore drawn that the oil is less poisonous for animals than for man. It should be considered, however, in this connection that the essential oil of

nutmeg is very variable in character, and that some specimens may be practically free from myristicin, or even consist entirely of terpenes (compare *Ber. d. deutsch. chem. Ges.*, 1890, 23, p. 1804). The experiments of Jürss on birds and mammals were conducted by the subcutaneous injection of myristicin, in amounts varying from 2 c.c. to 6 c.c. per kilo of bodyweight in the case of guinea pigs, or 0.9 c.c. to 1.76 c.c. per kilo of bodyweight in the case of rabbits. The effects were manifested by a paralysis of the central nervous system, with a reduction of temperature, followed by death without convulsions. A post-mortem examination of the animals showed, among other phenomena, extensive degenerative changes in the liver, such as coagulative necroses, vacuolation of the protoplasm, and the abundant presence of fat, resembling the effects of phosphorus poisoning.

Although the above-noted experiments afford ample evidence that myristicin is a substance possessing a considerable degree of physiological activity, it is also evident that the results are hardly comparable with the symptoms produced in man by the administration of relatively small amounts of nutmeg. If, for example, two nutmegs, an amount which is known to be capable of producing serious effects in man, be considered as weighing 10 grammes, they would contain on an average not more than about 1.0 gramme of essential oil, of which a very small proportion is myristicin. On the other hand, the toxic effects produced in guinea pigs weighing 500 grammes and in rabbits weighing from 1300 to 2200 grammes respectively were obtained by the subcutaneous injection of myristicin in amounts many times greater than are contained in two nutmegs, and even considerably exceeding the total amount of essential oil contained in the latter (compare also *Semi-annual Report* of Schimmel & Co., Leipzig, Oct., 1904, p. 103). From a consideration of these facts, it appeared possible that the narcotic effects produced in man by the nutmeg might not be due solely to the essential oil or the myristicin contained therein, and it was, therefore, with the object of elucidating this question that a complete study of the constituents of the nutmeg was undertaken.

Some considerable time after beginning this investigation a paper was published by Dr. A. R. Cushny (*loc. cit.*) on the subject of "nutmeg poisoning." It was noted in this communication that some years ago Dr. G. B. Wallace had undertaken an examination of the pharmacological action of nutmeg on animals and the separa-

tion of its poisonous constituent, the results having been published in 1903 in "Contributions to Medical Research," dedicated to V. C. Vaughan, Ann Arbor, Michigan. For the purpose of completeness it is desirable that the following brief abstract of the recent paper by Cushny should be included in this account of the subject.

"The nutmeg contains from 3 to 8 per cent. of volatile oil, and when this has been extracted from it the residue produces no effect whatever on animals, while small doses of the oil itself induce characteristic effects. The oil contains several terpenes and small quantities of higher boiling substances which can be separated by fractional distillation.[1] The terpenes are devoid of action except in enormous quantities, while the fraction boiling at 150° C. at 14 mm. pressure [2] proved to be a powerful poison."

Wallace conducted experiments with the high-boiling fraction of the oil on frogs, rabbits, and cats, and the following observations and conclusions drawn therefrom are further noted by Cushny, as follows:

"The cat is much more susceptible to the action than the rabbit, as is very generally the case with drugs acting on the central nervous system. About 0.4 gramme per kilo of the highest distillate given *per os* causes restlessness with weak spasmodic movements and tremor resembling that seen in carbolic acid poisoning, and profuse salivation. The restlessness passes into quiet with persistence of the tremor, incoördination of the movements, weak reflexes and partial anæsthesia. The pupils are dilated. Soon a stage of stupor, gradually deepening, sets in, the respiration is labored and feeble, and finally ceases some eight to twelve hours after the ingestion of the poison. In many cases, however, after some hours of stupor, a gradual improvement begins, and in fifteen hours from the taking of the poison the animal appears fairly normal save for unusual quietness and disinclination to move about. This improvement is only temporary, however, the cat again becoming weaker and more depressed, eating nothing and paying no attention to its surroundings, until coma returns, followed by death in 36–72 hours from the time the oil was taken."

"The symptoms in mammalia are thus, as in the frog, to be attributed to action on the central nervous system, which is depressed

[1] Compare Power and Salway. *Journ. Chem. Soc.*, 1907, 91, pp. 2037–2058.

[2] According to the results of our investigation of the essential oil of nutmeg (*loc. cit.*), this fraction would consist chiefly of myristicin.

for the most part, but exhibits some indication of stimulation in the form of restlessness, slight convulsive movements, and tremor. Animals, therefore, correspond very closely to man in their reactions to nutmeg poison."

"Many volatile oils induce fatty degeneration of the liver and other organs, but nutmeg poison has little or no action in this direction."

"Wallace's results do not indicate any useful purpose which nutmeg might serve in therapeutics, but are of interest in drawing attention to the possibility of serious poisoning from one of our common domestic flavoring agents."

The above record of experiments would appear to have established the fact that the narcotic properties of nutmeg are to be attributed to myristicin, and that much smaller amounts of the latter substance are required to produce the characteristic symptoms of nutmeg poisoning when administered by the mouth to a cat than when injected subcutaneously into the guinea pig or rabbit, as indicated by Jürss (*loc. cit.*). It may be noted, however, that the statement by Cushny, that nutmeg poison has little or no action in inducing fatty degeneration of the liver, is quite at variance with the observations of Jürss, and is not confirmed by the results of the experiments conducted by Dale, as recorded in the latter part of this paper.

EXPERIMENTAL.

In the beginning of this investigation it was thought possible that the narcotic action of nutmeg might be due to the presence of either small amounts of an alkaloid or of a soluble toxic protein. Special tests were therefore made for both of these classes of substances, but with negative results. For the further systematic investigation of the subject it was decided to make a complete study of (I) the essential oil, (II) the expressed oil or fat, and (III) the "press-cake" remaining after the removal of the latter, as all the constituents of the nutmeg would be included in these products.

I. *The Essential Oil of Nutmeg.*

A complete account of our investigation of this product, which was specially distilled for us from Ceylon nutmegs by Messrs. Stafford Allen & Sons, of London, has already been published (*Journ. Chem. Soc.*, 1907, 91, 2037), and therefore need not be

specially considered here. The opportunity may, however, be taken of presenting a few comments on the requirements made for this essential oil by the United States and British Pharmacopœias.

In the "United States Pharmacopœia" (8th revision) the specific gravity of this oil was given as 0.862 to 0.910 at 25° C., and in the list of additions and corrections to June 1, 1907, these figures were altered to 0.884 to 0.924. It is evident, however, that in this alteration an error has been made, and that the limits were intended to be placed at 0.864 to 0.924 at 25° C. (compare the *Semi-annual Report* of Schimmel & Co., Leipzig, April, 1906, p. 71). The "British Pharmacopœia" requires a specific gravity of 0.870 to 0.910 at 15.5° C., the German 0.890 to 0.930, and the Belgian 0.865 to 0.920 at 15° C. The last-mentioned limits would appear to be those most in accordance with normal products of distillation.[1] In this connection it is of interest to note that the present "German Pharmacopœia" (4th edition, 1900) has adopted for the essential oil of nutmeg ("Aetherisches Muskatnussöl") the Latin title of *Oleum Macidis*. This not only involves an etymological inaccuracy, but also the assumption that the essential oils of nutmeg and mace are identical in character and composition, which has not as yet been proved to be the case. In the second (1882) and third (1890) editions of the "German Pharmacopœia" *Oleum Macidis* was correctly defined as mace oil ("Mascisöl"), and the last-mentioned title and definition have been adopted by the "Swedish Pharmacopœia" (*Pharmacopœa svecica,* ed. VIII) with the following requirements: specific gravity at 15° C. = 0.855 — 0.930; optically dextrogyrate; soluble in 3 parts of alcohol (see *Semi-annual Report* of Schimmel & Co., April, 1902, p. 73).

The "United States Pharmacopœia," in its latest edition, has introduced a requirement for oil of nutmeg, evidently adapted from the "British Pharmacopœia," which is as follows: "When 2 or 3 c.c. of oil are evaporated on a water-bath, no residue which crystallizes on cooling should be left." The purpose of this test, as stated in the "British Pharmacopœia," is to ensure the "absence of the concrete oil of nutmeg." It is likely, however, to involve the exclusion of constituents of a normal essential oil which are not without considerable value, for any crystalline residue which would be obtained from a genuine oil under these conditions would

[1] Compare Allen and Brewis, *Pharm. Journ.*, 1901, 66, p. 328.

consist of myristic acid, and this usually accompanies the highest boiling constituents of the oil in the process of distillation. In order, therefore, to exclude these very small amounts of myristic acid, it would be necessary that the essential oil should represent only its more volatile constituents, consisting chiefly of terpenes, and it thus becomes evident that the requirement is a thoroughly irrational one.

II. *The Expressed Oil of Nutmeg.*

This product was obtained by the expression of 23.7 kilogrammes of Ceylon nutmegs, the operation having been kindly conducted for us by Messrs. Stafford Allen & Sons, of London. An account of its complete investigation is recorded in the *Journ. Chem. Soc.*, 1908, 93, p. 1653, to which reference may be made.

III. *Examination of the "Press-cake" from Nutmeg.*

The so-called "press-cake," resulting from the expression of the above-mentioned 23.7 kilogrammes of nutmegs, amounted to about 16 kilogrammes. After being finely ground, it was mixed with purified sawdust, and successively extracted in a large Soxhlet apparatus with (A) light petroleum (b. p. 30–40° C.) and (B) alcohol.

(A.) *The Petroleum Extract.*

This consisted of a nearly colorless, solid fat, amounting to 2800 grammes, or 17.5 per cent. of the total press-cake. It was expected to contain, although in different proportions, the same substances as had previously been found by us in the expressed oil of nutmeg (*loc. cit.*), which proved to be the case.

A quantity (250 grammes) of the fat extracted by petroleum was hydrolized by heating for an hour on a water-bath with an alcoholic solution of 80 grammes of potassium hydroxide. The greater part of the alcohol was then removed, water added, and the alkaline, aqueous mixture extracted repeatedly with ether. The combined ethereal liquids were washed with a little water, dried with anhydrous sodium sulphate, and the ether removed, when about 10 grammes of a thick, yellow oil were obtained. This oil, when treated with an equal volume of dilute alcohol, deposited a small amount of a solid, which was collected, and crystallized from a mixture of

alcohol and ethyl acetate. Colorless leaflets were thus obtained, which melted at 134–135° C., and afforded the color reactions characteristic of the phytosterols.

After removing the alcohol from the liquid from which the phytosterol had originally been deposited, the residual oily product was distilled under a pressure of 10 mm., and fractions collected which boiled between 70–200° and 200–280° C./10 mm. respectively. The first of these fractions consisted of a mixture of various constituents of the essential oil of nutmeg, while the second fraction, on redistillation, boiled for the most part at 270–274° C./10 mm., and, at the ordinary temperature, formed a yellow, transparent, extremely viscid liquid, which showed no tendency to crystallize. On analysis it gave the following result:

0.2523 gave 0.6274 CO_2 and 0.1546 H_2O. C = 67.8; H = 6.8

$C_{18}H_{22}O_5$ requires C = 67.9; H = 6.9 per cent.

This substance was evidently identical with the compound $C_{18}H_{22}O_5$, which had previously been isolated from the expressed oil of nutmeg, and was fully described in connection with the latter product (*loc. cit.*). It possessed no apparent physiological activity.

The Fatty Acids.—The alkaline liquid from which the unsaponifiable material had been removed, as above described, was acidified with sulphuric acid and distilled with steam, but the distillate only contained a small amount of myristic acid. The contents of the distillation flask were then extracted with ether, the ethereal solution being washed, dried, and the ether removed. A quantity of fatty acids was thus obtained, which was distilled under 15 mm. pressure, when more than 90 per cent. of the material passed over at 196–197°, the remainder distilling from 197–240° C./15 mm. The portion boiling at 196–197° C./15 mm. melted at 54° C., and was found to consist of pure myristic acid.

0.5087 required 4.45 c.c. $\frac{N}{2}$ KOH for neutralization.

Acid value = 245.

$C_{14}H_{28}O_2$ requires an acid value of 246.

The fraction 197–240° C./15 mm. was only small in amount and contained some unsaturated acid, since it absorbed bromine in chloroform solution. On digesting it with alcohol it deposited a

very small quantity of a solid substance. The latter, after recrystallization from hot alcohol, melted at 74–75° C., and was identified as cerotic acid, which had previously been isolated by us from the expressed oil of nutmeg.

(B.) *The Alcohol Extract.*

This was a dark brown mass, amounting to 2300 grammes, or about 14.4 per cent. of the total press-cake. It was mixed with water, and the mixture distilled with steam until all the volatile substances present had been removed.

Volatile Constituents of the Alcohol Extract.

The aqueous distillate, which contained some oil floating on the surface, was extracted with ether, the ethereal solution being washed with a little water, dried with calcium chloride, and the ether removed. A quantity (about 26 grammes) of a pale yellow oil was thus obtained, which possessed an aromatic, and also somewhat pungent odor. Its density was 0.9362 at 20° C., and the optical rotation + 2° 59′ in a 100 mm. tube. The presence of furfural was indicated by the odor, and by the production of a deep red color when tested with aniline in acetic acid solution.

The essential oil was first extracted with a 10 per cent. solution of sodium carbonate. This removed about 1 gramme of a solid substance which, after recrystallization from alcohol, melted at 53–54° C., and was identified as myristic acid. The oil was subsequently extracted with a 5 per cent. solution of sodium hydroxide. On acidifying the alkaline liquid, and extracting with ether, a small quantity (about 0.5 gramme) of an oil was obtained which possessed a strong odor of eugenol, and yielded a crystalline benzoyl derivative melting somewhat indefinitely between 84 and 98° C. This phenolic product evidently consisted of a mixture of eugenol and *iso*eugenol, these substances having previously been identified by us as constituents of the essential oil of nutmeg (*loc. cit.*).

After the above treatment the oil was distilled under the ordinary pressure. It commenced to pass over at 190° C., the temperature gradually rising to 265° C. The amount of this essential oil was much too small for a complete examination, and it would naturally be expected to contain the same substances as had previously been

identified in the normal product obtained by the direct distillation of nutmegs. The last portions of the distillate were, however, specially tested for myristicin, the presence of which was established by the formation of the crystalline bromo-derivative, melting at 128–129° C.

The aqueous distillate, from which the essential oil had been removed by extraction with ether, as above described, had an acid reaction. It was therefore neutralized with baryta, and the solution concentrated, when three successive crops of crystals were obtained, amounting in all to 4 grammes. Each of these barium salts, after drying at 110° C., was analyzed, with the following results:

(*a*) 0.3787 of salt gave 0.3388 $BaSO_4$. Ba = 52.6
(*b*) 1.2626 " " 1.1381 $BaSO_4$. Ba = 53.0
(*c*) 1.0017 " " 0.9040 $BaSO_4$. Ba = 53.1
$(C_2H_3O_2)_2$ Ba requires Ba = 53.7 per cent.

It is thus evident that the volatile acid consisted chiefly of acetic acid.

Non-volatile Constituents of the Alcohol Extract.

After the removal of the volatile substances by distillation with steam, as above described, there remained in the distillation flask a reddish-brown, aqueous liquid (*a*) and a large quantity of a very dark colored resin (*β*). The latter was separated and thoroughly washed with water, the washings being added to the aqueous liquid.

Examination of the Aqueous Liquid (*a*).

The aqueous liquid, together with the washings from the resin, was concentrated to a convenient bulk. It was first tested for the presence of an alkaloid, but, as in the previously mentioned preliminary test with powdered nutmeg, the result was negative. The liquid was subsequently extracted several times with ether, the combined ethereal liquids being washed, dried, and the ether removed, when about 20 grammes of a semi-solid, dark colored, resinous substance was obtained. This was redissolved in ether, and the ethereal liquid extracted successively with solutions of sodium carbonate and sodium hydroxide, but this treatment removed only

substances of a resinous character. The ethereal liquid was finally washed until free from alkali, and the ether removed, when about 0.5 gramme of a solid substance was obtained. The latter, after recrystallization from alcohol, melted at 54° C., and was identified as trimyristin.

The aqueous liquid, after extraction with ether, was treated with a solution of basic lead acetate, which yielded a voluminous brown precipitate. The latter was collected, washed, suspended in water, and decomposed by hydrogen sulphide. On filtering the mixture a reddish-brown liquid was obtained, which, when concentrated under diminished pressure, yielded only a resinous product. It gave a deep green color with ferric chloride, and appeared to consist chiefly of tannic and coloring matters.

The filtrate from the basic lead acetate precipitate was deprived of the excess of lead by means of hydrogen sulphide, again filtered, and the liquid concentrated under diminished pressure. A large quantity (about 1000 grammes) of a thick syrup was thus obtained, but after standing for a long time it deposited nothing crystalline. It was optically inactive, contained an abundance of sugar, and readily yielded an osazone which, after a few crystallizations from pyridine, melted at 212–213° C., and was evidently *d*-phenylglucosazone. A portion of the syrupy liquid was dried on prepared sawdust, and the mixture successively extracted in a Soxhlet apparatus with ether, ethyl acetate, and alcohol. The ether removed nothing, and the other solvents yielded only syrupy extracts from which nothing crystalline could be obtained. Another portion of the original syrupy liquid was heated for some time with dilute sulphuric acid, when a little furfural was produced, but there was no evidence of the presence of a glucoside.

Examination of the Resin (β).

The resinous matter which had been separated from the aqueous liquid, as previously described, formed, when dry, a black, brittle solid, and amounted to 490 grammes. It was dissolved in alcohol, and intimately mixed with purified sawdust. The mixture was then thoroughly dried, and extracted successively in a Soxhlet apparatus with light petroleum (b. p. 40–60° C.), ether, chloroform, ethyl acetate, and alcohol, when the following amounts of extract, dried at 100° C. were obtained:

Petroleum	extracted	47	grammes or	9.6	per cent.
Ether	"	66	" "	13.5	"
Chloroform	"	33	" "	6.7	"
Ethyl Acetate	"	55	" "	11.2	"
Alcohol	"	170	" "	34.7	"
		371	grammes or	75.7	per cent.

It is evident that by this treatment a considerable proportion of the original resin had been rendered insoluble.

Petroleum Extract of the Resin.

This was a soft, dark brown mass. It was dissolved in ether and the ethereal solution extracted, first with small successive portions of aqueous sodium carbonate, and afterwards with a solution of sodium hydroxide. The sodium carbonate extracts were of a dark brown color, and, when acidified, yielded soft, resinous solids. The latter were distilled under diminished pressure, when a small fraction was collected between 210 and 230° C./20 mm., which became crystalline on cooling. After recrystallization from alcohol, it melted at 52–53° C., and proved to be myristic acid. The sodium hydrate extract, when acidified, yielded a light yellow solid, which was readily soluble in hot, but not in cold alcohol, and was deposited from its hot solution in an amorphous state.

The portion of the petroleum extract which was not soluble in alkalies amounted to about 30 grammes. It was hydrolized by heating on a water-bath with an alcoholic solution of 12 grammes of potassium hydroxide. After the removal of the alcohol, water was added, and the alkaline mixture extracted with ether, the ethereal solution being washed, dried, and the ether removed. A quantity (about 5 grammes) of unsaponifiable material was thus obtained, which was distilled under diminished pressure, and the following fractions collected: 160–175°; 175–280°; 280–310° C./15 mm. Only the highest fraction, 280–310° C./15 mm., was sufficient in amount for further examination. This was a yellow, viscid product which, on digesting with dilute alcohol, yielded a very small amount of solid substance. The latter, after crystallization from a mixture of alcohol and ethyl acetate, melted at 135°, and yielded the color reactions characteristic of the phytosterols.

The above-mentioned aqueous, alkaline liquid, after extraction with ether, was acidified with sulphuric acid and distilled with steam, but the only volatile product was a little myristic acid. The contents of the distillation flask were then extracted with ether, the ethereal solution being washed, dried, and the ether removed. A quantity of solid acids was thus obtained, which was distilled under diminished pressure to remove some resinous matter. The greater portion passed over at 205° C./20 mm., and consisted of practically pure myristic acid, melting at 53° C. From a smaller fraction, collected between 205 and 250° C./20 mm., a small quantity of cerotic acid, melting at 74–76° C., was isolated. Some unsaturated acids were also present in the mixture.

Ether Extract of the Resin.

This was a soft, reddish-brown solid. It was digested with an amount of ether insufficient to dissolve the whole, and the sparingly soluble portion separately examined. This latter portion was a brownish, brittle mass, which was readily soluble in hot, but only moderately soluble in cold alcohol. It was systematically fractionated from alcohol, but the deposits all appeared to be amorphous. In order to ascertain whether a crystalline acetyl compound could be obtained from this product, it was heated with acetic anhydride and anhydrous sodium acetate for several hours. The mixture was then treated with water, when a solid substance separated, which was collected, washed with water, and dried on a porous plate. On fractionating this substance from hot alcohol, the first few deposits, representing the greater portion of the material, were quite amorphous. The mother-liquors, however, on standing for some time, yielded a small quantity (about 0.2 gramme) of a crystalline substance, which was separated from some amorphous matter by filtration through muslin. The crystalline substance was thus obtained in flat plates, melting at 163–164° C., and, after drying at 105° C., was analyzed.

0.1016 gave 0.2580 CO_2 and 0.0889 H_2O. C = 69.3; H = 9.7.

It was then recrystallized from methyl alcohol, when, after drying at 105° C., it melted at 164–166° C., and was again analyzed.

0.0706 gave 0.1798 CO_2 and 0.0596 H_2O. C = 69.5; H = 9.4

$C_{27}H_{44}O_6$ requires C = 69.8; H = 9.5 per cent.

The substance afforded a color reaction similar to that characteristic of the phytosterols. Thus, when dissolved in chloroform with a little acetic anhydride, and a drop of concentrated sulphuric acid added, a pink color was produced which rapidly changed to blue and finally to green.

The composition and character of the above-described substance render it evident that it is diacetylipuranol, $C_{23}H_{38}O_4$ $(CO.CH_3)_2$. The dihydric alcohol, ipuranol, $C_{23}H_{38}O_2$ $(OH)_2$, was first isolated in these laboratories from the resin of *Ipomœa purpurea,* Roth (*Amer. Journ. Pharm.,* 1908, **80,** p. 264), and subsequently from olive bark (*Journ. Chem. Soc.,* 1908, **93,** p. 907).

The above-mentioned ethereal solution of the more readily soluble portion of the ether resin was extracted, first with small successive portions of a saturated solution of sodium carbonate, and subsequently with a 10 per cent. solution of sodium hydroxide. The first sodium carbonate extract formed a thick, dark brown emulsion of an insoluble sodium compound which could not be filtered. It was, therefore, directly acidified, when a yellow solid was obtained, which was collected and washed with water. The attempts to obtain it in a crystalline form were unsuccessful, and it also yielded nothing crystalline on acetylation. The subsequent sodium carbonate extracts were similar in character and behavior to that above described. The sodium hydrate extracts were dark in color, and, on acidification, yielded brown, amorphous products. After extracting the ethereal solution with the above-mentioned alkalies, it was washed, dried, and the ether removed, but only a small amount of a pale yellow, amorphous product was obtained.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

The portion of resin extracted by chloroform was a reddish-brown solid, while the portions removed by ethyl acetate and by alcohol respectively were soft, black masses. Nothing of a crystalline character could be obtained from any of these products. In order to ascertain whether the alcohol extract of the resin contained anything of a glucosidic nature, a quantity (50 grammes) of it was heated for several hours in alcoholic solution with such an amount of sulphuric acid that the latter represented 5 per cent. of the mixture. After the removal of the greater portion of the alcohol, water was added, and the mixture distilled with steam. A small amount of a volatile oily product was thus obtained, which was found to contain

furfural. The distillation flask then contained a quantity (35 grammes) of a black resin, together with an aqueous liquid of a reddish color. The resinous matter was separated by filtration, and carefully examined, but nothing crystalline could be obtained from it. The filtered aqueous liquid was first extracted with ether, which, however, removed only a little amorphous coloring matter. It was then treated with an amount of baryta just sufficient for the removal of the sulphuric acid, and the filtered liquid concentrated under diminished pressure. A dark colored product was thus obtained which reduced Fehling's solution, but no osazone could be prepared from it.

In considering the results of this investigation, it may be noted that the only constituents of the petroleum and alcohol extracts from the "press-cake" of nutmeg which had not previously been identified in either the essential oil or the expressed oil were the following: sugar, tannic acid and coloring matters, resins, and a very small amount of the crystalline alcohol, ipuranol, $C_{23}H_{38}O_2(OH)_2$.

Physiological Tests.

In order to obtain confirmation of the statements which have previously been recorded that the narcotic effects produced by nutmeg are due to the essential oil or the myristicin contained therein, and also to ascertain whether any of the other products obtained in the course of this investigation possessed physiological activity, a considerable number of tests were conducted for us by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories. Many of these tests were performed prior to the publication of the observations by Professor Cushny on the subject of nutmeg poisoning, to which reference has been made in the introductory portion of this paper.

It was found by Dr. Dale that nutmeg itself, when administered to a cat, in doses of 5 grammes, has a very marked effect. Thus a cat weighing 2640 grammes was given 5 grammes of nutmeg at 2.30 P.M. A small amount of this was vomited during the night, but the cat seemed practically well on the following day. On the second day after administration, however, the animal was found to be very sluggish. It could walk when roused, but very quickly dropped into a semi-comatose condition, and at 3 P.M. on this day it died. Apart from a slight congestion of the intestinal mucous membrane, the only post-mortem abnormality was a fatty degenera-

tion of the liver. In another case, in which 10 grammes of nutmeg were given, no effect except slight malaise and some salivation could be observed until the third day after administration, when the cat was found in a state of very deep coma, and shortly afterward died. Another cat, to which 5 grammes of nutmeg were given, died on the morning of the fourth day after administration. The liver again showed marked fatty degeneration, and the urine contained much bile and a little albumin. The kidneys were not noticeably abnormal.

In connection with the above results it may be noted that the dog appears to be comparatively insensitive to the toxic action of nutmeg, since doses amounting to as much as 20 grammes of the substance, and even 10 c.c. of myristicin, have been given by the mouth to this animal without any perceptible effect. Injections of the essential oil and of myristicin intravenously did, indeed, cause acute symptoms of incoördination and, in some instances, complete unconsciousness; but the value of such observations is seriously diminished by the consideration that the insoluble oil will produce multiple emboli, certainly in the lungs, and possibly also in the cerebral capillaries, insofar as it passes into the lungs and gets into the general circulation. Pulmonary hemorrhage was actually the cause of death in these cases.

With regard to the action of myristicin, $C_{11}H_{12}O_3$, the high-boiling constituent of the essential oil of nutmeg, to which, in accordance with the observations of Wallace, the narcotic effects produced by nutmeg are attributed by Cushny, as also independently by Jürss (*loc. cit.*), the following experiments may be noted.

Quantities of myristicin which were appreciably greater than the amount of this substance contained in a toxic dose of nutmeg, for example, 0.1 to 0.2 c.c., when given by the mouth to a cat, produced no apparent effect. A dose of 1 c.c. of myristicin, however, produced results which were not dissimilar to those produced by 5 to 10 grammes of nutmeg. Thus a cat to which 1 c.c. of myristicin was given by the mouth survived without marked symptoms until the third day after administration, when it was found lying in a semi-conscious condition. The fatty degeneration of the liver, and staining of the urine and all the tissues with bile pigment, were the only noticeable abnormalities post mortem. Another cat, to which an equal dose was given, survived until the seventh day after administration, but the changes observed post mortem were similar in character to those above described.

These results, whether produced by nutmeg itself, or by myristicin in doses up to 1 c.c. of the latter, clearly differ from the recorded effects of nutmeg on man. By the administration of rather larger doses of myristicin to the cat, some light was thrown on this discrepancy. Thus 1.5 c.c. of myristicin, given by the mouth to a cat of 3 kilogrammes, produced after a few hours a condition not unlike that described by Wallace, as reported by Cushny. The animal showed considerable excitement, together with some incoördination, and avoided obstacles imperfectly. The pupils were dilated. No actual stupor or narcosis, however, was observed, but the excitement was succeeded on the following day by a condition of unusual quietness. The second day after administration the cat became deeply jaundiced, comatose, and died. A post-mortem examination showed very advanced fatty degeneration of the liver. Another cat, to which 2 c.c. of myristicin were given, showed marked excitement and incoördination about half an hour after administration. It then became unconscious and lay narcotized for about three hours, but subsequently recovered consciousness, and the primary effects gradually disappeared. In this case again, after an interval of a day without symptoms, jaundice and coma appeared, and on the third day after administration the cat died. The primary effects—excitement, incoördination and narcosis—are not markedly different from the effects reported to be produced by nutmeg in man. Apart from the question of dosage, the difference, in any case, is not greater than that observed in other drugs affecting principally the brain. On the other hand, the remote effects of myristicin, including the terminal coma, may with considerable probability be regarded as secondary to the degenerative changes in the liver. In man the dose necessary to produce narcosis is too small to lead to these remote bad results, while in the much less sensitive cat a dose which is large enough to cause the primary cerebral symptoms causes also extensive liver changes, and is therefore ultimately fatal.

The main discrepancy between the results produced by nutmeg on the one hand and those produced by myristicin on the other is that due to dosage. It would be quite reasonable to attribute all the effects of nutmeg on the cat to myristicin, but for the fact that the dose of nutmeg sufficient to cause death in a few days represents a quantity of myristicin which, given by the mouth, produces no appreciable effect. It seems possible, however, that the discrepancy may be explained by a consideration of the conditions of absorption. Thus the failure to obtain an effect with small doses of myristicin

may be due to its being only imperfectly absorbed when given in a pure state, and passing out to a large extent in the fæces. A small dose of myristicin might, therefore, be expected to be effective if injected hypodermically, for although the absorption of such a substance from the subcutaneous tissue would be very slow, none at least would leave the body without passing through the circulation. It was found, in fact, that a dose of 2 minims (about 0.12 c.c.) of myristicin, when injected hypodermically into a cat, produced a very slow, but ultimately extensive degeneration of the liver, the latter effect being manifested during life by wasting and jaundice. This slow degeneration is what might be expected when a substance so sparingly soluble as myristicin has to be absorbed from the connective-tissue spaces.

The other products from nutmeg which were subjected to physiological tests comprised the following:

1. A *viscid substance,* boiling at 270–280° C. under 15 mm. pressure, and agreeing in composition with the formula $C_{18}H_{22}O_5$, which was separated from the unsaponifiable constituents of the expressed oil of nutmeg (*loc. cit.*).

2. The *resins* obtained from the "press-cake."

3. The *aqueous liquid* obtained, as described in this paper, from the alcoholic extract of the "press-cake," after the separation of the resins.

The viscid substance (1) was given to a cat in doses of 0.5 and 1.0 gramme respectively, but no physiological effect could be observed. The resins (2) and the aqueous liquid (3) likewise produced no noticeable effects when administered in amounts corresponding to many times the toxic dose of nutmeg. All these products must therefore be regarded as physiologically inactive.

With consideration of the results above described there would appear to be no doubt that the narcotic property of nutmeg is correctly attributed to myristicin, $C_{11}H_{12}O_3$, and it may be assumed that the latter substance when associated with the other constituents of the nutmeg is in a condition much more favorable for absorption than when in a pure state. As in the case of many other narcotics, the lower animals are much less sensitive than man to the direct action of nutmeg on the cerebral functions.

In conclusion, we desire to express our best thanks to Dr. H. H. Dale for having conducted the large number of physiological experiments involved in this investigation.