supply and demand of these components in the cell metabolism, and more probably serve as an indication of a pattern of feed-back in the cell. Such a role would be in harmony with the suggestions of Stent and Brenner[12], and of Kurland and Maaløe[13], that the transfer-RNA molecules, or the activating enzymes themselves not saturated with their amino-acids, could serve as repressors of RNA synthesis. The changing spectra of other cell metabolites, operating in like fashion, could also have important significance in the overall regulation and control of metabolism in the cell.

The metabolic pathways leading to the precursors used for macromolecular synthesis in the cell are closely integrated at the lowest levels and largely comprise what Davis[14] has called the amphibolic area of cell metabolism. Our knowledge of the interplay and overall patterns of feed-back mechanisms is still rudimentary, but evidence is available of the possibility for integrated controls of different pathways, for example of nucleotides by amino-acids and vice versa[15–18]. The description by Magasanik[18] of the elegant control of the histidine and guanylic acid biosynthetic pathways by purine and amino-acid components, respectively, is an outstanding example of how feed-back mechanisms might be integrated.

The possibilities offered by an integrated network based on twenty channels of amino-acid feed-back and five or six channels of nucleotide feed-back lead to a system that would suffice to co-ordinate a wide range of metabolic activity, and serve to link the amphibolic metabolism with the biosynthesis of the vital macromolecules, proteins and nucleic acids. In such a system, feed-back mechanisms could operate back from a master template, to order and modulate the supplies of precursors required for the ultimate duplication of the template. The arrangement, linked to cell intermediates and connected to other areas of cell metabolism, could satisfactorily account for the phenotypic variations of the genotype observed in cellular metabolism and growth under different conditions.

In recent articles in *Nature*, Dean and Hinshelwood[20–22] have discussed the possible integrations and implications of cell reactions, cell organization and cell regulation. The work described in this article, in demonstrating a characteristic and organized behaviour of intracellular pools, lends support to their general thesis and emphasizes also the lack of information available from contemporary investigations of cell metabolism in relation to the neglected but important question of the overall supply of building units for the architecture of the cell.

The investigation of metabolic pools is an empirical exercise; their nature, origin, behaviour and complexity are unknown. It would appear that a closer investigation of metabolic pools, and of other aspects of metabolism *in vivo*, might now be attempted with advantage, possibly by using some of the methods described here, in a systematic manner.

I thank Drs. C. G. Youngs and B. M. Craig, of this laboratory, for the determinations of amino-acids and fatty acids by gas chromatography, and E. G. Darichuk for technical assistance.

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POSSIBLE IMPLICATION OF MYRISTICIN AS A PSYCHOTROPIC SUBSTANCE

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WITHIN the past decade there has been a rapid expansion of knowledge of psychotropic medicine. The development of the ataraxics, and more recently of the 'psychic energizers', has depended on chemical syntheses in areas almost totally unknown in Nature.

A third field of investigation concerns those psychotropic materials known as the hallucinogenic drugs. Substances that could 'to an extent duplicate the syndrome of insanity' or that might 'permit one's capacities to transcend the limits of the sensorium' have long been in existence, but serious study of them, under the respective classifications of psychotomimetics and psychedelics, has only very recently been started. Most of these latter chemicals, in contrast to the tranquillizers and antidepressants above, come at least originally from natural sources. The pursuit and exploitation of the folk-lore that describes the many intoxicants of Nature has resulted in a disproportionately small number of new structural families. In many instances it has become apparent that the active material in an intoxicating plant is the same as, or is closely related to, a previously known system. Thus a small handful of chemical individuals, disguised by minor chemical distortions, account for the pharmacological effectiveness of a large variety of plants.

Investigations of the Amazon intoxicant ayahuasca (cappi, yaje) have led to a study of the genus *Banisteriopsis*. The analysis of these vines has revealed a series of alkaloids related to harmine which, with its hydrogenation products harmaline and tetrahydroharmine, is claimed to be the central stimulant present in *Prestonia amazonicum*. Harmine has also been isolated from the seeds and roots of the biologically active *Peganum harmala*, of both a different family and a different continent (*Zygophyllaceae*, rather than *Malpighiaceae;* Asia, rather than South America). Two additional intoxicating plants, of yet different genera (*Tetrapterys methystica* and *Mascagnia psilophylla*), probably embody this same alkaloid group.

The extracts of *P. amazonicum*, mentioned above, have also been found to contain *N*,*N*-dimethyl-5-hydroxytryptamine (bufotenine). This presumed psychotomimetic also occurs in *Lespedeza bicolor* and is one of the toxic principals in the snuffs made from the seeds of *Piptadenia peregrina*, *P. colubrina* and *P. macrocarpa*. This latter genus serves as yet another connecting link, for from its various species has been isolated *N*,*N*-dimethyltryptamine. The implication of this indole as the responsible agent in a narcotic drink concocted from

the closely related plant, *Mimosa hostilis*, must be questioned, as the purified alkaloid is effective only on parenteral administration.

Mescaline is usually associated exclusively with the peyotl button from *Anhalonium lewinii* from which it was originally isolated, but more recent investigations into other cacti, *Gymnocalycium gibbosum* and *Opuntia cylindrica*, have revealed that here, too, the same phenethylamine is a major component.

The search for the sacred Mexican intoxicating mushrooms known as teonanacatl successfully led to the investigation of several members of the genus, *Psilocybe*. One of the most potent examples, *P. mexicana*, has afforded a new hallucinogenic chemical, psilocybin. This compound, the phosphate of a positional isomer (*N*,*N*-dimethyl-4-hydroxytryptamine) of bufotenine (v. s.), has now been shown to be responsible for the narcotic effects of a large number of active mushrooms of the same genus. It has been shown to be present in *Stropharia cubensis* and is suspected to occur in the genus *Panaeolus*.

d-Lysergic acid diethylamide, LSD, is perhaps the most widely studied of all the hallucinogens and was first prepared by synthetic modification of alkaloids derived from the ergot fungi. The lysergic acid structure was long thought to be unique to this plant phylum, but recent analysis of the native (Mexican) drugs ololiuqui and tlitliltzen has led to the investigation of various *Convolvulaceae* (specifically the morning glory plants *Rivia corymbosa* and *Ipomoea violacea*), wherein the intoxicating chemicals were also found to be amides of lysergic acid.

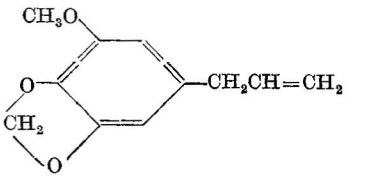
Thus, research into the active components of a score of psychotropic plant sources has revealed perhaps a half-dozen chemicals and their closely related analogues. It is possible that a similar set of circumstances, outlined later, may implicate the simple chemical myristicin (3-methoxy-4,5-methylenedioxyallylbenzene) in a comparable role.

An intoxicating snuff, yakee or paricá, is used by Indians in Colombia, and the origins of this material have been established to be a species of the genus *Virola*, *vis.*, *V. calophylla*, *V. calophylloidea*, and possibly *V. elongata*. These trees are members of the family Myristicaceae, and the essential oil myristicin has been mentioned as a possible suspect in the explanation of the biological activity. Secondly, increasing attention is being drawn, in the medical literature, to the use of nutmeg as a mentally disorganizing material. The seed of *Myristica fragrans*, on ingestion, produces cycles of delirium and giddiness. The components generally believed culpable lie in the non-terpinacious volatile oil fraction, of which the primary component is myristicin. Very recently a new, synthetic, psychotomimetic has been described, 3-methoxy-4,5-methylenedioxyamphetamine (MMDA). This base possesses the precise carbon and oxygen skeleton of myristicin, from which it can be theoretically derived, by the addition of a molecule of ammonia.

Thus, the natural essential oil myristicin may serve a psychotogen role in being a possible component of the virola tree, as a probable active agent in the nutmeg, and as a potential *in vivo* origin of MMDA. The following discussion is intended to present a complete definition of myristicin, both chemically, botanically, and pharmacologically. Further, the known biological effectiveness of it, its cogeners, its botanically related analogues, and the oils in which these have been found, is outlined and is discussed in the light of possible involvement in human intoxication.

Chemical Definition

In early descriptions of natural products, the name 'myristicin' referred to the solids that crystallized from old samples of oil of nutmeg on prolonged standing. These are now known to be myristic acid, $CH_3(CH_2)_{12}COOH$ (ref. 1). Semmler, in his initial investigations of the structures of components of oil of nutmeg[2], used the term to refer to a substance he had isolated after base isomerization and which is now known to be isomyristicin. The name was then applied to the material as found in nutmeg, and is at present used in this sense to represent 3-methoxy-4,5-methylenedioxyallylbenzene, (I).



I

Myristicin has usually been isolated from natural oils by fractional distillation, as it is crystallized only with difficulty even at very low temperatures. The first insight into its structure was achieved by Semmler, who demonstrated that the base-isomerized material from oil of nutmeg could be oxidized to a nine-carbon aromatic acid, the methoxy methylenedioxy ether of gallic acid. Misleading elemental analyses suggested the presence of a four-carbon olefinic chain. A decade later, Thoms[3], working with material isolated from oil of mace, correctly established the side-chain as being an allyl group, in analogy to the then established ether from parsley, apiole. His confirmation of Semmler's further work established that the latter had been working with the conjugated isomer, isomyristicin.

Two routes have been employed in the synthesis of myristicin. The first, reported in 1939 by Trikojus and White[4], used the monoallyl ether of 3-methoxy catechol. Claisen rearrangement yielded 5-allyl-3-methoxy catechol which on reaction with methylene iodide afforded myristicin. A second synthesis of myristicin used eugenol as a starting material[5]. Reaction with hexamine yielded the corresponding salicylaldehyde which was oxidized to the catechol described above. The over-all yield of myristicin was 8 per cent.

Myristicin reacts with bromine to form a tetrabromo derivative (m.p. 130° C) which is frequently used for characterization; the oxidation with permanganate may yield either myristicin aldehyde (m.p. 130°) or myristinic acid (m.p. 208°–210° C). Interaction with methyl magnesium iodide leads to the opening of the methylenedioxy ring with formation of 3-methoxy-4-ethoxy-5-hydroxy allylbenzene[6]. This substance, myristinol*, retains the allyl group intact as is demonstrated by its normal conversion to the conjugated isomyristinol.

Treatment of myristicin with either potassium hydroxide in alcohol or metallic sodium converts the allylic side-chain into the conjugated propenyl group. This latter compound, isomyristicin (m.p. 44° C), is convertible into both a dibromo (m.p. 109° C) and a tetrabromo product (m.p. 156° C) (ref. 3). The oxidation of isomyristicin with ozone yields myristicinaldehyde; oxidation with permanganate leads to the corresponding acid as well. Reaction with nitrous acid leads to a pseudo-nitrosite, which is further convertible through a β-nitro styrene to the corresponding phenyl acetone[7].

Myristicin is a clear, mobile, colourless oil that does not normally freeze even at very low temperatures, although a recent report[8] describes its purification by repeated crystallization at −30° C. It is best isolated from oil of parsley by vacuum distillation (b.p. 173°/40 mm, 126°/4 mm) and shows an $n_D^{20°}$ 1·5407 and a $d^{20°}$ 1·442. It has a faintly aromatic odour often reminiscent of the botanical origin. This is due, without doubt, to the inefficiencies of

* Myristinol (occasionally mylistinol) is not to be confused with myristicol, the name given to a fraction, b.p. 212°–218°, from oil of nutmeg. This alcohol (Wright, *J. Chem. Soc.*, **26**, 549; 1873) has been shown to be a mixture of 1-terpinen-4-ol, borneol, and α-terpineol.

vacuum distillation. Although generally believed to be stable on storage, gradual changes in a synthetic sample have been determined by a bio-assay technique[9]. The physical properties of myristicin as isolated from oil of nutmeg must allow for the presence of elemicin as a concomitant contaminant[10].

Botanical Definition

The most important plant family containing myristicin is the Umbelliferae (syn. Ammiaceae or Apiaceae), which embraces many of the rooty vegetables and common herbs. Of the some 250 genera known in this family, about 10 per cent have appeared in the literature with some form of systematic analysis of components. Myristicin has been shown to be present in the species of each of six of these listed directly below; closely related polyalkoxy aromatics have been observed in at least three additional genera. Several of the parsley plants (*Petroselinum hortense*, *P. sativum*) yield essential oils rich in myristicin, the latter possessing the distinct advantage of yielding a distillation cut, unlike that from oil of nutmeg, that is free of elemicin. The oil of lovage that is derived from the Scotch lovage (or sea parsley) *Levisticum scoticum* contains myristicin as a major component. The rhizomes have been reported to yield well over 1 per cent of their total weight as myristicin[11]. The more common lovage, *L. officinale*, contains eugenol, but no aromatic nucleus more highly substituted. Myristicin has not been reported as a component of most of the more common fennel plants, that is, *Foeniculum vulgare* (in its several varieties, known as bitter, sweet, and Roman fennel), *Crithmum maritimum* (sea fennel), and *Phellandrium aquaticum* (water fennel). The genus *Ridolfia* has been found to be an excellent source, however, in the plant known as harvest fennel (*R. segetum*). After the removal of acidic and carbonyl-containing components, the essential oil consists of 33 per cent myristicin. The commercial dill plant *Anethum graveolens* yields essential oils derived either from the above-ground portions (known as dill-weed or dill-herb oil) or from the seeds. The dill-weed oil contains sizable amounts of myristicin as well as several related aromatics listed below. The edible variety of parsnip (*Pastinaca sativa*) has recently been shown[12] to possess properties of an insecticide and of an insecticide synergist. Analysis of a non-polar solvent extraction revealed that myristicin was the active component, although it was present in the whole parsnip to the extent of only 0·02 per cent. Many species of the genus *Oenanthe* (relatives of the dropwart) have undergone analysis as to their composition, namely, *Oe. crocata*, *Oe. stolonifera*, *Oe. californica* or *sarmentosa*, *Oe. aquatica* and *Oe. phellandrium*. The first of these species has been the most thoroughly studied because of its extreme toxicity to both animals and man (see discussion in the portion on toxicology and pharmacology), although *Oe. crocata* has never been reported to contain myristicin, but *Oe. stolonifera* has[13]. The compound is located in the fruit, and represents perhaps 1 per cent of the total weight.

In the family Labiatae (syn. Lamiaceae or Menthaceae), the genus *Orthodon* alone has been observed to contain myristicin. *O. grosseseratum* Kudo contains about 0·2 per cent volatile oils, which contain a small amount of myristicin. *O. asaroniferum* Fujita, so named because of an appreciable amount of asarone in its 0·3 per cent volatile oil fraction, has been equated to *O. asaniferum* by a virtually identical oil composition, which includes a small amount of myristicin[14].

Analysis of the 'Nepal camphor wood' of India, *Cinnamomum glanduliferum* (fam. Lauraceae), has revealed three components, safrole, elemicin and myristicin[15]. Identification of the latter was made by comparison of the tetrabromide with a reference specimen.

The only plant classified under the family Myristicaceae that has been documented as containing myristicin is the nutmeg tree. This source (*Myristica fragrans* as well as many additional species) yields through its seed (the nutmeg) or the sheath of the seed (mace) a volatile oil fraction that contains about 4 per cent myristicin. If the source of the drug yakee (paricá) can be assigned to trees of the genus *Virola* (as mentioned in the introduction) and the presence of myristicin demonstrated, then the aforementioned species will constitute a second occurrence in the family Myristicaceae.

Within these botanical families there are frequent occurrences of many compounds that may be considered as close analogues of myristicin. The term analogue here defines aromatics containing at least three oxygen functions and a three-carbon olefinic chain. The propenyl analogue of myristicin itself, isomyristicin, is known to accompany myristicin in *A. graveolens*. It is most unlikely that it could be generated from myristicin in the course of isolation of the essential oil fraction, as other oils of the Umbelliferae, isolated in similar ways and containing allyl compounds equally easily isomerized (see following), are not found to contain the propenyl isomer.

Elemicin, 3,4,5-trimethoxyallylbenzene, is the analogue of myristicin, in which the methylenedioxy group has been replaced with two methoxy groups. It has been mentioned earlier as a congeneric contaminant of myristicin in the oil of nutmeg. Further, it is one of the principal components of yet another of the orthodon oils, *O. elemiciniferum*. It has yet to be demonstrated in any member of the family Umbelliferae. The positional conjugated isomer of elemicin, asarone, is 2,4,5-trimethoxypropenylbenzene. It occurs in the orthodon group, as mentioned here, as the definitive component of *O. asaroniferum*. In the family Umbelliferae, it is found in the oil of carrot (*ex Daucus carota*). The strict isomer of myristicin and elemicin, namely, 2,4,5-trimethoxyallylbenzene, is unknown in Nature.

Among the more highly substituted analogues of myristicin, both 2,3,4,5-tetramethoxyallylbenzene and apiole* (2,5-dimethoxy-3,4-methylenedioxyallylbenzene) are observed as components of the oil of parsley, *P. sativum*[3]. The positional isomer of apiole, 2,3-dimethoxy-4,5-methylenedioxyallylbenzene (dillapiole), accompanies myristicin in *Anethum graveolens*. It also occurs in *A. sowa* (dill-seed oil) as well in the sea-fennel *Crithmum maritimum*. In the family Labiatae, *Orthodon formosanum* contains only 0·1 per cent oil, but this is 65 per cent dillapiole.

Pharmacological Definitions

The pharmacology of these materials may be divided into three groupings: that of botanicals known to contain myristicin, that of isolated myristicin concentrates, and that of chemicals closely allied (in both molecular structure and in natural origin) to myristicin.

Plants of the genus *Oenanthe* have long been known to be highly toxic both to animals and to man. Oenanthotoxin has been isolated as the toxic principal of *Oe. crocata* and has been established as a multiply unsaturated diol[16]. The toxic symptoms are characterized as initial nervous and intestinal disturbance followed by emesis, mydriasis, cyanosis and convulsions. In humans, a lethal dose is effective within 2 h of ingestion. The purified material, oenanthotoxin, shows a pharmacological spectrum parallel to that of a tincture of the natural tuber but cannot be the sole toxic principle, as the extract devoid of any oenanthotoxin still produces toxic symptoms in man[17]. A non-toxic ketone, crocatone(II), has been isolated which bears a close resemblance to myristicin. Another species of water parsley, *Oe. sarmentosa*, is reported to be non-toxic[18], but no attempt has been made to establish the presence of myristicin. Thus, one must conclude that any responses to myristicin as in these plants would be obscured by the presence of oenanthotoxin.

* The term apiole, representing the compound 2,5-dimethoxy-3,4-methylenedioxyallylbenzene, is not to be confused with the same word (often without the final 'e') used to describe the crude green oil of parsley.

The parsnip, *P. sativa*, has only recently been shown to contain myristicin[12]. It has long been known that, in sensitized individuals, contact with the juices leads to actinic dermatitis. Various furocomarins have been isolated and two of them display biological activity; these are pastinacin, which shows spasmolytic and vasodilating properties, and xanthotoxin, which has been used in the treatment of vitiligo (non-congenital leukodermia)[19]. It appears that, here again, any responses of the myristicin present would be masked by active, but unrelated, congenors.

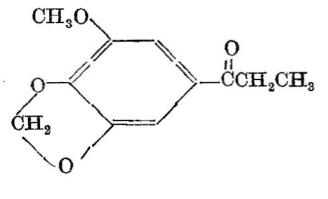
The pharmacology of *A. graveolens* (dill) is virtually unknown. The oil has been recommended as a remedy for hiccoughs and emesis, and as an aqueous solution, as a carminative. Toxicity due to excess is unreported.

The most frequently investigated form of parsley is, rather than the distilled oil, the oleoresin which is obtained by extraction. This fraction is called 'apiol', 'liquid apiol', or green oil of parsley. This natural extract received a clouded reputation in the 1930's when a popular claim of effectiveness as an abortifacient coincided with a commercial product which contained some 30 per cent *o*-cresyl phosphate as an impurity. The polyneurites, paralyses and deaths associated with its misuse during this period have been blamed on this contaminant. The uncontaminated extract produces a fall in blood pressure, tremors, and weakness of extremities similar to nutmeg poisoning. This is discussed later.

Although various fennels have long been established in the medical world, the only one that contains myristicin, *R. segetum*, has a totally unknown pharmacology. Similarly, studies on the genus *Orthodon* have been conducted solely as a guide to taxonomic arrangement. *L. scoticum*, too, is unexplored although the related lovage, *L. officianele*, apparently dates back for centuries in medical history.

The seed of the myristica has long been enveloped in a folklore of emmanogogic and ecbolic potency, and consequently there is a large body of literature available concerning its misuse. As the animal and human pharmacology have recently been reviewed[21], only a generalized syndrome of human intoxication will be described. The ingestion of about 5 g of the whole seed (approximately one large nutmeg) leads after a period of a few hours to a more or less severe physical collapse, characterized by a weak pulse, hypothermia, clamminess of the extremities, giddiness, vertigo, nausea and a feeling of congestion and pressure either in the chest or abdomen. For some 12 h there is an alternation of delirium and stupor, and this is usually resolved by a heavy sleep. For a period of several days there may be headaches and perhaps spells of dizziness. Cases resulting from the ingestion of powdered nutmeg seem to parallel this general syndrome.

It is of interest to note that two of the components of *A. lewinii*, the source of mescaline, possess the same aromatic substitution pattern as that of myristicin. The isoquinolines lophophorine and anhalonine (III; R = CH_3, R = H, respectively) are natural components of the native cactus. Their pharmacologies are virtually unknown.



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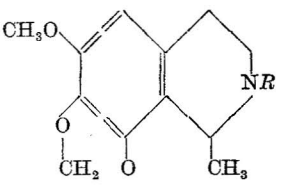


III

The only myristicin concentrates that have received any pharmacological evaluation have been obtained from nutmeg. Animal responses have been summarized[21] and reflect, apart from liver disruption, a modest hypotensive response. The first of the two reports on effectiveness in humans describes a total dose of 400 mg 2 or 3 h following ingestion there were suggestions of elation or of anxiety in several of the experimental subjects[22]. The second report involves a preliminary employment in diagnosed mental illness[23] but does not elaborate on the psychotropic effectiveness.

Apiole is the most carefully investigated of any of the related polyalkoxylated aromatic propenes. The syndrome of human poisoning includes, besides a transient intoxication, headaches, indigestion, loss of appetite and fever. The isomeric isoapiole, as a pure chemical, produces similar effects in human subjects. There is heart excitement and an expanded pulse becoming dicrotic on higher dosages (0·6–0·8 g). Again, the intoxication is short-lived although other of the toxic phenomena may persist for several days[24].

Of the several tri- or higher alkoxylated alkyl- or propenyl benzenes mentioned, only isomyristicin, tetramethoxyallylbenzene, and recently *cis*- and *trans*-asarone have received any pharmacological investigation. None of these has been reported to have been explored in human subjects, although recent studies in primates have shown *trans*-asarone to possess tranquillizing effects similar to chlorpromazine. Dosages of 5 mg/kg produced a catatonic indifference lasting several days[25].

Theoretical Modes of Action

The possibility must be considered that myristicin *per se* is not the form in which the substance is found in Nature. Thus, the physical isolation of the oil from nutmeg, parsley, etc., may employ techniques sufficiently strenuous to liberate myristicin from a precursor 'protomyristicin'. This natural form may then be either the actual toxicant in the instance of human ingestion, or may generate the active product *in situ*, perhaps at a site or in a concentration not otherwise attainable. These speculations are in accord with the inability to imitate nutmeg intoxication with synthetic myristicin. On the other hand, the isolated apiole from parsley has an effectiveness not enjoyed by the whole plant. This explanation can only be supported by an increasingly careful analysis of the nature source of these materials.

An appealing explanation of the action of myristicin involves the potential addition of a molecule of ammonia to the allyl chain. Such a reaction would readily explain the position of the synthetic drug MMDA, IV (ref. 26). Amination of the analogous ether elemicin has been shown to yield 3,4,5-trimethoxy amphetamine(TMA) *in vitro* by way of a bromo intermediate[27]. Further, a similar addition with an available substituted amine would lead easily to a corresponding N-substituted amphetamine.

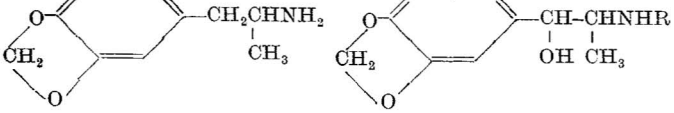
The facile isomerization of an aromatic allyl group to the conjugated propenyl counterpart is assumed to occur in the intact organism[28]. Thus the production of an aromatic acid as a metabolic product of allyl compounds presumes such a preliminary isomerization (that is, safrol → piperonylic acid). The propenyl analogue, isomyristicin, would again be capable of accepting either ammonia or amines as is true with the allyl isomer here, leading to the *in situ* generation of substituted amphetamines. Many other types of reactions at such a double bond are possible, such as oxidation or the addition of non-nitrogenous nucleophiles (alcohols→ethers, mercaptans→thioethers).

Hydrogenation of the double bond in either the allyl or propenyl isomer is improbable. Such a mechanism is not known in human detoxication, and in those instances wherein an olefinic material may be compared directly with the saturated counterpart, the latter is less potent and more slowly acting (for example, anaesthetic ether, barbiturates).

Oxidation could occur either on the ring of myristicin, or on the side-chain with or without chain cleavage. Ring oxidation, a well-recognized biological mechanism, would lead to a demethylated analogue either of apiole or

of dill-apiole. In the light of the known human effectiveness of apiole, this route has appeal, although its mode of action in turn must still be explained in terms of the present discussion of myristicin. Chain oxidation would lead to either 3-methoxy-4,5-methylenedioxyphenyl acetic acid or the corresponding benzoic acid. Both types of products have frequently been observed in the metabolism of three-carbon chains, but in no instance has either type represented anything other than a biological end-product. Hydroxylation of the chain would lead, in the instance of the propenyl isomer, to the enol form of the corresponding propiophenone or the phenylacetone. The former has been mentioned here (crocatone) and is a non-toxic component found in *Oenanthe*. Recently, however, the acetophenone analogue of crocatone was synthesized and found to be of comparatively high toxicity to mice[29]. Using the transaminase enzyme system, the phenyl acetone isomer could easily be converted again to the MMDA. This process has been mentioned in connexion with elemicin[21].

Recently, propenyl aromatics have been found to react readily, after epoxidation, with aliphatic amines, to form various isomers of ephedrine[30]. A parallel reaction with isomyristicin would lead to a mixture of isomers of the corresponding ethanolamines (V):



IV V

Such compounds are not known with the methylenedioxy substitution although analogous trimethoxy compounds have been synthesized[31].

A metabolic pathway which must be considered as a potential explanation of the function of myristicin in the body involves hydrolytic de-alkylation of the ring. Mention has been made here of the easy disruption of the methylenedioxy heterocycle, and a similar reaction *in vivo* employing existing hydrolytic enzymes may explain the increased potency of a methylenedioxy group over the corresponding dimethoxy analogue.

Summary

The purpose of this review has been to delineate the limits of the present knowledge concerning the aromatic ether, myristicin. An appealing suggestion exists that, as a potential factor in two natural intoxicants and as a chemical precursor to a synthetic one, it may be implicated as the responsible agent. Preliminary human pharmacology suggests that it may indeed possess psychotropic properties, but this is yet to be expanded on, and is so far quantitatively inadequate in the explanation of the effectiveness of nutmeg or of paricá.

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SYNTHESIS OF VIRUS AND MACROMOLECULES BY RUBELLA-INFECTED CELLS

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RUBELLA virus grows *in vitro* in a wide variety of mammalian cell types. A recent report[1] describes the growth of this virus in clones of a baby hamster kidney cell line (*BHK* 21) with the production of virus titres which permit an investigation of one-step growth and of virus host-cell interaction.

Rubella virus was concentrated from *BHK* 21 or RK_{13} cell supernatants[1,2] by dialysis against 'Carbowax 20-*M*' until an approximately ten-fold concentration was obtained. The virus was then dialysed overnight against phosphate buffered saline and centrifuged for 4 h at 4° C and 25,000 r.p.m. in an '*SW*39' Spinco rotor, on a cushion of 0·7 M sucrose in phosphate buffered saline. In this way, most of the serum was removed. The sucrose as well as 1 ml. of the adjacent supernatant solution were taken out by a syringe and dialysed against phosphate buffered saline. By these concentration procedures, virus titres were increased fifty or one hundredfold, up to between 10^7 and 10^9 TCD_{50}/ml.

The cultures were infected with this virus-concentrated pool at a multiplicity of 5 or 10 TCD_{50} per cell. Fig. 1 shows the growth curves of cell-associated and extracellular rubella virus in two cell lines after infection with 5–10 TCD_{50} per cell. The virus multiplication is presented as an increase of titre above the virus present intracellularly by 10 h after infection. Although the biological characteristics of the two cell lines[3,4] differ considerably, no basic difference was observed in the time-lapse for the appearance of the new virus. It has been shown by Parkman *et al.*[5] that a true diploid cell culture, such as that of an African green monkey kidney inoculated with 10^4–10^5 InD_{50}, responds in the same manner, the end of the eclipse period occurring at about 12 h after infection. This growth curve also seems to be valid for a continuous line of Rhesus monkey kidney cells (LLC-MK_2)[6]. However, with the same input multiplicity of infection, the maximum intracellular titres obtained in the four systems vary considerably, being 10^4 TCD_{50} in green monkey kidney, 10^5 TCD_{50}