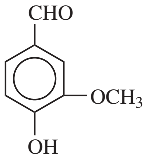
VANILLIN

1. Introduction

Vanillin (3-methoxy-4-hydroxy-benzaldehyde [121-33-5]), a natural product, can be found in the form of its β-D-glucoside (glucovanillin) in vanilla beans. The curing process, including the hydrolysis of its β-D-glucoside, leads to vanillin, at concentrations of ~2%. The biosynthesis of vanillin β-D-glucoside remains incompletely understood.

Vanillin can be extracted with water, alcohol, or other organic solvents. Approximately 250 by-products have been identified in natural vanilla, out of which 26 are present at levels in excess of 1 part per million (ppm). The balance of all these products contributes to the subtle taste of vanilla beans. The vanilla bean contains ~2% vanillin, but the 10% extract prepared from beans has several times the strength of a solution of 2% vanillin. For this reason, the U.S. Food and Drug Administration (FDA) regulations state that one part of vanilla beans is equivalent to 0.07 parts vanillin in flavor strength. The best known natural source of vanillin is the vanilla plant, *Vanilla planifolia* A., which belongs to the orchid family. It is cultivated mainly in Mexico, Madagascar, Reunion, Java, and Tahiti.

The long and expensive process of extracting vanillin from vanilla beans yields a product that has an inconsistent quality. The demand for this universally popular flavoring cannot be satisfied by vanilla beans alone. For technological and economic reasons, the consumption of naturally occurring vanilla has gradually given way to synthetic vanillin. Synthetic vanillin is identical to that contained in the pod, but differs in smell and flavor from natural vanillin as a result of the various compounds in the natural extract that do not exist in artificial vanillin. These other compounds represent only 2% of the extract; the remaining 98% is vanillin. Vanillin is the common name for 3-methoxy-4-hydroxy-benzaldehyde [121-33-5] (**1**).

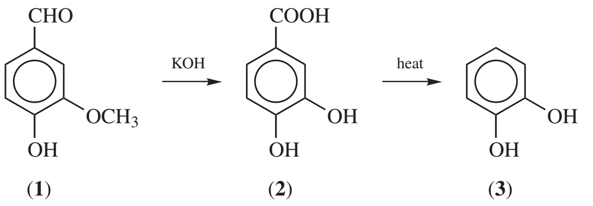


(**1**)

2. Production

Vanillin was observed long before it was reported in chemical literature, as it crystallizes on the surface of vanilla beans after harvesting, processing, and storage. The first report in the literature was probably made by Bucholtz in 1816. Some years later, Bley referred to vanillin as vanilla camphor. In 1858, Gobley crystallized out vanillin from alcoholic solutions of vanilla bean extract and succeeded in obtaining it in a relatively pure form. He reported its composition to be $C_{10}H_6O_2$. Its correct analysis, $C_8H_8O_2$, was established in 1872 by Carles, who also gave its correct melting point, 81°C (177.8°F).

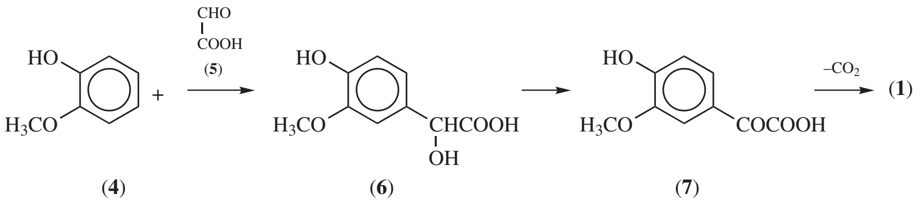
In 1874, Tiemann and Haarmann examined the structure of vanillin and reported it to be 3-methoxy-4-hydroxybenzaldehyde. This was not a difficult task because, on treatment with potassium hydroxide, vanillin (**1**) gave protocatechaic acid [99-50-3] (**2**), which, in turn, was decarboxylated to catechol [120-80-9] (**3**) by dry distillation (eq. 1). As both compounds were known at that time, the position of the substituent groups in vanillin was established. Finally, Reimer synthesized vanillin from guaiacol [90-05-1] and thus proved the identity of its structure. In 1894, Rhône-Poulenc began producing vanillin on an industrial scale. Since then, many other producers have entered into vanillin production, often only to leave it behind.



(1)

The manufacture of vanillin shows the progress made in the chemistry and chemical engineering of the substance. Most commercial vanillin is synthesized from guaiacol; the remainder is obtained by processing waste sulfite liquors. Preparation by oxidation of isoeugenol is of historical interest only.

2.1. Preparation from Guaiacol. Several methods can be used to introduce an aldehyde group into an aromatic ring. Condensation of guaiacol (**4**) with glyoxylic acid (**5**), followed by oxidation of the resulting mandelic acid (**6**) to the corresponding phenylglyoxylic acid (**7**) and decarboxylation continues to be a competitive industrial process for vanillin synthesis (eq. 2).



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Guaiacol is synthesized from catechol, which is prepared by acid-catalyzed hydroxylation of phenol with hydrogen peroxide (see HYDROQUINONE, RESORCINOL, AND CATECHOL).

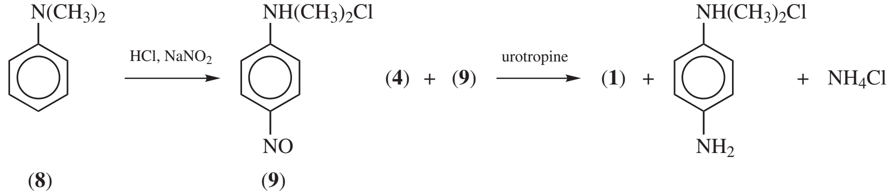
Glyoxylic acid is obtained as a by-product in the synthesis of glyoxal from acetaldehyde (qv), and it can also be produced by oxidation of glyoxal with nitric acid. Condensation of guaiacol with glyoxylic acid proceeds smoothly in alkaline media. Crude vanillin is obtained by acidification and simultaneous decarboxylation of the 4-hydroxy-3-methoxyphenyl glyoxylic acid solution. Commercial grades are obtained by vacuum distillation and subsequent recrystallization.

This process has the advantage that, under the reaction conditions, the glyoxyl radical enters the aromatic guaiacol ring almost exclusively para to the phenolic hydroxyl group. Tedious separation procedures are thus avoided.

2.2. Preparation from *ortho*-Chloronitrobenzene.

In this route, still used in Asia, Guaïacol is manufactured from *ortho*-chloronitrobenzene. Nitroanisole and anisidine are the main intermediate products.

Vanillin is then produced by condensation of guaiacol with a dimethyl aniline (**8**) nitroso derivative (**9**), and urotropine. The reaction gives also para-amino dimethyl aniline (**10**) as side product (eq. 3).



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Purification of vanillin needs several steps of distillation and recrystallization. Wastewater treatment is a concern, due to the toxic nature of the raw materials and side products. This process is the less environmental friendly, and would not be considered as sustainable. Furthermore, chlorinated impurities can be found in the final product prepared with this process, despite the purification steps.

2.3. Preparation from Waste Sulfite Liquors.

The starting material for vanillin production can also be the lignin (qv) present in sulfite wastes from the cellulose industry. The concentrated mother liquors are treated with alkali at elevated temperature and pressure in the presence of oxidants. The vanillin formed is separated from the by-products, particularly acetovanillone, 4-hydroxy-3-methoxyacetophenone, by extraction, distillation, and crystallization.

A large number of patents describe various procedures for the mainly continuous hydrolysis and oxidation processes, as well as for the purification steps required to obtain high grade vanillin. Lignin is degraded either with sodium hydroxide or with calcium hydroxide solution and simultaneously oxidized in air in the presence of catalysts. When the reaction is completed, the solid wastes are removed. Vanillin is extracted from the acidified solution with a solvent, eg, butanol or benzene, and reextracted with sodium hydrogen sulfite solution. Reacidification with sulfuric acid followed by vacuum distillation yields technical-grade vanillin, which must be recrystallized several times to obtain food-grade vanillin. Water, associated with small amounts of an alcohol, is used as the solvent in the last crystallization step.

The process starting from lignin has faced serious problems, such as reduced availability and environmental impact. The availability is reduced because the new process for making paper paste yields less liquor. As a result, it is likely that the larger companies will not reinvest in new factories to process liquors to meet demand. The process's environmental impact is also problematic because > 160 tons of caustic waste are produced for every ton of vanillin manufactured.

Many impurities are present in vanillin produced by the lignin process, principally 5-formylvanillin, *para*-coumaric acid, *para*-hydroxybenzaldehyde, syringic aldehyde and syringic acid, and acetovanillone (4-hydroxy-3-methoxy-acetophenone). The last mentioned is the main impurity present in vanillin from lignin. It can be found, although only rarely, at levels of up to 1000 ppm. Its relatively strong odor is responsible for the typical odor of the head space observed in lignin vanillin samples.

In contrast to vanillin from lignin, the principal impurity found in vanillin from guaiacol and glyoxylic acid is 5-methyl vanillin, typically present at levels of ~ 100 ppm in Rhovanil Extra Pure (Rhodia), although levels as high as 3000 ppm have been found in samples from other producers. This impurity is completely odorless.

No residual guaiacol can be found in vanillin produced by the guaiacol and glyoxylic acid process. In contrast to vanillin from lignin, vanillin from guaiacol is extremely consistent in quality owing to the consistency of the supply source, and shows no variation in taste, odor, or color.

2.4. Production by Biotechnology. Some work has been made recently in order to produce vanillin by biotechnology. Depending on the nature of the starting raw material, and the process used, the produced vanillin may be considered as natural. Isotopic deviation determination can be used to check the “naturallity” of one product. For example, Rhovanil Natural (Rhodia) clearly meets the definitions for natural flavours as described by the European and U.S. regulation (88/388 and FDA–CFR21).

3. Specifications

The physical properties of Rhovanil Extra Pure vanillin of Rhodia, the leading company in this area, are shown in Table 1.

3.1. Solubility. Solubility in water is $< 2\%$; the solubility in ethanol is given by the ratio 1 part vanillin to 2 parts alcohol. Certain manufacturing processes require that the product be in liquid form. Depending on the application, the solvent must be chosen in accordance with the manufacturing process and regulation requirements. The solubility of the Rhovanil vanillin in water–ethanol, water–propylene glycol, and water–glycerol solutions are shown in Figure 1. In addition, the influence of temperature and solvent concentration are important in maximizing the vanillin concentration.

3.2. Particle-Size Distribution. Particle size, crystal shape, and distribution of vanillin are important and greatly affect parameters, eg, taste, flavor, solubility, ease of dispersion in solvent, flowability of the powder, caking effect, and production of dust.

3.3. Taste and Flavor. The taste effect is generally sweet, but depends strongly on the base of preparation. For tasting purposes, vanillin is often evaluated in ice-cold milk with $\sim 12\%$ sugar. A concentration of 50 ppm in this medium is clearly perceptible. Vanilla is undoubtedly one of the most popular flavors; its consumption in the form of either vanilla extracts or vanillin is almost universal.

The food flavor industry is the largest user of vanillin, an indispensable ingredient in chocolate, candy, bakery products, and ice cream. Commercial vanilla extracts are made by macerating 1 part of vanilla beans with 10 parts of 40–50% alcohol. Although vanillin is the primary active ingredient of vanilla beans, the full flavor of vanilla extract is the result of the presence of not only vanillin, but also other ingredients, especially little-known resinous materials that contribute greatly to the quality of the flavor.

It is easy to smell a difference in the quality of vanillins from different origins, but it is normally difficult to taste the same difference, provided the various samples are of good quality. Vanillin is sensitive to contamination with other crystalline odor chemicals. Adherent or absorbed odor on the crystals perceptibly affects the odor of vanillin from a fiber-drum or other large containers.

So far, several blind-test studies have been conducted to detect possible differences in odor and taste (retroolfaction) of vanillin from lignin and guaiacol, respectively. In none of these trials did the taste panel observe significant differences between lignin and guaiacol vanillin. However, a difference in odor can be observed in the head space of pure vanillin containers, ie, top note. But no difference in top note is observed between vanillin from lignin and vanillin from guaiacol after acetovanillone is added to the latter, at the level present in the lignin vanillin. In fact, guaiacol-based vanillin has the real, unadulterated odor of pure vanillin, in contrast to lignin-based vanillin, whose odor is influenced by the presence of acetovanillone at variable levels.

3.4. Available Grades. Rhovanil Extra Pure is the trade name of the food-grade vanillin of Rhodia, worldwide leader in the diphenols area. The following grades are commercially available: Rhovanil Extra Pure crystallized, Rhovanil Fine Mesh, Rhovanil Free Flow.

Rhovanil Extra Pure is the standard mesh, multipurpose quality of food-grade extra pure vanillin. Its broad particle-size distribution shows a versatile granulometry, compatible with a wide range of granulometric profiles from any other ingredients, and allows a homogeneous powder mixability, even at low content in a given blend.

Rhovanil Fine Mesh, a specially calibrated extra pure vanillin that avoids demixing with other very fine dry ingredients, eg, sucrose, flour, and dextrose, provides a faster dissolution rate at lower stirring, at lower temperature, in low acidity medium, or in viscous liquids.

Rhovanil Free Flow is obtained by adding an anticaking agent (0.5% max) to the extra-pure vanillin. The flowability is increased, making it particularly suitable for self-dispensing equipment (instant beverage), while both mixability and dispersion/dissolution ratios remain as good as the standard Rhovanil Extra Pure vanillin.

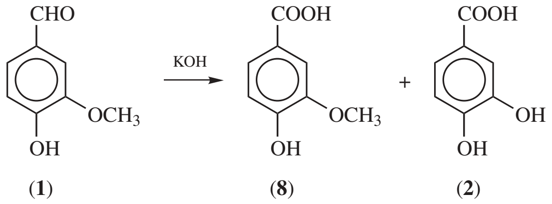
Other Producers. Several key producers of vanillin from guaiacol stopped their productions. For example, UBE Industries, based in Japan, stopped its vanillin and ethylvanillin productions at the end of 2004. Borregaard (Norway) is no more producing vanillin from guaicaol, but only from lignin (EuroVanillin Supreme).

Since 2000, three out of the five major Chinese producers stopped due to the environmental and safety constraints and economic reason: in China, all producers, except Rhodia, are operating the polluting ONCB process. Rhodia is the only company with a broad range of vanillin grades and having tree units of production based in France, the United States, and China. Competitors of Rhodia generally have only one standard quality at their disposal.

4. Chemical Properties

Vanillin is a compound that possesses both a phenolic and an aldehydic group. It is capable of undergoing a number of different types of chemical reactions. Addition reactions are possible owing to the reactivity of the aromatic nucleus.

On distillation at atmospheric pressure, vanillin undergoes partial decomposition with the formation of catechol. This reaction was one of the first to be studied and contributed to the elucidation of its structure. Exposure to air causes vanillin to oxidize slowly to vanillic acid. When vanillin is exposed to light in an alcoholic solution, a slow dimerization takes place with the formation of dehydrodivanillin. This compound is also formed in other solvents. When fused with alkali (eq. 4), vanillin (**1**) undergoes oxidation and/or demethylation, yielding vanillic acid [121-34-6] (**8**) and/or protocatechaic acid (**2**).



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Reduction of vanillin by means of platinum black in the presence of ferric chloride gives vanillyl alcohol in excellent yields. In 1875, Tiemann reported the reduction of vanillin to vanillin alcohol by using sodium amalgam in water. The yields were poor, however, and there were a number of by-products. High yields of vanillin alcohol have been obtained by electrolytic reduction.

Because vanillin is a phenol aldehyde, it is stable to autooxidation and does not undergo the Cannizzarro reaction. Numerous derivatives can be prepared by etherification or esterification of the hydroxy group and by aldol condensation at the aldehyde group. All three functional groups in vanillin are highly reactive. The hydroxy group can be methylated and acetylated. Acetals and mercaptals have also been prepared.

5. Applications

In flavor formulations, vanillin is used widely either as a sweetener or as a flavor enhancer, not only in imitation vanilla flavor, but also in butter, chocolate, and all types of fruit flavors, root beer, cream soda, etc. It is widely acceptable at different concentrations; 50–1000 ppm is quite normal in these types of finished products. Concentrations up to 20,000 ppm, ie, 1 part in 50 parts of finished goods, are also used for direct consumption, eg, toppings and icings. Ice cream and chocolate are among the largest outlets for vanillin in the food and confectionery industries, and their consumption is many times greater than that of the perfume and fragrance industry.

Vanillin, being an aldehyde, is able to form acetals and hemiacetals. Therefore, in flavor formulations using high concentrations of vanillin in conjunction with carriers, eg, propylene glycol, a gas layer chromatography (glc) analysis often shows a reduced vanillin peak after storage of the compounded flavor, and the presence of new peaks indicating acetal formation. Addition of $\sim 0.5\%$ of water to the formula reverses the reaction, ie, there is a reduction of acetal, and the reappearance of vanillin peaks.

5.1. Food Flavoring Compounds. When vanillin is not used as a single flavoring ingredient, it is a key part of flavor compounding. At least 30% of food-grade vanillin consumed in the world is through flavoring compounds. Flavor compounding requires expertise to develop well-balanced, complex flavors, such as fruit flavors, by mergers of multiples and different single flavors. Single flavors can be classified according to the stage of flavor perception, incorporating both direct olfaction and retronasal olfaction at 35°C. Head note describes the first fugacious impression; intermediary note, a more sustained impression when the food is in front of the nose; body note, an impression more solid or dense when the food is in the mouth; and queue or long-lasting note, the feelings or sensations after deglutition. These four principal notes correspond to various components.

The work of several flavors always corresponds to an optimized work of assembling these four notes into a given direction, starting with a basis or a body note. Vanillin and ethylvanillin belong to the category of body notes. Vanilla extracts, owing to > 200 minor components in addition to vanillin, are considered a queue note.

Baking. In the industrial production of dry cookies, cakes, and pastries, the vanillin content ranges between 20 and 50 g/100 kg of dough. Often, vanillin is added at the dry stage of dough preparation as the flour and sugar are being mixed. In fact, it is better to take advantage of the properties of fatty ingredients that are excellent at retaining flavor. Better results can be obtained by incorporating vanillin into the fatty ingredients, as is the case, eg, during creaming or topping.

In fat-free recipes, where this method cannot be practiced, it is possible to add and mix vanillin powder with eggs. An alternative to vanillin powder for highly mechanized processes is to use a vanillin solution or liquid flavor; the solvent used is either ethanol- or monopropylene glycol-based.

Chocolate. Vanillin is added during the manufacturing process, in powder form, in average amounts of 20 g/100 kg of the finished product. However, this amount varies according to the quality of the chocolate being made. Chocolate having a high melting point, eg, the type of chocolate used in icing, is more strongly flavored, ~ 25 g/100 kg of mixture. Chocolate with a low melting point is less strongly flavored, ~ 15 g/100 kg of mixture.

Vanillin can be added either in the first stage of production during crushing and before conching, or just before conching, or in the last steps of conching. In the first two cases, some flavor may be lost if the temperature is raised to > 80°C.

An example of the last case is by dissolving the vanillin in cocoa butter, which is added to adjust the product's melting point.

Although the vanillin concentration is a matter of taste depending on different factors in each individual case, the following concentrations are generally accepted:

Vanillin flavoring	g/100 kg
dark chocolate	15–60
milk chocolate	5–30

Confections. Main applications are sugared almonds, caramel, nougat, and sweets. For sugared almonds and caramel, vanillin is mixed into the sugar in the dry phase of the recipe. For nougat, vanillin is added during the liquid phase of manufacturing. In sweets, vanillin is added in the form of a 10% ethanol solution.

The appropriate concentration of vanillin depends on the nature of the finished product and the flavor desired. Therefore, in caramel for which vanillin is used to give a pleasant, but not pronounced taste, 15 g of vanillin is sufficient for 100 kg of confectionery. However, if a pronounced taste is required, at least 40 g of vanillin is required for 100 kg of the finished product. The vanillin flavoring can be incorporated either in liquid extract form or in the form of vanillin-flavored sugar, or even by mixing the required amount of a special finer mesh grade, such as Rhovanil Fine Mesh (Rhodia) with a little sugar and adding this to the rest of the ingredients. If the process requires the mixture to be oven-baked, the vanillin should only be added at the end of the cooking to avoid losses caused by evaporation. Although the concentration may be different in individual cases, the following may serve as a rough guide:

Vanillin flavoring	g/100 kg
soft-center sweets	5–15
other sweets	15–30
caramel	15–55
chewing gum	15–45
nougat	40–55

Vanillin is used in flavored milk, desserts, yogurts, sorbets, and ice cream. Generally, vanillin is used in liquid form either in ethanol solution with a vanillin concentration up to 400 g/L or in monopropylene glycol with a vanillin concentration to 300 g/L. Both concentrations are given for a temperature of 20°C to avoid recrystallization problems.

The content of vanillin, in relation to the product to be flavored, is ~5 g/100 kg of the finished product. Flavoring is carried out by adding the appropriate quantity of vanillin solution during one of the product's mixing stages. To obtain an even distribution of the flavoring in the product, it is best to add the flavoring solution as early as possible during the manufacturing process.

Vanillin Sugar. This product is prepared by dry mixing or impregnating the sugar with a vanillin alcohol solution and evaporating the alcohol. However, modern techniques increasingly involve grinding the sucrose and vanillin mixture very finely.

Vanillin sugar can also be prepared by mixing 20 g of vanillin with 980 g of sieved sugar to remove any lumps and then placing the mixture in an air-tight container for the amount of time necessary for the synthetic vanillin aroma and taste to develop. This method of preparation is used for smaller volumes or household needs. Vanillin sugar can be used for flavoring all dry preparations. This method of dry flavoring can also be used for dispersing agents other than sugar, eg, flour, cornstarch, or any other suitable ingredient used in the food industry.

Beverages. Vanillin confers a pleasant note to liqueur flavoring and improves the flavor of fortified wines by giving them a greatly enhanced bouquet. For example, vanillin is used for flavoring grenadine as well as chocolate-flavored drinks.

5.2. Animal Feed. Vanillin is used as a palatability enhancer to make animal feed more appetizing by flavor-masking minerals with off-taste. Approximately 5 g of vanillin/100 kg of feed is added when preparing feed for lambs and pigs in order to increase feed intake and stimulate the growth of the animals. Vanillin is added during the manufacturing process either by mixing into the dry ingredients or in its liquid form. Increasingly, vanillin is also used as a substitute for aniseed.

5.3. Perfumes and Cosmetics. The uses of vanillin in international perfumery are many. In aldehydic perfumes, vanillin provides the powdery impression given by the background smell, usually up to 2% in the perfume concentrate. In fruity notes, vanillin enhances the various fruity constituents (0.1–0.5% in pears; up to 2% in peaches). When vanillin is combined with some floral notes, eg, heliotrope and orchid, which actually contain strong vanilla impressions, amounts of 2–5% are possible. However, with notes, eg, rose, orange flower, and jonquil, the addition of 0.1–2% vanillin can bring warmth and elegance. In woody families, eg, fougère and chypre, and also in spicy perfumes, the harsh impression also needs the fine, smooth aroma provided by vanillin traces.

In some cases, especially when the family is directed more toward Oriental fragrances, the use of vanillin can be up to 5%. These types of perfumes include Spanish fougère, sweet or fruity chypres, woody Oriental, or spicy Oriental notes. Vanillin, when used together with coumarin and nitro-musks, can have a concentration of up to 10%.

In detergent perfumes, the stability of vanillin is not always certain. It depends on the association made with other raw materials. The use of high concentrations of vanillin in soap perfumery can cause discoloring effects; over time, dark or black spots appear on the soap and foaming power is reduced. In some cases, however, the use of ethylvanillin [121-32-4] is possible.

Vanillin is also useful as a deodorant to mask the unpleasant odor of many manufactured goods. As a masking agent for numerous types of ill-smelling mass-produced industrial products, particularly those of synthetic rubber, plastics, fiber glass, inks, etc, vanillin finds extensive use. The masking effect requires only traces of vanillin, which odor is perceptible in dilutions of 2×10^{-7} mg/m^3 of air.

5.4. Pharmaceutical Products. Rhodia offers a flaked technical-grade vanillin, Vaniltek, to be used in pharmaceutical applications. The single largest use for vanillin is as a starting material for the manufacture of an antihypertensive drug having the chemical name of Methyldopa or L-3-(3,4-dihydroxyphenyl)-2-methylalanine.

L-Dopa and Trimethoprim are two other drugs that can be made from vanillin. L-Dopa is used for the treatment of Parkinson's disease; Trimethoprim is an antiinfective agent used mainly for urinary tract infections and certain venereal diseases. Also, Mebeverine, an antispasmodic agent, and Verazide, a generic antitubercular agent, are drugs that can be made from vanillin or its derivatives.

Papaverine, used to treat heart diseases as a vasodilator, is a drug that was originally made from vanillin, but has since been made from veratrole and *ortho*-1,2-dimethoxybenzene. Vanillin is also used as a pharmaceutical excipient.

Antimicrobial Effect. Vanillin itself has some bacteriostatic properties, and therefore has been used in formulations to treat dermatitis. More specifically, it has been reported that, at dosages between 500 and 1000 ppm, vanillin and to a lesser extent ethyl vanillin, showed clear antimicrobial effects. Both compounds are more effective against fungi and nonlactic Gram-positive than against Gram-negative bacteria. Effectiveness was greater at pH 6 than at pH 8, which suggests that such antimicrobial activity is reinforced in foods with unfavorable pH conditions, osmotic pressure, and growth of temperature (cf. acid foods).

5.5. Agrochemical Products. Hydrazones of vanillin have been shown to have a herbicidal action similar to that of 2,4-D, and the zinc salts of dithiovanillic acid. Made by the reaction of vanillin and ammonium polysulfide in alcoholic hydrochloric acid, dithiovanillic acid is a vulcanization inhibitor. 5-Hydroxymercurivanillin, 5-acetoxymercurivanillin, and 5-chloromercurivanillin have been prepared and found to have disinfectant properties.

Ripening Agent. A new potential use for vanillin is as a ripening agent to increase the yield of sucrose in sugarcane by the treatment of the cane crop a few weeks before harvest.

5.6. Industrial Applications. The antiultraviolet protection properties of vanillin have been patented and look promising for the plastics and cosmetics (suncreams) industries.

Other uses for vanillin include the prevention of foaming in lubricating oils, as a brightener in zinc coating baths, as an activator for electroplating of zinc, as an aid to the oxidation of linseed oil, as an attractant in insecticides, as an agent to prevent mouth roughness caused by smoking tobacco, in the preparation of syntans for tanning, as a solubilizing agent for riboflavin, and as a catalyst to polymerize methyl methacrylate.

6. Analysis

6.1. Identification. When a solution of ferric chloride is added to a cold, saturated vanillin solution, a blue color appears that changes to brown upon warming to 20°C for a few minutes. On cooling, a white to off-white precipitate (dehydrodivanillin) of silky needles is formed. Vanillin can also be identified by the white to slightly yellow precipitate formed by the addition of lead acetate to a cold aqueous solution of vanillin.

6.2. Determination. Various classical techniques are used for the analysis of vanillin, including colorimetric, gravimetric, spectrophotometric, and chromatographic [thin-layer chromatography (tlc), gas chromatography (gc), and (hplc) high performance liquid chromatography] methods. The *Food Chemical's Codex* (FCC) prescribes infrared (ir) spectrophotometry for identifying and testing vanillin. However, more vanillin analyses are made by either gc or hplc.

Gas chromatography is a widely used method for analyzing vanillin products, vanilla extracts, and compounded flavors. It can also be used to monitor the vanillin manufacturing process. It is ideal for detecting many trace impurities associated with manufacturing either lignin vanillin or guaiacol vanillin. It can determine the levels (ppm) of impurities in the vanillin finished product. Many commercial chromatographic column packings are available for analyzing vanillin.

In the 1990s, hplc has become widely used in the flavor and fragrance industry to measure vanillin and other phenolic compounds. Routine methods have been developed that are particularly adapted to thermosensitive products, eg, vanillin and its derivative products, with elution gradient and uv detection at given wavelengths. Certain critical impurities can thus routinely be traced to very low (10 ppm) concentrations.

7. Health and Safety Factors

Vanillin is listed in the *Code of Federal Regulations* by the (FDA) as Generally Recognized As Safe (GRAS) substance. The Council of Europe and the FAO/WHO Joint Expert Committee on Food Additives have both given vanillin an unconditional Acceptable Daily Intake (ADI) of 10 mg/kg.

Vanillin has a low potential for acute and chronic toxicity, with a reported oral LD_{50} in rats of 1580–3300 mg/kg. Dietary doses up to 20,000 ppm administered to rats for 2 years resulted in no adverse toxicologic or carcinogenic effects. Vanillin is classified as a GRAS substance by FEMA. Consequently, at levels normally found in the human diet, vanillin would present no significant health or carcinogenic risk to humans.

Vanillin is known to cause allergic reactions in people previously sensitized to balsam of Peru, benzoic acid, orange peel, cinnamon, and clove, but vanillin itself is not an allergic sensitizer.

Vanillin has been reported to be a bioantimutagen, demonstrating the ability to protect against mutagenic effects by enhancement of an error-free postreplication repair pathway. Vanillin has been reported to be nonmutagenic in bacterial systems, but conflicting results in mammalian systems leave no clear indication of the SCE-inducing potential of vanillin.

Vanillin was reported to be nonteratogenic in chicks. No mammalian teratology studies were found in the literature. One study of the influence of vanillin on MNNG teratogenicity indicated that vanillin could enhance or reduce MNNG teratogenicity, depending on the specific endpoint examined and the teratogenic mechanism involved.

BIBLIOGRAPHY

"Vanillin" in *ECT* 1st ed., Vol. 14, pp. 603–611, by D. M. C. Reilly, Food Machinery and Chemical Corp.; in *ECT* 2nd ed., Vol. 21, pp. 180–196, by D. G. Diddams, Sterling Drug Inc., and J. K. Krum, The R. T. French Co.; in *ECT* 3rd ed., Vol. 23, pp. 704–717, by J. H. Van Ness, Monsanto Co.; in *ECT* 4th ed., Vol. 24, pp. 812–825, by L. J. Esposito, K. Formanek, G. Kientz, F. Mauger, V. Maureaux, G. Robert, and F. Truchet, Rhône-Poulenc; "Vanillin" in *ECT* (online), posting date: December 4, 2000, by L. J. Esposito, K. Formanek, G. Kientz, F. Mauger, V. Maureaux, G. Robert, and F. Truchet, Rhône-Poulenc.

GENERAL REFERENCES

S. Arctander, ed., *Perfume and Flavor Chemicals (Aroma Chemicals)*, Montclair, N.J., 1969.
K. Bauer, in D. Garbe, ed., *Common Fragrance and Flavor Materials: Preparation, Properties and Uses*, VCH, Weinheim, Germany, 1985.
P. Z. Bedoukian, ed., *Perfumery and Flavoring Synthetics*, Allured Publishing Corp.
G. S. Clark, *Perfum. Flavor* **15** (Mar.–Apr. 1990).
J. M. Jay and G. M. Rivers, *J. Food Safety* **6**, 129 (1984).
H. Priefert, J. Rabenhorst, and A. Steinbüchel, *Appl. Microbiol. Biotechnol.* **56**, 296 (2001).
S. Ramachandra Rao and G. A. Ravishankar, *J. Sci. Food Agric.* **80**, 289 (2000).
Ullmann's Encyclopedia of Industrial Chemistry, 5th ed., Vol. A 11, VCH, Weinheim, Germany, 1988, pp. 199–200.

JEAN-PAUL VIDAL
Rhodia

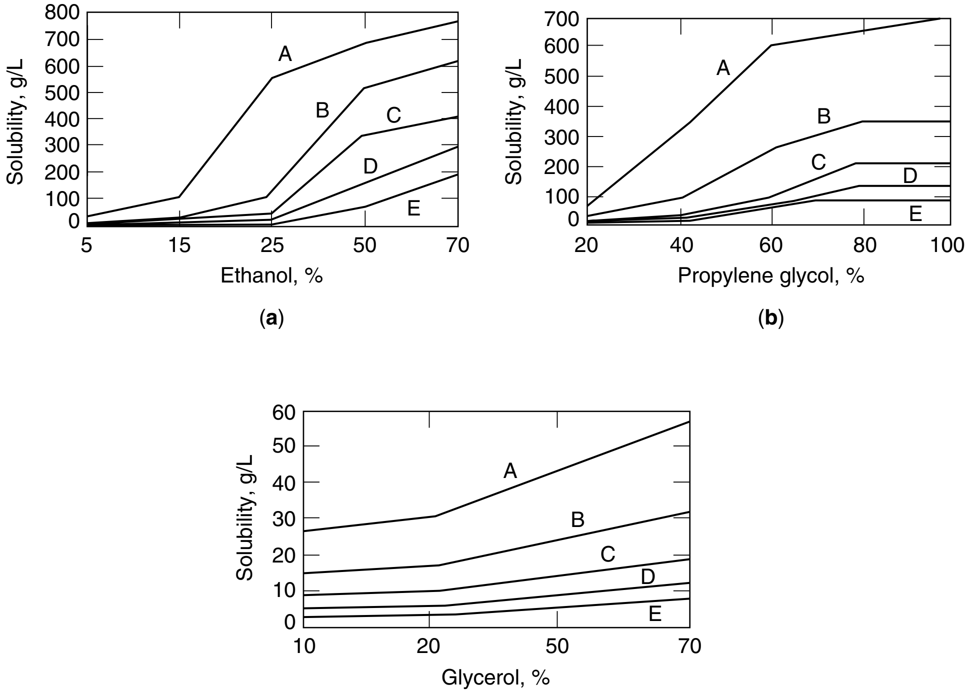
Table 1. **Physical Properties of Rhovanil[a] Extra Pure Vanillin**

Property	Value
white to off-white nonhygroscopic crystalline powder	
melting point, capillary, °C	81–83
assay, %	99.96 min
bulk density	~0.6
flash point, °C	153
boiling point, °C	
at 101.3 kPa[b]	284–285
10 mmHg	154
sublimation,[c] °C	70

[a] Trademark of Rhodia.

[b] To convert kPa to mmHg, multiply by 7.5.

[c] At normal pressure.



(**a**)

(**b**)

(**c**)

Fig. 1. Solubility of vanillin in (**a**) ethanol solutions, (**b**) propylene glycol solutions, and (**c**) glycerol solutions, where A is 40°C; B, 30°C; C, 20°C; D, 10°C; and E, 0°C.