

methylguanidine can scarcely be regarded as playing an important part in the mechanism of "meat poisoning" for such poisoning is due to meat that is not badly decomposed. The nature of the toxic agent will be considered in another paper.

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[CONTRIBUTION FROM THE ESSENTIAL OILS LABORATORY, DRUG DIVISION, BUREAU OF CHEMISTRY.]

THE CONSTITUTION OF CAPSAICIN, THE PUNGENT PRINCIPLE OF CAPSICUM.

BY E. K. NELSON.

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The pungent principle of Capsicum was first isolated by Thresh,¹ who assigned to it the name capsaicin. Thresh, however, did not throw any light on the constitution of the substance and overlooked the presence of nitrogen, giving the formula $C_9H_{14}O_2$.

Micko² improved the method of extracting capsaicin and obtained it as a crystalline substance of extreme pungency, melting at 63.5° and possessing the properties of a phenol. On the basis of analyses and molecular-weight determinations, he ascribed to it the formula $C_{13}H_{28}NO_3$. This is an impossible formula. His analyses agree well with the formula $C_{18}H_{27}NO_3$. Micko found one hydroxyl and one methoxyl group. He prepared a benzoyl derivative, melting at 74° , which was not pungent. On treating an alcoholic solution of capsaicin with platinic chloride and hydrochloric acid and allowing the mixture to evaporate spontaneously he noted a vanilla-like odor. This suggests the presence of a vanillin residue in capsaicin.

Micko isolated capsaicin from paprika (*Capsicum annuum*) and from cayenne pepper (*Capsicum fastigiatum*). From one kg. of cayenne pepper he extracted 5.5 g. of crude capsaicin, which was 20 times the amount he found in paprika.

Nelson³ extracted capsaicin by Micko's method and obtained 2.13 g. of the pure crystalline substance, melting at 64.5° , from 1.5 kg. of African capsicum.

Preparation of Capsaicin.

In order to prepare material for this investigation Micko's method of extraction was used. A considerable difference was observed in the amounts of capsaicin recoverable from different lots of cayenne pepper obtained on the market. In one case, only 12 g. of crude capsaicin was obtained from 40 pounds of the pepper. From 50 pounds of a very hot

¹ *Pharm. J. and Trans.*, [3] 7, 21, 259, 473 (1876-77); 8, 187 (1877-78).

² *Z. Nahr. Genussm.*, 1, 818 (1898); 2, 411 (1899).

³ *J. Ind. Eng. Chem.*, 2, 419 (1910).

cayenne pepper 77 g. of crude capsaicin was obtained, which, on recrystallizing 3 times, yielded about 50 g. of the pure substance, melting at 65° . Capsaicin is purified by crystallizing from petroleum ether containing 10% of ethyl ether. An alcoholic solution of capsaicin was found to be optically inactive.

Optical Crystallographic Data.¹—*In ordinary light*, under the microscope, capsaicin shows overlapping plates which have, in part, rectangular outlines, but which break up readily into angular irregular fragments. The crystal system is probably monoclinic. Refractive indices [${}_{D}^{20}$] (determined by the immersion method in potassium-mercuric iodide solutions): $\alpha = 1.520$, $\beta = 1.540$, $\gamma = 1.580$, $\gamma - \alpha = 0.060$, all ± 0.005 . α and β are most often obtained, but γ is not difficult to find. *In parallel polarized light*: The birefringence is strong, moderately thin plates showing lower second order colors, although thin ones show first order grays. Extinction on rectangular plates is parallel to the edges, but on those tilted up seems to be inclined, reaching 20° . There is not definite elongation. *In convergent polarized light*: Partial biaxial figures are frequently visible, the axial angle being moderate in size, $2E =$ about 50° . The sign is positive and the dispersion rather strong, with $2E_{\gamma} < 2E_{\beta}$.

EXPERIMENTAL.

Methyl Capsaicin.

In order to stabilize capsaicin so that it would yield definite products on oxidation, the hydroxyl was methylated. Capsaicin (1.42 g.) was dissolved in 25 cc. of 5% sodium hydroxide solution, 3 cc. of dimethyl sulfate added, and the mixture shaken for 30 minutes. 10 cc. of 5% sodium hydroxide solution was then added to dissolve unchanged capsaicin and the methyl compound was separated by filtration and recrystallized from dil. alcohol. The yield was 1.46 g., or 98.3%. Methyl capsaicin is only moderately pungent, melting at $77-78^{\circ}$. Determination of methoxyl groups by Zeisel's method:

Subs., 0.2043, 0.2507: AgI, 0.2978, 0.3625.

Calc. for $C_{17}H_{22}NO(CH_3O)_2 \cdot CH_3O$, 19.31. Found: 19.26, 19.10.

Optical Crystallographic Data.—*In ordinary light*, methyl capsaicin is found to consist of minute needles more or less grouped into bundles, and curved or bent. The crystal system appears to be monoclinic. Refractive indices [${}_{D}^{20}$] (determined by the immersion method in potassium-mercuric iodide solutions): $\alpha = 1.55$, $\beta = 1.58$, $\gamma = 1.60$, $\gamma - \alpha = 0.05$, all ± 0.01 . β is usually obtained lengthwise, and a mean of α and γ crosswise. *In parallel polarized light*: The birefringence is strong, high first order colors, white to yellow, being shown even by rather slender

¹ The crystallographic measurements in this paper were made by Dr. Edgar T. Wherry, of the Bureau of Chemistry.

needles. The extinction is strongly inclined, reaching practically 45° . The elongation is mostly negative. *In convergent polarized light*: The crystals are too minute to yield definite figures, but the class is clearly biaxial.

Oxidation of Methyl Capsaicin.

Methyl capsaicin (1.46 g.) was suspended in 50 cc. of 2 *N* sodium carbonate solution and to the mixture, boiling under a reflux condenser, 190 cc. of 3% potassium permanganate solution was slowly added. Ammonia was evolved. The solution was filtered from manganese dioxide, concentrated and acidified, when a difficultly soluble acid separated, which was recrystallized from boiling water. The yield was 0.4 g. The melting point of the dry acid was $180-181^\circ$. Admixture with veratric acid caused no depression in the melting point. On titration with alkali 0.333 g. required 3.66 cc. of 0.5 *N* sodium hydroxide giving 182 as the neutralizing equivalent.

Calc. for $C_9H_{10}O_4$: C, 59.31; H, 5.48. Found: C, 59.23; H, 5.55.

Veratric acid is therefore a product of the oxidation of methyl capsaicin, which indicates that a vanillin residue is present in capsaicin.

In addition to veratric acid, volatile acids appear to be formed, but, as the amount was small, they were not investigated at this time.

Acid Hydrolysis of Methyl Capsaicin.

As capsaicin and methyl capsaicin appear to resist the action of acids and alkalies remarkably well at ordinary temperatures, the hydrolysis of methyl capsaicin was conducted in an autoclave.

Methyl capsaicin (2.5 g.) was dissolved in 125 cc. of 50% methyl alcohol and 5 cc. conc. hydrochloric acid added. The solution was heated at 125° for 3 hrs. Alcohol and volatile substances were then removed by steam distillation and the acid solution, after extraction with ether to remove a small amount of unchanged methyl capsaicin, was evaporated to dryness, leaving a crystalline residue amounting to 1.35 g. This was recrystallized from hot alcohol. Analysis:

Subs., 0.2016: AgCl, 0.1419.

Subs., 0.1427: CO_2 , 0.2776; H_2O , 0.0914.

Calc. for $C_9H_{13}NO_2 \cdot HCl$: C, 53.04; H, 6.92; N, 6.87; Cl, 17.43. Found: C, 53.05; H, 7.14; N, 6.82; Cl, 17.39.

This substance proved to be identical with the hydrochloride of veratryl amine (3,4-dimethoxy-benzylamine hydrochloride), described by Juliusberg,¹ as no depression in melting point was observed on admixture with the hydrochloride of veratryl amine prepared by reducing methyl-vanilline oxime. A crystallographic examination also shows the compound to be the same.

Optical Crystallographic Data.—*In ordinary light*, 3,4-dimethoxybenzyl-

¹ *Ber.*, 40, 120 (1907).

amine hydrochloride is seen to be well crystallized in rods and narrow plates. The crystal system is monoclinic. Refractive indices $[\text{D}]^{20}$ (determined by the immersion method in organic liquids): $\alpha = 1.505$, $\beta = 1.670$, $\gamma = 1.700$; $\gamma - \alpha = 0.195$, all ± 0.005 . Those usually obtained are β and intermediate values between α and γ . *In parallel polarized light*: The birefringence is extreme, the colors being mostly of the fourth order. The extinction of the plates may be straight, but when they are tilted up to any extent is strongly inclined, reaching 40° . The elongation is either positive or negative. *In convergent polarized light*: Partial biaxial figures are seen on practically all of the plates, one optic axis being nearly perpendicular to them. The plane of the optic axes lies perpendicular to the surface of the plates, and parallel to their long sides. The acute bisectrix is, however, very difficult to obtain, as it lies at an angle of about 40° from the perpendicular to the plates. The axial angle $2E$ is about 60° and the sign negative. Dispersion is rather weak, with $2E_r < 2E_v$.

From the distillate containing volatile products of hydrolysis, ether extracted a pleasant-smelling oil which proved to be an ester formed by the action of methyl alcohol on an acid product of hydrolysis. The yield of this ester was small, on account either of loss from the autoclave, which was not quite tight, or of chemical changes. It was later found that the acid could be better isolated by an alkaline hydrolysis.

Acid Hydrolysis of Capsaicin.

The conditions under which the acid hydrolysis of capsaicin was carried out were identical with those described for methyl capsaicin. A crystalline hydrochloride was obtained, from which the free base was separated by dissolving in water and adding just sufficient 10% sodium hydroxide solution to neutralize the hydrochloric acid. The liberated base separates rapidly in needles, which, freed from water of crystallization, melt at $132-133^\circ$. On admixture with 3-hydroxy-4-methoxybenzylamine (vanillyl amine), the synthesis of which will be described later, no depression in melting point occurs. The crystallographic properties of the hydrochloride are identical with those of vanillyl-amine hydrochloride.

Synthesis of Vanillyl-amine.

Vanillin oxime (7 parts by weight) is dissolved in 20 parts of alcohol and reduced with 160 parts of 2.5% sodium amalgam, 14 parts of glacial acetic acid being gradually added, so as to maintain an acid reaction in the mixture. The temperature of the reaction should not exceed $50-60^\circ$. The mixture is diluted with water and extracted with ether to recover unchanged oxime. An excess of hydrochloric acid (sufficient to liberate all the acetic acid and convert the base into a hydrochloride) is then added, and the solution is evaporated to dryness. The hydrochloride

of the base is dissolved in boiling absolute alcohol and filtered from sodium chloride. It crystallizes from the alcohol on cooling and is recrystallized from boiling alcohol. The yields averaged about 65%.

Optical Crystallographic Data.—*In ordinary light*, 3-hydroxy-4-methoxybenzylamine hydrochloride (vanillyl-amine hydrochloride) crystallizes in rods which break up into angular fragments. System probably monoclinic. Refractive indices $[\frac{D}{D}]$ (determined by the immersion method in organic liquids): $\alpha = 1.510$, $\beta = 1.705$, $\gamma = 1.735$, $\gamma - \alpha = 0.225$, all ± 0.005 . α is frequently seen in one direction, and an intermediate value between β and γ in the other. *In parallel polarized light*: The birefringence is extreme, most of the grains showing pale hues of the fourth or fifth order. Extinction is often inclined, reaching a maximum of 35° . Elongation is usually negative. *In convergent polarized light*: Partial biaxial figures are often seen. The axial angle is moderately large, $2E = 70^\circ$. The sign is negative. Dispersion is strong, with $2E_r < 2E_v$.

The free base is isolated by adding to a solution of the hydrochloride in lukewarm water an amount of 10% sodium hydroxide solution just sufficient to neutralize the hydrochloric acid. An excess will unite with the phenolic hydroxyl and redissolve the base. On standing, the base separates almost completely in fine needles, and, being very slightly soluble in water, can be washed on the filter. The melting point of the dried crystals is $131-133^\circ$. The material for analysis was dried at 110° .

Subs., 0.1903, 0.1592: CO_2 , 0.4399, 0.3652; H_2O , 0.1220, 0.1056.

Calc. for $\text{C}_8\text{H}_{11}\text{NO}_2$: C, 62.70; H, 7.24; N, 9.14. Found: C, 62.98, 62.56; H, 7.12, 7.36; N, 9.11, 9.07.

The crystals of vanillyl amine, freed from adhering moisture by drying in a desiccator, weighed and dried to constant weight at 110° , lost weight equivalent to two molecules of water.

Free vanillyl amine is very unstable, being easily decomposed by caustic alkalies and even by boiling water. In a dry form it keeps very well in the dark. In the light it gradually becomes yellow.

Optical Crystallographic Data.—3-Hydroxy-4-methoxy-benzylamine (vanillyl amine) is unsuited to complete optical characterization, as it is decidedly unstable, losing its water of crystallization at once on removal from the saturated aqueous solution. The following properties could be observed, however, on the crystals suspended in such a solution: *In ordinary light*: Crystallized in rods, probably belonging to the rhombic system. Refractive indices could not be determined. *In parallel polarized light*: The birefringence is very strong, second order colors being exhibited. The extinction is straight, and the elongation positive. *In convergent polarized light*: Biaxial, but definite figures are rarely obtained, the acute bisectrix being apparently the long axis of the rods.

Alkaline Hydrolysis of Capsaicin.

Twenty g. of capsaicin was mixed with 80 cc. of 25% sodium hydroxide solution and heated in a nickel vessel to 180° in an autoclave for 30 minutes. Ammonia was split off and the base was decomposed but the acid (previously detected as a product of acid hydrolysis) was almost quantitatively recovered. The reaction mixture was diluted with water, saturated with carbon dioxide, and extracted with ether. A quantity of resinous and colored products of the decomposition of vanillyl amine is thus separated.

The alkaline solution has a dark color, due, no doubt, to the presence of pyrocatechin or other phenolic decomposition products from the amine. It was evaporated to dryness and the sodium salt of the acid separated from sodium carbonate by boiling alcohol.

The residue left on evaporating the alcohol was dissolved in water, acidified with dil. sulfuric acid and distilled with steam. This operation required some time, as the acid distils slowly with steam. The distillates were extracted with ether and the solvent carefully evaporated. The yield was 11.1 g.

The acid was purified by distillation from a small flask at atmospheric pressure. It distils without decomposition at 258–261° (corr.). On titration with alkali, 0.5339 g. required 6.21 cc. 0.5 *N* sodium hydroxide, giving 172 as the neutralizing equivalent.

Subs., 0.1583, 0.1248, 0.1296: CO₂, 0.4075, 0.3231, 0.3338; H₂O, 0.1517, 0.1213, (lost).

Calc. for C₁₀H₁₈O₂: C, 70.53; H, 10.66. Found: C, 70.20, 70.60, 70.24; H, 10.74, 10.88.

The acid absorbs two atoms of bromine, yielding a dibromide, which, recrystallized from ethyl acetate, melts at 57–59°.

Hydrogenation by Paal's method, using colloidal palladium, showed 2.018 g. of the acid to absorb about 250 cc. of hydrogen (reduced to 0° and 760 mm.). The absorption was very slow and continued for 30 hours. The theoretical absorption for one double bond in C₁₀H₁₈O₂, using 2.018 g. of substance, is 264.8 cc. at 0° and 760 mm. The acid therefore absorbs two atoms of hydrogen, and is a decylenic acid.

The boiling point of the saturated acid obtained by hydrogenation is about 260°, and its melting point 24–25°. Admixture with capric acid from coconut oil causes immediate liquefaction. Therefore the saturated acid formed on hydrogenating the acid from capsaicin is not capric acid but an isomeric decylic acid.

The amide of the acid obtained from capsaicin is a beautifully crystalline substance. It was prepared in the following manner: 1.2 g. of the acid was mixed with 0.4 g. of phosphorus trichloride and allowed to stand for 12 hours. It was then added drop by drop to 7.5 cc. of conc. ammonium

hydroxide, cooled in a mixture of ice and salt. The amide, which separated, was washed with 2% sodium hydroxide solution and recrystallized from dil. alcohol. The melting point was 96–97° (corr.).

Optical Crystallographic Data.—The amide of the decylenic acid from capsaicin forms beautifully pearly leaflets which are not, however, adapted for optical crystallographic measurement. *In ordinary light*, it is seen to be crystallized in overlapping plates, without definite crystal outline. The mean refractive index (determined by the immersion method in organic liquids) is approximately 1.55. *In parallel polarized light*: The birefringence is weak, only blue-gray interference colors being shown. There is no definite elongation nor extinction. *In convergent polarized light*, the crystals yield a somewhat hazy, biaxial interference figure, with the axial angle $2E$ about 60° and the sign positive.

The decylenic acid from capsaicin crystallizes when cooled in a mixture of ice and salt and melts at about –5°. It is scarcely soluble in water and the silver and barium salts are insoluble in cold, and very difficultly soluble in boiling water.

A decylenic acid was prepared by Wallach¹ from menthone oxime, and found to boil at 257–261°, but the melting point of the amide (104–105°) differs from that of the decylenic acid from capsaicin. Wallach² has also prepared a decylenic acid from tetrahydro-carvone isoxime. This acid boils at 257–260°, but the amide melts at 63–64°. The recorded data on other decylenic acids are so meager that the identity of the acid from capsaicin with some one of them can, at the present time, be neither established nor disproved.

Constitution of Capsaicin.

Capsaicin is a condensation product of vanillyl amine (3-hydroxy-4-methoxybenzylamine) and a decylenic acid. Its constitution is therefore represented by the following formula:



The synthesis of substances analogous to capsaicin is at present in progress. Some of the substances thus far prepared are extremely pungent. Their description is reserved for a later communication.

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WASHINGTON, D. C.

¹ *Ann.*, 278, 312 (1894); 296, 120 (1897); 312, 199 (1900).

² *Ibid.*, 312, 204 (1900); 323, 325 (1902).