

Piperanine, a Pungent Component of Black Pepper

James T. Traxler¹

The isolation of piperanine, a new pungent component of black pepper (*Piper nigrum* L.) oleoresin, is described, and its structure is shown by synthesis

to be *trans*-5-(3,4-methylenedioxyphenyl)-2-pentenoic acid piperidide.

According to Dewein (1955), the pungency of black pepper (*Piper nigrum* L.) was attributed by Oerstedt (1821) to the presence of piperine, the structure of which was later proven to be *trans*-5-(3,4-methylenedioxyphenyl)-2,4-pentadienoic acid piperidide (Ladenburg and Scholtz, 1894). Further investigations into the pungency of this spice were reviewed by Rogers in 1966, who noted the observations by several early workers that unidentified materials other than piperine contributed to the pungency of black pepper. In an organoleptic comparison of the pungency of Malabar black pepper fixed oil with an equivalent amount of piperine based on analyses by the ultraviolet (uv) method of Genest *et al.* (1963), similar observations also were made in this laboratory, and an investigation of the additional pungency was undertaken.

ISOLATION AND IDENTIFICATION

Malabar black pepper oleoresin (100 g) (Kalamazoo Spice Co., Lot J-434) was mixed with 500 ml of distilled water and the mixture was distilled under a Clevinger trap, replacing water as needed, until no more oily distillate was obtained (15 hr). The distillate was extracted with ether, which was then dried and concentrated to give 18.75 g of light yellow volatile oil.

The aqueous residue from the steam distillation was extracted continuously with ether for 25.5 hr and the extract was chilled to give, in several crops, 26.67 g of crude piperine, identified by infrared (ir), determined with a Perkin-Elmer Model 21 spectrometer, and thin-layer chromatography (tlc) on silica gel G plates developed with 20% acetone in Skellysolve F. Recrystallization from ethyl acetate gave pure piperine, mp 129.2–130.5° C [Ruegheimer (1882) reports mp 128–129.5° C for piperine]. All melting points were determined with a Thomas-Hoover capillary apparatus and melting points below 100° C are corrected; those above 100° C are not. The mother liquors remaining from the piperine crystallization were combined and concentrated to give 58.18 g of black viscous fixed oil.

A solution of 19.94 g of the fixed oil in a minimum amount of Skellysolve F was added to a 2-cm column prepared from

200 g of Davidson Grade 923 silica gel containing 5% water, washed with Skellysolve F, and eluted with 15% benzene in Skellysolve F, benzene, 0 to 100% ether in benzene and 0 to 100% methanol in ether. A linear solvent composition gradient obtained with the apparatus of Bock and Ling (1954) was used where indicated. A total of 396 tubes was collected (20 ml each) and the tubes were combined on the basis of their tlc into 20 fractions, which were rated at a dilution of 1×10^4 cm³/g on an arbitrary scale of 0–4 for pungency, using the Schoville method described in U.S. Federal Spice Specifications (1962). Pungent material had been eluted with 7.5 to 28.5% ether in benzene. The two most pungent fractions (4.34 g) were combined and were shown by tlc to be composed mainly of piperine. Crystallization from ethyl acetate gave 1.98 g, mp 125.3–129.0° C. The mother liquor from this crystallization was combined with the other two pungent fractions to give 6.81 g of pungent material, which was rechromatographed on a similar column prepared from 150 g of silica gel, with linear solvent compositions varying from 5 to 100% ether in benzene and from 0 to 100% methanol in ether. A total of 187 tubes (23 ml each) was collected; these were combined to give 13 fractions on the basis of tlc. The pungency of these fractions was rated at 2×10^4 cm³/g; the pungent material (five fractions, 4.78 g) had been eluted with 18.5 to 46.0% ether in benzene. This material was rechromatographed on coupled (top, 2 cm, 100 g; bottom, 0.75 cm, 25 g) silica gel columns with linear solvent composition varying from 10 to 100% ether in benzene followed by 0.8% methanol in ether to give a total of 396 tubes (11 ml each), which were combined on the basis of tlc into 14 fractions. The seven pungent fractions (3.30 g) had been eluted with 11.2 to 33.0% ether in benzene.

Since the available data indicated that the chromatographic system used above was decreasing in effectiveness, a search for a new system was undertaken. It was found that partition chromatography on cellulose gave a more effective separation when used with a solvent system composed of dimethylformamide (DMF), water, acetone, and Skellysolve F [12:1:8:36; upper layer, mobile phase (69% v/v); lower layer, stationary phase (31% v/v)]. The two middle fractions (1.07 g) of the pungent material from the previous separation were dissolved in 15 ml of acetone and added to 5.0 g of Whatman CF-11 cellulose powder which had been treated with 0.4 g of DMF in 20 ml of acetone and air-dried. This material was then tamped into place on top of a column 23 mm

Dwight P. Joyce Research Center, Glidden-Durkee, Division of SCM Corp., Strongsville, Ohio 44136

¹ Present address: Growth Sciences Center, International Minerals and Chemical Corp., Libertyville, Illinois 60048

in diameter containing 50 g of cellulose powder which had adsorbed 3.7 g of DMF after equilibration with the stationary phase. Elution was carried out with mobile phase, collected in 240 tubes (11 ml each) which were combined on the basis of tlc to give ten fractions. The pungency of these fractions was rated as above; the pungent material was contained in three fractions (tubes 8 to 72, 1.03 g). A similar separation on the same column was carried out with the combined second, fourth, and fifth pungent fractions (1.05 g) from the last silica gel fractionation to give, after treatment as above, 11 fractions. The pungent material (tubes 19 to 76, 0.62 g) was found in five fractions. These cellulose powder fractions were found to give characteristic colors with the sulfuric-chromic acid reagent used for visualization of the tlc chromatograms. Thus the pungent fractions showed spots at R_f 0.31 (piperine, yellow), 0.37 (blue and/or purple), 0.40 (purple), and/or 0.42 (yellow). The five fractions from the previous separations which contained little or no piperine were combined to give 0.32 g of material which was rechromatographed on a 0.75×47 cm column of cellulose powder, prepared as above. The 5-ml portions of eluent were combined on the basis of tlc to give ten fractions. The six pungent fractions accounted for a total of 0.31 g (tubes 10 to 37). The first three pungent fractions (0.22 g) from the above separation, the main components of which had R_f 0.37 (blue spot) and 0.42 (purple spot), were rechromatographed on the previously used column. The eighty 2-ml portions of eluent were combined on the basis of tlc to give five fractions, the second and third of which contained pungent material (tubes 36 to 48). The most pungent second fraction showed one spot on tlc (blue color, R_f 0.37) and gave, on concentration, 31.8 mg of viscous, colorless liquid. This material, another pungent component of black pepper, was designated piperanine. The third fraction, also pungent, was shown by tlc and organoleptic examination to consist of crude piperanine, contaminated by another nonpungent material. Because of the large number of components present in the original oleoresin with R_f values close to that of piperanine, it is not possible to calculate the concentration of this material in the original fixed oil, even though the average recovery in the first six chromatographic separations was $99.9 \pm 4.1\%$.

The cryoscopic molecular weight of piperanine in benzene solution was 302, and chemical tests (Feigl, 1956) showed it to contain nitrogen and to produce, after hydrolysis, a carboxylic acid and a volatile, probably secondary amine. The acid portion of the molecule was also shown to contain a formaldehyde precursor by means of the sulfuric-chromotropic acid test (Feigl, 1956).

After the synthesis of piperanine had been completed,

Table I. Ultraviolet Spectra

Item	Compound	λ_{\max} (m μ)	$\epsilon_{\max} \times 10^{-3}$
1	Piperanine ^a	233 (s) ^b	5.92
		283	1.47
2	Piperine ^c	310 (s)	19.8
		343	37.5
3	3,4-Methylenedioxytoluene ^d	236	1.66
		284	1.53
4	Item 1-Item 3	243 (s)	2.63
5	Piperidyl crotonamide ^e	236 (s)	26.1
6	Item 3 + Item 5 ^f	231 (s)	6.40
		284	1.65

^a $c = 6.4 \times 10^{-5}$, ^b s = shoulder, ^c Genest *et al.* (1963), ^d $c = 3.07 \times 10^{-4}$, ^e $c = 8.4 \times 10^{-5}$, ^f $c = 5.7 \times 10^{-5}$.

Table II. Nmr Spectral Data for Piperanine

Peak ^a	Multiplicity ^b	Integration
6.65	m ^c	7.0 ^e
6.25	t ^d	
5.91	s	
3.45	m ^c	3.95
2.55 ^f	m	4.30
1.62	b	6.15

^a In ppm downfield from tetramethylsilane. ^b m = multiplet, s = singlet, t = triplet, b = broad, ^c Unresolved, ^d Apparent triplet, $J \cong 16$ Hz. ^e Seven protons assigned to peaks in the range 7.0-5.5 ppm. See text. ^f Approximate center of unresolved multiplet.

the natural material, which had been stored in carbon tetrachloride solution after unsuccessful crystallization attempts, was crystallized from 1:1 ether:Skellysolve F solution after seeding with synthetic piperanine to give mp $73.5-77.0^\circ$ C, undepressed on admixture with synthetic material.

The ir spectrum of piperanine was determined at several concentrations in carbon tetrachloride and as a liquid film. Significant bands in these spectra included 1661 s (conjugated ketone or amide), 1626 s (conjugated and/or aromatic double bond), 1517 m, 1506 s, and 1493 s (1,2,4-substituted aromatic ring), 1218 s, 1186 s, 1139 m, 1122 m, 1099 m, and 813 s (1,2,4-aromatic C-H), 1244 s (aryl-O-), 1040 s (CH₂-O-), and 966 m (*trans* olefin) cm⁻¹. Since the 1244 and 1040 cm⁻¹ bands are also present in the ir spectra of piperine, piperonal, safrole, 3,4-methylenedioxybenzyl alcohol, and 3,4-methylenedioxytoluene, they would seem to be characteristic of the methylenedioxy group.

The uv spectra of piperanine and of other compounds of interest were determined (1 = 1 cm, 95% ethanol) with a Beckman DU spectrophotometer equipped with a Sargent model SR recorder and are listed in Table I. Items 1, 2, and 3 further substantiate the structural indications obtained from the ir spectra; *i.e.*, piperanine resembles but is less conjugated than piperine and may have two independent chromophors. The uv spectrum of 3,4-methylenedioxytoluene, bp $93-94^\circ$ C (22 mm) [Sabetay and Sandulesco (1928) report bp $80-81^\circ$ C (14 mm)], prepared by Clemmenson reduction of piperonal with zinc amalgam according to Martin (1942), as well as the subtraction spectrum obtained by cancellation of the 283 m μ band of piperanine with 3,4-methylenedioxytoluene, are also shown in Table I, Items 3 and 4, respectively. The 233 m μ band of piperanine may therefore result from the coincidence of the electron transfer band of the aromatic chromophor with that of the α,β -unsaturated system.

As a further test of the above spectroscopic and chemical evidence, crotonoyl chloride, bp $72-73^\circ$ C (96 mm) [Fuson *et al.* (1936) report bp $124-126^\circ$ C], and piperidine were combined to give, in 83.6% yield, piperidyl crotonamide. The product was purified by elution from silica gel with 75% ether in Skellysolve F and showed significant ir bands (CCl₄) at 3003 (w), 1664 (s), 1618 (s), and 964 (m) cm⁻¹. The uv absorptions for piperidyl crotonamide and of a solution containing equimolar amounts of 3,4-methylenedioxytoluene and piperidyl crotonamide are given in Table I, Items 5 and 6, respectively, the latter spectrum being superimposable (except for a small base line difference) upon that of piperanine.

Further evidence for the structural characteristics of piperanine from the nuclear magnetic resonance (nmr) spectrum, determined in CDCl₃ with a Varian Associates Model A-60 spectrometer, is given in Table II. Because of overlap in

the integration curve, the peaks in the 7.0–5.5 ppm range were used to establish a proportionality basis for the remainder of the integration. The multiplet at 6.65 ppm is assigned to aromatic protons, of which there are three, according to ir and uv data. The apparent triplet centered at 6.25 ppm is assigned to two olefinic protons, whose configuration is probably *trans* [$J \cong 16$ Hz, Silverstein and Brassler (1963)]. The singlet at 5.91 ppm is well established [Goodwin *et al.* (1959); Stothers (1963)] as a result of the two methylene protons in the methylenedioxyphenyl group which, according to earlier evidence, is present in the molecule. The broad 4-proton unresolved multiplet at 3.45 ppm most probably represents the protons of the two methylene groups bonded to nitrogen in the amide portion of the molecule (Bacca *et al.*, 1962). A chemical shift into the region occupied by the pattern at 2.55 ppm indicates that these four protons may be allylic and/or benzylic methylene protons, and are therefore assigned to carbon atoms connecting the two chromophoric groups, a situation which would result in the partial structure $\text{Aryl-CH}_2\text{CH}_2\text{CH}=\text{CHC}=\text{O}$. A detailed analysis of this region was not possible, but the value of δ corresponds to that expected (Stothers, 1963) for protons on carbons α and β to a conjugated olefinic group and β and α , respectively, to a benzene ring. The 6-proton singlet at 1.62 ppm is assigned to methylene groups in the alkyl portion of the amide. Since earlier evidence indicates that the amide is disubstituted, and since no methyl proton peaks appear in the nmr spectrum, the amide nitrogen is evidently part of a six-membered ring. Thus **5** represents a suitable hypothesis for the structure of piperanine.

SYNTHESIS

As proof of the structure suggested by the studies discussed above, piperanine was synthesized by the sequence outlined in Figure 1. The hydroboration of safrole (**1**) with diborane (prepared *in situ* according to Zweifel and Brown, 1963), followed by treatment with alkaline hydrogen peroxide gave, in 90% yield, 3-(3,4-methylenedioxyphenyl)propanol (**2**), bp 101–107° C (0.1–0.2 mm) [Ramart-Lucas and Amagat (1932) report bp 180° C (13 mm) for this material prepared in 50% yield by chemical reduction of the corresponding amide].

3-(3,4-Methylenedioxyphenyl)propionaldehyde (3). To a solution of 8.5 g (0.05 mol) of 3-(3,4-methylenedioxyphenyl)propanol (**2**) in 125 ml of freshly distilled dimethylsulfoxide was added 2.5 g (0.025 mol) of anhydrous phosphoric acid, followed by 20.6 g (0.10 mol) of freshly distilled dicyclohexylcarbodiimide [bp 92–93° C (0.2 mm); Albertson (1962) reports bp 148–152° C (11 mm)], and the solution, which be-

came cloudy almost immediately, was stirred overnight at room temperature. The mixture was filtered, the filtrate was added to an equal volume of water, and this mixture was extracted with ether. The extract was washed with water, dried, and concentrated to give 7.65 g (91.3%) of viscous liquid, bp 105–108° C (0.6 mm). Tlc of the 2,4-dinitrophenylhydrazone of this material indicated small amounts of contaminants, so the aldehyde was purified through the bisulfite derivative to give 4.0 g of colorless viscous liquid, with significant ir bands (neat) at 2730 (w), 1735 (s), 1497 (s), 1510 (s), 1250 (s), and 1041 (s) cm^{-1} . The semicarbazone of this material was prepared in high yield and had, after one recrystallization from 95% EtOH, mp 192–195° C. Fournneau and Tiffeneau (1905) report mp 196° C for 3-(3,4-methylenedioxyphenyl)propionaldehyde semicarbazone obtained from the distillate of safrole oxide.

trans-5-(3,4-Methylenedioxyphenyl)-2-pentenoic Acid (4). Carboethoxymethylenetriphenylphosphorane (5.6 g, 0.02 mol), prepared according to Considine (1962), mp 121–125° C (89.5%), was dissolved in 25 ml of dry benzene and, to the resulting yellow solution, was added dropwise at room temperature 3.4 g (0.02 mol) of 3-(3,4-methylenedioxyphenyl)propionaldehyde in 5 ml of dry benzene. At the end of the small exotherm, the solution was heated and stirred under reflux for 5 hr, cooled, and added to 40 ml of Skellysolve F. This mixture was then filtered and concentrated to give 4.41 g (92.0%) of a light yellow liquid with significant ir bands (neat) at 1720 (s), 1655 (m), 1510 (s), 1498 (s), 1248 (s), 1190 (s), 1040 (s), and 975 (w) cm^{-1} .

The ester was hydrolyzed by stirring under reflux with 1.12 g (0.02 mol) of KOH in 50 ml of water for 2.5 hr. The reaction mixture was extracted with ether, acidified with concentrated HCl, again extracted with ether, which was then washed, dried, and concentrated to give 3.02 g (72%) of slightly yellow crystals, mp 128–129.5° C, neutral equivalent 212 (calcd, 210), with significant ir bands (Nujol) at *ca.* 2600 (m), 1695 (s), 1650 (m), 1510 (s), 1497 (s), 1250 (s), 1040 (s), and 980 (m) cm^{-1} . Fittig and Buri (1883) reduced piperinic acid with sodium amalgam in HCl to give α -hydropiperinic acid, mp 78° C and β -hydropiperinic acid, mp 130–131° C. Later Fittig and Weinstein (1885) concluded on the basis of fragmentary chemical evidence that the latter was probably 5-(3,4-methylenedioxyphenyl)-2-pentenoic acid. Tlc examination showed the above product to be homogeneous and chromogenic (blue color) with $\text{H}_2\text{SO}_4\text{-CrO}_3$. Two additional crops (0.26 g total) of somewhat less pure material were obtained from the mother liquor of the first crystallization. The residue (0.81 g) remaining from the third crop was examined for the presence of the *cis* isomer by column and tlc, but no material corresponding to this structure was found.

trans-5-(3,4-Methylenedioxyphenyl)-2-pentenoic Acid Piperide (5). The acid chloride was prepared by the reaction of 1.10 g (0.005 mol) of the acid (**4**) with 1.1 ml (1.04 g, 0.013 mol) of oxalyl chloride in benzene and was added in 10 ml of benzene to a solution of 1.0 ml (0.012 mol) of freshly distilled piperidine in 20 ml of benzene at 40° C. After stirring at 40° C for 1 hr, the solution was heated to reflux, cooled to room temperature, washed with water, 5% K_2CO_3 , water, dilute (1:10) HCl, and water to neutral. The solution was then concentrated to give 1.44 g (100%) of viscous light yellow oil which crystallized very slowly on storage at *ca.* 10° C, mp 70.5–72.3° C. This material was purified by elution from silica gel with 75% ether in Skellysolve F (96.2% recovery) to give mp 78.5–79.1° C.

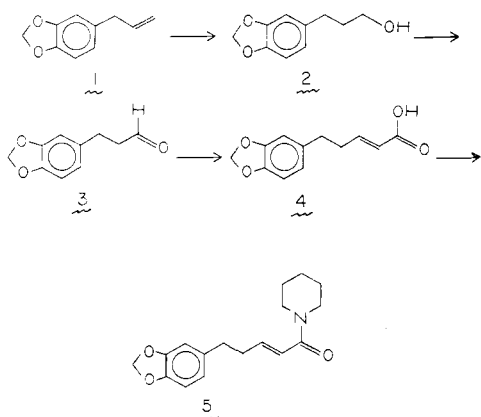


Figure 1. Synthesis of piperanine

Anal. Calcd for C₁₇H₂₁NO₃: C, 71.05; H, 7.37; N, 4.87. Found: C, 71.27; H, 7.34; N, 4.86.

The identity of **5** with piperanine isolated from Malabar black pepper was established by ir, uv, and nmr, as well as mixture melting point, *R_f* on tlc and color with H₂SO₄-CrO₃. The pungency of piperanine was found by the usual organoleptic method to be approximately one-half that of piperine.

ACKNOWLEDGMENT

The author expresses his gratitude to Helma Ralston, Analytical Section, who carried out the tlc and the piperine analyses. He also thanks the management of the Glidden-Durkee Division of SCM Corporation, especially A. E. Thomas, for permission to publish these results.

LITERATURE CITED

- Albertson, N. F., *Org. React.* **12**, 212 (1962).
Bacca, N. S., Johnson, L. F., Schoolery, J. N., "High Resolution NMR Spectral Catalog," Spectrum 572, Varian Associates, Palo Alto, 1962.
Bock, R. M., Ling, N., *Anal. Chem.* **26**, 1543 (1954).
Considine, W. J., *J. Org. Chem.* **27**, 647 (1962).
Dewein, H., *Seifen-Fette-Wachse* **81**, 489 (1955).

- Feigl, F., "Spot Tests in Organic Analysis," 5th ed., Elsevier, New York, N.Y., 1956, pp 90, 336, 260, 190.
Fittig, R., Buri, E., *Justus Liebigs Ann. Chem.* **216**, 171 (1883).
Fittig, R., Weinstein, L., *Justus Liebigs Ann. Chem.* **227**, 31 (1885).
Fourneau, E., Tiffeneau, M., *C.R. Acad. Sci., Paris* **141**, 662 (1905).
Fuson, R. C., Christ, R., Whitman, G. M., *J. Amer. Chem. Soc.* **58**, 2450 (1936).
Genest, C., Smith, D. N., Chapman, D. G., *J. Agr. Food Chem.* **11**, 508 (1963).
Goodwin, S., Smith, A. F., Valasquez, A. A., Horning, E. C., *J. Amer. Chem. Soc.* **81**, 6209 (1959).
Ladenburg, A., Scholtz, M., *Chem. Ber.* **27**, 2958 (1894).
Martin, E. L., *Org. React.* **1**, 155 (1942).
Oerstedt, *Schweigers J. Chem. Phys.* **29**, 80 (1821).
Ramart-Lucas, M., Amagat, P., *Bull. Soc. Chim. Fr.* (4)51, 126 (1932).
Rogers, J. A., "Flavor Chemistry," A.C.S. Monograph 56, R. F. Gould, Ed., 1966, pp 213-216.
Ruegheimer, L., *Chem. Ber.* **15**, 1391 (1882).
Sabetay, S., Sandulesco, G., *Bull. Soc. Chim. Fr.* (4)43, 906 (1928).
Silverstein, R. M., Brassler, G. C., "Spectrophotometric Identification of Organic Compounds," 2nd ed., Wiley, New York, N.Y., 1967, p 144.
Stothers, J. B., "Techniques of Organic Chemistry," Vol. XI, Part I, K. W. Bentley, Ed., Interscience, New York, N.Y., 1963, pp 199, 202, 245.
U.S. Federal Spice Specifications EE-S-631d, U.S. Army Quartermaster Corps, 1962, p 17.
Zweifel, G., Brown, H. C., *Org. React.* **13**, 30 (1963).

Received for review February 24, 1971. Accepted May 19, 1971.