the plane of the image from *all* sources (scattering by lens inclusions, reflections from lens mounts and the inside of the "blackened" microscope tube and stops, lens aberrations, and the multiple reflections between the lens surfaces) to considerably less than 3% of the focused illuminating intensity for most oil immersion systems. For transmittances which fall in the range of those of the *quantitative* studies already published (viz., *10–15*), this error has been negligible beside the other known errors of the method. It cannot have any relation to the validity of the conclusions these authors have drawn from their data.

Naora has used the expression $\frac{(1-\bar{r})}{1+(m-1)\bar{r}}$ as representative of their total flux, imaging, $(1-\bar{r})^m$, plus glare flux through a lens system with m air-glass interfaces of average reflectance $\bar{r}$ (*9*). He has assumed that equal fractions of the total glare flux and imaging flux fall on a given area of the image. (It is important to note that this equation applies only to the case of m plane air-glass interfaces, parallel to one another, and for the case of perfectly normal incidence.)

There are at least two ways in which the above theoretical approximation deviates seriously from the actual situation in microscopical systems. First, very few surfaces in such an optical train are plane elements perpendicular to the optical axis. As a result, all off-axis flux contributes glare diverging from the element at which it "originates" in such a way that the following element usually intercepts less of the solid angle of glare flux than of the focused imaging flux. (As an extreme example, between an objective and an ocular, up to 99% of the glare flux may be lost outside the aperture of the ocular, whereas all the imaging flux is passed if the field of illumination is set equal to the field limited by the ocular field diaphragm in accord with standard microscopical practice. The same holds for glare developed in the ocular with reference to the photocell entrance pupil or a photographic plate [*7*]). Thus, for small but finite[1] condenser apertures, Naora's calculations represent a large overestimate. As noted above, most microspectrophotometric work is carried on with a small condenser aperture. Second, if a large condenser aperture is chosen for microphotometric studies, as in Naora's case (condenser N.A. equal to objective N.A., in the range of 1.25 [*9, 16*]), most rays in the system meet the glass-air interfaces at angles very different from the normal. Under these conditions, $\bar{r}$ is increased (4), and therefore the glare flux is also increased. This, plus the increase in aberration glare as the illuminating aperture is increased, may account for the large amount of glare demonstrated experimentally by Naora's system with even small illuminated fields.

When specimens with high extinctions are studied, additional precautions will be in order, as Naora has indicated. His approach (that of Schwarzschild and Villiger) of limiting the illumination entirely to the minute area measured is the simplest solution when the convenience of larger illuminated fields of view may be abandoned. Special precautions will be even more important, however, in terms of distributional error and the aberration glare of the lens system. The curve-correction method for distributional error (*17*) adequately corrects for both glare and inhomogeneous distribution of chromophore.

[1] Not so small as to constitute a close approach to a condition where all the flux can be considered to be contained in an infinitesmal on-axis pencil. As this latter condition is approached, glare again increases toward Naora's computed values.

References

1. Rheiberg, J. *English Mech.*, **76**, 463 (1903).
2. Pollister, A. W., Himes, M., and Ornstein, L. *Federation Proc.*, **10**, 629 (1951).
3. Pollister, A. W. *Lab. Investigation*, **1**, 231 (1952).
4. Beck, C. *J. Roy. Microscop. Soc.*, 399 (1922).
5. ———. *The Microscope*. London: R. & J. Beck, Ltd. (1938).
6. Belling, J. *The Use of the Microscope*. New York: McGraw-Hill (1930).
7. Shillaber, C. P. *Photomicrography*. New York: Wiley (1944).
8. Schwarzschild, K., and Villiger, W. *Astrophys. J.*, **23**, 286 (1906).
9. Naora, H. *Science*, **115**. 248 (1952).
10. Swift, H. H. *Physiol. Zoöl.*, **23**, 169 (1950).
11. Ris, H., and Mirsky, A. E. *J. Gen. Physiol.*, **33**, 125 (1949).
12. Alfert, M. *J. Cellular Comp. Physiol.*, **36**, 381 (1951).
13. Leuchtenberger, C., Vendrely, R., and Vendrely, C. *Proc. Natl. Acad. Sci. U. S.*, **37**, 33 (1951).
14. Pollister, A. W., Swift, H. H., and Alfert, M. *J. Cellular Comp. Physiol.*, **38**, Suppl. 1, 101 (1951).
15. Harrington, N. J., and Koza, R. W. *Biol. Bull.*, **101**, 138 (1951).
16. Naora, H. *Science*, **114**, 279 (1951).
17. Ornstein, L. *Lab. Investigation*, **1**, 250 (1952).

Manuscript received March 18, 1952.

Study of Irritants Related to Piperine[1]

Torsten Hasselstrom, Harold W. Coles, and Norene E. Kennedy

Pioneering Research Laboratories,
U. S. Army Quartermaster Corps,
Philadelphia, Pennsylvania

The piperidine nucleus joined by the amide linkage to an unbroken nine-carbon chain produces a peppery pungency taste in compounds of quite different composition, such as pelargonylpiperidide (*1*), 2-phenylthiophene-5-carboxy-piperidide (*2*), and the piperine of black pepper, *Piper nigrum*. However, the pleasant bite of this spice has been duplicated only by the piperidides of β-cinnamenyl-acrylic acid, the 5-phenylpentenoic acid, and 5-phenyl-*n*-valeric acid, of which 5-phenyl-*n*-valeroyl piperidide has the most pungent taste (*3*).

It is known that the pharmacologic activity of certain compounds containing piperidine is increased by substitution in the piperidine ring. It was of interest,

[1] Thanks are due to Donald K. Tressler, scientific director, the Quartermaster Food and Container Institute of the Armed Forces, Chicago, Ill., for his interest in this work; to David Peryam, of the same institute, for outlining the taste-testing procedure used; and to the Evans Research and Development Corporation, New York, for carrying out the panel testing.

TABLE 1

CHARACTERISTIC DATA FOR THE 5-PHENYL-*n*-VALEROYLAMIDES

Compound	Formula	Boiling point	Nitrogen (%)	
			Calcd	Found
5-Phenyl-*n*-valeroyl-2-methylpiperidide*	$C_{17}H_{25}ON$	163.5°–165.5°/250 μ	5.40	5.72; 5.71
" 3-methylpiperidide†	$C_{17}H_{25}ON$	145°–146°/350 μ	5.40	5.15; 5.11
" 4-methylpiperidide*	$C_{17}H_{25}ON$	120°–122°/90 μ	5.40	5.48; 5.48
" pyrrolidide*	$C_{15}H_{21}ON$	126°–129°/1–3 μ	6.06	6.44; 6.45
" 3-methylpyrrolidide*	$C_{16}H_{23}ON$	97.5°–100.5°/80 μ	5.71	5.32; 5.33
N-5-phenyl-*n*-valeroylphthalimide†	$C_{19}H_{17}O_3N$	MP, 125°–126°/(Uncorr)	4.56	4.50; 4.51
5-Phenyl-*n*-valeroylisobutylamine†	$C_{15}H_{23}ON$	153.3°–155.0°/250 μ	6.00	5.58; 5.48

* Analyses by Jean M. Marino, Pioneering Research Laboratories.
† Analyses by Micro-Tech Laboratories, Skokie, Ill.

therefore, to ascertain whether the taste characteristics of the piperinelike amides could be changed or intensified by the substitution of methyl piperidines (pipecolines) and other amines for the piperidine and yet retain a pleasant peppery bite, without off-flavor.

The procedure recommended by Staudinger and Schneider (*3*) was followed for the preparation of the acid amides, with the minor modification that the crude amide ether solution was washed with dilute hydrochloric acid and sodium carbonate to remove excess starting materials before rectification in vacuum. The 5-phenyl-*n*-valeric acid was employed as the acid component, and the amines were prepared by methods previously described in the literature.

N-5-phenyl-*n*-valeroyl phthalimide was prepared by refluxing potassium phthalimide and 5-phenyl-*n*-valeroyl chloride in benzene solution, subsequently recrystallizing the separated organic solid from the same solvent. The properties of the acid amides appear in Table 1.

The taste tests were conducted on a taste-free 0.1% water infusion, which was prepared by dissolving the synthetic bite principle in ether or ethanol, and adding enough of the solution to ether-extracted black pepper pulp to make a 5% concentration of bite materials. The solvent was then evaporated, and the residue dispersed in water to the extent of 0.1%. These synthetic samples were compared to a 0.1% dispersion of natural malabar pepper in taste-free water. A taste testing panel of eight members carried out the testing (Table 2).

TABLE 2

PEPPERY-BITE AND TASTE-FLAVOR RATING ON CERTAIN 5-PHENYL-*n*-VALEROYLAMIDES

Compound	Peppery bite strength			Flavor (quality rating)			Flavor (subjective rating)		
	Strong (control)	Moderately weak	Weak or none	Least	Moderate	Most	Slightly pleasant	Neutral or slightly unpleasant	Definitely unpleasant
5-Phenyl-*n*-valeroyl piperidide		x		x				x	
" -2-methylpiperidide*		x			x			x	
5-Phenyl-*n*-valeroyl-3- " *		x		x			x		
" 4- " *	x				x		x		
" 4-pyrrolidide*		x			x			x	
" 3-methylpyrrolidide*		x		x			x		
N-5-phenyl-*n*-valeroylphthalimide*			x	x				x	
5-Phenyl-*n*-valeroylisobutylamide*			x	x				x	
Pelargonylpiperidide		x				x			x

* New.

The result of this work (*4*) shows that a peppery-bite taste more pleasant than natural piperine or 5-phenyl-*n*-valeroyl piperidide was accomplished by the substitution of the pipecolines and methyl pyrrolidines for piperidine.

References

1. ASANO, M., and KANEMATSU, T. *J. Pharm. Soc. Japan*, **531**, 375 (1926).
2. STEINKOPF, W., and GORDING, R. *Biochem. Z.*, **292**, 368 (1937).
3. STAUDINGER, H., and SCHNEIDER, H. *Ber.*, **56**, 699 (1923).
4. Paper presented at 75th Anniversary Mtg., Am. Chem. Soc., New York (Sept. 11, 1951).

Manuscript received March 12, 1952.