References and Notes

1. Geol. Assoc. Can., Glacial Map of Canada (1958).
2. W. Blake, Jr., *Can. Dept. Mines Tech. Surv. Geol. Surv. Can. Paper 63-28* (1963).
3. J. D. Ives, *Geograph. Bull. Can. No. 12* (1958); O. H. Løken, *Rept. to Arctic Inst. N. Amer.* (1964) (unpublished).
4. J. D. Ives and J. T. Andrews, *Geograph. Bull. Can. No. 19* (1963).
5. V. W. Sim, *Can. Geographer No. 17* (1960).
6. Unpublished maps prepared for Glacial Map of Canada (*1*). Glacial features were mapped from aerial photographs of northern Canada by Falconer in 1955 as part of a program supervised by J. T. Wilson, University of Toronto.
7. B. G. Craig, *Can. Dept. Mines Tech. Surv. Geol. Surv. Can. Paper 61-5* (1961); *Can. Dept. Mines Tech. Surv. Geol. Surv. Can. Geol. Surv. Bull. 99* (1964); W. Dyck and J. G. Fyles, *Radiocarbon* **4**, 24 (Yale Univ. Press, New Haven, 1962).
8. V. K. Prest, *Can. Dept. Mines Tech Surv. Geol. Surv. Can. Paper 63-6* (1963).
9. J. Terasmae and O. L. Hughes, *Science* **131**, 1444 (1960).

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Optical Second-Harmonic Generation in Crystalline Amino Acids

Abstract. *Optically active amino acids contain many highly efficient optical second-harmonic generators. When light from a ruby laser at 6943 Å falls on the crystalline amino acids with sufficient intensity, the second harmonic of the light at 3471 Å can be observed. Although the symmetry requirements for optical second-harmonic generation are always met by isomerically pure optically active substances, there is considerable variation in efficiency for the generation of the second harmonic, ranging from almost zero to greater than that of potassium dihydrogen phosphate, the most efficient known.*

Our earlier investigations (*1*) of two-photon absorption in organic materials showed that glass surfaces bearing finger smudges emitted ultraviolet light in the region between 3400 and 3700 Å when excited by light from a ruby laser with peak powers of the order of 100 watts. We considered the possibility that the emission might come from second-harmonic generation by amino acids whose presence in finger smudges is well known (*2*). Crystals capable of generating second harmonics of the ruby laser light must have a unit cell with no center of inversion (*3*), and this requirement is met by crystals of isomerically pure D- or L-amino acids because these molecules themselves are dissymmetric. We found that L-tryptophan as well as many other optically active amino acids are relatively efficient generators of optical second harmonics, the conversion efficiency being of the same order of magnitude as that of the best-known inorganic second-harmonic generator, potassium dihydrogen phosphate (KH_2PO_4). This behavior is almost certainly characteristic of most crystals of optically active molecular compounds of which the amino acids and peptides form a large and biologically important subclass.

It appears that the generation in the amino acids of ultraviolet-light quanta from red-light quanta may be of biologic interest. Although the conversion efficiency at light intensities ordinarily encountered in nature is extremely small, the phenomenon may provide a source of internally produced quanta of electromagnetic radiation of sufficient energy to damage nucleic acid chains; multiquantum processes such as optical harmonic generation or two-photon absorption may play some role in the generation of mutations by light of wavelengths which are normally considered biologically inactive on a one-photon basis.

The experimental arrangement used for the comparative measurements is shown schematically in Fig. 1. The laser was a Raytheon LH 5 head modified for use with an external reflecting prism and a chemical Q-switch consisting of Vd-phthalocyanine dissolved in nitrobenzene to give an optical density of approximately 0.32 at 6943 Å over a path length of 1 mm. The laser gave output pulses with a peak power of about 10 Mw and a halfwidth of 30 to 40 nsec. A Corning 2-60 glass filter in front of the laser prevented the escape of pumping light. The laser power was monitored after attenuation in 1 cm of concentrated $NiSO_4$ solution by an RCA 925 phototube operating with an anode voltage of 225 into a 50-ohm load. Laser output was focused by a lens with a focal length of 15 cm, and the samples were placed 7 cm behind this lens. Thus, the laser light focused partially on the sample into an area of roughly 0.25 cm^2. The light scattered from the sample normal to the direction of the laser beam was picked up at a distance of 25 cm, after it had passed through one 4-70 and three 7-37 Corning glass filters, by an RCA 1P28 photomultiplier operating from a 1000-volt supply and into a 50-ohm load; the filters ensured that only ultraviolet light of a narrow spectral region was picked up by the photomultiplier. Signals from the phototube and photomultiplier were displayed on the lower and upper beam of a Tektronix 555 dual-beam oscilloscope in positive and negative polarity, respectively (Fig. 2); the delay between the peaks reflected the electron transit time in the photomultiplier.

To establish that the ultraviolet signals were second harmonic and were not caused by some other luminescence of different origin, the exact wavelength and size of the bandwidth of the emitted light were determined spectroscopically (*4*) for L-tryptophan and L-leucine by means of photographic detection and also, in the case of L-tryptophan, by photoelectric detection at the output of a Beckman DU spectrophotometer converted for use as a monochromator. In both cases the linewidth was so narrow that its resolution was limited by the capability of the instrument. A very narrow linewidth at twice the laser frequency is a definite indication of harmonic generation.

Each sample was in the form of a layer of powder, 0.1 mm thick, pressed between a microscope glass slide and a cover glass. The plane of the layer was placed at an angle of 45 deg to the incident laser beam. Sample geom-

Table 1. Second-harmonic conversion efficiencies relative to potassium dihydrogen phosphate equaling 1.0.

Compound	D	L	DL
	Amino acids		
Alanine	0.40	0.20	0.40
α-Amino *n*-butyric acid	.30		
Arginine		1.50	
Asparagine HCl	.02		
Aspartic acid	.70	1.10	
Cysteine HCl	.04		
Cystine		0.02	
Ethionine	.30		
Glutamic acid	.70	.30	
Glycine			.20
Histidine HCl		.01	
Homocysteine thiolactone HCl	.20		
Isoleucine		.80	
Leucine		.90	.03
Lysine HCl		.60	
Methionine	.70	.60	.03
Phenylalanine	.15	.05	
Proline		.20	
Serine	.23	.22	.02
Tryptophan		.80	.05
Threonine	1.25	1.15	
Tyrosine	0.003	0.01	.06
Tyrosine HCl		0.02	
Valine	1.70	1.10	.01
	Sugars		
Sucrose	0.2		
Dextrose	.1		
Saccharose	.2		
D-Glucose	.1		

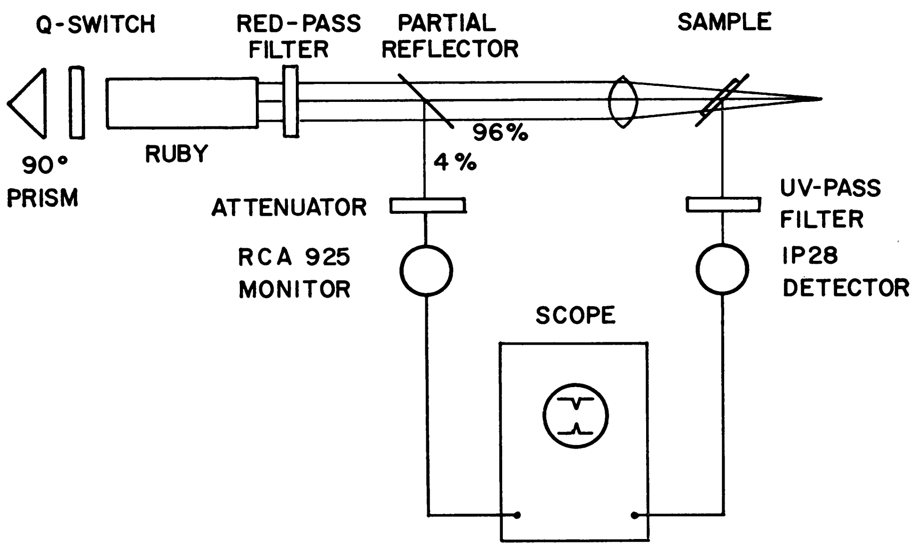

Fig. 1. Experimental arrangement for comparative measurements of optical second-harmonic generation (schematic).

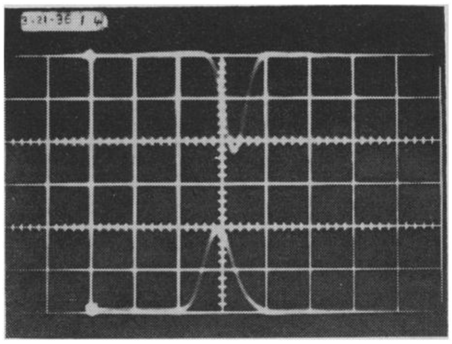

Fig. 2. Typical signal; sweep, 40 nsec/cm. Upper trace, second-harmonic signal (negative polarity). Lower trace, red-light monitor (positive polarity).

etry was such that the beam could be assumed to cause multiple scattering of any ultraviolet light generated in the sample, and uniform distribution of ultraviolet light emitted from the axis of the incident laser light. Reproducibility of the data and apparent independence of the results on minor changes in the geometry of the sample and in the angle of detection seemed to justify this assumption. The samples used were L-, D-, and DL-amino acids (*5*), as well as KH_2PO_4 (used as a reference standard) and some optically active sugars.

Table 1 shows the relative conversion efficiencies at constant laser power, with KH_2PO_4 taken to be unity. The values are only indications of orders of magnitude because the nonuniformity of such crystalline powder samples may produce inaccuracies ranging up to a factor of 2 in individual cases; this is indicated by the variation in value between some D- and L-isomers.

Apart from alanine, no DL- racemic mixtures showed significant second-harmonic generation. Because the small signals observed fell into the range of other luminescence phenomena associated with the laser pulse, they were considered to be noise. Except for phenylalanine, which showed a marginal harmonic generation in its D-isomer, whenever the D-isomer was an efficient second-harmonic generator, then so was the L-isomer. Conversion efficiencies ranged from almost zero to higher than that of KH_2PO_4 and did not seem to correlate with the electronic structure of the particular amino acid. For example, the only amino acids with π-electrons are tyrosine, tryptophan, and phenylalanine; of these, tyrosine showed no second-harmonic generation, phenylalanine showed only marginal generation, and tryptophan was comparable with KH_2PO_4. The optically active sugars showed a second-harmonic generation efficiency an order of magnitude less than the more efficient of the amino acids.

According to the theory of optical second-harmonic generation (*3*), the magnitude of the effect is determined by the size of the coefficient of the quadratic term in the formal expansion of the electric polarization, *P*, in terms of the electric field, *E*, produced by the light. This expansion may be written

$$\mathbf{P} = \chi' \mathbf{E} + \chi'' \mathbf{E}\,\mathbf{E} + \ldots ,$$

where χ' and χ'' are the coefficients of the linear and square terms, respectively. For anisotropic materials, χ' and χ'' are second- and third-order tensors, respectively. From symmetry consideration it follows that only structures having no inversion symmetry can have a nonvanishing χ'' (*3*). This is a necessary but not a sufficient condition which is automatically satisfied by all pure L- and pure D-isomers —all those that have been investigated by x-ray diffraction (*6*) crystallize with $P\,2_12_12_1$ space-group symmetry. All but one of the DL- racemic mixtures so far investigated by x-ray diffraction, however, crystallize in space groups with a center of inversion; by necessity their unit cells must contain equal numbers of L- and D- molecules in symmetrical arrangement; the exception is DL-alanine which does not have a center of inversion (*7*), but which crystallizes in a form similar to the pure D- and the pure L- forms. This exceptional behavior of alanine is borne out by the large amount of second-harmonic generation in the DL-form, an amount approximately the same as that in D- and L- forms.

The differences in conversion efficiencies between the D- and L- forms of certain amino acids is currently attributed to the crudeness of the measurements. A real difference cannot be ruled out; accurate determination of the coefficients of χ'' on single crystals should be attempted.

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References and Notes

1. W. L. Peticolas, J. P. Goldsborough, K. E. Rieckhoff, *Phys. Rev. Letters* **10**, 43 (1963); W. L. Peticolas and K. E. Rieckhoff, *J. Chem. Phys.* **39**, 1347 (1963).
2. R. J. Block, E. L. Durrum, G. Zweig, *Paper Chromatography and Paper Electrophoresis* (Academic Press, New York, 1955).
3. P. A. Franken and J. F. Ward, *Rev. Mod. Phys.* **35**, 23 (1963).
4. Engis model 1700 spectrograph.
5. Grade A, from Cal-Biochem.
6. J. D. Bernal, *Z. Krist.* **78**, 363 (1931); J. C. Kendrew and M. P. Perutz, *Ann. Rev. Biochem.* **26**, 332 (1957); B. Dawson and A. McL. Mathieson, *Acta Cryst.* **4**, 475 (1951); D. P. Shoemaker, R. E. Barieau, J. Donohue, C. S. Lu, *ibid.* **6**, 241 (1953); A. McL. Mathieson, *ibid.* **5**, 332 (1952); Sakutaro Hirokawa, *ibid.* **8**, 637 (1955); J. Donohue, L. R. Lavine, J. S. Rollett, *ibid.* **9**, 655 (1956).
7. R. E. Marsh, private communication.
8. We thank R. E. Marsh, California Institute of Technology, for information on the crystal structure of the amino acids, Fred Rodgers for sample preparation, Ken Foster for certain of the measurements, and Dick DeBrunce for x-ray diffraction patterns on some of our samples.

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