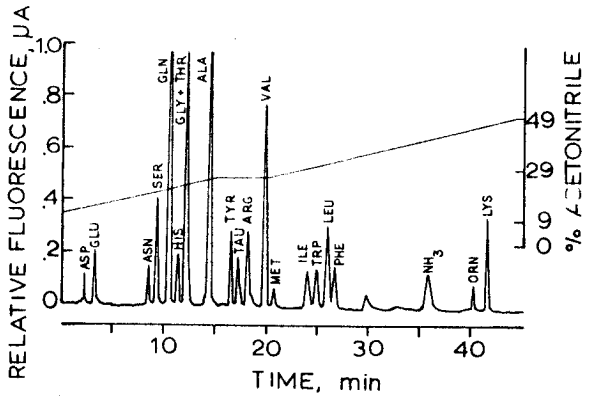

Figure 3. Profile of the derivatized amino acids extracted from a human serum sample. Chromatographic conditions as in Figure 1. See Experimental section for the handling of the serum sample

0.052; arginine, 0.051; valine, 0.062; methionine, 0.050; isoleucine, 0.034, tryptophan, 0.048; leucine, 0.038; phenylalanine, 0.044; ornithine, 0.018, and lysine, 0.037. Studies are currently in progress to determine the linearity of this response, the detection limits, and other optimum quantitative parameters.

Figure 3 shows a profile of the derivatized amino acids extracted from a human serum sample. It can be seen that 20 amino acids commonly present in human serum (with the exception of glycine and threonine) are adequately separated. These preliminary results suggest that the *o*-phthaldialdehyde/ethanethiol amino acid derivatives offer the specificity, that is not afforded by PTH or dansyl amino acids, that will allow the analysis of primary amino acids in complex biological fluids. Further quantitative studies on various biological matrices are presently in progress.

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Solvent-Dependent Photolysis for Identification of Lysergic Acid Diethylamide and Other Indolamines

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It has been recognized for some time that LSD and other indolamines are degraded by ultraviolet irradiation. Stoll and Schlientz (*1*) found that the irradiation of LSD in acetic acid solution results in the addition of water across the 9–10 double bond, the compound formed being called "lumi-LSD". In a series of four papers, Hellberg (*2–5*) described the preparation of a number of lumi derivatives of ergot alkaloids by irradiation in acidic aqueous solutions. Genest and Farmilo (*6*) applied the photolysis of indolamines to the identification of LSD, noting that LSD can be distinguished from ergot alkaloids by directly irradiating a spot of the indolamine at the origin of a thin-layer chromatographic plate prior to development. Andersen (*7*) reported that the photolysis of LSD in chloroform resulted in products which are clearly distinguishable by TLC from those obtained from the irradiation of ergot alkaloids.

Although the identification of LSD and ergot alkaloids may be achieved in a comparison mode by these procedures, the techniques are susceptible to the legitimate criticism that, with the exception of the lumi derivatives, the chemical nature of the photolysis products are not known. These procedures will remain entirely empirical and, therefore, scientifically suspect until the products are reproducibly prepared, isolated, and their structures elucidated. This will require, as an initial step, the rigorous standardization of photolysis conditions. One previous attempt has been made in this regard. Niwaguchi and Inoue (*8*) conducted time courses of the photolysis products of LSD in acidic and alkaline aqueous solution and in several organic solvents. The present work attempts to systematically correlate the photolysis products of LSD and other indoles with solvent, irradiation time, wavelength, and a critically determined value for the intensity of the ultraviolet source. The latter is a particularly important factor, since the actual intensity may deviate substantially from the manufacturer's stated value, and, additionally, it is virtually impossible for one group of workers to attempt to verify or reproduce the work of another group without quantitative data pertaining to the ultraviolet source.

EXPERIMENTAL

Ultraviolet Source for Irradiation in Solution. The ultraviolet source for the irradiation of the indolamines in solution was a Ultraviolet Products Photochemical Quartz Lamp #-PCQ9G-1 equipped with a 60-Ap, 115-V, Model #SCT-4 Power Supply (Ultraviolet Products, Inc., 5100 Walnut Grove Avenue, San Gabriel, Calif., 91778). Eighty-five percent of the irradiation emitted from this lamp is at 2537 Å.

Reaction Vessel and Stirrer. The ultraviolet source was 65 mm long with a ⅋ 24/40 male joint. The reaction vessel was constructed from a 25-mm diameter open tube with a ⅋ 24/40 female joint; the opposite end was flame sealed. The volume of

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Table I. Results of Ferrioxalate Actinometry

irradiation time, s	Fe^{2+} formed, μmol	intensity, Einsteins/s
5.4	3.16	4.68×10^{-7}
10.1	5.11	4.05×10^{-7}
14.8	7.77	4.20×10^{-7}
	mean intensity	4.31×10^{-7}

the resulting vessel with the lamp inserted but without a stirring bar was 35 mL. In the photolysis experiments, a one-half inch magnetic stirring bar was used, powered by a conventional stirring motor.

Materials. With the exception of LSD and *N,N*-dimethyltryptamine, the indoles were all USP. The LSD and dimethyltryptamine were portions of illicit seizures, the identity of which was confirmed by infrared spectroscopy and gas chromatography. The solvents were all analytical reagent grade. The *p*-dimethylaminobenzaldehyde was Eastman Practical grade.

Chemical Actinometry. A quantitation of the ultraviolet energy emitted from the lamp and absorbed into solution under the reaction conditions was achieved by means of ferrioxalate actinometry. The method used was that of Parker (*9, 10*). A sulfuric acid solution of potassium ferrioxalate is irradiated, reducing the iron to the ferrous state, which is then reacted with 1,10-phenanthroline to form a red-colored complex. This red complex is determined spectrophotometrically, the absorbance being a function of the intensity of the irradiation.

Thin-Layer Chromatography. Following irradiation, the indoles were subjected to thin-layer chromatography. Commercial Silica Gel G plates (Macherey-Nagel & Co., distributed by Brinkmann Instruments, Inc., Cantiague Road, Westbury, N.Y., 11590) of 250-μm thickness were used. Thirty microliters of the solution to be analyzed were spotted on the plate and the plate was developed with chloroform/methanol (9:1). Visualization was achieved by a 2537-Å ultraviolet light and by spraying with a solution of *p*-dimethylaminobenzaldehyde (200 mg *p*-dimethylaminobenzaldehyde plus 2 mL concentrated HCl in 20 mL ethanol).

Irradiation. Five milligrams of the indole to be studied was dissolved in 50 mL of solvent. Thirty-five milliliters of the solution was placed in the reaction vessel and irradiated with stirring for time intervals as discussed below. Prolonged irradiation resulted in the elevation of solution temperature from ambient to approximately 40 °C, but did not exceed 40 °C in any experiment.

RESULTS AND DISCUSSION

By ferrioxalate actinometry, the mean intensity of the irradiation was found to be 4.3×10^{-7} Einsteins/s, or 2.6×10^{17} quanta/s (Table I). This is approximately 8% of the total lamp intensity of 2.5 W as rated by the manufacturer. The intensity measured by actinometry is that intensity delivered to the reaction mixture. The quantum yield for reaction will be affected by the solute concentration, solvent absorption, and solvent–solute interaction.

Chloroform and methanol were selected for the solvent–irradiation studies because of their differing polarities and because they are widely used in thin-layer chromatographic developing solvents for the analysis and identification of indoles.

A time course with LSD was initially run to determine the irradiation time producing the maximum number of spots as seen by thin-layer chromatography. Prolonged irradiation will

Table II. R_fs Relative to LSD of Indoles Irradiated in Chloroform (The italic superscripts refer to the appearance of the spot as indicated in the legend below)

Spot no.	LSD	indole	dimethyltryptamine	lysergic acid	ergonovine	ergotamine
1	0.00^{i}	$0.09^{l,q}$	0.00^{u}	0.00^{u}	$0.12^{h,r,t,w}$	$0.03^{j,s}$
2	$0.09^{f,t}$	0.17^{h}	$0.03^{f,r,t}$	$0.06^{h,r,t}$	0.32^{f}	$0.38^{k,q}$
3	0.16^{u}	0.29^{n}	$0.09^{f,t}$	0.12^{k}	0.44^{k}	$0.47^{k,q}$
4	$0.34^{h,q,s}$	$0.40^{m,t}$	0.29^{u}		$0.65^{f,q}$	0.56^{k}
5	0.65^{f}	$0.49^{l,t}$			0.85^{b}	0.76^{k}
6	0.74^{f}	$0.91^{c,r}$				$0.97^{h,r,t}$
7	$0.90^{h,r,t}$	$1.54^{f,t}$				$1.15^{f,r}$
8	$1.00^{h,r,t}$	$1.66^{o,t}$				$1.32^{k,q}$
9	1.33^{f}	1.77^{g}				$1.47^{k,q}$
10	1.50^{k}					$1.74^{f,q}$
11	1.62^{c}					

[a] Red fluorescence by 2537 Å; [b] red-orange fluorescence by 2537 Å; [c] orange fluorescence by 2537 Å; [d] yellow fluorescence by 2537 Å; [e] yellow-green fluorescence by 2537 Å; [f] green fluorescence by 2537 Å; [g] blue fluorescence by 2537 Å; [h] purple fluorescence by 2537 Å; [i] brown fluorescence by 2537 Å; [j] grey fluorescence by 2537 Å; [k] white fluorescence by 2537 Å; [l] red, visible with unaided eye following TLC development; [m] orange, visible with unaided eye following TLC development; [n] yellow, visible with unaided eye following TLC development; [o] purple, visible with unaided eye following TLC development; [p] brown, visible with unaided eye following TLC development. [q] faint, minor spot; [r] strong fluorescence; [s] diffuse fluorescence; [t] *also* visible by *p*-dimethylaminobenzaldehyde, purple; [u] *only* visible by *p*-dimethylaminobenzaldehyde, purple; [v] very faint, diffuse purpose by *p*-dimethylaminobenzaldehyde; [w] spot extends from origin to 0.24 RRf.

Table III. R_fs Relative to LSD of Indolamines Irradiated in Methanol (The italic superscripts are the same as in Table II)

Spot no.	LSD	indole	dimethyltryptamine	lysergic acid	ergonovine	ergotamine
1	$0.00^{k,q}$	0.18^{h}	0.08^{u}	$0.00^{d,t}$	0.00^{c}	0.00^{p}
2	$0.05^{d,r}$	1.46^{c}		0.06^{g}	$0.03^{d,t}$	$0.03^{d,r}$
3	0.13^{u}	1.54^{u}		0.15^{e}	$0.06^{h,t}$	$0.15^{d,q}$
4	0.44^{u}	1.62^{g}		0.18^{d}	$0.12^{h,t}$	$0.39^{e,q}$
5	0.74^{c}			$0.55^{d,r}$	$0.30^{g,t}$	$0.79^{k,q,t}$
6	$0.92^{h,r,t}$			$0.70^{c,r}$	0.39^{e}	$0.91^{k,q}$
7	$1.00^{h,r,t}$			$0.85^{c,q}$	$0.55^{c,q}$	$1.06^{k,q,v}$
8	$1.18^{c,q}$			1.33^{c}	0.79^{c}	$1.30^{d,r}$
9	1.36^{f}			1.42^{e}	0.94^{c}	$1.52^{a,q}$
10	1.46^{c}			$1.73^{d,q}$	1.06^{c}	1.61^{b}
11	1.64^{c}					
12	1.74^{c}					

diminish the number of spots resolvable by thin-layer chromatography. In chloroform, the irradiation times were 0, 0.5, 1, 2, 4, 6, 8, and 10 min, respectively. A time of 2 min (at 4.3×10^{-7} Einsteins/s) was found to produce the maximum number of photolysis products from LSD. Irradiation of chloroform alone for 6 min or more gave a single product with yellow-green fluorescence as visualized by ultraviolet light of 2537 Å at a R_f of 0.27 relative to LSD. In methanol, the irradiation times were 0, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 20, 24, 28, 32, 36, and 40 min. No products were detected from irradiation of methanol alone. A time of 12 min maximized the number of photolysis products from LSD.

Irradiation times of 2 min in chloroform and 12 min in methanol were then used to compare the photolysis products of LSD with those from other indoles.

Tables II and III illustrate the chromatographic mobility of the various photolysis products of common indolamines, expressed as the R_f relative to LSD. Table II indicates the products observed from irradiation in chloroform, and Table III indicates the products observed from irradiation in methanol.

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Identification of the Acid Monomer in Elastomeric Latices

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The polymerization of an unsaturated carboxylic acid as a third monomer into styrene-butadiene, acrylonitrile-butadiene and other rubber latices, at the 1% level, generally gives several improvements to the polymer. These include greater abrasion resistance, oil resistance, and increased stability to filler loading.

The identification of the acid monomer is a difficult one. Using infrared spectroscopy, for example, carboxylated rubber generally shows only a typical acid carbonyl or carboxylate absorption because of the low concentration of the acid.

A simple ion-exchange technique has been found to isolate monomeric carboxylic acid in a pure form from the serum or water-soluble portion of the latex. Since polymerization of these monomers is incomplete, there is adequate material for identification. The ion-exchange conditions described retain the monomeric carboxylic acid until such other latex components as emulsifiers, antioxidants, and polymerization residues are discarded. These polymerization residues include homopolymerized carboxylates. The desired acid monomer is then eluted and identified.

EXPERIMENTAL

Procedure. A 25-mL buret with a glass-wool plug is partially filled with about 10 mL of AG-3-X4A anion-exchange resin (Bio-Rad Laboratories, Richmond, Calif.). The resin is converted to the hydroxyl form with 30 mL of 0.5 N sodium hydroxide solution, and then the excess base is washed out with distilled water.

Enough of the latex sample is taken so that only several milliequivalents of free acid is present. This is generally about 25 mL of latex. The latex is then coagulated with methanol, or methanol and dilute hydrochloric acid if stronger coagulation conditions are needed, the polymer removed, and the serum brought to a pH between 3 and 5. Alternatively, a film prepared from the latex is extracted with water and brought to the pH range described above. The serum or extract is concentrated to about 25 mL and put through the ion-exchange resin bed. Additional washings of 50 mL of distilled water are put through the resin and the total eluate is discarded.

Thirty-five milliliters of 1 N hydrochloric acid are now used to bring the acid off the column.

Nonvolatile acids can be identified by taking the acid wash to dryness and obtaining an infrared spectrum of the residue. Fumaric, itaconic, and other solid acids are amenable to this treatment. Volatile acids can be identified by gas chromatography of the acid wash. Acrylic and methacrylic acids are typical, often-used volatile acids.

RESULTS AND DISCUSSION

The procedure described here could readily be adapted to obtain a quantitative value for the residual, unpolymerized acids in latices.

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