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QUANTITATIVE MICRODETERMINATION BY THIN LAYER CHROMATOGRAPHY OF THE PHOTOPRODUCTS INVOLVED IN THE PHOTOCYCLIZATION OF *cis*-3-STYRYLPYRIDINE

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SUMMARY

The quantitative determination of the products involved in the photocyclization of *cis*-3-styrylpyridine by thin layer chromatography is described. The method may find wide application in following the photoreaction pathway at any time by the quantitative determination of the two photocyclization products and of the two *cis* and *trans* isomers. The determination is obtained by spectrophotometric microanalysis. Using a concentration of $5.5 \cdot 10^{-4}$ and a 254 mμ exciting line, the ratio of the two benzoquinolines is constant at about 5.3 in favour of the 5,6-benzoisoquinoline. It increases with irradiation at 313 mμ.

Thin layer chromatography (TLC) in conjunction with quantitative spectrometric microanalysis was investigated for the determination of the photocyclization products of *cis*-3-styrylpyridine. For the latter purpose an analytical method was necessary which allows the separate determination of the various products involved in the reaction. In a previous paper[1], it was shown that in the photocyclization of *cis*-3-styrylpyridine, in addition to 5,6-benzoisoquinoline(II), as found by LOADER[2], small amounts of 7,8-benzoquinoline(I) are also formed (Fig. 1).

The purpose of the present investigation was to establish the ratio of 5,6-benzoisoquinoline to 7,8-benzoquinoline as a function of time. The influence of the wavelength of the exciting light was also studied. The analysis of the irradiated solution was complicated by the presence of the two geometric isomers since *cis–trans* photoisomerization takes place in parallel with the photocyclization[3]. The photocyclization of the stilbenes[4], styrylpyridines[1,2], styrylthiophenes[5] and azobenzenes[6] is known and has been intensively studied during the last few years. However only a few authors were interested in the formation of cyclized isomeric photoproducts as a consequence either of the presence in the ring of a heteroatom as described in Fig. 1 or of the presence of a substituent as described in Fig. 2. Of these authors, MALLORY[4], LEWIS[6] and CARRUTHERS[5] on investigating the latter type of isomerism found that the different isomeric products were formed in nearly equivalent quantities.

PERKAMPUS[7], LOADER[8], and the present authors[1,9], examined the former type of isomerism (different position of the heteroatom), and found that the different

isomeric products were formed in highly variable amounts, in agreement with our preliminary results[1]. Some authors explained the larger yield of one product with respect to another on the basis of theoretical calculations of charge density and of localization energy in the different reactive positions both in the ground state[8,10] and in the first excited state[9,11]. In order to compare the experimental data with the theoretical calculations an accurate analytical procedure was developed. In particular we employed TLC using Eastman Chromagram sheets instead of the usual silica

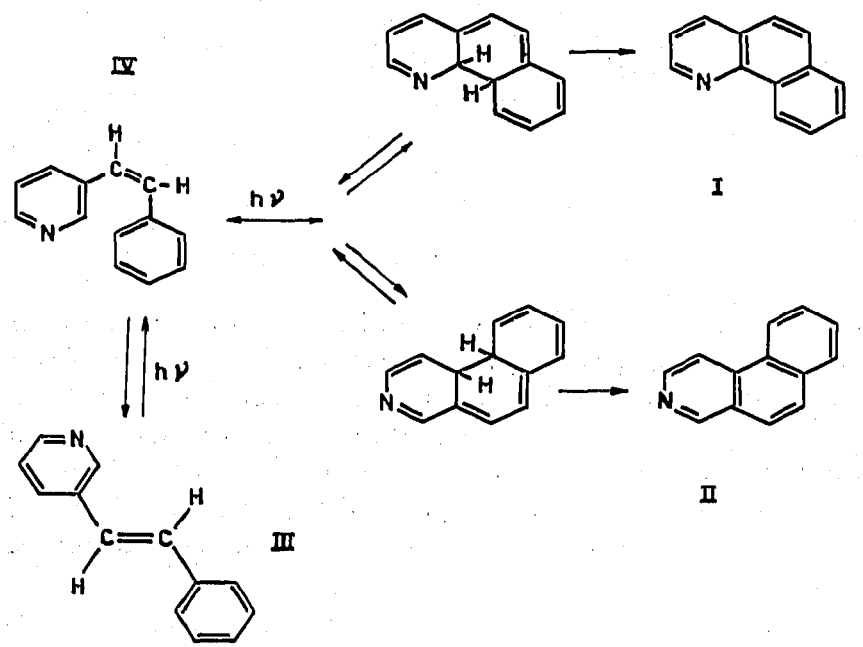

Fig. 1. Scheme of photocyclization and isomerization of 3-styrylpyridine.

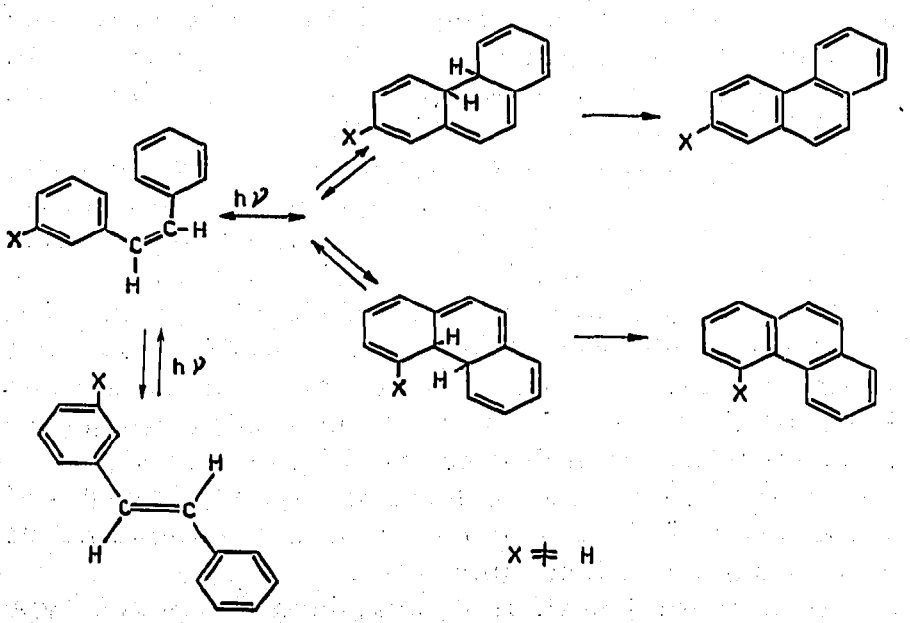

Fig. 2. Scheme of photocyclization of 3-substituted stilbene.

glass plates. We preferred to use these Chromagram sheets because of their high reproducibility and sensitivity, and because of the facility with which the separated components can be recovered. This fact is very important for a quantitative analysis.

EXPERIMENTAL

Preparation of standard compounds

The 3-styrylpyridines, *cis* and *trans*, the 7,8-benzoquinoline and the 5,6-benzoisoquinoline were prepared as described by GALIAZZO[1,12]. In all cases the purity of the products was also determined by I.R. and U.V. spectra, and by C, H and N analyses.

Materials

Eastman Chromagram sheets (K 301 V). These are precoated sheets of polyethylene terephthalate coated with a layer of about 100 μ silica gel with polyvinyl alcohol as a binder.

Chromatography tanks. Desaga GmbH, Heidelberg.

Micropipettes. A. E. Pedersen, Denmark.

Solvents. Ethyl ether, methanol, *n*-hexane (Erba) specially prepared for chromatography and tested for absorption in the ultraviolet. Dimethylformamide was dried over phosphorus pentoxide and was then fractionally distilled in the dark and under reduced pressure immediately before use.

Spectrophotometer. Beckman DU.

Quartz cell. U.V. sources, 4 cm path-length. Mineralight, low pressure mercury lamp equipped with a 254 mμ filter; Osram, high pressure (HBO-200) mercury lamp with 313 mμ filter.

Procedure

The *cis*-3-styrylpyridine in *n*-hexane (about 10^{-4} mole/l) was irradiated in a quartz cell with a U.V. lamp for times varying from ½ h to 15 h. A fixed support kept the cell at a constant distance.

The spotted amounts of irradiated solutions were chosen such that they have an optical density between 0.600 and 0.200 for every separated compound. When the volumes of solution were too large, the solvent was evaporated under reduced pressure to a final volume of a few microliters (about 10 μl).

After irradiation, duplicate samples of the solution, containing the three photoproducts and the reagent, were spotted in an appropriate quantity by means of a micropipette. At the same time known amounts of the test compounds corresponding to each component of the reaction mixture were spotted. The sheets were placed in the chromatography tank, previously saturated with the solvent vapours, ethyl ether–dimethylformamide (99:1) for 1 h[13–15]. The chromatograms were developed by the usual ascending technique. The sheets were removed when the solvent front reached 10 cm: running time 60 min. The spots were located under 366 mμ U.V. light, cut out from the sheets in strips of identical size and extracted with 5 ml of methanol. After 15 min the solution was centrifuged at 15,000 r.p.m. for 10 min to remove the last traces of silica gel, the optical density was measured at the absorption maximum of each compound, employing cells of 1 cm path-length. The complete spectrum of every compound was measured simultaneously. The amounts of the substances were

calculated by reference to data obtained with known amounts of control, after subtracting the reading given by an equal area of unstained sheet at locations on the plate with precisely equivalent R_F values.

RESULTS AND DISCUSSION

The R_F values are given in Table I. Since these compounds have high extinction coefficients, a suitable spectrophotometric reading can also be made with quite low concentrations. Table I shows that, within the limits of accuracy of the measurements, Beer's law is obeyed up to a concentration at least as high as $6.1 \cdot 10^{-5} M$. Undoubtedly silica gel is the most suitable absorbent in the quantitative analysis of the

TABLE I

RECOVERY OF THE REACTION PRODUCTS FROM SILICA GEL LAYERS AND R_F VALUES[a]

	ε max 10^4 [g]	*Absorbance*			*Percent recovery*	$R_F \times 100$[h]
		Sample	*Blank*	*Net*		
7,8-Benzoquinoline[b]	2.38					82
Solution determined	(max = 265 mμ)					
directly[c]		0.537		0.537	100	
Spotted on plate and						
developed		0.515	0.011	0.504	94	
		0.511	0.008	0.503	94	
		0.513	0.015	0.498	93	
5,6-Benzoisoquinoline[b]	5.38					42
Solution determined	(max = 249.5 mμ)					
directly[d]		0.404		0.404	100	
Spotted on plate and						
developed		0.381	0.000	0.381	94	
		0.380	0.005	0.375	93	
		0.375	0.003	0.372	92	
trans-3-Styrylpyridine[b]	2.34					54
Solution determined	(max = 292 mμ)					
directly[e]		0.523		0.523	100	
Spotted on plate and						
developed		0.487	0.003	0.484	93	
		0.494	0.006	0.488	94	
		0.487	0.007	0.480	92	
cis-3-Styrylpyridine[b]	1.05					67
Solution determined	(max = 277 mμ)					
directly[f]		0.352		0.352	100	
Spotted on plate and						
developed		0.332	0.007	0.325	92	
		0.340	0.009	0.331	94	
		0.339	0.010	0.329	93	

[a] Eluent system: ethyl ether–dimethylformamide.
[b] Stock solution: 7,8-benzoquinoline, 10.094 mg; 5,6-benzoisoquinoline, 13.457 mg; *trans*-3-styrylpyridine, 10.058 mg; *cis*-3-styrylpyridine, 10.150 mg; respectively, were dissolved in methanol in a 10 ml volumetric flask.
[c] Aliquot of 20 μl was diluted to 5 ml.
[d] Aliquot of 10 μl was diluted to 5 ml.
[e] Aliquot of 20 μl was diluted to 5 ml.
[f] Aliquot of 30 μl was diluted to 5 ml.
[g] Average of six determinations.
[h] Average of ten determinations.

TABLE II

LIGHT-INDUCED (254 mμ) CYCLIZATION AND ISOMERIZATION OF *cis*-3-STYRYLPYRIDINE. PERCENTAGE AND RATIO OF THE REACTION PRODUCTS AS A FUNCTION OF THE TIME

Conc. ≈ $5 \cdot 10^{-4}$ *M*. Compound I = 7,8-benzoquinoline; compound II = 5,6-benzoisoquinoline; compound III = *trans*-3-styrylpyridine; compound IV = *cis*-3-styrylpyridine.

Time (min)	*Percentage of the reaction products (weight %)*				*Compound II / Compound I*	*Total percent recovery*
	Compound I	*Compound II*	*Compound III*	*Compound IV*		
30	1.43	7.63	18.07	69.27	5.3	96.40
120	3.85	21.09	20.90	45.65	5.4	91.49
210	5.85	32.14	15.30	37.00	5.5	90.29
300	7.40	39.90	11.61	31.99	5.4	90.90
420	9.38	50.10	7.30	24.24	5.3	91.02
525	10.70	58.77	4.54	17.60	5.5	91.61
735	12.76	67.10	1.75	8.26	5.3	89.87
900	13.45	69.92	0.50	5.63	5.2	89.50

TABLE III

LIGHT-INDUCED (313 mμ) CYCLIZATION AND ISOMERIZATION OF *cis*-3-STYRYLPYRIDINE: PERCENTAGE AND RATIO OF THE REACTION PRODUCTS AS A FUNCTION OF THE TIME

Conc. ≈ $5 \cdot 10^{-4}$ *M*. Compound I = 7,8-benzoquinoline; compound II = 5,6-benzoisoquinoline; compound III = *trans*-3-styrylpyridine; compound IV = *cis*-3-styrylpyridine.

Time (min)	*Percentage of the reaction products (weight %)*				*Compound II / Compound I*	*Total percent recovery*
	Compound I	*Compound II*	*Compound III*	*Compound IV*		
210	3.35	25.13	4.59	58.17	7.5	91.24
300	4.47	34.17	4.17	48.86	7.6	91.67
330	4.80	36.69	3.51	46.68	7.6	91.68
525	7.83	59.75	1.70	21.24	7.6	90.52
735	9.48	72.63	0.44	7.48	7.6	90.03

compounds studied[17,18]. The uniformity of coating thickness of the sheet assured very precise measurements. Moreover there is no need for scraping and collecting the adsorbent, because it remains firmly attached to the solvent-resistant support. The adsorbed compound was eluted by immersing the entire cut-out piece in methanol. Notwithstanding the high eluting efficiency of methanol, the substances were not completely extracted from the adsorbent, but an appreciable and reproducible fraction was recovered as shown in Table I. Other workers have found that the analytical blanks are generally high and not reproducible owing to impurities contained in the silica gel and to silica particles which remain in suspension in the eluate[19].

In order to obtain constant and low values for the blanks, we removed areas equal in size to those occupied by the sample spots and at locations on the sheet with precisely equivalent R_F values[20]. Then the eluate was centrifuged as described earlier in order to eliminate the traces of finely divided silica gel[19]. Table I shows that low blanks were obtained in this manner. However, to reduce further any error resulting from this procedure, the corresponding standard compounds were simultaneously run through the procedure and were used to establish the content of unknown samples[21].

The results obtained by irradiating at 254 mμ a $5.52 \cdot 10^{-4}$ *M* *cis*-3-styrylpyridine solution in *n*-hexane are given in Table II. The data allow us to estimate the ratio of the two photocyclization products. This ratio is constant at 5.3 in favour of the 5,6-benzoisoquinoline at any time during the reaction. These data are in good agreement with the theoretical calculations.

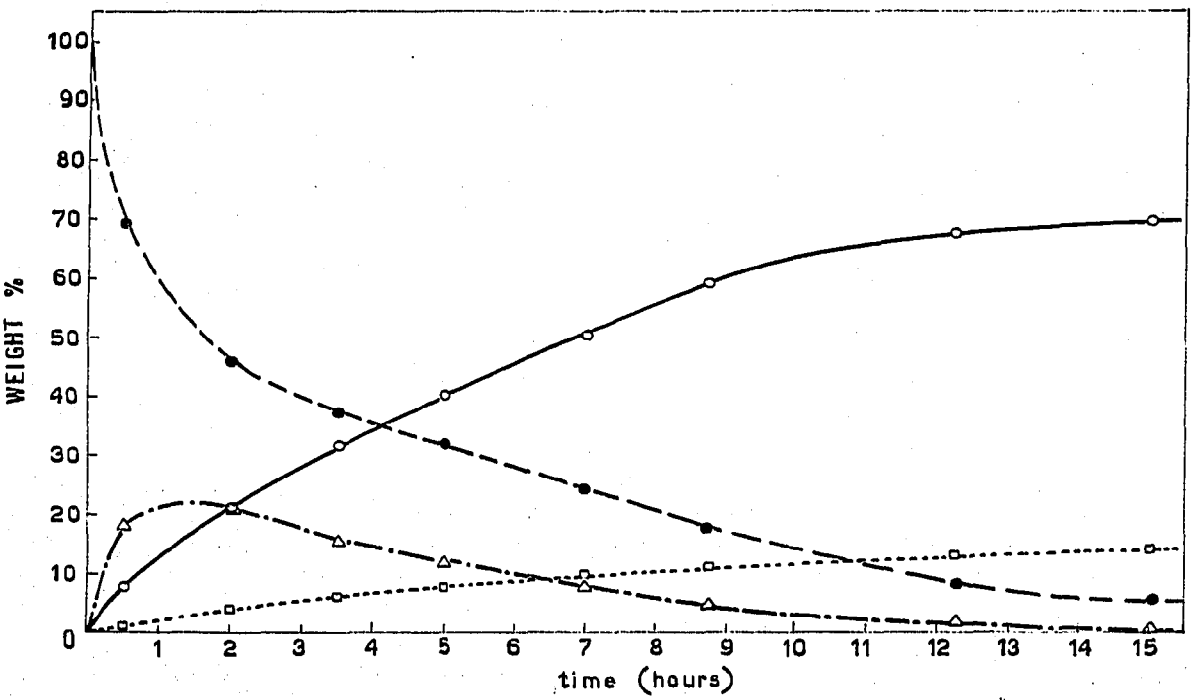

Fig. 3. Percentage of the reaction products as a function of time: □ 7,8-benzoquinoline; ○ 5,6-benzoisoquinoline; △ *trans*-3-styrylpyridine; ● *cis*-3-styrylpyridine.

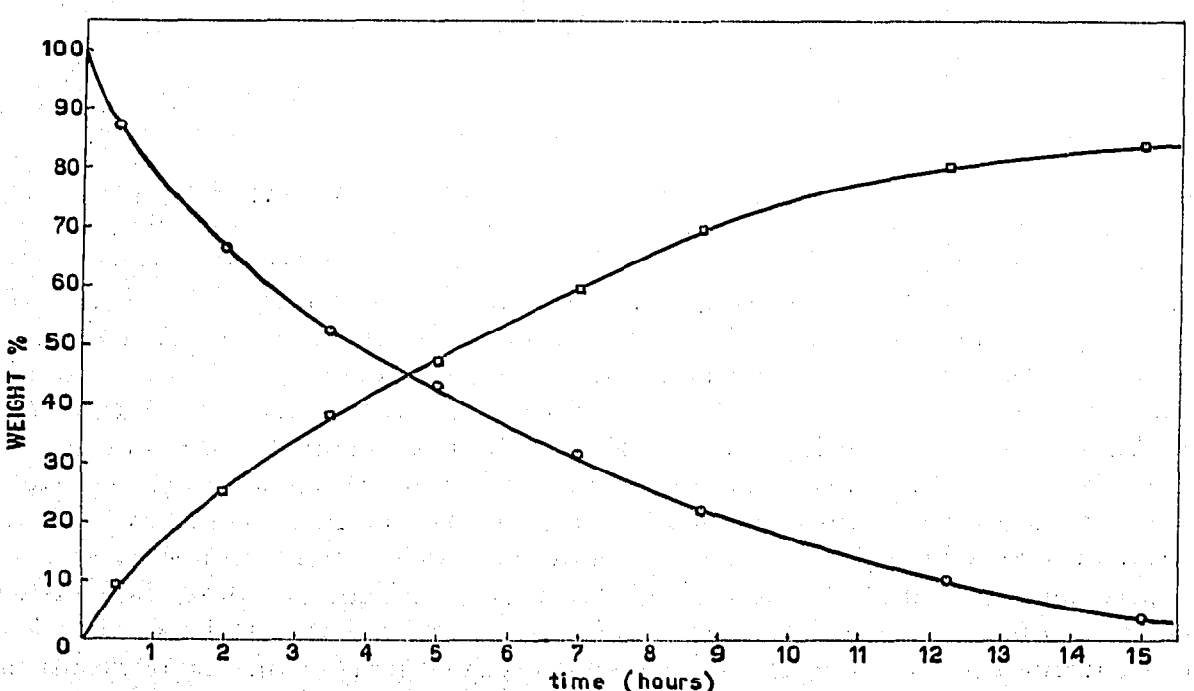

Fig. 4. Extent of photocyclization and photoisomerization: □ 7,8-benzoquinoline and 5,6-benzoisoquinoline, ○ *trans*- and *cis*-3-styrylpyridine.

The recovery of the compounds decreased with time (see Tables II and III). This is due to the increasing yield of the decomposition products which are often formed in many photochemical reactions.

The ratio of the *cis* and *trans* styrylpyridines is not constant with time of irradiation probably because of the fact that a photostationary composition of the two geometrical isomers is not reached owing to the competitive photocyclization of the *cis* compound[3].

The data are plotted in Figs. 3 and 4. On the basis of the plots in Fig. 4 it would appear that the total amount of the cyclization products formed is almost identical with the amount of the *cis–trans* styrylpyridines which disappears.

When the irradiation was carried out under the same conditions but using the 313 mμ, Hg line, the ratio of the two benzoquinolines was markedly increased in favour of the 5,6-benzoisoquinoline, as shown in Table III. The different ratio could indicate the formation of two intermediate dihydrobenzoquinolines in photochemical equilibrium with the reagent *cis*-3-styrylpyridine (see also ref. 16). Presumably the two possible forms of the derivatives have a different absorption spectrum and consequently a different photochemical equilibrium.

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