

## Thin-layer chromatography of isomeric styryl-pyridines and their photocyclization compounds

During the last few years the *cis-trans* photoisomerization of ethylenic derivatives, stilbenes and of styryl-pyridines (stilbazoles) has been the subject of many investigations<sup>1-12</sup>.

The particular importance of these reactions lies in the possibility of obtaining from the *trans* compounds, by irradiation, the corresponding *cis* isomers and derivatives of cyclo-butane, phenanthrene, benzoquinoline and benzoisoquinoline, these being difficult to obtain through the usual chemical reactions<sup>13-15</sup>. Moreover some derivatives of these compounds can be used in the chemotherapy of cancer<sup>16</sup>.

In previous work we studied the chemical and photochemical behaviour of a new series of styryl-pyridines<sup>17-19</sup>. However, the structural analogies, the instability of some isomers and the formation of the cyclic derivatives from side reactions gave rise to troubles regarding the identification and the separation of the photoproducts.

This work deals with the separation by thin-layer chromatography on silica gel of isomeric styryl-pyridines and their cyclic photoproducts.

Thin-layer chromatography due to its selectivity, provides a rapid and guaranteed method of overcoming the difficulties.

### Experimental

#### Materials and methods

Layer: Silica Gel G (E. Merck, A. G., Darmstadt).

Solvents: Merck, Erba, specially prepared for chromatography.

Spreader, chromatography tanks: Desaga GmbH, Heidelberg.

Thickness of the layer: 250  $\mu$ .

The coated plates were left to dry overnight at room temperature. The samples were dissolved in methanol or acetone (optimal concentration of solution: 0.5-1 %) and applied to the plate following the usual procedures.

Among the solvent systems examined the best results were obtained with:

(A) ethyl ether-dimethylformamide (99:1),

(B) *n*-butanol-glacial acetic acid-water (80:20:20).

The ascending technique was used and the plates were removed from the tank when the solvent front reached 15 cm. The time required to cover this distance was about 20 min with solvent (A) and 160 min with solvent (B). Saturation of the tank atmosphere is of particular importance in order to obtain the maximum reproducibility of results and the most satisfactory separation<sup>20</sup>.

Because the *cis-trans* isomerization reaction is particularly sensitive, the solvent systems and the separation conditions used were checked spectrophotometrically to ensure that they did not promote any interconversion between the products examined.

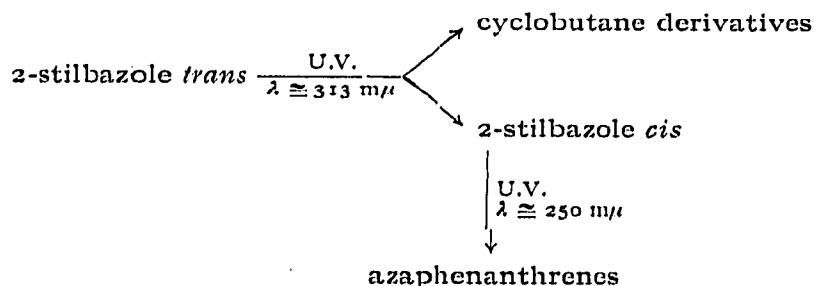
*Detection methods.* Some of the compounds could be localized in U.V. light, through a Wood's filter. However, this method has some disadvantages, *viz.* *cis* derivatives do not fluoresce if they are pure. The fluorescence is actually due to the presence of cyclic products, whose formation could be attributed to the higher photosensitivity of the *cis* derivatives.

The use of the iodine-azide (FEIGL's<sup>21</sup> reagent) gave a useful method of chemical detection for all the substances examined. Spraying with FEIGL's reagent we obtained

fleeting brown spots which could be stabilized by spraying with a dilute solution of starch.

### Results

The photoisomerization reaction of *trans*-stilbazoles to the *cis* isomers often gives cyclic compounds as by-products. For instance, the *trans* isomers can be transformed by irradiation into derivatives of cyclobutane, while the *cis* isomers could give rise to various derivatives of azaphenanthrene<sup>22-24</sup>. The transformation of 2-stilbazole when irradiated is shown in the following scheme:



The expected presence of these cyclic products has been shown for the first time by thin-layer chromatography<sup>24</sup>.

Many of the compounds were subsequently identified by comparison of infrared and ultraviolet spectra and of chromatographic behaviour of the pure compounds obtained unambiguously by appropriate chemical synthesis. Chemical and physical experiments to confirm finally the above mentioned structures are in progress<sup>25</sup>.

In Tables I, II and III the  $R_F$  values of isomeric 2-, 3- and 4-stilbazoles are reported. The related isomers, variously substituted, are shown on the same line for comparison. The values of  $R_F$  reported show that the *cis* and *trans* components are clearly separated from each other.

After having separated the components of a mixture, a spectrophotometric determination can be performed after using extraction techniques.

The maxima of the U.V. absorption and the  $\log \epsilon$  of the molar extinction coefficients are also given. A more detailed description of the U.V. properties is reported elsewhere<sup>18</sup>.

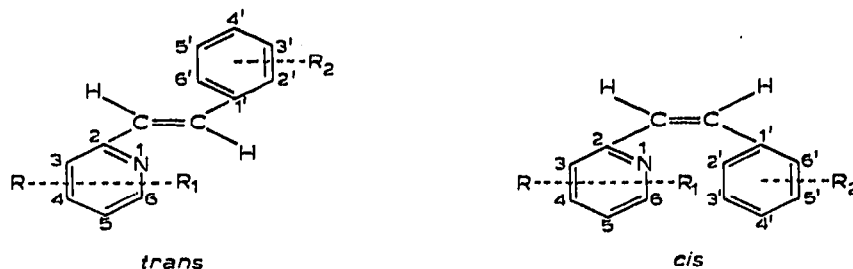
The  $R_F$  values of substituted derivatives of cyclobutane, which are obtained by photochemical dimerization from the corresponding 2- and 4-*trans*-stilbazoles, are given in Tables IV and V. Table V gives  $R_F$  values in solvent B. This solvent is used owing to the poor solubility of this class of compounds in solvent A. This permitted us to separate them selectively from the other types of compounds examined, but did not allow the separation of the derivatives from each other. This could be overcome by using solvent (B), either in monodimensional or in bidimensional separations.

In Table VI, the  $R_F$  values of derivatives of azaphenanthrene obtained by irradiation of 2- and 3-*cis*-stilbazoles are reported.

In Table VII, the  $R_F$  values of the *trans* compounds and the corresponding *cis* isomers are given together with the  $R_F$  values of the cyclobutane (see Tables IV and V) and of the azaphenanthrene derivatives (see Table VI) that can be obtained, as side products, in the course of the irradiation of the *trans* isomers.

TABLE I

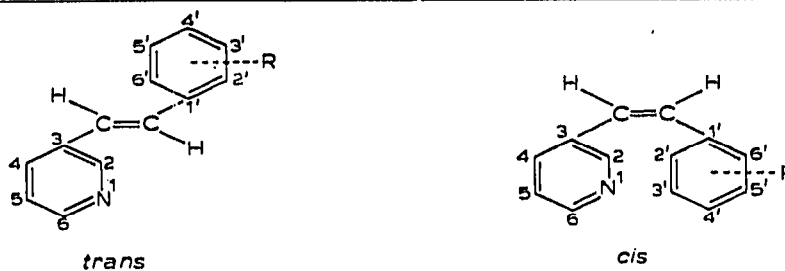
PROPERTIES OF ISOMERIC 2-STILBAZOLES  
Solvent system A.



No.	R	R <sub>1</sub>	R <sub>2</sub>	$\lambda$ max n-hexane (m $\mu$ )	log $\epsilon$ n-hexane	R <sub>F</sub> × 100		$\lambda$ max n-hexane (m $\mu$ )	log $\epsilon$ n-hexane
						trans	cis		
1	H	H	H	226; 314	4.050; 4.435	76	68	285	3.990
2	H	H	4'CH <sub>3</sub>	230; 320	4.104; 4.487	77	72	222; 292	4.183; 4.100
3	3CH <sub>3</sub>	H	H	228; 277; 322	4.104; 4.203; 4.412	82	63	257	3.987
4	4CH <sub>3</sub>	H	H	227; 303; 312	4.154; 4.435; 4.405	73	61	285	4.023
5	5CH <sub>3</sub>	H	H	228; 390; 319	4.020; 4.322; 4.430	75	90	290	4.018
6	6CH <sub>3</sub>	H	H	228; 277; 317	4.081; 4.223; 4.424	83	76	292	4.033
7	3CH <sub>3</sub>	6CH <sub>3</sub>	H	229; 275; 329	4.127; 4.200; 4.395	68	86	238	3.992
8	4CH <sub>3</sub>	6CH <sub>3</sub>	H	229; 278; 315	4.081; 4.165; 4.343	70	80	222; 285	4.193; 4.006

TABLE II

PROPERTIES OF ISOMERIC 3-STILBAZOLES  
Solvent system A.

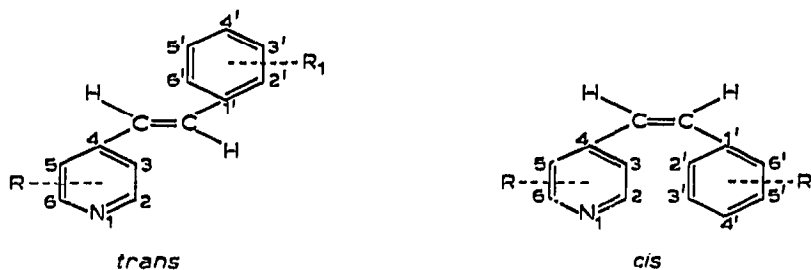


No.	R	$\lambda$ max n-hexane (m $\mu$ )	log $\epsilon$ n-hexane	R <sub>F</sub> × 100		$\lambda$ max n-hexane (m $\mu$ )	log $\epsilon$ n-hexane
				trans	cis		
9	H	277; 300	4.050; 4.380	49	59	270	4.030
10	4'CH <sub>3</sub>	228; 310	4.050; 4.390	45	73	225; 273	4.250; 4.020
11	4'Cl	227; 305	4.090; 4.425	40	61	225; 273	4.255; 4.090
12	4'Br	225; 312	4.022; 4.430	43	69	224; 273	4.254; 4.097
13	4'I	229; 317	4.065; 4.490	48	62	225; 275	4.150; 4.009
14	4'CH <sub>3</sub> O	229; 320	4.070; 4.420	34	51	228; 287	4.191; 4.058
15	4'NO <sub>2</sub>	326; 335	4.470; 4.475	29	45	307	4.040

TABLE III

PROPERTIES OF ISOMERIC 4-STILBAZOLES

Solvent system A.



No.	R	R <sub>1</sub>	$\lambda$ max n-hexane (m $\mu$ )	log $\epsilon$ n-hexane	$R_F \times 100$		$\lambda$ max n-hexane (m $\mu$ )	log $\epsilon$ n-hexane
					trans	cis		
16	H	H	228; 295	4.184; 4.475	41	53	270	3.895
17	3CH <sub>3</sub>	H	220; 300	4.130; 4.377	45	55	265	4.060
18	H	3'CH <sub>3</sub>	230; 299	4.232; 4.483	44	59	283	4.084
19	H	4'NO <sub>2</sub>	320	4.432	27	40	222; 307	4.119; 4.126
20	H	4'CH <sub>3</sub>	228; 301	4.164; 4.454	45	58	223; 295	4.190; 4.120
21	H	4'CH <sub>3</sub> O	231; 318	4.110; 4.451	40	48	229; 293	4.251; 4.044
22	3CH <sub>3</sub>	4'CH <sub>3</sub>	227; 302	4.096; 4.392	46	54	221; 267	4.204; 4.103

TABLE IV

 $R_F$  VALUES OF DIMERS FROM *trans* 2-STILBAZOLES

Solvent system A.

	R	R <sub>1</sub>	$R_F \times 100$
	H	H	70
	3 CH <sub>3</sub>	H	93
	5 CH <sub>3</sub>	H	72
	6 CH <sub>3</sub>	H	74
	3 CH <sub>3</sub>	6 CH <sub>3</sub>	97

TABLE V

$R_F$  VALUES OF DIMERS FROM *trans* 4-STILBAZOLES  
Solvent system B.

	$R$	$R_1$	$R_F \times 100$
	H	2'CH <sub>3</sub>	47
	H	4'CH <sub>3</sub>	50
	H	4'Cl	54
	3 CH <sub>3</sub>	2'CH <sub>3</sub>	41
	3 CH <sub>3</sub>	4'CH <sub>3</sub>	45

TABLE VI

$R_F$  VALUES OF AZAPHENANTHRENE DERIVATIVES FROM *cis* 2- AND 3-STILBAZOLES  
Solvent system A.

	$R$	$R_1$	$R_F \times 100$
	H	H	57
	H	2 CH <sub>3</sub>	66
	H	4 CH <sub>3</sub>	55
	2 CH <sub>3</sub>	4 CH <sub>3</sub>	65
	-	-	96
	-	-	79

TABLE VII

$R_F \times 100$  VALUES OF COMPOUNDS OBTAINED BY PHOTOISOMERIZATION AND PHOTOCYCLIZATION OF 2-, 3-, AND 4-STILBAZOLES

Solvent system A.

<i>2-Stilbazoles</i>					
<i>R</i>	<i>R</i> <sub>1</sub>	<i>Trans</i>	<i>Cis</i>	<i>R<sub>F</sub></i> ( <i>Dimer</i> )	<i>R<sub>F</sub></i> ( <i>Cyclic</i> )
H	H	76	68	70	57
3 CH <sub>3</sub>	H	82	63	93	57
4 CH <sub>3</sub>	H	73	61	—	55
5 CH <sub>3</sub>	H	75	90	72	—
6 CH <sub>3</sub>	H	83	76	74	66
3 CH <sub>3</sub>	6 CH <sub>3</sub>	68	86	97	66
4 CH <sub>3</sub>	6 CH <sub>3</sub>	70	80	—	65
<i>3-Stilbazoles</i>					
H		49	59	—	79 96
<i>4-Stilbazoles</i>					
H	4'CH <sub>3</sub>	45	58	50*	—
3 CH <sub>3</sub>	4'CH <sub>3</sub>	46	54	45*	—

\* Solvent system B.

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## Partition and ion-exchange thin-layer chromatography of water-soluble fluorescent compounds\*

It is essential for quantitative studies of energy transfer that the energy acceptors be free from impurities that either emit fluorescence or absorb strongly in the spectral regions of interest. In preparation for a study of excitation energy transfer between molecules in solution, twelve fluorescent water-soluble compounds have been tested in fourteen thin-layer chromatography systems to find satisfactory methods for analysis and for preparative separations.

Thin-layer chromatography (TLC) is a useful technique for the separation of impurities that would prove troublesome in energy transfer studies, since fluorescent compounds and compounds that absorb ultraviolet light strongly are easily seen on developed chromatograms. Reports in the literature<sup>1</sup> generally indicate silica gel as the adsorbent of choice for the analyses of such fluorescent compounds as anthranilic acid, vitamins B<sub>2</sub> and B<sub>6</sub>, fluorescein, and rhodamine B, although an ion-exchange resin has been used for separation of B vitamins<sup>2</sup>. Air-dried silica gel layers are also reported to give good results on occasion<sup>3</sup>, suggesting that separation may sometimes depend on partition as well as on adsorption, and that layers of unmodified and ion-exchange cellulose might be useful. Also WOLLENWEBER<sup>4,5</sup> and GÄNSHIRT *et al.*<sup>6</sup> have reported the use of cellulose layers for the chromatography of synthetic food colors. In addition, adaptation of successful thin-layer systems to column chromatography should be relatively much easier with cellulose than with air-dried silica gel with its poorly defined water content.

The results to be discussed below indicate that partition and ion-exchange TLC on cellulose is useful for the analysis of a variety of fluorescent water-soluble compounds. Adaptation to column chromatography has not yet been attempted.

### Methods

The layers were either spread in the laboratory (with Bio-Sil A silicic acid, Bio-Sil CM cellulose for TLC, or Serva DEAE cellulose for TLC) or purchased pre-coated (Eastman Chromagram Sheet, Brinkmann silica gel HF, or Brinkmann MN 300 cellulose). Layers prepared in the laboratory were made by mixing the silica gel or cellulose powder with water in a blender, then spreading the slurry on glass plates with an adjustable-thickness spreader (Research Specialties Co.).

The compounds to be studied were dissolved in water or alcohol (except for

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