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Fluorescence Studies on Some 6,7-Substituted 3,4-Dihydroisoquinolines Formed from 3-Hydroxytyramine (Dopamine) and Formaldehyde

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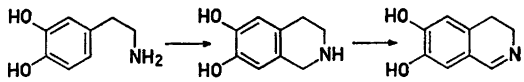
Primary catecholamines such as dopamine and noradrenaline enclosed in a protein layer as well as in tissues readily condense with formaldehyde in a protein-promoted reaction, yielding strongly fluorescent 6,7-dihydroxy-3,4-dihydroisoquinolines. This reaction can be used for the histochemical demonstration of biogenic monoamines. Earlier investigations indicated that the tautomeric quinoidal form is responsible for the fluorescence. In order to establish the exact structure of the quinone, the spectral properties of several synthetic 6,7-substituted 3,4-dihydroisoquinolines were determined. The data show that the 6-quinone structure is the fluorescent tautomeric form of 6,7-dihydroxy-3,4-dihydroisoquinoline.

Primary catecholamines, such as dopamine and noradrenaline, readily condense with formaldehyde in a Pictet-Spengler reaction forming 1,2,3,4-tetrahydroisoquinolines, even when present in a dried protein layer. The tetrahydroisoquinolines are, however, immediately dehydrogenated and converted to their corresponding 3,4-dihydroisoquinolines.[1–4] The latter step is promoted by proteins and certain amino acids, *e.g.* glycine and alanine.[3]



This reaction can be used for the histochemical demonstration of biogenic monoamines, by means of fluorescence microscopy (see review by Hillarp *et al.* 1965 [5]). *m*-Hydroxyphenylethylamines react similarly in principle, forming 6-hydroxy-3,4-dihydroisoquinolines.[6] Investigations [3,6] suggested that in the pH range 6 to 10 and also in the protein layer, the 6,7-dihydroxy-3,4-dihydroisoquinolines exist predominantly in their tautomeric quinoidal form which seems to be responsible for the intense fluorescence at 480 mμ, the

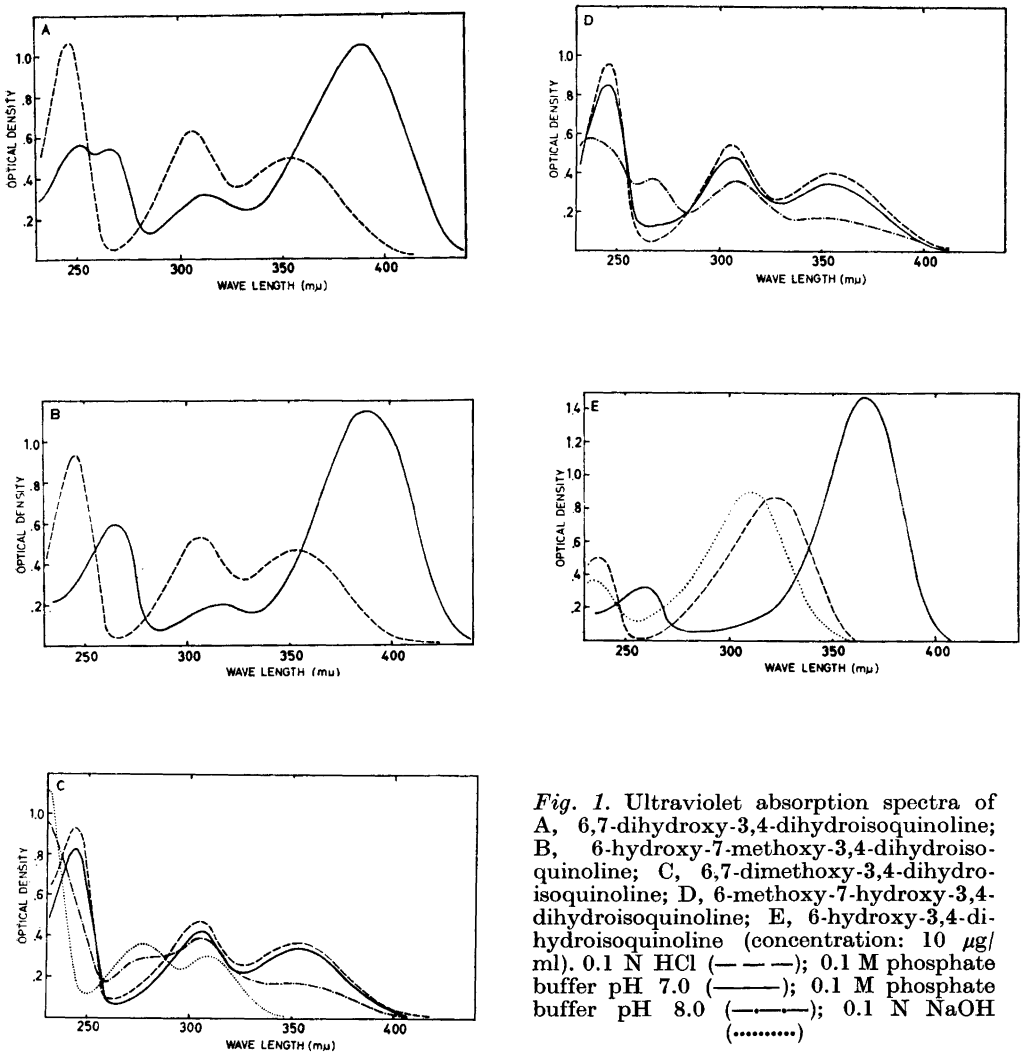
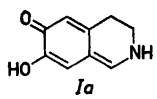
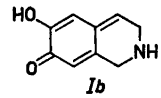


Fig. 1. Ultraviolet absorption spectra of A, 6,7-dihydroxy-3,4-dihydroisoquinoline; B, 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline; C, 6,7-dimethoxy-3,4-dihydroisoquinoline; D, 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline; E, 6-hydroxy-3,4-dihydroisoquinoline (concentration: 10 μg/ml). 0.1 N HCl (— — —); 0.1 M phosphate buffer pH 7.0 (———); 0.1 M phosphate buffer pH 8.0 (—·—·—); 0.1 N NaOH (·····)

quinoidal form being of the type (Ia). In another investigation [7] it was suggested that the structure should rather involve the hydroxy group in position 7 (Ib).



Ia



Ib

In order to elucidate whether the tautomeric quinoidal form is of the type (Ia) or of the type (Ib), the spectral characteristics of several 6,7-substituted 3,4-dihydroisoquinolines have been studied.

MATERIAL AND METHODS

6-Hydroxy-3,4-dihydroisoquinoline hydrobromide, 3-methyl-6-hydroxy-3,4-dihydroisoquinoline hydrobromide, 6,7-dihydroxy-3,4-dihydroisoquinoline hydrobromide and 6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride were synthesized by Dr. H. Corrodi (synthesis see Refs. 2, 3, 6).

The isomers 6-hydroxy-7-methoxy- and 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline (bases) were a gift from F. Hoffmann-La Roche & Co. AG, Basel (through Dr. H. Bruderer; synthesis see Ref. 8).

The substances did not contain any significant amounts of oxidized products, as judged from the spectral and thin layer chromatographical analyses.

Stock solutions were prepared by dissolving the appropriate compound (1.0 mg/ml) in glass-distilled water and stored at −20°C. All salts were calculated as the free base.

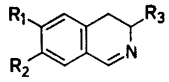
Ultraviolet absorption spectra between 220 and 500 mμ were obtained with a Beckman DU spectrophotometer. Excitation and emission spectra were determined in an Aminco-Bowman spectrophotofluorometer equipped with a Mosely Model 1 Recorder. All measurements were made using a 1/16 inch defining slit and a 1P28 photomultiplier. The light source was a 150 W Hanovia Xenon lamp. All absorption, excitation and emission wavelengths quoted in this paper are instrument readings.

The spectra were examined in 0.1 N HCl, 0.1 N NaOH and 0.1 M buffers (acetic acid-acetate, phosphate, glycine-NaOH) of various pH's. All the substances studied exhibited a fairly pronounced quenching above 1 μg/ml. The solutions were therefore examined at concentrations of about 1 μg/ml or less, where the fluorescence intensity was proportional to concentration.

Solutions were examined for fluorescence and absorption immediately after they had been prepared and fluorescence intensity was always determined within 10—20 sec of exposure to the exciting light to avoid possible photodecomposition and temperature effects. All measurements were made at room temperature.

RESULTS

6,7-Dihydroxy-3,4-dihydroisoquinoline (I) and 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline (II). The ultraviolet absorption spectra of 6,7-dihydroxy-3,4-dihydroisoquinoline and 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline are practically identical throughout the whole pH range. In 0.1 N HCl there are three absorption peaks (see Fig. 1 and Table 1). Up to pH 4 the spectra are unchanged but above pH 5 they undergo characteristic and similar changes for both substances. The most prominent change is the appearance of an absorption peak at 390 mμ, which reaches a maximum at about pH 8. With a further increase of pH up to 13, the absorption at 390 mμ is considerably diminished.



I: $R_1 = R_2 = OH$; $R_3 = H$
II: $R_1 = OH$; $R_2 = CH_3O$; $R_3 = H$
III: $R_1 = R_2 = CH_3O$; $R_3 = H$
IV: $R_1 = CH_3O$; $R_2 = OH$; $R_3 = H$
V: $R_1 = OH$; $R_2 = R_3 = H$
VI: $R_1 = OH$; $R_2 = H$; $R_3 = CH_3$

The dihydroxy compound has almost no or very weak fluorescence in the pH range 1—5, but above pH 5 a fluorescence maximum at 480 mμ appears, which gradually increases with increasing pH. The main activation peak is at

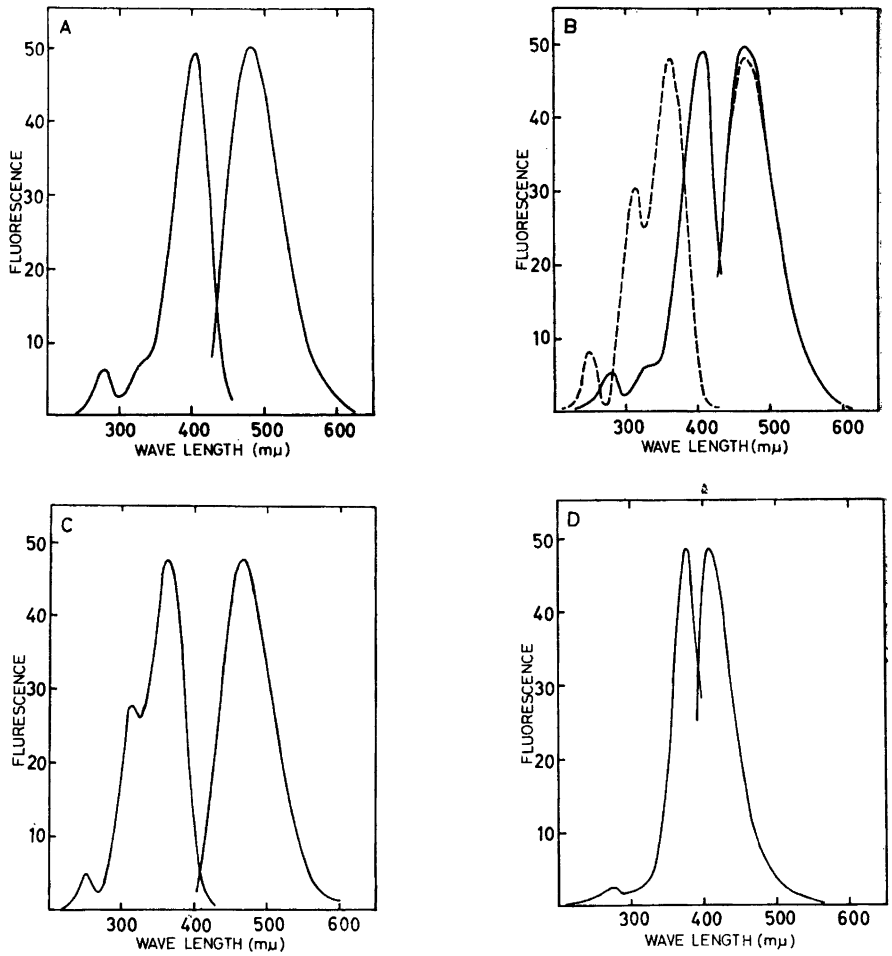


Fig. 2. Excitation and emission spectra of A, 6,7-dihydroxy-3,4-dihydroisoquinoline; B, 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline; C, 6,7-dimethoxy-3,4-dihydroisoquinoline; D, 6-hydroxy-3,4-dihydroisoquinoline (concentration 1 μg/ml). 0.1 N HCl (— — —); 0.1 M phosphate buffer pH 7.0 (———).

400 mμ. The fluorescence intensity reaches a maximum at about pH 8; above this pH, the emission intensity weakens, falling to a small value at higher pH's (see Figs. 2 and 4).

In 0.1 N HCl 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline exhibits a fairly strong fluorescence with a main excitation maximum at 365 mμ and an emission peak at 465 mμ. At about pH 5 the excitation maximum gradually turns to 405 mμ, but still with the maximum fluorescence at 465 mμ (see Figs. 2 and 5). Maximum fluorescence intensity is attained at about pH 8 (see Fig. 3). The fluorescence decreases when increasing pH above 8, and at pH 11

Table 1. Absorption and fluorescence of the various 3,4-dihydroisoquinolines.
Concentration: 10 μg/ml (absorption); 1 μg/ml (fluorescence).

Compound	Solvent	Absorption max. (mμ)	Excitation max. (mμ)	Emission max. (mμ)	Relative fluorescence intensity*
6,7-Dihydroxy-3,4-dihydroisoquinoline	0.1 N HCl	247, 307, 355	320	510	0.002
	Buffer pH 7	251, 266, 312, 390	400	480	1.0
	0.1 N NaOH	235, 328	355	505	
6-Hydroxy-7-methoxy-3,4-dihydroisoquinoline	0.1 N HCl	245, 306, 355	365	465	9.2
	Buffer pH 7	265, 315, 390	405	465	21.3
	0.1 N NaOH	245, 328	350	465	
6,7-Dimethoxy-3,4-dihydroisoquinoline	0.1 N HCl	244, 305, 354	365	470	1.1
	Buffer pH 7	244, 305, 353	365	470	9.3
	0.1 N NaOH	229, 276, 309	320	470	
6-Methoxy-7-hydroxy-3,4-dihydroisoquinoline	0.1 N HCl	245, 305, 355	320	510	0.03
	Buffer pH 7	245, 305, 355	320	510	0.03
	0.1 N NaOH	245, 280, 340	350	515	
6-Hydroxy-3,4-dihydroisoquinoline	0.1 N HCl	236, 323	335	410	0.07
	Buffer pH 7	259, 365	375	410	8.9
	0.1 N NaOH	233, 312	370	410	
3-Methyl-6-hydroxy-3,4-dihydroisoquinoline	0.1 N HCl	238, 323	360	460	0.02
	Buffer pH 7	260, 366	375	410	6.8
	0.1 N NaOH	232, 310	370	410	

* The relative fluorescence intensities are corrected for comparison on a molar basis.

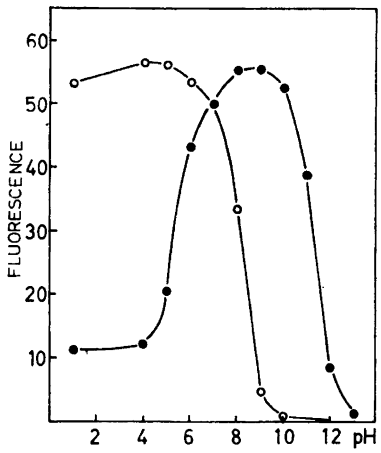


Fig. 3. Variation of fluorescence intensity with pH of 6-hydroxy-7-methoxy (●) and 6,7-dimethoxy- (○) -3,4-dihydroisoquinoline.

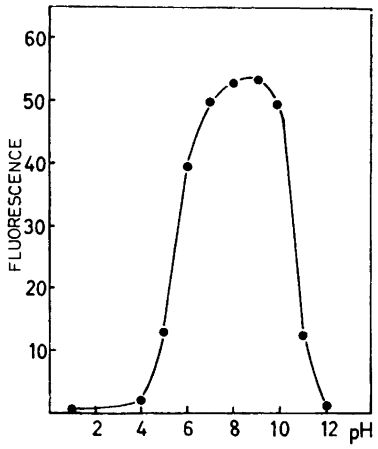


Fig. 4. Variation of fluorescence intensity with pH of 6,7-dihydroxy-3,4-dihydroisoquinoline.

the compound exhibits very weak fluorescence at 465 mμ. In 0.1 N NaOH the excitation maximum has changed to 350 mμ, still with emission at 465 mμ, but the intensity is weaker. The above described pH dependent spectral changes are entirely reversible.

6,7-Dimethoxy-3,4-dihydroisoquinoline (III) and 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline (IV). 6,7-Dimethoxy- and 6-methoxy-7-hydroxy-3,4-dihydroisoquinoline have almost similar ultraviolet absorption properties at all pH's. The spectra are practically identical from pH 1 to 7, and in this pH-range they also resemble those of 6,7-dihydroxy- and 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline between pH 1 and 4.5 (see Fig. 1). Above pH 7.5 they undergo reversible and typical changes. The absorption peak at 355 mμ gradually decreases and disappears (see Fig. 1).

6,7-Dimethoxy-3,4-dihydroisoquinoline shows high fluorescence intensity up to pH 7 with the spectral characteristics: the main excitation peak at 365

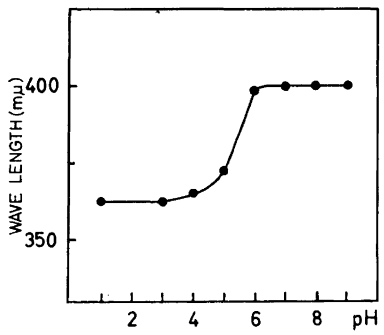


Fig. 5. Variation of excitation maximum of 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline with pH.

mμ and the emission maximum at 470 mμ. Above pH 7.5 the fluorescence intensity decreases, disappearing at higher pH values (see Fig. 3).

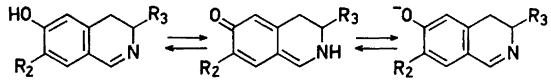
6-Methoxy-7-hydroxy-3,4-dihydroisoquinoline exhibits very weak fluorescence compared to the other 3,4-dihydroisoquinolines investigated and, to obtain more accurate wavelength values, the concentration had to be increased up to at least 10 μg/ml. Maximal excitation and emission (see Table 1).

6-Hydroxy-3,4-dihydroisoquinoline (V) and 3-methyl-6-hydroxy-3,4-dihydroisoquinoline (VI). In 0.1 N HCl 6-hydroxy-3,4-dihydroisoquinoline and its 3-methyl derivative have two absorption peaks; one at 236 mμ and the other at 323 mμ. Above pH 6—6.5 the spectra show typical, drastic changes with the appearance of a strong absorption peak at 365 mμ. This peak increases with pH, reaching a maximum at pH 8—9. Above pH 9 the absorption gradually decreases and in 0.1 N NaOH the absorption characteristics are similar to those in acid solution (see Fig. 1).

The two 6-hydroxy compounds exhibit only weak fluorescence in acid solution. This emission is at all pH's 410 mμ (except for VI at pH 1) and increases at higher pH's attaining a maximum around pH 8. The main activation peak is then at 375 mμ. Above pH 8 the fluorescence intensity falls becoming very weak above pH 12; *cf.* results in Ref. 6.

DISCUSSION

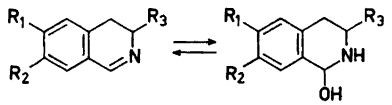
The results show that all the 3,4-dihydroisoquinolines investigated and having a free hydroxy group in position 6, have the same type of pH dependent spectral changes. The appearance of an absorption peak at a higher wavelength and increasing fluorescence intensity at pH's above 4 and 5, with a maximum at approximately pH 8, is no doubt due to transition of the 6-hydroxy-3,4-dihydroisoquinoline into its tautomeric quinoidal form (I→Ia, II→IIa and V→Va).



I,II,V *Ia,IIa,Va* *I,II,Vb*

The quinone structure has been shown to be responsible for the fluorescence for polycyclic phenols as well.[9] Since 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline, where the 7-hydroxy group is blocked, behaves like its dihydroxy derivative, the quinone structure of the latter must be of the type (Ia). This is also supported by the finding that 6-hydroxy-3,4-dihydroisoquinolines have the same pH dependent spectral properties in principle. An increase of pH above 8, results in a decrease and almost disappearance of the longwave absorption and fluorescence. This is probably due to ionization of the hydroxy group in position 6; see also Ref. 6.

The 6,7-dimethoxy- and 6-methoxy-7-hydroxy compounds behave almost identically. The typical spectral changes above pH 7.5 (decrease in absorption at 355 mμ and fluorescence at 470 mμ for III) are certainly due to a transition into the carbinolamine structure (III→IIIa and IV→IVa).[3]



III, IV IIIa, IVa

Concerning the relative fluorescence intensities of the various 3,4-dihydroisoquinolines, the strongest fluorescence is exhibited by those substituted with 6-hydroxy-7-methoxy, 6-hydroxy, and 6,7-dimethoxy groups, whereas those substituted with 6,7-dihydroxy and 6-methoxy-7-hydroxy groups show much weaker fluorescence intensity. Thus, the 7-hydroxy-substitution seems to quench or weaken the emission of a quinone structure of the type (Va). This explains to some extent why 3-O-methylated catecholamines in a dried protein layer (*e.g.* metanephrine, normetanephrine and 3-methoxytyramine) give weak fluorescence when exposed to formaldehyde vapour.[10] Another reason for this is that a 3-methoxy group of a phenylethylamine activates less than a hydroxy group,[11] thus resulting in a lower yield in the Pictet-Spengler reaction.

From the data presented in this paper it can be concluded that the quinoidal form of 6,7-dihydroxy-3,4-dihydroisoquinoline is of the type (Ia). In Ref. 7 the structure (Ib) has been suggested, but with the reaction conditions used a further reaction must have taken place. This has also been proposed by the authors. The strongly fluorescent products formed in these experiments do not seem to have the spectral characteristics of (IV). Preliminary studies have shown that a reaction of this type can occur with catecholamines and 1,2,3,4-tetrahydro- and 3,4-dihydroisoquinolines having a free hydroxy group in position 4 and 7, respectively.[10]

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REFERENCES

1. Falck, B., Hillarp, N.-Å., Thieme, G. and Torp, A. *J. Histochem. Cytochem.* **10** (1962) 348.
2. Corrodi, H. and Hillarp, N.-Å. *Helv. Chim. Acta* **46** (1963) 2425.
3. Corrodi, H. and Hillarp, N.-Å. *Helv. Chim. Acta* **47** (1964) 911.
4. Corrodi, H. and Jonsson, G. *J. Histochem. Cytochem.* **13** (1965) 484.
5. Hillarp, N.-Å., Dahlström, A. and Fuxe, K. *Release of biogenic amines* (Eds.: U. S. von Euler, S. Rosell and B. Uvnäs), Pergamon, Oxford 1966, p. 32.
6. Corrodi, H. and Jonsson, G. *Helv. Chim. Acta* **49** (1966) 798.
7. Bell, C. E. and Somerville, A. R. *Biochem. J.* **98** (1966) lc.
8. Bruderer, H. and Brossi, A. *Helv. Chim. Acta* **48** (1965) 1945.
9. Williams, R. T. *J. Roy. Inst. Chem.* **83** (1959) 611.
10. Jonsson, G. *Unpublished observations.*
11. Whaley, W. M. and Govindachari, T. R. *Org. Reactions* **6** (1951) 151.

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