

Studies on the Fluorophore Forming Reactions of Various Catecholamines and Tetrahydroisoquinolines with Glyoxylic Acid

LEIF-ÅKE SVENSSON,^a ANDERS BJÖRKLUND^b and OLLE LINDVALL^b

^aResearch and Development Department, AB DRACO, * Fack, S-221 01 Lund 1, Sweden and ^bDepartment of Histology, University of Lund, Biskopsgatan 5, S-223 62 Lund, Sweden

Strongly fluorescent 2-carboxymethyl-3,4-dihydro-7-hydroxyisoquinolin-6-ones are formed in high yields when catecholamines are reacted with glyoxylic acid. Formation of the fluorophores has been found to take place in two steps; *i.e. via* virtually non-fluorescent tetrahydroisoquinoline-1-carboxylic acids, which react to give the fluorophores in a subsequent, rapid reaction with glyoxylic acid. The rates of reaction (pseudo first-order) with glyoxylic acid for 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, and 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid show that introduction of a carboxyl group at either C₁ or C₃ in a tetrahydroisoquinoline highly facilitates the reaction with glyoxylic acid. This behaviour is discussed in terms of a mechanism involving both intramolecular acid catalysis by the C₁ or C₃-carboxyl groups during dehydration of the carbinolamine intermediate, and facilitation of the prototropic shifts of the resulting Schiff's base by decarboxylation.

The versatility of glyoxylic acid (GA) as a new histochemical reagent for fluorescence visualization of different biogenic amines, is now becoming well established.¹ Knowledge of the reaction mechanisms underlying the fluorophore-forming reaction is of great importance, not only as a basis for the specificity of the histochemical GA method, but also because such knowledge may offer possibilities both of increasing the yield of the fluorophores as well as favouring reaction with a single, or a group of compounds, by merely changing the reaction conditions in the proper direction. Therefore,

a detailed investigation of the reaction of GA with representative biogenic amines has been undertaken. The reaction mechanism for formation of fluorophores from tryptamine after reaction with GA has recently been elucidated² and found to involve an initial Pictet-Spengler reaction between GA and tryptamine, followed by a second reaction with GA by the initially formed 1,2,3,4-tetrahydro- β -carboline-1-carboxylic acid (I), yielding a fluorescent 2-carboxymethyl-3,4-dihydro- β -carbolinium compound. Moreover, the great difference in rate of reaction with GA as observed for I and 1,2,3,4-tetrahydro- β -carboline, suggests that one of the important features of the -1-COOH group in I may be to act as an intramolecular acid catalyst, and it was proposed that also catecholamines should react with GA in a similar way.

In this paper we report the results from a study of the fluorophore forming reactions of dopamine, noradrenaline, and some derivatives of 1,2,3,4-tetrahydroisoquinoline with GA and analogous carbonyl compounds. Some kinetic measurements have also been performed as an attempt to establish the role of the C₁- or C₃-carboxyl group as intramolecularly operating catalysts.

EXPERIMENTAL

Instrumentation. Infra red spectra (in KBr) were recorded on a Perkin-Elmer 157G infra red spectrophotometer and NMR-spectra on a Varian T-60 instrument. Chemical shifts in D₂O are usually given together with the shift for the DOH-signal on an arbitrary scale,

* Subsidiary of AB ASTRA, Sweden.

while in other solvents the chemical shifts refer to TMS as an internal standard. Mass spectral analyses were performed on a LKB 9000 instrument, usually operated at 70 eV. Kinetic measurements were performed on a Zeiss DMR 21 ultra violet spectrophotometer, equipped with a HETO ultra-thermostat (Birkerød, Denmark), set at 30.0°C.

Kinetic measurements. The kinetic measurements were performed by rapidly adding a small volume (usually 100 μ l) of the appropriate amine ($\sim 10^{-3}$ M stock solution) to a thermostated cuvette, containing 0.1 M glyoxylic acid (3.0 ml). After stirring for a few seconds by blowing air through the solution, the time versus extinction curve was recorded at an appropriate wavelength. The rates were usually followed to completion and the pseudo-first-order rate constants were calculated in the conventional manner. The method of Guggenheim,³ was, however, used to calculate constants from the slower reactions. In all cases, excellent first order kinetics was observed, and the rate constants obtained from triplicate runs were usually reproducible within $\pm 5\%$ of the mean value.

Thin layer chromatography (TLC) was performed on Merck Fertigplatten F₂₅₄, silica gel and cellulose, and the solvent systems used were ethanol-2 M HOAc, 1:1, saturated with boric acid; ethanol-HOAc, 1:1; butanol-HOAc-H₂O, 4:1:5.

Materials. All solvents and other commercially available reagents were of *p.a.* quality.

Ethyl glyoxylate was prepared according to Ref. 4.

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, VIII, was prepared according to Ref. 5. Mass spectrum $M^+ = 209$.

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline, X, was prepared by reacting dopamine-HBr (0.23 g) and formaldehyde (0.2 ml, 33% solution) in ethanol (5 ml) for 20 h at room temperature. After precipitation with diethyl ether/CHCl₃, 0.2 g X, m.p. 260°C (decomp.) was obtained. NMR (D₂O): δ 3.09 (m, 2 H), 3.60 (m, 2 H), 4.90 (DOH), and 6.84 (m, 2 H).

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, VII. Dopamine-HBr (0.24 g) was dissolved in a small volume of water, whereafter glyoxylic acid monohydrate (0.1 g) dissolved in 1 ml of water was added dropwise with stirring during 15 min. pH was then adjusted to ~ 5 and the zwitterionic form of VII precipitated; IR: $\nu_{C=O}$ 1640 cm⁻¹. Yield: 0.17 g. Recrystallization from ethanolic hydrogen chloride/diethyl ether gave the hydrochloride salt of VII. IR: $\nu_{C=O}$ 1740 cm⁻¹. NMR (D₂O): δ 3.04 (t, 2 H), 3.64 (m, 2 H), 4.90 (DOH), 5.25 (s, 1 H), 6.80 (s, 1 H), and 7.14 (s, 1 H).

4,6,7-Trihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, III, was prepared in the same manner as described for VII above.

The zwitterionic form of III had an IR spectrum identical to that of a sample of II, (kindly supplied by Dr. Gagnault). After exposure of the KBr pellet to HCl gas or crystallization from ethanolic hydrogen chloride, the IR spectrum displays a $\nu_{C=O}$ at 1730 cm⁻¹. NMR (DMSO-*d*₆ + CF₃COOH): δ 3.97 (m, 2 H), 4.74 (m, 1 H), 5.17 (s, 1 H), and 7.00 (s, 2 H).

2-Carboxymethyl-3,4-dihydro-7-hydroxy-isoquinolin-6-one, IV. Dopamine hydrobromide (0.2 g) and glyoxylic acid monohydrate (0.5 g) in *tert*-butyl alcohol (25 ml) and water (2 ml) was allowed to stand at room temperature for 20 h. The zwitterionic form of IV was then precipitated by the addition of diethyl ether. Yield: 0.2 g. The zwitterion was then transformed into IV-HCl by recrystallization from ethanolic hydrogen chloride-diethyl ether. IR: ν 1730, 1650 cm⁻¹. NMR (DMSO-*d*₆): δ 3.10 (t, 2 H), 4.00 (t, 2 H), 4.96 (s, 2 H), 6.95 (s, 1 H), 7.33 (s, 1 H), 9.25 (s, 1 H).

When ethanol was used as a solvent a small crop of IV-ethyl ester could be isolated from the mother liquor. NMR (DMSO-*d*₆): δ 1.27 (t, 3 H, $J = 7$ Hz), 3.10 (t, 2 H), 4.02 (t, 2 H), 4.27 (q, 2 H, $J = 7$ Hz), 5.04 (s, 2 H), 7.00 (s, 1 H), 7.34 (s, 1 H), 9.23 (s, 1 H). IR: ν 1740, 1640 and 1220 cm⁻¹.

2-Carboxymethyl-3,4-dihydro-4,7-dihydroxyisoquinoline-6-one, V, and **2-carboxymethyl-3,4-dihydro-7-hydroxyisoquinolin-6-one-3-carboxylic acid, VI**, were prepared in the same manner as IV.

V: IR: ν 1755, 1735, 1650 cm⁻¹. NMR (DMSO-*d*₆): δ 4.07 ("d", 2 H), 4.90 ("t", 1 H), 4.97 (s, 2 H), 7.21 (s, 1 H), 7.44 (s, 1 H), 9.25 (s, 1 H).

VI: IR: ν 1740, 1640 and 1620 cm⁻¹. NMR (D₂O + CF₃COOD): δ 4.20 (d, 2 H), 5.53 (s, 2 H), 5.60 (m, 1 H), 6.47 (DOH), 7.57 (s, 1 H), 7.93 (s, 1 H) and 9.40 (s, 1 H).

2,4-Dicarboxymethyl-7-hydroxyisoquinolin-6-one, IX. To 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid hydrochloride (1.3 g), dissolved in DMSO (20 ml) was added a solution of GA (2 g) in DMSO (5 ml). The orange coloured reaction mixture was allowed to stand at room temperature for 5 days, when NMR showed that all starting material had disappeared. The product was then isolated as yellow crystals by precipitation with diethyl ether and acetonitrile. NMR (DMSO-*d*₆ + CF₃COOD): δ 4.04 (s, 2 H), 5.45 (s, 2 H), 7.40 (s, 1 H), 7.62 (s, 1 H), 8.31 (s, 1 H), 9.40 (s, 1 H). IR: ν 1720 and 1630 cm⁻¹. MS: *m/e* 203, 189, 175, 161, 147, 140, 132.

2-(1-[Trifluoromethyl]-ethyl)-7-hydroxy-3,4-dihydroisoquinolin-6-one, XII. A solution of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrochloride (0.2 g) and trifluoroacetone (0.5 g) in DMF (10 ml) was heated in a closed vessel at 80°C for 20 h. Diethyl ether was then added to the brown-red solution and the hygroscopic precipitate collected. Mass spectrum: *m/e* 259 (M^+), 230, 192, 190, 179,

178, 177, 162, 153, 148, 136, 129.5 (M^{2+}) and 124.

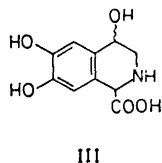
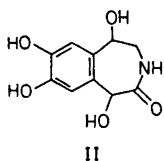
When this reaction was performed in the NMR-sample tube, the C_1 -proton appeared at δ 9.3 and the $-CH_3$ protons as a doublet at δ 1.8 ($J = 8$ Hz).

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid ethyl ester, XV. To dopamine-HBr (0.2 g) dissolved in water (1 ml) was added ethyl glyoxylate (0.1 g). The mixture was then allowed to stand for 20 h at room temperature. EtOH was then added and the mixture evaporated to dryness. The residue was dissolved in MeCN, filtered and to the filtrate was added Et₂O to precipitate the ester. Yield: 150 mg, m.p. 192°C (uncorr., decomp.). IR: $\nu_{C=O}$ 1730 cm^{-1} . NMR (DMSO-*d*₆) δ 1.40 (t, 3 H, $J = 7$ Hz), 2.92 (m, 2 H), 3.45 (m, 2 H), 4.30 (q, 2 H, $J = 7$ Hz), 5.30 (s, 1 H), 6.67 (s, 1 H), and 6.90 (s, 1 H).

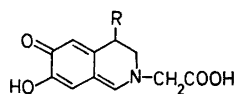
RESULTS AND DISCUSSION

A. Elucidation of the structures of the GA-catecholamine fluorophores

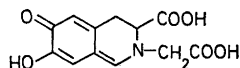
The reactions of noradrenaline and other similar compounds with GA in water solution have been investigated by Fournau and co-workers.⁶ They found that when equimolar amounts of noradrenaline and GA were allowed to react under "physiological" conditions, a 77 % yield of a compound claimed to be the 1,5,7,8-tetrahydroxy-tetrahydrobenzazepine-3-one-2, II, was obtained, together with a mixture of *cis*- and *trans*-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid, III.



We found, however, that when dopamine, noradrenaline, and Dopa are allowed to react with GA in polar solvents (water, alcohols, wet DMF and DMSO), compounds with the structure IV and V and VI are formed in high yield. In contrast to the tetrahydroisoquinolines, compounds IV, V, and VI have strongly fluorescent properties, which is the basis for the use of this reaction as a histochemical tool.



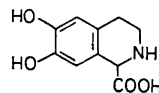
IV R = H
V R = OH



VI

On the other hand, we have not been able to demonstrate the formation of compounds like II, the formation of which also seems highly unlikely under the actual reaction conditions.

Formation of IV and V has been found to proceed *via* a rapid further reaction with GA by the initially formed tetrahydroisoquinoline-1-carboxylic acids; *i.e.* III and VII.

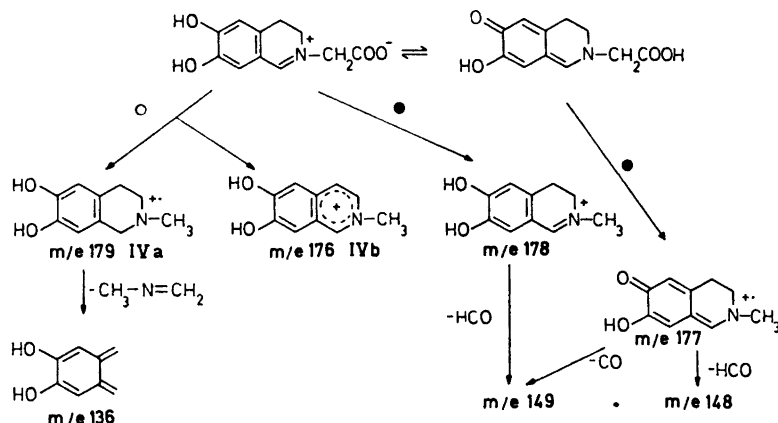


VII

The rates of fluorophore formation are rather slow in aqueous solution, while the reactions proceed very efficiently in dipolar aprotic solvents like DMF and DMSO.

Preparatively, compounds IV and V could be synthesized in high yields (~ 90 %) (see Experimental) after reaction of dopamine and noradrenaline, respectively, with GA in *t*-BuOH or in DMF containing 4 % water. If other alcohols were used as solvents in the reaction, esters of IV and V with these alcohols were concomitantly formed. The yields of the esters formed were usually below 25 %. The proposed structures for IV and V are in excellent agreement with their IR and NMR spectroscopic properties.

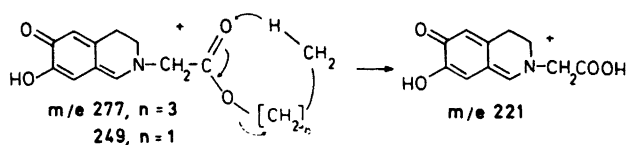
Some support for the proposed structure can also be obtained from the mass spectrum of IV, although any molecular ion cannot be detected, probably due to thermally induced decarboxylation of IV in the mass spectrometer. Generally 3,4-dihydroisoquinoline derivatives undergo decomposition and disproportionation when heated,⁷ for instance in the direct inlet



Scheme 1. O, Disproportionation and decarboxylation in the direct inlet of the mass spectrometer followed by electron impact; ●, Decarboxylation in direct inlet followed by electron impact.

system of the mass spectrometer, and the spectrum will thus also contain fragmentation processes originating from the disproportionated products, e.g. IVa and IVb (see Scheme 1). Thus in the mass spectra of IV, the most abundant ions are found at m/e 179, 178, 177, 176, 149, 148, and 136, where the ions at m/e 176–9 may have been formed by loss of CO_2 from IV and its disproportionated products (see Scheme 1).

of IV and V were also recognized in the spectrum. These observations make it highly unlikely that a compound like II is formed from dopamine and noradrenaline in their reactions with GA in water solution. In our opinion, the compounds claimed to have the structure II should instead be formulated as the zwitterionic forms of the tetrahydroisoquinoline-1-carboxylic acids, III and VI, respectively, because both an authentic sample of the compound claimed



Scheme 2.

Further mass spectroscopic support for the structures IV is found in the mass spectra of the butyl and ethyl esters of IV, where a McLafferty rearrangement of the molecular ions from the esters gives rise to a common ion at m/e 221, while the rest of the spectra is quite similar to that obtained from IV (Scheme 2).

The spectral properties of V are quite similar to those observed for IV, the presence of an -OH group at C_4 in V being reflected in the NMR and mass spectra.

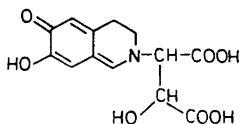
When studying the reactions of dopamine and noradrenaline with GA in D_2O in the NMR probe, formation of VI and III, respectively, were observed, and when the reaction was allowed to proceed for a longer time, formation

to have the structure II and the zwitterionic form of III reveals the C_1 -H as a singlet located at δ 4.50 ($\text{DMSO}-d_6$) while after addition of CF_3COOH the signal for the C_1 -H is shifted to δ 5.17. Such a behavior is to be expected from a structure like III, but not from a structure like II.*

Side reaction. When either dopamine or VII is allowed to react with an excess GA in neat dipolar aprotic solvents, two additional compounds are formed together with IV, as revealed by TLC. These compounds have

* *Added in proof.* At GC-MS analysis of the TMS-derivatives of III, and the compound claimed to have structure II, both compounds gave identical retention times and mass spectra with characteristic fragment ions of an isoquinoline structure.

fluorescent properties similar to those observed for IV, one of the products formed has been identified as the following structure:⁸



B. Reaction product from 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (VIII)

The reaction of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (VIII) with GA, the kinetics of which is also discussed below, is suggested to follow a reaction path, involving the generation of a reactive 1,2-dihydroisoquinoline intermediate,⁹ which then undergoes a further reaction with GA (Scheme 3).

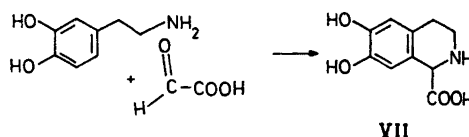
Compound IX is formed in almost quantitative yield (see Experimental), and any accumulation of intermediates could not be detected when the reaction was followed in the NMR sample tube.

C. Kinetics of the reaction of dopamine and some tetrahydroisoquinoline derivatives with GA

The kinetics of the reaction between GA and dopamine and VII at 30°C has been investigated

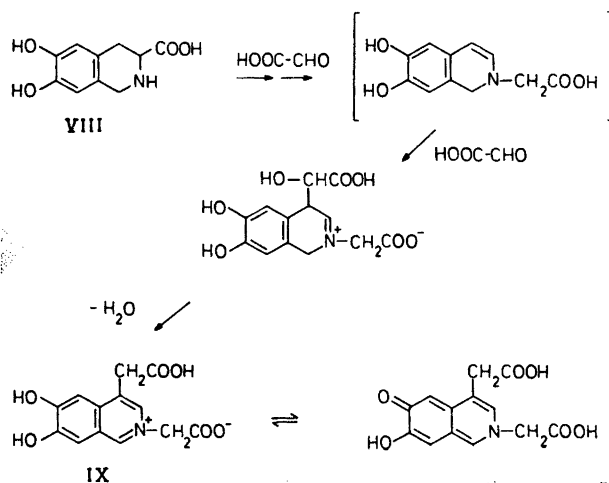
and in order to evaluate the influence of the -1-COOH- group on the rates, the reactions of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (X) and VIII with GA have also been investigated. All reactions were carried out in an excess of GA in order to obtain pseudo-first order conditions. Relevant rate constants are shown in Table I, where also the rate constant for the reaction of 1,2,3,4-tetrahydro- β -carboline-1-COOH, (I) with GA is given for comparison.

From the rate constants in Table I it is evident that after the introduction of a -COOH group in X, at either C₁ as in VII or at C₃ as in VIII, considerable enhancement of the reactions with GA is obtained. It can also be seen that the first step in the reaction of dopamine with GA in EtOH, *i.e.* formation of VII, (Scheme 4) is slower than its further reaction with GA.



Scheme 4.

When the reaction of dopamine with GA was performed in neat DMF, only about 15% of the calculated absorbance at 380 nm was reached. This may be due to concomitant formation of the presumably colourless lactone XI, the formation of which ought to be



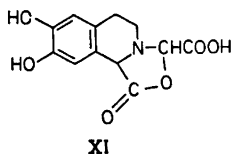
Scheme 3.

Table 1. Pseudo-first order rate constants, k_{obs} min^{-1} , at 30.0 °C for the reactions of I, VII, VIII, X, and dopamine with different carbonyl compounds.

Compound	VII				VIII	X	Dopamine	I	
Solvent	EtOH	DMF	DMF + 4 % H ₂ O	DMF + 0.1 M HCOOH	DMF	DMF	EtOH	DMF	EtOH
Reagent									
GA	0.13 ^a	38	38	—	0.15 ^d	$\ll 10^{-4b}$	0.026 ^e	0.7 ^f	0.037
GA-ethyl ester	—	18	—	—	—	—	—	—	—
FA	—	0.065 ^b	—	0.065 ^c	—	$\ll 10^{-4g}$	—	—	—
CF ₃	—	0.02	—	—	—	—	—	—	—
CH ₃ -C=O									

^a Half the actual conc. of VII gave the same value; ^b measured at = 420 nm; ^c measured at = 278 nm; ^d measured at = 350 nm; ^e measured at = 294 nm, the isosbestic point for GA and IV; ^f rate constant for formation of VII; ^g DMF + 0.1 M HCOOH, estimated value; ^h estimated value.

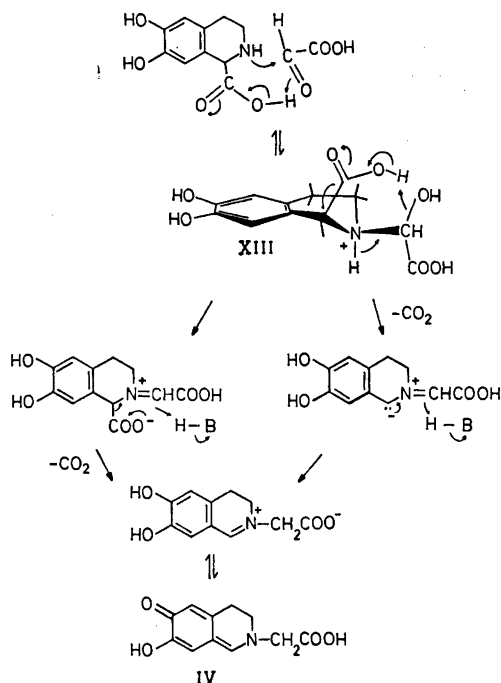
suppressed when the concentration of water in the DMF solution is increased.



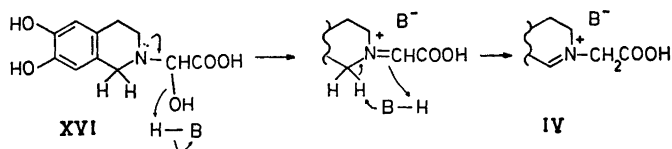
The absorption *vs.* *t*-curve, however, obeyed first order kinetics and gave a rate constant of 0.7 min^{-1} . The same value was obtained in DMF containing 4 % water, where the calculated absorbance also was reached. The value of 0.7 min^{-1} for the rate constant may be taken as an approximative value for the rate constant for formation of VII from dopamine and GA. This is a fair approximation since the following step in the reaction is very fast (38 min^{-1}).

The dramatic increases in rates of reaction with GA, as observed after introduction of a -COOH group at C₁, (VII), or at C₃, (VIII), in the 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (X), is in the opposite direction to what should be expected from the increased steric hindrance from the introduced carboxyl groups and from the hence lessened nucleophilicity of the nitrogen atoms in VII and VIII compared to that in X. The high rates may therefore be due to a combination of intramolecular carboxyl group catalysis in the de-

hydration of the carbinolamine intermediate and facilitation of prototropy in the Schiff's base by decarboxylation of the suitably situated carboxyl group (see Scheme 5).



Scheme 5.



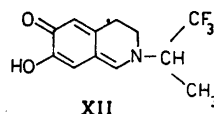
Scheme 6.

Thus, the differences in rates observed for VII and VIII in their reactions with GA are in the proper direction and may partly reflect the somewhat weaker acidity, and hence lessened catalytic efficiency of the 3-COOH group. But also the increased conformational flexibility of the 3-COOH group in VIII compared with the 1-substituted one in VII, which is obvious by inspection of molecular models, may affect the rate in the direction observed. The difference in pK_a -values for VII and VIII is supposed to be of the same order as for phenylalanine and phenylglycine, which have $pK_a=1.80$ and 2.16 , respectively.¹⁰ The pK_a -values for the ammonium functions in VII and VIII were found to be 3.4 and 3.3 , respectively.¹¹ Although strongly basic amines generally attack carbonyl groups without acid catalysis,¹² the juxtaposition of an amino and an acid function as in VII and VIII, may well be an effectively operating catalytic system in reactions with carbonyl compounds.^{13,14}

That the carboxyl group in GA is unimportant as a catalyst in this system is obvious from the small difference in rates observed for VII in its reactions with GA and GA ethyl ester. The more prominent rate difference observed for VII in its reaction with GA and FA respectively, will mainly be caused by the increased electrophilicity of the carbonyl carbon atom in GA, caused by the presence of the electron withdrawing carboxyl group. The presence of FA-polymers may, however, also be considered as a rate-affecting factor. Intermolecular acid catalysis seems to be unimportant in the reactions of VII with FA, since the rates are not significantly affected by the presence of 0.1 M formic acid.

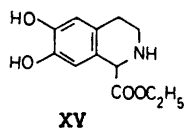
A support for the assumption that intramolecular acid catalysis is involved in the dehydration of the carbinol-amine is found in the reactions of VII with hexa- and trifluoroacetone. Although these carbonyl com-

pounds, owing to the strongly electron-withdrawing properties of the CF_3 -groups usually form very stable carbinolamines,¹⁵ those formed in the reaction with VII are rapidly dehydrated to yield fluorophores of the same type as those obtained from GA and FA, *i.e.* XII.



Considering the further fate of the carbinol-amine intermediate (XIII), the fluorophores (IV) will be formed after decarboxylation and prototropy, according to Scheme 5. The decarboxylation reaction may take place concerted with, or following dehydration; neutralization of the positive charge on the nitrogen and gain of resonance stabilization being the driving forces for the prototropic shifts.

The involvement of decarboxylation in the further reaction of the carbinolamine (XIII) as depicted above, may be advantageous over that of the carbinolamine derived from X and GA (*e.g.* XIV) where the prototropic shifts in the Schiff's base have to solely rely on gain of resonance stabilization, probably assisted by a general base (Scheme 6). However, even if the decarboxylation reaction thus seems to play an important role in the prototropic reaction, decarboxylation alone cannot account for the dramatic rate enhancement obtained when going from X to its carboxyl substituted derivatives VII and VIII. Extremely low reactivity is also observed for the ethyl ester of VII, (XV), which in fact, does not display any visible reaction with GA when monitored in the NMR probe for two days. It thus seems like dehydration of the carbinol-amine intermediate must also be an important step in the reaction leading to IV and V, but that this reaction requires an efficient catalyst,



most probably supplied by the 1- or 3-COOH groups in VII and VIII.

REFERENCES

1. a. Axelsson, S., Björklund, A., Falck, B., Lindvall, O. and Svensson, L. Å. *Acta Physiol. Scand.* 87 (1973) 57; b. Lindvall, O. and Björklund, A. *Histochemistry* 39 (1974) 97; c. Lindvall, O., Björklund, A. and Svensson, L. Å. *Histochemistry* 39 (1974) 197.
2. a. Svensson, L. Å. *Experientia* 28 (1972) 874; b. Björklund, A., Lindvall, O. and Svensson, L. Å. *Histochemie* 32 (1972) 113.
3. Guggenheim, E. A. *Phil. Mag.* 2 (1926) 538.
4. Kelly, T. R., Schmidt, T. E. and Haggerty, J. G. *Synthesis* 4 (1972) 544.
5. Brossi, A., Focella, A. and Teitel, S. *Helv. Chim. Acta* 55 (1972) 15.
6. a. Fourneau, J. P., Gagnault, C., Jacquier, R., Stoven, O. and Davy, M. *Chim. Ther.* 4 (1969) 67; b. Gagnault, J. C. *Diss.* University of Montpellier (1969).
7. Brodrick, C. I. and Short, W. F. *J. Chem. Soc.* (1949) 2587.
8. Hansson, C. and Wickberg, B. *Personal communication.*
9. Battersby, A. R. and Binks, R. *J. Chem. Soc.* (1955) 2888.
10. Perrin, D. D., Ed., *Dissociation Constants of Organic Bases in Aqueous Solution*, Butterworths, London 1965.
11. Hugosson, S. AB Draco. *Personal communication.*
12. Jencks, W. P. *Catalysis in Chemistry and Enzymology*, Mc Graw, New York 1969, p. 52.
13. Hine, J., Cholod, M. S. and Chess, Jr. W. K. *J. Amer. Chem. Soc.* 95 (1973) 4270.
14. French, T. C., Auld, D. S. and Bruice, T. C. *Biochemistry* 4 (1965) 77.
15. Zeifman, Yu. V., Gambaryan, N. P. and Khunyants, I. L. *Izv. Akad. Nauk. SSSR Ser. Khim.* (1965) 450.

Received September 17, 1974.