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Addition of Amines to Methyl 3-Dehydroquinate and 3-Dehydroshikimate

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Abstract : Reaction of 3-dehydroquinate compounds **1-3** with primary amines is studied in CH_2Cl_2 and MeOH. The reaction yields 3-dehydroshikimate **4** (in CH_2Cl_2) and(or) 3-alkylamino 4-hydroxybenzoate derivatives **6** or **7**. The results support a Schiff base formation and an intramolecular catalysis by a specific diamine [2-(2-(aminoethyl)pyridine]. The effects of the imine intermediate and of the solvent on the aromatization reaction are discussed.

The shikimic acid pathway,[1] leading to the biosynthesis of aromatic aminoacids is specific to microorganisms and plants. It has attracted much interest at various levels : primary structure determination of the different enzymes [2] involved, study of the reaction mechanisms,[3] synthesis of substrate analogs and of inhibitors.[4]

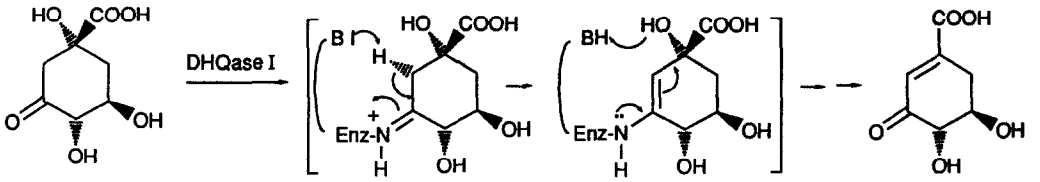
The enzyme 3-dehydroquinase catalyses the third step of this process, which is the transformation of 3-dehydroquinic acid (3-DHQ) into 3-dehydroshikimic acid (3-DHS). Three classes of biosynthetic 3-dehydroquinases are known : the *E. Coli* enzyme is monofunctional,[5] the *Neurospora crassa* enzyme occurs in a pentafunctional polypeptide chain [6] and the plant enzyme acts in a bifunctional complex with shikimate dehydrogenase.

Two DHQase activities have been found in certain plants. The first one (DHQase I) is associated with shikimate oxidoreductase (SHOrase) and is involved in the shikimic acid pathway ; the second one (DHQase II) is associated with quinate oxidoreductase (QOrase) and is involved in the catabolic pathway leading to the protocatechuic acid.

The two DHQases interact to regulate aromatic aminoacids.[7] The enzymic reaction in DHQase I proceeds through a multistep mechanism [8] involving :

- formation of a Schiff base between the substrate's ketone function and a primary amine (supposedly a lysine of the active site) ;
- enantioselective deprotonation of the pro(R) proton on C-2 ;
- β-elimination of the hydroxy group on C-1 leading to 3-dehydroshikimic acid (scheme 1) ;

The proposal of an imine intermediate is founded on a well precedented series of experiments. Recently, direct spectroscopic evidence of an imine intermediate by electrospray spectroscopy has been reported. [9]

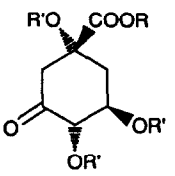


Scheme 1

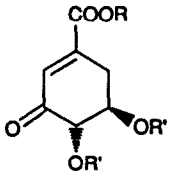
Although studies on the hydrolysis of α,β unsaturated Shiff bases [10] and on the formation of iminium ions derived from β-acetoxy-ketone [11] have been reported and proposed as models of DHQase, no results on the reactivity of 3-dehydroquinic acid towards amines have yet been evaluated. We thus present our first results on the role of the amine and the solvent in this reaction.

Results

The reactivity of 3-dehydroquinic (**1-3**) and 3-dehydroshikimic (**4, 5**) acid derivatives [12] toward primary amines of varying basicity were studied. Two aminoacids methyl glycinate, L-lysine N-a and L-lysine methylester N-ε BOC protected (**g, h, i**) were studied. For comparative purposes the action of morpholine and triethylamine were also included.



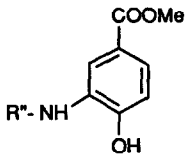
1 R = Me, R' = $SiMe_3$
2 R = Me, R' = H
3 R = R' = H



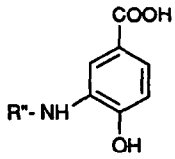
4 R = Me, R' = $SiMe_3$
5 R = Me, R' = H

Among the existing methods for Schiff bases formation [13] we have chosen the one based on the use of molecular sieves.[14] It can be applied successfully to sterically hindered and medium size ring ketones, while allowing a wide choice of solvents. This method is preferable when working in CH_2Cl_2 with 3-DHQ acid which lactonises on heating. Reactions are thus carried out at room temperature with one equivalent of amine versus substrate either in methylene chloride (non-protic apolar solvent) or in methanol (polar protic solvent). The reaction is controlled by thin layer chromatography (TLC) on silica plates till reactants are seen to disappear or remain constant.

We first studied the reaction of amines with DHQ compounds (**1-3**) (Table 1). The final products from the reaction with primary amines in methylene chloride were identified after purification as being the protected methyl-3-dehydroshikimate **4** and the corresponding methyl 3-alkylamino 4-hydroxy benzoates **6d-6f**. However the same reaction when undertaken in methanol yields only one product (50-70%) which is the corresponding aromatic amine derivatives (**6d-6i** and **7g-7i**). Other products present could not be identified ; they are probably polycondensation or degradation products of phenolic derivatives.



6d-6i



7g-7i

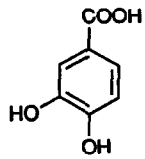


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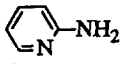
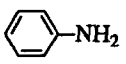
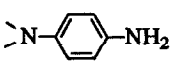
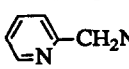
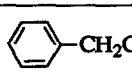
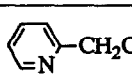
Table 1 : Reaction of 3-dehydroquinic acid derivatives with amines in CH_2Cl_2 and MeOH

Entry		Amine	15 (pK_A)	substrate	Reaction time (h) CH_2Cl_2	MeOH	Yields of products * (%) 1	4	6 or 7	8
1	a		(-7.60)	1	72		100			
2	b		(4.60)	1	72		100			
3	c		(6.71	1	72		100			
4				2		72	97		3	
5	d		(8.62)	1	24			44	42	
6						24			70	
7	e		(9.08)	1	24			41	34	
8						18			54	
9	f		(9.52)	1	2			50	47	
10						2			55	
11	g	$HCl.NH_2CH_2COOMe$	(7.73)	1		0.5			65	
12				3		0.5			55	
13	h	$HCl.NH_2(CH_2)_4CH$(COOMe, NHBOC)	(10.79)	2		12			65	
14	i	$BOC\text{-}NH(CH_2)_4\text{-}CH$(COOMe, NH_2HCl)	(8.90)	2		12			60	
15				3		48			65	
16	j		(8.33)	1	72		90	10		
17						72	40			40
18	k	Et_3N	(10.75)	1	78			100		
19						18				100

* Yields of products are determined after HPLC purification. Satisfactory spectroscopic data were obtained for all new compounds described in this paper

The reaction of morpholine and triethylamine are also included. The reaction products are either the protected methyl 3-dehydroshikimate **4** (reaction in CH_2Cl_2) or the methyl protocatechuate **8** (in methanol).

The action of primary amines with methyl 3-dehydroshikimate compounds **4** and **5** was also studied. The reaction product was identified as being the corresponding aromatic amine derivatives **6**. It was the only one in methanol solution, while it was found together with the starting compound when methylene chloride was used (table 2). Triethylamine reacts only in methanol to yield photocatechuic acid **8**.

Table 2 - Reaction of 3-dehydroshikimic acid derivatives with amines

			Time reaction (h)		Yields of products (%)		
Entry	Amine	Substrate	CH_2Cl_2	MeOH	**4** or **5**	**6**	**8**
20	**e**	**4**	24		60	40	
21				12	-	42	
22	**g**	**5**	24		70	27	
23				12	5	63	
24	**k**	**4**	72		100		
25				18			100

Discussion

Aromatic amines (**a**, **b**, **c**) do not react with 3-dehydroquinic acid derivatives under our experimental conditions (table 1, entries 1-4). The low basicity, as shown by their pK_A values, renders them weak nucleophiles toward the substrate's ketone group. Further more the sterically hindered ketone function, due mainly to the dehydroquinic structure may prevent reaction with these amines.

The action of other primary amines with the DHQ compounds **1-3**, does not yield protocatechuic acid but the corresponding aminoaromatic derivatives **6** which clearly indicates the formation of a Shiff base intermediate.

Considering the complexity of the system for a detailed mechanistic study, we shall discuss here the qualitative aspects of our results.

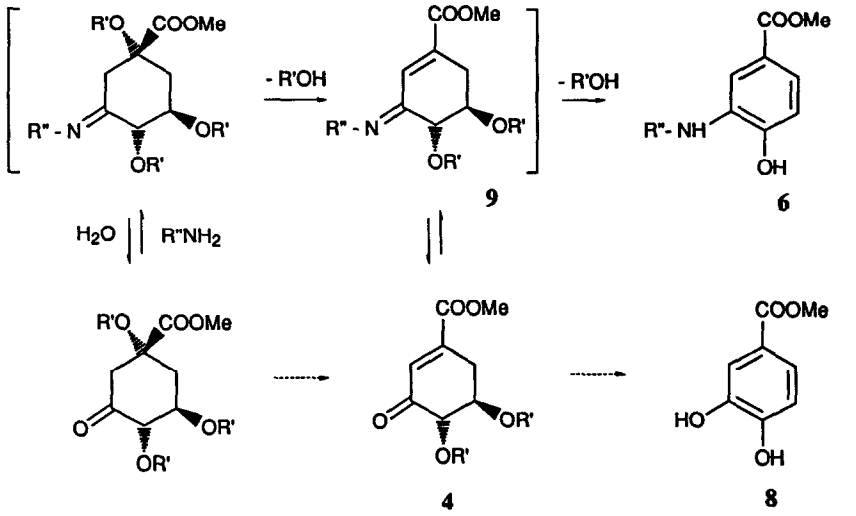
- 2-(2-Aminoethyl)pyridine reacts extremely rapidly towards trisilylated methyl 3-dehydroquinate **1** compared to 2-(aminomethyl)pyridine **d** and 2-phenylethylamine **e** (table 1 entries 9, 10 versus 5-8).
- Despite very close pK_A's morpholine reacts very slowly with **1** compared to 2-aminomethyl pyridine **d**. Whereas reaction of **l** with morpholine yields barely a 10% overall transformation into protected methyl 3-DHS after three days, the reaction of **1** with **d** is complete in 24 hours (table 1, entries 16, 5 respectively).
- Triethylamine, the strongest base used, reacts much more slowly with **1** than 2-(2-aminoethyl)pyridine **f** does ($pK_A = 9.62$) (table 1, entries 18, 9 respectively).

These results tend to support the mechanism shown in Scheme 3 for the action of primary amines with the methyl 3-dehydroquinate.

The primary amines **d**, **e**, **f** react in methylene chloride with protected methyl 3-dehydroquinate **1** to form a Schiff base which is not observed in the given experimental conditions. The deprotonation at C-2 followed by C-1 dehydroxylation yields the ene-imine intermediate **9** (scheme 2). The latter is either

transformed into methyl 3-alkylamino 4-hydroxy benzoate **6** or is rapidly hydrolysed to give methyl 3-DHS **4**. This last reaction (**9** → **4**) is apparently balanced since the action of a primary amine on methyl DHS **4** yields the corresponding aminoaromatic derivative **6** (table 2 entries 20, 22) along with starting material.

Among the primary amines 2(2-aminoethyl)pyridine **f** reacts rapidly with **1**. In this case deprotonation of the C-2 hydrogen atom α to the imine function may be catalysed by the pyridine's tertiary amino group.



Scheme 2

The effect of the intramolecular catalysis depends on the ring size in the transition state - no catalysis is observed with 2(aminomethyl) pyridine (table 1 entry 5). This is in agreement with the studies of Hine et al. [16] on the dedeuteriation of $^2H_6$ acetone using diamines of the $H_2N(CH_2)_nN(Me)_2$ type. They report an intramolecular base catalysis by the tertiary amino group for n= 2 and n = 3, the maximum effect being observed for n = 3.

Methyl 3-dehydroshikimate compound **4** reacts in methylene chloride with primary amines to yield in addition to the starting product the corresponding aminoaromatic derivatives **6**. On the other hand it does not react at all with triethylamine (methyl protocatechuate is not observed) (table 2). It is clear that under our experimental conditions formation of the Schiff base is necessary for the second dehydration step to occur (scheme 3).

When switching from a polar non protic solvent (CH_2Cl_2) to a polar protic one (MeOH) only the methyl 3-alkylamino 4-hydroxybenzoate **6** or its corresponding acid are isolated for reactions with primary amines ; methyl 3-DHS or acids **4**, **5** are not observed when MeOH is used.

Methanol probably catalyses both dehydrations by protonation of the C-1, C-5 silyloxy groups yielding compounds **6** or **7**. Formation of the iminium ion $\rangle C{=}\overset{+}{N}H\text{-}R$ in polar solvents may also facilitate deprotonation of allylic C-6 hydrogen atom and the subsequent elimination reaction leading to the aromatic amine derivatives **6** or **7**.

Although we must be cautious in extrapolating these results to the biological system of a shikimic acid pathway we can note i) a C-1 dehydroxylation mechanism favored by the formation of a Shiff base, ii) an important role played by intramolecular catalysis, iii) that 3-dehydroshikimate which is the normal product of the enzymic reaction, is only observed in an apolar aprotic solvent in competition with the aromatic compounds which are the only ones obtained in methanol.

Aromatization of the imine intermediate **9** in organic media constitutes the main qualitative difference between the reaction studied in organic solvents and the enzymic one. It seems likely that even in the aprotic non polar methylene chloride, diffusion of water molecules may catalyze both hydrolysis of the imine function and the dehydration aromatization reaction. While in the enzymic environment the water molecule confined to the proximity of the $\geq C=\overset{+}{N}H$-enz may favor the ene imine ketone conversion leading to 3-DHS.This observation suggests that the microenvironment surrounding the C_5-C_6 carbon frame of the substrate 3-DHQ in the active site of DHQase could be hydrophobic.

Further studies on derivatives without hydroxyl group on C-5 or specifically labelled on C-6 could allow more detailed information on this mechanism.

References

1. Ganem, B. *Tetrahedron* **1978**, *34*, 3353-3383 ; Bartlett, P.A. *Rec. Adv. Phytochem.* **1986**, *20*, 119-146 ; Walsh, C.T.; Jun, L.; Rusnak, F.; Saikatani, M. *Chem. Rev.* **1990**, *90*, 1105-1129.
2. Dunkan, K.; Chaudhuri, S.; Campbell, M.S.; Coggins, J.R. *Biochem. J.* **1986**, *238*, 465-483.
3. Pompliano, P.L.; Reimer, L.M.; Myrvold, S.; Frost, J.W. *J. Am. Chem. Soc.* **1989**, *111*, 1866-1871. Anderson, K.S.; Sikorski, J.A.; Johnson, K.A. *Biochemistry* **1988**, *27*, 7395-7406. Montchamp, J.L.; Piehler, L.T.; Frost, J.W. *J. Am. Chem. Soc.* **1992**, *114*, 4453-4459. Alberg, D.G.; Lauhon, C.T.; Nyfeler, R.; Fässler, A.; Bartlett, A.A. *J. Am. Chem. Soc.* **1992**, *114*, 3535-3546.
4. Montchamp, J.L.; Frost, J.W. *J. Am. Chem. Soc.* **1991**, *113*, 6296-6298. Bugg, T.D.H.; Abell, C.; Coggins, J.R. *Tetrahedron Lett.* **1988**, 6779-6782, 6783-6786. Bowles, S.: Campbell, M.M.; Sainsbury, M.; Davies, G. *Tetrahedron Lett.***1989**, *30*, 3711 ; *Tetrahedron* **1990**, *46*, 3981. Leroy, J.; Fisher, N.; Wakselman, C. *J. Chem. Soc., Perkin I* **1990**, 1281-1296.Chahoua, L.; Baltas, M.; Gorrichon, L.; Tisnès, P.; Zedde, C. *J. Org. Chem.* **1992**, *57*, 5793-5802.
5. Gilchrist, D.G.; Kosage, T. *"Aromatic aminoacid biosynthesis and its regulation"* in *"The Biochemistry of plants"* Academic Press, **1981**, *5*, 507.
6. Coggins, J.R. *"Herbicides and Plant Metabolism"*, A.D. Dodge (Ed) Cambridge University Press **1989**, 97-112.
7. Boudet, A.M.*Plant and Cell Physiol.* **1980**, *21*, 785-792.
8. Duncan, K.; Chaudhuri, S.; Campbell, M.S.; Coggins, J.R. *Biochemistry J.* **1986**, *238*, 475-483. Turner, M.J.; Smith, B.W.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1975**, 52-55. Haslam, E.; Turner, M.J.; Sargent, D.; Thompson, R.S. *J. Chem. Soc.* **1971**, 1489-1495. Butler, J.R.; Alworeth, W.L.; Nugent, M.J. *J. Am. Chem. Soc.* **1974**, *96*, 1617-1618. Vaz, A.D.N.; Butler, J.R.; Nugent, M.J. *J. Am. Chem. Soc.* **1974**, *97*, 5914-5915.
9. Shneier, A.: Kleanthous, G.; Deka, R.; Coggins, J.R.; Abell, C. *J. Am. Chem. Soc.* **1991**, *113*, 9416-9418.
10. Brault, M.; Pollack, R.M.; Bevins, C.L. *J. Org. Chem.* **1976**, *41*, 346-350.
11. Ferran, H.E.; Roberts, R.D.; Jacob, J.N.; Spencer, T.A. *J. Chem. Soc., Chem. Com.*. **1978**, 49-50.
12. Compounds **1-5** are synthesized according to : Delfourne, E.; Despeyroux, P.; Gorrichon, L.; Véronique, J. *J. Chem. Res.* **1991**, (S) 56-57), (M) 532- 544.
13. O'Donnell, M.J.; Bennett, W.D.; Bruder, W.A.; Jacobsen, W.N.; Knuth, K.; Leclef, B.; Po, R.L.; Bordwell, F.G.; Mrozack, S.R.; Kripe, T.A. *J. Am. Chem. Soc.* **1988**, *110*, 8520-8525.
14. Taguchi, K.: Westheimer, F.H. *J. Org. Chem.* **1971**, *36*, 1570-1572.
15. Perrin, D.D. *"Dissocciation constants of organic bases in aqueous solution"* Butterworths scientific publication, London **1965**.
16. Hine, J.; Cholod, M.S.; King, R.A. *J. Am. Chem. Soc.* **1974**, *96*, 835-845.