NADH Model Studies. Part 2.[1] Cationic Hydrogenations using Acridan Derivatives as Hindered NADH Models.

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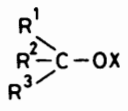
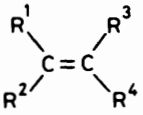
Various aryl alcohols (**5**), arylethylenes (**6**), and trityl ethers (**7**) are found to undergo smooth reduction in dichloromethane at room temperature when treated with an equimolar amount of the acridans (**3**) and trifluoroacetic acid. The limiting acidity value (H_0) for the medium in which the compounds (**3**) are completely protonated and hence not suitable as NADH models was also determined spectrophotometrically. Kinetic studies of reduction of (**5**) with (**3**) in dilute sulphuric acid, or Hantzsch ester (**2**) in glacial acetic acid, indicated that during reduction steric hindrance is important in less acidic media whereas the reduction of cations performed with trifluoroacetic acid is not affected by steric factors.

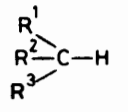
1,4-Dihydropyridines such as (**1**) or (**2**) have often been employed as model compounds for the redox coenzyme NADH. Reductions of a large variety of substrates in a manner analogous to the biochemical processes have frequently been demonstrated using these models.[2] The pH of the medium in such reactions has invariably been near neutral. However, there are certain biochemical processes, especially during double bond reductions[3] in steroid biosynthesis, where unmistakably highly electrophilic conditions prevail at the catalytic site of the enzyme, near the 1,4-dihydronicotinamide moiety of NADH. Such electrophilic conditions are essential to activate the substrate, and the acid labile dihydronicotinamide in the coenzyme survives these, protected by the hydrophobic protein envelope. It is apparent, therefore, that in order to mimic such processes *in vitro*, the NADH model compounds used should be such as to survive in a highly electrophilic environment, which compounds such as (**1**) and (**2**) do not. In this context, we report the design of highly acid stable NADH models and their use in the reduction of transient cationic species generated in acidic media.



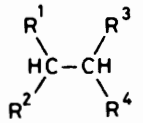
(**5**) X = H
(**7**) X = R^4



(**6**)



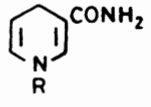
(**8**) **a**; R^1 = R^2 = R^3 = Ph
b; R^1 = R^2 = Ph, R^3 = *p*-$MeOC_6H_4CO$
c; R^1 = Ph, R^2 = R^3 = *p*-$MeOC_6H_4CO$
d; R^1 = R^2 = R^3 = *p*-$MeOC_6H_4CO$
e; R^1 = R^2 = Ph, R^3 = Me
f; R^1 = Ph, R^2 = H, R^3 = *p*-$MeOC_6H_4CO$
g; R^1 = R^2 = Ph, R^3 = H



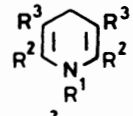
(**9**) **a**; R^1 = R^2 = R^3 = Ph, R^4 = H
b; R^1 = Ph, R^2 = R^3 = H, R^4 = *p*-$MeOC_6H_4CO$

Results and Discussion

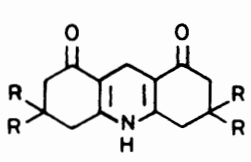
In view of the acid lability of the usual NADH models[4] such as (**1**) or (**2**), we planned to use the hindered acridan derivatives[5] (**3**) for the reduction of carbenium ions. Not only are compounds (**3**) easily prepared,[6] but they were also found to be stable for long periods in concentrated sulphuric and trifluoroacetic acids when, as a result of protonation of the oxygen [indicated by a large (*ca.* 50 nm) red shift in the electronic absorption spectrum of (**3**) in concentrated sulphuric acid] the species (**4**) is formed. Regeneration of (**3**) occurs when the concentrated acid solution is poured into cold water. In contrast, similar species obtained from (**1**) and (**2**) undergo attack by water, other nucleophiles, a second unprotonated molecule to give disproportionation, or ring-opening reactions.[4] This suggests that the 1,4-dihydropyridine functions in (**3**) are protected by the outer rings from attack by nucleophiles or other unprotonated molecules.



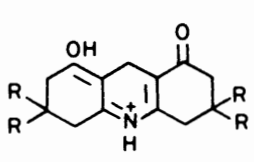
(**1**)



(**2**) **a**; R^1 = H, R^2 = Me, R^3 = CO_2Et
b; R^1 = H, R^2 = Me, R^3 = Ac
c; R^1 = R^2 = Me, R^3 = Ac



(**3**) **a**; R = H
b; R = Me



(**4**)

Solutions of (**3**) in concentrated sulphuric or trifluoroacetic acid rather slowly reduced the trityl cation, generated from triphenylmethanol (**5a**), to triphenylmethane. It was observed that (**3**) in dichloromethane (2 ml) containing trifluoroacetic acid (0.2 ml) does not show any red shift, indicating lack of protonation, whereas triarylmethanols and 1,4-diarylethylenes produce carbenium ions. When (**3**) (1 mmol) was added to a solution of triphenylmethanol (1 mmol) in dichloromethane containing trifluoroacetic acid, a rapid decolourization of the solution took place and triphenylmethane was formed. The unprotonated compound (**3**), as expected, reduced the cation more rapidly than did (**4**) in strong acids. Consequently, various alcohols, olefins, and ethers capable of giving carbenium ions

Table 1. Reduction of arylmethanols (**5**), arylethylenes (**6**), and trityl ethers (**7**) using the acridan

Compd.	R^1	R^2	R^3	R^4	pK_R+ [a]	Product	Yield (%)	m.p.[b] (°C)	b.p.[b] (°C)
(**5a**)	Ph	Ph	Ph		−6.44	(**8a**)	89	92 (94)[g]	
(**7a**)	Ph	Ph	Ph	Me	−6.44	(**8a**)	93		
(**7b**)	Ph	Ph	Ph	Et	−6.44	(**8a**)	92		
(**7c**)	Ph	Ph	Ph	CH_2Ph	−6.44	(**8a**)	92		
(**5b**)	Ph	Ph	*p*-$MeOC_6H_4$		−3.20	(**8b**)	87	61 (62—63)[h]	
(**5c**)	Ph	*p*-$MeOC_6H_4$	*p*-$MeOC_6H_4$		−0.89	(**8c**)	86	96 (96—97)[i]	
(**5d**)	*p*-$MeOC_6H_4$	*p*-$MeOC_6H_4$	*p*-$MeOC_6H_4$		−0.82	(**8d**)	85	57 (56—58)[i]	
(**5e**)	Ph	Ph	Me		−10.40	(**8e**)	85[c]		265 (268—272)[j]
(**6a**)	Ph	Ph	H	H	−10.40	(**8c**)	90		
(**5f**)	Ph	Ph	CH_2Ph		−11.50[d]	(**9a**)	84	52 (54—55)[k]	
(**6b**)	Ph	Ph	H	Ph	−11.50	(**9a**)	90		
(**5g**)	Ph	*p*-$MeOC_6H_4$	H		−7.90	(**8f**)	87		110 (112—114)[i]
(**5h**)	Ph	Ph	H		−12.30	(**8g**)	87		260 (261—262)[l]
(**5i**)	Ph	Me	Me		*e*		*f*		
(**5j**)	Ph	Me	H		*e*		*f*		
(**5k**)	Ph	H	H		*e*		*f*		

[a] *Ref.* 7. [b] Known compounds, literature values in parentheses. [c] Olefin isolated. [d] Estimated from the values for $Ph_2C^+CH_2Me(-11)$. [e] Expected to be more negative than −15. [f] No products isolated. [g] D. C. Sayles and M. S. Kharasch, *J. Org. Chem.* 1961, **26**, 4210. [h] L. D. Bethell and V. Gold, *J. Chem. Soc.*, 1956, 1905. [i] T. Mukaiyama, K. Naracaka, and H. Hokonoki, *Bull. Chem. Soc. Jpn.*, 1970, **43**, 2549. [j] J. S. Reichart and J. A. Nieuland, *J. Am. Chem. Soc.*, 1923, **45**, 3090. [k] R. Walonsky and N. Maoz, *J. Org. Chem.*, 1973, **38**, 4040. [l] D. I. Davies and C. Waring, *J. Chem. Soc.*, 1968, 2332.

under these conditions were reduced using (**3**) (Table 1). The limiting acidity value (H_0)[7] for the medium in which the compounds (**3**) are completely protonated, and hence unsuitable as NADH models, was determined spectrophotometrically using aqueous sulphuric acid solutions of different concentrations. Thus, compound (**3a**; R = H) is completely protonated ($\lambda_{max.}$ 440 nm) in 70% aqueous sulphuric acid solution (H_0, −3.95) and almost unprotonated ($\lambda_{max.}$ 410 nm) in 10% aqueous sulphuric acid (H_0, 0.72). Compound (**3a**; R = H) is, therefore, expected to reduce easily cations which are formed at H_0 values higher than −3.95.

The ease of formation of arylmethyl cations can be judged from their pK_R+ values.[7] The cation from (**5h**) (pK_R+ −13.3) is reduced whereas those obtained from (**5i**—**k**) with pK_R+ more negative than the limiting value around −13.3 are not reduced; the concentration necessary to produce a cation from (**5h**) is known to be 95% H_2SO_4.

Alcohols possessing two or more groups on the α carbon (**5a**—**d**) are reduced, whereas those having only one are generally not reduced unless the aryl group has an electron-releasing group at the *para*-position. Side reactions competing with the process of reduction appear to be either polymerization of less hindered cations, or loss of a proton from an α-carbon wherever possible. For instance, in the case of the alcohol (**5e**), initially, 1,1-diphenylethylene was isolated instead of 1,1-diphenylethane. The olefin however was successfully reduced using a higher concentration of the acid. Aliphatic tertiary cations in equilibrium with the corresponding alkenes probably induced polymerization of the latter and none of the reduction products could be detected.

Interestingly, the olefins, *e.g.* (**6a**), as well as the alcohols, (**5**) are also reduced in weak acids such as acetic acid. The kinetics of such reductions in glacial acetic acid indicate that the olefin (**6a**) and the corresponding alcohol (**5e**) are reduced at similar rates, although the rates for the olefin with different models were somewhat lower than those of the corresponding alcohol. The reduction of the olefin in the presence of weak acid is of great biological importance since during steroid biosynthesis double bonds are reduced by NADPH assisted by carboxylic groups.

The utility of tricyclic hindered NADH models in acidic media is evident from the comparison of rates of decomposition of unhindered models with those of the hindered ones. The tricyclic models are less reactive than the monocyclic Hantzsch ester models, owing mainly to the greater electron-withdrawing nature of the carbonyl group compared with that of the ester group in the Hantzsch ester (**2a**). The tricyclic models are also less reactive than the monocyclic models (**2b**) and (**2c**) containing carbonyl groups, owing most probably to the steric hindrance of the *gem*-dimethyl groups. These conclusions are borne out by the kinetic data discussed below.

Whereas all the hindered compounds (**3**) are unaffected even in 50% aqueous sulphuric acid for up to 24 h, the Hantzsch ester (**2a**; R^1 = H, R^2 = Me, R^3 = CO_2Et) has a half-life of only 4 min in 10% aqueous sulphuric acid and *N*-benzyl-1,4-dihydronicotinamide (**1**) has a half life of 2 min in acetic acid diluted (40%) with acetonitrile. Owing to the relative stability of compounds (**2**) in glacial acetic acid [1] we were interested to see whether the alcohol (**5a**) would undergo reduction in this medium, in which it did not show any absorptions characteristic of a trityl cation. However, the alcohol underwent reduction smoothly in glacial acetic acid on treatment with compounds (**2**). The same carbinol is not reduced by (**3b**; R = Me) in glacial acetic acid, but is reduced slowly by (**3b**; R = Me) in 10% aqueous sulphuric acid, with increasing rate as the acidity increases to 30% aqueous sulphuric acid. Since trityl cation formation is observed in neither glacial acetic acid nor dilute (up to 30%) sulphuric acid, reduction of the trityl alcohol must involve a solvent-assisted bimolecular mechanism for reduction, with the steric hindrance of the acridan model dominating the reduction, as seems evident from the reduction in acetic acid by compounds (**2**) and the complete absence of reduction by compounds (**3**). Against this, the reduction of preformed tertiary cations (with TFA in dichloromethane) is not much affected by steric factors, the main considerations being the ease of formation and stability of the cation. This difference is clearly shown by the ease of reduction of the dimethylphenyl alcohol (**5i**) in acetic acid with (**2a**), and the absence of its reduction in dichloromethane–TFA due to non-formation of the cation. Furthermore, the steric hindrance of the hindered models appears to be detrimental to reduction only in weakly acidic media, owing probably to the bimolecular nature of the reduction, but not in the case of strongly acidic conditions involving a preformed cation. This observation is important

Table 2. Kinetic studies of reductions of arylmethanols (**5**) using NADH models.

Substrate	Model compd.	Conditions[a]	$K_{obs.} \times 10^3$	Substrate	Model compd.	Conditions	$K_{obs.} \times 10^3$
(**5a**)	(**2a**)	A	9.23	(**5a**)	(**3b**)	A	0.80
(**5e**)	(**2a**)	A	1.86	(**5a**)	(**3b**)	B	0.71
(**5i**)	(**2a**)	A	2.44	(**5a**)	(**3b**)	C	0.86
(**5l**)	(**2a**)	A	1.13	(**5a**)	(**3b**)	D	3.06
(**5a**)	(**2b**)	A	2.79	(**5a**)	(**3b**)	E	3.18
(**5e**)	(**2b**)	A	0.62	(**5a**)	(**3b**)	F	5.14
(**5i**)	(**2b**)	A	5.00	(**6a**)	(**2a**)	A	0.72
(**5l**)	(**2b**)	A	1.25	(**6a**)	(**2b**)	A	0.17
(**5a**)	(**2c**)	A	1.91	(**6a**)	(**2c**)	A	0.59
(**5e**)	(**2c**)	A	4.38				
(**5i**)	(**2c**)	A	7.50				
(**5l**)	(**2c**)	A	4.09				

[a] See Experimental section. [b] Compound (**5**; R^1 = Ph, R^2 = α-naphthyl, R^3 = Me).

from a biological point of view in the sense that the mechanism of reduction of olefinic double bonds catalyzed by weakly acidic groups at the active site of enzymes should also be concerted bimolecular, thus circumventing the need for highly reactive preformed cations. Strongly acidic groups such as sulphonic *etc.*, necessary for the latter course, are not encountered in the enzymes concerned.

Experimental

Column chromatography was carried out using silica gel (60—200 mesh) and benzene or benzene–ethyl acetate (90:10) as the eluant. T.l.c. was performed with silica gel (Merck silica GF_{254}) in benzene. Dichloromethane was used after distillation over phosphorus pentaoxide. Trifluoroacetic acid was obtained from Fluka and used as supplied. Arylmethanols were prepared by Grignard methods. Triphenylmethanol (**5a**) was prepared by the reaction of methyl benzoate with phenylmagnesium bromide. Similarly, (**5b**) was prepared by the reaction of benzophenone with 4-methoxyphenylmagnesium bromide and compounds (**5c**—**j**) were prepared by analogous procedures starting either from the ester or the ketone, depending upon the availability of the starting materials. Olefins were prepared[9] by the dehydration of the corresponding alcohols with sulphuric acid. Ethers were prepared[10] by the reaction of aryl alcohols with methyl iodide or ethyl iodide in the presence of sodium hydride.

General Procedure for the Reduction.—The substrate (1 mmol) was taken up in dichloromethane (15 ml), and trifluoroacetic acid (0.05 ml) was added to generate the cation; the acridans (**3**) (1 mmol) were then added. The room temperature reaction was followed using t.l.c. When the reaction was found to be complete, dichloromethane was evaporated off and the residue, after either elution through a chromatographic column or p.l.c., was analysed. The products were characterized by comparison of spectroscopic properties with those of authentic samples prepared by established alternative procedures.

General Procedure for the Kinetic Studies.—Rate measurements (Table 2) were performed by u.v.-visible spectrophotometry using a Shimadzu Graphicord UV-240 spectrophotometer. The reaction chamber was thermostatted to 25.00 ± 0.05 °C by means of water circulating from a Shimadzu TB-85 thermobath. Equal volumes of stock solutions of compound and substrate required for the reduction were mixed rapidly and the changes in absorbance corresponding to reduction of substrate were recorded as a function of time. Reactions were followed under pseudo-first order conditions and plots of $\ln(A - A_t)$ *versus* time were linear over 78% of the reaction. The reaction medium was either glacial acetic acid (method A) or dilute sulphuric acid (methods: B, 10%; C, 20%; D, 30%; E, 35%; and F, 40%).

Acknowledgements

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References

1 Part 1, S. Singh, V. K. Sharma, S. Gill, and R. I. K. Sahota, *J. Chem. Soc., Perkin Trans. 1*, 1985, 437.
2 R. J. Kill and D. A. Widdowson, 'Bioorganic Chemistry,' ed. E. E. van Tamelen, Academic Press, New York, 1978, vol. IV, pp. 239—275.
3 D. C. Wilton, K. A. Munday, S. J. M. Skinner, and M. Akhtar, *Biochem. J.*, 1968, **106**, 803; M. Akhtar, K. A. Munday, A. D. Rahimtula, I. A. Watkinson, and D. C. Wilton, *Chem. Commun.*, 1969, 1287; I. A. Watkinson, D. C. Wilton, K. A. Munday, and M. Akhtar, *Biochem. J.*, 1971, **121**, 931.
4 C. C. Johnson, J. L. Gardner, C. H. Suelter, and D. E. Melzler, *Biochemistry*, 1963, **2**, 689; C. S. Y. Kim and S. Chaykin, *ibid.*, 1968, **7**, 2339; S. Shinkai, R. Ando, and T. Kunitake, *Bull. Chem. Soc. Jpn.*, 1975, **48**, 1914.
5 Preliminary communication, S. Singh, S. China, V. K. Sharma, and S. S. Sachdev, *J. Chem. Soc., Chem. Commun.*, 1982, 453.
6 E. I. Stankevich and G. Vanags, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 1961, 233 (*Chem. Abstr.*, 1963, 58, 4508f).
7 N. C. Deno, P. T. Groves, and G. Saines, *J. Am. Chem. Soc.*, 1959, **81**, 5790.
8 N. C. Deno, J. J. Jaruzelski, and A. Schriescheim, *J. Am. Chem. Soc.*, **77**, 3044; E. M. Arnett and T. C. Hofelich, *ibid.*, 1983, **105**, 2889.
9 C. F. H. Allen and S. Converse, *Org. Synth.*, Coll. Vol. I, p. 226.
10 B. A. Stoochnoff and N. L. Benoiton, *Tetrahedron Lett.*, 1973, 21.

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