Tetrahedron, Vol. 37, pp. 1697 to 1702, 1981
Printed in Great Britain.

0040-4020/81/091697-06$02.00/0

MULTIFUNCTIONAL CATALYSIS—X

"TAILOR-MADE" CATALYSTS FOR THE ISOMERISATION OF Δ-5 CHOLESTENONE

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(*Received in France* 25 *May* 1980)

Abstract—"Tailor-made" dicarboxylic acids and dihydroxynaphthalene-triethylamine complexes catalyse the isomerisation of Δ-5-cholestenone. The reaction is faster and of lower kinetic order than with simple bifunctional catalysts; the activation entropy is more favourable, but the activation enthalpy is higher, due possibly to steric and electronic interference, between the catalysing functions, which are absent in the corresponding isomerases.

The enzymatic conversion of Δ-5 3β-hydroxy steroids into the corresponding Δ-4 3-oxo steroids is an important step in the biosynthesis of steroidal hormones. Extracts of mammalian steroidogenic tissues and cultures of micro-organisms have yielded two enzyme activities specific to this conversion, namely oxidoreductase 1.1.1.5.1. and isomerase 5.3.3.1.[1-11] Pure crystalline Δ-5 3-oxo steroid isomerase obtained from *Pseudomonas testosteroni*[12-18] is characterised by a very high turnover number (8.7×10^6), and has been the object of numerous studies, focusing in particular on the nature of the active sites and the reaction mechanism.[19-27]

The isomerisation of Δ-5 3-oxo steroids is also catalysed by acids and bases in aqueous media.[22,28]

In the course of our work on multifunctional catalysis, we had already studied the catalysis of this reaction in an aprotic and apolar medium (benzene) by bifunctional systems, in particular phenol-tertiary amine mixtures,[29,30] carboxylic,[31] and phosphinic acids.[32]

The activation parameters for the isomerisation of Δ-5 androstendione-3,17 and Δ-5 cholestenone by enzyme, acid or base (monofunctional) and bifunctional catalysts are compared in Table 1; bifunctional catalysts give an activation enthalpy close to that of the enzyme, and lower than those of the monofunctional catalysts, but the activation entropy is highly unfavourable compared to that of the enzyme.

The reaction mechanisms of the two types of bifunctional catalysts studied, as inferred from their reaction kinetics, involve highly organised transition states in which respectively three and two molecules of catalyst participate (Fig. 1).

It might be expected that by decreasing the number of species involved in the transition state, and thus reducing the number of degrees of freedom of the system, the activation entropy of the isomerisation should be made less negative.

Accordingly, molecules containing several functions (diphenols and diacids) were envisaged.

Dihydroxynaphthalene-triethylamine

One of the several known dihydroxynaphthalenes seemed an appropriate starting material for building such a system. It had already been discovered that in catalysis using phenol-triethylamine mixtures, the most acidic phenols gave the least unfavourable activation entropies. Accordingly, nitro-derivatives of 1,8-dihydroxynaphthalene were prepared by nitration to give a mixture of isomers.[33] However the original structural attributions of the products were not confirmed by us. NMR data indicated clearly that 2,5-dinitro 1,8-dihydroxy naphthalene (m.p. 225°) and 4,5-dinitro, 1,8-dihydroxy naphthalene (m.p. 170°) had been obtained.

Unfortunately, these derivatives were found to be insufficiently soluble in benzene to enable a meaningful kinetic study to be made. Indeed, the insufficient solubility of most of the substituted or unsubstituted dihydroxynaphthalenes tried, led us to limit ourselves to the study of just one, namely 1,7-dihydroxynaphthalene.

The rate law observed for the isomerisation of Δ-5

Table 1. Activation parameters for the isomerisation of Δ-5 3-oxo-steroids by various catalysts in aqueous (a) or benzene (b) solution

Substrate	Δ-5 androstenedione-3,17				Δ-5 cholestenone
Catalyst	Enzyme(22)$_a$	Acid(22)$_a$	Base(22)$_a$	CF_3CO_2H(31)$_b$	ArOH-NEt_3(29)$_b$
$\Delta H^{\neq}$ Kcal.mole^{-1}	5.0 ± 0.1	14.0 ± 0.1	11.0 ± 0.1	8 ± 2	5.7 ± 0.5
$\Delta S^{\neq}$ e.u.	-16.8 ± 0.5	-19.6 ± 0.4	-15.5 ± 0.4	-60 ± 10	-53 ± 1
$\Delta G^{\neq}$ Kcal.mole^{-1} 25°C	10.0 ± 0.1	19.8 ± 0.1	16.0 ± 0.1	26 ± 2	21.5 ± 0.5

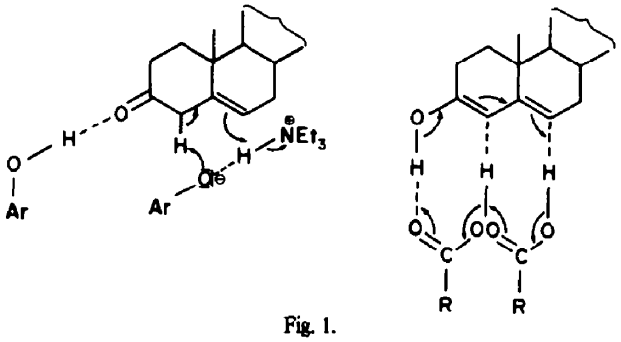

Fig. 1.

cholestenone by a mixture of monophenol and triethylamine takes the form: $v = k'$ (Δ-5 cholestenone) (complex) (free phenol); the complex consists of one molecule of phenol and one molecule of tertiary amine.

Replacing the monophenol by a diphenol, we would expect the following rate law $v = k'$ (Δ-5 cholestenone) (complex), where the complex carries a free phenol function.

The formation of 1,7-dihydroxynaphthalene-triethylamine complexes in benzene brings about a shift of the UV absorption band of the proton donor (N) which is proportional to the concentration of the proton acceptor (T).

$$N + T \rightleftarrows NT_1, K_1$$

$$NT_1 + T \rightleftarrows NT_2, K_2$$

when $T \gg N$

$$N + 2T \rightleftarrows NT_2, K, K = K_1 \times K_2.$$

With a very large excess of triethylamine (500–3000 times the concentration of naphthalenediol), the overall equilibrium constant K may be determined graphically using the Beer–Lambert law[34] according to Benesi and Hildebrand.[35]

The rigorous determination fof K_1 is more complicated. However, at low triethylamine concentrations, we can assume to a first approximation that the concentration of complex NT_2 is negligible and that only the first equilibrium is present (see Experimental, Table A).

The two forms of the first equilibrium are both possible and we have no way of differenciating between the two. If N = H–A–H, H–A–H + T⇄H–A ... H ... T or T ... H ... A–H or NT_1.

Although we have no detailed informations about their geometry, we assume that the two forms have comparable reactivities in the isomerisation.

The rate law obtained (Table B), shows that the rate of isomerisation is indeed proportional to the complex NT_1 produced in the first equilibrium:

$$v = k_{ex}(\Delta\text{-5 cholestenone}) = k'(\Delta\text{-5 cholestenone})(NT_1).$$

By analogy with the catalysis of the reaction by phenol-amine mixtures, we may infer the following reaction mecanism, (Fig. 2), with the formation of an hydrogen bond between the carbonyl group of the steroid and the free hydroxyl group of the catalyst.

No precision as to the stereospecificity of the displaced protons can be given, as the necessary studies involving deuterium incorporations are going on.

The activation parameters were determined (Table C),

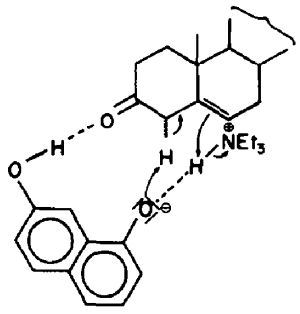

Fig. 2.

giving:

$$\Delta H^{\neq} = 7.7 \text{ kcal mole}^{-1} \text{ and } \Delta S^{\neq} = -51 \text{ e.u.}$$

The activation free energy is less favourable than with the phenoltriethylamine mixture; the activation entropy is less negative, but the activation enthalpy is higher.

It may be that the most important factor in the mechanism of isomerisation put forward is the association between the aryl hydroxy group and the 3-carbonyl group which positions the catalyst favourably for the transfer of a proton to C_6.

Since the acidity of the second acid function of a bifunctional compound such as a diphenol is weaker than that of the first, the association between the 3-carbonyl and the free phenol function will be much weaker in the case of naphthalenediol than with an ordinary phenol.

Thus a more favourable entropy factor is achieved by linking the two phenol functions, but this is offset by a higher activation enthalpy due to the electronic effects arising from their close proximity.

Dicarboxylic acids

Here again, rigid *cis*-diacids of sufficient strength, e.g. maleic, phthalic or *meso*-diphenyl-succinic acids, were found to be insufficiently soluble in benzene.

Tetrafluorosuccinic acid (TFSA) and hexafluoroglutaric acid (perfluoroglutaric acid (PFGA) proved to be both sufficiently acidic and sufficiently soluble in benzene to give a useful set of results.

Experimental rate-constants k_{ex} indicated a rate law involving only one molecule of diacid (Table D), confirming our starting hypothesis.

$$v = k_{ex}(\Delta\text{-5 cholestenone}) = k' (\Delta\text{-5 cholestenone})(\text{diacide}).$$

The activation parameters for the two diacids were

Table 2. Activation parameters for the catalysis of the isomerisation by diacids and monoacids in benzene. Half-reaction times (τ_h) and pKa of acids in methanol

Catalyst	$\Delta H^{\neq}$ Kcal.mole^{-1}	$\Delta S^{\neq}$ e.u.	$\Delta G^{\neq}$ Kcal.mole^{-1}, 25°C	pK_1 , pK_2	τ_h
TFSA	13.8 ± 0.5	-20 ± 1	19.7 ± 0.5	4.6 , 6.1	6
PFGA	13.0 "	-23 "	19.5 "	4.6 , 5.3	9
CF_3CO_2H	9.4 "	-25 "	16.8 "	4.6	63
CCl_3CO_2H	9.0 "	-27 "	17.0 "	4.9	80
$CHCl_2CO_2H$	8.3 "	-30 "	17.7 "	6.4	409
$CH_2BrCHBrCO_2H$	4.0 "	-50 "	19.9 "	7.2	2750

calculated and compared with those for four monoacids (Table 2).

(The activation parameters for trichloroacetic acid had already been calculated[31] from experimental rate constant k_{ex}. Here, parameters in terms of k' were needed in preference to those calculated from k_{ex} (Table E) to enable a better comparison, since k' has different units for diacids and monoacids. k' was determined for each of the four monoacids).

It is immediately apparent from Table 2 that the diacids improve the entropy factor but give a less favourable activation enthalpy.

A plot of $\Delta H^{\neq}$ vs $\Delta S^{\neq}$ for monoacids shows a linear relationship (Fig. 3).

If the points corresponding to the enzyme and the two diacids are added, the former is situated well below the straight line, while the latter, due to the high $\Delta H^{\neq}$ value are situated some way above it.

This effect may be due to the difference in acidity of the two functions in the same molecule. In the monoacids, the acidity is the determining factor; pKa values in methanol were determined for both mono- and diacids by potentiometry at 25° (Table 2): the most acidic acid gives the lowest activation free energy.

For the diacids, the relatively lower reactivity of the second function may be responsible for the higher activation enthalpy. The differences between the pK_2 of TFSA and PFGA and between their respective $\Delta H^{\neq}$ values are consistent with this interpretation.

However, another important factor may be involved, namely the conformational strain undergone by the catalyst in the transition state, assuming that both acid functions act simultaneously.

Thus, if we construct a model of the transition state for TFSA for example (Fig. 4), it is reasonable to postulate a

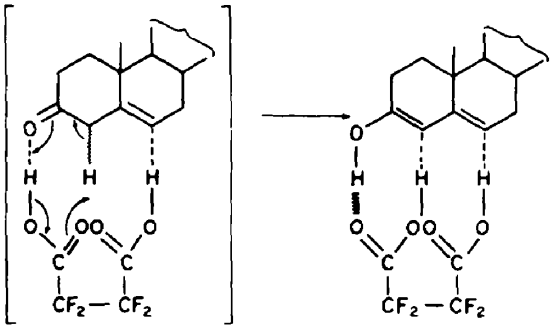

Fig. 4.

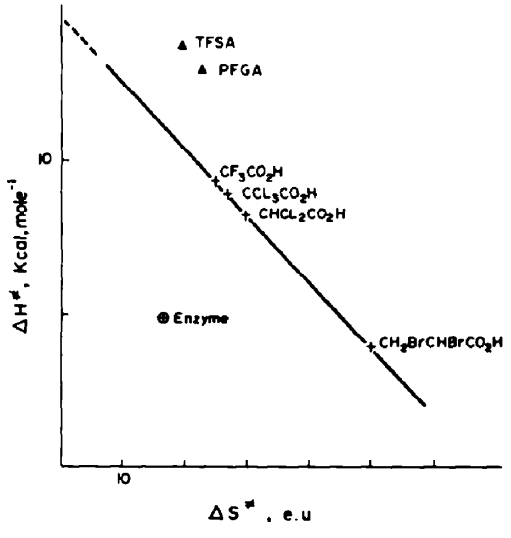

Fig. 3.

degree of enolisation at C_3, the formation of a hydrogen bond between the enol and one of the acid functions (schematically IIIII), and of two other weak interactions at C_4 and C_6 (schematically . . . . .). In this position, the diacid molecule must adopt a pratically eclipsed conformation.

The enthalpy required for such a conformational strain can be roughly evaluated from data in the literature. The values reported for the rotation of fluoroethane (3.3–3.4 kcal mole^{-1}) and of hexafluoro ethane (3.9–4 kcal mole^{-1})[36] can be taken as minimum values, which, if subtracted from $\Delta H^{\neq}$ for the two diacids, gives values of activation enthalpy closer to those found for the monoacids with similar pKa (see Table 2).

If this interpretation is correct, a diacid having a greater conformational flexibility should be a good "tailor-made" catalyst. The extension of the carbon chain from TFSA to PFGA is evidently not sufficient to achieve such a flexibility.

CONCLUSION

The aim of the present work was to prepare "tailor-made" catalysts carrying several active functions

at appropriate distances in order to lower the activation entropy of the reaction under study. A lowering of activation entropy was indeed observed in the case of dicarboxylic acids, but was offset by an increase in activation enthalpy. An analogous effect was similarly observed with naphthalenediol as compared with ordinary phenols.

This counterbalancing effect could be due to the proximity of the active functions, both as a result of electronic interactions which considerably lower the acidity of the second function, and for the diacids as a result of conformational strain in the transition state. Such factors are likely to be absent in an enzyme catalyst, where the coils of the peptide chains can assure the proximity of concerting functional groups which are nevertheless far apart along the carbon chain and so pratically independent electronically.

This result thus seems to suggest one answer to the question recently asked by Luisi: "Why are enzymes macromolecules?"[37]

Another factor may be involved, namely the fit between the active sites on the catalyst and those on the substrate, which may not be as good in our multifunctional catalysts as in the enzyme.

Although the activation parameters for the multifunctional catalysts are not very obviously more favorable than those of the simple bifunctional catalysts, the isomerisation takes place very much faster. The lower reaction order brings about a 100-fold reduction in the half-reaction time for equivalent concentrations of naphthalenediol-triethylamine and phenol-triethylamine. The reaction with diacids is also very much faster than with monoacids (Table 2).

Thus, the multifunctional catalysts studied in the present work represent an improvement upon the simple bifunctional catalysts previously studied, and indicate directions for further research.

EXPERIMENTAL

Synthesis of nitro-derivatives of 1,8-dihydroxy naphthalene

The hydroxyl groups were first blocked with methylene sulphate.[38] Nitration[33] of 1,8-methylenedioxy naphthalene gave a mixture of isomers separated by chromatography on silica gel Merck 0.062/0,2 mm (eluant cyclohexane–ethyl acetate 6/4).

The structures proposed[33] by the authors in 1936 are not confirmed by NMR spectra (recorded on a JEOL C60 HL in $CDCl_3$ with TMS internal standard). Product A: $R_f = 0.59$; p = 27%; m.p. = 178° after recrystallisation from EtOH. Carnero and Calvet[33] assign the structure 4,5-dinitro 1,8-methylenedioxy naphthalene. ^1H NMR ($CDCl_3$): $\delta = 5.98$ ppm (s, 2H) methylene protons; $\delta = 7.36$ ppm (d, 1H) $\delta = 8.73$ ppm (d, 1H); J = 9 Hz; $\delta = 8.35$ ppm (s, 2H) and 2 weak peaks at 8.20 and 8.50 ppm. In C_6D_6 2 doublets were clearly obtained (J = 10.5 Hz). Jackman and Sternhell[39] indicate that these values correspond to 2,5-dinitro 1,8-methylenedioxynaphthalene. Product B: $R_f = 0.36$; p = 60%; m.p. = 204° after recrystallisation from EtOH. ^1H NMR ($CDCl_3$): $\delta = 5.93$ ppm (s, 2H) methylene protons; $\delta = 7.4$ ppm (d, 2H), (d, 2H), $\delta = 8.57$ ppm (d, 2H); J = 9 Hz. This isomer is the symmetrical *para–para* derivative 4,5-dinitro 1,8-methylenedioxy naphthalene, not the *ortho–ortho* derivative claimed by Carnero and Calvet.[33]

The products were deblocked[33] and purified by chromatography on a column of silica gel (acetone–hexane 10.8/9.2).

"Tailor-made" catalysts

Commercial 1,7-dihydroxynaphthalene was recrystallised 3 times from methanol after chromatography on Merck 60 PF silica gel (eluant benzene–methanol (9/1) m.p. = 175–177°. Tetrafluorosuccinic acid, obtained by hydrolysis of the corresponding commercial anhydride, was recrystallised from anhydrous benzene and dried over P_2O_5 (m.p. = 114–115°). Triethylamine, perfluoroglutaric acid and the carboxylic acids were high-purity grade commercial products and were rigourously anhydrous.

Physical measurements

1,7-Dihydroxy naphthalene-triethylamine equilibrium mixture. The instrument used was a UV-visible Cary 15 spectrophotometer with 1 cm thermostatted cells. Naphthalenediol concentrations were of the order of 10^{-4} M in anhydrous benzene. For the determination of K, triethylamine concentrations varied from 0.1 to 0.6 M, and for K_1 from 10^{-4} to 10^{-3}M.

These values give the following enthalpies of formation;

$$\text{for } N + T \rightleftarrows NT_1 \quad Hf_1 = -5.9 \text{ kcal mole}^{-1}$$

$$\text{for } N + 2T \rightleftarrows NT_2 \quad Hf_2 = -5.3 \text{ kcal mole}^{-1}$$

The average value of K_2, calculated from the above, is 2 l mole^{-1}. However, given the low accuracy inherent in the method of determination of K and K_1, it is not possible to give an exact value to K_2 at each temperature.

Dissociation of carboxylic acids in anhydrous methanol. A scale of acidity of the different catalysts used in a non-aqueous medium was obtained by potentiometry. Each acid was titrated against 0.01 M tetrabutylammonium methylate in methanol distilled over

Table A. Variations of the overall dihydroxynaphthalene-triethylamine equilibrium constant K and the first equilibrium constant K_1, as a function of temperature

T°C	25	30	35	40	45
$K_1{}^2$.mole^{-2}	280	230	210	190	150
K_1 l.mole^{-1}	150	130	110	93	80

Table B. Rates of isomerisation of Δ-5 cholestenone in benzene at 40° as a function of the concentration of 1,7 dihydroxy naphthalene (N) and triethylamine (T), in mole l^{-1}

N x 10^3	0.4	0.8	1.6	2.0	2.5	6.4	6.4	6.4	6.4
T x 10^2	0.4	0.8	1.6	2.0	2.5	6.4	0.64	0.064	20
$k_{ex_s}{}^{-1}$ x 10^7	0.9	1.5	2.8	3.0	4.0	9.1	4.2	1.2	10.1

Table C. Variation of the rate constant of the isomerisation of Δ-5 cholestenone in benzene, as a function of temperature

T°C	25	30	35	40	45
$k'_{s^{-1}}$.l.mole^{-1} x 10^4	0.89	1.18	1.38	1.68	2.27

Table D. Rate of isomerisation of Δ-5 cholestenone in benzene at 40°, as a function of ATFS concentration, C, in mole l^{-1}

C x 10^4	7.0	5.6	3.5	2.8	1.4	1.7	0.6
$k_{ex_{s^{-1}}}$ x 10^5	4.3	3.4	3.2	1.8	0.8	0.4	0.3

Table E. Variation of rate constant of the isomerisation of Δ-5 cholestenone in benzene, as a function of temperature, for different carboxylic acid catalysts

Catalyst	k' T°C 15	20	25	30	35	40	45
TFSA	$k'_{s^{-1}}$.l.mole^{-1} x 10^2 0.71	1.37	2.14	3.50	4.28	6.00	8.43
PFGA	k' " " x 10^2 0.70	1.06	1.46	----	3.46	4.66	6.40
CF_3CO_2H	$k'_{s^{-1}}.l^2.mole^{-2}$ ----	2.22	3.02	4.35	5.55	6.04	9.20
CCl_3CO_2H	k' " " " ----	2.19	2.44	----	4.08	6.22	7.50
$CHCl_2CO_2H$	k' " " ----	0.36	----	0.58	0.80	0.99	1.20
$CH_2BrCHBrCO_2H$	k' " " x 10^2 ----	6.00	7.00	8.00	8.80	----	----

Mg. The titration was monitored with a Tacussel Ariès 10,000 millivoltmeter at 25°. The measuring electrode was a glass-H^+ electrode and the reference was a calomel electrode with a liquid junction consisting of a 0.1 M solution of tetraethylammonium iodide in methanol. Before each measurement, calibration was performed using a salicylate buffer (acid 0.01 M-sodium salt 0.01 M, in methanol) of pH = 7.53.[40]

$$pK = \frac{E - E'}{k} + pH\ ref - \log \gamma \pm$$

where E = half-neutralisation potential
E' = reference buffer potential
(k = 2.303 RT/F = 0.05916 V at 25°).

Polarimetry. Kinetic measurements were made using an automatic Perkin–Elmer PE 141 polarimeter (mercury J line) according to Ref. 29.

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