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Theory and example of a small-molecule motor

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epoc ABSTRACT: Conceptual principles for operation of a molecular motor stipulate that such a species must consist of an energy-consuming catalyst that exhibits within its mechanism temporal overlap of successive catalytic cycles, so as to induce irreversible intramolecular motion during operation. The exothermic hydration reaction of Me-COCH=C=NCMe$_3$ brought about by catalyst $HO_2CCH_2OCMe(CO_2H)_2$ qualifies as such a process, in the form of serial conversion between canonical carboxylic anhydride structures within the catalyst. The steady-state kinetic behavior and inactivation by methanol demonstrate the operation of an appropriate repetitive catalytic loop in this case. Copyright © 2003 John Wiley & Sons, Ltd.
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KEYWORDS: small-molecule motor; molecular motor; catalyst; kinetics

INTRODUCTION

The simplest type of 'molecular motor' would be a single chemical entity for which internal motion in a specific sense can be induced to occur repetitively and automatically. The designated atom movements need to be distinguished from the ordinary thermal vibrations, rotations and tautomerizations that organic molecules continuously experience. Although most organic molecules in solution are constantly changing shape, methods are only now being consciously developed for engendering systematic oscillatory motion that might be captured for performing work on such a nanoscale.[1] The challenge is to fashion a molecular mechanism that realizes this objective. Of course, living organisms have solved the problem. However, the biochemical resolution is for the most part macromolecular.[2] We seek a reductionist answer: can one recognize and constructively exploit the minimally essential features of such a molecular machine? For example, a reciprocating motor might be a molecule that could be chemically forced to alternate regularly in a controlled (induced) fashion between two related covalent structures that the molecule would otherwise not adopt. Another type of motor might entail counter-rotation exclusively in one dihedral sense, about a designated covalent sigma bond connecting two molecular halves (turrets), with that motion driven chemically in a particular torsional direction.[3] Practical realization of these objectives requires a dissipative system; specifically, provision of an expendable reservoir of free energy is required, such that its controlled release drives the assemblage in one of the manners stipulated. Any nano-construct achieving this dynamic behavior (i.e. 'motor') is required to be a chemical catalyst, as strictly defined. Within the course of channeling a spontaneous energy flow, for example, in the nature of degradation of a chemical high-potential 'fuel,' the catalyzing species is to suffer according to plan a repeated systematic intramolecular shuttling or twisting, which is the designated goal. The requirement for catalyzed energy expenditure with consequent forced repetitive operation distinguishes a true molecular motor from simpler 'molecular switches' and similar devices that have been reported previously. Initially, one need not address conversion of that energy release into useful work. The immediate objective (as typified here) is only to counter entropy locally, namely, to defeat the inherent and thermodynamically dictated propensity of freely interconverting molecular conformers to proceed with an equal flux in both directions (i.e., both clockwise and counterclockwise in the case of a counter-rotator) (An attempt has been described[4] to prepare a unidirectional rotator in the form of a 'ratchet' device, which serially catches after each fraction of a turn, so that ordinary thermal energy suffices to drive the process. The device proved unsuccessful, as indeed was obliged to be the case, since all such spontaneous and thermoneutral microscopic processes are necessarily reversible).

RESULTS

Design

As a demonstration of feasibility, a simple oscillatory

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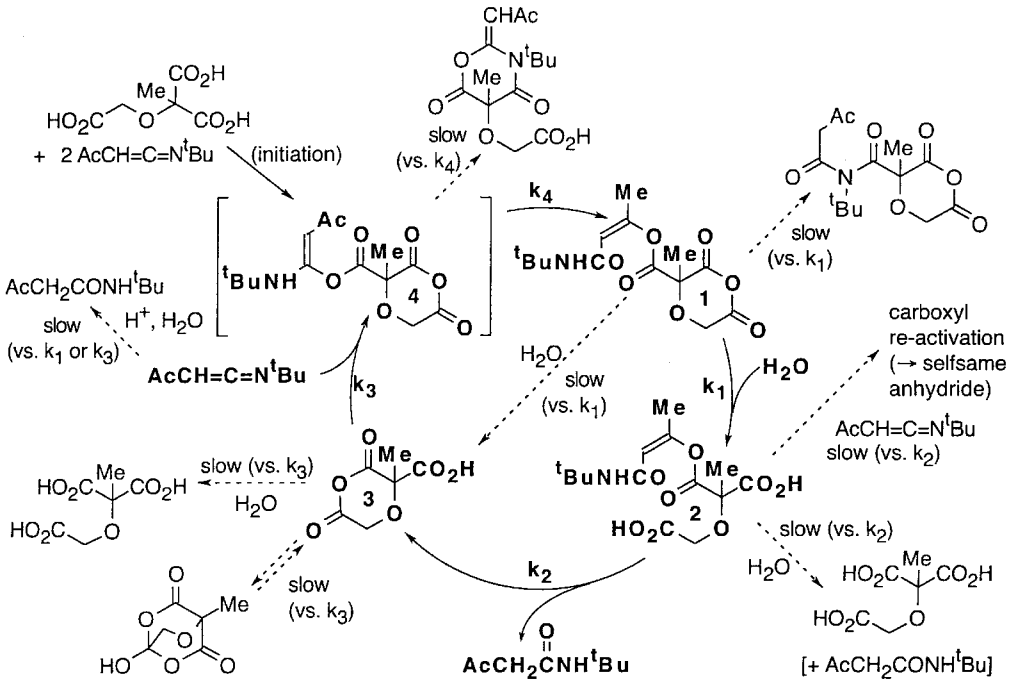


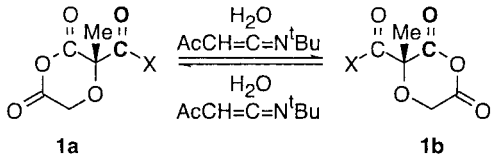
Scheme 1

motor that spontaneously operates in solution has been assembled and examined. Analysis of it serves to illustrate some of the principles and techniques entailed in successful operation of a molecular machine of the type sought. The 'fuel' is an acylketenimine (MeCOCH=C=NCMe$_3$), provided in excess, which in aqueous medium undergoes exothermic hydration to the corresponding carboxamide (MeCOCH$_2$CONHCMe$_3$), through a stepwise process that may be conveniently monitored spectrophotometrically. That conversion is catalyzed by a tricarboxylate 'motor,' HO$_2$CCH$_2$OCMe(CO$_2$H)$_2$, which oscillates between two anhydride forms as a consequence of this energy expenditure. The key to successful operation lies in the establishment of appropriate relative rates for individual steps within a catalytic loop, as presented in Scheme 1. The working cycle is indicated by the structures lettered in bold face (**1**–**4**), as will be fully examined. The lighter-faced species branching off from this core process represent initiation (upper-left of the diagram), and also potential side reactions (dashed arrows) that must be considered, but which in actuality are not of sufficient consequence to vitiate motor function. Evidence for successful operation is provided subsequently. The chemical mechanism will be inspected first.

The overall catalyzed reaction produced by the loop is AcCH=C=N^{t}Bu + H$_2$O → AcCH$_2$CONH^{t}Bu. For satisfactory motor operation, the cardinal kinetic relationship within Scheme 1 is k_2, $k_4 > k_3$[AcCH=C=N^{t}Bu] $> k_1$[H$_2$O], for which adequate reaction conditions may in fact be found, as subsequently described. Under these appropriate circumstances, and specifically because step **1** → **2** is rate limiting, the prevalent species during the steady state consists of the carboxy-activated cyclic anhydride (upper-right structure labelled **1** within the loop). The catalytic cycle proceeds in partly aqueous medium with a spontaneous hydrolysis of the carboxy–anhydride linkage (step **1** → **2**). This ring-opening reaction takes place more readily than hydrolysis of the activated enol ester moiety of **1**, as suggested by previous mechanistic investigations of acylketenimines[5] and structurally related carbodiimides[6] and by prototype rate measurements. The released activated-acyl dicarboxylate (lower-right structure **2** within the loop) then rapidly recyclizes to a six-membered ring anhydride (**3**) with release of ketocarboxamide catalysis product (step **2** → **3**). This transformation can be estimated to be very fast. The loop is completed with consumption of acylketenimine 'fuel' by reconstitution of the original activated anhydride through an amino enol ester cyclic anhydride (via step **3** → **4**) and subsequent acyl group migration (which is known to be rapid)[7] giving back the original anhydride (step **4** → **1**). Successful motor function requires that this latter carboxyl reactivation (specifically, bimolecular step **3** → **4**) be faster than the anhydride hydrolysis (step **1** → **2**), but slower than the preceding cyclization (step **2** → **3**). Under such circumstance, the catalytic cycle will proceed as indicated, without intervention of free tricarboxylic acid, and without activation of carboxyl groups except in the third step. Evidence for motor operation is exhibited in subsequent Figures.

But what justification can be provided for dubbing this catalyst as a reciprocating motor? The net consequence of

one traversal of the afore-described loop is to exchange the geminal carboxyls of the catalyst within otherwise equivalent cyclic anhydrides (**1a** → **1b**). A second traversal of the cycle returns the molecule to its original state (**1b** → **1a**). Energy released in hydration of acylketenimine is being directed so as to shuttle the —OCH_2CO_2— moiety back and forth between otherwise equivalent anhydride linkages, in an epimerization reaction. As summarized in **1a** and **1b**, serial consumption of two acylketenimines shifts the bold-faced carbonyl and then returns it to the starting position. So long as fuel is being consumed, this interchange process continues; when the reserve of acylketenimine becomes exhausted, it ceases. The motor function consists of this repetitive and energetically forced interconversion of transient molecular structures.



1a **1b**

Evidence that the motor operates as described

An indication of successful performance comes from kinetic examination of substrate conversion, which provides energy for the process. Because the acylketenimine reactant absorbs light in the ultraviolet region more strongly than does the hydration-product amide, the water addition may be conveniently followed spectrophotometrically. Figure 1 displays a reaction progress curve for an individual acylketenimine hydration experiment (solvent aqueous dioxane, 25 °C, pH 5.5, concentration of AcCH=C=N^{t}Bu initially 2 M). It certifies true catalytic behavior, as monitored by consumption of acylketenimine 'fuel.' The ratio of substrate to catalyst employed indicates an average of 11 turnovers taking place in the example provided, although a greater number is attainable with a diminished amount of catalyst, which remains active at the finish. The reaction velocity has been observed to depend directly upon catalyst concentration but, as demonstrated by the plot, it is of nearly zero order with respect to substrate acylketenimine through 90% of completion, which is an expected kinetic consequence of catalytic step **1** → **2** being rate-limiting (i.e. slow hydrolysis of cyclic anhydride intermediate, not directly involving a substrate molecule). In order to estimate the pertinent rate constants, the reaction progress curve shown has been fitted to an appropriate kinetic expression for Scheme 1 by the method of least squares, in inverse mode, as described in the Supplementary Material. The initial, nearly linear portion of the curve corresponds to a rate constant of 0.024 ± 0.01 s^{-1}, referring to the substrate-loaded catalyst **1** (k_1). That value satisfactorily matches an independent rate constant for spontaneous cyclic anhydride hydrolysis, as determined stoichiometrically for separately prepared analogue **5** under the reaction conditions, $k_w = 0.066 \pm 0.002$ s^{-1}, a value which does not depend on pH. These observations confirm that hydrolytic ring opening is the slow step during catalysis. Towards the terminus of the trace presented in Fig. 1 a serious curvature is noted, as the reactant becomes exhausted and the ultraviolet absorption of product levels off at its final value. Depletion of substrate has led to a change in rate-limiting step, attributable to the bimolecular reaction of AcCH=C=N^{t}Bu + **3** → **4** becoming slower than solvolytic step **1** → **2**, owing to dilution. The curve-fitting process alluded to previously yields a rate constant of 0.46 ± 0.02 M^{-1} s^{-1} for the final decay of substrate (k_3). This value adequately matches an independently measured rate constant of 0.125 ± 0.002 M^1 s^{-1} for the bimolecular reaction of non-catalytic model imidocarboxylic acid **6** with the substrate acylketenimine under reaction conditions approximating that of the catalytic reaction (aqueous dioxane, pH 5.5). The initial, zero-order portion of the curve presented in Fig. 1, comprising 90% of the reaction, is not perfectly straight. This is a consequence of a competing spontaneous hydration of acylketenimine, not involving the catalytic cycle, as may be detected in a blank reaction. The fitted rate constant for this latter process is 0.00017 ± 0.00003 s^{-1}, a value similar to that measured for the control reaction in absence of tricarboxylate catalyst, which may also include other minor competing reactions of substrate as well. For Fig. 1, by calculation 96% of the substrate hydration occurs through the agency of the catalyst, with the remaining 4% of the acylketenimine being hydrated in a competing spontaneous H_2O addition, as depicted by

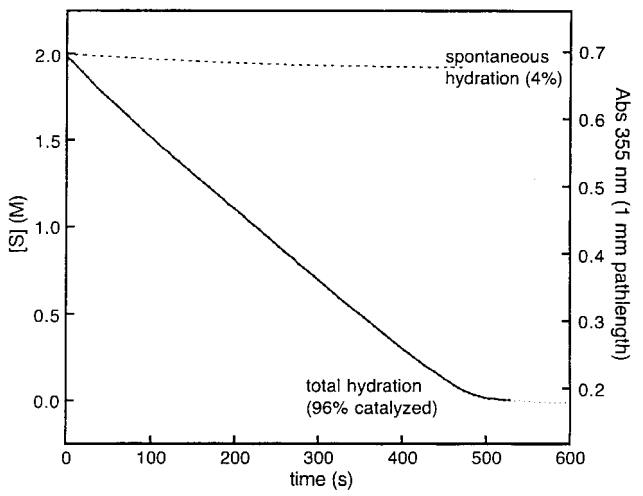
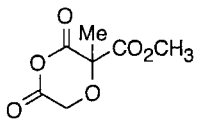


Figure 1. Hydration consumption of substrate *N-tert*-butylacetylketenimine catalyzed by $HO_2CCH_2OCMe(CO_2H)_2$, as measured spectrophotometrically. Initial substrate concentration, 2.0 M; catalyst concentration, 0.18 M; solvent, aqueous dioxane; apparent pH, 5.5; temperature, 25 °C

the dashed line at the top of the plot. Intramolecular steps **2** → **3** (k_2) and **4** → **1** (k_4) should be more rapid than either of the detected catalytic steps, in order to yield the observed kinetic behavior. Previous studies in an analogous case have indicated that step **4** → **1** is too rapid to measure, even at −60 °C[7] (the same catalytic behavior would follow were step k_4 rate limiting). Likewise, step **2** → **3** must be fairly fast,[8] because if that were not so then all three carboxyl groups of the catalyst would become converted to enol esters, with loss of repetitive action as in the following demonstration. We conclude that the kinetic behavior illustrated in Fig. 1 is satisfactorily accommodated by the main loop of Scheme 1.



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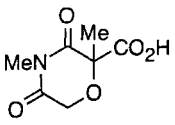


6

Methanol deliberately incorporated into the reaction mixture in minor amount serves as an inactivator. By replacing H_2O in step **1** → **2**, it converts the catalyst into an inoperative ester **7**, a species independently isolated (R = H) and characterized as an ineffectual catalyst. Reaction progress curves for two such kinetic runs are presented in Fig. 2. Inclusion of 0.0045 mole fraction of CH_3OH (vs available H_2O) progressively retards catalysis. Raising the mole fraction to 0.023 effectively halts

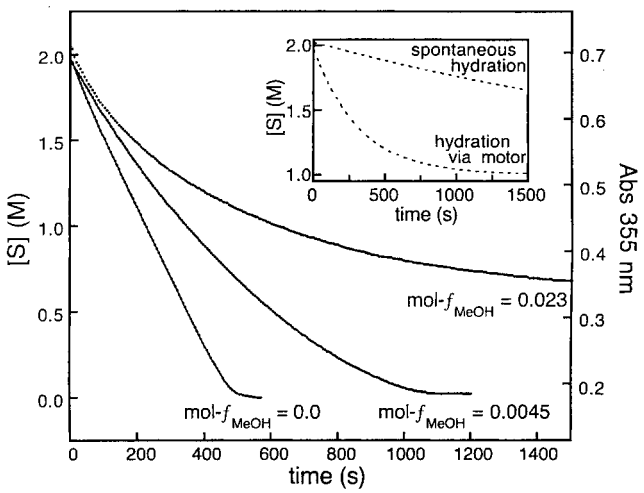
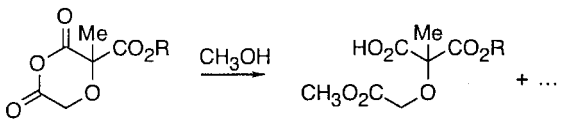


Figure 2. Demonstration of catalyst poisoning in hydration of acylketenimine as brought about by $HO_2CCH_2OCMe(CO_2H)_2$, carried out in absence and in presence of methanol (mole fractions relative to H_2O as indicated). Inset: partitioning of substrate consumption in the instance of CH_3OH mole fraction 0.023; approximate division into that consumed by tricarboxylate catalyst (exhausted after half of the substrate has been converted, owing to formation of methyl ester **7**), and into the portion of acylketenimine hydrated spontaneously (upper slow exponential decay, continuing unabated)

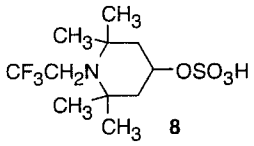
the catalytic reaction short of completion (although the competing spontaneous hydration of ketenimine continues). Calculations based on the efficiency of inactivation suggest that methanol reacts with the cyclic anhydride approximately eightfold more rapidly than does water on a molar basis. Such evidence, in conjunction with prototype rate constants previously described for the individual steps of the catalytic loop (k_1 and k_3), substantiates the designated mode of operation of the motor in alcohol-free solution.



(R = H, enolacetoacet-^{t}Bu-amide) **7**

Included on the periphery of Scheme 1 are a number of side reactions (lighter-faced type) that called for consideration when implementing the motor. By inspection it may be seen that each branch directed away from the catalytic loop, designated by a dashed arrow, would in some fashion vitiate the motor function. Since the catalyst does repetitiously operate successfully, most such side reactions may be excluded as kinetically insignificant. Others can be discounted by rate measurements for model reactions, as previously indicated. The one side reaction which we are at present unable to exclude is racemization of **3** via a bicyclic hemi-*ortho*-anhydride (lower-left branch-off from cycle in Scheme 1). Latent diversions of reaction intermediates are of consequence, for they potentially could be fatal to the catalytic process. By way of illustration, initial attempts to employ a carbodiimide in place of an acylketenimine as 'fuel' for the motor apparently succumbed to catalyst destruction by intramolecular *N*-acylation of an *iso*urea intermediate by the cyclic anhydride, in the manner typified by the potential diversion of **4** in the upper-center of Scheme 1. Hence, recourse was made to *N-tert*-butylacetylketenimine, which escapes that fate (**4** → **1** faster). The necessity for avoiding such faults is a major impediment as regards the design of molecular motors, since even minor side reactions can deplete a repetitively operating catalyst. As another example of design complication, control of pH presented a serious problem encountered in developing this system. The bimolecular activation step **3** → **4** is in actuality catalyzed by acid (H^+), as is also the competing spontaneous hydration of acylketenimine. Buffering of the kinetic reaction solutions was necessary, but many common buffers of an appropriate pK_a were found to react by nucleophilic addition to the acylketenimine (present in excess), with obstruction of kinetic analysis. The problem was solved by preparation of a new buffer, 1-trifluoroethyl-2,2,6,6-tetramethyl-4-piperidinol bisulfate (**8**), pK_a 4.54 ± 0.03 in aqueous solution, the nitrogen of which is sufficiently sterically hindered as not to react with the substrate, and

within which the solubility-conferring bisulfate anion is non-nucleophilic. By employment of this buffer, a fixed pH for the acylketenimine-consuming reaction could be selected that yielded satisfactory relative rates for individual steps within the catalytic loop of Scheme 1 (i.e. with step $k_3[H^+]$ rendered sufficiently faster than k_1, which latter conversion has no acid dependence).



8

DISCUSSION

Strategy for molecular motor design

The potential significance of this catalytic investigation is that it allows us to articulate formally a theory of molecular motors, one that the previous minimal case exemplifies. We identify three essential characteristics exhibited by such a motor, which may serve as a guide for the purposes of the invention of suchlike devices: the first attribute is consumption of energy in the form of a chemical (or other) fuel, so as to drive the process. The second attribute is catalysis of that energy dissipation involving two or more discrete chemical steps with structurally distinct intermediates, such that a repetitive molecular movement within the catalyst may be identified (i.e. motor function corresponds to serial conversion between intermediates). The third essential attribute is poly-phasic convolution yielding overlap of successive catalytic cycles, such that the first step of an ensuing cycle commences prior to completion of the final step of the immediately preceding cycle. This last requirement is the unique and critically defining feature of a nano-scale motor. Molecular chemical processes are microscopically reversible, and no accessible counterpart to physical momentum exists that can be exploited to sustain motion between successive catalytic turnovers (i.e. no 'memory' effect, as with a macroscopic flywheel). The 'relaxed state' of any simple, mono-phasic catalyst immediately after completion of its energy-expenditure cycle generally will allow undoing of work accomplished during the cycle (e.g. random molecular motions thermally reversing the change accomplished). It is essential to void this 'dead time' by having the succeeding cycle commence within a poly-phasic motor prior to completion of the previous cycle. That was achieved as efficiently as possible within the foregoing example, by arranging relative rate constants: $k_2,\ k_4 > k_3[\mathrm{AcCH{=}C{=}N^tBu}] > k_1[H_2O]$. In that manner, the possibility of an undesired re-formation of the selfsame cyclic anhydride (motor reversal) was specifically avoided, by acylketenimine activation of the next carboxyl prior to hydrolysis of an existing cyclic anhydride, with that latter step only subsequently finishing release of latent energy from the previously consumed acylketenimine. Maintenance of a continuous chemical dis-equilibrium within the catalyst-motor provides a thermodynamic bias, debarring mechanical relapse at any time during motor operation. The microscopic reversibility that generally characterizes molecular transformations is so counteracted, thereby yielding the processive vectorial change that we equate with motor function. To help visualize this, release of Gibbs free energy (chemical potential) as a function of time during the catalytic process in the case of the reciprocating motor is portrayed graphically in Fig. 3.

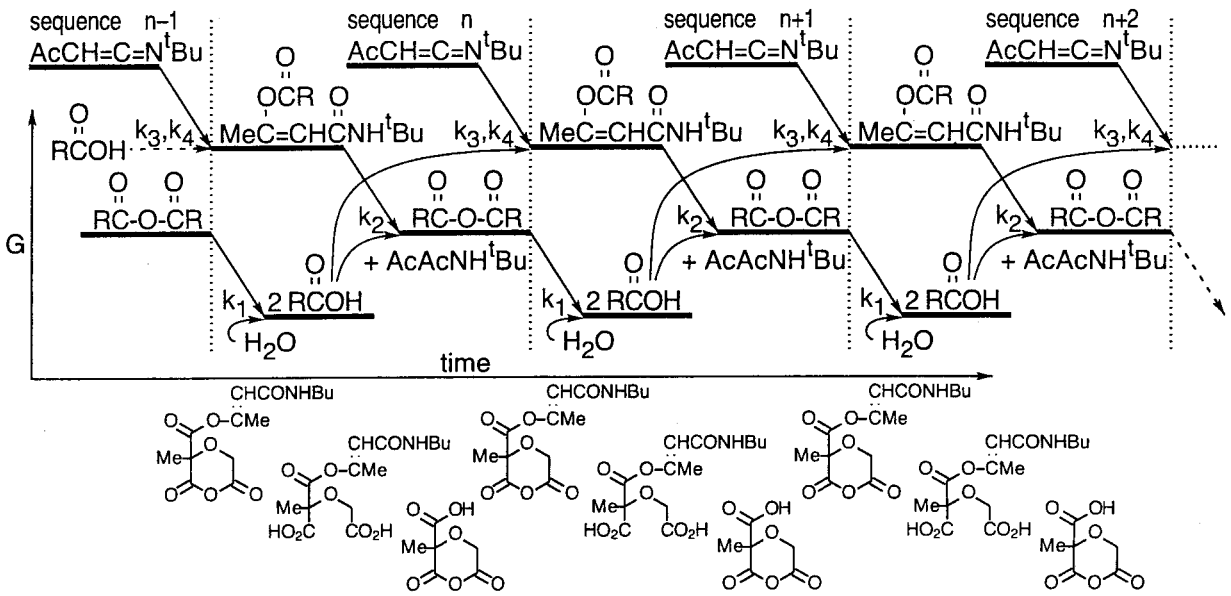


Figure 3. Diagram showing cascade of free energy in course of catalysis by reciprocating motor

Energy dissipation from each molecule of 'fuel' is represented by downward-sloping arrows connecting successive intermediates. With regard to a specific reaction sequence designated by integer n, energy dissipation commences (in the form of initiating step **3** → **4**) before that from sequence $n-1$ has finished (by ultimate step **1** → **2**), and this pattern persists for succeeding cycles ($n+1$, …). In the diagram, vertical divisions correspond to the transitory arrest point within the individual sequence detailed in Scheme 1 (created by the loop's rate-limiting step), with intermediates also summarized below the time-axis of the free-energy diagram.

We believe that any true molecular motor must in some manner exhibit this last catalytic feature (third attribute), as was recognized previously by Jencks in a biochemical context.[9] The challenge in molecular motor design is to incorporate this abstract precept into nanostructures having a desired function. Of overriding importance is the principle that a stipulated molecular motion should arise from a continuous discharge of chemical potential, in consequence of a poly-phasic overlap of energy-consumptive catalytic cycles. Concern for the management of energy flow is as critical as molecular structure assembly, in constructing a nano-scale motor.

CONCLUSION

A theory of molecular motors has been formulated and a 'bare-bones' specimen has been constructed and operated. The present motor does not actually accomplish any work, other than the overcoming of entropy in generating unidirectional conformational changes within the catalytic molecule. The plainness of the example provided ought not to obscure the conceptual advance. We regard it as a design virtue that the intrinsic objective can be achieved with such a compact molecule.

EXPERIMENTAL

Materials. The catalyst structure was readily secured synthetically: $HO_2CCH_2OCMe(CO_2H)_2$ obtained from EtO_2CCH_2OH and $BrCMe(CO_2Et)_2$ plus base, followed by saponification:[10]

2-Carboxymethoxy-2-methylmalonic acid. A solution of 25.3 g (0.1 mol) of diethyl 2-bromo-2-methylmalonate and 10.9 g (0.105 mol) of ethyl glycolate in 100 ml of dry tetrahydrofuran was stirred at −10 °C. To that mixture 2.28 g (0.105 mol) of sodium hydride were added portionwise. After the addition, the stirred mixture was gradually brought to room temperature over a period of 1 h, followed by 1 h of reflux. Salts were separated by filtration and the solvent was removed under reduced pressure. The residue was dissolved in chloroform, which was washed successively with 10% hydrochloric acid, saturated sodium bicarbonate and water. The solution was then dried over anhydrous sodium sulfate. Fractional distillation afforded 18.1 g (65.5%) of triethyl 2-oxa-1,3,3-butanetricarboxylate as a colorless oil, b.p. 110–120 °C (0.2 mm Hg); ^{1}H NMR (200 MHz, $CDCl_3$), δ 4.35 (2 H, s), 4.24 (4 H, q, J = 7 Hz), 4.20 (2 H, q, J = 7 Hz), 1.68 (3 H, s), 1.28 (6 H, t, J = 7 Hz), 1.27 (3 H, t, J = 7 Hz). A solution of 18 g (65 mmol) of this triester in 60 ml of methanol was stirred at 0 °C and slowly treated with 100 ml of 10% sodium hydroxide in methanol. The mixture was allowed to come to room temperature, and was stirred overnight. The obtained white precipitate was filtered, washed with methanol and dried under reduced pressure. The white powder was dissolved in a minimum amount of water and was acidified with sulfuric acid. The solution was then extracted continuously with diethyl ether for 24 h. The extract was concentrated under reduced pressure, giving 10.9 g (87%) of 2-carboxy-methoxy-2-methylmalonic acid as a colorless oil which eventually crystallized, m.p. 129 °C; ^{1}H NMR (500 MHz, CD_3COCD_3), δ 4.38 (2 H, s), 1.66 (3 H, s); ^{13}C NMR (125 MHz, CD_3COCD_3), δ 171.11, 170.07, 82.03, 64.33, 21.04. Anal. Calcd for $C_6H_9O_7$ $[M+H]^+$: *m/z* 193.0348. Found: 193.0356 (LSIMS, glycerol). The substance could not be dried for elemental analysis without decarboxylation.

Substrate *N-tert*-butylacetylketenimine was obtained from commercially available 2-*tert*-butyl-5-methylisoxazolium perchlorate by treatment with alkali.[5] Catalyst surrogates **5** and **6** (models) and catalytically inert ester **7** and novel buffer **8** were obtained as follows.

*3-Methoxycarbonyl-3-methyl-[1,4]-dioxane-2,6-dione (**5**).* To a stirred and cooled (0 °C) solution of 4.0 g (20.8 mmol) of 2-carboxymethoxy-2-methylmalonic acid in 50 ml of methylene chloride, 4.4 g (21 mmol) of trifluoroacetic anhydride were added dropwise. The mixture was allowed to come to room temperature and was stirred for 30 min. The solution was then concentrated. While under reduced pressure, a white solid precipitated out. The rest of the solvent and trifluoroacetic acid were carefully decanted and the precipitate was washed twice with 1:1 methylene chloride–hexane solution. The solid was then dried under reduced pressure. We obtained 3.37 g (93%) of 3-carboxy-3-methyl-[1,4]-dioxane-2,6-dione (**3**) as a white solid, m.p. 145 °C; ^{1}H NMR (500 MHz, CD_3COCD_3), δ 4.85 (1 H, d, J = 18 Hz), 4.78 (1 H, d, J = 18 Hz), 1.80 (3 H, s); ^{13}C NMR (125 MHz, CD_3COCD_3), δ 167.60, 164.02, 163.64, 78.72, 62.63, 21.59; IR, ν (cm^{-1}) 1827, 1778, 1720. A stirred and cooled (0 °C) solution of 1.58 g (9.1 mmol) of this material in 30 ml of diethyl ether was treated with 15 ml (10.7 mmol) of a 3% diethyl ether solution of diazomethane. After the addition the mixture was immediately subjected to solvent stripping and the

residue was then distilled, yielding 1.64 g (96%) of 3-methoxycarbonyl-3-methyl-[1,4]-dioxane-2,6-dione (**5**) as a colorless oil, b.p. 100–105 °C (0.2 mm Hg); ^{1}H NMR (400 MHz, CD_3COCD_3), δ 4.79 (1 H, d, J = 18 Hz), 4.73 (1 H, d, J = 18 Hz), 3.85 (3 H, s), 1.75 (3 H, s); ^{13}C NMR (100 MHz, CD_3COCD_3), δ 167.36, 163.92, 163.42, 78.75, 62.57, 53.78, 21.96. This substance allowed an independent estimate of the first-order hydrolysis rate for **1**.

*2-Carboxy-2,4-dimethylmorpholine-3,5-dione (**6**).* To 50 ml of a 1 M solution of methylamine in dioxane at 0 °C were added with stirring 20 ml of a 1 M solution of 3-carboxy-3-methyl-[1,4]-dioxane-2,6-dione (**3**) in dioxane. After the addition was complete, external cooling was removed and mixture was stirred for 15 min at room temperature. Next, excess methylamine and solvent were removed under reduced pressure. The oil obtained was dissolved in 50 ml of diethyl ether and washed 3× with 20 ml portions of 1 M hydrochloric acid. The solution was then dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting crude oil was purified by silica chromatography with ethyl acetate as eluent. We obtained 2.34 g (57%) of 2-(*N*-methylaminocarbonylmethoxy)-2-methylmalonic acid as a colorless oil; ^{1}H NMR (400 MHz, CD_3COCD_3), δ 4.16 (2 H, s), 2.76 (3 H, s), 1.65 (3 H, s). A solution of 1.02 g (5 mmol) of this material in 20 ml of diethyl ether was stirred at room temperature while it was treated with 1.07 g (5.1 mmol) of trifluoroacetic anhydride. The mixture became warm, and was stirred at room temperature for 20 min. Then, solvent was removed under reduced pressure and the concentrate obtained was purified by silica chromatography with ethyl acetate as eluent. We obtained 0.58 g (62%) of 2-carboxy-2,4-dimethylmorpholine-3,5-dione (**6**) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$), δ 4.64 (1 H, d, J = 17.5 Hz), 4.54 (1 H, d, J = 17.5 Hz), 3.23 (3 H, s), 1.80 (3 H, s); ^{13}C NMR (125 MHz, $CDCl_3$), δ 171.27, 169.43, 168.92, 65.20, 26.49, 22.65. This substance allowed an independent estimate of the rate of activation for **3** (for bimolecular reaction with AcCH═C═N^{t}Bu). It was converted into a dicyclohexylamine salt for elemental analysis, white crystals, m.p. 147 °C. Anal. Calcd for $C_{19}H_{32}N_2O_5$: C, 61.93; H, 8.75; N, 7.60. Found: C, 61.82; H, 8.89; N, 7.60%.

*2-(Methoxycarbonylmethoxy)-2-methylmalonic acid (**7**).* A solution of 0.174 g (1 mmol) of 3-carboxy-3-methyl-[1,4]-dioxane-2,6-dione (**3**) in 10 ml of methanol was stirred at room temperature for 3 min. Removal of excess methanol under reduced pressure resulted in 0.204 g (99%) of 2-(methoxycarbonylmethoxy)-2-methylmalonic acid (**7**) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$), δ 10.20 (2 H, s), 4.31 (2 H, s), 3.73 (3 H, s), 1.64 (3 H, s); ^{13}C NMR (100 MHz, $CDCl_3$), δ 171.61, 171.40, 81.68, 64.11, 52.47, 20.64. The spectral symmetry present in **7** confirms that the less-hindered carbonyl of the cyclic anhydride moiety in **3** was the site of nucleophilic addition. The substance was an ineffectual catalyst. Anal. Calcd for $C_7H_{10}O_7$: C, 40.78; H, 4.89. Found: C, 40.76; H, 4.98%.

*1-(2,2,2-Trifluoroethyl)-2,2,6,6-tetramethyl-4-piperidinol bisulfate (**8**, buffer).* A solution of 15.1 g (50 mmol) of perfluoro-*n*-butanesulfonyl fluoride in 20 ml of methylene chloride was stirred and chilled to − 78 °C while treated with a solution of 5.1 g (50.5 mmol) of triethylamine and 5 g (50 mmol) of 2,2,2-trifluoroethanol in 20 ml of methylene chloride. After addition the mixture was allowed to warm to room temperature, and was washed with 1 M hydrochloric acid, 1 M sodium hydroxide and water. The solution was dried over anhydrous sodium sulfate, and was concentrated under reduced pressure. Distillation of the residue gave 17.0 g (89%) of 2,2,2-trifluoroethyl perfluoro-*n*-butanesulfonate as a colorless oil, b.p. 141 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 4.73 (q, J = 7 Hz). A mixture of 15.3 g (40 mmol) of this material and 6.3 g (40 mmol) of 2,2,6,6-tetramethyl-4-piperidinol was stirred at 130 °C for 2 h. The resulting material was purified by silica chromatography with 1:1 ethyl acetate–hexane as eluent. We obtained 4.4 g (46%) of 1-(2,2,2-trifluoroethyl)-2,2,6,6-tetramethyl-4-piperidinol as white crystals, m.p. 128 °C; ^{1}H NMR (400 MHz, $CDCl_3$), δ 3.99 (1 H, m), 3.13 (2 H, q, J = 9 Hz), 1.86 (2 H, d, J = 12 Hz), 1.46 (2 H, t, J = 11.5 Hz), 1.15 (6 H, s), 1.06 (6 H, s); ^{13}C NMR (100 MHz, $CDCl_3$), δ 63.89, 57.15, 49.92, 46.82, 34.51, 22.79. A solution of 2.4 g (0.01 mol) of this material and 1.8 g (0.0113 mol) of pyridine–sulfur trioxide complex was refluxed in 25 ml of toluene for 15 h. The crude product precipitated from the chilled reaction mixture and was filtered. Recrystallization from water gave 2.9 g (91%) of 1-(2,2,2-trifluoroethyl)-2,2,6,6-tetramethyl-4-piperidinol bisulfate as white crystals, m.p. 222 °C; ^{1}H NMR (500 MHz, D_2O), δ 4.74 (1 H, m), 4.10 (2 H, q, J = 9 Hz), 2.34 (2 H, d, J = 12 Hz), 1.94 (2 H, t, J = 13 Hz), 1.43 (12 H, s); ^{13}C NMR (125 MHz, D_2O), δ 69.79, 69.15, 46.05, 42.73, 39.82, 28.88, 21.30. Anal. Calcd for $C_{11}H_{20}F_3NO_4S$: C, 41.37; H, 6.31; N, 4.39. Found: C, 41.23; H, 6.32; N, 4.36%.

Measurements. Estimates of pH for kinetic runs are glass-electrode meter readings in buffered aqueous dioxane (1:1, v/v), referenced to calibration standards in water alone. Catalytic kinetic runs as represented by Figs 1 and 2 were monitored spectrophotometrically at a suitable wavelength employing a 1 mm cell, with absorption measurements serially recorded at 6 s intervals subsequent to mixing of reactants. In most instances recrystallized **3** was the form in which the catalyst was introduced to initiate reaction. Solvent was maintained at 50% aqueous in runs that were 2 M in substrate, by reducing appropriately the amount of dioxane co-solvent.

Hydrolysis of control substance **5** was followed similarly by observing an exponential decay in the spectral absorption of the anhydride (265 nm) following addition to aqueous dioxane, yielding an independent estimate of the catalytically limiting rate constant. The velocity did not depend on pH. The characteristic rate constant of carboxyl activation was separately estimated by employing control substance **6** to combine with substrate acylketenimine under pseudo-first-order conditions (**6** in excess) by the same technique. The addition reaction was found to be acid catalyzed for pH values that provide an ionized carboxyl of model **6** (measured pK_a 2.65 ± 0.05 in aqueous dioxane by pH dependence of ^{13}C NMR); the inferred mechanism entails addition of the carboxylate anion to an transiently protonated acylketenimine ($pK_a < 2$ for conjugate acid of substrate).[6] Throughout, tolerances listed are standard errors from least-squares analysis.

Supplementary material

Derivation of rate equations employed in fitting kinetic data is available at the epoc website at http://www.wiley.com/epoc.

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