of exchange at the H_a position must be due to an important difference in the geometry of the enzyme–substrate complexes. We believe that the enantiomer having the configuration similar to that of a native peptide substrate is incorporated properly into the active site so that its 3-methylene group may be within reach of the catalytic groups of the enzyme (see Scheme I). On this basis, (−)-**1** has been assigned the *R* configuration and (+)-**1** the *S* configuration.

In contrast to what was observed for the H_a position, no significant exchange at the H_b site was observed for either of the enantiomers of **1**. This observation suggests that the exchange reaction at the H_a position in (*R*)-(−)-**1** proceeds highly stereospecifically with retention of configuration at the methylene carbon. It is improbable that complete inversion would be taking place because in this case both the H_a and H_b positions would have undergone exchange by the end of the reaction. Therefore, we envision that H_a, which faces basic group(s) in the active site, is predominantly abstracted and that the enolate intermediate formed is reprotonated on the same side with respect to its olefinic plane.

Turning to the kinetics of the exchange process, the reaction which we have observed can be treated as illustrated in eq 3. If we assume that the K_m value for **1**-*d* is the same as that for **1**-d_2, where **1**-*d* corresponds to **1** containing one deuterium at the methylene position, it can be easily shown that the rate expression for the exchange reaction is that given in eq 4. As predicted by eq 4, we have found that the exchange reaction follows an apparent first-order rate law and that the observed first-order rate constant, k_{obsd}, shows the expected inverse dependence on the initial substrate concentration up to substrate concentrations as high as 4.5×10^{-3} M. From the slope of a plot[9] of k_{obsd} vs. $1/[\mathbf{1}\text{-}d_2]_{initial}$, the k_{cat} value for the exchange of deuterium for hydrogen at the H_a position of (−)-**1**-d_2 is calculated to be 3.7×10^{-4} s^{-1} at pH 7.5. We have not been able to as yet obtain accurate measurements of the K_m value in exchange experiments. However, it may be reasonable to estimate K_m from the value of K_i measured for the inhibition by (−)-**1** of the CPA_γ catalyzed hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactate (see above). Using the assumption that $K_m \approx K_i$, k_{cat}/K_m for exchange at the H_a position of **1**-d_2 is estimated to be 3.4 M^{-1} s^{-1} at pH 7.5, 25.0 °C. A direct comparison of the enzyme's catalytic effect on the proton-abstraction reaction with the behavior of a suitable model system is not feasible at the present time. However, if one considers the catalytic effect of acetate on hydrogen–deuterium exchange in acetone, for example, the second-order rate constant k_{OAc^-} is 2.5×10^{-7} M^{-1} s^{-1} at 25.0 °C.[10,11] Since the pK_a for the ionization of the γ-carboxylic acid group of Glu-270 is thought to be in the vicinity of 6.5 and since $\beta_B = 0.88$ for carboxylate ion catalyzed exchange in the case of acetone,[12] one can estimate that a model for the carboxylate component of the CPA active site would give catalysis of exchange with a rate constant of $\sim 10^{-4}$–10^{-5} M^{-1} s^{-1}. On this basis we estimate that CPA_γ catalyzes the hydrogen–deuterium exchange reaction of **1** at least 10^4–10^5 times more effectively than an appropriate carboxylate ion model system would.

$$\mathrm{E} + \mathbf{1}\text{-}d_2 \underset{K_m}{\rightleftharpoons} \mathrm{E}\cdot\mathbf{1}\text{-}d_2 \xrightarrow{k_{cat}} \mathrm{E} + \mathbf{1}\text{-}d \qquad (3)$$

$$v = \frac{k_{cat}[\mathrm{E}_0][\mathbf{1}\text{-}d_2]}{[\mathbf{1}\text{-}d_2]_{initial} + K_m} \qquad (4)$$

In this communication we have demonstrated that the active site of a hydrolytic enzyme is capable of catalyzing stereospecifically an enolization reaction. Compared with enzymic hydrolytic reactions which can involve the formation of several intermediates (e.g., tetrahedral adducts and an anhydride in the case of CPA catalysis), the enolization of a ketonic substrate is a relatively simple process. The study of such enolization reactions at the active sites of enzymes involved in acyl and phosphoryl transfer may allow us to examine the catalytic properties of enzyme-bound nucleophilic groups without having to consider the complications of the formation and breakdown of a multiplicity of intermediates along the reaction pathway. Studies of this type are underway in our laboratory.

Acknowledgment. We are grateful to the National Institute of Arthritis, Metabolic and Digestive Diseases for support of this research. The NMR instrument used in this work was partly financed by the National Cancer Institute (PHS CA-14599) via the University of Chicago Cancer Research Center and by the National Science Foundation.

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(6) After incubation at 25 °C for 36 h no appreciable decrease of the enzymic activity of CPA_γ was observed in the hydrolysis of *O*-(*trans*-*p*-chlorocinnamoyl)-L-β-phenyllactic acid.
(7) The reaction was quenched by adding concentrated hydrochloric acid to a sample aliquot removed from the enzymic solution. Precipitated **1** was extracted using ethyl acetate. After the solution was dried over sodium sulfate, the solvent was removed by evaporation, and the NMR spectrum was recorded in deuteriochloroform solution.
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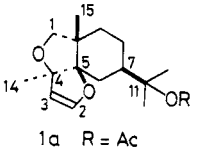
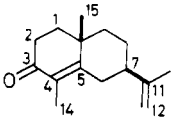
Synthesis of Phytuberin[1]

Sir:

Phytuberin[2] (**1a**) is a novel sesquiterpene qualified as a representative phytoalexin[3] together with rishitin[4] in the genus *Solanum* and characterized structurally by the presence of two fused hydrofuran rings. The proposed biogenesis[2b,5] suggested that the ring system in **1a** would be formed via oxidative cleavage of a C-1–C-2 bond of a hypothetical intermediate with an eudesmane skeleton. We describe herein a stereoselective synthesis of **1a**, which in its critical stages mimics the



1a R = Ac
1b R = H



2

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most likely biogenetic pathway.[5b] Our synthesis starts with (+)-α-cyperone[6a,7a] (**2**), easily prepared from commercially available (−)-carvone via (−)-*trans*-carone[6b] and (−)-α-santonin.[7b]

Oxidation of **2** with lithium diisopropylamide (LDA) in tetrahydrofuran (THF) and then with the molybdenum peroxide complex MoO_5·Py·HMPA (MoOPH)[8] afforded an ~2:1 inseparable mixture of 2β- and 2α-hydroxycyperones[9] (**3a** and **3b**) (62%), which were converted into the *tert*-butyldimethylsilyl ethers (**4a** and **4b**) (95%) by treatment with *tert*-butyldimethylsilyl chloride and imidazole.[10] The β- and α-configurations were assigned to the 2-hydroxyl groups of **3a** and **3b** on the basis of the NMR spectra:[9b] δ 1.34 and 1.22 (total 3 H (2:1), each s, 15-H) (δ_{calcd} 1.32 and 1.22 for **3a** and **3b**).[11] Treatment of the mixture (**4**) with lithium tri-*sec*-butylborohydride in THF (−78 °C, 2 h)[12] resulted in stereoselective reduction of only **4a**, giving 3β-ol (**5a**), mp 72–73 °C (55%), with unreacted **4b** (30%) (acetate of **5a**, δ 3.90 (dt, J = 10, 4.5, and 4.5 Hz, 2-H) and 5.30 (dd, J = 4.5 and 2 Hz, 3-H)). Reduction of **4b** with lithium aluminium hydride ($LiAlH_4$) (−78 °C, 15 min) also gave only 3β-ol (**5b**) (80%) (acetate of **5b**, mp 102–104 °C, δ 3.90 (m, 2-H, but dd, J = 10 and 4.5 Hz, on irradiation at δ 5.40) and 5.40 (d, J = 8 Hz, 3-H)). Alcohol **5a** was oxidized with *tert*-butyl hydroperoxide in the presence of bis(acetylacetonato)oxovanadium(IV) ($VO(acac)_2$)[13] to 4β,5β-epoxy-3β-ol (**6a**) and then converted into the 3β-mesylate (**7a**, 84% from **5a**). Epoxy mesylate **7a**, when submitted to the Birch reduction,[14] underwent reductive elimination to yield Δ^3-5β-ol (**8a**, 95%), which on hydrolysis in a 1:1:2 mixture of acetic acid, water, and THF (room temperature, 16 h) gave Δ^3-2β,5β-diol (**9a**), mp 170–171 °C (90%) (δ 4.18 (br dd, J = 10 and 6 Hz, 2-H) and 5.52 (br s, W_H = 5 Hz, 3-H)). Likewise, **5b** was transformed (via **6b**, **7b**, and **8b**) into Δ^3-2α,5β-diol (**9b**, 50% from **5b**) (δ 4.30 and 5.72 (each br, W_H = 16 and 7.5 Hz, 2- and 3-H)). Oxidation of **9a** and **9b** with manganese(IV) oxide[15] afforded the same α,β-unsaturated ketone, mp 117–119 °C, $[\alpha]^{20}_D$ +44.8° ($CHCl_3$) (85 and 90%), whose spectral data were identical with those of natural (+)-β-rotunol[16] (**10a**).[17]

Conversion of **10a** into **1a** was commenced by oxidation by the aforementioned Vedejs procedure (LDA and MoOPH in THF)[8] to give 1β,5β-diol (**11**, 57%), which formed the acetonide, as a single product (δ 3.60, br s, 1-H). In view of many unsuccessful attempts for oxidative cleavage of the C-1–C-2 bond leaving the isopropenyl group intact, **11** was converted into the 11,12-epoxide (**12**) by treatment with *m*-chloroperbenzoic acid in a heterogeneous mixture of 5% aqueous sodium hydrogencarbonate and dichloromethane (10 °C, 3 h), which was immediately reduced with $LiAlH_4$ in a 5:2 mixture of dimethoxyethane (DME) and THF in an inverse addition manner to yield 1β,2ξ,5β-11-tetraol (**13**, 56% from **11**). Smooth oxidative cleavage of the 1,2-diol system was achieved by treatment with lead(IV) acetate in pyridine (room temperature, 0.5 h).[18] The reaction proceeded as expected, giving the desired formyl γ-lactone (**14**, 80%) in one step (ν^{film}_{max} 2710, 1755, 1720, and 1630 cm^{-1}; δ 2.16 (s, 14-H) and 9.36 (s, 1-H)). Treatment of **14** with diisobutylaluminium hydride (3.5 equiv) in DME (−78, −20, and 0 °C, each 0.5 h)[19] effected reduction of two carbonyl groups followed by smooth cyclization, affording a hydrofuran alcohol, oil, $[\alpha]^{20}_D$ −40.6° (EtOH) (83%), which was converted to the acetate, oil, $[\alpha]^{20}_D$ −38.8° (EtOH) (84%), with acetic anhydride and 4-(*N*,*N*-dimethylamino)pyridine in triethylamine (room temperature, 16 h).[20] The alcohol and acetate were identified as phytuberol[2] (**1b**) and phytuberin[2] (**1a**), respectively, by direct comparison of the synthetic and natural samples. The result indicates completion of the first synthesis of phytuberin (**1a**) and also establishes the undecided absolute configuration (*R*) at C-7 of **1a**.

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Supplementary Material Available: Physical properties and spectral data of compounds (9 pages). Ordering information is given on any current masthead page.

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(17) (+)-α-Rotunol[16] (**10b**) was prepared as follows. Treatment of tosylhydrazone of **2** with methyllithium gave diene **15** (73% from **2**), which on oxygenation with triplet oxygen in the presence of tungsten(VI) chloride (dark, −78 °C, 2 h), followed by treatment of the resulting peroxide (**16**) with alumina (room

15 16

temperature, 16 h), afforded α,β-unsaturated ketone, mp 102–104 °C, $[\alpha]^{20}_D$ +53° ($CHCl_3$) (88%), whose spectral data were identical with those of natural sample of **10b**. Cf. D. H. R. Barton, R. K. Haynes, P. D. Magnus, and I. D. Menzies, *J. Chem. Soc., Chem. Commun.*, 511 (1974).
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The Dianion of Dimethyl Cyclobut-3-ene-1,2-dicarboxylate. Aromaticity vs. Coulombic Repulsion

Sir:

That monocyclic anions and dianions containing $[4n + 2]$ π electrons are unusually stable and diatropic is now well authenticated. However, as the ring size decreases, the charge density per atom increases, and at high charge densities coulombic repulsion may become the dominant factor. Thus in small, multiply charged species, such as the cyclobutene dianion **1**, electron repulsion may outweigh the stabilization normally associated with the aromatic state. A number of attempts to prepare derivatives of **1** have been unsuccessful,[2,3] but Pettit and co-workers[4] provided evidence for the preparation of **1** itself by treatment of 3,4-dichlorocyclobutene with excess sodium naphthalide and quenching with MeOD, when 4% 3,4-dideuteriocyclobutene was isolated. We now report the preparation of the dimethyl cyclobut-3-ene-1,2-dicarboxylate dianion **3** and describe spectroscopic and chemical data which indicate that this anion does *not* benefit from aromatic delocalization.

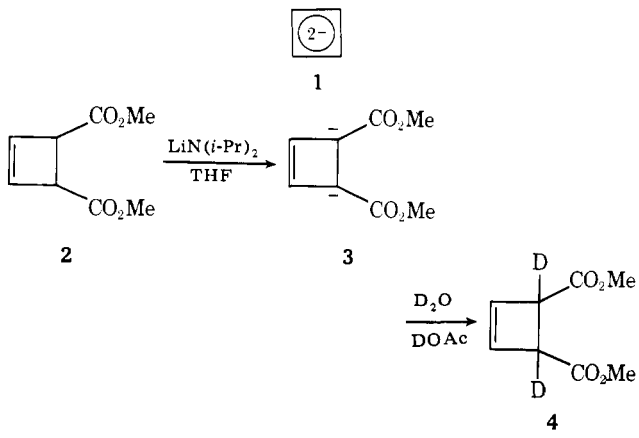
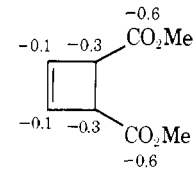
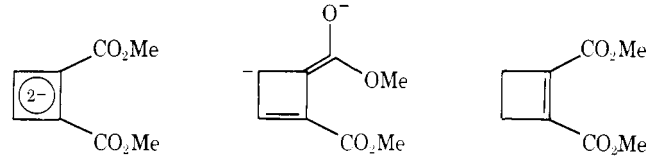


Chart I. Charge Distribution in **3**



Addition of dimethyl cyclobut-3-ene-*cis*-1,2-dicarboxylate (**2**)[5] to 2.5 equiv of $LiN(i\text{-}Pr)_2$ in THF-d_8 at −75 °C under argon gave a solution of the dianion **3**, stable at room temperature. The ^1H NMR spectrum showed two signals as broad singlets at δ 5.94 (2 H) and 3.73 (6 H),[6] and the ^{13}C NMR spectrum showed signals at 150.4 (C=O), 121.1 (C-3,4), 93.2 (C-1,2), 53.6 and 50.9 (OCH_3). The ^{13}C NMR assignments are based on peak multiplicity under offset decoupling and selective decoupling of the methoxyl protons.[7,8] Addition of the dianion solution to rapidly stirred excess D_2O in dioxane containing 5 equiv of AcOD gave a 1:1 mixture of *cis*- and *trans*-dimethyl 1,2-dideuteriocyclobut-3-ene-1,2-dicarboxylate (**4**) in 50% yield.[9] When the dianion was generated in the same way but in the presence of HMPA, quenching gave mainly (90%) *trans*-**4**.[10,12] Comparison of the ^{13}C NMR chemical shifts of **3** with those of **2** and dimethyl cyclobut-1-ene-1,2-dicarboxylate (**5**),[9,14] assuming a chemical-shift relationship of 160 ppm per electron,[15] gave the charge distribution shown in Chart I, suggesting that 40% of the charge is located in the four-membered ring with 10% on C-3,4. The presence of two methoxy signals in the ^{13}C NMR spectrum of **3** reveals a substantial barrier to rotation of the methoxyl groups between nonequivalent positions in the anion, a finding not unexpected from the charge distribution in the dianion and earlier observations by others.[16]

The ^1H NMR spectrum of **3** shows a small upfield shift of 0.27 ppm for the ring protons compared with that of the olefinic protons of **2**.[12,17] This is somewhat less than would be predicted from the ^{13}C NMR charge densities (δ 1.0 assuming a chemical-shift relationship of 10 ppm per electron)[18] and could be due to a small diatropic contribution from **3a**, to which the ^{13}C NMR spectrum is not susceptible.[19,20] In order to quantify any

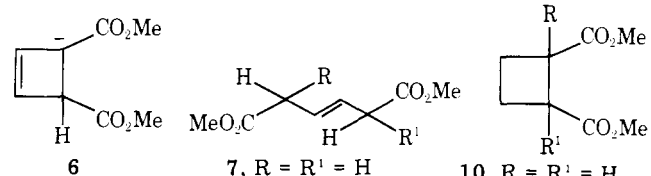


3a **3b** **5**

contribution from **3a**, the pK_a for the formation of the dianion **3** from the monoanion **6** was estimated by equilibration of **3** with hydrocarbons of known pK_a.

$$D^{2-} + RH \rightleftharpoons DH^- + R^- \qquad (1)$$

This pK_a value was then compared with those obtained in a similar manner for the formation of the dianions of dimethyl but-2-ene-1,4-dicarboxylate and dimethyl cyclobutane-1,2-dicarboxylate (**8** and **11**) from the corresponding monoanions



6 **7**, R = R¹ = H; **8**, R = R¹ = –; **9**, R = H; R¹ = – **10**, R = R¹ = H; **11**, R = R¹ = –; **12**, R = H; R¹ = –

9 and **12**. Solutions of the dianions were prepared by adding the ester (1.0 mmol) in THF (0.5 mL) to $LiN(i\text{-}Pr)_2$ (2.0

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