Table I. Kinetic Constants for Substrates and Inactivators

Substrate	V_{max} nm min^{-1} mg^{-1}	K_M, M	$t_{1/2}$ for inactivation	R^a
Benzylamine	140	1×10^{-3}	∞	—
Ethyl glycinate	150	5×10^{-6}	∞	—
Phenyl glycinate[4]	270	1.5×10^{-5}	1.4 min	80
p-Nitrophenyl glycinate[4b]	90	1×10^{-5}	0.3 min	10
Phenyl 3-aminopropionate[4,5]	30	1×10^{-4}	∞	—

[a] R = (moles O_2 consumed)/(moles enzyme inactivated). Reaction rates were determined by measuring O_2 consumption with a Clark type electrode. Reactions were carried out (at 23°) in 0.4 ml of 50 m*M* potassium phosphate buffer pH 7.0, except for *p*-nitrophenyl glycinate where 100 m*M* potassium phosphate buffer pH 6.0 was used. Specific activity of enzyme 270–400[7] at 23°.

with time, indicating enzyme inactivation. Further addition of substrate at this point results in no additional oxygen consumption. Addition of enzyme, however, leads to resumption of oxygen uptake with the same initial rate as that observed after the first enzyme addition. Inactivation, therefore, is not due to accumulation in solution of a compound enzymically derived from phenyl glycinate. This conclusion was confirmed by measuring the kinetics of phenyl glycinate inactivation in a separate experiment. The inactivation was found to be first order in enzyme for over four half-times. The rate of inactivation by phenyl glycinate is decreased competitively by ethyl glycinate (a noninactivating substrate). "R" (Table I) is independent of phenyl glycinate concentration and is not affected by ^2H substitution in the α-position.[6] These results suggest that oxidation and inactivation occur from the same binding site and may have one or more chemical steps in common.

When the enzyme is inactivated with phenyl [1-^{14}C]glycinate,[7] and then passed through Sephadex G-25 at 2° to remove small molecules, 1.8–2.1 mol of radioactivity are bound per mole of enzyme,[8] and no enzymic activity is observed. In addition, if the enzyme is inactivated with a mixture of phenyl [1-^{14}C]glycinate and phenyl [2-^{3}H]glycinate,[9] one atom of ^3H is lost per molecule of phenyl glycinate incorporated. These data probably indicate loss of one α-proton upon incorporation rather than a selection against the ^3H speciies, because there is no such selection in the oxidation of ethyl glycinate.[6]

By a process similar to that shown in eq 1, abstraction of a proton adjacent to a cyano group (instead of an ester group) could lead to the formation of a ketenimine, which is also expected to be a reactive species. Therefore, the effect of 2-aminoacetonitrile, NH_2CH_2CN, upon the enzyme was also investigated. The compound showed no substrate activity, but was an effective inhibitor. At 2.5×10^{-5} *M*, $t_{1/2}$ for inactivation was 0.8 min. In other respects, the inactivation resembled that of phenyl glycinate. Experiments with [1-^{14}C]aminoacetonitrile[10] showed that the enzyme became covalently labeled.[11] The rate of inactivation was decreased in the presence of substrates. Aminoacetonitrile inactivates rabbit plasma amine oxidase in vivo. Since concentrations of aminoacetonitrile which completely inactivate the plasma enzyme have no effect on the activity of the mitochondrial amine oxidase (a flavoprotein), the compound can be used in vivo to study the role of these enzymes in the metabolism of amines.[12] It is interesting to note that aminoacetonitrile is one of the most effective lathrogenic agents known.[13] An amine oxidase, believed to be mechanistically similar to plasma amine oxidase, is involved in the cross-linking of collagens.

The results described show that phenyl glycinate and aminoacetonitrile irreversibly inactivate plasma amine oxidase by covalent modification of the enzyme. The results are consistent with α-proton abstraction followed by elimination and reprotonation to form ketene-like intermediates, but until the process is examined in more detail, other mechanisms cannot be ruled out.

Acknowledgments. This work was supported by National Science Foundation Grant No. GB 31952 to R.H.A., National Institutes of Health Training Grant No. GM 212 to R. S., and National Institutes of Health Special Fellowship 5F02 GM 40063-02 to A.M.

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(7) Synthesized from [1-^{14}C]glycine; final specific activity = 2.2×10^{12} dpm/mol. See ref. 4a, 4b, 5.
(8) The molecular weight of the enzyme is 170,000 per dimer (F. M. Achee, C. Chervenka, R. A. Smith, and K. T. Yasunobu, Biochemistry **7**, 4329 (1968); calculations are based on specific activity = 600 for pure enzyme (H. Yamada and K. T. Yasunobu, *J. Biol. Chem.*, **237**, 1511 (1962)). The specific activity of the enzyme used in these experiments ranged from 220 to 400.
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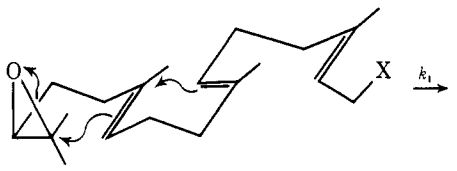
Total Biogenetic-Type Synthesis of (±)-Isopimaradienols and (±)-Araucarol

Sir:

Previous nonenzymic di- and triterpenoid terminal epoxide[1] cyclizations have invariably generated tri-, tetra- and pentacyclic systems in which the methyl substituted polyene backbone of the starting material is preserved. In a significant departure from this pattern, we have now observed that the "rearranged", pimaradien-3β-ol/isopimaradien-3-β-ol type (**4**)[2] is produced directly from an all trans 14,15-oxidogeranylgeranyl ester (**1**) by means of a specific sequence involving appearance of the exocyclic methylene bicycle **2**, loss of allylic X, and further cyclization (**3**), an overall process closely related to that by which the same category of tricycles is believed to be formed enzymically from a geranylgeranyl species or terminal epoxide thereof.[3]

Timing of the various chemical events (k_1:k_2:k_3) is crucial—starting material **1** and anticipated, unisolated intermediates **2** and **3** are subject to a number of side reactions, many of which have been observed in similar systems, and all of which must be minimized in order that significant amounts of **4** be produced in one reaction vessel. For exam-



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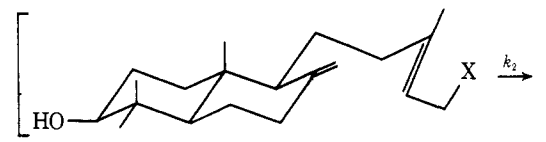

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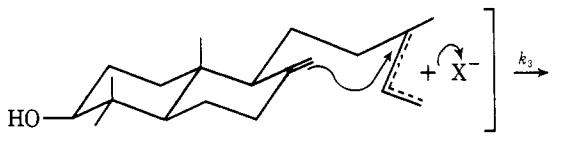

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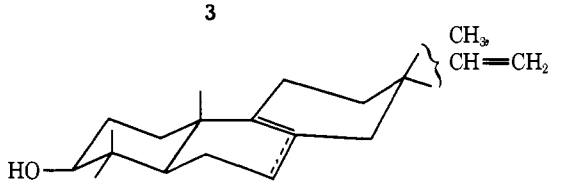
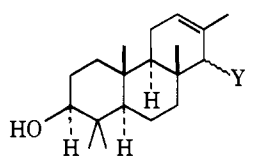
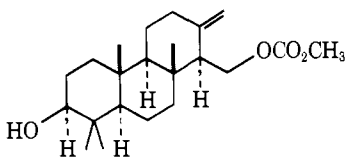


4

ple, if loss of X occurs (a) much more rapidly from **1** than k_1 or (b) much more slowly from **2** than $k_2 - k_3$, undesired cyclizations or other phenomena could prevail. Furthermore, the single catalyst and solvent combination utilized obviously must be suitable for all three reactions. Opportunity for control is offered in the choice of catalyst, solvent, and leaving group X. Although the acetate or 3,5-dinitrobenzoate in various solvents was shown not to serve the desired purpose, the above requirements were met with the methylcarbonate (**1**, X = $OCOOCH_3$), conveniently prepared by treatment of 14,15-oxidogeranylgeraniol[4] with methyl chloroformate. Exposure of the carbonate to $BF_3(C_2H_5)_2O$ in CH_3NO_2 at 0° for 0.5 hr provided, after work-up with aqueous $NaHCO_3$ and subsequent preparative TLC (silica gel with ethyl acetate–hexane), four major fractions: R_f 0.44 (A, 19%), 0.40 (B, 11%), 0.28 (C, 36%), and 0.23 (D, 17%). Fraction D was shown by $AgNO_3$–silica gel TLC to consist of approximately equal amounts of two components, assigned structures **5a** and **6** on the basis of ep-

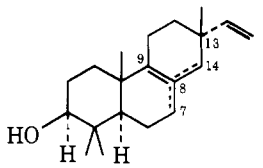


5a, Y = α-$CH_2OCO_2CH_3$
b, Y = β-$CH_2OCO_2CH_3$

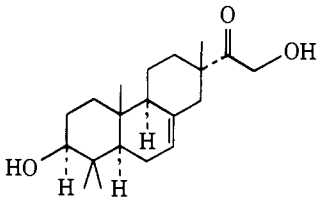


6

oxide cyclization precedents[1] as well as NMR spectral properties, including consonance with those of methyl epidrimenyl[5] carbonate. In that its NMR spectral properties tallied with those of methyl drimenyl[5] carbonate and the mass spectrum of the corresponding diol was in accord with that reported for authentic material,[1c] C is considered to possess structure **5b.** Component B, a complex mixture which did not possess 3β-OH functionality, was not investigated further. Preliminary VPC analysis of A suggested the presence of pimaradienols, and, through a combination of multiple elution $AgNO_3$–silica gel TLC (isopropyl alcohol–benzene), HPLC, and preparative VPC(OV225 on Gas Chrome Q), they were isolated and identified by VPC, NMR, and mass spectral comparison with authentic specimens. Approximately 65% of A consisted of nearly equal amounts of (±)-pimara-8(9),15-dien-3β-ol (C-13 isomer of **7b**)[6] and (±)-isopimara-8(9),15-dien-3β-ol (**7b**). An addi-



7a, $\Delta^{7(8)}$
b, $\Delta^{8(9)}$
c, $\Delta^{8(14)}$



8

tional 5% was shown to consist of (±)-isopimara-7(8),15-dien-3β-ol (**7a**), indistinguishable from the natural product.[10,11] While $BF_3(C_2H_5)_2O$–benzene or 85% H_3PO_4 cyclization produced similar types, the yields were not as satisfactory; and $BF_3(C_2H_5)_2O$–CH_3CN, $SNCl_4$–CH_3NO_2, $SNCl_4$–$(C_2H_5)_2O$, or $C_6H_2(NO_2)_3OH$–CH_3NO_2 did not effect formation of pimaradienols.

Through the method used by Wenkert and Kumazawa for the isopimaradienes,[8] dienols **7a, 7b,** or **7c** could be converted by dry HCl–$CHCl_3$ at 0° to an equilibrium mixture consisting by VPC analysis of 13% **7a,** 79% **7b,** and 8% **7c.** The isomerization of **7a** and **7b** by such means thus constitutes the total synthesis of (±)-isopimara-8(14),15-dien-3β-ol (**7c**), both antipodes of which occur naturally.[9a,b] Oxidation of **7a** with $KMnO_4$–K_2CO_3 in $(CH_2OCH_3)_2$–Ac_2O,[12] followed by saponification of intermediary acetate with KOH–MeOH, gave rise in good yield to (±)-araucarol (**8**),[10] isolated by preparative TLC and found indistinguishable from the natural product on the basis of chromatographic as well as NMR and mass spectral comparison.

Despite the propensity for formation of product type **5–6** under nonenzymic conditions, this cyclization course does not appear to take place in nature. On the other hand, the ubiquitous pimaradiene system can also be generated from acyclic precursor in the laboratory, as is demonstrated herein, although predominately as the thermodynamically most stable $\Delta^{8(9)}$ isomer, again not found in nature. We suggest therefore that chemical functions of enzymes involved in pimarane synthesis include (a) forestalling the production of types **5–6,** (b) increasing the pimarane yield beyond that which can be realized nonenzymically, and (c) producing the less stable $\Delta^{7(8)}$ or $\Delta^{8(14)}$ isomer, by kinetically controlled proton removal at C-7 or C-14.

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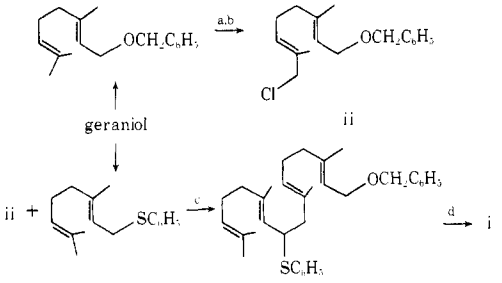
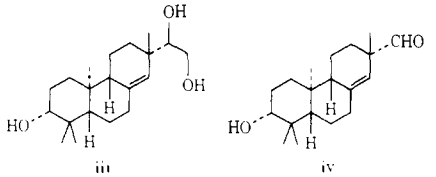


where (a) SeO_2–95% EtOH; (b) MeLi, TsCl, LiCl; (c) C_4H_9Li–THF; (d) Li/Na–$EtNH_2$.
(5) (a) P. A. Stadler, A. Eschenmoser, H. Schinz, and G. Stork, *Helv. Chim. Acta*, **40**, 2191 (1957); (b) R. Lees, Ph.D. Dissertation, Stanford University, 1972, and references therein.
(6) Comparison compound was prepared by sodium periodate cleavage of darutigenol (iii) to aldehyde (iv),[7] followed by conversion with methylene triphenylphosphine to 13-epi **7c**, which was transformed by treatment with HCl–$CHCl_3$ to 13-epi **7b**.[8]



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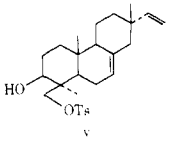

(12) Cf. K. B. Sharpless, R. F. Lauer, O. Repic, A. Y. Teranishi, and D. R. Williams, *J. Am. Chem. Soc.*, **93**, 3303 (1971).

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Deuterium Isotope Effects in the Solvolysis of Benzal Chlorides. II. Evidence for a Change in Mechanism in the Hydrolysis of *o*-Carboxybenzal Chloride in Water and Water–Dioxane Mixtures

Sir:

The relevance of the mechanisms of neighboring group participation to problems of enzymatic catalysis has been documented and continues to be a topic of interest to physical organic chemists.[1]

In this communication we wish to report that *o*-carboxybenzal chloride (I) hydrolyzes in water and in dioxane–water mixtures containing greater than 40% (by volume) dioxane by two distinct mechanisms.

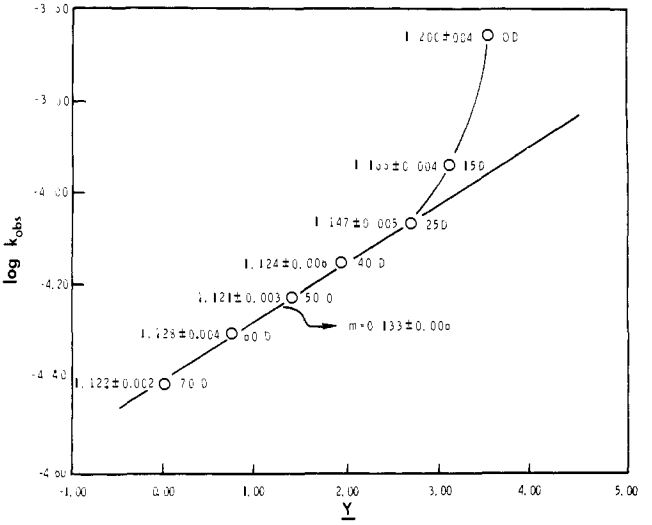
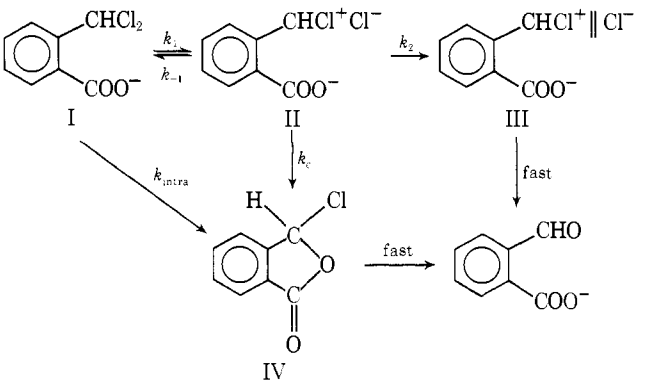


Figure 1. First-order rate constants for the hydrolysis of *o*-carboxybenzal chloride determined spectrophotometrically by monitoring the appearance of aldehyde at 257 mμ at 25° as a function of solvent polarity, Y. [NAOH] = 0.10 *M* for all solvents except water. For water [NAOH] = 0.20 *M*. Numbers to the left of each datum point refer to the α-D isotope effect determined under these conditions. Numbers to the right of each datum point refer to the volume per cent dioxane in the solvent, e.g., 70 D = 70% dioxane–30% water = 70 ml of dioxane + 30 ml of water.

In water the hydrolysis of I involves rate determining interconversion of ion-pair intermediates (k_2, Scheme I)

Scheme I



whereas in 40–70% aqueous dioxane the rate-determining step is intramolecular capture of the intimate ion-pair (II) by the neighboring carboxylate ion (k_c, Scheme I).

The α-D isotope effect observed for the sodium salt of I in water (1.200 ± 0.004, Figure 1) is the *maximum* value for benzal chlorides[2] and we, like Shiner,[3] interpret this result in terms of a transition state involving no covalent attachment of leaving group or incoming nucleophile (k_2, Scheme I). As expected for this mechanism the α-D effect for the lithium salt of I in water is unchanged, 1.197 ± 0.006. Addition of 0.20 *M* $LiClO_4$ in this solvent results in a modest rate increase (the expected normal salt effect, k_{salt}/k_0 = 1.09) and an unchanged α-D effect, 1.198 ± 0.009. Thus, in water, $k_{-1} > k_2$, $k_2 > k_c$, and k_2 is rate-limiting.

In the less polar dioxane–water mixtures the situation is quite different. In the range 40–70% dioxane the α-D effect for the sodium salt of I is much smaller, 1.124 ± 0.005. This α-D effect is consistent with a transition state involving substantial covalent bonding at the isotopically labeled position. For example, the α-D effect for rate limiting attack of solvent on the solvent-separated ion pair in the hydrolysis of *p*-methoxybenzal chloride in 85% aqueous diox-