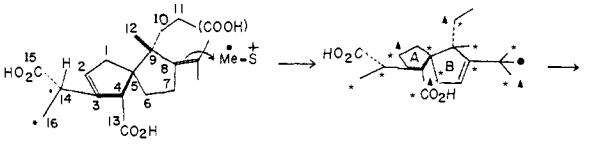
Table I. Incorporation of Labeled Precursors by the Cotton Wick Method [a]

| Precursor | No. of plants | Period of administration, days | % incorporation into GB[b] |
|---|---|---|---|
| Sodium [2-$^{14}$C]acetate (*) 1.0 mCi | 120 | 14 | 0.0033 |
| [2-$^{14}$C]MVA (rac) (▲) 0.3 mCi | 50 | 7 | 0.0048 |
| [Me-$^{14}$C]Methionine (●) 0.3 mCi | 80 | 5 | 0.0027 |

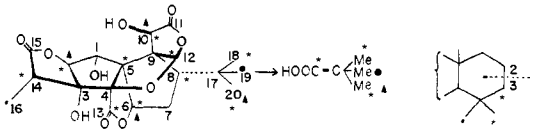
[a] Five-month plants of 20–25 cm height were employed. [b] Based on ginkgolide B (GB) recrystallized to constant specific activity.

Kuhn–Roth oxidation of GB **1**[1b] gave pivalic acid and acetic acid from 16-Me, which were separated by column chromatography and identified by tlc[7] and paper chromatography,[8] coupled with scintillation counting. The radioactive acids were submitted to Schmidt oxidation, the pivalic acid giving *tert*-butylamine (recrystallized to constant specific activity as its 2,4-dinitrophenyl benzoate, mp 182°) and carbon dioxide (trapped as barium carbonate). It was found that the radioactivity was distributed in the ratio of 2.2:1.0 (*tert*-$BuNH_2$ dinitrobenzoate–$BaCO_3$, see **6**). This clearly showed that, assuming a terpenoid origin for the ginkgolides, *the tert-Bu group could not have simply originated by a fission between C-2 and C-3 as in* **7**. The activity in acetic acid was distributed in a ratio of 1.2:1.0 between the methyl and carboxyl groups ($MeNH_2$ dinitrobenzoate–$BaCO_3$) (for discussion, see below.)

Mevalonate (denoted by ▲) was also incorporated (Table I) thus establishing *the terpenoid nature of the ginkgolides*. Acetic acid was not labeled; on the other hand, the pivalic acid was found to contain 95% of the activity expected from one MVA unit, provided the four $C_5$ units are evenly distributed.[9]



**4** **5**



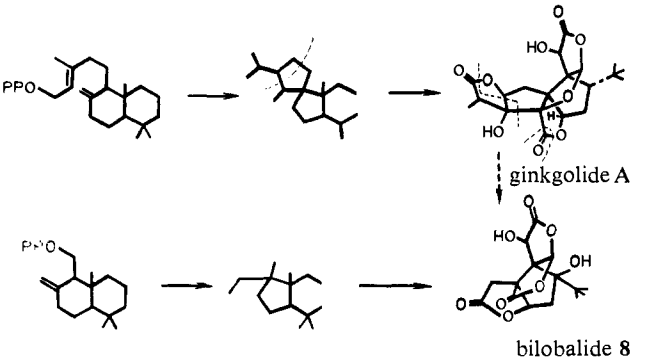
**1** **6** **7**

The ginkgolides were next administered with methionine (denoted by ●) in order to clarify the origin of the *tert*-Bu group. As shown in Table I, methionine was incorporated as expected, the radioactivity being exclusively located in the pivalic acid resulting from oxidation of GB.

The evidence mentioned above leads to Scheme I for ginkgolide biosynthesis. The precursor could be an entpimaradienone cation, *e.g.*, **2**, which undergoes three major modifications: (i) migration of the Me group from C-3 to C-14 (ginkgolide numbering is employed in **2**–**5**) which is in agreement with the even distribution of activity in the acetic acid resulting from acetate incorporation; (ii) formation of the characteristic spiro[4.4]nonane moiety with the correct stereochemistry at C-9; and (iii) formation of the *tert*-Bu group initiated by cleavage of the bond adjacent to the *gem*-dimethyl group, a cleavage frequently encountered in terpenoids.[10,11] Intermediacy of a structure such as **4** or **5** is necessary to explain the inversion at C-8 from going to **2** to the ginkgolides **1**.

Clearly bilobalide **8**[2] should be a sesquiterpene. Its biogenesis (Scheme II) can be explained either as

Scheme II. Relation between the Ginkolides (*e.g.*, Ginkolide A) and Bilobalide **8**[a]




[a] The latter can either be derived from GA, by losing the five carbons indicated by dotted lines in the GA structure, or from farnesol.

being a pentanorginkgolide, or a genuine sesquiterpene derivable from farnesyl pyrophosphate in a manner similar to that shown for the ginkgolides.[12]

(7) A. Lynes, *J. Chromatogr.*, **15**, 108 (1964).
(8) E. P. Kennedy and H. A. Barker, *Anal. Chem.*, **23**, 1033 (1951).
(9) Although rare, preferential labeling has been observed, *e.g.*, coriamyrtin (sesquiterpene): M. Biollaz and D. Arigoni, *Chem. Commun.*, 633 (1969).
(10) D. Arigoni, D. H. R. Barton, R. Bernasconi, C. Djerassi, J. S. Mills, and R. Wolff, *J. Chem. Soc.*, 1900 (1960); G. H. Whitham, *ibid.*, 2016 (1960).
(11) K. Habaguchi, M. Watanabe, Y. Nakadaira, and K. Nakanishi, *Tetrahedron Lett.*, 3731 (1968).
(12) We acknowledge support by the Ministry of Education (Japan) and USPHS Grant No. CA 11572.

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*Received April 22, 1971*

## A Novel Palladium(II)-Catalyzed Cis–Trans Isomerization of Enol Propionates and Vinyl Halides[1]

*Sir:*

There have been many reports of double bond and cis–trans isomerization catalyzed by metal salts.[2] Mechanisms suggested for these reactions include intermolecular hydride transfer *via* metal hydrides, intramolecular hydride transfer *via* π-allyl hydrides, and reversible π-allyl complex formation.[2c] This communication will describe a Pd(II)-catalyzed cis–trans

(1) Hercules Research Center Contribution No. 1549.
(2) For recent reviews see: (a) N. R. Davies, *Rev. Pure Appl. Chem.*, 17, 83 (1967); (b) E. W. Stern, *Catal. Rev.*, 1, 125 (1967); (c) F. R. Hartley, *Chem. Rev.*, 69, 799 (1969).

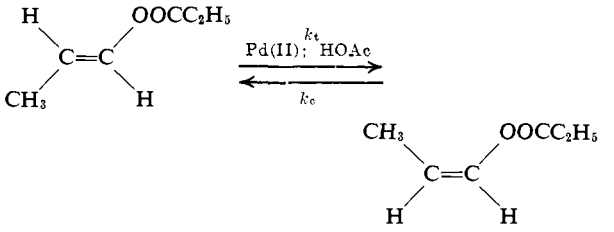
**Table I.** Rates of Isomerization without Exchange of the Cis and Trans Isomers of the Enol Propionate of Propionaldehyde at Various $[Pd(II)]_t$ and $[Cl]_t$[a]

| $[Pd(II)]_t$, $M \times 10^2$ | $[Cl]_t$, $M \times 10^2$ | $[Li_2Pd_2Cl_6]$,[b] $M \times 10^2$ | $[LiCl]$,[b] $M \times 10^2$ | $k_{obsd}$,[c] $sec^{-1} \times 10^6$ | $\frac{k_{obsd}[LiCl]}{[Li_2Pd_2Cl_6]}$, $sec^{-1} \times 10^5$ |
|---|---|---|---|---|---|
| 0.667 | 3.82 | 0.319 | 1.65 | 7.55 | 3.91 |
| 0.667 | 5.32 | 0.308 | 2.85 | 4.01 | 3.72 |
| 5.04 | 21.33 | 2.40 | 4.8 | 19.7 | 3.94 |
| 0.33 | 7.28 | 0.138 | 4.96 | 1.05 | 3.78 |
| 2.24 | 18.68 | 0.99 | 8.23 | 3.40 | 2.82 |
| 2.668 | 29.28 | 1.12 | 12.65 | 3.33 | 3.77 |
| 2.24 | 48.68 | 1.01 | 20.3 | 1.54 | 3.78 |
| 2.24 | 88.68 | 0.706 | 31.2 | 1.15 | 5.08 |

[a] Rates were measured at 25° by glc determination of the cis–trans ratio at various times during the isomerization. Data were treated in the usual fashion for first-order runs approaching equilibrium (A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, Wiley, New York, N. Y., 1960, p 186). [b] These values were calculated from values of equilibrium constants determined in a previous study (P. M. Henry and O. Marks, *Inorg. Chem.*, **10**, 373 (1971)). [c] $k_{obsd} = k_t + k_c$ for a given set of reaction conditions. Values of individual rate constants can be readily calculated (see Frost and Pearson, footnote *a*) from the equilibrium mixture which is 68% trans.

isomerization which apparently does not occur by any of these mechanisms.

During the study of enol acetate exchange[3] with acetic acid using deuterium labeling, it was found that *cis*- or *trans*-propenyl acetate isomerized without exchange with solvent. A further study of this isomerization without exchange was undertaken using propionate

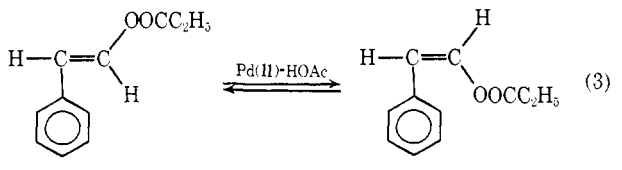



(1)

esters. Some typical values of the rate constants for various total Pd(II) concentrations ($[Pd(II)]_t$) and total chloride concentrations ($[Cl]_t$) are listed in Table I. The fact that the ratio in the last column of the table remains constant with varying $[Li_2Pd_2Cl_6]$ and [LiCl] indicates that the rate expression for the isomerization is given by eq 2 where $k$ ($= k_t + k_c$) is $3.5 \times 10^{-5}$

$$\text{rate} = \frac{k[Li_2Pd_2Cl_6][\text{enol propionate}]}{[LiCl]} \qquad (2)$$

$sec^{-1}$ at 25°. The value of the corresponding rate constant for the isomerization of the cis and trans isomers of the enol propionate of phenylacetaldehyde was also measured and found to have a value of $3.5 \times 10^{-6}$ $sec^{-1}$ at 25°.




(3)

*cis*- and *trans*-1-chloro- and 1-bromopropenes are also isomerized without exchange of halide with the LiCl in solution. During the course of all three cis–trans isomerizations no allylic isomers were formed.

Strong acid had little effect on all three isomerizations. At $[Pd(II)]_t = 0.048$ *M* and a $[Cl]_t$ of 0.37 *M*, reaction mixtures containing 1 *M* $CF_3COOH$ gave rates of isomerization only about 30% faster than reaction mixtures without added strong acid.

The cis isomers of 1-propen-1-ol propionate and the propenyl halides were allowed to equilibrate in $CH_3COOD$ which was 0.05 *M* in $[Pd(II)]_t$ and 0.3 *M* in $[Cl]_t$. The trans isomers were collected by glc and analyzed for deuterium content by mass spectrometry. The propionate contained 1% deuterium and the halides less than 0.2%. *cis*-1-Propen-1-ol propionate was isomerized to an equilibrium mixture in $CH_3COOH$ containing 0.024 *M* $[Pd(II)]_t$ and 0.13 *M* $[Cl]_t$ in the presence of a sixfold excess of ethylene-$d_4$. The trans isomer was analyzed as above and found to contain less than 0.5% deuterium.

Of the three mechanisms suggested for cis–trans isomerization, the two involving $\pi$-allyl complexes can be eliminated since the phenylacetaldehyde enol propionates, which cannot form $\pi$ allyls, were isomerized. The metal hydride mechanism could involve either palladium(II) hydride of palladium(IV) hydride. The palladium(II) hydride could involve formation of Pd(0) by a small amount of oxidation of the organic substrate followed by generation of palladium(II) hydride by oxidative addition of $H^+$ to Pd(II). The palladium(IV) hydride would be formed by oxidative addition to Pd(II). These two mechanisms seem unlikely for the following reasons. (1) The Pd(0) would have to be formed by reduction of the Pd(II). This would require induction periods and give poor kinetics. The clean kinetics and lack of induction periods argue against this mechanism. (2) There was little or no exchange with solvent when $CH_3COOD$ was used as solvent. (3) The lack of appreciable strong acid catalysis argues against hydride formation by oxidative addition.[4] (4) No double bond isomerization to allylic products was observed. (5) No deuterium exchange from $C_2D_4$ during isomerization was observed.

To further test the feasibility of hydride mechanisms, palladium(II) hydride was formed *in situ* by the reaction of diethylmercury with Pd(II) salts[6] and its effect on

(3) (a) P. M. Henry, *Amer. Chem. Soc., Div. Petrol. Chem., Prepr.*, 14 (2), B15 (1969); (b) P. M. Henry, *J. Amer. Chem. Soc.*, in press; (c) all reactions studied at 25°.

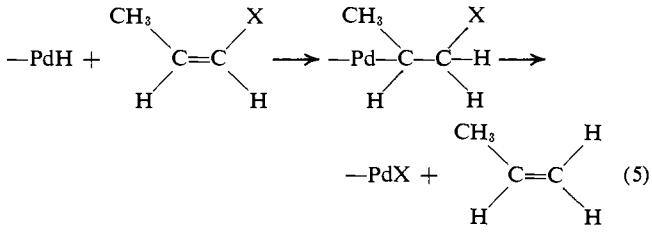
(4) A Pd(II)-catalyzed isomerization postulated to proceed *via* hydrides was found to display acid catalysis.[5]

(5) R. Cramer and R. V. Lindsey, Jr., *J. Amer. Chem. Soc.*, **88**, 3534 (1966).

(6) There seems little doubt that mercury alkyls exchange with $PdCl_2$ and that Pd(II) alkyls eliminate palladium(II) hydride: see, for instance, G. Calvin and G. E. Coates, *J. Chem. Soc.*, 299 (1951).

$$(C_2H_5)_2Hg + -PdCl \longrightarrow C_2H_5HgCl + -PdC_2H_5 \longrightarrow -PdH + C_2H_4 \quad (4)$$

the enol propionate tested. It was found that no isomerization occurred but rather the enol propionate disappeared. Propylene was detected as one of the products in the gas phase of the reaction vessel. This product was most likely formed by palladium(II) hydride addition followed by elimination of Pd(II) and acetate, chloride, or bromide (X = OAC, Cl, or Br).



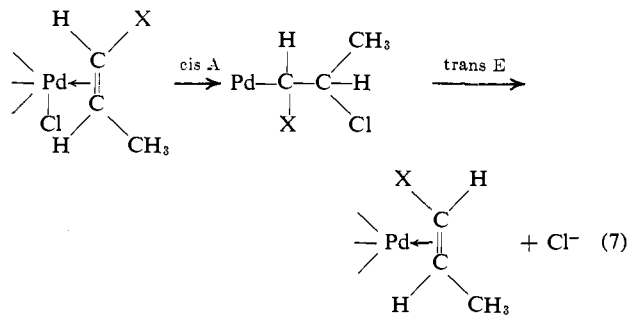
(5)

It thus appears that the mechanisms usually considered for Pd(II)-catalyzed double bond isomerizations are not operative in the present examples. It is noteworthy that the rate expression for enol propionate isomerization is consistent with a Pd(II) dimer $\pi$ complex being the species which performs the isomerization. The exact mechanism whereby the $\pi$ complex

$$Li_2Pd_2Cl_6 + \text{enol ester} \rightleftharpoons Li_2Pd_2Cl_5(\text{enol ester}) + LiCl \quad (6)$$

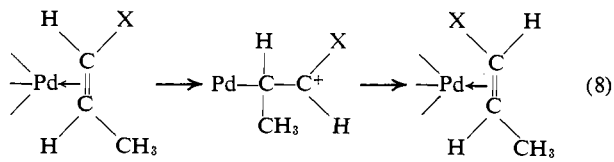
causes the isomerization is uncertain.

Possibilities include nonspecific chloropalladation (A = addition, E = elimination)



(7)

or arrangement of the $\pi$ complex to a carbonium ion with sufficient lifetime for rotation. Studies aimed at elucidating the exact mechanism are presently under-



(8)

way. Whatever the mechanism, this type of isomerization will have to be considered as a possible route in the cis–trans isomerization of other olefins.

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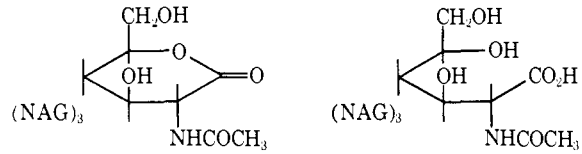
## The Role of Strain in Catalysis by Lysozyme[1]

*Sir:*

We have prepared the lactone I derived from tetra-*N*-acetylchitotetraose[2] and have determined the association constant for its binding to lysozyme. We explain



I II

NAG = *N*-acetylglucosamine

why the magnitude of this association constant is evidence for the importance of strain in catalysis by lysozyme.

I was prepared in the following way. A solution of tetra-*N*-acetylchitotetraose[3,4] (0.005 *M*) was oxidized to the acid anion of II with iodine (0.01 *M*) in KI (0.05 *M*)–$K_2CO_3$ (0.045 *M*) at 4° in the dark.[5] After 3.5 hr, when titration of an acidified aliquot with sodium thiosulfate showed that 1 mol of iodine per mole of sugar had been reduced, the reaction mixture was adjusted to pH 2 with $H_2SO_4$, and the excess iodine was extracted with benzene. The extracted aqueous solution was adjusted to pH 7 with KOH, concentrated, and chromatographed on a Bio-Gel P-2 column, which separated the potassium salt of II (KII) from the other ions. Titration of KII gave a p$K_a$ of 3.6 for II. Passage of KII through a cation exchange resin (Bio-Rad AG50WX-8, $H^+$ form) and evaporation of the eluent to dryness yielded a mixture of solid I and II. Colorimetric tests for reducing sugar[6] and amine[7] showed that the mixture contained less than 2% unreacted tetraose or deacetylated product. The lactone content and equivalent weight of the mixture were determined by rapid titration to pH 5.5, at which pH the half-time for hydrolysis of I is greater than 30 min, followed by titration to pH 9, at which pH the half-time for hydrolysis of I is less than 1 min: 23 mol % I and equivalent weight, 832 (theoretical value, 843). The concentration of I was routinely measured with the neutral hydroxylamine–ferric chloride test.[8,9]

The interaction of I with lysozyme was studied by its alteration of the protein fluorescence[10] and its inhibition of lytic activity[11] (Figure 1). The lactone was identified as by far the more potent compound in the mixture of I and II by comparing the concentration dependences of the changes in fluorescence and lytic activity before and after hydrolysis of I (see Figure 1).

(1) Supported by National Science Foundation Grant No. GB 12848.
(2) *O*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-gluconic acid δ lactone.
(3) J. A. Rupley, *Biochem. Biophys. Acta*, **83**, 245 (1964).
(4) M. A. Raftery, T. Rand-Meir, F. W. Dahlquist, S. M. Parsons, C. L. Borders, Jr., R. G. Wolcott, W. Beranek, Jr., and L. Jao, *Anal. Biochem.*, **30**, 427 (1969).
(5) R. W. Jeanloz and E. Forchielli, *J. Biol. Chem.*, **188**, 361 (1951).
(6) J. T. Park and M. J. Johnson, *ibid.*, **181**, 149 (1949).
(7) H. Rosen, *Arch. Biochem. Biophys.*, **67**, 10 (1957).
(8) W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, **83**, 1743 (1961).
(9) T. E. Couling and R. Goodey, *Biochem. J.*, **119**, 303 (1970).
(10) S. S. Lehrer and G. D. Fasman, *Biochem. Biophys. Res. Commun.*, **23**, 133 (1966).
(11) G. Gorin, S.-F. Wang, and L. Papapavlou, *Anal. Biochem.*, **39**, 113 (1971).