around ten. Thus the effect of the substituents in the gas phase is ten times larger. While attenuation in aqueous solution is always observed, the ρ factor is normally around four, i.e., considerably smaller.[4,5,10]

The largest deviation between gaseous and aqueous values is observed for p-OH benzoic acid which is much stronger in the gas phase. (See Figure 1.) In a study of the gas phase acidity of substituted phenols[6] plots similar to those shown in Figure 1 but involving the phenols were made. Taking $\sigma^- = 0.728$ for the p-CO_2H substituent (Jaffé[11]) it could be estimated with help of these plots that the acidity of the hydroxy proton in p-OH benzoic acid should be higher than that of the carboxy proton and close to that observed for p-OH benzoic acid. This explains why this acid does not fit in the benzoic series.

Ortho substituents have long been known to have specific or "anomalous" effects in solution. Thus the aqueous acidities of ortho-substituted benzoic acids are generally much higher than those of the para compounds. Some of these effects have been partially explained. Thus the direct conjugation by π donating substituents as illustrated in resonance structure I, which weakens the para-substituted benzoic acids, is assumed to be ineffective in ortho position since steric hindrance causes the carboxy group to twist out of the phenyl ring plane. The lack of resonance stabilization in the neutral ortho acid results in increased acidity relative to the para-substituted isomer. As was pointed out earlier in the gas phase resonance, structure I does not make a marked contribution and the benzoic acids with π donating ortho and para substituents should have more similar gaseous acidities. That this is the case is easily verified from Table I. The o-OH benzoic acid, or, as was established above, the o-COOH phenol, is an exception and has a much larger gaseous acidity than the para isomer. This must be due to stabilization of the ortho anion by strong intermolecular hydrogen bonding involving the remaining acidic proton and the negative center.

The very much higher aqueous acidity of o-NO_2 and o-CN substituted acids relative to the para isomers seems less well understood. It is interesting to note (Figure 1) that in the gas phase p-NO_2 and o-NO_2 benzoic acids have roughly similar acidities, the para isomer being the stronger acid.

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A Novel Substrate for Prenyltransferase. Formation of a Nonallylic cis-Homofarnesyl Pyrophosphate

Sir:

The substrate specificity of prenyltransferase (farnesyl pyrophosphate synthetase EC 2.5.1.1) is relatively low with respect to the structure of the allylic pyrophosphate, and a number of allylic pyrophosphates have been found to act as substrates to react with isopentenyl pyrophosphate (3-methylbut-3-enyl pyrophosphate, 1a)

in the reaction catalyzed by either liver or pumpkin enzyme.[1–7] However, the structural requirement for the condensing partner has been shown to be so stringent that in a series of homologs of 1 only 3-ethylbut-3-enyl pyrophosphate (1b) can act as an artificial substrate in place of the genuine substrate 1a.[8,9] This fact has stimulated us to explore other artificial substrates of the isopentenyl pyrophosphate type, and we have examined the substrate specificity of this enzyme with respect to homologs of 2. In this paper we report the finding that among 2a, 2b, and 2c only 2b is reactive and that the enzymatic reaction of 4-methylpent-4-enyl pyrophosphate (2b) with geranyl pyrophosphate (3) results in an exclusive formation of the cis isomer of a nonallylic homofarnesyl pyrophosphate (4).
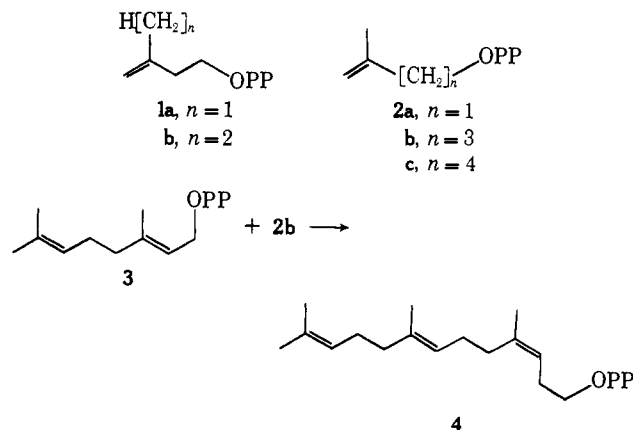
Compounds 2a, 2b, and 2c were prepared from the corresponding alcohols by phosphorylation as usual and were purified by selective crystallization from a solvent system of n-propyl alcohol–ammonia–water (6:3:1).[10] The incubation mixture for the enzymatic reaction contained, in a final volume of 5 ml, 125 μmol of Tris–HCl buffer, pH 7.5, 25 μmol of $MgCl_2$, 125 nmol of [^{3}H]-geranyl pyrophosphate (3), 500 nmol of a homolog to be examined, and 0.5 mg of prenyltransferase purified from pig liver according to the method of Holloway and Popják.[1] The mixture was kept at 37° for 5 hr and was then treated with alkaline phosphatase, and the radioactive materials were extracted with light petroleum and analyzed by radio-glpc with a 1-m column of Silicon OV-17 1.5% at linear programmed temperature at a rate of 4°/min from 100 to 200°.

The material thus derived from the incubation of [^{3}H]-3 with either 2a or 2c showed no radioactivity peak other than that for geraniol recovered from the starting substrate, but the material from the reaction of [^{3}H]-3 with 2b gave a radioactivity peak at a retention time of

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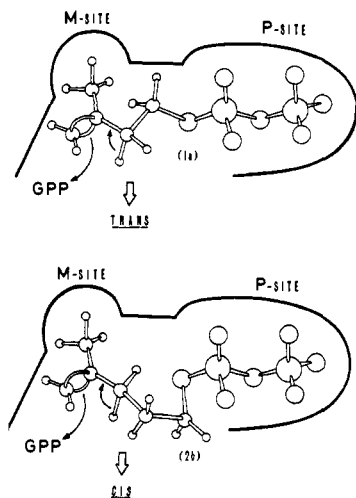(10) B. K. Tidd, J. Chem. Soc. B, 1186 (1971).

Figure 1.

1.16 relative to that for *trans,trans*-farnesol, suggesting a formation of a homolog of farnesol. Glpc–mass spectrometric analysis of the material derived from non-labeled **3** with **2b** also showed a peak at a retention volume reasonable for a homofarnesol (1.35 relative to that for *trans,trans*-farnesol on a 1-m column of Silicon OV-1 1% at 150°) and the mass spectrum for this peak exhibited a parent ion at m/e 236 ($C_{16}H_{28}O$) with an intensity of 1.9% relative to the base peak at 69. Peaks were also observed at 218 (M − 18), 205 (M − 31), 193 (M − 43), 167 (M − 69), 137 ($C_{10}H_{17}$) with relative intensities of 0.4, 0.4, 1.6, 2.0, and 5.8, respectively.

For a further identification, *cis*- (**5a**) and *trans*-homofarnesol (**6a**) were chemically synthesized essentially according to the method of Lucius[11] as modified by Watanabe,[12] and the enzymatically derived material was compared with the authentic specimens by glpc–mass spectrometery. Both the retention time and the mass spectrum of this material were completely identical with cis isomer **5a** in various conditions with Silicon OV-1. Since a better glpc separation was observed with the acetates (**5b** and **6b**) on Carbowax 20M, the product of

5a, X = OH
b, X = OAc

6a, X = OH
b, X = OAc

R =
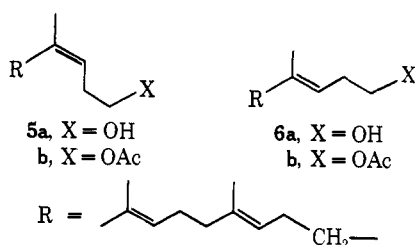
the enzymatic reaction was converted into the acetate. The glpc–mass spectrometry of the acetate also clearly revealed the identity with the cis isomer (**5b**) and denied the presence of the trans isomer (**6b**). Thus, the formation of **4** from **2b** and **3** was proved. The yield was *ca.* 50% based on **3**.

The exclusive formation of the cis isomer (**4**) catalyzed by the prenyltransferase which is responsible for the synthesis of the trans isomer of farnesyl pyrophosphate can be explained by assuming that the binding site for **1a** contains an M site and P site which must be filled up simultaneously with the methyl group

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and pyrophosphate moiety of the **1a** molecule, respectively, to hold C-4 and *pro-R* H of C-2 at the fixed position. In the case of the homolog (**2b**) possessing an extra methylene, both of the methyl and pyrophosphate groups can fit exactly in M site and P site when the methylene chain is lifted to take such a conformation that the cis condensation is indispensable (Figure 1).

We have observed an abnormal reaction with the other homolog (**1b**) catalyzed by isopentenyl pyrophosphate isomerase and proposed a mechanism involving two essential sites for the methyl and pyrophosphate moieties.[13] It is noteworthy that in the prenyltransferase reaction **1b** acts as a substrate normally and that **2b** acts abnormally in the stereochemical relation concerning the double bond formation. The reason for the normal reaction of **1b** may be as follows. The binding of the methyl and the pyrophosphate group with a conformation leading to the cis product results in the shift of the reacting points (C-4 and C-2) to a wrong position, whereas the binding with a conformation leading to the trans product can hold, though with a little distortion, these reacting points within the right position. In other words, M site may have a capacity for accommodating up to an ethyl group.

It is also of interest that the reactivity of **2b** with dimethylallyl pyrophosphate was almost negligible as compared with that of geranyl pyrophosphate (**3**). This marked difference will add support to our proposal[6] that there are involved in the prenyltransferase two separate sites for the reaction of dimethylallyl pyrophosphate with **1a** and the reaction of **3** with **1a**.

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Rapid Intramolecular Rearrangements in Pentacoordinate Transition Metal Compounds. V. The Coupling of Olefin Rotation and Berry Pseudorotation in Tetracarbonyliron–Olefin Complexes[1]

Sir:

Variable temperature ^{13}C nmr studies on metal carbonyl complexes are proving to be a valuable probe into structural and dynamic behavior in solution. However, for $Fe(CO)_5{}^2$ and several of its derivatives (*e.g.*, (norbornadiene)$Fe(CO)_3,^{2c}$ [$(CH_3)_2PCH_2CH_2P$-$(CH_3)_2$]$Fe(CO)_3,^{2d}$ [$(C_6H_5)_2PCH_2P(C_6H_5)_2$]$Fe(CO)_3,^3$ $R_3PFe(CO)_4,^{2c,4}$ (η^4-cyclooctatetraene)$Fe(CO)_3,^5$ and (diene)$Fe(CO)_3{}^6$) the activation energy for intramolecu-

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