enzyme, and the lactone. This equilibrium mixture then slowly yields the final acid product by a nonenzymatic hydrolysis of the lactone. At high enzyme concentrations ($>10^{-5}$ M) the reaction is further complicated by the acceleration of the spontaneous hydrolysis by the protein.

The rates of α-chymotrypsin-catalyzed hydrolysis of *p*-nitrophenyl phenylacetate (III) and *p*-nitrophenyl *m*-nitrophenyl acetate (IV) have been measured for comparison. These compounds also readily acylate the enzyme as measured by a burst of *p*-nitrophenol at 400 mμ. The kinetics of acylation have been followed on a stopped-flow instrument. The second-order acylation rate constants (k_2/K_s) have been determined. For compound III the rate constants of enzymatic hydrolysis have been calculated from turnover experiments ($S_0 > E_0$). For compound IV the acyl enzyme has been generated *in situ* by mixing stoichiometric amounts of enzyme and substrate and the deacylation rate constant (k_3) determined by measuring the time dependence of the recovery of the enzyme activity (Table I).

Finally, the rate constants for the uncatalyzed hydrolysis (k_{sp}) of all four compounds have been measured at pH 7.2 (Table I).

Our results suggest the following conclusions: (1) α-chymotrypsin is able to react with esters of carboxylic acids which are constrained in the *cis* configuration. Comparison of the acylation rate constants of *cis* and *trans* esters show, however, that the enzyme is unable to display its full specificity for the acyl group in the *cis* compound; *e.g.*, compound V acylates the enzyme some 60 times faster than its *cis* analog II, while in deacylation, when the acyl enzymes from both compounds can assume a *trans* configuration, the rate constants for the two systems are different by less than a factor of 2. Furthermore, the nonenzymatic hydrolysis of I and II is faster than that of their *trans* analogs III and V, although the difference is much less pronounced than in the case of the aliphatic lactones. This then seems to indicate that the enzyme reacts with its substrate whenever possible in the *trans* form[5] and that distortion of the substrate[6] is not a major factor in the acylation reaction of the enzyme. (2) The acyl enzymes derived from I and II are ideally suited for the role of "reporter molecules";[7] they are bound covalently but still reversibly to the very center of the active site. The spectral and kinetic behavior of the acyl enzymes indicate that in the acyl enzyme II the phenol group is located far away from the catalytic site, and from its pK we conclude that its environment does not differ much from the solvent. The behavior of the acyl enzyme derived from I shows that the phenol group must be constrained in the neighborhood of the catalytic site. The acid group perturbing the ionization of the phenol is then in all likelihood the histidine-57 of the active center. (3) The deacylation of the acyl enzyme from I indicates that the phenolic group remains in a sterically favorable position for attack of the carbonyl function. This is the first example of which we are aware where a reactive function introduced into an enzyme molecule has been demonstrated to act as an intramolecular nucleophile.

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Formation of Carbonium Ions by Cl^+ Transfer from Antimony Pentachloride to Olefins

Sir:

Two distinct routes lead to the formation of carbonium ions from olefins. (1) Electron transfer from an olefin to a suitable acceptor yields radical cations which dimerize into dimeric dicarbonium ions, *e.g.*

$$Ph_2C{=}CH_2 + SbCl_5 \longrightarrow Ph_2\overset{+}{C}CH_2CH_2\overset{+}{C}Ph_2$$ [1]

or $(p\text{-}Me_2NC_6H_4)_2C{=}CH_2 + (p\text{-}BrC_6H_4)_3N\cdot{}^+ClO_4^-$ gives the analogous dimethylamino derivative.[2] (2) Transfer of a positive moeity from a suitable donor to olefin converts the latter into carbonium ion, *e.g.*

$$Ph_2C{=}CH_2 + C(NO_2)_4 \longrightarrow Ph_2\overset{+}{C}CH_2NO_2 + \bar{C}(NO_2)_3$$ [3]

It seems that $SbCl_5$ may act not only as an electron acceptor[1,4] but also as a Cl^+ donor. A colored species, λ_{max} 540 mμ, is formed when a solution of $(p\text{-}CH_3OC_6H_4)_2C{=}CH_2$ (I) is rapidly mixed with a large excess of $SbCl_5$. The spectrum reveals a shoulder at 500 mμ, and this absorption may appear as a peak if the mixing is poor or if the excess of $SbCl_5$ is small. In fact, only a peak at λ_{max} 500 mμ appears if the olefin is in large excess.

The peak at λ_{max} 540 mμ is *not* due to $[^+C(C_6H_4OCH_3)_2CH_2{-}]_2$ or $C^+(C_6H_4OCH_3)_2CH_3$. The former dicarbonium ion was prepared by allowing I to react with $(BrC_6H_4)_3N\cdot{}^+,ClO_4^-$, and the latter carbonium ion was formed by allowing $HOC(C_6H_4OCH_3)_2CH_3$ to react with $SbCl_5$ or by adding trifluoroacetic acid to $(CH_3OC_6H_4)_2C{=}CH_2$. In these preparations the resulting carbonium ions absorb at λ_{max} 500–505 mμ. Moreover, the nmr spectrum of the species absorbing at λ_{max} 540 mμ (shown in Figure 1) reveals a signal due to $>^+C{-}CH_2{-}$ protons at δ 5.4 ppm, while the CH_2 or CH_3 protons of the carbonium ions mentioned above absorb at δ 3.4 ppm.

We proved that the species absorbing at λ_{max} 540 mμ is $^+C(C_6H_4OCH_3)_2CH_2Cl$ (II). (1) $(CH_3OC_6H_4)_2C{=}CHCl$ was isolated from the solution absorbing at 540 mμ after quenching with ammonia. Apparently

$$\overset{+}{C}(C_6H_4OCH_3)_2CH_2Cl + NH_3 \longrightarrow NH_4^+ + (CH_3OC_6H_4)_2C{=}CHCl$$

Its identity with synthetic sample[5] was confirmed by ir

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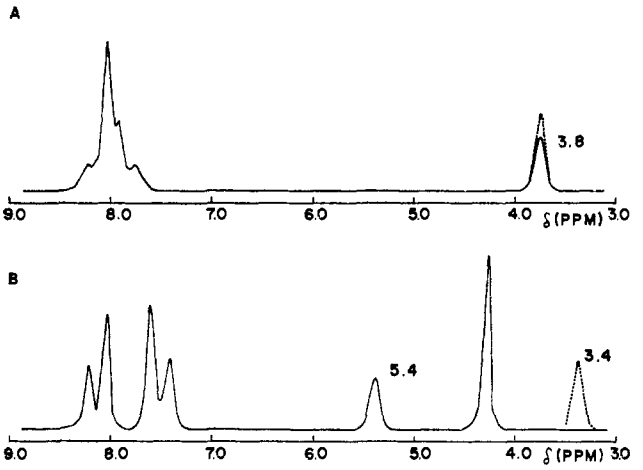

Figure 1. (A) Nmr spectrum of $^{+}C(Ph)_2CH_2CH_2C^{+}(Ph)_2$ and of $^{+}C(Ph)_2CH_3$. Both spectra are identical in the δ 8 ppm region (aromatic protons) but differ in their intensity in the δ 3.8 ppm region $-CH_2-C^{+}$ (or $^{+}C-CH_3$). Solid line corresponds to the ratio H (aromatic):H (aliphatic) 10:2 (dimer); the dotted line to the ratio 10:3. (B) Nmr spectrum of $^{+}C(C_6H_4OCH_3)_2CH_2Cl$ (II): δ 5.4 ppm, $>C^{+}-CH_2Cl$; δ 4.1 ppm, $-OCH_3$. The dimer, $^{+}C(C_6H_4OCH_3)_2CH_2CH_2C^{+}(C_6H_4OCH_3)_2$, or $^{+}C(C_6H_4OCH_3)_2CH_3$ do not show absorption at δ 5.4 ppm but at δ 3.4 ppm. Otherwise the spectrum is the same as that of II.

analysis and by its melting point. (2) A solution of $(CH_3OC_6H_4)_2C{=}CHCl$ reacts with CF_3COOH and gives colored species absorbing at λ_{max} 540 mμ with *no* shoulder at 500 mμ. (3) The nmr spectrum of $(CH_3OC_6H_4)_2C{=}CHCl$ in concentrated sulfuric acid is identical with that of II (although all the lines are slightly shifted because of the difference in the environment). No absorption is seen at δ 3.4 ppm.

The reaction of $SbCl_5$ with I is visualized as

$$SbCl_5 + (CH_3OC_6H_4)_2C{=}CH_2 \longrightarrow SbCl_4^- + \overset{+}{C}(C_6H_4OCH_3)_2CH_2Cl$$

i.e., as a transfer of Cl^+ from $SbCl_5$ to the olefin. Its detailed mechanism is now under investigation. The existence of $SbCl_4^-$ ions is demonstrated by the preparation of salts such as $(NH_4)^+ SbCl_4^-$ and its analogs.

The participation of free chlorine in the process is ruled out. The chlorine may be formed by the reaction $SbCl_5 \rightleftarrows SbCl_3 + Cl_2$; however, the reaction discussed above was not inhibited by the addition of $SbCl_3$ to $SbCl_5$ solution. In fact, a small excess of $SbCl_3$ was added to a solution of $SbCl_5$, and the mixture was chilled to $-70°$ after being kept at room temperature for several hours. On mixing it with a solution of I, cooled also to $-70°$, the conversion to $^{+}C(C_6H_4OCH_3)_2CH_2Cl$ took place in less than a second and the spectrophotometric analysis showed that the reaction was quantitative.

The hypothetical reaction

$$I + SbCl_5 \longrightarrow ClC(C_6H_4OCH_3)_2CH_2Cl + SbCl_3$$

followed by

$$SbCl_5 + ClC(C_6H_4OCH_3)_2CH_2Cl \longrightarrow SbCl_6^- + \overset{+}{C}(C_6H_4OCH_3)_2CH_2Cl$$

cannot be ruled out yet, although we believe it is improbable under our conditions. The problem may be solved by kinetic studies.

In the presence of an excess of I, the carbonium ion reacts as

$$ClCH_2\overset{+}{C}(C_6H_4OCH_3)_2 + I \longrightarrow ClCH_2C(C_6H_4OCH_3)_2CH_2\overset{+}{C}(C_6H_4OCH_3)_2$$

The latter carbonium ion absorbs most probably at 500 mμ.

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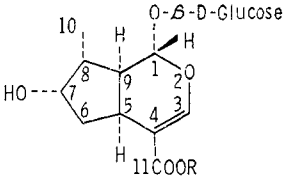
Mechanism of Secoiridoid Monoterpene Biosynthesis

Sir:

In the biosynthesis of the nontryptamine moiety of indole alkaloids in *Vinca rosea*, both the intermediacy of loganin (**2**), an iridoid monoterpene glucoside, and the precursor relationship of sweroside (**3**), a secoiridoid monoterpene glucoside, have been demonstrated.[1-3] Since these glucosides have been found to be of mevalonoid origin, a general biogenetic scheme envisages their biosynthesis to proceed by the customary isoprenoid mechanism to geranyl pyrophosphate followed by eventual conversion of the iridoid monoterpene to the secoiridoid type.[4-8] We now have direct evidence to support both these hypotheses.

In the biosynthesis of all-*trans* isoprenoid compounds thus far examined, isomerization of isopentenyl pyro-



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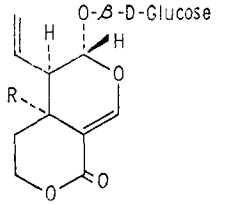
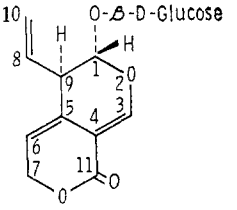
2 R = CH_3



3 R = H
4 R = OH



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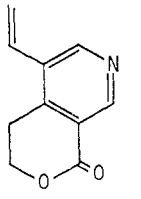

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