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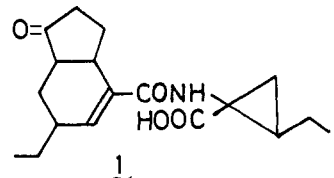
Received April 26, 1976

The Structure of Coronatine

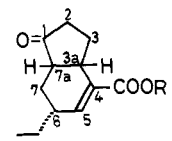
Sir:

Coronatine (**1**), produced by *Pseudomonas coronafacience var. atropurpurea*, is a toxin which induces chlorosis on the leaves of Italian ryegrass; it also expands potato cells at concentrations of 1×10^{-7} mol/l.[1] In this communication, we forward structure **1** for coronatine on the basis of spectroscopic data of the derivatives and x-ray crystallographic analysis of coronafacic acid (**2b**).

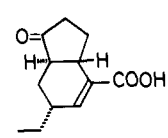


1

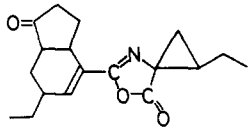
Coronatine (**1**), $[\alpha]^{20}_D$ +68.4° (*c* 2.2, CH_3OH), mp 151–153 °C was formulated as $C_{18}H_{25}O_4N$ (*m/e* M^+ found, 319.1753; calcd, 319.1731) and shows the following spectral data, UV λ_{max}^{EtOH} 208 nm (ϵ 8378); IR ν_{max}^{KBr} 1740 (five-membered ring C=O), 1620 (C=C), 3270, 1645, 1525 cm^{-1} (-CONH-); NMR (90 MHz) $\delta_{Me_4Si}^{CD_3COCD_3}$ 0.94 (6 H, t, J = 7 Hz, CH_2CH_3), 3.15 (1 H, br. q, -CHCO), 6.50 (1 H, s, =CH). The high resolution mass spectrum of **1** indicates that coronatine consists of two fragments, $C_{12}H_{15}O_2$ (*m/e* found, 191.1075; calcd, 191.1071) and $C_6H_{10}O_2N$ (*m/e* found, 128.0682; calcd 128.0710), which are bonded to each other by an amide linkage. Other ion peaks below *m/e* 191 are quite similar to those of coronafacic acids, **2a** and **2b**, which were



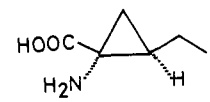
2a R=H
5 R=CH_3



2b



3



4

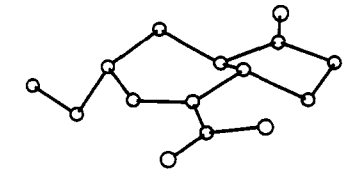


Figure 1. The molecular configuration.

isolated directly from the culture broth. In fact, hydrolysis of coronatine gave an acid, whose R_f value on TLC is identical with that of **2a**, and an α-amino acid which is identified as coronamic acid (**4**).

Treatment of coronatine with acetic anhydride–pyridine afforded anhydrocoronatine **3**, *m/e* 301 (M^+), whose IR spectrum exhibited peaks at 1800, 1640, 1605 cm^{-1} assignable to an azlactone moiety. Since the NMR spectrum of **3** (and **1** also) shows the presence of two ethyl groups (δ 0.98, 6 H, t, J = 7 Hz), in which one arises from coronafacic acid part, the amino acid, coronamic acid, must be depicted as **4** (plane structure) by considering the degree of unsaturation. The structure **4** including stereochemistry (NH_2/CH_2CH_3 trans) was also confirmed by the synthesis of *dl*-coronamic acid.[2]

Coronafacic acids exist as two stereoisomers, **2a** ($C_{12}H_{16}O_3$ (*m/e* M^+ 208): mp 125–126 °C, $[\alpha]^{20}_D$ +119.1° (*c* 3.3, CH_3OH), IR ν_{max}^{KBr} 3250, 1720, 1640, 1403 cm^{-1}; NMR (90 MHz) $\delta_{Me_4Si}^{CDCl_3}$ 1.00 (3 H, t, J = 7 Hz, $-CH_2CH_3$), 3.15 (1 H, quintet, -CHCO-), 7.28 (1 H, br s, =CH), 11.58 (1 H, br s, -COOH)) and/or **2b** ($C_{12}H_{16}O_3$ (*m/e* M^+ 208), mp 141–142 °C, IR ν_{max}^{KBr} 3250, 1730, 1625 cm^{-1}; NMR (90 MHz) $\delta_{Me_4Si}^{CDCl_3}$ 1.00 (3 H, t, J = 7 Hz, $-CH_2CH_3$), 7.11 (1 H, dd, J = 4 Hz, 2 Hz, =CH), 11.08 (1 H, br s, -COOH), depending on the conditions of recrystallization. The isomer **2b** is easily convertible through enolisation to **2a**.[3] Esterification of each of **2a** and **2b** by methanolic HCl afforded the same methyl ester **5**: *m/e* 222 (M^+); IR ν_{max}^{film} 1740, 1715, 1645, 1405 cm^{-1}. The UV spectrum ($\lambda_{max}^{CH_3OH}$ 217 nm, ϵ 8558) of **2b** is compatible with α,β-unsaturated acid, whose double bond is located in a six-membered ring.[4] The IR spectra (1740 cm^{-1} in $CHCl_3$, five-membered ring ketone) of **2a** and **2b**, D_2O treatment of **2b** (*m/e* M^+ 211), and the NMR spectrum of **2a** (δ 3.15 quintet, J = 10 Hz, 7 Hz, 6 Hz, $COC(CH)H\text{-}CH_2$) suggest a 1-hydrindanone structure for coronafacic acids. Decisive structure including relative configuration of **2b** was obtained by x-ray analysis. Coronafacic acid (**2b**) crystallizes in the orthorhombic space group $P2_12_12_1$ with unit-cell dimensions a = 8.727 (4), b = 16.437 (6), and c = 7.638 (4) Å; there are four molecules in the unit cell. Intensities of 1144 independent reflections with 2θ values up to 140° were collected on an automatic, four-circle diffractometer using Cu Kα radiation monochromatized with a LiF crystal. The structure was solved by the direct method[5] on the basis of 242 $|E|$-values above 1.30, and refined by the block-diagonal-matrix least-squares method with anisotropic temperature factors for oxygen and carbon atoms. The hydrogen positions were obtained from a difference Fourier map. Further refinement of the structure including these hydrogen atoms reduced the R-value to 4.5%.[6] The molecular configuration obtained is shown in Figure 1. The molecule contains a trans-fused, bicyclic system, both rings in which are approximately in the envelope form. Thus, the structure of coronafacic acid has been established as **2b**, and plane structure of coronatine is depicted as **1**. Stereochemical and synthetic studies of coronatine are in progress.

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The Determination of the Rate-Limiting Step in a Proton Transfer Reaction from the Breakdown of the Swain–Schaad Relation

Sir:

Streitwieser et al. showed[1] that for the base-catalyzed exchange of C–L bonds (where L = H, D, or T), the amount of internal return could be measured from the breakdown of the Swain–Schaad relation[2] which connects the deuterium isotope effect (k_D/k_H) and the tritium isotope effect (k_T/k_H):

$$(k_T/k_H) = (k_D/k_H)^{1.44} \tag{1}$$

When two or more transition states are partially rate limiting, eq 1 does not hold exactly and Northrop has recently suggested[3] that this breakdown could be used to investigate the details of enzyme catalyzed reactions. We show here that although the treatment can be applied to any sequence of catalytic steps, the breakdown is generally small. Before trying to use this approach one must therefore ask whether the experimental data will be sufficiently precise to enable useful information to be obtained.

Consider the simple two-step processes shown in Scheme I, where the second step is a proton transfer. The observed

Scheme I

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Fractionation factors for D	ϕ_S	ϕ_1	ϕ_2
Fractionation factors for T	Φ_S	Φ_1	Φ_2

second-order rate constant, $k_{1,2}$, is given by

$$k_{1,2} = k_1/(1 + \kappa) \tag{2}$$

where

$$\kappa = k_{-1}/k_2 \tag{3}$$

The quantity κ describes the free energy difference between transition states 1 and 2 and it is the value of κ that we wish to obtain from the breakdown of the Swain–Schaad relation.

The effects of isotopic substitution are best described by fractionation factors[4] because a fractionation factor relates to a particular species. We are mainly interested in transition states 1 and 2 and so we define the mixed fractionation factors $\phi_{1,2}$ and $\Phi_{1,2}$ where

$$\frac{(k_{1,2})_D}{(k_{1,2})_H} = \frac{\phi_{1,2}}{\phi_S} \tag{4}$$

$$\phi_{1,2} = \frac{1 + \kappa}{\phi_1^{-1} + \kappa\phi_2^{-1}} \tag{5}$$

$$\Phi_{1,2} = \frac{1 + \kappa}{\Phi_1^{-1} + \kappa\Phi_2^{-1}} \tag{6}$$

and

$$\Phi_n = \phi_n^{1.44} \text{ where } n = 1 \text{ or } 2$$

If the first step is rate limiting, $\kappa \ll 1$ and $\phi_{1,2} \rightarrow \phi_1$; if the second step is rate limiting, $\kappa \gg 1$ and $\phi_{1,2} \rightarrow \phi_2$. For isotopic substitution with deuterium, $\phi_{1,2}$ can be determined from eq 4 since the other quantities can all be measured experimentally. For isotopic substitution with tritium, $\Phi_{1,2}$ is determined by measuring the isotopic content of the reactant or product as a function of the extent of reaction.[5]

This treatment can be extended to the general case shown in Scheme II. The proton transfer occurs at the jth step (EI $\rightleftarrows$ EJ) and the only irreversible step is the loss of product Z. We can show that $\phi_{1,2,\ldots,z}$, the observed fractionation factor ($= \phi_A(k_{obsd})_D/(k_{obsd})_H$), is given by

Scheme II

$$E + A \underset{\vec{k}}{\overset{\bar{k}}{\rightleftharpoons}} EA \cdots EI \underset{k_{-j}}{\overset{k_j}{\rightleftharpoons}} EJ \cdots EZ \xrightarrow{\vec{k}} E + Z$$

ϕ_A $\qquad$ ϕ_j

$$\phi_{1,2,\ldots,z} = \frac{1 + \kappa'}{\bar{\phi}^{-1} + \kappa'\phi_j^{-1}}$$

where

$$\frac{1}{\kappa'} = \frac{k_j}{\bar{k}} + \frac{k_{-j}}{\vec{k}}$$

$$\bar{\phi} = \frac{1 + \kappa''}{\phi_{1,2\ldots j-1}^{-1} + \kappa''\phi_{j+1,\ldots,z}^{-1}}$$

and

$$\kappa'' = \bar{k}k_{-j}/\vec{k}k_j$$

The rate constants $\bar{k}$ and $\vec{k}$ describe the rates of EI to E + A and of EJ to E + Z, respectively.[6] Since the equations for the general case have the same form as those for the simple two-step reaction, we continue our discussion using the simpler Scheme I.

Now, if c is the percentage breakdown of the Swain–Schaad relationship, as derived from the experimentally observed quantities $\phi_{1,2}$ and $\Phi_{1,2}$, we may write:

$$1 + \frac{c}{100} = \frac{\phi_{1,2}}{\Phi_{1,2}^{0.69}} = \frac{(k_{1,2})_D}{(k_{1,2})_H}\left(\frac{\Phi_S}{\Phi_{1,2}}\right)^{0.69} \tag{7}$$

where $0.69 = 1.44^{-1}$.

The quantity c is calculated from experimental values of the isotope effects and does *not* depend on any fractionation in reactants or intermediates. From eq 5, 6, and 7 we obtain:

$$\frac{c}{100} = \frac{(1 + \kappa)^{0.31}\,[1 + \kappa(\phi_1/\phi_2)^{1.44}]^{0.69}}{1 + \kappa\phi_1/\phi_2} - 1 \tag{8}$$

Since ϕ_2 describes a proton transfer and ϕ_1 does not, $\phi_1/\phi_2 > 1$. Most primary deuterium isotope effects[7] lie in the range 3