

Supernumerary larval molts in *Galleria mellonella* could be produced by injection of 2000–4000 TU of each compound per animal. This dosage level is equivalent to that required to obtain a similar response with the natural juvenile hormone. Further biological experiments with these compounds in insects of different orders are currently in progress.

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### Steroids and Steroidases. V.<sup>1</sup> On the Mechanism of Action of the $\Delta^5$ -3-Ketoisomerase of *Pseudomonas testosteronei*

Sir:

During our investigations on the  $\Delta^5$ -3-ketoisomerase of *P. testosteronei*, which effects the isomerization of  $\Delta^5$ -3-keto steroids to the corresponding  $\Delta^4$ -3-ketones, it became of interest to compare the acid-, base-, and enzyme-catalyzed reactions.

Previous studies on base-catalyzed isomerizations of  $\Delta^5$ -3-keto steroids have been hindered by multiple product formation.<sup>2</sup> However, when solutions of buffers, such as Tris-HCl, pH range 8.5–9, or aqueous sodium hydroxide, pH > 10, were used, clean isomerizations to  $\Delta^4$ -3-ketones were observed. Using aqueous sodium hydroxide solutions in the pH range 10.6–11.7, androst-5-ene-3,17-dione isomerized with over-all second-order kinetics, the reaction being first order with respect to both steroid and hydroxide ion concentrations.

Loss of the C<sub>4</sub> hydrogen atom has been shown to be the rate-determining step for both acid- and enzyme-catalyzed isomerizations of androst-5-ene-3,17-dione.<sup>3</sup> A comparison of the pH 10 isomerization of androst-5-ene-3,17-dione with that of its 4,4-dideuterio analog showed a primary kinetic isotope effect of 3.2, thus indicating C<sub>4</sub> proton abstraction to be rate determining for the base-catalyzed reaction also.

The  $\Delta^5$ -3-ketoisomerase of *P. testosteronei* is the most active enzyme known,<sup>4</sup> and the rates of the acid-, base-, and enzyme-catalyzed reactions of androst-5-ene-3,17-dione are in the ratio 1:700:(17 × 10<sup>6</sup>). In an attempt to ascertain where the enzymic isomerization gains its advantage over those catalyzed by acid and base, the

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(3) H. J. Ringold and S. K. Malhotra, *J. Am. Chem. Soc.*, **87**, 3228 (1965).

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activation parameters were determined.<sup>5</sup> As seen from the values recorded in Table I, the facility of the enzymic process is mainly due to an extremely low enthalpy of activation. The differences in the entropies of activation for the three processes are not very great, and the  $\Delta S^\ddagger$  value of  $-16.8 \text{ cal deg}^{-1} \text{ mole}^{-1}$  for the enzymic catalysis is consistent with the development of charge separation in the transition state as required by the mechanism proposed by Ringold and Malhotra.<sup>3</sup>

Table I. Activation Parameters for the Isomerization of Androst-5-ene-3,17-dione<sup>a</sup>

|   | HCl,<br>pH 0.88 <sup>b</sup> | Catalyst<br>Tris-HCl,<br>pH 8.82 <sup>b</sup> | Enzyme <sup>c</sup> |
|---|------------------------------|---|---------------------|
| Enthalpy of activation, $\Delta H^\ddagger$ ,<br>kcal mole <sup>-1</sup>                    | 14.0 ± 0.1                   | 11.4 ± 0.1                                    | 5.0 ± 0.1           |
| Entropy of activation, $\Delta S^\ddagger$ ,<br>cal deg <sup>-1</sup><br>mole <sup>-1</sup> | -19.6 ± 0.4                  | -15.5 ± 0.4                                   | -16.8 ± 0.4         |

<sup>a</sup> Data obtained with 1.6% aqueous methanol solutions and a steroid concentration of 0.05  $\mu\text{mole/ml}$ . <sup>b</sup> Temperature range 15–40°. <sup>c</sup> Temperature range 15–30°.

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(5) Direct comparison of the activation parameters for the acid-, base-, and enzyme-catalyzed reactions is considered to be reasonable since the same rate-determining step is involved in each case.

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### Correlations between Carbon-13 and Boron-11 Chemical Shifts. I. The Alkanes and Analogous Boron-Nitrogen Compounds<sup>1</sup>

Sir:

Chemical shift data for carbon-13 and boron-11 nuclei are not very extensive because of a number of well-known features which are unfavorable toward magnetic resonance measurements.<sup>2</sup> A clear correlation of chemical shift between the two nuclei in related types of compounds would have considerable value from both a practical as well as a theoretical viewpoint.

The amine boranes and the closely related compounds, the diborazanes and cycloborazanes, may be viewed as the inorganic analogs of the alkanes in which one or more carbon-carbon linkages have been replaced by a corresponding number of isoelectronic boron-

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Table I. Boron-11 and Carbon-13 Chemical Shift Data for Some Boron-Nitrogen Compounds and Their Analogous Alkanes

| No. | Boron compound   | Carbon compound <sup>a</sup>  | C <sup>13</sup> , ppm <sup>b</sup> | B <sup>11</sup> , ppm <sup>c</sup>                      |
|-----|--|---|------------------------------------|---|
| 1   | H <sub>3</sub> BNH <sub>3</sub>  | H <sub>3</sub> C*CH <sub>3</sub>  | 122.80 <sup>d</sup>                | 23.8, <sup>e</sup> 24.0 <sup>h</sup>                    |
| 2   | H <sub>3</sub> BNH <sub>2</sub> ( <i>t</i> -C <sub>4</sub> H <sub>9</sub> )                        | H <sub>3</sub> C*CH <sub>2</sub> ( <i>t</i> -C <sub>4</sub> H <sub>9</sub> )                        | 120.03 <sup>d</sup>                | 22.1 <sup>g</sup>                                       |
| 3   | H <sub>3</sub> BNH <sub>2</sub> CH <sub>3</sub>  | H <sub>3</sub> C*CH <sub>2</sub> CH <sub>3</sub>  | 113.10 <sup>d</sup>                | 20.5, <sup>g</sup> 19.6 <sup>h</sup>                    |
| 4   | H <sub>3</sub> BNH(CH <sub>3</sub> ) <sub>2</sub>  | H <sub>3</sub> C*CH(CH <sub>3</sub> ) <sub>2</sub>  | 104.35 <sup>d</sup>                | 14.7, <sup>g</sup> 15.0, <sup>h</sup> 15.4 <sup>i</sup> |
| 5   | H <sub>3</sub> BN(C <sub>2</sub> H <sub>5</sub> ) <sub>3</sub>                                     | H <sub>3</sub> C*C(C <sub>2</sub> H <sub>5</sub> ) <sub>3</sub>                                     | 104.73 <sup>e</sup>                | 14.3 <sup>h</sup>                                       |
| 6   | (CH <sub>3</sub> ) <sub>3</sub> BNH <sub>3</sub>   | (CH <sub>3</sub> ) <sub>3</sub> C*CH <sub>3</sub>   | 100.80 <sup>d</sup>                | 8.7 <sup>h</sup>  |
| 7   | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>3</sub>   | H <sub>3</sub> C*C(CH <sub>3</sub> ) <sub>3</sub>   | 97.23 <sup>d</sup>                 | 8.5, <sup>h</sup> 8.1 <sup>i</sup>                      |
| 8   | (CH <sub>3</sub> ) <sub>3</sub> BNH <sub>2</sub> CH <sub>3</sub>                                   | (CH <sub>3</sub> ) <sub>3</sub> C*CH <sub>2</sub> CH <sub>3</sub>                                   | 98.26 <sup>d</sup>                 | 7.5 <sup>h</sup>  |
| 9   | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> NH <sub>3</sub>                   | H <sub>3</sub> C*C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>                   | 99.77 <sup>d</sup>                 | 10.2 <sup>i</sup>                                       |
| 10  | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> NH <sub>2</sub> CH <sub>3</sub>   | H <sub>3</sub> C*C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>   | 99.44 <sup>e</sup>                 | 12.1 <sup>i</sup>                                       |
| 11  | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> NH(CH <sub>3</sub> ) <sub>2</sub> | H <sub>3</sub> C*C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> | 99.13 <sup>e</sup>                 | 12.4 <sup>i</sup>                                       |
| 12  | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> N(CH <sub>3</sub> ) <sub>3</sub>  | H <sub>3</sub> C*C(CH <sub>3</sub> ) <sub>2</sub> CH <sub>2</sub> C(CH <sub>3</sub> ) <sub>3</sub>  | 98.82 <sup>e</sup>                 | 11.65 <sup>i</sup>                                      |
| 13  | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> NH <sub>3</sub>                   | H <sub>3</sub> CC(CH <sub>3</sub> ) <sub>2</sub> C*H <sub>2</sub> CH <sub>3</sub>                   | 92.02 <sup>d</sup>                 | 2.9 <sup>i</sup>  |
| 14  | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> NH <sub>2</sub> CH <sub>3</sub>   | H <sub>3</sub> CC(CH <sub>3</sub> ) <sub>2</sub> C*H <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>   | 82.63 <sup>e</sup>                 | 3.18 <sup>i</sup>                                       |
| 15  | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> NH(CH <sub>3</sub> ) <sub>2</sub> | H <sub>3</sub> CC(CH <sub>3</sub> ) <sub>2</sub> C*H <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> | 75.73 <sup>e</sup>                 | -3.0 <sup>i</sup>                                       |
| 16  | H <sub>3</sub> BN(CH <sub>3</sub> ) <sub>2</sub> BH <sub>2</sub> N(CH <sub>3</sub> ) <sub>3</sub>  | H <sub>3</sub> CC(CH <sub>3</sub> ) <sub>2</sub> C*H <sub>2</sub> C(CH <sub>3</sub> ) <sub>3</sub>  | 71.06 <sup>e</sup>                 | -3.45 <sup>i</sup>                                      |
| 17  | (H <sub>2</sub> BNH <sub>2</sub> ) <sub>3</sub>  | (H <sub>2</sub> C*CH <sub>2</sub> ) <sub>3</sub>  | 100.30 <sup>f</sup>                | 11.8 <sup>k</sup>                                       |

<sup>a</sup> C\* indicates carbon corresponding to observed boron. <sup>b</sup> C<sup>13</sup> shifts relative to benzene. <sup>c</sup> B<sup>11</sup> shifts relative to BF<sub>3</sub>·(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>O. When necessary, values were converted (ref 2, p 973) by  $\delta_{\text{BF}_3 \cdot (\text{C}_2\text{H}_5)_2\text{O}} = \delta_{\text{B}(\text{OCH}_3)_3} - 17.4$ . <sup>d</sup> Observed value from ref 4. The values are reported to be accurate to  $\pm 0.07$  ppm. <sup>e</sup> Value calculated by Grant and Paul's method. <sup>f</sup> J. J. Burke and P. C. Lauterbur, *J. Am. Chem. Soc.*, **86**, 1870 (1964). <sup>g</sup> R. Schaeffer, "Progress in Boron Chemistry," Vol. 1, H. Steinberg and A. L. McCloskey, Ed., The Macmillan Co., New York, N. Y., 1964, p 417. <sup>h</sup> C. W. Heitsch, *Inorg. Chem.*, **4**, 1019 (1965). <sup>i</sup> W. D. Phillips, H. C. Miller, and E. L. Muettterties, *J. Am. Chem. Soc.*, **81**, 4496 (1959). <sup>j</sup> G. A. Hahn and R. Schaeffer, *ibid.*, **86**, 1503 (1964). <sup>k</sup> K. W. Bøddeker, S. G. Shore, and R. K. Bunting, *ibid.*, **88**, 4396 (1966). <sup>l</sup> D. F. Gaines and R. Schaeffer, *ibid.*, **86**, 1505 (1964).

nitrogen units.<sup>3</sup> Comparison of the boron-11 chemical shifts in these boron-nitrogen compounds with the corresponding carbon-13 chemical shifts in the analogous alkanes reveals a striking correlation.

Table I contains chemical shift data for a number of boron-nitrogen compounds and their analogous isoelectronic alkanes. The carbon-13 shifts in parts per million relative to benzene are either the experimentally observed values recorded by Grant and Paul<sup>4</sup> or calculated values based on their empirical method which was shown to predict values for a number of linear and branched alkanes with a standard deviation of  $\pm 0.30$  ppm. The boron-11 values in parts per million relative to boron trifluoride etherate are all observed values obtained from the literature and are reported to be accurate to  $\pm 0.5$  ppm.

Figure 1 shows a plot of the carbon-13 shift *vs.* the boron-11 shift for each analogous compound. A linear relationship is evident. A least-squares analysis using only the ten observed carbon-13 shifts yields the equation

$$\delta_{\text{C}^{13}} = 1.44\delta_{\text{B}^{11}} + 86.0 \text{ (ppm from benzene)}$$

Carbon-13 chemical shift values calculated from the boron-11 values by this equation have an average deviation of 1.96 ppm from the observed values. When all carbon-13 values are used, the least-squares analysis yields

$$\delta_{\text{C}^{13}} = 1.71\delta_{\text{B}^{11}} + 81.0 \text{ (ppm from benzene)}$$

which predicts the carbon-13 values with an average deviation in chemical shift of 2.51 ppm. Since the total range of carbon-13 shifts is over 50 ppm, the results strongly confirm the linear correlation of chemical shift between the two nuclei in these compounds as well as the general validity of Grant and Paul's empirical relationships.

It is interesting to note that the correlation is apparently valid for three types of boron-nitrogen com-

pounds: the amine boranes (1-8 in Table I) in which one carbon-carbon unit is replaced by a boron-nitrogen unit; the diborazanes (9-16 in Table I) in which two carbon-carbon units are replaced by two boron-nitrogen units; and for cycloborazane (17 in Table I) in which three carbon-carbon units have been replaced

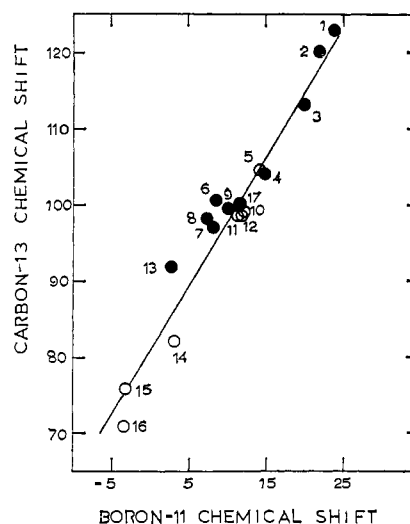


Figure 1. A plot of carbon-13 chemical shifts for a number of alkanes *vs.* the corresponding boron-11 shifts in the analogous boron-nitrogen compound. Numbers refer to the compounds as listed in Table I. Solid circles represent observed values of carbon-13 shift; open circles represent values calculated by the method of Grant and Paul.<sup>4</sup> Where more than one boron-11 value was reported, the average was used.

by three boron-nitrogen units in a cyclic arrangement. We are presently obtaining additional boron-11 nmr data and are examining the theoretical implications of the chemical shift correlation and will report later in more detail.

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