Vf-B ("Stevane B") is identical with (−)-β-dihydrokaurene, a by-product in the hydrogenation of (−)-kaurene.[10] Since the absolute configuration of gibberic acid appears secured[11] the interconversion of steviol to (−)-kaurene provides chemical evidence for the stereochemistry of the latter at positions 8 and 13.[12]

(10) Private information by Professor Briggs.
(11) G. Stork and H. Newmann, THIS JOURNAL, **81**, 3168 (1959).
(12) L. H. Briggs, B. F. Cain and B. R. Davis, *Tetrahedron Letters*, in press.

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RECEIVED OCTOBER 8, 1959

## THE RELATION BETWEEN AMINO ACID COMPOSITION AND DENATURATION OF VERTEBRATE COLLAGENS

*Sir:*

A direct correlation has been shown between the hydroxyproline content of vertebrate collagens and their thermal stability as expressed by the shrinkage temperature.[1] This phenomenon has been explained by the ability of the hydroxy group of hydroxyproline to form hydrogen bonds.[1] However, hydroxyproline (and proline) can participate in protein structure in at least one other manner. The pyrrolidine rings of the imino acids can direct the geometry of a polypeptide chain in regions in which they occur.[2,3,4] This arises from the double bond character of the peptide link, the rigidity of the N–$C_\alpha$ bond in the pyrrolidine ring, and restricted rotation about the $C_\alpha$–C=O bond adjacent to the pyrrolidine ring.[4] Since the stability of the collagen molecule, according to this view, would be in part a function of *total* imino acid, it is of interest to examine the proline and hydroxyproline contents and the shrinkage temperatures of different collagens.

These data are presented in Fig. 1 for all the vertebrate collagens for which complete amino acid analyses and shrinkage temperatures are available. The numerical data from which the graph was taken have been published in part[5]; the remainder will be published elsewhere.[6] It is readily apparent that proline, hydroxyproline, and their total are related in some manner to shrinkage temperature; the single regression coefficients are all statistically significant. It is not obvious whether the sum (or any other function) of the two imino acids provides a better explanation of the variation in shrinkage temperature than either imino acid alone. This can be determined by calculating multiple regression coefficients; these values, with their standard errors, are obtained: 0.434 ± 0.091 (proline) and 0.246 ± 0.079 (hydroxyproline). Both are significantly different from zero ($P < 0.001$ and $P < 0.02$, respectively), but they do not differ significantly from each other. Therefore, it can be concluded that the variation in shrinkage temperature of vertebrate collagens is associated with both proline and hydroxyproline and with each independent of the other. That is, the two imino acids together provide a better explanation of the variation in shrinkage temperature than either one alone. Also, the two imino acids do not have a different effect on shrinkage temperature. Employing total imino acid, a regression coefficient of 0.332 ± 0.039 ($P << 0.001$) is obtained.

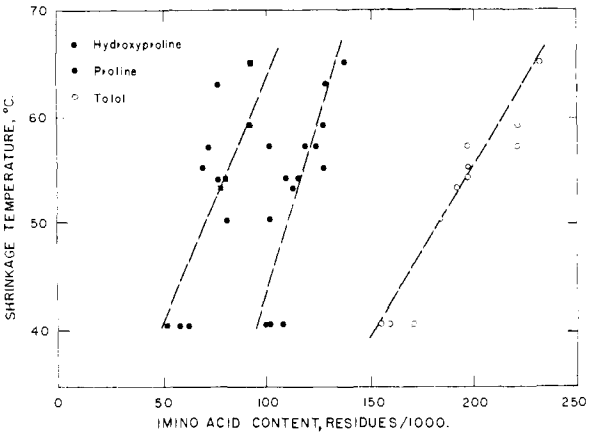


Fig. 1.—The hydrothermal shrinkage temperatures of vertebrate collagens plotted as a function of imino acid content. The dash lines indicate the single regression lines calculated for each group of values.

Thus it seems likely that the varying stabilities exhibited by vertebrate collagens are related to the pyrrolidine ring content rather than the hydroxy group of hydroxyproline. The hydroxy group of hydroxyproline need not play a unique role since the total content of hydroxy groups (hydroxyproline, hydroxylysine, serine, and threonine) of vertebrate collagens is essentially constant.[5,6]

A detailed presentation of these results together with some implications with regard to collagen structure will be the subject of a forthcoming paper.[6]

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## STRUCTURE AND PROPERTIES OF PROPARGYLENE $C_3H_2$[1]

*Sir:*

It was suggested that the two varieties of bivalent carbon[2–6] are distinguished by their singlet

(1) K. H. Gustavson, "The Chemistry and Reactivity of Collagen," Academic Press, Inc., New York, N. Y., 1956, Chap. 9.
(2) W. F. Harrington, *Nature*, **181**, 997 (1958).
(3) W. F. Harrington and M. Sela, *Biochim. et Biophys. Acta*, **27**, 24 (1958).
(4) P. H. von Hippel and W. F. Harrington, *ibid.*, in press.
(5) J. E. Eastoe and A. A. Leach, "Recent Advances in Gelatin and Glue Research," Ed. by G. Stainsby, Pergamon Press, New York, 1958, p. 173.
(6) K. A. Piez and J. Gross, *J. Biol. Chem.*, in press.

(1) This work was supported by the Office of Ordnance Research, Contract No. DA-36-061-ODR-607.
(2) P. S. Skell and A. Y. Garner, THIS JOURNAL, **78**, 3409 (1956).
(3) P. S. Skell and R. C. Woodworth, *ibid.*, **78**, 4496, 6247 (1956), **81**, 3383 (1959).
(4) P. S. Skell and A. Y. Garner, *ibid.*, **78**, 5430 (1956).
(5) P. S. Skell and R. M. Etter, *Chem. and Ind.*, 624 (1958).
(6) R. M. Etter, H. S. Skovronek and P. S. Skell, THIS JOURNAL, **81**, 1008 (1959).