

part due to a refractive index change) as evidence that van der Waals interaction between a hydrophobic region of BPA and the coupled anthracene is greater at pH 6 than at pH 2.

Although the pH-dependent spectral perturbation can be satisfactorily explained by postulating that the anthracene chromophore is more enfolded in the structure of the protein at pH 6 than at pH 2, the direct cause of the perturbation may be due to additional factors other than an increase in the refractive index of the environment of the chromo-

phore *per se*. It is conceivable that when the chromophore is enfolded in the protein, it is placed in juxtaposition to one or more of the numerous protein groups which contain double bonds and that the proximity of such groups, on the basis of the interpretation of Lauer and Horio, contributes to the spectral perturbation. Then too, there is the possibility that the perturbation is influenced by electrostatic interaction of protein groups with the chromophore.

LAFAYETTE, INDIANA

COMMUNICATIONS TO THE EDITOR

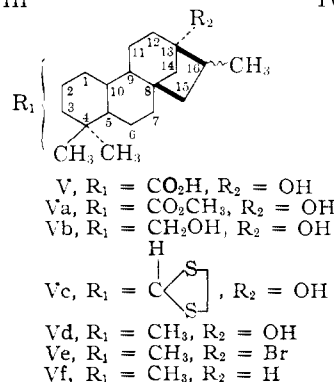
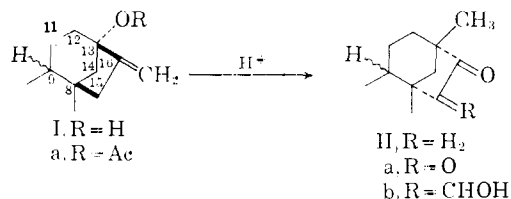
THE STRUCTURE AND STEREOCHEMISTRY OF STEVIOL AND ISOSTEVIOL

Sir:

We are now proposing structures I and II for steviol¹ and isosteviol.¹ Isosteviol (II) with SeO₂ gives IIa, m.p. 272–274°, quinoxaline derivative, m.p. 237–238°; II (methyl ester), sodium methoxide, methyl formate and benzene give IIb, m.p. 166–167°; ozonization of I (methyl ester) yields ketol III, m.p. 224–227° [α]_D -101 ± 2.1°² and ketoacid IV, m.p. 178–181°, [α]_D -69.5 ± 1.0°. The rotatory dispersion curves³ of III and of the corresponding ozonization product of allogibberic acid, the ketonorlogibberic acid,⁴ are practically superimposable. Also the rotatory dispersion curves³ of gibberic acid⁵ and isosteviol⁶ are coincident, which leaves no doubt about the steric identity of the ring fusion of the five- and six-membered rings in the two pairs, allogibberic acid-gibberic acid and steviol-isosteviol.³

Hydrogenation (Pd-C) of steviol (I) gives dihydrosteviol V-A, m.p. 210–212°, [α]_D -41.5 ± 1.6°, while stevioside⁷ (PtO₂) and subsequent hydrolysis yields V-B,⁸ m.p. 209–212°, [α]_D -78.8 ± 1.0° (hydrate, 1 H₂O). Va-A, m.p. 107–112°, [α]_D -63.8 ± 2°; Va-B, m.p. 132–134°, [α]_D -100.5 ± 2°. Vb-A (LiAlH₄, T.H.F., 6 hr. reflux), m.p. 214–216°, [α]_D -40.4 ± 2.0°; Vb-B, m.p. 187–188.5°, [α]_D -65.6 ± 1.4°; Vb-A and Vb-B gave with CrO₃-pyridine the respective (solid) aldehydes which were immediately converted to Vc-A, m.p. 166–168°, [α]_D -35.4 ± 1.7° and Vc-B, m.p. 170–171°, [α]_D -59.3 ± 1.8°. Desulfurization with Raney nickel (W-6) gave Vd-A,

m.p. 147–148°, [α]_D -24.4 ± 1.4°, and Vd-B, m.p. 152–154°, [α]_D -51.7 ± 1.3°. The replacement of the tertiary hydroxyl group by bromine (PBr₃, ether) gave, respectively, Ve-A, m.p. 110–112°, [α]_D -17.0 ± 1.2°, and Ve-B, m.p. 117–119°, [α]_D -66.1 ± 0.9°. Raney nickel hydrogenolysis gave Vf-A ("Stevane A") m.p. 87.5–88.5°, [α]_D -31.9 ± 0.8° and Vf-B, m.p. 47°/54–55°, [α]_D -66.8 ± 1.3°.



Professor Briggs kindly compared (melting points, infrared, X-ray powder patterns) Vf-A with (-)- α -dihydrokaurene (m.p. 86–87°, [α]_D²¹ -32° CHCl₃)⁹ and found them identical. Apparently

(9) J. Simonsen and D. R. H. Barton, "The Terpenes," University Press, Cambridge, 1952, Vol. III, p. 339.

- (1) E. Mosettig and W. R. Nes, *J. Org. Chem.*, **20**, 884 (1955).
- (2) All rotations measured in CHCl₃, *c* approx. 1.0 to 20.
- (3) We are indebted to Prof. C. Djerassi for this information and additional measurements; for details see C. Djerassi, "Optical Rotatory Dispersion," McGraw Hill Book Co., New York, N. Y., 1960.
- (4) T. B. C. Mulholland, *J. Chem. Soc.*, 2693 (1958).
- (5) B. E. Cross, J. F. Grove, J. MacMillan and T. B. C. Mulholland, *ibid.*, 2520 (1958).
- (6) C. Djerassi, R. Riniker and B. Riniker, *THIS JOURNAL*, **78**, 6362 (1956).
- (7) E. Vis and H. G. Fletcher, *ibid.*, **78**, 4709 (1956).
- (8) A and B are arbitrary designations.

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 secured¹¹ 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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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 α bond in the pyrrolidine ring, and restricted rotation about the C α -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 part⁵; 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 $0.246 \pm$

(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.

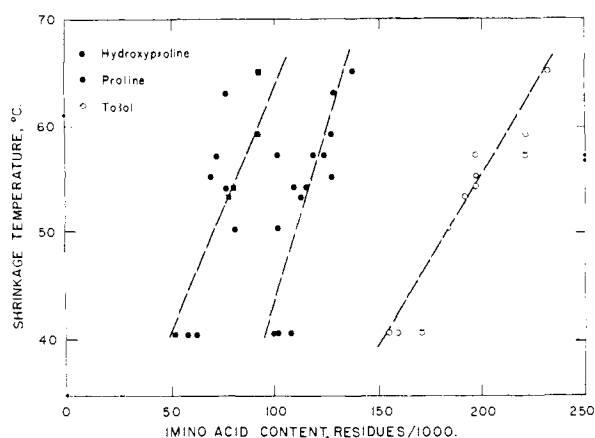


Fig. 1.—The hydrothermal shrinkage temperatures of vertebrate collagens plotted as a function of imino acid content. The dash lines indicate the single regression lines calculated for each group of values.

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.

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

I am indebted to Mr. Nathan Mantel for the statistical analysis and to Dr. Jerome Gross and Dr. W. F. Harrington for helpful discussions.

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STRUCTURE AND PROPERTIES OF PROPARGYLENE C $_3$ H $_2$ ¹

Sir:

It was suggested that the two varieties of bivalent carbon²⁻⁶ are distinguished by their singlet

(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).