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 *p*H 6 than at *p*H 2.

Although the *p*H-dependent spectral perturbation can be satisfactorily explained by postulating that the anthracene chromophore is more enfolded in the structure of the protein at *p*H 6 than at *p*H 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 chromophore *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

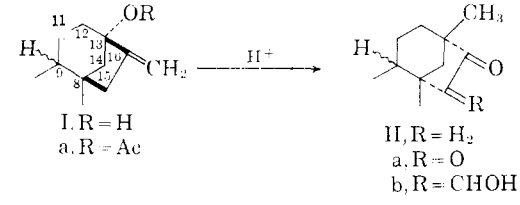
COMMUNICATIONS TO THE EDITOR

THE STRUCTURE AND STEREOCHEMISTRY OF STEVIOL AND ISOSTEVIOL

Sir:

We are now proposing structures I and II for steviol[1] and isosteviol.[1] Isosteviol (II) with SeO_2 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° $[\alpha]D$ −101 ± 2.1°[2] and ketoacid IV, m.p. 178–181°, $[\alpha]D$ −69.5 ± 1.0°. The rotatory dispersion curves[3] of III and of the corresponding ozonization product of allogibberic acid, the ketonorallogibberic acid,[4] are practically superimposable. Also the rotatory dispersion curves[3] of gibberic acid[5] and isosteviol[6] 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]

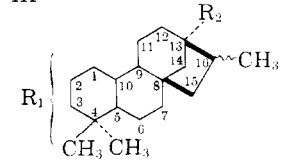
Hydrogenation (Pd–C) of steviol (I) gives dihydrosteviol V-A, m.p. 210–212°, $[\alpha]D$ −41.5 ± 1.6°, while stevioside[7] (PtO_2) and subsequent hydrolysis yields V-B,[8] m.p. 209-212°, $[\alpha]D$ −78.8 ± 1.0° (hydrate, 1 H_2O). Va-A, m.p. 107–112°, $[\alpha]D$ −63.8 ± 2°; Va-B, m.p. 132–134°, $[\alpha]D$ −100.5 ± 2°. Vb-A ($LiAlH_4$, T.H.F., 6 hr. reflux), m.p. 214–216°, $[\alpha]D$ −40.4 ± 2.0°; Vb-B, m.p. 187–188.5°, $[\alpha]D$ −65.6 ± 1.4°; Vb-A and Vb-B gave with CrO_3–pyridine the respective (solid) aldehydes which were immediately converted to Vc-A, m.p. 166–168°, $[\alpha]D$ −35.4 ± 1.7° and Vc-B, m.p. 170–171°, $[\alpha]D$ −59.3 ± 1.8°. Desulfurization with Raney nickel (W-6) gave Vd-A, m.p. 147–148°, $[\alpha]D$ −24.4 ± 1.4°, and Vd-B, m.p. 152–154°, $[\alpha]D$ −51.7 ± 1.3°. The replacement of the tertiary hydroxyl group by bromine (PBr_5, ether) gave, respectively, Ve-A, m.p. 110–112°, $[\alpha]D$ −17.0 ± 1.2°, and Ve-B, m.p. 117–119°, $[\alpha]D$ −66.1 ± 0.9°. Raney nickel hydrogenolysis gave Vf-A ("Stevane A") m.p. 87.5–88.5°, $[\alpha]D$ −31.9 ± 0.8° and Vf-B, m.p. 47°/54–55°, $[\alpha]D$ −66.8 ± 1.3°.



I, R = H
a, R = Ac

II, R = H_2
a, R = O
b, R = CHOH

III IV



V, R_1 = CO_2H, R_2 = OH
Va, R_1 = CO_2CH_3, R_2 = OH
Vb, R_1 = CH_2OH, R_2 = OH
Vc, R_1 =  , R_2 = OH
Vd, R_1 = CH_3, R_2 = OH
Ve, R_1 = CH_3, R_2 = Br
Vf, R_1 = CH_3, R_2 = H

Professor Briggs kindly compared (melting points, infrared, X-ray powder patterns) Vf-A with (−)-α-dihydrokaurene (m.p. 86–87°, $[\alpha]^{21}D$ −32° $CHCl_3$)[9] and found them identical. Apparently

(1) E. Mosettig and W. R. Nes, *J. Org. Chem.*, **20**, 884 (1955).

(2) All rotations measured in $CHCl_3$, *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.

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

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_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 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.

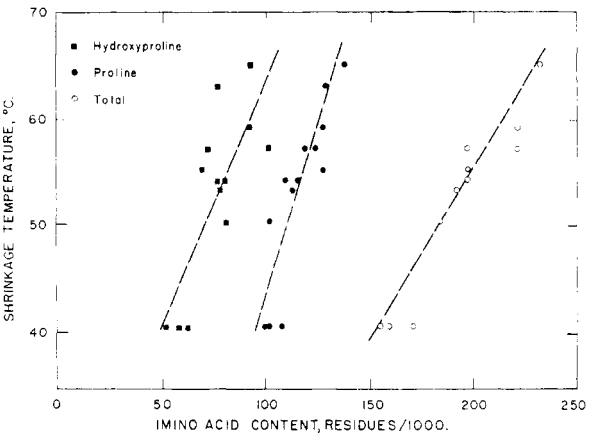

Fig. 1.—The hydrothermal shrinkage temperatures of vertebrate collagens plotted as a function of imino acid content. The dash lines indicate the single regression lines calculated for each group of values.

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]

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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RECEIVED OCTOBER 22, 1959

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).