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.

NATIONAL INSTITUTES OF HEALTH
NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
BETHESDA, MARYLAND
FRED DOLDER
HEINZ LICHTI
ERICH MOSETTIG
PETER QUITT

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.

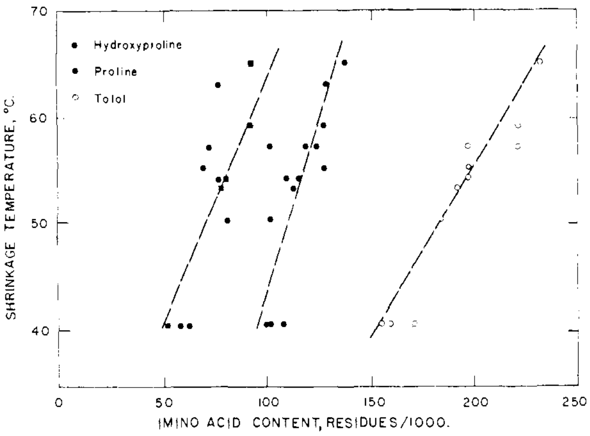


Fig. 1.—The hydrothermal shrinkage temperatures of vertebrate collagens plotted as a function of imino acid content. The dash lines indicate the single regression lines calculated for each group of values.

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.

NATIONAL INSTITUTE OF DENTAL RESEARCH
NATIONAL INSTITUTES OF HEALTH
BETHESDA 14, MD.
KARL A. PIEZ

RECEIVED OCTOBER 22, 1959

---

(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.

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) 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).

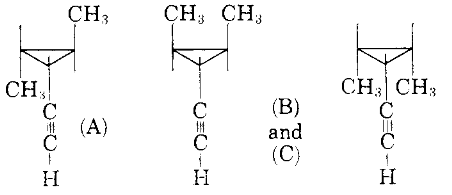
(5) P. S. Skell and R. M. Etter, *Chem. and Ind.*, 624 (1958).

(6) R. M. Etter, H. S. Skovronek and P. S. Skell, THIS JOURNAL, **81**, 1008 (1959).

and triplet state natures, from which derives their respective chemical properties, the singlet state species, carbenes, having non-radical properties (for the main part electrophilic) and undergoing stereospecific *cis* addition to olefins, the triplet state species, methylenes, undergoing non-stereospecific additions to olefins. The bivalent carbon species examined thus far have been characterized as: *carbenes*, CH_2,[3] $CHCH_2CH_3$,[7] CBr_2,[2,4] CCl_2,[8] CHCOOEt,[3,5] $CHCOCH_3$[10]; and *methylenes*, $(C_6H_5)_2C$,[5] oxygen atoms (ground state).[11]

Symmetry considerations employed to rationalize the stability of triplet $(C_6H_5)_2C$ suggested that $H—C—C{\equiv}C—H \longleftrightarrow H—C{\equiv}C—C—H$, with three sp hydridized carbon atoms, would also be in a triplet state, and thus a methylene.

Propargylene, C_3H_2, has been generated by photolysis of diazopropine (from reactions of N-nitroso-N-proparglyurea with aqueous base). If propargylene is generated in the presence of *cis*-2-butene, three isomeric 2,3-dimethyl-1-acetylenylcyclopropanes (A,B,C) are produced in ratios 1:2.5:4.0. In the presence of *trans*-2-butene the identical products (retention times and infrared spectra of the pure compounds) are obtained in ratios 63:2.3:1. Absorption bands at 3.05–3.10 and 4.70–4.77 μ, uptake of 2.0 moles of H_2 (platinum catalyst), identity of tetrahydro (A) with the product from *trans*-2-butene and ethylcarbene, and (B) and (C) with the products from *cis*-2-butene and ethyl carbene, oxidation of (A), to the known *trans*-2,3-dimethylcyclopropane carboxylic acid,[2] makes possible structure assignments



(A)

(B) and (C)

The pattern of relative rates of addition of diphenylmethylene,[6] propargylene and ground state oxygen atom[11] are not identical, emphasizing that the occurrence or absence of stereospecificity in olefin additions is the best criterion for distinguishing methylenes and carbenes.

Molecular orbital considerations indicate that linear or nearly linear[12] C_3H_2, having three sp hydridized carbon atoms and six electrons for distribution among the p-orbitals, is a cylindrically symmetrical compound with two low lying π-orbitals which accounts for four electrons. The next higher molecular orbitals are nearly equivalent[12] and non-bonding, so that they are occupied singly by the two remaining electrons, localizing one electron on each of the terminal carbons. Hund's stabilization of the order of 30–50 kcal. is realized when the two non-bonding electrons have parallel spins, thus rationalizing the triplet nature of propargylene.

DEPARTMENT OF CHEMISTRY PHILIP S. SKELL
THE PENNSYLVANIA STATE UNIVERSITY
UNIVERSITY PARK, PENNSYLVANIA JOHANN KLEBE

RECEIVED NOVEMBER 25, 1959

---

[7] P. S. Skell and J. F. Klebe, unpublished results. Ethyl carbene is generated by copper sulfate catalyzed decomposition of 1-diazopropane. Reactions with *cis*- and *trans*-2-butenes are stereospecific.

[8] W. von E. Doering and W. A. Henderson, Jr., THIS JOURNAL, **80**, 5274 (1958).

[9] W. von E. Doering and P. LaFlamme, *ibid.*, **78**, 5447 (1956).

[10] P. S. Skell and R. M. Etter, unpublished results.

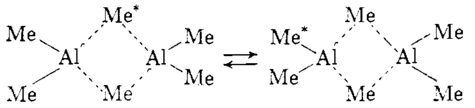
[11] S. Sato and R. J. Cvetanović, *Can. J. Chem.*, **37**, 953 (1959). See also for references to earlier work with oxygen atoms by Cvetanović and co-workers.

[12] H. A. Jahn and E. Teller, *Proc. Roy. Soc., London*, **A161**, 220 (1957).

PROTON MAGNETIC RESONANCE SPECTRUM OF ALUMINUM TRIMETHYL DIMER

Sir:

It has been shown using X-ray diffraction data[1] that aluminum trimethyl exists in the solid state as a dimer with two methyl groups forming bridges between the aluminum atoms. The compound also appears to be predominantly dimeric in benzene solution[2] and in the gaseous state.[3] It is therefore surprising that the room temperature proton magnetic resonance spectrum[4] of the liquid consists of a single peak rather than a pair of signals with relative intensities 1:2 corresponding to protons in bridging and in terminal methyl groups, respectively. This indicates either that all the protons are magnetically equivalent or that the two species are involved in a rapid exchange process which may be represented as



(1)

To decide between these possibilities we have examined the proton resonance spectrum at 56.445 mc. of a liquid solution containing approximately 12 mole per cent. of Al_2Me_6 in cyclopentane, between 25 and −75°. At the low temperature we observed the two signals expected for the bridge structure. We detected no further splitting of the smaller peak, showing that the protons in the bridging methyl groups remain magnetically equivalent at −75°. Using the solvent resonance as an internal standard we found chemical shifts of +1.04 p.p.m. and +2.17 p.p.m., respectively, for the protons of the bridging and the terminal methyl groups. As the temperature increases the peaks first broaden and eventually coalesce to a single, sharp peak at +1.79 p.p.m., precisely the weighted average of the low-temperature shifts. This behavior is characteristic of an exchange process.[5]

We are studying the detailed temperature-dependence of this spectrum in order to obtain the activation energy of reaction (1) as accurately as possible. The data already obtained indicate that this energy is between 6 and 14 kcal./mole. The heat of dissociation of the dimer is 20.2 kcal./mole at 100 to 150° in the gaseous phase.[3] We conclude that $AlMe_3$ monomer probably is not formed as an intermediate in (1) but that the ex-

---

[1] P. H. Lewis and R. E. Rundle, *J. Chem. Phys.*, **21**, 986 (1953).

[2] K. S. Pitzer and H. S. Gutowsky, THIS JOURNAL, **68**, 2204 (1946).

[3] A. W. Laubengayer and W. F. Gilliam, *ibid.*, **63**, 477 (1941).

[4] J. A. Pople, W. G. Schneider and H. J. Bernstein, "High-resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, p. 296.

[5] Ref. 4, pp. 218 ff.