[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, PURDUE UNIVERSITY]

Perturbation of the Ultraviolet Absorption Spectrum of Anthracene Coupled to Bovine Plasma Albumin[1]

BY EDWARD J. WILLIAMS[2] AND JOSEPH F. FOSTER

RECEIVED APRIL 18, 1959

The perturbation of the absorption spectrum of anthracene attached to the sulfhydryl group of bovine plasma albumin (BPA) through a mercury atom has been examined utilizing differential spectrophotometry. *p*H-dependent changes in the differential spectrum of the anthracene chromophore betwen 320 and 400 mμ appear to be related to configurational alterations in the molecular structure of the protein and are similar to previously observed *p*H-induced changes in the differential spectrum of BPA in the region of tyrosyl absorption. The nature of the effect of *p*H on the differential spectrum is such as to suggest that the spectral perturbation is a manifestation of non-specific interaction of the anthracene with its protein environment, the character of the environment being dependent on the molecular configuration of the protein at a given *p*H. The results are interpreted in accord with a previous suggestion of the authors that differential spectra of this and other proteins are produced in large part by effects other than changes in tyrosyl hydrogen-bonding as has been frequently presumed.

Introduction

It has been proposed that studies of the absorption spectra of proteins may provide information pertaining to identification of tyrosyl hydrogen bonds in the macromolecules.[3] By differential spectrophotometric procedures small spectral perturbations due to alteration of *p*H have been found in insulin and ribonuclease and interpreted on the basis of tyrosyl-carboxylate hydrogen-bonding.[3,4] In a study of bovine plasma albumin by this technique the present authors[5] concluded that the shift in absorption spectrum associated with titration of the carboxyl groups results not from disruption of tyrosyl-carboxylate hydrogen bonds directly, but as an indirect consequence of coöperative structural alterations known to take place in the albumin molecule in this *p*H range. It was further suggested that the perturbation of the spectrum could result in part, and possibly in entirety, from effects (such as variations in the electronic polarizability of the environment of the tyrosyl residues) other than changes in the extent of tyrosyl hydrogen-bonding. For example, if the tyrosyl residues are immersed in the interior of the native protein in a region of relatively high electron polarizability and are exposed to the aqueous environment (having a lower polarizability) when the protein undergoes isomerization and/or expansion it might be predicted that a substantial blue shift in the tyrosyl spectrum would result,[6] as was observed.[5]

In an attempt to devise an experimental test of this alternate explanation of protein differential spectra the sulfhydryl group of BPA was coupled to a strongly absorbing aromatic chromophore which could neither participate in hydrogen-bonding nor protonic equilibria. This approach was suggested by a paper of Klotz and Ayers[7] in which they coupled a 4-(*p*-dimethylaminobenzeneazo)-phenyl group to the sulfhydryl of BPA through mercury for the purpose of studying spectrophotometrically the modification of its protonic equilibrium by the protein. Previous investigators have shown that the absorption spectra of certain aromatic mercurial compounds are perturbed when the aromatics are coupled to the sulfhydryl groups of proteins.[8-10]

In the present investigation anthracene–mercuric chloride was reacted with the sulfhydryl group of BPA and the *p*H dependence of the spectrum of the resultant conjugate studied by differential spectrophotometry. The anthracene chromophore was chosen because of its intense absorption at wave lengths well above the region where the aromatic residues of the protein exhibit appreciable absorption,[11] because its spectrum includes several sharp absorption maxima[12,13] and because anthracene contains no ionic or polar groups capable of hydrogen-bonding with the protein. Results of the investigation show that the perturbation of the absorption spectrum of anthracene attached to the protein is strongly *p*H-dependent in a manner which is in accord with the earlier suggestion that the perturbation of the absorption spectrum of tyrosyl residues in the protein may result from factors other than hydrogen-bonding.

Experimental

Anthracene–mercuric chloride was prepared by the procedure of Goswami and Das-Gupta.[14] Propionic acid was used as a solvent instead of glacial acetic acid to avoid running the reaction under pressure. After washing the greyish yellow product thoroughly with distilled water, it was recrystallized several times from glacial acetic acid and then from benzene. The precipitate was washed with absolute ethanol and finally with absolute ether and dried in a vacuum oven at 60°. The melting point was 241–246°, decomposition being observed during the melting process. Microanalysis yielded the following elemental composition (in %): Cl, 8.52 (8.59 calcd.); C, 39.62 (40.70 calcd.); H, 2.58 (2.19 calcd.); Hg, 49.28 (48.52 calcd.). There is some doubt concerning the accuracy of the carbon and hydrogen analyses and hence of the percentage of mercury which was obtained by subtracting the sum of the carbon, hydrogen and

(1) This investigation was supported in part by the National Cancer Institute, National Institutes of Health, Grant C-2248.

(2) Fellow of the Visking Corporation, 1957–1958. Present address, Marquette University Medical School, Milwaukee, Wisconsin.

(3) M. Laskowski, Jr., J. M. Widom, M. L. McFadden and H. A. Scheraga, *Biochim. et Biophys. Acta*, **19**, 581 (1956).

(4) H. A. Scheraga, *ibid.*, **23**, 196 (1957).

(5) E. J. Williams and J. F. Foster, THIS JOURNAL, **81**, 865 (1959).

(6) N. S. Bayliss and E. G. McRae, *J. Phys. Chem.*, **58**, 1002 (1954).

(7) I. M. Klotz and J. Ayers, THIS JOURNAL, **79**, 4078 (1957).

(8) S. F. Velick, *J. Biol. Chem.*, **203**, 563 (1952).

(9) P. D. Boyer, THIS JOURNAL, **76**, 4331 (1954).

(10) M. G. Horowitz and I. M. Klotz, *Arch. Biochem. Biophys.*, **63**, 77 (1956).

(11) P. Doty and E. P. Geiduschek, in Neurath and Bailey, "The Proteins," Vol. IA, Academic Press, Inc., New York, N. Y., 1953, p. 403.

(12) K. A. Friedel and M. Orchin, "Ultraviolet Spectra of Aromatic Compounds," John Wiley and Sons, New York, N. Y., 1951, Plate 388.

(13) N. Jones, *Chem. Revs.*, **41**, 353 (1947).

(14) M. Goswami and H. N. Das-Gupta, *Ind. Chem. Soc.*, **8**, 475 (1953).

chlorine percentages from one hundred. The chlorine analysis, however, is thought to be highly accurate.

Goswami and Das-Gupta reported a melting point of 181–183° (with decomposition) for their preparation of anthracene–mercuric chloride,[14] compared to our value of 241–246° (also with decomposition). This discrepancy may be due to the fact that the two products are different mono-substitution isomers. The results of Goswami and Das-Gupta, on the other hand, may be erroneous in view of the fact that their melting point value is considerably lower than that of anthracene (217°), while, for what seem to be analogous situations, phenyl mercuric chloride and naphthyl mercuric chloride (both the α and β-isomers) have melting points which are appreciably higher than those of benzene and naphthalene, respectively. The above-mentioned authors did not indicate how they purified their product.

No attempt was made to determine whether substitution occurred at the $\alpha(1,4,5,8)$, $\beta(2,3,6,7)$ or $\gamma(9,10)$ carbon atoms of anthracene, and it is not improbable that the product is actually a mixture of mono-substituted isomers. The separation of such a mixture into its constituents and the determination of spectral differences due to substitution at a particular anthracene carbon is in itself another problem.

Since anthracene–mercuric chloride is highly insoluble in water, BPA and cysteine–mercuric anthracene (BPA–HgA and C–HgA, respectively) were prepared by stirring an aqueous solution of BPA (1%) or cysteine hydrochloride (0.01 M) and a suspension of anthracene–mercuric chloride together at room temperature. The procedure was repeated at 5° and there was no noticeable temperature effect. At the end of the reaction period, insoluble material was removed and the solutions clarified by centrifugation in a Servall centrifuge. Protein solutions were centrifuged at 5° and cysteine solutions at room temperature (rotor temperature-45°). Plastic containers were used in all centrifuge runs. The concentration of BPA–HgA in the centrifuged reaction mixture was approximately 6×10^{-5} M. The centrifuged reaction mixtures, together with 0.1 M NaCl, NaOH and HCl solutions and deionized water were used to prepare sets of solutions for spectrophotometric analyses. The concentration of BPA–HgA in the solutions used for spectrophotometric analyses was approximately 4×10^{-6} M.

Purified anthracene was generously supplied by Dr. Samuel Resconich. All other chemicals were reagent grade and were used without further purification. Crystalline Sigma brand cysteine hydrochloride was used without further purification. BPA solutions were prepared from crystalline Armour brand BPA and were deionized[15] and defatted.[5]

Total and differential spectra[16] were obtained within four hours following preparation of the solutions using a Beckman DU spectrophotometer equipped with a photomultiplier attachment and 1-cm. quartz cuvettes. Thermospacers and a constant temperature bath (25°) were used to maintain a constant temperature in the cuvette compartment. pH measurements were made at room temperature with a Beckman Model G pH meter. In obtaining differential optical densities,[16] the spectrophotometer was first balanced at zero absorbance against a reference pH 2 solution and the optical density of each sample solution measured relative to that of the reference solution. Since the concentration of BPA–HgA (or C–HgA) was the same in both the reference and sample solutions, a positive differential optical density signified that the sample solution had a larger extinction coefficient than that of the reference solution, the extinction coefficient difference being related to the difference in the pH values of the sample and reference solutions.

Results and Discussion

Total spectra between 320 and 400 mμ for anthracene, anthracene–mercuric chloride, BPA–HgA and C–HgA are shown in Fig. 1. Although the spectra of anthracene–mercuric chloride, BPA–HgA and C–HgA are shifted to slightly longer wave lengths relative to the spectrum of anthracene, there is no indication that substitution of the anthracene nucleus has produced a gross perturbation of the anthracene spectrum such as is evident when the nucleus is substituted with a group capable of conjugative effects.[12] pH-dependent differential spectra of BPA–HgA are presented in Fig 2. Although the differential spectra in this figure have not been corrected for the differential absorption of BPA due to differences in pH, Fig. 3 indicates that this correction is significant only at high pH and in the shorter wave length region.

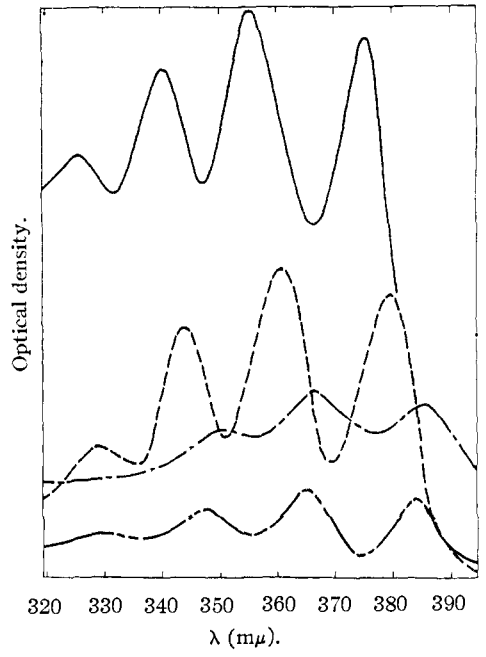


Fig. 1.—Absorption spectra of anthracene derivatives: ———, anthracene in cyclohexane; — —, anthracene–HgCl in cyclohexane; — · —, BPA–HgA in 0.02 M Cl^-; — · · —, cysteine–HgA in 0.02 M Cl^-.

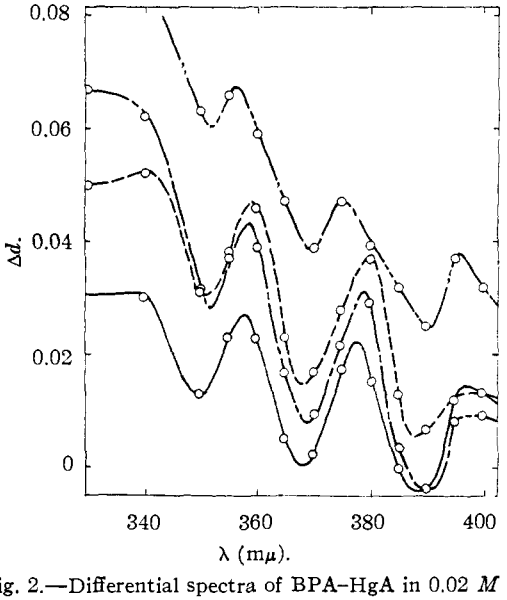


Fig. 2.—Differential spectra of BPA–HgA in 0.02 M Cl^-; pH of reference solution is 2: ———, pH 4.18; — —, pH 5.53; — · —, pH 7.25; — · · —, pH 10.6.

The concentration of BPA–HgA in the sets of solutions which were subjected to spectrophoto-

(15) H. M. Dintzis, Ph.D. Thesis, Harvard University, 1952.
(16) R. Bastian, *Anal. Chem.*, **21**, 972 (1949).

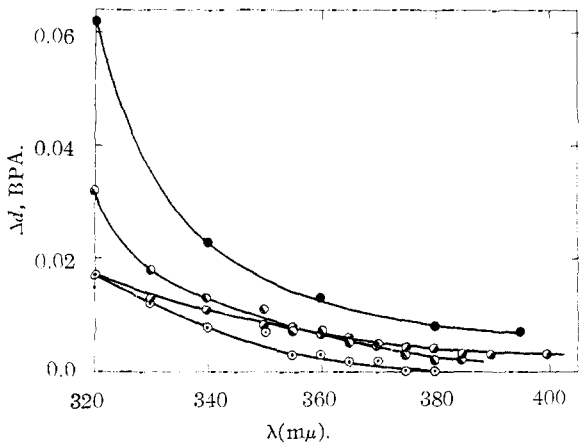


Fig. 3.—Differential spectra of 1% BPA, 0.02 M Cl^- solutions; pH of reference solution is 2: ⊙, pH 4; ◒, pH 6; ◐, pH 9; ●, pH 10.

metric analysis was of the same order of magnitude as that employed by Klotz and Ayers in their titration studies.[7] The concentration of BPA–HgA in the centrifuged reaction mixtures was estimated by dividing the optical densities of these solutions at the wave lengths of their primary absorption maxima (360–365 mμ) by 1×10^4 which is roughly the average value of the molar extinction coefficients of the mono-substituted derivatives listed in Table I. The spectra of these listed compounds exhibit little or no evidence of conjugation of the aromatic nucleus with the various substituent groups. Since there is no great variation in the extinction coefficient values of the mono-substituted derivatives and because of the close resemblance of the spectra of these derivatives both to each other and to the BPA–HgA and C–HgA derivatives, this admittedly crude method for estimating the concentration of the mercurials

TABLE I

SPECTRAL DATA FOR ANTHRACENE DERIVATIVES

	Solvent	λ_{max} (mμ)	$\epsilon_{max} \times 10^{-3}$
Anthracene[12]	Cyclohexane	323, 338, 357,[a] 374	7.0
	Ethanol	324, 339, 357,[a] 376	8.9
9-Methylanthracene[12]	Ethanol	332, 347, 365,[a] 386	8.3
1-Methylanthracene[17]	Isoöctane	325, 340, 360,[a] 375	8.9
9-Chloroanthracene[18]	Ethanol	330, 350, 365,[a] 385	10.0
1-Chloroanthracene[19]	Hexane	325, 355, 365,[a] 385	11.0
2-Chloroanthracene[19]	Hexane	320, 340, 365,[a] 385	9.0
BPA–HgA	pH 2, 0.02 Cl	330, 347, 363,[a] 385	
C-HgA	pH 2, 0.02 Cl	350, 367,[a] 388	

[a] Wave length of primary absorption maximum.

appears to be justifiable. On the basis of the foregoing method of estimation, it appears that approximately 0.40 mole of the 0.67 mole of sulfhydryl groups per mole of BPA have reacted. This value was obtained by dividing the estimated concentration of BPA–HgA in the reaction mixture (6×10^{-5} M) by the initial concentration of BPA (1.43×10^{-4} M for a 1% solution). Since each set of solutions for a given series of differential spectra measurements was prepared from the same

(17) M. L. Mosby, *J. Org. Chem.*, **18**, 966 (1953).

(18) A. Etienne and M. Legrand, *Bull. soc. chim. France*, **20**, 108 (1953).

(19) H. Conrad-Billroth, *Z. physik. Chem.*, **B38**, 133 (1936).

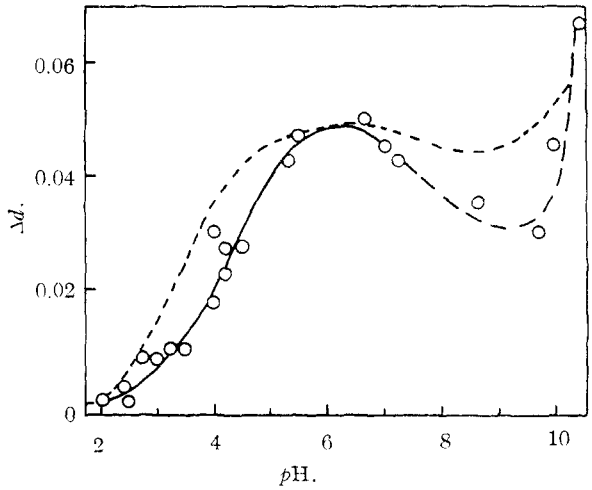


Fig. 4.— ———, BPA–HgA at 355–360 mμ (present results), dashed portion of curve represents uncertainty in d–pH relationship; — — BPA at 287 mμ (divided by 20) from Williams and Foster.[5]

BPA–HgA stock solution, the concentration of BPA–HgA was constant in each of the series of spectrophotometric runs.

It is evident from Fig. 2 that changes in pH exert a small effect on the absorption spectrum of the anthracene chromophore in BPA–HgA. In an attempt to correlate the pH-dependent differential spectrum of anthracene in BPA–HgA with pH-induced changes in the molecular structure of BPA, the differential optical density (Δd) at the wave length of maximum differential absorption (355–360 mμ) was plotted against pH (Fig. 4). For comparison there is plotted our previously published results of the pH dependence of the differential spectrum of BPA in the predominantly tyrosyl absorption region (287 mμ). The Δd value at 287 mμ has been divided by 20, in view of the approximately 20 tyrosyl residues per molecule of BPA, so that comparison is approximately on a per residue basis. The effect of pH on both differential spectra appears to be of the same order of magnitude and, as is aptly illustrated in this figure, is most pronounced at low and high pH, the pH regions where the BPA molecule is susceptible to conformational changes.[5,20–23] Interpretation of the Δd–pH relationship at alkaline pH values is somewhat questionable in view of the fact that a significant change in the spectra of alkaline solutions was detectable when they were examined 24 hr. after initial spectrophotometric analysis. This spectral change appeared to be promoted by exposure to light. No significant change in the spectra of solutions whose pH values were below pH 7 was detectable during the elapsed interval (1–4 hr.) between preparation of the solutions and completion of the spectrophotometric analysis. Although the BPA–HgA curve in Fig. 4 has not been corrected for differential BPA ab-

(20) C. Tanford, J. G. Buzzell, D. G. Rand and S. A. Swanson, THIS JOURNAL, **77**, 6421 (1955).

(21) J. T. Yang and J. F. Foster, *ibid.*, **76**, 1588 (1954).

(22) K. Aoki and J. F. Foster, *ibid.*, **79**, 3385 (1957).

(23) M. J. Kronman and J. F. Foster, *Arch. Biochem. Biophys.*, **72**, 205 (1957).

sorption, such a correction does not modify the shape of the Δd–pH curve to any great extent.

Although there is considerable evidence for believing that the molecular structure of BPA does not undergo pronounced modification between pH 4.3 and pH 10.5,[24-27] there are some data which indicate that small structural changes do occur in this pH region.[5,24,28-30] Hence the dip in the Δd–pH curves (Fig. 4) may be a reflection of these minor conformational changes.

Coggeshall and Pozefsky[31] have reported that the spectrum of naphthacene is shifted progressively red as the refractive index of solvent is increased. They also report that the anthracene spectrum undergoes a red shift when the solvent is changed from 2,2,4-trimethylpentane to benzene, benzene having the higher refractive index. This observation is in agreement with the statement of Bayliss[6,32] that for a non-polar compound in either a polar or non-polar solvent, the spectrum should be shifted to longer wave lengths when the refractive index (n) of the solvent is increased. This statement is based on the postulated effect of the electron polarizability of the solvent (proportional to n^2) on the light absorption characteristics of the solute species. Schnurmann and Maddoms,[33] however, claim that the shift is not a regular function of n or n^2 since they observed a red shift of the spectra of naphthacene and anthracene, along with those of other aromatic hydrocarbons, upon changing the solvent from isoöctane (n^{20}D 1.39) to ethanol (n^{18}D 1.36). Jones[13] also lists anthracene spectra in which the shift is bathochromic when ethanol replaces n-heptane (n^{20}D 1.39) as solvent.

Lauer and Horio[34] have reported that the anthracene spectrum is shifted red in accordance with the following solvent order: benzene $\geq$ acetic acid $\geq$ cyclohexene $\geq$ ethanol $\geq$ cyclohexane $\geq$ hexane. This order does not coincide with an order based either on systematic changes of refractive index or of dielectric constant. Lauer and Horio deduced from their study that solvents with double bonds produced larger red shifts than those without double bonds and that polar molecules were more effective than non-polar molecules.

Herington and Kynaston[35] obtained the difference spectrum of anthracene in cyclohexane relative to the spectrum in ethanol as standard and also in ethanolic 1 M lithium chloride relative to the spectrum in ethanol. These authors have interpreted their results as being indicative that a modification in the extent of interaction of solvent molecules with the anthracene chromophore occurs when the solvent is changed from ethanol to cyclohexane or when lithium chloride is added to ethanol. In view of this work, together with that of Schnurmann, *et al.*[33] (and also the work of Lauer and Horio),[34] it appears that there is some degree of electrostatic interaction between polar ethanol molecules and anthracene

The observation that Δd increases slightly with pH between pH 2 and pH 6 can be explained by postulating that the spectrum of the anthracene chromophore attached to BPA undergoes a small progressive red shift as the pH is raised above 2, since the maxima and minima of the pH-dependent differential spectrum are found at longer wave lengths than are the corresponding maxima and minima of the total spectrum. In view of the evidence that the molecular structure of BPA is most compact near the isoelectric point, becoming less compact upon the addition of acid,[20-23] one can speculate that the red spectral shift, which occurs when the pH is raised from 2 to 6, is due to the fact that the anthracene chromophore is more enfolded in the compact structure of the protein at pH 6 than in the expanded structure which exists at pH 2.

The refractive index of BPA is approximately 1.6,[11] while that of water is approximately 1.34. If the anthracene chromophore were enfolded in the structure of the protein, it should be located in an environment whose refractive index is greater than that of an aqueous environment. Therefore, in agreement with Coggeshall and Pozefsky's results, the observed increase in Δd at pH 6 (compared to pH 2) might be attributed to the increase in the refractive index of the environment of the anthracene chromophore due to its having been enfolded in the structure of the protein at pH 6.

An approximation of London's general equation[36] which describes the Van der Waals' interaction energy (ΔE) of non-polar molecules is

$$\Delta E = \frac{-3\alpha_A\alpha_B(I_A)(I_B)}{2r_{AB}^6(I_A + I_B)} \qquad (1)$$

in which α_A and α_B are the polarizabilities of the molecules, I_A and I_B are approximately the average energy difference for normal and excited states of the molecules and r_{AB} is the distance between molecules. In view of the fact that the polarizability is related to refractive index by the equation

$$\alpha = \frac{3}{4\pi N} \times \frac{M}{e} \times \left(\frac{n^2 - 1}{n^2 + 2}\right) \qquad (2)$$

it is apparent that ΔE for the interaction of a non-polar solute with a non-polar solvent is related to the refractive index of the solvent and in those cases where the red shift of the spectrum of a solute is related solely to changes in the refractive index of solvent, it can be concluded that there is a connection between the spectral shift and the variation in the Van der Waals' interaction energy. In applying this deduction to the present investigation, the postulated red shift of the anthracene spectrum as reflected in the change of the differential spectrum when the pH of a BPA–HgA solution is raised from 2 to 6 can be interpreted (assuming that the spectral change is at least in

(24) C. Tanford and J. G. Buzzell, *J. Phys. Chem.*, **60**, 225 (1956).
(25) J. M. Creeth, *Biochem. J.*, **51**, 10 (1952).
(26) G. Weber, *ibid.*, **51**, 155 (1952).
(27) H. J. Almquist and D. M. Greenberg, *J. Biol. Chem.*, **105**, 519 (1934).
(28) J. Jirgensons, *Arch. Biochem. Biophys.*, **39**, 261 (1952).
(29) I. M. Klotz and J. Ayers, THIS JOURNAL, **74**, 6178 (1952).
(30) S. Katz and I. M. Klotz, *Arch. Biochem. Biophys.*, **44**, 351 (1953).
(31) N. D. Coggeshall and A. Pozefsky, *J. Chem. Phys.*, **19**, 980 (1951).
(32) N. S. Bayliss, *ibid.*, **18**, 292 (1950).
(33) R. Schnurmann and W. F. Maddoms, *ibid.*, **19**, 1430 (1951).
(34) K. Lauer and M. Horio, *Ber. Deut. Chem. Ges.*, **69B**, 130 (1936).
(35) E. F. G. Herington and W. Kynaston, *J. Chem. Soc.*, 3137 (1952).
(36) D. F. Waugh, *Advances in Protein Chem.*, *IX*, 325 (1954).

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

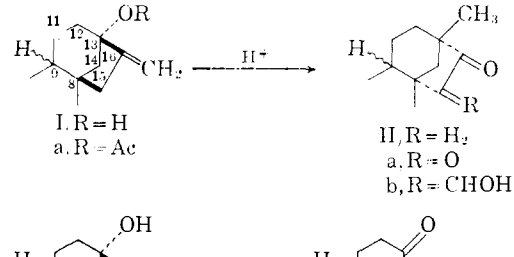
COMMUNICATIONS TO THE EDITOR

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]

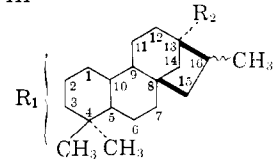
Hydrogenation (Pd–C) of steviol (I) gives dihydrosteviol V-A, m.p. 210–212°, $[\alpha]D$ −41.5 ± 1.6°, while stevioside[7] (PtO_2) and subsequent hydrolysis yields V-B,[8] m.p. 209-212°, $[\alpha]D$ −78.8 ± 1.0° (hydrate, 1 H_2O). Va-A, m.p. 107–112°, $[\alpha]D$ −63.8 ± 2°; Va-B, m.p. 132–134°, $[\alpha]D$ −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 = H₂
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.