THE HEAT OF DENATURATION OF DNA

Sirs:

Measurements of the changes in enthalpy accompanying the acid denaturation[1] of sodium desoxyribosenucleic acid (DNA) have been made by a previously described technique.[2] The DNA employed, (isolated from salmon testes by the method B of Simmons[3]) had a weight-average molecular weight[4] of 6.3×10^6. Determinations of the enthalpy changes on mixing solutions of DNA in 0.1 *M* NaCl with HCl in 0.1 *M* NaCl were made at 5, 25 and 40°. The results, shown in Fig. 1 for 25°, are qualitatively similar at all three temperatures: the entire enthalpy change occurs in the narrow *p*H range associated with the macromolecular configuration change.

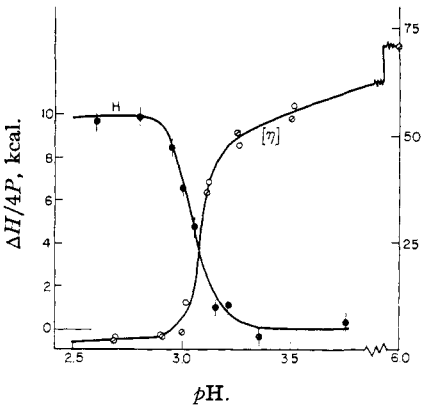

Fig. 1.—The apparent heat content and intrinsic viscosity of DNA as a function of *p*H at 25° and $\Gamma/2 = 0.1$: *M* ●: heat absorbed (per 4 moles nucleotide phosphorus) when solutions of DNA at *p*H 6 are mixed with HCl solutions to give the indicated final *p*H values; ○, ⊖: intrinsic viscosity (zero gradient) at the indicated *p*H.

The calorimetric data at *p*H values above and below the denaturation region are consistent with the view that the heats of ionization of the bases in both native and denatured DNA are close to zero. Independent evidence[1b,c] supports this conclusion with respect to denatured DNA. If we assume negligibly small heats of ionization, and enthalpy changes proportional to the extent of denaturation, then we may, with the aid of titration curves at each temperature, obtain the dependence of the extent of denaturation on the extent of proton binding. This is given in Fig. 2. It is seen that as many as two-thirds of the possible protons may be bound by the DNA molecule without causing any enthalpy change and without causing denaturation.

This fact is of interest in connection with the

(1) For recent investigations see (a) P. Doty, *J. Cell. Comp. Physiol.*, **49**, (Suppl. 1), 27 (1957); (b) L. F. Cavalieri and B. H. Rosenberg, THIS JOURNAL, **79**, 5352 (1957); (c) R. A. Cox and A. R. Peacocke, *J. Polymer Sci.*, **28**, 764 (1957); (d) A. R. Mathieson and S. Matty, *ibid.*, **28**, 747 (1957); (e) H. K. Schachman, *J. Cell. Comp. Physiol.*, **49**, (Suppl. 1), 71 (1957).

(2) A. Buzzell and J. M. Sturtevant, THIS JOURNAL, **73**, 2454 (1951).

(3) N. S. Simmons, A.E.C. Report, U.C.L.A., 184 (1952), and private communication.

(4) See E. P. Geiduschek, *J. Polymer Sci.*, in press, for additional properties.

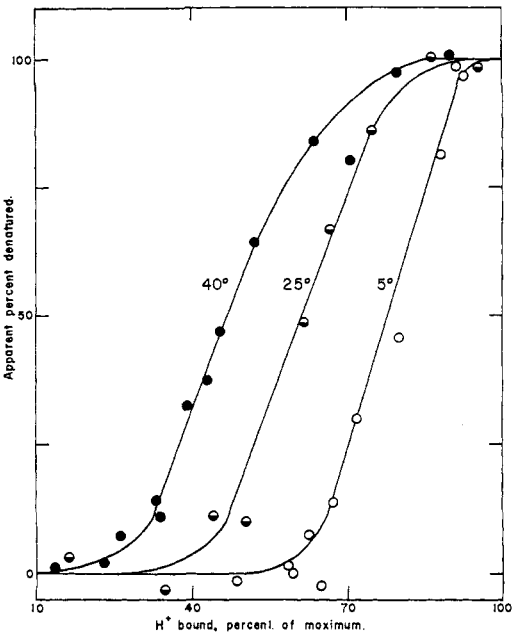

Fig. 2.—The extent of denaturation of DNA as a function of the temperature and the extent of proton addition to the bases. The extent of denaturation is computed on the assumption that the enthalpy change is proportional to the extent of denaturation.

helical structure[5] of DNA. It is to be expected that heat should be absorbed when a strong hydrogen bond is broken. If hydrogen bonds are responsible for maintaining the helical structure, then it would appear that protonation of the donor group in a hydrogen bond does not necessarily break the bond. Thus, the denaturation of DNA which takes place when a sufficient number of protons is bound, cannot be considered as evidence for the existence of hydrogen bonds between the base pairs in the native molecule.

(5) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

(6) Contribution No. 1479. This research was aided by grants from the National Science Foundation and the United States Public Health Service.

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KANAMYCIN. III. KANAMYCIN B

Sir:

Kanamycin fermentations yield a second antibiotic, designated kanamycin B,[1] similar in properties to kanamycin.

Kanamycin B has been isolated in pure form by countercurrent distribution of the salicylidene derivatives of the crude antibiotic mixture, using a methanol–water–chloroform–benzene (5:4:2:1) system, and by chromatography of the crude antibiotic on columns of Amberlite XE-64(NH_4^+)[2]

(1) M. J. Cron, D. L. Johnson, F. M. Palermiti, Y. Perron, H. D. Taylor, D. F. Whitehead and I. R. Hooper, THIS JOURNAL, **80**, 752 (1958).

(2) A product of the Rohm & Haas Co.

with 0.08 *N* ammonium hydroxide. Kanamycin B base is soluble in water, slightly soluble in the lower alcohols, insoluble in non-polar organic solvents, and decomposes over a wide range above 170°, $[\alpha]_D^{24}$ +135 (*c* 0.63, H_2O). It gives positive Molisch, Elson–Morgan and ninhydrin tests but gives negative reducing sugar tests and yields no furfural-like material after 40% sulfuric acid treatment at 100° for 100 min., in contrast to kanamycin. The infrared spectrum in KBr shows the following absorption maxima: 2.96, 3.44, 6.35, 6.48 (shoulder), 6.74, 6.85 (shoulder), 7.25, 7.45, 7.86, 8.08, 8.28, 8.76, 9.55, 9.65, 10.4, and 11.15.

Kanamycin B base was recrystallized repeatedly from 90% ethanol and dried to constant weight at 135° under vacuum. *Anal.* C, 44.7, 44.8; H, 7.46, 7.55; N, 12.56, 12.55; neut. eq., 106; mol. wt., 1170 (Rast method with urea as solvent).

Kanamycin B was characterized as a polyacetyl derivative, prepared from kanamycin B by acetic anhydride–pyridine acetylation. Acetylation of a mixture of the antibiotics and countercurrent distribution in a *n*-butanol–acetic acid–water system (4:1:5) also yielded polyacetylkanamycin B, $[\alpha]_D^{24}$ +107.8 (*c* 0.49, CH_3OH). *Anal.* C, 50.68, 51.05; H, 6.52, 6.61; N, 7.55, 7.68; O-acetyl, 25.4; total acetyl, 46.3; mol. wt., 2010, 2220 (Signer). N-Acetylkanamycin B was obtained by de-O-acetylation with Amberlite IR-410 (OH^-)[2] of the polyacetyl derivative and by acetylation of kanamycin B base in methanol with acetic anhydride. It decomposed gradually at 220° to 250°, $[\alpha]_D^{24}$ +150 (*c* 0.42, H_2O). *Anal.* C, 48.23, 47.97; H, 6.81, 6.78; N, 9.07, 9.14. Kanamycin B yields Schiff bases with aromatic aldehydes as does kanamycin.[1] The N-salicylidene derivative was obtained by treatment of the base in water with an alcoholic solution of salicylaldehyde. *Anal.* C, 58.15; H, 5.60.

Hydrolysis of N-acetylkanamycin B (1 *N* HCl, 40 min. reflux) yielded 2-deoxystreptamine, isolated as the di-N-acetyl derivative and confirmed by infrared comparison with an authentic sample,[1] and kanosamine,[3] isolated as the pentaacetate and confirmed by infrared comparison. Paper chromatograms of acid hydrolyzates of kanamycin B show, in addition to deoxystreptamine and kanosamine, an unidentified ninhydrin-positive reducing spot but no spot for 6-deoxy-6-amino-D-glucose (6-glucosamine).[3] The 6-glucosamine component of kanamycin yields a substance with an ultraviolet spectrum similar to furfural when treated with hot sulfuric acid. Kanamycin B does not give this product, confirming the absence of 6-glucosamine. Both kanamycin B and kanosamine on treatment with 80% sulfuric acid yield a product with the properties of an aminofurfural.

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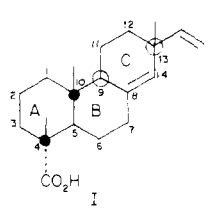
(3) M. J. Cron, O. B. Fardig, D. L. Johnson, H. Schmitz, D. F. Whitehead, I. R. Hooper and R. U. Lemieux, THIS JOURNAL, **80**, 2342 (1958).

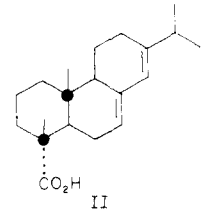
ON THE STEREOCHEMISTRY OF THE PIMARIC ACIDS

Sir:

In view of the stereochemical and possibly biogenetic relationship of pimaric and isopimaric acids (I) with other diterpenes,[1] elucidation of the configuration of especially their 13-substituents is most important.



I



II

Identical concd. sulfuric acid treatment of pimaric as well as isopimaric acids (I),—−30° for fifteen minutes,—gave the previously reported hydroxylactone,[2] m.p. 180–181°, I.R.($CHCl_3$) 5.75μ, an oily mixture of 5- and 6-membered lactones and abietic acid (II), identified by the characteristic 241 mμ ultraviolet absorption peak and the identity of the infrared spectrum and specific rotation of its di-*n*-amylamine salt with that of an authentic specimen.[3] *These results constitute the first chemical proof of the fact that isopimaric acid is not a racemate*[4] *and represent the first chemical conversion of a pimaradiene to an abietadiene, the final step in the generally accepted biogenesis of abietadienic diterpenes.*[1,5]

Sulfuric acid treatment of dihydropimaric acid at room temperature for ten minutes led to a 1.6:1 mixture of 5- and 6-membered lactones,[6] respectively. Similarly, dihydroisopimaric acid yielded a 1.6:1 mixture of a 5-lactone, m.p. 108–110°,[7] $[\alpha]_D$ −15°, and 6-lactone, m.p. 60–65°, $[\alpha]_D$ −40°. *The production of non-identical sets of lactones from the two dihydro acids under identical conditions of a reaction which perturbs all asymmetric centers except C-4 and 13 proves that the resin acids are at least 13-epimers.*

An infrared spectrophotometric product analysis of an acid-catalyzed equilibration of dihydropimaric acid—concentrated sulfuric acid at room temperature for nineteen hours—indicated the formation of 95 ± 0.6% 6-lactone (III) and 5 ± 0.6% 5-lactone (IV). Equilibration of pure 6-lactone gave the same reaction mixture. However, similar treatment of dihydroisopimaric acid led to a mixture of 96.4 ± 0.8% 6-isolactone (III) and 3.6 ± 0.8% 5-isolactone (IV). *Since the 6-lactones are the more stable products and since the change from the 5- to the 6-lactones involves among other things a conformational inversion at C-13, the system with lower 6-lactone content at equilibrium must have its bulkier*

(1) E. Wenkert, *Chemistry and Industry*, 282 (1955).
(2) E. E. Fleck and S. Palkin, THIS JOURNAL, **62**, 2044 (1940).
(3) G. C. Harris and T. F. Sanderson, *ibid.*, **70**, 334 (1948).
(4) *Cf.* O. Jeger and A. Brossi, *Helv. Chim. Acta*, **33**, 722 (1950).
(5) L. Ruzicka, *Experientia*, **10**, 357 (1953).
(6) (a) T. Hasselström and B. L. Hampton, THIS JOURNAL, **61**, 967 (1939); (b) Le-van-Thoi and J. Ourgaud, *Bull. soc. chim., France*, 1388 (1955).
(7) G. C. Harris and T. F. Sanderson, THIS JOURNAL, **70**, 2081 (1948).