hydrin formation, closure to the 9,11β-oxide and opening with hydrogen fluoride gave 6α,9α-difluoro-16α-methylprednisolone 21-acetate (III), m.p. 257–259° (dec.), $\lambda_{max}^{alc.}$ 238 mμ (ϵ 16,500). Satisfactory analyses were obtained for the compounds.

The biological effects of these new hydrocortisone analogs will be reported in detail elsewhere by members of the Upjohn Company Endocrinology Department. As examples of the type of potentiation of activity observed, 6α-fluoro-16α-methylhydrocortisone acetate (I), 6α-fluoro-16α-methylprednisolone acetate (II), and 6α,9α-difluoro-16α-methylprednisolone acetate (III) were, respectively, approximately 40, 160 and 700 times as active as hydrocortisone in the liver glycogen deposition assay.[11]

(11) R. O. Stafford, L. E. Barnes, B. J. Bowman and M. M. Meinzinger, *Proc. Soc. Exp. Biol. Med.*, **89**, 371 (1955). We are indebted to Mr. S. C. Lyster for these assays.

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THE STEREOCHEMISTRY OF ALLOGIBBERIC ACID AND OF GIBBERIC ACID

Sir:

We wish to outline the evidence which permits the assignment of the stereochemistry shown in I and II, respectively, to allogibberic acid and to gibberic acid, two acid rearrangement products of the plant growth hormone gibberellic acid.[1]

(1) The carboxyl group in allogibberic acid (I) must be *cis* to the two carbon bridge of the bicyclo[1,2,3]octane system. This follows from the fact that the diacid (III) obtained from I by Cross, *et al.*,[2,3] on ozonolysis followed by sodium bismuthate cleavage is known to give an anhydride, reconvertible to I on hydrolysis, on treatment with acetic anhydride. We have now shown that the C_6 epimer of III[3] gives the *same anhydride* as III when refluxed with acetic anhydride. This behavior is compatible only with a *cis* relationship of the two acid groups in III[4] and therefore the C_6 carboxyl and the two carbon bridge are *cis* to each other.

(2) The mechanism of the rearrangement of allogibberic acid into gibberic acid (I→II) is such as to require that the two-carbon bridge in gibberic acid have the opposite configuration from that which it occupies in allogibberic acid. This mechanistic consideration is compelling but since the evidence is contradictory[5] we have established this point by demonstrating that the rotatory dispersion curve of II is the mirror image of that of the ketone from the ozonolysis of I.[6]

(1) B. E. Cross, *J. Chem. Soc.*, 4670 (1954).

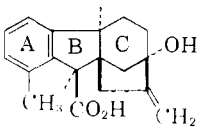
(2) B. E. Cross, J. P. Grove, J. MacMillan and T. P. C. Mulholland, *Chem. and Ind.*, 954 (1956).

(3) T. P. C. Mulholland, *J. Chem. Soc.*, 2693 (1958).

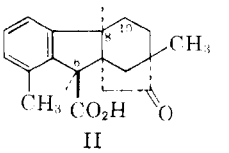
(4) H. H. Cook and R. P. Linstead, *ibid.*, 956 (1934); D. K. Banerjee, and S. K. Das Gupta, THIS JOURNAL, **74**, 1318 (1952).

(5) A. J. Birch, R. W. Rickards and J. H. Smith, *Proc. Chem. Soc.*, 192 (1958).

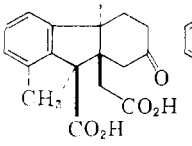
(6) We wish to thank Professor Djerassi for arranging t have the rotatory dispersion data taken on our compounds. We wish to thank Merck, Sharp and Dohme for a very generous gift of the gibberellic acid used in these studies.



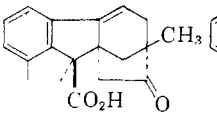
I



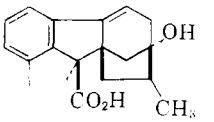
II



III



IV



V

This "inversion" of the two carbon bridge requires that the B/C junction be *cis* in one member of the gibberic–allogibberic acid pair while *trans* in the other. In view of this, it is illuminating that the catalytic hydrogenation of the $\Delta^{8,10}$ olefins derived from II and from the dihydro-derivative of I (IV and V, respectively)[2] results in the regeneration of the stereochemistry at C_8 present in the parent substance. The catalytic hydrogenation of these bicycloöctene systems has thus produced a *cis* B/C junction in one case and *trans* in the other. Put differently, the reduction has taken place *cis* to the two-carbon bridge in one substance and *trans* in the other. Since reduction *trans* to the two-carbon bridge takes place in only one of the two cases it must be that in which both the carboxyl and the bridge are on the same side of the plane. Since such a *trans* reduction regenerates the original stereochemistry, allogibberic acid must be I.

The structures I and II represent the relative stereochemistry of the four asymmetric centers in these molecules. It also can be shown to represent the *absolute* stereochemistry. The keto acid III, which we now know to have a *trans* B/C fusion, has a rotatory dispersion curve[6] which has the same sign of the Cotton effect as cholestanone or of the related (+)*trans*-8-methylhydrindanone.[7] The absolute stereochemistry of I and II is thus established.

(7) C. Djerassi, D. Marshall and T. Nakano, THIS JOURNAL, **80** 4853 (1958); C. Djerassi, *Record of Chemical Progress*, in press.

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C^{14}-HYBRIDS OF HUMAN HEMOGLOBINS. II. THE IDENTIFICATION OF THE ABERRANT CHAIN IN HUMAN HEMOGLOBIN S

Sir:

Both normal adult human hemoglobin and sickle cell hemoglobin (HbA and HbS) contain two each of two kinds of polypeptide chains.[1] The two α chains have the N-terminal sequence, val-leu, and the β chains the sequence val-his-leu.[2] In HbS, a valyl residue has been substituted in one kind of chain for a glutamyl residue in HbA.[3] We wish to report that substitution is in the β chain.

(1) H. S. Rhinesmith, W. A. Schroeder and L. Pauling, THIS JOURNAL, **79**, 4682 (1957), and unpublished data.

(2) H. S. Rhinesmith, W. A. Schroeder and N. Martin, *ibid.*, **80**, 3358 (1958).

(3) V. M. Ingram, *Nature*, **178**, 792 (1956); **180**, 326 (1957).

Both hemoglobins may be dissociated into two equal or nearly equal molecules by change in pH.[4,5,6,7] When a mixture of HbA and S is first taken to a dissociating pH and then returned to a non-dissociating pH, the lack of new species, either electrophoretically[7] or chromatographically,[8] suggests two possibilities: that heterologous recombination does not occur or that dissociation is asymmetric[7] $\alpha_2\beta_2 \rightleftarrows \alpha_2 + \beta_2$. According to Itano dissociation in acid solution is asymmetric and the expected hybrids do form.[9] When labelled and unlabelled hemoglobins are hybridized,[8] the hybrids contain both labelled and unlabelled chains and any peptide may be assigned to the proper chain. In the experiments to be described, the N-terminal peptides have been used to define the aberrant chain in HbS.

HbA* (7,000 c.p.m./mg.) and HbS* (2,600 c.p.m./mg.) were prepared by incubating the appropriate reticulocyte-rich bloods with uniformly C^{14}-labelled L-leucine.[8,10]

As required, both radioactive or non-radioactive hemoglobins were purified chromatographically[11] to prevent interference from minor hemoglobin components.

In Experiment I, oxyhemoglobin A* diluted 1:1 with HbA was mixed with an equal amount of HbS. The solution was dialyzed at 3° against 0.1 M sodium acetate buffer at pH 5.0 for 24 hr. and further dialyzed for 24 hr. prior to chromatography with Developer No. 12 at pH 7.22. Chromatographic separation of 50 mg. of the mixture then was carried out with Developer No. 1. The main portion of the zone of HbS (now radioactive, 850 c.p.m./mg.) was taken, combined with 150 mg. of HbS as a carrier, and dinitrophenylated. In Experiment II, HbS* was hybridized with HbA at pH 11.0 in 0.05 M sodium phosphate, 0.15 M NaCl at 3° for 24 hours. The N-terminal peptides were isolated chromatographically, estimated spectrophotometrically,[1,2] and assayed for radioactivity with these results.

	Hybrid HbS* from HbS and HbA*		Hybrid HbA* from HbS* and HbA	
	c.p.m./ μmole	μmoles	c.p.m./ μmole	μmoles
DNP-val-leu	47.5[a]	2.21	32.2	2.33
di-DNP-val-his-leu	6.2	0.52	3.4	1.08
di-DNP-val-his	7.2	1.09	1.8	0.56
Dinitroaniline	0.6	2.79	0.2	5.36

[a] Contents of planchette rechromatographed and reassayed: DNP val-leu, 47 c.p.m./μmole.

Because the N-terminal dipeptide DNP-val-leu is radioactive and the N-tripeptide di-DNP-val-his-leu is substantially inactive, the α chains must have exchanged and are the chains common to both hemoglobins. The β chains differ and are aberrant in HbS.

Thus, sickle cell anemia is associated with a mutation of the gene which controls the synthesis of the β chains of hemoglobin.

We wish to thank Miss Joan Balog for isolating the N-terminal peptides. This investigation was supported in part by a grant (H-3394) from the National Institutes of Health, United States Public Health Service.

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RECEIVED JANUARY 9, 1959

(4) E. O. Field and J. R. P. O'Brien, *Biochem. J.*, **60**, 656 (1955).

(5) U. Hasserodt and J. Vinograd, paper presented at the meeting of the American Chemical Society, New York, September 8–13, 1957. Hutchinson, M.S. Thesis, California Institute of Technology, Pasadena, 1957

(6) U. Hasserodt and J. Vinograd, *Proc. Nat. Acad. Sci.*, **45**, 12 (1959).

(7) S. J. Singer and H. A. Itano, *ibid.*, **44**, 522 (1958).

(8) W. D. Hutchinson and J. Vinograd, *Nature*, to be submitted.

(9) H. A. Itano, paper presented at the meeting of the American Chemical Society, Chicago, Ill., September 7–12, 1958.

(10) We wish to thank Dr. P. A. Sturgeon, Children's Hospital, Los Angeles, for supplying us with the blood samples and the hematological data. The blood for experiment I was obtained from an individual with an acquired hemolytic anemia. We wish to thank Professor H. M. Dintzis for aid in the preparation of the labelled hemoglobins.

(11) D. W. Allen, W. A. Schroeder and J. Balog, THIS JOURNAL, **80**, 1628 (1958).

BOOK REVIEWS

Advances in Enzymology and Related Subjects of Biochemistry. Volume XX. Edited by F. F. NORD, Fordham University, New York, N. Y. Interscience Publishers, Inc., 250 Fifth Ave., New York, N. Y. 1958. vii + 488 pp. 15.5 × 23.5 cm. Price, $12.50.

This publication is a continuation of an annual series of volumes started in 1941. A wide variety of topics is included, ranging from a possible relation between optical activity and aging to antibiotics and plant diseases. Much of the material is concerned with topics where modern chemical theory and methods can be applied to problems of enzyme chemistry.

The first chapter by W. Kuhn is concerned with the proposition that changes in optical activity of metabolites may be related to aging. The analysis seems to be sound but the basic assumptions appear most improbable to the reviewer. For instance it is assumed that although an enzyme brings about predominantly the synthesis of a given enantiomorph, there is always *some* synthesis of the mirror image. Another improbable assumption is that the enantiomorph not produced normally in appreciable quantity must somehow damage the organism. Few facts are given that might support the theory of change in optical activity and many of the references are out of date. In the opinion of the reviewer the thesis advanced in this chapter is most unconvincing.

The second chapter by H. Theorell is concerned with the mechanism of action of alcohol dehydrogenase, a DPN-requiring enzyme. The chapter summarizes a modern physicochemical approach to the problem of mechanism of action of this enzyme. One mechanism proposed previously by the author is critically examined and found to be inadequate and, although advances are reported, the problem is considered as still remaining not completely solved. A valuable discussion of the use of fluorescence in determining the dissociation constant of the complex between DPN and apo-enzyme is included.