two pieces of information in addition to the geometrical array in the triangular faces. First, adjoining planes are frequently visible in which the molecular arrangement is like that of an orthographic projection of (200) faces. Secondly, where molecules are lying on the triangular surfaces or where steps occur, the shadow lengths indicate that the molecules are approximately as thick as their diameter in the triangular faces. Apparently the molecules are approximately spherical, about 80 Å. diameter in the dry crystal.

With an assumed density of 1.30 for the crystal,[1] the calculated molecular weight is 290,000 agreeing well with values deduced by other methods.[1] Although the unit cell of edestin has not been measured by X-ray methods, the structure of a similar globulin from tobacco seed has been reported as face-centered cubic with $a = 123$ Å. in the dry crystal.[2] Although this value is a little larger than that obtained for edestin, the agreement appears satisfactory insofar as there may be actual differences between the two proteins or differences in hydration as examined.

(1) See E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold Publishing Corp., New York, N. Y., 1943.
(2) D. Crowfoot and I. Fankuchen, *Nature*, **141**, 522 (1938).

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RECEIVED JUNE 16, 1949

CRYSTALLIZATION OF VITAMIN B_{12b}

Sir:

The existence of more than one pink clinically-active pigment in liver extract was noted by Smith.[1] In the present study, a crystalline fraction was separated by chromatography having absorption spectrum maxima different from those at 278, 361 and 550 mμ which have been reported for vitamin B_{12}.[2,3] The biological activity of an impure preparation of this new fraction, obtained from liver extract, for chicks and in the microbiological assay was described elsewhere.[4] A similar fraction was obtained from cultures of *Streptomyces aureofaciens*[5] by adsorption with charcoal followed by elution and chromatography upon silicic acid columns.[6] Two characteristic pink bands were thus separated and were eluted. Fractional precipitation of the first of these with acetone yielded small rod-like red crystals which contained cobalt and phosphorus and which showed absorption spectrum maxima at 273, 351 and 525 mμ. The secondary "peaks" at 307 and 325 mμ, which are shown by vitamin B_{12}, were absent. The crystals were biologically active in the chick assay[4] and in the assay with *L. leichmannii* 313.[7] Since the term "vitamin B_{12a}" has recently been applied to a biologically active compound related to vitamin B_{12},[8] the term "vitamin B_{12b}" is suggested for the preparation described in the present investigation.

The second pink fraction had an absorption spectrum which was characteristic of vitamin B_{12}.[2,3] It was concentrated to yield needle-like crystals which appeared similar to those of vitamin B_{12}.

(1) Smith, *Nature*, **161**, 638 (1948).
(2) Ellis, Petrow and Snook, *J. Pharm. and Pharmacol.*, **1**, 60 (1949).
(3) Brink, Wolf, Kaczka, Rickes, Koniuszy, Wood and Folkers, THIS JOURNAL, **71**, 1854 (1949).
(4) Stokstad, Jukes, Pierce, Page and Franklin, *J. Biol. Chem.*, in the press (Sept. 1949).
(5) Duggar, *Annals N. Y. Acad. Sci.*, **51**, 175 (1948).
(6) Smith and Parker, *Biochem. J.*, **43**, viii (1948).
(7) Hoffman, Stokstad, Franklin and Jukes, *J. Biol. Chem.*, **176**, 1465 (1948).
(8) Kaczka, Wolf and Folkers, THIS JOURNAL, **71**, 1514 (1949).

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RECEIVED JULY 22, 1949

PROTEIN SYNTHESIS BY CHYMOTRYPSIN

Sir:

Bergmann and Fruton[1] showed that chymotrypsin can synthesize anilides. It has now been found that an insoluble protein forms when very small quantities of chymotrypsin are added to Witte peptone. The clear peptone solution gradually changes into a solid gel. The reaction appears to have an optimum *p*H very close to 7.00. Crude lima bean trypsin inhibitor and crystalline lima bean trypsin inhibitor cause temporary inhibition.[2] The synthetic protein is soluble in hot water. It is precipitated by trichloroacetic acid and by a saturated ammonium sulfate solution which has been adjusted to *p*H 7.00. It gives the usual protein color reactions and a pink biuret test.

TABLE I

Sample	Chymotrypsin	After 16 hours	After 40 hours	Insoluble protein N, mg.
1	50 gamma	Viscous solution	Solid gel	3.60
2	1 mg.	Solid gel	Heavy precipitate	9.84
3	None	Clear solution	Clear solution	None

Each sample contained 0.66 g. of Witte peptone in 2 cc. of distilled water. The chymotrypsin was dissolved in 0.25 cc. of distilled water. Sample 3 contained 0.25 cc. of distilled water instead of the enzyme solution. The *p*H was 7.00. The temperature was 37°. A generous quantity of salt-free crystalline chymotrypsin was very kindly furnished by Dr. M. Kunitz of the Rockefeller Institute for Medical Research. Identical results were obtained with a commercial preparation of crystalline chymotrypsin Worthington Biochemical Laboratory—4 × crystallized, ap. 50% $MgSO_4$). Three different Witte peptone preparations gave identical results. A few commercial peptones (not Witte peptone) were unsuitable for the protein synthesis.

(1) N. Bergmann and J. S. Fruton, *Ann. N. Y. Acad. Sci.*, **45**, 409 (1944).
(2) H. Tauber, B. B. Kershaw and R. D. Wright, *J. Biol. Chem.*, in press.

One cubic centimeter of hot 0.3 *M* trichloroacetic acid was added to each sample and the insoluble protein which formed was collected by centrifuging. It was washed three times with 2.5 cc. of 0.1 *M* trichloroacetic acid. Fifty gamma of chymotrypsin produced an insoluble protein containing 3.6 mg. of nitrogen per 0.66 g. of Witte peptone in forty hours. One milligram of chymotrypsin produced insoluble protein containing 9.84 mg. of nitrogen under similar conditions.

The protein synthesizing property of chymotrypsin is of biological importance because under optimum conditions this enzyme displays only weak proteolytic action.

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THE STRUCTURE OF THE BICYCLO[2,2,1]2-HEPTYL (NORBORNYL) CARBONIUM ION

Sir:

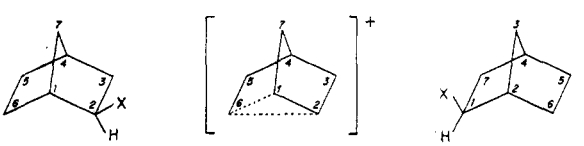
From a generalized viewpoint many rearrangements involve participation of electrons associated with a neighboring β-H,R, or Ar group in a unimolecular-type nucleophilic replacement process. Thus, in the Wagner–Meerwein rearrangement, ionization produces directly, or in a later stage, a rearranged ion or an ion with a bridged structure. The latter type formulation for a carbonium ion has been mentioned a number of times as a possibility or definitely proposed.[1] The same kinetic and stereochemical methods employed in the study of functional neighboring groups[2] are useful in this connection.

In the norbornyl system relative rates of acetolysis at 45° of *p*-toluenesulfonates or *p*-bromobenzenesulfonates are: *exo*-norbornyl (I, III, X = $OSO_2C_6H_4Br$), 350 > *endo*-norbornyl, 1 ≅ cyclohexyl. The driving force[2b] in the stereochemically favorable *exo*-isomer is a substantial fraction of that displayed by isobornyl chloride.

Exo-norbornyl *p*-bromobenzenesulfonate (I, III, X = $OSO_2C_6H_4Br$), m. p. 55.3–57.0°, prepared from *exo*-norborneol (I, III, X = OH), m p. 127.6–128.5° (reported[3] 128–129°) on acetolysis yields *exo*-norbornyl acetate (I, III, X = $OCOCH_3$) (identified as the *exo*-norbornyl 3,5-dinitrobenzoate, m. p. 103.7–105.0°, reported[3] 105°) with no evidence of any *endo*-norbornyl acetate or norbornylene in the product. Also, *endo*-norbornyl *p*-toluenesulfonate, m. p. 28.1–29 2° yields only the *exo*-norbornyl acetate and *exo*-norbornyl alcohol in acetolysis and hydrolysis (aqueous acetone or dioxane), respectively.

The total resolution of the *exo*-norborneol is still in progress but sufficient resolution has been effected for the type of stereochemical test previously carried out in the case of bromonium ions[2a] and similar species. *Exo*-norborneol, $[\alpha]^{22}D - 1.09°$ (chloroform, $c = 10.1$), prepared from acid phthalate, $[\alpha]^{23}D + 3.33°$ (chloroform, $c = 10.0$), and which gives an acetate, $[\alpha]^{23}D + 4.47°$ (acetic acid, $c = 5.36$), was converted to *p*-bromobenzenesulfonate, $[\alpha]^{25}D \cong + 1.29°$ (initially, in glacial acetic acid, $c = 20.08$). This *p*-bromobenzenesulfonate acetolyzes (with or without dissolved potassium acetate) to give completely inactive product, the activity of the solution disappearing at roughly the solvolysis rate. Examination of the concentrated product showed more precisely the completeness of the racemization under conditions where the fully survived activity would have been 100–200 times the experimental error.

The facts are at present best accommodated by the formulation of the intermediate ion from *exo*-norbornyl derivatives as II



I II III

which has a plane of symmetry through atoms 4, 5 and 6 and is therefore internally compensated. Attack at C-2 yields the original configuration, I; attack at C-1 yields the enantiomorph, III.

The solvolysis of the *endo*-norbornyl *p*-bromobenzenesulfonate is being subjected to the same kind of stereochemical scrutiny.

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RECEIVED JUNE 20, 1949

(1) *E. g.* (a) Winstein and Lucas, THIS JOURNAL, **60**, 836 (1938); (b) Nevell, de Salas and Wilson, *J. Chem. Soc.*, 1188 (1939); (c) Watson, "Annual Reports," 197 (1939); 120 (1941); (d) Eyring, *Ind. Eng. Chem.*, **35**, 511 (1943); (e) Dewar, *J. Chem. Soc.*, 406 (1946); (f) Walsh, *ibid.*, 89 (1947); (g) Arcus, *Chemistry and Industry*, 442 (1947).

(2) *E. g.* (a) Winstein and Lucas, THIS JOURNAL, **61**, 2845 (1939); (b) Winstein and Grunwald, *ibid.*, **70**, 828 (1948).

(3) Alder and Rickert, *Ann.*, **543**, 1 (1940).

(4) Postdoctoral Research Associate, 1948–1949.