4-Dimethylamino-2,2-diphenyl-3-methylbutanal Hydrochloride.—The reduction of 4-dimethylamino-2,2-diphenyl-3-methylbutanenitrile, 27.8 g. (0.1 mole), with lithium aluminum hydride in the above manner gave 7.5 g. of aminoaldehyde hydrochloride which was recrystallized from acetone–methanol, m.p. 187.8–192°.

Anal. Calcd. for: $C_{19}H_{23}NO\cdot HCl$: C, 71.81; H, 7.61; N, 4.40. Found: C, 71.75; H, 7.34; N, 4.47.

Catalytic reduction as indicated above gave 4-dimethylamino-2,2-diphenyl-3-methylbutanol hydrochloride, m.p. 200–201°.

Anal. Calcd. for $C_{19}H_{25}NO\cdot HCl$: C, 71.34; H, 8.19; Cl, 11.08. Found: C, 71.58; H, 8.39; Cl, 11.00.

Acknowledgment.—The authors are indebted to Messrs. M. E. Auerbach and K. D. Fleischer and staff for the analyses reported here.

STERLING–WINTHROP RESEARCH INSTITUTE
RENSSELAER, N. Y. RECEIVED JANUARY 4, 1951

COMMUNICATIONS TO THE EDITOR

PYRIDOXAL PHOSPHATE, THE COENZYME OF THIOETHER-CLEAVAGE

Sir:

In a previous report[1] the activation of certain preparations of the enzyme responsible for the cleavage of thioethers (*e.g.*, cystathionine with the formation of cysteine) by relatively large amounts of folic acid was described. Since that time, it has been found that derivatives of folic acid (conjugates and citrovorum factor) were without effect. The failure of these derivatives to activate the preparations and the limited results obtained in further studies with folic acid led us to consider other possibilities as to the identity of the dialyzable component. It has been found that minute amounts of pyridoxal phosphate[2] activated all preparations of the enzyme—fresh, aged or dialyzed. Maximal activation was obtained with 0.5 γ of pyridoxal phosphate per ml. of digest. Djenkolic acid,[3] 10 mg., and 1 ml. enzyme[4] in a total volume of 10 ml. 0.1 *M* sodium citrate were incubated for 30 minutes at 37° with amounts of pyridoxal phosphate varying from 0.1 to 10 γ per ml. With the fresh enzyme, maximal activity, 1.1 mg. of cysteine was obtained with 0.5 γ of pyridoxal phosphate; the control was 0.5 mg. of cysteine. After dialysis overnight against acetate buffer, 0.1 *M*, *p*H 5.5, the activity was reduced to 0.2 mg. of cysteine and was restored to 1.0 mg. of cysteine upon the addition of 0.5 γ of pyridoxal phosphate per ml. of digest. These amounts of pyridoxal phosphate are of the same order of magnitude as required for the transamination and decarboxylation enzymes and are compatible with the amounts predicted from the absorption spectrum of the enzyme.[1] It would appear, therefore, that pyridoxal phosphate is the coenzyme of the cleavage-enzyme.

When 10 mg. of pyridoxin and 50 mg. of adenosinetriphosphate were incubated in 10 ml. of saline with 1 ml. of homogenate of liver tissue (1 g. in 10 ml.) for 15 minutes, an apparent content of 5.5 γ of pyridoxal phosphate per ml. (activation of dialyzed enzyme) was found. The addition of folic acid was found to increase markedly the amount of coenzyme formed. It would appear probable, therefore, that the effects of folic acid and of adenosinetriphosphate[5] on the cleavage will be found to be concerned with the synthesis of pyridoxal phosphate or a closely related compound. It is of interest that the ultraviolet absorption of the purified enzyme[1] may be interpreted as that of protein and pyridoxal phosphate.[6]

These and related studies will be reported in detail in the near future.

(1) F. Binkley, THIS JOURNAL, **72**, 2809 (1950).
(2) Obtained from Dr. W. W. Umbreit.
(3) M. D. Armstrong and V. du Vigneaud, *J. Biol. Chem.*, **168**, 373 (1947). Djenkolic acid is an easily prepared substrate.
(4) F. Binkley and D. Okeson, *J. Biol. Chem.*, **182**, 273 (1950).
(5) F. Binkley, *J. Biol. Chem.*, **155**, 39 (1944).
(6) W. W. Umbreit, D. J. O'Kane and I. C. Gunsalus, *ibid.*, **176**, 629 (1948).

DEPARTMENTS OF PATHOLOGY AND BIOCHEMISTRY
UNIVERSITY OF UTAH COLLEGE OF MEDICINE
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FRANCIS BINKLEY
GERALD M. CHRISTENSEN

RECEIVED APRIL 30, 1951

"CITROVORUM FACTOR" ACTIVITY OF TETRAHYDROPTEROYLGLUTAMIC ACID

Sir:

The preparation of leucovorin (I),[1] 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid,[2,3] led to speculation as to its possible role in the transfer of "single-carbon fragments," following the suggestion which has been made for folic acid in such biological mechanisms.[4] It seemed feasible that I might be reversibly transformed to tetrahydropteroylglutamic acid (II) *in vivo* during such a process in which case II should have biological properties similar to those of I. II was synthesized by hydrogenation of 14.6 mg. of pteroylglutamic acid in 10 cc. of glacial acetic acid at room temperature, using 15 mg. of platinum oxide catalyst and a standard Ogg–Cooper micro-hydrogenation apparatus.[5] After 5.75 hours, reduction was complete; hydrogen uptake was 92.5% of the theoretical 2 moles. Subsequent operations were carried out under nitrogen to prevent oxidation. The catalyst was separated from the colorless solution of II by centrifugation, then aliquots were transferred to small test-tubes and vacuum-dried to a

(1) J. A. Brockman, Jr., *et al.*, THIS JOURNAL, **72**, 4325 (1950).
(2) E. H. Flynn, *et al.*, Abstracts of Papers, Am. Chem. Soc., 119th meeting, 18M (1951).
(3) B. Roth, *et al.*, in preparation.
(4) M. Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).
(5) B. L. O'Dell, *et al.*, *ibid.*, **69**, 250 (1947).

white solid at room temperature over potassium hydroxide for 16 hours. For the animal experiments oxygen-free sterile water was added to each tube, and the solution was injected immediately. II was assayed with *Leuconostoc citrovorum* 8081[1] by aseptic addition to the culture medium and had an activity corresponding to 4 to 8 mγ per unit, which is about 2.5% of that of leucovorin and 5000 times that of pteroylglutamic acid. The effect of II in reversing the toxic effects of 4-aminopteroylglutamic acid (III) was quite marked. Injections into mice were made three times weekly[6] using 10 or 12 mice per group. With 10γ of III, average survival time was 4.9 days; with 10γ of III and 10γ or 20γ of I, all mice survived the 8-day assay period with respective weight gains of 0.3 g. and 3.5 g.; with 10γ of III and 30γ or 100γ of II all mice survived with respective weight gains of 1.3 g. and 3.3 g.; with 10γ of III and 30γ or 100γ of 10-formylpteroylglutamic acid the average survival times were respectively 4.5 days and 5.7 days. The results indicated that II had about one-third of the activity of I in reversing III and were confirmed by a second experiment. The inactivity of 10-formylpteroylglutamic acid is in contrast to the activity of II. The biological activity of II needs consideration in evaluating the effect of ascorbic acid in increasing the production of "citrovorum factor" from pteroylglutamic acid by liver slices of rats.[7]

The present observations enable some speculation to be made on the mechanism of the action of III. The formation of an imidazolinium ring at *p*H 2 by condensation of the 5-CHO group with the 10-position was postulated for I.[8] If, however, III formed an analog of I by reduction and formylation *in vivo*, an imidazole ring might form by condensation of the 5-CHO group with the 4-NH_2 group which distinguishes III from pteroylglutamic acid, giving rise to a compound which in contrast to I would be unable to reversibly transfer the "single-carbon fragment" represented by the 5-CHO group.

(6) A. L. Franklin, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **67**, 398 (1948).

(7) C. A. Nichol and A. D. Welch, *Proc. Soc. Exp. Biol. & Med.*, **74**, 52 (1950).

(8) M. May, *et al.*, Abstracts of Papers, Am. Chem. Soc., 119th meeting, 5C, 1951.

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PEARL RIVER, NEW YORK E. L. R. STOKSTAD
T. H. JUKES

RECEIVED MAY 31, 1951

THE TRANSGLUCOSIDASE OF *ASPERGILLUS ORYZAE*[1]

Sir:

In this communication we are reporting preliminary studies on a carbohydrate-synthesizing enzyme present in the filtrate of the mold *Aspergillus oryzae*.[2] Evidence is presented which shows that this enzyme is a transglucosidase,[3] *i.e.*, an enzyme capable of transferring glucose residues.

The enzymic digests were prepared by mixing appropriate amounts of the carbohydrate substrates with the enzyme, allowing the reaction to proceed at room temperature, and removing aliquots of the reaction mixture at varying time intervals. Next, the enzyme activity in these aliquots was destroyed by heat and finally the qualitative composition of the digest aliquots was ascertained by paper chromatography procedures.[4]

From pure maltose, the transglucosidase synthesizes the disaccharide isomaltose,[5] the trisaccharides 6-(α-D-glucosyl) maltose[6] and 6-(α-D-glucosyl) isomaltose[6] and a tetrasaccharide of unknown constitution. The mechanism postulated for the synthesis of these carbohydrates is termed transglucosidation and involves a transfer of the terminal glucose residue of maltose to the 6-position of a co-substrate saccharide. Phosphorylation is apparently not involved since the enzyme is without action on glucose and glucose 1-phosphate substrates.

Evidence for a transglucosidation mechanism was obtained from experiments[7] with C^{14} labelled glucose.[8] In the tracer study the enzyme was allowed to act on maltose in the presence of a small amount of labelled glucose. Examination of the digest for reducing sugars by paper chromatography showed that the distribution of synthesized compounds was essentially identical with that obtained for pure maltose. A radiogram[8] of the products showed the isomaltose and the 6-(α-D-glucosyl) isomaltose to be radioactive. Evidently the glucosyl units of maltose are transferred to radio-glucose to yield radio-isomaltose. The radio-isomaltose, in turn, functions as a glucosyl acceptor molecule in the synthesis of radio-6-(α-D-glucosyl) isomaltose. The non-radioactive reducing saccharides in the digest result from enzyme action on non-radioactive substrates.

(3) M. Doudoroff, H. A. Barker and W. Z. Hassid, *J. Biol. Chem.*, **168**, 725 (1947).

(4) D. French, D. W. Knapp and J. H. Pazur, THIS JOURNAL, **72**, 5150 (1950).

(5) E. M. Montgomery, F. B. Weakley and G. E. Hilbert, *ibid.*, **71**, 1862 (1949).

(6) D. French, *Science*, **113**, 352 (1951).

(7) Carried out in coöperation with Dr. S. Aronoff and his associates, Botany Div. of the Institute for Atomic Research, Ames, Iowa.

(8) S. Aronoff and L. Vernon, *Arch. Biochem.*, **28**, 424 (1950).

DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE JOHN H. PAZUR
AMES, IOWA DEXTER FRENCH

RECEIVED MAY 17, 1951

SUBSTITUTED CYCLOÖCTATETRAENES FROM SUBSTITUTED ACETYLENES[1]

Sir:

We have found that copolymerization of mono- and disubstituted acetylenes with acetylene[2] leads to the formation of mono- and 1,2-disubstituted

(1) Journal Paper No. J-1949 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1116. Supported in part by a grant from the Corn Industries Foundation.

(2) Supplied by Dr. L. A. Underkofler, Chemistry Department Iowa State College, Ames, Iowa.

(1) Supported in part by the Office of Naval Research under Contract N5ori-07822, Project Designation NR-055-96. Presented at the Twelfth National Organic Chemistry Symposium, Denver, Colorado, June 14, 1951.

(2) Under conditions used for the polymerization of acetylene to cycloöctatetraene: (a) W. Reppe, O. Schlichting, K. Klager and T. Toepel, *Ann.*, **560**, 1 (1948); (b) A. C. Cope and L. L. Estes, Jr., THIS JOURNAL, **72**, 1128 (1950).

cycloöctatetraenes. Such copolymerizations apparently have not been described. It has been reported that phenylacetylene fails to polymerize, and that vinylacetylene yields a gel-like polymer,[3] although ref. 2a (p. 37) states that homologs of acetylene would lead to corresponding substituted cycloöctatetraenes.

In the copolymerizations, the substituted acetylene (20–50 g.) was included with the tetrahydrofuran solvent, nickel acetylacetonate and calcium carbide in a 1-l. stirred autoclave, which was pressured to 250–300 p.s.i. with acetylene and stirred and heated at 70–90° with periodic repressuring in the manner previously described[2b] for a reaction period of 7 to 12 hours. The product was steam distilled, and the substituted cycloöctatetraene was isolated from the steam distillate, or from the residue. The steam distillates contained benzene, cycloöctatetraene, an alkyl benzene (from copolymerization of the substituted acetylene with acetylene in a 1:2 ratio) and the substituted cyclooctatetraene (if volatile with steam). The less volatile substituted cycloöctatetraenes were isolated from the water-insoluble residue (largely cuprene) from the steam distillation by extraction with benzene in a Soxhlet apparatus. The substituted cycloöctatetraenes were isolated by fractional distillation, or through silver nitrate adducts, in yields of 16–25%. Phenylcycloöctatetraene and *n*-butylcycloöctatetraene were identified by direct comparison with authentic samples.[4] Methylcycloöctatetraene was isolated as a yellow liquid, b. p. 84.5° (67 mm.), $n^{25}D$ 1.5249, d^{25}_4 0.8978. (*Anal.* Calcd. for C_9H_{10}: C, 91.47; H, 8.53. Found: C, 91.17; H, 8.35). Quantitative reduction of methylcycloöctatetraene in the presence of platinum in acetic acid required 97% of four molar equivalents of hydrogen and yielded methylcycloöctane.

1,2-Dimethylcycloöctatetraene was isolated as a yellow liquid, b.p. 107° (96 mm.), $n^{25}D$ 1.5218 (*Anal.* Calcd. for $C_{10}H_{12}$: C, 90.85; H, 9.15. Found: C, 91.01; H, 9.21). Hydrogenation in the presence of 1% palladium on calcium carbonate in methanol resulted in the absorption of three molar equivalents of hydrogen and the formation of 1,2-dimethylcycloöctene, which was characterized by ozonization. Hydrogenation of the ozonide yielded 39% of decane-2,9-dione, which after recrystallization melted at 56–57°, and formed a dioxime (m.p. 131.5–132.5°). Both the diketone and dioxime were identical with authentic samples in melting point and mixed melting point.

Investigation of the preparation of substituted cycloöctatetraenes by the copolymerization of acetylene with substituted acetylenes, including derivatives containing various functional groups is being continued.

DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASS.

ARTHUR C. COPE
HUGH C. CAMPBELL

RECEIVED JUNE 12, 1951

(3) K. Kammermeyer, "Polymerization of Acetylene to Cycloöctatetraene," Hobart Publishing Co., Washington, D. C., 1947, p. 2 (based upon work of the group headed by Reppe).

(4) A. C. Cope and M. R. Kinter, THIS JOURNAL, **72**, 630 (1950); **73**, 3424 (1951); A. C. Cope and H. O. Van Orden, to be published.

ION-PAIR FORMATION IN ION EXCHANGE SYSTEMS

Sir:

When a movable, exchange ion forms an associated ion-pair with a fixed exchange group in an ion exchange resin, the formulation of the thermodynamic equilibrium constant must consider the unique conditions which exist in these systems. For example, consider the process, $A^- + R^+ = RA$, where A^- is a movable anion, R^+ the fixed exchange group, and RA the ion-pair. Since R^+ and RA are both fixed to the resin matrix, and are at finite distances of separation (7–10 Å.), they do not possess translational degrees of freedom and should be regarded as separate, solid phases. The exchanger system has four phases, the external solution (o), the internal solution phase (i), and the two solid phases R^+ and RA. Under these conditions, the dissociation constant $K_m = (m_{A^-})(\gamma_\pm)$, where m_{A^-} is the molality of the A^- ion in the solution phase,[1] and $\gamma_\pm$ the mean activity coefficient of R^+A^-; m_{R^+} and m_{RA} are set equal to unity.

Dissociation constants for this process have been written in the conventional manner as for solutions, $K'_m = (m_{A^-})(m_{R^+})/m_{RA}$.[2] A critical test of these equations is a comparison of calculated and experimental values for the variation in the selectivity coefficient, K_D, as a function of the fraction of the exchange capacity (X^i) taken up by an exchanging ion. Where ion-pair formation exists, the selectivity (which favors the ion-pair forming ion) should decrease as X^i_A increases according to K_m, but should increase according to K'_m.

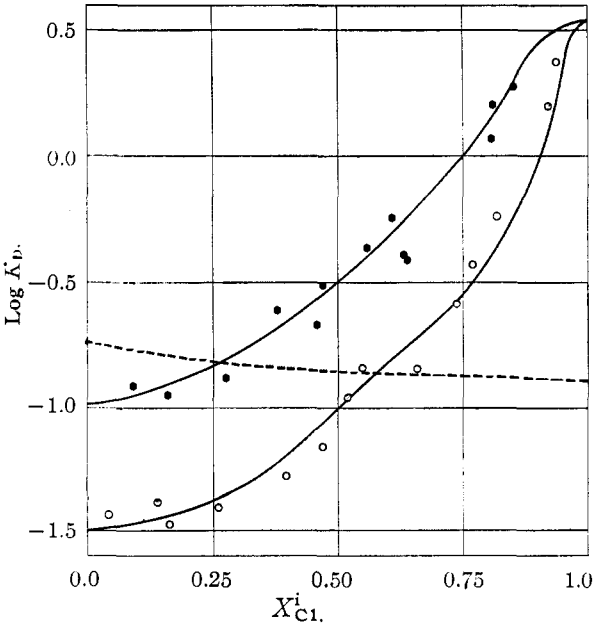

Fig. 1.—Variation in logarithm of K_D with fraction of exchange capacity in chloride state: experimental points for perchlorate–chloride exchange, O; for trichloroacetate–chloride exchange, ●; calculated curves by new theory (K_m) are thus ——; by old theory (K'_m) is – – –.

(1) H. P. Gregor, F. Gutoff and J. I. Bregman, *J. Colloid Sci.*, in press.

(2) D. K. Hale and D. Reichenberg, *Faraday Soc. Discussion*, **7**, 79 (1949).

A critical, experimental test of these equations has been made, by measuring $K_D = (Cl/A)_i \cdot (A^-/Cl^-)_o$ as a function of X^i_{Cl} for perchlorate-chloride and trichloroacetate-chloride exchange using Dowex-2, a quaternary base anion exchanger. (The quaternary ammonium perchlorates are somewhat insoluble; the trichloroacetate forms ion-pairs to a marked extent, while the chloride appears to form ion-pairs to a smaller extent). The curves shown in Fig. 1 were calculated assuming $K_m = 0.12$ for perchlorate, $K_m = 0.4$ for trichloroacetate, $K_m = 1.2$ for chloride. According to the theory the value of K_D at $X^i_A \longrightarrow 1$, is the same as K_m for that ion. Values taken for K'_m are 0.04 for perchlorate and trichloroacetate, and 0.4 for chloride. The resin phase contains about 250 g. of water per mole of exchange capacity. Pressure–volume effects were neglected in these calculations, since they are small for these systems.

The experimental points fit the calculated curve based upon K_m well; the K'_m curves obviously do not apply. For loosely cross-linked gels where translational degrees of freedom may exist, a trend from K_m to K'_m may be anticipated. A series of papers dealing with ion-pair phenomena is in preparation.

DEPARTMENT OF CHEMISTRY
POLYTECHNIC INSTITUTE OF BROOKLYN HARRY P. GREGOR
BROOKLYN 2, NEW YORK

RECEIVED MAY 14, 1951

ON THE STRUCTURE OF STABLE PENTABORANE

Sir:

We have found several arrangements of the boron atoms which appear to be capable, with appropriate disposition of the hydrogen atoms, of giving complete agreement with the electron diffraction pattern of gaseous B_5H_9, whereas neither the model reported by Bauer and Pauling[1] nor the planar five-membered ring proposed by Pitzer[2] is satisfactory. Of the satisfactory arrangements, however, only the tetragonal pyramid, originally proposed and later discarded in the original electron diffraction study[1] but recently further advocated by Pauling,[3] has the high symmetry suggested by the spectrum[4] and entropy.[5] For this arrangement, which we at first thought unsatisfactory,[6] the position and shape of the radial distribution peak at about 2.57 Å. (Fig. 1), as well as intensive analysis of the pattern by trial and error, eventually led us to the structure shown in Fig. 2, which is of the type that had been

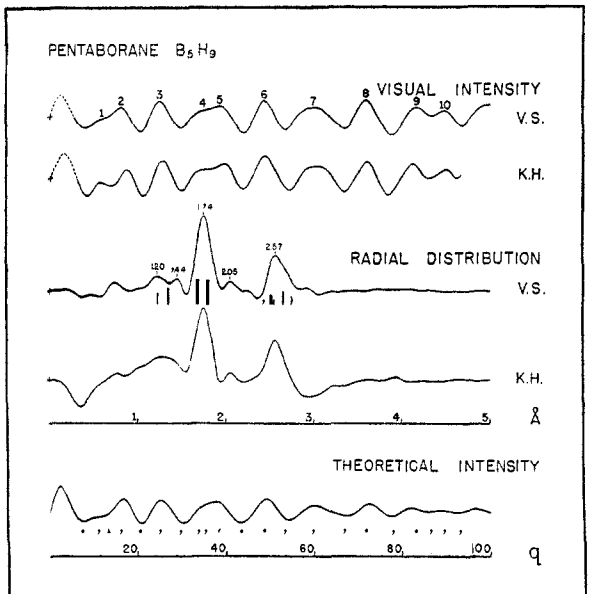

Fig. 1.—Visual, radial distribution and theoretical intensity curves. Theoretical intensity curve for the following model $B\text{-}H_{av}/B\text{-}B_{av} = 1.275/1.74$, $B_1\text{-}B_2/B_2\text{-}B_2 = 1.69/1.79$, $B_2\text{-}H_1/B_2\text{-}H_3 = 1.20/1.35$, $\angle B_1\text{-}B_2\text{-}H_1 = 125°$, external $\angle$ plane $B_1\text{-}B_2\text{-}B_2$, plane $B_2\text{-}B_2\text{-}H_3 = 190°$.

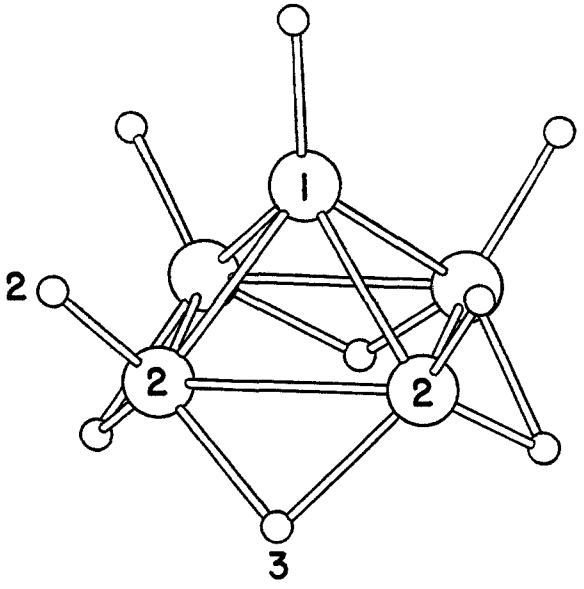

Fig. 2.—B_5H_9, diagram of the structure.

suggested to us by King and Lipscomb[7] on the attractive grounds outlined in the following communication by Dulmage and Lipscomb. The parameter values

$B_1\text{-}B_2 = 1.70$ Å.
$B_2\text{-}B_2 = 1.80$
$B_2\text{-}H_2 = 1.23$
$B_2\text{-}H_3 = 1.36$
$\angle B_1\text{-}B_2\text{-}H_2 = 120°$
external dihedral $\angle B_1B_2B_2\text{-}B_2B_2H_3 = 185°$,

which are surely close to the final values to be obtained from our data, give excellent agreement, qualitative and quantitative (12-feature average deviation for q/q_0, 0.008, with $\overline{q/q_0}$ adjusted to 1.000). Limits of error, not yet completely determined, are large for the two angle values and about ±0.03 Å. and ±0.05 Å. for the B–B and B–H distances, respectively.

(1) S. H. Bauer and L. Pauling, THIS JOURNAL, **58**, 2403 (1936).
(2) K. S. Pitzer, *ibid.*, **67**, 1126 (1945).
(3) Private communication.
(4) Private communications from Professor K. S. Pitzer and from Dr. G. C. Pimentel.
(5) W. J. Taylor, C. W. Beckett, J. Y. Tung, R. B. Holden, and H. L. Johnston, *Phys. Rev.*, **79**, 234 (1950).
(6)(a) V. Schomaker, *J. chim. phys.*, **46**, 252 (1949); (b) K. Hedberg, V. Schomaker, M. E. Jones, *Abstracts*, Chicago Meeting, A. C. S., September 1950. Substantially the present result was given at the meeting, however.
(7) M. V. King and W. N. Lipscomb, private communication.

We are indebted to Professor H. I. Schlesinger and Dr. I. Shapiro for samples of pentaborane and to the Office of Naval Research (Contract N6onr 24423) for support during most of this investigation.

CHEMISTRY DEPARTMENT KENNETH HEDBERG
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RECEIVED JUNE 7, 1951

THE MOLECULAR STRUCTURE OF PENTABORANE

Sir:

If one compares the recently-determined structures of boron carbide[1] and decaborane[2] on the one hand with the structure of calcium boride[3] and possible structures of pentaborane on the other, the most reasonable structure of B_5H_9 (M. V. King and W. N. Lipscomb, unpublished) that can now be predicted is a tetragonal pyramid of molecular symmetry C_{4v}, such as that shown in Fig. 1. Apparently a molecule of this structure was overlooked in the earlier electron diffraction study,[4] because a recent study by Hedberg, Jones and Schomaker[5] shows that the electron diffraction pattern is consistent with such a model, but is also not inconsistent with some quite different, but chemically less reasonable, models.

We have completed an X-ray diffraction study of single crystals of pentaborane. The crystals are pyroelectric, and belong to the tetragonal space group C_{4v}^9–I4mm., with two molecules in a unit cell of dimensions $a = 7.16$ Å. and $c = 5.38$ A. Evaluation of parameters from three-dimensional sections leads to the values:

B_2–B_2 = 1.77 ± 0.02 Å.	$H_3 \ldots H_3$ = 1.95 ± 0.09 A.
B_1–B_2 = 1.66 ± 0.02 Å.	∠ B_1B_2, H_3 = 115 ± 5°
B_1–H_1 = 1.21 ± 0.05 Å.	External dihedral angle between planes $B_1B_2B_2$ and
B_2–H_2 = 1.20 ± 0.07 Å.	$B_2B_2H_3$ = 190 ± 5°
B_2–H_3 = 1.35 ± 0.04 Å.	

As in decaborane the intermolecular approaches are all between hydrogen atoms. These approaches range from 2.46 to 2.96 Å. which appear reasonable in view of the very large temperature factor in the crystal. The diagonal of the base of the pyramid has the direction of the a axis. It may be noted that the average boron-boron distance in the molecule is apparently about two per cent. smaller in the solid state than in the gaseous state, a situation previously observed only in hexamethylenetetramine.[6]

The interatomic binding in pentaborane, which is similar to decaborane, is of the 'metallic' type and in such a case we may expect Pauling's relation[7] $r = r_1 - 0.3 \log n$ to be reasonably valid. If we choose a covalent radius for hydrogen equal to 0.36 Å., then the total bond order for the molecule, which should be twelve, will depend upon the choice of the boron single-bond radius. A value of 0.78 Å. for this boron radius satisfies these conditions, thus leading to a total bond order of 12.03. The individual bond orders are $n_{12} = 0.681$, $n_{22} = 0.447$, $n'_{11} = 0.764$, $n'_{22} = 0.794$ and $n'_{23} = 0.447$, where the prime refers to B–H bonds. These orders suggest that the hydrogen atoms are somewhat positively charged and the boron atoms are somewhat negatively charged. This distribution of charge indicates a dipole moment of about 0.6 Debye. Similar calculations lead to a single-bond radius for boron of 0.79 Å. from the diborane molecule, and of 0.81 Å. from the decaborane molecule.

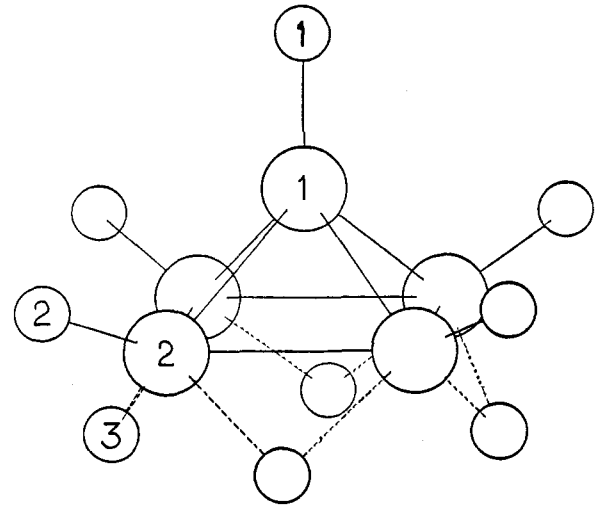

Fig. 1.—The pentaborane molecule: boron and hydrogen atoms are represented by large and small circles, respectively.

We wish to thank Dr. R. R. Miller of the Naval Research Laboratory and Dr. A. E. Newkirk and Dr. L. V. McCarty of the General Electric Company for samples of pentaborane. Support of this research by the Office of Naval Research and by a Fellowship (to W.J.D.) from the Minnesota Mining and Manufacturing Company is gratefully acknowledged.

(1) G. S. Zhdanov and N. G. Sevast'yanov, *Compt. rend. acad. sci. U.S.S.R.*, **32**, 432 (1941); H. K. Clark and J. L. Hoard, THIS JOURNAL, **65**, 2115 (1943).

(2) J. S. Kasper, C. M. Lucht, and D. Harker, *Acta Cryst.* 4, 436 (1950).

(3) L. Pauling and S. Weinbaum, *Z. Krist.*, **87**, 181 (1934).

(4) S. H. Bauer and L. Pauling, THIS JOURNAL, **58**, 2403 (1936).

(5) K. Hedberg, M. Jones, and V. Schomaker, private communication. A preliminary report on this determination was made at the Chicago meeting of the American Chemical Society, September, 1950. We are indebted to these authors for making available to us their results, described in the preceding letter.

(6) V. Schomaker and P. A. Shaffer, Jr., THIS JOURNAL, **69**, 1555 (1947); P. A. Shaffer, Jr., *ibid.*, **69**, 1557 (1947).

(7) L. Pauling, THIS JOURNAL, **69**, 542 (1947). The details of the present method of applying Pauling's relation were suggested to us by K. Hedberg and V. Schomaker. Following their suggestion also we have taken the revised covalent radius for hydrogen (V. Schomaker and D. P. Stevenson, THIS JOURNAL, **63**, 37 (1940)) corrected for the electronegativity difference between boron and hydrogen.

SCHOOL OF CHEMISTRY WILLIAM J. DULMAGE
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RECEIVED JUNE 5, 1951

PHOTOGRAPHY IN ELECTROPHORESIS OF HEMOLYZED SERA

Sir:

The occasional necessity for the examination by electrophoresis of hemolyzed sera, or of solutions of hemoglobin or other proteins having high optical density except for red or infrared light, has presented a technical problem in photography. The customarily used mercury vapor arc lamp has strong green and yellow lines, but its red intensity

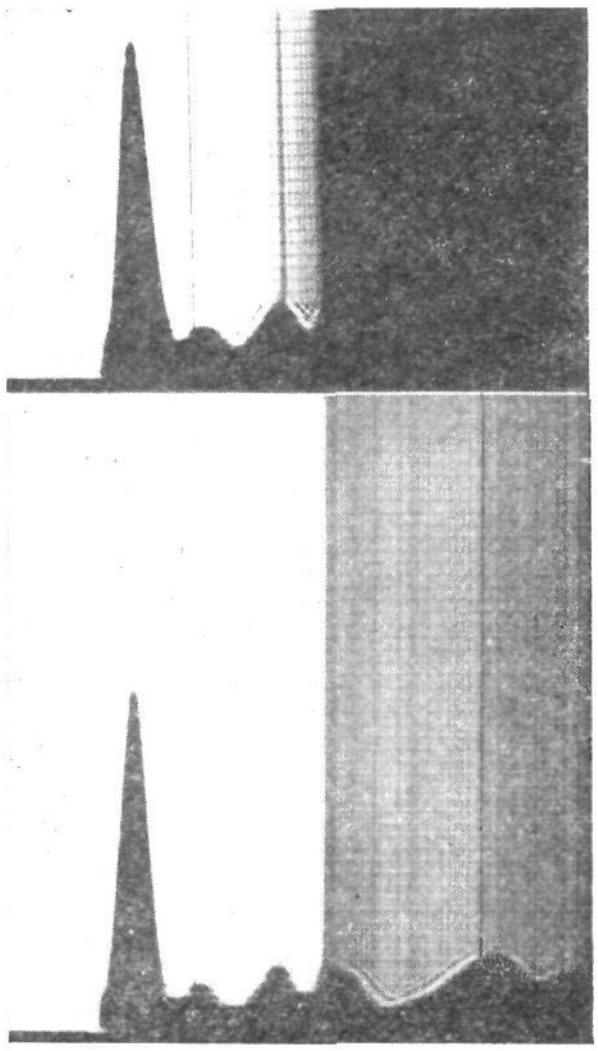

Fig. 1.

is relatively low. For this reason, special infrared sources have been employed,[1] and special adapters have been designed,[2] to permit the interchangeable use of overvoltage tungsten filaments illuminating a slit in the correct focal plane for the schlieren optical system. In an extended study of the sera of a leukemia patient,[3] the critical demand for the analysis of a serum sample which was irreplaceable, but had been hemolyzed when drawn led us to the accidental discovery of a very simple technique for photography, in the absence of a specially designed source of red light. We found that with a Wratten No. 105 (hemoglobin analysis) filter to reduce contrast, Eastman Spectroscopic Type 103F plates are sufficiently sensitive to the mercury red lines to permit satisfactory schlieren scanning photographs[4] in the normal exposure range. Moreover, the lens system used[5] is sufficiently achromatic that satisfactory longitudinal focusing is obtained without shifting the source slit. In the accompanying figure, the upper schlieren scanning photograph of a badly hemolyzed sample was made, after 7200 seconds electrophoresis at 6.29 volts/cm. in 0.1 molar sodium diethylbarbiturate buffer at *p*H 8.60, by use of a Wratten No. 77A (monochromat green) filter and a Kodaline CTC photographic plate. At the position conjugate to the incidence of hemoglobin in the cell, the pattern of the rising boundaries vanishes. The lower schlieren scanning diagram of the same boundaries was obtained with the 105 filter and 103F plate and permits the observation of the globulin and δ-boundaries in the cell. However, since hemoglobin refracts as well as absorbs light its quantitative effect upon the globulin components with which it is associated must not be overlooked in analyzing the pattern. The same light source, filter and photographic plate combination has been used to obtain continuous scanning records[6] of the chromatographic resolution of artificial mixtures of proteins containing hemoglobin as a component,[7,8] and also to obtain cylindrical lens schlieren diagrams in the ultracentrifuge[9] with purified human carbon monoxide hemoglobin solutions up to 2% concentration.

(1) H. P. Treffers and D. H. Moore, *Science*, **93**, 240 (1941).

(2) L. G. Longsworth, *Ind. Eng. Chem., Anal. Ed.*, **18**, 219 (1946).

(3) F. J. Gutter, J. Krevans, G. A. Moulton and G. Kegeles, *J. Nat. Cancer Inst.*, in press.

(4) L. G. Longsworth, THIS JOURNAL, **61**, 529 (1939).

(5) Klett Mfg. Co., New York, N. Y.

(6) G. Kegeles and H. A. Sober, Abstracts of 119th National Meeting, American Chemical Society, April, 1951.

(7) H. A. Sober, G. Kegeles and F. J. Gutter, Abstracts of 117th National Meeting, American Chemical Society, April, 1950.

(8) H. A. Sober and G. Kegeles, *Fed. Proc.*, **10**, 299 (1951).

(9) G. Kegeles and F. J. Gutter, THIS JOURNAL, in press.

NATIONAL CANCER INSTITUTE
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U. S. PUBLIC HEALTH SERVICE GERSON KEGELES
BETHESDA, MD. FREDERICK J. GUTTER

RECEIVED MAY 9, 1951

STEROIDS. XXII. THE SYNTHESIS OF 19-NOR-PROGESTERONE

Sir:

In 1944, Ehrenstein[1] reported the twelve-step degradation of strophanthidine in 0.07% yield to a resin, $[\alpha]_D$ +89°, believed to be 19-norprogesterone (IIIb). The material represented a mixture of stereoisomers, most likely possessing the "unnatural" configuration[2] at C-14(β) and C-17(α) and was reported[3] to exhibit the same biological activity as progesterone. Subsequent work[4] has shown that the "unnatural" configuration at C-14 and C-17 *per se* does not confer progestational activity and it remained, therefore, to be seen whether the lack of an angular methyl group at C-10 in IIIb was responsible for this pronounced biological effect, so surprising in view of the extreme specificity of this type of hormonal activity.[2a]

A modified[5] Birch reduction[6] on 3-methoxy-17-acetyl-1,3,5-estratriene (I)[7] produced $\Delta^{2,5(10)}$-19-nor-3-methoxy-20-hydroxypregnadiene (II), (m.p. 135–138°, $[\alpha]^{20}_D$ +88° (all rotations in chloroform), no selective absorption in the ultraviolet, free hydroxyl band in infrared. Calcd. for $C_{21}H_{32}O_2$: C, 79.69; H, 10.19; methoxy, 9.80. Found: C, 79.33; H, 10.47; methoxyl, 9.15), which without isolation upon boiling with alcoholic hydrochloric acid yielded Δ^4-19-norpregnen-20-ol-3-one (IIIa),

(1) M. Ehrenstein, *J. Org. Chem.*, **9**, 435 (1944).

(2) (a) M. Ehrenstein, *Chem. Rev.*, **42**, 457 (1948); (b) *J. Org. Chem.* **16**, 355 (1951).

(3) W. M. Allen and M. Ehrenstein, *Science*, **100**, 251 (1944).

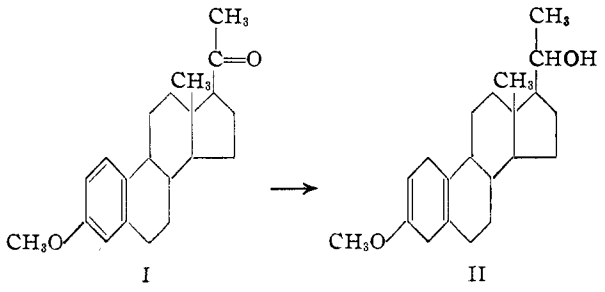
(4) Pl. A. Plattner, H. Heusser and A. Segre, *Helv. Chim. Acta*, **31**, 249 (1948).

(5) A. L. Wilds and N. Nelson, to be published. We are greatly indebted to Prof. A. L. Wilds, University of Wisconsin, for advance information on this modified procedure.

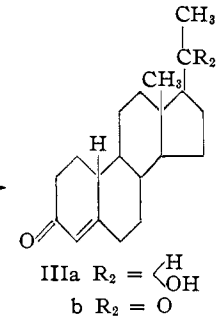
(6) A. J. Birch, *Quart. Rev.*, **4**, 69 (1950); *J. Chem. Soc.*, 2531 (1949).

(7) C. Djerassi, G. Rosenkranz, J. Iriarte, J. Berlin and J. Romo, THIS JOURNAL, **73**, 1523 (1951).

probably representing a mixture of 20-epimers; m.p. 174–177°, $[\alpha]^{20}D$ +42°, λ_{max}^{alc} 240 mμ (4.35), infrared bands (CS_2) at 3617 cm.$^{-1}$ (hydroxyl) and 1678 cm.$^{-1}$ (Δ^4-3-ketone). Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.45; H, 10.24. Chromium trioxide oxidation of IIIa in acetic acid solution afforded in 55% over-all yield (based on I) pure 19-norprogesterone (IIIb), m.p. 144–145°, $[\alpha]^{20}D$ +147°, λ_{max}^{alc} 240 mμ (4.36), infrared bands (CS_2) at 1706 cm.$^{-1}$ (20-ketone) and 1674 cm.$^{-1}$ (Δ^4-3-ketone). Calcd. for $C_{20}H_{28}O_2$: C, 79.95; H, 9.39. Found: C, 80.07; H, 9.28. The reddish-orange 3,20-bis-2,4-dinitrophenylhydrazone possessed m.p. 278–279°, $\lambda_{max}^{CHCl_3}$ 380 mμ (4.78).[8] Calcd. for $C_{32}H_{36}O_8N_8$: C, 58.17; H, 5.49; N, 16.95. Found: C, 58.28; H, 5.37; N, 16.57.



I II



IIIa $R_2 = \langle^{H}_{OH}$
b $R_2 = O$

19-Norprogesterone (IIIb) exhibits approximately the same activity as natural progesterone in rabbits. Since the mode of synthesis automatically establishes the "natural" configuration for all asymmetric centers with the possible exception of C-10,[9] the replacement of the angular methyl group at C-10 by hydrogen in progesterone does not reduce biological activity.[10] This observation is of considerable importance since if it should also apply to the cortical hormones, notably cortisone, it would considerably simplify the total synthesis of anti-arthritic substances. In fact, the present preparation of 19-norprogesterone (IIIb) constitutes the first total synthesis of a potent progestational hormone, since the starting methyl ether I[7] has been obtained[11] from estrone which has already been synthesized totally.[12]

Further work on 19-norsteroids, particularly of the cortical hormone series, is in progress.

JOINT CONTRIBUTION FROM THE
RESEARCH LABORATORIES OF SYNTEX, S. A.
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MEXICO CITY 17, D. F., AND G. ROSENKRANZ
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UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
TACUBA, D. F.

RECEIVED MAY 21, 1951

(8) Progesterone bis-dinitrophenylhydrazone shows $\lambda_{max}^{CHCl_3}$ 383 mμ (4.72) (C. Djerassi, *Anal. Chem.*, **20**, 880 (1948)).

(9) The hydrogen atom at C-10 most likely assumed the more stable "natural" β-configuration during the acid hydrolysis of II.

(10) The reason for the high biological activity of Ehrenstein's (ref. 1) mixture of 19-norprogesterones is still obscure since the presently described isomer IIIb could have been at best only a minor constituent of that mixture.

(11) L. Velluz and G. Muller, *Bull. Soc. Chim. France*, 166 (1950).

(12) G. Anner and K. Miescher, *Helv. Chim. Acta*, **31**, 2173 (1948); W. S. Johnson, D. K. Banerjee, W. P. Schneider and C. D. Gutsche, THIS JOURNAL, **72**, 1426 (1950).

CRYSTALLINE ALLETHRIN ISOMER

Sir:

The insecticide known as allethrin, now being produced commercially, is obtained by acylation of *dl*-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one[1] (*dl*-allethrolone) with a mixture of *dl-cis*- and *dl-trans*-chrysanthemum monocarboxylic acid chlorides.

Allethrin may be considered a mixture of four racemic forms (or eight individual optical and geometric isomers). Two racemic forms are esters of the *cis* acid and two of the *trans* acid.

When a sample of molecularly distilled allethrin was cooled to a low temperature, it crystallized in part, as likewise did samples of commercial allethrin kept at about 4°. Cold filtration and recrystallization from isooctane or pentane gave colorless crystals, m.p. 50.5–51°.

Anal.[2] Calcd. for $C_{19}H_{26}O_3$: C, 75.46; H, 8.67. Found: C, 75.41; H, 8.67.

Upon saponification of the crystalline product, *dl-trans*-chrysanthemum monocarboxylic acid was obtained, which, after recrystallization from pentane or nitromethane, melted at 55–56° and gave no depression in a mixture melting-point determination with the authentic acid.[3]

dl-Allethrolone when acylated with *dl-cis*-chrysanthemum monocarboxylic acid chloride furnished an ester mixture, b.p. 146–149° (0.4 mm.), $n^{25}D$ 1.5070,[4] which, on being cooled and seeded with the above-mentioned crystalline compound, could not be induced to crystallize. Acylation of *dl*-allethrolone with *dl-trans*-chrysanthemum monocarboxylic acid chloride furnished an ester mixture, b.p. 147–150° (0.4 mm.), $n^{25}D$ 1.5047,[4] which crystallized in part on being cooled and seeded. When 8.4 g. of this ester mixture was dissolved in 12.6 ml. of isooctane, cooled, and filtered on a cold-jacketed filter kept at about −30°, about half was obtained as the crystalline form. Removal of solvent from the filtrate *in vacuo* left 4.4 g. of oil, $n^{25}D$ 1.5050. The crystalline portion, when recrystallized from isooctane, melted at 50.5–51° and did not depress the melting point of the crystalline compound obtained from allethrin. The crystalline isomer will be called the α-*dl-trans* isomer, and the other isomer found concentrated in the filtrate, the β-*dl-trans* isomer of allethrin. Based on the yield, the concentrate of β-*dl-trans* isomer contained about 5% of dissolved α-*dl-trans* isomer.

The crystalline α-*dl-trans* isomer must consist of one of the racemic ester pairs, *d-trans* acid with *d*-allethrolone plus *l-trans* acid with *l*-allethrolone,

(1) M. S. Schechter, N. Green, and F. B. LaForge, THIS JOURNAL, **71**, 1517 (1949); **71**, 3165 (1949); *Agr. Chemicals*, **4** (6), 57 (1949).

(2) J. S. Ard, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture.

(3) I. G. M. Campbell and S. H. Harper, *J. Chem. Soc.*, 283 (1945).

(4) Compare, L. Crombie, A. J. B. Edgar, S. H. Harper, M. W. Lowe, and D. Thompson, *J. Chem. Soc.*, 3553 (1950).

or *d-trans* acid with *l*-allethrolone plus *l-trans* acid with *d*-allethrolone; the *β-dl-trans* isomer consists of the other pair.

Entomological tests[5] on house flies indicate the *α-dl-trans* isomer to be less effective and the *β-dl-trans* isomer to be more effective than allethrin.

The pure, crystalline *α-dl-trans* isomer of allethrin should serve as a useful reference standard in Peet-Grady, Campbell turntable, and other insecticide test methods, and for checking chemical analytical methods for substances of the pyrethrin or allethrin type.

(5) By W. A. Gersdorff, N. Mitlin, and J. H. Fales, Bureau of Entomology and Plant Quarantine.

BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE
AGRICULTURAL RESEARCH ADMINISTRATION
U. S. DEPARTMENT OF AGRICULTURE MILTON S. SCHECHTER
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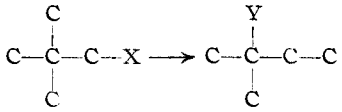
BENZOL PRODUCTS CO. A. ZIMMERLI
NEWARK, N. J. J. M. THOMAS

RECEIVED JUNE 19, 1951

NON-REARRANGEMENT REACTIONS OF THE NEOPENTYL-OXYGEN BOND. NEW SYNTHESES OF NEOPENTYL HALIDES

Sir:

Anionoid substitution reactions of neopentyl compounds have long been known to proceed with rearrangement of the carbon skeleton.[1] Indeed,



these changes are often cited in textbooks as classical simple examples of the Wagner–Meerwein rearrangement.

In a recent elegant series of papers, however, I. Dostrovsky, E. D. Hughes and C. K. Ingold[2] have demonstrated rearrangement for neopentyl bromide in S_N1 reactions and non-rearrangement in S_N2 reactions. This suggested to us that similar non-rearrangement reactions of the neopentyl–oxygen bond might be isolated by appropriate structural variations in neopentyl alcohol (which did not disturb the neopentyl–oxygen configuration) or by reaction conditions unfavorable to reaction of the alcohol by an S_N1 mechanism.

In this communication we wish to report what we believe to be the first unequivocal examples of reactions of the neopentyl–oxygen bond proceeding without rearrangement.

$$Me_3CCH_2OSiEt_3 + PBr_3 \xrightarrow{C_9H_7N\cdot HX} Me_3CCH_2Br + Et_3SiBr$$

$$Me_3CCH_2OSiEt_3 + SOCl_2 \xrightarrow{C_9H_7\cdot HX} Me_3CCH_2Cl + Et_3SiCl$$

$$Me_3CCH_2OH + PBr_3 + C_9H_7N \longrightarrow Me_3CCH_2Br + C_9H_7N\cdot HBr$$

Triethylneopentoxysilane, b.p. 67° (10 mm.), $n^{20}D$ 1.4189 (calcd. for $C_{11}H_{26}OSi$: Si, 13.84. Found: Si, 13.78) was prepared by the method of R. O. Sauer[3] using neopentyl alcohol, triethylchlorosilane, and quinoline, in a benzene solvent. Pure neopentyl bromide, b. 104° (733 mm.), $n^{20}D$ 1.4371, d^{20} 1.200, lit.[1c] b.p. 105°, $n^{20}D$ 1.4370, d^{20} 1.199; anilide, m.p. and mixed m.p. 130°; less than 0.5% reaction with NaOEt in EtOH at reflux for four hours; (calcd. for $C_5H_{11}Br$: Br, 52.9. Found: Br, 52.9) was formed in 85% yield by heating 2 moles of triethylneopentoxysilane with four moles of phosphorus tribromide at 173° for 16 hours in the presence of 3 g. of quinoline hydrochloride.[4]

A similar reaction using thionyl chloride and carried out at 115° for 23 hours gave a 61% yield of pure neopentyl chloride, b.p. 83° (725 mm.), $n^{20}D$ 1.4043, d^{20} 0.8659, lit.[1c] b.p. 83.5° (740 mm.), $n^{22}D$ 1.4043, d^{20} 0.865; completely inert to NaOEt in EtOH; anilide, m.p. and mixed m.p. 130° (calcd. for $C_5H_{11}Cl$: Cl, 33.27. Found: Cl, 32.58).

Neopentyl bromide, b.p. 104.5° (728 mm.), $n^{20}D$ 1.4370, d^{20} 1.200, was also prepared in 47% yield by heating a mixture of neopentyl alcohol (2 moles), quinoline (2.43 moles) and bromobenzene (900 cc.) at 181° for 24 hours.

From the above data it is now clear that use of neopentyl as a critical group in experiments aimed at determining the mechanism of cleavage of a carbon–oxygen bond in a given reaction[5] (based on the postulate[1a] that neopentyl–oxygen fission invariably gives rearrangement) are of little absolute value in the elucidation of such mechanisms.

A further consequence of the present work is that neopentyl bromide is now as readily available as other aliphatic bromides from reaction of the alcohol with phosphorus tribromide and quinoline.

The mechanisms and possible extensions of the above reactions are under investigation.

(1) (a) F. C. Whitmore, THIS JOURNAL, **54**, 3274 (1932); (b) F. C. Whitmore and H. S. Rothrock, *ibid.*, **54**, 3431 (1932); (c) F. C. Whitmore, F. L. Wittle and B. R. Harriman, *ibid.*, **61**, 1586 (1939).

(2) Hughes and Ingold, *J. Chem. Soc.*, 157 (1946).

(3) Sauer, THIS JOURNAL, **66**, 1707 (1944).

(4) The latter is a necessary catalyst whose broad function probably involves action as a good source of halide ions and in addition includes a labilizing effect on the Si–O–C grouping *via* electrophilic attack by quinolinium ions; *cf.* W. Gerrard and A. French, *Nature*, **159**, 263 (1947).

(5) *Cf.* A. Scattergood, W. H. Miller and J. Gammon, THIS JOURNAL, **67**, 2150 (1945); N. C. Deno and M. S. Newman, *ibid.*, **72**, 3852 (1950).

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THE PENNSYLVANIA STATE COLLEGE LEO H. SOMMER
STATE COLLEGE, PA. HERBERT D. BLANKMAN
PAUL C. MILLER

RECEIVED APRIL 11, 1951

THE NATURE OF THE INTERMEDIATE IN CARBONIUM ION-TYPE INTERCONVERSION REACTIONS OF CYCLOBUTYL, CYCLOPROPYLCARBINYL AND ALLYLCARBINYL DERIVATIVES[1]

Sir:

The striking ease of interconversion of cyclobutyl and cyclopropylcarbinyl derivatives in carbonium ion-type reactions[2] and the abnormally large solvolytic reactivities of cyclobutyl and cyclopropylcarbinyl halides[2] and sulfonate esters[3,4] have led to a

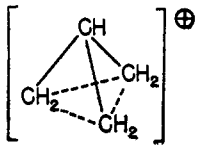
(1) Supported by the program of research of the U. S. Atomic Energy Commission.

(2) J. D. Roberts and R. H. Mazur, THIS JOURNAL, **73**, 2509 (1951).

(3) J. D. Roberts and V. C. Chambers, *ibid.*, **73**, 3176 (1951).

(4) C. G. Bergstrom and S. Siegel, Abstracts of the 119th Meeting of the American Chemical Society, Boston, Mass., April 4, 1951, p. 33M.

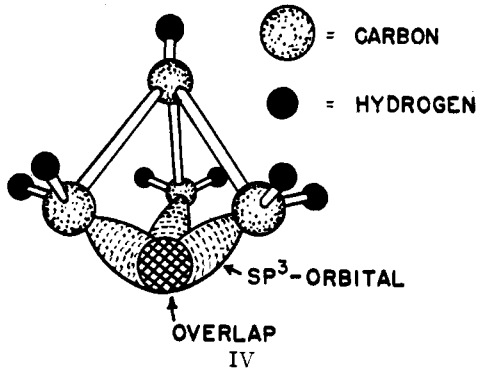
number of suggestions[2,4,5] regarding the nature of the cationic intermediate (or intermediates) which might be involved. Investigation of the course of the reactions using C^{14}-labeled cyclopropylcarbinyl derivatives reveals that the three methylene groups of the starting material achieve a degree of equivalence at some point between reactants and products which is well (although not uniquely) accounted for by a "non-classical" cationic intermediate of structure I.[4,5]



I

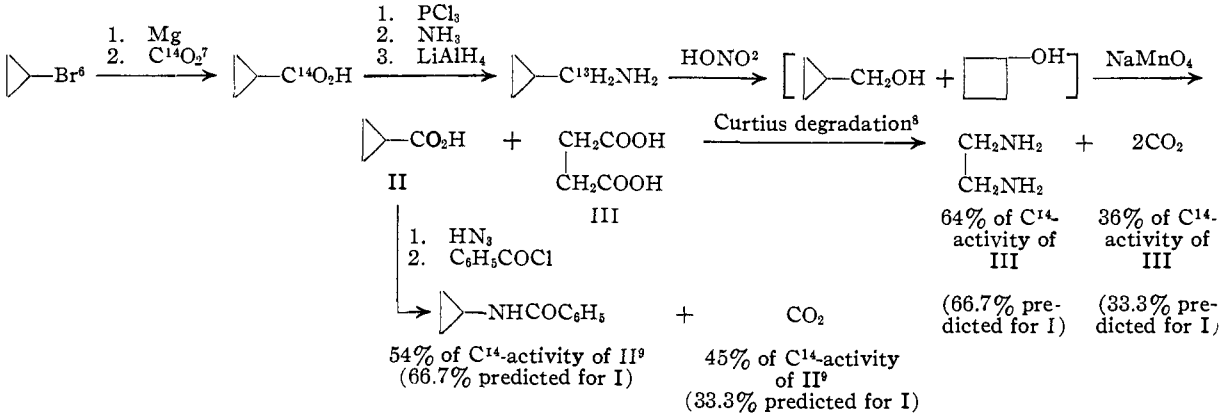
The main features of the experimental evidence for attainment of substantial equivalence between the methylene groups in the reaction of cyclopropylcarbinylamine with nitrous acid follow.

the extra electrons would have to go into less stable orbitals. It is significant that the experimental data, so far available,[2] indicate that cyclopropylcarbinyl and cyclobutyl derivatives are not interconverted in anion or free-radical reactions.



IV

A full report of this and related work will be presented in later papers.



Dr. M. J. S. Dewar (private communication) suggests that I can be very reasonably formulated by the molecular orbital theory if it is considered that all of the carbon atoms use the customary sp^3 orbitals and that the methinyl (CH) group is attached to the three methylene groups by the customary σ-bonds. The three extra sp^3 orbitals of each of the methylene groups are then positioned to overlap as shown in IV and can form one stable molecular orbital holding two electrons[10] and two vacant, considerably-less stable orbitals. This formulation is especially attractive since it permits prediction that structures analogous to II for the corresponding anion or free radical would be unfavorable because

(5) (a) V. C. Chambers, Ph.D. Thesis, M.I.T., October, 1950; (b) R. H. Mazur, Ph.D. Thesis, M.I.T., January, 1951; (c) R. B. Woodward (Harvard University), private communication.

(6) J. D. Roberts and V. C. Chambers, THIS JOURNAL, **73**, 3176 (1951).

(7) The carbon dioxide was prepared from barium carbonate-C^{14} supplied by the Oak Ridge National Laboratory on allocation from the U. S. Atomic Energy Commission. The radioactivity analyses were made by Miss Winifred Bennett and Miss Clare M. McGinnis.

(8) A. A. Benson and J. A. Bassham, THIS JOURNAL, **70**, 3939 (1948).

(9) The difference between predicted and found is likely to be due to some direct (non-carbonium ion) replacement of $-NH_2$ by $-OH$.

(10) A. D. Walsh, *Trans. Faraday Soc.*, **45**, 179 (1949); T. M. Sugden, *Nature*, **160**, 367 (1947); R. S. Mulliken, *J. Chem. Phys.*, **1**, 492 (1933). It is to be noted that the argument here is not rigorous since the three orbitals do not have D_{3h} symmetry.

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CAMBRIDGE 39, MASS. ROBERT H. MAZUR

RECEIVED MAY 25, 1951

THE VALENCE OF PRECIPITATING RABBIT ANTIBODY

Sir:

In the course of a quantitative ultracentrifugal and electrophoretic investigation of the soluble complexes formed between antigen and antibody in the region of antigen excess, we have obtained evidence that precipitating antibody to bovine serum albumin (BSA) is largely bivalent. Crystalline BSA was iodinated to an average degree of 5.1 I atoms per molecule (BSA-5I), and rabbit antisera were prepared against the un-iodinated BSA. The γ-globulin fraction of the pooled antisera was first purified by precipitation with $^1/_3$ saturated $(NH_4)_2SO_4$, and then a specific antigen-antibody precipitate was formed in the equivalence zone using BSA-5I as antigen. The precipitate was washed with cold saline and then was redissolved in antigen excess. Treatment of this solution with $^1/_2$ saturated $(NH_4)_2SO_4$ resulted in a precipitate that completely redissolved in buffered saline.

This final solution was analyzed for I and N, and thus the total antigen and total antibody content determined. Solutions in greater antigen excess were prepared by adding known amounts of BSA-5I to this solution.

Ultracentrifuge experiments were carried out in phosphate buffer, *p*H 7.6, $\mu = 0.1$, at 21 ± 1°. Several species of antigen-antibody complexes, as well as free antigen, appeared in the sedimentation diagrams, as was originally observed by Heidelberger and Pedersen[1] in similar systems. The faster-sedimenting complexes, present to a large extent in solutions in low antigen excess, were considerably diminished in solutions in high excess, and in the latter a single peak (*a* complex) became most prominent. The *a* complex appears to be the richest antigen-containing complex capable of being formed by the antibody in this system. Extrapolation of the sedimentation constants of the *a* complex to zero effective concentration yields the value $s^{w}_{20} = 8.7\ S$.

Electrophoresis experiments were performed in veronal buffer, *p*H 8.5, $\mu = 0.1$. Resolution of the free antigen, with its appropriate mobility, was readily achieved in both ascending and descending limbs, and the relative areas under the free antigen peak were accurately determined from the ascending pattern. The values so obtained agreed with the per cent. of free antigen evaluated from the ultracentrifuge diagrams. Resolution among the complexes was poor, but in solutions in which sufficiently large proportions of the *a* complex were present, a partial but definite separation of a faster-moving peak from the other complexes was observed. The relative area under this peak agreed with the per cent. of the *a* complex in the solution determined ultracentrifugally.

For several reasons which are too lengthy to be detailed here, it is unlikely that the *a* complex is the 3:1 antigen:antibody species. One reason is that such a complex, with $s^{w}_{20} = 8.7\ S$ and a molecular weight of 370,000, would be required to have a frictional ratio, f/f_0, of 2.1. This value suggests a molecular asymmetry that is larger than would be expected for a complex in which 3 antigen molecules were attached to a single antibody molecule. The *a* complex must therefore be either largely the 1:1 or 2:1 antigen:antibody species, or a mixture of about equal proportions of the two. However, 1:1 and 2:1 complexes should have observably different electrophoretic mobilities, and the fact that all of the *a* complex observed ultracentrifugally can be accounted for under a single peak electrophoretically makes it unlikely that the *a* complex is a mixture of the two species, and likely that it is rather largely one or the other.

The following considerations indicate that the *a* complex cannot be the 1:1 species. If the amount of free antigen in a given solution, determined electrophoretically, is subtracted from the total antigen present, the result is the total amount of antigen bound in all of the complexes in that solution. This, divided by the total antibody, and multiplied by the appropriate molecular weight factor, 2.3, gives $\overline{(AG/AB)}_{N,B}$, the *average* number of antigen molecules bound per antibody in *all* of the complexes in the solution. The results are presented in Table I. This average number rises well above unity as the antigen excess is increased. Solution II-2 contains about 33% of the complexes as the *a* complex, the other species in the solution being characterized by smaller antigen-antibody ratios. The *a* complex therefore cannot be the 1:1 species, since it must obviously be richer in antigen.

TABLE I

COMPOSITION OF ANTIGEN-ANTIBODY COMPLEXES

Solution	% Total antigen	% Free antigen	$\overline{(AG/AB)}_{N,B}$
I	34.7	8.6	0.92
II	35.8	12.4	0.82
I-1	50.0	28.1	1.01
I-2	62.2	42.6	1.19
II-2	68.1	50.9	1.24

We conclude that the major part of the *a* complex contains 2 antigen molecules and 1 antibody molecule, and that the antibody is therefore largely bivalent. Further studies with this and similar systems are in progress or are contemplated, and a detailed account of this investigation will be published shortly.

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RECEIVED MAY 28, 1951

(1) M. Heidelberger and K. O. Pedersen, *J. Exp. Med.* **65**, 393 (1937).

A NEW ROUTE TO HYDROPHENANTHRENE KETONES. THE SYNTHESIS OF THE C_{18} KETONE DERIVED FROM DEHYDROABIETIC ACID

Sir:

The presently available methods for the preparation of hydrophenanthrene ketones of type I are useful only in the special case where R′ = H,[1] while the synthesis of substances related to the resin acids from such intermediates would require that the R′ group be methyl. We now wish to report a simple general synthesis of these hydrophenanthrene ketones.

Alkylation of the sodium derivative of Hagemann's ester[2] in a 3:1 mixture of benzene and dimethylformamide with the required β-phenethyl bromide gave, in 70% yield, the following 2-substituted-3-methyl-4-carbethoxy-Δ²-cyclohexenones: 2-[β-phenethyl], b.p. 178–182° (0.4 mm.), dinitrophenylhydrazone (orange needles) m.p. 132–133° ($C_{24}H_{26}N_4O_6$: C, 61.79; H, 5.62; found: C, 62.19; H, 5.68), semicarbazone m.p. 167–168° ($C_{19}H_{24}N_3O_3$: C, 66.66; H, 7.07; found: C, 66.96; H, 7.46); 2-[β-*m*-isopropylphenethyl], b.p. 190–194° (0.4 mm.), dinitrophenylhydrazone (orange-red needles) m.p. 103° ($C_{27}H_{32}N_4O_6$: C, 63.76; H, 6.34; found: C, 64.09; H, 6.34). These were decarbethoxylated

(1) *Inter alia*, R. Robinson and J. Walker, *J. Chem. Soc.*, 747 (1936), 183 (1938); W. E. Bachmann, S. Kushner and A. C. Stevenson, THIS JOURNAL, **64**, 974 (1942).

(2) C. T. Hagemann, *Ber.*, **26**, 876 (1893); *cf.* L. I. Smith and G. F. Rouault, THIS JOURNAL, **65**, 631 (1943).

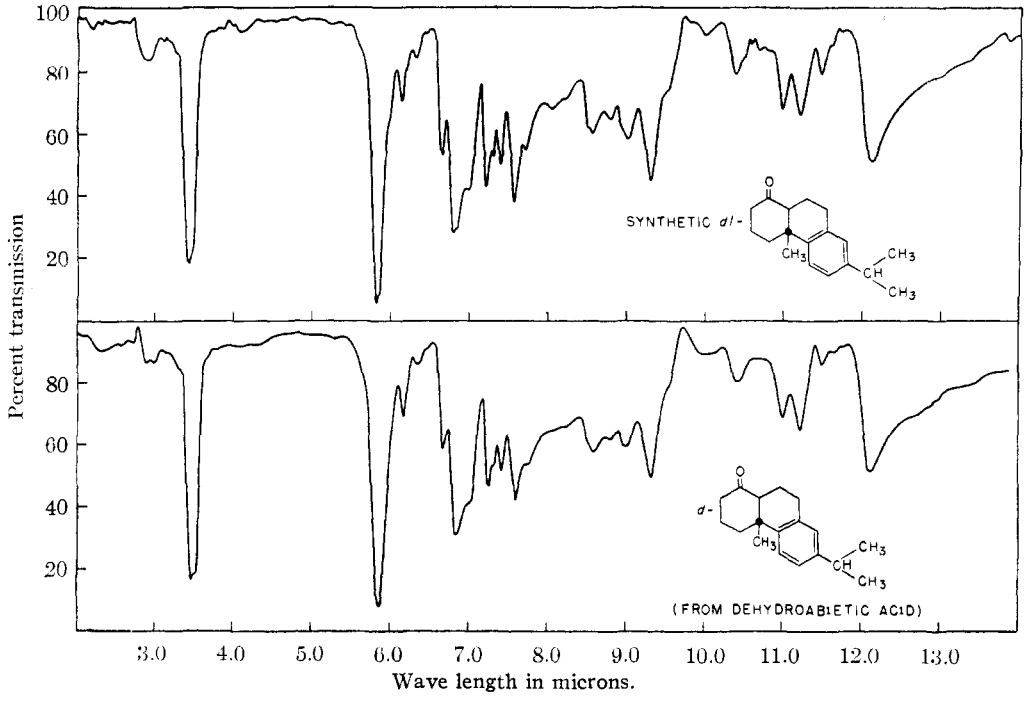


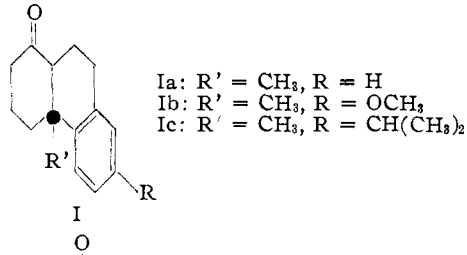
Fig. 1.—Infrared spectra in chloroform.

with alcoholic KOH under nitrogen in 80% yield to the following 2-[β-phenethyl]-3-methyl-Δ^2-cyclohexenones (II):

IIa (R = H), b.p. 140–143° (0.4 mm.), dinitrophenylhydrazone (red needles) m.p. 171–172° ($C_{21}H_{22}N_4O_4$: C, 63.94; H, 5.62; found: C, 64.22; H, 5.67), semicarbazone m.p. 185° ($C_{16}H_{21}N_3O$: C, 70.82; H, 7.80; found: 70.44; H, 7.81).

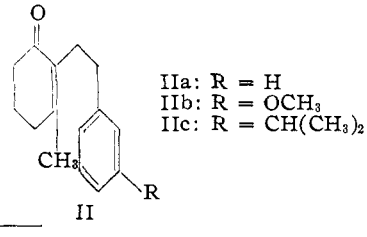
IIb (R = OCH_3), b.p. (bath temp.) 160–170° (0.5 mm.), dinitrophenylhydrazone (red prisms) m.p. 169–170° ($C_{22}H_{24}N_4O_5$: C, 62.25; H, 5.70; found: C, 62.25; H, 5.67), prepared from the corresponding Hagemann ester derivative described previously.[3]

IIc (R = $CH(CH_3)_2$), b.p. (bath temp.) 165–170° (0.6 mm.), dinitrophenylhydrazone (red platelets) m.p. 134° ($C_{24}H_{28}N_4O_4$: C, 66.04; H, 6.47; found: C, 66.21; H, 6.50), semicarbazone m.p. 142–143° ($C_{19}H_{27}N_3O$: C, 72.80; H, 8.68; found: 73.15; H, 8.76).



Ia: R' = CH_3, R = H
Ib: R' = CH_3, R = OCH_3
Ic: R' = CH_3, R = $CH(CH_3)_2$

I



IIa: R = H
IIb: R = OCH_3
IIc: R = $CH(CH_3)_2$

II

(3) J. A. Hogg, THIS JOURNAL **70**, 161 (1948).

Heating IIa with 85% phosphoric acid at 165–170° for 12 hours resulted in a 70% yield of 1-keto-12-methyl-1,2,3,4,9,10,11,12-octahydrophenanthrene (Ia), b.p. (bath temp.) 150–155° (0.5 mm.), dinitrophenylhydrazone (yellow needles) m.p. 193–195° (found: C, 64.01; H, 5.52), semicarbazone m.p. 227–228° (found: C, 71.13; H, 7.83). IIb on cyclization with phosphoric acid at 115–120° for 6 hours similarly produced Ib, b.p. (bath temp.) 165–170° (0.5 mm.), in 65% yield, dinitrophenylhydrazone (deep yellow prisms) m.p. 203° (found: C, 62.61; H, 5.78), semicarbazone m.p. 213–215° ($C_{17}H_{23}N_3O_2$: C, 67.75; H, 7.69; found: C, 67.87; H, 7.65). Cyclization of IIc was best effected by heating with a mixture of phosphoric acid (5 parts) and concd. sulfuric acid (1 part) at 120–125° for 12 hours when Ic, b.p. (bath temp.) 155–160° (0.3 mm.), was formed in 65% yield, dinitrophenylhydrazone (yellow needles) m.p. 189–190° (found: C, 66.41; H, 6.43), semicarbazone m.p. 225–226° (found: C, 73.23; H, 8.98).

Compounds IIa, IIb and IIc had the I.R. absorption of α,β-unsaturated ketones (6.0 and 6.1 μ), in contrast to Ia, Ib and Ic, which exhibited only saturated carbonyl absorption (5.85 μ). The tricyclic ketones have a *trans* decalone system as expected from their method of formation and demonstrated by the identity of the derivatives obtained before and after heating with alcoholic base.[4]

Dehydrogenation of the lithium aluminum hydride reduction product of Ia smoothly afforded phenanthrene, while dehydrogenation of the carbinol formed by the action of methyl lithium on Ic

(4) It is of considerable interest that on treatment with sulfuric acid the 4-carbethoxy derivative of IIb has been found to undergo *cyclodehydration* to a tetrahydrophenanthrene carboxylate rather than *cyclization* to a ketone.[3] This latter reaction has previously been attempted without success in related cases (see J. W. Cook and A. Cohen, *J. Chem. Soc.*, 1098 (1933); 1570 (1935)).

(regenerated from its semicarbazone) gave a 60% yield of retene, m.p. 99.5–100.5° (picrate m.p. 125.5–126.5°), undepressed with an authentic sample prepared from dehydroabietic acid.[5]

The structural identity of the synthetic *dl*-1-keto-7-isopropyl-12-methyl-1,2,3,4,9,10,11,12-octahydrophenanthrene (Ic) with the C_{18} *d*-ketone recently obtained from dehydroabietic acid[6] is confirmed by comparison of their infrared spectra (Fig. 1).

The extension of this work to the stereospecific synthesis of the resin acids is being actively pursued.

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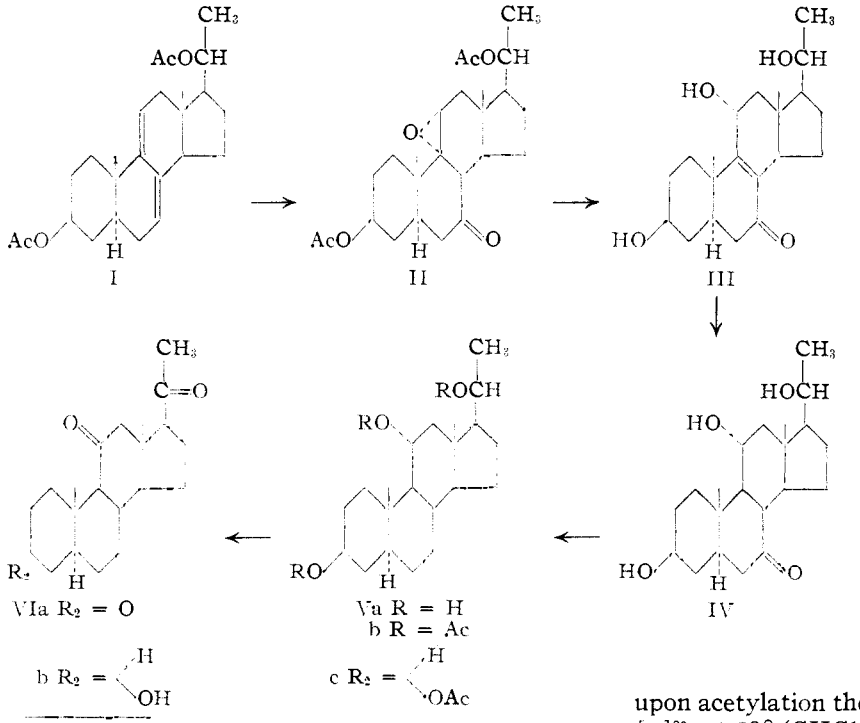
RECEIVED JUNE 15, 1951

(5) L. Ruzicka and H. Waldmann, *Helv. Chim. Acta*, **16**, 842 (1933).
(6) A. Brossi, H. Gutmann and O. Jeger, *ibid.*, **33**, 1730 (1950).
(7) National Institutes of Health Predoctoral Fellow, Harvard University, 1950–1951.

STEROIDS. XXIV.[1] INTRODUCTION OF THE 11-KETO AND 11α-HYDROXY GROUPS INTO RING C UNSUBSTITUTED STEROIDS

Sir:

The conversion of Δ^5-3β-hydroxy steroids to a number of $\Delta^{7,9(11)}$-dienes of the pregnane and sapogenin series with both the *allo* and *normal* configuration at C-5 has recently been reported from this Laboratory.[1–4] In two Communications[5,6] to the Editor, there is described the transformation of such $\Delta^{7,9(11)}$-dienes to 11-ketosteroids by way of Δ^8-unsaturated and thence saturated 7,11-diones. We should like to record herewith an alternate procedure for the synthesis of 11-oxygenated steroids from ring C unsubstituted steroids via $\Delta^{7,9(11)}$-dienes, which does not involve the above mentioned[5,6] intermediates. In addition to its versatility, the presently described method exhibits the attractive feature of representing a novel and convenient synthesis for the hitherto unknown 11α-hydroxyallopregnanes and sapogenins.[6a]



Performic acid oxidation of $\Delta^{7,9(11)}$-allopregnadiene-3β,20β-diol diacetate (I)[1] readily led to 9α,11α-oxidoallopregnane-3β,20β-diol-7-one diacetate (II) (m.p. 260–262° (all m.p.s are uncorrected), $[\alpha]^{20}D$ −55° ($CHCl_3$), no ultraviolet maximum, $\lambda_{max.}^{nujol}$ 1736 cm.$^{-1}$ (acetate) and 1718 cm.$^{-1}$ (7-ketone), no free hydroxyl band; found: C, 68.95; H, 8.39). Alkaline hydrolysis was accompanied by isomerization and afforded in high yield Δ^8-allopregnene-3β,11α,20β-triol-7-one (III) (m.p. 250–252°, $[\alpha]^{20}D$ −25° (EtOH), $\lambda_{max.}^{EtOH}$ 254 mμ, log ε 4. 11, $\lambda_{max.}^{nujol}$ 1662 cm.$^{-1}$, found: C, 72.52; H, 8.97; triacetate, m.p. 203–205°; found: C, 68.21; H, 8.06). Catalytic hydrogenation (palladized charcoal in ethanol solution) of III produced 78% of allopregnane-3β,11α,20β-triol-7-one (IV) (m.p. 246–248°, $[\alpha]^{20}D$ −112° (EtOH), no ultraviolet maximum, $\lambda_{max.}^{nujol}$ 1718 cm.$^{-1}$; found: C, 71.58; H, 10.01). Wolff–Kishner reduction gave allopregnane-3β,11α,20β-triol(Va) (m.p. 253–255°, $[\alpha]^{20}D$ −28° (EtOH), no carbonyl band in infrared; found: C, 74.63; H, 10.83), which formed a triacetate (Vb) (m.p. 162–164°, $[\alpha]^{20}D$ −16° ($CHCl_3$), $\lambda_{max.}^{CS_2}$ 1736 cm.$^{-1}$ (acetate), no free hydroxyl band; found: C, 70.47; H, 9.26). Chromium trioxide oxidation of the triol Va led to the known[7] allopregnane-3,11,20-trione (VIa) (m.p. 211–213°, $[\alpha]^{20}D$ +129° (EtOH); found: C, 76.19; H, 9.39), which on Raney nickel reduction smoothly yielded allopregnane-11,20-dione-3β-ol (VIb) (m.p. 192–194°, $[\alpha]^{20}D$ +99° ($CHCl_3$); found: C, 75.44; H, 9.55) and upon acetylation the acetate (VIc)[8] (m.p. 143–144°, $[\alpha]^{20}D$ +89° ($CHCl_3$), $\lambda_{max.}^{CS_2}$ 1736 and 1710 cm.$^{-1}$, no

(1) Paper XXIII, J. Romo, G. Rosenkranz and C. Djerassi, THIS JOURNAL, in press.
(2) R. Yashin, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, (1951) in press.
(3) G. Rosenkranz, J. Romo, E. Batres and C. Djerassi, *J. Org. Chem.*, **16**, 298 (1951).
(4) C. Djerassi, J. Romo and G. Rosenkranz, *ibid.*, **16**, 754 (1951).
(5) L. F. Fieser, J. E. Herz and W. Huang, THIS JOURNAL, **73**, 2397 (1951).
(6) E. M. Chamberlin, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, *ibid.*, **73**, 2396 (1951).

(6a) W. T. Long, T. W. Marshall, and T. F. Gallagher, *J. Biol. Chem.*, **165**, 197 (1946), have prepared 11a-hydroxy compounds in the bile acid series.
(7) M. Steiger and T. Reichstein, *Helv. Chim. Acta*, **21**, 161 (1938), prepared the trione VIa by degradation of corticosterone and reported m.p. 212–216°, $[\alpha]^{20}D$ +133° (EtOH). A mixed melting point determination, kindly performed by Prof. T. Reichstein, further confirmed the identity of the two specimens.
(8) Ref. 5 records m.p. 141–143°, $[\alpha]D$ +88°.

free hydroxyl band; found: C, 73.24; H, 8.88). The presently described procedure is equally applicable to the steroidal sapogenins as exemplified by the performic acid oxidation of $\Delta^{7,9(11)}$-22-isoallospirostadien-3β-ol acetate[3] to 9α,11α-oxido-22-isoallospirostan-3β-ol-7-one acetate (m.p. 295–297°, $[\alpha]^{20}D$ −128° ($CHCl_3$), $\lambda_{max.}^{nujol}$ 1736 and 1718 cm.$^{-1}$; found: C, 71.74; H, 8.94). Analogous transformations of this oxidoketone to 11-oxygenated 22-isoallospirostan-3β-ols have already been completed and will be reported shortly in a detailed paper.

Since the starting diol (I)[5] has been prepared from both diosgenin[3,4] and Δ^5-pregnen-3β-ol-20-one[4] (which is also available from stigmasterol), the above described experiments constitute the conversion of the two most abundant plant steroids into 11-oxygenated pregnane derivatives.

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RECEIVED JUNE 11, 1951

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ACYLALKYLCARBONATES AS ACYLATING AGENTS FOR THE SYNTHESIS OF PEPTIDES

Sir:

Mixed anhydrides of carbonic with carboxylic acids have been found to be excellent acylating agents for the preparation of amides. In particular, anhydrides between branched chain alkyl carbonic acids and N-substituted amino acids or peptides react readily at low temperature with amino acid or peptide esters, or with a salt of an amino acid, to give the corresponding peptide or higher peptide in good yield. The by-products of the reaction, carbon dioxide and an alcohol, are readily removed and the peptide is obtained initially in a very high state of purity.

For peptide synthesis, the over-all reaction is given by

$$\text{X-NHCH(R)COOCOOR''} + \text{H}_2\text{NCH(R')COOR''} \longrightarrow \text{X-NHCH(R)CONHCH(R')COOR''} + \text{R'''OH} + \text{CO}_2\uparrow$$

where X is a blocking group, R and R′ are amino-acid residues and R″ is an esterifying or salt forming group. Best results have been obtained when R‴ is a *s*- or isobutyl radical.

The mixed anhydrides are formed by treating *s*- or isobutylchlorocarbonate with a solution of the triethylamine salt of an N-substituted aminoacid or peptide in an inert solvent as toluene or chloroform at 0 to −10°. The reaction is complete in 25–30 minutes. A solution of the amino acid or peptide ester to be acylated, also in an inert solvent, is then added and the reaction mixture is allowed to warm to room temperature and stand overnight. Carbon dioxide evolution begins immediately upon addition of the base and is substantially complete after several hours. In some cases, the formed N-substituted peptide ester crystallizes directly from the reaction mixture and is essentially pure after washing with water to remove triethylamine hydrochloride. More generally, the reaction mixture is washed with water and with dilute sodium bicarbonate solution, dried and diluted with petroleum ether to crystallize the product.

Amino acids may be used in this procedure by preparing a solution in one equivalent of 2 *N* alkali and adding this to the preformed mixed anhydride. The heterogeneous mixture is then stirred rapidly for 1–2 hours and the aqueous phase is separated, extracted with ether and acidified to precipitate the formed peptide acid.

In general, *s*-butylchlorocarbonate gave slightly higher yields than the isobutyl isomer. Peptide ethyl esters prepared using these reagents to form the reactive mixed anhydrides include those of carbobenzoxyglycyl-L-tyrosine[1] (68%); m.p. 129–130°, $[\alpha]^{24}D$ +19.3° (*c* = 10, ethanol); dicarbobenzoxy-L-lysylglycine[2] (64%), m.p. 89–90°, $[\alpha]^{24}D$ −12.0° (*c* = 4, ethanol); carbobenzoxy-L-leucyl-L-tyrosine[3] (63%), m.p. 116–118°, $[\alpha]^{24}D$ −14.9° (*c* = 10, ethanol); phthalylglycyl-L-leucine[3] (61%), m.p. 142–143°, $[\alpha]^{24}D$ −23.2° (*c* = 5, ethanol); carbobenzoxyglycyl-DL-phenylalanylglycine[3] (83%), m.p. 134–135° (from carbobenzoxyglycyl-DL-phenylalanine and ethyl glycinate); phthalyl-DL-phenylalanylglycylglycine[3] (67%), m.p. 164–165° (from phthalyl-DL-phenylalanine and ethyl glycylglycinate) and carbobenzoxyglycyl-DL-phenylalanyl-DL-phenylalanylglycylglycine[3] (59%), m.p. 188–193° (from carbobenzoxyglycyl-DL-phenylalanine and ethyl DL-phenylalanylglycylglycinate).

Peptide acids prepared by the free aminoacid procedure include carbobenzoxyglycyl-DL-phenylalanine[4] (63%), m.p. 160–162°; carbobenzoxyglycyl-DL-valine[5] (49%), m.p. 127–128° and 146–147° and carbobenzoxy-DL-alanyl-DL-phenylalanine[5] (50%), m.p. 145–146°.

ADDED IN PROOF. We have just received a publication by R. A. Boissonnas (*Helv. Chim. Acta*, **34**, 874 (1951)) on this same general subject matter.

(1) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 405 (1937).
(2) M. Bergmann, *et al.*, *Z. physiol. Chem.*, **224**, 26 (1934).
(3) Carbon, hydrogen and nitrogen analysis was satisfactory.
(4) H. Neurath, *et al.*, *J. Biol. Chem.*, **170**, 221 (1947).
(5) T. Wieland and R. Sehring, *Ann.*, **519**, 122 (1950).

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RECEIVED MAY 31, 1951

THE TOTAL SYNTHESIS OF SOME NATURALLY OCCURRING STEROIDS

Sir:

We have resolved methyl *dl*-3-keto-$\Delta^{4,9(11),16}$-etiocholatrienate[1] by the following method. Reduction of the keto-ester with sodium borohydride in ethanol gave a mixture of the corresponding 3-α and 3-β-hydroxy-esters. Treatment with excess digitonin,[2] followed by decomposition of the precipitated complex, gave material enriched in the desired *d*-3-β-hydroxy-ester. Further resolution was achieved by two repetitions of this procedure, and

(1) Woodward, Sondheimer, Taub, Heusler and McLamore, THIS JOURNAL, **73**, 2403 (1951).
(2) *Cf.* Windaus, Klänhardt and Weinhold, *Z. physiol. Chem.*, **126**, 308 (1923).

the product was oxidized by the Oppenauer method. Chromatographic purification of the resulting impure keto-ester, followed by several crystallizations, then furnished pure methyl *d*-3-keto-$\Delta^{4,9(11),16}$-etiocholatrienate, m.p. 188–191°, $[\alpha]_D$ + 182° ±5° ($CHCl_3$). This keto-ester has been obtained by the degradation of Compound F[1], and had m.p. 187–191°, $[\alpha]_D$ +177 ±5° ($CHCl_3$); mixed m.p. showed no depression.

Hydrogenation of the *d*-keto-ester with platinum in acetic acid, and oxidation of the resulting saturated product with chromium trioxide in acetic acid, gave a mixture from which methyl 3-ketoetio*allo*cholanate (I), m.p. 177–180°, was isolated after chromatography and crystallization. An authentic sample of (I)[3,4] had m.p. 178–180°, and there was no depression in m.p. on admixture. The infrared spectra of the two samples were also identical.

The saturated keto-ester (I) has previously been converted to the Δ^4-compound,[4] which we have now hydrolyzed to the free acid, 3-keto-Δ^4-etiocholenic acid, m.p. 240–243°. In view of the conversion of the latter to desoxycorticosterone,[5] and progesterone,[6] this reaction sequence constitutes a total synthesis of these hormones.

Alkaline hydrolysis of the keto-ester (I) has given us the corresponding acid, 3-ketoetioallocholanic acid, m.p. 256–259°, which has previously been transformed into methyl 3-α-acetoxyetiallocholanate.[7] This compound has been converted to androsterone,[8] which in turn has been transformed *via* androstanedione[9] into androstenedione,[10] and thence into testosterone.[11] The conversion of progesterone to androstanedione has also been described,[12] and this constitutes another route to testosterone.

(3) Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 1040 (1937).

(4) Djerassi and Scholz, THIS JOURNAL, **69**, 2404 (1947).

(5) Wilds and Shunk, *ibid.*, **70**, 2427 (1948).

(6) Riegel and Prout, *J. Org. Chem.*, **13**, 933 (1948); Reichstein and Fuchs, *Helv. Chim. Acta*, **23**, 684 (1940).

(7) Plattner and Fürst, *ibid.*, **26**, 2260 (1943).

(8) Dalmer, v. Werder, Honigmann and Heyns, *Ber.*, **68**, 1814 (1935).

(9) Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 185 (1934).

(10) Djerassi and Scholz, *J. Org. Chem.*, **13**, 697 (1948); Rosenkranz, Mancera, Gatica and Djerassi, THIS JOURNAL, **72**, 4077 (1950).

(11) *Inter al.*, Miescher and Fischer, *Helv. Chim. Acta*, **22**, 158 (1939).

(12) Marker, Kamm, Jones and Oakwood, THIS JOURNAL, **59**, 614 (1937).

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THE TOTAL SYNTHESIS OF CHOLESTEROL

Sir:

Cholesterol is the characteristic sterol of higher animals. It was isolated from gall stones by Conradi in 1775 and thus was the first member of the steroid family to be discovered. However it was not until 1932 that its correct structure (apart from stereochemical refinements) was proposed, mainly due to the brilliant researches of Windaus dating from 1903, and to those of Wieland dating from 1912. We now wish to record the total synthesis of cholesterol.

Methyl 3-ketoetio*allo*cholanate (I) has been obtained previously by total synthesis.[1] In view of conversions already described,[2] essentially the only remaining stage in a synthetic route from (I) to cholesterol is the homologation of 3-β-acetoxy*nor*-Δ^5-cholenic acid to 3-β-acetoxy-Δ^5-cholenic acid. The rather cumbersome nature of this scheme however led us to employ a more direct approach. Reduction of (I) with sodium borohydride in ethanol gave crude methyl 3-β-hydroxyetio*allo*cholanate, purified through the digitonide. The pure ester, m.p. 168–170° (undepressed on admixture with an authentic sample), was hydrolyzed to the corresponding hydroxy-acid, m.p. 249–251°, which was then acetylated. Treatment with thionyl chloride gave the acid chloride, m.p. 134–136°, which with excess cadmium-methyl yielded crude 3-β-acetoxy*allo*pregnanone-20, m.p. 139–144°. The latter reacted with excess isohexylmagnesium bromide,[3] and the gummy product, containing 20-hydroxycholestanol-3 and probably the C-20 epimer, was dehydrated (at C-20) and acetylated (at C-3) by boiling with acetic acid and then with acetic acid–acetic anhydride.[3] The reaction mixture was hydrogenated in the presence of a platinum catalyst. Chromatographic purification of the saturated material gave crude cholestanol-3 acetate, which on crystallization readily yielded the pure ester, m.p. 109–110°,[4] undepressed on admixture with an authentic sample (m.p. 110°). The infrared spectra of the two samples were also identical. Alkaline hydrolysis of the synthetic acetate furnished cholestanol-3, m. p. 142–142.5°,[4] undepressed on admixture with an authentic specimen (m.p. 142–143°). Cholestanol-3 has already been oxidized to cholestanone-3,[5] which in turn has been converted *via* Δ^4-cholestenone-3[6] into cholesterol[8]; the total synthesis of the latter is therefore complete.

Cholesterol has previously been converted into a number of other compounds of interest, the most important of which perhaps is vitamin D_3.

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(1) Woodward, Sondheimer and Taub, THIS JOURNAL, **73**, 3547 (1951).

(2) Djerassi and Scholz, *ibid.*, **69**, 2404 (1947); Reich and Lardon, *Helv. Chim. Acta*, **29**, 671 (1946); Butenandt and Schmidt-Thomé, *Ber.*, **71**, 1487 (1938); Steiger and Reichstein, *Helv. Chim. Acta*, **20**, 1164 (1937); MacPhillamy and Scholz, *J. Biol. Chem.*, **178**, 37 (1949); Ruzicka, Plattner and Pataki, *Helv. Chim. Acta*, **25**, 425 (1942); Plattner and Pataki, *ibid.*, **26**, 1241 (1943); Kuwada and Yogo, *J. Pharm. Soc. (Japan)*, **57**, 963 (1937); Riegel and Kaye, THIS JOURNAL, **66**, 723 (1944).

(3) *Cf.* Butenandt and Cobler, *Z. physiol. Chem.*, **234**, 218 (1935).

(4) These m.p's. were taken in a capillary. All others were taken on a Kofler micro hot-stage.

(5) *Inter al.* Bruce, *Organic Syntheses*, Coll. Vol. II, 139 (1943).

(6) Butenandt and Wolff, *Ber.*, **68**, 2091 (1935); Ruzicka, Plattner and Aeschbacher, *Helv. Chim. Acta*, **21**, 866 (1938). This transformation could probably be more conveniently carried out by use of the general method for converting *allo*steroids to Δ^4-3-ketosteroids recently developed (Ref. 7).

(7) Rosenkranz, Mancera, Gatica and Djerassi, THIS JOURNAL, **72**, 4077 (1950).

(8) Reich and Lardon, *Helv. Chim. Acta*, **29**, 671 (1946); Dauben and Eastham, THIS JOURNAL, **72**, 2305 (1950); Birch, *J. Chem. Soc.*, 2325 (1950).