useful material for the recovery of a large number of heavy metals which form insoluble sulfides.

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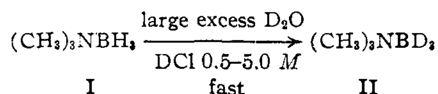
RECEIVED JANUARY 16, 1963

A RAPID AND QUANTITATIVE EXCHANGE OF THE BORON HYDROGENS IN TRIMETHYLAMINE BORANE WITH D_2O[1]

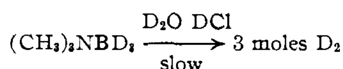
Sir:

Close examination of the slow acidic hydrolysis of trimethylamine borane[2–4] and the most noteworthy absence of a boron–hydrogen kinetic isotope effect[3] has demonstrated now that the boron hydride hydrogens are *exchanging* with the protons of the solvent.



$$\underset{\text{I}}{(CH_3)_3NBH_3} \xrightarrow[\text{DCl } 0.5\text{–}5.0\ M \text{ fast}]{\text{large excess } D_2O} \underset{\text{II}}{(CH_3)_3NBD_3}$$

The homogeneous reaction of I in dilute deuteriochloric acid first produces II which can be extracted with ether or allowed to hydrolyze slowly to produce pure deuterium gas.



$$(CH_3)_3NBD_3 \xrightarrow[\text{slow}]{D_2O\ DCl} 3 \text{ moles } D_2$$

In keeping with these data the hydrolysis of trimethylamine borane-d_3 in hydrochloric acid produces only hydrogen gas.

No other simple derivatives of diborane have been reported to exchange with heavy water.[5] As I has been used to reduce carbonyl compounds and hydroborate olefins,[6–8] the exchange reaction now allows convenient reductive deuteriations using heavy water as the source of deuterium. Norcamphor, benzophenone, cyclohexanone and acetone have been reduced to the α-deuterio alcohols using II and boron trifluoride etherate.[7] Deuterioborations have been performed in refluxing toluene.[8] Trimethylamine amine borane-d_3 also has been converted into sodium borodeuteride using sodium methoxide in diglyme.

Large amounts of trimethylamine-d_3 are obtained readily using this procedure: sulfuryl chloride (0.50 ml.) is vigorously stirred for twenty minutes with 20 ml. of heavy water. Trimethylamine borane (Callery Chemical Co., 1.000 g., 13.9 mmole) dissolved in fifty ml. of ether is vigorously stirred with the acidic heavy water at 25°. The exchange reaction is followed by infrared analysis. The extent of hydrolysis is determined manometrically. After six hours the deuterium content of the amine borane is 98% while less than 6% has hydrolyzed. The ether layer is dried over potassium carbonate.[9] Evaporation *in vacuo* leaves a white residue which is sublimed. The product weighs 0.997 g. (m.p. 94°, undepressed with I, 98.1% deuterium by mass spectrum).

These new procedures should greatly facilitate the synthesis of compounds labeled with deuterium or tritium with known stereochemistry to aid in the elucidation of reaction mechanisms.

Acknowledgment.—The present study was supported by a Frederick Gardner Cottrell grant from the Research Corporation and a grant from the Sloan Foundation. Discussion with Prof. R. W. Parry is appreciated.

(1) Boron Hydrides. VI. Paper V, R. E. Davis and J. A. Gottbrath, *J. Am. Chem. Soc.*, **84**, 895 (1962).
(2) G. E. Ryschkewitsch, *ibid.*, **82**, 3290 (1960).
(3) R. E. Davis, C. L. Kibby and C. G. Swain, *ibid.*, **82**, 5950 (1960).
(4) R. E. Davis, *ibid.*, **84**, 892 (1962).
(5) Decaborane will produce $B_{10}H_{10}D_4$: G. A. Guter and G. W. Schaeffer, *ibid.*, **78**, 3546 (1956); R. Atterberry, *J. Phys. Chem.*, **62**, 1457 (1958); R. J. F. Palchak, J. H. Norman and R. E. Williams, *J. Am. Chem. Soc.*, **83**, 3380 (1961). The $B_{12}H_{12}^{-2}$ ion will completely exchange with heavy water (E. L. Muetterties, R. D. Menifield, H. C. Miller, W. H. Knoth, Jr., and J. R. Downing, *ibid.*, **84**, 2506 (1962)). Neither has found application as a useful reducing agent in organic chemistry.
(6) E. C. Ashby, *ibid.*, **81**, 4791 (1959).
(7) W. M. Jones, *ibid.*, **82**, 2528 (1960).
(8) H. C. Brown, K. J. Murray, L. J. Murray, J. A. Snover and G. Zweifel, *ibid.*, **82**, 4233 (1960).
(9) This solution can be used directly since the amount of II can be calculated from the amount of gas produced.
(10) Alfred P. Sloan Fellow, 1962–1964.
(11) This study was begun by Miss A. E. Brown as an undergraduate research problem.
(12) National Science Foundation Predoctoral Cooperative Fellow, 1961–1963.

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RECEIVED JANUARY 14, 1963

ISOLATION OF ANTIBODY BY MEANS OF AN IMMUNOLOGICAL SPECIFIC ADSORBENT

Sir:

As the immunochemist strives for a better understanding of the physicochemical factors involved in antigen–antibody interactions, the need for purified reactants becomes apparent. In his search he has devised a number of non-specific and specific techniques to obtain purified preparations of antibody. The non-specific methods are based on the fractionation of serum to provide a purified preparation of γ-globulin, and include such methods as ammonium sulfate[1,2] or alcohol fractionation,[3] electrophoresis[4] and column chromatography on DEAE-cellulose.[5] Although these techniques provide fairly pure γ-globulin preparations the specific immune γ-globulin desired may be only a small percentage of the total protein in the purified preparation. Hence, one obtains a good yield but low purity with respect to antibody.

The specific methods of antibody purification involve the removal of antibody by precipitation with the specific soluble antigen or reaction of antiserum with antigen in some insoluble state, and subsequent dissociation of the antibody from the precipitate by one of several methods.[6–9] Many of these specific methods depend upon some special property (*i.e.*, insolubility of the antigen in high salt concentration, mercurial salts, etc.) of the particular antigen–antibody system and therefore limit their applicability. The more general method of alkaline dissociation of antigen–antibody precipitates often leads to denaturation of the antibody, and particularily upon prolonged contact with the antibody. The method of acid dissociation[10] of antigen–antibody precipitates at pH 3.0 has been used by many investigators, and seems to be the most applicable. However, the soluble protein antigens must be rendered insoluble by some other procedure such as

(1) R. A. Kekwick, *Biochem. J.*, **34**, 1248 (1940).
(2) E. J. Cohn, T. L. McMeekin, J. L. Oncley, J. M. Newell and W. L. Hughes, *J. Am. Chem. Soc.*, **62**, 3386 (1940).
(3) E. J. Cohn, J. A. Luetscher, J. L. Oncley, S. H. Armstrong and B. D. Davis, *ibid.*, **62**, 3396 (1940).
(4) A. Tiselius, *Biochem. J.*, **31**, 1464 (1937).
(5) H. A. Sober, F. G. Gutter, M. M. Wyckoff and E. A. Peterson, *J. Am. Chem. Soc.*, **78**, 756 (1956).
(6) M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **64**, 161 (1936).
(7) M. Heidelberger and E. A. Kabat, *ibid.*, **67**, 181 (1938).
(8) L. A. Steinberger and D. Pressman, *J. Immunol.*, **65**, 65 (1950).
(9) D. H. Campbell, R. H. Blaker and A. B. Pardee, *J. Am. Chem. Soc.*, **70**, 2496 (1948).
(10) D. H. Campbell and F. Lanni in D. M. Greenberg, "Amino Acids and Proteins," 1st ed., Charles C. Thomas, Springfield, Ill., Chapter XI, 1951.

selective precipitation,[11] or modification of the antigen.[8,12]

An approach to the isolation of antibody against a soluble protein antigen was suggested by Campbell, Leuscher, and Lerman.[13] They coupled a soluble protein antigen to an insoluble, modified (*p*-aminobenzyl-) cellulose by means of a diazonium bond. This provided an insoluble protein antigen which could specifically combine with antibody and upon acidification to pH 3.2 be separated by centrifugation into soluble antibody and insoluble antigen. This method provided a yield of 57% of the total antibody present in the original serum as determined by quantitative precipitation with specific antigen. The purity (precipitable antibody/total protein) of the soluble material recovered by specific adsorption and subsequent elution was 90%.

This problem of immune adsorbents was re-investigated in our Laboratory as a result of commercially available *p*-aminobenzylcellulose, Cellex-PAB, (Bio-Rad Lab., Richmond, Calif.) and the coupling of a simple haptenic material, arsanilic acid, to cellulose (ICN Corp., City of Industry, Calif.). The method employed in our study involves the use of an antigen "fixed" by a diazo bond to the commercially available cellulose to which the *p*-aminobenzyl group has been coupled. After exposure of the appropriate antiserum to the antigen-coupled Cellex, the column was washed prior to elution of antibody with a glycine–HCl buffer pH 3.0. Utilizing this method we have isolated rabbit antibodies to (1) bovine serum albumin (BSA), (2) whole ragweed pollen extracts (WRE), (3) a highly purified Timothy pollen fraction[14] and (4) a simple hapten (arsanilate).

In order to couple the protein antigens to the Cellex-PAB diazotization was carried out at 0° with 2 *N* HCl and 14% $NaNO_2$ for 1 hour. The diazotized Cellex-PAB was filtered and washed successively with 5% sodium acetate, 5% urea, and distilled water. Coupling of the antigen was accomplished by addition of the diazotized Cellex to the protein solution at pH 8, and the suspension was magnetically stirred overnight at 2°. The non-reacting antigen was recovered by filtration, and the Cellex was further coupled with β-naphthol to block any unreacted free diazonium sites. The antigen-coupled Cellex was washed successively with distilled water, glycine–HCl buffer, pH 3.0, and was then readjusted to pH 7.25 with 0.1 *N* NaOH. The immune adsorbent then was poured as a slurry into a 1 × 10 cm. column, packed by gravity at 2°, and washed with an appropriate buffer (pH 7.25).

The isolation of purified rabbit anti-whole ragweed pollen extract (AWRE) antibodies from a whole ragweed-coupled Cellex (WRE) column was accomplished in the following manner. To 1 g. samples of WRE coupled-Cellex was added 20 ml. of rabbit immune serum containing 50 mg. of antibody protein precipitable by WRE. The time required for the serum to pass through the column was 1–2 hours. The column was then washed with 50–100 ml. of a citrate–phosphate buffer, pH 7.25, in order to remove all non-specific proteins. The specifically bound antibody then was dissociated from the immune adsorbent with a glycine–HCl buffer, pH 3.0. Five 5-ml. fractions were collected, and adjusted to pH 7.4 with 0.1 *N* NaOH. The presence of antibody in the collected fractions was determined by ring tests and a quantitative precipitin test. The recovery of protein from the eluted fractions varied from 24–35 mg., which represented a yield of 46–70%, and 83% of the recovered protein was precipitable with specific antigen.

The same general procedure was used in the isolation of rabbit antibodies directed against bovine serum albumin, arsanilate hapten, and a highly purified Timothy pollen fraction. It also has been applied to the isolation of human reaginic antibody from non-treated Timothy sensitive patients,[15] and these results will be presented in more detail in a subsequent publication.

This work was supported by a grant from the U. S. Public Health Service; Grant No. AI-01339.

(11) S. I. Epstein, P. Doty and W. C. Boyd, *J. Am. Chem. Soc.*, **78**, 3306 (1956)

(12) S. J. Singer, J. E. Fothergill and J. R. Shainoff, *ibid.*, **81**, 2277 (1959).

(13) D. H. Campbell, E. Leuscher and L. S. Lerman, *Proc. Natl. Acad. Sci. U. S.*, **37**, 575 (1951).

(14) A. Malley, A. Lietze and C. E. Reed, *J. Allergy*, **33**, 84 (1962).

(15) A. Malley, *Federation Proc.*, **21**, No. 2, 14 (1962).

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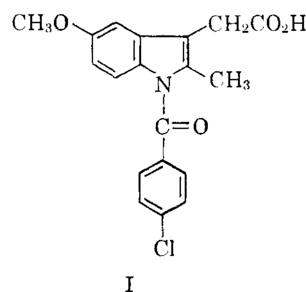
RECEIVED DECEMBER 19, 1962

NON-STEROID ANTI-INFLAMMATORY AGENTS

Sir:

We wish to report a new class of anti-inflammatory and antipyretic agents, substituted indole acetic and propionic acids. Of some three hundred and fifty indole derivatives studied, one member of the series, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid (I), designated as indomethacin, has demonstrated a high degree of anti-inflammatory activity both in the granuloma inhibition assay[1] and in the foot-edema



I

assay.[2] Indomethacin was equally active in intact or adrenalectomized animals and was also active by local application directly to the cotton pellets. Its anti-inflammatory potency relative to phenylbutazone varied from ten to eighty-five times depending on the test employed.[3] Fever induced in rabbits by intravenous injection of pyrogenic lipopolysaccharide prepared from *E. coli* was effectively blocked by I subcutaneously administered. Antipyretic potency of I was approximately 10 times that of aminopyrine and 20 times phenylbutazone, with duration of action resembling the latter compound. In animals indomethacin is relatively free of activities referable to central nervous, autonomic or cardiovascular systems.

For the synthesis of indomethacin, 5-methoxy-2-methylindole-3-acetic acid[4] was converted to its anhydride with dicyclohexylcarbodiimide in tetrahydrofuran. The anhydride was treated with zinc chloride and *t*-butanol to give *t*-butyl 5-methoxy-2-methyl-3-indolylacetate, m.p. 110–111°. Acylation of the *t*-butyl ester with *p*-chlorobenzoyl chloride afforded *t*-butyl 1-(*p*-

(1) R. Meier, W. Schuler and P. Desaulles, *Experientia*, **6**, 469 (1950), as modified by C. A. Winter and C. C. Porter, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 515 (1957).

(2) C. A Winter, E. A. Risley and G. W. Nuss, forthcoming publications.

(3) Preliminary clinical reports of indomethacin indicate that its potency in man is about 0.25–0.5 that of cortisone.

(4) E. Shaw, *J. Am. Chem. Soc.*, **77**, 4319 (1955).