

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 fraction¹⁴ 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% NaNO₂ 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.

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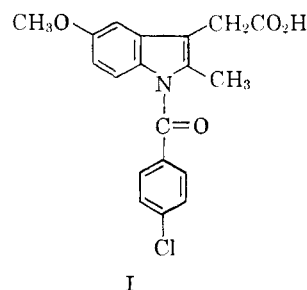
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CONTRIBUTION NO. 2920
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NON-STEROID ANTI-INFLAMMATORY AGENTS

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 assay¹ and in the foot-edema



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 acid⁴ 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*-

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(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).

chlorobenzoyl)-5-methoxy-2-methyl-3-indolylacetate, m.p. 103–104°. (*Anal.* Calcd. for $C_{23}H_{24}NO_4Cl$: C, 66.74; H, 5.84. Found: C, 67.04; H, 5.93.) Since the N-benzoyl group was rather labile toward acidic or alkaline cleavage, the acylated *t*-butyl ester was pyrolyzed at 210° to give indomethacin, m.p. 153–154°. (*Anal.* Calcd. for $C_{19}H_{16}NO_4Cl$: C, 63.78; H, 4.50; N, 3.91. Found: C, 63.14; H, 4.65; N, 4.10) $\lambda_{max}^{E:OH}$ 230 m μ (20,800), inf. 260 m μ (16,200), and 319 m μ (6,290).

The mechanism of the anti-inflammatory activity of these compounds is not yet understood. The activ-

ity observed with adrenalectomized animals would indicate that the pituitary-adrenal axis is probably not involved. Further studies are in progress.

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BOOK REVIEWS

Tables of Chemical Kinetics. Homogeneous Reactions. (Supplementary Tables.) National Bureau of Standards Monograph 34. C. H. STAUFFER, Project Director. Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. 1961. 459 pp. 23 × 29 cm. Price, \$2.75.

This monograph contains tables of chemical kinetics supplementary to the previously published National Bureau of Standards Circular 510 and Supplements 1 and 2. The present supplement contains information pertaining to substitution, exchange and elimination reaction types.

The user of these tables would probably be wise to browse generally through the volume before seeking specific information, since the classification system will not be immediately obvious to all chemists. For example, nucleophilic displacements on carbon attached to the nitrogen of a quaternary ammonium ion are listed as eliminations from a Vth group element. Reactions involving the transfer of hydrogen, activated by electron-withdrawing substituents, from carbon to a base are also listed as elimination reactions. The reader will, however, experience very little difficulty in accommodating himself to the classification system used and should realize that no system would be equally acceptable to all chemists.

The present compilation is incomplete and more material in the groups covered is being prepared for future publication. As in previous volumes, only the chief American and British periodicals are covered completely, but articles cited as references in these primary sources are also included. All of the tables cover this literature through 1955 and some contain references as recent as 1959.

The tables contain appended comments which are concise, judicious and pertinent. This is a careful and critical compilation and a welcome addition to the previously published tables.

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Absorption Spectra and Chemical Bonding in Complexes. By C. K. JØRGENSEN, Cyanamid European Research Institute, Cologne, Geneva, Switzerland. Addison-Wesley Publishing Company, Inc., Reading, Mass. 1962. xii + 352 pp. 15 × 22.5 cm. Price, \$10.00.

The inorganic chemist with a major interest in transition metals and their complexes, the "non-nuclear" physicist, and the spectroscopist will find Jørgensen's "Absorption Spectra and Chemical Bonding in Complexes" one of the most useful and instructive volumes published to date in transition metal chemistry. It is most fortunate for those in the United States with research interests in the above-defined areas to have a book written by a chemist of Jørgensen's stature in English.

Some of the expected differences (U. S. *vs.* Continental) in notation are worth mentioning, although the reviewer feels that certain of the notations used are more typical of the author than of general practice in Europe. The reader must acclimate himself to the Kayser (K or $ks.$ = 1 cm.⁻¹) as an energy unit. The author has been consistent in converting all energy terms to the kilokayser. Another not completely familiar term is "nephelauxetic" to describe an electron expansion effect leading to cation and

anion series resembling the spectrochemical series. A full chapter is devoted to this effect. The symbol U is used for electrochemical potential with the signs reversed from the Latimer convention. In place of ΔF the symbol ΔG is used for free energy change. The Rydberg constant is referred to as "ry," a logical choice since no confusion is introduced with the gas law constant. The schematicism of a line for an electron pair in Lewis octet structure is referred to as "valency stroke," a term not familiar to this reviewer. Shell and subshell seem to be used interchangeably in elementary electron structure. There are some instances of unusual notation of complex species such as for the nickel(II) and cobalt(III) complexes written in the form of $Ni(CN)_2, NH_3, C_6H_6$ and $Co(NH_3)_6, SO_4^+$.

The author makes no apology for the mathematics included and used in developing bonding, structure and spectra, which in a manner sets this volume apart from another recent treatment on transition metals. The adherents of hybridization and valence bond theory will have strong feelings about Jørgensen's treatment since it points up the incompatibility of these concepts with absorption spectra interpretation; also that hybridization is only one very special case of molecular orbital theory. Incidentally, the author extends a plea for a wider adoption of visible and ultraviolet spectra in interpreting bonding in view of the relative simplicity of the instrumentation and use of aqueous solutions.

What can and what cannot be accomplished by M.O. treatment is clearly defined in some eleven "theses." The M.O. treatment of metal-metal bonds is not considered because of the "great complexities" arising. The reader is thus left with the feeling that interpreting spectra of complexes is free of these complexities.

The bibliography, though quite complete, is not always of high utility. The listing of all references is at the end of the volume by a number. On many occasions specific references are made by author with no number, making it difficult for the reader to decide which, if any, of several articles should be consulted. It is surprising in one instance that Bailar's work in the use of activated charcoal for complex cobalt systems is not mentioned, though Bjerrum's work on this subject is.

Although, in general, the clarity of presentation is beyond criticism, there is a disturbing number of instances where sentence structure is chopped by the too frequent use of phrases and ideas set off by parentheses. General effectiveness of presentation would be improved by devoting the full sentence to such thoughts.

There is an abundance of tremendously useful information incorporated into tables not found in any other single source. A useful interrelation is found of the various U (or E_0) values with the oxidation states of ions obtained in a 1 *M* non-complexing acid solution. To illustrate, for chromium the (II) state exists at -0.5 v., the (III) state at 0.0 +0.5 and +1.0 v., while the (VI) state is obtained at +1.5 v. Such information is given for 97 elements. Tables with spectral summary data are available in a form of high utility for general interpretation. Spectra are tabulated (among other ways) according to the three transition arrangements 3d^q, 4d^q and 5d^q, with increasing q -values for each group and also in accord with increasing oxidation number of the metal. References to the original literature are given in these tables in a rather unusual fashion. When the author agrees with the data a certain superscript notation is used, different from that used when there is a disagreement.