have made theoretical calculations of the $1/R^3$ force modifications of the spectrum to be expected for myristamide (using both of the angles given in Fig. 1). These calculations support the interpretation of the 2120–2250 Å. absorption as a crystal transition, in that the long-wave-length allowed component is predicted to occur in the *a* axis direction, split off by *ca.* 100 to 150 Å., just as observed (and regardless of which angle is chosen).

We have also made quantum-theoretical calculations of the direction of the transition moment for an amide model. In the model an amide is regarded as a perturbed allyl anion[5] with the nitrogen represented by a deepening of the coulomb potential on one of the end carbon atoms and the oxygen considered as being the same as carbon. The result is that the direction should be close to the nitrogen–oxygen line, but inclined toward the carbon–nitrogen line. Of the two values found in our experiments the one with an angle of 9.1° from the nitrogen–oxygen line is thus favored. Both the experimental and theoretical aspects of this investigation will be reported in detail in a forthcoming paper.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON

D. L. PETERSON[6]
W. T. SIMPSON

RECEIVED JUNE 13, 1955

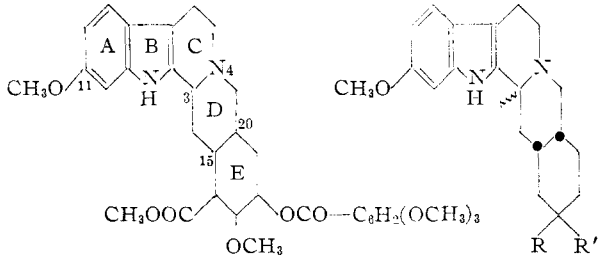
(5) Calculation was facilitated by use of full configuration interaction A.S.M.O. calculations on allyl anion (H. D. Hunt, D. L. Peterson and W. T. Simpson, to be published).

(6) National Science Foundation Predoctoral Fellow, 1954–1955.

THE D/E *CIS* RING JUNCTURE OF RESERPINE

Sir:

Reserpine, the potent hypotensive agent obtained from various species of Rauwolfia, has been assigned the structure I.[1,2] Because of its proxi-



I

IIa, b

mate relation to deserpidine (formula I with the 11-methoxyl replaced by −H), Schlittler, *et al.*, surmised that reserpine too possesses a D/E *cis* (allo) ring juncture[3]; and Wintersteiner, *et al.*,[4] on the basis of the intramolecular N-4 quaternization of methyl reserpate tosylate, inferred the same relationship. We wish to report for this view conclusive confirmation obtained through an 11-methoxyalloyohimbane (IIa, R = R′ = H) (reserpane) synthesis which is stereochemically unambiguous insofar as the C_{15}-C_{20} asymmetric centers are concerned.[5]

6-Methoxytryptamine[6] (obtained through the route: 6-methoxyindole → 6-methoxygramine methosulfate → 6-methoxyindoleacetonitrile → 6-methoxytryptamine) was alkylated in boiling dimethylformamide by ethyl *dl-cis*-2-bromomethylcyclohexaneacetate,[7,8] affording the lactam of *dl-cis*-N-(β-3′-indolylethyl)-2-aminomethylcyclohexaneacetic acid (III) (benzene solvate), m.p. 72.5–74.0° (Calcd. for $C_{20}H_{26}N_2O_2 \cdot C_6H_6$: C, 77.19; H, 7.97. Found: C, 77.02; H, 7.95). Heating III with phosphorus oxychloride in benzene, followed by platinum-catalyzed reduction of the unisolated Δ^3-ring-closed product, yielded the desired *dl*-allo base IIa, which melted at 209–210° after crystallization from methanol. Infrared spectral comparison of chloroform solutions of IIa and a reserpane derived by reduction from reserpone (IIb, R, R′ = O)[9] showed the two substances to be, apart from the racemic nature of the former, identical.[10]

Publication of our views on the nature of the remaining asymmetric centers in reserpine and deserpidine, including evidence relating thereto, is anticipated.

Acknowledgment.—The authors wish to express their gratitude to the Department of Health, Welfare and Education for financial support (Grant No. G-3892) and to S. B. Penick and Co. for a gift of reserpine.

(5) The matter of the stereochemistry at C_3 in our synthetic product is deferred for the present.

(6) S. Akabori and K. Saito, *Ber.*, **63B**, 2245 (1930).

(7) G. Stork and R. Hill, THIS JOURNAL, **76**, 949 (1954).

(8) Unpublished results obtained in this Laboratory.

(9) C. F. Huebner, H. B. MacPhillamy, A. F. St. André and E. Schlittler, THIS JOURNAL, **77**, 472 (1955).

(10) We should like to thank Dr. Schlittler and Dr. St. André for their assistance in establishing the identity of the two specimens. In addition, our base IIa was shown to be identical with material obtained by them *via* a different synthetic route.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

EUGENE E. VAN TAMELEN
PAUL D. HANCE
KENNETH V. SIEBRASSE
PAUL E. ALDRICH

RECEIVED MAY 3, 1955

POLY-GLUTAMYL PTERIDINE COENZYMES

Sir:

It was recently demonstrated that the conversion of serine to glycine by a bacterial extract is dependent upon DPN, Mn^{++}, pyridoxal phosphate, orthophosphate and catalytic levels of a new coenzyme, Co C.[1,2] Co C is isolated from *Clostridium cylindrosporum*, and substitutes for but is not identical with known folic acid derivatives. By means of fractional acetone precipitation, chromatography on cellulose columns, and repeated paper chromatography in various solvent systems,[3] five groups of pteridine derivatives with Co C activity have been separated in relatively pure form from extracts of

(1) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Müller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

(2) N. Neuss, H. E. Boaz and J. W. Forbes, THIS JOURNAL, **76**, 2463 (1954).

(3) H. B. MacPhillamy, L. Dorfman, C. F. Huebner, E. Schlittler and A. F. St. André, *ibid.*, **77**, 1071 (1955).

(4) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, *ibid.*, **77**, 2028 (1955).

(1) B. E. Wright, *Biochim. et Biophys. Acta*, **16**, 165 (1955).

(2) B. E. Wright, *Fed. Proc.*, **14**, 308 (1955).

(3) B. E. Wright and E. R. Stadtman, unpublished data.

TABLE I

Abbreviations: P_{stable}, $P_{tot.-1}$ — P_{10} plus P_{labile}; P_{10}, phosphate liberated after 10 minutes of boiling in 1.0 N HCl, minus P_{labile}. P_{labile}, phosphate fully liberated after 20 minutes under conditions of orthophosphate determination by the Fiske–SubbaRow method. This phosphate is stable in the Lowry–Lopez method. No inorganic phosphate was present in any sample.

Co C type	Relative coenzymatic activity[18] Type I = 100%	$\lambda_{Max.}$ mμ	$\epsilon_{Max.}$[a]	Moles pentose	P_{total}[16]	P_{stable}	P_{10}	P_{labile}	Amino acids present[c] Moles glutamic[a]	Moles total other amino acids[a]	Other amino acid in Order of Concn.
I	100	286	27,900	None	None	None	None	None	2.6	0.37	Glycine, serine
II	80	268	25,000	1.0	1.28	1.0	0.25	None	2.7	0.50	Glycine, alanine, serine
III	50	265	24,000	0.85	1.0	1.0	None	None	>1.3[b]	>0.19	Glycine, serine
IV	40	280	27,900	None	None	None	None	None	6.1	2.0	Glycine, "x," serine
V	40	265	26,000	0.85	4.05	2.0	1.05	1.05	5.95	3.9	Alanine, "x," glycine, serine

[a] The molecular extinction coefficient of the unphosphorylated types I and IV is assumed to be that for C.F. (= 27,900); the others are calculated on stable phosphate content. Values for labile phosphate, pentose and amino acid content per mole are based on these molecular extinction coefficients. [b] Part of this sample was lost. [c] Amino acids were determined quantitatively after acid hydrolysis by conversion to their DNP derivatives and subsequent chromatography[14]; pentose was determined according to the procedure of Mejbaum.[15]

C. cylindrosporum.[4] These fractions are considered to be polyglutamyl pteridine compounds which contain other amino acids as parts of the molecule. Chemical data characterizing various types of Co C are given in Table I, which includes a triglutamyl derivative (I), a triglutamyl and a diglutamyl form with one pentose and one stable phosphate (II) and (III), a hexa- or heptaglutamate derivative (IV), and a hexa- or heptaglutamate with one pentose, two stable and two labile phosphates (V). All types have a blue fluorescence except V, which is yellow in color and fluorescence. The fluorescence is enhanced under alkaline conditions.

Co C types I–V are listed in Table I in order of decreasing coenzymatic activity on a molar basis. Compound I is the most fully characterized. It has the ultraviolet spectrum of C.F. (with a maximum at 286) at neutral *p*H and shows the isoleucovorin characteristics (maximum at 355) in 0.1 *N* HCl.[5] The infrared spectrum is practically superimposable upon that for C.F.,[6] suggesting that I is closely related to triglutamyl C.F., some species of which contain amino acids other than glutamic acid.[7a] Microbial assay of the Co C derivatives gave additional evidence for a pteridine structure.[8]

Compounds II, III and V keep the same ratio of pentose to ultraviolet absorption after chromatography in two solvent systems (pyridine–propanol–H_2O, 1:1:1 and EtOH–M/1 NH_4OH, 2:1). Preliminary evidence that pentose and phosphate are an integral part of these coenzymes was obtained by showing that when compound III (R_f = 0.32 in EtOH solvent system) is treated with phosphomonoesterase, about 50% can be recovered as a new compound (R_f = 0.57) which retains one pentose per mole. This compound has an ϵ_{max} = 260 mμ and is not coenzymatically active. No pentose was recovered from the chromatogram of the incubated sample at R_f = 0.32 nor from the control sample at R_f = 0.57.

These cofactors containing varying amounts of glycine and serine, and in some cases alanine and an unidentified amino acid, "x," which is not identical with any amino acid normally found in protein hydrolysates.[12] Chromatographic evidence shows that besides glycine, alanine and "x" are also formed from serine in the presence of Dowex-treated, dialyzed enzyme. The enzymatic conversion of serine to glycine, alanine and compound "x" are all dependent on the presence of Co C. It is clear that the Co C types listed in Table I represent mixtures of species with respect to the amino acids other than glutamic acid. This is not surprising in view of the fact that only in the last stage of purification did the tri- and hexa-glutamates separate. Quantitative ninhydrin determinations on I, II and IV before hydrolysis revealed one free amino group

(4) It is now clear that the forms of Co C described previously[1] correspond to compounds I and IV, Table I. The forms with 280 absorption arise at least in part from the 260 absorbing forms during purification.

(5) D. B. Cosulich, B. Roth, J. M. Smith, Jr., M. E. Hultquist and R. P. Parker, THIS JOURNAL, **74**, 3252 (1952).

(6) Infrared records of Co C I and C.F. were generously run by Dr. D. Anderson of Eastman Kodak Company.[7]

(7) D. H. Anderson and N. B. Woodall, *Anal. Chem.*, **25**, 1906 (1953).

(7a) It is possible that this compound is similar to one described by Ericson,[7b] in spite of differences in activity for *Streptococcus faecalis R*.

(7b) L. E. Ericson, *Arkiv. för Kemi*, **6**, 503 (1953).

(8) When various forms of Co C were assayed for microbiological activity using *Streptococcus faecalis R* and *Leuconostoc citrovorum*,[9] activity was found only for the latter organism, and the growth curve was unlike that for C.F.[10] Digestion with γ-glutamic acid carboxypeptidase[11] gave significant increases of C.F. activity.

(9) M. Silverman and B. E. Wright, unpublished data.

(10) M. E. Swendseid, P. D. Wright, F. H. Bethell, *Proc. Soc. Exper. Biol. and Med.*, **80**, 689 (1952).

(11) H. Kazenko and M. Laskowski, *J. Biol. Chem.*, **173**, 217 (1948).

(12) An unidentified dicarboxylic acid was described by Ratner, Blanchard and Green,[13] in a PAB conjugate containing 10–11 glutamyl residues.

(13) S. Ratner, M. Blanchard and D. E. Green, *J. Biol. Chem.*, **164**, 691 (1946).

(14) A. L. Levy, *Nature*, **174**, 129 (1954).

(15) W. Mejbaum, *Z. physiol. Chem.*, **258**, 117 (1939).

(16) The stable phosphate on compound II and one phosphate of V (not P_{labile}, which is liberated during orthophosphate determination) is removed by partially purified human semen phosphomonoesterase. Kornberg's purified potato pyrophosphatase[17] releases one phosphate from V suggesting a free pyrophosphate linkage. Combining the pyrophosphatase and monoesterase removes phosphate quantitatively from V.

(17) A. Kornberg and W. E. Pricer, Jr., *J. Biol. Chem.*, **182**, 763 (1950).

(18) % activity = μM. glycine produced per hour × 1000 when 0.005 μM. of Co C is incubated with 4 units of enzyme under previously defined assay conditions.[1]

on IV but none on I and II. After hydrolysis, total ninhydrin values corresponded to those obtained for the DNP derivatives.

Acknowledgments.—I would like to thank Dr. A. Kornberg for the purified nucleotide pyrophosphatase, Dr. L. Heppel for the phosphomonoesterase, and Dr. R. Redfield for assistance in chromatographic techniques.

LABORATORY OF CELLULAR PHYSIOLOGY
NATIONAL HEART INSTITUTE BARBARA E. WRIGHT
NATIONAL INSTITUTES OF HEALTH
BETHESDA 14, MARYLAND

RECEIVED APRIL 16, 1955

BOOK REVIEWS

Advances in Carbohydrate Chemistry. Volume 9. By MELVILLE L. WOLFROM, Editor, R. STUART TIPSON, Assistant Editor, and E. L. HIRST, Associate Editor for the British Isles. Academic Press, Inc., Publishers, 125 East 23rd Street, New York 10, N. Y. 1954. xviii + 426 pp. 16 × 23 cm. Price, $10.50.

It is most appropriate that this, the latest volume of a distinguished series, should begin with a biographical sketch of the late C. S. Hudson, written by the present editor, M. L. Wolfrom. Not only did Professor Hudson play a major part in the development of carbohydrate chemistry in this country but he was also closely associated in various capacities with this series of reviews from its inception until his death in 1952.

The present volume, like its predecessors, reminds the writer of the medieval *Speculum Alchemiae*, for it is a kind of mirror, a mirror of the state of carbohydrate chemistry, and affords even the most casual reader an opportunity to see what areas of this fertile field are being tilled most intensively. In this light two aspects of the latest volume seem particularly worthy of note. First, several of the contributions illustrate the gratifying extent to which modern theories of the mechanism of organic reactions have been applied in the carbohydrate field and, second, the volume, taken as a whole, caters to a surprisingly broad spectrum of interests. The organic chemist concerned with reaction mechanisms will find a chapter by R. U. Lemieux ("Some Implications in Carbohydrate Chemistry of Theories Relating to the Mechanisms of Replacement Reactions"), one by Clinton E. Ballou ("Alkali Sensitive Glycosides") and a third by Mary Grace Blair ("The 2-Hydroxyglycals"). The worker in natural products will turn especially to Ballou's chapter as well as to one by Dexter French entitled "The Raffinose Family of Oligosaccharides." If he is among the increasing number who deal with the uronic acids, G. O. Aspinall's chapter on "The Methyl Ethers of Hexuronic Acids" will prove a useful compilation of important data which have heretofore been widely dispersed in the literature. He will also find a contribution by Robert S. Teague entitled "The Conjugates of D-Glucuronic Acid of Animal Origin" which will, of course, appeal as well to the biochemist and physiologist. One interest of the sugar technologist is represented by "Color and Turbidity of Sugar Products" written by R. W. Liggett and Victor R. Deitz. The chemist interested in the industrial utilization of carbohydrates will find a contribution by J. V. Karabinos and Marjorie Hindert on "Carboxymethylcellulose" while laboratory workers in several fields will note much of practical value in a review on the "Paper Chromatography of Carbohydrates and Related Compounds" by George N. Kowkabany. The present volume is, incidentally, the first of the series in which an attempt has been made to use the new carbohydrate nomenclature [*Chem. Eng. News*, 31, 1776 (1953)] throughout.

Taken as a whole, this volume, together with the preceding ones, forms a set which is of great utility to chemists generally and invaluable to those specializing in the carbohydrate field.

NATIONAL INSTITUTES OF HEALTH
BETHESDA 14, MARYLAND HEWITT G. FLETCHER, JR.

The Chemistry of Lipids of Biochemical Significance. By J. A. LOVERN. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1955. xiii + 132 pp. 11 × 17 cm. Price, $1.75.

This relatively short monograph is intended to give an over-all general picture of lipid chemistry and therefore does not fully cover either subject matter or bibliography. In general only major points are presented with a minimum amount of experimental detail. The monograph brings to focus many important aspects of the lipids in a manner that the reader is not bothered with less important minutia. Although the text covers lipid structure, preparation and analysis, a considerable portion is devoted to the distribution, dynamic state and biochemical functions of the lipids. Emphasis is placed on the phosphatides. The carotenoids and fat-soluble vitamins are not included in the monograph. It is the opinion of the reviewer that the title of the book might have been more appropriately chosen since a large part of the monograph deals with topics other than lipid chemistry.

The author gives constructive comments on lipid nomenclature and classification and indicates the need for more uniform and discrete terminology. The interrelationships of the various lipid classes are stressed and an attempt is made to integrate the entire lipid subject.

The reader will find the personal comments of the author interesting, helpful and provocative. An excellent evaluation of the various methods of lipid preparation and analysis is presented including an informative discussion on lipid extraction and purification. In addition, there is a stimulating discourse on the limitations of radioactive isotope techniques for the study of lipid metabolism. Topics such as lipid complexes with proteins and carbohydrates, and lipid digestion, absorption and biosynthesis are briefly covered.

The subject matter of the book is well presented and should have particular appeal to those interested in obtaining the essential highlights of the biochemistry of the major lipids. Moreover, the fine integration of the material should make the monograph especially useful to the beginner in the lipid field.

DEPARTMENT OF BIOCHEMISTRY
THE UNIVERSITY OF ROCHESTER G. V. MARINETTI
MEDICAL CENTER
ROCHESTER 20, NEW YORK

The Vitamins. Chemistry, Physiology, Pathology. Volume III. Edited by W. H. SEBRELL, JR., Director, National Institutes of Health, Bethesda, Maryland, and ROBERT S. HARRIS, Professor of Biochemistry of Nutrition, Massachusetts Institute of Technology, Cambridge, Massachusetts. Academic Press, Inc., Publishers, 125 East 23rd Street, New York 10, N. Y. 1954. xi + 665 pp. 16.5 × 23.5 cm. Price, $15.00.

This is the third and last volume of a series. It is, of course, difficult to review adequately one-third without reference to the other two thirds. In the whole series the vitamins are presented in alphabetical order so the biochemist, chemist and clinician, to whom the series is directed,