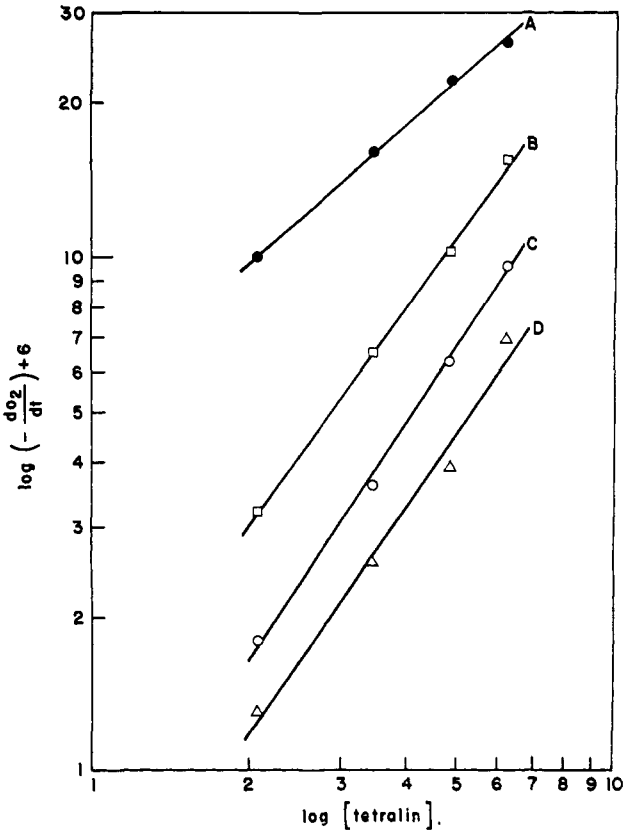

Fig. 1.---Dependence of rate of oxygen absorption on tetralin concentration at 60° and 37 × 10^{-3} *M* AIBN: A, no phenol, slope = 0.90; B, 7.6 × 10^{-4} *M* phenol, slope = 1.42; C, 23 × 10^{-4} *M* phenol, slope = 1.53; D, 38 × 10^{-4} *M* phenol, slope = 1.48.

assumptions and the further approximations

$$k_3[\mathrm{RH}] \gg k_5[\mathrm{AOH}]$$

and

$$k_6[\mathrm{RH}] \gg k_7[\mathrm{RO_2}\cdot],$$

then

$$-\frac{d\mathrm{O_2}}{dt} = k_3\left(\frac{k_6R_i}{2k_5k_7}\right)^{1/2}\frac{[\mathrm{RH}]^{3/2}}{[\mathrm{phenol}]^{1/2}}$$

Thus, the kinetic expression obtained for the phenol–tetralin system is consistent with a mechanism in which chain transfer,[5,6] reaction 6, is of considerable importance, with no necessity of invoking a peroxy radical–inhibitor complex. Other inhibitors whose kinetic behavior has been interpreted in terms of "complex formation" (*viz.*, N-methylaniline,[1] diphenylamine,[3] trialkylamines[4]) are substances which, like phenol, would give relatively unreactive monofunctional radicals and which therefore could restart chains by a similar chain transfer mechanism.

(5) W. A. Waters and C. Wickham-Jones, *J. Chem. Soc.*, 812 (1950).
(6) A. F. Bickel and E. C. Kooyman, *ibid.*, 2215 (1956).

CHEMISTRY DEPARTMENT
SCIENTIFIC LABORATORY
FORD MOTOR COMPANY
DEARBORN, MICHIGAN

LEE R. MAHONEY
FRED C. FERRIS

RECEIVED JUNE 13, 1963

The Selective Acetylation of Terminal Hydroxyl Groups in Deoxyribo-oligonucleotides[1]

Sir:

As a part of work on the sequential analysis of nucleic acids, recently, methods for the labeling of terminal phosphomonoester groups in polynucleotides have been reported from this Laboratory.[2,3] The present communication describes a method for the selective acetylation of the free hydroxyl groups, on the terminal nucleosides in deoxyribopolynucleotides. The approach thus promises to be complementary to those developed previously[2,3] for the labeling of end groups in nucleic acids.

TABLE I
RESULTS OF ACETYLATION OF 3′-HYDROXYL GROUPS IN MONO- AND OLIGONUCLEOTIDES

Compound[4]	Concentration (μmole/ml.)	Ac_2O (mmoles)	Yield	Assay
pT	0.72	1	63	1
d-pA	.70	1	85	1
d-pG	.70	1	88	1
d-pC	.70	1	71	1
		2	81	1
		3	96	1
pTpTpTpT	.18	1	55	1, 2
		3	70	1, 2
d-pApApApA	.18	1	75	1, 2
		3	80	1
d-pCpCpCpC	.18	1	73	2
		3	87	2
d-pApApApApApApA	.035	1	62	2

A solution of the mononucleotide or oligonucleotide (Table I) (0.07–1.4 μmoles of the sodium or ammonium salt) in water (2.0 ml.) was treated with acetic anhydride (0.1–0.3 ml.) at room temperature, the mixture being stirred by a magnetic stirrer. Acetic anhydride was added in portions of 0.01 ml., the pH of 7 maintained by the continuous addition of 4 *N* sodium hydroxide from a microsyringe. After the completion of the addition (about 15 min.), the nucleotidic material was freed from sodium acetate either by extensive dialysis against water (for oligonucleotides) or by passage of the total solution through a column of pyridinium Dowex-50 ion-exchange resin and lyophilization of the resulting solution. Two types of assays were used for following the extent of the acetylation reaction. In assay 1, the mixture was chromatographed in the solvent *n*-butyl alcohol–acetic acid–water (5–2–3) or ethyl alcohol–0.5 *M* ammonium acetate (pH 3.8) (7–3, v./v.). In the case of mononucleotides and the tetranucleotides pTpTpTpT[4] and *d*-pApApApA,[4] the 3′-*O*-acetyl derivatives were clearly resolved from the starting materials. The assay 2 involved incubation of the mixture with the *Escherichia coli* phosphodiesterase[5] in order to degrade the unchanged oligonucleotide to mono- and dinucleotides and to determine the acetylated product by paper chromatography. It has been demonstrated recently that acetylation of the 3′-hydroxyl group confers complete resistance on the oligonucleotide towards this enzyme.[6] The results are given in Table I. In general, the yields were high and probably can be increased further. It is worth noting that the yields in the case of the tetranucleotides and the one heptanucleotide studied showed no decrease.

Previous experience[7] of acylation of nucleotides in

(1) This work has been supported by grants from the National Cancer Institute of the National Institutes of Health, The National Science Foundation, Washington and The Life Insurance Medical Research Fund, New York, N. Y.

(2) R. K. Ralph, R. J. Young, and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 1490 (1962).
(3) R. J. Young and H. G. Khorana, *ibid.*, **85**, 244 (1963); U. L. Raj-Bhandary, R. J. Young, and H. G. Khorana, *Federation Proc.*, **22**, 350 (1963).
(4) The system of abbreviations is as in current use in *J. Biol. Chem.* and previously described. H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest," John Wiley and Sons, Inc., New York, N. Y., 1961.
(5) I. R. Lehman, *J. Biol. Chem.*, **235**, 1479 (1960).
(6) A. Falaschi, J. Adler, and H. G. Khorana, *ibid.*, in press.
(7) (a) H. G. Khorana, A. F. Turner, and J. P. Vizsolyi, *J. Am. Chem. Soc.*, **83**, 686 (1961); (b) R. K. Ralph and H. G. Khorana, *ibid.*, **83**, 2926 (1961).

anhydrous pyridine had shown the relative ease of N-acylation, particularly in the case of the N^6-amino group of the cytosine ring.[7a,8] In the present work, acetylation of the amino groups in the pyrimidine and purine rings was carefully looked for but none was detected. The most significant aspect of the aqueous acetylation technique described is its selectivity in acetylation of the terminal hydroxyl groups. Further, although most of the experiments so far have been carried out with oligonucleotides bearing 3′-hydroxyl (sec.) groups, preliminary work shows that the technique is promising also for the substitution of the terminal 5′-hydroxyl groups in polynucleotides.

Currently, we are investigating the use of C^{14}-labeled acetic anhydride in this technique.

(8) H. Schaller, G. Weimann, B. Lerch, and H. G. Khorana, *J. Am. Chem. Soc.*, in press.

INSTITUTE FOR ENZYME RESEARCH
THE UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

ALEXANDER STUART
H. G. KHORANA

RECEIVED JUNE 19, 1963

BOOK REVIEWS

Progress in the Chemistry of Organic Natural Products. Volume XX. Edited by L. ZECHMEISTER, California Institute of Technology. Springer-Verlag, Molkerbastei 5, Wien, Austria. 1962. xiii + 509 pp. 16 × 23.5 cm. Price, $23.00; leatherbound, $24.00.

"Zechmeister's Fortschritte" appeared first in 1938 and ever since has held a very prominent position among reports of progress in organic chemistry. All the succeeding volumes contain valuable reviews on novel classes of natural products, modern experimental techniques and articles of general biochemical interest. The present twentieth volume reaches the same high quality as its forerunners.

The recent, hectic development of the chemistry of the ubiquinones and plastoquinone is reviewed by Schindler. These lipophilic compounds, which are structurally related to the vitamins of the E- and K-groups, play an important role in biological electron transport systems, but, like acyclic oligoterpenes such as solanesol, they are also very intriguing from a purely chemical point of view. Careful investigations of other "unattractive" lipid fractions so often discarded in phytochemical studies are bound to be rewarding.

Mors, Taveira Magalhães and Gottlieb discuss styryl- and phenyl-α-pyrones from the unrelated genera *Piper* (Piperaceae) and *Aniba* (Lauraceae). The biosynthesis of the benzene ring of these compounds poses an interesting problem. A related substance, hispidin, has recently been isolated from a fungus, and a β-pyridyl analog, anibine, from an *Aniba* species.

Harborn contributes a chapter on anthocyanins and their glycosides. Recently the presence of these pigments in mosses (*Bryum*) has been definitely established. Hence anthocyanins have now been shown to occur in all "higher" plant divisions. Their absence in the angiosperm order Centrospermae, where they are replaced by betanidin and similar substances, is a matter of great systematic interest.

The at first weird-looking *Lycopodium* alkaloids now form a homogeneous, albeit isolated group of alkaloids confined to the ancient pteridophyte order Lycopodiales. The elucidation of their structures is largely due to Canadian chemists including Wiesner, the author of a chapter on these alkaloids. Conroy's hypothesis on the acetate origin of these alkaloids is also interesting in view of the occurrence of nicotine in some *Lycopodium* species. Narayanan has written an ambitious review on the steroidal alkaloids first isolated from *Veratrum* species, which are characteristic of a few related genera of the chemotaxonomically interesting family Liliaceae, renowned for its large variety of steroidal constituents.

The numerous nitrogen-containing metabolites of fungal origin are amply reviewed by Birkinshaw and Stickings. An outstanding contribution on aminosugars has been written by Baschang. Much of our knowledge in this field is the result of recent studies on antibiotics. The brillant work of R. Kuhn and his collaborators in Heidelberg on milk oligosaccharides and brain gangliosides is well summarized, and the chapter on aminosugars from polysaccharides and glycoproteids provides a vision of what is to be expected from future investigations in this immensely important field. There are no less than 430 references. Various uses of the ultracentrifuge technique in the study of macromolecules and viruses are discussed in an important contribution by Vinograd and Hearst.

A paper by Freudenberg on the structure of the lignins mainly deals with the views presently held in Heidelberg but is clearly influenced by current ideas in other centers of lignin research. A comparison with the article by the same author in Volume 11 of "Zechmeister" is recommended. Less emphasis is now laid upon coniferin and syringin which are rare natural compounds, and the insinuating *Araucaria* cross section is omitted. One must admire the interminable patience with which Freudenberg has struggled with the lignin problem for about forty years, and the great experimental skill with which the Heidelberg school has developed the reviewer's dehydrogenation hypothesis of 1933. (Discussed in more detail in "Research" 1950, a paper which is seldom referred to in lignin literature). The author, unfortunately does not strictly differentiate between "biosynthesis" and "biogenetic" theories, and the presumptuous statement on page 63 "Erst durch die Auffindung der oligomeren Zwischenprodukte der Biosynthese . . . wurde es möglich definierte Angaben über die Struktur grosser Teile des Naturstoffs zu machen" contains an overstatement obvious to every critical reader.

Still more speculative, but probably more universally enjoyable, is Horowitz and Miller's article on Current Theories on the Origin of Life, which ends with a chapter on "Space Research and the Origin of Life." May time be granted to Professor Zechmeister to include an article on extraterrestric natural products in a future volume of this excellent series.

Talking about space research, I have found that out of the 459 text pages of the present volume no less than about 70 are devoted to literature references containing the full titles of the relevant papers. Much space could be saved omitting the titles as well as by using Arabic instead of Roman numerals. Moreover, at least this reviewer finds it much easier to apprehend 88 than LXXXVIII.

DEPARTMENT OF CHEMISTRY
THE ROYAL INSTITUTE OF TECHNOLOGY
STOCKHOLM 70, SWEDEN

HOLGER ERDTMAN

Ionization Constants of Acids and Bases. A Laboratory Manual. By ADRIEN ALBERT, D.Sc. (London), F.R.I.C., F.A.A. Professor of Medical Chemistry in the Australian National University, Canberra, and E. P. SERJEANT, University of New South Wales, Sydney. John Wiley and Sons, Inc., 440 Park Avenue South, New York 16, N. Y. 1962. xxi + 179 pp. 14 × 20 cm. Price, $3.75.

The authors of this book state that "it is intended for those, who without previous experience, wish to determine an ionization constant." Judging the book in the framework of this stated aim, I believe the authors have been partially successful in writing a book which will accomplish this goal. The authors present extensive experimental details and numerical examples to illustrate the calculation of dissociation constants by potentiometric, spectrophotometric, and conductometric methods, and also touch upon the use of solubility techniques. Seven pages are devoted to a description of zwitterions, and a concise description of the effect of structure on the ionization constants of organic and inorganic acids and bases is presented in Chapter 8, along with a well documented compilation of 400 dissociation constants. The last chapter is a clear treatment of the application of the potentiometric pH method for the determination of stability constants of metal chelates.

In an introductory book it is obvious that compromises must be made in the interest of brevity. In this book, since it is a laboratory manual, considerably heavier emphasis has been placed upon the details of obtaining the experimental data than on the theory underlying the treatment of these data. In nearly all cases, however, enough literature references are given so that the interested student can expand on the theoretical background. There are several glaring omissions, *e.g.*, in the last chapter the discussion of other methods of determining complex ion stability constants is completely inadequate. Only seventy words are devoted to the mention of ligand and metal ion exchange, spectrophotometric, indicator, polarographic, and metal ion indicator electrode methods, giving only one literature refer-