The suggested formation of the phosphoranyl radical, IV, *via* ring-opening rearrangement of the initial radical addition product, III, rather than by direct addition of a radical to a trivalent phosphorus derivative[3] is certainly a unique involvement of such an intermediate.

Reaction 5 in which the phosphoranyl radical intermediate IV is trapped is closely related to one of the steps proposed[4] for reactions of trialkyl phosphites with CCl_4 (eq. 12 and 13) and also in the free-radical reactions of solutions of *n*-BuSH, $(EtO)_3P$, and CCl_4.[5] In the latter case, with equimolar portions of these reactants the following radical chain sequence was proposed (product yields in parentheses).

$$RS\cdot + (EtO)_3P \longrightarrow \underset{\text{VI}}{RS\dot{P}(OEt)_3} \quad (7)$$

$$VI \longrightarrow R\cdot + \underset{\text{VII (18\%)}}{(EtO)_3PS} \quad (8)$$

$$R\cdot + RSH \longrightarrow RH + RS\cdot \quad (9)$$

$$VI + CCl_4 \longrightarrow \underset{\text{VIII}}{RS\overset{+}{P}(OEt)_3\bar{Cl}} + Cl_3C\cdot \quad (10)$$

$$VIII \longrightarrow \underset{\text{IX (60\%)}}{RS\overset{O}{\overset{\|}{P}}(OEt)_2} + EtCl \quad (11)$$

$$Cl_3C\cdot + P(OEt)_3 \longrightarrow Cl_3C\dot{P}(OEt)_3 \xrightarrow{CCl_4} Cl_3C\overset{+}{P}(OEt)_3\bar{Cl} \quad (12)$$

$$X \longrightarrow EtCl + \underset{\text{XII (22\%)}}{Cl_2C\overset{O}{\overset{\|}{P}}(OEt)_2} \quad (13)$$

Substitution of $BrCCl_3$, a better chain-transfer agent, for CCl_4 should favor step 10 at the expense of scission step 8. Thus with $BrCCl_3$ and excess *n*-BuSH, which would be predicted to react with trichloromethyl radicals produced in (10) and hence to depress formation of XII *via* (12) and (13), an almost quantitative conversion of triethyl phosphite to IX was obtained.[5]

$BrCCl_3$ is evidently also a very efficient chain-transfer agent in the oxyphosphorane system, as the obvious β-scission cleavage alternative for IV is suppressed.

$$IV \longrightarrow (CH_3O)_3PO + Cl_3C-\dot{C}(CH_3)-\overset{O}{\overset{\|}{C}}-CH_3 \quad (14)$$

Even with equal amounts of $BrCCl_3$ and I diluted in chlorobenzene solvent at 116–120°, no appreciable buildup of trimethyl phosphate was noted by v.p.c. However the reaction was carried to only about 60% completion, and reaction 14 may become competitive at more reduced concentrations of reactants. These results emphasize the facility of IV formation *via* step 4 in competition with 1,2 addition.[6]

The most intense bands in the infrared of II are found at 1730 (C=O), 1290 (P=O), 1050, and 1010 cm.$^{-1}$ (POC). A weak band at 1355 cm.$^{-1}$ is evidence for the CH_3CO group. Further support for structure II is found in the n.m.r. spectrum, measured at 60 Mc.p.s. with assignments as shown in Figure 1. The two isolated methyls bonded to carbon appear as 3-H singlets at τ 7.98 and 7.49. The methoxyls, however, are magnetically nonequivalent and appear as a pair of doublets of equal intensity at τ 6.20 and 6.13 (Δ = 3.9 c.p.s.). The coupling constant J_{PH} for each is 11.5 c.p.s. The nonequivalence almost certainly results from the asymmetric carbon center in the molecule. Although long-range asymmetry effects have been noted previously,[7] we believe that a chemical shift difference of 4 c.p.s. is quite large[7a] for hydrogens separated from the interacting asymmetric carbon center by a distance of five bonds.[8] In addition we are unaware of any similar long-range effect of an asymmetric carbon center in a trialkyl phosphate molecule. These effects are being further evaluated in conjunction with more extensive studies of reactions of $BrCCl_3$ and other addenda with unsaturated oxyphosphoranes such as I.

Acknowledgment. The author thanks Dr. D. M Grant for helpful discussions of the n.m.r. data. This work was supported in part by the Petroleum Research Fund (2439-A4).

(3) See, for example, C. Walling and M. S. Pearson, *J. Am. Chem. Soc.*, 86, 2262 (1964), and previous papers; also J. I. G. Cadogan, *Quart Rev.* (London), 16, 208 (1962), for cases which illustrate the increasingly numerous postulations of phosphoranyl radical intermediates.

(4) C. E. Griffin, *Chem. Ind.* (London), 415 (1958); J. I. G. Cadogan and W. R. Foster, *J. Chem. Soc.*, 3071 (1961).

(5) P. J. Bunyan and J. I. G. Cadogan, *ibid.*, 2953 (1962).

(6) In contrast, vinylene carbonate readily undergoes radical-initiated 1,2-polymerization [N. D. Field and J. R. Schaefgen, *J. Polymer Sci.*, 58, 533 (1962), and references cited] as well as copolymerization without ring opening [H. L. Narder and C. Schuerch, *ibid.*, 44, 129 (1960)]. Decarboxylation on attempted copolymerization with maleic anhydride, however, has been reported: K. Hayashi, *Kyoto Daigaku Nippon Kagakuseni Kenkyusho Koenshu*, 15, 69 (1958).

(7) (a) G. M. Whitesides, D. Holtz, and J. D. Roberts, *J. Am. Chem. Soc.*, 86, 2628 (1964), have reported chemical shift differences for isopropyl methyls in a series of compounds containing the asymmetric center CH_3—CH—C_6H_5 at distances of three, four, five, six, and seven bonds from the isopropyl methyl hydrogens. In benzene the magnetic nonequivalences noted were correspondingly 8.0, 0.9, 0.5, 1.8, and 0.8 c.p.s. (b) F. Ramirez, O. P. Madan, and S. R. Heller, *ibid.*, 87, 731 (1965), note a magnetic nonequivalence of 10 c.p.s. for a pair of methoxyls of a ketophosphonate in which the carbon attached to phosphorus is asymmetric (the asymmetric center here being four bonds removed from the affected hydrogens). (c) Rather long-range effects of asymmetric phosphorus centers have also been reported: T. H. Siddall and C. A. Prohaska, *ibid.*, 84, 3467 (1962).

(8) The relatively large chemical shift difference noted in our case may be due to one or several of the obvious geometric and electronic differences between II and the ether, $(CH_3)_2CHCH_2O(CH_3)CHC_6H_5$, studied by Roberts.[7a] It is also interesting that Roberts and co-workers report an even greater nonequivalence for isopropyl methyl hydrogens six bonds removed from the asymmetric center than for those five bonds away.

Wesley G. Bentrude
Department of Chemistry, University of Utah
Salt Lake City, Utah
Received August 2, 1965

Separation of Nucleoside Mixtures on Dowex-1 (OH^-)[1]

Sir:

We wish to report a fractionation method of high resolving power applicable to certain nucleosides as well as derivatives and analogs thereof.

Existing column methods for nucleosides employ either ion-exchange or partition as the basis of separation. In the former, fractionation is achieved by exploiting differences in the pK_a values of the purine and pyrimidine residues[2-4] or differences in the borate-

(1) This investigation was supported in part by a research grant (No. GB-882) from the National Science Foundation. The author thanks Miss Gloria Herold for excellent technical assistance.

(2) W. E. Cohn, *J. Am. Chem. Soc.*, 72, 1471 (1950).

(3) W. Andersen, C. A. Dekker, and A. R. Todd, *J. Chem. Soc.*, 2721 (1952).

(4) N. G. Anderson, J. G. Green, M. L. Barber, and Sr. F. C. Ladd, *Anal. Biochem.*, 6, 153 (1963).

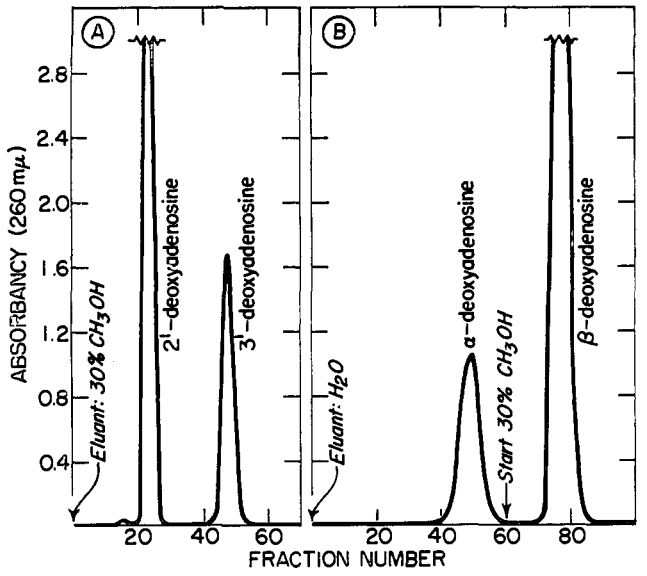

Figure 1. Dowex 1-X2 (OH^-) separations of nucleosides. For conditions, see legend to Table I.

complexing potential of the nucleosides.[5] In the partition method, solubility differences are employed.[6,7] Both methods rely heavily on variations in the properties of the heterocyclic base moieties.

Since the sugar hydroxyl groups of nucleosides are known to have pK_a values in the range 12–17, ion exchange at high pH was examined as a means of fractionating those nucleosides having no dissociable hydrogen on the aglycone residue. Precedent for such fractionation existed in the ionophoretic studies of Frahn and Mills[8] which revealed that certain polyols and sugar derivatives migrated extensively in 0.1 *N* sodium hydroxide. More recently, Austin, *et al.*,[9] have separated isomeric glycosides on the hydroxide form of strongly basic anion-exchange resins but have not commented on the physical basis of the separation. Similar columns have been used for the resolution of the antibiotic complexes, neomycin and catenulin,[10] which also have glycosidic components.

Utilizing the method of Austin, *et al.*,[9] but employing alcohol–water mixtures as eluents we have achieved the separation of a variety of structurally related glycosylamines. In Table I are listed pairs or groups of substances which have been resolved and the conditions employed in each case. Application of the technique to complex mixtures resulting from various types of hydrolysis of nucleic acids is facilitated by complete retention of purines, pyrimidines, free sugars, and nucleotides at the top of the column during elution of nucleosides by aqueous methanol. The resolution of a synthetic mixture of the four major nucleosides is shown in the table. Separation of 100-mg. amounts of each component has been achieved on a column of comparable dimensions.

That fractionation is based primarily on ion-exchange rather than adsorption, partition, or ion exclusion is seen from Figure 1A and expt. 1 of the table. The adenine derivatives are eluted in the order 2′,3′-isopropylideneadenosine, 2′-deoxyadenosine, 3′-deoxyadenosine, adenosine, as one would predict from existing information regarding the pK_a values of the sugar hydroxyl groups.[11] It is apparent that ease of

Table I. Nucleoside Mixtures Resolved[a]

Expt.	Compds. separated[b]	Elution solvent, ml.[c]	Source of compd.[k]
1	2′,3′-Isopropylidene adenosine	30% CH_3OH, 90	*f*
	2′-Deoxyadenosine	30% CH_3OH, 190	*f*
	3′-Deoxyadenosine	30% CH_3OH, 375	*g*
	Adenosine	30% CH_3OH, 380 + 60% CH_3OH, 450	*f*
2	2′-Deoxycytidine	30% CH_3OH, 140	*f*
	Cytidine	30% CH_3OH, 725	*f*
3	2′-Deoxy-9-α-D-ribofuranosyladenine	H_2O, 570	*h*
	2′-Deoxy-9-β-D-ribofuranosyladenine	H_2O, 600 + 30% CH_3OH, 120	*f*, *h*
4	1-β-D-Ribofuranosylcytosine[d]	30% CH_3OH, 695	*f*
	1-β-D-Xylofuranosylcytosine[d]	30% CH_3OH, 800	*i*
	1-β-D-Arabinofuranosylcytosine[e]	30% CH_3OH, 1000 + 0.1 *M* NH_4HCO_3, 350	*l*
5	9-β-D-Ribofuranosyladenine	55% CH_3OH, 600	*f*
	9-β-D-Psicofuranosyladenine	55% CH_3OH, 750	*j*
6	Cytidine	30% CH_3OH, 710	*f*
	Adenosine	30% CH_3OH, 1185 + 60% CH_3OH, 250	*f*
	Uridine[e]	30% CH_3OH, 1185 + 60% CH_3OH, 275 + 0.1 *M* NH_4HCO_3, 285	*f*
	Guanosine[e]	30% CH_3OH, 1185 + 60% CH_3OH, 275 + 0.1 *M* NH_4HCO_3, 430	*j*

[a] Mixtures of pure nucleosides (1–3 mg. of each component) were dissolved in 3–5 ml. of 30% methanol and applied to a 1.6 × 20 cm. column of Bio-Rad AG 1-X2 (OH^-, 200–400 mesh) previously equilibrated with 30% methanol. Elution schedules are as shown. Identification was accomplished by measuring areas under the peaks since a known amount of each component was applied and recoveries were quantitative. Purity of eluted components was ascertained by determination of D_{280}/D_{260} ratios and comparison with literature values. [b] Compounds within each group are listed in order of elution. [c] Volume given is that which will completely elute the compound. Conditions of elution are not necessarily optimal. [d] Only partially resolved under the conditions employed. [e] Compounds were displaced from the column wth bicarbonate ion. [f] Commercial sample. [g] W. W. Lee, A. Benitez, C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **83**, 1906 (1961). [h] C. Pederson and H. G. Fletcher, *ibid.*, **82**, 5210 (1960). [i] J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *ibid.*, **79**, 5060 (1957). [j] W. Schroeder and H. Hoeksema, *ibid.*, **81**, 1767 (1959). [k] The author expresses his gratitude to Drs. L. Goodman, H. G. Fletcher, J. J. Fox, and H. Hoeksema for providing samples for this study. [l] E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 84 (1959).

(5) W. E. Cohn in "The Nucleic Acids," Vol. I, E. Chargaff and J. N. Davidson, Ed., Academic Press Inc., New York, N. Y., 1955, p. 273.
(6) P. Reichard and B. Estborn, *Acta Chem. Scand.*, **4**, 1047 (1950).
(7) R. H. Hall, *J. Biol. Chem.*, **237**, 2283 (1962).
(8) J. L. Frahn and J. A. Mills, *Australian J. Chem.*, **12**, 65 (1959).
(9) P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, 5350 (1963).
(10) H. Maehr and C. P. Schaffner, *Anal. Chem.*, **36**, 104 (1964).

elution of members of this group is related to the number of sugar hydroxyl groups and the extent of their dissociation. The ability of methanol–water mixtures to readily elute substances only slowly desorbed by water is due to the greater ionization of methanol (in water) as compared to water.[12] The effectiveness of a series of alcohols (aqueous) as eluents for cytidine was found to parallel roughly the published pK_a values,[12] *i.e.*, 2,2,2-trifluoroethanol > glycerol > ethylene glycol > methanol > ethanol > 1-propanol.

Recent progress in synthetic methodology has made available a large number of nucleosides and nucleoside analogs.[13–15] In the absence of those anchimeric effects leading to exclusive formation of the β-anomer, synthetic methods give mixtures of α- and β-anomers the total resolution of which is often difficult. The ease of resolution of such anomeric pairs on Dowex-1 (OH^-) is seen in Figure 1B and expt. 3 of the table. The separation of isomeric nucleosides resulting from the hydrolytic opening of 2′,3′-epoxide, 2,2′-anhydride, and other synthetic intermediates has also been achieved and has simplified the preparation of certain antimetabolites and chemotherapeutic agents. A specific example is the resolution of the mixture of cytidine and cytosine arabinoside obtained on hydrolysis of 2,2′-anhydro-1-β-D-arabinosylcytosine.[16]

Other applications of the method which are currently being investigated include (1) the quantitative isolation of certain O- and N-methylated nucleosides from soluble and ribosomal RNA's and (2) the resolution of alkali-stable derivatives of nucleosides which are employed as intermediates in the synthesis of nucleotides and polynucleotides.

Since the method is based on ion exchange, the amount of material which can be resolved is limited only by the dimensions of the column and the capacity of the resin employed. The use of volatile solvents for elution greatly simplifies product isolation.

(11) (a) R. Kuhn and H. Sobotka, *Z. physik. Chem.*, **109**, 65 (1924); (b) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

(12) P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **82**, 795 (1960).

(13) See reviews in A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides," Academic Press Inc., New York, N. Y., 1963, Chapter 2; T. L. V. Ulbricht, *Angew. Chem. Intern. Ed. Engl.*, **1**, 476 (1962).

(14) (a) T. Sato, T. Simadate, and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1440, 1442 (1960); (b) Y. Ishido and T. Sato, *Bull. Chem. Soc. Japan*, **34**, 347, 1374 (1961).

(15) M. J. Robins, W. A. Bowles, and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 1251 (1964).

(16) See Table I, footnote *l*.

Charles A. Dekker
Department of Biochemistry
University of California, Berkeley, California
Received February 27, 1965

Book Reviews

Die Nucleinsäuren. Eine einführende Darstellung ihrer Chemie. Biochemie und Funktionen. By EBERHARD HARBERS. Institut für Medizinische Physik und Biophysik der Universität Göttingen. Georg Thieme Verlag. Postbach 732, Herdweg 63, 7000 Stuttgart 1, Germany. 1964. xii + 303 pp. 18 × 26 cm. DM 68.

This book offers a good guide to fundamental facts in nucleic acid biochemistry and structure. At the same time, it covers much of what is valuable and interesting in present-day work at the molecular (*i.e.*, macromolecular) biological frontiers. The approach is critical enough to distinguish in general between supported and provisional models, yet the style remains lively and agreeable. Illustrative material is very good, and the bibliography contains over two thousand items, fifty per cent of them from the years 1960–1963 and forty per cent from the preceding decade.

The work achieves considerably more critical evaluation and comprehensiveness than its origin as an expanded lecture series would ordinarily lead one to expect. The emphasis is concisely centered upon accumulated fact (and hypothesis) and only to a very limited extent upon a historical development of the several topics. On the other hand, the approach is naturalistic rather than intellectual; the nucleic acids are of interest because they exist and have significant roles in living cells—not as triumphant theoretical contributions of the human mind. The authors have not detracted from the brilliance of the mental powers focused on a DNA fiber in a darkened room or the beauty of the patterns derived when they clearly point out that an eminently measurable photographic plate and a clear monochromatic X-ray beam had first to operate in that dark chamber.

In fact, the section on physical studies of nucleic acids, contributed by W. Müller, is admirably thorough in presenting the physical, thermodynamic, and hydrodynamic approaches to nucleic acid structure. The principles and strengths generally taken for granted and the weaknesses commonly overlooked in the modern work or omitted from most short compendia are described here. The section on biochemistry of constituents by the same author is brief but includes essential reactions and an introduction to th newer chemical synthetic methods. The section by G. F. Domagk on metabolism of nucleotides is extensive and even includes a brief summary of nucleotide coenzyme functions. Harbers himself has contributed main sections dealing with localization in tissues, metabolism of nucleic acids, biosynthesis, and information transfer. These are remarkably up to date and objective in reporting a field which moves so fast. The level reached is that of the exceedingly well-informed biochemist or molecular biologist of 1963; perspective and critical judgment are only slightly weaker on the latest additions to the story. The newest physical studies on nucleic acids, ribosomes, etc., appear here rather than in the chapter on physical methods.

If this reviewer were to attempt the classical "sampling in depth" for his own specialty, information transfer, he would have mainly to report a satisfactory breadth, with a certain relative lack of emphasis. Whereas 30 pages are devoted to tumor biochemistry, two-thirds of it to drug therapy, only 3 pages are given to the transformations (with 1 for transduction and 10 for virus chemistry) that within the past two decades convinced everyone of the importance of nucleic acids. The genetic coding mechanisms are well-covered elsewhere, so if there is any weak emphasis, it is on the biological side of transfer. One misconception might be noted, although it is a frequently misinterpreted subject: heat inactivation of transforming agents is pictured as it once was, as inactivation of genetic function, whereas it has been shown in papers actually referred to as inactivation of ability of DNA to be incorporated into cells, without loss of linkages or intrinsic activity.

It may be surprising too that in a work that presents nucleic acids as works of nature rather than of man, so little attention is paid to the overwhelming and empirical fact that base compositions of nucleic acids of different species are so different. No tables are given, and the fact itself slips in in two or three lines of text almost unnoticed, as the underlying assumption toward which certain chemical and physical searches for homologies are directed.