was dissolved in pyridine (30 ml) and PhCOCl (2.8 g, 20 mmol) was added dropwise under cooling. The mixture was stirred at room temperature for 15 hr and the solvent was concentrated into a small volume *in vacuo* at 50°. The residue was diluted with H_2O and neutralized with aqueous $NaHCO_3$ solution to give a crystalline material (2.94 g, 81%), which was recrystallized from a large amount of EtOAc: mp 175°. *Anal.* ($C_{21}H_{17}NO_5$) C, H, N.

3-Benzoyloxy-5-benzoyloxymethyl-4-chloro-2-methylpyridine (9). Dibenzoate 8 (3.6 g) and $POCl_3$ (10 ml) were refluxed for 18 hr. Excess $POCl_3$ was evaporated *in vacuo*, and the residue was diluted with ice–H_2O, neutralized with aqueous dilute $NaHCO_3$ solution, and extracted with EtOAc. The extract was dried (Na_2SO_4) and the solvent was removed to give a colorless crystalline product, which was recrystallized from MeOH to produce 3.3 g (87%) of **9**: mp 126°. *Anal.* ($C_{21}H_{16}NO_4Cl$) C, H, N, Cl.

4-Chloro-3-hydroxy-5-hydroxymethyl-2-methylpyridine (10). Na (0.35 g) was dissolved in absolute MeOH (50 ml) and to this solution was added the 4-chloro compound **9** (1.9 g). The mixture was refluxed for 15 hr, the solvent was removed *in vacuo*, and the residue was diluted with H_2O (20 ml), acidified with dilute HCl, and then shaken with ether. The aqueous layer was separated, neutralized with aqueous dilute $NaHCO_3$ solution, and extracted with EtOAc. The extract was dried (Na_2SO_4) and the solvent was removed to leave an oil, which gradually crystallized. Recrystallization from EtOAc–ether gave 0.24 g (27.6%) of the product (**10**): mp 203–204°. *Anal.* ($C_7H_8NO_2Cl$) C, H, N, Cl.

3-Benzoyloxy-5-benzoyloxymethyl-2-methylpyridine (11). A solution of the 4-chloro compound **9** (3.8 g) in MeOH (500 ml) was hydrogenated in the presence of 5% Pd/C (2 g). After 30 min, the theoretical volume of H_2 was absorbed, and the solvent was removed *in vacuo*. The residue was again dissolved in EtOAc and the solution was washed with aqueous dilute $NaHCO_3$ solution and H_2O and dried (Na_2SO_4). Evaporation of the solvent left the crystalline solid, which was recrystallized from EtOAc and *n*-hexane to afford 3.1 g (89.4%) of an analytically pure product, mp 85–86°. *Anal.* ($C_{21}H_{17}NO_4$) C, H, N.

4-Deoxymethylpyridoxol (3-Hydroxy-5-hydroxymethyl-2-methylpyridine, 12). A solution of the dibenzoate **11** (1.15 g) in 2 *N* HCl (20 ml) was refluxed for 3 hr, cooled, and shaken with ether. The aqueous layer was concentrated *in vacuo* into dryness to leave a crystalline product. Recrystallization from EtOH–ether afforded 0.49 g (83.8%) of **12**: mp 167–168° (lit.[5] mp 168–170°). *Anal.* ($C_7H_{10}NO_2Cl$) C, H, N, Cl.

Acknowledgments. We wish to express our gratitude to Dr. G. Sunagawa, Director of these Laboratories, and to Dr. K. Murayama, Assistant Director, for their encouragement and discussion. We are also indebted to Miss F. Takeuchi, Messrs. T. Sakamoto, and K. Sato for their technical assistance.

References

(1) Y. Morisawa, M. Kataoka, T. Watanabe, N. Kitano, and T. Matsuzawa, *J. Med. Chem.*, **17**, 1083 (1974).
(2) (a) D. A. Karnofsky, C. C. Stock, L. P. Ridgway, and P. A. Patterson, *J. Biol. Chem.*, **182**, 471 (1950); (b) H. W. Coover and N. J. Bowman, U. S. Patent 2,481,573; *Chem. Abstr.*, **44**, 668 (1950).
(3) J. L. Green, Jr., and J. A. Montgomery, *J. Med. Chem.*, **6**, 294 (1963).
(4) (a) D. Heyl, E. Luz, and S. A. Harris, *J. Amer. Chem. Soc.*, **73**, 3437 (1951); (b) W. Korytnyk and B. Paul, *J. Heterocycl. Chem.*, **2**, 144 (1965).
(5) (a) L. A. Perez-Medina, R. P. Mariella, and S. M. McElvain, *J. Amer. Chem. Soc.*, **69**, 2574 (1947); (b) T. Yoshikawa, F. Ishikawa, Y. Omura, and T. Naito, *Chem. Pharm. Bull.*, **13**, 873 (1965).
(6) Abstracts of the 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 1969, MEDI 11.
(7) W. Korytnyk and B. Paul, *J. Med. Chem.*, **13**, 187 (1970).

Communications to the Editor

Synthesis and Metabolic Behavior of the Suggested Active Species of Isophosphamide Having Cytostatic Activity

Sir:

Isophosphamide (**1**) is an experimentally effective antitumor agent structurally related to cyclophosphamide and is thought to exert cytotoxicity after *in vivo* metabolic transformation.[1] Some recent studies[2-4] have suggested that the activation of isophosphamide, like that of cyclophosphamide,[5-9] is caused by the enzymatic C-4 hydroxylation in animal liver. In a recent communication,[10] we have described the first chemical synthesis of the active metabolite of cyclophosphamide by a new route which may be regarded as a general method leading to 4-functionalized 1,3,2-oxazaphosphorinanes. We now apply the method to the synthesis of the suggested active species of isophosphamide and wish to report that the synthetic active species exhibited comparable antileukemic activities in both *in vivo* and *in vitro* experiments to that of cyclophosphamide and that there were significant differences not only in *in vivo* activity but also in *in vivo* metabolic behavior between isophosphamide and its active form.

O-3-Butenyl *N,N'*-bis(2-chloroethyl)phosphorodiamidate (**3**)† was prepared in 70% yield by reacting $POCl_3$ with 3-buten-1-ol and 2-chloroethylamine in CH_2Cl_2. Ozonolysis of **3** in aqueous acetone, followed by treatment with 30% H_2O_2, gave 4-hydroperoxyisophosphamide (**4**) in *ca.* 30% yield: mp 113–114° (with violent decomposition); ir_{max} (KBr) 3268, 3193, 2995, 2963, 2949, 2927, 2858, 2837, 1435, 1322, 1239, 1193, 1160, 1117, 1059, 1040, 990, 934, 879, 826, 800, 770, 744 cm^{-1}; nmr (DMSO-d_6, TMS) δ 2.09 (2 H, m, C_5-H), 2.81–4.10 (8 H, m, $2CH_2CH_2Cl$), 4.30 (2 H, m, C_6-H), 4.96 [1 H, d of t, J(P,C_4-H) = 19.0 Hz, J(C_4-H,C_5-H) = 3.0 Hz, C_4-H], 4.98 [1 H, d of t, J(P,-NH) = 19.0 Hz, J(NH,CH_2) = 5.6 Hz, NH], 11.65 (1 H, s, OOH). By the action of Fe^{2+} ($FeSO_4$) or Cu^+ (CuCl), **4** was converted into 4-ketoisophosphamide (**5**)[4] in excellent yield, while treatment of **4** with triethyl phosphite in CH_2Cl_2 at 0° resulted in the quantitative formation of 4-hydroxyisophosphamide (**2**) as fine needles: mp 74–75° dec; ir_{max} (KBr) 3313, 3285, 2990, 2950, 2931, 2888, 2860, 1444, 1313, 1262, 1234, 1215, 1195, 1109, 1063, 1044, 975, 921, 887, 810, 772, 743, 715 cm^{-1}; nmr (D_2O, DSS) δ 1.99 (2 H, m, C_5-H), 2.75–3.90 (8 H, m, $2CH_2CH_2Cl$), 4.0–4.9 (2 H, m, C_6-H), 5.05 [1 H, d of t, J(P,C_4-H) = 18.0 Hz, J(C_4-H,C_5-H) = 3.5 Hz, C_4-H]. In contrast to the behavior of 4-hydroperoxycyclophosphamide,[11] treatment with aqueous alkali (Na_2CO_3) converted **4** into a cyclic peroxide **6** in good yield: mp 127–129° dec; ir_{max} (KBr) 3200, 2975, 2928, 2920, 2867, 1467, 1428, 1300, 1255, 1215, 1162, 1136, 1080, 1062, 1026, 985, 917, 880, 861, 792 cm^{-1}; nmr (DMSO-d_6, TMS) δ 1.84 (2 H, m, C_5-H), 2.7–3.8 (7 H, m, $NHCH_2CH_2Cl$, NCH_2), 5.34 [1 H, d of t, J(P,C_4-H) =

†All the new compounds described in this communication gave correct elemental analyses (C, H, N, P, Cl).

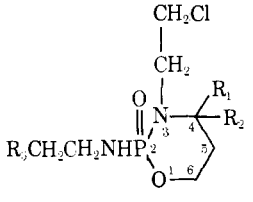
Table I. Comparative Antileukemic Activity (*in Vivo*) and Host Toxicity of the C-4 Oxidized Isophosphamide and Cyclophosphamide Derivatives

Compound	Antileukemic activity: Dose, mg/kg iv[a]	ILS, %[b]	Survivors over 30 days	Host toxicity,[c] LD_{10}, mg/kg iv
4-Hydroxyisophosphamide (**2**)	50	200	4/10	200
	100	>270	10/10	
	200	220	6/10	
4-Hydroperoxyisophosphamide (**4**)	50	200	4/10	200
	100	>270	10/10	
	200	−20	0/10	
Isophosphamide (**1**)	50	45	0/10	470
	100	180	1/10	
	500	200	4/10	
4-Hydroxycyclophosphamide	50	100	2/10	180
	100	270	8/10	
	200	200	7/10	
4-Hydroperoxycyclophosphamide	50	150	3/10	180
	100	>270	10/10	
	200	−5	0/10	
Cyclophosphamide	50	125	2/10	310
	100	235	8/10	
	300	200	7/10	

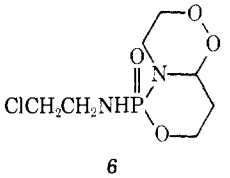
[a] Administered at 24 hr after inoculation of L1210 cells. [b] Increase of life span in dying animals over control. [c] Toxicity against DS mice.

18.9 Hz, $J(C_4\text{-H},C_5\text{-H})$ = 4.5 Hz, C_4-H], 3.8–4.8 (4 H, m, $2OCH_2$).

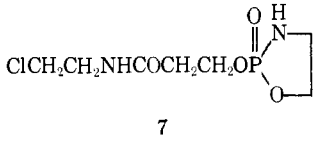
As expected, the synthetic 4-hydroxyisophosphamide (**2**) was active *in vitro*, giving an ED_{50} of 0.88 μg/ml against L1210 leukemia cells (on 42 hr of exposure), which was comparable to that of 4-hydroxycyclophosphamide (0.87 μg/ml). **4** was also active *in vitro* with essentially the same potency as **2**. More striking activity of **2** and **4** was found in the *in vivo* antileukemic effect which suggests that they will be rival candidates against the cyclophosphamide derivatives. Thus, as shown in Table I, both **2** and **4** exhibited a greater activity against L1210 leukemia in BDF_1 mice, inoculated (ip) with 5×10^5 cells, at 50 mg/kg (iv) of dose and they gave a complete inhibition at 0.5 of the LD_{10} dose, although the ILS value decreased slightly for **2** and markedly for **4** at the LD_{10} dose (200 mg/kg iv) as in the cases of cyclophosphamide derivatives. It is also striking that there was a greater difference between the *in vivo* activity of the C-4 oxidized isophosphamides (**2** and **4**) and isophosphamide (**1**) than between the C-4 oxidized cyclophosphamides and cyclophosphamide, which suggests that the *in vivo* activation of isophosphamide is inefficient as compared to cyclophosphamide.



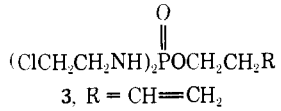
1. $R_1 = R_2 = H$; $R_3 = Cl$
2. $R_1 = OH$; $R_2 = H$; $R_3 = Cl$
4. $R_1 = OOH$; $R_2 = H$; $R_3 = Cl$
5. R_1, $R_2 = O$; $R_3 = Cl$
12. R_1, $R_2 = O$; $R_3 = OH$
13. R_1, $R_2 = O$; $R_3 = OCH_2C_6H_5$



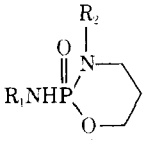
6



7



3. R = CH=CH$_2$
8. R = COOH



9. $R_1 = H$; $R_2 = CH_2CH_2Cl$
10. $R_1 = CH_2CH_2Cl$; $R_2 = H$
11. $R_1 = COCH_3$; $R_2 = CH_2CH_2Cl$

The *in vivo* metabolic behavior of **2** and **4** was also found to be different from that of isophosphamide. Thus, administration of **2** to rabbits resulted in the excretion of carboxylic acid (**8**)[4] as the sole characterizable urinary metabolite, while **4** was metabolized into **8** (major) and a new 1,3,2-oxazaphospholane derivative **7** (minor), accompanied by a small quantity of **5** (**8**:**7**:**5** = 40:8:1).‡ On the other hand, two N-dechloroethylated compounds (**9** and **10**, minor) and **8** (major) were isolated with recovery of a considerable amount of **1** as the urinary metabolites of isophosphamide (**1**) (**8**:**9**:**10**:**1** = 10:5:3:4),‡ but, in contrast to the result of Hill, *et al.*,[4] **5** was not isolated. The structure of the metabolites **7** (mp 159–161°) and **10** (mp 109–110°) was assigned on the basis of spectral data. For **7**: ir_{max} (Nujol) 3260, 3200, 3080, 1659, 1563, 1363, 1299, 1225, 1135, 1076, 1043, 1030, 1015, 936, 929 cm^{-1}; nmr (DMSO-d_6, TMS) δ 2.0–4.6 (12 H, m, $6CH_2$), 5.17 (1 H, br, ring NH), 7.63 (1 H, br, CONH). For **10**: ir_{max} (Nujol) 3195, 1220, 1203, 1090, 1045, 980, 905, 785 cm^{-1}; nmr ($CDCl_3$, TMS) δ 1.83 (2 H, m, C_5-H), 2.83–3.80 (8 H, m, $NHCH_2$, $NHCH_2CH_2Cl$), 4.35 (2 H, m, C_6-H), and confirmed by direct comparison with synthetic specimens. The structure of metabolite **9** was confirmed by acetylation to **11**: mp 118–119°; ir_{max} (Nujol) 3200, 3160, 1690, 1323, 1245, 1115, 1050, 988, 950, 930, 877, 790 cm^{-1}; nmr ($CDCl_3$, TMS) δ 1.8–2.4 (2 H, m, C_5-H), 2.10 (3 H, d, J = 1.9 Hz, $COCH_3$), 2.8–3.9 (6 H, m, $CH_2NCH_2CH_2Cl$), 4.57 (2 H, m, C_6-H), 9.17 [1 H, br d, J(P,NH) = ~8 Hz, NH], which was also identical with a synthetic specimen. The metabolite **7** is considered to be formed from **5**, which is possibly produced directly from **4** *via* an intermediate **12** by the intramolecular nucleophilic displacement at the phosphorus atom. In fact, **5** was shown to be metabolized into **7**, and the synthetic species **12**, which was generated by catalytic reduction of **13**, was found to be readily converted into **7** on alkali treatment.

These results lead to the conclusion that the activation of isophosphamide takes place in a similar way to that of cyclophosphamide by C-4 hydroxylation, but it is metabolized less efficiently *in vivo*. This may be attributed to the fact that the C-4 oxidation is in competition with oxidation of the side-chain carbons leading to the N-dechloroethylated metabolites.§ Detailed biological evaluations of **2** and **4** are in progress.

References

(1) N. Brock, *Laval Med.*, **39**, 696 (1968).

‡The ratio represents the relative weight of the metabolites isolated. The total amount of the purified metabolites was 15–20% of the administered. The actual ratio of **8** present in the urine may be greater than the value shown here, since the amount of loss during the isolation procedure was always larger for **8** than for the other metabolites. Separation of the urinary metabolites was carried out according to the same procedure used for the isolation of cyclophosphamide metabolites.[12]

§It is known that the *in vivo* oxidation of cyclophosphamide in sheep occurs at C-4 and also at the side chain.[13]

(2) L. M. Allen and P. J. Creaven, *Cancer Chemother. Rep., Part 1*, **56**, 603 (1972).
(3) R. A. Alarcon, J. Meienhofer, and E. Atherton, *Cancer Res.*, **32**, 2519 (1972).
(4) D. L. Hill, W. R. Laster, Jr., M. C. Kirk, S. El Dareer, and R. F. Struck, *Cancer Res.*, **33**, 1016 (1973).
(5) N. Brock and H.-J. Hohorst, *Arzneim.-Forsch.*, **13**, 1021 (1963); N. Brock and H.-J. Hohorst, *Cancer*, **20**, 900 (1967).
(6) H.-J. Hohorst, A. Ziemann, and N. Brock, *Arzneim.-Forsch.*, **21**, 1254 (1971).
(7) N. E. Sladek, *Cancer Res.*, **31**, 901 (1971).
(8) D. L. Hill, W. R. Laster, Jr., and R. F. Struck, *Cancer Res.*, **32**, 685 (1972).
(9) M. Colvin, C. A. Padgett, and C. Fenselau, *Cancer Res.*, **33**, 915 (1973).
(10) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino, and K. Yamaguchi, *J. Amer. Chem. Soc.*, **95**, 985 (1973).
(11) R. F. Struck, M. C. Thorpe, W. C. Coburn, Jr., and W. R. Laster, Jr., *J. Amer. Chem. Soc.*, **96**, 313 (1974); A. Takamizawa, S. Matsumoto, and T. Iwata, *Tetrahedron Lett.*, 517 (1974).
(12) A. Takamizawa, Y. Tochino, Y. Hamashima, and T. Iwata, *Chem. Pharm. Bull.*, **20**, 1612 (1972).
(13) J. E. Bakke, V. J. Feil, C. E. Fjelstul, and E. J. Thacker, *J. Agr. Food Chem.*, **20**, 384 (1972).

Akira Takamizawa,* Saichi Matsumoto
Tsuyoshi Iwata, Yoshihiro Tochino
Ken Katagiri, Kenji Yamaguchi, Osamu Shiratori
Shionogi Research Laboratory
Shionogi and Company, Ltd.
Fukushima-ku, Osaka 553, Japan
Received April 29, 1974

Book Reviews

Laboratory Course in Organic Chemistry. 2nd Edition. By David Hirsch Rosenblatt and George Thomas Davis. Allyn and Bacon, Boston, Mass. 1973. xxvii + 452 pp. 28 × 21 cm. $7.95.

This manual is a companion to the Morrison and Boyd textbook of organic chemistry, and the text makes frequent references to page citations in "M & B." The student is presented with much material to read and digest, in addition to the experimental directions, *per se*. The manual is written in a rather informal, conversational style. The preparation of organic compounds is stressed and is used to introduce ir, uv, nmr, and chromatographic methods. However, mass spectroscopy is not mentioned, and the authors have made no attempt to provide electronic rationalizations for the reactions which the students perform in the laboratory. The equation for each reaction is shown in its simplest terms, and the student is referred to pages in Morrison and Boyd for the "nature of the reaction." The authors have made liberal use of footnotes, which are frequently separated from the textual material by several pages. This format may be undesirable in a manual for undergraduates. Each experiment concludes with a set of questions based on the experiment and/or the textual material accompanying it. This reviewer questions the value of inclusion of these, since "canned" questions generally generate "canned" answers.

Extensive (perhaps too much) space is devoted to simple laboratory apparatus; pictures of a beaker, an iron ring, and a graduated cylinder seem unnecessary, as do many of the diagrams of simple equipment set-ups. In contrast, discussions of some of the more esoteric topics are not always easy to follow. A terse exposition of the Hansch approach to biological activity seemed out of place and is likely to baffle a beginning organic chemistry student.

The chapter on the library was not satisfying and will perhaps not be maximally helpful to the student. The authors' list of primary source journals in organic chemistry was mystifying for its omissions as well as for some of the journals listed. The authors omitted mention of Theilheimer's series in their rather extensive compilation of reference works for synthetic organic chemistry.

The flexible binding of the manual seems somewhat flimsy; a hard cover might be more suitable for a book which will obviously receive hard usage, both in the laboratory and as a text.

Overall, the manual seems to be a good one, and it is worth considering for use in a beginning organic chemistry laboratory, although this reviewer is not certain that it is the best one available.

Division of Medicinal Chemistry and Natural Products
College of Pharmacy, The University of Iowa
Iowa City, Iowa 52242
Joseph G. Cannon

Computer-Assisted Instruction in Chemistry. Parts A and B (Computers in Chemistry and Instrumentation. Volume 4). Edited by J. S. Mattson, H. B. Mark, Jr., and H. C. MacDonald, Jr. Marcel Dekker, New York, N. Y. 1974. 23.5 × 16 cm. Part A: xi + 271 pp. $24.50. Part B: xv + 258 pp. $26.50.

This two-part set is an attempt by the editors to fulfill a need for a comprehensive treatise on the "state of the art" of computer-assisted instruction (CAI). The work has been divided into two parts, with Part A presenting general approaches and Part B treating specific applications. CAI, like computer programming in general, is not a subject which is easily put into a book, especially one written for those who are unfamiliar to the field. In this respect, the authors and editors have done a respectable job of communicating the essence of the subject, although some phases of the work are stronger than others.

Much of Part A consists of material which perhaps has somewhat questionable application to the field of education. Chapter 1 is a concise introduction to the subject and briefly (7 pages) discusses some possible uses of CAI and problems which can occur. Chapter 2 attempts to present the reader with the picture of a complete and well-integrated system of CAI, allowing him to appreciate the really sophisticated projects which can be accomplished under ideal circumstances (in this case, at the University of Pittsburgh). It does in fact do this, although one is left with the feeling that such a system is well beyond the level of anyone who may find the use of such a system desirable. The third chapter is a rather detailed discussion of the use of analog and hybrid computers. The editors attempt to justify its inclusion on the basis of its future potential, and there is probably some merit in this. One might also suggest that such a topic would be more timely in a more advanced volume. Chapter 4 presents some interesting uses of interactive computing during actual classroom sessions through the projection of teletype output. A portion of the chapter is devoted to design description and listings of some actual programs, mostly for freshman chemistry use. Chapter 5 is another one which has questionable value in a book such as this, although the topic is an important one. It is a survey of information storage and retrieval by computerized systems. Such a topic is again justified by the editors as being potentially important to both students and instructors. Chapter 6, the final chapter in Part A, is probably the most important and useful chapter in the book. It is a brief but very informative discussion of practical considerations—primarily, "does it work?" and "how much will it cost?."

Part B is devoted to the more practical considerations of computers in education and would seem to be somewhat more valuable to those working in the field or contemplating it. Chapter 1 of this second part illustrates the laboratory and classroom use of the APL language, as applied to a general chemistry course. Although APL is an IBM language, it has now become more generally avail-