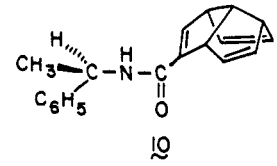
with concentrated hydrochloric acid at 130° (sealed tube, 11 hr) or, more simply, at the reflux temperature (28 hr) to afford the crystalline acid lactone **6**, mp 157.5–158°,[5] in 95 and 82% yields, respectively. Its pair of olefinic hydrogens appear in the nmr spectrum ($CDCl_3$) at δ 5.63 (br d, 1 H, J = 5.5 Hz) and 5.42 (br d, 1 H, J = 5.5 Hz), while the >C*H*O– proton is seen as a multiplet at 5.2–4.85 (the two >C*H*CO– are obscured by the other methine protons). Thus, the exo nature of the carboxyl group could not be unequivocally established spectroscopically but is rather inferred on thermodynamic grounds, our inability to achieve double lactonization, and the nonepimerizable nature of the derived (CH_2N_2) methyl ester. Treatment of **6** in carbon tetrachloride–benzene solution (1:1) under nitrogen with lead tetraacetate and iodine under conditions of concomitant irradiation from a 250-W tungsten lamp source[6] produced **7**, mp 153–154°,[5] in 56% yield.

Mild hydrolysis and dehydroiodination of **7** was achieved concurrently by treatment with potassium carbonate in 1:1 tetrahydrofuran–water at 25° for 32 hr. Subsequent direct methylation of the resulting crystalline hydroxy acid (mp 152–153°)[5] with excess diazomethane gave 68% of the hydroxy ester **8**[5] as a clear oil showing infrared maxima at 3500 and 1720 cm^{-1}.

Introduction of the third double bond was effected by direct dehydration of **8** with ethyl(carboxysulfamoyl)-triethylammonium hydroxide inner salt[7] (dry THF, 25°, 60 min, N_2 atmosphere, 56% yield) or somewhat more conveniently by conversion of **8** to its mesylate (mp 105–106°)[5] and exposure of this derivative to a slurry of neutral alumina (activity I) in dichloromethane[8] (25°, 12 hr, 57% overall yield). The nmr spectrum ($CDCl_3$) of **9a** shows a downfield olefinic proton absorption at δ 6.64 (H_3), multiplets of area 4 at 5.95–5.5 and 4.1–3.7, and a three-proton singlet at 3.73. Saponification of **9a** (KOH, aqueous ethanol) proceeded quantitatively to give **9b**, mp 131–133°.[5,9]

In design, therefore, our synthesis proceeds formally by reorganization of the eight ring atoms in cyclooctatetraene with interposition of two carbon atoms (and the carboxyl substituent) from tetracyanoethylene. The susceptibility of **5** to acidic hydrolysis occurs with unusual facility for a 1,1,2,2-tetranitrile,[10] a finding of utmost significance to the scheme. Although loss of a double bond does occur during the conversion of **5** to **6**, the subsequent four-step elaboration of **9a** comprises a simple sequence for introduction of the second and third olefinic centers.

Resolution of **9b** was achieved by fractional crystallization of the (+)-(*R*)-α-phenethylamine[11] salts from methanol–water (4:1). The less soluble diastereomer exhibited $[\alpha]^{25}_{365}$ −97.8° (*c* 0.3, ethanol). Acidification of this salt afforded (−)-**9b**, mp 112–113°, $[\alpha]^{25}D$ −12.3°, $[\alpha]^{25}_{365}$ −339° (*c* 0.5, ethanol), which was shown to be enantiomerically pure (≥95%) by conversion to amide **10**[12] through stepwise treatment with oxalyl chloride[13] and (+)-(*R*)-α-phenethylamine in benzene containing pyridine (86% yield). Whereas the amides prepared from a partially resolved sample of **9b** ($[\alpha]^{25}_{365}$ +13°) exhibited a distinct pair of methyl doublets in the pmr spectrum (C_6D_6, $\Delta\nu$ = 2.7 Hz at 60 MHz; diastereomeric ratio *ca.* 55:45), pure **10** ($[\alpha]^{25}D$ −37.0°



10

(*c* 0.7, ethanol)) showed only the upfield methyl doublet at δ 1.24.[14]

Enantiomerically homogeneous (−)-**9b** is characterized by a lone electronic absorption maximum at 224 nm (ε 6230) and a negative Cotton effect: $[\theta]_{278}$ 0, $[\theta]_{224}$ −79,800, $[\theta]_{210}$ 0, Γ/2 = 20 nm[15] (*c* 0.05, ethanol, 25°).[16]

(5) All new substances reported were shown to possess the correct molecular composition by combustion analysis (±0.3% of theory).

(6) D. H. R. Barton, H. P. Faro, E. P. Serebryakov, and N. F. Woolsey, *J. Chem. Soc.*, 2438 (1965); T. Sakan and K. Abe, *Tetrahedron Lett.*, 2471 (1968); U. Scheidegger, J. E. Baldwin, and J. D. Roberts, *J. Amer. Chem. Soc.*, **89**, 894 (1967); for a review, consult R. A. Sheldon and J. K. Kochi, *Org. React.*, **19**, 279 (1972).

(7) E. M. Burgess, H. R. Penton, Jr., and E. A. Taylor, *J. Amer. Chem. Soc.*, **92**, 5224 (1970); *J. Org. Chem.*, **38**, 26 (1973).

(8) C. Mercier, P. Soucy, W. Rosen, and P. Deslongchamps, *Syn. Commun.*, 3, 161 (1973); G. H. Posner, R. J. Johnson, and M. J. Whalen, *J. Chem. Soc., Chem. Commun.*, 281 (1972).

(9) Identical in all respects to a sample provided by Dr. Fukunaga.[3]

(10) W. J. Middleton, R. E. Heckert, E. L. Little, and C. G. Krespan, *J. Amer. Chem. Soc.*, 80, 2783 (1958); H. E. Simmons, private communication.

(11) W. Leithe, *Chem. Ber.*, **64**, 2827 (1931); G. Fodor and G. Csepreghy, *Tetrahedron Lett.*, **No. 7**, 16 (1959); E. Benedetti, P. Corradini, and C. Pedone, *J. Organometal. Chem.*, **18**, 203 (1969).

(12) In the absence of absolute configuration data, the diastereomeric identity of **10** remains, of course, an unresolved issue.

(13) Ch. R. Engel and G. Just, *Can. J. Chem.*, **33**, 1515 (1955); F. Reber, A. Lardon, and T. Reichstein, *Helv. Chim. Acta*, **37**, 45 (1954).

(14) Attempts to establish the enantiomeric composition of (−)-**9b** by conversion (CH_2N_2) to ester **9a** and application of the chiral shift reagent tris(3-trifluoromethylhydroxymethylene)-*d*-camphoratoeuropium(III) [H. L. Goering, J. N. Eikenberry, and G. S. Koermer, *J. Amer. Chem. Soc.*, **93**, 5913 (1971)] did not result in "resonance doubling" (100 MHz) of the signals due either to the methyl group or the downfield shifted olefinic proton H_3.

(15) C. Djerassi and E. Bunnenberg, *Proc. Chem. Soc., London*, 299 (1963).

(16) This work was assisted financially by grants from the National Science Foundation and Eli Lilly and Co.

(17) National Institutes of Health Postdoctoral Fellow, 1972–1974.

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Cyclophosphamide. Complete Inhibition of Murine Leukemia L1210 *in Vivo* by a Fenton Oxidation Product

Sir:

Cyclophosphamide,[1] an effective agent against many animal and human tumors, is oxidized *in vivo* by the mixed function oxidase of liver microsomes to produce a cytotoxic form of the drug.[2,3] A recent communication[4] prompts us to report our results on the chemical synthesis of a stable, crystalline, oxidation product of cyclophosphamide that is cytotoxic *in vitro*, is able to

(1) H. Arnold, F. Bourseaux, and N. Brock, *Arzneim.-Forsch.*, **11**, 143 (1961).

(2) D. L. Hill, W. R. Laster, Jr., and R. F. Struck, *Cancer Res.*, **32**, 658 (1972), and references cited therein.

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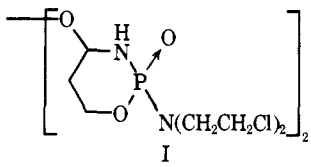
(4) A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino, and K. Yamaguchi, *J. Amer. Chem. Soc.*, **95**, 985 (1973).

completely inhibit leukemia L1210 *in vivo*, and is different from the oxidized cyclophosphamide derivatives described in the prior report.

Fenton oxidation of cyclohosphamide[5,6] yielded two products that react chemically as both aldehydes and alkylating agents, as shown by reaction with fuchsin aldehyde reagent[7] and with 4-(*p*-nitrobenzyl)pyridine.[8] The oxidation was performed in the following manner. Cyclophosphamide (1 g) and $FeSO_4 \cdot 7H_2O$ (1.25 g) in 30 ml of water at 5° were treated dropwise with 0.5 ml of 30% H_2O_2 at such a rate that the temperature remained below 10°; after stirring 4 hr at 5°, the solution was extracted with $CHCl_3$. Thin-layer chromatography of the extract on silica gel in acetone–chloroform (3:1) demonstrated that the products (R_f 0.65 and 0.85) could be separated from unreacted cyclophosphamide (R_f 0.45) and 4-ketocyclophosphamide[9,10] (R_f 0.60). Both products were isolated by column chromatography of the extract on silica gel in acetone–chloroform (3:1) in yields of 4 and 6%, respectively.

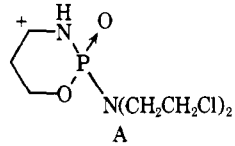
Elemental, pmr, ir, and mass spectral analysis of the faster migrating product (R_f 0.85), obtained as crystals from ether (mp 112.3°, Mettler FP-1, rate of heating 2°/min), are consistent with the ring-closed structure of 4-peroxycyclophosphamide (I): pmr_{TMS}^{DMSO} (Varian XL-



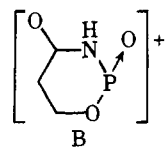
I

4-peroxycyclophosphamide

100) δ 1.6–2.4 (4 H, m, C_5–H_2), 3.0–3.8 (16 H, m, $[CH_2CH_2Cl]_2$), 3.8–4.6 (4 H, m, C_6–H_2), 5.22 (5.10 and 5.38, 2 H, d of q, $J_{H,P}$ = 27.6 Hz, converts to d of t upon addition of D_2O), 6.26 (2 H, t, NH, disappears on addition of D_2O); ir^{KBr} (cm^{-1}) 3210, 2960, 2935, 2895, 1450, 1430, 1355, 1340, 1330, 1315, 1240, 1220, 1200, 1125, 1105, 1095, 1050, 975, 955, 885, 815, 725, 650, 530, 490, 445; mass spectrum: *m/e* 259 (2 Cl, A, relative abundance 35), 141 (2 Cl, $[HN(CH_2CH_2Cl)_2]^+$, relative abundance 50), 135 (no Cl, B, relative abundance 100).



A

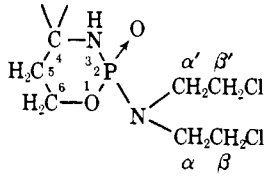


B

The slower migrating component (R_f 0.65) was obtained as a white, crystalline solid from chloroform. Pmr data (DMSO, XL-100), which differed slightly from reported[4] data, were consistent for 4-hydroperoxycyclophosphamide:[11] δ 1.94 (2 H, m, C_5–H_2), 3.1–3.8 (8 H, m, $[CH_2CH_2Cl]_2$), 3.8–4.6 (2 H, m, C_6–H_2), 4.7–5.15 (1 H, d of q, $J_{H,P}$ = 24.8, C_4–H), 5.86 (1 H, d of d, NH), 11.54 (1 H, s, OOH); ir^{KBr} (cm^{-1}) 3315, 3110, 2955, 2800, 1460, 1430, 1325, 1240, 1215, 1200, 1160, 1145, 1090, 1045, 985, 960, 930, 840, 780, 745, 710, 650, 545, 530, 500, 410. Elemental analysis was in agreement (±0.22%) with 4-hydroperoxycyclophosphamide. A significant difference, however, was observed between the melting point of our product (131–133° with subsequent darkening and explosive decomposition, Kofler Heizbank) and that previously reported (107–108°).[4,12]

Further support for the structures assigned to 4-peroxy- and 4-hydroperoxycyclophosphamide was obtained from the ^{13}C nmr spectra (Table I).

Table I. ^{13}C Nmr Chemical Shifts (ppm at 25.16 MHz, solvent $CDCl_3$, TMS internal reference)



Compound	C_4	C_5	C_6	$C\alpha\alpha'$	$C\beta\beta'$
Cyclophosphamide	41.48	25.84	67.78	48.95	42.33
4-Hydroperoxycyclophosphamide	86.60	27.78	63.44	48.97	42.15
4-Peroxycyclophosphamide	86.85	28.33	62.76	49.19	41.97
4-Ketocyclophosphamide	170.42	33.48	62.07	48.46	41.85

Washing 4-hydroperoxycyclophosphamide in $CHCl_3$ with 1 *N* KOH effected rapid conversion to 4-peroxycyclophosphamide (I). Treatment of I with hydrogen peroxide in aqueous DMSO resulted in slow regeneration of 4-hydroperoxycyclophosphamide plus the formation of a small amount of 4-ketocyclophosphamide.

When BDF_1 mice, implanted intraperitoneally (IP) or intravenously (IV) with L1210 leukemia cells, were treated IP with 4-peroxycyclophosphamide on day 2 after innoculation of test animals with leukemic cells, the results recorded in Table II were obtained.

The oxidation product was also effective *in vitro*, giving an ED_{50} of 0.4 μg/ml against human epidermoid cells, thus comparing closely with prior results from this laboratory for enzymatically activated cyclophosphamide.[2]

Because the effectiveness of cyclophosphamide depends on prior activation in the liver, 4-peroxycyclophosphamide may prove useful against some forms of cancer in humans with impaired liver function.

(5) R. M. Rauen, K. Norporth, and E. Golovinsky, *Naturwissenschaften*, **54**, 589 (1967).

(6) C. Benckhuijsen, J. Van Der Steen, and J. G. Westra, Vortrag VII, International Chemotherapy Kongress, Prague, 1971, B-3/9.

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(8) O. M. Friedman and E. Boger, *Anal. Chem.*, **33**, 906 (1961).

(9) H. Arnold and F. Bourseaux, Vortrag, a.d. 2 International Symposium on Pharmaceutical Chemistry, Munster/Westf., 1968, p 22.

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(11) Takamizawa, *et al.* (ref 4), mention in a footnote the possibility of dimerization or decomposition of 4-hydroperoxycyclophosphamide but report no attempt to demonstrate the presence of or to characterize any dimerization or decomposition products.

(12) The significant difference in melting point between our product and that reported by Takamizawa, *et al.*, is reconcilable by regulating the rate of heating using Mettler FP-1 apparatus. The following melting points, all with decomposition, were observed: 108.1° at 10°/min; 93.4° at 2°/min; 71.4° at 0.2°/min.

Table II. Comparative Inhibition of L1210 Leukemia *in Vivo* by 4-Peroxycyclophosphamide and by Cyclophosphamide

Compound	Innoculum of leukemic cells	Dose (mg/kg)	Increase in life span (%) (in dying animals)	30-day survivors
4-Peroxycyclo-phosphamide	10^6 (IP)	250	175	3/10
		200	100	2/10
Cyclophosphamide		300	93	0/10
		200	87	1/10
		133	50	0/10
4-Peroxycyclo-phosphamide	10^7 (IV)	250	200	0/10
		200	175	0/10
Cyclophosphamide		312	237	0/10
		156	162	0/10

Acknowledgment. This work was supported by Contracts NIH-NCI-C-73-3712 and NIH-NCI-C-71-2098 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare. Spectral and elemental analytical data were obtained by the Molecular Spectroscopy Section of Southern Research Institute. We thank Mrs. Jean Carpenter for determination of the ED_{50} of 4-peroxycyclophosphamide against human epidermoid cells.

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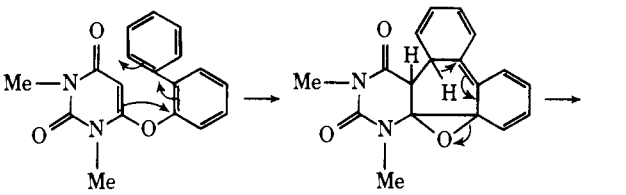
Photocyclodehydration of 6-*o*-Biphenyloxy-1,3-dimethyluracil

Sir:

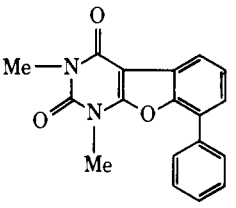
Photochemical cyclodehydrogenation of stilbenes[1] and related compounds[2] and photocyclodehydrohalogenation of their substituted ortho iodo derivatives[3] has been reported to produce mostly a six-membered[1-3] and rarely a five-membered ring.[4]

In general,[5] photocyclodehydrogenation occurs through a cyclic system,[1] while photocyclodehydrohalogenation occurs through an aromatic radical intermediate.[4]

In an effort to further extend our understanding of the mechanism of photocyclization reactions, we have investigated the photolytic behavior of 6-*o*-biphenyloxy-1,3-dimethyluracil (**1**) and 5-iodo-6-*o*-biphenyloxy-1,3-dimethyluracil (**2**). These two compounds are of particular interest in two ways. First, the mode of their cyclization process may be different thus leading to distinct products. In addition, since they exhibit two alternative positions for cyclization, that is ring closure between C_5 of the pyrimidine nuclei and C_6' or C_8' of the biphenyloxy group thus leading to a five- or seven-membered ring formation, respectively, competition between the two may exist.



1, R = H; R' = 2-phenylphenoxy
2, R = I; R' = 2-phenylphenoxy
3, R = H; R' = Cl
7, R = H; R' = 2-iodophenoxy



6

The synthesis of **1**[6] (mp 154–155°; nmr (CCl_4) δ 3.12 (s, NMe, 3 H), 3.28 (s, NMe, 3H), 4.52 (s, C_5H, 1 H), 7.38 (m, aromatic H, 9 H); mass spectrum *m/e* 308 (M^+)) was accomplished by replacement of the chlorine of 6-chloro-1,3-dimethyluracil[7] (**3**) with an *o*-biphenyloxy group using a solution of sodium *o*-biphenyloxide. Its iodo derivative (**2**)[6] (mp 172–173°; nmr ($CDCl_3$) δ 3.24 (s, NMe, 3 H), 3.42 (s, NMe, 3 H), 7.18–7.62 (m, aromatic H, 9 H); mass spectrum *m/e* 434 (M^+)) was obtained by acetomercuration followed by iodination[7] of **1**.

Irradiation of **1** in benzene in 16 hr near room temperature in a cylindrical quartz cell or quartz tube utilizing the light from a Hanovia 100-W mercury lamp led to the tetracyclic **4**[6,8,9] (mp162–163°; nmr ($CDCl_3$) δ 3.52 (s, NMe, 3 H), 3.75 (s, NMe, 3 H), 7.94 (m, 4 H, $H_C + H_B + H_C' + H_B'$), 8.13 (dd, $J = 8.1, J = 1.5$ Hz, H_A'), 8.40–8.73 (m, 2 H, $H_D + H_D'$), 9.78 (dd, $J = 8.2$, $J = 2.5$ Hz, 1 H, H_A); mass spectrum *m/e* 290 (M^+)) while

(1) A. Bomber, K. A. Muszkat, and E. Fischer, *Isr. J. Chem.*, **10**, 765 (1972).
(2) C. E. Loader and C. J. Timmons, *J. Chem. Soc.*, 1678 (1967).
(3) J. Blum, F. Grauer, and E. D. Bergmann, *Tetrahedron*, **25**, 3501 (1969).
(4) W. A. Henderson, R. Lopresti, and A. Zweig, *J. Amer. Chem. Soc.*, **91**, 6049 (1969); W. A. Henderson and A. Zweig, *Tetrahedron Lett.*, 625 (1969).
(5) Contrary to these, suggested mechanisms have also been reported: R. Srinivasan and J. N. C. Hsu, *J. Amer. Chem. Soc.*, **93**, 2816 (1971); G. DeLuea, G. Martelli, P. Spagnolo, and M. Tiecco, *J. Chem. Soc.*, 2504 (1970).
(6) Elemental, nmr, uv, and mass spectra analysis are in complete agreement with the proposed structures.
(7) W. Pfleiderer and H. Deiss, *Isr. J. Chem.*, 603 (1968).
(8) The same substance was obtained from the irradiation of 5,6-diiodo-1,3-dimethyluracil in benzene, which will be reported in the near future.
(9) There is a certain similarity between the nmr of **4** and phenanthrene: F. A. Bovey, "Nuclear Magnetic Resonance Spectroscopy," Academic Press, New York, N. Y., 1969, p 68.