Synthesis and Absolute Configuration of the Optically Active Forms of 2-[Bis(2-chloroethyl)amino]-4-methyltetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide (4-Methylcyclophosphamide)

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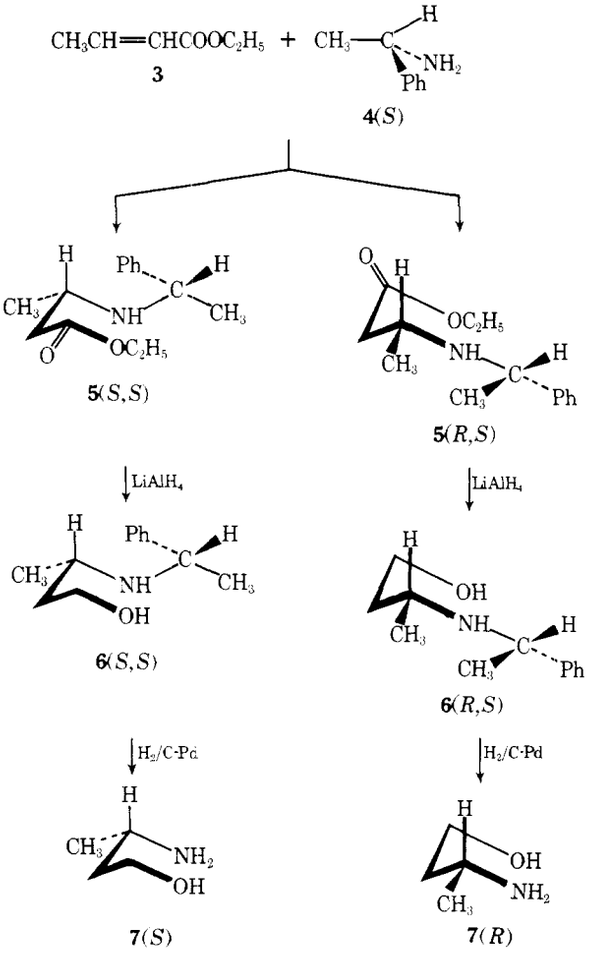
Received September 9, 1976

The cytochrome P450 mediated oxidation of the antitumor agent 2-[bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide (cyclophosphamide **1**) to give the 4-hydroxy derivative is well documented[1] and has prompted many detailed investigations.[2] 4-Methylcyclophosphamide (**2**) is of interest since the methyl group prevents further oxidative metabolism of the 4-hydroxy derivative.[3] The isolation and configurational assignment of the cis and trans forms of 4-methylcyclophosphamide (**2**) has been described recently by Struck et al.[4] We now report evidence which indicates that these configurational assignments are erroneous and also describe the synthesis of the optically active forms of *cis*- and *trans*-4-methylcyclophosphamide.

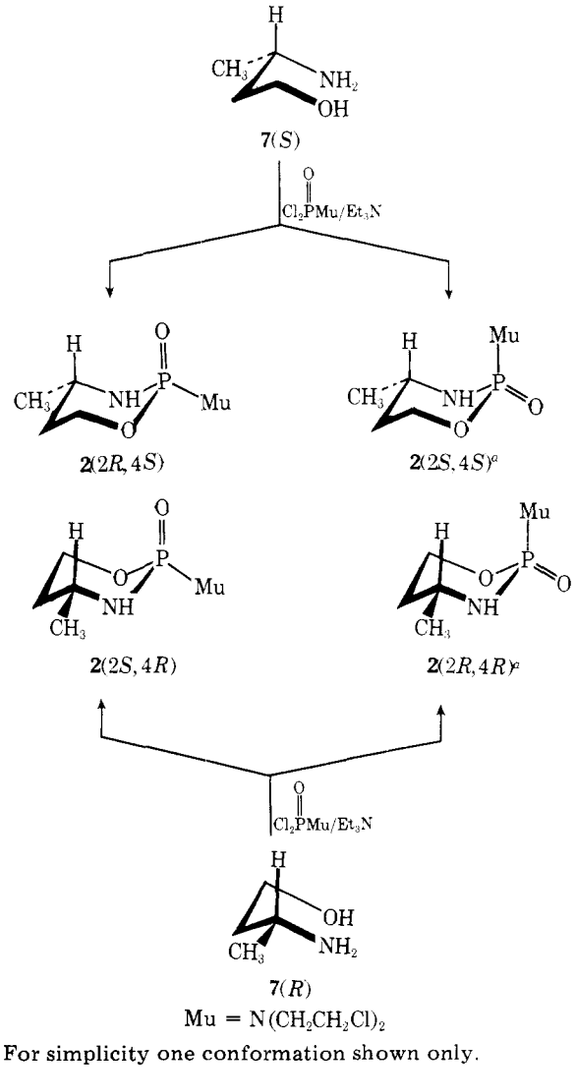
The synthesis of the four optically active forms of 4-methylcyclophosphamide (**2**) was based on (+)- and (−)-3-aminobutan-1-ol (**7**) as depicted in Scheme I. Treatment of ethyl crotonate (**3**) with (−)-1(*S*)-phenylethylamine [**4**(*S*)] gave the separable diastereoisomers of ethyl 3-*N*-[1(*S*)-methylbenzyl]aminobutyrate (**5**) having readily determinable absolute configurations at the newly introduced asymmetric carbon atom (C-3).[5] These were reduced in two stages to give the requisite enantiomers of 3-aminobutan-1-ol (**7**). Condensation of each of these enantiomers with *N,N*-bis(2-chloroethyl) phosphoramidic dichloride gave in each case a mixture of two separable isomers of 4-methylcyclophosphamide, designated "fast" and "slow" according to their relative mobilities on TLC. Since the absolute configuration of the starting materials **7** is known, the four enantiomeric 4-methylcyclophosphamides have predetermined configurations at C-4.

Determination of the absolute configurations at phosphorus for the pairs of diastereoisomers **2** for which the absolute configuration at C-4 is known is equivalent to the assignment of cis and trans geometry to the "fast" and the "slow" isomers **2**. Contrary to the assignments made by Struck et al.[4] for the faster migrating racemic **2** (mp 72–74 °C) and the slower migrating racemic **2** (mp 102 °C) we assign, taking the example of the fast and the slow products derived from 3-(*R*)-aminobutan-1-ol, the spatial arrangement 4-Me_{eq}-2NR_{2eq} (trans) to the slower migrating **2** (and hence the configuration 2*S*,4*R*) and 4-Me_{eq}-2NR_{2ax} ⇌ 4-Me_{ax}-2NR_{2eq} (cis) to the faster migrating **2** (2*R*,4*R*) on the basis of the following arguments: (1) **2** (2*S*,4*R*) absorbs at lower field in its ^{31}P NMR spectrum ($\delta_{^{31}P}$ −13.5 ppm, external H_3PO_4) than **2** (2*R*,4*R*) ($\delta_{^{31}P}$ −11.0 ppm);[6a] (2) **2**(2*S*,4*R*) exhibits a lower ν_{PO} value (1218 cm^{-1} in

Scheme I



Scheme II



[a] For simplicity one conformation shown only.

CCl_4) than **2**(2*R*,4*R*) (1231 cm^{-1});[6b,7] (3) The value (2.9 Hz) of $^4J_{PNCCH_3}$ (^1H NMR) for **2**(2*S*,4*R*) is higher than that (1.9 Hz)[8] for **2**(2*R*,4*R*).

The conformational stability of **2**(2*S*,4*R*) was established on the basis of the data $^3J_{POCH_{eq}}$ = 22.75 (^1H NMR), $^3J_{PNCC_5}$ = 3.2, and $^3J_{PNCCH_3}$ = 12.1 Hz (^{13}C NMR). Although analysis of the ^1H NMR spectrum of **2**(2*R*,4*R*) was not possible because of its complexity, the ^{13}C NMR spectrum gave the data $^3J_{PNCC_5}$ = 7.0 and $^3J_{PNCCH_3}$ = 7.6 Hz, which suggested rapid equilibrium of two or more conformations of **2**(2*R*,4*R*). The assignment 4-Me_{eq}-2NR_{2eq} to **2**(2*S*,4*R*) and its conformational stability is consistent with the known equatorial preference of the 4-methyl and 2-dialkylamino groups in dioxa- and oxazaphosphorine ring systems.[9a,b]

Arguments analogous to those applied to the products from 3-(*R*)-aminobutan-1-ol enabled the assignment of absolute configuration to the products from the 3-(*S*) isomer, namely, **2**(2*S*,4*S*) and **2**(2*R*,4*S*).

The metabolism and antitumor activity of the optically active forms of *cis*- and *trans*-4-methylcyclophosphamide are being investigated. In this context it is interesting that the (+) and (−) forms of cyclophosphamide[10] exhibit markedly different antitumor activities against an experimental mouse tumor (ADJ/PC6A) and undergo differential metabolism in man.[11] Configuration around phosphorus would therefore appear to be an important factor in metabolic transformations of this clinically important drug.

Experimental Section

All melting points and boiling points are uncorrected. Solvents and commercial reagents were distilled and dried by conventional methods before use.

^1H NMR spectra were recorded at 60 MHz with a JEOL C-60H spectrometer equipped with Hetero-Spin-Decoupler SNH-SD-HC or at 80 MHz with a Tesla BS 487 C spectrometer with Me_4Si as an internal standard. ^{31}P NMR spectra were obtained on the first-mentioned instrument at 24.3 MHz with external H_3PO_4 as the reference. Negative chemical shift values are reported for compounds absorbing at lower fields than H_3PO_4. ^{13}C spectra were measured at 22.63 MHz with a Bruker HX-72 system using the FT technique. Chemical shifts are related to internal Me_4Si. Mass spectra were obtained on a LKB 9000S spectrometer at 70 eV ionizing energy. Optical activity measurements were made with a Perkin-Elmer 241 MC photopolarimeter. Product purities were determined from integrated ^1H and ^{31}P NMR spectra or TLC (silica gel 60, F254). Silica gel for column chromatography was 100–200 mesh.

Starting Materials. 1(*S*)-Phenylethylamine had bp 94–95 °C (28 mm), $n^{25}D$ 1.5240, $[\alpha]^{20}D$ −37.0° (neat). 1-Phenylethylamine was resolved into optical antipodes according to ref 14.

(−)-Ethyl 3(*S*)-*N*-[1(*S*)-Methylbenzyl]aminobutyrate [5(*S,S*)] and Its 3(*R*),1(*S*) Isomer [5(*R,S*)]. A solution of ethyl crotonate (**3**, 25 g, 0.22 mol) and 1-(*S*)-phenylethylamine [**4**(*S*)] (22 g, 0.18 mol) in ethanol (50 mL) was heated under reflux for 6 h, then concentrated. Distillation of the residue gave unchanged **4**(*S*) [15 g, bp 35 °C (0.1 mm)] and the mixture of diastereoisomers (**5**) [12 g, bp 99 °C (0.1 mm), $n^{25}D$ 1.4968]. To the recovered 4(*S*) was added a further 10 g of **3** and the foregoing procedure was repeated to yield a further 8 g of **5**. A portion (20 g) of the resulting equimolar mixture of **5**(*S,S*) and **5**(*R,S*) was fractionated on a column of silica gel using benzene–dioxane–acetone (40:2:1) as eluent, to give first the **5**(*S,S*) isomer [8 g (after distillation); bp 76–77 °C (0.05 mm); $[\alpha]^{25}D$ −48.5° (*c* 7.4, benzene); $n^{25}D$ 1.4924; R_f 0.26 (TLC, benzene–dioxane–acetone, 20:2:1); mass spectrum M^+ *m/e* 235 (1), 105 (100). Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.50; H, 8.93; N, 5.96. Found: C, 71.23; H, 8.80; N, 5.80%], followed by the **5**(*R,S*) isomer [7 g; bp 76–77 °C (0.05 mm); $[\alpha]^{25}D$ −35.2° (*c* 6.8, benzene); $n^{25}D$ 1.4943; R_f 0.19; mass spectrum M^+ *m/e* 235 (0.2), 105 (100). Anal. Found: C, 71.42; H, 8.95; N, 5.88].

3(*S*)-*N*-[1(*S*)-Methylbenzyl]aminobutan-1-ol [6(*S,S*)] and Its 3(*R*),1(*S*) Enantiomer [6(*R,S*)]. A solution of **5**(*S,S*) (7 g, 0.03 mol) in dry ether (30 mL) was added to a solution of $LiAlH_4$ (2.4 g, 0.063 mol) in ether (75 mL). After heating under reflux for 5 h followed by standing overnight at room temperature water was added, followed by 10 M NaOH (12.5 mL). The ether layer was separated and the aqueous phase further extracted with ether (2 × 25 mL). The combined extracts were dried (KOH) and concentrated to an oil which was distilled to give **6**(*S,S*): 5.05 g, 88%; bp 82–87 °C (0.1 mm); $[\alpha]^{25}D$ +13.6° (*c* 4.9, benzene); $n^{25}D$ 1.5200. Anal. Calcd for $C_{12}H_{19}NO$: C, 74.62; H, 9.83; N, 7.25. Found: C, 74.30; H, 9.63; N, 7.13.

By an identical procedure **5**(*R,S*) afforded **6**(*R,S*) except that this product was crystallized from *n*-hexane: yield 4.90 g, 85%; mp 59–60 °C; $[\alpha]^{25}D$ −89.0° (*c* 4.4, benzene). Anal. Found: C, 74.50; H, 9.71; N, 7.22.

3(*S*)-Aminobutan-1-ol [7(*S*)] and Its Enantiomer [7(*R*)]. A solution of **6**(*S,S*) (11.5 g, 0.06 mol) in 96% ethanol (30 mL) was added to a suspension of 10% Pd/C (0.5 g) in ethanol (50 mL) in an atmosphere of H_2. The stirred mixture was maintained at 50 °C until the theoretical volume of H_2 was consumed (ca. 2 days). Catalyst was removed by filtration, the filtrate was concentrated, and the residue was distilled to give **7**(*S*): 3.66 g, 69%; bp 80 °C (20 mm); $[\alpha]^{25}D$ +10.5° (*c* 5.1, ethanol).

By an identical procedure, **6**(*R,S*) was converted into 3(*R*)-aminobutanol [**7**(*R*)]: 70%; bp 80 °C (20 mm); $[\alpha]^{25}D$ −11.2° (*c* 5.1, ethanol).

Physical and chemical properties of **7**(*R*) and **7**(*S*) were identical with those reported for racemic **7**.[12]

Preparation of the Four Diastereoisomers of 4-Methylcyclophosphamide [2(*S*)-Bis[(2-chloroethyl)amino]-4(*S*)-methyltetrahydro-2*H*-1,3,2-oxazaphosphorine 2-Oxide [2-(2*S*,4*S*)(*cis*)], the (2*R*,4*S*)(*trans*), the (2*R*,4*R*)(*cis*), and the (2*S*,4*R*)(*trans*) Isomers]. To a solution of 3(*S*)-aminobutan-1-ol [**7**(*S*), (3.66 g, 0.04 mol)] in dry dioxane (100 mL) containing triethylamine (9.1 g, 0.09 mol) was added a solution of *N,N*-bis(2-chloroethyl)phosphoroamidic dichloride[13] (10.6 g, 0.04 mol) in dioxane (100 mL). After stirring for 15 h at room temperature, Et_3N·HCl was removed by filtration. The concentrated filtrate was applied to a column of silica gel (400 g) which was eluted with acetone–chloroform (3:1) to give first the faster migrating (TLC in the same solvent) cis isomer [**2**(2*S*,4*S*)] obtained as colorless crystals from ether [1.80 g, 31.8%; mp 83–83.5 °C; $[\alpha]^{25}D$ +16.5° (*c* 3.0, methanol); $\delta^{31}P$ −11.0 ppm ($CDCl_3$); R_f 0.57] followed by the slower moving trans isomer [**2**(2*R*,4*S*)] which crystallized from light petroleum [1.45 g, 25.6%; mp 56–57 °C; $[\alpha]^{25}D$ +7.7° (*c* 3.0, methanol); $\delta^{31}P$ −13.5 ppm ($CDCl_3$); R_f 0.48].

Similarly prepared from 3(*R*)-aminobutan-1-ol [**7**(*R*)] were the cis isomer [**2**(2*R*,4*R*)] [1.50 g, 26.5%; mp 83–84 °C (from ether); $[\alpha]^{25}D$ −17.8° (*c* 2.2, methanol); $\delta^{31}P$ −11.0 ppm ($CDCl_3$); R_f 0.57] and the trans isomer [**2**(2*S*,4*R*)] [1.10 g, 19.5%; mp 56–57 °C (from light petroleum); $[\alpha]^{25}D$ −8.3° (*c* 3.0, methanol); $\delta^{31}P$ −13.5 ppm ($CDCl_3$); R_f 0.48].

Acknowledgments. This work was supported by the Polish Academy of Sciences. The authors are grateful to Dr. R. F. Struck of Southern Research Institute, Birmingham, Ala., for reading the manuscript and for stimulating comments.

Registry No.—(2*S*,4*S*)-**2**, 61520-80-7; (2*R*,4*R*)-**2**, 61520-81-8; (2*R*,4*S*)-**2**, 61520-82-9; (2*S*,4*R*)-**2**, 61520-83-0; **3**, 10544-63-5; (*S*)-**4**, 2627-86-3; (*S,S*)-**5**, 61477-36-9; (*R,S*)-**5**, 61477-37-0; (*S,S*)-**6**, 61477-38-1; (*R,S*)-**6**, 60920-20-9; (*S*)-**7**, 61477-39-2; (*R*)-**7**, 61477-40-5; *N,N*-bis(2-chloroethyl)phosphoroamidic dichloride, 127-88-8.

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Epimerization of Acyclic Diastereomers. 2.[1] Bis(alkylphenylcarbinyl) Ether

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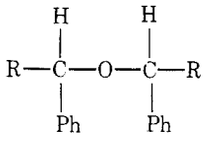
Received June 11, 1976

The epimerization of cyclic compounds has been extensively investigated[2-11] and it has been shown that the thermodynamic stabilities of cyclic diastereomers differed considerably in several cases.[2-5,10,11] On the other hand, thermodynamic stabilities of acyclic diastereomers do not differ by much in general.[12,13] However, in one case, i.e., 2,4-dichloropentane, a large energy difference between two diastereomers has been reported by Billups et al.[14,15] and calculated by MacMahon et al., assuming Lennard–Jones type interactions between nonbonded atoms.[16,17] In the preceding paper,[1] we reported the notable stability of the *dl* isomer compared to the meso isomer in bis(α-phenylethyl) ether and suggested that this stability of the *dl* isomer could not be explained entirely by steric factors (nonbonded interactions).

In the present report the even greater preference of the *dl* isomers of bis(alkylphenylcarbinyl) ether compared to the meso isomers is shown and the source of this preference of *dl* isomers is described.

Results and Discussion

Epimerization of three compounds (Ia, Ib, and Ic) catalyzed by boron trifluoride etherate in carbon tetrachloride or nitrobenzene was carried out.



Ia, R = Et
b, R = *n*-Bu
c, R = cyclohexyl

As reported before,[1] an epimerization of bis(alkylphenylcarbinyl) ether is accompanied by an elimination reaction. The results of epimerization of Ia in carbon tetrachloride or nitrobenzene are shown in Figures 1 and 2. One might suppose that the meso isomer would be preferentially destroyed and that epimerization of the ether would not take place. To clarify this point, a reaction of Ia consisting of 3.5% *dl* and 96.5% meso isomers in carbon tetrachloride was carried out and the result is shown in Figure 3. This figure indicates that epimerization of the ether takes place in carbon tetrachloride giving an ether consisting of 100% *dl* isomer at prolonged time. The results of epimerization reactions of Ia, Ib, and Ic in carbon tetrachloride and nitrobenzene are summarized in Table I. The epimerization reaction of Ib was also started with equal concentrations of the *dl* and meso isomers, and a large excess of the meso isomer. A separation of isomers of Ic was unsuccessful. Each run was allowed to continue until the composition of the ether was constant. In the determination of the ether composition after epimerization of Ic in nitrobenzene by means of NMR (see Experimental Section), the signals of the methine proton doublet of the meso isomer overlapped with those of unknown by-products, making the isomeric composition uncertain. Since the reactions of Ia, Ib, and Ic in carbon tetrachloride were slow, boron trifluoride etherate was used in higher concentration than in nitrobenzene.

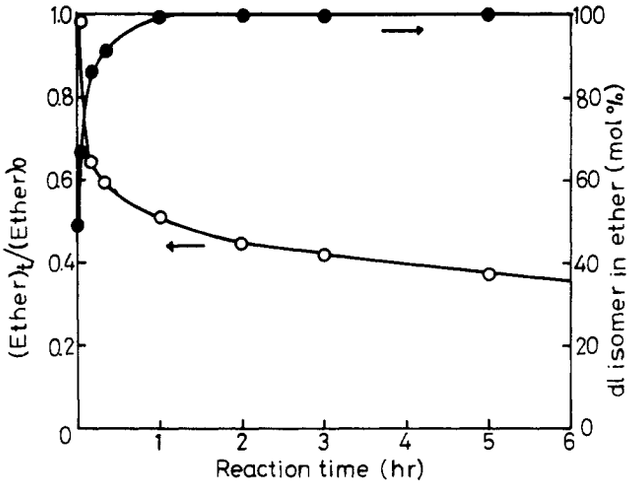


Figure 1. Epimerization and elimination of bis(ethylphenylcarbinyl) ether in carbon tetrachloride at 25 °C. Ether consisting of equal moles of *dl* and meso isomer was used. Ether, 0.05 mol/L; BF_3OEt_2, 0.04 mol/L. ○, decrease of ether; ●, mol % of *dl* isomer in ether.

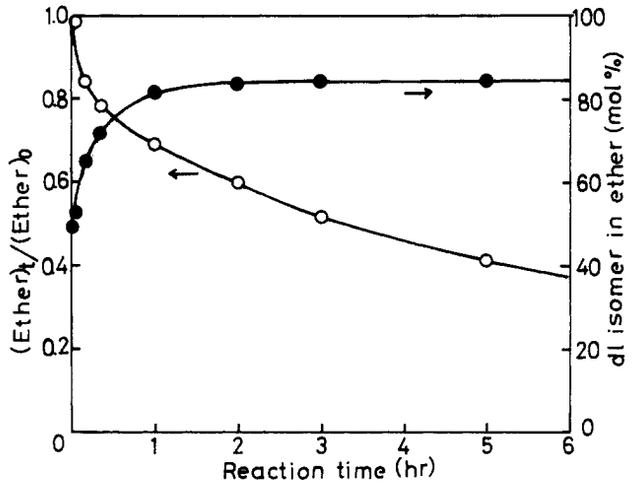


Figure 2. Epimerization and elimination of bis(ethylphenylcarbinyl) ether in nitrobenzene at 25 °C. Ether consisting of equal moles of *dl* and meso isomer was used. Ether, 0.05 mol/L; BF_3OEt_2, 0.01 mol/L. ○, decrease of ether; ●, mol % of *dl* isomer in ether.

Steady-state values of ether composition shown in Table I do not depend on the composition of starting ether and do not change even after prolonged reaction time. These results indicate that destruction of both isomers (*dl* and meso isomers) and epimerization of isomers took place. Since the rate of the epimerization reaction is faster than that of the destruction, the reaction of the ether with boron trifluoride etherate should give the steady-state composition of ether. Although, strictly speaking, the product compositions in Table I are not equilibrated but kinetically controlled, these values would indicate thermodynamic stabilities of these isomers. Generally speaking, the difference in thermodynamic stabilities between two isomers is even greater in the less polar solvent than in the polar solvent. Therefore, although the destruction of ether in carbon tetrachloride seriously competes