PHOSPHONOMYCIN ANALOGS: SYNTHESIS OF DIETHYL ESTERS OF cis- and trans-(2-METHYL-3-AZIRIDINYL) PHOSPHONATES AND DERIVATIVES FROM PHENYL ISOCYANATE

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Cis- and *trans*-isomers of diethyl (2-methyl-3-aziridinyl)phosphonate have been synthesized and characterized via proton magnetic resonance (PMR) analysis and by conversion to solid derivatives by reaction with phenyl isocyanate. The esters are related to phosphonomycin. Attempts to hydrolyze the esters or the derivatives to the exact nitrogen analog of phosphonomycin resulted in extensive polymerization.

MATERIALS AND METHODS

Diethyl propadienylphosphonate (Compound A)

The procedure used was a modification of that reported in the literature (1). To a mixture of diethyl phosphorochloridite (compound B) (31.3 g; 0.2 mole) and triethylamine (20.25 g; 0.2 mole) in 300 ml of dry benzene was added, under dry nitrogen, propargyl alcohol (11.5 g; 0.2 mole) in 50 ml of benzene, over the course of 30 min with cooling and stirring. The reaction temperature was maintained at 0—10 C. After the addition, the reaction mixture was stirred at 5—10 C for 1 hr and then warmed to 40 C and left at that temperature for 1.5 hr. After cooling to room temperature, 200 ml of water was added and the benzene layer was separated. The aqueous layer was extracted with benzene (2 x 50 ml) and the benzene extracts were combined, washed with saturated salt solution, and dried ($MgSO_4$). Evaporation of benzene in a rotary evaporator without heating gave a yellow oil (32 g). Infrared (IR) (film): 5.13 μ (-CH=C=CH_2) and 8 μ (P→O). Since compound A is known to rearrange on storage or on heating (1), it was used without purification for the next step.

Hydrogenation of diethyl propadienylphosphonate (compound A) and synthesis of diethyl *cis*-propenylphosphonate (compound C)

Crude A (32 g) from the previous step was divided into two 16-g batches. Each batch was dissolved in 125 ml of dry benzene and hydrogenated at room temperature using 1 g of 10% palladium on charcoal as catalyst at 20 p.s.i. in a Parr hydrogenation unit. The hydrogen intake was complete in 1.5 hr. Both batches were mixed together and the catalyst filtered off. Evaporation of benzene and distillation of the residue using a 4-inch column gave pure compound C (2-5), b.p. 42-43 C, (0.025 mm), 18.3 g (56.8%, based on A); IR (film) 6.14 μ (C=C), 7.99 μ (P→O).

Bromination of diethyl *cis*-propenylphosphonate

The olefin C (17.8 g; 0.1 mole) was dissolved in 100 ml of glacial acetic acid, and 16 g (0.1 mole) of bromine in 100 ml of glacial acetic acid was added in the course of 1 hr with stirring at room temperature. Stirring was continued for another 20 hr at room temperature. The acetic acid was distilled off under aspirator, and the residue, after dilution with 400 ml of water, was extracted with chloroform. The chloroform extracts were washed with water and dried ($MgSO_4$). Evaporation of the $HCCl_3$ gave 31.2 g (92%) of crude diethyl *threo*-1, 2-dibromopropylphosphonate (compound D), which was used directly in the next step without further purification.

Diethyl (1-bromopropenyl)-phosphonate (compound E)

Crude diethyl *threo*-1, 2-dibromopropylphosphonate (compound D) (31.2 g; 0.092 mole) obtained from the previous reaction was dissolved in 250 ml of dry $HCCl_3$ and dry ammonia gas was passed

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through the solution with stirring and cooling in water. After passing NH_3 for 30 min, the precipitate of ammonium bromide was filtered off and washed (50 ml of $HCCl_3$). The chloroform solution was washed first with water and then with saturated salt solution and dried ($MgSO_4$). Evaporation of $HCCl_3$ gave an oil, which upon distillation using, an 8-inch Vigreux column, gave 20.42 g (87.2%) of compound E, b.p. 75 C (0.1 mm) (86%); IR (film) 6.15 μ (C=C), and 7.95 μ (P→O).

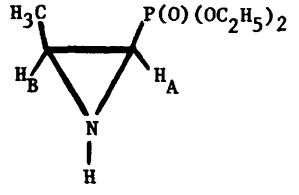
Diethyl *cis*- (compound F) and diethyl *trans*-(2-methyl-3-aziridinyl)-phosphonate (compound G)

The unsaturated ester E (19.4 g; 0.075 mole) was placed in a thick-walled Pyrex tube (22" long, 1" diameter) and cooled in a dry ice-acetone bath. Approximately 20 ml of liquid NH_3 was condensed in the tube, and the tube was sealed and left at room temperature for 16 hr. The tube was opened to atmosphere (after cooling in dry ice) and the excess NH_3 was allowed to evaporate. To the residue, 100 ml of dry $HCCl_3$ was added and the NH_4Br was filtered off; the NH_4Br was washed with $HCCl_3$ and the $HCCl_3$ extracts were washed with water and dried ($MgSO_4$). Evaporation of $HCCl_3$ resulted in a yellow oil which was distilled using a short path distillation unit to give a mixture of compounds F and G, b.p. 71-72 C (0.05 mm), 10.5 g (71%); IR (film) 2.88 μ (N-H), 3.09 μ (hydrogen bonded N-H) and 8.03 μ (P→O). The presence of two sets of doublets in the NMR ($HCCl_3$, 60 MHz) for the ring methyl group indicated the presence of compounds F and G in the mixture. Thin layer chromatography on neutral alumina, using $HCCl_3$ as developing solvent, showed two close spots.

Column chromatography of 1 gm of the mixture of compounds F and G on 30 g of neutral alumina (activity 1) column gave 188 mg of compound G (in 1:1 $HCCl_3$-benzene elution) and 220 mg of compound F (in 3:2 $HCCl_3$-C_6H_6 elution) and 272 mg of intermediary fractions containing F and G. Large-scale chromatography resulted in high losses of products due to strong column adsorptivity which required exceptionally large amounts of solvent to cause disassociation. Consequently, some structural changes occurred in compounds F and G, probably due to cleavage of the $P(O)(OC_2H_5)_2$ group on the column. Stripping of the column with C_2H_5OH: H_2O (9:1) gave a colored oil which rapidly decomposed a 10% $NaHCO_3$ solution. Thus, it is suggested that hydrolysis occurred to give acidic material which probably contained P-OH.

Unfortunately, the samples of esters colored on standing even a short time; this prevented adequate elemental analysis. Consequently, a fresh mixture of compounds F and G was used at once to prepare the derivatives H and I. Fresh samples of compounds F and G were immediately subjected to PMR analysis with the following results.

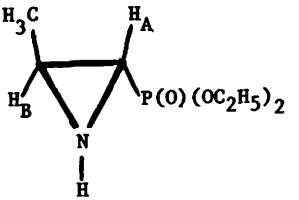
100 MHz NMR of compound F in $DCCl_3$ (in δ ppm):



Compound F

δ 1.33 (t, J = 7 Hz, OCH_2CH_3), δ 1.46 (d, J = 6 Hz, ring CH_3), a pair of doublets at δ 1.8 and 1.95 [H_A, $J_{AB(cis)}$ = 6Hz, J_{PCH_A} = 15 Hz], multiplet δ 2.3 (H coupled by CH_3 and *cis* coupled to H_A), and a multiplet at δ 4.14 (OCH_2CH_3).

100 MHz NMR of G in $DCCl_3$ (in δ ppm):



Compound G

δ 1.34 (t, J = 7 Hz., OCH_2CH_3), multiplet at δ ≃ 1.26 merged with the 1.34 triplet (ring CH_3 coupled by H_B and probably long-range coupling from P or

H_A), a pair of doublets centered at δ 1.89 and 1.96 [H_A, $J_{AB(trans)}$= 3 Hz, $J_{P\text{-}CH_A}$ = 7 Hz], multiplet at $\delta \simeq$ 2.38 ($H\beta$ coupled by CH_3 and *trans* coupling with H_A), and a multiplet at δ 4.12 (OCH_2CH_3, coupled by CH_3 and P).

Addition of the mixture of compounds F and G to phenyl isocyanate

A mixture of compounds F and G [1.93 g; 0.01 mole] was dissolved in 5 ml of dry benzene and added to 600 mg (0.005 mole) of phenyl isocyanate (freshly distilled) and stirred at room temperature (protected from moisture) for 10 min and then warmed at 60-65 C for 20 min. The solution was diluted with hexane until the solution turned turbid. On cooling the solution, 867 mg of solid m.p. 97-104 C was obtained (crude compound I). The filtrate was concentrated to 5 ml, mixed with 600 mg (0.005 mole) of phenyl isocyanate, warmed on a water bath for 30 min, and cooled. Hexane was added to this solution until it turned turbid and cooled. White, needle-shaped crystals were obtained, 1.065 g, m.p. 139-144 C (crude compound H). From the filtrate a solid, m.p. 100-137 C, (mixture of compounds H and I) (909 mg) was obtained. Thus, a total of 2.841 g (88%) of the addition products was isolated. Crude compound I (867 mg), upon 3 recrystallizations from benzene-hexane, gave 756 mg of pure compound I, m.p. 100-102 C.

Similarly crude compound H (1.065 g), after 4 recrystallizations from benzene-hexane, gave 828 mg of pure compound H, m.p. 149-151 C.

Mother liquors from these two recrystallizations and 909 mg of solid melting at 100-137 C (mixture of compounds H and I) were mixed together and gave, after a number of fractional crystallizations, 328 mg, m.p. 100-102 C, of compound I and 540 mg, m.p. 149-151 C, of compound H. There was also obtained 188 mg of a solid, m.p. 96-125 C (a mixture of compounds H and I). Thus, total yields of pure compounds H and I were 1.176 g (36.5%) and 1.08 g (33.4%), respectively.

μ Infrared spectra of both compounds H and I show N-H stretching at 3.08μ, C=O stretching at 5.95μ, and P→O stretching at 8μ. NMR (100) MHz) analysis of compound H (δ ppmm $DCCl_3$) shows two sets of triplets centered at δ 1.35 and 1.37 [J = 7 Hz, $P(O)(OCH_2CH_3)_2$; the two sets may be due to nonequivalence of the two ethoxy groups arising from the asymmetric carbon and restricted rotation around C-P bond due to hindrance from the *cis* CH_3 group and N-substituent]. A doublet centered at $\delta \simeq$ 1.36 (partly merged with the CH_3 triplets of OCH_2CH_3), the components of which were further split by the long-range P coupling (ring methyl coupled to H_B, J = 7 Hz, and long-range coupling with P), a pair of doublets centered at δ 2.49 and 2.67 [H_A, $J_{AB(cis)}$= 3.5 Hz, $J_{P\text{-}CH_A}$ = 18 Hz], multiplet at $\delta \simeq$ 3.24 (H_A coupled to CH_3 and H_A), a multiplet at δ 4.22 [$P(O)(OCH_2CH_3)$], multiplets at δ 7, 7.24 and 7.54 (phenyl ring protons) and a singlet (broad) at 9.26 μ (N-H) are observed. Similar analysis of compound I shows a triplet at δ 1.35 [J = 7 Hz, $P(O)\text{-}OCH_2CH_3)_2$ (the corresponding CH_3 absorption in the case of *cis* form compound H was a set of two triplets). This may be due to accidental equivalence in the *trans* form of compound I. There is also observed an unresolved pair of two doublets at δ 1.56 (ring CH_3, coupled to H and long-range coupling with P), a pair of doublets centered at δ 2.7 and 2.83 [H_A, $J_{AB(trans)}$ 1.5 Hz; $J_{P\text{-}CH_A}$ = 13.5 Hz] (a multiplet overlapping with the peaks of H_A centered at δ 2.73 [H_B coupled to CH_3 and trans H_A], a multiplet at δ 4.2 [$P(O)(OCH_2\text{-}CH_3)_2$], multiplets at δ 7.03, 7.26 and 7.46 (aromatic ring protons), and a broad singlet at δ 8.22 (N.H).

Reaction of ester H with potassium hydroxide in solution

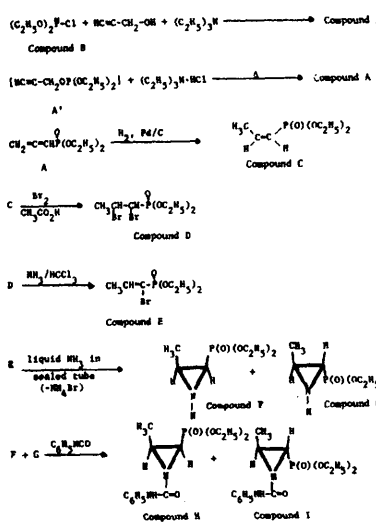
A suspension of 200 mg of compound H in a solution of 200 mg of KOH dissolved in 4 ml of H_2O and 3 ml of ethanol was warmed on a water bath for 30 min. Removal of solvents was accomplished under vacuum. A solid residue containing excess KOH was dried under vacuum and dissolved in D_2O. Analysis of the NMR spectrum permits an assignment of a tentative structure, compound J, as described in the Discussion. All attempts to purify the organic portion of the solid residue, both with recrystallization and chromatography, fail-

ed. Longer reaction times or increased concentrations of KOH resulted in destruction of the ring system.

RESULTS AND DISCUSSION

Because of our long interest (2-4) in the chemistry of aziridines as chemotherapeutic agents and because of a recent patent (5), we are prompted to reveal our results on the synthesis of esters of nitrogen analogs of phosphonomycin and certain reactions thereof. The basic reaction sequence is as shown under Scheme I.

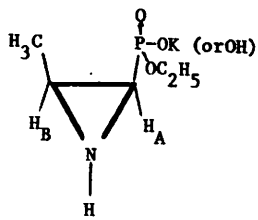
Scheme I



The overall yield of diethyl *cis*-propenylphosphonate (compound E) is 51.4% based on the propargyl alcohol used in the initial step. Bromination of C in acetic acid gave the dibromide compound D, which on dehydrobromination using ammonia gas, gave the vinyl bromide E. Treatment of the vinyl bromide E with liquid ammonia in a sealed tube gave a mixture of diethyl *cis*-(2-methyl-3-aziridinyl)phosphonate F and diethyl *trans*-(2-methyl-3-aziridinyl)phosphonate G (71%). Separation of compounds F and G could be effected by column chromatography over neutral alumina but only with exessive loss, possibly due to hydrolysis of the P-ester groups and rearrangements. The 100 MHz NMR spectrum of compound F gave a value of 6 Hz (2) for the *cis* coupling of the aziridine ring hydrogens and a geminate P-C-H coupling of 15 Hz. In the case of the trans isomer G, the trans coupling of ring hydrogens was 3 Hz (2) and geminate P-C-H coupling was 7 Hz. The two isomers colored upon standing and satisfactory elemental analysis was not achieved. However, reaction with phenyl isocyanate gave solid derivatives H and I which were adequately characterized.

The phenyl isocyanate products H and J prepared from the mixture of aziridines F and I, respectively, could be easily separated by fractional crystallization of the mixture. It was also found that the *trans* aziridine I reacts faster with phenyl isocyanate than does the *cis* form. Thus, treatment of one equivalent of an approximate 1:1 mixture of compounds F and I with 0.5 equivalent of C_6H_5NCO first gives the adduct I. After separating the adduct I, further addition of 0.5 equivalent of C_6H_5NCO yields the adduct H. The 100 MHz NMR spectrum of compound H shows that the OC_2H_5 groups are nonequivalent, which is likely the primary result of the presence of the asymmetric carbon atom in the ring. Comparison of J_{AB} for isomers F (*cis*) and G (*trans*) shows values of 6 Hz and 3 Hz, respectively. The corresponding derivatives H and I had J_{AB} values of 3 Hz and 1.5 Hz, respectively. This is strong evidence for preservation of the ring structure during derivatization (cf. 2).

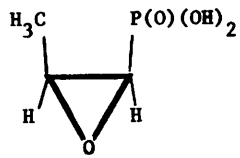
Attempted basic hydrolysis of the *cis* isomeric derivative I in H_2O:C_2H_5OH gave only a complex mixture. PMR analysis (in D_2O) at 100 MHz of a solid residue revealed signals as follows (these δ values are approximate since the zero point, the usual TMS peak, varies by a few Hz when the ^2H lock is used): δ 0.75-1.00 (6H, $2CH_3$ groups appears to be a triplet and complex doublet overlapping), 1.18 (1H, H_A, d, with J_{PCH_A} = 15 Hz, split by H-H coupling — $J_{H_AH_B}$ = 3 Hz, 1.85 (1H, H_B, m), and 3.52 (2H, CH_2, quintet). These data suggest the structure J for the organic component of the mixture. The extreme



Compound J

insolubility of the material in other organic solvents prevented further NMR analysis. All attempts to purify it by chromatography on alumina or ion exchange resins failed and produced an intractable polymeric material. These results differ from those published (5) on the hydrolysis of the methyl ester of compound G since, with NaOH, the monosodium compound was obtained. This latter salt was converted by *Aspergillus niger* to the free acid (5). Unfortunately, all attempts using simple chemical hydrolysis with freshly prepared compound F or G resulted in the production of polymeric materials in our laboratory.

In summary, the *cis* and *trans* ethyl esters



Compound K

of the nitrogen analog of phosphonomycin (K) have been prepared. Attempts to hydrolyze the esters to the corresponding phosphonic acid (the exact nitrogen analog) gave primarily polymeric materials. Derivatives of the esters were made from reaction with phenyl isocyanate. Characterization of the derivatives via PMR analysis revealed coupling patterns similar to those in the esters, which strongly implies that the stereochemistry of the ring protons is intact.

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