Compound I gives the two characteristic bands at 1170 cm.$^{-1}$ and 1130 cm.$^{-1}$ of weaker intensity due to the presence of the 3,4,5-trisubstituted aromatic ring. Compound II shows in its spectra two bands at 1170 and 1130 cm.$^{-1}$ of the 3,4,5-trisubstituted aromatic ring, and the peak at 1630 cm.$^{-1}$ of weak intensity is due to the presence of the aromatic tertiary amine structure in the molecule. Compound III, being a 1,2,3-trisubstituted aromatic compound, shows absorption bands at 1000 and 1170 cm.$^{-1}$. Since there is a C-methyl group in the molecule, a peak at 1350 cm.$^{-1}$ due to CH deformation is observed. Compound IV, being a 2,4,5-trisubstituted derivative, gives absorption bands at 1000 and 1150 cm.$^{-1}$ and CH deformation for the C—CH_3 group at 1350 cm.$^{-1}$. With compound V, the bands of absorption at frequencies 1130 and 1000 cm.$^{-1}$ and 1170 to 1130 cm.$^{-1}$ clearly reveal that this compound contains two aromatic nuclei, both of which are trisubstituted.

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Synthesis and Chemotherapeutic Effects of Ethyl Bis-(2,2-dimethyl)-ethylenamido Phosphate. A Preliminary Report

By Z. F. CHMIELEWICZ, T. J. BARDOS, A. MUNSON, H. L. BABBITT, and J. L. AMBRUS

The title compound, a new analog of the experimental anticancer agent, AB-132, was synthesized and tested against a spectrum of transplanted animal tumors. It was found to have significant chemotherapeutic activity in all of the tumor systems studied. In these assays, the new compound was a much more effective antitumor agent than AB-132.

ETHYL BIS(2,2 - DIMETHYL - 1 - AZIRIDINYL)PHOSPHINYL CARBAMATE (AB-132) was synthesized in this laboratory several years ago (1). This compound, although only moderately effective against various transplanted tumors in rodents, nevertheless showed promising therapeutic activity in the clinical testing against various forms of human cancer, particularly, in conjunction with X-irradiation (2–5). In view of these results, it appeared of interest to synthesize the title compound (I), in which the urethan moiety of AB-132 is replaced by an ethoxy group.

RESULTS AND DISCUSSION

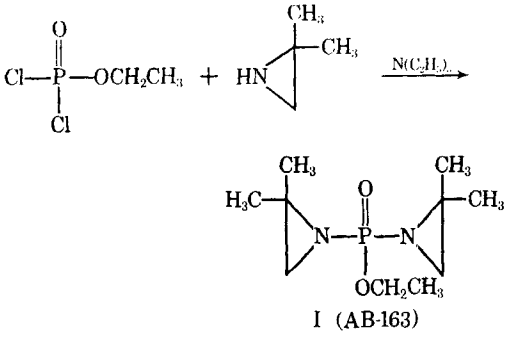
Ethyl bis(2,2-dimethyl)ethylenamido phosphate (I), designated as AB-163, was synthesized by the reaction of ethyl dichlorophosphate with 2,2-dimethylaziridine (in the presence of triethylamine) and purified by distillation under high vacuum, as described under *Experimental*. (Scheme I.)

The compound, a colorless, viscous oil, is readily soluble in water but, like other 2,2-dimethylaziridine derivatives, it undergoes rapid hydrolysis; it gradually decomposes even on exposure to air (humidity and carbon dioxide) and has to be stored in sealed containers. Its NMR spectrum shows the characteristic β-phosphorus splitting of the methylene protons, both in the aziridine rings and the ester group, and a singlet for the methyl substituents of the tertiary ring carbon.

In chemical alkylation studies, using 4-(*p*-nitrobenzyl)pyridine as a model nucleophile (6), AB-163 demonstrated a higher initial rate of alkylation compared to AB-132 (Fig. 1). Its higher chemical reactivity was paralleled by its greater toxicity. The LD_{50} of AB-163 in mice was 159 mg./Kg. (i.p.), in comparison to the 600 mg./Kg. LD_{50} value for AB-132.

The results of some of the antitumor studies are shown in Figs. 2–5. Details of the methods used in the animal assays will be published elsewhere.

Figure 2 shows the effects of various doses of AB-163 against Ehrlich ascites tumor in ICR/Ha mice. Treatment was started 24 hr. after inoculation, and the drug was administered intraperitoneally for 9 consecutive days. The animals were sacrificed on the 10th day and tumor cell counts were taken. Tumor inhibition was expressed as the per cent difference between the mean number of cells in the control group and the test group. At the optimal dose level (30 mg./Kg.), 95% inhibition was observed.



I (AB-163)

Scheme I

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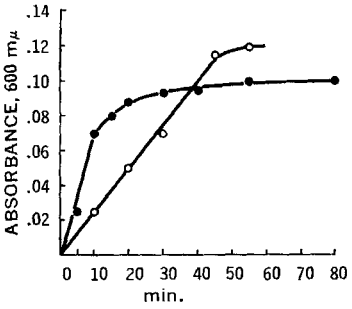

Fig. 1—Comparative reaction rates of ethyl bis(2,2-dimethyl-1-aziridinyl)phosphinyl carbamate (AB-132) and ethyl bis(2,2-dimethyl)ethylenamido phosphate (AB-163) with 4-(p-nitrobenzyl)pyridine, at 80°. Standard initial concentrations: compound, 0.2 μmole/ml.; reagent, 240 μmole/ml. Key: O, AB-132; ●, AB-163.

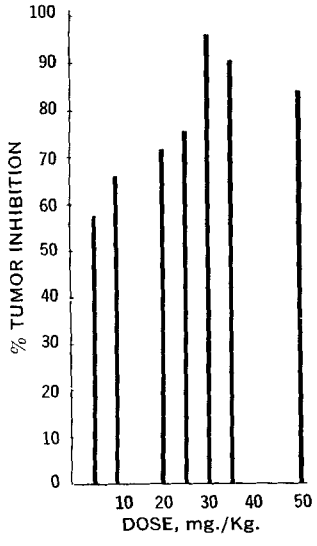

Fig. 2—Effect of AB-163 against Ehrlich ascites tumor (E-2) in ICR/Ha mice.

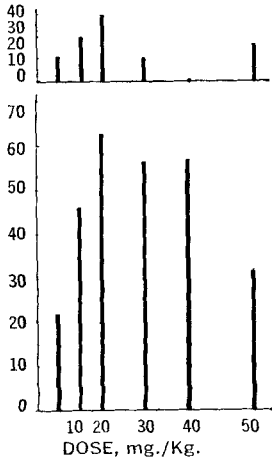

Fig. 3—Effect of AB-163 against sarcoma 180 in ICR/Ha mice. Top: per cent of animals without tumor, at various dose levels indicated on bottom abscissa. Bottom: per cent tumor inhibition in the remaining animals, at each of these dose levels.

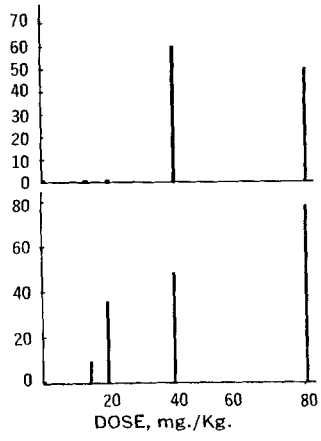

Fig. 4—Effect of AB-163 against adenocarcinoma 755 in 57B16 mice. Top: per cent of animals without tumor, at various dose levels indicated on bottom abscissa. Bottom: per cent tumor inhibition in the remaining animals, at each of these dose levels.

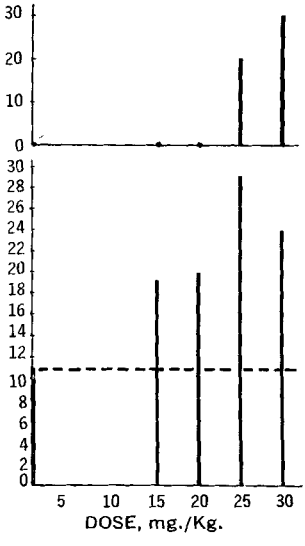

Fig. 5—Effect of AB-163 against leukemia L1210 in BDF1 mice. Top: per cent of animals surviving for 45 days, at various dose levels indicated on bottom abscissa. Bottom: per cent mean survival time of the remaining animals, at each of these dose levels. (Drug administered 1–11 days.)

Figure 3 shows the effect of the compound against sarcoma 180 in ICR/Ha mice. The drug was administered intraperitoneally for 7 consecutive days starting 24 hr. after tumor implantation. At the optimal dose range (15–25 mg./Kg.), 20–30% of the animals did not develop tumors while in the rest of the animals the tumors were 57–63% smaller (by weight) as compared to the controls.

Figure 4 illustrates the results obtained against adenocarcinoma 755 in 57B16 mice. Treatment was continued daily from day 1 to 11 following tumor implantation. At 40–80 mg./Kg. dose levels, 55–60% of the treated animals had no tumors

while the rest of the treated animals showed 48–77% inhibition of tumor growth as compared to the controls.

Figure 5 shows the effect of AB-163 against leukemia L1210 in BDF1 mice. In this experiment treatment was continued daily from day 1 to 11 post-inoculation. At optimal dose range, 25–30 mg./Kg., 20–30% of the animals survived for more than 45 days and are considered "cures." The remaining animals showed an average increase of survival time of 107–170%.

These results demonstrate that AB-163 is a much more effective compound against a spectrum of transplanted tumors than was AB-132(2).

EXPERIMENTAL

Into a 1-L. round-bottom flask equipped with a mechanical stirrer, pressure equalized addition funnel, thermometer, and a calcium chloride drying tube was introduced a solution of 15.6 Gm. (0.22 mole) of 2,2-dimethylaziridine, 22.2 (0.22 mole) of triethylamine in 350 ml. of toluene. The content of the flask was cooled to 0° by immersion in a mechanically refrigerated bath maintained at −3° to 0°, and a solution of 16.3 Gm. (0.1 mole) of ethyl dichlorophosphate in 100 ml. of toluene was slowly added. The reaction mixture was stirred for 24 hr. and then slowly warmed to room temperature and filtered. The precipitate contained nearly the theoretical amount of pure triethylamine hydrochloride. The filtrate was concentrated in a flash evaporator under reduced pressure, and the crude product was obtained as a viscous oil. The material was purified by high vacuum distillation and the analytically pure sample was obtained by collecting the fraction boiling at 70–71°/0.03–0.04 mm.; yield 17.3 Gm. (74%). This distillation had to be conducted with care because of the tendency of the material to decompose on overheating.

Anal.—Calcd. for $C_{10}H_{21}N_2O_2P$: C, 51.72; H, 9.05; N, 12.07. Found: C, 51.60; H, 9.18; N, 11.94.

Infrared absorption bands (Beckman IR-8): $\nu_{max.}^{CHCl_3}$ 2990, 1460 (broad), 1385, 1375, 1340, 1265, 1210 (broad), 1145, 1105, 1035, 960, 840 cm.$^{-1}$.

NMR absorption bands (in CCl_4, with tetramethylsilane as internal standard): 8.78 triplet (ester CH_3); 8.65 singlet (CH_3 ring-substituents); 8.05 doublet, P_H = 14 cps. (ring CH_2); 6.05, multiplet (ester CH_2).

Alkylation Studies—Comparative alkylating activities of I and AB-132 were determined at 80° by a previously described method (6).

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Determination of Ephedrine, Phenobarbital, and Theophylline in Tablets by Gas Chromatography

By MILTON ELEFANT, LESTER CHAFETZ, and JOSEPH M. TALMAGE

A gas chromatographic procedure is described for the rapid, specific, and precise determination of ephedrine, phenobarbital, and theophylline in tablets. Ephedrine is converted to benzaldehyde by periodate oxidation prior to chromatography, while the phenobarbital and theophylline are chromatographed directly after simple tablet disintegration in a co-solvent. Adaptation of the method to single tablet analysis is described.

THE PROBLEM of devising a convenient and accurate assay for combinations of ephedrine, a barbiturate, and theophylline or its complexes has become classical in the literature of pharmaceutical analysis. The literature has been reviewed by Connors (1) and, more recently, by Foreman and Blake (2). Most of the solutions described so far require lengthy and tedious liquid–liquid extractions, ion-exchange chromatography, and/or liquid–liquid partition chromatographic separations followed by quantitation of the separated drugs by gravimetry, titrimetry, or spectrophotometry. Since gas chromatography combines separation with quantitation, it appeared to be a singularly attractive means for assay of a tablet declaring ephedrine HCl, phenobarbital, and theophylline.

Gas chromatographic methods have been successfully applied to assay of barbiturates (3–7) and ephedrine (8–11); however, mixtures of ephedrine, phenobarbital, and theophylline present more difficulties in gas chromatography than the assay of the individual drugs because of their differences in functionality, polarity, and dose. The polar, strongly basic amine, ephedrine, tails badly on columns which give good separation and symmetrical peaks for the weakly acidic barbiturate and xanthine drugs. Ephedrine is quantitatively oxidized by periodate, and this observation has been made the basis of quantitative assays of the alkaloid by Wickström (12), who determined the acetaldehyde formed by colorimetry, and by Chafetz (13),

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