enimine, 115 g (0.84 mole) of powd, anhyd K_2CO_3, and 950 ml of abs EtOH was obtained[2] 133 g (86%) of the 1-substituted aziridine: bp 110–115° (0.3 mm); glpc 97%. The nmr spectrum was as expected. *Anal.* ($C_{13}H_{19}NO$) H, N; C: calcd, 76.05; found, 75.25.

3-[4-(*p*-Methoxyphenyl)butyl]-2-methylthiazolidine Hydrochloride (12). A soln of 9.4 g (0.034 mole) of **9** and 15 g (0.34 mole) of acetaldehyde in 100 ml of 50% aqueous MeOH was heated under reflux for 3 hr. The solvent was removed under vacuum, and the residue was treated with satd aqueous K_2CO_3. The thiazolidine base was extd into Et_2O, and the soln was dried ($MgSO_4$) and treated with dry HCl. The Et_2O, containing an oily HCl salt, was evapd leaving 4.0 g of light yellow solid which was recrystallized from MeCN to give 1.9 g (19%) of **12**: mp 133–135°. The nmr spectrum was as expected.

1-Octylaziridine. To 75 ml of cold (5°) ethylenimine was slowly added 31 ml of 1.6 *M* BuLi in heptane (1-aziridinyllithium in Et_2O has been prepd[11]). The mixt, protected from moisture, was stirred at 5° for 10 min before adding dropwise 9.7 g (0.05 mole) of 1-bromooctane. The cooling bath was removed, and the reaction was allowed to proceed for 1 hr longer. The solvent was removed under reduced pressure, and the residue (24.7 g) was extd with several portions of hexane. The combined exts were filtered through Celite and concd to give 8.7 g of liquid: glpc 88%. Distn afforded 7 g (90%) of 1-octylaziridine: bp 87° (15 mm); glpc 99%. The nmr spectrum was as expected. *Anal.* ($C_{10}H_{21}N$) C, H, N.

Reaction of *p*-(4-Bromobutyl)anisole with 1-Aziridinyllithium. To 125 ml of cold (5°) ethylenimine was slowly added under N_2 100 ml of 1.6 *M* BuLi in hexane. The mixt was stirred at 5° for 10 min before adding dropwise 20 g (0.082 mole) of neat *p*-(4-bromobutyl)anisole. The ice bath was removed, and the mixt was stirred overnight at room temp. The mixt was concd under reduced pressure, and the residue was extd with six portions of hexane. The combined exts were dried ($MgSO_4$) and concd to give 10.7 g of light yellow liquid. An additional 2.1 g of liquid was extd into Et_2O. The 10.7-g sample was distd giving the following fractions: 3.3 g, bp 80–83° (0.1 mm) and glc 60:40; 2.7 g, bp 84–89° (0.1 mm) and glc 50:50; and 3.4 g, bp 89–90° (0.1 mm) and glc 30:70. The total quantity distd was 9.4 g (56%). Ratios of the two components in the three fractions calculated from nmr spectra and based on the structural identification given below were within 5–10% of the glc ratios. A portion of the 2.7-g fraction was sepd by preparative glc (Loenco, Prepmatic; 10% SE30 column, 3/8 × 48 in.; programmed, 125–225° at 6°/min) to give 160 mg of liquid having the longer retention time, and shown by ir and nmr spectra to be the expected alkylation product, 1-[4-(*p*-methoxyphenyl)butyl]aziridine (**5**). The component with the shorter retention time amounted to 99 mg and was shown to be 1-(α-ethyl-*p*-methoxyphenethyl)aziridine (**6**): nmr (CCl_4) δ 6.83 (m, 4, aromatic H's), 3.68 (s, 3, CH_3O), 2.64 (d, 2, J = 6 Hz, $PhCH_2$), and 0.6–1.7 ppm [m, 10, $CH(C_2H_5)N(CH_2)_2$];** and mass spectrum (70 eV) *m/e* (rel intensity) 205 (6), 176 (7), 147 (8), 134 (8), 121 (55), 91 (16), 84 (100), 78 (28), 56 (38). *Anal.* ($C_{13}H_{19}NO$) C, H, N. Trials using a much higher ratio of 1-aziridinyllithium to aralkyl bromide did not result in any appreciable change in the ratio of the two products. None of the rearrangement product (**6**) was formed when **5** was treated with butyllithium in ethylenimine–hexane as solvent. The starting aziridine (**5**) was recovered unchanged.

1-(α-Ethyl-*p*-methoxyphenethyl)aziridine (6). To 60 ml of cold (5°) ethylenimine was slowly added under N_2 9.5 ml of a 13.3% soln of BuLi in heptane. The mixt was stirred at 5° for 15 min before adding dropwise 8.8 g (0.054 mole) of *p*-(3-butenyl)anisole.[9] The mixt was stirred for 18 hr at room temp and then concd under vacuum. The residue was extd with several portions of hexane, and the combined exts were filtered through Celite. The filtrate was concd under vacuum, and the crude oil was distd to give 6.5 g (58%) of **6**: bp 95–100° (0.1 mm); glc 99%; ir and nmr spectra were identical with the sample obtained by preparative glc (see above). A prepn using BuLi and *p*-(3-butenyl)anisole in a molar ratio of 2:1 resulted in only a 44% yield of the same product.

1-Phenethylaziridine. Freshly distd styrene (20.8 g, 0.20 mole), 30 ml of 15% BuLi, and 180 ml of ethylenimine were allowed to react as described for the prepn of **6**, except that the soln of BuLi in ethylenimine was kept at 5° for 30 min before adding the styrene. Crude liquid (30.4 g) was distd to give 24.1 g (82%) of 1-phenethylaziridine: bp 57° (1 mm) [lit.[6] bp 89° (6 mm)]; and glc 100%. The nmr spectrum was as expected.

In our work H's on α-carbons of 1-substituted aziridines are shifted upfield to *ca.* δ 2.2 ppm. The large upfield shift in the case of **6 was unexpected.

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L-Asparaginol and Derivatives. Synthesis and Screening Data†

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The tumor growth inhibitory activity of L-asparaginase from several sources has been widely investigated.[1-6] This enzyme catalyzes the deamidation of L-asparagine, an amino acid essential for the growth of certain L-asparaginase sensitive tumors.[7] It has been found that tumors which are resistant to this enzyme synthesize L-asparagine from L-aspartic acid with the help of a synthetase.[8] In order to develop substances capable of interfering with the utilization of L-asparagine in sensitive as well as resistant tumors, the synthesis of L-asparagine analogs was undertaken.

The antitumor activity *in vivo* of *N*-carbobenzyloxy-L-asparagine has been described.[9] An analog of L-asparagine, *N*-hydroxyasparagine, was reported, although no biological data were shown.[10]

The synthesis of a series of aminoalkyl adenylates from the corresponding amino alcohols was described by Boissonas, *et al.*,[11] they were intended as inhibitors of the aminoacyl-tRNA synthetase specific for each particular amino acid. These authors did not report the synthesis of

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Table I. Screening Data of L-Asparaginol (V) and Adenosine 5'-L-Asparaginol-1-yl (Phosphate (VI)

Tissue culture	Compound	Dosage μg/ml	Inhibition, %	
P815/L-Asparaginase	L-Asparaginol	300	100	
		100	64	
		30	10	
L5178Y	L-Asparaginol	300	81	
		100	25	
		30	6	
	Adenosine 5'-L-asparaginol-1-yl phosphate	1000	82	
		200	65	
		100	57	
In vivo (mouse Leukemia)	Compound	Dosage, mg/kg (5 X qd)	ILS[a] %	Weight change, g (6 days)
L5178Y	L-Asparaginol	200	0	+2.0
		100	−13	+5.0
		50	−9	+2.5
	Adenosine 5'-L-asparaginol-1-yl phosphate	200	−17	+5.0
		100	−13	+4.0
		50	−4	+0.5
		Dosage, mg/kg (9 X qd)		
L5178Y/CA 55/L-Asparaginase	L-Asparaginol	200	−6	+2.3
		100	+5	−0.5
	Adenosine 5'-L-asparaginol-1-yl phosphate	100	−3	−0.2
		Dosage, mg/kg (5 X qd)		
P815	L-Asparaginol	500	−7	+2.7
P815/Ara-C	L-Asparaginol	500	0	+3.8

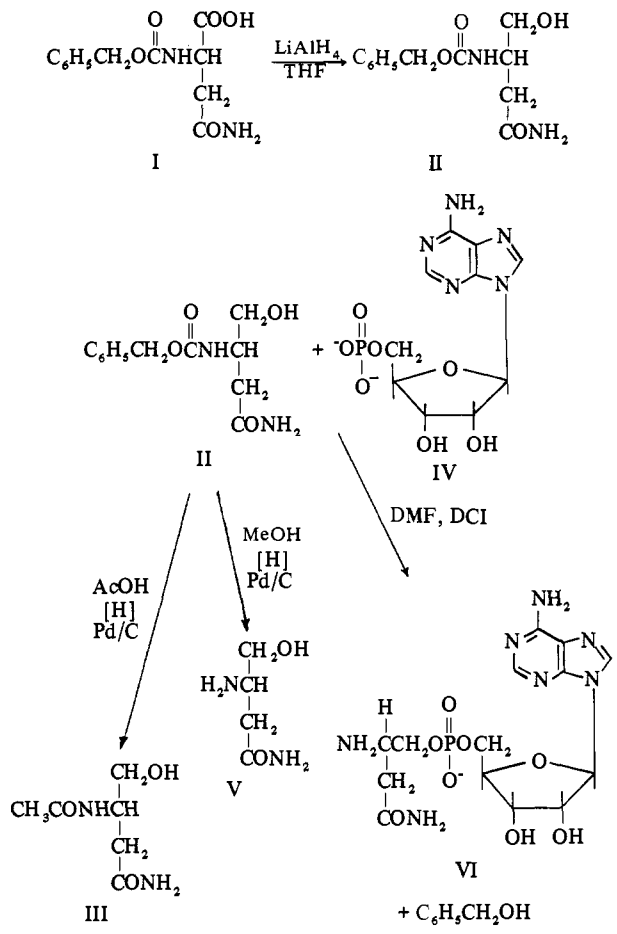
[a] ILS = increase in life span.

the corresponding amino alcohol of L-asparagine. The preparation of L(-)-3-amino-4-hydroxybutyramide (L-asparaginol) was carried out by Harnden and Yellin[12] but an elementary analysis for L-asparaginol (as the CF_3COOH salt) could not be obtained because of its extreme hygroscopicity.‡

We wish to report the synthesis of both L(-)-3-amino-4-hydroxybutyramide (L-asparaginol) and L-asparaginol adenylate and the results obtained from their screening.

Treatment of *N*-carbobenzyloxy-L-asparagine (I) with $LiAlH_4$ in tetrahydrofuran (Scheme I) by a modification of the method of Vogl and Pöhm[14] for the synthesis of amino alcohols led to the preparation of *N*-carbobenzyloxy-L(-)-3-amino-4-hydroxybutyramide (*N*-carbobenzyloxy-L(-)-asparaginol (II). Compound II was catalytically hydrogenated (Pd/C) in AcOH-H_2O-MeOH or MeOH to yield respectively *N*-acetyl-L-asparaginol (III) and L-asparaginol (V). Condensation of adenosine 5'-monophosphate, Na salt (IV), with an equivalent of II and *N,N'*-carbonyldiimidazole after 9 days at 50° gave adenosine 5'-asparaginol-1-yl phosphate (L-asparaginol adenylate) (VI). As a by-product of the reaction, benzyl alcohol was obtained, which was isolated as the 3,5-dinitrobenzoate. The displacement of the *N*-carbobenzyloxy group in these conditions is an unusual reaction that appears to be specific for the reaction conditions leading to VI. When the *N*-carbobenzyloxy derivatives I and II (with the exclusion of AMP) reacted under conditions identical with the one leading to VI, no displacement of

Scheme I



‡While this manuscript was being prepared, the synthesis of the hydrochloride of V from *N*-carbobenzyloxy-L-asparagine methyl ester by reduction with $Ca(BH_4)_2$ was reported by Keresztesy, *et al.*[13]

the carbobenzyloxy group was observed and subsequently no benzyl alcohol could be detected.

Experimental Section§

N-Carbobenzyloxy-L(-)asparaginol (II). Lithium aluminum hydride (1.2 g, 31.6 mmoles) was added to tetrahydrofuran (THF) (160 ml, distilled from $LiAlH_4$) and stirred. *N*-Carbobenzyloxy-L-asparagine[15] (I, 6.0 g, 22.6 mmoles) was added slowly, and the mixture was stirred for 2 days at 25°. To decompose excess $LiAlH_4$, EtOAc (10 ml) was added dropwise, then ether (100 ml), and finally 2 *N* HCl (10 ml). The reaction mixture was filtered after stirring for 15 min. The filtrate was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The resulting oily residue was recrystallized from EtOAc, yielding 0.97 g of colorless needles, mp 142–143°. The precipitate, containing Al oxides and starting material, was treated with 0.5 *N* NaOH (20 ml) and filtered. The filtrate was acidified to pH 1 with concd HCl, upon which unchanged starting material (I) was isolated (1.03 g), yield of *N*-carbobenzyloxy-L(-)-asparaginol (II), 23%. *Anal.* ($C_{11}H_{14}N_2O_3$) C, H, N.

N-Acetyl-L(-)asparaginol (III). A solution of *N*-carbobenzyloxy-L(-)asparaginol (II, 190 mg, 0.85 mmole) in MeOH (2 ml), AcOH (2 ml), and H_2O (2 ml) containing 5% Pd/C (150 mg) was hydrogenated at atmospheric pressure. After absorption of the theoretical amount of H_2, the catalyst was removed by filtration; toluene odor was noticed. The filtrate was evaporated to dryness under reduced pressure. An oily residue was obtained from which, after solution in EtOAc and standing at 0° for 3 days, 57 mg (37%) of colorless needles, mp 126°, was obtained. Recrystallization from EtOAc raised the mp to 134°, ir KBr disk 1050 (OH), 1550 (CH_3CONH), and 1660 cm^{-1} ($CONH_2$). *Anal.* ($C_6H_{12}N_2O_3 \cdot H_2O$) C, H, N.

L(-)-3-Amino-4-hydroxybutyramide (L-Asparaginol) (V). A solution of *N*-carbobenzyloxy-L(-)asparaginol (II, 6.0 g, 0.027 mole) in MeOH (240 ml) with 10% Pd/C (0.4 g) was hydrogenated until H_2 uptake ceased. The solution was filtered and the filtrate was evaporated at 25°, first at 10 mm, then at 0.05 mm. The resulting white crystalline material was taken up in EtOH (30 ml), filtered, and collected (0.3 g), mp 93–95° dec. The filtrate was added to EtOAc (350 ml), and the solution decanted from the yellow oil which separated. This solution was concentrated *in vacuo* until crystallization began to occur. White crystals were collected (1.7 g), mp 95–98° dec. An additional crop (0.4 g), mp 93–95° dec, was obtained by extracting the yellow oil with EtOH, addition of EtOAc, charcoal treatment, and concentration, total yield, 2.4 g (75%). An analytical sample of this material was obtained by recrystallization from EtOAc, mp 98–100° dec, $[\alpha]^{24}D$ −23° (H_2O); ir KBr disk 1050 (OH), 1660 and 1600 ($CONH_2$), 3400 cm^{-1} (NH_2). *Anal.* ($C_4H_{10}N_2O_2$) C, H, N.

Adenosine 5′-L(-)Asparaginol-1-yl Phosphate Na Salt (L-Asparaginol Adenylate) (VI). *N,N′*-Carbonyldiimidazole (DCI) (0.50 g, 3.1 mmoles) was added to a stirred mixture of adenosine 5′-monophosphate, Na salt (NaAMP) (III, 0.50 g, 1.5 mmoles), in dry DMF (15 ml). Solution was effected after 30 min, and *N*-carbobenzyloxy-L(-)-asparaginol (II, 0.36 g, 1.5 mmoles) was added. The solution was kept at 50° for 9 days. The DMF was removed *in vacuo* at 60°, the gummy residue was extracted 3 times with Et_2O, and the ethereal extracts were discarded. The remaining solid was chromatographed on a cellulose column, using 70% aqueous 1-PrOH as an eluent. The solvent from the first 300 ml was removed *in vacuo* and the residue was dissolved in H_2O (3 ml) and precipitated by the addition of EtOH (50 ml) to give white needles (0.40 g, 60%), mp 168–170° dec. A sample was prepared for analysis by futher chromatography, dissolving the resulting residue from evaporation of the eluate in H_2O and reprecipitating with EtOH, $[\alpha]^{25}D$ −26.8° (H_2O); ir KBr disk 1650, 1600 ($CONH_2$), 1570 and 820 (NH_2), 1240 (P=O), 1160, 1100 (POC), 1070 (COH, sugar). *Anal.* ($C_{14}H_{21}N_7O_8PNa$) C, H, N, P.

Isolation of Benzyl Alcohol from the Preparation of VI. A condensation reaction was carried out in the same conditions as above but on a larger scale using 6.5 g of DCI and 6.5 g of NaAMP and 5 g of II. After removal of the DMF from the reaction mixture, the gummy residue was extracted 3 times with Et_2O, the residue being used for the preparation of VI. The ether extracts were combined, and the ether was removed *in vacuo*. The residue was distilled at 10 mm, and the 100–110° fraction was collected (2.5 g, 95%). The uv spectrum of this product was identical with benzyl alcohol; 3,5-dinitrobenzoate, mp 111–112° (lit.[16] 113°) and ir identical with that of an authentic sample.

Reaction of compounds I and II, DCI and DMF (in the absence of NaAMP), in proportions similar to those used for the synthesis of VI failed to yield any benzyl alcohol.

Screening Data. When L-asparaginol (V) and its adenylate (VI) were administered intraperitoneally (ip) in normal BDF_1 mice for 8 days no toxicity was found at dosage levels up to 400 mg/kg.[17,18]

In tissue culture, with P815 leukemia cells resistant to L-asparaginase 100% inhibition was observed when L-asparaginol (V) was administered at 300 μg/ml; at lower dosages compound V was less effective (Table I).

When tested *in vivo* in several mouse leukemias (L5178Y, L5178Y resistant to cytosine arabinoside and L-asparaginase, P815 and P815 resistant to cytosine arabinoside) no antitumor activity up to a dosage of 500 mg/kg, given ip, was observed. No weight loss was encountered.

It can be speculated from the *in vivo* results that V is inactive because it may be oxidized to L-aspartic acid, or be poorly absorbed into the cell and thus could not prevent L-asparagine from being converted to its adenylate. The lack of inhibitory activity of compound VI *in vivo* could be also attributed to its poor absorption.

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§ The infrared data were obtained with a Perkin-Elmer Model 137B infracord spectrophotometer and the uv spectra with a Beckman DBG spectrophotometer. Melting points were determined with a Thomas-Hoover apparatus and were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Synthesis and Alkylation of Tetrahydrofuro[3,4-*c*]pyrazolols

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Phenylbutazone and oxyphenbutazone are antiinflammatory-analgetic pyrazoles which are frequently used in the treatment of rheumatoid arthritis.[1] As a continuation