Elution of the column with Et_2O gave 6.1 g (28%) of orange crystals, mp 64–66° Recrystallization (cyclohexane) gave light tan crystals of 3a,4,5,6-tetrahydro-3a-methyl-2-phenylcylcopentapyrazol-3(2*H*)-one (**18**), mp 68–69°. *Anal.* ($C_{13}H_{14}N_2O$) C, H, N; ir, 5.85 μ (C=O); nmr (DMSO-d_6) τ 8.62 (s, 3, CCH_3), 8.44–7.16 (m, 6, CH_2), and 3.00–205 (5, phenyl).

Elution of the column with Et_2O–MeOH (4:1) gave 2.8 g (13%) of tan crystals, mp 123–127°. Recrystallization (EtOH) gave colorless crystals of 1,4,5,6-tetrahydro-1-methyl-2-phenylcyclopentapyrazol-3(2*H*)-one (**6**), mp 127–128° (lit.[3] mp 128°). *Anal.* ($C_{13}H_{14}N_2O$) C, H, N; ir, 6.01 μ (C=O).

B. With Methyl *p*-Toluenesulfonate.—A solution of 2.0 g (0.01 mol) of **10**, 20 ml of DMF, 0.44 g (0.01 mol) of 55% NaH dispersion, and 1.86 g (0.01 mol) of methyl *p*-toluenesulfonate was heated at 120° with stirring for 2 hr. The solution was cooled, diluted with 100 ml of H_2O, and extracted with Et_2O. The Et_2O solution was dried ($MgSO_4$) and concentrated to a brown oil which was chromatographed on silica gel. Eluted with cyclohexane–Et_2O (9:1) was 0.9 g (42%) of an oil. Short-path distillation at 115° (0.2 mm) gave 2,4,5,6-tetrahydro-3-methoxy-2-phenylcyclopentapyrazole (**13**) as a pale yellow. *Anal.* ($C_{13}H_{14}N_2O$) C, H, N; nmr (DMSO-d_6) τ6.04 (s, 3, OCH_3); ir (liq film), no CO band below 6.15 μ.

C. With Allyl Chloride.—A solution of 4.0 g (0.02 mol) of **10**, 1.53 g (0.02 mol) of allyl chloride, 1.08 g (0.02 mol) of NaOMe, and 50 ml of EtOH was heated under reflux with stirring for 18 hr, and then concentrated to dryness. The residue was taken up in H_2O, and the mixture was extracted with Et_2O. The Et_2O solution was dried ($MgSO_4$) and concentrated to a yellow oil which was chromatographed on alumina.

Elution of the column with C_6H_6 gave 1.5 g (31%) of a yellow oil. Evaporative distillation at 110° (0.05 mm) gave 3a-allyl-3a,4,5,6-tetrahydro-2-phenylcylopentapyrazol-3(2*H*)-one (**19**) as a colorless oil. *Anal.* ($C_{15}H_{16}N_2O$) C, H, N; ir (liq film) 5.82 μ (C=O).

Elution of the column with Et_2O–MeOH (1:1) gave 1.0 g (21%) of brown crystals, mp 92–93°. Recrystallization (cyclohexane) gave tan crystals of 1-allyl-1,4,5,6-tetrahydro-2-phenylcyclopentapyrazol-3(2*H*)-one (**7**), mp 93–94°. *Anal.* ($C_{15}H_{16}N_2O$) C, H, N; ir, 6.04 μ (C=O).

Benzylation of 2-(*p*-Bromophenyl)-2,4,5,6-tetrahydrocyclopentapyrazol-3-ol (**11**).—A solution of 2.8 g (0.01 mol) of **11**, 1.7 g (0.01 mol) of $PhCH_2Br$, 0.54 g (0.01 mol) of NaOMe, and 25 ml of EtOH was heated under reflux for 15 hr, and then concentrated to dryness. The residue was taken up in H_2O, and the mixture was extracted with Et_2O. The Et_2O solution was dried ($MgSO_4$) and concentrated to a yellow oil which was chromatographed on alumina.

Elution of the column with C_6H_6 gave 1.2 g (32%) of colorless crystals, mp 85–95°. Recrystallization (hexane) provided colorless crystals of 3a-benzyl-2-(*p*-bromophenyl)-3a,4,5,6-tetrahydrocyclopentapyrazol-3(2*H*)-one (**20**), mp 110–111°. *Anal.* ($C_{19}H_{17}BrN_2O$) C, H, Br, N; ir, 5.83 μ (C=O).

Elution of the column with MeOH gave 1.0 g (27%) of tan crystals, mp 113–115°. Recrystallization (cyclohexane) gave colorless needles of 1-benzyl-2-(*p*-bromophenyl)-1,4,5,6-tetrahydrocyclopentapyrazol-3(2*H*)-one (**8**), mp 117–118°. *Anal.* ($C_{19}H_{17}BrN_2O$) C, H, Br, N; ir, 5.98 μ (C=O).

Benzylation of 2-(*p*-Fluorophenyl)-2,4,5,6-tetrahydrocyclopentapyrazol-3-ol (**12**).—The procedure used for the benzylation of **11** was employed. From 2.2 g (0.01 mol) of **12**, 1.7 g (0.01 mol) of benzyl bromide, 0.54 g (0.01 mol) of NaOMe, and 25 ml of EtOH was obtained an oil which was chromatographed on alumina.

Elution of the column with hexane–Et_2O (3:1) gave 0.09 g (3%) of colorless crystals, mp 75–76°. Recrystallization (hexane) provided colorless crystals of 3-benzyloxy-2-(*p*-fluorophenyl)-2,4,-5,6-tetrahydrocyclopentapyrazole (**14**), mp 77°. *Anal.* ($C_{19}H_{17}FN_2O$) C, H, F, N; ir, no CO band below 6.20 μ.

Elution of the column with hexane–Et_2O (1:1) provided 0.51 g (17%) of colorless crystals, mp 90–94°. Recrystallization (hexane) gave colorless crystals of 3a-benzyl-2-(*p*-fluorophenyl)-3a,4,5,6-tetrahydrocyclopentapyrazol-3(2*H*)-one (**21**), mp 98–99°. *Anal.* ($C_{19}H_{17}FN_2O$) C, H, F, N; ir, 5.86 μ (C=O).

Elution of the column with Et_2O–MeOH (49:1) provided 0.83 g (27%) of yellow crystals, mp 90–92°. Recrystallization (cyclohexane) gave colorless crystals of 1-benzyl-2-(*p*-fluorophenyl)-1,4,5,6-tetrahydrocyclopentapyrazol-3(2*H*)-one (**9**), mp 112°. *Anal.* ($C_{19}H_{17}FN_2O$) C, H, F, N; ir, 6.00 μ.

2,4,5,6-Tetrahydro-3-methoxy-1-methyl-2-phenylcyclopentapyrazolium Iodide (**24**).—A solution of 4.0 g (0.02 mol) of **13** and 6 ml of MeI was allowed to stand at room temperature for 15 hr. The solution was diluted with 200 of Et_2O and filtered to give 1.45 g (20%) of crystals, mp 108–110°. Recrystallization (MeOH–Et_2O) gave colorless crystals, mp 109–110° dec. *Anal.* ($C_{14}H_{17}IN_2O$) C, H, I, N.

Pyrolysis of 2,4,5,6-Tetrahydro-3-methoxy-1-methyl-2-phenylcyclopentapyrazolium Iodide (**24**).—A solution of 170 mg of **24** and 4 ml of MeOH was heated under reflux for 24 hr. Evaporation of the solvent left 87 mg of **6**, mp 123–125°. Ir and tlc examination of the product failed to indicate the presence of **18**.

Puromycin Analogs. Aminoacyl Derivatives of 9-(3′-Amino-3′-deoxy-β-D-arabinofuranosyl)adenine[1]

LINDA V. FISHER, WILLIAM W. LEE, AND LEON GOODMAN

Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025

Received January 5, 1970

Puromycin (**1**), an antibiotic with antitumor activity,[2] has been used as an important biochemical tool in the study of protein synthesis. Many structural analogs of **1** have been prepared in an effort to exploit the biological properties of the antibiotic. These include analogs where other aminoacyl groups replace the *p*-methoxyphenylalanine moiety of **1**,[3-5] compounds where the *p*-MeO of **1** is replaced by other substituents,[6] the derivative where the 3′-aminoribofuranose of **1** is replaced by 3′-aminoglucopyranose,[7] and the 2′-*O*-Ac derivative[8] of **1**. In an extension of these investigations, we have prepared a number of aminoacyl derivatives of 9-(3-amino-3-deoxy-β-D-arabinonofuranosyl)adenine (**3**),[9] a 2′ epimer of **2**. See Table I.

The aminoacyl derivatives **4** of **3** were prepared by standard methods of peptide synthesis, the method of choice for each compound being dependent on the relative ease of formation and facility of purification from by-products. The choice of solvents was severely restricted by the low solubility of **3**, with DMF solvent mixtures generally being the most useful. As in the work of Baker, *et al.*,[3] unblocked **3** could be used in these coupling reactions. In contrast, blocking of the OH groups was essential to successful aminoacylation of 1-(3-amino-3-deoxy-β-D-glucopyranosyl)uracil.[10]

The two coupling procedures used were A, the mixed anhydride method using isobutyl chloroformate[11]

(1) This work was performed under the auspices of Chemotherapy, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH-43-64-500. The opinions expressed here are those of the authors and not necessarily those of Chemotherapy.

(2) B. L. Hutchings, *Chem. Biol. Purines, CIBA Found. Symp.*, **1956**, 777 (1957).

(3) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Amer. Chem. Soc.*, **77**, 1 (1955).

(4) R. H. Symons, R. J. Harris, L. P. Clarke, J. F. Wheldrake, and W. H. Elliott, *Biochem. Biophys. Acta*, **179**, 248 (1969).

(5) A. O. Hawtrey, S. I. Bielron, and S. H. Eggers, *Tetrahedron Lett.*, 1693 (1967).

(6) A. M. Small, H. M. Kissman, J. P. Joseph, and M. J. Weiss, *J. Med. Pharm. Chem.*, **2**, 375 (1960).

(7) F. W. Lichtenthaler and H. P. Albrecht, *Angew. Chem.*, **80**, 440 (1968).

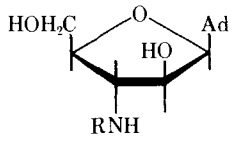
(8) H. Neumann, V. E. Shashoua, J. C. Sheehan, and A. Rich, *Proc. Nat. Acad. Sci. U. S.*, **61**, 1207 (1968).

(9) A. P. Marcinez, D. F. Calkins, E. J. Reist, W. W. Lee, and L. Goodman, *J. Heterocycl. Chem.*, **7**, in press.

(10) H. A. Friedman, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, **32**, 3775 (1967).

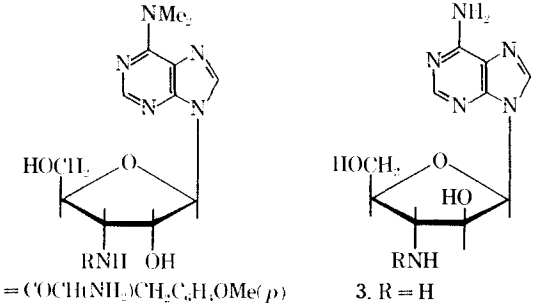
(11) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, **89**, 5012 (1967).

TABLE I



Compd	R[a]	Method[b,c]	Yield,[c] %	Mp, °C[c]	Solv[d]	Spec rot[e] [α]	Spec rot[e] C	Spec rot[e] Solv	Chromatography[f] tlc	Chromatography[f] pc	Formula[g]
5	Z-L-Phe	A	51	191–196	M	−13.5	0.72	P	0.71 A	0.88 D	$C_{27}H_{29}N_7O_6 \cdot 0.2H_2O$
6	L-Phe		78	134–137	W	−35.9	2.23	P	0.23 A	0.44 D	$C_{19}H_{23}N_7O_4 \cdot 1.5H_2O$
7	Z-L-Ala	A	49 (73)	190–192	W–M	−28.2	0.99	P	0.52 B		$C_{21}H_{25}N_7O_6 \cdot 0.75H_2O$
8	L-Ala		27 (89)	211–216	E–W	−11.5	1.00	P		0.23 D	$C_{13}H_{19}N_7O_4 \cdot H_2O$
9	Z-D-Ala	A (B)	30 (56[h])	205–213	E–W	−6.9[h]	0.99	P	0.58 A	0.82 D	$C_{21}H_{25}N_7O_6 \cdot 0.5H_2O$
10	D-Ala		70	223–234	M	+5.95	0.20	W		0.27 D	$C_{13}H_{19}N_7O_4 \cdot 0.25H_2O$
11	O_2N-Z-L-Arg	C (B)	11 (56)	138–146 (135–147)	W–M	−14.4	1.00	ME	0.60 B	0.85 D	$C_{24}H_{30}N_{11}O_8 \cdot CH_3OH \cdot H_2O$[i]
12	L-Arg		62	117–146	F	−18.7	0.27	W		0.55 E	$C_{16}H_{26}N_{10}O_4 \cdot 2.75C_2H_4O_2$[k]
13	Z,Z-L-Lys	A	15 (64)	160–161	G	−26.1	0.99	P	0.75 A	0.89 D	$C_{22}H_{28}N_8O_8$
14	L-Lys		30 (59)	187–190 (185–195)	E–W	−26.9	0.85	P		0.13 D	$C_{16}H_{26}N_8O_4 \cdot 0.5H_2O$
15	By-BOC-L-Glu	A (B, D)	47	108–115	W	−33.1	0.94	P	0.55 C		$C_{27}H_{35}N_7O_8 \cdot 0.4H_2O$
16	L-Glu		7 (43)	167–174	W	+26.8	0.44	W		0.24 D	$C_{15}H_{21}N_7O_6 \cdot 4H_2O$
17	Z-L-Phe-L-Ala	A (A, B, C)	29 (63)	185–195[j]	W	−7.3[k]	0.99	P	0.50 A	0.89 D	$C_{30}H_{34}N_8O_7 \cdot H_2O$
18	L-Phe-L-Ala		55 (59)	208–228	E	−41.2	0.50	DMF		0.38 D	$C_{22}H_{28}N_8O_5 \cdot 0.5H_2O$
19	Z-*p*-MeO-L-Phe	A	30 (62)	240–243	M	−24.6	1.00	P	0.50 C		$C_{28}H_{31}N_7O_7 \cdot 0.5H_2O$
20	*p*-MeO-L-Phe		49 (55)	125–127 (123–127)	W	−46.1	0.96	P		0.44 D	$C_{20}H_{25}N_7O_5 \cdot 0.75H_2O$

[a] Standard abbreviations are used for the amino acids: Z = benzyloxycarbonyl; By = γ-benzyl ester; BOC = *N*-*t*-butoxycarbonyl; *p*-MeO = *p*-methoxy. [b] Methods of synthesis: A, mixed anhydride; B, DCC–NHS; C, DCC–aq pyridine; D, water-soluble carbodiimide. [c] Method of synthesis, yield data and melting point values are for analytical samples except values in parentheses which are for homogeneous product suitable for the next reaction. [d] Crystallization or reprecipitation solvents are: E, ethanol; E–W, ethanol–water; F, hydrogenolysis solvent of ethanol–acetic acid–water was evaporated and residue foam triturated with acetone isopropanol to give the analytical sample; G, ethyl acetate; M, methanol; W, water. [e] The specific rotation values are degrees followed by concentration (C, %) and solvent: P = pyridine, W = water, ME = methoxyethanol. [f] Thin-layer chromatography (tlc) on silica gel utilized these solvent systems: A, $CHCl_3$–MeOH (1:1); B, *n*-PrOH–EtOAc–H_2O (3:2:1); C, $CHCl_3$–MeOH (3:1). Paper chromatography utilized solvent D, *n*-BuOH–HOAc–H_2O (4:1:5), and E, 5% disodium hydrogen phosphate. [g] Product prepared by method B has $[\alpha]D$ −3.2°. [h] Solvents present in nmr spectrum. [i] When the γ-benzyl ester was removed first, the intermediate, **4**, R = BOC-L-Glu, was obtained, which analyzed correctly for a solvate with 0.75 MeOH and 0.75 H_2O, but was not further characterized. [j] Softens at 155°. [k] This rotation, and other properties for the analytical sample of **17** was prepared from Z-L-Phe-L-Ala and **3** (see Discussion). Other samples of **17** prepared from 2-L-Phe and **8** by methods A and B had $[\alpha]D$ −12.0° and −11.4°, respectively (see Experimental Section). [l] All compounds were analyzed for C, H, N.



1. R = $COCH(NH_2)CH_2C_6H_4OMe(p)$
2. R = H

3. R = H
4. R = aminoacyl

and B, the dicyclohexylcarbodiimide (DCC)–*N*-hydroxysuccinimide (NHS) method.[12,13] Representative examples of each of these methods are described in the Experimental Section. Attempts to use a water-soluble carbodiimide[14] gave much less satisfactory results.

Racemization during certain of these peptide syntheses was a problem as shown by the significant differences in rotation of the blocked D-alanyl nucleoside **9** prepared by methods A and B. The product from method B gave the larger rotation difference from the 2-alanyl compound **7** and we assume that this method caused less racemization. Method B has been shown to be especially good in maintaining optical purity.[13] In method A, although DMF as the solvent for mixed anhydride formation induces racemization, it is safe for the reaction of a preformed mixed anhydride.[11] In preparing the dipeptide nucleoside **17**, the rotation data were in accord with expectations that the coupling of *N*-benzyloxycarbonyl-L-phenylalanine with **8** was preferable to the coupling of *N*-benzyloxycarbonyl-L-phenylalanyl-L-alanine[15] with **3** by method A.

Catalytic hydrogenolysis with 5% Pd–C was used generally to remove the blocking groups to afford the final nucleoside peptides, all obtained as solvated solids. HOAc was added to aid in the deblocking of the arginyl derivative **11**. The blocked glutamyl nucleoside **15** was first hydrogenolyzed to remove the γ-benzyl ester, then the *t*-butoxycarbonyl group was removed by a brief treatment (5 min) with trifluoroacetic acid at room temperature. Trial experiments with the aminonucleoside **3** showed it to be stable in trifluoroacetic acid for brief periods; but after 1 hr there was a detectable decrease in the rotation and after 4 days considerable adenine had formed. The original plan to couple another amino acid to **16** was deferred when trifluoroacetic acid treatment of **15** gave a product that did not seem very tractable and when several of the nucleoside peptides **4** gave negative preliminary testing results.

All the aminoacyl derivatives and some of the intermediates (all compounds in Table I except **5**, **7**, **9**, and **11**) were screened for antitumor activity in the mouse leukemia L-1210 system by Chemotherapy, National Cancer Institute, according to its protocol.[16] These compounds were inactive at a dose of 400 mg/kg per day.

Experimental Section[17]

9-[3-Deoxy-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-alanylamino)-β-D-arabinofuranosyl)adenine (17).—A solution of 90 mg (0.27 mmol) of the alanyl nucleoside **8**, 81 mg (0.27 mmol) of *N*-benzyloxycarbonyl-L-phenylalanine and 31 mg (0.27 mmol) of *N*-hydroxysuccinimide in 2 ml of dry DMF was stirred and cooled in an ice–salt bath. To this was added 55 mg (0.27 mmol) of DCC. The mixture was stirred for 2 hr at room temp, cooled, diluted with 2 ml of water, and filtered to remove the dicyclohexylurea. The filtrate was evapd to dryness *in vacuo*, partitioned between 15 ml of EtOAc–BuOH (2:1) and 10 ml of H_2O, the H_2O being reextracted with 5 ml more of the organic solvents. The combined organic phase was washed several times with 10% $KHCO_3$ solution, once with H_2O, dried, and evapd *in vacuo* to give 69% of a homogeneous (by tlc) solid foam. This was taken up in a hot solution of 20 ml of H_2O and 4 ml of MeOH, filtered, and the filtrate allowed to cool. There was deposited 64 mg (41%) of a white amorphous solid which, after drying, had $[\alpha]^{22}D$ −11.4° (*c* 1.00, pyridine), and other properties like those listed in Table I for **17** prepared by other procedures.

9-[3-(Benzyloxycarbonyl-*p*-methoxyphenyl-L-alanylamino)-3-deoxy-β-D-arabinofuranosyl]adenine (19).—Using the procedure suggested by Anderson, *et al.*,[11] the mixed anhydride was prepared from 2.5 ml (18.2 mmol) of Et_3N, 2.4 ml (18.2 mmol) of isobutyl chlorocarbonate, 40 ml of EtOAc, and 5.97 g (18.2 mmol) of *N*-benzyloxycarbonyl-*p*-methoxyphenyl-L-alanine[18] in an ice–salt bath, and stirred for 15 min. Meanwhile, 3.3 g (12.5 mmol) of the aminonucleoside **3** was dissolved by warming in 110 ml of dry DMF. This solution was cooled, added to the mixed anhydride in EtOAc and the mixture was stored at *ca.* 4° for 27 hr. The mixture was filtered, washed with 10 ml of DMF, and the combined filtrates evapd to dryness *in vacuo*. The residue was treated with 20 ml of H_2O and again evapd to a gummy solid. This was triturated with 150 ml of H_2O, then with 50 ml of Et_2O to afford 7.5 g of a white solid, R_f 0.50 in solvent C (tlc) with four trace spots of contaminants. Recrystallization from MeOH (800 ml concd to 350 ml and chilled) afforded, after washing with 30 ml of Et_2O, 4.5 g of white solid, mp 231–238° (62% yield), homogeneous by tlc with R_f 0.5 in solvent C. One more MeOH crystallization of similar material from an earlier run gave the anal sample of **19**, mp 240–243°; other properties in Table I.

9-[3-Deoxy-3-(L-phenylalanylamino)-β-D-arabinofuranosyl]-adenine (6).—A solution of 1.3 g (2.28 mmol) of 9-[3-(benzyloxycarbonyl-L-phenylalanylamino)-3-deoxy-β-D-arabinofuranosyl)-adenine (**5**) in 100 ml of 95% EtOH was hydrogenated in the presence of 0.3 g of 5% Pd–C for 3 hr at 60° and 1 atm. After standing overnight at ambient temperature, the reaction mixture was filtered through Celite,[18] the Celite washed successively with three 10-ml portions of 95% EtOH, 10 ml of MeOH, and 10 ml of H_2O. The combined filtrate and washes were evapd to afford 0.94 g of product, mp 130–136°. Recrystallization from 115 ml of boiling H_2O and drying at 56° for 15 hr (<1 mm), afforded 0.77 g (78%) of **6** needles, mp 134–137°; $\lambda_{max}^{pH\ 1}$ 257 mμ (ε 16,600); $\lambda_{max}^{pH\ 7}$ 258 (17,000); $\lambda_{max}^{pH\ 13}$ 259 (17,700).

Acknowledgment—We are endebted to Mr. Osborne P. Crews, Jr. and his staff for the large scale preparation of intermediates and to Dr. Peter Lim and his staff for the spectra and paper chromatography.

(12) F. Weygand, D. Hoffman, and E. Wünsch, *Z. Naturforsch.*, **21b**, 426 (1966).

(13) J. E. Zimmerman and G. W. Anderson, *J. Amer. Chem. Soc.*, **89**, 7151 (1967).

(14) (a) D. G. Knorre and T. N. Shubina, *Zh. Obshch. Khim.*, **36**, 656 (1966); (b) J. C. Sheehan and J. J. Hlavka, *J. Org. Chem.*, **21**, 439 (1956).

(15) W. Grassmann, E. Wunsch, and A. Riedel, *Chem. Ber.*, **91**, 455 (1958).

(16) *Cancer Chemother. Rep.*, **25**, 1 (1962).

(17) Melting points were determined on a Fisher–Johns apparatus and are corrected. Optical rotations were measured at ambient temperatures with a Perkin-Elmer Model 141 automatic polarimeter. Paper chromatograms were run by the descending technique on Whatman No. 1 paper. Tlc was run on silica gel HF (E. Merck AG Darmstadt). The solvent systems are listed in Table I. All spots were detected by uv light and also sometimes with ninhydrin spray. All solutions were dried with $MgSO_4$ (anhyd) and were concd *in vacuo* with a bath temp of less than 50° unless otherwise noted. Celite is a diatomaceous earth product of Johns–Manville. Samples were dried *in vacuo* (<1 mm) at 56° for 15 hr before analysis. Analytical results are within ±0.4% of the calculated values.

(18) (a) R. P. Rivers and J. Lerman, *J. Endocrinol.*, **5**, 223 (1948); (b) H. E. Carter and J. W. Hinman, *J. Biol. Chem.*, **178**, 403 (1949).

Alkylation of 5-Substituted Tetrazoles

LELAND HUFF AND RONALD A. HENRY[1]

Chemistry Division, Code 605, Naval Weapons Center, China Lake, California 93555

Received January 12, 1970

A series of antihypertensive aminoethyltetrazoles, prepared by the alkylation of 5-alkyl- or 5-aryltetra-

(1) To whom communications should be directed.