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

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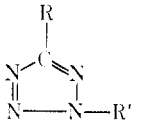
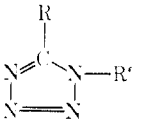
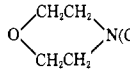
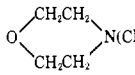
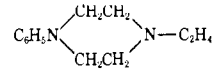
A series of antihypertensive aminoethyltetrazoles, prepared by the alkylation of 5-alkyl- or 5-aryltetra-

(1) To whom communications should be directed.

TABLE I
PRODUCT DISTRIBUTION FROM ALKYLATION REACTIONS


2 isomer


1 isomer

R	R'	Yield from alkylation, %	Isomer	Eluting solvent[a]	% in mixture	Mp, °C	Formula	Analyses[b]	Pmr, CH_2 attached to tetrazole N, τ(CDCl₃)
C_6H_5	$C_6H_5CH_2$	78	2	C_6H_6	71.5	70–71[c]	$C_{14}H_{12}N_4$	C, H, N	
			1	$CHCl_3$	28.5	92–93[d]	$C_{14}H_{12}N_4$	C, H, N	
$C_6H_5CH_2$	$C_6H_5CH_2$	91	2	C_6H_6	56[e]	41–42[d]	$C_{15}H_{14}N_4$	N	4.35 (s)
			1	C_6H_6	44	76[f]	$C_{15}H_{14}N_4$	N	4.64 (s)
C_6H_5		96	2	95:5 C_6H_6–EtOH	78[g]	Liquid[h]	$C_{14}H_{19}N_5O$	C,[i] H, N	5.24 (t, J = 6.5 Hz)
			1	EtOH	8	Liquid[j]	$C_{14}H_{19}N_5O$		5.35 (t, J = 6.5 Hz)
$C_6H_5CH_2$		89.5	2	95:5 C_6H_6–EtOH	50.5[g]	Liquid[k]	$C_{15}H_{21}N_5O$	N,[l] equiv wt	5.34 (t, J = 6.5 Hz)
			1	EtOH	35.5	88–89[m]	$C_{15}H_{21}N_5O$	C,[n] H, N	5.73 (t, J = 6.5 Hz)
	C_2H_5	76	2	95:5 C_6H_6–EtOH	57	67–68[o,p]	$C_{25}H_{32}N_6$	N	5.47 (q, J = 7 Hz)
			1	95:5 C_6H_6–EtOH	43	83–84[o,q]	$C_{25}N_{32}N_6$	N	5.63 (q, J = 7 Hz)

[a] The chromatographic column was packed with 2:1 silica gel (Matheson Coleman and Bell, Micro-Sized)–celite. [b] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements or functions were within ± 0.4% of the theoretical value. [c] Recrystallized from 75% aq AcOH. [d] Recrystallized from cyclohexane. [e] The ratio of isomers was essentially the same whether the alkylation was performed in absolute EtOH or in 65% aq EtOH. [f] Recrystallized from 80:20 C_6H_{12}–C_6H_6. [g] An unidentified material, amounting to 14% of the alkylation product, was eluted from the column before the 2 isomer. [h] The maleate melted at 143.5–145° after recrystallization from MeOH: reported,[2] mp 143–144.5°. *Anal.* ($C_{18}H_{23}N_5O_5$) C, H, N. The picrate melted at 156–158° after recrystallization from $CHCl_3$. *Anal.* ($C_{20}H_{22}N_8O_8$) C, H, N. [i] C: Calcd, 61.52; found, 62.08. [j] The picrate melted as 152–154° after recrystallization from $CHCl_3$; a mixture melting point with 2 isomer picrate was 130–135°. *Anal.* ($C_{20}H_{22}N_8O_8$) C, H, N. The ir spectra of the isomeric picrates were different. [k] The picrate, recrystallized from $CHCl_3$–Et_2O, melted at 139–141°. *Anal.* ($C_{21}H_{24}N_8O_8$) C, H, N. [l] N: Calcd, 24.37; found, 23.89. [m] The picrate, recrystallized from $CHCl_3$, melted at 167–168°; a mixture melting point with picrate from the 2-isomer was 125–132°. *Anal.* ($C_{21}H_{24}N_8O_8$) C, H, N. [n] C: Calcd, 62.69; found, 62.21. [o] The melting point was taken on the solid material recovered after evaporation of the eluting solvent; this material was also analyzed without further recrystallization. [p] The dihydrochloride was obtained as felted needles, decomposing at 214–216°, after recrystallization from *i*-PrOH–HCl; reported[2] mp 202–204°. *Anal.* ($C_{25}H_{34}Cl_2N_6$) N, Cl. The dipicrate from absolute EtOH melted at 151–153°. *Anal.* ($C_{37}H_{38}N_{12}O_{14}$) C, H, N. [q] The dihydrochloride from *i*-PrOH–HCl contained one *i*-PrOH of crystallization and decomposed indefinitely at 180–185°. *Anal.* ($C_{18}H_{32}Cl_2N_6O$), N, Cl. Attempted desolvation at 68°, 25 mm, also effected the loss of one HCl; the monohydrochloride melted at 176–178°. *Anal.* ($C_{15}H_{22}ClN_6$) N, Cl. The dipicrate melted at 137–139° after recrystallization from 95% EtOH; admixture with the 2 isomer dipicrate depressed the melting point to 128–132°. *Anal.* ($C_{37}H_{38}N_{12}O_{14}$) N.

zoles, was previously reported.[2] The alkylation products were always assigned the 2 isomer structure. Such an arbitrary assignment of structure without further proof is not justified since there is substantial evidence in the literature[3] that the alkylation of 5-substituted tetrazoles furnishes a mixture of 1 and 2 isomers, the ratio of which is dependent on the nature of the 5 substituent. Although the 2 isomer is the predominant one (80–90%) obtained with 5-aryltetrazoles,[4] the ratio of isomers can approach unity with 5-alkyltetrazoles.[5,6] Steric factors can also play a role in the ratio of isomers formed.[6] Furthermore, since 1 isomers are more polar[7] and consequently less soluble than the corresponding 2 isomers,[5] recrystallization of a mixture of isomers, especially one containing nearly equal amounts, could concentrate the 1 isomer in the initial precipitate. For these reasons, there is considerable uncertainty about the structure assignments and/or the purity for some of the compounds described by Hayao, *et al.*[2]

In order to demonstrate that mixtures of isomers are formed, several 5-substituted tetrazoles, as their anions, have been alkylated in EtOH or aq EtOH, the recovered product separated by column chromatography, and the isomers characterized. The results are summarized in Table I. Two of the present examples duplicate those reported[2] previously. In one example, the maleate obtained from chromatographically purified 2-[3-(*N*-morpholino)propyl]-5-phenyltetrazole had a melting point in agreement with that reported; in this case, the ratio of 2 to 1 isomer in the alkylated product was almost 10:1. In the other example, the dihydrochloride of purified 2-ethyl-5-[2-(4-phenylpiperazinyl)ethyl]tetrazole had a melting point substantially higher than that reported, which suggests that the derivative purported to be the 2 isomer was probably a mixture of isomers. The ratio of isomers in the alkylated product in this latter example was 1.3:1.

Experimental Section

The following procedure is typical of those used to prepare and separate the isomeric 1,5- and 2,5-disubstituted tetrazoles listed in Table I.

(2) S. Hayao, H. J. Havera, W. U. Strycker, T. J. Leipzig, and R. Rodriguez, *J. Med. Chem.*, **10**, 400 (1967).

(3) See F. R. Benson [*Heterocycl. Compounds*, **8**, 53 (1967)] for summary of earlier references.

(4) B. Elpern, *J. Amer. Chem. Soc.*, **75**, 661 (1953); R. A. Henry, *ibid.*, **73**, 1470 (1951).

(5) R. A. Henry and W. G. Finnegan, *ibid.*, **76**, 923 (1954); R. N. Butler and F. L. Scott, *J. Org. Chem.*, **31**, 3182 (1966); J. Cobeb, W. B. Finnegan, and R. A. Henry U. S. Patent 3073731 (1963).

(6) R. Raap and J. Howard, *Can. J. Chem.*, **47**, 813 (1969).

(7) M. H. Kaufman, F. M. Ernsberger, and W. S. McEwan, *J. Amer. Chem. Soc.*, **78**, 4197 (1956).

Dried Na salt of 5-[2-(4-phenyl-1-piperazinyl)ethyl]tetrazole[2] (8.41 g, 0.03 mol) in 40 ml of abs EtOH was refluxed with 4.75 g (0.0305 mol) of EtI for 16 hr. After concentrating to about 20 ml, the solution was diluted with 35 ml of H_2O and the pH adjusted to the phenolphthalein endpoint with base. Extraction with five 20-ml portions of Et_2O, followed by drying and evaporation of the extracts, gave 6.55 g (76%) of solid product. Two, well-separated spots of approximately equal size developed in tlc (Eastman Chromatogram Sheet 6060, Silica Gel; 95:5 C_6H_6–abs EtOH). This mixture was chromatographed on 90 g of 2:1 silicic acid–Celite using 95:5 C_6H_6–abs EtOH as the eluting solvent. Between 450 and 500 ml of solvent was required to remove the 2-isomer, which came off the column first.[8] The product (3.76 g) recovered by evaporating the eluting solvent crystallized spontaneously, mp 67–68°. Although a portion (0.6 g, mp 83–84°) of the 1 isomer was later eluted from the column by the same solvent mixture (300 ml), the balance of this isomer was more conveniently recovered by extruding the column packing, boiling the latter with a large volume of the solvent, filtering hot, and evaporating the extract.

Derivatives were prepared from the separated isomers by conventional methods.

(8) This assignment is made for three reasons: a 2 isomer is less polar than the 1 isomer;[7] the melting point of a 2 isomer is usually lower than that for the 1 isomer; and the chemical shift for the protons on a methylene group attached to tetrazole ring nitrogen is always at slightly lower field for the 2 isomer than that for the 1 isomer.[6]

Aminoadamantane Derivatives as Potential Insect Chemosterilants

JAMES E. OLIVER AND JERRY B. STOKES

Entomology Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705

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Since the development of 1-aminoadamantane as an antiviral agent,[1] and subsequent reports that the adamantyl moiety enhances biological activity in a variety of classes of compounds,[2] there has been a marked interest in the synthesis and biochemistry of a wide variety of adamantyl-substituted and closely related compounds.

We wish to describe 16 derivatives[3] of 1-aminoadamantane that were tested as candidate chemosterilants[4] against three species of insects. Our selection of compounds was influenced by the occasional biological activity—*e.g.*, chemosterilant, antitumor, antiviral—associated with ureas and semicarbazides and their thio analogs, thiosemicarbazones, and related hydrazine derivatives. In addition, we incorporated into some of these compounds a dimethylamino group, another functionality frequently associated with chemosterilant activity.[5] Their mode of preparation is shown in Table I.

(1) H. J. Eggers and I. Tamm, *Annu. Rev. Pharmacol.*, **6**, 239 (1966) and ref therein.

(2) K. Gerzon, D. J. Tobias, Sr., R. E. Holmes, R. E. Rathbun, and R. W. Kattau, *J. Med. Chem.*, **10**, 603 (1967) and previous papers.

(3) (a) Compounds **1**, **2**, and **12**, had been reported previously; E. I. duPont de Nemours and Co., Netherlands Application 6,403,294, April 23, 1965 [*Chem. Abstr.*, **63P** 9837*h* (1956)]; (b) Compounds **7**, **13**, and **14** were reported at about the time this work was completed: S. Sallay and S. J. Childress, U.S. Patent 3,406,180, Oct 15, 1968 [*Chem. Abstr.* **70**, 11223 (1969)]. Melting points of **7** and **13** agreed with those reported; the abstract did not contain a melting point for **14**.

(4) A. B. Borkovec, "Insect Chemosterilants," Interscience, New York, N. Y., 1966.

(5) P. H. Terry and A. B. Borkovec, *J. Med. Chem.*, **10**, 118 (1967); A. B. Borkovec and A. B. DeMilo, *ibid.*, **10**, 457 (1967); and unpublished observations.

Chemistry.—Compounds **5**, **7**, and **9** were obtained from the reaction of 1-adamantyl isothiocyanate with Me_2NH, N_2H_4, and 1,1-dimethylhydrazine, respectively. Compounds **4**, **8**, **10**, and **11** were similarly prepared by treating Me_2NH, 1,1-dimethylhydrazine, ethyl carbazate, and semicarbazide, respectively, with 1-adamantyl isocyanate. This isocyanate has been reported,[6] but our synthesis *via* a Schmidt reaction (Experimental Section) provides a particularly convenient preparation on a laboratory scale. With one exception described below, the reactions of nucleophiles with both the isocyanate and the isothiocyanate proceeded smoothly at or below room temperature in any of a variety of solvents, *e.g.*, CH_2Cl_2, THF, C_6H_6, Et_2O. EtOH could be used at low temperatures, although heating the isocyanate with EtOH in refluxing C_6H_6 afforded the carbamate **12**[3a] in high yield. Work-up consisted of simply collecting the solid product if it had separated from solution, or evaporating the reaction mixture to dryness and recrystallizing the residue from an appropriate solvent. A typical example, the preparation of **10**, is described in the Experimental Section. 4-(1-Adamantyl)semicarbazide (**6**) could not be prepared cleanly from 1-adamantyl isocyanate and N_2H_4, and was instead obtained from alkaline hydrolysis of **10**. 4-(1-Adamantyl)thiosemicarbazones (**13**–**16**) were prepared from **7** and the appropriate carbonyl compounds.

Results

In general these adamantyl compounds have not been highly effective as insect chemosterilants against house flies, *Musca domestica* L., screw-worm flies, *Cochliomyia hominivorax* (Coquerel), or boll weevils, *Anthonomus grandis* Boheman. Although **3**, **6**, **7**, and **16** reduced oviposition in some tests when fed to mixed sexes of house flies at a concentration of 1% of the diet,[7] the activity was not sustained at lower concentrations or in male sterilant tests. None of the compounds exhibited significant activity against boll weevils. Compounds **3** and **4** reduced egg hatch when fed to screw-worm flies[8] (1% of diet), and **1** prevented oviposition at this concentration.

Experimental Section[9]

1-Adamantanecarbonyl Azide and 1-Adamantyl Isocyanate.—A solution of 1-adamantanecarbonyl chloride (50 g, 0.24 mol) in Me_2CO (150 ml) was added dropwise to a cold (0–5°) solution of NaN_3 (20 g, 0.31 mol) in H_2O (75 ml) and Me_2CO (50 ml). The mixture was stirred in the cold for 20 min then extracted with Et_2O (3 × 150 ml). The combined Et_2O portions were washed (H_2O, aq $NaHCO_3$, aq NaCl) and then dried. The solvent was stripped at 15–20°, and the azide was obtained as a white solid

(6) (a) H. Stetter and C. Wulff, *Chem. Ber.*, **95**, 2302 (1962); (b) M. Paulshock and J. C. Watts, U.S. Patent 3,203,970, August 31, 1965 [*Chem. Abstr.*, **64**, PC 615*g* (1966)].

(7) Screening tests on house flies were performed by G. C. LaBrecque and associates, of this Division in Gainesville, Fl.; *cf.* R. L. Fye, G. C. LaBrecque, and H. K. Gouck, *J. Econ. Entomol.*, **59**, 485 (1966).

(8) Screening tests on screw-worm flies were performed by M. M. Crystal and associates, of this Division in Mission, Texas; *cf.* M. M. Crystal, *J. Econ. Entomol.*, **57**, 726 (1964).

(9) Melting points were obtained on a Büchi melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer Model 137 NaCl prism spectrophotometer. $MgSO_4$ was employed as a drying agent. 1-Adamantanecarbonyl chloride and 1-adamantyl isothiocyanate were purchased from the Aldrich Chemical Co. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.