TABLE I

N-5-*p*-BROMOANILINOMETHYLHYDANTOINS

Substituted hydantoin	Mp, °C[a]	% yield[b]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
5,5-Dicyclopropyl[d]	210.5–212[e]	89	$C_{16}H_{18}BrN_3O_2$	52.76	4.98	11.54	52.98	5.11	11.46
5,5-Diethyl	121–123[f]	70	$C_{14}H_{18}BrN_3O_2$	49.42	5.33	12.35	49.63	5.49	11.95
5,5-Dimethyl	196–196.5	76	$C_{12}H_{14}BrN_3O_2$[g]						
5,5-Diphenyl	190–192[h]	77	$C_{22}H_{18}BrN_3O_2$[i]	60.56	4.16	9.63	60.36	4.40	9.81
5,5-Di-*n*-propyl	154.5–155[h]	82	$C_{16}H_{22}BrN_3O_2$	52.18	6.02	11.41	52.48	6.20	11.25
5-Ethyl-5-phenyl	160–160.5[h]	85	$C_{18}H_{18}BrN_3O_2$	55.68	4.67	10.82	55.79	4.88	10.63
5,5-Hexamethylene	200–200.5[j]	42	$C_{16}H_{20}BrN_3O_2$	52.47	5.50	11.47	52.41	5.59	11.45
Hydantoin[d]	178.5–179[j]	90	$C_{10}H_{10}BrN_3O_2$	42.27	3.55	14.79	42.36	3.74	14.94
Menthonespiro[d]	246–246.5[j]	85	$C_{19}H_{26}BrN_3O_2$	55.89	6.42	10.29	55.87	6.23	10.11
5-*p*-Methoxyphenyl	168–168.5[h]	87	$C_{17}H_{16}BrN_3O_3$	52.32	4.13	10.77	52.40	4.29	10.88
5-Methyl-5-*p*-chlorophenyl[k]	199[j]	74	$C_{17}H_{15}BrClN_3O_2$	49.96	3.70	10.28	50.16	3.75	10.27
5-Methyl-5-isobutyl	166–166.5[h]	86	$C_{15}H_{20}BrN_3O_2$	50.86	5.69	11.86	51.16	5.92	11.93
5-Methyl-5-pentyl	134[h]	86	$C_{16}H_{22}BrN_3O_2$	52.18	6.02	11.41	52.18	5.99	11.42
5-Methyl-5-phenyl	152.5–153[h]	50	$C_{17}H_{16}BrN_3O_2$	54.56	4.31	11.23	54.37	4.53	11.31
5-Methyl-5-(2-thienyl)	155–156[h]	89	$C_{15}H_{14}BrN_3O_2S$	47.38	3.71	11.05	47.26	3.96	11.06
5,5-Pentamethylene[d]	223.5–224.5[h]	84	$C_{15}H_{18}BrN_3O_2$	51.15	5.15	11.93	51.16	5.37	11.53
5-Phenyl[d]	186–186.5	82	$C_{16}H_{14}BrN_3O_2$	53.35	3.92	11.67	53.61	4.13	11.51
5,5-Tetramethylene[d]	190.5–191[j]	77	$C_{14}H_{16}BrN_3O_2$	49.72	4.77	12.42	49.93	5.03	12.55
5-(*p*-Tolyl)	181–182[j]	81	$C_{17}H_{16}BrN_3O_2$	54.56	4.31	11.23	54.58	4.53	11.08

[a] All melting points were determined using a Mel-Temp apparatus and are corrected. [b] The products as obtained from the reaction mixture were of high purity, and the percentage yield reported is of unrecrystallized product. [c] Carbon and hydrogen analyses were conducted by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Nitrogen analyses were by the semimicro Kjeldahl method and were determined by Victor Krimsley and Eric Steinberg of this laboratory. [d] The product precipitated out of solution shortly after refluxing had begun, and the reaction mixture was refluxed for 30 min. [e] Recrystallized from aqueous acetone. [f] Recrystallized from aqueous ethanol. [g] This compound was reported.[2] [h] Recrystallized from ethanol. [i] Lit.[2] mp 194.5–195.5°, after repeated recrystallizations. [j] Recrystallized from ethanol–dimethylformamide. [k] A mixture of ethanol and dimethylformamide was used as the reaction solvent.

the method described by Goodson and co-workers.[3] Equimolar quantities of the hydantoin, *p*-bromoaniline, and formaldehyde were refluxed for 1 hr or less in ethanol solvent following the procedure previously described.[2] The products were obtained upon filtering and cooling the filtrate and are reported in Table I.

Acknowledgment.—The author wishes to thank the members of his 1964-1965 organic chemistry class who prepared the compounds reported herein as part of a special laboratory exercise.

(3) L. H. Goodson, I. L. Honigberg, J. J. Lehman, and W. H. Burton, *J. Org. Chem.*, **25**, 1920 (1960).

Quinoxaline Studies. XIII. N-(2-Quinoxaloyl)-α-amino Acids[1,2]

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Several physiologically active polypeptides, such as levomycin,[3] actinoleukin,[4] echinomycin,[5] and quinomycin,[6,7] have been shown to possess one or more quinoxaloyl moieties. This prompted the preparation of a series of N-(2-quinoxaloyl)-α-amino acids (Table I) for testing as antitumor agents.

(1) We gratefully acknowledge support of this work under National Institutes of Health Grant GM-11968.

(2) Paper XII of this series: E. Brill and H. P. Schultz, *J. Org. Chem.*, **29**, 579 (1964).

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(4) M. Ueda, Y. Tanigawa, Y. Ogami, and H. Umezawa, *J. Antibiotics* (Tokyo), **A7**, 125 (1954).

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(6) T. Yoshida, K. Katagiri, and S. Yokozawa, *J. Antibiotics* (Tokyo), **A14**, 330 (1961).

(7) J. I. Shoji and K. Katagiri, *ibid.*, **A14**, 335 (1961).

Experimental Section

Ultraviolet absorption spectra were determined with a Bausch and Lomb 505 spectrophotometer at concentrations of 5 mg/l. of 95% ethanol. Amino acids utilized were of CP grade, purchased from Mann Research Laboratories.

2-(D-*arabino*-Tetrahydroxybutyl)quinoxaline Monohydrate.—A solution of 1505 g of sucrose, 432 g of *o*-phenylenediamine, 1600 ml of water, and 480 ml of acetic acid was stirred and refluxed for 2 hr while air was bubbled through the solution. Cooling the solution at 10° for 12 hr gave 213 g (19.9%) of light brown crystals, mp 189–190° (lit. mp 188°,[8] mp 190–191°[9]). The material was used without further purification.

2-Quinoxalinecarboxylic Acid.—Sodium hydroxide (140 g) was dissolved in a solution of 200 ml of 30% H_2O_2 and 800 ml of water at 5°. After warming the solution to room temperature, 107 g of 2-(D-*arabino*-tetrahydroxybutyl)quinoxaline monohydrate was added, and the mixture was stirred while warmed carefully to 75°, at which time the reaction became self-sustaining. The temperature was carefully maintained at 80 ± 1° by use of an ice bath until vigorous reaction ceased (about 30 min); stirring and heating at 80° of the light yellow solution was continued for 1 hr. (If the solution were dark and/or if solid were present, 25-ml portions of 30% H_2O_2 solution were added with continued stirring and heating until the solution was a clear, light yellow color.) The solution was transferred to a large beaker, stirred, and cooled to 0°, and then neutralized with 111 ml of cold (0°) H_2SO_4. The precipitate was filtered, then redissolved in a solution of 30 g of KOH in 500 ml of water. The basic solution was treated with 5 g of decolorizing carbon and of filter aid, filtered, and then added to a stirred, cold (0°) solution of 30 ml of H_2SO_4 in 1 l. of water. The precipitation was repeated to give 46 g (66%) of light yellow material, mp 200–201° dec. The product could be recrystallized from hot water or ethanol, mp 215° dec (lit.[8] mp 210°). However, the per cent yield and quality of 2-quinoxaloyl chloride obtained in the subsequent procedure was the same whether precipitated or recrystallized material was used.

N-(2-Quinoxaloyl)-L-α-alanine.—A mixture of 2.94 g of L-α-alanine, 180 ml of water, 9.9 g of $NaHCO_3$, and 6.35 g of quinox-

(8) K. Maurer and B. Scheidt, *Ber.*, **67**, 1980 (1934).

(9) D. Horton and M. J. Miller, *J. Org. Chem.*, **30**, 2457 (1965).

TABLE I

N(-2-QUINOXALOYL)-α-AMINO ACID DERIVATIVES

N-(2-Quinoxaloyl) derivative of	Formula	Calcd, %				Found, %				Reaction time, hr	Yield, %	Recrystn solvent[a]	Mp, °C dec	[α]D, deg (t, °C) (c 1, aq 5% $NaHCO_3$)	$\lambda_{max}^{95\%\ EtOH}$, mμ (ε × 10^{-3})	$\lambda_{max}^{95\%\ EtOH}$, mμ (ε × 10^{-2})
		C	H	N	S	C	H	N	S							
Ammonia	$C_9H_7N_3O$	Known[b]	...	...	...	...	...	...	...	3	85	E	203.5–204[b]	...	207 (1.77), 243 (3.75)	316 (6.73), 324 (6.81)
L-Alanine	$C_{12}H_{11}N_3O_3$	58.8	4.52	17.1	...	58.9	4.53	17.3	...	3	59	W	237–238	+123.7 (26.0)	206 (0.85), 244 (2.01)	317 (3.69), 328 (3.80)
D-Alanine	$C_{12}H_{11}N_3O_3$	58.8	4.52	17.1	...	58.9	4.40	17.3	...	3	56	W	236–237	−132.6 (26.0)	206 (0.85), 244 (2.01)	317 (3.69), 328 (3.80)
DL-α-Aminobutyric acid	$C_{13}H_{13}N_3O_3$	60.2	5.05	16.2	...	59.9	5.08	16.3	...	6	73	E + W	216.5–217.5	...	207 (1.71), 244 (3.29)	319 (5.28), 327 (5.68)
L-Aspartic acid	$C_{13}H_{11}N_3O_5$	54.0	3.83	14.5	...	53.9	4.08	14.4	...	5	51	W	199–205	+50.6 (27.5)	206 (2.90), 243 (2.57)	318 (9.13), 326 (9.71)
L-Cysteine	$C_{12}H_{11}N_3O_3S$	52.0	4.00	15.2	11.6	51.5	3.85	15.3	11.8	5	26	DMF + W	213.5–214.5	−132.8 (27.0)	206 (2.48), 244 (3.92)	319 (6.46), 328 (6.69)
L-Cystine-(N,N′-bis-2-quinoxaloyl)	$C_{24}H_{20}N_6O_6S_2$	52.2	3.65	15.2	11.6	52.2	3.68	14.9	...	7	46	E (abs)	207.0–207.5	−84.6 (27.0)	207 (4.55), 244 (7.03)	320 (13.3), 327 (12.0)
DL-Ethionine	$C_{15}H_{17}N_3O_3S$	56.4	5.37	13.2	10.0	56.8	5.45	13.4	9.8	3	51	E + W	181.5–182.0	...	206 (2.64), 245 (3.98)	278 (8.07), 318 (6.40), 328 (6.93)
L-Glutamic acid	$C_{14}H_{13}N_3O_5$	55.4	4.32	13.9	...	55.1	4.51	13.7	...	8	38	E + W	214–215	+29.7 (20.0)	207 (2.16), 244 (3.72)	320 (5.51), 328 (5.39)
L-Glutamine	$C_{14}H_{14}N_4O_4$	55.6	4.67	18.5	...	55.6	4.81	18.4	...	5	53	W	198.0–198.5	+46.0 (24.5)	207 (2.28), 244 (3.82)	318 (7.05), 328 (7.40)
Glycine	$C_{11}H_9N_3O_3$	Known[c]	...	...	...	...	...	...	...	3	65	W	230–231[c]	...	207 (2.46), 244 (5.51)	320 (10.0), 327 (10.1)
L-Isoleucine	$C_{15}H_{17}N_3O_3$	62.7	5.96	14.6	...	62.5	6.15	14.8	...	4	74	E + W	188–189	+90.7 (20.0)	206 (1.55), 244 (3.56)	318 (6.17), 328 (3.80)
L-Leucine	$C_{15}H_{17}N_3O_3$	62.7	5.96	14.6	...	62.7	6.12	14.4	...	5	56	E + W	212.5–214	+59.0 (22.0)	207 (2.83), 244 (4.90)	320 (8.86), 327 (8.86)
L-Methionine	$C_{14}H_{15}N_3O_3S$	55.1	4.95	13.8	10.5	54.9	5.05	13.9	10.7	5	62	E + W	193–195	+9.3 (20.0)	206 (2.29), 244 (3.72)	320 (6.47), 327 (6.89)
DL-Norleucine	$C_{15}H_{17}N_3O_3$	62.7	5.96	14.6	...	62.8	5.92	14.7	...	3	74	E + W	169–169.5	...	206 (2.89), 244 (3.87)	318 (6.23), 326 (6.95)
DL-Norvaline	$C_{14}H_{15}N_3O_3$	61.5	5.53	15.4	...	61.5	5.74	15.4	...	3	55	W	189.5–190	...	207 (1.71), 244 (2.88)	319 (4.34), 327 (4.68)
L-Phenylalanine	$C_{18}H_{15}N_3O_3$	67.3	4.71	13.1	...	67.2	4.81	13.1	...	8	72	E + W	160–161	−26.7 (22.5)	207 (2.59), 244 (4.47)	319 (8.21), 327 (8.39)
L-Serine	$C_{12}H_{11}N_3O_4$	55.2	4.24	16.1	...	55.3	4.40	15.9	...	8	50	A + P	186.5–187[d]	+75.7 (26.0)	206 (2.11), 244 (2.44)	319 (5.99), 327 (6.13)
D-Serine	$C_{12}H_{11}N_3O_4$	55.2	4.24	16.1	...	55.2	4.46	15.9	...	8	46	A + P	186–187[d]	−87.8 (26.0)	206 (2.11), 244 (2.44)	319 (5.99), 327 (6.13)
L-Threonine	$C_{13}H_{13}N_3O_4$	56.7	4.76	15.3	...	56.7	4.65	15.2	...	3	73	E + W	212-212.5	+109.0 (27.5)	207 (2.03), 244 (3.30)	319 (5.82), 327 (5.46)
L-Valine	$C_{14}H_{15}N_3O_3$	61.5	5.53	15.4	...	61.3	5.79	15.0	...	4	87	E + W	196.5–197.5	+96.1 (22.0)	207 (2.35), 244 (5.03)	317 (8.85), 327 (9.42)
DL-Valine	$C_{14}H_{15}N_3O_3$	61.5	5.53	15.4	...	61.7	5.39	15.4	...	4	87	E + W	208–209	...	207 (2.35), 244 (5.03)	317 (8.85), 327 (9.42)

[a] A, acetone; DMF, dimethylformamide; E, 95% ethanol; P, 30–60° petroleum ether; W, water. [b] I. Yosioka and H. Otomasu [*Chem. Pharm. Bull.* (Tokyo), **5**, 277 (1957)] give mp 200°. [c] Lit.[10] mp 226° dec. [d] Lit.[10] mp 224° dec for DL-serine.

aloyl chloride[10] (mp 113–115°) was stirred at 25° for 3 hr. The colorless mixture turned dark red in 30 min then became light yellow in 3 hr. Various amino acids required 3–8 hr for this color change. The solution was treated with decolorizing carbon and filter aid, filtered, and brought to pH 4 with concentrated HCl, cooled at 10° for 12 hr, and filtered to give 6.94 g (85.5%) of white powder, mp 230–231°, $[\alpha]^{26}D$ +99.7° (*c* 1, aqueous 5% $NaHCO_3$). The product was recrystallized from hot water to give 4.80 g (59.4%) of analytically pure material, mp 237–238°, $[\alpha]^{26}D$ +123.7° (*c* 1, aqueous 5% $NaHCO_3$).

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The Acetylation of 1-(β-D-Arabinofuranosyl)cytosine[1]

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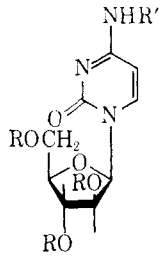
The tri-*O*-acetate (II) and the tetraacetate (III) of 1-(β-D-arabinofuranosyl)cytosine (I) have been prepared as compounds that may be pharmacologically more useful forms of I which has antitumor properties.[2] The tri-*O*-acetate was obtained by selective acetylation of the hydrochloride of I under mild conditions. Standard acetylation conditions readily converted I to III.



I, R = R′ = H
II, R = Ac; R′ = H
III, R = R′ = Ac

Experimental Section[3]

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)cytosine (II).—A solution of acetic–trifluoroacetic anhydride was prepared by mixing 1.90 g (91 mmoles) of trifluoroacetic anhydride and 30 ml (0.52 mole) of acetic acid and allowing this solution to stand at room temperature for 30 min. The solution was cooled and slowly added (over about 10 min) to a solution of 3.0 g (10.7 mmoles) of I·HCl in 35 ml of trifluoroacetic acid[4] so that the temperature did not rise above 10°. After 30 min at 10° and about 12 hr at room temperature,[5] the clear solution was cooled and the temperature maintained below 25° during treatment with 5.5 ml of absolute ethanol to decompose excess anhydride. After 0.5 hr at room temperature, the solution was evaporated *in vacuo* (water-bath temperature 40–50°). The residue was dissolved in 60 ml of water and neutralized to pH 6 with solid $NaHCO_3$. The aqueous solution[6] was extracted with two 450-ml portions of ethyl acetate[7] which were combined, dried, and concentrated to about 90 ml to afford 2.36 g (60%) of highly crystalline II, mp 189–190°, homogeneous by thin layer chromatography. Evaporation of the ethyl acetate filtrate afforded 0.82 g (21%) of amorphous product, mostly II with a trace of less acylated material. A third ethyl acetate extract of the aqueous solution afforded 0.47 g more of material that was composed of II and less acylated material in about equal amounts.

Recrystallization of product from a previous run with ethyl acetate afforded the analytical sample of II: mp 189.0–189.5°; λ_{max}^{Nujol} (μ) 2.90, 3.00, 3.20 (N–H), 5.72 (C=O of acetate); $\lambda_{max}^{pH\ 1}$ 276 mμ (ε 13.8 × 10³); $\lambda_{max}^{pH\ 7}$ 233 mμ (ε 8.1 × 10³), 270 (9.1 × 10³);[8] $\lambda_{max}^{pH\ 13}$ 274 mμ (ε 10.0 × 10³); [α] +68°. The methyl protons of the *O*-acetates were located between τ 7.85 and 8.01. It was homogeneous by thin layer chromatography[3] and by paper chromatography in these solvents: A, R_{Ad} 1.44, and B, R_{Ad} 2.45.

Anal. Calcd for $C_{15}H_{19}N_3O_8$: C, 48.8; H, 5.19; N, 11.4. Found: C, 48.5; H, 5.22; N, 11.5.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)-N^4-acetylcytosine (III).—A solution of 4.70 g of I·HCl in 180 ml of dry pyridine was treated with 18.0 ml of acetic anhydride (slight warming). The resultant solution was left overnight (about 20 hr) at room temperature and was then evaporated *in vacuo* (bath temperature 45–50°). The residue was treated with 20 ml of absolute ethanol, diluted with 150 ml of toluene, and again evaporated to dryness. The residue was partitioned between 300 ml of ethyl acetate and 75 ml of water. The ethyl acetate layer was washed with water, dried, concentrated to 50 ml, and then diluted 10-fold with ether. The product which crystallized was collected, washed with ethyl acetate–ether (1:10), and dried to afford 5.86 g (85%) of III, mp 171–172°, homogeneous by thin layer and paper chromatography. Recrystallization from ethyl acetate–ether afforded the analytical example of III: mp 171–171.5°; λ_{max}^{Nujol} (μ) 3.15, 3.23 (N–H), 5.72 (acetate); $\lambda_{max}^{pH\ 1}$ 247 mμ (ε 11.2 × 10³), 302 (9.0 × 10³); $\lambda_{max}^{pH\ 7}$ 247 mμ (ε 16.4 × 10³), 298 (8.6 × 10³);[8] $\lambda_{max}^{pH\ 13}$ 275 mμ (ε 10.5 × 10³); [α] + 87°. The methyl protons of the *O*-acetates appeared at τ 7.84–8.01; the *N*-acetyl, at τ 7.67. It moved as a single spot in thin layer chromatograms (R_f 0.90) and on paper in these solvents: A, R_{Ad} 1.75, and C, R_{Ad} 1.35.

Anal. Calcd for $C_{17}H_{21}N_3O_9$: C, 49.6; H, 5.15; N, 10.22. Found: C, 49.5; H, 5.23; N, 9.86.

Acknowledgment.—The authors thank Dr. Peter Lim and his staff for the ultraviolet and infrared spectra, optical rotations, and paper chromatography.

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(2) (a) R. W. Talley, and V. K. Vaitkevicius, *Blood*, **21**, 352 (1963); (b) J. S. Evans, E. A. Musser, L. Bostwick, and G. D. Mengel, *Cancer Res.*, **24**, 1285 (1964); (c) J. E. Evans, L. Bostwick, and G. D. Mengel, *Biochem. Pharmacol.*, **13**, 983 (1964).

(3) Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Anhydrous $MgSO_4$ was used as the drying agent. Paper chromatograms were run by the descending technique on Whatman No. 1 paper and the spots were located in R_{Ad} units with adenine at 1.00. The solvent systems were: A, 1-butanol–water (saturated); B, water; C, 1-butanol–acetic acid–water (5:2:3). Thin layer chromatograms were run on silica gel HF plates with a solvent system of methanol–$CHCl_3$ (1:4). The spots on plates and paper were detected by ultraviolet light. Optical rotations were determined in 1% solutions in *N,N*-dimethylformamide at 21.6° using the sodium D line. The nmr spectra were determined with a Varian A-60 spectrometer, using $CDCl_3$ solutions containing 4% $Si(CH_3)_4$ as internal standard. The spectra of II and III were compatible with their structures.

(4) The hydrochloride of I was soluble in trifluoroacetic acid but not in acetic acid, acetic anhydride, mixtures of these two, or in the solution of acetic–trifluoroacetic anhydride.

(5) Aliquots were periodically examined by thin layer chromatography for completeness of reaction. Compound II (R_f 0.66) was readily distinguished from less acylated products (R_f <0.5).

(6) In one preparation of II, the product was left in water overnight. The yield of II was low, suggesting that considerable amounts of II had hydrolyzed.

(7) Ethyl acetate cannot be replaced by $CHCl_3$. It was inefficient for extracting the quite water-soluble II from the aqueous phase.

(8) J. Beránek and J. Píťha, *Collection Czech. Chem. Commun.*, **29**, 625 (1964), have reported the ultraviolet spectra in ethanol for 2′,3′,5′-tri-*O*-acetylcytidine (λ_{max} 243 and 268 mμ) and N^4-acetyl-2′,3′-5′-tri-*O*-acetylcytidine (λ_{max} 249 and 299 mμ).