Table III. Activity of 6α,9α-Difluoroprednisolone Derivatives in the Vasoconstriction Test in Humans[a]

Compd	Relative potency[c]
Betamethasone 17-valerate[b]	1
6α,9α-Difluoroprednisolone	<0.1
3	1
4	1
6	1
12	1
13	1
14	1
16	2
17	3
18	3
19	3.5
20	3
21	2
22	3.5
23	3
24	1.5
25	3
26	2
27	2.5
28	2
29	1
30	2

[a]Each compd was texted on 24 subjects at least at three dose levels (0.015–0.18 mcg). [b]0.1 μg induced 50% of maximum blanching score. [c]Compds of Tables I and II not listed here displayed potency <1.

30) exhibited [*vs.* the corresponding Δ^4 compounds (**3**, **6**, **12**, **29**, respectively)] either no advantage or an advantage by far lower than that reported for 6α,9α-difluoroprednisolone 21-acetate systemically given.[10]

Experimental Section§

17,21-Methyl Ortho Esters (1–7). The procedure of Gardi, *et al.*,[1] was used. Isomeric mixts were obtd in almost quant yield which could be used directly in the subsequent step. Analytical samples of Table I were obtd by chromatog on Al_2O_3 and crystn. This processing enhanced the content of the more polar isomer from 50% to about 90% (tlc evidence only). No attempt was made to isolate pure isomers.

17-Monoesters (8–13). The following modification of the original procedure of Gardi, *et al.*,[2,3] was used. To a soln of the proper 17,21-methyl ortho ester (1 g) in MeOH (20 ml), NaOAc buffer (8 ml), prepd by mixing AcOH, 0.1 *N* soln (90 ml), and NaOAc, 0.1 *M* soln (10 ml), was added so to obtain a pH near 5. The reaction mixt was refluxed for 1 hr, concd under reduced pressure, and worked up in the usual way.

17,21-Diesters (14–30). The relevant 17-monoesters were acylated by treatment with the proper anhydride in pyridine at about −5° overnight. Products were isolated as usual and crystd.

Acknowledgment. The authors are indebted to Dr. C. Pedrali for the spectral determinations. We wish to thank Mr. G. Villa for chemical assistance and Mr. P. Beretta for assistance in the biological evaluations.

References

(1) R. Gardi, R. Vitali, and A. Ercoli, *Gazz. Chim. Ital.*, **93**, 413 (1963).
(2) R. Gardi, R. Vitali, and A. Ercoli, *Tetrahedron Lett.*, 448 (1961).
(3) R. Gardi, R. Vitali, and A. Ercoli, *Gazz. Chim. Ital.*, **93**, 431 (1963).
(4) A. W. McKenzie and R. M. Atkinson, *Arch. Dermatol.*, **89**, 741 (1964).
(5) E. Shapiro, L. Finckenor, H. Pluchet, L. Weber, C. H. Robinson, E. P. Oliveto, H. L. Herzog, I. I. A. Tabachnick, and E. Collins, *Steroids*, **9**, 143 (1967).
(6) A. Ercoli, R. Gardi, G. Celasco, and G. Falconi, *Int. Congr. Hormonal Steroids, 3rd*, 363 (1970).
(7) K. D. Jaitly, J. Wieriks, and J. Lens, *ibid.*, 365 (1970).
(8) A. Ercoli, G. Falconi, R. Gardi, and R. Vitali, *J. Med. Chem.*, in press.
(9) J. A. Hogg, G. B. Spero, J. L. Thompson, B. J. Magerlein, W. P. Schneider, D. H. Peterson, O. K. Sebek, H. C. Murray, J. C. Babcock, R. L. Pederson, and J. A. Campbell, *Chem. Ind. (London)*, 1002 (1958).
(10) W. E. Dulin, F. L. Schmidt, and S. C. Lyster, *Proc. Soc. Exp. Biol. Med.*, **104**, 345 (1960).
(11) A. Ercoli, R. Gardi, and R. Vitali, German Patent Application 2031205 (June 26, 1969).
(12) L. Salce, G. G. Hazen, and E. F. Shoenewaldt, *J. Org. Chem.*, **35**, 1681 (1970).
(13) H. Selye, *Proc. Soc. Exp. Biol. Med.*, **82**, 328 (1953).
(14) A. W. McKenzie and R. B. Stoughton, *Arch. Dermatol.*, **86**, 608 (1962).
(15) G. Falconi and G. Rossi, *Int. Congr. Hormonal Steroids, 3rd*, 373 (1970).
(16) G. Falconi and G. Rossi, *Arch. Dermatol.*, in press.
(17) G. DiPasquale, C. L. Rassaert, and E. McDougall, *Steroids*, **16**, 663 (1970); 679 (1970); G. DiPasquale and L. Tripp, *ibid.*, **16**, 693 (1970).

§Melting points were taken in a capillary apparatus and are uncorrected. Optical rotations were detd in dioxane at 24° at a concn of about 0.5%. Uv were detd in 95% EtOH, and ir in Nujol mull. Absorption bands of these spectra were as expected. Tlc of analytical samples were done on silica gel GF with C_6H_6–Me_2CO 8:2 (1–7, 14–30) and 6:4 (8–13). Compounds 1–7 appeared as couples of closely moving spots, compounds 8–30 were homogeneous on tlc. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Pyrido[3,2-*g*]pteridines. 1. Chemistry and Growth-Inhibitory Activity of Some 1*H*,3*H*-2,4-Dioxopyrido[3,2-*g*]pteridines (9-Azaalloxazines)†

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Recently we have been engaged in the synthesis and biological evaluation of some aza analogs of riboflavine, including 3*H*,10*H*-2,4-dioxo-7,8-dimethyl-10-[D(−)-ribityl]-pyrido[3,2-*g*]pteridine ("8-azariboflavine").‡ In connection with this program, we have also prepared some closely related 1*H*,3*H*-2,4-dioxopyrido[3,2-*g*]pteridines (9-azaalloxazines)§ for evaluation of their tumor growth-inhibitory action in selected *in vitro* and *in vivo* bioassay systems and for study of the chemistry of the azaalloxazine ring system. Except for one paper,[2] which reported the synthesis of 9-azaalloxazine (**1**) and 7-chloro-9-azaalloxazine (**2**) without spectral data and, in the case of **2**, with incomplete analytical data, nothing is reported in the literature on the chemistry and biology of this type of compound.

The compounds listed in Table I were obtained when the appropriately substituted diaminopyridine was condensed with alloxan monohydrate in glacial AcOH at room temp in the presence of $B(OH)_3$. With the exception of 2,3-diaminopyridine, which was obtained from a commercial source, the

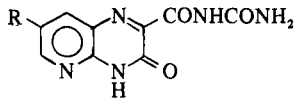
†This investigation was supported in part by Research Grant C6516 and Research Career Development Award K3-CA-22,151 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service

‡A brief account of this work has appeared.[1]

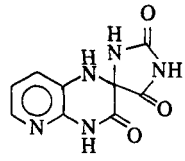
§The Chemical Abstracts numbering sequence for the pyrido[3,2-*g*]pteridine ring system is used in this report. The convenient expression "9-azaalloxazine" for 1*H*,3*H*-2,4-dioxopyrido[3,2-*g*]-pteridine derives from the common use of "alloxazine" for the benzo analog, 1*H*,3*H*-2,4-dioxobenzo[*g*]pteridine.

various ortho diamines required for these condensations were prepared according to procedures already described in the literature.

Boric acid, which is known to possess unusual catalytic properties for the formation of alloxazines and isoalloxazines from alloxan and *o*-phenylenediamines,[3] appears equally efficacious in promoting the formation of 9-azaalloxazines. Except in the preparation of **1**, where a stoichiometric equivalent of $B(OH)_3$ was required for the reaction, the quantity of catalyst needed was not critical. However, the presence of catalyst was essential. We found, as did Ziegler[2] before us, that alloxan monohydrate and 2,3-diaminopyridine in glac AcOH in the absence of $B(OH)_3$ gave the carboxyureidopyridopyrazinone **7a** rather than **1**. In contrast to Ziegler, we experienced no difficulty in the preparation of **2**, or of **3**-**6** for that matter, in the presence of a catalytic quantity of $B(OH)_3$. Ziegler claimed that alloxan and 2,3-diamino-5-chloropyridine in the presence of $B(OH)_3$ gave **7b**.# We believe that Ziegler's supposed sample



7a, R = H
7b, R = Cl



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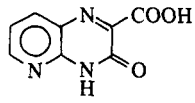


9

of **7b** may actually have been a hydrated form of **2**; the two products can be differentiated easily only by means of their electronic spectra at pH 1 (see below).

Condensation of alloxan with 2,3-diaminopyridine in aqueous acidic or neutral media is known[4] to give a mixture of the carboxyureide **7a** and the spirohydantoin **8**. We have now found that, when condensed in aqueous alkali, alloxan and 2,3-diaminopyridine gave 3-keto-3,4-dihydropyrido-[2,3-*b*]pyrazine-2-carboxylic acid (**9**). Although **1** is hydrolyzed in base to **7a**, as described below, and **7a** is known[4] to give **9** on treatment with warm dil NaOH, the formation of **9** from alloxan and the diamine in base does not appear to be due to hydrolysis of **1**; we could uncover no evidence for the formation of **1** during the course of the reaction. Rather, the formation of **9** appears to be analogous to the formation of 7-keto-7,8-dihydropteridine-6-carboxylic acids from alloxan and 4,5-diaminopyrimidines in base, as described by Taylor and Loux.[5] These investigators suggest that alloxan is hydrolyzed in base to alloxanic acid and that alloxanic acid exists in equilibrium in alk solution with the monoureide of ketomalonic acid, which then reacts with the diamine.[5]

Compds **1**-**6** are bright yellow to mustard yellow solids which emit dark green fluorescence even in highly dilute solution. They turn black when heated above 245° but do not melt up to 325°. Like many pteridine derivatives, they are insoluble in H_2O and in most organic solvents. Their insolubility in AcOH provided a convenient means of purification for several of the products; compds **1**-**4** were suspended in boiling glac AcOH, which leached out the more soluble impurities. The azaalloxazines are soluble in base (pH 9 or higher) due to anion formation. However, purification methods which involved aqueous base/acid precipitation returned hydrated products from which H_2O of hydration could not always be removed (*e.g.*, the hemihydrate of **2**, Table I).

When sampled as KBr disks, the azaalloxazines showed characteristic ir absorption signals for associated NH (3.10 and 3.28 μ), the aromatic pyridine nucleus (6.25 μ), and, most predominantly, a C=O doublet (5.79 and 5.88 μ); the higher wavelength band of the doublet was always stronger, as is characteristic of 6-membered cyclic imides.[6] The electronic spectra of the azaalloxazines were similar to that of the parent compd **1**: λ_{max}^{EtOH} (log ϵ) 243 (4.48), 292 shoulder (3.70), and 374 (4.10) nm; $\lambda_{max}^{pH\,1}$ 241 (4.50), 305 (3.82), 372 (4.16), and 386 shoulder (4.11) nm; $\lambda_{max}^{pH\,10}$ 268 (4.64), 340 (3.72), and 443 (4.01) nm. The bathochromic shift observed at pH 10 is due to formation of the anion, which results in an extension in conjugation.

Compds **1**-**6** are stable toward acids and weak oxidizing agents. However, the azaalloxazine system is attacked by vigorous oxidizing agents, such as hot $KMnO_4$ or hot H_2CrO_4-AcOH; no identifiable products could be recovered from these reactions. Treatment of **1** with H_2O_2 in AcOH gave no reaction, although at least one alloxazine, under similar conditions, is known to give a 5,10-di-*N*-oxide.[7] It should be mentioned that we have previously[8,9] noted the inability of other pyrido[2,3-*b*]pyrazine derivatives to give *N*-oxides, although the corresponding quinoxaline *N*-oxides are formed easily. Treatment of a fine suspension of **1** or **3** in hot H_2O with sodium dithionite or of a dil solution of **1** or **2** in EtOH with H_2 in the presence of Pt resulted in very pale yellow, nonfluorescing solutions of the dihydro derivatives. The reduction products were too unstable to isolate, being rapidly reoxidized to the azaalloxazine upon exposure to air.

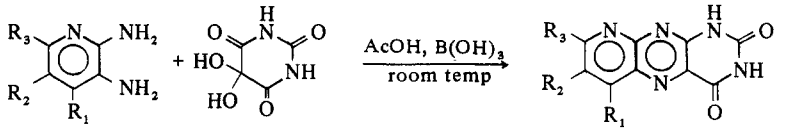
On paper chromatography, **1**-**6** each moved as a single spot in 1-BuOH-AcOH-H_2O and 1-BuOH satd with H_2O solvent systems. However, with 2-PrOH-NH_3-H_2O (3:1:5 by vol) as the developing system, each product showed 2 distinct yellow spots. The second, slower moving component was initially interpreted as being an impurity in the original sample, but was later recognized to arise from alk hydrolysis of the azaalloxazine system. A sample of the hydrolysis product recovered from the chromatography of **1** on paper was found to be identical with samples of **7a** prepared according to the procedure of Clark-Lewis and Thompson.[4] The uv spectra of **7a** in alcohol and pH 10 are very similar to those of **1**. However, as noted above, **7a** is easily differentiated from **1** by means of its electronic spectrum at pH 1. Under acidic conditions, **7a** cyclizes readily to **8**,[4] which exhibits a uv spectrum ($\lambda_{max}^{pH\,1}$ 247 and 311 nm) consistent with that of a simple substituted pyridinediamine, whereas the electronic spectrum of **1** does not change appreciably in going from alcohol to pH 1 media.

Treatment of **1**-**6** with boiling 1 *N* NaOH resulted in opening of the pyrimidine ring and saponification of the ureide, affording, after neutralization, the pyridopyrazinonecarboxylic acids **9**-**14**, respectively (Table II). The products were identical with samples of **9**-**14** prepared by condensation of the corresponding pyridinediamines with the disodio salt of ketomalonic acid in dil NaOH.

Attempts to utilize the azaalloxazines prepared during this investigation for the synthesis of other pyrido[3,2-*g*]-pteridine derivatives were unsuccessful. The oxo functions proved remarkably resistant to chlorination, except under extremely forcing conditions when **2** and **3** showed evidence

#Boron trifluoride-etherate catalyst was claimed necessary for the preparation of **2**.[2]

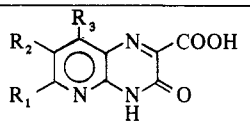
Table I. 1*H*,3*H*-2,4-Dioxopyrido[3,2-*g*]pteridines (9-Azaalloxazines)



Compd No.	R_1	R_2	R_3	Yield, %	Decomposition point, °C	Purification	Molecular formula	Analysis
1[a]	H	H	H	94	>310	AcOH	$C_9H_5N_5O_2$	
2[a]	H	Cl	H	74	>325	AcOH	$C_9H_4ClN_5O_2$	C, H, Cl, N
					293	NH_4OH/AcOH pptn	$C_9H_4ClN_5O_2 \cdot 0.5H_2O$	C, H, Cl, N
3	H	Br	H	83	245	AcOH	$C_9H_4BrN_5O_2$	C, H, Br, N
4	CH_3	Br	H	79	250	AcOH	$C_{10}H_6BrN_5O_2$	C, H, Br, N
5	H	Br	CH_3	50	270	MeOH	$C_{10}H_6BrN_5O_2 \cdot CH_3OH$	C, H, Br, N
6	CH_3	Br	CH_3	81	285	DMSO	$C_{11}H_8BrN_5O_2$	C, H, Br, N

[a] Previously reported by Ziegler.[2]

Table II. 3-Keto-3,4-dihydropyrido[2,3-*b*]pyrazine-2-carboxylic Acids[a]



Compd No.	R_3	R_2	R_1	Prepn[b]	Yield, %	Decomposition point, °C	Molecular formula	Analysis
9[c]	H	H	H	A	40	229-232[d]	$C_8H_5N_3O_3$	
				B	72			
				C	47			
10	H	Cl	H	A	43	238-239	$C_8H_4ClN_3O_3$	C, H, Cl, N
				B	77			
11	H	Br	H	A	46	242-245	$C_8H_4BrN_3O_3$	C, H, Br, N
				B	80			
12	CH_3	Br	H	A	30	221-223	$C_9H_6BrN_3O_3$	C, H, Br, N
				B	73			
13	H	Br	CH_3	A	36	180-183	$C_9H_6BrN_3O_3$	C, H, Br, N
				B	94			
14	CH_3	Br	CH_3	A	37	208-210	$C_{10}H_8BrN_3O_3$	C, H, Br, N
				B	49			

[a] The compds were purified by pptn from dil aq NaOH by the addn of 2 *N* HCl. [b] A = Azaalloxazine in boiling 1 *N* NaOH; B = pyridinediamine + disodio ketomalonate in boiling 1 *N* NaOH; C = pyridinediamine + alloxan monohydrate in boiling 1 *N* NaOH. [c] Previously prepd by Clark-Lewis and Thompson[4] by hydrolysis of ethyl 3-keto-3,4-dihydropyrido[2,3-*b*]pyrazine-2-carboxylate and by hydrolysis of **7a**. [d] Lit.[4] decomp point: 232°.

of polychlorination. Although satisfactory analyses could not be obtained, product samples showed high Cl content on microchemical analysis and no aromatic proton signals in the nmr; ir spectra showed weak, but still present, imide carbonyl absorption. Attempted thiation of **1-6** led only to tarry material.

Biological Activity. Four compds (**1**, **2**, **4**, and **6**) have been investigated for activity against KB (human epidermoid carcinoma) cells in culture;[10] **1**, **2**, and **6** were found to be active at a 50% inhibiting dose (ID_{50}) of 2.8, 1.5, and 2.8 μg/ml, respectively.

Compds **1**, **3**, **4**, and **6** have been examined for *in vivo* antitumor activity against 2 or more transplantable mouse tumors following the standard assay procedures employed at The Children's Cancer Research Foundation.** The agents were administered ip as suspensions in DMSO and H_2O. At the nontoxic dose of 5 mg/kg given daily beginning the first day after tumor implantation, **3** produced significant inhibition of tumor growth in animals bearing the P1534 lymphatic leukemia; this tumor growth inhibition was accompanied by a 72% increase in mean survival time of treated animals, as compared to the control group. Again at nontoxic dosages, **1**, **3**, and **4** produced slight to moderate inhibition of growth of the C1498 myelogenous leukemia; there was no concomitant increase in the survival time of tumor-bearing animals in these assays. None of the compounds was effective in increasing the survival time of mice bearing the highly refractory L1210 leukemia.

We are indebted to Dr. George E. Foley and his associates for the *in vitro* assays and to the late Dr. Charlotte L. Maddock and Miss Barbara Brown for the *in vivo* antitumor data.

Experimental Section††

Uv absorption spectra were measured with Cary Model 11 and Model 15 spectrophotometers. Ir spectra were detd with a Perkin-Elmer Model 137B spectrophotometer. Paper chromatog was done by the descending technique on Whatman No. 1 paper and spots were visualized by examination under uv (366 nm) light. Melting points were taken by the capillary method in a modified Wagner-Meyer melting point apparatus[12] at a rate of heating of 2°/min and are corrected for stem exposure; decompn points are not reproducible unless conditions are rigidly controlled.

Pyridinediamines. 2,3-Diaminopyridine was purchased from Aldrich Chemical Company, as was 2-amino-5-chloropyridine, which

**The following experimental tumor systems were used: L1210 ascitic lymphatic leukemia in the BDF/1 hybrid; P1534 lymphatic leukemia in the DBA/2 inbred; C1498 myelogenous leukemia in the C57B1/6 inbred; and DBRB mammary adenocarcinoma in the DBA/1 inbred strain. The assay procedures have been described.[11]

††Microchemical analyses were performed by Scandanavian Microanalytical Laboratory, Herlev, Denmark, and by Galbraith Microanalytical Laboratories, Knoxville, Tenn; found values are within ±0.4% of theory.

was converted into 2,3-diamino-5-chloropyridine in 2 steps *via* the procedure of Vaughan, *et al.*[13] The following starting materials were obtd from Reilly Tar and Chemical Corporation: 2-aminopyridine, 2-amino-4-methylpyridine, 2-amino-6-methylpyridine, and 2-amino-4,6-dimethylpyridine. Bromination of 2-aminopyridine by the method described by Case[14] afforded 2-amino-5-bromopyridine, which was nitrated and reduced by means of the Vaughan procedure[13] to give 2,3-diamino-5-bromopyridine. 2,3-Diamino-5-bromo-4-methylpyridine and 2,3-diamino-5-bromo-6-methylpyridine were prepd according to the procedure of Israel and Day.[8] The method of Grayboyes and Day[15] was used to obtain 2,3-diamino-5-bromo-4,6-dimethylpyridine.

Condensation of Pyridinediamines with Alloxan Monohydrate. 1*H*,3*H*-2,4-Dioxopyrido[3,2-*g*]pteridines (1-6). The following procedure was used to prepare **1-6**, except that a full equiv of $B(OH)_3$ was used for the prepn of **1**.

A soln containing 4.8 g (0.03 mole) of alloxan monohydrate and 0.5 g (0.008 mole) of $B(OH)_3$ in 100 ml of glac AcOH was combined quickly with a soln of 0.03 mole of the pyridinediamine in 100 ml of glac AcOH and the resulting reaction soln was stirred at room temp for 2-3 hr. At the end of the reaction, the solid present was collected, washed well with cold H_2O, and then dried (NaOH pellets) The product was purified according to the method indicated in Table I. Purified samples showed single spots on paper chromatog in 2 solvent systems [1-BuOH-AcOH-H_2O (4:1:1) and 1-BuOH satd with H_2O].

As indicated by microchem analysis, **2** formed a hemihydrate on purification by base/acid pptn; the hemihydrate was stable to prolonged drying at 100° under vacuum. The existence of **2** in this sample was confirmed by mass spectral analysis which showed two peaks in a 3:1 relationship at *m/e* 249 and 251, consistent with the natural isotope distribution of ^{35}Cl and ^{37}Cl in **2**. Similarly, the mass spectrum of the methanolate of **5**, formed on crystn of **5** from MeOH, showed peaks of essentially equal intensity at *m/e* 307 and 309, consistent with the natural abundance of ^{79}Br and ^{81}Br in the parent compd.

2-Carboxyureido-3,4-dihydropyrido[2,3-*b*]pyrazin-3-one (7a). A soln of 1.81 g (0.017 mole) of 2,3-diaminopyridine in 75 ml of glac AcOH was combined with a soln of 2.7 g (0.017 mole) of alloxan monohydrate in 75 ml of glac AcOH. The reaction soln was stirred at room temp overnight. The yellow solid was sepd, washed with AcOH, and dried; mp 280-283° dec [lit.[2] mp 273-280° dec; 283-285° dec[4]]; uv: λ_{max}^{EtOH} 225, 277, and 361 nm; $\lambda_{max}^{pH\ 10}$ 232, 260 (sh), 308, and 396 nm.

3-Keto-3,4-dihydropyrido[2,3-*b*]pyrazine-2-carboxylic Acids (9-14). Method A. Hydrolysis of 1-6. A sample of the corresponding azaalloxazine was boiled with 1 *N* NaOH (60 ml/g) for 5 hr. The pale yellow soln was cooled to below room temp and the pH was adjusted to 1 by the addn of 3 *N* HCl (CO_2 evoln). The pale yellow to green solid was purified by several pptns from 1 *N* NaOH soln (charcoal) by the addn of HCl.

Method B. Pyridinediamine and Disodio Ketomalonate in Base. A soln contg equimolar quantities of the pyridinediamine and the disodium salt of ketomalonic acid in 1 *N* NaOH was refluxed overnight. The soln was then acidified (to pH 1) and the product collected and purified by several base/acid pptns.

Method C. 2,3-Diaminopyridine and Alloxan in Base. Preparation of 9. A soln of 2.5 g (0.014 mole) of 2,3-diaminopyridine and 2.44 g (0.014 mole) of alloxan monohydrate in 100 ml of 1 *N* NaOH was boiled for 16 hr. The soln was cooled and a small quantity of inorganic material was sepd. The pale yellow filtrate was acidified to pH 1 by the addn of 4 *N* HCl and the ppt of **9** was collected.

References

(1) M. Israel, N. Muhammad, N. Tirosh, L. C. Jones, and E. J. Modest, *Int. Cong. Heterocycl. Chem., 3rd.*, 109 (1971).
(2) J. B. Ziegler, *J. Amer. Chem. Soc.*, **71**, 1891 (1949).
(3) (a) R. Kuhn, K. Reinemund, F. Weygand, and R. Ströbele, *Ber.*, **68**, 765 (1935); (b) R. Kuhn and F. Weygand, *ibid.*, **68**, 1282 (1935).
(4) J. W. Clark-Lewis and M. J. Thompson, *J. Chem. Soc.*, 430 (1957).
(5) E. C. Taylor and H. M. Loux, *J. Amer. Chem. Soc.*, **81**, 2474 (1959).
(6) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962, p 47.
(7) H. G. Petering, U. S. Patent 2,973,359 (1961).
(8) M. Israel and A. R. Day, *J. Org. Chem.*, **24**, 1455 (1959).
(9) M. Israel, L. C. Jones, and E. J. Modest, *J. Heterocycl. Chem.*, in press.
(10) (a) G. E. Foley and H. Eagle, *Cancer Res.*, **18**, 1012 (1958); (b) H. Eagle and G. E. Foley, *ibid.*, **18**, 1017 (1958).
(11) C. L. Maddock, G. J. D'Angio, S. Farber, and A. H. Handler, *Ann. N. Y. Acad. Sci.*, **89**, 386 (1960).
(12) E. C. Wagner and J. F. Meyer, *Ind. Eng. Chem., Anal. Ed.*, **10**, 584 (1938).
(13) J. R. Vaughan, Jr., J. Krapcho, and J. P. English, *J. Amer. Chem. Soc.*, **71**, 1885 (1949).
(14) F. Case, *ibid.*, **68**, 2576 (1946).
(15) H. Graboyes and A. R. Day, *ibid.*, **79**, 6421 (1957).

Nucleoside Peptides. 4. Synthesis of Certain 1-(*N*-4-Aminoacyl-4-amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronic acid)cytosine Derivatives Related to Blasticidin S

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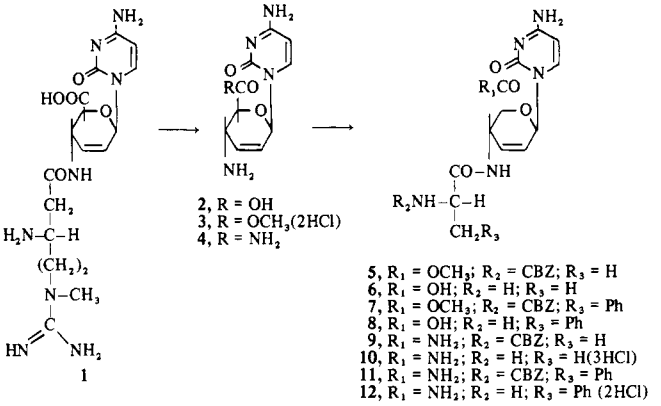
The antibiotic blasticidin S (**1**, Scheme I) has been studied

Scheme I



extensively (for a recent review of blasticidin S see ref 1) and been found to be a potent inhibitor of protein biosynthesis.[2] The present study was initiated to investigate possible changes in biological properties of this antibiotic by the replacement of the amino acid (blasticidic acid) portion of blasticidin S with several commonly occurring amino acids. These studies follow a general program for the synthesis of nucleoside peptides as potential medicinal agents.[3a-c]

The starting material, cytosinine (**2**, Scheme I), was prepared by alkaline hydrolysis of **1** according to the procedure of Yonehara and coworkers.[4] In our hands cytosinine was not absorbed on an Amberlite IRC-50 column; therefore, this procedure was modified by the absorption of cytosinine (**2**) on an IRA-410 (OH^- form) column and eluting the desired product in 46% yield with acetic acid. The preparation of 1-(4-amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranuronic acid methyl ester)cytosine (**3**) has also been previously described[5] by treatment of cytosinine with methanol containing 3% hydrogen chloride. It was found, however, that 10% methanolic hydrogen chloride was necessary for the complete conversion of **2** to **3**.

The action of dicyclohexylcarbodiimide (DCC)[6] on a mixture of *N*-carbobenzyloxy(CBZ)-L-alanine *N*-hydroxysuccinimide (NHS),[7,3c] and **3** provided 1-(4-[(*S*)-2'-CBZ-aminopropionylamido]-2,3,4-trideoxy-β-D-*erythro*-2-eno-