Table II—Analysis of Spiked Plasma Samples for Clobazam

Mixture	Concentration Added, ng/ml	Concentration Found[a], ng/ml	Accuracy, %
1 Clobazam	200	196.90 ± 2.30	1.55
2 Clobazam	400	409.75 ± 15.00	2.44
3 Clobazam	600	608.00 ± 28.00	1.33
4 Clobazam	1200	1211.75 ± 29.81	0.98
5 Clobazam	300	285.45 ± 6.43	4.85
N-Desmethylclobazam	300		
6 Clobazam	600	604.00 ± 23.00	0.67
N-Desmethylclobazam	600		
7 Clobazam	400	389.30 ± 2.20	2.68
Diazepam[b]	400		
8 Clobazam	400	373.35 ± 11.35	6.66
Chlordiazepoxide hydrochloride[c]	400		
9 Clobazam	400	368.85 ± 6.85	7.79
Oxazepam[d]	400		
10 Clobazam	400	398.00 ± 29.00	0.50
Clidinium bromide[e]	400		
11 Clobazam	400	376.00 ± 20.00	6.00
Aspirin	400		

[a] Based on duplicate samples. [b] Valium, Hoffmann-La Roche, Nutley, N.J. [c] Librium, Hoffmann-La Roche, Nutley, N.J. [d] Serax, Wyeth Laboratories, Radnor, Pa. [e] Quarzan, Hoffmann-La Roche, Nutley, N.J.

The other known metabolites of clobazam, in which hydroxy and hydroxy and methoxy substituents are present on the drug molecule, have not been shown in these laboratories to form fluorophores and should not interfere with the analysis of clobazam.

Initial extractions of clobazam from spiked plasma samples were performed using ether (9) (percent extraction = 85.58 ± 7.59%). Analysis of a 1-ml plasma sample spiked with 400 ng each of clobazam and *N*-desmethylclobazam with ether as the extraction solvent indicated that the clobazam concentration was 608.95 ± 19.05 ng/ml (n = 2). Assay of a plasma sample containing only the metabolite (400 ng/ml) gave 215.40 ± 0.40 ng/ml (n = 2), expressed as clobazam concentration. These data establish that the fluorophore formed from the desmethyl metabolite interferes with the clobazam analysis because of concurrent extraction of drug and metabolite from the plasma sample. Thus, partitioning studies were performed to find an organic solvent that would selectively extract clobazam from the desmethyl compound.

Chloroform, benzene, hexane, cyclohexane, petroleum ether, heptane, carbon tetrachloride, methylene chloride, and ethyl acetate were investigated. Hexane provided the best selectivity for the extraction of clobazam over that of the *N*-desmethyl metabolite at equimolar concentrations. The percent extraction of clobazam from plasma with hexane was 51.27 ± 2.00%.

Application of the fluorometric assay to the determination of spiked plasma samples containing clobazam and to plasma samples containing mixtures of clobazam and *N*-desmethylclobazam, some 1,4-benzodiazepines, or other drugs is presented in Table II. Determination of clobazam in these samples was achieved with accuracy in the range of 0.50–7.8%.

Figure 3 shows a plasma level–time profile after oral administration of a single 40-mg dose of clobazam to a healthy adult male. The data, obtained by using the described fluorometric procedure, indicate that the method should be applicable to studies where plasma levels of the intact drug are desired.

REFERENCES

(1) A. C. Parrot and I. Hindmarch, *IRCS Med. Sci. Clin. Pharmacol. Ther.*, **3**, 177 (1975).

(2) R. G. Borland and A. N. Nicholson, *Br. J. Clin. Pharmacol.*, **2**, 215 (1975).

(3) P. A. Berry, R. Burtles, D. Grubb, and M. V. Hoare, *ibid.*, **1**, 346 (1974).

(4) Clinical Investigator Literature, Hoechst-Roussel Pharmaceuticals, Somerville, N.J., May 1973.

(5) S. Fielding and I. Hoffman, *Br. J. Clin. Pharmacol.*, in press.

(6) S. Rossi, O. Pinola, and R. Maggi, *Chim. Ind.*, **51**, 479 (1969).

(7) P. Hajdu and D. Damm, *Arzneim.-Forsch.*, **26**, 2141 (1976).

(8) J. J. Vallner, T. E. Needham, I. L. Honigberg, J. T. Stewart, W. J. Brown, J. A. Kotzan, and H. W. Jun, *J. Clin. Pharmacol.*, **18**, 319 (1978).

(9) P. Hajdu, M. Uihlein, and D. Damm, *Arzneim.-Forsch.*, in press.

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Effect of *N*-Trifluoroacetyl Derivatives of Amino Acids and Amino Acid Analogs on Microbial Antitumor Screen

THEODORE T. OTANI ˣ and MARY R. BRILEY

Received August 11, 1978, from the *Laboratory of Pathophysiology, National Cancer Institute, Bethesda, MD 20014.* Accepted for publication September 28, 1978.

Abstract □ Eighteen trifluoroacetyl derivatives of amino acids and of amino acid analogs were prepared and tested for growth-inhibitory activity using a *Lactobacillus casei* system as a prescreen for antitumor activity. Of the compounds tested, the trifluoroacetyl derivatives of *o*-, *m*-, and *p*-fluorophenylalanine and of β-3-thienylalanine showed modest activity; trifluoroacetyl derivatives of phenylalanine and of β-2-thienylalanine showed marginal activity. The activity exhibited by the active trifluoroacetyl compounds was equal to that noted for most active chloroacetyl derivatives reported previously, as judged by comparison of their activity with that of chloroacetyl-*m*-fluorophenylalanine. No reversal of inhibition was noted when a representative of these inhibitors was challenged with a corresponding natural metabolite, both as a free amino acid and as a noninhibitory acylated compound.

Keyphrases □ Amino acids and analogs—*N*-trifluoroacetyl derivatives, effect on microbial antitumor screen □ Antitumor activity—18 trifluoroacetyl derivatives of amino acids and amino acid analogs, growth inhibitory activity using *Lactobacillus casei* system

An alteration in the biological behavior of certain amino acids, both the naturally occurring and the "unnatural," as measured by growth-inhibitory capacity in a microbial system, was noted upon acylation (1–3). The extent of such activity varied with the nature of the acyl group and the amino acid moiety (3). While *N*-acetyl and *N*-propionyl derivatives of these amino acids showed no appreciable growth-inhibitory activity, the *N*-chloroacetyl derivatives showed modest activity (3), particularly with those amino acids that are normally considered to be essential for

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Table I—Purity Data of *N*-Trifluoroacetyl Derivatives

N-Trifluoroacetyl Derivative	Melting Point		Optical Rotation $[\alpha]_D^{21-24}$		Van Slyke Nitrous Acid Determination[c], % amino nitrogen		Empirical Formula	Analysis, %[d]		
	Reported	Observed[a]	Reported	Observed[b]	Calc.	Obs.			Calc.	Obs.
L-Alanine	66–68°[e]	62–64°	−60.7° (W)	−54.6° (W)[f]	0	2	$C_5H_6F_3NO_3$	C	32.44	32.03
								H	3.27	3.38
								N	7.57	7.28
L-Aspartic acid	—	164–165°	—	−18.0° (W)	0	0	$C_6H_6F_3NO_5$	C	31.45	31.40
								H	2.64	2.74
								N	6.11	6.19
o-Fluoro-DL-phenylalanine	—	117–119°	—	—	0	7	$C_{11}H_9F_4NO_3$	C	47.32	47.40
								H	3.24	3.45
								N	5.03	5.23
m-Fluoro-DL-phenylalanine	—	117–119°	—	—	0	0	$C_{11}H_9F_4NO_3$	C	47.32	47.26
								H	3.24	3.24
								N	5.01	5.15
p-Fluoro-DL-phenylalanine	—	143°	—	—	0	1	$C_{11}H_9F_4NO_3$	C	47.32	47.64
								H	3.24	3.16
								N	5.01	5.01
Glycine	117–119°[e]	115–117°	—	—	0	0	$C_4H_4F_3NO_3$	C	28.08	28.08
								H	2.36	2.49
								N	8.19	8.32
β-Hydroxy-DL-norleucine AB[g]	—	179–180°	—	—	0	3	$C_8H_{12}F_3NO_4$	C	39.51	38.89
								H	4.98	5.21
								N	5.76	6.25
L-Isoleucine	—	71–72°	—	+4.2° (A)[h]	0	1	$C_8H_{12}F_3NO_3$	C	42.29	42.40
								H	5.32	5.40
								N	6.17	6.40
L-Leucine	72–75°[e]	76–77°	−39.5° (A)	−26.2° (A)[f]	0	0	$C_8H_{12}F_3NO_3$	C	42.29	42.37
								H	5.32	5.41
								N	6.17	6.16
DL-Methionine	95–96°[e]	91–96°	—	—	0	1	$C_7H_{10}F_3NO_3S$	C	34.28	34.32
								H	4.11	4.36
								N	5.71	5.74
DL-Norleucine	79–82.5°[e]	79–81°	—	—	0	1	$C_8H_{12}F_3NO_3$	C	42.29	42.50
								H	5.32	5.34
								N	6.17	6.10
DL-Norvaline	—	62°	—	—	0	0	$C_7H_{10}F_3NO_3$	C	39.44	39.46
								H	4.73	4.54
								N	6.57	6.85
L-Phenylalanine	120–122°[e]	120–121°	+17.4° (A)[e]	+15.8° (A)	0	5	$C_{11}H_{10}F_3NO_3$	C	50.58	49.95
								H	3.86	3.85
								N	5.36	5.46
D-Phenylalanine hemihydrate	120–122°[e]	120–122°	−17.2° (A)[e]	+14.8° (A)[i]	0	3	$C_{11}H_{12}F_3NO_{3\text{-}1/2}$	C	48.89	48.59
								H	4.10	3.86
								N	5.18	5.32
DL-Phenylalanine	127–128°[e]	127–128°	—	—	0	1	$C_{11}H_{10}F_3NO_3$	C	50.58	50.90
								H	3.86	4.01
								N	5.36	5.68
β-2-Thienyl-DL-alanine	—	128–130°	—	—	0	0	$C_9H_8F_3NO_3S$	C	40.45	40.51
								H	3.02	3.04
								N	5.24	5.46
β-3-Thienyl-DL-alanine hemihydrate	—	115–118°	—	—	0	0	$C_9H_9F_3NO_{3\text{-}1/2}S$	C	39.13	39.43
								H	3.28	3.06
								N	5.07	5.15
DL-Tyrosine dihydrate	—	194°	—	—	0	2	$C_{11}H_{14}F_3NO_6$	C	42.18	41.90
								H	4.50	3.92
								N	4.47	4.45
L-Tyrosine	192.5–193.5°	195–198°	+45.0° (WB)	+48.6° (WB)	0	1	$C_{11}H_{10}F_3NO_4$	C	47.66	47.55
								H	3.64	3.76
								N	5.05	5.12
L-Valine	86–88°	85–86°	−15.2° (W)	−15.2° (W)	0	1	$C_7H_{10}F_3NO_3$	C	39.44	39.47
								H	4.73	4.85
								N	6.57	6.82

[a] Melting points (uncorrected) were determined on a Fisher-Johns melting point block (Fisher Scientific Co., Silver Spring, Md.). [b] Optical rotation was determined on a Rudolf polarimeter (Rudolf Research, Fairfield, N.J.), model 80, sodium lamp, using 100-mm tubes (bore size of 3 mm) and a sample capacity of 0.7 ml. (W) = water, (A) = absolute ethanol, (WB) = water and equivalent amount of sodium hydroxide, c = 2. [c] Van Slyke nitrous acid determination of primary amino nitrogen (8) made on a 1-ml sample containing an equivalent of 0.3 mg of amino nitrogen (when hydrolyzed). [d] Elemental analyses were performed by the Microanalytical Laboratory, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Md. [e] *Cf.*, Ref. 10. [f] Not optically pure but predominantly the desired enantiomer. [g] Mixture of diastereomers A and B, A being the isomer moving faster and B being the isomer moving slower in a methyl ethyl ketone–1-butanol–ammonia–water system (2). [h] Probable racemization during preparation. [i] Rotation value, measured as hemihydrate.

growth and with those that are analogs of essential amino acids (3).

The ability of fluoro derivatives of metabolites to interfere and disrupt the normal metabolic pathway of analogous metabolites is well known. The fluoroacetates, the fluorocitrates as inhibitors of specific steps in the citric acid cycle, and the interference of nucleic acid metabolism by fluorouracil and its role in cancer chemotherapy are examples. The anomalous behavior of the trifluoroacetyl derivatives of certain amino acids, especially the straight-chain amino acids, as substrates of hog renal acylase where there was no optical specificity in the enzymatic hydrolysis was reported (4, 5).

In view of these observations, coupled with earlier

Table II—Effect of *N*-Trifluoroacetyl Derivatives of Amino Acids and Amino Acid Analogs on Growth of *L. casei* 7469 [a]

N-Trifluoroacetyl Derivative	Concentration Equivalent at 1 mg/ml, m*M*	Inhibition[b], % 0.1 mg/ml[c]	0.5 mg/ml[c]	1.0 mg/ml[c]
L-Alanine	5.40	0	0	+1[d]
L-Aspartic acid	4.36	3	4	2
o-Fluoro-DL-phenylalanine	3.58	3	9	17
m-Fluoro-DL-phenylalanine	3.58	4	10	22
p-Fluoro-DL-phenylalanine	3.58	4	9	19
Glycine	5.84	+4	+3	+3
β-Hydroxy-DL-norleucine AB	4.11	+2	5	+3
DL-Isoleucine	4.40	1	0	0
L-Leucine	4.40	1	1	2
DL-Methionine	4.08	+2	+1	3
DL-Norleucine	4.40	+5	+2	3
DL-Norvaline	4.69	+2	+1	0
DL-Phenylalanine	3.83	+1	8	12
β-2-Thienyl-DL-alanine	3.74	+3	3	13
β-3-Thienyl-DL-alanine hemihydrate	3.62	+2	8	23
DL-Tyrosine dihydrate	3.19	1	6	6
L-Tyrosine	3.61	+2	+1	5
L-Valine	4.69	3	5	4
Trifluoroacetic acid[e]	8.77	4	0	4

[a] For details, see text and Ref. 3. [b] Turbidity readings of the inoculated control tubes (containing no test compound) were 154–194 Klett units. At least three duplicate determinations were made for each compound. The duplicate values in each determination agreed within ±5 Klett units. The extent of variation in these experiments has been discussed (*cf.*, 3). The standard deviation of a compound showing a mean value of 20% inhibition, for example, was 2.7 and the standard error was 0.95. [c] Final concentration in assay system. [d] The + indicates stimulation of growth. [e] Free acid.

findings of the alteration of certain biological properties of amino acids upon chloroacetylation, the trifluoroacetyl derivatives of these amino acids were studied to note their action on the growth of a microbial system selected as a prescreen for antitumor action.

EXPERIMENTAL

The free amino acids, except for the β-hydroxynorleucines which were prepared in this laboratory (6), were obtained commercially and were recrystallized from water–ethanol before use. Their purity was checked by paper chromatography in at least four solvent systems (*cf.*, 2), by elemental analyses, and, where applicable, by optical rotation measurements. The *N*-trifluoroacetyl derivatives were prepared by acylation with freshly distilled trifluoroacetyl chloride[1] in freshly distilled trifluoroacetic acid[1] in an anhydrous system according to the method of Weygand and Geiger (7).

The purity of the products was checked by melting-point determination, elemental analysis, optical rotation measurements, and Van Slyke nitrous acid determination of primary amino nitrogen (8). The acyl compounds liberated no nitrogen upon treatment with nitrous acid, as determined by the Van Slyke nitrous acid determination of primary amino nitrogen, indicating that the compounds contained no appreciable amount of the unacylated amino acids. Chloroacetyl-*m*-fluoro-DL-phenylalanine was prepared in this laboratory (*cf.*, 3). Data on the purity of these compounds are summarized in Table I.

The preparation of the test solution for the microbiological assay and details of the assay were described previously (1). The microorganism, *Lactobacillus casei* 7469[2], was carried on agar[3] as stab cultures and subcultured in broth[3]. The assay medium, as described previously (1), was a riboflavin-supplemented riboflavin assay medium. Growth was determined turbidimetrically after 19 hr as described (1). Inhibition is expressed as the percentage of growth and is 100 times the ratio of the difference of the turbidity readings of the control and experimental tubes to the turbidity reading of the control tube (*cf.*, 1).

Special care was taken to check the final pH of the assay system, the test compounds being neutralized to pH 6 before being added to the test system. The pH of the assay system containing the maximum quantity of the test compound was within 0.05 pH unit of the control system, which contained water in place of the test solution.

In studies where the inhibitors were challenged by the natural metabolite or by the acyl derivative of the natural compounds, the medium was two times the normal concentration so that the final concentration, after the addition of the inhibitor, the challenging compound, and an appropriate volume of water, was equal to that of the normal assay system. Both the inhibitor and the challenging compounds were neutralized with appropriate volumes of 0.1 *N* NaOH to bring the pH of the solutions to 6. The final pH of the system was determined, as before, in a tube containing equivalent amounts of the test solution, the reversing agent, the medium, and water as in the corresponding experimental tubes. The final pH of these tubes was also within 0.05 pH unit of the control tubes.

[1] Eastman Organic Chemicals, Rochester, N.Y.
[2] American Type Culture Collection, Rockville, Md.
[3] Micro assay culture agar (Difco B319) and micro inoculum broth (Difco B320), Difco Laboratories, Detroit, Mich.

Table III—Comparison of the Effect of Equimolar Concentrations of *N*-Acyl Derivatives of Amino Acids and Amino Acid Analogs on Growth of *L. casei* 7469 [a]

N-Acyl Amino Acid	Inhibition[b], %
N-Trifluoroacetyl-*o*-fluoro-DL-phenylalanine	18
N-Trifluoroacetyl-*m*-fluoro-DL-phenylalanine	20
N-Trifluoroacetyl-*p*-fluoro-DL-phenylalanine	23
N-Trifluoroacetyl-DL-phenylalanine	16
N-Trifluoroacetyl-L-phenylalanine	23
N-Trifluoroacetyl-D-phenylalanine	20
N-Trifluoroacetyl-β-2-thienyl-DL-alanine	24
N-Trifluoroacetyl-β-3-thienyl-DL-alanine	26
N-Chloroacetyl-*m*-fluoro-DL-phenylalanine	23
Trifluoroacetic acid[c]	1

[a] Maximum growth in inoculated control tube containing no test compound, measured turbidimetrically, was 166 Klett units. For explanation of extent of variations of values, see footnote of Table II. [b] Concentration was 4.47 μmoles/ml and was the final concentration in the assay system. For details of assay, see text. [c] Free acid.

RESULTS AND DISCUSSION

The trifluoroacetyl derivatives of 18 amino acids and amino acid analogs were prepared and tested for growth-inhibitory activity using a *L. casei* system as a prescreen for possible antitumor activity (9). Such a system was selected because of the rapidity with which the test could be made with a minimal amount of the test compound. Of the compounds tested, four showed moderate, but distinct, activity (Table II): the *N*-trifluoroacetyl derivatives of *o*-fluoro-DL-phenylalanine, *m*-fluoro-DL-phenylalanine, *p*-fluoro-DL-phenylalanine, and β-3-thienyl-DL-alanine. The trifluoroacetyl derivatives of β-2-thienyl-DL-alanine and of DL-phenylalanine showed marginal activity at 1 mg/ml final concentration.

Because of the uncertainty of the Klett readings of about 5%, the inhibition of 1–6% shown by some compounds (Table II) was considered to be insignificant. When the activity of these compounds was tested on an equimolar basis (Table III), the growth-inhibitory activity of these compounds was in the same range. The molar concentration selected was equivalent to 1 mg of *N*-chloroacetyl-β-hydroxy-D-norleucine B/ml (*cf.*, 3). The activity of the trifluoroacetylfluorophenylalanines was about that of the chloroacetyl counterparts (Table III) (*cf.*, 3). Unlike the chloroacetyl derivatives, which showed activity with both the essential amino acids and with analogs of essential amino acids, the activity of the trifluoroacetyl derivatives was confined to the analogs of phenylalanine.

Where the pure enantiomers were tested, neither optical isomer showed greater or lesser activity than the racemic mixture (Table III). Hence, the inhibition at the level of the inhibitor does not appear to involve an enzymatic reaction. The nonspecificity probably is not due to the trifluoroacetyl radical, as it is in the nonspecific hydrolysis of trifluoroacetyl amino acids by hog renal acylase (4, 5), since similar nonspecificity in growth inhibition was observed with the chloroacetyl compounds (*cf.*, 3). The participation of a racemase, however, is not ruled out.

The trifluoroacetyl derivative of a natural amino acid, phenylalanine, showed growth-inhibitory activity, suggesting that the trifluoroacetyl moiety had some function in this activity. Since free *o*-fluorophenylalanine showed little activity in the system used here, the activity probably is not due to the hydrolytic release of the free *o*-fluorophenylalanine (*cf.*, 3). Furthermore, it is unlikely that the hydrolytic release of free trifluoroacetic acid could be a factor since free trifluoroacetic acid present in equimolar quantity exhibited no inhibition (Table III).

Earlier experiments (3) showed that there was no appreciable hydrolysis of the test compounds during incubation with chloroacetyl-DL-norleucine. Apparently, the activity of the trifluoroacetyl derivatives

Table IV—Effect of Phenylalanine and *N*-Acetylphenylalanine on the Inhibition [a] (Percent) by Trifluoroacetyl-*p*-fluoro-DL-phenylalanine

Reversing Agent [b]	*N*-Trifluoroacetyl-*p*-fluoro-DL-phenylalanine			
	0	1.43 m*M*	3.58 m*M*	7.16 m*M*
None	0	6	21	40
L-Phenylalanine (6.05 m*M*)	+4	9	22	41
N-Acetyl-L-phenylalanine (4.82 m*M*)	8	12	25	47

[a] Maximum growth of controls containing no inhibitor was 141 Klett units. [b] Final concentration of 1 mg/ml. Figures in parentheses are molar equivalents.

of these amino acids and amino acid analogs is independent of the activity of the free parent amino acid. While free *p*-fluoro-DL-phenylalanine and β-3-thienyl-DL-alanine showed considerable activity, their corresponding trifluoroacetyl derivatives did not show as much activity; *o*-fluro-DL-phenylalanine, *m*-fluoro-DL-phenylalanine, and β-2-thienyl-DL-alanine, which showed no activity as free amino acids, showed considerable activity as the trifluoroacetyl derivatives (*cf.*, 3).

It is difficult to propose any mechanism of such inhibition. Interference with phenylalanine metabolism may be involved, since all trifluoroacetyl compounds showing activity are analogs of phenylalanine.

A study was made to note if the inhibition could be overcome or reversed by the addition of suitable quantities of the natural metabolite. In a series of experiments, the active trifluoroacetyl derivative was challenged by various levels of a natural metabolite and of a noninhibitory acyl derivative. No significant degree of reversal was observed with either phenylalanine or acetylphenylalanine (Table IV). This observation, however, does not rule out the involvement of phenylalanine because the area of inhibitory action could be beyond the point of participation of these compounds as free amino acids or as simple acyl derivatives in its metabolic pathway. More sophisticated studies are required to learn the mechanism of inhibition.

In view of the involvement of the trifluoroacetyl derivatives of only phenylalanine-related compounds in this screening system, consideration of these compounds as potential inhibitors of melanomas, wherein the metabolism of phenylalanine is believed to be intimately associated, may be of value. These compounds are now being prepared in larger quantities in preparation for testing in mammalian systems, especially in melanomas.

REFERENCES

(1) T. T. Otani, *Cancer Chemother. Rep.*, **38**, 25 (1964).
(2) T. T. Otani and M. R. Briley, *J. Pharm. Sci.*, **65**, 534 (1976).
(3) *Ibid.*, **67**, 520 (1978).
(4) W. S. Fones and M. Lee, *J. Biol. Chem.*, **201**, 847 (1953).
(5) *Ibid.*, **210**, 227 (1954).
(6) T. T. Otani and M. Winitz, *Arch. Biochem. Biophys.*, **102**, 464 (1963).
(7) F. Weygand and R. Geiger, *Chem. Ber.*, **89**, 647 (1956).
(8) D. D. Van Slyke, *J. Biol. Chem.*, **83**, 425 (1929).
(9) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Guirard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N.Y. Acad. Sci.*, **76**, 413 (1958).
(10) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Wiley, New York, N.Y., 1961.

Stability-Indicating Assay for Vidarabine

WEN-HAI HONG ˣ and DALE H. SZULCZEWSKI

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Abstract □ A physicochemical procedure for the analysis of vidarabine in aqueous parenteral formulations was needed to assure potency and to define stability. Concurrent with the development of this method, its decomposition products and route in aqueous solution were determined. A quantitative procedure was developed to determine intact drug in the presence of decomposition products, and the results obtained were validated by microbial assay. Spectral (UV and polarimetric) and TLC evidence indicated that, in aqueous solution, hydrolysis without racemization occurs, yielding adenine and arabinose. The sensitivity of the method to decomposition is improved by ion-exchange separation of adenine and drug before UV measurement. Analysis of partially decomposed solutions of the drug by both ion-exchange and microbiological methods gave comparable results.

Keyphrases □ Vidarabine—stability-indicating assay, analysis in aqueous parenteral formulations, behavior in aqueous solutions, analysis in the presence of decomposition products □ Parenterals—analysis of vidarabine stability □ Stability—vidarabine in aqueous parenteral formulations

Vidarabine (9-β-D-arabinofuranosyladenine, Vira-A or Ara-A) is an antiviral agent with marked activity *in vitro* and *in vivo* against a wide spectra of DNA viruses (1–8). It also inhibits DNA synthesis in bacteria (9), in mouse fibroblasts in cell culture (10), and in experimental tumors in mice (11, 12).

Since the dosage form being developed is essentially aqueous, information pertinent to the drug's chemistry in an aqueous system is of value for predicting characteristics of the finished formulation and designing a stability-indicating assay. This paper presents a physicochemical procedure for the analysis of vidarabine in aqueous parenteral suspension needed to assure potency and define stability.

EXPERIMENTAL

Chemicals and Reagents—All chemicals used in the preparation of 0.2 *M* borate buffer and 0.1 *M* phosphate buffer were reagent grade and were used without further purification. The hydrochloric acid solutions were prepared from a prestandardized volumetric solution[1]. Vidarabine was an experimental batch[2]; adenine[3] was obtained from a commercial source.

Instrumentation—A direct-reading digital pH meter[4] was used. UV spectra were obtained using a recording UV spectrophotometer[5]. The specific rotation was determined using a polarimeter[6].

Degradation of Vidarabine in Acidic Solution—A 1% solution of vidarabine was prepared in 0.1 *N* HCl, and 2-ml aliquots were sealed in glass ampuls. The ampuls were heated at 100°, withdrawn periodically, and cooled to room temperature; then the specific rotation was measured

[1] Acculute, Anachemia Chemicals.
[2] Rx X lot 41020, Warner-Lambert/Parke-Davis, Detroit, MI 48232.
[3] Lot E-1528, Mann Research Laboratories, New York, N.Y.
[4] Sargent, model DR.
[5] Cary model 11 or 14.
[6] Perkin-Elmer model 141.

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