under nitrogen with diborane-d_6 gas generated from 1 g. (55 mmoles) of lithium aluminum deuteride in 50 ml. of ether and 4.8 g. of freshly distilled boron trifluoride etherate in 10 ml. of dry ether. After the generation of the diborane-d_6 was complete, the THF solution was stirred at room temperature under nitrogen for 1 hr. The excess diborane-d_6 was destroyed by the addition of ice chips to the reaction mixture after which it was diluted to 100 ml. with water. The THF was removed under reduced pressure whereupon the product crystallized. The isolated product was washed thoroughly with water, air dried, and recrystallized from ethanol giving 1.2 g. of material, m.p. 196.5–199.8°. N.m.r.[16] confirmed the structure and the absence of hydrogen at the α-ethyl position: 131 (s) (CH_3), 168 (singlet) (β-CH_2), 3821 s) (Me_2ArH_2), and 470 c.p.s. (s) (I_2ArH_2).

(16) N.m.r. spectrum was determined on a 5–10% solution in CDCl at 60 Mc. with a Varian A-60 spectrometer, employing tetramethylsilane as an internal reference. Frequencies are reported in cycles per second relative to tetramethylsilane as 0 c.p.s.

3,5-Diiodo-4-(3,5-dimethyl-4-hydroxyphenoxy)phenethyl Alcohol Diacetate (2).—A 1.0-g. sample of the thyroethanol **1** was dissolved in 10 ml. of pyridine and 1 ml. of acetic anhydride and allowed to stand at room temperature overnight. The reaction mixture was poured into 100 ml. of water and allowed to stand until an amorphous solid formed. The product (1.1 g.) was filtered, washed with water, and dried *in vacuo*, 60°. Recrystallization from Skellysolve B gave 930 mg. of a substance, m.p. 137.5–139.0°. A sample was recrystallized for analysis[17]: m.p. 139.5–140.5°; ν_{max} 1753, 1740, 1595, and 1537 cm.$^{-1}$; λ_{max}^{EtOH} 223 mμ (ε 35,850), 272 (2650), and 279 sh (2350).

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The Synthesis of Tenuazonic and Congeneric Tetramic Acids

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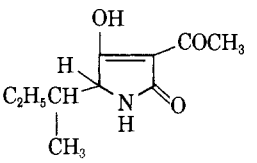
The structure of tenuazonic acid as 3-acetyl-5-*sec*-butyltetramic acid has been verified by total synthesis from L-isoleucine and diketene. A new series of crystalline-N-acetoacetylamino acids is described. For the purpose of correlating structure *vs.* biological activity, a series of tetramic acids having various substituents at the 1-, 3-, and 5-positions has been synthesized. An enhancement of the *in vitro* antibacterial activity of N-substituted tetramates has been confirmed.

Tenuazonic acid and several related tetramates have been synthesized from amino acids and diketene for study in human tumor and other biological systems.

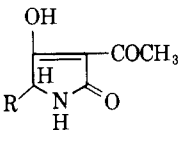
Hadacidin[2] was recently discovered as a new growth-inhibitory substance in human tumor systems, and further research led to the discovery of another crystalline human antitumor substance which was identified[3] as the known tenuazonic acid (I).[4,5] Recently Miller, *et al.*,[6] reported that synthetic tenuazonic acid showed antiviral activity at rather high dose levels but that it was inactive against bacteria and yeast. We were interested in varying the substituents on the tetramic acid skeleton of tenuazonic acid in order to make possible a study of the effect of these changes on their biological activities.

Our synthesis of tenuazonic acid differs slightly from that of Lacey.[7] In this process, we were able to isolate and characterize as crystalline compounds a new series of N-acetoacetylamino acids which are given in Table I. This was the basis for the synthesis of the substituted tetramic acids which are described in Table II, in which variations have been made in the alkyl group at position 5 and substitutions have been made at position 1 (N). Several 3-acetyltetramic acids having the following groups in position 5, benzyl, isopropyl, methylthioethyl, ethyl, phenyl, dimethyl, *n*-butyl, methyl, hydrogen and isobutyl, have already been described.[8] There was no N-substitution on these compounds.

3-Acetyl and 3-acetyl-5-methyltetramic acids have been synthesized by Lacey[7] who allowed the methyl ester of glycine and DL-alanine to react with diketene, and then carried out the cyclization to give II and III.



I



II, R = H
III, R = CH_3

Since Lacey did not start with optically active amino acids, his products could not reveal the stereochemistry of C-5. The product which we synthesized by these reactions and L-isoleucine was identical in all respects with tenuazonic acid (I).

Table III lists a few tetramic acids in which the acetyl group at position 3 has been replaced by other carbonyl functions.

The tumor-inhibiting properties of tenuazonic acid against a human tumor growing on chick embryos are described by Gitterman, *et al.*[9] The activities of the substituted tetramates in this system are described[10]

(1) To whom inquiries should be addressed.

(2) E. A. Kaczka, C. O. Gitterman, E. L. Dulaney, and K. Folkers, *Biochemistry*, **1**, 340 (1962).

(3) E. A. Kaczka, C. O. Gitterman, E. L. Dulaney, M. C. Smith, D. Hendlin, H. B. Woodruff, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **14**, 54 (1964).

(4) C. E. Stickings, *Biochem. J.*, **72**, 332 (1959).

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TABLE I

ACETOACETYLAMINO ACIDS

RCHCOOH

R'NCOCH$_2$COCH$_3$

No.	Amino acid	R'	Formula	Mol. wt.	M.p., °C.	Calcd., %			Found, %		
						C	H	N	C	H	N
1	Isoleucine	H	$C_{10}H_{17}NO_4$	215.24	120–121	55.80	7.91	6.50	55.87	7.91	6.50
2	DBED salt[a] N-Me isoleucine	CH_3	$C_{38}H_{68}N_4O_8$	698.91	...	65.30	8.36	8.02	65.52	8.14	7.94
3	N-Benzylvaline	Benzyl	$C_{16}H_{21}NO_4$	291.36	...	65.95	7.27	4.81	65.67	7.01	5.05
4	3,3-Diethylalanine	Benzyl	$C_{18}H_{25}NO_4$	319.39	129–131	67.68	7.89	4.38	67.78	7.96	4.11
5	Proline		$C_9H_{13}NO_4$	199.20	120–123	54.26	6.58	7.03	53.99	6.23	7.17
6	Phenylalanine	H	$C_{13}H_{15}NO_4$	249.19	112–114	62.65	6.07	5.62	61.52	5.92	5.02
7	L-Valine	H	$C_7H_{15}NO_4$	201.15	124–125	53.73	7.51	6.96	53.96	7.40	6.97
8	DL-Trytophane	H	$C_{15}H_{16}N_2O_4$	288.22	163–166	62.50	5.55	9.72	62.96	5.42	8.07
9	Methionine	H	$C_9H_{15}NO_4S$	232.28	55–58	46.33	6.48	6.01	46.30	6.18	6.47
10	L-Aminobutyric	H	$C_8H_{13}NO_4$	187.19	75–77	51.33	7.00	7.48	51.69	6.78	7.57
11	L-Aminophenylacetic	H	$C_{12}H_{13}NO_4$	235.16	138–142	61.29	5.57	5.96	61.49	5.76	5.82
12	L-Aminoisobutyric	H	$C_8H_{13}NO_4$	187.19	145–148	51.33	7.00	7.48	51.51	6.81	7.80
13	L-Amino-*n*-caproic	H	$C_{10}H_{17}NO_4$	215.24	98–101	55.80	7.91	6.50	56.42	7.97	6.19
14	Alanine	H	$C_7H_{11}NO_4$	173.17		48.50	6.41	8.09	48.46	6.35	8.16
15	Glycine	H	$C_6H_9NO_4$	159.14	107–109	45.28	5.70	8.80	45.22	5.46	9.17
16	α-Amino-*n*-heptanoic	H	$C_{11}H_{19}NO_4$	229.27		57.69	8.35	6.11	57.77	8.12	6.38
17	1-Amino-1-cyclopentylcarboxylic	H	$C_{10}H_{15}NO_4$	213.23	157–160	56.33	7.09	6.57	56.36	6.80	6.31
18	Leucine	H	$C_{10}H_{17}NO_4$	215.24	124–126	55.75	7.96	6.50	55.63	7.81	6.53
19	1-Amino-3-ethylpentanoic	H	$C_{11}H_{19}NO_4$	229.27	115–117	57.69	8.35	6.11	57.92	8.30	5.99
20	Pseudoleucine	H	$C_{10}H_{17}NO_4$	215.24	148–150	55.75	7.96	6.50	55.90	7.70	6.30
21	O-Methylthreonine	H	$C_9H_{15}NO_5$	217.22	107–108	49.76	6.90		46.88	6.25	
22	Isoleucine	Benzyl	$C_{17}H_{23}NO_4$	305.36		66.86	7.59	4.58	67.13	7.73	4.33
23	*dl-allo*-Isoleucine	H	$C_{10}H_{17}NO_4$	215.24		55.75	7.96	6.50	55.92	8.06	6.64
24	D-Isoleucine	H	$C_{10}H_{17}NO_4$	215.24	112–114	55.75	7.96	6.50	56.07	7.92	6.49
25	α-Methylisoleucine	H	$C_{11}H_{19}NO_4$	229.27	139–141	57.69	8.35	6.11	57.60	8.62	6.26
26	5,5,5-Trifluoronorvaline	H	$C_9H_{12}F_3NO_4$	255.19	129–130	42.36	4.74	5.49	42.64	4.85	5.41
28	α-Amino-*n*-heptanoic	Benzyl	$C_{18}H_{25}NO_4$	319.41	100–101	67.68	7.89	4.39	67.18	7.89	4.37

[a] From dibenzylethylenediamine.

in an accompanying paper. His report shows that this activity is quite specific for tenuazonic acid. Gitterman also discovered that tenuazonic acid and other tetramic acids have a low order of antibacterial activity by the agar plate method and that N-substituted tetramates have a greatly enhanced antibacterial activity *in vitro*. This enhanced antibiotic activity seems to be fairly general for N-substituted tetramates.

This work was confirmed by the results tabulated in Table IV. We wish to thank Dr. E. O. Stapley and Mrs. Ann Germain for these assays (Table IV) which were carried out by the general method as published.[11] The compounds were assayed as aqueous solutions by agar plate diffusion employing soaked 6.35-mm. disks or droplets of 0.02 ml. at a concentration of 2 mg./ml. Thin plates using 5 ml. of nutrient agar plus 0.2% of yeast extract/Petri dish were seeded with appropriate microorganisms and incubated overnight at 25°.

It will be seen from Table IV that most of the tetramic acids unsubstituted on the N have a low order of antibacterial activity (Table II, **1, 17, 20, 23,** and **26,** and Table III, **1** and **2**). Substitutions of the N with a benzyl group generally broadens the antibiotic spectrum and enhances their activities (Table II, **3, 4, 22, 27,** and **28**, and Table III, **4**). Unlike the egg tumor test, the antibiotic activity is not restricted to the 5-*sec*-butyltetramates.

Dr. Betty M. Frost and Miss M. E. Valiant of the Merck Institute have found that these N-substituted compounds were toxic to mice at single intraperitoneal doses of 2.5 mg. and that they did not show any *in vivo* antibacterial activity up to this dosage level.

(11) E. O. Stapley, *Appl. Microbiol.* **6**, 392 (1958).

Experimental

Sodium Tenuazonate.—Acetoacetyl-L-isoleucine was prepared by the direct acetoacetylation of the sodium salt of L-isoleucine in aqueous solution by dropwise addition of diketene; acidification, extraction with ether, and recrystallization from ether yielded the product, m.p. 120–121°, yield 76%. For the analysis, see Table I, **1**. The ultraviolet absorption spectrum of the free acid exhibited a shoulder at 2480 Å. in methanol. A peak at 2735 Å. was exhibited in alkaline solution; $[\alpha]_{546}$ −122° (*c* 2.0, methanol). The methyl ester of acetoacetyl-L-isoleucine was prepared in ether solution by adding an ether solution of diazomethane to the acid; $[\alpha]_{546}$ +7° (*c* 2.0, methanol). This ester was condensed[7] to give the sodium salt of tenuazonic acid in 71% yield; $[\alpha]_{546}$ −96.7° (*c* 2.0, methanol). The ultraviolet absorption spectrum showed bands at 2800 Å. ($E^{1\%}_{1\,cm}$ 573) and 2410 Å. ($E^{1\%}_{1\,cm}$ 400).

The sodium salt of tenuazonic acid (5 g.) was converted[4] to the copper salt with $Cu(OAc)_2$. After four recrystallizations from methanol and water, the optical rotation had changed from $[\alpha]_{546}$ −76.5 to −122° (*c* 2.0, methanol); yield 1.2 g.; lit.[4] $[\alpha]_{546}$ −124°.

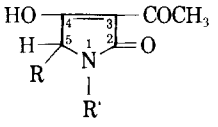
The copper salt was decomposed with H_2S, and the solution was neutralized with $NaHCO_3$. The freeze-dried sodium tenuazonate weighed 1.07 g. (85%), $[\alpha]_{546}$ −119.0° (*c* 2.0, methanol), $[\alpha]_{578}$ −101.5° (*c* 2.0, methanol). The spectrum showed bands at 2800 Å. ($E^{1\%}_{1\,cm}$ 684) and 2400 Å. ($E^{1\%}_{1\,cm}$ 548) for the sodium salt and 2760 Å. ($E^{1\%}_{1\,cm}$ 645) for the free acid. For the analysis, see Table II, **1.**

The following synthesis of 1-benzyl-3-acetyl-5-(3-amyl)tetramic acid (II, 4) is typical for the N-benzyl analogs described in Table II.

3-(3-Amyl)hydantoin.—A mixture of 50 g. of 2-ethylbutyraldehyde with 50 g. of NaCN, 50 g. of $NaHSO_3$, and 200 g. of ammonium carbonate in 150 ml. of methanol and 150 ml. of water were heated overnight at 60°. The reaction mixture was diluted with water, filtered, and dried to yield 52 g. of the hydantoin, m.p. 180–181°.

TABLE II

1,5-DISUBSTITUTED 3-ACETYLTETRAMATES

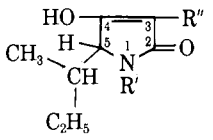


No.[a]	R	R'	Formula	Mol. wt.	M.p., °C.	Calcd. % C	Calcd. % H	Calcd. % N	Found % C	Found % H	Found % N	λ_{max}^{MeOH}, A. ($E_{1cm}^{1\%}$) pH 7	pH 11	pH 1
1	*sec*-Butyl	H	$C_{10}H_{14}NNaO_3$	219.21		54.78	6.44	6.39	55.10	6.40	6.00	2800 (684)	2400 (548)	2760 (645)
2	*sec*-Butyl	CH_3	$C_{11}H_{16}KNO_3$	249.36		53.03	6.46	5.62	53.21	6.35	5.43	2465 (450)	2860 (486)	2815 (450)
3	Isopropyl	Benzyl	$C_{16}H_{18}K_{1.5}NO_3$	331.		58.00	5.44	4.23	58.73	5.46	4.19	2470	2840	2820
4	3′-Amyl	Benzyl	$C_{18}H_{22}KNO_3 \cdot H_2O$	357.48		60.47	6.77		60.68	6.50		2480	2850	2830
5	1,5-Trimethylene		$C_{24}H_{42}N_4O_6$ DBED salt[b]	602.74	151–153	67.75	7.03	9.30	67.43	7.23	9.34	2490 (235)	2800 (232)	2780 (218)
16	*n*-Amyl	H	$C_{11}H_{17}NO_3$	211.25	98–99	62.54	8.11	6.63	62.65	7.90	6.87	2380	2780	2760
17	Spirotetramethylene	H	$C_{10}H_{13}NO_3Na \cdot 1.5H_2O$	246.22	162–164	49.25	6.15	5.74	49.85	5.83	5.64	2410	2800	2770
19	3′-Amyl	H	$C_{11}H_{17}NO_3$	211.25	78	62.54	8.11	6.63	62.24	7.82	6.76	2400 (537)	2775 (753)	2760 (624)
20	*t*-Butyl	H	$C_{10}H_{15}NO_3$	197.24	62.6	60.89	7.67	7.11	61.09	7.87	7.43	2425 (605)	2850 (740)	2780
21	Ethylidene	H	$C_5H_5NO_3$	167.16	198–201	57.48	5.43	8.38	57.27	5.36	8.46	2320 (631)	2630 (1349)	2640
22	*sec*-Butyl	Benzyl	$C_{17}H_{21}NO_3$	287.35	56–57	71.12	7.32	4.80	71.06	7.25	4.73			
			$C_{50}H_{62}N_4O_6$ DBED salt[b]	815.08		73.68	7.67	6.98	74.23	7.78	6.98	2480	2850	2830
23	*allo*-*sec*-butyl	H	$C_{36}H_{50}N_4O_6 \cdot H_2O$ DBED salt[c]	652.8	137–141°	66.21	8.03	8.58	66.07	8.23	8.62	2410	2800	2770
24	D-*sec*-butyl	H	$C_{36}H_{50}N_4O_6 \cdot 1.5H_2O$[d]	661.82	135–140	65.34	8.07	8.48	64.93 65.21	7.78 7.53	8.48	2410	2801	2701
25	5-Methyl-5-*sec*-butyl	H	$C_{11}H_{17}NO_3$	211.25	182–230 sub.	62.54	8.11	6.63	62.02	8.07	7.33	2400 (385)	2780 (515)	2750
26	3′,3′,3′-Trifluoro-*n*-propyl	H	$C_9H_{10}NO_3F_3$	237.18	120–121	45.57	4.25	5.91	45.55	4.43	5.97	2400 (462)	2775 (575)	2750
27	3′,3′,3′-Trifluoro-*n*-propyl	Benzyl	$C_{16}H_{16}NO_3F_3$	327.30	73–76	58.71	4.92	4.28	58.48	5.06	4.31	2810 (423)	2450 (464)	2810 (404)
28	*n*-Amyl	Benzyl	$C_{18}H_{22}NO_3Na$[e]	301.37		66.86	6.86	4.33	64.55 64.36	7.00 6.63	4.25	2470	2840	2820
29	Phenyl	Benzyl	$C_{19}H_{17}NO_3$	307.33		74.25	5.58	4.56	74.40	5.67	4.18	2490	2860	2830
30	*n*-Hexyl	H	$C_{12}H_{19}NO_3$	225.28		63.97	8.48	6.22	64.09	8.21	5.88	2400 (446)	2790 (567)	
31	Phenyl	Phenyl	$C_{18}H_{15}NO_3$	293.33		73.70	5.16	4.77	74.19	5.40	4.83			

[a] Numbers refer to corresponding precursors listed in Table I. [b] DBED salt is prepared by treatment with dibenzylethylenediamine which neutralizes 2 moles of tetramic acid. [c] No attempt was made to obtain optically pure material. This compound should have the same configuration as isotetramazonic acid; see ref. 4. [d] This compound did not crystallize as the free acid, the DBED salt, or sodium salt. Analyses were performed on amorphous vacuum-dried material. The λ_{max} showed the characteristic shift from two maxima in basic solution to one peak in acid solution.

TABLE III

1,3-DISUBSTITUTED 5-*sec*-BUTYLTETRAMATES



No.	R¹	R²	Formula	M.p., °C.	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
1	H	$CONH_2$	$C_9H_{14}N_2O_3$	164–165	54.52	7.12	14.13	54.52	6.83	14.17
2	H	CN	$C_9H_{12}N_2O_2$	212–214	60.23	6.94	15.54	59.98	6.71	15.57
3	Benzyl	$CONH_2$	$C_{16}H_{20}N_2O_3$[a]	145–148	66.64	6.95	9.72	66.22	6.73	9.68
4	Benzyl	COOEt	$C_{18}H_{22}NO_4Cu_{0.5}$		60.53	6.21	3.92	60.48	6.17	3.67
5	H	H	$C_8H_{13}NO_2$[b]	108–112	61.89	8.43	9.03	61.22	8.13	9.02

[a] $\lambda_{max}^{CH_3OH}$ 2330 Å. ($E_{1\ cm}^{1\%}$ 451), 2700 Å. ($E_{1\ cm}^{1\%}$ 327). [b] $\lambda_{max}^{CH_3OH}$ 2310 and 2620 Å., $\lambda_{max}^{pH\ 1}$ 2390 Å.

TABLE IV

ANTIBACTERIAL ACTIVITY OF TETRAMIC ACIDS

Microorganism	Merck culture no.	Zones of inhibition (diameters in mm.)[a] II[b] 1	2	3	4	17	20	22	23	26	27	28	31	III[b] 1	2	4
Escherichia coli	M.B. 60	11	00	07	7	10	8	00	12	00	00	00	07	07	07	00
Bacillus sp.	M.B. 633	11H	23	26	28	15	15	27	13	10H	26	31	21	07	28	20
Proteus vulgaris	M.B. 1012	00	00	07	07	15	00	00	00	00	16H	00	07	07	07	00
Pseudomonas aeruginosa	M.B. 979	00	00	07	07	00	00	00	00	00	00	00	07	07	07	00
Serratia marcescens	M.B. 252	00	00	07	07	12	00	00	00	00	00	00	07	07	07	00
Staphylococcus aureus	M.B. 108	13	13H	25	28	14H	10	28	09	00	24	34	19	07	25	17
Bacillus subtilis	M.B. 964	11	16	28	28	16	11	29	12	00	28	35	22	07	07	19
Sarcina lutea	M.B. 1101	00	11	19	24	00	00	23	00	00	29	29	20	07	07	15
Staphlococcus aureus (resistant to Streptomycin)	M.B. 698	10H	00	22	23	00	07	28	10	00	22	32	14	07	07	13
Streptococcus faecalis	M.B. 753	00	13	15	17	00	00	20	00	00	22H	22	10	07	07	00
Alcaligenes faecalis	M.B. 10	10	12	12	09	00	00	15	12	00	13H	10	07	07	07	00
Brucella branchiseptica	M.B. 965	08	00	11	08	00	00	18	11	00	20	11H	07	07	07	14
Salmonella gallinarum	M.B. 1287	11	00	07	07	11H	10H	00	10H	00	11H	10	07	07	07	00
Vibrio percolans	M.B. 1272	10	11H	10	10	00	00	15	10	00	16	21	11	13	07	13
Xanthomonas vesicatoria	M.B. 815	10	00	07	07	11H	09H	00	09	00	10H	00	07	07	07	00

[a] H = hazy. [b] The numbers refer to compounds listed in Tables II and III.

β,β-Diethylalanine.—Thirty grams of 3-(3-amyl)hydantoin was refluxed overnight in 125 ml. of 48% HBr. The reaction mixture was cooled and filtered, and the precipitate was neutralized in acetone solution with NH_4OH. The first crop was mainly NH_4Br. The amino acid was obtained from the second crop in a yield of 12 g. It was recrystallized from water solution by slowly adding 1 vol. of acetone.

Anal. Calcd. for $C_7H_{15}NO_2 \cdot H_2O$: C, 56.82; H, 10.55; N, 8.62. Found: C, 56.89; H, 10.14; N, 8.88.

N-Benzyl-β,β-diethylalanine.—A mixture of 5.5 g. of diethylalanine, 3.6 g. of benzaldehyde, and 1.9 g. (1 equiv.) of KOH in 150 ml. of methanol was hydrogenated with palladium on carbon. After removal of the catalyst, the solution was neutralized with 1 equiv. of concentrated HCl (2.85 ml.) and filtered quickly to remove KCl. The N-benzyldiethylalanine crystallized at once from the filtrate and was filtered and washed with water and dried; yield 6.86 g. (85%), m.p. 240–242°.

Anal. Calcd. for $C_{14}H_{21}NO_2$: C, 71.45; H, 8.99; N, 5.95. Found: C, 71.76; H, 11.44 (?); N, 5.32.

N-Acetoacetyl-N-benzyl-β,β-diethylalanine.—A solution of 2.35 g. (0.01 mole) of N-benzyldiethylalanine and 0.01 mole of NaOH in 25 ml. of water was cooled in an ice bath while 1.8 g. of diketene was added with agitations. After 1 hr. the solution was extracted with ether, and the crystalline acetoacetyldiethylalanine was recovered by acidifying the solution with dilute HCl. After recrystallization from ethyl acetate it melted at 129–131°. See Table I, **4**, for analyses.

Sodium 1-Benzyl-3-acetyl-5-(3-amyl)tetramate.—The N-acetoacetyldiethylalanine from 0.01 mole of amino acid was esterified in ether solution in the cold with an excess of ethereal diazomethane. After removal of the excess diazomethane and the ether, the oily residue was taken up in 15 ml. of benzene and treated with 15 ml. of methanol containing 0.0115 mole of freshly prepared $NaOCH_3$. This solution was refluxed on a steam bath for 3 hr. The benzene was decanted and the residue was triturated with ether to give a crude solid sodium salt. This salt was dissolved in water, neutralized with H_2SO_4, and extracted into ether. The extracts were washed, dried, and back extracted with 1 equiv. of $NaHCO_3$, and the solution was lyophilized. The over-all yield from the amino acid was between 20 and 30%. See Table II, **4**, for ultraviolet spectra and analysis of K salt.

N-Methylisoleucine.—The N-tosyl derivative of isoleucine was methylated with methyl iodide according to the general procedure given by Greenstein and Winitz.[12] As infrared analyses showed presence of some NH in the product, repeated methylations were carried out with an excess of methyl iodide to give a mixture of esters. Countercurrent extractions in separatory funnels between petroleum ether (b.p. 40–60°) and a solution containing 10% of water in methanol yielded the fully methylated product in the petroleum ether fraction. An 80% yield of the pure N-methylisoleucine was obtained by hydrolyzing 2.96 g. of the ester in 5 ml. of glacial acetic acid and 50 ml. of water in a shaker bomb at 150° for 2 hr.

Anal. Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41; N, 9.65. Found: C, 57.94; H, 10.09; N, 9.78.

Acetoacetylation as described previously yielded an oily N-acetoacetyl derivative which was isolated as the crystalline dibenzylethylenediamine (DBED) salt. See Table I, **2**, for the analysis.

Esterification with diazomethane and condensation with $NaOCH_3$ was carried out as described in the general procedure. The potassium salt was obtained from the free tetramic acid in ether solution by adding potassium 2-ethylhexanoate. Washing with ether and acetone yielded a crystalline product. See Table II, **2**, for the analysis and data; $[\alpha]_{578}$ +26.5° (*c* 2, CH_3OH); $[\alpha]_{546}$ +30.0°.

(12) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1961, p. 2757.

Pseudoleucine (**Table I, 20**).—Twenty-two grams of the oxime of α-keto-β,β-dimethylbutyric acid[13] was hydrogenated in 500 ml. of glacial acetic acid with 1 g. of PtO_2 catalyst. The reduction was complete after 54 hr.; yield 16.5 g., 84%; m.p. 300–307° (subl.).

Anal. Calcd. for $C_8H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68. Found: C, 54.85; H, 10.37; N, 10.51.

N-Benzyl-5,5,5-trifluoronorvaline.—5,5,5-Trifluoronorvaline[14] (0.513 g., 0.003 mole) was reductively benzylated in 26 ml. of methanol with benzaldehyde as described previously. After removal of the catalyst and neutralization with dilute HCl, the product crystallized immediately; yield 0.65 g. (83%), m.p. 276°.

Anal. Calcd. for $C_{12}H_{14}F_3NO_2$: C, 55.17; H, 5.40; N, 5.36. Found: C, 54.97; H, 5.21; N, 5.98.

1,5-Diphenyl-3-acetyltetramic Acid (**Table II, 31**).—This acid was prepared from ethyl N-phenyl-α-phenylglycinate which was obtained from ethyl α-chlorophenylacetate and aniline hydrochloride as described by Bischoff.[15] The acetoacetyl derivative was oily and was directly ring closed to yield the tetramate.

N-Benzylamino Acids.—There were a number of references in the literature to N-benzylvaline.[16] However, the N-benzyl derivatives of the amino acids described below seem to be new. They were all prepared by the catalytic hydrogenation of the amino acid potassium salt with benzaldehyde in methanol.

N-Benzyl-L-isoleucine, m.p. 256–258°.

Anal. Calcd. for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.64; N, 6.32. Found: C, 70.38; H, 8.59; N, 5.90.

N-Benzylaminoheptanoic Acid.

Anal. Calcd. for $C_{14}H_{21}NO_2$: C, 71.47; H, 8.99; N, 5.95. Found: C, 70.78; H, 8.83; N, 5.84.

N-Benzyl-α-phenylglycine, m.p. about 225°.

Anal. Calcd. for $C_{15}H_{15}NO_2$: C, 74.62; H, 6.24; N, 5.80. Found: C, 74.11; H, 6.07; N, 5.80.

N-(2-Ethylbutyl)isoleucine.—This compound was prepared from 2-ethylbutyraldehyde and isoleucine by the reductive coupling method; m.p. 183–184°, after recrystallization from water. Only 140 mg. was obtained from 6.55 g. of L-isoleucine.

Anal. Calcd. for $C_{12}H_{25}NO_2$: C, 66.89; H, 11.70; N, 6.50. Found: C, 66.15; H, 11.80; N, 6.08.

3-Cyano-4-*sec*-butyltetramic Acid (**Table III, 2**).—Isoleucine ethyl ester (6.8 g.) was acetylated with cyanoacetylhydrazide in ether solution in the cold to yield 4.7 g. of oily N-cyanoacetylisoleucine ethyl ester. This ester was condensed with $NaOCH_3$ in methanol and benzene at reflux temperature for 3 hr. The sodium salt (4 g.) in aqueous solution was acidified and the crystalline ester was recrystallized from ethyl acetate. See Table III, **2**, for analyses.

3-Carboxamido-4-*sec*-butyltetramic acid (**Table III, 1**).—The 3-cyano derivative (0.7 g.) was dissolved in 5 ml. of concentrated H_2SO_4 and allowed to stand overnight. Upon dilution with ice and water, 0.7 g. of the amide was obtained in pure state. Upon titration with standard alkali, a neutralization equivalent of 197 was obtained (calcd. 198); acidification of the solution yielded recovered crystalline tetramic acid. See Table III, **1**, for analyses.

1-Benzyl-3-carbethoxy-5-*sec*-butyltetramate Copper Salt (**Table III, 4**).—N-Benzylisoleucine was esterified by adding it to an ethanol solution to which a 100% excess of thionyl chloride had been added. The crystalline ester hydrochloride was obtained by adding benzene to an ether solution; m.p. 185°. This salt (29 g.) in 100 ml. of pyridine was cooled in an ice bath and treated dropwise with 15.4 g. of carbethoxyacetyl chloride (prepared from monopotassium monoethyl malonate). After 0.5 hr., the mixture was concentrated and taken up in ice water, and the oil was extracted into ether. After washing with dilute HCl and water, and drying ($MgSO_4$), 17 g. (47%) of the diethyl ester of N-carboxyacetylisoleucine was recovered as an oil.

The diester was treated with 1.26 g. of sodium dissolved in 15 ml. of ethanol and refluxed for 3 hr. in 50 ml. of benzene. The precipitated sodium salt was dissolved in water, acidified, and extracted into ether. After washing and drying and removal of the ether, the yield was 12.3 g. (86%), m.p. 185–192° (with sublimation). The product was purified by treating the sodium salt in solutions with cupric acetate solution. See Table III, **4**, for analyses.

1-Benzyl-3-carboxamido-5-*sec*-butyltetramic Acid (**Table III, 3**).—One gram of the crude carbethoxytetramate was dissolved in 4 ml. of liquid NH_3 and 7.5 ml. of ethanol and heated for 12 hr. at 100°. The solution was concentrated, and the residue was dissolved in water, acidified, and extracted with ethyl acetate. This was dried, concentrated, and recrystallized from ether. See Table III, **3**, for analyses.

5-*sec*-Butyltetramic Acid (**Table III, 5**).—An attempt to prepare the 3-carbethoxytetramate from N-carbethoxyacetylisoleucine ethyl ester (2.25 g.) and $NaOCH_3$ resulted in the production of the decarboxylated sodium 5-*sec*-butyltetramate in a yield of 0.9 g. Acidification yielded the free acid which was recrystallized from petroleum ether (b.p. 40–60°). See Table III, **5**, for analytical data.

(13) E. Abderhalden, W. Fause, and E. Hause, *Z. physiol. chem. Hoppe-Seylers*, **228**, 187 (1934).

(14) H. M. Walborsky, M. Baum, and D. F. Loncrini, *J. Am. Chem. Soc.*, **77**, 3637 (1955).

(15) C. A. Bischoff, *Ber.*, **30**, 2303 (1897).

(16) P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).