of the acetonitrile by ethyl acetate; 1.22-g. yield (53.5%), m.p. 158.5–160.5°. The C^{14}-labeled protected tripeptide was prepared by the carbodiimide method also.[1a]

Glycyl-α-aminoisobutyryl-L-alanine.—Hydrogen was passed through a solution of 1.37 g. (0.03 mole) of the protected tripeptide in 50 ml. of absolute ethanol containing 2.0 ml. of glacial acetic acid for 4 hr. in the presence of 0.5 g. of fresh palladium black with agitation by means of a Vibro-Mixer.[9] The product was obtained as shiny plates and recrystallized from water–acetone; 0.66-g. yield (95%), m.p. 238.5–240° dec., $[\alpha]^{26}D$ −26.3° (*c* 0.457, 1 *N* HCl); R_f 0.34 in 2-butanol–formic acid–water (75:15:10) and 0.70 in phenol–water (80:20), descending 15 hr., Whatman No. 1 paper.

Anal. Calcd. for $C_7H_{17}N_3O_4$: C, 46.74; H, 7.41; N, 18.17. Found: C, 46.78; H, 7.56; N, 17.57.[10]

The C^{14}-labeled free tripeptide was prepared by a similar procedure; specific activity was 16.8 μc./mmole.[1a]

Carbobenzoxyglycyl-α-aminoisobutyryl-L-valine Benzyl Ester.—To a solution of 1.47 g. (0.005 mole) of carbobenzoxyglycyl-α-aminoisobutyric acid in 100 ml. of acetonitrile was added 1.03 g. (0.005 mole) of dicyclohexylcarbodiimide; the mixture was stirred at room temperature for 1 hr. A fresh filtered solution of L-valine benzyl ester in acetonitrile [from 1.83 g. (0.005 mole) of L-valine benzyl ester benzenesulfonate and 0.75 ml. (0.005 mole) of triethylamine] was added; stirring was continued for 20 hr. at room temperature. The acetonitrile was removed *in vacuo*, and the residue was taken up in ethyl acetate and worked up as usual. Recrystallization from hot absolute ethanol and ethanol–ether yielded 1.60 g. (66%) of protected tripeptide, m.p. 114–116.5°, $[\alpha]^{26}D$ −26.7° (*c* 0.457, ethanol). A portion was recrystallized from ethanol–ether for analysis, m.p. 115.5–118°.

Anal. Calcd. for $C_{26}H_{33}N_3O_6$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.66; H, 6.98; N, 8.59.

Glycyl-α-aminoisobutyryl-L-valine.—Catalytic hydrogenolysis was carried out as described above on 0.97 g. (0.002 mole) of the protected tripeptide. To remove any acetate associated with the free tripeptide obtained, the product was dissolved in 20 ml. of water containing a sixfold molar excess of ammonia, lyophilized, redissolved in water, and lyophilized three more times. Recrystallization from water–alcohol–acetone yielded 0.50 g. (96%) of shiny plates, m.p. 240.5–241.5°, $[\alpha]^{26}D$ −11.0° (*c* 1.486, 1 *N* HCl); R_f 0.49 in 2-butanol–formic acid–water (75:15:10) and 0.86 in phenol–water (80:20), descending 15 hr.

Anal. Calcd. for $C_{11}H_{21}N_3O_{24}$: C, 50.95; H, 8.94; N, 16.21. Found: C, 50.99; H, 8.70; N, 16.02.

(9) The Vibro-Mixer was obtained from Fisher Scientific Co.

(10) A second portion of the carbobenzoxytripeptide benzyl ester was dissolved in methanol and subjected to hydrogenolysis as above, but in the absence of acetic acid. The melting point of the free tripeptide obtained was identical with that of the analytical sample and with that of an equal mixture of the two preparations.

3,6-Bis-*p*-dimethylaminobenzylidene-2,5-diketopiperazine[1]

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Received August 19, 1964

In preparing compounds containing systems of conjugated double bonds by reaction of dialkylaminobenzaldehyde with suitable ring compounds,[2] we attempted unsuccessfully to condense *p*-dimethylaminobenzaldehyde with 2,5-diketopiperazine. The desired compound was obtained, however, by use of the methiodide salt of this aldehyde. A mixture of 11.6 g. of the quaternary salt, 2.2 g. of 2,5-diketopiperazine, 4.0 g. of sodium acetate, and 12.0 g. of acetic anhydride was heated 3 hr. in an oil bath at 170°. The resulting insoluble product was washed with hot water and with hot methanol, then recrystallized twice from dimethylformamide. The tan crystals melted at 340°[3]; they formed an orange solution in acetic acid which became colorless on addition of a little concentrated HCl.

Anal. Calcd. for $C_{22}H_{24}N_4O_2$: C, 70.20; H, 6.41. Found: C, 69.98; H, 6.18.[4]

Walker 256 tumor screening test showed C/T 0.85 at 400 mg./kg.[5]; tissue culture screening test against KB cells *in vitro*: ED_{54}, 43 γ/ml.[6]

(1) This research was supported by a United States Public Health Service Grant CA-03717-5 from the National Cancer Institute.

(2) C. T. Bahner, J. Wilson, M. West, G. Browder, J. G. Goan, C. Cook, J. Fain, E. Franklin, and A. Myers, *J. Org. Chem.*, **22**, 683 (1957).

(3) Corrected for stem exposure; determined by use of Thiele tube.

(4) Average of two analyses by Weiler and Strauss, Oxford, England.

(5) We are grateful to Professor Alexander Haddow, Mr. J. E. Everett, and Mr. B. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single i.p. injection in arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor bearing animals were sacrificed approximately 8 days later and the average weights of tumors in treated and untreated hosts reported as the ratio C/T.

(6) Results of the standard KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center.

The Preparation of 4-Substituted Benzothiadiazines

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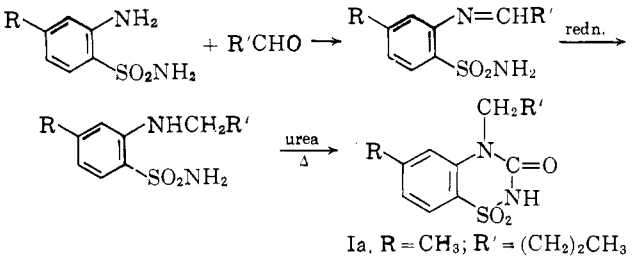
During our attempts to prepare cyclic analogs (I) of tolbutamide, a publication[2] appeared setting forth the synthesis of Ia. We have prepared Ia and twelve other 4-substituted analogs by an alternate route according to Scheme I.

SCHEME I



Ia, R = CH_3; R′ = $(CH_2)_2CH_3$

In only one instance (**15**), when using 10% palladium on carbon in acetic acid solution, was catalytic reduction of a Schiff base successful. When the Schiff bases were refluxed in acetic acid with either di- or trimethylamine borane for 0.5 hr., pure reduction products were obtained in yields ranging from 84–99%. Schiff bases (**12–14**) prepared from *para*-substituted benzaldehydes did not give pure products by this procedure.

The 4-pyridylethyl compound (**21**) was prepared by addition of 4-vinylpyridine to *o*-aminobenzenesulfonamide. All cyclizations were carried out by heating the N-substituted sulfonamides with urea at 200–205°.[3] The N-pyridylethylsulfonamide was heated at lower temperatures (*ca.* 170°) since at 200° decomposition occurred with loss of 4-vinylpyridine.

Experimental[4]

Schiff Bases.—These were prepared by mixing equimolar amounts of the appropriate aniline and aldehyde in ethanol. The mixtures were allowed to stand from 7–24 hr. at room temperature, and the solvent was removed. Yields, analyses, recrystallization solvents, and melting points are indicated in Table I.

(1) Author to whom inquiries should be addressed.

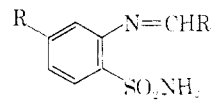
(2) D. L. Simmons, J. M. Dodsworth, and F. L. Chubb, *Can. J. Chem.*, **41**, 804 (1963).

(3) D. V. Park and R. T. Williams, *J. Chem. Soc.*, 1760 (1950).

(4) Melting points were determined on Fisher-Johns block with a calibrated thermometer. Analyses were performed by Midwest Microlab, Inc.

TABLE I

SCHIFF BASES

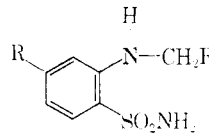


No.	R	R′	Yield, %	Recrystn. solvent[a]	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1	H	*n*-Propyl	85	Et–H	106–107	$C_{10}H_{14}N_2O_2S$	53.09	53.04	6.24	6.22	12.38	12.16
2	CH_3	*n*-Propyl	69	E–H	141–145	$C_{11}H_{16}N_2O_2S$	54.99	54.88	6.71	6.94	11.66	11.78
3	H	Phenyl	34.5	B–H	136–138	$C_{13}H_{12}N_2O_2S$	55.99	60.50	4.65	4.85	10.77	10.65
4	H	Cyclohexyl	91	I–H	194–198	$C_{13}H_{18}N_2O_2S$	58.63	58.56	6.81	6.85	10.52	10.46
5	H	Benzyl	78	E	166–168	$C_{14}H_{14}N_2O_2S$	61.31	61.12	5.15	5.18	10.21	10.20
6	CH_3	Benzyl	57	B	150–151	$C_{15}H_{16}N_2O_2S$	62.49	62.54	5.59	5.74	9.72	9.66
7	H	2-Pyridyl	80	E	158–160	$C_{12}H_{11}N_3O_2S$	55.17	54.94	4.24	4.20	16.09	16.38
8	H	3-Pyridyl	86	M	220–221 dec.	$C_{12}H_{11}N_3O_2S$	55.17	55.25	4.24	4.16	16.09	16.25
9	H	4-Pyridyl	72	M	195–201	$C_{12}H_{11}N_3O_2S$	55.17	54.63	4.24	4.46	16.09	15.87
10	CH_3	4-Pyridyl	95	MC–M	249–252 dec.	$C_{13}H_{13}N_3O_2S$	56.72	56.91	4.76	5.04	15.27	15.04
11	H	*n*-Hexyl	99	Ea–H	107–110	$C_{13}H_{20}N_2O_2S$	58.19	58.28	7.51	7.55	10.44	10.43
12	CH_3	4-Hydroxyphenyl	32	I	161–166	$C_{13}H_{14}N_2O_3S$	57.93	58.21	4.86	5.05	9.65	9.59
13	H	4-Hydroxy-3-methoxyphenyl	57	Ea–B	177–179	$C_{14}H_{14}N_2O_4S$	54.90	54.70	4.61	4.79	9.15	9.08
14	H	4-Dimethylaminophenyl	70	E	142–147	$C_{15}H_{17}N_3O_2S$	59.39	59.66	5.65	5.79	13.86	13.97

[a] B = benzene, D = dimethylformamide, E = ethanol, Ea = ethyl acetate, Et = ether, H = hexane, I = 2-propanol, M = methanol, MC = Methyl Cellosolve, W = water.

TABLE II

SULFAMYLANILINES



No.	R	R′	Reducing Agent	Yield, %	Recrystn. solvent[a]	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
15	H	*n*-Propyl	Pd–C, H_2	50	B–H	80–83	$C_{10}H_{16}N_2O_2S$	52.62	52.57	7.07	6.95	12.27	12.37
16	CH_3	*n*-Propyl	TMAB[b]	95	B	108–109[c]	$C_{11}H_{18}N_2O_2S$	54.53	54.40	7.49	7.32	11.56	11.51
17	H	Phenyl	TMAB[b]	99	B–H	117–118	$C_{13}H_{14}N_2O_2S$	59.68	59.53	5.61	5.38	10.68	10.87
18	H	Cyclohexyl	TMAB[b]	99	E–W	72–75	$C_{13}H_{20}N_2O_2S$	58.19	58.54	7.51	7.57	10.44	10.20
19	H	Benzyl	TMAB[b]	93	E	127	$C_{14}H_{16}N_2O_2S$	60.86	60.83	5.84	5.83	10.14	10.21
20	CH_3	Benzyl	TMAB[b]	98	E	85–90	$C_{15}H_{18}N_2O_2S$	62.05	62.06	6.25	6.53	9.65	9.56
21	H	4-Pyridylethyl[d]	...	53	M	187–188	$C_{13}H_{15}N_3O_2S$	56.31	56.54	5.45	5.43	15.16	14.95
22	H	2-Pyridyl	DMAB[e]	79	E	153–156	$C_{12}H_{13}N_3O_2S$	54.75	54.95	4.98	4.97	15.96	16.26
23	H	3-Pyridyl	TMAB	91	E	159–161	$C_{12}H_{13}N_3O_2S$	54.75	54.70	4.98	5.21	15.96	16.18
24	H	4-Pyridyl	TMAB	88	D–Et	182–184	$C_{12}H_{13}N_3O_2S$	54.75	54.99	4.98	5.24	15.96	15.82
25	CH_3	4-Pyridyl	TMAB	70	M	163–166	$C_{13}H_{15}N_3O_2S$	56.31	56.46	5.45	5.57	15.16	15.29
26	H	*n*-Hexyl	TMAB	95	H	59–62	$C_{13}H_{22}N_2O_2S$	57.76	58.01	8.20	8.30	10.36	10.29

[a] See Table I. [b] Trimethylamine borane. [c] Lit.[2] m.p. 112°. [d] By addition of 4-vinylpyridine to *o*-aminobenzenesulfonamide; see Experimental. [e] Dimethylamine borane.

Reduction of Schiff Bases. N-Butyl-2-sulfamylaniline (15).—A solution of 3.0 g. (0.135 mole) of N-butylidene-2-sulfamylaniline (1) in 250 ml. of acetic acid was hydrogenated with 1.0 g. of 10% palladium on carbon at 50° at an initial pressure of 4 atm.; the required amount of hydrogen was taken up in about 15 hr. Filtration and removal of solvent left a residue (2.0 g.) which was recrystallized twice by dissolving in a minimum amount of boiling benzene and adding hexane to incipient turbidity to give 100 mg., m.p. 80–83° (Table II).

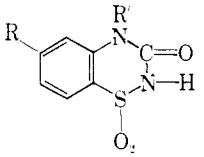
N-Butyl-5-methyl-2-sulfamylaniline (16).[2]—A mixture of 14.0 g. (0.058 mole) of N-butylidene-5-methyl-2-sulfamylaniline (2) and 35 ml. of acetic acid was stirred, and 1.9 g. (0.026 mole) of trimethylamine borane (Callery Chemicals) in 10 ml. of acetic acid was added in one portion. The clear solution was refluxed for 0.5 hr. Addition of water resulted in crystallization of the product to yield 13.4 g. (95%), m.p. 111–112°. Two crystallization from benzene failed to raise the melting point; the final m.p. was 108–109°.

N-(4-Pyridylethyl)-2-sulfamylaniline (21).—A mixture of 2.1 g. (0.02 mole) of 4-vinylpyridine, 3.4 g. (0.02 mole) of sulfamylaniline, and 1.2 g. (0.02 mole) of acetic acid was refluxed in 10 ml. of absolute ethanol for 41 hr. The colorless solid was filtered off and washed with water to yield 2.9 g. (53%), m.p. 170–183°. Two crystallizations from methanol raised the m.p. to 187–188°.

4-Butyl-6-methyl-3-oxo-3,4-dihydro-1,2,4-benzothiadiazine 1,1-Dioxide (28).—An intimate mixture of 14.0 g. (0.058 mole) of **16** and 3.9 g. (0.065 mole) of urea was heated at 200–205° for 0.5 hr. in a stream of anhydrous nitrogen. The residue was partitioned between 1 *N* NaOH and chloroform. The alkaline extract was acidified with 1 *N* HCl. An oily material separated which gradually became crystalline to yield 9.7 g. (62.5%), m.p. 171–172° (Table III).

TABLE III

BENZOTHIADIAZINES



No.	R	R′	Yield, %	Recrystn. solvent[a]	M.p., °C.	Formula	—Carbon, %— Calcd.	Found	—Hydrogen, %— Calcd.	Found	—Nitrogen, %— Calcd.	Found
27	H	*n*-Butyl	45	W	139–140	$C_{11}H_{14}N_2O_3S$	51.96	51.76	5.55	5.45	11.02	10.86
28	CH_3	*n*-Butyl	62.5	B	171–172[b]	$C_{12}H_{16}N_2O_3S$	53.72	53.70	6.01	6.07	10.44	10.57
29	H	Benzyl	50	M	205–207	$C_{14}H_{12}N_2O_3S$	53.33	58.56	4.20	4.44	9.27	9.68
30	H	Cyclohexylmethyl	37	E–H	202–204	$C_{14}H_{18}N_2O_3S$	57.13	56.94	6.17	5.99	9.52	9.33
31	H	Phenethyl	82.5	E–H	160	$C_{15}H_{14}N_2O_3S$	59.60	59.71	4.67	4.65	9.27	9.43
32	CH_3	Phenethyl	58	B	155–156	$C_{16}H_{16}N_2O_3S$	60.75	61.02	5.10	5.17	8.86	8.68
33	H	4-Pyridylethyl	20	D	249–252 dec.	$C_{14}H_{13}N_3O_3S$	55.44	55.16	4.32	4.95	13.86	13.99
34	H	2-Pyridylmethyl	59	D-H-Et	259–264 dec.	$C_{13}H_{11}N_3O_3S$	53.98	53.83	3.83	4.04	14.53	14.36
35	H	3-Pyridylmethyl	65	D	289–290 dec.	$C_{13}H_{11}N_3O_3S$	53.98	53.53	3.83	3.97	14.53	14.63
36	H	3-Piperidylmethyl[c]	20	W	332 dec.	$C_{13}H_{17}N_3O_3S$	52.87	52.98	5.80	6.02	14.23	14.15
37	H	4-Pyridylmethyl	92	D	300	$C_{13}H_{11}N_3O_3S$	53.98	53.82	3.83	4.03	14.53	14.41
38	CH_3	4-Pyridylmethyl	48	D-H-Et	297–307 dec.	$C_{14}H_{13}N_3O_3S$	55.44	54.39	4.32	4.64	13.86	13.72
39	H	*n*-Heptyl	89	B–H	92–93	$C_{14}H_{20}N_2O_3S$	56.74	56.60	6.80	7.14	9.45	9.24

[a] See Table I. [b] Lit.[2] m.p. 173–174°. [c] By catalytic reduction of **35** with PtO_2.

3-Oxo-4-(4-pyridylethyl)-3,4-dihydro-1,2,4-benzothiadiazine 1,1-Dioxide (33).—A mixture of 21.5 g. (0.078 mole) of **21** and 4.65 g. (0.078 mole) of urea was heated for 1 hr. at 150–170° under a stream of nitrogen. The residue was heated with ethanol and cooled, and a colorless solid was filtered off, yield 6.0 g., m.p. 239–245°. Crystallization from methanol, then from a minimum amount of hot dimethylformamide to which ether was added to incipient turbidity gave 2.1 g., m.p. 253–256°. When heated at 200–205°, the product obtained analyzed for loss of vinylpyridine.

The Synthesis of N-Substituted 2-Aminoethanethiosulfuric Acids

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Almost all aminoalkylthiosulfuric acids whose pharmacological properties have been reported in the literature thus far have a primary amino group. A series of N-monosubstituted 2-aminoethanethiosulfuric acids was, therefore, synthesized to determine the effect of the presence of secondary amino groups in alkyl thiosulfates (Bunte salts) on their biological activity. The procedures employed in their synthesis were method A, used when the direct alkylation of the sodium salt of 2-aminoethanethiosulfuric acid was feasible, and method B, used mainly to prepare those Bunte salts in which the amino group is attached to a secondary carbon atom of an alkyl chain. This latter method involved the conversion of an N-alkylaminoethanol to the bromide hydrobromide which was allowed to react subsequently with one equivalent of sodium thiosulfate in aqueous or aqueous–ethanolic solution.

Experimental[1]

Method A. N-Alkylaminoethanethiosulfuric Acids.—To 0.25 mole of sodium hydroxide dissolved in 300 ml. of warm 95% ethanol was added rapidly with stirring a hot solution of 0.25 mole of 2-aminoethanethiosulfuric acid[2] in 25 ml. of water. To the mixture, which was then heated under reflux, there was added dropwise over 1.5 hr. 0.20 mole of the primary alkyl bromide. Heating and stirring were continued for 4.5 hr. after the complete addition of the halide. About 150 ml. of ethanol was then distilled from the mixture and replaced with an equal volume of water. The solution was neutralized with glacial acetic acid and cooled overnight causing the crystalline product to separate. The Bunte salt was collected by filtration, air-dried, and recrystallized several times to remove the contaminant resulting from dialkylation.

Method B. 2-(*sec*-Alkylamino)ethanols.—These compounds were prepared from 2-aminoethanol and *sec*-alkyl bromides by the method previously described[3] except that xylene was used as the solvent instead of benzene. A mixture of 0.5 mole of the alkyl bromide, 1.5 moles of 2-aminoethanol, and 150 ml. of xylene was heated under reflux with stirring for 21–37 hr. The two layers were separated and the lower layer containing mainly 2-aminoethanol was washed with three 20-ml. portions of benzene. The benzene extracts and the xylene layer were combined, washed with three 20-ml. portions of water, and dried over anhydrous magnesium sulfate. The solvents were removed on a steam bath by the use of an aspirator and the residue was distilled under reduced pressure through a Vigreux column. Yields ranged from 56–70%.

N-Alkylaminoethyl Bromide Hydrobromides.—These compounds were prepared according to the procedure of Cortese[4] by treating the corresponding N-alkylaminoethanol with 48% hydrobromic acid and by slowly distilling the water formed in the course of the reaction.

(1) Melting points are uncorrected and were determined on a Fisher-Johns melting point apparatus. The rate of heating influences the melting and decomposition points of aminoalkyl Bunte salts. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and by Mr. Joseph Alicino, Metuchen, N. J.

(2) H. Bretschneider, *Monatsh.*, **81**, 372 (1950).

(3) J. B. Wright, E. H. Lincoln, R. V. Heinzelmann, and J. H. Hunter, *J. Am. Chem. Soc.*, **72**, 3536 (1950).

(4) F. Cortese, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943 p. 91.