of 6.5 g. of 2-chloro-4,5-diamino-6-trifluoromethylpyrimidine (XXVII), 70 ml. of ethyl orthoformate, and 70 ml. of acetic anhydride was heated under reflux for 2 hr. The reaction mixture was evaporated *in vacuo* and to the residue was added 150 ml. of 5% sodium hydroxide solution. The mixture was stirred until clear, treated with Darco, and filtered. The filtrate was acidified with hydrochloric acid and the resulting colorless prisms were collected; yield, 6 g. (90%). Recrystallization from methanol–benzene gave colorless prisms, m.p. 240°.

Anal. Calcd. for $C_6H_2ClF_3N_4$: C, 32.36; H, 0.90; Cl, 15.74; F, 25.62. Found: C, 32.23; H, 1.20; Cl, 15.72; F, 25.15.

2-Chloro-6,8-bis(trifluoromethyl)purine (XXIX).—A solution of 10 ml. of trifluoroacetic acid, 10 ml. of trifluoroacetic anhydride, and 1.0 g. (0.0047 mole) of 4,5-diamino-2-chloro-6-trifluoromethylpyrimidine[1a] (XXVII) was heated under reflux for 2 hr. The mixture was concentrated almost to dryness and the residue was diluted with 5 ml. of 5% sodium hydroxide. This mixture was extracted with ethyl acetate and the extract was dried over sodium sulfate. Concentration provided 0.25 g. (22%) of white crystals of XXIX. Vacuum sublimation gave an analytical sample as colorless prisms, m.p. 149°.

Anal. Calcd. for $C_7HClF_6N_4$: C, 28.93; H, 0.34. Found: C, 28.92; H, 0.55.

6-Chloro-2-trifluoromethylpurine (XXXIV).—This compound was prepared from XXXI in the manner described for the synthesis of XXVIII. Crystallization from water gave an 83% yield of colorless prisms, m.p. 200–201°.

Anal. Calcd. for $C_6H_2ClF_3N_4$: C, 32.36; H, 0.90; Cl, 15.74; F, 25.62. Found: C, 32.58; H, 1.22; Cl, 16.00; F, 25.50.

6-Mercapto-2-trifluoromethylpurine (XXXV).—A solution of 2 g. (0.009 mole) of 6-chloro-2-trifluoromethylpurine (XXXIV) and 0.8 g. (0.011 mole) of thiourea in 20 ml. of methanol was heated under reflux for 3 hr. The solvent was removed and a small amount of water was added to the residue. Insoluble crystals (0.7 g., m.p. 239°) were filtered. The filtrate was made basic with sodium hydroxide solution and then acidified to give another crop of crystals for a total yield of 1.2 g. Recrystallization from aqueous methanol gave colorless plates of XXXV, m.p. 274–275°.

Anal. Calcd. for $C_6H_3F_3N_4S$: C, 32.73; H, 1.36; S, 14.54. Found: C, 32.70; H, 1.58; S, 14.98.

Synthesis of the 6-Alkylamino-2-trifluoromethylpurines (XXXVI–XXXIX).—A mixture of 0.5 g. (0.002 mole) of 6-chloro-2-trifluoromethylpurine (XXXIV), 20 ml. of methanol, and an excess of 70% ethylamine was heated under reflux for 2 hr. The mixture was concentrated and the residue was crystallized from water to give 0.22 g. of 6-ethylamino-2-trifluoromethylpurine (XXXVII). The other 6-alkylaminopurines (Table V) were prepared in the same manner.

5-Chloro-7-trifluoromethylthiazolo[5,4-*d*]pyrimidine (XLII).—A solution of 3 g. of 5-amino-2-chloro-4-mercapto-6-trifluoromethylpyrimidine (XLI)[1a] in 150 ml. of ethyl orthoformate was heated under reflux for 2 hr. The mixture was concentrated *in vacuo* and the residue was treated with 5% sodium hydroxide and extracted with ether. Concentration provided 2.8 g. of XLII. Purification data for this compound and the identically prepared 7-chloro-5-trifluoromethylthiazolo[5,4-*d*]pyrimidine (XLVII) are included in Table VI.

2-Amino-5-anilino-7-trifluoromethylthiazolo[5,4-*d*]pyrimidine (XLV).—To a solution of 4.7 g. (0.018 mole) of 2,4-dichloro-5-nitro-6-trifluoromethylpyrimidine (XL) in 30 ml. of acetic acid was added 1.7 g. (0.018 mole) of potassium thiocyanate. The mixture was stirred for 30 min. and 3.4 g. (0.036 mole) of aniline was then added dropwise, with cooling. After an additional 20 min., 3 g. of iron powder and 100 ml. of acetic acid were added. The mixture was stirred at 60° for 1.5 hr. and filtered. The filtrate was poured into 300 ml. of water and 0.55 g. of XLV was collected (Table VI).

Bis(2-trifluoromethylthiazolo[5,4-*d*]pyrimidyl 5-Disulfide (XLIX).—A mixture of 20 ml. of trifluoroacetic acid, 20 ml. of trifluoroacetic anhydride, and 2 g. of 5-amino-2,4-dimercaptopyrimidine (XLVIII) was heated under reflux for 2 hr. The reaction mixture was evaporated under reduced pressure and the residue was diluted with 40 ml. of water. The water-insoluble solid was dissolved in ethyl acetate. Concentration gave yellow-green crystals which were crystallized from methanol to provide 1.3 g. (22%) of XLIX as colorless plates, m.p. 150–151°.

Anal. Calcd. for $C_{12}H_2F_6N_6S_4$: C, 30.51; H, 0.42. Found: C, 30.43; H, 0.52.

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Hydrazino Acids. II. Alkyl-, Aralkyl-, and Hydroxyalkyl-α-hydrazino Acids

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The reaction of α-halo aliphatic acids with some substituted hydrazines was studied. Ethyl-, butyl-, phenethyl-, and 1,1-dimethylhydrazines have been observed to yield 1,1-disubstituted α-hydrazino acids, while isopropyl-, β-hydroxyethyl-, β-hydroxypropyl-, and benzylhydrazines gave 1,2-disubstituted products. 1-Isopropyl- and 1-benzyl-α-hydrazino acids were prepared by reduction of the corresponding nitrosoamino acids.

The preliminary screening results of some hydrazino and 1-methylhydrazino aliphatic acids tested on Sarcoma 180 in mice at the Sloan Kettering Institute for Cancer Research, N. Y., justified further synthetic exploration in the field of hydrazino acids.

In the first paper of this series,[1] methylhydrazine has been shown to react with halo acids to give α-(1-methylhydrazino) acids. Numerous attempts have now been made to obtain α-(2-methylhydrazino) acids but most of them proved unsuccessful. These included the reduction of the methylene derivatives of some α-hydrazino acids with sodium borohydride, the reduction of the methylhydrazone of pyruvic acid and ethylidene-α-hydrazino acids in the presence of platinum oxide catalyst, the reaction of chloroamino acids with methylamine and the reaction of 1-methyl-2-benzoyl hydrazine with α-halo acids. However, the methylation of the benzylidene derivatives of α-hydrazino acids with dimethyl sulfate led to the desired compounds in low yields. By this method we prepared the α-(2-methylhydrazino)propionic (I) and caproic (II) acids. Although an attempt to repeat these preparations failed, we record the results in the experimental part.

Other alkylhydrazines, owing to the electron-releasing properties of the alkyl group,[2] were expected to behave

(1) A. Carmi, G. Pollak, and H. Yellin, *J. Org. Chem.*, **25**, 44 (1960).

(2) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, New York, N. Y., 1953, p. 316.

TABLE I

$R_1CHCOOH$
|
R_2NNHR_3

Compd.	R_1	R_2	R_3	Yield, %	M.p. dec., °C.	Formula	Nitrogen % calcd.	Nitrogen % found
I	CH_3	H	CH_3	16.5[a]	132–134	$C_4H_{10}N_2O_2$	23.71	23.81
II	C_4H_9	H	CH_3	19.5[a]	123–126	$C_7H_{16}N_2O_2$	17.49	17.88
III	CH_3	C_2H_5	H	34.0[b]	141–143	$C_5H_{12}N_2O_2$	21.20	20.92
IV	CH_3	C_4H_9	H	50.0[b]	131–132	$C_7H_{16}N_2O_2$	17.49	17.28
V	C_2H_5	C_4H_9	H	40.0[b]	146–147	$C_8H_{18}N_2O_2$	16.08	16.02
VI	CH_3	H	$(CH_3)_2CH$	61.0[b]	203–204	$C_6H_{14}N_2O_2$	19.20	19.20
VII	C_2H_5	H	$(CH_3)_2CH$	76.0[c]	212–213	$C_7H_{16}N_2O_2$	17.49	17.51
VIII	CH_3	$(CH_3)_2CH$	H	21.5[b]	197–198	$C_6H_{14}N_2O_2$	19.20	18.96
IX	C_2H_5	$(CH_3)_2CH$	H	27.0[b]	191–193	$C_7H_{16}N_2O_2$	17.49	17.76
X	H	H	$C_6H_5CH_2$	38.8[a]	146–147	$C_9H_{12}N_2O_2$	15.55	15.57
XI	CH_3	H	$C_6H_5CH_2$	28.1[a]	159–160	$C_{10}H_{14}N_2O_2$	14.43	14.70
XII	C_2H_5	H	$C_6H_5CH_2$	40.4[d]	211–213	$C_{11}H_{16}N_2O_2$	13.46	13.80
XIII	H	$C_6H_5CH_2$	H	33.5[b]	149–150	$C_9H_{12}N_2O_2$	15.55	15.44
XIV	C_2H_5	$C_6H_5C_2H_4$	H	37.0[a]	146–148	$C_{12}H_{18}N_2O_2$	12.60	12.60[e]
XV	C_2H_5	H	$C_6H_5C_2H_4$	2.0	184–186	$C_{12}H_{18}N_2O_2$	12.60	12.40
XVI	C_2H_5	H	HOC_2H_4	13.6[a]	133–134	$C_6H_{14}N_2O_3$	17.27	17.03
XVII[f]				12.4[a]	185–187	$C_6H_{14}N_2O_3$	17.27	17.27
XVIII	C_3H_7	H	HOC_2H_4	14.7[a]	166–168	$C_7H_{16}N_2O_3$	15.90	15.73
XIX	C_2H_5	H	HOC_3H_6	16.2[a]	168–169	$C_7H_{16}N_2O_3$	15.90	15.99

[a] Crystallized from 2-propanol. [b] Crystallized from absolute ethanol. [c] Crystallized from aqueous ethanol. [d] Crystallized from water. [e] *p*-Nitrobenzal derivative, m.p. 124–126°. *Anal.* Calcd. for $C_{19}H_{21}N_3O_4$: N, 11.80. Found: N, 11.67. [f] α-(2-Hydroxyethylhydrazino)isobutyric acid.

similarly to methylhydrazine and undergo with α-halo acids substitutions at the alkyl-bearing nitrogen. We found this to be the case with unbranched alkylhydrazines. Ethyl- and butylhydrazine reacted with α-bromopropionic and α-bromobutyric acids to give the corresponding α-(1-alkylhydrazino) acids (III–V). The structures of these compounds were deduced by the reductive cleavage of the N–N bond with hydrogen in the presence of a large excess of Raney nickel in water, which yielded ammonia and the corresponding alkylamino acids.

However, the reaction of isopropylhydrazine with α-bromobutyric acid led to substitution at the unsubstituted amino nitrogen, though in small yield (8.3%), presumably owing to steric effects.[3] This compound (VII), whose structure has been deduced by reductive cleavage with Raney nickel, as well as some of its homologs, were also prepared in good yields by hydrogenation of the isopropylidene α-hydrazino acids (VI, VII) or esters in the presence of platinum oxide catalyst.

In order to obtain the α-(1-isopropylhydrazino) acids (VIII, IX), the reduction of the corresponding N-nitroso amino acids was studied. Isopropylamino acids known in the literature were obtained readily and in excellent yields by a method similar to that used by us in the preparation of hydrazino acids, namely by the treatment of α-halo acids with a large excess of isopropylamine, and passing the reaction mixture through a strong cation-exchange resin. The N-nitroso derivatives were prepared in good yields by treating the acidified isopropylamino acids with sodium nitrite (Table III).

Attempts to use the usual reduction methods on the nitroso derivatives led in most cases to practically complete cleavage of the N–N bond.[4] We found that cadmium in acid media could reduce the nitroso derivatives XXVII and XXVIII with little cleavage of the N–N bond, whereas XXVI was completely cleaved. A partial reduction of XXVI was possible by using granulated zinc.

Unsymmetrical dimethylhydrazine, owing to the very strong electron donating properties of the methyl groups, gave with α-halo acids products substituted at the methyl bearing nitrogen, *viz.*, α-(1,1-dimethylhydrazinium) aliphatic acids or inner anhydrides of the betaine type. This was proved by subjecting the products to reductive cleavage with Raney nickel whereupon ammonia and the corresponding dimethylamino acids were obtained. The α-1,1-dimethylhydrazinium derivatives of acetic, propionic, butyric, isobutyric, and isocaproic acids were prepared (Table II). Some of these quaternary hydrazinium compounds were also obtained by direct exhaustive methylation of α-hydrazino acids with dimethyl sulfate.

Phenethylhydrazine and benzylhydrazine were selected to test the effect of aralkyl groups on this type of reaction. Phenethylhydrazine, similar to unbranched aliphatic hydrazines, reacted with α-bromobutyric acid to give almost exclusively α-(1-β-phenethylhydrazino)-butyric acid (XIV). The α-2-analog was obtained only as a by-product in 2% yield. Benzylhydrazine, on the other hand, was found to lead to α-(2-benzylhydrazino)-acid in 30–40% yield (X–XII); this is contrary to the reaction of benzyl chloride with benzylhydrazine which gives unsymmetrical dibenzylhydrazine.[5] The α-(1-benzylhydrazino)acetic acid (XIII) was obtained similarly to the 1-isopropyl analog by the reduction of the corresponding N-nitroso compound (XXVIII).

Unsymmetrical dibenzylhydrazine, unlike the corresponding dimethyl compound, failed to react with α-bromobutyric acid. Even after refluxing 48 hr., the reactants were recovered unchanged.

(3) H. C. Brown and N. R. Eldred, *J. Am. Chem. Soc.*, **71**, 445 (1949).

(4) H. Zimmer, L. F. Audrieth, M. Zimmer, and R. A. Howe, *ibid.*, **77**, 790 (1955); L. A. Carpino, A. A. Santielli, and R. W. Murray, *ibid.*, **82**, 2728 (1960).

(5) J. H. Biel, A. E. Drukker, T. F. Mitchell, E. P. Sprengeler, P. A. Nuhfer, A. C. Conway, and A. Horita, *ibid.*, **81**, 2805 (1959).

TABLE II

$$\left[\begin{array}{c} R_1CR_2COOH \\ | \\ (CH_3)_2NNH_2 \end{array}\right]^{+}OH^{-}$$

Compd.	R_1	R_2	Type	Yield, %	M.p., dec., °C.	Formula	Nitrogen, % calcd.	Nitrogen, % found
XX	H	H	Hydroxide	69.5[a]	222	$C_4H_{12}N_2O_3$	20.58	20.66
XXI	CH_3	H	Betaine	69.0[b,c]	185	$C_5H_{12}N_2O_2$	21.20	[d]
XXII	C_2H_5	H	Hydroxide	43.2[b,e]	186–187	$C_6H_{16}N_2O_3$	17.08	17.03
XXIII	CH_3	CH_3	Hydroxide	55.5[f]	186–187	$C_6H_{16}N_2O_3$	17.08	17.05
XXIV	$(CH_3)_2C_2H_3$	H	Betaine	31.0[f]	202–203	$C_8H_{18}N_2O_2$	16.08	15.92

[a] Crystallized from absolute ethanol. [b] Crystallized from 2-propanol. [c] Yield by B: 41.0%. [d] Hygroscopic; analyzed as chloride. *Anal.* Calcd. for $C_5H_{13}ClN_2O_2$: Cl, 21.08. Found: Cl, 20.92. [e] Yield by B: 49.0%.

TABLE III

$$\begin{array}{c} R_1CHCOOH \\ | \\ R_2NNO \end{array}$$

Compd.	R_1	R_2	Yield, %	M.p., °C.[a]	Formula	Nitrogen, % calcd.	Nitrogen, % found
XXV	H	$(CH_3)_2CH$	82	138	$C_5H_{10}N_2O_3$	19.17	18.96
XXVI	CH_3	$(CH_3)_2CH$	72	140–141	$C_6H_{12}N_2O_3$	17.75	17.77
XXVII	C_2H_5	$(CH_3)_2CH$	71	112–114	$C_7H_{14}N_2O_3$	16.08	16.41
XXVIII	H	$C_6H_5CH_2$	95	146–149[b]	$C_9H_{10}N_2O_3$		

[a] All compounds crystallized from water. [b] Lit. m.p. 148–149° [W. Baker, W. D. Ollis, and V. D. Pool, *J. Chem. Soc.*, 307 (1949)].

On the basis of the pK values of ethylamine and ethanolamine it was anticipated that β-hydroxyalkyl hydrazines would lead to α-(2-β-hydroxyalkyl)hydrazino acids. This was found to be the case in the reaction of β-hydroxyethylhydrazine with α-bromobutyric, isobutyric and valeric acids (XVI–XVIII), as well as in the reaction of 1-hydrazino-2-propanol with α-bromobutyric acid (XIX). The structures were also proved by reductive cleavage with Raney nickel; in the presence of ethanol, the N-ethyl ethanolamine and corresponding N-ethylamino acids were obtained,[6] whereas hydrogenation with Raney nickel in water led to the corresponding α-amino acids.

The products of β-hydroxyethylhydrazine with chloroacetic and α-bromopropionic acid were also prepared, but found to be very unstable at room temperature, and their analyses showed deviations from the calculated values.

All compounds listed in Table I, with the exception of XII and XIV, are freely soluble in water, giving practically neutral solutions. In presence of air they decompose slowly. The 1,1-disubstituted hydrazino acids decolorize iodine solutions readily and form hydrazones when treated with carbonyl reagents. The 1,2-substituted products do not form hydrazones and their reducing properties are considerably less pronounced. The compounds listed in Table II are also freely soluble in water, are quite stable, do not form hydrazones, and fail to decolorize iodine solutions.

All the compounds synthesized in this and in the previous[1] report were screened at the Sloan Kettering Institute for Cancer Research. The data[7] on the evaluation in tumor growth retardation have shown that most unsubstituted α-hydrazino aliphatic acids had a slight nonreproducible activity in Sarcoma 180 (S-180) tests. Thus, α-hydrazinoacetic acid had a ± rating at a dose level of 6 mg./kg. and death rate 0/5 against S-180, as compared with α-hydrazinocaproic acid (±, 16 mg./kg., 2/5), α-hydrazinovaleric acid (±, 60 mg./kg., 0/5), and α-hydrazinodiethylacetic acid (±, 125 mg./kg., 0/5). Some of the methyl substituted hydrazino aliphatic acids, such as α-(1-methylhydrazino) butyric acid (±, 60 mg./kg., 0/5), and α-(1-methylhydrazino) isobutyric acid (±, 125 mg./kg., 0/5), also showed a slight activity. All the compounds described in this paper, with the exception of α-(1 benzylhydrazino)acetic acid (XIII) (±⁺, 250 mg./kg., 1/5) were inactive.

When tested against Adenocarcinoma 755, α-hydrazinoacetic acid had a ±⁻ rating at a dose level of 6 mg./kg. and death rate 0/5 as compared with α-(2-benzylhydrazino) propionic acid (XI) (±⁻, 250 mg./kg., 0/5), α-(2-benzylhydrazino)butyric acid (XII) (±⁻, 250 mg./kg., 0/5), and ethyl α-(2-isopropylhydrazino)-acetate hydrochloride (±⁻, 500 mg./kg., 0/5).

Further study of related structures and their testing is in progress.

Experimental[8]

α-(2-Methylhydrazino)propionic Acid (I).—A solution of 10.4 g. (0.1 mole) of α-hydrazinopropionic acid in 75 ml. water was shaken for 15 min. with 11 g. (0.11 mole) of benzaldehyde. The resulting solid was filtered, washed with 100 ml. of water, and the wet cake dissolved in 25 ml. of hot benzene. The water layer was separated and the benzene layer yielded on cooling 15.15 g. (80%) of the benzylidene derivative, pale yellow crystals, m.p. 99–102° dec. It was dissolved in 50 ml. of thiophene-free benzene, 11.0 g. (0.087 mole) of dimethyl sulfate was added and the solution refluxed for 5 hr. Water was added and benzene and benzaldehyde steam-distilled until a clear distillate was obtained. The residual liquor was treated with 1.5 ml. of benzaldehyde, left overnight and filtered through a filter aid. The filtrate was passed through a Duolite A 7[9] column (alkaline form), the ef-

(6) K. H. Shah, B. D. Tilak, and K. Venkataraman, *Proc. Indian Acad. Sci.*, **28A**, 145 (1948); *cf. Org. Syn.*, **36**, 21 (1956); R. G. Rice and E. J. Kohn, *J. Am. Chem. Soc.*, **77**, 4052 (1955); C. Ainsworth, *ibid.*, **78**, 1636 (1956).

(7) The authors are indebted to Drs. C. C. Stock, D. A. Clarke, and R. K. Barclay, Sloan Kettering Institute, for conducting these tests. The procedures and rating scales were published in *Cancer Res. Suppl. No. 1*, **13**, 91 (1953); *ibid., Suppl. No. 2*, **15**, 179 (1955).

(8) Melting points were taken in capillary tubes with a partial immersion thermometer, using a Büchi–Totolli melting point apparatus, and are not corrected. Microanalyses by the Microanalytical Department, Weizmann Institute of Science, Rehovoth, Israel, and Drs. Weiler and Strauss, Oxford, England.

(9) Manufactured by Chemical Process Co., Redwood City, Calif.

fluent evaporated to dryness, the residue washed with 15 ml. of ether and dissolved in 10 ml. of hot 2-propanol. The solution was kept in the refrigerator for 2 days, the crystals were filtered, washed with 7 ml. of 2-propanol and dried at 50°, giving 1.5 g. (16.5%) of I, m.p. 132–134°.

α-(1-Butylhydrazino)propionic Acid (IV).—The compound was prepared essentially according to **Method B** described in the previous publication.[1] The structure was elucidated in the following manner: One gram of the pure substance was dissolved in 100 ml. water and hydrogenated at room temperature in presence of Girdler nickel catalyst G 49 B.[10] After 10 hr. shaking, when the absorption of hydrogen ceased, the catalyst was filtered off, the solution passed through an Amberlite IRC 50[11] column (acid form), and the effluent evaporated to dryness *in vacuo*. The residue was crystallized from 150 ml. of methanol and then from 25 ml. of 96% ethanol, giving 0.25 g. of N-butylalanine, m.p. (closed capillary) 290–295° dec.

Anal. Calcd. for $C_7H_{15}NO_2$: N, 9.66. Found: N, 9.75.

α-(2-Isopropylhydrazino)butyric Acid (VII). A. From Isopropylhydrazine.—A solution of 2.45 g. of isopropylhydrazine oxalate in 120 ml. of water was passed through an Amberlite IRA 410 column (alkaline form) and the first 400 ml. containing 0.146 mole of isopropylhydrazine was collected. α-Bromobutyric acid (5.0 g., 0.03 mole) was added to the effluent and left to stand 4 days. The solution was worked up as usual, yielding 0.4 g. (8.3%) of VII, m.p. 212–213° (85% ethanol).

A solution of 0.4 g. of VII in 25 ml. of water was hydrogenated in the presence of 1 g. of Raney nickel (W-2) to give 0.2 g. of α-aminobutyric acid m.p. 296–298° (95% ethanol). A mixture m.p. with an authentic sample showed no depression.

Anal. Calcd. for $C_4H_9NO_2$: N, 13.55. Found: N, 13.70.

B. From α-Hydrazinobutyric Acid.—A solution of 2.35 g. (0.02 mole) of α-hydrazinobutyric acid in 100 ml. of water was treated with 1.5 ml. (0.02 mole) of acetone and 1.15 ml. (0.02 mole) of glacial acetic acid and hydrogenated in presence of 0.1 g. of platinum oxide catalyst. When the absorption of hydrogen ceased, the catalyst was filtered and the solution passed through a Duolite C 20 column and worked up as usual. The substance was purified by crystallizing from aqueous ethanol, giving 2.5 g. (76%) of VII, m.p. 212–213°. A mixture m.p. with the product prepared by A showed no depression.

Ethyl (2-Isopropylhydrazino)acetate Hydrochloride.—A solution of 15.45 g. (0.1 mole) of ethyl hydrazinoacetate hydrochloride in 300 ml. water was treated with 6.0 g. (0.104 mole) of acetone and hydrogenated in the presence of 0.2 g. of platinum oxide catalyst at atmospheric pressure until the absorption ceased. The catalyst was filtered, the filtrate evaporated to dryness, and the residue crystallized from 20 ml. of absolute ethanol, giving 9.8 g. (50%) of ethyl (2-isopropylhydrazino)acetate hydrochloride, m.p. 99°. The sample for analysis was recrystallized from 2-propanol.

Anal. Calcd. for $C_7H_{17}ClN_2O_2$: N, 14.23. Found: N, 14.40.

An attempt to hydrolyze the ester hydrochloride with Duolite C 20 (acid form) resin in order to recover the parent acid gave a yellowish crystalline product which decomposed very quickly in presence of air.

α-Isopropylaminobutyric Acid.—To a mixture of 120 g. (2 moles) of isopropylamine and the same weight of crushed ice was added in one portion 57 g. (0.33 mole) of α-bromobutyric acid. After a few days the reaction mixture was distilled to dryness, redissolved in water, passed through a Duolite C 20 column and worked up as usual.[12] The residue was dissolved in 200 ml. of water, decolorized with charcoal and, owing to strong bumping, concentrated stepwise *in vacuo* almost to dryness, yielding 39.5 g. (81%) of product, m.p. 280° (sublimation).

N-Nitroso-α-isopropylaminobutyric Acid (XXVII).—To a solution of 34.5 g. (0.25 mole) of isopropylaminobutyric acid in 100 ml. of 10% hydrochloric acid and 220 g. of crushed ice was added dropwise a solution of 21 g. (approx. 0.3 mole) of sodium nitrite in 100 ml. of water. The reaction mixture was heated and kept for 1 hr. at 70–80°, filtered and cooled in the refrigerator overnight. The crystals were filtered, washed with water, and dried in a vacuum desiccator, giving 34.0 g. of XXVII, m.p. 112–114°. On extracting the mother liquors with ether, a second crop of 5.4 g. was obtained, giving a total yield of 90%.

α-(1-Isopropylhydrazino)butyric Acid (IX).—A solution of 28.0 g. (0.16 mole) of XXVII in 250 ml. of 5% hydrochloric acid was added to about 150 g. of granulated cadmium, the mixture heated in a boiling water bath, and stirred for 5 hr. The liquid was decanted from the cadmium granules and passed through a Duolite C 20 column and worked up as usual. The crude product was boiled up with 150 ml. of absolute ethanol (the greater part, consisting of α-aminobutyric acid, remained undissolved), filtered and cooled, giving 6.4 g. (27%) of IX, m.p. 191–193° (ethanol).

α-(1-Isopropylhydrazino)propionic Acid (VIII).—To a suspension containing 15.4 g. (0.096 mole) of XXVI and 25 g. of granulated zinc was added during 2 hr. with stirring 29 ml. of glacial acetic acid at 25–30°. The reaction mixture was then heated gradually to 80°, cooled to room temperature, and the excess zinc filtered. The solution was passed through a Duolite C 20 column and worked up as usual, but a pure product could not be obtained by repeated crystallization. A solution of the crude product in 100 ml. water was shaken with 4 ml. of benzaldehyde, left to stand overnight, and extracted with ether. The ethereal extract was heated with 20 ml. of 10% hydrochloric acid and steam distilled. The residual solution was filtered, passed through a Duolite C 20 column, and worked up as usual. The residue was dissolved in water, decolorized with charcoal, the solution evaporated to dryness, and the colorless solid crystallized twice from absolute ethanol, giving 2.2 g. (16%) of VIII, m.p. 197–198° dec.

(1-Carboxypropyl)dimethylhydrazinium Hydroxide (XXII). A. From Dimethylhydrazine.—α-Bromobutyric acid (16.7 g., 0.1 mole) was added to 24 g. (0.4 mole) of unsymmetrical dimethylhydrazine with external cooling and the reaction mixture left to stand for 2 days. The excess dimethylhydrazine was distilled off, the residue dissolved in 250 ml. of water, passed through a Duolite C 20 column, and worked up as usual, giving 12.7 g. of a white product, which, on recrystallization from 40 ml. of 2-propanol, yielded 8.0 g. (49%) of XXII, m.p. 186–187°.

A solution of 0.8 g. of XXII in 50 ml. of water was hydrogenated in the presence of 3 g. Raney nickel catalyst, giving 0.4 g. of α-N,N-dimethylaminobutyric acid, m.p. 178–179° (96% ethanol) (lit.[13] m.p. 179°).

Anal. Calcd. for $C_6H_{13}NO_2$: N, 10.6. Found: N, 10.3.

B. From α-Hydrazinobutyric Acid.—Dimethyl sulfate (30.0 g., 0.24 mole) was added dropwise, with slight cooling to a solution of 5.9 g. (0.05 mole) of α-hydrazinobutyric acid and 14 g. of potassium hydroxide in 25 ml of water, followed by a second portion of 14 g. of potassium hydroxide. The reaction mixture was brought to the boil, cooled, and filtered from the separated potassium sulfate. The filtrate was passed through a Duolite C 20 column and worked up as usual. The product was crystallized from 14 ml. of 2-propanol giving 1.4 g. of XXII, m.p. 185–187°. On addition of ether to the filtrate, a second crop of 1.8 g. was obtained, giving a total yield of 39%. A mixture m.p. with the product prepared by A showed no depression.

α-(2-Benzylhydrazino)acetic Acid (X).—Chloroacetic acid (9.4 g., 0.1 mole) was added gradually with cooling and stirring to a mixture of 65.0 g. (0.54 mole) of benzylhydrazine and 50 ml. of water. The mixture was left to stand at room temperature for 7 days, diluted with 200 ml. of water, and passed through an Amberlite IRC 50 column (acid form). The effluent was concentrated to 500 ml., passed through a Duolite C 20 column and worked up as usual. The residue was crystallized from 50 ml. of 2-propanol to give 7.0 g. (38.8%) of X, m.p. 144–146° dec. A second crystallization from 2-propanol raised the m.p. to 146.5–147° dec.

α-(2-Benzylhydrazino)butyric Acid (XII).—α-Bromobutyric acid (16.7 g., 0.1 mole) was added gradually with stirring to a mixture of 61 g. (0.5 mole) of benzylhydrazine and 50 ml. of water and allowed to stand at room temperature for 1 day. The white precipitate, was filtered, washed with water until free from bromide ions, and dried at 50°. The crude product was crystallized from 160 ml. of water, yielding 4.4 g. (40.4%) of XII, m.p. 211–213° dec.

XII, on reductive cleavage with Raney nickel (W-2) in ethanol as described under XVI, gave benzylamine hydrochloride (m.p.

(10) Product of Girdler Catalysts, Louisville Ky.; equally suitable is Raney nickel catalyst W-2, prepared according to R. Mozingo, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 181.

(11) Manufactured by the Permutit Co., Ltd., Permutit House, Gunnersbury Avenue, London W. 4.

(12) The concentration of large volumes of eluates was very conveniently carried out in the cyclone evaporator of Messrs. Quickfit and Quartz Ltd., Heart of Stone, Staffordshire, England.

(13) M. Duvillier, *Bull. Soc. Chim., France* **35**, 156 (1906).

and mixture m.p. with authentic sample, 257°) and α-ethylaminobutyric acid m.p. 248–250° dec. in good yields.

α-[2-(β-Hydroxyethyl)hydrazino]butyric Acid (XVI).—α-Bromobutyric acid (33.4 g., 0.2 mole) was added gradually with cooling to a solution of 60.8 g. (0.8 mole) of β-hydroxyethylhydrazine, b.p. 100–105° (1 mm.) in 100 ml. of water, and the mixture allowed to stand at room temperature for 5 days. The solution was passed through a Duolite C 20 column and eluted as usual. The eluate was concentrated to 200 ml. and passed through an Amberlite IRC 50 column, the effluent evaporated to dryness *in vacuo* and the residue crystallized from 300 ml. of 2-propanol, giving 4.4 g. (13.6%) of XVI, m.p. 133–134° dec.

Reductive Cleavage with Raney Nickel. In Ethanol.—A suspension of 1.9 g. of XVI in 50 ml. of ethanol and 10 g. of Raney nickel catalyst was refluxed for 5 hr. in a reaction flask equipped with an ethanolic HCl trap. The catalyst was filtered, the alcoholic liquors distilled into the ethanolic HCl, and the latter evaporated to dryness. The product (0.8 g.) was crystallized from a mixture of ethanol–ether (1:1), giving ethylaminoethanol hydrochloride, m.p. 225–230°.

Anal. Calcd. for $C_4H_{12}ClNO$: N, 11.24; Cl, 28.51. Found: N, 11.50; Cl, 28.20. The Raney nickel catalyst was combined with the alcohol residue, boiled with 150 ml. of water, and filtered. The green solution was diluted with 300 ml. of water and passed through an Amberlite IRC 50 column, the effluent evaporated to dryness and the residue crystallized from methanol, giving 0.4 g. of N-ethylaminobutyric acid, m.p. 248–252° dec. (sublimation).

Anal. Calcd. for $C_6H_{13}NO_2$: N, 10.68. Found: N, 10.80.

In Water.—XVI (1.6 g.) in 100 ml. of water was hydrogenated in the presence of 5 g. of Raney nickel catalyst at atmospheric pressure. The filtrate was neutralized with 8 ml. of 10% hydrochloric acid, evaporated to dryness, the residue dissolved in 50 ml. of water, filtered, and passed through an Amberlite IRC 50 column. The effluent was passed through a Duolite C 20 column and eluted with 400 ml. of 4% ammonia. The eluate was evaporated to dryness and the residue crystallized from 100 ml. of aqueous ethanol, giving 0.4 g. of α-aminobutyric acid, m.p. 298–300° dec. (sublimation).

Anal. Calcd. for $C_4H_9NO_2$: N, 13.58. Found: N, 13.25.

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Phosphorylated Alkylating Agents Related to DL-Phenylalanine[1]

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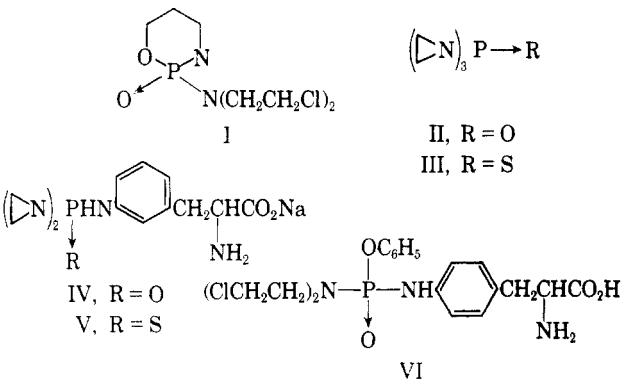
A group of alkylating agents has been prepared which use 3-(*p*-aminophenyl)propionic acid and 3-(*m*- and *p*-aminophenyl)-DL-alanine as the carrier moiety for a number of phosphorylated and thiophosphorylated ethylenimines and bis(2-chloroethyl)amines. Methods were devised for removing the blocking groups used in these syntheses so that the alkylating groups remained intact. Several of the thiophosphorylated ethylenimines were active antitumor agents according to testing results with Walker 256 carcinosarcoma.

A number of phosphorylated alkylating agents, among which are 2-[bis(2-chloroethyl)-amino]-2*H*-1,3,2-oxazaphosphorinane 2-oxide[2] (Cytoxan) (I), tris-(1-aziridinyl)phosphine oxide[3] (TEPA) (II), and tris-(1-aziridinyl)phosphine sulfide[4] (thio-TEPA) (III), have been synthesized and used clinically[5a–c] against cancer. It has been suggested[5d,e] that I and its analogs have latent activity until hydrolyzed by the phosphamidases, present in high concentrations in tumor tissue, to release the cytotoxic nitrogen mustard. A number of modifications of I, II, and III have been synthesized with the phosphorylated alkylating groups attached to various carrier moieties in an attempt to obtain greater selectivity of antitumor action. Because of the importance of nitrogen mustards of the phenylalkanoic acids (*e.g.*, chlorambucil)[6] and of phenylalanine (*e.g.*, sarcolysin),[7] it was considered of interest to use these aromatic moieties as carriers for the phosphorylated alkylating groups. This article describes the preparation and antitumor evaluation of a number of such compounds typified by IV, V, and VI.



Knunyants, *et al.*,[8] have prepared examples of these compounds based on phenylacetic acid and phenylalanine in which certain of the functional groups were left in the blocked state. We were interested in preparing compounds which possessed the free carboxyl and free amino acid functional groups.

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