The major constituent of the ketone was established as the 6- rather than the 7-keto derivative by formation of a monobenzal derivative, no dibenzal derivative being isolated.

The ketone, 470 mg. (3.17 mmol.) was dissolved in 5 ml. of 5% potassium hydroxide in methanol[9] and 1.1 ml. of benzaldehyde was added. After 20 hr. the mixture was diluted with 11 ml. of water and extracted with 10 ml. of hexane. The hexane layer was dried over magnesium sulfate and concentrated. Excess benzaldehyde was removed by maintaining the residue at 60° (0.25 mm.) for several hours. The cooled semicrystalline residue was recrystallized twice from 3 ml. portions of hexane to give 0.151 g. (0.639 mmol., 20.1%) of pale yellow monobenzal derivative, m.p. 74.0–77.0°.

Anal. Calcd. for $C_{17}H_{16}O$: O, 6.77. Found: O, 6.63.

The infrared spectra of all compounds encountered in this investigation were measured routinely and supported the proposed structures. In particular, these nortricyclene derivatives absorbed at 12.3–12.4 microns, as first noted by Roberts and co-workers.[10]

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α-Hydrazino- Acids. I. α-Hydrazinoaliphatic Acids and α-(1-Methylhydrazino)aliphatic Acids

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An improved method for the preparation of α-hydrazino-aliphatic acids is presented. The reaction is extended to the preparation of α-(1-methylhydrazino)-aliphatic acids, the structures of which are proved. The ultraviolet spectra are described.

Thiele[1] prepared α-hydrazinoisobutyric acid by the action of acetone semicarbazone on hydrocyanic acid. He also synthetized[2] α-hydrazinopropionic acid starting from acetaldehyde. Traube *et al.*[3] described the preparation of a series of α-hydrazino-aliphatic acids by the reduction of the isonitramino acids. Darapski *et al.*,[4] Bailey,[5] and Berger[6] obtained these compounds by the action of α-bromo acids on excess hydrazine in alcohol or in water.

Our interest in these compounds and their derivatives stems from their possible use as antimetabolites, especially in cancer chemotherapy.

We found the Darapski method convenient for the preparation of some of the higher members, as these hydrazino acids crystallize directly from the aqueous reaction mixture in fair yield. However, the preparation of the α-hydrazino acetic, propionic, and isobutyric acids is extremely cumbersome, requiring a Fischer esterification and a subsequent tedious saponification with barium hydroxide.

We found that by the use of ion exchange resins the isolation of these compounds is considerably simplified and the yields increased. When a weak anion exchanger is used, the addition of acetone is necessary to bind the hydrazine in the form of the ketazine, thus making the acid available for absorption on a weak anion exchanger (Method A).

The hydrazino acids are also absorbed on strong cation exchange resins, from which they can be eluted with ammonia and recovered by evaporation of the effluent (Method B). The yields by both methods are almost identical.

However, hydrazineacetic acid is not obtained directly in the highest purity by these methods. The preparation of the ester hydrochloride is still required, but saponification is conveniently carried out by a strong cation exchanger.

Berger[6] made a thorough study of the reaction between α-bromo-α-ethylbutyric acid and hydrazine and obtained α-hydroxy-α-ethylbutyric acid as the only product. He also failed to prepare the hydrazino diethylacetic acid by other methods and attributed the "non-formation" of this hydrazino acid to the influence of the tertiary carbon

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TABLE I

α-1-METHYLHYDRAZINO ACIDS

Parent Acid[a]	M.P.,[b] °C.	Yield, %	Formula	Analysis N, % Calcd.	Analysis N, % Found[c]	m-Nitrobenzal Hydrazones M.P., °C.[b]
1. Acetic[d]	153–154	54	$C_3H_8N_2O_2$	26.9[e]	26.9	128–130
2. Propionic[d]	145	42	$C_4H_{10}N_2O_2$	23.7	23.7	115–117
3. n-Butyric[f]	122–124	47	$C_5H_{12}N_2O_2$	21.2	21.4	82–83
4. Isobutyric[f]	163–165	20	$C_5H_{12}N_2O_2$	21.2	21.2	121–123
5. n-Valeric[f]	123–124	28	$C_6H_{14}N_2O_2$	19.2	19.0	83–85
6. Isovaleric[f]	188–191	33	$C_6H_{14}N_2O_2$	19.2	19.3	74–75
7. n-Caproic[f]	130–132	27	$C_7H_{16}N_2O_2$	17.5	17.5	88–90
8. Isocaproic[g]	146–148	25	$C_7H_{16}N_2O_2$	17.5	17.8	85–87

[a] Procedures for representative compounds are described in the experimental part. [b] Uncorrected. [c] Analyses by Mr. E. Meier of the Microanalytical Department, Weizmann Institute of Science, Rehovoth, Israel. [d] Crystallized from absolute ethanol. Calcd.: C, 34.7; H, 7.74. Found: C, 34.5; H, 7.74. [f] Crystallized from isopropyl alcohol. [g] Crystallized from water.

atom in the α-position. The ready formation of α-hydrazino isobutyric acid was regarded by Berger as an exception.

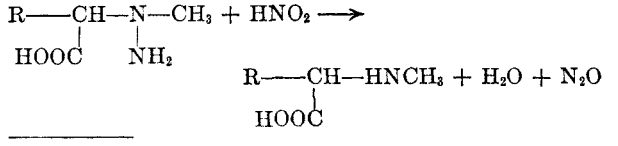
On repeating Berger's experiment and using our procedure we obtained α-hydrazino-α-ethylbutyric acid in fair yield, while the α-hydroxy acid and α-ethylcrotonic acid were side products. The latter seems to be formed directly by the dehydrobromination of α-bromo-α-ethylbutyric acid.

Working with a large excess of hydrazine in the cold the yield of α-hydrazino-α-ethylbutyric acid increases considerably.

α-Hydrazino-α-ethylbutyric acid shows properties similar to those of α-hydrazinoisobutyric acid.[1] At pressures of *ca.* 1 mm. it sublimes without decomposition (also observed by us in the case of hydrazinoisobutyric acid).

The above procedure was extended to the reaction between α-halo acids and methylhydrazine. Considering the electron-donating properties of the methyl group[7] it was expected that substitution would occur at the methyl bearing nitrogen. However, in view of the known exceptions to this rule,[8] we proceeded to prove the structure of the compounds by independent means. Attempts to reduce the N—N linkage with chromous chloride, titanous chloride and other reducing agents proved unsuccessful.

One line of evidence relies on the reaction with nitrous acid, in which gas is evolved and the corresponding *N*-methylamino acid isolated from the solution in almost theoretical yield. This was proved for α-(1-methylhydrazino)-acetic and -propionic acids. In analogy to the action of nitrous acid on *N,N*-diethylhydrazine[9] the reaction may be represented by the following equation:



Confirmation of the structure was obtained by the reductive cleavage of the N—N bond with a large excess of Raney nickel in alcohol, a method used by Ainsworth and by Hinman.[10] All the compounds tested, *viz.* α-(1-methylhydrazino)-acetic, -propionic and -butyric acids gave ammonia in 80–90% yield.

The above proves conclusively that the compounds obtained by reaction of the halo acids with methylhydrazine have both substituents on the same nitrogen and are essentially free from isomers.

In view of this evidence it is surprising that it was not always possible to obtain the common hydrazones with benzaldehyde, salicylaldehyde etc. However, the *m*-nitrobenzaldehyde derivatives were readily obtained in good yield.

We have prepared the α-(1-methylhydrazino) acids described in Table I. All the compounds listed dissolve readily in water, giving practically neutral solutions, as can be expected from zwitter ions. The compounds are unstable in presence of air and reduce iodine and Fehling's solutions readily.

All the α-(1-methylhydrazino)-aliphatic acids and some α-hydrazino acids were submitted to the Sloan Kettering Institute for Cancer Research, New York, for tests.

Ultraviolet Spectra. The absorption of α-hydrazino and α-(1-methylhydrazino)-aliphatic acids was measured in the region between 220–350 mμ. Aliphatic acids, amino acids, and various hydrazine derivatives show no characteristic absorption in this region.[11–14] The α-hydrazino acids fall in line with these compounds (λ_{max} 276–278 mμ, ϵ_{max} 3–5).

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However the straight chain α-(1-methylhydrazino)-acids unexpectedly possess an appreciable absorption coefficient, and the absorption maximum shifts to longer wave lengths with increasing molecular weight (λ_{max} 285–332 mμ, ϵ_{max} 69–140). The corresponding branched chain acids absorb at shorter wave lengths and with much lower intensity (λ_{max} 299–325 mμ, ϵ_{max} 6–63).

EXPERIMENTAL[15]

Ethyl hydrazineacetate hydrochloride. To 500 g. (5 mol.) of 32% aqueous hydrazine was added gradually 94.5 g. (1 mol.) monochloroacetic acid. After 48 hr., 85 g. (approx. 2 mol.) sodium hydroxide was added and the solution distilled *in vacuo* to dryness (4 mol. of hydrazine was recovered). The residue was treated with 650 ml. ethanolic HCl (approx. 30% w./v.), refluxed gently, cooled, and saturated with gaseous HCl for 2 hr. The solution was boiled with 500 ml. absolute alcohol and filtered hot. The ester hydrochloride crystallized, yielding 103 g. (66.5%), m.p. 150–152° (lit. 150–153°[3]).

Hydrazineacetic acid. A solution of 46.5 g. (0.3 mol.) ethyl hydrazineacetate hydrochloride in 350 ml. water was refluxed with stirring in the presence of 15 g. Duolite C 20[16] (acid form) for 3 hr. The filtered solution was passed through a Duolite A 7[16] column (alkaline form), the effluent concentrated *in vacuo* to 50 g. and added dropwise into 250 ml. absolute alcohol. The crude acid was recrystallized from 20 ml. hot water and dried *in vacuo* over P_2O_5. The yield was 10 g. (37%), m.p. 152° (lit. 152° dec.[3]). From the mother liquor an additional 10 g., m.p. 145–147° was precipitated by ethanol.

Method A: α-hydrazinopropionic acid. To 150 ml. (2.3 mol.[17]) of 50% aqueous hydrazine was added gradually 51 g. (0.33 mol.) of α-bromopropionic acid and the mixture was allowed to stand for 24 hr. Excess hydrazine was distilled *in vacuo*. The residue was dissolved in 600 ml. water and 100 ml. acetone was added, lowering the *p*H from 8 to 4. The solution was passed through a Duolite A 7 column and concentrated *in vacuo* to dryness. The residue was dissolved in 40 ml. water and added dropwise into 500 ml. of absolute alcohol. The yield was 15 g. (48%), m.p. 182° (lit. 180°[5]). A second crop of 4 g. was obtained from the mother liquors. The *m*-nitrobenzal derivative crystallized from aqueous alcohol, m.p. 118–120°.

Method B: α-hydrazinoisobutyric acid. To 100 ml. (0.5 mol.) 16% aqueous hydrazine was added gradually 16.7 g. (0.1 mol.) of α-bromoisobutyric acid and allowed to stand for 48 hr. Excess hydrazine was distilled *in vacuo*, the residue dissolved in 200 ml. water and passed through a Duolite C 20 column. On evaporating the acid effluent 1.1 g. of a crystalline solid, m.p. 77–79° was isolated, which was identified as α-hydroxyisobutyric acid. After washing the column with water to neutrality, 700 ml. 4% ammonia was passed and the eluate concentrated to dryness. The solid residue was dissolved in 15 ml. water and 50 ml. absolute alcohol added, giving 3.6 g. (30.5%) product, m.p. 238–240° (lit. 237°[4]).

Method B: α-hydrazino-α-ethylbutyric acid. One tenth of a mole (19.5 g.) α-bromo diethylacetic acid was added dropwise with cooling to 64 g. (1 mol.) of 50% aqueous hydrazine. The reaction mixture was worked up as described under α-hydrazinoisobutyric acid. The acid effluent yielded 0.8 g. (6%) α-hydroxy-α-ethylbutyric acid, m.p. 79–80° (lit. 79–80°[18]). A mixed m.p. with an authentic sample showed no depression. From the alkaline eluate 10.3 g. (70%) α-hydrazino-α-ethylbutyric acid was obtained, m.p. (closed capillary) 225°.

Anal. Calcd. for $C_6H_{14}N_2O_2$: C, 49.3; H, 9.7; N, 19.2; O, 21.9. Found: C, 48.8; H, 9.7; N, 19.5; O, 22.4.

The *m-nitrobenzal derivative* melted at 112–114°.

Berger's procedure: α-hydrazino-α-ethylbutyric acid. To 32 g. (0.5 mol.) 50% aqueous hydrazine and 43 ml. of water was added dropwise 30 g. (0.154 mol.) α-bromodiethylacetic acid. The temperature rose to 70°. After 24 hr. the mixture was acidified with dilute hydrochloric acid to Congo red and 4.2 g. of an oil separated. This oil was brominated in carbon tetrachloride at −10°, whereby 2.95 g. of bromine was absorbed, giving, after crystallization from ether-ligroin, 2.5 g. of 2,3-dibromo-2-ethylbutyric acid, m.p. 116–117° (lit. 116.5°[19]), identified by a mixed m.p. with an authentic sample. The aqueous layer was extracted with ether, which on evaporation left 8.1 g. residue. By crystallization from petroleum ether 5.3 g. α-hydroxydiethylacetic acid was isolated. From the petroleum ether mother liquors 0.9 g. of an oil was recovered, which yielded on bromination 2.3 g. 2,3-dibromo-2-ethylbutyric acid.

The extracted aqueous layer was then passed through Duolite C 20 (acid form) and worked up as described under "Method B," yielding 8.3 g. (37%) of α-hydrazino α-ethylbutyric acid, m.p. 225°, identical with the compound obtained above.

Method A: α-(1-methylhydrazino)acetic acid. To 36 g. (0.76 mol.) methylhydrazine in 200 ml. water was added gradually 13.5 g. (0.143 mol.) monochloroacetic acid and left at room temperature for 4–5 days. The solution was worked up as described under α-hydrazinopropionic acid. The product was crystallized from absolute ethanol. Yield 8 g. (54.5%); m.p. 153–154°.

Method B: α-(1-methylhydrazino)isovaleric acid. To 18.5 g. (0.4 mol.) methylhydrazine in 200 ml. water was added gradually 18.2 g. (0.10 mol.) α-bromoisovaleric acid and left at room temperature. After 3 days, the solution was worked up as described under method B for α-hydrazinoisobutyric acid. The product was crystallized from 20 ml. water. Yield 33%.

Reaction of α-(1-methylhydrazino)propionic acid with sodium nitrite. A solution of 2.6 g. (0.022 mol.) α-(1-methylhydrazino)propionic acid in 20 ml. water was treated with 2 ml. acetic acid and 2.5 g. (0.018 mol.) sodium nitrite in 10 ml. water. After 30 min. the solution was made up to 50 ml. and passed through a column of Duolite C 20 (acid form). The alkaline eluate was concentrated *in vacuo*. A solid was obtained, which was identified by mixed m.p. (300–303° subl.) as *N*-methylalanine. Yield 1.5 g. (63%).

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(15) All melting points are uncorrected.

(16) Manufactured by Chemical Process Co., Redwood City, Calif., U.S.A.

(17) The concentrations of hydrazine and methylhydrazine have, as far as we could observe, little effect on the yields.

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