When the purity of squalene is examined by comparison of its specific activity with that of its hexahydrochloride, it was found in earlier work that the hexahydrochloride had a higher specific activity which varied considerably from that of the crude squalene from which it was derived.[5] The additional purification employed in these experiments reduces this difference to approximately 8%.

The fact that no color was observed in 90 seconds in the Liebermann–Burchard reaction on the sterol digitonides of human scalp skin tissue indicates that this material contains less than 10% of sterols, that are "fast-acting." Baumann and Moore[17] have found 22 to 36% of fast acting sterols in rat skin tissue lipids. The color developed at 33 minutes for human skin tissue indicates a cholesterol content of 66%. The amount of digitonin precipitable material available was insufficient for a purification of cholesterol *via* its dibromide derivative. However, in order to controvert the argument for the precursor relationship of squalene to cholesterol, less than 10% of the digitonin precipitable matter would have to be cholesterol and the remainder non-radioactive material, assumptions which do not appear likely.

Of the minor components of the human skin tissue lipid unsaponifiable matter, the fraction of the chromatograph corresponding to the wax alcohols contained radioactivity. This suggests that the alcohols are at least in part derived from acetate. This fraction is being investigated further.

In both expts. I and II a small but definite quantity of lipid was found in the petroleum ether eluate of the silica gel chromatograph. In the analysis of human hair fat[22] this material was found to consist of saturated hydrocarbons.[23] Since no radioactivity could be detected in this material, it must be assumed that under the conditions of this experiment, acetate is not utilized as such for the synthesis of their carbon skeletons. Further investigation is needed to decide whether these materials are truly endogenous.

(22) N. Nicolaides and S. Rothman, *J. Invest. Dermatol.*, **21**, 9 (1953).

(23) Schwenk, *et al.*, (ref. 8) also separated non-radioactive hydrocarbons from squalene in their pig liver perfusion experiments.

CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

1,2,4-Triazole-3-alanine

BY R. G. JONES AND C. AINSWORTH

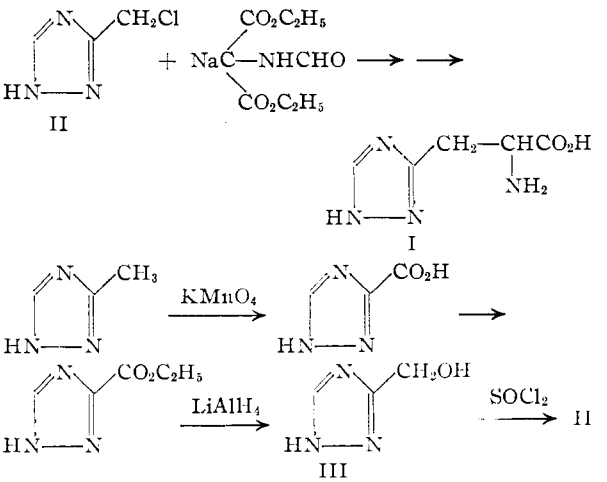
RECEIVED OCTOBER 25, 1954

1,2,4-Triazole-3-alanine has been synthesized and found to be an antagonist of histidine when tested against certain strains of *E. coli*. 1,2,4-Triazole and formalin gave 3-hydroxymethyl-1,2,4-triazole in moderate yield.

A number of α-aminocarboxylic acids related to histidine have been synthesized in this Laboratory and tested for chemotherapeutic activity against bacteria, viruses and cancers. One objective has been to find antagonists of histidine. Of some twenty compounds in which the imidazole nucleus of histidine was replaced by variously 1-substituted imidazoles,[1] thiazoles,[2] pyrazoles[3] and other hetero cycles, only one compound, 2-thiazolealanine,[2] has shown any appreciable activity as a growth inhibitor for *E. coli*. The inhibition was reversed by addition of histidine to the culture medium. This communication reports the synthesis of another amino acid, 1,2,4-triazole-3-alanine (I), which appears to be a specific histidine antagonist when tested against certain strains of *E. coli*.

Compound I was obtained in 70% yield by the reaction of 3-chloromethyl-1,2,4-triazole (II) with the sodium derivative of N-formylaminomalonic ester according to previously published directions for this general method.[4] The chief problem encountered in the synthesis of I was the preparation of the intermediate 3-hydroxymethyl-1,2,4-triazole (III) from which II was obtained by reaction with thionyl chloride.

The first method used in obtaining III was a straightforward series of reactions starting from 3-methyl-1,2,4-triazole. The latter was oxidized with permanganate to 1,2,4-triazole-3-carboxylic acid. This was esterified, and the ester was reduced with lithium aluminum hydride. Although reasonably good yields were obtained in each step, this sequence was cumbersome because of the number of steps involved.



A second and more direct synthesis of III was from the readily available 1,2,4-triazole (IV)[5] by

(1) R. G. Jones and K. C. McLaughlin, THIS JOURNAL, **71**, 2444 (1949).

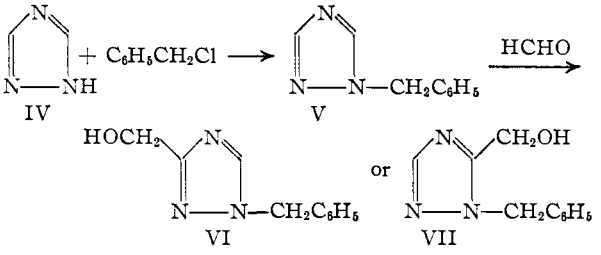
(2) R. G. Jones, E. C. Kornfeld and K. C. McLaughlin, *ibid.*, **72**, 4526 (1950).

(3) R. G. Jones, *ibid.*, **71**, 3994 (1949).

(4) H. R. Snyder, J. F. Shekleton and C. D. Lewis, *ibid.*, **67**, 310 (1945).

(5) C. Ainsworth and R. G. Jones, *ibid.*, **77**, 621 (1955).

condensation with formaldehyde. In initial experiments IV was converted to 1-benzyl-1,2,4-triazole (V) which, by heating with aqueous formaldehyde, gave a good yield of a single hydroxymethyl-1-benzyl-1,2,4-triazole. Presumably the structure is either VI or VII. Structure VII is preferred because of analogy with 1-benzyl-2-hydroxymethylimidazole[6] which is the product obtained from 1-benzylimidazole and formaldehyde.



After the reaction of V with formalin had been studied, it was found that IV also underwent condensation with formalin when heated in a closed vessel to give a moderate yield (about 30%) of III.

A third but quite unsatisfactory route leading to I was based on 3-ethoxymethyl-1,2,4-triazole. This was cleaved by boiling with hydrobromic acid. The resulting hydroxymethyl or bromomethyl compound was not purified but was treated with thionyl chloride followed by sodium formylaminomalonic ester as described above. The over-all yield of I was poor. Unexpectedly, 3-methoxymethyl- and 3-phenoxymethyl-1,2,4-triazole were not cleaved by boiling with 30% hydrogen bromide in acetic acid.

Acknowledgments.—The authors are grateful to Mrs. Marjorie J. Mann for valuable assistance, to W. L. Brown, H. L. Hunter, G. M. Maciak and G. Beckmann for the analyses, and to Dr. H. E. Boaz for physical-chemical measurements.

Experimental[7]

3-Methyl-1,2,4-triazole.—1-Acetylthiosemicarbazide was prepared by the addition of 393 g. (5 moles) of acetyl chloride to a stirred suspension of 455 g. (5 moles) of thiosemicarbazide and 5 l. of dry pyridine. The temperature was maintained between −5 and 0° throughout the addition. After standing at room temperature overnight most of the pyridine was removed by evaporation under reduced pressure. To effect cyclization to 3-methyl-1,2,4-triazole-5-thiol the residue was dissolved in 3 l. of methanol, and 540 g. (10 moles) of sodium methylate was added. The mixture was heated overnight on the steam-bath, and then the solvent was removed by evaporation under reduced pressure. This residue was dissolved in 1 l. of water, the solution was decolorized with charcoal, and 1 l. of concentrated hydrochloric acid was added; a solid precipitated. It was collected on a filter, washed with water and air-dried; the yield was 475 g. (83%). A sample was recrystallized from water and obtained as shiny plates, m.p. 282–283° (lit.[8] m.p. 260–261°).

Anal. Calcd. for $C_3H_5N_3S$: C, 31.29; H, 4.38; S, 27.85. Found: C, 31.26; H, 4.64; S, 27.85.

The mercapto group of 3-methyl-1,2,4-triazole-5-thiol was removed by nitric acid oxidation of 100-g. portions according to the procedure for the preparation of 3β-phthalimidoethyl-1,2,4-triazole.[9] Since 3-methyl-1,2,4-triazole was soluble in water the basified reaction mixture was evaporated to dryness by heating under reduced pressure. The residue was extracted with ethanol. After evaporation of the solvent the product was distilled under reduced pressure and 44–65 g. (61–90% yield) of 3-methyl-1,2,4-triazole was obtained. It came over at 138° (8 mm.) and solidified on standing, m.p. 94° (lit.[10] m.p. 95°).

Ethyl 1,2,4-Triazole-3-carboxylate.—1,2,4-Triazole-3-carboxylic acid was prepared from a solution of 83 g. (1 mole) of 3-methyl-1,2,4-triazole, 3 l. of water and 316 g. (2 moles) of potassium permanganate. After heating on the steam-bath overnight the manganese dioxide was removed by filtration. The filtrate was concentrated to about 150 ml. by heating under reduced pressure. It was placed in a large beaker with 500 g. of ice, and concentrated hydrochloric acid was added to *p*H 1. The solid which separated was collected on a filter, washed with water and air-dried. The yield of 1,2,4-triazole-3-carboxylic acid was 60%. It melted with decomposition at 135–137° (lit.[11] m.p. 137° dec.).

A mixture of 34 g. (0.3 mole) of 1,2,4-triazole-3-carboxylic acid and 500 ml. of ethanol saturated with hydrogen chloride at 0° was allowed to stand at room temperature for three days. After removal of the solvent the residue was treated with 200 ml. of saturated aqueous sodium bicarbonate solution and the solid ester which separated was collected by filtration. An additional quantity of ester was obtained by extraction of the filtrate with two 200-ml. portions of ethyl acetate. The product was recrystallized from ethanol and obtained as plates, m.p. 178°, yield 26 g. (61%).

Anal. Calcd. for $C_5H_7N_3O_2$: C, 42.55; H, 5.00; N, 29.78. Found: C, 42.33; H, 4.81; N, 29.79.

When the mixture was heated during esterification, the yield was much lower due to decarboxylation of the acid.

3-Hydroxymethyl-1,2,4-triazole Hydrochloride (III). (a) Lithium Aluminum Hydride Reduction of Ethyl 1,2,4-Triazole-3-carboxylate.—To a suspension of 8.5 g. (0.225 mole) of lithium aluminum hydride and 200 ml. of tetrahydrofuran was added a solution of 32 g. (0.225 mole) of ethyl 1,2,4-triazole-3-carboxylate in 500 ml. of hot tetrahydrofuran. After heating under reflux for two hours, 50 ml. of 50% aqueous methanol was added cautiously. The resulting mixture was filtered and the filter cake was extracted with 500 ml. of hot methanol followed by 500 ml. of hot water. The combined extracts, including the tetrahydrofuran, were evaporated to dryness by heating under reduced pressure. The residue was dissolved in 500 ml. of ethanol and saturated with carbon dioxide by the addition of Dry Ice. Evaporation of the mixture to dryness by heating under reduced pressure left a residue that was extracted with 200 ml. of hot ethanol. Addition of hydrogen chloride followed by anhydrous ether to this ethanol solution caused 3-hydroxymethyl-1,2,4-triazole hydrochloride to separate as white plates, m.p. 150–153°. The yield was 23 g. (75%).

Anal. Calcd. for $C_3H_5N_3O{\cdot}HCl$: C, 26.58; H, 4.46. Found: C, 26.74; H, 4.62.

(b) **1,2,4-Triazole and Formalin.**—A solution of 6.9 g. (0.1 mole) of 1,2,4-triazole[5] and 20 ml. of formalin was heated at 130° in a sealed tube for 20 hours. The mixture was distilled under reduced pressure and 3 g. (30% yield) of 3-hydroxymethyl-1,2,4-triazole came over at about 140° (1 mm.). It was converted to the hydrochloride and recrystallized from ethanol–ether, m.p. 150°.

Anal. Calcd. for $C_3H_5N_3O{\cdot}HCl$: N, 31.00. Found: N, 31.12.

The 3-hydroxymethyl-1,2,4-triazole hydrochloride samples prepared by the two methods described above had identical infrared absorption spectra.

3-Chloromethyl-1,2,4-triazole Hydrochloride (II).—A mixture of 6.8 g. (0.05 mole) of 3-hydroxymethyl-1,2,4-triazole hydrochloride and 100 ml. of thionyl chloride was heated under reflux for three hours. The reaction mixture was cooled and 500 ml. of dry ether was added causing precipitation of a white solid. The yield was 7.7 g. (100%). A sample was recrystallized from ethanol–ether mixture, m.p. 115–116°.

Anal. Calcd. for $C_3H_4ClN_3{\cdot}HCl$: C, 23.40; H, 3.27; N, 27.29. Found: C, 23.55; H, 3.38; N, 27.42.

1,2,4-Triazole-3-alanine (I).—To a solution formed from 2.3 g. (0.1 g. atom) of sodium and 100 ml. of absolute eth-

(6) R. G. Jones, THIS JOURNAL, **71**, 383 (1949).
(7) Melting points were determined with a Fisher–Johns apparatus.
(8) M. Freund, *Ber.*, **29**, 2486 (1896).
(9) C. Ainsworth and R. G. Jones, THIS JOURNAL, **75**, 4915 (1953).
(10) G. Pellizzari, *Gazz. chim. ital.*, [2], **41**, 36 (1911).
(11) J. A. Bladin, *Ber.*, **25**, 744 (1892).

anol was added 10.2 g. (0.05 mole) of diethyl formylaminomalonate. With stirring 7.7 g. (0.05 mole) of 3-chloromethyl-1,2,4-triazole hydrochloride dissolved in 100 ml. of ethanol was added. During the addition the temperature was maintained below 10°. After standing for six hours at room temperature, the alcohol was removed by warming under reduced pressure, and the residue was dissolved in 200 ml. of 2 *N* hydrochloric acid. The solution was extracted with ethyl acetate to remove any unchanged diethyl formylaminomalonate. The aqueous layer was made basic with sodium carbonate and extracted with ether followed by ethyl acetate. After evaporation of the organic extracts the oil remaining was heated on the steam-bath overnight with 50 ml. of concentrated hydrochloric acid. The solvent was removed by heating under reduced pressure and the resulting amino acid hydrochloride dissolved in 100 ml. of ethanol. Addition of 5 ml. of aniline caused the amino acid to separate. The solid was collected on a filter and dissolved in 5 ml. of hot water to which was added about 100 ml. of ethanol. 1,2,4-Triazole-3-alanine separated as a white solid, m.p. 263–264° dec., pK'_a 2.1, 8.4 and 10.7 (water). The yield was 5.5 g. (70%).

Anal. Calcd. for $C_5H_8N_4O_2$: C, 38.46; H, 5.16; N, 35.88. Found: C, 38.38; H, 5.28; N, 35.48.

3-Phenoxymethyl-1,2,4-triazole.—1-Phenoxyacetyl thiosemicarbazide was obtained from phenoxyacetyl chloride[12] and thiosemicarbazide in dry pyridine according to the procedure for the preparation of 1-β-phthalimidopropionyl thiosemicarbazide.[9] It was recrystallized from water and obtained as needles, m.p. 215° dec. The yield was 80%.

Anal. Calcd. for $C_9H_{11}N_3O_2S$: C, 47.98; H, 4.92; N, 18.66. Found: C, 47.85; H, 4.72; N, 18.41.

3-Phenoxymethyl-1,2,4-triazole-5-thiol was prepared by ring closure of 1-phenoxyacetyl thiosemicarbazide with sodium methylate according to the procedure for obtaining 3-β-phthalimidoethyl-1,2,4-triazole-5-thiol.[9] It was recrystallized from water and obtained as plates, m.p. 224–225°, yield 65%.

Anal. Calcd. for $C_9H_9N_3OS$: C, 52.15; H, 4.38. Found: C, 51.80; H, 4.17.

3-Phenoxymethyl-1,2,4-triazole was prepared by nitric acid oxidation of the above thiol according to the procedure for the preparation of 3β-phthalimidoethyl-1,2,4-triazole.[9] It was recrystallized from benzene–petroleum ether and obtained as needles, m.p. 85°. The yield was 50%.

Anal. Calcd. for $C_9H_9N_3O$: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.95; H, 5.18; N, 24.14.

The hydrobromide salt was recrystallized from ethanol–ether and obtained as prisms, m.p. 172–175°.

Anal. Calcd. for $C_9H_9N_3O\cdot HBr$: C, 42.21; H, 3.94. Found: C, 42.38; H, 4.06.

A solution of 6 g. of 3-phenoxymethyl-1,2,4-triazole and 100 ml. of 30% hydrogen bromide in acetic acid was heated under reflux overnight. After removal of the solvent by heating under reduced pressure the residue was recrystallized from ethanol–ether. The product (5 g.) was identical with 3-phenoxymethyl-1,2,4-triazole hydrobromide above.

3-Methoxymethyl-1,2,4-triazole.—By the same procedure used above methoxyacetyl chloride[13] was condensed with thiosemicarbazide. The product was ring closed directly with sodium methylate and 3-methoxymethyl-1,2,4-triazole-5-thiol was obtained in 54% over-all yield. It was recrystallized from 95% ethanol and separated as plates, m.p. 185–187°.

Anal. Calcd. for $C_4H_7N_3OS$: C, 33.09; H, 4.86; S, 22.09. Found: C, 33.29; H, 4.88; S, 21.72.

3-Methoxymethyl-1,2,4-triazole was distilled under reduced pressure and came over at about 130° (1 mm.). It solidified on standing. A sample was recrystallized from ether–petroleum ether and obtained as needles, m.p. 65–66°. The yield was 40%.

Anal. Calcd. for $C_4H_7N_3O$: C, 42.47; H, 6.24. Found: C, 42.55; H, 6.21.

3-Ethoxymethyl-1,2,4-triazole.—3-Ethoxymethyl-1,2,4-triazole-5-thiol was prepared by the same procedure as the above homolog starting from ethoxyacetyl chloride.[13] It was recrystallized from ethyl acetate–petroleum ether and obtained as prismatic needles, m.p. 130–131°. The over-all yield was 42%.

Anal. Calcd. for $C_5H_9N_3OS$: S, 20.14. Found: S, 20.29.

3-Ethoxymethyl-1,2,4-triazole was distilled under reduced pressure, b.p. 118° (0.5 mm.). It solidified on standing, m.p. 54°.

Anal. Calcd. for $C_5H_9N_3O$: N, 33.05. Found: N, 33.08.

A solution of 20 g. of 3-ethoxymethyl-1,2,4-triazole and 300 ml. of 48% hydrobromic acid was heated under reflux overnight. After removal of the solvent the residue was heated for two hours with thionyl chloride. The excess thionyl chloride was removed by evaporation under reduced pressure, and the resulting residue was dissolved in 25 ml. of cold ethanol. Addition of 100 ml. of ether caused the product to separate as an oil which on long standing solidified. The yield of hygroscopic solid was about 8 g. It was added to a cold solution formed from 2.3 g. of sodium, 10.2 g. of diethyl formylaminomalonate and 100 ml. of ethanol. 1,2,4-Triazole-3-alanine was isolated by the same procedure reported above. The yield was 1.5 g.

1-Benzyl-1,2,4-triazole (V).—A solution formed from 25 g. (1.1 g. atom) of sodium, 200 ml. of ethanol, 69 g. (1 mole) of 1,2,4-triazole[5] and 190 g. (1.5 moles) of benzyl chloride was heated to boiling and then stirred for one hour without external heating. The mixture was heated under reflux for one hour and then allowed to stand overnight at room temperature. Sodium chloride was removed by filtration and the filtrate was distilled under reduced pressure. The product came over at 115–120° (1 mm.) and solidified on standing, m.p. 54–55°. The yield was 110 g. (70%).

Anal. Calcd. for $C_9H_9N_3$: N, 26.40. Found: N, 26.35.

The hydrochloride was recrystallized from methanol–ether and obtained as plates, m.p. 166–167° (capillary).

Anal. Calcd. for $C_9H_9N_3\cdot HCl$: C, 55.25; H, 5.15. Found: C, 55.05; H, 5.14.

3- or 5-Hydroxymethyl-1-benzyl-1,2,4-triazole (VI or VII).—A solution of 7.8 g. (0.05 mole) of 1-benzyl-1,2,4-triazole and 20 ml. of formalin was heated overnight at 130–140°. The excess formalin was removed and the residue was distilled under reduced pressure. Following a forerun boiling near 120° (1 mm.), the bulk came over near 160° (1 mm.). This was converted to the hydrochloride, which was recrystallized from methanol–ether and obtained as prisms, m.p. 148–149° (capillary), yield 7 g. (62%).

Anal. Calcd. for $C_{10}H_{11}N_3O\cdot HCl$: C, 53.22; H, 5.36; N, 18.62. Found: C, 53.29; H, 5.39; N, 18.51.

Essentially the same yield was obtained when the reactants were heated in a sealed vessel on the steam-bath for three days.

INDIANAPOLIS 6, INDIANA

(12) K. W. Rosenmund and F. Zetzsche, *Ber.*, **56**, 1481 (1923).

(13) B. Rothstein, *Bull. soc. chim.*, **51**, 838 (1932).