cology Division for providing the pharmacological results.

Experimental

9-Ethynyl-9-hydroxyfluorene.—A solution of 36 g. (0.2 mole) of fluorenone in 150 ml. of dimethylacetal was added rapidly to a suspension of sodium acetylide prepared from 9.2 g. (0.4 g. atom) of sodium,[10] in 400 ml. of liquid ammonia. A slow stream of acetylene was passed through the reaction mixture for 3 hours while stirring and cooling, and then 32 g. (0.6 mole) of ammonium chloride was added. After one hour, the ammonia was distilled out, 300 ml. of ether added, and the mixture stirred for 30 minutes. The ether was filtered off, the filter cake re-extracted and the filtrates dried with potassium carbonate. Filtration and evaporation yielded 41.2 g. of crude product. Recrystallization from methanol–water (charcoal) gave 30.9 g. (75%) of white crystals, m.p.[11] 109–110°.

Anal.[12] Calcd. for $C_{15}H_{10}O$: C, 87.35; H, 4.89. Found: C, 87.45; H, 4.89.

9-Ethynyl-9-hydroxyfluorene-4-carboxylic Acid.—A suspension of 33.6 g. (0.15 mole) of fluorenone-4-carboxylic acid[13] in 200 ml. of methylal was added to sodium acetylide which had been prepared from 8.1 g. (0.35 g. atom) of sodium in 500 ml. of liquid ammonia. After stirring 5 hours, the cooled mixture was allowed to stand overnight. The ammonia was evaporated and dilute hydrochloric acid added with cooling until the mixture was decidedly acid. The insoluble solid (30.5 g.) was separated and dissolved in saturated sodium bicarbonate. Cautious acidification yielded the product (12%) which melted with darkening at 170° and completely decomposed at 282°.

Anal. Calcd. for $C_{16}H_{10}O_3$: C, 76.79; H, 4.03. Found: C, 76.84; H, 4.16.

Trichloromethylethynylcarbinol.—Sodium acetylide was prepared from 13.8 g. (0.6 g. atom) of sodium in 500 ml. of liquid ammonia. The ammonia was replaced by 300 ml. of dry ether, the mixture cooled in a Dry Ice–acetone bath and 73.7 g. (0.5 mole) of trichloroacetaldehyde added over one hour with stirring. The reaction mixture was cooled and stirred overnight. Upon reaching room temperature, water (300 ml.) was added slowly with rapid stirring. The ether layer was separated and the aqueous fraction re-extracted. The extracts were combined and dried with magnesium sulfate.

Filtration, removal of the ether and distillation yielded 23.4 g. (27%), b.p. 77–81° (16 mm.), $n^{22}D$ 1.5029, d^{25} 1.467. Titration of the ethynyl hydrogen by the method of Hanna and Siggia[14] yielded 60% of the theoretical yield; molar refraction found 34.91, calcd. 34.61 (the value for trichloromethyl group was calculated from trichloroethanol).

Anal. Calcd. for $C_4H_3OCl_3$: C, 27.70; H, 1.74; Cl, 61.32. Found: C, 27.52; H, 1.78; Cl, 61.4.

The *p*-nitrophenylurethan, m.p. 150–153°, was prepared in the usual manner.

Anal. Calcd. for $C_{11}H_7N_2O_4Cl_3$: C, 39.15; H, 2.09. Found: C, 39.41; H, 2.21.

An attempt to prepare diethynylmethylcarbinol from ethyl acetate and sodium acetylide by a similar procedure resulted in reddish oils, apparently extremely susceptible to air oxidation, which resisted purification by distillation.

Allylcyclopropylmethylcarbinol.—The compound prepared from allylmagnesium bromide and methylcyclopropyl ketone following the procedure of Favorskaya[15] was obtained in 31% yield, b.p. 113–117° (193–195 mm.).

Anal. Calcd. for $C_8H_{14}O$: C, 76.14; H, 11.18. Found: C, 75.56; H, 10.80.

The phenylurethan m.p. 48–49.5° (pentane), was prepared in the usual manner.

Anal. Calcd. for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81. Found: C, 73.45; H, 7.74.

Benzylcyclopropylmethylcarbinol.—Prepared as above in 47% yield, b.p. 112–115° (8 mm.).

Anal. Calcd. for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 82.18; H, 9.05.

The phenylurethan, m.p. 95–97° (pentane), was prepared in the usual manner.

Anal. Calcd. for $C_{19}H_{21}NO_2$: C, 77.26; H, 7.17. Found: C, 77.14; H, 7.23.

(10) C. D. Hurd and W. D. McPhee, THIS JOURNAL, **69**, 240 (1947).

(11) Melting points are not corrected.

(12) Analyses by Drs. Weiler and Strauss, Oxford, England.

(13) E. R. Atkinson and H. J. Lawler, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 222.

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A Non-enzymatic Duplication of the "Fatty Acid Cycle"[1]

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The "fatty acid cycle," which governs fat metabolism *in vivo*, has been duplicated using simplified model compounds containing the structural elements which are considered important to the activity of coenzyme A. The reactions include Claisen-type self-condensation of N,S-diacetylthioethanolamine (I) to N-acetyl-S-acetoacetylthioethanolamine (III), reduction of the latter to N-acetyl-S-β-hydroxybutyrylthioethanolamine (IV), dehydration of IV to N-acetyl-S-crotonylthioethanolamine (V), and hydrogenation of V to N-acetyl-S-butyrylthioethanolamine (VI). The structures of III, V and VI were ascertained by independent syntheses. The compounds prepared are of interest as models of intermediates involved in the enzymatic cycle.

The role of coenzyme A (CoA) in fat metabolism has been the object of numerous investigations. Recently, the combined efforts of Lynen[3] and Lipmann[4] have resulted in the formulation of a "fatty acid cycle," in part anticipated by Barker,[5] consisting of four reversible, enzyme-catalyzed steps. In the first of these, two molecules of S-acetylCoA (Ia) are condensed by β-ketothiolase to give one molecule of S-acetoacetylCoA (IIIa) and one molecule of CoA (IIa). The acetothioacetate (IIIa) is reduced by β-ketohydrase to S-β-hydroxybutyryl-CoA (IVa). Dehydration catalyzed by crotonase

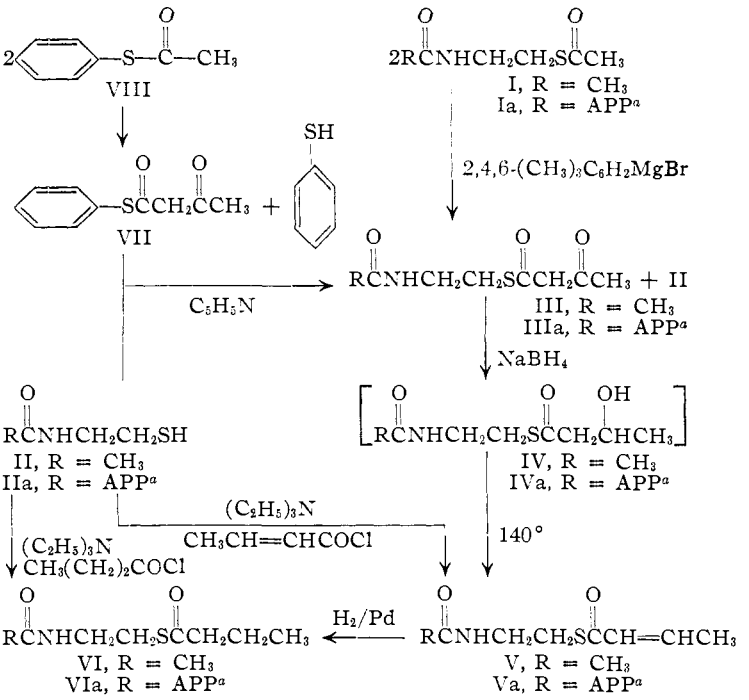
(1) This work was supported by a grant from the Office of Naval Research, Washington, D. C.

(2) Abstracted from part of the Ph.D. Dissertation of Curt W. Beck, January, 1955.

(3) F. Lynen, Acetyl-coenzyme A and the "fatty acid cycle," Harvey Lectures, Series XLVIII, 1952–1953, p. 210, 1953.

(4) F. Lipmann, "Biosynthetic Mechanisms," Harvey Lectures, Series XLIV, 1948–1949, p. 99, 1950.

(5) H. A. Barker in "Phosphorus Metabolism," edited by W. D. McElroy and B. Glass, The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, pp. 241 ff.



[a] APP = 3-adenyl-pyrophosphoryl-4'-pantothenyl.

leads in the third step to S-crotonylCoA (Va), which is finally hydrogenated in the presence of ethylene reductase to S-butyrylCoA (VIa). Thus a four-carbon chain has been built up from two acetate units, and recycling the product, *i.e.*, reaction with a new molecule of S-acetylCoA (Ia), etc., will lead eventually to even-numbered fatty acids of any chain length. Reversal of the cycle, conversely, serves to break down ingested fatty acids into acetate units.

The biochemical interest attached to this series of reactions has prompted us to duplicate the "fatty acid cycle" in the absence of enzymic catalysts. N,S-Diacetylthioethanolamine (I) was used as a model compound for S-acetylCoA (Ia), being sufficiently closely related to the presumed functional part of the coenzyme thiolacetate (Ia) without possessing those structural features which might be too sensitive to withstand the conditions of conventional chemical methods. The Claisen-type self-condensation of I, catalyzed by mesitylmagnesium bromide, gave about 30% of N-acetyl-S-acetoacetylthioethanolamine (III). The low yield probably is due to side reactions of the amide group with the Grignard reagent,[6] since thiolacetates without amide functions have given acetothiolacetates in our hands in yields as high as 70%, using the same catalyst. Compound III also was prepared by independent synthesis from N-acetylthioethanolamine[7] (II) and S-acetoacetylthiophenol (VII), using a general method of intermolecular transacylation from aromatic to aliphatic sulfhydryl groups as developed by T. Wieland.[8] Wieland has reported[9] use of VII, but has published no information about the compound save that it was obtained from thiophenol and diketene. In the present work, S-acetoacetylthiophenol was prepared by Claisen condensation of S-acetylthiophenol (VIII). The product was purified by distillation, but contained considerable amounts of thiophenol, probably arising from thermolytic decomposition. The distillation residue contained a corresponding amount of dehydroacetic acid. A similar pyrolysis of acetothiolacetates has been reported[10] and is in direct analogy to a known reaction of oxygen esters, *e.g.*, ethyl acetoacetate.[11]

Reduction of the β-keto group of III was effected smoothly by use of sodium borohydride at a controlled *p*H of 3.5 ± 0.5. The resulting N-acetyl-S-β-hydroxybutyrylthioethanolamine (IV) was not isolated but dehydrated spontaneously during distillation, yielding 60% over-all of N-acetyl-S-crotonylthioethanolamine (V). Compound V was prepared independently by acylation of N-acetylthioethanolamine[7] (II) with crotonyl chloride in the presence of triethylamine, and shown to be identical with the product obtained from III.

Hydrogenation of the thiolcrotonate V over palladium yielded N-acetyl-S-butyrylthioethanolamine (VI). The latter also was prepared independently by acylation of II with butyryl chloride in the presence of triethylamine, and found identical with the hydrogenation product.

Experimental

S-Acetylthiophenol (VIII).—The compound was prepared by the method of Michler.[12] After storage in a refrigerator for several weeks, acetylthiophenol completely solidified, forming large, colorless crystals, m.p. 16–17°.

S-Acetoacetylthiophenol (VII).—To a solution of isopropylmagnesium bromide (prepared from 7.3 g. (0.30 gram-atom) of magnesium turnings and 23.4 ml. (30.7 g., 0.250 mole) of isopropyl bromide in 400 ml. of ether), cooled to −25 to −30° in a Dry Ice–acetone bath, a solution of 38.05 g. (0.250 mole) of freshly distilled S-acetylthiophenol (VIII) in 100 ml. of ether was added dropwise with stirring. A heavy white precipitate formed, and precipitation continued after addition of the thiolester was complete. Cooling was continued for two additional hours, after which the cold mixture was acidified by dropwise addition of 260 ml. of 2.4 *N* hydrochloric acid. The ethereal layer was washed with saturated sodium chloride solution, dried over calcium chloride, and the solvent evaporated under reduced pressure at room temperature. The residue was distilled at 45° (4 mm.), yielding 11.7 g. (85%) of thiophenol. The pot residue, which gave a strong enol test with ferric chloride, was distilled in a short-path still at 60° and 0.15 mm. pressure, yielding 11.90 g. (49%) of crude VII over a period of six days. The non-volatile residue was identified as dehydroacetic acid. The distillate was found to contain an appreciable amount of thiophenol.

Anal. Calcd. for $C_{10}H_{10}O_2S$: C, 61.83; H, 5.19. Found: C, 63.12; H, 5.41.

N-Acetyl-S-acetoacetylthioethanolamine (III) by Transacylation.—To a solution of 1.2 g. (0.01 mole) of N-acetylthioethanolamine[7] (II) in 10 ml. of dry benzene, 1.95 g. (0.01 mole) of crude S-acetoacetylthiophenol (VII) was added, followed by 5 drops of pyridine. After storage at

(6) F. Runge, "Organometallverbindungen," Wissenschaftliche Verlags. G.m.b.H., Stuttgart, 1944; pp. 495 ff., 569 ff.
(7) R. Kuhn and G. Quadbeck, *Ber.*, **84**, 844 (1951).
(8) T. Wieland and H. Köppe, *Ann.*, **588**, 15 (1954).
(9) T. Wieland and L. Rueff, *Angew. Chem.*, **65**, 186 (1953).
(10) R. B. Baker and E. E. Reid, THIS JOURNAL, **51**, 1567 (1929).
(11) F. Arndt, *Org. Syntheses*, **20**, 26 (1940).
(12) W. Michler, *Ann.*, **176**, 177 (1875).

room temperature for 3 hours, the solvent was removed under reduced pressure, and the residue distilled at 40° and 0.1 mm. pressure, yielding 1.0 g. (91%) of colorless liquid identified as a mixture of thiophenol and diphenyl disulfide by infrared spectrum. The residue was extracted with ether, and the insoluble portion recrystallized at −80° from ethanol–acetone–pentane, yielding 1.3 g. (64%) of yellowish solid, m.p. 39–42°.

Recrystallization at −80° from ethanol–ether–pentane raised the melting point to 45–47°. On storing the mother liquor in a refrigerator for several days, an additional crop of colorless crystals separated, m.p. 61–63°. The product gave a strong enol test with ferric chloride and formed a green, water-soluble complex with cupric acetate.

Anal. Calcd. for $C_8H_{13}NO_3S$: C, 47.27; H, 6.45; N, 6.89. Found: C, 47.57; H, 6.44; N, 6.90.

N-Acetyl-S-acetoacetylthioethanolamine (III) by Claisen Condensation of N,S-Diacetylthioethanolamine (I).—A 1-l. three-necked flask, equipped with stirrer, dropping funnel, reflux condenser, and gas inlet tube, and flushed with prepurified nitrogen, was charged with 3.28 g. (0.135 gram-atom) of dried magnesium turnings and 50 ml. of dry ether. After initiating reaction by addition of 1 ml. of isopropyl bromide, a solution of 25 g. (0.125 mole) of mesityl bromide in 50 ml. of dry ether was added over a period of 3 hours. After standing at room temperature for 8 hours, the Grignard reagent was cooled to −25°, and a solution of 10.08 g. (0.0625 mole) of N,S-diacetylthioethanolamine[7] (I) in 100 ml. of dry tetrahydrofuran was added dropwise over a period of 1.5 hours. A light-colored precipitate formed, which darkened on storage at room temperature overnight. The product was decomposed by dropwise addition of 125 ml. of 1.2 *N* hydrochloric acid at 0°. The yellowish organic layer was washed with saturated sodium chloride solution, dried over calcium chloride, and the solvents evaporated at room temperature in a nitrogen stream. The crude product gave a strong enol test with ferric chloride, but gave no immediate sulfhydryl test with sodium nitroprusside.

On cooling to −80° for 2 hours, 4.10 g. (32.3%) of a yellow solid, m.p. 35–39°, separated from the crude product. Three recrystallizations from ethanol–ether–pentane raised the melting point to 57–60°. A mixed m.p. with N-acetyl-S-acetoacetylthioethanolamine was 57–61° (undepressed). The infrared spectra of the condensation product and of N-acetyl-S-acetoacetylthioethanolamine were identical.

The aqueous layer of the decomposed reaction mixture was diluted to 250 ml. and aliquot portions were titrated with 0.1 *N* iodine in 95% ethanol. Duplicate titrations indicated the presence of 0.00875 mole of free mercaptan (28%), *i.e.*, N-acetylthioethanolamine (II).

N-Acetyl-S-crotonylthioethanolamine (V) by Acylation of N-Acetylthioethanolamine (II).—To a solution of 15.45 g. (0.130 mole) of N-acetylthioethanolamine[7] (II) and 18 ml. (13.10 g., 0.130 mole) of triethylamine in 50 ml. of dry benzene, cooled to 0°, a solution of 14.00 g. (0.135 mole) of crotonyl chloride (Eastman Kodak Co.) in 50 ml. of dry benzene was added dropwise with stirring. The addition required 30 minutes, during which time the solution turned orange and a yellow precipitate formed. Stirring was continued at room temperature overnight. The solid product was collected by filtration, yielding 16.5 g. (92.5%) of triethylammonium chloride.

The filtrate was flash-evaporated on a steam-bath, and the red-brown residue fractionated in a Holzmann column, yielding 9.20 g. (38%) of product, b.p. 130–137° (0.5 mm.). Redistillation in a 2.5 inch Vigreux column yielded a uniform fraction, b.p. 150–152° (2 mm.), $n^{26}D$ 1.5232.

Anal. Calcd. for $C_8H_{13}NO_2S$: C, 51.31; H, 7.00; N, 7.48. Found: C, 51.47; H, 7.07; N, 7.78.

N-Acetyl-S-crotonylthioethanolamine (V) from N-Acetyl-S-acetoacetylthioethanolamine (III).—To a solution of 2.03 g. (0.01 mole) of N-acetyl-S-acetoacetylthioethanolamine (III) in 100 ml. of 80% aqueous dioxane, cooled in an ice–water bath, a solution of 0.100 g. (0.00264 mole) of sodium borohydride in 10 ml. of ice-cold water was added dropwise with stirring. The *p*H of the reaction mixture was held at 3.5 ± 0.5 by simultaneous addition of 1 *N* sulfuric acid. Stirring was continued for one hour at room temperature. After adding a further 5 ml. of 1 *N* sulfuric acid, the solution was concentrated to about 10 ml. under reduced pressure, 50 ml. of water was added, and the aqueous solution extracted with five 10-ml. portions of methylene chloride. After drying the extract over anhydrous sodium sulfate, and removing the solvent under reduced pressure, the remaining yellow oil was fractionated in a short Vigreux column, yielding 1.15 g. (61.5%) of product, b.p. 140–150° (0.7 mm.). Redistillation afforded a fraction of b.p. 135–138° (0.5 mm.), $n^{25}D$ 1.5226. The infrared spectra of the product and of N-acetyl-S-crotonylthioethanolamine (V) were identical.

N-Acetyl-S-butyrylthioethanolamine (VI) by Acylation of N-Acetylthioethanolamine (II).—To a stirred solution of 2.4 g. (0.02 mole) of N-acetylthioethanolamine[7] (II) and 2.02 g. (0.02 mole) of triethylamine in 50 ml. of dry benzene, cooled in an ice–water-bath, a solution of 2.35 g. (0.022 mole) of *n*-butyryl chloride (Eastman Kodak Co.) in 30 ml. of dry benzene was added dropwise over a period of 20 minutes. Stirring was continued for an additional 2 hours. Filtration yielded 2.8 g. (101%) of triethylammonium chloride. The colorless filtrate was concentrated under reduced pressure and fractionated, yielding 2.65 g. (70%) of N-acetyl-S-butyrylthioethanolamine (VI), b.p. 141.5–143° (0.5 mm.), $n^{25}D$ 1.4977. After storage in a refrigerator for several days, the product solidified, m.p. 22–23°.

Anal. Calcd. for $C_8H_{15}NO_2S$: C, 50.76; H, 8.00; N, 7.40. Found: C, 50.94; H, 8.20; N, 7.53.

N-Acetyl-S-butyrylthioethanolamine (VI) by Hydrogenation of N-Acetyl-S-crotonylthioethanolamine (V).—A suspension of 6.5 g. of 29.8% palladium-on-charcoal catalyst in 50 ml. of dry, purified dioxane was pre-reduced at room temperature and atmospheric pressure. N-Acetyl-S-crotonylthioethanolamine (V) (1.87 g., 0.01 mole) was added and hydrogenation resumed. An additional 4.2 g. of pre-reduced catalyst was added in small portions as required to overcome the effects of catalyst poisoning. At the end of 6 hours, hydrogenation was 92% complete. The reaction was allowed to proceed for an additional 16 hours, at which time 0.98 equivalent of hydrogen had been consumed, and addition of fresh catalyst had no effect.

The catalyst was removed by filtration, washed with dioxane and the combined filtrates were concentrated under reduced pressure. The residual yellowish oil was fractionated, yielding 1.2 g. (63.5%) of colorless liquid, b.p. 150–152° (2.3 mm.), $n^{25}D$ 1.4980. On storage in a refrigerator, the product solidified, m.p. 21–23°. A mixed melting point with N-acetyl-S-butyrylthioethanolamine (VI) was 21–23° (undepressed). The infrared spectra of the hydrogenation product and of compound VI were identical.

Anal. Calcd. for $C_8H_{15}NO_2S$: C, 50.76; H, 8.00. Found: C, 50.63; H, 7.80.

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