TABLE I

MAXIMAL EXTINCTION COEFFICIENTS FOR THE 6-μ NITRO BAND

Compound	Band, μ	E_{max}[a]	No. of nitro groups	E_{max} per nitro group
2,5,5-Trinitro-3-aza-4-oxa-2-hexene[2,5]	6.30	1060	2	530
	6.40	550	1	550
2-Chloro-5,5-dinitro-3-aza-4-oxa-2-hexene[2,6]	6.30	860	2	430
2,2,3,3-Tetranitrobutane	6.30	1600	4	400
3,3,4,4-Tetranitrohexane	6.31	1620	4	405
2,2-Dinitropropane[7,8]	6.40	993	2	496
Ethanenitrolic acid[9]	6.44	365	1	365
N-Acetylethanenitrolic acid	6.41	439	1	439

[a] Absorbance per centimeter for a 1 M solution in chloroform, slit width 0.050 μ.

The two tetranitro compounds, 2,2,3,3-tetranitrobutane and 3,3,4,4-tetranitrohexane, are not reported in the literature. The compound[4] bearing the name 2,2,3,3-tetranitrobutane has been assigned the structure 2,5,5-trinitro-3-aza-4-oxa-2-hexene.[2,5] The structure of 2,2,3,3-tetranitrobutane was confirmed by synthesis from two starting compounds, dimethylglyoxime and 2,3-dinitro-2-butene, followed by reduction to 2,3-diaminobutane. Rearrangements in the two syntheses and the reduction seems unlikely.

Experimental[10]

Infrared Spectra.—Infrared spectra were measured on a double beam recording spectrometer, a modified Perkin–Elmer model 12B using a sodium chloride prism. For solutions in chloroform, matched cells in which one contained only solvent gave spectra free of solvent bands. The E_{max} given in Table I is the absorbance of the band maximum per centimeter in one molar solution, calculated from the formula $E_{max} = \log(I_0/I)CL$ for a spectral slit width of 0.050 μ.

2,2,3,3-Tetranitrobutane. A. From Dimethylglyoxime.—Six grams of dimethylglyoxime (recrystallized from glacial acetic acid) was added slowly to 25 ml. of fuming nitric acid (sp. gr. 1.5) previously cooled to 5°. The reaction mixture was allowed to stand one-half hour in the cooling bath and then 25 ml. of fuming sulfuric acid (containing 30% sulfur trioxide) was added dropwise with stirring so that the temperature did not rise above 10°. After 15 minutes the red solution was poured into crushed ice and allowed to stand overnight. The white waxy solid was collected, washed with water and dried; yield 1.51 g. (12%), m.p. 159° dec. An analytical sample was prepared by recrystallization from dilute ethanol, followed by sublimation at 90° (0.5 mm.), m.p. 162.2–163.0° dec.

Anal. Calcd. for $C_4H_8N_4O_8$: C, 20.18; H, 2.54; N, 23.52. Found: C, 20.52; H, 2.76; N, 23.39.

B. From 2,3-Dinitro-2-butene.—One gram of 2,3-dinitro-2-butene[11] in a Carius tube was covered with 2 ml. of liquid nitrogen dioxide (dried by passing through phosphorus pentoxide on glass wool) at −77°. The tube was sealed and heated to 85° for 75 hours. Hexane was added after allowing the excess nitrogen dioxide to boil away and upon cooling white crystals of 2,2,3,3-tetranitrobutane precipitated. The crystals were washed with cold hexane and dried. Sublimation at 90° (0.4 mm.) gave 0.51 g. (31%) of pure 2,2,-3,3-tetranitrobutane, m.p. 159–160° dec.; mixed m.p. with the product from (A) above, no depression.

Reduction of 2,2,3,3-Tetranitrobutane.—Catalytic reduction of 2,2,3,3-tetranitrobutane in absolute ethanol with Adams catalyst under one atmosphere of hydrogen gave a 70% yield of 2,3-diaminobutane, isolated as the hydrochloride. The diamine was identified by converting the hydrochloride to the known dibenzamide, m.p. 295–297°.[12]

3,3,4,4-Tetranitrohexane.—By adding nitrogen dioxide to 3,4-dinitro-3-hexene[11] using the method just described (B, above) white waxy crystals of 3,3,4,4-dinitrohexane were obtained in 32% yield, m.p. 106.0–107.0°.

Anal. Calcd. for $C_6H_{10}N_4O_8$: C, 27.07; H, 3.79; N, 21.05. Found: C, 27.44; H, 3.95; N, 20.44.

The tetranitrohexane was identified by catalytic reduction to 3,4-diaminohexane in 70% yield and conversion to the dibenzamide, m.p. 330–332°.[12]

N-Acetylethanenitrolic Acid.[13,14]—The sodium ethanenitrolate prepared from 10.0 g. of ethanenitrolic acid[9] was added slowly with stirring to an ice-cold solution of 6.3 g. of acetyl chloride in 100 ml. of ether. After stirring for 45 minutes the sodium chloride was removed, the filtrate was washed with 20 ml. of saturated sodium bicarbonate solution and dried over calcium chloride. Part of the ether was removed in an atmosphere of nitrogen at reduced pressure. Upon adding hexane and cooling with a Dry Ice-bath, 5.64 g. (50%) of N-acetylethanenitrolic acid precipitated. Recrystallization from ether gave an analytical sample, m.p. 24.0–25.0°. Infrared spectrum (0.013 M solution in chloroform, μ): 5.58 (strong),[15] carbonyl; 6.01 (weak), C=N; 6.44, 7.44 (medium), nitro.

Anal. Calcd. for $C_4H_6N_2O_4$: C, 32.88; H, 4.14; N, 19.18. Found: C, 33.18; H, 4.29; N, 19.26.

(4) A. J. Miller and H. Hunt, *J. Phys. Chem.*, **49**, 20 (1945).

(5) Or an alternate structure, 2,4,4-trinitro-3-aza-2-pentene 3-oxide.

(6) Or an alternate structure, 2-chloro-4,4-dinitro-3-aza-2-pentene 3-oxide.

(7) A value E_{max} 430 for 2,2-dinitropropane with a spectral slit width of 0.034 μ was obtained by Dr. John F. Brown, Jr., General Electric Co., Schenectady, N. Y. The authors are grateful to Dr. Brown for valuable talks in connection with this work.

(8) H. W. Jacobson, Ph.D. Thesis, Purdue University, 1942.

(9) V. Meyer, *Ann.*, **175**, 96 (1875); V. Meyer and E. J. Constam, *ibid.*, **214**, 329 (1882); H. Wieland, *ibid.*, **353**, 82 (1907).

(10) Melting points given to tenths of a degree are corrected. Analyses by S. M. Nagy, Microchemical Laboratory, Massachusetts Institute of Technology, Cambridge, Mass.

(11) E. M. Nygaard and T. T. Noland, U. S. Patent 2,396,282 March 12, 1946).

(12) L. B. Clapp, J. F. Brown, Jr., and L. Zeftel, *J. Org. Chem.*, **15**, 1043 (1950).

(13) P. Grammaticakis, *Compt. rend.*, **223**, 741 (1946); **224**, 1067 (1947). Grammaticakis has presented spectral evidence in the ultraviolet region on a number of oximes related to the compound under discussion which suggests that direct acylation of an oxime leads to N-acylation. Evidence from thermal decomposition and hydrolysis[14] leading to an O-acyl assignment in this ethanenitrolate derivative appears less reliable for the purpose of structure assignment. Grammaticakis suggested that N-acyl oximes are converted to O-acyl oximes during hydrolysis. See also infrared data below and footnote 15.

(14) V. Meyer, *Ber.*, **27**, 1600 (1894); J. U. Nef, *Ann.*, **280**, 284 (1894); L. W. Jones, *Am. Chem. J.*, **20**, 1 (1898).

(15) Interpretation of the position of the strong band found at a shorter wave length (5.58 μ) than would be expected in a normal ester carbonyl band (5.68–5.81 μ) in the light of recent work by E. J. Hartwell, R. E. Richards and H. W. Thompson, *J. Chem. Soc.*, 1436 (1948), led the authors to conclude that the N-acyl structure assignment is more tenable than an O-acyl structure.

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The 2,4-Dinitrophenylhydrazones of Some Hindered Ketones

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The 2,4-dinitrophenylhydrazones of 2,6-dimethyl-, 2,4,6-trimethyl- and 2,6-dimethyl-4-*t*-butylacetophenones previously have not been reported. The general impression is probably that they cannot be made directly because of the steric effect of the *ortho*-situated methyl groups.[1] This impression must now be revised because the 2,4-

(1) P. De Jong, *Rec. trav. chim.*, **61**, 539 (1942); L. H. Schwartzman and B. B. Corson, THIS JOURNAL, **76**, 781 (1954); C. O. Guss, *ibid.*, **75**, 3177 (1953).

dinitrophenylhydrazones of the above ketones have been made by direct methods in this Laboratory. Previous negative results may be accounted for by the tendency of the derivatives to supersaturate in solvents until seed crystals were available, by relatively unfavorable equilibria, and perhaps by difficulty in distinguishing readily between derivative and reagent. A modified qualitative test, to be published elsewhere, apparently helps to surmount these difficulties. In the qualitative test, a more highly concentrated solution of the reagent is used, and the derivative is washed thoroughly with hot, 10% hydrochloric acid.

The formation of 2,4-dinitrophenylhydrazones of some hindered ketones suggests the possibility of the formation and isolation of other derivatives such as the oximes. Report on this work will be made later.

Experimental[2]

2,4,6-Trimethylacetophenone-2,4-DNH.—The derivative was made by refluxing a mixture of 1 g. each of ketone and 2,4-dinitrophenylhydrazine, 5 ml. of concentrated hydrochloric acid, 5 ml. of water and 40 ml. of ethanol. As the solution cooled, seeds from a previous smaller run of a qualitative nature were added. The crystals obtained were washed thoroughly with hot, 10% aqueous hydrochloric acid and then with water. The yield was 1.2 g., 57%, of amber-colored, transparent prisms of m.p. 148–149° (previous softening) after two recrystallizations from a mixture of 25 ml. of methanol and 4 ml. of ethyl acetate.

Anal. Calcd. for $C_{17}H_{18}N_4O_4$: N, 16.37. Found: N, 16.21.

4-*t*-Butyl-2,6-dimethylacetophenone-2,4-DNH.—This compound, made in a similar manner, was recrystallized from methylcyclohexane as small, yellow-orange needles, m.p. 164.5–166° (previous softening); nearly quantitative crude yield.

Anal. Calcd. for $C_{20}H_{24}N_4O_4$: N, 14.57. Found: N, 14.46.

Recrystallization from methanol gave yellow needles of wide melting range (150–162°). After being evacuated at 2 mm. and 78°, the yellow needles melted at 163.5–164.5 (previous softening).

2,6-Dimethylacetophenone-2,4-DNH.—The derivative was made in 28% yield from 0.75 g. of 2,6-dimethylacetophenone.[3] From the filtrate, 0.5 g., 66% of ketone, was recovered by extraction with petroleum ether (b.p. 30–60°). The derivative was recrystallized twice from methylcyclohexane as yellow needles, m.p. 158.5–159.5°.

Anal. Calcd. for $C_{16}H_{16}N_4O_4$: N, 17.07. Found: N, 16.90.

Benzoylmesitylene[4] failed to give a 2,4-DNH, or a readily isolable one, under the above conditions and under the forcing conditions (concentrated sulfuric acid and dioxane) of Josten.[5]

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(2) All melting points are corrected. Analyses were by Clark Microanalytical Laboratory, Urbana, Ill.

(3) This ketone was made in two steps from 2,6-dimethylaniline (Distillation Products, Inc.). The yield of 2,6-dimethylbenzonitrile, m.p. 88.5–89.5°, was 16%. We are indebted to Mr. James R. Cox, Jr., and Mr. C. G. Carlile for this synthesis. The addition of the nitrile to methylmagnesium iodide in anisole gave a 34% yield of ketone, b.p. 69–70° at 2 mm., $n^{25}D$ 1.5132.

(4) We are indebted to Dr. R. C. Fuson, University of Illinois, for a sample of this ketone.

(5) W. Josten, *Ber.*, **71**, 2230 (1938); N. O. V. Sonntag, S. Linder, E. I. Becker and P. E. Spoerri, THIS JOURNAL, **75**, 2283 (1953).

The Biosynthesis of Radioactive β-Hydroxyisovaleric Acid in Rat Liver[1-3]

BY JOSEPH L. RABINOWITZ

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Previous reports by this and other laboratories on the biosynthesis of cholesterol have established the formation of β-hydroxy-β-methylglutaric acid,[4,5] β-methylglutaconic acid,[6] and senecioic acid[7,8] from labeled acetate in cell-free homogenates[9,10] and particle-free extracts of rat liver.[11] Further investigation has revealed the presence of another radioactive acid which has been found to be β-hydroxyisovaleric acid. It is probable that this acid was derived from HMG by an enzymatically catalyzed decarboxylation. β-Hydroxyisovaleric acid was isolated from cell-free homogenates and particle-free extracts of rat liver,[4,11,12] after incubation with 2-C^{14}-NaOAc or with 3′-C^{14}-HMG by means of carrier technique. Results are shown in Table I and the details are described in the experimental part. In Table II the incorporation of different substrates into HIV is shown for both cell-free homogenate and aqueous extracts of rat liver. As expected, HMG proved to be a much better precursor of HIV than acetate. The distribution of isotope in HIV (derived from 2-C^{14}-HOAc) paralleled the observations of other workers in the field.[5,8] The results of the degradation of HIV obtained from 3′-C^{14}-HMG pointed to the utilization of some intact HMG; but also to a great deal of breakdown and equilibration of HMG with HOAc and probably AcAcOH prior to utilization. Table III shows the results obtained. Although a relatively small amount of C^{14} is found in carbons 1 and 3 in

TABLE I

INCORPORATION OF 2-C^{14}-NaOAc INTO CHOLESTEROL AND β-HYDROXYISOVALERIC ACID BY RAT LIVER HOMOGENATES

Each flask contained 5 ml. of cell-free homogenate; the additions were 1 mg. each of AMP, DPN and 2-C^{14}-NaOAc (1.5×10^5 c.p.m./mg. C). Following incubation for 3 hours at 37°, 0.5 mg. of carrier cholesterol and 30 mg. of carrier HIV was added to each flask. Gas phase was O_2.

	Radioactivity recovered as $BaCO_3$, c.p.m./mg. C				
Experiment	HIV	Cholesterol	Cu salt of HIV	Ag salt of HIV	Paper chromatographed HIV
1	1590	220	1410	1540	1430
2	1970	375	..	..	..

(1) Supported by a grant from the American Heart Association.

(2) The radioactive materials were obtained on allocation from the United States Atomic Energy Commission.

(3) The following abbreviations are used: AMP = adenosine-5′-monophosphate; ATP = adenosinetriphosphate; DPN = diphosphopyridine nucleotide; HMG = β-hydroxy β-methylglutaric acid; MG = β-methylglutaconic acid; SA = senecioic acid; HIV = β-hydroxyisovaleric acid; NaOAc = sodium acetate; AcAcOH = acetoacetic acid.

(4) J. L. Rabinowitz and S. Gurin, *J. Biol. Chem.*, **208**, 307 (1954).

(5) H. Rudney, THIS JOURNAL, **76**, 2595 (1954).

(6) J. L. Rabinowitz and S. Gurin, *ibid.*, **76**, 5168 (1954).

(7) J. L. Rabinowitz, *ibid.*, **76**, 3037 (1954).

(8) H. Rudney, *Federation Proc.*, **13**, 2861 (1954).

(9) M. Rabinowitz and D. M. Greenberg, *Arch. Biochem. Biophys.*, **40**, 472 (1952).

(10) N. L. R. Bucher, THIS JOURNAL, **75**, 498 (1953).

(11) J. L. Rabinowitz and S. Gurin, *Biochim. Biophys. Acta*, **10**, 345 (1953).

(12) B. Bachhawat, W. G. Robinson and M. J. Coon, THIS JOURNAL, **76**, 3098 (1954).