or 150% of the theoretical amount). The canary-yellow crystalline hydrazone formed immediately. It was filtered off, washed with a little glacial acetic acid, then with ether. Recrystallized from chloroform–petroleum ether, the hydrazone melted at 189–190°. A mixed melting point with an authentic specimen of O-*p*-nitrobenzoylglycolic aldehyde 2,4-dinitrophenylhydrazone[20] gave no depression.

Anal. Calcd. for $C_{12}H_{11}O_8N_5$: C, 46.3; H, 2.9; N, 18.0. Found for the hydrazone obtained from the bis-O-*p*-nitrobenzoate ester of: (a) D'-methoxydiethylene glycol: C, 46.3; H, 3.2; N, 17.7, and of (b) L'-methoxydiethylene glycol: C, 46.5; H, 3.1; N, 18.1.

ST. PAUL, MINNESOTA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Two 3-Epimeric Ketononoses[1]

BY M. L. WOLFROM AND HARRY B. WOOD, JR.

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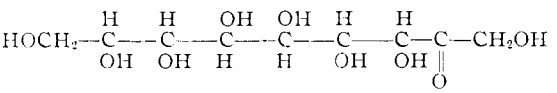
The synthesis of D-*erythro*-L-*manno*-nonulose (D-*arabino*-L-*tagato*-nonose, I) and of D-*erythro*-L-*gluco*-nonulose (D-*arabino*-L-*sorbo*-nonose, II), the first known ketononoses, is described. They were prepared by acetylation of the corresponding 2-epimeric aldoöctonic acids to give the fully acetylated acids which were converted to their acyl chlorides and these with diazomethane yielded the diazomethyl ketones. The latter were transformed into the *keto*-acetates which on deacetylation yielded the ketononoses (amorphous). These sugars were non-fermentable by yeast (*Saccharomyces*).

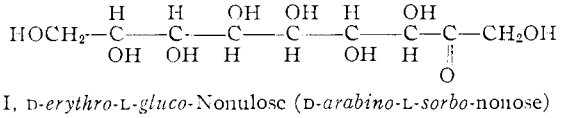
In continuation of our work on the preparation of higher ketoses, we report herein the synthesis of two sugars, the first known ketononoses, designated D-*erythro*-L-*manno*-nonulose (D-*arabino*-L-*tagato*-nonose, I) and D-*erythro*-L-*gluco*-nonulose (D-*arabino*-L-*sorbo*-nonose, II) from D-*erythro*-L-*manno*-octonic acid and D-*erythro*-L-*gluco*-octonic acid, respectively. The reaction sequence employed was

$$R{-}CO_2H \xrightarrow{SOCl_2} RCOCl \xrightarrow{CH_2N_2} R{-}CO{-}CHN_2 \xrightarrow{HOAc + Cu^{++}} R{-}CO{-}CH_2OAc \xrightarrow{Ba(OH)_2} CH_2OH{-}(CHOH)_6{-}CO{-}CH_2OH$$

wherein R = $CH_2OCOCH_3{-}(CHOCOCH_3)_6{-}$.

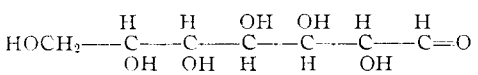


I, D-*erythro*-L-*manno*-Nonulose (D-*arabino*-L-*tagato*-nonose)



II, D-*erythro*-L-*gluco*-Nonulose (D-*arabino*-L-*sorbo*-nonose)

The starting point of our synthesis was D-mannose, which was converted to D-*glycero*-D-*galacto*-heptose



either through the cyanohydrin reaction[2] with subsequent reduction of the aldonolactone to the aldose stage,[3] or, more directly, through the nitromethane procedure.[4] The configuration of D-*glycero*-D-*galacto*-heptose was established by Peirce[5] and the sugar was characterized further by Montgomery and Hudson.[6] One of the two possible aldoöctonic acids (D-*erythro*-L-*manno*-octonic acid), obtainable through the cyanohydrin reaction on D-*glycero*-D-*galacto*-heptose, was isolated by Fischer and Passmore.[3] Its configuration was established by Peirce[5] and confirmed by Hudson and co-workers.[7] The 2-epimer of this acid (D-*erythro*-L-*gluco*-octonic acid) was described first by Karabinos, Hann and Hudson,[8] who further characterized these 2-epimeric aldoöctonic acids and described the crystalline heptaacetate of D-*erythro*-L-*manno*-octonic acid.

In the D-*erythro*-L-*manno*-nonulose series, all of the intermediates in our synthetic scheme, except the *keto*-octaacetate, were obtained in crystalline form. In the D-*erythro*-L-*gluco*-nonulose structure, the acyl halide and the *keto*-acetate were not crystallized. Unfortunately, the two final products, the unsubstituted ketononoses I and II, resisted crystallization. The molecular rotations of the ketononoses are those predictable from their structures[9]; that of D-*erythro*-L-*gluco*-nonulose ($270 \times -47.2° = -12{,}700$) falls between the values $-14{,}100$ and $-10{,}800$ established for L-*gluco*-heptulose[10] and D-*glycero*-L-*gluco*-octulose,[11] respectively, of like top structure, while that of D-*erythro*-L-*manno*-nonulose ($270 \times -20° = -5{,}400$) compares favorably with those, $-6{,}100$ and $-4{,}100$, of the ketoheptose[9,12] and ketoöctose,[11] respectively, of like structure.

The diazomethyl ketones were obtained in pure form only by the application of chromatographic techniques, utilizing silicate columns. Their contaminants were probably the methyl esters of the acetylated octonic acids, the syntheses of which are described. The infrared spectra of the diazomethyl ketones were determined. The bands characteristic

(1) Paper No. 15 in the series entitled "The Action of Diazomethane upon Acylic Sugar Derivatives"; previous communication, M. L. Wolfrom, J. M. Berkebile and A. Thompson, THIS JOURNAL, **74**, 2197 (1952).

(2) E. Fischer and J. Hirschberger, *Ber.*, **22**, 365 (1889).

(3) E. Fischer and F. Passmore, *ibid.*, **23**, 2226 (1890).

(4) J. C. Sowden and R. Schaffer, THIS JOURNAL, **73**, 4662 (1951).

(5) G. Peirce, *J. Biol. Chem.*, **23**, 327 (1915).

(6) Edna M. Montgomery and C. S. Hudson, THIS JOURNAL, **64**, 247 (1942).

(7) R. M. Hann, W. D. Maclay, A. E. Knauf and C. S. Hudson *ibid.*, **61**, 1268 (1939).

(8) J. V. Karabinos, R. M. Hann and C. S. Hudson, *ibid.*, **75**, 4320 (1953).

(9) M. L. Wolfrom and P. W. Cooper, *ibid.*, **72**, 1345 (1950).

(10) G. Bertrand and G. Nitzberg, *Compt. rend.*, **186**, 925 (1928); W. C. Austin, THIS JOURNAL, **52**, 2106 (1930).

(11) M. L. Wolfrom and P. W. Cooper, *ibid.*, **71**, 2668 (1949).

(12) F. B. LaForge, *J. Biol. Chem.*, **28**, 511 (1917).

of the nitrogen function[13] were located at 4.75 μ and 6.10 μ.

The fermentability of the ketononoses described in this work is of interest since Fischer and Passmore[3] reported a positive fermentation test on a nonose prepared from D-*erythro*-L-*manno*-octose. The identity of the Fischer nonose remains unknown since R. Hagenbach,[14] working with Fischer, repeated the earlier work and obtained a nonose which was non-fermentable. Neither of our ketononoses was found to be fermented by *Saccharomyces*. Should the biochemical behavior of these ketoses parallel that of D-fructose, then this finding would further eliminate the two 2-epimeric aldononoses corresponding to each of the ketononoses and thus, in turn, all of the possible nonoses obtainable from D-*glycero*-D-*galacto*-heptose by the Fischer–Kiliani cyanohydrin higher aldose synthesis.

Experimental

Hepta-*O*-acetyl-D-*erythro*-L-*manno*-octonyl Chloride.—A mixture of 0.9 g. of cadmium hydroxide and 100 ml. of water was heated to boiling and 2.0 g. of D-*erythro*-L-*manno*-octonic acid,[3,8,15] m.p. 171–172°,[16] $[\alpha]^{20}D$ +4 → −31° (70 hr.; *c* 1.0, water), was added. The reaction was stirred and the cadmium hydroxide slowly dissolved. Heating was continued for 4 hr. and the volume was maintained at a constant level by the addition of water. After filtering the hot solution, the filtrate was evaporated under reduced pressure to 25 ml. and approximately 200 ml. of methanol was added, whereupon the cadmium salt separated as a fine, amorphous powder. The solid was filtered, washed with methanol and ether, and dried under reduced pressure at 100° over phosphorus pentoxide; yield 2.5 g.

An amount of 5 g. of the cadmium salt was acetylated with acetic anhydride and hydrogen chloride according to the general procedure of Ladenburg and co-workers.[17] The crude sirup obtained was extracted with chloroform and crystallized from ether–petroleum ether to give hepta-*O*-acetyl-D-*erythro*-L-*manno*-octonic acid[8]; yield 5.7 g., m.p. 141–144°, $[\alpha]^{23}D$ +3° (*c* 3.18, chloroform).

An amount of 10 g. of the above hepta-*O*-acetyl-octonic acid was dissolved in 200 ml. of dry benzene and treated with 15 ml. of purified[18] thionyl chloride. The solution was refluxed for 3 to 4 hr. or until only a trace of hydrogen chloride was detected at the top of the reflux column by ammonia. At the end of the reaction period, the clear solution was evaporated under reduced pressure to remove the excess thionyl chloride. As the solution became concentrated, beautiful birefringent plates formed; yield 6.18 g. Several recrystallizations from benzene–petroleum ether gave pure material; m.p. 138–141°, $[\alpha]^{20}D$ +12.8° (*c* 3.7, chloroform). The product was readily soluble in acetone, chloroform and benzene, and was insoluble in water and petroleum ether.

Anal. Calcd. for $C_{22}H_{29}O_{15}Cl$: C, 46.45; H, 5.14. Found: C, 46.47; H, 5.15; Cl, present.

1-Deoxy-1-diazo-*keto*-D-*erythro*-L-*manno*-nonulose Hepta-acetate.—A solution of 3 g. of hepta-*O*-acetyl-D-*erythro*-L-*manno*-octonyl chloride in 150 ml. of dry ether was poured slowly with stirring into 150 ml. of ether containing 1.5 g. of diazomethane.[19] There was a moderate evolution of nitrogen gas during the addition and subsequent precipitation of the product. The mixture was allowed to stand at 0° for 4 hr. and was then permitted to warm gradually to room temperature. The volume of the reaction mixture was reduced to 50 ml. by passing a stream of dry air through the solution and the crystalline diazomethyl ketone was removed by filtration and washed with a small amount of cold ether and then with petroleum ether; yield 1.2 g., m.p. 151–156°.

The crystalline diazomethyl ketone was purified by chromatographic techniques. Two grams of the crystalline product was dissolved in 20 ml. of benzene and placed on a chromatographic column, 270 × 44 mm. (diam., adsorbent dimensions) containing 100 g. of Magnesol[20]–Celite[21] (5:1 by wt.) and developed with 400 ml. of benzene–ethanol (100:1 by vol.). The main zone, indicated by the alkaline permanganate streak[22] and containing the diazomethyl ketone, was near the bottom of the column. After elution from the cut zone by 250 ml. of acetone, 1.3 g. of material was obtained. Like other diazomethyl ketones, it possessed a slight yellow color. Pure material was obtained on recrystallization from benzene–petroleum ether; m.p. 156–157°, $[\alpha]^{27}D$ +12.7° (*c* 3.0, chloroform). The infrared absorption spectrum of the substance showed medium strong bands at 4.75 and 6.10 μ. The *keto*-acetate (see below) infrared spectrum showed a complete loss of these lines.

Anal. Calcd. for $C_{23}H_{30}O_{15}N_2$: C, 48.08; H, 5.26; N, 4.88. Found: C, 47.90; H, 5.44; N, 4.85.

Methyl Hepta-*O*-acetyl-D-*erythro*-L-*manno*-octonate.—One gram of hepta-*O*-acetyl-D-*erythro*-L-*manno*-octonic acid was dissolved in dry ether (20 ml.) and added to an ether solution of diazomethane (slightly in excess of 1 mole) and allowed to stand at room temperature for 8 hr. After evaporation (dry air jet) to a sirup, the substance was dissolved in 25 ml. of ether (decolorizing carbon) and petroleum ether was added to opalescence. The ester crystallized on standing overnight at ice-box temperature; yield 0.8 g. Three recrystallizations from ether–petroleum ether gave pure material; m.p. 172–173°, $[\alpha]^{21}D$ +10.5° (*c* 1.5, chloroform).

Anal. Calcd. for $C_{23}H_{32}O_{16}$: C, 48.91; H, 5.71. Found: C, 48.70; H, 5.82.

***keto*-D-*erythro*-L-*manno*-Nonulose Octaacetate.**—A solution of 5.6 g. of 1-deoxy-1-diazo-*keto*-D-*erythro*-L-*manno*-nonulose heptaacetate in 80 ml. of glacial acetic acid containing a trace of cupric acetate (0.015 g.)[23] was heated to reflux whereupon the solution changed in color from green to yellow and a vigorous evolution of nitrogen occurred. The reaction mixture was refluxed for 3 min. longer and then was concentrated under reduced pressure at 50° to 15 ml. This solution was diluted with 75 ml. of chloroform, washed five times with water, dried over anhydrous sodium sulfate, decolorized with carbon, and evaporated under reduced pressure to a sirup. Since Wolfrom and Thompson[23] had shown that considerable deacetylation takes place in this general procedure, the sirup was subjected to acetylation by treatment with 65 ml. of acetic anhydride containing 0.7 g. of zinc chloride. After standing overnight at room temperature, the acetic anhydride was hydrolyzed with 200 g. of ice and water and the acetate was extracted with chloroform. The chloroform solution was washed with water, dried and evaporated under reduced pressure to a sirup; yield 6.1 g. The sirup failed to crystallize.

The sirup (2 g.) was dissolved in 20 ml. of benzene and placed on a chromatographic column (270 × 44 mm., diam.) containing approximately 100 g. of Magnesol–Celite (5:1) and developed with 400 ml. of benzene–ethanol (100:1). The contents of the principal zone[22] near the center of the column, after elution with 800 ml. of acetone and evaporation (dry air jet), failed to crystallize. This procedure was twice repeated, but still the material failed to crystallize; yield 1.1 g. The pure material was obtained after dissolving in acetone (decolorizing carbon) and drying the resulting amorphous material under reduced pressure at 56°;

(13) Compare D. Anderson, R. J. W. Le Fèvre and J. Savage, *J. Chem. Soc.*, 445 (1947); N. Sheppard and G. B. B. M. Sutherland, *ibid.*, 453 (1947); S. A. Fusari, R. P. Frohardt, A. Ryder, T. H. Haskell, Doris W. Johannessen, Carole C. Elder and Q. R. Bartz, THIS JOURNAL, **76**, 2878 (1954); S. A. Fusari, T. H. Haskell, R. P. Frohardt and Q. R. Bartz, *ibid.*, **76**, 2881 (1954).

(14) E. Fischer, "Untersuchungen über Kohlenhydrate und Fermente," Vol. 1, J. Springer, Berlin, 1909, p. 582; C. S. Hudson, *Advances in Carbohydrate Chem.*, **1**, 5 (1945).

(15) An authentic specimen, kindly furnished by the late Dr. C. S. Hudson, then at the National Institutes of Health, Bethesda, Md.

(16) All melting points were corrected.

(17) K. Ladenburg, M. Tishler, J. W. Wellman and R. D. Babson, THIS JOURNAL, **66**, 1217 (1944).

(18) D. L. Cottle, *ibid.*, **68**, 1380 (1946).

(19) A. F. McKay, *ibid.*, **70**, 1974 (1948); L. I. Smith, *Chem. Revs.*, **23**, 193 (1938); A. F. McKay, W. L. Ott, G. W. Taylor, M. N. Buchanan and J. F. Crooker, *Can. J. Research*, **28B**, 683 (1950).

(20) Westvaco Chemical Division of Food Machinery and Chemical Corp., South Charleston, West Virginia.

(21) No. 535, Johns-Manville Co., New York, N. Y.

(22) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

(23) M. L. Wolfrom and A. Thompson, *ibid.*, **68**, 1453 (1946).

$[\alpha]^{21}D$ +16.8° (*c* 4.5, chloroform). The acetate was readily soluble in chloroform and acetone, moderately soluble in ether, benzene and ethanol and was insoluble in water and petroleum ether. It reduced Benedict solution rapidly.

Anal. Calcd. for $C_{25}H_{34}O_{17}$: C, 49.50; H, 5.65. Found: C, 49.86; H, 5.63.

D-*erythro*-L-*manno*-**Nonulose** (D-*arabino*-L-*tagato*-**Nonose**, I).—To a solution of 14 g. of barium hydroxide octahydrate in 80 ml. of water at 0°, there was added 3.7 g. of *keto*-D-*erythro*-L-*manno*-nonulose octaacetate. The solution was shaken occasionally until the acetate had dissolved, which required approximately 3 hr., and was then kept at 0° for an additional 60 min. The barium ion was removed by precipitation with oxalic acid and subsequent filtration of the barium oxalate formed. The filtrate was passed, at the rate of 220 ml. per 30 min., through a 200 × 30 mm. (diam.) column containing 75 g. of cation exchange resin (Amberlite IR-100-H[24]). The effluent was then passed at the same rate over a like amount of anion acceptor resin (Duolite A-4[25]). The resulting solution (500 ml.) was concentrated under reduced pressure to a thick sirup; yield 0.98 g., $[\alpha]^{25}D$ −20° (*c* 3.7, water). The amorphous material possessed a sweet taste and rapidly reduced Benedict solution. It exhibited a positive Molisch test[26] and a negative Seliwanoff reaction.

Anal. Calcd. for $C_9H_{18}O_9$: C, 40.00; H, 6.72. Found: C, 39.90; H, 6.82.

A 2% aqueous solution of the above substance was spotted on a paper strip (Whatman No. 1) and developed descendingly in a cabinet for 72 hr. with 1-butanol/ethanol/water: 40/11/19. Indication with 1% aqueous solutions (applied successively and with intermediate drying) of sodium metaperiodate and potassium permanganate[27] showed only one bright yellow spot on a pink background, changing slowly to a brown spot on a gray background.[28]

Hepta-*O*-acetyl-D-*erythro*-L-*gluco*-octonic Acid.—An amount of 20 g. of D-*erythro*-L-*gluco*-octonic acid,[8,15] m.p. 170–172°, $[\alpha]^{20}D$ +4° (7 min.) → −17° (7 days, *c* 3.1, water), was converted to the cadmium salt as described above for its 2-epimer; yield 27 g. An amount of 22.8 g. of the salt was acetylated as described above except that the acetate was extracted with chloroform from the aqueous acetic acid solution without concentration. The sirup obtained from the chloroform extract crystallized and the hepta-*O*-acetyl-D-*erythro*-L-*gluco*-octonic acid was further purified by crystallization from ether–hexane; yield 26 g., m.p. 129–131°, $[\alpha]^{20}D$ +14.8° (*c* 3.9, chloroform).

Anal. Calcd. for $C_{22}H_{30}O_{18}$: C, 48.00; H, 5.49. Found: C, 47.80; H, 5.47.

1-Deoxy-1-diazo-*keto*-D-*erythro*-L-*gluco*-Nonulose Heptaacetate.—Hepta-*O*-acetyl-D-*erythro*-L-*gluco*-octonic acid was converted to the acid chloride as described above for its 3-epimer. The acyl chloride could not be brought to crystallization and the isolated amorphous product (11.1 g.) was converted to the diazomethyl ketone in the manner described above. The crude, crystalline product (9.2 g.) was purified chromatographically as described above. The sectioned main zone, located in the bottom half of the column, was eluted with acetone; yield 6.43 g. Pure material was obtained on two recrystallizations from benzene–petroleum ether; m.p. 74–76°, $[\alpha]^{20}D$ −26.5° (*c* 3, chloroform). This diazomethyl ketone formed light yellow needles. The infrared absorption spectra showed the two medium strong bands (4.73 and 6.08 μ) cited above as characteristic for the 3-epimeric diazomethyl ketone.

Anal. Calcd. for $C_{23}H_{30}O_{15}N_2$: C, 48.08; H, 5.26; N, 4.88. Found: C, 47.99; H, 5.39; N, 4.72.

Methyl Hepta-*O*-acetyl-D-*erythro*-L-*gluco*-octonate.—This ester was prepared from its acid (1.00 g.) and diazomethane as described above for its 2-epimer and the product was isolated in the same manner; yield 0.53 g. Pure material was obtained on recrystallization from benzene–petroleum ether; yield 0.36 g., m.p. 114–116°, $[\alpha]^{21}D$ +9.9° (*c* 3.2, chloroform).

Anal. Calcd. for $C_{23}H_{32}O_{16}$: C, 48.91; H, 5.71. Found: C, 49.11; H, 5.87.

***keto*-D-*erythro*-L-*gluco*-Nonulose Octaacetate.**—This substance was prepared from 5.6 g. of 1-deoxy-1-diazo-*keto*-D-*erythro*-L-*gluco*-nonulose heptaacetate as described above for the synthesis of its 3-epimer. The reacetylated product was isolated in the same manner and the final material was amorphous; yield 4.01 g., $[\alpha]^{20}D$ +7.5° (*c* 6.1, chloroform). The *keto*-D-*erythro*-L-*gluco*-nonulose octaacetate was readily soluble in acetone and chloroform, moderately so in ether and ethanol, and was insoluble in water and petroleum ether. It reduced hot Benedict solution.

Anal. Calcd. for $C_{25}H_{34}O_{17}$: C, 49.50; H, 5.65. Found: C, 49.70; H, 5.61.

D-*erythro*-L-*gluco*-**Nonulose** (D-*arabino*-L-*sorbo*-**Nonose**, II).—*keto*-D-*erythro*-L-*gluco*-Nonulose octaacetate (4 g.) was deacetylated with barium hydroxide solution as described above for the synthesis of D-*erythro*-L-*manno*-nonulose and the product was isolated in the same manner; yield 1.2 g., $[\alpha]^{23}D$ −47.2° (*c* 1.24, water).

The white amorphous product was soluble in hot glacial acetic acid, methanol and water, but was insoluble in ethanol and acetone. It reduced hot Benedict solution and possessed a sweet taste. It showed positive Molisch[26] and negative Seliwanoff reactions. The product has so far resisted crystallization. It was found to be chromatographically homogeneous when placed on paper strips[28] as described above for D-*erythro*-L-*manno*-nonulose.

Anal. Calcd. for $C_9H_{18}O_9$: C, 40.00; H, 6.72. Found: C, 40.12; H, 6.62.

Non-fermentability of D-*erythro*-L-*manno*-Nonulose (I) and D-*erythro*-L-*gluco*-Nonulose (II) by Yeast.—Samples of the two ketononoses were forwarded to Professor Carl C. Lindegren,[29] who made the following report.

"We tested the nonoses on six different yeasts of widely differing potentials but obtained no reaction from any. This may be taken to indicate that *Saccharomyces* generally will not ferment your nonoses under relatively anaerobic conditions."

We previously had forwarded a sample of the D-*erythro*-L-*gluco*-nonulose to Dr. S. C. Pan,[30] who reported as follows (italics represent our changes).

"It was not fermented by either a suspension of bakers' yeast at *p*H 5 or by a growing culture of the distillers' yeast NRRL[31] 132. Recoveries of 100% were obtained in both cases as determined by reducing power toward Somogyi's copper tartrate–phosphate reagent. The most readily fermentable sugars (D-glucose, maltose, sucrose, D-fructose and D-mannose) are rapidly fermented under the former conditions while the difficultly fermentable sugars (maltotriose, isomaltose, *panose* and D-galactose) are also fermented under the latter conditions. Since negative results were obtained in both cases, I would classify this ketononose as a non-fermentable (*by Saccharomyces*) sugar."

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(24) The Resinous Products and Chemical Co., Philadelphia, Pa.

(25) Chemical Process Co., Redwood City, Calif.

(26) A. W. Devor, THIS JOURNAL, **72**, 2008 (1950).

(27) R. U. Lemieux and H. F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

(28) Paper strip chromatography by Mr. J. N. Schumacher of this Laboratory.

(29) Director, Biological Research Laboratory, Southern Illinois University, Carbondale, Ill.

(30) Then at the Research Laboratories of Joseph E. Seagram and Sons, Inc., Louisville, Ky.

(31) Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Ill.