tion of sodium hydroxide (50 g. in 250 ml.) was refluxed for five minutes, cooled, extracted with ether and acidified with concd. hydrochloric acid. The resulting organic material was removed by extraction with ether and distilled to give 44 g. of material, b. p. 20–56° (1 mm.). The distillate (35 g.) was distilled from a Claisen flask with the evolution of carbon dioxide to give 16 g. of liquid, $n^{20}D$ 1.4190–1.420. Redistillation of 6 g. of this material gave 4 g. of the aldehyde (XIX), b. p. 103–109°, $n^{20}D$ 1.4163, d^{20}_{20} 0.863.

A 2,4-dinitrophenylhydrazone was prepared and after two recrystallizations from ethanol melted at 116–117.5°.[16]

Anal. Calcd. for $C_{11}H_{22}N_4O_4$: C, 50.19; H, 4.59. Found: C, 50.21; H, 4.38.

Ethyl β-Ethoxy-α-allylacrylate.—A mixture of 104 g. (0.53 mole) of the β-acetoxyacrylate (XVIII), 150 g. (3.26 moles) of anhyd. ethanol and 1 g. of *p*-toluenesulfonic acid was fractionated through a four-foot packed column to give 120 g. of a mixture consisting of ethanol, ethyl acetate and water; b. p. 70–80°, $n^{20}D$ 1.3642. The residue was fractionated through a one-foot packed column to give 71 g. (73%) of the β-ethoxyacrylate.

Allyl Diallylmalonaldehydate (XXI).—Fractionation of 99 g. (0.5 mole) of the β-acetoxyacrylate (XVIII), 116 g. (2 moles) of allyl alcohol, 1 g. of *p*-toluenesulfonic acid and 2 g. of β-naphthol (polymerization inhibitor) gave 81.5 g. of a mixture consisting of allyl alcohol, allyl acetate and water. The residue was taken up in ether and washed with water. The ether was removed on the steam-bath and the oily residue was heated at 200–220° for one and a half hours. Distillation from a Claisen flask gave 75 g. (72%) of the malonaldehydate (XXI), b. p. 71–72° (0.3 mm.), $n^{20}D$ 1.4539, d^{20}_{20} 0.997, *MR* 56.33 (calcd. 57.98), sapon. equiv., 105 (calcd. as a dibasic acid 104).

A 2,4-dinitrophenylhydrazone was prepared and recrystallized from ethanol; m. p. 96–97°. *Anal.* Calcd. for $C_{18}H_{20}N_4O_6$: N, 14.42. Found: N, 14.65.

Allyl Diallylacetate (XXII).—A mixture of 34 g. (0.17 mole) of the malonaldehydate (XXI) and 17 g. (0.3 mole) of potassium hydroxide dissolved in 20 ml. of water was stirred at room temperature for three hours and extracted with ether. The extracts were dried over anhydrous magnesium sulfate and distilled to give, after removal of ether, 19 g. (62%) of the acetate (XXII), b. p. 52–58 (2 mm.), $n^{20}D$ 1.4440, d^{20}_{20} 0.904, *MR* 52.54 (calcd. 53.25), sapon. equiv. 172 (calcd. 180).

The aqueous portion from above was acidified and distilled to give a distillate, b. p. 90–100°, which had a sharp odor and reduced mercuric oxide and accordingly indicates the presence of formic acid.

Diallylacetic Acid (XXIII).—A mixture of 9 g. (0.05 mole) of the acetate (XXII) and 4 g. of sodium hydroxide dissolved in 30 ml. of water was refluxed and stirred for thirty minutes. Acidification of the aqueous solution gave an oil which was taken up in ether and dried over anhydrous magnesium sulfate. Distillation gave, after removal of ether, 6 g. (86%) of the acid (XXIII), b. p. 115–120° (3 mm.), $n^{20}D$ 1.4510, d^{20}_{20} 0.9532, neut. equiv., 140 (calcd. 140).[17]

Summary

1. Allyl type alcohols transetherify with ethyl β-ethoxyacrylate (I) and ethyl β,β-diethoxypropionate (II) to give the corresponding β-alloxyacrylates.

2. The β-alloxyacrylates undergo a Claisen rearrangement to α-formylpentenoates.

3. A method for the preparation of allyl allylacetate (XIII) and methallyl methallylacetate (XII) is presented.

4. Methyl α,α-dimethoxysuccinate with allyl alcohol in the presence of a sodium alkoxide catalyst has been found to yield allyl oxalate (XVII) and allyl allylacetate (XIII).

5. Ethyl β-alloxyacrylate (III) rearranges in the presence of acid anhydrides to give ethyl β-acyloxy-α-allylacrylates.

6. The β-acyloxyacrylates undergo alcoholysis to yield alkyl β-alkoxy-α-allylacrylates.

7. Application of this reaction to allyl alcohol produced allyl diallylmalonaldehydate (XXI).

(16) Hurd and Pollack, THIS JOURNAL, **60**, 1905 (1938) report m. p. 120°.

(17) V. Auwers and Moosbrugger, *Ann.*, **387**, 167 (1912).

PHILADELPHIA, PA. RECEIVED JUNE 8, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Condensation of Nitromethane with D-Erythrose and 2,4-Benzylidene-D-erythrose[1]

BY JOHN C. SOWDEN

Since the introduction five years ago[2] of the aldehyde–nitromethane condensation reaction as a synthetic tool in the carbohydrate series, this method of lengthening the sugar chain has been applied to various pentoses, both unsubstituted and as the benzylidene derivatives, as well as to D-glucose and 4,6-benzylidene-D-glucose. Carbohydrate C-nitroalcohols have been prepared from D- and L-arabinose,[3] 3,5-benzylidene-D-arabinose,[2] 2,4-benzylidene-L-xylose[4] and 4,6-benzylidene-D-glucose.[5] Acetylated carbohydrate C-nitroölefins have been prepared from D- and L-arabinose,[3] D- and L-xylose,[6] D-ribose[6] and D-glucose.[6] In general, it has been found that the yields of the nitro compounds are higher from the pentose series than from the hexose series.[7] This agrees with previous observations that the condensation reaction gives decreasing yields with increasing complexity or

(1) A preliminary communication outlining the main features of this work appeared in THIS JOURNAL, **71**, 1897 (1949).

(2) Sowden and Fischer, *ibid.*, **66**, 1313 (1944).

(3) Sowden and Fischer, *ibid.*, **69**, 1963 (1947); Sowden, *Science*, **109**, 2827 (1949); *J. Biol. Chem.*, **180**, 55 (1949).

(4) Sowden and Fischer, THIS JOURNAL, **67**, 1713 (1945).

(5) Sowden and Fischer, *ibid.*, **68**, 1511 (1946).

(6) Sowden and Fischer, *ibid.*, **69**, 1048 (1947).

(7) Carbohydrate C-nitroalcohols have recently been prepared in this Laboratory by Mr. Robert Schaffer from D-mannose and D-galactose. In each instance the yields are considerably higher than for D-glucose but somewhat lower than the average yield from the pentoses listed above. Experimental details will be reported in the near future.

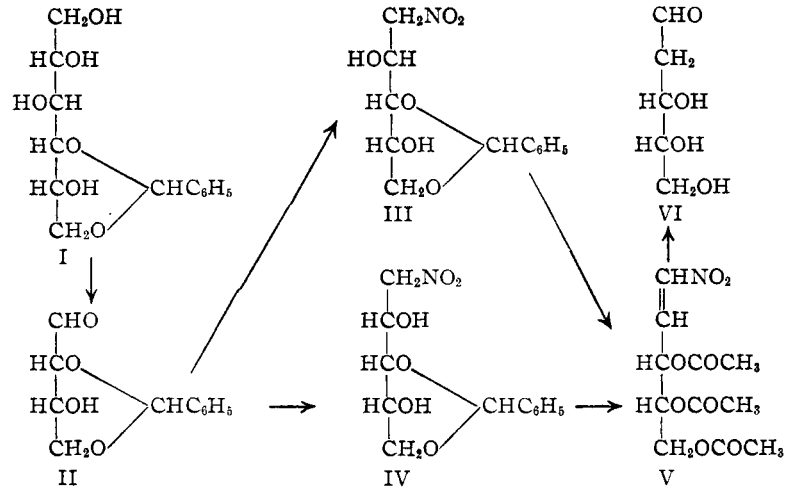
chain length of either component.[8] Thus, it was of interest to apply the synthesis to the tetrose series of sugars in the expectation that here it would be even more fruitful than with the pentoses.

D-Erythrose was chosen as the most attractive starting material since the nitroölefin-desoxy-sugar synthesis[6] leads in this instance to the important natural sugar D-*erythro*-2-desoxypentose.

The earlier methods of preparing erythrose, depending on the degradation of arabinose,[9] lead to impure sirups containing only small amounts of the tetrose. For this reason, a more reliable preparation of D-erythrose was devised: 4,6-benzylidene-D-glucose was reduced catalytically to 4,6-benzylidenesorbitol (I). Cleavage with sodium metaperiodate then gave amorphous 2,4-benzylidene-D-erythrose (II) which, on acid hydrolysis, gave sirupy D-erythrose of good purity. While this work was in progress, the preparation of D-erythrose from 4,6-ethylidene-D-glucose by a similar series of reactions was reported by Hockett, Collins and Scattergood.[10] In view of the low and uncertain yield of 4,6-benzylidene-D-glucose obtainable by the method of Zervas,[11,12] 4,6-ethylidene-D-glucose appears to be preferable for the preparation of D-erythrose.

The condensation of 2,4-benzylidene-D-erythrose with nitromethane under alkaline conditions gave the nitroalcohols, 3,5-benzylidene-1-nitro-1-desoxy-D-arabitol (III) and 3,5-benzylidene-1-nitro-1-desoxy-D-ribitol (IV) in a combined yield of 64%. This mixture of isomers was readily separated into the two pure components by virtue of a rather remarkable difference in their solubilities in chloroform. Hydrolysis of the benzylidene group from either isomer followed by acetylation gave the corresponding crystalline nitroalcohol tetraacetates. Either of the tetraacetates when boiled in benzene solution with sodium bicarbonate then gave D-*erythro*-triacetoxy-1-nitropentene-1 (V). This acetylated nitroölefin was also obtained from sirupy D-erythrose in 44% yield by application of the appropriate reactions without the isolation of intermediate compounds.

D-*erythro*-2-Desoxypentose (VI), the sugar occurring naturally in the "desoxyribose" nucleic acids was obtained from the acetylated nitroölefin (V), by hydrogenation of the double bond followed by deacetylation and hydrolysis of the *aci*nitroalcohol sodium salt with sulfuric acid.



Experimental

4,6-Benzylidenesorbitol.—A solution of 12.8 g. of 4,6-benzylidene-D-glucose,[11,12] m. p. 185–186°, in 150 cc. of 95% ethanol was shaken with hydrogen in the presence of 1.5 g. of platinum oxide (Adams catalyst) at room temperature and an initial pressure of 50 p. s. i. The reduction was complete in eighteen hours with the absorption of approximately one mole-equivalent of hydrogen. After filtration of the catalyst, concentration of the solution yielded 10.5 g. (82%) of 4,6-benzylidenesorbitol, m. p. 131–134°. Recrystallization from ethanol by the addition of ether yielded the pure compound,[1] m. p. 132–133°.

4,6-Hexahydrobenzylidenesorbitol.—When water was used in place of 95% ethanol in the reduction of 4,6-benzylidene-D-glucose as described above, approximately four mole-equivalents of hydrogen were absorbed in twenty-four hours. The product, isolated in 80% yield, was 4,6-hexahydrobenzylidenesorbitol. After recrystallization from ethanol by the addition of ether and petroleum ether, the pure compound showed m. p. 124–125° and $[\alpha]^{22}D$ −24.2° in water, *c* 5.3.

Anal. Calcd. for $C_{13}H_{24}O_6$ (276.3): C, 56.5; H, 8.76. Found: C, 56.5; H, 8.63.

2,4-Benzylidene-D-erythrose Phenylhydrazone.—Two grams of 4,6-benzylidenesorbitol was treated with a solution containing 3.15 g. of sodium metaperiodate and 0.6 g. of sodium bicarbonate in 40 cc. of water. After standing two hours at room temperature, the solution was concentrated to dryness at reduced pressure. Absolute ethanol was added to the residue and the concentration to dryness repeated. The residue was extracted several times with warm ethyl acetate and, after washing with water and drying with sodium sulfate, the extract was concentrated at reduced pressure. Treatment of the amorphous residue with phenylhydrazine in a small volume of ethanol gave 1.85 g. (84%) of 2,4-benzylidene-D-erythrose phenylhydrazone, m. p. 143–146°. After recrystallization from ether by the addition of petroleum ether, the colorless hydrazone showed m. p. 146–147° and $[\alpha]^{25}D$ 64.5° in absolute chloroform, *c* 2.

Anal. Calcd. for $C_{17}H_{18}O_3N_2$ (298.3): C, 68.5; H, 6.08; N, 9.40. Found: C, 68.8; H, 6.14; N, 9.68.

2,4-Benzylidene-D-erythrose Benzylphenylhydrazone.—Prepared in 85% yield in the same manner as the phenylhydrazone, the benzylphenylhydrazone showed m. p. 156–157° and $[\alpha]^{25}D$ 20.5° in absolute chloroform, *c* 2.2.

(8) Hass and Riley, *Chem. Revs.*, **32**, 373 (1943).

(9) Wohl, *Ber.*, **32**, 3667 (1899); Ruff, *ibid.*, **32**, 3672 (1899); Deulofeu and Selva, *J. Chem. Soc.*, 225 (1929).

(10) Hockett, Collins and Scattergood, 113th National Meeting, American Chemical Society, April 19–23, 1948, Chicago, Ill.

(11) Zervas, *Ber.*, **64**, 2289 (1931).

(12) It has recently been observed in this Laboratory that the benzylidenation of D-glucose by the method of Zervas, ref. 11, leads not only to 4,6-benzylidene-D-glucose but also to a second crystalline monobenzylidene-D-glucose. The structure of the new isomer, which melts at 176–177° and does not reduce Fehling solution, is being investigated at present.

Anal. Calcd. for $C_{24}H_{24}O_8N_2$ (388.5): C, 74.2; H, 6.23; N, 7.22. Found: C, 74.2; H, 6.22; N, 7.54.

3,5-Benzylidene-1-nitro-1-desoxy-D-arabitol and 3,5-Benzylidene-1-nitro-1-desoxy-D-ribitol.—Thirty grams of 4,6-benzylidenesorbitol was converted to amorphous 2,4-benzylidene-D-erythrose by oxidative cleavage with sodium metaperiodate as described above. The erythrose derivative was dissolved in a mixture of 250 cc. of absolute methanol and 75 cc. of nitromethane and 6 g. of sodium in 300 cc. of absolute methanol added. After standing ten hours at room temperature, the solution was treated with 16.5 cc. of glacial acetic acid and concentrated to a small volume at reduced pressure. Water was added and the concentration repeated. The resulting partly crystalline residue was taken up with ether and water and the ether layer was separated and washed with a small volume of cold water. After drying and concentration at reduced pressure, the ether solution yielded a crystalline mass. The crystals were extracted with chloroform at room temperature, leaving a residue of 7.0 g. of nearly pure 3,5-benzylidene-1-nitro-1-desoxy-D-arabitol, m. p. 138–142°. Concentration of the chloroform extract to dryness followed by extraction of the residue with anhydrous ether yielded as a residue an additional 1.7 g. of this material. Recrystallization from water yielded the pure isomer,[1] m. p. 145–146°. Concentration of the ether extract to dryness gave 10.6 g. of crystalline material which on recrystallization from ether and petroleum ether gave pure 3,5-benzylidene-1-nitro-1-desoxy-D-ribitol,[1] m. p. 106–107°. The yield of crystalline nitroalcohols was 19.3 g. (64%).

1-Nitro-1-desoxy-D-arabitol.—Five grams of 3,5-benzylidene-1-nitro-1-desoxy-D-arabitol was heated for one hour at 65–70° (stirring) with a solution containing 40 cc. of water, 10 cc. of ethanol and 0.15 cc. of sulfuric acid. After removal of the resulting benzaldehyde by distillation *in vacuo* and of the sulfuric acid by ion-exchange (Duolite A-4), concentration of the solution to dryness gave a crystalline residue. Recrystallization from absolute ethanol yielded 2.85 g. (85%) of 1-nitro-1-desoxy-D-arabitol,[1] m. p. 147–148°.

Acetylation of the nitroalcohol at room temperature with acetic anhydride containing a trace of sulfuric acid gave the tetraacetate,[1] m. p. after recrystallization from absolute ethanol 123–125°, in 96% yield.

1-Nitro-1-desoxy-D-ribitol Tetraacetate.—Hydrolysis of 3,5-benzylidene-1-nitro-1-desoxy-D-ribitol as described above for the arabitol derivative gave amorphous 1-nitro-1-desoxy-D-ribitol in 90% yield. Acetylation then gave the crystalline tetraacetate in 85% yield, m. p. after recrystallization from absolute ethanol 64–65°; $[\alpha]^{25}D$ −7.8° in chloroform, *c* 6.

Anal. Calcd. for $C_{18}H_{19}O_{10}N$ (349.3): C, 44.7; H, 5.48. Found: C, 44.9; H, 5.36.

D-Ribose and D-Arabinose Benzylphenylhydrazones.—Hydrolysis of sodium 1-*aci*nitro-1-desoxy-D-arabitol with sulfuric acid as described previously[2–6] gave D-arabinose, isolated in 70% yield as the benzylphenylhydrazone,[13] m. p. 170–172°, without recrystallization.

Similarly, amorphous 1-nitro-1-desoxy-D-ribitol gave 72% of D-ribose benzylphenylhydrazone,[14] m. p. 127–128°.

D-*erythro*-Triacetoxy-1-nitropentene-1.—Either of the nitroalcohol tetraacetates described above when refluxed in benzene solution with sodium bicarbonate[6] gave the acetylated nitroölefin[1] in 60–80% yield.

The acetylated nitroölefin was also prepared from sirupy D-erythrose, obtained from 4,6-benzylidene-D-glucose, without the isolation of intermediate products: A mixture of 24.8 g. of 4,6-benzylidene-D-glucose and 15.5 g. of sodium bicarbonate was treated with a cold solution of 40 g. of sodium metaperiodate in 550 cc. of water. After standing one hour at room temperature the solution was concentrated to dryness at reduced pressure and the residue was extracted several times with warm ethyl acetate. Concentration of the extract at reduced pressure left a dry amorphous residue weighing 17 g. This residue was refluxed for ninety minutes with 200 cc. of 0.1 *N* sulfuric acid. The resulting benzaldehyde was removed by distillation at reduced pressure and the sulfuric acid was removed by ion-exchange (Duolite A-4). Concentration of the resulting solution at reduced pressure then gave 9.4 g. (85%) of colorless sirupy D-erythrose. (An aliquot of the sirup, on treatment with benzylphenylhydrazine, gave D-erythrose benzylphenylhydrazone,[15] m. p. 100–102° without recrystallization, in 84% yield.) A solution of 8.3 g. of D-erythrose sirup in 75 cc. of anhydrous methanol and 30 cc. of nitromethane was treated with 2.5 g. of sodium in 75 cc. of anhydrous methanol. After a few seconds a copious precipitation of the sodium nitroalcohols occurred. After six hours the solution was cooled to 0°, an equal volume of dry ether was added, and the sodium nitroalcohols were filtered and washed rapidly with a cold mixture of methanol and ether, ether and petroleum ether. After dissolution in 250 cc. of ice-water, the sodium was removed by ion-exchange (Amberlite IR-100-AG). Concentration of the effluent, finally in high vacuum, then yielded 9.3 g. of partly crystalline, mixed nitroalcohols. The nitroalcohols were acetylated with 75 cc. of acetic anhydride containing 5 drops of sulfuric acid by heating for five minutes on the steam-bath. After stirring with ice-water to destroy excess acetic anhydride, the acetylated nitroalcohols were extracted with chloroform. Concentration of the extract followed by several distillations with benzene to remove acetic acid left a partly crystalline mass. This was taken up in 250 cc. of dry benzene and the solution was refluxed with 18 g. of sodium bicarbonate for ninety minutes. Filtration followed by concentration then left a light colored sirup which crystallized rapidly when seeded with D-*erythro*-triacetoxy-1-nitropentene-1. Filtration with cold ethanol yielded 8.75 g. (44% based on D-erythrose) of the acetylated nitroölefin,[1] m. p. 63–65°.

D-*erythro*-2-Desoxypentose.—A solution of 2.2 g. of D-*erythro*-triacetoxy-1-nitropentene-1 in 50 cc. of absolute ethanol was shaken with hydrogen at room temperature and pressure in the presence of 0.2 g. of freshly-prepared palladium black.[16] The hydrogenation was interrupted after twenty minutes when 1.05 mole-equivalents of hydrogen had been absorbed and the rate had become slow. The sirup obtained after filtration and concentration was stirred with a mixture of 40 cc. of 1 *N* sodium hydroxide solution and 10 cc. of ethanol. After the sirup was dissolved, the resulting solution was added dropwise to a stirred mixture of 5 cc. of sulfuric acid and 7.5 cc. of water at 0°. The reaction mixture was then diluted with ice-water and neutralized by stirring with solid barium carbonate. After filtration, a few drops of glacial acetic acid were added to the filtrate and it was concentrated at reduced pressure to a sirup. The sirup was taken up in a small volume of 75% ethanol and 1.5 cc. of benzylphenylhydrazine added. Slow evaporation of this solution then gave 1.4 g. (59%) of D-*erythro*-2-desoxypentose benzylphenylhydrazone.[17] After recrystallization from aqueous ethanol, the pure hydrazone melted at 127–128° and showed $[\alpha]^{25}D$ −17.7° in pyridine, *c* 2.

Cleavage of the hydrazone with benzaldehyde as described by Meisenheimer and Jung[18] gave D-*erythro*-2-desoxypentose in 82% yield as a colorless sirup showing $[\alpha]^{28}D$ −50° in water, *c* 1. After standing several days in a desiccator, the sugar crystallized spontaneously and was washed by filtration with cold isopropyl alcohol. After recrystallization from a few drops of isopropyl alcohol, the desose melted at 83–85° and showed $[\alpha]^{25}D$ −56° in water, *c* 1. Mutarotation was not observed.

Cleavage of the hydrazone with formaldehyde by the method of Ruff and Ollendorf[13] gave colorless sirups in near quantitative yield which also crystallized spontaneously. However, if the desose sirups obtained in this way

(13) Ruff and Ollendorf, *Ber.*, **32**, 3234 (1899).

(14) Levene and Jacobs, *ibid.*, **42**, 1198 (1909).

(15) Ruff, *ibid.*, **32**, 3672 (1899).

(16) Tausz and Putnoky, *ibid.*, **52**, 1573 (1919).

(17) Levene and Mori, *J. Biol. Chem.*, **83**, 803 (1929).

(18) Meisenheimer and Jung, *Ber.*, **60**, 1462 (1927).

were repeatedly evaporated with water on the steam-bath to constant weight to remove all traces of polymeric formaldehyde, they became somewhat colored, their specific rotation decreased to as little as −15° and crystallization could no longer be induced.

Summary

The condensation of nitromethane with D-erythrose and with 2,4-benzylidene-D-erythrose has been studied. The latter yielded the corresponding benzylidene nitroalcohols in the crystalline state in a combined yield of 64%. D-*erythro*-Triacetoxy-1-nitropentene-1, prepared from either of the two nitroalcohols, or directly from D-erythrose without the isolation of intermediates, was converted to D-*erythro*-2-desoxypentose, the sugar occurring naturally in certain nucleic acids.

SAINT LOUIS, MISSOURI RECEIVED JULY 15, 1949

[CONTRIBUTION FROM THE DEPARTMENTS OF NUTRITION AND VIROLOGY, MEDICAL RESEARCH DIVISION, SHARP AND DOHME, INC.]

Competitive Antagonism of Ribonucleic and Desoxyribonucleic Acids in the Nutrition of *Lactobacillus bifidus*[1]

BY HELEN R. SKEGGS, JOHN SPIZIZEN AND LEMUEL D. WRIGHT

Recent studies with lactobacilli have demonstrated instances of a relationship between their nutritive requirements and desoxyribonucleic acid or certain of its constituents. Thymine desoxyriboside was reported by Shive, *et al.*,[1a] to replace vitamin B_{12} in the nutrition of *Lactobacillus lactis*. Skeggs, *et al.*,[2] reported similar findings for *Lactobacillus leichmannii*. Further studies by Kitay, *et al.*,[3] indicated that thymidine is not specific in its ability to substitute for essential factors in the nutrition of various lactobacilli. *Lactobacillus bifidus*[1a] (ATCC 4963) has been found[4] to be capable of utilizing either vitamin B_{12} or thymidine for growth in an otherwise complete medium. However, *Lactobacillus bifidus* also has been found to respond to intact desoxyribonucleic acid in the absence of either vitamin B_{12} or thymidine. While the growth factor requirements of this organism are not specific, it was felt that it might prove to be a useful instrument in a search for a compound that would inhibit utilization of desoxyribonucleic acid (DNA).

Experimental and Results

The basal medium employed is shown in Table I. *Lactobacillus bifidus* was carried in skim milk medium (Difco) containing 1% Bacto tryptose. Inocula were prepared by suspending 0.1 ml. of a twenty-four-hour culture in 10 ml. of sterile physiological saline. Each tube received one drop of this suspension as seed. All tests were carried out in 10-ml. volumes, of which 5 ml. was the double-strength medium. Tests were autoclaved at 120° for fifteen minutes prior to aseptic addition of samples and seeding, then incubated at 37° for from twenty-four to seventy-two hours. Results were obtained at twenty-four hours by measuring the turbidity produced with a Klett–Summerson photoelectric colorimeter or at seventy-two hours by titration of acid production with 0.1 *N* sodium hydroxide and brom thymol blue as indicator.

TABLE I

COMPOSITION OF DOUBLE STRENGTH BASAL MEDIUM

Casein hydrolysate[a]	1.0 g.
Tryptophan	20 mg.
Cystine	20 mg.
Adenine	1 mg.
Guanine	1 mg.
Xanthine	1 mg.
Uracil	1 mg.
Salts A[b]	1 ml.
Salts B[b]	1 ml.
Na acetate (anhyd.)	1.2 g.
Glucose	4 g.
Biotin	1 γ
Pyridoxine	400 γ
Pyridoxal	400 γ
Riboflavin	200 γ
Nicotinic acid	200 γ
Pantothenic acid	200 γ
Thiamine	200 γ
Folic acid	100 γ
p-Aminobenzoic acid	100 γ
Tween 80	0.2 ml.
Distilled water to 100 ml.	

[a] Univ. Texas Pub. No. 4137, 82, 1941. [b] E. E. Snell and L. D. Wright, *J. Biol. Chem.*, 139, 675 (1941).

Lactobacillus bifidus responded to DNA over a range of from 5 to 50 micrograms per tube as shown in Fig. 1. Increased amounts of DNA allowed no further growth. The response of the organism to DNA was the same whether the DNA was autoclaved in the test medium or added aseptically prior to seeding of the test. Various com-

(1) This organism has been reclassified as *Lactobacillus acidophilus* and is now designated as such by the American Type Culture collection. However, other strains of *L. acidophilus* do not, in our hands, exhibit the characteristics described here for culture 4963.

(1a) W. Shive, R. E. Eakin, W. M. Harding, J. M. Ravel and J. E. Sutherland, THIS JOURNAL, 70, 2299 (1948).

(2) H. R. Skeggs, J. W. Huff, L. D. Wright and D. K. Bosshardt, *J. Biol. Chem.*, 176, 1459 (1948).

(3) E. Kitay, W. S. McNutt and E. E. Snell, *ibid.*, 177, 993 (1949).

(4) H. R. Skeggs, J. Spizizen and L. D. Wright, "The Use of *Lactobacillus bifidus* in the Study of Antagonists of Desoxyribonucleic Acid," Abstract 3rd Meeting-in-Miniature, Philadelphia, p. 27, 1949.