D,L-*xylo*-3-*Hexulose*. Ten milliliters of 0.5*N* aqueous hydrochloric acid was added to a solution containing 10 g. of the anomeric mixture of 1,2:4,5-di-*O*-isopropylidene-D,L-*xylo*-3-hexulofuranose in 100 ml. of methanol. After 3 days at room temperature, the solution was neutralized with silver carbonate. The inorganic solids were removed by filtration and the filtrate was concentrated to a sirup under reduced pressure. When the sirup was dissolved in 30 ml. of ethanol and cooled, the crystalline sugar precipitated from solution. The crude product weighed 2.5 g. and melted at 115–119° (with slight decomposition). An analytical sample of D,L-*xylo*-3-hexulose (m.p. 123–124°, with decomposition) was obtained after two recrystallizations from ethanol.

Anal. Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.72. Found: C, 39.96; H, 6.89.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY,[3] UNIVERSITY OF WISCONSIN]

myo-Inositol-2-C^{14} and -4,5-C^{14}, and a Novel Degradation Reaction of *myo*-Inosose-2 Ethylene Disulfone

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The nitroinositol synthesis of Grosheintz and Fischer was used to prepare two varieties of *myo*-inositol, specifically labeled with C^{14} at position 2 and positions 4 and 5, respectively. The *myo*-inositol-2-C^{14} was extensively purified by chromatography. *myo*-Inosose-2 ethylene disulfone was synthesized. The alkaline dealdolization of this sulfone gives two products, one of which was partially characterized. Periodate degradation of the sulfone yields formic acid and 1,3-dithiolane 1,1,3,3-tetroxide, and is thus a convenient method for isolating C-2 from labeled *myo*-inositol.

When the senior author began to plan, some years ago, to undertake investigations of *myo*-inositol[4] metabolism, it was clear that some phases of this work would require inositol labeled with C^{14} at known positions in the ring. At the time (1955), the only known synthesis which was potentially capable of yielding *myo*-inositol so labeled was the nitroinositol synthesis of Grosheintz and Fischer.[5] We undertook the further development of this synthesis, and were able to prepare first *myo*-inositol-2-C^{14} then subsequently a *myo*-inositol-4,5-C^{14} (see formula VIIIa).

In connection with this work, certain degradation reactions were investigated which might serve for the isolation of individual carbon atoms from *myo*-inositol. Such reactions would be useful in tracer studies of inositol biosynthesis. The periodate oxidation of the ethylene disulfone (XI) of *myo*-inosose-2 (IX) was one of the reactions examined, and it was found to follow a novel course. Some information on the alkaline degradation of this sulfone was also obtained.

After our synthesis of *myo*-inositol-2-C^{14} was completed, Posternak and colleagues independently carried out a similar synthesis and described it fully.[6] We therefore propose to discuss only certain steps which were executed differently in the two laboratories, and in which our procedure appears to offer advantages. In its original form,[5] the synthesis involved the conversion of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (I) to 1,2-*O*-isopropylidene-D-xylofuranopentodialdose (III) and the alkaline condensation of the latter with excess nitromethane to give, after hydrolysis to remove the isopropylidene group, a mixture of 6-nitro-6-deoxy sugars (IV). Under appropriate conditions, the mixture IV cyclized to a nitrodeoxyinositol, which was reduced to an amine designated "aminodesoxyinositol III". Posternak[7] in 1950 identified the amine as *scyllo*-inosamine (VIa), and thus also established the configuration of the nitroinositol as Va. He found that free *scyllo*-inosamine could be deaminated with nitrous acid to a mixture of products including *myo*-inositol in 8–12% yield.

Two problems had to be solved to make the nitroinositol synthesis a feasible one for the preparation of carbon-labeled inositol: 1) When the label was to be introduced as nitromethane, the wastage of this compound at the condensation step had to be reduced; and 2) the yield in the deamination step had to be increased several fold.

In the Posternak synthesis[6] of *myo*-inositol-2-C^{14}, the molar ratio (7:1) of nitromethane and dialdose used in the condensation was that of the

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(4) The cyclitols discussed in this paper are named and numbered according to the system of H. G. Fletcher, Jr., L. Anderson, and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

(5) J. M. Grosheintz and H. O. L. Fischer, *J. Am. Chem. Soc.*, **70**, 1476, 1479 (1948).

(6) T. Posternak, W. H. Schopfer, and R. Huguenin, *Helv. Chim. Acta*, **40**, 1875 (1957).

(7) T. Posternak, *Helv. Chim. Acta*, **33**, 1597 (1950).

original workers and most of the excess nitromethane was recovered. Fresh nitromethane, unlabeled in the later stages, was added, and the condensation repeated, fifteen times in all, so that most of the labeled nitromethane was converted to condensation products. The mixture of isopropylidene-nitro sugars from each condensation was crystallized and combined lots were hydrolyzed to IV and cyclized to Va. Our approach was to carry out a single condensation using 1.7 molar equivalents of nitromethane, hydrolyze, and cyclize the resulting syrup directly. And although the method of repeated condensations is theoretically superior, the radioactive yield in the cyclized product (measured as the acetate Vb in our procedure) was the the same in both cases—*ca.* 8.5% based on nitromethane.

A comparison of the two procedures suggests that our success in making up for the low yield inherent in the use of a low nitromethane dialdose ratio was the result of acidifying the condensation mixture before removing the solvents and unused nitromethane, and then bypassing the crystallization of the mixed nitrosugars. If this conclusion is correct, improved yields could be obtained by combining our procedure with Posternak's method of operation. Continued repetition of the condensation (eighteen to twenty hours each time) would be tedious, but probably worth the effort in view of the high cost of nitromethane-C^{14}. Dilution with unlabeled nitromethane could be avoided if the initial scale of operation were sufficiently large.

The key to improvement of the yield in the deamination step turned out to be the blocking (with acetyl groups) of the hydroxyl functions in the inosamine VIa. The suggestion that the *O*-acetylated inosamine might be cleanly deaminated, in contrast to the unsubstituted compound, came from work done by Wintersteiner and Klingsberg[8] on streptamine (1,3-diamino-1,3-dideoxy-*scyllo*-inositol) and certain of its acyl derivatives. To test the possibility, penta-*O*-acetyl-*scyllo*-inosamine hydrochloride was synthesized[9] and treated with silver nitrite. Treatment of the reaction residue with acetic anhydride gave *myo*-inositol hexaacetate in better than 75% yield. Posternak *et al.*[6] obtained similar results.

In the Posternak synthesis, *scyllo*-inosamine (VIa) was first obtained by reducing the cyclization product Va, then converted to the hydrochloride of the penta-*O*-acetate (VIb) in three steps *via* the *N*-salicylidene derivative, as described by Anderson and Lardy.[9] We were able to reduce by two the number of steps required for transforming Va to VIb by the expedient of acetylating Va and reducing the acetate, Vb, directly to VIb. As noted by Iselin and Fischer,[10] the hydrogenation of penta-*O*-acetylnitrodeoxyinositols is difficult, but the difficulty was overcome by using the Raney nickel W-6 of Billica and Adkins[11] as the catalyst.

myo-Inositol doubly labeled in the ring was prepared from D-glucose-3,4-C^{14}. The problem of securing a good radioactive yield in this synthesis is one of efficiently converting bicarbonate-C^{14} to glucose-3,4-C^{14}, and this in turn to the monoacetone derivative (II-3,4-C^{14}). Glucose-3,4-C^{14} has to date been obtained only by biosynthesis, usually with radioactive yields of a fraction of 1%. It was therefore of interest to investigate the claim, by Palmstierna and Ehrensvärd,[12] that improved conversions of $C^{14}O_2$ to glucose (polysaccharide) could be accomplished by cells of *Escherichia coli* B suspended in a nitrogen-free lactate medium. The radioactive yield in the experiments described by the Swedish workers was about 1%, and the statement was made that "it does not seem impossible to step up the yield to 20%" (by recycling unused $C^{14}O_2$). The glucose-3,4-C^{14} used in our synthesis was prepared by the procedure of Palmstierna and Ehrensvärd, but in our hands the radioactive yield was 0.07%, only slightly better than that obtained by the standard rat-liver glycogen method.

An interesting feature of the inositol prepared from D-glucose-3,4-C^{14} is that it is asymmetrically labeled. Ordinary, unlabeled *myo*-inositol is a *meso* compound, with a single plane of symmetry, which passes through carbons 2 and 5. The configurations about carbons 4 and 6 are enantiomorphic, as are those about carbons 1 and 3. The asymmetry arises in the present case from the fact that one of the carbons of the first named enantiomorphic pair is labeled, while the other is not. In naming the labeled inositol,[4] counterclockwise numbering is chosen so as to give the labeled atoms the lowest numbers. The compound may then be called L-*myo*-inositol-4,5-C^{14}.

For final purification, the synthetic labeled *myo*-inositols were chromatographed on cellulose powder columns. This procedure gave products with satisfactory melting points and no impurities detectable by paper chromatography. However, it was felt that further rigorous tests of purity would be advisable. It seemed likely that in the deamination of the *scyllo*-inosamine derivative VIb, where the principal product is formed by inversion of configuration, a portion of the material would react with retention. The result would be contamination of the final product by *scyllo*-inositol ("scyllitol," XVII), which is very difficult

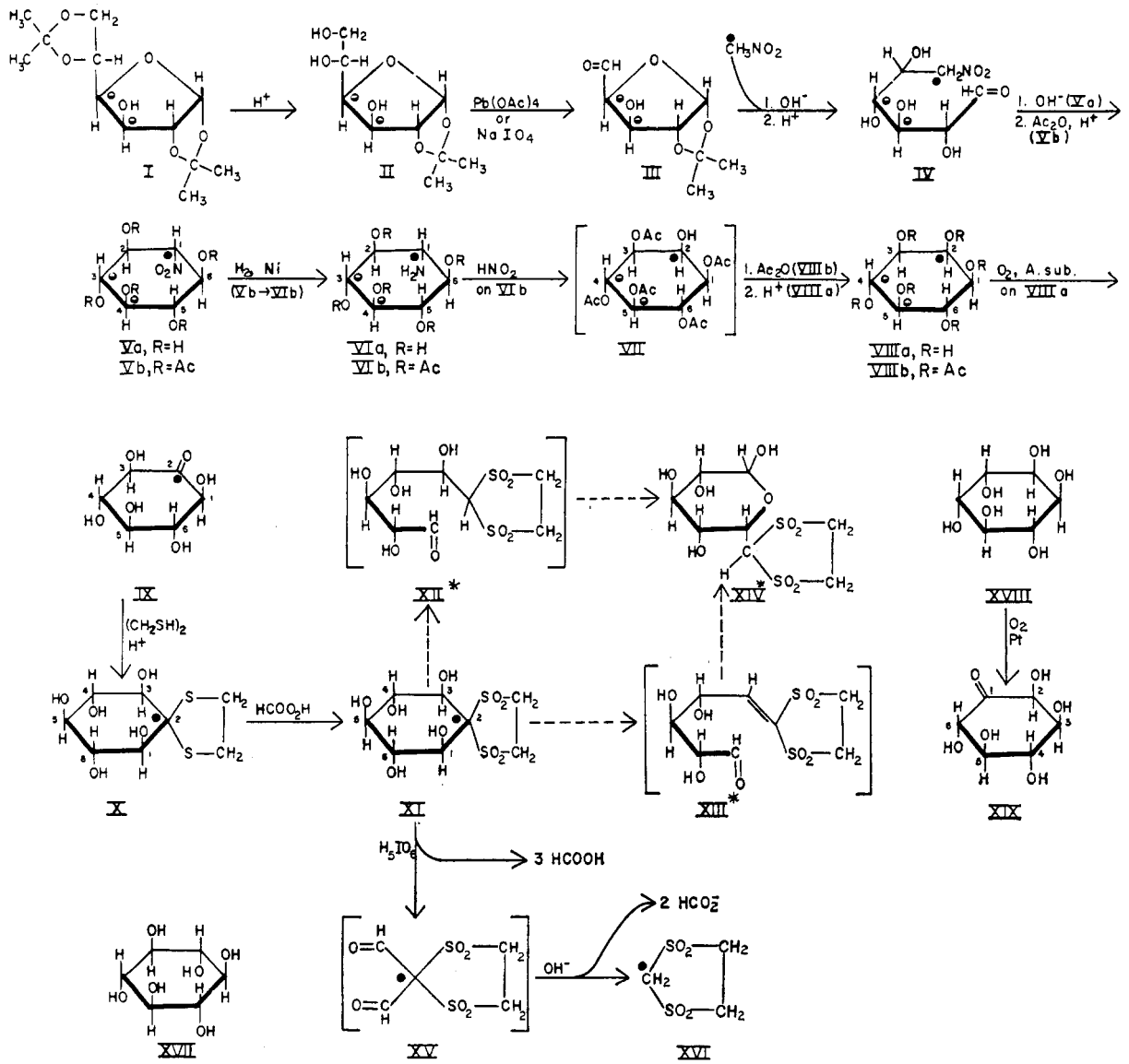
(8) O. Winstersteiner and Anna Klingsberg, *J. Am. Chem. Soc.*, **73**, 2917 (1951).

(9) L. Anderson and H. A. Lardy, *J. Am. Chem. Soc.*, **72**, 3141 (1950).

(10) B. Iselin and H. O. L. Fischer, *J. Am. Chem. Soc.*, **70**, 3946 (1948).

(11) H. R. Billica and H. Adkins, *Org. Syntheses*, **29**, 24 (1949).

(12) H. Palmstierna and G. Ehrensvärd, *Acta Chem. Scand.*, **10**, 691 (1956).



● Indicates labeled atoms derived from nitromethane-C^{14}
◒ Indicates labeled atoms derived from D-glucose-3,4-C^{14}
* Indicates racemic mixture, only one enantiomorph shown

to separate from *myo*-inositol on cellulose or filter paper. Chromatography of a portion of the purified *myo*-inositol-2-C^{14} on Dowex-1 borate, which does separate the two isomers, gave a peak emerging ahead of the *myo*-inositol and containing about 4% of the radioactivity of the sample. *scyllo*-Inositol was tentatively identified as one of several substances constituting this peak, but it was present, if at all, in very small amounts. The deamination of VIb thus proceeds almost exclusively by inversion.

In considering the inosose disulfones (see formula XI) as possible intermediates for the carbon-by-carbon degradation of *myo*-inositol, we had in mind the alkaline dealdolization procedure ("sulfone degradation") of MacDonald and Fischer.[13,14] For maximal utility, the disulfone used should be derived from an asymmetric inosose, and only one of the bonds at the sulfonylated carbon atom should be cleaved. If a single product could be formed in this cleavage in good yield, and if the product could be further degraded one carbon at a time, the degradation would distinguish between the enantiomorphic carbons (1 and 3, 4 and 6) on either side of the plane of symmetry of the molecule.

In an attempt to explore this possibility, the optically active ketone D-*myo*-inosose-1 (XIX) was prepared[15] by the catalytic air oxidation of L-inositol (XVIII). The inosose was treated with

(13) D. L. MacDonald and H. O. L. Fischer, *J. Am. Chem. Soc.*, **74**, 2087 (1952).

(14) D. L. MacDonald and H. O. L. Fischer, *J. Am. Chem. Soc.*, **77**, 4348(1955).

1,2-ethanedithiol in concentrated hydrochloric acid, but no dithioacetal could be isolated. Further work was accordingly conducted with the *meso* ketone *myo*-inososе-2 (IX), which, as noted by MacDonald and Fischer,[14] does react readily with 1,2-ethanedithiol. The dithioacetal, X, was easily oxidized to the ethylene disulfone XI with performic acid.

The dealdolization of the *meso* disulfone XI did proceed with cleavage of only one bond,[16] but under the conditions tried the reaction was not complete, and two products were formed. One of the products was isolated crystalline, and one was obtained only as a syrup. The crystalline compound is isomeric with the starting material XI, and has no carbonyl absorption in the infrared or ultraviolet. These and its other properties (see Experimental) are compatible with the formula XIV. The syrupy companion, which has an absorption maximum between 280 and 290 mμ, is probably XII or XIII, either of which is a likely product of the cleavage of XI. Presumably XIV is formed *via* XII or XIII in a secondary reaction. Further characterization of these compounds was not attempted because the dealdolization of XI appeared to have little practical use

Hough and Taylor[17] oxidized with periodate a diethyl disulfone which they obtained from D-xylose, and reported the presence of almost one molar equivalent of nonvolatile (with steam) acid in the reaction mixture. Attempts to isolate the acid yielded only bis(ethylsulfonyl)methane. The English workers suggested that the nonvolatile acid was the enol of 2,2-bis(ethylsulfonyl)acetaldehyde, and that this latter was converted to the isolated sulfonylmethane by loss of carbon monoxide. We found that the inosose ethylene disulfone XI consumed the expected amount (four molar equivalents) of periodate, and that 1,3-dithiolane 1,1 3 3-tetroxide (XVI) and nearly five molar equivalents of bariumformate could be isolated directly from the reaction mixture after titration with barium hydroxide. These results suggest that 2,2-ethylenedisulfonylmalondialdehyde (XV) is formed as expected by the action of periodate, but that it decomposes, *via* 2,2-ethylenedisulfonylacetaldehyde, to XVI and two moles of formate. The decomposition, however, is clearly a hydrolysis, not a decarbonylation. The explanation is presumably the same as for the hydrolysis of trihalogenoacetaldehydes and trihalogenomethylketones during the haloform reaction—the carbonyl –α-carbon bonds are weakened by the strong inductive effect of the two sulfonyl groups. Neutral or mildly alkaline conditions are apparently required; Barker and MacDonald[18] have recently reported that the aldohexose diethyl disulfones, when oxidized with periodate and acidified, yield only three molar equivalents of steam volatile (formic) acid. The bis(ethylsulfonyl)acetaldehyde formed in the periodate oxidation is thus stable to aqueous acid, in concordance with the results of Hough and Taylor.

The degradation just described afforded an easy method for isolating carbon 2 from a labeled inositol. Accordingly, we subjected a sample of our *myo*-inositol-2-C^{14} to this degradation to check for possible positional randomization of the C^{14}. Such randomization was scarcely expected, but in view of the somewhat capricious nature of the nitrous acid deamination could not be completely excluded. The result of the degradation was that all of the radioactivity, within experimental error, was found in the dithiolane tetroxide. Negligible amounts were found in the formate. It could thus be concluded that there is no randomization of carbon atoms during the synthesis of *myo*-inositol by the nitroinositol route.

EXPERIMENTAL

Melting points were determined in capillary tubes. The thermometer used has been calibrated against Anschütz thermometers calibrated by the National Bureau of Standards. Microanalyses by the Micro-Tech Laboratories, Skokie, Ill.

Nitromethane-C^{14} was prepared from carbon dioxide, generated from 20 mmoles of barium carbonate containing 20 mc. of $BaC^{14}O_3$, by the reaction sequence:

$$CO_2 \xrightarrow{LiAlH_4} CH_3OH \xrightarrow{HI} CH_3I \xrightarrow{AgNO_2} CH_3NO_2$$

Directions for the successive steps are found in the literature[19–21]; in each case these were adapted as needed. The apparatus used in carrying out the reactions was specially constructed for the purpose. Its design[22] was patterned largely after that of the equipment employed by Melville, Rachele and Keller[20] in the synthesis of methyl-C^{14} iodide. An important feature was that the receiver in which each intermediate was collected served as the vessel from which that intermediate was introduced into the set-up for the next reaction. The successive products were transferred by vaporizing them into a carrier stream of dry nitrogen. No vacuum was used at any time.

The final product weighed 1.04 g. This represents a yield of about 80% overall[23] from barium carbonate, after allow-

(15) G. G. Post, M. S. thesis (with L. Anderson), University of Wisconsin, 1957.

(16) By contrast, the diethyl disulfone under the same conditions breaks down to bis(ethylsulfonyl)methane and xylopentodialdose (Rdf. 14).

(17) L. Hough and T. J. Taylor, *J. Chem. Soc.*, 1212 (1955).

(18) R. Barker and D. L. MacDonald, *J. Am. Chem. Soc.*, **82**, 2297 (1960).

(19) R. F. Nystrom, W. H. Yanko, and W. G. Brown, *J. Am. Chem. Soc.*, **70**, 441 (1948). These authors' description of the lithium aluminum hydride reduction is meager. The careful study of Cox, *et al.* (Ref. 24) should be consulted for fuller information.

(20) D. B. Melville, J. R. Rachele, and Elizabeth B. Keller, *J. Biol. Chem.*, **169**, 419 (1947).

(21) J. C. Sowden, *J. Biol. Chem.*, **180**, 55 (1949).

(22) The authors would be glad to furnish rough drawings on request.

(23) About 15% of the methyl iodide is converted to methyl nitrite in a side reaction. As the methyl nitrite is recovered, its C^{14} could be recycled.

ance is made for traces of visible, immiscible impurity and for a few per cent of nitroethane which must be presumed to have been present as a contaminant.[24]

*1,2-O-Isopropylidene-*D*-xylofuranopentodialdose* (III). Difficulty was experienced in preparing 1,2-*O*-isopropylidene-D-glucofuranose (II) by published methods, and modifications of one of these had to be worked out. The details are omitted, however, in view of the fact that carefully checked directions for preparing this compound have recently appeared.[25] Six grams of the isopropylideneglucose was cleaved to syrupy III (5.5 g.) as described by Grosheintz and Fischer,[5] and a portion of the product was immediately condensed with the $C^{14}H_3NO_2$.

1-Nitro-1-deoxy-scyllo-inositol-1-C^{14} *pentaacetate* (Vb-1-C^{14}). The isopropylidenexylodialdose (1.9 g.) was dissolved in absolute ethanol, the whole of the nitromethane was added, and the volume was made up to 10 ml. with absolute ethanol. The solution was made alkaline to *p*H paper with 2*N* sodium methoxide, a small excess was added (4.2 ml. in all), and the mixture was set aside at room temperature for 18 hr. It was then neutralized with a few drops of glacial acetic acid, and volatile components were removed *in vacuo*. The heavy, amber syrup was taken up in 35 ml. of chloroform, and the solution was cooled in ice and washed with four portions (1.5–0.5 ml.) of water. The combined water layers were extracted with six 1-ml. portions of chloroform, then discarded. The combined chloroform solutions were evaporated *in vacuo*.

The residue was dissolved in 7.0 ml. of 0.0968*N* sulfuric acid and heated at 80° for 80 min. in a water bath. Barium hydroxide (7.4 ml., 0.0876*N*, 0.96 molar equivalents) was added to the magnetically stirred solution, and the barium sulfate was filtered off. The still acid solution of nitro sugars (IV-6-C^{14}) was then evaporated *almost* to a syrup *in vacuo*.

Ten milliliters of water was used to dissolve the nitrosugars. The solution was brought to *p*H 9 ("pHydrion" paper) with 3.8 ml. of 0.988*N* sodium hydroxide, and set aside at room temperature for 48 hr. It was then neutralized with glacial acetic acid and deionized over a small column (12 meq.) of Amberlite IR-100 (H^+), and the effluent was evaporated in vacuum to give crude nitrodeoxy-*scyllo*-inositol.

Nine milliliters of acetic anhydride was added to the nitroinositol, and, after the mixture was chilled in ice, two drops of concd. sulfuric acid. The mixture was shaken vigorously until all the solid had dissolved, then left at room temperature. After exactly 1 hr., the completely precipitated pentaacetate was collected on a sintered glass funnel and washed in turn with acetic anhydride, ethanol, water, and ethanol. The product weighed 0.599 g. and melted at 257° with some decomposition (lit.[5] m.p. 258–259° dec.). Yield, 8.4% from nitromethane, 14% from isopropylidenexylodialdose.

scyllo-Inosamine-1-C^{14} *penta-O-acetate* (VIb-1-C^{14}). Raney nickel W-6[11] was prepared on a 20-g. scale in an apparatus of appropriate size.[22] The nitroinositol pentaacetate from the preceding operation was dissolved in 35 ml. of purified dioxane,[26] 2 ml. of catalyst suspension ($\approx$0.7 g.) was added, and the mixture was stirred magnetically under hydrogen at atmospheric pressure. Additional catalyst was put in at 45 min. (2 ml.) and again at 9 hr. (1 ml.) when hydrogen uptake was lagging. At 12 hr., the theoretical amount (111 ml.) of hydrogen had been consumed. The catalyst was centrifuged off and washed with 20 ml. of dioxane, woich was added to the original supernatant. Evaporation of the dioxane gave a beautifully white, powdery residue weighing, when not quite dry, 0.57 g. (theoretical, 0.56 g.). The product was completely soluble in 0.25*N* aqueous hydrochloric acid.

myo-Inositol-2-C^{14} (VIIIa-2-C^{14}). The *myo*-inositol hexaacetate obtained by deaminating VIb-1-C^{14} to (presumably) VII-2-C^{14} and acetylating the latter melted, after crystallization from boiling ethanol, over the range 205–212° (lit.[27] m.p. 216–217°) and weighed 0.352 g. The residue resulting from the acid hydrolysis of the hexaacetate was taken up in 1.5 ml. of water, applied to a column of Whatman cellulose powder *ca.* 4 × 40 cm., and chromatographed with acetone-water 4:1 (v/v).[28] Fractions of 40-ml. size were collected and aliquots were evaporated on planchets for counting. Three fast running impurities gave peaks between fractions 11 and 41; the inositol peak comprised fractions 63 to 95. These were pooled, the tubes were rinsed with boiling water, and the combined solutions boiled down to a volume of 200 ml. on a hot plate. Aliquots were removed for assays, and the solution was then taken to dryness in vacuum. The mother liquor from the crystallization of the hexaacetate was hydrolyzed and chromatographed separately.

Microbiological assay[29] indicated that a total of 144 mg. of *myo*-inositol had been recovered from the chromatograms. The actual combined residue weight was 141 mg. The yield was thus 55% from nitrodeoxyinositol pentaacetate, 7.8% overall from isopropylidenexylodialdose. As the absolute specific activity of the product was not determined, an exact radioactive yield cannot be given, but the molar yield of inositol was 3.9% in terms of barium carbonate taken as starting material.

The combined residues were taken up in water and treated with charcoal, and the volume reduced to 1 ml. By successive additions, over a period of 5 days, of 95% ethanol in the total amount of 8 ml., there was obtained 120 mg. of crystalline *myo*-inositol melting at 224–225° (lit.[27] 225°) and showing only one spot on paper chromatography with acetone-water (9:1). This spot had the same R_f as authentic *myo*-inositol run on the same paper.

Ion exchange chromatography[30,31] *of myo-inositol-2-*C^{14}. A portion (1.03 mg.) of the inositol was dissolved in 1 ml. of 0.007*M* sodium tetraborate and put on a column of Dowex-1 X-8, 50–100 mesh, borate form, 1 × 20 cm. Elution was with 0.021*M* sodium tetraborate, fraction volume 10 ml. Radioactivity appeared in the first eight fractions, and in the region (fractions 40–55) known to be characteristic of *myo*-inositol by experiments with authentic, unlabeled material. The column was eventually flushed with 5% hydrochloric acid, but no further radioactivity was removed. The *myo*-inositol peak contained 868,000 c.p.m. (thin-window Geiger tube) and the fast running peak (impurities) 35,400 c.p.m., or 4% of the total radioactivity.

The fast running peak was concentrated, and then freed of borate by passage through Dowex-50 (H^+), reconcentration, acidification to *p*H 1 with hydrochloric acid, and brief heating before final passage through Dowex-1 (OH^-). The recovery of radioactivity in these operations was complete. The material, estimated to amount to 22–25 μg., was divided into two approximately equal portions. One portion, chromatographed on Whatman no. 1 paper with acetone-water (9:1), was resolved into one major spot[32] with R_{pinitol} 0.5, and at least three minor spots, R_{pinitol} 0.1, 0.32 and 0.65. Authentic *scyllo*-inositol run on the same paper had R_{pinitol} 0.32. The second portion was assayed microbiologically for *myo*-inositol. It gave no response.

(24) J. D. Cox, H. S. Turner, and R. J. Warne, *J. Chem. Soc.*, 3167 (1950).

(25) E. R. Blakley, *Biochem. Preparations*, **7**, 39 (1960).

(26) L. F. Fieser, *Experiments in Organic Chemistry*, 2d ed., D. C. Heath and Co., 1941, p. 368.

(27) S. J. Angyal and L. Anderson, *Advances in Carbohydrate Chem.*, **14**, 192, 193 (1959).

(28) L. Anderson, *et al.*, *J. Am. Chem. Soc.*, **79**, 1171 (1957).

(29) L. Atkin, *et al.*, *Ind. Eng. Chem., Anal. Ed.*, **15**, 141 (1943).

(30) F. C. Charalampous and Pamela Abrahams, *J. Biol. Chem.*, **225**, 575 (1957).

(31) We thank Mrs. Maryanne Yu-Tsao for assistance with these operations.

(32) Spots were developed with 5% ammoniacal silver nitrate.

D-*1-Nitro-1-deoxy-scyllo-inositol-3,4,C*^{14} *pentaacetate* (Vb-3,4-C^{14}). The *E. coli* "glycogen" used as starting material (see discussion, above) had a specific activity of 5.83 × 10^5 c.p.m. per millimole (glucose unit) when counted at about 10% efficiency. A degradation with *Leuconostoc mesenteroides*[33] showed that the radioactivity was distributed as follows (figures are per cent of total radioactivity): C-1, 5.2; C-2, 6.7; C-3, 30; C-4, 55; C-5, 0.9; C-6, 2.6. A portion of the "glycogen," after dilution with oyster glycogen, was hydrolyzed with 1*N* sulfuric acid and the acid was neutralized with calcium carbonate. After further dilution with unlabeled glucose, the filtrate was freeze-dried. The slightly gummy residue of glucose-3,4-C^{14} was made granular by dissolving it in warm methanol, adding dry isopropyl alcohol, and evaporating the solvents in a stream of dry air (operations repeated several times).

The powdered glucose was acetonated[34] by shaking it for 5 hr. with forty-five volumes of dry acetone containing 4% (v/v) sulfuric acid; the process was repeated three times to dissolve all the sugar. One gram of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose-3,4-C^{14} (I-3,4-C^{14}) was obtained from the acetone solutions. This was converted to 0.71 g. of the monoisopropylidene derivative (II-3,4-C^{14}) by hydrolysis according to Mehltretter, *et al.*[34] Syrupy 1,2-*O*-isopropylidene-D-xylofuranopentodialdose-3,4-C^{14} (III-3,4-C^{14}) was obtained by cleaving the II in aqueous solution with sodium metaperiodate,[35] freeze-drying the reaction mixture, extracting the residue with chloroform, and evaporating the chloroform in a stream of dry air. The nearly colorless product (calculated weight, 0.61 g.) was taken up in 1.8 ml. of 95% ethanol, treated with 1 ml. (1.13 g., 5.7 molar equivalents) of nitromethane and 0.2 ml. of 2*N* sodium methoxide in methanol, and set aside at room temperature for 18 hr.

The subsequent workup of the condensation product, the cyclization, and the acetylation were carried out almost exactly as described above for nitrodeoxy-*scyllo*-inositol-1-C^{14} pentaacetate. We omitted the step of dissolving the original condensation product in chloroform and extracting the solution with water. The product weighed 0.34 g. (25% yield based on monoisopropylideneglucose).

L-*myo-Inositol-4,5-C*^{14} (VIIIa-4,5-C^{14}). The steps Vb → VIb → VII → VIIIb → VIIIa were accomplished essentially as described above for *myo*-inositol-2-C^{14}. The use of a larger initial ratio (8:1 by weight) of Raney nickel W-6 to Vb facilitated the hydrogenation to *scyllo*-inosamine penta-*O*-acetate (VIb-3,4-C^{14}). No effort was made to recover the inositol in the mother liquor from the crystallization of the hexaacetate VIIIb or to do ion-exchange chromatography on the mother liquors from the inositol itself. The twice-recrystallized product weighed 55 mg. (8% overall yield from diisopropylideneglucose) and had a specific activity of 9000 c.p.m. per mmole, counted at *ca.* 10% efficiency. It melted at 220–223°, and had the same R_f as authentic *myo*-inositol on paper (acetone-water system).

As noted in the discussion (above), the positions of the principal labeled atoms (4 and 5) in this inositol are such as to make the molecule asymmetric. In addition, because the label was not distributed symmetrically in the two halves of the precursor glucose, these positions are not equally labeled. Thus C-4 of the inositol (from C-4 of glucose) has 1.8 times the activity of C-5 (from C-3 of glucose). Carbons 1,3, and 6 of the inositol (from carbons 1,5, and 2 of glucose, respectively) are also slightly labeled.

myo-Inosose-2 ethylene disulfone (XI). One half gram of *myo*-inosose-2 ethylene dithioacetal (X)[14] in 25 ml. of water was treated at room temperature with 50 ml. of performic acid solution[36] and let stand *ca.* 15 hr. The white product, obtained by evaporating the solution to dryness in vacuum, was recrystallized from water and water-isopropyl alcohol for analysis. The pure compound decomposed over the range 225–290° and gave a single spot,[32] R_f 0.48, on ascending chromatography on Whatman no. 1 paper in acetone-water (7:3).

Anal. Calcd. for $C_8H_{14}O_9S_2$ (318.32): C, 30.18; H, 4.43. Found: C, 30.08; H, 4.61.

Alkaline degradation of the disulfone XI. A solution of 2 g. of XI in 250 ml. of water was brought to *p*H *ca.* 10.5 with a few drops of concd. ammonium hydroxide and set aside at room temperature for 4 hr. Evaporation of the solution to dryness in vacuum gave a syrup, which was dissolved in 60 ml. of water. An aliquot of this solution, chromatographed on paper as described for XI (above), gave three strong spots having, respectively, R_f 0.48 (starting material), R_f 0.63, and R_f 0.80. Refrigeration of the solution and the daily addition of a few milliliters of ethanol over a period of a week brought about the deposition of 340 mg. of crystals of the R_f 0.63 compound. It was obtained analytically and chromatographically pure by three recrystallizations from 10 parts of warm 1:1 water-ethanol. The crystals were small, white prisms which decomposed over the range 230–280°.

Anal. Calcd. for $C_8H_{14}O_9S_2$(318.32): C, 30.18; H, 4.43. Found: C, 30.02; H, 4.42.

The compound had no absorption maximum in the ultraviolet. Its infrared spectrum differed from that of XI, but showed no peak in the carbonyl region (1700 cm.$^{-1}$). When it was treated with excess sodium metaperiodate, consumption of the reagent[37] leveled off after 12 hr. at a value of 3.1 molar equivalents. At this time, 2 equivalents of titratable acid, presumed to be formic, had been liberated per mole of sample. The structure XIV is tentatively proposed.

Periodate degradation of the disulfone XI. The disulfone (300 mg., 0.95 mmole), periodic acid (H_5IO_6, 985 mg., 4.32 mmole), and water (30 ml.) were shaken gently at room temperature until a homogeneous solution resulted (*ca.* 3 hr.). At 20 hr. this solution was treated with 2 drops of ethylene glycol. On titration 1 hr. later, 8.58 meq. of barium hydroxide was required to neutralize the iodic and formic acids to the methyl red end point. The precipitated barium iodate was filtered off and the filtrate was concentrated to dryness *in vacuo*. The residue was then extracted with three 40-ml. portions of boiling acetone, and the acetone-insoluble barium formate recrystallized from water with the addition of acetone. The yield was 517 mg. (4.64 moles per mole of XI).

The acetone extracts, on evaporation to dryness, yielded a compound which was characterized as 1,3-dithiolane 1,1,-3,3-tetroxide (XVI) on the basis of the following data: 1) After recrystallization from 100 parts of hot ethanol, the compound (70 mg.) melted at 204–205° (lit.[38] m.p. 204–205°). 2) The C—H analysis conformed to the theoretical for XVI. 3) Chlorination gave a derivative melting at 222–229° (lit.[38] for the 2,2-dichloro derivative, m.p. 222–223° dec.).

Compound XVI was obtained in yields of 50–60% when simultaneous recovery of the formic acid was not attempted

Test of the isotope distribution in myo-inositol-2-C^{14}. The labeled compound (0.34 mg.) was diluted with 2 g. of unlabeled *myo*-inositol and oxidized to *myo*-inosose-2 (IX-2-C^{14}) with *Acetobacter suboxydans*.[39] From the first crop (725 mg.) of the inosose the ethylene disulfone was obtained as before. An amount (188 mg.) of the disulfone was treated with a slight excess of periodic acid as described above. At 23 hr., slightly over 1 mole of barium carbonate per mole of periodic

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acid was added, and shaking was continued for 3 more hr. The insoluble barium salts were filtered off and washed with water and acetone, and the combined filtrate and washings were concentrated *in vacuo* to 5 ml.

Barium formate (200 mg.) was obtained from the concentrate by the addition of 50 ml. of acetone. It was purified by three crystallizations from water-acetone, then steam distillation from acid solution, and reconversion to the barium salt. The pure salt was plated on copper planchets from water solution.

The aqueous acetone filtrate from the original precipitation of the barium formate was concentrated to dryness. 1,3-Dithiolane 1,1,3,3-tetroxide was extracted from the residue with boiling acetone and crystallized from isopropyl alcohol. After three recrystallizations, it was plated from acetone solution with collodion as a binder. The following activities were found (corrected for selfabsorption):

	c.p.m. per mmole diluted inositol
All carbons (counted as disulfone)	38,300
Carbon 2 (dithiolane tetroxide)	37,500
Carbons 1,3,4,5,6 (barium formate)	500

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Convenient New Synthesis of Pregnenolone-4-C^{14}

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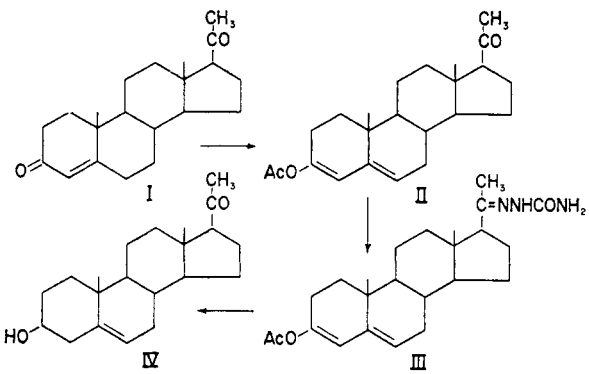
A convenient new synthesis of pregnenolone-4-C^{14} from progesterone-4-C^{14} is described.

Although progesterone-4-C^{14} has been widely used as a substrate for the study of steroid biosynthesis, the Δ^4-3-keto moiety in ring A of progesterone (I), in fact, already represents an advanced step in the biosynthetic pathway from pregnenolone (IV) which contains a Δ^5-3β-ol system. The various metabolic pathways concerned with the Δ^5-double bond and the 3β-hydroxyl group could not be elucidated with progesterone-4-C^{14}, and for a better understanding of these metabolic processes, pregnenolone-4-C^{14} would be the logical substrate.

The previously published method for the synthesis of pregnenolone-4-C^{14} by Milan Uskokovic, *et al.*[1] was rather involved and the over-all yield was very low. Since progesterone-4-C^{14} is readily available, it was decided to transform it to pregnenolone-4-C^{14} by a suitable synthesis which forms the subject of the present paper. The various stages involved in the synthesis were first carried out with nonradioactive material, and when optimum conditions were established the synthesis was repeated with the radioactive steroid.

Progesterone (I) was converted to its 3-enol acetate (II) in 67% yield by treating with acetic anhydride and acetyl chloride as described by Westphal.[2] It has been shown previously by Wendler, *et al.*[3] that, where the reduction of a particular ketonic group was not desirable it could be effectively protected as its semicarbazone derivative, and that the subsequent reduction of the semicarbazone derivative with lithium borohydride



does not reduce the semicarbazone linkage whereas it reduces other carbonyl functions. Consequently, the protected ketonic function could readily be regenerated by removing the semicarbazone group with pyruvic acid. Accordingly, the 20-keto group in II was then protected as the 20-semicarbazone by treating II in pyridine solution with semicarbazide hydrochloride dissolved in aqueous methanol to give 3-acetoxy-$\Delta^{3,5}$-pregnadiene-20-one-20-semicarbazone (III) in 85% yield. The enol acetate function in III was then reduced with lithium borohydride[4] in tetrahydrofuran-dimethylformamide solution to give a mixture of 3α- and 3β-hydroxy-Δ^5-pregnen-20-ones as their semicarbazones. Without further purification the semicarbazone group was removed with pyruvic acid to give a mixture of 3α- and 3β-hydroxy-Δ^5-pregnene-20-ones. The 3β-hydroxy-Δ^5-pregnene-20-one (IV) was selectively isolated in 55% yield through a digitonide formation and subsequent split of the digitonide with pyridine. The over-all

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(4) See L. F. Fieser and M. Fieser, *Steroids*, Reinhold, New York, 1959, p. 439.