the temperature increases and local or general overheating may occur.

For the determination of the reliability of the ultrasonic method, 15 parallel samples were treated at 35 W/cm² for 5 min and analyzed for ADH and IDH. Standard deviations were 3.1 % for ADH and 7.5 % for IDH.

In comparative experiments, freezing and thawing was found to be inefficient when dilute (5—10 %) suspensions of yeast were used. Only 5 % of the ADH was liberated after 12 successive repetitions. After grinding for 10 min in a Potter-Elvehjem homogenisator, or for 7.5 min (50 000 rpm) in the Bühler homogenisator with glass beads (ø 0.2 mm), less than 5 % of ADH was extracted. Better results have been obtained by using more concentrated yeast suspensions.[11] By experience, grinding of baker's yeast with an equal volume of quartz sand in a cone shaped all-glass homogenisator results in complete breakage of cells, but only 30 % of the ADH activity can be extracted. Acetone dried yeasts have 60 % of the activity of the ultrasonically treated material.

This work has been partly supported by a grant from *Neste Oy:n säätiö*, which is gratefully acknowledged.

1. Ohlmeyer, P., Kutscha, W. and Rüssman, W. *Z. physiol. Chem.* **330** (1962) 1.
2. Gehring, L. B. and Miettinen, J. K. *Suomen Kemistilehti* **B 32** (1959) 21.
3. Edebo, L. *Acta Pathol. Microbiol. Scand.* **52** (1961) 361.
4. Hübener, H. J., Gollmick, H. J., Tesser, K., Lippert, W. and Rossberg, L. *Biochem. Z.* **331** (1959) 410.
5. Neppiras, E. A. and Hughes, D. E. *Biotechnol. Bioeng.* **6** (1964) 247.
6. Oppenrooth, W. F. E. *Brauwissenschaft* **11** (1958) 113.
7. Beisenherz, G., Boltze, H. J., Bücher, Th., Czok, R., Garbade, K. H., Meyer-Ahrendt, E. and Pfleiderer, G. *Z. Naturforsch.* **8b** (1953) 555.
8. Schmidt, E., Schmidt, F. and Wildhirt, E. *Klin. Wochschr.* **36** (1958) 172.
9. Ochoa, S. In Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*, Academic, New York 1955, Vol. I, p. 699.
10. Hughes, D. E. *J. Biochem. Microbiol. Technol. Eng.* **3** (1961) 405.
11. Lamanna, C. and Mallette, M. F. *J. Bacteriol.* **67** (1954) 503.

Received November 18, 1966.

Synthesis of 1,3-Di-*O*-acetyl-glycerol from Glycerol and Acetic Anhydride and a Method to Determine the Ratio of 1,2-Di-*O*-acetyl-glycerol and 1,3-Di-*O*-acetyl-glycerol in a Mixture

LEIF HOLMQUIST

Pharmaceutical Chemistry Section, Research Institute of National Defence, Dept. 1, Sundbyberg 4, Sweden

Several authors have reported methods for the preparation of di-*O*-acetylglycerol;[1-6] however, it seems doubtful that the product obtained by these methods was pure di-*O*-acetyl-glycerol. Furthermore, the relative occurrence of 1,2-di-*O*-acetyl- and 1,3-di-*O*-acetyl-glycerol was not determined. Langenbeck and Bollow[7] have described a method to synthesize 1,3-di-*O*-acetyl-glycerol *via* 2-amino-propan-1,3-diol but no details of the method of synthesis were given. The diester was characterized by hydrolysis and by acetylation to 1,2,3-tri-*O*-acetyl-glycerol. Smith[3] claimed that he got pure di-*O*-acetyl-glycerol by direct acetylation of glycerol with acetic anhydride but gave no experimental details. It is not clear whether 1,2-di-*O*-acetyl- and/or 1,3-di-*O*-acetyl-glycerol was obtained. The isolated product was analysed by hydrolysis and by determination of the number of acetyl groups. A mixture of mono-*O*-acetyl-glycerol, di-*O*-acetyl-glycerol and tri-*O*-acetyl-glycerol could give an apparently pure product.

We have examined the possibility of synthesizing 1,3-di-*O*-acetyl-glycerol by direct acetylation of glycerol with acetic anhydride with and without the presence of catalytic pyridine. The reactions have been carried out at different temperatures and with different proportions of acetic anhydride and glycerol. The reaction mixtures were studied by thin layer chromatography. The isolated diesters have been submitted to NMR analysis. Tri-*O*-acetyl-glycerol gives a characteristic quintet at 4.88 τ ($J \approx 5$ cps), which derives from the methine proton of the $CH\text{-}OOC\text{-}CH_3$ group. The methylene protons of the $CH_2\text{-}OOC\text{-}CH_3$ group give signals between 5.60 and 6.00 τ. The methylene protons of the $CH_2\text{-}OH$ group in 1-mono-*O*-acetyl-glycerol give a

Table 1. Values of τ and signal intensities of a mixture of 1,3-di-*O*-acetyl- and 1,2-di-*O*-acetyl-glycerol.

Mixture	τ ppm	Signal intensity mm
$CH_2(-O-CO-CH_3^a)-CH(-OH)-CH_2(-O-CO-CH_3^a)$ (A)	$H^a = 7.89$	85
$CH_2(-O-CO-CH_3^a)-CH(-O-CO-CH_3^b)-CH_2^c-OH$ (B)	$H^b = 4.74$	3
	$H^c = 6.26$	6

$$\% \text{ B in the mixture} = \frac{3 \times 6 \times 100}{85} = 21.$$

doublet at 6.26 τ ($J \approx 5$ cps). The methine proton of the CH-OH group and the methylene protons of the CH_2-OOC-CH_3 group in 1,3-di-*O*-acetyl-glycerol give signals between 5.60 and 6.00 τ. The methyl group gives a singlet at 7.89 τ.

With the aid of this information we have been able to determine the ratio of 1,2-di-*O*-acetyl- and 1,3-di-*O*-acetyl-glycerol by measuring the signal intensities at 4.74 τ or 6.26 τ and at 7.89 τ (Table 1).

In the reaction between glycerol and acetic anhydride, mono-*O*-acetyl-glycerol, di-*O*-acetyl-glycerol, and tri-*O*-acetyl-glycerol were formed even at room temperature. In order to get pure diester it was necessary to use an excess of acetic anhydride to completely eliminate the formation of mono-*O*-acetyl-glycerol. Without pyridine as a catalyst the reaction between glycerol and acetic anhydride was very slow at 25°C and at 40°C. At 25°C mono-*O*-acetyl-glycerol was not eliminated after 8 days although an excess of acetic anhydride was used. With pyridine, the reaction was rather fast even at 25°C; however, the isolated di-*O*-acetyl-glycerol contained more 1,2-di-*O*-acetyl-glycerol than in experiments without pyridine. The synthesis of 1,3-di-*O*-acetyl-glycerol that is described in this paper gives a product that contains less than 10 % (by weight) 1,2-di-*O*-acetyl-glycerol. No other impurities could be detected with NMR or chromatographic techniques.

Experimental. Synthesis. To 24.5 g of distilled acetic anhydride was added 10.8 g of anhydrous glycerol. The mixture was stirred vigorously for 4 h at 70°C, then 6 g of acetic anhydride was added and the reaction allowed to proceed for 20 h at 70°C with continued stirring. The reaction mixture was evaporated at 60°C (10—15 mm Hg) to remove all the acetic acid formed. 30 ml of water was added to the colourless oily residue and the solution was shaken with 3 × 10 ml of benzene to remove tri-*O*-acetyl-glycerol. The benzene phase was discarded and the water phase was evaporated at 60°C (10—15 mm Hg). The residue was taken up in 50 ml of dry ethyl ether and passed through a 1 × 20 cm column of Merck's Molecular sieve 3 Å rods. The ether was evaporated at 30°C (10—15 mm Hg). The colourless residue was chromatographically pure di-*O*-acetyl-glycerol which NMR showed to consist of 21 % 1,2-di-*O*-acetyl-glycerol and 79 % 1,3-di-*O*-acetyl-glycerol. Yield: 10 g (48 %).

10 g of di-*O*-acetyl-glycerol obtained in this way was allowed to react with 3 g of acetic anhydride for 24 h at 70°C. The mixture was stirred vigorously. After evaporation of the reaction mixture as described above 15 ml of water was added and the solution was extracted with 3 × 5 ml of benzene. The water phase was evaporated and the residue dried as described above. The resulting pure di-*O*-acetyl-glycerol was shown by NMR analysis to contain less than 10 % of 1,2-di-*O*-acetyl-

glycerol. Yield: 4.6 g (48 %). (Found: C 47.6; H 6.82. Calc. C 47.7; H 6.88).

Thin layer chromatography. Mono-*O*-acetyl-glycerol, di-*O*-acetyl-glycerol, and tri-*O*-acetyl-glycerol were separated on glass plates coated with SiO_2 (Merck HF_{254} nach Stahl) activated by heating 30 min at 120°C. Dry ethyl ether was used as solvent. The esters were detected by spraying the chromatogram with iodine in chloroform. Time for separation was 15 min.

Substance	R_F-value
Mono-*O*-acetyl-glycerol	0.15
Di-*O*-acetyl-glycerol	0.42
Tri-*O*-acetyl-glycerol	0.62

NMR analysis. The glycerol esters were dissolved in deuterochloroform and run with TMS as internal reference in a Varian A-60A Analytical NMR Spectrometer.

1. *Beilsteins Handbuch der Organischen Chemie* II 147, Springer, Berlin 1920.
2. Fischer, E. *Ber.* **2** (1920) 1639.
3. Smith, L. *Z. physik. Chem.* **102** (1922) 54.
4. Simons, F. L. *J. Am. Chem. Soc.* **48** (1926) 1992.
5. Schuette, H. A. and Sah, P. P. T. *J. Am. Chem. Soc.* **48** (1926) 3161.
6. Levene, P. A. and Walti, A. *J. Biol. Chem.* **77** (1928) 685.
7. Langenbeck, W. and Bollow, M. *Naturwiss.* **42** (1955) 389.

Received December 8, 1966.

Methyl 6-Deoxy-6-nitro-D-glucopyranosides

BENGT LINDBERG and SIGFRID SVENSSON

Institutionen för organisk kemi, Stockholms Universitet, Stockholm, Sweden

During structural studies on branched dextrans a method was sought which would selectively remove the terminal non-reducing glucose residues. These are the only residues which contain primary hydroxyl groups. Replacement of the primary hydroxyls with a sequence of tosyl groups, iodide groups, and finally strong electron attracting groups would render the modified sugar residues alkali labile.[1,2] In a previous communication [1] the synthesis and alkaline degradation of methyl 6-deoxy-6-*p*-tolyl-sulphonyl-α- and -β-D-glucopyranoside was reported. The present paper reports the synthesis of the analogous 6-deoxy-6-nitro-derivatives. The synthesis of methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-nitro-α-D-glucoside by reaction of the corresponding 6-deoxy-6-iodo-derivative with sodium nitrite in an aprotic solvent has previously been reported by Sugihara *et al.*[3] During the course of this work, Baer and Rank [2] have reported the synthesis of methyl 6-deoxy-6-nitroglycosides from 6-deoxy-6-nitrosugars by Fischer synthesis and have studied the alkaline degradation of these substances.

Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-nitro-α- and β-D-glucosides were formed in about 50 % yield by treatment of the corresponding 6-deoxy-6-iodo-derivatives with sodium nitrite in dimethylsulphoxide [4] at 60°. Phloroglucinol was added to decompose the nitrite ester formed simultaneously, as devised by Kornblum *et al.*[5] The nitro derivatives could also be prepared from the corresponding 6-*O*-tosylates but this reaction was less satisfactory. When 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose was treated under the same conditions, no reaction was observed and under more drastic conditions severe decomposition of the product occurred. The low reactivity of primary tosylates and iodides in the galactose series is well known and has been assigned to steric hindrance by the axial substituent in the 4-position.[3,6]

The acetates of the methyl 6-deoxy-6-nitro-glucosides cannot be deacetylated because of the alkaline lability of the free glucosides. Reaction of the methyl 6-deoxy-6-iodo-glucosides with sodium nitrite gave mixtures which made the isolation of the desired product difficult; however, the methyl 6-deoxy-6-nitro-glucosides could be prepared when the free hydroxyls were protected by reaction with 2,3-dihydro-4-H-pyrane. This protecting group was later removed by mild acid treatment.

The inductive and steric effects of the nitro group should render the 6-deoxy-6-nitro-hexosides more stable to acid hydrolysis than the corresponding hexosides. In this connection, methyl β-D-glucopyranoside was hydrolysed about 5 times faster than methyl 6-deoxy-6-nitro-β-D-glucopyranoside in 4 M sulphuric acid at 80°.