

462. Nucleotides. Part XXXVII.* The Structure of Uridylic Acids *a* and *b*, and a Synthesis of Spongouridine (3- β -D-Arabo-furanosyl-uracil).

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Monoacetylation of 5'-*O*-acetyluridine yields a crystalline diacetate, from which by phosphorylation with dibenzyl phosphorochloridate followed by removal of protecting groups uridylic acid *a* is obtained. The diacetate is shown to be 3' : 5'-di-*O*-acetyluridine by the following transformations which prove the structures of the intermediates : 3' : 5'-Di-*O*-acetyl-2'-*O*-toluene-*p*-sulphonyluridine \longrightarrow *O*² : 2'-cyclouridine \longrightarrow 3- β -D-arabofuranosyluracil. Uridylic acid *a* is, therefore, uridine-2' phosphate. 3- β -D-Arabo-furanosyluracil is identical with spongouridine, a nucleoside occurring naturally in sponges.

EARLIER¹ in this series the structure of adenylic acid *a* obtained from alkaline hydrolysates of ribonucleic acids was rigidly established by showing its identity with adenosine-2' phosphate, synthesised from 3' : 5'-di-*O*-acetyladenosine by a series of reactions which did not involve group migration. The orientation of the diacetyladenosine used was determined by *O*-toluene-*p*-sulphonylation followed by methylation. It seemed desirable to apply similar methods to provide unequivocal proof of the orientation of the *a* and *b* nucleotides derived from uridine and cytidine. Reactivity of the 6-amino-group led to complications in attempting to prepare appropriate partially acylated cytidines^{2,3} and attention was therefore turned to uridine.

Acetylation of 2' : 3'-*O*-isopropylideneuridine⁴ yielded its 5'-*O*-acetate from which, by hydrolysis with dilute acetic acid, 5'-*O*-acetyluridine was obtained. The same monoacetyluridine was also prepared by partial deacetylation of 2' : 3' : 5'-tri-*O*-acetyluridine with methanolic ammonia. When treated with acetic anhydride (1 mol.) in pyridine solution 5'-*O*-acetyluridine gave a mixture separable by countercurrent distribution into a crystalline diacetyluridine, some triacetyluridine, and some unchanged monoacetyl compound.

Phosphorylation of the diacetyluridine with dibenzyl phosphorochloridate gave a crude dibenzyl diacetyluridine phosphate from which benzyl groups were removed by hydrogenolysis and acetyl groups by subsequent treatment with methanolic ammonia. Removal of protecting groups in this order ensured that no phosphoryl migration could occur.¹ The crude product contained, in addition to much uridine, a uridine phosphate. Ion-exchange chromatography showed that only one nucleotide was present, corresponding to uridylic acid *a*.⁵ Experiments described below show conclusively that the diacetyluridine used was 3' : 5'-di-*O*-acetyluridine (I) so that the derived phosphate, uridylic acid *a*, is, in fact, uridine-2' phosphate (II). This provides synthetic proof of structure of the isomeric uridylic acids isolated from alkaline hydrolysates of ribonucleic acids, and confirms an earlier degradative⁶ study which showed that uridylic acid *b* is uridine-3' phosphate. The structural studies on cytidylic acids *a* and *b* by physical⁷ and degradative⁶ methods and the established relation⁸ between cytidylic and uridylic acid *b* find further confirmation in the present experiments.

It was originally intended to orient the diacetyluridine by a procedure analogous to that used in the adenosine series.¹ Toluene-*p*-sulphonylation in pyridine afforded an

* Part XXXVI, *J.*, 1956, 1546.

¹ Brown, Fasman, Magrath, and Todd, *J.*, 1954, 1448.

² Brown, Todd, and Varadarajan, preceding paper.

³ See also Kenner, Reese, and Todd, *J.*, 1955, 855.

⁴ Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113.

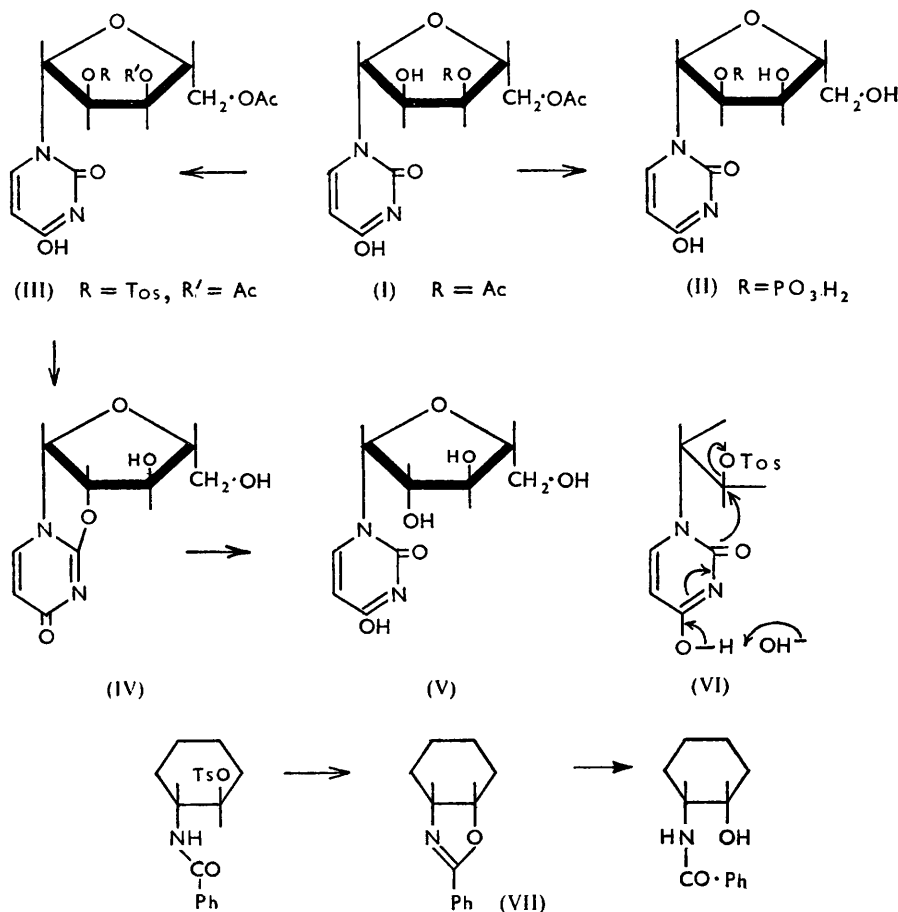
⁵ Cohn, *J. Amer. Chem. Soc.*, 1950, **72**, 2811.

⁶ Baron and Brown, *J.*, 1955, 2855.

⁷ Cavalieri, *J. Amer. Chem. Soc.*, 1952, **74**, 5804; Fox, Cavalieri, and Chang, *ibid.*, 1953, **75**, 4315; Michelson and Todd, *J.*, 1954, 34.

⁸ Brown, Dekker, and Todd, *J.*, 1952, 2715.

amorphous product which on the basis of its properties was a diacetyl-toluene-*p*-sulphonyl-uridine. Treatment with methanolic ammonia yielded, not the expected toluene-*p*-sulphonyluridine, but a product which gave analytical values corresponding to an anhydro-uridine together with ammonium toluene-*p*-sulphonate. The anhydro-compound was high-melting, and very soluble in water, but sparingly so in alcohols, and its ultraviolet absorption (λ_{max} , 249.5—251 $\text{m}\mu$) differed substantially from that of uridine (λ_{max} , 260 $\text{m}\mu$). Mild hydrolysis with dilute sulphuric acid afforded a new nucleoside, isomeric with uridine. It had the same R_F value as uridine on paper chromatograms, but did not form a borate complex on electrophoresis in 0.1M-sodium borate, nor did it give a positive reaction to the periodate spray reagent.⁹ These indications that the substance had a *trans*-glycol system were confirmed by degradation.



Hydrogenation of the nucleoside over platinum¹⁰ or, better, reduction with sodium and methanol in liquid ammonia,¹¹ followed by mild acid hydrolysis, yielded a sugar which was identified as arabinose and was distinguished from the other aldopentoses by paper chromatography: this identity was confirmed by conversion into its *p*-bromophenylosazone and toluene-*p*-sulphonylhydrazone. The new nucleoside must therefore be 3- β -D-arabofuranosyluracil.

⁹ Buchanan, Dekker, and Long, *J.*, 1950, 3165.

¹⁰ Levene and La Forge, *Ber.*, 1912, **45**, 619.

¹¹ Burke, *Chem. and Ind.*, 1954, 1393.

The shift in the ultraviolet absorption maximum during the formation of the anhydro-uridine suggested an analogy with the conversion of the 2' : 3'-*O*-isopropylidene-5'-*O*-toluene-*p*-sulphonyl derivatives of cytidine and adenosine, by heat, into the corresponding cyclonucleoside toluene-*p*-sulphonates, a process of intramolecular alkylation.¹² Formation of *O*² : 3'- and *O*² : 5'-cyclouridine by a reaction between the corresponding iododeoxy-thymidine and silver acetate also produced a hypochromic spectral shift.¹³ Experiments subsequent to those described in this paper have also shown that 3' : 5'-di-*O*-methane-sulphonylthymidine with methanolic ammonia gives a product formulated as 5'-*O*-methane-sulphonyl-*O*² : 3'-cyclouridine.¹³

Our observations find a simple explanation if it is assumed that the anhydro-uridine is *O*² : 2'-cyclouridine (3-*O*² : 2'-anhydro-β-D-arabofuranosyluracil) (IV). Its formation from 3' : 5'-di-*O*-acetyl-2'-*O*-toluene-*p*-sulphonyluridine (III) with expulsion of toluene-*p*-sulphonate ion, due to nucleophilic attack of the neighbouring 2-carbonyl function, has a simple analogy. Thus *trans*-2-benzamidocyclohexyl toluene-*p*-sulphonate is converted by sodium acetate in dry ethanol into the corresponding *cis*-isomer,¹⁴ in which reaction the oxazoline (VII) is an isolable intermediate.¹⁵ In the present instance nucleophilic attack should be aided by electron release from the acidic nuclear hydroxyl group as in (VI).

No such simple explanation of inversion at C₍₂₎ would be possible if the *O*-toluene-*p*-sulphonyl group in the diacetyl-toluene-*p*-sulphonyluridine had been at C₍₃₎; participation by the vicinal *cis*-acetoxy-function would in this case have been necessary either directly or by formation of a 2' : 3'-epoxide, both processes which can be excluded since concomitant production of a xylofuranosyl derivative was not observed.

We conclude, therefore, that the original diacetate was 3' : 5'-di-*O*-acetyluridine and hence that the derived phosphate was uridine-2' phosphate.

In subsequent experiments it was found preferable to prepare the monotoluene-*p*-sulphonyl derivative from 5'-*O*-acetyluridine directly. This yielded crystalline 5'-*O*-acetyl-2'-toluene-*p*-sulphonyluridine, the structure of which was apparent from its conversion by methanolic ammonia into *O*² : 2'-cyclouridine. Further work on the chemistry of both *O*² : 2'- and *O*² : 5'-cyclouridine will be published later. Meanwhile, the conversion of the former cyclouridine by acid into 3-β-D-arabofuranosyluracil constitutes a synthesis, and simultaneously a proof of structure, of the naturally occurring spongouridine. Bergmann and Burke¹⁶ isolated this nucleoside from sponges and as a result of degradative studies concluded that it was uracil β-arabofuranoside. Through the kindness of Drs. Bergmann and Burke we have been able to compare a specimen of the natural material with the synthetic arabinoside, and find them identical.

The advantage of the present synthetic route over the more conventional pyrimidine nucleoside synthesis *via* acetohalogeno-sugars is that the β-configuration at C_(1') is retained, whereas Bristow and Lythgoe¹⁷ found that condensations involving acetobromo-D-arabofuranose led to α-glycosides.

EXPERIMENTAL

R_F values quoted refer to paper chromatography on Whatman No. 1 paper with the butan-1-ol-acetic acid-water (5 : 2 : 3) solvent system.

2' : 3' : 5'-*Tri-O*-acetyluridine.—Uridine (3.0 g.; dried at 110° *in vacuo* over phosphoric oxide) was mixed with freshly distilled acetic anhydride (20 c.c.), and a few drops of dry pyridine were added. On gentle warming a vigorous reaction occurred and the solid dissolved. The solution was set aside overnight, methanol (40 c.c.) was added, and the mixture concentrated to small bulk at <40°. Water (150 c.c.) was added, and the solution carefully neutralised with sodium hydrogen carbonate, then decanted from a small amount of resinous material and kept at 0° for several hours. The *triacetate* was collected and recrystallised from ethanol. It formed

¹² Clark, Todd, and Zussman, *J.*, 1951, 2952.

¹³ Michelson and Todd, *J.*, 1955, 816.

¹⁴ McCasland, Clark, and Carter, *J. Amer. Chem. Soc.*, 1949, **71**, 637.

¹⁵ Winstein, Goodman, and Boschan, *ibid.*, 1950, **72**, 2311.

¹⁶ Bergmann and Burke, *Angew. Chem.*, 1955, **67**, 127; *J. Org. Chem.*, 1955, **20**, 1501.

¹⁷ Bristow and Lythgoe, *J.*, 1949, 2306.

colourless rectangular prisms, m. p. 128—130° (3.1 g.), R_p 0.93 (Found: C, 49.1; H, 5.2; N, 7.7. $C_{15}H_{18}O_9N_2$ requires C, 48.7; H, 4.9; N, 7.6%).

5'-O-Acetyl-2':3'-O-isopropylideneuridine.—2':3'-*O*-isopropylideneuridine⁴ (2.0 g.) was dissolved in dry pyridine (15 c.c.), and freshly distilled acetic anhydride (2.5 c.c.) added. After 4 hr. at room temperature, methanol (15 c.c.) was added, with cooling. Evaporation at reduced pressure below 50°, followed by repeated evaporation with added ethanol, gave an oil which was dissolved in hot ethanol (10 c.c.). *5'-O-Acetyl-2':3'-O-isopropylideneuridine* slowly separated as prisms (1.9 g.), m. p. 146—147° (Found: C, 51.6; H, 5.5; N, 8.6. $C_{14}H_{18}O_7N_2$ requires C, 51.5; H, 5.6; N, 8.6%). Traces of acetic acid in the acetic anhydride or of water in the pyridine, or longer reaction time, led to hydrolysis of the isopropylidene residue and formation of triacetyluridine.

5'-O-Acetyluridine.—(a) The above isopropylidene acetate (1.9 g.) was refluxed in aqueous acetic acid (75 c.c. of 20%) for 1.3 hr. Removal of solvents under reduced pressure and evaporation of the residual gum with ethanol gave a product which after two recrystallisations from ethanol was obtained as rectangular rods and had m. p. 160—162° (1.3 g.). This material was satisfactory for further work but paper chromatography revealed traces of uridine in it, which could not be removed by repeated recrystallisation. Pure *5'-O-acetyluridine* was obtained by countercurrent distribution in the ethyl acetate–water system. It then melted at 163—164° and had R_p 0.66 (Found: C, 45.7; H, 5.6; N, 9.5. $C_{11}H_{14}O_7N_2$ requires C, 46.2; H, 4.9; N, 9.8%). Light absorption in 95% EtOH: λ_{max} , 261 m μ , λ_{min} , 230 m μ (ϵ 8467, 1702 respectively). This substance has previously been described as a glass prepared from benzylideneuridine.^{18,19}

(b) Triacetyluridine (6 g.) was dissolved in cold methanol (2 litres), and saturated methanolic ammonia (40 c.c.) added. After 1.5 hr. a stream of air was passed through the solution to remove most of the ammonia, and the solution was then concentrated under reduced pressure. The residue contained only uridine and *5'-acetyluridine*, as shown by paper chromatography. These were separated by countercurrent distribution (20.5 c.c. phases; 100 stages), uridine appearing in tubes 1—3 and *5'-O-acetyluridine* in tubes 6—11. The latter (1.92 g.), recrystallised from 95% ethanol, had m. p. and mixed m. p. 163—164°.

3':5'-Di-O-acetyluridine.—*5'-O-Acetyluridine* (6.1 g.) was dissolved in anhydrous pyridine (50 c.c.), and freshly distilled acetic anhydride (2.18 c.c.; 1.1 mol.) added to the solution, which was then set aside at room temperature overnight. Ethanol (50 c.c.) was added and solvents were removed under reduced pressure below 55°. Evaporation with additions of ethanol removed last traces of pyridine and acetic acid. The residue, dissolved in water saturated with ethyl acetate, was placed in the first 3 tubes (20.5 c.c. phase) of a countercurrent distribution apparatus and submitted to 100 transfers with the ethyl acetate–water solvent system. Tubes 5—14 contained *5'-acetate* (recovered, 2.5 g.), 25—46 contained diacetate, and 70—91 contained uridine triacetate (2.13 g.), the distribution being found by estimating the optical density (at 260 m μ) of diluted samples from each tube.

The contents of tubes 25—46 were pooled and evaporated under reduced pressure. The residual oil was dissolved in ethanol (5 c.c.) and set aside for several days in the refrigerator; colourless needles of *3':5'-di-O-acetyluridine* (1.18 g.) separated. Recrystallised from ethanol the diacetate had m. p. 138—140° after sintering at 135°, and R_p 0.83 (Found: C, 47.8; H, 4.5; N, 8.7. $C_{13}H_{16}O_8N_2$ requires C, 47.5; H, 4.9; N, 8.5%). It was easily soluble in water and pyridine, fairly readily in chloroform and ethanol and sparingly in ethyl acetate and benzene.

Uridine-2' Phosphate.—A solution of *3':5'-di-O-acetyluridine* (1.0 g.) in dry pyridine (20 c.c.) was treated for 4 hr. at –30°, moisture being excluded, with dibenzyl phosphorochloridate prepared from dibenzyl phosphite (4 g.; 5 mol.), then set aside overnight at room temperature. Crushed ice and sodium hydrogen carbonate were added, the solution was extracted with chloroform (150 c.c.), and the extract washed with water and dried (Na_2SO_4). Chromatography showed the presence of unchanged diacetyluridine together with a phosphorus-containing substance, presumably its 2'-(dibenzyl phosphate) (R_p 0.97). Evaporation of the chloroform and removal of last traces of pyridine gave a gum which was hydrogenated overnight in 75% ethanol (30 c.c.) over a mixture of palladium oxide and palladised charcoal. Paper chromatography showed the appearance of a new substance, presumably *3':5'-di-O-acetyluridine-2' phosphate* (R_p 0.70). The solution was concentrated and ethanolic ammonia (100 c.c.) added. After 12 hr. solvent was removed and the residue dissolved in water. Paper chromatography showed the presence of uridylic acid in addition to uridine. A portion of the solution was brought to

¹⁸ Michelson and Todd, *J.*, 1949, 2476.

¹⁹ Brown, Haynes, and Todd, *J.*, 1950, 3299.

pH 10 and analysed by ion-exchange chromatography on a column (15 × 1 cm.) of Dowex-2 resin (formate).⁸ A single peak was observed corresponding in position to that of uridylic acid *a*, established by prior standardisation with authentic samples of the *a* and *b* acids.

*O*²: 2'-cycloUridine.—The above diacetate (0.2 g.) and toluene-*p*-sulphonyl chloride (0.51 g., 4.4 mol.) were dissolved in dry pyridine (10 c.c.). After 15 hr. at room temperature the solution was poured on crushed ice (100 g.), with stirring. After 2 hr. the mixture was extracted with chloroform, and the extract washed with sodium hydrogen carbonate solution and water and then evaporated under reduced pressure. Evaporation with ethanol removed residual pyridine, giving a yellow oil. This was dissolved in hot ethanol (charcoal) and filtered. The colourless filtrate was concentrated and on cooling deposited a waxy solid (0.21 g.) which was clearly the required toluene-*p*-sulphonate as it ran as a single spot on paper chromatograms (*R*_F 0.97). Further purification could not be effected.

A solution of the solid (0.2 g.) in methanol (15 c.c.) was mixed with saturated methanolic ammonia (15 c.c.) and set aside at room temperature overnight. Removal of solvent gave a crystalline residue which was triturated with ethanol (4 c.c.) and collected. Recrystallisation from ethanol gave *O*²: 2'-cycloUridine as colourless rods (0.12 g.), m. p. 234–236° (decomp.), *R*_F 0.51. The substance, which was easily soluble in water, gave a negative periodate-Schiff reaction with the reagent of Buchanan, Dekker, and Long⁹ (Found: C, 47.5; H, 4.9. N, 12.3. C₉H₁₀O₅N₂ requires C, 47.8; H, 4.5; N, 12.4%). Light absorption in water: λ_{max}, 249.5–251, 223–223.5 mμ, λ_{min}, 234 mμ (ε_{max}, 7860, 7860; ε_{min}, 5990).

The alcoholic mother-liquors from a number of preparations were mixed and evaporated. Ammonium toluene-*p*-sulphonate crystallised and the acid was characterised as its *S*-benzylthiuronium salt, m. p. and mixed m. p. 180°.

5'-*O*-Acetyl-2'-*O*-toluene-*p*-sulphonyluridine.—Toluene-*p*-sulphonyl chloride (2.02 g., 1.1 mol.) was added to a solution of 5'-*O*-acetyluridine (2.76 g.) in dry pyridine (15 c.c.), and the mixture set aside overnight. Ethanol (40 c.c.) was added, solvents were removed, and water was added to the red viscous residue. After 12 hr. the pale yellow solid was collected, washed with water, and triturated with ethanol (10 c.c.), and the colourless crystalline product collected. 5'-*O*-Acetyl-2'-*O*-toluene-*p*-sulphonyluridine separated from ethyl acetate as thin colourless plates (1.93 g.), m. p. 173–175°, *R*_F 0.95 (Found: C, 49.0; H, 4.2; N, 6.6. C₁₈H₂₀O₈N₂S requires C, 49.1; H, 4.6; N, 6.4%). Light absorption in 95% EtOH: λ_{max}, 260, 226.5–227.5 mμ, λ_{min}, 243–244 mμ (ε_{max}, 8280, 13,300; ε_{min}, 5680).

Treatment of the toluene-*p*-sulphonate (150 mg.) with methanolic ammonia, as before, yielded *O*²: 2'-cycloUridine (75 mg.) in rods, m. p. and mixed m. p. 234–236° (decomp.) (Found: C, 48.2; H, 4.7; N, 12.4%). Its identity with the material prepared as above was further established by comparison of infrared spectra and by X-ray crystallographic unit-cell determination kindly carried out by Dr. M. M. Woolfson, Cavendish Laboratory, Cambridge.

3-β-D-Arabinofuranosyluracil (*Spongouridine*).—A solution of *O*²: 2'-cycloUridine (300 mg.) in 0.1N-sulphuric acid (15 c.c.) was heated at 100° for 1 hr., cooled, and passed through a column (6 × 1.5 in.) of Amberlite IR-4B resin (OH form). The column was washed with distilled water (350 c.c.), and eluate and washings were taken to dryness under reduced pressure. The colourless glassy residue was dissolved in hot anhydrous methanol (5 c.c.). On cooling, the *arabinoside* separated as colourless prisms (112 mg.) which had m. p. 220–221° after one further recrystallisation, [α]_D²⁰ +131.1° (c 0.63 in H₂O) (Found: C, 43.9; H, 5.2; N, 11.4. C₉H₁₂O₆N₂ requires C, 44.3; H, 5.0; N, 11.5%). Light absorption in H₂O: λ_{max}, 262.5–263.5 mμ, λ_{min}, 230–231 mμ (ε_{max}, 10,500; ε_{min}, 2000).

On paper chromatograms, it had the same *R*_F value (0.52) as uridine but did not give a positive periodate-Schiff spray reaction. It travelled on paper electrophoresis (230 v) in 0.1M-sodium tetraborate at 0.19 times the rate of uridine. The infrared spectrum in Nujol mulls differed from that of uridine.

Spongouridine, kindly supplied by Dr. D. F. Burke, was compared with the above *arabinoside*. The m. p. and mixed m. p. was 220–221°. They behaved identically on paper chromatograms and on paper electrophoresis. Infrared spectra were identical.

2': 3': 5'-Tri-*O*-acetyl-3-β-D-arabinofuranosyluracil.—The synthetic *arabinoside* (90 mg.) was acetylated with acetic anhydride and pyridine in the usual way. The *triacetate* crystallised from dilute ethanol in thin plates, m. p. 129–130° depressed to 105–115° in admixture with uridine *triacetate* (Found: C, 48.9; H, 5.3; N, 7.7. C₁₅H₁₈O₉N₂ requires C, 48.7; H, 4.9; N, 7.6%). It had *R*_F, 0.93.

D-Arabinose Toluene-*p*-sulphonylhydrazone.—The general method of Easterby *et al.*²⁰ was

²⁰ Easterby, Hough, and Jones, *J.*, 1951, 3416.

followed. A solution of D-arabinose (100 mg.) in methanol (10 c.c.) was mixed with toluene-*p*-sulphonylhydrazine (700 mg.) in methanol (10 c.c.) and heated for 1 hr. On concentration to 3 c.c. and cooling to 0°, crystals separated. The *hydrazone* crystallised from methanol in thick prisms, m. p. 149–150° (Found: C, 45.4; H, 5.7; N, 8.6. $C_{12}H_{18}O_6N_2S$ requires C, 45.3; H, 5.7; N, 8.8%).

D-Arabinose from the Synthetic Uracil Arabinoside.—(a) The synthetic arabofuranosyluracil (100 mg.) was hydrogenated in water (12 c.c.) and ethanol (3 c.c.) in presence of platinum oxide at room temperature and pressure. After 6 hr. 10 c.c. of hydrogen had been absorbed (theor.). The ultraviolet absorption of the solution, together with paper chromatography, showed complete conversion into the dihydro-derivative. Removal of catalyst and evaporation gave a residue which was refluxed with sulphuric acid (15 c.c. of 0.2N) for 1.5 hr. After removal of sulphate ions by barium hydroxide solution and barium ions by carbon dioxide, the solution was filtered through Hyflo-Supercel, and studied on paper chromatograms. In three solvent systems (a) butan-1-ol–water, (b) butan-1-ol–acetic acid–water (5:2:3), and (c) the upper layer of pyridine–ethyl acetate–water (1:2:2), the sugar present corresponded to arabinose and was distinguishable from ribose, xylose, and lyxose. Paper electrophoresis in 0.1M-borate buffer also confirmed the identity of the sugar.

(b) The above catalytic reduction is rather capricious and the following chemical method²¹ is preferable. The arabinoside (300 mg.) was finely powdered and added to liquid ammonia (50 c.c.) with stirring. Methanol (10 c.c.) and sodium (0.3 g.) were added alternately in small quantities during 10 min. After evaporation of the ammonia and methanol, the residue was dissolved in water (10 c.c.), and the solution passed through a column (8 × 1 in.) of Dowex-50 resin (H form). The column was washed with distilled water, and eluate and washings were taken to dryness. Paper chromatograms showed the presence of arabinose in the residue, which was then treated with *p*-bromophenylhydrazine in methyl cellosolve–2N-acetic acid,²¹ and the osazone was isolated. It had m. p. 171–173° (decomp.), alone or in admixture with authentic D-arabinose *p*-bromophenylosazone.

In a second experiment the sugar was treated with toluene-*p*-sulphonylhydrazine. The product had m. p. and mixed m. p. 149–150°.

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²¹ Brown, Magrath, and Todd, *J.*, 1954, 1442.