693. *The Configuration of the* 2-C-*Hydroxymethylpentonic Acids.*

By R. J. Ferrier.

2-*C*-Hydroxymethyl-L-*ribo*-, -L-*arabino*-, -D-*xylo*-, and -D-*lyxo*-pentono-γ-lactone have been prepared and characterised. The configurations previously assigned to hamamelose and other 2-*C*-hydroxymethyl-carbohydrates from optical-rotational evidence are confirmed.

The four 2-hydroxymethylpentonic acids which are potentially separable by chromatography were required as their lactones for comparison with compounds derived from photosynthetic intermediates that were tentatively assigned to this class.[1] The reactions here employed to yield the required branched-chain acids have been reported previously,[2] but only the phenylhydrazides of the products have been fully described and their stereochemical relations have not been ascertained. We have avoided the use of these phenylhydrazides as we found them to be unsuitable crystalline derivatives.

The ketopentoses, D-xylulose (D-*threo*-pentulose) and L-ribulose (L-*erythro*-pentulose)

[1] Moses and Calvin, *Proc. Nat. Acad. Sci. U.S.A.*, 1958, **44**, 260.
[2] Schmidt and Heintz, *Annalen*, 1934, **515**, 77.

obtained by the oxidation of D-arabitol and ribitol with *Acetobacter suboxydans*,[3] were treated with aqueous sodium cyanide, the pH being maintained at 9·3 by the addition of glacial acetic acid.[4] The solutions were made alkaline and heated under a current of air till the cyanohydrins were hydrolysed (*i.e.*, till all ammonia had been evolved). Removal of the contaminating ions and solvent gave mixtures of syrupy lactones.

The products from L-ribulose yielded crystalline derivatives (acetates A and B) on acetylation with acetic anhydride in pyridine solution. These were separated by gas–liquid partition chromatography [5] and were thus shown to be present in the ratio A : B = 2·0 : 1. The two acetates were also separated by fractional crystallisation but only small amounts of the minor component (B) were obtained because of co-crystallisation of the isomeric pair. Acetates A and B, on deacetylation, gave lactones A and B which ran together (and with the unacetylated mixture) in most chromatographic solvents but at different rates in a phenol–water solvent. The possibility that the pair are γ- and δ-lactones of one acid is precluded by their regeneration in chromatographically unchanged and separable form from the derived sodium salts. Barker, Bourne, Pinkard, and Whiffen [6] have shown γ- and δ-lactones of sugar acids have absorptions in the regions 1760—1790 and 1720—1760 cm.$^{-1}$, respectively, and so lactones A and B which have carbonyl absorption at 1782 and 1780 cm.$^{-1}$, are both five-membered. For similar reasons acetates A and B are believed to possess five-membered rings. Lactone A was smoothly converted into acetate A (gas-chromatographically pure) under the conditions used in the original acetylation, so isomerisation did not occur at that stage. The infrared spectrum of lactone A was unaltered when it was regenerated from the derived sodium salt. This, and the equivalent weight of the compound, eliminate the possibility that methyl esters were formed during the deacetylation. Methyl D-arabonate was found to have an absorption maximum at 1731 cm.$^{-1}$.

The lactones derived from D-xylulose were readily separated chromatographically into pure lactones C and D which were regenerated with the same chromatographic properties from their derived salts. Infrared spectroscopy again indicated that they had the γ-lactone structure.

The yields of formaldehyde obtained from the four lactones A—D on complete oxidation with periodate showed that they all possessed the required branched structures.

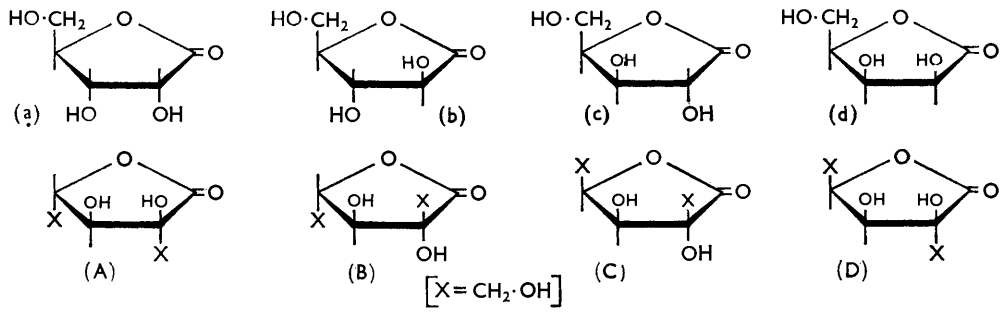
With the four known crystalline D-pentonic acid γ-lactones as models, it was found (see Table) that *cis-vic*-diol systems were much more readily oxidised by periodate than were the *trans*-isomers. The Table shows that lactones A and D contain a 1,2-*cis*-diol group and they are therefore assigned the indicated *ribo*- and *lyxo*-configuration, respectively. It is of interest that in both the pentono-γ-lactone and the 2-hydroxymethylpentono-γ-lactone series the isomers with the *arabino*-configuration are less readily oxidised than the *xylo*-compounds, and ribonolactone less readily than lyxonolactone. It is suggested that in the more readily oxidised compounds the transition state is stabilised by the 4-hydroxymethyl group. Differences in the rates of oxidation of the branched lactones are large, so the presence of a primary-tertiary system [$C_{(2)}(OH)$–CH_2·OH] does not invalidate this method of differentiating between cyclic *cis*- and *trans*-diols. This is in accord with the results of Woods and Neish [7] who showed that (*a*) the exocyclic diol system of D-α-fructoheptonic lactone (I) is initially attacked almost specifically by the periodate ion, and (*b*) the exocyclic diol and the *cis*-2,3-diol in D-mannono-γ-lactone (II) react at similar rates. In addition we have shown that several acyclic 1,2-diol systems reduce periodate at about the same rate as γ-lactones with *cis*-1,2-glycol groups (and therefore much more rapidly than the corresponding *trans*-systems). The relative

[3] Moses and Ferrier, *Biochem. J.*, 1962, **83**, 8; Hann, Tilden, and Hudson, *J. Amer. Chem. Soc.*, 1938, **60**, 1201.
[4] Gorin and Perlin, *Canad. J. Chem.*, 1958, **36**, 480.
[5] Ferrier, *Chem. and Ind.*, 1961, 831.
[6] Barker, Bourne, Pinkard, and Whiffen, *Chem. and Ind.*, 1958, 658.
[7] Woods and Neish, *Canad. J. Chem.*, 1954, **32**, 404.

Properties of the γ-lactones.

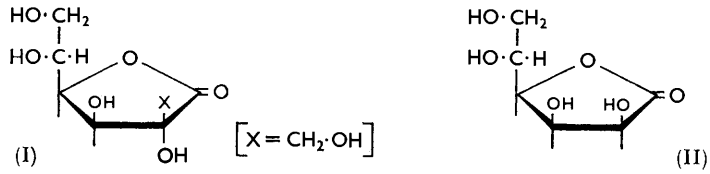
	D-ribono-	D-arabono-	D-xylono-	D-lyxono-	A	B	C	D
Time (min.) *	23	750	300	15	9	850	325	9
$10^3k/2{\cdot}303$ †	70	41	130	70	91	24	119	44
Mobility: ‡ system A	0·92	1·00	0·99	0·76	0·85	0·86	1·00	0·74
system B	0·97	0·94	0·95	1·00	0·99	0·92	0·96	1·00
Structure	a	b	c	d	A	B	C	D

* Time for redn. of 0·5 mol. of $5{\cdot}6 \times 10^{-5}$M-periodate at 25°. † k = First-order velocity constant for hydrolysis at pH 8 and 37° (min.$^{-1}$). ‡ Chromotographic mobility relative to the fastest in each series: A = BuOH–EtOH–H_2O; B = PhOH–H_2O.



resistance to oxidation of lactones B and C indicates that in the oxidation solutions they had not undergone hydrolysis and that they did not contain a *cis*-1,2-diol.

Steric interactions between substituents on lactone rings could be expected to influence their stability towards hydrolysis, but it was shown that other important factors,



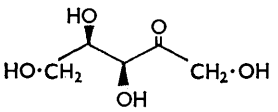
presumably dependent chiefly on hydrogen bonding, effected the rates of ring opening with alkali, so that kinetic studies of these rates were alone insufficient as a basis for configurational assignment (see Table). The measurements did, however, show that in both the pentono-γ-lactone and the branched-chain γ-lactone series the compounds with the *arabino*- and the *lyxo*-configuration were more stable than their epimers. The *lyxo*-isomers in particular are relatively more stable than would be expected on purely steric grounds.

Chromatographic properties are also distinctive (see Table). In a neutral solvent the isomers with the *arabino*- and *xylo*-configuration move faster than their epimers; however, the converse holds in a phenolic solvent (inversion of relative mobilities of carbohydrate derivatives has been noted before [8] when phenol-containing solvents have been used).

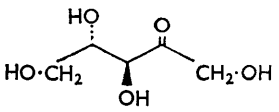
The proportions of lactones A and B, and C and D, obtained during the syntheses from ribulose and xylulose, respectively, were next determined by carrying out the addition with $K^{14}CN$. These were found to be 2·0 : 1 (which confirms the ratio obtained by analysis of the acetates) and 3·5 : 1, respectively, and are consistent with the results of attack by the cyanide ion on the acyclic ketopentoses.[9] The shielding of one side of the carbonyl grouping to approach by the nucleophile is greater in xylulose than in ribulose; in the latter the two secondary hydroxyl groups are on opposite sides of the planar carbon chain, and therefore stereospecificity during addition would be less. In both cases the

[8] Isherwood and Jermyn, *Biochem. J.*, 1951, **48**, 515.
[9] Ferrier and Overend, *Quart. Rev.*, 1959, **13**, 265.

protective effect of the 3-hydroxyl group would be expected to exceed that of the 4-hydroxyl group and so the isomers with *trans*-2,3-diol systems in the derived 2-carboxypentitols (*i.e.*, *cis*-diols in the 2-*C*-hydroxymethylpentonic acids; Fischer projection formulæ) would be expected to predominate.



D-Xylulose



L-Ribulose

Lactone A corresponded chromatographically with the lactone of hamamelonic acid which had been prepared from natural hamamelose and by Burton, Overend, and Williams [10] from their configurationally characterised synthetic L-hamamelose; lactone A, moreover, yielded ammonium L-hamamelonate. Lactone C was identical with Woods and Neish's 2-*C*-hydroxymethyl-D-xylono-γ-lactone.[7] These findings indicate the correctness of the configurations assigned previously to hamamelose [2,11] and the substituted xylonic acid [7] on the basis of optical-rotational rules. Further, the configurations of the 2-*C*-hydroxymethyl-D-*arabo*-hexonic acids [7,11] (D-α- and D-β-fructoheptonic acid) are confirmed, since the 2-*C*-hydroxymethyl-D-xylono-γ-lactone was derived after the removal of C-6 from the D-*gluco*-isomer. Sowden and Strobach [12] have recently shown by chemical means that α-D-glucosaccharinic acid is 2-*C*-methyl-D-ribonic acid and have so clarified an issue which was not readily resolvable by reference to optical-rotational evidence.[13]

Experimental

Paper partition chromatography was carried out with the systems (A) Whatman No. 1 paper (untreated), butan-1-ol–ethanol–water (4 : 1 : 5 v/v; upper layer), and (B) Whatman No. 4 paper (washed with oxalic acid), phenol–water (72 : 28 w/w). Optical rotations were measured at 24° ± 2°.

L-*Ribulose*.—Ribitol (adonitol) was subjected to bacterial oxidation by *Acetobacter suboxydans* which converted the pentitol quantitatively into the pure pentulose in the absence of buffer or growth factors. The substrate (5 g.) was incubated for 3 days at 28° with cells (about 300 mg. dry wt.) suspended in distilled water (150 ml.) under oxygen. The reaction was shown to be complete by the magnitude and constancy of the optical rotation of the solution after removal of the cells at the centrifuge. Evaporation of the supernatant liquid gave L-ribulose, $[\alpha]_D$ +16·2° (*c* 2 in H_2O), as a pale yellow, chromatographically pure syrup, identical in mobility (system A) with ribulose, $[\alpha]_D$ +16·6° (in H_2O), prepared by isomerisation of L-arabinose.[14]

D-*Xylulose*.—This ketose was prepared similarly by bacterial oxidation of D-arabitol. The product had $[\alpha]_D$ −29° (*c* 2 in H_2O) [Levene and Tipson give $[\alpha]_D$ −33° (*c* 2 in H_2O) for D-xylulose [14]] and was found to contain small amounts of an aldopentose (probably xylose) as impurity when examined chromatographically. Pure xylulose, $[\alpha]_D$ −32·7° (*c* 1·5 in H_2O), was obtained by conversion of the crude product into its mono-*O*-isopropylidene derivative m. p. 67—68°, $[\alpha]_D$ −6·6° (*c* 6 in H_2O), and subsequent removal of the ketal ring. Mono-*O*-isopropylidene-D-xylulose is reported [14] as having m. p. 70—71°, $[\alpha]_D$ −6·5 (*c* 2 in H_2O).

Treatment of L-*Ribulose with Sodium Cyanide*.—A solution of sodium cyanide [2·1 g. (1·3 mol.) in water (100 ml.)] was added with stirring to L-ribulose [5·0 g. (1·0 mol.) in water (100 ml.)]. The pH was maintained at 9·3 by addition of glacial acetic acid [2·0 ml. (1·0 mol.) was required]. The solution was then made 0·1N in sodium hydroxide and heated on a steam-bath under an air-current till all the ammonia had been evolved (about 5 hr.). Omission of this procedure gave products containing large proportions of unhydrolysed cyanohydrins. After cooling of the solution the cations were removed by passage down a column of Dowex 50 cation-exchange

[10] Burton, Overend, and Williams, *Proc. Chem. Soc.*, 1962, 181.
[11] Schmidt and Weber-Molster, *Annalen*, 1934, **515**, 43.
[12] Sowden and Strobach, *J. Amer. Chem. Soc.*, 1960, **82**, 3707.
[13] Sowden, *Adv. Carbohydrate Chem.*, 1957, **12**, 35.
[14] Levene and Tipson, *J. Biol. Chem.*, 1936, **115**, 731.

resin (H^+ form). Evaporation of the eluate gave a clear pale yellow syrup which was redissolved in water (100 ml.) and again taken to dryness. Heating the resultant syrup under a vacuum at 60° for 3 hr. gave a product (5·2 g., 88%) containing free hexonic acid [2% (direct titration); N, <0·3%; equiv., 178 (hexonic acid lactone 178)]. Chromatography in system A revealed one lactone component (hydroxylamine–ferric chloride spray [15]).

Tetra-O-*acetyl*-2-C-*hydroxymethyl*-L-erythro-*pentonolactones*.—The lactone mixture (3·5 g.) obtained from L-ribulose was dissolved in pyridine (20 ml.) and left overnight at room temperature with acetic anhydride (15 ml.). Pouring the mixture on ice gave a syrup, part of which was removed, washed with ice–water, and dried, while the remainder was allowed to crystallise during 2 hr. The syrupy sample was shown by gas–liquid partition chromatography [Wilkins "Aerograph" instrument; column, 0·6 cm. internal diameter; Apiezon L grease (20%) on fire brick; 200°; helium carrier gas, 75 ml. per min.] to contain two compounds (I and II) having respective retention times of 25·5 and 19·8 min. Areas under the peaks showed that I and II were present in the ratio 2·0 : 1. The solid product (2·5 g.) was fractionally crystallised from ethanol and two fractions (A, 1·0 g.; B, 0·1 g.) were obtained which were each shown to be pure by gas-chromatography and to correspond respectively with components I and II. The remaining unresolved fraction crystallised repeatedly from ethanol as a mixture of A and B (gas-chromatography).

Acetate A, later shown to be *tetra*-O-*acetyl*-2-C-*hydroxymethyl*-L-*ribono*-γ-*lactone*, after two recrystallisations from ethanol, had m. p. 102—103°, $[\alpha]_D$ −101° (*c* 1·6 in $CHCl_3$) (Found: C, 48·6; H, 5·4; Ac, 50·2. $C_{14}H_{18}O_{10}$ requires C, 48·5; H, 5·2; Ac, 49·7%). Acetate B (*tetra*-O-*acetyl*-2-C-*hydroxymethyl* L-*arabono*-γ-*lactone*), crystallised from ethanol, had m. p. 92—93°, $[\alpha]_D$ −21° (*c* 1·2 in $CHCl_3$) (Found: C, 48·5; H, 5·3; Ac, 48·4%). Acetates A and B had strong broad absorption bands at 1745—1765 and 1737—1756 (ester C=O) and sharp medium bands at 1800 and 1787 $cm.^{-1}$, respectively (γ-lactone C=O).

Deacetylation of Acetates A and B.—The acetates were left overnight at room temperature with methanolic 0·02N-sodium methoxide. One volume of water was added to each solution which was then treated with Dowex-50 resin (H^+ form). The solvents were removed and the residual colourless syrups were heated for 3 hr. at 60° under a vacuum to promote lactonisation. Yields were >95%.

2-C-*Hydroxymethyl*-L-*ribono*-γ-*lactone* (*Lactone A*).—After several months the *lactone* had crystallised completely. Recrystallisation from acetonitrile–chloroform gave plates, m. p. 88—89°, $[\alpha]_D$ −74° (*c* 2·1 in H_2O) (Found: C, 40·2; H, 5·7%; equiv., 178. $C_6H_{10}O_6$ requires C, 40·4; H, 5·7%; equiv. 178), $\nu_{max.}$ 1782 $cm.^{-1}$ (lactone C=O). Complete oxidation with periodate yielded 1·95 mol. of formaldehyde.[16] Adding an excess of aqueous ammonia to an aqueous solution of the lactone caused an immediate change in the optical rotation, finally to $[\alpha]_D$ +4·8° (*c* 1·2 in aq. N-NH_3) for the ammonium salt. The crystalline ammonium salt was isolated and after recrystallisation from aqueous ethanol had m. p. 150—151°. Freudenberg and Blummel [17] give $[\alpha]_D$ −3·9°, m. p. 152°, for ammonium D-hamamelonate. The lactone was indistinguishable chromatographically (system B) from the lactone of hamamelonic acid obtained by oxidation of natural hamamelose with bromine water and also from the hamamelonic lactone of Burton, Overend, and Williams.[10] The ammonium salt obtained by these authors caused no depression in m. p. of our salt.

2-C-*Hydroxymethyl*-L-*arabono*-γ-*lactone* (*Lactone B*).—The syrup had $[\alpha]_D$ −57° (*c* 1·6 in H_2O), equiv. 184, and $\nu_{max.}$ 1780 $cm.^{-1}$ (C=O), and was shown by titration to contain a negligible amount of free acid. Formaldehyde (1·94 mol.) was produced on oxidation with periodate. Hydrolysis with aqueous ammonia gave a solution of ammonium 2-*C*-hydroxymethyl-L-arabonate, $[\alpha]_D$ −11·5° (*c* 1·2 in aq. N-NH_3); no crystalline salt was isolated. The lactone was indistinguishable chromatographically in system A from lactone A but the two were resolved in system B (see Table).

Tetra-O-*acetyl*-2-C-*hydroxymethyl*-L-*ribono*-γ-*lactone from Lactone A*.—Lactone A and acetic anhydride in pyridine gave a product which was gas-chromatographically pure and corresponded in retention volume to acetate A. Crystallisation from ethanol gave tetra-*O*-acetyl-2-*C*-hydroxymethyl-L-ribono-γ-lactone, $[\alpha]_D$ −102° (*c* 1·6 in $CHCl_3$), m. p. 103—104° alone or mixed with acetate A.

[15] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 5859.
[16] O'Dea and Gibbons, *Biochem. J.*, 1953, **55**, 580.
[17] Freudenberg and Blummel, *Annalen*, 1924, **440**, 45.

Treatment of D-*Xylulose with Sodium Cyanide.*[4]—Pure D-xylulose (0·76 g.) was treated with sodium cyanide (titration of the liberated alkali indicated complete reaction), and the lactone mixture was prepared as described above. Chromatography in system A revealed two lactones (C and D) having relative mobilities 1 : 0·74 (lactones A and B travelled at an intermediate rate; see Table). Efforts to prepare suitable derivatives failed. The lactones were separated on a column of cellulose powder (solvent A) and finally obtained pure after chromatography on Whatman No. 3 MM paper (system A).

2-C-*Hydroxymethyl*-D-*xylono*-γ-*lactone* (*Lactone C*).—The fraction which travelled faster on chromatograms crystallised gradually and was purified by washing it with ethanol on a porous tile. It had m. p. 117—119°, $[\alpha]_D$ +106° (*c* 0·4 in H_2O). Wood and Neish [7] report m. p. 120°, $[\alpha]_D$ +107° (*c* 0·3 in H_2O), for their 2-*C*-hydroxymethyl-D-xylono-γ-lactone. Their compound caused no depression in the m. p. of lactone C and the two were identical chromatographically (system A). Lactone C had an absorption maximum at 1775 cm.$^{-1}$ and liberated 1·92 mol. of formaldehyde on complete periodate oxidation. Ammonium 2-*C*-hydroxymethyl-D-xylonate, prepared in solution by dissolving lactone C in aqueous ammonia, had $[\alpha]_D$ −12·7° (*c* 1·5 in aq. N-NH_3). Woods and Neish give −14·2° (*c* 1·3 in H_2O).

2-C-*Hydroxymethyl*-D-*lyxono*-γ-*lactone* (*Lactone D*).—The chromatographically slower lactone prepared from D-xylulose did not crystallise; it had $[\alpha]_D$ +46° (*c* 2·3 in H_2O), equiv. 180, and $\nu_{max.}$ 1778 cm.$^{-1}$. Complete oxidation with periodate liberated 1·85 mol. of formaldehyde. The ammonium salt produced by dissolving lactone D in aqueous ammonia had $[\alpha]_D$ −2·2° (*c* 3 in aq. N-NH_3).

Measurement of Initial Rates of Oxidation of the Lactones.—The periodate consumption of the lactones was followed spectrophotometrically [18] in 4·7 × 10^{-5}M-aqueous solutions containing 1·2 mole of sodium metaperiodate per mole of lactone. At this concentration and at 25° the rates were convenient and the optical densities of the solutions could be measured without dilution.[19]

Measurement of the Rates of Hydrolysis of the Lactones.—The rates were measured at 37° by automatic addition to pH 8·0 of 0·0455N-aqueous sodium hydroxide to the lactone solutions (a solution of 8·4 × 10^{-7} mole in water, initially brought to pH 8·0). The first-order velocity constants were calculated from the automatically recorded plots of titres against time and are the average of two determinations (maximum discrepancy 7%).

Addition of $K^{14}CN$ *to Ketopentoses.*—The two ketopentoses (2 mg.) in sodium acetate buffer (0·05 ml.; 0·6M; pH 9·3) were mixed with solutions of potassium cyanide (1 mg.; 2·0 μc/mg.; in 0·05 ml. of the same buffer). After 1 hr., the solutions were made 0·1N in sodium hydroxide and heated on a steam-bath for 2 hr. The cations were removed with Dowex 50 resin, and the anions by repeated evaporation to dryness with water. After chromatographic separation of the lactones which were applied to the papers from hydrochloric acid solution, detected by radioautography, and identified by their mobilities relative to known non-radioactive markers, their relative abundances were determined by direct counting of both sides of the appropriate areas of the chromatograms, a Scott tube being used (counting time 1 min.).

(*a*) L-*Ribulose.* The derived lactones were separated in the conditions employed for the non-radioactive isomers and together comprised 94% of the radioactive material on the chromatograms. The two lactones were incompletely resolved but the slight overlap caused an error of <5% in the ratio of their abundances.

(*b*) D-*Xylulose.* Although the radioactive lactones C and D were completely resolved, other unidentified components contributed 18% to the total activity on the paper.

The author is indebted to Professor Melvin Calvin, who suggested this investigation, for his interest and for laboratory facilities, to Dr. N. R. Williams for informing him of his related synthetic work and for samples, and to Dr. A. C. Neish for a specimen of his 2-*C*-hydroxymethylxylono-γ-lactone. The work was sponsored by the U.S. Atomic Energy Commission and was carried out during the tenure of a N.A.T.O. Fellowship awarded by D.S.I.R. The Governors of Birkbeck College are thanked for granting leave of absence.

LAWRENCE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA,
BERKELEY 4, CALIFORNIA, U.S.A.
[Permanent address: DEPARTMENT OF CHEMISTRY, BIRKBECK COLLEGE,
MALET STREET, LONDON, W.C.1.] [*Received, March 5th,* 1962.]

[18] Aspinall and Ferrier, *Chem. and Ind.*, 1957, 1216.
[19] Dixon and Lipkin, *Analyt. Chem.*, 1954, **26**, 1092.