273. *Carbohydrate Sulphuric Esters. Part I. Glucose and Galactose Sulphates.*

By E. G. V. Percival and T. H. Soutar.

The *barium galactose monosulphate* prepared by the direct interaction of chlorosulphonic acid and galactose is shown to be different from *barium galactose* 6-*sulphate*. The velocity of hydrolysis in acid solution for six hexose and hexoside monosulphates has been determined, but the differences in rate are not sufficient to warrant the use of this method as a means of differentiation between related hexose sulphates. Alkaline hydrolysis is shown to be very rapid in the case of the reducing hexose sulphates but slow for barium diacetone galactose 6-sulphate. Two new *anhydromethylhexosides* have been isolated.

Although glucose sulphates have been investigated by Ohle (*Biochem. Z.*, 1922, **131**, 601; 1923, **136**, 428), Soda (*ibid.*, 1923, **135**, 621) and others, little attention has been paid to galactose ethereal sulphates. Hassid (*J. Amer. Chem. Soc.*, 1933, **55**, 4163) has, however, described a polygalactose ethereal sulphate derived from a species of alga and there exists evidence that Carrageen mucilage contains galactose sulphate residues (Haas and co-workers, *Biochem. J.*, 1921, **15**, 469, *et seq.*; Dillon and O'Colla, *Nature*, 1940, **145**, 749; Percival and Buchanan, *ibid.*, p. 1020), so it seemed of interest to examine the properties of simple galactose sulphates.

According to Ohle (*loc. cit.*), on treatment of monoacetone glucose and diacetone glucose respectively with chlorosulphonic acid in pyridine, the sulphate residue enters in the first case position 6 and in the second position 3, and Soda and Nagai (*J. Chem. Soc. Japan*, 1935, **56**, 1258) also consider that by direct interaction between glucose and chlorosulphonic acid in the presence of pyridine substitution occurs in the main at the primary alcohol residue, although polysulphates are also formed. The same considerations do not appear to apply to galactose, however, for the *galactose monosulphates* (A) prepared by direct treatment have different properties from similar salts (B) prepared from *barium diacetone galactose* 6-*sulphate* by hydrolysis with acetic acid (Table I).

TABLE I.

	Galactose monosulphates (A).	Galactose 6-sulphates (B).
Barium salt	$[\alpha]_D^{18°}$ +46°	$[\alpha]_D^{18°}$ +56°
Brucine salt	$[\alpha]_D^{18°}$ $-5° \longrightarrow -11°$	$[\alpha]_D^{18°}$ $+5° \longrightarrow 1°$

Furthermore it has been found possible to condense *barium galactose* 6-*sulphate* with acetone to regenerate the barium diacetone galactose 6-sulphate, whereas the barium galactose sulphate (A) under the same conditions yielded diacetone galactose.

Assuming (A) to be pyranose, it follows that the sulphate group is attached to either C_2, C_3, or C_4, since the possibility of substitution on C_1 is excluded owing to the mutarotation and the high reducing power (as estimated by alkaline hypoiodite) exhibited by the barium salt.

The rates of hydrolysis at 100° with N/10-hydrochloric acid were determined for a number of sulphuric esters as the barium salts and the mean values for the velocity constants calculated for a unimolecular reaction are recorded in Table II.

TABLE II.

	$k_{100°} \times 10^6$.		$k_{100°} \times 10^6$
Barium glucose sulphate	1635	Barium diacetone galactose 6-sulphate	1481
Barium galactose sulphate (A)	1601	Barium α-methylglucoside sulphate ...	1060
Barium galactose 6-sulphate (B) ...	1324	Barium α-methylgalactoside sulphate	1010

It is clear that the variations are not sufficient to warrant the use of this method to differentiate between different hexose sulphuric esters. Levene and Meyer (*J. Biol. Chem.*, 1922, **53**, 437) found for barium diacetone glucose 3-sulphate and barium monoacetone glucose sulphate $k_{75°} \times 10^6 = 600$ and 400 respectively in N/10-hydrochloric acid, and Soda and Nagai (*loc. cit.*) for barium glucose sulphate $k_{80°} \times 10^6 = 400$ in N/10-sulphuric acid.

Very different results are obtained on alkaline hydrolysis. Both the barium glucose monosulphate and the barium galactose monosulphates liberated barium sulphate immediately with N/10-sodium hydroxide at 100°, the reactions being complete within 5 minutes. At the same time extensive decomposition occurred, no identifiable osazones could be prepared from the hydrolysate, and an immediate precipitate of iodoform was formed in the cold on treatment with iodine and alkali. Evans and his co-workers (*J. Amer. Chem. Soc.*, 1928, **50**, 2543) have described the alkaline fission of sugars with the formation of products such as pyruvaldehyde and lactic acid, and it may be that the process is accelerated in the cases here described.

In contrast with this result, barium diacetone galactose sulphate was untouched by 2N-sodium hydroxide at 100° for several hours, but that the absence of a reducing group is not the only operating factor is shown by the fact that, although the glycoside sulphates are not hydrolysed by any means as rapidly as those of the reducing sugars, complete removal of the sulphuric ester residue is achieved within 6 hours in contact with a saturated solution of barium hydroxide at 100°. By this treatment barium α-methylglucoside sulphate yielded a product, m. p. 105—106°, $[\alpha]_D^{18^\circ}$ + 52° in water, and barium α-methylgalactoside sulphate a product, m. p. 105—106°, $[\alpha]_D^{18^\circ}$ + 50·2°, which analysis showed to be *anhydromethylhexosides*. These substances are being investigated more fully.

EXPERIMENTAL.

Barium Glucose Sulphate.—To glucose (10 g.) suspended in dry pyridine (75 c.c.), a solution of chlorosulphonic acid (3·4 c.c.) in alcohol-free chloroform (25 c.c.) was added during 20 minutes at − 10° with vigorous stirring. After standing overnight, the solvents were removed at 40°/15 mm. and the resulting syrup, dissolved in water, was shaken for 8 hours with lead oxide and barium carbonate. After filtration and extraction with ether, an excess of silver sulphate solution was added, the excess of silver ion being removed by hydrogen sulphide in the presence of barium carbonate. The filtered solution was concentrated at 40°/15 mm. to 20 c.c. and after filtration poured into alcohol (2 l.) to yield a crude salt (8·5 g.) containing *ca.* 50% of free glucose. An aqueous solution of brucine sulphate (7·3 g.) was added to a solution of this salt and after removal of barium sulphate the solution was concentrated at 40°/15 mm. until crystals appeared. Further crops were obtained on concentration and addition of acetone. The crude product was repeatedly extracted with alcohol and recrystallised from water (yield, 9·5 g.). It showed $[\alpha]_D^{18^\circ}$ −4° (5 mins. in water, *c* 0·3); −6° (24 hours) [Found: C, 53·5; H, 5·7; N, 4·5; S (hydrolysis), 4·8; (fusion) 5·0. Calc. for $C_6H_{12}O_9S,C_{23}H_{26}O_4N_2$: C, 53·2; H, 5·8; N, 4·3; S, 4·9%].

This brucine salt (8·5 g.) was decomposed by an excess of saturated barium hydroxide solution, the excess being removed by a current of carbon dioxide, followed by filtration and extraction with chloroform. Concentration, further filtration, and precipitation in alcohol yielded an amorphous, deliquescent, white solid (2·9 g.), which showed $[\alpha]_D^{14^\circ}$ + 33·4° in water (*c*, 2·4). 1 G. required 56·4 c.c. of N/10-iodine (Bergmann and Machemer, *Ber.*, 1930, **63**, 316). Calc., 61·0 c.c. [Found: Ba, 20·9; S, 9·5. Calc. for $(C_6H_{11}O_9S)_2Ba$: Ba, 20·95; S, 9·8%].

A similar experiment in which double the quantity of chlorosulphonic acid was used yielded brucine glucose sulphate (15·3 g.). $[\alpha]_D^{16^\circ}$ −4° (5 mins. in water, *c* 0·25); −6° (24 hours) (Found: C, 53·1; H, 6·0; N, 4·9%). This was converted into the barium salt (5·4 g.), $[\alpha]_D^{15^\circ}$ + 32·4° in water (*c*, 1·6) (Found: Ba, 21·9; S, 9·9%).

Barium Galactose Sulphate.—Following the above procedure, galactose (10 g.) yielded a *brucine* salt (15 g.) as before, which crystallised from water in rosettes of needles and showed $[\alpha]_D^{17^\circ}$ −5° (5 mins. in water, *c* 0·35); −11° (24 hours) [Found: C, 52·8; H, 6·0; N, 4·3; S (hydrolysis), 4·6; (fusion), 4·8. $C_6H_{12}O_9S,C_{23}H_{26}O_4N_2$ requires C, 53·2; H, 5·8; N, 4·3; S, 4·9%). This compound (13 g.) yielded a *barium galactose monosulphate* (5·2 g.), which showed $[\alpha]_D^{18^\circ}$ + 46° in water (*c*, 1·7). 1 G. required 58·4 c.c. of N/10 iodine (Bergmann and Machemer, *loc. cit.*). Calc., 61·0 c.c. [Found: Ba, 21·1; S, 9·3. $(C_6H_{11}O_9S)_2Ba$ requires Ba, 20·95; S, 9·8%].

Barium Diacetone Galactose 6-*Sulphate.*—Pure diacetone galactose (10 g.) in dry pyridine (50 c.c.) was treated at −10° with chlorosulphonic acid (2·6 c.c.) in chloroform as before. Barium carbonate was added immediately, and the mixture kept overnight; the solvents were then removed at 40°/15 mm. in the presence of barium carbonate. Chlorides were eliminated as before and the aqueous solution of the barium salt finally obtained was evaporated to dryness at 40°/15 mm., and the residue dissolved in chloroform, filtered, and precipitated in light petroleum (b. p. 60—80°). The white, amorphous, non-reducing powder (4·6 g.) showed $[\alpha]_D^{14^\circ}$ −35·7° in water (*c*, 7·3); −42·4° in chloroform (*c*, 8·2) [Found: Ba, 16·8; S, 7·6. $(C_{12}H_{19}O_9S)_2Ba$ requires Ba, 16·85; S, 7·85%].

Barium Galactose 6-*Sulphate.*—Barium diacetone galactose sulphate (10 g.) was heated at 100° for 3 hours with acetic acid (200 c.c.; 1%). Evaporation to dryness at 35°/15 mm., solution of the residue in water, filtration, and precipitation in alcohol gave the crude salt (7 g.), $[\alpha]_D^{14^\circ}$ + 40° in water (*c*, 3·6) (Found: Ba, 17·5; S, 7·5%).

Brucine Galactose 6-*Sulphate.*—This was obtained as before as an amorphous powder (8·8 g.) on precipitation from aqueous solution by acetone and showed $[\alpha]_D^{18^\circ}$ + 5° (30 mins. in water, *c* 0·5); + 1° (24 hours) (Found: C, 52·8; H, 5·8; N, 4·5. $C_6H_{12}O_9S,C_{23}H_{26}O_4N_2$ requires C, 53·2; H, 5·9; N, 4·3%).

On recrystallisation from aqueous acetone, transparent stout prisms were obtained which effloresced in dry air and appeared to be the *dihydrate.* They had $[\alpha]_D^{18^\circ}$ + 5° (30 mins. in water, *c* 0·5); + 1° (24 hours) (Found: C, 50·4; H, 5·9; N, 4·2. $C_6H_{12}O_9S,C_{23}H_{26}O_4N_2,2H_2O$

requires C, 50·5; H, 5·9; N, 4·1%). On treatment with barium hydroxide *barium galactose* 6-*sulphate* was obtained (2·5 g.) as a white, amorphous, deliquescent powder, $[\alpha]_D^{18^\circ}$ + 56° in water (*c*, 4·6). 1 G. required 57·9 c.c. of N/10-iodine (Bergmann and Machemer, *loc. cit.*). Calc., 61·0 c.c. [Found : Ba, 20·8; S, 9·4. $(C_6H_{11}O_9S)_2Ba$ requires Ba, 21·0; S, 9·8%].

The above barium salt (2 g.) was shaken with acetone (60 c.c.) containing sulphuric acid (0·7 c.c.) for 8 hours. After neutralisation with barium carbonate and evaporation in the presence of barium carbonate, followed by solution of the residue in chloroform and precipitation in light petroleum, barium diacetone galactose 6-sulphate, $[\alpha]_D^{16^\circ}$ −36° in water (*c*, 6·0), was obtained, from which the characteristic brucine salt, with properties identical with those described above, was isolated by suitable treatment.

On the other hand, barium galactose sulphate (B) on similar treatment underwent hydrolysis and only diacetone galactose could be isolated. Such a hydrolysis did not take place when barium diacetone galactose 6-sulphate was used.

Barium α-Methylglucoside Sulphate.—α-Methylglucoside (10 g.) in pyridine (100 c.c.) was treated with chlorosulphonic acid (6·8 c.c.) in chloroform (20 c.c.) as previously described. The white solid produced on precipitation from aqueous solution by alcohol was continuously extracted with hot alcohol and the resulting product had $[\alpha]_D^{15^\circ}$ + 90° in water (*c*, 0·4) [Found : Ba, 19·1; OMe, 8·9. Calc. for $(C_7H_{13}O_9S)_2Ba$: Ba, 19·6; OMe, 9·1%].

Barium α-Methylgalactoside Sulphate.—Prepared as above, this *salt* had $[\alpha]_D^{17^\circ}$ + 142° in water (*c*, 0·5) [Found : Ba, 19·2; OMe, 8·6. $(C_7H_{13}O_9S)_2Ba$ requires Ba, 19·6; OMe, 9·1%].

The Acid Hydrolysis of the Barium Ethereal Sulphates.—A weighed quantity of the barium salt (*ca.* 0·5 g.) was made up to 100 c.c. with hydrochloric acid (0·1012N) containing barium chloride (3%). 10 C.c. portions were introduced into tubes, which were then sealed and immersed in boiling water for the period of time indicated. At the end of each period each tube was cooled rapidly, and the barium sulphate weighed. The hydrolysis constant was calculated from $k = 1/t.\log a/(a - x)$, where a is the weight of barium sulphate that would be precipitated on complete hydrolysis and x the weight deposited in t minutes.

t.	x.	$a - x$.	$k \times 10^6$.	t.	x.	$a - x$.	$k \times 10^6$.	t.	x.	$a - x$.	$k \times 10^6$.
Barium glucose sulphate.				*Barium galactose sulphate.*				*Barium diacetone galactose 6-sulphate.*			
240	0·0229	0·01439	1723	180	0·0187	0·01751	1753	180	0·0185	0·01992	1584
300	0·0255	0·01179	1666	240	0·0219	0·01431	1680	240	0·0218	0·01662	1517
420	0·0293	0·00799	1593	300	0·0246	0·01161	1646	300	0·0247	0·01372	1489
480	0·0308	0·00649	1619	420	0·0280	0·00821	1535	360	0·0272	0·01122	1485
600	0·0334	0·00389	1636	480	0·0295	0·00671	1525	420	0·0288	0·00962	1429
720	0·0348	0·00249	1632	600	0·0318	0·00441	1524	540	0·0319	0·00652	1430
				720	0·0334	0·00281	1542	600	0·0331	0·00532	1431
		Mean	1635			Mean	1601			Mean	1481
Barium galactose 6-sulphate.				*Barium α-methylglucoside sulphate.*				*Barium α-methylgalactoside sulphate.*			
180	0·0165	0·02085	1401	120	0·0097	0·02802	1076	120	0·0095	0·0272	1090
240	0·0191	0·01825	1300	180	0·0134	0·02432	1059	180	0·0129	0·02362	1052
300	0·0221	0·01525	1299	240	0·0169	0·02082	1075	240	0·0155	0·02102	1000
420	0·0267	0·01065	1365	300	0·0201	0·01762	1103	300	0·0181	0·01842	991
480	0·0285	0·00885	1303	420	0·0244	0·01332	1076	360	0·0201	0·01642	965
540	0·0299	0·00745	1296	480	0·0249	0·01282	976	420	0·0225	0·01402	990
600	0·0312	0·00615	1305	540	0·0277	0·01002	1056	480	0·0243	0·01222	989
								540	0·0259	0·01062	993
		Mean	1324			Mean	1060			Mean	1010

Alkaline Hydrolysis.—Barium glucose monosulphate was treated at 100° with carbonate-free sodium hydroxide (0·1N). Caramelisation was rapid and quantitative experiments showed that within 5 minutes all the sulphate residue was removed. Similar results were obtained with the galactose sulphates. In no case could identifiable products be obtained on acidification and treatment with phenylhydrazine. On addition of iodine and alkali a precipitate of iodoform was produced in the cold.

Barium diacetone galactose 6-sulphate resisted hydrolysis by 2N-sodium hydroxide at 100° during 6 hours.

Alkaline Hydrolysis of Barium α-Methylglucoside Sulphate [With R. B. DUFF].—The salt (1·5 g.), dissolved in water (50 c.c.), was heated for 5 hours at 100° with barium hydroxide (15·8 g.). After filtration and treatment with carbon dioxide, followed by filtration and evaporation to dryness at 30°/15 mm., the residue was extracted with alcohol. On removal of solvent crystallisation ensued and recrystallisation from light petroleum–ethyl acetate yielded long needles of an *anhydromethylhexoside*, m. p. 105—106°, $[\alpha]_D^{18°} + 52°$ in water (*c*, 1·5) (Found : C, 48·0; H, 6·9; OMe, 17·4. $C_7H_{12}O_5$ requires C, 47·7; H, 6·9; OMe, 17·6%).

Alkaline Hydrolysis of Barium α-Methylgalactoside Sulphate [With R. B. Duff].—Similar treatment yielded, after recrystallisation from ethyl acetate–light petroleum, long needles of an *anhydromethylhexoside*, m. p. 105—106°, mixed m. p. with the corresponding product from the glucoside 65—80°; $[\alpha]_D^{18°} + 50·2°$ in water (*c*, 1·1) (Found : C, 48·1; H, 6·9; OMe, 17·4. $C_7H_{12}O_5$ requires C, 47·7; H, 6·9; OMe, 17·6%).

Thanks are expressed to the Carnegie Trust for the Universities of Scotland, the Earl of Moray Endowment, and Imperial Chemical Industries Ltd. for grants.

The University, King's Buildings, Edinburgh.

[*Received, September* 16*th*, 1940.]